RX-0201 (Archexin)

A Phase I Trial of RX-0201 (Archexin; akt-1 Antisense) in Patients with An Advanced Cancer

J. Marshall[1], J. Posey[2], J. Hwang[1], S. Malik[1], R. Shen[1], K. Kazempour[3], L.R., White[3], K.M. Fraser[4], C.G. Chang[4], C.H. Ahn[4].
[1]Lombardi Cancer, Washington, DC, [2]Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL, [3]Amarex Clinical Research, Germantown, MD,
[4]Rexahn Pharmaceuticals, Inc., Rockville, MD.

ABSTRACT

Background: AKT-1, the expression protein product of akt-1 proto-oncogene, plays a vital role in cancer progression by stimulating proliferation and inhibiting apoptosis of cancer cells. RX-0201 (Archexin), a 20-mer oligonucleotide with sequence complementary to akt-1 mRNA, is designed to inhibit the expression of akt-1 within cancer cells in cancer patients (Pts). Non-clinical studies conducted on RX-0201 demonstrated that RX-0201 bears significant *in vitro* and *in vivo* anti-cancer activities with favorable safety.

Purpose: The objectives of the current phase I trial were to determine the maximum tolerated dose (MTD) and to establish safety and pharmacokinetic (PK) profiles of RX-0201 in pts with an advanced cancer.

Method: RX-0201 was administered to pts with an advanced cancer by up to 2 cycles of continuous infusion; each cycle of infusion lasted for 14 days; the infusion phase was followed by a 7-day rest phase. Rapid dose escalation had been used until at least a grade (G) 2 toxicity was observed, and then a traditional dose escalation followed. Pts entry criteria included Karnofsky Performance Status score ≥ 70, advanced cancer, tumor accessible for paired biopsy, and a signed informed consent.

Results: 17 cancer pts were enrolled and treated at a dose level ranging from 6 to 315 mg/m^2/d on D1-14, repeated q 21d.

Safety: Compound-related G 3 fatigue was observed in two pts at the 315 mg/m^2/d dose; three Pts dosed at 250 mg/m^2/d were not noted with any G3 toxicity. No other significant, compound-related, adverse events were observed in those 17 Pts who participated.

Pharmacokinetics: On Day 1, a gradual, dose-proportioned increase in plasma concentration of RX-0201 was noted from 48-315 mg/m^2/d with respective volume of distribution increased from 70.1 L/m^2 to 116 L/m^2 proportionally; C_{ss} increased approximately proportionally to dose. On Day 15, an apparent mono-phasic or bi-phasic decline was observed from C_{max} (observed at the end of infusion (T_{max})). Clearance of RX-0201 was independent of dose or cycle, with value ranged from 24.2 to 42 L/h.m^2; mean terminal half-lives at the dose of 250-315 mg/m^2/d ranged from 1.9-4 hrs. At 250 mg/m^2/d, the estimated volumes of distribution, the mean C_{max}, mean AUC_{last}, and mean C_{ss} for both Cycle 1 and Cycle 2 were comparable; which indicates absence of system drug from Cycle 1 at the start of Cycle 2 infusion.

Conclusion: **MTD** of RX-0201 is 315 mg/m^2/d in Pts with an advanced cancer when the compound is administered in the current continuous infusion regimen. 250 mg/m^2/d will be used as the dose for the follow-on phase IIa trial. Sponsored by Rexahn Pharmaceutical Inc. {www.Rexahn.com}

INTRODUCTION

AKT-1 (Archexin), a protein product of akt-1 proto-oncogene, plays a vital role in cancer progression by stimulating proliferation and inhibiting apoptosis of cancer cells. RX-0201, a 20-mer oligonucleotide with sequence complementary to akt-1 mRNA, is designed to inhibit the expression of akt-1 within cancer cells in cancer patients. Non-clinical studies conducted on RX-0201 demonstrated that RX-0201 bears significant *in vitro* and *in vivo* anti-cancer activities with favorable safety.

OBJECTIVES:

- To characterize the safety in cancer pts,
- To determine the maximum tolerated dose,
- To recommended Phase II trial dose in patients,
- To establish Pharmacokinetics profile,
- To observe AKT-1 expression in tumor biopsy samples.

METHODS

PHASE I TRIAL DESIGN:

RX-0201 was administered to pts with an advance cancer for up to 2 cycles (See Fig. 1). Each treatment cycle consisted of a 14-day continuous infusion phase of RX-0201 followed by a 7-day rest period. Phase I trial of RX-0201 is an open-label, single-arm, dose escalation study to determine safety and tolerability and to recommend a Phase II dose level.

Figure 1: Treatment Outline

ELIGIBILITY CRITERIA:

- Histologically confirmed diagnosis of solid tumor or lymphoma.
- Standard therapies are either ineffective or not tolerated.
- Measurable or evaluable tumors.
- Karnofsky performance status of ≤70.

ESCALATION STRATEGY:

The initial starting dose was 6 mg/m^2/d. Doses doubled until the occurrence of ≥ Grade 2 treatment-related toxicity. After the initial ≥ Grade 2 treatment-related toxicity, the dose increased by up to 66% for the next dose level and by up to 33% for subsequent dose levels. (See Table 1)

Table 1: Dose Escalation

Escalation	
Dose Level 1	6.0 mg/m^2/day
	↓ (up to a doubling of the dose until Grade 2 toxicity)
Dose Level X	yy mg/m^2/day (≥ Grade 2 toxicity)
Dose Level X1	up to a 66% increase
Dose Level X2	up to a 33% increase
Dose Level X3	up to a 33% increase (until experiencing DLTs)

This dose escalation schedule was followed until the MTD was determined.

Pharmacokinetics: Blood samples were collected from each subject into tubes containing heparin at the following time points: Cycle I: Pre-dose, 1, 2, 3, 4, 6 hours post infusion on Day 1; on Day 8; prior to end of infusion and 1, 2, 3, 4, and 6 hours on Day 15; and 24 hours post-infusion completion (on Day 16). Cycle II (if applicable): Pre-dose on Day 1. Plasma samples generated were store frozen and shipped to analysis site where they were stored frozen before measurement. Measurement of RX-0201 was conducted using a validated LC-MS/MS method. Plasma concentration-time data were subjected to non-compartmental pharmacokinetic evaluation using WinNonlin 1.5 Model 201 (Pharsight Corp).

Immunohistochemistry: Tumor biopsy samples were collected into neutral-buffered formalin (NBF) during screening. NBF-fixed tumor samples were embedded in paraffin and a 5-µm section was made from each sample. Paraffin tissue sections were stain immunohistochemically for AKT-1 with mouse anti-human AKT-1 monoclonal antibody (MAB17751, R&D Systems, Inc.; Minneapolis, MN) following the following procedure: deparaffinized in xylene; hydrated from 100% alcohol gradient to water; antigen retrieval (steam); incubated with 3% H_2O_2, blocking serum, and primary antibody sequentially; rinsed and incubated with horse anti-mouse HRP (ImmPRESS Universal Antibody Kit, Vector Labs); rinsed and incubated with DAB substrate (Vector Labs); counterstained with heatoxylin; dehydrated through a gradient of alcohol to xylene; and mounted.

RESULTS

Seventeen patients were successfully enrolled and treated at a dose level of RX-0201 ranging from 6 to 315 mg/m^2/d on D1-14, repeated q 21d (when applicable). (See Table 2, 3, & 4) Compound-related G3 fatigue was observed in two pts at the 315 mg/m^2/d dose; three Pts dosed at 250 mg/m^2/d were not noted with any G3 toxicity. (See Table 5 & 6) No other significant, compound-related, adverse events were observed in those 17 Pts participated.

Table 2: Patient Demographics

Median Age (Range)	61 (47-73)
Gender M/F	6/11
Cancer Stage I/II/III/IV	1/3/1/12

Table 3: Patient Enlistment

Dose Level mg/m^2/d	Patients Enlisted
6	1
12	2
24	1
48	1
96	1
192	4
250	3
315	4

Table 4: Patient Dose Chart

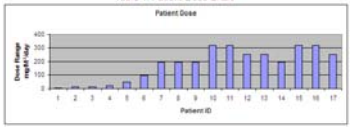


Table 5: Adverse Events

	Dose group (mg/m^2/day) Severity/Grade						
Observations	6	12	24	48	96	192	250	315
ALKALINE PHOSPHATASE 162 HIGH								2
ALOPECIA			1					
ALT 55 - CLINICAL CHEM RESULTS								2
ANEMIA		1						
ANOREXIA								1, 2
ARTHRALGIAS				1	1			
AST CLINICAL CHEM RESULTS								2
BB TEST ON HIGH BB INCREASE								2
CHILLS							1	1
COMPLEMENT FACTOR ELEVATED CH50						2		
COUGH								1
CREATININE (INCREASE)					1			
DECREASE IN BREATH SOUNDS			1			1		
DECREASED (TASTE IN MOUTH)								
DIZZINESS					1			1
DYSPNEA (RESPIRATORY)					1			
DYSPNEA WITH EXERTION								1
ELEVATED CH50						2		
FATIGUE	2	2	1		1	1,2,3	1	1,2,3
FATIGUE/WEAKNESS								
FEVER								1
HEADACHE					1			
HEEL PAIN				1				
LEUKOCYTOPENIA		1	1					
LOSS OF APPETITE							1	
MUSCLE CRAMPS (MYALGIA)				1				
NAUSEA		1						1, 2
PLATELET COUNT 96 LOW								2
RIGHT SHOULDER PAIN								1
VOMITING		1						3

Note – Mild -1, moderate -2, Severe -3

Table 6: Karnofsky Score

Dose Group (mg/m^2/D)	Pre Score	Post Score
6	80	80
12	85	80
24	80	60
48	100	100
96	90	80
192	90	80
250	90	73
315	85	80

Figure 2: Individual Plasma RX-201 Concentrations on Day 1, during Cycle 1, at Doses of 6, 12, 24, 48, and 96 mg/m^2/day

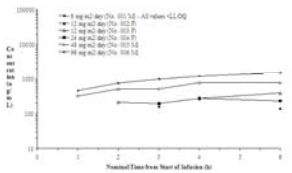


Figure 3: Individual Plasma RX-201 Concentrations on Day 15, during Cycle 1, at Doses of 6, 12, 24, 48, and 96 mg/m^2/day

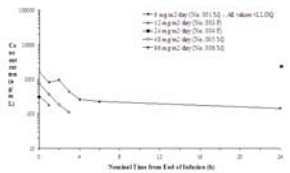


Figure 4: Mean Plasma RX-201 Concentrations on Day 1 at Doses of 192, 250, and 315 mg/m^2/day

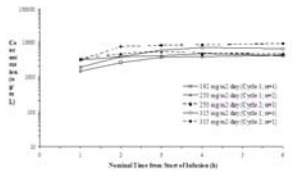
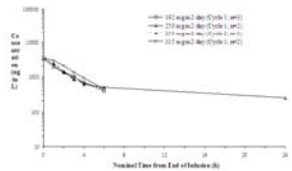


Figure 5: Mean Plasma RX-201 Concentrations on Day 15 at Doses of 192, 250, and 315 mg/m^2/day

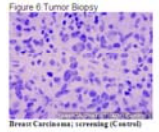


Plasma concentration of RX-0201 at dose of: 6 mg/m^2/d were <LLOQ; 12 and 24 mg/m^2/d concentration were insufficient; and 48 to 315 mg/m^2/d had gradual increase in concentration through 6 hrs post-dose; at 24hrs post-dose, the concentration of RX-0201 reached steady state; and on Day 15, a mono-phasic or bi-phasic decline from C_{max} was noted. (See Figs 2-5 and Table 7)

Table 7: RX-201 Pharmacokinetic Parameters in Patients with Solid Tumors

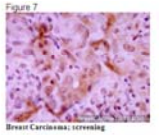
Immunohistochemistry analysis results showed AKT-1 was present in cytoplasm and the nucleus of cancer cells. (See Figures 6-11)

Figure 6 Tumor Biopsy



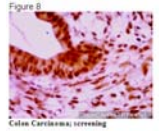
Breast Carcinoma; screening (Control)

Figure 7



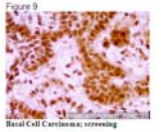
Breast Carcinoma; screening

Figure 8



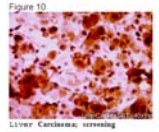
Colon Carcinoma; screening

Figure 9



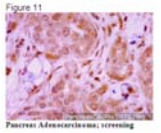
Basal Cell Carcinoma; screening

Figure 10



Liver Carcinoma; screening

Figure 11



Pancreas Adenocarcinoma; screening

CONCLUSION

- No significant dose-limiting toxicity was noted, except for Grade 3 fatigue, which was noted only at 315 mg/m^2/day.
- MTD for RX-0201 after up to 2 cycles of continuous RX-0201 infusion was 315 mg/m^2/day in pts with an advanced cancer; Phase II trial dose was set at 250 mg/m^2/day and will be dosed for up to 8 cycles.
- Continuous intravenous infusion of RX-0201
  Initial phase: Dose-proportioned increase in plasma concentration of RX-0201;
  Steady status: Increase proportionally to dose;
  Termination phase: An apparent mono-phasic or bi-phasic decline.
  Between cycles: No systemic drug residue of RX-0201 from Cycle I was found in the blood before Cycle II started.