

HHMI Undergraduate Research Project

Jody Gordon - Summer 2012

**“The Impact of Dietary Calcium on Rates of
Breast Cancer Metastasis to Bone”**

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of the OSU Skeletal Biology Laboratory

Why does cancer and metastasis research interest me?

- As a researcher, I want to understand the how and why.
- As a pre-medical student, cancer and metastasis is a topic I'll deal with throughout my career.
- As an individual, I'm personally interested in any opportunity where science can advance medicine, decreasing suffering and increasing quality of life.

Background:

Cancer:

- 2nd highest leading cause of death in US
- Causes 1 in 4 deaths in the US
- Kills 1,600 people in US every day
- Causes more deaths annually in the US than the next five leading causes of death *combined*

Economic Impact:

Direct Medical Care Costs of Cancer:

- 2007: \$103.8 billion
- 2010: \$124.6 billion
- 2020: \$158 billion (CDC estimated)

An additional 125 billion is also lost annually due to cancer deaths and incapacity.

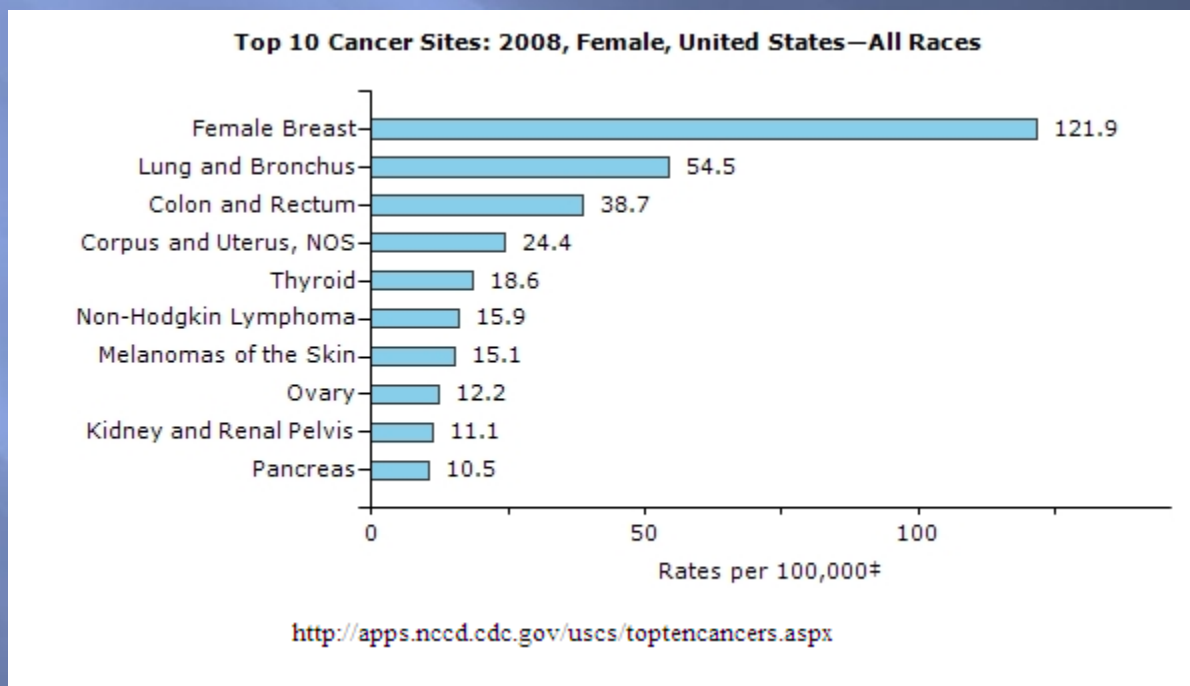


http://www.hcplive.com/conferences/asco/costs_of_cancer_care/

<http://www.cancer.gov/aboutnci/servingpeople/cancer-statistics/costofcancer>

Breast Cancer (BC)

- Most common type of cancer affecting women
- Will affect 1 in 8 US women in their lifetime
- 2nd highest cause of cancer mortality in US women



- Late stage breast cancer is highly prone to metastasis.
- Prognosis for metastatic BC is poor.
- 90% of the deaths attributed to breast cancer are from metastases.

Common metastasis locations:

- Bone (present in 70% of stage IV cancer patients)
- Lungs
- Lymph nodes
- Liver
- Brain



Resultant symptoms:

- Chronic pain
- Hypercalcemia (elevated blood Calcium levels)
- Pathologic fractures (especially of weight bearing bones) due to osteolysis or abnormal bone turnover
- Compression of the spinal cord and nerve roots (possibly resulting in paralysis)

One of these occurs on average every 3 to 6 months for a BC patient. Thus, breast cancer metastasis to bone results in a severe reduction in the quality and duration of life for women with BC.

- **Mechanisms mediating BC metastasis to bone are not well understood**
 - Lack of appropriate animal models
 - Lack of non-invasive methods for detecting tumors
 - Immune compromised animals and surgical trauma cause abnormal bone turnover



http://www.criver.com/EN-US/PRODSERV/BYTYPE/RESMODEOVER/RESMOD/Pages/Athymic_Nude_Mouse.aspx

This study addresses historic problems by:

- Using non-immune compromised mice (“Balb/c” strain) to investigate BC pathology in bone using the highly malignant “4T1” mouse cancer cell line
- Using a minimally invasive tibial injection approach for site-specific metastasis evaluation



Experimental approach:

- Administered 4T1 cancer cells
- Monitored cancer progression in living mice with non-invasive methods
- Performed necropsy, currently evaluating tumor burden, bone turnover and degree of osteolysis using assortment of quantitative and qualitative techniques.

Hypothesis:

Parathyroid hormone (PTH) is directly involved in BC metastasis, as well as the severity of metastatic tumors.

Background:

- ▣ Excess PTH, caused by insufficient dietary calcium, may be an important and preventable factor affecting BC metastasis to bone.
- ▣ PTH levels increase with age in most women, largely due to insufficient dietary calcium.
- ▣ The goal of the research is to test this hypothesis in mice that have BC, and are fed diets containing adequate or insufficient calcium.

Predictions:

- Compared to mice fed adequate calcium, mice fed a low calcium diet will experience a higher degree of destructive bone pathology.
- Once established in bone, malignant tumors will grow more rapidly and be more prone to metastasize again.

Specific Goal:

Determine the effect of elevated bone turnover on BC growth following minimally invasive implantation of 4T1 cell in bone.

Experimental Setup

Group	Sacrifice Date		
	Day 5	Day 10	Day 21
Normal Diet	9 mice	11 mice	12 mice
Low Calcium	9 mice	11 mice	12 mice
Group	Sacrifice Date		
	Day 10		
No cell control (normal diet)	6 mice		
No cell control (low calcium diet)	6 mice		

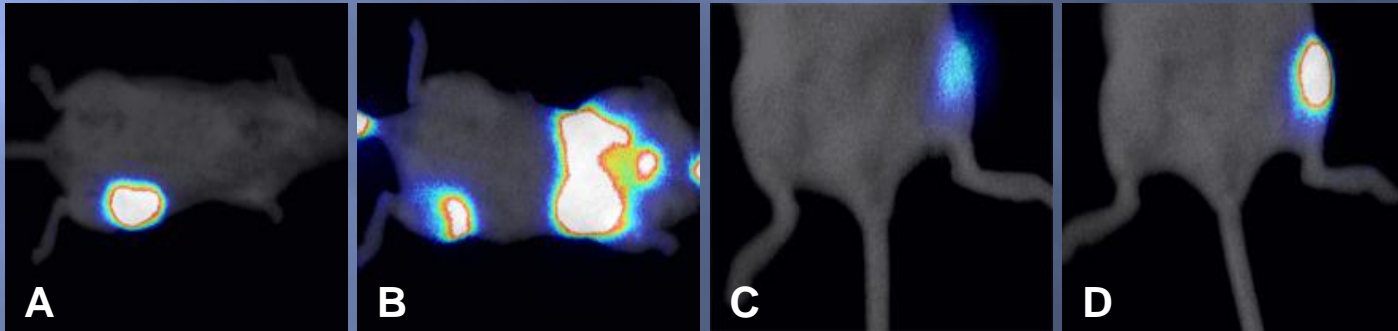
Data Collection and Analysis Techniques

- ▣ Bioluminescent Imaging
- ▣ Microscopic computerized tomography
- ▣ Histomorphometry

Data Collection and Analysis Techniques

Bioluminescent Imaging, “BLI”

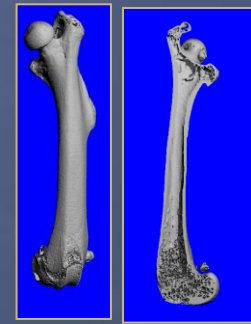
- ▣ 4T1 cells tagged with firefly derived Luciferase
- ▣ Following Luciferin injection, location and size of tumors can be non-invasively visualized with a sensitive camera



Only the 4T1 cancer cells express light

Data Collection and Analysis Techniques

Microscopic computerized tomography, “ μ CT”

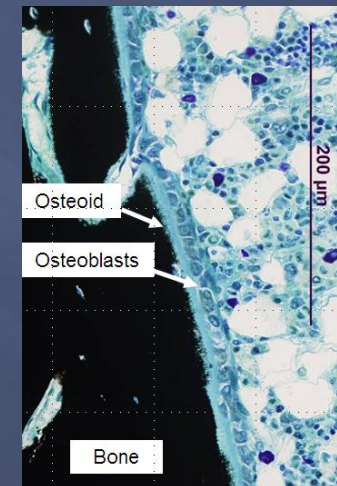
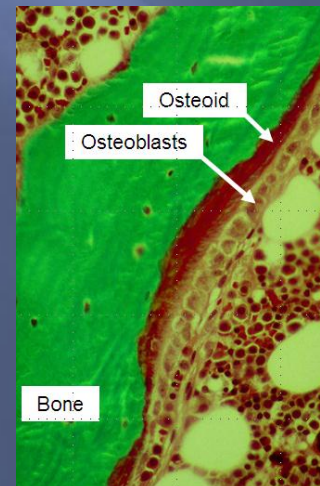
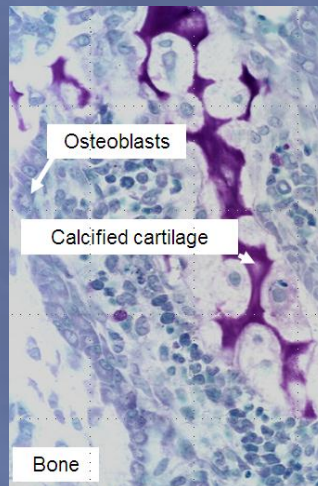


- Allows precise 3D evaluation of bone mass and architecture
- Measures:
 - Total bone volume
 - Cancellous bone volume
 - Cancellous bone architecture
 - Cortical bone volume
 - Cortical bone architecture

Data Collection and Analysis Techniques

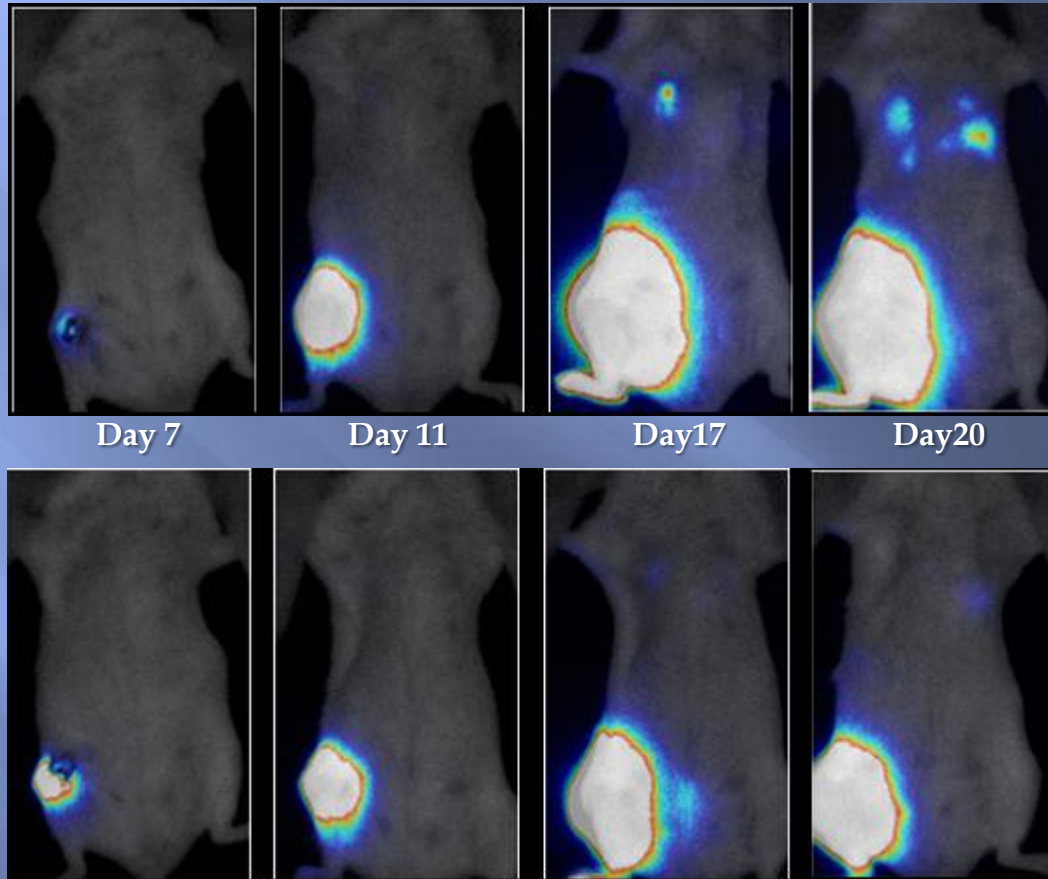
Histomorphometry

- ▣ Provides critical information that cannot be obtained by less invasive methods
- ▣ Only method for direct analysis of bone cellular activity
- ▣ Measurement of:
 - Bone quality
 - Bone growth
 - Bone turnover



Results:

Metastatic progression of 4T1 cells monitored by BLI

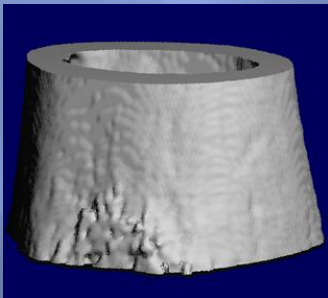


Mice fed low calcium diet experienced greater degree of growth and metastasis of 4T1 primary tumors.

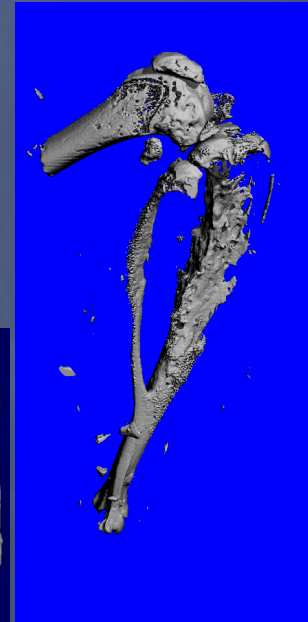
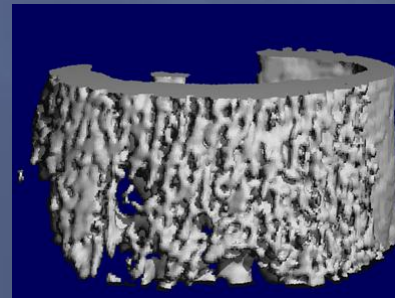
Results:

μ CT 3D reconstructions of control and treatment groups at same levels in bone

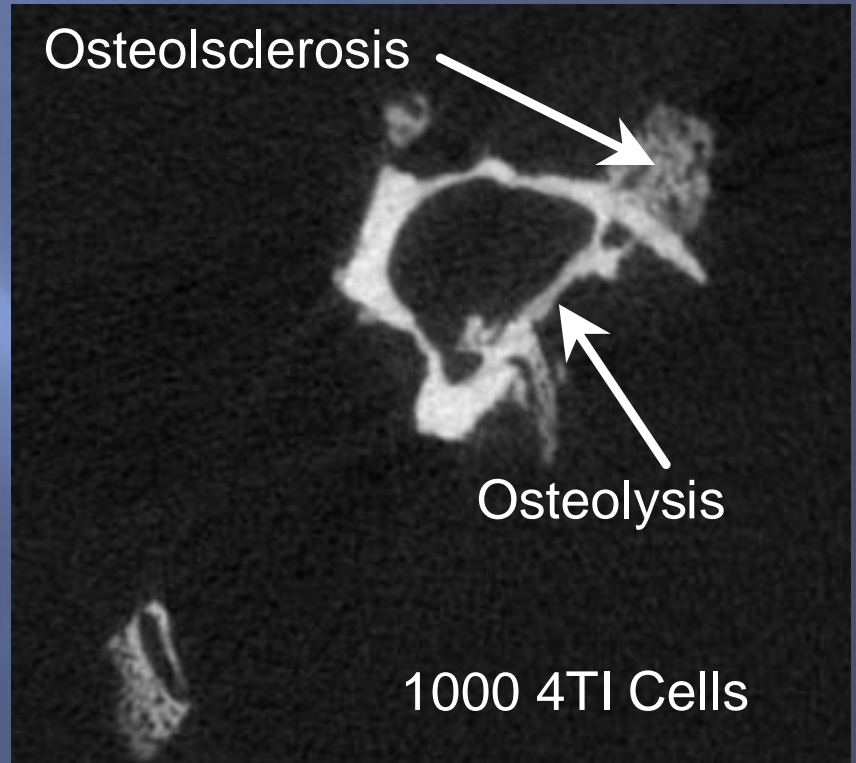
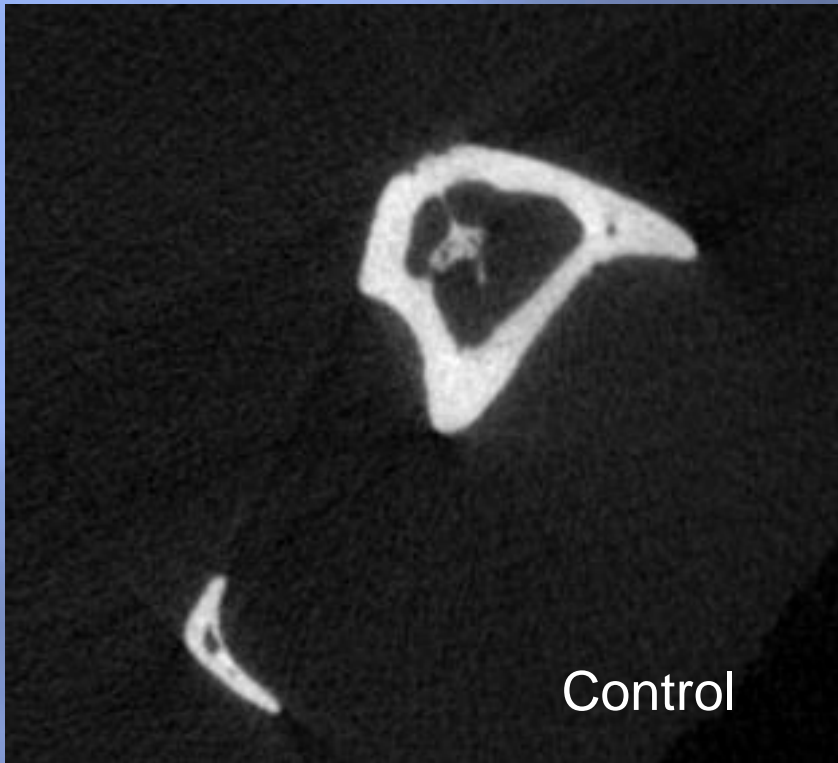
Control



4T1
Treatment



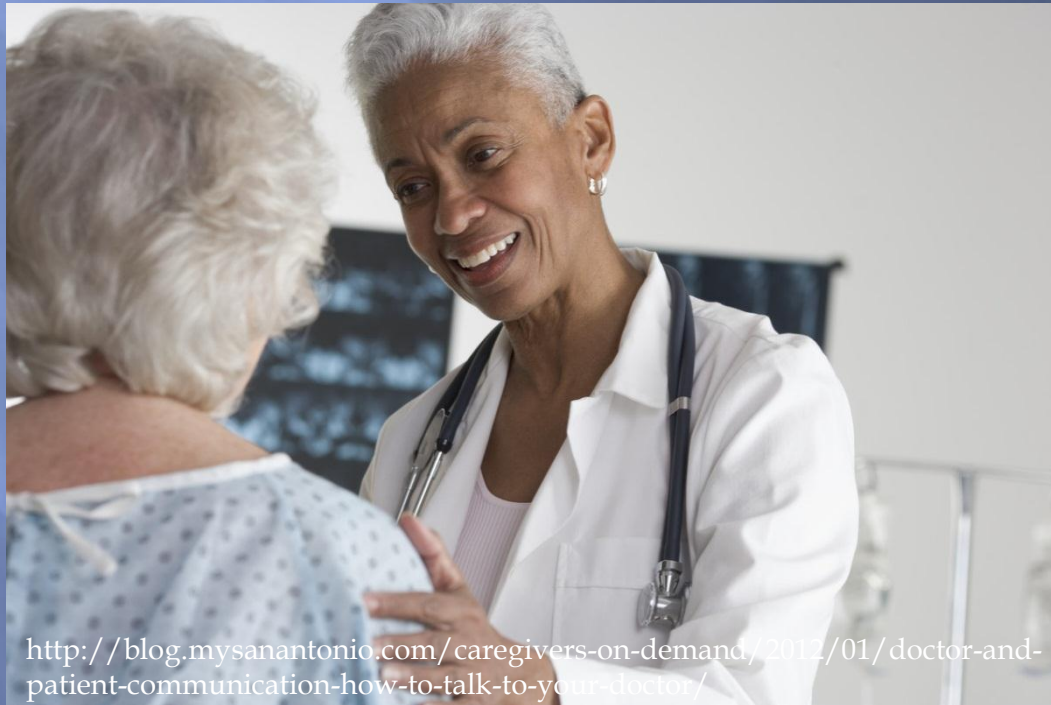
Results:





- If this novel explanation proves accurate, increasing calcium levels by eating calcium rich foods or by taking calcium supplements may reduce the spread of breast cancer to bone and subsequent metastasis to soft tissues in women living with cancer.

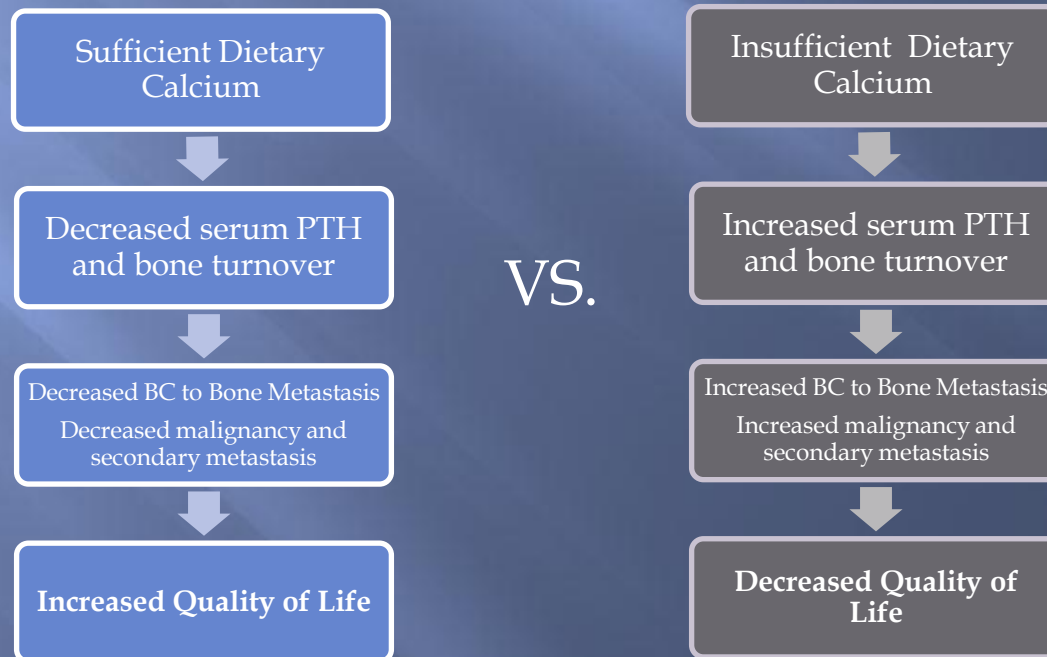
- The results of these studies could quickly alter the way the medical community views the spread of BC, and these experimental findings could easily be implemented in clinical practice.



<http://blog.mysanantonio.com/caregivers-on-demand/2012/01/doctor-and-patient-communication-how-to-talk-to-your-doctor/>

Overall Impact:

If simple dietary changes – which improve calcium homeostasis and consequently reduce PTH levels and bone turnover rate – can reduce metastasis of breast cancer to bone, we will have made a major impact on one of the most debilitating aspects of the progression of BC, one that causes intolerable pain and greatly diminishes quality of life for BC patients and survivors.



Acknowledgements:

I would like to extend my sincere thanks to:

- ▣ Dr. Urszula Iwaniec and Dr. Russell Turner, my mentors in the Skeletal Biology Lab (as well as my coworkers: Dawn, Ken, Bailey, Gino, Jessica, Roy, and Carmen)
- ▣ Dr. William Helferich of the University of Illinois
- ▣ The OSU HHMI Undergraduate Research Program, for research funding and a valuable and enjoyable experience
- ▣ OSU's Director of Undergraduate Research, Dr. Kevin Ahern, for savvy program administration, unflinchingly honest presentation critiques, and the zany humor and enthusiasm we know so well
- ▣ Dr. Carol Rivin, for making the introduction and referral that made all of this possible for me