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The potential role of miRNAs in multiple myeloma therapy

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Abstract

Introduction: MicroRNAs (miRNAs) are short non-coding RNAs that are crucial players as post-transcriptional regulators of messenger RNAs (mRNAs). miRNA deregulation has been associated to the pathogenesis of several human malignancies, since they might potentially regulate relevant pathways involved in cancer onset and progression. Therefore, targeting the miRNA network could represent a promising therapeutic strategy for human cancer.

Area covered: This review summarizes recent findings on miRNAs as therapeutics or therapeutic targets against multiple myeloma (MM) and its microenvironment, including the challenges to overcome in the next future for the clinical application of this innovative therapeutic approach.

Expert Commentary: The rising body of advanced preclinical evidence on the biological activity of miRNAs in the pathobiology of MM strongly supports the therapeutic potential of treatment for this still incurable disease. However, translation of this therapeutic strategy for MM patients requires the development of optimized delivery systems and efficient integration of "omics" data with clinical evidence, to precisely identify MM patients that may benefit from a novel miRNA-based therapy.

Key words: miRNA, non-coding RNA, multiple myeloma, experimental therapeutics, miRNA therapeutics
1. Introduction

Multiple myeloma [MM] is a hematologic malignancy of post–germinat center terminally differentiated B cells accounting for about 10% of all hematologic malignancies. It is characterized by proliferation of malignant clones in the bone marrow [BM], associated to skeletal destruction, serum monoclonal gammopathy, immune suppression, and end-organ damage [1]. Despite huge advances in the treatment of this disease by drugs such as proteasome inhibitors [PIs] and immunomodulators (IMiDs), which have significantly improved the outcome of patients [2], MM still remains an incurable malignancy [3]. A deeper molecular understanding of its pathogenesis is therefore urgently needed to identify new molecular targets and to design therapeutic agents patient-tailored [4, 5]. In this context, there is growing evidence that links hallmarks of MM to deregulation of non-coding RNAs, including miRNAs [6].

We review here the role of miRNA in MM pathogenesis, the rationale for innovative miRNA-based new therapeutics and the most relevant and advanced findings from pre-clinical studies and early clinical trials.

1.1. Overview on miRNA network in cancer

miRNA genes represent approximately 1% of the human genome [7] and their biogenesis is a multi-step process [8]. They are transcribed by the RNA polymerase II in pri-miRNA hairpin that is cleaved by Drosha enzyme, in a 70-100 bp pre-miRNA double strand product with two-nucelotide overhang at 3’ end. By exportin 5, this precursor is exported from nucleus to the cytoplasm where the RNase III enzyme Dicer interacts with the 5’ and 3’ ends of the hairpin and cuts the loop, producing a 20-22-bp miRNA/miRNA* duplex. Only one strand of the duplex is incorporated into the RNA-Induced Silencing Complex (RISC) that drives the binding of the mi-RNA ‘seed’ sequences (2~7nt long) to consensus sequences within 3’UTR site in mRNA targets. The other strand, called the passenger strand due to lower steady state levels, is normally degraded. Exceptionally, both strands of the duplex are functional and target different mRNA populations. The active RISC miRNA/mRNA binding represses translation or induces mRNA degradation, depending on the degree of complementarity between miRNA and its target sequence [9].

Overall, miRNAs play substantial roles in post-transcriptional negative regulation of half of the protein-coding genes within the human genome, including oncogenes...
and tumor suppressors. Indeed, miRNAs represent critical regulators of tumor initiation, progression, and dissemination [10], acting as “oncomiRs” if their targets are tumor suppressors and their expression is upregulated in cancer cells, or as “tumor suppressor miRNAs” [TS-miRs], if their targets are oncogenes and their expression is downregulated in tumor cells [11]. Furthermore, deregulation of a variety of miRNAs has been associated with specific clinical-pathological traits of different malignancies [12].

1.2. The rationale of miRNA-based therapeutic strategies in cancer

Two opposite strategies (Figure 1) can be developed to regulate miRNA aberrant expression in cancer: the replacement of downregulated miRNAs or the inhibition of upregulated miRNAs [13, 14].

1.2.1 Replacement approach

miRNA replacement aims to restore miRNAs, which are downregulated and/or selectively deleted. This strategy relies on previous characterization of the biologic function of the designated miRNA, which must be able to suppress tumor growth and progression by targeting critical mRNA involved in survival and proliferation of cancer cells. Restoring miRNA levels can be obtained by infection of viral vectors overexpressing a specific miRNA [15] or by transient transfection of double-stranded miRNAs, known as miRNA mimics, consisting in 22-mer oligonucleotides bearing the same sequence of mature miRNA or its precursor. To this aim several types of non-viral vectors were developed, including polymeric vectors, lipid-based carriers [positively and negatively charged or neutral] and inorganic materials [16].

1.2.2 miRNA inhibition approach

miRNA inhibition approach relies on targeting selected miRNAs that are upregulated and support proliferation and survival of cancer cells. To this aim antisense oligonucleotides are widely used as miRNA inhibitors (antagomirs, AMOs), specifically designed to anneal in a complementary, according to Watson-Crick base-pairing rules, to the ‘sense’ miRNA strand inducing RNAse-H-mediated degradation. AMOs may carry some chemical modifications to enhance stability and affinity for the miRNA target. For instance: a] modification in the 2-OH residue of the ribose by O-methyl (2 -OMe) or O-methoxyethyl (2 -MOE) group [17]; b] Locked
Nucleic Acid [LNAs], where RNA nucleotide bring an extra bridge connecting the 2' oxygen and 4' carbon that locks the ribose in the 3'-endo conformation, enhancing base stacking and increasing hybridization properties. These oligonucleotides are mainly synthesized with a phosphorothioate [PS] backbone, where a sulfur atom substitutes a non-bridging oxygen atom. This modification confers resistance to nuclease degradation. LNA-PS oligonucleotides are highly soluble in water and stable in biofluids, with optimal bio-distribution within tissues and long-lasting knockdown of the target in vivo [18, 19].

In addition to antisense oligonucleotides, other strategies have been developed to inhibit oncogenic miRNA functions: 1) miRNA sponges, transcripts that contain multiple tandem binding sites to a miRNA of interest, which acts as an miRNA decoy preventing their binding to target mRNA [20]; 2) miR-MASKs, modified antisense oligonucleotides complementary to miRNA binding sites on the mRNA target [21], which mask the miRNA binding site and selectively inhibit the interaction of the miRNA with the specific mRNA target, in order to antagonize repression; 3) gapmers, oligonucleotides containing LNA nucleotides that are able to sequester their RNA target in highly stable DNA:RNA heteroduplexes, and bringing a central nucleotide “gap” of DNA lead to RNase-H-mediated target degradation [22].

Furthermore, indirect strategies could be employed to modulate miRNA expression. Recent evidence supports the possibility to modulate the machinery that contributes to specific miRNA maturation and degradation processes, by using selective small molecules inhibitors (Small Molecular Inhibitors of Specific miRNAs, SMIR) [23], identified by efficient screening of chemical libraries.

1.3. Clinical trial evaluation of miRNA therapeutics

miRNA-based therapeutic strategies are evaluated in several clinical trials for the treatment of different disease, including cancer [24]. The first miRNA-based therapeutic named Miravirsen (SPC3649), reached phase II clinical trials for the treatment of hepatitis C virus (HCV) infection. Miravirsen is an LNA-ASOs which blocks miR-122 interaction with HCV RNA, thus promoting virus destruction [25]. MRX34, a liposome-formulated mimic of miR-34a, was the first agent investigated in a Phase I clinical trial in patients with advanced solid tumors [26]. MRX34, infused intravenous (IV) with a biweekly or daily schedule, showed a relevant activity in hepatocellular carcinoma, renal cell carcinoma and melanoma. However, multiple
immune-related severe adverse events were registered and MRX34 development to phase II clinical trial was halted.

miR-16 mimics have been also recently evaluated in a Phase I clinical trial for patients with Malignant Pleural Mesothelioma and Advanced Non-Small Cell Lung Cancer that have failed standard therapy [27]. These miRNA mimics, were delivered by IV infusion using a bacterial-derived (EDV) packaging [28] and were conjugated with an EGFR-targeting antibody. After treatment, 27% of patients had progressive disease, 68% had stable disease and 5% had a partial response. On the basis of these results, this trial is expected to continue to phase II.

MRG-106, a synthetic LNA antimiR of miR-155, is presently investigated in an ongoing phase I trial, for patients with cutaneous T-cell lymphoma of the mycosis fungoides (MF) sub-type. Preliminary results presented by Foss et al. at ASCO meeting in 2017 demonstrated that intratumoral injection of MRG-106 in MF patients was well-tolerated with promising therapeutic response in cutaneous lesions, as revealed by reduction in neoplastic cell density and depth, together with decrease in systemic symptoms, such as pruritus.

2. miRNA deregulation in MM

Deregulation of miRNA expression can occur because of gene amplification or deletion, mutations, methylation, transcription and miRNA processing alteration [29]. Integrated analyses are therefore required for a better comprehension of the biological role of individual miRNA in cancer.

miRNAs expression profiling has been extensively performed in MM to identify potential candidates linked to development and progression of the disease. To this aim, different methodological approaches were used to process various sample types (MM cell lines, bone marrow plasma-cells [BMPC] isolated from patients, biological fluids), from target tools such as quantitative reverse transcription PCR [qPCR] to high-throughput techniques, such as microarray and next generation sequencing [30, 31].

The first implication of deregulated miRNA expression in MM, was reported by Loffler et al. in 2007 [32]. In this study the authors demonstrated that miR-21 transcription is under IL-6 control through a mechanism involving STAT3, and that miR-21 ectopic expression makes MM cells independent from the IL-6 growth stimulus. Pichiorri et al. [33] performed an integrative miRNA-mRNA analysis in MM-
derived cell lines and CD138\(^+\) BMPCs from MM subjects, monoclonal gammopathy of undetermined significance [MGUS] and normal donors. They identified a miRNA signature associated with transformation of normal PCs to clinical MM via MGUS. Microarray data analyses demonstrated that miR-32, miR-21, miR-17-92, miR-106b-25 cluster, and miR-181a/b were upregulated in both MM cell lines and primary tumors versus normal PCs. miR-106b-25 cluster, miR-181a/b, and miR-21 were upregulated also in MGUS patients with respect to normal PCs, while miR-32 and the miR-17-92 cluster were highly expressed only in MM patients, suggesting that these miRNAs could be involved in MM pathogenesis. In a further study, Roccaro et al. [34] profiled miRNAs in CD138\(^+\) cells isolated from BM of 15 relapsed/refractory MM patients, 3 MM cell lines and BM of healthy donors. MM patients showed increased miR-221, miR-222, miR-382 and miR-181a/b levels compared with controls, while miR-15 and miR-16 levels resulted decreased in MM patients or absent in patients carrying chromosome 13 deletion. Alterations of DNA copy number could also account for miRNAs deregulation in MM. Some studies have correlated miRNA expression patterns with cytogenetic abnormalities known to be of biological or prognostic relevance in MM. In particular, Gutierrez et al. [35] investigated the association between miRNA deregulation and chromosomal rearrangements in 60 MM patients. miR-1 and miR-133a, both mapped in 18q11, were overexpressed in t[14;16] while miRNAs located in 13q in MM patients with monosomy 13q were downregulated as consequence of haplo-insufficiency. In another study, Lionetti et al. [36] identified 16 deregulated miRNAs in MM mapped to chromosomal regions previously reported as relevant in MM pathogenesis. These included miR-92b (1q22), miR-26a (12q14.1), miR-21 (17q23.1), miR-22 and miR-324 (17p13.3), miR-106b cluster and miR-25 (7q22.1), miR-15a and miR19a (13q14.3), miR130b and miR-185 (22q11.21). In addition to copy number changes, Amodio et al. [37] demonstrated that epigenetic mechanisms drive the silencing of miR-29b in MM cell lines such as aberrant deacetylation of miR-29a/b-1 promoter by histone deacetylases (HDACs) or negative regulation of miR-29b by H3K27 methyltransferase EZH2 [38]. Similarly, Fulciniti et al. showed that promoter methylation could be one of mechanism involved in miR-23b downregulation observed in myeloma cells [39]. Furthermore, single nucleotide polymorphisms within miRNA-binding sites (miRSNPs) in target genes may modify miRNA–mRNA interactions and alter protein
expression. In this context, De Larrea et al. [40] investigated the role of miRSNPs on survival and progression in MM patients undergoing autologous stem cell transplantation. They found two miRSNPs correlated with longer OS. The first was rs3660, located in the 3′-UTR of KRT81 gene and inside the miRNA-binding site for seven miRNAs, previously reported to be upregulated in MM (miR-17, miR-20a, miR-20b, miR-93, miR-106a, miR-106b, and miR-519d). The authors demonstrated that rs3660 enhances the binding of miRNAs to the complementary sequence resulting in a reduction of KRT81 expression levels. The second miRSNP was rs11077, located in the 3′-UTR of XPO5 gene encoding a protein involved in the export of pri-miRNAs from nucleus to cytoplasm. It was demonstrated that inefficient XPO5 activity impairs miRNA processing and miRNA target regulation. The 3′-UTR analysis revealed that rs11077 was not included in any miRNA-binding sites, but the nucleotide change creates a binding site for miR-4763-5p.

The role of key regulators in miRNA processing, such as Dicer and Drosha enzymes, in the pathogenesis of monoclonal gammopathy has been also investigated by Sarasquete et al. [41]. The authors evaluated the expression of Dicer and Drosha in 102 MM, 23 smoldering myeloma (SMM) and 26 MGUS patients and found upregulation of Dicer in MGUS compared with SMM and MM, suggesting an involvement of this enzyme in the progression and prognosis of monoclonal gammopathy.

As for cellular miRNAs, in the recent years several studies have documented the clinical relevance of circulating miRNAs (c-miRNAs) for diagnostic, prognostic and response to therapy markers in a variety of cancers, including MM [42]. Manier et al. [43] examined the prognostic significance of circulating exosome miRNAs in newly diagnosed MM. They identified by RNA sequencing a signature of 22 c-miRNAs, selected on the base of their biological relevance in MM, and they used them to screen serum samples from 156 MM patients by qRT-PCR array. Univariate and multivariate analysis, the latter after adjusting for the International Staging System (ISS) and adverse cytogenetics, evidenced that let-7b and miR-18a were significantly associated with both Progression Free Survival (PFS) and Overall Survival (OS).

Although c-miRNAs are attractive, exist a lack of consistent finding in different studies, probably due to technical differences in high-throughput platforms used, miRNA-array content and statistical analysis. Moreover, c-miRNA profiling in biofluid
samples, such as plasma, serum, urine, is affected by a range of pre-analytical and analytical challenges in experimental design, from sample collection to profiling and data analysis. Small sample size is another critical factor, thus statistically relevant miRNAs require a further validation in greater and independent prospective cohorts before being validated as clinically actionable biomarkers.

2.1. Preclinical findings of miRNA therapeutics in MM
Since miRNAs deregulation is involved in MM pathogenesis, it is conceivable that their enforced expression or inhibition may be used as a therapeutic tool to equilibrate their pathologic downregulation or upregulation respectively [44-50].

Indeed, several preclinical in vitro and in vivo findings of miRNA-based therapy in MM has been produced, thus confirming that miRNAs modulation is a promising and innovative therapeutic approach to target different pathways involved in myelomagenesis.

Here we report pre-clinical data (Table 1) regarding the perturbations induced by miR-based therapeutic strategy on hallmarks [51] of MM cells and their microenvironment: sustaining proliferative signaling, evading growth suppression, fostering genomic instability, inducing angiogenesis, activating invasion and metastasis, promoting inflammation in cancer milieu, triggering bone disease (Figure 2).

2.2 Sustaining proliferative signaling and survival
The miR-106b/25 cluster, encoding for 3 mature miRNAs [miR-106b, miR-93, and miR-25] was found upregulated in MGUS and MM patients, as compared to healthy subjects [33]. Gu et al. [52] demonstrated that a tiny anti-miR-106b/25 cluster seed-directed induced a significant reduction on cell viability and decreased colony formation in MM cells by reducing p38-MAPK dependent signaling.

Morelli et al. [53] found that miR-125b is downregulated in TC (translocation and cyclin) 2/3 molecular MM subgroups and in established MM cell lines. Moreover miR-125b expression was reduced also by bone marrow microenvironment secreted TNF, IGF-1 and IL-6, important survival factors for malignant plasmacells [54, 55]. Finally, the authors showed that miR-125b replacement exerts strong anti-MM activity in vitro and in vivo via targeting IRF4-addiction on malignant PCs.

miRNA 15a and 16-1, are located in chromosome 13q14, a genomic region often
deleted in MM [56]. Roccaro et al. demonstrated that miR-15a and miR-16-1 were significantly decreased in MM compared to healthy PCs. Consistently, the authors showed that transfection of MM cells with pre-miR-15a and -16-1 induced cell cycle arrest and growth inhibitory activity in vitro and in vivo, by targeting pro-survival factors such as AKT serine/threonine-protein-kinase (AKT3), ribosomal-protein-S6, MAP-kinases, and NF-kB-activator MAP3KIP3 [34].

The miR-17-92 cluster is a master regulator in Myc-driven MM pathogenesis. Morelli et al. [57] developed a first-in-class inhibitor of this oncogenic cluster as locked nucleic acid gapmeR (MIR17PTi), which prevents biogenesis of all six mature miRNAs [miR-17/-18a/-19a/-20a/-19b1/-92a1]. Importantly, treatment with MIR17PTi induced apoptosis and inhibited MM growth in clinically relevant in vivo MM models, via the impairment of pro-survival Myc/miR-17-92 feed-forward loops.

2.3. Evading growth suppressor and resisting cell death

miR-21 plays a key role in tumor progression and is significantly upregulated in several human cancers, including MM [58]. Leone et al. [59] showed that miR-21 targeting induced in vitro and in vivo myeloma cell growth inhibition. Mechanistically, miR-21 inhibition led to upregulation of its tumor suppressor targets (PTEN, Rho-B, and BTG2), which finally induced functional impairment of pro-survival signaling in MM cells.

miR-221/222 cluster promotes tumorigenesis by inhibiting tumor suppressor genes in several malignancies [60], including MM. In particular, Di Martino et al. reported that miR-221/222 are upregulated in MM patients, specifically in TC2, TC4 and in a subgroup of TC3 MM, including MM harboring t(4;14) translocation. Consistently, miR-221/222 inhibition resulted in efficient anti-tumor activity both in vitro and in murine models of MM [61]. Importantly, these growth inhibitory effects were correlated to strong upregulation of validated miR-221-222 target such as p27Kip1, PUMA, PTEN and p57Kip2. These authors developed a novel chemically modified miR-221 inhibitor, the LNA-i-miR-221, with promising in vivo results [62]. The LNA-i-miR-221, a 13-mer oligonucleotide, combines high-affinity-LNA nucleotides with PS modified backbone proprieties, conferring high stability versus exo- and endonuclease cleavage, thereby enabling prolonged biological effects after in vivo treatment [19].

Tian et al. [63] demonstrated that miR-33b overexpression induced significant cell
death and apoptosis in MM cells, decreased number of colonies and of migrating cells and inhibited tumor growth and prolonged survival \textit{in vivo}. At molecular level, these effects seem to be related to direct targeting of PIM-1, a serin/threonine kinase that prevents the association of Bad with Bcl2 and Bcl-xl, blocking Bad-induced cell apoptosis.

The tumor suppressor p53 acts as a potent transcription factor and can be activated in response to different cellular stresses, thereby promoting apoptosis, cell cycle arrest, and senescence. \textit{TP53} mutation is rarely detected at diagnosis in MM, thus several evidence showed that re-activation of p53 could represent a promising therapeutic strategy [64]. Recently, miRNAs have been reported to be directly involved in p53 activity and in its regulatory loop. Among these, miR-194-2-192 and miR-215-194-1 clusters, miR-125-5p, miR-214 and miR-34a, seem to significantly interact with p53 pathways in MM.

Indeed, Pichiorri et al [65], showed that miR-192, 194, and 215, which are downregulated in a subset of MMs patients, were transcriptionally activated by p53 interfering at the same time with p53/MDM2 autoregulatory loop. Mechanistically, enforced expression of miR-192, 194, and 215 led to direct downregulation of MDM2 levels and this was accompanied by higher activation of p53 and p21. Importantly, ectopic overexpression of miR-192, 194, and 215 in MM cells enhanced the therapeutic activity \textit{in vitro} and \textit{in vivo} of MDM2 inhibitors (MI-219, Nutlin-3a) by increasing their p53-activating effects.

An increased expression of miR125a-5p in MM cells is associated to a subset of MM patients carrying the t(4;14) translocation [36]. On these basis, Leotta et al. [66] showed that miR-125-5p inhibitor in wild-type p53 MM cells dampened cell growth, increased apoptosis and reduced cell migration by the activation of p53 tumor-suppressor pathway, including activation of a subset of p53-induced miRNAs, such as miR-192 and miR-194.

miR-214 directly targets p28/gankyrin (encoded by PSMD10), an oncprotein which functions as a negative regulator of p53 by binding to MDM2/HDM2 complex, thus enhancing p53 degradation. Consistently, gankyrin down-regulation induced by miR-214 ectopic expression led to an increase of \textit{P53} transcriptional signaling which finally translated into tumor suppressor activity this miRNA in MM [67].

miR-34a transcription is induced by TP53 in response to cell stress [68]. Di Martino et al. reported a significant antitumor effect of miR-34a enforced expression \textit{in vitro}
and in vivo in MM. In fact, treatments with synthetic miR-34a mimics formulated in a Neutral Lipid Emulsion (NLE) induced strong inhibition of tumor growth with complete tumor regression in 50% of MM-xenograft mice and a dramatic prolongation of survival [69]. Mechanistically, miR-34a counteracted anti-apoptotic and pro-survival regulators such as BCL2, CDK6 and NOTCH1, which resulted significantly downregulated at both the mRNA and protein level. Moreover, Wu et al. demonstrated that the overexpression of miR-34a in MM cells and in cancer stem cells was associated to inhibition of cell proliferation in vitro and in vivo through inhibition of transforming growth interaction factor 2 (TGIF2) [70]. Based upon the high anti-proliferative activity of miR-34a mimics in MM, a nanotechnology-based delivery system, to overcome the biopharmaceutical issues of the administration of nucleic acids, was developed. Specifically, Di Martino et al. used the Stable Nucleic Acid Lipid Particles (SNALPs) technology, which confers better stability in serum and is characterized by high encapsulation and efficient transfection, to delivery miR-34a in vivo [71]. Importantly the authors demonstrated the efficacy of this strategy, based on efficient systemic delivery and significant anti-tumor effects of miR-34a mimics in MM xenografts in mice [72]. In addition, Cosco et al. performed miR-34a delivery by chitosan/PLGA nanoplexes in MM murine xenograft. Nanoplexes provided protection of miR-34a mimics from ribonuclease in systemic treatment, high grade of transfection efficacy in vitro, and significant anti-tumor activity in vivo [73].

The miRNA-29 family consists of miR-29a, miR-29b, and miR-29c, among which miR-29b is the most aberrantly expressed in cancer, including MM [74]. Amodio et al. showed that enforced expression of miR-29b downregulated major tumor promoting or anti-apoptotic mRNA targets, including CDK6, MCL-1, SP1 [4], as well as mRNAs coding for epigenetic regulators [75, 76], such as HDAC4 [37] and DNMT3A/B [77], thus triggering apoptosis and tumor-growth inhibition. Importantly, mir-29b replacement increased also antmyeloma activity of Bortezomib [4, 78]. Indeed, mir-29b enforced expression reduced the proteasome's peptidase activity and aggresome-autophagosome formation, by targeting the proteasome activator pa200 (encoded by PSME4).

2.4. Fostering genome instability & mutation

MM is characterized by deep genomic instability, which is the driver of karyotypic
aberrations observed in malignant PCs. Qin et al. [79] demonstrated that miR-137 ectopic expression overcame chromosomal instability in MM cells, by targeting AURKA, a serine/threonine kinase which plays an essential role in CHK1/2 regulation and DNA damage response. Preliminary results by our group shed new lights on mechanism of MM-related genomic instability. Indeed, MM cells seems to rely on DNA Ligase III (LIG3)-driven DNA repair to accumulate new genetic changes, which drive disease progression and drug-resistance. Interestingly, replacement with miR-22 mimics negatively regulates LIG3 activity reducing genomic instability and increasing unrepaired DNA damage, which finally led to anti-MM activity in vitro and in vivo [80].

2.5. Inducing angiogenesis
MM pathogenesis and progression are strongly supported by aberrant angiogenic events [81]. Raimondi et al. [82] showed that enforced expression of miR-199a-5p led to downmodulated expression of HIF-1α as well as of other pro-angiogenic factors (VEGF-A, IL-8, and FGFb) in hypoxic MM cells and reduced endothelial cells migration, which lastly resulted in significant tumor-growth inhibition in vivo. MiR-15/16a plays a relevant role even in the context of the BM microenvironment. By MiR-15a and miR-16 replacement, it was induced direct targeting of VEGF mRNA and was inhibited MM cell-dependent endothelial cells growth and capillary formation in vitro, together with tumor growth and angiogenesis reduction in vivo [83].

2.6. Activating invasion and metastasis
MiR-199a-5p seem to be implicate also in aberrant invasion and metastasis processes [84]. In particular, transfection of MM cells with miR-199a-5p mimics induced a reduction of critical component of chemotaxis machinery such as VCAM-1 and ICAM-1, DDR1, metalloproteinase MMP2 and CXCR4, which in turn resulted in reduced cellular migration [82].

Another miRNA implicated in cell migration is miR-30. MM cell proliferation, survival and migration seems to be influenced by activity of Wnt pathway component such as TCF, β-catenin and BCL9. Zhao et al. [85] demonstrated that miR-30-5p replacement inhibits MM cell proliferation, invasion, and migration by targeting BCL9. Importantly, the authors showed that in vivo systemic delivery of lipid-
conjugated miR-30, decreased tumor burden and metastasis in murine MM xenografts.

2.7. Tumor promoting inflammation
Dendritic cells (DCs) play a key role in regulating tumor inflammation in several malignancies, including MM [86]. Botta et al. [87] demonstrated that MM reprograms the DCs functional phenotype to sustain plasma cells growth, by downregulating miR-29b. Importantly, miR-29b antagonized polarization of DCs to a pro-inflammatory phenotype and this effect mainly relied on inhibition of NF-κB, STAT3, MAPK and JUN activity. In addition, miR-29b enforced expression, produced a decrease of IL-23 and other pro-inflammatory cytokines/chemokines that in turn impaired attraction of inflammatory cells such as polarization and expansion of Th17 lymphocytes.

2.8 Avoiding immune destruction
Increasing evidence highlights the relevance of natural killer [NK] cells in immune responses against multiple myeloma cells, especially in response to immunomodulatory drugs or following allogeneic stem cell transplantation. In this scenario, miRNAs could play a critical regulatory role taking into account their ability to modulate expression of ligands involved in NK-driven anti-tumor immunity [88].
Indeed, Abruzzese et al. [89] found that miR-125-b replacement was able to upregulate expression of NKG2D ligand on myeloma cells, thus potentially increasing sensitivity to cytotoxic activity of NK cells. Mechanistically, this effect was related to selective targeting of IRF4 [53], a transcriptional repressor of NK ligand MICA.

2.9. Triggering bone disease
Bone disease (BD) is a highly symptomatic condition and has a relevant impact on myeloma patients’ outcome. MM-related BD is produced by cell- and humoral-mediated (cytokines, chemokines, exosomal microRNA) interaction [90, 91] of MM cells with bone-marrow stromal cells [BMSCs], that led to high levels of RANK ligand (RANKL), a pro-osteoclast (OCL) factor, and low levels of osteoprotegerin (OPG), the main bone protective factor [92].
Pitari et al. [93] showed that the interaction between MM cells and BMSCs strongly enhanced miR-21 expression. Importantly, inhibition of miR-21 in stromal cell line increased OPG production, decreased RANKL production, and restored RANKL/OPG ratio in BMSCs co-cultured with MM cells, thus favoring bone apposition and impairing the resorbing activity of mature OCLs. At molecular level, these effects seem to be related to upregulation of inhibitor of activated STAT3 (PIAS3), a validated miR-21 target, which in turn decreased STAT3-dependent induction of RANKL production [94]. Furthermore, Rossi et al. [95] observed a progressive downregulation of miR-29b during OCL differentiation. Consistently, miR-29b enforced expression was able to overcome the pro-osteoclastic effect of MM cells. In particular, OCLs overexpressing miR-29b were impaired in their resorptive activity compared to control OCLs in the presence of MM cells. In addition, miR-29b treated OCLs are less responsive to RANKL stimulation and showed reduced levels of pro-osteolytic enzymes. These effects were mainly mediated by direct targeting of c-FOS, a crucial factor for differentiation, maturation and resorbing activity of OCLs [96].

3. Conclusions
Since the discovery of miRNAs in 1993 [97], the understanding of their role in cancer onset and progression has dramatically increased. As for other cancer types, the dissection of MM miRNA network is leading to deep knowledge of MM vulnerabilities. Indeed, large preclinical evidence indicates the therapeutic potential of miRNAs in MM [4, 98]. However, outstanding challenges [14, 99] remain to be overcome: 1) there still exists the need to develop better chemical designs and delivery options, to achieve sustained target inhibition; 2) a full assessment of off-target effects needs to be evaluated, since miRNAs can regulate the expression of multiple genes; 3) the identification of the best miRNA candidate or miRNA targets for each disease type is crucial.

4. Expert Commentary
The biologic complexity of MM pathogenesis requires an individualized approach to reach an effective therapeutic goal. In this scenario miRNA-based treatment of MM
represent an innovative approach, since *in vitro* and *in vivo* pre-clinical data had demonstrated a significant anti-MM activity. However, several challenges will need to be overcome in the next future. Development of selective miRNA-based strategy must deal with complexity of miRNA “targetome”, which might include oncogenes and tumor suppressors, as well as a number of targets not involved in cancer. To this aim, novel technologic tools will permit to dissect and integrate the complex data deriving from human miRNome, genome, transcriptome and proteome, to successful design the clinical translation of this therapeutic approach. For these reasons, major efforts will be also necessary for the adoption in the clinic of sound strategies based on “integrOmics” data obtained from diverse technology platforms [5], in the final aim to translate wet biologic information to an improved “standard of care” for MM patients.

In our opinion, the advent of next-generation sequencing [NGS] technologies [100], will provide important novel information allowing us to better understand which MM patients are more likely to benefit from miR-based therapy. Indeed, precision oncology can be achieved by designing the specific miRNA mimic or inhibitor based on patient’s individual miRNA expression profile. We are also confident that development of nanotechnologies [101] can overcome the biological barriers for miRNA delivery and finally exploit the great potential of miRNA therapeutics. Indeed, many obstacles, such as low cellular uptake, immunogenicity, elimination by nuclease or phagocytic cells, limit the delivery of miRNA to cancer cells. In this field, new hopes arise from latest generation of delivery systems which consists of “smart” nanoparticle, whose size and property are changeable due to different stimuli, such as low pH or low partial oxygen pressure. This strategy will allow to achieve high and selective penetration of miRNA-containing delivery vesicles into the tumor microenvironment [102, 103]. Moreover, the promising pharmacokinetic properties of chemically modified miRNA-inhibitors, such as LNA-oligonucleotides, provides challenging tools for further investigation and clinical translation [19].

An exciting opportunity is the integration of miRNA therapeutics in the currently available treatment of MM, to reduce the risk of drug resistance by targeting compensatory pathway [104, 105] or drug absorption, distribution, metabolism and excretion (ADME)-related genes [106-108]. In addition, an intriguing strategy could be based on the use of miR-based therapeutics to reverse MM-mediated immune
paralysis. Indeed, several findings demonstrated that miRNAs are able to polarize the immune-response through molecular reprogramming of immune microenvironment [88].

In conclusion, despite the outstanding challenges, we expect that an increasing number of synthetic miRNA/anti-miRNA will progress through clinical trial in MM patients in the next future, to successful translate miRNA therapeutics from bench to bedside.

5. Five-year view
The introduction of immunomodulatory drugs and proteasome inhibitors as well as the use of novel monoclonal antibodies in the therapeutic setting of MM [109] have resulted in prolonged survival of patients. However, MM remains an incurable disease, and there is an urgent need for new therapeutic options. miRNA therapeutics might arise as a promising strategy for implementation of “precision oncology” to the standard treatment of this important disease. Therefore, future efforts have to overcome the limits with actually precludes the clinical application of this innovative therapeutic approach. Biological and technologic advancements will allow in the next years to identify MM patients which could benefit from a miR-based therapy, providing at the same time novel nanotech-systems to deliver miRNA-therapeutics into MM microenvironment.

Taking into account these considerations, in our opinion a potential future option could be an integrated therapeutic strategy between novel agents (immunomodulatory and proteasome inhibitors) and miRNA-based agents, selected on accurate molecular profiling of the individual disease status, to finally achieve a personalized approach to the treatment of this still incurable disease with the best cost/benefit balance.

6. Key issues
- Multiple Myeloma is a plasma-cell dyscrasia characterized by clonal expansion of plasma cells in the bone marrow, which leads to osteolytic bone disease, infections, renal insufficiency, and bone marrow failure
- miRNA profiling studies provided evidence of a widespread deregulation of miRNA in MM, thus highlighting their role in the pathogenesis of the disease
• Preliminary *in vitro* and *in vivo* studies demonstrated that miR-based therapeutics exert anti-MM activity

• Efficient translation of miR-based approach to clinical setting of MM therapy requires an optimized integration of “omics” data and innovative delivery systems, which therefore represents critical issues to be addressed in the next future

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**Declaration of interest**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

**Reviewer disclosures**

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

**References**
Papers of special note have been highlighted as:

* of interest
** of considerable interest


83. Sun CY, She XM, Qin Y, et al. miR-15a and miR-16 affect the angiogenesis of multiple myeloma by targeting VEGF. Carcinogenesis. 2013 Feb;34:426-35.


Figure legends

**Figure 1: miRNA-based therapeutic strategies.** Therapeutic modulation of miRNA biogenesis and activity, obtained by [A] restoration of Tumor suppressor miRNAs [miRNA replacement approach] or by [B] targeting OncomiRs [miRNA inhibition approach]
Figure 2: miRNAs and MM hallmarks. The cartoon illustrates how miRNAs deregulation contributes to MM onset and progression by promoting hallmarks of the disease.
<table>
<thead>
<tr>
<th>miRNA</th>
<th>Expression</th>
<th>Target</th>
<th>Hallmark</th>
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**Table 1: miRNAs deregulation in Multiple Myeloma**

p38 MAPK, p38 mitogen-activated protein kinase; BCL2, B-cell lymphoma 2; CDK6, Cell division protein kinase 6; NOTCH1, Notch homolog 1, translocation-associated; TP53, Tumor protein p53; IRF4, Interferon regulatory factor 4; AKT3, RAC-gamma serine/threonine-protein kinase; rp-S6, Ribosomal protein S6; VEGF, Vascular endothelial growth factor; BCL9, B-cell CLL/lymphoma 9 protein; PTEN, Phosphatase and tensin homolog; PIAS3, E3 SUMO-protein ligase PIAS3; PUMA, p53 upregulated modulator of apoptosis; PIM-1, Proto-oncogene serine/threonine-protein kinase Pim-1; AURKA, Aurora kinase A; MCL1, Induced myeloid leukemia cell differentiation protein; SP1, Transcription factor Sp1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; STAT3, Signal transducer and activator of transcription 3; HIF1α, hypoxia-inducible factor 1, alpha subunit; DDR1, Discoidin domain receptor family, member 1; PSMD10, 26S proteasome non-ATPase regulatory subunit 10; PSME4, Proteasome activator complex subunit 4; MYC, [v-myc myelocytomatosis viral oncogene homolog]; LIG3, DNA Ligase III;