Artificial microRNAs as Emerging Gene Therapy Platforms for the Treatment of Incurable Monogenic Disorders: Molecular Design and Translational Potential

Dr. Lucas Meyer[1]*
Prof. Hana Suzuki[2]

[1] University of Zurich, Institute of Molecular Medicine and Gene Therapy, Zurich, Switzerland
[2] Kyoto University, Department of Genetic Engineering and RNA Therapeutics, Kyoto, Japan

Abstract

Well-designed artificial miRNAs (amiRNAs) are as effective as short hairpin RNAs (shRNAs) but produce 10–80 times less siRNA. They enable long-term silencing and are safer than other RNAi triggers. They are suitable instruments for gene therapy techniques, especially for incurable monogenic diseases. In clinical studies, stereotactic injection of AAV5 directly into the striatum is the most effective approach. Intravenous injections would not only make patients more comfortable, but would also reduce the cost of complex brain surgery.In terms of structure, biogenesis, and expression levels, Ami RNAs are more "natural" than other gene therapy methods. They also utilise the cell's native protein machinery and do not produce irreversible alterations, unlike genome editing technologies. The amount of time spent on a technology determines its level of progression. ASOs have an edge in this regard, as seen by the number of authorized medicines. Perhaps RNAi is just around the corner.

1 Introduction

RNA interference (RNAi) is a posttranscriptional process in which small interfering RNAs (siRNAs) or endogenous microRNAs (miRNAs) interact with protein partners to control cellular gene expression by degrading complimentary transcripts and/or inhibiting translation (Bartel, 2004; Elbashir et al., 2001; Zeng et al., 2003). Researchers have utilized this natural event to build technology that allows the expression of any gene of interest to be blocked by short, tailored RNAs (McManus et al., 2002; Silva et al., 2005). Synthetic siRNAs, vector-based short hairpin RNAs (shRNAs), and artificial miRNAs (amiRNAs) are three types of RNAi tools that are processed in cells by miRNA biogenesis machinery to create mature siRNAs (Rao et al., 2009; Silva et al., 2005). RNAi triggers based on vectors differ in a number of ways. RNA polymerase III (Pol III)-driven shRNAs contain a stem-loop structure similar to natural miRNA precursors (pre-miRNAs) (Ma et al., 2014; Sheng et al., 2020). The stem of a 60 nucleotide (nt) shRNA is formed by a target-specific siRNA sequence, which is released after single-step processing in the cytoplasm by the RNase DICER. Second-generation shRNAs (Silva et al., 2005), also known as amiRNAs, miRNA mimics (Rossi, 2008), shRNA-miRs (Silva et al., 2005), or pri-miRNA-like shRNAs (Ros & Gu, 2016), enter the miRNA biogenesis pathway early and are processed by the RNases DROSHA and DICER in two steps, similar to most endogenous (McBride et al., 2008; Zeng et al., 2002). Unlike shRNAs, amiRNAs have an siRNA sequence contained inside a complex endogenous pri-miRNA backbone produced by RNA polymerase II (Pol II) promoters (McManus et al., 2002; Zeng et al., 2002). As a result of the low amounts of siRNAs, which are closely regulated by endogenous machinery, amiRNAs are regarded as safe and effective RNAi triggers (R. Boudreau, et al., 2009; McBride et al., 2008). ShRNAs and amiRNAs, which are delivered to cells as viral vectors, have a longer silencing effect than synthetic siRNAs or antisense oligonucleotides (ASOs) (Sliva & Schnierle, 2010; Van den Haute et al., 2003). Furthermore, tissue-specific RNA Pol II promoters and viral vectors with particular serotypes can be used to specifically produce amiRNAs in a tissue of interest. This property is critical in clinical applications because it decreases the risk of nonspecific effects (Evers et al., 2018; Pfister et al., 2017). Despite this, shRNAs are still the most often employed RNAi molecules in mammalian cells because of their simpler design and more predictable cellular processing effects (Moore et al., 2010). Plant research, on the other hand, has made extensive use of amiRNAs to investigate gene functions, crop improvement, and antiviral medicines (Carbonell & Dars, 2019; Schwab et al., 2006).

Studies on miRNA formation laid the framework for amiRNA design, and numerous new papers on the significance of sequence and structure in pri-miRNA processing have appeared in recent years (Fang & Bartel, 2015; Jin et al., 2020; Kwon et al., 2019; Liu et al., 2018; Wang et al., 2020). This might help to enhance amiRNA technologies, which have previously been used in biomedical research (Brendel et al., 2020; Spronck et al., 2019) and show promise in the clinic (Brendel et al., 2020). (NCT03282656, 2020; NCT04120493, 2020).

RNAi technology must address other aspects of gene therapy, such as delivery and safety, notwithstanding the obstacles of rational amiRNA creation. AmaRNAs have been effectively employed in preclinical research for a variety of illnesses, and the success of a newly launched clinical study for Huntington's disease (HD) might pave the way for future amiRNA-based treatments if they are found to be effective and safe.

We have provided a comprehensive and up-to-date review of miRNA biogenesis in order to create effective and specific amiRNAs.We compiled and synthesized research on the use of amiRNAs in the treatment of neurological illnesses, malignancies, viral infections, and other disorders. We also go through the existing constraints and hurdles that RNAi technology must overcome before amiRNAs can be successfully used in the clinic.

2 Biogenesis of miRNA

MiRNA biogenesis is a multi-step process that begins with RNA Pol II transcription of miRNA genes, which produces pri-miRNAs (Cai et al., 2004; Lee et al., 2002). Approximately half of all miRNAs now discovered are encoded in the introns of coding or noncoding genes, with exons being encoded less frequently (intragenic). Furthermore, there is a subset of intergenic miRNAs that have their own promoters and are not controlled by the host gene. MiRNA genes can be monocistronic, coding for only one miRNA, or polycistronic, coding for many miRNAs. Long pri-miRNAs have a local stem-loop structure that is processed by either a canonical or noncanonical route to produce a mature 22 nt miRNA (Bartel, 2018).

2.1 Biogenesis of canonical miRNAs

The Microprocessor complex, which consists of the nuclear RNase III enzyme DROSHA and two copies of the double-stranded RNA (dsRNA)-binding protein DiGeorge Syndrome Critical Region 8, catalyzes the first step in the canonical route (DGCR8). RIIIDa and RIIIDb, two highly conserved RNAse III domains found in DROSHA, cleave the 3p- and 5p-strands of pri-miRNAs, respectively. This phase produces pre-miRNAs, which are 70 nt stem-loop precursors with 2 nt overhangs at the 3′ end and a phosphate group at the 5′ end (Lee et al., 2003). The Exportin-5 (XPO5)/Ran-GTP complex then transports pre-miRNAs from the nucleus to the cytoplasm (Yi et al., 2003). The exact method by which pri-miRNA recognizes and selects cleavage sites is unknown. Recent research, however, has shed light on this mechanism (Kwon et al., 2019; Li et al., 2020; Nguyen et al., 2020; Roden et al., 2017). The miRNA sequence is contained within a stem-loop structure that includes a basal junction, a 13 nt lower stem, a 22 nt upper stem, and a loop. Furthermore, specific sequence patterns found in various sections of the pri-miRNA have an impact on its processing. Through its dsRNA-binding domain, DGCR8 attracts pri-miRNAs (dsRBD). Following that, DROSHA's detection of the UG motif at the basal junction and DGCR8's detection of the apical UGU motif the placement of pri-miRNAs. The cofactor SRSF3, which binds with the CNNC motif in the 3′ RNA-flanking region and recruits DROSHA to the basal junction, also plays a role in DROSHA's proper orientation (Kim et al., 2018; Nguyen et al., 2019).

The inclusion of a mismatched GHG motif (mGHG) inside the lower stem of the pri-miRNA is critical for DROSHA cleavage precision. In general, distances of 13 base pairs (bp) (on the 5′ side) and 11 bp (on the 3′ side) from the basal junction indicate the cut site. The Microprocessor identifies pri-miRNA and picks the cleavage site, according to cryo-EM structures of human DROSHA and DGCR8 in association with a pri-miRNA (Partin et al., 2020).

Pre-miRNAs are processed in the cytoplasm by a complex that includes the RNAse III-like enzyme DICER, its cofactor TAR RNA binding protein (TRBP), and protein kinase R-activating protein (PACT) (Chendrimada et al., 2005; Grishok et al., 2001; Hutvágner et al., 2001; Lee et al., 2006; Lee e In this process, DICER acts as a molecular ruler by binding the basal ends of pre-miRNA with its PAZ domain and measuring a distance of 22 nt from the 5′ phosphate end to the pre-miRNA terminal loop (Park et al., 2011). Pre-miRNAs are cleaved by two catalytic RNase III domains, which remove the loop and create an incomplete 22-nt miRNA duplex with distinctive 2 nt 3′ overhangs (Zhang et al., 2004). The RNA-induced silencing complex (RISC) is formed when the miRNA duplex is loaded onto an Argonaute protein (AGO1-4) with the help of chaperone proteins (HSC70/HSP90) (Iwasaki et al., 2010). AGO then unwinds the RNA duplex, removing the passenger strand from the complex. The strands are chosen in part depending on the thermodynamic stability of the miRNA duplex's '5' end. As a result, some miRNAs come from the 5′ ends, whereas others come from the 3′ ends. The less stable strand is loaded onto AGO first and then acts as a guide RNA, directing the activated RISC (miRISC) to the corresponding target region in mRNA (Khvorova et al., 2003; Liu et al., 2004). Translation is inhibited, mRNA is destabilized, and/or target mRNA is degraded as a result of this. The nucleotides 2–8 from the miRNA's 5′ end, known as the "seed sequence," determine the selectivity of miRISC-target interactions (Bartel, 2018).

2.2 Noncanonical miRNAs

Some miRNAs have a noncanonical biogenesis process. Mirtrons, for example, which are produced from short intronic hairpins, do not require the use of a microprocessor. Spliceosomes and debranching enzymes handle mirtrons, resulting in the generation of pre-miRNAs, which are exported to the cytoplasm by XPO5 and cut into 22 nt RNAs by DICER (Okamura et al., 2007; Wen et al., 2015). Small nucleolar RNA-derived miRNAs (snoRNAs) and tRNA-derived miRNAs are two further types of DROSHA-independent miRNAs.

Noncanonical miRNAs that are not reliant on DROSHA/DGCR8 include miR-320 and miR-484 (M. Xie et al., 2013). Endogenous short hairpin RNAs are hairpin structures formed by their transcripts (endo-shRNAs). They lack the flanking sequences that conventional pri-miRNAs have, which are useful for microprocessor identification of pri-miRNAs. Endo-shRNAs are thought to be produced directly via transcription and capped at the 5′ end, according to research. For nuclear transport, endo-shRNAs require XPO1 rather than XPO5. Only 3p- miRNAs are loaded onto AGO proteins after DICER-mediated cleavage produces 20 nt RNAs (Kim et al., 2016; M. Xie et al., 2013).

Muscle-specific miR-1, one of the most conserved miRNAs, does not require DROSHA for processing. In zebrafish, mouse, and human genomes, two copies of the miR-1 gene code for 70 nt precursors (miR-1-1 and miR-1-2), which are processed by DICER to create identical 3′ arm-derived mature miRNAs (J.-F. Chen et al., 2006; Zhao et al., 2005).

The only known example of a miRNA that can be processed without DICER is vertebrate-specific miR-451, which relies on AGO's slicing activity. The Microprocessor complex cleaves pri-miR-451 in the nucleus after transcription. This results in a hairpin with a stem length of 17 bp, which is too short for DICER to identify and cleave. The pre-miRNA is immediately loaded onto AGO2, which cleaves the stem's 3′ strand at 10/11 nt from the end (Cheloufi et al., 2010). Furthermore, translation initiation factor 1A (eIF1A) has been found to bind directly to AGO2 and enhance miR-451 biogenesis (T. Yi et al., 2015). AGO2 cleaves a 30 nt product, which is then trimmed by poly (A)-specific ribonuclease (PARN) to provide a mature miRNA of 22–26 nt. The miRNA duplex is placed on RISC during maturation, which unwinds the strands into guide and passenger strands (Cheloufi et al., 2010; J.-S. Yang et al., 2010; Yoda et al., 2013). Interestingly, miR-451 and the DICER-dependent miR-144 are both encoded in the same main transcript (Dore et al., 2008). According to a recent study, miR-144 recruits a microprocessor and transfers it to miR-451, making its processing easier (Shang et al., 2020). Furthermore, miR-144 inhibits conventional miRNA processing during erythropoiesis by targeting DICER in a negative feedback loop. Otherwise, miR-451 processing is unaffected by DICER (Kretov et al., 2020).

Both the conventional and noncanonical miRNA biogenesis processes produce a diverse pool of miRNAs that vary mostly at their 3′ ends. The inaccurate cutting of pri- and pre-miRNA by the RNases DROSHA and DICER, as well as posttranscriptional alterations, are the major sources of diversity. The functional consequence of 5′ end heterogeneity is a shift in the seed sequence, which causes distinct mRNAs to be regulated. The silencing efficiency and stability of 3-IsomiRs in cells can vary.Because it can consolidate and increase miRNA-target pairing, especially with inadequate seed complementarity, the interaction between the 3′ end of a miRNA and its target is critical for binding specificity (M. J. Moore et al., 2015).

3 Structural aspects of artificial life miRNA

AmiRNAs are made up of a pri-miRNA scaffold and a siRNA insert. The consequences of replacing the miRNA sequence with an exogenous siRNA sequence are difficult to anticipate, since the sequence and structure of a pri-miRNA substantially impact its processing. Furthermore, posttranscriptionally, miRNA synthesis is regulated at numerous levels, with a variety of cis- and trans-acting factors modulating the process (Ha & Kim, 2014; Michlewski & Cáceres, 2019). Improper AmiRNA design can lead to inefficient processing, the creation of siRNA variants with different seed sequences, or the activation of arm switching, which causes the passenger strand of siRNA to be released (Medley et al., 2020).

The inherent variability of miRNAs derived from a single precursor (isomiRs) may be favorable to cells since it allows for the control of a large number of transcripts (Neilsen et al., 2012). The basic concepts of RNAi technology are precision and safety, and poor processing of amiRNAs raises the possibility of off-target effects (Galka-Marciniak et al., 2016). To guarantee effective and specific processing of an amiRNA, the backbone of the pri-miRNA, the length of the stem and surrounding sequences, the placement of the siRNA insert, and the presence of structural and sequence motifs must all be carefully considered. The properties of naturally occurring miRNAs are applied to amiRNAs to increase their processing and, as a result, their silencing efficacy (Ros et al., 2019; Ros & Gu, 2016).

RNAi Technology's nonspecific effects

RNAi technologies can have unforeseen consequences, including off-target activity, saturation of the miRNA synthesis pathway, and an immunological response, in addition to silencing specific targets. These side effects can make it difficult to assess phenotypic effects in gene-silencing research and can cause toxicity (Olejniczak et al., 2016).

Nonspecific interactions between siRNAs and other transcripts containing complementary sequences cause sequence-dependent off-target effects. Using bioinformatics to detect potential off-target interactions during siRNA design (e.g., BLAST NCBI and siSPOTR) can considerably minimize this risk. Even partial sequence complementarity (inside the "seed" region) to some transcripts' 3′ UTRs can cause miRNA-like gene silence. Nonspecific suppression of complimentary transcripts might potentially be caused by excess passenger strand or siRNA variations.

Overexpression of an RNAi trigger, which competes with endogenous miRNAs for the Microprocessor complex, XPO5, DICER, or AGO proteins, causes the miRNA synthesis pathway to become saturated. The processed siRNA content may be evaluated quantitatively and qualitatively with respect to the endogenous miRNA content using small RNA sequencing analysis. Weaker promoters, amiRNAs as RNAi triggers, or the lowest effective dosage of viral vector can all help to overcome this impact.

Immune reaction Exogenous RNAi triggers can activate cellular foreign RNA and DNA sensors, which detect pathogen-associated molecular patterns (PAMPs). The length, structure, concentration, and cellular location of the molecule all play a role in this impact, which results in the generation of proinflammatory cytokines and interferons. TLR9, AIM2, and ZBP1 sensors, for example, identify foreign DNA that has been introduced into cells through plasmid or viral vectors.

AmiRNAs have a double-stranded stem bordered by two single-stranded basal segments (5p- and 3p-) and an apical loop, similar to endogenous pri-miRNAs. The stem may be separated further into an upper stem that covers about 22 bp from the terminal loop and a lower stem that covers approximately 13 bp from the basal segments. The basal and apical junctions are the borders between these dsRNA and ssRNA areas.

The presence of basal (CNNC and UG) and apical (UGU) motifs, as well as stem length and apical loop size, all impact miRNA processing, and the combination of these properties results in mature miRNAs. There are no general principles for the optimal design of amiRNAs, since each pri-miRNA is unique in terms of its structure and sequence features. Years of miRNA biogenesis research and high-throughput analysis of hundreds of thousands of pri-miRNA variations, on the other hand, have enabled the development of simple criteria for the efficient and specific processing of amiRNAs (Fang & Bartel, 2015).

3.1 Basal segments with only one strand

For pri-miRNA processing, the existence of basal segments and their single-stranded nature are essential. Processing of pri-miR-16 mutants lacking one or both basal regions was shown to be impaired or totally eliminated in vitro (Han et al., 2006). The cleavage of pri-miRNA mutants with double-stranded basal regions by DROSHA was also prevented (Han et al., 2006). The better the pri-miRNA processing is, the longer the flanking sequences are (Zeng & Cullen, 2005). The length of endogenous pri-miRNAs' basal regions varies greatly and can reach several hundred nucleotides. In amiRNA construction, substantially shorter sequences are employed for practical reasons. In vitro processing of minimum pri-miR-16-1 and pri-miR-30a sequences including 20 nt outside of the DROSHA cleavage site, for example, was discovered (Han et al., 2004). When transcribed from the H1 promoter in transfected HEK293T cells, however, a minimum pri-miR-31 sequence proved inefficient for mature miRNA synthesis (Zeng & Cullen, 2005). To guarantee efficient processing, libraries of miR-30-based constructs were created using 125 nt flanking regions from original transcripts (Silva et al., 2005; Stegmeier et al., 2005).

The nucleotide sequence of basal segments isn't significant for processing in general (Zeng & Cullen, 2005). The CNNC motif, which may be found in the 3′ basal section of 60 percent of sample pri-miRNAs, is an exception (Auyeung et al., 2013). This motif interacts with SRSF3, causing DROSHA to be recruited at the basal junction and pri-miRNA processing to be stimulated. Only when CNNC is 17 nt from the Microprocessor cleavage site does this impact occur (Kim et al., 2018). The single-stranded basal segments are recognized by the Microprocessor complex, which measures 11 bp from the basal junctions (Han et al., 2006).

3.2 stem with two strands

Asymmetric modifications, which can generate single mismatches or internal loops, and symmetric modifications, which are mostly single nucleotide bulges, distinguish Pri-miRNAs in terms of stem length and the range of motifs that disrupt the stem structure. Furthermore, G:U wobble base pairs may be found at various spots along the stem of natural pri-miRNA hairpins. Internal loops within the lower stem are normally fully base-paired, and thus suppress miRNA expression (Nguyen et al., 2020). The optimum length of the stem is one of the most significant characteristics of amiRNAs.

The stem structure of naturally occurring pri-miRNAs is incomplete, consisting of three 35 1 bp helical twists. Previous research has shown that for the microprocessor complex, a stem length of 33 bp is ideal (Han et al., 2006; Nguyen et al., 2015). The ideal range of the pri-miRNA stem length was expanded to 36 3 bp in the Lu group's study.They altered the 35-bp stem of miR-125b by inserting/deleting 1–4 bp on the apical loop, on the basal side, or in the center, and found that 4 bp changes lowered processing efficiency by 80%. Changes of 3 bp, on the other hand, had no effect on processing. Their findings show that there is a range of ideal stem lengths for effective pri-miRNA processing (Roden et al., 2017).

Sequence motifs play a significant role in pri-miRNA processing, as they aid microprocessors in putting pri-miRNAs with non-optimal stem lengths in the cleavage site (Auyeung et al., 2013). Pri-miRNAs having a bulge at locations 5–9 nt from the stem's base were shown to be more abundant in several studies (Fang & Bartel, 2015; Han et al., 2006; Roden et al., 2017). The existence of this bulge in the context of the GHG motif defines the DROSHA cleavage site, according to a study of 40,000 pri-miRNA variants (Fang & Bartel, 2015). The GHG motif contributes to a unique RNA structure that favors the creation of a four-way junction, according to cryo-EM structures (Partin et al., 2020). DROSHA also identifies the UG motif in some pri-miRNAs' 5′ basal junction. Roden et al. also discovered bulge-depleted areas at locations 16–21 and 28–32 nt from the stem's base (Roden et al., 2017). These areas may interact with DGCR8, according to the scientists, and bulges within these areas may disrupt processing.

Pri-miRNAs encode miRNAs in the stem structure's 5′, 3′, or both strands. In contrast to the 3′ miRNAs, which are released by DROSHA and DICER, DROSHA defines 5′ miRNAs. DROSHA is reported to cut miRNAs more precisely than DICER, resulting in miRNAs with a predicted seed sequence. The siRNA guide strand is frequently placed into the 5′ amiRNA arm as a result (Silva et al., 2005). Furthermore, the majority of amiRNA stems are precisely base-paired, making amiRNA creation and analysis easier. However, such highly organized structures' transcription might be prematurely halted, resulting in inert viral vectors (J. Xie et al., 2017, 2020).

3.3 Loops at the end

For pri-miRNA identification, placement, and cutting, unstructured terminal loops are required. Furthermore, XPO5 recognizes pre-miRNAs and shRNA loops, which are required for nuclear-cytoplasmic trafficking. The effective processing of amiRNAs is ensured by terminal loops and basal segments produced from natural pri-miRNA scaffolds. Pri-miRNAs typically have loops of 3–23 nt in length (Zeng & Cullen, 2003), and pri-miRNAs with loops of > 10 nt are effectively processed (Ma et al., 2013). Large loops (> 15 nt) are less effectively digested because they might resemble the basal segment, resulting in cleavage from the incorrect end of the pri-miRNA (Han et al., 2006).

The use of the apical UGU motif in combination with basal motifs aids in the appropriate orientation of the cleavage site and improves the processing of non-optimal hairpins (Fang & Bartel, 2015). In cleavage site selection, however, basal components are more significant than the apical motif. The UGU motif is found in more than 30% of pri-miRNAs, and DGCR8 identifies it. For DGCR8 activation and terminal loop recognition, the heme-binding region (HBR) is required (Nguyen et al., 2015; Partin et al., 2017).

4 Scaffolds of artificial miRNA

The Pri-miRNA scaffold controls how amiRNA is processed in a cell and how much siRNA is produced, which is an effector molecule in this system. The activated RISC is directed to this region by the siRNA guide strand, which has perfect complementarity to the target mRNA and promotes AGO2 cleavage of the mRNA. MiR-26a was the first miRNA whose parts were exploited for siRNA expression. McManus et al. demonstrated that a class II hairpin containing a 9-nt miR-26a loop efficiently suppressed target genes in human cells (McManus et al., 2002). The miR-26a loop was also tweaked, with a single C base removed to prevent unexpected structural folding. There was also an asymmetric bulge inside the stem structure, 19 nt of unbroken RNA duplex, and a 5 nt GC clamp in the construct. The researchers found that even minor alterations in the structure of the hairpins resulted in variations in silencing activity. Further research has revealed that the design of miRNA hairpin mimics is crucial to their ability to silence miRNAs.

4.1 Pri-miR-30a

For the first time, Zeng et al. showed that the mature miRNA-binding region of the original hsa-miR-30a transcript may be substituted for a heterologous sequence without affecting activity (Zeng et al., 2002). Pri-miR-30a is now one of the most well-known and widely utilized siRNA scaffolds. It is a member of the miR-30 family, which includes miR-30a, miR-30b, miR-30c, miR-30d, and miR-30e, all of which play a role in tissue and organ development as well as disease pathogenesis (L. Mao et al., 2018). The stem of Pri-miR-30a has an asymmetrical 2 nt bulge and G:U wobble base pairs, as well as sequence motifs including the basal CNNC and UG motifs and the apical UGU (G) motif. Both the 5′ and 3′ ends produce mature 22 nt miRNAs, although the 5′ products predominate.

Many studies have improved MiR-30a-based amiRNA constructs. The length of the flanking sequences, the preservation of structural and sequence features, and the selection of the best promoter for cell expression were the key considerations for these creations. The more an amiRNA's structure resembles that of pri-miR-30a, the more effective the silencing is. The original loop sequence with an apical UGU (G) motif is seen in most miR-30a-based amiRNAs. The lengths of the flanking sequences varied from 20 to 125 nt, but no clear findings were drawn about their effects on processing or silencing efficiency.

Although the properties of endogenous miRNAs are preserved in amiRNA constructions, the number of emerging siRNAs remains lower than that of siRNAs released from shRNAs. This has a direct impact on the effectiveness of silence. Fellmann et al. (2013) built an experimental miR-30 backbone called miR-E to optimize the processing of miR-30a-based amiRNAs. A guide strand was put on the 3′ arm of the synthetic miR-30 stem, which was precisely base-paired. In addition, the apical UGU (G) motif was changed to AGU by replacing two conserved base pairs surrounding the loop from CU/GG to UA/UA (G). The inclusion of XhoI/EcoRI restriction sites for shRNA cloning also modified the highly conserved 3′ region of the basal stem. The efficiency of amiRNA knockdown was improved as a result of these changes. The amount of mature siRNA was 10–30 times higher than that of miR-30-based amiRNA that had not been changed.

A large-scale library of amiRNAs was generated using the miR-30 scaffold. Each target is represented by six distinct amiRNAs in this collection, which covers the majority of human (34,711) and mouse (32,628) genes (Chang et al., 2006; Silva et al., 2005). The PRIME (potent RNAi utilizing microRNA expression) vector, produced by the same group, provides for the control of amiRNA production by a tetracycline (Tet)—responsive promoter (Stegmeier et al., 2005). This approach enables the detection of amiRNA in cells through the coexpression of reporter genes and the manipulation of gene knockdown through the use of varied doxycycline concentrations (DOX). The PRIME constructs are divided into three types: (1) the TET-ON system, in which DOX activates transcription from the TET promoter by binding to the reverse tet-controlled transcriptional activator (rtTA); (ii) the TET-OFF system, which is active in the absence of DOX; and (iii) the Tet-repressor-based expression system (TREX). Other lentiviral vectors, such as pSLIK (single lentivector for inducible knockdown; Shin et al., 2006) or constructs controlled by the ubiquitin C (UbiC) promoter (Xia et al., 2006; Zhou et al., 2005), have been created to produce Tet-responsive gene expression knockdown (reviewed in Calloni & Bonatto, 2015).

4.2 miR-155 (Pri-miR)

A miRNA backbone produced from mouse or human pri-miR-155 in the exon of a noncoding RNA and produced from the B-cell integration cluster is another extensively utilized miRNA backbone (BIC). Humans, mice, and chickens all have high levels of MiR-155, which has a role in hematopoiesis, inflammation, and immunity. It is abundant in the thymus and spleen, although it has also been found in other tissues (Faraoni et al., 2009; Mashima, 2015).

The 13-nt loop in Pri-miR-155 lacks the UG, GHG, CNNC, and UGU motifs, and the loops produced by humans and mice differ by 3 nt. Bulges and G:U wobbling base pairs can be seen on the hairpin stem. The mature miRNA has a length of 24 nucleotides and is produced by the 5′ arm. The 3′ arm also produces a functional miRNA with a length of 22 nt (Y. Wang et al., 2018). Chung et al., 2006; X. Liu et al., 2012; Pfister et al., 2017) have shown that Pri-miR-155 cassettes may successfully produce single or multiple amiRNAs.

Many studies utilize the commercial plasmid pcDNA6.2-miR, which comprises flanking sequences from mouse pri-miR-155 ranging from 30 to 40 nucleotides long. The cytomegalovirus (CMV) promoter controls the expression of the matching amiRNA. In cultivated cells and in vivo, this construct effectively silences the expression of a gene of interest. In cellular and animal models of cancer, viral, and neurological illnesses, MiR-155-based amiRNAs have been employed (X. Liu et al., 2012; Murphy et al., 2013; Sharma et al., 2018). Human pri-miR-155 has also been employed as a backbone for siRNA. Using the hsa-miR-155 scaffold and the HTT siRNA insert, the Mueller group created two amiRNA constructs. The U6 promoter was used to drive one of the constructs, which produced one copy of the amiRNA. The chimeric CMV-chicken ß-actin (CBA) promoter drove the second construct, which produced two copies of the amiRNA. In contrast to the amiRNA driven by the Pol II promoter, which caused toxicity and aberrant behavior in mice, the construct created from the U6 promoter generated the amiRNA at a supraphysiologic level. The presence of guide strand preponderance was observed when miR-155-based siRNA was expressed (Pfister et al., 2017). A modified loop and 50 nt flanking sequences obtained from hsa-pri-miR-155 were included in the construct. An intron might potentially be introduced with an amiRNA cassette based on mmu-miR-155 (Chung et al., 2006; Du et al., 2006). A nearly 500-nt sequence from the mouse BIC's third exon, including the miR-155 sequence (149 nt), is inserted in an expression vector dubbed synthetic inhibitory BIC-derived RNA in this cassette (SIBR). A 22-nt duplex code for the appropriate siRNA was introduced in lieu of the mature miR-155. The simian CMV IE94 promoter (sCMV) controls the construct, and the cassette terminates with SV40 late polyadenylation. Northern blot analysis indicated that the vector's 22 nt product was expressed. The silencing effectiveness of several genes, such as the kinases B-Raf and c-Raf, was validated using luciferase assays and western blot analysis. Multiple copies of this miRNA cassette (up to eight copies) can be included to augment the inhibition of a single target mRNA (Chung et al., 2006).

4.3 miR-451 (Pri-miR)

Noncanonical miR-451 is found in mature erythrocytes and has a role in erythropoiesis. The miRNA-451 gene is found on chromosome 17, around 100 nucleotides downstream of the DICER-dependent miR-144 gene, in an intergenic region. The pri-miR-451 structure has a 33-bp stem and a 4-nt loop, both of which are seen in mature miR-451. The pre-miRNA is generated by DROSHA-mediated cleavage, which has a highly structured 17 bp stem and is cleaved by AGO2 to create 30 nt intermediates. These intermediates are reduced to mature miRNAs of 20–26 nt (Pan et al., 2015; Yoda et al., 2013).

Because the passenger strand is not formed during biogenesis, this miRNA is a promising weapon with few off-targets (Kretov et al., 2020). This backbone was successfully employed by Konstantinova's lab to express siRNA targeting the HTT gene, and a phase I/II clinical study for this amiRNA in HD began this year. In this construct, the original pri-miRNA structure has been retained, and the guide siRNA strand has been used in lieu of the mature miR-451. The flanking sequences are 200 nt long and contain EcoRV and BamHI restriction sites (J. Miniarikova et al., 2016, 2017). In vitro and in vivo experiments, the CMV or CMV-chicken ß–actin-rabbit beta-globin (CAG) promoters were used to drive the amiRNA cassette. A human growth hormone polyadenylation (hGH polyA) signal is also included in the amiRNA cassette. The results revealed that the processing pattern might vary depending on the siRNA sequence, resulting in siRNAs varying in length from 19 to 31 nt (J. Miniarikova et al., 2016).

4.4 Other amiRNA scaffolds

Other pri-miRNAs, while less common than pri-miR-30a and pri-miR-155, have been employed as the backbone for amiRNA creation. Mouse pri-miR-33, for example, is involved in cholesterol absorption and synthesis and is encoded within intron 16 of the SREBF2 gene. MiR-33 has been shown to influence HDL synthesis and cellular cholesterol export in the liver (Rayner et al., 2010). mmu-pri-miR-33 was discovered to have almost all of the characteristics of an ideal miRNA, including a 35-bp stem, an UG motif in the basal junction region, mGHG in the stem, UGU (G) in the loop, and a CNNC motif in the 3′ flanking sequence. This backbone was improved by Gao et al. to silence Apob and PC-1 (Gao et al., 2008).

The constructs were made up of a pre-miRNA loop and 100 nt flanking sequences generated from pri-miR-33 and were driven by the CB promoter. The passenger strand of the hairpin stem has bulges to match the natural structure. Bulges in the miR-33 scaffold greatly enhance rAAV genome integrity as compared to entirely complementary stems, which cause shortened genomes during vector manufacture. The constructs efficiently cause gene silencing at a level equivalent to the matching shRNA in cultivated cells and animals. Furthermore, miR-33-based amiRNAs were digested correctly, resulting in a large number of guide strands compared to passenger strands (J. Xie et al., 2020). This backbone, as well as the design guidelines that went into it, were employed in studies of osteoporosis treatments. It efficiently silences expression of the major osteoclast regulators RANK (receptor activator for nuclear factor B) and cathepsin K in vivo investigations (Yang et al., 2020).

The amiRNA backbone is made out of human pri-miR-31. The gene for it is found on chromosome 9. MiR-31 is engaged in a number of signaling pathways that have distinct impacts on various malignancies (Yu et al., 2018). Within a 17-nt loop, natural pri-miR-31 has an apical UGU motif and a CNNC motif in its 3′ flanking region. The majority of mature miRNAs are derived from the '5' arm.Based on this pri-miRNA, Arbuthnot and colleagues created an amiRNA (Ely et al., 2008, 2009).

The native sequence of pri-miR-31 was preserved, with the exception of the apical UGU motif, which was changed to GGU. On each strand of the cassette, wild-type flanking sequences of 51 nt were present. Sequences obtained from a previously developed shRNA to suppress the hepatitis B virus were used to replace the guide and passenger strands. The U6 or CMV promoters were used to drive the construction. The production of 21 nt products matched to mature miR-33 was validated by Northern blot analysis. This shuttle can form heterogeneous fragments, as evidenced by the presence of 20 and 22 different products. The silencing effectiveness obtained in the cellular model was validated in in vivo tests.

The hsa-miR-122 backbone, whose sequence is taken from a liver-specific noncoding RNA, was optimized by the same group. The pri-miR-122 transcript is around 4.5 kb long (Thakral & Ghoshal, 2015). A 12 nt loop with a UGU motif, a hairpin stem with symmetric bulges, a basal CNNC motif, and mature miRNA generated mostly from the 5′ arm make up Pri-miR-122. Mir-122 enhances hepatitis C virus replication and is linked to cholesterol metabolism and hepatocellular cancer (Jopling, 2012). When driven by U6 or CMV promoters, anti-HBV amiRNA constructs effectively suppress HBV. Cellular processing of the amiRNAs yielded 21-nt products and was more efficient for CMV-driven constructs than for U6 promoter-driven constructs (Ely et al., 2008).

Hsa-miR-21 (Choi et al., 2015; Yue et al., 2010), gga-miR-126 (S. C.-Y. Chen et al., 2011), mmu-miR-144 (Walder et al., 2011), hsa-miR-221 (X. Huang & Jia, 2013), and miR-223 are examples of miRNA backbones utilized (Guda et al., 2015).

4.5 clusters of miRNA

Approximately 40% of human miRNA genes are grouped together in clusters (Altuvia et al., 2005). Two or more miRNA genes are transcribed in the same direction in such clusters. A transcription unit or miRNA in the other direction does not separate these genes. Intergenic regions have more miRNA clusters than introns and exons. The grouping of miRNA genes with comparable biological roles has been proposed to boost the transcription efficiency of the miRNA genes within the cluster. Furthermore, genes found in miRNA clusters have the ability to influence a wide range of biological activities (Kabekkodu et al., 2018). The miR-17–92 cluster, for example, naturally encodes six different miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a; Concepcion et al., 2012; Y. P. Liu et al., 2008). The virus's activity might be inhibited by the production of four anti-HIV siRNAs from the miR-17–92 cluster backbone. This polycistronic design improves silencing activity when compared to a conventional building (Y. P. Liu et al., 2008).Another group suggested that the miR-106b cluster may be used to effectively limit viral activity. This tri-cistronic cluster expresses miR-106b, miR-93, and miR-25 endogenously. siRNAs targeting HIV-1's tat and rev transcripts replaced endogenous mature miRNA sequences (Aagaard et al., 2008).

Researchers designed amiRNAs produced from polycistronic transcripts to achieve greater silencing efficacy than a single amiRNA because of the localisation of miRNAs within introns. The miR-30 shuttle was one of the earliest polycistronic constructs. Two tandem shRNAs with flanking sequences obtained from miR-30 were expressed by the CMV promoter in the related investigation. Surprisingly, a construct containing two copies of the identical shRNA inserted in the backdrop of naturally occurring pri-miRNA performed worse than one containing a single amiRNA (Zhou et al., 2005). The usage of the miR-155 backbone to create tandem amiRNAs targeting separate genes, on the other hand, has yielded promising results. The CMV promoter was also used to drive this construction. Even more intriguing, inserting up to eight amiRNAs targeting the same gene proved to be more successful than embedding just one copy. The secondary structure of one helical turn right before the DROSHA cleavage site must be preserved to ensure proper processing of the chimeric transcript (Chung et al., 2006). Due to the mutability of viruses, polycistronic amiRNAs may be particularly effective in the treatment of viral illnesses. The usage of two miR-155-based amiRNAs targeting conserved regions in the human immunodeficiency virus (HIV) with tolerance for wobble base pairing resulted in a synergistic antiviral impact. The stem-loop structure and flanking sequences from pri-miR-155 were preserved (Son et al., 2008).

5 miRNA delivery by artificial means

Viral vectors are the most popular delivery mechanism for amiRNAs. This system consists of adenoviruses (Ads), lentiviruses (LVs), and adeno-associated viruses (AAVs) of various serotypes with expression cassettes in place of pathogenic genes. AmiRNAs can be successfully delivered to a tissue of interest and can enable stable or regulated production of siRNA by mixing different types of vectors and promoters.

5.1 Vectors of viral infection

5.1.1 Adenoviral vectors

Adenoviruses are members of the Adenoviridae family and are the most common type of non-enveloped virus. The adenovirus genome is a linear dsDNA with a length of between 26 and 48 kb. The replication mechanism of the host is used to replicate in the nucleus of vertebrate cells. Interaction with the receptors CD46 for group B human adenovirus serotypes and coxsackievirus adenovirus receptor (CAR) for all other serotypes results in binding to the cell. Interestingly, human Ad virus-associated (VA) RNA suppresses shRNA transport and RISC activity by competing for binding to XPO5 and DICER, therefore inhibiting RNAi (Andersson et al., 2005; Lu & Cullen, 2004).

Adenoviral vectors are simple to work with and can infect both dividing and nondividing cells without integrating their cargo into the host DNA. They have a high vector titer and may be generated in large quantities. The viral E1 gene is absent from first-generation Ad vectors, which are capable of packing 8 kb. The E2, E3, and E4 genes are missing in the second-generation Ad, which reduces the immunological response. Only the packing signal and inverted terminal repeats are present in third-generation Ad vectors, which lack all viral protein-coding genes (ITRs). The lack of viral proteins minimizes the likelihood of eliciting immunostimulatory responses, allowing the transgene to be expressed for longer (H.-H. Chen et al., 1997; Herrera-Carrillo et al., 2017). In vitro and in vivo, these vectors are employed to deliver shRNA and amiRNA expression cassettes to cells, and studies have shown that target gene expression is decreased (Ibriimovi, Kneidinger, et al., 2013; Pan et al., 2015).

5.1.2 Lentiviral vectors

Lentiviruses are a kind of retrovirus that belongs to the retrovirus family. Their genome is made up of two single-stranded RNA copies. Human immunodeficiency virus type 1 is the most well-known lentivirus (HIV-1). Trans-activation of transcription and Rev-mediated export of spliced and unspliced HIV-1 transcripts from the nucleus are carried out by standard retroviral proteins such as Gag, Pol, Env, and the regulatory protein Tat. Only the regulatory sequences remain in lentiviral vectors, which have a capacity of about 10 kb. By integrating into the host genome, LVs may transduce both dividing and nondividing cells, allowing for persistent transgene expression. As a result, transcriptional silence might last for a long period. Lentiviruses can be pseudotyped by combining their envelope proteins with those of other viruses. This permits the virus to infect cells that are normally resistant to infection, such as hematopoietic and embryonic stem cells. In vitro and in vivo, an LV expressing three amiRNAs targeting the Bcl-Abl oncogene was demonstrated to effectively change leukemogenic potency (McLaughlin et al., 2007). AMIRNAs given by LV and targeting osteopontin reduced cell growth in hepatocellular cancer (Sun et al., 2008). In vitro findings using LV expressing anti-influenza virus amiRNAs that efficiently reduced influenza virus generation were also achieved (S. C.-Y. Chen et al., 2011).

5.1.3 Adeno-related viral vectors are vectors that are associated with adenoviruses.

The Parvoviridae family includes adeno-associated viruses. They are not enclosed, and their genome is 4.7 kb long and made up of single-stranded DNA. The DNA strands might be positive (sense) or negative (anti-sense). The rep and cap genes have two ITRs and two open reading frames in the genome. Cap encodes the capsid protein, while Rep encodes a protein required for the AAV life cycle. When a helper virus, such as adenovirus or herpesvirus, is present, productive infection ensues. AAVs establish latency by integrating into chromosome 19q13.4 in the absence of a helper virus (Daya & Berns, 2008).

The Rep protein is missing from the viral genome in recombinant AAVs (rAAVs) and can be provided in trans. As a result, rAAV integration is less efficient than wild-type AAV integration. There are 11 distinct AAV serotypes, each with its own unique host cell tropism and immunological characteristics. AAVs can be pseudotyped, allowing them to target cells and tissues that aren't natural targets. Antibodies targeting particular capsids have been created to prevent capsids from being neutralized, which increases in vivo transduction efficiency (Dhungel et al., 2020; Wu et al., 2006).

Because of their lack of pathogenicity in humans, capacity to allow long-term episomal expression, and little carcinogenic potential and inflammatory response, AAVs are attractive delivery vectors (D. Wang et al., 2019). AAVs are the most widely used platform for gene therapy delivery, and rAAVs are now being tested in a number of clinical studies (D. Wang et al., 2019). In preclinical investigations into neurological illnesses, malignancies, muscular dystrophies, and viral infections, AAVs are routinely employed. AAVs may infect postmitotic cells, and rAAV2, rAAV5, and rAAV9 injection resulted in efficient neuronal cell transduction in mouse, sheep, and pig brains. The ability of recombinant AAV9 to effectively transduce osteoclasts has been employed to treat osteoporosis. In mice, systemic injection of rAAV9 containing amiRNAs to mute the expression of key osteoclast regulators increased bone mass significantly (Y.-S. Yang et al., 2020).

5.2 Expression cassettes

AmiRNAs can be produced from their own promoters, inserted in introns, or found in the 3′ UTRs of protein-coding genes (e.g., GFP). They're usually transcribed from Pol II promoters such as the CMV promoter, CBA promoter, CAG promoter, UbiC promoter, phosphoglycerate kinase (PGK) promoter, and elongation factor 1 alpha (EF1A) promoter, resulting in transcripts with a 5′ cap and poly (A) tail. RNA Pol III promoters like U6 and H1 are also employed to create amiRNA constructs, although they are far more "natural" promoters for shRNA production. RNA The Pol III termination signal (typically 5–6 Ts) results in a 5′ PPP and 3′ poly (U) tail in Pol III-derived transcripts. Stretches of 4 Ts should be avoided in Pol III-regulated amiRNAs due to the possibility of premature transcription termination. Furthermore, Pol II promoters allow for the polycistronic production of numerous amiRNAs, which is beneficial in the treatment of viral infections.

When choosing a promoter, there are various factors to consider, like the promoter's strength and activity in the target cell or tissue. In six human cell lines, direct evaluation of the efficacy of an LV-derived amiRNA generated from five distinct Pol II promoters indicated a favorable relationship between promoter strength, siRNA expression level, and protein target knockdown (Lebbink et al., 2011). Furthermore, the efficacy of silencing was discovered to be cell type dependent. Strong viral promoters, such as the CMV promoter, are not efficiently expressed in lymphohematopoietic cells like T, B, and monocytic cells, despite their efficiency in fibroblasts and cancer cell lines (Lebbink et al., 2011).

The CMV and MND promoters, meanwhile, were shown to produce transgenic expression in a small percentage of primary cortical neurons, but the UbiC and PGK promoters ensured very high transgene expression in these cells. Despite this, transgenic expression induced by the CMV promoter was strong in cerebellar granule cells and neuroblastoma cultures. The PGK, CMV, or MND promoters were the best choices for primary astrocyte cultures (M. Li et al., 2010).

Pol III promoters have also been tried in miR-30 based cassettes, despite the fact that Pol II controls pri-miRNAs. The Hannon lab cloned two distinct amiRNA cassettes downstream of the U6, H1, tRNA-val, MSCV-LTR, and CMV promoters and found that the U6 and CMV promoters had the most consistent suppression (Silva et al., 2005). Instead, the Davidson group showed that amiRNA vectors driven by the U6 promoter were more effective than amiRNA vectors controlled by the CMV promoter (R. L. Boudreau et al., 2008). These discrepancies might indicate that, in addition to promoter type, additional factors impact amiRNA processing and silencing effectiveness.

Oversaturation of the miRNA biogenesis pathway, for example, can cause toxicity when promoters are excessively powerful, especially in the setting of shRNA expression. Furthermore, because off-target effects are directly associated with the number of RNAi triggers, the price paid for longer expression may be decreased silencing specificity. As a result, using the lowest effective dosage of vectors and tissue-specific or inducible promoters rather than powerful ubiquitous promoters is a good alternative to safe RNAi treatment techniques. Compared to shRNAs, amiRNAs are more "natural" and safer, and even at a 10-fold larger vector dose, they do not overwhelm the miRNA machinery (Bauer et al., 2009; R. L. Boudreau et al., 2009; McBride et al., 2008).

Reporter genes are generally cotranscribed from the vector to aid in the tracking of amiRNAs in cells. According to certain research, inserting an amiRNA cassette immediately upstream of the reporter gene in a monocitronic construct can result in poor marker protein translation (Dickins et al., 2005). To guarantee optimal expression, the reporter gene should be positioned between the CMV promoter and the miR-30-based amiRNA, or it should have its own promoter (Stegmeier et al., 2005). Without sacrificing knockout efficiency, a miR-21-based amiRNA may be directly controlled by a Pol II promoter or downstream of a reporter gene (Yue et al., 2010).

6 Neurodegenerative disorders therapeutic tools

When neurons in the brain or peripheral nervous system lose their function and eventually die, this is known as a neurodegenerative disease. The increasing buildup of defective proteins in cells is commonly linked to these disorders. HD, spinocerebellar ataxias (SCAs) types 1, 2, 3, 6, 7, and 17, Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) are examples of this type of condition (ALS). Current treatments for this type of disease focus on symptom relief and maintaining the quality of life rather than correcting the underlying pathologic process.

Polyglutamine diseases are a subset of polyglutamine diseases.

The development of unstable CAG triplet repeats in the coding areas of unrelated genes causes the genetically inherited autosomal dominant illnesses HD and Sare (Buijsen et al., 2019; Duyao et al., 1993). More than 40 CAG repeats are seen in completely penetrant alleles, but this is a disease-specific characteristic. Toxic proteins with the polyglutamine (polyQ) domain are the key pathogenic component in this type of illness (Zoghbi & Orr, 2000). Aggregates of polyQ-containing proteins may induce neuronal degeneration in specific brain areas associated with each illness, such as the striatum and cerebral cortex (HD), cerebellum, basal ganglia, brainstem, and spinal cord (SCA3), and retina (SCA7) (Novak & Tabrizi, 2010; Paulson, 2009; Ross, 2002; Seidel et al., 2012; Sittler et al., 2018).

Due to the monogenic nature of many illnesses, RNAi technology offers a significant potential to reduce harmful protein levels (Ashizawa et al., 2018; J. Miniarikova et al., 2018). The transport of RNAi triggers to the CNS, as well as silencing specificity and selectivity toward the mutant version, are the primary hurdles. Patients would benefit from long-term expression of a therapeutic molecule following a single injection, since an effective therapy should most likely be started before the symptoms appear and persist for decades. As a result of their high potency, safety, and long-term expression as episomal vectors, AAV-based amiRNAs are perfect options, as evidenced by several preclinical investigations. The AMT-130 chemical, which targets exon 1 of the human huntingtin (HTT) gene and started phase I/II clinical trials this year, is the most advanced research. After a single intrastriatal injection of rAAV5 at two dosages, the safety, tolerability, and effectiveness of AMT-130 will be assessed in 26 HD patients (NCT04120493).

To limit the potential of off-target effects due to passenger strand activity, a 21-nucleotide siRNA sequence is placed into the noncanonical pri-miR-451 scaffold. Many constructs, including human miR-1-2, miR-16-1, miR-26a-1, miR-101-1, miR-122, miR-135b, miR-155, miR-203a, miR-335, and miR-451a, with 200 nt 5′ and 3′ flanking regions, were analyzed to find Pri-miR-451. A luciferase reporter system was used to assess silencing effectiveness and passenger strand activity. In vitro and in vivo, miR-451-based amiRNA was processed into 30 nt species, achieving 60% and 30% of all reads, respectively, according to next generation sequencing (NGS) data. The second most common variation had a 31-nt species (which accounted for 20% of all readings in vitro) or a 23-nt species (which accounted for 30% of all readings in vivo) (J. Miniarikova et al., 2016). There was no full-length passenger strand found. In several cellular and animal models of HD, such as cultured human neurons, rodents, transgenic minipigs, and nonhuman primates, the silencing efficacy and safety of AMT-130 were investigated, and a drop in total huntingtin level of 40%–80% was seen (Evers et al., 2018; Keskin et al., 2019; J. Miniarikova et al., 2017; Spronck et al., 2019).

Pri-miR-30a was employed as a siRNA scaffold in one of the first investigations documenting the usage of amiRNAs against the HTT gene. Despite the significant disparity in expression levels, experiments in HEK293 cells and mouse models revealed that amiRNA produced under the U6 promoter was virtually as efficient as the equivalent shRNA in reducing HTT transcripts (R. L. Boudreau et al., 2009; Dufour et al., 2014; McBride et al., 2008; Monteys et al., 2015). In addition, unlike shRNAs, AAV-amiRNA was not harmful in the mouse striatum. The SCA1 model and miR-30-based amiRNA were used by the same group to corroborate these findings (R. L. Boudreau et al., 2008). AAV2/1 containing U6-amiRNA was injected into the rhesus putamen to assess the safety of partial wild-type huntingtin suppression in an animal model that is more similar to humans in terms of size and structure. AmiRNA infusion reduced HTT transcripts by 45 percent without causing motor skill deficiencies, an immunological response, neuronal death, or gliosis (McBride et al., 2011). When produced in vivo under the control of the CBA promoter, AAV-amiRNA constructs were likewise effective, lowering mutant huntingtin levels by 50% and Htt aggregates in the brains of YAC128 mice (Stanek et al., 2014). MiR-155-based constructs targeting the HTT transcript were used to compare the safety and efficiency of amiRNAs produced by the U6 and CBA promoters. AmiRNA was injected into the striatum of an HD mouse model using a self-complementary AAV9 vector, and the human HTT mRNA was decreased by 50%. The amiRNA was synthesized at supraphysiologic levels in the U6 constructs, and cellular processing resulted in a high amount of the passenger strand. 6 months following injection, this led to behavioral problems and striatal damage (W. Liu et al., 2016; Pfister et al., 2017). CBA-amiRNA, on the other hand, did not cause any toxicity (Keeler et al., 2016; Pfister et al., 2017). Transgenic sheep expressing full-length human HTT cDNA with 73 CAG repeats from the human HTT promoter were used to demonstrate the construct's efficiency and safety. HTT mRNA and protein levels were reduced by 40%–80% depending on the brain area six months after injection (Pfister et al., 2018).

Effective RNAi treatment for Sis is not as far along as it is for HD. The majority of techniques employ pri-miR-30a as a backbone for a sequence that targets a specific area of ATXN genes. In the instance of SCA1, pseudotyped AAV2 vectors (AAV2/1 and AAV2/5) were used to deliver U6-amiRNA to the deep cerebellar nuclei of model mice (Keiser et al., 2013, 2014). This resulted in a considerable decrease in ATXN1 mRNA and protein levels, as well as improved molecular and behavioral results (Keiser et al., 2013, 2014, 2016). The identical design was injected into the rhesus deep cerebellar nuclei, which lowered ATXN1 mRNA by more than 30% and was well tolerated (Keiser et al., 2015).

For the treatment of SCA7, the same group created AmiRNA. This construct was also driven by the U6 promoter and packed into the AAV2/1 vector, much like the previous one. Without causing considerable toxicity, injection into the deep cerebellar nuclei of SCA7 mice led to a 50 percent drop in ATXN7 mRNA and a 35 percent drop in ATXN7 protein (Ramachandran, Boudreau, et al., 2014). SCA7 also affects the retina of the eye, and injecting miR-30-based AAV-amiRNA into this structure lowered ATXN7 levels in SCA7 animals without affecting retinal or visual function (Ramachandran, Bhattarai, et al., 2014).

In the instance of SCA3, amiRNAs with the backbones of pri-miR-124 and pri-miR-451 produced promising outcomes. The U6 promoter was used to produce amiRNA in the first construct (Rodrguez-Lebrón et al., 2013). The therapeutic RNA was delivered through AAV2/1 into the deep cerebellar nuclei of SCA3 transgenic mice. The ATXN3 mRNA level was lowered by around 70%, while the ATXN3 protein level was lowered by around 35%. Furthermore, ATXN3 nuclear accumulation was decreased. This discovery is significant because nuclear accumulation of proteins with polyQ stretches or even fragments is regarded to be a characteristic event in the pathogenesis of polyQ disorders (Koch et al., 2011). The second study used a luciferase reporter system and human iPSC-derived neurons to create and select pri-miR-451-based amiRNA constructs driven by the CAG promoter. Three amiRNA candidates were found to be expressed within the range of natural miRNA expression and had no effect on endogenous miRNA levels, according to small RNA sequencing. Furthermore, the authors evaluated bioinformatics predictions and RNA sequencing findings and found no significant changes in gene expression following treatment with the three amiRNAs. These findings show that the dangers of miRNA biogenesis pathway saturation and off-target consequences are low. The amiRNAs were then delivered into the deep cerebellar nuclei of SCA3 knock-in mice using AAV5 vectors. Depending on the brain area, ATXN3 mRNA levels were lowered by 15%–40%, while ATXN3 protein levels were lowered by 64%. (Raygene Martier, Sogorb-Gonzalez, Stricker-Shaver, et al., 2019).

6.2 Frontotemporal dementia and ALS

ALS is a kind of motor neuron disease that is caused by the degeneration and death of motor neurons. Stiff muscles, muscular twitching, and steadily deteriorating weakness are all indications of ALS. About 20% of familial ALS cases are caused by mutations in the superoxide dismutase (SOD1) gene, which is linked to neurodegeneration. It has been shown that the synthesis of mutant SOD1 protein might be harmful. As a result, RNAi-mediated suppression of SOD1 gene expression might be used as an ALS treatment. In a mouse model and nonhuman primates, miR-155-based amiRNAs successfully knocked down SOD1. The first method employed a single-stranded AAV9 vector to produce two tandem amiRNAs under the control of the CBA promoter. SOD1 mRNA levels in the spinal cord were reduced by up to 50% and in the heart and gastrocnemius muscles by more than 80% after bilateral injection into the cerebral lateral ventricles of ALS SOD1G93A animals (Stoica et al., 2016). The identical amiRNA, packaged in the rAAVrh10 vector and expressed by the CAG or U6 promoter, was injected intrathecally into the marmosets at the lumbar level. Treatment with U6-amiRNA resulted in higher silencing efficiency (decreases of 93 percent in the lumbar region, 65 percent in the thoracic region, and 92 percent in the cervical cord region) than treatment with CAG-amiRNA (decreases of 93 percent in the lumbar region, 65 percent in the thoracic region, and 92 percent in the cervical cord region) (Borel et al., 2016).

The amiRNA was then produced by three separate promoters, the H1, U6, and CBA promoters, and delivered to the macaques through an intrathecal lumbar injection. The H1 promoter was used to silence SOD1 expression in the lumbar area, which resulted in a 93 percent reduction in SOD1 expression. The decline in SOD1 transcript levels ranged from 40% to 90% depending on the promoter and brain area studied. RNA sequencing validated the construct's safety, revealing that the amiRNA was digested properly and that the guide sequence was 100-fold more abundant than the passenger strand. Furthermore, no off-target silencing was discovered (Borel et al., 2018).

Frontotemporal dementia (FTD) is one of the most frequent kinds of dementia in adults under the age of 65, with 40% of FTD cases being hereditary (Ratnavalli et al., 2002). An intronic hexanucleotide repeat expansion inside chromosome 9 open reading frame 72 (C9orf72), which is the most common cause of both disorders, is involved in both ALS and FTD. RNA foci and dipeptide repeat (DPR) proteins are produced by sense and antisense repeat-containing transcripts, which cause cellular toxicity and neuron death. R. Martier, Liefhebber, Garca-Osta, et al., 2019; R. Martier, Liefhebber, Miniarikova, et al., 2019) found that a miR-101-based amiRNA construct delivered to iPSC neurons and the mouse model as the AAV5 vector lowered C9orf72 mRNA levels by 60% and 40%, respectively. A substantial reduction in RNA foci was also seen in ALS/FTD mice following bilateral injection of amiRNA into the striatum (R. Martier, Liefhebber, Garca-Osta, et al., 2019).

6.3 Alzheimer's disease

Alzheimer's disease (AD) is a degenerative disease that causes people to lose their memories and other cognitive abilities. Alzheimer's disease is caused by a combination of age-related changes in the brain as well as genetic, environmental, and lifestyle factors. The buildup of plaques consisting of amyloid-A, an insoluble cleavage product of amyloid precursor protein (APP), in the brain is one biochemical feature of Alzheimer's disease (Tiwari et al., 2019). Because some studies have connected Alzheimer's disease to lipid metabolism in the brain, targeting the acyl-CoA: cholesterol acyltransferase 1 (ACAT1) enzyme might be one way to treat the condition. As a result, cellular and animal models of AD were used to evaluate miR-155-based amiRNA targeting the Acat1 (Soat1) gene. The CAG promoter-driven construct was delivered into the dorsal hippocampus as an AAV2 vector, resulting in a 45 percent reduction in ACAT activity in the total mouse brain. Furthermore, knocking down Acat1 reduced the amount of A and human APP (Murphy et al., 2013).

7 Diseases caused by viral infections

AmiRNAs have also been utilized to treat viral infections. Viruses, a category of pathogenic agents that can't multiply outside of their host, may cause a variety of serious illnesses (Girardi et al., 2018). Because viruses are difficult to eliminate due to their ease of transmission across species, which may lead to pandemics, and the ease with which viral genome modifications (mutations) occur, there is still a rising demand for precise therapies. Chikungunya virus, HIV-1, hepatitis B, influenza, dengue virus, West Nile virus, and Japanese encephalitis virus have all been shown to be repressed by amiRNAs (Gao et al., 2008; Karothia et al., 2020; Sharma et al., 2018; P. Xie et al., 2013; T. Zhang et al., 2012).

Despite its numerous benefits, using amiRNAs as therapeutic agents in the treatment of viral illnesses may have a number of drawbacks. One such issue is that viruses can evolve quickly, making it impossible to silence a target gene indefinitely. As a result, highly conserved sections of the viral genome must be targeted, and several amiRNA sequences must be expressed in a single vector. To avoid viral escape due to the formation of evasive mutations, all studies have utilized several amiRNAs. Furthermore, some viral proteins have been shown to have a viral suppressor of RNAi (VSR) function (Maillard et al., 2019).G.H. Samuel and colleagues discovered this phenomena in the yellow fever virus (YFV) in 2016. They observed that the YFV capsid protein suppresses RNA silencing by interfering with DICER. VSR activity is also widely conserved in the C proteins of other therapeutically significant flaviviruses, according to the author (Samuel et al., 2016).

AmiRNAs are typically integrated inside the pri-miR-155 backbone, produced under the CMV promoter, and supplied to cell cultures by lipofection, according to an analysis of articles on the use of amiRNAs for the treatment of viral illnesses. All of the researchers employed the interferon response as their only technique for an off-target investigation. Except for four investigations, cellular processing of amiRNAs was not investigated (S. C.-Y. Chen et al., 2011; Choi et al., 2015; Ely et al., 2009; Maepa et al., 2017). Anti-HBV multiple amiRNA cassettes were developed by Ely et al. (2009) to effectively limit viral replication while avoiding off-target effects. Pri-miR-31, miR-30a, and miR-122 were employed as siRNA shuttles in this study, and they were expressed under the strong Pol II promoter. A conserved area inside the HBV X (HBx) ORF was targeted with amiRNA sequences. The authors confirmed around 90% knockdown of the target by measuring secreted HBV surface antigen (HBsAg) levels. Analysis of the interferon response ruled out nonspecific effects. The concentration of IFN-mRNA in transfected cells was tested, and the findings revealed no immunostimulation. Northern blot analysis was used to examine amiRNA processing. The results demonstrated that pri-miR-31 may be used to make Pol II trimeric cassettes. Furthermore, co-injecting animals with HBV plasmid and miR-31-based amiRNA resulted in a significant HBsAg decrease. The capacity of pri-miR-31 to act as a shuttle for trimeric amiRNAs to effectively silence HBV replication in vivo was supported by these findings.

H. Zhang et al. have also undertaken in vivo experiments (2015). One of their experiments was aimed at using amiRNAs to inhibit influenza A virus replication by targeting highly conserved areas (the M1, M2, or nucleoprotein genes). The authors emphasized their research's value by pointing out the limitations of currently available viral vaccinations. Current viral vaccines, for example, do not provide cross-protection against antigenic variation strains and do not properly protect sick or elderly individuals. Because of its tissue tropism and high gene expression in cells of the lower respiratory tract, recombinant Ad was employed to deliver drugs to cells and mice. Designed amiRNAs inhibited viral replication by up to 80% in transduced HEK293T cells. Coexpression of the most effective therapeutic drugs in one vector, however, did not improve inhibition. Zhang and colleagues have shown that amiRNA may be used to provide cross-protection. Not only did vaccinated mice develop resistance to fatal influenza (A/PR8), but they also developed resistance to two heterotypic viruses (H9N2 and H5N1). The scientists concluded their research by indicating that using amiRNAs to target conserved areas of the influenza virus might be a novel strategy to avoid viral infection.

Another method employs a combinatorial gene expression cassette that includes the gene producing HSV thymidine kinase, as well as several amiRNAs targeted to preterminal proteins (HSV-TK). The CMV promoter (amiRNAs) and the E4 promoter were responsible for gene expression (HSV-TK). This construct was used to stop adenovirus replication in A549 cells. Ganciclovir (GCV), an antiherpetic prodrug, requires HSV-TK to be converted to its active form. When triggered, ganciclovir acts as a competitive nucleotide analog, inhibiting viral and cellular DNA synthesis. The authors incorporated this cassette into a replication-deficient Ad vector, which is interesting (in which the E1 and E3 genes have been deleted). Such Ad vectors guarantee that a therapeutic substance is delivered to the same area as the wild-type Ad. Treatment of cells with a single amiRNA, WT virus, and GCV at the same time resulted in a larger reduction in the number of Wt Ad genome copies than treatment without GCV. The copy number difference between these two variations was nearly 1.7 orders of magnitude. Because the combination of GCV with multiple amiRNAs was more efficient than the combination of the highest dose of GCV with a single amiRNA, the dose of GCV required was reduced by inserting additional copies of the amiRNA. Ibriimovi, Lion, et al. (2013) claimed that the created combinatorial amiRNA/HSV-TK cassette could be a useful tool for inhibiting Ad replication and spread.

Saha et al. (2016) investigated combinatorial therapy, combining amiRNA treatment with several antivirals (chloroquine, ribavirin, and mycophenolic acid) to inhibit Chikungunya virus replication in Vero cells. These combinations significantly decreased viral replication, but only the combination of amiRNA and chloroquine decreased replication more effectively than amiRNA or the antiviral alone. The scientists hypothesized that combining these two chemicals would stop the viral life cycle in its early and late phases. The potentially harmful effect of ribavirin-amiRNA was justified by the possibility of ribavirin interfering with the RNAi pathway.

8 Cancer

Cancer, along with cardiovascular illnesses, is one of the leading causes of mortality globally, according to the World Health Organization. Carcinogenesis can be caused by a variety of factors, including hereditary abnormalities as well as biological, physical, or chemical carcinogens (Schulz, 2007). Breast, prostate, lung, and colorectal cancers are the most frequent malignancies, according to the GLOBOCAN 2018 study. Every year, the number of cancer patients rises, and future predictions are unreliable. As a result, the most effective treatments are continually sought. Despite the availability of a wide range of cancer medicines, many malignancies remain incurable, and treatment outcomes are often disappointing. This is due to a combination of tumor metastasis, heterogeneity, and treatment resistance (S. Wang et al., 2012).

The RNAi treatment has attracted the attention of many scientists. AmiRNAs have so far been developed to treat breast, pancreatic, gastric, and cervical malignancies, as well as melanoma and hepatocellular carcinoma (Bonetta et al., 2015; Z. Li et al., 2006; Liang et al., 2007; C. Liu et al., 2016; X. Liu et al., 2012). The miR-155 backbone and CMV promoter were employed in almost all of these amiRNAs. To treat pancreatic ductal cancer, pri-miR-155-based amiRNAs targeting the vascular endothelial growth factor receptor (VEGFR) were developed. VEGFRs are overexpressed in cancer cells and play a vital role in angiogenesis regulation. Vectors were created that included amiRNAs that were identical to the sequences of all three kinds of VEGFRs (VEGFR1, VEGFR2, and VEGFR3). Researchers also created an amiRNA vector that includes all three cassettes simultaneously. Transfecting pancreatic cell lines and injecting SW1990 cells into athymic nude mice were used to assess the vectors' efficiency. Each amiRNA considerably lowered VEGFR1, 2, and 3 mRNA and protein levels by >50%, and the triple-amiRNA vector dramatically lowered the expression of all three genes. Furthermore, transfected cells proliferated substantially less than mock-treated cells. Their capacity to invade was reduced by up to 80%, and apoptosis was boosted in both the early and late phases. Treatment with the triple-amiRNA vector and cisplatin fully stopped tumor development in mice. The scientists did not look into amiRNA processing or off-target consequences in any manner, but they did examine the morphology and functionality of the mouse pancreas to ensure that the amiRNAs were safe. Despite a few drawbacks, the authors believe that therapy with numerous amiRNAs has a lot of potential (J. Huang et al., 2017).

Targeting p21, a protein that inhibits p53-mediated apoptosis, was done in a similar way using three amiRNAs. In HEK293T cells, the scientists confirmed that a tandem array inhibited the induction of p21. Furthermore, in cancer cells, simultaneous reduction of p21 and production of p53 results in an increase in apoptosis. Experiments were also carried out in real time. The therapeutic Ad-p53/amiRNA-p21 injection resulted in a decreased tumor volume. The scientists also demonstrated that p21 suppression in the absence of p53 overexpression raises the risk of cancer development, which should be taken into account in future research (Idogawa et al., 2009).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), eukaryotic translation initiation factor 4E (eIF4E), and DNA polymerase were all targeted by amiRNAs using Pri-miR-30a as a shuttle. The goal of this study was to see if knocking down critical genes for cell survival had an anticancer impact on hepatocellular cancer. To assure safety, all of the constructs were produced under the tissue-specific AFP promoter and recombinant Ad was used to deliver them to cells and animals. The results revealed that target genes were effectively knocked down, which had an anticancer impact. Furthermore, infecting cells with all amiRNAs at the same time greatly reduced the expression of the target genes. The authors discovered that ATP generation and protein synthesis were slowed, as well as cell cycle arrest. The cells had a lower survival rate than the control cells. The administration of amiRNA targeted at GAPDH caused the tumor to be destroyed in the mice. The scientists also said that though this therapy plan may have certain limits, their findings propose a unique therapeutic approach despite these limitations (C. Mao et al., 2015).

AmiRNAs have been shown to target genes involved in tumor growth and metastasis pathways in a number of additional investigations. For example, CDH17-specific amiRNA was delivered to gastric cancer cells via recombinant Ad. The cadherin CDH17 is linked to tumor differentiation and lymph node metastasis. Because the target gene was downregulated when cells were treated with amiRNA, cancer cell motility and proliferation were inhibited (J. Zhang et al., 2011).

Notably, AmiRNA therapy can be used in conjunction with other forms of treatment, such as radiation. This method uses amiRNA to target either a vital factor for nonhomologous end joining (XRCC4) or a vital factor for homologous recombination repair (XRCC4) in brain tumor and lung cancer cells (XRCC2). The cells were then subjected to X-rays or carbon ion radiation. In vitro and in vivo, tumor cells were sensitized to high-LET radiation when only the XRCC2 or HRR pathways were targeted, indicating that this combination has therapeutic promise (Zheng et al., 2013).

9 Additional diseases

AMIRNAs have been used to treat Pelizaeus–Merzbacher disease (PMD), osteoporosis, cardiovascular disorders, sickle cell disease (SCD), myotonic dystrophy (DM1), facioscapulohumeral muscular dystrophy (FSHD), hyperalgesia, familial hypercholesterolemia (FH), and alcohol abuse, in addition to neurodegenerative diseases, viral infections, and cancers. Overexpression of the proteolipid protein 1 (Plp1) gene in oligodendrocytes causes PMD, a hypomyelinating leukodystrophy. Currently, this X-linked illness is incurable. H. Li et al. (2019) suggested that scAAV-mediated gene-specific suppression might be used to treat PMD. The corpus striatum and internal capsule of mice were injected with a Pri-miR-155-based amiRNA targeting Plp1 produced under the human CNP promoter (selected because of its size and strong activity in oligodendrocytes). Plp1 mRNA and protein levels in oligodendrocytes were lowered by 50% when compared to controls. In addition, the researchers looked at off-target effects by evaluating the expression of eight genes that had 60 percent or greater complementarity with amiRNA seed sequences.

The scientists established the safety of their treatment method based on the lack of a substantial difference in the expression of these genes and the fact that their downregulation did not impact the expression of other oligodendrocyte-associated genes. In addition, scientists noticed that treated mice had a longer lifetime, gained weight, and had better motor capabilities. The number of mature oligodendrocytes rose, and astrogliosis and microgliosis were suppressed, despite the fact that the myelin structure was retained.

Y.-S. Yang et al. (2020) presented an intriguing method of off-target suppression.They designed amiRNAs with the pri-miR-33 backbone to target two genes linked to osteoporosis progression: cathepsin K (which degrades type 1 collagen) and RANK (a tumor necrosis factor, crucial for the differentiation of monocytes into osteoclasts). The amiRNAs were injected into 2-month-old mice through an AAV9 vector. Both amiRNAs decreased expression of the rank and ctsk genes by around 60%, according to femur analysis. There was also an increase in trabecular bone mass. According to the Yang group, treatment with the amiRNA targeted at rank resulted in a decrease in osteoclast differentiation while maintaining osteoblast activity.Targeting CTK, on the other hand, decreased bone resorption and increased bone production (owing to osteoblast activity), but had no effect on osteoclasts. Finally, the authors concluded that targeting ctsk held more promise than targeting rank, and they used this amiRNA in subsequent tests.The authors changed the viral capsid to reduce off-target effects, which can occur since cathepsin K is present in numerous nonskeletal organs, including in the heart and in the skin. They introduced two distinct peptide patterns to the N-terminus of the VP2 capsid protein component, either (Asp-Ser-Ser) 6 or (Asp) 14. These motifs have been found to direct liposomes to either osteoblast-enriched or osteoclast-enriched surfaces. The scientists demonstrated that adding these alterations did not diminish viral effectiveness and concluded that adding the (Asp-Ser-Ser) 6 motif increased bone-homing rAAV9 specificity by reducing transduction to other tissues. The amiRNA was then packaged into a modified capsid and tested for therapeutic potential.Injections of this drug protected ovariectomized mice's femurs from bone loss. Virus injection decreased ctsk expression and enhanced trabecular bone mass in the femur and lumbar vertebrae in a rat model of senile osteoporosis. The authors verified the clinical efficacy of a rAAV-mediated therapeutic agent for the treatment of postmenopausal and senile osteoporosis, which has benefits over traditional antiosteoporotic medicines, in this study (Y.-S. Yang et al., 2020).

Among cardiovascular illnesses, amiRNAs have been used to treat hypertension and heart failure (Fan et al., 2012; Größl et al., 2014). The first study's goal was to see if AT1aR (angiotensin II type 1 receptor, which is overexpressed in spontaneously hypertensive rats) amiRNA based on mir-155 might lower blood pressure and promote cardiovascular remodeling in spontaneously hypertensive rats. Injecting rAd into the brain lowered target expression, which led to lower arterial blood pressure, better myocardial and vascular remodeling, and hypertension protection (Fan et al., 2012).

Another study that was intended to enhance decreased Ca2+ homeostasis—a major cause of heart failure—showed that amiRNAs outperformed shRNAs in terms of safety. The authors previously discovered the toxicity of shRNA targeted to phospholamban (PLB). As a result, they created an amiRNA based on miR-155 that was targeted to PLB produced under a heart-specific promoter. Because of its selectivity for cardiac cells, the AAV6 vector was utilized as a delivery vehicle. Despite lower amiRNA expression, both RNAi triggers lowered PLB expression and increased Ca2+ transport in the same way. Größl et al. (2014) found that shRNA boosted the expression of interferon-regulated and proinflammatory genes, but artificial miRNA did not (proteomic study).

A phase 1 clinical study for SCD is now underway (NCT03282656). Patients are being sought for a therapy that involves autologous transplantation of bone marrow-derived CD34+ HSC cells that have been transduced with an LV-containing amiRNA that targets the BCL11A gene. Downregulation of this gene activates -globin, which then generates fetal hemoglobin, according to previous studies (HbF; Brendel et al., 2016; Guda et al., 2015; Sankaran et al., 2008). As a consequence, the sickling of red blood cells, which is a defining feature of SCD, is avoided. The therapeutic agent is a miR-223-based amiRNA that is produced under the Pol II promoter (-globin) and packed into an improved LV vector (BCH-BB694) with better titer characteristics (Brendel et al., 2020). The scientists obtained effective knockdown of the target in transduced CD34+ derived erythroid cells, -globin induction, and a more than 50% increase in HbF level using this method (Guda et al., 2015). They also established the therapy's safety in preclinical testing and its suitability for clinical trials (Brendel et al., 2020).

The use of miR-30-based amiRNAs for illnesses affecting muscle tissue, such as DM1 and FSHD, has also yielded promising outcomes (Bisset et al., 2015; Wallace et al., 2012, 2017). The acid-sensing ion channel 3 (ASIC3) gene was downregulated using miR-144-based amiRNA to cure hyperalgesia (Walder et al., 2011). In preclinical investigations into familial hypercholesterolemia (Kerr et al., 2016) and even into alcohol consumption, amiRNAs with a miR-155 backbone were successfully employed (Baek et al., 2010).

10 Final remarks

RNA interference (RNAi) is a potent tool for studying gene function, and siRNA/shRNA remain the most popular agents for knocking down gene expression over time. The lesser interest in amiRNAs and their utilization compared to shRNA is likely due to their more complicated design and less predictable processing, which may result in poorer silencing effectiveness. Well-designed amiRNAs, on the other hand, are as effective as shRNAs (although producing 10–80 times less siRNA), enable long-term silencing, and, most importantly, are safer than other RNAi triggers. AmiRNAs are suitable instruments for gene therapy techniques because of these characteristics, especially for well-defined and incurable monogenic diseases.

Because our understanding of the laws driving miRNA production is always growing, rational AmiRNA design appears to be becoming easier. The efficacy of amiRNAs as therapeutic RNAi triggers for the treatment of various illnesses has been proven in a number of preclinical investigations. However, the specificity of amiRNA processing and amiRNA expression level were not investigated in the great majority of studies. To estimate the risk of off-target effects, detailed information on the siRNA variants produced by amiRNA processing, as well as the contribution of passenger and guide strands, is required. Because it does not create a passenger strand, DICER-independent pri-miR-451 has an advantage over other pri-miRNA backbones in this aspect. It does, however, generate '3' interneurons, which can interact with cells in a nonspecific manner.The relationship between mature siRNA levels and endogenous miRNA levels can be used to anticipate the danger of the miRNA synthesis pathway being saturated. According to research, using tissue-specific Pol II promoters for amiRNA production reduces the risk. To get the whole safety profile of amiRNA therapies, a thorough examination of the transcriptome deregulation caused by off-target interactions is required. NGS should be used in an appropriate cellular and/or animal model, and additional procedures such as qRT-PCR and western blotting should be used to corroborate the results. It's very hard to avoid disrupting the cells' physiological circumstances through viral transduction and foreign material overexpression. In terms of structure, biogenesis, and expression levels, amiRNAs are more "natural" than other gene therapy techniques.

Gene therapy has a number of challenges, including the safety of treatment instruments and delivery concerns. The creation of viral vectors has progressed in recent years. Nonetheless, the efficient transport of amiRNAs to particular tissues, such as deep brain regions, remains a difficulty. In clinical studies, stereotactic injection of AAV5 directly into the striatum is presently the most effective approach to delivering HTT-targeting amiRNAs. Intravenous injections would not only make patients more comfortable, but would also reduce the cost of complex brain surgery.Unlike genome editing methods like CRISPR-Cas9 or transcription activator-like effector nucleases (TALENs), amiRNA expression cassettes are substantially smaller and may be packed into nearly any viral vector. They also utilise the cell's native protein machinery and do not produce irreversible alterations, unlike genome editing technologies.

These results reveal that while all currently employed treatment techniques, such as ASOs, RNAi, and genome editing, have their benefits, they also have their drawbacks. The amount of time spent on a technology determines its level of progression, and ASOs have an edge in this regard, as seen by the number of authorized medicines. Perhaps RNAi is just around the corner.

References

Aagaard, L., Zhang, J., von Eije, K. J., Li, H., Sœtrom, P., Amarzguioui, M., & Rossi, J. J. (2008). Engineering and optimization of the mir-106b-cluster for ectopic expression of multiplexed anti-HIV RNAs. Gene Therapy, 15(23), 1536–1549. https://doi-org.ezproxy.its.uu.se/10.1038/gt.2008.147

Andersson, M. G., Haasnoot, P. C. J., Xu, N., Berenjian, S., Berkhout, B., & Akusjärvi, G. (2005). Suppression of RNA interference by adenovirus virus-associated RNA. Journal of Virology, 79(15), 9556– 9565. https://doi-org.ezproxy.its.uu.se/10.1128/JVI.79.15.9556-9565.2005

Ashizawa, T., Öz, G., & Paulson, H. L. (2018). Spinocerebellar ataxias: Prospects and challenges for therapy development. Nature Reviews. Neurology, 14(10), 590– 605. https://doi-org.ezproxy.its.uu.se/10.1038/s41582-018-0051-6

Auyeung, V. C., Ulitsky, I., McGeary, S. E., & Bartel, D. P. (2013). Beyond secondary structure: Primary-sequence determinants license pri-miRNA hairpins for processing. Cell, 152(4), 844– 858. https://doi-org.ezproxy.its.uu.se/10.1016/j.cell.2013.01.031

Abdelhamid, H. N., M. Dowaidar, M. Hällbrink, and Ü. Langel. 2019. “Cell Penetrating Peptides-Hierarchical Porous Zeolitic Imidazolate Frameworks Nanoparticles: An Efficient Gene Delivery Platform.” SSRN Electron. J. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3435895.

Abdelhamid, Hani Nasser, Moataz Dowaidar, and Ülo Langel. 2020. “Carbonized Chitosan Encapsulated Hierarchical Porous Zeolitic Imidazolate Frameworks Nanoparticles for Gene Delivery.” Microporous and Mesoporous Materials: The Official Journal of the International Zeolite Association 302 (August): 110200. https://doi.org/10.1016/j.micromeso.2020.110200.

Abdelhamid, Hani Nasser, Moataz Dowaidar, Mattias Hällbrink, and Ülo Langel. 2020. “Gene Delivery Using Cell Penetrating Peptides-Zeolitic Imidazolate Frameworks.” Microporous and Mesoporous Materials: The Official Journal of the International Zeolite Association 300 (June): 110173. https://doi.org/10.1016/j.micromeso.2020.110173.

Ahmad, Almeman, Khalaf Hassan, Rasool Semaab, Moataz Dowaidar, and Al Orainy Mohammad. 2013. “The Impact of CYP2C19 Polymorphism on Platelet Reactivity for Guiding Clopidogrel Treatment and Cost Analysis.” Journal of the Saudi Heart Association 25 (2): 107. https://doi.org/10.1016/j.jsha.2013.03.005.

Algahsham, Abdullah, Ahmad A. A. Settin, Ahmad Ali, and Hisham Ismail. n.d. “Association of MTHFR C677T and A1298C Polymorphisms with Hypertension among Saudi Subjects from Qassim Region.” International Journal of Health Sciences 6 (1). Accessed June 18, 2021. http://ijhs.org.sa/index.php/journal/article/view/312.

Algasham, Abdullah, Hisham Ismail, Moataz Dowaidar, and Ahmad A. Settin. 2011. “Methylenetetrahydrofolate Reductase (MTHFR) and Angiotensin Converting Enzyme (ACE) Gene Polymorphisms among Saudi Population from Qassim Region.” International Journal of Health Sciences 5 (2 Suppl 1): 3–4. https://www.ncbi.nlm.nih.gov/pubmed/23284552.

Alghasham, Abdullah, Ahmad A. Settin, Ahmad Ali, Moataz Dowaidar, and Hisham Ismail. 2012a. “Association of MTHFR C677T and A1298C Gene Polymorphisms with Hypertension.” International Journal of Health Sciences 6 (1): 3–11. https://doi.org/10.12816/0005968.

Alghasham, Abdullah, Ahmad Ali, Hisham Ismail, Moataz Dowaidar, and Ahmad A. Settin. 2012. “CYP2J2 -50 G/T and ADRB2 G46A Gene Polymorphisms in Saudi Subjects with Hypertension.” Genetic Testing and Molecular Biomarkers 16 (9): 1027–31. https://doi.org/10.1089/gtmb.2012.0006.

Ali, Ahmad, Abdullah Alghasham, Hisham Ismail, Moataz Dowaidar, and Ahmad Settin. 2013. “ACE I/D and eNOS E298D Gene Polymorphisms in Saudi Subjects with Hypertension.” Journal of the Renin-Angiotensin-Aldosterone System: JRAAS 14 (4): 348–53. https://doi.org/10.1177/1470320312459976.

Ali, Ahmed A. A., Nahla M. Wassim, Moataz Dowaidar, and Ahmed E. Yaseen. 2013b. “Association of eNOS (E298D) and CYP2J2 (−50G/T) Gene Polymorphisms with Hypertension among Egyptian Cases.” The Journal of Basic & Applied Zoology 66 (4): 234–41. https://doi.org/10.1016/j.jobaz.2012.12.001.

Ali, Ahmed A. A., Nahla M. Wassim, Moataz M. Dowaidar, and Ahmed E. Yaseen. 2013a. “Genetic Polymorphism of CYP2D6 Gene among Egyptian Hypertensive Cases.” The Journal of Basic & Applied Zoology 66 (4): 228–33. https://doi.org/10.1016/j.jobaz.2012.12.002.

Aljarallah, Badr, Ahmed Ali, Moataz Dowaidar, and Ahmad Settin. 2011. “Prevalence of α-1-Antitrypsin Gene Mutations in Saudi Arabia.” Saudi Journal of Gastroenterology: Official Journal of the Saudi Gastroenterology Association 17 (4): 256–60. https://doi.org/10.4103/1319-3767.82580.

Altuvia, Y., Landgraf, P., Lithwick, G., Elefant, N., Pfeffer, S., Aravin, A., Brownstein, M. J., Tuschl, T., & Margalit, H. (2005). Clustering and conservation patterns of human microRNAs. Nucleic Acids Research, 33(8), 2697– 2706. https://doi-org.ezproxy.its.uu.se/10.1093/nar/gki567

Baek, M. N., Jung, K. H., Halder, D., Choi, M. R., Lee, B.-H., Lee, B.-C., Jung, M. H., Choi, I.-G., Chung, M.-K., Oh, D.-Y., & Chai, Y. G. (2010). Artificial microRNA-based neurokinin-1 receptor gene silencing reduces alcohol consumption in mice. Neuroscience Letters, 475(3), 124– 128. https://doi-org.ezproxy.its.uu.se/10.1016/j.neulet.2010.03.051

Bartel, D. P. (2004). MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell, 116(2), 281– 297. https://doi-org.ezproxy.its.uu.se/10.1016/s0092-8674(04)00045-5

Bartel, D. P. (2018). Metazoan MicroRNAs. Cell, 173(1), 20– 51. https://doi-org.ezproxy.its.uu.se/10.1016/j.cell.2018.03.006

Bauer, M., Kinkl, N., Meixner, A., Kremmer, E., Riemenschneider, M., Förstl, H., Gasser, T., & Ueffing, M. (2009). Prevention of interferon-stimulated gene expression using microRNA-designed hairpins. Gene Therapy, 16(1), 142– 147. https://doi-org.ezproxy.its.uu.se/10.1038/gt.2008.123

Bisset, D. R., Stepniak-Konieczna, E. A., Zavaljevski, M., Wei, J., Carter, G. T., Weiss, M. D., & Chamberlain, J. R. (2015). Therapeutic impact of systemic AAV-mediated RNA interference in a mouse model of myotonic dystrophy. Human Molecular Genetics, 24(17), 4971– 4983. https://doi-org.ezproxy.its.uu.se/10.1093/hmg/ddv219

Bonetta, A., Mailly, L., Robinet, E., Travé, G., Masson, M., & Deryckere, F. (2015). Artificial microRNAs against the viral E6 protein provoke apoptosis in HPV positive cancer cells. Biochemical and Biophysical Research Communications. 465, 658– 664. https://doi-org.ezproxy.its.uu.se/10.1016/j.bbrc.2015.07.144

Borel, F., Gernoux, G., Cardozo, B., Metterville, J. P., Toro Cabreja, G. C., Song, L., Su, Q., Gao, G. P., Elmallah, M. K., Brown, R. H., & Mueller, C. (2016). Therapeutic rAAVrh10 mediated SOD1 silencing in adult SOD1G93A mice and nonhuman Primates. Human Gene Therapy, 27(1), 19– 31. https://doi-org.ezproxy.its.uu.se/10.1089/hum.2015.122

Borel, F., Gernoux, G., Sun, H., Stock, R., Blackwood, M., Brown, R. H., & Mueller, C. (2018). Safe and effective superoxide dismutase 1 silencing using artificial microRNA in macaques. Science Translational Medicine, 10(465), eaau6414. https://doi-org.ezproxy.its.uu.se/10.1126/scitranslmed.aau6414

Boudreau, R. L., McBride, J. L., Martins, I., Shen, S., Xing, Y., Carter, B. J., & Davidson, B. L. (2009). Nonallele-specific silencing of mutant and wild-type Huntingtin demonstrates therapeutic efficacy in Huntington's disease mice. Molecular Therapy: The Journal of the American Society of Gene Therapy, 17(6), 1053– 1063. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2009.17

Boudreau, R. L., Monteys, A. M., & Davidson, B. L. (2008). Minimizing variables among hairpin-based RNAi vectors reveals the potency of shRNAs. RNA, 14(9), 1834– 1844. https://doi-org.ezproxy.its.uu.se/10.1261/rna.1062908

Boudreau, R., Martins, I., & Davidson, B. L. (2009). Artificial microRNAs as siRNA shuttles: Improved safety as compared to shRNAs in vitro and in vivo. Molecular Therapy: The Journal of the American Society of Gene Therapy, 17(1), 169– 175. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2008.231

Brendel, C., Guda, S., Renella, R., Bauer, D. E., Canver, M. C., Kim, Y.-J., Heeney, M. M., Klatt, D., Fogel, J., Milsom, M. D., Orkin, S. H., Gregory, R. I., & Williams, D. A. (2016). Lineage-specific BCL11A knockdown circumvents toxicities and reverses sickle phenotype. The Journal of Clinical Investigation, 126(10), 3868– 3878. https://doi-org.ezproxy.its.uu.se/10.1172/JCI87885

Brendel, C., Negre, O., Rothe, M., Guda, S., Parsons, G., Harris, C., McGuinness, M., Abriss, D., Tsytsykova, A., Klatt, D., Bentler, M., Pellin, D., Christiansen, L., Schambach, A., Manis, J., Trebeden-Negre, H., Bonner, M., Esrick, E., Veres, G., … Williams, D. A. (2020). Preclinical evaluation of a novel lentiviral vector driving lineage-specific BCL11A knockdown for sickle cell gene therapy. Molecular Therapy—Methods & Clinical Development, 17, 589– 600. https://doi-org.ezproxy.its.uu.se/10.1016/j.omtm.2020.03.015

Buijsen, R. A. M., Toonen, L. J. A., Gardiner, S. L., & van Roon-Mom, W. M. C. (2019). Genetics, mechanisms, and therapeutic progress in polyglutamine spinocerebellar ataxias. Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics, 16(2), 263– 286. https://doi-org.ezproxy.its.uu.se/10.1007/s13311-018-00696-y

Cai, X., Hagedorn, C. H., & Cullen, B. R. (2004). Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA (New York, N.Y.), 10(12), 1957– 1966. https://doi-org.ezproxy.its.uu.se/10.1261/rna.7135204

Calloni, R., & Bonatto, D. (2015). Scaffolds for artificial miRNA expression in animal cells. Human Gene Therapy Methods, 26(5), 162– 174. https://doi-org.ezproxy.its.uu.se/10.1089/hgtb.2015.043

Carbonell, A., & Daròs, J.-A. (2019). Design, synthesis, and functional analysis of highly specific artificial small RNAs with antiviral activity in plants. Methods in Molecular Biology (Clifton, N.J.), 2028, 231– 246. https://doi-org.ezproxy.its.uu.se/10.1007/978-1-4939-9635-3_13
Crossref

Chang, K., Elledge, S. J., & Hannon, G. J. (2006). Lessons from nature: MicroRNA-based shRNA libraries. Nature Methods, 3(9), 707– 714. https://doi-org.ezproxy.its.uu.se/10.1038/nmeth923

Cheloufi, S., Dos Santos, C. O., Chong, M. M. W., & Hannon, G. J. (2010). A Dicer-independent miRNA biogenesis pathway that requires ago catalysis. Nature, 465(7298), 584– 589. https://doi-org.ezproxy.its.uu.se/10.1038/nature09092

Chen, H.-H., Mack, L. M., Kelly, R., Ontell, M., Kochanek, S., & Clemens, P. R. (1997). Persistence in muscle of an adenoviral vector that lacks all viral genes. Proceedings of the National Academy of Sciences of the United States of America, 94(5), 1645– 1650.

Chen, J.-F., Mandel, E. M., Thomson, J. M., Wu, Q., Callis, T. E., Hammond, S. M., Conlon, F. L., & Wang, D.-Z. (2006). The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nature Genetics, 38(2), 228– 233. https://doi-org.ezproxy.its.uu.se/10.1038/ng1725

Chen, S. C.-Y., Stern, P., Guo, Z., & Chen, J. (2011). Expression of multiple artificial microRNAs from a chicken miRNA126-based lentiviral vector. PLoS One, 6(7), e22437. https://doi-org.ezproxy.its.uu.se/10.1371/journal.pone.0022437

Chendrimada, T. P., Gregory, R. I., Kumaraswamy, E., Norman, J., Cooch, N., Nishikura, K., & Shiekhattar, R. (2005). TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature, 436(7051), 740– 744. https://doi-org.ezproxy.its.uu.se/10.1038/nature03868

Choi, J.-G., Bharaj, P., Abraham, S., Ma, H., Yi, G., Ye, C., Dang, Y., Manjunath, N., Wu, H., & Shankar, P. (2015). Multiplexing seven miRNA-based shRNAs to suppress HIV replication. Molecular Therapy, 23(2), 310– 320. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2014.205

Chung, K.-H., Hart, C. C., Al-Bassam, S., Avery, A., Taylor, J., Patel, P. D., Vojtek, A. B., & Turner, D. L. (2006). Polycistronic RNA polymerase II expression vectors for RNA interference based on BIC/miR-155. Nucleic Acids Research, 34(7), e53. https://doi-org.ezproxy.its.uu.se/10.1093/nar/gkl143

Concepcion, C. P., Bonetti, C., & Ventura, A. (2012). The miR-17-92 family of microRNA clusters in development and disease. Cancer Journal (Sudbury, Mass.), 18(3), 262– 267. https://doi-org.ezproxy.its.uu.se/10.1097/PPO.0b013e318258b60a

Daya, S., & Berns, K. I. (2008). Gene therapy using Adeno-associated virus vectors. Clinical Microbiology Reviews, 21(4), 583– 593. https://doi-org.ezproxy.its.uu.se/10.1128/CMR.00008-08

Dhungel, B. P., Bailey, C. G., & Rasko, J. E. J. (2020). Journey to the center of the cell: Tracing the path of AAV transduction. Trends in Molecular Medicine. https://doi-org.ezproxy.its.uu.se/10.1016/j.molmed.2020.09.010

Dickins, R. A., Hemann, M. T., Zilfou, J. T., Simpson, D. R., Ibarra, I., Hannon, G. J., & Lowe, S. W. (2005). Probing tumor phenotypes using stable and regulated synthetic microRNA precursors. Nature Genetics, 37(11), 1289– 1295. https://doi-org.ezproxy.its.uu.se/10.1038/ng1651

Dore, L. C., Amigo, J. D., dos Santos, C. O., Zhang, Z., Gai, X., Tobias, J. W., Yu, D., Klein, A. M., Dorman, C., Wu, W., Hardison, R. C., Paw, B. H., & Weiss, M. J. (2008). A GATA-1-regulated microRNA locus essential for erythropoiesis. Proceedings of the National Academy of Sciences of the United States of America, 105(9), 3333– 3338. https://doi-org.ezproxy.its.uu.se/10.1073/pnas.0712312105

Dowaidar, M., J. Regberg, D. A. Dobchev, and T. Lehto. 2017. “Refinement of a Quantitative Structure–activity Relationship Model for Prediction of Cell-Penetrating Peptide Based Transfection Systems.” International Journal of. https://link.springer.com/content/pdf/10.1007/s10989-016-9542-8.pdf.

Dowaidar, Moataz, and Ahmad Settin. 2010. “Risk of Myocardial Infarction Related to Factor V Leiden Mutation: A Meta-Analysis.” Genetic Testing and Molecular Biomarkers 14 (4): 493–98. https://doi.org/10.1089/gtmb.2010.0017.

Dowaidar, Moataz, and Moataz Dowaidar. 2018. “Chimeric Gene Delivery Vectors : Design, Synthesis, and Mechanisms from Transcriptomics Analysis.”

Dowaidar, Moataz, H. A. Ismail, A. A. Alghasham, M. M. Dowaidar, and A. A. Settin. 2011. “Polymorophisms in MTHF and Ace Genes and the Association with Hypertension among Saudi Population from Qassim Region.” Egyptian Journal of Biochemistry and Molecular Biology 29 (1). https://doi.org/10.4314/ejbmb.v29i1.67382.

Dowaidar, Moataz, Hani Nasser Abdelhamid, Mattias Hällbrink, Krista Freimann, Kaido Kurrikoff, Xiaodong Zou, and Ülo Langel. 2017. “Magnetic Nanoparticle Assisted Self-Assembly of Cell Penetrating Peptides-Oligonucleotides Complexes for Gene Delivery.” Scientific Reports 7 (1): 9159. https://doi.org/10.1038/s41598-017-09803-z.

Dowaidar, Moataz, Hani Nasser Abdelhamid, Mattias Hällbrink, Ülo Langel, and Xiaodong Zou. 2018. “Supplemental Material for Chitosan Enhances Gene Delivery of Oligonucleotide Complexes with Magnetic Nanoparticles–cell-Penetrating Peptide.” SAGE Journals. https://doi.org/10.25384/SAGE.7105436.V1.

Dowaidar, Moataz, Hani Nasser Abdelhamid, Mattias Hällbrink, Ülo Langel, and Xiaodong Zou. 2018. “Chitosan Enhances Gene Delivery of Oligonucleotide Complexes with Magnetic Nanoparticles-Cell-Penetrating Peptide.” Journal of Biomaterials Applications 33 (3): 392–401. https://doi.org/10.1177/0885328218796623.

Dowaidar, Moataz, Hani Nasser Abdelhamid, Mattias Hällbrink, Xiaodong Zou, and Ülo Langel. 2017a. “Graphene Oxide Nanosheets in Complex with Cell Penetrating Peptides for Oligonucleotides Delivery General Subjects.” Biochimica et Biophysica Acta, General Subjects. https://pubag.nal.usda.gov/catalog/5734174.

Dowaidar, Moataz. 2017. “In-Silico Design of Peptide-Based Transfection Systems, in-Vitro Validation, and up-Take Pathways Investigation.” Department of Neurochemistry, Stockholm University.

Du, G., Yonekubo, J., Zeng, Y., Osisami, M., & Frohman, M. A. (2006). Design of expression vectors for RNA interference based on miRNAs and RNA splicing. The FEBS Journal, 273(23), 5421– 5427. https://doi-org.ezproxy.its.uu.se/10.1111/j.1742-4658.2006.05534.x

Dufour, B. D., Smith, C. A., Clark, R. L., Walker, T. R., & McBride, J. L. (2014). Intrajugular vein delivery of AAV9-RNAi prevents neuropathological changes and weight loss in Huntington's disease mice. Molecular Therapy, 22(4), 797– 810. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2013.289

Duyao, M., Ambrose, C., Myers, R., Novelletto, A., Persichetti, F., Frontali, M., Folstein, S., Ross, C., Franz, M., & Abbott, M. (1993). Trinucleotide repeat length instability and age of onset in Huntington's disease. Nature Genetics, 4(4), 387– 392. https://doi-org.ezproxy.its.uu.se/10.1038/ng0893-387

Elbashir, S. M., Lendeckel, W., & Tuschl, T. (2001). RNA interference is mediated by 21- and 22-nucleotide RNAs. Genes & Development, 15(2), 188– 200.

Ely, A., Naidoo, T., & Arbuthnot, P. (2009). Efficient silencing of gene expression with modular trimeric Pol II expression cassettes comprising microRNA shuttles. Nucleic Acids Research, 37(13), e91. https://doi-org.ezproxy.its.uu.se/10.1093/nar/gkp446

Ely, A., Naidoo, T., Mufamadi, S., Crowther, C., & Arbuthnot, P. (2008). Expressed anti-HBV primary microRNA shuttles inhibit viral replication efficiently in vitro and in vivo. Molecular Therapy: The Journal of the American Society of Gene Therapy, 16(6), 1105– 1112. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2008.82

Evers, M., Miniarikova, J., Juhas, S., Vallès, A., Bohuslavova, B., Juhasova, J., Skalnikova, H. K., Vodicka, P., Valekova, I., Brouwers, C., Blits, B., Lubelski, J., Kovarova, H., Ellederova, Z., van Deventer, S. J., Petry, H., Motlik, J., & Konstantinova, P. (2018). AAV5-miHTT gene therapy demonstrates broad distribution and strong human mutant Huntingtin lowering in a Huntington's disease Minipig model. Molecular Therapy: The Journal of the American Society of Gene Therapy, 26(9), 2163– 2177. https://doi-org.ezproxy.its.uu.se/10.1016/j.ymthe.2018.06.021

Fan, Z.-D., Zhang, L., Shi, Z., Gan, X.-B., Gao, X.-Y., & Zhu, G.-Q. (2012). Artificial microRNA interference targeting AT 1a receptors in paraventricular nucleus attenuates hypertension in rats. Gene Therapy, 19(8), 810– 817. https://doi-org.ezproxy.its.uu.se/10.1038/gt.2011.145

Fang, W., & Bartel, D. P. (2015). The menu of features that define primary microRNAs and enable de novo design of microRNA genes. Molecular Cell, 60(1), 131– 145. https://doi-org.ezproxy.its.uu.se/10.1016/j.molcel.2015.08.015

Faraoni, I., Antonetti, F. R., Cardone, J., & Bonmassar, E. (2009). miR-155 gene: A typical multifunctional microRNA. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1792(6), 497– 505. https://doi-org.ezproxy.its.uu.se/10.1016/j.bbadis.2009.02.013

Fellmann, C., Hoffmann, T., Sridhar, V., Hopfgartner, B., Muhar, M., Roth, M., Lai, D. Y., Barbosa, I. A. M., Kwon, J. S., Guan, Y., Sinha, N., & Zuber, J. (2013). An optimized microRNA backbone for effective single-copy RNAi. Cell Reports, 5(6), 1704– 1713. https://doi-org.ezproxy.its.uu.se/10.1016/j.celrep.2013.11.020

Galka-Marciniak, P., Olejniczak, M., Starega-Roslan, J., Szczesniak, M. W., Makalowska, I., & Krzyzosiak, W. J. (2016). SiRNA release from pri-miRNA scaffolds is controlled by the sequence and structure of RNA. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms, 1859(4), 639– 649. https://doi-org.ezproxy.its.uu.se/10.1016/j.bbagrm.2016.02.014

Gao, Y.-F., Yu, L., Wei, W., Li, J.-B., Luo, Q.-L., & Shen, J.-L. (2008). Inhibition of hepatitis B virus gene expression and replication by artificial microRNA. World Journal of Gastroenterology: WJG, 14(29), 4684– 4689. https://doi-org.ezproxy.its.uu.se/10.3748/wjg.14.4684

Gestin, Maxime, Moataz Dowaidar, and Ülo Langel. 2017. “Uptake Mechanism of Cell-Penetrating Peptides.” Advances in Experimental Medicine and Biology 1030: 255–64. https://doi.org/10.1007/978-3-319-66095-0_11.

Girardi, E., López, P., & Pfeffer, S. (2018). On the importance of host MicroRNAs during viral infection. Frontiers in Genetics, 9, 439. https://doi-org.ezproxy.its.uu.se/10.3389/fgene.2018.00439

Grishok, A., Pasquinelli, A. E., Conte, D., Li, N., Parrish, S., Ha, I., Baillie, D. L., Fire, A., Ruvkun, G., & Mello, C. C. (2001). Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control C. elegans developmental timing. Cell, 106(1), 23– 34. https://doi-org.ezproxy.its.uu.se/10.1016/s0092-8674(01)00431-7

Größl, T., Hammer, E., Bien-Möller, S., Geisler, A., Pinkert, S., Röger, C., Poller, W., Kurreck, J., Völker, U., Vetter, R., & Fechner, H. (2014). A novel artificial MicroRNA expressing AAV vector for phospholamban silencing in cardiomyocytes improves Ca2+ uptake into the sarcoplasmic reticulum. PLoS One, 9(3), e92188. https://doi-org.ezproxy.its.uu.se/10.1371/journal.pone.0092188

Guda, S., Brendel, C., Renella, R., Du, P., Bauer, D. E., Canver, M. C., Grenier, J. K., Grimson, A. W., Kamran, S. C., Thornton, J., de Boer, H., Root, D. E., Milsom, M. D., Orkin, S. H., Gregory, R. I., & Williams, D. A. (2015). MiRNA-embedded shRNAs for lineage-specific BCL11A knockdown and hemoglobin F induction. Molecular Therapy: The Journal of the American Society of Gene Therapy, 23(9), 1465– 1474. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2015.113

Ha, M., & Kim, V. N. (2014). Regulation of microRNA biogenesis. Nature Reviews Molecular Cell Biology, 15(8), 509– 524. https://doi-org.ezproxy.its.uu.se/10.1038/nrm3838

Han, J., Lee, Y., Yeom, K.-H., Kim, Y.-K., Jin, H., & Kim, V. N. (2004). The Drosha-DGCR8 complex in primary microRNA processing. Genes & Development, 18(24), 3016– 3027. https://doi-org.ezproxy.its.uu.se/10.1101/gad.1262504

Han, J., Lee, Y., Yeom, K.-H., Nam, J.-W., Heo, I., Rhee, J.-K., Sohn, S. Y., Cho, Y., Zhang, B.-T., & Kim, V. N. (2006). Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell, 125(5), 887– 901. https://doi-org.ezproxy.its.uu.se/10.1016/j.cell.2006.03.043

Herrera-Carrillo, E., Liu, Y. P., & Berkhout, B. (2017). Improving miRNA delivery by optimizing miRNA expression cassettes in diverse virus vectors. Human Gene Therapy Methods, 28(4), 177– 190. https://doi-org.ezproxy.its.uu.se/10.1089/hgtb.2017.036

Huang, J., Mei, H., Tang, Z., Li, J., Zhang, X., Lu, Y., Huang, F., Jin, Q., & Wang, Z. (2017). Triple-amiRNA VEGFRs inhibition in pancreatic cancer improves the efficacy of chemotherapy through EMT regulation. Journal of Controlled Release, 245, 1– 14. https://doi-org.ezproxy.its.uu.se/10.1016/j.jconrel.2016.11.024

Huang, X., & Jia, Z. (2013). Construction of HCC-targeting artificial miRNAs using natural miRNA precursors. Experimental and Therapeutic Medicine, 6(1), 209– 215. https://doi-org.ezproxy.its.uu.se/10.3892/etm.2013.1111

Hutvágner, G., McLachlan, J., Pasquinelli, A. E., Bálint, E., Tuschl, T., & Zamore, P. D. (2001). A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science (New York, N.Y.), 293(5531), 834– 838. https://doi-org.ezproxy.its.uu.se/10.1126/science.1062961

Ibrišimović, M., Kneidinger, D., Lion, T., & Klein, R. (2013). An adenoviral vector-based expression and delivery system for the inhibition of wild-type adenovirus replication by artificial microRNAs. Antiviral Research, 97(1), 10– 23. https://doi-org.ezproxy.its.uu.se/10.1016/j.antiviral.2012.10.008

Ibrišimović, M., Lion, T., & Klein, R. (2013). Combinatorial targeting of 2 different steps in adenoviral DNA replication by herpes simplex virus thymidine kinase and artificial microRNA expression for the inhibition of virus multiplication in the presence of ganciclovir. BMC Biotechnology, 13, 54. https://doi-org.ezproxy.its.uu.se/10.1186/1472-6750-13-54

Idogawa, M., Sasaki, Y., Suzuki, H., Mita, H., Imai, K., Shinomura, Y., & Tokino, T. (2009). A single recombinant adenovirus expressing p53 and p21-targeting artificial microRNAs efficiently induces apoptosis in human cancer cells. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, 15(11), 3725– 3732. https://doi-org.ezproxy.its.uu.se/10.1158/1078-0432.CCR-08-2396

Ismail, H. A., A. A. Alghasham, M. M. Dowaidar, and A. A. Settin. 2011. “Polymorophisms in MTHF and Ace Genes and the Association with Hypertension among Saudi Population from Qassim Region.” Egyptian Journal of Biochemistry and Molecular Biology 29 (1). https://doi.org/10.4314/ejbmb.v29i1.67382.

Iwasaki, S., Kobayashi, M., Yoda, M., Sakaguchi, Y., Katsuma, S., Suzuki, T., & Tomari, Y. (2010). Hsc70/Hsp90 chaperone machinery mediates ATP-dependent RISC loading of small RNA duplexes. Molecular Cell, 39(2), 292– 299. https://doi-org.ezproxy.its.uu.se/10.1016/j.molcel.2010.05.015

Jin, W., Wang, J., Liu, C.-P., Wang, H.-W., & Xu, R.-M. (2020). Structural basis for pri-miRNA recognition by Drosha. Molecular Cell, 78(3), 423– 433.e5. https://doi-org.ezproxy.its.uu.se/10.1016/j.molcel.2020.02.024

Jopling, C. (2012). Liver-specific microRNA-122. RNA Biology, 9(2), 137– 142. https://doi-org.ezproxy.its.uu.se/10.4161/rna.18827

Kabekkodu, S. P., Shukla, V., Varghese, V. K., Souza, J. D., Chakrabarty, S., & Satyamoorthy, K. (2018). Clustered miRNAs and their role in biological functions and diseases. Biological Reviews, 93(4), 1955– 1986. https://doi-org.ezproxy.its.uu.se/10.1111/brv.12428

Karothia, D., Kumar Dash, P., Parida, M., Bhagyawant, S. S., & Kumar, J. S. (2020). Vector derived artificial miRNA mediated inhibition of West Nile virus replication and protein expression. Gene, 729, 144300. https://doi-org.ezproxy.its.uu.se/10.1016/j.gene.2019.144300

Keeler, A. M., Sapp, E., Chase, K., Sottosanti, E., Danielson, E., Pfister, E., Stoica, L., DiFiglia, M., Aronin, N., & Sena-Esteves, M. (2016). Cellular analysis of silencing the Huntington's disease gene using AAV9 mediated delivery of artificial micro RNA into the striatum of Q140/Q140 mice. Journal of Huntington's Disease, 5(3), 239– 248. https://doi-org.ezproxy.its.uu.se/10.3233/JHD-160215

Keiser, M. S., Boudreau, R. L., & Davidson, B. L. (2014). Broad therapeutic benefit after RNAi expression vector delivery to deep cerebellar nuclei: Implications for spinocerebellar ataxia Type 1 therapy. Molecular Therapy, 22(3), 588– 595. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2013.279

Keiser, M. S., Geoghegan, J. C., Boudreau, R. L., Lennox, K. A., & Davidson, B. L. (2013). RNAi or overexpression: Alternative therapies for spinocerebellar ataxia Type 1. Neurobiology of Disease, 56, 6– 13. https://doi-org.ezproxy.its.uu.se/10.1016/j.nbd.2013.04.003

Keiser, M. S., Kordower, J. H., Gonzalez-Alegre, P., & Davidson, B. L. (2015). Broad distribution of ataxin 1 silencing in rhesus cerebella for spinocerebellar ataxia type 1 therapy. Brain, 138(12), 3555– 3566. https://doi-org.ezproxy.its.uu.se/10.1093/brain/awv292

Keiser, M. S., Monteys, A. M., Corbau, R., Gonzalez-Alegre, P., & Davidson, B. L. (2016). RNAi prevents and reverses phenotypes induced by mutant human ataxin-1. Annals of Neurology, 80(5), 754– 765. https://doi-org.ezproxy.its.uu.se/10.1002/ana.24789

Kerr, A., Tam, L., Cioroch, M., Hale, A., Douglas, G., Channon, K., & Wade-Martins, R. (2016). A novel combinatorial non-viral vector to treat familial hypercholesterolaemia (FH). Atherosclerosis, 252, e238. https://doi-org.ezproxy.its.uu.se/10.1016/j.atherosclerosis.2016.07.018

Keskin, S., Brouwers, C. C., Sogorb-Gonzalez, M., Martier, R., Depla, J. A., Vallès, A., van Deventer, S. J., Konstantinova, P., & Evers, M. M. (2019). AAV5-miHTT lowers Huntingtin mRNA and protein without off-target effects in patient-derived neuronal cultures and astrocytes. Molecular Therapy. Methods & Clinical Development, 15, 275– 284. https://doi-org.ezproxy.its.uu.se/10.1016/j.omtm.2019.09.010

Khvorova, A., Reynolds, A., & Jayasena, S. D. (2003). Functional siRNAs and miRNAs exhibit strand bias. Cell, 115(2), 209– 216. https://doi-org.ezproxy.its.uu.se/10.1016/s0092-8674(03)00801-8

Kim, N., Nguyen, T. D., Li, S., & Nguyen, T. A. (2018). SRSF3 recruits DROSHA to the basal junction of primary microRNAs. RNA, 24(7), 892– 898. https://doi-org.ezproxy.its.uu.se/10.1261/rna.065862.118

Kim, Y.-K., Kim, B., & Kim, V. N. (2016). Re-evaluation of the roles of DROSHA, Exportin 5, and DICER in microRNA biogenesis. Proceedings of the National Academy of Sciences, 113(13), E1881– E1889. https://doi-org.ezproxy.its.uu.se/10.1073/pnas.1602532113

Koch, P., Breuer, P., Peitz, M., Jungverdorben, J., Kesavan, J., Poppe, D., Doerr, J., Ladewig, J., Mertens, J., Tüting, T., Hoffmann, P., Klockgether, T., Evert, B. O., Wüllner, U., & Brüstle, O. (2011). Excitation-induced ataxin-3 aggregation in neurons from patients with Machado-Joseph disease. Nature, 480(7378), 543– 546. https://doi-org.ezproxy.its.uu.se/10.1038/nature10671

Kretov, D. A., Walawalkar, I. A., Mora-Martin, A., Shafik, A. M., Moxon, S., & Cifuentes, D. (2020). Ago2-dependent processing allows miR-451 to evade the global microRNA turnover elicited during erythropoiesis. Molecular Cell, 78(2), 317–328. https://doi-org.ezproxy.its.uu.se/10.1016/j.molcel.2020.02.020

Kwon, S. C., Baek, S. C., Choi, Y.-G., Yang, J., Lee, Y.-S., Woo, J.-S., & Kim, V. N. (2019). Molecular basis for the single-nucleotide precision of primary microRNA processing. Molecular Cell, 73(3), 505– 518.e5. https://doi-org.ezproxy.its.uu.se/10.1016/j.molcel.2018.11.005

Lebbink, R. J., Lowe, M., Chan, T., Khine, H., Wang, X., & McManus, M. T. (2011). Polymerase II promoter strength determines efficacy of microRNA adapted shRNAs. PLoS One, 6(10), e26213. https://doi-org.ezproxy.its.uu.se/10.1371/journal.pone.0026213

Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Rådmark, O., Kim, S., & Kim, V. N. (2003). The nuclear RNase III Drosha initiates microRNA processing. Nature, 425(6956), 415– 419. https://doi-org.ezproxy.its.uu.se/10.1038/nature01957

Lee, Y., Hur, I., Park, S.-Y., Kim, Y.-K., Suh, M. R., & Kim, V. N. (2006). The role of PACT in the RNA silencing pathway. The EMBO Journal, 25(3), 522– 532. https://doi-org.ezproxy.its.uu.se/10.1038/sj.emboj.7600942

Lee, Y., Jeon, K., Lee, J.-T., Kim, S., & Kim, V. N. (2002). MicroRNA maturation: Stepwise processing and subcellular localization. The EMBO Journal, 21(17), 4663– 4670. https://doi-org.ezproxy.its.uu.se/10.1093/emboj/cdf476

Li, H., Okada, H., Suzuki, S., Sakai, K., Izumi, H., Matsushima, Y., Ichinohe, N., Goto, Y.-I., Okada, T., & Inoue, K. (2019). Gene suppressing therapy for Pelizaeus-Merzbacher disease using artificial microRNA. JCI Insight, 4(10). https://doi-org.ezproxy.its.uu.se/10.1172/jci.insight.125052

Li, M., Husic, N., Lin, Y., Christensen, H., Malik, I., McIver, S., LaPash Daniels, C. M., Harris, D. A., Kotzbauer, P. T., Goldberg, M. P., & Snider, B. J. (2010). Optimal promoter usage for lentiviral vector-mediated transduction of cultured central nervous system cells. Journal of Neuroscience Methods, 189(1), 56– 64. https://doi-org.ezproxy.its.uu.se/10.1016/j.jneumeth.2010.03.019

Li, S., Nguyen, T. D., Nguyen, T. L., & Nguyen, T. A. (2020). Mismatched and wobble base pairs govern primary microRNA processing by human microprocessor. Nature Communications, 11(1), 1926. https://doi-org.ezproxy.its.uu.se/10.1038/s41467-020-15674-2

Li, Z., Wenhua, Z., Zhao, W., Baohe, Z., Yulong, H., Junsheng, P., Shirong, C., & Jinping, M. (2006). Inhibition of PRL-3 gene expression in gastric cancer cell line SGC7901 via microRNA suppressed reduces peritoneal metastasis. Biochemical and Biophysical Research Communications, 348, 229– 237. https://doi-org.ezproxy.its.uu.se/10.1016/j.bbrc.2006.07.043

Liang, Z., Wu, H., Reddy, S., Zhu, A., Wang, S., Blevins, D., Yoon, Y., Zhang, Y., & Shim, H. (2007). Blockade of invasion and metastasis of breast cancer cells via targeting CXCR4 with an artificial microRNA. Biochemical and Biophysical Research Communications. 363, 542– 546. https://doi-org.ezproxy.its.uu.se/10.1016/j.bbrc.2007.09.007

Liu, C., Wang, S., Zhu, S., Wang, H., Gu, J., Gui, Z., Jing, J., Hou, X., & Shao, Y. (2016). MAP3K1-targeting therapeutic artificial miRNA suppresses the growth and invasion of breast cancer in vivo and in vitro. Springerplus, 5, 11. https://doi-org.ezproxy.its.uu.se/10.1186/s40064-015-1597-z

Liu, J., Carmell, M. A., Rivas, F. V., Marsden, C. G., Thomson, J. M., Song, J.-J., Hammond, S. M., Joshua-Tor, L., & Hannon, G. J. (2004). Argonaute2 is the catalytic engine of mammalian RNAi. Science (New York, N.Y.), 305(5689), 1437– 1441. https://doi-org.ezproxy.its.uu.se/10.1126/science.1102513

Liu, W., Pfister, E. L., Kennington, L. A., Chase, K. O., Mueller, C., DiFiglia, M., & Aronin, N. (2016). Does the mutant CAG expansion in huntingtin mRNA interfere with exonucleolytic cleavage of its first exon? Journal of Huntington's Disease, 5(1), 33– 38. https://doi-org.ezproxy.its.uu.se/10.3233/JHD-150183

Liu, X., Fang, H., Chen, H., Jiang, X., Fang, D., Wang, Y., & Zhu, D. (2012). An artificial miRNA against HPSE suppresses melanoma invasion properties, correlating with a Down-regulation of chemokines and MAPK phosphorylation. PLoS One, 7(6), e38659. https://doi-org.ezproxy.its.uu.se/10.1371/journal.pone.0038659

Liu, Y. P., Haasnoot, J., ter Brake, O., Berkhout, B., & Konstantinova, P. (2008). Inhibition of HIV-1 by multiple siRNAs expressed from a single microRNA polycistron. Nucleic Acids Research, 36(9), 2811– 2824. https://doi-org.ezproxy.its.uu.se/10.1093/nar/gkn109

Liu, Z., Wang, J., Cheng, H., Ke, X., Sun, L., Zhang, Q. C., & Wang, H.-W. (2018). Cryo-EM structure of human dicer and its complexes with a pre-miRNA substrate. Cell, 173(5), 1191– 1203.e12. https://doi-org.ezproxy.its.uu.se/10.1016/j.cell.2018.03.080

Lu, S., & Cullen, B. R. (2004). Adenovirus VA1 noncoding RNA can inhibit small interfering RNA and MicroRNA biogenesis. Journal of Virology, 78(23), 12868– 12876. https://doi-org.ezproxy.its.uu.se/10.1128/JVI.78.23.12868-12876.2004

Ma, H., Wu, Y., Choi, J.-G., & Wu, H. (2013). Lower and upper stem-single-stranded RNA junctions together determine the Drosha cleavage site. Proceedings of the National Academy of Sciences of the United States of America, 110(51), 20687– 20692. https://doi-org.ezproxy.its.uu.se/10.1073/pnas.1311639110

Ma, H., Wu, Y., Dang, Y., Choi, J.-G., Zhang, J., & Wu, H. (2014). Pol III promoters to express small RNAs: Delineation of transcription initiation. Molecular Therapy. Nucleic Acids, 3(5), e161. https://doi-org.ezproxy.its.uu.se/10.1038/mtna.2014.12

Maepa, M. B., Ely, A., Grayson, W., & Arbuthnot, P. (2017). Sustained inhibition of HBV replication in vivo after systemic injection of AAVs encoding artificial antiviral primary microRNAs. Molecular Therapy - Nucleic Acids, 7, 190– 199. https://doi-org.ezproxy.its.uu.se/10.1016/j.omtn.2017.04.007

Maillard, P. V., van der Veen, A. G., Poirier, E. Z., & Reis e Sousa, C. (2019). Slicing and dicing viruses: Antiviral RNA interference in mammals. The EMBO Journal, 38(8). https://doi-org.ezproxy.its.uu.se/10.15252/embj.2018100941

Mao, C., Liu, H., Chen, P., Ye, J., Teng, L., Jia, Z., & Cao, J. (2015). Cell-specific expression of artificial microRNAs targeting essential genes exhibit potent antitumor effect on hepatocellular carcinoma cells. Oncotarget, 6(8), 5707– 5719. https://doi-org.ezproxy.its.uu.se/10.18632/oncotarget.3302

Mao, L., Liu, S., Hu, L., Jia, L., Wang, H., Guo, M., Chen, C., Liu, Y., & Xu, L. (2018). miR-30 family: A promising regulator in development and disease. BioMed Research International, 2018. https://doi-org.ezproxy.its.uu.se/10.1155/2018/9623412

Martier, R., Liefhebber, J. M., García-Osta, A., Miniarikova, J., Cuadrado-Tejedor, M., Espelosin, M., Ursua, S., Petry, H., van Deventer, S. J., Evers, M. M., & Konstantinova, P. (2019). Targeting RNA-mediated toxicity in C9orf72 ALS and/or FTD by RNAi-based gene therapy. Molecular Therapy. Nucleic Acids, 16, 26– 37. https://doi-org.ezproxy.its.uu.se/10.1016/j.omtn.2019.02.001

Martier, R., Liefhebber, J. M., Miniarikova, J., van der Zon, T., Snapper, J., Kolder, I., Petry, H., van Deventer, S. J., Evers, M. M., & Konstantinova, P. (2019). Artificial MicroRNAs targeting C9orf72 can reduce accumulation of intra-nuclear transcripts in ALS and FTD patients. Molecular Therapy. Nucleic Acids, 14, 593– 608. https://doi-org.ezproxy.its.uu.se/10.1016/j.omtn.2019.01.010

Martier, R., Sogorb-Gonzalez, M., Stricker-Shaver, J., Hübener-Schmid, J., Keskin, S., Klima, J., Toonen, L. J., Juhas, S., Juhasova, J., Ellederova, Z., Motlik, J., Haas, E., van Deventer, S., Konstantinova, P., Nguyen, H. P., & Evers, M. M. (2019). Development of an AAV-based microRNA gene therapy to treat Machado-Joseph disease. Molecular Therapy - Methods & Clinical Development, 15, 343– 358. https://doi-org.ezproxy.its.uu.se/10.1016/j.omtm.2019.10.008

Mashima, R. (2015). Physiological roles of miR-155. Immunology, 145(3), 323– 333. https://doi-org.ezproxy.its.uu.se/10.1111/imm.12468

McBride, J. L., Boudreau, R. L., Harper, S. Q., Staber, P. D., Monteys, A. M., Martins, I., Gilmore, B. L., Burstein, H., Peluso, R. W., Polisky, B., Carter, B. J., & Davidson, B. L. (2008). Artificial miRNAs mitigate shRNA-mediated toxicity in the brain: Implications for the therapeutic development of RNAi. Proceedings of the National Academy of Sciences of the United States of America, 105(15), 5868– 5873. https://doi-org.ezproxy.its.uu.se/10.1073/pnas.0801775105

McBride, J. L., Pitzer, M. R., Boudreau, R. L., Dufour, B., Hobbs, T., Ojeda, S. R., & Davidson, B. L. (2011). Preclinical safety of RNAi-mediated HTT suppression in the rhesus macaque as a potential therapy for Huntington's disease. Molecular Therapy, 19(12), 2152– 2162. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2011.219

McLaughlin, J., Cheng, D., Singer, O., Lukacs, R. U., Radu, C. G., Verma, I. M., & Witte, O. N. (2007). Sustained suppression of Bcr-Abl-driven lymphoid leukemia by microRNA mimics. Proceedings of the National Academy of Sciences of the United States of America, 104(51), 20501– 20506. https://doi-org.ezproxy.its.uu.se/10.1073/pnas.0710532105

McManus, M. T., Petersen, C. P., Haines, B. B., Chen, J., & Sharp, P. A. (2002). Gene silencing using micro-RNA designed hairpins. RNA, 8(6), 842– 850.

Medley, J. C., Panzade, G., & Zinovyeva, A. Y. (2020). microRNA strand selection: Unwinding the rules. WIREs RNA, e1627. https://doi-org.ezproxy.its.uu.se/10.1002/wrna.1627

Michlewski, G., & Cáceres, J. F. (2019). Post-transcriptional control of miRNA biogenesis. RNA, 25(1), 1– 16. https://doi-org.ezproxy.its.uu.se/10.1261/rna.068692.118

Miniarikova, J., Evers, M. M., & Konstantinova, P. (2018). Translation of microRNA-based Huntingtin-lowering therapies from preclinical studies to the clinic. Molecular Therapy: The Journal of the American Society of Gene Therapy, 26(4), 947– 962. https://doi-org.ezproxy.its.uu.se/10.1016/j.ymthe.2018.02.002

Miniarikova, J., Zanella, I., Huseinovic, A., van der Zon, T., Hanemaaijer, E., Martier, R., Koornneef, A., Southwell, A. L., Hayden, M. R., van Deventer, S. J., Petry, H., & Konstantinova, P. (2016). Design, characterization, and Lead selection of therapeutic miRNAs targeting Huntingtin for development of gene therapy for Huntington's disease. Molecular Therapy. Nucleic Acids, 5, e297. https://doi-org.ezproxy.its.uu.se/10.1038/mtna.2016.7

Miniarikova, J., Zimmer, V., Martier, R., Brouwers, C. C., Pythoud, C., Richetin, K., Rey, M., Lubelski, J., Evers, M. M., van Deventer, S. J., Petry, H., Déglon, N., & Konstantinova, P. (2017). AAV5-miHTT gene therapy demonstrates suppression of mutant huntingtin aggregation and neuronal dysfunction in a rat model of Huntington's disease. Gene Therapy, 24(10), 630–639. https://doi-org.ezproxy.its.uu.se/10.1038/gt.2017.71

Abdelhamid, H. N., M. Dowaidar, M. Hällbrink, and Ü. Langel. 2019. Cell Penetrating Peptides-Hierarchical Porous Zeolitic Imidazolate Frameworks Nanoparticles: An Efficient Gene Delivery Platform. SSRN Electron. J. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3435895.

Abdelhamid, Hani Nasser, Moataz Dowaidar, Mattias Hällbrink, and Ülo Langel. 2020. Gene Delivery Using Cell Penetrating Peptides-Zeolitic Imidazolate Frameworks. Microporous and Mesoporous Materials: The Official Journal of the International Zeolite Association 300 (June): 110173. https://doi.org/10.1016/j.micromeso.2020.110173.

Abdelhamid, Hani Nasser, Moataz Dowaidar, and Ülo Langel. 2020. Carbonized Chitosan Encapsulated Hierarchical Porous Zeolitic Imidazolate Frameworks Nanoparticles for Gene Delivery. Microporous and Mesoporous Materials: The Official Journal of the International Zeolite Association 302 (August): 110200. https://doi.org/10.1016/j.micromeso.2020.110200.

Ahmad, Almeman, Khalaf Hassan, Rasool Semaab, Moataz Dowaidar, and Al Orainy Mohammad. 2013. The Impact of CYP2C19 Polymorphism on Platelet Reactivity for Guiding Clopidogrel Treatment and Cost Analysis. Journal of the Saudi Heart Association 25 (2): 107. https://doi.org/10.1016/j.jsha.2013.03.005.

Algahsham, Abdullah, Ahmad A. A. Settin, Ahmad Ali, and Hisham Ismail. n.d. Association of MTHFR C677T and A1298C Polymorphisms with Hypertension among Saudi Subjects from Qassim Region. International Journal of Health Sciences 6 (1). Accessed June 18, 2021. http://ijhs.org.sa/index.php/journal/article/view/312.

Algasham, Abdullah, Hisham Ismail, Moataz Dowaidar, and Ahmad A. Settin. 2011. Methylenetetrahydrofolate Reductase (MTHFR) and Angiotensin Converting Enzyme (ACE) Gene Polymorphisms among Saudi Population from Qassim Region. International Journal of Health Sciences 5 (2 Suppl 1): 3–4. https://www.ncbi.nlm.nih.gov/pubmed/23284552.

Alghasham, Abdullah, Ahmad Ali, Hisham Ismail, Moataz Dowaidar, and Ahmad A. Settin. 2012. CYP2J2 -50 G/T and ADRB2 G46A Gene Polymorphisms in Saudi Subjects with Hypertension. Genetic Testing and Molecular Biomarkers 16 (9): 1027–31. https://doi.org/10.1089/gtmb.2012.0006.

Alghasham, Abdullah, Ahmad A. Settin, Ahmad Ali, Moataz Dowaidar, and Hisham Ismail. 2012a. Association of MTHFR C677T and A1298C Gene Polymorphisms with Hypertension. International Journal of Health Sciences 6 (1): 3–11. https://doi.org/10.12816/0005968.

Moataz Dowaidar. 2012b. Association of MTHFR C677T and A1298C Gene Polymorphisms with Hypertension. International Journal of Health Sciences 6 (1): 3–11. https://doi.org/10.12816/0005968.

Ali, Ahmad, Abdullah Alghasham, Hisham Ismail, Moataz Dowaidar, and Ahmad Settin. 2013. ACE I/D and eNOS E298D Gene Polymorphisms in Saudi Subjects with Hypertension. Journal of the Renin-Angiotensin-Aldosterone System: JRAAS 14 (4): 348–53. https://doi.org/10.1177/1470320312459976.

Ali, Ahmed A. A., Nahla M. Wassim, Moataz M. Dowaidar, and Ahmed E. Yaseen. 2013. Genetic Polymorphism of CYP2D6 Gene among Egyptian Hypertensive Cases. The Journal of Basic & Applied Zoology 66 (4): 228–33. https://doi.org/10.1016/j.jobaz.2012.12.002.

Ali, Ahmed A. A., Nahla M. Wassim, Moataz Dowaidar, and Ahmed E. Yaseen. 2013b. Association of eNOS (E298D) and CYP2J2 (−50G/T) Gene Polymorphisms with Hypertension among Egyptian Cases. The Journal of Basic & Applied Zoology 66 (4): 234–41. https://doi.org/10.1016/j.jobaz.2012.12.001.

Moataz Dowaidar. 2013. Association of eNOS (E298D) and CYP2J2 (−50G/T) Gene Polymorphisms with Hypertension among Egyptian Cases. The Journal of Basic & Applied Zoology 66 (4): 234–41. https://doi.org/10.1016/j.jobaz.2012.12.001.

Aljarallah, Badr, Ahmed Ali, Moataz Dowaidar, and Ahmad Settin. 2011. Prevalence of α-1-Antitrypsin Gene Mutations in Saudi Arabia. Saudi Journal of Gastroenterology: Official Journal of the Saudi Gastroenterology Association 17 (4): 256–60. https://doi.org/10.4103/1319-3767.82580.

Dowaidar, M., J. Regberg, D. A. Dobchev, and T. Lehto. 2017. Refinement of a Quantitative Structure–activity Relationship Model for Prediction of Cell-Penetrating Peptide Based Transfection Systems. International Journal of. https://link.springer.com/content/pdf/10.1007/s10989-016-9542-8.pdf.

Dowaidar, Moataz. 2017. In-Silico Design of Peptide-Based Transfection Systems, in-Vitro Validation, and up-Take Pathways Investigation. Department of Neurochemistry, Stockholm University.

Moataz Dowaidar. 2018. Chimeric Gene Delivery Vectors : Design, Synthesis, and Mechanisms from Transcriptomics Analysis. Department of Biochemistry and Biophysics, Stockholm University. https://www.diva-portal.org/smash/record.jsf?pid=diva2:1242000.

Moataz Dowaidar. Cardiometabolic Conditions Could Be Related to Vitamin D Deficiency. The Genetic Determinants That Affect Vitamin D Pathways May Be Solved with Nanomedicines. https://doi.org/10.31219/osf.io/nqewr.

Moataz Dowaidar. Different Insulin Resistance and Inflammation Pathways Are Influenced by Genetic Factors in Metabolic Syndrome. Gene Therapy Enables Early Recognition and Treatment of the Genetic Factors. https://doi.org/10.31219/osf.io/gqwj2.

Moataz Dowaidar. Gene Therapy Has Been Shown to Be Valuable for Understanding Complex Disease Pathophysiologies. The Medical Profession as a Whole Will Have to Invest in Specialized Investigations. https://doi.org/10.31219/osf.io/8fg9y.

Moataz Dowaidar. Genetic and Epigenetic Discoveries Hold Promising Avenues in Cardiovascular Prevention and Management (CVDs). Key Nucleic Acids Are Being Researched and Developed for Medicinal Use. https://doi.org/10.31219/osf.io/hk7pe.

Moataz Dowaidar. Genome Editing Can Now Be Carried out in an Isogenic Setting. It Can Be Effectively Transmitted to Somatic Tissues in Mice, but Not to Humans. Despite These Doubts, CRIS Has Great Potential as a Medical Promise. https://doi.org/10.31219/osf.io/4rn3v.

Moataz Dowaidar. Genome-Wide Association Experiments Have Uncovered a Slew of Cardiometabolic Trait-Associated Variants. This Information Can Be Useful in the Implementation of New Diagnostic and Treatment Strategies. https://doi.org/10.31219/osf.io/4vws8.

Moataz Dowaidar. Genome-Wide Association Studies (GWAS) Have Revolutionized Our View of Human Health and Disease Genetics and Offered Novel Gene Therapy Targets. https://doi.org/10.31219/osf.io/rvm3z.

Moataz Dowaidar. Metabolic Syndrome_ the Presence of Inflammatory Mechanisms in Abdominal Obesity Is Undeniable, Gene Therapy Using Nanoparticles and Adenoviruses Technologies Is Promising. https://doi.org/10.31219/osf.io/2j5xt.

Moataz Dowaidar. miRNAs May Be Used as Preventive Agents for Metabolic Diseases in the near Future. Understanding the Interplay between pro-Adipogenic_ and Anti-Ad Pipogenic miRNA' Could Lead to New Biomarkers. https://doi.org/10.31219/osf.io/3dr8c.

Moataz Dowaidar. Nanomedicine Has Elegantly Attempted to Cure Multiple Gene Polymorphisms and Mutations in Cardiovascular Diseases Using Gene Therapy Techniques. https://doi.org/10.31219/osf.io/d3x8g.

Moataz Dowaidar. Thrombosis Pathways and Therapeutic Strategies. https://doi.org/10.31219/osf.io/57vyz.

Moataz Dowaidar. What Genomic Research Has Told Us about the Obesity and Its Possible Gene Therapy Targets. https://doi.org/10.31219/osf.io/ym49s.

Moataz Dowaidar. Exosomes Can Make the Use of Circulating miRNA as a Biomarker More Feasible. The Aim of Gene Therapy Should Be to Learn Everything There Is to Know about miRNA Activity. https://doi.org/10.31219/osf.io/edkua.

Moataz Dowaidar. Anti-Sense Pathways Have Been Generated Using siRNA. The Liver and Other Often Used Organs Will Now Be Targeted. https://doi.org/10.31219/osf.io/m6xvp.

Moataz Dowaidar. CrisPR/CRIS Systems Are Highly Effective and Useful for Genomic Manipulation. Despite This, Cardiac Treatment Remains Difficult due to Existing Genome Editing and Delivery Processes. https://doi.org/10.31219/osf.io/3nwzd.

Moataz Dowaidar. Discoveries in Gene-Environment Interactions That Influence CVD, Lipid Traits, Obesity, Diabetes, and Hypertension Appear to Be Able to Influence Gene Therapy. https://doi.org/10.31219/osf.io/cr5af.

Moataz Dowaidar. Genome Editing's Potential Target Diseases in the Cardiovascular Field. https://doi.org/10.31219/osf.io/gc23p.

Moataz Dowaidar. Key Genetic Factors in the Metabolic Syndrome Predisposition Which May Be a Therapeutic Options by Gene Therapy. https://doi.org/10.31219/osf.io/f38sk.

Moataz Dowaidar. miRNA Can Be a Part of Both the Onset and Cure of Coronary Heart Disease. https://doi.org/10.31219/osf.io/teqh8.

Moataz Dowaidar. Preclinical Studies and Clinical Trials Have Sparked Interest in Certain Biological Medications for Atherosclerotic Coronary Heart Disease. https://doi.org/10.31219/osf.io/ts8mh.

Moataz Dowaidar. Researchers Would Be Able to Develop a Detailed Picture of Chromatin in Disease, Which Would Be Useful for Gene Therapy. https://doi.org/10.31219/osf.io/m9z48.

Moataz Dowaidar. The Cardiometabolic-Based Chronic Disease Model Lays the Foundations for Accurate, Evidence-Based Preventive Targeting and Gene Therapy. https://doi.org/10.31219/osf.io/up9z4.

Moataz Dowaidar. 2D MOFs Have Unique Features for Biological Applications. They Can Be Utilized for Gene Therapy, Bioimaging, Biosensing, Photodynamic Therapy, and Tissue Engineering. https://doi.org/10.31219/osf.io/4q9ct.

Moataz Dowaidar. 3D Bioprinting for Enhanced Vascularization, and Gene Editing to Provide a More Favorable Immunological Response Are Just Some of the Potential Uses of Carbon Materials. https://doi.org/10.31219/osf.io/v2xy8.

Moataz Dowaidar. Anderson–Fabry Disease Can Be a Target for Gene Therapy. https://doi.org/10.31219/osf.io/tcgka.

Moataz Dowaidar. Antisense Oligonucleotides (ASOs) and CRISPR Systems Are Promising Gene Therapy Treatments for Alzheimer's Disease. https://doi.org/10.31219/osf.io/ws796.

Moataz Dowaidar. Any Alteration in PPAR Genomic Sequence, Splicing Pattern, or PTM Is Likely to Cause Major Alterations in Its Function. In Personalized Medicine, Such Data Becomes More Significant in Gene Therapy Design. https://doi.org/10.31219/osf.io/y8n79.

Moataz Dowaidar. Applying Genome-Wide Association Technology to Brain Diseases Enables the Discovery of lncRNas Targets for Gene Therapy. https://doi.org/10.31219/osf.io/hm4eu.

Moataz Dowaidar. Autophagy and Proteostasis Adjustment Role in Normal Brain Function and Neurodegenerative Disorders. https://doi.org/10.31219/osf.io/m4yra.

Moataz Dowaidar. Basal Ganglia-Cerebellar and Brainstem-Cerebellar Circuits May Interact Improperly with Dystonia. Linking Network Disruptions to Cell Failure Will Enable Understanding Pathophysiology and Designing Gene Therapy Methods. https://doi.org/10.31219/osf.io/8w35s.

Moataz Dowaidar. Blood Products Are Used to Treat a Multitude of Diseases, so the Blood Transfusion System Needs to Be Enhanced. CRISPR/Cas9 Has Made It Viable to Make HLA Class I-Deleted Blood Products to Avoid Rejection. https://doi.org/10.31219/osf.io/egr3n.

Moataz Dowaidar. Calixarenes (CAs) Are Promising in Biomedicine, Biosensing, Bioimaging and Gene Delivery Systems. https://doi.org/10.31219/osf.io/n9vjy.

Moataz Dowaidar. CAR T Cell Research Has Quickly Advanced from the Bench to the Clinic and Back. The Results of the Trials Have Revealed New Mechanisms. https://doi.org/10.31219/osf.io/f9wm7.

Moataz Dowaidar. CAR T-Cell Treatment Remains Clinically Challenging. Therapeutic Strategies May Be Designed to Cut off Immunotherapy Utilizing Safety Switches. https://doi.org/10.31219/osf.io/s7x4y.

Moataz Dowaidar. Central Nervous System Gene Therapy Has Entered a New Development Paradigm. New Techniques Are Being Employed for a Wide Range of Illness Indications and Pathways. https://doi.org/10.31219/osf.io/j49wz.

Moataz Dowaidar. Chronic Obstructive Pulmonary Condition (COPD) Is a Prevalent, Preventable, and Curable Illness with Persistent Respiratory Symptoms and Airflow Limitation. https://doi.org/10.31219/osf.io/vkdut.

Moataz Dowaidar. CircRNAs Have the Potential to Aid in the Diagnosis and Treatment of Lipid Diseases. https://doi.org/10.31219/osf.io/y3hp4.

Moataz Dowaidar. Clinical Symptoms, Underlying Pathogenesis, and the Prospect of Tailored Therapies Have All Benefited from Genetic Discoveries in Parkinson's Disease. https://doi.org/10.31219/osf.io/pdzqb.

Moataz Dowaidar. Code Distribution of siRNA for Cancer Genes such as p53 and Bcl2 Family Genes Has Demonstrated Efficacy in Killing Cancer Cells. Nanoparticles Can Produce a Surface Where Numerous Drugs May Be Coupled, Allowing Combinatory Treatment. https://doi.org/10.31219/osf.io/hvcse.

Moataz Dowaidar. Cognitive Deficiencies Pathophysiology Are Mainly an Unknown Area. Curing the Neurological Conditions Could Be an Objective for Gene Therapy. https://doi.org/10.31219/osf.io/23xf8.

Moataz Dowaidar. CRISPR-Based Gene Editing Is Presently Being Tried in Many Clinical Trials. https://doi.org/10.31219/osf.io/qbngx.

Moataz Dowaidar. CRISPR–Cas9 Gene Editing as a Tool for Developing Immunotherapy for Cancer. https://doi.org/10.31219/osf.io/dvr4t.

Moataz Dowaidar. CRISPR/Cas System Research Has Advanced Significantly in Biological sciences.There Are Still Many Challenges to Effective Delivery before Efficient Gene Editing May Be Achieved. https://doi.org/10.31219/osf.io/mc26v.

Moataz Dowaidar. CRISPR/Cas9 Genome Editing Technology Applications in Biological and Biomedical Fields. https://doi.org/10.31219/osf.io/ctqbe.

Moataz Dowaidar. Critical Limb Ischemia Potential Gene Therapy Strategies. https://doi.org/10.31219/osf.io/aqcpt.

Moataz Dowaidar. Deep Learning Algorithms for scRNAseq Analysis Have Yielded Positive Results, but There Are Still More Promising Ways That Need to Be Developed for Regenerative Medicine. https://doi.org/10.31219/osf.io/dh2pt.

Moataz Dowaidar. Depression May Be Epigenetically Controlled by miRNAs Making It a Diagnostic or Gene Therapy Target. https://doi.org/10.31219/osf.io/fw65m.

Moataz Dowaidar. Dermatophytes: Role of Host Genetics in the Development of Illness. https://doi.org/10.31219/osf.io/mf3bu.

Moataz Dowaidar. Developments in Biomedical Technology Will Increase the Importance of mRNA in Treating Brain Tumors, as Well as Other Malignancies. https://doi.org/10.31219/osf.io/tvj5x.

Moataz Dowaidar. Downstream Processing of Virus, Virus-like Particles and Nanoparticulate Inclusion Bodies to Be Used as Gene Delivery Vehicles for Human Gene Therapy Applications. https://doi.org/10.31219/osf.io/exa3q.

Moataz Dowaidar. Dravet Syndrome Is a Severe Developmental and Epileptic Encephalopathy. Fenfluramine and Gene Therapy Are Promising. https://doi.org/10.31219/osf.io/zvq8y.

Moataz Dowaidar. Exosomes' Function in Cardiovascular Protection and Neovascularization Implies That They Might Be Used to Treat Ischemia and Atherosclerotic Cardiovascular Diseases. https://doi.org/10.31219/osf.io/2h8c7.

Moataz Dowaidar. Ferropsis Cell Death Can Cause Complications That May Be Difficult to Detect and Quantify: Autophagy Role and Possible Therapeutics. https://doi.org/10.31219/osf.io/zd2jg.

Moataz Dowaidar. Following the Discovery of Anti-MDA5 Ab, the Clinical Understanding of Dermatomyositis Has Been Improved. https://doi.org/10.31219/osf.io/j2t5f.

Moataz Dowaidar. For the Treatment of Cystic Fibrosis, RNA Medicines, Gene Transfer Therapies, and Gene Editing Treatments Have Potential. https://doi.org/10.31219/osf.io/6afzm.

Moataz Dowaidar. Frontotemporal Dementia Is a Complex Disorder with a Wide Spectrum of Clinical Symptoms. Personalized Medicine and Gene Therapy Are Promising Strategies for Treatment. https://doi.org/10.31219/osf.io/gh4x7.

Moataz Dowaidar. G6PD Deficiency Is a Common Genetic Trait That Can Protect Heterozygotes from Dying from Malaria. https://doi.org/10.31219/osf.io/g2kza.

Moataz Dowaidar. Gastric Cancer Is the World's Second-Largest Death Cause. Peptides Can Be Used to Deliver Radiation or Other Fatal Chemicals to Tumors. https://doi.org/10.31219/osf.io/eu5mj.

Moataz Dowaidar. Gene Doping May Be Possible for Lifestyle Enhancement. https://doi.org/10.31219/osf.io/8xkm5.

Moataz Dowaidar. Gene Expression Assays Gather Evidence That They Can Provide Useful Therapeutic Information in Young Women. https://doi.org/10.31219/osf.io/d372s.

Moataz Dowaidar. Gene Therapy and Genome-Editing Treatments That Can Protect Patients from Coronary Artery Disease Are under Investigation. https://doi.org/10.31219/osf.io/xqgf8.

Moataz Dowaidar. Gene Therapy Approaches for Hemophilia A and B. https://doi.org/10.31219/osf.io/ufc4g.

Moataz Dowaidar. Gene Therapy for the Central Nervous System Has Been Initiated. This Expansion Will Require Some Degree of Simplicity in Delivery Processes. https://doi.org/10.31219/osf.io/hdy5q.

Moataz Dowaidar. Gene Therapy for the Treatment of Spinal Muscular Atrophy. https://doi.org/10.31219/osf.io/kpz5f.

Moataz Dowaidar. Gene Therapy May Benefit Inherited Ichthyoses with Concurrent Fungal Infections and Severe Ich Thyroidoses. https://doi.org/10.31219/osf.io/zxmun.

Moataz Dowaidar. Gene Therapy May Target APOE for Alzheimer's Disease. https://doi.org/10.31219/osf.io/3y52k.

Moataz Dowaidar. Gene Therapy Promises Accurate, Targeted Administration and Overcoming Drug Resistance in Diverse Cancer Cells. https://doi.org/10.31219/osf.io/j34n6.

Moataz Dowaidar. Gene Therapy Targeting FVIII, FIX for Haemophilia Treatment. https://doi.org/10.31219/osf.io/qcbwp.

Moataz Dowaidar. Gene Therapy Targeting PRMT5 May Be Useful in Immunotherapy. https://doi.org/10.31219/osf.io/gkw8j.

Moataz Dowaidar. Gene Therapy Using Extracellular Vesicles Loaded with miRNA Derived from Bone Marrow Mesenchymal Stem Cells Is a Cell-Free Medication Delivery Method Used in a Variety of Diseases. https://doi.org/10.31219/osf.io/3znvw.

Moataz Dowaidar. Genetic Engineered MSCs Are Attractive Possibilities for Regenerative Stem-Cell Therapy to Treat Several Liver Diseases. https://doi.org/10.31219/osf.io/4cfrd.

Moataz Dowaidar. Genetic Variants Shared between Alzheimer's Disease and Parkinson's Disease Have Been Discovered in Blood and Brain Samples. Somatic Mosaicism Might Function as an Accelerator. https://doi.org/10.31219/osf.io/tr58n.

Moataz Dowaidar. Genome-Wide Association Studies Promise to Discover Novel Indicators of Hypertension. Endothelin-Related SNPs Are Currently in Clinical Trials. https://doi.org/10.31219/osf.io/2n4wa.

Moataz Dowaidar. Gingival and Intraventricular Haemorrhages Are Severe Newborn Diseases Causing Damage to White Matter and Neurological Dysfunction in Surviving Newborns Who Can Benefit from Gene Therapy. https://doi.org/10.31219/osf.io/qb84p.

Moataz Dowaidar. Glioblastoma Therapeutic Approaches Were Established Utilizing Contemporary Discoveries in Delivering Medicines to the Brain as Smart Nanoparticles for Focused Therapy. https://doi.org/10.31219/osf.io/db4f6.

Moataz Dowaidar. Haemophilia Gene Therapy Is in Clinical Studies, Making Continuous Safety and Efficacy Testing a Key Emphasis. https://doi.org/10.31219/osf.io/sa8ny.

Moataz Dowaidar. Hematopoietic Stem Cell Transplantation and Gene Therapy Are the Sole Treatments for Sickle Cell Disease and Other Hemoglobinopathies. https://doi.org/10.31219/osf.io/v8xqc.

Moataz Dowaidar. Huntington's Disease Gene Therapy and Nanomedicines May Be Available Shortly. https://doi.org/10.31219/osf.io/rxvgd.

Moataz Dowaidar. Hybrid Gene Therapy Designed to Fully Understand the Underlying Molecular Cancer Process May Be a Feasible Option. https://doi.org/10.31219/osf.io/ajyfd.

Moataz Dowaidar. Hydrogels Are Promising Considering Their Incredible Capacity to Modify, Encapsulate and Co-Deliver Medicinal Compounds, Cells, Biomolecules, and Nanomaterials. https://doi.org/10.31219/osf.io/px3qy.

Moataz Dowaidar. Immune Evasion Is Linked to Histone Variation Malfunction. Gene Therapy Could Provide Tools for Targeting Histone Variant Deposition as a Critical Part of Its Pharmacology. https://doi.org/10.31219/osf.io/kjm76.

Moataz Dowaidar. Implementing the Human Artificial Chromosome Gene Therapy Platform Remains Challenging, but Continuous Animal Model Research Will Advance the Platform Closer to Clinical Trials. https://doi.org/10.31219/osf.io/a53f7.

Moataz Dowaidar. Inflammatory Breast Cancer Remains the Most Aggressive Form of Breast Cancer. A Multimodality Therapeutic Plan Has Shown Improved Survival Results. https://doi.org/10.31219/osf.io/cr935.

Moataz Dowaidar. Inherited Immunohematological and Metabolic Diseases Have the Potential to Improve Significantly, or Be Cured, Using Haematopoietic Stem Cell Transplantation Gene Therapy. https://doi.org/10.31219/osf.io/ukbnm.

Moataz Dowaidar. Insulin and IGF-1 Receptors Mutations Can Lead to Targets for Gene Therapy in Diabetes, Obesity, and Metabolic Syndrome. https://doi.org/10.31219/osf.io/s86x5.

Moataz Dowaidar. Integrating High-Throughput Genetics and Neuroimaging Technologies Promises Greater Information on Neurobiological Anomalies in Neurodegenerative Diseases. https://doi.org/10.31219/osf.io/hpgyz.

Moataz Dowaidar. Intravitreal and Subretinal Injections Currently Deliver Most Gene Therapy, Including siRNA for Eye Illnesses. Non-Viral Vectors May Provide Targeting. https://doi.org/10.31219/osf.io/rjkhy.

Moataz Dowaidar. LncRNA Regulating Reprogramming Glucose Metabolism Has Become One of the Most Tempting Antineoplastic Targets for Gene Therapy. https://doi.org/10.31219/osf.io/hqma5.

Moataz Dowaidar. lncRNAs Are Upregulated and Downregulated in OS Cells. Angiogenesis, Metastasis, Cell Signaling, Autophagy, and Death Are among Biological Processes That RNAs Play a Role in. https://doi.org/10.31219/osf.io/48n7q.

Moataz Dowaidar. Magnetic Nanoparticles Are Widely Used in Drug Delivery, Imaging, Diagnosis, and Targeting. It Has Promises for the Treatment of Inflammatory Disorders such as Rheumatoid Arthritis. https://doi.org/10.31219/osf.io/p2gme.

Moataz Dowaidar. Many miRNAs Participate in Inflammatory Regulation and Bone Metabolism. Overexpression of miR21 and miR155 Releases Proinflammatory Cytokines. https://doi.org/10.31219/osf.io/2wuvp.

Moataz Dowaidar. MiR490's Diagnostic Capacity Was Demonstrated in Various Cancer Kinds and Diseases, Adding to Its Clinical Value. https://doi.org/10.31219/osf.io/wysre.

Moataz Dowaidar. miRNAs Have an Impact on Xeno-Infectious Diseases by Influencing Host And/or Infection Factors. https://doi.org/10.31219/osf.io/7qewx.

Moataz Dowaidar. Mutations in MED12 Lead to Mental Retardation, Including Opitz–Kaveggia Syndrome, Ohdo Syndrome, Lujan–Fryns Syndrome, and Psychosis. It's a Target for Gene Therapy. https://doi.org/10.31219/osf.io/cyns8.

Moataz Dowaidar. Nanocarriers Can Be Used to Control the Activity of Genome Editing in a Spatiotemporal Way by Using Stimulusresponsive Nanocarriers. https://doi.org/10.31219/osf.io/nua89.

Moataz Dowaidar. Nanomaterials Were Formed into Various Shapes, with Functionalization Aimed at Various Internalization Processes. Their Nanoscale Size Allows Drugs to Reach Cells or Extracellular Environments. https://doi.org/10.31219/osf.io/p2ajv.

Moataz Dowaidar. Nanomedicine Is Offering Promising Strategies for Tumor Blockade Treatment. https://doi.org/10.31219/osf.io/yzxuq.

Moataz Dowaidar. Network Medicine Might Lead to New Treatments for Dyslipidemia. It Will Be a Challenging Method to Implement in a Clinical Context. https://doi.org/10.31219/osf.io/nksbw.

Moataz Dowaidar. Neuroinflammation Caused by Activated Microglia and Astrocytes Can Contribute to the Progression of Pathogenic Damage to Substantia Nigra Neurons, Playing a Role in Parkinson's Disease Progression. https://doi.org/10.31219/osf.io/ac896.

Moataz Dowaidar. Neurologists Rarely Perform Genetic Testing for Parkinson's Disease. Evidence Suggests That Many Patients with Major Genetic Variants Go Undiagnosed. https://doi.org/10.31219/osf.io/ykpb2.

Moataz Dowaidar. Neuronal Intranuclear Hyaline Inclusion Disease Is a Neurodegenerative Condition Which Can Be a Target for Gene Therapy. https://doi.org/10.31219/osf.io/upgqd.

Moataz Dowaidar. New Therapies Aim at Restoring the Molecular, Morphological, and Functional Integrity of Parkinson's Specific Brain Circuits. https://doi.org/10.31219/osf.io/dvyxc.

Moataz Dowaidar. Not All lncMIRHGs Are 'Junk Transcripts,'. LncM IRHG Loci May Make Both Functional miRNAs and lncRNAs, Which Can Work Together or Separately. https://doi.org/10.31219/osf.io/a567w.

Moataz Dowaidar. Nrf2 Signaling Pathways Are Part of a Wider Network of Signaling Pathways Regulating Thymoquinone Therapeutic Actions Which Need Innovative Formulations and Delivery Methods. https://doi.org/10.31219/osf.io/u2fa7.

Moataz Dowaidar. Omics Should Be Integrated with Genomics to Uncover Molecular Networks and Tissue and Single-Cell Epigenetic Changes. With These Findings, Targeted Pseudoexfoliation Syndrome and Glaucoma Gene Therapy Procedures May Be Viable. https://doi.org/10.31219/osf.io/48fj5.

Moataz Dowaidar. Ophthalmic Gene and Cell Therapies. https://doi.org/10.31219/osf.io/n84m9.

Moataz Dowaidar. P21 Is a Flexible, Multi-Functional Protein. It Governs Various Tumor Cell Activities, Including Autophagy. p21 Is a Possible Radiotherapy Target. https://doi.org/10.31219/osf.io/ydkca.

Moataz Dowaidar. Parkinson's Disease Simulating Complexity via Improving the Identification of Significant Genetic Alterations and Environmental Contaminants Should Be a Priority. https://doi.org/10.31219/osf.io/pmcu9.

Moataz Dowaidar. Patient-Specific Microphysiology Systems Are Likely to Become a Crucial Aspect of Translational Research and Precision Medicine. https://doi.org/10.31219/osf.io/bc8fr.

Moataz Dowaidar. Patients with PMD Who Are Thoroughly Screened by Genomic Medicine Have a Considerable Chance of Benefiting Greatly from Whole-Genome Sequencing. https://doi.org/10.31219/osf.io/dajft.

Moataz Dowaidar. Polydopamine Nanoparticles' Activity and Long-Term Stability Should Be Fully Studied for Gene Therapy Applications. https://doi.org/10.31219/osf.io/x4nej.

Moataz Dowaidar. Potential Therapeutics for Primary Mitochondrial Disorders. https://doi.org/10.31219/osf.io/6pz5k.

Moataz Dowaidar. Potentials of Medicinal Nanostructured Diamond Particles and Coatings. https://doi.org/10.31219/osf.io/h68xz.

Moataz Dowaidar. Preclinical Investigations Revealed Possibilities for Salmonella Tumor Treatment. Bacteria Can Also Be Coupled to Nanomaterials Enabling Drug-Loading, Photocatalytic And/or Magnetic Properties, Using the Bacteria's Net Negative Charge. https://doi.org/10.31219/osf.io/embqk.

Moataz Dowaidar. Research into P2X Purinergic Receptor Function in Tumor Growth Has Made Substantial Progress with Potential Gene Therapy Targeting. https://doi.org/10.31219/osf.io/r34fs.

Moataz Dowaidar. RNA Therapies Hold Great Promise for Treating Cancer. High-Throughput Screening Techniques Have Facilitated the Development of RNA Treatments. https://doi.org/10.31219/osf.io/9vxrb.

Moataz Dowaidar. RNAi Treatment Has Been Shown to Successfully Modify Human-Related Target Gene Expression, Including Cancer. It Has the Capacity to Control Non-Standard Oncogenes, such as Oncogenic lncRNAs. https://doi.org/10.31219/osf.io/bwqep.

Moataz Dowaidar. RNAs Hold a Lot of Potential When It Comes to Druggable Molecular Targets. https://doi.org/10.31219/osf.io/2dtxg.

Moataz Dowaidar. Shadow Enhancers' Objective Seems to Be to Establish Robust Growth Patterns Independent of Genetic or Environmental Stress. https://doi.org/10.31219/osf.io/qfnkp.

Moataz Dowaidar. Sickle Cell Disease Hematopoietic Stem Cell Gene Therapy with Globin Gene Addition Is Promising. https://doi.org/10.31219/osf.io/j5fkb.

Moataz Dowaidar. Single-Gene Mutations in mtDNA-Associated Proteins Are Unlikely to Be the Main Cause of Sporadic Parkinson's Disease. Cumulative Genetic Variation in Numerous Genes May Be Important in Neurodegeneration and PD Risk. https://doi.org/10.31219/osf.io/89qte.

Moataz Dowaidar. Small Nuclear Ribonucleoproteins (snRNPs) Based Gene Therapy. https://doi.org/10.31219/osf.io/c43r9.

Moataz Dowaidar. Studying the Pathologic Mechanisms of Osteoporosis and the Bone Microenvironment May Help Researchers Better Know the Etiology of Rheumatoid Arthritis, Periodontitis, and Multiple Myeloma, as Well as Other Inflammatory and Autoimmune Disorders. https://doi.org/10.31219/osf.io/t3z6y.

Moataz Dowaidar. Suicide Gene Therapy May Be Effective in the Treatment of Malignant Glioma. https://doi.org/10.31219/osf.io/vdkst.

Moataz Dowaidar. Synuclein Is a Protein That Is Expressed in Brain Tissue. The Specific Missense Mutation (SNCA) Found in a Family with Parkinson's Disease Is the Cause. Other Diseases Include Alzheimer's Disease and REM Sleep Behavior Disorder. https://doi.org/10.31219/osf.io/bs8rc.

Moataz Dowaidar. Systems Biology Is a Method for Analyzing Massive Amounts of Multidimensional Data Generated by Omics Technologies. Cross-Validation of the Various Technological Platforms Is Critical. https://doi.org/10.31219/osf.io/p8vkd.

Moataz Dowaidar. Targeting Mitochondria and Especially Taz Gene Mutation Induces CL May Give Novel Therapeutic Alternatives for Treating Barth Syndrome. https://doi.org/10.31219/osf.io/unfpy.

Moataz Dowaidar. The Ability to Combine Multiple mRNA Antigens Targeting Multiple Pathogens Simultaneously, and the Robust Immune Responses Are Confirmed in Several Clinical Studies. https://doi.org/10.31219/osf.io/6qksx.

Moataz Dowaidar. The Cubic Polyhedral Oligomeric Silsesquioxanes Based Hybrid Materials Have a Wide Variety of Applications, Including Drug Administration, Gene Therapy, Biological Imaging, and Bone Regeneration. https://doi.org/10.31219/osf.io/9peq8.

Moataz Dowaidar. The Development of Tissue Replacement Therapies and Drug Discovery Was a Critical Milestone in Advancing Regenerative Medicine. https://doi.org/10.31219/osf.io/w9bsm.

Moataz Dowaidar. The Epidemic of COVID-19 Prompted Widespread Use of mRNA Vaccinations. https://doi.org/10.31219/osf.io/jqws5.

Moataz Dowaidar. The Most Useful and Commonly Available Acute Rejection Surveillance Strategies Are Routine Monitoring of Myocardial Function and Donor-Specific Anti-HLA Abs Monitoring. https://doi.org/10.31219/osf.io/ebw68.

Moataz Dowaidar. The Protease MBTPS2 Is an Important Regulator of Several Cellular Processes, Especially in Health and Sickness. https://doi.org/10.31219/osf.io/qyn6h.

Moataz Dowaidar. The Sigma 1 Receptor (S1R) Is a Potential Therapeutic Target for the Treatment of Huntington's Disease. https://doi.org/10.31219/osf.io/mcefx.

Moataz Dowaidar. The Use of a Network Medicine Approach Might Result in Innovative Strategies for Lowering Coronary Heart Disease and CV Risks. https://doi.org/10.31219/osf.io/eakg8.

Moataz Dowaidar. The Vasoconstrictor Endothelin System Involvement in Chronic Kidney Diseases Pathogenesis Is Now the Most Often Employed Treatment Method. https://doi.org/10.31219/osf.io/cnkqy.

Moataz Dowaidar. The VPS35-D620N Mutation Is Associated with Parkinson's Disease and Can Be a Target for Gene Therapy. https://doi.org/10.31219/osf.io/83sxr.

Moataz Dowaidar. Therapeutics Including Gene Therapy for Osteoarthritis as a Concept. https://doi.org/10.31219/osf.io/7zsqy.

Moataz Dowaidar. Tissue Hypoxia Has Been Established as a Master Regulator for Alternative Splicing, with Substantial Clinical Consequences and Possibilities for Gene Therapy Targeting. https://doi.org/10.31219/osf.io/5pbw4.

Moataz Dowaidar. To Rectify Alzheimer's Disease Etiology, Excessive Mitochondrial Division Might Be Stopped or Mitophagy Might Be Promoted. https://doi.org/10.31219/osf.io/6kdxw.

Moataz Dowaidar. Transcriptomics Is a Rapidly Growing Field That Generates New Data That May Be Used on Its Own or in Combination with Existing Clinical Data for Development of New Therapeutics, Including Gene Therapy. https://doi.org/10.31219/osf.io/kfr6a.

Moataz Dowaidar. Tumor Microenvironment Has Clinical Significance in Terms of Prognosis and Therapy Prediction. https://doi.org/10.31219/osf.io/4dz8q.

Moataz Dowaidar. Using AAV as a Gene Delivery Vector in the Neural System Is Effective in Several Animals, such as Nonhuman Primates. https://doi.org/10.31219/osf.io/ut4fa.

Moataz Dowaidar. Using Pre-Existing Datasets to Combine Published Information with New Metrics Would Help Researchers Construct a Broader Picture of Chromatin in Disease. https://doi.org/10.31219/osf.io/gsqv5.

Moataz Dowaidar. Virus-like Particles Are Good Nanocarriers for Liquid Biopsy Probes, Imaging Contrast Agents, and Anticancer Medications. https://doi.org/10.31219/osf.io/xbtka.

Moataz Dowaidar. ZEB1 Controls the Expression of ICAM1, Promoting Monocyte-Macrophage Adhesion and Hence the Formation of Atherosclerotic Lesions. https://doi.org/10.31219/osf.io/kzjqg.

Moataz Dowaidar. Gene Therapy Development and Legislation. https://doi.org/10.31219/osf.io/mwb2n.

Moataz Dowaidar. Next-Generation Sequencing Is Now Utilized to Identify Genetic Abnormalities and Develop Gene Therapy. https://doi.org/10.31219/osf.io/em7xp.

Moataz Dowaidar. Nucleic Acid Designs, Artificial Intelligence for Screening Nanomaterials, and Enhanced Characterization Methods Are Needed to Make Nanomedicine More Successful. https://doi.org/10.31219/osf.io/2w5aq.

Moataz Dowaidar. Potential Strategies for Cancer Gene Therapy. https://doi.org/10.31219/osf.io/atcqz.

Moataz Dowaidar. Quantitative Groups Will Be Critical to the Success of Future Gene Therapy Programs. https://doi.org/10.31219/osf.io/v97ht.

Moataz Dowaidar. The Treatment of Major Human Illnesses with Recombinant Adeno-Associated Virus (rAAV) Has Shown Tremendous Promises. https://doi.org/10.31219/osf.io/uwa4e.

Moataz Dowaidar. Carbon Nanotubes Have Enormous Potential in Gene Therapy. https://doi.org/10.31219/osf.io/9bcxk.

Moataz Dowaidar. Charge-Alteration-Based Approaches Can Address the Evolving Needs of Nucleic Acid-Based Gene Therapy, Charge Reversal Techniques Are Also Promising. https://doi.org/10.31219/osf.io/zwq5h.

Moataz Dowaidar. Chromosome X, the Most Explored Genome-Editing Chromosome, Presents Possibilities for Hemophilia A Treatments. https://doi.org/10.31219/osf.io/6vsdz.

Moataz Dowaidar. Clinical Investigations Show That siRNA May Be Used to Treat a Variety of Disorders, Including Cancer. https://doi.org/10.31219/osf.io/fcsgq.

Moataz Dowaidar. Cyclodextrins as Potential Gene Therapy Vectors. https://doi.org/10.31219/osf.io/zhtsc.

Moataz Dowaidar. Development of Specialized Carriers Capable of Delivering Effective RNAi and siRNA Gene Therapy. https://doi.org/10.31219/osf.io/3ykwm.

Moataz Dowaidar. Gene Therapy Can Target Mutations such as BRAF, Which Have Been Shown to Make Tumors More Susceptible to Autophagy Suppression. https://doi.org/10.31219/osf.io/3gwra.

Moataz Dowaidar. Gene Therapy Vectors Should Enable CRISPR Systems to Accumulate at Disease Sites and Successfully Penetrate Nuclei. https://doi.org/10.31219/osf.io/xzmnc.

Moataz Dowaidar. Nanoformulations Can Be Utilized to Deliver Effective siRNA to Tumor Cells to Decrease Gene Expression. https://doi.org/10.31219/osf.io/zvukc.

Moataz Dowaidar. Neuronal Ceroid Lipofuscinosis Therapeutics. https://doi.org/10.31219/osf.io/75vcp.

Moataz Dowaidar. Nonviral Gene Delivery Vectors for Transfection of the CAR Gene for CAR-T Cell Therapy. https://doi.org/10.31219/osf.io/ckxh5.

Moataz Dowaidar. Potential HIV Gene Therapy Strategies. https://doi.org/10.31219/osf.io/e5hm2.

Moataz Dowaidar. Research on Cell Sources for Brain Cell Replacement Methods Has Gained Major Importance. Cell and Gene Therapy Are Potentially Intriguing New Domains of Regenerative Medicine. https://doi.org/10.31219/osf.io/g835b.

Moataz Dowaidar. RNAi-Based Gene Therapy Provides a Wide Variety of Applications. Safe, Biodegradable Nano Delivery Vectors Are Still Needed. https://doi.org/10.31219/osf.io/s2zhn.

Moataz Dowaidar. Strategies for Treating Multiple Sclerosis with Gene Therapy. https://doi.org/10.31219/osf.io/sycn6.

Moataz Dowaidar. The Combination of Unique Biomolecules and Nanoparticles Has Shown Successful Gene Therapy Treatment Approaches for Non-Small Cell Lung Cancer Treatment. https://doi.org/10.31219/osf.io/yeq5z.

Moataz Dowaidar. Understanding Why the Same Gene Delivery Vector Behaves Differently in Different Cell Types Is Essential for Developing More Adaptable Transfection Systems. https://doi.org/10.31219/osf.io/6q8af.

Moataz Dowaidar. AAV9 Is Considered the Most Efficient AAV Serotype Targeting Blood-Brain Barriers. To Enhance Effective Gene Therapy for CNS Illnesses, Testing Novel Vectors with More Efficient Crossing Capabilities Is Vital. https://doi.org/10.31219/osf.io/7bf5s.

Moataz Dowaidar. Artificial miRNAs Are Potential Gene Therapy Tools, Especially for Incurable Monogenic Disorders. https://doi.org/10.31219/osf.io/d5rnm.

Moataz Dowaidar. Breakthroughs in mRNA Modification and Nanoparticle-Based Delivery Vehicles Facilitate Gene Therapy Strategies. https://doi.org/10.31219/osf.io/ky7dt.

Moataz Dowaidar. CRISPR/Cas9-Mediated Genome Editing Has Demonstrated Significant Promise for Genetic Correction in Autologous Hematopoietic Stem/progenitor Cells (HSPCs) and Induced Pluripotent Stem Cells (iPSCs). https://doi.org/10.31219/osf.io/xk54r.

Moataz Dowaidar. Gene Therapy Vectors for Targeting the Heart. https://doi.org/10.31219/osf.io/gcbhf.

Moataz Dowaidar. Liposomes Can Minimize Cardiotoxicity, Address Drug Resistance, and Improve Overall Drug Release Profiles in Breast Cancer. https://doi.org/10.31219/osf.io/tn56d.

Moataz Dowaidar. Liposomes with Cerasome-Forming Lipids as Gene Therapy Vectors. https://doi.org/10.31219/osf.io/zjn6v.

Moataz Dowaidar. Nanomaterials Combine Multiple Therapeutic Approaches for Cancer Cell Multidrug Resistance, Ferroptotic Cell Death Is Promising in Various Cancers. https://doi.org/10.31219/osf.io/7bg9t.

Moataz Dowaidar. Nanomedicines for Enhanced Permeability and Retention (EPR)-Stratified Patients Have the Potential to Improve Treatment Outcomes. https://doi.org/10.31219/osf.io/xrcb2.

Moataz Dowaidar. RNA-Based Gene Therapy for Manipulating the Neuroinflammatory Cascade Closely Linked to Neurodegeneration Can Help Reduce Disease Development. https://doi.org/10.31219/osf.io/2hswv.

Moataz Dowaidar. Targeted Chemical Nucleases Have a Wide Range of Untapped Applications in Biological Fields, Including Gene Therapy. https://doi.org/10.31219/osf.io/6bexs.

Moataz Dowaidar. Bacterial Nanoparticles Can Deliver Proteins, Medications, Enzymes, and Genes to Diagnose and Cure Numerous Illnesses. https://doi.org/10.31219/osf.io/7gyna.

Moataz Dowaidar. Exosomal miRNA Diagnostic and Gene Therapy Tools. https://doi.org/10.31219/osf.io/aknrc.

Moataz Dowaidar. Gene Modification Research Has Potential, from Diagnostic to Therapeutic Levels. The Most Promising Metabolic Pathways Include the TGF-1 Signaling System, Inflammation and Protein Transport. https://doi.org/10.31219/osf.io/5ert4.

Moataz Dowaidar. Gene Therapy Using MnO2 Nanoparticles. https://doi.org/10.31219/osf.io/xmwjs.

Moataz Dowaidar. Gene-Regulatory Elements May Change the Amount, Timing, or Location of Gene Expression, Cis-Regulation Therapy Platforms Might Become a Gene Therapy to Treat Many Genetic Diseases. https://doi.org/10.31219/osf.io/xc5a2.

Moataz Dowaidar. Hemophilia Therapeutics. https://doi.org/10.31219/osf.io/gu74x.

Moataz Dowaidar. Mesenchymal Stem Cells Strategies in Cancer Immunotherapy. https://doi.org/10.31219/osf.io/dkv6w.

Moataz Dowaidar. Nanomaterials Can Inhibit Planktonic and Biofilm Bacteria and Can Be Used as Topical Therapy for Mouth and Wound-Related Infections. https://doi.org/10.31219/osf.io/aqd2e.

Moataz Dowaidar. New Technologies to Improve CAR T Cell Generation and Biomanufacturing Will Lead to Safer, More Therapeutically Effective Cells. https://doi.org/10.31219/osf.io/un8gp.

Moataz Dowaidar. Ocular Gene Therapy Strategies. https://doi.org/10.31219/osf.io/7en3k.

Moataz Dowaidar. Peripheral Nerve Injury Therapeutics, Including Electrical Stimulation, Stem Cell Treatments, and Synthetic Neural Scaffolds, Have Shown Promising Preclinical and Even Clinical Results with Potential Regenerative Treatment. https://doi.org/10.31219/osf.io/m8cs9.

Moataz Dowaidar. Photothermal and Photodynamic Photoactivation of Nanomaterials-Based Prodrugs Are Two Key Methods for NIR Light-Mediated Photoactivation. https://doi.org/10.31219/osf.io/2bh3r.

Moataz Dowaidar. Quantum Dots Have the Potential to Be Used in Gene Therapy. https://doi.org/10.31219/osf.io/bdeg6.

Moataz Dowaidar. Sickle Cell Disease Has Emerged as a Public Health Concern. Some Drugs May Conflict with Curative Therapies, yet They May Be Useful as a Bridge to HSCT and Gene Therapy. https://doi.org/10.31219/osf.io/6kufh.

Moataz Dowaidar. Stimulator of Interferon Genes (STING)-Activating Nanoparticles Can Be Employed as a Tool for Controlled Immune Activation. https://doi.org/10.31219/osf.io/2ez7a.

Moataz Dowaidar. CRISPR/Cas9 Has Introduced New Gene Therapy Possibilities for Muscular Dystrophies. https://doi.org/10.31219/osf.io/ug8v4.

Moataz Dowaidar. Degradable Branched Polycationic Systems Are Promising Gene Therapy Vectors. https://doi.org/10.31219/osf.io/utypf.

Moataz Dowaidar. Developing Nanotechnology Platforms for Peptide-Based Combinatory Cancer Gene Therapy Will Likely Have a Significant Influence on the Development of Personalized Cancer Medicines. https://doi.org/10.31219/osf.io/zbrkj.

Moataz Dowaidar. Exosomes May Prevent Cardiac Attacks, Heart Failure, and Cardiomyopathy. https://doi.org/10.31219/osf.io/agm3k.

Moataz Dowaidar. 2021gr. Exosomes Potential Therapeutics. https://doi.org/10.31219/osf.io/mhwt3.

Moataz Dowaidar. Gene Therapy Using miRNA Treatment Suppresses the Expression of Bone-Forming Defective Genes and Raises the Expression of Genes That Become Dormant during Bone Building. https://doi.org/10.31219/osf.io/tcka3.

Moataz Dowaidar. Genome-Editing Is Promising for Producing Therapeutically Relevant Animal Models for Possible Therapies for Rare Human Diseases. https://doi.org/10.31219/osf.io/dehr9.

Moataz Dowaidar. Human Corneal Endothelial Cells Grafts to Replace Cadaveric Donor Corneas. https://doi.org/10.31219/osf.io/p9x7e.

Moataz Dowaidar. Hybrid Nanotechnology and Peptide Nucleic Acid Could Improve the Effectiveness of Gene Therapy by Increasing Its Cell Permeability. https://doi.org/10.31219/osf.io/d8wzt.

Moataz Dowaidar. In Prenatal Stem Cell Transplantation and in Utero Gene Therapy, a Wide Spectrum of Genetic Diseases Can Be Diagnosed and Treated before Birth. https://doi.org/10.31219/osf.io/sa3vz.

Moataz Dowaidar. Magnetic Iron Oxide Nanoparticles Have Potential on Gene Therapy Effectiveness and Biocompatibility. https://doi.org/10.31219/osf.io/f3hm4.

Moataz Dowaidar. Neurotrophin Gene Therapy May Be Able to Treat Individuals with Noise-Induced Hearing Loss or Neural Presbyacusis. https://doi.org/10.31219/osf.io/spkxh.

Moataz Dowaidar. Plant Viral Nanoparticles Can Be Used in Biological Systems for Loading and Transporting Cargo. https://doi.org/10.31219/osf.io/txdka.

Moataz Dowaidar. Polydopamine May Be Easily Functionalized with a Range of Nanomaterials for Synergistic Cancer Therapy, in Addition to Its Exceptional Photothermal Effects. https://doi.org/10.31219/osf.io/cq942.

Moataz Dowaidar. Tumor-Targeted Drug Delivery Systems for Anticancer Therapies Can Selectively Provide an Appropriate Cytotoxic Payload to Cancer Cells, Reducing the Side Effects of Chemo. https://doi.org/10.31219/osf.io/683nj.

Dowaidar, Moataz, Hani Nasser Abdelhamid, Mattias Hällbrink, Krista Freimann, Kaido Kurrikoff, Xiaodong Zou, and Ülo Langel. 2017. Magnetic Nanoparticle Assisted Self-Assembly of Cell Penetrating Peptides-Oligonucleotides Complexes for Gene Delivery. Scientific Reports 7 (1): 9159. https://doi.org/10.1038/s41598-017-09803-z.

Dowaidar, Moataz, Hani Nasser Abdelhamid, Mattias Hällbrink, Ülo Langel, and Xiaodong Zou. 2018. Supplemental Material for Chitosan Enhances Gene Delivery of Oligonucleotide Complexes with Magnetic Nanoparticles–cell-Penetrating Peptide. SAGE Journals. https://doi.org/10.25384/SAGE.7105436.V1.

Dowaidar, Moataz, Hani Nasser Abdelhamid, Mattias Hällbrink, Xiaodong Zou, and Ülo Langel. 2017. Graphene Oxide Nanosheets in Complex with Cell Penetrating Peptides for Oligonucleotides Delivery General Subjects. Biochimica et Biophysica Acta, General Subjects. https://pubag.nal.usda.gov/catalog/5734174.

Moataz Dowaidar. 2017. Graphene Oxide Nanosheets in Complex with Cell Penetrating Peptides for Oligonucleotides Delivery. Biochimica et Biophysica Acta, General Subjects 1861 (9): 2334–41. https://doi.org/10.1016/j.bbagen.2017.07.002.

Dowaidar, Moataz, and Moataz Dowaidar. 2018. Chimeric Gene Delivery Vectors : Design, Synthesis, and Mechanisms from Transcriptomics Analysis.

Moataz Dowaidar. Addiction Biology Research on miRNAs, and Their Role in the Pathophysiology of Addiction Is Enabling Gene Therapy Opportunities. https://doi.org/10.31219/osf.io/z5wyt.

Moataz Dowaidar. Aptamers Targeting Vascular Endothelial Growth Factor Molecular Regulation as Potential Therapists. https://doi.org/10.31219/osf.io/a8qpr.

Moataz Dowaidar. Arrhythmogenic Cardiomyopathy Is a Set of Hereditary Cardiac Muscle Disorders Where Various Etiologies Converge. Most ACM Patients Do Not Have a Genetic Diagnosis. https://doi.org/10.31219/osf.io/pztv3.

Moataz Dowaidar. Autophagy, Immunological Response, and Inflammation All Rely on the TRIM Family Proteins. TRIM-Based Therapeutics for Inflammatory Illnesses Including Diabetes and Diabetic Comorbidities Are Promising. https://doi.org/10.31219/osf.io/y4g6e.

Moataz Dowaidar. Biogenic Particles Can Be Multiantigenic, Immunostimulative and Activate Innate Immunity While Suppressing Tumor Development. https://doi.org/10.31219/osf.io/q2kby.

Moataz Dowaidar. Biological Medications for Interventional Pain Have a Lot of Clinical Data behind Them. It Is Fair to Assume They Will Replace Steroid-Based Interventional Techniques, Providing Patients with Longer Relief. https://doi.org/10.31219/osf.io/4y5fm.

Moataz Dowaidar. Carbon Nanofibers Assist in the Manufacture of Prosthetic Joints, Promote Tissue, Organ, Nerve Regeneration and Development, and Improve Anticancer Therapy Impact and Chemosensitization for a Range of Tumor Types. https://doi.org/10.31219/osf.io/z3ucn.

Moataz Dowaidar. Emerging Therapy Options May Help Patients with RAG Deficiency, Especially Those with Severe Immune Dysregulation. https://doi.org/10.31219/osf.io/v5tjg.

Moataz Dowaidar. Exosomes as Promising Gene Therapy Tools Still Need to Be Researched and Manufactured More Efficiently. https://doi.org/10.31219/osf.io/nw4z7.

Moataz Dowaidar. Focus on Exosomes Could Help Make the Use of Circulating miRNA as Biomarkers More Practical. A Detailed Understanding of miRNA Behavior Should Be a Subject of Gene Therapy. https://doi.org/10.31219/osf.io/uan6x.

Moataz Dowaidar. Gene-Free Viral-like Particles (VLPs) Offer a Safer Alternative to Inactivating or Weakening Viral Strains for Traditional Vaccines. VLP-Based Vaccinations without Adjuvants Have Been Found to Promote Humoral and Cellular Immunity. https://doi.org/10.31219/osf.io/9dvut.

Moataz Dowaidar. Given the Importance of mTOR Signaling in a Number of Illnesses, It Looks Suitable to Use miR 99 Family Members as a Therapeutic Intervention to Deal with These Illnesses by Using Gene Therapy Tools. https://doi.org/10.31219/osf.io/8cwgh.

Moataz Dowaidar. HMGB1 Has Sparked a Lot of Attention as a Model DAMP Molecule Involved in Inflammation, Inflammatory Diseases, and Cancer. https://doi.org/10.31219/osf.io/5qx36.

Moataz Dowaidar. Nucleic Acid Nanocarriers Can Be Programmable, Spatially Adjustable and Biocompatible, Minimizing Systemic Toxicity and Improving Pharmacodynamics. https://doi.org/10.31219/osf.io/wr237.

Moataz Dowaidar. Osteoporosis Is a Prominent Source of Morbidity and Mortality in the Elderly, Particularly in Postmenopausal Women. Long Noncoding RNAs (lncRNAs) Have Been Found to Be Important Regulators and Possible Gene Therapy Targets. https://doi.org/10.31219/osf.io/ghfpt.

Moataz Dowaidar. Polycomb Genes Role in Cancer Pathophysiology Is Offering Targets for Therapeutics Including Gene Therapy. https://doi.org/10.31219/osf.io/sfvej.

Moataz Dowaidar. RNA Sequencing and Microarray Analysis Are Helpful Techniques to Detect Obesity-Related lncRNAs. LncRNA Can Alter Cholesterol Metabolism and Can Be a Target for Gene Therapy. https://doi.org/10.31219/osf.io/3fb6w.

Moataz Dowaidar. Sepsis-Associated Acute Kidney Damage Is a Disease That Affects the Patient's Quality of Life. It Should Be a Target for Gene Therapy. https://doi.org/10.31219/osf.io/49k7q.

Moataz Dowaidar. The Gene Expression Profiling Gives an in-Depth Insight of Breast Cancer Heterogeneity, Better than a Single Protein or Gene Expression. It Is Time to Include It in the Daily Routine. https://doi.org/10.31219/osf.io/xhyd7.

Moataz Dowaidar. The Nanomedicine System Has Successfully Inhibited Tumor Neovascularization Using Gene Silencing, Chemotherapy, Photothermal Therapy, and Other Therapies. https://doi.org/10.31219/osf.io/rk2bf.

Moataz Dowaidar. The Therapeutic Application of a Nucleic Acid Sequence to Patients' Diseased Organs Is Currently Available. https://doi.org/10.31219/osf.io/pqsbf.

Moataz Dowaidar. Triple-Negative Breast Cancer, Which Lacks the Expression of Hormone Receptors and HER2, Has a Worse Prognosis. Massive Parallel Sequencing Is Capable of Reliably Breaking down the Intra-Tumor and Inter-Tumor Heterogeneity. https://doi.org/10.31219/osf.io/pvk7u.

Dowaidar, Moataz, H. A. Ismail, A. A. Alghasham, M. M. Dowaidar, and A. A. Settin. 2011. Polymorophisms in MTHF and Ace Genes and the Association with Hypertension among Saudi Population from Qassim Region. Egyptian Journal of Biochemistry and Molecular Biology 29 (1). https://doi.org/10.4314/ejbmb.v29i1.67382.

Dowaidar, Moataz, Hani Nasser Abdelhamid, Mattias Hällbrink, Ülo Langel, and Xiaodong Zou. 2018. Chitosan Enhances Gene Delivery of Oligonucleotide Complexes with Magnetic Nanoparticles-Cell-Penetrating Peptide. Journal of Biomaterials Applications 33 (3): 392–401. https://doi.org/10.1177/0885328218796623.

Dowaidar, Moataz, and Ahmad Settin. 2010. Risk of Myocardial Infarction Related to Factor V Leiden Mutation: A Meta-Analysis. Genetic Testing and Molecular Biomarkers 14 (4): 493–98. https://doi.org/10.1089/gtmb.2010.0017.

Gestin, Maxime, Moataz Dowaidar, and Ülo Langel. 2017. Uptake Mechanism of Cell-Penetrating Peptides. Advances in Experimental Medicine and Biology 1030: 255–64. https://doi.org/10.1007/978-3-319-66095-0_11.

Ismail, H. A., A. A. Alghasham, M. M. Dowaidar, and A. A. Settin. 2011. Polymorophisms in MTHF and Ace Genes and the Association with Hypertension among Saudi Population from Qassim Region. Egyptian Journal of Biochemistry and Molecular Biology 29 (1). https://doi.org/10.4314/ejbmb.v29i1.67382.

Settin, Ahmad A., Abdullah Algasham, Moataz Dowaidar, and Hisham Ismail. 2009. Methylene Tetrahydrofolate Reductase and Angiotensin Converting Enzyme Gene Polymorphisms Related to Overweight/obesity among Saudi Subjects from Qassim Region. Disease Markers 27 (2): 97–102. https://doi.org/10.3233/DMA-2009-0660.

Settin, Ahmad A., Abdullah Alghasham, Ahmad Ali, Moataz Dowaidar, and Hisham Ismail. 2012. Frequency of Thrombophilic Genetic Polymorphisms among Saudi Subjects Compared with Other Populations. Hematology 17 (3): 176–82. https://doi.org/10.1179/102453312X13376952196575.

Settin, Ahmad, Ibrahem S. Abu-Saif, Rizk El-Baz, Moataz Dowaidar, Rabab Abu-Al Kasim, and Shaimaa Shabana. 2007a. Diagnosis of Sex Chromosome Disorders and Prenatal Diagnosis of Down Syndrome Using Interphase Fluorescent In-Situ Hyperidization Technique. International Journal of Health Sciences 1 (2): 203–9. https://www.ncbi.nlm.nih.gov/pubmed/21475429.

Settin, Ahmad, Abdullah Algasham, Moataz Dowaidar, and Hisham Ismail. 2011. Methylene Tetrahydrofolate Reductase (MTHFR) and Angiotensinogen Converting Enzyme (ACE) Gene Polymorphisms Related to Overweight and Obesity among Saudi Patients in Al Qassim. International Journal of Health Sciences 5 (2 Suppl 1): 24–25. https://www.ncbi.nlm.nih.gov/pubmed/23284565.

Settin, Ahmad, Hala Almarsafawy, Ahmad Alhussieny, and Moataz Dowaidar. 2008a. Dysmorphic Features, Consanguinity and Cytogenetic Pattern of Congenital Heart Diseases: A Pilot Study from Mansoura Locality, Egypt. International Journal of Health Sciences 2 (2): 101–11. https://www.ncbi.nlm.nih.gov/pubmed/21475491.

Settin, Ahmad, Moataz Dowaidar, Rizk El-Baz, Ayman Abd-Al-Samad, Ibrahim El-Sayed, and Mahmoud Nasr. 2008. Frequency of Factor V Leiden Mutation in Egyptian Cases with Myocardial Infarction. Hematology 13 (3): 170–74. https://doi.org/10.1179/102453308X316158.

Venit, Tomas, Moataz Dowaidar, Maxime Gestin, Syed Raza Mahmood, Ülo Langel, and Piergiorgio Percipalle. 2020. Transcriptional Profiling Reveals Ribosome Biogenesis, Microtubule Dynamics and Expression of Specific lncRNAs to Be Part of a Common Response to Cell-Penetrating Peptides. Biomolecules 10 (11): 1567. https://doi.org/10.3390/biom10111567.

Dowaidar, M. Cell-Penetrating Peptides Uptake Pathways and Role in Drug Deliverywith Potentials for Gene Therapy and Vaccine Development . Preprints.org 2023, 2023070889. https://doi.org/10.20944/preprints202307.0889.v1

Monteys, A. M., Wilson, M. J., Boudreau, R. L., Spengler, R. M., & Davidson, B. L. (2015). Artificial miRNAs targeting mutant Huntingtin show preferential silencing in vitro and in vivo. Molecular Therapy - Nucleic Acids, 4, e234. https://doi-org.ezproxy.its.uu.se/10.1038/mtna.2015.7

Moore, C. B., Guthrie, E. H., Huang, M. T.-H., & Taxman, D. J. (2010). Short hairpin RNA (shRNA): Design, delivery, and assessment of gene knockdown. Methods in Molecular Biology (Clifton, N.J.), 629, 141– 158. https://doi-org.ezproxy.its.uu.se/10.1007/978-1-60761-657-3_10

Moore, M. J., Scheel, T. K. H., Luna, J. M., Park, C. Y., Fak, J. J., Nishiuchi, E., Rice, C. M., & Darnell, R. B. (2015). MiRNA–target chimeras reveal miRNA 3′-end pairing as a major determinant of Argonaute target specificity. Nature Communications, 6(1), 8864. https://doi-org.ezproxy.its.uu.se/10.1038/ncomms9864

Murphy, S. R., Chang, C. C., Dogbevia, G., Bryleva, E. Y., Bowen, Z., Hasan, M. T., & Chang, T.-Y. (2013). Acat1 knockdown gene therapy decreases amyloid-β in a mouse model of Alzheimer's disease. Molecular Therapy, 21(8), 1497– 1506. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2013.118

NCT03282656. (2020). Clinical Trial Registration study/NCT03282656. clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/study/NCT03282656

NCT04120493. (2020). Clinical Trial Registration No. NCT04120493. Available from clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/NCT04120493

Neilsen, C. T., Goodall, G. J., & Bracken, C. P. (2012). IsomiRs—The overlooked repertoire in the dynamic microRNAome. Trends in Genetics: TIG, 28(11), 544– 549. https://doi-org.ezproxy.its.uu.se/10.1016/j.tig.2012.07.005

Nguyen, H. M., Nguyen, T. D., Nguyen, T. L., & Nguyen, T. A. (2019). Orientation of human microprocessor on primary MicroRNAs. Biochemistry, 58(4), 189– 198. https://doi-org.ezproxy.its.uu.se/10.1021/acs.biochem.8b00944

Nguyen, T. A., Jo, M. H., Choi, Y.-G., Park, J., Kwon, S. C., Hohng, S., Kim, V. N., & Woo, J.-S. (2015). Functional anatomy of the human microprocessor. Cell, 161(6), 1374– 1387. https://doi-org.ezproxy.its.uu.se/10.1016/j.cell.2015.05.010

Nguyen, T. L., Nguyen, T. D., Bao, S., Li, S., & Nguyen, T. A. (2020). The internal loops in the lower stem of primary microRNA transcripts facilitate single cleavage of human microprocessor. Nucleic Acids Research, 48(5), 2579– 2593. https://doi-org.ezproxy.its.uu.se/10.1093/nar/gkaa018

Novak, M. J. U., & Tabrizi, S. J. (2010). Huntington's disease. BMJ (Clinical Research Ed.), 340, c3109. https://doi-org.ezproxy.its.uu.se/10.1136/bmj.c3109

Okamura, K., Hagen, J. W., Duan, H., Tyler, D. M., & Lai, E. C. (2007). The mirtron pathway generates microRNA-class regulatory RNAs in drosophila. Cell, 130(1), 89– 100. https://doi-org.ezproxy.its.uu.se/10.1016/j.cell.2007.06.028

Olejniczak, M., Urbanek, M. O., Jaworska, E., Witucki, L., Szczesniak, M. W., Makalowska, I., & Krzyzosiak, W. J. (2016). Sequence-non-specific effects generated by various types of RNA interference triggers. Biochimica et Biophysica Acta, 1859(2), 306– 314. https://doi-org.ezproxy.its.uu.se/10.1016/j.bbagrm.2015.11.005

Pan, Y., He, B., Chen, J., Sun, H., Deng, Q., Wang, F., Ying, H., Liu, X., Lin, K., Peng, H., Xie, H., & Wang, S. (2015). Gene therapy for colorectal cancer by adenovirus-mediated siRNA targeting CD147 based on loss of the IGF2 imprinting system. International Journal of Oncology, 47(5), 1881– 1889. https://doi-org.ezproxy.its.uu.se/10.3892/ijo.2015.3181

Park, J.-E., Heo, I., Tian, Y., Simanshu, D. K., Chang, H., Jee, D., Patel, D. J., & Kim, V. N. (2011). Dicer recognizes the 5′ end of RNA for efficient and accurate processing. Nature, 475(7355), 201– 205. https://doi-org.ezproxy.its.uu.se/10.1038/nature10198

Partin, A. C., Ngo, T. D., Herrell, E., Jeong, B.-C., Hon, G., & Nam, Y. (2017). Heme enables proper positioning of Drosha and DGCR8 on primary microRNAs. Nature Communications, 8, 1737. https://doi-org.ezproxy.its.uu.se/10.1038/s41467-017-01713-y

Partin, A. C., Zhang, K., Jeong, B.-C., Herrell, E., Li, S., Chiu, W., & Nam, Y. (2020). Cryo-EM structures of human Drosha and DGCR8 in complex with primary MicroRNA. Molecular Cell, 78(3), 411– 422.e4. https://doi-org.ezproxy.its.uu.se/10.1016/j.molcel.2020.02.016

Paulson, H. L. (2009). The spinocerebellar ataxias. Journal of Neuro-Ophthalmology: The Official Journal of the North American Neuro-Ophthalmology Society, 29(3), 227– 237. https://doi-org.ezproxy.its.uu.se/10.1097/WNO0b013e3181b416de

Pfister, E. L., Chase, K. O., Sun, H., Kennington, L. A., Conroy, F., Johnson, E., Miller, R., Borel, F., Aronin, N., & Mueller, C. (2017). Safe and efficient silencing with a pol II, but not a pol lII, promoter expressing an artificial miRNA targeting human Huntingtin. Molecular Therapy. Nucleic Acids, 7, 324– 334. https://doi-org.ezproxy.its.uu.se/10.1016/j.omtn.2017.04.011

Pfister, E. L., DiNardo, N., Mondo, E., Borel, F., Conroy, F., Fraser, C., Gernoux, G., Han, X., Hu, D., Johnson, E., Kennington, L., Liu, P., Reid, S. J., Sapp, E., Vodicka, P., Kuchel, T., Morton, A. J., Howland, D., Moser, R., … Aronin, N. (2018). Artificial miRNAs reduce human mutant Huntingtin throughout the striatum in a transgenic sheep model of Huntington's disease. Human Gene Therapy, 29(6), 663– 673. https://doi-org.ezproxy.its.uu.se/10.1089/hum.2017.199

Ramachandran, P. S., Bhattarai, S., Singh, P., Boudreau, R. L., Thompson, S., LaSpada, A. R., Drack, A. V., & Davidson, B. L. (2014). RNA interference-based therapy for spinocerebellar ataxia type 7 retinal degeneration. PLoS One, 9(4), e95362. https://doi-org.ezproxy.its.uu.se/10.1371/journal.pone.0095362

Ramachandran, P. S., Boudreau, R. L., Schaefer, K. A., La Spada, A. R., & Davidson, B. L. (2014). Nonallele specific silencing of ataxin-7 improves disease phenotypes in a mouse model of SCA7. Molecular Therapy, 22(9), 1635– 1642. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2014.108

Rao, D. D., Vorhies, J. S., Senzer, N., & Nemunaitis, J. (2009). siRNA vs. shRNA: Similarities and differences. Advanced Drug Delivery Reviews, 61(9), 746– 759. https://doi-org.ezproxy.its.uu.se/10.1016/j.addr.2009.04.004

Ratnavalli, E., Brayne, C., Dawson, K., & Hodges, J. R. (2002). The prevalence of frontotemporal dementia. Neurology, 58(11), 1615– 1621. https://doi-org.ezproxy.its.uu.se/10.1212/wnl.58.11.1615

Rayner, K. J., Suárez, Y., Dávalos, A., Parathath, S., Fitzgerald, M. L., Tamehiro, N., Fisher, E. A., Moore, K. J., & Fernández-Hernando, C. (2010). MiR-33 contributes to the regulation of cholesterol homeostasis. Science (New York, N.Y.), 328(5985), 1570– 1573. https://doi-org.ezproxy.its.uu.se/10.1126/science.1189862

Roden, C., Gaillard, J., Kanoria, S., Rennie, W., Barish, S., Cheng, J., Pan, W., Liu, J., Cotsapas, C., Ding, Y., & Lu, J. (2017). Novel determinants of mammalian primary microRNA processing revealed by systematic evaluation of hairpin-containing transcripts and human genetic variation. Genome Research, 27(3), 374– 384. https://doi-org.ezproxy.its.uu.se/10.1101/gr.208900.116

Rodríguez-Lebrón, E., Costa, M. d. C., Luna-Cancalon, K., Peron, T. M., Fischer, S., Boudreau, R. L., Davidson, B. L., & Paulson, H. L. (2013). Silencing mutant ATXN3 expression resolves molecular phenotypes in SCA3 transgenic mice. Molecular Therapy, 21(10), 1909– 1918. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2013.152

Ros, X. B.-D., & Gu, S. (2016). Guidelines for the optimal design of miRNA-based shRNAs. Methods (San Diego, Calif.), 103, 157– 166. https://doi-org.ezproxy.its.uu.se/10.1016/j.ymeth.2016.04.003

Ros, X. B.-D., Kasprzak, W. K., Bhandari, Y., Fan, L., Cavanaugh, Q., Jiang, M., Dai, L., Yang, A., Shao, T.-J., Shapiro, B. A., Wang, Y.-X., & Gu, S. (2019). Structural differences between Pri-miRNA paralogs promote alternative drosha cleavage and expand target repertoires. Cell Reports, 26(2), 447– 459. https://doi-org.ezproxy.its.uu.se/10.1016/j.celrep.2018.12.054

Ross, C. A. (2002). Polyglutamine pathogenesis: Emergence of unifying mechanisms for Huntington's disease and related disorders. Neuron, 35(5), 819– 822. https://doi-org.ezproxy.its.uu.se/10.1016/s0896-6273(02)00872-3

Rossi, J. (2008). Expression strategies for short hairpin RNA interference triggers. Human Gene Therapy, 19(4), 313– 317. https://doi-org.ezproxy.its.uu.se/10.1089/hum.2008.026

Saha, A., Bhagyawant, S. S., Parida, M., & Dash, P. K. (2016). Vector-delivered artificial miRNA effectively inhibited replication of Chikungunya virus. Antiviral Research, 134, 42– 49. https://doi-org.ezproxy.its.uu.se/10.1016/j.antiviral.2016.08.019

Samuel, G. H., Wiley, M. R., Badawi, A., Adelman, Z. N., & Myles, K. M. (2016). Yellow fever virus capsid protein is a potent suppressor of RNA silencing that binds double-stranded RNA. Proceedings of the National Academy of Sciences of the United States of America, 113(48), 13863– 13868. https://doi-org.ezproxy.its.uu.se/10.1073/pnas.1600544113

Sankaran, V. G., Menne, T. F., Xu, J., Akie, T. E., Lettre, G., Van Handel, B., Mikkola, H. K. A., Hirschhorn, J. N., Cantor, A. B., & Orkin, S. H. (2008). Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science (New York, N.Y.), 322(5909), 1839– 1842. https://doi-org.ezproxy.its.uu.se/10.1126/science.1165409

Schulz, W. (2007). Molecular biology of human cancers: An advanced student's textbook. Springer. https://doi-org.ezproxy.its.uu.se/10.1007/978-1-4020-3186-1

Schwab, R., Ossowski, S., Riester, M., Warthmann, N., & Weigel, D. (2006). Highly specific gene silencing by artificial microRNAs in arabidopsis. The Plant Cell, 18(5), 1121– 1133. https://doi-org.ezproxy.its.uu.se/10.1105/tpc.105.039834

Seidel, K., Siswanto, S., Brunt, E. R. P., den Dunnen, W., Korf, H.-W., & Rüb, U. (2012). Brain pathology of spinocerebellar ataxias. Acta Neuropathologica, 124(1), 1– 21. https://doi-org.ezproxy.its.uu.se/10.1007/s00401-012-1000-x

Settin, Ahmad A., Abdullah Algasham, Moataz Dowaidar, and Hisham Ismail. 2009. “Methylene Tetrahydrofolate Reductase and Angiotensin Converting Enzyme Gene Polymorphisms Related to Overweight/obesity among Saudi Subjects from Qassim Region.” Disease Markers 27 (2): 97–102. https://doi.org/10.3233/DMA-2009-0660.

Settin, Ahmad A., Abdullah Alghasham, Ahmad Ali, Moataz Dowaidar, and Hisham Ismail. 2012. “Frequency of Thrombophilic Genetic Polymorphisms among Saudi Subjects Compared with Other Populations.” Hematology 17 (3): 176–82. https://doi.org/10.1179/102453312X13376952196575.

Settin, Ahmad, Abdullah Algasham, Moataz Dowaidar, and Hisham Ismail. 2011. “Methylene Tetrahydrofolate Reductase (MTHFR) and Angiotensinogen Converting Enzyme (ACE) Gene Polymorphisms Related to Overweight and Obesity among Saudi Patients in Al Qassim.” International Journal of Health Sciences 5 (2 Suppl 1): 24–25. https://www.ncbi.nlm.nih.gov/pubmed/23284565.

Settin, Ahmad, Hala Almarsafawy, Ahmad Alhussieny, and Moataz Dowaidar. 2008a. “Dysmorphic Features, Consanguinity and Cytogenetic Pattern of Congenital Heart Diseases: A Pilot Study from Mansoura Locality, Egypt.” International Journal of Health Sciences 2 (2): 101–11. https://www.ncbi.nlm.nih.gov/pubmed/21475491.

Settin, Ahmad, Ibrahem S. Abu-Saif, Rizk El-Baz, Moataz Dowaidar, Rabab Abu-Al Kasim, and Shaimaa Shabana. 2007a. “Diagnosis of Sex Chromosome Disorders and Prenatal Diagnosis of Down Syndrome Using Interphase Fluorescent In-Situ Hyperidization Technique.” International Journal of Health Sciences 1 (2): 203–9. https://www.ncbi.nlm.nih.gov/pubmed/21475429.

Settin, Ahmad, Moataz Dowaidar, Rizk El-Baz, Ayman Abd-Al-Samad, Ibrahim El-Sayed, and Mahmoud Nasr. 2008. “Frequency of Factor V Leiden Mutation in Egyptian Cases with Myocardial Infarction.” Hematology 13 (3): 170–74. https://doi.org/10.1179/102453308X316158.

Shang, R., Baek, S. C., Kim, K., Kim, B., Kim, V. N., & Lai, E. C. (2020). Genomic clustering facilitates nuclear processing of suboptimal Pri-miRNA loci. Molecular Cell, 78(2), 303– 316.e4. https://doi-org.ezproxy.its.uu.se/10.1016/j.molcel.2020.02.009

Sharma, H., Tripathi, A., Kumari, B., Vrati, S., & Banerjee, A. (2018). Artificial microRNA-mediated inhibition of Japanese encephalitis virus replication in neuronal cells. Nucleic Acid Therapeutics, 28(6), 357– 365. https://doi-org.ezproxy.its.uu.se/10.1089/nat.2018.0743

Sheng, P., Flood, K. A., & Xie, M. (2020). Short hairpin RNAs for strand-specific small interfering RNA production. Frontiers in Bioengineering and Biotechnology, 8. https://doi-org.ezproxy.its.uu.se/10.3389/fbioe.2020.00940

Shin, K.-J., Wall, E. A., Zavzavadjian, J. R., Santat, L. A., Liu, J., Hwang, J.-I., Rebres, R., Roach, T., Seaman, W., Simon, M. I., & Fraser, I. D. C. (2006). A single lentiviral vector platform for microRNA-based conditional RNA interference and coordinated transgene expression. Proceedings of the National Academy of Sciences, 103(37), 13759– 13764. https://doi-org.ezproxy.its.uu.se/10.1073/pnas.0606179103

Silva, J. M., Li, M. Z., Chang, K., Ge, W., Golding, M. C., Rickles, R. J., Siolas, D., Hu, G., Paddison, P. J., Schlabach, M. R., Sheth, N., Bradshaw, J., Burchard, J., Kulkarni, A., Cavet, G., Sachidanandam, R., McCombie, W. R., Cleary, M. A., Elledge, S. J., & Hannon, G. J. (2005). Second-generation shRNA libraries covering the mouse and human genomes. Nature Genetics, 37(11), 1281– 1288. https://doi-org.ezproxy.its.uu.se/10.1038/ng1650

Sittler, A., Muriel, M.-P., Marinello, M., Brice, A., den Dunnen, W., & Alves, S. (2018). Deregulation of autophagy in postmortem brains of Machado-Joseph disease patients. Neuropathology: Official Journal of the Japanese Society of Neuropathology, 38(2), 113– 124. https://doi-org.ezproxy.its.uu.se/10.1111/neup.12433

Sliva, K., & Schnierle, B. S. (2010). Selective gene silencing by viral delivery of short hairpin RNA. Virology Journal, 7. https://doi-org.ezproxy.its.uu.se/10.1186/1743-422X-7-248

Son, J., Uchil, P. D., Kim, Y. B., Shankar, P., Kumar, P., & Lee, S.-K. (2008). Effective suppression of HIV-1 by artificial bispecific miRNA targeting conserved sequences with tolerance for wobble base-pairing. Biochemical and Biophysical Research Communications, 374(2), 214– 218. https://doi-org.ezproxy.its.uu.se/10.1016/j.bbrc.2008.06.125

Spronck, E. A., Brouwers, C. C., Vallès, A., de Haan, M., Petry, H., van Deventer, S. J., Konstantinova, P., & Evers, M. M. (2019). AAV5-miHTT gene therapy demonstrates sustained Huntingtin lowering and functional improvement in Huntington disease mouse models. Molecular Therapy. Methods & Clinical Development, 13, 334– 343. https://doi-org.ezproxy.its.uu.se/10.1016/j.omtm.2019.03.002

Stanek, L. M., Sardi, S. P., Mastis, B., Richards, A. R., Treleaven, C. M., Taksir, T., Misra, K., Cheng, S. H., & Shihabuddin, L. S. (2014). Silencing mutant Huntingtin by Adeno-associated virus-mediated RNA interference ameliorates disease manifestations in the YAC128 mouse model of Huntington's disease. Human Gene Therapy, 25(5), 461– 474. https://doi-org.ezproxy.its.uu.se/10.1089/hum.2013.200

Stegmeier, F., Hu, G., Rickles, R. J., Hannon, G. J., & Elledge, S. J. (2005). A lentiviral microRNA-based system for single-copy polymerase II-regulated RNA interference in mammalian cells. Proceedings of the National Academy of Sciences of the United States of America, 102(37), 13212– 13217. https://doi-org.ezproxy.its.uu.se/10.1073/pnas.0506306102

Stoica, L., Todeasa, S. H., Cabrera, G. T., Salameh, J. S., ElMallah, M. K., Mueller, C., Brown, R. H., & Miguel, S.-E. (2016). AAV delivered artificial microRNA extends survival and delays paralysis in an amyotrophic lateral sclerosis mouse model. Annals of Neurology, 79(4), 687– 700. https://doi-org.ezproxy.its.uu.se/10.1002/ana.24618

Sun, B.-S., Dong, Q.-Z., Ye, Q.-H., Sun, H.-J., Jia, H.-L., Zhu, X.-Q., Liu, D.-Y., Chen, J., Xue, Q., Zhou, H.-J., Ren, N., & Qin, L.-X. (2008). Lentiviral-mediated miRNA against osteopontin suppresses tumor growth and metastasis of human hepatocellular carcinoma. Hepatology, 48(6), 1834– 1842. https://doi-org.ezproxy.its.uu.se/10.1002/hep.22531

Thakral, S., & Ghoshal, K. (2015). MiR-122 is a unique molecule with great potential in diagnosis, prognosis of liver disease, and therapy both as miRNA mimic and antimir. Current Gene Therapy, 15(2), 142– 150.

Tiwari, S., Atluri, V., Kaushik, A., Yndart, A., & Nair, M. (2019). Alzheimer's disease: Pathogenesis, diagnostics, and therapeutics. International Journal of Nanomedicine, 14, 5541– 5554. https://doi-org.ezproxy.its.uu.se/10.2147/IJN.S200490

Van den Haute, C., Eggermont, K., Nuttin, B., Debyser, Z., & Baekelandt, V. (2003). Lentiviral vector-mediated delivery of short hairpin RNA results in persistent knockdown of gene expression in mouse brain. Human Gene Therapy, 14(18), 1799– 1807. https://doi-org.ezproxy.its.uu.se/10.1089/104303403322611809

Venit, Tomas, Moataz Dowaidar, Maxime Gestin, Syed Raza Mahmood, Ülo Langel, and Piergiorgio Percipalle. 2020. “Transcriptional Profiling Reveals Ribosome Biogenesis, Microtubule Dynamics and Expression of Specific lncRNAs to Be Part of a Common Response to Cell-Penetrating Peptides.” Biomolecules 10 (11): 1567. https://doi.org/10.3390/biom10111567.

Walder, R. Y., Gautam, M., Wilson, S. P., Benson, C. J., & Sluka, K. A. (2011). Selective targeting of ASIC3 using artificial miRNAs inhibits primary and secondary hyperalgesia after muscle inflammation. Pain, 152(10), 2348– 2356. https://doi-org.ezproxy.its.uu.se/10.1016/j.pain.2011.06.027

Wallace, L. M., Liu, J., Domire, J. S., Garwick-Coppens, S. E., Guckes, S. M., Mendell, J. R., Flanigan, K. M., & Harper, S. Q. (2012). RNA interference inhibits DUX4-induced muscle toxicity in vivo: Implications for a targeted FSHD therapy. Molecular Therapy, 20(7), 1417– 1423. https://doi-org.ezproxy.its.uu.se/10.1038/mt.2012.68

Wallace, L. M., Saad, N. Y., Pyne, N. K., Fowler, A. M., Eidahl, J. O., Domire, J. S., Griffin, D. A., Herman, A. C., Sahenk, Z., Rodino-Klapac, L. R., & Harper, S. Q. (2017). Pre-clinical safety and off-target studies to support translation of AAV-mediated RNAi therapy for FSHD. Molecular Therapy. Methods & Clinical Development, 8, 121– 130. https://doi-org.ezproxy.its.uu.se/10.1016/j.omtm.2017.12.005

Wang, D., Tai, P. W. L., & Gao, G. (2019). Adeno-associated virus vector as a platform for gene therapy delivery. Nature Reviews Drug Discovery, 18(5), 358– 378. https://doi-org.ezproxy.its.uu.se/10.1038/s41573-019-0012-9

Wang, J., Lee, J. E., Riemondy, K., Yu, Y., Marquez, S. M., Lai, E. C., & Yi, R. (2020). XPO5 promotes primary miRNA processing independently of RanGTP. Nature Communications, 11(1), 1845. https://doi-org.ezproxy.its.uu.se/10.1038/s41467-020-15598-x

Wang, S., Shu, J.-Z., Cai, Y., Bao, Z., & Liang, Q.-M. (2012). Establishment and characterization of MTDH knockdown by artificial MicroRNA interference—Functions as a potential tumor suppressor in breast cancer. Asian Pacific Journal of Cancer Prevention: APJCP, 13(6), 2813– 2818. https://doi-org.ezproxy.its.uu.se/10.7314/apjcp.2012.13.6.2813

Wang, Y., Yan, L., Zhang, L., Xu, H., Chen, T., Li, Y., Wang, H., Chen, S., Wang, W., Chen, C., & Yang, Q. (2018). NT21MP negatively regulates paclitaxel-resistant cells by targeting miR-155-3p and miR-155-5p via the CXCR4 pathway in breast cancer. International Journal of Oncology, 53(3), 1043– 1054. https://doi-org.ezproxy.its.uu.se/10.3892/ijo.2018.4477

Wen, J., Ladewig, E., Shenker, S., Mohammed, J., & Lai, E. C. (2015). Analysis of nearly one thousand mammalian mirtrons reveals novel features of dicer substrates. PLoS Computational Biology, 11(9), e1004441. https://doi-org.ezproxy.its.uu.se/10.1371/journal.pcbi.1004441

Wu, Z., Asokan, A., & Samulski, R. J. (2006). Adeno-associated virus serotypes: Vector toolkit for human gene therapy. Molecular Therapy: The Journal of the American Society of Gene Therapy, 14(3), 316– 327. https://doi-org.ezproxy.its.uu.se/10.1016/j.ymthe.2006.05.009

Xia, X.-G., Zhou, H., & Xu, Z. (2006). Multiple shRNAs expressed by an inducible pol II promoter can knock down the expression of multiple target genes. BioTechniques, 41(1), 64– 68. https://doi-org.ezproxy.its.uu.se/10.2144/000112198

Xie, J., Mao, Q., Tai, P. W. L., He, R., Ai, J., Su, Q., Zhu, Y., Ma, H., Li, J., Gong, S., Wang, D., Gao, Z., Li, M., Zhong, L., Zhou, H., & Gao, G. (2017). Short DNA hairpins compromise recombinant adeno-associated virus genome homogeneity. Molecular Therapy, 25(6), 1363– 1374. https://doi-org.ezproxy.its.uu.se/10.1016/j.ymthe.2017.03.028

Xie, J., Tai, P. W. L., Brown, A., Gong, S., Zhu, S., Wang, Y., Li, C., Colpan, C., Su, Q., He, R., Ma, H., Li, J., Ye, H., Ko, J., Zamore, P. D., & Gao, G. (2020). Effective and accurate gene silencing by a recombinant AAV-compatible microRNA scaffold. Molecular Therapy, 28(2), 422– 430. https://doi-org.ezproxy.its.uu.se/10.1016/j.ymthe.2019.11.018

Xie, M., Li, M., Vilborg, A., Lee, N., Shu, M.-D., Yartseva, V., Šestan, N., & Steitz, J. A. (2013). Mammalian 5′-capped MicroRNA precursors that generate a single MicroRNA. Cell, 155(7), 1568– 1580. https://doi-org.ezproxy.its.uu.se/10.1016/j.cell.2013.11.027

Xie, P., Xie, Y., Zhang, X., Huang, H., He, L., Wang, X., & Wang, S. (2013). Inhibition of dengue virus 2 replication by artificial microRNAs targeting the conserved regions. Nucleic Acid Therapeutics, 23(4), 244– 252. https://doi-org.ezproxy.its.uu.se/10.1089/nat.2012.0405

Yang, J.-S., Maurin, T., Robine, N., Rasmussen, K. D., Jeffrey, K. L., Chandwani, R., Papapetrou, E. P., Sadelain, M., O'Carroll, D., & Lai, E. C. (2010). Conserved vertebrate mir-451 provides a platform for Dicer-independent, Ago2-mediated microRNA biogenesis. Proceedings of the National Academy of Sciences, 107(34), 15163– 15168. https://doi-org.ezproxy.its.uu.se/10.1073/pnas.1006432107

Yang, Y.-S., Xie, J., Chaugule, S., Wang, D., Kim, J.-M., Kim, J., Tai, P. W. L., Seo, S., Gravallese, E., Gao, G., & Shim, J.-H. (2020). Bone-targeting AAV-mediated gene silencing in osteoclasts for osteoporosis therapy. Molecular Therapy - Methods & Clinical Development, 17, 922– 935. https://doi-org.ezproxy.its.uu.se/10.1016/j.omtm.2020.04.010

Yi, R., Qin, Y., Macara, I. G., & Cullen, B. R. (2003). Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes & Development, 17(24), 3011– 3016. https://doi-org.ezproxy.its.uu.se/10.1101/gad.1158803

Yi, T., Arthanari, H., Akabayov, B., Song, H., Papadopoulos, E., Qi, H. H., Jedrychowski, M., Güttler, T., Guo, C., Luna, R. E., Gygi, S. P., Huang, S. A., & Wagner, G. (2015). EIF1A augments Ago2-mediated dicer-independent miRNA biogenesis and RNA interference. Nature Communications, 6, 7194. https://doi-org.ezproxy.its.uu.se/10.1038/ncomms8194

Yoda, M., Cifuentes, D., Izumi, N., Sakaguchi, Y., Suzuki, T., Giraldez, A. J., & Tomari, Y. (2013). PARN mediates 3′-end trimming of Argonaute2-cleaved precursor microRNAs. Cell Reports, 5(3), 715– 726. https://doi-org.ezproxy.its.uu.se/10.1016/j.celrep.2013.09.029

Yu, T., Ma, P., Wu, D., Shu, Y., & Gao, W. (2018). Functions and mechanisms of microRNA-31 in human cancers. Biomedicine & Pharmacotherapy, 108, 1162– 1169. https://doi-org.ezproxy.its.uu.se/10.1016/j.biopha.2018.09.132

Yue, J., Sheng, Y., Ren, A., & Penmatsa, S. (2010). A miR-21 hairpin structure-based gene knockdown vector. Biochemical and Biophysical Research Communications, 394(3), 667– 672. https://doi-org.ezproxy.its.uu.se/10.1016/j.bbrc.2010.03.047

Zeng, Y., & Cullen, B. R. (2003). Sequence requirements for micro RNA processing and function in human cells. RNA (New York, N.Y.), 9(1), 112– 123. https://doi-org.ezproxy.its.uu.se/10.1261/rna.2780503

Zeng, Y., & Cullen, B. R. (2005). Efficient processing of primary microRNA hairpins by Drosha requires flanking nonstructured RNA sequences. The Journal of Biological Chemistry, 280(30), 27595– 27603. https://doi-org.ezproxy.its.uu.se/10.1074/jbc.M504714200

Zeng, Y., Wagner, E. J., & Cullen, B. R. (2002). Both natural and designed micro RNAs can inhibit the expression of cognate mRNAs when expressed in human cells. Molecular Cell, 9(6), 1327– 1333. https://doi-org.ezproxy.its.uu.se/10.1016/S1097-2765(02)00541-5

Zeng, Y., Yi, R., & Cullen, B. R. (2003). MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proceedings of the National Academy of Sciences, 100(17), 9779– 9784. https://doi-org.ezproxy.its.uu.se/10.1073/pnas.1630797100

Zhang, H., Kolb, F. A., Jaskiewicz, L., Westhof, E., & Filipowicz, W. (2004). Single processing center models for human dicer and bacterial RNase III. Cell, 118(1), 57– 68. https://doi-org.ezproxy.its.uu.se/10.1016/j.cell.2004.06.017

Zhang, H., Tang, X., Zhu, C., Song, Y., Yin, J., Xu, J., Ertl, H. C. J., & Zhou, D. (2015). Adenovirus-mediated artificial MicroRNAs targeting matrix or nucleoprotein genes protect mice against lethal influenza virus challenge. Gene Therapy, 22(8), 653– 662. https://doi-org.ezproxy.its.uu.se/10.1038/gt.2015.31

Zhang, J., Liu, Q.-S., & Dong, W.-G. (2011). Blockade of proliferation and migration of gastric cancer via targeting CDH17 with an artificial microRNA. Medical Oncology (Northwood, London, England), 28(2), 494– 501. https://doi-org.ezproxy.its.uu.se/10.1007/s12032-010-9489-0

Zhang, T., Cheng, T., Wei, L., Cai, Y., Yeo, A., Han, J., Yuan, Y. A., Zhang, J., & Xia, N. (2012). Efficient inhibition of HIV-1 replication by an artificial polycistronic miRNA construct. Virology Journal, 9, 118. https://doi-org.ezproxy.its.uu.se/10.1186/1743-422X-9-118

Zhao, Y., Samal, E., & Srivastava, D. (2005). Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature, 436(7048), 214– 220. https://doi-org.ezproxy.its.uu.se/10.1038/nature03817

Zheng, Z., Wang, P., Wang, H., Zhang, X., Wang, M., Cucinotta, F. A., & Wang, Y. (2013). Combining heavy ion radiation and artificial microRNAs to target the homologous recombination repair gene efficiently kills human tumor cells. International Journal of Radiation Oncology, Biology, Physics, 85, 466– 471. https://doi-org.ezproxy.its.uu.se/10.1016/j.ijrobp.2012.04.008

Zhou, H., Xia, X. G., & Xu, Z. (2005). An RNA polymerase II construct synthesizes short-hairpin RNA with a quantitative indicator and mediates highly efficient RNAi. Nucleic Acids Research, 33(6), e62. https://doi-org.ezproxy.its.uu.se/10.1093/nar/gni061

Zoghbi, H. Y., & Orr, H. T. (2000). Glutamine repeats and neurodegeneration. Annual Review of Neuroscience, 23, 217– 247. https://doi-org.ezproxy.its.uu.se/10.1146/annurev.neuro.23.1.217