Phase I Trial of Cobomarsen, a MiR-155 Inhibitor, in Patients with Aggressive HTLV-1 Associated ATLL: Disease Stabilization and Biomarker Analysis

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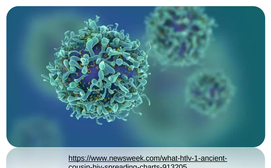
BACKGROUND

Cobomarsen (MRG-106) miR-155-5p Inhibitor

- Cobomarsen is a chemically synthesized, phosphorothioate oligonucleotide, 14 nucleotides in length, that contains a mixture of deoxyribonucleotides and 2'-O, 4'-C-methylene-β-d-ribonucleotides (LNA).
- Genome-wide expression analysis demonstrates that cobomarsen regulates numerous genes implicated in cell cycle and apoptosis, consistent with the pharmacologic impact on cell survival.
- A subset of these genes has been identified as potentially translatable biomarkers to monitor cobomarsen activity in clinical samples.

ATLL Epidemiology

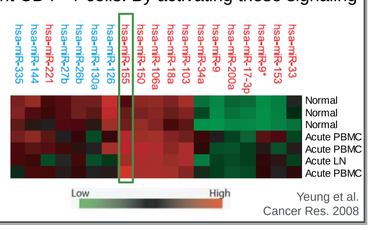


https://www.newsweek.com/what-htlv-1-ancient-cousin-hiv-spreading-charts-913205

- Adult T-cell leukemia/lymphoma (ATLL) is a mature, peripheral T-cell neoplasm caused by human T-cell leukemia virus type 1 (HTLV-1).[1]
- HTLV-1 establishes lifelong latency in human T cells (20-40 years). [2]
- Malignant transformation leading to ATLL occurs in HTLV-1–infected individuals with a cumulative lifetime risk of 2.1% for women and 6.6% for men. [2]

Role of MicroRNA-155 in ATLL

- Increased expression of miR-155-5p enhances the growth of HTLV-1 infected T cells, suggesting an important role for miR-155-5p in the pathogenesis of ATLL.[3]
- Tax promotes the activation of multiple survival pathways, including NFκB and JAK/STAT in virally infected malignant CD4+ T cells. By activating these signaling pathways, Tax promotes the binding of transcription factors, such as NFκB and AP-1, in the promoter region of miR-155-5p, thereby increasing the transcription of this onco-miR.[4]
- miR-155 is upregulated in leukemic tumor cells from patients with HTLV-1-associated ATLL.[5]



Yeung et al. Cancer Res. 2008

PRECLINICAL PHARMACOLOGY

Cobomarsen Reduces Proliferation and Induces Apoptosis in a Dose-Dependent Manner in an ATLL Cell Line

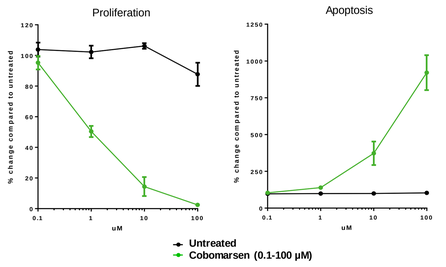


Figure 1. HTLV-1 positive HuT102 cells were grown in medium containing varying concentrations of cobomarsen, ranging from 0.1 to 100 μM. Cell number (based on ATP measurement) and caspase 3/7 activity were measured after 8 days of exposure to cobomarsen. Day 8 was selected from previous time course experiments. Caspase activity was normalized to ATP levels, as all cells have a low basal level of caspase activity. All data was normalized to the signal of untreated cells collected at the same timepoint.

- The HuT102 T-cell lymphoma cell line has been reported to be HTLV-1 positive.[6] miRagen has demonstrated that miR-155 expression is overexpressed in this cell line and that cobomarsen inhibits cellular proliferation and induces apoptosis after 8 days of treatment *in vitro.* Cobomarsen treatment induces a gene expression signature in HuT102 cell lines including direct and downstream targets of miR-155, demonstrating target engagement and mechanism of action.[7]

CLINICAL TRIAL DESIGN

- Open-label, dose-ranging, multiple dose study of SC or IV administration of cobomarsen in subjects with HTLV-1 associated ATLL, acute, lymphomatous, chronic, and smouldering subtypes.
- Subjects received 3 loading doses the first week of Cycle 1 followed by weekly dosing until subject becomes intolerant, develops clinically significant side effects, progresses, or the trial is terminated.
- Primary objective is to characterize safety and tolerability of cobomarsen in the ATLL population. Secondary and exploratory outcomes included PK, clinical efficacy and effect on biomarkers of cell proliferation and activation.

Primary Endpoint

- Safety and tolerability as assessed by incidence, severity and dose-relationship of AEs and changes in ECG and laboratory parameters by dose.

Secondary Endpoint

- Phase 2 Dose identification based on risk/benefit analysis.
- Main efficacy endpoints:
  -PFS, CR, PR, OR, OS

Exploratory Endpoints

- Pharmacokinetic profile.
- Changes in biomarkers of drug activity in cell proliferation and cell activation.
- Changes in peripheral blood immune cells.
- Changes in HTLV-1 pro-viral load.

Main Inclusion Criteria

- ≥ 18 years of age.
- ECOG performance status of ≤ 2.
- Confirmed diagnosis of ATLL (any stage of disease).
- Intolerant, relapsed or refractory ATLL to at least one prior therapy.

Main Exclusion Criteria

- Candidates for any therapy known to provide clinical benefit.
- Eligibility for hematopoietic stem cell transplant.

HISTORICAL COHORT

- A retrospective historical cohort was established based on a literature search of papers which reported median survival time (MST) from diagnosis and PFS.
- PubMed was searched from 12 April 2009 - 12 April 2019 for reports of human clinical trials with the terms “Adult T-Cell Leukemia/Lymphoma” OR “Adult T Cell Leukemia-Lymphoma” OR “Adult T-Cell Leukemia OR ATL OR ATLL”.
- A total of 556 papers were identified and screened by title, abstract and full text and were excluded if they were reviews, reported only in vitro data, were case reports or letters to the editor, or did not report any survival data.
- A total of 16 papers from Japan, USA and Europe were included.

DEMOGRAPHICS

- 15 patients with aggressive ATLL at diagnosis (10 lymphomatous, 4 acute, and 1 chronic unfavorable) have received at least 3 doses of cobomarsen.
- Cobomarsen was administered at 600 mg (n=8), 900 mg (n=4) or 1200 mg (n=3) doses.
- Of the 15 patients in the study, 9 were actively relapsing and 6 had received chemotherapy or other prior therapy and had a PR or CR, but with minimum residual disease (RD) at time of screening.
- RD was determined by either measurable circulating ATL cells in blood by flow cytometry or residual abnormal lymph nodes on CT scan.

Table 1. Subject Demographics

Demographic	600 mg (8)	900 mg (4)	1200 mg (3)	Total (15)
Sex				
Male	5 (63%)	2 (50%)	0 (0%)	7 (47%)
Age				
Mean (SD)	52 (10)	60 (12)	56 (5)	55 (10)
Race				
Black	7 (88%)	4 (100%)	3 (100%)	14 (93%)
Not reported	1 (11%)	0 (0%)	0 (0%)	1 (7%)
ATLL Subtype				
Acute	3 (38%)	1 (25%)	0 (0%)	4 (27%)
Lymphomatous	4 (50%)	3 (75%)	3 (100%)	10 (67%)
Smoldering	1 (13%)	0 (0%)	0 (0%)	1 (7%)
Prior Systemic Therapies per Subject				
Mean (SD)	7 (3)	10 (3)	9 (10)	8 (5)
Prior Skin Directed Therapies per Subject				
Mean (SD)	3 (2)	0 (0)	1 (0)	3 (2)

Data Cutoff: 17 October 2019

SAFETY

- There were 196 reported AEs at the time of data cut off with the majority being mild or moderate in severity
- Of the 196 reported AEs, only 43 were considered ‘possibly related’ by the Investigator.
- The most common AEs, reported in > 20% of all ATLL patients were diarrhea, nausea, vomiting, pruritus, fatigue, upper respiratory infection, cough and some laboratory abnormalities, including increased AST, ALT, hypercalcemia and anemia.
- 14% of the AEs (occurring in 8 patients) were Grade 3 or 4 and most resolved within 11 days.
- 2 of these (1.0%), occurring in the same patient, were possibly related to cobomarsen and considered serious (SAE). The SAEs (localized edema and dermatitis exfoliative generalized) resolved and the patient discontinued study treatment and has had no further events reported.
- There were 9 other SAEs reported, all judged related to the underlying disease.
- There have been no drug-related fatalities and no SAEs in patients treated with the 600 mg or 900 mg doses.

BASELINE DISEASE CHARACTERISTICS AND RESPONSE IN PATIENTS WITH RESIDUAL DISEASE

- 6 patients had measurable residual disease at screening including circulating ATL cells in 5 patients (4 with ≥ 5% ATL cells and 1 with ~3% ATL cells), 2 of which also had measurable node involvement, and 1 with only residual node involvement.
- Studies of prognostic factors in ATLL have shown that the initial response to treatment along with the expression of Ki-67 and CCR7 on ATLL cells is associated with aggressive disease and has negative prognostic significance.[8,9] Therefore these prognostic factors were assessed in RD patients. For cell markers, patients were considered positive for negative prognostic factors if the circulating ATL cells expressed > 20% Ki-67+ or ≥ 30% CCR7+. All patients had at least one negative prognostic factor at baseline.
- The median duration of treatment with cobomarsen in this group of patients was 384 days (range 115 days - 692 days), with 3 patients still on treatment at the time of data analysis (October 17, 2019).
- The median PFS from Day 1 until progression or data cut off (October 17, 2019) was 12.6 months (range 4.6 months – 24.5 months).
- The MST from diagnosis until death or data cut off was 26.0 months (range 13 months - 76 months).

Table 2. Subject baseline disease characteristics and duration of cobomarsen treatment

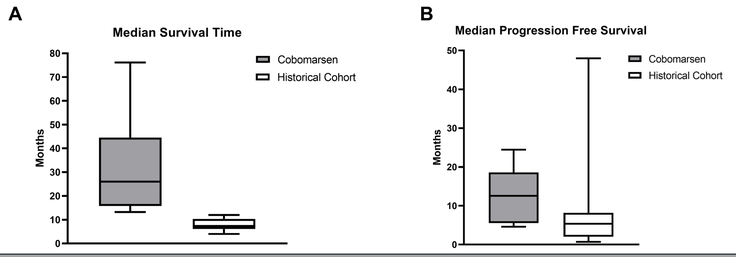
Subject	Residual Compartment at Screening	Prior Therapies	Duration of Study Participation Days (Months)	Survival from Diagnosis (Months)	PFS on Cobomarsen (Months)	Prognostic Factors (PF)		
						Initial response to Tx	Biomarkers at Baseline	# of negative PF
101-008[a]	Blood	Interferon α2b; Zidovudine, Lenalidomide; EPOCH	692 (23)	34	24.5[b]	PR	57% CCR7 35% Ki-67+	3
101-010	Lymph nodes and blood	CHOPE	520 (17)	30	16.6	PR	27% CCR7 16% Ki-67+	1
101-012	Blood	CHOPE	301 (10)	22	9.1[c]	CR	36% CCR7 23% Ki-67+	2
101-014	Blood	CHOPE; Radiation	154 (5.1)	17	4.6	CR	18% CCR7 21% Ki-67+	1
117-001[a]	Lymph nodes and blood	EPOCH; Brentuximab	172 (5.7)	13	5.9	PR	36% CCR7 25% Ki-67+	3
119-001[a]	Lymph nodes	AZT; VCAP+VECP+AP; Gemcitabine + Oxyplatin; Mogamulizumab; Valproic Acid + AZT; Pralatrexate; Alemtuzumab; AZT + VPA	467 (15)	76	16.0	PD	36% CCR7[d]	1
Median			**384 (12.6)**	**26.0**	**12.5**			

Abbreviations: PFS= progression free survival; CR = complete response; PR= partial response; PD = progressive disease; Tx=treatment
[a] Patient is still on study at time of data cut off (October 17, 2019)
[b] Per Tsukasaki criteria[10], patient progressed in the blood via flow cytometry which showed circulating ATLL cells rose from 540/uL to 1334/uL. Imaging studies showed no nodal disease or splenomegaly. The PI considers the patient to be clinically stable and the patient has continued treatment. PFS is calculated based on the data cutoff date (not date of progression) because the patient remains on the study drug and is considered by the PI to have stable disease
[c] This patient had new pulmonary lesions by CT scan after 6 months in the study. The patient remained on cobomarsen monotherapy for 2 more months, when they were taken off cobomarsen due to disease progression
[d] Baseline CCR7+ value was collected on Day 27 and baseline Ki-67+ was not collected

MST AND PFS in RD COBOMARSEN TREATED PATIENTS VS. HISTORICAL COHORT

- The MST for all the aggressive ATLL patients (n = 12 papers) was 7.4 months (range 4 months – 12 months).
- When assessing the acute and lymphomatous subtypes separately (n = 8 papers), the MST was 6.8 months (range 2 months – 9.9 months) and 10.4 months (range 8.6 months – 15 months), respectively.
- The median PFS was 5.4 months (range 0.7 months – 48 months). The wide range is affected by inclusion of a cohort of 16 acute ATLL patients that obtained a CR after first line treatment with AZT/IFN and had a PFS of 48 months. Excluding this cohort of patients, the median PFS is 4.7 months (range 0.7 months – 11 months).
- When the 6 RD patients treated with cobomarsen were compared with the historical cohort, the cobomarsen treated patients had a longer MST (26 months vs. 7.4 months) and median PFS (12.5 months vs. 5.5 months).

Figure 1. Median Survival Time and Median Progression Free Survival of Cobomarsen Treated Patients with Residual Disease compared to Historical Cohort



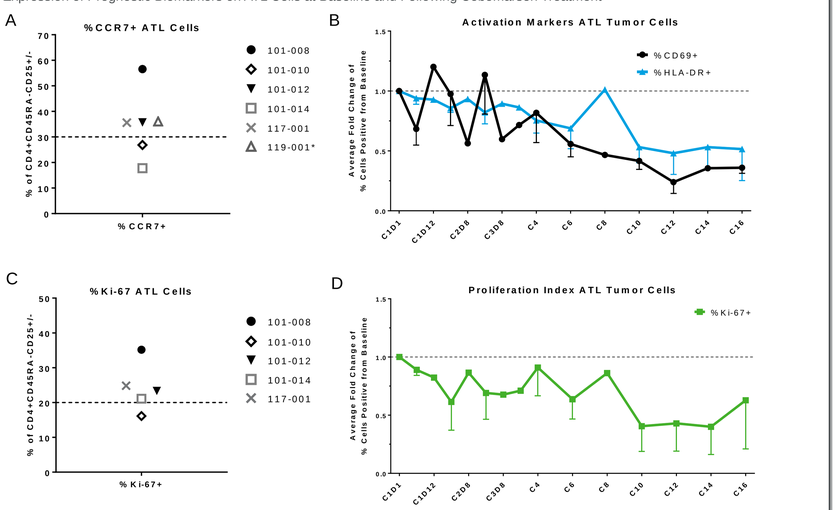
COBOMARSEN EFFECT ON PROGNOSTIC BIOMARKERS

- Prognostic biomarker data is only available for 5 of 6 patients, as the chronic unfavorable patient did not have baseline immunophenotyping results.
- Three (3) of the 5 evaluable patients had ≥ 30% of abnormal cells expressing CCR7.
- Four (4) of the 5 evaluable patients had > 20% of the circulating abnormal cells staining positive for Ki-67.

Cobomarsen’s Effect in Actively Proliferating ATL Cells (Ki-67)

- Following cobomarsen treatment, the percentage of actively proliferating ATL cells (as measured by Ki 67+ staining) decreased in all 5 evaluable patients.
- The expression of CD69 and HLA-DR on ATL cells decreased in all 5 of the evaluable RD patients and was maintained in 2 patients for at least 16 months.
- There was no significant change in CCR7 expression on ATL cells, except at the time of progression.

Figure 2: Expression of Prognostic Biomarkers on ATL Cells at Baseline and Following Cobomarsen Treatment



A) CCR7+ ATL cells quantitated at baseline prior to cobomarsen treatment. *The percentage of CCR7+ cells was assessed for Subject 119-001 at Day 27 post cobomarsen treatment and should not be affected by cobomarsen treatment. The Day 1 baseline sample was not collected for subject 119-001, so this subject was excluded in the data analysis for panels B-D. B) Average fold change (±SEM) from the pretreatment time point (Day 1) in the percentage of ATL cells expressing CD69 and HLA-DR activation markers. C) Ki-67+ proliferating ATL cells quantitated at baseline prior to cobomarsen treatment. D) Average fold change (±SEM) from the pretreatment time point (Day 1) in the percentage of ATL cells expressing Ki-67.

BIOMARKERS CORRELATE WITH CLINICAL STABILIZATION AND DISEASE PROGRESSION

- After 18 months of treatment, patient 101-010 started to relapse after a long period of stabilization, and discontinued treatment.
- At the time of discontinuation, the patient had an increase in Ki-67+ and CD69+ ATL cells in the periphery (Figure 3b and 3c).
- Notably, the increase in the proliferation and activation markers on the ATL cells correlated with a new population of ATL cells with increased expression of CCR7, while losing expression of both CD127 and CD25, consistent with the phenotype of aggressive ATL cells[9] (Figure 3a).
- These data demonstrate that the proliferation and activation markers, Ki-67 and CD69, correlated with clinical disease stabilization in patients during cobomarsen treatment and with disease progression.

Figure 3: Immunophenotype of ATL Cells over the Course of Cobomarsen Treatment for Subject 101-010

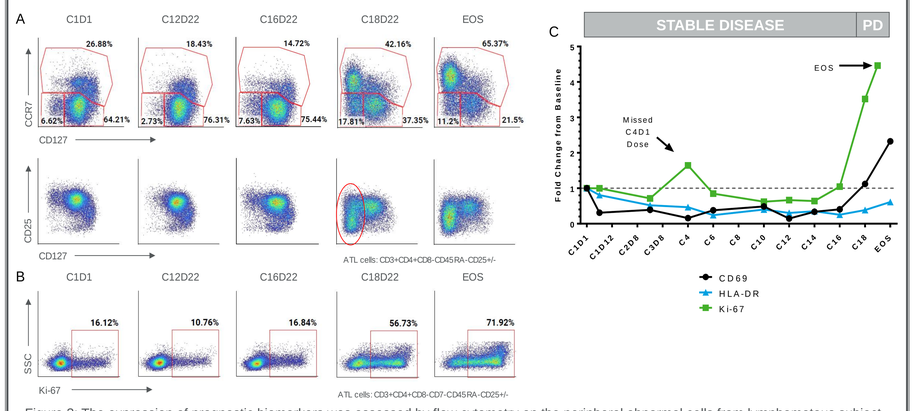


Figure 3: The expression of prognostic biomarkers was assessed by flow cytometry on the peripheral abnormal cells from lymphomatous subject 101-010 over the course of cobomarsen treatment. ATL cells were gated as CD3+ CD4+ CD8- CD45RA- CD25+/-. A) The percentage of ATL cells positive for CCR7, CD127 and CD25. The red oval shows the new aggressive ATL cell population that has lost expression of both CD127 and CD25. B) Ki-67+ ATL cells over the course of treatment and at the End of Study (EOS) visit. C) Fold change from the pretreatment time point (Cycle # and Day # [C#D#]) in the percentage of ATL cells expressing CD69 (black), HLA-DR (blue) and Ki-67 (green) quantitated over multiple cycles of cobomarsen treatment.

CONCLUSIONS

- Cobomarsen is well tolerated up to the 900 mg dose level and has a favorable safety profile over 1 year of dosing on a weekly schedule.
- When compared to a historical cohort of over 6,000 ATLL patients, the MST and PFS for patients treated with cobomarsen was favorable, irrespective of treatment comparator.
- Flow cytometric analysis of circulating ATL cells from aggressive ATLL patients demonstrated that cobomarsen treatment decreased proliferation and the expression of activation markers, thus providing a biological mechanism for the prolonged clinical disease stabilization observed in these patients.
- The preliminary results are encouraging and miRagen will continue to explore cobomarsen for the treatment of both acute and lymphomatous ATLL patients, especially in those with residual disease after front line therapy.

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