

Anti-Myeloma activity of HuLuc63 alone and in combination with bortezomib

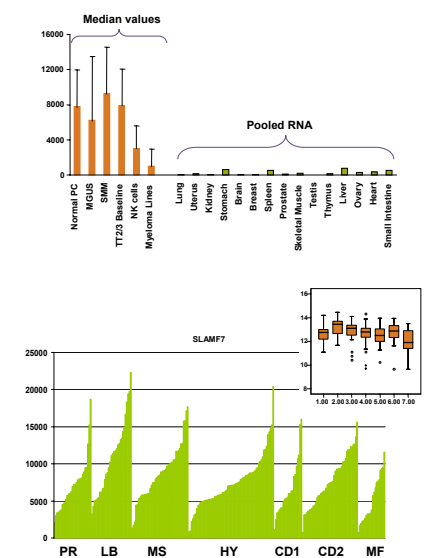
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ABSTRACT

HuLuc63 is a humanized monoclonal antibody (mAb) that targets the cell surface glycoprotein CS1 (CD2 subset 1, CRACC, SLAMF7, CD319). We have shown that CS1 is expressed on normal plasma cells, a subset of lymphocytes and at high levels on myeloma cells from multiple myeloma (MM) patients. HuLuc63 treatment of mice with MM xenograft tumors resulted in significant *in vivo* anti-tumor activity. HuLuc63 mediates anti-MM activity via Fc interaction with natural killer (NK) cells, suggesting that antibody-dependent cellular cytotoxicity (ADCC) is the main mechanism of action. Bortezomib (Velcade®) has been observed to down-modulate myeloma surface expression of MHC class I, an inhibitor of NK function. The purpose of this study was to examine whether using HuLuc63 in combination with bortezomib provided therapeutic benefit. The effect of HuLuc63 and bortezomib treatment on expression of CS1 in myeloma cell lines and xenograft tumors was examined by flow cytometry and immunohistochemistry (IHC) respectively. CS1 protein expression on the OPM2 myeloma cell line did not significantly change when treated with HuLuc63, bortezomib or with both agents *in vitro* and *in vivo*. The combination of HuLuc63 with bortezomib was tested for anti-myeloma activity *in vitro* using ADCC assays and *in vivo* for anti-tumor activity in a mouse xenograft model. Pre-treatment with bortezomib significantly enhanced HuLuc63-mediated ADCC towards OPM2 cells using NK effector cells from healthy donors. In the autologous setting, *in vitro* pre-treatment with bortezomib also increased HuLuc63-mediated killing when using purified NK cells from a MM patient to target the patient's own myeloma cells. Finally, *in vivo* anti-tumor activity of HuLuc63 was also enhanced by co-treatment with bortezomib. Using sub-optimal doses of HuLuc63 (1 mg/kg given twice a week), combination treatment with bortezomib enhanced the anti-tumor effects in OPM2 models by 40-50%. These effects are likely due to the combined anti-tumor effects of HuLuc63 and bortezomib. These pre-clinical studies support the use of HuLuc63 as a new therapeutic for the treatment of MM and suggest that bortezomib co-treatment may add to the anti-myeloma activity of HuLuc63. HuLuc63 is currently being evaluated in a phase I clinical study as monotherapy for the treatment of relapse/refractory MM.

CS1 is highly and uniformly expressed in MM

Figure 1. Gene expression profiling of CS1

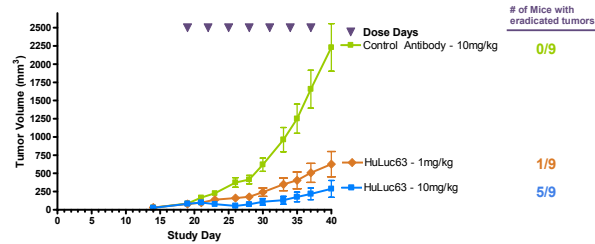


CS1 is uniformly and highly expressed in plasma cells from healthy donors, patients with monoclonal gammopathies of undetermined significance (MGUS), smoldering myeloma (SMM), and over 500 patients with frank MM at diagnosis. NK cells expressed significantly lower levels of CS1 mRNA. CS1 expression is high in all 7 molecular subtypes of MM, which include groups characterized by activating translocations involving c-MAF/MAFB (MF), CCND1 (CD1), CCND3 (CD2), and MMSET/FGFR3 (MS), as well as groups characterized by hyperdiploidy (PR), low-bone disease (LB) and higher gene expression associated with proliferation (PR).

RESULTS

HuLuc63 exhibits dose-dependent anti-tumor activity

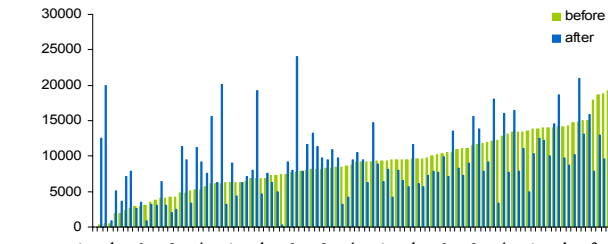
Figure 2. OPM2 xenograft model



HuLuc63 was tested for *in vivo* anti-tumor activity using the human OPM2 MM xenograft model. OPM2 bearing SCID mice were treated with 1 or 10 mg/kg HuLuc63, or isotype control antibody at 10 mg/kg. HuLuc63 treatment resulted in decreased tumor volumes in both dose cohorts, although the magnitude of anti-tumor activity was larger at the higher dose.

CS1 expression persists in MM patients after bortezomib treatment

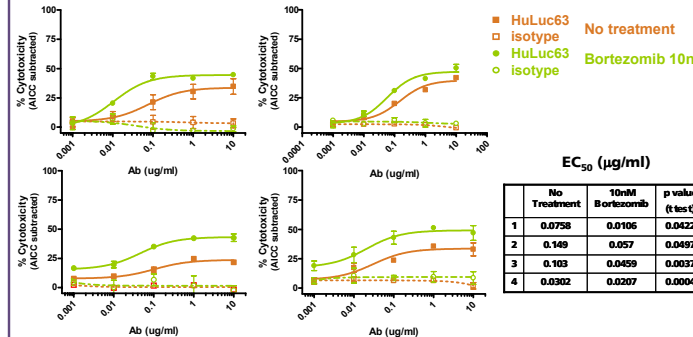
Figure 4. Gene expression profiling of CS1 before and after bortezomib treatment



Plasma cells were purified from MM patient bone marrow samples before and 48 hrs after bortezomib treatment. CS1 levels were assessed by gene expression profiling using the Affymetrix DNA microarray technology.

Bortezomib decreases the EC₅₀ for HuLuc63-mediated ADCC *in vitro*

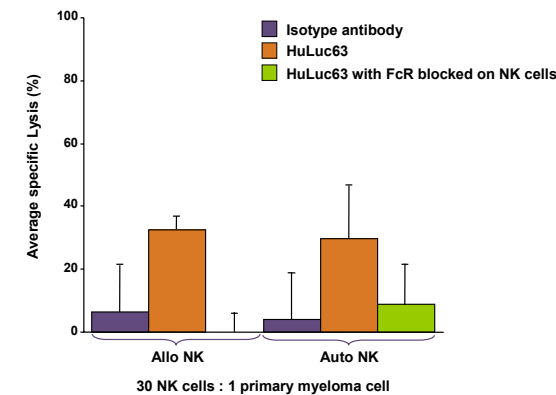
Figure 6. HuLuc63-mediated ADCC with and without pre-treatment with bortezomib



OPM2 cells were pretreated with vehicle control or bortezomib (10 nM) for 18 hrs and were then subjected to HuLuc63 mediated ADCC using human NK cells from healthy donors. HuLuc63 was used at doses ranging from 0.001-10 µg/ml. The results show that bortezomib pre-treatment significantly decreased the EC₅₀ for HuLuc63-mediated ADCC *in vitro*. Examples are shown for 4 different donors.

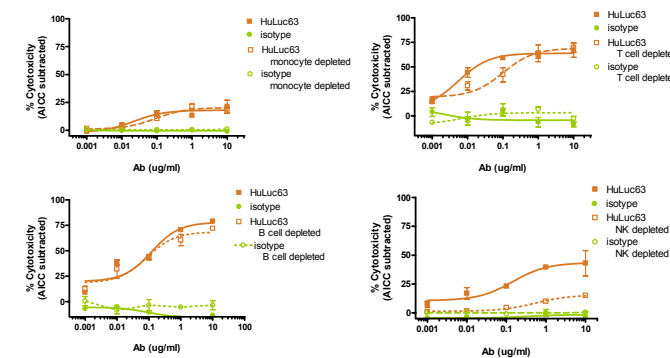
HuLuc63 eliminates myeloma cells via NK-mediated ADCC

Figure 3a. HuLuc63 mediates ADCC *in vitro* towards primary myeloma



HuLuc63 mediated ADCC of Cr-51 labeled primary myeloma cells by purified allogeneic or autologous NK cells. Shown is the average specific lysis from multiple chromium release assays using as effectors purified NK cells from healthy allogeneic donors (5 assays) or autologous NK cells (6 assays). HuLuc63-mediated ADCC was inhibited by blocking the Fc receptor (CD16) on NK cells with an anti-CD16 antibody.

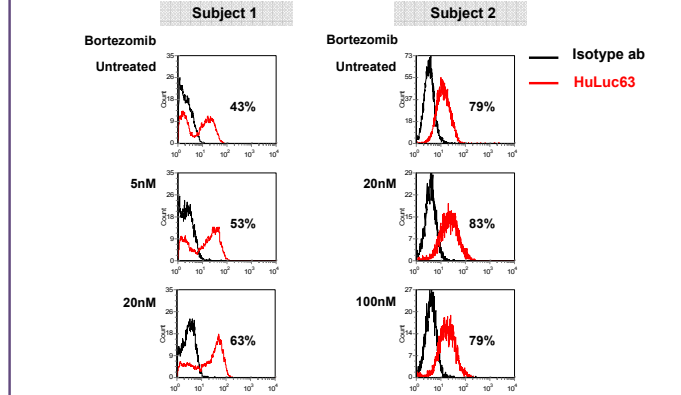
Figure 3b. Depletion of NK cells decreases HuLuc63-mediated ADCC



HuLuc63 mediated ADCC of Cr-51 labeled OPM2 cells by human PBMCs. PBMC preparations that were depleted of monocytes (A), B cells (B) or T cells (C) did not exhibit a significant decrease in effecting HuLuc63-mediated ADCC. However, depletion of NK cells (D) significantly impaired HuLuc63-mediated ADCC, indicating that HuLuc63 mediates myeloma cell killing via NK cells.

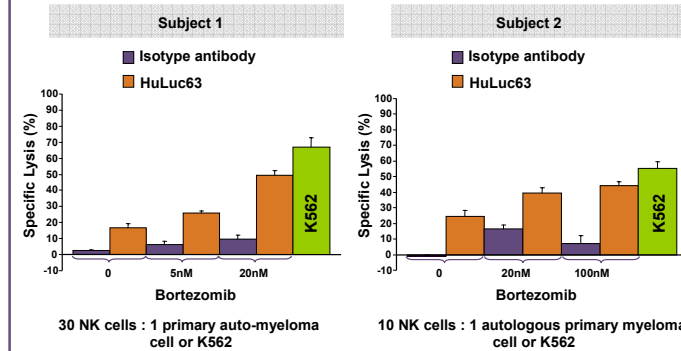
Bortezomib enhances HuLuc63-mediated ADCC of MM patient samples *in vitro*

Figure 5a. Cell surface CS1 on myeloma cells is not reduced after bortezomib treatment



Primary myeloma cells were purified from patient BM, treated with bortezomib (at doses ranging from 5-100 nM) or vehicle for 16-18 h and tested for CS1 expression by flow cytometry. Examples are shown for 2 different MM patients. Propidium iodide was used to exclude dead cells from the analysis. Histogram subtraction (HuLuc63-Isotype control) was used to calculate the percent of cells positive for CS1. CS1 levels on the myeloma cell surface were not reduced after bortezomib treatment.

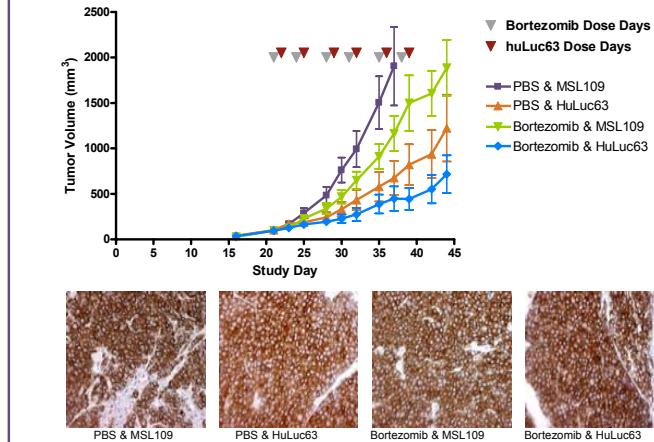
Figure 5b. Bortezomib pre-treatment enhances HuLuc63-mediated ADCC



The primary myeloma cells shown in Figure 5a were also subjected to HuLuc63 mediated ADCC using autologous NK cells from the same MM patients. HuLuc63 was used at 10 µg/ml. The results show that bortezomib pre-treatment significantly enhanced the *in vitro* ADCC activity mediated by HuLuc63.

Bortezomib enhances HuLuc63-mediated anti-tumor activity *in vivo*

Figure 7. HuLuc63 and bortezomib combination treatment in the OPM2 model



OPM2 tumor-bearing mice were treated with sub-optimal doses of HuLuc63 (1 mg/kg), or isotype control antibody twice weekly for three weeks. Bortezomib was given twice a week at 0.75 mg/kg to mice receiving either isotype control antibody or HuLuc63. IHC analysis of tumors with the non-competing anti-CS1 antibody 1G9 demonstrated that *in vivo* CS1 protein expression was not affected by any of the treatments. The results show significant anti-tumor activity of HuLuc63 alone and in combination with bortezomib. Mice in the combination treatment group exhibited on average 40-50% smaller tumors than in the HuLuc63 monotherapy group, and 60-70% smaller tumors than in the bortezomib group.

CONCLUSIONS

- ❖ HuLuc63 exhibits dose-dependent anti-tumor activity towards MM xenografts *in vivo*.
- ❖ HuLuc63 eliminates myeloma cells by NK cell-mediated ADCC.
- ❖ CS1 expression is uniform and high in MM, even after treatment with bortezomib.
- ❖ Bortezomib enhances the anti-myeloma activity of HuLuc63 *in vitro* and *in vivo*.
- ❖ These data support a combination therapy approach of HuLuc63 with bortezomib for the treatment of multiple myeloma.