Small Molecule Drugs Targeting Non-Coding RNAs as Treatments for Alzheimer’s Disease and Related Dementias

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Abstract: Despite the enormous burden of Alzheimer’s disease and related dementias (ADRD) on patients, caregivers, and society, only a few treatments with limited efficacy are currently available. While drug development conventionally focuses on disease-associated proteins, RNA has recently been shown to be druggable for therapeutic purposes as well. Approximately 70% of the human genome is transcribed into non-protein-coding RNAs (ncRNAs) such as microRNAs, long ncRNAs, and circular RNAs, which can adopt diverse structures and cellular functions. Many ncRNAs are specifically enriched in the central nervous system, and their dysregulation is implicated in ADRD pathogenesis, making them attractive therapeutic targets. In this review, we first detail why targeting ncRNAs with small molecules is a promising therapeutic strategy for ADRD. We then outline the process from discovery to validation of small molecules targeting ncRNAs in preclinical studies, with special emphasis on primary high-throughput screens for identifying lead compounds. Screening strategies for specific ncRNAs will also be included as examples. Key challenges—including selecting appropriate ncRNA targets, lack of specificity of small molecules, and general low success rate of neurological drugs and how they may be overcome—will be discussed throughout the review.

Keywords: non-coding RNA; Alzheimer’s disease and related dementias; small molecules; drug discovery; high-throughput screens; miRNA; lncRNA; circRNA

1. Introduction

1.1. ncRNAs as Novel Therapeutic Targets for Treating ADRD

Dementia, the progressive decline in memory and cognitive function, is a major public health problem that affects 40 to 50 million people worldwide [1]. Alzheimer’s disease (AD) is the most common dementia diagnosis and is characterized by the accumulation of Aβ plaques and tau neurofibrillary tangles. In addition to classic AD pathology, a majority of dementia cases also exhibit distinct histologic changes and are further classified as frontotemporal dementia (FTD), Lewy body dementia (LBD), vascular contributions to cognitive impairment and dementia (VCID), and multiple etiology dementias (MED) [2]. Collectively known as Alzheimer’s disease and related dementias (ADRD), they exert devastating burdens on patients, caregivers, and society. Yet, there is currently no effective, disease-modifying treatment for ADRD. The five drugs approved for treating ADRD by the US Food and Drug Administration (FDA), including donepezil, galantamine, rivastigmine (cholinesterase inhibitors), memantine (N-methyl-D-aspartate receptor antagonist), and Aducanumab (Aβ monoclonal antibody), only have modest clinical benefits [3]. Discovering new, more effective therapies for ADRD will, undoubtedly, require innovation on many fronts.

RNAs have been extensively studied for their roles in protein biogenesis. Messenger RNAs (mRNAs) are the intermediary codes between DNA and proteins. Other housekeeping RNAs, including ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), small nucleolar RNAs (snoRNAs), and small nuclear RNAs (snRNAs), participate in modifying and translating mRNAs into proteins. However, recent advances in RNA capturing, sequencing,
and annotation have revealed new classes of regulatory non-coding RNAs (ncRNAs) that further enrich the complexity of gene regulations. Whereas 1.5–2% of the human genome is transcribed into mRNAs, ~70% of the genome is pervasively transcribed into ncRNAs [4]. These ncRNAs are broadly divided into small ncRNAs (snRNAs, <200 nucleotides), including microRNAs (miRNAs) and Piwi-interacting RNAs (piRNAs), and long ncRNAs (lncRNAs, >200 nucleotides), including linear and circular lncRNAs (circRNAs). Table 1 includes some classes of ncRNAs relevant to this review. Physiologically, and particularly in the central nervous system (CNS), ncRNAs play diverse and critical roles in cell division, migration, metabolism, neuronal plasticity, and cell death, through regulating genome folding, gene modifications, transcription, protein synthesis, and scaffolding of protein complexes (for review, see [5]). Notably, many ncRNAs are highly enriched in specific brain regions or cell types [6,7] and are only found in primates and humans [8], supporting the hypothesis that ncRNAs accelerate primate brain evolution and the acquisition of higher-order cognition [9]. Correspondingly, the dysregulation of various ncRNAs contributes to neurodegenerative diseases (for review, see [10]). As such, ncRNAs constitute a large, attractive, and still underexplored pool of possible therapeutic targets for treating ADRD.

### Table 1. Different classes of ncRNAs.

| Category                                      | Description                                                                                                                                                                                                 |
|-----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **Small non-coding RNAs:** snRNAs of <200 nucleotides. | MicroRNAs: ncRNAs of 18–25 nucleotides that facilitate the degradation or inhibit the translation of mRNA targets through imperfect complementary base pairings.  
Piwi-interacting RNAs: ncRNAs of 26–31 nucleotides that facilitate the silencing of transposons in germline cells, may also have other functions in somatic cells.  
tRNA-derived small RNAs: tRNA-derived fragments of 14–30 nucleotides and tRNA halves of 30–50 nucleotides produced from precursor or mature tRNAs. Emerging evidence suggests that they function as signaling molecules in stress responses and as regulators of gene expression. |
| **Long non-coding RNAs (lncRNAs):** ncRNAs of >200 nucleotides, with largely elusive functions. They can be further classified by the genomic loci from which they are transcribed. | Intergenic lncRNAs: lncRNAs transcribed from regions not overlapping with protein-coding genes.  
Intronic lncRNAs: lncRNAs produced from introns of protein-coding genes.  
Sense-overlapping lncRNAs: lncRNAs transcribed from regions overlapping with introns and exons of protein-coding genes.  
Bidirectional lncRNAs: lncRNAs transcribed from the same promoters of protein-coding genes, but in the opposite direction.  
Antisense lncRNAs: lncRNAs transcribed from the antisense RNA strands of protein-coding genes.  
Enhancer RNAs: lncRNAs transcribed from genomic enhancer regions.  
Circular RNAs: Closed single-stranded lncRNAs produced by back-splicing, in which the 5’ and 3’ termini of linear RNAs are covalently joined by spliceosome-mediated splicing. Most known circRNAs are transcribed from protein-coding genes.  
Pseudogene transcripts: RNAs transcribed from DNA sequences that resemble protein-coding genes but lack the ability to produce functional proteins. These transcripts may be processed into siRNAs or function as endogenous miRNA sponges. |

1.2. Small Molecule Drugs for Modulation of ncRNAs

Extensive work has gone into developing synthetic oligonucleotides to target mRNAs and ncRNAs, including antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), miRNA mimics and inhibitors, and others (for review, see [11]). However, major hurdles concerning immunogenicity, specificity, and delivery persist [11]. Particularly, delivery of oligonucleotides to tissues other than the liver remains poor, and the brain is further protected by the blood-brain barrier (BBB), which blocks the entry of many compounds [12]. Alternatively, gene therapy utilizing viral vectors can supplement,
knockdown, or edit ncRNAs [13]. While potentially allowing for more durable ncRNA modulation, gene therapy also comes with major challenges regarding immunogenicity, delivery, transgene expression in off-target cell populations, and off-target genome editing [13].

In parallel to advancing oligonucleotide and gene therapies for the CNS [13–15], small molecules comprise another approach to target ncRNAs. Small molecules typically have low molecular weights (usually <1 kDa) and are administered orally for distribution throughout the body. Their small size, negligible charge, and hydrophobicity allow them to pass the cell membrane and bind to intracellular targets to exert systemic effects [16]. For example, ibuprofen, a non-steroidal anti-inflammatory drug, can readily cross the BBB [17]. In addition to better, “drug-like”, pharmacokinetics and pharmacodynamics (PK/PD), small molecules are typically easier and cheaper to synthesize and modify than synthetic oligonucleotides and viral vectors. Furthermore, as many small molecules are already on the market or in clinical trials with known safety and toxicity profiles, those with newly discovered ncRNA-targeting properties can be repurposed, therefore saving critical time and costs [18].

While targeting disease-relevant proteins with small molecules has long been an integral part of drug discovery, targeting RNAs, and specifically ncRNAs, is an emerging field, with standard rules remaining to be established [4]. Compared to proteins, RNAs have limited chemical diversity, are highly negatively charged, and possess few hydrophobic pockets for conventional drug binding [4,19]. Yet, RNAs can fold into secondary, tertiary, and quaternary structures, with internal loops, bulges, hairpins, and triple helices providing potential binding sites for small molecules [19]. For example, bacterial riboswitches are naturally-occurring RNA regulatory segments that bind metabolites to regulate transcription [19]. In 2020, risdiplam, a small molecule, was the first direct RNA-splicing modifier approved by the FDA for treating spinal muscular atrophy (SMA) [19,20]. Risdiplam directly binds the survival of the motor neuron 2 (SMN2) mRNA-spliceosome complex to facilitate exon 7 inclusion and increase SMN protein expression. Small molecules binding to ncRNAs has also been identified as a potential treatment for bacterial infections and cancer [21–24]. Furthermore, small molecules may regulate ncRNAs without direct binding, for example, by influencing their biogenesis or protein partners as described in Section 2.

In the next section, we will discuss several high-throughput screens (HTS) for discovering small molecules that target ncRNAs and outline the discovery and validation process and their limitations. Special consideration for targeting the CNS for treating ADRD will also be included as appropriate.

2. Discovery and Validation of Small Molecules Targeting ncRNAs

A small molecule may regulate an ncRNA by binding directly to the ncRNA to change its conformation; or by initiating a signaling cascade that alters its biogenesis, splicing, function, or stability; or by interfering with the formation of the ncRNA-protein complex. Methods utilized to discover drugs that target proteins have been repurposed for targeting RNAs with varying degrees of success. So far, most studies have focused on small molecules that target bacterial or viral RNAs or ncRNAs in cancer [20]. Small molecules that bind to and modify the splicing of pre-mRNAs (for example, SMN2 or microtubule associated protein tau (MAPT)) have also been investigated [25–27]. However, we are not aware of any study that uses small molecules to target ncRNAs in neurodegenerative diseases. We posit that small molecules that target ADRD-relevant ncRNAs can be discovered using similar methods (Figure 1). However, more so than other diseases, small molecules that target the CNS and potentially require chronic administration need to satisfy additional stringent criteria, including BBB permeability, acceptable general and brain-specific PK/PD, high specificity, and low toxicity.
Figure 1. Methods for discovering small molecules modulating the expression level and activity of ncRNAs. (A) In the cell-based reporter assays, a fluorescent or luminescent reporter is used to measure ncRNA-expression level or activity: (i) if the reporter is under the control of a ncRNA promoter region, small molecules that modulate the reporter may transcriptionally modulate the ncRNA; (ii) if the reporter gene is under the control of a region interacting with an ncRNA, small molecules that modulate the reporter may regulate the expression level or activity of the ncRNA; (iii) for variants of an ncRNA produced from alternative splicing, small molecules that modulate the in-frame expression of the reporter gene may regulate alternative splicing to favor the production of a specific variant. (B) Various biochemical assays can be used to discover small molecules that bind directly to ncRNAs to modulate their configuration and function: (i) in the small molecule microarray assay, small molecules are immobilized on glass slides and then incubated with fluorophore-bound ncRNAs. After unbound ncRNAs are washed away, fluorescence signals may indicate binding between a small molecule and the ncRNA; (ii) in the fluorescent indicator displacement (FID) assay, the ncRNA is first reversibly attached to a fluorescent indicator. Small molecules that bind to and displace the indicator result in a loss of fluorescent signal; (iii) in the automated ligand identification system (ALIS), the ncRNA of interest is first incubated with various small molecules. RNA-small molecule complexes are then separated from unbound RNAs and small molecules by size-exclusion chromatography, followed by treatment under harsh conditions to release the small molecules from the complexes. The small molecules are then identified by liquid chromatography-mass spectrometry (LC-MS); (iv) computational methods can also screen millions of small molecules to predict those that are more likely to bind to a particular ncRNA. The Inforna platform utilizes known RNA motif–small molecule interactions to predict compounds for specific RNA secondary structures. RNA structures produced by X-ray crystallography or nuclear magnetic resonance (NMR) can also be used to facilitate small molecule–RNA docking. (C) Several assays can also be used to discover small molecules that modulate RNA-protein interaction: (i) in the fluorescence resonance energy transfer (FRET) assay, the protein can be tagged with a fluorophore, whereas the ncRNA is tagged with either an activator or a quencher; (ii) in the catalytic enzyme-linked click chemistry assay (cat-ELCCA), the protein is immobilized, while the RNA is tagged with a handle such as a 5′-trans-cyclooctene (TCO) that can be converted into horseradish peroxidase (HRP) using click chemistry; (iii) in the fluorescence polarization assay, small molecules bound to proteins rotate more slowly than unbound molecules, resulting in more highly polarized light emission. Small molecules that modulate the fluorescent, or luminescent, or polarized light intensity in these assays may also modulate ncRNA-protein interaction. (D) High throughput ncRNA-seq can be used to directly identify ncRNAs that are differentially expressed in cellular models treated with small molecules. This approach also enables the construction of ncRNA networks altered by a particular small molecule.
2.1. Selection of Compound Libraries and Cellular or Animal Models

The distinct advantage of small molecules over synthetic oligonucleotides or viral vectors is the wealth of knowledge, the variety of compounds, and the relatively low cost of synthesis and modification. For example, the ZINC15 database lists 230 million purchasable small molecules (as of November 2021), with each compound annotated for its chemical property, biological activity, and commercial availability [28]. The selection of the size and diversity of a screening library relies on various factors, including cost, quality, availability, chemical diversity, assay sensitivity, single or multiple concentrations, and estimated hit rate, as well as specific properties of the ncRNA target [29]. To maximize potential novel discoveries, large libraries (>50,000) of compounds of diverse chemical structures can be utilized. However, it should be noted that existing libraries are still optimized for protein binding, not RNA binding [19]. For example, a recent screen for direct binders using the Merck Diversity Library (~50,000 compounds) has an average hit rate of 0.05% for proteins and 0.01% for RNAs, while the Functionally Annotated Library (~5100 compounds) has an average hit rate of 1.54% for proteins and 0.04% for RNAs [23]. Alternatively, more focused specialty libraries can be utilized, including ZINC15’s substances active in cells (~34,000 compounds), Selleck’s Approved Drug Library (~3000 compounds), Sigma-Aldrich’s Library of Pharmacologically Active Compounds (LOPAC, 1280 compounds), or a recently curated list of BBB-permeable compounds (~5000 compounds, [30]). In addition, libraries of chemical fragments (<300 Da) can be used to detect fragments with mM binding affinity to guide syntheses of higher-affinity compounds [29]. Finally, while computational predictions of small molecule-RNA binding remain to be improved, they can be used to narrow down millions of compounds to a few thousand for experimental validation.

Few drugs discovered through academic preclinical research advance to human clinical testing, and, of those, the vast majority fail at various stages. Between 2010 and 2017, only 7% of new drugs that entered phase I clinical trials eventually made it to the market, with drugs targeting the nervous system having the lowest success rate, at only 3% [31]. Particularly, the success rate for disease-modifying treatments for ADRD is still 0%. Selecting the appropriate cellular and animal models, either in the primary screen or in follow-up validation studies, is critical for reducing the failure rate. For example, most cell-based screens still utilize a single immortalized cell line grown in media designed for rapid growth and on artificial 2D substrates [32]. In contrast, neurons are primarily non-dividing and grow in complex 3D environments with other neurons, astrocytes, oligodendrocytes, and microglia from diverse lineages. As such, more relevant cellular models for screening or validation include primary or induced pluripotent stem cell (iPSC)-derived neuronal and glial cells, organotypic brain slices, and brain organoids [32]. However, such models are also more heterogenous and challenging for screen design, and so far, they are rarely utilized in HTSs. Specific neuronal-like cell lines with neurodifferentiation potentials, such as SH-SY5Y cells, may be considered a compromise between technical feasibility and disease relevance. Subsequently, the most promising small molecules need to be tested in animal models to establish brain PK/PD and efficacy at the molecular, cellular, and behavioral levels. Determining adequate replication and group sizes, potential sex-specific effects, evidence of target engagement, treatment timing, and testing in multiple disease-relevant models is critical for enhancing reproducibility and translatability to clinical studies [33].

2.2. Cell-Based Reporter Assays for Discovering Regulators of ncRNAs

Most regulatory ncRNAs, including many miRNAs [34], antisense transcripts (AS), and IncRNAs [35], are transcribed similarly to mRNAs by RNA polymerase II. circRNAs are produced from the processing of pre-mRNAs [35]. Therefore, similar to mRNAs, these ncRNAs are regulated by mechanisms involving promoters, transcription factors, enhancers, and suppressors. International collaborative efforts, including the Encyclopedia of DNA Elements (ENCODE) [36] and the Functional Annotation of the Mammalian Genome (FANTOM) [37] consortia, have extensively annotated regulatory elements of
the genomes of various species, allowing for the prediction of regulatory regions of ncRNAs. For example, the promoters of 1357 human and 804 mouse miRNAs were recently annotated [38]. In promoter-driven screens (Figure 1A(i)), the promoter of an ncRNA of interest can be cloned into plasmids to control the expression level of a fluorescent or luminescent reporter. Upon the addition of small molecules, the intensity of the reporter is used as a readout for promoter activity—with lower intensity indicating reduced activity and vice versa. A recent screen of 52,041 compounds identified 739 compounds that decreased huntingtin (HTT) promoter-driven luciferase activity and 52 that increased HTT-AS promoter-driven luciferase activity in HEK293 cells [39]. Seventeen of those compounds were shown to reduce HTT protein levels but at concentrations also toxic to cortical-like neurons differentiated from iPSCs from Huntington’s disease patients.

The reporter assay can also be used to measure ncRNA activity by placing the reporter gene under the control of the region interacting with the ncRNA of interest, for example, the 3′ untranslated region containing miRNA binding sites (Figure 1A(ii)). The intensity of the reporter is then used as the readout for ncRNA activity. Such screens have been used to identify small molecules that modify the activity of miR-21 [40–42], miR-34a [43], and miR-122 [44] in cancer cells. Alternatively, a reporter assay can be used to screen for RNA splicing modifiers similar to risdiplam. Splicing is important for producing variants of ncRNAs with distinct functions, including variants that gain or lose coding potentials [45]. In particular, the production of circRNAs requires the back-splicing of linear transcripts [35,45]. In the splicing reporter assay, the inclusion or exclusion of an exon results in the in-frame expression of the reporter gene, allowing for the identification of compounds that regulate the splicing process (Figure 1A(iii)). A reporter system was recently used to identify molecular elements critical for back-splicing [46], which may be repurposed to screen for small molecules that promote circRNA expression. Additionally, with the knowledge of the regulatory sequences of ncRNAs, the putative transcription factors that regulate the ncRNAs can be predicted. For example, the TransmiR database lists the possible transcription factors for many miRNAs based on published studies, chromatin immunoprecipitation—RNA-seq (ChIP-seq) data, and predicted binding motifs [47]. Knowing the pathways that regulate these transcription factors, in turn allows for the selection of specific classes of small molecules for smaller-scale, targeted screening.

A possible challenge for cell-based reporter assays is the technical difficulty of obtaining uniform transfection efficiency and expression level of the reporter gene in miniaturized formats, especially for heterogeneous systems such as primary cultures, organotypic slices, and brain organoids. For example, compared to immortalized cell lines, primary neurons have lower transfection efficiency and show reduced survival or viability following transfection [48]. Reporter signals may also be confounded by small molecules that directly inhibit or enhance the reporters, for example, small molecules that inhibit luciferase [49]. In addition, the mechanism of actions (MoAs) of positive hits needs to be determined. Ideally, the MoAs of positive hits should be novel or related to disease-relevant pathways [50]. However, the hits may also have non-specific MoAs or MoAs resulting in toxic effects downstream.

2.3. Biochemical Assays for Discovering Direct Binders of ncRNAs

Much work has been invested in identifying direct binders of ncRNAs, inspired by decades of research for small molecules that bind proteins. Recent biochemical assays and computational predictions have successfully identified small molecules that directly bind ncRNAs to modify their life cycles and functions. Depending on the screening methods, the compound libraries, the target ncRNAs, and the selection criteria, between 0.01–9% of compounds tested were identified as positive hits from the primary HTSs [20,23].

In the small molecule microarray assay (Figure 1B(i)), small molecules are spatially arrayed and covalently bound to glass slides and then incubated with the fluorescently labeled RNA of interest [51]. After washing, glass slides are imaged with a fluorescence scanner to identify positive hits, for example, compounds that bind to and modulate riboswitch
activity [52]. In the fluorescent indicator displacement (FID) assay (Figure 1B(ii)) [53], the 
RNA target is first incubated with a fluorescent indicator that reversibly binds RNA, fol-
lowed by incubation with a library of small molecules. Compounds that bind to the RNA 
and displace the fluorescent indicator result in the loss of fluorescence signal. However, 
compounds that bind the RNA without displacing the indicator may not be detected as pos-
itive hits. FID using SYBR Green II as the indicator was employed to compare the binding 
efficiency of compounds that bind the lncRNA metastasis-associated lung adenocarcinoma 
transcript 1 (MALAT1) triple helix motif [21]. In the automated ligand identification system 
(ALIS) (Figure 1B(iii)), [23], RNA-small molecule complexes are first separated from un-
bound components by size-exclusion chromatography. Small molecules are then released 
from the complexes and identified by liquid chromatography-mass spectrometry (LC-MS). 
A recent screen of 42 RNAs of diverse classes against 50,000 small molecules identified 
direct binders for 40 RNAs, with an average hit rate of 0.01% per RNA [23]. Notably, 
30 RNAs had one or more selective binder that did not bind any other RNA in the screen. 
The discovery of direct RNA-small molecule interactions has also provided the basis 
for bioinformatics tools that facilitate in silico predictions of novel small molecules that 
bind to specific RNAs sequences (Figure 1B(iv)). For example, the Informa platform incor-
porates known RNA motif–small molecule interaction to predict lead compounds for RNA 
secondary structures [54]. Informa was used to identify lead compounds that target miR-96, 
which were optimized into TargaprimiR-96, which binds pri-miR-96 to inhibit its processing 
into the oncogenic mature form [24]. Deeper analyses of known RNA-small molecule inter-
actions further suggest that these compounds tend to contain aromatic amine-containing 
heterocycles or amidine-like motifs conducive for stacking and hydrogen-bonding interac-
tions with their RNA targets [23,55]. Additionally, protein crystal structures obtained from 
X-ray diffraction and deposited into the Protein Data Bank (PDB) allow for computatio-
ナル docking of millions of small molecules to facilitate structure-based drug discovery [56]. 
The PDB also contains crystal structures of RNA-small molecule complexes (PDB ID: 3C44, 
HIV-1 extended duplex RNA bound to paromomycin HIV-1 [57]; PDB ID: 4QLM, ydaO 
riboswitch bound to c-di-AMP [58]). Nevertheless, compared to proteins, RNAs are often 
more dynamic with more disordered regions, making them harder to crystalize [19]. For 
short and disordered RNAs, nuclear magnetic resonance (NMR) spectroscopy utilizes 
magnetic fields to determine their structures and possible conformational changes induced 
upon binding to small molecules (PDB ID: 5UZT, pre-miR21 structure [59]). The increase in 
the number of solved and deposited RNA-small molecule complexes is expected to improve 
in silico predictions. Both X-ray diffraction and NMR can also be used to validate direct 
RNA-small molecule interaction of positive hits from the primary screen. Other valida-
tion methods include surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), 
cryoEM, and chemical probing (for review, see [19]). In reverse, Chemical Cross-Linking 
and Isolation by Pull-down to Map Small Molecule–RNA Binding Sites (Chem-CLIP-Map-
Seq) can be used to confirm the specificity of RNA-small molecule interaction [60]. Briefly, 
a small molecule conjugated to beads is crosslinked to its RNA targets, which are then 
identified through RNA-seq, therefore providing information about the specificity of the 
interaction between the small molecule and the RNA of interest. 

While selective RNA binders are expected to act more specifically than indirect regula-
tors, a limitation is that the binding of small molecules to ncRNAs almost always inhibits 
their functions, either by blocking their maturation [24], inducing their degradation by 
endogenous RNases, or interfering with their structure or activity [61] (for review, see [62]). 
On the other hand, we have not found studies of small molecules that stabilize and enhance 
the functions of ncRNAs through direct binding, necessitating further studies. In addition, 
as most regulatory ncRNAs are expressed at much lower levels than rRNA, tRNAs, and 
snRNAs [63], direct small molecule binders may be sequestered away by the more abun-
dant RNA species even if the latter has a much lower binding affinity, therefore requiring 
higher concentrations for the desired effect.
2.4. Assays for Discovering Small Molecules Modulating RNA–Protein Interaction

The life cycle and functions of ncRNAs are intertwined with their protein partners. For example, miRNA maturation and export involves Drosha, DiGeorge critical region 8 (DGCR8), Exportin 5 (Exp5), Dicer, and the transactivating response RNA-binding protein (TRBP) [34]. miRNAs’ function then requires loading the guide miRNA strand into the RNA-induced silencing complex (RISC) composed of Argonaute. Other proteins bind to and regulate more specific clusters of miRNAs (see [34] for review). For example, Lin28 binds and negatively regulates the let-7 family, miR-9, miR-107, and miR-143. The lncRNA HOX antisense intergenic RNA (HOTAIR) recruits proteins in the polycomb repressive complex 2 (PRC2) to catalyze H3K27me3 markers to modulate transcription. Interfering with the interaction between ncRNAs and their RNA binding proteins (RBPs) may also be a therapeutic strategy to modulate the abundance and functions of ncRNAs.

Various fluorescence- or chemiluminescent-based methods can be used to detect small molecules that interfere with RNA–RBP interaction [64]. In the fluorescence resonance energy transfer (FRET) assay (Figure 1C(i)), the RBP can be tagged with a fluorophore, and the RNA of interest can be tagged with either a quencher or an activator. Second, in the catalytic enzyme-linked click chemistry assay (cat-ELCCA) (Figure 1C(ii)), the protein is immobilized, whereas the RNA is tagged with a handle. Using click chemistry, the RNA is then conjugated with horseradish peroxidase (HRP), allowing for chemiluminescent detection upon the addition of an HRP substrate. Third, in the fluorescence polarization (FP) assay (Figure 1C(iii)), the RNA is tagged with a fluorophore that rotates rapidly in its unbound state. The binding of RNA to a larger RBP reduces the rotation rate, resulting in increased polarized light emission. In these assays, small molecules that change fluorescent or chemiluminescent intensity, or polarized light emission, are candidate modulators of RNA–RBP interaction. FRET [65], FP [66], and cat-ELCCA [67] assays were used successfully to identify small molecules that disrupt Lin28/let-7 interaction.

Small molecules that interfere with RNA-protein binding can do so either by binding to the RNA or the protein. Notably, the three studies that discovered small molecules disrupting Lin28/let-7 interaction also demonstrated that these compounds bound to Lin28, not let-7 [65–67]. However, small molecules that bind to proteins with ubiquitous functions may act non-specifically. For example, small molecules that bind Dicer [68] or Argonaute [68,69] likely affect general miRNA activity, leading to non-specific downstream effects.

2.5. RNA-seq for Discovering Small Molecules That Modulate ncRNA Levels and Networks

The various methods described above focus on examining a single ncRNA or a small group of closely related ncRNAs. Applying RNA-seq to discover drugs targeting ncRNAs promises comprehensive, unbiased data on many ncRNAs. Furthermore, sequencing does not rely on artificial reporters and can be more easily used for neuronal cells that are difficult to transfect with reporter constructs. However, besides cost concerns, compared to mRNA-seq, ncRNA-seq, particularly for lncRNA, remains more challenging because of their low abundance, lack of evolutionary conservation, and incomplete genomic annotations [70]. While methods are being developed for more accurate transcriptomic studies of snRNAs, lncRNAs, and circRNAs [71], and a mouse tissue atlas of sncRNAs was recently published [72], we are not aware of any ncRNA-seq-based efforts for small molecule discovery specifically. Nevertheless, recent advances in next-generation sequencing have made it cheaper and faster to sequence complete sets of RNA transcripts in HTS drug-discovery platforms. For example, the Digital RNA with pertUrbation of Genes (DRUG-seq) platform was used to profile 433 compounds across 8 doses at ~$2–4 per sample [73]. More recently, targeted organoid sequencing (TORNADO-seq) was used to profile the effects of 320 compounds on 206 genes in wildtype and tumor intestinal organoids at $5 per sample [74]. These new developments suggest that ncRNA-seq for small molecule drug discovery will become more feasible in the near future.
In addition, compared to synthetic oligonucleotides, which can recognize specific target sequences through complementary base pairing, small molecules are more likely to act non-specifically. Beyond the ncRNA of interest, small molecules probably perturb other mRNAs and ncRNAs, potentially leading to adverse effects. The adverse effects of some drugs, for example, cognitive impairment following chemotherapy [75], may be outweighed by their clinical benefits or may be addressed by the post-hoc development of combination therapy counteracting the side effects. As such, knowing how small molecules affect pathways other than the intended targets can help predict and alleviate adverse effects before entering human clinical testing. Notably, microarray gene expression data was used to predict adverse drug effects, therefore informing go/no-go decisions in the early stage of drug discovery [76], suggesting that RNA-seq data can not only be used for the primary HTSs but also for subsequent functional and specificity validation assays.

2.6. Validation and Optimization of Candidate Small Molecules from Primary HTSs

Figure 2 outlines the process from discovering small molecule candidates to validation assays, then to obtaining data that may support progress to human clinical trials. Ideally, the initial screen of several thousands of small molecules identifies a few dozen to hundreds of small molecules as promising hits. Compounds that generate false positives such as pan-assay interference (PAIN) compounds [77], non-specific DNA/ RNA intercalators [20], and luciferase inhibitors [78] should be considered critically and excluded using orthogonal assays at the early stage of the pipeline. Subsequently, the remaining candidate compounds can be resynthesized and validated through a battery of functional assays, further optimized, and then tested in animal models to obtain preclinical data.

Briefly, for the validation process, the expected MoA between a small molecule and an ADRD-related ncRNA should first be verified. For direct binders, the binding site between a small molecule and an ncRNA can be determined by X-ray crystallography, cryoEM, or NMR, while the stoichiometry and thermodynamic properties of the interaction can be determined using ITC and SPR. Chem-CLIP-Map-Seq can also be used to determine if the small molecule may interact with other structurally similar RNAs. For non-binders, the mechanism of ncRNA modulation should be determined, for example, whether the small molecule transcriptionally modulates the ncRNA or whether it promotes the processing of the ncRNA. Next, to determine the specificity of the small molecule, RNA-seq comparing cellular models treated with the small molecule or with relevant oligonucleotides should be performed, as oligonucleotides are often more specific than small molecules. Most importantly, the small molecule should show desirable functional effects, for example, decreasing amyloid aggregates or improving neuronal survival with minimal toxicity in relevant ADRD cellular models.

Small molecules identified in the primary screen can be further optimized with medicinal chemistry (for example, through structure–activity relationship (SAR) analysis) to improve potency, efficacy, and specificity and to minimize toxicity. Subsequently, small molecules can then be tested in several animal models to establish evidence of CNS entry and general and brain-specific PK/PD. Inefficient drug delivery to the CNS is a major challenge for treating ADRD, as well as glioma, stroke, and traumatic brain injury [79]. Some small, hydrophobic drugs can cross the BBB. For example, risdiplam is taken orally and crosses the BBB to increase functional SMN proteins in the CNS [80]. However, if oral administration does not lead to sufficient levels of small molecules in the CNS, other routes of delivery can be considered, including intravenous infusion, intranasal delivery, intrathecal delivery to the cerebrospinal fluid, intraventricular infusion, and focused ultrasound to transiently disrupt the BBB [79]. For the selected delivery route, maximally tolerated dose, target engagement, and efficacy at the molecular, cellular, and behavioral levels can be established. A small molecule with extensive evidence of safety and efficacy in animal models may then be considered a candidate for advancement into human clinical testing.
3. Examples of ADRD-Relevant ncRNAs and Screening Strategies

3.1. Selecting ncRNA Targets and Screening Strategies

Similar to selecting a compound library, selecting appropriate ncRNA targets and screening strategies is critical and involves many factors. Generally, for any disease, the ncRNA target should directly contribute to the pathophysiology of a disease, and modification of this ncRNA can reverse or delay the course of the disease without significantly...
Genes 2021, 12, 2005

impacting physiological functions [81]. For ADRD specifically, the timing of therapeutic interventions is also important. Interventions can be categorized as primary prevention before the first pathophysiologically changes occur, secondary prevention when pathophysiological changes start to accumulate but the patient is cognitively normal, or symptomatic treatment when the patient starts to show mild cognitive impairment and eventually progresses to full-blown ADRD [80]. With primary diagnosis and prevention remaining challenging, secondary prevention may currently be the desirable sweet spot where ADRD can be detected up to 20 years before noticeable and irreversible cognitive decline occurs, though symptomatic treatment is also an essential clinical need. As such, ncRNA targets for secondary prevention are likely to be dysregulated before extensive cell deaths occur. In Table 2, we list various ncRNAs that have been shown to be dysregulated in human ADRD patients, the pathways implicated in ADRD pathology, as well as evidence of therapeutic potential in cellular and animal models. These include various miRNAs that have been extensively studied and validated. Others such as circRNAs, piRNAs, and enhancer RNAs (eRNAs) were discovered more recently and are therefore less well-studied but may emerge as promising candidates with further study.

Table 2. Some ncRNAs dysregulated in ADRD with evidence for therapeutic potential.

| RNA Species | Evidence of Dysregulation in Human ADRD | Signaling Pathways and Genes Affected | Therapeutic Application and Potential |
|-------------|----------------------------------------|--------------------------------------|-------------------------------------|
| **Small ncRNAs** | | | |
| miR-132 | Downregulated in AD | Aβ homeostasis: ITPKB, ERK1/2, BACE1 [87] | miR-132 viral overexpression or mimics rescued peroxide, glutamate, and Aβ toxicity in primary rodent and human neurons [82,92] |
| | hippocampus, prefrontal cortex, temporal cortex [82–89], significantly associated with Braak score in some studies | Tau homeostasis: MAPT, CAPN2, RHOFOX1, GSK3β, EP300, PTPβ, ITPKB [82,87,92,93] | |
| | Downregulated in FTD | Neuronal apoptosis: PTEN, FOXO3a [82] | |
| | frontal cortex [90,91] | Neurogenesis and synaptic plasticity: p250GAP, MeCP2, BDNF [94–96] | Viral overexpression and mimics rescued hippocampal cell death, tau homeostasis [92,93], hippocampal adult neurogenesis, and behavioral deficits in various AD mouse models [93,96] |
| miR-107 | Downregulated in AD | Aβ homeostasis: BACE1 [97,98] | miR-107 mimics rescued glutamate and Aβ toxicity in primary rodent and human neurons [92] |
| | neocortex and temporal cortex [97,98] | Tau homeostasis: APP, Aβ42 [99–102] | Viral overexpression of miR-107 sponge in mouse hippocampus induced memory deficits [101] |
| miR-101 | Downregulated in AD | Aβ homeostasis: BACE1, APP, Aβ42 [99–102] | Overexpression of miR-195 rescued lysosomal defects in iPSC-derived neurons from ADRD patients [102] and decreased Aβ plaque in N2a cells [104] |
| | temporal cortex [99,100] | Lyosomal defects: SYNJ1 [103] | Viral overexpression in mouse models decreased Aβ plaque, tau hyperphosphorylation and rescued cognitive deficits in ApoE4/’’ mice [103] |
| miR-195 | Downregulated in AD | Aβ homeostasis: BACE1 [104] | Overexpression of miR-195 rescued lysosomal defects in iPSC-derived neurons from ADRD patients [102] and decreased Aβ plaque in N2a cells [104] |
| | parietal cortex tissue [105] | Lyosomal defects: SYNJ1 [103] | Viral overexpression in mouse models decreased Aβ plaque, tau hyperphosphorylation and rescued cognitive deficits in ApoE4/’’ mice [103] |
| miR-34a | Upregulated in AD | Aβ homeostasis: ADAM10 [106] | miR-34a mimics exacerbated, whereas miR-34a inhibitor protected against glutamate and Aβ toxicity in primary rodent and human neurons [92] |
| | temporal cortex [88,105] | Tau homeostasis: PTPA [106] | Overexpression of miR-34a induces rapid cognitive impairment in mouse model [106] |
| | | Synaptic plasticity: VAMP, SYT1, HCN1, NR2A, Gria1, SIRT1 [105,106] | |
| miR-26b | Upregulated in AD | Tau homeostasis: CDK5 [107] | miR-26b inhibition protects mouse and human primary neurons against peroxide and Aβ toxicity [92,107] |
| | temporal cortex [107] | Aberrant cell cycle entry: RB1 [107] | |
| miR-203 | Upregulated in FTD | Genes in the neurodegeneration-associated synaptic (NAS) module: BCL2L2, DGKB, MAPK10, VSNL1 [108] | Overexpressing miR-203 in mouse primary cortical neurons increased apoptosis [108] |
| | frontal cortex [108] | | Upregulation of miR-203 and corresponding downregulation of predicted targets in the cortex of FPRS0 tau mice [108] |
| **piRNAs** | Various piRNA dysregulated in AD prefrontal cortex [109,110] | | |
### Table 2. Cont.

| RNA Species | Evidence of Dysregulation in Human ADRD | Signaling Pathways and Genes Affected | Therapeutic Application and Potential |
|-------------|----------------------------------------|---------------------------------------|---------------------------------------|
| **Linear IncRNAs** | | | |
| BACE1-AS (β Secretase Antisense Transcript) | Upregulated in AD cerebellum, hippocampus, cortex, and serum [111–113] | Aβ homeostasis: BACE1, Aβ42 [111–114] | Knockdown BACE1-AS in SH-SY5Y cells reduced Aβ toxicity [113] |
| | | | Hippocampal injection of BACE1-AS inhibitors reduced neuronal death in APP/PS1 mice [113] |
| | | | Activation of NRF2 by sulforaphane inhibited BACE1 and BACE1-AS transcription and rescued cognitive deficits in 5xFAD mice [114] |
| NEAT1 (Nuclear Enriched Abundant Transcript 1) | Upregulated in AD temporal cortex and hippocampus [89,115,116] | Autophagy: NEDD4L, PINK1 [117] | Overexpression of NEAT1 exacerbated peroxide injury in N2A cells [119] |
| | | | Hippocampal knockdown of NEAT1 with siRNA improved memory in aged mice and vice versa [118] |
| | | | Viral knockdown of NEAT1 rescued memory deficit in APP/PS1 mice [117] |
| I7A | Upregulated in AD cortex [120] | Neurogenesis: nestin [121] | Overexpression of I7A in SH-SY5Y cells increased Aβ secretion [120] |
| | | | Neuronal activity: miR-7 sponge, c-Fos promoter to suppress c-Fos expression [118] |
| | | | Downregulation of I7A in SH-SY5Y cells decreased apoptosis and Aβ-42 secretion [121] |
| BC200 | Upregulated in AD cortex and hippocampus [122] | Aβ homeostasis: APP [123] | Hippocampal overexpression of rodent homolog BC1 increased APP and Aβ expression and impaired memory, whereas inhibiting BC1 was neuroprotective [123] |
| **Enhancer RNAs** | Various enhancer RNAs dysregulated in AD prefrontal cortex [124] | | |
| **circRNAs** | | | |
| CDR1-AS (Cerebellar Degeneration-Related Protein 1 Antisense Transcript) | Downregulated in AD temporal cortex and hippocampus [125,126] | Aβ homeostasis: BACE1, APP [128] | CDR1-AS overexpression promoted lysosomal degradation of BACE1 and APP proteins in HEK293 and SH-SY5Y cells [128] |
| | | | Hippocampal overexpression of CDr1-as KO mice showed impaired sensorimotor gating, but normal memory acquisition [130] |
| | | | Neuronal activity: miR-7 sponge, c-Fos promoter to suppress c-Fos expression [118] |
| | | | Downregulation of I7A in SH-SY5Y cells decreased apoptosis and Aβ-42 secretion [121] |
| cieHOMER1 (circ-Homer Protein Homolog 1) | Downregulated in AD parietal cortex [127] | Aβ homeostasis: EGR1, ARC [129,130] | Hippocampal knockdown of BACE1-AS and CDR1-AS KO neurons showed dysfunctional synaptic neurotransmission [130] |
| cieKCNN2 (circ-PotassiumCalcium-Activated Channel Subfamily N Member 2) | Downregulated in AD parietal cortex [127] | Aβ homeostasis: BACE1, APP [128] | Hippocampal overexpression of rodent homolog BC1 increased APP and Aβ expression and impaired memory, whereas inhibiting BC1 was neuroprotective [123] |
| ciecDOCK1 (circ-Dedicator Of Cytokinesis 1) | Upregulated in AD parietal cortex [127] | Aβ homeostasis: APP [123] | Hippocampal overexpression of rodent homolog BC1 increased APP and Aβ expression and impaired memory, whereas inhibiting BC1 was neuroprotective [123] |

**Target gene abbreviations:** ADAM10: ADAM Metallopeptidase Domain 10; APP: Amyloid β Precursor Protein; ARC: Activity Regulated Cytoskeleton Associated Protein; BACE1: β-Secretase 1; BCL2L2: BCL2-Like 2; BDNF: Brain Derived Neurotrophic Factor; CAPN2: Calpain 2; CDK5: Cyclin-Dependent Kinase 5; DGKB: Diacylglycerol Kinase β; EGR1: Early Growth Response 1; ERK1: Extracellular Signal-Regulated Kinase 1/2; EP300: E1A Binding Protein P300; FOXO3a: Forkhead Box D3; GSK-3β: Glycogen Synthase Kinase 3 β; HCN1: Hyperpolarization-Activated Cyclic Nucleotide Gated Potassium Channel 1; ITPKB: Inositol-3-phosphate 3-Kinase B; LC3B: microtubule-associated proteins 1A/1B light chain 3B; MAPK10: Mitogen-Activated Protein Kinase 10; MAPT: Microtubule Associated Protein Tau; MeCP2: Methyl-CpG Binding Protein 2; NEDD4L: NEDD4-Like E3 Ubiquitin Protein Ligase; NR2A: NMDA receptor subunit 1; NR2F2: Nuclear Factor Erythroid 2-related Factor 2; p53: p53; p53: GTPase-activating protein; PINK1: PTEN Induced Kinase 1; PP2B: Protein Phosphatase-2B; PTPA: Protein Phosphatase 2 Phosphatase Activator; PTEN: Phosphatase And Tensin Homolog; RB1: Retinoblastoma-associated protein; RBFOX1: RNA Binding Protein Fox-1 Homolog 1; SIRT1: NAD+ Dependent Deacetylase Sirtuin-1; SIRT1: NAD+ Dependent Deacetylase Sirtuin-1; SYNJ1: Synaptojanin 1; SYT1: Synaptotagmin 1; VAMP: Vesicle-Associated Membrane Protein 1; VSNL1: Visinin-Like 1.
In addition, the screening strategy selected will depend on the ncRNA of interest. For example, IncRNAs may be more amenable to direct binders than mature miRNAs, which are likely too short to possess well-defined binding pockets. In the following section, we briefly discuss the screening strategies for discovering small molecule drugs, using three relatively well-validated ncRNAs as examples.

3.2. Upregulating miR-132, a miRNA Commonly Downregulated in ADRD

We and other labs have shown that miR-132 is a key miRNA downregulated in the early stages of ADRD before significant neuronal death occurs [82–89]. miR-132 overexpression via viral vectors or oligonucleotide mimics exerts neuroprotective effects, delays neurodegeneration, and rescues behavioral deficits in several animal models of ADRD [92,96]. However, targeting miRNAs through a direct small-molecule binder would more likely lead to miR-132 inhibition, as seen for small molecules binding to precursors of other miRNAs [24,61,132]. Since miR-132 has a well-defined promoter region regulated by transcription factors including the cAMP-response element-binding protein (CREB), the brain-derived neurotrophic factor (BDNF), and the RE1-silencing transcription factor (REST) [133], a promoter-driven luciferase reporter assay may identify small molecules that transcriptionally upregulate miR-132. Alternatively, high-throughput miRNA-seq may also be utilized to identify small molecules that upregulate miR-132 either directly or indirectly.

3.3. Inhibiting BACE1-AS, a lncRNA Upregulated in ADRD

β-secretase 1 antisense transcript (BACE1-AS) is a lncRNA and the antisense transcript of BACE1 and is upregulated in various brain regions of ADRD patients [111,112]. BACE1-AS binds to and stabilizes BACE1 mRNA, resulting in increased BACE1 protein expression, enhanced APP processing, Aβ1–42 production, and plaque formation. Therefore, reducing BACE1-AS expression level or functional activity may alleviate Aβ plaque. Another lncRNA, MALAT1, was successfully targeted with direct and selective small molecule binders through small-molecule microarray [22] and FID assays [21]. As such, identifying small molecules that directly bind various regions of BACE1-AS may decrease its expression level or inhibit its binding to BACE1 mRNA to reduce Aβ plaque load.

3.4. Modulating the Splicing Efficiency of circRNAs Dysregulated in ADRD

circRNAs are highly stable and usually exhibit tissue- or cell type-specific expression, therefore making them promising therapeutic targets [134]. Cerebellar degeneration-related protein 1 antisense RNA (CDR1-AS) is one of the first and most well-studied circRNAs. The CDR1-AS sequence contains several miR-7 binding sites to sequester miR-7 away from its targets, therefore inhibiting miR-7 activity [129]. CDR1-AS is highly expressed in human and mouse brains [129] and is reported to be downregulated in the hippocampus and temporal cortex of AD patients [126], although a different study reported a small upregulation in AD parietal cortices [127]. Two separate studies also found a downregulation of circHOMER1 and circKCNN2 and an upregulation of circDOCK1 in AD frontal and parietal cortices [127,131]. To identify small molecules that modulate the back-splicing, and hence biogenesis, of circRNAs, the splice sites of these RNAs can be cloned into a plasmid in which the successful back-splicing and circularization of the RNA results in the in-frame expression of a reporter [46]. As such, small molecules that modulate the reporter intensity may also be able to modulate circRNA expression.

4. Conclusions and Future Perspectives

Despite intensive research and significant advances in our understanding of ADRD genetics and pathophysiology, a disease-modifying treatment with significant clinical benefits has still not been identified. Directly modulating well-established ADRD targets, including Aβ, PSEN1, PSEN2, BACE1, Tau, and APOE, have yielded limited success [135]. Regulatory ncRNAs, which have only been discovered, annotated, and investigated in
the last two decades, represent novel therapeutic targets for treating ADRD. This review describes various methods to identify small molecules that modulate ncRNAs as potential ADRD treatments. Going forward, some key challenges are selecting the most relevant ncRNA targets, the potential off-target effect of small molecules, and the low translatability from basic research to clinical testing for neurological diseases. For the first challenge, more studies into novel, brain-enriched, and specific ncRNAs, their physiological functions, and how they are dysregulated in the early stages of ADRD will provide information on ADRD-relevant targets. Notably, most ADRD-associated genetic variants identified in genome-wide association studies are located in non-coding regions [136], suggesting that some variants maybe transcribed into aberrant ncRNAs that can be targeted therapeutically. For non-specificity, medicinal chemistry can help with optimizing the specificity, safety, and BBB-permeability. Finally, performing screen and validation studies in ADRD-relevant cellular and animal models is important for reproducibility and maximizing translatability. Combining the extensive knowledge of ADRD pathophysiology and small molecules with the emerging field of ncRNA biology will perhaps lead to novel, truly disease-modifying medications for ADRD.

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Abbreviations

| Acronym | Description |
|---------|-------------|
| AD | Alzheimer’s disease |
| ADRD | Alzheimer’s disease and related dementias |
| ALIS | Automated ligand identification system |
| APP | Amyloid-β precursor protein |
| AS | Antisense transcript |
| ASO | Antisense oligonucleotide |
| BACE1-AS | β-secretase 1 antisense transcript |
| BBB | Blood–brain barrier |
| BDNF | Brain-derived neurotropic factor |
| Cat-ELCCA | Catalytic enzyme-linked click chemistry assay |
| CDR1-AS | Cerebellar degeneration-related protein 1 antisense RNA |
| Chem-CLIP-Map-Seq | Chemical Cross-Linking and Isolation by Pull-down to Map Small Molecule-RNA Binding Sites |
| ChIP-seq | Chromatin immunoprecipitation-RNA-seq |
| circRNA | Circular RNA |
| CREB | cAMP response element-binding protein |
| DGCR8 | DiGeorge critical region 8 |
| DRUG-seq | Digital RNA with perturbation of Genes |
| ENCODE | Encyclopedia of DNA Elements |
| Exp5 | Exportin 5 |
| FANTOM | Functional Annotation of the Mammalian Genome |
| FDA | Food and Drug Administration |
FID Fluorescent indicator displacement
FP Fluorescence polarization
FRET Fluorescence resonance energy transfer
FTD Frontotemporal dementia
HD Huntington’s disease
HOTAIR HOX antisense intergenic RNA
HTS High-throughput screen
HTT Huntingtin
ITC Isothermal titration calorimetry
LBD Lewy body dementia
LC-MS Liquid chromatography-mass spectrometry
lincRNA Long non-coding RNA
MALAT1 Metastasis-associated lung adenocarcinoma transcript 1
MED Multiple etiology dementias
miRNA MicroRNA
MoA Mechanism of action
mRNA Messenger RNA
ncRNA Non-coding RNA
NMR Nuclear magnetic resonance
PAIN Pan-assay interference
PDB Protein data bank
PK/PD Pharmacokinetics and pharmacodynamics
PRC2 Polycomb repressive complex 2
PSEN1 Presenilin 1
PSEN2 Presenilin 2
RBP RNA binding protein
REST RE1-silencing transcription factor
RISC RNA-induced silencing complex
rRNA Ribosomal RNA
shRNA Short hairpin RNA
siRNA Small interfering RNA
SMN2 Survival of motor neuron 2
sncRNA Small ncRNA
snRNA Small nuclear RNA
SPR Surface plasmon resonance
TORNADO-seq Targeted organoid sequencing
TRBP Transactivating response RNA-binding protein
tRNA Transfer RNA
VCID Vascular contributions to cognitive impairment and dementia

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