

# Phase I Pharmacokinetic Study of CR011-vcMMAE, an Antibody Toxin Conjugate Drug, in Patients with Unresectable Stage III/IV Melanoma

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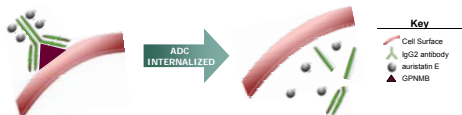
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## BACKGROUND

**Background:** Using transcript profiling, CuraGen has identified glycoprotein NMB (GNMB) as a potentially novel therapeutic target for melanoma. GNMB which is highly expressed in human melanoma tissue is a predominantly intracellular glycoprotein with a small extracellular component. A fully human monoclonal antibody (CR011) was raised against the extracellular domain of GNMB and conjugated to the cytotoxic agent monomethyl auristatin E (MMAE) via a valine-citrulline dipeptide linker. (Figure 1). CR011-vcMMAE has potent anti-tumor activity *in vitro* (IC<sub>50</sub> 1.25-5 µg/ml) and in melanoma xenograft mouse models (C<sub>max</sub> 15 µg/ml at the ED<sub>50</sub>).

We conducted a Phase I multi-center study to evaluate the safety, maximum tolerated dose (MTD), and pharmacokinetics (PK) of CR011-vcMMAE in patients with metastatic malignant melanoma.

FIGURE 1: CR011-vcMMAE MECHANISM OF ACTION



CR011-vcMMAE is administered intravenously. Following administration, the CR011-vcMMAE ADC binds to GNMB expressed on the cell surface and is internalized. Intracellular enzymes cleave MMAE from the IgG2 antibody releasing the activated cytotoxic auristatin E.

## STUDY DESIGN AND METHODS

### Study Design

- Phase I open-label multi-center, dose-escalation study

### Objectives

- To evaluate the dose-limiting toxicities (DLT) and determine the maximum tolerated dose (MTD) of CR011-vcMMAE
- To assess the pharmacokinetic (PK) profile

### Selected Entry Criteria

- Histologically/cytologically confirmed unresectable Stage III or IV melanoma
- Karnofsky PS ≥70%
- ≤ 1 prior line of cytotoxic therapy; prior cytokines permitted
- No active brain metastases

### Treatment

- CR011-vcMMAE administered as a single IV infusion over 90 min on day 1 of a 21-day cycle x 4 cycles
- Patients demonstrating tumor shrinkage may be treated beyond 4 cycles

### Dose Escalation

- Sequential dose cohorts of 3-6 patients
- Doses escalated by 100% until Grade 2 or higher toxicity observed in 1 patient, after which doses escalated by 40% dose increments until MTD is achieved
- Only dose limiting toxicities occurring in Cycle 1 are considered for MTD

Cohort	Dose (mg/kg)	n
1	0.03	3
2	0.06	3
3	0.12	3
4	0.24	3
5	0.48	3
6	0.96	3
7	1.34	3
8	1.88	4

### Dose Limiting Toxicities (DLT)

- Grade 4 neutropenia lasting > 5 days or associated with a fever > 100.5 °F
- Grade 4 thrombocytopenia
- Any Grade 4 non-hematologic toxicity excluding alopecia
- Any Grade 3 non-hematologic toxicity excluding nausea, vomiting, rash, arthralgias or myalgias, and fatigue

### Assessments

- Safety assessments performed every cycle
- Plasma PK for CR011-vcMMAE assessed during Cycles 1 and 2
- Tumor assessments every 2 cycles

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## DEMOGRAPHICS

- 25 patients have been treated as of 16 October 2007

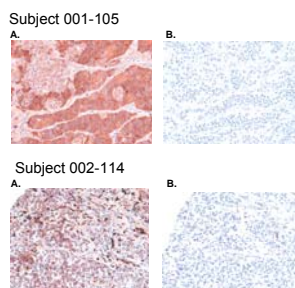
TABLE 1: PATIENT CHARACTERISTICS

Patient Characteristics	N <sup>o</sup> of Patients
<b>Gender</b>	
Male	15
Female	10
<b>Age (yrs)</b>	
Median	63
Range	47 – 80
<b>Race</b>	
White	21
Non-white	4
<b>Stage<sup>1</sup></b>	
III, unresectable	7
IV	13
<b>Duration of disease (yrs)</b>	
Median	1.8
Range	0.1 – 5.5
<b>Prior systemic therapy<sup>1,2</sup></b>	
None	2
Any chemotherapy	10
Any cytokine	6
Vaccine	2
Other investigational agent	3

- Data unavailable for 5 patients
- Patients may have received combination therapy

## IHC ANALYSIS OF PATIENT SPECIMENS

- Patient specimens were available from 2 patients
- Immunohistochemical analysis of tumor specimens show specific expression of GNMB antigen in melanoma tissue (A, B), demonstrating the presence of the antibody target in patient's tumors.



Immunohistochemistry analysis performed by Mosaic Laboratories (Lake Forest, CA). Resected cancer specimens were stored in 10% neutral buffered formalin. Samples were processed and paraffin embedded.

Panel A. Specimen stained with Biotinylated anti-CR011, human IgG2.  
Panel B. Isotype control consisting of Biotinylated isotype, human IgG2.

## SAFETY (n=25)

- Overall, CR011-vcMMAE has been well-tolerated
- Most adverse events have either been Grade 1 or Grade 2
- Drug-related Grade 3 or 4 events have been reported in 2 patients
- No DLTs have been observed in the first 8 cohorts (up to 1.88 mg/kg)

TABLE 2: DRUG-RELATED ADVERSE EVENTS WITH FREQUENCY ≥ 2

	Any Grade n (%)	Grade 1 n (%)	Grade 2 n (%)	Grade 3 or 4 n (%)
Rash	5 (20%)	3 (12%)	2 (8%)	0 (0%)
Fatigue	5 (20%)	4 (16%)	1 (4%)	0 (0%)
Pruritus	4 (16%)	3 (12%)	1 (4%)	0 (0%)
Anorexia	3 (12%)	2 (8%)	1 (4%)	0 (0%)
Headache	3 (12%)	3 (12%)	0 (0%)	0 (0%)
Vomiting	3 (12%)	3 (12%)	0 (0%)	0 (0%)
Neutropenia	2 (8%)	0 (0%)	0 (0%)	2 (8%)
Chills	2 (8%)	1 (4%)	1 (4%)	0 (0%)
Nausea	2 (8%)	2 (8%)	0 (0%)	0 (0%)
Diarrhea	2 (8%)	2 (8%)	0 (0%)	0 (0%)

TABLE 3: TREATMENT-EMERGENT ADVERSE EVENTS ≥ GRADE 2 REGARDLESS OF CAUSALITY

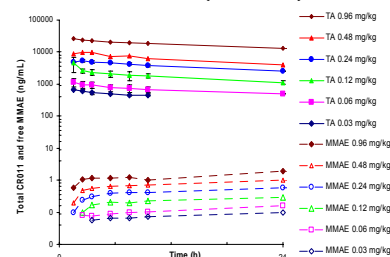
Patient	Cohort	Dose (mg/kg)	Adverse Event
1102	1	0.03	Grade 2 pain, edema
1109	3	0.12	Grade 4 thrombosis (SAE) <sup>1</sup>
2110	3	0.12	Grade 2 pain, fatigue, nausea, anorexia
2115	5	0.48	Grade 2 anorexia, nausea, vomiting
2116	5	0.48	Grade 2 fatigue, pain
1117	6	0.96	Grade 2 hot flashes
1118	6	0.96	Grade 2 nausea, vomiting, diarrhea
2119	6	0.96	Grade 2 nausea
2120	7	1.34	Grade 4 neutropenia, Grade 3 leukopenia. Treatment delay x2. Dose reduction to 0.96 mg/kg
1122	7	1.34	Grade 2 rash, pruritus
3123	8	1.88	Grade 4 neutropenia. Grade 2 rash, alopecia, anorexia, fatigue. Laboratory: Grade 3 hypokalemia (no AE reported)
3126	8	1.88	Grade 2 chills
2124	8	1.88	Grade 2 neutropenia

<sup>1</sup> 55 y/o man with metastatic melanoma and history of deep vein thrombosis 5 months prior to event; warfarin discontinued 4 days before event. SAE in cycle 2 of therapy and assessed as unrelated to study drug

## PHARMACOKINETICS

- Non-compartmental analysis was performed on concentrations of total CR011 antibody (TA) and free MMAE and PK results are reported for the first six cohorts (up to 0.96 mg/kg)
- Following 90 minute IV infusion, CR011 serum concentrations decline in an apparent first order manner
- Terminal phase half-life of CR011 increases with dose and is approximately 38 hours at 0.96 mg/kg
- Free MMAE concentrations are approximately 4 logs lower than that of total antibody at 24 hours
- C<sub>max</sub> at the 0.96 mg/kg dose (24.8 µg/ml) approaches levels expected to be in the active range extrapolated from *in vivo* melanoma xenograft mouse studies
- At doses through 0.96 mg/kg, cycle 2 exposure similar for both total CR011 and free MMAE as cycle 1

FIGURE 2: SERUM CONCENTRATIONS OF TOTAL ANTIBODY AND FREE MMAE TO 24 HOURS FROM CYCLE 1 (Mean ± SE)



Total antibody CR011 (TA) in patient serum was quantitated with an ELISA based on capture with immobilized antigen, GNMB. Free MMAE was determined using LC-MS/MS

TABLE 4: PHARMACOKINETICS OF TOTAL CR011 (Mean ± SD)

Dose (mg/kg)	n	T <sub>1/2</sub> (hr)	C <sub>max</sub> (ng/mL)	AUC <sub>0-24</sub> (hr*ng/mL)
0.03	3	16.7 ± 5.0	680 ± 37	4320 ± 715
0.06	3	16.0 ± 1.7	1150 ± 484	10635 ± 7826
0.12	3	27.6 ± 16.4	4741 ± 3103	42868 ± 4963
0.24	3	20.6 ± 3.0	5433 ± 707	71268 ± 27948
0.48	3	24.5 ± 13.8	10010 ± 505	144830 ± 6674
0.96	3	38.1 ± 5.3	24840 ± 6102	1793823 ± 492501

T<sub>1/2</sub> = Half-life of the terminal phase, C<sub>max</sub> = Maximum plasma concentration, AUC<sub>0-24</sub> = Area under the plasma until last measurable point. PK assessment conducted during Cycles 1 and 2. Samples obtained pre-infusion, 30 minutes during infusion, and at 1, 2, 4, 6, 8, and 24 hours post infusion.

## TREATMENT SUMMARY

- Of 25 patients currently enrolled, 22 patients have had evaluation of tumor response
- To date 7 patients have demonstrated stable disease (SD)
- A 51 year old woman in cohort 4 (0.24 mg/kg) has completed 11 cycles of CR011-vcMMAE and demonstrates SD with 20% shrinkage of perirectal lymphadenopathy. Patient is ongoing on study.
- A 59 year old woman in cohort 7 (1.34 mg/kg) with multiple liver metastases has received 6 cycles of CR011-vcMMAE and demonstrates SD with some tumor shrinkage. Patient previously received 4 months of biochemotherapy and had PD upon study entry. Patient is ongoing on study.

TABLE 5: RESPONSE AND TREATMENT DURATION BY COHORT

Cohort No. 1	Dose level mg/kg	N <sup>o</sup> . Pts	N <sup>o</sup> . Treatment Cycles	Ongoing	DLT	Best response
1	0.03	3 pts	2-4	0	0	2 SD; 1 PD
2	0.06	3 pts	2-3	0	0	3 PD
3	0.12	3 pts	2-4	0	0	3 PD
4	0.24	3 pts	2-11	1	0	1 SD (11 cycles tx ongoing); 2 PD
5	0.48	3 pts	2-4	0	0	3 PD
6	0.96	3 pts	2-4	0	0	3 PD
7	1.34	3 pts	4-6	1	0	3 SD
8	1.88	4 pts	2-3	3	0	2 SD; 2*

Pts = patients; DLT = dose limiting toxicity; SD = stable disease; PD = progressive disease.  
<sup>1</sup> Dose-escalation is ongoing. \* Not yet evaluable.

## CONCLUSIONS

- 25 patients have been treated in this dose escalation study in patients with metastatic melanoma, the first human clinical trial of CR011-vcMMAE
- GNMB cell surface glycoprotein target was expressed in melanoma tumor specimens from 2 of 2 patients on study
- CR011-vcMMAE has been safe and well-tolerated with no dose-limiting toxicities observed to date in 8 cohorts (0.03 to 1.88 mg/kg). Enrollment is ongoing in cohort 9 (2.63 mg/kg)
- CR011-vcMMAE can be administered at doses > 1 mg/kg which are consistent with the active range extrapolated from *in vivo* xenograft animal models
- Neutropenia is the most common Grade 3 / 4 adverse event and appears dose-dependent
- PK studies show dose proportional exposure of the anti-GNMB antibody, and minimal free MMAE exposure
- No objective responses have been observed to date, however 4 patients displayed tumor shrinkage of up to 20%, and 6 patients had SD for ≥ 4 cycles (range 4 – 11+)
- Upon establishing the maximum tolerated dose the trial will expand into a Phase II study and assess the activity of CR011-vcMMAE in up to 32 patients with unresectable Stage III and IV malignant melanoma

## ACKNOWLEDGEMENTS

The patients and their families.