

Pharmacokinetics of CR011-vcMMAE, an Antibody-Drug Conjugate, in a Phase I Study of Patients with Advanced Melanoma

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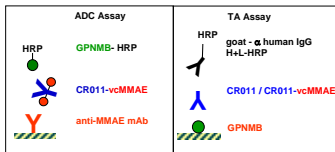
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INTRODUCTION

- CR011, an IgG₁ antibody, targets the extracellular domain of GPNMB, a glycoprotein expressed in melanoma and other cancers (ca. 75 kDa).
- CR011 is conjugated to the dolastatin-like tubulin inhibitor monomethylauristatin-E (MMAE) via a valine-citrulline enzyme-cleavable linker.
- Dose escalation studies established a maximum tolerated dose of 1.88 mg/kg q3w.
- The relatively short half-life (< 48 hours) suggested more frequent dosing could be achieved without drug accumulation. A Phase I study to evaluate the pharmacokinetics and toxicity of CR011-vcMMAE and its by-products in more frequent schedules was initiated.

ANALYTICAL METHODS

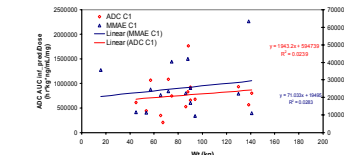
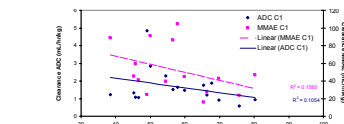
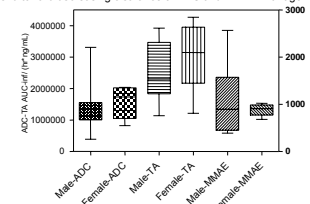
- Validated ELISAs were used to quantify the intact antibody-drug conjugate (ADC) and total antibody (TA) CR011 component with sensitivities of 40 ng/mL (ADC) and 400 ng/mL (TA).



- Free MMAE was quantitated using LC-MS/MS methods with a sensitivity of 50 pg/mL.
- Total and Free GPNMB assays were developed using capture antibodies which did not compete with the CR011 binding site (total) and which did (free). Detection was afforded with rabbit polyclonal anti-GPNMB. Both assays exhibited sensitivities of 0.4 ng/mL.
- Baseline GPNMB was quantitated in pre-infusion sera from 38 patients from the dose escalation portion of the trial.
- The development of antibodies against CR011-vcMMAE was determined from the upper bounds of the 95% confidence interval of the background OD of 31 pre-dose patient serum samples in a bridging ELISA between immobilized and HRP-conjugated CR011-vcMMAE using a floating cut-point assay with a sensitivity of 50 ng/mL. Samples were collected at baseline and day 1 pre-infusion in subsequent cycles.

PK by DEMOGRAPHICS (1.88 mg/kg q3w)

- Pharmacokinetic parameters at 1.88 mg/kg for ADC and free MMAE were graphed against patient age, weight and gender (n=15).
- No obvious trends were indicated in either weight or gender.
- Trend toward decreasing clearance of ADC and MMAE with age.

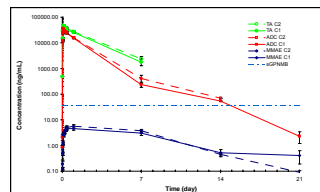


PHARMACOKINETIC METHODS

- Noncompartmental analysis using WinNonlin version 5.2 (model 202) was used in estimation of terminal phase kinetics of antibody-drug conjugate (ADC) and total CR011 antibody (TA).
- Noncompartmental analysis of free MMAE was performed with MMAE equivalent dosing and Model 200.
- AUC estimates were estimated with the linear trapezoidal (Linear Interpolation) method.

SUMMARY PK at 1.88 mg/kg q3w

- Concentrations of CR011-vcMMAE antibody-drug conjugate (ADC), total CR011 antibody (TA), and free MMAE are graphed over the 3 week interval at 1.88 mg/kg q 3 week dose (mean ± SEM; n=16).
- Total antibody is cleared more slowly than the ADC.
- Free MMAE reaches maximum concentration between 24 and 48 hours, then declines along with conjugate.
- Levels of both ADC and TA exceed levels of sGPNMB for more than one week post dosing.



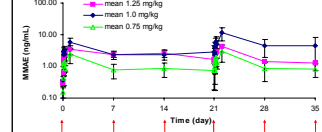
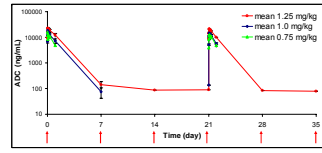
Analyte	Patient (n)	Half Life (hr)	C _{max} (ng/mL)	AUC ₀₋₂₄ (ng·h/mL)	Cl _{CR} (mL/kg)	V _d (mL/kg)
ADC	16	28.4 (13.6)	36.3 (10.7)	1433.4 (662.2)	1.7 (1.0)	48.2 (24.2)
TA	16	40.7 (24.2)	59.0 (24.3)	2334.5 (973.1)	0.82 (0.36)	33.9 (17.3)
MMAE	15	69 (30.2)	0.005 (2.8)	0.084 (0.0916)	54 (27.7)	5422 (3745.9)

Noncompartmental PK analysis (1.88 mg/kg q3w); Mean ± SD.

PK: ALTERNATE DOSING SCHEDULES

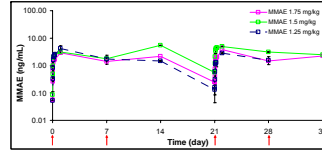
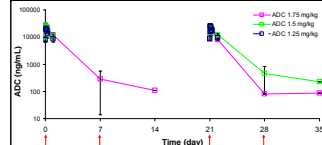
Weekly Schedule

- Concentrations represent mean ± SEM of patients dosed with 0.75 mg/kg/w (n=3); 1.0 mg/kg/w (n=7); and 1.25 mg/kg/w (n=3)
- Dense PK was performed on Day 1 of each cycle only. Pre-dose samples were taken on Days 8 and 15 of each 3-week cycle.
- ADC levels proportional with dose.
- Minimal increase in peak levels or overall exposure noted for cycle 2 suggests little accumulation of drug or free MMAE within range studied.



Two out of Three Week Schedule

- Concentrations represent mean ± SEM of patients dosed with 1.25, 1.50, and 1.75 mg/kg (n=3 each).
- Dense PK was performed on Day 1 of each cycle only. Pre-dose sample was taken on Day 8 and an additional sample was taken on Day 15 of each cycle.
- Minimal increase in peak levels or overall exposure noted for cycle 2 suggests little accumulation of drug or free MMAE within range studied.



Arrows represent dosing with CR011-vcMMAE. Trough concentrations, which were below the lower limit of quantitation were not graphed or included in calculation of means.

ALTERNATE DOSING SCHEDULES: CLINICAL DATA

- Preliminary data from this ongoing study are presented
- Data cutoff: April 30, 2009
- Median Duration of Follow-up: 6 weeks
- 28 patients treated
- 19 patients had post-treatment activity assessments as of data cutoff

Weekly Schedule (n=15)

Dose (mg/kg)	3-wk Cumulative Dose (mg/kg)	n	DLT (n)	Objective Response (n)
0.75	2.25	3	0	1
1.25	3.75	5	1	2

- 1.25 mg/kg/w was discontinued after 5 patients due to dose limiting toxicities at the highest dose in the 2/3 week schedule (described below)
- 1.0 mg/kg/w was declared the MTD
- Of 11 evaluable patients, 3 objective responses were observed: 1 confirmed

Two out of Three Week Schedule (n=13)

Dose (mg/kg)	3-wk Cumulative Dose (mg/kg)	n	DLT (n)	Objective Response (n)
1.25	2.5	3	0	0
1.5	3	4	0	1
1.75	3.5	6	3	0

- 1.25 mg/kg in 2/3 wk was not tolerated
- 1.5 mg/kg in 2/3 wk cohort is currently enrolling to complete n of 6
- Of 8 evaluable patients, 1 confirmed objective response was observed

Summary of Dose Limiting Toxicities

Dose	Age	Gender	Events
Weekly Schedule			
1.0 mg/kg qw	58	F	Grade 3 pruritic rash and grade 3 neutropenia
1.25 mg/kg qw	72	M	Acute renal failure, hyperglycemia, refractory hypotension, death
Two out of Three Week Schedule			
1.75 mg/kg 2/3 weeks	80	M	Toxic epidermal necrolysis, diarrhea, fever, neutropenia, renal failure, refractory hypotension, multiorgan system failure, death
1.75 mg/kg 2/3 weeks	64	M	Grade 3 pruritic rash and grade 3 hyperglycemia
1.75 mg/kg 2/3 weeks	56	M	Grade 3 rash

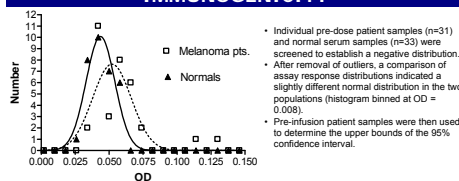
Most Common Adverse Events (n=28)

Event	n (%)
Rash	22 (79%)
Fatigue	20 (71%)
Pruritus	19 (68%)
Diarrhea	14 (50%)
Neuropathy	11 (39%)

CONCLUSIONS

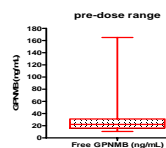
- Pharmacokinetic studies of CR011-vcMMAE 1.88 mg/kg q3w have shown that the half-life for total antibody is approximately 40 hours, and that of conjugated antibody is approximately 28 hours.
- Concentrations of CR011-vcMMAE exceed levels of soluble GPNMB for over 1 week after dosing.
- Data show that treatment emergent immunogenicity to CR011-vcMMAE is not significant.
- Using more frequent dosing, higher cumulative doses over a three week period were administered than with 1.88 mg/kg q3w dosing.
- Dermatologic toxicities, fatigue and diarrhea were the most common toxicities across all three schedules (q3w, qw and 2/3 weeks).
- Preliminary activity data show 4 objective responses in patients on the more frequent dosing schedules. Additional patients will be enrolled at the MTD to define activity.

IMMUNOGENICITY



- Individual pre-dose patient samples (n=31) and normal serum samples (n=33) were screened to establish a negative distribution.
- After removal of outliers, a comparison assay response distributions indicated a slightly different normal distribution in the two populations (histogram binned at OD = 0.008).
- Pre-infusion patient samples were then used to determine the upper bounds of the 95% confidence interval.
- Of the 245 total samples run, 4.1% were found to be positive, approximating the theoretical false positive rate.
- 59 patients from the dose-escalation and Phase II portion of the study were analyzed. Three patients had a positive response at baseline (OD > cut-point).
- Two patients (3.4%) exhibited a single positive response (at Cycle 3 and 5, respectively), but each tested negative in subsequent cycles.
- Two patients (3.4%) had a single positive OD (at Cycles 2 and 4, respectively), with no subsequent testing performed to date.
- Determination of specificity of the response is ongoing.

SOLUBLE GPNMB



Distribution	GPNMB
Range (ng/mL)	11-165
Mean (ng/mL)	34.8
Median (ng/mL)	22.2
SD (ng/mL)	34.4
SEM (ng/mL)	5.6

- Soluble GPNMB was quantitated in baseline serum samples selected at random from 38 patients in the dose-escalation phase.
- Maximum GPNMB observed in this group of patients was 165 ng/mL (2.2 nM), with 75% of patients having less than 40 ng/mL (0.5 nM).
- Administration of CR011-vcMMAE at 1.88 mg/kg q3w, results in mean day 8 concentrations of total CR011 antibody of 11.6 ± 3.4 nM, or 5 fold excess over mean baseline sGPNMB levels.