CDMI
CENTER FOR DISRUPTIVE MUSCULOSKELETAL INNOVATIONS

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

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NEED*:** Understanding the control of bone mass has yielded essential therapies to treat bone fragility and other skeletal diseases, including those that target parathyroid hormone or sclerostin ***(Figure 1). However, *at least half of people with fragility fractures do not have low bone mass, pointing to the importance of bone quality. Currently, therapies for bone quality simply do not exist.* This is significantly because the cellular mechanisms controlling bone quality were unknown.

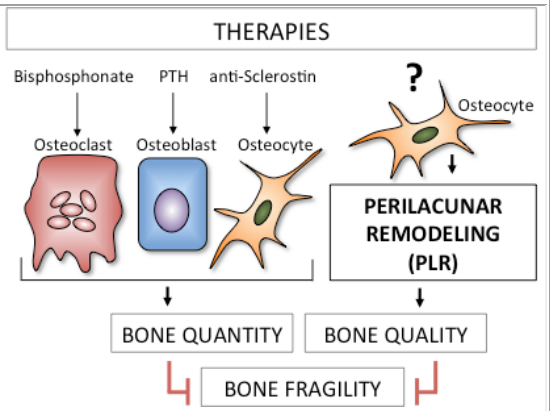

Figure 1: *Schematic of therapies targeting bone cells to improve skeletal health.*

In 2012, we discovered that osteocytes control bone quality through a process called Osteocyte-Mediated Bone REmodeling (OMBRE). OMBRE is the process by which osteocytes secrete acid, matrix metalloproteases (MMPs), and other factors to continually remodel the local bone matrix. OMBRE maintains the mechanical quality of bone matrix and the canalicular channels that provide vascular support, both of which support bone and cartilage function. Work by our group and others reveals that OMBRE is a fundamental mechanism in bone homeostasis.

Just as disruption of other fundamental cellular processes causes disease, disruption of OMBRE compromises skeletal health. We have implicated OMBRE as a causal factor in bone fragility and **osteonecrosis**. Our recent data suggests an important role for OMBRE in **bone fragility** in **aging** and **diabetes**, as well as in **osteoarthritis**. Therefore, *agents that control OMBRE have great and completely untapped therapeutic potential for treating skeletal disease or for improving orthopaedic and dental implant fixation.*

***INDUSTRIAL RELEVANCE*:** Using the in vitro OMBRE assays developed through our 2016-2017 CDMI project, we propose an unbiased screen of FDA-approved compounds to identify agents that control OMBRE. The current effort is important to industry for three reasons. First, this screen has potential to identify current drugs that can be repurposed as OMBRE-regulators for the treatment of skeletal disease with a relatively short regulatory pathway. Second, this screen will reveal the extent to which currently used medications regulate OMBRE, which could produce unanticipated (positive or negative) side effects on skeletal health. Third, this screen will advance our fundamental understanding of OMBRE, an essential next step in developing improved therapies that target this cellular process.

PROJECT OBJECTIVE AND AIMS: This project promotes the development of new therapies to improve bone quality and prevent bone fragility and other common musculoskeletal diseases by targeting osteocyte-mediated bone remodeling (OMBRE) or perilacunar remodeling ***(Figure 1)***. *This* ***project objective*** *is to screen a library of FDA-approved small molecule compounds to identify agents that regulate OMBRE in vitro*. In partnership with M. Arkin, PhD, the Director of the UCSF Small Molecule Development Center (SMDC), we have developed two in vitro assays to quantify functional OMBRE outcomes that are suitable for high throughput screening (HTS). Specifically, we aim to:

Aim 1: Validate functional OMBRE assays in a high-throughput screen (HTS) format.
Using established metrics of HTS assay performance, we will optimize and validate assays in a 384-well HTS format using OCY454 osteocyte-like cells, which robustly express markers of mature osteocytes, such as SOST. The primary screen monitors intracellular acidification using a pH-sensitive fluorescent dye, whereas the secondary screen monitors OMBRE gene expression using Taqman arrays.

Aim 2: Perform high throughput screen for OMBRE regulatory compounds.
We will employ HTS primary and secondary screens to identify OMBRE regulatory compounds from among a library of 2000 FDA & European-approved small molecule compounds.

Aim 3: Identify and validate lead OMBRE-regulatory compounds for in vitro analysis.
Following detailed statistical analyses of HTS results, we will cherry pick 'hits' from Aim 2 for detailed in vitro validation. Validated compounds will be evaluated in vivo in subsequent studies.

METHODS*:*

Aim 1: Validate functional OMBRE assays in a high-throughput screen (HTS) format.
We will adapt two in vitro OMBRE assays, developed with CDMI support, to meet standard HTS design parameters in a 384-well format. The primary screen will evaluate intracellular pH (pHi), a key functional outcome. For example, OMBRE agonists, including SOST, TGF-beta and PTH, induce intracellular acidification of MLOY4 cells. This drop in pHi is detected as a shift in the wavelength of a pH-sensitive intracellular fluorescent dye (5-(and-6)-carboxy SNARF-1, AM), a change that is detectable in a fluorescence plate reader in a 384 well format.

The secondary screen will evaluate the induction of prototypical OMBRE genes using Taqman array plates. In an HTS format, the effect of 16 compounds can be screened on 5 genes (MMP13, MMP14, cathepsin K, carbonic anhydrase 2 (CA2), and the ATPase Atp6v0d) and one housekeeping gene (RPL19) in OCY454 cells. All have established roles in OMBRE in vivo[8-11,34].

Assays will be optimized for OCY454 osteocyte-like cells, which show superior fidelity to native osteocytes relative to other osteocytic cell lines. We will assess several factors that must be optimized to conduct screens with suitable quality and consistency, as determined using HTS metrics such as Z-Prime, Z-Factor, and the coefficient of variation[67]. Since the SMDC compound libraries are dissolved in DMSO, both assays will be validated in cells treated with negative (0.5% DMSO) or positive (5 ng/ml TGF beta in 0.5% DMSO) controls in replicates proscribed by the SMDC.

Aim 2: Perform high throughput screen for OMBRE regulatory compounds.
Validated assays from Aim 1 will be used to screen a library of FDA and European-approved drugs. This library of approximately 2000 compounds, with relevant positive and negative controls, is screened in seven 384 well plates. This is a standard first step for new HTS screens, because it has many significant advantages. First, the smaller-scale screen reduces the technical risk for new assays. Second, a 'hit' from this screen could have enormous translational value. 'Repurposing' an existing drug presents an easier and faster regulatory path, given that the pharmacokinetics, pharmacodynamics, and mechanisms of action are already established for other indications. Third, this screen may reveal unknown side

effects of commonly used drugs on OMBRE, which could affect musculoskeletal health. Finally, even if this screen does not yield an ideal OMBRE ant/agonist, the results will guide the choice of much larger compound libraries for subsequent screens. Larger screens would be conducted under the guidance of the SMDC using the same approach described here for this small library.

Assays are performed on liquid handling robots using bar-coded plates, and results are processed using Pipeline Pilot software, which calculates standard values such as % inhibition or % activation relative to reference controls. The SMDC will advise on the statistical analysis and the selection of hits for Aim 3.

Aim 3: Identify and validate lead OMBRE-regulatory compounds for in vitro analysis.
The criteria for choosing a hit is based on the initial potency scored in the screens, the lack of known toxic effects associated with the compound, and the lack of promiscuous non-specific activity demonstrated by the compound in other SMDC screens. The SMDC will provide aliquots of the hit compounds for in vitro validation in OCY454 cells using a more extensive range of doses (1 µM, 10 µM, or 30 µM) and time points (i.e. 1h, 6h, 12h, 24h, 72h). Outcome measures include the pHi assay and qRT-PCR. The most promising candidate OMBRE regulators will be tested for their off-target effects.

Anticipated Results: At the conclusion of the in vitro validation, we anticipate that we will have a prioritized list of 2-6 compounds that are candidates for subsequent in vivo analyses. These 'first generation' OMBRE regulators will lay the foundation for the development of more specific and effective therapies. Independently of these translational implications, the compounds identified here will have significant impact as much-needed research tools to advance our understanding of OMBRE mechanisms and their deregulation in musculoskeletal disease.

MILESTONES*:*

- Aim 1: Validated HTS OMBRE assays & screening plan for FDA approved compound library – 3/30/2018
- Aim 2: List of lead OMBRE regulatory compounds for in vitro validation – 7/30/2018
- Aim 3: Validated list of OMBRE regulatory compounds for in vitro and in vivo analysis – 9/30/18

DELIVERABLES*:*

- *December 2017 – Conference call*
- *Spring 2018 – Spring Symposium @ UT (conference call)*
- *June 2018 – Conference call*
- *August 1, 2018 - List of lead OMBRE regulatory compounds for in vitro validation*
- *September 2018 – Fall Symposium @ UCSF*
- *November 2, 2018 - Final written report including results and prioritized list of validated OMBRE regulatory compounds*

General Budget Outline:

Personnel	$	24,000
Tissue Culture Supplies	$	6,000
Chemicals and Supplies	$	6,364
Total Direct	$	36,364
Indirects (10%)	$	3,636
Total	$	40,000

Start Date:	**End Date**:
October 2, 2017	September 30, 2018