



# Novel Approaches to Targeting Tumour Growth

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# 'Designer Biomimetic Vectors for the Delivery of Nucleic Acids



**Principle Investigator: Dr Helen McCarthy**

Co-investigator: Tracy Robson

# iNOS Gene Therapy



## Nitric oxide—A novel therapeutic for cancer

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ARTICLE INFO

**ABSTRACT**  
 Much research over the past two decades has focused on understanding the complex interactions of nitric oxide (NO) in both physiological and pathological processes. As with many other aspects of NO biology, its precise role in tumour pathophysiology has been the cause of intense debate and we now know it participates in numerous signalling pathways that are crucial to the malignant character of tumours. This article highlights experimental evidence that NO is a cancer suppressor and that inhibition of NO synthesis, and subsequent up-regulation of iNOS, is a potential therapeutic target. The evidence presented here suggests that iNOS gene therapy could be a novel approach to cancer treatment.

THE JOURNAL OF CLIMATE MEDICINE  
 Published online 15 March 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jcsm.202

## Use of the radiation-inducible WAF1 promoter to drive iNOS gene therapy as a novel anti-cancer treatment

Jenny Worthington<sup>1</sup>  
 Helen O. McCarthy<sup>1,4</sup>  
 Elaine Barrett<sup>1</sup>  
 Catherine Adams<sup>1</sup>  
 Tracy Robinson<sup>1</sup>  
 David G. Hirst<sup>1,4</sup>

### Abstract

**Background:** Inducible nitric oxide synthase (iNOS) gene therapy has been identified as a potential anti-tumour strategy. A major problem common to all gene therapy strategies is ensuring expression in the target tissue. In this study we report on the use of the X-ray-inducible WAF1 promoter to achieve targeting of iNOS expression to the tumour volume.

Gene Therapy (2008) 15, 495–503  
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 www.nature.com/gt

## ORIGINAL ARTICLE

### p21<sup>(WAF1)</sup>-mediated transcriptional targeting of inducible nitric oxide synthase gene therapy sensitizes tumours to fractionated radiotherapy

H.O. McCarthy<sup>1,4</sup>, J. Worthington<sup>2</sup>, E. Barrett<sup>1</sup>, E. Castro<sup>2</sup>, M. Boyd<sup>1</sup>, R. Mairs<sup>1</sup>, C. Ward<sup>1</sup>, S.R. McKewen<sup>1</sup>, D.G. Hirst<sup>1</sup> and T. Robson<sup>1</sup>

<sup>1</sup>School of Pharmacy, McClay Research Centre, Queen's University Belfast, Northern Ireland, UK; <sup>2</sup>Centre for Molecular Biomedicine, University of East Anglia, Norwich, Norfolk, UK and <sup>3</sup>Targeted Therapy Group, Division of Cancer Science and Molecular Pathology, Glasgow University, Cancer Research UK Astute Laboratories, Glasgow, UK

Cancer gene therapy that utilizes toxic transgene products requires strict transcriptional targeting to prevent adverse normal tissue effects. We report on the use of a promoter derived from the cyclin dependent kinase inhibitor, p21<sup>(WAF1)</sup>, to control transgene expression. We demonstrate that this promoter is relatively silent in normal cells (L1210, F98, HMEC-1) compared to the almost constitutive expression obtained in tumour cells (DU145, LNCaP, HT29 and MCF-7). Varying p53 status, a characteristic that will be important for gene therapy protocols, in addition, we found that the p21<sup>(WAF1)</sup> promoter could be further induced by both external radiation (up to eight-fold in DU145 cells), intracellularly irradiated radioisotopes (<sup>125</sup>I-APMAGE; up to 3.5-fold

in SK-N-BE(2c) cells) and hypoxia (DU145 cells). We have previously demonstrated that iNOS gene therapy in vivo by using inducible nitric oxide synthase (iNOS) to generate the potent oxidant (NO<sup>•</sup>). Here, we report that the schedule of p21<sup>(WAF1)</sup>-driven iNOS gene therapy sensitized both p53 wild-type and p53 mutant HT29 tumours to five Gy and highlight the utility of this p21 approach. Gene Therapy (2007) 14, 248–255. doi:10.1007/s12016-007-9018-2 published online 28 September 2008

THE JOURNAL OF CLIMATE MEDICINE  
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## Human osteocalcin: a strong promoter for nitric oxide synthase gene therapy, with specificity for hormone refractory prostate cancer

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 Jonathan A. Coulter<sup>1,4</sup>  
 Jenny Worthington<sup>2</sup>  
 Tracy Robinson<sup>1</sup>  
 David G. Hirst<sup>1,4</sup>

<sup>1</sup>School of Pharmacy, McClay Research Centre, Queen's University Belfast, Northern Ireland, UK; <sup>2</sup>Centre for Molecular Biomedicine, University of East Anglia, Norwich, Norfolk, UK; <sup>3</sup>Centre for Drug Research, Department of Pharmacy, Loughborough University, Loughborough, Leicestershire, Leicestershire LE11 3TU, UK; <sup>4</sup>Centre for Drug Research, Department of Pharmacy, Loughborough University, Loughborough, Leicestershire, Leicestershire LE11 3TU, UK

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<sup>2</sup>These authors contributed equally to this work.

### Abstract

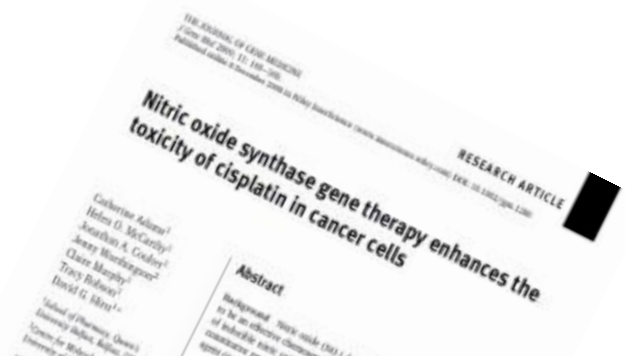
**Background:** Gene therapy has been identified as a promising treatment strategy for hormone refractory prostate cancer (HRPC). We report, for the first time, the use of the human osteocalcin (hOC) promoter to control inducible nitric oxide synthase (iNOS) transgene expression in HRPC.

**Methods:** Human prostate carcinoma cells (PC3, DU145, LNCaP), colon cancer cells (HT29) and human embryonic kidney cells (HEK293T) were transfected with either constitutively driven iNOS (iNOS-C) or hOC-driven iNOS (iNOS-hOC), in control, basal, or high levels of hOC expression. hOC expression was measured using a hOC-specific antibody, and transgene activity.

**Results:** Transfection of the hOC/iNOS plasmid increased iNOS protein and total nitric oxide (NO) in PC3 and DU145 cells, but not HEK293T or HT29. Transfection with iNOS-hOC or hOC-iNOS resulted in an additional approximately 10-fold increase in iNOS protein in the HRPC cells, but not in the non-prostate cells. However, transfection with iNOS-hOC did not result in a significant increase in NO in the HRPC cells.

**Conclusions:** Utilizing the osteocalcin-specific promoter of the hOC gene to co-express with the iNOS gene, we have demonstrated a specific and targeted expression in the endogenous endogenous prostate cancer cells using hOC/iNOS, in contrast to control and endogenous independent cells. Furthermore, the levels of hOC gene expression were comparable with those more generalized with constitutively driven iNOS. The data obtained from this study provide a basis for further development of hOC/iNOS gene therapy strategies to target HRPC. Gene Therapy (2008) 15, 504–511. doi:10.1007/s12016-008-9020-2

**Keywords:** iNOS; gene therapy; osteocalcin; hormone refractory prostate cancer; transcriptional targeting



Gene Therapy (2008) 15, 495–503  
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 www.nature.com/gt

## ORIGINAL ARTICLE

### The radiation-inducible pE9 promoter driving inducible nitric oxide synthase radiosensitizes hypoxic tumour cells to radiation

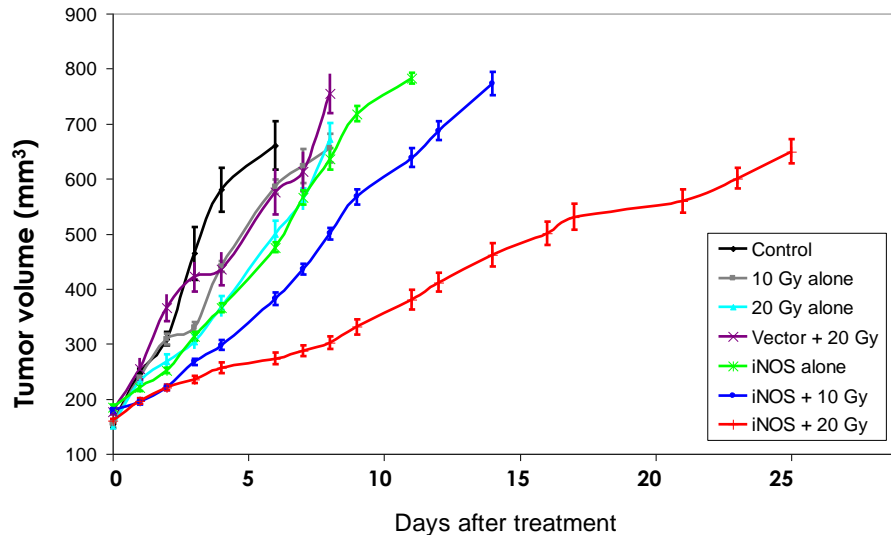
J.A. Coulter<sup>1,4</sup>, H.O. McCarthy<sup>1,4</sup>, J. Worthington<sup>2</sup>, T. Robson<sup>1</sup>, S. Scott<sup>3</sup> and D.G. Hirst<sup>1,4</sup>

<sup>1</sup>School of Pharmacy, McClay Research Centre, Queen's University Belfast, Northern Ireland, UK; <sup>2</sup>Centre for Molecular Biomedicine, University of East Anglia, Norwich, Norfolk, UK and <sup>3</sup>Medical School of Pharmacy, University of Kent, Kent, UK

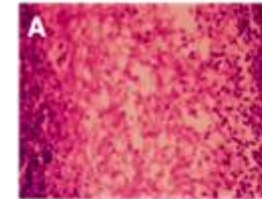
our-specific alignment of irradiated the high levels of the this radiation dose of 10 Gy (P < 0.01). The combination of both therapies resulted in a significant 4.25 day growth delay compared to the gene therapy treatment alone (P < 0.001). In summary, we have demonstrated the potential of the pE9/iNOS construct for radiosensitizing radio-resistance conferred by tumour cell hypoxia in vitro and in vivo, with greater tumour growth delay observed following the treatment with the gene therapy construct as compared with radiotherapy alone. Gene Therapy (2008) 15, 495–503. doi:10.1007/s12016-008-9020-2 published online 7 February 2008

# iNOS radiogenic therapy

Cytotoxicity and radiosensitisation of RIF-1 tumours *in vivo*.



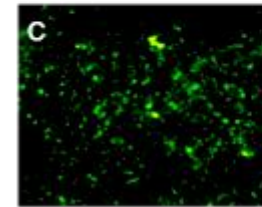
Necrosis and apoptosis (*in vivo*)



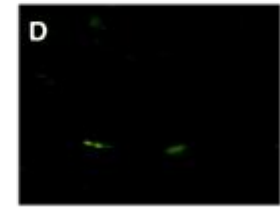
H & E tumour 24 h after CMV/iNOS



H & E of control tumour.

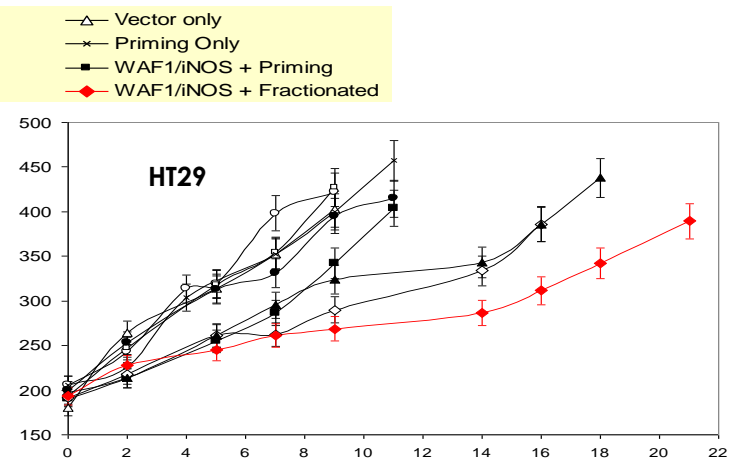
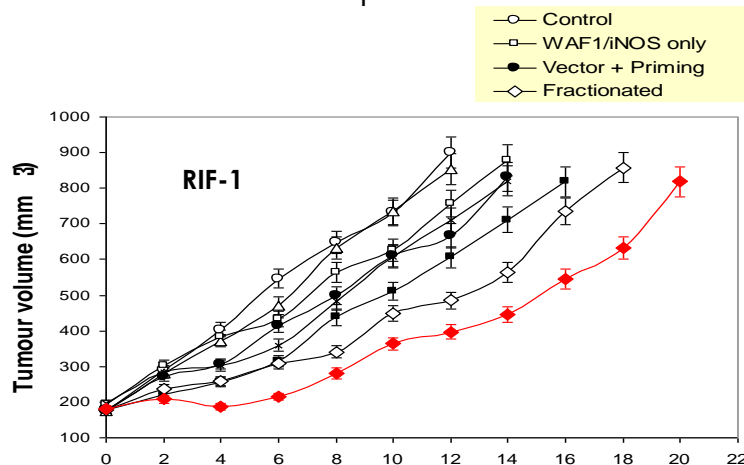


Tunel (CMViNOS)



Tunel (control)

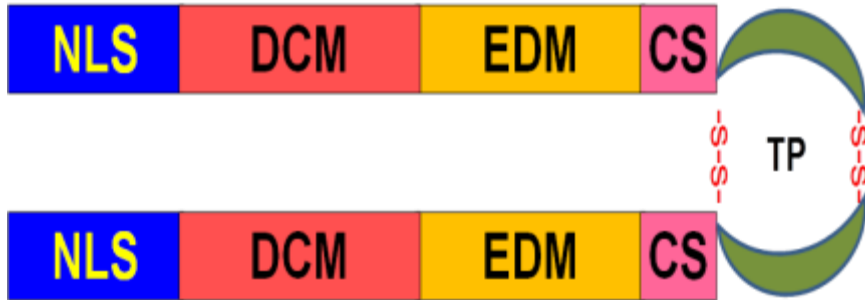
Also effective in fractionated protocols



**Conclusion: NO production by activation of the transfected iNOS gene kills tumour cells and sensitises them to radiation *in vivo*.**

From Worthington et al. 2002, *Gene therapy*;  
McCarthy et al. 2007 *Gene Therapy*

# Designer Biomimetic Vector



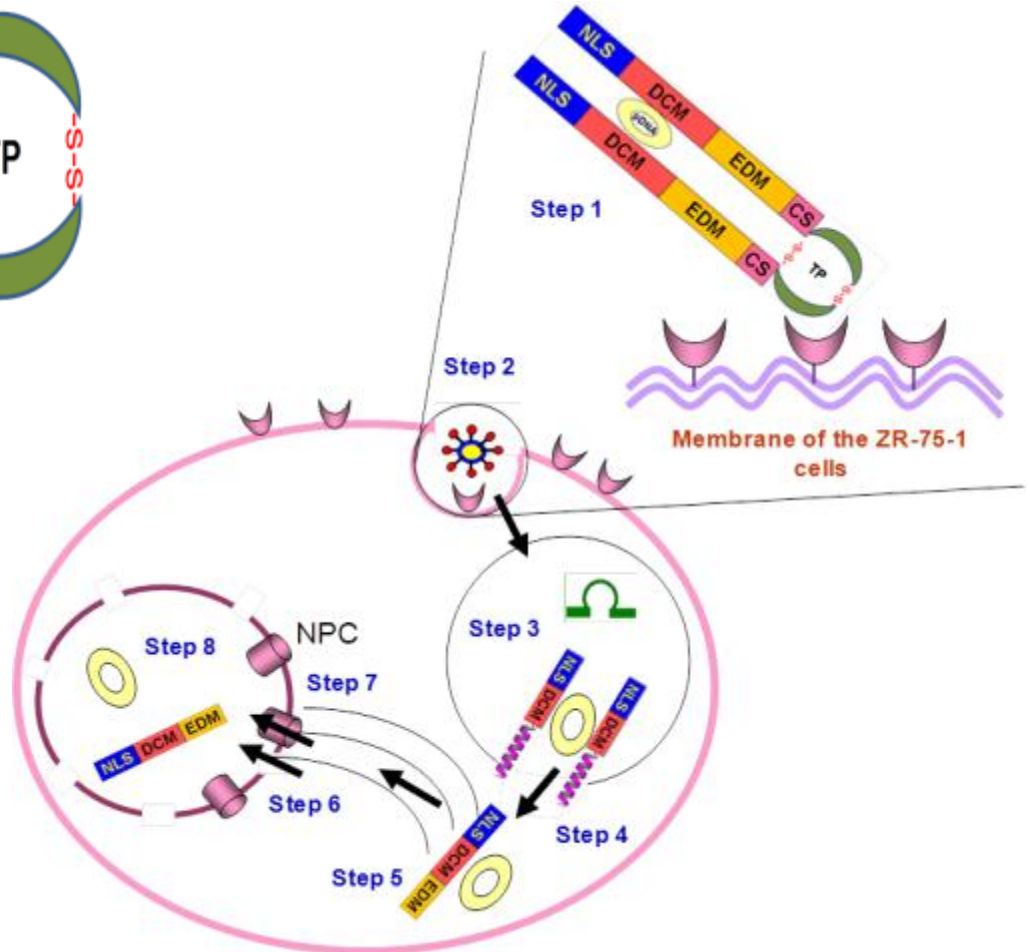
NLS – Nuclear Localisation Signal

DCM – DNA Condensing Motif

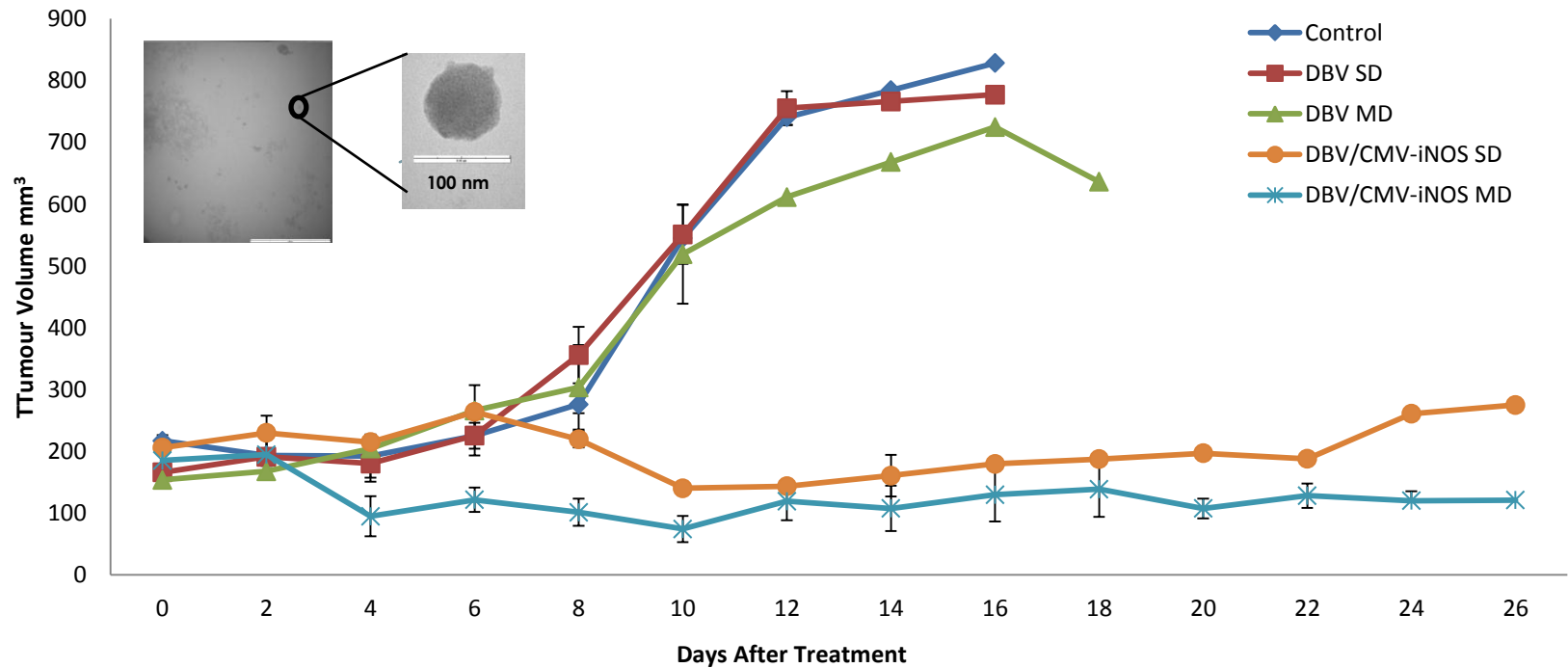
EDM- Endosomal Disruption Motif

TP- Targeting Peptide

CS – Cathepsin Substrate



# Systemic Delivery of DBV/CMV-iNOS *In Vivo*





# Acknowledgements



**Dr Helen McCarthy**  
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Dr Marie Migaud  
Prof Tracy Robson  
Prof David Hirst



Dr Brenda Canine  
Dr Al Wang



Ms Zahra Karjoo-Diarkhan  
Dr Arash Hatefi



# Functionalised GNPs as Radiosensitisers

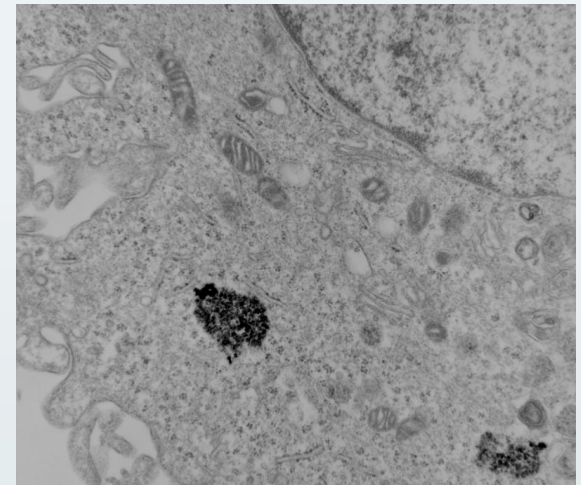
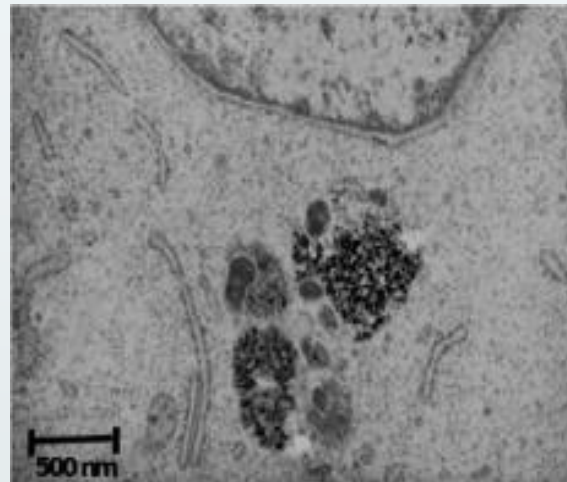
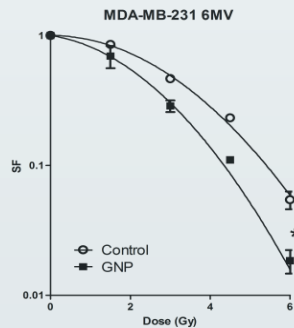
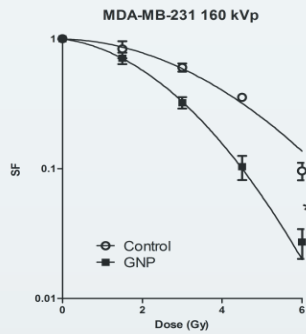
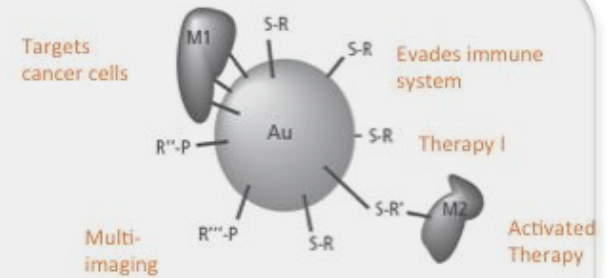
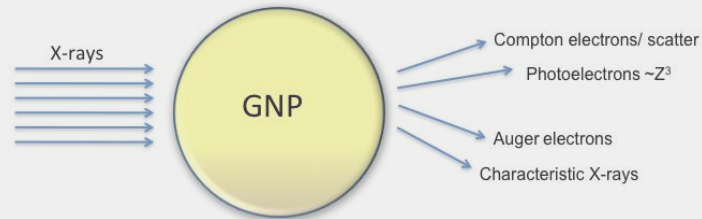
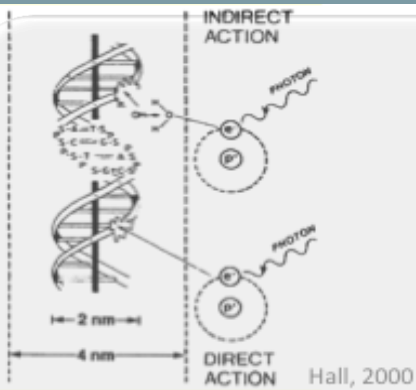
**Principle Investigator: Dr Jonathan Coulter**

Co-investigators: Fred Currell, Kevin Prise, Joe O'Sullivan,  
Alan Hounsell, Helen McCarthy, Marie Migaud





# Functionalised GNPs as Radiosensitisers



Typical accumulation of 1.9 nm GNPs

# The therapeutic and diagnostic potential of FKBP1; a novel anti-cancer protein

**PI: Professor Tracy Robson**

Co-Inv: Dr Helen McCarthy

Inhibition of tumour growth and effects on chemo and radiosensitivity

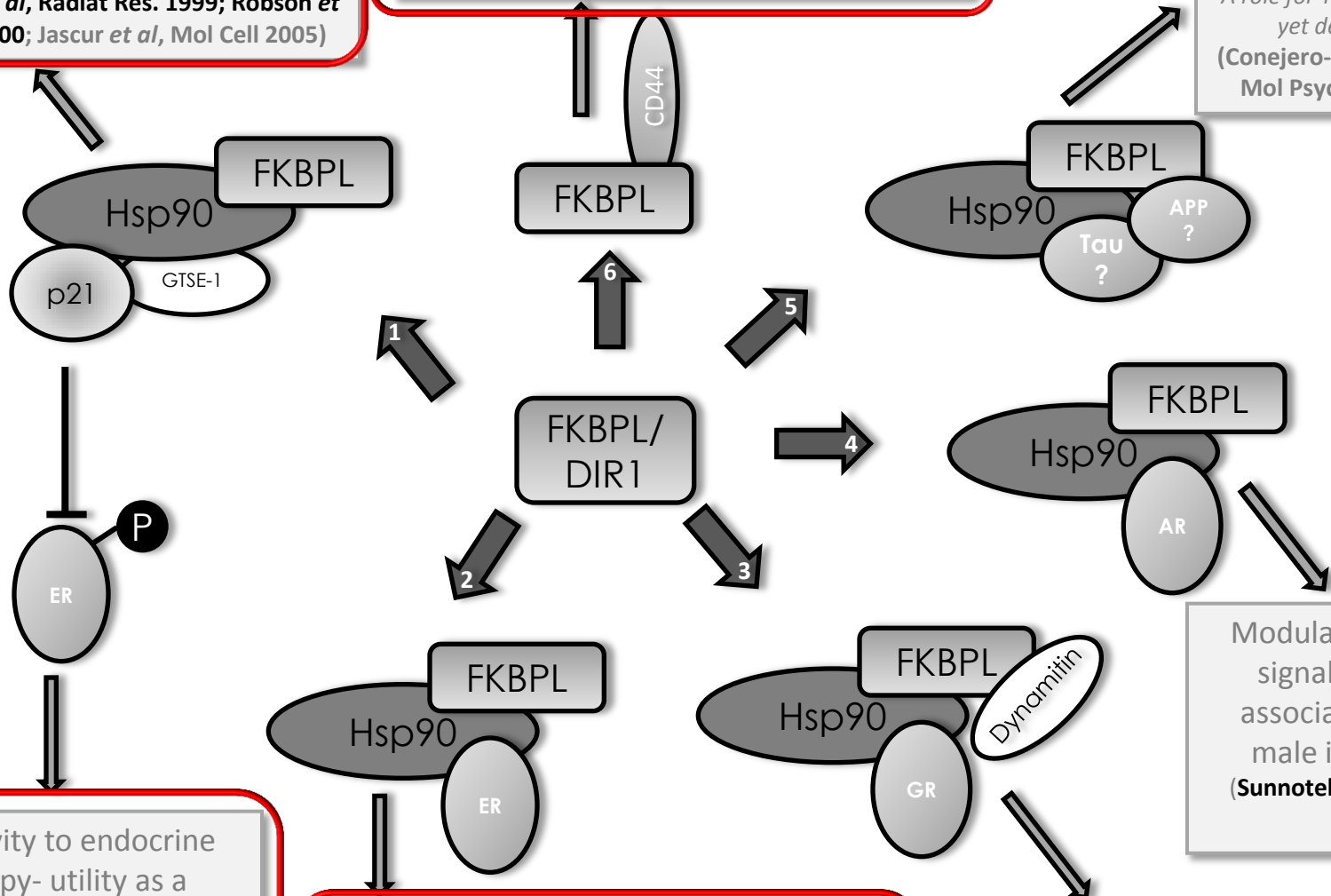
(Robson *et al*, *Radiat Res*. 1999; Robson *et al* *IJRB* 2000; Jascur *et al*, *Mol Cell* 2005)

A potent secreted anti-angiogenic protein

(Valentine *et al.*, *Clin Cancer Res*. 2011)

Associated with neuroprotection

A role for Tau and APP not yet determined  
(Conejero-Goldberg *et al.* *Mol Psychiatry* 2011)



Sensitivity to endocrine therapy- utility as a predictive marker of response to therapy

(McKeen *et al.*, *Cancer Res*, 2010)

Modulation of ER signalling, inhibition of ER positive breast cancer growth, correlation with breast cancer outcome

(McKeen *et al.*, *Cancer Res*, 2010; McKeen *et al.*, *Biochem Soc Trans* 2011)

Modulation of AR signalling and association with male infertility

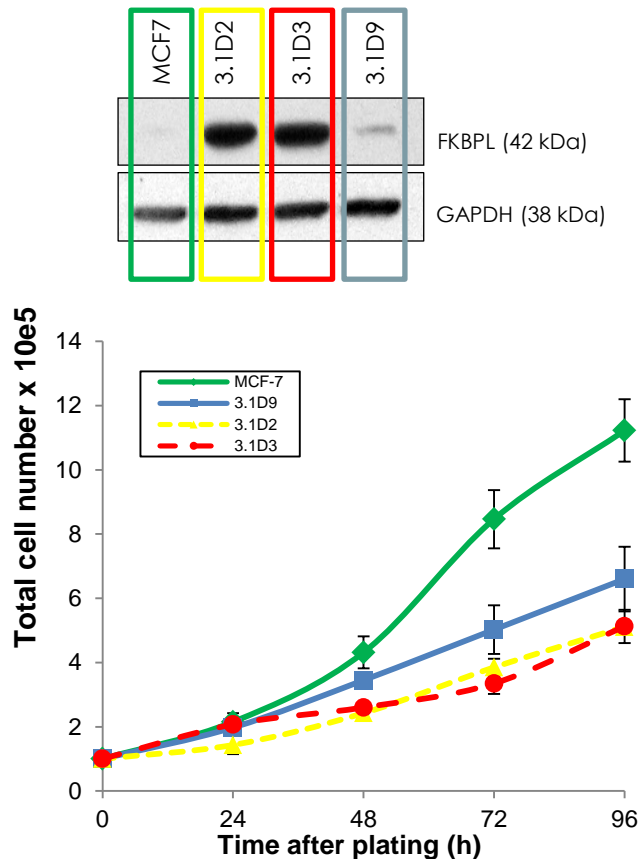
(Sunnotel *et al.*, 2010)

Modulation of GR signalling

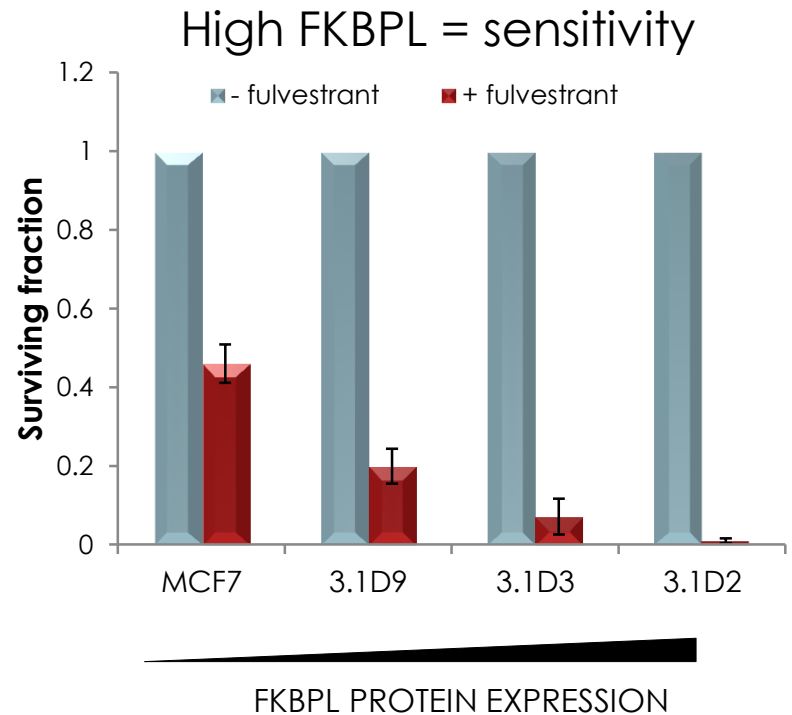
(McKeen *et al.*, *Endocrinol* 2008)

# The diagnostic potential of FKBPL

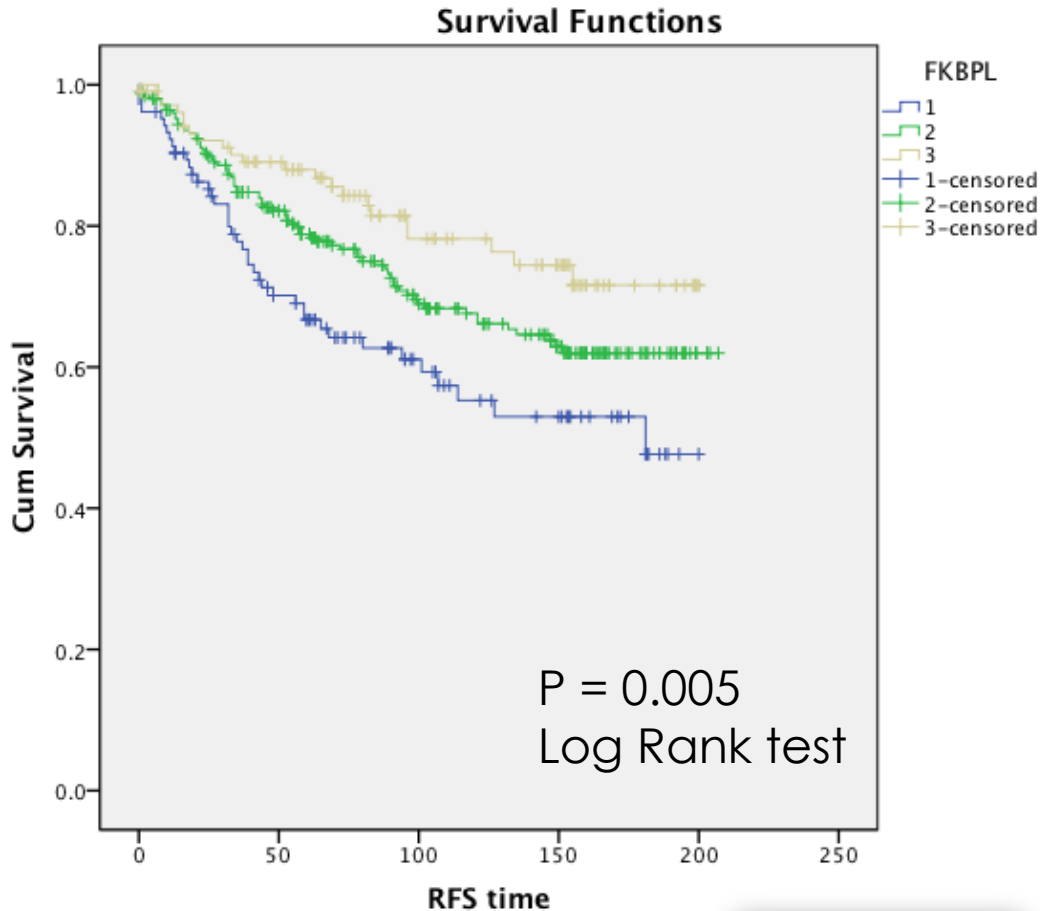
Increased FKBPL slows breast cancer cell proliferation



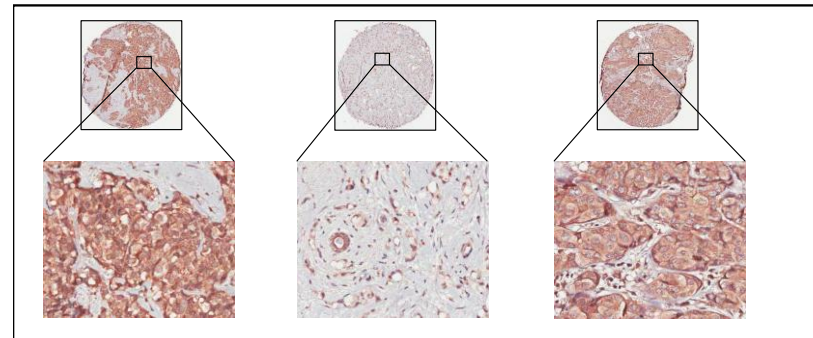
FKBPL modulates the response to endocrine therapy



# FKBPL increases recurrence free survival



## Screening breast cancer TMA for FKBPL

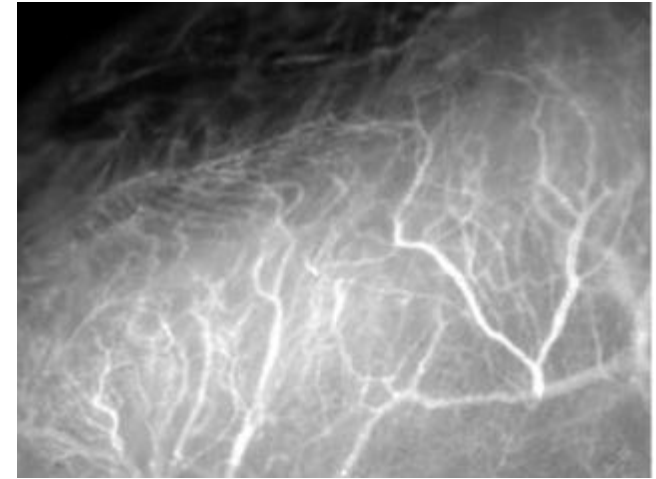
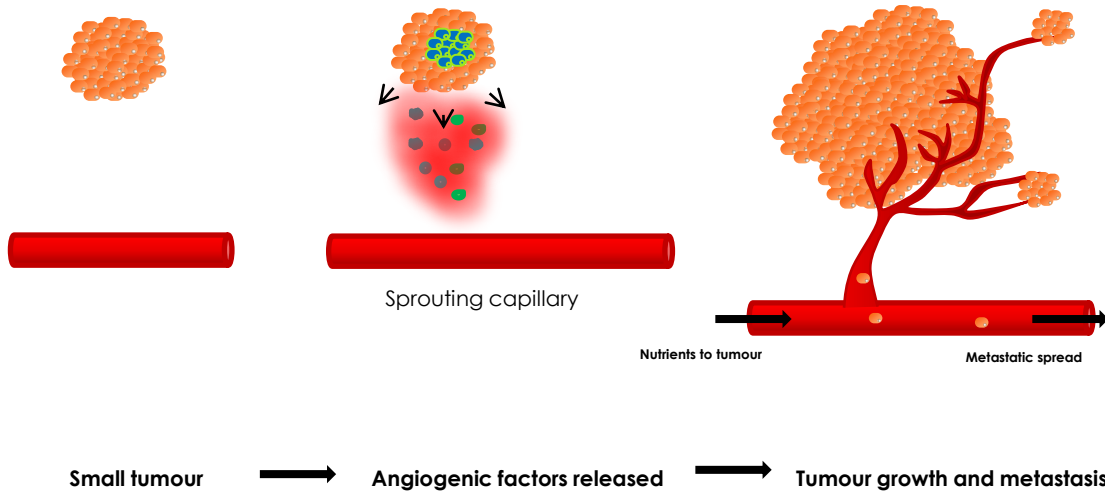


FKBPL	Total N	N of Events	Censored	
			N	Percent
1	104	41	63	60.6%
2	256	76	180	70.3%
3	105	22	83	79.0%
Overall	465	139	326	70.1%

**High FKBPL also correlated with:**

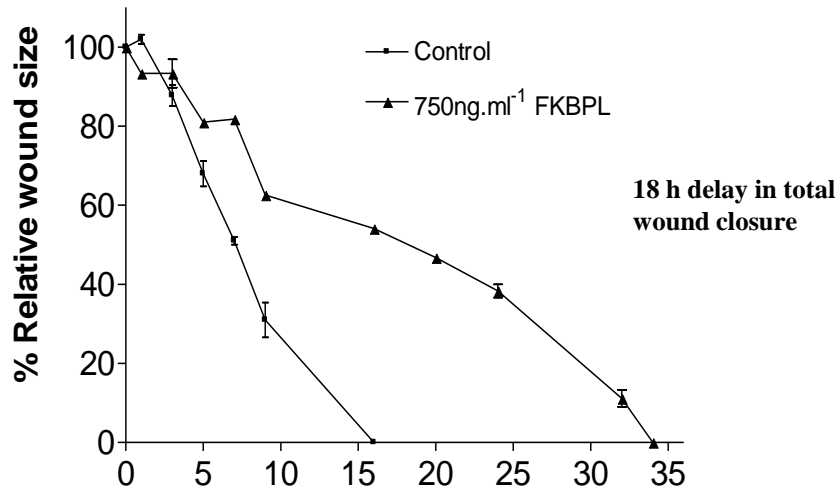
- Small tumour size (P= 0.023)
- Low grade (P=0.001)

**Chi-squared test/Pearson's correlation coefficient**

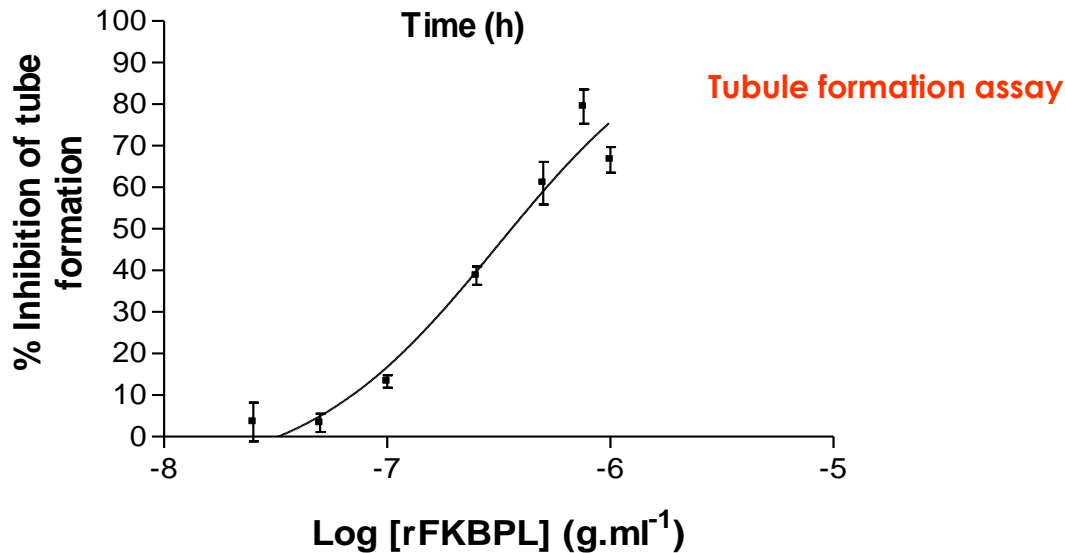
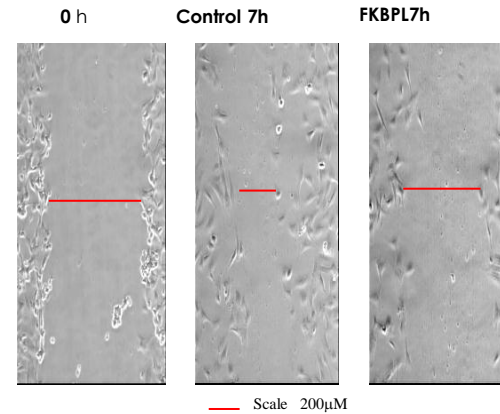


# The therapeutic potential of FKBPL; an anti-angiogenic protein

# FKBPL is an anti-angiogenic protein;



## HMEC-1 migration assay



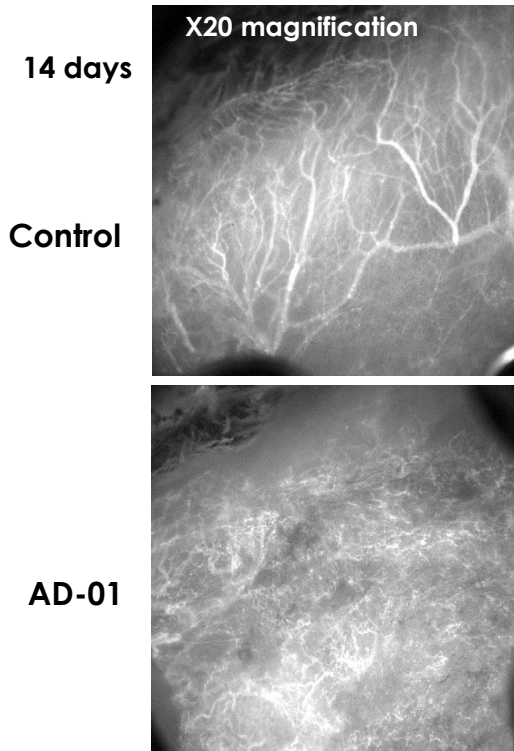
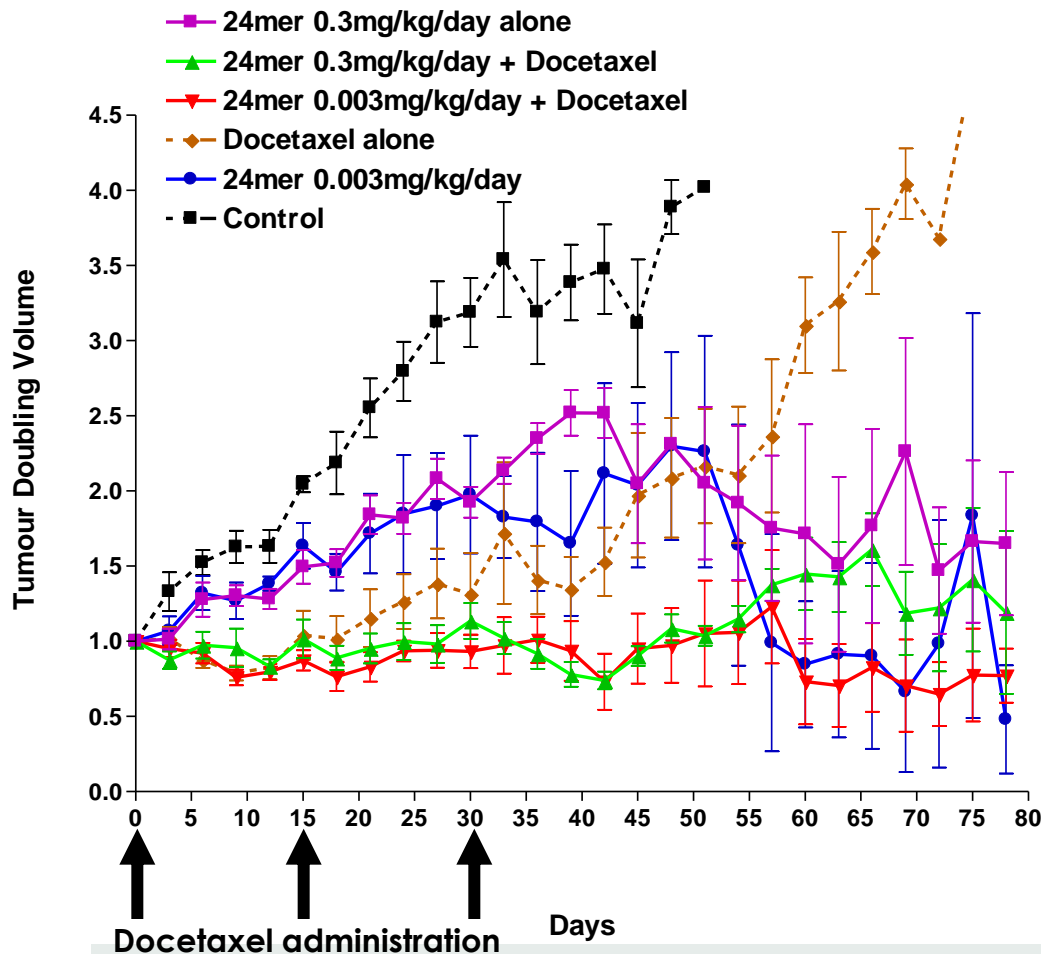


# AD-01, a 24mer FKBPL peptide inhibits tumour xenograft growth and prevents angiogenesis *in vivo*

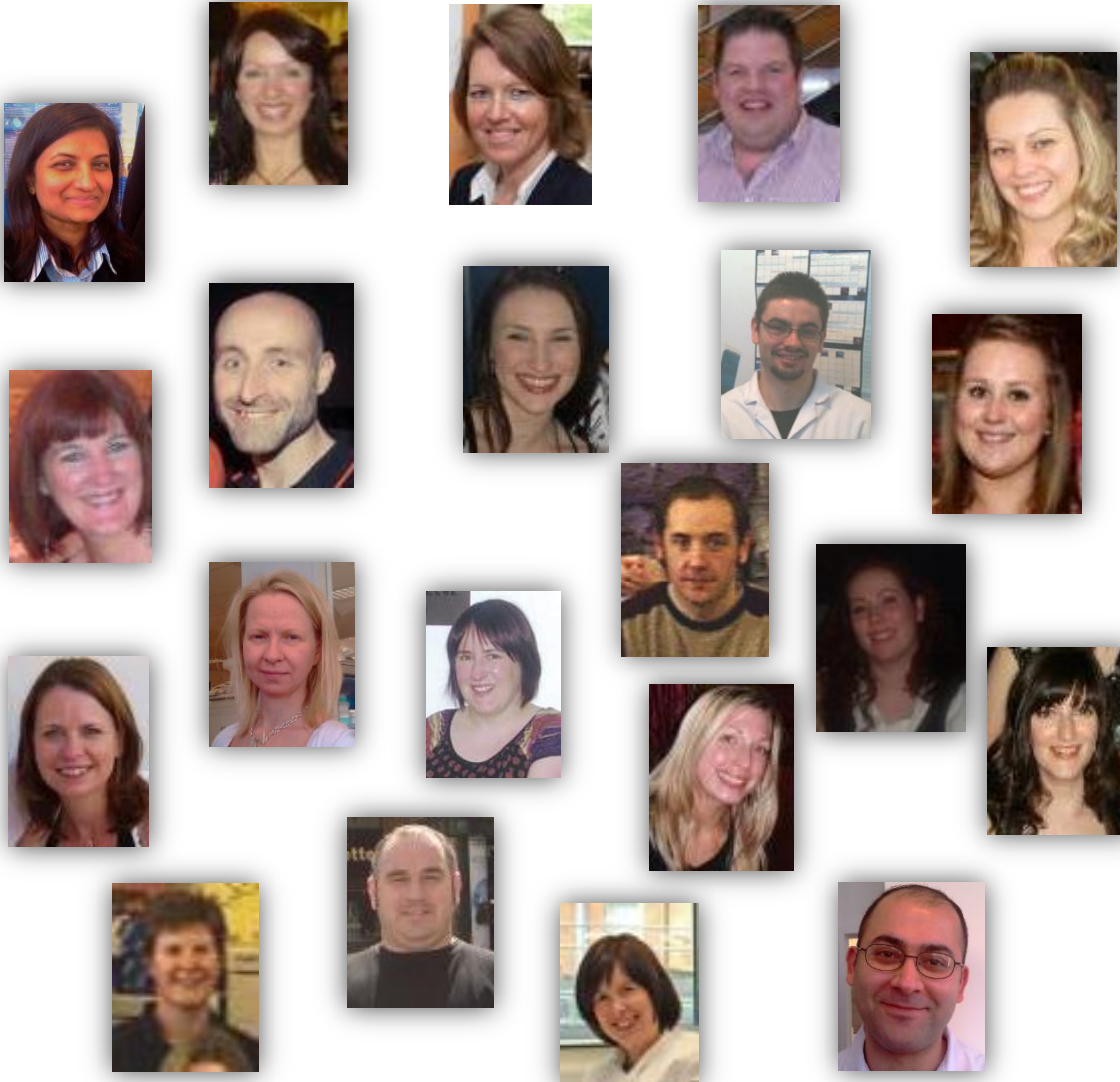
Tumour model: DU145 human prostate

Treatment: 24mer, docetaxel

Dosage: 24mer I.P. daily; 0.3mg/kg and 0.003mg/kg; Docetaxel 20mg/kg once in 15 days in 3 cycles



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