



Figure 6 *In vivo* activity of hGal9. (a) Average tumor volumes of hGal9-untreated (only given PBS) and -treated mice during the treatment period. (b) Untreated IM9 inoculated nude mice (left) and those treated with hGal9 for 10 days (right).

potential limitation for recapitulating MM, which normally represents multiple tumors in systemic BM sites. It is urgently desired to explore the anti-myeloma effect of hGal9 at multiple BM sites.

In conclusion, we have shown the activity of hGal9 against myeloma cell lines and primary human MM cells both *in vitro* and *in vivo*, and that the activation of JNK/p38-H2AX pathways is involved in this anti-myeloma activity. Further clinical study of hGal9 as a new anti-MM agent thus certainly seems to be warranted.

Conflict of interest

The authors declare no conflict of interest.

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References

- Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, Feizi T et al. Galectins: a family of animal beta-galactoside-binding lectins. *Cell* 1994; **76**: 597–598.
- Barondes SH, Cooper DN, Gitt MA, Leffler H. Galectins: structure and function of a large family of animal lectins. *J Biol Chem* 1994; **269**: 20807–20810.
- Brewer CF. Binding and cross-linking properties of galectins. *Biochem Biophys Acta* 2002; **1572**: 255–262.
- Wada J, Ota K, Kumar A, Wallner EJ, Kanwar YS. Developmental regulation, expression, and apoptotic potential of galectin-9, a beta-galactosides binding lectin. *J Clin Invest* 1997; **99**: 2452–2461.
- Perillo NL, Marcus ME, Baum LG. Galectins: versatile modulators of cell adhesion, cell proliferation, and cell death. *J Mol Med* 1998; **76**: 402–412.
- Rabinovich GA. Galectins: an evolutionarily conserved family of animal lectins with multifunctional properties; a trip from the gene to the clinical therapy. *Cell Death Differ* 1999; **6**: 711–721.
- Liu FT. Galectins: a new family of regulators of inflammation. *Clin Immunol* 2000; **97**: 79–88.
- Matsumoto R, Matsumoto H, Seki M, Hata M, Asano Y, Kanegasaki S et al. Human ecalectin, a variant of human galectin-9, is a novel eosinophil chemottractant produced by T lymphocytes. *J Biol Chem* 1998; **273**: 16976–16984.
- Seki M, Oomizu S, Sakata K, Arikawa T, Watanabe K, Ito K et al. Galectin-9 suppresses the generation of Th17, promotes the induction of regulatory T cells, and regulates experimental autoimmune arthritis. *Clin Immunol* 2008; **127**: 78–88.
- Dai SY, Nakagawa R, Itoh A, Murakami H, Kashio Y, Abe H et al. Galectin-9 induces maturation of human monocyte-derived dendritic cells. *J Immunol* 2005; **175**: 2974–2981.
- Kageshita T, Kashio Y, Yamauchi A, Seki M, Abedin MJ, Nishi N et al. Possible role of galectin-9 in cell aggregation and apoptosis of human melanoma cell lines and its clinical significance. *Int J Cancer* 2002; **99**: 809–816.
- Lu LH, Nakagawa R, Kashio Y, Ito A, Shoji H, Nishi N et al. Characterization of galectin-9-induced death of Jurkat T cells. *J Biochem* 2007; **141**: 157–172.
- Okudaira T, Hirashima M, Ishikawa C, Makishi S, Tomita M, Matsuda T et al. A modified version of galectin-9 suppresses cell growth and induced apoptosis of human T-cell leukemia virus type I-infected T-cell lines. *Int J Cancer* 2007; **120**: 2251–2261.
- Makishi S, Okudaira T, Ishikawa C, Sawada S, Watanabe T, Hirashima M et al. A modified version of galectin-9 induces cell cycle arrest and apoptosis of Burkitt and Hodgkin lymphoma cells. *Br J Haematol* 2008; **142**: 583–594.
- Nobumoto A, Nagahara K, Oomizu S, Katoh S, Nishi N, Takeshita K et al. Galectin-9 suppresses tumor metastasis by blocking adhesion to endothelium and extracellular matrices. *Glycobiology* 2008; **18**: 735–744.
- Kiziltepe T, Hideshima T, Ishitsuka K, Ocio EM, Rajc N, Catley L et al. JS-K, a GST-activated nitric oxide generator, induces DNA double-strand breaks, activates DNA damage response pathways, and induces apoptosis *in vitro* and *in vivo* in human multiple myeloma cells. *Blood* 2007; **110**: 709–718.
- Kuroda J, Kamitsuyu J, Kimura S, Ashihara E, Kawata E, Nakagawa Y et al. Anti-myeloma effect of homoharringtonine with concomitant targeting of the myeloma-promoting molecules, Mcl-1, XIAP, and beta-catenin. *Int J Hematol* 2008; **87**: 507–515.
- Nishi N, Itoh A, Fujiyama A, Yoshida N, Araya S, Hirashima M et al. Development of highly stable galectins: truncation of the linker peptide confers protease-resistance on tandem-repeat type galectins. *FEBS Lett* 2005; **579**: 2058–2064.
- Kuroda J, Kimura S, Segawa H, Sato K, Matsumoto S, Nogawa M et al. p53-independent anti-tumor effects of the nitrogen-containing bisphosphonate zoledronic acid. *Cancer Sci* 2004; **95**: 186–192.
- Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DCS et al. Bim and Bad mediate imatinib-induced killing of Bcr/Abi¹ leukemic cells and resistance due to their loss is overcome by a BH3 Mimetic. *Proc Natl Acad Sci USA* 2006; **103**: 14907–14912.

- 21 Lu C, Zhu F, Cho YY, Tang F, Zykova T, Ma WY *et al*. Cell apoptosis: requirement of H2AX in DNA ladder formation, but not for the activation of caspase-3. *Mol Cell* 2006; **23**: 121–132.
- 22 Sluss HK, Davis RJ. H2AX is a target of the JNK signaling pathway that is required for apoptotic DNA fragmentation. *Mol Cell* 2006; **23**: 152–153.
- 23 Lu C, Shi Y, Wang Z, Song Z, Zhu M, Cai Q *et al*. Serum starvation induces H2AX phosphorylation to regulate apoptosis via p38 MAPK pathway. *FEBS Lett* 2008; **582**: 2703–2708.
- 24 Xu F, Sharma S, Gardner A, Tu Y, Raitano A, Sawyers C *et al*. Interleukin-6-induced inhibition of multiple myeloma cell apoptosis: support for the hypothesis that protection is mediated via inhibition of the JNK/SAPK pathway. *Blood* 1998; **92**: 241–251.
- 25 Hideshima T, Mitsiades C, Akiyama M, Hayashi T, Chauhan D, Richardson P *et al*. Molecular mechanisms mediating antimyeloma activity of proteasome inhibitor PS-341. *Blood* 2003; **101**: 1530–1534.
- 26 Anderson KC. Lenalidomide and thalidomide: mechanisms of action—similarities and differences. *Semin Hematol* 2005; **42**: S3–S8.
- 27 Chauhan D, Anderson KC. Mechanism of cell death and survival in multiple myeloma (MM): therapeutic implications. *Apoptosis* 2003; **8**: 337–343.
- 28 Hideshima T, Catley L, Yasui H, Ishitsuka K, Raju N, Mitsiades C *et al*. Perifosine, an oral bioactive novel alkylphospholipid, inhibits Akt and induces *in vitro* and *in vivo* cytotoxicity in human multiple myeloma cells. *Blood* 2006; **107**: 4053–4062.
- 29 Carew JS, Nawrocki ST, Reddy VK, Bush D, Rehg JE, Goodwin A *et al*. The novel polyamine analogue CGC11093 enhances the antimyeloma activity of bortezomib. *Cancer Res* 2008; **68**: 4783–4790.
- 30 Hideshima T, Akiyama M, Hayashi T, Richardson P, Schlossman R, Chauhan D *et al*. Targeting p38 MAPK inhibits multiple myeloma cell growth in the bone marrow milieu. *Blood* 2003; **101**: 703–705.
- 31 Mitsiades CS, Ocio EM, Pandiella A, Maiso P, Gajate C, Garayoa M *et al*. Aplidin, a marine organism-derived compound with potent antimyeloma activity *in vitro* and *in vivo*. *Cancer Res* 2008; **68**: 5216–5223.
- 32 Feng R, Oton A, Mapara MY, Anderson G, Belani C, Lentzsch S. The histone deacetylase inhibitor, PXD101, potentiates bortezomib-induced anti-multiple myeloma effect by induction of oxidative stress and DNA damage. *Br J Haematol* 2007; **139**: 385–397.
- 33 Shimizu T, Nakazato T, Xian MJ, Sagawa M, Ikeda Y, Kizaki M. Resveratrol induces apoptosis of human malignant B cells by activation of caspase-3 and p38 MAP kinase pathways. *Biochem Pharmacol* 2006; **71**: 742–750.
- 34 Kashio Y, Nakamura K, Abedin MJ, Seki M, Nishi N, Yoshida N *et al*. Galectin-9 induces apoptosis through the calcium-calpain-caspase-1 pathway. *J Immunol* 2003; **170**: 3631–3636.
- 35 Jimbo A, Fujita E, Kourouk Y, Ohnishi I, Inohara N, Kuida K *et al*. ER stress induces caspase-8 activation, stimulating cytochrome c release and caspase-9 activation. *Exp Cell Res* 2003; **283**: 156–166.

Supplementary Information accompanies the paper on the Leukemia website (<http://www.nature.com/leu>)

