Preclinical Results Supporting Therapeutic Development of MRG-106, an Oligonucleotide Inhibitor of miR-155, in CTCL

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Abstract

Treatment-resistant hematological malignancies remain an area of high unmet need and novel therapeutic approaches will be required. microRNAs are small (~ 22 nt) non-coding RNAs that act as negative regulators of gene expression. These small RNAs impact expression of a substantial fraction of the genome, and have powerful effects on cellular phenotypes and physiological processes. miR-155-5p is a well-described oncomiR associated with poor prognosis in multiple malignancies, particularly lymphoma and leukemia. Cutaneous T-cell lymphoma (CTCL) is a rare hematological malignancy with limited treatment options and a strong mechanistic link to increased miR-155-5p. Because of the accessibility of cutaneous lesions, CTCL provides a unique opportunity to determine if inhibition of miR-155-5p has therapeutic potential in lymphomas associated with elevated miR-155-5p.

We optimized a LNA-modified oligonucleotide inhibitor of miR-155-5p, MRG-106, based on the ability to de-repress canonical miR-155-5p targets in multiple cell types *in vitro*. In mycosis fungoides (MF) cell lines, MRG-106 does not require additional formulation to achieve maximum pharmacodynamic efficacy. Inhibition of miR-155-5p resulted in transcriptome changes consistent with miR-155-5p target gene modulation, reduction in cell proliferation, and activation of the programmed cell death pathway. The gene expression and phenotypic effects were inhibitor dose-dependent and sequence-specific. Based on an informatics approach for the expression profiling of MF cell lines treated with MRG-106, a set of 600 genes was identified to represent the translational pharmacodynamic biomarker signature, both direct and downstream of miR-155-5p.

GLP preclinical safety studies have been completed in rats and non-human primates, demonstrating an acceptable safety profile for MRG-106. We plan to initiate a 4-week first-in-human clinical trial in CTCL (MF) patients. The trial design is two-part, with Part A testing the effect of direct intra-tumoral injection of MRG-106 into plaque and nodular skin lesions, and Part B testing the effect of systemic (subcutaneous) administration of higher doses of MRG-106. An important objective of Part A is to profile the pharmacodynamic effect of MRG-106 on the miR-155-5p gene expression signature, establishing a PK/PD model to guide future development. The primary objective of Part B is to establish the safety, tolerability, PK and skin deposition of MRG-106 after systemic delivery. Exploratory objectives include measures for clinical response, immune system effects, and biomarker validation.

microRNAs are novel therapeutic targets that act through the regulation of systems biology

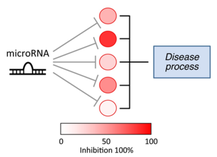


Figure 1.
- The unique objective of microRNA-targeted therapy is achieving disease modification by restoring systems homeostasis.
- microRNAs regulate complex biological systems and this role is amplified in disease states and biological stress.
- The pharmacology of microRNA-targeted therapies is intrinsically focused on disease relevant pathways.
- Recent appreciation of the role of miRNAs as a "*molecular switch*" that constrains differentiation and maintains adaptive and maladaptive phenotype.

The biogenesis and therapeutic modulation of microRNAs

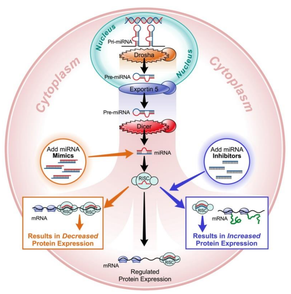


Figure 2.
- MicroRNAs are transcribed from unique loci or reside within introns of protein-coding genes. After transcription in the nucleus, the pri-microRNA is cleaved by the ribonuclease Drosha.
- After Drosha cleavage, the pre-microRNA is exported to the cytoplasm by the nuclear export protein complex Exportin-6.
- The cytoplasmic pre-microRNA is further cleaved by Dicer.
- After Dicer cleavage, the mature microRNA of 16-22 nucleotides in length is assembled into the RISC protein-RNA complex.
- microRNAs function by binding to short sequences located in the 3' UTR of mRNA transcripts.
- The RISC complex contains the microRNA that identifies the target mRNA and protein factors that prevent the conversion of the mRNA to protein.
- Therefore, the function of the microRNA is to repress protein translation.
- microRNA-based therapeutics can act by either increasing the level of endogenous microRNA (mimicry) or by inhibiting the microRNA's activity (inhibitor, or antimiR)

miR-155 plays a key role in inflammation and oncogenesis

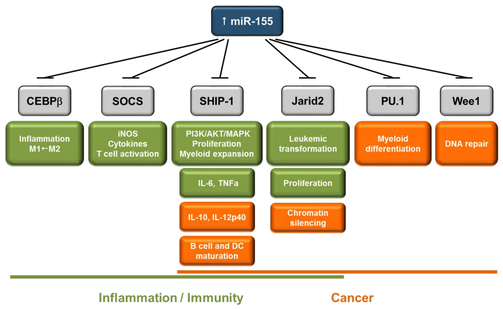
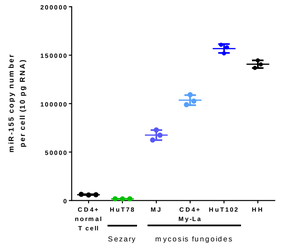


Figure 3.
- miR-155 is one of the first microRNAs shown to be upregulated in a variety of cancers, including lymphomas and leukemias.
- miR-155 expression is driven by STAT3 and NFκB.
- miR-155 expression is increased with immune cell activation.
- The direct gene targets of miR-155 (containing miR-155 binding sites in the 3'UTR) reflect the systems biology of this microRNA in inflammation and oncology.

miR-155 is elevated in mycosis fungoides cell lines and skin biopsies

Figure 4.
- Several publications have shown that miR-155 is elevated in skin biopsies of patients with mycosis fungoides (Ref. 1-4)
- Elevated miR-155 is mechanistically relevant to MF pathogenesis (Ref. 5-6)
- One publication showed that miR-155 is unchanged in circulating cells of patients with Sézary syndrome (Ref. 7)
- Quantitation of miR-155 copy number by real time PCR in MF cell lines showed >50-fold increase in miR-155 compared to normal CD4+ T cells, with copy numbers ranging from 75,000 to 175,000 per cell.
- A cell line derived from a Sézary syndrome patient did not have elevated miR-155.



miR-155 inhibition results in direct target gene regulation

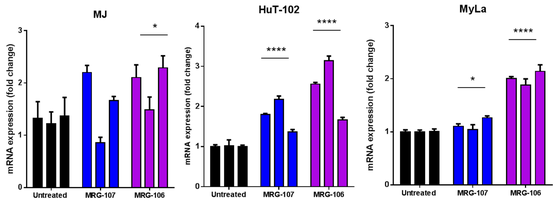


Figure 5.
- The indicated MF cell lines were treated with miR-155 inhibitors in the absence of agents to enhance cellular penetration such as cationic lipids or peptide conjugates (unfacilitated uptake).
- MRG-106 and MRG-107 are inhibitors to miR-155.
- After treatment for four days with the indicated compound, cells were harvested and RNA isolated. Expression of three direct gene targets was measured by real time PCR and normalized to the untreated cells' expression.
- MRG-106 appeared to have a greater magnitude of target de-repression compared to MRG-107

Inhibitor design can influence uptake properties

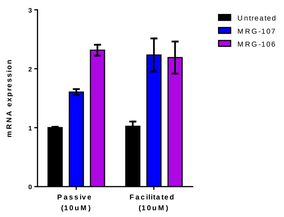


Figure 6.
- MRG-106 or MRG-107 was tested in cells with unfacilitated or facilitated uptake. Facilitated uptake was performed using Amaxa nucleofection.
- MRG-107 showed an approximately 1.5-fold change in direct target de-repression with passive uptake and a 2-fold change with nucleofection, suggesting that MRG-107 activity in the cell is limited with unfacilitated uptake.
- In contrast, MRG-106 showed similar activity with unfacilitated and facilitated uptake, suggesting that MRG-106 is optimized with regard to uptake.

Direct target gene regulation is specific for miR-155 inhibition

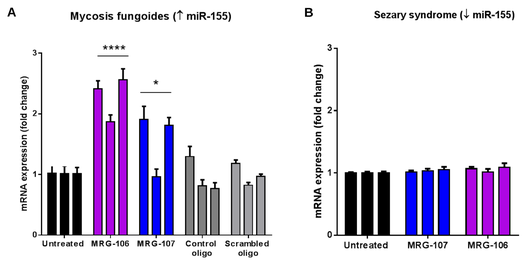


Figure 7.
- Panel A: The MJ mycosis fungoides cell line was treated with miR-155 inhibitors MRG-106 or MRG-107, or two control oligos. Target de-repression was only observed with miR-155 inhibitors, therefore demonstrating the specificity of the gene changes to the miR-155 inhibitor sequence.
- Panel B: A Sézary syndrome cell line does not show the gene expression changes measured in the MF cell lines, suggesting that the high miR-155 copy number may contribute to the measureable target de-repression.

Inhibition of miR-155 results in reduction in cell number and activation of programmed cell death

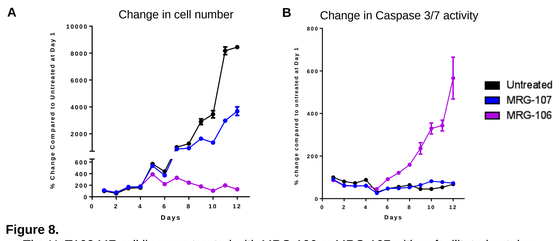


Figure 8.
- The HuT102 MF cell line was treated with MRG-106 or MRG-107 with unfacilitated uptake. Cells were removed daily for measurements of ATP and Caspase 3/7 activity.
- Panel A: Cellular ATP levels were measured as a surrogate for cell number. As cells are cultured in the presence of MRG-106, cell number did not increase over time, compared with untreated cells or MRG-107-treated cells.
- Panel B: Caspase 3/7 activity was measured. Cells treated with MRG-106 had higher enzymatic activity, demonstrating that treatment with MRG-106 activated programmed cell death. In comparison, untreated cells or MRG-107-treated cells did not show activation of Caspase 3/7.
- These results demonstrate that MRG-106 has a phenotypic effect on MF cells, and that these effects can provide therapeutic benefit.

Direct target regulation correlates with phenotypic changes

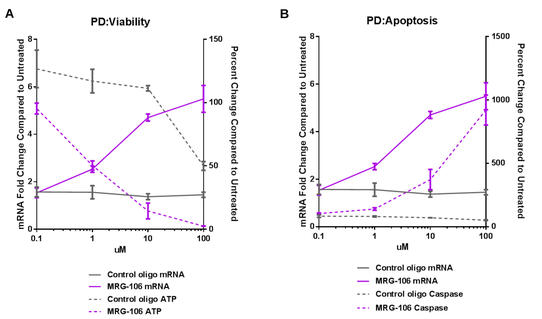
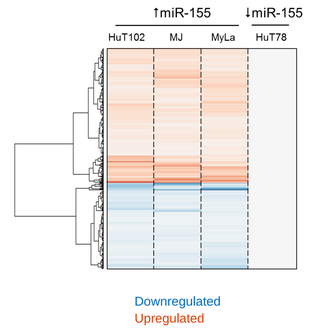


Figure 9.
- Panels A and B: Direct target changes in response to a dose-curve of MRG-106 or control oligo was plotted on the left y-axis (0.1, 1, 10, and 100 uM MRG-106). Cells were harvested for mRNA analysis after four days of MRG-106 treatment.
- Panel A: Cellular ATP levels were measured after eight days of MRG-106 or control oligo treatment over a dose-curve. The change in ATP levels relative to the untreated cells was graphed on the right y-axis.
- Panel B: Caspase 3/7 activity was measured after eight days of MRG-106 or control oligo treatment. The change in Caspase 3/7 activity relative to untreated cells was graphed on the right y-axis.
- These results demonstrate that increased target de-repression inversely correlates with a decrease in cell number, or directly correlates with activation of apoptosis. Therefore, gene expression changes in response to MRG-106 are predictive of cellular changes.

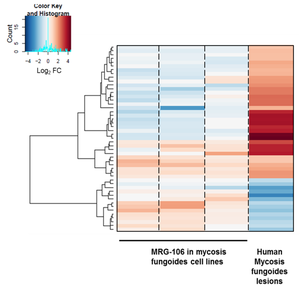
Microarray profiling identifies a translational pharmacodynamic gene signature of MRG-106 activity

Figure 10.
- RNA from three mycosis fungoides cell lines treated with MRG-106 was microarray profiled. 600 genes were identified to be significantly changed with MRG-106 treatment in all three cell lines.
- The heat map showed that the signature contains both up- and downregulated transcripts.
- Microarray profiling of the Sézary syndrome cell line treated with MRG-106 did not result in changes in any of the gene identified in the MF cell lines
- This gene signature contains miR-155. direct targets, genes associated with apoptosis and cell cycle, therefore confirming the on-target mechanism and cellular phenotype consequences of inhibiting miR-155.



Gene signature of MRG-106 activity normalizes gene expression changes in mycosis fungoides lesions

Figure 11.
- Transcripts published to be changed when comparing microarray profiling data of myosis fungoides lesions to normal skin are shown in the fourth column of the heat map (Ref. 8).
- The changes in three mycosis fungoides cells lines in response to MRG-106 was shown in the first three columns of the heat map.
- It is visually apparent that the gene signature associated with MF skin biopsies is reversed in response to MRG-106 in the cell lines, suggesting that miR-155 inhibition may reverse the pathologic gene signature and restore homeostasis..



MRG106-11-101: Phase 1 study in patients with mycosis fungoides

Two part study:
- *Part A - Intra-tumoral (local) delivery of MRG-106 to maximize local dosing*
- *Part B - Systemic subcutaneous delivery to determine maximum tolerated dose*

Objectives:
- *Primary:*
  - Investigate the safety and tolerability of multiple intratumoral or subcutaneous injections of MRG-106
- *Secondary:*
  - Characterize the pharmacokinetic profile of MRG-106
- *Exploratory:*
  - Pharmacodynamic profile of MRG-106 in skin
  - Cellular changes in lesion biopsy
  - Visual changes in lesion morphology

Currently recruiting.
For more information, please go to www.clinicaltrials.gov

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Disclosures: All the authors are employed by and have equity ownership in miRagen Therapeutics, Inc.