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Non-coding RNAs in disease: from mechanisms to therapeutics

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Nemeth, K., Bayraktar, R., Ferracin, M., Calin, G.A. (2024). Non-coding RNAs in disease: from mechanisms to therapeutics. NATURE REVIEWS GENETICS, 25(3), 211-232 [10.1038/s41576-023-00662-1].

Availability:

This version is available at: <https://hdl.handle.net/11585/978659> since: 2024-08-22

Published:

DOI: <http://doi.org/10.1038/s41576-023-00662-1>

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1 **Non-coding RNAs in disease: from mechanisms to therapeutics**

2

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13

14

15 **Abstract** | Non-coding RNAs (ncRNAs) are a heterogeneous group of transcripts that, by
16 definition, are not translated into proteins. Since their discovery, ncRNAs have emerged
17 as important regulators of multiple biological functions across a range of cell types and
18 tissues, and their dysregulation has been implicated in disease. Notably, much research
19 has focused on the link between microRNAs (miRNAs) and human cancers, although
20 other ncRNAs, such as long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs),
21 are also emerging as relevant contributors to human disease. In this Review, we
22 summarize our current understanding of the role of miRNAs, lncRNAs and circRNAs in
23 cancer and other major human diseases, notably cardiovascular, neurological and
24 infectious diseases. We further discuss the potential use of ncRNAs as biomarkers of
25 disease and therapeutic targets.

26

27 **Table of Contents (ToC) blurb (~40 words max)**

28 **In this review, the authors describe our current knowledge of the role of miRNAs,**
29 **lncRNAs and circRNAs in disease, with a focus on cardiovascular, neurological,**
30 **infectious diseases, and cancer. They further discuss the potential use of ncRNAs**
31 **as disease biomarkers and as therapeutic targets.**

32

33 **[H1] Introduction**

34 The majority of the human genome (76–97%) encodes for RNAs that are not translated
35 into proteins, termed non-coding RNAs (ncRNAs)¹⁻³. Since their discovery, the biological
36 importance of ncRNAs has become increasingly apparent, shifting the perspective of
37 RNA as a simple intermediary of protein synthesis towards RNA as a functional molecule
38 with essential roles in the regulation of gene expression and genome organization. The
39 functional relevance of one class of ncRNAs in particular, microRNAs (miRNAs), has
40 received much attention, with important roles in a myriad of cellular processes, including
41 muscle differentiation and cardiac development^{4,5}, as well as neural stem cell
42 differentiation and neurogenesis^{6,7}. Compelling evidence further implicated dysregulated
43 miRNAs in human diseases, particularly human cancers, such as by functioning as
44 oncogenes and/or tumor suppressors⁸. miRNAs have also been found to be differentially
45 expressed in a range of other human pathologies, including cardiovascular^{9,10},
46 neurological^{6,7}, and infectious diseases¹¹. Most recently, their involvement in SARS-Cov-
47 2 infection was demonstrated¹².

48 Over the years, high-throughput sequencing and other technologies have led to
49 the identification of a wide range of ncRNAs of different types and sizes^{4,5}. These include
50 ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs
51 (snoRNAs), transfer RNAs (tRNAs) and more recently miRNAs, long ncRNAs (lncRNAs),
52 circular RNAs (circRNAs), heterogeneous nuclear RNA (hnRNAs), PIWI-interacting
53 RNAs (piRNAs).

54 Long ncRNAs and circular RNAs are recognized as essential regulators in a variety
55 of biological processes. Similar to miRNAs, dysregulation of lncRNAs and circRNAs has

56 been associated with various human diseases¹³⁻¹⁶. One of the biggest challenges in the
57 field today is to elucidate the diverse functions and mechanisms of action of ncRNAs,
58 which is essential for defining their clinical relevance and exploiting their potential use as
59 biomarkers or therapeutic targets.

60 Here, we review the role of ncRNAs in human diseases that account for the highest
61 mortality worldwide, including cardiovascular diseases, cancer, neurodegenerative and
62 infectious diseases, such as COVID-19. We place a focus on the disease-related ncRNAs
63 that have received the most research focus: miRNAs, lncRNAs, and circRNAs. Readers
64 are referred to other review articles for insights into additional classes of ncRNAs and
65 their potential role in diseases¹⁷⁻²⁰. We first provide a brief overview of the different ncRNA
66 mechanisms and physiological roles, and then discuss the impact of ncRNA dysregulation
67 in human disease. Finally, we review the use of ncRNAs as diagnostic and prognostic
68 markers and targets of new therapeutic strategies.

69

70 **[H1] Mechanisms of action and functions of ncRNAs**

71 ncRNAs act through diverse mechanisms on target genes and interact with each other,
72 creating a complex and dynamic regulatory RNA network²¹. Variations in the expression
73 of a given ncRNA can affect the expression of other ncRNAs, altering many cellular
74 processes including gene expression, RNA splicing, editing, intracellular transport, and
75 translation²².

76

77 **[H2] microRNAs**

78 miRNAs are short ncRNAs that were first identified thirty years ago in *Caenorhabditis*
79 *elegans* (*C. elegans*)^{23,24}. To date, more than 38,000 miRNAs from 271 species, including
80 2,654 human mature miRNAs, have been annotated in the miRNA archive [miRBase](#)
81 (v22.1)²⁵. Functionally, it is predicted that the majority of the human transcriptome is under
82 miRNA regulation²⁶. The complexity of such regulation is demonstrated by the fact that a
83 single miRNA can target hundreds of different messenger RNAs (mRNAs) and that
84 multiple miRNAs can target a single mRNA²⁷. Overall, miRNAs have a function in every

85 fundamental biological process, including cell proliferation, differentiation, and embryonic
86 development, and their tissue-specific functions have been demonstrated²⁸⁻³⁰.

87 The classic function of miRNAs involves binding to the 3' untranslated region (UTR)
88 of target mRNAs, leading to their degradation or translational repression³¹. This process
89 requires miRNA association with an Argonaute (Ago) protein, which is the core
90 component of the RNA-induced silencing complex (RISC). Once loaded onto an Ago
91 protein, a miRNA can guide the RISC to a complementary target mRNA for translational
92 repression or mRNA degradation. miRNAs also have the ability to inhibit protein
93 expression by binding to coding regions (CDS) or the 5' UTR of mRNA molecules. For
94 example, CDS-located miRNA interaction sites (miR-134, miR-296 and miR-470) in
95 Nanog, Oct4 and Sox2, modulate embryonic stem cell differentiation³⁰.

96 Although miRNAs typically inhibit gene expression, there are instances in which they
97 instead boost translation³². For example, human miR-369 has been shown to activate
98 translation via a mechanism that involves direct binding to *TNF- α* and *FXR1*³². Moreover,
99 let-7 miRNA has been shown to upregulate the translation of its target mRNAs during cell
100 cycle arrest and to repress translation in actively proliferating cells, indicating that miRNA
101 function alters between repression and activation during cell cycle³². Additional ways in
102 which miRNAs activate genes include their attachment to the CDS or the 5' UTR of
103 mRNAs^{33,34}. Despite the fact that these alternative miRNA mechanisms of action are less
104 well studied, there is increasing evidence of their cellular relevance (**Figure 1**).

105 In addition to regulating transcription within the cells in which they are produced,
106 miRNAs can act as intercellular communication molecules through their secretion in
107 extracellular vesicles or by acting as hormones^{35,36}. Moreover, secreted miRNAs can
108 directly target Toll-like receptor (TLR) proteins by acting as their ligands³⁷, a mechanism
109 that activates TLR signaling transduction pathways and induces an immune response³⁸⁻
110 ⁴¹. Recent studies have also revealed their interaction with non-Ago proteins [G], although
111 the mechanism is poorly understood (**Figure 1**).

112

113 **[H2] Long non-coding RNAs**

114 lncRNAs are a large and highly diverse class of ncRNAs that are >200 nucleotides in
115 length⁴². The first lncRNAs identified in eukaryotes, *H19* and *Xist*, were discovered long
116 before the genomic era^{43,44}; however, it took considerable time to recognize their broad
117 biological functions. Although many lncRNAs have been identified to date, only a handful
118 have been functionally characterized. The human [GENCODE](#) project estimated that there
119 are 16,000 human lncRNAs, whereas the current version of the [NONCODE](#) database
120 (v6.0) has annotated 96,411 human lncRNA genes, generating 173,112 lncRNA
121 transcripts, an amount several times larger than the number of coding genes (estimated
122 at around 20,000)^{45,46}.

123 lncRNAs can be transcribed in sense or antisense directions from various genomic
124 regions, including introns or exons of overlapping protein-coding genes, intergenic
125 regions (lincRNAs), pseudogenes (pseudogene-derived lncRNAs), transcribed
126 ultraconserved elements (T-UCRs), telomeres (telomeric repeat-containing RNAs),
127 centromeric repeats (centromeric lncRNAs), ribosomal DNA loci (promoter and pre-rRNA
128 antisense, PAPAS), promoters (promoter-associated lncRNAs, PALRs), enhancers
129 (eRNAs) and 3'-UTRs (UTR-associated RNAs)⁴⁷. Similar to mRNAs, lncRNAs can be
130 spliced; however, they usually contain fewer exons, are often retained in the nucleus and
131 their abundance can be 10 times lower than mRNAs^{48,49}. lncRNAs often show high tissue
132 specificity and their expression alters dynamically during development⁵⁰.

133 The diversity of lncRNAs is also reflected in their function, which includes genomic,
134 transcriptional, and translational regulation of neighboring and distant genes^{22,51-53}.
135 lncRNAs can directly interact with DNA, forming R-loops⁵⁴, and can associate with
136 enhancers or promoters, activating or suppressing their function⁵⁵⁻⁵⁸. By forming a
137 complex with proteins, lncRNAs can also bind to the DNA and regulate chromatin by
138 recruiting chromatin modifiers to the promoter region of their target genes⁵⁹⁻⁶¹.

139 As well as associating with DNA, lncRNAs can interact with various other RNAs,
140 including mRNAs, circRNAs, and miRNAs. They can further influence RNA splicing and
141 act as **miRNA sponges [G]** and thereby inhibit the target-repressing function of
142 miRNAs^{62,63}. In addition, through their interaction with proteins, lncRNAs can serve as

143 scaffolds or guides to promote the colocalization of proteins or facilitate protein-protein
144 interactions^{64,65} (**Figure 2**).

145

146 **[H2] Circular RNAs**

147 CircRNAs are generated by back-splicing of linear transcripts and can be derived from
148 exons, introns, exon-intron junctions, or intergenic regions of the genome⁶⁶⁻⁶⁸. Their
149 circular structure makes circRNAs unsuitable for further processing, reducing
150 susceptibility to exonuclease activity compared to linear RNAs, which results in a high
151 degree of stability⁶⁹. circRNA expression is often unrelated to the expression of their host
152 genes⁷⁰, and due to their stability, they can be more abundant than their associated linear
153 mRNA⁷¹. With regards to their localization, circRNAs usually accumulate in the
154 cytoplasm⁷²; however, they are also present in the nucleus, and similarly to lncRNAs,
155 circRNAs can also bind to the DNA and form circR-loops {Conn, 2023 #337}. Although
156 the turnover of circRNAs is largely unknown⁷⁰, it most likely involves secretion via
157 exosomes⁷³.

158 CircRNAs can interact with miRNAs, mRNAs, or RNA-binding proteins (RBPs),
159 activate or repress gene expression, or act as miRNA or protein sponges⁷⁴. The
160 complexity of the RNA network is well illustrated by the fact that circRNAs can sequester
161 miRNAs and thereby indirectly influence the expression of their mRNA targets⁷⁴.
162 circRNAs can also function as protein 'enhancers', either by forming a circRNA-protein
163 complex⁷⁵, acting as protein scaffolds⁷⁶, or recruiting proteins to a specific loci or
164 subcellular compartment that facilitates their colocalization and thereby influencing
165 protein-protein interactions^{77,78}. (**Figure 3**).

166

167 **[H2] ncRNA-encoded peptides**

168 Despite their original non-coding classification, it was uncovered in the last decade that
169 some ncRNAs contain **short open reading frames [G]** (sORFs) that encode **small**
170 **regulatory peptides (sPEPs) [G]**, or micropeptides, consisting of less than 100 amino
171 acids (AA)⁷⁹⁻⁸¹. The first identified miRNA-encoded sPEPs (miPEP), miPEP171b (9 AA)

172 and miPEP165a (18 AA), were described in plants in 2015⁸². Both pri-miR-171b and pri-
173 miR-165a miRNA precursors encode small proteins that enhance the accumulation of
174 their corresponding mature miRNAs, leading to downregulation of their target mRNAs.
175 After their discovery in plants, several studies have reported human sPEPs derived from
176 ncRNAs and their potential roles in diseases⁸³⁻⁸⁵.

177 **Ribosomal profiling [G]** experiments have uncovered many unexpected associations
178 between ncRNAs and ribosomes. Combined with the development of various
179 computational methods, these experiments have led to the discovery of thousands of
180 sORFs and many ncPEPs^{86,87}. Several of these ncPEPs have been experimentally
181 validated⁸⁸. For example, a muscle-specific lncRNA is translated into the 35 AA protein
182 DWORF, which was shown to regulate intracellular calcium signaling in heart tissue⁸⁷.
183 Moreover, the lncRNA *Linc00116* encodes a small peptide (56 AA), MTLN, that supports
184 protein complex assembly in the mitochondria and inhibits the production of reactive
185 oxygen species, thereby enhancing respiratory efficiency⁸⁹. The identification of novel
186 ncPEPs is ongoing, and their investigation can be facilitated by databases such as
187 [FuncPEP](#)⁹⁰, which currently lists 112 functional sPEPs encoded by ncRNAs and provides
188 details on the ncRNA 'host' transcripts. Another database, [SPENCER](#), annotates cancer-
189 associated sPEPs encoded by ncRNAs⁹¹.

190 Recent studies showed that, similarly to miRNAs and lncRNAs, some circRNAs can
191 also encode proteins^{92,93}. Owing to the lack of the 5' end, translation initiation from
192 circRNAs requires N⁶-methyladenosine (m⁶A) modification⁹⁴ or an IRES, which is usually
193 rare in eukaryotic transcripts but has been identified in eukaryotes through systematic
194 investigations^{95,96}. Like the general function of many circRNAs, the function of their
195 encoded peptides is largely unknown. Several hypotheses have been put forward about
196 the role of translated circRNAs⁹⁷, including the generation of rapidly degraded peptides
197 that regulate immune surveillance, acting analogous to lncRNA-encoded proteins and,
198 therefore, participating in nonsense-mediated mRNA decay, or inhibiting the translation
199 of other RNAs by sequestering ribosomes⁹⁷. Regardless of the precise biological role, it
200 has been hypothesized that translated circRNAs might have evolutionarily conserved
201 functions, as their sequence is highly conserved across different species⁹³.

202

203 **[H1] ncRNA dysregulation in human disease**

204 Because their regulatory functions are crucial for normal cell activities, it is not surprising
205 that dysregulation of ncRNAs leads to human disease^{8,98}. Indeed, perturbations in ncRNA
206 biology have been linked to a wide range of conditions, including cancer, cardiovascular
207 diseases, neurological disorders, infectious diseases, and sepsis (**Figure 4**). Generally,
208 in diseased tissues, ncRNAs are dysregulated as a consequence of genomic structural
209 and copy number variations, epigenetic modifications, or transcription factor alterations
210 ^{99,100}. Several databases, such as [The Human MicroRNA Disease Database](#)¹⁰¹ for
211 miRNAs, and [LncRNADisease](#)^{102,103} for lncRNAs and circRNAs, can be useful resources
212 for up-to-date information on disease-related ncRNAs. Ultimately, gaining a deeper
213 understanding of the involvement of ncRNAs in disease could pave the way for the
214 development of innovative diagnostic and therapeutic approaches.

215

216 **[H2] Non-coding RNAs in cancer**

217 The first evidence of ncRNA involvement in human cancer came in 2002 from genetic
218 studies of patients with chronic lymphocytic leukemia (CLL)¹⁰⁴, the most common type of
219 leukemia in the Western world²⁷⁷. Loss of chromosome region 13q14 is a common feature
220 observed in CLL and is often the only genetic abnormality that is found in leukemic cells.
221 Notably, the 13q14 region harbors genes encoding precursors of miR-15a and miR-16-1,
222 which were later characterized as tumour suppressors through their targeting of *BCL-2*¹⁰⁴
223 and *MCL1*¹⁰⁵. Soon after these discoveries, other miRNA-encoding loci were shown to be
224 frequently located in the fragile regions of chromosomes^{106,107} and lost or disrupted in
225 various cancer types^{108,109,52}.

226 Due to the complexity of miRNA regulation, one of the biggest challenges is
227 understanding whether miRNA dysregulation is the cause or consequence of the disease.
228 Nonetheless, pan-cancer analyses have uncovered that certain miRNAs, such as the
229 oncogenic miR-21 and miR-155, or the tumour suppressors miR-16 and miR-145, are
230 commonly dysregulated in several types of cancer^{110,111}. These studies have identified

231 miRNA signatures that are consistent across 15 different cancer types and indicate a
232 major role in regulating the particular hallmarks of cancer. For example, miR-210, miR-
233 21-3p, and let-7a-3p were associated with hypoxia gene signatures^{110,111}. The miR-29
234 family regulates the DNA demethylation pathway members TET1 and TDG^{110,111}. miR-21,
235 which was shown to be overexpressed in a large variety of cancers^{112,113}, is involved in
236 therapy resistance¹¹⁴ and tested as a cancer biomarker¹¹⁵. miR-324 has an oncogenic role
237 both in malignant cells and the surrounding tumor microenvironment (TME), specifically
238 in neurons in mouse models of oral cancers¹¹⁶, where miR-324 (in conjunction with miR-
239 21 and opposition of miR-34a) promotes neuritogenesis.

240 Certain miRNAs can function as either an oncogene or a tumor suppressor, depending
241 on tumor type, tumor stage, and the tumor microenvironment (TME), which further
242 emphasizes that miRNA functions need to be investigated in a context-dependent
243 manner^{110,117}. For example, dysregulation of miR-324 was described in various cancer
244 types including colorectal and gastric cancers, and its oncogene and tumor suppressor
245 functions have been both demonstrated depending on the cellular context¹¹⁸. Meanwhile,
246 several lncRNAs and circRNAs, including the lncRNA *MALAT1* can act as a sponge for
247 miR-324¹¹⁸. miRNAs from the *let-7* family have been also shown to exhibit dual
248 functionality, acting as tumor suppressors in cancer cells while concurrently exerting
249 oncogenic effects within the TME¹¹⁹. Recently, an estrogen-driven mechanism was
250 discovered in which estrogen receptor-positive breast cancer cells eliminate the tumor
251 suppressor members of let-7 family via extracellular vesicles, and these have oncogenic
252 effects through the immunostimulatory (M1) macrophage activation and polarization in
253 the TME³⁶. Similarly, miR-21 released inside extracellular vesicles by glioblastoma cells
254 was demonstrated to act on microglial cells of the TME changing the levels of target
255 genes, including *Btg2*, and consequently their phenotype¹²⁰.

256 Sequence conservation suggests positive selection during evolution and is therefore
257 an important hint of potential functionality. Plenty of lncRNAs are transcribed from
258 genomic regions that are perfectly conserved between humans, mice, and rats, termed
259 ultraconserved elements (UCEs)¹²¹. However, newly-evolved, human, or primate-specific
260 elements (pyknons) are also an interesting topic of research, and their non-coding
261 transcripts were found to have a role in cancer progression¹²².

262 After their discovery, miRNA-encoded peptides (miPEPs) gained intense research
263 interest, with several studies reporting the involvement of human ncPEPs in growth and
264 development, as well as disease. More recently, the pri-miR-34a-encoded miPEP133
265 (133 AA) has been demonstrated to positively regulate its own pri-miRNA in human
266 cancer cell lines, in which it functions as a tumour suppressor¹²³. In contrast, pri-miR-31-
267 encoded miPEP31 (44 AA) decreases the expression of miR-31 by binding to its promoter
268 region, inhibiting transcription initiation. miPEP31 is highly expressed in regulatory T cells
269 (Treg), promotes Treg cell differentiation, and suppresses experimental autoimmune
270 encephalomyelitis¹²⁴. Pri-miR-155-encoded miPEP155 (17 AA) does not influence the
271 expression of pri-miR-155 but increases the expression of the oncogenic *Rictor* and
272 *EGFR* genes in HeLa cells¹²⁵. Pri-miR-147b-encoded MOCCI micropeptide seems to
273 have a role in the immune response to viral infection¹²⁶, whereas miPEP200a (187 AA)
274 and miPEP200b (54 AA), encoded by pri-miR-200a and pri-miR-200b, respectively,
275 inhibited the migration of prostate cancer cells in vitro¹²⁷.

276 A lncRNA-encoded peptide identified to have a role in cancer is HOXB-AS3 (53 AA),
277 translated from the lncRNA *HOXB-AS3*, which was shown to suppress colon cancer
278 growth; its loss is a critical oncogenic event in metabolic reprogramming¹²⁸. Several
279 lncRNA-encoded micropeptides have been further associated with cancer, such as the
280 *LINC00665*-encoded CIP2A-BP (52 AA) and *LNC00908*-encoded ASRPS (60 AA), both
281 of which inhibit breast cancer progression^{129,130}.

282 Through their roles in development, apoptosis, stress responses and cell cycle
283 regulation, circRNAs-encoded peptides could be important in cancer initiation and
284 progression⁹⁴. Indeed, several circRNAs-encoded proteins, such as FBXW7-185aa,
285 SHPRH-146aa, and PINT-87aa were shown to suppress glioma tumorigenesis^{84,131,132}.
286 Moreover, a recent study proposed a tumor suppressor role for the circRNA-encoded
287 protein circFGFR1p (87 AA), which negatively regulates the FGFR1 oncoprotein⁹⁷. Future
288 research should focus extensively on the functions of ncRNA-encoded peptides and their
289 coding mechanisms. Although there are still many challenges for such research,
290 advances in coding prediction tools and genomics technologies should facilitate the
291 progress.

292

293 **[H2] ncRNAs in cardiovascular diseases**

294 Cardiovascular diseases (CVDs), including myocardial infarction, atherosclerosis, heart
295 failure, and cardiac hypertrophy, remain the leading cause of mortality and morbidity in
296 the world¹³³. ncRNAs are relevant for heart physiological activity and are involved in CVD
297 processes through their functions in regulating apoptosis, proliferation, migration, cardiac
298 remodeling, fibrotic responses and cardiac hypertrophy^{134,135}. Emerging studies have
299 revealed that miRNAs are involved in the pathogenesis of CVDs. A notable example is
300 miR-21, which is upregulated in humans and mice with cardiac allograft vasculopathy
301 (CAV), which is a complication of heart transplantation that limits long-term survival¹³⁶.
302 Moreover, miR-21 is also overexpressed in a mouse model of cardiac fibrosis caused by
303 myocardial infarction, and correlated with attenuated *TGFβRIII* levels¹³⁷. Silencing of miR-
304 21 via antagomir-21 could disrupt CAV and prolong cardiac allograft survival¹³⁶ as well as
305 reduce hypertrophy and fibrosis and restore impaired cardiac function¹³⁸. It was also
306 recently demonstrated that anti-miR-21 treatment successfully suppressed miR-21 and
307 improved cardiac function in a pig model of ischemia–reperfusion injury with reduced
308 cardiac fibrosis and hypertrophy¹³⁹.

309 miR-122 is abundantly expressed in various cardiovascular cell types. Upregulation
310 of miR-122 was shown in patients with systolic dysfunction, cardiovascular fibrosis and
311 cardiovascular remodeling¹⁴⁰. Mechanistically, miR-122 was shown to directly inhibit the
312 anti-apoptotic protein Xiap in a mouse model of cardiovascular disease, promoting
313 endothelial cell apoptosis¹⁴¹. Moreover, circulating miR-122 level correlated negatively
314 with cardiac function and has been shown to be an important indicator with both predictive
315 and prognostic value for cardiac recovery¹⁴⁰. In the same study, it was revealed that miR-
316 122 inhibits the expression of the anti-apoptotic *BCL2* and thereby decrease the viability
317 of cardiomyocytes¹⁴⁰.

318 The exact role of lncRNAs in CVDs has recently started to be elucidated. Notably,
319 cardiac mesoderm enhancer-associated ncRNAs (CARMNs) are among the most
320 thoroughly annotated lncRNAs. These lncRNAs are predominantly expressed in smooth
321 muscle cells (SMCs) and are significantly upregulated in post-myocardial infarction^{142,143}.

322 Analysis of publicly available transcriptomic datasets has revealed a reduction in the
323 expression of CARMNs in cerebral arteries with aneurysms and human atherosclerotic
324 arteries¹⁴². Downregulation of CARMNs in human coronary artery SMCs resulted in
325 enhanced cell proliferation and migration in vitro, and significantly attenuated the
326 expression level of SMC-specific marker genes including *MYH11*, *ACTA2*, *CNN1*,
327 and *TAGLN*¹⁴². Furthermore, RNA immunoprecipitation assays confirmed that *CARMN*
328 could interact with *MYOCD* (Myocardin), an activator of SMC-specific genes and
329 transcriptionally active in cardiomyocytes as well as in SMCs¹⁴².

330 Adult mammalian hearts fail to regenerate after ischemic injury, primarily due to a
331 decline in cardiomyocyte mitosis. Yet, the specific molecular mechanisms that account
332 for the non-dividing nature of adult cardiomyocytes remain largely unknown¹⁴⁴. Cardiac
333 regeneration-related lncRNA (*CAREL*) is increased in expression in postnatal hearts and
334 has been associated with regeneration during cardiac injury¹⁴⁴. Indeed, the capacity for
335 cardiomyocytes to proliferate, an important step in regeneration, was attenuated in
336 transgenic mice that overexpress *CAREL*¹⁴⁴. In contrast, knocking down of *CAREL* in
337 cardiomyocytes by adenoviral shRNA increased the proliferation of cardiomyocytes and
338 enhanced cardiac regeneration after injury. Further experiments using biotin-avidin pull-
339 down and luciferase assays in human embryonic kidney cells (HEK293) and neonatal
340 cardiomyocytes have revealed that *CAREL* acts as a competing endogenous RNA to
341 miR-296, a positive regulator of cardiac replication and regeneration¹⁴⁴. Other lncRNAs,
342 such as *ZFAS1*¹⁴⁵, *SNHG3*¹⁴⁶, *ExACT1*¹⁴⁷, *MIAT*¹⁴⁸, *CPhar*¹⁴⁸, *Mhrt779*¹⁴⁸, *H19*¹⁴⁹ and
343 *CPR*¹⁵⁰, have been implicated in myocardial ischemia-reperfusion injury, aortic valve
344 calcification, pathological hypertrophy, and heart failure, atherosclerosis in carotid
345 arteries, physiological cardiac hypertrophy, pulmonary arterial hypertension, and
346 cardiomyocyte proliferation, respectively.

347 It has also been reported that several circRNAs have an important role in heart failure
348 and myocardial infarction. Global circRNA profiling demonstrated that the ultraconserved
349 *circ-INSR*, derived from the gene encoding the insulin receptor (*INSR*), regulates
350 mitochondrial functions in cardiomyocytes under doxorubicin stress¹⁵¹. In human and
351 mouse failing heart tissue, the expression of *circ-INSR* was diminished, inducing
352 apoptosis of cardiomyocytes and impairing metabolic activity¹⁵¹. Consistent with this

353 observation, overexpression of *circ-INSR* successfully decreased doxorubicin-induced
354 DNA damage and apoptosis of primary rat cardiomyocytes¹⁵¹.

355 In a recent study, researchers identified and investigated the function of *circHIPK3*,
356 derived from exon 2 of the *HIPK3* gene within mouse heart. *CircHIPK3* negatively
357 regulates the RBP Hur at the post-transcriptional level, which leads to the destabilization
358 of *p21* mRNA in a rat cardiomyocyte cell line (H9C2) and primary mouse
359 cardiomyocytes¹⁵². In addition, *circMAP3K5* is known to be associated with SMC
360 differentiation by sponging miR-22-3p and thereby inducing the expression of *TET2*¹⁵³.

361 A recent study demonstrated the protective effects of *circSlc8a1* (a circular antisense
362 RNA) in the context of heart injury, highlighting the important role of *circSlc8a1* in
363 preserving physiological heart function¹⁵⁴. The experimental induction of cardiac-specific
364 expression of *cA-circSlc8a1* in mice resulted in profound phenotypic alterations, notably
365 characterized by a substantial increase in body weight, hepatic steatosis, and impaired
366 cardiac function¹⁵⁴. Another study demonstrated that the *circNlgn*, along with its translated
367 product Nlgn173, is a mediator of doxorubicin-induced cardiomyocyte fibrosis¹⁵⁵. Silencing
368 endogenous *circNlgn* has been found to reduce doxorubicin-induced cardiomyocyte
369 apoptosis and enhance cardiomyocyte viability. Moreover, the silencing of *circNlgn*
370 effectively inhibits collagen deposition and enhances the expression of fibrosis markers.
371 These findings suggest that targeting *circNlgn* could potentially alleviate the adverse
372 effects associated with doxorubicin, particularly its impact on fibrosis development¹⁵⁵.

373 In summary, ncRNAs have emerged as crucial regulators of cardiovascular
374 diseases^{156 157}, highlighting a promising research area that warrants further exploration.
375 Future research should continue to elucidate the molecular mechanisms and potential
376 therapeutic applications of ncRNAs, while large-scale, multicenter studies will have a
377 crucial role in validating their translational feasibility from the laboratory to the clinic.

378

379 **[H2] Non-coding RNAs in neurodegenerative disorders**

380 Neurodegenerative disorders, such as Alzheimer disease, Parkinson disease,
381 amyotrophic lateral sclerosis (ALS), and Huntington disease, are characterized by

382 degeneration and loss of neurons in specific areas of the brain and the spinal cord.
383 Neurodegenerative disorders are currently irreversible and tend to worsen over time with
384 no effective treatment available; they are thus associated with severe morbidity and are
385 considered one of the leading causes of death by the World Health Organization (WHO).

386 Dysregulation of miRNA expression has been frequently observed in the central
387 nervous system and is a powerful modulator of the onset of neurodegeneration¹⁵⁸. It has
388 been shown that increased expression of miR-29b-3p in striatal medium spiny neurons
389 (MSN) is associated with age and contributes to the degeneration of MSNs in Huntington
390 disease by directly targeting the 3'-UTR of *STAT3*¹⁵⁹. Downregulation of *STAT3*
391 diminished autophagy and increased apoptosis in patient-derived MSNs. In Huntington
392 disease-MSNs, administration of anti-miR-29b-3p reduced neural cell death, whereas the
393 depletion of *STAT3* counteracted the therapeutic effect of anti-miR-29b-3p treatment¹⁵⁹.
394 In other instances, miR-520f-3p, miR-135b-3p, miR-4317, miR-3928-5p, and miR-8082
395 have been found to be significantly differentially expressed in patients with Huntington
396 disease compared to the control group¹⁶⁰. Also, several miRNAs have been associated
397 with Alzheimer disease through directly regulating disease-associated risk factors,
398 including beta-site amyloid precursor protein cleaving enzyme 1 (*BACE1*), amyloid
399 protein precursor (*APP*) cleavage, and presenilin-1 (*PSEN1*)¹⁶¹. Moreover, an elevated
400 miR-543 level was found in the white matter tissue of patients with early-stage Parkinson
401 disease, associated with a decreased level of *SIRT1* protein, a potential target of miR-
402 543. Subsequent in vitro experiments confirmed *SIRT1* as a direct target of miR-543 and
403 the upregulation of miR-543 resulted in transcriptional downregulation of *SIRT1* in a
404 neuroblastoma cell line and foetal astrocytes¹⁶².

405 As many lncRNAs (~40%) are expressed in a brain-specific manner¹⁶³, experimentally
406 altering their expression might lead to important insights into neuronal development and
407 the pathogenesis of neurodegenerative disorders. However, only a small proportion of
408 lncRNAs have been studied with regard to their role in neurodevelopment and brain
409 function. For example, the lncRNA *RUS*, located upstream of the *Slitrk3* gene, is
410 predominantly expressed in neural tissues, and its level increases during the
411 differentiation of neural stem cells into neurons¹⁶⁴. Depletion of *RUS* resulted in
412 proliferation arrest and induced apoptosis in mouse embryonic cortical neural stem

413 cells¹⁶⁴. Another example is the lncRNA *TUNA*, which has been implicated in the neural
414 differentiation of mouse embryonic stem cells¹⁶⁵. When *TUNA* is depleted, embryonic
415 stem cell proliferation is compromised, although pluripotency is maintained. The *TUNA*-
416 RNA binding proteins complex was detected at the promoters of important regulators of
417 embryonic stem cell differentiation, including *Nanog*, *Sox2* and *Fgf4*. Single knockdown
418 of each of these RBPs led to inhibition of neural differentiation of mouse ESCs, similar to
419 the effect of *TUNA* knockdown¹⁶⁵.

420 The role of circRNAs in the molecular pathogenesis of neurodegenerative disorders
421 and brain aging was recently reviewed¹⁶⁶. Several circRNAs associated with
422 neurodegenerative diseases were shown to act as miRNA sponges, such as *circHDAC9*,
423 *circSAMD4A*, *circDLGAP4*, and *circSLC8A1*¹⁶⁶. The mechanisms of action of these
424 circRNAs during normal conditions remain unknown, and it is therefore difficult to
425 determine their exact role in disease development. In rat spinal cord injury (SCI) models,
426 the expression of *circRNA-2960* was found to be significantly enriched and it was
427 suggested that *circRNA-2960* might exacerbate secondary damage to the spinal cord.
428 Mechanistically, *circRNA-2960* inhibits its target miR-124, a molecule that prevents
429 secondary injuries from SCI and promotes injury recovery. The regulation of miR-124
430 expression by *circRNA-2960* could therefore represent a crucial mechanism that
431 influences the prognosis of SCI¹⁶⁷. Overall, despite clear evidence of aberrant expression
432 of circRNAs in neurological disorders¹⁶⁸⁻¹⁷⁰, the functional significance of these alterations
433 remains to be thoroughly investigated. Because each ncRNA could be involved in
434 different pathways in neurons, further studies at the level of singular targets of specific
435 ncRNAs are warranted to discover ncRNAs that could serve as biomarkers and
436 therapeutic targets for neurodegenerative disorders.

437

438 **[H1] ncRNAs in infectious diseases and sepsis**

439 Many studies have assessed how ncRNAs are involved in immune defense against
440 microbial infections^{171,172}. When considered collectively, ncRNAs act as positive or
441 negative regulators to encourage a balanced immune response for an effective defense

442 against pathogens¹⁷³. In **Box 1** we highlight the example of miR-155, which is functionally
443 involved in many types of diseases, and discuss its therapeutic use.

444 miRNAs have been functionally connected to the cellular response during microbial
445 infections. For example, miR-718, which is encoded from the 5' UTR of *IRAK1*, was
446 shown to have an anti-inflammatory function through targeting PTEN¹⁷⁴. IRAK1 is an
447 important component of the TLR signaling pathways and thereby has a role in innate
448 immunity, whereas PTEN downregulation by miR-718 decreases proinflammatory
449 cytokine production through its downstream target molecules¹⁷⁴. Pre-miR-718 is highly
450 conserved across mammals and decreased miR-718 expression was shown to be
451 associated with *Neisseria gonorrhoeae* infection¹⁷⁴. It was hypothesized that miR-718 can
452 help to evade recurrent bacterial infections and lower the lipopolysaccharide (LPS)-
453 induced mortality rate by establishing LPS-induced tolerance¹⁷⁴. On the same TLR
454 pathway, let-7i directly binds to and downregulates *TLR4*¹⁷⁵, participating in the immune
455 response against *Cryptosporidium parvum*, a parasite that causes intestinal and biliary
456 infections.

457 It was shown that *MALAT1* lncRNA is a key player in controlling macrophage M1/M2
458 polarization¹⁷⁶. Briefly, *MALAT1* expression is upregulated in LPS-treated macrophages,
459 which differentiate towards a proinflammatory M1 phenotype, and it is downregulated in
460 IL-4-treated cells, which differentiate in the M2 subtype. Notably, *MALAT1* knockdown
461 decreases LPS-induced M1 macrophage activation, whereas IL-4-induced M2
462 differentiation and a macrophage profibrotic phenotype are increased by *MALAT1*
463 knockdown¹⁷⁶. Consistent with these observations, an independent study found that the
464 expression of *Mirt2* lncRNA is elevated upon activation of the LPS-p38-Stat1 and LPS-
465 IFN- α/β -Stat1 pathways in mouse macrophages¹⁷⁷. Increased levels of *Mirt2* upon LPS
466 treatment inhibited the K63-ubiquitination of TRAF6 and relieved inflammatory responses
467 after TLR4 activation¹⁷⁷. Other early experiments found that treating M2 microglia cells
468 with IL-4 caused a dramatic decrease in the expression of lncRNA *GAS5* compared with
469 resting microglia¹⁷⁸. Mechanistically, *GAS5* negatively regulates the transcription of *IRF4*
470 by binding PRC2 to inhibit M2 polarization¹⁷⁸. Recently, it was reported that ablation of
471 the mouse lncRNA *Malat1* activates the antioxidant pathway and alleviates sepsis¹⁷⁹.

472

473 [H2] Sepsis and the balance between human and viral miRNAs

474 Sepsis, the final stage of full-body disequilibrium to pathogenic bacterial, viral or fungal
475 infections, remains a leading cause of human death and currently has no pathogenesis-
476 specific therapy¹⁸⁰. Since the initial discovery of downregulated miR-150 in peripheral
477 blood cells and plasma from patients with septic shock¹⁸¹, a substantial number of
478 dysregulated cellular miRNAs in sepsis have been identified¹⁸². Cellular overexpression
479 of miR-150 was sufficient to inhibit the pre-pro-B cell to pro-B cell transition by targeting
480 MYB and FOXP1, respectively^{183,184}. The miR-212 and miR-132 cluster was found to
481 have a similar effect through negative regulation of FOXP1 and SOX4, respectively¹⁸⁵. In
482 another study, the loss of function of the miR-15 family, which comprises the miR-
483 15a/miR-16-1, miR-15b/miR-16-2, and miR-497/miR-195 clusters, reduced normal pre-B
484 differentiation by directly targeting cyclin E1 and D3¹⁸⁶. Another recent study reported that
485 miR-146a-5p is highly enriched in *Leishmania donovani*-infected bone marrow-derived
486 macrophages (BMDMs), and positively correlates with dose and time of infection, which
487 was further examined in an in vitro mouse model¹⁸⁷. In infected BMDMs, downregulation
488 of miR-146a-5p led to a decrease in *Arg1* expression and abundance of iNOS. It was
489 observed that silencing BRD4 effectively restored miR-146a-5p expression and M2
490 polarization marker expressions in infected BMDMs¹⁸⁷. Another therapeutically important
491 sepsis-related miRNA, miR-93-5p, was uncovered by analyzing mouse and baboon
492 models of sepsis, in addition to human peripheral blood mononuclear cells (PBMCs)
493 obtained from patients with sepsis. In an in vivo mouse sepsis model, inhibition of miR-
494 93-5p reduced inflammatory monocytes and increased circulating effector memory T
495 cells, resulting in longer survival¹⁸⁸.

496 Although investigation of a single miRNA can yield insight into its biological function,
497 it does not capture the complex, interconnected network of miRNAs that control cell
498 biology and disease. In patients with sepsis, the miRNA network exhibits significantly less
499 connection when compared to that of healthy controls. Perhaps explaining this
500 observation, several miRNAs, including miR-16, miR-29a, miR-146, miR-155, and miR-

501 182, were reported to be 'sponged' by their protein coding targets in patients with
502 sepsis¹⁸⁹.

503 Unexpectedly, it was discovered that Kaposi sarcoma virus (KSV)-produced miRNAs
504 are differentially expressed in sepsis and may be used for diagnostic and therapeutic
505 purposes. Specifically, elevated levels of miR-K-10b and miR-K12-12* play a functional
506 role in sepsis as agonists of TLR8, leading to cytokine dysregulation characteristic of a
507 cytokine storm¹⁹⁰. Moreover, the viral Epstein Barr miR-BHRF-1 and the KSV miR-K12-
508 12 were detected in plasma during the early systemic response to injury and were
509 associated with unfavorable outcomes in polytrauma patients¹⁹¹. Starting from these
510 observations, we suggest considering non-human (particularly viral) ncRNAs when using
511 next-generation sequencing (NGS) methods to screen for ncRNAs involved in sepsis and
512 other human diseases. Although virus-encoded-ncRNAs have largely been linked to
513 immune evasion, virus life cycle regulation and virus-induced tumorigenesis¹⁹², there is a
514 considerable gap in understanding the specific mechanisms and processes underlying
515 these functions.

516

517 [H2] SARS-CoV-2 infection and ncRNAs

518 Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome
519 coronavirus 2 (SARS-CoV-2), has led to hundreds of millions of confirmed cases and
520 millions of deaths all over the world in the last few years¹⁹³. The importance of ncRNAs
521 in infectious diseases prompted scientists to swiftly investigate their potential role in
522 COVID-19 soon after the beginning of the pandemic¹⁹⁴. As a result, several studies
523 published in the last three years demonstrate the involvement of miRNAs and lncRNAs
524 in COVID-19^{12,195-197}.

525 A recent study reported that SARS-CoV-2 expresses a miRNA-like small RNA, termed
526 CoV2-miR-O7a, which is derived from the coding region of the *ORF7a* transcript¹⁹⁵.
527 CoV2-miR-O7a is associated with Ago proteins, seems to influence interferon signaling
528 pathways, and may contribute to SARS-CoV-2 pathogenesis¹⁹⁵. It has also been shown
529 that lncRNAs, such as *CHROMR*, are overexpressed in patients with SARS-CoV-2

530 infection¹⁹⁸. Depletion of *CHROMR* resulted in attenuated interferon-stimulated gene
531 expression and the sequestration of the nuclear repressor complex, IRF-2/IRF2BP2¹⁹⁸.
532 In addition, other lncRNAs, such as *PIRAT* and *LUCAT1* have been shown to be
533 upregulated in patients with COVID-19 and were implicated in the progression of the
534 disease. *PIRAT* appears to be preferentially expressed in myeloid cells and has been
535 connected to tissue infiltration in infectious and inflammatory diseases¹⁹⁷. Through the
536 inhibition of alarmin expression, *PIRAT* creates a negative feedback loop with PU.1,
537 located in the nucleus of human monocytes¹⁹⁷. Consistent with this finding, negative
538 feedback regulation was observed between *LUCAT1* and Jack-STAT-dependent IFN
539 immunity¹⁹⁹.

540 Coronavirus transcriptomes seem to contain additional components that contribute to
541 the intensified inflammatory responses observed in patients with SARS-CoV-2, SARS,
542 and Middle East Respiratory Syndrome (MERS) ²⁰⁰. circRNAs encoded by coronavirus
543 genomes have been identified and are implicated in viral pathogenesis²⁰⁰. For instance,
544 two SARS-CoV-2 circRNAs contain IRES signals and have the potential for translation²⁰⁰.
545 Moreover, in a study utilizing the human-pathogenic MERS-CoV as a model, the
546 interactions between circRNAs and key components of the host cell competing
547 endogenous RNA network were demonstrated, revealing several differentially expressed
548 circRNAs during coronavirus replication²⁰¹. Downregulation of circFNDC3B and
549 circCNOT1 resulted in a substantial reduction in MERS-CoV viral load in human lung
550 cancer cells (Calu-3) and fibroblast cells (HFL1), potentially related to the downregulation
551 of their target genes, specifically *MAP3K9* and *USP15*²⁰¹. Further research is needed to
552 identify clinically applicable ncRNA signatures and explore the role of ncRNA in
553 immunological and peripheral system regulation. However, a signature composed of 6
554 lncRNAs including *NRIR*, *BISPR*, *MIR155HG*, *FMR1-IT1*, *USP30-AS1*, and *U62317.2*
555 has been shown to be associated with the regulation of SARS-CoV-2 infection¹⁹⁶.
556 Moreover, the importance of coronavirus infections as a source of interacting RNA and
557 identifying novel drug targets for patients affected by SARS-CoV, SARS-CoV-2, and
558 MERS patients remain important areas of investigation.

559

560 **[H1] ncRNAs as disease biomarkers**

561 Interest among the scientific community in the use of ncRNAs as disease biomarkers rely
562 on several critical observations and considerations. First, relevant changes in ncRNA
563 expression or activity have been detected in pathological tissues, rendering these
564 molecules good indicators of underlying disease state or specific disease features²⁰².
565 Second, technologies are available for quantifying small and long ncRNAs in different
566 tissue types and across different preservation methods (such as fresh frozen and fixed
567 tissue samples). These span from PCR-based assays, through hybridization-based
568 methods to NGS-based technologies. Finally, it is possible to quantify specific ncRNA
569 molecules in cellular and subcellular compartments of diseased cells, as well as in
570 extracellular compartments (such as extracellular vesicles, body fluids including urine,
571 saliva, cerebrospinal fluid, synovial fluid, placenta, and breast milk)²⁰³, which makes these
572 molecules suitable for liquid biopsy applications. MicroRNAs were recently found to be
573 selectively inserted in extracellular vesicles, that also display a moderate content of small
574 ncRNAs, the distribution and composition of which depends on the size and isolation
575 method. Comprehensive review articles have recently been published covering the main
576 discoveries on biomarker ncRNAs and human diseases^{8,204-210}.

577 Several considerations should be made regarding studies on lncRNA in liquid biopsy
578 samples. The levels of lncRNA in plasma and serum are particularly low compared to
579 intracellular levels, their detection can be challenging, and their stability can be a
580 concern²¹¹⁻²¹³. Therefore, the preanalytical steps should be well-standardized, and the
581 appropriate reference genes should be selected carefully²¹¹⁻²¹³. Furthermore, the
582 examination of genome-wide miRNA expression profiles in both healthy and diseased
583 aging individuals has revealed that age has a significant impact on blood miRNA
584 composition, potentially compounding the interpretation of results in the older
585 population^{214,215}.

586

587 **[H2] ncRNAs as biomarkers in cancer**

588 Each year, thousands of papers are published on the differential expression of ncRNAs
589 in various types of cancer. Although many of these studies suggest that the identified

590 dysregulated ncRNAs have potential as biomarkers and/or therapeutic targets, only a
591 small fraction will reach in clinical trials. The [NIH Early Detection Research Network](#)
592 ([EDRN](#)) and the [ClinicalTrials.gov](#) database are useful to track the hundreds of ongoing
593 clinical trials of ncRNAs as potential biomarkers for cancer and other diseases. **Table 1**
594 provides examples of clinical trials that examine the use of ncRNAs as biomarkers of
595 disease; a comprehensive summary of clinical trials at [ClinicalTrials.gov](#) is provided in
596 **Supplementary Table 1**.

597

598 *[H3] ncRNAs as tissue biomarkers*

599 Among the small ncRNA biomarkers most broadly tested in cancer tissues is miR-21-5p,
600 which has been validated as a diagnostic or prognostic biomarker in most frequent
601 cancers, such as lung²¹⁶, breast²¹⁷, colorectal^{218,219}, and CLL²²⁰. miR-21 is also released
602 in circulation and detectable in extracellular vesicles, as described in the next section.
603 miR-506-3p is another miRNA that has demonstrated significant prognostic potential
604 across multiple cancer types, including ovarian, pancreatic, and gastric cancer²²¹⁻²²³.
605 Likewise, miR-31-3p has predictive potential and has been shown to have clinical
606 relevance in metastatic colorectal cancer (CRC). Specifically, low levels of miR-31-3p
607 have been identified as a predictor of response to anti-EGFR therapy in two clinical
608 studies^{224,225}. Currently, miR-10b-5p is the subject of investigation in an ongoing multi-
609 site clinical trial on gliomas as a potential biomarker (NCT01849952) and has been
610 identified as a potential therapeutic target in advanced glioblastoma²²⁶.

611 Regarding lncRNA biomarkers, the most studied lncRNA in cancer tissues is *HOTAIR*.
612 *HOTAIR* is involved in chromatin reprogramming, and its expression has been tested as
613 a diagnostic or prognostic and predictive biomarker in ovarian²²⁷, colorectal²²⁸, breast²²⁹,
614 esophageal²³⁰, and pancreatic²³¹ cancer. *MALAT1* has been identified as an early
615 prognostic marker for metastasis development in surgically removed lung cancer²³², while
616 *CCAT1* and *CCAT2* have well-characterized mechanistic roles and serve as prognostic
617 biomarkers for CRC^{233,234}. The detection of *PCA3* in urine is an FDA-approved diagnostic
618 biomarker for prostate cancer. Furthermore, the combined measurement of *PCA3* and

619 the *TMPRSS2:ERG* gene fusion in prostate biopsy can improve the detection of prostate
620 cancer²³⁵.

621

622 *[H3] ncRNA biomarkers in liquid biopsies and extracellular vesicles*

623 Circulating ncRNA biomarkers gained much attention in biomedical research when it was
624 discovered that several tumour-associated miRNAs, including miR-155 and miR-21, were
625 elevated in the serum of patients with diffuse large B cell lymphoma (DLBCL)²³⁶.
626 Additionally, serum levels of miR-141 were found to distinguish patients with prostate
627 cancer from healthy controls. It subsequently became clear that miRNAs are stable in a
628 large variety of biological fluids²⁰³.

629 As aberrant miR-21-5p expression is associated with inflammatory events, increased
630 levels of this miRNA in serum or plasma have been reported in many human conditions,
631 spanning from cancer to cardiac disorders. In cancer, there is now convincing data on the
632 value of miR-21-5p as a biomarker for gastric, esophageal and prostate cancer^{218,237-239}.
633 Among the circulating miRNAs with the confirmed diagnostic value, miR-371-3p is highly
634 promising; indeed, a prospective multicentric study on testicular germ cell tumours²⁴⁰
635 (teratoma excluded), revealed that miR-371-3p had a detection accuracy greater than
636 90%, higher than standard-of-care biomarkers, including alpha-fetoprotein in surgically
637 resected tumours²⁴¹. ncRNA biomarkers can be particularly useful in diseases that are
638 difficult to diagnose, and where no coding gene-based biomarkers have been
639 identified²⁴².

640 A frequently explored opportunity in biomarker studies of circulating miRNAs is the
641 use of miRNA signatures, composed of miRNAs that are expressed concordantly. Two
642 studies have proposed miRNA signatures consisting of 13 miRNAs for early detection of
643 lung cancer, to be used alone^{243,244} or in combination with low-dose Computer
644 Tomography (CT) scan²⁴⁵, with promising results in clinical trials. Another group validated
645 an eight miRNA signature for the early diagnosis of esophageal squamous cell
646 carcinoma²³⁹ in different prospective cohorts. A circulating miRNA signature composed
647 of five miRNAs can distinguish more aggressive prostate cancers²⁴⁶.

648 The levels of miR-25-3p and miR-92a-3p were tested as prognostic biomarkers in
649 patients with liposarcoma since they are secreted by liposarcoma cells through
650 extracellular vesicles. The secreted miRNAs indeed act as proinflammatory signals for
651 tumour-associated macrophages²⁴⁷.

652 Detection of unmethylated fragments of the lncRNA *XIST* in plasma has been
653 associated with testicular cancer presence²⁴⁸ and proposed as a testicular germ-cell
654 tumour biomarker²⁴⁹. Moreover, the lncRNA *ANRIL* was found to be upregulated in bone
655 marrow mononuclear cells of patients with acute myeloid leukemia (AML) compared to
656 healthy donors and suggested to serve as a valuable prognostic biomarker for AML²⁵⁰.

657

658 **[H2] ncRNAs biomarkers in cardiovascular diseases**

659 ncRNAs with a diagnostic role in cardiovascular disease have been extensively
660 investigated²⁰⁶. Cardiac tissue is not easily accessible; therefore, biomarkers of cardiac
661 disorders are commonly tested in the blood or blood derivatives (plasma and serum)²⁵¹.
662 A prospective study tested the association between extracellular miRNA levels and heart
663 failure risk in approximately 2,400 individuals²⁵². Three plasmatic miRNAs (miR-17, miR-
664 20a, and miR-106b) were associated with heart failure, such that individuals with higher
665 levels of these miRNAs had a 15% reduction in long-term incident heart failure, after
666 adjustments for other risk factors.

667 Cardiac tissue-specific miR-1, miR-133, and miR-208 (collectively known as myomirs)
668 have been tested as circulating biomarkers in different settings. These miRNAs are
669 collectively released to the circulation upon heart failure²⁵³ and were proposed as
670 diagnostic biomarkers to distinguish coronary artery disease (CAD, the primary cause of
671 mortality in the United States), acute coronary syndrome, and heart failure²⁵⁴. As
672 prognostic biomarkers, circulating miR-132, miR-140-3p, and miR-210 were validated as
673 survival predictors in CAD on 1112 individuals using a multivariate model²⁵⁵.

674 The detection of various lncRNAs in the bloodstream suggests that they are either
675 protected from RNase-mediated degradation, similar to miRNAs or that they originate
676 from a plentiful source with a continuous release. Two circulating lncRNAs, *ZFAS1* and

677 *ICDR1AS*, have recently been identified as independent predictors of myocardial
678 infarction²⁵⁶. However, the precise origins and mechanisms linking these lncRNAs to
679 myocardial infarction remain uncertain, as they were derived from whole blood²⁵⁶.
680 Conversely, decreased levels of the lncRNA *HOTAIR* were observed in the plasma of
681 myocardial infarction patients and were described to be cardioprotective through
682 interaction with miR-1²⁵⁷.

683 CircRNAs are present in abundance in bodily fluids such as blood, urine, and
684 extracellular vesicles. These molecules have high stability and exhibit differential
685 expression patterns in response to stress stimuli²⁵⁸. The circRNA *MICRA*, also known as
686 *circ-ZNF609*, was found to be significantly reduced in the blood of individuals who have
687 experienced myocardial infarction and serves as a promising prognostic biomarker for left
688 ventricular dysfunction following myocardial infarction^{259,260}. Furthermore, circRNA
689 microarray analysis of PBMCs from patients with CAD has revealed
690 *hsa_circRNA_0001879* and *hsa_circRNA_0004104* as potential diagnostic biomarkers
691 for this condition²⁶¹. Besides the lncRNAs and circRNAs mentioned above, additional
692 transcripts have been suggested as potential circulating biomarkers in CVD^{262,263}. The
693 testing of lncRNAs and circRNAs as biomarkers in CVD is still at an early stage, although
694 their above-mentioned dysregulation points toward potential usefulness in the near future,
695 as it was recently reviewed^{264,265}. A large proportion of the findings that suggest lncRNAs
696 and circRNAs may serve as biomarkers for CVD are based on studies that involve limited
697 numbers of participants and therefore require independent validation in larger studies.

698

699 **[H2] ncRNAs biomarkers in neurodegenerative diseases**

700 As obtaining nervous tissue from living individuals can be challenging, body fluids are
701 often used as the most reliable source of ncRNAs. Several studies investigated the use
702 of circulating small RNAs as Alzheimer disease biomarkers, tested either in cerebrospinal
703 fluid (CSF), serum, or plasma, and derived extracellular vesicles. Serum miRNA
704 signatures to differentiate frontotemporal lobar degeneration²⁶⁶ or other forms of
705 dementia²⁶⁷ and Alzheimer disease were proposed. The neuronal-released miR-181a-5p
706 was proposed as a circulating prognostic biomarker for ALS²⁶⁸. In this study, the authors

707 performed longitudinal monitoring of miR-181 in 252 patients subdivided into discovery
708 and validation cohorts, and the potential of plasma miR-181 in predicting patients' death
709 risk was assessed.

710 A panel of lncRNAs, quantified in PBMCs, was proposed as a diagnostic biomarker
711 for multiple sclerosis²⁶⁹. Two of these lncRNAs, *NRON* and *TUG1*, were validated in an
712 independent cohort. As for discrimination of multiple sclerosis subtypes, both CSF- and
713 blood-circulating miRNAs have been investigated. Specifically, high levels of CSF miR-
714 181c were associated with conversion from clinically isolated syndrome to relapsing-
715 remitting (recovering) multiple sclerosis²⁷⁰. Serum miR-191-5p and miR-128-3p were
716 associated with progressive forms (no recovery) of multiple sclerosis²⁷¹.

717 A total of 4,060 circRNAs with differential expression levels were identified in PBMCs
718 derived from patients diagnosed with Alzheimer disease²⁷². In silico analysis showed that
719 the top 10 dysregulated circRNAs were strongly associated with various risk factors of
720 AD, including inflammation, metabolism, and immune responses. These findings suggest
721 that these circRNAs might play a potential role in the diagnosis of AD²⁷². As another
722 example, high expression levels of three circRNAs, *circFUNDC1*, *circPDS5B*, and
723 *circCDC14A*, were found in patients with acute ischemic stroke (AIS) compared with
724 healthy controls²⁷³. The elevated expression levels of these circRNAs were found to be
725 positively correlated with infarct volume. These findings suggest that the three circRNAs
726 may serve as potential biomarkers for the diagnosis of AIS²⁷³.

727

728 [H1] Non-coding RNA therapeutics

729 The use of RNA-based therapies has emerged as a promising treatment approach for
730 human diseases and vaccine development. Certain endogenous ncRNAs can regulate
731 the expression of genes involved in human diseases, and their dysregulated expression
732 can contribute to the onset of disease, highlighting the potential of these ncRNAs as
733 targets for drug development. ncRNAs play a dual role in cancer as either oncogenes or
734 tumour suppressors, leading to the abnormal inhibition or degradation of their target
735 mRNAs, and as a result, they serve as both direct therapeutic targets and potential
736 therapeutic candidates for cancer treatment. In this respect, the utilization of miRNA-

737 based therapeutics offers dual advantages. Firstly, as natural molecules found within
738 human cells, miRNAs possess pre-existing mechanisms for their processing and
739 downstream target selection, in contrast to artificial chemotherapy compounds or
740 Antisense Oligonucleotides (ASOs). Secondly, miRNAs target multiple genes within a
741 single pathway, leading to a more comprehensive and specific response. Several recent
742 reviews have extensively discussed a range of approaches to modulate the therapeutic
743 potential of ncRNAs, including the use of siRNAs, ASOs, shRNAs, anti-miRNAs, miRNA
744 mimics, miRNA sponges, therapeutic circRNAs, and CRISPR-Cas9-based gene
745 editing^{52,274-278}. Currently, several ongoing clinical trials investigate the specific targeting
746 of miRNAs for therapeutic purposes (**Table 2**); it is expected that similar clinical trials for
747 lncRNAs and circRNAs will begin in the near future.

748

749 ***[H2] Approaches of non-coding RNA therapeutics***

750 Currently, two main approaches exist for ncRNA-based therapeutic interventions,
751 depending on the desired molecular outcome. The first is ncRNA antagonism, which
752 involves inhibiting or repressing the expression or function of target ncRNA transcripts,
753 often achieved by antisense RNAs. The second approach, known as ncRNA replacement
754 therapy, aims to restore the expression or function of the target ncRNA and mostly
755 involves the introduction of small RNAs.

756 One example of the former approach is Replarsen (MRG-201), a molecule designed
757 to mimic the activity of miR-29, which was shown to reduce the expression of proteins
758 involved in skin fibrosis²⁷⁹. MRG-201 was investigated in a phase 2, double-blind,
759 placebo-controlled study (NCT03601052) to explore the efficacy, safety and tolerability of
760 the drug following intradermal injection in individuals with a history of scar fibrosis
761 (keloids). Furthermore, based on the reports that MRG-201 reduced fibrosis in animal
762 models, a peptide-conjugated MRG-229 mimic was developed as a potential therapy in
763 humans with idiopathic pulmonary fibrosis²⁸⁰. After detailed anti-fibrotic activity tested in
764 multiple models, including TGF- β 1-treated human lung fibroblasts (NHLFs) and human
765 precision-cut lung slices (hPCLS), in vivo bleomycin studies and toxicology in rats and
766 non-human primates, the outcomes supports further clinical development²⁸⁰.

767 A recent example of directly targeting ncRNA transcripts is a phase 1 randomized,
768 double-blind, placebo-controlled study to assess the safety, pharmacokinetics, and
769 pharmacodynamic properties of CDR132L, an antisense oligonucleotide-based inhibitor
770 of miR-132, in patients with stable heart failure of ischaemic origin (NCT04045405). This
771 trial described the linear plasma pharmacokinetics of miR-132, with no signs of
772 accumulation, and was associated with cardiac functional improvements²⁸¹.

773 Additional developments in ncRNA-related therapeutics are of particular interest. A
774 new therapeutic option is to target the downstream pathways of master regulator ncRNAs,
775 including their target coding genes. As an example, the miR-15a/16-1 cluster is an
776 essential player in CLL pathogenesis by targeting key anti-apoptotic proteins, BCL2 and
777 MCL1²⁸². When this cluster is downregulated or deleted, as in CLL, the downstream
778 coding genes are upregulated, and the malignant cells lose anti-apoptotic potential and
779 survive for longer periods. This mechanism had made CLL a deadly disease, until the
780 development of Venetoclax (ABT199), a BCL-2 homology 3 (BH3) mimetic that
781 specifically inhibits Bcl-2. Its use has fundamentally changed the natural history of the
782 disease. Today, the therapeutic combination of Venetoclax and inhibitors of Bruton
783 tyrosine kinase (such as Ibrutinib) increase progression-free survival and overall survival
784 rates at 24 months to 95% and 98%, respectively²⁸³. As the downregulation of miR-15a/16
785 cluster is frequent also in other human cancers²⁸⁴, the identification of patients with
786 genomic deletions or mutations and/or with reduced expression of miR-15a/16 can
787 identify patients who might respond well to Venetoclax or other novel BH3 mimetics.

788 Due to different mechanisms of action, a specific microRNA can be considered either
789 a drug or a drug target in different pathologic conditions. For example, miR-16 was
790 discovered as a tumour suppressor miRNA and therefore restoration by a miR-16 mimetic
791 constitutes a suitable therapeutic strategy in cancers where this miRNA has reduced
792 expression. For example, the safety and activity of miR-16-loaded minicells in patients
793 with recurrent malignant pleural mesothelioma showed an acceptable safety profile and
794 early signs of activity²⁸⁵. By contrast, recent studies showed that endothelium-targeted
795 deletion of the miR-15a/16-1 cluster ameliorates blood-brain barrier dysfunction in
796 ischemic stroke²⁸⁶ and poststroke angiogenesis and improves long-term neurological
797 recovery²⁸⁷. In this setting, the use of anti-miR-16 agents can result in adequate

798 therapeutic progress. Another promising therapeutic target is miR-21, which, besides its
799 relevance to other diseases, has recently been demonstrated to be upregulated in
800 pulmonary macrophages of both patients with COVID-19 and mice exhibiting acute
801 inflammatory lung injury. The inhibition of miR-21 (using RCS-21) reversed the
802 pathological activation of the macrophages and prevented pulmonary dysfunction and
803 fibrosis after acute lung damage in the mouse model²⁸⁸. The development of small-
804 molecule inhibitors (SMIs) of miRNAs, which directly bind and inhibit the activity of an
805 oncogenic or disease-causing miRNA (**Box 1**), has important advantages over the use of
806 oligonucleotides, such as superior metabolic stability, solubility and bioavailability^{276,289-}
807 ²⁹¹. By use of **small molecule inhibitors [G]**, effective inhibition of oncomiRs (such as miR-
808 10b), as well as the upregulation of some important tumour suppressor targets (such as
809 PTEN) has been achieved in pre-clinical studies²⁹². In addition, two small molecules
810 targeting specifically the triple-helical element for nuclear expression in *Malat1* RNA, but
811 not other similar structures present in the lncRNA *Neat1*, were identified by high-
812 throughput screening. The compounds significantly reduced *Malat1* levels and activation
813 of its downstream genes and induced the phenotypic attenuation of mammary gland
814 organoids branching²⁹³. This approach has great therapeutic potential as it can be
815 developed against small RNAs with very similar structures (as miR-21 and miR-10b), as
816 well as any large RNA that has a known secondary structure and that is involved in any
817 non-cancer disease including infectious diseases. An example of a potentially targetable
818 RNA is the genome of SARS-CoV-2, which is the largest single-stranded RNA virus
819 known to infect humans²⁹⁴.

820

821 **[H2] Challenges of non-coding RNA therapeutics**

822 Although ncRNAs have demonstrated therapeutic potential in vitro and in vivo, their
823 limited bioavailability in vivo presents a major challenge to their clinical translation²⁷⁵. To
824 overcome this obstacle, advanced drug delivery strategies are urgently required. To
825 address the problems of a short half-life, off-target effects, and low transfection efficiency
826 associated with RNA delivery, various ncRNA carriers and systems have been proposed
827 and extensively investigated, including several types of nanoparticles, ncRNA

828 modification, and the oncolytic adenovirus strategy. These strategies represent promising
829 approaches to enhance the delivery and efficacy of ncRNA-based therapies in vivo²⁷⁵.

830 In parallel to improving delivery systems, challenges regarding the safety of ncRNA
831 therapies need to be addressed. Despite ongoing clinical trials evaluating miRNA
832 therapeutics for the treatment of human diseases, immune-related side effects still
833 present a significant challenge²⁹⁵. To illustrate, a phase I clinical study involving MRX34,
834 a liposomal miR-34a mimic, was prematurely terminated due to severe immune-related
835 side effects that resulted in the unfortunate deaths of four patients²⁹⁶. However, there is
836 still uncertainty regarding the specific cause of the clinical effects (including both toxicity
837 and anti-tumor activity) observed in MRX34. Serious adverse events (SAEs) attributed to
838 the treatment were predominantly observed later in the treatment cycle, occurring after
839 the completion of daily MRX34 infusions. These SAEs included sepsis, hypoxia, cytokine
840 release syndrome, and hepatic failure, which collectively suggest a pattern indicative of
841 immune-mediated toxicity²⁹⁶. It is unclear whether these effects are attributable to the
842 targeted gene-suppressing activity of the miR-34a nucleotide, a non-specific
843 inflammatory response triggered by the dsRNA present in the MRX34 formulation, or
844 possibly another underlying mechanism²⁹⁶. Considering the administration of
845 dexamethasone pre-medication and the absence of similar SAEs associated with the
846 same liposomal carrier used for a different investigational oligonucleotide drug, it is not
847 likely that the severe toxicities observed in MRX34 were caused by the liposome
848 carrier^{297,298}. Moreover, the immune-related toxicities observed, along with the
849 unconventional response patterns occasionally seen with other immune-activating agents
850 such as CTLA-4 and PD-1/L1 immune checkpoint inhibitors, indicate an immune-
851 mediated mechanism underlying the clinical effects of MRX34^{299,300}.

852

853 [H1] Conclusions and outlook

854 Recent advancements in comprehensive functional genomics have improved our
855 understanding of the mechanism of action of ncRNAs on fundamental pathways related
856 to human diseases, thereby enhancing our knowledge of the clinical manifestations and
857 natural history of human diseases including cancer. However, understanding the full

858 range of ncRNA functions in human diseases still necessitates extensive investigation
859 and clarification. For example, the amount of ncRNA exploration in infectious diseases
860 could be widely expanded and such knowledge be used to prevent (through innovative
861 biomarkers or vaccines) or control (through new therapeutics) future pandemics. This
862 review has exclusively focused on the ncRNAs that have garnered the most attention in
863 the literature. It is important to recognize that many other ncRNA types were not
864 addressed here and therefore the impact of the entire ncRNA landscape in human
865 diseases is even broader. Conducting large-scale expression screens and clinically
866 evaluating ncRNAs can aid in identifying new non-coding transcripts that have a role in
867 human diseases, potentially serving as therapeutic targets or biomarkers.

868 An essential question is: where is the field heading in the near future? We
869 anticipate at least three major areas of development. First, we need to catalog and
870 annotate all ncRNAs from each human cell type and body fluid in populations of millions
871 of individuals from different races and ages, and possibly at the single-cell level. We are
872 witnessing the start of such a huge and beneficial effort³⁰¹. From a technological point of
873 view, ncRNA quantification methods and data analysis methods are already available, so
874 the bottleneck for this goal is more related to coordination and funding rather than
875 technology availability. Such a catalog will be essential for understanding new
876 mechanisms of diseases and even more so for biomarker development. Second, there is
877 a need to advance proteomics methodologies capable of accurately and consistently
878 identifying micropeptides ranging from 10–20 amino acids in length. These extremely
879 short peptides originated from ncRNAs could be in a far greater number than what is
880 currently known thus holding a more extensive biological relevance than previously
881 assumed. If this is the case, then we will witness another major transformation in the
882 ncRNA paradigm that will offer the scientific community an additional large category of
883 ‘micro’ molecules to examine in-depth, carrying relevant translational implications.
884 Another pivotal potential advancement will be the development of secure and effective
885 ncRNA therapeutics. A revolutionary therapeutic breakthrough on par with the targeted
886 efficacy of Gleevec or the immunotherapeutic impact of anti-PD1/PD-L1 drugs could
887 substantially propel the field forward. One promising avenue in this domain involves

888 harnessing artificial intelligence to facilitate high-throughput development and
889 investigation of small molecules that bind to coding and non-coding RNAs.

890

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1843
1844

1845 **Acknowledgements**

1846 The authors would like to dedicate this review to Deborah Silverman, PhD, and to Angelo
1847 Veronese, PhD, for their passion to science and love for life, who will both be sorely
1848 missed! G.A.C. is a Felix L. Haas Endowed Professor in Basic Science. Work in G.A.C.'s
1849 laboratory is supported by NCI grants 1R01 CA182905-01 and 1R01CA222007-01A1,
1850 NIGMS grant 1R01GM122775-01, DoD Idea Award W81XWH-21-1-0030, a Team DOD
1851 grant in Gastric Cancer W81XWH-21-1-0715, a Chronic Lymphocytic Leukemia
1852 Moonshot Flagship project, a CLL Global Research Foundation 2019 grant, a CLL Global
1853 Research Foundation 2020 grant, a CLL Global Research Foundation 2022 grant, The
1854 G. Harold & Leila Y. Mathers Foundation, two grants from Torrey Coast Foundation, an
1855 Institutional Research Grant and Development Grant associated with the Brain SPORE
1856 2P50CA127001. The M.F. laboratory is supported by the Italian Association for Research
1857 on Cancer (AIRC) under IG 2021 - ID. 25789 project.

1858

1859 **Author contributions**

1860 The authors contributed equally to all aspects of the article.

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1862 **Competing interest's statement**

1863 G.A.C. is one of the scientific founders of Ithax Pharmaceuticals. The other authors
1864 declare no competing interests.

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1866

1867 **Peer review information**

1868 *Nature Reviews Genetics* thanks Howard Chang, who co-reviewed with Hyerim Yi, and
1869 the other, anonymous, reviewers for their contribution to the peer review of this work.

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1871

Commentato [MA1]: Copy editor and MPS: Please leave names written out in full in dedication.

1872 **Related links**

1873

1874 FuncPEP <https://bioinformatics.mdanderson.org/Supplements/FuncPEP/database.html>

1875 miRbase <https://www.mirbase.org/>

1876 NIH Early Detection Research Network <https://edrn.nci.nih.gov>

1877 GENECODE <https://www.gencodegenes.org/>

1878 NONCODE <http://www.noncode.org>

1879 SPENCER <http://spencer.renlab.org>

1880 The Human MicroRNA Disease Database <http://www.cuilab.cn/hmdd>

1881 LncRNADisease <http://www.manut.net/lncrnadisease>

1882

1883 **Supplementary information**

1884 Supplementary information is available for this paper at <https://doi.org/10.1038/s415XX-XXX->

1885 XXXX-X

1886

1887 **Table 1.** Representative examples of clinical trials investigating ncRNA biomarkers in cancer,
 1888 cardiovascular disease, infectious diseases and neurogenerative disorders. For each miRNA, example
 1889 trials were chosen based on a high number of study participants. COPD: Chronic Obstructive Pulmonary
 1890 Disease.

NCT Number	Conditions	ncRNA	n=	Scope of the study
Cancer				
NCT02618538	Breast cancer	miRNAs	26,600	Investigating whether circulating miRNAs are significantly altered in the plasma of patients with cancer compared to matched healthy controls.
NCT05633342	Various cancer types	miRNAs	15,000	Characterizing intra-cellular multi-omic profiles of cancer and adjacent healthy tissues to aid the selection of circulating cancer biomarkers.
NCT02338167	Breast cancer	miRNAs	13,500	Discovery of biomarkers that predict progression free survival of patients with breast cancer. Biomarkers include gene expression profiling of the primary tumor and the corresponding metastases, somatic mutations, germline genetic variation, epigenetic changes and miRNA variation.
NCT03830619	Lung cancer	lncRNAs	1,000	Analysis of the sensitivity and specificity of serum exosome ncRNA as a biomarker for the diagnosis of lung cancer.
NCT05397548	Gastric cancer	lncRNA-GC1	700	Investigating whether circulating, exosomal lncRNA-GC1 can be used to monitor gastric cancer.
NCT05647941	Gastric cancer	lncRNA-GC1	700	Investigating whether lncRNA-GC1 can serve as a non-invasive biomarker for monitoring the neo-adjuvant chemotherapy response to personalized medicine for gastric cancer.
NCT04584996	Pancreatic cancer, biliary tract cancer	circRNAs	186	Defining the circRNA expression profile of pancreatic ductal adenocarcinoma (PDAC) tissues compared to controls, in an attempt to identify circRNA PDAC biomarkers.
NCT04464122	Neuroendocrine tumors	circRNAs	60	Identifying new circRNA biomarkers from tumor-educated platelets (TEPs) for the diagnosis and evaluation of treatment response in pulmonary and gastro-entero-pancreatic neuroendocrine neoplasms.
NCT05771337	Breast cancer	Circ-ELP3	80	Investigating the diagnostic value of <i>hsa_circ_0001785</i> (Circ-ELP3) and <i>hsa_circ_100219</i> (Circ-FAF1) in serum samples of patients with breast cancer.
Cardiovascular Diseases				
NCT05766046	Lung cancer, Cardiovascular diseases, COPD	miRNAs	7,324	Developing a diagnostic test analyzing miRNAs from blood of patients with cardiovascular diseases and lung cancer.
NCT03049254	Various cardiovascular conditions	miRNAs	6,000	Investigating blood-based biomarkers that predict disease onset, disease progression, and the likelihood of arrhythmia.
NCT04189029	Heart failure	miRNAs, lncRNAs	2,620	A prospective multicenter study to decipher phenotypic variability within patients with heart failure and preserved left ventricular ejection fraction.
NCT03170830	Acute Myocardial Infarction	circRNA-Uck2	178	Evaluating the diagnostic value of circRNA-Uck2 in acute myocardial infarction.
NCT02297776	Cardiac Arrest	miRNA, circRNA	160	Evaluating circRNA and miRNA plasma biomarkers for their ability to estimate the extent of brain injury after cardiac arrest.
NCT03076580	Cardiomyopathies	miRNAs, lncRNAs	2,000	A multi-omics study of cardiomyopathies patients, aiming to determine genetic risk factors and serial biomarkers of cardiomyopathies in diagnosis and prognosis.
NCT03225183	Cardiovascular Disease, Hypertension	lncRNAs	1,700	Characterizing the relationship between of lncRNAs and cardiovascular diseases and risk factors.
Neurological Diseases				
NCT05418023	Autism spectrum disorder, developmental delay	miRNAs	6,604	Validating a salivary miRNA diagnostic test for autism spectrum disorder.
NCT04961450	Amyotrophic Lateral Sclerosis, Frontotemporal Dementia, Motor Neuron Disease	miRNAs	2,500	Investigating miRNA biomarkers in blood, saliva, feces, cerebrospinal fluid, muscle tissue and nerve tissue of patients with motor neuron disease and frontotemporal dementia.
NCT04509271	Alzheimer disease	miRNAs	1,300	Investigating miRNA biomarkers for the diagnosis of mild cognitive impairment due to Alzheimer disease.
NCT03152630	Dementia	lncRNA	600	Investigating early and prognosis diagnosis of vascular dementia.
NCT04807738	Multiple sclerosis	lncRNA	110	Studying the effect of virtual reality on upper limb function and postural stability in people with multiple sclerosis; lncRNA biomarkers were analyzed to assess the biological effect of rehabilitation intervention.
NCT05341453	Spinal muscular atrophy	lncRNA	16	A randomized controlled trial is aimed to discover Studying the effect of physiotherapy and hippotherapy effect and efficacy on children with SMA with efficacy assessed in part using measurement of lncRNA in blood.
NCT05098340	Acute Ischemic Stroke	circRNAs	500	Analyzing the expression pattern of circRNAs in patients with acute ischemic stroke and healthy controls, to identify detection and prognosis biomarkers.
NCT04175691	Acute Stroke, Ischemic Stroke	miRNAs, lncRNAs, circRNAs	500	Analyzing the expression pattern of ncRNAs in patients with acute ischemic stroke and healthy controls, to identify detection and prognosis biomarkers.

Commentato [MA2]: [Au: Please clarify how lncRNAs were studied in this clinical trial.]

Commentato [FN3R2]: MFerracin: George I don't know here- The lncRNA is just the target of the disease, I don't think it is correct to cite the study among the biomarkers

Commentato [FN4R2]: In this trial the efficacy of physiotherapy and hippotherapy is analyzed on children with SMA by biomedical measures, by molecular **biological markers (lncRNA) in blood** and by surface electromyography (EMG). The primary goal of this study is to compare two physiotherapeutic approaches - the recommended form of classical physiotherapy and the method on a neurophysiological basis - hippotherapy.

I think this is a good example of how ncRNA biomarkers can be used to monitor therapy, however, we can change this to another example for lncRNAs if you prefer so.

Commentato [MA5R2]: OK – how is this for a description?

Commentato [MOU6R2]: George: this is fine for me

NCT04230785	Acute and Ischemic Stroke, Endovascular Treatment	ncRNAs	300	Analyzing the expression pattern of ncRNAs in patients with acute ischemic stroke before and/or after endovascular treatment.
Infectious Diseases and Sepsis				
NCT01780298	COPD	miRNAs	739	Investigating biomarkers for the differentiation of participants with COPD and three matched control groups: one of non-smoking subjects (never smoked), one of ex-smokers and one of current smokers.
NCT03280576	Sepsis	miRNAs	556	Analyzing the expression levels of miRNAs isolated from plasma, circulating exosomes and blood cells by next-generation sequencing to characterize epigenetic influences on progranulin plasma levels.
NCT05398952	Post viral fatigue, viral myocarditis	miRNAs, lncRNAs, circRNAs	2,000	Examining circulating ncRNA biomarkers in patients with post-COVID-19 persisting symptoms to identify new diagnostic and prognostic biomarkers.

1891
1892

1893 **Table 2. Examples of clinical trials investigating miRNA-targeting therapies.**

Related disease	Study ID	Treatment	Target(s)	Scope of the study	Phase
Cutaneous T-cell Lymphoma, Mycosis Fungoides (CTCL, MF)	NCT03837457	A synthetic locked nucleic acid-modified oligonucleotide inhibitor of miR-155 (MRG-106)	miR-155	Evaluation of MRG-106 impact on skin lesion severity, disease symptoms, quality of life, and the duration of stable or improved disease status, while ensuring no evidence of disease progression.	2
Cutaneous T-cell Lymphoma, Mycosis Fungoides (CTCL, MF)	NCT03713320	A synthetic locked nucleic acid-modified oligonucleotide inhibitor of miR-155 (MRG-106)	miR-155	Comparison of the effects of MRG-106 to vorinostat, a drug that has been approved for the treatment of CTCL.	2
Liver Cancer, Small Cell Lung Cancer, Lymphoma, Melanoma, Multiple Myeloma, Renal Cell Carcinoma, Non-Small Cell Lung Cancer	NCT01829971	A liposomal miR-34a mimic (MRX34)	Multiple oncogenic genes (such as, MEK1, MYC, PDGFR- α , CDK4/6, BCL2, WNT 1/3, NOTCH1, CD44)	Evaluation of MRX34 safety on patients with primary liver cancer, selected solid tumors, and hematologic malignancies.	1
Malignant Pleural Mesothelioma, Non-Small Cell Lung Cancer	NCT02369198	Targeted minicells containing a miR-16 mimic (TargomiRs)	EGFR-expressing cancer cells with an anti-EGFR bispecific antibody	Evaluating the safety, optimal dosing, and activity of TargomiRs in patients with malignant pleural mesothelioma	1
Colorectal Cancer	NCT03362684	Cetuximab, FOLFOX	miR-31-3p and miR-31-5p	Identifying the prognostic role of miR-31-3p and miR-31-5p in stage III colon cancer, specifically their potential as indicators of patient outcomes in the context of anti-EGFR therapy.	3
Coronary Heart Disease, Acute Myocardial Infarction	NCT02850627	Tongguan capsule	Global regulation of miRNA levels	Assessing the impact of Tongguan capsule on miRNA profiles in patients.	4
Preclinical Alzheimer Disease	NCT02045056	Gemfibrozil	miR-107	Examining the safety and efficacy of Gemfibrozil in regulating miR-107 levels as a potential strategy for	Early 1

				Alzheimer Disease prevention.	
<i>Organ Protection</i>	NCT05503043	Lidocaine	MiR-135a, Rock2, Add1	Investigating the impact of intravenous lidocaine on serum miR-135a levels and its downstream proteins (Rock2 and Add1) in patients.	NA
<i>Alport Syndrome</i>	NCT03373786	RG-012 (lademirsén)	miR-21	Assessing the impact of RG-012 on renal miR-21.	1
<i>Keloid</i>	NCT03601052	An oligonucleotide mimic of miR-29b (MRG-201/Replarsén)	Multiple multiple factors involved in the fibrotic response (eg, collagen)	Assessing the efficacy of Replarsén in preventing or reducing Keloid formation	2
<i>Endometriosis</i>	NCT05331053	Atorvastatin	Global regulation of miRNA levels	Investigating the role of miRNAs (let7-a, let7-b, let7-g, miR-98, miR-590) in driving elevated LOX-1 receptor expression and function in endometriosis.	4

1894

1895

1896 **Figure legends**

1897

1898 **Figure 1. The classic and non-classic functions of miRNAs.** | **a)** The classic function
1899 of miRNAs is to target the 3' UTR of sequence-specific mRNAs, causing mRNA
1900 degradation or translational repression, as illustrated for miR34a targeting *PDL1*
1901 transcripts. **b)** Certain miRNAs can target the 5' UTR or coding sequence (CDS) of their
1902 target mRNA, resulting in either mRNA degradation, translational repression, or even
1903 increased translation of their target. For example, miR-24 can bind both to the 3' and 5'
1904 UTR of *Jab1* mRNA causing its posttranscriptional inhibition, whereas miR-10a binds to
1905 the 5' UTR of ribosomal-encoding mRNAs, such as *Rps16*, and enhances their
1906 translation. **c)** miRNAs can act as mediators of intracellular communication by being
1907 secreted via extracellular vesicles (EVs) and acting as hormones³⁹. On immune cells,
1908 miRNAs can directly target Toll-like receptor (TLR) proteins by acting as their ligands, in
1909 turn activating TLR signaling pathways and inducing an immune response^{38,40 41}. For
1910 example, let-7i can target TLR4, whereas miR-21 and miR-29a can target TLR8. **d)** Some
1911 miRNAs can also interact with non-Ago proteins, so-called miRNA-binding proteins
1912 (miRBPs), which can work in cooperation or competition with Ago, thereby enhancing or
1913 silencing miRNA function on its target molecule. Examples include miR-1 and the
1914 TNRC6B miRBP, and miR-21 and PDCD4 miRBP, respectively. miRNA can also be
1915 transported between Ago2 and miRBP; however this mechanism is less studied and not
1916 currently well understood. **e)** Some pri-miRNAs encode regulatory peptides that can
1917 influence the expression of the mature miRNA.

1918

1919 **Figure 2. The main functions of lncRNAs.** | **a)** DNA interaction. lncRNAs can directly
1920 bind to DNA, forming R-loops, or can have a role in chromatin regulation in a complex
1921 with DNA-binding proteins. For instance, GADD45A can bind to the R loop formed by the
1922 lncRNA *TARID* at the TCF21 promoter, triggering local DNA demethylation by recruiting
1923 TET1 to the DNA⁵⁹. *SWINGN* lncRNA, which is transcribed from an enhancer, modulates
1924 the activation of *GAS6* oncogene by binding to SWI/SNF tumor suppressor complex and
1925 influences its ability to drive epigenetic activation of specific promoters⁶⁰. *Xist* lncRNA,

1926 which is responsible for X chromosomal inactivation, binds to SAF-A chromatin-
1927 interacting protein and is thereby able to localize to sites on the X chromosome. *Xist*
1928 directly binds to SHARP and the resulting complex recruits SMRT to these DNA regions
1929 and recruits HDAC3 to the X chromosome or induces HDAC3 enzymatic activity, which
1930 results in chromatin compaction and transcription silencing⁶¹. **b)** Various RNA
1931 interactions. lncRNAs can interact with mRNAs and affect translation, RNA stability or
1932 block miRNA binding sites and thereby inhibit the effect of miRNAs. For example, the
1933 lncRNA *GAS5* interacts with the translation initiation complex eIF4F, by directly binding
1934 to eIF4E and decreasing the translation of c-Myc³⁰². The *TINCR*–*STAU1* complex seems
1935 to mediate the stabilization of different mRNAs, such as *KRT80*³⁰³. *PTB-AS* substantially
1936 increases *PTBP1* mRNA levels by directly binding to its 3' UTR and blocking miRNA
1937 binding sites³⁰⁴. **c)** Sponge activity by miRNA interaction. *MALAT1* can act as a miRNA
1938 sponge for miR-34c and thereby upregulate *SATB2* expression and alleviate the
1939 symptoms of osteoporosis in mice⁶². *HOTAIR* can sponge the tumor-suppressor miR-
1940 222-3p and thereby contribute to ovarian cancer progression⁶³. **d)** Protein interactions.
1941 lncRNAs can interact with proteins and act as their scaffolds or guides. For example,
1942 NFAT1 kinases are scaffolded by the lncRNA *NRON*⁶⁴, whereas *HOTAIR* specifically
1943 binds to YBX1, and promotes YBX1 nuclear translocation^{64,65}. **e)** Some lncRNAs can
1944 harbor 'coding' activity and produce micropeptides. For example, *LINC0065* lncRNA can
1945 be translated to the CIP2a-BP micropeptide.

1946

1947 **Figure 3. The main functions of circRNAs.** | **a)** CircRNAs have the potential to serve
1948 as sponges for miRNAs, as demonstrated by *circTDRD3*, which harbors target sites for
1949 miR-1231. **b)** CircRNAs can interact with specific mRNAs and regulate their stability
1950 and/or translation. An example is *circZNF609* interaction with *CKAP5* mRNA. **c)**
1951 CircRNAs can undergo translation and produce small peptides, as demonstrated by
1952 *circCDYL2* translation into Cdy12-60aa small peptide, which is approximately 7 kDa. **d)**
1953 CircRNAs that contain motifs capable of binding to RNA-binding proteins possess the
1954 capacity to act as decoys or sponges for proteins, consequently modulating their activity.
1955 CircRNAs harboring motifs that facilitate binding between an enzyme and its substrate
1956 can act as scaffolds, enabling the co-localization of the two molecules and optimizing

1957 reaction kinetics. CircRNAs can interact with gene promoters, recruit TET1 demethylase,
1958 and initiate significant demethylation of CpG islands within the DNA. Additionally,
1959 circRNAs can bind to U1 snRNP and subsequently engage with the RNA polymerase II
1960 transcription complex, enhancing protein expression.

1961

1962 **Figure 4. Examples of the different mechanisms of ncRNAs in human diseases. | a)**
1963 *H19* lncRNA acts as a miRNA sponge in breast cancer tissue and thereby reduces the
1964 level of miR200a and miR200b in tissues and circulation, resulting in ARF protein
1965 accumulation that facilitates epithelial-mesenchymal transition (EMT). **b)** *Dwarf* lncRNA
1966 has a heart-specific expression and is down-regulated in ischemic failing human hearts.
1967 Expression changes in DWORF small peptide, which is encoded by *Dwarf* lncRNA and
1968 acts by regulating the Sarcoendoplasmic Reticulum Calcium ATPase (SERCA) calcium
1969 pump in myocytes, have a potential role in heart failure. **c)** *Cdr1as* circRNA contains 73
1970 binding sites for let-7 and thereby acts as a miRNA sponge. As a consequence, reduced
1971 let-7 levels cause decreased UBE2A and increased SNCA protein levels that contribute
1972 to amyloid beta plaque accumulation and thereby to Alzheimer Disease. **d)** SARS-CoV-
1973 2 integrates and increases miR-2392 expression, promoting COVID-19 disease
1974 progression.

1975

1976 **Figure 5. NcRNAs are important biomarkers and therapeutic targets.**

1977 **A.** Various ncRNA species can be detected and analyzed in standard biopsy samples
1978 and liquid biopsy specimens through various techniques including quantitative PCR
1979 (qPCR), droplet digital PCR (ddPCR), RNA Sequencing or in situ Hybridization. All types
1980 of ncRNA species can be isolated from blood cells, serum, plasma, extracellular vesicles,
1981 urine, saliva, breast milk, and cerebrospinal fluid among others. They have the potential
1982 to serve as diagnostic and prognostic biomarkers as well as to help monitor the diseases
1983 treatment and outcomes. **B.** Representative examples of the two main types of ncRNA
1984 therapies e investigated in pre-clinical and clinical stages. In response to cardiomyocyte
1985 stress, miR-132 is upregulated in the cardiac tissue of patients with cardiac stress or
1986 injury. Intravenous infusion of CDR132L containing antisense miR-132 is under clinical

1987 investigation to improve cardiac function. MesomiR 1 is a miR-16 mimic encapsulated in
1988 minicells that aim to restore the level of miR-16 tumour suppressor in cancer cells and is
1989 under clinical investigation to treat malignant pleural mesothelioma.

1990

1991 **Box 1. miR-155 regulation of the immune system and therapeutic use.**

1992 The involvement of ncRNAs in the various facets of immune function is an extensively
1993 studied area³⁰⁵⁻³⁰⁷. Various well-characterized miRNAs, such as miR-146, miR-150 and
1994 miR-155, have been reported to be involved in regulating lymphocyte, monocyte and
1995 macrophage phenotypes, respectively³⁰⁸. From a clinical perspective, the most advanced
1996 therapeutic applications are related to miR-155, which has important roles in T and B cell
1997 proliferation and cytokine production³⁰⁹⁻³¹². Overexpression of miR-155 in activated T
1998 helper (Th) cells can participate in Th2-mediated airway inflammation through targeting
1999 of sphingosine receptor S1PR1³¹³. In addition, miR-155 is required for optimal
2000 proliferation of regulatory T cells (Treg) in vitro³¹⁴, which suggests an important role in
2001 regulating T-cell expansion. In vivo studies also showed that miR-155 acts in regulating
2002 interferon (IFN) responsiveness and the CD8⁺ T cell response against pathogens³¹¹.
2003 Furthermore, overexpression of miR-155 has been shown to enhance cytokine
2004 responsiveness, engraftment, cytokine production and anti-tumour function³¹⁰. Consistent
2005 with these findings, overexpression of miR-155 directly suppresses SHIP1 levels, while
2006 enhancing Polycomb repressor complex 2 (PRC2) activity by promoting the expression
2007 of the PRC2-associated factor PHF19³¹⁰.

2008 miR-155 has a central role in regulating serine/threonine kinase (Akt)-dependent
2009 M1/M2 activation of macrophages³¹⁵. In addition, miR-155 overexpression enabled
2010 successful reprogramming of tumour-associated macrophages (TAMs) into pro-
2011 inflammatory M1 macrophages³¹⁶. Notably, miR-155 may contribute to the development
2012 of resistance to chemotherapy, as evidenced by its role in the cross-talk between
2013 neuroblastoma cells and human monocytes in chemoresistance, involving cell-to-cell
2014 communications with malignant cells via an exosomal miR-21/TLR8-NF- κ B/exosomal
2015 miR-155/TERF1 signaling pathway³¹⁷. Clinical applications related to suppressing miR-
2016 155 for cancer therapy targeted to the TME were developed for DLBCL³¹⁸ using the
2017 attachment of nucleic acid anti-miRs to a peptide with a low pH-induced transmembrane
2018 structure (pHLIP). Further combinatorial therapeutics of anti-miR-155 with chemotherapy
2019 for the treatment of lung cancers was reported using the non-toxic DOPC (1,2-dioleoyl-
2020 sn-glycero-3-phosphocholine) Food and Drug Administration (FDA)-approved

2021 nanoliposomes³¹⁹. Cobomarsen (also known as MRG-106) is a locked nucleic acid (LNA)-
2022 based inhibitor of miR-155, used for the treatment of cutaneous T-cell lymphoma (CTCL),
2023 mycosis fungoides (MF) subtype. Promisingly, it is likely that this drug could be
2024 repurposed for any other non-cancer type of disease in which miR-155 has abnormally
2025 high expression, and is pathogenetically involved, such as autoimmune inflammatory
2026 disorders³²⁰. A phase I trial demonstrated that Cobomarsen was well-tolerated, had
2027 clinical activity and had the potential to improve patients' quality of life³⁰¹.

2028

2029 **Glossary terms**

2030 **Small peptides (sPEP)**

2031 Small peptides, also called micropeptides, are polypeptides that are encoded by small
2032 open reading frames (sORFs) and consist of less than 100-150 amino acids, sometimes
2033 translated from ncRNAs.

2034 **Non-Ago proteins**

2035 Argonaute (Ago) proteins are interactor partners of small ncRNAs, such as miRNAs and
2036 siRNAs, which facilitate their target binding and thereby their effector mechanisms. It was
2037 recently uncovered that miRNAs can interact with other, non-Ago proteins as well.

2038 **Sponge**

2039 RNA molecules such as circRNAs that can bind and sequester RNAs or proteins and
2040 thereby inhibit their effects.

2041 **Ribosome profiling**

2042 A deep-sequencing-based method that reveals ribosome-associated mRNAs, thereby
2043 predicting regions subjected to translation.

2044 **Small-molecule inhibitor (SMI)**

2045 Compounds smaller than 500 Da developed to target any portion of a target molecule
2046 and to cause its inhibition.

2047 **Short open reading frame (sORF)**

2048 Also known as small ORF, these are 100 nucleotide-long putative protein-coding sites,
2049 which were previously overlooked as non-relevant regions.

2050