

HuLuc63 in Combination Regimens with Conventional and Targeted Therapies Has Additive and Synergistic Anti-tumor Activity in Pre-clinical Models of Myeloma

Audie Rice, Myles Dillon, Shihao Chen, Anne van Abbema, Tina Emek, Debra Chao, and Daniel E. H. Afar
Department of Research, PDL BioPharma, Redwood City, CA, USA

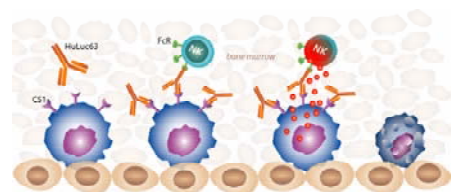
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ABSTRACT

HuLuc63 is a humanized monoclonal antibody that targets CS1 (CD2 subset 1, CRACC, SLAMF7, CD319), a cell surface glycoprotein that is highly and universally expressed on myeloma cells^{1,2}. In preclinical studies, we have shown that HuLuc63 treatment of mice with multiple myeloma (MM) xenograft tumors resulted in significant *in vivo* anti-tumor activity that is mediated at least in part by an antibody-dependent cellular cytotoxicity (ADCC) mechanism of action^{1,3,4,5}. The goals of this study were: (a) to evaluate the role of the NK cell in HuLuc63 mediated tumor inhibition *in vivo*; (b) to determine whether HuLuc63 in combination with a panel of drugs having distinct modes of action (dexamethasone, thalidomide, bevacizumab, melphalan, and bortezomib) could result in additional therapeutic benefit and provide a rationale for the design of future clinical trials. The results indicate that anti-tumor activity of HuLuc63 was significantly higher in mice having the normal number of functional NK cells. In addition, depletion of NK cells significantly reduced the anti-tumor efficacy of HuLuc63. Both results corroborate that the mechanism of action of HuLuc63 involves NK cell-mediated ADCC. In mouse pre-clinical combination studies with HuLuc63, addition of dexamethasone resulted in a statistically significant increase in anti-tumor activity over either agent alone ($p < 0.04$). Combination with thalidomide only showed a slight enhancement of HuLuc63-mediated tumor inhibition, albeit not statistically different from HuLuc63 alone. Co-treatment of HuLuc63 with the anti-VEGF anti-angiogenic monoclonal antibody bevacizumab resulted in a significant increase in tumor inhibition ($p < 0.05$) over either antibody alone. The strongest anti-myeloma activity was observed when HuLuc63 was combined with bortezomib, which appeared to result in a synergistic inhibition of tumor cell growth in mice. *In vitro* studies suggest that bortezomib pre-treatment of myeloma cells results in enhanced HuLuc63-mediated ADCC^{5,6}. However, the mechanism of action of this synergy is not yet known. HuLuc63 is currently in a Phase I clinical trial for the treatment of MM⁷.

Targeting myeloma with HuLuc63

Figure 1. HuLuc63 targets CS1 on myeloma cells



- CS1, a cell surface glycoprotein, is expressed on > 95% of myeloma cells
- HuLuc63 specifically targets CS1 and recruits NK cells
- HuLuc63-recruited NK cells eliminate myeloma cells through ADCC

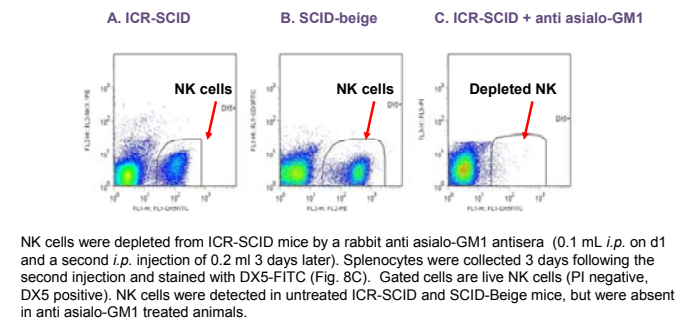
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RESULTS

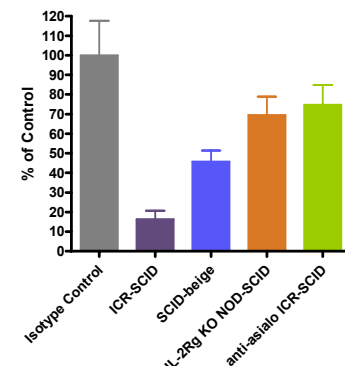
NK cell depletion in SCID mice

Figure 2. Depletion of NK cells in ICR-SCID mice by α -asialo-GM1 antibody treatment



HuLuc63 anti-tumor activity *in vivo* is mediated by NK cells

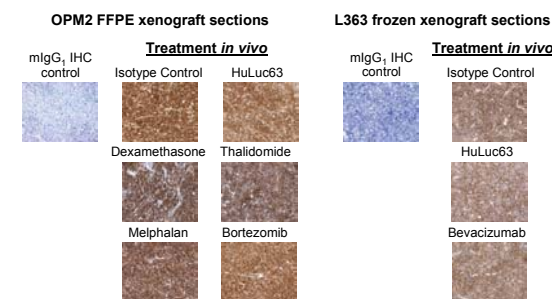
Figure 3. NK depletion or deficiency results in diminished activity with HuLuc63



The anti-tumor activity of HuLuc63 was evaluated in mice with normal NK cell function (ICR-SCID) and compared to the anti-tumor activity seen in mice that had been either depleted of NK or are deficient in NK cell function (SCID-beige and NOD-SCID/IL-2R γ KO mice). For depletion of NK, SCID mice received anti asialo-GM1 three days prior to the start of HuLuc63 or isotype control antibody treatment (10 mg/kg) then 2x per week for the duration of the study. Average tumor volumes were normalized to the isotype control group within each study and plotted as percent control for comparisons between the different animal models. As expected, the anti-tumor activity of HuLuc63 was significantly higher in mice having the normal number of functional NK cells.

Anti-myeloma agents do not reduce CS1 expression on myeloma xenografts

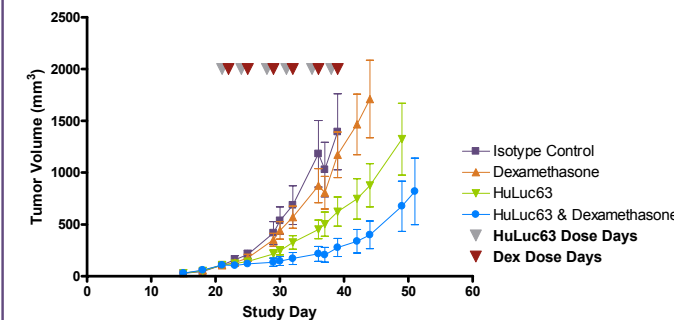
Figure 4. IHC analysis of CS1 expression in myeloma xenografts



CS1 expression on tumor xenograft samples was evaluated by formalin fixed paraffin embedded IHC (or frozen sections for the bevacizumab study) using 1G9, a mouse IgG, monoclonal antibody against the intracellular domain of CS1. In all studies the expression of CS1 remained uniformly high on OPM2 and L363 xenograft tumor sections irrespective of treatment regimen.

Dexamethasone increases anti-tumor activity of HuLuc63

Figure 5. Sub-optimal doses of HuLuc63 in combination with Dex in OPM2 model

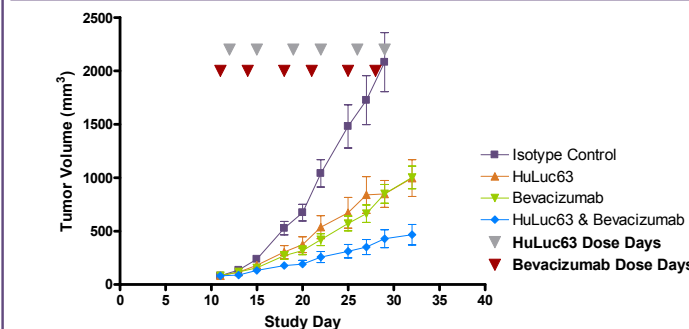


OPM2 bearing SCID mice (n=10 per group) were treated with either 1.0 mg/kg isotype control, 10 mg/kg dexamethasone, 1.0 mg/kg HuLuc63, or the combination of HuLuc63 and dexamethasone. The dose of 1 mg/kg of HuLuc63 is considered a sub-optimal dose with respect to anti-tumor efficacy⁶. The combination of dexamethasone with HuLuc63 showed a statistically significant increase in anti-tumor activity over either agent alone ($p < 0.04$).

*For all studies statistical analyses were performed using an ANOVA with a Tukey's Studentized Range Test correction after a square root transformation to make the group variances more uniform.

HuLuc63 shows increased tumor killing in combination with anti-angiogenesis inhibitor bevacizumab

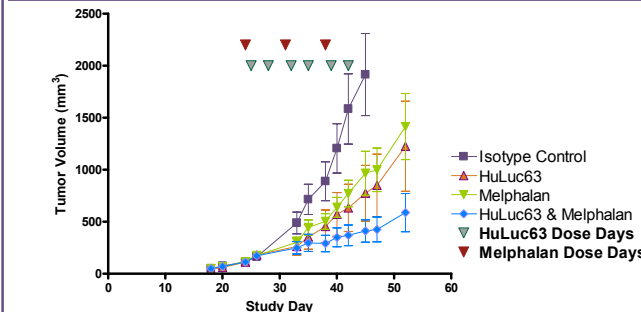
Figure 6. HuLuc63 combination study with bevacizumab in L363 model



L363 bearing SCID mice (n=12 per group) were treated with either 10 mg/kg isotype control, 10 mg/kg bevacizumab, 10 mg/kg HuLuc63, or the combination of both HuLuc63 and bevacizumab at 10 mg/kg each. Co-treatment of HuLuc63 with bevacizumab resulted in a significant increase in tumor inhibition ($p < 0.05$) over that observed with either antibody when used as a single agent.

HuLuc63 combination with Melphalan

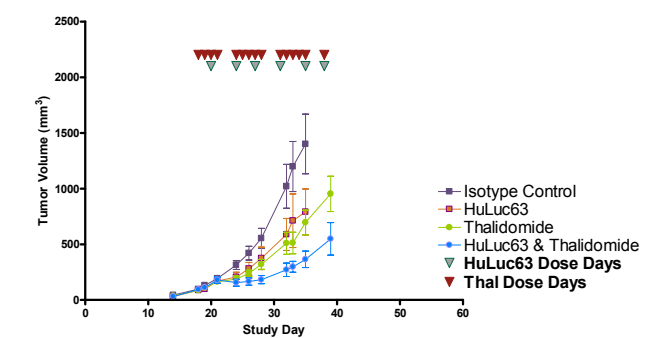
Figure 7. Sub-optimal doses of HuLuc63 in combination with melphalan in OPM2 model



The combination of Melphalan with HuLuc63 showed a slight increase in anti-tumor activity over either treatment alone but did not reach statistical significance. All the treatment groups were statistically different from the isotype control group ($p < 0.05$) but not from each other. Melphalan was dosed at 9 mg/kg; HuLuc63 and isotype control antibody were dosed at 1.0 mg/kg 2x per week for 3 weeks.

HuLuc63 in combination with Thalidomide shows enhanced anti-tumor effect

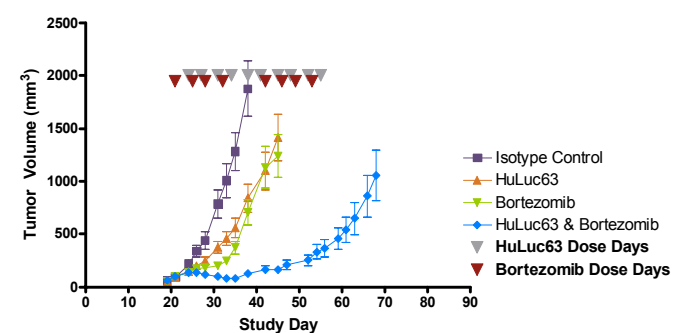
Figure 8. Sub-optimal doses of HuLuc63 in combination with thalidomide in OPM2 model



Thalidomide (50 mg/kg *i.p.*) was tested in combination with HuLuc63 (1.0 mg/kg) in the OPM2 xenograft model. The combination showed a slight enhancement of tumor inhibition but did not reach a statistically significant increase over that of HuLuc63 alone.

HuLuc63 in combination with bortezomib has potent anti-tumor activity

Figure 9. Sub-optimal doses of HuLuc63 in combination with bortezomib in OPM2 model



The strongest anti-myeloma activity was observed when HuLuc63 was combined with bortezomib, which appeared to result in a synergistic inhibition of OPM2 MM tumor cell growth. HuLuc63 was dosed twice weekly for 5 weeks at 1.0 mg/kg and bortezomib dosed at 1 mg/kg for two dosing cycles, each cycle consisting of twice weekly dosing for 2 weeks followed by a week of rest. The HuLuc63 and bortezomib combination group was statistically different from either treatment alone and from the isotype control group ($p < 0.001$). It was also found that the separate treatments of HuLuc63 alone and the bortezomib alone were statistically different from the isotype control group ($p < 0.001$) but not from each other.

CONCLUSIONS

- HuLuc63 inhibits myeloma cell growth by NK cell-mediated ADCC *in vivo*
- Strategies to increase NK cell number or activation state in conjunction with HuLuc63 treatment may increase anti-tumor activity
- Enhanced tumor killing is obtained when HuLuc63 is used in combination with conventional or targeted therapies
- None of the agents tested negatively affected the CS1 expression level on myeloma cells or diminished the anti-myeloma activity of HuLuc63.
- These data support investigation of potential combination therapies utilizing HuLuc63 with different classes of approved drugs to enhance its anti-myeloma effects.