Preliminary Results of a Phase 1 Trial Evaluating MRG-106, a Synthetic microRNA Antagonist (LNA antimiR) of microRNA-155, in Patients with CTCL

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Abstract

Introduction and Objectives:

microRNAs are small, non-coding RNAs that regulate expression of multiple genes which impact physiological processes and cellular phenotypes. miR-155-5p is a well-described onco-miR with a strong mechanistic link to cutaneous T-cell lymphoma (CTCL). A LNA-modified oligonucleotide inhibitor of miR-155-5p, MRG-106, was selected based on its ability to de-repress canonical miR-155-5p targets in multiple mycosis fungoides (MF) cell lines in vitro. In preclinical models, MRG-106 showed significant pharmacodynamic activity without requiring additional formulation. The objective of this first-in-human study is to evaluate the safety, tolerability, pharmacokinetics and preliminary efficacy of MRG-106 in patients with mycosis fungoides (MF).

Methodology:

This Phase 1 trial employs a dose-escalation design to evaluate both intratumoral and subcutaneous administration of MRG-106 at doses of 75 mg and up to 900 mg per injection, respectively. Patients were required to be ≥ 18 years old, have a confirmed diagnosis of MF, be clinical stage I-III with plaques or tumors, be on a stable treatment regimen or without any concomitant therapy for MF, and have no other major illness. The first 6 patients were dosed with four or five 75 mg intratumoral injections of MRG-106 over 2 weeks. In addition, 4 patients received saline injections in a second lesion on the same schedule. Skin biopsies were taken from MRG-106 and saline treated lesions for molecular, bioanalytical, and histological analyses, before the first dose and after the last dose.

Results:

Six patients (5M/1F, median age 61 years, 5 Caucasian/ 1 African-American) were dosed intratumorally. All tolerated the administrations well with only minimal injection site reactions noted in three patients. One patient was discontinued from the trial due to rapid progression of disease, which was considered not related to the study drug. There were no clinically significant adverse events or laboratory abnormalities. To date, the first cohort of 6 patients has either completed the dosing period (5 patients) or discontinued due to progressive disease (1 patient).

All patients showed a reduction in the baseline Composite Assessment of Index Lesion Severity (CAILS) score in both MRG-106-treated and saline-treated lesions. The maximal reduction was on average 55% [range: 33% to 77%] in the MRG-106 treated lesion and 43% [range: 22% to 75%] in the saline treated lesions. In all the subjects that completed dosing, the MRG-106 treated lesions had a CAILS score reduction of ≥ 50% which was maintained to the end of study; in contrast, a ≥ 50% reduction was observed in only one saline treated lesion. Most patients noted a marked decrease in systemic pruritus. Histological examination of pre-treatment and post-treatment biopsies of the same lesion injected with MRG-106 from five evaluable patients revealed that one patient had a complete loss of the neoplastic infiltrate, two patients had a reduction in neoplastic cell infiltrate density and depth, one patient had fewer CD30+ large atypical cells, and one patient demonstrated no change.

After the first dose, MRG-106 had a median $t_{1/2}$ in plasma of 4.8 hours and a mean C_{max} of 1.2 µg/mL. The drug was detectable 24 hours after the last dose in the MRG-106-injected lesions that were biopsied. Gene expression analysis of the pre- and post-treatment biopsies showed transcript changes consistent with the expected mechanism of action of MRG-106.

Conclusions:

These promising preliminary results in this first-in-human study in 6 MF patients show that intratumoral injection of MRG-106 was well-tolerated, and demonstrated encouraging therapeutic improvements in cutaneous lesions, based on CAILS scores and histological findings. In addition, reductions in CAILS scores in other lesions as well as decreases in systemic symptoms such as pruritus were observed. Preliminary biomarker analysis indicates that MRG-106 induces transcriptional changes consistent with on-target activity and molecular proof of concept. The trial is ongoing.

Study Objectives and Design

Primary Objective:

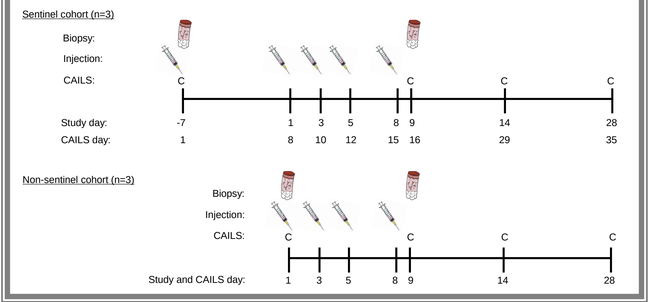
The primary objective of the study is to investigate the safety and tolerability of multiple intratumoral (Part A) and subcutaneous (Part B) injections of MRG-106 in patients with CTCL, MF sub-type.

Secondary Objectives:

- Characterize the pharmacokinetic profile of MRG-106

Exploratory Objectives:

- Characterize the pharmacodynamic (PD) profile of MRG-106
- Evaluate changes in pathohistology of biopsied tissue
- Determine the effect of MRG-106 on skin assessment scores using the Composite Assessment of Index Lesion Severity (CAILS) and the modified Severity Weighted Assessment Tool (mSWAT) [mSWAT for Part B patients only]
- Investigate the effects of MRG-106 on immunity, in Part B



Demographic and Clinical Characteristics of Patients Who Received One or More Doses of MRG-106

Characteristic	MRG-106-treated (n = 6)
Age, years Median (Range)	61 (50-64)
Sex Male	5
Female	1
Race White	4
African American- Hispanic	1
	1
Stage at screening IB	1
IIA	2
IIB	3
Prior systemic treatments Median (range)	3 (1-6)
Reported concomitant therapies Topical	1 (0-2)
Systemic	1 (0-1)

All Adverse Events

Patient	AE	Start (Study day)	Stop (Study day)	Grade	Relatedness
102-003	Pain during injection	Day 1	Day 6	1	Definitely
102-003	Burning sensation during injection	Day 1	Day 6	1	Definitely
110-001	Tingling at injection site	Day 1	Day 8	1	Definitely
101-001	Pruritus	Day 12	Day 27	2	Possibly
101-001	Sore on hand	Day 13	Day 27	1	Possibly
101-001	Pruritus	Day 36	UNK	2	Possibly
102-001	Erythema	Day 2	Day 6	1	Possibly
102-001	Skin inflammation	Day 2	Day 9	1	Possibly
105-001	Nausea	Day 1	Day 9	1	Possibly
101-001	WBC Decreased	Day 29	UNK	2	Possibly
110-001	Neutropenia	Day 2	Day 6	2	Possibly
110-001	Prolonged PTT	Day 2	Day 6	2	Possibly
110-001	Elevated CK	Days 8	UNK	3	Not related
102-001	Bruising (saline injection site)	Day 1	Day 6	1	Not related
102-001	Pruritus	Day 6	Day 8	1	Not related
102-001	Dry skin	Day 8	Day 9	1	Not related
107-001	Orthopnea (SAE)	Day 16	Day 20	1	Not related
107-001	Cellulitis (SAE)	Day 16	Day 30	3	Not related
107-001	DVT	Day 9	UNK	2	Not related
107-001	Hypercalcemia (SAE)	Day -7 (pre-dose)	Day 7	4	Not related
107-001	Hypophosphatemia	Day 7	Day 16	1	Not related
105-001	AST increased*	Day 3	Day 7	1	Not related
105-001	AST increased*	Day 9	Day 15	1	Not related
105-001	Hyperglycemia	Day 1	UNK	1	Not related

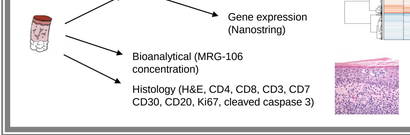
*elevated ALT and AST (HEX) at Baseline

Adverse Events Occurring at the Injection Site

Patient	AE	Start (Study day)	Stop (Study day)	Grade	Relatedness
102-003	Pain during injection	Day 1	Day 6	1	Definitely
102-003	Burning sensation during injection	Day 1	Day 6	1	Definitely
110-001	Tingling at injection site	Day 1	Day 8	1	Definitely
102-001	Erythema	Day 2	Day 6	1	Possibly
102-001	Skin inflammation	Day 2	Day 9	1	Possibly
102-001	Bruising (saline injection site)	Day 1	Day 5	1	Not related

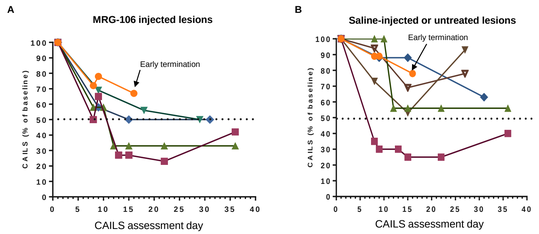
Injection site reactions are common for oligonucleotide therapies, occurring in over 70% of clinical trial subjects for most oligonucleotides (van Meer et al., 2016). Because of the known class effect, the adverse events occurring at the injection site are separately listed in this table for ease of review. Of note, this data represents updated findings that were not available at the time of abstract submission.

Exploratory Analyses of Mycosis Fungoides Lesion Biopsies



Preliminary Efficacy of Intratumoral Injection of MRG-106

Patient	# of Doses	Study Days with Drug Injection	MRG-106 Treated: CAILS Score (Max/Min)	MRG-106 Treated: Maximal % Reduction in CAILS	Saline or Untreated: CAILS Score (Max/Min)	Saline or Untreated: Maximal % Reduction in CAILS
107-001	3	-7, 1, 2	18 → 12	33%	18 → 14	22%
102-001	5	-7, 1, 3, 5, 8	26 → 6	77%	20 → 5	75%
101-001	5	-7, 1, 3, 5, 8	12 → 4	67%	9 → 5	44%
105-001	4	1, 3, 5, 8	16 → 8	50%	Not Done	-
102-003	4	1, 3, 5, 8	12 → 6	50%	8 → 5	37%
110-001 (1)	4	1, 3, 5, 8	Not Done	-	15 → 8	47%
110-001 (2)	4	1, 3, 5, 8	Not Done	-	36 → 25	31%

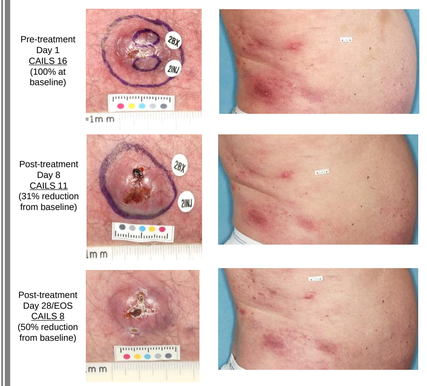


The individual lesion Composite Assessment of Index Lesion Severity (CAILS) score was obtained by adding the severity score of each of the following categories: erythema, scaling, plaque elevation, and surface area. The maximum score achievable is 50. The maximum (baseline) CAILS score and the minimum score recorded for each monitored lesion, as well as the calculated maximum percentage change, is shown. The change over time in CAILS scores (normalized to 100% at baseline) is presented graphically. Panel A shows the scores for MRG-106-injected lesions and Panel B shows the score for the saline-injected or un-injected lesions.

All lesions showed improvement in the CAILS score. A reduction in CAILS score of ≥ 50% (consistent with the definition of clinical response (Olsen et al., 2011)) was observed in the MRG-106 treated lesions in all four evaluable subjects who completed dosing. In contrast, a ≥ 50% reduction was observed in only one saline treated lesion. The MRG-106 injected lesion responses were maintained to the End of Study visit (either 28 days or 35 days after the first dose). The full duration of response was not formally measured in this Phase 1 safety study.

Photographic Example of Clinical Response for a Patient on Study

50-year old male patient (ID 105-001) with stage IIB mycosis fungoides showed improvement in skin lesions after four intratumoral injections of MRG-106.



Pharmacokinetic Characterics of Intratumoral Injection of MRG-106

Cohort	Patient #	T_{max} (h)	C_{max} (µg/mL)	$t_{1/2}$ (h)	AUC_{inf} (µg*hr/mL)	Cl/F (L/h)
Sentinel	101-001	1	1.15	4.84	11.2	6.71
	102-001	1	0.721	4.12	5.88	12.8
	107-001	0.5	2.28	4.29	7.63	9.83
	Mean	N/A*	1.38	4.41	8.23	9.77
	SD	N/A	0.805	0.378	2.7	3.02
Non-sentinel	102-003	0.5	1.95	5.42	7.79	9.63
	105-001	1	0.562	UND†	UND	UND
	110-001	0.5	0.782	10.7	5.08	14.8
	Mean	N/A	1.1	8.06	6.44	12.2
	SD	N/A	0.746	3.73	1.91	3.62

* Not calculated for categorical variables
† UND= Undetermined due to undefined terminal elimination rate constant

Noncompartmental PK parameter estimates after the first intratumoral (IT) dose of MRG-106, out to 24-hours post dose, show quick systemic uptake from the tumor tissue with a T_{max} of 0.5-1 hour, the median half-life measurement was 4.8 hours and a mean C_{max} of 1.2 µg/mL for all patients. Plasma clearance is biphasic with a short, initial distribution phase half-life (0-24 hours), followed by a longer elimination phase half-life of days to weeks as compound is cleared from tissues and returns to the plasma for clearance (data not shown). This distribution and elimination profile is consistent with what is known for heavily modified single-strand oligonucleotide therapeutics. Observed T_{max} and half-life values are consistent to what was measured in subcutaneously dosed non-human primate GLP-toxicology studies.

Histological Findings and Changes in Pruritus after 8 or 15 days of MRG-106 Treatment

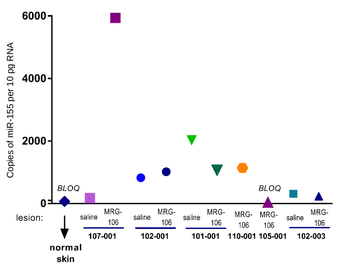
Pt ID:	102-001		101-001		110-001		105-001		102-003	
Bx type:	Baseline	Post-tx	Baseline	Post-tx	Baseline	Post-tx	Baseline	Post-tx	Baseline	Post-tx
Lesion type and immuno-phenotype	Plaque, CD3+ CD4+ CD8- CD7- CD20		Plaque CD3+ CD4- CD8- CD7- CD20-		Plaque CD3+ CD4+ CD8- CD7- CD20- CD30- (minor subset +)		Tumor CD3+ CD4- CD8- CD7 (+ on minor subset) CD20-		Plaque CD3+ CD4+ CD8- CD30+ (5-10%), CD7 (focal)	Slightly reduced CD30+ subset (~5%)
CD4:CD8 ratio	10:1	10:1	2:1 (tumor CD4 negative)	2:1 (tumor CD4 negative)	10:1	8:1	8:1 (tumor CD4 negative)	5:1 (tumor CD4 negative)	5-8:1	5-8:1
Tumor cells (% of lymphoid cells)	40-50%	20-30%	60-70%	50-60%	80-90%	80%	50-60%	0%	70-80%	70-80%
Ki67+	~5%	< 5%	10-15%	10-15%	~5%	10-15%	5-10%	< 5%	5-10%	~5%
Cleaved caspase 3	Rare cells positive	Rare cells positive	Negative	Negative	Negative	Rare cells positive	Negative	Rare cells positive	Rare cells positive	Very rare cells pos.
Infiltrate density	Very dense, confluent infiltrate	Dense infiltrate	Mild (dermis); Moderate to marked (epidermis)	Mild (dermis); Moderate (epidermis)	Mild to moderate	Mild to moderate	Moderate to marked (dermis); Mild (epidermis)	Mild	Mild to moderate	Mild to moderate

Patient ID#	102-001	101-001	110-001	105-001	102-003
Pruritus (anecdotal)	No change reported	Reduced	Reduced	Reduced	No change reported

Baseline and post-treatment biopsies of the MRG-106-injected lesion were taken from 5 of 6 subjects. H&E and immunohistochemical staining for CD4, CD8, CD7, CD3, CD20, Ki67, and cleaved caspase 3 was performed followed by interpretation by a blinded hematopathologist.

Anecdotal improvements in pruritus were reported for three of the five patients who completed dosing.

miR-155 Copy Number in Baseline Lesion Biopsies



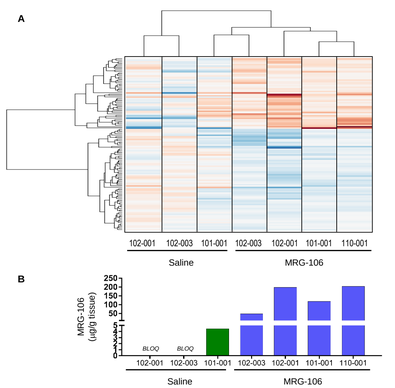
miR-155 was quantitated by qPCR calibrated against a standard curve. The levels of miR-155 in baseline biopsies varied from below limit of quantitation (patient 105-001) to 5936 copies/10 pg RNA (patient 107-001). miR-155 in normal skin is typically below the limit of quantitation of the assay.

Gene Expression Changes Common to Mycosis Fungoides Lesion Biopsies with Intratumoral Injection of MRG-106

MRG-106 treatment of three mycosis fungoides cell lines previously identified 587 transcripts with changed expression levels compared to untreated cells (Seto et al. 2015). 122 of these 587 genes were found to be consistently up-regulated or consistently down-regulated in 4 of the 5 patients' biopsies collected after treatment with MRG-106.

Panel A shows the heat map of the fold-change of 122 genes in MRG-106 treated lesion biopsies normalized to the pre-treatment biopsy taken from the same lesion. Red indicates transcripts that were up-regulated and blue indicates transcripts that were down-regulated with MRG-106 treatment. Two-way hierarchical clustering was performed on the signatures, revealing two clusters of samples: The first cluster represents the gene expression of lesions injected with saline, whereas the second cluster represents the expression in all lesions injected with MRG-106 and one saline-injected lesion.

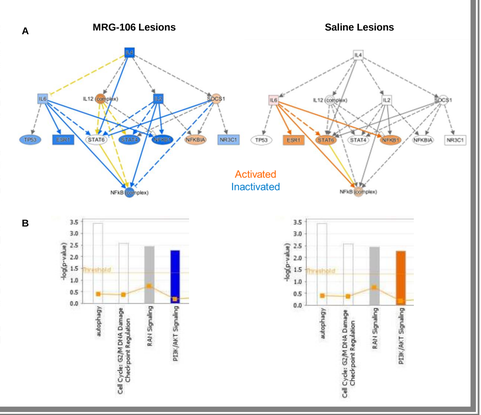
Panel B shows a bar graph of the MRG-106 quantification in the biopsies, as µg of MRG-106 per gram of tissue. One saline-treated biopsy demonstrated a gene signature that showed some similarity to the MRG-106 common signature, consistent with the detectable amount of MRG-106 in the tissue (101-001, green bar). This could suggest distal distribution of the drug from the MRG-106-treated lesion.



MRG-106 Treatment Inactivates the STAT, NFκB, and PI3K/AKT Pathways

Ingenuity Pathway Analysis (IPA) identified the biological context of the gene expression analysis. IPA analysis of the common signature that was changed with MRG-106 treatment in four biopsies were associated with inactivation of the STAT, NFκB, and PI3K/AKT pathways, consistent with the mechanism of miR-155 inhibition (Panel A, left). In contrast, these pathways are activated in the saline-treated lesions (Panel A, right).

IPA's Canonical Pathway Analysis was utilized to identify pathways enriched in the common set of genes regulated by MRG-106. Shown in Panel B are the top four pathways enriched in the common gene signature, according to p-value (the higher number on the bar graph indicates greater enrichment; the threshold line indicates the p-value threshold above which enrichment is considered significant). Of these four pathways, the PI3K/AKT pathway is predicted to be inhibited (blue color) following MRG-106 treatment, based on the differential regulation of the genes associated with this pathway. In contrast, the PI3K/AKT pathway is predicted to be activated (orange color) in saline-treated lesions. Additionally, the gene signature in the MRG-106 treated lesions reflected increased cell death (not shown).



Conclusions

- Intratumoral injection of MRG-106 was well-tolerated with generally minor injection site reactions.
- Intratumoral injection of MRG-106 led to encouraging therapeutic improvements in cutaneous lesions, based on CAILS scores and histological findings.
- Intratumoral injection of MRG-106 reduced CAILS scores in lesions not injected with MRG-106 as well as anecdotal decrease in pruritus.
- After the first dose, MRG-106 had a median half-life in plasma of 4.8 hours, and a mean C_{max} of 1.2 µg/mL for all patients treated in Part A.
- MRG-106 was detectable 24 hours after the last dose in the biopsies of MRG-106-injected lesions.
- A common gene signature in four MRG-106-injected lesion biopsies was identified. The signature was enriched for factors in the STAT, NFκB, and PI3K/AKT pathways.
- Assessment of higher doses and different routes of administration are on-going.

References

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Olsen et al. (2011) *J. Clin. Oncol.* Clinical end points and response criteria in mycosis fungoides and Sézary syndrome: a consensus statement of the International Society for Cutaneous Lymphomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer.

Seto et al. (2015) ASH abstract: Preclinical Results Supporting Therapeutic Development of MRG-106, an Oligonucleotide Inhibitor of miR-155, in CTCL.

ClinicalTrials.gov Identifier: NCT02580552

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