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*High-throughput screening for  
osteocyte-mediated bone remodeling  
(OMBRE) regulatory compounds*

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[WWW.NSFCDMI.ORG](http://WWW.NSFCDMI.ORG)

# Bone Fragility – beyond osteoporosis

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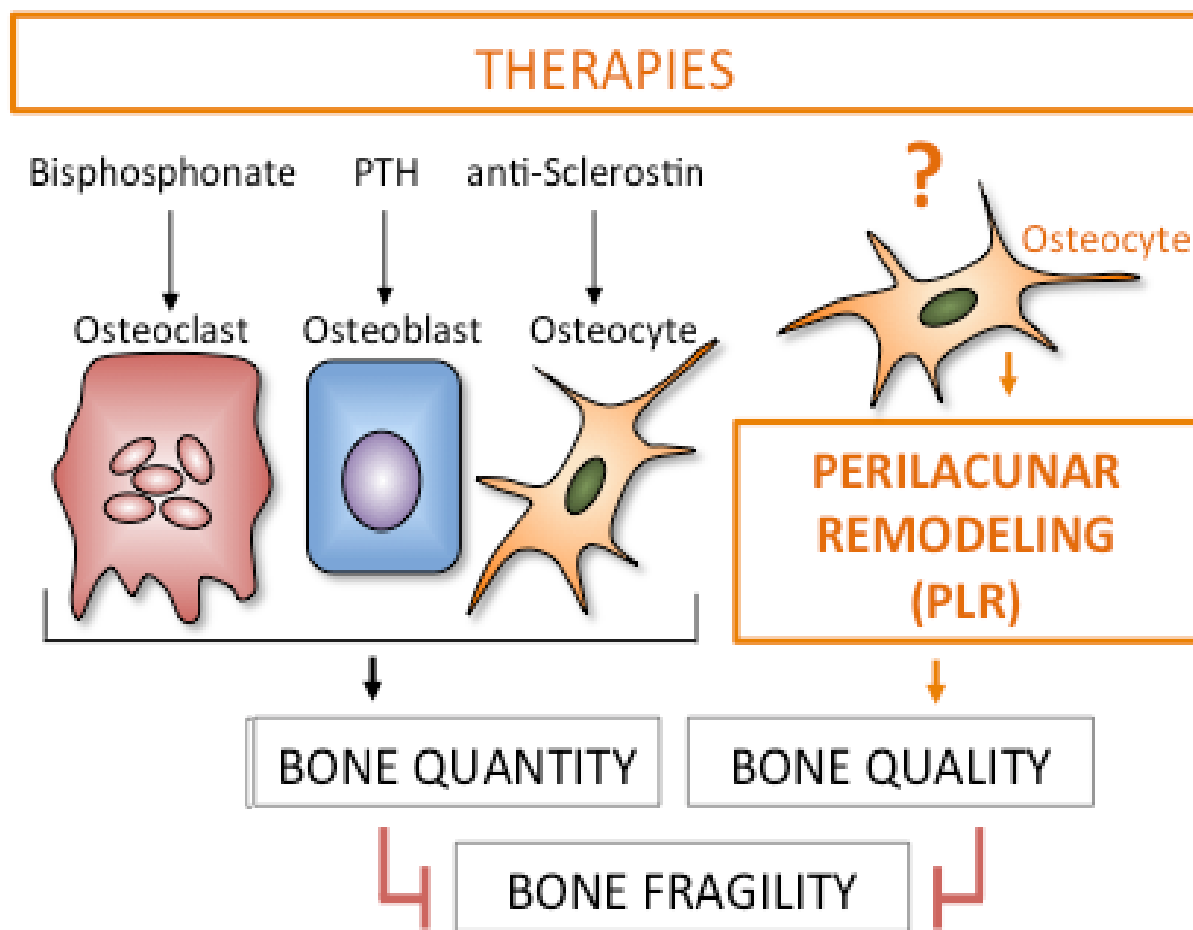
Healthy bone

Osteoporotic bone

**At least half of fragility fractures occur in individuals with normal bone mass.**

*- Wainwright, JCEM 2005*

# Osteocyte-Mediated Bone Remodeling (OMBRE)

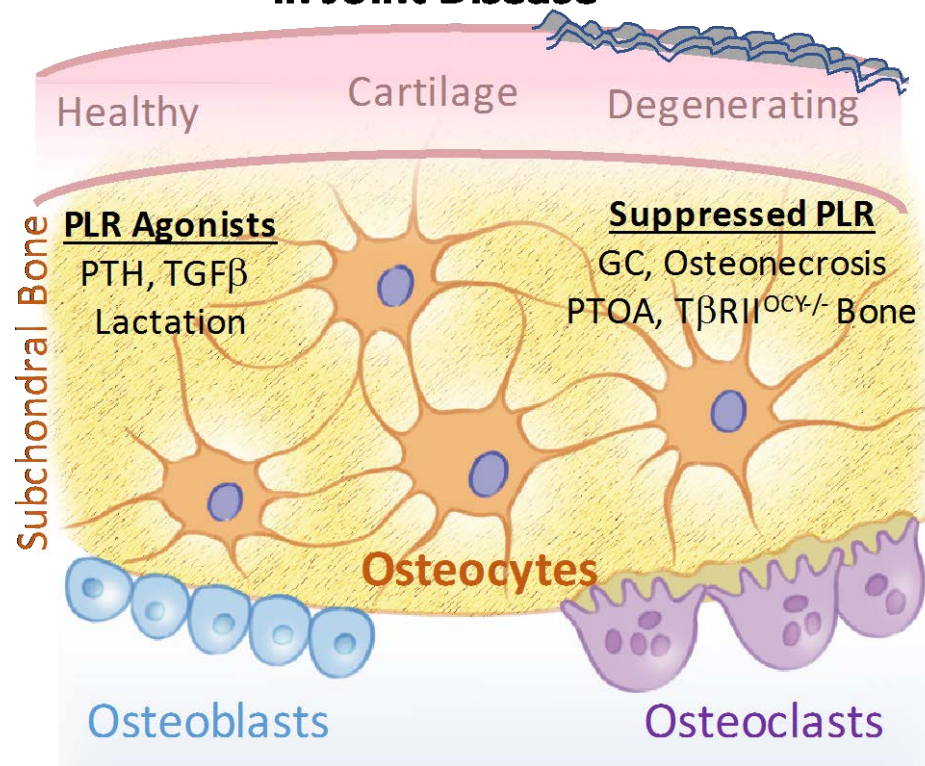




# Clinical Need and Industrial Relevance

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## Osteocytic Perilacunar Remodeling (PLR) in Joint Disease



**Steroid Use**  
**Osteonecrosis**  
**Osteoarthritis**  
**Bone Fragility-**  
**Aging**  
**Diabetes**

Agents that control OMBRE have therapeutic potential for treating skeletal diseases.

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**Knowledge Gaps:** role of OMBRE in skeletal disease, OMBRE therapies

1. Are there current bioactive **drugs that can be used** as OMBRE-regulators for treating skeletal diseases?
2. Advance **fundamental understanding of OMBRE** to develop improved therapies for skeletal diseases.

## Project Aims

This project aims to screen a library of bioactive small molecule compounds to identify agents that regulate OMBRE in vitro.

***Aim 1: Validate functional OMBRE assays in a high-throughput screen (HTS) format.***

*- currently, there is no validated in vitro PLR assay*

***Aim 2: Perform high throughput screen for OMBRE regulatory compounds.***

***Aim 3: Identify and validate lead OMBRE-regulatory compounds for in vitro analysis.***

## *Validate in vitro OMBRE HTS assay*

**Aim 1:** Functional pHi assay

Gene expression screening

## *Identify OMBRE regulatory compounds*

**Aim 2:** Bioactive drug screening

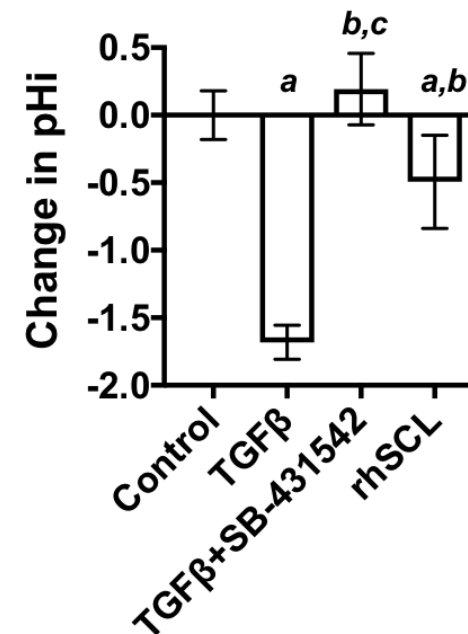
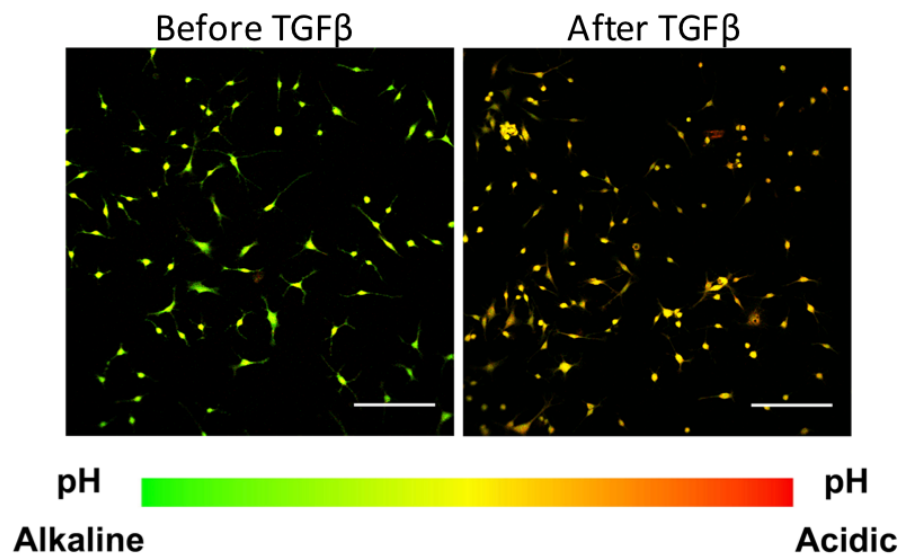
**Aim 3:** Validate OMBRE-regulatory compounds

# Primary Screen – pH Assay

## Validate *in vitro* OMBRE HTS assay

### Aim 1: Functional pH Assay

Gene expression screening



Change in intracellular pH will be visually and quantitatively evaluated.



# Primary Screen – pH Assay

## *Validate in vitro OMBRE HTS assay*

### **Aim 1: Functional pH Assay**

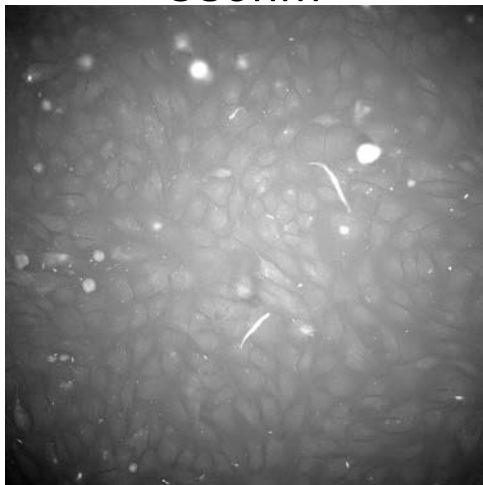
#### Gene expression screening

Ran initial 384w plate for adapting intracellular pH assay to HTS format.  
OCY454 cells, untreated and varying doses of TGFb.

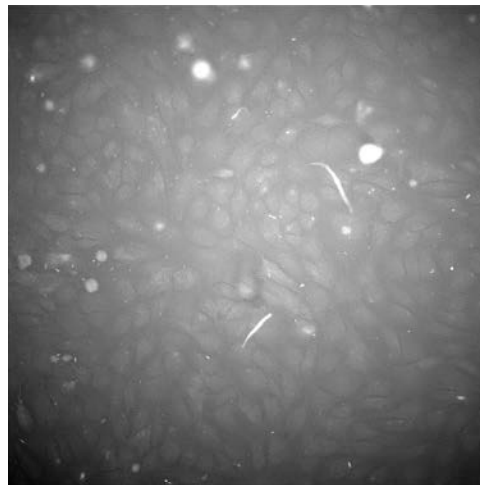
Output:

Image at emission wavelength 580nm, and 640 nm.

580nm



640nm



Segment cells.

Determine average fluorescent intensity.

Calculate ratio of 580nm/640nm.  
(Higher ratio corresponds to acidification.)

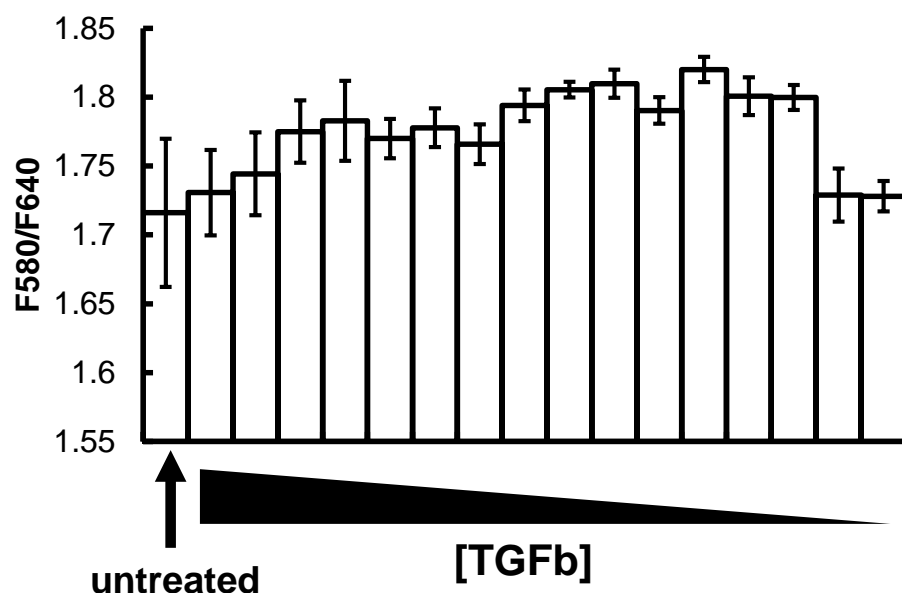
Apparent shift in intensity detected with TGFb.

## Validate *in vitro* OMBRE HTS assay

### Aim 1: Functional pH Assay

#### Gene expression screening

Increased F580/F640 corresponds to acidification.



**Z-prime > 0**

Measures degree of separation between positive and negative control

**%CV < 20%**

Measures degree of variation around mean value of negative control

**%CV = 3.12%**

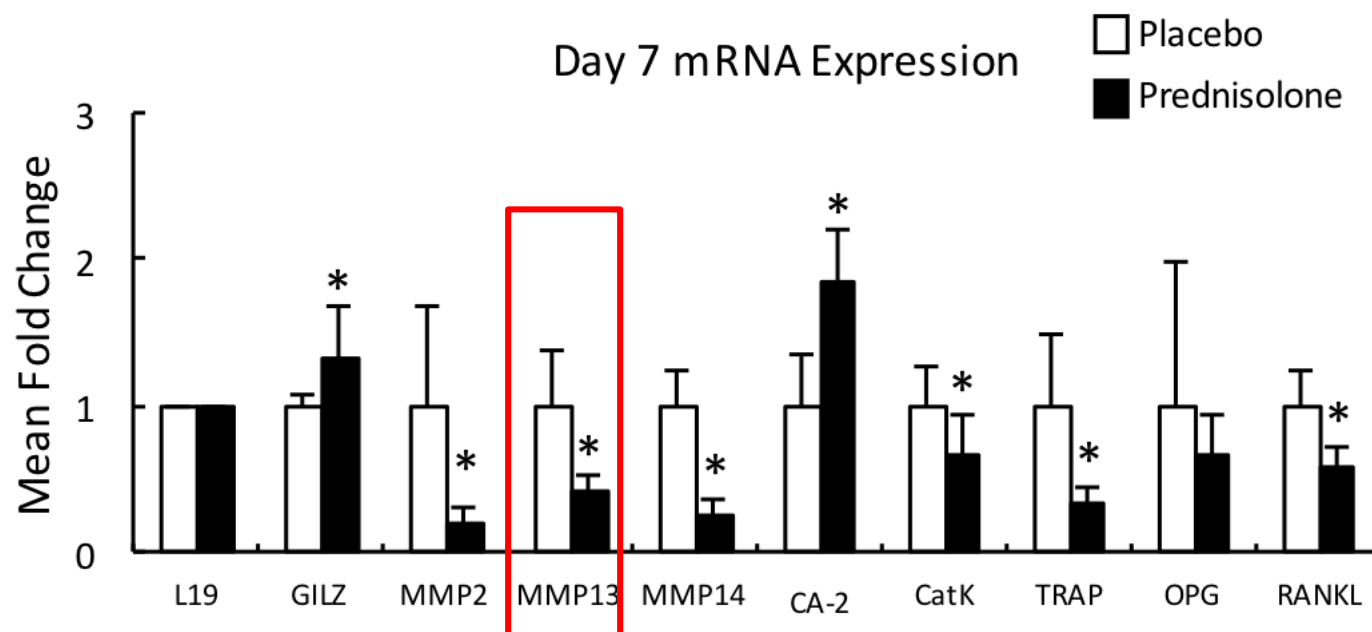
Values for untreated samples are tight enough for a robust HTS assay.

# Secondary Screen- Gene Expression

## *In vitro OMBRE assay*

### **Aim 1:** Functional pH Assay

### Gene expression screening



**MMP13 is a reliable OMBRE marker, so ELISA will be used to detect expression in a HTS format, based on hits from the primary screen.**

# Milestones & Timeline

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*August 2018*

***Primary screen optimized***

**Aim 2**



***Lead compounds identified in primary screen***

***Secondary screen optimization, and hit identification***

*September 2018*

*Fall Symposium @ UCSF*

**Aim 3**

***Validate key lead compounds from secondary screen for in vitro and in vivo analysis***

*November 2018*

*Final Report*



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## Contributors

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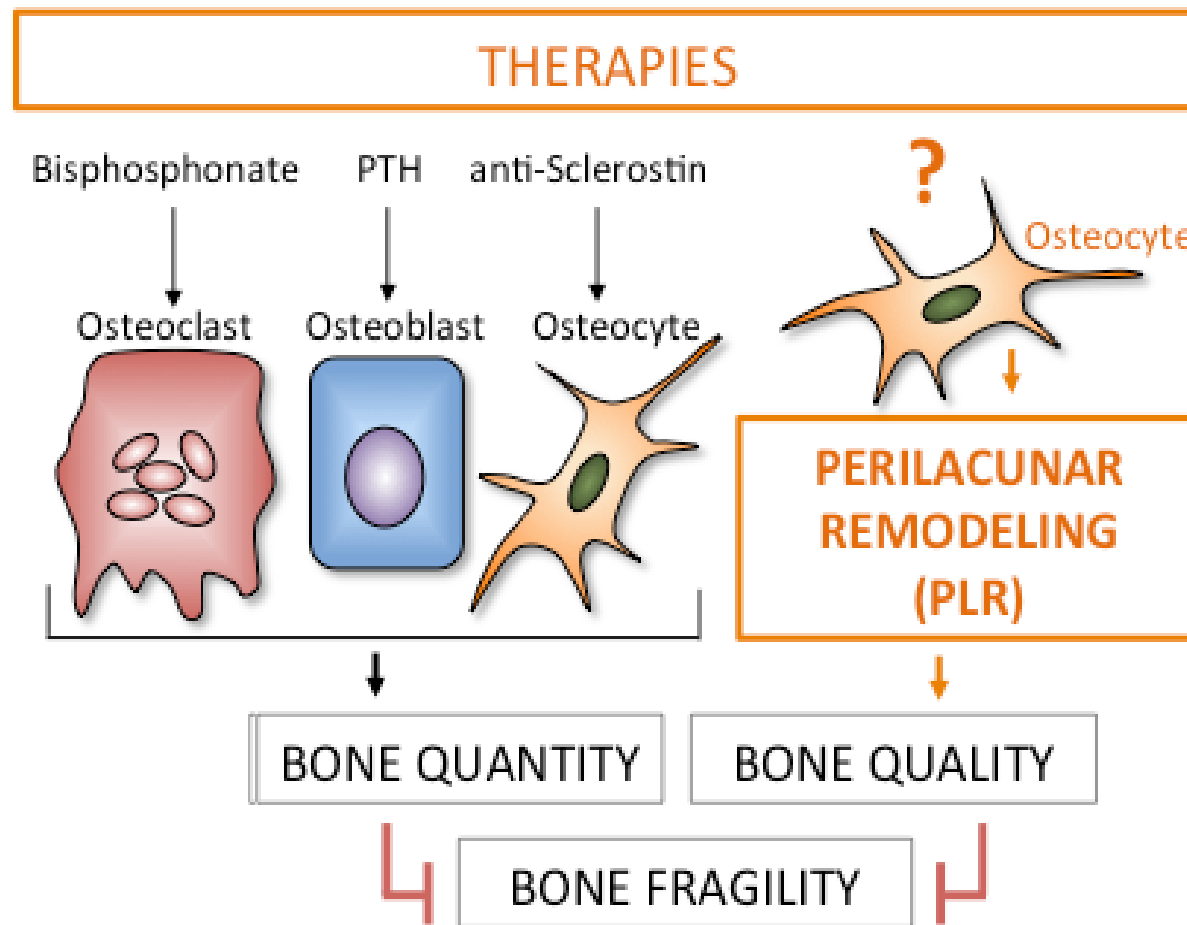
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Steroid Use  
Osteonecrosis  
Osteoarthritis  
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Diabetes