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ADVANCED REVIEW

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microRNA-based diagnostic and therapeutic applications in cancer medicine

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Abstract

It has been almost two decades since the first link between microRNAs and cancer was established. In the ensuing years, this abundant class of short noncoding regulatory RNAs has been studied in virtually all cancer types. This tremendously large body of research has generated innovative technological advances for detection of microRNAs in tissue and bodily fluids, identified the diagnostic, prognostic, and/or predictive value of individual microRNAs or microRNA signatures as potential biomarkers for patient management, shed light on regulatory mechanisms of RNA–RNA interactions that modulate gene expression, uncovered cell-autonomous and cell-to-cell communication roles of specific microRNAs, and developed a battery of viral and nonviral delivery approaches for therapeutic intervention. Despite these intense and prolific research efforts in preclinical and clinical settings, there are a limited number of microRNA-based applications that have been incorporated into clinical practice. We review recent literature and ongoing clinical trials that highlight most promising approaches and standing challenges to translate these findings into viable microRNA-based clinical tools for cancer medicine.

This article is categorized under:

RNA in Disease and Development > RNA in Disease

KEYWORDS

cancer, clinical trials, diagnostics, microRNA, miR, miRNA, noncoding RNA, therapeutic, tumor

1 | INTRODUCTION

microRNAs (miRNAs) are an evolutionarily conserved gene class of short noncoding regulatory RNAs (Bartel, 2018; Fromm et al., 2015; Gebert & MacRae, 2019; Sempere, 2019). The discovery, detection, biology, and clinical applications of miRNAs are intertwined with those of small interfering RNAs (siRNAs) (Sempere, 2019; Titze-de-Almeida et al., 2017). Accolades to these classes of short noncoding RNAs include Breakthrough of the Year 2002 for new roles for RNAs from Science magazine; the Nobel Prize in Physiology or Medicine 2006 to Andrew Fire and Craig Mello for their discovery of RNA interference—gene silencing by double-stranded RNA; the 2008 Albert Lasker Award for Basic Medical Research to Victor Ambros, Gary Ruvkun, and David Baulcome for their discovery of an unanticipated world

of tiny RNAs that regulate gene activity; and the 2015 Breakthrough Prize to Victor Ambros and Gary Ruvkun for their co-discovery of microRNAs. The recent clinical success and FDA approval of two siRNA drugs, patisiran, and givosiran (Roberts et al., 2020), have paved the way for similar miRNA-based drugs to reach the clinic in the near future. Unlike exogenous siRNAs, miRNAs are single stranded RNAs that result from the transcription and processing of endogenous longer precursor RNAs. Briefly, most primary miRNA transcripts are transcribed by RNA polymerase II from which a stereotypical stem-loop hairpin precursor RNA is cleaved in the nucleus by the RNAse III enzyme Drosha, a core component of the microprocessor complex (Bartel, 2018; Gebert & MacRae, 2019). Once in the cytoplasm, the RNAse III enzyme Dicer cleaves the pre-miRNA releasing the mature and biologically active miRNA strand, which is loaded in the Argonaute-containing miRNA-induced silencing complex (miRISC) (Bartel, 2018; Gebert & MacRae, 2019). In humans and other mammals, the mature miRNA guides the miRISC to partially complementary site(s) on the 3′UTR of target mRNAs, which leads to Argonaute-mediated recruitment of adaptor protein TNRC6 and subsequent recruitment of deadenylase complexes such as CCR4-NOT, that primarily triggers mRNA decay and secondarily, and to a lesser extent, translational repression (Bartel, 2018; Gebert & MacRae, 2019). Generally, miRNAs can interact with hundreds of target mRNAs and more abundant miRNAs more significantly decrease protein output of a larger number of their target genes (Bartel, 2018; Thomson & Dinger, 2016). Collectively, miRNA-mediated regulation can affect up to 60% of the transcriptome of a given cell having a global influence on protein output and cell function (Bartel, 2018; Gebert & MacRae, 2019; Thomson & Dinger, 2016).

A myriad of regulatory mechanisms that affect miRNA activity have been associated with carcinogenic processes. Many of these regulatory mechanisms disrupt miRNA biogenesis at different stages that ultimately alter the levels of mature and biologically active miRNA molecules: copy number alterations at the chromosomal or region-specific levels, epigenetic and transcriptional regulation, nuclear export, RNA processing and stability (Gebert & MacRae, 2019; Peng & Croce, 2016; Rupaimoole & Slack, 2017; Sempere & Kauppinen, 2009). Generally, levels of mature miRNA expression correlate well with miRNA activity (Anfossi et al., 2018; Gebert & MacRae, 2019; Thomson & Dinger, 2016). Hence, profiling of miRNA expression in cancer has served not only to identify differentially expressed miRNAs that could have diagnostic, prognostic, and/or predictive value, but also to uncover the etiological role of specific miRNAs relevant to cancer initiation, progression, and/or metastasis (Anfossi et al., 2018; Graveel et al., 2015; Peng & Croce, 2016; Rupaimoole & Slack, 2017; Sempere, 2014b; Sempere & Kauppinen, 2009). Nonetheless, it is worth mentioning some regulatory mechanisms that can affect miRNA activity without significantly altering miRNA expression such as isomer formation (Bartel, 2018; Gebert & MacRae, 2019), post-translational modifications of miRISC components (Gebert & MacRae, 2019), 3′UTR shortening of target mRNAs (Hoffman et al., 2016; Mao et al., 2020; Mayr & Bartel, 2009), and RNA–RNA interactions (Anfossi et al., 2018; Gebert & MacRae, 2019; Thomson & Dinger, 2016). These diverse classes of noncoding RNAs can modulate the activity of miRNAs by either serving as precursors, stabilizers, decoys or sponges (Anfossi et al., 2018; Gebert & MacRae, 2019; Thomson & Dinger, 2016). Several studies provide experimental evidence that specific RNA–RNA interactions can have a profound effect on miRNA activity and cancer phenotype (Anfossi et al., 2018; Gebert & MacRae, 2019; Thomson & Dinger, 2016) via miRNA sequestration and target displacement as postulated by the endogenous competing RNA (ceRNA) hypothesis (Poliseno et al., 2010; Salmena et al., 2011). Additional mechanisms may be at play since a single ceRNA may not be able to displace a sufficiently large number of miRNA molecules to have a biologically detectable effect on a particular cognate target gene (Bartel, 2018; Gebert & MacRae, 2019; Thomson & Dinger, 2016).

This advanced review focuses on the most promising approaches and standing challenges to translate recent cancer research findings into viable microRNA-based clinical tools for cancer medicine. We conducted a systematic PubMed search for articles that included the terms microRNA, Cancer, or Tumor, Diagnostic or Therapeutic in their Title or Abstract sections (microRNA[Title/Abstract] AND (Cancer[Title/Abstract] OR Tumor[Title/Abstract]) AND (Diagnostics[Title/Abstract] OR Therapeutic[Title/Abstract]) AND "2015/08/01"[Date–Entry]: "2020/11/01"[Date–Entry]) as well as active clinical trials registered at clinicaltrials.gov. Earlier studies of great significance may be cited directly via reference to original publication or indirectly via recent reviews in that topic area.

2 | MICRORNA-BASED DIAGNOSTICS

Seminal studies (Lu et al., 2005; Volinia et al., 2006) of miRNA expression profiling in healthy control and tumor tissues identified a core set of miRNAs (let-7, miR-10b, -15, -16, -17-5p, -20a, -21, -29b, -34, -126, -145, -155, -221) with altered expression in multiple hematological and solid tumors, whereas altered expression of other miRNAs was specific to a

particular cancer type or subtype (Barbarotto et al., 2008; Peng & Croce, 2016; Sempere, 2014b; Sempere et al., 2010). These initial discoveries were followed by more refined and focused studies to exploit the differential expression of miRNAs as diagnostic, prognostic, and/or predictive indicators. We review some of the largest retrospective studies conducted to illustrate the potential of specific miRNAs or miRNA signatures to improve patient management and how these studies have been received and could be implemented in the clinic based on current clinical trials registered at clinicantrials.gov. There is a clear trend in the recent literature and on-going clinical trials to develop miRNA-based noninvasive clinical assays using blood as starting material, whereas initial discovery, diagnostic, and prognostic studies mainly used tissues from diagnostic biopsies or surgical procedures.

2.1 | Technological advances for microRNA detection

The specific and sensitive detection of the short sequence of a mature miRNA presents some challenges that have been overcome by different technological advances (Figure 1). Classic hybridization-based detection methods, such as northern blot and RNA protection assays, require a large amount of total RNA (>1 μg) for analysis and are low-throughput. Capture-probe microarray and bead platforms were major technological advances in the mid 2000s for high-throughput miRNA expression analysis in bulk tumor tissue samples (Liu et al., 2004; Lu et al., 2005; Nelson et al., 2004; Volinia et al., 2006). A reverse transcription quantitative-polymerase chain reaction (RT-qPCR) assay emerged shortly after as a highly sensitive method for detection of miRNA expression from a small amount of starting materials (>25 pg; Chen et al., 2005) and quickly became an orthogonal and gold-standard method for validation of differential miRNA expression. This RT-qPCR assay overcame short size challenge by providing an extended sequence in a stem-loop primer with

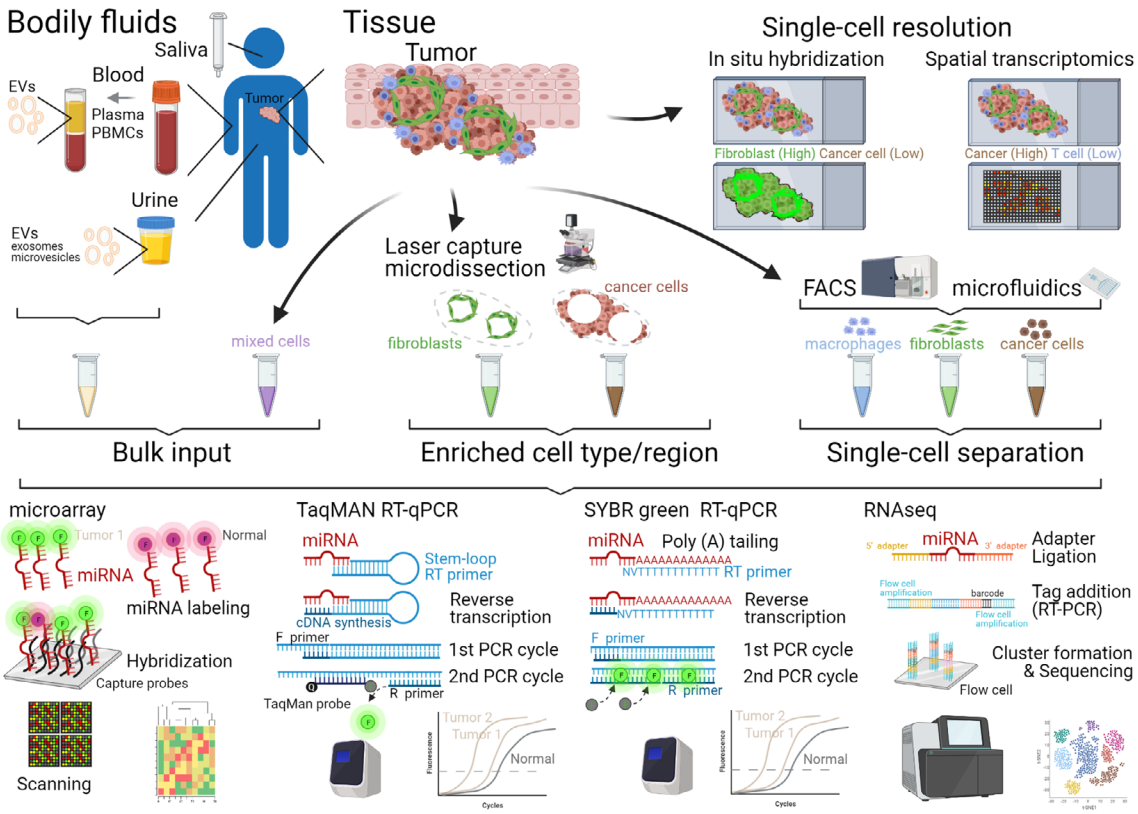

FIGURE 1 Biological source and detection technologies for miRNA expression analysis. Sensitive and specific detection technologies enable detection of miRNAs from tumor tissue or bodily fluids. These biological samples can be used as bulk input for miRNA analysis or can be further processed with different methodologies to refine the cellular (e.g., cancer cells vs. immune cells in tissue samples) or circulating source (e.g., extracellular vesicles vs. cell-free in plasma samples) of miRNAs. Key steps of detection and/or readout of each detection technology are shown (see Tables 1 and 2 for more details on studies applying these technologies). Molecules and constructs not drawn to scale. EVs, extracellular vesicles; FACS, fluorescence-activated cell sorting; F or R primer, forward or reserve primer; PBMCs, peripheral blood mononuclear cells

a secondary structure conformation that enables only reverse transcription of the miRNA sequence with a complementary and size-match 3′ ending (C. Chen et al., 2005), which was followed by real-time qPCR with a miRNA-specific TaqMan® probe (Chen et al., 2005). Other RT-qPCR assays overcame the short size challenge by extending the miRNA sequence via enzymatic addition of a poly(A) tail or another known sequences, two-tailed RT primer with partial complementarity to the miRNAs at both ends and an internal hairpin to extend the sequence; miRNA levels are quantitated by qPCR assay with a TaqMan® probe or a DNA intercalating dye such as SYBR® Green (Forero et al., 2019). In general, these different RT-qPCR assays produce similar readouts, but there are some discrepancies due to the terminal sequence of the miRNA (e.g., tolerance for isomers) and enzymatic preference for some bases at specific positions (Anfossi et al., 2018; Forero et al., 2019; Graveel et al., 2015). The original stem-loop and other RT-qPCR assays on an array card format can be used as a high-throughput and sensitive method to analyze the expression of up to 384 known miRNAs. There are other methods such as NanoString nCounter microRNA assay with high-throughput capability up to 800 miRNAs and high sensitivity (Foye et al., 2017). Next generation RNA sequencing (RNAseq) analysis is a more recent technology that has some advantages in the discovery setting since it provides deep expression analysis of well-annotated miRNAs and their variants (e.g., isomers) as well as unknown miRNAs, but it is also a more resource-intensive technology (Andres-Leon et al., 2016; Aparicio-Puerta et al., 2020; Lu et al., 2018). Likely technical advances in spatial transcriptomics, single-cell RNAseq, and microfluidic sorting (Drula et al., 2020; Eng et al., 2019; Nagarajan et al., 2020; Rodriques et al., 2019; Wang et al., 2019; Yoosuf et al., 2020) will soon enable detection of miRNA expression at an unprecedented level of single-cell, and even single-extracellular vesicle, resolution.

Independent studies with similar intent for clinical application of altered miRNA expression have often reported discordant results (Graveel et al., 2015; Jarry et al., 2014). The detection method (e.g., RNAseq, microarray chips, and RT-qPCR), normalization and analytical tools, sample preparation (e.g., fresh, frozen, or fixed), source and quality of starting RNA material (e.g., bulk tissue vs. sorted cells, serum vs. plasma), patient characteristics (e.g., sex, age, ethnicity, stage, treatment history), sample size, study design (e.g., single cohort, vs. training and validation set), and statistical tools can affect the miRNA expression readout (Anfossi et al., 2018; Bahnassy et al., 2018; Drula et al., 2020; Foye et al., 2017; Graveel et al., 2015; Jarry et al., 2014; Nik Mohamed Kamal & Shahidan, 2019; Sempere et al., 2017; Skjefstad et al., 2018). Thus, it is important to understand the influence of all these variables in experimental design and analysis among studies to identify and translate the most robust protocols and informative miRNAs into clinical assays.

2.1.1 | Considerations for microRNA detection in tissues

Total RNA extraction from bulk normal or tumor tissue is the most common approach for analysis of miRNA expression using standard detection methods (Figure 1). When RNA is extracted from snap-frozen tissue, it is typically difficult to estimate the content of cancer cell and other cell types of the tumor microenvironment (TME) vis-a-vis immune cells and reactive stroma or remaining residual normal tissue. Improved methods for extracting RNAs from formalin-fixed paraffin embedded (FFPE) tissues not only opened the possibility for conducting large retrospective studies with archival tissue blocks, but also for selecting tissue sections with higher content of cancer cells. However, these bulk tissue analyses cannot determine the specific cell source(s) of altered miRNA expression, which may lead to result misinterpretation (Kent et al., 2014; Nielsen, 2012; Sempere, 2014b; Sempere et al., 2010, 2020; Svoronos et al., 2016). Intra- and intertumoral heterogeneity can affect detected levels of a miRNA without a true change in miRNA expression but rather a change in the number of miRNA-expressing cells present. Lower detected levels of a miRNA can reflect an etiological relevant downregulation in cancer cells or simply a loss or decreased representation of an expressing cell type in the tumor mass (e.g., adipocytes in breast cancer, acinar cells in pancreatic ductal adenocarcinoma, smooth muscle cells of the muscularis mucosa in colorectal cancer; Andrew et al., 2014; Kent et al., 2014; Kjaer-Frifeldt et al., 2012; Nielsen et al., 2011; Sempere et al., 2007, 2010). Similarly, higher detected levels of a miRNA can reflect an etiologically relevant upregulation in cancer cells or simply a gain or increased representation of an expressing cell type in the tumor mass (e.g., tumor-associated macrophages in breast cancer, myofibroblasts in pancreatic ductal adenocarcinoma and colorectal cancer; Preis et al., 2011; Sempere et al., 2007, 2010). Different methods have been deployed to detect miRNA expression more precisely at a single-cell level. Laser capture microdissection of discrete tissue regions followed by quantitative analysis revealed that altered expression of some miRNA in neoplastic cells correlates with the stage of malignant transformation (Caponi et al., 2013; du Rieu et al., 2010; Han et al., 2017; Paterson et al., 2013; Smith et al., 2015), whereas altered expression of other miRNAs occurs in fibroblasts or other

noncancer cell types of the TME (Bumrungthai et al., 2015; Han et al., 2017; Kent et al., 2014; Nielsen et al., 2011). In situ hybridization (ISH) assays with locked nucleic acid (LNA)-modified DNA probes enables detection of miRNA expression at single-cell resolution (Figure 1). While ISH assay is not as sensitive or quantitative as other methods, it uses workflow compatible with clinical immunohistochemical assays and can provide compartment-specific diagnostic and prognostic information (Nielsen, 2012; Sempere, 2014a, 2014b; Sempere et al., 2020; Warford, 2016). Other approaches to determine miRNA expression in individual cells or cell pools enriched for specific cell-type markers require physical separation methods (Figure 1) such as magnetic bead pull down, fluorescence-activated cell sorting (FACS) or microfluidics (Hoefig & Heissmeyer, 2010; Li et al., 2019; Petriv et al., 2010; Wu et al., 2013).

2.1.2 | Considerations for microRNA detection in bodily fluids

Blood and other bodily fluids (e.g., stool, urine, and saliva) are readily accessible by noninvasive means and thus can provide convenient and longitudinal measurements of circulating miRNA levels. Blood has been more frequently used as starting material than other bodily fluids to detect changes in miRNA levels associated with a specific cancer condition (Tables 1 and 2). miRNAs can be present in a variety of forms in blood: free, in protein complexes, or encapsulated in extracellular vesicles, but also in circulating immune cells, erythrocytes or platelets (Nik Mohamed Kamal & Shahidan, 2019; Sempere et al., 2017; Wu et al., 2017). Cell-free circulating miRNAs may derive from lysed cancer cells or other cells in the TME due to inflammation and immune responses. Extracellular vesicle-bound miRNAs may derive from cell-to-cell communications between cancer cells and TME or between immune cells mounting response against the tumor, may be shed from cancer cells as unwanted content (e.g., tumor suppressive miRNAs; Anfossi et al., 2018; Nik Mohamed Kamal & Shahidan, 2019; Sempere et al., 2017). miRNA levels can also vary in peripheral blood mononuclear cells (PBMCs) either as a change in the representation of lymphocytes and monocytes and/or activation of these immune cell types in response to the tumor (Ma, Lin, et al., 2015; Mishra et al., 2015; Mosallaei et al., 2020; Wang et al., 2013). Thus, blood samples need to be processed in different ways depending on the informative source of miRNA origin, and promptly and consistently to avoid unintended contamination from other sources such as lysed erythrocytes (haemolysis; Anfossi et al., 2018; Graveel et al., 2015; Jarry et al., 2014; Schwarzenbach, 2017). Plasma or serum has been used as starting material for miRNA analysis, but their content of cell-free and EV-bound miRNAs varies significantly (Graveel et al., 2015; Jarry et al., 2014; Nik Mohamed Kamal & Shahidan, 2019; Wang et al., 2012). Data normalization presents a greater challenge in blood and other bodily fluid studies than in tissues because the origin of the testing miRNAs and reference miRNA or other RNAs may be different and unrelated in this cell-free medium (Anfossi et al., 2018; Graveel et al., 2015; Jarry et al., 2014). A generally accepted approach to address this challenge is to start with an identical sample volume, use an exogenous spike-in RNA such as Cel-miR-39, and use a panel of relatively stable miRNAs for that diseased state rather than a single gene reference such as small nuclear U6 RNA (Anfossi et al., 2018; Jarry et al., 2014). Even with this best practice approach, some patient-to-patient variables can affect normalization. For example, hydration status of the patient and fluid retention due to medications or other conditions can alter miRNA concentration (e.g., blood and urine) and provide an inaccurate readout based on volume normalization. Similarly, there is no universal set of stable housekeeping miRNAs that can be used for normalization in all cancer types or that remain stable regardless of age, sex, or other variables of each individual patient.

2.2 | Large retrospective studies

We highlight in broad strokes some of the largest retrospective studies that illustrate the versatility and potential of altered expression of specific miRNAs or miRNA signatures to provide actionable information to improve patient management. Table 1 provides a comprehensive summary of large retrospective studies that included more than 200 individuals.

2.2.1 | Diagnostic value of microRNA for disease classification

The utility of differential miRNA expression for separating malignant from benign conditions, cancer staging, and/or cancer typing has been extensively evaluated in tissue samples. Differential diagnosis based only on visual examination

TABLE 1 Retrospective studies evaluating microRNAs as diagnostic, prognostic, and/or predictive indicators

miRNA(s)	Source	Technology	Cancer site	Study design	Clinical application (s)	References
miR-34a	Tissue (TMA)	LNA ISH	Bladder	Population-based ($n = 229$)	Prognostic value of epithelial cell expression	Andrew et al. (2014)
miR-21	Tissue (TMA)	LNA ISH	Bladder	Testing ($n = 232$)	Prognostic value of both epithelial and stroma cell expression in urothelial carcinoma (80% of the bladder)	Ohno et al. (2016)
miR-222	Tissue (Frozen)	RT-qPCR	Bladder	Testing ($n = 387$)	Diagnostic value for separating from malignant from normal tissue and prognostic value	Tsikrika et al. (2018)
miR-658,-762,-4281, -4649-5p,-4665-3p,-4736, -6836-3p	Blood (Serum)	3D-Gene® Human miRNA Oligo Chip	Bone and soft tissue	Discovery ($n = 161$), training ($n = 276$), validation ($n = 356$)	Diagnostic value of miRNA signature for early disease detection (sarcomas) and for separating malignant tumors from benign conditions	Asano et al. (2019)
miR-1908	Tissue (Frozen)	RT-qPCR	Bone	Testing ($n = 424$)	Prognostic value in osteosarcoma	Lian et al. (2016)
miR-1915-3p,-3679-5p, -4763-3p	Blood (Serum)	3D-Gene® Human miRNA Oligo Chip	Brain	Training ($n = 300$), validation ($n = 280$)	Diagnostic value of miRNA signature for early disease detection (diffuse glioma)	Ohno et al. (2019)
miR-1246,-1307-3p,-1364, -6861-5p,-6875-5p	Blood (Serum)	3D-Gene® Human miRNA Oligo Chip	Breast	Training ($n = 2081$), validation ($n = 2549$)	Diagnostic value of miRNA signature for early disease detection	Shimomura et al. (2016)
let-7b-5p,miR-16-5p, -19a-3p,-19b-3p,-20a-5p, -25-3p,-92a-3p,-93-5p, -106a-5p,-223-3p, -425-5p,-451a	Blood (Serum)	qRT-PCR	Breast	Discovery ($n = 48$), training ($n = 64$), testing ($n = 246$), validation ($n = 72$)	Diagnostic value of miRNA signature for early disease detection	Zou, Xia, et al. (2020)
miR-629-3p,-4710	Blood (Serum)	3D-Gene® Human miRNA Oligo Chip	Breast	Training ($n = 460$) and validation ($n = 461$)	Diagnostic value of miRNA signature in combination with clinicopathological factors for detection of lymph node metastasis	Shiino et al. (2019)
miR-26a-5p,-29c-3p,-29c-5p,-30b-5p, -148a-3p,-361-3p,-645, -652-5p,-934	Tissue (FFPE)	qRT-PCR	Breast	Training ($n = 318$), testing ($n = 318$), validation ($n = 204$)	Diagnostic value of miRNA signature in combination with clinicopathological factors for detection of lymph node metastasis	Xie et al. (2018)
miR-21,-96,-139,-141,-145, -182,-183,-200a,-429	Tissue (TCGA)	Bioinformatics (RNAseq)	Breast	TCGA data ($n = 1214$)	Diagnostic value of miRNA signature for early disease detection and for separating malignant tumors from normal tissue	Xiong et al. (2017)

TABLE 1 (Continued)

miRNA(s)	Source	Technology	Cancer site	Study design	Clinical application (s)	References
miR-24-3p	Blood (Plasma), Tissue (TCGA)	Nanostring, Bioinformatics (miRNAseq)	Breast	Testing ($n = 230$), TCGA validation ($n = 1024$)	Prognostic value and predictive value of metastatic disease	Khodadadi-Jamayran et al. (2018)
miR-21	Tissue (TMA)	LNA ISH	Breast	Validation ($n = 901$)	Prognostic value of tumor-associated fibroblast expression based on ER/PR/HER2 tumor classification	MacKenzie et al. (2014)
Let-7b	Tissue (TMA)	LNA ISH	Breast	Population-based ($n = 2033$)	Prognostic value of epithelial cell expression based on ER/PR/HER2 tumor classification	Quesne et al. (2012)
miR-148b-3p,-190b,-429	Tissue (TCGA)	Bioinformatics (miRNAseq)	Breast	TCGA data ($n = 1187$)	Prognostic value of miRNA signature	Dai et al. (2019)
miR-205	Tissue, TMA	LNA ISH	Breast	Population-based ($n = 1686$)	Prognostic value of epithelial cell expression based on histological tumor classification	Quesne et al. (2012)
miR-210	Tissue, Frozen	TaqMan RT-qPCR	Breast	Validation ($n = 219$)	Prognostic value of miRNA expression	Camps et al. (2008)
miR-221	Tissue	ISH	Breast	Validation ($n = 377$)	Prognostic value of epithelial cell expression	Hanna, Wimberly, et al. (2012)
miR-301	Tissue (TMA, TCGA/METABRIC)	LNA ISH, bioinformatics (miRNAseq, microarrays)	Breast	Testing ($n = 380$), TCGA validation ($n = 634$), METABRIC validation ($n = 1262$)	Prognostic value of epithelial cell expression	Zheng, Huang, et al. (2018)
miR-493	Tissue, TMA	LNA ISH	Breast	Validation ($n = 382$)	Prognostic value of epithelial expression in triple negative breast cancer	Yao et al. (2018)
miR-145,-200c,-218	Tissue, TCGA	Bioinformatics	Cervix	Validation ($n = 254$)	Prognostic value of miRNA signature	Liang et al. (2017)
miR-144, −200b-3p,-451	Stool	RT-qPCR	Colon, rectum	Training ($n = 60$), testing ($n = 175$)	Diagnostic value of erythrocyte-specific miRNAs in the normalizer for early disease detection as a modified fecal occult blood test.	Wu et al. (2017)
miR-18a,-221	Stool	TaqMan RT-qPCR	Colon, rectum	Testing ($n = 595$)	Diagnostic value of miRNA signature for early disease detection	Yau et al. (2014)
miR-21,-320,-498	Tissue	TaqMan RT-qPCR	Colon, rectum	Testing ($n = 224$)	Prognostic value of miRNA signature	Bahnassy et al. (2018)

(Continues)

TABLE 1 (Continued)

miRNA(s)	Source	Technology	Cancer site	Study design	Clinical application (s)	References
miR-10b	Tissue (Frozen/FFPE)	RT-qPCR	Colon, rectum	Testing ($n = 492$)	Prognostic value and predictive value of metastatic disease	Jiang et al. (2016)
miR-21	Tissue, FFPE	LNA ISH	Colon, rectum	Population-based ($n = 764$)	Prognostic value of tumor-associated fibroblast expression	Kjaer-Frifeldt et al. (2012)
miR-21	Tissue, TMA	LNA ISH	Colon, rectum	Population-based ($n = 277$)	Prognostic value of tumor-associated fibroblast expression	Kang et al. (2015)
miR-181c	Tissue (Frozen, FFPE)	TaqMan RT-qPCR	Colon, rectum	Discovery ($n = 10$), training ($n = 80 \times 2$[matched Frozen and FFPE]), validation ($n = 57$)	Concordant prognostic value in frozen and FFPE tumor tissues of stage II cases	Yamazaki et al. (2017)
let-7a-2, miR-32,-181a-1, -197,-328,-505,-652	Tissue (TCGA)	Bioinformatics (RNAseq)	Colon, rectum	TCGA data ($n = 337$)	Prognostic value of miRNA signature	Xu et al. (2017)
miR-99a,-137,-499a,-548k,-654,-3619,-3170	Tissue (TCGA)	Bioinformatics (miRNAseq)	Head and Neck	TCGA data ($n = 569$)	Prognostic value of miRNA signature in head and neck squamous cell carcinoma	Lu et al. (2019)
let-7b-5p, miR-24-3p,-140-3p,-192-5p, -223-3p	Blood, Serum	RT-qPCR	Nasopharyngeal	Discovery ($n = 60$), testing ($n = 304$), validation ($n = 82$)	Diagnostic value of miRNA signature for early disease detection	Zou, Zhu, et al. (2020)
miR-20a-5p,-25-3p,-30a-5p, -92a-3p,-132-3p,-185-5p, -320a,-324-3p	Blood (Plasma)	TaqMan RT-qPCR	Liver	Training ($n = 85$), testing ($n = 64$), validation ($n = 149$)	Diagnostic value of miRNA signature for early disease detection	Wen, Han, et al. (2015)
miR-21	Blood (Serum)	RT-qPCR	Liver	Training ($n = 80$), validation ($n = 453$)	Diagnostic value of miRNA signature for early disease detection	Guo et al. (2017)
miR-26a,-26b	Tissue (FFPE)	Multiplex RT-qPCR	Liver	Training ($n = 129$), testing ($n = 119$)	Predictive value of treatment response to interpheron-alpha	Ji et al. (2013)
miR-101-3p,-101-5p	Tissue (TCGA)	Bioinformatics (miRNAseq)	Liver	TCGA data ($n = 414$)	Diagnostic value for early disease detection and for separating hepatocellular carcinoma from normal liver tissue	Yang, Pang, et al. (2018)
miR-10b,-195	Tissue (TCGA, GEO datasets)	Bioinformatics (miRNAseq, microarrays)	Liver	TCGA data ($n = 421$), GEO datasets ($n = 803$)	Prognostic value of miRNA signature	Nagy et al. (2018))
miR-17-5p	Blood, Serum	RT-qPCR	Lung	Validation ($n = 275$)	Diagnostic value for early disease detection and prognostic value	Chen et al. (2013)
miR-20a,-24,-25,-145,-152, -199a-5p,-221,-222,-223, -320	Blood, Serum	TaqMan RT-qPCR	Lung	Training ($n = 310$), validation ($n = 310$)	Diagnostic value of miRNA signature for early disease detection	Chen et al. (2012)

TABLE 1 (Continued)

miRNA(s)	Source	Technology	Cancer site	Study design	Clinical application (s)	References
miR-21	Tissue, TMA	LNA ISH	Lung	Validation ($n = 335$)	Prognostic value of tumor-associated fibroblast and epithelial cell expression	Stenvold, Donnem, Andersen, Al-Saad, Valkov, et al. (2014)
miR-21-5p,-30d-5p	Tissue (TCGA, GEO datasets)	Integrated bioinformatics	Lung	Training ($n = 2251$), TCGA validation ($n = 423$)	Prognostic value of miRNA signature	Li et al. (2017)
miR-451	Blood (Plasma)	3D-Gene® Human miRNA Oligo Chip	Lung	Discovery ($n = 6$), validation ($n = 309$)	Prognostic value of exosomal miRNA levels	Kanaoka et al. (2018)
miR-126	Tissue	LNA ISH	Lung	Validation ($n = 312$)	Prognostic value of epithelial cell expression	Donnem et al. (2011)
miR-143,-145	Tissue, TMA	LNA ISH	Lung	Validation ($n = 553$)	Sex-specific prognostic value of stromal expression (miR-143 in females and miR-145 in males)	Skjefstad et al. (2018)
miR-155	Tissue, TMA	LNA ISH	Lung	Validation ($n = 320$)	Prognostic value of epithelial cell expression	Donnem et al. (2011)
miR-182	Tissue, TMA	LNA ISH	Lung	Validation ($n = 305$)	Prognostic value of epithelial cell expression	Stenvold, Donnem, Andersen, Al-Saad, Busund, et al. (2014)
miR-205	Tissue, Blood	Meta-analysis	Lung	Aggregate ($n = 1231$)	Diagnostic value for separating malignant tumors from normal lung tissue	Li, Sun, et al. (2017)
miR-205	Tissue, Blood	Meta-analysis	Lung	Aggregate ($n = 756$)	Prognostic value	Li, Sun, et al. (2017)
miR-210	Tissue, TMA	LNA ISH	Lung	Validation ($n = 259$)	Prognostic value of stromal cell expression	Eilertsen et al. (2020)
miR-873,-1293,-1914	Tissue (TCGA)	Bioinformatics (miRNAseq)	Lung	TCGA data ($n = 528$)	Prognostic value of miRNA signature in lung adenocarcinoma	Zheng, Mao, et al. (2018)
miR-10b	Blood (PBMC)	RT-qPCR	Lung	Testing ($n = 393$)	Prognostic and predictive value of treatment response to chemotherapy	Yang, Wang, et al. (2018)

(Continues)

TABLE 1 (Continued)

miRNA(s)	Source	Technology	Cancer site	Study design	Clinical application (s)	References
miR-16,-17,-20a,-21,-29b, -32,-101,-125b,-155-5p, -181,-196a,-204,-372,-373, -455-5p,-1246	Tissue (FFPE, Frozen	Meta-analysis	Mouth	Aggregate ($n = 1200$)	Prognostic value of miRNA signature in oral squamous cell carcinoma	Troiano et al. (2018)
miR-744-5p,-3196,-6794-5p,-6799-5p,-6820-5p,-8073	Blood, Serum	3D-Gene® Human miRNA Oligo Chip	Esophagus	Training ($n = 566$), validation ($n = 4965$)	Diagnostic value of miRNA signature for early disease detection	Sudo et al. (2019)
miR-18a,-20b,-106a,-223-3p, -486-5p,-584	Blood (Plasma)	RT-qPCR	Esophagus	Discovery ($n = 30$), training ($n = 84$), testing ($n = 214$), validation ($n = 99$)	Diagnostic value of miRNA signature in exosomes for early disease detection	Zhou et al. (2017)
miR-375	Tissue, TMA	LNA ISH	Esophagus	Validation ($n = 249$)	Prognostic value of epithelial cell expression	Li et al. (2013)
miR-27-3p	Blood (PBMC)	TaqMan RT-qPCR	Pancreas	Discovery ($n = 60$) and validation ($n = 292$)	Diagnostic value of miRNA expression in PBMCs in combination with CA-19-9 in serum for early disease detection	Wang et al. (2013)
let-7b-5p,miR-19a-3p, -19b-3p,-25-3p,-192-5p,-223-3p	Blood, Serum	RT-qPCR	Pancreas	Training ($n = 72$), testing ($n = 164$), validation ($n = 60$)	Diagnostic value of miRNA signature for early disease detection	Zou et al. (2019)
miR-24,-130b,-135b,-148a, -196	Tissue (FFPE, FNA)	RT-qPCR	Pancreas	Training ($n = 95$), validation ($n = 228$)	Diagnostic value of miRNA signature for separating PDAC from chronic pancreatitis and benign conditions	Brand et al. (2014)
miR-182-5p,-375-3p	Blood (Plasma)	RT-qPCR	Prostate	Training ($n = 113$), validation ($n = 304$)	Diagnostic value for early disease detection and prognostic value	Bidarra et al. (2019)
miR-27a-3p,-29b-3p,-4286	Blood (Serum)	RT-qPCR	Prostate	Training ($n = 155$), validation ($n = 100$)	Diagnostic value of miRNA signature in combination with clinicopathological factors for early disease detection	Lyu et al. (2019)
miR-148,-375	Urine	TaqMan RT-qPCR	Prostate	Training ($n = 166$), testing ($n = 134$)	Diagnostic value for early disease detection in combination with PSA levels	Stuopelyte et al. (2016)
miR-21	Tissue (TMA)	LNA ISH	Prostate	Validation ($n = 478$)	Prognostic value of stromal cell expression	Melbo-Jorgensen et al. (2014)
miR-182	Tissue (TMA)		Prostate	Validation ($n = 461$)	Prognostic value of epithelial cell expression	Baumann et al. (2019)

TABLE 1 (Continued)

miRNA(s)	Source	Technology	Cancer site	Study design	Clinical application (s)	References
let-7i-3p,miR-30d-3p,-106a-5p,-133a-3p, -185-5p,-210-3p, -221-3p,-222-3p, -615-3p	Tissue (FFPE, TCGA)	RT-qPCR, bioinformatics (miRNAseq)	Prostate	Training ($n = 198$), validation ($n = 159$), TCGA validation ($n = 350$)	Prognostic value of miRNA signature in combination with DNA methylation status of *AOX1*, *COL4A6*, and *PROM1* in prostate cancer	Strand et al. (2019)
miR-205	Tissue (TMA)	LNA ISH	Skin	Validation ($n = 297$)	Prognostic value of melanoma cell expression	Hanna, Hahn, et al. (2012)
miR-15b-5p,-16-5p,-150-5p, -374b-3p	Tissue (FFPE)	RT-qPCR	Skin	Training ($n = 92$), testing ($n = 119$), validation ($n = 45$)	Prognostic value and predictive value for metastatic disease	Hanniford et al. (2015)
miR-25	Blood (serum)	RT-qPCR	Stomach	Testing ($n = 318$)	Diagnostic and value for early disease detection and prognostic value	Kong et al. (2019)
miR-200c	Blood, tissue	Meta-analysis	Stomach	Aggregate ($n = 452$)	Diagnostic value for early disease detection	Huang et al. (2019)
miR-200c	Blood, Tissue	Meta-analysis	Stomach	Aggregate ($n = 935$)	Prognostic value	Huang et al. (2019)
let-7e, miR-21,-26a,-125b, -126,-148a,-222	Blood (plasma)	TaqMan RT-qPCR	Stomach	Discovery ($n = 27$), training ($n = 170$), validation ($n = 169$)	Prognostic value of miRNA signature in Stage II and III cases	Liu, Zhang, et al. (2017)
miR-29b-1-5p,-31-5p,-138-1-3p,-139-5p,-146b-5p,-155,-204-5p,-222-3p, -375,-551b-3p	Tissue (FNA)	RT-qPCR (miR*Inform* Thyroid Test)	Thyroid	Training ($n = 240$), validation ($n = 109$)	Diagnostic value of miRNA signature for separating thyroid cancer from benign conditions	Labourier et al. (2015)
miR-23a-3p,-31-5p,-125b-5p,-138-5p,-146b-5p,-152-3p,-181c-5p,-200c-3p, -222-3p,-342-3p,-345-5p, -346,-375,-424-3p,-486-5p,-551b-3p,-3074-5p,-574-3p,-5701,MID-16582,-20094, -50969,-50971,-50976	Tissue (FNA)	RT-qPCR (RosettaGX Reveal assay)	Thyroid	Discovery ($n = 82$), training ($n = 375$), validation ($n = 201$)	Diagnostic value of miRNA signature for separating thyroid cancer from benign conditions	Lithwick-Yanai et al. (2017)
miRNA classifier (64 miRNA signature)	Tissue (FFPE)	Microarrays (miRview mets^2 assay)	Various	Training ($n = 1282$), validation ($n = 509$)	Diagnostic value of miRNA classifier for organ site assignation of cancers of unknown primary origin	Meiri et al. (2012)

(Continues)

TABLE 1 (Continued)

miRNA(s)	Source	Technology	Cancer site	Study design	Clinical application (s)	References
miRNA classifier (up to 32 miRNA signature)	Tissue (TCGA, GEO datasets)	Bioinformatics (miRNAseq, microarrays)	Various	Training ($n = 3578$), testing ($n = 3024$), validation ($n = 504$)	Diagnostic value of miRNA classifier for organ site assignation of cancers of unknown primary origin	Tang et al. (2018)

Note: Unless otherwise noted, cancer type arising from cancer site is as follows: urothelial carcinoma from bladder, breast carcinoma from breast, colorectal adenocarcinoma from colon and/or rectum, endometrial carcinoma from endometrium, esophageal squamous cell carcinoma from esophagus, nonsmall cell lung cancer (adenocarcinoma or squamous cell carcinoma) from lung, pancreatic adenocarcinoma from pancreas, prostatic adenocarcinoma from prostate, hepatocellular carcinoma from liver, and cutaneous melanoma from skin. Total number of analyzed samples (n) from healthy controls, site-specific cancer, benign conditions, and/or (matched) normal sample of same organ site, and/or other-organ site(s) cancer. Samples from same case analyzed in different forms (e.g., frozen vs. FFPE tissue, serum vs. exosomal fraction from blood) were counted as independent samples. To harmonize cohort/population studies, we used definitions that mostly matched those attributed in the original study: Discovery set is designed for selection of candidate miRNAs after high-throughput profiling; Training set is designed for refining number of miRNAs in test and/or scoring system; Testing set is designed for assessing performance of miRNA(s), may include cases from Discovery and/or Training set; Validation set is designed for validating performance of miRNA(s) test in an independent cohort of patients not included in the Discovery, Training and/or Testing set(s). Population-based set consists of consecutive cancer cases for a determined period of time.

Abbreviations: FFPE, formalin-fixed paraffin-embedded tissue; FNA, fine-needle aspirate tissue biopsy; LNA ISH, in situ hybridization assay with locked nucleic acid-modified oligonucleotide probes; METABRIC, Molecular Taxonomy of Breast Cancer International Consortium; miRNAseq, small RNA sequencing; PBMC, peripheral blood mononuclear cells; RT-qPCR, reverse transcription quantitative-polymerase chain reaction; TCGA, the cancer genome atlas; TMA, tissue microarray.

TABLE 2 On-going clinical trials evaluating microRNAs as diagnostic, prognostic, and/or predictive indicators

miRNA(s)	Source	Technology	Cancer site	Study design	Clinical application(s)	Trial ID
Unspecified	Tissue (frozen)	Microarrays, miRNAseq	Bone and soft tissue	Discovery and testing ($n = 1000$ estimated)	Prognostic value in pediatric sarcomas, osteosarcomas, neuroblastomas	NCT01050296
Unspecified	Tissue, blood	Microarrays	Bone marrow	Discovery and testing ($n = 529$ actual)	Diagnostic value of miRNA signatures in acute myeloid leukemia	NCT00900224
Unspecified	Tissue, blood	Microarrays	Bone marrow	Discovery and testing ($n = 735$ estimated)	Diagnostic value of miRNA signatures in acute myeloid leukemia	NCT00898092
miR-10b	Tissue, Blood, CSF	RT-qPCR?	Brain	Testing ($n = 200$ estimated)	Prognostic and predictive value in gliomas	NCT01849952
Unspecified	Tissue (Frozen)	miRNAseq	Brain	Discovery and testing ($n = 640$ estimated)	Prognostic value in glioblastomas	NCT03770468
Unspecified	Blood	Unspecified	Breast	Discovery and testing (n = 506 estimated)	Predictive value for recurrence risk reduction by interventional diet, exercise and vitamin D regimen	NCT02786875
Unspecified	Blood	Unspecified	Breast	Discovery and testing ($n = 3500$ estimated)	Prognostic value in metastatic disease	NCT02338167
Unspecified	Blood (Extracellular vesicles)	Unspecified	Breast	Discovery and testing ($n = 370$ estimated)	Prognostic and prediction for treatment response to anti-HER2 therapy	NCT02514681
Unspecified	Tissue (FFPE)	RT-qPCR	Breast	Discovery and testing ($n = 1000$ estimated)	Prognostic value in early stage hormone-receptor positive breast cancer cases	NCT02918084
Unspecified	Tissue, Blood	Unspecified	Breast	Discovery ($n = 217$ actual)	Predictive value for treatment response to anti-HER2 therapy in combination with PET imaging (scheduled)	NCT01957332
Undisclosed	Blood (Plasma)	RT-qPCR	Breast	Validation ($n = 300$ estimated)	Predictive value for treatment response to anti-HER2 therapy	NCT02656589
Unspecified	Tissue	Microarrays	Breast	Discovery and testing ($n = 550$ estimated)	Predictive value of miRNAs for risk stratification of lobular cancer in situ	NCT00581750
5-miRNA, 8-miRNA signatures	Tissue	RT-qPCR?	Colon, rectum	Validation ($n = 200$ estimated)	Diagnostic value of miRNA signature for detection of lymph node metastasis	NCT04150081
Unspecified	Blood (Plasma)	RT-qPCR	Colon, rectum	Discovery and testing ($n = 200$ estimated)	Predictive value of miRNAs in neoadjuvant treatment of rectal cancer	NCT03962088
miR-20a-5p, -21,-103a-3p, -106b-5p, -143-5p,-215	Tissue	RT-qPCR	Colon, rectum	Interventional validation ($n = 430$ estimated)	Prognostic value of miRNAs in Stage II colorectal cancer	NCT02466113

(Continues)

TABLE 2 (Continued)

miRNA(s)	Source	Technology	Cancer site	Study design	Clinical application(s)	Trial ID
miR-20a-5p,-21,-103a-3p,-106b-5p,-143-5p,-215	Tissue	RT-qPCR	Colon, rectum	Validation ($n = 630$ estimated)	Prognostic value of miRNAs in Stage II colorectal cancer	NCT02635087
Unspecified	Tissue?	Unspecified	Colon, rectum	Discovery and testing ($n = 1000$ estimated)	Diagnostic and prognostic value of miRNAs	NCT03309722
miR-34b-5p, -34c-5p, -34c-3p,-184, -375,	Blood (Plasma)	RT-qPCR?	Endometrium	Validation ($n = 443$ estimated)	Diagnostic value of miRNA signature for detection of lymph node metastasis	NCT03776630
Unspecified	Blood (Serum, Plasma)	Unspecified	Gastrointestinal tract	Discovery and testing ($n = 6300$ estimated)	Diagnostic and prognostic value in colorectal, pancreatic, and gastroesophageal cancer	NCT04363983
miR-371	Blood (Plasma)	Unspecified	Germ cell	Testing ($n = 956$ estimated)	Diagnostic value for early disease detection of testicular cancer	NCT04435756
Unspecified	Tissue	NGS	Germ cell	Discovery and testing ($n = 200$ estimated)	Diagnostic and prognostic value in testicular stromal tumors	NCT01970696
Unspecified	Saliva, Blood, Tissue (FNA)	RT-qPCR	Head and Neck	Discovery and testing ($n = 462$ estimated)	Diagnostic or prognostic value of miRNA signature in matched bodily fluids or tissue samples (squamous cell carcinoma)	NCT04305366
Unspecified	Blood (Serum)	Unspecified	Head and Neck	Discovery ($n = 370$ estimated)	Prognostic value of miRNA signature in oral cancer	NCT03202810
Unspecified	Blood (Exosomes)	Unspecified	Lung	Discovery ($n = 200$ estimated)	Predictive value of miRNA signature for treatment response to immunotherapy treatment (scheduled)	NCT04427475
Unspecified	Blood (Exosomes)	Unspecified	Lung	Discovery ($n = 800$)	First line of screening for lung cancer detection in combination with high-resolution CT imaging (scheduled)	NCT04629079
Unspecified	Blood	Unspecified	Lung	Discovery and testing ($n = 286$)	Prognostic and predictive value in metastatic lung cancer	NCT03721120
Unspecified	Blood	Unspecified	Lung	Discovery and testing ($n = 1000$ estimated)	Diagnostic and prognostic value in early stage cases	NCT03397355
24-miRNA signature	Blood (Plasma)	Microfluidic card	Lung	Interventional, active surveillance of smokers ($n = 4119$ actual)	First line of screening for lung cancer detection	NCT02247453
Hummingbird microRNA profile	Blood	HMBDx microRNA Test	Lung	Testing ($n = 479$ actual)	First line of screening for lung cancer detection in combination with low-dose CT imaging (scheduled)	NCT03452514

TABLE 2 (Continued)

miRNA(s)	Source	Technology	Cancer site	Study design	Clinical application(s)	Trial ID
Unspecified	Blood (Plasma)	HTG EdgeSeq miRNA Assay	Lung	Discovery and testing ($n = 900$ estimated)	Predictive value of treatment response to targetable molecular alteration or immunotherapy	NCT02511288
16-miRNA and 45-miRNA signatures	Blood (Plasma)	Unspecified	Lung	Testing ($n = 2000$ estimated)	First line of screening for lung cancer detection in combination with low-dose CT imaging	NCT04323579
Unspecified	Blood (Plasma)	Unspecified	Esophagus	Discovery and testing ($n = 200$ estimated)	Predictive value of miRNA signature in combination with number of circulating tumor cells	NCT02812680
miR-192-5p,-194-5p,-215-5p	Exfoliated cells, Blood (Serum)	Digital RT-qPCR	Esophagus	Testing ($n = 220$ estimated)	Diagnostic value of miRNA signature for Barrett's Esophagus	NCT02464930
Unspecified	Bile (Exosomes)	Digital RT-qPCR	Esophagus	Testing ($n = 220$ estimated)	Diagnostic value of miRNAs for early cancer detection	NCT02464930
Unspecified	Blood	Unspecified	Pancreas	Discovery and testing ($n = 5000$ estimated)	Diagnostic, prognostic and predictive value	NCT03311776
Unspecified	Blood	Unspecified	Pancreas and Gall Bladder	Discovery and testing ($n = 500$ estimated)	Diagnostic value of miRNA signature compared to or in combination with other biomarkers (e.g., CA 19-9)	NCT02531607
Unspecified	Unspecified	RNAseq, nanostring, RT-qPCR	Pancreas	Discovery and testing ($n = 200$)	Prognostic and predictive value in pancreatic ductal adenocarcinoma and/or neuroendocrine tumors	NCT03840460
Unspecified	Blood (Serum)	miRNAseq	Pancreas	Discovery and testing ($n = 629$ estimated)	Predictive value of miRNA signature for malignant progression from Multiple Endocrine Neoplasia to Neuroendocrine Tumor	NCT03048266
Unspecified	Tissue, Blood, Urine, Saliva	miRNAseq	Prostate	Discovery ($n = 3000$ estimated)	Exploratory molecular profiling studies	NCT02594202
Unspecified	Blood	Unspecified	Prostate	Discovery and testing ($n = 330$ estimated)	Prognostic value for clinical relapse-free survival	NCT02745587
Unspecified	Blood (Exosomes)	miRNAseq	Prostate	Discovery and testing ($n = 600$ estimated)	Diagnostic value of exosomal miRNAs for early disease detection in combination with PSA and MR imaging	NCT03694483
X chromosome-linked miRNAs	Unspecified	Unspecified	Prostate	Discovery and testing ($n = 329$ actual)	Prognostic and predictive value for treatment response to hypofractionated radiotherapy	NCT01444820

(Continues)

TABLE 2 (Continued)

miRNA(s)	Source	Technology	Cancer site	Study design	Clinical application(s)	Trial ID
let-7c,miR-16, -25,-141, -151-3p -187, -188-5p, -196b, -200c, -200b,-375	Tissue	Unspecified	Prostate	Interventional, scheduled MR imaging and biopsy ($n = 628$ estimated)	Diagnostic value for early disease detection in combination with PSA and PHI	NCT04283032
Unspecified	Urine (Exosomes)	NGS	Prostate	Discovery and validation ($n = 240$ estimated)	Diagnostic value of exosomal miRNAs for separating pathological significant and insignificant prostate cancer	NCT03911999
miR-200b	Blood (Plasma)	RT-qPCR?	Ovary	Validation ($n = 443$ estimated)	Prognostic value of miR-200b changes in matched pre- and post-treatment samples	NCT03776630
Unspecified	Tissue	NGS	Ovary	Discovery and testing ($n = 200$ estimated)	Diagnostic and prognostic value in ovarian stromal tumors	NCT01970696
Unspecified	Blood	Unspecified	Pelvis	Discovery and testing ($n = 500$ estimated)	Prognostic and predictive value in cervical, endometrial, and ovarian cancer	NCT03622983
Unspecified	Tissue (Frozen)	NGS, RT-qPCR	Stomach	Discovery and validation ($n = 800$ estimated)	Predictive value of miRNA signature to chemotherapy treatment	NCT03253107
KRAS variants (DNA)	Saliva	DNA sequencing	Various	Validation ($n = 15{,}000$ estimated)	Role of *KRAS*-variant and miRNA binding site mutation in cancer risk, prevention, and treatment	NCT02253251
Unspecified	Blood (Exosomes)	Unspecified	Various	Discovery and testing ($n = 1000$ estimated)	Diagnostic, prognostic and/or predictive value of miRNAs in combination with circulating DNA	NCT04530890
Unspecified	Blood (Plasma)	Unspecified	Various	Discovery and testing ($n = 388$ estimated)	Diagnostic value for cancer detection in patients with serious illness but who have no organ specific symptoms	NCT01709539
Unspecified	Tissue	NGS	Various	Discovery and testing ($n = 300$ estimated)	Predictive value of treatment response in patients who are exceptional responders	NCT02243592

Note: This table includes only clinical studies with a status of "Active, not recruiting," "Recruiting," or "Enrolling by invitation," and with at least 200 participants by study design (estimated or actual participants). Unless otherwise noted, these are all observational prospective studies. Interventional study indicates that participants receive a treatment and/or other procedure (e.g., imaging) that is related to intended clinical application of miRNA(s) test. Unless otherwise indicated cancer type arising from cancer site is as follows: breast carcinoma from breast, colorectal adenocarcinoma from colon and/or rectum, esophageal squamous cell carcinoma from esophagus, nonsmall cell lung cancer (adenocarcinoma or squamous cell carcinoma) from lung, pancreatic adenocarcinoma from pancreas, prostatic adenocarcinoma from prostate.
Abbreviations: CSF, cerebrospinal fluid; CT, computed tomography; MR, magnetic resonance; NGS, next generation sequencing; RT-qPCR, reverse transcription quantitative-polymerase chain reaction; miRNAseq, small RNA sequencing; PET, positron emission tomography; PHI, prostate health index; PSA, prostate-specific antigen.

of tissue material obtained by tissue biopsy of fine needle aspirate (FNA) can often be challenging, especially when tissue sample is limited and/or lacks architectural context. Differential diagnosis studies based on miRNA expression in FNA samples from thyroid (Labourier et al., 2015; Lithwick-Yanai et al., 2017) and pancreas (Brand et al., 2014; Szafranska-Schwarzbach et al., 2011) led to the first commercialization of miRNA-based laboratory-developed tests (see Section 2.3). miRNA signatures have also shown diagnostic value in assigning organ site to cancers of unknown primary origin, which can improve patient management and outcome by matching patients with treatment options for that organ site (Meiri et al., 2012; Tang et al., 2018). miRNA signatures correlate with clinical parameters such as tumor size, lymph node involvement, and stage (Table 1). A 9-miRNA signature (miR-26a-5p, -29c-3p, -29c-5p, -30b-5p, -148a-3p, -361-3p, -645, -652-5p, -934) is associated with regional metastatic disease in early stage breast cancer patients (Xie et al., 2018). This miRNA signature along with other clinical parameters could be useful to guide what patients may elect to not have an axillary (sentinel) lymph node biopsy if at low risk of having metastatic spread to the lymph nodes and what patients should have said biopsy if at high risk. More recently, blood-based analyses have been used to determine the diagnostic utility of miRNAs for separating benign from malignant tumors or diagnosing a specific cancer type or subtypes (Table 1). A two-circulating miRNA signature (miR-629-3p, -4710) is associated with regional metastatic disease in breast cancer patients (Shiino et al., 2019). Curiously, there is no overlap between these diagnostic tissue-based (Xie et al., 2018) and serum-based miRNA signatures (Shiino et al., 2019), which may arise from technical (e.g., RT-qPCR vs. microarray) or biological differences (e.g., most differentially expressed miRNA in tissues may not be proportionally represented in circulation). Diagnosis of sarcomas represent a challenge due to their relative rarity and diversity of histological subtypes. A seven-circulating miRNA signature (miR-658, -762, -4281, -4649-5p, -4665-3p, -4736, -6836-3p) detected in serum samples can distinguish sarcoma patients regardless of subtype from other bone and soft tissue benign conditions as well as healthy controls (Asano et al., 2019).

2.2.2 | Diagnostic value of microRNA for early cancer detection

The utility of differential miRNA expression for early cancer detection has been extensively evaluated in blood as starting material. There is a recent trend to specifically interrogate miRNA levels within the exosomal/EV fraction of blood, but there is not a clear consensus as to whether serum or plasma is a more appropriate source for miRNA analysis. Tissue analysis from previous studies or from matched individuals in concurrent study has often guided the selection of most informative miRNAs to be evaluated (Table 1). A five-circulating miRNA (miR-1246, -1307-3p, -1364, -6861-5p, -6875-5p) and a 12-circulating miRNA (let-7b-5p, miR-16-5p, -19a-3p, -19b-3p, -20a-5p, -25-3p, -92a-3p, -93-5p, -106a-5p, -223-3p, -425-5p, -451a) signature analyzed in serum samples can distinguish breast cancer patients from individuals with benign breast conditions as well as healthy controls (Shimomura et al., 2016; Zou, Xia, et al., 2020). A five-circulating miRNA (miR-744-5p, -3196, -6794-5p, -6799-5p,-6820-5p, -8073) signature analyzed in serum samples (Sudo et al., 2019) and a six-circulating miRNA (miR-18a, -20b, -106a, -223-3p, -486-5p, -584) signature analyzed in plasma samples (Zhou et al., 2017) can distinguish esophageal cancer patients from healthy controls. Concordant levels of miR-223-3p and miR-584 in matched tissue, plasma, and the exosomal fraction of plasma from the same patients suggest that these two miRNAs are actively secreted by tumor cells (Zhou et al., 2017). Puzzlingly, there is more overlap between the 12-circulating miRNA breast cancer signature and the six-circulating miRNA esophageal cancer signature than there is between organ site–specific signatures.

Other bodily fluids have been used to study the diagnostic value of miRNA expression. The choice of these bodily fluids is informed and restricted by anatomic location of the organ(s) being interrogated such as saliva for upper digestive system (Setti et al., 2020) and stool for lower digestive system (Rashid et al., 2020), sputum for upper respiratory system (X. Zhang et al., 2019), and urine for urinary system (Kutwin et al., 2018; Paiva et al., 2020). A three-miRNA (miR-144-5p, miR-200b-3p, miR-451a) and a two-miRNA (miR-18a and -221) signature detected in stool samples can distinguish colorectal cancer patients from healthy controls (C. W. Wu et al., 2017; Yau et al., 2014). The lack of overlap between these signatures is expected since the three-miRNA signature is tailored to detect erythrocyte-expressed miRNAs (miR144-5p and -451a) as a modified fecal occult blood test whereas the other signature interrogates tumor-induced miRNA changes. A two-miRNA (miR-148 and -375) signature detected in urine samples can distinguish prostate cancer patients from individual with benign prostate conditions and healthy controls (Stuopelyte et al., 2016). The combination of this miRNA signature and serum levels of prostate-specific antigen (PSA) provides a better diagnostic performance; this is good example of how integrating existing clinical indicators and new miRNA biomarkers can enhance diagnostic power.

2.2.3 | Prognostic value of microRNA for treatment selection and patient management

The utility of differential miRNA expression for identifying patients with a worse clinical outcome regardless of treatment regimen has been most extensively studied in tissue samples. Especially in the setting of prognostic studies, ISH detection has flourished as a powerful research tool to extract contextual information based on altered miRNA expression at single-cell resolution within tumor lesions. Moreover, an ISH detection assay can obtain information from hundreds of tumor samples at a time in rapid and cost-effective fashion using tissue microarrays (Table 1). Depending on the cancer type and the specific miRNA(s), altered expression in cancer cells has been reported to be more informative than in other cell types of the TME or vice versa (Table 1). Using similar ISH assays, several groups have reported that stromal (mostly cancer-associated fibroblast) expression of miR-21 carries more prognostic information than cancer cell expression in breast, colorectal, pancreatic, and prostate cancer (Kadera et al., 2013; Kang et al., 2015; Kjaer-Frifeldt et al., 2012; MacKenzie et al., 2014; Melbo-Jorgensen et al., 2014; Nielsen et al., 2011). miR-143 and miR-145 are closely linked in a gene cluster, but they can be differentially expressed in some cell types and contexts (Kent et al., 2014; Sempere et al., 2004). Stromal expression of miR-143 in female lung cancer patients and miR-145 in male lung cancer patients is associated with clinical outcome (Skjefstad et al., 2018). This sex-specific survival effect of stromal expression is highly correlated with steroid hormone receptor status in the tumor tissues, suggesting a regulatory interaction and crosstalk between these two miRNAs and sex hormones (Skjefstad et al., 2018). There are some limitations with these retrospective studies, either using ISH or other detection methods. miRNA expression may be correlated with known prognostic indicators (e.g., tumor size) and stratification of cases to match clinical parameters decreases statistical power. Similarly, a patient with a worse prognosis determined by standard clinical parameters would likely have received a more aggressive treatment, which may confound the interpretation of altered miRNA expression.

2.2.4 | Predictive value of microRNA for treatment response and treatment selection

The utility of differential miRNA expression for identifying responders has been evaluated in tissue samples collected prior to or in blood samples before and after specific treatment. While many miRNA-based predictive studies have been reported, we find only a few meet our inclusion criterion of participant number (Table 1). This type of study is more challenging due to the need to include a large sample size for each specific treatment arm to have robust statistical power for analysis. A case in point is the association of stromal expression of miR-21 with poor treatment response to 5-fluorouracil, but not gemcitabine in pancreatic cancer patients (Donahue et al., 2014). This study started with 538 patients recruited into RTOG-9704 clinical trial comparing 5-fluorouracil to gemcitabine before and after chemoradiation in an adjuvant setting (NCT00003216). Attrition occurred at different levels, from not meeting inclusion criteria, to lack of tissue materials for analysis or poor tissue preservation determined postanalysis, and thereby the final analysis was limited to about 90 cases in each treatment arm (Donahue et al., 2014). Some of these studies also blur the line between a prognostic and predictive indication based on miRNA expression. For example, if miRNA expression is associated with metastatic disease, it is difficult to dissociate a direct effect on tumor response from expected poorer clinical performance of more advanced cases with more extended tumor burden. A case in point is the association of miR-10b expression in PBMCs with treatment response to chemotherapy in advanced stage lung cancer (Yang, Wang, et al., 2018). Lower levels of miR-10b expression in PBMCs in pretreatment samples correlated with complete or partial response to treatment. In both responders and nonresponders miR-10b levels were lower in PBMCs in post-treatment relative to pretreatment samples (Yang, Wang, et al., 2018). miR-10b levels were higher in patients with adenocarcinomas vs. other subtypes, with lymph node metastasis versus regional metastasis-free, and without distant metastasis versus metastatic disease (Yang, Wang, et al., 2018). Thus, these associations may confound the pure predictive value of the miR-10b assay.

2.3 | Laboratory developed tests for miRNA-based diagnosis

A laboratory develop test (LDT) is a type of in vitro diagnostic that is designed and performed by a single laboratory under Clinical Laboratory Improvement Amendments (CLIA) regulations. Asuragen and Rossetta Genomics were first companies in bringing miRNA-based LDTs to market (Bonneau et al., 2019). These LDTs were rigorously validated in

clinical trials (Brand et al., 2014; Labourier et al., 2015; Lithwick-Yanai et al., 2017; Szafranska-Schwarzbach et al., 2011). Asuragen miRInform Pancreas LDT has an improved sensitivity and specificity compared to cytological analysis of the same fine-needle aspiration (FNA) specimens (82.6 vs. 78.8% and 96.1 vs. 69.2%, respectively) to correctly identify PDAC or benign conditions based on altered expression of miR-24,-130b,-135b,-148a,-196 (Brand et al., 2014). The most successful miRNA-based LDT is a 10-miRNA signature (now TyraMIR®, Table 1) used in combination with Asuragen miRInform Thyroid test (now ThyGeNEXT®) that detects a DNA/mRNA mutation panel (Labourier et al., 2015). The combined information of miRNA expression and mutation status provides an 89% sensitivity and 85% specificity to correctly identify malignant or benign cases from FNAs of solid thyroid nodules, improving preoperative diagnosis based on cytology alone (Labourier et al., 2015). Similarly, Rosetta Genomics developed RosettaGX Reveal™ LDT that can correctly identify malignant and benign cases from FNAs of solid thyroid nodules with an 85% sensitivity and 72% specificity solely based on a 24-miRNA signature (Lithwick-Yanai et al., 2017; Table 1). Rosetta Genomics miRView™ mets is a LDT based on the differential expression of 64 miRNAs to assign most likely organ site for cancers of unknown primary. In a validation set of 509 independent cases, miRView™ correctly identified the tissue of origin for up to 90% of the cases (Meiri et al., 2012). These early successes seemed to have paved the way for miRNA-based diagnosis in the clinic, however, this has not yet been realized. There are probably many contributing factors to this, including, but not limited to, competition with already established clinical biomarkers, coverage by insurance companies, the lack of mechanistic link or incomplete understanding between altered miRNA expression and the underlying disease. Rosetta Genomics and Asuragen have been since acquired or merged with other companies (Bonneau et al., 2019) reflecting the challenges of LDT profitability. TyraMIR® + ThyGeNEXT® LDT is being offered by Interpace Diagnostics based on Asuragen's assets for differential diagnosis of thyroid cancer or benign condition (ThyGeNEXT +ThyraMIR, 2020). Similarly, Genoptix acquired some assets of Rosetta Genomics in 2018, but miRView™ mets and Reveal™ tests are not currently being offered (Genoptix, 2019); Genoptix was subsequently acquired by NeoGenomics.

2.4 | On-going clinical trials

More than 150 clinical studies are registered at clinicaltrials.gov in which the value of a miRNA or miRNA signature is being investigated for a variety of clinical applications from early disease detection to treatment response (ClinicalTrials.gov, 2020). These clinical trials are different in scope, cancer site, and clinical applications, but there is a general trend for the desirable use and perceived value of miRNA analysis in bodily fluids, mainly blood, as a noninvasive tool to inform clinical decisions. We only consider for detailed discussion studies that include more than 200 participants (Table 2). Many of these clinical trials are exploratory or have combined discovery and validation phases, whereas some have a more advanced design to validate the clinical utility of a published miRNA signature. Hummingbird diagnostics (Hummingbird, 2020) and Toray Molecular Laboratory (Toray, 2020) are two of the companies conducting validation trials with the hope to bring to market miRNA-based blood test for early detection of lung, breast, and other cancer types.

2.4.1 | Diagnostic value of microRNA for early cancer detection

In several clinical studies (Table 2), the utility of miRNA-based blood analysis for early detection is conducted in conjunction with a primary screening tool such as imaging (e.g., breast and lung cancer) or a more established biomarker (e.g., PSA in prostate cancer and CA 19-9 for pancreatic cancer). In 2017, Toray launched a multi-institutional prospective clinical trial with 2000 estimated participants to determine if serum miRNA signature can be used to stratify the risk of the individual to be diagnosed with breast cancer or a benign condition in subsequent tissue biopsies after receiving an abnormal breast imaging finding classified as BI-RADS® 3, 4, or 5 (Barke, 2019). While the primary goal is to complement information of screening mammography and minimize the number of unwarranted call backs for diagnostic imaging and biopsies, implicit in this study is the possibility of using this serum miRNA signature for early detection of breast cancer either alone or by enhancing imaging findings. Hummingbird diagnostics and other sponsors have similar on-going clinical trials to assess the utility of circulating miRNAs to assist or complement the information of low dose computed tomography (CT) scans for early detection and diagnosis of lung cancer.

2.4.2 | Prognostic and predictive value of microRNA for patient management, treatment selection, and/or treatment response

While there are several clinical trials investigating the prognostic or predictive value of miRNAs, the potential use of these miRNAs is exploratory and not integral to the clinical study. This likely reflects the fact that known mechanisms of action of current treatments (e.g., chemotherapy, targeted therapies, immunotherapy) are not directly linked or dependent on miRNA activity. Several miRNAs have been shown to regulate cellular processes such as apoptosis, proliferation, migration, epithelial-to-mesenchymal transition, antigen presentation, and immunosuppression (see Section 3) that ultimately affect treatment response and clinical outcome. Because the same miRNA can be implicated in multiple cellular processes and lead to different regulatory outcomes depending on the disease context, this can create challenges for correctly interpreting and acting on the information provided by these miRNA biomarkers.

3 | MICRORNA-BASED THERAPEUTICS

The frequent expression dysregulation of a core set of cancer-associated miRNAs (e.g., let-7, miR-10b, -15, -16, -17-5p, -20a, -21, -29b, -34, -155, -221) lends support to their etiological involvement, but are these miRNAs drivers, mediators, or mere passengers in the processes of tumor formation, progression, and metastasis? A large body of literature of in vitro functional studies supports a phenotypic contribution of specific miRNA activities. We will focus our discussion on rigorous in vivo studies that complement or expand on the type of questions and mechanistic insights that can be addressed with in vitro cell models. These in vivo studies utilize genetically engineered, chemically induced, and/or transplantable cancer models in which miRNA activity can be modulated by genetic or pharmacological approaches (Bajan & Hutvagner, 2020; Fornari et al., 2019; Forterre et al., 2020; Pal & Kasinski, 2017). Genetic approaches include genetic engineering of the host animals or viral delivery systems (Fornari et al., 2019; Pal & Kasinski, 2017). Typically, the range of activity manipulation afforded by genetic engineering can be more sophisticated and precise than viral delivery systems. Retroviruses and lentiviruses are powerful research tools but with a limited translational potential, whereas adeno-associated viruses have favorable properties to make it into the clinic. While none of the on-going clinical trials with miRNA drugs uses a viral delivery system, viral approaches to replenish miRNA expression may be a viable option. Viral approaches to inhibit miRNA activity are more cumbersome, requiring a squelching strategy such as miRNA sponge to sequester miRNA molecules away from target sites or a combination with CRISPR system to delete miRNA locus, which currently do not seem viable in a clinical setting. Pharmacological approaches typically include chemically modified oligonucleotides that can be administered unconjugated, or conjugated to or encapsulated in a nanoparticle carrier (Bajan & Hutvagner, 2020; Forterre et al., 2020), but there are also some efforts to develop small molecule modulators with drug-like properties (Fan et al., 2019; Monroig-Bosque et al., 2018; Wen, Danquah, et al., 2015). Currently, chemically modified oligonucleotide approaches are the only ones that have been or are being evaluated in clinical trials.

3.1 | Mouse models causally link miRNA activity to carcinogenesis

Global or conditional modulation of the activity of a miRNA or multiple miRNAs by genetic deletion of a miRNA or miRNA gene cluster for loss-of-function studies and enforced or inducible expression of a miRNA or miRNA gene cluster for gain-of-function studies have causally linked specific miRNAs to the initiation and/or progression of cancer in hematological and solid tumors (Table 3). The majority of global KO mouse lines of cancer-associated miRNA or miRNA gene clusters are viable and do not exhibit overt developmental defects (Bartel, 2018; Park et al., 2010). Nonetheless, there are some notable exceptions to organismal viability, and many of these global KO mice have a spectrum of mild to severe phenotypes related to immune cell function (Bartel, 2018; Park et al., 2010). It is important to note that miRNA-mediated regulation in immune cells may lead to different outcomes in the context of hematological malignancies in which a specific immune cell subtype constitutes the neoplastic cells versus solid tumors in which specific immune cell subtype(s) and their interactions with the neoplastic cells can be tumor-promoting (tumorigenic) or tumor-restraining (tumoricidal). We describe for some of the most studied miRNAs, their intrinsic role as tumor suppressive or oncogenic (oncomiR) actors in the neoplastic cells as well as their potential contribution or compounded effect in other cell types of the TME.

TABLE 3 Genetic or viral modulation of miRNA activity in in vivo cancer models

miRNA modulation	Approach	Model	Cancer site	Overall phenotype	miRNA role	References
miR-20a loss	Ex vivo viral transduction, enforced expression of inhibitory sequence	Intravenous injection of LM7 osteosarcoma cells expressing BLOCK-iT™ Pol II miR-20a RNAi Expression Vector	Bone	Decrease in metastatic nodules in the lung	Oncogenic, promotes distant colonization	Huang et al. (2012)
miR-15,-16 loss	Genetic, global KO	Mir-15a~-16-1$^{-/-}$	Bone marrow, spleen	Develops lymphoproliferative disorders, including CLL	Tumor suppressive, B-cell intrinsic function	Klein et al. (2010)
miR-15,-16 loss	Genetic, global KO	Mir-15b~-16-2$^{-/-}$	Bone marrow, spleen	Develops B cell malignancies, including CLL	Tumor suppressive, B-cell intrinsic function	Lovat et al. (2015)
mir-15,-16 loss	Genetic, global KO	Mir-15a~-16-1$^{-/-}$; Mir-15b~-16-2$^{-/-}$	Bone marrow, spleen	Develops myeloid proliferative disorders, including AML	Tumor suppressive, Myeloid cell-intrinsic function. Complete loss of activity of miR-15/-16 family members shifts cancer type susceptibility	Lovat et al. (2018)
miR-15,-16 gain	In vivo viral transduction, enforced expression	Systemic delivery lentiviral vector expressing miR-15 and miR-16 in NZB de novo model of CLL	Bone marrow, spleen	Decrease in tumor burden	Tumor suppressive, reduced viability of transduced malignant B-1 cells	Kasar et al. (2012)
miR-17,-18, -19a,-20, -19-1b gain	Ex vivo viral transduction, enforced expression	Engrafted Eμ-Myc cells expressing Mir-17~-19-1b	Bone marrow	Increase in overall tumor burden and shorter overall survival	Oncogenic, accelerates Myc-driven B-cell lymphoma formation, and progression	He et al. (2005)
miR-19a, -19b-1 gain	Ex vivo viral transduction, enforced expression	Engrafted Eμ-Myc cells transduced with minigene expressing Mir-19a~-19-1b	Bone marrow	Decrease in overall survival	Oncogenic, accelerates Myc-driven B-cell lymphoma formation and progression	Olive et al. (2009)
miR-19a, -19b-1 loss	Genetic, global KO	Eμ-Myc;miR-19a~-19b-1$^{-/-}$	Bone marrow	Increase in overall survival	Oncogenic, accelerates Myc-driven B-cell lymphoma formation, and progression	Han et al. (2015)
miR-34a, -34b,-34c loss	Genetic, global KO	Eμ-Myc; Mir-34a$^{-/-}$;Mir-34b~-34c$^{-/-}$	Bone marrow	No difference in tumor incidence	Neutral, miR-34 activity dispensable in cancer model known to be restrained by p53 function	Concepcion et al. (2012)
miR-155 gain	Genetic, enforced expression in B cell	Eμ-Mir-155	Bone marrow, spleen	Develops B cell malignancies, including ALL	Oncogenic, B-cell intrinsic function	Costinean et al. (2006)

(Continues)

TABLE 3 (Continued)

miRNA modulation	Approach	Model	Cancer site	Overall phenotype	miRNA role	References
miR-155 loss	Genetic, global KO	FLT3-ITD;Mir-155$^{-/-}$	Bone marrow	Decrease in tumor burden	Oncogenic, likely myeloid cell-intrinsic function	Wallace et al. (2017)
miR-10b loss	Genetic, global KO	MMTV-PyMT;Mir-10b$^{-/-}$	Breast	Delay in tumor formation, decreased in distant metastasis	Oncogenic, phenotype presumed to be driven by miR-10b activity in epithelial cells	Kim, Diverly, et al. (2016)
miR-21 loss	Genetic, global KO	MMTV-PyMT;Mir-21$^{-/-}$, C3(1)-TAg;Mir-21$^{-/-}$	Breast	Significant but slight delay in tumor formation, no change in tumor multiplicity	Tumor promoting, modest effect; cell type-specific activity not determined	Sempere LF, unpublished observations
miR-155 loss	Genetic, global KO	Brca1$^{L/L}$;Trp53$^{L/L}$;Mir-155$^{-/-}$; *K14-Cre*	Breast	No change in overall survival	Oncogenic activity in epithelial cells is negated by tumor suppressive activity in the TME	Kim, Song, et al. (2016)
miR-155 loss	Ex vivo viral transduction, enforced expression of inhibitory sequence	Bone marrow engraftment of HSP cells expressing miRT against miR-155 in MMTV-PyMT	Breast	Increase in tumor growth and overall tumor burden	Tumor suppressive in the TME, miR-155 activity induces tumoricidal phenotype of macrophages	Zonari et al. (2013)
miR-17,-18, -19a,-20, -19-1b,-92 gain	Ex vivo viral transduction, enforced expression	Orthotopic implant of Ink4c$^{-/-}$; Ptch1$^{+/-}$ granule neuron progenitors cells expressing Mir-17~-92	Brain	Increase in tumor initiation, progression	Oncogenic, accelerates medulloblastoma	Uziel et al. (2009)
miR-31 loss	Genetic, conditional KO in epithelial cells	Apc$^{Min/+}$;Mir-31$^{L/L}$;Villin-Cre	Colon	Increase in tumor multiplicity and tumor burden	Tumor suppressive, cell-autonomous role in epithelial cells	Liu, Bai, et al. (2017)
miR-34a and/or miR-34b–34c loss	Genetic, global KO	Apc$^{Min/+}$;Mir-34a$^{-/-}$ and/or Apc$^{Min/+}$;Mir-34b~-34c$^{-/-}$	Colon	Increase in tumor burden, decreased in overall survival	Tumor suppressive, phenotype is enhanced when all miR-34 family members are deleted	Jiang and Hermeking (2017)
miR-155 loss	Genetic, global KO	Chemically-induced carcinogenesis in Mir-155$^{-/-}$	Colon	Decrease in tumor multiplicity and tumor growth	Tumor-promoting, miR-155 activity likely tumorigenic in the TME	Chen et al. (2015)
miR-155 loss	Genetic, global KO	Subcutaneous implant of MC38 colon cancer cells in Mir-155$^{-/-}$	Colon	Decrease in tumor multiplicity and tumor growth	Tumor-promoting in the TME, miR-155 activity dampens anti-tumor T cell-mediated responses	Chen et al. (2015)

TABLE 3 (Continued)

miRNA modulation	Approach	Model	Cancer site	Overall phenotype	miRNA role	References
miR-17,-18, -19a,-20, -19-1b,-92 gain	Genetic, conditional overexpression in retinal cells	$Rb^{L/L}$;$p107^{-/-}$;CAG-LSL-Mir-17–92;Pax6α-Cre	Eye	Increase in tumor initiation, progression and metastatic spread to the brain	Oncogenic, accelerates retinoblastoma	Conkrite et al. (2011)
miR-15,-16 loss	Genetic, global KO	Subcutaneous implant of H-22 liver cancer cells in Mir-15a~-16-$1^{-/-}$	Liver	Decrease in tumor growth	Tumor promoting in the TME, macrophage-mediated tumorigenic response	Jia et al. (2018)
miR-15,-16 loss	Genetic, global KO	Subcutaneous implant of H-22 liver cancer cells in Mir-15a~-16-$1^{-/-}$	Liver	Increase in tumor growth	Tumor suppressive in the TME, B-cell mediated anti-tumor response	Jia et al. (2019)
miR-21 loss	Genetic, global KO	Subcutaneous implant of Heps liver cancer cells in Mir-$21^{-/-}$	Liver	Increase in tumor growth	Tumor suppressive in the TME, T cell-mediated anti-tumor response	He et al. (2017)
Let-7b gain	In vivo viral transduction, enforced expression	LSL-$Kras^{G12D/+}$ plus nasal inhalation of adenoviral vectors expressing let-7b and Cre	Lung	Decrease in tumor growth and tumor burden	Tumor suppressive, likely cell-autonomous role in epithelial cells based on adenoviral delivery	Esquela-Kerscher et al. (2008)
Let-7g gain	In vivo viral transduction, enforced expression	LSL-$Kras^{G12D/+}$;$Tp53^{L/L}$ plus intratracheal lentiviral vectors expressing let-7 g and Cre	Lung	Decrease in tumor multiplicity and tumor burden	Tumor suppressive, likely cell-autonomous role in epithelial cells based on lentiviral delivery	Kumar et al. (2008)
Let-7g gain	In vivo viral transduction, enforced expression	LSL-$Kras^{G12D/+}$ plus nasal inhalation of lentiviral vector expressing let-7g and Adeno-Cre	Lung	Decrease in tumor growth and tumor burden	Tumor suppressive, likely cell-autonomous role in epithelial cells based on lentiviral delivery	Trang et al. (2010)
miR-21 loss	Genetic, global KO	Latent 2 $Kras^{G12D}$;Mir-$21^{-/-}$	Lung	Delay in tumor initiation, decrease in tumor multiplicity and overall tumor burden	Oncogenic, phenotype presumed to be driven by miR-10b activity in epithelial cells	Hatley et al. (2010)
miR-21 gain	Genetic, global overexpression	Latent 2 $Kras^{G12D}$; CAG-Mir-21	Lung	Increase in tumor multiplicity and overall tumor burden	Oncogenic, phenotype presumed to be driven by miR-10b activity in epithelial cells	Hatley et al. (2010)
miR-21 loss	Genetic, conditional KO in macrophages	Orthotopic implant of LLC lung cancer cells in Mir-$21^{L/L}$;LysM-Cre	Lung	Decrease in tumor growth	Tumor promoting in the TME, macrophage-mediated tumorigenic response	Sahraei et al. (2019)

(Continues)

TABLE 3 (Continued)

miRNA modulation	Approach	Model	Cancer site	Overall phenotype	miRNA role	References
miR-21 loss	Genetic, global KO	Subcutaneous implant of LLC1 lung cancer cells in Mir-21$^{-/-}$	Lung	Decrease in tumor growth	Tumor promoting in the TME, macrophage-mediated tumorigenic response	Xi et al. (2018)
miR-30b loss	In vivo CRISPR/Cas9-mediated KO via viral transduction	LSL-Kras$^{G12D/+}$;Tp53$^{L/L}$ plus nasal inhalation of lentiviral CRISPR/Cas-Cre vector	Lung	Increase in tumor multiplicity and overall tumor burden	Tumor suppressive, likely cell-autonomous role in epithelial cells based on lentiviral delivery	Hong, Yao, et al. (2020)
miR-34a gain	In vivo viral transduction, enforced expression	LSL-Kras$^{G12D/+}$;LSL-Tp53$^{R172H/+}$ plus intratracheal lentiviral miR-34a minigene and Adeno-Cre	Lung	Delay in tumor initiation and progression	Tumor suppressive, likely cell-autonomous role in epithelial cells based on lentiviral delivery	Kasinski and Slack (2012)
miR-34a loss	Genetic, global knockout	LSL-Kras$^{G12D/+}$;Tp53$^{L/+}$;Mir-34a$^{-/-}$ plus nasal inhalation of Adeno-Cre	Lung	Increase in tumor burden	Tumor suppressive	Okada et al. (2014)
miR-146a loss	In vivo CRISPR/Cas9-mediated KO via viral transduction	LSL-Kras$^{G12D/+}$;Tp53$^{L/L}$ plus nasal inhalation of lentiviral CRISPR/Cas-Cre vector	Lung	Increase in tumor multiplicity and overall tumor burden	Tumor suppressive, likely cell-autonomous role in epithelial cells based on lentiviral delivery	Hong, Yao, et al. (2020)
miR-155 loss	Genetic, global KO	Subcutaneous implant of LLC lung cancer cells in Mir-155$^{-/-}$	Lung	Increase in tumor growth	Tumor suppressive in the TME, miR-155 activity reduces MDSC infiltration	Wang et al. (2015)
miR-155 loss	Genetic, global KO	Subcutaneous implant of LLC1 or LLC1-OVA lung cancer cells in Mir-155$^{-/-}$	Lung	Decrease in tumor growth	Tumor-promoting in the TME, miR-155 activity required for MDSC-mediated Treg induction and dampening anti-tumor T cell-mediated responses	Chen et al. (2015)
miR-190b loss	In vivo CRISPR/Cas9-mediated KO via viral transduction	LSL-Kras$^{G12D/+}$;Tp53$^{L/L}$ plus nasal inhalation of lentiviral CRISPR/Cas9– Cre vector	Lung	Increase in tumor multiplicity and overall tumor burden	Tumor suppressive, likely cell-autonomous role in epithelial cells based on lentiviral delivery	Hong, Yao, et al. (2020)
miR-190b gain	In vivo viral transduction, enforced expression	LSL-Kras$^{G12D/+}$;Trp53$^{L/L}$ plus nasal inhalation of lentiviral miR-190b/Cre vector	Lung	Decrease in tumor multiplicity and overall tumor burden	Tumor suppressive, likely cell-autonomous role in epithelial cells based on lentiviral delivery	Hong, Yao, et al. (2020)
miR-21 gain	Genetic, Inducible expression in Nestin-expressing cells	LSL-Tet^{OFF}Mir-21;Nestin-Cre	Lymph nodes, spleen	Develops pre-B-cell lymphoma	Oncogenic, oncogenic addition (tumor regresses when enforced expression is discontinued)	Medina et al. (2010)

TABLE 3 (Continued)

miRNA modulation	Approach	Model	Cancer site	Overall phenotype	miRNA role	References
miR-31 loss	Genetic, global KO (Rat)	Chemically-induced carcinogenesis in Mir-31$^{-/-}$	Esophagus	Prevents tumor formation	Tumor promoting	Fong et al. (2020)
miR-17,-18, -19a,-20, -19-1b, -92 loss	Genetic, conditional KO in pancreatic epithelia	LSL-Kras$^{G12D/+}$;Tp53$^{L/+}$;Mir-17–92$^{L/L}$;Pft1a-Cre	Pancreas	Delay in tumor formation, no change in overall survival	Neutral to oncogenic, epithelial activity may affect MAPK signaling, and metastatic spread	Quattrochi et al. (2017)
miR-21 loss	Genetic, global KO	LSL-Kras^{G12D};Tp53$^{L/+}$;Mir-21$^{-/-}$Pdx1-Cre	Pancreas	Faster tumor initiation and progression, and shorter overall survival	Tumor suppressive in the TME, stromal activity required for myofibroblast formation (presumed tumor-restraining)	Schipper et al. (2020)
Mir-29a loss	Genetic, global KO	Ela1-TAg;Mir-29a$^{-/-}$	Pancreas	No change in tumor initiation and overall survival	Neutral	Dooley et al. (2017)
miR-34a loss	Genetic, conditional KO in pancreatic epithelia	LSL-Kras$^{G12D/+}$;Mir-34a$^{L/L}$;Pft1a-Cre	Pancreas	Increase in tumor initiation and progression	Tumor suppressive	Hidalgo-Sastre et al. (2020)
miR-216 or miR-217 loss	Genetic, global KO	Ela1-Kras$^{G12D/+}$;Mir-216a$^{-/-}$ or Mir-217$^{-/-}$	Pancreas	No change in tumor growth	Neutral	Sutaria et al. (2019)
miR-17,-18, -19a,-20, -19-1b, -92 gain	Ex vivo viral transduction, enforced expression	Subcutaneous implant of PC-3 or M12 prostate cancer cells transduced with minigene expressing Mir-17~-92	Prostate	Decrease in tumor growth	Tumor suppressive	Ottman et al. (2016)
miR-21 loss	Genetic, global KO	Chemical-induced carcinogenesis in Mir-21$^{-/-}$	Skin	Delay in tumor formation and decrease in tumor multiplicity	Oncogenic, phenotype presumed to be driven by miR-21 activity in keranocytes	Ma et al. (2011)
miR-21 loss	Genetic, global KO	Subcutaneous implant of B16 melanoma cells in Mir-21$^{-/-}$	Skin	Decrease in tumor growth	Tumor promoting in the TME, macrophage-mediated tumorigenic response	Xi et al. (2018)
miR-155 loss	Genetic, conditional KO in T cells	Subcutaneous implant of B16F10 or B16F10-OVA melanoma cells in Mir-155$^{-/-}$	Skin	Increase in tumor growth	Tumor suppressive in the TME, miR-155 activity in T cells is required for anti-tumor response, reduces MDSC infiltration	Ekiz et al. (2019) and Huffaker et al. (2017)

(Continues)

TABLE 3 (Continued)

miRNA modulation	Approach	Model	Cancer site	Overall phenotype	miRNA role	References
miR-155 loss	Genetic, adoptative transfer of KO T cells	Subcutaneous implant of B16F10 melanoma cells in mice with Mir-155$^{-/-}$ T-cells	Skin	Increase in tumor growth, decrease in overall survival	Tumor suppressive in the TME, miR-155 activity in T cells is required for anti-tumor response	Dudda et al. (2013)
miR-155 gain	Ex vivo viral transduction, enforced expression in T cells	Subcutaneous implant of B16F10 melanoma cells in animals overexpressing miR-155 in T cells	Skin	Decrease in tumor growth, increase in overall survival	Tumor suppressive in the TME, miR-155 activity in T cells is required for anti-tumor response	Dudda et al. (2013) and Martinez-Usatorre et al. (2019)
miR-155 loss	Genetic, global KO	Subcutaneous implant of B16F10 melanoma cells in Mir-155$^{-/-}$	Skin	Increase in tumor growth	Tumor suppressive in the TME, miR-155 activity reduces MDSC infiltration	Wang et al. (2015)
miR-21 loss	Genetic, global KO	Subcutaneous implant of S180 fibrosarcoma cells in Mir-21$^{-/-}$	Soft tissue	Increase in tumor growth	Tumor suppressive in the TME, T cell-mediated anti-tumor response	He et al. (2017)
miR-21 loss	Genetic, global KO	Tp53$^{-/-}$;Mir-21$^{-/-}$	Various	Slight delay in tumor formation, no change in overall survival	Neutral to slightly oncogenic. Slight change in incidence of p53-depedent cancer spectrum	Ma et al. (2013)
miR-34a, -34b,-34c loss	Genetic, global KO	Mir-34a$^{-/-}$;Mir-34b~-34c$^{-/-}$	Various	No spontaneous tumor formation	Neutral, lymphomas, and sarcomas arise in p53-deficient mice	Concepcion et al. (2012)
miR-34a, -34b,-34c loss	Genetic, global KO	1-Gy irradiation of Mir-34a$^{-/-}$; Mir-34b~-34c$^{-/-}$	Various	No radiation-induced tumor formation	Neutral, lymphomas and sarcomas arise in p53-deficient mice	Concepcion et al. (2012)

Note: Unless otherwise noted, experiments were conducted in mice.

Abbreviations: ALL, acute lymphoblastic leukemia or lymphoblastic lymphoma; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; HSP, hematopoietic stem and progenitor; L, conditional "floxed" allele, flanked by Lox P sites; LSL, Lox-STOP-Lox cassette; MDSC, myeloid-derived suppressive cells; miRT, miRNA target sequence complementary to an miRNA seed region, acts as miRNA sponge; NZB, New Zealand Black; TME, tumor microenvironment.

3.1.1 | Mir-15~Mir-16 gene family

Chromosomal deletion of *MIR15A–MIR16-1* gene cluster in chronic lymphocytic leukemia (CLL) was the first causal link between miRNA function and human cancer (Calin et al., 2002). *MIR15A–MIR16-1* gene cluster maps to the minimal region at chromosome 13q14.3 which is frequently deleted in CLL cases and expression of miR-15 and miR-16 is low in these cases (Calin et al., 2002). Enforced expression of miR-15a and miR-16-1 in MEG-01 leukemic cells completely inhibits tumor formation in a xenograft model (Calin et al., 2008). Similarly, enforced expression of miR-15 and miR-16 in B-1 cells derived from New Zealand Black mouse model of CLL or systemically in this de novo CLL model significantly reduced tumor growth (Kasar et al., 2012; Salerno et al., 2009). Global KO of the *Mir-15a~Mir-16-1* gene cluster in mice leads to CLL (Klein et al., 2010). Similarly, global KO of the *Mir-15b~Mir-16-2* gene cluster in mice leads to CLL (Lovat et al., 2015). These studies indicate the requirement of minimal tumor suppressive activity miR-15 and miR-16 family members to keep CLL in check. Intriguingly and somewhat puzzlingly, global double KO of *Mir-15a~Mir-16-1* and *Mir-15b~Mir-16-2* gene clusters and consequently complete loss of activity of any miR-15 or miR-16 family members leads to acute myeloid leukemia (AML; Lovat et al., 2018). This mechanistic link to myeloproliferative disorders prompted the authors to analyze miR-15 and miR-16 expression in AML patients and their results suggest that restoring miR-15 and miR-16 activity could be an attractive therapeutic option in AML (Lovat et al., 2020). Collectively, these studies identified anti-apoptotic *BCL2* and *MCL1*, and proliferative *Cyclin D1* as key targets of miR-15 and miR-16 family members (Lovat et al., 2018; Pekarsky et al., 2018). Enforced expression of miR-15/-16 in cell lines derived from an array of solid tumors consistently inhibits cell growth and viability in vitro through these key targets (Bonci et al., 2008; Cai et al., 2012; Reid et al., 2013). In in vivo mouse models of solid tumors using the global KO of *Mir-15a~Mir-16-1* strain, the intrinsic tumor suppressive role of these miRNAs in neoplastic cells is less obvious and their activity in immune cells seems to drive tumor initiation and progression. A role of miR-15 and miR-16 in macrophage polarization and regulatory B cells influences tumor growth in transplantable hepatic cancer and chemically-induced colitis (prompt to develop colon cancer) models (Jia et al., 2018; Jia et al., 2019). A similar role of miR-15 and miR-16 in macrophage polarization has been observed in ex vivo experiments with multiple myeloma models (Khalife et al., 2019).

3.1.2 | Mir-17~Mir-92 gene cluster (OncomiR-1)

The *MIR17~MIR92* gene cluster was first identified due to frequent amplification of this chromosomal region in B-cell lymphomas (Mogilyansky & Rigoutsos, 2013; Olive et al., 2013). *MIR17~MIR92* gene cluster contains six miRNAs: miR-17, -18a, -19a, -20a, -19b-1, and 92a-1. Enforced expression of this miRNA gene cluster (lacking miR-92) in conjunction with c-Myc expression enhanced tumor development in a transplantable B-cell lymphoma model demonstrating for the first time the oncogenic activity of miRNA(s) and hence its coining as oncomiR-1 (L. He et al., 2005). Similarly, gain-of-function approaches show that enforced expression of all or specific miRNAs in this cluster can initiate or enhance tumor development of B-cell and T-cell malignancies as well as retinoblastoma and medulloblastoma in mouse models (Mogilyansky & Rigoutsos, 2013; Olive et al., 2013). Global KO of *Mir-17~-92* gene cluster results in perinatal lethality with severe skeletal abnormalities resembling the clinical manifestation of patients with autosomal dominant Feingold syndrome due to heterozygous microdeletion of the *MIR17~MIR92* gene cluster (Mogilyansky & Rigoutsos, 2013; Olive et al., 2013). Thus, the therapeutic window for inhibiting all members of this cluster is a concern due to developmental and physiological defects. Exquisite genetic dissection of individual miRNAs in this cluster reveals distinct roles for individual miRNAs in B cell development and Myc-driven tumorigenesis (Han et al., 2015). Transcriptional regulation by Myc and E2F family members are major drivers of gene cluster expression. Several gene cluster members target tumor suppressive *BIM*, *PTEN*, *p21*, and/or *p57*, while some of these miRNAs and other miRNAs in the cluster seemingly restrain its oncogenic activity by downregulating expression of E2Fs and other transcription activators (Mogilyansky & Rigoutsos, 2013; Olive et al., 2013). Previous gain-of-function experiments of enforced expression suggested that miR-19a and miR-19-1b activity was the main oncogenic driver in the gene cluster to cooperate with Myc oncogene in B-cell lymphoma; genetic ablation of *Mir-19a* and *Mir-19b-1* loci significantly extends overall survival in the aggressive *Eμ-Myc* mouse model of B-cell lymphoma (Han et al., 2015). This study further demonstrates the oncogenic role of miR-19a and miR-19b in B-cell lymphoma pointing to the possibility that their selective inhibition may be a viable option in Myc-driven lymphoma and in other Myc-driven cancers. Context of the activity for *Mir-17~-92* gene cluster members is important and therapeutic intervention may lead to ineffective or paradoxical outcomes. Pancreatic ductal adenocarcinoma (PDAC) is among the most aggressive and lethal types of solid

tumors. Deletion of the *Mir-17~-92* gene cluster within the pancreatic epithelial cell lineages in the well-established KRas-driven p53-deleted mouse model of PDAC (KPC model; Table 3) has little impact on overall survival, but may influence metastatic spread to the liver (Quattrochi et al., 2017). Enforced expression of all *Mir-17~-92* gene cluster members in several human prostatic cancer cell lines induces an epithelial phenotype and increases drug sensitivity in in vitro studies, and inhibits tumor growth in in vivo xenograft mouse models (Ottman et al., 2016).

3.1.3 | Mir-21 gene

miR-21 is among the most highly expressed miRNAs and is frequently upregulated in most cancer types (Bautista-Sanchez et al., 2020). miR-21 has a role in the regulation of carcinogenesis, tissue fibrosis, and inflammatory responses (Bautista-Sanchez et al., 2020; Sheedy, 2015). Oncogenic activity of miR-21 is mainly driven within cancer cells by downregulation of key anti-proliferative and/or pro-apoptotic target genes such as *PTEN*, *PDCD4*, *RECK*, and *Sprouty1/2* (Bautista-Sanchez et al., 2020). However, miR-21 also engages these targets in other cell types of the TME such as fibroblasts, T cells, and macrophages. Thus, the regulatory interactions of miR-21 with these and additional targets depending on signaling inputs, predominant cell type(s) at play, and mutational landscape can have distinct and somewhat opposite effects in tumor formation and evolution in different cancer types. Enforced expression of miR-21 enhances tumor formation and increases tumor burden in a KRas-driven lung cancer model (Hatley et al., 2010). While this enforced expression of miR-21 under a ubiquitous promoter alone is not sufficient to cause lung cancer or other cancers (Hatley et al., 2010), enforced expression of miR-21 under Nestin promoter induces pre-B cell lymphoma and the malignant cells are addicted to continuous miR-21 expression (Medina et al., 2010). Interestingly, enforced expression of miR-21 in this model is not sufficient to induce neuroblastomas, glioblastomas, or other Nestin-expressing brain tumors; *MIR21* gene is locally amplified in a proportion of these tumor types in human patients (Calin et al., 2004). Thus, the difference in dose and timing of expression in different cell types may explain the unique susceptibility of B cells to become malignant in these mouse models. Global *Mir-21*KO animals are viable and have been crossed into several genetic models, used as recipients of transplantable tumor, and subjected to chemically induced protocols of carcinogenesis. Global loss of miR-21 activity significantly delays tumor formation and decreases tumor burden in a KRas-driven lung cancer model (Hatley et al., 2010) and a chemically induced skin cancer model (X. Ma et al., 2011), but has a modest effect in lymphoma- and sarcoma-prone *Tpr53*KO model (X. Ma et al., 2013) as well as in viral protein-driven breast cancer models (Table 3). In human breast cancer tumors, miR-21 expression is predominantly upregulated in the cancer-associated fibrobasts (CAFs) and carries more robust prognostic information, especially in triple-negative breast cancer (MacKenzie et al., 2014). It is possible that miR-21 has a CAF-specific tumor-promoting role that is not accurately captured or required in these viral protein-driven mouse models, known to have a relatively low desmoplastic reaction. In contrast, the KRas-driven KPC model recapitulates several aspects of human PDAC including a profound desmoplastic reaction. Global loss of miR-21 activity in this KPC model somewhat paradoxically accelerates tumor initiation and progression that results in a significantly much shorter overall survival (Schipper et al., 2020). While the effects could be compounded by the activity of miR-21 in different cell types, an obvious absence in this profoundly remodeled TME are myofibrotic CAFs (Schipper et al., 2020), which have recently emerged as a tumor-restraining barrier in PDAC (Helms et al., 2020). Tumor transplantation experiments demonstrate the requirement of stromal miR-21 activity for formation of myofibroblasts (Schipper et al., 2020). This pro-fibrotic role of miR-21 has also been observed in chemically induced models of colitis (Shi et al., 2013) and pancreatitis (Ma, Conklin, et al., 2015) as well as in a diet-induced model of nonalcoholic steatohepatitis (Rodrigues et al., 2017). Studies in other cancer models have also shown a range of immune cell–dependent tumoricidal to tumor promoting effects of miR-21 activity. Global loss of miR-21 activity reduces tumor growth by polarizing macrophages toward a tumoricidal phenotype in transplantable melanoma and lung cancer models (Sahraei et al., 2019; Xi et al., 2018). In contrast, global loss of miR-21 activity enhances tumor burden in transplantable hepatocarcinoma and fibrosarcoma models due to diminished anti-tumoral T responses (W. He et al., 2017). Collectively, these mouse model studies suggest a complex, cell type-specific, and organ site-dependent role of miR-21 and that indiscriminate inhibition of miR-21 activity may lead to unintended overall tumor promoting effects.

3.1.4 | Mir-34 gene family

miR-34 family members are located at two chromosomal locations. In humans, miR-34a transcript is processed from 1p36, a region frequently deleted in tumors, and miR-34b~miR-34c polycistronic transcript is processed from 11q23

(Jain & Barton, 2012; Slabakova et al., 2017). miR-34 family members are transcriptionally upregulated by p53 and originally identified as important mediators of p53-dependent DNA damage response (Agostini & Knight, 2014; Jain & Barton, 2012). Cell line studies uncovered a strong tumor suppressive role of miR-34 via coordinated inhibition of a large number of (proto)oncogenic target genes involved in cell cycle regulation and proliferation (*Cyclin D1*, *CDK4*), apoptosis (*BCL2*, *SIRT1*), cancer cell stemness (e.g., *CD44*, *Nanog*), and oncogenic signaling (e.g., *MET*, *MYC*) (Agostini & Knight, 2014; Bader, 2012; Slabakova et al., 2017). Intratracheal delivery of lentiviral particles carrying miR-34a-expressing minigene in an aggressive KRas-driven p53-mutated mouse model of lung cancer was among the first studies to show therapeutic efficacy in vivo (Kasinski & Slack, 2012). Most of these in vitro and in vivo studies relied on enforced expression or replenishment of miR-34 molecules, and collectively provided overwhelming support for the clinical development and evaluation of miR-34 replacement therapy in cancer (Agostini & Knight, 2014; Bader, 2012). Indeed, a phase I clinical study started in 2013 to evaluate the safety of miR-34 mimic drug, MRX34 ([Agostini & Knight, 2014]; see Section 3.4.3 for more details). Soon after, independent groups showed that mice with complete loss of miR-34 activity in a global double KO of *Mir-34a* and *Mir-34b~-34c* genes have virtually no phenotypic consequences and p53-depedent tumor suppressive pathways were functioning robustly in several in vitro transformation assays and in vivo cancer models (Choi et al., 2011; Concepcion et al., 2012; Jiang & Hermeking, 2017). Functional redundancy with miR-449 might in part offer an explanation, though miR-34 family members are expressed at higher levels than miR-449 family members in most tissue types (Concepcion et al., 2012; Song et al., 2014; J. Wu et al., 2014). Mice with complete loss of miR-34 and miR-449 activity in a global triple KO of *Mir-34a*, *Mir-34b~-34c*, and *Mir-449a~-449c* genes die perinatally and a minimum activity level of miR-34 and miR-449 is required for proper brain development, ciliogenesis, and spermatogenesis (Song et al., 2014; J. Wu et al., 2014). The requirement of a minimum combined activity of miR-34 and miR-449 has not yet been formally tested in cancer models, and this may prove difficult due to organismal viability issues. More-complex-than-anticipated regulatory loops between miR-34 family members and p53 with the involvement of distinct inputs in different cancer contexts and cell types may be at play (Navarro & Lieberman, 2015; Slabakova et al., 2017). A point in case is the requirement of miR-34a activity in a KRas-driven lung cancer model to enhance p53 via downregulation of p53 repressor HDM4 (Okada et al., 2014). Global loss of miR-34a activity has no effect in tumor initiation or progression in this KRas-driven lung cancer model with intact p53 activity. However, reduced p53 activity by genetic deletion of one of the *Tpr53* alleles (haploinsufficiency) exposes a requirement of miR-34a activity in potentiating p53-depedent tumor-suppressive functions (Okada et al., 2014). Global KO of *Mir-34a* or *Mir-34b~-34c* genes moderately increases tumor burden and decreases overall survival of the Apc^{Min} genetic mouse model of colon cancer (Jiang & Hermeking, 2017). Global double KO of *Mir-34a* and *Mir-34b~-34c* genes further enhances these phenotypes indicating that a collective tumor-suppressive function of miR-34 family members is at play in this model, but whether cooperation or interaction with p53 is required for this was not directly investigated (Jiang & Hermeking, 2017).

3.1.5 | Mir-155/BIC gene

miR-155 is located at 21q21.3 region in the human genome and it is processed from host gene *MIR155HG* also known as *BIC* (B cell integration cluster). The *BIC* gene is evolutionarily conserved and is a common integration site for avian leucosis virus in chickens that leads to B cell lymphomas (Bayraktar & Van Roosbroeck, 2018). Enforced expression of miR-155 in B cells under the *Eμ* promoter is sufficient to cause B cell malignancies, including acute lymphoblastic leukemia and high-grade lymphomas, in transgenic mice (Costinean et al., 2006). Global KO of *Mir-155* gene in mice leads to impaired B and T cell responses (Thai et al., 2007), which indicate important physiological roles in development and differentiation of hematopoietic cell lineages, inflammation and protective immunity against pathogens (Bayraktar & Van Roosbroeck, 2018; O'Connell et al., 2012). These impaired B and T cell responses can in part be due to an intrinsic role of miR-155 in these cell types, but also by a role of miR-155 in other immune cell types. Global KO of *Mir-155* gene significantly diminishes overall tumor burden of AML in the FLT3-ITD mouse model (Wallace et al., 2017). The extent of miR-155 activity in myeloid cells can paradoxically lead to overall tumor suppressive or oncogenic effects in AML cell line models (Narayan et al., 2017). The role of miR-155 in solid tumors may be also compounded by effects in the neoplastic cells as well as in immune cells. Global KO of *Mir-155* gene in a *Brca1*-deficient mouse model of breast cancer has no impact on overall survival (Kim, Song, et al., 2016). Allograft transplantation reveals an oncogenic role of miR-155 in the cancer cells that is counteracted by a tumoricidal role in the TME, which limits the recruitment and infiltration of tumor-promoting myeloid-derived suppressive cells (MDSCs) (S. Kim et al., 2018; Kim, Song, et al., 2016).

While not addressed with a genetic approach, miR-155 can have a cancer cell–intrinsic role in chemoresistance and/or radioresistance in breast, colorectal, lung, and other cancer types (Bayraktar & Van Roosbroeck, 2018). Opposite tumor-promoting or tumoricidal roles of miR-155 in MDSCs or tumor-associated macrophages have been observed in different solid tumor models (Chen et al., 2015; Dueck et al., 2014; Li et al., 2014; Wang et al., 2015; Zonari et al., 2013). Moreover, conditional deletion of *Mir-155* gene exclusively within the T cell lineages uncovered a T-cell specific activity of miR-155 for mounting an anti-tumor response in transplantable melanoma models (Dudda et al., 2013; Ekiz et al., 2019; Huffaker et al., 2017). In a complementary approach, enforced expression of miR-155 in $CD8^+$ T-cells enhanced their in situ antigen-specific response in similar transplantable melanoma mouse models (Dudda et al., 2013; Martinez-Usatorre et al., 2019).

3.2 | Therapeutic strategies and considerations for modulating miRNA activity in cancer

Two main features make miRNAs attractive candidates for cancer therapy: the ability of influencing protein output and function of many direct miRNA target genes with a single miRNA drug and the chemical synthesis of RNA-based miRNA activity modulators (Figure 2). There are functional and technical considerations and challenges for replenishing the activity of a tumor suppressive miRNA or inhibiting the activity of an oncogenic miRNA. Replenishing miRNA activity or even supplementing the activity above physiological levels can have a potent effect in the down-regulation of many direct target genes, but it could also have off-target effects similar to siRNAs in the downregulation of unintended mRNAs and/or ectopic effects in unintended cell types. In contrast, inhibiting miRNA activity can be

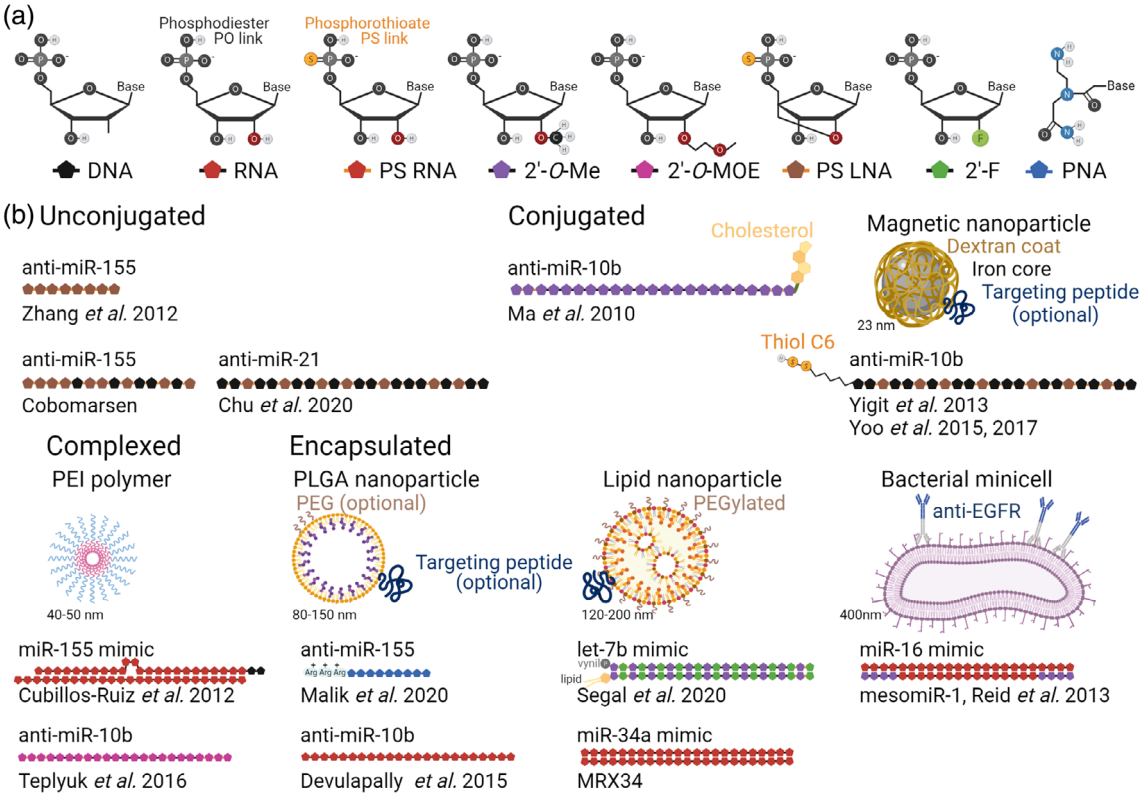

FIGURE 2 Chemical modifications and delivery technologies. (a) Chemical structure of most common RNA analogue modifications incorporated in antisense miRNA inhibitor or double-stranded mimics. (b) Representative examples of different chemical modification and delivery technologies (see Tables 4 and 5 for more details). Pattern and location of chemical modifications are representative of that particular approach and are approximation (in some cases the exact modified sequence is not fully disclosed). For miRNA mimics, top strand depicts the guide strand (mature miRNA) and bottom strand the passenger strand. Molecules and constructs not drawn to scale. 2′-F, 2′-Fluoro; 2′-*O*-me, 2′-*O*-methyl; 2′-*O*-MOE, 2′-*O*-methoxyethyl; Arg, arginine; LNA, locked nucleic acid; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic acid); PNA, peptide nucleic acid; PO, phosphodiester; PS, phosphorothioate

more precise since an inhibitor will bind with high affinity to the intended miRNA sequence, but the extent of downregulation of target genes may be more modest due to redundant regulation by other miRNAs or mechanisms. Mimic compounds used to replenish miRNA activity are generally double-stranded RNA (dsRNA) molecules (Figure 2) that need to interact with and be processed by the cellular machinery as siRNAs do, which limits the type of chemical modifications that they can incorporate and may unintentionally affect processing of other miRNAs by saturating the miRISC processing capacity (Bajan & Hutvagner, 2020; Lee et al., 2020; Roberts et al., 2020). In contrast, anti-miRNA inhibitors are typically single-stranded molecules that directly bind to the complementary miRNA sequence in the cytoplasm, which allows them to have more types of chemical modifications and utilize more cell internalization routes without the need of nanoparticle conjugation or encapsulation (Bajan & Hutvagner, 2020; Lee et al., 2020). Recent FDA approval of two RNA-based drugs, patisiran and givosiran, provides a confidence boost for clinical development and evaluation of similar miRNA-based drugs (Figure 2). Patisiran is an siRNA targeting mutant transthyretin (*TTR*) mRNA to treat transthyretin amyloidosis and is delivered intravenously as cargo in a liposomal nanoparticle (Urits et al., 2020). Givosiran is an siRNA targeting delta-aminolaevulinic acid-synthase 1 (*ALAS1*) mRNA to treat acute hepatic porphyria and is delivered subcutaneously as an *N*-acetylgalactosamine (GalNAc) conjugate (Neeleman et al., 2020; Springer & Dowdy, 2018). Lessons learned from these and other clinical experiences with siRNAs provide valuable information for the design of chemical modifications, complex formation, and delivery strategies aimed at increasing the stability, biodistribution, targeted delivery, and cell penetration of miRNA-based drugs (Bajan & Hutvagner, 2020; Forterre et al., 2020; Lee et al., 2020; O'Neill & Dwyer, 2020; Roberts et al., 2020; Springer & Dowdy, 2018) .

3.3 | microRNA-based therapy in preclinical models

Table 4 provides a comprehensive list of approaches describing the development of miRNA-based drugs systemically delivered in in vivo models. We highlight pros and cons of some of these approaches and their therapeutic efficacy. Chemical modifications (Figure 2) to stabilize and protect from RNAse degradation include replacement of the labile 2′OH group by 2′-*O*-methyl, 2′-*O*-methoxyethyl, or 2′-Fluoro and/or replacement or mixing of the phosphodiester backbone with phosphorothioates (Bajan & Hutvagner, 2020; Roberts et al., 2020). In addition to increasing stability, chemical modifications can increase affinity for complementary sequence including locked nucleic acid (LNA) and peptide nucleic acid (PNA; Bajan & Hutvagner, 2020; Roberts et al., 2020). The chemically modified oligonucleotide or the nanoparticle vehicle can be decorated with targeting peptides (e.g., iRGD, penetratin) and other moieties (e.g., folate, cholesterol) for preferential interaction and accumulation at tumor sites (Forterre et al., 2020; Lee et al., 2020).

3.3.1 | anti-miR-10b inhibitors

miR-10b was first described as an important mediator of pro-invasion and pro-metastatic programs in cell line models of breast cancer via downregulation of HOXD10, a homeobox family transcription factor that represses expression of pro-metastatic *RHOC* gene (Ma et al., 2007). Follow-up studies focused on the pro-invasion and pro-metastatic function of miR-10b and uncovered other key target genes, including *BIM*, *KLF4*, *PTEN*, *TBX5*, and *TIAM1* (Sheedy & Medarova, 2018). Treatment with anti-miR-10b antisense chemically-modified oligonucleotide (AMO) conjugated to cholesterol prevented seeding and initial formation of metastatic lung lesions, but had no effect in the growth of the primary tumor or established metastatic lesions in an orthotopically implanted mouse model of breast cancer (Ma et al., 2010). In contrast, another study using a very similar approach and therapeutic agent observed a significant inhibition of primary tumor growth (Monroig-Bosque et al., 2018). Treatment with anti-miR-10b AMO conjugated to dextran-coated iron oxide magnetic nanoparticles shows therapeutic efficacy against established regional and distant metastatic lesions in immunocompetent and immunocompromised orthotopically implanted mouse models of breast cancer (Yigit et al., 2013; Yoo et al., 2015; Yoo et al., 2017). These magnetic nanoparticles have intrinsic imaging capabilities; the biodistribution and tumor accumulation of this anti-miR-10b nanodrug can be monitored in vivo by magnetic resonance imaging. While this nanodrug accumulates both in primary tumor and metastatic lesions, it has a minimal effect on growth kinetics of the primary tumor (Yigit et al., 2013). These studies have led to the clinical development of the anti-miR-10b nanodrug, TTX-MC138, by Transcode Therapeutics (Table 5). The timing, dose, tumor penetration, and/or potency of these different formulations may explain in part discordant effects of tumor growth

TABLE 4 Pharmacological modulation of miRNA activity in in vivo cancer models

miRNA modulation	Chemistry	Delivery technology	Model	Cancer site(s)	Therapeutic benefit	References
miR-155 inhibition	8-mer AMO (Fully LNA, fully PS backbone)	Unconjugated	Bone marrow engraftment, BCWM1 B-cell lymphoma cells	Bone marrow	Inhibits tumor growth, decreases overall tumor burden, upregulates expression of direct target genes *CEBPβ*, *MAFB*, *SOCS1*, and others	Zhang et al. (2012)
miR-10b inhibition	AMO (2′-*O*-MOE, partial PS backbone)	in vivo-jetPEI® complex (intracranial injection) or unconjugated (IV injection)	Orthotopic, GBM8 glioblastoma cells	Brain	Inhibits primary tumor growth and increases overall survival. Effect is enhanced by combination treatment with doxorubicin	Teplyuk et al. (2016)
miR-10b and miR-21 inhibition	AMO (?)	PLGA nanoparticle with tumor-penetrating cRGD	Subcutaneous, U87-MG glioblastoma cells	Brain	Inhibits tumor growth. Effect is enhanced by combination treatment with temozolomide	Malhotra et al. (2018)
miR-21 inhibition	AMO (2′-*O*-Me)	Exosome with tumor-penetrating T7 peptide	Orthotopic, C6 glioblastoma cells	Brain	Inhibits tumor growth, upregulates expression of direct target genes *PTEN* and *PDCD4*	G. Kim et al. (2020)
miR-21 inhibition, miR-100 replacement	AMO for miR-21, ssRNA? for miR-100	Gold-iron oxide nanoparticles with PEG-T7 peptide (intranasal delivery)	Orthotopic, U87-MG glioblastoma cells	Brain	Inhibits tumor growth, extends overall survival. Effect is enhanced by combination treatment with temozolomide	Sukumar et al. (2019)
miR-21 inhibition	AMO (2′-*O*-Me)	Star-branched PLA/PDMAEMA copolymers	Subcutaneous, LN229 glioma cells	Brain	Inhibits tumor growth, upregulates expression of direct target gene *PTEN*. Effect is enhanced by combination treatment with doxorubicin	Qian et al. (2014)
miR-21 inhibition	AMO (partial LNA)	Lipid nanoparticle with chlorotoxin peptide	Orthotopic, GL261 glioma cell	Brain	Inhibits tumor growth in combination treatment with sunitinib	Costa et al. (2015)
miR-21 inhibition	AMO (partial LNA)	Three-way junction-based RNA (2′-F modified) nanoparticle with folate	Orthotopic, patient-derived GBM30 glioblastoma cells	Brain	Inhibits tumor growth, upregulates expression of direct target genes *PTEN* and *PDCD4*, and increases overall survival	Lee et al. (2017)
miR-34a replacement	Invitrogen™ Pre-miR™ miRNA mimic	Silica nanoparticle with anti-GD_2 antibody	Subcutaneous, NB1691 or SK-N-AS neuroblastoma cells	Brain	Inhibits tumor growth	Tivnan et al. (2012)
miR-10b inhibition	AMO (2′-*O*-Me, partial	Liposomal nanoparticle		Breast	Inhibits primary tumor growth	Monroig-Bosque et al. (2018)

TABLE 4 (Continued)

miRNA modulation	Chemistry	Delivery technology	Model	Cancer site(s)	Therapeutic benefit	References
	PS backbone)		Orthotopic, MDA-MB-231 breast cancer cells			
miR-10b inhibition	AMO (2′-*O*-Me, partial PS backbone)	Cholesterol conjugation	Orthotopic, 4T1 breast cancer cells	Breast, Lung (distant metastasis)	Prevents formation of metastatic lung lesions. No effect on primary tumor growth or established lung metastasis	Ma et al. (2010)
miR-10b inhibition	AMO (Partial LNA)	Dextran-coated iron oxide magnetic nanoparticle with tumor targeting cRGD peptide (IV injection)	Orthotopic, MDA-MB-231 breast cancer cells	Breast, lymph node (regional metastasis)	Prevents formation of lymph node metastases, inhibits growth of established lymph node metastases. No effect on primary tumor growth	Yigit et al. (2013)
miR-10b inhibition	AMO (Partial LNA)	Dextran-coated iron oxide magnetic nanoparticle	Orthotopic, MDA-MB-231 breast cancer cells	Breast (primary tumor removed), lymph nodes and lung	Inhibits growth of established lymph node metastases, prevents lung metastases, increases overall survival. Effect is enhanced by combination treatment with low-dose doxorubicin	Yoo et al. (2015)
miR-10b inhibition	AMO (Partial LNA)	Dextran-coated iron oxide magnetic nanoparticle	Orthotopic, 4T1 breast cancer cells	Breast (primary tumor removed), lymph nodes and lung	Inhibits growth of established lymph node and lung metastases, increases overall survival. Effect is enhanced by combination treatment with low dose doxorubicin	Yoo et al. (2017)
miR-10b inhibition	AMO (partial PS backbone)	PLGA-*b*-PEG nanoparticle with targeting uPA peptide	Subcutaneous, MDA-MB-231 breast cancer cells	Breast	Inhibits tumor growth. Effect is enhanced by combination treatment with anti-miR-21 AMO	Devulapally et al. (2015)
miR-21 inhibition	AMO (partial PS backbone)	PLGA-*b*-PEG nanoparticle with targeting uPA peptide	Subcutaneous, MDA-MB-231 breast cancer cells	Breast	Inhibits tumor growth. Effect is enhanced by combination treatment with anti-miR-10b AMO	Devulapally et al. (2015)
miR-21 inhibition	AMO (partial LNA)	Three-way junction-based RNA (2′-F modified) nanoparticle with EGFR or CD133 targeting aptamer	Orthotopic, MDA-MB-231 breast cancer cells	Breast	Inhibits tumor growth and upregulates expression of direct target genes *PTEN* and *PDCD4*	Shu et al. (2015) and Yin et al. (2019)

(Continues)

TABLE 4 (Continued)

miRNA modulation	Chemistry	Delivery technology	Model	Cancer site(s)	Therapeutic benefit	References
miR-210 inhibition	AMO (miniPEG-γPNA)	PLGA nanoparticle	Subcutaneous, HeLa cervical cancer cells	Cervix	Inhibits tumor growth and increases overall survival	Gupta et al. (2017)
miR-34a replacement	dsRNA mimic (RNA?)	Amphoteric liposome	Orthotopic, Hep3B or HuH7 liver cancer cells	Liver	Inhibits tumor growth, downregulates expression of direct target genes *MET*, *MYCN*, *TRA2A*, and others	Daige et al. (2014)
miR-122 replacement	ssRNA (?)	Megamer-based nanoparticles (PEG, PAMAM)	Subcutaneous, HuH7 liver cancer cells	Liver	Inhibits tumor growth	Wu et al. (2019)
miR-122 replacement, miR-21 inhibition	ssRNA (?) for miR-122, AMO for miR-21	PLGA-*b*-PEG nanoparticle with ultrasound-guided delivery	Subcutaneous, HepG2 or Hepa1–6 liver cancer cells	Liver	Inhibits tumor growth. Effect is enhanced by combination treatment with doxorubicin	Chowdhury et al. (2018), Mullick Chowdhury et al. (2016), and Wischhusen et al. (2020)
miR-34a replacement	Dharmacon mimic	Lipid emulsion	Subcutaneous, U2932 lymphoma cells	Lymph node	Inhibits tumor growth	Craig et al. (2012)
miR-155 inhibition	AMO (partial LNA, full PS backbone; Cobomarsen)	Unconjugated?	Subcutaneous, U2932 lymphoma cells	Lymph node	Inhibits tumor growth and upregulates expression of direct targets *SOCS1*, *WEE1*, and others	Anastasiadou et al. (2020)
Mir-155 inhibition	Short AMO (PNAs)	PLGA nanoparticle	Subcutaneous, U2932 lymphoma cells	Lymph node	Inhibits tumor growth	Malik et al. (2020)
Let-7b replacement	Invitrogen™ Pre-miR™ miRNA mimic	siPORTamine complex	Subcutaneous, H460 lung cancer cells	Lung	Inhibits tumor growth	Trang et al. (2010)
Let-7b replacement	Dharmacon mimic	Neutral lipid emulsion	Genetic, LSL-$Kras^{G12D/+}$ plus intranasal Adeno-Cre	Lung	Inhibits tumor growth and decreases overall tumor burden	Trang et al. (2011)
Let-7b replacement	AccuTarget miRNA mimic	Cationic copolymer (PEG_{5K}, VE, DET)	Subcutaneous, A549 lung cancer cells	Lung	Inhibits tumor growth. Effect is enhanced by combination treatment with paclitaxel	Dai et al. (2016)

TABLE 4 (Continued)

miRNA modulation	Chemistry	Delivery technology	Model	Cancer site(s)	Therapeutic benefit	References
Let-7b replacement	Invitrogen™ Pre-miR™ miRNA mimic	Neutral lipid emulsion or lipid-based NOV340	Genetic, LSL-$Kras^{G12D/+}$; LSL-$Tp53^{L/L}$ plus intratracheal Adeno-Cre	Lung	Inhibits tumor growth, decreases overall tumor burden, and increases overall survival. Effect is enhanced by combination treatment with miR-34a mimics	Kasinski et al. (2015)
Let-7b replacement	dsRNA mimic (2′-F, 2′-*O*-Me, partial PS backbone)	Lipid (DCA, EPA or cholesterol) conjugation	Subcutaneous, HCC827 lung cancer cells	Lung	No effect on tumor growth, but decreases proliferation rate and downregulates direct target *HMGA2*	Segal et al. (2020)
miR-16 replacement	dsRNA mimic (2′-*O*-Me on passenger strand only)	Minicells with anti-EGFR antibody	Subcutaneous, MSTO-211H mesothelioma cells	Lung	Inhibits tumor growth	Reid et al. (2013)
miR-34a replacement	Invitrogen™ Pre-miR™ miRNA mimic	Lipid nanoparticle	Subcutaneous, H460 or A549 lung cancer	Lung	Inhibits tumor growth, downregulates expression of direct target genes *CDK4* and *MET*	Wiggins et al. (2010)
miR-34a replacement	Dharmacon mimic	Neutral lipid emulsion	Genetic, LSL-$Kras^{G12D/+}$ plus intranasal Adeno-Cre	Lung	Inhibits tumor growth and decreases overall tumor burden.	Trang et al. (2011)
miR-34a replacement	Invitrogen™ Pre-miR™ miRNA mimic	Neutral lipid emulsion or NOV340 lipid-based delivery agent	Genetic, LSL-$Kras^{G12D/+}$; LSL-$Tp53^{L/L}$ plus intratracheal Adeno-Cre	Lung	Inhibits tumor growth, decreases overall tumor burden, and increases overall survival. Effect is enhanced by combination treatment with let-7b mimic	Kasinski et al. (2015)
miR-21 inhibition	AMO (partial LNA)	PEGylated porous silicon nanoparticle with tumor-penetrating CGKRK peptide	Subcutaneous, COV-318 ovarian cancer cells	Ovary	Inhibits tumor growth	Bertucci et al. (2019)
Mir-155 replacement	dsRNA Dicer substrate	in vivo-jetPEI® complex	Peritoneal, ID8 or ID8-*Defb29/Vegf-A* ovarian cancer cells	Ovary (metastatic)	Inhibits tumor growth, reprograms tumor-associated dendritic cells, and increases overall survival. Effect is enhanced by combination treatment with anti-CD40 agonistic antibody	Cubillos-Ruiz et al. (2012)

(Continues)

TABLE 4 (Continued)

miRNA modulation	Chemistry	Delivery technology	Model	Cancer site(s)	Therapeutic benefit	References
miR-21 inhibition	AMO (Partial LNA, fully PS backbone)	Unconjugated	Genetic, LSL-$Kras^{G12D/+}$; LSL-$Tp53^{R172H/+}$;Pdx1-Cre	Pancreas	Prevents or delays the formation of advanced intraepithelial lesion and invasive carcinoma	Chu et al. (2020)
miR-21 inhibition	miRVana® anti-miR-21 inhibitor	PEGylated lipid nanoparticle with tandem tumor-penetrating iRG and cell-penetrating transportan peptides	Subcutaneous, mouse or patient-derived cancer cells	Pancreas	Inhibits tumor growth	Gilles et al. (2018) and Gilles et al. (2019)
miR-634 replacement	miRVana® miRNA mimic	Lipid nanoparticle	Subcutaneous, BxPC-3 pancreatic cancer cells	Pancreas	Inhibits tumor growth	Gokita et al. (2020)
miR-34a replacement	Invitrogen™ Pre-miR™ miRNA mimic	Chitosan nanoparticle	Subcutaneous, PC3MM2 prostate cancer cells	Prostate	Inhibits tumor growth, downregulates expression of direct target genes *AXL*, *MET*, *MYC*	Gaur et al. (2015)
miR-34a replacement	Dharmacon mimic	LPH nanoparticle with tumor targeting scFv	Lung metastasis, B16F10 melanoma cells	Skin (metastatic)	Inhibits tumor growth	Chen et al. (2010)
miR-155 inhibition	AMO (PNAs)	PLGA nanoparticle with cell-penetrating penetratin peptide	Subcutaneous, LSL-$Mir\text{-}155^{tTA}$; NesCre8 B-cell lymphoma cells	Spleen	Inhibits tumor growth	Babar et al. (2012)

Note: Unless otherwise noted, therapeutic agent was intravenously delivered.

Abbreviations: 2′-F, 2′-Fluoro; 2′-*O*-Me, 2′-*O*-methyl; 2′-*O*-MOE, 2′-*O*-methoxyethyl; AMO, antisense miRNA oligonucleotide; cRGD, Cyclic 3-aminoacid (RGD) peptide has high affinity for αVβ3 integrin; DCA, dososanoic acid; dsRNA, Double-stranded RNA; DET, diethylenetriamine; EPA, eicosapentaenic acid; GD_2, disialoganglioside GD2 glycolipid; iRGD, Cyclic 9-aminoacid (CRGDKGPDC) peptide has high affinity for αVβ3 integrin; LNA, locked nucleic acid; LPH, liposome-polycation-hyaluronic acid; mini-PEG, Hydrophilic diethylene glycol moiety; PDMAEMA, poly-dimethylaminoethyl methacrylate; PAMAM, polyamidoamine; PEG, polyethylene glycol; PEG_{5K}, polyethylene glycol 5000; PLA, polylactic acid; PLGA, Poly(lactic-co-glycolic acid); PNA, peptide nucleic acid; PS, phosphorothioate; scFv, single-chain antibody fragment; T7, TfR-binding 7-aminoacid (HAIYPRH) peptide; uPA, urokinase plasminogen activator; VE, vitamin E.

TABLE 5 Clinical candidates and clinical trials evaluating microRNAs as therapeutic agents against cancer

miRNA modulation	Drug name	Sponsor	Chemistry	Delivery technology	Phase	Clinical application(s)	Trial identifier
miR-10b inhibition	RGLS5579	Regulus Therapeutics	AMO (2′-*O*-MOE?, partial PS backbone?)	?	Safety and dose escalation	Treatment of glioblastoma	Clinical candidate nomination (Regulus, 2019)
miR-10b inhibition	TTX-MC138	Transcode Therapeutics	AMO (partial LNA, partial? PS backbone	Dextran-coated iron oxide magnetic nanoparticle	Safety and dose escalation	Treatment of metastatic breast cancer	Scheduled in 2021–2022 (Transcode, 2020)
miR-16 replacement	mesomiR1 (TargomiR)	Asbestos Diseases Research Foundation	dsRNA mimic (2′-*O*-Me on passenger strand only))	Bacterial minicells with anti-EGFR bispecific antibody	Safety, Tolerability and early signs of efficacy (phase 1)	Treatment of recurrent malignant pleural mesothelioma and nonsmall cell lung cancer	NCT02369198 (completed)
miR-34a replacement	MRX34	Mirna Therapeutics	dsRNA mimic (RNA?)	Liposome	Safety, Tolerability and Pharmacokinetics (phase 1)	Treatment of primary liver cancer or other selected solid tumors or hematologic malignancies	NCT01829971 (terminated)
miR-34a replacement					Pharmacodynamics and pharmacokinetics (phase 1b)	Treatment of advanced melanoma	NCT02862145 (withdrawn)
miR-155 inhibition	Cobomarsen (MRG-106)	miRagen Therapeutics	AMO (partial LNA, full PS backbone)	Unconjugated?	Safety, Tolerability and Pharmacokinetics (phase 1)	Treatment of certain lymphomas and leukemias, including CTCL mycosis fungoides subtype, CLL,D DLBCL, and ATLL	NCT02580552 (completed)
miR-155 inhibition					Efficacy and Safety against active comparator (phase 2)	Treatment of mycosis fungoides subtype CTCL, SOLAR clinical trial	NCT03713320 (active, not recruiting)
miR-155 inhibition					Efficacy and Safety (phase 2)	Treatment of mycosis fungoides subtype CTCL, PRISM clinical trial	NCT03837457 (terminated, study no longer needed)

Abbreviations: 2′-*O*-Me, 2′-*O*-methyl; 2′-*O*-MOE, 2′-*O*-methoxyethyl; AMO, antisense miRNA oligonucleotide; ATLL, adult T-cell leukemia/lymphoma; CLL, chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; LNA, locked nucleic acid; PS, phosphorothioate.

inhibition at primary and distant metastatic sites. Global KO of *Mir-10b* gene in an aggressive viral protein-driven genetic model of breast cancer delays and reduces primary tumor growth in addition to decreasing multiplicity of metastatic lung lesions (Kim, Siverly, et al., 2016). Thus, this result suggests perhaps a different requirement for miR-10b activity depending on the driver mutations of breast cancer tumor subtypes.

miR-10b is also highly expressed in glioblastoma, the most aggressive and lethal form of brain cancer. Anti-miR-10b AMO treatment or genetic ablation of *Mir-10b* gene by CRISPR editing technology in in vitro cell models and in vivo orthotopic models reduces cancer cell growth and viability, increases apoptotic rate, and/or reduces migratory and invasive behavior (El Fatimy et al., 2017; Gabriely et al., 2011; Guessous et al., 2013; Teplyuk et al., 2016). Systemic delivery of an unconjugated anti-miR-10b AMO achieves a similar therapeutic efficacy as the same AMO complexed with in vivo-jetPEI® transfection agent injected intracranially in an orthotopic mouse model of glioblastoma (Teplyuk et al., 2016). This study has led to the clinical development of RGLS5579 by Regulus Therapeutics (Table 5).

3.3.2 | anti-miR-21 inhibitors

Several groups have used different encapsulation (e.g., exosomes and liposomes) approaches to effectively deliver anti-miR-21 AMO to the brain to treat glioblastoma in mouse and rat models (Costa et al., 2015; Kim et al., 2020; Qian et al., 2014). Decoration of the delivery vehicle with folate or tumor-penetrating peptides (i.e., T7 and chlorotoxin) enhances the tumor accumulation of the anti-miR-21 AMO (Costa et al., 2015; Kim et al., 2020; Lee et al., 2017). Folate and anti-miR-21 AMO are directly conjugated to different strand ends of a three-way junction-based RNA nanoparticle (Lee et al., 2017). These three-way junction-based RNA nanoparticles can be readily functionalized with other targeting moieties. For example, functionalization with EGFR or CD133 aptamers (instead of folate) enhances the delivery of anti-miR-21 AMO to the tumor site in an orthotopic mouse model of breast cancer (Shu et al., 2015; Yin et al., 2019). Treatment with nanocomplex loaded with MirVana® miR-21 inhibitor and decorated with tumor- and cell-penetrating tandem peptides suppresses tumor growth in immunocompetent and immunocompromised subcutaneously implanted mouse models of PDAC (Gilles et al., 2018; Gilles et al., 2019). Treatment with an unconjugated anti-miR-21 AMO in KRas-driven p53-mutated genetically engineered model of PDAC (variant KPC model) at a young age, when only precursor lesions (pancreatic intraepithelial neoplasia) have formed, prevents the development of more advanced and invasive lesions (Chu et al., 2020). This is one of the few studies showing tumor delivery of miRNA-based therapeutic agent in a genetic model, which more closely approximates to the TME of human tumors than do transplantable models with a less stiff stroma and more leaky vasculature. This study also introduces the interesting concept of using miRNA drugs for cancer interception. This study evaluated the therapeutic efficacy for cancer prevention after only 6 weeks of treatment by analyzing the pancreata from euthanized animals; longer treatment course or the potential consequences of prolonged miR-21 inhibition were not examined. There could be some challenges with identifying individuals at high-risk and when to optimally start such preventative treatment as well as unintended consequences of a prolonged period of miRNA inhibition for cancer interception. Unintended tumor promoting effects due to prolonged period of miRNA inhibition has been reported in particular for miR-21 in PDAC and in general for other miRNAs (e.g., miR-122 in liver cancer) using miRNA KO strains (Hsu et al., 2012; Schipper et al., 2020). Thus, more research is needed in this emerging area to understand if the dose and frequency of miRNA activity modulation for cancer interception may need to be adjusted with respect to the use of the same miRNA-based drug(s) for cancer treatment in which maximal potency in an acute shorter period would likely be required and desirable.

3.3.3 | miR-34 mimics

Several groups used different liposomal formulations to effectively deliver chemically-modified dsRNA miR-34 mimics to the lung to treat lung cancer in KRas-driven genetic models (Kasinski et al., 2015; Trang et al., 2011) as well as metastatic melanoma in an immunocompetent mouse model (Chen et al., 2010). These studies show the capability of these liposomal formulations to deliver the therapeutic agent to a more challenging and complex tumor context than that of orthotopically or subcutaneously implanted models of lung cancer and other cancer types (Table 4). Decoration of the delivery vehicle with tumor targeting single-chain antibody fragments further enhances accumulation of the miR-34 mimic in distant melanoma metastatic lesions to the lung (Y. Chen et al., 2010).

3.4 | Clinical trials with miRNA drugs and promising clinical candidates

Table 5 provides a comprehensive list of clinical trials evaluating miRNA-based drugs for cancer treatment. We highlight key findings, challenges, successes, and failures. We also touch briefly on other clinical trials with miRNA drugs for noncancer applications (Bonneau et al., 2019) that may inform and have implications for cancer treatment. miR-122 exemplifies the promises and challenges of clinical implementation of a robust miRNA candidate (Bonneau et al., 2019). miR-122 is expressed at high levels and almost exclusively in hepatocytes. Mechanistic studies in mouse and nonhuman primate models strongly supported targeting miR-122 as therapeutic strategy against Hepatitis C virus (HCV). An unconjugated anti-miR-122 LNA-modified AMO (miravirsen) developed by Santaris Pharma was successful at eliminating viral load of HCV genotype 1b as monotherapy in a phase 2b clinical trial (Janssen et al., 2013). Santaris Pharma was acquired by Roche in 2014. Further clinical development of miravirsen is uncertain, at least in part, due to concerns of sequence variants with innate and acquired resistance (Li, Van Pham, et al., 2016; Ottosen et al., 2015), broader activity spectrum of competitors such as HARVONI®, and, to a lesser extent, the risk of cancer development (Hsu et al., 2012; Li et al., 2016).

3.4.1 | miR-16 mimic

The majority (60%) of malignant pleural mesotheliomas do not respond to current chemotherapy treatments. A dsRNA miR-16 mimic loaded in bacterial minicells (EnGeneIC Dream Vectors) showed therapeutic efficacy in a subcutaneously implanted xenograft mouse model of mesothelioma (Reid et al., 2013). The minicells were decorated with bispecific anti-EGFR antibody to enhance delivery to mesothelioma tumors, which highly expressed EGFR (Reid et al., 2013). These positive results with this innovative nanoparticle (targomiR) led to a phase 1 clinical trial (NCT02369198) designed to evaluate the safety and tolerability in patients with malignant pleural mesothelioma refractory to chemotherapy (van Zandwijk et al., 2017). This miR-16 mimic targomiR (mesomiR-1) has an acceptable safety profile and weekly treatment with 5×10^9 mesomiR-1 nanoparticles was established as the maximum tolerated dose (van Zandwijk et al., 2017). Preliminary assessment of treatment efficacy indicates an overall objective response of 5% (1/22 participants had a partial response). These results are encouraging and supportive of a phase 2 clinical trial designed to assess treatment efficacy of mesomiR-1 as monotherapy or in combination with standard chemotherapy.

3.4.2 | anti-miR-21 inhibitor

Despite the numerous preclinical studies supporting an oncogenic role of miR-21 in many cancer contexts, there are no on-going clinical trials evaluating miR-21-based therapies for cancer treatment. Dichotomy of the role(s) of miR-21 in cancer cells and distinct elements of the TME and the complexity and context of these roles are clearly a challenge for identifying tumor types and subtypes that could benefit from miR-21 activity modulation. Strategies and technologies to impart compartment or cell type-specific modulation should be considered to overcome this challenge. An on-going phase 2 clinical trial with an anti-miR-21 AMO (RG-102 also known as lademirsen) for amelioration of fibrosis and renal function in patients with Alport syndrome (NCT02855268) could provide valuable information for its application in cancer treatment in contexts where fibroblast reprogramming could be beneficial.

3.4.3 | miR-34 mimic

The miR-34a mimic, MRX34, was the first miRNA replacement drug to enter the clinic (Agostini & Knight, 2014; Bader, 2012). MRX34 was administered intravenously as cargo in a liposomal nanoparticle in patients with primary tumor or metastatic lesions in the liver, other solid tumors, or hematological malignancies to investigate its safety, pharmacokinetics and pharmacodynamics (NCT01829971). This study was halted and eventually terminated due to five severe immune reactions that resulted in the death of four participants (Hong, Kang, et al., 2020). A contemporaneous second clinical trial (NCT02862145) was designed to mitigate immune-mediated toxicity by pretreatment with dexamethasone (Hong, Kang, et al., 2020), but it was withdrawn due to early termination of the other clinical trial. Evaluation of clinical data suggests that miR-34a mimic specifically caused this immune-mediated toxicity in patients since

the same liposome formulation and other dsRNA compounds were well tolerated in other clinical trials (Hong, Kang, et al., 2020). This was an unexpected finding given the rigorous toxicology studies conducted in mouse and other animal models, including nonhuman primates (Hong, Kang, et al., 2020). Nonetheless, animal models may not fully recapitulate all aspects of human disease at molecular (e.g., repertoire of target genes, signaling pathways, and ligands), cellular (e.g., TME composition and immune response), and/or physiological (e.g., desmoplastic reaction, hypoxia, enhanced permeability and retention [EPR] effect) levels. Immune-mediated toxicities of MRX34 are similar to those caused by immune checkpoint inhibitors (Hong, Kang, et al., 2020), suggesting an on-target effect of miR-34a mimic on immune cells that puts them on overdrive. In this heavily pretreated and diverse patient cohort the overall objective response was 4% (3/85 participants had a partial response to treatment; Hong, Kang, et al., 2020). A better understanding of on-target effects of miR-34a in cancer and immune cells could inform a more selective application of this miRNA replacement therapy in a more focused subset of cancer types. Mirna Therapeutics Inc canceled further development of MRX34 and was subsequently acquired by Synlogic Therapeutics in 2017. MRX34 or a modified formulation does not appear to be in the pipeline of Synlogic Therapeutics (Synlogic, 2020).

3.4.4 | anti-miR-155 inhibitor

Cutaneous T cell lymphoma (CTCL) is a relatively rare cancer. Mycosis fungoides (MF) is the most common subtype of CTCL. In vitro functional studies and altered expression data in patient samples supported an etiological role of miR-155 in MF (Moyal et al., 2013; Seto et al., 2018). Cobomarsen (also known as MRG-06) is a chemically-modified anti-miR-155 AMO functionalized for preferential uptake by MF and $CD4^+$ T cells (Seto et al., 2018). Safety and tolerability of cobomarsen was evaluated in a successfully completed phase I clinical trial (NCT02580552) that included patients with MF, adult T-cell leukemia/lymphoma, CLL, and diffuse large B-cell lymphoma (DLBCL). A phase 2 clinical trial was initiated to evaluate the efficacy of cobomarsen for the treatment of MF (NCT03713320). In July 2020, cobomarsen received orphan drug designation for treatment of CTCL (miRagen, 2020a). However, preliminary analysis of this phase 2 clinical trial failed to show a treatment effect of cobomarsen over control arm receiving vorinostat treatment. miRagen Therapeutics recently decided to discontinue further internal development of cobomarsen (miRagen, 2020b). Genetic studies of *Mir-155*KO mice (Table 3), therapeutic efficacy of cobomarsen and other anti-miR-155 AMOs in in vivo animal models (Table 4), and good safety profile of cobomarsen in patients (Table 5) supports its clinical evaluation in other hematological malignancies, especially B cell lymphomas. Anecdotal evidence from a relapsing DLBCL patient enrolled in the phase 1 study (NCT02580552) further supports a therapeutic effect of cobomarsen (Anastasiadou et al., 2020).

4 | CONCLUSION

PubMed queries on studies from the last 15 years focusing on diagnostic (about 2700 entries) or therapeutic development (about 6660 entries) of miRNA-based applications indicate great interest and activity on understanding the molecular mechanism of miRNA action and implementing delivery methods for patient treatment. However, these efforts have not yet fully translated to the clinical setting, in which the overwhelming number of current clinical trials are investigating miRNA-based diagnostic applications (about 150 registered trials at clinicaltrials.gov; Table 2) and only a handful are directly evaluating miRNA-based therapeutic applications (Table 5) in cancer medicine. Some challenges with the diagnostic application of circulating miRNA signatures are related to their specificity for a particular cancer type or even cancer per se since these miRNAs can be altered in other physiological (e.g., pregnancy) and pathological conditions (e.g., chronic inflammation, COVID-19 comorbidities), and their diagnostic performance compared to other investigational and clinically established biomarkers. We provided some examples of added value of miRNA biomarkers and strategies to combine or complement existing clinical indicators rather than attempting to replace them. Clinical success of drug treatment has been greatly enhanced by the use of companion diagnostic tests for patient selection (e.g., HER-2 overexpression for anti-HER2 antibody treatment in breast cancer, BRAF mutations for BRAF inhibitor treatment in colon cancer and melanoma, cancer cell-expressing PD-L1 for immune checkpoint therapy in lung cancer and others). We described robust tissue-based technologies for rapid and sensitive detection of miRNA expression at a single-cell resolution that could serve as companion diagnostic tests for miRNA-based drugs. These miRNA-based companion diagnostic tests could provide clinically actionable information based on not only significant changes of miRNA

expression, but also the specific cell type(s) with altered expression. miRNA-based drugs in clinical trials have shown activity in the kidney, lung, and other organ sites demonstrating the therapeutic potential of various systemic delivery technologies to go beyond the liver. Some of these systemic delivery technologies such as gold nanoparticles and iron oxide magnetic nanoparticles have intrinsic imaging capabilities, whereas others can gain imaging capabilities via conjugation of an imaging agent; image guidance could be instrumental for noninvasive monitoring of miRNA-based drug accumulation at tumor sites. Gained knowledge from cell type–specific mechanistic studies in in vivo mouse models (Tables 3 and 4) should guide clinical evaluation of miRNA modulation in cancer cells or other cells in the TME depending on the cancer type. To this end, targeting peptides and other moieties should be further exploited in future clinical studies to deliver the miRNA drug to the most etiologically relevant cellular compartment in order to maximize therapeutic benefit.

RESEARCH RESOURCES

Figures were created with BioRender.com.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Lorenzo Sempere: Conceptualization; data curation; methodology; project administration; resources; visualization; writing-original draft; writing-review & editing. **Asfar Azmi:** Data curation; validation; writing-review & editing. **Anna Moore:** Data curation; funding acquisition; methodology; resources; writing-review & editing.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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