Investigating the effect of zoledronic acid treatment on myeloma cell homing to bone *in vivo*

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Introduction

Multiple myeloma is a clonal plasma cell malignancy characterised by accumulation of malignant plasma cells in the bone marrow. Accumulation of these abnormal cells in the bone marrow results in multiple osteolytic lesion which causes bone pain and pathological fractures. Previous works have shown that the myeloma cell activates osteoclast thereby increasing osteoclastic bone resorption which causes bone disease in myeloma. This results in the release of bone growth factors into the surrounding environment which makes the bone a suitable niche for the survival and proliferation of myeloma cell thereby establishing a vicious cycle (Fig 1). So we hypothesised that **blocking bone resorption with zoledronic acid will prevent the colonisation of myeloma cells in bone.**

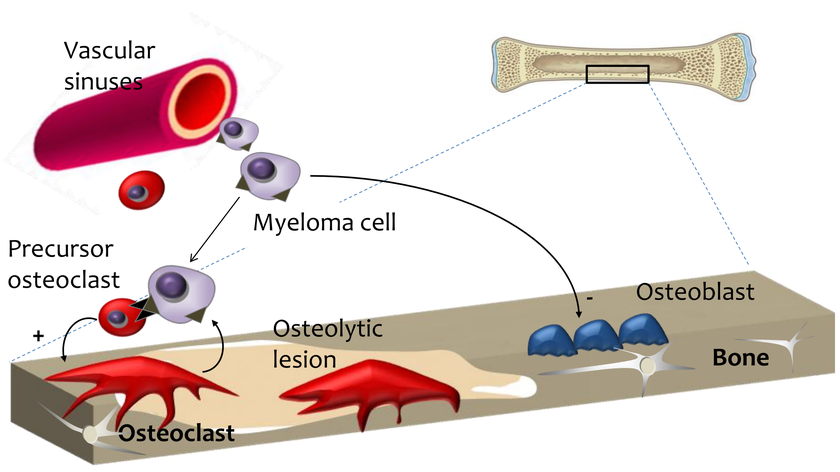
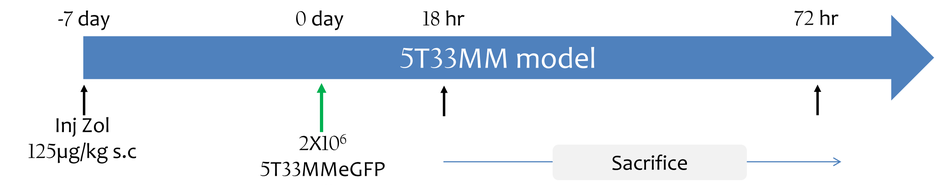


Fig 1: Osteoclast and myeloma cell interaction. Myeloma cells secrete tumour derived growth factors such as osteoclast activating factors (OAFs) that activates osteoclast which induces bone resorption. Increased bone resorption in turn releases bone derived growth factors such as TGF-β and IL-6 in the bone microenvironment which supports the myeloma cells survival and proliferation thereby establishing a vicious cycle.

Aim

To investigate the effect of zoledronic acid treatment on the homing and colonisation of myeloma cells in the 5T33MM murine myeloma model.

Study Design



C57BLKalwRij mice were treated with a single dose of zoledronic acid (125µg/kg s.c.) or PBS. One week after treatment, all mice were injected with 2X10^6 5T33MM-eGFP cells via the tail vein and were sacrificed after 18 and 72 hr. The response to zoledronic acid treatment was assessed by static histomorphometry for osteoclast in bone, serum levels of TRAP5b for osteoclastic activity and micro-computed tomography for assessing structural changes. Multiphoton microscopy was used to quantify the number of 5T33-eGFP cells in the bone.

Results

I.Single dose zoledronic acid treatment showed a reduction in the number of osteoclast.

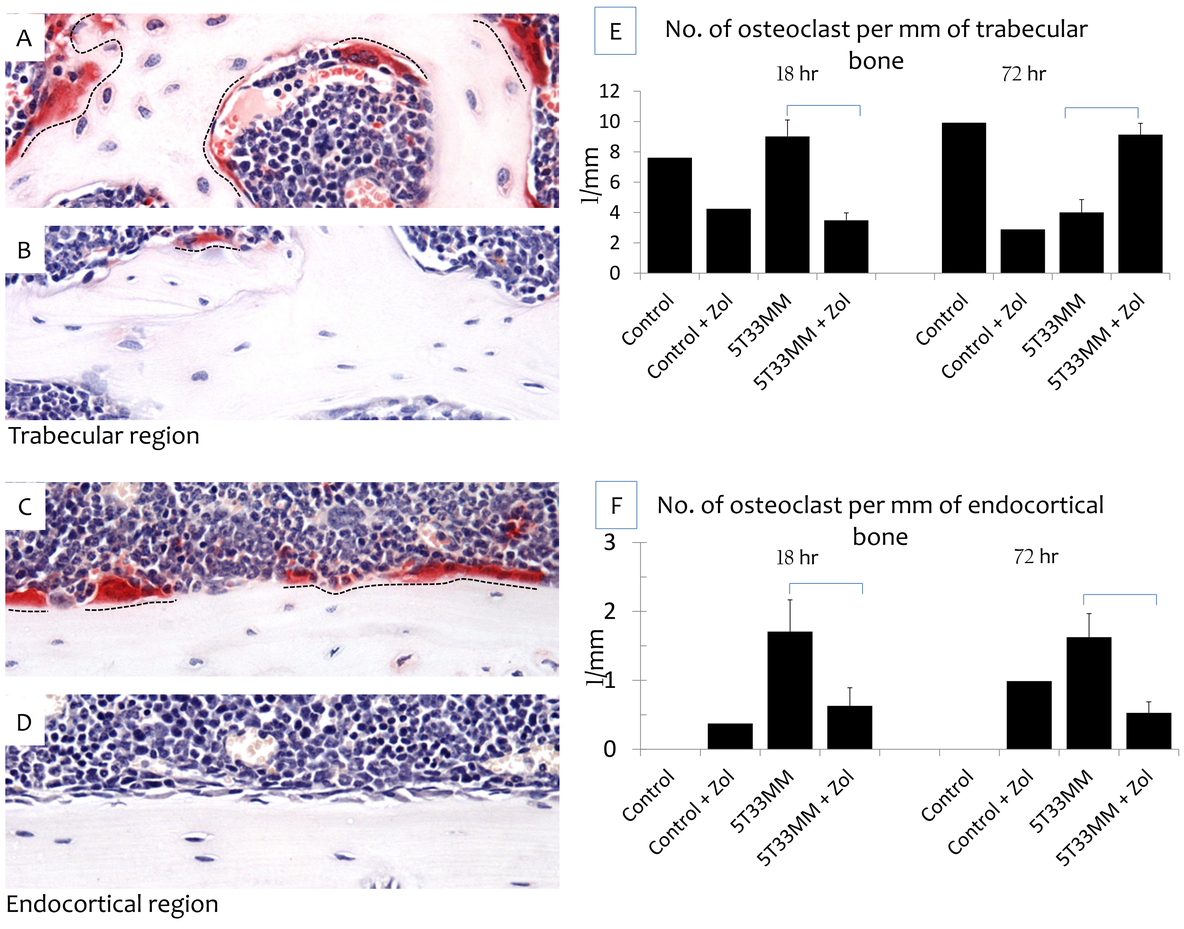


Fig 2: The effect of zoledronic acid on the number of osteoclast in the 5T33MM myeloma model. TRAP stained section of femoral metaphysis of 5T33MM + vehicle (A, C) and zoledronic acid treated group (B, D) showing osteoclast occupying the bone surface. E, F shows the number of osteoclast on the bone surface between the vehicle and zoledronic acid treated group in both the trabecular and endocortical regions.

II.Zoledronic acid showed significant reduction in serum levels of both TRAP5b and PINP

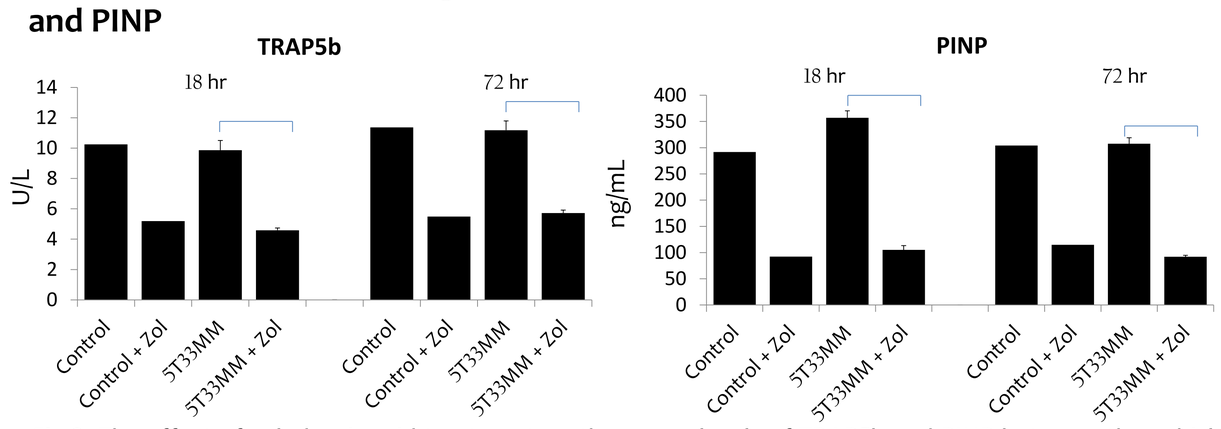


Fig 3: The effect of zoledronic acid treatment on the serum levels of TRAP5b and PINP between the vehicle treated and zoledronic acid treated 5T33MM myeloma model after 18hr and 72 hr post tumour cell injection.

III. Zoledronic acid treatment showed greater trabecular bone volume.

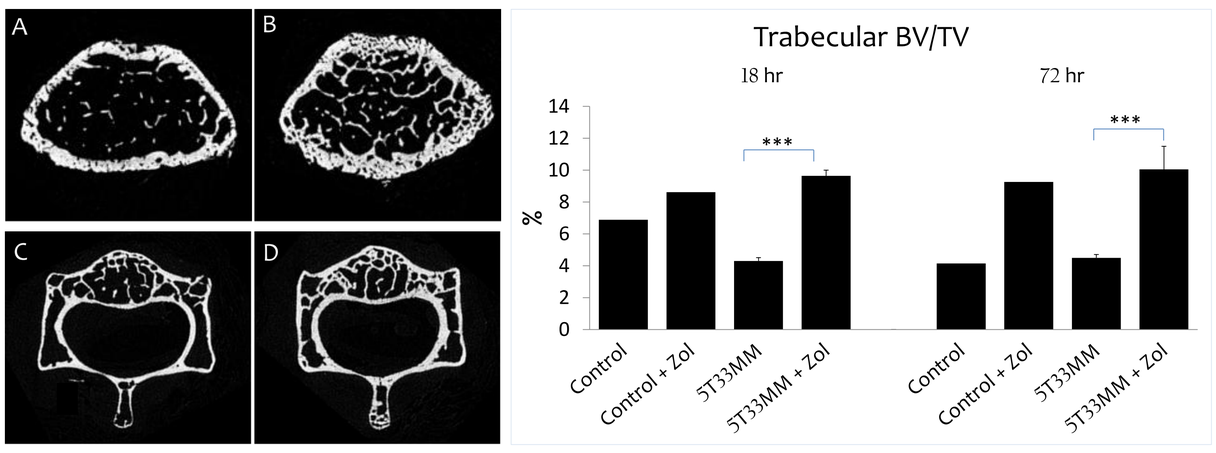


Fig 4: A, B, C and D shows representative micro computed tomographic cross section image of femur and vertebrae of vehicle treated control and zoledronic acid treated 5T33MM myeloma model. Graph shows significant greater percentage trabecular bone volume (BV/TV) in zoledronic acid treated group when compared with vehicle treated control group.

IV.Zoledronic acid treatment showed no reduction in the number of 5T33MM cells homing to bone after 18 and 72 hr post tumour cell injection.

Mice treated with Zoledronic acid or control (PBS) were injected with 2X10^6 5T33MM-eGFP cells and the proximal metaphysis of tibia was analysed after 18 and 72 hr for the presence of tumour cells in the bone. The tibiae were scanned to a depth of 70 µm. Spectral (lambda) finger printing confirmed the GFP signals (Fig 6: Panel A).

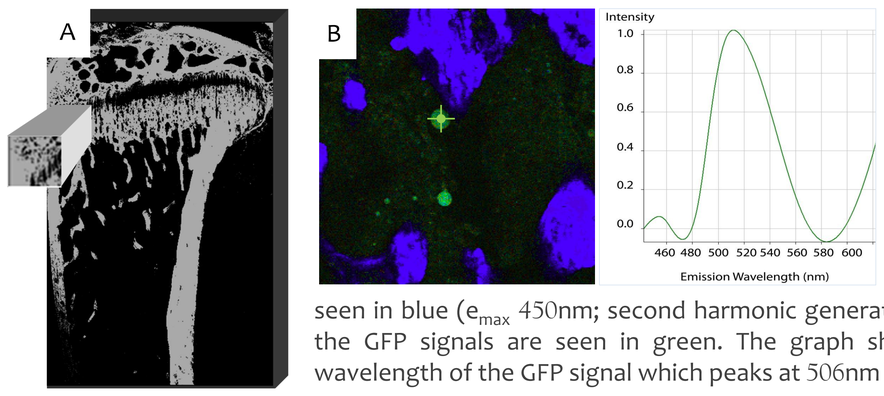


Fig 5: Panel A shows the cut section of the proximal tibial metaphysis (region of interest) scanned using the multiphoton microscopy.

Panel B shows the lambda spectral scan of the bone with GFP signal .The bone is seen in blue (e_{max} 450nm; second harmonic generation from the collagen) while the GFP signals are seen in green. The graph shows the intensity over the wavelength of the GFP signal which peaks at 506nm (e_{max}).

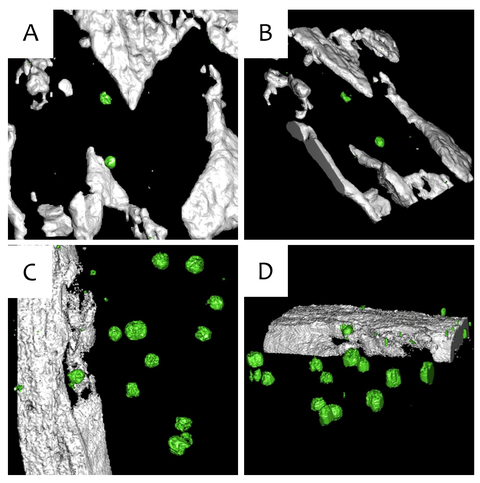


Fig 6 shows reconstructed 3D images of 5T33MM-eGFP cells in bone scanned by multiphoton microscopy. Images A and C shows 5T33MM-eGFP cell adjacent to trabecular and endocortical bone. Images C and D shows rotated 3D images of the same. The bone is seen in white and the GFP labelled myeloma cell in green.

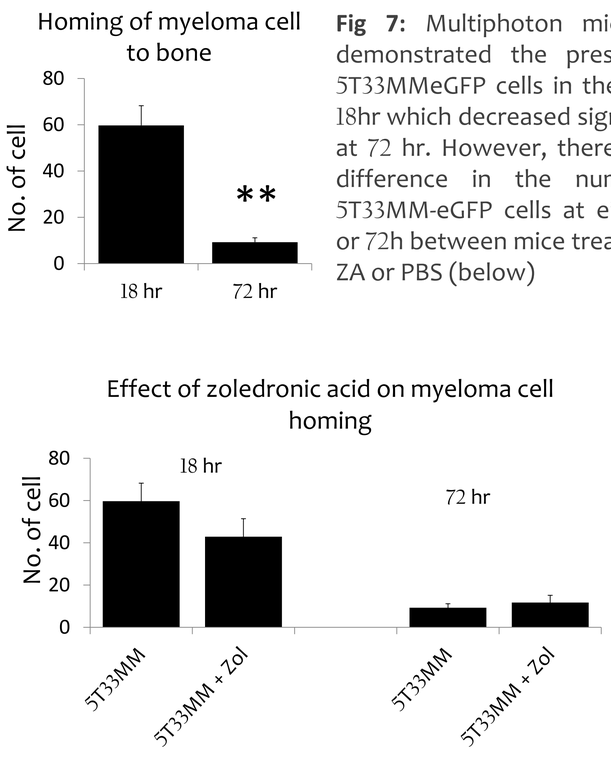


Fig 7: Multiphoton microscopy demonstrated the presence of 5T33MMeGFP cells in the tibia at 18hr which decreased significantly at 72 hr. However, there was no difference in the number of 5T33MM-eGFP cells at either 18h or 72h between mice treated with ZA or PBS (below)

Conclusion

- Single dose zoledronic acid significantly inhibited osteoclastic bone resorption after 7 days following treatment.
- significant numbers of tumour cells appear to home to the bone marrow after 18hr, however only a small proportion are present at 72h reflecting extravasation and migration out of the vasculature.
- ZA does not appear to have an effect on tumour cell homing at these early time points.