

## Abstract

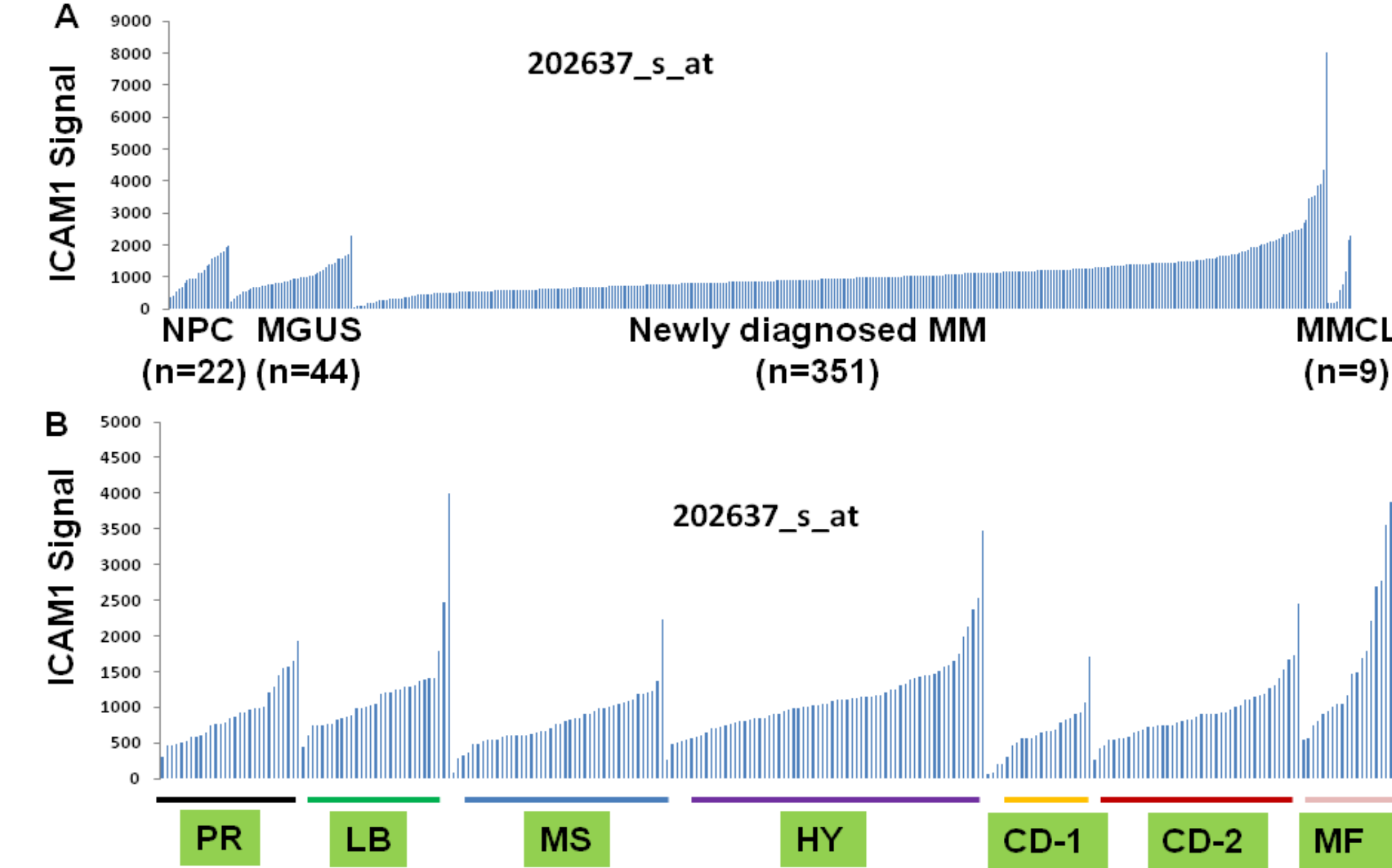
**Background:** Myeloma (MM) - stromal cell-cell interactions play a crucial role in MM cell growth, survival, and drug resistance. Intercellular adhesion molecule-1 (ICAM-1) is up-regulated in different cancers, including MM, and represents one of the major adhesion molecules mediating tumor-stromal cell-cell contacts. Previous investigations have shown that ICAM antibodies induce antitumor effects in SCID mice xenografted with MM cell lines. Since optimal anti-tumor effects were observed with the use of intact antibody, ADCC and/or CDC are likely involved. We have examined the anti-MM activity in the SCID-hu mouse model of a specific humanized ICAM1 antibody (BI-505, BioInvent, Sweden) using primary MM cells.

**Materials and Methods:** The expression of ICAM1 was examined on plasma cells from 22 donors, 351 newly diagnosed MMs, and 9 MM cell lines using Affymetrix U133 Plus2 chips. Human fetal femurs and tibias, obtained at 17 to 22 gestational weeks, were cut into fragments and implanted subcutaneously in 16 SCID mice (SCID-hu) at age 6 to 8 weeks. Four weeks after bone implantation, approximately  $5 \times 10^6$  CD138+ plasma cells from MM patients were injected directly into the human fetal bone of the SCID-hu mice in a final volume of 30 to 40  $\mu$ l of phosphate-buffered saline (PBS). Human immunoglobulin (hlg) levels measured by ELISA were used as an indicator of myeloma cell growth. Four weeks after injection of tumor cells, when hlg level reached 10  $\mu$ g/mL or higher in 2 consecutive samples, the mice were divided into four groups (n=4); control group (no injected MM cells and no drug treatment); the other 3 groups were injected with MM cells: the isotype control group (isotype IgG 2mg/kg, i.p., twice weekly), BI-505 group (2mg/kg, i.p., twice weekly) and bortezomib group (1mg/kg, i.p., twice weekly). Bone remodeling was detected by X-radiography and by measuring bone mineral density (BMD). Tumor cells were detected by immunohistochemistry using the CD138 antibody.

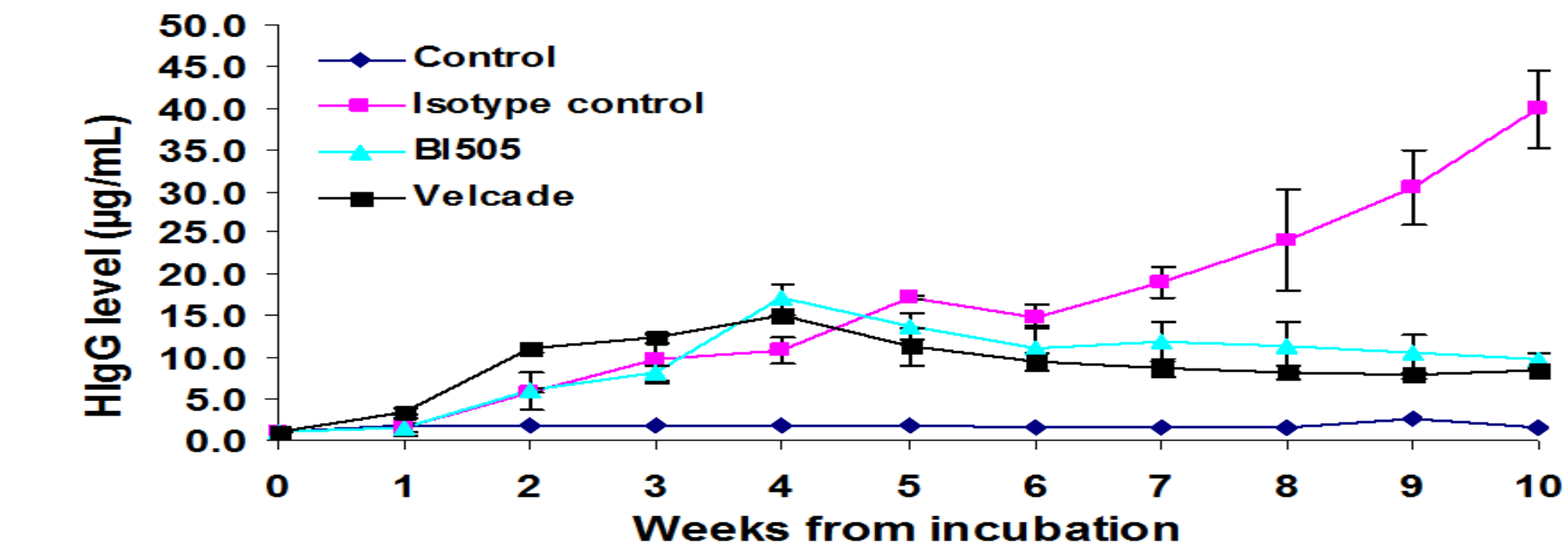
**Results:** Expression of ICAM1 was present in normal plasma cells, MM cells and MM cell lines. Interestingly, the MAF group, a subgroup with t(14;16) and t(14;20), showed significantly higher expression than the other subtypes of MM, indicating a possible target subgroup for antibody treatment. With a follow-up of 10 weeks, the tumor burden in the mice treated with BI-505 or bortezomib was significantly lower compared with the isotype control group ( $P$ : BI505 = 0.008;  $P$ : bortezomib < 0.01). Also, the number of MM tumor cells staining with the CD138 antibody was significantly less in samples treated with either BI-505 or bortezomib than in the isotype control group ( $P$  < 0.01). In addition, 6 weeks after injection of tumor cells, X-rays showed severe bone resorption in the isotype-control group, while there was no obvious bone resorption in the fetal bones after treatment with BI-505 or bortezomib. The BMD ( $0.0715 \pm 0.006$  g/cm<sup>2</sup>) of isotype control was significantly lower than that in the other 3 groups including control:  $0.1278 \pm 0.006$  g/cm<sup>2</sup>, BI-505 group:  $0.102 \pm 0.0064$  g/cm<sup>2</sup>, and bortezomib group:  $0.106 \pm 0.0059$  g/cm<sup>2</sup>. The number of TRAP-positive cells was significantly higher in the isotype control group than in the other 3 groups ( $P$  < 0.01).

**Conclusion:** ICAM1 expression is up-regulated in all MM subtypes, but most in the MF subgroup. The ICAM1 antibody BI-505 significantly inhibits primary MM cell growth and bone destruction in the SCID-hu mouse model to the same degree as velcade.

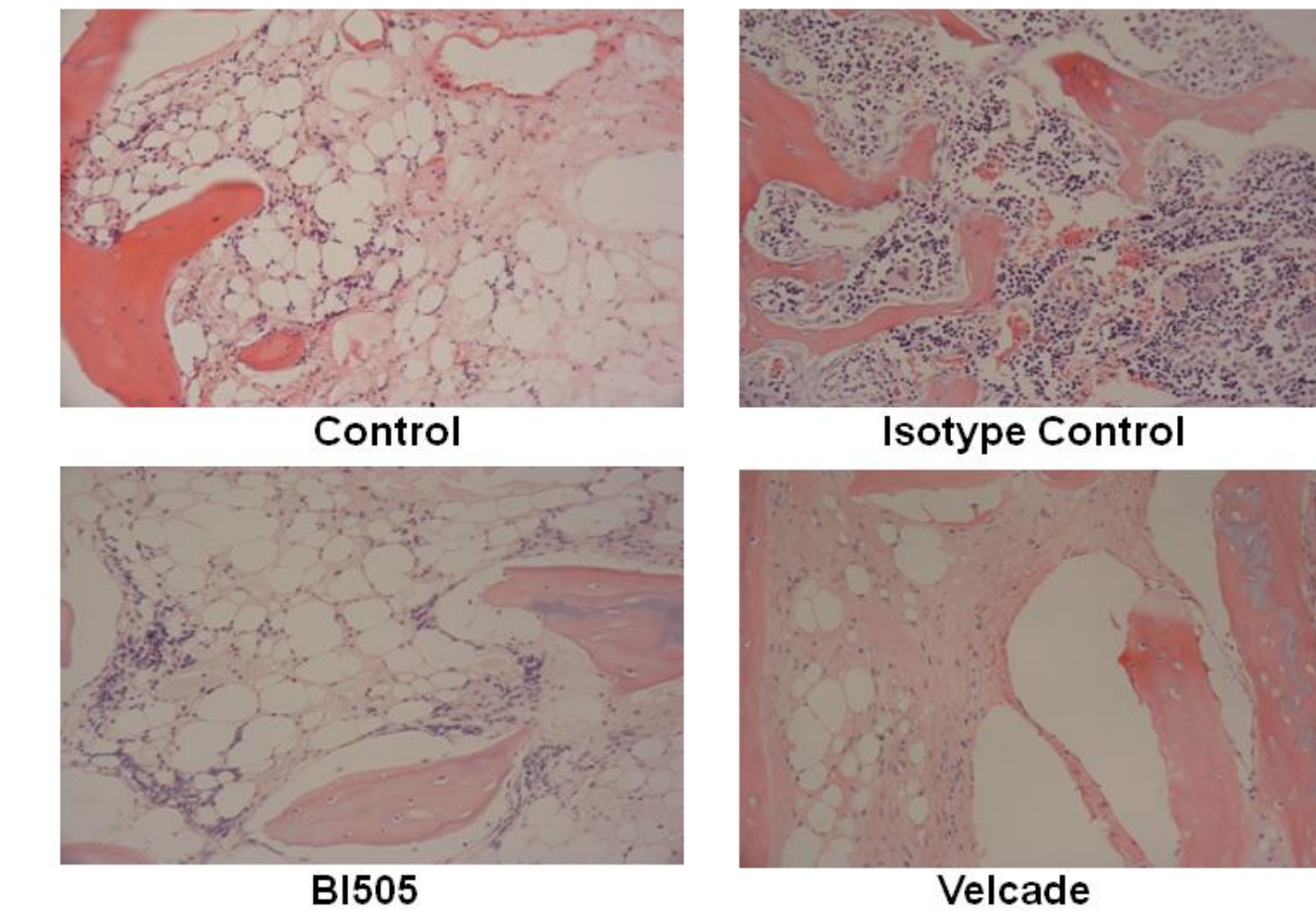
## Results



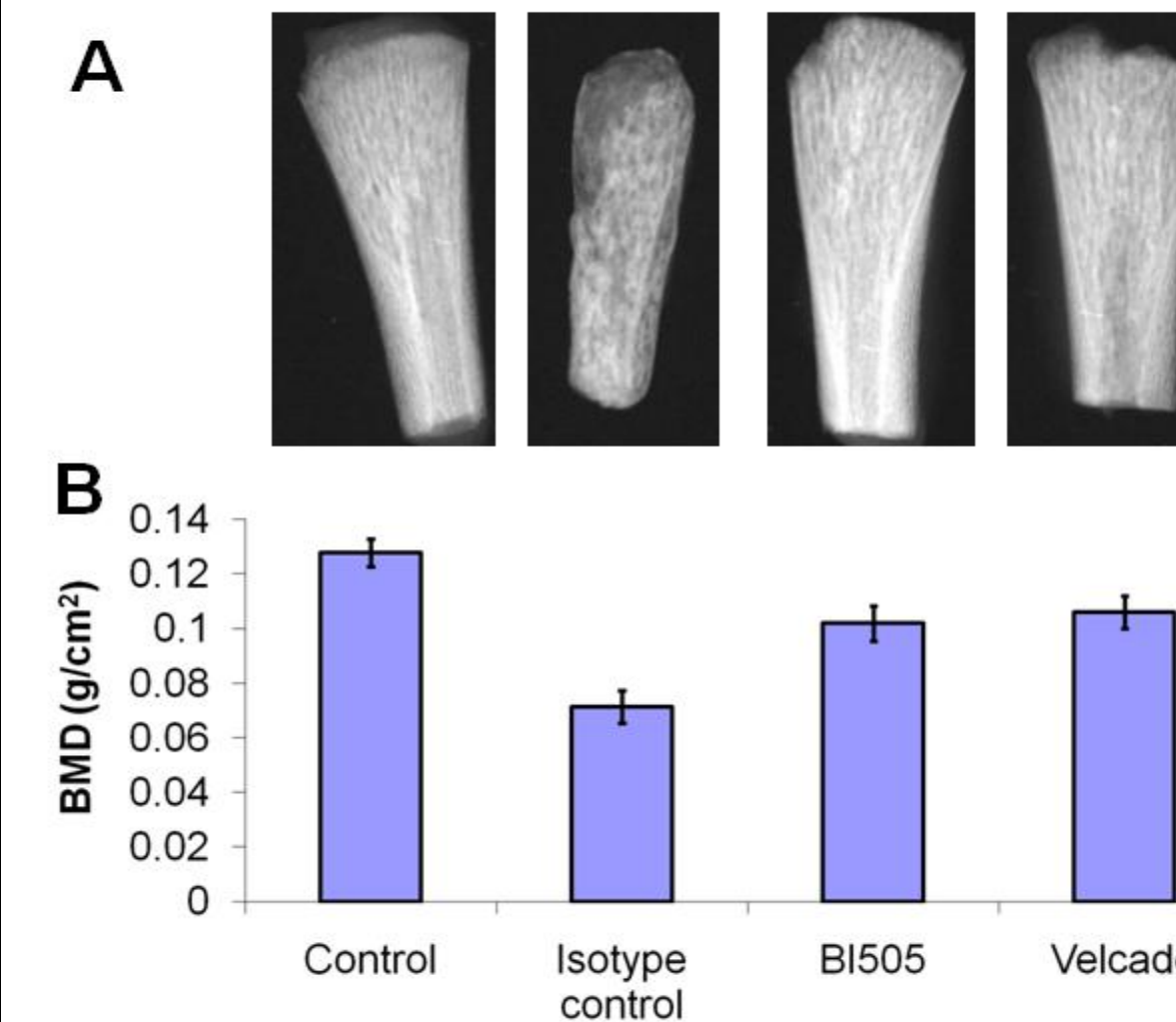
**Figure 1. The expression of ICAM1 is increased in a subset of newly diagnosed MM patients.** (A) The expression of ICAM1 was examined in plasma cells from 22 donors (NPC), 44 monoclonal gammopathy of undetermined significance (MGUS), 351 newly diagnosed MMs, and 9 MM cell lines (MMCL) using Affymetrix U133 Plus2 chips. ICAM1 expression is increased in a subset of newly diagnosed MM patients compared with NPC, MGUS and MMCLs. (B) The expression of ICAM1 was compared in seven genetic myeloma subgroups (Zhan et al, Blood 2006), the MAF (MF) group with either t(14;16) or t(14;20) showed significantly higher expression than the other subtypes of MM.



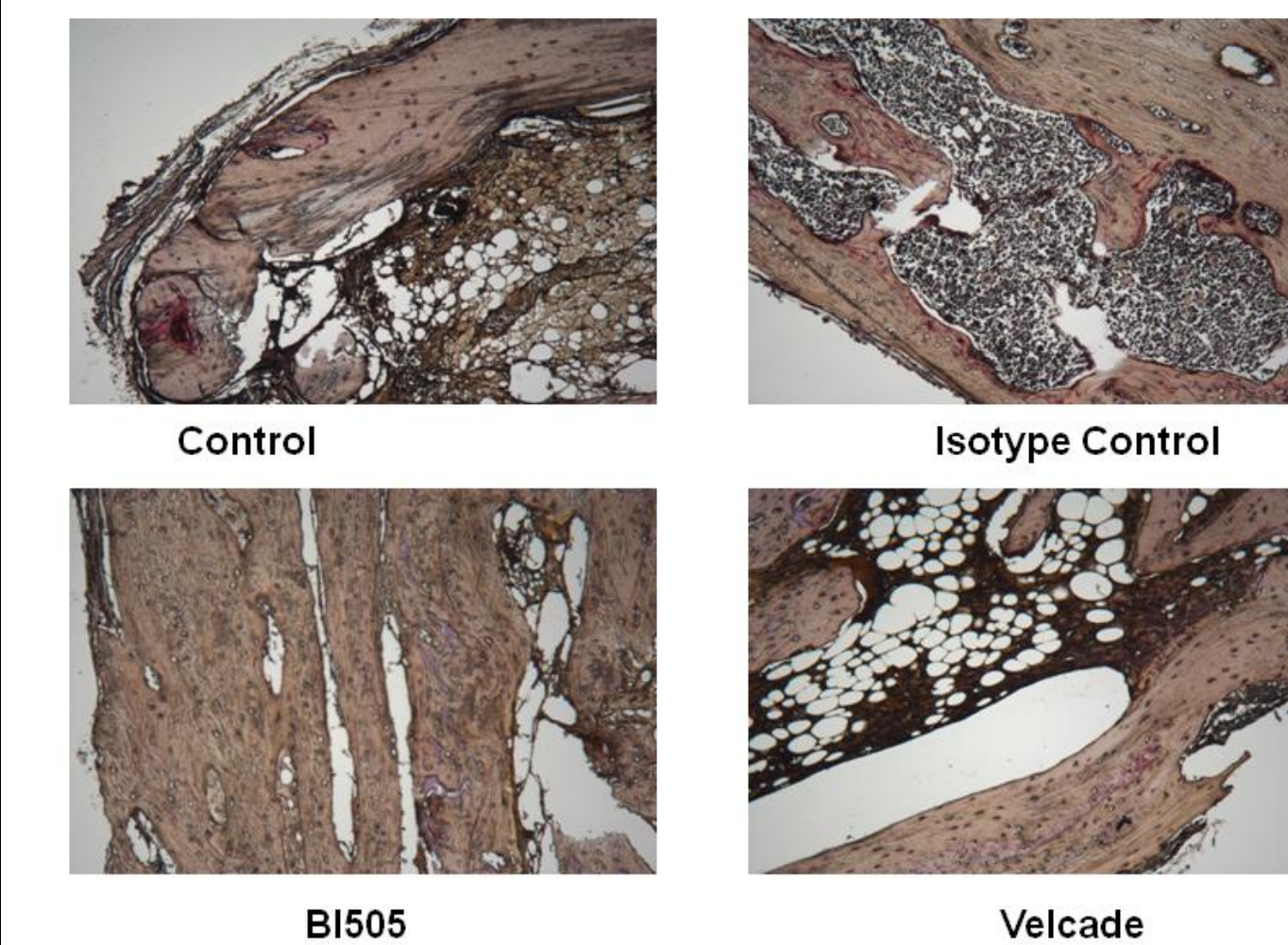
**Figure 2. The specific human ICAM-1 antibody BI-505 inhibits primary myeloma cell growth in the SCID-hu mouse model.** Human fetal femurs and tibias, obtained at 17 to 22 gestational weeks, were cut into fragments and implanted subcutaneously in 16 SCID mice (SCID-hu) at age 6 to 8 weeks. Four weeks after bone implantation, 2 to  $14 \times 10^6$  bone marrow cells from MM patients, containing >20% plasma cells were injected directly into the human bone of SCID-hu mice in a final volume of 30 to 40  $\mu$ l of phosphate-buffered saline (PBS). Mouse sera were serially monitored for human immunoglobulin (hlg) and these levels were used as an indicator of myeloma cell growth by ELISA. The mice were divided into four groups (n=4), Control group (not injected myeloma cells and no drug treatment), Isotype control group (injected myeloma cells with Isotype IgG treatment, 2mg/kg, i.p., twice weekly), BI505 group (treated with BI-505, 2mg/kg, i.p., twice weekly) and the Velcade group (1mg/kg, i.p., twice weekly). Tumor burden detected by hlg levels showed significantly decreased in the groups treated with BI505- and velcade compared with the group-treated Isotype control group ( $P$  < 0.05) while there is no hlg level change in control group.



**Figure 3. Hematoxylin/eosin staining was performed for quantitation of total infiltrated nucleated cells in implanted bone.** Representative pictures show significantly lower numbers of nucleated cells in BI-505 treated, and velcade treated mice compared with isotype control treated mice.



**Figure 4. BI-505 inhibits bone damage induced by myeloma cells in the SCID-hu mice.** (A) X-radiography evaluation for bone remodeling. After treated for 6 weeks, all mice in four groups were sacrificed, and the implanted human bones were taken out and fixed in 10% formalin solution. Radiographs were taken with an AXR Minishot-100 beryllium source instrument (Associated X-Ray Imaging Corp., Haverhill, MA, USA) used a 100s exposure at 30 kV. As shown in this Figure, severe bone resorption was found in the Isotype-control group of 6 weeks after injection of tumor cells, while there is no obvious bone resorption change in the groups treated with BI505 and Velcade. (B) Measurement of bone mineral density (BMD). BMDs of implanted human bones in SCID-hu mice revealed that the BMD in Isotype control ( $0.0715 \pm 0.006$  g/cm<sup>2</sup>) was significantly lower than that in other three groups (Control:  $0.1278 \pm 0.006$  g/cm<sup>2</sup>; BI505:  $0.102 \pm 0.0064$  g/cm<sup>2</sup>; and Velcade:  $0.106 \pm 0.0059$  g/cm<sup>2</sup>).



**Figure 5. Quantitation of bone marrow osteoclasts.** Trap staining for detection of osteoclasts was performed on primary and contralateral bones harvested from SCID-hu mice at end of the experiment. There were significantly greater numbers of osteoclasts (purple stain) in isotype control treated compared with BI-505 or velcade treated SCID-hu mice.

## Acknowledgements

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