

Abstract # 1509

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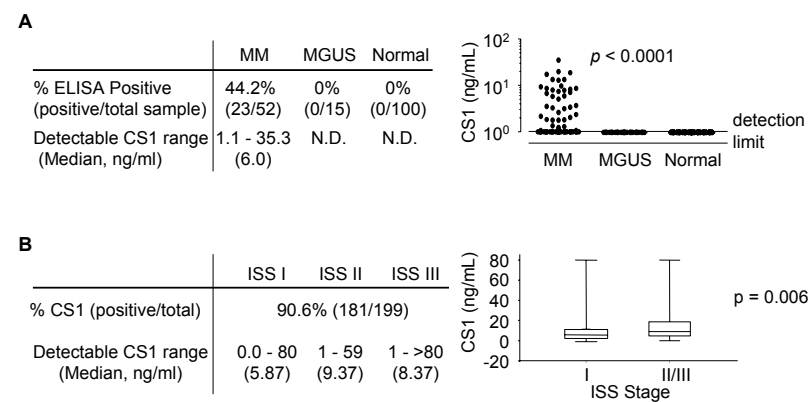


## ABSTRACT

The humanized anti-CS1 mAb HuLuc63 is currently undergoing Phase 1 safety study in patients with relapsed/refractory multiple myeloma. HuLuc63 induces significant antibody-dependent cellular cytotoxicity (ADCC) against autologous CS1-expressing patient MM cells (Tai Blood 2007 Oct 9). Here we examined whether soluble CS1 is detectable in MM patient sera and affects HuLuc63-induced cytotoxicity against MM cells. Using a sandwich ELISA, CS1 was detected in 44% (23/52) of MM patient sera with a range of 1.2 to 35.3 ng/ml. Immunoprecipitation of CS1 from ELISA-positive and -negative samples using anti-CS1 mAbs (HuLuc63 and ChLuc90) followed by immunoblotting with HuLuc63 and anti-CS1 mAb 1G9, identified two forms of CS1 in MM patient sera. CS1 was detected in MM patient sera, but not in sera from MGUS patients (n=15) or healthy donors (n=100). In additional sera of 199 MM patients with newly diagnosed MM, 90% (181/199) of sera had detectable CS1 (1 to >80 ng/ml). Median CS1 levels for International Stage System (ISS) I (n=100), II (n=53), and III (n=46) patients were 5.87, 9.37, and 8.37 ng/ml, respectively. The correlation between ISS and CS1 was moderate (spearman correlation coefficient = 0.197, p=0.005). Patients with ISS II/III had significantly higher CS1 levels compared with those with ISS I (median 9.0 vs. 5.9 ng/ml, p=0.006), suggesting a correlation of serum CS1 with active MM. As patients with ISS II and III require therapy, while those with ISS I do not, these results suggest that circulating CS1 may indicate need for therapy. Preliminary data also show that levels of circulating CS1 appeared to correlate with the percent plasma cells in the bone marrow of MM patients, suggesting that serum CS1 may be a marker for myeloma tumor burden. In mouse studies, HuLuc63 exhibited significant dose-dependent anti-tumor activity, leading to reduced or eliminated human myeloma xenograft tumors. Anti-myeloma activity of HuLuc63 in mice correlated with sustained serum levels of >2 µg/ml of antibody. At the molar level, this is above the levels of circulating CS1 protein observed in MM patients. Optimal anti-tumor activity was observed at sustained antibody levels of >70 µg/ml. Thus, if one assumes that one CS1 molecule binds one antibody molecule, our data suggests that serum CS1 will be an unlikely antibody sink in patients treated with optimal doses of HuLuc63. Since NK cells also express CS1, albeit at lower levels than myeloma cells, we next determined the effects of HuLuc63 on NK cell function. Pretreatment of NK cells with HuLuc63 for 3 days did not alter NK-mediated ADCC against CS1-expressing myeloma cells via HuLuc63. Moreover, recombinant CS1-Fc at levels <200 ng/ml did not significantly inhibit HuLuc63-induced ADCC against myeloma cells. Together, these studies strongly support continued clinical investigation of HuLuc63 in MM.

## Soluble CS1 correlates with stage of disease

**Fig. 2 Serum level of circulating CS1 is detected only in myeloma patients**



(A) CS1 ELISA was performed on serum samples from MM (n=52), MGUS (n=15), and normal donors (n=34). P value was calculated by 2 test from 2 x 2 contingency table. p < 0.0001 (B) CS1 ELISA was performed in additional serum samples from newly diagnosed MM patients (n = 199). Patients with ISS I (n = 100) had significantly lower levels of CS1 than ISS II (n = 53) and III (n = 46) (p = 0.006).

## Soluble CS1 as a potential marker for tumor burden

**Table 1. Levels of circulating CS1 correlate with plasma cell numbers in bone marrow**

case #	sCS-1 (ng/mL)	% of plasma cells	years post diagnosis
1	1.0	0	1.5 yrs
15	1.0	0	N/A
35	1.0	5	newly diagnosed
8	1.0	5	2 yrs
39	1.0	10	2 yrs
40	1.0	10	4.5 yrs
41	1.4	10	4 yrs
27	1.0	15	newly diagnosed
36	1.0	20	5 yrs
25	1.0	20	4 yrs
32	3.2	20	2.5 yrs
38	1.0	40	3 yrs
31	3.5	40	newly diagnosed
10	13.5	40	4 yrs
34	1.9	50	5 yrs
42	1.0	60	6.8 yrs
20	1.0	60	newly diagnosed
37	9.8	60	newly diagnosed
19	35.3	60	newly diagnosed
13	1.0	70	1 yr
14	1.1	70	7.5 yrs
22	5.8	70	2 yrs
11	8.3	70	11 yrs
44	8.7	70	4 yrs
45	6.0	80	newly diagnosed
43	8.7	80	3 yrs
29	9.3	95	0.7 yr

CS1 ELISA was performed on serum samples from 27 MM patients. In parallel, bone marrow (BM) samples from the same patients were analyzed for % plasma cells (PCs). The results indicate a positive correlation between serum CS1 levels and % PCs at initial sample submission.

Spearman's Rho=0.61  
p=0.0006, N=27.

Note: Patients with CS1 values <1 were coded "1" in the summary statistics (median, range) and the correlation analysis.

## RESULTS

### Serum levels of HuLuc63 mediating anti-MM activity in mice

**Table 2. Circulating levels of HuLuc63 during a dose-range study in OPM2 model**

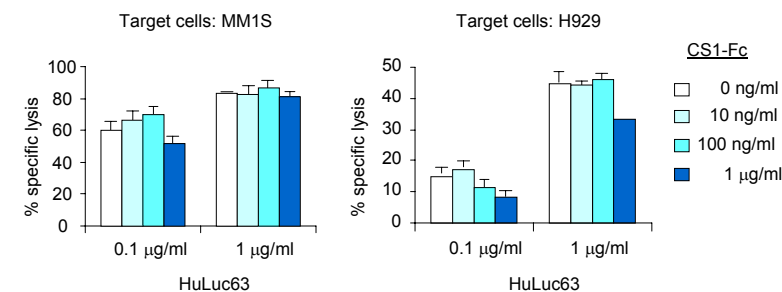
Dose Group (mg/kg)	Protocol Timepoint	N	HuLuc63 Mean Result (µg/mL)	% tumor growth inhibition (P values)
Baseline	Mice not dosed	20	BQL	
Placebo	C1max	5	BQL	0
Placebo	C1min	4	BQL	
Placebo	C6min	5	BQL	
Placebo	C7max	4	BQL	
Placebo	Terminal	9	BQL	
0.1	C1max	5	0.48	18 (0.67)*
0.1	C1min	4	0.13	
0.1	C6min	5	0.43	
0.1	C7max	3	0.87	
0.1	Terminal	8	0.30	
0.5	C1max	5	3.55	59 (0.003)
0.5	C1min	4	1.88	
0.5	C6min	5	6.90	
0.5	C7max	4	13.0	
0.5	Terminal	9	7.05	
1.0	C1max	5	8.25	72 (<0.001)
1.0	C1min	4	3.15	
1.0	C6min	5	18.9	
1.0	C7max	4	23.8	
1.0	Terminal	9	13.9	
5.0	C1max	5	43.1	81 (<0.001)
5.0	C1min	4	34.0	
5.0	C6min	5	127.0	
5.0	C7max	4	176.0	
5.0	Terminal	9	109.0	
10.0	C1max	5	98.0	87 (<0.001)
10.0	C1min	3	68.5	
10.0	C6min	5	272.0	
10.0	C7max	4	429.0	
10.0	Terminal	9	257.4	

BQL = below quantifiable limits.  
\*P value in brackets.

The OPM2 xenograft model was used to determine the range of HuLuc63-mediated anti-tumor activity, and to correlate activity with the levels of HuLuc63 in the circulation. Mice with OPM2 tumors were treated with control antibody at 10 mg/kg or HuLuc63 at 0.1, 0.5, 1, 5 and 10 mg/kg. Blood samples were collected from the animals at various time points and processed to serum. The concentrations of HuLuc63 in the serum samples were measured using an ELISA. The treatment groups consisted of HuLuc63 at doses of 0.1, 0.5, 1, 5, and 10 mg/kg. The control group received isotype control antibody at 10 mg/kg. Dosing was once every 3 days for a total of 7 doses. Blood was collected at 8 hours after the first dose (C1 max), immediately before the second dose (C1 min), immediately before the 7th dose (C6 min), 8 hours after the last dose (C7 max), and one dose-interval after the last dose (terminal bleed). The results showed that minimal anti-tumor activity correlated with HuLuc63 serum levels of 2-13 µg/ml. Optimal anti-tumor activity correlated with levels of 68-429 µg/ml.

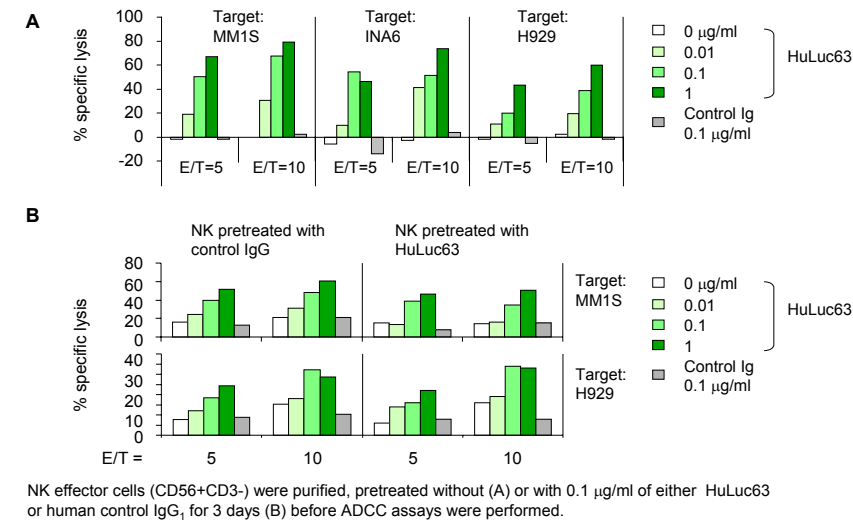
### Low levels of serum CS1 did not alter HuLuc63-mediated ADCC

**Fig. 3 CS1-Fc at physiological serum levels did not block HuLuc63-mediated ADCC**



ADCC was performed by incubating calcein-AM-labeled target MM cells with human NK effector cells at an E/T ratio of 5/1, in the presence of HuLuc63 with varying concentrations of recombinant CS1-Fc fusion protein (2, 20, 200 ng/ml).

**Fig. 4 HuLuc63 pre-treatment of NK cells did not affect ADCC against MM cells**



NK effector cells (CD56+CD3-) were purified, pretreated without (A) or with 0.1 µg/ml of either HuLuc63 or human control IgG, for 3 days (B) before ADCC assays were performed.

## SUMMARY & CONCLUSIONS

- ❖ CS1, the therapeutic target for HuLuc63, is a potential biomarker for MM
- ❖ CS1 is detectable at low levels (<200 ng/ml) only in the sera of myeloma patients, and not in sera of MGUS or healthy donors
- ❖ Serum CS1 correlates with stage of disease and tumor burden
- ❖ Patients with active disease (ISS II and III) exhibit higher levels than those with ISS I, suggesting that higher levels of circulating CS1 may indicate need for therapy.
- ❖ Soluble CS1 at physiological levels did not significantly block HuLuc63-induced ADCC against MM cells, suggesting that serum CS1 will be an unlikely antibody sink in patients treated with optimal doses of HuLuc63.
- ❖ HuLuc63 inhibits tumor growth in a dose-dependent manner in xenograft mouse models.
- ❖ Maximal in vivo efficacy in mice correlates with HuLuc63 serum levels of 70-430 µg/ml, suggesting that potential clinical activity in MM patients may be observed at doses that achieve sustained levels of HuLuc63 in this range.

## ACKNOWLEDGEMENTS

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### DISCLOSURE STATEMENT

All authors, except A.G.R., M.D., L.J., I.C., D.E.H.A. (paid employees of PDL Biopharma) have no relevant conflicts of interest to disclose.