THE CANCER AND BONE SOCIETY (CABS) 2023 CONFERENCE

Eric P. Newman Education Center (EPNEC)
at Washington University in St. Louis
St. Louis, MO, USA
June 7 – 10, 2023

The Cancer and Bone Society Board would like to thank the following sponsors for their generous contributions to the CABS 2023 conference

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HISTORY OF CABS

The Cancer and Bone Society (CABS) was initially founded in 2004 by Greg Mundy, Robert Rubens, Etsuro Ogata, Herbie Fleisch, and Jack Martin, with Rob Coleman appointed as the inaugural chairman. The primary mission of CABS was to formally run the cancer-induced bone disease (CIBD) meetings, initiated in 1991, and to promote international collaborations across the cancer and bone field in both clinical and basic research. The CIBD meetings continued to be held every 1-2 years, alternating between the US and Europe to promote international involvement. CABS ran these meetings as an independent society from 2004 until 2011, before becoming incorporated into the International Bone and Mineral Society (IBMS). The CIBD meetings continued under the direction of IBMS through 2015.

In 2016, CABS once more become an independent entity and established a new CABS Board. CABS now manages and facilitates the CIBD meetings as they were originally introduced in 1991 and, where appropriate, partner with other societies that have an interest in bone and cancer. CABS believes these partnerships will enhance research and education in this rapidly developing area of biology and promote the translation of increasing scientific knowledge into clinical practice.

CABS BOARD

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CABS 2023 AWARD WINNERS

Young Investigator Awards
Douglas Faget, PhD
Déja Grant
Diego Grinman, PhD
Japneet Kaur, PhD
Ganesh Rout, PhD
Yang Yu, MS

Outstanding Abstract Awards
Janet Brown, FRCP, MD
Julie O'Neal, PhD
Lei Shi, PhD
Xinming Su, PhD

High Scoring Abstract Awards
Rozita Bagheri-Yarmand, PhD
Nadia Benabdallah, PhD
Natalie Bennett
Ryan Bishop, PhD
Emma Briggs, MS, MEng
Breelyn Karno
Subhashis Pal, PhD
Jennifer Zarrer, PhD

CABS 2023 Distinguished Service Award
G. David Roodman, MD, PhD

CABS 2023 SCIENTIFIC PROGRAM

Wednesday, June 7, 2023

Registration desk opens 4:00pm - 6:00pm

5:00pm - 7:00pm Young Investigator Networking Session and Reception, *Great Rooms, EPNEC*

ALL CAREER STAGES ARE WELCOME AND ENCOURAGED TO ATTEND

5:00pm - 5:05pm Welcome by the CABS President

5:05pm - 6:00pm Round Table Professional Development Discussions

6:00pm - 7:00pm Networking Reception *(Margaritas, Beer, Taco Bar, Chips, Salsa)*

Thursday, June 8, 2023

Registration desk opens 7:30am - 2:00pm

All lectures are held in the EPNEC Auditorium

Posters are in the EPNEC Great Rooms

8:00am - 8:45am Networking Breakfast, *EPNEC Lobby (Breakfast may be brought into Auditorium)*

8:45am - 8:55am Welcome and Opening Comments, *Julie Rhoades, PhD, CABS President*

8:55am - 9:05am Welcome to St. Louis, *Katherine Weilbaecher, MD, Local Organizer*

9:05am - 10:20pm Plenary Symposium: Seminal Advances in Mechanisms of Dormancy and the Bone Marrow Microenvironment

> *Chairs: Julie Rhoades, PhD, Vanderbilt University Medical Center, Nashville, TN, USA and Jesus Delgado-Calle, PhD, University of Arkansas Medical School, Little Rock, AR, USA*

> 9:05pm - 9:30am Dissecting myeloma cell dormancy one cell at a time, *Peter Croucher, PhD, Garvan Institute, Sydney, Australia*

> 9:30am - 9:55am Age related changes drive breast tumorigenesis, *Sheila Stewart, PhD, Washington University in St. Louis, St. Louis, MO, USA*

> 9:55am - 10:20am Bone marrow niches as targets of bone metastasis and sources of immunosuppression in breast cancer, *Xiang Zhang, PhD, Baylor College of Medicine, Houston, TX, USA*

10:20am - 11:00am BREAK, *Coffee, tea, and refreshments in EPNEC Lobby*

11:00am - 4:20pm Immune Oncology in the Bone Metastatic Setting: Part I

> *Chairs: Xiang Zhang, PhD, Baylor College of Medicine, Houston, TX, USA and Kristin Kwakwa, PhD, Washington University in St. Louis, St. Louis, MO, USA*

> 11:00am - 11:25am Systemic effects of bone cells and bone derived factors on breast cancer progression, *Roberta Faccio, PhD, Washington University in St. Louis, St. Louis, MO, USA*

> 11:25am - 11:50am Immune checkpoint signaling in bone metastatic disease: Mechanistic revelations from the skeletal niche, *Rachelle Johnson, PhD, Vanderbilt University Medical Center, Nashville, TN, USA*

> 11:50am - 12:00pm BoHFAB: The impact of adjuvant zoledronate therapy on bone health in postmenopausal patients with early breast cancer, *Janet Brown, University of Sheffield, Sheffield, UK *Outstanding Abstract Award*

> 12:00pm - 12:10pm The roles of the FL/FLT3 axis in the development of cancer-induced bone pain mediated by prostate cancer, *Yang Yu, Wake Forest University, Wake Forest, NC, USA *YI Award Winner*

**12:10pm - 1:30pm LUNCH*, ** *EPNEC Lobby*

> *1:00pm - 1:25pm Optional: Stretch your legs on a tour of the Washington University in St. Louis Medical Center campus or Musculoskeletal Research Center! Meet tour guides in the foyer.

**CABS Board Meeting - Board members please get lunch and proceed to 309 EPNEC.

1:30pm - 2:20pm Breakthrough Topics in Bone Metastasis

Chairs: Theresa Guise, MD, MD Anderson, Houston, TX, USA and Shreya Patel, Rush University, Chicago, IL, USA

1:30pm - 1:55pm Moving on bone metastases: Exercise as medicine, *Tarah Ballinger, MD, Indiana University, Indianapolis, IN, USA*

1:55pm - 2:20pm Denosumab in bone metastatic patients – Is it safe to stop treatment?, *Michelle McDonald, PhD, Garvan Institute, The University of Sydney, Sydney, Australia*

2:20pm - 2:30pm Cancer and Bone Society 2023 Distinguished Service Award Presentation to G. David Roodman, MD, PhD

2:30pm - 3:15pm BREAK*, *Coffee, tea, and refreshments in EPNEC Lobby*

*2:35pm - 3:00pm Optional: Stretch your legs on a tour of the Washington University in St. Louis Medical Center campus or Musculoskeletal Research Center! Meet tour guides in the foyer.

3:15pm - 4:05pm Bone Anabolic Therapies in Metastasis

Chairs: Katherine Weilbaecher, MD, Washington University in St. Louis, St. Louis, MO, USA and Michelle McDonald, PhD, Garvan Institute, The University of Sydney, Sydney, Australia

3:15pm - 3:40pm To Wnt or not to Wnt: That is the question in Cancer in Bone, *Jesus Delgado-Calle, PhD, University of Arkansas Medical School, Little Rock, AR, USA*

3:40pm - 4:05pm Anabolic therapy: Should it be used in bone metastasis?, *Hanna Taipaleenmaki, PhD, Ludwig-Maximilians-University of Munich, Munich, Germany*

4:05pm - 4:35pm BREAK, *Coffee, tea, and refreshments in EPNEC Lobby*

4:35pm - 5:00pm Lightning Talks (High Scoring Abstract Award Winners)

Chairs: Claire Edwards, PhD, Oxford University, Oxford, UK and Jenna Ollodart, Wake Forest University, Wake Forest, NC, USA

4:35pm - 4:40pm Acid ceramidase (ASAH1) is a key mediator of drug resistance in relapsed/refractory multiple myeloma, *Ryan Bishop, The Moffitt Cancer Center, Tampa, FL, USA*

4:40pm - 4:45pm Homing of intestinal NK and Th1 cells to the tumor site restrains melanoma bone growth in a gut microbiome dependent manner, *Subhashis Pal, Emory University, Atlanta, GA, USA*

4:45pm - 4:50pm IL-17A supports osteoblasts to become cancer-associated fibroblasts in breast cancer bone metastases, *Jennifer Zarrer, University Medical Center, Hamburg, Germany*

4:50pm - 4:55pm Elucidating the Effect of Glutamine Metabolism in Breast-to-Bone Metastasis, *Breelyn Karno, Vanderbilt University, Nashville, TN, USA*

4:55pm - 5:00pm Activating RET mutations in medullary thyroid cancer cells promotes osteoblastic bone metastasis by inhibiting osteoclastogenesis and stimulating osteoblast activity, *Rozita Bagheri-Yarmand, MD Anderson, Houston, TX, USA*

5:00pm - 5:05pm Mechanical loading of breast cancer laden bones alters osteocyte mediated bone loss in vivo, *Emma Briggs, University of Colorado Boulder, Boulder, CO, USA*

5:05pm - 5:10pm Downstream Effects of Gli2 Targeting on Tumor-Immune Cell Crosstalk in Bone Metastatic Breast Cancer, *Natalie Bennett, Vanderbilt University, Nashville, TN, USA*

5:10pm - 5:15pm Radium-223 Treated Primary Patient Bone Biopsy Analysis: Macro to Microscale Analyses and Dosimetry, *Nadia Benabdallah, Washington University in St. Louis, St. Louis, MO, USA*

5:00pm - 7:00pm Poster Session and Wine/Cheese Reception*, EPNEC Great Rooms, with hot hors d'oeuvres*

Friday, June 9, 2023

Registration desk opens 7:30am - 12:00pm

All lectures are held in the EPNEC Auditorium

Posters are in the EPNEC Great Rooms

8:15am - 9:00am Networking Breakfast, *EPNEC Lobby (Breakfast may be brought into Auditorium)*

9:00am - 10:15am Plenary Symposium: Imaging the Bone Metastatic Niche

Chairs: *Hanna Taipaleenmaki, PhD, Ludwig-Maximilians-University of Munich, Munich, Germany and Peter Croucher, PhD, Garvan Institute, Sydney, Australia*

9:00am - 9:25am A macrophage-dependent vascular doorway in breast tumors that both induces cancer stem cells and injects them into the systemic circulation, *John Condeelis, PhD, Albert Einstein College of Medicine, Bronx, NY, USA*

9:25am - 9:50am The trajectory of prostate cancer metastasis: Insights from PSMA imaging, *Ken Pienta, MD, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

9:50am - 10:15am Perspective of using routine bone metastasis qCT to predict bone strength, *Cyrille Confavreux, MD, PhD, Centre Hospitalier Universitaire de Lyon, Lyon, France*

10:15am – 11:00am BREAK, *Coffee, tea, and refreshments in EPNEC Lobby*

*10:20am - 10:45am Optional: Stretch your legs on a tour of the Washington University in St. Louis Medical Center campus or Musculoskeletal Research Center! Meet tour guides in the foyer.

11:00am - 12:10pm Immune Oncology in the Bone Metastatic Setting: Part II

Chairs: Ken Pienta, MD, Johns Hopkins School of Medicine, Baltimore, MD, USA and Giulia Furesi, PhD, Washington University in St. Louis, St. Louis, MO, USA

11:00am - 11:25am Acid signaling promotes immune suppression in breast cancer bone metastases, *Katherine Weilbaecher, MD, Washington University in St. Louis, St. Louis, MO, USA*

11:25am - 11:50am Tumor transcriptional network drives cancer plasticity and immune inhibitory landscape, *Julie Rhoades, PhD, Vanderbilt University Medical Center, Nashville, TN, USA*

11:50am - 12:00pm Chemotherapy-induced adipocyte senescence triggers bone loss through RANKL-mediated osteoclastogenesis, *Ganesh Raut, PhD, Washington University in St. Louis, St. Louis, MO, USA *YI Award Winner*

12:00pm - 12:10pm PTHrP biological domains mediate breast cancer bone osteolysis via TGF-β/SNAI2/ZEB1 pathways; *Déja Grant, Vanderbilt University, Nashville, TN, USA *YI Award Winner*

12:10pm - 1:40pm LUNCH and Interactive Town Hall Debate Forum: All conference attendees are invited to debate, discuss, and briefly present critical topics in small groups, *EPNEC Great Rooms*

Chairs: Rachelle Johnson, PhD, Vanderbilt University Medical Center, Nashville, TN, USA and Celia Soto, MS, University of Rochester, Rochester, NY, USA

12:10pm - 12:25pm Collect buffet lunch in lobby and bring into Great Rooms

12:25pm - 12:30pm Group Guidelines and Instructions

12:30pm - 1:40pm Topic Discussions and Presentations

1:40pm - 2:00pm BREAK*, *Coffee, tea, and refreshments in EPNEC Lobby*

*1:40pm - 2:00pm Optional: Stretch your legs on a tour of the Washington University in St. Louis Medical Center campus or Musculoskeletal Research Center! Meet tour guides in the foyer.

2:00pm - 2:10pm IFMRS and NCRI Updates, *Claire Edwards, PhD, CABS Past-President*

2:10pm - 2:55pm Young Investigator Plenary Oral Presentations, Session I

Chairs: Yusuke Shiozawa, MD, PhD, Wake Forest University, Wake Forest, NC, USA and Trupti Trivedi, PhD, MD Anderson Cancer Center, University of Texas, Houston, TX, USA

2:10pm - 2:25pm Senolytics deplete senescent osteocytes and improve bone health in metastatic breast cancer, *Japneet Kaur, PhD, University of Arkansas Medical School, Little Rock, AR, USA *YI Award Winner*

2:25pm - 2:40pm Targeting stromal p38MAPKalpha triggers innate-adaptive anti-tumor immunity and sensitizes metastatic breast cancer to immunotherapy, *Douglas Faget, PhD, Washington University in St. Louis, St. Louis, MO, USA *YI Award Winner*

2:40pm - 2:55pm Cancer-Associated Hypercalcemia Signals Through the Hindbrain to cause Anorexia, *Diego Grinman, PhD, Yale University, New Haven, CT, USA *YI Award Winner*

2:55pm - 3:15pm BREAK, *Coffee, tea, and refreshments in EPNEC Lobby*

3:15pm - 4:15pm Speed Mentoring / Networking, *EPNEC Great Rooms*

Pitch your translational ideas to clinical trialists and clinical researchers or speed network with senior cancer and bone translational scientists!

4:15pm Sessions End

6:00pm - 9:00pm Dinner and Gala at the City Museum, *Casual dress recommended, https://www.citymuseum.org/*

5:30pm Shuttle to City Museum leaves from Chase Park Plaza Hotel

5:45pm Shuttle to City Museum leaves from DoubleTree Hotel

Shuttles will run continuously; last shuttle at 9:00pm.

All lectures and panels are held in the EPNEC Auditorium

Posters are in the EPNEC Great Rooms

8:15am - 9:00am Networking Breakfast, *EPNEC Lobby (Breakfast may be brought into Auditorium)*

9:00am - 10:15am Cellular Profiling and Thinking Beyond the Bone

Chairs: Jitesh Pratap, PhD, Rush University Medical Center, Chicago, IL, USA and Logan Northcutt, Vanderbilt University, Nashville, TN, USA

9:00am - 9:25am Role of RON kinase in immune-modulation of bone metastasis, *Alana Welm, PhD, Metastasis Research Society, President-Elect, University of Utah, Salt Lake City, UT, USA*

9:25am - 9:50am Profiling the tumour-bone microenvironment, *Claire Edwards, PhD, Oxford University, Oxford, UK*

9:50am - 10:15am Beyond seed and soil: The adaptive strategies that drive lethal cancer, *Sarah Amend, PhD, Johns Hopkins School of Medicine, Baltimore, MD, USA*

10:15am - 10:30am BREAK, *Coffee, tea, and refreshments in EPNEC Lobby*

10:30am - 11:00am PLENARY ORAL PRESENTATIONS, Session II

Chairs: Alana Welm, PhD, University of Utah, Salt Lake City, UT, USA and Greg Clines, MD, PhD, University of Michigan, Ann Arbor, MI, USA

10:30am - 10:40am Bone-derived excess TGF-ß release is associated with cognitive dysfunction due to oxidation of RyR, Ca2+ leak and mitochondrial dysfunction, *Lei Shi, PhD, MD Anderson Cancer Center, Houston, TX, USA *Outstanding Abstract Award*

10:40am - 10:50am Anti-myeloma efficacy of BCMA CAR-iNKT is enhanced with a long-acting IL-7, rhIL-7hyFc, *Julie O'Neal, PhD, Washington University in St. Louis, St. Louis, MO, USA *Outstanding Abstract Award*

10:50am - 11:00am CDK4/6 inhibitor and doxorubicin synergistically inhibit breast cancer bone metastasis and enhance T-cell targeted therapy; *Xinming Su, PhD, Washington University in St. Louis, St. Louis, MO, USA *Outstanding Abstract Award*

11:00am - 11:45am Treating Bone Metastases: Ask the Doctor! Clinical Panel

Panelists: Theresa Guise, MD, Bone and Mineral Endocrinologist, MD Anderson Cancer Center, Houston TX, USA; Katherine Weilbaecher, MD, Breast Cancer Medical Oncologist, Washington University School of Medicine, St. Louis, MO, USA; Russell Pachinsky, MD, Prostate Cancer Medical Oncologist, Washington University School of Medicine, St. Louis, MO, USA

11:45am - 12:00pm Award Presentations / Closing Remarks

12:00pm Boxed lunch

ORAL ABSTRACTS

Young Investigator Award Winners
and
Outstanding Abstract Award Winners
in
Program Order

OUTSTANDING ABSTRACT AWARD WINNER

BoHFAB. The impact of adjuvant zoledronate therapy on bone health in postmenopausal patients with early breast cancer

Janet Brown[1], Margaret Paggiosi[1], Emma Rathbone[1], David Dodwell[2], David Cameron[3], Walter Gregory[4], Richard Eastell[1], Robert Coleman[1].

[1]University of Sheffield, UK, [2]Leeds Teaching Hospitals Trust UK, [3]University of Edinburgh UK, [4]University of Leeds, UK.

Background. Adjuvant bisphosphonates, usually given over 3-5 years, have become widely used in post-menopausal patients with intermediate-to-high risk, early breast cancer with the aim of reducing distant recurrence. One of the main trials to underpin the adoption of adjuvant bisphosphonates was the randomised Phase III AZURE trial in which 3,300 patients with stage II/III breast cancer received zoledronate plus standard therapy or standard therapy alone for 5 years, with a further 5-year follow-up.

Purpose. To evaluate the impact of adjuvant zoledronate on bone health of a sub-group of AZURE patients over the 5-year follow-up period.

Methods. 229 women (122 zoledronate/107 control), who had completed the AZURE trial within the previous 3 months, were recruited from 20 UK sites. BMD (lumbar spine, femoral neck, total hip) and bone turnover biomarkers, urinary αCTX, βCTX (relative to creatinine), serum PINP, TRACP5b, were assessed at sub-study entry and thereafter at 12, 24 months (60 months for BMD).

Results. Mean BMD (±SD) at study entry in zoledronate and control arms respectively was: lumbar spine 1123 (±201) and 985 (±182) mg/cm2 (p<.0001); femoral neck 847 (±130) and 782 ((±120) mg/cm2 (p<.0001). The BMD differences at baseline persisted throughout the 5-year follow-up with little change in mean BMD (see figure). Baseline median (IQR) biomarkers were all significantly (p<.001)) suppressed in the zoledronate arm (see Table) and remained suppressed, though with some offset at 24 months.

Conclusions. Adjuvant zoledronate results in a sustained increase in BMD and suppression of bone turnover. Most patients after receiving adjuvant bisphosphonates are unlikely to require additional bone-targeted therapy to prevent bone loss.

Characteristic	Zoledronate	Control
Baseline (month 0)		
uαCTX/Cr(µg/mmol)	0.14 (0.08, 0.19)	0.68 (0.45, 0.97)
uβCTX/Cr(µg/mmol)	0.63 (0.40, 0.89)	2.17 (1.47, 3.06)
sP1NP(ng/mL)	16.8 (14.4, 21.6)	45.8 (31.1, 61.3)
sTRACP5b(U/L)	1.9 (1.7, 2.1)	2.8 (2.3 to 3.1)

YOUNG INVESTIGATOR AWARD WINNER

The roles of the FL/FLT3 axis in the development of cancer-induced bone pain mediated by prostate cancer

Yang Yu, Sun Hee Park, Jenna Ollodart, Kelly Contino, Laiton R Steele, Yusuke Shiozawa

Department of Cancer Biology and Comprehensive Cancer Center, Medical Center Blvd, Wake Forest University Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC, 27157-1082, USA.

Cancer-induced bone pain (CIBP) is the most common and intractable symptom in prostate cancer (PCa) patients with bone metastases, and impairs patients' quality of life. The currently established treatments for CIBP are non-steroidal anti-inflammatory drugs and opioids. However, these treatments provide sufficient pain control in only half of patients with CIBP and have serious side effects. Thus, there is an urgent need to develop more effective and safer therapies for CIBP. A kinase microarray comparing dorsal root ganglia (DRG) sensory neurons harvested from mice with and without CIBP revealed that activated FMS-like tyrosine kinase 3 (FLT3) is elevated in the DRGs of mice with CIBP. In the same mouse model, bone marrow from mice with CIBP was found to express higher levels of FLT3 ligand (FL). Further, analysis of 497 PCa tissue samples from TCGA database showed that the bone pain gene signature was highly enriched in patients with high FL levels compared to those with low FL. Recent studies have demonstrated that FL induces peripheral neuropathic pain, and FLT3 inhibitors alleviate FL-mediated peripheral neuropathic pain. However, little is known regarding the role of the FL/FLT3 axis in the development of CIBP and whether this axis can be targeted as a CIBP treatment. Since pathological neurite outgrowth of sensory nerves near bone metastatic sites is known to be one of the mechanisms contributing to CIBP, the effect of FL on neurite outgrowth was assessed using in vitro nerve sprouting assays. Surprisingly, FL itself failed to induce DRG nerve sprouting; however, when DRGs were treated with nerve growth factor (NGF), known to induce nerve sprouting, FL enhanced NGF-mediated nerve sprouting. FLT3 inhibitors significantly reduced FL + NGF-induced nerve sprouting. Moreover, phosphoproteomic analysis in DRGs treated with FL revealed (i) enrichment of signaling pathways associated with neurite outgrowth, including NGF; reelin; neurotrophin/Trk receptor; and synaptogenesis signaling, and (ii) that FLT3 activation in DRGs may phosphorylate downstream RGS10, a G protein regulator involved in pain. Together, our findings suggest that (i) FL from bone metastatic PCa activates FLT3 in DRGs, which may result in CIBP and (ii) FL induces CIBP by enhancing NGF-mediated nerve sprouting. Although further studies are undoubtedly needed, the FL/FLT3 axis can be a potential therapeutic target for PCa-mediated CIBP.

YOUNG INVESTIGTOR AWARD WINNER

Chemotherapy-induced adipocyte senescence triggers bone loss through RANKL-mediated osteoclastogenesis

Ganesh Kumar Raut[1], Zhangting Yao[1], Xianmin Luo[1], Qihao Ren[1], Taylor Malachowski[1], Douglas V. Faget[1] and Sheila A. Stewart[1,2,3,4*]

[1]Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110, USA. [2]Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA. [3]Siteman Cancer Center, Washington University School of Medicine, St. Louis, MO 63110, USA. [4]ICCE Institute, Washington University School of Medicine, St. Louis, MO 63110, USA.

Despite the central role chemotherapy plays in prolonging survival, the toxicities associated with its use negatively impact quality of life and in some cases, can be so severe that patients forego further life preserving treatments. Recently we demonstrated that chemotherapy-induced senescence drives bone loss by limiting mineralization of new bone and increasing bone resorption. However, the underlying mechanism of action is not fully understood. Therefore, it is critical that we understand the mechanisms that drive these toxicities and develop approaches to mitigate their severity. Using a vossicle model in which neonatal vertebral bones from *INK-ATTAC* pups (allow to selectively kill senescent cells) were transplanted into wildtype adult mice, we found that the elimination of senescent cells in donor vossicles protects from chemotherapy-induced bone loss, indicating that chemo-induced senescence is a local type, not a systemic. To understand the mechanism that contributed to therapy-induced bone loss, we used scRNA-seq and co-immunofluorescence staining to determine which bone resident cells underwent senescence in response to chemotherapy and how their gene expression was impacted. Results showed that chemotherapy induced senescence in bone marrow adipocytes that expressed RANKL and resulted in bone loss.

YOUNG INVESTIGATOR AWARD WINNER

PTHrP biological domains mediate breast cancer bone osteolysis via TGF-β/SNAI2/ZEB1 pathways

Déja Grant[1,2], Julia Ahn[2], Courtney Edwards[3,4], Jasmine A. Johnson[3,5] and Rachelle W. Johnson[3-5]

[1] Department of Biochemistry, Cancer Biology, Neuroscience, & Pharmacology, Meharry Medical College, [2] Vanderbilt University, [3] Vanderbilt Center for Bone Biology, Vanderbilt University Medical Center, Vanderbilt University, [4] Graduate Program in Cancer Biology, [5] Department of Medicine, Division of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, TN

Breast cancer cells frequently metastasize to bone, where they may proliferate or enter a dormant state. Parathyroid hormone-related protein (PTHrP) expressed by breast cancer cells promotes tumor outgrowth in bone by increasing bone resorption and stimulating tumor cell exit from dormancy. PTHrP has multiple biological domains that determine its autocrine, paracrine, and intracrine functions, but the role of the PTHrP nuclear localization signal (NLS) and C-terminus is not well understood. To assess the role of PTHrP biological domains in breast cancer bone colonization, we stably expressed full-length secreted PTHrP, PTHrP with deletion of the NLS, or PTHrP with deletion of the NLS and C-terminus in human MCF7 breast cancer cells, which normally lay dormant in bone. MCF7 cells expressing these proteins were termed FLSEC, DNLS, and DNLS+CTERM mutant cell lines, respectively, and inoculated into athymic nude mice by intracardiac injection to facilitate bone colonization. Osteolytic lesion area and number assessed by radiography, and tumor burden in the bone marrow quantified by flow cytometry were significantly higher in mice inoculated with DNLS (5.9-fold, $p<0.05$) and DNLS+CTERM (4.19-fold, $p<0.05$) mutant cell lines compared to control MCF7 cells. To determine which downstream signaling pathways were enriched by the PTHrP mutants, we performed RNAseq and Gene Set Enrichment Analyses on each cell line, which revealed significant enrichment for epithelial-to-mesenchymal (EMT) markers and specifically ZEB1 as a core driver gene in the DNLS+CTERM mutant. Upon validation at the molecular level, we found that the EMT markers E-cadherin and vimentin were unchanged; however, TGF-β2 was increased in all PTHrP mutant cell lines, and SNAI2 (Slug) was significantly down-regulated in DNLS and DNLS+CTERM mutants ($p<0.0023$-0.0005), while ZEB1 was significantly upregulated in DNLS+CTERM cells ($p<0.05$), compared to MSCV controls. Crosstalk within the TGF-β/Slug/ZEB-1 signaling pathway is exceedingly complex, with bi-directional regulation, depending on the cell type and context. It is also well established that TGF-β positively regulates PTHrP; however, our data suggest that down regulation of Slug may be important for breast tumor progression in bone downstream of PTHrP, and this pathway may or may not converge on ZEB1. Taken together, our data indicate that PTHrP-mediated osteolysis involves the NLS and C-terminal domains signaling through EMT-associated pathways.

YOUNG INVESTIGATOR AWARD WINNER

Senolytics deplete senescent osteocytes and improve bone health in metastatic breast cancer

Kaur J[1], Adhikari M[1], Sabol HM[1], Anloague A[1], Khan S[1], Barnes L[2], Stambough JB[2], Palmieri M[3], ReyesCastro O[3], Ambrogini E[3], Almeida M[3], O'Brien CA[3], Nookaew I[4,5], Delgado-Calle J[1,5].

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Breast cancer (BCa) bone metastases are typically incurable and cause a devastating bone disease. The bone microenvironment fosters BCa progression and is an important therapeutic target. Osteocytes (Ots) are the most abundant bone cells, but their role in BCa metastasis is not well established. Here, we injected murine E0771 BCa cells or PBS in 8-wk-old female NuTRAP reporter mice crossed with Dmp1-8kbCre mice and, after 2 wks, used scRNAseq of purified GFP+ cells and histology to define the impact of BCa metastases on Ots. Unbiased transcriptomic analyses revealed three main cell clusters: Ots, osteoblasts (Obs), and pre-osteoblasts (pre-Obs). BCa cells decreased the Ob population and increased pre-Obs, but did not affect Ots. Ots from BCa metastases showed 1) upregulation of genes enriched in senescence, senescence-associated secretory phenotype (SASP), and inflammatory response GO terms, 2) upregulation of *Spp1, Mmp13, Vegfa, Timp2*, 3) increased senescence/SASP score, and 4) higher number of p16+-RANKL+ Ots compared to naïve mice, suggesting increased Ot osteoclastogenic potential. Further, histological analyses showed higher mRNA *p16+* Ots in BCa-bearing bones. Using *in vitro* cultures, we established that conditioned media (CM) derived from murine E0771 or human MDA-MB-231 BCa cells increased senescence-associated β-galactosidase activity and upregulated senescence-related genes *p16, p21, Mmp13, IL6,* and *Vegfa* in Ocy-454 Ot-like cells. Remarkably, we found that bone colonization by human MDA-MB-231 BCa cells led to similar gene expression changes in *ex vivo* organ cultures with authentic Ots established with human bone. To define the impact of cellular senescence on BCa bone metastases, we treated 8-wk-old female mice with intratibial BCa tumors with a cocktail (D+Q) of the senolytics Dasatinib (5 mg/kg) and Quercetin (50mg/kg) once a wk, starting two days after tumor inoculation. D+Q, known to eliminate senescent cells, prevented the increase in p16+ Ots induced by BCa cells but did not affect tumor burden. In contrast, D+Q decreased the number of lytic lesions and mitigated the bone loss induced by BCa tumors. Our results demonstrate that metastatic BCa cells generate a microenvironment conducive to cellular senescence in bone cells, including Ots, which contribute to osteolytic disease and support using senolytic drugs as an adjuvant therapy to prevent BCa-induced bone loss.

YOUNG INVESTIGATOR AWARD WINNER

**Targeting stromal p38MAPKalpha triggers innate-adaptive anti-tumor immunity
and sensitizes metastatic breast cancer to immunotherapy**

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Metastatic breast cancer remains an intractable disease that is poorly responsive to therapy. Moreover, once tumor cells reach the bone, the disease is considered incurable and treatments are only palliative. For this reason, new therapies that restrain metastatic growth are desperately needed. We have previously found that limiting stromal p38MAPKα signaling reduced the growth of bone metastases, however the mechanism behind this effect remains poorly understood. Here, we show that p38MAPKα inhibition (p38i) reprograms the metastatic tumor microenvironment (TME) in the bone to limit tumor progression in a CD4+ T cell, IFNγ, and macrophage dependent manner. To further increase the efficacy of p38i, we utilized a stromal labeling approach and single cell RNA sequencing (scRNAseq) to identify immunotherapeutic targets in the metastatic setting. Informed by these analyses, we combined p38i and an OX40 agonist antibody that synergized to significantly reduce metastatic growth and increase overall survival in clinically relevant mouse models. Intriguingly, patients with breast cancer harboring our p38i metastatic stromal signature had a better overall survival rate that was further increased when tumors displayed increased mutational load. Thus, we asked if our combinatorial approach was effective in antigenic breast cancer. The combination of p38i, anti-OX40, and cytotoxic T cell engagement cured mice of metastatic disease and led to long-term sustained immunologic memory. Our findings demonstrate that analyses of the stromal compartment can lead to the rationale design of effective anti-metastatic therapies that target the stromal compartment and produce immunologic memory.

YOUNG INVESTIGATOR AWARD WINNER

Cancer-Associated Hypercalcemia Signals Through the Hindbrain to cause Anorexia

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Cancer-associated hypercalcemia is a frequent complication that portends a poor prognosis. Hypercalcemia can arise from skeletal metastases or in patients with few or no bone lesions as part of the syndrome known as humoral hypercalcemia of malignancy (HHM). HHM is caused by tumor secretion of parathyroid hormone-related protein (PTHrP) and is defined by a constellation of biochemical abnormalities, including elevated calcium and suppressed PTH levels. Patients with hypercalcemia experience nausea, anorexia and weight loss, however, the molecular mechanism explaining these symptoms is unknown. To investigate the nature of the metabolic alterations in HHM, we developed an inducible mouse model of PTHrP overexpression in PyMT-derived mammary tumors (Tet-PTHrP;PyMT). Administering doxycycline (Dox) to TetPTHrP;PyMT mice activates human PTHLH cDNA expression in mammary tumors, causing elevated circulating PTHrP levels and severe hypercalcemia associated with weight loss, fat loss and reduced food intake, recreating the clinical HHM syndrome. Pair feeding experiments demonstrated that weight loss and changes in body composition were caused by reduced food intake. Therefore, we aimed to understand how PTHrP overexpression caused anorexia. We performed immunofluorescence for cFos, a marker of neuronal activation, in sections of the mouse brain and found that tumor PTHrP production activated the area postrema (AP), nucleus tractus solitarius (NTS) and parabrachial nucleus (PBN), defining a well-described brain circuit that suppress appetite. To distinguish whether PTHrP or elevations in calcium accounts for the reduced appetite, we treated Tet-PTHrP;PyMT mice on Dox with a blocking antibody against the type 1 PTH/PTHrP receptor (PTHR1), and 2 different antiresorptives (rhOPG-Fc, zoledronic acid). Treatment with anti-PTHR1, rhOPG-Fc or zoledronic acid blocked bone resorption preventing the raise in calcium levels, cFos activation and anorexia. On the contrary, treating wild type mice with either calcium or cinacalcet, an allosteric activator of the calcium-sensing receptor (CaSR), inhibits food intake and activation of the same brain areas. CaSR is expressed in the AP and CaSR+ neurons showed increased cFos in animals that overexpress PTHrP or treated with either calcium or cinacalcet. Overall, we conclude that cancer induced hypercalcemia contributes to weight loss by activating a hindbrain, anorexigenic circuit, defining a new link between tumor, bone and brain.

OUTSTANDING ABSTRACT AWARD WINNER

Bone-derived excess TGF-ß release is associated with cognitive dysfunction due to oxidation of RyR, Ca2+ leak and mitochondrial dysfunction

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Cancer-related cognitive impairment occurs in up to 30% of patients before treatment. Long-term survivors of bone cancer at particularly increased risk of cognitive deficits. Recent studies have shown that the skeleton is an active endocrine organ that secretes many kinds of bone derived factors, including transforming growth factor ß (TGF-β), and contributes to the pathophysiology of many diseases, such as cachexia and Alzheimer's disease. In this study, we measured a biomarker of neuronal damage neurofilament light chain (sNfL) in the serum and found sNfL in patients present with bone metastases (117±18 pg/ml) was significantly higher than controls (13±4 pg/ml). Similarly, bone turnover marker β-CTX (P=0.002) and platelet-poor plasma TGF-ß (P=0.036) were significantly increased in patients with bone metastasis. We also investigated the relevance of bone-derived TGF-β with disease progression. In patients, TGF-ß concentration was positively correlated with β-CTX (R^2=0.67, P<0.0001) and sNfL (R^2=0.85, P<0.0001). This suggests that higher dementia risk correlates with excessive amounts of TGF-β release. Using a transgenic mouse model of Camurati Engelmann Disease (CED), a nonmalignant metabolic bone disorder associated with increased TGF-ß activity and bone destruction, we identified that TGF-ß mediated oxidation of Ca^{2+} channels in the brain, which may cause cognitive dysfunction. We found that CED mice exhibited a decline in novel object recognition and cognition compared to littermate controls. This is associated with increased phosphosmad2/3 expression, persistent activation of microglia and astrocyte, ß-amyloid plaque formation and subsequent synaptic loss. Mechanistically, we showed that TGF-β1 increased transcription of NADPH oxidases and subsequently oxidized RyR2 chancel. Furthermore, RyR2 channel remodeling induced intracellular Ca^{2+} leakage that causes mitochondrial Ca^{2+} overload and induce apoptosis leading to neuron damage, which impairs synaptic plasticity and causes memory and behavioral deficits. Thus, a feed-forward cycle of pathological Ca^{2+} handling is created between ER and mitochondria, which underlies TGF-ß-driven cognitive dysfunction. These findings suggest that excess TGF-ß release by pathological bone destruction induces oxidative stress, ER Ca^{2+} leak and neurodegeneration and could explain cognitive impairment observed clinically with bone metastasis. Targeting this pathway may improve survival in patients with bone metastases.

OUTSTANDING ABSTRACT AWARD WINNER

Anti-myeloma efficacy of BCMA CAR-iNKT is enhanced with a long-acting IL-7, rhIL7hyFc

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Multiple myeloma (MM), a malignancy of mature plasma cells, remains incurable. Immunotherapy, including genetic modification of T-cells to express a chimeric antigen receptor (CAR-T) is transforming myeloma therapy. Optimal myeloma CAR target proteins are highly expressed on myeloma cells and have limited/no expression on normal cells. B-cell maturation antigen (BCMA) is the lead protein target for myeloma CAR-T therapy because of high expression on most MM with limited expression on normal cell types resulting in favorable "on target-off tumor" toxicity. The response rate to autologous BCMA CAR-T therapy is high; however, it is not curative and is associated with risk of cytokine release syndrome (CRS) and immune effector cell associated neurotoxicity syndrome (ICANS). Outcomes in BCMA CAR-T treated patients may improve with allogeneic CAR-T which offer higher cell fitness and reduced time to treatment. However, to prevent risk of graft versus host disease (GvHD), allogenic BCMA CAR-T requires genetic deletion of the T-cell receptor (TCR) which has potential for unexpected functional or phenotype changes. Invariant natural killer T (iNKT) cells have an invariant TCR that do not cause GvHD and as a result can be used in allogeneic setting without need for TCR gene editing. We demonstrate significant anti-myeloma activity of BCMA CARiNKT in a xenograft mouse model of myeloma. We also found that a long-acting IL-7, rhIL-7- hyFc significantly prolonged survival and reduced tumor burden in BCMA CAR-iNKT treated mice in both a primary and a re-challenge setting. Further, in CRS *in vitro* assays, where effector cells, tumor targets, and primary immature dendritic cells (IDC) are co-cultured, CAR-iNKT induced less IL-6 secretion by the IDC than CAR-T, suggesting the potential for reduced likelihood for CAR-iNKT cell therapy to induce CRS in patients. Together, BCMA CAR-iNKT demonstrate anti-myeloma efficacy that can be potentiated with rhIL-7-hyFc and BCMA CARiNKT may have a safer profile than CAR-T.

OUTSTANDING ABSTRACT AWARD WINNER

CDK4/6 inhibitor and doxorubicin synergistically inhibit breast cancer bone metastasis and enhance T-cell targeted therapy

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CDK4/6 inhibitor combined with endocrine therapy has successfully treated advanced estrogen receptor-positive (ER+) breast cancer; however, drug resistance is an emerging cause of breast cancer–related death. Overcoming CDK4/6 inhibitor treatment resistance is an urgent problem. Here, we show that in triple-negative 4T1 and luminal B ER+ PyMT-BO1 breast tumor cell line derived mammary fat pad (MFP) models, CDK4/6 inhibitor LEE011 single-agent treatment decreased primary tumor burden. We observed increased CD8+ T cell numbers in the spleen and bone marrow but not in the primary tumor tissues after LEE011 therapy. Depletion of CD8+ T cells before PyMT-BO1 implantation in the mammary fat pad diminished the LEE011 treatment effect, suggesting LEE011 works partially through CD8+ T cell function. In the bone colonization model derived from both 4T1 and PyMT-BO1 cells, LEE011 single-agent treatment had little effect on bone tumor burden, indicating treatment resistance in the bone tumor microenvironment. In vivo, LEE011 therapy for 10 days did not change trabecular bone volume and bone mineral density, suggesting that LEE011 may not modulate osteoclast function to promote bone tumor growth. To better treat bone metastases, we screened anti-breast cancer therapies that could synergize with LEE011. We identified that doxorubicin could synergize with LEE011 to inhibit tumor cell proliferation. LEE011 and doxorubicin combination treatment caused a sharp drop in cell numbers in the S phase and more cells arrested in the G1 and G2 phase; however, the combination treatment only slightly enhanced tumor cell apoptosis, and did not significantly alter the DNA damage marker expression. We found that doxorubicin plus LEE011 treatment groups showed the lowest bone tumor burden and tumor-associated bone loss than the vehicle, LEE011, or doxorubicin alone in the PyMT-BO1 bone colonization model and likewise lowest tumor burden in the MFP model. Histology analysis of tumor tissues showed that the number of ARG1+ and Ki67+ cells significantly decreased from the combination treatment groups. Given the effect of LEE011 on CD8+ T-cells in vivo, we evaluated the combination of LEE011 plus doxorubicin on T-cell therapy in the immune therapy-resistant

PyMT-BO1 bone colonization model. In the bone colonization model established with OVA257-264 expressing PyMT-BO1 cells, LEE011 and doxorubicin combination treatment significantly enhanced the tumor-specific OT-1 T-cell therapy efficacy to decrease bone tumor burden and prolong survival. Taken together, the combination therapy of CDK4/6 inhibitor LEE011 and chemotherapy agent doxorubicin showed promising results in preclinical breast cancer mouse models.

LIGHTNING TALK ORAL ABSTRACTS

High Scoring Abstract Awards
(Lightning Talks)
with
Poster Numbers

HIGH SCORING ABSTRACT AWARD WINNER

#1   Acid ceramidase (ASAH1) is a key mediator of drug resistance in relapsed/refractory multiple myeloma

Ryan T. Bishop[1], Tao Li[1], Raghunandan R. Alugubelli[2], Mostafa Nasr[1,3], Karl J. Nyman[3], Praneeth Sudalagunta[4], Mark Meads[1,2], Jeremy Frieling[1], Niveditha Nerlakanti[1,3], Marilena Tauro[1], Oliver Hampton[5], Bin Fang[6], Steven Grant[7], John Koomen[6,8], Ariosto Siqueira Silva[4], Kenneth H. Shain[1,9] and Conor C. Lynch[1*]

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Despite the growing armamentarium of treatments including proteasome inhibitors (PIs), immunomodulatory imides (IMiDs) and cellular and non-cellular immunotherapies MM remains incurable, due to the emergence of drug resistance that causes patient relapse. To develop therapies that significantly extend overall survival, a clearer understanding of the mechanisms driving drug-resistance is urgently required. Analysis of RNA-seq data derived from 672 MM Moffitt patients revealed that sphingolipid metabolism genes and in particular acid ceramidase (ASAH1), are significantly enriched in relapsed/refractory MM (RRMM) and predict poor overall survival in PI-treated patients (median overall survival 552 vs 1226 days). Next, we queried potential mechanisms of treatment resistance using gene-set enrichment analysis (GSEA). ASAH1 High patients displayed upregulation of gene regulation of apoptosis. Further, PI-resistant cell lines displayed elevated levels of ASAH1, and total and phosphorylated levels of anti-apoptotic proteins MCL-1 and BCL2 compared to PI-sensitive parental cells. Genetic and pharmacological inhibition of ASAH1 significantly decreased MM viability and reduced MCL-1/BCL-2 expression. Further, ASAH1 inhibition resensitized resistant MM cells to PI treatment. Importantly, we recapitulated our findings in CD138+ patient MM cells ex vivo with ceranib-2/PI treatment being synergistic in the majority (>75%) of patient samples tested. In vivo, ceranib-2 treatment limited PI-resistant MM tumor growth and extended overall survival of NSG mice. Further, ASAH1 depletion resensitized MM cells to PI treatment in vivo and loss of MCL-1 and BCL-2 expression was confirmed ex vivo. Total and pSTY proteomic profiling revealed endogenous phosphatase PP2A inhibitor, SET as a candidate mechanism. Further, ASAH1 depletion of ceramide promoted SET inhibition of PP2A phosphatase activity thus facilitating the increased expression and activity of the prosurvival proteins, MCL-1 and BCL-2. Notably, SET was elevated in PMRC RRMM patient samples, correlating with ASAH1 expression, and predicting poorer overall survival. In conclusion, these preclinical studies suggest ASAH1 is a potential therapeutic target for the treatment of RRMM. Future studies to develop novel and

potent ASAH1 inhibitors for the treatment resistant MM, in combination with current standard of care therapies are on-going and we believed warranted based on our emerging data.

HIGH SCORING ABSTRACT AWARD WINNER

#2   Homing of intestinal NK and Th1 cells to the tumor site restrains melanoma bone growth in a gut microbiome dependent manner

Subhashis Pal[1,2], Daniel S. Perrien[1,2], Tetsuya Yumoto[3], Roberta Faccio[4], M. Neale Weitzmann[1,2,5], and Roberto Pacifici[1,2,6]

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Malignant melanoma is an aggressive type of skin cancer that can lead to osteolytic bone metastasis. Antibiotics, which are frequently prescribed to patients with cancer, affect the composition of the gut microbiome and subsequently changes the immune response. We postulated that intestinal immune cells would be altered by antibiotic-induced gut microbiome loss, speeding up the formation of bone metastasis. We used intracardiac or intratibial injections of B16-F10 melanoma cells and found that gut microbiome depletion caused by treatment with broad spectrum antibiotics (Abx) increased bone metastasis growth compared to non-Abx treated control mice. Peyer's patches (PPs) and bone marrow (BM) cells within tumor lesions were analyzed using flow cytometry, and the results showed that microbiome depletion prevented the growth of natural killer (NK) cells and T helper 1 (Th1) cells caused by melanoma as well as their migration from the gut to tumor bearing bones. According to our finding, intestinal NK and Th1 cells expand and migrate to the BM in response to the development of cancer. Antibiotics mediated gut microbiome depletion reduced NK and Th1 cell migration from the gut to the tumor site by ~ 8-fold when it was measured directly using Kaede mice, a strain that expresses a photo-convertible fluorescent protein enabling direct tracking of intestinal lymphocytes. S1PR5 and S1PR1 receptors respectively mediate the exit of NK cells and Th1 cells from the gut. Pharmacological blockade of either S1PR5-mediated egress of NK cells or S1PR1-mediated egress of Th1 cells from the intestine mimicked the effects of antibiotics, preventing expansion of NK cells and Th1 cells in the BM and causing accelerated bone metastasis growth, demonstrating the functional relevance of immune cell trafficking. The chemokine ligand CXCL9, which is expressed by BM cells, and the CXCL9 receptor CXCR3, which is expressed by NK and Th1 cells, control the influx of circulating NK and Th1 cells to the tumor site. The frequency of tumor NK and Th1 cells was lowered, and tumor growth was accelerated by either global ablation of CXCR3 or antibody-mediated neutralization of CXCL9. According to our study, immunological control of melanoma metastasis and tumor growth in bone is affected by processes of microbiota-mediated gut-bone crosstalk. Thus antibiotic-induced microbiome changes in melanoma patients may have detrimental clinical effects.

HIGH SCORING ABSTRACT AWARD WINNER

#3   IL-17A supports osteoblasts to become cancer-associated fibroblasts in breast cancer bone metastases

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Cancer-associated fibroblasts (CAFs) reside inside tumors providing a micro-milieu that supports tumor growth and metastatic spread. However, little is known about the presence of CAFs in bone metastases. In breast cancer bone metastases osteoclasts are activated, while the abundance of osteoblasts (OB) on adjacent bone surfaces is diminished. To test the hypothesis whether OBs might abandon the bone surface to become CAFs within metastases, we induced breast cancer bone metastasis by intracardiac injection of 4T1 breast cancer cells into female *Osx-cre-ERt+;Ai9* mice. Using this lineage-tracing model, we performed single cell RNA sequencing (scRNAseq) of tdTomato-positive (tdTom+) OBs in cancer-bearing and healthy control animals. Bioinformatic analysis revealed a distinct cluster of tdTom+ OBs in metastases-bearing mice that express genes related to tumor microenvironment, fibrosis and proliferation of CAFs (e.g. *Cxcl12, Mmps, Pdgfr-α, Pdgfr-β, Col1*). Furthermore, histological analysis demonstrated elongated tdTom+ OBs within metastases while only a few OBs were present on nearby bone surfaces. Consistently, MC3T3-E1 and primary OBs acquired a spindle shape morphology upon stimulation with medium conditioned by 4T1 or MDA-MB-231 breast cancer cells (CCM), which was accompanied with an increased OB migration. Furthermore, in an ex vivo co-culture model, OBs in the vicinity of cancer cells became elongated and migrated in-between tumor cells. Quantitative PCR, scRNAseq and histological analyses revealed a reduction of OB marker genes (*Runx2, Osx*) and an increase of CAF-associated markers (*Pdgfr-α, Pdgfr-β*) in CCM-stimulated OBs and cancer-associated tdTom+ OBs. In addition, CCM promoted OBs to remodel the extracellular matrix, which is characteristic of CAFs. To elucidate the mechanisms transitioning OBs to CAFs, we performed RNA sequencing and identified an activated IL-17A signaling pathway in CCM-stimulated OBs compared to controls, which was confirmed by immunoblot and qRT-PCR analyses of downstream targets (*C/ebp, Ccl2*). Furthermore, recombinant IL-17A promoted OB migration and acquisition of a CAF phenotype while inhibition of IL-17A signaling using an anti-IL-17 antibody restored the CCM-induced morphological changes and OB migration. Together, these results demonstrate that IL- 17A supports OBs to become CAFs in breast cancer bone metastases, which might contribute to metastatic growth.

HIGH SCORING ABSTRACT AWARD WINNER

#4   Elucidating the Effect of Glutamine Metabolism in Breast-to-Bone Metastasis

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Bone-metastatic tumors will develop in 65-75% of metastatic breast cancer (MBC) and are associated with high morbidity and mortality in patients. However, despite the prevalence of bone metastases in MBC, we lack a firm understanding of what makes MBC cells so bonetropic. One important factor in a circulating tumor cell's ability to reach a secondary site and develop into a macro-metastatic lesion is the ability to adapt metabolically to new environments with varying nutrient availability and stressors. Breast tumors, particularly triple-negative (TNBC), HER2+ and advanced ER+ subtypes, have been shown to be very reliant on glutamine metabolism due to the upregulation of a gene called GLS1 (hereafter referred to as GLS). In this study, we aimed to characterize the function of GLS and glutamine metabolism on expansion of breast cancer cells within the bone microenvironment. We found that bone tumors failed to develop after inoculation with breast cancer cells carrying a GLS loss-of-function mutation. Our data also indicates that GLS knockout in various breast cancer cell lines leads to a decrease in bone destruction in vivo. Pharmacological inhibition or deletion of GLS leads to a "senescent-like" phenotype that suggests suggesting that GLS may be required for breast-to-bone metastasis. Together, this work suggests that glutamine metabolism may promote bone metastasis in breast cancer, raising the possibility of using pre-existing cancer therapies targeting glutamine addiction to ameliorate bone metastatic burden in breast cancer.

HIGH SCORING ABSTRACT AWARD WINNER

#5   Activating RET mutations in medullary thyroid cancer cells promotes osteoblastic bone metastasis by inhibiting osteoclastogenesis and stimulating osteoblast activity

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Bone metastases (BM) are a source of increased mortality and significant morbidity in patients with medullary thyroid cancer (MTC), with ~50% survival at five years post-diagnosis. MTC causes osteoblastic, osteolytic, or mixed lesions, yet the underlying mechanisms are unknown. The *RET* proto-oncogene encodes a transmembrane tyrosine kinase receptor and is the driver oncogene in ~60% of MTC cases where a germline or somatic activating mutation results in ligand-independent constitutive activation of the receptor. RET mutations are the second most mutated cancer-predisposing gene in the germline of patients with osteosarcoma. However, the role of RET mutations in the genesis of bone metastases lesions is unknown. We show that *RET* mutations promote an osteoblastic phenotype in mice implanted with patient-derived MTC cells by increasing bone formation and decreasing bone resorption.

We found that the radiographic appearance of osteoblastic lesions differed between the type of RET mutations (C634W and M918T). MTC cells with RET^{C634W} mutation showed a four-fold increase in bone mineral density, trabecular bone volume, number, and thickness compared to nontumor-bearing femur, whereas MTC-RET^{M918T} mutation showed an increased cortical thickness and porosity with reduced medullary area compared to the non-tumor-bearing femur. MTC cells formed tumors with extensive mineralized tissue, composed of bone matrix surrounded by osteoblast like cells and osteoid. TRAP staining revealed that osteoclast numbers per long bone were significantly reduced in MTC injected femur compared to the control. The knockdown of RET in the MTC-RET^{M918T} model significantly reduced cortical thickness and increased the number of osteoclasts per bone surface as determined by micro-CT and histomorphometry analysis. The secreted factors by MTC cells, including osteoprotegerin (OPG), inhibited osteoclast formation from primary bone marrow cells and Raw264 cells.

Moreover, the MTC conditioned media (CM) increased osteoblast differentiation and mineralization of the primary culture of preosteoblasts isolated from calvaria, long bones, and preosteoblast MC3T3 cells, inhibited by RET knockdown. The expression of alkaline phosphatase, osteocalcin, OPG, and OSX increased in preosteoblast incubated with MTC-CM. These results suggest that MTC induces osteoblastic bone metastasis due to decreased bone resorption and increased bone formation.

HIGH SCORING ABSTRACT AWARD WINNER

#6   Mechanical loading of breast cancer laden bones alters osteocyte mediated bone loss in vivo

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Background: Roughly 70% of breast cancer patients develop bone metastases that cause pain, bone loss, and increased fracture risk. Mechanical loading has an osteoprotective role during metastasis, reducing secondary tumor formation and bone loss, and reducing sclerostin (SOST) expression in healthy bone. The key mechanosensing bone cell is the osteocyte (OCy), which guides remodeling via osteoblasts and osteoclasts in response to loading. OCys can also remodel their microenvironment through perilacunar remodeling. However, few studies have specifically investigated OCys, especially perilacunar remodeling, during loading and bone metastasis. We hypothesize that loading following intratibial breast cancer cell inoculation will inhibit osteocyte-driven perilacunar bone loss.

Materials and methods: Mouse Models: Female mice (16wko FVBn, n=16) received intratibial injection of breast cancer cells (FVBn, 100k cells) in both left and right tibiae. After 5 weeks of tumor growth, mice received high-magnitude (8.1N, n=8) or low-magnitude (2.6N, n=8) cyclic compressive loading on the left tibia for two weeks (M-F, (4s loading at 4Hz + 3s rest) x 50 cycles). Mice were sacrificed at 18wko, tibiae were isolated and fixed in 4% PFA. **Imaging:** µCT imaging was used to evaluate injection success and gross bone loss. nanoCT was performed on a subset (n=11, Res=0.62µm) to evaluate OCy perilacunar remodeling. **Histology:** Tibiae were processed for sclerostin immunohistochemical staining. SOST+ OCys within bone were counted and normalized to bone area. **Statistics:** One-way ANOVA, Students t-test (p=0.05)

Results: High magnitude loading accelerated bone loss for bones with developed tumors (reduced cortical/cancellous bone volumes). However, bone volume was similar for low-loading and unloaded groups, unlike prior studies where loading preceded or was concurrent with tumor inoculation. Preliminary data indicates that the percentage of SOST+ OCys decreased with high and low magnitude loading as expected, with changes more noticeable in trabecular bone. This was accompanied by a slight increase in lacunar sphericity (a phenotype associated with aging and altered mechanosensitivity), which may explain the reduced mechano-response in the low magnitude group. Next steps include increased examination of lacunar morphometric data to identify changes within bone and lesion types.

HIGH SCORING ABSTRACT AWARD WINNER

#7   Downstream Effects of Gli2 Targeting on Tumor-Immune Cell Crosstalk in Bone Metastatic Breast Cancer

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Bone is the most common site of breast cancer metastasis, and bone metastases drastically impact patient outcomes through skeletal-related events (SREs), such as fracture, pain, and spinal cord compression. Bone resorption inhibitors delay time to SRE but do not improve survival, and other breast cancer treatments are not curative for bone metastases. Tumor-induced bone disease (TIBD) is driven by cancer cell overexpression of the transcription factor Gli2, which induces bone breakdown, and Gli2 inhibition reduces TIBD. The fundamental studies of Gli2 in TIBD have focused primarily on its effects on bone destruction via crosstalk between tumor cells and bone remodeling cells, but studies have yet to identify other roles for Gli2 in the bone metastatic microenvironment. However, recent research has identified a role for Gli2 in modulating expression of immunosuppressive cytokines in other cancers. This suggests a role for Gli2 in tumor-immune crosstalk. Therefore, we hypothesize that tumoral Gli2 drives expression of genes that induce M2-like macrophages and other suppressive immune populations. We evaluated this hypothesis by analyzing gene expression correlation to Gli2 in human primary breast tumor bulk RNA sequencing data and in similar analyses of bone-tropic MDA-MB-231 breast cancer cells treated with a Gli inhibitor (GANT58). Our human data shows significant correlation between Gli2 and 212 immune response-related genes (gene list from GO: 0006955). For example, the Spearman's correlation between Gli2 and TGFBR3, STING1, AXL, and CSF1 is 0.60, 0.47, 0.46, and 0.45, respectively. We found that GANT58 treatment of bone-tropic MDA-MB-231 breast cancer cells results in 1972 differentially expressed genes by DESeq2 analysis of bulk RNA sequencing data. Gene Ontology analysis demonstrates over 1.8-fold enrichment of biological processes related to immune system development, hemopoiesis, and lymphocyte activation. Our results also show that treatment with conditioned media from GANT58-treated tumor cells leads to increased macrophage activation. We predict that current ongoing studies using tumor cells with overexpressed or knocked down Gli2 will demonstrate a similar phenotype outside of the context of pharmacologic inhibition and will induce M2-like macrophage polarization in coculture. These findings suggest a potential role of Gli2 inhibition as an immunemodulating cancer therapy, which may help to overcome therapeutic resistance in bone metastasis.

HIGH SCORING ABSTRACT AWARD WINNER

#8   Radium-223 Treated Primary Patient Bone Biopsy Analysis: Macro to Microscale Analyses and Dosimetry

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In nuclear medicine and radiation oncology, dosimetry has an important role in guiding clinical trial design to maximize the likelihood of a successful, minimally toxic intervention. For alpha particle-emitting radiopharmaceuticals, personalized dosimetry is challenging because of the short range of alpha particles and the challenges in noninvasive imaging. Existing small-scale dosimetry methods predominately rely on standardized anatomical models of healthy tissues and a uniform distribution of emitters in the source volume. To overcome these problems, we investigated the microscopic distribution of ^{223}Ra in bone biopsies from patients suffering from metastatic castration-resistant prostate cancer (mCRPC).

Bone biopsies and blood were collected from seven mCRPC patients 24h after injection of ^{223}Ra. The ^{223}Ra activity in each biopsy and blood sample was measured with a gamma-counter and evaluated with a highpurity Germanium detector. Microstructure images of the tissue were imaged for each biopsy by μCT. The samples were then cryosectioned and autoradiography imaging was performed on the sections using a Cyclone PhosphorImager. The same sections were stained with hematoxylin and eosin. Autoradiographic and histological images were finally segmented and registered with an automated procedure using machine learning. The autoradiographies were calibrated using a range of known activity of ^{223}Ra on each phosphor sheet. Dosimetry calculations were done based on the MIRD formalism.

Our study showed disparities between the biopsy of each patients. We observed that ^{223}Ra was still present in the blood 24h after injection. There is a correlation between the bone volume present in the biopsy sample and the activity measured. Microdistribution analysis confirmed localized high-activity regions in a background of low-activity tissue. Indeed, the autoradiography revealed that the ^{223}Ra was localized in the bone and predominantly to the surface of the bone, with little activity in the marrow cavity.

In conclusion, we have determined the activity concentrations in patient-derived samples and evaluated microstructural characteristics and their role in activity and dose distribution. These results provide the first patient sample microdosimetry values from a primary source. This work will help to improve the current small-scale dosimetry methods and demonstrate that autoradiographic and pathological samples can be used to provide direct and personalized dosimetric data.

POSTER ABSTRACTS

#9 Inhibition of the Fatty Acid Binding Proteins (FABPs) in Myeloma Cells modifies their Lipid Composition

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Background: Multiple Myeloma (MM) is an incurable blood neoplasm with only a 53% 5-year survival rate. We have recently identified a new potential vulnerability for MM by blocking the Fatty Acid Binding Proteins (FABPs) in MM cells. FABPs have been show to support cancerous growth in breast and prostate cancer. Last year, we showed that inhibiting the FABPs is also beneficial in MM models (https://www.biorxiv.org/content/10.1101/2022.07.01.498411v1.full). Here, we build on this by testing the hypothesis that lipid profiles of myeloma cells change when FABPs are inhibited, which, if true, could help us determine how the inhibition of the FABPs affects MM cells. Our objective was to understand the changes FABP inhibitors have on lipid composition of myeloma cells.

Methods: Three biological replicates of GFP+/Luc+MM.1S human myeloma cells were treated with either the FABP inhibitors BMS309403 (50 µM), SBFI-26 (50 µM) or the combination of both, or vehicle, for 24 hours. Then, lipid species were isolated using a water-free dichloromethane/methanol approach and analyzed with mass spectrometry, with three technical repeats per biological sample, using negative and positive ion mode.

Results: Principal component analysis (PCA) showed distinct clustering of the 4 treatment groups in both positive and negative ion modes. The most upregulated and downregulated lipids in response to each of the FABP inhibitor treatments were plotted with volcano plots using p-values and fold changes. In response to both the BMS309403 and the combination treatments, ceramides (Cer) were significantly upregulated while dihexosylceramides (Hex2Cer) and triacylglycerols (TAGs) were downregulated in MM cells. SBFI-26 induced different effects on lipids, suggesting that the changes in the combination were driven mostly by the effects of BMS309403.

Conclusions: The subgroupings of lipids revealed a direct effect of FABP inhibition on lipid metabolism pathways. The upregulation of Ceramides is of great interest and will be further examined, because ceramide metabolism has been implicated in other cancers as important for cell survival. The upregulation of triacylglyerols in MM cells treated with the pharmacological inhibitors may also be telling of FABP effects on energy storage pathways in Myeloma or other cancers.

#10   Investigating the role of non-canonical Hedgehog signaling in paclitaxel-resistant bone- metastatic breast cancer

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Paclitaxel (PTX) is a first-line treatment for patients diagnosed with late-stage breast cancer, but despite its value as a cancer therapeutic it is associated with multidrug resistance (MDR). MDR most often develops through overexpression of the ATP-binding cassette (ABC) drug transporters which efflux peptides, xenobiotics, metabolites, or ions depending on subfamily. Therapeutics for MDR targeting ABC transporters have been in clinical trials since the 1980s but have yielded limited success due to lack of tissue specificity and modulation of chemotherapeutic pharmacokinetics, resulting in increased drug exposure and likelihood of adverse side effects. Thus, the development of tumor-specific inhibitors of ABC transporters remains an important objective for the treatment of MDR.

The literature suggests that the bone microenvironment confers drug resistance, as breast cancer cells display chemoresistance and upregulation of ABC transporters when grown on 3D bone-mimetic scaffolds. Our preliminary data supports this notion; in an intracardiac model of bone-metastatic breast cancer, the IC_{50} of paclitaxel was increased by more than a thousand-fold (from 3 nM to 10 uM) in comparison to parental cell lines.

One potential mechanism for this is a downstream effector of the Hedgehog (Hh) signaling pathway called Gli2 (Glioma-associated oncogene 2). Its expression in healthy tissues is limited to embryogenesis, but Gli2 is non-canonically activated through TGF-β signaling and is overexpressed in bone-metastatic tumors by TGF-β released from the bone matrix during remodeling. Gli2 activates secretion of parathyroid hormone-related protein (PTHrP), inducing osteoblast secretion of RANKL, in turn promoting osteoclastogenesis and bone resorption. As a result, Gli2 is a key mediator of the vicious cycle of tumor-induced bone disease (TIBD).

Interestingly, Gli2 has also been implicated in MDR in several cancers, including ovarian, prostate, and melanoma, which occurs through upregulation of the ABC transporter genes *MDR1* (*ABCB1*), *ABCC1*, and *ABCG2*. However, the role of Gli2-induced transcription in PTX resistant breast cancer has yet to be elucidated. Given that Gli2 promotes a bone-metastatic phenotype in breast cancer and is also associated with overexpression of drug transporters, we hypothesize that Gli2 is the mechanism by which bone-metastatic breast cancer develops MDR. This transcriptional mechanism merits further investigation, as Gli2 is a promising therapeutic target for the treatment of bone-metastatic and PTX-resistant breast cancer.

#11   Bone marrow adipocyte-induced increases in lipid content and fatty acid binding proteins may support proliferation in myeloma cells

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Purpose/Background: The importance of bone marrow adipocytes (BMAds) in affecting the colonization and survival of cancer cells, including multiple myeloma (MM) cells, in the bone marrow has been a recent area of intense research. BMAds increase with both obesity and aging, two prominent risk factors for MM and many other cancers. To determine whether BMAds can support MM cells, we grew these cells together i and characterized phenotypes with implications for myeloma cell proliferation and survival.

Methods/Approach: To investigate the impact of BMAds on MM cells, MM1S[luc+] human myeloma cells were cultured in conditioned media from human BMAds (BMAd-CM). MM cells were assessed at 24 and 72 hours using BLI and flow cytometry (Ki67+, Cell Cycle) to determine the effects of BMAd-CM on MM cell proliferation. Flow cytometry was also used to assess reactive oxygen species (ROS; CellRox) and lipid content (LipidSpot). MM cells were also collected for RNA and protein extraction, to be used for qPCR, ELISA, and mass spectrometry proteomics.

Results: BMAd-CM increased MM1S proliferation at 24 hours by BLI (n=8, $p<0.001$) and Ki67+ ($p<0.05$), and increased the proportion of cells in the G2/M phase of cell cycle ($p<0.05$). Preliminary results suggest increases in lipid and ROS in the BMAd-CM-treated cells compared to MM.1S cells alone. Based on our prior work, we tested the hypothesis that FABPs (fatty acid binding proteins) may contribute to these effects. The expression of *FABP5* in MM1S cells increased in response to BMAd-CM exposure at 24 hours (qPCR, $p<0.05$) and elevated FABP5 protein was also detected by mass spectrometry ($p<0.05$). In addition, gene expression and protein data from MM1S cells treated with BMAd-CM for 72 hours revealed an increase in FABP4/FABP4 (pcr: $p<0.01$, ELISA: $p<0.0001$). We next tested whether FABP small molecule inhibitors (BMS309403and SBFI-26) could alter MM proliferation and/or survival. BMS309403 consistently induced a marked decrease in MM cell number ($p<0.0001$) with very little effect on apoptosis. However, when MM.1S cells were treated with SBFI-26, cell number was significantly reduced ($p<0.0001$) and apoptosis was increased ($p<0.05$) in both conditions.

Conclusions: Recently FABP5 has been associated with poor prognosis in myeloma patients, and here we provide evidence that BMAds support MM cell proliferation, in part through upregulation of the fatty acid binding proteins in MM cells, and suggest a new role for FABP-mediated support of myeloma cells.

#12 Potential Role of Bone Metastasis-Associated Fibroblasts in Prostate Cancer-Induced Bone Pain

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Prostate cancer (PCa) bone metastasis is a disease that is not only incurable but also negatively affects patient quality of life due to one of its painful complications, cancer-induced bone pain (CIBP). CIBP presents a clinical challenge, as currently established treatments (e.g. non-steroidal anti-inflammatory drugs, opioids) have limited efficacy and serious side effects. Therefore, more effective and safer therapies are needed to treat CIBP. To develop such agents, we must understand the underlying molecular mechanisms of CIBP. Bone is an innervated organ, and it has been suggested that the interaction between the bone marrow cells (e.g. bone metastatic cancer cells, hematopoietic cells, bone cells) and sensory nerves is crucial for the development of CIBP. However, little is known about the detailed mechanisms whereby bone marrow cells are involved in CIBP development. To bridge this knowledge gap, we performed single-cell RNA sequencing (scRNAseq) of bone marrow cells obtained from a mouse model of bone metastasis, where C57BL6 mice were intrafemorally inoculated with cancer cells. Prior to scRNAseq, using this bone metastasis model, CIBP (guarding behaviors) was compared between mice with murine prostate cancer cells (RM-1, B6-Myc Cap0, Tramp C1) and melanoma cells (B16-F10), which served as negative control of CIBP (based on prior experience). Mice with RM-1 developed more CIBP than other tumor-bearing mice, which showed little CIBP. Thereafter, bone marrow cells were collected and were subjected to scRNAseq. Populations of bone marrow cells were then clustered through a dimension-reduction analysis to establish cell populations (e.g. immune cells, fibroblasts, etc.). There were relatively similar proportions of immune cells and erythrocytes among all the cancer groups; however, a larger proportion of fibroblasts was observed in only the RM-1 group. Interestingly, this fibroblast population observed in mice with RM-1 had increased expression of nerve growth factor and bradykinin receptor 2, known to relate to nociception, compared to the other cancer-bearing mice. Further, an ingenuity pathway analysis of the differentially expressed genes in the fibroblast population from RM-1 mice revealed significance in pathways related to axonal guidance and myelination signaling. Overall, although further studies are clearly warranted, our findings suggest bone metastasis-associated fibroblasts have a potential role in PCa CIBP development.

#13   3D printed bioactive glasses porous scaffolds with high strength for the repair of long-bone segmental defects

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The clinical treatment of long bone segmental defects (LBSD) remains a challenge worldwide due to the deficiency of bone transplantation donors. Developing bone tissue engineering scaffolds similar to cortical in strength and good biological activity is a feasible solution. In this work, bioactive glasses with different ratios of copper and magnesium (Cu/MgBGs) were fabricated, and then processed by 3D printing and sintered at 700 °C to obtain porous BGs scaffolds with high strength and no crystallization. The compressive strength of Cu/MgBGs scaffolds exceeded 67.05±13.01 MPa (Cu0/Mg8.0) and even reached 109.27±8.18 MPa for Cu1.0/Mg7.0 in the presence of a high porosity (50.99±1.2%), which matched the strength of cortical bone (90-150 MPa). The mineralization experiments confirmed that the scaffolds had good bioactivity before and after sintering. *In vitro* experiments showed that different ratios of Cu/Mg in BGs had a decisive role in osteogenesis and angiogenesis. Cu0.5/Mg7.5 group had an optimal osteogenic performance while Cu1.0/Mg7.0 group was best for angiogenesis. Also, the antibacterial experiment illustrated that the scaffolds had a good bacterial killing rate of above 95.29±1.02%, which could effectively avoid the infection and broadened the application scenario of the scaffolds. In vivo animal experiments revealed that Cu0.5/Mg7.5 scaffold group had the best bone repair effect on rabbits of radius segmental defect with the highest volume and quality of new bone formation. Therefore, Cu0.5/Mg7.5 BGs scaffolds provided an alternative option for the treatment of LBSD.

Key words: Bioactive glasses, 3D Printing; High strength, Osteogenesis and angiogenesis, Long bone segmental defects (LBSD) repair

#14 Determining how prostate cancer cell heterogeneity promotes bone metastasis using fluid-walled microfluidics

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Prostate cancer (PCa) bone metastases cause significant morbidity and indicate progression to incurable disease. To combat advanced PCa, it is important to understand which traits make PCa cells more likely to metastasise to bone. As such, functional analysis of single cells is critical to identify key mechanisms driving bone metastasis. We used microfluidic technology to approach this question in three facets: (1) characterise heterogeneity in the PCa cell line LNCaP (2) pick single LNCaP cells into bone microenvironment models to assess diversity in survival and proliferation and (3) develop new co-culture assays to study the interactions between PCa and bone cells. We used a microfluidic device (iotaSciences) to print custom fluid-walled microfluidic circuits for single-cell cloning, single-cell picking, and co-culture assay development. Bone metastatic cell lines have previously been derived from LNCaP, indicating the presence of subclones with the potential to drive bone metastasis. We performed microfluidic single-cell cloning of LNCaP, deriving seven clonal lines. Functional characterisation demonstrated clonal heterogeneity, with differences in morphology, growth rates, migration, cell cycle and cellular metabolic reduction activity. Transcriptomic profiling identified differential expression in clinically important PCa-related genes (e.g., *KLK2&3*) and the *MYC* targets pathway. Using a novel microfluidic single-cell picking approach, single cells were isolated and deposited into distinct bone microenvironments (HS-5 vs HS-27A bone marrow stromal cells), revealing diversity in single-cell survival and proliferation within each. Average colony size was greater for cells picked into LNCaP than HS-5 ($p<0.01$) and media-only ($p<0.05$) conditions but did not differ significantly from those in HS-27A. In co-culture studies, increased migration towards stromal cell populations was observed when PCa cells were co-cultured with stromal cells. Overall, we identified clonal populations of PCa cells with distinct metastatic characteristics and molecular signatures. Our microfluidic approaches provide advantages over existing methods: we avoid the uncertainty of dilution cloning and the high shear stress of FACS. The compatibility with continuous live-cell imaging enables detailed study of dynamic cellular interactions in the tumour-bone microenvironment, revealing novel insights into the biology underlying PCa metastasis to and survival within bone.

#15   Single-cell discovery and multi-omic characterization of therapeutic targets in multiple myeloma

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Multiple myeloma (MM) is a highly refractory hematological cancer for which targeted immunotherapy has shown promise but remains hindered by the challenge of identifying specific yet broadly representative tumor markers. We analyzed 53 bone marrow (BM) aspirates from 41 MM patients using an unbiased, high-throughput pipeline for therapeutic target discovery via single-cell transcriptomic profiling, yielding 38 MM marker genes encoding cell-surface proteins and 15 encoding intracellular proteins. We highlight 20 candidate genes not yet under clinical study, 11 of which are previously uncharacterized in their therapeutic potential. We cross-validated our findings using bulk RNA-sequencing, flow cytometry, and proteomic mass spectrometry of MM cell lines and patient BM, finding high overall concordance across data types. Independent discovery using bulk RNA-sequencing reiterated top candidates, further affirming the ability of single cell transcriptomics to accurately capture marker expression despite limitations in sample size or sequencing depth. We further examined target dynamics and heterogeneity using both transcriptomic and immuno-imaging methods. Overall, we present a robust and broadly applicable strategy for identifying tumor markers to better inform the development of targeted cancer therapy.

#16   Variable Fluid Stresses May Alter Breast Cancer Expression in a 3D Perfusion Model of Bone Metastasis

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Introduction: Approximately 70% of breast cancers metastasize to bone[1], and only 12% of patients with skeletal breast cancer metastases demonstrate a 5-year survival rate after diagnosis[2]. Breast cancer cells are mechanosensitive; thus, skeletal mechanics has a considerable impact on their behavior. While the bone mechanical environment has been generally well-characterized, bone fluid stresses (vascular and interstitial) are often neglected in this characterization. Consequently, it is unknown how breast cancer cells react to those stresses and how that reaction may vary with loading magnitude. Thus, the objective of this study is to determine whether breast cancer cell expression adapts to changing fluid shear in bone using bone-mimicking scaffolds as 3D models of skeletal perfusion. We hypothesize that these cells will overexpress cancer-associated markers in response to higher fluid stresses within these scaffolds.

Materials and Methods: _Bioreactor-induced perfusion:_ Bone-mimicking scaffolds seeded with bone-homing breast cancer cells (MDA-231-MB1, Rhoades Lab) underwent a 3-day loading period. Perfusion was applied (1 hr/d, 1 Hz unsteady flow) at 1, 5, and 15 mL/min (control = static). After the loading period, scaffolds were incubated in low-serum media (1% FBS) for 24 hours. Incubation media was broadly screened for cytokines (R&D Human XL Cytokine Array). _Scaffold imaging and perfusion simulation:_ Micro-CT images of the scaffolds were taken (n=3, Xradia 520 Versa) and meshed (Synopsys Simpleware). These meshes were inputs for computational fluid dynamics (CFD) models to simulate loading (OpenFOAM). Ultrasound imaging of particle flow during perfusion generated flow profiles to aid wall shear stress (WSS) computation via CFD. _Statistical analysis:_ All loading groups were normalized to static controls. Low- and high-magnitude loading groups were compared using two-sample t-tests ($p<0.05$).

Conclusions: Interstitial WSSs estimated by CFD differ significantly between low and high flow rates. Preliminary data from breast cancer cell cytokine profiles show that pro-tumor proteins (e.g., MIP3β, RAGE, CD14) are elevated in response to higher magnitudes of applied flow ($p<0.1$).

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#17   Evolution of Epidemiology of Bone metastasis in France: a rising issue

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Background: Bone metastases (BM) are responsible for severe skeletal complications associated with altered quality of life and drug interruption. BM require dedicated care. Progress in oncology has considerably improved patient prognosis but there is no reliable recent BM epidemiology data in France. OPTIMOS study aimed to assess and describe incident BM patients in France over a 10-year period (from 2009 to 2018).

Methods: We accessed the EGB (*Echantillon Généraliste des Bénéficiaires*) French National Health Insurance database corresponding to 1/97 of the whole population. The algorithm identified BM patients either through the BM CIM-10 hospitalization code or through the onset of a skeletal-related event (SRE) in patients with cancer: pathologic fracture, cimentoplasty, spondyloplasty, spinal cord compression, palliative radiotherapy, orthopaedic surgery (preventive or curative) and malignant hypercalcemia. Inclusion period spanned between 2009 and 2018. Patients below 18, not affiliated to the general health scheme (about 20% of the French population), patients with primary sarcoma or with prevalent bone metastasis during the 3 years preceding inclusion date, have been excluded.

Results: We identified 6,663 new BM patients over 10 years. Sex ratio was balanced (47% of female). Mean (std) age was 69.7 yrs (13.2) and Charlson index was high (≥5) in 83% of patients. The most frequent primary cancer sites were breast (15.8%), prostate (13.4%), lung (12.6%), and digestive organs (10.6%). The median [Q1-Q3] follow-up was 1.3 yrs [0.3-3.4] mainly interrupted by death (63.5%) or end of the study (35.6%). Among BM patients, 4,737 patients with SRE at inclusion or over the follow-up were identified. Incidence tended to increase over time from 401 new patients in 2009 to 908 in 2018 (x 2.3) mainly because of SRE (from 176 to 681 (x3.9)). By comparison, the number of patients included through BM diagnosis code was stable (236/year in average).

Conclusions: Despite the huge progress in oncology over the last 10 years, BM concern a high number of patients estimated to 775,573 new cases over 10 years in the whole French population. The burden of SRE (x3.9) is preoccupant and urges the development of specific strategies to fight this issue and improve oncologic patients.

#18 **Inhibition of *de novo* serine synthesis in osteosarcoma depletes cysteine and drives dependency upon exogenous cystine import through system xCT to maintain redox balance**

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Objective: Osteosarcoma (OS) is the most common malignant bone tumor in children and adults. Standard of care for OS depends on surgical resection coupled with systemic chemotherapy and high dose methotrexate. The toxicity of this chemotherapy regimen and poor prognosis in the metastatic setting highlight a need for targeted therapeutics. A large subset of OS over expresses phosphoglycerate dehydrogenase (PHGDH), the rate limiting enzyme of *de novo* serine synthesis. Elevated PHGDH is correlated with reduced overall survival and disease-free survival in OS. Therefore, we sought to understand the metabolic mechanisms underlying *de novo* serine dependency in OS.

Methods: Multi-omics screening of NCT503 (a PHGDH inhibitor) treated OS cells was used to identify mechanisms of *de novo* serine synthesis dependency. sgRNA cocktails were used to generate KOs of metabolic enzymes and xCT. ROS probes were measured using the IncuCyte S3 with NCT503 treatment to evaluate oxidative stress. Live cell imaging analysis was used to quantify cell death and proliferation in response to KO and inhibition of PHGDH and cystine import.

Results: Metabolomics revealed significant changes in GSH metabolism upon PHGDH inhibition. Disruption in the GSH/GSSG ratio with PHGDH inhibition occurred concomitantly with increased ROS, suggesting that loss of *de novo* serine synthesis results in oxidative stress. Serine can contribute to GSH synthesis through the transsulfuration pathway. PHGDH inhibition results in reduced transsulfuration pathway products (cysteine) and increased substrates, suggesting reduced flux. KO of transsulfuration enzymes recapitulates the growth arrest induced by PHGDH inhibition. Additionally, PHGDH inhibition results in increased expression of xCT and import of cystine. Combined inhibition of PHGDH and xCT results in cell death *in vitro*.

Conclusions: *De novo* serine synthesis is necessary for the maintenance of redox homeostasis in OS. Serine enters the transsulfuration pathway to produce cysteine and subsequently GSH. In the absence of serine incorporation into the transsulfuration pathway cells become dependent upon exogenous cystine import through xCT to maintain redox balance. Combined inhibition of PHGDH and xCT results in cell death *in vitro*. The transsulfuration pathway is an infrequently utilized pathway in normal cells; therefore, targeting this pathway is a promising strategy for the development of highly specific therapeutics in OS.

#19 RANKL independent osteolysis in Adult T cell leukemia via small extracellular vesicles

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Adult T cell Leukemia (ATL), caused by chronic infection of human CD4+ T cells with HTLV-1, is associated with hypercalcemia and osteolytic lesions. Osteolysis in a mouse model of HTLV-1 infection was only partially blocked by anti-RANKL antibodies in vivo. HTLV-1 infected T cells (HTLV/T) produce small extracellular vesicles (sEVs), previously shown to facilitate infection by promoting cell-cell contact of T cells and to target mesenchymal stromal cells. We hypothesized that these sEVs also mediate bone loss by targeting the osteoclast (OC) lineage. A panel of clonal HTLV/T (n=9) were generated by co-culturing lethally irradiated HTLV-1 producer cells with human peripheral blood mononuclear cells (hPBMCs) and PBMCs from ATL (ATL/P) (n=3) patients were isolated. To examine their direct effects on OCs, supernatants from HTLV/T lines and ATL/P were added to mouse bone marrow macrophages (mBMMs) and hPBMCs cultured in osteoclastogenic conditions. HTLV/T supernatants were variable in their ability to stimulate OC differentiation, but showed similar effects on murine and human cultures. ATL/P were consistent in their stimulation of osteoclastogenesis of mBMMs. Expression of RANKL and OPG mRNAs by HTLV/T was also variable, but we found no correlation between OC numbers and the RANKL/OPG ratio, suggesting an alternative mechanism. Small EVs isolated from both HTLV/T and ATL/P were taken up by mBMMs and carried most of the OC stimulatory activity of supernatants, and were devoid of RANKL. TEM revealed absence of viral particles in sEVs suspension. Pre-treatment of HTLV/T cells by exosome generation inhibitor GW4869 blunted the OC stimulatory activity of supernatant. LC-MS/MS followed by statistical analysis of HTLV/T sEVs showed upregulated expression of cytoskeletal proteins suggesting their effect on OC function. Micro RNA profiling of HTLV/T sEVs with high osteoclastogenic activity revealed micro-RNAs with known function in osteoclastogenesis. Select HTLV/T lines were injected into tibia of immunodeficient NCG mice and led to osteolysis, but the effect was independent of RANKL expression level. Our study suggests that solely targeting RANKL may not be an effective treatment approach for preserving bone mass in ATL, while broader OC-targeting therapies such as bisphosphonates could be more efficacious.

#20 Investigating the response of prostate cancer cells to bone-derived growth factor gradients via fluid-wall microfluidic culture systems

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Osteoblasts and bone marrow adipocytes are known to impact prostate cancer (PCa) bone metastases, acting as sources of bone morphogenic proteins (BMPs) and adiponectin (ADPN), respectively. BMP concentration gradients are essential in embryogenesis, dictating many developmental events; less is known about the role of BMP gradients in bone, especially related to metastatic cancer cells. Studies found that BMPs have multiple effects in PCa, however, the impact of cellular proximity and osteoblast (BMP)/adipocyte (ADPN) crosstalk is poorly understood. We hypothesize that the impact of BMP signalling in bone metastatic PCa depends on the proximity of PCa cells to osteoblasts and adipocytes and therefore the balance of exposure to BMPs and ADPN. To investigate, we utilised conventional cell culture methods as well as fluid-walled microfluidic devices. Our microfluidic approach involves the creation of custom circuits wherein external pumps flow compounds over adherent cells, creating quantifiable secretory concentration gradients that can be spatially and temporally manipulated. Using microfluidics, we expose PCa cells to a dynamic, multifactorial circulatory environment more reflective of the in vivo bone microenvironment. Treatment of LNCaP and C42B PCa cells with BMP-2 or -7 resulted in a significant reduction in growth rates, as assessed by real-time imaging (BMP-2: 49%; BMP-7: 48%, both $p<0.05$). Treatment with a BMPR antagonist and with ADPN prevented the growth reduction. LNCaP cells were transduced with a Fluorescent Ubiquitination Cell Cycle Indicator (FUCCI) plasmid to enable real-time cell cycle analysis. Treatment with BMP-2 induced a loss of cells in S and G2M phases and an accumulation in G1. Treatment with a BMPR antagonist reversed these changes to control levels, whereas treatment with ADPN partially decreased the accumulation in G1 but had no effect on loss of cells from S and G2M. LNCaP cells were plated in microfluidic chambers and exposed to BMP-2 or control at a flowrate of 5uL/hr. Live-dead staining confirmed cell viability within the chambers and did not show increased cell death after BMP treatment. Reduced cell confluence in alignment with the predicted BMP diffusion gradient was observed. Taken together, we exploit microfluidic technology to highlight the impact of BMP gradients on PCa growth and show that ADPN can prevent the response of PCa cells to BMPs, revealing a previously unrecognised crosstalk between BMP and ADPN signalling.

#21 PD-1 blockade induces bone loss and reduces bone strength in a breast cancer bone metastasis model

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Immune checkpoint inhibitor (ICI) therapy, which enables anti-tumor immunity through blocking immunoregulatory receptor-ligand interactions [e.g., programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1)], has revolutionized cancer treatment; however, ICIs have lower efficacy in patients with bone metastases and there are reports of rheumatologic toxicities and increased fracture risk in patients receiving ICI therapy. This suggests that PD-1 blockade in a tumor setting negatively affects the bone, but the mechanism of bone loss is unknown. We challenged female wildtype (WT) mice and global PD-1 knockout (KO) mice (n=7/group) as well as WT female mice treated with IgG or an α-PD-1 inhibitor (n=7- 10/group) with E0771 mouse mammary carcinoma cells via intracardiac injection. We assessed bone structure of the distal femoral metaphysis by µCT and biomechanics of the femoral diaphysis by 3-point bending and found significant decreases in BV/TV (p=0.0279) and ultimate strength (p=0.0052) in PD-1 KO mice compared to WT controls. Mice treated with α-PD-1 showed significant decreases in BMD (p=0.0359), ultimate strength (p=0.0019), and ultimate force (p=0.0069) as compared to IgG treated controls. We next performed flow cytometry of the bone marrow to analyze changes in T cell subsets during PD-1 blockade and found that CD8 effector memory T cells were significantly expanded (p=0.0274) in α-PD-1 treated mice compared to IgG treated controls. Since CD8 T cells secrete cytokines that regulate bone remodeling, we investigated bone turnover markers as potential mechanisms for the observed decrease in trabecular bone. We saw a significant increase in serum CTX-1 levels (p=0.0411) in E0771-inoculated PD-1 KO mice compared to WT controls, suggesting increased bone resorption; PD-1 KO mice also displayed significant increases in *Gzmb* (p=0.0003) and *Sema4D* (p=0.0012), both of which have anti-osteoblastic effects, and in *Ifng* (p=0.0018), which has pro-osteoclastogenic effects in settings of chronic inflammation. Similarly, E0771-inoculated mice treated with α-PD-1 had significant increases in *Sema4D* (p=0.0021) and *Gzmb* (p=0.0134) compared to IgG treated controls. Taken together, these data suggest that the trabecular bone loss observed in tumor bearing mice with PD-1 blockade may be due in part to upregulation of pro-osteoclastogenic and anti-osteoblastic factors produced by CD8 effector memory T cell expansion.

#22   Investigating the TGF-β/integrin β3 signaling axis as a mediator of chemoresistance in breast cancer bone metastasis

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Bone metastases are a common complication of breast cancer and cause significant comorbidities that reduce patient survival. This is partly due to intrinsic and acquired resistance mechanisms of tumor cells residing in the bone microenvironment. Our lab previously demonstrated that the integrin beta 3 subunit (ITGB3) is overexpressed by patient breast cancer bone metastases relative to matched primary tumors, through a transforming growth factor beta (TGF-β)-dependent mechanism. Targeted delivery of the chemotherapeutic docetaxel (DTX) using αvβ3-nanoparticles significantly decreased bone tumor burden and osteolytic lesions. We recently showed that ITGB3 expression is induced by chemotherapy and loss of ITGB3 sensitized boneresiding tumor cells to chemotherapy treatment. In the current study, we performed *in vivo* RNA sequencing on wild-type and *Itgb3* knockout tumor cells isolated from bones and kidneys of the same mice. We identified several DNA repair and TGF-β pathway genes that were uniquely downregulated in wild-type bone tumors even prior to chemotherapy. Since TGF-β signaling has also been implicated in chemotherapy resistance and regulation of DNA repair, we hypothesized that TGF-β signaling contributes to integrin β3-mediated chemoresistance in breast cancer bone metastases. We established CRISPR-derived TGF-β receptor 2 (*Tgfbr2*) knockout PyMT-BO1 (luminal B) or 4T1 (triple-negative) tumor cells in bone and surprisingly found that these tumors were not more susceptible to DTX treatment compared to wild-type tumors as measured by bioluminescence imaging. Moreover, immunohistochemistry analyses showed that *Tgfbr2* knockout bone tumors still expressed phosphorylated SMAD3 as well as low levels of ITGB3. *In vitro* treatment with a potent inhibitor of SMAD2/3 phosphorylation (SD-208) sensitized these cells to DTX, suggesting SMADs may be important regulators of integrin β3-mediated chemoresistance rather than TGF-β receptor signaling. Ongoing experiments include mouse models using double CRISPR *Itgb3/Tgfbr2* knockout as well as SMAD luciferase reporter cell lines, validating key DNA repair and TGF-β pathway genes identified from our RNA sequencing screen, and combinatorial treatments with DTX and SMAD inhibitors delivered via αvβ3- nanoparticles to target chemoresistant cells in bone. Ultimately, these findings can be used to evaluate other aspects of drug resistance in bone metastasis that may help improve patient outcomes.

#23 Elucidating the Role of Bone Marrow Stiffness in Estrogen Signaling in Metastatic Breast Cancer to the Bone

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Bone metastasis is highly prevalent in breast cancer (BC) patients with metastatic disease, with 70% of patients succumbing to the disease having detectable metastases after autopsy. Metastasized BC cells in the bone marrow form osteolytic lesions and decrease quality of life for patients. There are several studies elucidating the mechanism of metastasized BC cells' involvement of bone destruction, however, it is unclear how the microenvironment plays a role in this process. Recent studies have shown that as BC cells experience increased stiffnesses in their microenvironment in their primary site, there is greater expression of proteins related to mesenchymal transition. However, these processes have not been studied when cells experience stiffnesses relative to the bone marrow, which can be ten times higher than the primary site. Estrogen receptor (ER) status also plays a pivotal role in patient survival with metastatic disease, but how ER expression and function changes in the bone metastatic site remains unclear. Clinical studies have indicated that both ER+ and ER- patients develop bone metastases, however, there is minimal work to establish how the physical factors of the bone marrow can affect ER sensitivity after metastasis. We hypothesize that higher stiffness ranges of the bone marrow can increase BC invasiveness and decrease estrogen receptor sensitivity of metastasized tumor cells. To analyze this question, we seeded ER+ (MCF7s) and ER- (MDAs) cells on CytoSoft® Rigidity Plates that mimicked the stiffness of the bone marrow (0.5 – 64 kPa). After 2 days in culture, RNA was extracted, and the expression of genes associated with osteolysis were probed (*ITGB3, TGFB-RII, Gli2*, and *PTHLH*). Protein expression was also conducted by western blot analysis for the proteins associated with these genes. Additionally, we probed the expression of ER and phosphorylated ER (pS118ERa) using western blot at varying stiffnesses. Overall, this work gives insight into how mechanical properties of the bone marrow can potentially regulate estrogen signaling after metastasis. In the future, we plan to evaluate tumor cell proliferation and expression of osteolytic genes when given aromatase inhibitors at different stiffnesses. Overall, this work will elucidate the physical factors that alter tumor cells' behavior when metastasized to the bone.

#24   Mechanical Loading-Induced Changes to the Bone Microenvironment Control Bone Metastatic Breast Cancer Lesions

Blayne Sarazin, Emma Briggs, Meredith Provera, Philip Owens, Maureen Lynch

Breast cancer preferentially metastasizes to bone, resulting in osteolysis and dismal patient prognosis. Applied mechanical forces are potentially anti-tumorigenic while also stimulating bone formation. Cyclic tibial loading provided anti-tumorigenic to the bone microenvironment of the loaded tibia. Subsequent studies showed that loading has a dose-dependency effect, where lower magnitude loads are anti-tumorigenic while high loads no longer protect bone from tumor progression. Primary breast cancer patients have a 90%, 5-year survival rate that drastically drops to 13%-28% upon bone metastasis. Our goal was to determine if mechanical loading prior to the arrival of breast cancer cells will pre-condition the bone microenvironment to resist cancer colonization and subsequent tumor growth. We also determine if the impacts of loading are dose-dependent.

The left tibiae of 11wko FVBn female mice (n=8 per group) were subjected to low (2.6 N) or high (8.1 N) tibial compression for two weeks (M-F, 4 peak loads applied at 4 Hz followed by a 3 sec rest, total 50 cycles). Right tibiae served as nonloaded controls. Following loading, both tibiae were intratibially injected with breast cancer cells (FVBn, 100k cells), and tumor growth was permitted for 5 weeks. Tibiae were then dissected of soft tissue, fixed for histology, and imaged with microCT.

Despite final tumor volume being insensitive to force level, scoring of bone lesions (osteolytic, mixed, osteoblastic) across both groups revealed that limbs receiving low loads more often formed sclerotic lesions whereas limbs receiving high loads more often formed osteolytic lesions. Thus, tibiae receiving low loads, with scleroses, would have greater strength than those with osteolysis. MicroCT analysis revealed that bone volumes were elevated in nonloaded tibiae. Histological analysis of sclerostin showed no significant differences.

Mechanical loading has been shown to have positive physical outcomes, but our data indicates that loading impacts the lesion type formation once tumor cells localize to the skeleton. Furthermore, this data shows that cancer could be interfering with bones' innate ability to respond to loading. More work is needed to evaluate the mechanisms through which exercise impacts lesion formation, specifically whether or not there exists a limit at which exercise begins to have a negative impact on tumor-induced bone disease and whether bone metastasis interferes with bone mechanosensitivity.

#25 Regulation of Osteoblast Migration, Secretome, and Metabolic Programming by Prostate Cancer

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Osteosclerotic bone metastasis is a dynamic and symbiotic relationship between prostate cancer cells and osteoblasts. How osteoblasts respond during the first encounter, when prostate cancer cells arrive in bone, is uncertain. We hypothesized that an initial step is to recruit local and circulating osteoblasts to the metastatic bone niche. To test the hypothesis, GFP-expressing mouse long-bone primary osteoblast cultures were placed on optically opaque 8 µm pore cell culture inserts with one of the human prostate cancer cell lines ARCaPM, ARCaPE, C4-2B, LNCaP, and 22Rv1 cultured below. Osteoblast migration through the membrane was monitored by fluorescence microscopy in hypoxia. ARCaPM and ARCaPE cells markedly increased migration, while the remaining cell lines repelled osteoblast migration. These data suggested that prostate cancers possess different degrees of osteoblast tropism. We next performed single-cell RNA-seq (scRNA-seq) of osteoblast cultures that migrated through the membrane on their own (Migrat OB) and cultures that migrated in the presence of ARCaPM cells plated below (Migrat OB+PrCa), using the 10X Genomics platform. Significant fold increases in osteoblast gene expression were detected in *Nos2* (~1200X), *Il13ra2* (~900X), *Cxcl5* (~70X), *Cxcl3*, *Cxcl1*, *Rarb*, *Mmp13*, and *Bmp7* in the Migrat OB+PrCa compared to the Migrat OB group. Culturing mouse osteoblasts in ARCaP$_M$-conditioned media (CM) increased osteoblast secretion of CXCL5 (73.0 µg/mL vs. 4.9 µg/mL). CXCL5 has pro-metastatic actions, and it has been found that men with prostate cancer bone metastasis have increased serum concentrations. An increase in nitric oxide (NO), a product of inducible nitric oxide synthase encoded by *Nos2*, was also detected in osteoblast cultures treated with ARCaP$_M$ CM (24.6 µM vs. 0.5 µM). NO has numerous biologic actions and was recently reported to increase osteoblast anabolism through the regulation of glycolysis. We next determined the transcriptomic differences in osteoblast glycolytic enzymes and found an increase in *Pfkfb3, Pfkp, Aldoa, Tpi1, Gapdh, Pgk1*, and *Ldha* in Migrat OB+PrCa vs. Migrat OB groups. An increase in the osteoblast energy supply via glycolysis would be critical for promoting the osteosclerotic response, especially in the hypoxic environment of bone metastasis. We report here how prostate cancer changes the osteoblast tropism, secretome, and metabolic programming to support prostate cancer and osteoblast growth.

#26 Regulating Breast Cancer Phenotype Under the Reign of 3D Bone Endosteal Mimics

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Solid cancers originating in the breast, prostate, and lung tend to metastasize to bone, typically indicating a short-term prognosis for the patients. Once deployed in bone, these tumor cells harness the microenvironment, shift to a quiescent mode and following proper ques, reactivated and initiate a vicious cycle that often leads towards bone deformation or destruction. These processes that take place under the reign of the bone marrow niches by which they can gain an increased tumorigenicity are not yet fully understood. Here, we introduce a new threedimensional model which closely resembles the endosteal niche that can be used to study cellular and molecular cues during the development of bone metastasis. Using this model, we showed that the mineral phase may have an important role on cellular characteristics such as, proliferation rates and tumorigenicity. We also suggest that by affecting key regulatory stimuli, such as, calcium intake, Wnt and CD44 related pathways, the endosteal microenvironment can take a pivotal role in osteomimicry and long-term dormancy of invading breast cancer cells. New insights on the interplay between these signaling cues and their effects tumor progression will be discussed. A better understanding of the molecular signaling mechanisms involved in the tumor development and bone metastasis may contribute for the development of new therapeutic strategies.

#27   First-in-Human Evaluation of Safety and Dosimetry of ^{64}Cu-LLP2A for PET Imaging

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Background: There remains an unmet need for molecularly targeted imaging agents in multiple myeloma (MM). The integrin, very late antigen-4 (VLA4), is differentially expressed in malignant MM cells as well as in the pathogenic inflammatory microenvironmental cells. [^{64}Cu]Cu-CB-TE1A1P-LLP2A (^{64}Cu-LLP2A) is a VLA4 targeted, high-affinity radiopharmaceutical with promising utility for managing patients diagnosed with MM. Here, we evaluated safety and human radiation dosimetry of ^{64}Cu-LLP2A for potential use in MM patients.

Methods: Single dose [[nat]Cu]Cu-LLP2A (Cu-LLP2A) tolerability and toxicity study was performed in CD-1 (Hsd:ICR) male and female mice. ^{64}Cu-LLP2A was synthesized in accordance with the good manufacturing practice compliant procedures. Three MM and six healthy participants underwent ^{64}Cu-LLP2A-PET/CT or PET/MR scans up to three time points to help determine tracer biodistribution, pharmacokinetics and radiation dosimetry. Time-activity curves were plotted for each participant. Mean organ absorbed doses and effective doses were calculated using the Organ Level INternal Dose Assessment (OLINDA) software. Tracer bioactivity was evaluated *via* cell binding assays and metabolites from human blood samples were analyzed with analytical radio-high performance liquid chromatography. When feasible, VLA4 expression was evaluated in the biopsy tissues using 14-color flow cytometry.

Results: 150-fold mass excess of the desired imaging dose was tolerated well in male and female CD-1 mice (no observed adverse effect level (NOEL)). Time-activity curves from human imaging data showed rapid tracer clearance from blood via kidneys and bladder. The effective dose of ^{64}Cu-LLP2A in humans was 0.036 ± 0.006 mSv/MBq, and spleen had the highest organ uptake of 0.142 ± 0.034 mSv/MBq. Among all tissues, the red marrow demonstrated highest residence time. Image quality analysis supports early imaging time (4-5 h post injection of the radiotracer) as optimal. Cell studies showed statistically significant blocking for the tracer produced for all of the human studies ($82.42 \pm 13.47\%$). Blood metabolism studies confirmed a stable product peak (> 90%) up to 1 h post-injection of the radiopharmaceutical. No clinical or laboratory adverse events related to ^{64}Cu-LLP2A were observed in the human participants.

Conclusions: ^{64}Cu-LLP2A exhibited a favorable dosimetry and safety profile for use in humans.

#28   Identification of key proteins within prostate cancer derived exosomes and their role in priming the pre-metastatic niche

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Rational/Hypothesis: Bone is a common site of prostate cancer (PCa) metastasis with approximately 80% of patients developing bone metastases. There is considerable evidence that PCa cells release 30-150nm diameter vesicles (termed exosomes or EVs) which prime the pre-metastatic niche within bone to receive incoming cancer cells. Micro-#RNAs within EVs are key in this reprogramming We have previously applied a novel RNA-tracking methodology to identify the pivotal micro-RNAs within PCa EVs which are transferred to osteoblasts. Extending this work, we have applied proteomic techniques to identity the protein changes inside osteoblasts in response to EV-derived micro-RNAs from benign prostate epithelial cells and bone metastatic PCa cells. In addition, we have looked at the proteomic composition of EVs from these two cell types.

Objectives: To identify the proteins which differ in expression level within EVs during the development of bone metastatic ability in PCa. In addition, to study the effects of EVs upon protein expression within osteoblasts. This work will identify a crucial mechanism by which PCa cells prime bone, identifying potential circulating biomarkers (within PCa-EVs), as well as potential drug targets (proteins which alter in bone resident osteoblasts as well as PCa-EVs).

Methodology: Pulsed SILAC (Stable Isotope Labelling by amino acids in Cell-culture, pSILAC) was used to identify the intra-cellular proteins altering within human osteoblasts in response to EVderived micro-RNAs (miR-17-5p / miR-27-5p). In addition, we have applied Label-free quantification-MS (LFQ-MS) to the proteins present within EVs from benign prostate epithelia cells (PNT1A) and the PCa cell line PC3.

Results: pSILAC analysis of osteoblasts +/- miR-17-5p quantified 3977 proteins of which 858 proteins were differentially regulated in response to micro-RNA (+/- 1.3-fold cut off, p-value < 0.05, n = 4). pSILAC analysis of osteoblasts +/- miR-27-5p resulted in identification and quantification of 3932 proteins with 258 proteins differentially expressed (+/- 1.3 fold cut off, p-value < 0.05, n = 4). Bioinformatic analysis revealed that miR-17-5p regulated proteins clustered within cellular metabolism and angiogenesis, whilst miR-27-5p regulated proteins were involved in cell motility and cell shape regulation.

LFQ-MS of PCa EVs identified 928 proteins with 24 proteins shoring a statistically significant difference (p< 0.05, n = 4). The differential EV proteins included new and novel regulators of cell-cell signalling.

Bioinformatic analysis was conducted within both data sets looking at gene expression levels.

For many candidate proteins, the relevant gene expression levels predicted the probability of disease recurrence within PCa patients.

Conclusion(s): Proteomic techniques have identified key pathways regulated within osteoblasts by incoming prostate cancer derived exosomes and their micro-RNAs as well as difference in protein composition of EVs within PCa. These proteins offer targets for development of future therapies.

#29   A Novel Control Mechanism of Endosomal Trafficking in Bone Metastatic Breast Cancer Cells

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Bone metastasis remains a leading problem in the treatment of cancer. Cancer cells metastasizing to the bone often increase osteoclast activity, releasing growth factors and nutrients that aid in the further fueling of tumor growth. A potentially important aspect of this process is the regulation of growth factor signaling through endosomal and secretory vesicle trafficking on microtubules (MT). Previously we reported that aberrant Runx2 expression in bone metastatic breast cancer increases autophagy and MT stability, via acetylation of α-tubulin. In this study, we examine the impact of Runx2-dependent MT stability on the endosomal pathway and secretory activity through confocal microscopy and biochemical approaches. Subcellular distribution of early endosome formation and maturation was evaluated using early endosome antigen 1 (EEA1), syntaxin-6, Rab5, and Rab7. Rab5 regulates maturation and recruits Rab7. Inhibition of Runx2 in MDA-MB-231 decreases expression of these proteins, especially EEA1 and Rab7. Confocal microscopy shows a reduction in the number of large endosomal puncta and changes in cytosolic distribution, with a tendency towards a more polar distribution centered closer to the nucleus. These findings were validated in Runx2 knockout mouse calvarial osteoblasts. Since secretory activity relies on MTs for trafficking, we assessed IL-6 secretion in bone metastatic cells. IL-6 secretion is increased in MDA cells during normal growth conditions. Interestingly, IL-6 secretion increases during glucose starvation and treatment with MT-targeting chemotherapeutics, whereas Runx2 knockdown shows decreased secretion. Furthermore, in colony formation assays we saw inhibition of Runx2 sensitizes MDA-MB-231 to docetaxel and vinblastine. These findings suggest a novel Runx2-dependent control mechanism for the intracellular trafficking of endosomes and secretory activity within bone metastatic breast cancer cells. As endosomal pathways regulate internalization and recycling of growth factors, our findings provide novel insights into metastatic responses in the bone microenvironment. Our results indicate that inhibition of Runx2 may sensitize metastatic tumors to MT targeting agents and prevent pro-tumoral cellular activity through alterations in endosomal signaling. Importantly, Runx2, MT acetylation, and endosomal protein levels may serve as potential markers to help stratify patients and targets for effective treatment for bone metastatic disease.

#30   Fractures in metastatic hormone-sensitive prostate cancer (mHSPC). Results from two phase 3 trials from the STAMPEDE platform.

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Introduction and Objective: Long-term ADT is recommended for men with mHSPC: bone loss and increased fracture risk are known complications. The STAMPEDE trial compared ADT ± docetaxel (Doc) ± zoledronic acid (ZA): adding ZA did not improve overall survival (OS) but long-term effects of bisphosphonate treatment on skeletal health was not formally collected as part of the trial. Accessible, high quality, routinely-collected healthcare data (RCHD) enables analysis of outcome data beyond standard trial follow-up to evaluate long-term treatment toxicity and clinical efficacy of ZA as a bone protective agent in mHSPC. The Hospital Episode Statistics (HES) database provides RCHD for patients in England and Wales.

Methods: HES data up to 2018 were obtained for patients (pts) randomised 2:1 between ADT (Arm A) and ADT+ZA (Arm B), or ADT+Doc (Arm C) or ADT+Doc+ZA (Arm E). ZA (4 mg) was six 3-weekly cycles, then 4-weekly for 2 years. Clinical fracture events were identified using a bespoke pre specified coding framework of International Classification of Diseases (ICD 10) diagnosis and Classification of Interventions and Procedures (OPCS 4) procedure codes. Multivariate Cox regression models were developed to adjust for age, N stage, WHO performance status, Gleason score and NSAID use to determine impact of ZA on fracture risk.

Results: Connected datasets were available for 2,145 eligible pts (796 M0, 1,349 M1) of 2,962 (72%). Overall, 5-year fracture incidence was 6.4%; higher in M1 pts (9.6% in M1 vs 2.1% in M0, $p<0.0001$), and lower amongst M1 pts allocated ZA (4.55% with ZA vs 12.9% without ZA, $p<0.0001$). ZA significantly reduced fracture risk in M1 pts (HR 0.36, 95% CI 0.22- 0.57, $p<0.0005$) but not in M0 pts (HR 0.67, 95% CI 0.32-1.39, $p=0.28$).

Conclusions: By accessing RCHD, we evaluated long-term fracture events amongst patients in STAMPEDE. Fracture rates were clinically notable in M1 and reduced significantly in pts allocated ZA with ADT±Doc. These data support using bone protective agents to reduce clinically significant fractures in mHSPC.

#31 A Phase I/II trial of ATI-2231 with Phase Ia in advanced soild tumor malignancies followed by Phase Ib/II in combination with capecitabine in patients with hormone receptor-positive (HR+) and HER2-negative metastatic breast cancer

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Introduction: Bone metastases from breast cancer lead to bone pain and skeletal related events (SRE) and reduce survival. Chemotherapy for metastatic HR+ HER2- breast cancer after resistance to endocrine therapy has limited anti-tumor effects due to tumor cell resistance and is associated with toxicity and bone loss. Although bisphosphonates and denosumab reduce SREs, these drugs do not improve overall survival and have side effects such as osteonecrosis of the jaw. Using pre-clinical models, we reported that p38MAPK-MK2 (MK2) pathway activation modulates therapy-induced bone loss and p38MAPK-MK2 activation in stromal cells leads to immune suppression and promotes the growth of breast cancer cells in bone. Inhibition of stromal cell MK2 activity limited expression of pro-tumorigenic and immune suppressive factors, reduced bone loss and reduced metastatic tumor growth in bone. We hypothesize that MK2 inhibition inhibits disease and chemotherapy-induced bone loss and improves progression free survival (PFS).

Methods: This is a phase I/II trial designed to examine the safety and efficacy of the MK2 inhibitor ATI-2231 (Aclaris) plus Capecitabine (CPB) in patients with metastatic ER+ HER2- breast cancer. The initial "3+3" dose-escalation phase Ia study will determine safety, tolerability, and Recommended Phase 2 Dose (RP2D) of ATI-2231 monotherapy in all solid tumor types. This will be followed by the Phase Ib trial to determine safety, tolerability, and RP2D of ATI-2231 plus CPB in patients with metastatic ER+ HER2- breast cancer. The Phase II trial will assess if ATI-2231 combined with CPB is more effective than CPB and SOC antiresportive agents in reducing serum C-telopeptide crosslink (CTX) level and prolonging PFS. A sample size of 42 in SOC versus 84 in the two ATI-2231 arms (42 each of RP2D-L1 and RP2D-L2 arm) gives evaluable N for sCTX % reduction of 39 and 80, respectively, assuming a ~5% attribution rate. The sample size will achieve 88.68% power to detect the expected difference of 15.7% (18% vs. 33.7%, StdDev=28%) for % reduction of sCTX between the SOC arm versus the ATI-2231 arms, based on 1-sided two sample t-test with, at a 5% alpha level. We will conduct an extensive biomarker study to assess bone turnover, inflammatory and immune changes induced by ATI-2231 and correlate these changes with disease progression and bone loss.

#32   **Identification of proteins regulating the metastatic spread of breast cancer to bone: Proteomic analysis of the role of the pro-metastatic transcription factor TWIST1 and the gene target micro-RNA (miR-10b).**

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Background/Introduction: The skeleton is the most common site of secondary disease in breast cancer with over 70% of patients with advanced disease developing bone metastases. Spread to bone results in skeletal related events (SREs) including spinal cord compression, bone fractures and hypercalcemia. There is an urgent clinical need for biomarkers to predict those patients at greatest risk of developing bone metastasis. In addition, we need to discover new drug targets within this process. In this regard, we previously identified a key role for the transcription factor TWIST1, and its gene target micro-RNA-10b (miR-10b) within cancer spread to bone.

Purpose: We identified the proteins regulated by miR-10b within a bone-homing variant of the MDA-MB-231 cell line (B02), both inside the cell and within the secreted fraction of cells. These proteins have considerable promise to act as potential biomarkers and/or drug targets.

Methods: The proteins altering within BO2 in response to miR-10b were quantified using Pulsed SILAC (Stable Isotope Labelling by Amino acids in Cell-culture). BONCAT (Bio-Orthogonal Non-Canonical Amino Acid Tagging) has identified the secreted proteins altering due to miR-10bin B02 cells.

Results: Pulsed SILAC analysis quantified 5412 proteins within BO2 cells of which 30 proteins were up-regulated and 119 proteins down-regulated by miR-10b (+/- 1.3-fold cut off, p-value < 0.05, n = 4 biological replicates). These included proteins involved in regulation of cell-motility and cell-cell communication. BONCAT analysis of BO2 cells quantified 545 secreted proteins of which 17 proteins were up-regulated and 31 proteins were down-regulated +/- miR-10b – (+/- 1.3-fold cut off, p < 0.05, n = 4 biological replicates). The differentially secreted proteins included known regulators of cancer-induced bone remodelling as well as novel cell-signalling proteins that could play key roles within metastatic spread to bone. These proteins are being further validated by immunohistochemistry (IHC) within tissue microarrays (TMAs) from breast cancer patients (intracellular proteins), as well as by ELISA and targeted-mass spectrometry within patient-derived serum samples.

Conclusion(s): Novel proteins with roles in breast cancer spread to bone were identified. We are using well-established biomarker translation techniques to advance these proteins towards clinical utility.

#33   PTHrP biological domains differentially regulate breast tumor intracrine signaling independent of nuclear localization

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Parathyroid Hormone Related Protein (PTHrP) is an important paracrine regulator of tumor-induced osteolysis; however, the PTHrP molecule contains regions with intracrine function in breast cancer cells. These regions include a nuclear localization sequence (NLS) and carboxyl-terminus that negatively regulate prostate tumor progression. However, conflicting clinical and *in vivo* data suggest a multiplicity of pro-tumorigenic and tumor-suppressive roles for PTHrP, and regions of the protein remain uncharacterized. We therefore hypothesize that the PTHrP biological domains may have unique and opposite functions in breast cancer progression. To investigate this, we generated MCF7 cell lines that overexpress full-length PTHrP or different mutant forms of PTHrP that lack the NLS or the NLS and Cterminus. Following orthotopic inoculation into 6-week old female athymic nude mice, tumor cells expressing PTHrP that lacks the NLS grew faster and formed significantly larger tumors (p<0.0002), while tumor cells expressing PTHrP that lacks the NLS *and* C-terminal region grew slower and formed significantly smaller tumors (p<0.0021) compared to control MCF7 cells. Immunofluorescence of the PTHrP mutant tumor cells in culture revealed that deletion of the PTHrP NLS did not preclude PTHrP from nuclear entry in MCF7 cells, suggesting that the NLS is not the primary nuclear entry signal in breast cancer cells, and that intracrine regulation of pro-dormancy genes like leukemia inhibitory factor receptor (LIFR) by nuclear PTHrP may still occur. Indeed, *in vivo* staining of primary DNLS tumors confirmed a downregulation of LIFR. We next conducted *in silico* analyses to determine if regions other than the NLS were contributing to the maintenance of intracrine signaling. DP-bind predicted a high DNA binding affinity (9/12 residues) in a "gap" region between the PTHrP NLS and C-terminal region enriched with lysine and arginine, and which AlphaFold predicted to have a novel alpha-helix structure when the NLS is removed. This high DNA binding affinity, similar to structures found in transcription factors, suggests a potential for the "gap" region to bind to DNA that is structurally enriched by the deletion of the NLS. This phenomenon may explain the phenotypic changes seen in the DNLS mutant lines. Taken together, these results indicate that the historically named biological domains of PTHrP may take on novel and unique functions in breast cancer that contribute to tumor progression.

#34 The effect of dickkopf-1 (DKK-1) on prostate cancer growth and bone metastasis using the canine osteoblastic Probasco cell line

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Abstract: Osteoblastic bone metastasis is the predominant phenotype observed in advanced prostate cancer and is associated with high patient mortality and morbidity. WNT signaling is necessary for both bone formation and tumor progression. Dickkopf-1 (DKK-1), a secretory antagonist of the canonical WNT/β-catenin signaling pathway, plays a complex role in bone metastases by either promoting or decreasing tumor growth and metastasis. The present study explored the role of DKK-1 in prostate tumor progression, metastasis, and tumor-bone interactions using the osteoblastic, bone-metastatic canine prostate cancer cell line, Probasco. Previously, we found that DKK-1 can inhibit bone mineralization of mouse calvaria by Probasco conditioned medium (CM). DKK-1 was stably expressed in Probasco cells (Probasco+DKK-1), which were injected into the tibias or left ventricle of athymic mice. Intratibial tumors and bone metastases were monitored by bioluminescent imaging, microCT, and histopathology. Our results showed that Probasco+DKK-1 bone metastases had increased tumor growth, increased osteoclastic bone resorption, and decreased intramedullary woven bone formation *in vivo. In vitro*, DKK-1 had an autocrine effect on Probasco cells, which altered the cell morphology, increased cell proliferation, and induced EMT. Mechanistically, DKK-1 had little effect on the canonical WNT/ β-catenin pathway while dramatically down-regulated the non-canonical WNT/JNK pathway, which inhibited caspase-dependent apoptosis in Probasco+DKK-1 cells. To investigate the effect of Probasco+DKK-1 on bone cells, primary murine osteoblasts and osteoclasts were treated by CM collected from cancer cells. DKK-1 did not inhibit the mineralization of osteoblasts nor stimulate the activity of osteoclasts directly, but the expression of RANKL was significantly greater in Probasco+DKK-1 CM-treated osteoblasts. Therefore, the Probasco+DKK-1 cells enhanced the activity of osteoclasts indirectly via the regulation of osteoblasts, which induced bone resorption. In conclusion, DKK-1 promoted prostate tumor growth by stimulating cell proliferation and inhibiting apoptosis in a canonical WNTindependent manner and attenuated the osteoblastic activity of prostate cancer. These findings provide new insights into the molecular mechanisms of DKK-1 in prostate cancer progression.

#35 Defining short-form RON mediated anti-tumor immunity in breast cancer bone metastasis.

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Bone is the most common site of metastasis and the usual first site of recurrence in metastatic breast cancer. Bone metastasis is incurable and causes severe pain with significant bone loss in breast cancer patients. Current treatments for bone metastasis aim to diminish bone loss; however, most metastases still progress on current therapies, making it critical to identify new therapeutic targets. We have identified RON tyrosine kinase as a target for treating metastatic breast cancer. RON is the receptor for the macrophage-stimulating protein (MSP) and has tumor-intrinsic and -extrinsic roles. The *MST1R* (Ron) gene gives rise to two transcripts coding for full-length RON (FL-RON) and short-form RON (SF-RON) isoforms, where SF-RON lacks the ligand-binding domain for MSP and is constitutively active. In breast cancer lung metastasis models, deletion of kinase activity from both RON isoforms in the host eradicates tumor growth through increased anti-tumor immunity. More recently, we identified SF-RON as the main isoform mediating tumor-associated immunity in these models, with loss of host SFRON promoting anti-tumor function of T cells. In the bone, loss of host kinase activity from both isoforms of RON protects against bone loss, but its role in anti-tumor immunity is unknown. We aim to delineate the role of SF-RON-mediated anti-tumor responses in the bone, where immunotherapy is often ineffective. In SF-RON knockout mice (RonSF$^{-/-}$), tumor cells injected into the bone grow initially but are eradicated at later time points. Preliminary analyses suggest the importance of immune-mediated clearance of tumors in RonSF-/- mice, as depletion of CD8+ T cells rescued tumor growth by approximately 50%. To gain insight into the role of immune cell populations in this anti-tumor response, we utilized immunohistochemistry and flow cytometry to characterize specific immune cells in bone metastasis. Initial results suggest increased infiltration of T cells, B cells, and NK cells into bone metastases of RonSF-/- mice compared to wild-type controls. Notably, these immune cell populations are all known to play a significant role in the anti-tumor response in other models. Future work will utilize depletion studies to further investigate the role of T cells, B Cells, and NK cells in the SF-RON-mediated tumor clearance in the bone. This work will help elucidate the role of host SF-RON in breast cancer bone metastasis and its potential as a potent immunotherapeutic target.

#36 Novel therapeutic development with serine biosynthesis inhibition and molecularly targeted drugs against the aggressive osteosarcoma

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Osteosarcoma is the most common primary malignant bone tumor in both children and adults. The treatment landscape for osteosarcoma, including high-dose methotrexate, doxorubicin, and cisplatin, has not altered in over 40 years. Methotrexate (MTX) is a metabolic inhibitor that interferes with cell growth by inhibiting dihydrofolate reductase (DHFR), an enzyme involved in the body's use of folic acid. The dose required of MTX for histologic response are toxic and can result in kidney injury or liver toxicity, identifying less toxic therapies that inhibit the same pathway is highly desired. Given the importance of DHFR inhibition for OS, the upstream target phosphoglycerate dehydrogenase (PHGDH), was explored due to the sensitivity of osteosarcoma for MTX. The expression PHGDH, rate-limiting enzyme in the biosynthesis of serine from glucose has been demonstrated to be upregulated in a wide variety of distinct cancers including osteosarcoma, and upregulated PHGDH status in OS patients correlates with patient poor prognosis. We have demonstrated inhibition of PHGDH by NCT-503 resulted in series of metabolic alternations that affect one-carbon metabolism and nucleotide biosynthesis and caused significant attenuation of cellular proliferation, but not cell death in OS when NCT-503 is used as a single therapy because of mTORC1 activation caused by subsequent metabolic changes. Combined treatment of NCT-503 and non-rapalog mTOR1 inhibitor perhexiline, but not rapamycin sensitizing cell death, but its molecular mechanism was unclear. In addition, an alternative non-rapalog inhibitor ALPI3MT55, a novel PI3/mTOR inhibitor and investigated cell death induction mechanism synergically induced by the combined treatment of NCT-503 and non-rapalog mTORC1 inhibitors in OS. Combined treatment NCT-503 and perhexiline/ALPI3MT55 caused significant transcriptional upregulation of pro-apoptotic genes PUMA through activation of AMPK / inhibition of AKT signaling and subsequent activation and nuclear transition of FOXO3. Furthermore, the FOXO3 activation repress c-Myc expression by promoting GSK3 dependent proteasomal degradation in OS. These findings offer new insights of a triple targeted therapeutic strategy for PHGDH highly expressed aggressive OS.

#37 Lactate-GPR81 Signaling in Acute Myeloid Leukemia (AML) Contributes to Bone Marrow Microenvironment Dysfunction, Macrophage Polarization, and Leukemia Cell Growth

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Acute myeloid leukemia (AML) has one of the lowest cancer survival rates (~29% at five years) due to bone marrow dysfunction and relapse following treatment. Using metabolomics, we found that metabolite levels are elevated in the bone marrow of AML patients at diagnosis compared to healthy controls, including lactate (mmol/L = 3.62 vs 1.31, $p < 0.05$, n = 5). We hypothesized that elevated lactate contributes to leukemia progression in ways similar to the solid tumor microenvironment, such as by polarizing macrophages to an immunosuppressive M2-like phenotype and promoting cancer cell growth and chemoresistance; also, we researched effects to hematopoiesis. To test this, we used: (i) a murine model of blast crisis chronic myelogenous leukemia (bcCML), which recapitulates the metabolomic analysis of the human AML BMME, and (ii) C57BL/6J wild type or transgenic mice with a global knockout of the lactate receptor GPR81. In bcCML, leukemia-associated macrophages (LAMs) were found to overexpress the mannose receptor CD206, an M2-like marker. GPR81 signaling contributed to this phenotype in vivo (fold-change CD206 MFI compared to non-leukemic = 4.98 vs 2.06, $p < 0.05$, n = 11). RNA sequencing of LAMs found multiple upregulated T cell pathways. Next, leukemic progression was substantially lessened by mid-stage disease when bcCML was initiated using GPR81-/- leukemia cells (% leukemia in bone = 45.54 vs 11.75, $p < 0.05$, n = 5). Also, the leukemia stem cell ability of bcCML cells was lost when the cells lacked GPR81 signaling (2-3 vs. >5 self-repopulating CFU passages, n = 2). Bone marrow support for hematopoiesis was assayed by utilizing murine hematopoietic stem and progenitor cells (HSPCs) cocultured with a stromal monolayer of mesenchymal stem cells (MSCs) and macrophages; exposure to physiologically-relevant elevated lactate levels (10 mmol/L) reduced HSPC colony-forming ability in methylcellulose-containing media (fold-change CFU-C = 0.41, $p < 0.001$, n = 14). Elevated lactate also reduced MSC colony-forming ability (fold change area 0.63, $p < 0.005$, n = 17). This research identifies lactate as a critical driver of AML progression, highlighting GPR81 as an exciting and novel therapeutic target for both LAMs and AML cells. Furthermore, as lactate production is a hallmark of cancer, this mechanism is potentially applicable to multiple malignancies with bone marrow involvement including additional types of leukemia as well as bone marrow metastases of solid tumors.

#38   Cholesterol increases bone marrow tumour burden in myeloma in vivo and promotes myeloma cell viability and bortezomib resistance ex vivo.

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Multiple myeloma (MM) is characterized by an expansion of malignant plasma cells in the bone marrow (BM) and destructive osteolysis. MM is preceded by the non-malignant condition monoclonal gammopathy of undetermined significance (MGUS). Understanding MGUS progression and development of MM is key for patient management. Diet-induced obesity is known to induce a MM-like condition in vivo however the mechanisms underlying this are poorly understood. Our aim was to determine the effect of cholesterol on MM development. A 2% cholesterol diet was used to increase circulating cholesterol in C57Bl/KaLwRij mice. Mice under this diet received either a) cholesterol diet 4 weeks prior to 5TGM1-GFP inoculation or b) cholesterol diet 4 weeks prior to inoculation and continued for the entire experiment. After 4 weeks, cholesterol had no effect on body weight but increased liver weight and total serum cholesterol due to high LDL levels. Mice continuously on the cholesterol diet had increased BM tumour burden (p<0.005) associated with higher lipid content of MM cells. Additionally, these mice showed a reduction in cortical bone density, indicative of an increase in myeloma bone disease. No significant differences were detected in those mice where cholesterol treatment was halted at time of tumour inoculation. The direct effect of cholesterol was confirmed with mice on cholesterol diet from time of tumour inoculation. These mice showed a 100% increase in tumour burden. No changes were seen on spleen tumour burden, suggesting a bone specific effect of the cholesterol diet. MM1s and JJN3 MM cells cultured with delipidized FBS had a 50% reduction in viability after 72 hours (p<0.001). Rich cholesterol content lipoproteins (LDL) but not VLDL restored MM cell viability, suggesting that cholesterol is responsible for the lipid-depletion effect. Similar results were found using 5TGM1 cells. Moreover, myeloma cell lines pretreated with LDL in vitro for 3 hours prior to treatment with the proteasome inhibitor bortezomib for 24h showed no response to bortezomib when they were cultured under metabolic stress (no FBS) (p<0.001). LDL-mediated resistance to bortezomib was confirmed ex vivo using 5TGM1 cells together with bone marrow cells isolated from C57Bl/KaLwRij mice. Taken together, our results show that cholesterol promotes myeloma. Increased serum cholesterol results in higher lipid content in myeloma cells, ultimately increasing BM tumour burden. Pretreatment with a cholesterol diet did not alter disease progression suggesting a direct pro-tumourigenic effect of cholesterol. Moreover, LDL addition to myeloma cells prevents them from bortezomib-mediated apoptosis. These results identify cholesterol as a mediator of myeloma pathogenesis and bortezomib resistance and provide new directions for dietary or pharmacological intervention strategies.

#39   Bone-derived Dickkopf-Related Protein 1 supports primary breast cancer growth and alters hematopoiesis

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Dickkopf-related protein 1 (Dkk1), a Wnt/β-catenin signaling inhibitor best known for suppressing bone formation and increasing bone resorption, is upregulated in many cancer types and correlates with poor prognosis. Dkk1 has been recently shown to create an immune suppressive environment by altering multiple immune cell subsets depending on the cancer models. However, the role of Dkk1 in breast cancer tumor progression and the mechanisms by which Dkk1 can affect so many immune cell types remain unclear. We found that mice bearing the PyMT or EO771 breast cancer lines have elevated Dkk1 serum levels and that the bone is the primary source of Dkk1 during tumor progression. To determine the effects of bone-derived Dkk1, we orthotopically injected mice overexpressing Dkk1 in the osteoblasts (2.3kbCol1α1Dkk1Tg, referred to as OBDkk1Tg) with PyMT tumor cells and observed increased tumor growth and systemic alterations to immune populations, with increased myeloid suppressor cells in blood and spleen and reduced NK cells. Conversely, Dkk1 neutralization or Dkk1 ablation in the osteoblasts (tTA-TetOFFOsx-Cre;Dkk1$^{fl/fl}$, referred to as OBDKk1ckO) reduced primary tumor growth and restored anti-tumor immunity. To determine if Dkk1 modulates anti-tumor immune responses by altering hematopoiesis, we first performed noncompetitive bone marrow transplantation at steady state, using mice overexpressing or lacking Dkk1 in the osteoblasts as donors transplanted into WT recipients. Hematopoietic and progenitor cells (HSPCs) from OBDkk1Tg mice had better engraftment than those from OBDkk1cKO, suggesting that bone-derived Dkk1 can impact HSPC functionality. To investigate if Dkk1 affects HSPCs during tumor progression, we treated mice bearing EO771 or PyMT tumors with αDkk1 or IgG control antibody. HSPCs were increased in bone marrow of tumor-bearing mice receiving IgG compared to no tumor controls, especially in the EO771 model. While αDkk1 reduced tumor growth in both models, HSPC frequencies were only reduced in EO771-injected mice, suggesting different mechanisms regulating hematopoiesis depending on the breast cancer subtype. Together this data demonstrates that bone-derived Dkk1 exerts systemic effects modulating tumor immunity by altering bone marrow hematopoiesis in a cancer-type specific manner, highlighting the existence of a bone-tumor crosstalk to promote tumor progression.

#40   Bone metastatic cancer derived stem cell factor induces sensory nerve sprouting *in vitro* and pain behavior *in vivo* through c-kit receptor activation

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Of the 34,500 patients who die from prostate cancer (PCa) each year, up to 90% present with bone metastasis. This incurable disease state is often accompanied by debilitating cancer-induced bone pain (CIBP), a condition which drastically diminishes quality of life. However, the mechanisms behind CIBP are not well understood, and in turn, CIBP management poses a major clinical challenge. To elucidate the mechanisms behind CIBP, an antibody based signaling pathway array on the dorsal root ganglion (DRGs), a hub of sensory neurons responsible for nociceptive signaling, of mice intrafemorally inoculated with PCa cells was conducted. Interestingly, CD117 (c-kit) was activated in the DRGs of bone-tumor bearing mice compared with non-tumor bearing controls. C-kit, is known to be involved in the development of chronic pain via activation by its ligand, stem cell factor (SCF). Further, bone marrow derived SCF has been implicated in PCa pre-metastatic niche formation. Consistent with this notion, (i) bone marrow SCF expression was elevated in mice with bone metastasis compared to non-tumor bearing controls, (ii) SCF expression positively correlated with bone pain gene signatures in PCa patient samples, and (iii) c-kit was expressed in murine DRGs. Next, whether SCF could induce nerve sprouting, a mechanism of neuropathic pain, through c-Kit was determined using our previously established primary DRG culture model. In these assays, (i) SCF treatment significantly enhanced nerve sprouting, (ii) cancer conditioned media induced sprouting in an SCF dependent manner, and (iii) c-kit inhibition significantly reduced DRG nerve sprouting. The SCF levels were then down-regulated in LL/2 and RM-1 cells, two murine cell lines with high SCF expression. While no effects on cellular proliferation were observed, SCF down-regulation did reduce both nerve spouting *in vitro* and CIBP *in vivo*. To elucidate the downstream mechanisms behind SCF-mediated nerve sprouting and CIBP, murine DRGs were treated with SCF. Western blotting revealed that while SCF treatment activates c-kit, pathways known to be downstream of this axis [AKT, ERK, p38] were not activated. Proteomic analysis identified downstream fibroblast growth factor 1 (FGF1), known for its role in neural activation. Collectively, these results suggest that the SCF/c-kit axis is involved in CIBP by inducing nerve sprouting, and targeting this axis or downstream molecules may serve as a much needed therapy for CIBP.

#41   Discovery of novel tubulin code in bone metastasis of breast cancer.

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Bone metastasis (BM) of breast cancer (BC) causes bone loss and death. Crosstalk between BC cells and bone resident cells releases growth factors from the bone matrix causing aggressive tumor growth. A potentially important aspect of this process includes the internalization of growth factor receptors and secretion via vesicular trafficking that relies on microtubules (MTs). Therefore, it is critical to understand how specific changes in MTs might control these processes. Composed from heterodimers of α- and β-tubulin, MT's properties, and functions derive from the tubulin isotypes and post-translational modifications (e.g., acetylation), a concept known as the 'tubulin code' (TC). Alterations of the TC are associated with resistance to MT targeting chemotherapeutics and the worst outcome in BC patients. However, the specific change in the TC and its reprogramming in BM is currently unknown. Here, we examined the TC using the RNA-Seq database on BC cell lines (n=50), xenograft models of BM, matched primary and BM patient samples (n=30), and mass spectrometry of MTs in BM BC cells. Our RNA-Seq, qRT-PCR, and western blot analysis discovered a differential expression of isotypes specifically TUBB2 (β2) and TUBB3 (β3) levels and their integration in the MT polymer. We found increased expression of β2 and reduced β3 expression in metastatic cells compared to nonmetastatic BC cells, suggesting specific MTs cytoskeletal changes during cancer cell adaptation in bone. Acetylation modification of α-tubulin (Ac-α-Tub) promotes MT stability. During vesicular trafficking, MTs are at risk of breaks due to repetitive bending forces from vesicles and Ac-α-Tub helps prevent this breakage. IHC of BM tumors shows strong Ac-α-Tub positive cells compared to their primary tumors. Our studies revealed that Ac-α-Tub levels and protein levels of β2 and β3 isotypes are increased via Runx2. We and others previously reported that Runx2 promotes BM. Mass spectrometry of MT cytoskeletal from BM cells revealed that Runx2 regulates the interaction of HSP90 and HDAC6 with α-tubulin. To conclude, our findings suggest a novel regulation of the tubulin code by increasing the stability of the microtubule to support intracellular vesicular trafficking in bone metastasis. Importantly, our mechanistic studies suggest that targeting the Runx2-HDAC6-HSP90 axis and tubulin isotypes may sensitize BM tumors to MT-targeting chemotherapeutic agents and inhibit tumor growth in bone.

#42 Local and systemic effects of Dickkopf-1 during breast cancer progression by limiting NK cell-mediated killing

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Elevated levels of the Wnt inhibitor dickkopf-1 (Dkk1) are detected in breast cancer patients and correlate with the progression of bone metastases. We found that breast cancer patients with progressive metastatic bone disease unresponsive to standard-of-care therapies have higher Dkk1 levels than patients with stable disease. Intriguingly, serum Dkk1 levels do not always correlate with Dkk1 expression in the cancer cells. Thus, it is important to understand the source and role of Dkk1 in breast cancer to better identify patients that could benefit from Dkk1 targeted therapies.

In this study, we used mice orthotopically injected with PyMT, EO771, and 4T1 breast cancer lines. Although Dkk1 was not expressed in these cells, Dkk1 serum levels were upregulated in all models. Strikingly, Dkk1 neutralizing antibody (α-Dkk1) significantly reduced tumor growth in the primary site and in the bone compared to IgG, in all models.

To understand the source of Dkk1 in the tumor-bearing mice, we examined Dkk1 transcripts in the bone and tumor stroma. Dkk1 was highly expressed by the osteoblasts (OBs) in the bone and to a less extent by the CAFs in the tumor stroma. To address the role of bone- versus CAF-derived Dkk1, we generated mice with specific deletion of Dkk1 in the OBs (OsxCre;Dkk1$^{fl/fl}$), or CAFs (Fsp1Cre;Dkk1$^{fl/fl}$ and aSMACreERT2;Dkk1$^{fl/fl}$). To our surprise, all models showed a significant reduction in primary tumor growth. Furthermore, WT mice co-injected with PyMT and Dkk1 KO CAFs showed reduced tumor growth compared to mice co-injected with Dkk1 WT CAFs. These results suggest systemic and local effects of Dkk1.

To understand how Dkk1 supports tumor growth, we performed bulk RNAseq on tumor cells isolated from IgG and α-Dkk1 treated mice. Intriguingly, immune response-related pathways were upregulated in the α-Dkk1 treated tumors. Supporting the involvement of immune cells, α-Dkk1 did not reduce tumor growth in the NSG immune-compromised mice. Next, we treated tumor-bearing mice with α-Dkk1 and depleting antibodies against T or NK cells. Surprisingly, only NK cell depletion abrogated α-Dkk1 anti-tumor effects. Further confirming that Dkk1 directly affects NK cells, we found that the NK cell killing efficiency against PyMT tumor cells in vitro was significantly reduced by recombinant Dkk1.

In sum, our data show that Dkk1 exerts local and systemic effects to promote tumor progression by modulating the killing efficiency of NK cells.

#43   Long-chain Acyl-CoA Synthetases Support Multiple Myeloma Cell Proliferation, Survival and Mitochondrial Function

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Multiple myeloma (MM) is a defined by the clonal expansion of malignant plasma cells in the bone marrow and has a 5-year survival rate of 57.9%. Obesity correlates with a poor treatment response in MM patients and an increased incidence of MM. However, the mechanism of how dysfunctional fatty acid (FA) metabolism contributes to MM is unknown. Therefore, there is a critical need to understand how FA metabolism contributes to support MM. FA metabolism alterations have been shown to support cell proliferation, migration, and drug resistance in other blood cancers and solid tumors. Thus, we hypothesized that FA metabolism is important to supporting MM cell proliferation or survival.

Candidate FA metabolism genes that support MM cells were identified in the Hallmark FA Metabolism gene set within the Cancer Dependency Map, a genome-wide CRISPR screen of essential human genes. We found that the long-chain acyl-CoA synthetase (ACSL) family members, which contribute to both catabolic and anabolic FA metabolism, support MM cell line fitness. We therefore hypothesized that the ACSL family supports MM cell survival or proliferation. To test this hypothesis, we measured MM cell proliferation, apoptosis, mitochondrial number and membrane potential, cell cycle progression, and respiration parameters.

We treated 5 distinct human MM cell lines with Triacsin C (TriC), which is an inhibitor of four of the five human ACSLs (ACSL1, 3, 4 and 5). TriC decreased MM cell proliferation (p<0.0001, two-way ANOVA Tukey's multiple comparisons test is used throughout) mitochondrial number and membrane potential and increased apoptosis (p<0.001) in a dose-dependent manner starting at 48 hrs after TriC exposure. A metabolic flux assay of MM.1S cells treated with 1.0 µM TriC for 24 hours showed significantly decreased basal, maximal, ATP-dependent respiration, and mitochondrial ATP production rate (p<0.001).

Taken together, our data suggest that ACSLs support MM cell proliferation, survival, respiration, and mitochondrial function. Recent studies have shown mitochondrial respiration in MM cells is positively correlated with drug resistance to the BCL-2 inhibitor, venetoclax. Future studies will test whether ACSL inhibition sensitizes MM cells to venetoclax and utilize unbiased proteomics and RNA-seq in TriC treated cells to uncover the mechanistic connection between the ACSLs and mitochondrial function in MM cells.

#44 A Novel Osteolineage-derived (Osterix +) Stromal Population Contributes to Breast Cancer Progression

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Osterix (Osx) is an osteogenic marker required for bone mineralization and osteoblast differentiation. Surprisingly, we detected Osx expression in stromal cells from various breast tumor lines (PyMT, E0771, and 4T1) injected orthotopically in adult mice and in the tumor-associated stroma advanced breast carcinoma patients. However, the identity, function, and origin of these extra-skeletal Osx+ cells remain unclear. To trace adult osteolineage-derived cells *in vivo*, we crossed the R26R-tdTomato (TdT) reporter line with Sp7- tTA,tetO-EGFP-Cre (Osx;Cre) mice and the resulting TdTOsx+ mice were treated with doxycycline until weaning to activate the Cre postnatally. By using a combination of immune fluorescence, flow cytometry, and RNAseq analyses, we determined that CD45negTdTOsx+ cells residing in the tumor stroma of PyMT orthotopic tumors have an elongated morphology typical of cancer-associated fibroblasts (CAF), express CAF markers, such as alpha-SMA, Fsp1, PDGFRβ, and PDGFRα, Ly6C, and CD146, and genes associated with skeletal development. To understand their role in tumor progression, CD45negTdTOsx+ cells were sorted from the primary PyMT tumors and co-injected with tumor cells into WT recipient mice. Strikingly, TdTOsx+ cells significantly increased tumor growth compared to mice injected with tumor cells alone. To determine the origin of these tumor-infiltrating osteolineage cells, we first examined their presence in circulation and observed a 5-fold increase in their numbers in tumor-bearing mice compared to no tumor controls. Importantly, CD45.1 WT mice transplanted with the bone marrow (BM) from TdTOsx+ donor mice and orthotopically inoculated with PyMT cells, showed presence of TdTOsx+ cells at the primary tumor site, indicating that these extraskeletal osteolineage cells originate from a BM population. Finally, to determine whether Osx represents a new marker for bone marrow-derived CAFs or drives their protumorigenic properties, we ectopically expressed Osx or vector control in normal mammary fibroblasts (MMFs). Osx+ MMFsshowed upregulation of matrix-related genes, as well as immune regulatory factors, such as Dickkopfrelated protein1, and had significantly higher ability to support PyMT growth in co-injection experiments compared to control MMFs. In sum, our data demonstrate the presence of bone marrow-derived osteolineage Osx+ cells in extra-skeletal tumors, which have tumor-promoting effects and may represent a new subset of CAFs.

#45   Fatty acid binding proteins may modulate myeloma in *Fabp4/5* double knockout mouse

Author/Presenter: Haylee Duval

Mentor: Dr. Michaela Reagan, Heather Fairfield

Multiple myeloma is an incurable cancer characterized by uncontrolled proliferation of plasma cells that collect in the bone marrow. Multiple myeloma patients frequently suffer from immunodeficiency, bone pain, decreased kidney function, and an increased risk for bone fractures, among other symptoms. As such, the urgency to find potential, targetable factors that could aid in remedial action against multiple myeloma is pressing. Fatty acid binding proteins four and five (*Fabp4/5*) may participate in creating a favorable microenvironment for multiple myeloma tumor cells. *Fabp4*, specifically, is highly expressed in adipose tissue. Because of the pre-established link between an increase in adipose tissue (such as in obesity and with aging) and an increased risk of developing multiple myeloma, we hypothesize that targeting *Fabp4* may further the field of cancer research. Similarly, the same may be true for *Fabp5*, with high levels of *Fabp5* expressivity associated with negative myeloma patient outcomes, including an increased risk of relapse and decreased survival odds. To investigate the relationship between *Fabp4/5* expression and the viability of myeloma cells, we are using a novel genetic mouse model with genomic deletion of *Fabp4/5*. To determine whether there are phenotypic differences between cohorts of male and female mice aged eight and twelve weeks, we have begun collecting tissue types of interest from double knockout ($^{-/-},^{-/-}$) heterozygous, ($^{-/+},^{-/+}$), and wildtype ($^{+/+},^{+/+}$) mice. These will be assessed using histologic staining, micro-computed tomography-based bone parameters, complete blood counts, and real-time quantitative reverse transcription polymerase chain reaction (qRT- PCR). Preliminary results suggest that pups from heterozygous breeding crosses are born at the expected Mendelian ratios, with linkage between the *Fabp4* and *Fabp5* alleles on mouse chromosome 3. qRT-PCR of a subset of samples indicates a trend for reduced *Fabp4* and *Fabp5* gene expression in adipose tissues collected from heterozygous mice. Following this characterization, VK*MYC mouse myeloma cells will be introduced to each cohort. To monitor tumor burden, we will measure serum monoclonal Ig, (M-spike), which can be detected by serum protein electrophoresis (SPEP) or IgG ELISA, and survival will be assessed. Combined, these studies will be used to determine if there is a link between *Fabp4/5* expression in the myeloma microenvironment and multiple myeloma cell viability.

#46   cAMP-Mediated Acid Signaling Regulates Tumor-Myeloid-Bone Cell Crosstalk in the Bone-Tumor Microenvironment

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Conflict of interest: The authors have declared that no conflict of interest exists.

The physiological pH of bone marrow has been reported as low as 6.7, which may contribute to metastatic breast cancer severity and resistance to therapies, especially since rapid tumor metabolism makes the pH of the solid tumor microenvironment (TME) as low as 6.4. Tumor cells sense extracellular pH through GPCRs which signal through GαS (*Gnas*) to activate adenylyl cyclase to produce cyclic AMP (cAMP). Breast tumors have high levels of arginase 1 (Arg1) positive tumor infiltrating macrophages (TIMs) and we previously described that GM-CSF signaling through p38/MAPK and STAT3 and extracellular proton signaling through cAMP/CREB are both necessary for Arg1 expression. In the current study, we further explore the role of extracellular acid sensing in tumor and bone cells in the bone TME. We have developed an *in vitro* culture system to modulate pH without the use of CO2 using 2-(N-morpholino) ethanesulfonic acid (MES). 4T1 and bone colonizing PyMT-BO1 BC cell lines were cultured at pH 6.4, 6.8 and 7.2 and GM-CSF production was elevated with increasing culture acidity. We used CRISPR/Cas9 to knock out *Gnas* in these cell lines and found that tumor cells grown in acidic conditions no longer had increased GM-CSF. Likewise, *Gnas -/-* bone marrow macrophages (BMMs) derived from *Gnas^fl/fl* LysM-Cre +/- mice had decreased Arg1 expression when exposed to acid and GM-CSF. We hypothesize that breast tumors utilize self-generated protons in a feed-forward manner to produce more GM-CSF and maintain immune suppression by sustaining Arg1+ myeloid cells. These data suggest that tumor cells and TIMs can sense acid through GαS with functional consequences on immune suppression. We will compare tumor growth in bone, characterize immune infiltrates and evaluate resistance to checkpoint inhibitor therapy in wild type and *Gnas -/-* cells inoculated in Arg1-YFP mice. Further, we will use lactate dehydrogenase (*Ldha*) -/- cells that do not acidify the TME as controls. Since the bone TME is acidic and a common site of BC metastasis, we will next evaluate the role of GαS/cAMP in osteoclasts. Wild type and *Gnas^fl/fl* LysM-Cre +/- BMMs will be used for osteoclast differentiation and function testing in MES-buffered medium. *Gnas^fl/fl* LysM-Cre +/- mice will be implanted with bone colonizing tumors and osteoclast and bone parameters will be analyzed by IHC and μCT. Understanding GαS/cAMP mediated acid-sensing pathways in bone metastases may allow for improved efficacy of immune therapy.

#47 Chemotherapy-induced microenvironmental changes promote dormant breast cancer cell growth in the adipose tissue

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Despite the high 5-year survival rate for early-stage breast cancer patients, late recurrence of the disease remains a major challenge. Breast cancer can recur in approximately 20-25% of patients months to decades after an initial diagnosis and "cure" and there are few biomarkers that predict which patients will recur. Unfortunately, this means clinicians and their patients must take a frustrating wait-and-see approach. Notably, disseminated tumor cells (DTCs) are frequently detected in early-stage breast cancer patients, and their presence predicts a higher chance of recurrence. However, why some patients with DTCs recur while others do not remains a mystery. Previous work has suggested that changes in the tumor microenvironment (TME) play an important role in the maintenance of tumor cell dormancy. When assessing the impact of doxorubicin (DOXO), a chemotherapeutic agent often used to treat breast cancer patients, on the TME, we noted that DOXO treatment led to the activation of dormant breast cancer cells in the visceral adipose tissue. Moreover, in accordance with the clinical observation that obese patients suffer a higher risk of cancer recurrence, we found that diet-induced obesity further exacerbated DOXO's ability to reactivate dormant breast cancer cells in adipose tissue and this reactivation was associated with robust macrophage infiltration. Further investigation unveiled macrophages activate focal adhesion kinase pathway in dormant breast cancer cells. Given tumor cells can metastasize to visceral fat depots in breast cancer patients, our findings provide an important model to determine what drives dormant tumor cell growth in fat tissue. To understand why macrophages were recruited to the adipose tissue and how they led to dormant tumor cell reactivation, we profiled the microenvironmental changes in visceral fat from Veh- and Doxo- treated mice using single-cell RNA-sequencing and found that the infiltrating macrophages exhibited unique pro-inflammatory and lipid regulatory phenotypes. Our study provides novel insights into how the TME impacts breast cancer dormancy and also highlights the importance of adipose tissue as a unique reservoir that potentiates cancer relapse.

#48   N-cadherin in Tumor-Associated Osx+ Cells is Anti-Tumorigenic

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Tumor growth and metastases are dependent on interactions between tumor cells and tumor microenvironment (TME) cells. In mouse breast tumors, we previously identified tumor-associated Osx+ cells (TAOC), which by RNA-Seq exhibit a non-mineralizing osteogenic signature. We also reported that genetic deficiency of *Cdh2*, which encodes the adhesion protein N-cadherin (Ncad), in TAOC leads to hyperactivation of PI3K and β-catenin signaling pathways; and mouse breast tumors grow larger in Osx-driven conditional *Cdh2* knockout mice. However, the mechanisms by which Ncad in TAOC restrains tumor growth are unclear. Here we show that Ncad-deficient TAOC up-regulate Tgf-β1 production, an established pro-tumorigenic factor, by multiple molecular mechanisms. Since TAOC are rare, we created Cdh2-deficient MC3T3-E1 cells (*Cdh2* KO) using CRISPR-Cas9 gene editing. We found that PI3K-Akt-β-catenin signaling cascade is hyperactivated in *Cdh2* KO cells in response to Tgf-β1, confirming that Ncad negatively regulates this signaling pathway. In addition, *Tgfb1* mRNA was up-regulated by exposure to Tgf-β1, and such response was enhanced in *Cdh2* KO cells relative to control cells. Likewise, in promoter-luciferase reporter systems, Tgf-β1 stimulated Sp1 and Lef1 transcriptional activity to a larger extent in *Cdh2* KO cells relative to control cells, and enhanced *Sp1* and *Lef1* binding to the *Tgfb1* promoter. *In vitro* and *ex vivo*, Co-culture of MC3T3-E1 cells with breast cancer cells (BCC) generated larger tumor spheroids relative to BCC alone, and *Cdh2* KO MC3T3-E1 cells remarkably enhanced the pro-tumorigenic effect in the spheroids and after injection in the mouse mammary tissue *in vivo*. The pro-tumorigenic effect of MC3T3-E1 cells was abolished by *TGFBR1* ablation. Thus, Ncad in TAOC is anti-tumorigenic by restraining autocrine production of Tgf-β1 interference via interference with the PI3K-Akt-β-catenin signaling.

#49   Complete estrogen-deprived athymic nude mice are susceptible to changes in metabolism and musculoskeletal function mediated by a high-fat diet

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Athymic nude mice are immunodeficient, thus commonly used as suitable models for tumor xenograft studies. However, previous studies have shown that these mice are resistant to diet-induced weight gain and metabolic changes. We found that a low-fat diet (LFD) may not serve as an appropriate control diet for a high-fat diet (HFD). In order to answer important questions related to cancer, bone, and metabolism research, we developed a HFD-sensitive athymic nude mouse model and appropriate control diet for HFD. We characterized the impact of HFD on bone, muscle, fat, and other metabolic parameters in a mouse model of complete estrogen deprivation generated by ovariectomy and daily aromatase inhibitor treatment. Twelve-week-old mice were fed either a HFD (60%fat), LFD (10%fat), or regular chow diet (RCD,18%fat) for 16w. A subset of 8 mice per group were euthanized at 6,11, and 16w timepoints following experimental diet feeding. Our data indicate that, compared to RCD, HFD-fed mice developed glucose intolerance after 3w, insulin resistance and impaired insulin secretion were noted after 8 and 11w, respectively. Significant reduction in forelimb grip strength was observed after 8w (p<0.001) and ex-vivo extensor digitorum longus muscle contractility was reduced (p<0.01) after 11w of HFD. Mice fed with HFD did not show changes in body weight after 16w, compared to LFD and RCD. However, accumulation of body fat mass (p<0.05) and gonadal fat mass (p<0.05) began after 11w and continued up to 16w of HFD feeding compared to RCD. A decrease in tibial BMD started at 11w of HFD feeding and continued to decline up to 16w as compared to RCD (p<0.01). In contrast, mice fed a LFD started gaining body weight, body fat, and gonadal fat with a reduced lean mass as early as 6w after beginning the LFD. Both LFD and HFD had similar fat content when compared with RCD. Our data showed that when a LFD was used as an experimental control the statistical effects of HFD-mediated weight gain were insignificant; however, when an RCD was used as a control diet HFD-mediated fat gain was statistically significant. In athymic nude mice both HFD and LFD are equally abnormal, thus a RCD serves as a more accurate control group for studies involving a HFD. Our data conclude that, unlike estrogen-replete athymic nude mice, complete estrogen deprivation in athymic nude mice confers sensitivity to diet-induced changes in metabolism and musculoskeletal function.

#50 Investigating the role of EphA2 in breast cancer bone metastasis

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Despite remarkable advances in the treatment of primary breast cancer, metastatic breast cancer remains largely incurable. Metastasis to bone is the most common site of breast cancer, affecting approximately 70% of patients. Breast-to bone metastasis are predominately osteolytic which does not only reduce the quality of life of the patient but may also lead to other complications including bone pain, tumor-induced fracture and hypercalcemia. Although recent therapeutic advances in breast cancer have improve survival, breast cancer bone metastasis remains a challenge. Therefore, novel treatment options are urgently needed to target aggressive primary breast cancers before they disseminate, and metastasis once dissemination has occurred. EphA2 is a receptor tyrosine kinase that promotes tumor growth and metastasis of breast cancer to the lungs. However, its role in bone metastasis is unclear. Here, we examined how tumor-specific EphA2 affects breast-to-bone metastasis and evaluates its role in osteoclasts differentiation and osteolytic bone disease. By generating EpHA2 deficient cell lines in 4T1 and MDA-MB-231TNBC models, we observed a significant decrease in osteolytic bone lesions. Furthermore, we observed a decrease in osteoclast development in EphA2 knockdown cells compare to their wild type counterparts. Further analysis showed that EphA2 regulates osteoclast differentiation through an IL-6– dependent axis. These studies and previous studies from our group suggest that EphA2 inhibition decreases primary breast cancer growth and reduce breast-to-bone metastasis. Further elucidation of EphA2's role in regulating immune recruitment in the bone microenvironment will advance our understanding on how to better target EphA2 in tumor induce bone disease.

#51 High dimensional profiling of human prostate metastatic bone marrow microenvironment using mass cytometry

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Background: Bone is the most common site of distant metastases in prostate cancer patients and tends to be refractory to immunotherapy. Metastatic hormone sensitive prostate cancer (mHSPC) and metastatic castration-resistant prostate cancer (mCRPC) represent two advanced forms of PCa, with mHSPC almost uniformly progressing to mCRPC. To date, detailed analysis of the bone marrow (BM) tumor microenvironment (TME) from mHSPC and mCRPC patients has not been studied and compared using an advanced mass cytometry (CyTOF) approach. Here, we present high dimensional data describing/comparing the phenotype of BM microenvironment from these patients. Furthermore, we show the effects of chemerin (a novel chemoattractant known to recruit lymphocytes to TME) on bone marrow cells from patients with mCRPC.

Methods: We performed a comprehensive assessment of BM from 5 patients with treatment-naïve mHSPC, 3 with mCRPC (having prior androgen-deprivation therapy (ADT) + docetaxel) and 3 with mCRPC (prior ADT only), using CyTOF comprised of 39 different antibodies/markers. Furthermore, we performed CyTOF on control and chemerin-treated BM cells and identified changes in leukocyte subsets as well as markers of activation and exhaustion.

Results: Preliminary analyses (n=2) revealed that CD3+ T-cells (including CD4+ and CD8+) constituted the highest proportion (median, range: 34%, 26-42%) of raw BM, followed by CD19+ B-cells (28%, 26-29%), CD3-CD19-HLA-DR-CD11b+CD33+ myeloid-derived suppressor cells (MDSC: 17%, 10-24%), CD3-CD19-CD56-HLA-DR+CD11c+CD14+CD68+ monocytes (4%, 1-7%), CD3-CD19-CD11b+CD68+ macrophages (4%, 1-7%), and CD3-CD19- CD14-CD56+ natural killer (NK) cells (3%, 1-5) besides small proportion (2% or less) of TCRγδ+ T-cells, dendritic cells, and NKT cells. Following BM stimulation with chemerin, a pronounced reduction of immunosuppressive M2 macrophages (20%) and MDSC (5%) was seen.

Conclusion: This is the first comprehensive study to evaluate the composition of BM TME from mHSPC vs. mCRPC patients using high dimensional CyTOF analysis and serves as an important reference source for further intervening BM microenvironment. Furthermore, our data is the first to show that the addition of chemerin to BM from PCa could potentially reduce immunosuppressive microenvironment in PCa patients.

#52 Computational Modeling of Signaling Pathways and Cell-Cell Interactions Driving Tumor-Induced Bone Disease

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The complex microenvironment of the bone/bone marrow poses challenges for understanding tumor-induced bone disease (TIBD), a painful condition that arises in cancer patients when tumors metastasize to the bone. There are many different cell types, including osteoclasts, osteoblasts, immune cells, and fibroblasts that interact in complex ways within the dynamical system of the bone/bone marrow microenvironment. Moreover, multiple signaling pathways operate simultaneously within cells in the microenvironment. In tumor cells, for example, signaling through TGF-β and Wnt drives responses to drugs and to microenvironmental changes caused by bone resorption and release of growth factors. Despite decades of research over which multiple potential targets have been identified, there are still no targeted therapies for treating patients with TIBD. Frustratingly, therapies predicted to both reduce tumor burden and prevent bone destruction, based on the current "vicious cycle" model of TIBD, have not been as successful as expected. There is a compelling need, therefore, for novel therapies against TIBD that improve patient survival without deleterious side effects. Here, we present computational models that integrate current knowledge of TIBD at the cell population and intracellular levels to elucidate mechanisms of signaling pathway crosstalk and microenvironmental cell-cell interactions contributing to TIBD. These include models (i) of the bone/bone marrow microenvironment that includes tumor cells, osteoclasts, osteoblasts, and additional cell types, including fibroblasts, stem cells, and immune cells, and (ii) integrating multiple intracellular signaling pathways regulating Gli2 expression in tumor cells, including the TGF-b, integrin b3, EGFR, Hedgehog, and Wnt pathways. These models will facilitate *in silico* predictions of single-cell and population-level drug responses, which can be tested *in vitro* and *in vivo*. Ultimately, these models may help identify novel combination drug targets, leading to improved therapies that both reduce tumor burden and prevent bone destruction in patients with TIBD.

#53   Limiting metastatic breast cancer progression by targeting MK2 stromal signaling

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Breast cancer is now the most common cancer among women in the US and metastasis to distant organs is the major cause of death in these patients. Among metastatic sites, bone is the most frequent destination of breast cancer metastasis and once in the bone, it is refractory to therapy. In addition, bone metastasis is associated with significant skeletal related events that greatly impact quality of life. Thus, there is a significant need to develop novel therapies that extend survival and improve quality of life. Previously we found that MK2 inhibition (MK2i) limits metastatic growth by targeting the stromal compartment. To determine which stromal cells are targeted by MK2i, we isolated stromal cells from within a metastatic bone lesion and carried out single cell RNA sequencing (scRNA-Seq). Using this approach, we found numerous gene expression changes in both the immune (CD45+) and non-immune (CD45-) populations upon MK2i. To determine which cell(s) was targeted by MK2i we used an antibody depletion strategy and found that MK2 inhibition does not rely on T cells or macrophages. Besides that, we observed *tnfsf4* (OX40 ligand, OX40L) is absent upon MK2i on scRNA-Seq analysis. Interesting OX40L activates immune response. Using that approach to contribute with therapeutic strategies, we combine MK2i with an OX40 agonist antibody, which synergized to reduce metastatic growth and improve overall survival. Because previous work demonstrated that T cells were not effective against PyMT tumor cells we introduced OVA to ask how our combination therapy would work in the presence of a CD8 T cell response. When we treated mice barring OVAexpressing PyMT-BO1 cells, we found that MK2i plus OX40L treatment abolished metastatic growth and established T cell memory. Finally, to determine the target of MK2i, we returned to our scRNASeq data and found evidence of vascular cancer-associated fibroblasts (vCAF), inflammatory CAFs (iCAFs), matrix CAFs (mCAF), and p16 high CAFs that may be senescent inside the metastatic lesion. We are now testing the putative role of CAFs in metastatic tumor growth in the bone. To accomplish this, we are generating full knockout MK2 mice to elucidate the mechanism of MK2 inhibitor and establish its target cell type. Our data demonstrate that MK2i is as effective as chemotherapy at limiting metastatic progression and is more efficient when combined with immunotherapy.
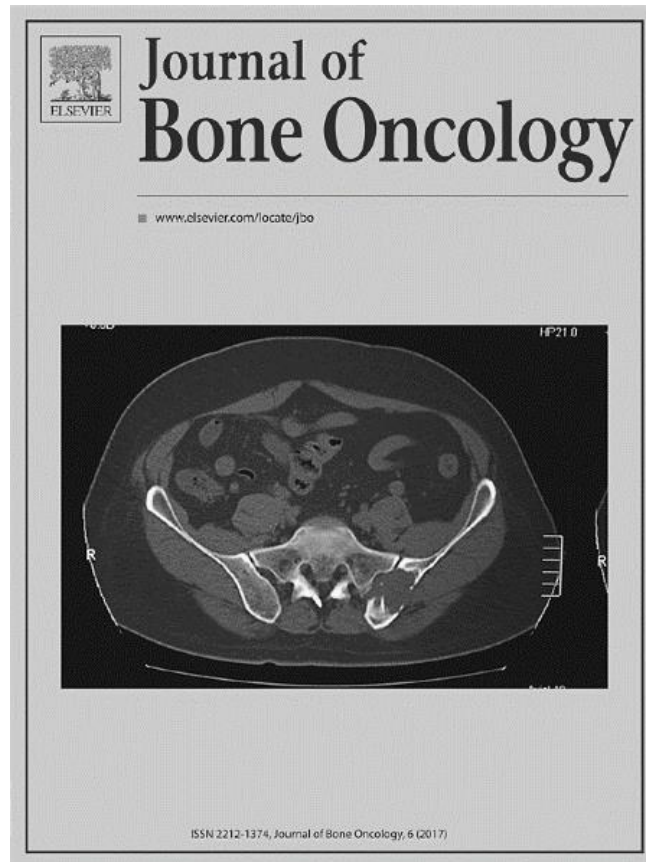
Abstracts from the CABS 2023 conference will be published as a supplement in the Journal of Bone Oncology

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