



# RNA therapeutics for mood disorders: current evidence towards clinical trials

Marguerite Le Marois, Eleni Tzavara, El Chérif Ibrahim, Olivier Blin, Raoul Belzeaux

## ► To cite this version:

Marguerite Le Marois, Eleni Tzavara, El Chérif Ibrahim, Olivier Blin, Raoul Belzeaux. RNA therapeutics for mood disorders: current evidence towards clinical trials. Expert Opinion on Investigational Drugs, 2021, 10.1080/13543784.2021.1928073 . hal-03361479

**HAL Id: hal-03361479**

**<https://hal.science/hal-03361479>**

Submitted on 5 Oct 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



## RNA therapeutics for mood disorders: current evidence towards clinical trials

Marguerite Le Marois, Eleni Tzavara, El Chérif Ibrahim, Olivier Blin & Raoul Belzeaux

To cite this article: Marguerite Le Marois, Eleni Tzavara, El Chérif Ibrahim, Olivier Blin & Raoul Belzeaux (2021): RNA therapeutics for mood disorders: current evidence towards clinical trials, Expert Opinion on Investigational Drugs, DOI: [10.1080/13543784.2021.1928073](https://doi.org/10.1080/13543784.2021.1928073)

To link to this article: <https://doi.org/10.1080/13543784.2021.1928073>



Accepted author version posted online: 08 May 2021.



Submit your article to this journal [↗](#)



Article views: 29



View related articles [↗](#)



View Crossmark data [↗](#)

**Publisher:** Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

**Journal:** *Expert Opinion on Investigational Drugs*

**DOI:** 10.1080/13543784.2021.1928073

RNA therapeutics for mood disorders: current evidence towards clinical trials

**Authors:**

Marguerite Le Marois<sup>1,2</sup>

Eleni Tzavara<sup>3,4</sup>, MD, PhD

El Chérif Ibrahim<sup>2,3</sup>, PhD

Olivier Blin<sup>1</sup>, MD, PhD

Raoul Belzeaux<sup>2,3,4</sup>, MD, PhD

**Affiliation:**

1. Aix Marseille Univ, APMH, INSERM, Inst Neurosci Syst, UMR 1106, University Hospital Federation DHUNE, Service de Pharmacologie Clinique et Pharmacovigilance, Marseille, France

2. Aix Marseille Univ, CNRS, Inst Neurosci Timone, Marseille, France.

3. Fondation FondaMental, Créteil, France.

4. Pôle de psychiatrie, Hôpital Sainte-Marguerite, AP-HM, Marseille, France.

**Corresponding author:** Raoul Belzeaux, MD, PhD

[Raoul.BELZEAUX@ap-hm.fr](mailto:Raoul.BELZEAUX@ap-hm.fr)

Hôpital Sainte-Marguerite, 270 boulevard Sainte-Marguerite, 13009 Marseille, FRANCE.

Phone number: +33(0)491746119

Fax number: +33(0)491745578

## **Abstract**

**Introduction** Mood disorders are severe yet frequent psychiatric disorders worldwide, comprising major depressive disorder (MDD) and bipolar disorders (BD). Their treatment remains poorly effective. For the past decades, various pathophysiological processes have been explored and growing evidence for epigenetic mechanisms has emerged. Consequently, a great interest in a novel pharmacological class for the treatment of mood disorders arose: RNA therapeutics.

**Areas covered** We conducted a systematic review of RNA therapeutics — antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs) and micro-RNA (miRNA) therapeutics — for the treatment of mood disorders studied in pre-clinical animal models listed in PubMed, in clinical trials registered in ClinicalTrials.gov and available on the market by combining literature search and Food and Drug Administration (FDA) and European Medicine Agency (EMA) online databases. Eighteen pre-clinical studies investigated the antidepressant effects of RNA therapeutics. However, even though there is an increasing number of marketing authorizations and clinical trials for the past twenty years, no RNA therapeutic reached the clinical development pipeline for the treatment of psychiatric disorders yet.

**Expert opinion** Several promising RNA therapeutics have been tested in pre-clinical studies for depressive disorders, whereas no molecule has been developed for BD. There are several issues to address before reaching clinical development and new challenges include stratifying patient population and predicting therapeutic response.

**Keywords:** depression , antisense oligonucleotide , small interfering RNA , animal model , epigenetic

## Article Highlights

- Up to 30% of patients suffering from mood disorders are resistant to conventional treatments
- RNA-based drugs acting on epigenetic events, called RNA therapeutics, are on the rise
- A systematic review of preclinical studies assessing RNA therapeutics on depression-like behaviour of animal models revealed several promising molecules: five ASOs, six shRNAs, eight siRNAs, one miRNA mimic and one anti-miRNA
- One promising delivery strategy using small molecules binding to specific neurotransmitter transporters and conjugated to RNA therapeutics ensures selective delivery to the targeted neurons after intranasal administration
- While phase I, II and III studies evaluate several RNA therapeutics for the treatment of various neurological disorders, no clinical trial tested RNA therapeutics for the treatment of mood disorder
- Several issues regarding efficacy, toxicity, safety and economic matters must be addressed before RNA therapeutics can reach clinical development for the treatment of mood disorders

## 1. Introduction

Mood disorders, comprised of major depressive disorder (MDD) and bipolar disorders (BD), are frequent psychiatric disorders with a lifetime prevalence of 16.9% in the general population (1). They are characterized by frequent variations of mood, energy and behaviour, associated with sleep and appetite disturbance. These are severe disorders with frequent psychiatric and addictive comorbidities, poor quality of life and poor global health associated with premature death. Indeed, they are the main cause of suicide behaviour and suicide death (2). The severity of mood disorders is unfortunately aggravated by poor treatment efficacy. The main classes of drugs used in both inpatient and outpatient settings for mood disorders are antidepressants, lithium, anticonvulsants and antipsychotics. Antidepressants, the main pharmacological treatment of depression, are ineffective in more than 30% of patients (3). Similarly, lithium, the main pharmacological treatment of BD, could be ineffective in up to 30% of patients (4). Several classes of antidepressants exist, including selective serotonin reuptake inhibitors (SSRI), serotonin and norepinephrine reuptake inhibitors (SNRI), tricyclic antidepressants and monoamine oxidase inhibitors. Side effects are variable according to the different classes, with usually fewer side effects for SSRIs and SNRIs than other antidepressants. Moreover, in mood disorders, medication adherence is an issue for 20% to 60% of patients (5).

Furthermore, there is a significant delay in the onset of treatment action. Antidepressants are generally considered to have very few clinical effects within the first two weeks of treatment and 6 weeks are required for the treatment to be fully efficient (6).

Consequently, a new therapeutic perspective is warranted. The first psychotropic medications, most of which are still in use, were discovered by serendipity after the second world war, such as chlorpromazine, the first antipsychotic described by Delay and Denicker in Paris (7). Subsequently, their pharmacological mechanisms have been partially elucidated,

antidepressants and antipsychotics act on monoaminergic systems (7,8), while the mechanisms of action of lithium remain largely unclear (9). Most of antidepressants are inhibitors of mono-amine recapture (mainly serotonin and noradrenalin) while antipsychotics are dopamine receptor antagonists (mainly of the D2 subtype). To this date, all main pharmacological treatments available have been developed from these early compounds and their purported mechanism of action.

In parallel, the pathophysiology of mood disorders has been progressively and more precisely described. Monoaminergic neurotransmission, including serotonergic and dopaminergic systems, are the main targets of the large majority of antidepressants and antipsychotics, and have been largely studied (7,8). Additionally, several other pathophysiological hypotheses emerged over the years, notably on the implication of brain-derived neurotrophic factor (BDNF), hippocampal neurogenesis, neuroendocrine systems including pituitary-thyroid and corticotropic axes, neuroinflammation, glutamatergic neurotransmission and synaptic plasticity, among others, all possibly related and influenced by external factors such as stress (10,11). Diverse brain regions may be involved in the development of depressive symptoms, such as the hippocampus, prefrontal cortex, nucleus accumbens (NAc), ventral tegmental area (VTA), locus coeruleus (LC), basolateral amygdala (BLA) and dorsal raphe nucleus (DR) (11).

Interestingly, epigenetic deregulation is one of the major biological processes associated with mood disorders. Mood disorders are associated with both genetic and environmental factors, and epigenetic mechanisms are probably involved in the gene-environment interaction underlying mood disorders' pathophysiology. They are modifications that cause heritable changes in gene expression without modifying the DNA sequence, encompassing covalent changes to DNA, such as DNA methylation, and post-translational modifications of histone N-terminal tails, as well as non-transcriptional gene-silencing mechanisms, including

micro-RNAs (miRNAs). These modifications were described in mood disorders in a large amount of work (12–14), leading to a new therapeutic interest in RNA therapeutics.

In this paper, we conducted a systematic review of RNA therapeutics for the treatment of mood disorders studied in pre-clinical animal models, in clinical trials and available on the market.

## 2. What are RNA-based therapeutic compounds?

RNA therapeutics are RNA-based drugs acting on epigenetic events. The different types of RNA therapeutics currently in development are described in the following sections (Figure 1).

### 2.1. Antisense oligonucleotides

The majority of RNA therapeutics used in the clinic are antisense oligonucleotides (ASOs) (15). They are single-stranded synthetic RNA or DNA sequences, highly modified, that bind their target mRNA or pre-mRNA by complementary Watson-Crick base pairing, resulting in different splicing events, protein translation inhibition, or transcript degradation (Figure 1.1) (16). For example, nusinersen is an 18-mer phosphorothioate 2'-O-methoxyethoxy antisense oligonucleotide with all cytidines methyl-modified at the 5-position that binds to a regulatory sequence in intron 7 of the SMN2 pre-mRNA, leading to the production of the functional SMN protein (17). To that extent, it was approved in the United States (US) and the European Union (EU) in 2017 for the treatment of spinal muscular atrophy (SMA).

### 2.2. Short interfering RNAs

Short interfering RNAs (siRNAs) are another category of RNA therapeutics on the rise. They are small double-stranded RNA sequences that trigger the RNA interference (RNAi) pathway. One strand of the siRNA duplex binds to an Argonaute 2 (Ago2) protein and is



loaded into the ribonucleoprotein complex RISC (RNA-induced silencing complex). Within the RISC, the siRNA binds to the target mRNA by perfect complementarity with a high selectivity, which leads to the degradation of the transcript (Figure 1.2) (18).

### 2.3. Short hairpin RNAs (shRNAs)

Short hairpin RNAs (shRNAs) are DNA plasmids that enter cell nuclei, where they are transcribed in RNA with a characteristic stem-loop structure that enters the endogenous miRNA biogenesis pathway. As they can encode two or more siRNAs or miRNAs, shRNAs allow a multiplex approach to treatment that could reduce the risk encountered by RNAi molecules to compete with endogenous miRNAs and saturate RISC machinery (Figure 1.3) (19,20).

### 2.4. Micro-RNA (miRNA) therapeutics

MiRNAs are endogenous single-stranded short RNA sequences that bind to an Ago protein forming the RISC, and binding to target transcripts with imperfect complementarity, leading to translational repression or transcript destabilization and degradation (21,22). They are involved in many various biological processes, and as miRNAs can bind to a multitude of RNA targets, miRNA therapeutics are powerful tools that can regulate entire gene networks underlying polygenic, complex disorders (23). MiRNA therapeutics can be divided into two categories: molecules enhancing miRNA expression, and molecules inhibiting miRNA expression and/or function.

#### 2.4.1 MiRNA replacement therapeutics

MiRNA mimics are synthetic double-stranded RNA molecules matching the corresponding endogenous miRNA sequence to restore its altered expression in specific disorders (Figure 1.4) (24).

Other replacement tools are shRNA plasmids coding for specific miRNAs. As their production respect endogenous cell processing rate, they minimize the potential toxicity caused by miRNA mimics (25).

#### 2.4.2 Anti-miRNAs

MiRNA inhibitors or anti-miRNAs (anti-miRs) are designed to suppress the function of a specific miRNA involved in disease development (Figure 1.5). They are of two types: antagomiRs and miRNA sponges.

AntagomiRs are single-stranded, highly modified synthetic ASOs that bind with high affinity to target mature miRNAs, resulting in their sequestration and inhibiting their function (26).

MiRNA sponges or decoys are short, synthetic RNA constructs containing high-affinity miRNA binding sites to which the miRNA will preferably bind, competing with the endogenous miRNA target transcripts. Thus, this strategy notably allows reducing the activity of a specific set of miRNAs (27).

#### 2.5. RNA aptamers

RNA aptamers are short, single-stranded RNA sequences folded into stable 3D conformations, that bind to proteins with high specificity and affinity, inhibiting their function (Figure 1.6) (28,29).

### 3. RNA therapeutics in pre-clinical development for the treatment of mood disorders

This review aimed to identify all the published articles that have studied the effect of RNA therapeutic administration in animals using validated behavioural tests for mood disorders. The article selection process was adapted from the PRISMA statement – Preferred Reporting Items for Systematic Reviews and Meta-analyses (30). Search terms were “mood disorder” and “RNA therapeutic”. A systematic electronic literature search using the database PubMed was conducted, last updated on 2 April 2021. The search was not limited by date restrictions but excluded unpublished literature in Pubmed. In addition, we used the articles reviewed to search for other relevant publications. Two reviewers (M.L.M. and R.B.) performed the total search independently and included the articles based on eligibility criteria. Studies were included when: 1. An animal model was used (Table 1); 2. At least one RNA therapeutic was administered to animals; 3. Mood disorder symptoms were assessed with validated behavioural tests; 4. The administered RNA therapeutic had a significant effect on behavioural tests exploring mood changes in animals. Articles were excluded when they were: 1. Reviews, editorial and comments; 2. Retracted. Only animal studies using behavioural tests (Table 1) were selected, as mood disorders are complex and multifactorial pathologies that cannot be modelled in cells. Figure 2 is an overview of the study search and selection process. The primary search of the electronic databases yielded 414 results, without any duplicates. Exclusion based on title, abstract and eligibility criteria narrowed the articles down to the seventeen included in this review. The discrepancies from the title and abstract search were discussed and resolved to lead to consensus. One relevant publication (31) was added based on one of the reviewed articles (32). Figure 3 shows the number of selected pre-clinical studies for each RNA therapeutic type.

Two assessors (M.L.M. and R.B.) independently extracted the data from the eighteen included articles. Any disagreements were resolved through discussion. The following information was extracted from the articles: the animal model used, type of RNA therapeutic

administered, therapeutic target, route of administration, effect on therapeutic target's expression in the investigated tissues, behavioural tests conducted and results, behavioural test controls, animal control groups, reversibility of the treatment, toxicity of the molecule, comparison to reference treatment, and whether the observer in behavioural tests was blinded or not for treatment (Tables 2 and 3). The quality assessment of each study was based on those data. The risk of bias in this review was assessed as low using ROBIS (33), a rigorously developed tool designed specifically to assess the risk of bias in systematic reviews.

The selected studies used various validated rodent models of depression based on the development of a depressive-like phenotype -characterized by well-established behavioural tests- after stress exposure, mimicking depressive phenotypes and neuronal changes similar to those observed in humans (Table 1). They are widely used, including in our hands (34,35), and have been extensively reviewed elsewhere (36–39). A widely-used model of depressive-like behaviours in mice is the unpredictable chronic mild stress (UCMS) procedure, consisting of randomly exposing animals to various transient psychosocial stressors of mild intensity. Based on another type of stress, social defeat stress (SDS) is performed by putting animals in physical interaction with a novel aggressor animal for few minutes every day in chronic SDS (CSDS) or every 3 days in intermittent SDS (ISDS) procedure. A different procedure is repeated restraint stress (RRS), obtained by individually restraining animals against back-and-forth movement into well-ventilated conical tubes. Depressive-like behaviours can also be induced through pharmacological interventions, such as dopamine depletion or chronic exposure to low dosages of corticosterone (40).

Depressive-like behaviours in rodents can be assessed by well-established tests that measure distinct yet interrelated aspects of negative affects (36–39) (Table 1). Anhedonia can

be measured by a reduced preference for sucrose over water in the sucrose preference test (SPT). Behavioural despair is assessed by greater immobility times in the tail suspension test (TST) and the forced swim test (FST). To exclude any influence on TST and FST results, the locomotor activity of the animals must be controlled. Reduced social behaviour is evaluated in the social interaction test, expressed a reduced time spent in social interaction with other animals (41), and the U-field test, with a reduced number of interactions with social target animals (42). Increased latency period to consume palatable food placed in the center of an open field after 24h of food deprivation is used as an index of anxiety- and depressive-like behaviours in the novelty-suppressed feeding test (43). Furthermore, as anxiety-like behaviours are commonly associated with a depressive phenotype, they are frequently explored in the elevated plus maze (EPM) (37) and open field paradigms and in the light/dark transition test (44).

### 3.1. Antisense oligonucleotides (ASOs)

In a study published in 2018, Sanna *et al.* (45) tested three ASOs directed against neuronal embryonic lethal abnormal vision (nELAV) proteins HuB, HuC and HuD. These are neuron-specific RNA binding proteins, which are key regulators of mRNA trafficking and metabolism and thus post-transcriptional gene expression. They play an important role in neuronal development and survival and have been found dysregulated in several neurological disorders.

They administered the ASOs by intracerebroventricular (i.c.v) injections and demonstrated the sequence-specific knockdown of the HuB, HuC and HuD in hippocampus whole cell lysate. Anti-HuB and anti-HuC ASOs had antidepressant-like effects comparable to that induced by amitriptyline, used as a reference drug, but did not show any anxiolytic effect on

UCMS-exposed mice. Conversely, anti-HuD ASO did not show any antidepressant-like effect but had anxiolytic effects on UCMS-exposed mice.

In a study published in 2008, Galeotti *et al.* (46) explored the role of three different isoforms of inositol 1,2,5-triphosphate receptor (InsP3R) expressed in the brain, InsP3R1, InsP3R2 and InsP3R3, in depressive-like behaviours assessed in the FST. InsP3Rs allow intracellular  $\text{Ca}^{2+}$  release. Since  $\text{Ca}^{2+}$  levels were found elevated in patients with affective disorders, InsPRs may contribute to the pathophysiology of mood disorders.

They administered to mice, by i.c.v injections, ASOs inhibiting the expression of InsP3R1, InsP3R2 and InsP3R3 and observed the silencing of their respective target in the cerebellum, hippocampus and cortex. Anti-InsP3R1, anti-InsP3R2 and anti-InsP3R3 showed antidepressant-like effects in a dose-dependent manner and with a maximum effect comparable to that induced by amitriptyline and clomipramine in the FST. The antidepressant-like effects of the three ASOs were reversible, as they started to decrease from the 36<sup>th</sup> hour after the end of the treatment and completely disappeared after 7 days, excluding any irreversible toxicity on cerebral structures. However, higher doses of the anti-InsPR ASOs induced signs of cerebral toxicity or death.

### 3.2. Small interfering RNAs (siRNAs)

In 2016, Ferrés-Coy *et al.* (31) tested the antidepressant effect of a siRNA directed against the serotonin transporter (SERT) after intranasal (i.n.) administration in mice. The siRNA (C-SERT-siRNA) was chemically conjugated with the SSRI sertraline, which binds to SERT with high specificity and high affinity, to selectively downregulate SERT expression in the serotonergic (5-HT) neurons, where SERT is differentially expressed. Given the structural

complexity of the compound (47), the specific role of the conjugated sertraline in the molecule's activity should be further explored.

After intranasal administration, the sertraline-conjugated siRNA was internalized into 5-HT neurons by endocytosis probably through SERT in the midbrain, more efficiently in the DR than in the median raphe nucleus (MnR). The molecule was absent from cells in olfactory bulbs and the dorsal hippocampus, supporting its SERT-modulated accumulation in the neurons.

The authors confirmed molecular inhibition of SERT in DR after intranasal administration of 30 µg/day for 1, 4, or 7 days in mice, with a maximal effect after 7-day treatment. The effect was not dose-dependent after the i.n. administration of 10, 30 or 100 µg/day for 7 days. Moreover, i.n. administration of 30 µg/day of C-SERT-siRNA for 7 days doubled extracellular 5-HT concentrations in the caudate putamen and reduced serotonergic 5-HT<sub>1A</sub>-autoreceptor expression and function in the DR more rapidly and efficiently than fluoxetine administered intraperitoneally (i.p.).

After i.n. administration of 30 µg/day of C-SERT-siRNA for 7 days, recovery of SERT expression to control values were obtained 7 and 15 days after the last administration, showing the reversibility of the C-SERT-siRNA effect. To evaluate the molecule's safety, neuronal degeneration, astrogliosis and immune responses were explored through immunostaining; there was no effect on these parameters.

They next tested the treatment on a model of depression: mice were exposed to low oral dosages of corticosterone for 28 or 49 days and developed depressive-like behaviours that were reversed by the i.n. administration of 30 µg/day of C-SERT-siRNA for 7 days, similar to treatment by fluoxetine administered i.p. for 28 days. Fluoxetine did not show any antidepressant-like effect when administered for 7 days.

Furthermore, they showed that 7-day treatment with C-SERT-siRNA and 28-day treatment, but not 7-day treatment with fluoxetine, promotes proliferation and maturation of cellular precursors, dendritic complexity and expression of neuroplasticity-associated genes in the hippocampus. These modifications are well known to be associated with clinical antidepressant action.

Bortolozzi and colleagues (47), from the same research team, had already used a similar strategy in a study published in 2012. They developed a siRNA directed against the serotonergic 5-HT<sub>1A</sub> receptor (C-1A-siRNA) in order to suppress negative feedback mechanisms mediated by 5-HT<sub>1A</sub>-autoreceptors responsible for an increased depression susceptibility and a reduced response to antidepressant treatment. Similarly, the siRNA was conjugated to sertraline by covalent bond to concentrate the molecule selectively in 5-HT neurons.

Both i.c.v infusion into the dorsal third ventricle (D3V) and i.n. administration of 30 µg of C-1A-siRNA in mice led to a significant decrease in 5-HT<sub>1A</sub>R levels into dorsal and median raphe nuclei but did not alter post-synaptic 5-HT<sub>1A</sub>R density or other genes expressed by 5-HT neurons, showing a selective effect on pre-synaptic 5-HT<sub>1A</sub>R. Furthermore, C-1A siRNA administration enhanced the effect of i.p. injection of fluoxetine on extracellular 5-HT concentrations in the medial prefrontal cortex.

I.c.v infusion of C-1A-siRNA into D3V of mice did not upregulate inflammatory cytokines nor apoptotic genes, excluding any off-target or toxic side effects of the drug on these parameters.

Both i.c.v infusion of 30 µg of C-1A-siRNA into D3V and i.n. administration of 30 µg or 100 µg of C-1A-siRNA induced antidepressant-like effects on mice behaviour in the FST and



TST, comparable to those observed with i.p. injection of fluoxetine, with a dose-effect relation. The EPM test did not show any effect on anxiety-like behaviour.

This study was the first to use the conjugation of a siRNA to sertraline in order to target a specific neuronal population in the brain. Bortolozzi *et al.* developed a siRNA targeting selectively, safely and with a high efficiency 5-HT<sub>1A</sub>-autoreceptors, leading to antidepressant-like effects. Furthermore, the i.n. administration provides ease of use in human. It was therefore the first study to show the feasibility of using RNAi strategies for the treatment of mood disorders.

In a study published in 2015, Choi *et al.* (48) explored molecular mechanisms associated with the thyrotropin-releasing hormone (TRH) system underlying stress-induced depression. They found that RRS in mice persistently increased TRH and TRH receptor-1 (TRHR1) in the BLA, specifically in mice developing severe depressive-like behaviours, compared to resilient and control mice. They injected either TRH peptide or taltirelin -a TRH receptor agonist- directly in the BLA of non-stressed mice; both were sufficient to produce depressive-like behaviours. They next explored the signalling downstream of TRH receptor activation and identified p-ERK1/2 as a probable important mediator of the TRH receptor activation in the BLA involved in the stress-induced development of depressive-like behaviours.

Thus, they separately infused two siRNA targeted against TRH (siRNA-*Trh*) and TRHR1 (siRNA-*Trhr1*) bilaterally in the BLA of stressed mice. 48h after the administration, the stress-induced increase in the expression of the corresponding transcript and their downstream partners previously observed was suppressed and depressive-like behaviours were reversed. The effects of siRNA-*Trh* and siRNA-*Trhr1* administration disappeared 14 days after the administration, confirming their reversibility.

In 2015, Kim *et al.* (49) investigated the underlying mechanisms involved in the treatment of depression by physical exercise. Mice were subjected to RRS and developed depressive-like behaviours; concomitantly levels of hypocretin/orexin (Hcr/Orx), melanin-concentrating hormone (MCH) and phospho-CaMKII $\alpha$  were increased in the amygdala, notably in BLA neurons. Mice were then forced to exercise on a running wheel for 60 minutes daily for 21 consecutive days, which reversed depressive-like behaviours and decreased expression of Hcr/Orx and MCH in the amygdala, in a similar manner that imipramine did. It is important to note that as SPT results are not expressed in the percentage of the total volume of fluid intake, their interpretability can be questioned.

They injected either a siRNA targeting Hcr/Orx (siHcr) or a siRNA targeting MCH (siMCH) in the BLA of mice previously subjected to RRS, which reduced their respective molecular target levels in the BLA and reversed the stress-induced depressive-like behaviours.

Similarly, they injected a siRNA directed against CaMKII $\alpha$  (siCaMKII $\alpha$ ) in the BLA of stressed mice, which had the same antidepressant-like effects. Curiously, the silencing of CaMKII $\alpha$  in the BLA by si-CaMKII $\alpha$  was not verified. Furthermore, the effects of siCaMKII $\alpha$  on mice's locomotor activity were not explored and thus cannot be excluded, contrary to siHcr and siMCH.

In 2011, Kim *et al.* (50) studied the implication of the anterior cingulate cortex (ACC) in anxiety disorders and identified neurabin as a potential novel molecular target for anxiolytic treatment. As their findings with neurabin-siRNA were inconclusive, further studies should be conducted to explore this novel therapeutic tool.

### 3.3. Short hairpin RNAs (shRNA)

Several studies tested shRNAs to explore the pathophysiology of depression (42,51–54) and provided evidence of antidepressant effects (Table 2). However, as they all use invasive routes of administration and lack reference treatment and control of reversibility (Table 3), translation from animal to human use of such shRNAs is questionable.

In a study published in 2011, Fanous *et al.* (55) investigated the consequences of depleting VTA brain-derived neurotrophic factor (BDNF) on stress-induced behavioural and weight impairments. They infused bilaterally in VTA an adeno-associated viral vector (AAV) containing shRNA against BDNF (shRNA-BDNF) to rats and exposed them to CSDS procedure. The *in vivo* effect of shRNA on BDNF levels was verified 3 weeks after the surgery. Reduction in VTA BDNF levels had disappeared 14 weeks after the surgery, indicating the reversibility of shRNA-BDNF effect on BDNF expression *in vivo*.

Treatment with shRNA-BDNF attenuated weight deficits accompanying CSDS exposure and enhanced long-term weight gain -50 days after surgery. It also increased social behaviours in both non-stressed and stressed rats seven weeks after surgery, suggesting that shRNA-BDNF infusion in rats' VTA augmented stress resilience. Further studies must be conducted to validate that BDNF depletion was the actual main regulator of social behaviour in this experiment.

#### 3.4. Micro-RNA (miRNA) therapeutics

Five studies explored the therapeutic potential of targeting miRNAs, by either their overexpression or their inhibition, for the treatment of MDD.

In 2019, Lou *et al.* (56) published a study exploring the potential role of miR-124 in depressive-like behaviours and microglial activation. They subjected mice to UCMS protocol

and confirmed the development of a depressive-like phenotype. They found a decreased miR-124 expression in the hippocampus of UCMS mice and a negative correlation between hippocampal miR-124 levels and depressive-like behaviours.

They next injected bilaterally a lentivirus expressing miR-124 (miR-124 lentivirus) directly in the hippocampi of mice and confirmed hippocampal overexpression of miR-124 one week later. Mice that were injected with miR-124 lentivirus and then subjected to UCMS showed resilience to develop depressive-like behaviours and prevented the neuroinflammation observed in the hippocampus of UCMS mice and the LPS-induced microglial activation *in vitro*.

Finally, they further explored molecular mechanisms underlying these processes by *in vitro* luciferase reporter and transfection assays and found that miR-124 negatively regulates STAT3, a member of the signal transducer and activator of transcription (STAT) family, by directly targeting its 3' untranslated (UTR) region. Moreover, they validated that this molecular axis was crucial for *in vitro* LPS-induced microglial activation.

Antidepressant effects of overexpressing miR-124 had already been highlighted by Higuchi *et al.* in 2016 (57). Similarly, they exposed mice to UCMS protocol and validated their depressive-like behaviours, which were all reversed by oral administration of imipramine during 3 weeks. They then explored the expression of 13 miRNAs known to be enriched in the brain and to be implicated in neuronal function, synaptic plasticity and/or psychiatric disorders. They found reduced levels of pri/pre-miR-124 and mature miR-124 in the hippocampus of UCMS mice. Thus, they injected an AAV expressing pre-miR-124 (AAV-miR-124) into the hippocampus of mice and confirmed its effect on miR-124 levels. AAV-miR-124 did not affect the behaviour of non-stressed mice, but induced resilience to the development of depressive-like behaviours in UCMS mice. However, as mice's locomotor

activity was not explored, results should be interpreted with caution. Conversely, downregulation of miR-124 through bilateral injection of anti-miR-124 oligonucleotide in the hippocampus of mice next subjected to RRS enhanced their susceptibility to depression-like behaviours. Moreover, Higuchi *et al.* demonstrated that miR-124 downregulation in the hippocampus contributed to stress-induced changes in dendritic morphology and spine of DG granule neurons, which was prevented by the bilateral injection of AAV-miR-124 in the hippocampus. They next identified histone deacetylase 4 and 5 (HDAC4/5) and GSK3 $\beta$  as targets of miR-124 and showed their involvement in the development of the depressive-like phenotype by infusing selective inhibitors of either HDAC4/5 or GSK3 $\beta$  in mice hippocampus, which had antidepressant-like effects in behavioural tests.

In a first work, Selvamani and Sohrabji showed that a single intravenous (IV) injection of miR-363-3p 4 hours after occlusion of the middle cerebral artery (MCAo) reduced infarct volume and improved sensory-motor performances in middle-aged female, but not male rats. In animal models, MCAo resulted in a late depressive-like phenotype, mimicking the post-stroke depression observed in human. Thus, in a study published in 2019 (58), Panta *et al.* investigated the antidepressant potential of miR-363-3p in middle-aged female rats subjected to MCAo. They administered miR-363-3p mimic by tail vein injection to MCoA female rats and excluded any locomotor, motor strength, or cognitive impairments 3 months after the stroke that could have interfered with behavioural tests. Nevertheless, the effect of miR-363-3p mimic on miR-363-3p expression was not confirmed and should have been explored by molecular assays. MiR-363-3p mimic displayed persistent antidepressant-like effects up to 100 days after stroke on behavioural tests, prevented cytokine circulating levels elevation observed after stroke and normalized BDNF circulating levels 100 days after stroke.

In a study published in 2015, Li *et al.* (59) explored the role of miR-182 in depression. They subjected rats to UCMS and found that stress increased miR-182 and decreased BDNF levels in a time-dependent manner. They next modulated miR-182 expression in the hippocampus of rats by injecting bilaterally a lentivirus expressing either miR-182 (LV-miR-182) or a siRNA directed against miR-182 (LV-si-miR-182). Overexpression of miR-182 after LV-miR-182 injection or downregulation of miR-182 after LV-si-miR-182 injection in the hippocampus were confirmed by RT-qPCR. They then subjected injected rats to UCMS protocol and assessed behavioural changes. Hippocampal overexpression of miR-182 enhanced UCMS-induced depressive-like behaviours and decreased levels of BDNF and its downstream targets CREB1 and pCREB1. Conversely, LV-si-miR-182 reduced depressive-like behaviours and increased hippocampal levels of BDNF, CERB1 and pCERB1. Finally, they validated direct interaction between miR-182 and BDNF 3'UTR by luciferase reporter assay and confirmed BDNF, CERB1 and pCERB1 expressions regulation by miR-182 in cultured neuronal cells.

In 2011, Launay *et al.* (60) directly injected fluoxetine into the raphe of mice for 3 days and found a decreased endogenous level of miR-16 and an increase in SERT and bcl-2 protein levels in the hippocampus, with SERT upregulation mainly localized in glutamatergic neurons. It also enhanced neuronal maturation. All these hippocampal changes were eliminated by the concomitant hippocampal infusion of miR-16.

They thus injected anti-miR-16 in the hippocampus of mice for 3 days, which produced the same hippocampal changes as fluoxetine injection into raphe. Moreover, it reduced the time of immobility of unstressed mice in the FST and alleviated the depressive-like behaviours - deterioration of coat state and reductions in body weight gain and sucrose preference- induced by UCMS protocol to the same extent as fluoxetine infusion into raphe. However, it is

important to notice that researchers did not confirm the molecular effect of anti-miR-16 on miR-16 expression in the hippocampus, and did not assess mice locomotor activity that could have influenced the FST.

Finally, they demonstrated that fluoxetine treatment provoked S100 $\beta$  release by the raphe, which partially relayed fluoxetine response in the hippocampus via the locus coeruleus, and that BDNF, Wnt2 and 15-Deoxy-delta12,14-prostaglandin J2 (15d-PGJ2) acted all three in association and synergistically in the hippocampus by decreasing miR-16.

#### 4. RNA therapeutics in clinical trials

We reviewed RNA therapeutics currently in clinical trials using the online database ClinicalTrials.gov, searching separately for “antisense oligonucleotide”, “siRNA”, “antagomiR”, “antimiR”, “miRNA mimic” or “shRNA”, with the last update on 25 November 2020. One reviewer (M.L.M.) performed the total search and included the articles based on eligibility criteria. Studies were included when: 1. At least one RNA therapeutic was administered; 2. The RNA therapeutic was tested for the treatment of a mood disorder. One assessor (M.L.M.) extracted the data from all the 210 clinical trials reviewed. The following information were extracted from the articles: condition(s), administered drug(s) and clinical phase. No clinical trial evaluating RNA therapeutics for the treatment of mood disorders was found in the database.

The development of RNA therapeutics is on the rise: at the end of the year 2020, more than two hundred clinical trials evaluate them for the treatment of various pathologies listed. Among those trials, 50% are phase I studies, 40% are phase II studies and 10% are phase III studies.

However, no RNA therapeutic is currently evaluated in clinical studies for the treatment of mood disorders or other psychiatric disorders while 12 molecules are tested in 22 clinical

trials for neurologic conditions, of which 59% are phase I, 27% are phase II and 14% are phase III studies. By intrathecal injection, RNA therapeutics can easily reach the central nervous system and treat complex multifactorial brain pathologies. Notably, seven ASOs are tested for Huntington's disease and progressive supranuclear palsy. Thus, clinical development of RNA-based drugs for mood disorders is likely to be feasible.

## 5. Licensed RNA therapeutics

Since the first licensed RNA therapeutic (fomivirsen), approved by Food and Drug Administration in 1998 and by European Agency for the Evaluation of Medicinal Products in 1999, eleven RNA therapeutics were approved in the US or the EU, and eight of them are currently marketed (61). Current marketing statutes were checked using the Drugs@FDA and European Medicine Agency online databases on 11 December 2020. Most target rare, gene-specific related diseases; only one is used for the treatment of a neurological disorder -spinal muscular amyotrophy- through intrathecal administration. None is used for mood disorders or other psychiatric disorders.

## 6. Conclusion

RNA therapeutics are on the rise: a growing number of RNA therapeutics have obtained marketing authorization in the US and/or in the EU for the past twenty years and more than 200 clinical trials involve RNA therapeutics. No RNA therapeutic reached the clinical development pipeline for the treatment of psychiatric disorders yet, but intrathecal injection enables rapid administration to CNS and several molecules are clinically tested to treat complex multifactorial neurological pathologies. RNA therapeutics for mood disorders have thus good chances to reach clinical stages of development.



To this date, however, RNA therapeutics for the treatment of mood disorders are only assessed in pre-clinical studies. All are using stress-induced animal models of depression; no RNA-based molecule developed to treat bipolar disorders specifically has been tested in animals yet. However, due to our restrictive keywords literature research, we may have missed poorly indexed but interesting papers on pre-clinical development of RNA therapeutics for mood disorders, as well as relevant clinical trials not registered on ClinicalTrials.gov database.

## 7. Expert opinion

This review listed several promising therapeutic RNAs developed in pre-clinical studies for depressive disorders; no molecule is currently tested for BD. The most promising molecule is the C-SERT-siRNA (31), which showed antidepressant effects by i.n. administration with a shorter delay of action than fluoxetine. I.n. administration is a clinically feasible administration route that presents several assets such as rapid absorption, high bioavailability, and direct nose-to-brain transport, bypassing the blood-brain barrier. However, it requires complex formulation strategies, and toxicity due to the molecule and the excipients can occur -either locally or at a systemic level (62). Interestingly, the same team recently focused on a miRNA targeting both 5-HT<sub>1A</sub> receptor and SERT. They also developed a conjugated siRNA against TASK3 potassium channel as a potential antidepressant therapy (63).

Despite the great possibilities that offer RNA therapeutics by modulating epigenetic events involved in various complex and polygenic pathologies, their development for the treatment of mood disorders raises several challenges (Figure 4). The translation from animal models to clinical efficacy remains a hurdle both for CNS drugs and RNA therapeutics. As proposed by Morgan (64) the principle of “three Pillars of survival” states that the drug is delivered to the

target site, that the target is occupied at the required level and that functional modulation of the target is achieved. The fourth cornerstone is to establish that there is a clinically significant therapeutic effect (65). In this context, it is important to keep in mind that stress-induced animal models of depression and behavioural tests remain limited and only partially reproduce the clinical condition, which presents a high heterogeneity of phenotypes (36–39).

First of all, there is no unique target gene involved in the pathophysiology of mood disorders and it can be difficult to get efficiency and possible considerable off-target effects can occur in case of poor selectivity for the molecular target. This is even more relevant that RNA therapeutics, as they act on epigenetic mechanisms, may have complex biological effects. It is thus very important to control those parameters in pre-clinical studies, while it may be complicated to detect all off-target effects.

Secondly, the route of administration is another main issue. Pre-clinical studies mainly inject RNA therapeutics locally in specific brain areas by stereotaxic surgeries on animals, but this mode of administration can certainly not be used in clinical practice. Intravenous and intranasal administration can be replicable in humans, but the effects are then global on the brain, if not systemic. An intrathecal injection is quite invasive, poorly accepted by the patients, especially in repeated procedures are required. Drug vectorization has undergone considerable development over the last few years and might be relevant in this case.

Thirdly, access to the “target site” remains a challenge. As they lack secondary or tertiary structures, small RNA therapeutics are more easily introduced to cells than large RNA molecules, but they are negatively charged and can have difficulty crossing the cell membrane. Also, their lifetime in blood circulation is often compromised as they can be degraded by nucleases and immune cells. Efficient delivery of RNA-based drugs to the specific targeted tissue can thus be quite difficult. Moreover, they can trigger an immune response, sometimes resulting in severe adverse effects. Therefore, to ensure efficient uptake

of the drug in the targeted tissue and to reduce toxicity, a variety of selective delivery agents are in development for RNA therapeutics, such as lipid or polymeric nanoparticles, ligand-based targeting molecules, viral vectors and bacterial minicells, alongside chemical modifications designed to increase stability and binding affinity (18). In the brain, the promising conjugated technology developed by Bortolozzi *et al.* (47) could be a good strategy to deliver RNA therapeutics to a specific region and/or cell type after systemic administration. Similar technology is used by miCure Therapeutics, a biotech company specialized in miRNA therapeutics and diagnostics for CNS disorders -including MDD- which develops small molecules binding to a specific neurotransmitter transporter and conjugated to miRNAs to ensure cell-specific delivery in the brain. Producing good manufacturing procedures for clinical-grade RNA therapeutics is however far from obvious as it is not a simple and cheap technology. Complex manufacturing processes, delivery vehicles, potential impurities and excipients, and potential immunogenicity are among the challenges faced by industry for this promising technology (66).

Fourthly, significant therapeutic effects of RNA therapeutics solely based on published results in animal remain unpredictable. They need to be replicated and the use of several animal models for the same molecule would help to further reach clinical development.

Lastly, toxicology and safety studies are required to evaluate the possible toxicity of the RNA therapeutic on different tissues and brain cells. Duration of the effect and potential reversibility must also be demonstrated before trials in human.

On another note, RNA therapeutics also raise economic challenges. In 2018, patisiran was the first siRNA drug put on the market by Alnylam for hereditary transthyretin amyloidosis, a disease affecting about 50,000 patients worldwide. Its cost was set at \$450,000 per patient per year, which is quite conform to other drugs indicted for rare disorders. This elevated cost was

based on the duration (16 years) and investment (\$2.5 billion) required for Research and Development, according to Alnylam, and included a money-back guarantee for insurers (67). Comparatively, nusinersen costs \$625,000 to \$750,000 in the first year, and then around \$375,000 every year after, likely for the rest of a patient's life. As this ASO is indicated for the treatment of SMA, the market is much larger than for patisiran.

However, despite their elevated production and marketing cost, RNA therapeutics have largely reached the point of profitability: between 2014 and 2020, the global market of RNA therapeutics had a compound annual growth rate of 28.4% and is expected to reap \$1.2 billion by 2020 (68).

Today, most RNA therapeutics have been developed as orphan medicines for rare and very severe diseases, including some CNS disorders. Mood disorders, however, are quite frequent (1) and although there is still room for improvement, several easily administered and affordable pharmacological treatments are widely available. The affordability of RNA therapeutics for the treatment of mood disorders might therefore be questionable. Stratifying patient populations according to the probability of therapeutic success, identifying clinical or biological markers to predict therapeutic efficacy, and potentially restricting RNA therapeutics selectively to treatment-resistant patients with a clear biological phenotype represent the new challenges.

## **Funding**

This paper is not funded.

## **Declaration of Interests**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials

discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

### Reviewer Disclosures

Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.

### References

*Papers of particular interest are identified (\*=of importance, \*\*= of considerable importance) and annotated with a brief sentence explaining why the reference is considered to be of interest.*

1. National Comorbidity Survey [Internet]. [cited 2020 Dec 14]. Available from: <https://www.hcp.med.harvard.edu/ncs/>
2. Gili M, Castellví P, Vives M, de la Torre-Luque A, Almenara J, Blasco MJ, et al. Mental disorders as risk factors for suicidal behavior in young people: A meta-analysis and systematic review of longitudinal studies. *J Affect Disord*. 2019 Feb 15;245:152–62.
3. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and Longer-Term Outcomes in Depressed Outpatients Requiring One or Several Treatment Steps: A STAR\*D Report. *Am J Psychiatry*. 2006;13.
4. Del Matto L, Muscas M, Murru A, Verdolini N, Anmella G, Fico G, et al. Lithium and suicide prevention in mood disorders and in the general population: A systematic review. *Neurosci Biobehav Rev*. 2020;116:142–53.
5. Consoloni J-L, M'Bailara K, Perchec C, Aouizerate B, Aubin V, Azorin J-M, et al. Trajectories of medication adherence in patients with Bipolar Disorder along 2 years-follow-up. *J Affect Disord*. 2021 Mar 1;282:812–9.
6. Leuchter AF, Cook IA, Hunter AM, Korb AS. A new paradigm for the prediction of antidepressant treatment response. *Dialogues Clin Neurosci*. 2009;11(4):435–46.
7. Wong M-L, Licinio J. From monoamines to genomic targets: a paradigm shift for drug discovery in depression. *Nat Rev Drug Discov*. 2004 Feb;3(2):136–51.
8. Di Giovanni G, Svob Strac D, Sole M, Unzeta M, Tipton KF, Mück-Šeler D, et al. Monoaminergic and Histaminergic Strategies and Treatments in Brain Diseases. *Front Neurosci*. 2016;10:541.
9. Malhi GS, Outhred T. Therapeutic Mechanisms of Lithium in Bipolar Disorder: Recent Advances and Current Understanding. *CNS Drugs*. 2016;30(10):931–49.
10. Ménard C, Hodes GE, Russo SJ. Pathogenesis of depression: Insights from human and rodent studies. *Neuroscience*. 2016 May;321:138–62.
11. Krishnan V, Nestler EJ. The molecular neurobiology of depression. *Nature*. 2008 Oct;455(7215):894–902.
12. Karsli Çeppioglu S. Epigenetic Mechanisms in Psychiatric Diseases and Epigenetic Therapy. *Drug Dev Res*. 2016;77(7):407–13.
13. Penner-Goeke S, Binder EB. Epigenetics and depression. *Dialogues Clin Neurosci*. 2019 Dec;21(4):397–405.
14. Camkurt MA, Karababa İF, Erdal ME, Kandemir SB, Fries GR, Bayazit H, et al. MicroRNA dysregulation in manic and euthymic patients with bipolar disorder. *J Affect Disord*. 2020 Jan;261:84–90.
15. Bennett CF. Therapeutic Antisense Oligonucleotides Are Coming of Age. *Annu Rev*

Med. 2019 Jan 27;70:307–21.

16. Bennett CF, Baker BF, Pham N, Swayze E, Geary RS. Pharmacology of Antisense Drugs. 2016;28.
17. Stein CA, Castanotto D. FDA-Approved Oligonucleotide Therapies in 2017. Mol Ther. 2017 May 3;25(5):1069–75.
18. Bajan S, Hutvagner G. RNA-Based Therapeutics: From Antisense Oligonucleotides to miRNAs. 2020;27. *\*\*This article is a high-quality and detailed review of the different types of RNA therapeutics.*
19. Grimm D. The dose can make the poison: lessons learned from adverse in vivo toxicities caused by RNAi overexpression. Silence. 2011;2(1):8.
20. Liu YP, von Eije KJ, Schopman NC, Westerink J-T, Brake O ter, Haasnoot J, et al. Combinatorial RNAi Against HIV-1 Using Extended Short Hairpin RNAs. Mol Ther. 2009 Oct;17(10):1712–23.
21. O'Carroll D, Schaefer A. General Principles of miRNA Biogenesis and Regulation in the Brain. Neuropsychopharmacology. 2013 Jan;38(1):39–54.
22. Bartel DP. MicroRNAs: Target Recognition and Regulatory Functions. Cell. 2009 Jan 23;136(2):215–33.
23. Bonneau E, Neveu B, Kostantin E, Tsongalis GJ, De Guire V. How close are miRNAs from clinical practice? A perspective on the diagnostic and therapeutic market. EJIFCC. 2019 Jun 24;30(2):114–27.
24. Bader AG, Brown D, Winkler M. The Promise of MicroRNA Replacement Therapy. Cancer Res. 2010 Sep 15;70(18):7027–30.
25. Boudreau RL, Monteys AM, Davidson BL. Minimizing variables among hairpin-based RNAi vectors reveals the potency of shRNAs. RNA. 2008 Jan 9;14(9):1834–44.
26. Broderick J, Zamore P. microRNA Therapeutics. Gene Ther. 2011 Dec;18(12):1104–10.
27. Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. Nat Methods [Internet]. 2007 Sep [cited 2020 Dec 8];4(9). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3857099/>
28. Keefe AD, Pai S, Ellington A. Aptamers as therapeutics. Nat Rev Drug Discov. 2010;9(7):537–50.
29. Allemailem KS, Almatroudi A, Alsahli MA, Basfar GT, Alrumaihi F, Rahmani AH, et al. Recent advances in understanding oligonucleotide aptamers and their applications as therapeutic agents. 3 Biotech. 2020 Dec;10(12):551.
30. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol. 2009 Oct;62(10):1006–12.
31. Ferrés-Coy A, Galofré M, Pilar-Cuellar F, Vidal R, Paz V, Ruiz-Bronchal E, et al. Therapeutic antidepressant potential of a conjugated siRNA silencing the serotonin transporter after intranasal administration. Mol Psychiatry. 2016 Mar;21(3):328–38. *\*\*This article is a robust pre-clinical study on a siRNA compound with a high level of proof and translational value.*
32. Artigas F, Bortolozzi A. Therapeutic Potential of Conjugated siRNAs for the Treatment of Major Depressive Disorder. Neuropsychopharmacology. 2017 Jan;42(1):371–371.
33. Whiting P, Savović J, Higgins JPT, Caldwell DM, Reeves BC, Shea B, et al. ROBIS: A new tool to assess risk of bias in systematic reviews was developed. J Clin Epidemiol. 2016 Jan;69:225–34.
34. Apazoglou K, Farley S, Gorgievski V, Belzeaux R, Lopez JP, Grenier J, et al. Antidepressive effects of targeting ELK-1 signal transduction. Nat Med. 2018

May;24(5):591–7.

35. Belzeaux R, Gorgievski V, Fiori LM, Lopez JP, Grenier J, Lin R, et al. GPR56/ADGRG1 is associated with response to antidepressant treatment. *Nat Commun.* 2020 Dec;11(1):1635.
36. Cryan JF, Slattery DA. Animal models of mood disorders: recent developments. *Curr Opin Psychiatry.* 2007 Jan;20(1):1–7.
37. Nestler EJ, Hyman SE. Animal Models of Neuropsychiatric Disorders. *Nat Neurosci.* 2010 Oct;13(10):1161–9.
38. Krishnan V, Nestler EJ. Animal Models of Depression: Molecular Perspectives. *Curr Top Behav Neurosci.* 2011;7:121–47.
39. Planchez B, Surget A, Belzung C. Animal models of major depression: drawbacks and challenges. *J Neural Transm.* 2019 Nov;126(11):1383–408. *\*This article is a very interesting and informative review of animal models of depression.*
40. Dieterich A, Yohn CN, Samuels BA. Chronic Stress Shifts Effort-Related Choice Behavior in a Y-Maze Barrier Task in Mice. *J Vis Exp JoVE [Internet].* 2020 Aug 13 [cited 2020 Dec 15];(162). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7646533/>
41. Kaidanovich-Beilin O, Lipina T, Vukobradovic I, Roder J, Woodgett JR. Assessment of Social Interaction Behaviors. *J Vis Exp JoVE [Internet].* 2011 Feb 25 [cited 2020 Dec 15];(48). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3197404/>
42. Seo J-S, Park J-Y, Choi J, Kim T-K, Shin J-H, Lee J-K, et al. NADPH Oxidase Mediates Depressive Behavior Induced by Chronic Stress in Mice. *J Neurosci.* 2012 Jul 11;32(28):9690–9.
43. Blasco-Serra A, González-Soler EM, Cervera-Ferri A, Teruel-Martí V, Valverde-Navarro AA. A standardization of the Novelty-Suppressed Feeding Test protocol in rats. *Neurosci Lett.* 2017 Sep 29;658:73–8.
44. Takao K, Miyakawa T. Light/dark transition test for mice. *J Vis Exp JoVE.* 2006 Nov 13;(1):104.
45. Sanna MD, Quattrone A, Galeotti N. Antidepressant-like actions by silencing of neuronal ELAV-like RNA-binding proteins HuB and HuC in a model of depression in male mice. *Neuropharmacology.* 2018 Jun;135:444–54.
46. Galeotti N, Vivoli E, Norcini M, Bartolini A, Ghelardini C. An antidepressant behaviour in mice carrying a gene-specific InsP3R1, InsP3R2 and InsP3R3 protein knockdown. *Neuropharmacology.* 2008 Dec;55(7):1156–64.
47. Bortolozzi A, Castañé A, Semakova J, Santana N, Alvarado G, Cortés R, et al. Selective siRNA-mediated suppression of 5-HT1A autoreceptors evokes strong antidepressant-like effects. *Mol Psychiatry.* 2012 Jun;17(6):612–23. *\*\*This article is a robust pre-clinical study presenting a high level of proof and translational value on a siRNA compound associated with an innovative delivery system.*
48. Choi J, Kim J, Kim T-K, Park J-Y, Lee J-E, Kim H, et al. TRH and TRH receptor system in the basolateral amygdala mediate stress-induced depression-like behaviors. *Neuropharmacology.* 2015 Oct;97:346–56.
49. Kim T-K, Kim J-E, Park J-Y, Lee J-E, Choi J, Kim H, et al. Antidepressant effects of exercise are produced via suppression of hypocretin/orexin and melanin-concentrating hormone in the basolateral amygdala. *Neurobiol Dis.* 2015 Jul;79:59–69.
50. Kim SS, Wang H, Li X-Y, Chen T, Mercaldo V, Descalzi G, et al. Neurabin in the anterior cingulate cortex regulates anxiety-like behavior in adult mice. *Mol Brain.* 2011;4(1):6.
51. Aceto G, Colussi C, Leone L, Fusco S, Rinaudo M, Scala F, et al. Chronic mild stress alters synaptic plasticity in the nucleus accumbens through GSK3 $\beta$ -dependent modulation of

Kv4.2 channels. *Proc Natl Acad Sci*. 2020 Apr 7;117(14):8143–53.

52. Liu Y, Li M, Fan M, Song Y, Yu H, Zhi X, et al. Chromodomain Y-like Protein-Mediated Histone Crotonylation Regulates Stress-Induced Depressive Behaviors. *Biol Psychiatry*. 2019 Apr;85(8):635–49.

53. Li S-X, Han Y, Xu L-Z, Yuan K, Zhang R-X, Sun C-Y, et al. Uncoupling DAPK1 from NMDA receptor GluN2B subunit exerts rapid antidepressant-like effects. *Mol Psychiatry*. 2018 Mar;23(3):597–608.

54. Aurbach EL, Inui EG, Turner CA, Hagenauer MH, Prater KE, Li JZ, et al. Fibroblast growth factor 9 is a novel modulator of negative affect. *Proc Natl Acad Sci*. 2015 Sep 22;112(38):11953–8.

55. Fanous S, Terwilliger EF, Hammer Jr. RP, Nikulina EM. Viral depletion of VTA BDNF in rats modulates social behavior, consequences of intermittent social defeat stress, and long-term weight regulation. *Neurosci Lett*. 2011 Sep;502(3):192–6.

56. Lou D, Wang J, Wang X. miR-124 ameliorates depressive-like behavior by targeting STAT3 to regulate microglial activation. *Mol Cell Probes*. 2019 Dec;48:101470.

57. Higuchi F, Uchida S, Yamagata H, Abe-Higuchi N, Hobara T, Hara K, et al. Hippocampal MicroRNA-124 Enhances Chronic Stress Resilience in Mice. *J Neurosci*. 2016 Jul 6;36(27):7253–67.

58. Panta A, Pandey S, Duncan IN, Duhamel S, Sohrabji F. Mir363-3p attenuates post-stroke depressive-like behaviors in middle-aged female rats. *Brain Behav Immun*. 2019 May;78:31–40.

59. Li Y, Li S, Yan J, Wang D, Yin R, Zhao L, et al. miR-182 (microRNA-182) suppression in the hippocampus evokes antidepressant-like effects in rats. *Prog Neuropsychopharmacol Biol Psychiatry*. 2016 Feb;65:96–103.

60. Launay JM, Mouillet-Richard S, Baudry A, Pietri M, Kellermann O. Raphe-mediated signals control the hippocampal response to SRI antidepressants via miR-16. *Transl Psychiatry*. 2011 Nov;1(11):e56–e56. *\*This article is an interesting review of licensed RNA therapeutics market share and investment data.*

61. Wang F, Zuroske T, Watts JK. RNA therapeutics on the rise. *Nat Rev Drug Discov*. 2020 Jul;19(7):441–2.

62. Katare YK, Piazza JE, Bhandari J, Daya RP, Akilan K, Simpson MJ, et al. Intranasal delivery of antipsychotic drugs. *Schizophr Res*. 2017 Jun;184:2–13.

63. Fullana MN, Ferrés-Coy A, Ortega JE, Ruiz-Bronchal E, Paz V, Meana JJ, et al. Selective Knockdown of TASK3 Potassium Channel in Monoamine Neurons: a New Therapeutic Approach for Depression. *Mol Neurobiol*. 2019 Apr 1;56(4):3038–52.

64. Morgan P, Van Der Graaf PH, Arrowsmith J, Feltner DE, Drummond KS, Wegner CD, et al. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discov Today*. 2012 May;17(9–10):419–24.

65. Blin O, Davies CH, Lu B. Clinical innovation for neurodegenerative diseases. *Clin Invest*. 2012 Jul;2(7):663–5.

66. Promising mRNA tech comes with regulatory, CMC headaches [Internet]. *Endpoints News*. [cited 2020 Dec 14]. Available from: <https://endpts.com/promising-mrna-tech-comes-with-regulatory-cmc-headaches/>

67. Alnylam Receives Approval of ONPATPRO™ (patisiran) in Europe [Internet]. Investor Relations | Alnylam Pharmaceuticals, Inc. [cited 2020 Dec 14]. Available from: <https://investors.alnylam.com/press-release?id=23066>

68. RNA Based Therapeutics Market Size, Share and Analysis Report 2020 [Internet]. [cited 2020 Dec 14]. Available from: <https://www.alliedmarketresearch.com/rna-based-therapeutics-market>



## Tables

<b>Animal models of depression</b>	<b>Etiological hypothesis in human</b>
Repeated restraint stress	Adulthood life-stress events
Social defeat stress	Adulthood life-stress events
Unpredictable chronic mild stress	Adulthood life-stress events
Chronic exposure to low dosages of corticosterone	Dysregulation of the HPA axis

<b>Behavioural tests</b>	<b>Parameters measured</b>	<b>Depressive-like symptoms explored</b>
Sucrose preference test	Sucrose consumption/total consumption (%)	Anhedonia
T-maze cost/benefit task	Number of trials in which climbing high barrier for high reward was chosen (vs low barrier for low reward)	Anhedonia
Elevated plus maze	Number of entries and time spent in each arm (open vs closed)	Anxiety
Light-dark box test	Time spent in the light chamber (s); number of transitions between chambers	Anxiety
Open field test	Time spent in the center (s)	Anxiety
Novelty-suppressed feeding test	Latency to feed (s)	Anxiety
Coat state score	Observed coat state evaluated with a quantitative scale attributing a score for a clean coat and another one for a dirty coat or in an abnormal state, and assessing different body parts	Apathy/depressed mood
Tail suspension test	Immobility time (s)	Behavioural despair
Forced swim test	Immobility time (s)	Behavioural despair
Bodyweight gain	Bodyweight gain (g)	Changes in eating behavior (loss or gain of appetite)
U-field sociability test	Time spent in the social target zone vs time spent in the non-target zone (s)	Social withdrawal
Social interaction test	Time spent in the interaction zone vs time spent in the avoidance zone (s)	Social withdrawal

Table 1: Animal models and behavioural tests of depressive-like phenotypes used in the selected pre-clinical studies from our literature review.

Type of molecule	Therapeutic target	Expected effect on therapeutic target's expression	Route of administration	Animal model	Behavioural tests		Observers blinded for animal groups	Reference
					Name of the test	Effects observed with RNA therapeutic administration		
ASO	HuB	↓	I.c.v injection	UCMS mice	TST	Anti-HuB, Anti-HuC: ↓ immobility time (control and stressed mice) Anti-HuD: no effect	Yes	Sanna et al., 2018
	HuC	↓			SPT	Anti-HuB, Anti-HuC: ↑ % of sucrose consumption (stressed mice) Anti-HuD: no effect		
	HuD	↓			Light-dark box test	Anti-HuB, Anti-HuC: no effect Anti-HuD: ↑ time spent in the light chamber and number of transitions (stressed mice)		
	InsP3R1	↓	I.c.v injection	WT mice	FST	↓ immobility time in a dose-dependent manner	No	Galeotti et al., 2008
	InsP3R2	↓						
	InsP3R3	↓						
	siRNA	SERT	↓	Intranasal administration	Chronic corticosterone administration in mice	SPT	↑ % of sucrose consumption (stressed mice)	Yes
NFST						↓ latency to feed (stressed mice)		
TST						↓ immobility time (stressed mice)		
Trh		↓	Injection in BLA	RRS mice	U-field test	↑ sociability index (stressed mice)	No	Choi et al., 2015
Trhr1		↓			TST	↓ immobility time (stressed mice)		
					FST	↓ immobility time (stressed mice)		

	Hcrt/Orx	↓	Injection in BLA	RRS mice	U-field test	↑ % of time spent in the target zone and ↓ % of time spent in the non-target zone (stressed mice)	No	Kim (T-K) et al., 2015
	MCH	↓			TST	↓ immobility time (stressed mice)		
	CaMKIIα	↓			FST	↓ immobility time (stressed mice)		
	5-HT1AR	↓	Injection in D3V	WT mice	EPM	No effect	No	Bortolozzi et al., 2012
			Intranasal administration		TST	↓ immobility time		
					FST	↓ immobility time		
	Neurabin	↓	Injection in ACC	WT mice	FST	No effect	No	Kim (S) et al., 2011
					EPM	↑ number of entries and percent of time in the open arms		
	shRNA	GSK3β	↓	Injection in NAc shell	UCMS mice	FST	↓ immobility time (stressed mice)	No
EPM						↑ time spent in open arms and ↓ time spent in the closed arms (stressed mice)		
SPT						↑ % of sucrose consumption (stressed mice)		
CDYL		↓	Injection in PL	CSDS mice	SIT	↑ social interaction ratio (stressed mice)	Yes	Liu et al., 2019
					SPT	↑ % of sucrose consumption (stressed mice)		
DAPK1		↓	Injection in mPFC	UCMS rats	SPT	↑ % of sucrose consumption (stressed rats)	Yes	Li (S-X) et al., 2018
					FST	↓ immobility time (stressed rats)		
FGF9		↓	Injection in DG	WT rats	FST	No effect	No	Aurbach et al., 2015
					EPM	↑ % of time spent in open arms and ↓ % of time spent in the closed arms		
p47phox		↓	Injection in DG	RRS mice	TST	↓ immobility time (stressed mice)	No	Seo et al., 2012

					FST	↓ immobility time (stressed mice)		
	BDNF	↓	Injection in VTA	ISDS rats	SIT	↑ % of time spent in social interaction zone (stressed rats)	No	Fanous et al., 2011
					Bodyweight gain	↑ grams gain (stressed rats)		
<b>miRNA therapeutics</b>								
miRNA lentivirus	miR-124	↑	Injection in hippocampus	UCMS mice	SPT	↑ % of sucrose consumption (stressed mice)	No	Lou et al., 2019
					TST	↓ immobility time (stressed mice)		
					FST	↓ immobility time (stressed mice)		
					SIT	↑ interaction time (stressed mice)		
miRNA mimic	miR-363-3p	↑	Tail vein injection	MCAo rats	T maze cost/benefit task test	↑ % of high barrier choice (MCAo rats)	Yes	Panta et al., 2019
					SIT	↑ time spent in social interaction zone (MCAo rats)		
					FST	No ↑ immobility time after MCAo		
miRNA AAV	miR-124	↑	Injection in hippocampus	UCMS mice	SIT	↑ interaction time (stressed mice)	No	Higuchi et al., 2016
					NFST	↓ latency to feed (stressed mice)		
					SPT	↑ % of sucrose consumption (stressed mice)		
					FST	No effect		
siRNA lentivirus	miR-182	↓	Injection in hippocampus	UCMS rats	NFST	↓ latency to feed (stressed rats)	No	Li (Yuefeng) et al., 2016
					SPT	↑ % of sucrose consumption (stressed rats)		
					FST	↓ immobility time (stressed rats); ↑ swimming time (control and stressed rats)		

anti-miRNA	miR-16	↓	Injection in hippocampus	UCMS mice	FST	↓ immobility time (non-stressed mice)	No	Launay et al., 2011
					SPT	↑ % of sucrose consumption (stressed mice)		
					Bodyweight gain	↑ grams gain (stressed mice)		
					Coat state score	↓ Coat state score (stressed mice)		

Table 2: RNA therapeutics developed in pre-clinical studies using animal models for the treatment of mood disorders. ↓: reduced; ↑: increased; 5-HT<sub>1A</sub>R: 5-HT<sub>1A</sub> receptor; AAV: adeno-associated viral vector; ACC: anterior cingulate cortex; NAc: nucleus accumbens; ASO: antisense oligonucleotide; BDNF: brain derived neurotrophic factor; BLA: basolateral amygdala; CaMKII $\alpha$ : Calcium/calmodulin-dependent protein kinase type II subunit alpha; CDYL: chromodain Y-like protein; CSDS: chronic social defeat stress; D3V: dorsal third ventricle; DAPK1: death-associated protein kinase 1; DG: dentate gyrus; EPM: elevated plus maze; FGF9: fibroblast growth factor 9; FST: forced swim test; GSK3 $\beta$ : glycogen-synthase kinase 3 $\beta$ ; Hcr/Orx: hypocretin/orexin; i.c.v: intracerebroventricular injection; InsP3R: inositol-1,4,5-triphosphate receptor; ISDS: intermittent social defeat stress; MCH: melanin-concentrating hormone; MCAo: middle cerebral artery occlusion; miRNA, miR: micro-RNA; mPFC: medial prefrontal cortex; NFST: novelty-suppressed feeding test; PL: prelimbic cortex; RRS: repeated restraint stress; shRNA: short hairpin RNA; siRNA: small interfering RNA; SERT: serotonin transporter; SIT: social interaction test; SPT: sucrose preference test; TRH: thyrotropin-releasing hormone; TRHR1: TRH receptor-1; TST: tail suspension test; UCMS: unpredictable chronic mild stress; WT: wild-type.

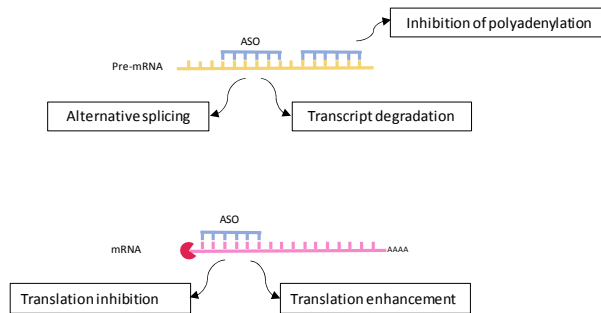
Type of molecule	Therapeutic target	Reference drug(s)	Effect on therapeutic target's expression in the investigated tissues	Exploration of locomotor activity	Reversibility of the effects	Toxicity of the RNA therapeutic	Reference
<b>ASO</b>	HuD	Amitriptyline	↓ in the hippocampus (WB)	Hole board test: no effect on spontaneous mobility and exploratory activity	Not explored	Not explored	Sanna et al., 2018
	HuB						
	HuC						
	InsP3R1	Amitriptyline, Clomipramine	↓ in the hippocampus, cortex and cerebellum (WB, IP and IHC)	Hole board test: no effect on spontaneous mobility and exploratory activity Rota road test: no effect on motor coordination	Yes: on target expression and FST	Signs of toxicity observed with higher doses of anti-InsP3Rs (e.g., tremors, convulsions, and death)	Galeotti et al., 2008
	InsP3R2						
	InsP3R3						
<b>siRNA</b>	SERT	Fluoxetine	↓ in the DR (ISH, autoradiography binding assay and IHC) and MnR (ISH and autoradiography binding assay)	Open field arena: no effect on the activation time, distance moved, slow/short and fast/large movement	Yes: on target expression	No neuronal degeneration, astrogliosis nor immune responses in the raphe nucleus after C-si-SERT i.n. administration	Ferrés-Coy et al., 2016
	Trh	None	↓ in the BLA (RT-qPCR and IHC)	No	Yes: on associated molecular effects and U-field test	Not explored	Choi et al., 2015
	Trhr1						
	Hcrt/Orx	None	↓ in the BLA (RT-qPCR)	U-field test: no effect on horizontal locomotions	Not explored	Not explored	Kim (T-K) et al., 2015
	MCH						
	CaMKIIα		Not demonstrated	No			
	5-HT1AR	Fluoxetine	↓ in raphe nuclei (ISH, autoradiography binding assay and RT-qPCR); no effect in mPFC and hippocampus (autoradiography binding assay)	No	Not explored	No inflammatory or apoptotic responses in midbrain raphe nuclei after C-1A-siRNA injection in D3V	Bortolozzi et al., 2012

	Neurabin	None	↓ in transfected primary cortical neuron culture (WB)	No	Not explored	Not explored	Kim (S) et al., 2011
<b>shRNA</b>	GSK3β	None	↓ in the MSNs of the NAc (single-cell RT-qPCR)	EPM: no effect on numbers of open and closed arm entrances	Not explored	Not explored	Aceto et al., 2020
	CDYL	None	↓ in the PL (WB)	SIT: no effect on distance travelled and total movement	Not explored	Not explored	Liu et al., 2019
	DAPK1	None	↓ in the mPFC (WB)	No	Not explored	Not explored	Li (S-X) et al., 2018
	FGF9	None	↓ in DG (ISH)	EPM: no effect on distance travelled	Not explored	Not explored	Aurbach et al., 2015
	p47phox	None	Indirect effect: no ↑ in the hippocampus of mice (RT-qPCR) after RRS protocol compared to before; no significant effect of the administration observed in the hippocampus of non-stressed mice	No	Not explored	Not explored	Seo et al., 2012
	BDNF	None	↓ in VTA (ELISA)	SIT: no effect on distance travelled	Yes: on target expression	Not explored	Fanous et al., 2011
<b>miRNA therapeutics</b>							
miRNA lentivirus	miR-124	None	↑ in the hippocampus (RT-qPCR)	No	Not explored	Not explored	Lou et al., 2019
miRNA mimic	miR-363-3p	None	Not demonstrated	Open field arena chamber: no effect on the number of beam breaks	Not explored	Not explored	Panta et al., 2019
miRNA AAV	miR-124	None	↑ in the hippocampus (NB)	No	Not explored	Not explored	Higuchi et al., 2016
siRNA lentivirus	miR-182	None	↓ in the hippocampus (RT-qPCR)	No	Not explored	Not explored	Li (Yuefeng) et al., 2016
anti-miRNA	miR-16	Fluoxetine	Not demonstrated	No	Not explored	Not explored	Launay et al., 2011

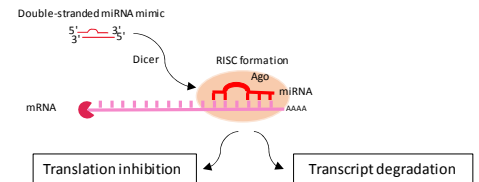
Table 3: Controls in pre-clinical studies using RNA therapeutics on animal models for the treatment of mood disorders. 5-HT<sub>1A</sub>R: 5-HT<sub>1A</sub> receptor; AAV: adeno-associated viral vector; ASO: antisense oligonucleotide; BLA: basolateral amygdala; BDNF: brain derived neurotrophic factor; C-1A-siRNA: sertraline-conjugated siRNA directed against 5-HT<sub>1A</sub> receptor; C-si-SERT: sertraline-conjugated siRNA directed against SERT; CaMKII $\alpha$ : Calcium/calmodulin-dependent protein kinase type II subunit alpha; CDYL: chromodain Y-like protein; DAPK1: death-associated protein kinase 1; D3V: dorsal third ventricle; DG: dentate gyrus; DR: dorsal raphe nucleus; ELISA: enzyme linked immunosorbent assay; EPM: elevated plus maze; FGF9: fibroblast growth factor 9; GSK3 $\beta$ : glycogen-synthase kinase 3 $\beta$ ; Hcr/Orx: hypocretin/orexin; IHC: immunohistochemistry; i.n.: intranasal; InsP3R: inositol-1,4,5-triphosphate receptor; IP: immunoprecipitation; ISH: *in situ* hybridization; MCH: melanin-concentrating hormone; miRNA, miR: micro-RNA; MnR: median raphe nucleus; mPFC: medial prefrontal cortex; NAc: nucleus accumbens; NB: Northern Blot; PL: prelimbic cortex; RRS: repeated restraint stress; RT-qPCR: reverse transcription-polymerase chain reaction; shRNA: short hairpin RNA; siRNA: small interfering RNA; SERT: serotonin transporter; SIT: social interaction test; TRH: thyrotropin-releasing hormone; TRHR1: TRH receptor-1; VTA: ventral tegmental area; WB: Western Blot.



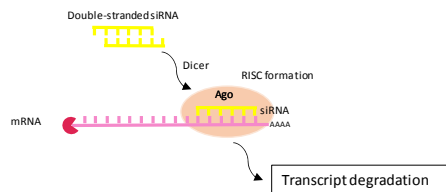
### 1. ASOs



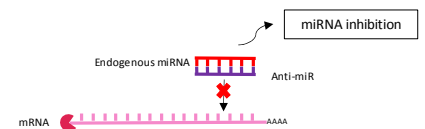
### 4. miRNA mimics



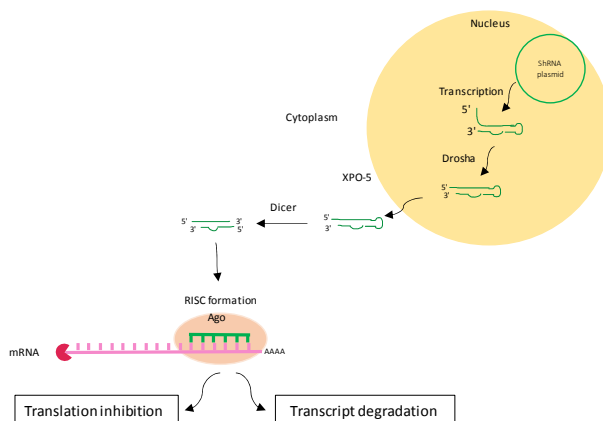
### 2. siRNAs



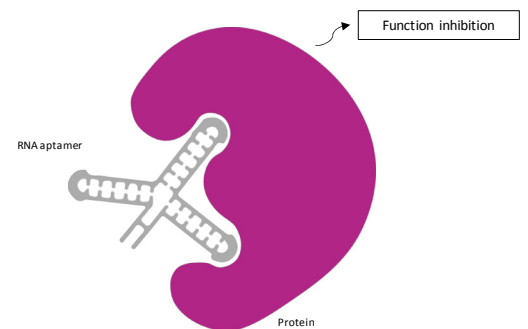
### 5. Anti-miRs



### 3. shRNAs



### 6. RNA aptamers



## Figures

Figure 1. Mechanisms of action of RNA therapeutics. Ago: Argonaute protein; ASOs: antisense oligonucleotides; anti-miRs: anti-micro-RNAs; miRNAs: micro-RNAs; mRNA: mature RNA transcript; pre-mRNA: pre-mature RNA transcript; RISC: RNA-induced

silencing complex; siRNAs: small interfering RNAs; shRNAs: short hairpin RNAs; XPO-5: exportin-5.

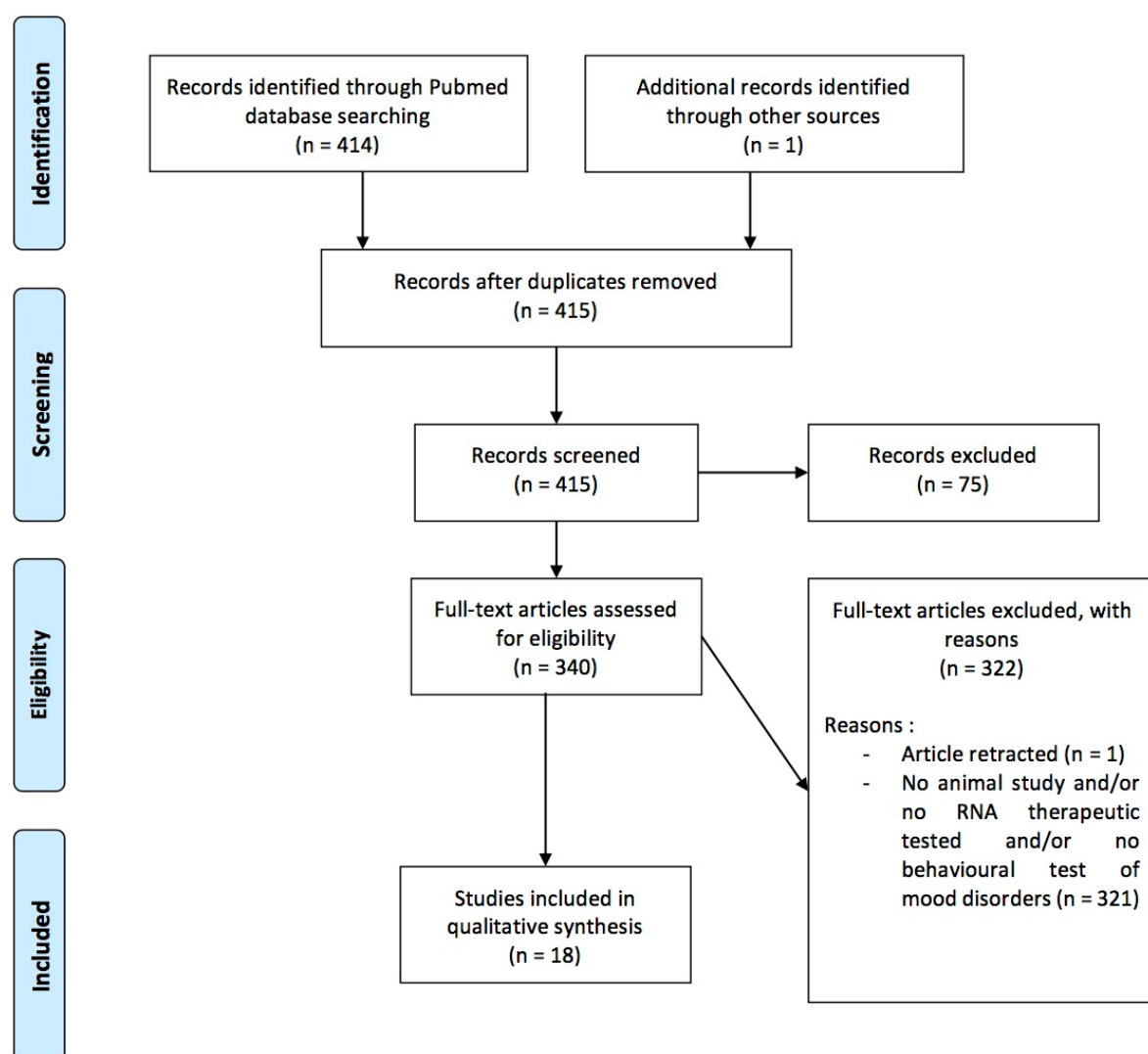


Figure 2. PRISMA flow diagram for pre-clinical study selection.

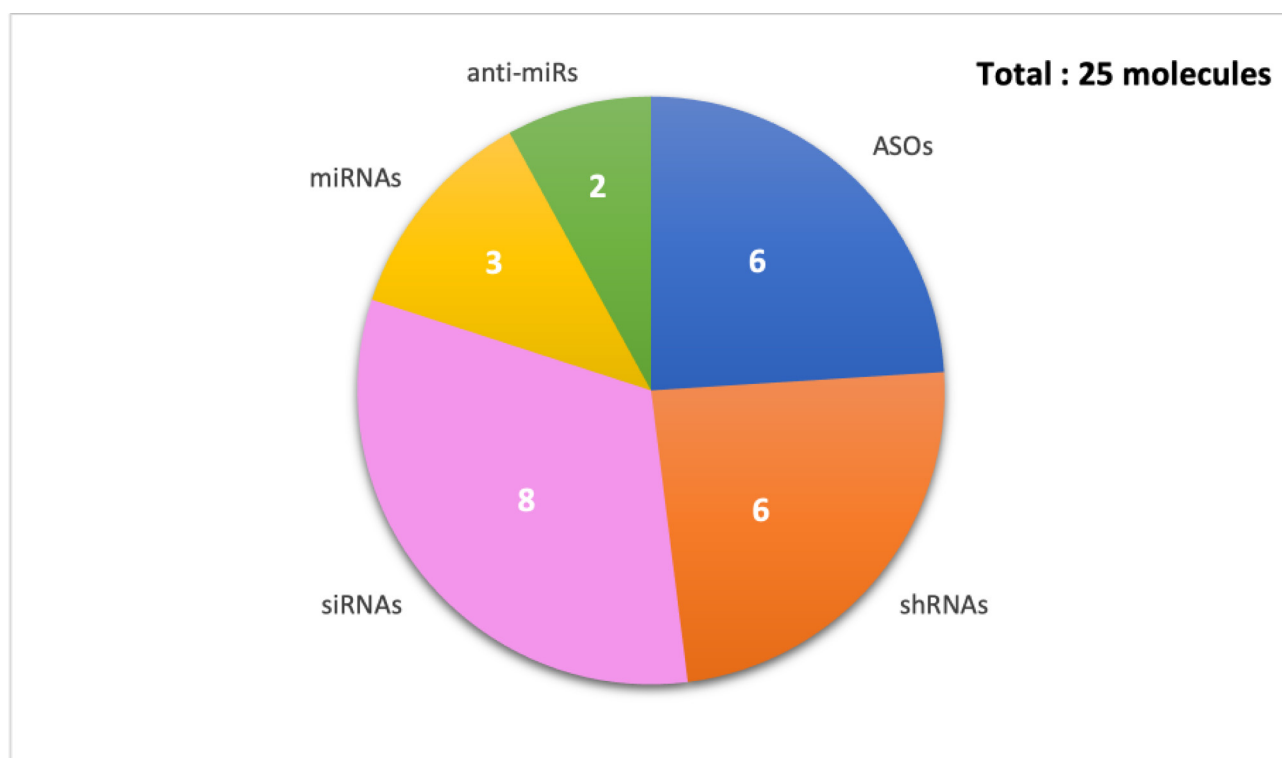
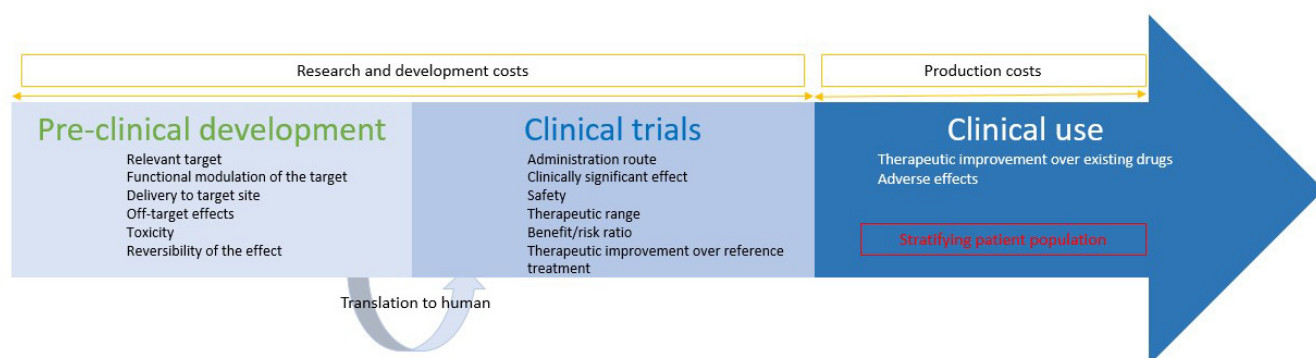


Figure 3. Graphic repartition of the number of selected pre-clinical studies for each RNA therapeutic type. ASOs: antisense oligonucleotides; anti-miRs: anti-micro-RNAs; miRNAs:



micro-RNAs; siRNAs: small interfering RNAs; shRNAs: short hairpin RNAs.

Figure 4. Schematic overview of major challenges for the development of RNA therapeutics.