Animal-specific modelling for the 3R in pre-clinical assessment: a bone drugs example

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About animal experimentation

Reduce, Refine, and Replace animal experimentation

National Centre for the Replacement, Refinement & Reduction of Animals in Research

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Cost to Develop New Drug Now Exceeds $2.5b

Source: Tufts Center for the Study of Drug Development, USA - 2014
Drug Discovery and Development: A LONG, RISKY ROAD

**PRE-DISCOVERY**

- DRUG DISCOVERY
- 5,000 – 10,000 COMPOUNDS
- 3 – 6 YEARS
- IND SUBMITTED

**PRECLINICAL**

- 250

**CLINICAL TRIALS**

- PHASE 1
- 20 – 80 VOLUNTEERS
- 6 – 7 YEARS
- PHASE 2
- 100 – 300 VOLUNTEERS
- PHASE 3
- 1,000 – 3,000 VOLUNTEERS

**FDA REVIEW**

- NDA SUBMITTED
- 0.5 – 2 YEARS

**LG-SCALE MFG**

- ONE FDA-APPROVED DRUG

Source: Pharmaceutical Research and Manufacturers of America
A mouse is a model
Bone drugs

Actonel (risedronate sodium) tablets

Reclast (zoledronic acid) injection
5 mg/100 mL
Solution for Intravenous Infusion
Dispense the accompanying Medication Guide to each patient.
1 bottle – Sterile Solution
Do not mix with calcium-containing solutions. Administer as a single intravenous solution through a separate verified infusion line.

Boniva (ibandronate sodium) tablets

FOSAMAX (alendronate sodium) tablets

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Pre-clinical testing

C57BL/6 – "Black-6"

BALB/c

Ovariectomy / Sham

Ovariectomy produces a systemic osteopenia similar to age-related osteoporosis

Sham surgery is required to normalise for the systemic effect of surgery

Proximal tibia cancellous bone well represents global skeletal changes (negligible spatial gradient)

Changes at 4 weeks are representative of the long-term response (negligible temporal gradient)

Cancellous bone 3D histomorphometry is the most reproducible measure of bone loss
In *vivo* imaging reduce animal experimentation
In vivo MicroCT

LONGITUDINAL MEASUREMENTS FOR MOUSE BONES

- Voxel size ~ 10 μm
- Whole tibia scanned ~30min
- Radiation dose ~ 500 mGy → 5% BMC
- Under anaesthesia
Whole body or whole bone

WHOLE BODY IN VIVO SCAN
(~70µm)

TIBIA IN VIVO SCAN
(~10µm)
In vivo MicroCT

LONGITUDINAL MEASUREMENTS: Proximal Trabecular Bone

Ovariectomized mice (model of accelerated bone resorption)
Results – traditional method

Sham

OVX

OVX + PTH

Longitudinal analysis: 34% reduction in the number of animals to discriminate BV/TV
Method: reproducibility

- Bone Mineral Content - BMC
- Densitometry calibration (HA equivalent)
- Mass = Volume * density
Results - reproducibility

## Results: sample size reduction

<table>
<thead>
<tr>
<th>Variable</th>
<th>Log scale residual SD</th>
<th>ICC point estimate</th>
<th>Theoretical sample size reduction (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard Morphometric parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV/TV</td>
<td>0.224</td>
<td>0.59</td>
<td>34% (20% to 53%)</td>
</tr>
<tr>
<td>Tb.Th</td>
<td>0.074</td>
<td>0.13</td>
<td>13% (6% to 28%)</td>
</tr>
<tr>
<td>Cort.Th</td>
<td>0.044</td>
<td>0.64</td>
<td>28% (18% to 41%)</td>
</tr>
<tr>
<td>Tb.N</td>
<td>0.105</td>
<td>0.27</td>
<td>7% (2% to 18%)</td>
</tr>
<tr>
<td><strong>BMC based parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tb.BMC</td>
<td>0.292</td>
<td>0.71</td>
<td>51% (35% to 68%)</td>
</tr>
<tr>
<td>Cort.BMC</td>
<td>0.064</td>
<td>0.87</td>
<td>63% (54% to 71%)</td>
</tr>
<tr>
<td>Tot.BMC</td>
<td>0.063</td>
<td>0.87</td>
<td>63% (54% to 71%)</td>
</tr>
</tbody>
</table>
Overall changes in BMC

Variation BMC in whole tibia with respect initial value

![Graph showing variation in BMC with respect to age and different conditions.]

- **Relative Tot ΔBMC [mgHA]**
- **Age [wks]**
- **WTAvg**
- **WTPTH2Avg**
- **SHAMAvg**
- **OVXAvg**
- **OVXPTH2Avg**
Accurate quantification of bone adaptation question validity of current animal models
Tissue adaptation – voxel-based

Rigid Registration plus Boolean operations

Scan1

Rigid Registration

Overlapped images

Scan1 and ScanN

ScanN

Boolean Operations

Schulte et al, Bone, 2011

Green:
- Only Scan1 = Resorbed bone
- Only ScanN = Formed bone
- Both Scans = Constant bone
Voxel-based method

Evaluation of accuracy of the method: “zero remodelling” case

14 wks C57BL/6 tibia still growing

Mouse age [wks]
Method: longitudinal study

Mouse 1 at time point 1

Mouse 1 at time point 2

Aligned Images

Registered images using rigid registration

Cropping box

Centre of mass

Medial (M)

Anterior (A)

Lateral (L)

Posterior (P)

Volume of interest (mouse 1, time point 1)

Volume of interest (mouse 1, time point 2)

Cross section a-a

Cross section b-b

Cross section c-c

Cross section d-d
% Changes BMC: SHAM vs WT groups

Mean relative percent difference [%] between Sham (N=7) and WT (N=5) groups

(* p<0.05, ** p<0.01)
% Changes BMC: OVX vs SHAM groups

Mean relative percent difference [%] between Ovx (N=7) and Sham (N=5) groups (* p<0.05, ** p<0.01)
% Changes BMC: WTPTH vs WT groups

Mean relative percent difference [%] between WTPth (N=5) and WT (N=5) groups

(* p<0.05, ** p<0.01)
Mean relative percent difference [%] between OvxPth (N=7) and WTPth (N=5) groups

(* p<0.05, ** p<0.01)
VPM predictors are discriminating
The future

VPM predictors

VPM Scaling

Analogy
Conclusions

• Pre-clinical models need to be “re-engineered” in order to produce reliable, reproducible quantifications of the process of interest

• Non-invasive methods and in silico technologies allow to observe over large anatomical spaces and over time, which is essential for a correct interpretation of the observations

• In silico technologies can drive a ”renaissance” of biological research based on the epistemology of physical sciences
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