I BIFOSFONATI
NEL PAZIENTE ONCOLOGICO ED EMATOLOGICO

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Meccanismi d'azione dei BPs: certezze ed ipotesi

Ruolo della Preclinica

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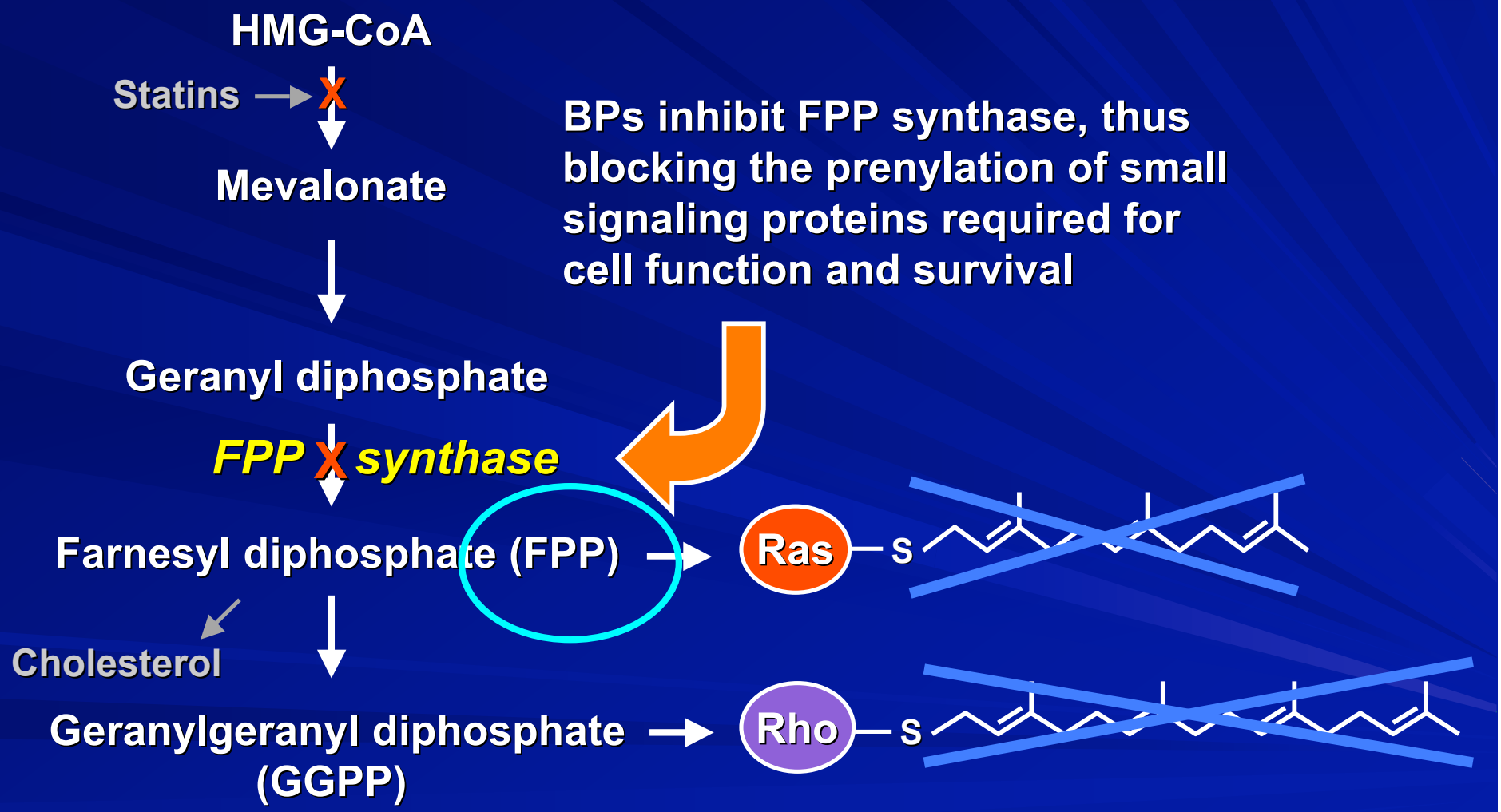
Molecular mechanism of action



Background

Translation from preclinical studies to clinical trials

- Direct antitumor effects (in vitro and animal models)
- Synergistic effects with cytotoxic and biological drugs (in vitro and animal models)
- Effects on the metastatic process (animal models)
- Effects on angiogenesis (in vitro, animal models and in humans)
- Stimulation of T gamma/delta lymphocytes (in vitro, and in humans immunomodulation)

Direct antitumor effects: in vitro evidences

Amino-bisphoshonates have demonstrated in vitro models the following activities:

Induction of apoptosis

Inhibition of cell proliferation

Inhibition of cell invasion

Inhibition of cell adhesion

Inhibition of cell viability/migration

Synergistic effects with cytotoxic drugs

Synergistic effects with biological drugs

In vitro antitumor evidences

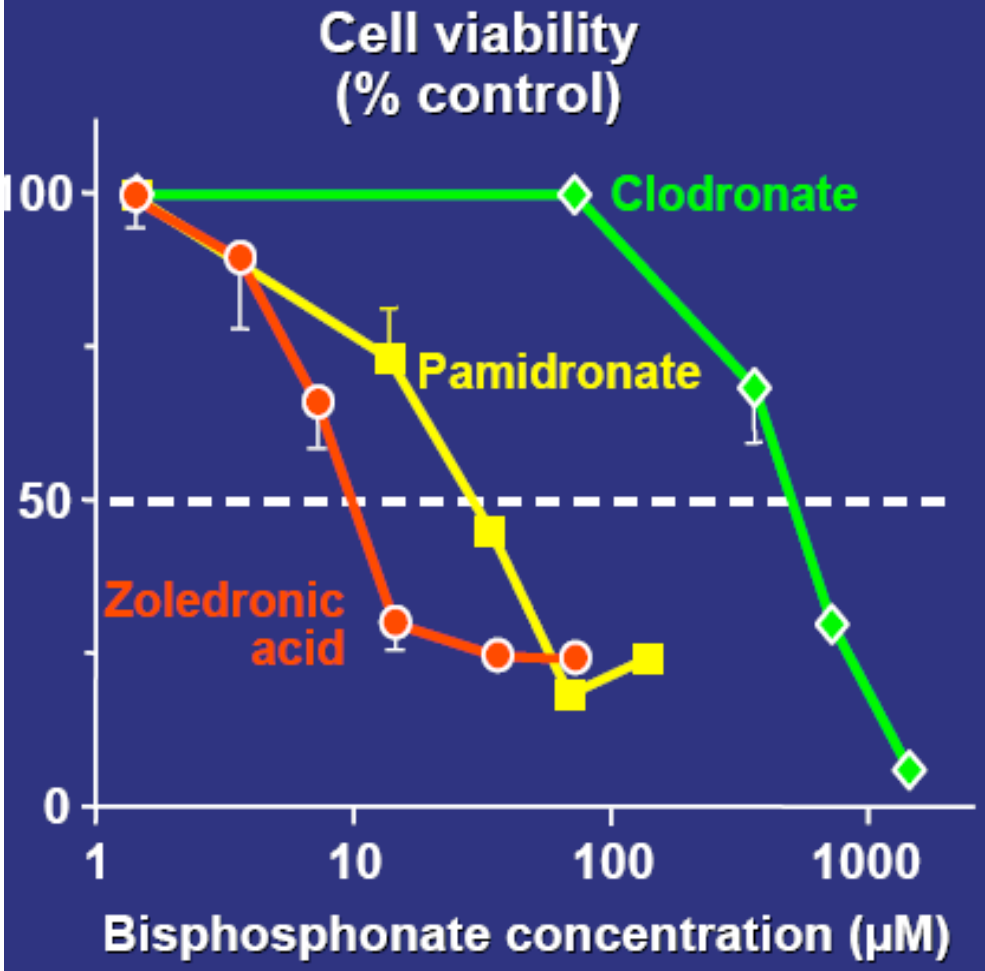
The potency of several N-BPs to inhibit cancer cell proliferation and increase apoptosis has been demonstrated in *breast, prostate, ovarian, bladder, hepatoma, osteosarcoma, leukemia, melanoma* as well as *myeloma cells*

Stesing B and Clezardin P. Cancer Letters, 2007

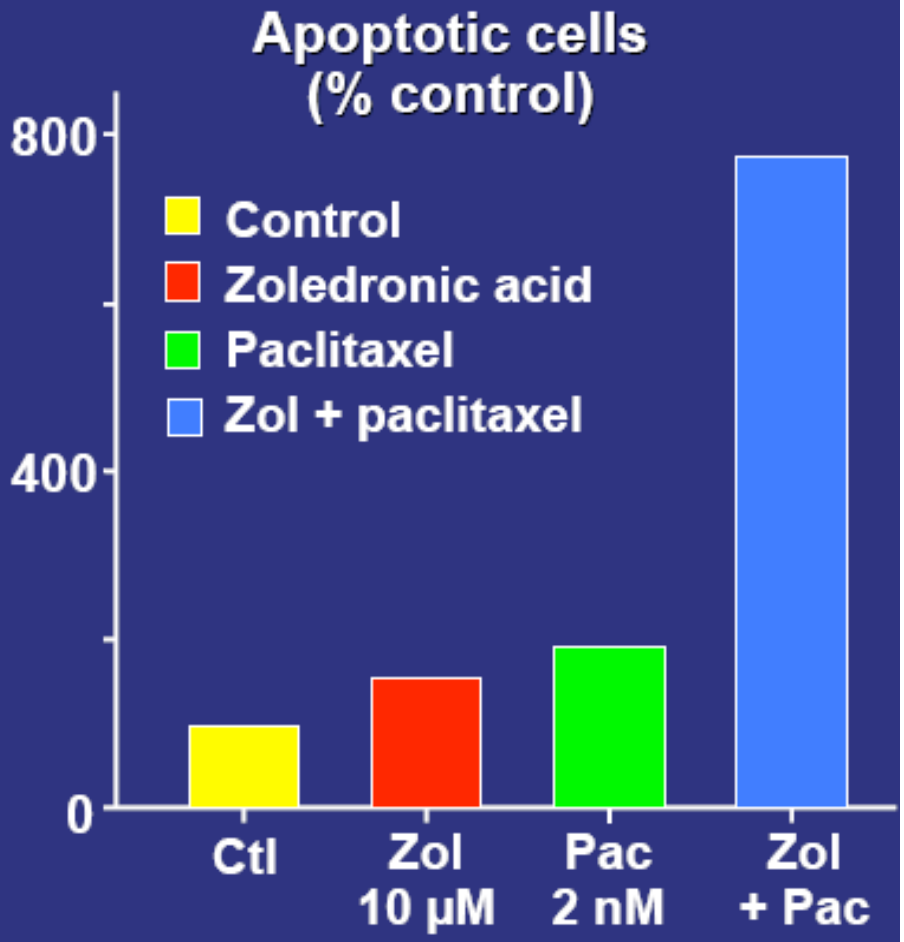
Preclinical evidences of antineoplastic effects on different cancer cell lines

Author (year)	Biphosph.	Cancer cell line	Type of effect
Neville-Webbe HL (2005)	ZOL	Breast cancer cell line Prostate cancer cell line	Induction of apoptosis Induction of apoptosis
Boissier S (2000)	ZOL	Breast cancer cell line Prostate cancer cell line	Inhibition of cell migration and cell adhesion
Donoyelle (2003)	ZOL	Breast cancer cell line Prostate cancer cell line	Inhibition of cell invasion
Matsumoto S (2005)	ZOL	Small cell lung cancer line	Induction of apoptosis Inhibition of cell proliferation
Dumon JC (2004)	ZOL	Prostate cancer cell line	Induction of apoptosis Inhibition of cell proliferation
Tassone P (2003)	ZOL	Pancreatic cancer cell line	Induction of apoptosis Inhibition of cell proliferation
Evdokiou A (2003)	ZOL	Human osteogenic sarcoma	Induction of apoptosis Inhibition of cell proliferation
Forsea AM (2004)	ZOL	Human melanoma cell line	Induction of apoptosis Inhibition of cell proliferation
Vorotnjak M (2004)	ZOL	Neuroblastoma cell line	Induction of apoptosis Inhibition of cell proliferation
Gordon S (2002)	ZOL/PAM	Plasma cells in multiple myeloma	Induction of apoptosis

BPs Decrease the Viability of Breast Cancer Cells and Interact Synergistically With Cytostatic Drugs

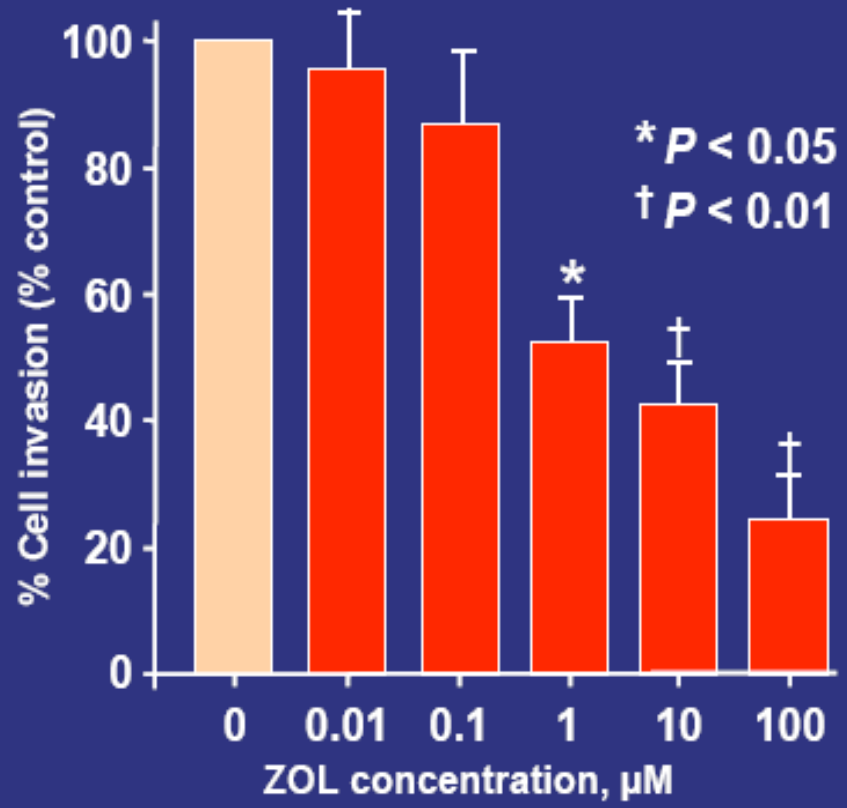


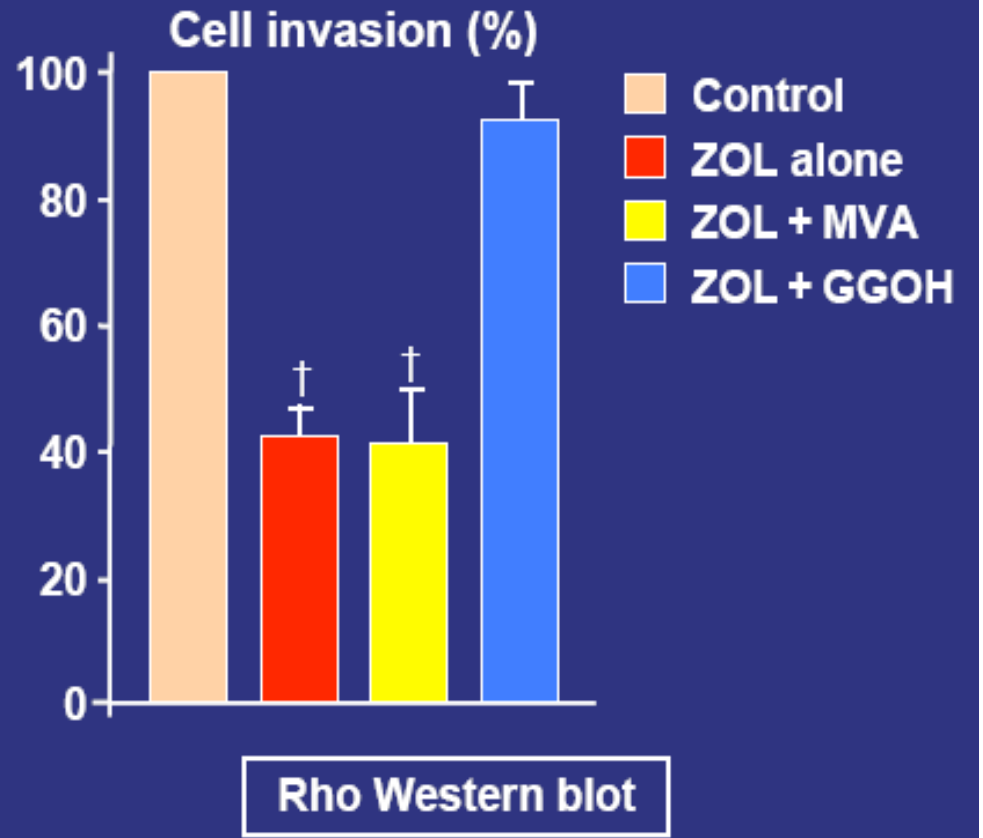
Senaratne et al, *Br J Cancer*, 2000

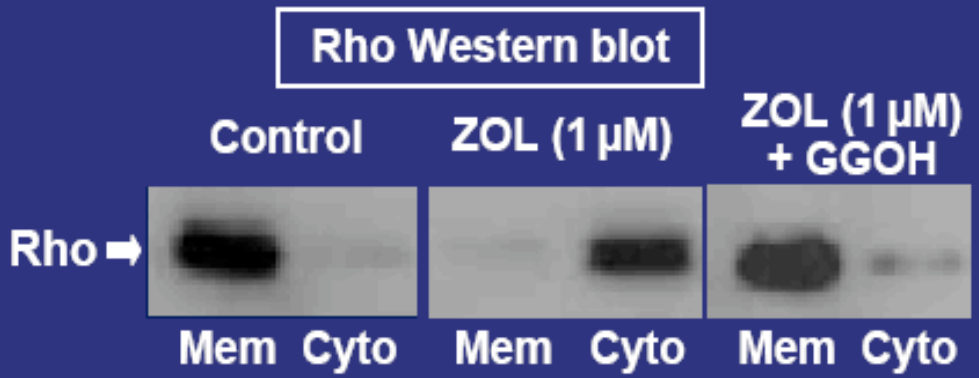


Jagdev et al, *Br J Cancer*, 2001

Zoledronic Acid Inhibits Invasion of MDA-MB-231 Human Breast Cancer Cells Through Matrigel In Vitro



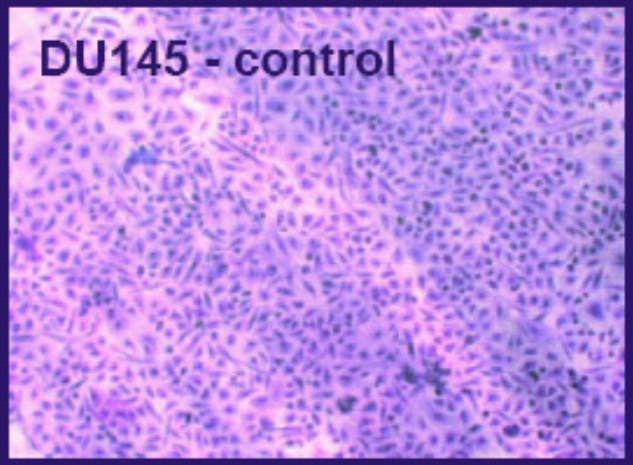


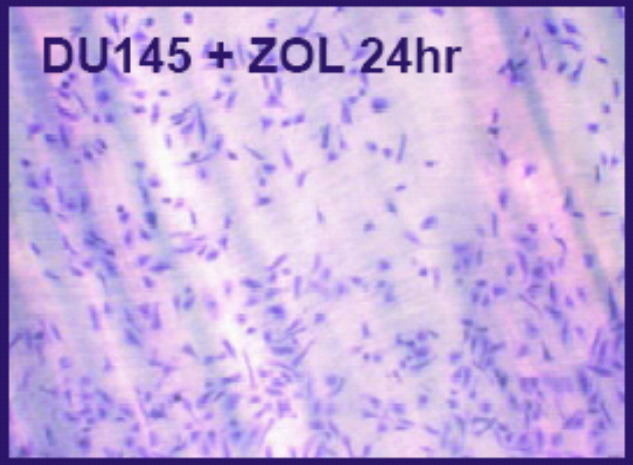


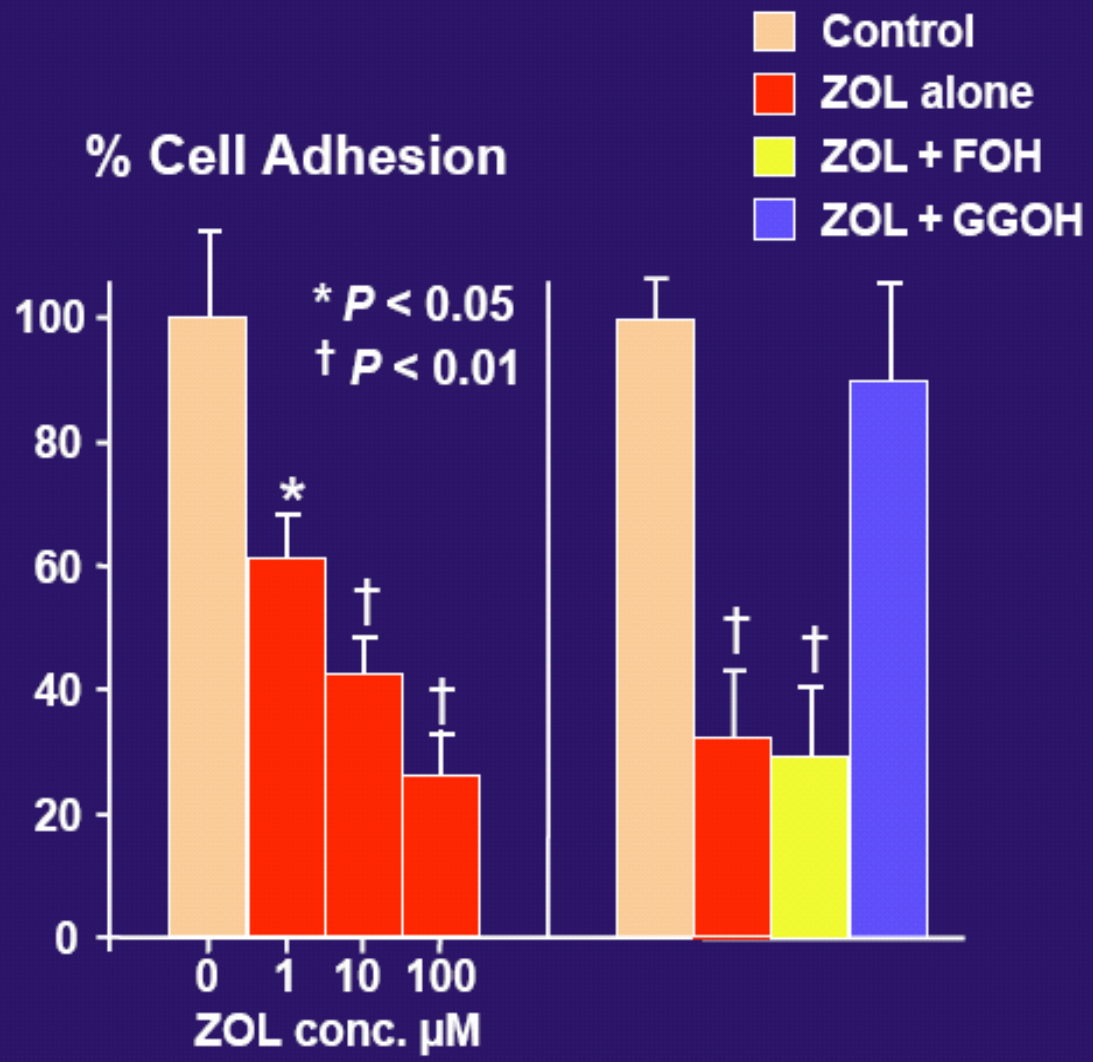
Denoyelle et al, *Br J Cancer*, 2003

Pretreatment of human prostate cancer cells with zoledronic acid inhibits adhesion to bone in vitro

Cell adhesion to dentine

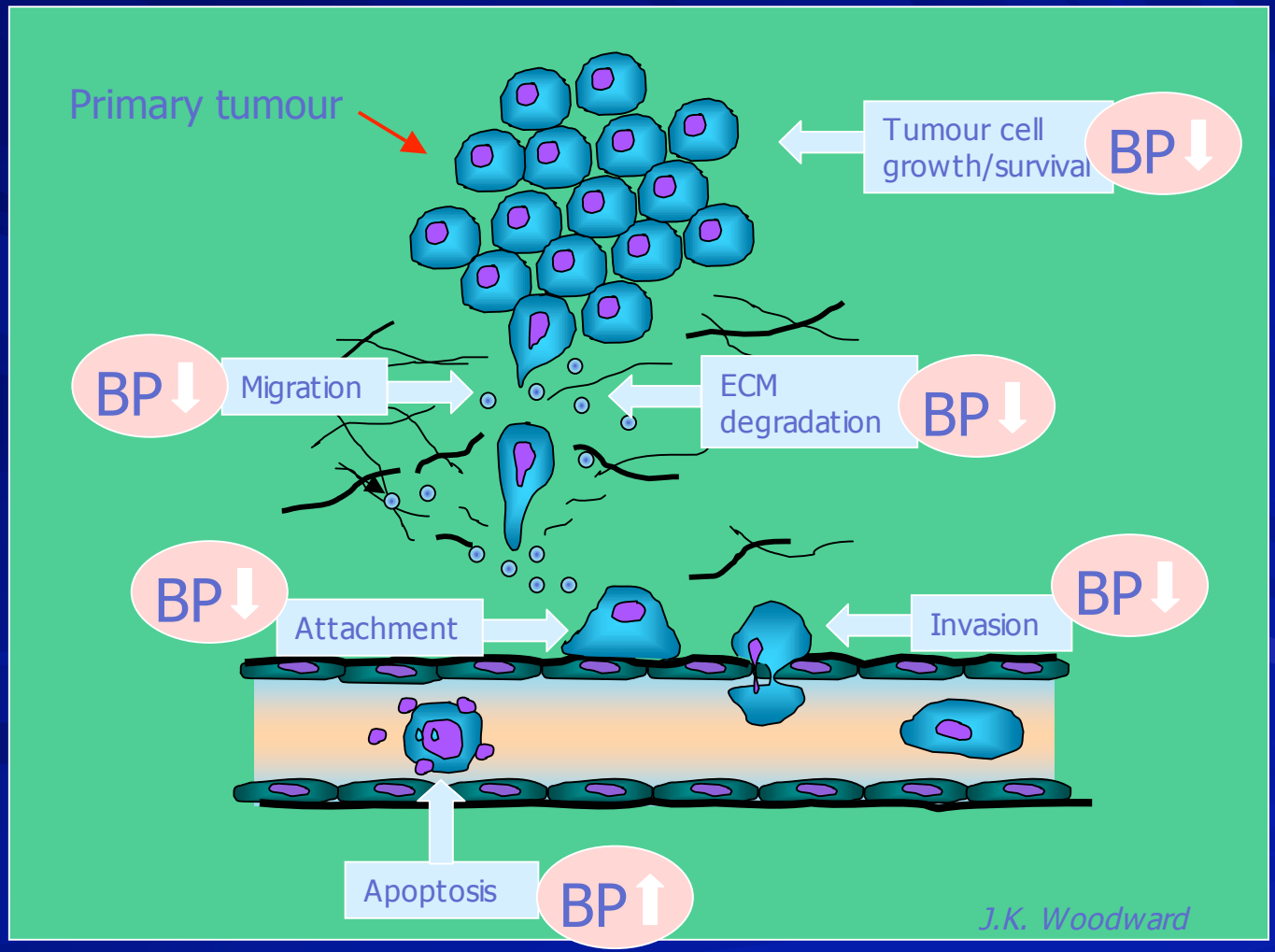






Coxon et al, *BJU Int*, 2004

Anti- tumour effects of BPs *in vitro*



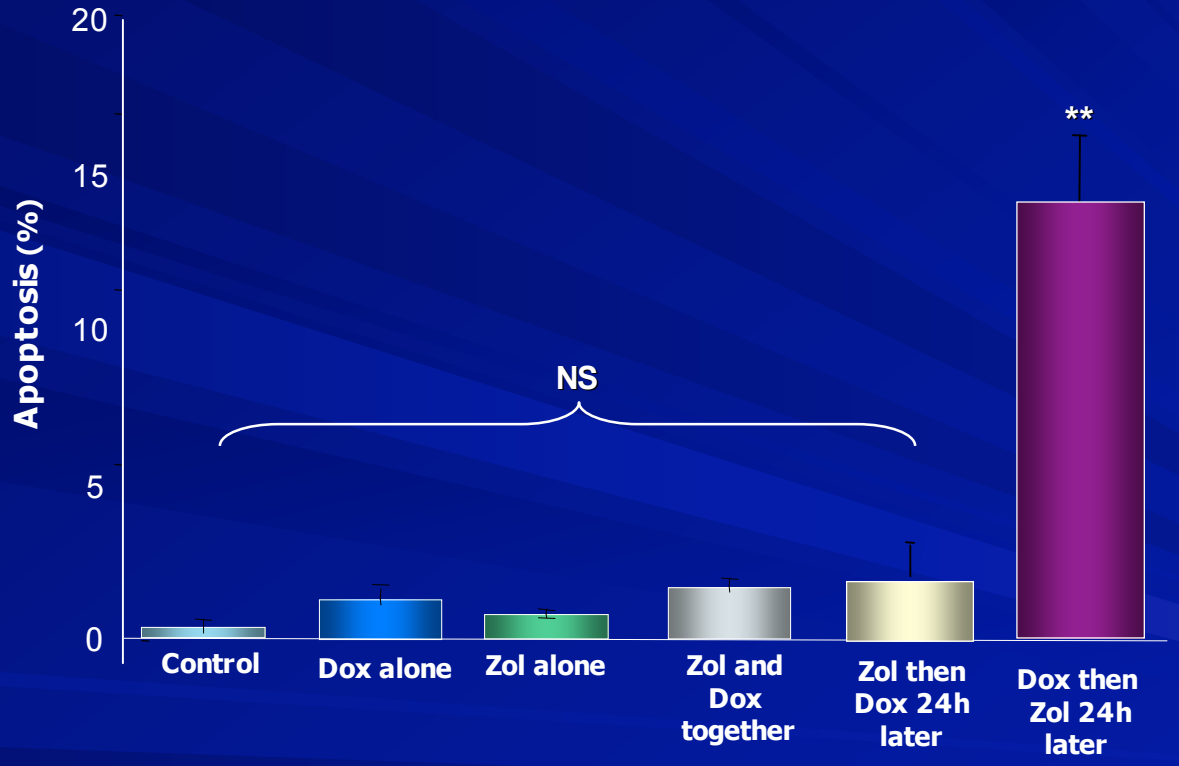
Zoledronic acid demonstrated synergistic effects with cytotoxic drugs

Santini D et al. NCPO, 2006

Author (year)	Biphosp.	Cancer cell line	Type of drug	Type of effect
Neville-Webbe HL (2005) Woodward J (2005)	ZOL	breast cancer cell lines, prostate cancer cell line	Doxorubicin (pro-apoptosis) (Inhibition of invasion)	Synergistic, timing and schedule-dependent
Evans (2005)	ZOL	breast cancer cell lines, prostate cancer cell line	Paclitaxel (pro-apoptosis)	Synergistic, timing and schedule-dependent
Morgan (2007)	ZOL	hormone resistant prostate cancer cell line PC-3	Docetaxel (pro-apoptosis)	Not Synergistic
Duivenvoorden WC (2007)	ZOL	mouse model of human breast cancer	Doxycycline	decreased tumour burden (synergistic)
Vogt U (2004)	ZOL	breast cancer cell line	epirubicin/cyclophosphamide/docetaxel/paclitaxel (growth inhibition)	Synergistic
Neville-Webbe HL (2005)	ZOL	Breast cancer cell line	Letrozole (pro-apoptosis)	Synergistic, timing and schedule-dependent
Trojan J (2005)	ZOL	Gastric cancer cell line	Gemcytabine oxaliplatin (pro-apoptosis)	Synergistic
Matsumoto S (2005)	ZOL	SCLC cell line	Paclitaxel/etoposide/cisplatinum/irinotecan (pro-apoptosis)	Synergistic
Tassone P (2000)	ZOL	human myeloma cell lines	Dexamethasone (pro-apoptosis, growth inhibition)	Synergistic
Hiraga T (2003)	ZOL	breast 4T1 murine breast carcinoma	UFT (pro-apoptosis)	Additive
Algur E (2005)	ZOL	IM-9 myeloma cells and C4-2 prostate cancer	RADIOTHERAPY	Synergistic

Zoledronic acid and doxorubicin – Does the Order of Exposure Matter?

Breast Cancer cells -24 h treatment with 0.05μM Doxorubicin /1h 25μM Zoledronic acid



For maximum cell death - Doxorubicin must be given prior to zoledronic acid

(HL Neville-Webbe *et al*, Int J Cancer 2005)

Is there also synergy *in vivo*?

In vivo Models of Breast Cancer

Subcutaneous

In Bone

Treat with doxorubicin and zoledronic acid - alone and in combination or sequence

Analysis of tumours

- Size/number
- Growth rate
- Apoptosis
- Proliferation
- Angiogenesis
- Histology
- DNA

Animals

- Survival?
- Morbidity?

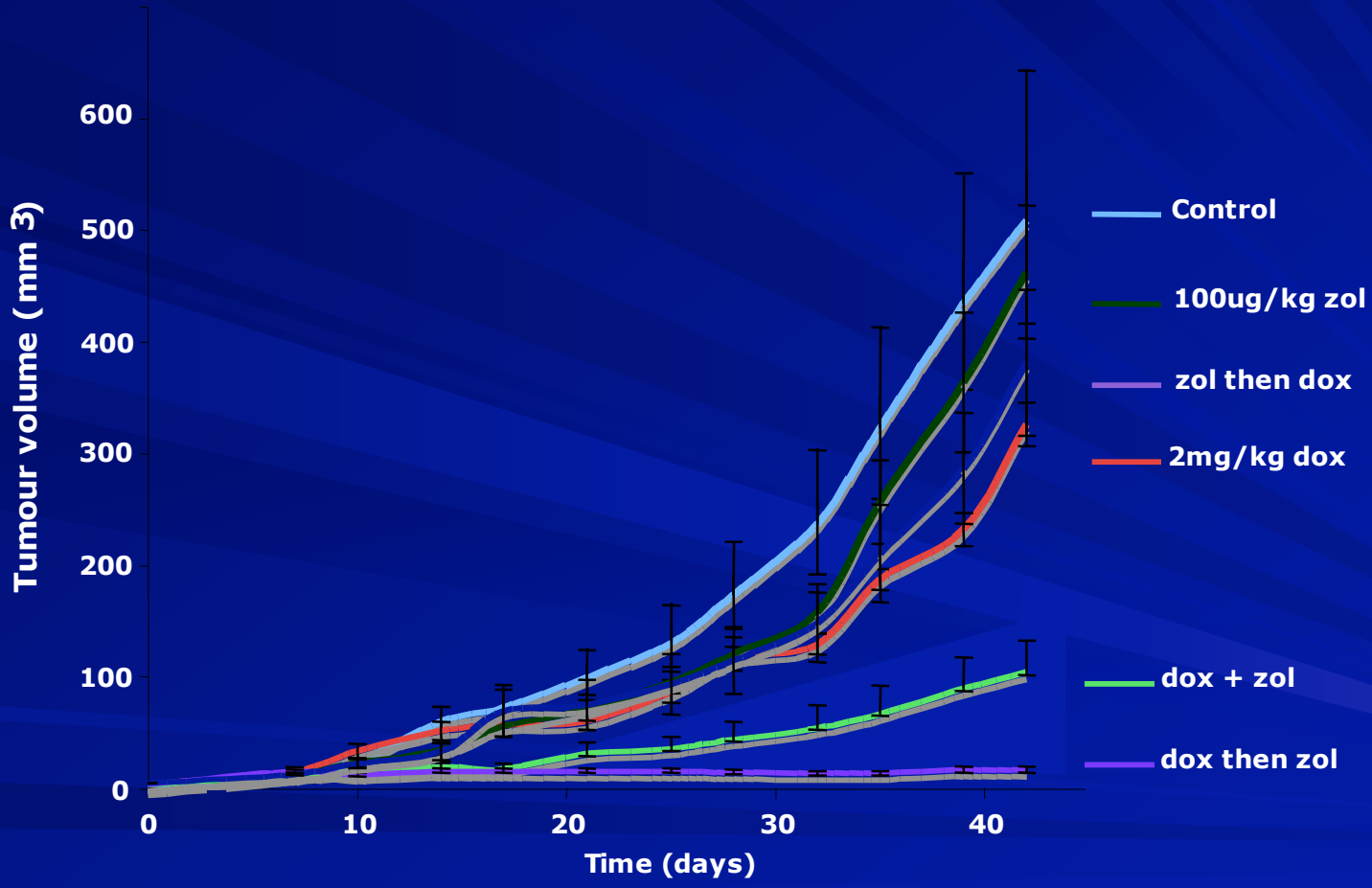
Analysis of bone

- Bone lesions - number/size
- Osteoclast/osteoblast number
- Bone structure/integrity
- Vascularisation
- Extra-osseous tumour growth

PD Ottewell, Clinical Cancer Research, in press

Effects of Dox and Zol on s.c. tumour growth in vivo

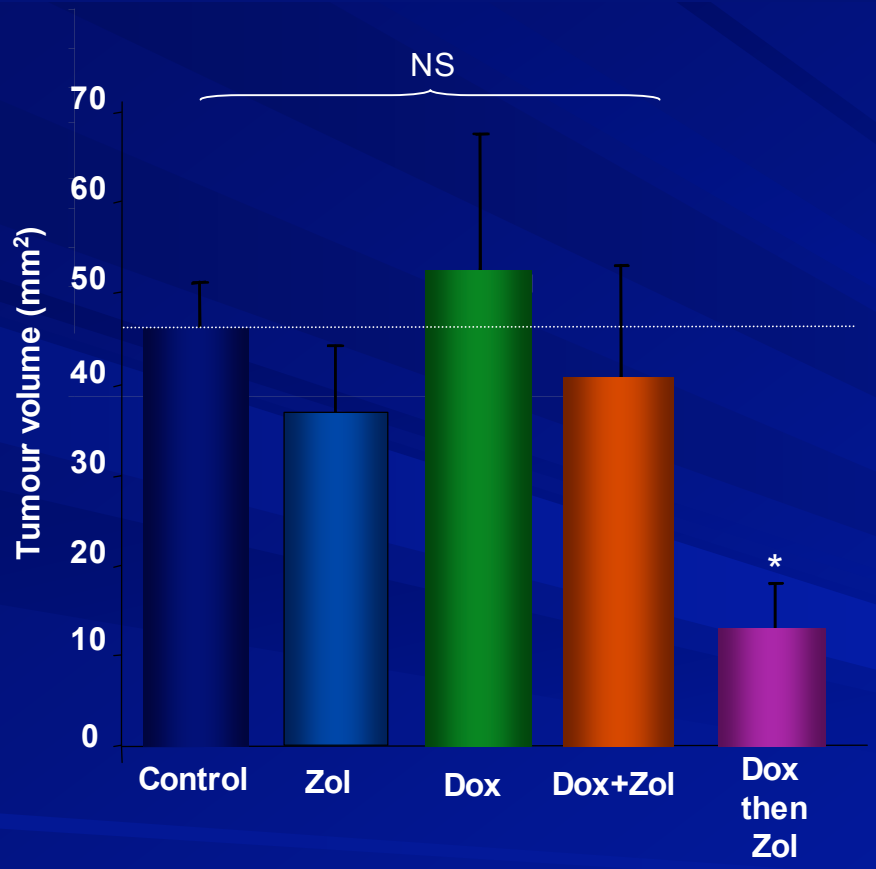
PD Ottewell, Clinical Cancer Research, in press



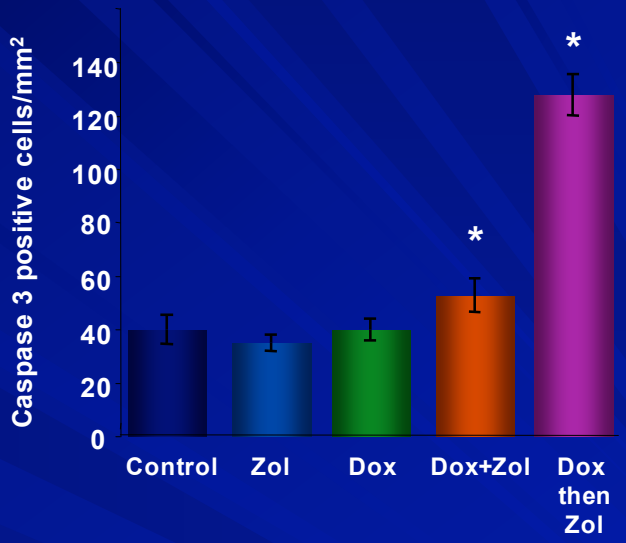
No effect of Dox or Zol alone –
Sequential treatment (dox followed by zol) eliminates tumour growth

Effects of Dox and Zol on BONE METASTASES

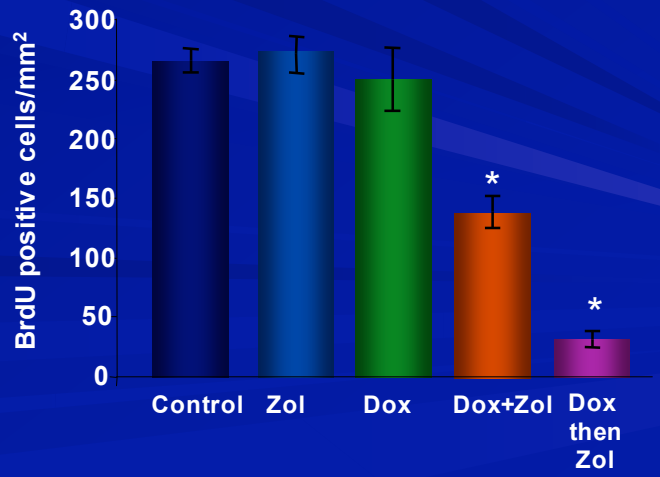
Tumour volume in bone



Tumour cell apoptosis



Tumour cell proliferation

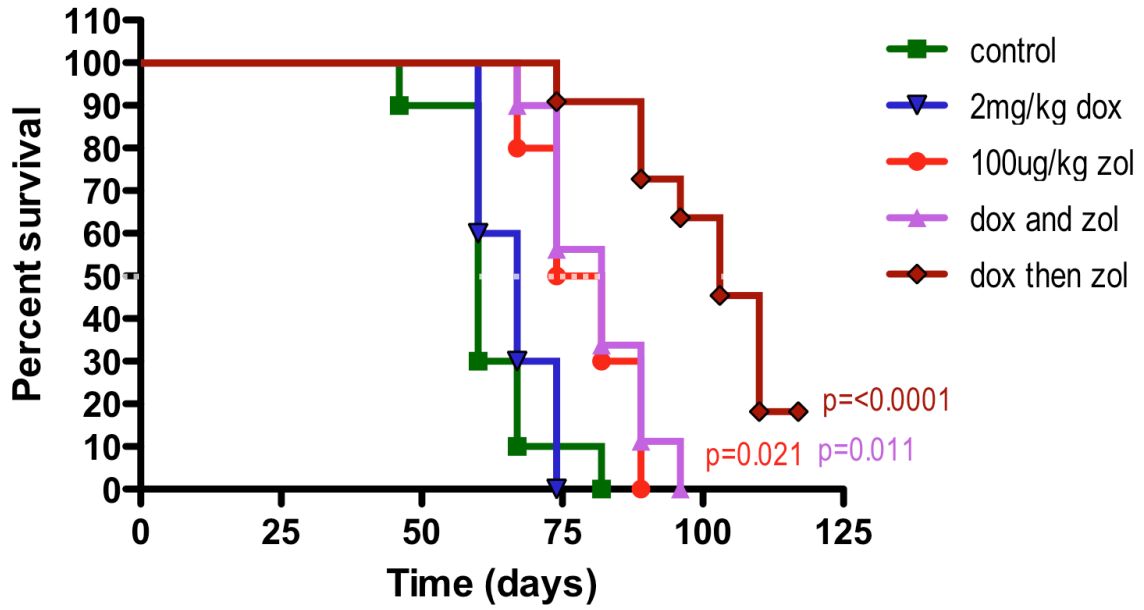


No effect on tumour growth with the single agents

Sequential treatment is superior to combined

PD Ottewell, Clinical Cancer Research, in press

Does combined treatment increase survival?



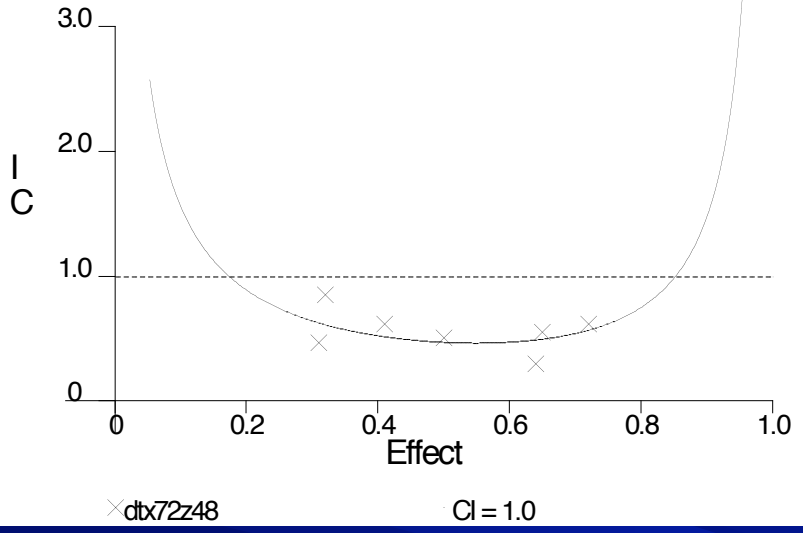
Zoledronic acid alone does improve survival (less bone pain)

Sequential treatment increases survival time significantly and is superior to combined

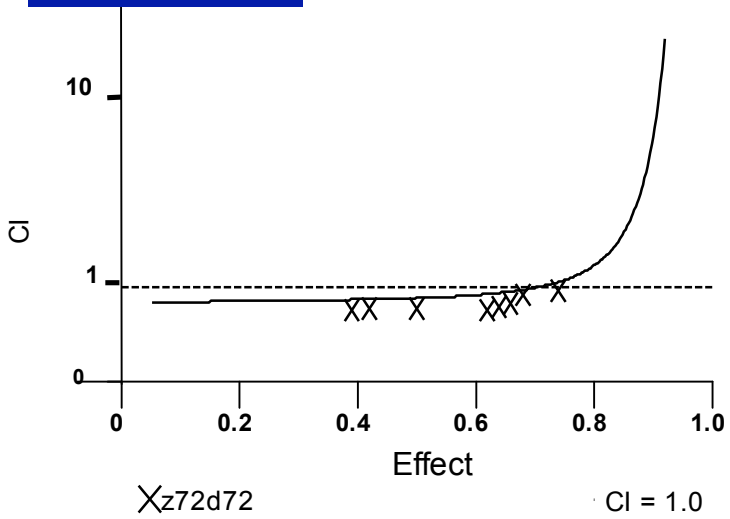
PD Ottewell, Clinical Cancer Research, in press

Prostate cancer cell line: DTX e ZOL synergism

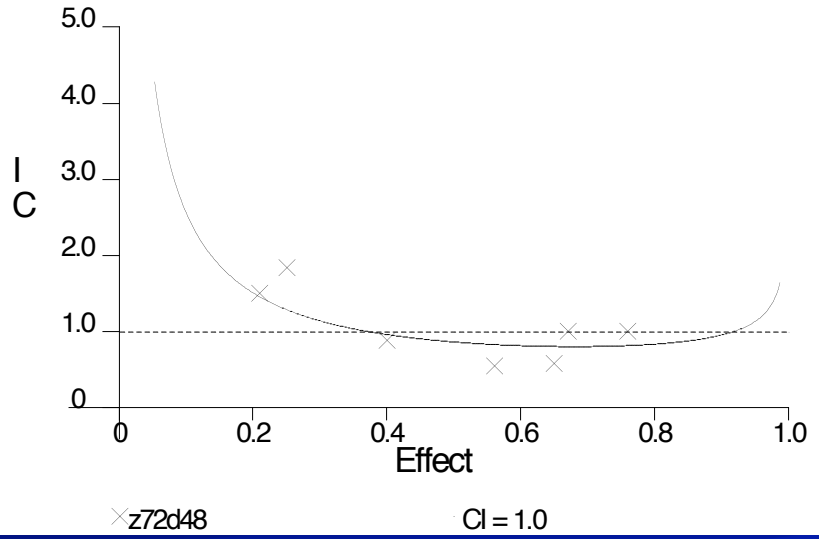
DTX →ZOL



DTX + ZOL



ZOL → DTX



	Effect	
	50%	75%
DTX → ZOL	0.475	0.623
DTX + ZOL	0.960	1.135
ZOL → DTX	0.873	0.822

CI

D. Santini & M. Caraglia, unpublished

Phase I study on the pharmacological combination between zoledronic acid and docetaxel in patients with hormone-refractory prostate cancer

ZANTE

Zoledronic ANd TaxoterE

Istituto Nazionale Tumori
Fondazione G. Pascale

UOC Farmacologia Sperimentale Oncologica M. Caraglia
UOC Oncologia Medica B
UOC Studi Clinici Controllati
UOC Urologia

DISEGNO DELLO STUDIO

Livelli	Sequenza A	Sequenza B
I	DTX 30 mg/m^2 → ZOL	ZOL → DTX 30 mg/m^2
II	DTX 40 mg/m^2 → ZOL	ZOL → DTX 40 mg/m^2
III	DTX 50 mg/m^2 → ZOL	ZOL → DTX 50 mg/m^2

M. Caraglia, data unpublished

Risultati clinici

- **Raggiunto il terzo livello di dose (altri 3 pazienti al completamento) senza raggiungere la MTD.**
- **Previsto un ulteriore livello di dose.**
- **Il protocollo è stato ben tollerato senza riscontro di tossicità grado 3 e 4 secondo CTC-NCI.**

Risposte

- **Livello I: 1 SD duratura (8 mesi); 2 SD della durata massima di 5 mesi.**
- **Livello 2: 1 RP sierologica e 2 SD della durata massima di 6 mesi.**
- **Livello 3: 3 SD con beneficio clinico (riduzione sostanziale del dolore e consumo analgesico e abolizione della richiesta trasfusionale) 2 RP cliniche.**

M. Caraglia, data unpublished

Zoledronic acid demonstrated synergistic effects with biological drugs

Santini D et al. NCPO, 2006

Preclinical evidences of synergistic effects with biological drugs

Author (year)	Biphosph.	Cancer cell line	Type of biological drug	Type of effect
Caraglia M (2004)	ZOL	lung H1355 cancer cells	farnesyl transferase inhibitor R115777 (Zarnestra)	Synergistic
Caraglia M (2004)	ZOL	Human epidermoid head and neck cancer cell line	farnesyl transferase inhibitor R115777 (Zarnestra)	Synergistic
Caraglia M (2005)	ZOL	- Human prostate cancer cell line (PC3) - Murine model of prostate cancer	farnesyl transferase inhibitor R115777 (Zarnestra)	Synergistic both in in vitro and in vivo models
Sonneman J (2007)	ZOL	prostate cancer cell lines LNCaP and PC-3	Histone deacetylase inhibitor (SAHA)	Synergistic
Kuroda J (2003)	ZOL	human leukemic Ph+ cell lines	Imatinib mesylate	Synergistic
Kimura S (2004)	ZOL	leukemic cell lines	Imatinib mesylate	Synergistic
Peterschmitt J (2007)	ZOL	- Breast cancer cell line - Murine model of osteolytic lesions by breadt cancer cell line	antibody against bone sialoprotein II	Synergistic both in in vitro and in vivo models
Witters LM (2003)	ZOL	HER-2/neu transfected breast cancer cell line	Anti-COX-2	Additive

Zoledronic acid demonstrated inhibitory effects on the metastatic process (animal models)

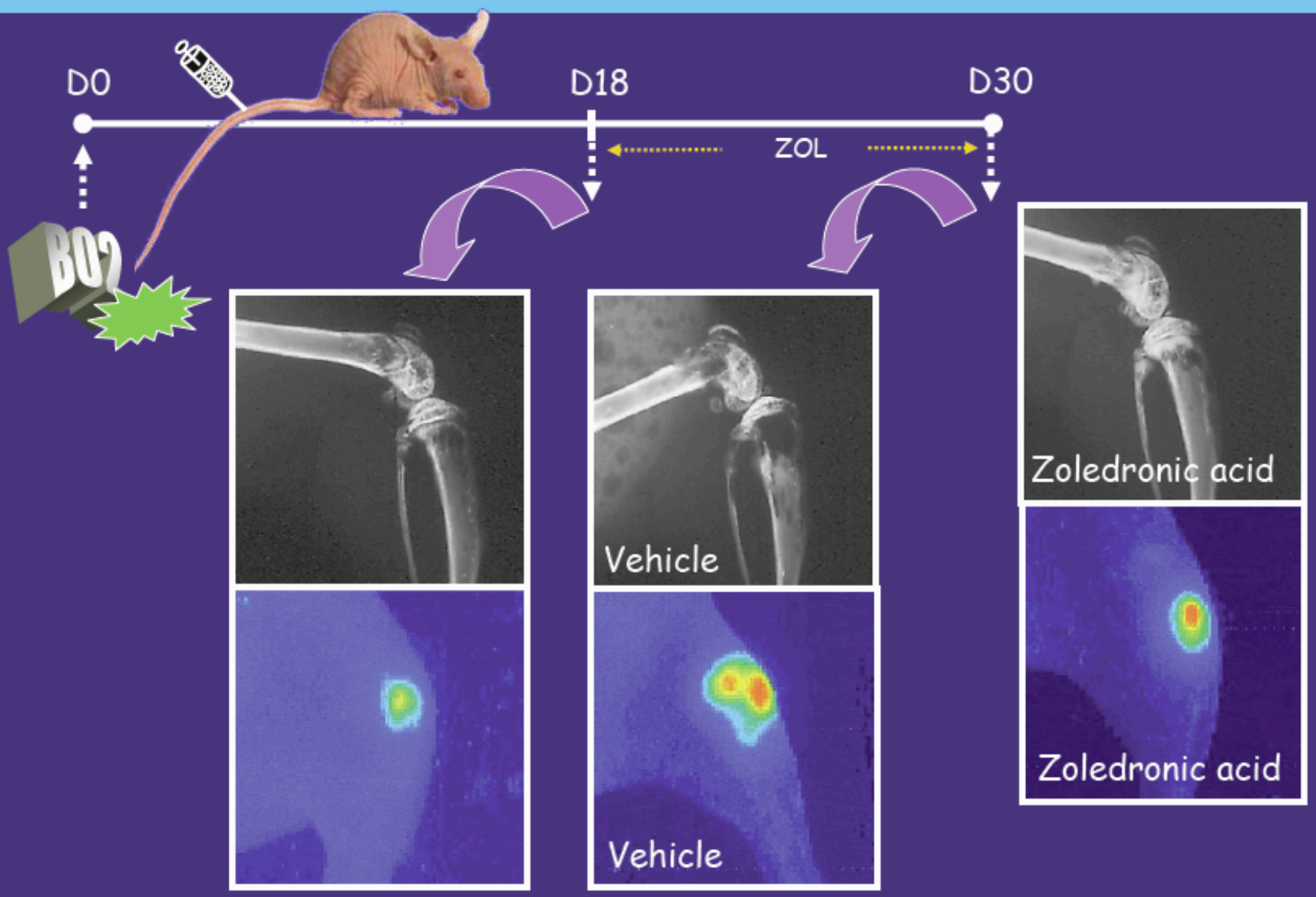
Santini D et al. NCPO, 2006

BPs demonstrated effects on the metastatic process

Preclinical evidences of antitumor effect IN VIVO

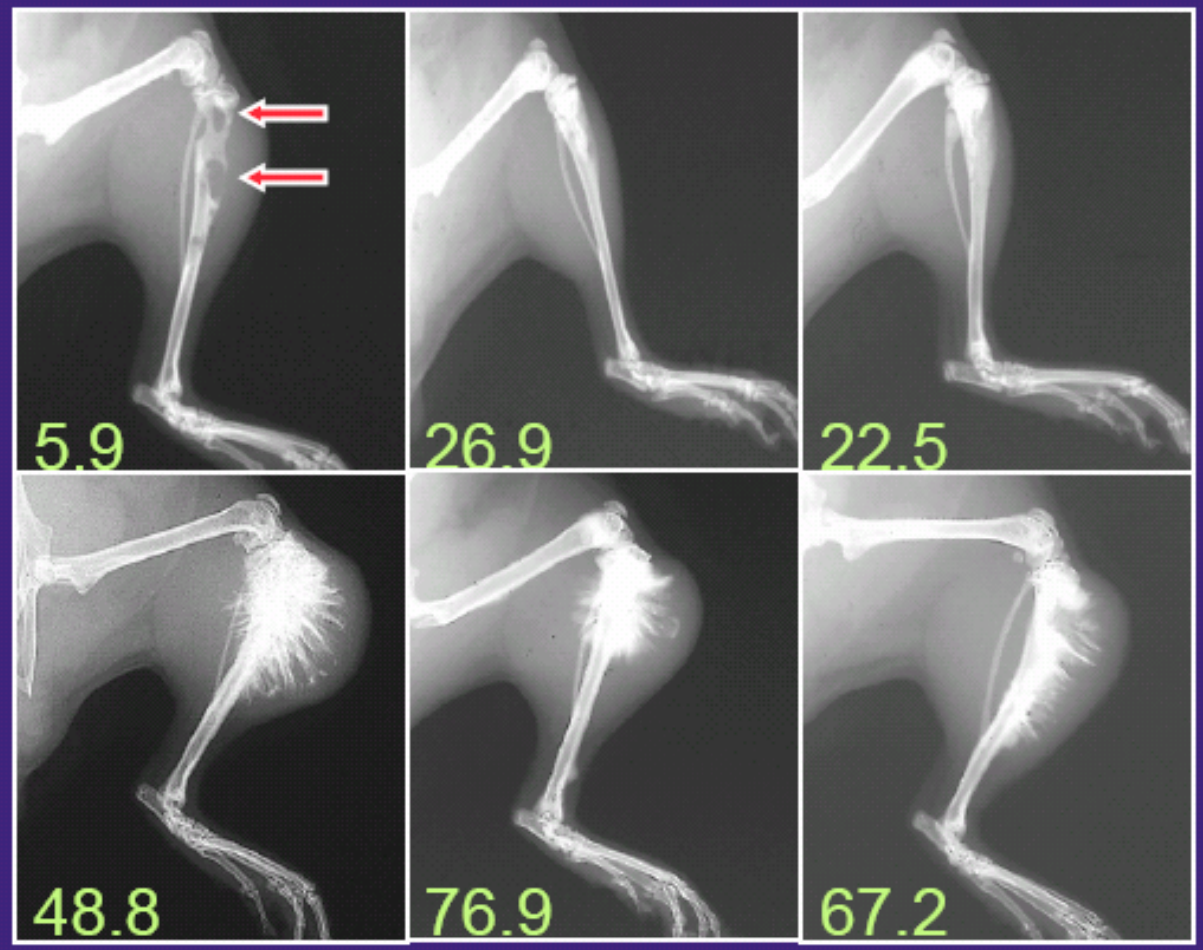
Study	Animal model	Bisphosphonate	Results
Yaccoby et al (2002)	Primary human myeloma cells	Zoledronic acid	inhibition of myeloma cell growth and survival
Croucher et al. (2003)	5T2 murine myeloma	Zoledronic acid	Zoledronic acid prevented the formation of bone lesions
Green et al. (2000)	MDA-MB-231 human breast cancer	Zoledronic acid	Inhibition of tumour-induced osteolysis
Peyruchaud et al. (2001)	MDA-MB-231 human breast cancer	Zoledronic acid	Inhibition of progression of established osteolytic lesions
Hiraga et al. (2004)	4T1 murine mammary carcinoma	Zoledronic acid	enhanced reduction of lung metastases
Nobuyuki et al. (2001)	4T1 murine mammary carcinoma	Zoledronic acid	Inhibition of metastases to bone and liver
Ory B. (2005)	Murine osteosarcoma model	Zoledronic acid	diminishes osteosarcoma-induced lung metastasis and prolongs survival
Corey et al. (2003)	PC-3 and LuCaP 23.1 prostate cancer	Zoledronic acid	Inhibition of growth of osteoblastic and osteolytic metastases
Sato K. (2006)	bladder cancer orthotopic model	Minodronic acid	anticancer effect on bone metastases
Gouin F et al. (2006)	rat chondrosarcoma model	Zoledronic acid	slows down rat primary chondrosarcoma development and increase overall survival
Wakchoure (2006)	mesothelioma tumor model	Zoledronic acid Risedronate	increased the median survival of mice with i.p. mesothelioma tumors in vivo.

Zoledronic Acid Inhibits the Progression of Breast Cancer Osteolytic Lesions in Animals



Peyruchaud O, *et al.* J Bone Miner Res 2001;16:2027-2034.

Zoledronic acid inhibits lytic and blastic lesions in mouse tibia bearing human prostate cancer cells



Corey et al, *Clin Cancer Res*, 2003

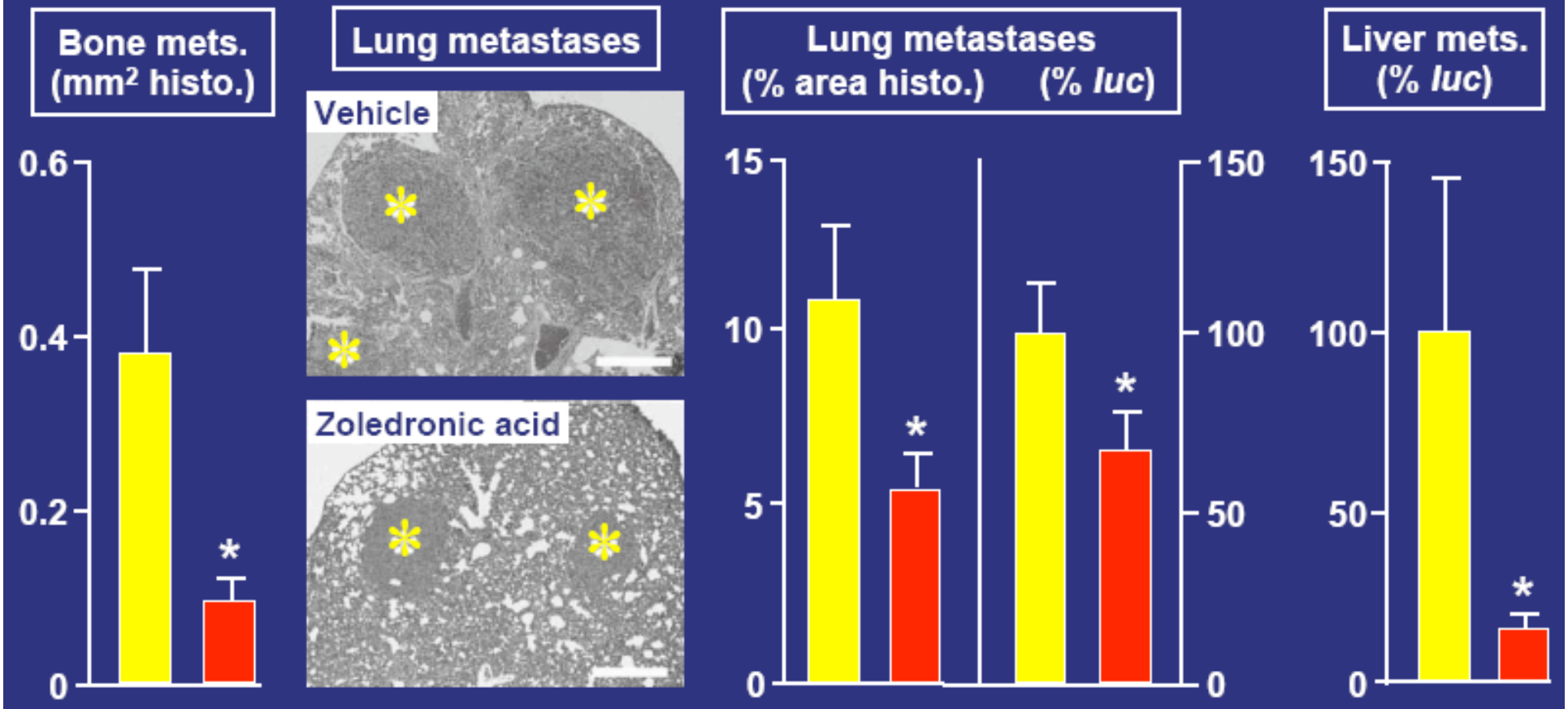
Preclinical evidences of antineoplastic effects on different animal models

In vivo prevention of bone metastases

These animal models provide convincing evidence of the potential of ZOLEDRONIC ACID

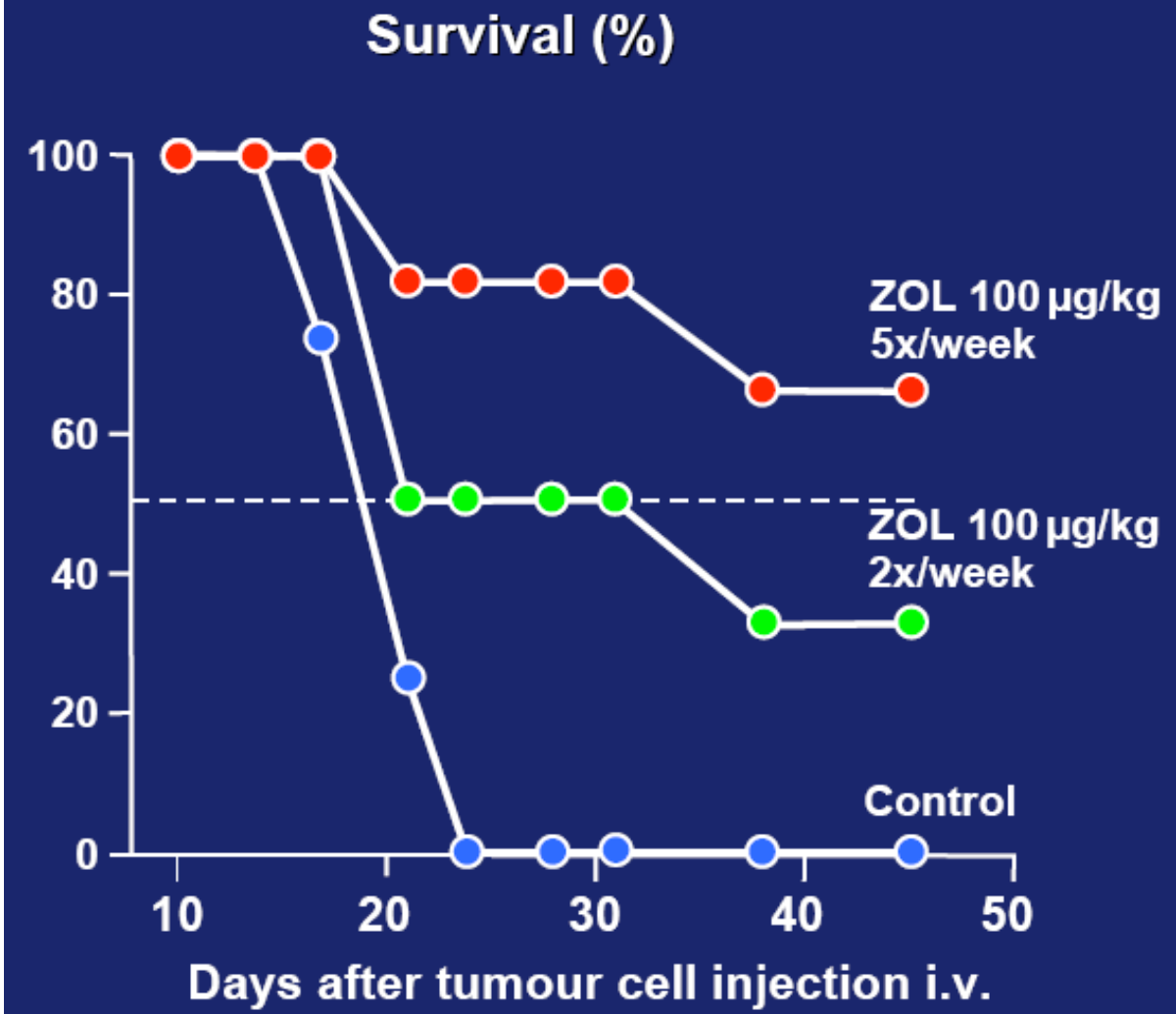
1. To induce tumor cell apoptosis in bone lesions
2. To reduce tumor burden in bone
3. To reduce the number of osteolytic lesions in tumor-bearing mice
4. To prevent formation and progression of bone metastases in a variety of tumor models
5. To reduce serum levels of tumor markers

Zoledronic acid inhibits bone, liver and lung metastases in the murine 4T1/*luc* syngeneic breast cancer model

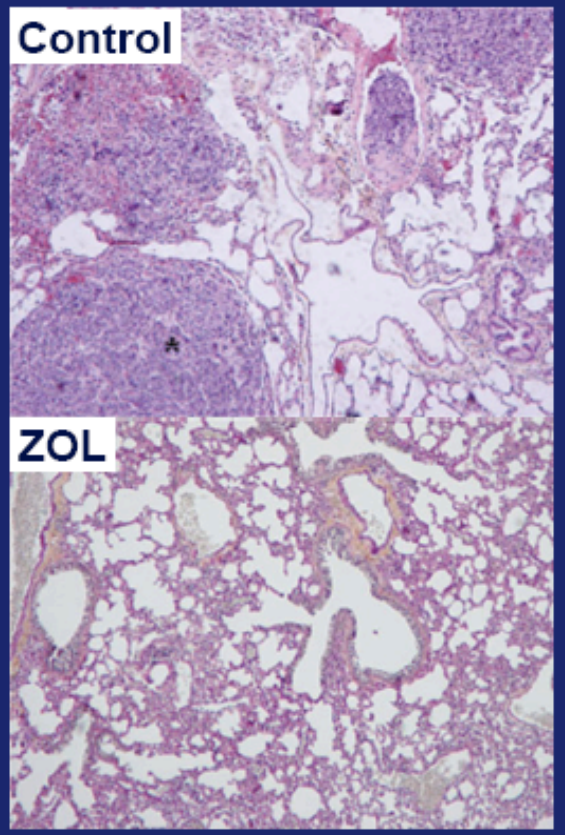


Hiraga et al, *Clin Cancer Res*, 2004

Zoledronic Acid Increases Survival in a Syngeneic Mouse Model of Lung Metastases From Osteosarcoma



Lung histology



Ory et al, *Cancer*, 2005

Preclinical evidences of antineoplastic effects on different animal models

In vivo prevention of extra bone metastases

- **Preliminary data from the 4T1 mammary tumor model have demonstrated that zoledronic acid can also inhibit visceral metastases**

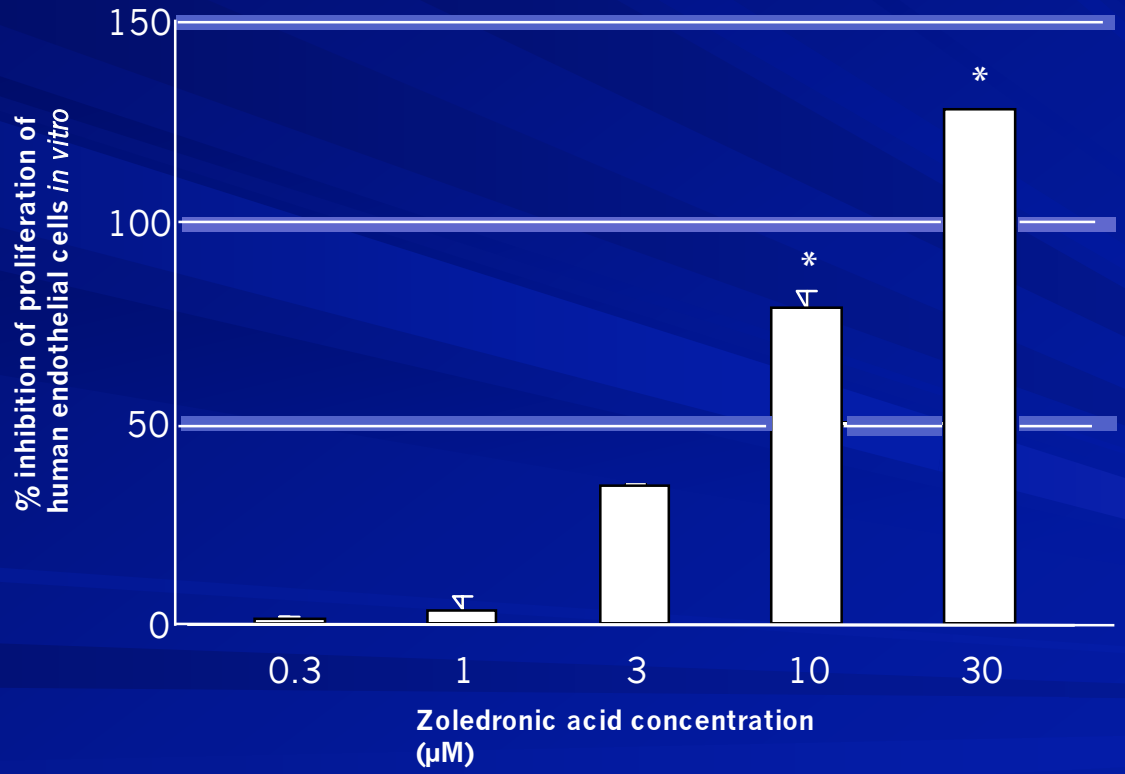
- **In the same model treatment at clinically relevant doses with zoledronic acid also prolonged survival of tumor-bearing mice**

- **Treatment of mice bearing 5T2 myeloma cells with zoledronic acid also resulted in a significantly longer disease-free survival time**

Zoledronic demonstrated effects on angiogenesis

Zoledronic Acid—Anti-angiogenic Potential

- Low doses of zoledronic acid inhibits proliferation of human endothelial cells *in vitro*

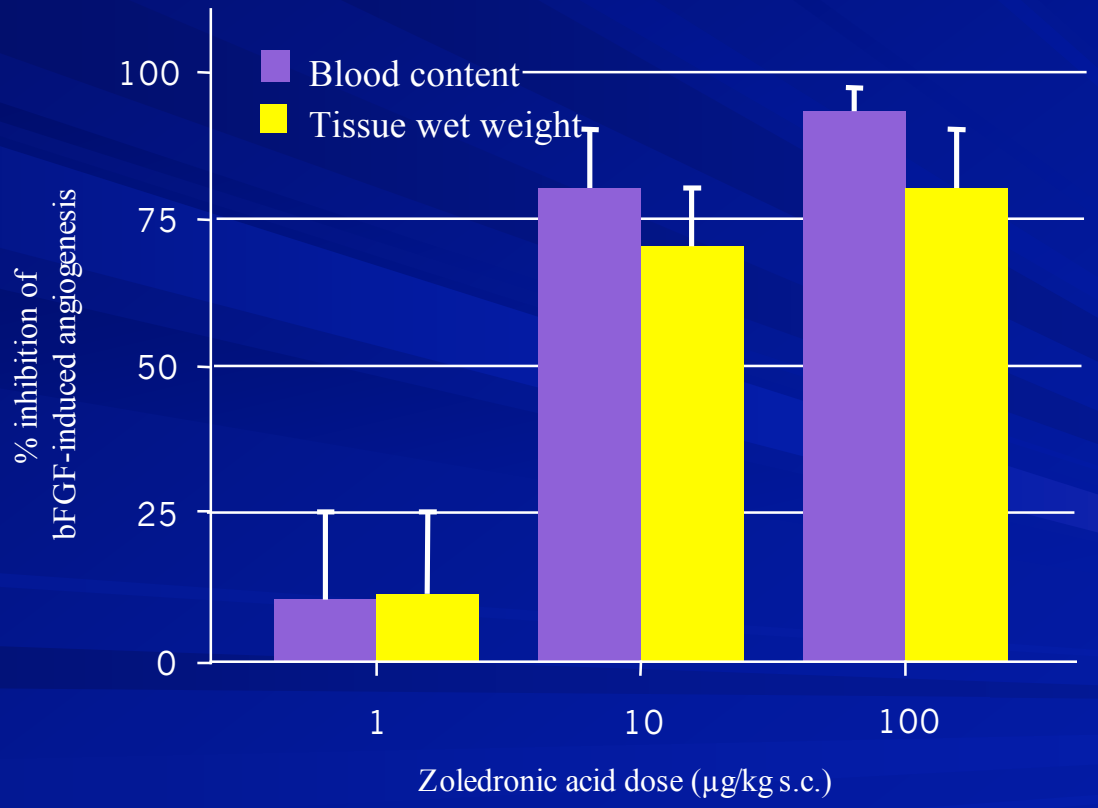


These points have reached statistical significance.

Wood J, et al. *Proc Am Soc Clin Oncol.* 2000.

Zoledronic Acid—Anti-angiogenic Potential

- Low doses of zoledronic acid inhibit angiogenesis in a mouse growth-factor implant model *in vivo*

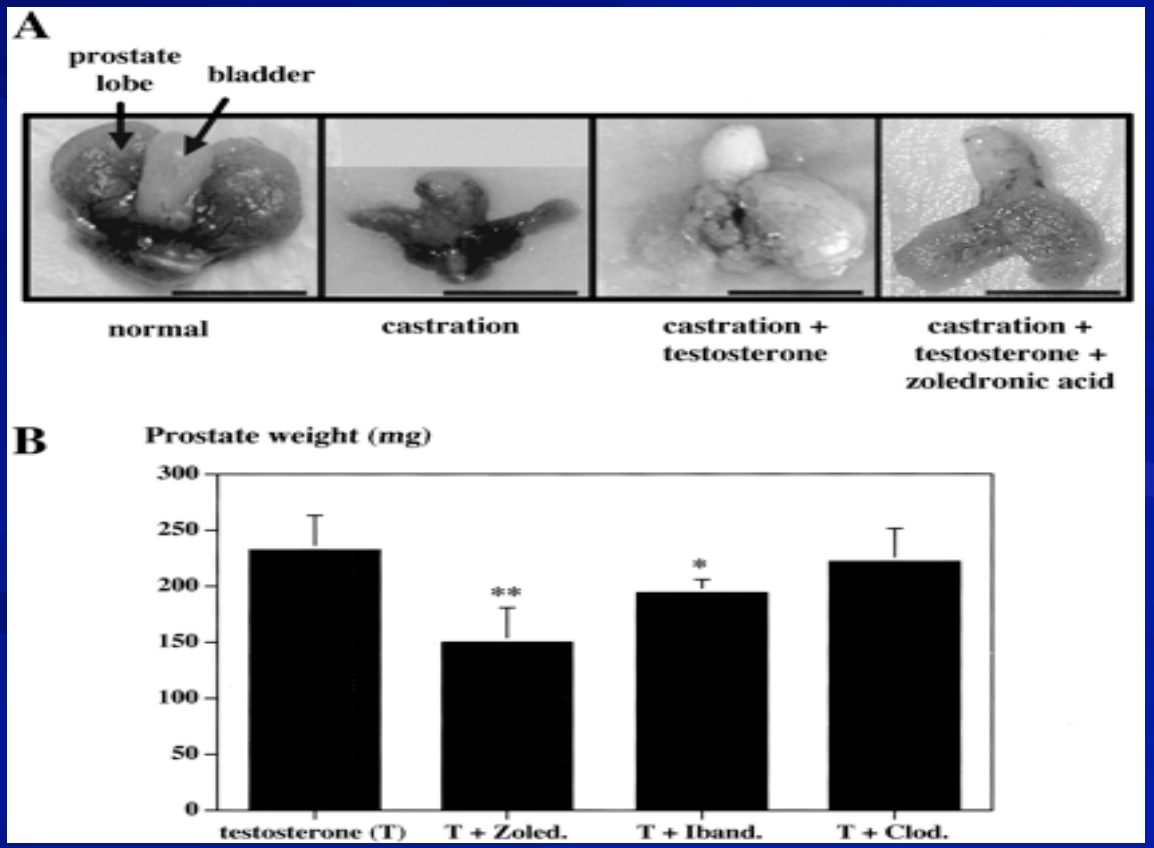


Wood J, et al. *Proc Am Soc Clin Oncol.* 2000.

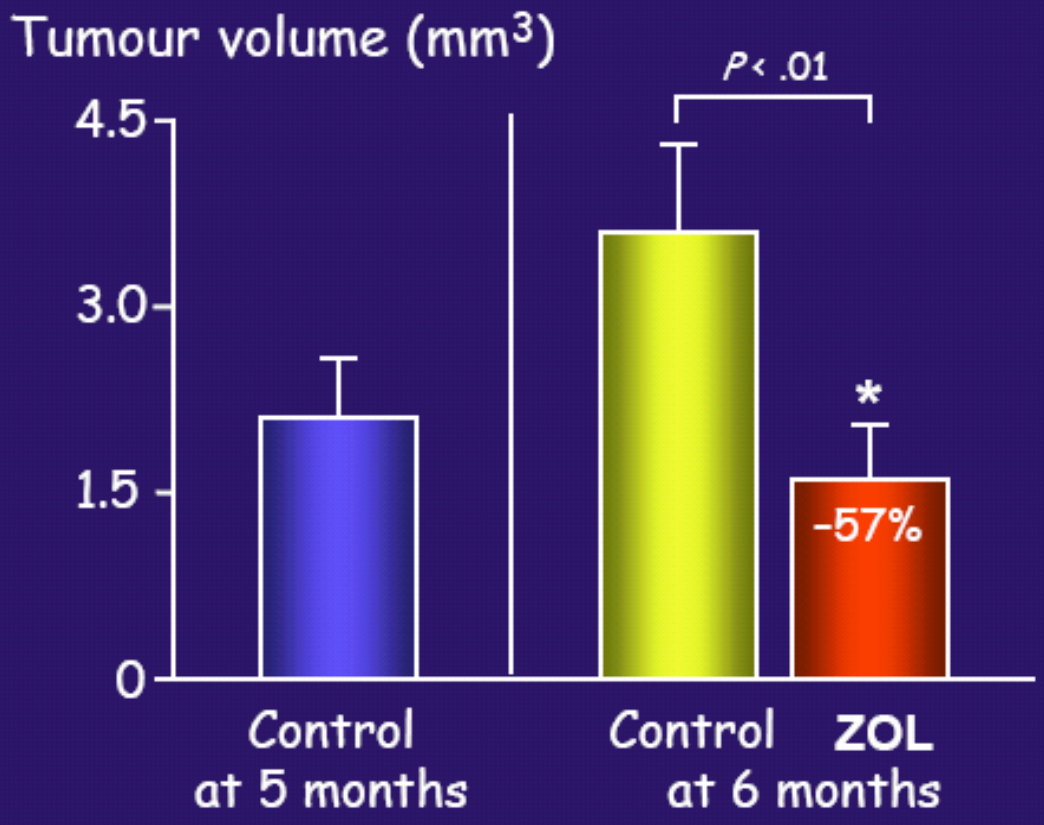
Bisphosphonates Inhibit Testosterone-stimulated Vascular Regrowth in the Ventral Prostate in Castrated Rats

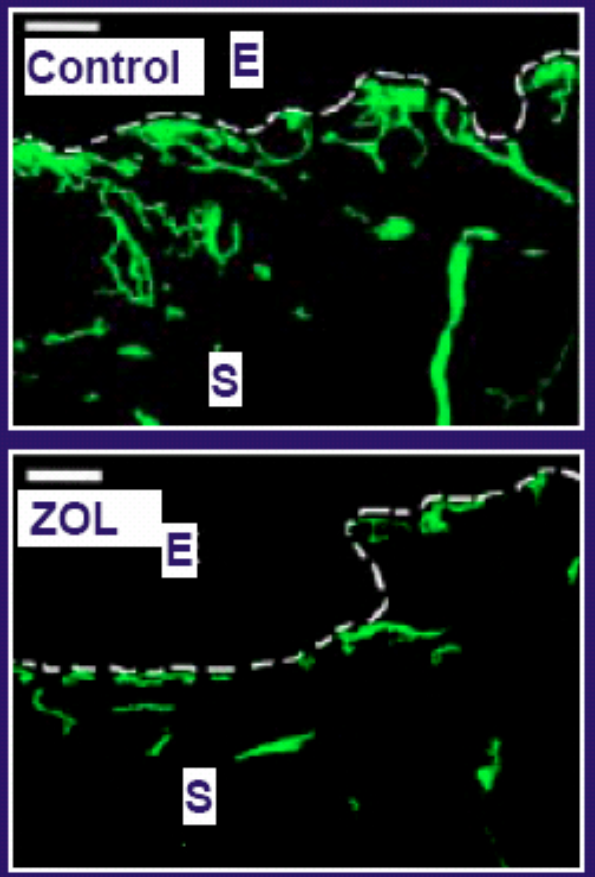
Fournier P et al Cancer Res. 2002

- **Blood vessel immunostaining on prostate tissue sections revealed that both ibandronate and zoledronic acid induced a 50% reduction of the revascularization of the prostate gland** *in animal model (rats)*



Zoledronic Acid Reduces Tumour Angiogenesis



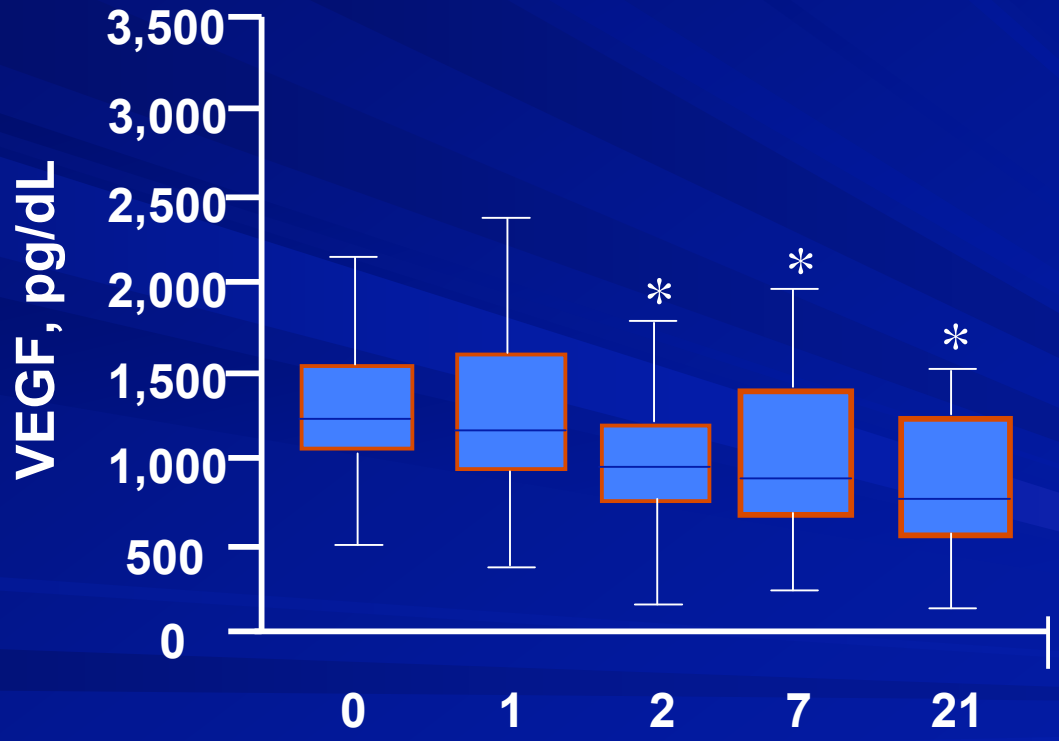


5-month-old mice bearing cervical squamous cell carcinomas were treated with vehicle or ZOL (100 µg/kg/d) in a tumour regression trial for 4 weeks.

Giraudo E, *et al.* J Clin Invest 2004;114:623-633.

Zoledronic Acid Decreased Circulating Plasma VEGF Levels in Patients With Metastatic Cancer

- 30 patients with metastatic cancer were administered a single dose of 4 mg zoledronic acid IV before chemotherapy



*$P < .05$.

Santini D, et al. *Clin Cancer Res*. 2003;9:2893-2897.

Background

- In the Daubine's study weekly regimens of ZA, (with total doses similar to that used in the clinical setting) were effective in demonstrating antitumor effect whereas monthly dosing was not
- Cytotoxic and biological drugs (especially when administered at low repeated doses) target tumor-stroma interactions and in particular angiogenesis, in cancer.
- Some metronomic-chemotherapy regimens induce sustained suppression of circulating proangiogenic cytokines such as VEGF levels
- All these pre clinical and clinical data should represent the rational basis to consider the metronomic administration of bisphosphonates as a new potential therapy targeting the endothelial-tumor-stroma behaviour

Patient Eligibility

Inclusion criteria

- Histologically confirmed solid cancer associated with scintigraphic and radiographic identification of bone metastases
- ECOG performance status ≤ 2
- Life expectancy > 3 months
- Neutrophil count > 2.0 × 10^9/L, platelet count > 100 × 10^9/L, normal hepatic function, serum creatinine < 1.5 times the upper limit of normal, and creatinine clearance > 60 mL/minute

Exclusion criteria

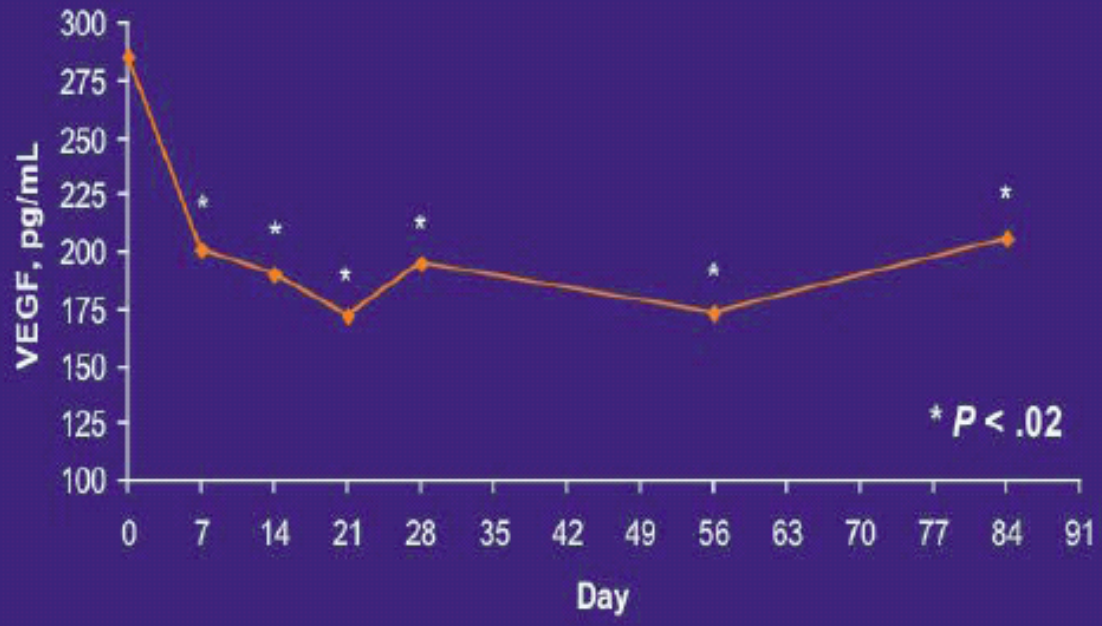
- Acute infections or inflammatory diseases during the 3 last months or fever (body temperature > 38.0°C) during the last week before study entry
- Chronic infections or inflammatory diseases
- Hormone therapy started ≤ 3 months before study entry
- Radiotherapy, chemotherapy, immunotherapy or growth factors during FIRST 4 WEEKS OR LAST 4 WEEKS of the study period
- Patients recently/simultaneously treated with steroids or bisphosphonates

Baseline Eastern Cooperative Oncology Group

Santini D, et al. *Clin Cancer Res.* 2007;13:4482-4486.

Results

- Patients with metastatic bone disease (N = 26) received zoledronic acid
  - 1 mg every week for 4 weeks followed by 4 mg every 28 days
  - VEGF assessment at baseline, and on days 1, 7, 14, 21, 28, 56, and 84



  - First clinical demonstration of possible anti-angiogenic effect of weekly doses of zoledronic acid

Santini D, et al. *Clin Cancer Res.* 2007;13:4482-4486.

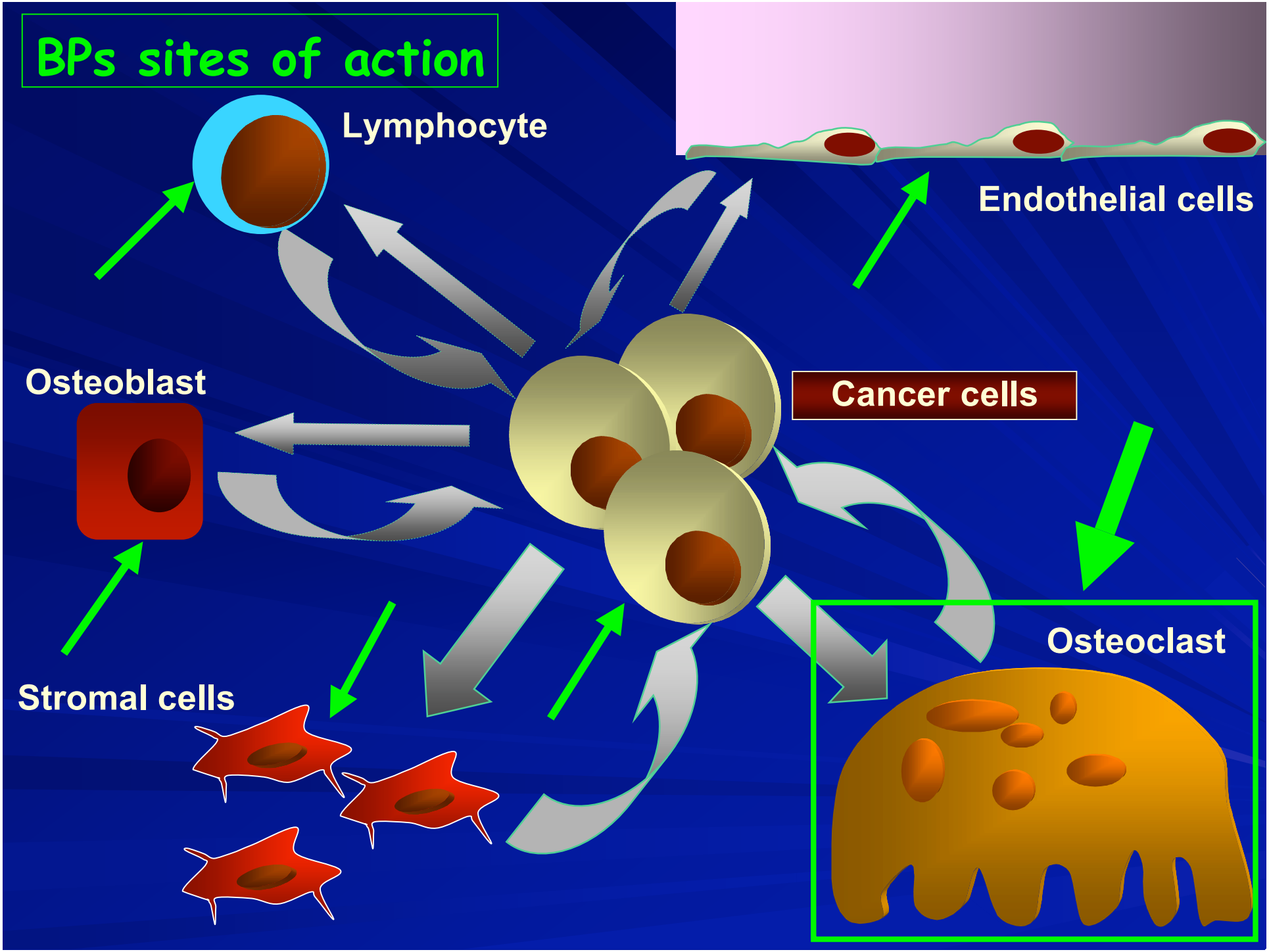
Conclusions (continued)

- These clinical data represent a rationale to consider the administration of bisphosphonates as a potential therapy targeting the endothelial-tumor-stroma environment

Future Perspectives

- Bisphosphonates may synergistically interact with antiangiogenic drugs (ie, antibodies against VEGF or VEGF receptor 2, or small tyrosine kinase molecules that inhibit multiple angiogenic receptors [PDGFR, VEGFR, EGFR])

VEGF = Vascular endothelial growth factor; PDGFR = Platelet-derived growth factor receptor; VEGFR = Vascular endothelial growth factor receptor; EGFR = Epidermal growth factor receptor.


BPs sites of action
Lymphocyte
Endothelial cells
Osteoblast
Cancer cells
Osteoclast
Stromal cells

Thank you very much for the attention