



**BONE**  
RESEARCH SOCIETY

# Annual Meeting



**ABERDEEN**

**3-5 July 2007**

## Final Programme & Abstracts

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**BONE**  
RESEARCH SOCIETY

# Annual Meeting

3-5 July 2007  
ABERDEEN, UK

*Local Organisers*  
David Reid (Chair)  
Richard Aspden  
Miep Helfrich  
Mike Rogers

[www.brsoc.org.uk](http://www.brsoc.org.uk)



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# Bone Research Society

The Society (formerly known as the Bone and Tooth Society) is the oldest and largest scientific society in Europe that is dedicated to further research into clinical and basic science problems related to mineralised tissues. The meeting attracts a wide audience from throughout the UK and, increasingly, from continental Europe and further afield. The presentations are traditionally a balance between clinical and laboratory-based studies. The participation of young scientists and clinicians is actively encouraged.

## Committee 2007

President: Jonathan Reeve (Cambridge)  
President Elect: Cyrus Cooper (Southampton)  
Secretary: Tim Arnett (London)  
Treasurer: Jonathan Tobias (Bristol)  
Kay Colston (London)  
Mark Cooper (Birmingham)  
Bronwen Evans (Cardiff)  
Miep Helfrich (Aberdeen)  
Richard Keen (London)  
David Marsh (London)

Student Representatives:  
Claire Clarkin (London)  
Nick Harvey (Southampton)

## Meeting Organiser

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## Membership Enquiries

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## NEXT YEAR'S BRS MEETING

**23-25 June 2008**

Manchester  
(jointly with the British Orthopaedic Research Society)



[www.brsoc.org.uk](http://www.brsoc.org.uk)

# Awards, Sponsors and Other Supporters

## New Investigator Awards

Awards were made by the Bone Research Society Committee according to marks given to blinded abstracts during the independent review process.

*Andrew Chantry (Sheffield):* OC22: A soluble activin type II receptor prevents myeloma bone disease

*Alireza Moayyeri (Cambridge):* (OC23): Height loss predicts fractures in middle aged and older men and women: the EPIC-Norfolk prospective population study

*Kirsten Petrie (Oxford):* OC11: An atypical mutation in the activin A receptor, type 1 gene (ACVR1) in a severely affected fibrodysplasia ossificans progressiva patient

*Debbie Scott (Aberdeen):* OC6: RANKL mutations are responsible for the defective osteoclast formation seen in six patients with osteoclast-poor osteopetrosis

## Major Sponsors:

**Alliance for Better Bone Health (Procter and Gamble Pharmaceuticals and sanofi aventis)**

**Amgen**

**Novartis Pharmaceuticals**

**Pfizer**

**ProStrakan**

**Roche/GlaxoSmithKline**

**Servier**

**Shire Pharmaceuticals**

## Other Supporters:

Abbott

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IDS

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Qados

Scanco

Technoclone



# Programme

## Tuesday 3 July

**07:30** Crombie and Kings Hall residents  
**Breakfast starts**

**08:55** Crombie and Kings Hall residents  
**Bus to AECC**

**09:30**  
**COFFEE, registration, poster hanging**

**10:25**  
**Opening and welcome**  
*David Reid (Aberdeen, UK) and Stephen Logan (Senior Vice-Principal, University of Aberdeen)*

**10:30**  
**Bone pain**  
*Chairs: Steven Goldring (New York, USA) and Jonathan Reeve (Cambridge, UK)*

- 10:30 IS1 ACID AND BONE  
*Tim Arnett (London, UK)*
- 11:00 IS2 THE ROLE OF THE ENDOCANNABINOID SYSTEM IN PAIN MODULATION  
*Ruth Ross (Aberdeen, UK)*
- 11:30 IS3 CANNABINOIDS AND BONE  
*Rob van t' Hof (Edinburgh, UK)*

**12:00**  
**Oral communications 1**  
*Chairs: Tim Arnett (London, UK) and Miep Helfrich (Aberdeen, UK)*

- 12:00 OC1 THE ENDOCANNABINOIDS 2-ARACHIDONOLYL GLYCEROL AND ANANDAMIDE ARE PRODUCED BY BONE CELLS AND STIMULATE BONE RESORPTION IN VITRO  
*SA Ridge\*, L Ford, GA Cameron, RA Ross, MJ Rogers*  
Bone & Musculoskeletal Research Programme, Institute of Medical Sciences, University of Aberdeen, Aberdeen UK
- 12:12 OC2 HYPOXIA-INDUCIBLE FACTOR AND HUMAN OSTEOCLASTS: EVIDENCE FOR A POSITIVE FEEDBACK LOOP REGULATING OSTEOCLAST FUNCTION  
*HJ Knowles\*, NA Athanasou*  
Nuffield Department of Orthopaedic Surgery, University of Oxford, Oxford, UK

- 12:24 OC3 INTRAUTERINE PROGRAMMING OF BONE-DELETERIOUS EFFECTS OF A LOW PROTEIN DIET IN LATE ADULTHOOD ON THE OSTEOGENIC ENVIRONMENT  
*SA Lanham\*<sup>[1]</sup>, C Roberts<sup>[1]</sup>, MJ Perry<sup>[2]</sup>, C Cooper<sup>[1]</sup>, ROC Oreffo<sup>[1]</sup>*

<sup>[1]</sup>Bone and Joint Research Group, Developmental Origins of Health and Disease, University of Southampton, Southampton, UK; <sup>[2]</sup>Department of Anatomy, University of Bristol, UK

- 12:36 OC4 DIFFERENTIAL EFFECT OF DOXORUBICIN AND ZOLEDRONIC ACID ON INTRA-OSSEOUS VS EXTRA OSSEOUS BREAST TUMOUR GROWTH IN VIVO  
*PD Ottewill\*<sup>[1]</sup>, B Deux<sup>[2]</sup>, H Mönkkönen<sup>[1]</sup>, RE Coleman<sup>[1]</sup>, P Clezardin<sup>[2]</sup>, I Holen<sup>[1]</sup>*

<sup>[1]</sup>Academic Unit of Clinical Oncology, School of Medicine and Biomedical Sciences, University of Sheffield, UK; <sup>[2]</sup>INSERM Research Unit 403, Faculté de Médecine Laënnec, 69372 Lyon Cedex 08, France

- 12:48 OC5 EVALUATION OF IN VIVO NEOVASCULARISATION IN ALLOGRAFT AND POLY D,L-LACTIC ACID TISSUE ENGINEERED SCAFFOLDS USING MICRO-COMPUTER TOMOGRAPHY  
*BJRF Bolland, JM Kanczler\*, DG Dunlop, ROC Oreffo*

Bone & Joint Research Group, Developmental Origins of Health and Disease, University of Southampton, Southampton SO16 6YD, UK

**13:00**

## LUNCH and posters

(ODD NUMBERS ATTENDED 13:30-14:30)

**14:30**

## Vesicular trafficking and bone disease

*Chairs: Matthew Gillespie (Melbourne, Australia) and Mike Rogers (Aberdeen, UK)*

- 14:30 IS4 VESICULAR TRAFFICKING IN OSTEOCLAST BIOLOGY  
*Fraser Coxon (Aberdeen, UK)*
- 15:00 IS5 DISEASES CAUSED BY DEFECTS IN VESICULAR TRAFFICKING AND ACIDIFICATION  
*Uwe Kornak (Berlin, Germany)*
- 15:30 IS6 CHLORIDE CHANNELS AS A TARGET FOR BONE THERAPY  
*Morten Karsdal (Herlev, Denmark)*

## Programme – Tuesday 3 July

16:00

### TEA

16:30

### Oral communications 2

*Chairs: Bronwen Evans (Cardiff, UK) and Uwe Kornak (Berlin, Germany)*

- 16:30 OC6 RANKL MUTATIONS ARE RESPONSIBLE FOR THE DEFECTIVE OSTEOCLAST FORMATION SEEN IN SIX PATIENTS WITH OSTEOCLAST-POOR OSTEOPETROSIS  
*DI Scott<sup>[1]</sup>, FP Coxon<sup>[1]</sup>, C Sobacchi<sup>[2]</sup>, A Frattini<sup>[2]</sup>, A Pangrazio<sup>[2]</sup>, M Guerrini<sup>[2]</sup>, L Susani<sup>[2]</sup>, A Teti<sup>[3]</sup>, C Messina<sup>[4]</sup>, G Errigo<sup>[4]</sup>, M Abinun<sup>[5]</sup>, A Cant<sup>[5]</sup>, N J Bishop<sup>[6]</sup>, P Grabowski<sup>[6]</sup>, RGM Bredius<sup>[7]</sup>, GMS Mancini<sup>[8]</sup>, PM Vezzoni<sup>[2]</sup>, A Villa<sup>[2]</sup>, MJ Rogers<sup>[1]</sup>, M Helfrich<sup>[1]</sup>*

<sup>[1]</sup> Department of Medicine and Therapeutics, University of Aberdeen, UK; <sup>[2]</sup> Institute of Biomedical Technologies, National Research Council, Segrate, Italy; <sup>[3]</sup> Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy; <sup>[4]</sup> Oncoematologia Pediatrica, Dipartimento di Pediatria, University of Padoa, Padoa, Italy; <sup>[5]</sup> Children's BMT Unit Newcastle General Hospital and School of Clinical Medical Sciences, Newcastle University, Newcastle upon Tyne, UK; <sup>[6]</sup> Sheffield Children's Hospital and Academic Unit of Child Health, University of Sheffield, Sheffield, UK; <sup>[7]</sup> Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands; <sup>[8]</sup> Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands

- 16:42 OC7 DOWNSTREAM SIGNALLING PATHWAYS OF NON-CANONICAL (RANKL-INDEPENDENT) PATHWAYS OF OSTEOCLAST FORMATION INDUCED BY TNF SUPERFAMILY MEMBERS LIGHT AND APRIL

*F Jones<sup>[1]</sup>, H Knowles<sup>[1]</sup>, NA Athanasou<sup>[2]</sup>*

<sup>[1]</sup> Botnar Research Centre, Institute of Musculoskeletal Sciences, Nuffield Department of Orthopaedic Surgery, University of Oxford, Nuffield Orthopaedic Centre, Windmill Road, Oxford, OX3 7LD, UK; <sup>[2]</sup> Department of Pathology, Nuffield Orthopaedic Centre, Nuffield Department of Orthopaedic Surgery, University of Oxford, Oxford, OX3 7LD, UK

- 16:54 OC8 MICE WITH A TRUNCATION MUTATION AFFECTING SEQUESTOSOME 1 EXHIBIT SEVERAL PHENOTYPIC FEATURES IN COMMON WITH PAGET'S DISEASE OF BONE

*JA Rojas<sup>[1]</sup>, A Daroszewska<sup>[1]</sup>, M Helfrich<sup>[2]</sup>, R Layfield<sup>[3]</sup>, R van't Hoff<sup>[1]</sup>, SH Ralston<sup>[1]</sup>*

<sup>[1]</sup> University of Edinburgh, Edinburgh, UK; <sup>[2]</sup> University of Aberdeen, Aberdeen, UK; <sup>[3]</sup> Institute of Neuroscience, University of Nottingham, Nottingham, UK

- 17:06 OC9 TARGETING OSTEOCLASTS IN RHEUMATOID ARTHRITIS VIA THE ALPHA(V)BETA(3) INTEGRIN

*F Brunton<sup>[1]</sup>, L Lee<sup>[1]</sup>, A Nissim<sup>[2]</sup>, AE Grigoriadis<sup>[3]</sup>, C Pitzalis<sup>[1]</sup>*

<sup>[1]</sup> Dept Rheumatology, KCL Guy's Hospital, London, UK; <sup>[2]</sup> William Harvey Research Institute, QMUL, London, UK; <sup>[3]</sup> Dept Craniofacial Dev, KCL Guy's Hospital, London, UK

- 17:18 OC10 SUPPRESSOR OF CYTOKINE SIGNALLING-2 (SOCS-2) EXPRESSION IN THE GROWTH PLATE: MODULATION BY PRO-INFLAMMATORY CYTOKINES.  
*VE MacRae<sup>[1]</sup>, S Pells<sup>[1]</sup>, SF Ahmed<sup>[2]</sup>, C Farquharson<sup>[1]</sup>*

<sup>[1]</sup> Roslin Institute, Midlothian, UK; <sup>[2]</sup> Royal Hospital for Sick Children, Glasgow, UK

17:30

### Close

17:40

Crombie and Kings Hall residents

Bus from AECC to Halls

18:30

Crombie and Kings Hall residents

Bus to Town and County Hall

19:00

Town and County Hall

Civic Reception

# Programme

## Wednesday 4 July

**06:45 Crombie and Kings Hall residents**  
**Breakfast starts**

**07:55 Crombie and Kings Hall residents**  
**Bus to AECC**

**08:30**

### Signalling pathways and bone formation

*Chairs: Brendon Noble (Edinburgh, UK) and Jon Tobias (Bristol, UK)*

08:30 IS7 CONTROL OF BONE REMODELING HOMEOSTASIS VIA THE AUTONOMOUS NERVOUS SYSTEM  
*Florent Elefteriou (Nashville, USA)*

09:00 IS8 DISCOVERY OF THE FIBRODYSPLASIA OSSIFICANS PROGRESSIVA (FOP) GENE  
*Matthew Brown (Brisbane, Australia)*

09:30 IS9 ROLE OF SCLEROSTIN IN BMP AND WNT SIGNALLING: IMPLICATIONS FOR BONE FORMATION  
*Wendy Balemans (Antwerp, Belgium)*

**10:00**

### Oral communications 3

*Chairs: Matthew Brown (Brisbane, Australia) and Claire Clarkin (London, UK)*

10:00 OC11 AN ATYPICAL MUTATION IN THE ACTIVIN A RECEPTOR, TYPE 1 GENE (ACVR1) IN A SEVERELY AFFECTED FIBRODYSPLASIA OSSIFICANS PROGRESSIVA PATIENT  
*KA Petrie\*, JJ Pointon, R Smith, RGG Russell, PW Wordsworth, JT Triffitt*  
Institute of Musculoskeletal Sciences, Botnar Research Centre, University of Oxford, Oxford OX120JH, UK

10:12 OC12 OSTEOCYTE SCLEROSTIN EXPRESSION: REGULATOR OF BMU BALANCE IN OSTEOARTHRITIS AND OSTEOPOROSIS?  
*N Loveridge<sup>[1]</sup>, J Power<sup>[1]</sup>, A Caballero-Alias<sup>[1]</sup>, R van Bezooijen<sup>[2]</sup>, S Papapoulos<sup>[2]</sup>, C Lowik<sup>[2]</sup>, KE Poole<sup>[1]</sup>, J Reeve<sup>[1]\*</sup>*  
<sup>[1]</sup>University of Cambridge, Cambridge, UK; <sup>[2]</sup>University of Leiden, Leiden, The Netherlands

10:24 OC13 INTERROGATING THE MECHANISMS CONTROLLING OSTEOCYTOGENESIS  
*M Prideaux\*[1,2], A Pitsillides<sup>[2]</sup>, L Bonewald<sup>[3]</sup>, N Loveridge<sup>[4]</sup>, C Farquharson<sup>[1]</sup>*  
<sup>[1]</sup>Roslin Institute, Edinburgh, UK; <sup>[2]</sup>Royal Veterinary College, London, UK; <sup>[3]</sup>School of Dentistry, University of Missouri-Kansas City, USA; <sup>[4]</sup>University of Cambridge, Cambridge, UK

10:36 OC14 SPONDYLOEPIPHYSEAL DYSPLASIA TARDA (SED)-ASSOCIATED SEDLIN MUTATIONS DISRUPT INTERACTIONS WITH C-MYC PROMOTER-BINDING PROTEIN 1 (MBP-1), PITUITARY HOMEOBOX 1 (PITX1) AND STEROIDOGENIC FACTOR 1 (SF1)  
*MA Nesbit\*[<sup>1</sup>], J Jeyabalan<sup>[1]</sup>, HA Ingraham<sup>[2]</sup>, RV Thakker<sup>[1]</sup>*

<sup>[1]</sup>Academic Endocrine Unit, Nuffield Department of Clinical Medicine, Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, UK; <sup>[2]</sup>Department of Physiology, University of California, San Francisco, California, USA

10:48 OC15 WNT SIGNALLING UPREGULATES OSTEOBLAST DIFFERENTIATION IN ZEBRAFISH: A NOVEL MODEL FOR STUDIES IN OSTEOBLASTOGENESIS  
*PM Elks\*, N Li, HH Roehl, PI Croucher*  
University of Sheffield, Sheffield, UK

**11:00**

### COFFEE

**11:30**

### Bone loss and inflammatory arthritis

*Chairs: Cyrus Cooper (Southampton, UK) and Richard Keen (London, UK)*

11:30 IS10 DETERMINANTS OF BONE DESTRUCTION IN RHEUMATOID ARTHRITIS  
*Steven Goldring (New York, USA)*

12:00 IS11 ROLE OF CYTOKINES AND BONE: USE OF IN VITRO MODELS  
*Matt Gillespie (Melbourne, Australia)*

12:30 IS12 MODULATION OF T CELL FUNCTION IN RHEUMATOID ARTHRITIS  
*Berent Prakken (Utrecht, Netherlands)*

**13:00**

### LUNCH and posters

(EVEN NUMBERS ATTENDED 13:30-14:30)

**14:30**

### Oral communications 4

*Chairs: Kay Colston (London, UK) and Florent Elefteriou (Nashville, USA)*

14:30 OC16 THE LOCAL AND SYSTEMIC EFFECTS OF GLUCOCORTICOID GENERATION IN SYNOVIUM  
*RS Hardy<sup>[1]\*</sup>, EH Rabbitt<sup>[1]</sup>, K Raza<sup>[2]</sup>, CD Buckley<sup>[2]</sup>, PM Stewart<sup>[1]</sup>, MC Cooper<sup>[1]</sup>*  
<sup>[1]</sup>Division of Medical Sciences, University of Birmingham, West Midlands, UK; <sup>[2]</sup>Division of Rheumatology, University of Birmingham, West Midlands, UK



## Programme – Wednesday 4 July

- 14:42 OC17 AUTO-INDUCTION OF IL-1BETA AND LOSS OF PROMOTER METHYLATION: A POSSIBLE EXPLANATION OF THE UNREMITTING PROGRESSION OF OSTEOARTHRITIS?  
*MB Gibson<sup>[1]</sup>, K Hashimoto<sup>[1,2]</sup>, HI Roach<sup>\*[1]</sup>*  
<sup>[1]</sup>Bone & Joint Research Group, University of Southampton, UK; <sup>[2]</sup>Tohoku University, Sendai, Japan
- 14:54 OC18 IDENTIFICATION OF LIPOCALIN 2, A NOVEL GLUCOCORTICOID RESPONSIVE GENE IN GROWTH PLATE CHONDROCYTES  
*HC Owen<sup>\*[1][2]</sup>, SF Ahmed<sup>[2]</sup>, C Farquharson<sup>[1]</sup>*  
<sup>[1]</sup>Bone Biology Group, Roslin Institute, Edinburgh, UK; <sup>[2]</sup>Bone & Endocrine Research Group, Royal Hospital for Sick Children, Glasgow, UK
- 15:06 OC19 CHILDHOOD PHYSICAL ACTIVITY IS ASSOCIATED WITH BONE MASS AT 4 YEARS  
*N Harvey<sup>\*[1]</sup>, K Westgate<sup>[2]</sup>, S Brage<sup>[2]</sup>, L Greenaway<sup>[1]</sup>, J Poole<sup>[1]</sup>, E Dennison<sup>[1]</sup>, H Inskip<sup>[1]</sup>, K Godfrey<sup>[1]</sup>, N Wareham<sup>[2]</sup>, U Ekelund<sup>[2]</sup>, C Cooper<sup>[1]</sup>*  
<sup>[1]</sup>MRC Epidemiology Resource Centre, Southampton, UK; <sup>[2]</sup>MRC Epidemiology Unit, Cambridge, UK
- 15:18 OC20 BODY MASS INDEX IS MORE PREDICTIVE OF THE OSTEOGENIC POTENTIAL OF BONE MARROW STROMAL CELLS THAN AGE IN MALES  
*RM McCann<sup>\*[1]</sup>, GR Jordan<sup>[1]</sup>, D Beverland<sup>[2]</sup>, SA Clarke<sup>[1]</sup>*  
<sup>[1]</sup>Trauma Research Group, Queen's University, Belfast, UK; <sup>[2]</sup>Department of Orthopaedic Surgery, Musgrave Park Hospital, Belfast, UK
- 15:30 OC21 GENETIC MANIPULATION OF HUMAN MESENCHYMAL PROGENITORS TO PROMOTE CHONDROGENESIS WITHIN POLYSACCHARIDE TEMPLATES  
*JC Babister<sup>\*</sup>, RS Tare, DW Green, S Inglis, ROC Oreffo*  
Bone & Joint Research Group, Developmental Origins of Health and Disease, University of Southampton, Southampton, SO16 6YD, UK
- 15:42 OC22 A SOLUBLE ACTIVIN TYPE II RECEPTOR PREVENTS MYELOMA BONE DISEASE  
*AD Chantry<sup>\*[1]</sup>, D Heath<sup>[1]</sup>, L Coulton<sup>[1]</sup>, O Gallagher<sup>[1]</sup>, H Evans<sup>[1]</sup>, J Seehra<sup>[2]</sup>, K Vanderkerken<sup>[3]</sup>, PI Croucher<sup>[1]</sup>*  
<sup>[1]</sup>Section of Musculoskeletal Science, University of Sheffield Medical School, Sheffield, UK; <sup>[2]</sup>Acceleron Pharma, Cambridge, MA, USA; <sup>[3]</sup>Department Hematology and Immunology, Vrije Universiteit Brussel (VUB), Brussels, Belgium

16:00

TEA

16:30

### Oral posters

*Chairs: Richard Aspden (Aberdeen, UK) and Helen MacDonald (Aberdeen, UK)*

- 16:30 OP1 SEGMENTAL BONE REGENERATION USING VASCULAR ENDOTHELIAL GROWTH FACTOR ENCAPSULATED POLY D,L-LACTIC ACID SCAFFOLDS AND HUMAN BONE MARROW STROMAL CELLS  
*JM Kanczler<sup>\*[1]</sup>, J Barry<sup>[2]</sup>, P Ginty<sup>[2]</sup>, SM Howdle<sup>[2]</sup>, KM Shakesheff<sup>[2]</sup>, ROC Oreffo<sup>[1]</sup>*  
<sup>[1]</sup> Bone & Joint Group, University of Southampton, UK; <sup>[2]</sup>School of Pharmacy & Chemistry, University of Nottingham, UK
- 16:35 OP2 CP55940, A NON-SELECTIVE CB1/CB2 AGONIST, STIMULATES BONE RESORPTION BY HUMAN OSTEOCLASTS IN VITRO  
*L Whyte<sup>\*</sup>, S Ridge, R Ross, MJ Rogers*  
Aberdeen OBRP Centre, Bone & Musculoskeletal Research Programme, University of Aberdeen, UK
- 16:40 OP3 DIETARY FLAVONOID INTAKE IS ASSOCIATED WITH BONE MINERAL DENSITY IN EARLY POSTMENOPAUSAL SCOTTISH WOMEN  
*AC Hardcastle<sup>\*[1,2]</sup>, JAM Kyle<sup>[2]</sup>, G Duthie<sup>[3]</sup>, G McNeill<sup>[2]</sup>, DM Reid<sup>[1,2]</sup>, HM MacDonald<sup>[1,2]</sup>*  
<sup>[1]</sup>Osteoporosis Research Unit, Health Sciences Building, University of Aberdeen, UK; <sup>[2]</sup>College of Medicine, University of Aberdeen, UK; <sup>[3]</sup>Rowett Research Institute, Aberdeen, UK
- 16:45 OP4 FEMORAL NECK MICROARCHITECTURE: DIFFERENCES BETWEEN CASES OF HIP FRACTURE AND CONTROLS  
*N Loveridge<sup>\*[1]</sup>, J Power<sup>[1]</sup>, H Kroger<sup>[2]</sup>, M Parker<sup>[3]</sup>, J Reeve<sup>[1]</sup>*  
<sup>[1]</sup>Bone Research Group, Cambridge, UK; <sup>[2]</sup>University Hospital, Kuopio, Finland; <sup>[3]</sup>District Hospital, Peterborough, UK
- 16:50 OP5 CHONDROCYTES IN OSTEOARTHRITIS DE-DIFFERENTIATE TO A PROGENITOR-LIKE INTERMEDIATE BEFORE RE-DIFFERENTIATING TO A COMPLEX AND MIXED PHENOTYPE  
*MA Da Silva, HI Roach<sup>\*</sup>*  
Bone & Joint Research Group, University of Southampton, General Hospital, Southampton, UK
- 16:55 OP6 ABSTRACT WITHDRAWN
- 17:00 OP7 OSTEOBLASTS PROTECT MULTIPLE MYELOMA CELLS FROM T-CELL-INDUCED APOPTOSIS  
*RM Locklin<sup>\*[1]</sup>, RGG Russell<sup>[1]</sup>, PI Croucher<sup>[2]</sup>, CM Edwards<sup>[3]</sup>*  
<sup>[1]</sup>Institute of Musculoskeletal Sciences, Botnar Research Centre, Nuffield Department of Orthopaedic Surgery, University of Oxford, Oxford, UK; <sup>[2]</sup>Division of Clinical Sciences, University of Sheffield Medical School, Sheffield, UK; <sup>[3]</sup>Department of Cancer Biology, Vanderbilt University Medical Center, Nashville, Tennessee, USA

## Programme – Wednesday 4 July

- 17:05 OP8 THE EXPRESSION AND REGULATION OF BIM IN OSTEOBLASTS UNDERGOING GROWTH FACTOR WITHDRAWAL  
*M Liang\**, RGG Russell, PA Hulley  
Botnar Research Centre, Nuffield Dept of Orthopaedic Surgery, University of Oxford, UK
- 17:10 OP9 NOVEL CONTROL OF GAG SYNTHESIS AND CHONDROGENESIS THROUGH SELECTIVE REGULATION OF UDPGD-MEDIATED MONOSACCHARIDE SUPPLY  
*CE Clarkin\**, S Allen, BT Wheeler, CPD Wheeler-Jones, AA Pitsillides  
The Royal Veterinary College, London, UK
- 17:15 OP10 THE EFFECT OF TENSILE FORCES ON THE DIFFERENTIATION OF MESENCHYMAL STEM CELLS  
*SB Mirza\**, M Greenwood, G Blunn  
Institute of Orthopaedics, University College London, UK
- 17:20 OP11 GENETIC MUTATIONS IN THE RAS/RAF/MAPKINASE PATHWAY RESULTS IN CHERUBISM  
*BD Idowu\**<sup>[1]</sup>, J Mangion<sup>[2]</sup>, RE Gale<sup>[3]</sup>, AM Flanagan<sup>[4]</sup>  
<sup>[1]</sup>Institute of Orthopaedics, UCL, London, UK; <sup>[2]</sup>MRC Clinical Sciences Centre, Imperial College London, UK; <sup>[3]</sup>Department of Haematology, UCL, London, UK; <sup>[4]</sup>Institute of Orthopaedics, UCL, London, UK

17.30

### KEYNOTE LECTURE

*Chair: C Duncan Rice*

Principal & Vice-Chancellor of the University of Aberdeen

### Ethical considerations and medical research: past, present and future

*Neva Haites (Aberdeen, UK)*

Head of the College of Life Sciences and Medicine

18:10

**Crombie and Kings Hall residents**

**Bus from AECC to Halls**

19:20

**Crombie and Kings Hall residents**

**Bus to Beach Ballroom**

19:45

**Beach Ballroom**

**Annual Dinner and Ceilidh**

Music from *Laverock*

# Programme

## Thursday 5 July

**07:00 Crombie and Kings Hall residents**  
**Breakfast starts**

**08:25 Crombie and Kings Hall residents**  
**Bus to AECC**

**09:00**  
**Contemporary issues in therapeutics of bone**  
*Chairs: Richard Eastell (Sheffield, UK) and Nick Harvey (Southampton, UK)*

09:00 IS13 ROLE OF BISPHOSPHONATES IN CANCER MANAGEMENT  
*Robert Coleman (Sheffield, UK)*

09:30 IS14 BISPHOSPHONATES IN THE MANAGEMENT OF PAGET'S DISEASE  
*Stuart Ralston (Edinburgh, UK)*

10:00 IS15 THERAPEUTIC OPTIONS AFTER BISPHOSPHONATES  
*Graham Russell (Oxford, UK)*

**10:30**  
**COFFEE**

**11:00**  
**Oral communications 5**  
*Chairs: Mark Cooper (Birmingham, UK) and Graham Russell (Oxford, UK)*

11:00 OC23 HEIGHT LOSS PREDICTS FRACTURES IN MIDDLE AGED AND OLDER MEN AND WOMEN: THE EPIC-NORFOLK PROSPECTIVE POPULATION STUDY  
*A Moayyeri<sup>[1]</sup>, RN Luben<sup>[1]</sup>, S Bingham<sup>[2]</sup>, A Welch<sup>[1]</sup>, NJ Wareham<sup>[3]</sup>, KT Khaw<sup>[1]</sup>*  
<sup>[1]</sup>Department of Public Health and Primary Care, Institute of Public Health, School of Clinical Medicine, University of Cambridge, Cambridge, UK; <sup>[2]</sup>MRC Dunn Human Nutrition Unit, Cambridge, UK; <sup>[3]</sup>MRC Epidemiology Unit, Cambridge, UK

11:12 OC24 AUTOLOGOUS CONDITIONED SERUM (ACS) COMPARED TO HYALURONAN AND SALINE-INJECTIONS FOR THE TREATMENT OF KNEE OSTEOARTHRITIS  
*C Moser<sup>[1,2]</sup>, AWA Baltzer<sup>[2]</sup>, R Krauspe<sup>[1]</sup>, P Wehling<sup>[2]</sup>*  
<sup>[1]</sup>Department of Orthopaedics, Heinrich Heine University Hospital Duesseldorf, Germany; <sup>[2]</sup>Centre for Molecular Orthopaedics, Königsallee 53-55, Duesseldorf, Germany

11:24 OC25 AN INTERNATIONAL MULTICENTER RANDOMIZED COMPARISON OF BALLOON KYPHOPLASTY AND NONSURGICAL CARE IN PATIENTS WITH ACUTE VERTEBRAL BODY COMPRESSION FRACTURES  
*D Wardlaw<sup>[1]</sup>, S Boonen<sup>[2]</sup>, L Bastian<sup>[3]</sup>, J Van Meirhaeghe<sup>[4]</sup>, AZ St Jan<sup>[4]</sup>*

<sup>[1]</sup>Department of Orthopaedics, Woodend Hospital, Eday Road, Aberdeen, UK; <sup>[2]</sup>UZ Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium; <sup>[3]</sup>Medizinische Hochschule Hannover, Unfallchirurgie, Carl Neuberg Str. 1, 30625 Hannover, Germany; <sup>[4]</sup>Dept Orthopaedics, Ruddershove 10, 8000 Brugge, Belgium

11:36 OC26 MUSCLE FUNCTION AND THE EFFECTS OF HYPOVITAMINOSIS D IN POST-MENARCHAL FEMALES  
*KA Ward<sup>[1]</sup>, G Das<sup>[2]</sup>, JL Berry<sup>[1]</sup>, SA Roberts<sup>[1]</sup>, R Rawer<sup>[3]</sup>, JE Adams<sup>[1]</sup>, MZ Mughall<sup>[4]</sup>*

<sup>[1]</sup>University of Manchester, Manchester, UK; <sup>[2]</sup>Central Manchester Primary Care Trust, Manchester, UK; <sup>[3]</sup>Novotec Medical, Manchester, UK; <sup>[4]</sup>Central Manchester & Manchester Childrens University Hospitals NHS Trust, Manchester, UK

11:48 OC27 EFFECT OF THE DUAL-SPECIFIC SRC/ABL KINASE INHIBITOR AZD0530 ON BONE TURNOVER IN PATIENTS WITH ADVANCED SOLID MALIGNANCIES  
*RA Hannon<sup>[1]</sup>, G Clack<sup>[2]</sup>, RB Iacona<sup>[3]</sup>, P Baker<sup>[2]</sup>, M Rimmer<sup>[2]</sup>, F Gossiel<sup>[1]</sup>, IC Smith<sup>[2]</sup>, A Robinson<sup>[2]</sup>, R Eastell<sup>[1]</sup>*

<sup>[1]</sup>Academic Unit of Bone Metabolism, University of Sheffield, Sheffield, UK; <sup>[2]</sup>AstraZeneca, Alderley Park, Macclesfield, UK; <sup>[3]</sup>AstraZeneca, Wilmington, Delaware, USA

**12:00**

### Clinical cases

*Chairs: Piet Geusens (Maastricht, Netherlands) and Stuart Ralston (Edinburgh, UK)*

12:00 CC1 SEVERE HIGH TURNOVER OSTEOPOROSIS OF UNKNOWN CAUSE IN A YOUNG MAN WITH AUTOIMMUNE HYPOTHYROIDISM; A NOVEL SYNDROME?  
*PL Riches<sup>\*</sup>, E McRorie, SH Ralston*  
Centre for Rheumatic Diseases, Western General Hospital, Edinburgh, UK

12:15 CC2 RESIDUAL (GHOST) SOCKETS IN INTRAVENOUS BISPHOSPHONATE USE - EVIDENCE OF POOR HEALING AND SLOW BONE TURNOVER  
*K Shetty<sup>\*</sup>, J Buoquot*  
University of Texas Health Science Center, Houston, US

12:30 CC3 BONE PAIN FROM CHRONIC SCLEROSING OSTEOMYELITIS SUCCESSFULLY TREATED WITH RISEDRONATE  
*SE Green<sup>[1]</sup>, RW Keen<sup>[2]</sup>*  
<sup>[1]</sup> Rheumatology Department, Ysbyty Gwynedd, Bangor, Gwynedd, LL57 2PW, UK; <sup>[2]</sup>Royal National Orthopaedic Hospital, Stanmore, Middlesex, HA7 4LP, UK

## Programme – Thursday 5 July

12:45 CC4 OSTEOCLAST ACTIVITY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) DURING THE FIRST YEAR OF TREATMENT

*RM Cox<sup>[1]</sup>, JW Gregory<sup>[1]</sup>, JH Davies<sup>[2]</sup>, MEM Jenney<sup>[3]</sup>, WD Fraser<sup>[4]</sup>, BAJ Evans<sup>\*[1]</sup>*

<sup>[1]</sup>Department of Child Health, School of Medicine, Cardiff University, Cardiff, UK; <sup>[2]</sup>Southampton University Hospital NHS Trust, Southampton, UK; <sup>[3]</sup>Paediatric Oncology, University Hospital of Wales, Cardiff, UK; <sup>[4]</sup>Clinical Chemistry, University of Liverpool, Liverpool, UK

13:00

### LUNCH

13:30

### BRS AGM

14:00

### Bone and rheumatoid arthritis

*Chairs: Barry Bresnihan (Dublin, Ireland) and David Reid (Aberdeen, UK)*

*Generously supported by an unrestricted educational grant from Amgen*

14:00

ASSESSMENT OF BONE LOSS IN THE PREDICTION OF LONG-TERM DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS  
*Paul Emery (Leeds, UK)*

14:30

ROLE OF RANK/RANKL PATHWAY IN THE BONE LOSS OF RHEUMATOID ARTHRITIS  
*Kurt Redlich (Vienna, Austria)*

15:00

POTENTIAL FOR RANKL INHIBITION IN THE MANAGEMENT OF RHEUMATOID ARTHRITIS  
*Piet Geusens (Maastricht, Netherlands)*

15:30

### Close

15:40

**Buses from AECC to  
Aberdeen Airport**

## Posters

- OP1** SEGMENTAL BONE REGENERATION USING VASCULAR ENDOTHELIAL GROWTH FACTOR ENCAPSULATED POLY D,L-LACTIC ACID SCAFFOLDS AND HUMAN BONE MARROW STROMAL CELLS  
*JM Kanczler<sup>[1]</sup>, J Barry<sup>[2]</sup>, P Ginty<sup>[2]</sup>, SM Howdle<sup>[2]</sup>, KM Shakesheff<sup>[2]</sup>, ROC Oreffo<sup>[1]</sup>*  
<sup>[1]</sup> Bone & Joint Group, University of Southampton, UK; <sup>[2]</sup>School of Pharmacy & Chemistry, University of Nottingham, UK
- OP2** CP55940, A NON-SELECTIVE CB1/CB2 AGONIST, STIMULATES BONE RESORPTION BY HUMAN OSTEOCLASTS IN VITRO  
*L Whyte\*, S Ridge, R Ross, MJ Rogers*  
Aberdeen OBRP Centre, Bone & Musculoskeletal Research Programme, University of Aberdeen, UK
- OP3** DIETARY FLAVONOID INTAKE IS ASSOCIATED WITH BONE MINERAL DENSITY IN EARLY POSTMENOPAUSAL SCOTTISH WOMEN  
*AC Hardcastle<sup>[1,2]</sup>, JAM Kyle<sup>[2]</sup>, G Duthie<sup>[3]</sup>, G McNeill<sup>[2]</sup>, DM Reid<sup>[1,2]</sup>, HM MacDonald<sup>[1,2]</sup>*  
<sup>[1]</sup>Osteoporosis Research Unit, Health Sciences Building, University of Aberdeen, UK; <sup>[2]</sup>College of Medicine, University of Aberdeen, UK; <sup>[3]</sup>Rowett Research Institute, Aberdeen, UK
- OP4** FEMORAL NECK MICROARCHITECTURE: DIFFERENCES BETWEEN CASES OF HIP FRACTURE AND CONTROLS  
*N Loveridge<sup>[1]</sup>, J Power<sup>[1]</sup>, H Kroger<sup>[2]</sup>, M Parker<sup>[3]</sup>, J Reeve<sup>[1]</sup>*  
<sup>[1]</sup>Bone Research Group, Cambridge, UK; <sup>[2]</sup>University Hospital, Kuopio, Finland; <sup>[3]</sup>District Hospital, Peterborough, UK
- OP5** CHONDROCYTES IN OSTEOARTHRITIS DE-DIFFERENTIATE TO A PROGENITOR-LIKE INTERMEDIATE BEFORE RE-DIFFERENTIATING TO A COMPLEX AND MIXED PHENOTYPE  
*MA Da Silva, HI Roach\**  
Bone & Joint Research Group, University of Southampton, General Hospital, Southampton, UK
- OP6** FRACTURE HEALING AND EARLY SYSTEMIC RESPOND MODIFIERS  
*I Pountos, T Georgouli, PV Giannoudis*  
Abstract withdrawn
- OP7** OSTEOBLASTS PROTECT MULTIPLE MYELOMA CELLS FROM T-CELL-INDUCED APOPTOSIS  
*RM Locklin<sup>[1]</sup>, RGG Russell<sup>[1]</sup>, PI Croucher<sup>[2]</sup>, CM Edwards<sup>[3]</sup>*  
<sup>[1]</sup>Institute of Musculoskeletal Sciences, Botnar Research Centre, Nuffield Department of Orthopaedic Surgery, University of Oxford, Oxford, UK; <sup>[2]</sup>Division of Clinical Sciences, University of Sheffield Medical School, Sheffield, UK; <sup>[3]</sup>Department of Cancer Biology, Vanderbilt University Medical Center, Nashville, Tennessee, USA
- OP8** THE EXPRESSION AND REGULATION OF BIM IN OSTEOBLASTS UNDERGOING GROWTH FACTOR WITHDRAWAL  
*M Liang\*, RGG Russell, PA Hulley*  
Botnar Research Centre, Nuffield Dept of Orthopaedic Surgery, University of Oxford, UK
- OP9** NOVEL CONTROL OF GAG SYNTHESIS AND CHONDROGENESIS THROUGH SELECTIVE REGULATION OF UDPGD-MEDIATED MONOSACCHARIDE SUPPLY  
*CE Clarkin\*, S Allen, BT Wheeler, CPD Wheeler-Jones, AA Pitsillides*  
The Royal Veterinary College, London, UK
- OP10** THE EFFECT OF TENSILE FORCES ON THE DIFFERENTIATION OF MESENCHYMAL STEM CELLS  
*SB Mirza\*, M Greenwood, G Blunn*  
Institute of Orthopaedics, University College London, UK
- OP11** GENETIC MUTATIONS IN THE RAS/RAF/MAPKINASE PATHWAY RESULTS IN CHERUBISM  
*BD Idowu<sup>[1]</sup>, J Mangion<sup>[2]</sup>, RE Gale<sup>[3]</sup>, AM Flanagan<sup>[4]</sup>*  
<sup>[1]</sup>Institute of Orthopaedics, UCL, London, UK; <sup>[2]</sup>MRC Clinical Sciences Centre, Imperial College London, UK; <sup>[3]</sup>Department of Haematology, UCL, London, UK; <sup>[4]</sup>Institute of Orthopaedics, UCL, London, UK
- P12** NITROGEN-CONTAINING BISPHOSPHONATES AND PREDICTION OF THEIR CLINICAL POTENCIES: DISSOCIATION OF TARGET ENZYME- AND HYDROXYAPATITE-BINDING AFFINITIES  
*Z Xia<sup>[1]\*</sup>, J Dunford<sup>[1]</sup>, MA Lawson<sup>[1]</sup>, JT Triffitt<sup>[1]</sup>, K Kavanagh<sup>[1]</sup>, U Oppermann<sup>[1]</sup>, BL Barnett<sup>[2]</sup>, FH Ebetino<sup>[2]</sup>, RGG Russell<sup>[1]</sup>*  
<sup>[1]</sup>The Oxford University Institute of Musculoskeletal Sciences, The Botnar Research Centre, Nuffield Orthopaedic Centre, Oxford, OX3 7LD, UK; <sup>[2]</sup>New Drug Development, Procter and Gamble Pharm, Cincinnati OH, USA; <sup>[3]</sup>Chemistry Department, University of Southern California, Los Angeles, CA, USA
- P13** MCL-1 IS AN IMPORTANT PRO-SURVIVAL FACTOR IN OSTEOCLASTS  
*K McConachie, D Tosh, MJ Rogers\**  
University of Aberdeen, Aberdeen, UK.
- P14** ADENOSINE STIMULATES MINERALISATION OF RAT MESENCHYMAL STEM CELLS IN VITRO  
*B Gharibi<sup>[1,2]</sup>, C Elford<sup>[1]</sup>, J Ham<sup>[2]</sup>, BAJ Evans<sup>[1]</sup>*  
<sup>[1]</sup>Department of Child Health, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK; <sup>[2]</sup>Centre for Endocrine and Diabetes Research, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK
- P15** COMPARTMENTALISATION OF GFP-TAGGED ER-ALPHA AND ER-GR CONSTRUCTS IN BONE (ROS) AND BREAST (MCF-7) CELLS  
*BS Maru<sup>[1]</sup>, MR Norman<sup>[1]</sup>, JH Tobias<sup>[2]</sup>, CA McArdle<sup>[1]</sup>*  
<sup>[1]</sup>Dorothy Hodgkin Building, University of Bristol, Whitson Street, Bristol, UK; <sup>[2]</sup>Rheumatology Unit, Bristol Royal Infirmary, Marlborough Street, Bristol, UK
- P16** OSTEOPENIA IN 129SV/EV SPARCTM1CAM MICE: BONE PHENOTYPIC CHARACTERIZATION AND GENE EXPRESSION CHANGES  
*FC Mansergh<sup>[1]</sup>, T Wells<sup>[1]</sup>, C Elford<sup>[2]</sup>, SL Evans<sup>[3]</sup>, MJ Perry<sup>[4]</sup>, MJ Evans<sup>[1]</sup>, BAJ Evans<sup>[2]</sup>*  
<sup>[1]</sup>School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3US, UK; <sup>[2]</sup>Department of Child Health, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK; <sup>[3]</sup>School of Engineering, Cardiff University, The Parade, Cardiff CF24 3AA, UK; <sup>[4]</sup>Department of Anatomy, University of Bristol, Bristol BS2 8EJ, UK
- P17** SB431542, A TGF-BETA TYPE 1 RECEPTOR INHIBITOR, PROMOTES C2C12 MYOTUBE HYPERTROPHY  
*K Watt\*, A Ratkevicius, H Wackerhage*  
Bone Research Group, Institute of Medical Sciences, University of Aberdeen, UK



## Posters

- P18 POLYMORPHIC VARIATION IN THE FPP SYNTHASE (FPPS) GENE AND EFFECTS ON BISPHOSPHONATE INHIBITION**  
*C Roemelt<sup>[1]</sup>, F McGuigan<sup>[2]</sup>, J Dunford<sup>[3]</sup>, O Albagha<sup>[4]</sup>, MJ Rogers<sup>[1]</sup>*  
<sup>[1]</sup>Bone & Musculoskeletal Research Programme, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK; <sup>[2]</sup>Clinical & Molecular Osteoporosis Research Unit, Malmö University Hospital Malmö, Sweden SE-205 02; <sup>[3]</sup>Structural Genomics Consortium, University of Oxford, Oxford OX3 7LD, UK; <sup>[4]</sup>Rheumatology Section, Molecular Medicine Centre, University of Edinburgh School of Molecular and Clinical Medicine, Western General Hospital, Edinburgh, EH4 2XU, UK
- P19 HUMAN OSTEOCLAST FORMATION AND ACTIVITY ON BONE SUBSTITUTE BIOMATERIALS**  
*V Perrotti<sup>[1,2]</sup>, BM Nicholls<sup>[1]</sup>, MA Horton<sup>[1]</sup>, A Piattelli<sup>[2]</sup>*  
<sup>[1]</sup>University College London, Bone and Mineral Centre, London, UK; <sup>[2]</sup>University of Chieti-Pescara, Dental School, Chieti, Italy
- P20 ZOLEDRONATE POTENTLY INHIBITS THE GROWTH, SURVIVAL AND ACTIVITY OF NORMAL RAT OSTEOBLASTS**  
*IR Orriss<sup>\*</sup>, JC Utting, ML Key, A Brandao-Burch, KW Colston, TR Arnett*  
 Department of Anatomy and Developmental Biology, University College London, London, UK
- P21 DIFFERENTIAL VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR EXPRESSION DURING OSTEOGENESIS**  
*G Kirmizidis<sup>[1]</sup>, JH Lakey<sup>[2]</sup>, MA Birch<sup>[1]</sup>*  
<sup>[1]</sup>Musculoskeletal Research Group, Newcastle University, Newcastle upon Tyne, UK; <sup>[2]</sup>Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, UK
- P22 BONE FORMATION AND FIBROCARILAGE REGENERATION AT BONE TENDON JUNCTION WITH ALLOGENEIC CULTURED CHONDROCYTE PELLET INTERPOSITION**  
*MWN Wong<sup>[1]</sup>, KO Tai<sup>[1]</sup>, KM Lee<sup>[2]</sup>, L Qin<sup>[1]</sup>, KS Leung<sup>[1]</sup>*  
<sup>[1]</sup>Department of Orthopaedics and Traumatology; <sup>[2]</sup>Lee Hysan Research Laboratory, The Chinese University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China
- P23 BCL-2-ASSOCIATED ATHANOGENE-1: A TRANSCRIPTIONAL REGULATOR MEDIATING CHONDROCYTE SURVIVAL AND DIFFERENTIATION DURING ENDOCHONDRAL OSSIFICATION**  
*RS Tare<sup>[1]</sup>, PA Townsend<sup>[2]</sup>, GK Packham<sup>[3]</sup>, S Inglis<sup>[1]</sup>, ROC Oreffo<sup>[1]</sup>*  
<sup>[1]</sup>Bone & Joint Research Group; <sup>[2]</sup>Human Genetics; <sup>[3]</sup>Cancer Sciences, University of Southampton, Southampton General Hospital, Southampton SO16 6YD, UK
- P24 NITRIC OXIDE REGULATES EXPRESSION OF CLASS 3 SEMAPHORINS IN MOUSE OSTEOBLASTS**  
*A Hughes<sup>[1]</sup>, M Helfrich<sup>[1]</sup>, SH Ralston<sup>[2]</sup>, MJ Rogers<sup>[1]</sup>*  
<sup>[1]</sup>Bone & Musculoskeletal Research Programme, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK; <sup>[2]</sup>Molecular Medicine Centre, Western General Hospital, Crewe Road West, Edinburgh, EH4 2XU, UK
- P25 DIFFERENTIAL EFFECTS OF ALPHA-HALOGENATION ON THE POTENCY OF BISPHOSPHONATES AND PHOSPHONOCARBOXYLATES FOR INHIBITION OF THEIR TARGET ENZYMES**  
*CA Stewart<sup>[1]</sup>, JE Dunford<sup>[2]</sup>, Z Xia<sup>[2]</sup>, R Baron<sup>[3]</sup>, MS Marmal<sup>[4]</sup>, BA Kashemirov<sup>[4]</sup>, CE McKenna<sup>[4]</sup>, FH Ebetino<sup>[5]</sup>, FP Coxon<sup>[1]</sup>*  
<sup>[1]</sup>Bone Research Group, Institute of Medical Sciences, University of Aberdeen, AB25 2ZD, UK; <sup>[2]</sup>Botnar Research Centre, University of Oxford, UK; <sup>[3]</sup>Imperial College, London, UK; <sup>[4]</sup>Dept. of Chemistry, University of Southern California, Los Angeles, CA, USA; <sup>[5]</sup>Procter & Gamble Pharmaceuticals, Cincinnati OH, USA
- P26 UN-COUPLING OF BONE TURNOVER MARKERS FOLLOWING GLUCOCORTICOID THERAPY FOR EXACERBATIONS OF INFLAMMATORY BOWEL DISEASE**  
*MH Kriel<sup>[1]</sup>, CS Probert<sup>[1]</sup>, WD Fraser<sup>[2]</sup>, JH Tobias<sup>[1]</sup>*  
<sup>[1]</sup>University of Bristol, Bristol, UK; <sup>[2]</sup>University of Liverpool, Liverpool, UK
- P27 BONE PROPHYLAXIS IN STEROID THERAPY: ARE WE DOING ENOUGH?**  
*T Dugal<sup>[1]</sup>, B Hameed<sup>[1]</sup>, S Jawed<sup>[2]</sup>*  
<sup>[1]</sup>St Georges Hospital Tooting, London; <sup>[2]</sup>St Thomas's Hospital London; <sup>[3]</sup>Kingston Hospital London
- P28 THE PHARMACOLOGY AND PUTATIVE FUNCTION OF BK CHANNELS IN HUMAN OSTEOBLAST-LIKE CELLS**  
*B Li<sup>[1]</sup>, BAJ Evans<sup>[2]</sup>, KT Wann<sup>[1]</sup>*  
<sup>[1]</sup>Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Ave, Cardiff CF10 3XF, UK; <sup>[2]</sup>Department of Child Health, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK
- P29 DETECTION OF ACOUSTIC EMISSIONS TO ASSESS PRESS-FIT STABILITY AND FRACTURE PROPAGATION DURING THE INSERTION OF A SIMULATED IMPLANT FOR CEMENTLESS HIP REPLACEMENT.**  
*BP Tweedie<sup>\*</sup>, AY Muir, HA Simpson*  
 Edinburgh Orthopaedic Engineering Centre, Chancellor's Building, University of Edinburgh, UK
- P30 GAS6 AND AXL RECEPTOR TYROSINE KINASE ARE EXPRESSED BY GROWTH PLATE CHONDROCYTES AND OSTEOBLASTS**  
*L Ronaldson<sup>[1,2]</sup>, A Canfield<sup>[2]</sup>, C Farquharson<sup>[1]</sup>*  
<sup>[1]</sup>Gene Function and Development, Roslin Institute, Roslin, Midlothian, UK; <sup>[2]</sup>Cardiovascular and Endocrine Sciences, School of Medicine, University of Manchester, Manchester, UK
- P31 THE USE OF SKELETAL AGE TO VERIFY CHRONOLOGICAL AGE IN YOUTH FOOTBALL PLAYERS**  
*M Johnson<sup>[1]</sup>, P Doherty<sup>[2]</sup>, AJ Freemont<sup>[3]</sup>*  
<sup>[1]</sup>Manchester United Football Club, Manchester, UK; <sup>[2]</sup>York St John University, York, UK; <sup>[3]</sup>Manchester University, Manchester, UK
- P32 EXTENT OF VITAMIN D INSUFFICIENCY IN YOUNG BRITISH WOMEN: INFLUENCE ON BONE HEALTH**  
*JL Berry<sup>[1]</sup>, DP Pattison<sup>[2]</sup>, AD Woolf<sup>[2]</sup>, SA Lanham-New<sup>[3]</sup>*  
<sup>[1]</sup>University of Manchester, UK; <sup>[2]</sup>University of Plymouth, UK; <sup>[3]</sup>University of Surrey, UK

## Posters

- P33 DOES HIP ARTHRITIS LIMIT THE LIFE EXPECTANCY IN THE ELDERLY: A COMPARATIVE STUDY OF OPERATED AND NON-OPERATED GROUPS OF PATIENTS SUFFERING FROM HIP OSTEOARTHRITIS**  
*SVM Karuppiah, A Dhaliwal, WM Ledingham*  
 Department of Orthopaedics, Grampian University Hospitals Trust, Woodend Hospital, Eday Road Aberdeen AB25 2ZN, Scotland, UK
- P34 THE SUBCELLULAR LOCALISATION OF FEO-RANK IS ALTERED WHEN CO-EXPRESSED WITH WILDTYPE RANK PROTEIN IN VITRO**  
*JC Crockett\*, M Helfrich, MJ Rogers*  
 Bone Research Group, Institute of Medical Sciences, University of Aberdeen, UK
- P35 ALTERED LIPID CONTENT IN OSTEOARTHRITIC FEMURS, ASSESSED USING MRI**  
*T Ahearn, JS Gregory\*, TW Redpath, RM Aspden, JD Hutchison, S Semple, D Younie, D Knight, FJ Gilbert*  
 University of Aberdeen, UK
- P36 CHANGES IN STRUCTURAL PROPERTIES LEAD TO REDUCED BONE STRENGTH IN GUNMETAL MICE**  
*SR Goodyear\*<sup>[1,2]</sup>, A Taylor<sup>[1]</sup>, FP Coxon<sup>[1]</sup>, IR Gibson<sup>[1,2]</sup>, JMS Skakle<sup>[2]</sup>, RPK Wells<sup>[2]</sup>, RM Aspden<sup>[1]</sup>*  
<sup>[1]</sup>Department of Orthopaedics, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK; <sup>[2]</sup>Department of Chemistry, University of Aberdeen, Meston Walk, Aberdeen, AB24 3UE, UK
- P37 INTER AND INTRA-OBSERVER REPEATABILITY OF KELLGREN-LAWRENCE GRADING FOR OSTEOARTHRITIS USING DXA IMAGES**  
*CD Campbell\*, JS Gregory, K Yoshida, N Basu, A Stewart, DM Reid, RM Aspden*  
 Bone Research Programme, University of Aberdeen, UK
- P38 ASSESSMENT OF OSTEOPOROSIS AND OSTEOARTHRITIS USING ACTIVE SHAPE AND ACTIVE APPEARANCE MODELS WITH DXA SCANS**  
*JS Gregory\*, RJ Barr, A Stewart, DM Reid, RM Aspden*  
 Bone Research Programme, University of Aberdeen, UK
- P39 ATYPICAL PHARMACOLOGY OF P2X7 RECEPTORS IN HUMAN OSTEOBLASTS**  
*SM Al-qallaf\*<sup>[1,2]</sup>, EJ Kidd<sup>[1]</sup>, BAJ Evans<sup>[2]</sup>*  
<sup>[1]</sup>Welsh School of Pharmacy, Cardiff University, Cardiff, UK; <sup>[2]</sup>Department of Child Health, School of Medicine, Cardiff University, Cardiff, UK
- P40 ENOS KNOCK OUT MICE SHOW REDUCED TRANSLOCATION OF BETA-CATENIN TO THE NUCLEUS WHEN STIMULATED WITH PULSATILE OR OSCILLATING FLUID FLOW**  
*C Huesa\*<sup>[1,2]</sup>, A Hughes<sup>[1]</sup>, RM Aspden<sup>[2]</sup>, MH Helfrich<sup>[1]</sup>*  
<sup>[1]</sup>Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, UK; <sup>[2]</sup>Department of Orthopaedics, University of Aberdeen, Aberdeen, UK
- P41 THE ROLE OF P21CIP1/WAF1 IN GLUCOCORTICOID INDUCED GROWTH RETARDATION**  
*HC Owen\*<sup>[1][2]</sup>, SF Ahmed<sup>[2]</sup>, C Farquharson<sup>[1]</sup>*  
<sup>[1]</sup>Bone Biology Group, Roslin Institute, Edinburgh, UK; <sup>[2]</sup>Bone & Endocrine Research Group, Royal Hospital for Sick Children, Glasgow, UK
- P42 VARIATION IN WNT-7A IS UNLIKELY TO BE A CAUSE OF FAMILIAL CONGENITAL TALIPES EQUINOVARUS**  
*G Liu<sup>[1]</sup>, J Inglis<sup>[1]</sup>, A Cardy<sup>[2]</sup>, D Shaw<sup>[1]</sup>, S Sahota<sup>[1]</sup>, L Sharp<sup>[3]</sup>, Z Miedzybrodzka\*<sup>[1]</sup>*  
<sup>[1]</sup>Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, Scotland, UK; <sup>[2]</sup>Department of Public Health, University of Aberdeen, Aberdeen, Scotland, UK; <sup>[3]</sup>National Cancer Registry Ireland, Cork, Ireland
- P43 EFFECTS OF AGEING ON CORTICAL AND TRABECULAR BONE IN RADIUS AND TIBIA: A HIGH-RESOLUTION PQCT STUDY**  
*S Kaptoge\*<sup>[1]</sup>, N Dalzell<sup>[1]</sup>, N Morris<sup>[2]</sup>, B Koller<sup>[3]</sup>, P Rueggsegger<sup>[3]</sup>, A Berthier<sup>[4]</sup>, L Braak<sup>[4]</sup>, J Reeve<sup>[1]</sup>*  
<sup>[1]</sup>Department of Medicine, University of Cambridge, Cambridge, UK; <sup>[2]</sup>Hethersett Surgery, Norfolk, UK; <sup>[3]</sup>Scanco, Zurich, Switzerland; <sup>[4]</sup>MEDES, Toulouse, France
- P44 BONE MARROW STROMAL CELLS AND BIOMIMETIC COLLAGEN-HYDROXYAPATITE SCAFFOLDS FOR SKELETAL TISSUE ENGINEERING**  
*JJ Dawson\*<sup>[1]</sup>, DA Wahl<sup>[2]</sup>, JT Czernuszka<sup>[2]</sup>, ROC Oreffo<sup>[1]</sup>*  
<sup>[1]</sup>University of Southampton, Southampton, UK; <sup>[2]</sup>Oxford University, Oxford, UK
- P45 A NOUVELLE METHOD OF APPLICATION OF TENSILE FORCES TO MESENCHYMAL STEM CELLS**  
*M Greenwood, SB Mirza\*, G Blunn*  
 Institute of Orthopaedics, University College London, London, UK
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*MM Beloti\*, GE Crippa, CR Cardoso, JS Silva, AL Ros*  
 University of Sao Paulo, Ribeirao Preto, SP, Brazil
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*SV Karuppiah\*<sup>[1]</sup>, AJ Johnstone<sup>[1]</sup>*  
<sup>[1]</sup>Robert Gordon University, Department of Engineering, Aberdeen, Scotland, UK
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*KES Poole, N Loveridge, S VEDI, JE Compston, J Reeve\**  
 Division of Bone Research, University of Cambridge, UK

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*SL Griffiths\*, SH Cartmell*  
Keele University, Institute of Science and Technology in Medicine, Guy Hilton Research Centre, Stoke, ST4 7QB, UK
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<sup>[1]</sup>Centre for MR Investigations, Hull Royal Infirmary, UK;  
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*X Hochet, RM Aspden\**  
Department of Orthopaedics, University of Aberdeen, Scotland, UK
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Department of Orthopaedic Surgery, Queen Alexandra Hospital, Portsmouth, UK
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University of Sheffield, Sheffield, UK
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University of Sheffield, UK
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<sup>[1]</sup>Department of Pharmacology, Hirosaki University School of Medicine, Hirosaki, Japan;<sup>[2]</sup>Department of Orthopedic Surgery, Hirosaki University School of Medicine, Hirosaki, Japan
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Inverclyde Royal Hospital, Greenock, UK
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*N Basu\*, AJ Black, DM Reid*  
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# Abstracts

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## IS1

### ACID AND BONE

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Elimination and buffering of the acid produced as a result of metabolism poses a fundamental problem for multicellular organisms. The skeletons of land vertebrates contain a massive reserve of base, which is ultimately available as a 'failsafe' to buffer H<sup>+</sup> if acid-base balance is not maintained within narrow limits. Systemic acidosis has many causes, including kidney or lung disease, anaerobic exercise, gastroenteritis, diabetes, diet, ageing and menopause. Local (tissue) acidosis can result from reduced vascular perfusion due to inflammation, infection, tumours, wounds, diabetes, ageing, or simply as a result of cell growth/metabolism (eg, in tumours). Normal extracellular pH in bone is probably 7.1 to 7.2. The deleterious action of acidosis on the skeleton has long been known but was thought to result from physico-chemical dissolution of bone mineral - ie, that the skeleton acts as a 'giant ion-exchange column' to buffer H<sup>+</sup> passively. We showed that bone resorption by cultured osteoclasts (OC) is stimulated directly by acid, and that OC are particularly H<sup>+</sup>-sensitive between pH 7.1 & 7.3. Below pH 7.0, the stimulatory effect plateaus, whereas above pH 7.4, resorption is 'switched off'. Similar responses occur in calvarial bone organ cultures, where H<sup>+</sup>-stimulated Ca<sup>2+</sup> release is almost entirely OC-mediated, with a negligible physico-chemical component. Acidification is the key initial requirement for OC to excavate resorption pits in all species studied to date; once activated, OC can be further stimulated by a wide range of agents including PTH, 1,25(OH)<sub>2</sub>D<sub>3</sub>, ATP and RANK ligand. Thus, extracellular H<sup>+</sup> may be regarded as the long-sought 'osteoclast activation factor'. We recently found that OC resorption is strongly activated at alkaline pH by low concentrations of capsaicin. The capsaicin receptor (TRPV1 or VR1) is a cation channel that is additionally activated by low pH (as well as by heat and endocannabinoids), and is thus a candidate receptor that could mediate OC activation. TRPV1 is also an important nociceptor, and there is increasing evidence that it plays a key role in mediating bone cancer pain. We also found that acidosis exerts a powerful, reciprocal inhibitory effect on the mineralisation of bone matrix trabeculae by cultured osteoblasts, with complete blockade at pH 6.9. This appears to be due to increased bone mineral solubility at low pH, along with selective inhibition of the expression/activity of alkaline phosphatase. However, osteoblast growth and collagen production are unaltered at pH values as low as 6.9. These results are consistent with the osteomalacia that can occur in chronic acidosis. Drugs that block acid-sensing receptors or shift acid-base balance in the alkaline direction may provide novel therapies for bone loss disorders.

## IS2

### THE ROLE OF THE ENDOCANNABINOID SYSTEM IN PAIN MODULATION

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The endocannabinoid system comprises two known receptors (CB1 and CB2); a family of endogenous ligands, the most important being arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG); and specific molecular machinery for the synthesis, transport, and inactivation of these ligands. CB1 receptors are highly expressed throughout the CNS and also in some peripheral tissues. They are found on pain pathways in brain and spinal cord and at the peripheral terminals of primary afferent neurons; they mediate analgesia in inflammatory, neuropathic and cancer pain models. There is ample evidence that the levels of endocannabinoids are altered in many pathophysiological situations. Inhibitors of fatty acid amide hydrolase (FAAH), the enzyme responsible for the rapid

intracellular hydrolysis of anandamide, are antinociceptive but do not display the CNS side-effects that are characteristic of direct CB1 receptor agonism. The CB2 receptor is primarily located on immune cells; it has been implicated in cell migration, differentiation, antigen processing and anti-tumour activity. More recently, CB2 receptors have been identified and functionally characterised in the brainstem. CB2 receptor-selective agonists are anti-nociceptive in models of hypersensitivity produced by peripheral inflammation and neuropathic pain. Whilst there is evidence that peripheral nerve injury induces CB2 protein expression in sensory neurones, CB2 receptor function and expression on neurones remains the subject of controversy. Anandamide also activates the TRPV1 receptor which is part of a family of transient receptor potential (TRP) channels, whose expression is associated with small diameter primary afferent fibres. This receptor is a non-selective cation channel that integrates multiple noxious stimuli and is associated with the pathophysiology of inflammatory hyperalgesia. Anandamide is a low intrinsic efficacy TRPV1 agonist that behaves as a partial agonist in tissues with low receptor reserve; whilst in tissues with high receptor reserve and in circumstances associated with certain disease states it behaves as a full agonist. Clearly, multiple receptor and enzyme targets encompassed within the endocannabinoid system represent potential targets for the treatment of chronic pain associated with both inflammation and nerve damage.

## IS3

### CANNABINOID AND BONE

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Over recent years, it has become apparent that neuro-endocrine signals play an important role in the regulation of bone metabolism. We have recently shown regulation of bone metabolism by the endocannabinoid system. The endocannabinoid system comprises two known receptors (CB1 and CB2), a family of endogenous ligands (endocannabinoids), and proteins for ligand synthesis, transport and inactivation. Endocannabinoids are involved in pain perception, learning, memory, inflammation, appetite, and motor function. CB1 is predominantly expressed in cells of the central nervous system, while CB2 is widely expressed in peripheral cells, including cells of the macrophage lineage. We have found expression of both CB1 and CB2 in bone marrow cells, osteoclasts and osteoblasts, although expression levels of CB1 were very low. In addition we have found the expression of NAPE-PLD and DAGL, enzymes which are critical for endocannabinoid synthesis, and FAAH, which is involved in endocannabinoid metabolism. These findings indicate that endocannabinoids may be synthesized within the bone environment, and may act directly on bone cells.

Both CB1 and CB2 antagonists are potent inhibitors of osteoclast formation and bone resorption in vitro, and ovariectomy (Ovx) induced bone loss in vivo, although CB2-inhibitors have an IC<sub>50</sub> that is almost 10 times lower than CB1-inhibitors. In addition, we found that CB1 KO mice have increased bone mass, and are protected from Ovx-induced bone loss. In contrast, Tam and co-workers recently showed that CB2 knockout mice develop age-related osteoporosis, and to our surprise, we recently observed that the CB2-agonist JWH133 prevented ovx-induced bone loss in vivo, while stimulating osteoclast formation in vitro. This could be due to the stimulation of bone formation by CB2 agonists, and we have indeed observed increased bone nodule formation in osteoblast cultures treated with endocannabinoids or CB2 agonists, and upregulated expression of collagen Type 1, BMP2 and osteocalcin.

As CB1 is predominantly expressed in the CNS, and CB1 antagonists are less potent osteoclast inhibitors than CB2 antagonists, CB1 regulates bone metabolism most probably via a neurogenic relay. CB2 on the other hand is abundantly expressed in the bone microenvironment and may regulate bone cell activity directly.



### IS4

#### VESICULAR TRAFFICKING IN OSTEOCLAST BIOLOGY

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Osteoclasts are highly dependent on vesicular transport processes to carry out their role of bone resorption. In particular, polarised transport of large numbers of acidic vesicles towards the membrane apposed to the bone surface is required for formation of the ruffled border, the resorptive organelle of the osteoclast, which provides a large surface area for the secretion of protons and proteolytic enzymes contained in these vesicles. The resulting acidic environment beneath the osteoclast enables dissolution of bone mineral, while the proteolytic enzymes degrade the bone matrix. It is clear that Rab family GTPases, key regulators of vesicular transport, play a crucial role in this transport process, since disruption of the post-translational prenylation of Rabs, either pharmacologically with an inhibitor of Rab geranylgeranyl transferase (RGGT) or physiologically as occurs in a mouse strain with defective RGGT activity, results in disrupted ruffled border formation and reduced resorption by osteoclasts. Although the exact role that Rab proteins play in the highly specialised function of osteoclasts remain poorly understood, it has been shown that both Rab3D and Rab7 are essential for ruffled border formation and bone resorption, the former regulating trafficking of post-TGN-vesicles, and the latter regulating transport of late endosomes to the ruffled border, which consequently has characteristics of a late endosomal membrane. Further evidence of the necessity for Rab7-positive vesicles in ruffled border formation is provided by the recent finding that a novel osteopetrosis-causing gene encodes a protein that normally co-localises with Rab7 on late endosomes in osteoclasts. The mutant form of this protein that causes osteopetrosis fails to localise to these vesicles, and as a consequence ruffled border formation and resorptive activity is defective. Vesicular transport also plays an important role in removing the breakdown products of resorption, which are endocytosed at the ruffled border, transported through the osteoclast by transcytosis, and released at a specialised membrane domain known as the functional secretory domain. However, mechanistic details regarding this trafficking route remain elusive.

### IS5

#### DISEASES CAUSED BY DEFECTS IN VESICULAR TRAFFICKING AND ACIDIFICATION

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As bone growth and remodeling require either secretion of large amounts of proteins for bone formation and endocytosis/transcytosis for bone resorption it is not surprising that defects in vesicle function can result in skeletal pathology. The osteoclast ruffled membrane is formed by massive fusion of acidic vesicles with the plasma membrane. Loss of proteins present in late endosomes and lysosomes, among them the chloride channel CIC-7, accordingly abolish ruffled border formation and bone resorption resulting in osteopetrosis. In the CNS CIC-7 deficiency leads to lysosomal storage and neurodegeneration. A similar phenotype has been found in the grey-lethal (gl) mutant. The mutated gene product, Ostm1, has been shown to be a beta subunit of CIC-7. In contrast, neurodegeneration is absent in the osteosclerotic (oc) mutant lacking the  $\alpha 3$  subunit of the v-type H<sup>+</sup>-ATPase, which colocalizes with CIC-7 in osteoclasts. Compared to the osteoclast the number of known vesicular defects in osteoblasts underlying skeletal dysplasias is much lower. Cranio-lenticulo-sutural dysplasia is one of the few bona fide phenotypes related to a defect in the secretory pathway. It is therefore very likely that more skeletal disorders linked to vesicular dysfunction will be discovered. Other possible examples and pathomechanisms will be discussed.

### IS6

#### ACIDIFICATION OF THE OSTEOCLASTIC RESORPTION LACUNAE ALLOWS INSIGHTS INTO THE COUPLING OF BONE FORMATION TO BONE RESORPTION - LESSONS LEARNED FROM CIC-7 AND H<sup>+</sup>-V-ATPASE MUTATIONS

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The chloride channel CIC-7 and the H<sup>+</sup>-V-ATPase are essential for osteoclastic bone resorption, as they mediate acidification of the lysosomes and the resorption lacuna. Patients with osteopetrosis caused by defective acidification of the resorption lacuna have severely decreased resorption, but increased numbers of TRACP positive osteoclasts with normal or even increased bone formation. This suggests that osteoclasts themselves and not their resorptive activity are important for sustaining bone formation.

We investigated whether 1) defective resorption due to attenuated acidification leads to increased osteoclast numbers and 2) whether osteoclasts secrete non-bone derived signals able to stimulate bone formation by the osteoblasts.

Human osteoclasts cultured on either plastic or bone in the presence of M-CSF and RANKL. We tested whether conditioned medium was able to induce bone nodule formation by MC3T3-E1 osteoblastic cells cultured under pro-osteoblastic conditions. We assessed bone formation by performing Alizarin red and Von Kossa stainings. We investigated osteoclast survival in the presence or absence of the V-ATPase inhibitor bafilomycin (20nM) on calcified and decalcified bones, and we measured osteoclast resorption and number, by CTX-I and scoring of multinuclear calcitonin receptor positive cells respectively.

We found that inhibition of the proton pump resulted in abrogation of acidification of the resorption lacuna, inhibition of bone resorption (95%), and increased osteoclast numbers (150%,  $p < 0.01$ ), but this did not occur on decalcified bone. Conditioned medium from osteoclasts dose dependently induced bone nodule formation with a maximal effect of 250%, which was comparable to the effect of Bone Morphogenetic Protein-2 (BMP-2).

In conclusion, we have shown that attenuation of acidification leads to increased numbers of osteoclasts, in alignment with the osteopetrotic patients. We show that osteoclasts secrete bone anabolic factors independent of resorptive activity. These results aid in the understanding of why patients with osteopetrosis caused by defective acidification of the resorption lacuna have increased bone formation despite lack of resorptive capacity, and indicate that inhibition of CIC-7 or the H<sup>+</sup>-V-ATPase, represents new approaches for drug development for osteoporosis, which may lead to inhibition of bone resorption without affecting bone formation.

### IS7

#### CONTROL OF BONE REMODELING HOMEOSTASIS VIA THE AUTONOMOUS NERVOUS SYSTEM

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The fields of neuroscience and bone biology have recently converged following the discovery that bone remodeling is directly regulated by the brain. This work has defined bone remodeling as one of the cardinal physiologic functions of the body, subject to homeostatic regulation and integrated with the other major physiologic functions by the hypothalamus. Central to this discovery was the definition of the adipocyte-derived hormone leptin as a regulator of both arms of bone remodeling, formation and resorption, through its action on the ventromedial hypothalamus and subsequently via the sympathetic nervous system to osteoblasts. Sympathetic nerves are indeed distributed within the bone microenvironment close to osteoblasts, which express a functional beta2-adrenergic receptor (beta2AR), and we have shown that both pharmacological and genetic blockade of beta2AR signaling in mice increases bone formation and decreases bone resorption. These findings indicate that norepinephrine, released from sympathetic nerves, acts directly on osteoblasts to exert a brake on their proliferation and function, and promotes the differentiation and function of osteoclasts. They also suggest that beta-blockers could be used as anabolic and anti-catabolic drugs for the treatment of low bone mass diseases, however this latter point will depend upon whether this neural regulatory arm of bone remodeling exists in human. Furthermore, based on the critical role



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of beta2AR signaling for osteoblast function, we hypothesized that this neural regulatory arm of bone remodeling could be involved in bone diseases in which autonomic tone or transduction of autonomic signaling in osteoblasts are impaired. Data illustrating current work on these implications will be presented at the meeting.

### IS8

#### DISCOVERY OF THE FIBRODYSPLASIA OSSIFICANS DYSPLASIA (FOP) GENE

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The identification of a mutation in the activin receptor gene, ACVR1, as the cause of fibrodysplasia ossificans progressiva (FOP) involved a global team of clinicians and researchers who over many years sifted through the limited available family resources to map the gene, eventually using just 5 families. Because FOP is (thankfully) extremely rare, is dominantly inherited, and because affected individuals infrequently have children, multicase families are uncommon. Several families were identified where there was recurrence in a child of a parent with mild evidence suggestive of FOP, consistent with parental mosaicism. Whether mosaicism for ACVR1 mutations is common is unknown, but it is more likely that this finding was due to ascertainment bias, in that mosaics with milder disease are more likely to reproduce. Identification of the FOP gene eventually resulted from concentration on a core set of families with classical FOP, and revising all the available linkage and sequencing data which inevitably contained errors which confused the process. Our linkage mapping was only able to restrict the FOP-linked region to 23.9 Mb of chromosome 2q, a massive region. Advances in sequencing technology are increasingly making sequencing such regions feasible, and will likely lead to the successful mapping of many such rare diseases where the critical region(s) is too great for current technologies. In the case of FOP, we were extremely fortunate that ACVR1 was the standout candidate gene in the region, leading to the eventual demonstration that all classical cases of FOP have a single mutation in the gene, R206H (617G\_A). Other mutations are being identified in atypical patients, but these too seem to cluster in the region encoding the GS domain of the ACVR1 protein, a region thought to be involved in binding of FKBP12, thought to inhibit receptor activation/signalling. The restricted genetic heterogeneity of FOP is encouraging that a single treatment will be developed which is universally effective in the condition. Many rare monogenic diseases have yet to be mapped, and the successful experience in FOP highlights that the likelihood of success in these other diseases is greatly increased by close, open, international collaboration between clinicians and scientists.

### IS9

#### ROLE OF SCLEROSTIN IN BMP AND WNT SIGNALING: IMPLICATIONS FOR BONE FORMATION

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Sclerostin is an osteocyte-specific secreted factor capable of inhibiting osteoblastic bone formation. Loss-of-function of this protein is associated with sclerosteosis and van Buchem disease, two similar sclerosing bone dysplasias characterized by a generalized increase in bone mass and density mainly affecting the skull and mandibular bones. Bone-specific over-expression of human sclerostin in mice results in low bone mass and decreased bone strength due to a significant reduction in osteoblast activity.

Based on homology, sclerostin belongs to the family of cystine-knot-containing growth factors. Its closest related homologues DAN and Cerberus are potent inhibitors of bone morphogenetic protein (BMP) signaling, suggesting a role for sclerostin as a potential BMP antagonist. Binding studies revealed interaction between sclerostin and BMPs, although affinities were much lower when compared to noggin, a potent BMP antagonist. Sclerostin has been shown to reduce late BMP responses, like BMP-stimulated ALP activity in vitro, however, the protein did not seem to affect early BMP responses, including Smad phosphorylation. This raised the question whether the effect of sclerostin on late BMP responses is indirectly through a different mechanism. Interestingly, we and others showed that sclerostin was able to decrease canonical Wnt signal transduction by direct binding to the Wnt co-receptors low-density lipoprotein

receptor-related protein 5 and 6 (LRP5/6). Our results also strongly indicate that activating mutations located in the first beta-propeller domain of LRP5, causing high-bone-mass conditions, interfere with sclerostin binding and modulation of canonical Wnt signaling. Recent data also suggest that sclerostin inhibits late BMP responses like ALP activity by directly antagonizing Wnt signaling.

In conclusion, sclerostin has been identified as an inhibitor of osteoblastic bone formation induced by BMPs. However, the protein does not act like a classical BMP-antagonist, but rather reduces BMP-induced bone formation by interfering directly with LRP5-mediated canonical Wnt signaling. This again demonstrates a cross-talk between BMP and Wnt signaling in the regulation of osteoblast maturation.

### IS10

#### DETERMINANTS OF BONE DESTRUCTION IN RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) represents an excellent model for dissecting the role of immune cells and proinflammatory molecules in regulating pathological bone remodeling. In this condition, the inflamed RA synovium produces a variety of immunomodulatory and proinflammatory cytokines that are potent regulators of bone cell function. These include factors that modulate the activity of both bone resorbing cells, as well as bone forming osteoblasts. RA is associated with several distinct patterns of bone loss, including focal articular and subchondral bone erosions, peri-articular osteopenia, and systemic osteoporosis. Studies by our group and others provide strong evidence that osteoclasts mediate the focal bone resorption at sites of joint destruction in RA. Although immunomodulatory factors that either activate or inhibit osteoclast differentiation and activity are produced in the inflamed synovium, the balance of these activities clearly favors osteoclastogenesis and bone resorption. Receptor activator of NF- $\kappa$ B ligand (RANKL) is among the osteoclast-inducing cytokines produced by the inflamed RA synovium. RANKL is a prototypical example of a cytokine that exhibits dual activities as an immunomodulatory factor and also regulates osteoclast differentiation and activity. Despite the extensive articular bone resorption, a unique finding in patients with RA is the absence of bone repair at sites of bone erosions. This suggests that the mechanisms that regulate the coupling of bone resorption and formation have been disrupted and that the process of bone formation is impaired. Recent studies have implicated the Wnt-pathway inhibitor Dickkopf-1 (DKK-1) in the suppression of articular bone repair in inflammatory arthritis suggesting that targeting this molecule may have therapeutic efficacy in RA (Diarra et al. Nat Med, 2007). Multiple other cytokines, similar to RANKL, possess dual roles as immunomodulators and factors involved in regulation of both bone resorbing and forming cells. These synovial-derived products also are released into the circulation where they may produce a generalized disturbance in bone remodeling leading to systemic bone loss. A better understanding of the activities and signal pathways by which these factors exert their biologic activities could lead to the development of new strategies for targeting the processes involved in joint inflammation and destruction in RA.

### IS11

#### ROLE OF CYTOKINES AND BONE: USE OF IN VITRO MODELS

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Several cytokines affect the skeleton and are required for normal turnover and normal bone mass in vivo, either modulating bone formation and or bone resorption. The study of genetically-altered mice (either loss or gain of function) have been useful to identify the fundamental physiological functions of several cytokines. Specifically, the architecture of bones of animals can be assessed by pQCT, microCT or by histomorphometry. Combined, these techniques provide valuable insights into the bone mass, bone length, disturbance of the growth plate, and perturbation of the cells in the bone microenvironment.

Many in vitro methods have been established with the view to replicate the in vivo processes of osteoblast differentiation, adipocyte formation or osteoclast formation. Several of these methods provide substantive information about each of these processes in isolation, however many do not allow for the potential cross talk between processes to be

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explored. An example is the ability to assess osteoblast differentiation concomitantly with osteoclast formation. In vitro assays for osteoblast differentiation usually requires cultures for 21 days, whilst osteoclast formation is assessed following 7 days of culture. Moreover, phenotypes readily identified in vivo may not be recapitulated in vitro using standard culture techniques of cell lines, primary cultures from wild type animals or from genetically modified animals.

Specifically, the in vivo effects of deletion of either IL-12, IL-18, IL-12+IL-18, IL-23 and osteoclast inhibitory lectin (OCIL) in mice strains will be addressed and compared with ex vivo and in vitro assays that were used to define their mechanisms of action.

### IS12

#### MODULATION OF T CELL FUNCTION IN RHEUMATOID ARTHRITIS

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Human autoimmune diseases are characterized by chronic, non-remitting inflammation. In the early phase of the chronic arthritis auto-reactive T cells initiate a cascade of events that leads to chronic inflammation. Following the initial phase of the disease, other cells such as macrophages and monocytes mainly mediate the ongoing non-specific inflammation. At this later stage, auto reactive T cells presumably do not play an important role in sustaining the chronic inflammation. Mechanisms underlying the perpetuation of this cascade of non-specific inflammation are still poorly understood. As a consequence treatment of the disease until now has been mainly focused on non-specific suppression of inflammation. In patients with chronic arthritis, such Juvenile Idiopathic Arthritis (JIA) and Rheumatoid Arthritis (RA) blockade of the TNF-alpha pathway has proven to be a very potent treatment option. On the other hand, such treatment is costly and fails to induce a long lasting remission of the disease. As a consequence long-term treatment with immune suppressive agents is necessary. This increases not only the costs of the treatment but also harbours considerable risk for long term side effects. Thus, the need for additive and/or alternative strategies is growing. Ideally such alternative treatment should be safe, not expensive and specifically modulate cells that are responsible for the inflammation and/or counter-regulation. Lately, a lot of attention has focused on the role of regulatory T cells for the control of autoimmunity. There are currently two well-characterized types of regulatory T-cells, Tr1 cells and CD4+CD25+ T regulatory cells (Tregs). The CD4+CD25+ Tregs is a heterogeneous group of cells identified by the expression of CD25 and the transcription factor foxP3 (5). We recently showed that these so-called naturally occurring T regulatory cells and regulatory T cells with specificity towards heat shock proteins (hsp's) may play a role in determining disease outcome in JIA (3, 6). Following autologous stem cell transplantation for arthritis T regulatory cell function is restored coinciding with a remission of the arthritis (1). Moreover oral treatment with a peptide (dnaJP1) derived from heat shock protein dnaJ, restored T regulatory cells in PBMC from patients with RA (4). Recently we identified 8 panDR binder peptides derived from hsp60 that are recognized by PBMC from a majority of patients with JIA(2). These peptides could be exquisite candidates for immune therapy in human arthritis. Thus, due to insufficient numbers of regulatory T-cells, feedback mechanisms fail, resulting in an unrestrained pro-inflammatory immune response and severe tissue damage in RA and JIA. A lack of a counter-regulatory mechanism based by regulatory T cells is at least in part responsible for the perpetuation of inflammation. Heat shock proteins may be instrumental in restoring the immunological balance and thus contribute to a long-lasting disease remission.

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### IS13

#### ROLE OF BISPHOSPHONATES IN CANCER MANAGEMENT

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There is increasing use of cancer treatments with adverse effects on bone health. These include ovarian suppression and aromatase inhibitors in early breast cancer and androgen deprivation therapy in prostate cancer. Both accelerated bone loss and an increased risk of fracture are seen with these frequently used interventions. The bisphosphonates are a safe and effective treatment for cancer treatment induced bone loss, but the choice of agent and optimum schedule of treatment remains under active investigation.

In metastatic bone disease, the bisphosphonates are an important component of multidisciplinary treatment. Multiple, randomised controlled trials over the past two decades have clearly demonstrated that bisphosphonates are effective in reducing skeletal morbidity from breast cancer and multiple myeloma. Zoledronic acid (Zometa™) is the most potent bisphosphonate, and has shown superior efficacy in hypercalcaemia of malignancy to pamidronate. In patients with breast cancer, a 20% additional reduction in the risk of a SRE with zoledronic acid over pamidronate has been shown, with additional advantages in terms of convenience. More recently, trials with zoledronic acid have shown that this agent is also able to significantly reduce skeletal morbidity across the range of solid tumours resulting in bone metastases including lung and endocrine resistant prostate cancers.

Attention is now focusing more on when to start treatment and the duration of treatment, as well as the use of bone markers to predict those patients most likely to benefit and to guide the treatment schedule. Recent studies have demonstrated that the risk of a skeletal related event (SRE) is strongly associated with the rate of bone resorption, as measured by specific biochemical markers that reflect the breakdown of type 1 collagen, and that the aim of bisphosphonate treatment in malignancy should be to restore the rate of bone resorption to normal. Serial measurements of biochemical markers of bone metabolism may be able to guide the schedule of administration. A large randomised trial to assess marker directed therapy (BISMARK) has recently started in the UK.

Bisphosphonates are generally well tolerated and, once bone metastases have developed, should be given throughout the course of the disease. However, monitoring of renal function is recommended to avoid the occasional clinically significant deterioration in renal function that may occur with potent aminobisphosphonates. Additionally a new complication of bisphosphonate treatment, osteonecrosis of the jaw (ONJ), has recently been described. The risk of ONJ is related to the presence of pre-existing dental problems requiring invasive procedures, concomitant treatments such as chemotherapy and corticosteroids that predispose to infection, and the duration of bisphosphonate treatment. The incidence in advanced breast cancer is probably around 1% per year of treatment. Good dental care and oral hygiene are recommended to reduce the incidence of ONJ.

It seems likely that the efficacy of bisphosphonates in metastatic disease has reached a therapeutic ceiling. Recently, the biological importance of the RANK ligand-RANK-OPG system in metastatic bone disease has been defined. Phase I/II studies of an antibody to RANK ligand (Denosumab) have been completed and registration studies are in progress across a range of clinical indications.

The clinical role of adjuvant bisphosphonates in early cancer has not been clearly defined. Studies with oral clodronate in early breast cancer suggest that they may delay the development of bone metastases and improve survival. However, the results from large adjuvant trials with clodronate (NSABP-34) and zoledronic acid (AZURE) are required to define clearly the role of bisphosphonates in the adjuvant setting. Both studies have completed accrual, and first efficacy results are anticipated in 2008.

**IS14****BISPHOSPHONATES IN THE MANAGEMENT OF PAGET'S DISEASE***SH Ralston\**

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Paget's disease of bone (PDB) is a common condition characterized by focal abnormalities of increased bone turnover leading to complications such as bone pain, deformity, pathological fractures, and deafness. Treatment of PDB is based on giving antiresorptive drugs to reduce bone turnover; and/or giving analgesics or NSAID for pain. Some experts believe that bisphosphonates should be given to normalise bone turnover in PDB in the hope that this will arrest disease progression but there is no direct evidence that this is the case. Indeed, the only randomised study that has been performed in which greater suppression of bone turnover has been accompanied a better symptomatic response is that of Reid and colleagues who compared Zoledronate with Risedronate in PDB. Zoledronate was more effective than Risedronate at improving some components of quality of life but with the exception of pain, the changes lay below the threshold that is considered clinically significant. The recently completed PRISM study was initiated to determine whether intensive bisphosphonate treatment conferred any advantage over symptomatic treatment in preventing complication of PDB or in affecting quality of life. This study involved 1324 PDB patients who were followed for a median of 36 months (range 24-60). Patients were randomised to receive symptomatic treatment where the aim was to control bone pain, or intensive bisphosphonate therapy where the aim was to normalise ALP. Normalization of ALP was achieved in 81% of the intensive group at 2 years compared with 63% in the symptomatic group ( $p < 0.001$ ) but there was no significant difference between the treatment groups in prevalence of fracture, requirement for orthopaedic surgery, progression of deafness or quality of life, although use of painkillers was greater in the symptomatic group. The PRISM study demonstrates that bisphosphonates improve pain in PDB but shows that they do not prevent complications. Current evidence suggest that symptomatic treatment for PDB should be adopted as the current standard-of-care until evidence emerges to show that the benefits of intensive treatment outweigh the greater treatment costs and the risks of adverse events.

**IS15****THERAPEUTIC OPTIONS AFTER BISPHOSPHONATES***G Russell\**

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Osteoporosis is the commonest disorder of bone. Since the early 1990s, there has been remarkable progress in introducing effective treatments that reduce vertebral fractures, and in many cases at other sites too, including importantly the hip.

Drugs that will benefit osteoporosis can achieve this in at least three possible ways, by inhibiting bone resorption, by stimulating bone formation, or a combination of both. Up until recently, all the major drugs used to prevent or treat osteoporosis are inhibitors of bone resorption, and thereby of remodelling. This includes bisphosphonates as the leading drugs, also the SERMs (Selective Estrogen Receptor Modulators), the first of which was raloxifene.

In the future, there is likely to be continued interest in the development of drugs that stimulate bone formation, as so-called anabolic agents. Developing alternate forms of PTH is ongoing, while 'calcilytic' drugs working via the Ca-sensing receptor to stimulate endogenous PTH secretion are proving to be a feasible approach. Apart from the androgen equivalents (SARMs) of SERMs, interesting opportunities arise from the discovery of the genetic basis of the high bone mass syndromes, including sclerosteosis. The targets lie in the BMP and wnt/LRP5/6 pathway. Blockade of SOST, an osteocyte derived protein that blocks BMPs and wnt signalling, by using antibodies has already been demonstrated experimentally to augment bone mass. A similar approach blocks dkk to activate wnt signalling.

**CC1****SEVERE HIGH TURNOVER OSTEOPOROSIS OF UNKNOWN CAUSE IN A YOUNG MAN WITH AUTOIMMUNE HYPOTHYROIDISM ; A NOVEL SYNDROME?***PL Riches\*, E McRorie, SH Ralston*

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A previously well 40 year old Caucasian man presented with a low trauma fracture of the clavicle and very low BMD (Spine T-score -6.6; hip T-score -2.9). He had a raised alkaline phosphatase (ALP) at 2610 u/l (normal <105) and autoimmune hypothyroidism with low T4 (< 5pmol/l), raised TSH (>65mU/l) and thyroid autoantibodies. Levels of PTH were low (8ng/l) and 25(OH)D levels were low/normal (35nmol/l). He required intravenous fluids for hypercalcaemia. Isotope bone scan showed increased tracer uptake throughout the skeleton but no focal lesions. A transiliac bone biopsy showed extremely high bone turnover and a mild mineralisation defect. A diagnosis of Coeliac disease was made by serology and duodenal biopsy. He was thought to have osteomalacia and was started on a gluten free diet, ergocalciferol 10,000 u/d and calcichew. Over the next 6 months he had further fragility fractures and spine BMD values fell to reach a T-score of -7.7. ALP remained high (1500 u/l) despite having had 6 months calcium and vitamin D and serum 25(OH)D was now high-normal. He was treated with Zoledronate 4mg on two occasions by intravenous infusion. This resulted in progressive reduction in ALP values to within the normal range. He had no more fractures and BMD improved dramatically to a T score of -2.5 in the spine and -1.3 at the hip. This patient was initially thought to have osteomalacia but had atypical features (hypercalcaemia, low PTH) and did not respond to vitamin D therapy. He responded dramatically to antiresorptive therapy which normalised bone turnover and increased BMD. Since TSH has been suggested to be a negative regulator of bone turnover, we speculated that our patient might have developed TSH receptor blocking antibodies but tests for these were negative. In summary this young man with autoimmune disease had severe high turnover osteoporosis of unknown cause which responded dramatically to antiresorptive therapy. As far as we are aware this is a unique clinical picture and emphasises the importance of fully investigating young men with idiopathic osteoporosis. At present, the cause of his profoundly increased bone turnover remains elusive.

**CC2****RESIDUAL (GHOST) SOCKETS IN INTRAVENOUS BISPHOSPHONATE USE - EVIDENCE OF POOR HEALING AND SLOW BONE TURNOVER***K Shetty\*, J Buoqurot*

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Background: Bisphosphonates are used to slow osteoclastic and osteoblastic activity in patients with metastatic disease (or the potential of same), myeloma, Paget disease and osteoporosis. Numerous recent examples have documented extremely poor healing of alveolar bone after relatively minor trauma (e.g. extraction), perhaps resulting in chronically exposed bone. It would seem logical that, even in cases with soft tissue healing, extraction sockets may remain radiographically visible for an extended period of time after surgery. However, no such case has been reported to date.

Objective: To report a clinical series of residual or ghost sockets (outline or lamina dura visible radiographically) in five patients on intravenous bisphosphonate use for metastatic cancer.

Methods: The cases were selected from the inpatient database of Medically Complex Patient Clinic at the University of Texas Health Sciences Center, Houston. The patients were radiographically and clinically followed after full mouth surgical extraction.

Results: The clinical series of patients demonstrated residual sockets or laminar rain remaining for a significant period (12 months) after extraction signifying chronic ischemia of jawbone marrow. The lower jaw in four of the five patients showed little remodeling, few trabeculae and 'ghost marrow,' a common radiographic feature of osteonecrosis of long bones.

Conclusions: Firstly, residual sockets may provide additional evidence of poor bone healing or very slow bone turnover in patients using bisphosphonates. Secondly: the presence of such a radiographic sign may alert the surgeon to potential healing problems in subsequent surgery or trauma of alveolar bone. Thirdly, it is clearly possible for



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adequate soft tissue healing to occur above ghost sockets. Finally, residual sockets are also seen in chronic ischemic bone disease without bisphosphonate. It is not known what the relative influence is of the bisphosphonates compared to local ischemia. This is the first study to report this phenomenon in the lower jaws of cancer patients undergoing chronic intravenous bisphosphonates therapy.

### CC3

#### BONE PAIN FROM CHRONIC SCLEROSING OSTEOMYELITIS SUCCESSFULLY TREATED WITH RISEDRONATE

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Chronic sclerosing osteomyelitis (CSO) is a term to describe single or multiple sclerotic non-pyogenic lesions that are predominantly located within the metaphyses, and often show a tendency for expansion. Long-term intractable pain can be a difficult management issue in CSO patients. Our typical case history highlights the long time course, and dramatic response to treatment with an oral bisphosphonate.

A 28 year old woman presented in 1999 with a 17 year history of intermittent pain in her left, dominant, upper arm occurring every 2 months. The episodes of pain lasted 7-10 days, with sleep disturbance and reduced function of the left arm. A diagnosis of chronic osteomyelitis was made on the radiographical appearances, but microbiology from biopsy specimens was negative. There was no consistent improvement in the pain with courses of oral antibiotic treatment. She became pain-free, however, during her two pregnancies in 2001 and 2002, and for 6 to 8 weeks post-partum. Pain also improved during 6 months of chemotherapy for breast cancer in 2003. In 2004, aged 34, her pain symptoms had recurred. Plain radiographs and isotope bone scan were performed and the open biopsy histology specimen from 2000 was reviewed. A diagnosis of CSO was again confirmed and the patient was started on oral risedronate 35mg once weekly.

By 18 months she was pain-free. Retrospectively, the patient scored her pain as 10/10 prior to starting risedronate, and after 6 months' treatment, this had fallen to 3/10. Repeat isotope bone scan performed 2 years after starting risedronate showed reduced uptake at the left humerus, suggesting reduced metabolic activity. Further imaging is awaited to determine if there has been a change in sclerosis. It is planned for the patient to continue on risedronate for at least 5 years.

This case demonstrates that oral risedronate, used in the dose licensed for postmenopausal osteoporosis, can have beneficial effects on pain in patients with CSO. This benefit was seen within 6 months, and was sustained at 2 years. In the presentation, a review will be given on the medical treatment options available for CSO, including the role for bisphosphonates.

### CC4

#### OSTEOCLAST ACTIVITY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) DURING THE FIRST YEAR OF TREATMENT

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Treatment for childhood ALL is associated with reduction in bone mineral density (BMD) and increase in serum markers of bone resorption. Current evidence reports a reduction in osteoblast activity with chemotherapeutic agents but the role of osteoclasts in this osteopenia is unclear. The aim of this study was to study the role of osteoclasts in the aetiology of this osteopenia.

Samples collected were from controls (20 normal children: mean (range) 8.7 (1.5-16) years) and patients (25 children with ALL: 6.8 (2-17) years). Serial samples from patients and a single sample from controls were used to measure (mean  $\pm$  SD) procollagen type I N terminal peptide (PINP), osteocalcin (Oc), cross-linked C terminal telopeptide of type I collagen (CTX) and parathyroid hormone (PTH), and to generate osteoclasts from blood mononuclear cells in vitro using M-CSF and RANKL. Osteoclast activity was determined by % resorption of a dentine slice (mean  $\pm$  SEM (range)). 15/25 patients had DEXA scans to monitor BMD.

Results were as follows (control; patient at induction wk 0 - 5; post induction wk 5 - 60 in each case:-

% resorption;	28.1 $\pm$ 5.89 (0 - 66.5);	4.68 $\pm$ 2.09 (0 - 37.9)**;	8.96 $\pm$ 1.7 (0 - 49.2)**
PINP mcg/l;	803 $\pm$ 276;	54.8 $\pm$ 42***;	54.8 $\pm$ 42***
Oc mcg/l;	164.9 $\pm$ 92.3	9.8 $\pm$ 5.8***;	81.2 $\pm$ 38*
CTX mcg/l;	1.85 $\pm$ 0.56;	1.07 $\pm$ 0.41**;	1.48 $\pm$ 0.6
PTH pmol/l;	2.35 $\pm$ 0.17;	8.2 $\pm$ 3.3***;	5.6 $\pm$ 3.4**

[\* p < 0.05, \*\*p < 0.01, \*\*\* p < 0.001 (patients vs. controls)]

BMD of hip, lumbar spine and whole body was reduced at diagnosis and remained low during the first year of treatment.

Bone markers demonstrate significant reduction in osteoblast activity in induction with less marked decreases in osteoclast activity. This data suggests that uncoupling of osteoblast and osteoclast activity could be the major contributor to loss of bone in ALL.

## OC1

### THE ENDOCANNABINOIDS 2-ARACHIDONOYL GLYCEROL AND ANANDAMIDE ARE PRODUCED BY BONE CELLS AND STIMULATE BONE RESORPTION IN VITRO

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The recent demonstration of abnormal bone phenotypes in cannabinoid receptor (CB1 and CB2) knockout mice implies a role for these receptors in bone physiology. However, the exact role of these receptors in bone is far from clear and it is not known whether their endogenous ligands, the endocannabinoids, are produced in the bone microenvironment. To address this we have measured the production of arachidonoyl ethanolamine (anandamide) and 2-arachidonoyl glycerol (2-AG) in primary bone cells and cell lines using LC-MS/MS. Endocannabinoids were extracted from cultured cells with methanol/acetonitrile and levels were normalised to total protein content. The extraction efficiency was >95% and the limit of quantification were 0.01pmol for anandamide and 25pmol for 2-AG. 2-AG was detected in mouse calvarial osteoblasts, osteoblast (MC3T3-E1 and MG-63), osteocyte (MLOY4) and macrophage cell lines (J774) and in human and mouse primary macrophages/osteoclast precursors and osteoclast-like cells. Anandamide was detected in calvarial osteoblasts, osteoblast and osteocyte cell lines but was not detectable in human and mouse primary macrophages or in osteoclasts. Addition of the calcitropic factor parathyroid hormone (PTH) caused a significant increase in the levels of 2-AG in the osteoblast-like cell line MC3T3-E1 (1.96 +/-0.59nmol/mg to 3.66 +/-1.03 nmol/mg protein) and a significant increase in anandamide levels in calvarial osteoblasts (2.25 +/-1.65 pmol/mg to 3.34 +/-2.56 pmol/mg protein). Treatment of mouse osteoclasts with the bacterial endotoxin LPS caused a significant increase in 2-AG levels (1.37 +/-0.44 nmol/mg to 4.81 +/-3.35 nmol/mg protein).

To study the role of these endocannabinoids in bone physiology, we investigated the effects of 2-AG and anandamide on the function of human osteoclasts derived from M-CSF-dependent peripheral blood monocytes. Treatment with 2-AG resulted in a 6.5-fold increase in bone resorption and a 2-fold increase in the number of F-actin rings in cultures of osteoclasts compared to the vehicle control. Similarly, treatment with anandamide resulted in a 3.5-fold increase in resorption and a 1.7-fold increase in the number of F-actin rings in osteoclast cultures. We conclude that 2-AG and anandamide can be produced locally by bone cells, can be regulated by osteotropic factors and are novel activators of bone resorption by human osteoclasts.

## OC2

### HYPOXIA-INDUCIBLE FACTOR AND HUMAN OSTEOCLASTS: EVIDENCE FOR A POSITIVE FEEDBACK LOOP REGULATING OSTEOCLAST FUNCTION

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The alpha subunit of the transcription factor Hypoxia-Inducible Factor (HIF) is over-expressed in many human cancers and commonly associated with a pro-tumourigenic phenotype. It is also important for the normal function of cells of the myeloid lineage. Giant cell tumour of bone (GCTB) contains numerous osteoclast-like multinucleated giant cells and a mononuclear component predominantly comprising macrophages and stromal osteoblast-like cells. We have investigated HIF expression in GCTB and its role in the regulation of osteoclast formation and function.

Expression of HIF-1 alpha, HIF-2 alpha and downstream target genes (Glut-1, BNIP3) was analysed by immunohistochemistry in a tissue array comprising 132 GCTB. Giant cells preferentially expressed HIF-1 alpha (40/132 HIF-1 alpha +ve, 26/132 HIF-2 alpha +ve), whereas HIF-2 alpha was predominant in the stromal population (95/132 HIF-2 alpha +ve, 57/132 HIF-1 alpha +ve). BNIP3 and Glut-1 showed a positive correlation with HIF-1 alpha and HIF-2 alpha expression.

Western blot analysis of lysates from normal human monocyte-derived osteoclasts, the osteoblastic cell line MG-63 and primary stromal cells derived from GCTB revealed HIF-1 alpha, HIF-2 alpha, BNIP3 and Glut-1 to be induced by hypoxia (0.1% O<sub>2</sub>, 16h) in culture. The HIF downstream target VEGF was also hypoxia-inducible as measured by ELISA (P < 0.05). As HIF is additionally stabilised by growth factors in some cell types we looked for an effect of the osteoclastogenic factors M-CSF and HGF. Both induced HIF-1 alpha,

HIF-2 alpha, BNIP3 and Glut-1 (25 ng/ml, 2-24h) in osteoclasts and stromal cells and additionally stimulated VEGF secretion in MG-63 (HGF 1327±211 versus 759±30 pg/106 cells [P < 0.05]; M-CSF 1133±186 versus 759±30 pg/106 cells [P < 0.05]) and primary stromal cells (M-CSF 1454±231 versus 916±149 pg/106 cells [P < 0.05]).

This is the first demonstration of HIF expression in either GCTB or human osteoclasts. Induction of HIF by factors that promote osteoclast differentiation / activity (M-CSF, HGF) and subsequent secretion of additional osteogenic agents (VEGF) suggests a potential positive feedback loop regulating osteoclast function. HIF expression in osteoclasts, osteoblasts and macrophages in vivo implies a potentially important role for the HIF pathway in osteoclast differentiation and the pathogenesis of GCTB.

## OC3

### INTRAUTERINE PROGRAMMING OF BONE-DELETERIOUS EFFECTS OF A LOW PROTEIN DIET IN LATE ADULTHOOD ON THE OSTEOGENIC ENVIRONMENT

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Evidence from epidemiological studies indicate skeletal growth is programmed during intrauterine and early postnatal life. This study has examined whether an age-related decrease in bone mass has, in part, a fetal origin using a rat model of maternal protein insufficiency.

Dams received either 18% (control) or 9% (low protein) diet during pregnancy, and the offspring studied at selected time points (4, 8, 12, 16, 20, 47, 75 weeks).

Alkaline phosphatase activity started low in control diet offspring at 4 weeks of age, reached peak levels from 8 to 20 weeks of age, then returned to low levels by week 47. In contrast, restricted diet offspring were at peak levels from 4 weeks of age. Peak levels were similar in both groups. Serum IGF-1 levels were lower in female restricted diet offspring at 4 weeks of age and serum osteocalcin was significantly higher at 4 weeks of age in male and female offspring from mothers fed the restricted diet, whereas serum 25-OH vitamin D was significantly lower in restricted diet males at 8, 12, and 20 weeks of age.

No differences were seen in the macro structure of the femur, tibia, vertebrae, or calveria. Using micro-CT, 75 weeks old female offspring from mothers fed a restricted protein diet during pregnancy had femoral heads with significantly thinner, less dense trabeculae, femoral necks with more closely packed trabeculae, altered density of the midshaft tibia, and vertebrae with thicker, wider spaced, more dense trabeculae, and thinner calveria. These same bones displayed altered strength characteristics when subjected to strength testing.

These data indicate that a low protein diet in utero is deleterious affecting the osteogenic environment in the offspring with effects that persist into late adulthood. Furthermore, offspring from mothers fed a restricted protein diet during pregnancy displayed significant differences in bone structure and density at various sites. These differences result in altered bone characteristics indicative of significantly altered bone turnover. These results illustrate the key role of the nutritional environment in early development on programming of skeletal development and demonstrate the consequences, in later life, for altered development.

## OC4

### DIFFERENTIAL EFFECT OF DOXORUBICIN AND ZOLEDRONIC ACID ON INTRA-OSSEOUS VS EXTRA OSSEOUS BREAST TUMOUR GROWTH IN VIVO

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Breast cancer patients may receive a chemotherapy agent as well as zoledronic acid to prevent tumour-induced bone disease. We have previously reported that sequential treatment with doxorubicin (dox) followed by zoledronic acid (zol) causes a synergistic inhibition of breast cancer cell growth in vitro, and inhibits s.c. breast tumour growth in vivo. The aims of the present study were: 1) To determine



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whether sequential treatment with clinically relevant doses of dox and zol is superior to combined treatment using an in vivo model of breast cancer-induced bone disease. 2) To establish whether treatment has a differential effect on intra-osseous vs extra osseous parts of the tumour.

MDA-MB-231/BO2 cells were injected via the tail vein in athymic mice, and tumour induced osteolytic lesions were detected by day 18. Mice were treated with saline, 100ug/kg zol, 2mg/kg dox, zol and dox simultaneously, or dox followed 24h later by zol. Animals were sacrificed on day 32. Tumour growth within the marrow cavity and outside the bone was measured, and apoptosis, proliferation and angiogenesis assessed at both sites using immunohistochemistry for caspase 3, BrdUrd and CD34, respectively. Effects on bone were evaluated following x-ray and uCT analysis.

Sequential treatment with dox then zol caused a significant inhibition of intra-osseous tumour growth compared to all other treatment groups. The reduction in tumour volume was associated with increased levels of tumour cell apoptosis, decreased tumour cell proliferation and angiogenesis. In contrast, none of the treatment schedules had any effect on extra-osseous tumour growth. Zol, alone or in combination with dox, resulted in significantly smaller osteolytic lesions compared with control or dox treated animals.

The use of dox followed by zol in the treatment of tumour induced bone disease causes significant reduction in intra-osseous tumour growth and bone lesions, but has no effect on extra-osseous tumour growth.

### OC5

#### EVALUATION OF IN VIVO NEOVASCULARISATION IN ALLOGRAFT AND POLY D,L-LACTIC ACID TISSUE ENGINEERED SCAFFOLDS USING MICRO-COMPUTER TOMOGRAPHY

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Vascularisation is critical to the development and functionality of tissue regeneration. Quantification and 3D visualization of new vessel networks remains a problem in tissue engineering constructs. We examined the potential of utilising a radio-opaque dye and micro-CT to visualize and quantify microvascular networks in 3D in vivo. We applied this technique to study neoangiogenesis in an impaction bone graft (IBG) model in vivo as proof of concept. Tissue engineered constructs were created with synthetic, Poly DL-Lactic Acid (PLA) and natural grafts (allograft).

Human bone marrow stromal cells (HBMSC), expanded in culture and labeled with a fluorescent probe (Vybrant) to measure cell viability, were seeded onto scaffolds (morsellised allograft or PLA) and impacted with a force equivalent to a standard femoral impaction (474J/m<sup>2</sup>). The impacted HBMSC/scaffolds (n=3) and scaffolds alone (n=3) were contained within capsules and implanted subcutaneously into nude mice for 4 weeks. Radiopaque dye (Microfil) was infused into all vessels via cardiac cannulation prior to removal of implants. Micro-CT imaging and immunohistochemistry was performed in all samples.

Abundant fluorescent staining was evident within all scaffold / HBMSC constructs indicating cell survival. Average number of vessels penetrating the capsules were 16.33 in allograft/HBMSC constructs compared to 3.5 (p=0.001) in allograft alone samples and 32.67 in PLA/HBMSC constructs compared to 7.67 (p=0.001) in PLA alone samples. Average total vessel volume within the capsules was 0.43mm<sup>3</sup> in allograft / HBMSC constructs compared to 0.04mm<sup>3</sup> (p=0.05) in allograft alone samples and 1.19mm<sup>3</sup> in PLA / HBMSC constructs compared to 0.12mm<sup>3</sup> (p=0.004) in PLA alone samples. Staining for Type-1 Collagen and Von Willebrand factor in living tissue engineered constructs confirmed osteogenic cell phenotype, and new blood vessel formation respectively.

HBMSC combined with either morsellised allograft or PLA can survive the forces of femoral impaction, differentiate along the osteogenic lineage and promote neovascularisation in vivo. Successful combined bone formation and neovascularisation in impacted tissue engineered constructs in vivo augers well for their potential use in IBG. This novel method utilising contrast enhanced 3D reconstructions in combination with immunohistochemistry enables quantification of new bone formation and neovascularisation in impacted tissue engineered constructs with widespread experimental application.

### OC6

#### RANKL MUTATIONS ARE RESPONSIBLE FOR THE DEFECTIVE OSTEOCLAST FORMATION SEEN IN SIX PATIENTS WITH OSTEOCLAST-POOR OSTEOPETROSIS

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Autosomal recessive malignant osteopetrosis (ARO) is a genetically heterogeneous group of bone diseases characterized by defects in osteoclast formation (osteoclast-poor), or, more commonly, osteoclast function. In about 30% of cases, including all the osteoclast-poor cases, the genetic basis of the disease is as yet unknown. The remainder are caused by loss of function mutations in the proton pump subunit ATP6a3, or the chloride channel ClC-7, or the 'grey-lethal' protein OSTM1.

We have recently found that a subset of ARO patients carry mutations in the RANKL gene. So far we have identified 3 different mutations in 6 patients from 4 unrelated families: an intronic mutation causing exon skipping, a frameshift mutation and a missense mutation. Interestingly, the same missense mutation was found in patients in two unrelated families. As predicted, when osteoclasts were generated from the patients' monocytes in the presence of synthetic wildtype RANKL, multinucleated, vitronectin receptor-positive osteoclasts formed normally in the cultures. Moreover, when cultured on dentine discs, the osteoclasts generated in vitro were able to form sealing zones and resorbed the dentine substrate as efficiently as control osteoclasts generated from monocytes from the unaffected parents. By contrast, the patients were all characterised by greatly reduced numbers of osteoclasts in bone biopsies (confirming that these are all cases of osteoclast-poor osteopetrosis) and a poor outcome, as bone marrow transplantation provided no improvement in bone remodelling despite successful engraftment. To further confirm that these RANKL mutations are solely responsible for the defective osteoclast formation in the patients, we have introduced the mutations into a bacterial expression plasmid containing wildtype RANKL, using site-directed mutagenesis. The ability of recombinant mutant and wildtype RANKL to stimulate osteoclast formation in cultures of peripheral blood mononuclear cells from normal donors is currently being evaluated, although preliminary data indicates that, as expected, the mutant forms of RANKL are unable to stimulate osteoclast differentiation. Together, these findings demonstrate that mutations in RANKL are a cause of osteoclast-poor osteopetrosis, and that such patients may benefit from early RANKL administration rather than bone marrow transplantation.

### OC7

#### DOWNSTREAM SIGNALLING PATHWAYS OF NON-CANONICAL (RANKL-INDEPENDENT) PATHWAYS OF OSTEOCLAST FORMATION INDUCED BY TNF SUPERFAMILY MEMBERS LIGHT AND APRIL

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A balance between bone resorption by osteoclasts and bone formation by osteoblasts is essential for maintaining normal bone structure. RANKL is a key growth factor required for osteoclast formation from circulating mononuclear phagocyte precursors. RANKL binds to its receptor RANK and, via interaction with various TRAFs (adaptor molecules), activates three key downstream signalling pathways: Akt, NFkappaB, and JNK. The Akt pathway, solely activated via TRAF6, is essential for the formation of fully functional osteoclasts. A number of TNF superfamily members such

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as LIGHT and TNF $\alpha$ , have been shown to substitute for RANKL to induce osteoclast formation. To determine the downstream signalling pathways of LIGHT, RAW264.7 cells were incubated (0-90 minutes) with RANKL (30ng/ml) or LIGHT (50ng/ml). JNK, NF $\kappa$ B, and Akt activation, detected by Western blotting, peaked at 5, 10, and 45 minutes respectively when incubated with RANKL. This was very similar to the LIGHT time course (JNK, 10 minutes; NF $\kappa$ B, 10 minutes; Akt, 30 minutes) suggesting LIGHT directly activates TRAF6. We also found that the downstream signalling of APRIL, another TNF-superfamily member, peaked at 5 minutes, 20 minutes, and 30 minutes for JNK, NF $\kappa$ B, and Akt respectively. We found that peripheral blood mononuclear cells cultured for up to 21 days in M-CSF (25ng/ml) and APRIL (5, 10, 25, and 50ng/ml) induced the formation of large numbers of TRAP+ and VNR+ multinucleated cells that formed F-actin rings. Our findings indicate that TNF superfamily members act via several downstream signalling pathways and show for the first time that LIGHT activates the Akt pathway via TRAF6. In addition, we have shown that another TNF superfamily member, APRIL, is capable of inducing the formation of osteoclastic cells and that it has the potential to enhance osteoclast activation and differentiation in the presence of other osteoclastogenic factors such as RANKL and LIGHT. Further investigation of the downstream signalling pathways of both LIGHT and APRIL will provide insight into the means by which these growth factors interact with canonical (RANKL-dependent) and non-canonical (RANKL-independent) pathways of osteoclast formation that play a role in pathological bone resorption.

### OC8

#### MICE WITH A TRUNCATION MUTATION AFFECTING SEQUESTOSOME 1 EXHIBIT SEVERAL PHENOTYPIC FEATURES IN COMMON WITH PAGET'S DISEASE OF BONE

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Paget's disease of bone (PDB) is characterized by focal increases in bone turnover and mutations affecting the Sequestosome 1 gene (SQSTM1) are an important cause of this condition, occurring in up to 40 percent of the patients with familial PDB.

We report upon our preliminary analysis of the skeletal phenotype of mice with a truncating mutation at codon 409 of the SQSTM1 gene which deletes most of the UBA domain and results in loss of ubiquitin binding.

Radiological analysis has shown femoral expansion in mutant mice by a mean plus minus SD of 8.63 plus minus 4.31% when compared with wild type (WT) ( $p < 0.0001$ ). Preliminary histomorphometric analysis showed a dramatic increase in bone turnover in 20 month old mice that are homozygous carriers of the mutation. There was a three fold increase in osteoclast number from 1.2 plus minus 0.5 mm<sup>2</sup> in WT to 4.5 plus minus 0.8 mm<sup>2</sup> in mutant ( $p < 0.01$ ) and a two fold increase in eroded surface from 6.1 plus minus 2.0% to 13.2 plus minus 1.8% ( $p < 0.01$ ). Osteoblast numbers were also increased from 15.2 plus minus 4.0 mm<sup>2</sup> in WT to 37.5 plus minus 5.0 mm<sup>2</sup> in mutant ( $p < 0.01$ ). Studies in vitro showed a 8.6 plus minus 14.4% increase in RANKL-induced osteoclast formation in mutant mice compared with WT ( $p = 0.03$ ). There was no difference in osteoblast growth between genotypes, but alkaline phosphatase activity was significantly lower in mutant compared with WT osteoblasts (46.5 plus minus 16.9% reduction;  $p < 0.001$ ). Analysis using microCT identified lytic lesions in the femur of 20 month old mutant mice whereas similar lesions have not yet been identified in WT.

Histological analysis is currently in progress. Osteoarthritis of the knee was also more common in mutant mice; 9/16 (56%) vs. 4/12 (33%), but the difference was not significant.

We conclude that mice carrying a truncating mutation of SQSTM1 gene exhibit several features that are reminiscent of PDB including increased bone turnover, lytic lesions, and osteoarthritis. Further studies are in progress to conduct more detailed characterization of the skeletal phenotype and define the mechanisms by which this mutation regulates bone cell activity.

### OC9

#### TARGETING OSTEOCLASTS IN RHEUMATOID ARTHRITIS VIA THE ALPHA(V)BETA(3) INTEGRIN

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Rheumatoid arthritis (RA) is an inflammatory arthropathy that culminates in joint destruction caused by deregulated osteoclast activity. Central to osteoclast attachment to bone and resorption is the integrin molecule alpha(v)beta(3). alpha(v)beta(3) mediates cell adhesion to extracellular matrix and serum proteins by recognition of the common RGD sequence. Mice lacking the alpha(v)beta(3) integrin have dysfunctional osteoclasts and accumulate bone mass with age. Thus, reagents targeted against alpha(v)beta(3) are potential therapeutics in RA. By combining the powerful platform technology of Phage display and novel synovial homing research we hope to obtain high affinity reagents which can target osteoclast reorption in the rheumatoid joint.

A C7C peptide phage display library and a human scFV antibody library were used to target the alpha(v)beta(3) integrin in vitro. Novel reagents identified were screened for their affinity to bind alpha(v)beta(3) and their antiresorptive effects were assessed in vitro on osteoclasts derived from murine bone marrow (BM) and human PBMCs cultured in MCSF and RANKL. Peptides were assessed against a positive control, the RGD inhibitor Echistatin.

Peptide phage display identified phage clones with affinity for alpha(v)beta(3). One peptide, CSPRGDHPC, inhibited RGD-dependent cell binding to vitronectin. Treatment of murine BM cells with this peptide showed a dose-dependent decrease in the number of multinucleated TRAP positive cells and resorption pits with a maximally effective dose of 10uM. Equimolar doses of a control peptide CDRPHSGC had no significant effects. Echistatin inhibited both murine and human differentiation and activity, although a 5-10 fold higher dose was required to see an inhibition in the number of human osteoclasts as identified by actin and VNR staining and an inhibition of resorption. We are currently assessing the effects of the CSPRGDHPC peptide on human osteoclasts.

Phage display technology can identify novel reagents against alpha(v)beta(3) with the capacity to inhibit osteoclast resorption in vitro, thus providing novel reagents for targeted drug delivery to the rheumatoid joint. Work is now in progress to select novel high affinity human antibody fragments using a scFV antibody phage display library which will be tested in murine models of inflammatory arthritis such as CIA and TNF $\alpha$  transgenic mice.

### OC10

#### SUPPRESSOR OF CYTOKINE SIGNALLING-2 (SOCS-2) EXPRESSION IN THE GROWTH PLATE: MODULATION BY PRO-INFLAMMATORY CYTOKINES.

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Abnormal growth patterns are commonly observed in children suffering from chronic inflammatory diseases, which are associated with the increased production of pro-inflammatory cytokines. Suppressor of Cytokine Signalling-2 (SOCS-2) negatively regulates the signal transduction of several cytokines and this study examined the role of SOCS2 in regulating bone growth, and the response of growth plate chondrocytes to pro-inflammatory cytokines. Initial studies indicated that SOCS2 was expressed by both murine primary chondrocytes and the ATDC5 chondrogenic cell line. Foetal (E14-E18) and neonatal (1 and 9-day-old) primary murine chondrocyte expressed SOCS-2 mRNA at similar levels whereas SOCS-2 protein expression was greater (78%) during chondrogenesis than terminal differentiation. IL-1 $\beta$  and TNF $\alpha$  (both 10ng/ml) exposure for 48h increased SOCS-2 protein expression in primary 1-day-old murine chondrocytes (79% and 65% increase respectively). 7-week-old female mice lacking SOCS-2 (SOCS2<sup>-/-</sup>) expression were significantly heavier (26%;  $P < 0.0001$ ) and longer (6%;  $P < 0.001$ ) compared to wild-type (WT) mice. The tibiae of the null mice were longer (8%;  $P < 0.001$ ), wider (18%;  $P < 0.001$ ) and had wider growth plates (24%;  $P < 0.001$ ) than WT mice. Proliferative and hypertrophic zones were significantly wider in SOCS2<sup>-/-</sup> mice compared to WT mice (10% and 14% respectively;  $P < 0.001$ ). The growth of

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metatarsals from 1-day-old female SOCS2<sup>-/-</sup> and WT mice over 8-days was inhibited by TNF $\alpha$  (47% and 33% respectively; both  $p < 0.001$ ) and IL-1 $\beta$  (71% and 86% respectively; both  $p < 0.001$ ) by comparable amounts. No effect of genotype was observed. In contrast, matrix gene expression by primary chondrocytes exposed to pro-inflammatory cytokines over 48h was dependent upon the genotype of the host animal. RT-PCR studies revealed that aggrecan expression was notably reduced only in chondrocytes from SOCS2<sup>-/-</sup> exposed to IL-1 $\beta$  and TNF $\alpha$ . Collagen Type X expression was markedly reduced in chondrocytes from both SOCS2<sup>-/-</sup> and WT mice whereas collagen Type II expression was not altered by cytokine exposure. This is the first study to show the expression of SOCS-2 in growth plate chondrocytes and its dramatic effect on suppressing skeletal growth. IL-1 $\beta$  and TNF $\alpha$  may mediate their effects through SOCS-2, influencing the growth of children with inflammatory diseases through a local effect at the growth plate.

### OC11

#### AN ATYPICAL MUTATION IN THE ACTIVIN A RECEPTOR, TYPE 1 GENE (ACVR1) IN A SEVERELY AFFECTED FIBRODYSPLASIA OSSIFICANS PROGRESSIVA PATIENT

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Fibrodysplasia Ossificans Progressiva (FOP) is a rare, heritable condition typified by progression of extensive ossification within skeletal muscle, ligament and tendon together with defects in skeletal development. The condition is easily diagnosed by the presence of shortened great toes and there is severe advancement of disability with age. FOP has been shown recently to result from a point mutation (G617A) in ACVR1 in all patients reported (1). We present here evidence for a further mutation in this gene in a FOP patient with severe atypical digit deficiencies and other clinical features. DNA was prepared from blood samples from healthy controls, from those with clear clinical phenotypes of FOP and from the severely affected atypical patient. PCR products were directly sequenced and restriction fragment length polymorphism confirmed the sequencing results. The ACVR1 G617A mutation previously described was present in all patients with characteristic features of FOP but was not present in controls or in the severe atypical phenotype. In the latter, the novel heterozygous mutation, G328W, was found within the ACVR1 gene. ACVR1 is a member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) receptor superfamily of serine/threonine kinases. TGF- $\beta$  type I receptors can be regulated through the binding of FKBP12 to their glycine-serine (GS) activation domains. This inhibits activation of the receptor, blocking further cell signalling. The G617A mutation causes an arginine to histidine change within the GS domain and has been proposed to confer constitutive activation, possibly by preventing FKBP12 binding and inhibition. However, the G328W mutation causes an arginine to tryptophan change within the kinase domain of the protein. The presence of this large change in amino acid structure within the kinase domain of ACVR1 could account for changes in activity of this receptor and result in the more severe phenotype observed in this patient.

(1) Nature Genetics 38:525-527, 2006. EM Shore, M Xu, GJ Feldman, DA Fenstermacher, T-J Cho, IH Choi, JM Connor, P Delai, DL Glaser, M Le Merrer, R Morhart, JG Rogers, R Smith, JT Triffitt, JA Urtizberea, M Zasloff, MA Brown, FS Kaplan.

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### OC12

#### OSTEOCYTE SCLEROSTIN EXPRESSION: REGULATOR OF BMU BALANCE IN OSTEOARTHRITIS AND OSTEOPOROSIS?

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Osteocytic sclerostin downregulates osteoblastic bone formation. Osteoarthritis of the hip (OA) is associated with increased bone formation in the intracapsular femoral neck while osteoporosis and subsequent femoral neck fracture (FNF) are associated with an inappropriate osteoblast response to increased bone resorption. To investigate whether sclerostin expression might mediate these disease effects, we analysed osteocytic expression in osteonal bone

multicellular units (BMUs) within the femoral neck cortex of patients with hip OA (5M, 5F; 49-92y), or FNF (5M, 5F; 73-87y) and post-mortem (PM) controls (5M, 6F; 61-90y). Sclerostin expression in individual osteocytes as evidenced by immunocytochemistry was assessed in the bone between the cement line and the canal surface in 623 BMUs. Adjacent sections were reacted for alkaline phosphatase activity (ALP) and each BMU classified as quiescent (no ALP), low bone formation (low ALP) or high (high ALP). Data are presented as the %osteocytes expressing sclerostin (scl+) and the mean distance of scl+ osteocytes from the canal surface. Sclerostin expression differed between BMUs with different levels of bone formation (quiescent: 90.0  $\pm$  1.5% (SE); low: 77.2  $\pm$  2.5%; high: 51.7  $\pm$  2.8%;  $p < 0.0001$ ) and between the 3 disease groups (OA: 67.5  $\pm$  2.2%; FNF: 76.5  $\pm$  2.1%, PM: 75.1  $\pm$  2.7%;  $p = 0.007$ ). This effect was most evident in the BMUs with high ALP (OA: 38.9  $\pm$  4.0%; FNF: 63.6  $\pm$  4.1%; PM: 52.6  $\pm$  6.0%;  $p = 0.007$ ). Forming BMUs were thicker or more mature in both OA and FNF, as evidenced by greater mean distances of scl+ osteocytes from canal surfaces (respectively +14%, +7%; Dunnett's test  $P < 0.0001$ ) but this did not explain the much greater distances (+56%, +42%  $P < 0.0001$ ) for scl- osteocytes. In logistic modelling, high ALP activity at BMU level was strongly dependent on the fraction of osteocytes sclerostin negative, with a significantly larger effect in OA and a smaller effect in FNF than in controls ( $P$  all  $< 0.0001$ ). Osteocyte distance from osteoid surface had little influence on this sclerostin effect. In conclusion, sclerostin expression is an important statistical determinant of mineralizing activity at BMU level and by regulating BMU balance might influence bone tissue loss or gain in disease.

### OC13

#### INTERROGATING THE MECHANISMS CONTROLLING OSTEOCYTOGENESIS

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Osteocytes, the most numerous cells in bone, appear to serve pivotal mechanomodulatory roles in directing bone remodelling in response to load-bearing. Despite this pivotal contribution, the genes controlling osteocyte formation from their osteoblast precursors (osteoctogenesis) remain largely undefined. To study the temporal expression pattern of a number of candidate genes associated with osteocytogenesis we have utilised the MLO-A5 cell line which has been shown to represent the post-osteoblast/pre-osteocyte phenotype (Kato et al. JBMR 2001). Such an approach will allow us to establish a hierarchical expression pattern of regulatory genes. We have used immunoblotting to monitor expression of five specific markers: E11, tissue non-specific alkaline phosphatase (TNAP), membrane-type 1 matrix metalloproteinase (MT1-MMP), MMP2 and sclerostin in MLO-A5 cells during a 12-day culture period and used Alizarin red staining to confirm mineralisation. For comparison, MLO-Y4 and MC3T3 cells were used as representative of the osteocyte and undifferentiated osteoblast phenotype, respectively. Our findings showed that E11, a recognised osteocyte marker, was predictably expressed by MLO-Y4 but not MC3T3 cells. Similarly, sclerostin and MT1-MMP were only expressed in MLO-Y4 cells, whereas expression of MMP2 and TNAP was observed in both. Consistent with transition between the post-osteoblast and pre-osteocyte phenotypes, E11 initially showed low expression levels on day-2 of culture (i.e. before mineralisation) that increased markedly in the MLO-A5 cells at later times. MMP2 expression increased over time in MLO-A5 cells and MT1-MMP expression was only apparent after day 10 (i.e. after onset of mineralisation). In contrast, expression of sclerostin and TNAP was seen at all stages. Our findings show that markers associated with the development of dendritic processes, namely E11, MT1-MMP and MMP2 increase in their expression during osteoblast-osteocyte transition, with E11 expression foremost during early osteocyte specification. Expression of sclerostin by MLO-A5 cells was unexpected as it is considered a characteristic marker of mature osteocytes and suggests a marked divergence between MLO-A5 cells and primary osteoblasts. Our finding that MLO-A5 cells retain high TNAP expression whilst they undergo overt osteocytogenesis suggests that down-regulation of TNAP is not key during this process. Rather, regulation of E11 production may be the primary driver of osteocytogenesis.



### OC14

#### **SPONDYLOEPHYPHYSEAL DYSPLASIA TARDA (SED)-ASSOCIATED SEDLIN MUTATIONS DISRUPT INTERACTIONS WITH C-MYC PROMOTER-BINDING PROTEIN 1 (MBP-1), PITUITARY HOMEBOX 1 (PITX1) AND STEROIDOGENIC FACTOR 1 (SF1)**

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Spondyloepiphyseal dysplasia tarda (SED) is an X-linked progressive osteochondrodysplasia that manifests at 10-14 years of age and is noticeable by a disproportionate short stature and barrel chest. Moderate epiphyseal dysplasia of the long bones, which may be associated with osteoarthritis of the femoral heads, often results in hip replacement before the age of 40 years. SED is caused by mutations of the SEDL gene, which is localised to Xp22, and encodes a 140 amino acid protein designated Sedlin. Sedlin interacts with the nuclear transcription factors c-myc promoter-binding protein 1 (MBP-1), pituitary homeobox 1 (Pitx1) and steroidogenic factor 1 (SF1). We have therefore investigated the hypothesis that SED-associated mutations of Sedlin may disrupt the interaction between Sedlin, MBP-1, Pitx1 and SF1.

Full-length Sedlin, MBP-1, Pitx1 and SF1 constructs tagged with cMyc were generated, together with a full-length HA-tagged Sedlin construct into which the SED mutations Asp47Tyr, Ser73Leu, Phe83Ser, Val130Asp and Gln131Stop were introduced by site-directed mutagenesis. These constructs were co-transfected into COS7 cells and cell lysates prepared for co-immunoprecipitation assays. These confirmed the interaction between Sedlin, MBP-1, Pitx1 and SF1, but revealed that the SED mutations did not disrupt the interactions. However, Sedlin was found to co-immunoprecipitate itself indicating that it may form a homodimer and thereby mask the effects of the SED mutations in these assays. The use of native gel electrophoresis demonstrated homodimerization of Sedlin, and the effects of the SED mutations were therefore investigated in a yeast two-hybrid system, as yeast does not endogenously express Sedlin. This revealed that all the SED mutations, except Asp47Tyr, lead to a loss of interaction with MBP-1, Pitx1 and SF1. Three-dimensional modelling studies of Sedlin revealed that Asp47Tyr resides on the surface whereas all the other mutant residues lie within the hydrophobic core of the protein, and hence are likely to affect the correct folding of Sedlin and thereby disrupt protein-protein interactions.

Thus, our studies are the first to demonstrate that Sedlin forms homodimers and that SED mutations cause a loss of interaction with MBP-1, Pitx1 and SF1, which may contribute developmental anomalies associated with SED.

### OC15

#### **WNT SIGNALLING UPREGULATES OSTEOBLAST DIFFERENTIATION IN ZEBRAFISH: A NOVEL MODEL FOR STUDIES IN OSTEOBLASTOGENESIS**

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Osteoporosis and cancer-induced bone diseases are associated with abnormal regulation of bone turnover. However, the signalling mechanisms involved in the regulation of bone turnover remain poorly defined. One pathway that has been implicated in osteoblast (OB) differentiation is the canonical Wnt signalling system. Wnts are a family of secreted proteins, which transduce signals via the receptor Frizzled and the coreceptor LRP5/6. Inhibitors of Wnt signalling block the formation of the Wnt/Frizzled/LRP complex. Wnt inhibitors, such as Dickkopf-1 (Dkk-1), have been implicated in regulating OB differentiation and may contribute to the development of bone disease; however, there are few whole organism models available to study these pathways. The aims of this study were to develop the zebrafish as a model of osteoblastogenesis and to determine the roles of canonical Wnt signalling and Dkk-1 in OB formation.

Osteoblastogenesis was followed spatially and temporally by examining the expression of Runx2, Osterix (Osx), Osteonectin (ON), and Collagen-I (Col-1) in zebrafish by in situ hybridisation. Mineralisation was visualised and characterised using wholemount alizarin red, calcein and von Kossa staining. Osx positive cells were first observed in the cleithrum (clavicle) at 36 hours post fertilisation

(hpf) and this was associated with new bone formation at 72hpf. Expression of Col-1 and ON was observed subsequently in other craniofacial bones. Mineralisation of each craniofacial element was observed between 72hpf and 10 days post fertilisation (dpf). Zebrafish were engineered to express Wnt-8 or Dkk-1 under the control of a heat-shock promoter. An IRES GFP was also expressed by the activated heat-shock promoter allowing easy sorting of positive transgenics. Increasing ambient temperature for 1 hour activated the heat-shock promoter, which was associated with increased GFP and either Wnt-8 or Dkk-1 expression. Elevated Dkk-1 from a single hour-long heat-shock at 48hpf caused a rapid (4hr) decrease in expression of Osx. In contrast, elevated Wnt-8 increased Osx expression. These data suggest that Wnt signalling promotes early OB differentiation and highlight the value of the zebrafish as a model of osteoblastogenesis.

### OC16

#### **THE LOCAL AND SYSTEMIC EFFECTS OF GLUCOCORTICOID GENERATION IN SYNOVIAL TISSUE**

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Therapeutic glucocorticoids are used in rheumatoid arthritis (RA) to reduce inflammation and bone destruction. We recently reported that primary synovial fibroblasts generate active glucocorticoids via expression of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1). This enzyme activates cortisol from inactive cortisone (and prednisolone from prednisone) and this activity is up-regulated by inflammation. We have now examined glucocorticoid metabolism in synovial tissue explants from patients with osteoarthritis (OA) and RA to determine whether synovial tissue metabolises glucocorticoids and to identify the local and systemic consequences of this activity.

Glucocorticoid metabolism was examined in synovial tissue taken from subjects with OA (n=8) or RA (n=12) during orthopaedic surgery using radiolabelled steroids and TLC. Immunohistochemistry and RT-PCR were used to identify the cellular distribution of enzymes. The functional consequences of enzyme activity on tissue IL-6 production were examined by ELISA. Systemic corticosteroid metabolism was examined in 31 RA patients and 9 controls using GC/MS.

All synovial biopsies had substantial capacity to activate glucocorticoids confirming 11 $\beta$ -HSD1 expression. In samples from patients with RA, synovial cortisol generation increased with the ESR of the tissue donor (r<sup>2</sup>=0.40). 11 $\beta$ -HSD1 activity had functional consequences with cortisone able to decrease synovial IL-6 production (36+9% OA; 31+15% RA; both p<0.05). Steroid inactivation was also apparent in synovium suggesting the presence of 11 $\beta$ -HSD2 and expression was localised to macrophages by immunohistochemistry. Synovial fluid analysis demonstrated lower (63%, p<0.05) cortisone levels compared to matched serum samples indicating net 11 $\beta$ -HSD1 activity. Urinary measures of total 11 $\beta$ -HSD1/2 activity demonstrated high levels of 11 $\beta$ -HSD1 activity in untreated RA patients compared to controls. In RA patients there was a significant correlation between urinary 11 $\beta$ -HSD1 activity and ESR (r<sup>2</sup>=0.16).

Synovial tissue metabolises glucocorticoids with the predominant effect glucocorticoid activation and this increases with inflammation. Endogenous glucocorticoid production in the joint is likely to have important consequences on inflammation and bone integrity. Importantly, high glucocorticoid levels would not impact on cells expressing 11 $\beta$ -HSD2 and this may contribute to persistence of inflammation and consequent bone destruction.

### OC17

#### **AUTO-INDUCTION OF IL-1 $\beta$ AND LOSS OF PROMOTER METHYLATION: A POSSIBLE EXPLANATION OF THE UNREMITTING PROGRESSION OF OSTEOARTHRITIS?**

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Osteoarthritis (OA) is characterised by progressive erosion of articular cartilage, which, once started, cannot be halted. The breakdown of cartilage is mediated by proteases, including MMP-3 and -13. These may initially be derived from the synovium but are also produced by OA chondrocytes, particularly in later stages of the disease. In normal articular chondrocytes, the proteases are silenced by DNA

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methylation at so-called CpG sites. In osteoarthritis, chondrocytes produce the enzymes and stably transmit the abnormal gene expression to daughter cells. This aberrant expression has been shown to be associated with an epigenetic 'un-silencing' via demethylation of specific CpG sites within the relevant promoter regions (Arthritis & Rheumatism 52:3110-24). Why and how this demethylation takes place is largely unknown.

The pro-inflammatory cytokine IL-1 $\beta$  is of potential importance in OA, since the cytokine upregulates MMPs in articular chondrocytes in vitro and synovitis could provide IL-1 $\beta$  in vivo. We investigated whether the IL-1 mediated induction of MMPs was associated with DNA demethylation, which might explain the progressive nature of OA. Chondrocytes were isolated from non-OA articular cartilage and grown in monolayer culture. The cells from each patient were divided into no-treatment versus IL-1 $\beta$  treated groups. When confluent, simultaneous RNA and DNA extraction was carried out. Expression levels were analysed by RT-PCR and the methylation status of specific CpG sites within the promoters of MMP-3, -13, and IL-1 $\beta$  was determined in the same samples, using the methylation-sensitive restriction enzymes and PCR. The pre-culture controls expressed type II collagen and low levels of MMP-3. All IL-1 treated samples expressed high levels of MMP-3, -13, and, surprisingly, IL-1 $\beta$  itself. The large increases in MMP-3 and IL-1 $\beta$  were associated with loss of methylation at specific CpG sites in the promoter of these mediators. The strongest correlation was seen between IL-1 $\beta$  expression and promoter demethylation. IL-1 $\beta$  thus induced its own expression, which corresponded to promoter demethylation at one specific CpG site. If the same applies in vivo, this data suggests that an initial inflammatory episode could set up an autocrine stimulatory loop as well as epigenetic changes. This may explain the unremitting progression of OA.

### OC18

#### IDENTIFICATION OF LIPOCALIN 2, A NOVEL GLUCOCORTICOID RESPONSIVE GENE IN GROWTH PLATE CHONDROCYTES

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Glucocorticoids (GC) are commonly used anti-inflammatory and immunosuppressive drugs. Long-term use can, however, result in marked growth retardation in children due to their actions on chondrocytes in the growth plate. To gain an insight into the mechanisms involved in GC-induced growth retardation, we performed Affymetrix microarray analysis of the murine chondrogenic cell line ATDC5, incubated with 10<sup>-6</sup>M dexamethasone (Dex) for 24h. Differential expression of selected genes was confirmed by qRT-PCR. Genes confirmed as down-regulated included Secreted frizzled-related protein and IGF-I, whilst upregulated genes included Serum/GC-regulated kinase, Connective tissue growth factor, and Lipocalin-2. Lipocalin-2 is an acute phase transport protein which we have shown is expressed in proliferative chondrocytes within the growth plate. In this study, qRT-PCR analysis confirmed that Lipocalin-2 gene expression increased in ATDC5 cells by 40-fold after 24h Dex. Expression further increased after 48h (75-fold) and 96h (84-fold) Dex and this response was Dex concentration-dependent. Western blotting confirmed that Dex also increased Lipocalin 2 protein expression. The Lipocalin-2 response was not blocked by cycloheximide or the p38 inhibitor SB203580, but was blocked by the GC-receptor antagonist RU-486 and the Nuclear-Factor kappaB (NFkB) inhibitor TLCK. The lack of a cycloheximide effect implies a direct action of Dex on Lipocalin-2 expression, which is consistent with the presence of a glucocorticoid response-element on the Lipocalin-2 promoter. Dex also caused an increase in Lipocalin-2 expression in primary murine chondrocytes at 48h (1.7-fold,  $p < 0.05$ ), and this was maintained for up to 72h. Proliferation in ATDC5 cells stably transfected to overexpress Lipocalin-2 was slower than control cells (49.1%,  $p < 0.05$ ) and Lipocalin-2 overexpression caused an increase in proteoglycans (65.9%,  $p < 0.05$ ) and collagen type-X expression (3.6-fold,  $p < 0.05$ ). Alkaline phosphatase activity and expression was unaffected. The effects of Lipocalin-2 overexpression on chondrocyte proliferation (63.9%,  $p < 0.05$ ) and collagen type-X expression (7.8-fold,  $p < 0.05$ ) were further exacerbated with the addition of 10<sup>-6</sup>M Dex. This synergistic effect may be explained by a further increase in Lipocalin-2 expression with 10<sup>-6</sup>M Dex in transfected cells (44.6%,  $p < 0.05$ ). These results suggest that Lipocalin-2 may mediate Dex effects on chondrocytes through an NFkB-dependent pathway, and therefore provides a potential novel mechanism for GC-induced growth retardation.

### OC19

#### CHILDHOOD PHYSICAL ACTIVITY IS ASSOCIATED WITH BONE MASS AT 4 YEARS

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Physical inactivity is an increasing problem amongst children. In addition to concerns regarding resulting obesity, the secular decrease in load-bearing activity may be associated with reduced accrual of bone mineral to peak, and thus increased risk of osteoporotic fracture in older age. In this study we utilised an ongoing longitudinal study of mothers and their children (Southampton Women's Survey) to examine the cross sectional relationship between childhood physical activity and contemporary bone mineral.

Children were recruited at 4 years old from the Southampton Women's Survey. They attended the Osteoporosis Centre at Southampton General Hospital for measurement of bone mass at whole body, lumbar spine and hip sites, together with assessment of diet, lifestyle, health and medications. At the end of the visit the children were fitted with an Actiheart combined accelerometer and heart monitor (Cambridge Neurotechnology Ltd, Cambridge, UK), which was to be worn for 7 days continuously. At the end of this period the monitor was posted back and correlation techniques were used to compare bone mass and measures of physical activity.

81 children (49 boys) took part. The mean (sd) age was 4.1 (0.1) years. They were all healthy term deliveries. There were strongly statistically significant associations between total daily energy expenditure adjusted for body mass index, and each of WB BA ( $r = 0.31$ ,  $p = 0.0045$ ), BMC ( $r = 0.32$ ,  $p = 0.0037$ ), BMD ( $r = 0.26$ ,  $p = 0.019$ ), lumbar spine BA ( $r = 0.22$ ,  $p = 0.049$ ) and BMC ( $r = 0.23$ ,  $p = 0.037$ ), and total hip BA ( $r = 0.31$ ,  $p = 0.0058$ ) and BMC ( $r = 0.29$ ,  $p = 0.010$ ). These associations were attenuated when energy expenditure was adjusted for weight or height alone.

In conclusion, levels of overall physical activity were positively associated with contemporary bone mineral, but the relationships were attenuated after adjustment for current body size. These results suggest that improving levels of physical activity in childhood may help to optimise accrual of bone mass, but further work examining the relationship between type of activity and site of effect is required.

### OC20

#### BODY MASS INDEX IS MORE PREDICTIVE OF THE OSTEOGENIC POTENTIAL OF BONE MARROW STROMAL CELLS THAN AGE IN MALES

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**Background & Objectives:** Mesenchymal stem cells (MSCs) reside in adult bone marrow. In vitro these cells have been shown to be multipotent and differentiate into many cell types including osteoblasts. Numerous studies have examined the ability of MSCs to aid regeneration and repair in animal models of bone defects and fracture repair and, clinically, many surgeons routinely use autologous bone marrow as part of their graft procedures. It is generally accepted that the proliferation, differentiation and migration of cells decreases with age, but there is controversy over whether the stem cell and osteoprogenitor population are similarly affected. To investigate this, we examined changes in osteogenic capacity of human bone marrow cells with two potential predictors-age and BMI.

**Methods:** Bone marrow samples were obtained with consent from 38 patients (22F & 16M; age range: 28-91) undergoing elective primary hip replacement at Musgrave Park Hospital. Patients with underlying disorders of bone metabolism, or those taking bisphosphonates or statins were excluded. 6mls bone marrow aspirate was collected from the femoral canal during surgery. Outcome measures were - total nucleated cell count, colony forming efficiency at d14, d17 and d21, alkaline phosphatase expression and expression of stem cell markers determined by flow cytometry, specifically percentage of cells in the CD34<sup>-</sup>, CD45<sup>-</sup>, CD105<sup>+</sup>, CD29<sup>+</sup>, CD44<sup>+</sup>, CD73<sup>+</sup> fraction.

**Results:** At all time points, there was a non-significant negative correlation between age and colony forming efficiency and stem cell



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marker expression. BMI had a much stronger positive relationship with many of the outcome measures. It correlated strongly with measures of colony area in males only (correlations between total colony area and BMI displayed p values of <0.001, 0.006 and 0.006 at day 14, 17 and 21 respectively). Regression analysis demonstrated that changes in BMI accounted for 60% of the variation in mean colony area in the male study population.

Conclusion: This study found no significant relationship between age and osteogenic potential in BMSCs for either gender. However, a gender-specific correlation with BMI was seen. BMI was highly predictive of CFU-F area measurements for males, suggesting an increase in the stem cell population in bone marrow with increasing BMI for this gender.

### OC21

#### GENETIC MANIPULATION OF HUMAN MESENCHYMAL PROGENITORS TO PROMOTE CHONDROGENESIS WITHIN POLYSACCHARIDE TEMPLATES

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Articular cartilage injury does not result in spontaneous repair due to the avascular, aneural and alymphatic nature of the tissue. Given the paucity of clinically viable cartilage formation regimes we have examined the potential of using a non-viral gene delivery and tissue engineering approach, whereby Sox-9 transduced human mesenchymal progenitors have been encapsulated within alginate/chitosan polysaccharide capsules and cultured in vitro and in vivo to promote chondrogenesis.

Human bone marrow stromal cells and articular chondrocytes were transduced using Amaxa Nucleofector technology with flag-tagged Sox-9 plasmid and encapsulated within alginate/chitosan templates containing TGF-beta-3 to promote chondrogenesis. Samples were also encapsulated with un-transfected cells and cells transfected with the empty vector pcDNA. Constructs were subsequently placed into Synthecon rotating-wall bioreactors or held in static conditions for up to 28 days. In addition, samples were examined in vivo using the sub-cutaneous implant model in SCID mice.

Successful Sox-9 transfection was demonstrated after 24 hours by western blot analysis using antibodies for anti-flag-Sox-9 and anti-Sox-9. After 7 days in static and bioreactor culture, regions of cell-generated matrix containing cartilage proteoglycans were demonstrated surrounding viable cells in Sox-9 transduced human bone marrow cells and articular chondrocytes samples, as demonstrated by alcian blue staining and Sox-9 immunohistochemistry. Further, after 28 days, in vitro and in vivo, samples encapsulated with Sox-9 transduced cells demonstrated large regions of cartilaginous matrix that comprised approximately 25% of the construct, as confirmed by positive alcian blue staining, Sox-9 and type-II collagen immunohistochemistry, which was absent in samples encapsulated with un-transfected cells. Extracted protein from in vivo constructs was further assessed by western blot analysis and positive expression of Sox-9 and type-II collagen was observed in Sox-9 transduced constructs which was absent in un-transfected cells.

These studies outline a tissue engineering approach utilizing a non-viral gene delivery method coupled with polysaccharide templates to promote cartilaginous construct formation. Overall, the regions of cartilage-like matrix were significantly increased in Sox-9 constructs in comparison with constructs encapsulated with un-transfected cells, confirming the presence of Sox-9 transduced cells promotes chondrogenesis. This has significant implications for skeletal regeneration and, ultimately, future therapies for human articular cartilage defects.

### OC22

#### A SOLUBLE ACTIVIN TYPE II RECEPTOR PREVENTS MYELOMA BONE DISEASE

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Multiple myeloma is associated with the development of bone disease characterised by increased osteoclast activity and a suppression of osteoblastic bone formation. Our understanding of the molecular

mechanisms responsible for increased osteoclastic activity has improved; however, our knowledge of the mechanism responsible for inhibiting bone formation remains poor. Equally, the effect of targeting bone formation rather than resorption is unknown. Recently, an antagonist of activin, a soluble form of the extra-cellular domain of the murine activin type II receptor, fused to a murine IgG-Fc fragment, (RAP-011) was shown to reverse ovariectomy-induced bone loss in vivo; however, the effect of this antagonist on myeloma bone disease is unknown. In the present study we investigated whether decreased bone formation contributes to the bone disease and whether RAP-011 prevents bone disease in the 5T2MM model of myeloma.

5T2MM cells injected into C57Bl/KaLwRij mice promoted a significant increase in osteoclast surface, the formation of osteolytic lesions and caused a significant decrease in bone area. Bone disease was associated with a decrease in osteoblast number (p<0.001), osteoblast surface (p<0.001) and a reduction in mineralization (p<0.01). Mice bearing 5T2MM cells were then treated with RAP-011 (10mg/kg, i.p. twice weekly), or a vehicle, from the time of 5T2MM injection, for a total of 12 weeks. MicroCT analysis of the proximal tibia and lumbar vertebrae demonstrated a 39% and 21% reduction in cancellous bone volume (p<0.001 and p<0.01) and a 37% and 15% reduction in trabecular number (p<0.01 and p<0.05) in 5T2MM-bearing mice compared to naive mice. RAP-011 completely prevented 5T2MM-induced decreases in trabecular volume and number in both tibia (p<0.001 and p<0.05) and vertebrae (p<0.01 and p<0.05) when compared to vehicle treated mice. Bone volume was 19% higher in the tibia (p=168) and 12% higher in vertebrae (p<0.05) of RAP-011 treated mice than naive non tumour bearing mice. RAP-011 prevented the development of osteolytic bone lesions (p<0.05), but had no effect on serum paraprotein or myeloma burden.

These data suggest that the soluble activin type II receptor construct, RAP-011, prevents the development of osteolytic disease and represents a novel therapeutic approach to treating myeloma bone disease.

### OC23

#### HEIGHT LOSS PREDICTS FRACTURES IN MIDDLE AGED AND OLDER MEN AND WOMEN: THE EPIC-NORFOLK PROSPECTIVE POPULATION STUDY

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With effective interventions available for fracture prevention, a major clinical issue is identification of individuals at greatest fracture risk for preventive interventions. Height loss among middle aged men and women can be easily measured in outpatient clinics and may contribute to fracture risk prediction. We aimed to assess measured height loss and fracture incidence in a prospective observational population study.

Height was measured in men and women in the Norfolk cohort of the European Prospective Investigation into Cancer (EPIC-Norfolk) between 1993 and 1997 and was repeated between 1997 and 2000. Incident fractures to 2006 were ascertained by hospital record linkage. Height loss and known risk factors of fracture were entered into Cox proportional hazards models to determine their independent contribution to the risk of fracture.

In 14,921 men and women aged 42-82 years, during follow-up period of 7.1 (SD 0.7) years, there were 390 fractures, including 122 hip fractures. Annual height loss in the group of fracture sufferers (1.8 mm, SD 0.3) was significantly greater than other participants (0.9 mm, SD 0.2; p<0.001). Participants with annual height loss >0.5 cm had an age and sex adjusted hazard ratio of any fracture of 1.76 (95%CI 1.16-2.67) and of hip fracture of 2.08 (95%CI 1.07-4.05) compared to those with no height loss. In multivariate models, 1 cm of height loss per year was associated with a hazard ratio of 1.86 (95% CI 1.28-2.72) for all fractures and 2.24 (95%CI 1.23-4.09) for hip fracture independent of age, sex, past history of fracture, smoking, body mass index, alcohol intake, and heel ultrasound measures. Annual height loss of 1 cm was comparable to having a past history of fracture and equivalent to being about 14 years older

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in chronological age in terms of magnitude of relationship with fracture risk.

Middle aged and older men and women with annual height loss >0.5 cm are at increased risk of hip and total fracture. Serial height measurements can easily be incorporated in routine clinical practice and may contribute to the development of practical fracture risk assessment tools.

### OC24

#### AUTOLOGOUS CONDITIONED SERUM (ACS) COMPARED TO HYALURONAN AND SALINE-INJECTIONS FOR THE TREATMENT OF KNEE OSTEOARTHRITIS

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**Background:** A new therapy, based on the intra-articular injection of autologous conditioned serum (ACS), is used in several European countries for osteoarthritis (OA) treatment. ACS is generated by incubating venous blood with medical grade glass beads. Peripheral blood leukocytes produce elevated amounts of endogenous anti-inflammatory cytokines such as interleukin-1 receptor antagonist (IL-1Ra) and growth factors that are recovered in the serum(1). ACS has been shown to improve the clinical lameness in horses significantly to enhance the healing of muscle injuries in animal models, and in human athletes. In the present study, the efficacy and safety of ACS was compared to intra-articular hyaluronan (HA), and saline in patients with confirmed knee OA.

**Methods:** In a prospective, randomized, patient- and observer-blind trial with three parallel groups, 376 patients with knee OA were included in an intention to treat (ITT-) analysis. Efficacy was assessed by patient-administered outcome instruments (WOMAC, VAS, SF-8, GPA) after 7, 13 and 26 weeks. The frequency and severity of adverse events were used as safety parameters.

**Results:** In all treatment groups, intra-articular injections produced a significant reduction in WOMAC-scores and weight-bearing pain (VAS). However, responses to ACS were far stronger. The superiority of ACS and either HA or saline was statistically significant for all outcome measures and all time points. No significant differences between HA treatment and saline injections ( $p>0.05$ , at all time points and all outcome measures) were recorded. Frequency of adverse events (AE) was comparable in the ACS- and the saline-group ( $p>0.05$ ).

**Conclusion:** The results demonstrate that ACS is effective and well tolerated in the management of chronic, idiopathic OA of the knee. So far, the efficacy of ACS is defined through improvement in clinical signs and symptoms, particularly pain. It remains to be determined whether they are disease-modifying, chondroprotective, or even chondroregenerative, sequelae.

**References:** 1.Meijer H, Reinecke J, Becker C, Tholen G, Wehling P. The production of anti-inflammatory cytokines in whole blood by physico-chemical induction. *Inflamm Res* 2003;52(10):404-7.

### OC25

#### AN INTERNATIONAL MULTICENTER RANDOMIZED COMPARISON OF BALLOON KYPHOPLASTY AND NONSURGICAL CARE IN PATIENTS WITH ACUTE VERTEBRAL BODY COMPRESSION FRACTURES

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**BACKGROUND** Balloon kyphoplasty is a minimally invasive surgery which aims to repair vertebral body compression fractures by expanding them and filling them with bone cement. The effect of balloon kyphoplasty on quality of life has not been tested in a randomized trial.

**METHODS** Patients with 1-3 non-traumatic vertebral compression fractures diagnosed within 3 months were randomly assigned to receive either balloon kyphoplasty (N=149) or usual nonsurgical care (N=151). Measurements of physical quality of life, back pain and function, and days of disability and bed rest were assessed at baseline and one month in the nonsurgical control group and one month after surgery in the balloon kyphoplasty group.

**RESULTS** Compared with the one-month changes in the nonsurgical group, participants assigned to receive balloon kyphoplasty had greater improvement in the physical component summary of the SF-36 questionnaire, (5.7 points, 95% confidence interval, 3.7 to 7.8;  $p<0.0001$ ), total EQ-5D score (0.17 points, 95% CI 0.7 to 0.27;  $p=0.0011$ ), Roland-Morris Disability (3.9 points; 95% CI 2.5 to 5.2;  $p<0.0001$ ) and ratings of pain (1.9 points, 95% CI 1.3 to 2.5;  $p<0.0001$ ). There was no difference between groups in the number of serious adverse events. There was one device-related (soft tissue hematoma), one procedure-related (postoperative urinary tract infection) and no bone cement-related serious adverse events.

**CONCLUSIONS** Compared to nonsurgical treatment, balloon kyphoplasty resulted in improved quality of life and reduced back pain and disability at one month after treatment (Clinicaltrials.gov number, NCT00211211).

### OC26

#### MUSCLE FUNCTION AND THE EFFECTS OF HYPOVITAMINOSIS D IN POST-MENARCHAL FEMALES

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Myopathy is the major clinical symptom of hypovitaminosis D, yet it has not been widely studied. We hypothesised that baseline serum 25-hydroxyvitamin D concentration (25(OH)D; a measure of an individual's vitamin D status) would be positively related to muscle power measured using the Leonardo jumping mechanography device(JM; Novotec Medical, Pforzheim, Germany). JM is designed to measure muscle force, velocity and power by from an individual's ground reaction forces.

Ninety-nine post-menarchal 12-14 year olds participated in the study. Height, weight and serum concentrations of 25(OH)D, parathormone (PTH) and calcium were measured. Each participant performed a counter movement jump with freely moving arms on the Leonardo ground reaction force platform. Instantaneous power (W) is measured from the force (N) and velocity (m/s) of jump. Each girl performed 3 maximal jumps and the jump with greatest height was taken for assessment.

Seventy-two out of 99 girls had suboptimal body stores of vitamin D with serum 25(OH)D concentration <15 ng/ml. Pearson's correlation coefficients were calculated to determine relationship between weight, height, serum 25(OH)D, PTH concentrations and jumping variables (muscle power, velocity of jump, height of jump). Data are given as correlation co-efficients and p values. Analysis of covariance was used to adjust for weight and test the effect of 25(OH)D upon muscle function.

There were positive correlations between 25(OH)D and jump height (0.28,  $p<0.001$ ), velocity (0.31,  $p<0.001$ ) and power (0.22,  $p<0.05$ ). Weight was negatively correlated to all parameters (jump height - 0.32,  $p<0.001$ , velocity -0.44,  $p<0.001$ , power -0.56,  $p<0.001$ ). Height was positively correlated to jumping height (0.26,  $P<0.05$ ) and power (0.44,  $p<0.001$ ). No correlations were found with PTH.

After correction for weight, there was a significant effect of 25(OH)D on velocity ( $p=0.003$ ), height ( $p=0.007$ ) and power ( $p=0.001$ ); PTH was of borderline significance for power ( $p=0.066$ ).

In conclusion, vitamin D status significantly affects muscle power through alteration in the velocity and height of the jump (probably through its effects on Type II muscle fibres); an effect of PTH was not clearly demonstrated. These effects persist after correction for body weight. These data highlight the importance of vitamin D status on muscle function in adolescent girls.

### OC27

#### EFFECT OF THE DUAL-SPECIFIC SRC/ABL KINASE INHIBITOR AZD0530 ON BONE TURNOVER IN PATIENTS WITH ADVANCED SOLID MALIGNANCIES

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AZD0530 is a novel, orally potent, once-daily, highly selective and dual-specific Src/Abl kinase inhibitor with potential for activity in a

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wide range of tumours. We have previously shown significant AZD0530 dose-dependent reduction in bone resorption in healthy males. The current Phase I study assesses the effect of AZD0530 on bone turnover in adult patients with a range of advanced malignancies refractory to standard therapy.

Patients were randomized into three parallel groups to receive AZD0530 50, 125 or 175 mg per day. After a single dose on day 1 and 6 days washout, patients received once-daily doses for 21 days. After overnight fast, serum and second-morning void urine were collected on day 1 predose and on days 2, 3, 17 and 28. Markers of bone resorption were serum cross-linked C-telopeptide of type I collagen (sCTX) and urinary cross-linked N-telopeptide expressed as a ratio to urinary creatinine (uNTX). An analysis of covariance model was fitted to the log-transformed baseline-scaled ratio data at day 28, with treatment as a fixed effect factor and log-transformed baseline (predose day 1) as a covariate.

There were significant reductions in sCTX in all treatment groups and in uNTX at 125 and 175 mg. At day 28, percent changes from baseline, derived from the adjusted geometric mean baseline-scaled ratios, for sCTX were -36.3% (95%CI=-57.6%,-4.4%; p=0.030;n=12), -61.7% (95%CI=-73.6%,-44.5%; p<0.001;n=14) and -74.5% (95%CI=-83.3%,-61.3%; p<0.001;n=11), in the 50, 125 and 175 mg treatment groups, respectively; equivalent values for uNTX were -12.7% (95%CI=-32.6%,13.1%; p=0.293;n=12), -48.3% (95%CI=-59.2%,-34.4%; p<0.001;n=14) and -50.1% (95%CI=-61.8%,-34.7%; p<0.001;n=11). There was a dose-response trend for reductions in biomarkers.

The significant reductions in the biomarkers are consistent with inhibition of osteoclast-mediated bone resorption via suppression of Src kinase activity. The >60% reduction in sCTX at the two highest doses of AZD0530 is similar to reductions reported in studies of bisphosphonate therapy. This suggests that AZD0530 may have therapeutic benefit in treating osteoclast-driven metastatic bone disease in addition to its inhibitory activity on Src within tumour cells. The trend for dose-dependent changes in biomarkers indicates that the maximum tolerated dose should be taken forward in further development.

## Abstracts - Oral Posters

### OP1

#### SEGMENTAL BONE REGENERATION USING VASCULAR ENDOTHELIAL GROWTH FACTOR ENCAPSULATED POLY D,L-LACTIC ACID SCAFFOLDS AND HUMAN BONE MARROW STROMAL CELLS

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Angiogenic factors such as vascular endothelial growth factor (VEGF) along with osteogenic factors play a prominent role in bone formation and bone healing. Bone fracture defects repair spontaneously with minimal treatment. However, in clinical settings where the defect is too large for natural repair, a replacement material is required to enhance the osteogenic healing process. The aim of this study was to determine if delivery of vascular endothelial growth factor (VEGF) from a biodegradable Poly D,L-lactic acid (PLA) scaffold with the addition of human bone marrow stromal cells. (HBMSC) could enhance the bone regeneration in a mouse-femur segmental defect.

Using supercritical CO<sub>2</sub> technology recombinant human VEGF was encapsulated into PLA monolith scaffolds. VEGF was added to PLA, lyophilised and then plasticised at 35°C under a pressure of 17.32 MPa. Upon release of the pressure, a porous monolith composite with the encapsulated rhVEGF is generated. These scaffolds were seeded with HBMC and implanted into a mouse (nu/nu) femur segmental defect (5mm) for four weeks (n=4 mice/group). The femur defect samples were analysed for an increase in bone regeneration using micro-computer tomography and histology (alcian blue and Sirius red staining for proteoglycans and collagen respectively).

The PLA/VEGF+HBMSC group showed significant bone regeneration in the femur-segmental defect compared to the PLA and PLA+HBMSC groups by indices of increased Bone Volume (BV mm<sup>3</sup>), trabecular number (Tb.N/mm) and reduced trabecular separation (Tb.Sp mm) in the defect region. BV±S.D: PLA= 10.82±3.76; PLA+HBMSC= 16.08±2.52; PLA/VEGF+HBMSC= 24.3±2.31. Tb.N/mm: PLA= 0.60±0.03; PLA+HBMSC=0.51±0.24; PLA/VEGF+HBMSC= 0.73±0.074; Tb.Sp mm: PLA= 1.55±0.09; PLA+HBMSC= 2.12±0.42; PLA/VEGF+HBMSC= 1.26±0.14. No differences were observed in trabecular thickness between the three groups. Histological examination of the PLA/VEGF+HBMSC group confirmed significant bone growth into the scaffold/defect region along with extensive staining of Sirius red in comparison to the other groups.

In conclusion, these studies demonstrate the ability to deliver, temporally, a combination of HBMSC and VEGF released from biocompatible scaffolds to sites of bone defects in a regulated manner, resulting in enhancement of the repair and formation of new tissue structures like bone. Thus, such cell and tissue engineering strategies offer tremendous therapeutic opportunities in orthopaedics and the wider tissue reparative arena.

### OP2

#### CP55940, A NON-SELECTIVE CB1/CB2 AGONIST, STIMULATES BONE RESORPTION BY HUMAN OSTEOCLASTS IN VITRO

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Cannabinoid receptors (CB1 and CB2) are G protein coupled receptors expressed in mammalian tissues and activated by endogenous cannabinoid ligands. Recent studies in mice have shown that cannabinoid receptors are present in bone, with both CB1 and CB2 knockout mice displaying altered bone phenotypes. Cannabinoid receptors are known to be expressed in human peripheral blood mononuclear cells but it is yet to be determined whether human osteoclasts express cannabinoid receptors. We sought to characterise the expression of CB1 and CB2 on human osteoclasts and to examine the effect of CP55940, a non-selective CB1/CB2 agonist.

Human osteoclasts were generated by culturing peripheral blood-derived monocytes from healthy donors with M-CSF and RANKL. Using real-time PCR and western blotting, both monocytes and osteoclasts generated in vitro were found to express CB1 and CB2 receptor mRNA and protein. The level of CB1 did not appear to change throughout osteoclast differentiation, whereas the level of



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CB2 appeared to decrease during differentiation (as has been reported during B cell differentiation).

To study the effect of CP55940, osteoclasts were generated on plastic (to study differentiation) or on dentine discs (to study resorptive activity) in the continual presence of vehicle or CP55940. In the presence of 1nM-1uM CP55940 there was no significant change in the number of VNR-positive osteoclasts (but, concentrations above 1uM caused a decrease in osteoclast number, attributed to cytotoxicity). However, 1nM-1uM CP55940 significantly increased the proportion of actively-resorbing osteoclasts (i.e. cells with actin rings) and increased resorption area e.g. at 1uM, cells with actin rings were 229% +/- 31 of control (P<0.05); resorption area was 334% +/- 50 of control (P<0.01), n = 7 experiments. This demonstrates for the first time that this non-selective CB1/CB2 agonist stimulates the activity of human osteoclasts. This finding is consistent with recent studies showing that a CB1 antagonist prevents bone loss in OVX mice.

Together, our observations confirm that CB1 and CB2 are expressed on human osteoclasts and suggest that endogenous ligands for these receptors may have direct effects on osteoclastic resorption. It remains to be determined whether the stimulatory effect of CP55940 is mediated via CB1 and/or CB2.

### OP3

#### DIETARY FLAVONOID INTAKE IS ASSOCIATED WITH BONE MINERAL DENSITY IN EARLY POSTMENOPAUSAL SCOTTISH WOMEN

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Flavonoids are bioactive polyphenols that are ubiquitously found in plants and are an integral part of the human diet. Little is known about the role of flavonoids on bone health in humans although flavonoids reduce osteoclast action in cellular models and less bone loss was observed in ovariectomised mice treated with hesperidin (Chiba et al 2003). The aim of the study is to investigate whether dietary flavonoids affect bone mineral density (BMD) in a group of Scottish postmenopausal women.

The subjects were women (mean age  $\pm$ SD, 54.7  $\pm$  2.2 y) that had been recruited in 1990-3 for the Aberdeen Prospective Osteoporosis Screening Study, the majority of whom returned 6.3  $\pm$  0.6 y later. A total of 898 subjects had bone scans (Norland XR6.2/6.3 DXA scanner) and filled in a food frequency questionnaire (FFQ) at both visits. This FFQ has been 'validated' for use with flavonoids using 4-d food diaries. The diets were analysed for flavonoid intake using a food composition database developed at the Rowett Research Institute (Kyle et al. 2002). The flavonoid contents of items that were in our FFQ but not in the Rowett database (e.g. swede) were obtained from the U.S. Department of Agriculture database.

The flavonoid intakes of the diet (mean  $\pm$ SD) had increased by 6% from 161 ( $\pm$ 86) to 170 ( $\pm$ 86) mg/d (p<0.001). BMD at the lumbar spine (LS) and femoral neck (FN) had decreased by 6% (p<0.001). Positive correlations were seen between flavonoid intakes and BMD at both sites and both visits, which were significant after adjustment for confounders (age, height, weight, menopausal status and HRT use) at baseline only (r=0.11, p<0.01 FN, r=0.07 p=0.03 LS). Using 1-way ANOVA, women in the highest quartile (Q4) of flavonoid intakes had significantly greater FN BMD at baseline than those in the lowest quartile, Q1 (FN BMD: Q4 0.90  $\pm$  0.13g/cm<sup>2</sup>, Q1 0.87  $\pm$  0.12 g/cm<sup>2</sup>, P=0.02). Future work will involve examining separate categories of flavonoids in relation to bone health.

Chiba, H. et al (2003) J. Nutr. 133, 1892-97

Kyle JAM, et al (2002) Proceedings of the Nutrition Society 61, 69A.

### OP4

#### FEMORAL NECK MICROARCHITECTURE: DIFFERENCES BETWEEN CASES OF HIP FRACTURE AND CONTROLS

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Although the risk of hip fracture is high in osteoporosis (T-score < -2.5), more fractures occur when T>-2.5 so bone distribution and porosity may be important. We analysed the micro-architecture of

femoral neck bone in biopsies from female cases of intracapsular hip fracture (n=50; age 63-95y, mean 78.8) and post-mortem controls (n=23; age 58-100y mean 80.9).

After embedding and staining with von Kossa, digital images (whole cross-sections, resolution 8x8 $\mu$ m; Surveyor) were analysed (ImageJ) using macros available from the author. The endosteal boundary was defined by dilating pores (160 $\mu$ m), filling in any enclosed spaces and eroding the image by the same distance. Images were analysed for cortical area (%Ct.Ar), cancellous bone area (%Cn.B.Ar), cortical porosity (%Ct.Po), canal density and mean canal area in 8 sectors derived from the centre of area. The ratio of major:minor diameter indicated proximity to the femoral head.

Total cross-sectional area was similar (cases 546.8 $\pm$ 11.7mm<sup>2</sup>; controls 548.1 $\pm$ 17.3mm<sup>2</sup>) although the cases had a lower major:minor ratio (cases: range 0.88-1.48 mean 1.19 $\pm$ 0.02; controls: range 0.94-1.42 mean 1.26 $\pm$ 0.03 p=0.021) indicating that they were nearer the femoral head. Least squares regression modelling analysed the effect of fracture (case or control) and sector. %Ct.Ar was dependent on fracture (p<0.0001, cases -15%), sector (p<0.0001) and position (p<0.0001). %Ct.Ar was significantly lower (p<0.03) in the anterior (-19%) infero-anterior (-14%), inferior (-11%) and infero-posterior (-20%) regions. %Cn.B.Ar was dependent on fracture (p<0.001, cases +15%), sector (p<0.017) and position (p=0.0005) and was significantly higher (p<0.006) in the inferior (38%) and infero-posterior (43%) sectors. %Ct.Po was dependent on sector (p<0.0001). Canal density was lower in the cases (p<0.0001) but canal area was higher (p=0.0007).

Trabecularisation of the inferior cortex is therefore a marked feature of women who proceed to hip fracture leading to reduced cortical bone in the lower femoral neck. Although, average porosity was little changed this disguised their prior merging into larger canals which had expanded sufficiently to redefine the surrounding bone as cancellous. These phenomena can be accounted for by an interaction of the combined effects of clustering of cortical remodelling with excess remodelling imbalance.

### OP5

#### CHONDROCYTES IN OSTEOARTHRITIS DE-DIFFERENTIATE TO A PROGENITOR-LIKE INTERMEDIATE BEFORE RE-DIFFERENTIATING TO A COMPLEX AND MIXED PHENOTYPE

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In osteoarthritis (OA), articular chondrocytes change phenotype to cells that express abnormal genes, including proteolytic enzymes. It has been proposed that this change represents differentiation to the hypertrophic stage, inasmuch as some hypertrophic markers (MMP-13 and type X collagen) are expressed in OA. Alternatively the change in phenotype may occur via an intermediate de-differentiation. To test the latter hypothesis, human articular cartilage was obtained from femoral heads of OA patients as well as a young (14-year) and aged non-OA patients. Paraffin sections were immunostained with the following markers: c-Myc, a cellular proto-oncogene that is associated with genomic instability, cell division or apoptosis; Nucleostemin, a coordinator of self-renewal, that is expressed in stem cells, marrow stromal and transformed cells; and Sox-9, the master gene for chondrocytic differentiation, which is switched off in hypertrophic chondrocytes. The staining pattern of c-myc, nucleostemin, and Sox-9 was compared with that of MMP-3, -9, -13 and ADAMTS-4. The cartilage of the 14 year-old male showed no sign of degradation and no immunostaining of proteases. In the very superficial zone of aged non-OA patients, very few surface chondrocytes were immunopositive for c-myc, nucleostemin and some proteolytic enzymes. In OA, progressively more chondrocytes became immunopositive for c-myc, nucleostemin and the proteolytic enzymes until all markers were present in the clonal chondrocytes of high-grade OA. RT-PCR confirmed that these factors were expressed by clonal superficial-zone OA chondrocytes, but not by control chondrocytes. Type X collagen was expressed in both control and OA chondrocytes. Early expression of c-myc may indicate temporary genomic instability, i.e. a gene expression pattern in flux. Because nucleostemin is found only in stem cells or cancer, the presence in clonal chondrocytes is surprising and suggests that chondrocytes undergo a de-differentiation to a progenitor-like intermediate. The re-activation of Sox-9 in late clones suggests reversion to an earlier chondrocytic differentiation stage. Overall, the findings suggest that

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OA chondrocytes, because they still retain some chondrocytic characteristics, display a complicated and mixed phenotype, arising from a progenitor-cell like intermediate stage, followed by re-differentiation. At the same time, the observations do not support the concept of continued differentiation to hypertrophic chondrocytes.

### OP6

#### FRACTURE HEALING AND EARLY SYSTEMIC RESPONSE MODIFIERS

I Pountos, T Georgioulis, PV Giannoudis

Abstract withdrawn

### OP7

#### OSTEOBLASTS PROTECT MULTIPLE MYELOMA CELLS FROM T-CELL-INDUCED APOPTOSIS

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Cells of the bone marrow microenvironment critically regulate the growth and survival of multiple myeloma cells with which they are in intimate contact. All cancer cells induce an immune response, a key element of which are T lymphocytes, whose activation leads to cell-mediated lysis of several tumour cell types including multiple myeloma. Tumour initiation and development is at least partly dependent on effective evasion of the anti-tumour effects of the immune system. However the mechanisms behind this are unknown. As myeloma cells are located within the bone marrow microenvironment, we investigated whether cells from this local environment could protect myeloma cells from the anti-tumour effects of the immune system.

The human T-cell line Jurkat E 6.1, or peripheral blood mononuclear cells (PBMCs), were activated by treatment for 10 minutes with 50 micro-g/ml PHA. Expression of TRAIL (TNF-related apoptosis-inducing ligand) by T-cells was analysed by RT-PCR and FACS. Multiple myeloma cells (NCI H929) were incubated for 24 hours with 20 % conditioned medium from quiescent or activated T-cells in the presence or absence of osteoblast conditioned medium. Apoptosis was analyzed using the nick translation assay.

PHA treatment induced T-cell activation, demonstrated by an increase in proliferation and cell size. Activation induced expression of TRAIL both at the mRNA level and on the cell surface. We have previously shown that TRAIL induces apoptosis of myeloma cells which can be blocked by a neutralising antibody to TRAIL or by rhOPG. In this study conditioned medium from activated T-cells increased apoptosis of myeloma cells by approximately 50 % at 24 hours compared to cultures incubated with conditioned medium from control T-cells. Addition of excess of a neutralising antibody to TRAIL or of 100 ng/ml rhOPG did not reduce the apoptotic effect of the T-cell conditioned medium. However, addition of conditioned medium from osteoblast-like cells reduced apoptosis to baseline levels. This study demonstrates that osteoblast-like cells release a soluble factor which inhibits T cell-induced apoptosis of myeloma cells, suggesting that osteoblasts may protect myeloma cells from the anti-tumour effects of the immune system, contributing to the growth and survival of myeloma cells within the bone marrow microenvironment.

### OP8

#### THE EXPRESSION AND REGULATION OF BIM IN OSTEOBLASTS UNDERGOING GROWTH FACTOR WITHDRAWAL

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Growth factor depletion contributes to cell death following vascular disruption and matrix degradation due to injury or disease in the skeleton. Osteoblasts respond to multiple growth factors, but undergo apoptosis when these factors are depleted following serum withdrawal. We have identified the pro-apoptotic BH3- only protein Bim as a potential regulator of apoptosis in osteoblasts. Bim protein levels are very low in healthy cells but accumulate from 2h after serum reduction, reaching a peak between 8-24h. Caspase 3 activity increased strongly from 4-16h, subsequent to Bim induction. The

upregulation of Bim at 8h was completely blocked by co-treatment with either actinomycin D or cycloheximide, indicating rapid, de novo protein synthesis. Real time RT-PCR revealed that Bim mRNA increased after serum depletion in a time-dependent manner and reached a peak between 6-24h, in agreement with protein data. Bim can be regulated either transcriptionally or post-translationally by the pro-survival kinases PKB and/or ERK depending on cell type. We found that both PKB and ERK became rapidly dephosphorylated following serum starvation in osteoblasts, preceding Bim upregulation. To establish which of these kinases regulate Bim in osteoblasts we treated cells with the MEK-ERK inhibitor, U0126, or the PI3K-PKB inhibitor, LY294002, and found that both induced strong expression of Bim protein. Moreover, inhibition of both PKB and ERK increased Bim gene transcription, indicating that survival kinases also regulate Bim mRNA levels in osteoblasts. Post-translational regulation of Bim is likely to occur via proteasomal degradation and indeed Bim protein markedly increased following treatment with the proteasome inhibitor MG132. Bak and Bax, the downstream effectors of Bim, are abundant in osteoblasts and levels remained unchanged during 24 h of serum reduction. However, immunoprecipitation data showed that serum withdrawal induced the conformational change associated with Bax activation. Knockdown of Bak or Bax protein levels alone by siRNA did not prevent apoptosis induced by growth factor withdrawal but knockdown of both Bak and Bax or Bim alone was partially protective. In conclusion, withdrawal of serum depletes growth factors in culture and osteoblasts respond to this by upregulating Bim and undergoing apoptosis.

### OP9

#### NOVEL CONTROL OF GAG SYNTHESIS AND CHONDROGENESIS THROUGH SELECTIVE REGULATION OF UDPGD-MEDIATED MONOSACCHARIDE SUPPLY

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Functional competence of all skeletal and repair tissues is reliant on the structural characteristics of their connective tissue compartments. In articular cartilage this is largely attained through structural moieties in the extracellular matrix that resist compressive as well as tensional loads engendered by mechanical use. Compressive resilience is dependent upon glycosaminoglycan (GAG) content and although GAG synthesis can be regulated at the level of monosaccharide supply, the mechanisms by which this is achieved are ill defined. UDP-glucose dehydrogenase (UDPGD) is a key enzyme catalysing the oxidation of UDP-glucose to UDP-glucuronic acid, an essential monosaccharide precursor required for hyaluronan (HA) and sGAG synthesis. Our objective was to examine the role of UDPGD in regulating GAG production and chondrogenesis. Herein, we have used retroviral (RCAS) transfection techniques to examine the effects of UDPGD overexpression on the behaviour of chick embryo articular surface (AS) cells, as well as chick marrow and limb bud micromass cultures. We found that media conditioned by UDPGD-transfected AS cells contained significantly greater HA and sGAG levels (vs. RCAS-GFP controls). UDPGD-overexpressing AS cells formed significantly larger HA-dependent pericellular coats, suggesting a rate-limiting role for UDPGD in both HA and sGAG synthesis. In addition, an inhibitor of HAS activity (4-MU) blocked control and UDPGD-dependent increases in HA release. Furthermore, we found that transfection with UDPGD resulted in significant increases in Alcian blue-positive nodule size, but not number, in micromass marrow culture. Similarly, chick limb bud micromass cultures overexpressing UDPGD also exhibited significant increases in sGAG production and chondrogenesis. These studies further confirm the rate limiting role of UDPGD during chondrogenesis. To test UDPGD involvement in the anabolic response to archetypal growth factors, consequent studies used AS cells and disclosed increases in UDPGD mRNA and protein expression in response to TGF-beta treatment that were associated with significant increases in sGAG production, HA release and pericellular coat formation. In conclusion, our results provide evidence that sGAG and HA synthesis are limited by UDPGD activity and that it plays a direct positive role in regulating chondrogenesis.



**OP10****THE EFFECT OF TENSILE FORCES ON THE DIFFERENTIATION OF MESENCHYMAL STEM CELLS**

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**Introduction:** There are 1 million cases of major skeletal defects that occur worldwide each year that lead to significant morbidity and disability and currently require bone grafting as the main mode of treatment. Limitations of bone-grafting include donor site morbidity, reduced osseointegration and risk of pathogen transmission to the host. There is considerable interest in finding ways of differentiating mesenchymal stem cells down the osteoblastic lineage to form bone tissue. We hypothesized that there is an optimum strain that promotes differentiation of mesenchymal stem cells into osteoblasts.

**Methods:** A bioreactor was developed that was capable of applying tensile forces across a culture strip in a graduated manner within a range of 1-4373me. Mesenchymal stem cells were grown on these strips and subjected to cyclical tensile strain at 1Hz. Cell morphology using Scanning Electron Microscopy, mineralization using specialized stains and expression of core binding factor1 (Cbfa1) was studied at various strain levels.

**Results:** Scanning Electron Microscopy revealed classic osteoblastic cells in the regions subjected to tensile force, especially in the region where average strain was 1312me. X-ray microanalysis revealed calcium deposits on the strip, indicating osteoblastic differentiation. Cbfa1 expression was greatest in the region with an average strain 1312 me followed by a region on the strip subjected to just fluid shear without any tension. Cbfa1 expression was significantly greater in cells subjected to tensile forces than unstrained controls at all levels of strain tested ( $p < 0.05$ ). Cbfa1 expression was further enhanced significantly by the addition of osteogenic factors ( $p < 0.05$ ). Significantly greater mineralization ( $p < 0.05$ ) occurred in the regions subject to tension with the greatest being in the region with an average strain of 1312 me.

**Conclusions:** Mechanical tensile forces especially in the range of up to 2173me promote differentiation of Mesenchymal Stem Cells into osteoblasts and encourage expression of the Cbfa1 gene. Tensile strain also promotes mineralization. Chemical factors in form of osteogenic media accelerate the differentiation of MSCs and encourages earlier production of osteoblast specific markers. Fluid shear appears to have a beneficial effect in stimulating differentiation into the osteoblast phenotype and, combined with tensile strain, may offer an even greater osteogenic stimulus.

**OP11****GENETIC MUTATIONS IN THE RAS/RAF/MAPKINASE PATHWAY RESULTS IN CHERUBISM**BD Idowu<sup>[1]</sup>, J Mangioni<sup>[2]</sup>, RE Gale<sup>[3]</sup>, AM Flanagan\*<sup>[4]</sup>

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Cherubism is a rare hereditary multicystic disease of the jaw characterised by its typical appearance. Microscopic examination reveals an osteoclast-rich lesion that is difficult to distinguish from giant cell-rich neoplasms. The disease is inherited as an autosomal dominant trait with variable expressivity and approximately 80% penetrance. It is associated with germline mutations in SH3BP2 and PTPN11.

We report on 50 families with Cherubism. Nine non-synonymous SH3BP2 point mutations (2 novel) in exon 9 were identified in 36 (72%) families: 24 with a family history, 5 with de novo mutations and 7 undetermined status. Four (8%) families had mutations (1 novel) in PTPN11. We then screened the mutation-negative cases for NF1, KRAS, HRAS, BRAF and MEK.

We report for the first time a NF1 mutation associated with Cherubism involving a splice donor site (IVS37+1). The child has mild signs of NF. Mutations in KRAS, HRAS, BRAF, and MEK were not detected. The remaining 9 families are currently being screened for mutations in SOS1. Since all the detected NF1, PTPN11, SH3BP2 and SOS1 mutations are involved in activation of the RAS/RAF/MAPK pathway, we speculate that somatic/oncogenic mutations resulting in activation of this signalling pathway are involved in the tumorigenesis of osteoclast-rich neoplasms, such as giant cell tumour of bone.

**P12****NITROGEN-CONTAINING BISPHOSPHONATES AND PREDICTION OF THEIR CLINICAL POTENCIES: DISSOCIATION OF TARGET ENZYME- AND HYDROXYAPATITE-BINDING AFFINITIES**Z Xia<sup>[1]\*</sup>, J Dunford<sup>[1]</sup>, MA Lawson<sup>[1]</sup>, JT Triffitt<sup>[1]</sup>, K Kavanagh<sup>[1]</sup>, U Oppermann<sup>[1]</sup>, BL Barnett<sup>[2]</sup>, FH Ebetino<sup>[2]</sup>, RGG Russell<sup>[1]</sup>

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Bisphosphonates (BPs) have pharmacological potencies that are dependent on both the binding affinities for bone mineral and inhibitory effects on the cellular activities of the major bone-resorbing cells, the osteoclasts. The cellular effects are mediated in the case of nitrogen-BPs (N-BP) by selective targeting of the enzyme Farnesyl Pyrophosphate Synthase (FPPS). The extent to which each property determines the potencies of individual BPs can be better defined by specific assays for FPPS activity and mineral binding. This study has employed a recently developed mineral binding assay that is highly reproducible and based on ceramic hydroxyapatite (HAP) column chromatography. Inhibitory potencies of a wide range of individual BPs on FPPS were measured by kinetic analyses. The results indicate that the N-BPs inhibit the enzyme by a competitive slow tight mechanism. From the use of a series of N-BPs, it is shown that the rank order for inhibitory potencies on FPPS is independent of the respective mineral binding affinities to HAP. Major determinants of mineral binding are confirmed to be the P-C-P group, an OH on the geminal carbon, and the structure and orientation of the nitrogen-containing substituent on the geminal carbon. For the inhibition of FPPS the PCP group is essential, together with specific orientation of the N in the R2 group relative to the enzyme-binding pocket. Examples include Risedronate and NE58051 (HomoRisedronate) which have similar retention times on the column, 9.97 and 9.67 min respectively, but show a great difference in FPPS enzyme inhibition with Ki's of 0.34nM and 79.9nM respectively. However, in the case of NE11807 (a 2-pyridyl amino-methane BP) and NE11808 (a pyridyl amino-ethane BP), whilst the enzyme inhibitions are similar (2.3 nM and 2.2 nM respectively), their retention times on HAP are very different (12.83 and 6.57 respectively). Molecular modelling studies indicate that different 3-D configurations of the N-BPs can account for the observed FPPS inhibitions and HAP-binding characteristics. In conclusion, the chemical structures that determine separately the FPPS inhibition and mineral binding activities of N-BPs are distinct, and each independently contribute to the overall observed clinical and pharmacological potencies of N-BPs in vivo.

**P13****MCL-1 IS AN IMPORTANT PRO-SURVIVAL FACTOR IN OSTEOCLASTS**

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Mcl-1 is an anti-apoptotic member of the Bcl-2 family and is expressed in cells of the myeloid lineage. However, the role of Mcl-1 in osteoclasts is unknown. Having recently found that increased Mcl-1 levels are associated with prevention of alendronate-induced osteoclast apoptosis by RANKL, we further investigated the role of Mcl-1 in mouse osteoclasts.

Osteoclasts were generated from mouse bone marrow macrophages by culturing cells with M-CSF + RANKL for 5 days. Mcl-1 protein increased in these cells after culture with RANKL. Removal of RANKL and M-CSF from cultures of multinucleated osteoclasts caused the appearance of morphologically apoptotic cells and the appearance of cleaved caspase-3 after about 6 hours. This was preceded by a decrease in the level of Mcl-1 protein, assessed by western blotting, such that Mcl-1 was undetectable after 12 hours. However, levels of Mcl-1 mRNA did not alter after cytokine starvation, suggesting that Mcl-1 is regulated at the translational and/or post-translational level in osteoclasts. In accord, cycloheximide (which rapidly induces osteoclast apoptosis) caused complete loss of Mcl-1 in osteoclasts after 6 hours, whilst the proteasome inhibitor MG132 increased Mcl-1 levels, indicating that continual protein synthesis and proteasomal degradation regulate Mcl-1 protein levels in osteoclasts. Decreased Mcl-1 levels during cytokine starvation in osteoclasts occurred much

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earlier than decreases in Bcl-2 or Bcl-XL protein levels, suggesting that loss of Mcl-1 is an early step in osteoclast apoptosis.

Mcl-1 protein levels and cell survival in cytokine-starved osteoclasts were restored by addition of either M-CSF, RANKL, TNF $\alpha$  or LPS. M-CSF and RANKL appear to maintain the expression of Mcl-1 via the mTOR signalling pathway (but not via p38 or ERK signalling), since rapamycin (but not SB203580 or U0126) prevented the ability of M-CSF or RANKL to maintain Mcl-1 expression.

Together, these observations demonstrate that Mcl-1 is a short-lived protein in osteoclasts and suggest that the pro-survival effect of M-CSF, RANKL, TNF $\alpha$  and LPS on osteoclasts is mediated, at least in part, by upregulation of Mcl-1 levels. This may be particularly relevant in conditions of inflammatory bone loss, where increased osteoclast survival may contribute to disease pathogenesis.

### P14

#### ADENOSINE STIMULATES MINERALISATION OF RAT MESENCHYMAL STEM CELLS IN VITRO

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P2 purinergic receptors have been implicated in bone health and disease for several years. There is, however, very little information on how the P1 (adenosine) purinergic receptor family interact with bone cells or their precursors. Our recent studies have investigated the effects of adenosine receptor agonists on mineralisation of rat derived bone marrow mesenchymal stem cells (MSCs) as they differentiate into osteoblasts in vitro. Furthermore, we have investigated the expression of CD73, responsible for the conversion of AMP to adenosine, in rat MSCs following up to 2 weeks of in vitro cell culture in non-differentiating medium, to explore the hypothesis that there are negative and positive populations in these cells in relation to this marker.

We have shown that adenosine (natural ligand for adenosine receptors; 100 $\mu$ M) significantly increased (by approximately 50%) mineralisation (after 10 days and assessed by Alizarin Red staining) in these cells. Since adenosine has a very short half-life, the medium in the adenosine treated and control cultures were replenished every weekday in these experiments. The differentiation medium used contained dexamethasone (10nM), ascorbate-2-phosphate (50  $\mu$ g/ml) and 2mM beta-glycerophosphate. Intriguingly, similar experiments with synthetic adenosine receptor agonists (NECA; universal adenosine receptor agonist: IB-MECA; specific A3 adenosine receptor agonist; both at 10 $\mu$ M) resulted in a decrease (by approximately 25%) in mineralisation. Other experiments with these compounds in the same differentiation medium but with 10mM beta-glycerophosphate also resulted in decreased mineralisation. FACS analysis of cells with mouse anti-rat CD73 monoclonal antibody showed that all cells expressed this protein.

In conclusion, we have shown for the first time a significant effect of adenosine on mineralisation of rat MSCs. Other adenosine receptor agonists, however, decreased mineralisation. In addition we have shown that similar results were obtained with rat derived MSCs in the presence of 2 or 10 mM beta-glycerophosphate. We are currently undertaking further experiments with a range of concentrations (100 $\mu$ M to 10nM) of adenosine, NECA and IB-MECA to address the disparity in the results obtained with the natural ligand when compared to those with synthetic adenosine receptor agonists.

### P15

#### COMPARTMENTALISATION OF GFP-TAGGED ER-ALPHA AND ER-GR CONSTRUCTS IN BONE (ROS) AND BREAST (MCF-7) CELLS

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Transcription activation assays are used extensively to screen for oestrogenic and anti-oestrogenic compounds but the effects observed are often dependent not only on the stimulus used, but also on cellular context and reporter. As an alternative strategy, GFP fusion proteins are increasingly used as reporters for protein function. Translocation of glucocorticoid receptor (GR)-GFP from the cytoplasm to the nucleus provides a convenient readout for GR activation but

this assay is not applicable to the oestrogen receptor (ER) that is mostly nuclear in unstimulated cells. Here we have generated vectors encoding the human ER $\alpha$  in tandem with GFP at the C-terminus (ER $\alpha$ -GFP), as well as a chimeric protein consisting of the full length human GR $\alpha$ , but with its ligand binding sequence replaced with that from the human ER $\alpha$ , again in tandem with GFP (ER/GR-GFP). These were transiently expressed in human breast cancer (MCF-7) and rat osteosarcoma (ROS) cells prior to assessment of fluorescence intensity and localisation using an InCell 1000 semi-automated image acquisition and analysis system. As expected, the ER $\alpha$ -GFP was primarily nuclear, with nuclear:cytoplasmic (N:C) ratios of 4.2 and 4.9 in MCF-7 and ROS cells, respectively, and this distribution was not measurably altered by 17  $\beta$ -oestradiol (E2, 10<sup>-7</sup>M) or the antagonist ICI 162,780 (ICI, 10<sup>-7</sup>M). In contrast, a much larger proportion of the ER/GR-GFP was cytoplasmic (N:C 0.9 and 1.0 in MCF-7 and ROS respectively) and the N:C ratio was increased approximately 2 fold by E2 (but not the GR agonist dexamethasone) in both cell types. This effect was dose-dependent (EC50 approx. 10<sup>-11</sup>M) and time-dependent (30 % increase at 4 hr and 100% increase at 16 hr at 10<sup>-8</sup>M). In MCF-7 but not ROS cells, ICI inhibited translocation of the ER $\alpha$ -GFP construct and interestingly, increased punctate staining in the cytoplasm was observed, where the antagonist increased the area of granular inclusions from approx. 3-12 $\mu$ m<sup>2</sup>/cell, and this effect was dose-dependently inhibited by E2 (E2 having an IC50 of 10<sup>-9</sup>M). We suggest that these simple and rapid readouts for occupancy of the ER ligand binding site may facilitate screening for ER agonist, antagonist and SERM effects in bone cells.

### P16

#### OSTEOPENIA IN 129SV/EV SPARCTM1CAM MICE: BONE PHENOTYPIC CHARACTERIZATION AND GENE EXPRESSION CHANGES

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The inactivation of Sparc (osteonectin) in mice causes low-turnover osteopenia, making these mice an ideal model for senile osteoporosis. Sparc null mutants have been generated twice, independently, via mutations in exons 4 and 6 of the Sparc gene. Previous studies have identified low turnover osteopenia in the exon 4 knockout. However, different mutations can produce differing outcomes and genetic background and/or linkage disequilibrium effects can influence phenotypic interpretation.

We have therefore evaluated the bone phenotype in the Sparctm1cam exon 6 knockout on a purebred 129Sv/Ev background at 4 and 9 months, using physical measurement, mechanical strength tests and DXA scanning. We have also quantified bone marrow adiposity and circulating leptin levels to assess adipose tissue regulation anomalies in these mice. Molecular phenotyping was undertaken using mouse HGMP NIA microarrays with cortical femur samples at various ages, using semi-quantitative RT PCR validation. 129Sv/EvSparctm1cam null mice have shorter femurs and decreased bone mineral density and bone mineral content. Increased mechanical fragility of bone was also evident. Increased body weight and levels of bone marrow adiposity, but decreased circulating leptin concentrations were identified at 4 months, but not at 9 months. Array studies have identified 6 genes (Sparc, Zfp162, Bysl, E2f4 and two uncharacterised ESTs) that are differentially regulated in 129Sv/EvSparctm1cam cortical femur versus 129Sv/Ev controls. We have also identified 429 ESTs expressed in normal bone.

We confirm that low turnover osteopenia is a feature of the Sparc null phenotype, regardless of the null mutation or the mouse strain. Circulating leptin concentrations were however reduced in the 129Sv/EvSparctm1cam null mouse, whereas they were increased in the previously reported Sparc exon 4 null animals. We have also identified genes that may contribute to the development of bone weakness in these 129Sv/EvSparctm1cam mice, and may therefore play a significant role in the pathophysiology of osteoporosis.

**P17**

## **SB431542, A TGF-BETA TYPE 1 RECEPTOR INHIBITOR, PROMOTES C2C12 MYOTUBE HYPERTROPHY**

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Myostatin, a member of the TGF-beta super family, is a key negative regulator of skeletal muscle growth and differentiation. Transgenic or natural knockout of the myostatin gene results in pronounced hypertrophy in various species including cattle, mice and humans. Myostatin achieves its atrophic effect by binding to TGF-beta type II and I receptors. Receptor binding by myostatin leads to phosphorylation of the transcription factors Smad2 and 3 at their C-terminal ends. SB431542 is marketed as an inhibitor of the TGF-beta type I receptor isoforms ALK4, 5 and 7 which mediate myostatin signalling. The aim of this study was to test if SB431542 inhibits myostatin-induced C-terminal phosphorylation of Smad2/3, promotes proliferation of C2C12 myoblasts and increases the size of C2C12 myotubes.

Treatment of C2C12 cells with 10µM of SB431542 prevented phosphorylation of

Smad2 at Ser465/467 induced by 0.25 µg/ml of recombinant human myostatin.

The inhibition of myostatin-induced Smad signalling by SB431542 was also apparent when an antibody raised against C-terminal Smad 3/1 phosphorylation was used. With this antibody, however, an unidentified protein of higher molecular weight was observed in all samples treated with SB431542. The functional effects of SB431542 were measured by culturing C2C12 myoblasts and myotubes in the presence of 10µM of SB431542. We measured cell numbers using flow cytometric analysis and analysed myotube width from varel-phase contrast light microscope images. SB431542 treatment did not increase C2C12 myoblast numbers suggesting that it did not stimulate proliferation.

However, SB431542 increased C2C12 myotube width after two days of treatment from (mean±SD; n=3 wells) 11.4±1.8 µm in control to 19.7±3.9 µm and after four days of treatment from 25.2±2.4 µm in control to 32.8±0.8 µm suggesting that SB431542 stimulated C2C12 myotube growth. In conclusion, SB431542 prevents myostatin-induced Smad2 and Smad3 C-terminal phosphorylation and stimulates C2C12 myotube growth. Thus SB431542 is a potential drug for the treatment of muscle wasting.

**P18**

## **POLYMORPHIC VARIATION IN THE FPP SYNTHASE (FPPS) GENE AND EFFECTS ON BISPHOSPHONATE INHIBITION**

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Nitrogen-containing bisphosphonates (N-BPs) are the frontline treatment for postmenopausal osteoporosis and other bone diseases. These drugs act by inhibition of the enzyme FPP synthase (FPPS) in osteoclasts. However, it appears that a considerable proportion of patients with osteoporosis do not benefit from N-BPs, and resistance to bisphosphonates has been documented in patients with Paget's disease. We have examined whether single nucleotide polymorphisms (SNPs) in the FPPS gene could influence the ability of N-BPs to inhibit this enzyme and affect the clinical response.

The FPPS gene was sequenced using an ET-terminator dye system, with DNA obtained from 100 volunteers from the APOSS (Aberdeen Prospective Osteoporosis Screening Study) population. Sequences were compiled in SeqMan for SNP identification.

Sequencing of the 11 exons of the FPPS gene revealed one novel (T to C) polymorphism in exon 11, causing an amino acid change from Val 364 to Ala (V364A), with an allele frequency of 0.08 for the C allele and 0.92 for the T allele. The minor C allele was only present in heterozygotes in the sampled population. A previously identified polymorphism (A to C) in intron 1 was confirmed, with an allele frequency of 0.25 for the C allele and 0.75 for the A allele.

The V364A polymorphism was introduced into a bacterial expression plasmid encoding His-tagged human FPPS. Wildtype and variant

recombinant proteins were then expressed and purified. Enzyme activity was assessed using <sup>14</sup>C-labelled IPP. Kinetic values for isoprenoid substrates GPP and IPP were found to be similar to published values (with Vmax between 20 to 24 umoles/min and a Km of 1.3 to 2 µM) but the enzymes did not appear to differ in the IC50 values for inhibition by a range of N-BPs.

In conclusion, we have identified a novel A/C polymorphism in exon 11 of the FPPS gene that appears to be present in approximately 8% of the population. Analysis of the variant enzyme shows no apparent difference in kinetics or inhibition by N-BPs compared to wildtype enzyme. Hence, common exonic polymorphisms in the FPPS gene do not appear to be responsible for the lack of response to N-BPs observed in some patients.

**P19**

## **HUMAN OSTEOCLAST FORMATION AND ACTIVITY ON BONE SUBSTITUTE BIOMATERIALS**

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The majority of studies on biomaterials have investigated the regenerative properties of synthetic materials, their osteoconductivity, and their ability to induce new bone formation from osteoblasts in both in vitro and in vivo models. However the ideal biomaterial for bone regeneration must be completely resorbable, which means that it should be remodelled through both new bone formation and resorption. This study aimed to determine whether human osteoclasts could be formed and subsequently activated on bone substitute materials.

Methods; Peripheral blood mononuclear cells from healthy volunteers were used to generate osteoclasts in vitro in the presence of M-CSF and RANKL, using Osteopontin (Biotech S.r.l., Arcugnano, Vicenza) Bio-Oss and a new screwable block prototype (Geistlich, Wohlen, Switzerland) as substrates, with bovine bone used as a positive control. Osteoclast morphology and activity were assessed by light and reflection microscopy (TRAP and Toluidine blue) and confocal microscopy (Vitronectin Receptor and Type I collagen). TRAP5b-ELISA was used to confirm the presence of osteoclasts by collecting the culture medium at various time points.

Results; Light and confocal microscopy revealed the formation of human osteoclasts on all substrates after 21 days. Osteoclasts on all biomaterials appeared well spread and they exhibited positive staining for F-actin and vitronectin receptor as assessed by confocal microscopy. Furthermore the presence of osteoclasts on Osteopontin and Bio-Oss was confirmed by TRAP5b-ELISA, detecting the amount of type 5b acid phosphatase secreted by mature osteoclasts in the culture mediums. Osteopontin exhibited a larger amount of TRAP released from the cultures than Bio-Oss, with statistically significant differences at the various time points. The presence of resorption pit-like surface irregularities, observed by immunofluorescence staining for type-I collagen, confirmed that there was osteoclast-mediated resorptive activity on all biomaterials. This was reinforced by reflective microscopy, after staining with toluidine blue.

Conclusions; These data show that human osteoclasts can be generated on alternative bone substitute biomaterials. This is the first evidence of human osteoclasts generation on such biomaterials. Moreover this study brings new insights into the understanding of the resorbability of biomaterials and can be considered a model for future comparison of biomaterials resorption rate.

**P20**

## **ZOLEDRONATE POTENTLY INHIBITS THE GROWTH, SURVIVAL AND ACTIVITY OF NORMAL RAT OSTEOBLASTS**

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Bisphosphonates are recognised not only as potent inhibitors of osteoclastic resorption but also for their toxicity to tumour cells. However, their actions on osteoblast function and survival are less well documented. We studied the effects of zoledronate, the most potent bisphosphonate, on primary osteoblast cultures derived from neonatal rat calvariae by trypsin/collagenase digestion. Osteoblasts were cultured up to 14d in DMEM supplemented with 2mM beta-glycerophosphate, 50 micrograms/ml ascorbate and 10nM dexamethasone. Bone nodule formation was measured by image analysis of alizarin red-stained cell



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layers; cell number, viability, alkaline phosphatase (ALP) activity and collagen production were quantified colorimetrically. In long-term osteoblast cultures, zoledronate exerted striking, dose-related cytotoxic effects. Treatment with 10 micromolar zoledronate resulted in 40% and 99% reductions in osteoblast number over the first 4d and 7d of culture, respectively. Treatment with 1 micromolar zoledronate did not affect osteoblast number during the first 7d, but decreased cell number by 40% at 14d. At lower doses, zoledronate potently blocked mineralised bone nodule formation: inhibitions of 65%, 95% and 100% were observed in cultures treated for 14d with 10nM, 100nM and 1 micromolar zoledronate, respectively. Light microscopy revealed that in osteoblast cultures treated with 10nM and 100nM zoledronate, abundant, collagenous matrix was deposited in characteristic 'trabecular' patterns but that mineralisation was selectively blocked. In cultures treated with 1 micromolar zoledronate, collagenous matrix deposition was decreased in line with reduced cell numbers. Quantitative analysis confirmed that total protein and soluble/deposited collagen production were unaffected by 10nM and 100nM zoledronate, whereas 1 micromolar zoledronate reduced collagen by 20-30%. ALP activity relative to cell protein was unaffected by 10nM zoledronate but was decreased by 35% and 80%, respectively in cultures treated with 100nM and 1 micromolar zoledronate. The IC50 in this system of the classical mineralisation inhibitor, pyrophosphate was 2 micromolar; thus zoledronate is about 200x more potent. In summary, our results show that chronic zoledronate treatment blocks bone formation in vitro via inhibition of mineralisation in the low nanomolar range and via a general cytotoxic action on osteoblasts in the low micromolar range. These findings raise the possibility that long-term exposure of osteoblasts to zoledronate in vivo could have adverse consequences for bone health.

### P21

#### DIFFERENTIAL VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR EXPRESSION DURING OSTEOGENESIS

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**Introduction:** Vascular endothelial growth factor (VEGF) has a multifaceted role in bone cell activity. It is implicated in the early stages of human osteoblastogenesis and aids bone repair by promoting angiogenesis and bone turnover. VEGF also acts as a chemotactic factor promoting migration of several cell types needed for bone formation. In addition, VEGF enhances bone cell differentiation. Consequently, VEGF is a good candidate for therapeutic strategies in bone healing and tissue engineering. In this study, experiments were performed to characterise osteoblast responsiveness to VEGF during osteogenesis.

**Methodology:** Osteoblasts were isolated from rat calvariae and treated with recombinant VEGF165. Cellular responses to VEGF including proliferation, differentiation and extra cellular matrix mineralization were assessed using an MTT assay, by measuring Alkaline Phosphatase activity and Von Kossa staining. Short term responses to VEGF treatment were also investigated by Western blotting with an antibody against phospho-p44/42. RT-PCR with primers for VEGFA, C, D, NP1, NP2 and VEGFR1, 2, 3 were used to follow transcript expression.

**Results-Discussion:** Treatment of primary rat osteoblasts with VEGF under osteogenic conditions for 14 days did not demonstrate any affect on the levels of alkaline phosphatase activity observed in each group. However, in longer term experiments, cultures exhibited significantly increased mineral deposition and enhanced bone nodule formation compared to controls when treated with VEGF (1ng/ml-10ng/ml - ANOVA, P=0.042). To characterise how VEGF influenced bone formation, its activity was assessed on immature bone cells. In proliferation assays VEGF had no effect on early osteoblasts though some evidence of signal transduction was observed with MAPK activation. To further clarify if VEGF responsiveness in our culture system was differentiation dependent, experiments were performed to analyse VEGF-related transcripts at 0, 7, 14, 21 days. RT-PCR identified transcripts for VEGF isoforms and receptors with apparent changes in mRNA abundance over the four week time points. VEGFR3 transcripts declined as osteoblast maturation progressed, while VEGFR2 seemed to be upregulated. Lastly, VEGFR1 mRNA levels remained unchanged. We speculate that osteoblast maturity dictates the ability to respond to VEGF and this is driven by differential VEGF receptor expression.

### P22

#### BONE FORMATION AND FIBROCARILAGE REGENERATION AT BONE TENDON JUNCTION WITH ALLOGENEIC CULTURED CHONDROCYTE PELLET INTERPOSITION

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Regeneration of fibrocartilage transition zone at healing bone tendon junction is essential to restore the gradual transition of material property from bone to tendon. We hypothesised that interposition of cultured chondrocyte pellet (CCP) during direct bone tendon junction repair might improve bone formation and fibrocartilage zone regeneration. Cultured chondrocyte pellets were prepared from chondrocytes released from 6-week-old rabbit rib cartilage and inserted as an interposition material during partial patellectomy repair in 18-week-old mature New Zealand White rabbits. The partial patellectomy repair was protected by a figure of eight wiring and external casting for 4 weeks. In the control group rabbits, the same partial patellectomy repair was performed without CCP interposition. Patella-patellar-tendon samples were harvested at 8 and 12 weeks after repair. The harvested samples were decalcified and processed for histomorphometric studies. Digitalized images were analyzed using MetaMorph image analysis system. The mean and maximum length of new bone formation, area of new bone formed, length of basophilic line formed, and length of fibrocartilage regeneration were measured. Quantitative data were analyzed using SPSS 14.0. Differences were compared using Mann-Whitney test. Statistical significance level was set at  $p < 0.05$ . The maximum new bone length at 12 weeks in the CCP insertion group measured 2.72 mm, compared with 1.52 mm in the control group ( $p=0.05$ , Mann-Whitney test). The mean bone length and the area of new bone formed, however, did not differ. Basophilic line formation was absent in all but one of the 8 week control samples. The length of fibrocartilage zone regenerated was significantly higher in the CCP insertion group than in the control group at both 8 weeks and 12 weeks (2.20 mm vs. 0.68 mm,  $p=0.05$ ; and 4.41 mm vs. 0.56 mm,  $p<0.05$ , respectively, Mann-Whitney test). We concluded that interposition of allogeneic culture chondrocyte pellet at direct bone tendon junction during repair could increase the amount of bone formation and fibrocartilage zone regeneration. Further studies are required to work out the mechanism of enhancement.

### P23

#### BCL-2-ASSOCIATED ATHANOGENE-1: A TRANSCRIPTIONAL REGULATOR MEDIATING CHONDROCYTE SURVIVAL AND DIFFERENTIATION DURING ENDOCHONDRAL OSSIFICATION

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BAG-1 is an anti-apoptotic protein identified by its ability to bind to BCL-2, HSP70-family molecular chaperones and nuclear hormone receptor family members. Murine cells express two BAG-1 isoforms namely, BAG-1L (50 kDa) and BAG-1S (32 kDa). Previously we have demonstrated expression of BAG-1 in the perichondrium, osteoblasts of primary spongiosa, osteocytes in bone shaft, bone marrow, growth plate and articular chondrocytes of long bones of normal BDF-1 mice. We also reported that BAG-1 was expressed at different stages of differentiation in growth plate chondrocytes during mouse bone development between 3 and 30 weeks. The aim of the present study was to delineate BAG-1 function in chondrocytes during endochondral ossification.

Murine chondrocytic ATDC5 cells were utilised in the present study as they exhibited robust expression of both BAG-1 isoforms and monolayer cultures of these cells over a period of 28 days in presence of insulin served to reproduce stages in chondrocyte differentiation observed during in vivo endochondral ossification. It was possible to demonstrate the anti-apoptotic role of BAG-1 in chondrocytes as over-expression of BAG-1 protected ATDC5 cells, which were subjected to heat-shock at 48°C for 30 minutes, against heat-shock-induced apoptosis. Independent of its anti-apoptotic function, BAG-1 was found to regulate the expression of genes important in chondrocyte hypertrophy and endochondral ossification. We were able to demonstrate by qPCR that the over-expressed BAG-1 proteins



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suppressed Type II collagen gene expression, and enhanced transcription of Runx-2 and Alkaline phosphatase in ATDC5 cells. The present study also elucidated the regulation of Bag-1 gene expression in chondrocytes. Although over-expression of BAG-1 had no effect on Sox-9 gene transcription, over-expression of SOX-9 enhanced expression of the Bag-1 gene. By co-transfecting CHO cells (devoid of endogenous Bag-1 or Sox-9 expression) with the human pSox-9 expression vector and the human Bag-1 gene promoter-Luciferase reporter construct, we were able to demonstrate that activity of the Bag-1 gene promoter was enhanced by SOX-9. These results indicate an important role for BAG-1 as a transcriptional regulator in the process of endochondral ossification, and modulation of BAG-1 expression plays an important role in mediating chondrocyte survival and turnover.

### P24

#### NITRIC OXIDE REGULATES EXPRESSION OF CLASS 3 SEMAPHORINS IN MOUSE OSTEOBLASTS

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Nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS) is an important regulator of bone metabolism. The targeted disruption of eNOS results in loss of bone due to a decrease in osteoblast (Ob) number and surface, with no changes observed in osteoclasts. Obs cultured from eNOS KO mice display a decreased growth rate, lower basal alkaline phosphatase (ALP) activity and a diminished ALP response to parathyroid hormone (PTH). It is not yet known what targets downstream of eNOS are responsible for the inhibition of Ob number and function. As part of the European STREP Framework 6 consortium (ANABONOS) we aimed to identify potential anabolic pathways associated with eNOS by comparing gene expression in eNOS WT and KO bone and in Obs.

Using Affymetrix arrays we measured changes in gene expression in cultured Obs. isolated from 4-day old eNOS WT and KO pups. Two replicates were hybridised per group (minimum of six mice per litter). Pairwise comparisons were performed between replicates and genes  $\geq 2$  fold up- or down-regulated with 100% concordance were identified. Array data was validated by quantitative PCR. We observed significant down regulation of several members of the class 3 semaphorin signaling molecules that are normally associated with axonal guidance, development and cancer.

Sema3a, 3d and 3e were significantly down regulated in eNOS KO Obs by 40%, 32% and 74% respectively. Furthermore, Sema3e expression was decreased in WT Obs by treatment with 0.5 mM N(G)-nitro-L-arginine methyl ester (L-NAME), an inhibitor of eNOS. In addition, expression of Sema3e was restored in eNOS KO Obs by the NO donor S-nitroso-N-acetyl-DL-penicillamine (SNAP, 20  $\mu$ M). Sema3e expression was stimulated by 10 nM vitamin D3 treatment and Sema3d expression increased with time in culture. Neither Sema3a, 3d or 3e expression increased in response to PTH. In conclusion, Sema3a, 3d and 3e represent a novel class of signalling molecules that may be involved in the normal function of Obs, but are differentially regulated by various osteotropic factors. Expression of all three of these semaphorins appears to depend upon functional eNOS, suggesting they may be mediators of the eNOS KO phenotype.

### P25

#### DIFFERENTIAL EFFECTS OF ALPHA-HALOGENATION ON THE POTENCY OF BISPHOSPHONATES AND PHOSPHONOCARBOXYLATES FOR INHIBITION OF THEIR TARGET ENZYMES

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Nitrogen-containing bisphosphonates (NBPs) inhibit bone resorption by inhibiting farnesyl diphosphate synthase (FPPS), thereby preventing the post-translational protein prenylation in osteoclasts. By contrast, 3-PEHPC (NE10790), a weakly anti-resorptive phosphonocarboxylate (PC) analogue of risedronate (RIS), acts by inhibiting Rab geranylgeranyl transferase (Rab GGTase), exclusively

preventing the prenylation of Rab proteins. In addition to enzyme inhibition, the anti-resorptive potency of NBPs and PCs is determined by their affinity for bone mineral. Most potent NBPs have a geminal hydroxyl (-OH) group that, with the phosphonate groups, contributes to bone affinity. However, the role of the -OH group in enzyme inhibition is unclear, and its role in PCs (in which one of the phosphonate group of BPs is substituted with a carboxylate group) is even less well characterised. We have therefore studied analogues of these compounds in which the -OH group has been substituted with the electronegative halogens fluorine, chlorine or bromine (halo-), or with hydrogen (desoxy-). We found that all the halo- and desoxy-analogues had reduced mineral affinity compared to the parent compounds. Desoxy-RIS was 4-fold less potent than RIS at inhibiting FPPS in vitro, while the halo- analogues also exhibited decreased potency, which became more pronounced as atomic size increases ( $F > Cl > Br$ ). These trends correlated with the ability of these compounds to inhibit Rap1A prenylation in J774 macrophages, and to reduce viable number of these cells. In contrast, desoxy-3-PEHPC and 3-PEHPC were equipotent at inhibiting Rab GGTase, inhibiting Rab prenylation and reducing cell viability. Interestingly, although the halo-3-PEHPC analogues and 3-PEHPC were equipotent for inhibition of Rab GGTase in vitro, these compounds showed a similar potency trend to the halo-RIS analogues in cell-based assays ( $F > Cl > Br$ ), with the fluorinated analogue more potent than 3-PEHPC and the others less potent. This data indicates that the -OH group plays a role in the interaction of NBPs with FPPS, an effect that cannot be explained simply by electronegativity, since substitution with halogens of similar electronegativity reduces potency. By contrast, the -OH group is not crucial for the interaction of 3-PEHPC with Rab GGTase and substitution with the halogen fluorine actually increases potency for inhibition of Rab prenylation.

### P26

#### UN-COUPLING OF BONE TURNOVER MARKERS FOLLOWING GLUCOCORTICOID THERAPY FOR EXACERBATIONS OF INFLAMMATORY BOWEL DISEASE

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We previously observed that inflammatory bowel disease (IBD) patients receiving an eight week reducing course of glucocorticoids (GC) for disease exacerbations undergo rapid bone loss. To investigate the relative contribution of disease activity and GC therapy to bone loss, we examined bone markers in 49 IBD patients, comprising 18 patients about to commence GC therapy following a disease exacerbation, and 31 patients with inactive disease. We used multivariable regression analysis to study the relationship between disease activity as reflected by Crohn's and Ulcerative Colitis clinical activity scores, bone resorption (serum CTX) and bone formation (PINP) markers. There was no association between either CTX or PINP and disease activity, suggesting IBD does not directly influence skeletal metabolism. Next, we examined the effect of GC therapy on bone metabolism in IBD, by comparing CTX and PINP levels immediately before, and one week after, commencement of GC therapy in the 18 patients with active disease. GC therapy increased CTX by 60 percent, but decreased PINP by 31 percent, suggesting profound un-coupling of bone formation from resorption,  $p$  less than 0.05 versus pre-GC by paired Student's  $t$ -test. Finally, we explored the role of interactions between GC therapy and IBD disease activity, by comparing changes in biomarkers in response to GC treatment across tertiles of disease activity. Broadly similar changes were observed in serum CTX and PINP, irrespective of disease activity tertile. For example CTX increased by 61, 61 and 78 percent in the three tertiles respectively, comparing pre-GC versus post-GC. For PINP the increase was 25, 34 and 30 percent respectively in the three tertiles. We conclude that GC therapy given for exacerbations of inflammatory bowel disease causes rapid and profound un-coupling of bone turnover, which we assume underlies the bone loss previously observed in this context. There was little evidence for an interaction with current levels of disease activity, suggesting that other factors contribute to individual variations in response.

**P27**

## **BONE PROPHYLAXIS IN STEROID THERAPY: ARE WE DOING ENOUGH?**

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Steroids are widely used in several conditions. Their effects on bone formation are mediated through local and endocrine mechanisms. Patients receiving steroids for more than three months may have a bone loss of 2-4% per year in spine and femur. Even small "physiological" doses of 7.5 mg have been shown to cause bone loss. This loss continues for up to one year even after cessation of treatment and is more prominent in trabecular bone. This is of enhanced importance in the elderly who may have a lower bone mineral density (BMD), thus increasing their risk of fracture whilst on steroids. After a hip fracture 20% patients die within the first year and 50% are incapacitated.

Bisphosphonates have shown reduction in fracture rates in patients on steroids in several studies. However, they remain underused in this group.

We present the findings of an audit done in a South London DGH. The aim of the audit was to assess the use of bisphosphonates in rheumatology patients exposed to oral prednisolone for more than three months. 50 patients were assessed on various doses of steroids. 36 patients were more than 65 years of age. 18 were not on bisphosphonates. Of these 9 patients were identified who were suitable but not receiving any. This matches with the findings of Reid et al in UK Consensus Group which showed the figures of bone prophylaxis in steroid use at 14%. 14 patients were below 65 and in this group 5 patients were not on Calcium and Vit D.

We suggest using minimum doses of steroids, regular reviews of repeat steroid prescriptions and using steroid sparing agents when possible. Bisphosphonates, calcium and Vit D should be offered to all patients above 65, as primary prevention, if suitable, while on steroids. They should be used as agents of secondary prevention in the presence of fragility fractures or low BMD (T score less than 1.5). Regular BMD estimations should be offered to all patients on long term steroids. Patients <65 need to have supplemental Calcium and Vit D with regular BMD. Bisphosphonates may not be needed. These measures should help to reduce the morbidity, mortality and deformity associated with reduced BMD and subsequent fractures of the hip and spine.

**P28**

## **THE PHARMACOLOGY AND PUTATIVE FUNCTION OF BK CHANNELS IN HUMAN OSTEOBLAST-LIKE CELLS**

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Ca-activated potassium channels have been identified, and classified according to their conductance and are called BK channel (100-300 pS), IK (25-100 pS), and SK (2-25 pS). BK channels are widely expressed in many tissues and are involved in diverse cellular functions. We have shown previously using both single channel electrophysiology and PCR that BK channels are present in both osteoblast-like MG63 and SaOS2 cells. Each BK channel exists as a tetramer composed of 4 alpha-subunits usually associated with beta-subunits. The type of beta subunit co-assembled with the alpha subunits modifies both the physiological and pharmacological properties of BK channels. Here we attempt to define in MG63 cells the subunit composition of BK channels, the pharmacology of the channel and the role of the channel in proliferation.

Single channel activity was studied using the conventional patch clamp technique. Growth of osteoblast-like MG 63 cells was measured by the MTS assay.

Openings of the BK channel were readily observed in cell-attached patches at resting membrane potential (circa -65 mV). The channel was voltage-dependent, activity increasing on depolarisation. In excised outside-out patches bath-applied tetraethylammonium chloride (TEA) at 500 microM, reduced markedly the open probability, and produced an apparent reduction in single channel current, due to flickery block. This effect was reversible on washout of TEA. Interestingly, both BK-selective blockers, iberiotoxin (Ibx- 5-60 nM) and tetrandrine (5-30 microM) also blocked the channel

reversibly in excised outside-out patches. Paxilline (10 microM) blocked reversibly when being applied internally. The channel was activated by external isopimaric acid (10 microM). In long term (72 hours) treatment with TEA and tetrandrine cell numbers were significantly increased at low (1 mM and 3 microM respectively), and reduced at high concentration (>10 mM and >10 microM). In contrast, Ibx (10 -300 nM) had no effect on cell numbers.

These data are consistent with a role for BK channels in cell growth in MG63 cells and current investigations are focusing on characterising further the pharmacology of the BK channel, its subunit composition, and its putative function in these osteoblast-like cells.

**P29**

## **DETECTION OF ACOUSTIC EMISSIONS TO ASSESS PRESS-FIT STABILITY AND FRACTURE PROPAGATION DURING THE INSERTION OF A SIMULATED IMPLANT FOR CEMENTLESS HIP REPLACEMENT.**

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Hip replacement is the cheapest and most successful form of joint replacement surgery and with current advancements in implant design and operative technique, cementless stem fixation is becoming more common. As a result of this, and due to the increased complexity of the procedure, the incidence of intraoperative femoral fractures is increasing. The rate has been reported to be as high as 27.8%, and literature also reports that fractures may often go unnoticed and undiagnosed on post-operative radiographs. This study was designed to utilise properties of sonic propagation through the implant-bone interface to indicate interference fit and bone failure. An instrumented, simulated implant was designed for mounting into a materials testing machine and testing was performed using animal bone material.

A push-pull solenoid assembly was arranged to act as an actuator on the proximal surface of the implant. The materials testing machine was programmed to load the bones to fracture and signal acquisition was performed by an array of piezoelectric accelerometers located on the implant and axially on the femoral shaft. The waveforms (collected in the time domain) were evaluated to determine the variation in signal transit times throughout the loading sequence. The peak-to-peak amplitudes of the initial waveform recorded at each accelerometer were also compared to identify changes in signal transmission with increasing implant fit. The materials testing machine also monitored implant displacement throughout loading and this was correlated with the previously mentioned data. Since the system analyses individual signal variations throughout insertion, it has the potential to be applied in many different clinical settings, where bone quality varies. However, due to the complexity of bone structure and variation in implant design, further experiments using human cadaver bone and anatomically correct implants needs to be carried out.

This study shows that changes in signal propagation across the implant-bone interface can be used to describe the process of implant insertion and can act as indicators for implant fit and material integrity. From the literature, it would seem that this would be most beneficial where intraoperative fractures are easily missed (minimally invasive surgery) and where fracture risk is high (revision surgery).

**P30**

## **GAS6 AND AXL RECEPTOR TYROSINE KINASE ARE EXPRESSED BY GROWTH PLATE CHONDROCYTES AND OSTEOBLASTS**

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Endochondral bone formation occurs at epiphyseal growth plates situated at the end of long bones; in which chondrocytes are spatially and temporally distributed into precise areas of proliferation and differentiation. The differentiated, hypertrophic chondrocyte secretes a collagen-rich matrix which mineralises over time. The mineralisation process is essential for normal bone growth and is a prerequisite for vascular invasion of the growth plate. However, the precise signals regulating chondrocyte differentiation and growth

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plate cartilage mineralisation are not yet fully understood. Axl receptor tyrosine kinase (Axl RTK) and its ligand, growth arrest specific gene 6 (Gas6) have been identified as modulators of the osteogenic differentiation and mineralisation of pericytes (Collett et al, Circ. Res 92:1123-9, 2003). Therefore, this present study aimed to determine if Gas6 and Axl RTK were expressed in the growth plate and if their expression was altered with the differentiation and mineralisation state of chondrocytes.

Tibiae were dissected from one week old mice, fixed, embedded in paraffin and immunostained with antibodies specific to Gas6 and Axl RTK. Both Gas6 and Axl RTK were found to be expressed by proliferating and hypertrophic chondrocytes of the growth plate, and osteoblasts at the chondro-osseous junction. The distribution of staining suggested that there was stronger expression of both Gas6 and Axl RTK in the proliferating chondrocytes than in those of the hypertrophic zone.

This work was extended using the ATDC5 cell line as an in-vitro model of growth plate chondrocytes. RNA was extracted from cell lysates which were collected at specific differential stages during time in culture. RT-qPCR analysis of the RNA showed that Gas6 and Axl RTK mRNA are expressed, confirming the immunostaining data, however this expression did not change during time in culture. The RT-qPCR analysis also showed that Collagen X expression was 158-fold higher at day 35 of culture when compared with expression at day 7.

These studies are the first to report the expression of both Gas6 and Axl RTK by osteoblasts and growth plate chondrocytes; and further studies will clarify the importance of this signalling pathway in chondrocyte differentiation and mineralisation.

### P31

#### THE USE OF SKELETAL AGE TO VERIFY CHRONOLOGICAL AGE IN YOUTH FOOTBALL PLAYERS.

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Background and objectives: In some Asian & African countries birth registration is not compulsory which has led to incorrect age grouping in age related football competitions, creating in some instances unfair advantage. It has been proposed that skeletal age should be used as an estimate of chronological age in international football competitions and that standard radiographs or an MRI examination of the epiphyseal fusion of the distal radius should be used for this purpose. This study evaluated the extent of agreement between skeletal age estimates prepared from such radiographs, and chronological age in youth footballers.

Method: A standard x-ray was taken of the left wrist of every academy boy, at Manchester United aged 9-16yrs, at the start of every season from 2001. Consents were gained from parents and boys and ethical approval was given by Manchester University. Skeletal age was determined by both the TW3 method and the Fels method.

Results: Over the six year period 276 boys were tested and the mean skeletal ages for the three approaches were chronological 11.75 (sd 2.35), Fels 12 (sd 3.14), TW3 11.26 (sd 2.94). Repeated measures ANOVA for skeletal age found significant mean differences between all three approaches ( $F=86$ ,  $p<0.001$ ). Pairwise Bonferroni comparison between approaches, found that all three approaches differed significantly from each other ( $p<0.05$ ). The extent of variability for any single year was up to 3years between TW3 and FELS.

Conclusions: The results showed that skeletal age measured over 6 years, varied significantly, according to the measurement technique used. The extent of variation for the average measurement over six years was 8 months between Fels and TW3 which questions the accuracy of either approach. Any situation that requires confirmation of bone age whether in sport or for medico legal purposes has to consider the approach used, because the two commonly-used approaches vary so significantly in the estimates obtained.

### P32

#### EXTENT OF VITAMIN D INSUFFICIENCY IN YOUNG BRITISH WOMEN: INFLUENCE ON BONE HEALTH

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There is currently no dietary reference value (DRV) for vitamin D in the UK. Recently, other countries have redefined their recommended dietary intake values for vitamin D, e.g. the USA adequate intake (AI) recommendation for 0-50 year olds is now set at 200IU/d, rising to 600IU/d for 70 years plus. It is likely that prolonged vitamin D insufficiency in early life leads to a low peak bone mass and may also increase the risk of osteoporosis in women in later life. The principal aim of this current investigation was to determine the extent of vitamin D insufficiency and the level of dietary vitamin D intake in young British women.

The British Women's Bone Health study was a cross-sectional investigation involving a total of 275 women aged 20-29 years. Subjects were recruited from GPs in Surrey and Cornwall using a two-stage random sampling process. Lifestyle, anthropometric and dietary information was collected via questionnaire, examination and estimated 7-d food diaries respectively. BMD was measured at the lumbar spine and femoral neck by dual energy x-ray absorptiometry. Food diaries were analyzed using the computer programme Diet 5. A fasted blood sample was taken for assessment of vitamin D status. 25-hydroxyvitamin D status (25OHD) was measured using extraction and straight phase HPLC. The principal aim of this current investigation was to determine the extent of vitamin D insufficiency and the level of dietary vitamin D intake. Mean values for 25OHD were 25.6 [12.6] ng/ml (n 99), with 11% being defined as vitamin D deficient (25OHD below 12ng/ml). Over 60% of UK women had vitamin D insufficiency (25OHD below 30ng/ml). All women were below 5µg/d for vitamin D intake. Median daily intakes of calcium were below the UK RNI values for women 19-50 years. Osteopenia was found in 18% and 8% of women at LS and FN respectively. Preliminary analysis of the data did not suggest that a lower bone density was associated with poor vitamin D status. Further analyses of the full dataset are required, but these data suggest extensive vitamin D insufficiency in young British women.

### P33

#### DOES HIP ARTHRITIS LIMIT THE LIFE EXPECTANCY IN THE ELDERLY: A COMPARATIVE STUDY OF OPERATED AND NON-OPERATED GROUPS OF PATIENTS SUFFERING FROM HIP OSTEOARTHRITIS

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Introduction: Hip osteoarthritis can be a debilitating disease restricting mobility causing pain resulting in poor quality of life in the elderly. The benefits of hip arthroplasty such as pain relief and better quality of life, may reflect as an increased life expectancy in the elderly population.

Objective: The aim of this study was to compare the life expectancy of two groups of patients more than or equal to 85 years of age, referred with severe disabling hip osteoarthritis; one group was managed medically (non-operated group) while the other was operated with total hip arthroplasty (operated group).

Patients and methods: 28 patients were managed medically (non-operated group) and 28 patients underwent joint replacement surgery (operated group). Records of patients were individually analysed and at the time of study, patients were interviewed by phone or in person.

Results: The average age of patients, at the time of clinical appointment, of both non-operated and operated groups were 91.4 years ( $\pm 1.3$ ). At the time of follow-up, 36% of the patients in the non-operated group were restricted in mobility and living in nursing homes while 96% of the patients in operated group, were mobile and living independent. Patients in the operated group had a significant improvement in pain relief ( $p<0.0001$ ) while patients in non-operated group continued to complain of hip pain and were on regular pain killers. The mean survival period of the non-operated group was 2.5years and operated group was 3.9years.



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Conclusion: The results of this study showed that there was an improved quality of life and longer survival period of elderly patients, suffering from osteoarthritis of the hip, after total hip arthroplasty surgery.

### P34

#### THE SUBCELLULAR LOCALISATION OF FEO-RANK IS ALTERED WHEN CO-EXPRESSED WITH WILDTYPE RANK PROTEIN IN VITRO

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Heterozygous insertion mutations in the signal peptide region of the RANK gene have been demonstrated to cause 3 related diseases that feature hyperactive osteoclasts: familial expansile osteolysis (FEO), early-onset Paget's disease and expansile skeletal hyperphosphatasia. It has been suggested that the osteoclast phenotype could be caused by overactivation of NFkB. We have recently shown in 293 cells, that, whilst overexpression of either wildtype (WT-RANK) or mutant proteins induces NFkB activation in the absence of RANKL, expression at physiological levels does not. Only cells expressing WT-RANK show RANKL-dependent activation of NFkB supporting our observations that, whereas WT-RANK is expressed at the plasma membrane and in the golgi, the mutant proteins localise throughout the cytosol to multilamellar extensions of the endoplasmic reticulum. Taken together, these data suggest that these are, in fact, inactivating mutations. It is therefore necessary to elucidate how these inactivating mutations can result in diseases characterised by osteoclast hyperactivity.

Since patients with these diseases are heterozygous for the mutations, they will possess one copy of each gene and express both WT-RANK and mutant RANK protein. RANK acts as a trimer and this raises the possibility that WT proteins could form heterotrimers with mutant proteins. If this occurred within the ER membrane following translation of the proteins then it is conceivable that this could alter the processing, and ultimately the localisation, of either protein. In this study, we examined the possibility that localisation of WT-RANK or FEO-RANK would be altered if the two proteins were co-expressed. GFP-tagged WT-RANK and FLAG-tagged FEO-RANK were co-transfected into 293 cells. Twenty-four hours post-transfection, immunofluorescence staining demonstrated that FEO-RANK was more prevalent in the golgi when expressed in combination with WT-RANK than when expressed alone. The two proteins showed some co-localisation, suggesting that WT- and FEO-RANK proteins may associate with each other.

These results suggest that co-expression of FEO- and WT-RANK proteins could result in FEO-RANK localising to the same subcellular compartment as the WT protein. Studies are underway to determine whether co-transfection results in a physical interaction between WT-RANK and FEO-RANK and what the implications of such an interaction would be for downstream signalling.

### P35

#### ALTERED LIPID CONTENT IN OSTEOARTHRITIC FEMURS, ASSESSED USING MRI

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Although Osteoarthritis (OA) has traditionally been thought of as a cartilage disease, it has recently been proposed that OA may be a systemic disorder with many precursors, including an altered lipid metabolism and changes in the regulation of cells that form the skeletal tissues. Previous studies have demonstrated an increased fat content in cancellous bone and a greater concentration of fatty acids in cartilage of OA patients. In this work we measure the lipid concentration in the femur of a group of OA patients and a cohort of normal volunteers using MRI.

Six patients, admitted for a total hip replacement and recruited prior to surgery, were age matched to 6 healthy volunteers (mean ages 71±6.2 and 69.3±7.9 respectively). Multi-slice coronal images of the pelvis were acquired using the Dixon 2-point technique, which calculates the percentage of the MRI signal that comes from fat. Regions of Interest (ROI) were drawn around the proximal femur and the average lipid calculated, using the assumption that lipid signal is greater than or equal to water signal in bone marrow.

A one-way ANOVA was performed with Bonferroni analysis of the left side of the volunteers, the patient OA side and the patient non-OA side.

The mean lipid percent measurements in the volunteers, the patient OA side and non-OA side were 82.5±1.7, 75.0±4.5 and 76.1±3.8 respectively. There was no significant difference between the affected and unaffected sides in the OA patients, but a significant difference was observed between the normal volunteers and both the patient OA (p<0.01) and non-OA sides (p<0.05).

We found an apparent reduction in the lipid concentration in the proximal femur in both the affected and unaffected sides in patients with OA. These observations support the theory that OA is not just a cartilage disease. However they are opposite to the expected trend and it may be that the percentage water (oedema) is also increasing in the OA patients. Further work using multiple observers, a larger cohort and more sophisticated analysis of the distribution of lipid throughout the femoral head is required to clarify these findings.

### P36

#### CHANGES IN STRUCTURAL PROPERTIES LEAD TO REDUCED BONE STRENGTH IN GUNMETAL MICE

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Gunmetal (gm/gm) mice have an autosomal recessive mutation in Rab geranylgeranyl transferase, resulting in a defect in prenylation of Rab proteins in certain cell types, particularly melanocytes, megakaryocytes and platelets. Impaired Rab prenylation disrupts vesicular trafficking in these cells, resulting in a coat colour defect, prolonged bleeding, thrombocytopenia and reduced platelet granule contents in gm/gm mice. We have recently found that osteoclasts derived from 28 week-old gm/gm mice have a Rab prenylation defect and reduced resorptive capacity in vitro, but paradoxically tibiae showed no trabecular bone phenotype and a reduced cortical thickness.

To determine whether this mild phenotype results in altered mechanical properties, and whether bones from these mice may have any alteration in chemical composition, 3-point bending studies and Raman spectroscopy were performed. Structural characteristics (cortical thickness, area and mean endosteal radius (endosteal perimeter/2.π)) were measured by micro computed tomography (micro CT).

Micro CT demonstrated that cortical area and thickness were reduced in gm/gm femora compared to +/gm femora (0.76 vs. 0.86 mm<sup>2</sup> (P=0.01)) and 0.17 vs. 0.21 mm (P=0.01) respectively). Conversely, the endosteal radius was larger in gm/gm femora (0.69 vs. 0.64 mm (P=0.087)). In agreement with our previous results, a similar trend was seen in tibiae but the differences were not significant. Three-point bending analysis showed that gm/gm femora have reduced stiffness compared to +/gm mice (88.5 vs 99.7 N/mm respectively (P=0.1)) and reduced strength (failure load 11.6 vs. 15.5 N (P<0.001)), however no such differences were seen in tibiae from the same mice (P>0.15). Analysis of the Raman spectra showed no differences between the groups.

Taken together, these results demonstrate that bones from gm/gm mice are less stiff and less strong than their heterozygous littermates, most likely due to the reduced cortical thickness and area rather than altered chemical composition. A larger endosteal diameter in gm/gm bones suggests that, rather than having an osteoclast defect in vivo, these mice may have increased osteoclast activity due to other effects in the bone microenvironment, such as reduced OPG production by megakaryocytes.

### P37

#### INTER AND INTRA-OBSERVER REPEATABILITY OF KELLGREN-LAWRENCE GRADING FOR OSTEOARTHRITIS USING DXA IMAGES

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Radiological signs of osteoarthritis (OA) are typically assessed on standard radiographs. We hypothesised that it may also be possible to use Kellgren & Lawrence (KL) scoring to grade OA of the hip from



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DXA images. This would provide a cost-effective method of assessment; reducing clinical time and expense, and radiation exposure to the patient. KL scores could also be used in the future in conjunction with bone mineral density in the assessment of OA.

Images of the hip were obtained from women in the general population aged over 55 using a Lunar Expert XL DXA scanner (GE Medical Systems). Subjects were part of the Aberdeen cohort of the Osteoporosis and Ultrasound Study (OPUS), a prospective European multicentre study. Author JSG selected 39 subjects that appeared to demonstrate the full range of OA. These were graded on 2 occasions at least 1 week apart by 3 observers (CDC, KY and NB) using the KL scoring system, where grade 0 is normal, and Grade 4 represents gross changes including loss of joint space, sclerosis and additional signs of OA. Author CDC graded 33 of the images in the first instance. We evaluated intraobserver and interobserver repeatability using weighted Kappa statistics.

The 39 images comprised five grade-0, eleven grade-1, thirteen grade-2, six grade-3 and four grade-4 scans. Intraobserver repeatability achieved a weighted kappa of 0.71-0.73 (standard error 0.11)).

Interobserver repeatability achieved a weighted kappa of 0.64-0.67 (standard error 0.10).

We have demonstrated that high-resolution DXA images may be used to assess the degree of OA of the hip using KL scores. The results have a similar intra and inter observer repeatability as that reported by other studies using radiographs.

Due to the reduced resolution of DXA images, joint space narrowing cannot be measured with the same precision as on film radiographs, however all the characteristic radiographic changes seen in OA were clearly visible on the DXA images. We would recommend that grading be done on an image that is displayed as large as possible, and that the graphical overlays marking the BMD regions and the edge of the bone are not visible.

### P38

#### ASSESSMENT OF OSTEOPOROSIS AND OSTEOARTHRITIS USING ACTIVE SHAPE AND ACTIVE APPEARANCE MODELS WITH DXA SCANS.

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Osteoporosis (OP) and Osteoarthritis (OA) are the most common musculoskeletal disorders in the UK. OP is diagnosed using Dual Energy Absorptiometry (DXA), which measures Bone Mineral Density (BMD), whilst OA is traditionally assessed using standard radiographs or, more recently, high resolution MRI.

Active Shape Modelling (ASM) models the variation in shape of a set of objects. Active Appearance Modelling (AAM) is an extension of ASM that incorporates the image (Texture) and how it correlates to shape (Appearance). Previously, using radiographs, we have found that the shape of the femur is strongly related to the development of OA and the risk of hip fracture in OP.

We present results from ASM and AAM models built using DXA scans, which enables the AAM to map the BMD distribution in the femur. The AAM was built using 465 images from postmenopausal women from the Aberdeen cohort of the Osteoporosis and Ultrasound (OPUS) study.

The first 10 Shape, Texture and Appearance variables were tested for correlations with Body Mass Index, BMD and age, known risk factors for OA and OP. The majority of AAM variables were not correlated with these risk factors ( $P > 0.05$ ). As expected, BMD was correlated to 6 Texture variables ( $r = 0.25-0.52$ ,  $P < 0.05$ ). The large number of variables that are independent of other risk factors indicates that they may add to the power of these risk factors for OA and OP.

A subset of 97 images was graded for OA using the Kellgren-Lawrence (KL) grade. Significant correlations were observed between KL grade and some Shape, Texture and Appearance variables ( $P < 0.05$ ). The ASM previously developed for OA radiographs was also applied to these images. The changes that had been observed with OA in radiographs were related to increasing KL scores in DXA images ( $P < 0.05$ ).

DXA is an attractive imaging modality as it quantifies BMD, emits a lower radiation dose than radiographs and is more widely available than MRI. By using ASM and AAM models, it may be possible to improve risk assessment for hip OA and fractures in the future by quantifying the shape of the femur and mapping the BMD distribution inside it.

### P39

#### ATYPICAL PHARMACOLOGY OF P2X7 RECEPTORS IN HUMAN OSTEOBLASTS

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The P2X7 receptor (P2X7R) is usually identified by the greater affinity of dibenzoyl ATP (DBzATP) compared to ATP. Prolonged activation of P2X7R results in the formation of a non-selective pore in the cell membrane permeable to molecules as large as 900 Da. It has been suggested that P2X7R has an atypical pharmacology in osteoblast cells as an hour rather than seconds is needed for pore formation. The aims of this project were to investigate P2X7R pharmacology in human osteoblasts and also to study expression of P2X2 and P2X4 receptors. We have previously confirmed P2X7R expression in two osteoblast cell lines (MG63, early differentiation; SaOS2, late differentiation).

We studied P2X7R function by measuring pore formation upon activation using the YO-PRO 1 (a dye that fluoresces on binding to nucleic acids) uptake method. Western blotting was performed to investigate the expression of other pore forming P2X receptors (P2X2 and P2X4) and hence their potential interference with the YO-PRO 1 uptake induced by P2X7Rs.

The expression of P2X4 but not P2X2 receptor protein was found. P2X7R agonists caused pore formation after a 5 minute incubation. Concentration-effect curves (CEC) for ATP in MG63 and SaOS2 cells gave EC50 values of  $0.36 \text{ mM} \pm 0.12$  and  $0.43 \text{ mM} \pm 0.15$ , respectively, while DBzATP gave EC50 values of  $0.5 \text{ mM} \pm 0.1$ , and  $0.36 \pm 0.15$ , respectively. CEC for ATPgammaS and 2MeSATP in SaOS2 cells gave EC50 values of  $0.6 \pm 0.09$  and  $0.42 \pm 0.09$ , respectively. Receptor desensitization experiments showed that the CEC for ATP was not affected by prior treatment with 3 mM ATP.

The affinities of the four agonists to induce pore formation and the lack of receptor desensitisation suggest that the P2X7R is likely to be mainly responsible for YO-PRO 1 uptake in these two osteoblast cell lines, although the P2X4 receptor could also be involved. The unexpectedly low affinity of DBzATP confirms earlier studies showing the atypical pharmacology of the P2X7R in osteoblast cells. Further experiments are required to elucidate the function of P2X7R in these cells.

### P40

#### ENOS KNOCK OUT MICE SHOW REDUCED TRANSLOCATION OF BETA-CATENIN TO THE NUCLEUS WHEN STIMULATED WITH PULSATILE OR OSCILLATING FLUID FLOW

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Fluid flow through the bone canaliculi has been theorized to produce enough fluid shear stress (FSS) to induce bone remodelling by stimulating osteocytes to rapidly produce a variety of signalling factors. These include nitric oxide (NO), via the enzyme endothelial NO synthase (eNOS), which in turn modulates the activity of all bone cells, including osteoblasts. eNOS knockout mice have a reduced bone mass mainly due to defective osteoblasts. FSS can also activate transcription and recently beta-catenin was shown to translocate to the nucleus in osteoblasts after exposure to 1 hour of FSS of 1 Pa. In this study we subjected osteoblasts to either pulsatile or oscillating fluid flow calculated to produce a peak shear stress on the cells of 1Pa. Translocation of beta-catenin to the nucleus was observed as a marker of the cellular response to FSS. Translocation was seen in 60% of cells and we found no significant difference in the response to pulsatile or oscillating flow. We then examined the response to FSS in primary osteoblasts from eNOS knockout mice and found a significant reduction (45% of cells) in which translocation of beta-catenin was observed. Again, this response was similar in both flow systems.

These experiments identify eNOS as an upstream regulator of beta-catenin translocation.

**P41****THE ROLE OF P21CIP1/WAF1 IN GLUCOCORTICOID INDUCED GROWTH RETARDATION**HC Owen<sup>\*[1][2]</sup>, SF Ahmed<sup>[2]</sup>, C Farquharson<sup>[1]</sup><sup>[1]</sup>Bone Biology Group, Roslin Institute, Edinburgh, UK; <sup>[2]</sup>Bone & Endocrine Research Group, Royal Hospital for Sick Children, Glasgow, UK

It has been shown that cell cycle genes play an important role in the coordination of chondrocyte proliferation and differentiation. The inhibitory effects of glucocorticoids (GCs) on chondrocyte proliferation are consistent with GCs disrupting cell cycle progression and promoting cell cycle exit. Progression of the cell cycle is promoted by the activation of cyclin-dependent kinases (CDKs). Cyclin-dependent kinase inhibitors (CDKIs) play an important role in maintaining growth arrest and cell differentiation by binding to, and inactivating, CDKs. Reports indicate that some CDKIs force cells to exit from the cell cycle and differentiate, and that the expression of the CDKI p21CIP1/WAF1 is increased in terminally differentiated cells. In this study we have used the chondrogenic ATDC5 cell line to examine changes in the expression of CDKIs during chondrocyte differentiation and after exposure to Dexamethasone (Dex). Increased expression of p21 mRNA and protein was observed during chondrocyte differentiation, whereas expression of other CDKIs remained unchanged. After exposure to Dex (10-6M) for 6h or 12h, p21 mRNA and protein levels increased compared to expression in control cells. To determine whether the increased expression of p21 with Dex was physiologically important, we studied skeletal growth in 3-week-old mice treated with 5mg/kg Dex for 7 days. At day 7, body weight and body length in Dex treated mice were significantly reduced compared to untreated mice (14.8%,  $p < 0.05$ ). Tibiae lengths were also significantly reduced (4.6%;  $p < 0.05$ ). Within the proximal tibiae, the epiphyseal growth plate in Dex mice was significantly narrower than controls (18.1%;  $p < 0.05$ ), due to a significant reduction in the width of both the proliferative and hypertrophic zones (15.2% and 20.1% respectively;  $p < 0.05$ ). In 4-week-old mice lacking a functional p21 gene, Dex caused a significant reduction in body weight when compared to saline control null mice (9.0%,  $p < 0.05$ ), but this was not significantly different from the reduction in body weight observed in Dex-treated wild-type littermates (9.4%). These findings suggest that p21 does not directly contribute to GC-induced growth retardation in vivo, although it will be interesting to examine the effects of Dex on the growth plate of p21 null mice

**P42****VARIATION IN WNT-7A IS UNLIKELY TO BE A CAUSE OF FAMILIAL CONGENITAL TALIPES EQUINOVARUS**G Liu<sup>[1]</sup>, J Inglis<sup>[1]</sup>, A Cardy<sup>[2]</sup>, D Shaw<sup>[1]</sup>, S Sahota<sup>[1]</sup>, L Sharp<sup>[3]</sup>, Z Miedzybrodzka<sup>\*[1]</sup><sup>[1]</sup> Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, Scotland, UK; <sup>[2]</sup> Department of Public Health, University of Aberdeen, Aberdeen, Scotland, UK; <sup>[3]</sup> National Cancer Registry Ireland, Cork, Ireland

Congenital talipes equinovarus (clubfoot/CTEV) is a common developmental disorder of the lower limb, with a prevalence of 1-4 per 1000 births worldwide. CTEV aetiology has been little studied and is poorly understood but genetic factors are thought to be important. Dietz (2005) suggested WNT7A as a candidate gene for CTEV on the basis of a genome-wide scan for linkage in a large multi-case family. The WNT gene family consists of at least 19 members; several of them are expressed in the limb, where they control patterning, outgrowth and/or differentiation (reviewed by Church and Francis-West, 2002). In mice and chicken, WNT7A provides a signal for pattern formation during limb development. In human, mutations in WNT7A have been found in individuals with a range of limb malformations including Fuhrmann syndrome and Al-Awadi/Raas-Rothschild/Schinzle Phocomelia syndrome. We further investigated the role of WNT7A using a family-based linkage approach in our large series of European multiplex CTEV families. We analysed the two microsatellite markers used in the Dietz study, of which one (D3S2385) is intragenic, and the other (D3S2403) is 700 kb 5' to the start of the gene. Additionally we typed a third marker (D3S1252) located 20 kb from the 3' end of the gene. Ninety-one clubfoot families, comprising 474 individuals of whom 191 were affected, were genotyped. Linkage analysis was conducted using the Linkage software package with different models of inheritance. No LOD score using any model approached 3. D3S2385 gave a LOD score of 1.5 at a recombination fraction of 0.2 when a plausible dominant model with 50% penetrance was applied. Our evidence to date suggests that the WNT7A gene is unlikely to be a major cause of familial CTEV.

**P43****EFFECTS OF AGEING ON CORTICAL AND TRABECULAR BONE IN RADIUS AND TIBIA: A HIGH-RESOLUTION PQCT STUDY**S Kaptoge<sup>\*[1]</sup>, N Dalzell<sup>[1]</sup>, N Morris<sup>[2]</sup>, B Koller<sup>[3]</sup>, P Rueggsegger<sup>[3]</sup>, A Berthier<sup>[4]</sup>, L Braak<sup>[4]</sup>, J Reeve<sup>[1]</sup><sup>[1]</sup> Department of Medicine, University of Cambridge, Cambridge, UK; <sup>[2]</sup> Hethersett Surgery, Norfolk, UK; <sup>[3]</sup> Scanco, Zurich, Switzerland; <sup>[4]</sup> MEDES, Toulouse, France

**BACKGROUND:** The steep rise in radius (Colles) fractures after menopause is unexplained. We wondered whether poor performance of SXA and DXA in prediction was due to their inability to detect trabecular disconnection or cortical porosity. The new Xtreme pQCT scanner (Scanco) provides a resolution approaching 0.1mm to redress these deficiencies. We recruited an unselected population-based sample of 55 men and 70 women aged 20-79.

**METHODS:** Each subject had measurements on the radius and tibia. The pQCT output includes: trabecular spacing, average bone density (D.Avg), trabecular bone density (D.Tb), meta-trabecular bone density (D.TbMeta), inner-trabecular bone density (D.TbInn), compact bone density (D.Comp) and mean cortical thickness (C.Th). We investigated cross-sectional age-related changes these parameters by fitting a regression model to test linear and quadratic effects of age, and assessed whether variability of these parameters was constant with age using the residual variance.

**RESULTS:** Age-related trends in trabecular spacing were not significant. In women, significant quadratic association with age were observed for: D.Avg (radius), D.Comp (both sites), and C.Th (radius); while significant inverse linear association with age were evident for: D.Avg (Tibia), D.TbMeta (both sites), and C.Th (radius). D.Tb and D.TbInn were linearly associated with age only at the Tibia ( $p < 0.010$ ). Uniquely, the residual variance for radius D.Comp increased exponentially across the decades of age ( $p = 0.001$ ), indicating that some developed greatly increased haversian canal diameter. In men, there was significant inverse linear association with age at radius and tibia for: D.Avg, D.TbMeta, D.Comp, and C.Th (all,  $p < 0.033$ ). D.Tb and D.TbInn were linearly inversely associated with age only at the Tibia ( $p < 0.05$ ), while in no case did the residual variance differ significantly across the decades of age (all,  $p > 0.144$ ).

**CONCLUSIONS:** Since cortical bone stiffness is reduced as a function of the cube of porosity, the radius cortex in a fall might become incompetent in women with low cortical density/high haversian canal diameter (difficult to assess with SXA or DXA). Interestingly there was no evidence of complementary effects (increased spacing) in trabecular bone. High-resolution pQCT is proving valuable in non-invasive micro-structural studies on the human skeleton in the investigation of bone quality and its determinants.

**P44****BONE MARROW STROMAL CELLS AND BIOMIMETIC COLLAGEN-HYDROXYAPATITE SCAFFOLDS FOR SKELETAL TISSUE ENGINEERING**JI Dawson<sup>\*[1]</sup>, DA Wahl<sup>[2]</sup>, JT Czernuszka<sup>[2]</sup>, ROC Oreffo<sup>[1]</sup><sup>[1]</sup> University of Southampton, Southampton, UK; <sup>[2]</sup> Oxford University, Oxford, UK

The extracellular matrix (ECM) is of defining importance for skeletal tissue engineering. ECM regulates cell proliferation and differentiation through direct cell receptor interaction, controlled diffusion of soluble factors and attenuated transmission of mechanical signals. The importance of collagen in ECM and its role in the temporal cascade of events leading to new bone from progenitors suggests it as a strong candidate material for tissue engineering scaffolds.

Using a biomimetic approach, type I collagen matrices of defined porosity, incorporating precipitated nano-sized, carbonate substituted hydroxyapatite (HA) crystals, were assessed for osteo- and chondrogenic conduction using unselected and STRO-1 immunoselected human bone marrow stromal cells (HBMSCs). HBMSCs were selected for expression of STRO-1 using magnetic activated cell sorting and culture expanded prior to dynamic seeding onto a collagen-HA composite (mean pore size = 135 micron) or pure collagen scaffold (mean pore size = 64 micron) to assess osteo- or chondrogenesis respectively. Cells were cultured in vitro in basal, osteogenic (BMP2, Ascorbate, Dexamethasone), or chondrogenic (TGF beta 3, Ascorbate, Dexamethasone) conditions for 21/28 days in comparison to a micromass culture chondrogenic model and calcium

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phosphate scaffold osteogenic model. Cell response was assessed by micro CT (VTEK, 5 micron resolution), histochemistry (Alcian blue/Sirius red, alkaline phosphatase [ALP]) and expression of osteo and chondrogenic markers by immunohistochemistry and RT-PCR.

Chondrogenesis was evident in pellet and collagen systems by an abundance of sox-9 expressing chondrocytes embedded in a proteoglycan and collagen II rich ECM. Regions of matrix immediately peripheral to the scaffold stained positive for BSP. By RT-PCR, collagen and pellet cultures expressed Collagen II, IX and XI, Aggrecan, Osteopontin and Osteonectin and were negative for Collagen I and Osteocalcin. Osteogenic differentiation on collagen-HA and calcium phosphate scaffolds was indicated by increased ALP activity and gene expression compared with basal conditions. Histology and micro CT established extensive penetration and matrix synthesis within the collagen-HA scaffold, areas of which were immunologically positive for BSP and osteocalcin.

Tailored biomimetic collagen matrices offer highly conductive scaffolds for growth and differentiation of HBMSCs. Tissue specific gene-expression and corresponding matrix synthesis indicate the potential of ECM cues for osteo and chondrogenesis.

### P45

#### A NOUVELLE METHOD OF APPLICATION OF TENSILE FORCES TO MESENCHYMAL STEM CELLS

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Application of forces to cells may affect cell metabolism, proliferation and differentiation. Regulation of bone formation may be affected by strain characteristics such as magnitude, frequency, duration of loading and total number of cycles. The optimal combination of mechanical loading parameters for bone formation remains undefined.

We have developed a bioreactor that is simple and inexpensive and provides an accurate method of application of tensile strain to cells in a graduated manner in the physiological range for bone.

A mechanical bioreactor was designed and manufactured out of acetyl-homopolymer, consisting of a chamber accommodating cell culture fluid and a perspex lid. The cell culture substrate consisted of rectangular strips machined out of Virgin Polystyrene. The strips were fixed at one end within the bioreactor with the treated surface upward-facing. A downward displacing force was applied to the other end of the culture strip by means of a vertically placed loading shaft attached to an actuator on a force-applying machine.

A system of applying tensile force to cells grown on treated virgin-polystyrene strips in culture fluid was produced. The system allowed forces to be accurately calculated in relation to an applied displacement. A mathematical system was used to calculate the strain at various points on the culture strip using the Euler-Bernoulli equation. The arrangement provided a method of applying tensile forces in a graduated manner across the length of the strip and the accurate determination of the amount of strain at each point along the strip. Mesenchymal stem cells were successfully grown on the strips and application of force caused a differentiation into osteoblastic cells.

This represents a nouvelle method of application of tensile force to cells. The system allows for investigation of the effects of tensile forces on cells using different frequencies, strain rates, different magnitudes of strain and investigation of the effects of total cycle number on cells. It represents one of the most accurate quantification of strain on cells and unlike most other systems, allows application of forces in the physiological range for bone.

### P46

#### REGULATION OF OSTEOGENIC MARKER GENE EXPRESSION BY GROWTH HORMONE IN OSTEOBLASTIC CELLS DERIVED FROM HUMAN ALVEOLAR BONE IS DONOR AGE-DEPENDENT

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Growth hormone (GH) is an important systemic regulator for longitudinal bone growth due to its action on precursor cells in the epiphyseal cartilage during childhood and adolescence. Additionally, there are evidences of a central role for GH in the maintenance of bone mass in adults by regulating bone remodelling through a complex interaction of circulating GH and insulin-like growth

factors. Our previous study demonstrated that the action of GH on key parameters of in vitro osteogenesis is influenced by cell donor age. We therefore hypothesized that GH regulates human osteoblastic differentiation in a donor age-dependent way. To test such hypothesis we investigated the effect of GH on osteogenic marker gene expression in osteoblastic cells from adolescent (13-15 year-old), young adult (18-35 year-old) and adult (36-49 year-old) donors. Cells were obtained by enzymatic digestion of alveolar bone fragments from three donors for each age range, and cultured in osteogenic medium until subconfluence. First passaged cells were cultured in 24-well culture plates (2 x 10<sup>4</sup> cells/well) in osteogenic medium without or with GH 100 ng/ml. At day 7, gene expression of type I collagen (COL-I), alkaline phosphatase (ALP), osteopontin (OPN), osteocalcin (OC), and Runx-2/Cbfa1 was assessed by quantitative real-time polymerase chain reaction. Comparisons were carried out using the non-parametric Kruskal-Wallis test for independent samples (level of significance: 5%). If Kruskal-Wallis test showed significance, the Fisher's test was performed. The effect of GH on gene expression of ALP and OC was significantly affected by age donor as follows: adult < young adult = adolescent. Although non-statistically significant, donor age influenced the effect of GH on gene expression of COL-I, OPN and Runx-2/Cbfa1, showing a tendency to be higher in cells from adolescents. In conclusion, we have demonstrated that the effect of GH on osteogenic marker gene expression in human osteoblastic cells is donor age-dependent. Such aspect must be taken into consideration to establish an efficient GH therapy.

### P47

#### HIGH DENSITY POLYETHYLENE AS A SUBSTITUTE FOR BONES IN BIOMECHANICAL STUDIES

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**Introduction:** The use of artificial bones (Sawbones) has become a popular substitute for cadaveric bones in orthopaedic implant testing. However, other commercially available materials such as stainless steel, aluminium and High density polyethylene (HDPE) maybe more suitable for implant testing. **Aim:** We aimed to test the suitability of HDPE, Aluminium and Stainless steel as an alternate material for artificial bones in orthopaedic implant testing.

**Materials and methods:** Cylinders of all four materials were made to represent different parts of the human femur, ie 50mmx5mm to represent the proximal diaphyseal cortical bone and 75mmx3mm to represent diaphyseal-metaphyseal junction cortical bone. Intramedullary (IM) nails were custom made from stainless steel alloys and the proximal end was clamped and connected to a load cell (Instron machine) and axial force applied. Each test was repeated thrice using different type of cylinder material and dimensions.

**Results:** In 50mmx5mm: For every 0.25KN increase in axial force, composite model using HDPE and sawbones had identical force-displacement curves. The IM nail displacement by 0.2mm at 0.25KN, 0.038mm at 0.50KN and 0.055mm at 0.75KN. Stainless steel and aluminium had similar force-displacement curves but variable when compared to HDPE. In 75mmx3mm: Similarly, for every 0.25KN increase in axial force, composite model using HDPE and sawbones had identical displacement of the intramedullary nail ie, 0.05mm at 0.25KN, 0.10mm at 0.50KN, 0.17mm at 0.75KN.

**Conclusion:** For IM nails implant testing, HDPE is comparable to artificial bones and hence maybe used as an alternate material for biomechanical testing.

### P48

#### CORTICAL AND TRABECULAR BONE FROM MICE COMPARED BY RAMAN SPECTROSCOPY

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Cortical bone forms the shafts of long bones and encases regions of trabecular bone, thereby combining strength with lightness where loads are less high. Trabecular bone is more metabolically active and it is still unclear whether its matrix has the same composition and properties as cortical bone matrix. To date, little work has been done comparing the bone types and, to our knowledge, none using Raman spectroscopy.



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We acquired Raman spectra from cortical and trabecular bone from 5 month old (skeletally mature) male mice (C57Bl6) using a Renishaw Via microscope. A 785 nm laser with a x63 immersion objective were used. Tibiae were sectioned axially to expose cortical and trabecular bone. Between 10 and 40 spectra were obtained from random sites for each sample. Spectra were pre-processed in Matlab; de-noised using a wavelet technique, background signal removed using an iteratively fitted polynomial and normalised to total mineral peak area. Principle components analysis (PCA) was performed on the resulting dataset. Spectra were averaged for each bone and peak parameters (intensity, centroid, width) were calculated by fitting a pseudo-Voigt profile to the major peaks.

Scores for PCA coefficients 1, 2, 3 and 5 were significantly different ( $P < 0.05$ ) between the bone types. Coefficient 1 clearly selected mineral peaks and scores (0.013 (cortical) and -0.018 (trabecular)  $P = 0.0002$ ) showed cortical bone had more mineral than trabecular bone. Sample orientation and laser beam polarisation effects make interpretation of parameters obtained from individual peaks difficult. More work is required to link peak parameters to structure and composition at each sample site.

PCA, examining the whole spectrum, showed a clear difference between bone types, and that cortical bone was more mineralised than trabecular bone. Sample orientation and beam polarisation effects vary between peaks and further analysis of these effects may enable us to separate structural and compositional information.

### P49

#### THE MECHANICAL, MATERIAL AND CHEMICAL PROPERTIES OF CORTICAL BONE FROM NNOS NULL MICE

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Nitric oxide (NO) is an important signalling molecule in many tissues. Neuronal nitric oxide synthase (nNOS) is one of the enzymes responsible for NO production in bone. nNOS knock out (KO) mice have been found to have high bone mineral density (BMD) and reduced bone turnover indices when compared to wild type (WT) controls but the effects on bone matrix properties are not known.

The mechanical properties of tibiae from 5 month-old male mice (6 KO, 10 WT) were measured using three-point bending. The density (by immersion) and speed of sound (ultrasound) of the cortical bone were measured and the elastic modulus calculated. Chemical content was obtained by Raman spectroscopy. Finally, bones were ashed to determine composition as water, organic and mineral content.

The ablation of nNOS increased the mechanical properties of cortical bone. Tibial stiffness (101 (KO) vs. 77 (WT) N/mm  $P = 0.003$ ), load at and work to the failure point (18.8 N (KO) vs. 13.6 N (WT),  $P < 0.001$  and 2.65 (KO) mJ vs. 1.47 (WT) mJ,  $P = 0.004$ ) were all significantly greater. No differences were found, however, between KO and control groups in density or modulus with  $P$  values being 0.35 or more. Comparisons between Raman spectra suggest KO bones contain more mineral than WT samples. When ashed, tibiae from KO mice contained more water (30.9 (KO) vs. 27.9 (WT) %) and mineral (54.3 (KO) vs. 40.8 (WT) %) and less organic material (14.8 (KO) vs. 31.3 (WT) %) than bones from WT animals ( $P$  values 0.045,  $< 0.001$  and  $< 0.001$  respectively).

The increase in mineral content in bones from nNOS knock out mice leads to stronger, stiffer and tougher bones when compared to WT controls. These results confirm the importance of nNOS in the development of bone strength.

### P50

#### THE EFFECTS OF ZOLEDRONATE ON ILIAC BONE REMODELLING IN STROKE PATIENTS

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The effects of zoledronate and stroke on human trans-iliac bone biopsies were evaluated with histomorphometry. Parameters were compared to four published reference ranges from healthy subjects without stroke.

Methods. Fourteen (3F, 11M) acute stroke patients (mean age 71±11) were randomly assigned to a single dose of zoledronate, ZOL 4 mg

(n5) or placebo, PBO (n9) within 5 weeks. Biopsies were obtained 10 weeks later (7 hemiplegic, 7 unaffected side). Hip DXA was performed at randomisation and 6 months later. HM measurements were made using light microscopy, a digitising pad and image analysis. Dynamic indices were calculated using fluorochrome double-labelled sections (n8). Osteoclasts and their precursors were identified on frozen sections using TRAP staining.

Results. Bone formation indices (OS/BS and MS/BS) were significantly lower in the ZOL and PBO groups compared to reference subjects irrespective of the side of biopsy. OS/BS for ZOL=  $7.7\% \pm 3.9$  and PBO=  $3.0\% \pm 1.7$ , MS/BS for ZOL=  $2.1\% \pm 0.8$  and PBO  $2.2\% \pm 1.2$  (\*significantly lower than four reference means by Dunnett's t-test  $p < 0.05$ ).

Unexpectedly, OS/BS was higher after ZOL than PBO ( $p = 0.008$ ) although mineralising surfaces were not different. There were no differences between hemiplegic and unaffected sides for any HM parameter despite asymmetric reductions in hip BMD. Thus, iliac crest HM parameters do not reflect side-to-side differences in hip BMD in stroke patients. Indices of bone resorption differed between ZOL and PBO groups; although ES/BS was similar (and similar to reference subjects) there were fewer osteoclasts/precursors after ZOL than after PBO (median 0.05mm<sup>2</sup> (IQR 0.01,0.17) vs. 0.35mm<sup>2</sup> (0.11,0.52),  $p = 0.023$ ).

Conclusion. We recently published data showing that a single dose of zoledronate prevented bone loss after stroke, measured by hip DXA. These new bone biopsy data suggest a mechanism for bone protection since 10 weeks after infusion, osteoclast numbers were reduced following zoledronate treatment. Stroke patients also had lower forming and mineralising surfaces than healthy reference subjects, indicating a deleterious effect of stroke/immobilisation on bone formation. These results help to explain the deleterious effects of stroke and immobility on the skeleton and provide a rationale for zoledronate therapy.

### P51

#### SERUM MARKERS OF BONE TURNOVER: A NOVEL APPROACH TO MONITORING THE NATURAL HISTORY OF CHARCOT OSTEOARTHROPATHY

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Charcot Osteoarthropathy is a destructive pathology leading to fractures, dislocations and deformity and is more common in Type 1 diabetic patients. The pathogenesis is unknown, but the disease is progressive and passes from an acute to a chronic condition.

Treatment by immobilisation in plaster casts is standard. At present, treatment needs to be monitored by imaging techniques (X-Ray, MRI and Bone scintigraphy). In this study, we evaluated serum markers of bone metabolism in Charcot osteoarthropathy. Serum bone markers were measured at regular time points as additional tests to determine their use in monitoring the bone disease as the condition passes from acute to chronic stages. Serum bone markers overcome the range of variables well known for urine samples.

We report a fifty year old female with type 1 diabetes who presented with hot swollen foot. X-ray was normal but technetium diphosphonate bone scan showed focal increased activity in the mid-foot and the diagnosis of Charcot osteoarthropathy was made. She was treated with a total contact cast. Serial measurements of bone turnover were made.

Serum Tartrate Resistant Acid Phosphatase 5b isoenzyme (TRAP) a bone resorption marker, and serum Bone Specific Alkaline Phosphatase (BAP) a bone formation marker were evaluated by an ELISA using an automatic sample processor (Triturus, Grifol), with our standard procedure for method evaluation. The assay uses an immunoenzymetric methodology with spectrophotometric detection. In order to compare the clinical effectiveness of BAP and TRAP measurements, results were converted to T-Scores using the formula  $T \text{ score} = \text{Observed value} - \text{mean of the reference range} / \text{SD reference range}$ .

At presentation, T-score (BAP) was 3.34 and T-score (TRAP) was 2.56. These scores then peaked at 3 month (4.44 and 2.96 respectively) and this was associated with fracture/dislocation on the radiograph. Over the next five months there was clinical resolution as the fracture/dislocation became stable and both scores decreased. At 10 months they reached 1.48 and 0.44 approaching normal limits for this patient group (post-menopausal females).

Serum markers of bone metabolism reflect changes observed by imaging techniques suggesting they may be useful in monitoring treatment response, hence potentially reducing radiation exposure.



**P52**

## THE USE OF STATINS IN TISSUE ENGINEERING TO ENHANCE HUMAN BONE CELL CULTURE

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The in vitro culture of human bone cells for tissue engineering has great potential as a therapy for patients suffering from bone loss as a result of trauma or disease. However, this relies on efficient differentiation and maturation of bone cell precursors and mature bone cells producing mineralised matrix. This study has looked at using statins, specifically simvastatin, for the novel use of enhancing human bone cell tissue engineered constructs.

Statins are commonly prescribed cholesterol lowering drugs, that also have beneficial side effects in enhancing bone differentiation, maturation and matrix formation, generally associated with an increase in BMP2 mRNA. Simvastatin has been added as a supplement to media in 2D and 3D primary human mesenchymal stem cell (hMSCs) and primary human osteoblasts seeded constructs. Realtime RT-PCR has been used to analyse gene expression, picogreen assays to determine proliferation and calcium assay and MicroCT for mineralisation data.

Our optimisation results in human osteoblasts have shown that simvastatin acts in a concentration dependent fashion on BMP2 expression. Concentrations of 5microM simvastatin produce the highest levels of BMP2 expression with an associated decrease in cell proliferation (both significant to  $p < 0.05$ ). Whilst lower concentrations of simvastatin have shown to have the opposite effect on cell proliferation, 0.0001 to 0.1microM showing increased proliferative effects. Dosages of 5microM simvastatin added to cultures of primary human mesenchymal stem cells (hMSC) and primary human osteoblasts, have been shown to significantly increase expression of osteopontin and BMP2, whilst significantly down regulating other bone related genes. Simvastatin treated cultures of osteoblasts and hMSC's seeded to PLLA scaffolds, also showed this significantly reduced proliferation of osteoblasts. MicroCT analysis however, highlighted a trend of increased volume of mineralized matrix being produced per osteoblast, not seen in hMSC's. Together these results suggest that statins play a role in altering the coupled regulation, of cell cycle progression and osteogenic differential gene expression. Therefore an optimised use of varied concentrations of simvastatin at different time points in 2D and 3D human bone cell culture could enhance proliferation and matrix mineralisation within tissue engineered constructs.

**P53**

## CULTURED OSTEOBLASTS FROM OSTEOARTHRITIC AND OSTEOPOROTIC PATIENTS DISPLAY DIFFERENTIAL GENE EXPRESSION PROFILES

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Characteristically, osteoarthritic bone is hypomineralized and increased in quantity with a more porous and disorganised structure compared to normal bone. The differences in bone quality indicate a change at the cellular level but little is known about the difference in the osteoblast phenotype. We have hypothesized that there is an abnormality in the osteoblasts that contributes to this condition. Osteoporosis (OP), in contrast, is a disease in which the bone is reduced in quantity, but more normally organized. By studying the profile of gene expression in osteoblasts from patients with these opposing bone phenotypes we aimed to elucidate alterations in normal signalling pathways that would help us understand the pathogenesis of osteoarthritis (OA), and osteoporosis (OP).

Microarrays were used to analyse differences in gene expression between primary osteoblasts derived from osteoporotic or osteoarthritic bone. Femoral heads were obtained from three female patients undergoing a hip replacement due to OA and three female patients having a hemi-arthroplasty following a fractured neck of femur attributed to OP. Osteoblast-like cells were cultured from fragments of trabecular bone and grown to 80% confluence. Total RNA was extracted by TRIzol and further purified via Qiagen RNeasy columns, RNA quality was confirmed by analysis on an Agilent Bioanalyzer. Samples were hybridised individually to Affymetrix HG-U133 Plus2 Genechips and data analysed using GCOS, DMT, Bioconductor and NetAffxTM software using OP patients as baseline.

The DAVID Bioinformatic resource was used to aid biological interpretation.

Preliminary statistical analysis revealed a significant number of genes that were consistently up or down regulated between disease types. Using Student's t-test we found 116 genes to be differentially expressed with a significance  $P < 0.05$  in both DMT and Bioconductor analysis. Biological interpretation of these genes has revealed pathways already implicated in bone metabolism, such as TGFbeta signalling, and genes which have previously been associated with OA cartilage, or other bone conditions.

These data support our hypothesis that there are significant differences in osteoblasts derived from OA and OP patients, and provide a starting point for further investigations into the biological processes involved.

**P54**

## BONE MARROW QUANTIFICATION USING 3.0 TESLA MAGNETIC RESONANCE IMAGING

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Introduction: Bone marrow consists of both red and yellow components, the proportions of which are thought to be related to the remodelling capacity of bone with implications for osteoporosis and the late effects of cancer treatment. Magnetic resonance spectroscopy (MRS) may be used to measure relative water (red marrow) to fat (yellow) ratio of bone marrow albeit at low spatial resolution (typically 1 cm<sup>3</sup>) and previous studies have often concentrated on one vertebral body of the lumbar spine. This work examines the normal variations of marrow content in terms of age, gender and skeletal site, using spectroscopic and high resolution fat mapping techniques at 3.0 Tesla.

Materials & Methods: A total of 16 normal subjects (aged between 8 and 57 years) were investigated on a 3.0 Tesla GE Signa system. Non-water suppressed spectra were acquired from single voxels in the calcaneus and multiple vertebral bodies within the lumbar spine (L1 to L5). Data was also correlated with bone mineral density (BMD) measured in six subjects using dual energy x-ray absorptiometry (DXA). Fat-water phantoms were constructed with known variations in fat content (0-100 %), to compare the accuracy and uniformity of four imaging techniques with MRS. From this, two of the techniques were subsequently used in the subjects to obtain in vivo fat fraction (FF) maps of lumbar spine.

Results: Fat content was an order of magnitude greater in the heel compared to the spine. Significant age-related increases and gender differences were demonstrated in the spine. Trends in vertebral bodies within the same subjects were also shown to be significant, with fat content increasing L5 greater than L1. Population coefficient of variation was greater for fat fraction compared to BMD. Phantom and in vivo results demonstrated one fat mapping technique to be superior (IDEAL) in terms of accuracy and uniformity and correlated significantly with MRS values.

Discussion: This work demonstrates significant age, gender and skeletal variations of marrow content which provides an important baseline for future clinical studies. The IDEAL imaging sequence provides a high resolution alternative to MRS for the quantification of the distribution of fat composition.

**P55**

## HABITUAL PHYSICAL ACTIVITY AND OSTEOARTHRITIS: HOW CAN WE BEST INVESTIGATE WHETHER THERE IS A LINK BETWEEN THEM?

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Osteoarthritis (OA) is the most common form of arthritis. Vigorous physical activity (PA) has been found to increase the risk of developing hip and/or knee OA. However the role of habitual PA in the development and progression of the disease is still controversial. The aim of this investigation was to validate the Aberdeen bone specific PA questionnaire (bsPAQ) in patients with OA of the hip and/or knee using a triaxial activity monitor (RT3, Stayhealthy, Inc), as the objective measure of PA.

The Aberdeen bsPAQ was designed to measure the amount of metabolic and mechanical aspects of an individual's habitual PA. To

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validate its use, 10 Scottish Caucasian women (mean age= 62.2 ± 6.4) suffering from hip and/or knee OA were recruited. Their results were combined with the results of 24 subjects who took part in a previous validation study. Subjects visited the Osteoporosis Unit twice; they were asked to wear the RT3 for 7 days, and fill in the bsPAQ and carry out three self-assessed anthropometric measurements (waist, hip and calf circumference), which I also measured and validated during their second visit

A significant correlation was observed between the metabolic component of PA measured by the bsPAQ and the RT3 ( $r=0.34$ ,  $p=0.05$ ). It was shown that 30% of the participants were classified into the same tertile of PA, while only 6% were grossly misclassified by the bsPAQ. There was also significant correlation for light activities ( $r=0.39$ ,  $p=0.02$ ) and the mechanical component of PA ( $r=0.35$ ,  $p=0.05$ ) measured by the two PA tools. Anthropometric measurements were accurately reported and measured by subjects.

In conclusion, the results obtained from the study suggested that the Aberdeen bsPAQ is a valid instrument in measuring PA in OA of the hip and/or knee.

### P56

#### SAMPLE SIZE REQUIREMENTS FOR BONE DENSITY PRECISION ASSESSMENTS AND EFFECT ON PATIENT MISCLASSIFICATION: A MONTE CARLO SIMULATION STUDY

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Bone density measurements are widely used for serial monitoring of patients with suspected or confirmed osteoporosis. A sample size of 30 degrees of freedom (df) for bone mineral density (BMD) precision studies, which is currently recommended by the International Society for Clinical Densitometry (ISCD), may be insufficient for reliably categorizing change among clinical patients. In this study, we used Monte Carlo simulation to evaluate the effect of precision study sample size on identifying change in clinical patients.

Data from the Manitoba Bone Density Program were used for the analyses. Precision estimates (root mean square standard deviations; RMS-SD) from 198 spine and 193 total hip scan-pairs were used to categorize change for 1420 patients undergoing BMD monitoring. Relative to this reference change fraction (RCF), cutoffs for RMS-SD were identified that gave specified deviations from the RCF (-25% to +25%). Confidence limits (95% and 80%) for these RMS-SD values (5 to 500 df) were estimated using 'bootstrap' samplings with 10,000 iterations. Level of deviation from RCF was derived for each sample size using simulation results for hip and spine BMD sites.

A sample size providing 140 df is needed to avoid over-diagnosing spine change by 5% and 150 df to avoid under-diagnosing spine change by 5% with 95% confidence limits. A sample size of 65 is sufficient to avoid 5% misclassification with 80% confidence in the lumbar spine. For the hip region, sample sizes of 110 and 50 are sufficient to avoid 5% misclassification with 95% and 80% confidence, respectively. A sample size of 30 df resulted in up to a 12.5% over-diagnosis and 10.0% under-diagnosis of spine or hip change based upon 95% confidence limits.

Assessing the effect of precision study sample size on categorizing change in monitored patients is neglected in current recommendations. Considerably larger sample size requirements are required if low levels of categorization error are to be achieved. Our method provides an alternative way of considering the trade-off between statistical power and feasibility constraints on precision studies.

### P57

#### WNT SIGNALLING IN BONE IN OSTEOARTHRITIS AND OSTEOPOROSIS

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Wnts are a family of secreted proteins traditionally associated with developmental processes. Wnt signalling has been implicated in high and low bone mass phenotypes. In osteoarthritis (OA) there is a massive proliferation of hypomineralized trabecular bone whereas osteoporosis (OP) results in bone loss. We have performed a pilot

study to analyze Wnt signalling in these diseases by profiling the expression of Wnt pathway genes in osteoblasts.

Femoral heads were obtained from consenting patients undergoing a total hip replacement for OA (N=5, aged 55-86) or a hemiarthroplasty following a fractured neck of femur for OP (N=5, aged 68-92). Primary osteoblasts were grown from bone chips. Total RNA was isolated and prepared for application as biotinylated cRNA to a Wnt pathway Oligo GE Array (Super Array Biosciences). Mean values of signal intensities were found for each disease group. The most highly expressed 30 genes (~25%) were examined in each group and the Log2(OA/OP) (signal log ratio, SLR) calculated.

The Wnt signalling pathway was clearly active. WISP2, GSK3A, AES and DVL1 were among the most highly expressed genes in both diseases. High SLR values were found in several genes, though differences did not quite reach statistical significance at  $P<0.05$ . Secreted frizzled related proteins SFRP4 (SLR 0.84) ( $P=0.056$ ), and SFRP3/FRZB (0.59) were higher in OA than OP. The Na/H transporter regulator SLC9A3R1/EBP50 (-0.85) and transducin-like enhancers of split TLE1 (-0.51) and TLE3 (-0.52) were more highly expressed in OP than OA. Wnts 16, 1 and 5a were the most highly expressed but only WNT5A showed any differential expression (SLR = 0.45,  $P=0.057$ ).

Wnt signalling is clearly active in elderly bone. SFRPs are extracellular inhibitors of Wnt signalling so it is curious that these are more highly expressed in OA which is characterized by bone proliferation. Higher levels of TLEs in OP would indicate lower levels of gene transcription through the canonical signalling pathway. The balance between these processes needs further investigation. Dishevelled-dependent Wnt5a signalling can affect commitment to cell lineage (via RHOA) and gene transcription (via MAP kinases and JNK). Higher levels could be a factor underlying the cellular changes seen in OA.

### P58

#### KEY DETERMINANTS OF BONE MINERAL DENSITY IN EXERCISING YOUNG WOMEN

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The study aims at determining which are the key determinants of bone mineral density in a group of young women undergoing intensive physical training.

A cohort of 44 dance students undergoing intensive physical training were compared with a control group of 28 sedentary eumenorrhoeic young women. Menstrual patterns in the past 12 months, basic anthropometric measurements, and body fat composition (by bio-electrical impedance) were measured. Hormonal profiles including fasting serum leptin levels were taken. All underwent dual energy X-ray absorptiometry to determine BMD of the lumbar spine and hip. The exercising women had lower BMI (18.8 Vs 20.3 kg/m<sup>2</sup>), lower body fat percentage (21.7% Vs 29.3%), and higher serum leptin levels (9.42 Vs 7.82 ng/ml) as compared to non-exercising controls. The exercising women had higher BMD values at the spine (1.00 Vs 0.92 mg/cm<sup>2</sup>) ( $p=0.027$ ) and hip sites (trochanter 0.76 Vs 0.66 mg/cm<sup>2</sup>, Ward's triangle 0.80 Vs 0.70 mg/cm<sup>2</sup>, neck of femur 0.96 Vs 0.83 mg/cm<sup>2</sup>) ( $p=0.001$ ). The correlation coefficients between a total BMD index and age ( $r=0.23$ ) and height ( $r=0.30$ ) were significant. A linear regression model to evaluate the effects of continuous variables on a total BMD index showed that height, BMI and body fat % are significant parameters ( $p=0.001$ ) while age, menarchal age, height and leptin levels were not. A logistic regression model showed that exercise ( $p=0.03$ ) high BMI ( $p=0.01$ ), and high body fat % ( $p=0.01$ ) was positively correlated with high total BMD (mean + 1SD), while menstrual status, leptin levels and body fat composition were excluded from the equation.

Height, body mass index, and body fat composition, and intensive exercises are the main determinants of BMD in this cohort of young women undergoing intensive dance training.

**P59**

## **METAL DEBRIS PRODUCED DURING TOTAL KNEE REPLACEMENT: DIFFERENCES BETWEEN METAL AND CERAMIC CUTTING JIGS**

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**Introduction:** Metal particles are generated during bone preparation in total knee arthroplasty (TKA). Material analysis has shown that the majority of the metal originates from the metallic cutting jigs and that several million particles are generated during each TKA. These may produce third-body wear, activate macrophages leading to osteolysis, and even cause hypersensitivity reactions. We aimed to investigate the mass of trace element debris produced in TKA and quantify any differences between metallic and ceramic-lined cutting jigs.

**Methods:** Following ethical committee approval, 10 patients undergoing TKA (Scorpio PS; Stryker Orthopaedics) were enrolled into the single surgeon study. 5 operations were carried out using metal cutting blocks, and 5 using cutting blocks with ceramic runners designed to ease saw blade passage. Debris was collected from the femoral cutting jig, saw blade and bone surfaces. These samples were then digested in tetramethylammonium hydroxide solution (TMAH) and nitric acid (NA), then analysed by 'Total Quant' quantitative analysis and Flame Atomic Absorption.

**Results:** Mean total debris for the ceramic group was  $69 \pm 39$ mg whereas the metal blocks produced  $90.5 \pm 42$ mg ( $p=0.39$ ). Quantitative analysis showed significantly more Nickel debris from the metal cutting blocks ( $32.8 \pm 26.4$ mg/g vs  $6.2 \pm 5.9$ mg/g,  $p<0.05$ ). Iron and chromium debris were found in much greater amounts from the metal cutting blocks ( $2802 \pm 1418$ mg/l vs  $1202 \pm 1061$ mg/l Fe, and  $392 \pm 224$ mg/l vs  $149 \pm 145$ mg/l Cr) but these differences did not quite reach statistical significance ( $p=0.078$  and  $0.076$  respectively).

**Discussion:** Significant quantities of metal debris are produced during TKA. The use of ceramic-lined cutting blocks can reduce total particulate production, especially that of nickel. This may have important implications in terms of reducing the risk of hypersensitivity reactions, in which nickel has been implicated. Differences in other metal particle production were also identified although not to statistically significant levels. Further research is required to understand the implications of this pilot study.

**P60**

## **LACK OF EFFECT OF ALENDRONATE THERAPY ON CIRCULATING OSTEOCLAST PRECURSOR CELL POPULATIONS AND THEIR OSTEOCLASTOGENIC CYTOKINE RECEPTORS**

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Circulating osteoclast precursor cells reside in the monocyte (CD14+) fraction of peripheral blood. Monocytes express key surface proteins including: the receptor for macrophage colony stimulating factor (M-CSFR); the adhesion molecule receptor (CD11b) and the type II receptor for tumour necrosis factor (TNFR1), which are involved in osteoclast differentiation. The effect of the bisphosphonate, alendronate, on circulating osteoclast precursors in vivo is currently unknown. The aim of the study was to determine the effect of alendronate therapy on circulating osteoclast precursors in a) 15 healthy postmenopausal women, ages 55 to 70 (mean 61) b) 15 untreated osteoporotic women, ages 66 to 83 (mean 71) c) 15 osteoporotic women taking alendronate therapy, ages 55 to 81 (mean 68).

Peripheral blood was drawn and the monocyte population was characterised using fluorescent labelled antihuman antibodies, CD14-FITC Ab, (Diacclone), M-CSFR-PE Ab (R & D Systems), alpha CD11b-APC Ab and alpha TNFR1- PE Ab (BD Pharmingen). The appropriate irrelevant fluorescent conjugated isotypes were used as negative controls. Serum crosslinked C-telopeptides of type I collagen (CTX) were measured by ELISA (Nordic Bioscience Diagnostics). The increase in mean fluorescence intensity (MFI) compared to isotype for each receptor and the percentage of receptor positive CD14+ cells was determined by flow cytometry (Becton Dickinson FACScaliburTM).

There were no significant differences in the number of circulating osteoclast precursor (CD14+/MCSFR+/CD11b+/TNFR1+) cells or receptor expression by these cells, between the 3 groups studied. CTX was 90% lower ( $p<0.01$ ) in women treated with alendronate than in control or osteoporotic women as expected. There was no significant

correlation between CTX and any of the markers of the osteoclast precursor population.

We conclude that circulating osteoclast precursors and the osteoclastogenic cytokine receptors, M-CSFR, CD11b and TNFR1 are not targets for the action of alendronate therapy.

**P61**

## **TUMOUR CELL-BONE MARROW STROMAL CELL INTERACTIONS MODIFY EXPRESSION OF CATHEPSIN K, ADAMTS-15, TIMP-3 AND OSTEOPROTEGERIN (OPG)**

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**Introduction:** Proteolytic enzymes are implicated in several key steps in the metastatic process, mediating tumour cell spread to distant sites such as bone. The enzymes involved, as well as their endogenous inhibitors, are produced both by tumour cells and by the surrounding non-malignant cells, and expression and enzyme activity is regulated by a number of complex cell-cell and cell-matrix interactions. We have investigated whether interactions between breast cancer cells and bone marrow stromal cells (BMSCs) affect gene expression of proteolytic enzymes and inhibitors by these cells.

**Methods:** MDA-MB-436 breast cancer cells transfected with GFP (MDA-G8 cells) and human BMSCs were grown individually or as co-cultures for 72h, and cells from the mixed population were subsequently separated using flow cytometry. Alternatively, MDA-G8 cells were cultured for 72h in BMSC conditioned medium. Real-time RT-PCR was then carried out to assess changes in gene expression of cathepsin K, ADAMTS-8, ADAMTS-15 and TIMP3 in the individual cell populations. Expression of the bone-related protein OPG was also analysed.

**Results:** Our initial results demonstrated that there was a decrease in expression of cathepsin K, TIMP3, ADAMTS-15 and OPG by BMSC following co-culture with tumour cells, compared to the levels detected when cells were cultured separately. This was accompanied by a corresponding increase in tumour cell expression of the same genes. No significant change in gene expression was detected in tumour cells grown in BMSC conditioned medium, indicating that the changes in gene expression were not mediated through soluble factors (or that such factors are not produced unless tumour cells and BMSC are in direct contact).

**Conclusions:** Our data demonstrate that expression of genes potentially involved in tumour spread to bone is modulated by direct contact between tumour cells and the cells of the bone microenvironment.

**P62**

## **A FUNCTIONAL RNAI SCREENING FOR RUNX2-REGULATED GENES CORRESPONDING TO ECTOPIC BONE FORMATION IN HUMAN SPINAL LIGAMENTS**

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Ossification of the posterior longitudinal ligament of the spine (OPLL) is characterized by ectopic bone formation in the spinal ligaments. OPLL enlarges with time and compresses spinal cord, causing a serious neurological symptoms. Recent molecular genetic reports have identified several candidate genes having susceptibility to OPLL. We have previously reported that osteoblast differentiation factors (Runx2 etc.) were already enhanced in OPLL cells. In this study, role of Runx2 in the ectopic ossification was investigated by RNA interference (using siRNAs targeted to Runx2) and genome-wide linkage analysis (microarray).

Tissue preparation was carried out under informed consent obtained from each patient. C3-C4 level ligamentum flavum were harvested aseptically from OPLL and non-OPLL (CSM; Cervical spondylotic myelopathy) patients during surgery to decompress the spinal cord for myelopathy. Harvested flavum were cultured by the explant method. Effects of induction of Runx2 by 10-7M dexamethasone (OS; osteogenic stimulation) and RNAi on gene expressions in OPLL cells were comprehensively analyzed by cluster analysis of oligo DNA



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microarray and confirmed by real-time PCR in OPLL cells, non-OPLL cells and osteoblast.

Microarray demonstrated 22 candidate genes regulated by Runx2 in OPLL cells. In addition to osteogenic markers, chondrogenic genes (Aggrecan-1, Col2a1) and angiopoietin-1 were significantly increased by OS induction and decreased by siRNAs for Runx2 in OPLL cells but not in non-OPLL cells. This observation was confirmed by real-time PCR. Furthermore, OS induction and Runx2-siRNAs inhibition of angiopoietin-1 expression were also observed in osteoblast.

In this study we paid attention to angiopoietin-1. Angiopoietin-1 revealed to be downstream of Runx2 both in OPLL cells and osteoblasts. Angiopoietin-1 was significantly expressed in OPLL cells than non-OPLL, suggesting that angiopoietin-1 is regulated by Runx2 in osteochondrogenesis or angiogenesis. Clinically, OPLL patients showed a bleeding tendency without dysfunction of platelet and coagulation factor after operation. However, the common systemic features of OPLL have been reported with less information about disease state. These data may become a key to elucidate disease state in OPLL between ectopic bone formation and angiogenesis. Angiopoietin-1 may play an important role in the disease state of OPLL.

### P63

#### THE PATTERN OF PROCALCITONIN IN UNCOMPLICATED TOTAL HIP AND KNEE ARTHROPLASTY AND ITS IMPLICATION IN PERIPROSTHETIC INFECTION

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**INTRODUCTION:** Two of the major complications of total knee and hip joint arthroplasty is peri-prosthetic infection and aseptic loosening. Since the early 1990s there has been much interest addressing whether procalcitonin (PCT) is a more specific marker in detecting early bacterial infection in the perioperative period. The aim of this study is therefore twofold: to illustrate the pattern of PCT in comparison to the routine inflammatory markers and, from this, to show if PCT could have a clinical role in periprosthetic infection.

**MATERIALS AND METHOD:** A prospective study over 6 months of fifty-nine patients undergoing either primary total hip or knee arthroplasty was performed. Serum blood samples for PCT, CRP, ESR and WCC were taken pre-operatively and on days 1, 3 and 5 post-operatively. The data was analysed by a statistician. PCT was measured using the Lumitest PCT-Q (Brahms Diagnostica, Germany).

**RESULTS:** The patient with a preoperative PCT of 5ng/ml had a preoperative CRP and ESR value of 56, and a normal WCC. The PCT was 0.5ng/ml on day 5. One of the patients who recorded a PCT of 10ng/ml had a CRP of 263mg/l on day 3 and 280mg/l on day 5. There was no relative abnormality his ESR and his WCC remained normal.

**DISCUSSION:** In summary, PCT may be a more reliable indicator of periprosthetic infection given the substantial proportion of patients with unexplained high CRP levels preoperatively. Also, because the surgery doesn't cause PCT to rise indicates that it could be used to monitor high risk patients in the immediate post-operative period. This is further supported by its rapid elevation in infection, and the fact that the CRP, ESR and WCC are still elevated during this period. This would further imply that PCT would be useful in monitoring patients after their revision surgery for periprosthetic infection when it is often difficult to know if the infection has been eradicated. A large multicentre study involving patients undergoing revision surgery for infection needs to be performed to validate these findings and assumptions.

### P64

#### THE IMPORTANCE OF PARATHYROID HORMONE (PTH) ASSESSMENT IN THE TREATMENT OF AUTOSOMAL DOMINANT HYPOPHOSPHATAEMIC RICKETS

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A recognised side effect of hypophosphataemic rickets is the development of secondary hyperparathyroidism. This is secondary to hypocalcaemia induced by the phosphate supplements with abnormal vitamin D metabolism. The recommended therapy for

hypophosphataemic rickets is therefore phosphate supplements plus super physiological doses of 1,25 dihydroxyvitamin D.

We present a case of hypophosphataemic rickets where the parents were unaware of the importance of vitamin D therapy and where PTH measurements were not performed.

JR presented in 1992 aged 2 years with leg bowing and abnormal epiphyses on X-ray. She was small for age and had a degree of frontal bossing. Her biochemistry was typical of hypophosphataemic rickets and is shown in the table. She was commenced on oral phosphate supplements and 1- $\alpha$ -calcidol. Regular monitoring of her serum phosphate(Ph) levels and alkaline phosphatase(ALK) occurred and she had annual renal ultrasounds. She remained small during childhood and suffered a degree of dental caries. No bone pain was experienced and only minimal leg bowing was present by age 10. Pubertal delay occurred and JR had early pubertal development around the age of 15 with an associated growth spurt.

She was transferred to the adult service aged 16 years. Her routine biochemistry including 25(OH)vitamin D and PTH were repeated. These are shown in the table. Although routinely prescribed 1- $\alpha$ -cacidol, her parents admitted that this was taken irregularly and they felt the emphasis had been on maximising her phosphate intake. X-rays of knees because of new pain suggested cupping and splaying of the metaphyses and Dual Energy X-ray Absorptiometry on a Lunar Prodigy scanner using paediatric software revealed a low BMD for age (L2-4 z score -2., Total Body z score -2.5)

	Ph mmol/l	A Ca mmol/l	ALK U/L	25(OH)D nmol/l	PTH ng/L
Normal Range	0.7-1.2	2.2-2.6	Varies with age	25-170	10-65
Age 2 years (diagnosis)	0.48	2.31	535	61	56
Age 16 years (adult service)	1.00	2.13	391	49	567

Regular measurement of PTH is valuable in ensuring efficacy of therapy in hypophosphataemia rickets and to monitor adherence to therapy. The importance of ongoing parental education is highlighted by this case.





# Speaker Profiles

## Tim Arnett

Tim Arnett graduated with a BSc in Biology from the University of East Anglia and gained his PhD at the Royal Postgraduate Medical School, working in the laboratory of Iain MacIntyre. He held postdoctoral positions at Columbia University and University College London before taking up a lectureship in the Department of Anatomy and Developmental Biology at UCL in 1986. In 1991-92, he undertook sabbatical work at the University of Texas. He was appointed Reader in Mineralised Tissue Biology at UCL in 2001. In addition to his work on the control of osteoclast and osteoblast function by extracellular pH and oxygen, he is interested in the role of extracellular nucleotides in bone. Tim Arnett is a past member of the editorial board of the *Journal of Bone & Mineral Research*, and currently serves on the editorial boards of *Calcified Tissue International* and *Endocrinology*; he was secretary of the Bone Research Society from 2004-7.

## Wendy Balemans

Wendy Balemans completed her studies as an Engineer in Biochemistry in 1994 and received her PhD in Biochemistry from the University of Antwerp in Belgium in 2002 where she worked on human genetics of sclerosing bone dysplasias. In the same year, she started a postdoctoral training in the Department of Genomic Technologies based at Janssen Pharmaceutica (Beerse, Belgium), which is part of Johnson & Johnson. There, she mainly focussed her research on target identification and validation for bipolar depression using mouse models. In 2003, she rejoined the Department of Medical Genetics at the University of Antwerp and obtained a postdoctoral research fellowship from the Flemish Fund for Scientific Research (F.W.O. Vlaanderen). Currently, her main research activities are directed towards the understanding of the role of canonical Wnt signaling in bone metabolism.

## Matt Brown

Matt Brown is a clinician-scientist who trained initially in medicine and rheumatology in Sydney, Australia before moving in 1994 to Oxford, England. Working first at the Wellcome Trust Centre for Human Genetics and then the Botnar Research Centre (University of Oxford Institute of Musculoskeletal Sciences), he pursued gene-mapping and genetic epidemiology studies in musculoskeletal diseases, including ankylosing spondylitis, rheumatoid arthritis, chondrocalcinosis and osteoporosis. He was appointed Professor of Musculoskeletal Sciences at University of Oxford in 2004 and was Deputy Director of the Botnar Research Centre from 2003-5. In 2005 Matt returned to Australia, taking a chair of

immunogenetics at University of Queensland, based at the Diamantina Institute of Cancer, Immunology and Metabolic Medicine in Brisbane. There he continues to work in musculoskeletal genetics, both in humans and in mouse models. He has published 73 original research papers in peer-reviewed journals, including in *Nature*, *Nature Genetics*, and *Science*.

## Robert Coleman

Professor Robert Coleman is Professor and Honorary Consultant Medical Oncologist at the Cancer Research Centre, Academic Unit of Clinical Oncology, Weston Park Hospital, Sheffield. He qualified in 1978 from the University of London and subsequently trained in Oncology at Guy's Hospital, London and Western General Hospital, Edinburgh before taking up his current post in 1991. He is Director of the Cancer Research Centre in Sheffield and the Research Lead for the North Trent Cancer Research Network in England.

Professor Coleman's research interests include cancer-induced bone disease and developments in the management of breast cancer. He is Chairman of the National Cancer Research Institute Breast Cancer Study Group in the UK, and President of the Cancer and Bone Society. Professor Coleman has authored over 250 publications and 300 abstracts and was the Editor for Cancer Treatment Reviews 1995-2005.

## Fraser Coxon

Fraser Coxon graduated in Applied and Human Biology from the University of Aston, then studied for a PhD in Bone Biology with Professor Graham Russell at the University of Sheffield. After the award of his Doctorate in 1997, he moved to the University of Aberdeen to work with Professor Mike Rogers on the mechanism of action of bisphosphonates as an MRC-funded Research Fellow and subsequently as an ARC Research Fellow. This work led to the identification of the enzyme target of these drugs and revealed the importance of prenylated small GTPases for osteoclast function. More recently he discovered a new class of bisphosphonate-related anti-resorptive compounds that selectively inhibit Rab GTPases, highlighting the critical role of these master regulators of vesicular transport in osteoclasts. This has also led to his interest in osteopetrosis, a bone disease in which osteoclasts are dysfunctional, often due to defects in vesicular trafficking pathways. Dr Coxon currently holds an RCUK Research Fellowship at the University of Aberdeen, and his current research is focused on the role that Rab GTPases play in regulating vesicular transport in osteoclasts, characterisation of the osteoclast defect in novel cases of osteopetrosis, and the pharmacology of novel bisphosphonate analogues.

## Speaker Profiles

### Florent Elefteriou

Florent Elefteriou graduated from Burgundy University with a Master in Biochemistry and obtained his PhD in 2000 from the Claude-Bernard University (Lyon, France) working on cell-extracellular interactions at the Institute of Protein Biology and Chemistry, in Dr Robert Garrone's group. He then held a post-doctoral position at Baylor College of Medicine (Houston, TX, USA) in Dr Gerard Karsenty's group before taking up an Assistant Professor position at UT-Health Science Center of San Antonio (TX, USA). Dr Elefteriou was appointed at Vanderbilt University (Nashville, TN, USA) in 2006 in the newly created Center for Bone Biology directed by Dr Gregory Mundy. Dr Elefteriou's work focuses on the regulation of bone remodeling by the nervous system, on bone cancer metastasis and on the skeletal defects of neurofibromatosis.

### Matthew Gillespie

Associate Professor Matthew Gillespie is an Associate Director of St Vincent's Institute of Medical Research (SVI) in Melbourne, where he is Head of the Bone, Joint and Cancer Unit. His research is focussed on actions of factors derived from breast cancers, and their relevance to breast cancer metastasis in bone, and how T cell-derived cytokines impact upon the formation and resorption of bone.

He has authored over 120 peer-reviewed publications. He is a Member of: the NHMRC Research Committee (Australia); Council and Science Advisory Committee of the Cancer Council of Victoria; Board of Directors of the International Bone and Mineral Society; Board of Directors the Australian and New Zealand Bone and Mineral Society. He is a member of the editorial boards for *Bone*, *Journal of Bone and Mineral Research*, and is an advisor for the *Journal of Oral Biosciences*.

### Steven Goldring

Steven R Goldring, MD, is the St Giles Chair and Chief Scientific Officer at Hospital for Special Surgery, Weill Medical College of Cornell University in New York City. He previously was a Professor of Medicine at Harvard Medical School and Chief of Rheumatology at New England Baptist Hospital and Beth Israel Deaconess Medical Center, Boston, Massachusetts. After receiving his MD from Washington University School of Medicine, St Louis, Missouri, he completed his medical residency training at Peter Bent Brigham Hospital and his rheumatology training at the Massachusetts General Hospital in Boston. His research interests focus on the cellular and molecular mechanisms involved in the regulation of physiological and pathological bone remodeling. He is the President and past Secretary-Treasurer of the

American Society of Bone and Mineral Research. He previously served as the Chairman of the Orthopaedics and Musculoskeletal Study Section at the National Institutes of Health and has been the Chairman of the Gordon Research Conference on the Molecular Biology of Bones and Teeth, Co-Chairman of the Keystone Conference on the Pathogenesis of Rheumatoid Arthritis and Vice-Chairman of the National Institutes of Health, Consensus Development Panel on Osteoporosis. Dr. Goldring is a co-recipient of the Carol Nachman Prize in Rheumatology and has received the Arthritis Foundation's James H. Fairclough, Jr. and Marian Ropes Awards and the Paget's Disease Foundation Research Award.

### Neva Haites

Neva Elizabeth Haites is originally from Australia where she studied Biochemistry and obtained a PhD. She studied Medicine in Aberdeen and completed her training in the UK. She is the Professor in Medical Genetics at the University of Aberdeen, Head of the College of Life Science and Medicine, Vice Principal of the University of Aberdeen and an Honorary Consultant Clinical Geneticist at Aberdeen Royal Hospitals NHS Trust. She is a member of the NHS Grampian Board and Chairs their Service Strategy and Redesign Committee.

Since 2004 she has been a member of the Human Fertilisation and Embryology Authority and Chairs their Scientific and Clinical Advances group and is a member of the Ethics and Law Committee. She recently became a member of the Biologics and Vaccines subgroup of the Commission for Human Medicines.

She has a special interest in inherited predisposition to cancer.

### Morten Karsdal

Morten A. Karsdal achieved his master of biotechnical engineering at the "Technical University of Denmark" in 1998. He achieved his PhD at the "Technical University of Southern Denmark" 2004, with special focus on the cell and molecular biology of bone. Dr Karsdal is presently the Head of Pharmacology at Nordic Bioscience, and has previously had various research positions at smaller biotech companies in Denmark. Morten Karsdal has more than 55 peer reviewed publications within the bone and cartilage field, and he has received numerous awards a range of conferences, including the ECTS, ASBMR, OARSI and NYAS meetings.

Dr Karsdal is presently involved in investigating a potential anabolic signaling from osteoclasts to osteoblasts and the role of the chloride channel ClC-7 in osteoclasts. Another of his main interests is the

## Speaker Profiles

interaction between bone and cartilage in the pathogenesis of osteoarthritis. Lastly, development of new biological models and biochemical assays for understanding of the disease and to monitor and identify potential treatments for bone and cartilage pathologies are major areas of interest.

### Uwe Kornak

Uwe Kornak finished his master in Biochemistry in 1996. His thesis was on the role of the *clc-7* chloride channel in brain and bone. This chloride channel turned out to reside in late endosomal and lysosomal function and to be functionally coupled with the v-type H<sup>+</sup>-ATPase. A loss of function abolishes bone resorption. In 2001 he finished medical school and went to Paris for a postdoc position at the Inserm U 606. Since 2003 he has been a subgroup leader at the Institute for Medical Genetics at the Charité Hospital in Berlin. His principal research interest has been the pathogenesis of recessive and dominant forms of osteopetrosis. He has subsequently become involved with developmental aspects of skeletogenesis and the role of different transcription factors like *Hoxd13*, *Runx2* and *Mef2c*. Recently, the scope of his interests was further broadened by investigations on Golgi function in bone homeostasis.

### Berent Prakken

Berent Prakken is professor of paediatric immunology at the University Medical Centre Utrecht, the Netherlands. He studied Medicine at the University of Groningen, specialized into paediatrics at the University of Utrecht. He did a fellowship in clinical immunology and started basic research at the Faculty of Veterinary Medicine in Utrecht (with prof. van Eden). He received his PhD cum laude in 1997 at the University of Utrecht. He continued his research at the University of California San Diego (with Professor Albani). Since 2000 he has headed up a research group that studies the regulation of inflammation in chronic inflammatory diseases. The main focus of his research is on the role of regulatory cells in the control of inflammation; the development of immune therapy for arthritis; and the role of heat shock proteins as targets for specific immune regulation. The work of his group has received numerous national and international awards. Prakken is scientific director of the Eureka Institute for Translational medicine ([www.eurekainstitute.org](http://www.eurekainstitute.org))

### Stuart Ralston

Stuart Ralston graduated in medicine from Glasgow University in 1978 and developed an interest in metabolic bone disease during postgraduate training with Dr Iain T Boyle at Glasgow Royal Infirmary.

Professor Ralston trained in general internal medicine and rheumatology in Glasgow between 1981 and 1988. He was appointed as a Wellcome Senior Clinical Research Fellow and Honorary Consultant at the University of Edinburgh between 1988 and 1990 and moved to Aberdeen to take up an appointment as Senior Lecturer in Medicine in 1991. He was appointed as Professor of Medicine and Bone Metabolism in 1996 and was Director of the Institute of Medical Sciences at Aberdeen between 2002 and 2004. Professor Ralston took up the ARC chair of Rheumatology at the University of Edinburgh in February 2005 and was appointed as Head of the School of Molecular and Clinical Medicine in November 2005. He is an Honorary Consultant Rheumatologist with Lothian Health Board and is lead clinician for Osteoporosis services within NHS Lothian.

Professor Ralston has published extensively on several aspects of bone disease including the genetics of osteoporosis; the pathogenesis and management of Paget's disease of bone; the role of Nitric Oxide in bone and the pathogenesis and management of cancer-associated bone disease. He has previously served on the Oliver Bird Committee of the Nuffield Foundation, the Heberden Committee of the British Society of Rheumatology, the Molecular and Cellular Medicine Board of the MRC, the Physiological Medicine and Infections Board of the MRC, the Physiology and Pharmacology panel of the Wellcome Trust and the Committee for Safety of Medicines. He is currently a member and vice chairman of the Research Subcommittee of the Arthritis Research Campaign. He acts as scientific advisor to the National Association for Relief of Paget's Disease and the Paget Foundation. He is a past president of the Bone and Tooth Society of the UK, and was President of the European Calcified Tissue Society between 1998-2005. He is currently joint editor-in-chief of *Calcified Tissue International*, associate editor of *Bone*; associate editor of *Endocrinology* and a member of the Editorial Board of the *Journal for Bone and Mineral Research*.

### Ruth Ross

Dr Ruth Ross is a senior lecturer in Pharmacology at The University of Aberdeen in Scotland. Her primary research focus is cannabinoid receptor pharmacology and the pharmacology of the endocannabinoids and related metabolites. This research is directed towards discovery of the role of the endocannabinoids in pain, inflammation, obesity and bone disorders and the development of small molecules, which modulate the various elements of the endocannabinoid system as novel therapeutics.



## Speaker Profiles

### Graham Russell

Graham Russell graduated with first-class honours in biochemistry from Cambridge and then worked in the MRC Unit in Leeds, gaining his PhD. He worked in Oxford, Bern and Harvard University before being appointed to the Chair of Human Metabolism and Clinical Biochemistry in Sheffield University.

Under his leadership that department became established as a major international centre for the study of basic and clinical research into bone diseases. He played a central role in the discovery of the biological effects of bisphosphonates, and in their evaluation for the treatment of bone disorders. Bisphosphonates are now the most widely-used drugs for the treatment of bone diseases throughout the world. His other research interests include bone cell biology - work which is directly concerned with the improvement of treatment of osteoporosis, Paget's disease and malignant disease of bone.

He has held many prestigious offices, including the Presidency of the International Bone & Mineral Society, and he was a founding Trustee and subsequent Chairman of the Council of Management of the National Osteoporosis Society (UK). He was the Heberden Orator of the BRS in 1993 and was the recipient of the John Johnson Award of the Paget's Foundation (USA) in 1997. In 2000 he was the first British scientist to receive the Neuman Award of the American Society of Bone & Mineral Metabolism.

His research team are now based in the Botnar Institute at the Oxford Nuffield Orthopaedic Centre, where they play a key role in the investigation of the cell biology and biochemistry of common bone

diseases, especially osteoporosis and malignant disease of bone, including studies of new treatments.

He was the Norman Collisson Professor of Musculoskeletal Sciences at Oxford University from 2001-2006, and since 2002 has been the first Director of the new Oxford University Institute of Musculoskeletal Sciences.

### Rob van 't Hof

Rob van 't Hof originally studied Biology at the University of Utrecht in the Netherlands. During his PhD at the University of Leiden, he developed an interest in the regulation of osteoclast formation and activity, with a special interest in the cross-talk between osteoblasts and osteoclasts. After his PhD, he moved to the University of Aberdeen to study the effects of Nitric Oxide (NO) on bone cells in the group of prof. Ralston. This led to the publication of several papers highlighting the importance of this small molecule as a local regulator of bone metabolism. In July 2005 Dr van 't Hof moved to the University of Edinburgh to take up his current post as senior lecturer in the Rheumatic Diseases Unit.

One of the enzymes that synthesises NO, neuronal NOS or nNOS, is predominantly expressed in neuronal cells. Dr van 't Hof found mice that lacking this enzyme have unusually high bone density. This started a new direction in his research in the regulation of bone turnover by the central nervous system (CNS). This has led to the discovery that endocannabinoids are important regulators of bone metabolism. Research is currently ongoing to study whether this is indeed due to actions of the endocannabinoids via the CNS.

## Exhibitor Profiles

### Abbott

Abbott is a global, broad-based health care company devoted to discovering new medicines, new technologies and new ways to manage health. Abbott scientists are applying innovative monoclonal antibody technology to discover and develop novel therapies to treat diseases of the immune system including rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease.

### Eli Lilly

Eli Lilly and Company is one of the world's largest research-based pharmaceutical companies, dedicated to creating and delivering innovative pharmaceutical healthcare solutions that enable people to live longer, healthier and more active lives. Our research and development efforts constantly strive to address urgent unmet medical needs.

Eli Lilly and Company was founded in 1876 in Indianapolis, USA, and has had a long history of producing endocrine products, dating all the way back to the collaboration with Banting and Best and the introduction of the world's first insulin product in 1922.

Another element of Lilly's endocrine portfolio is growth hormone replacement. Lilly manufactures recombinant human growth hormone (somatropin) at Speke near Liverpool, UK. A full range of products and services is provided for the healthcare professional to use with their patients on growth hormone replacement therapy for both adults and kids.

To assist in the therapeutic management of Osteoporosis, Lilly has two products each catering for different patient needs, namely Raloxifene and Teriparatide.

Finally Lilly continues to focus significant resources on research into the endocrine area. For additional information about any of our endocrine products or services please come and talk to us at the Lilly stand or log on to the company website -[www.lilly.com](http://www.lilly.com).

### Immunodiagnostic Systems (IDS)

Immunodiagnostic Systems (IDS) offers a range of esoteric immunoassay kits for clinical and research use. The company focuses in Bone & Mineral Metabolism and Growth Factor research, and engages in collaborative projects with researchers worldwide.

IDS is a leader in the field of Vitamin D analysis, offering both manual and automated 25(OH) Vitamin D methods, an award-winning 1,25-Dihydroxy Vitamin D RIA system, and now announces OCEIA 1,25(OH)<sub>2</sub> Vitamin D, an EIA employing the proven immunocapsule sample preparation.

IDS offers a full range of Bone & Skeletal products, including BoneTRAP® (Tartrate-Resistant Acid Phosphatase 5b), MouseTRAP™, RatTRAP™, Bone-Specific Alkaline Phosphatase (Ostase® BAP), Intact PTH, urinary DPD, RANKL & OPG for both clinical and research (animal) use.

IDS are also pleased to announce a NEW, UNIQUE Rat/Mouse PINP ELISA to quantify type I collagen formation in rat or mouse samples.

IDS OsteoSite™ antibodies include multiple forms of PTHrp, Cathepsin K, Osteoarthritis Matrix Antigen (a Collagen VI epitope) and a specific Osteoclast Antigen; the GroPep range has multi-species Growth Factor and GF-Binding Protein antibodies; and the Eurodiagnostica range has antibodies to many matrix antigens, neuropeptides and associated reagents.

Dentine discs are available for osteoclast activity studies in vitro, suitable for use in microtitre wells.

### Novartis

Novartis is a company committed to the discovery and development of medicines in areas of unmet clinical need. The therapeutic areas that Novartis works in include cardiovascular and metabolism, neuroscience, respiratory, arthritis, bone, infectious diseases, transplantation and immunology and oncology. Our product pipeline is one of the strongest in the pharmaceutical industry and in the UK alone we spend around £1million per week on research and development

### Nycomed

Nycomed is a Danish pharmaceutical company dedicated to meeting healthcare needs in Europe. The company provides specialist hospital products throughout the region and general practitioner and pharmacy medicines in some markets.

New products are sourced through licensing agreements and partnerships with research companies. Here Nycomed provides late-stage clinical development, registration and marketing. Nycomed employs about 3,500 people throughout Europe and Russia-CIS.

Nycomed has a strong heritage in osteoporosis with Calcichew and Calcichew D3 and now with Preotact and osteoporosis remains one of its key therapeutic areas for future development.

### Pfizer

Pfizer Inc, the world's largest research-based pharmaceutical company, discovers, develops, manufactures and markets prescription medicines in 11 therapeutic areas. Pfizer is also the world's largest animal health company.

Pfizer Inc employs approximately 105,000 colleagues worldwide, all of whom are devoted to working for a

## Exhibitor Profiles

healthier world. Pfizer conducts more biomedical research than any other corporation, and has 14,000 professionals working in six major R&D sites worldwide, including Sandwich in Kent. Pfizer's research investment in 2005 was more than \$7.4 billion.

In the UK, Pfizer Ltd has its UK business headquarters in Surrey and is the major supplier of medicines to the NHS.

### ProStrakan

ProStrakan Group plc is one of Europe's fastest growing speciality pharmaceutical companies. It is engaged in the development and commercialisation of prescription medicines for the treatment of unmet therapeutic needs in major markets. The company markets a range of products in major EU markets through its commercial operations based in the UK, Germany, France, Spain, Sweden and BeNeLux and has its headquarters and R&D activities in Galashiels, Scotland.

### Qados

Qados are the distributors of the Faxitron X ray imaging cabinets in the UK. These cabinets are widely used for imaging bone both during research or during routine work in the Histopathology Departments. Digital imaging options are also available from Qados, for example the EZ240 X ray scanner from NTB.

Qados also represents Cisbio International in the UK, Cisbio produce Quadramet which is for bone pain palliation after breast, prostate or bladder cancer treatment. If you would like to look at the complete qados portfolio please visit [www.qados.co.uk](http://www.qados.co.uk).

### Roche/GSK

#### *Roche in the UK*

Roche aims to improve people's health and quality of life with innovative products and services for the early detection, prevention, diagnosis and treatment of disease. Part of one of the world's leading healthcare groups, Roche in the UK employs nearly 2,000 people in pharmaceuticals and diagnostics. Globally Roche is the leader in diagnostics, the leading supplier of medicines for cancer and transplantation and a market leader in virology. Find out more at [www.rocheuk.com](http://www.rocheuk.com)

#### *GSK*

GSK, one of the world's leading research-based pharmaceutical and healthcare companies, is committed to improving the quality of human life by enabling people to do more, feel better and live longer. All trademarks used or mentioned in this release are legally protected.

### Scanco

Scanco Medical ([www.scanco.ch](http://www.scanco.ch)) is the leading manufacturer of state-of-the-art micro-CT scanners (research and clinical use). The scanners, together with built-in analysis and visualization software provide for a 3-dimensional, rapid, non-destructive and comprehensive quantitative measurements. Scanco Medical also offers contract based scanning services for academic and industrial groups at facilities in the USA or in Switzerland.

### Servier

Servier Laboratories Limited is the British Subsidiary of the Servier Research Group, a leading French based organisation specialising in ethical pharmaceuticals. Servier is currently within the top 15 largest pharmaceutical companies in the UK.

Servier's product portfolio in the UK focuses on the therapeutic areas of Cardiovascular disease, Diabetes, Osteoporosis and soon Depression. Servier's R&D pipeline is extremely healthy having the potential to submit one product for license every year for the next 8 to 10 years. Servier UK and the whole Servier Research Group is set for dramatic growth over this period. [www.servier.co.uk](http://www.servier.co.uk)

### Shire Pharmaceuticals

Shire Pharmaceuticals Group plc (Shire) is a global specialty pharmaceutical company with a strategic focus on meeting the needs of the specialist physician and currently focuses on developing projects and marketing products in the areas of central nervous system (CNS), gastrointestinal (GI), and renal diseases. Shire has operations in the world's key pharmaceutical markets (US, Canada, UK, France, Italy, Spain and Germany) as well as a specialist drug delivery unit in the US.

For further information on Shire, please visit the Company's website: [www.shire.com](http://www.shire.com)

### SkyScan

SkyScan is a fast growing company and one of the world's leading producers of micro-CT systems for a wide range of applications. SkyScan aims to bring to customers the newest technology, the best instrument quality and the highest level of support.

SkyScan can genuinely claim to be at the fore-front of the development of high performance micro-CT technology. Our research and development of 3D x-ray microscopy started in the early 1980s. This led to the first micro-CT imaging results being obtained in 1983-1987 and published in scientific journals and international conferences proceedings. Building on this early work, SkyScan was founded in 1996, and

## Exhibitor Profiles

within a year we were manufacturing a commercially available micro-CT scanner with spatial resolution in the micron range. In 2001 we produced the first high-resolution in vivo micro-CT scanner for small animal imaging. And in 2005 SkyScan became the world's only supplier of a laboratory nano-CT scanner with submicron spatial resolution. Responding to demand from the growing community of micro-CT users, we are continually active in research and development into new methods for non-destructive 3D microscopy.

### Technoclone

Technoclone Ltd is a specialist in the field of bone, cartilage and mineral metabolism immunoassays and is the exclusive distributor in the UK and Ireland of both the Metra® range of Bone Marker assay kits from Quidel, the Bone and Cartilage assay kits from Nordic Bioscience Diagnostics and Cartilage assays kits from Ibex Diagnostics.

The Nordic Bioscience range includes clinical tests for serum and urine CTX-I (CrossLaps®), N-MID Osteocalcin and urine CTX-II (CartiLaps®), as well as the preclinical RatLaps and CrossLaps for Culture CTX-I kits, Rat-MID Osteocalcin and serum and urine pre-clinical CartiLaps® (CTX-II) kits. Nordic Bioscience also supply bovine bone slices available in a range of sizes for different assay plates.

The Metra® range of ELISA assays includes a highly specific Bone Alkaline Phosphatase (BAP) assay, serum Osteocalcin, Collagen Type -1 C-Terminal propeptide (CICP), DPD and Total DPD, PYD, alpha-1 Helical Peptide and YKL-40, as well as a chromogenic Creatinine assay and other research reagents.

Details of new kits from both Nordic Bioscience and Quidel will be available at the BRS meeting including

- Metra® TRAcP 5b ELISA
- Alpha CrossLaps (CTX-I) for bone metastases
- Preclinical Total Aggrecan
- Preclinical PIINP for cartilage formation
- Preclinical Synovial Fluid CTX-II assay
- Metra® Osteoprotegerin

Technoclone also markets an extensive range of autoimmune assays in multiplex and ELISA format, Complement assays and research reagents and multiplex human cytokine assays.



# General Information

## Venue

Aberdeen Exhibition and Conference Centre -  
www.aecc.co.uk

**Registration, posters and exhibition:** Gordon Suite

**Lectures:** Fleming Auditorium

## Registration

Registration will be in the Concourse at the AECC.

The registration desk will be open as follows:

Tuesday 3 July:	09:30-17:30
Wednesday 4 July:	08:00-18:00
Thursday 5 July:	08.30-15:30

## Continuing Professional Development (CPD)

The meeting is approved for CPD credits. Please be sure to sign the register and collect a certificate if you would like to claim your points.

## Buses

For those staying on the University campus (King's Hall and Crombie Hall), buses will be provided to the AECC on the morning of 3, 4 and 5 July, returning at the end of each day.

## Pick-up points

**Mornings:** Outside the entrance to the reception at Crombie Hall (building 4 on the map).

**Evenings:** Outside the main reception of the AECC.

## Pick up times

From Halls to AECC:

08:55 on Tuesday morning

07:55 on Wednesday morning

## 08:25 on Thursday morning

From AECC to Halls:

17:40 on Tuesday evening

18:10 on Wednesday evening

There will be buses to Aberdeen Airport leaving the AECC at 15:40 on 5 July.

## Buses for social events

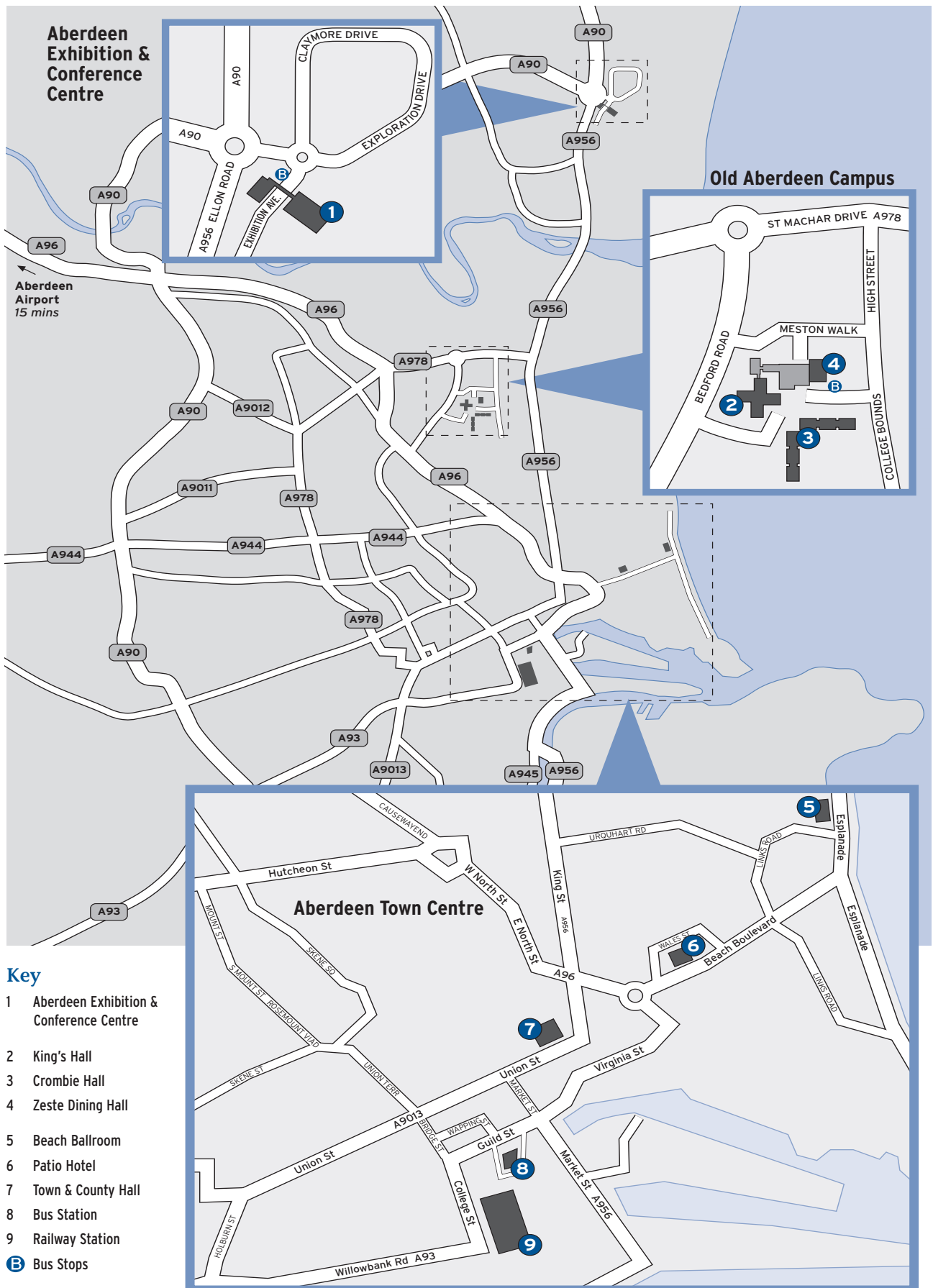
For those staying on the University campus (King's Hall and Crombie Hall), buses will be provided from outside the entrance to the reception at Crombie Hall to the Welcome Reception to be held at the Town and County Hall on Tuesday 3 July and to and from the Annual Dinner at the Beach Ballroom on Wednesday 4 July.

## Pick up times

Tuesday 3 July 18:30 (no return)

Wednesday 4 July 19:20 & returning at midnight

# Map





**BONE**  
RESEARCH SOCIETY