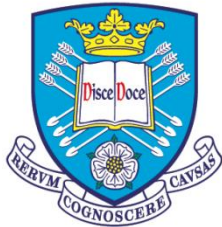
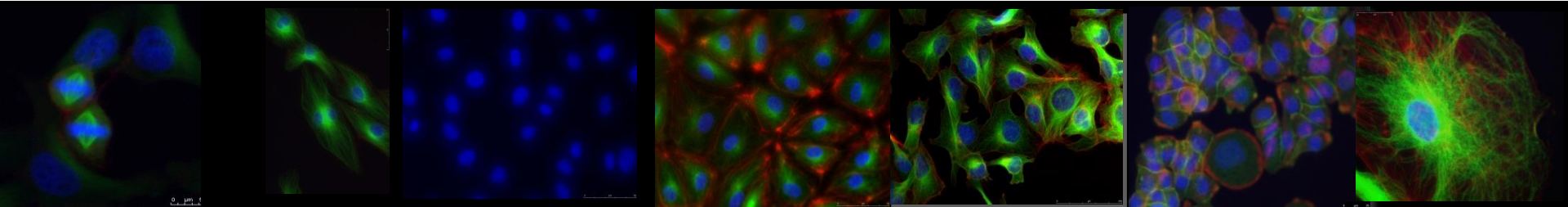


# OPG-Fc inhibits ovariectomy-induced growth of disseminated breast cancer cells in bone.



The  
University  
Of  
Sheffield.

Dr Penelope Ottewell





# Background

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- ~70% patients with late stage breast cancer develop bone metastasis.
- This condition is considered incurable with patients surviving 2-3 years after initial diagnosis of bone involvement.
- Current treatments are palliative; patients receive chemotherapy to treat their tumours and anti-resorptive agents to control their bone disease.
- **We have focused on optimising the use of anti-resorptive agents (zoledronic acid (ZOL) and OPG-Fc) to increase their anti-tumour properties.**

# Breast cancer bone metastasis

Tumour cells arrive in bone

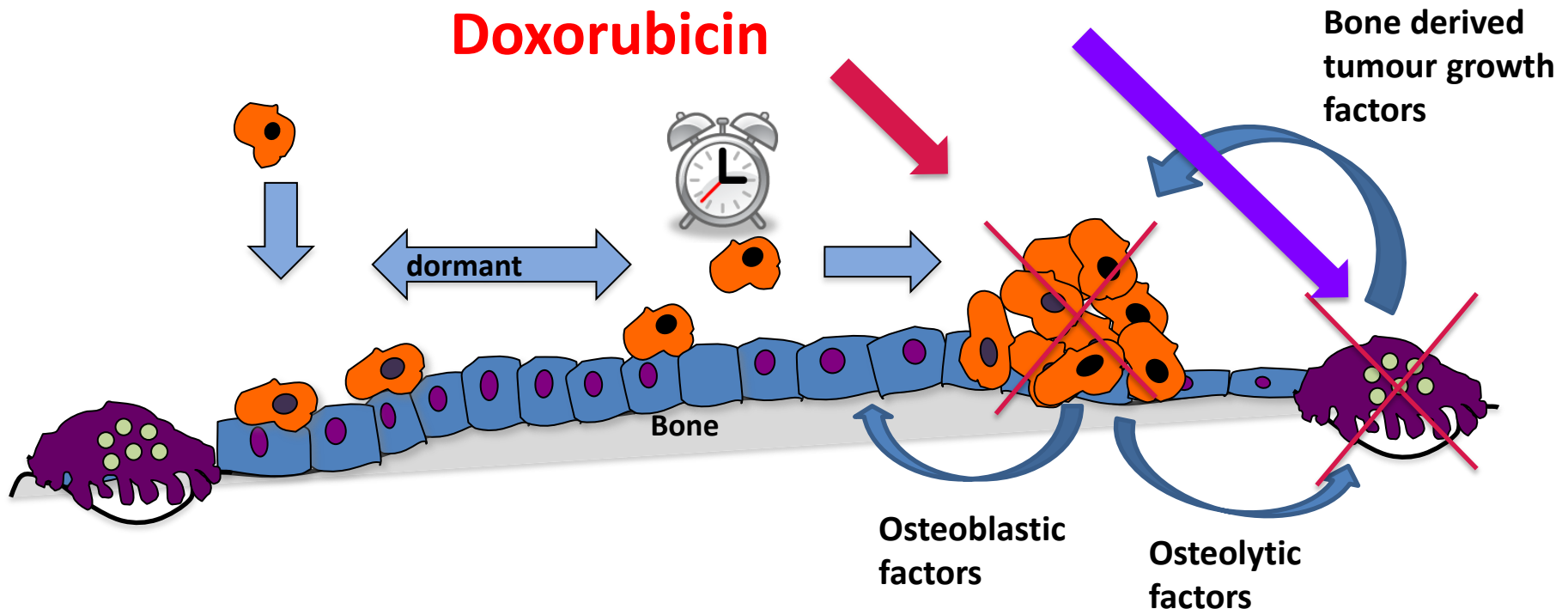
Tumour cells are triggered to 'wake up'

Tumour cells grow quickly....

and cause bone destruction

Zoledronic acid/OPG-Fc

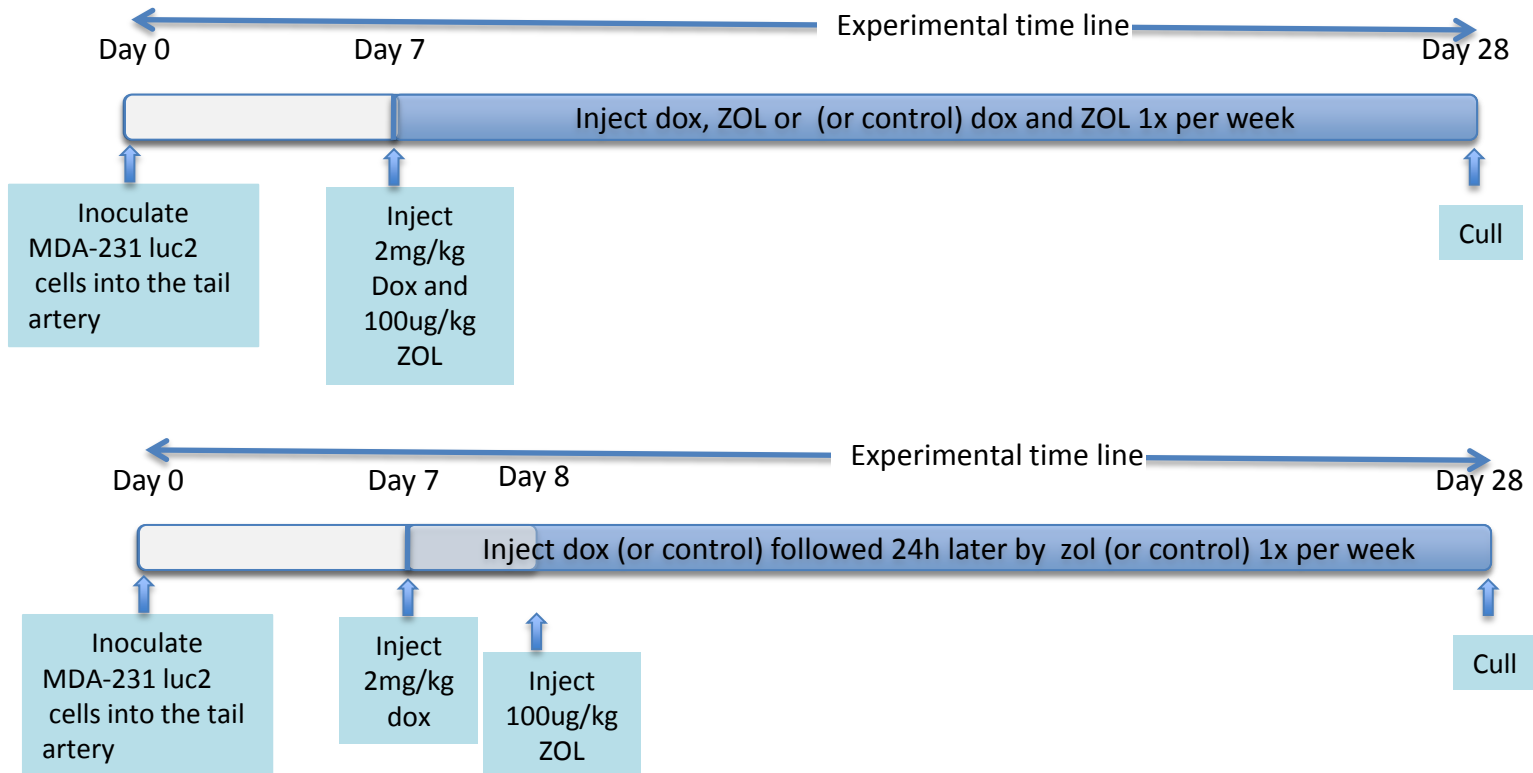
Doxxorubicin



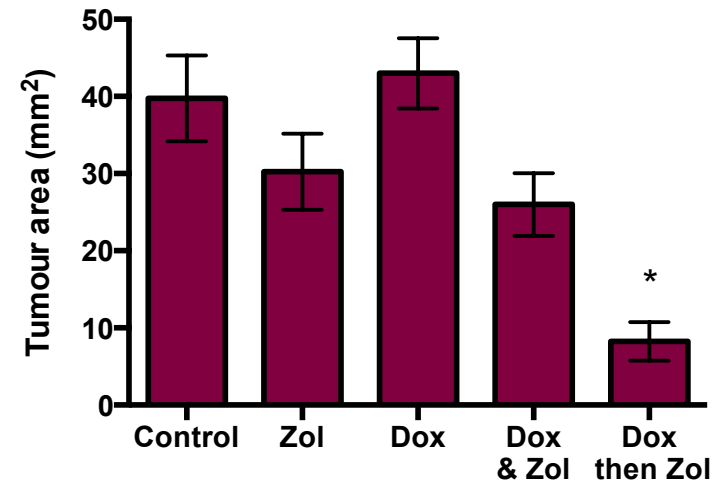
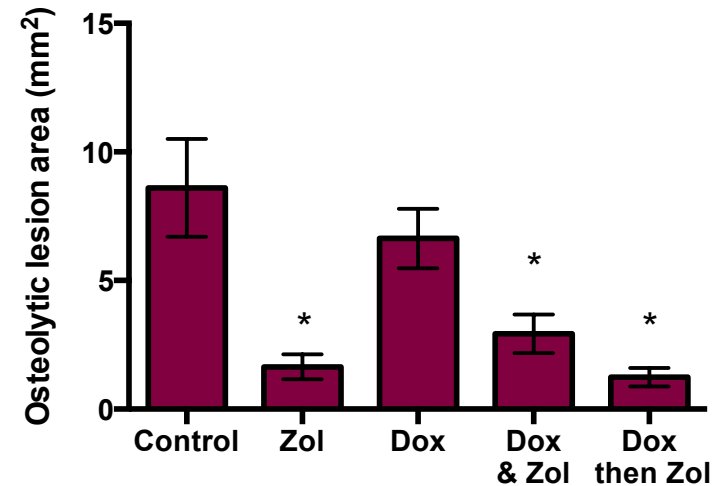
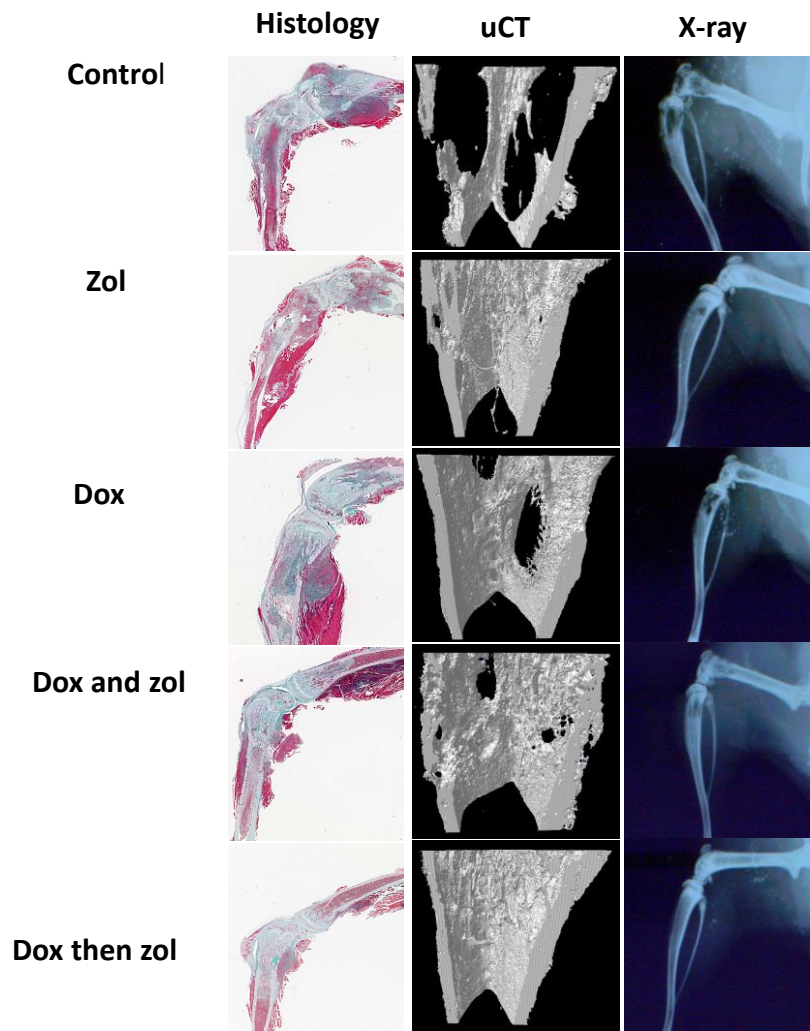
# Effects of adding zoledronic acid to doxorubicin on breast cancer bone metastases



6 week female BALB/c nude mice n=10/group



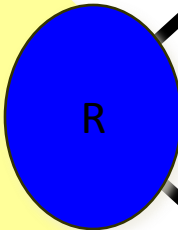
# Effects of doxorubicin and zoledronic acid on breast cancer growth in bone



Sequential administration with Dox followed by ZOL significantly reduced MDA-MB-231 breast cancer cell growth in bone .

# Clinical study design; AZURE

3,360  
Breast Cancer  
Patients  
Stage II/III



Standard therapy

Standard therapy +  
Zoledronic acid 4 mg

6 doses

Q3-4 weeks

8 doses

Q 3 months

5 doses

Q 6 months

Months

6

30

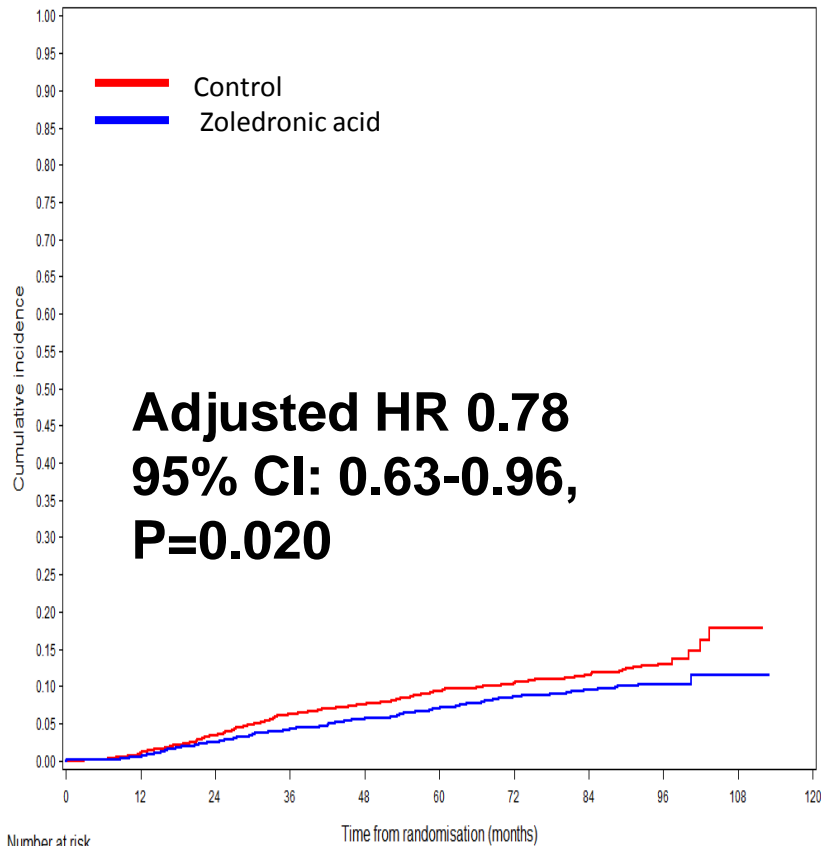
60

Zoledronic acid treatment duration 5 years

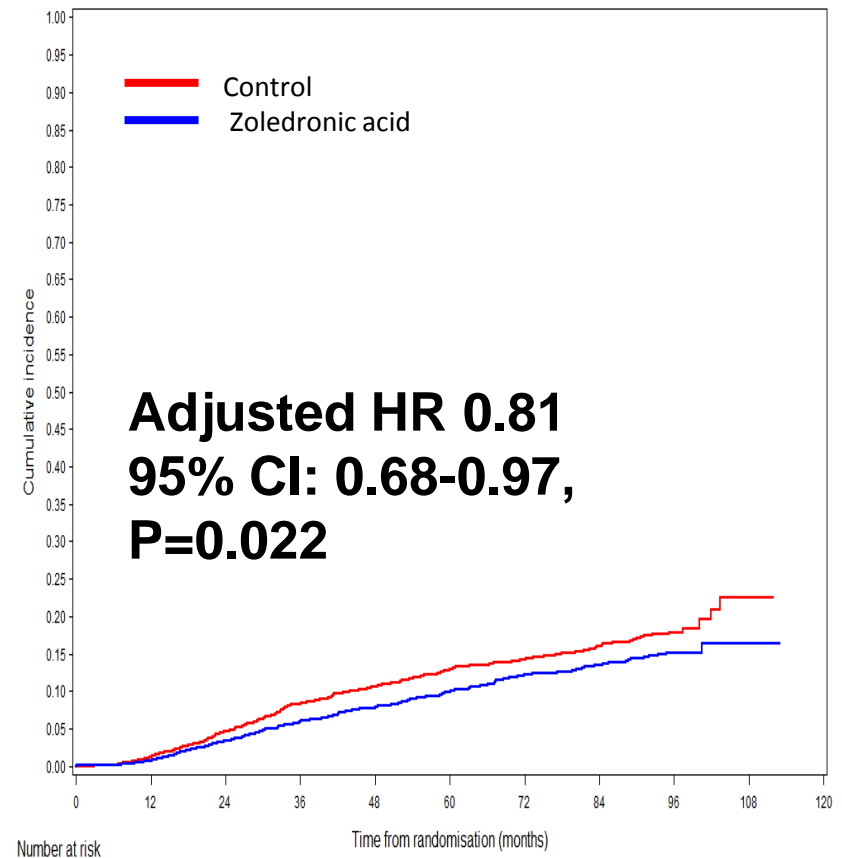
Countries	Centres	Patients
UK	123	2710
Eire	10	247
Australia	28	226
Spain	8	107
Portugal	1	32
Thailand	2	25
Taiwan	2	13

# Analysis of relapse in bone; AZURE

## Bone metastasis as first recurrence



## Bone metastasis at any time



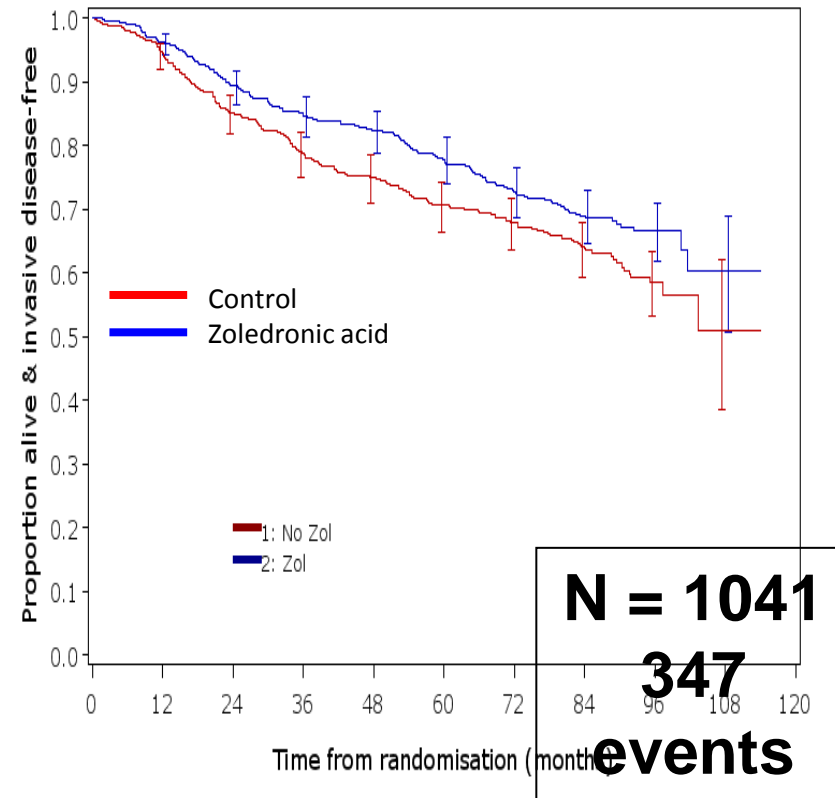
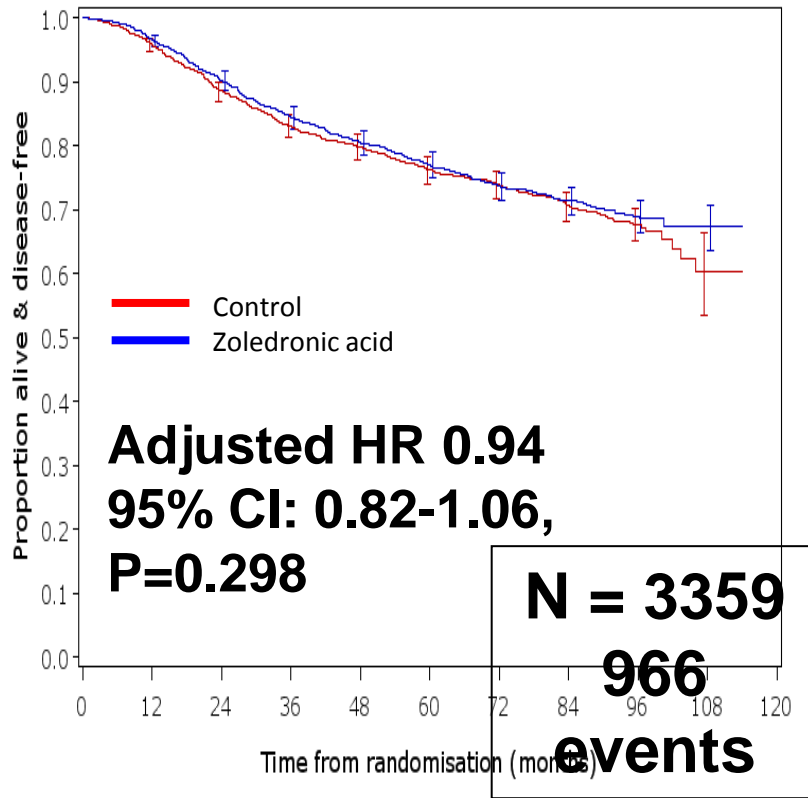
Adding zoledronic acid to standard of care delayed bone metastases



# Disease free survival and menopausal status; AZURE

## ITT analysis - DFS

## >5 years post-menopausal



No. at risk	0	12	24	36	48	60	72	84	96	108	120
Control	1678	1584	1452	1354	1283	1186	1079	762	231	17	0
ZOL	1681	1590	1467	1360	1281	1203	1090	781	250	16	0

No. at risk	0	12	24	36	48	60	72	84	96	108	120
Control	522	482	432	398	371	339	308	208	62	5	0
ZOL	519	488	444	416	401	371	325	239	84	8	0

Zoledronic acid provided significantly greater therapeutic benefit to post menopausal women,





# Emerging hypotheses from adjuvant trials

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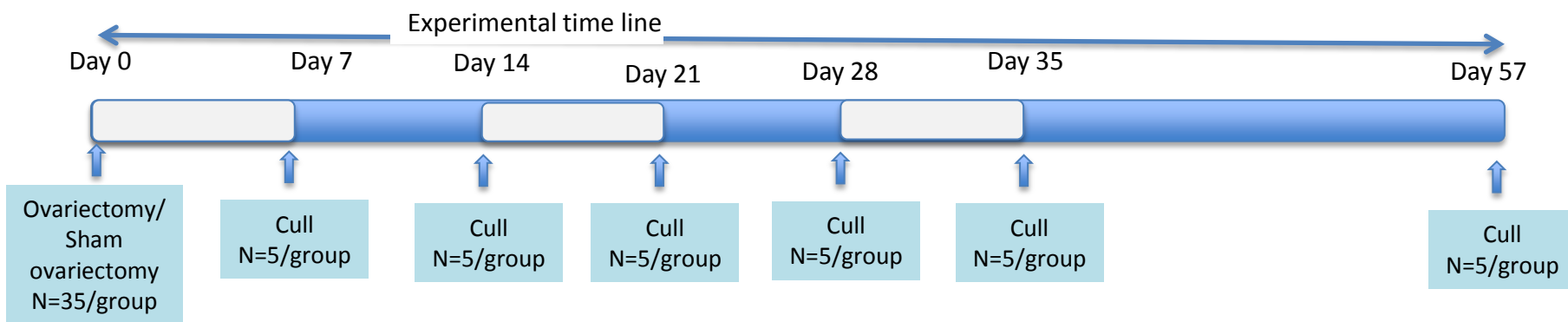
- Bisphosphonates predominantly reduce distant metastases rather than either local recurrence or contralateral disease
- Effects likely to be largest on bone recurrence
- Bisphosphonates only improve disease outcome in women who have low levels of reproductive hormones
  - Established natural menopause
  - Induced menopause at start of treatment

**Why do bisphosphonates specifically benefit patients with established menopause?**

# Modeling the pre-and post-menopausal bone environment in the laboratory



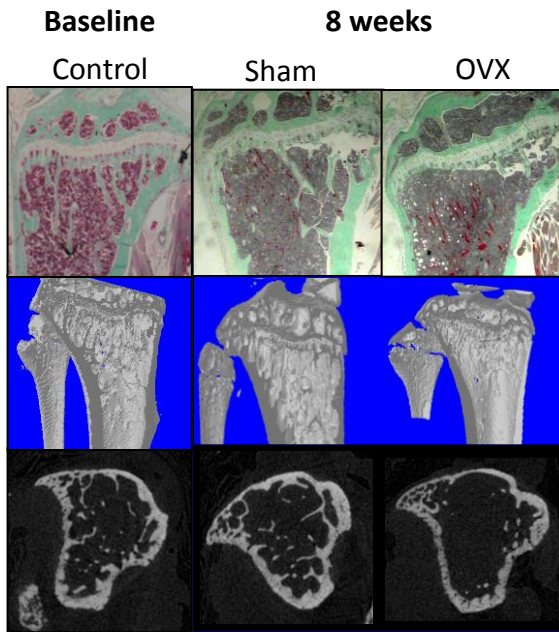
12 week female BALB/c nude mice n=5/group



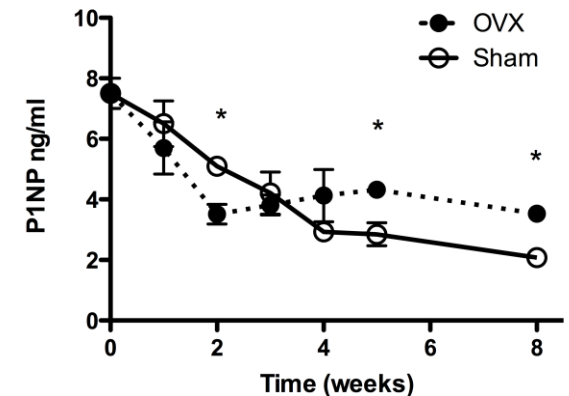
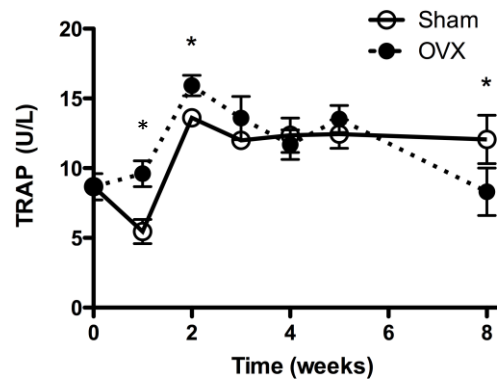
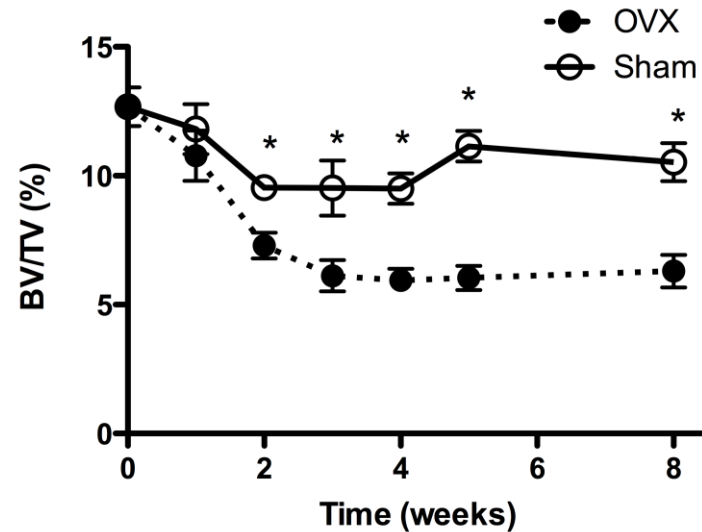
How does OVX alter the bone environment over time:

- Bone volume
- Osteoclast activity (TRAP ELISA)
- Osteoblast activity (P1NP ELISA)

# Bone microenvironment different under pre- and post-menopausal conditions

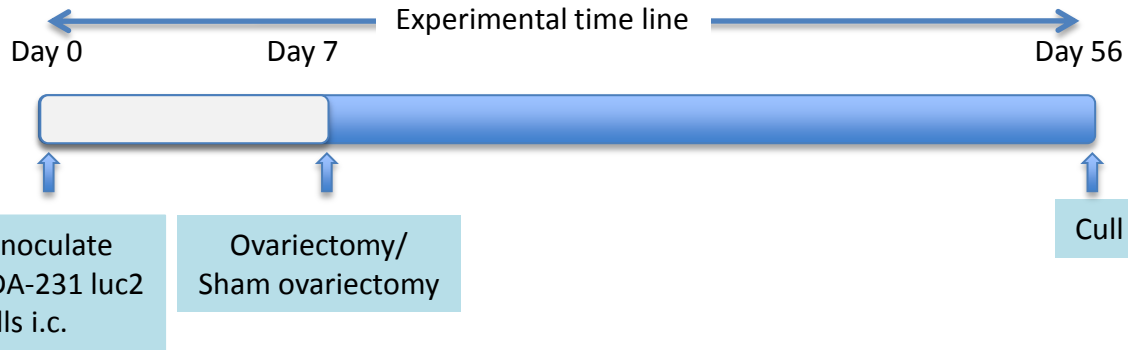


Ovariectomy reduces bone volume by increasing osteoclast induced bone resorption

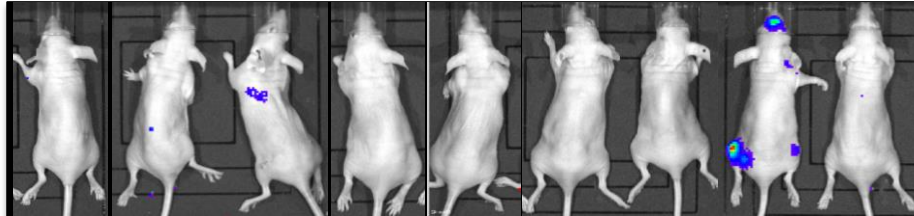


# Effects of ovariectomy on tumour cell proliferation in bone

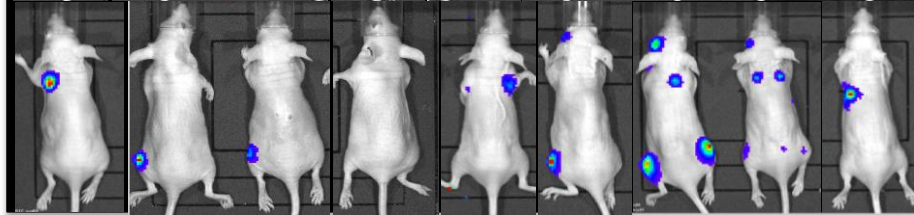
12-week old female BALB/c nude mice  
n=9/group



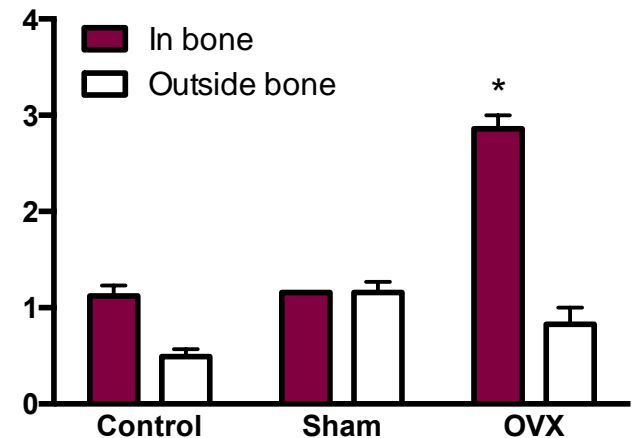
Sham



Ovariectomy



Number of tumours per mouse



Overiectomy stimulates proliferation of MDA-MB-231 cells disseminated in bone



# Summary/research question

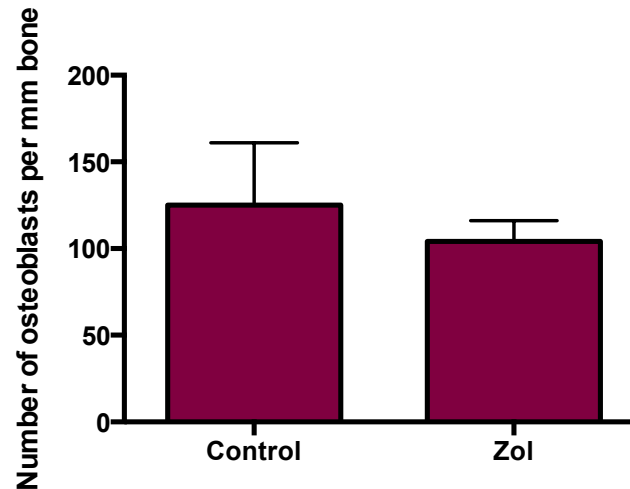
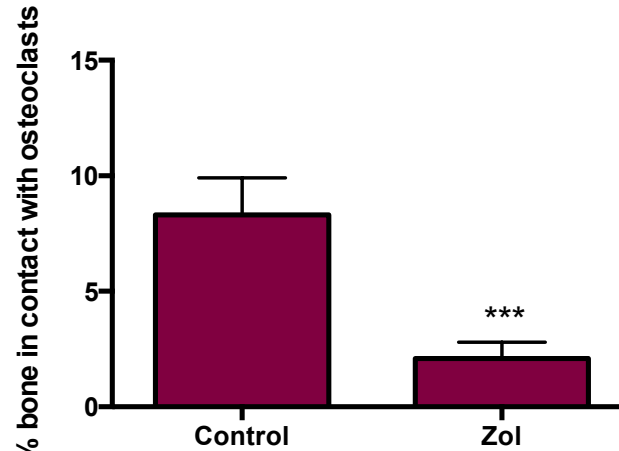
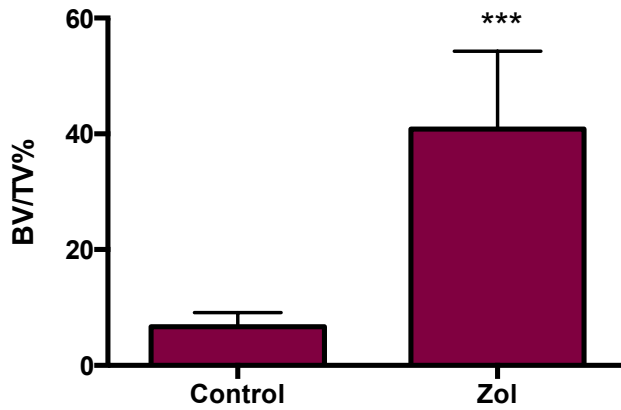
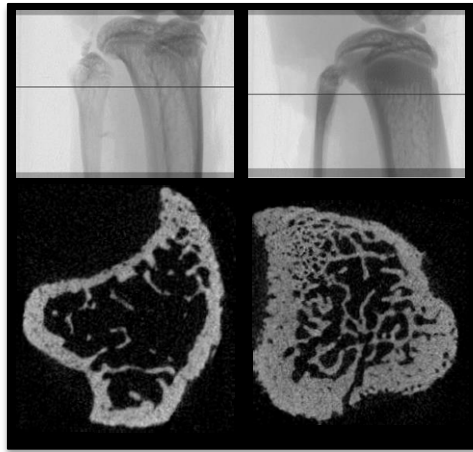
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- Mimicking the menopause with ovariectomy alters bone turnover.
- Ovariectomy stimulates proliferation of dormant breast cancer cells in bone.

- Can anti-resorptive agents prevent ovariectomy induced proliferation of breast cancer cells in bone?
- Do anti-resorptive agents have different anti-tumour effects in pre- and post- menopausal bone environments?

# Effects of ZOL on normal bone

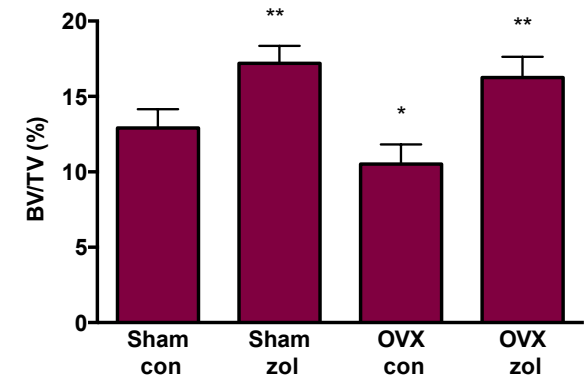
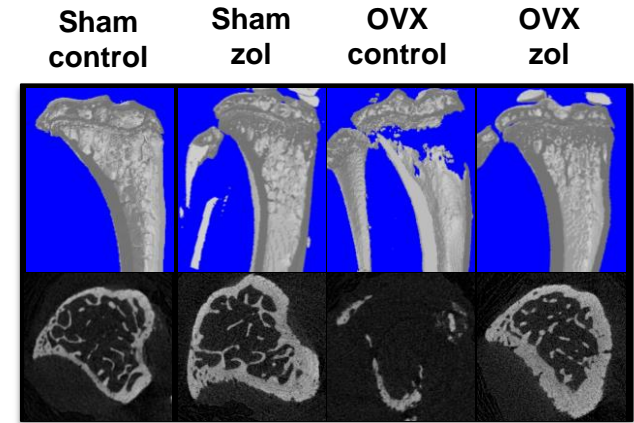
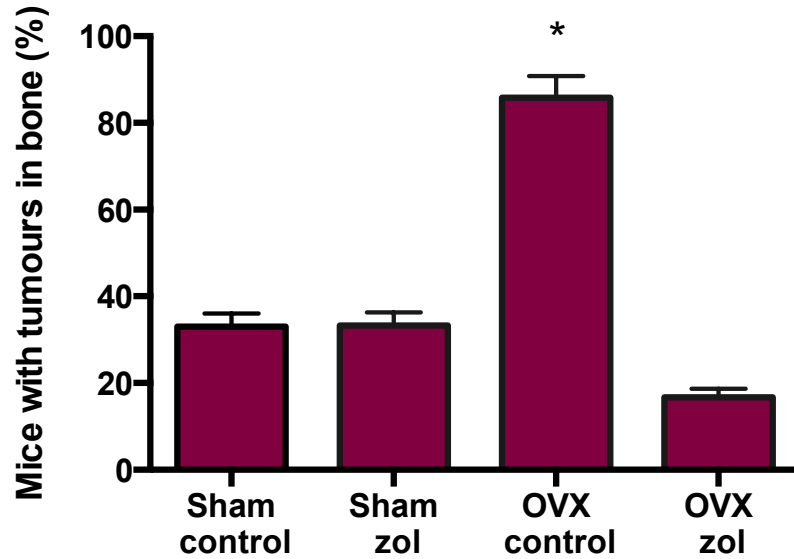
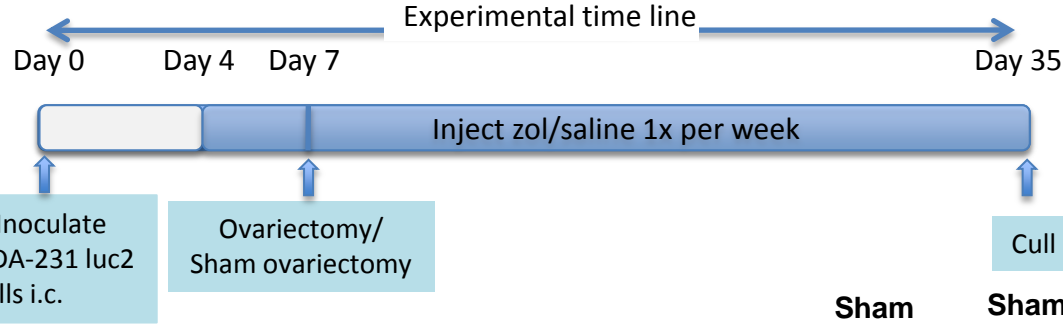
Control      Zol



ZOL inhibits bone turnover by reducing osteoclastic resorption of bone

# Effects of ZOL on ovariectomy induced tumour growth and bone resorption

12-week old female BALB/c nude mice  
n=10/group (X2)



ZOL inhibits OVX stimulated growth of disseminated breast cancer cells in bone but had no effect of spontaneous tumour development from these cells.



# Do other anti-resorptive agents prevent OVX induced bone metastases?

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## Denosumab in the clinic:

- Human specific monoclonal antibody that binds RANKL with high affinity blocking RANK/RANKL interactions essential for osteoclastogenesis.
- Superior to zoledronic acid for prevention of skeletal complications in patients with breast cancer bone metastasis.

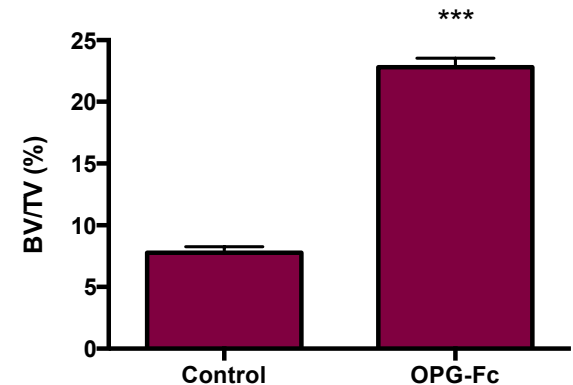
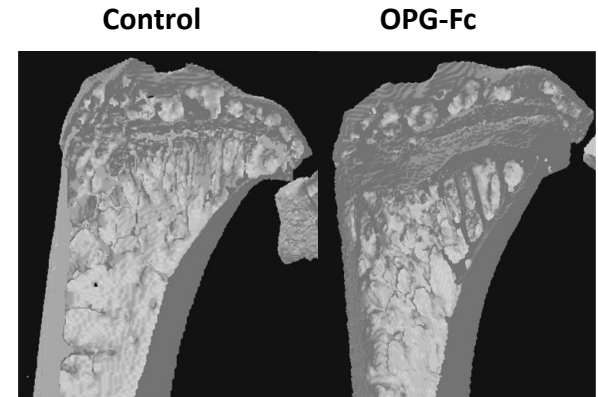
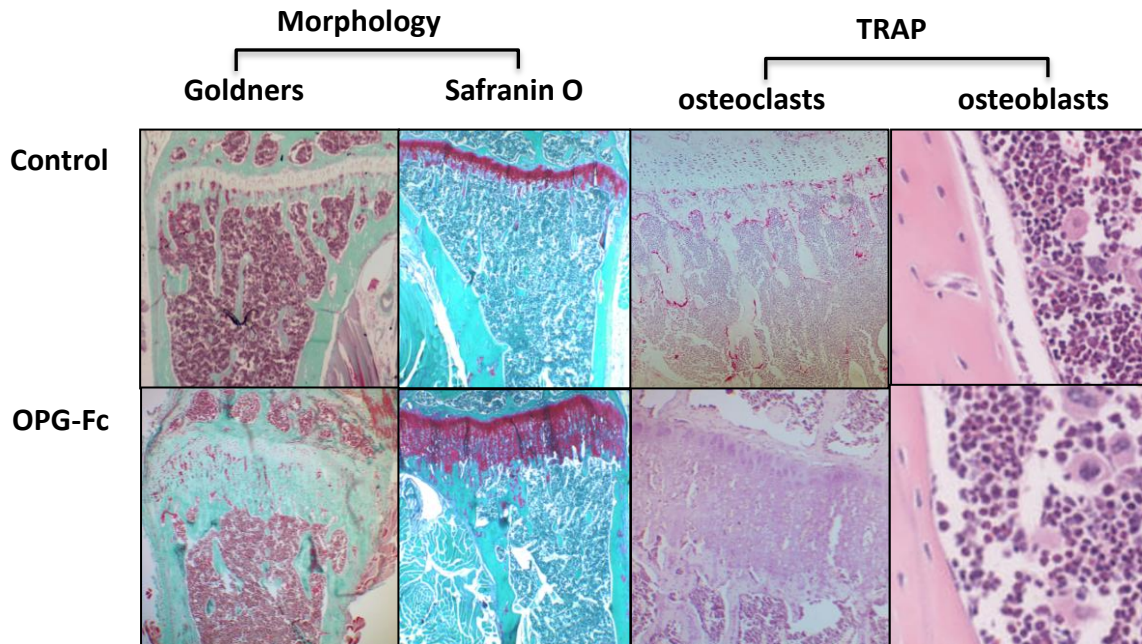
## OPG-Fc in the laboratory:

- Potent inhibitor of osteoclastogenesis that acts by preventing RANK/RANKL binding.

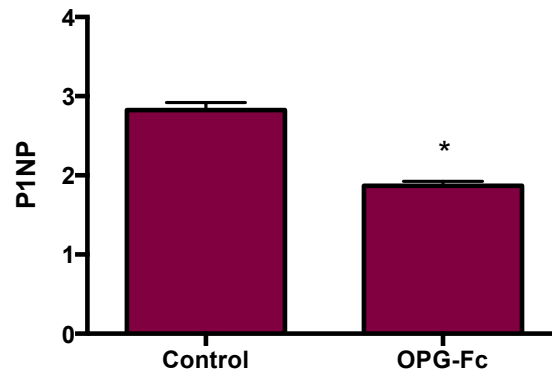
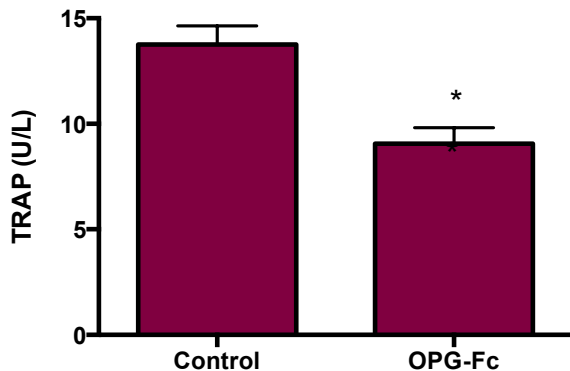
We investigated the effects of inhibiting OVX-induced bone resorption through targeting RANK/RANKL interactions in our *in vivo* model of disseminated breast cancer cells in bone.



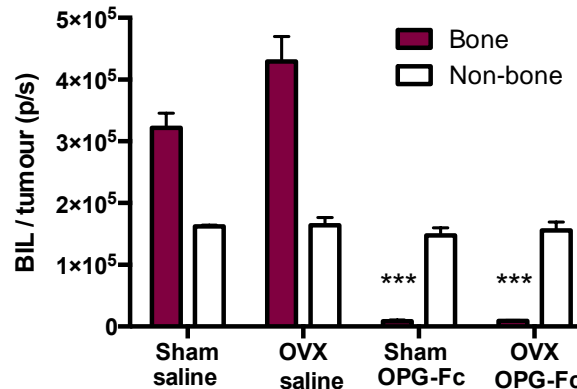
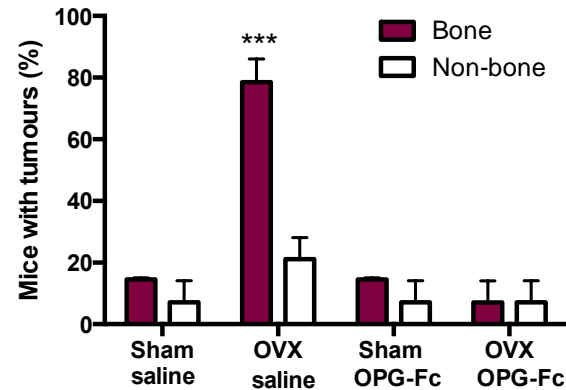
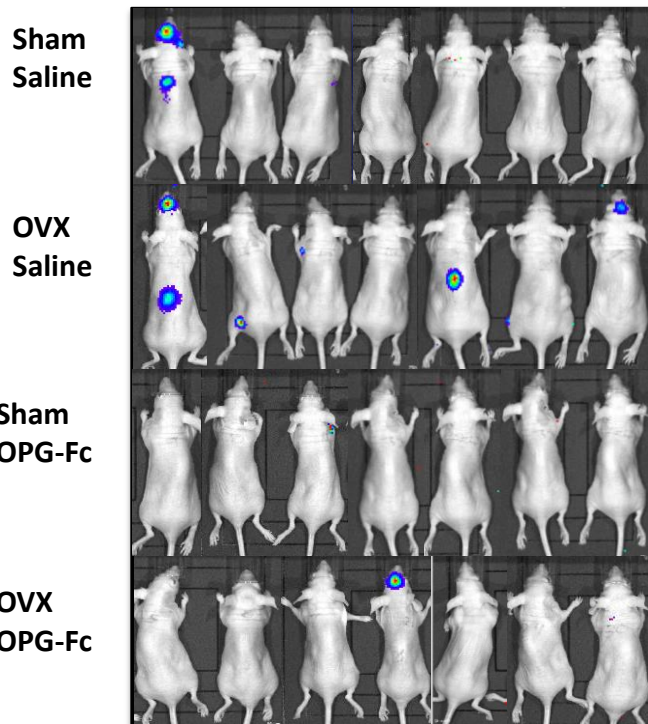
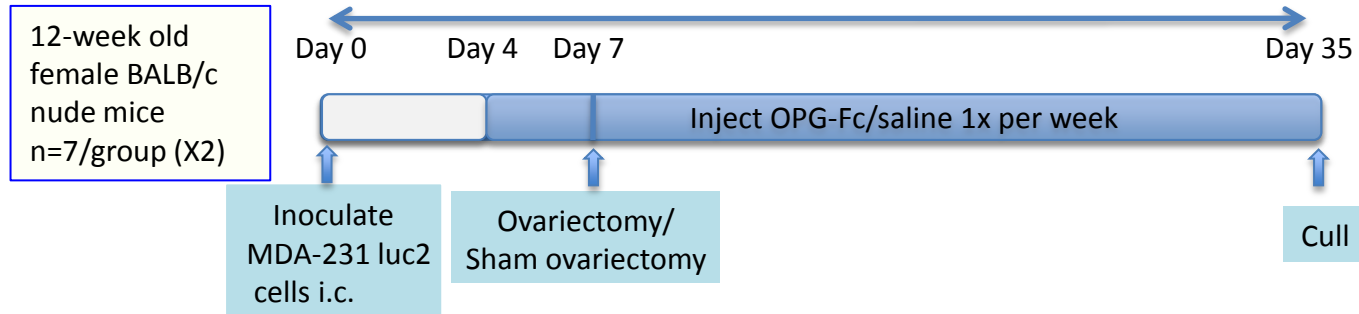
# Effects of OPG-Fc on bone



OPG-Fc inhibits osteoclastic bone resorption and osteoblastic bone formation



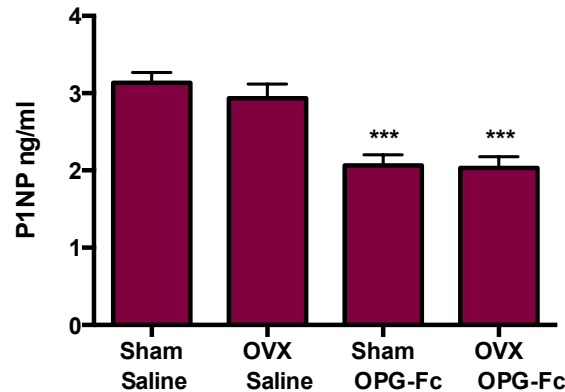
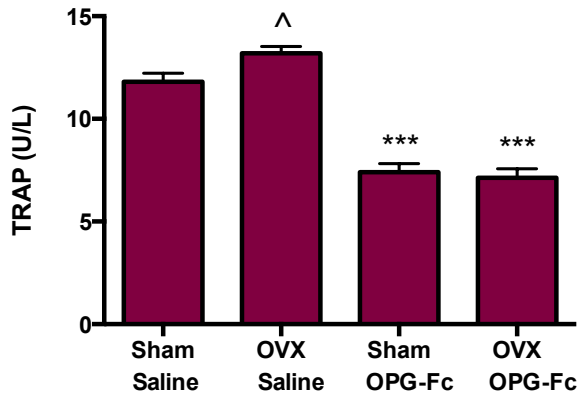
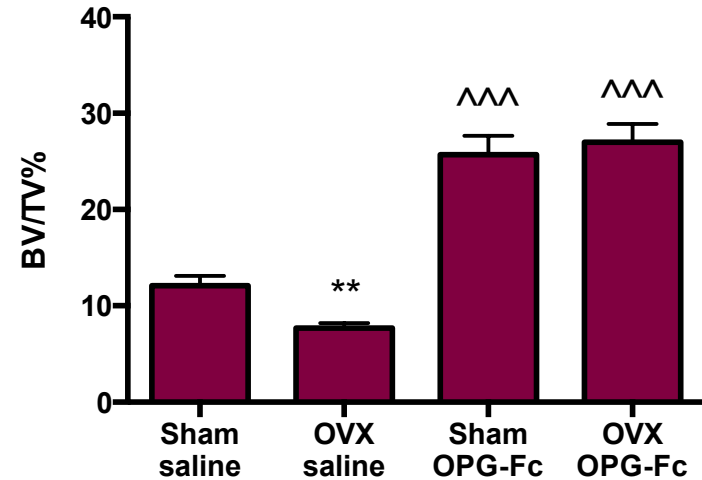
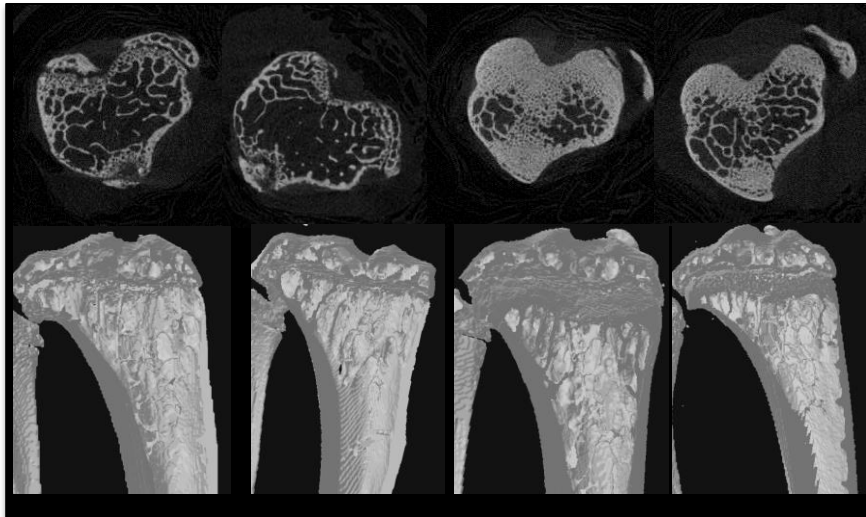
# Effects of OPG-Fc on growth of disseminated breast cancer cells in bone



OPG-Fc inhibits ovariectomy induced proliferation of disseminated MDA-MB-231 breast cancer cells in bone.

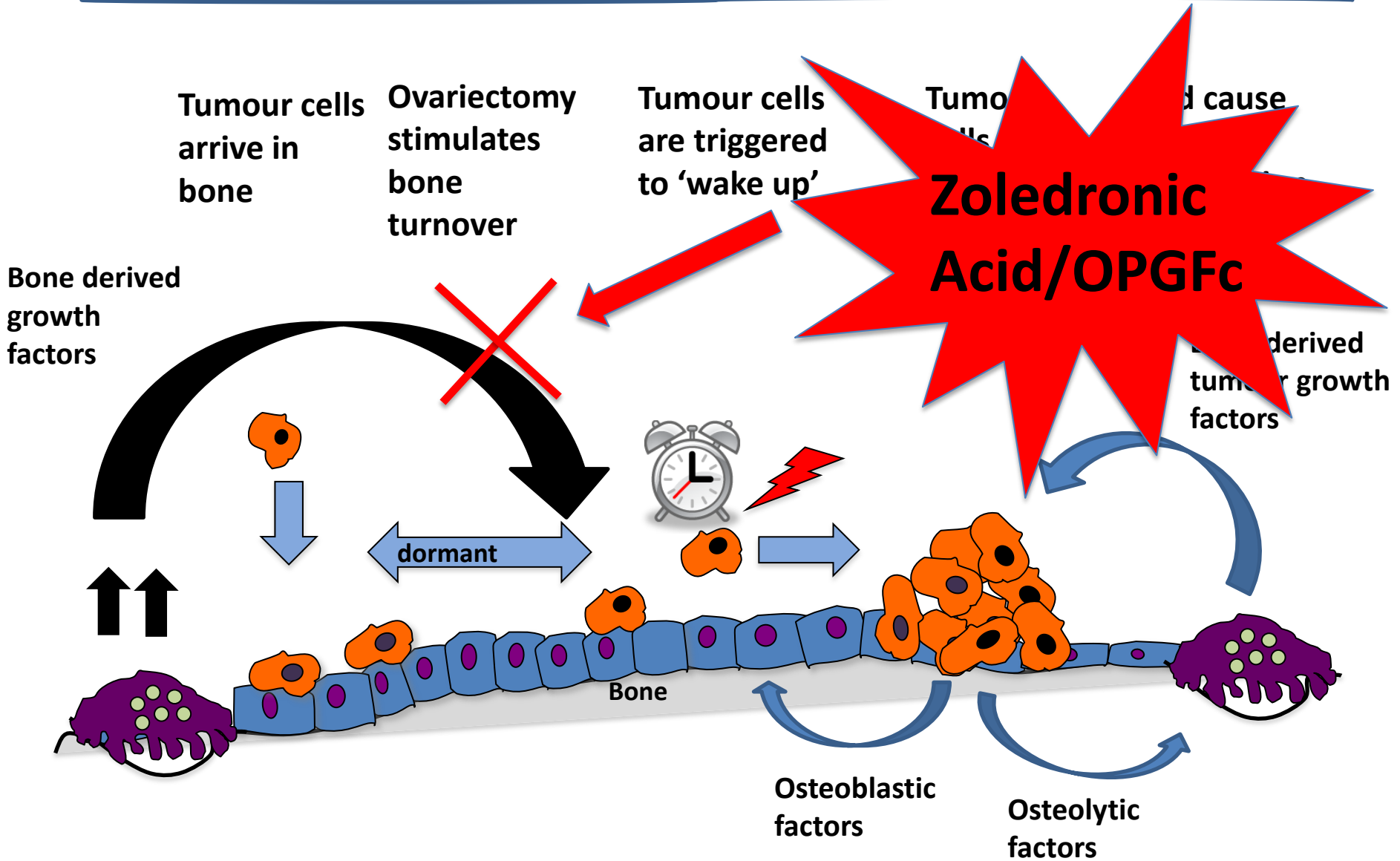
# Effects of OPG-Fc on tibiae of mice following tumour cell inoculation

Sham saline      OVX saline      Sham OPG-Fc      OVX OPG-Fc



OPG-Fc inhibits ovariectomy induced bone resorption leading to a net increase in bone volume and decrease in bone cell activity.

# Summary





# Conclusions

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- Cellular mechanisms responsible for driving tumour growth are different in pre- (sham) and post menopausal (OVX) bone metastasis models.
- Osteoclast mediated mechanisms drive progression of disseminated tumour cells only in the post-menopausal model.



- Inhibition of osteoclast activity with ZOL or disruption of RANK/RANKL interactions following administration of OPG-Fc inhibits OVX induced stimulation of dormant tumour cells in bone

Our data support early intervention with anti-resorptive therapy in a low oestrogen environment to prevent development of bone metastases.



# Acknowledgements

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**CANCER  
RESEARCH  
UK**