INSIGNEO

Institute for *in silico* Medicine

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

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NHS Foundation Trust

# About animal experimentation

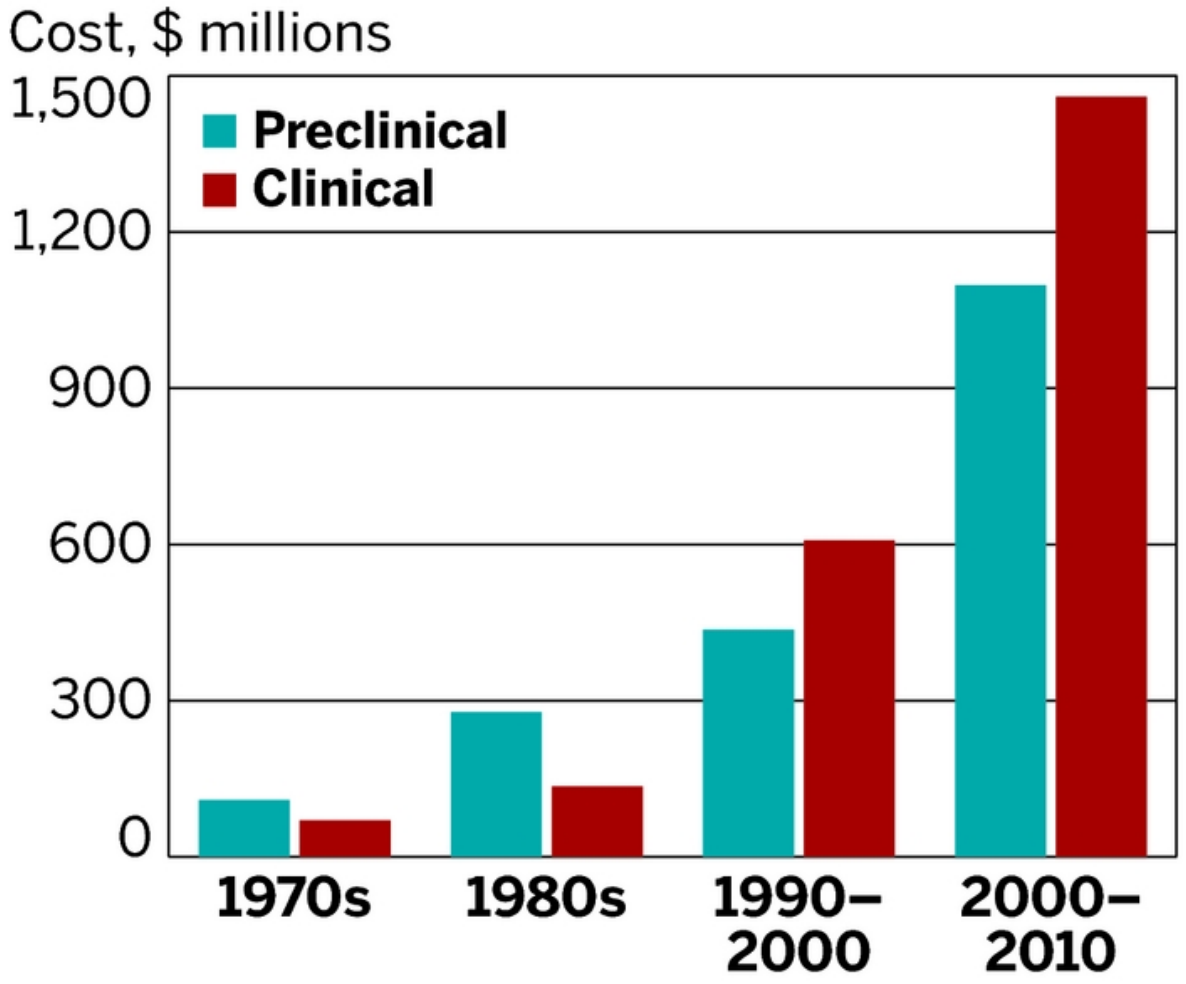


**Reduce, Refine, and Replace animal experimentation**

NC 3Rs

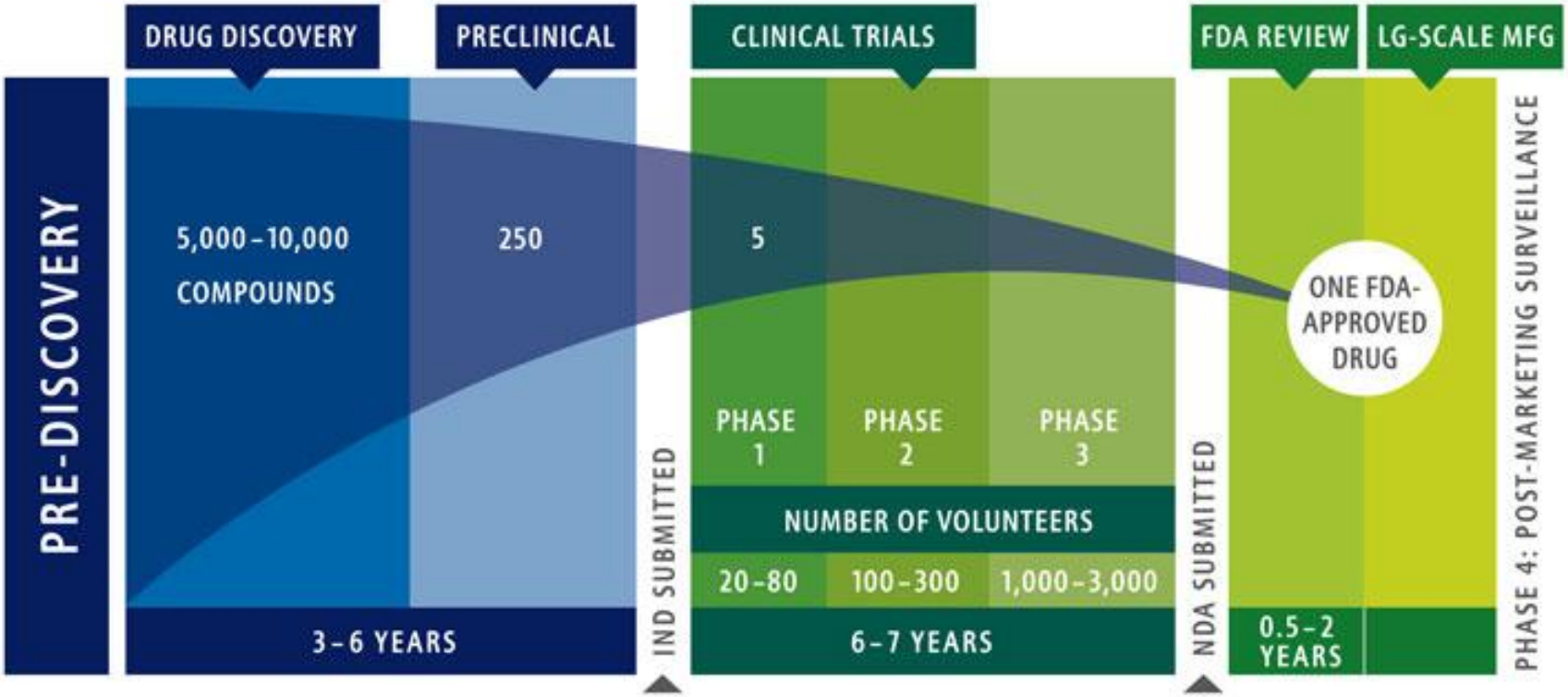
National Centre for the Replacement Refinement & Reduction of Animals in Research

# 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




Source: Pharmaceutical Research and Manufacturers of America

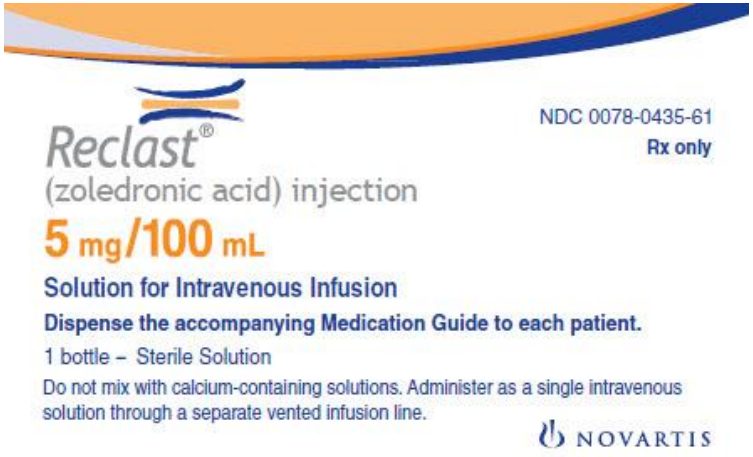
# A mouse is a model

Homology

Analogy

# Bone drugs

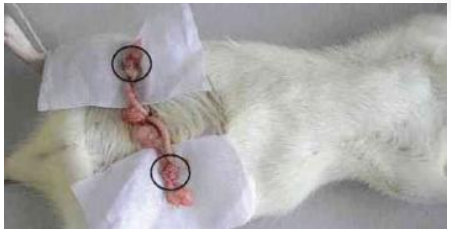
Actonel®
(risedronate sodium) tablets


FOSAMAX®
(alendronate sodium) tablets

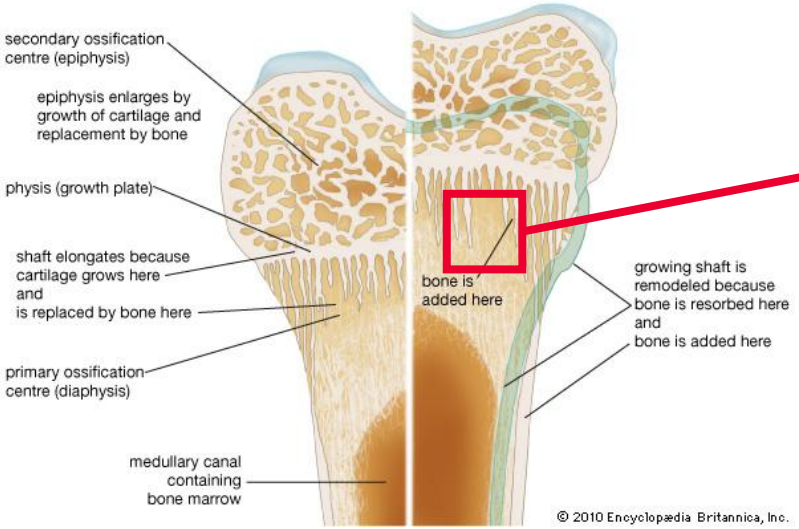
# Pre-clinical testing

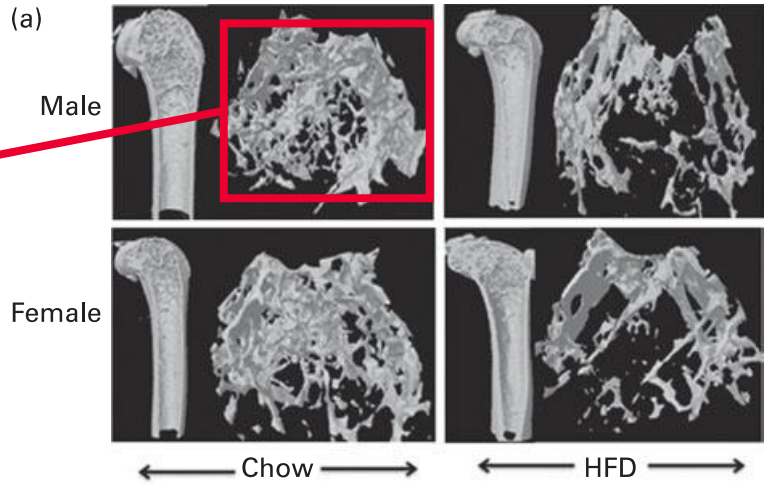
C57BL/6 – ”Black-6”



Ovariectomy / Sham

BALB/c




Br J Nutr. 2014 May 28;111(10):1811-21.

# Implicit assumptions

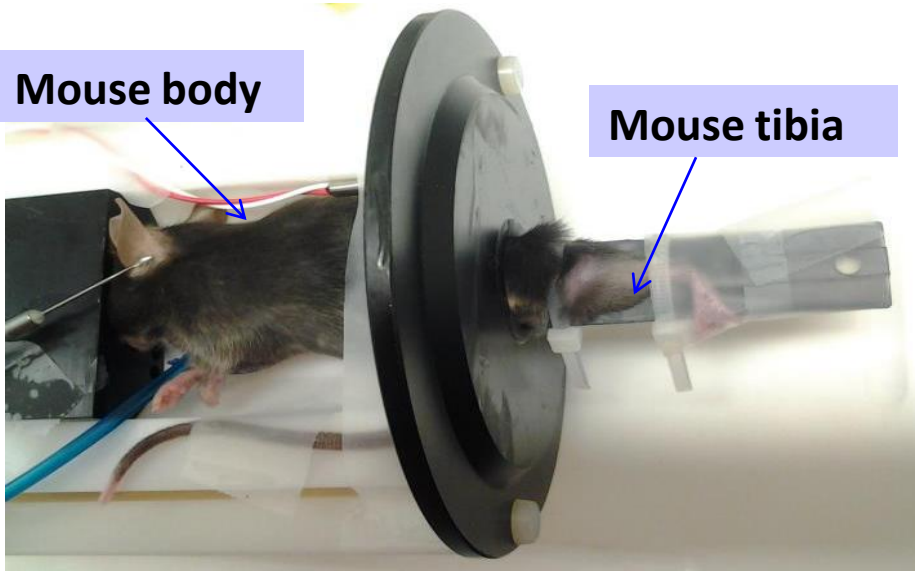
- Ovariectomy produces a systemic osteopenia similar to age-related osteoporosis
- Sham surgery is required to normalise for the systemic effect of surgery
- Proximal tibia cancellous bone well represents global skeletal changes (negligible spatial gradient)
- Changes at 4 weeks are representative of 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**

- Technol Health Care. 1998 6(5-6):339-50
- Voxel size ~ 10 µm
- Whole tibia scanned ~30min
- Radiation dose ~ 500 mGy ➔ 5% BMC
- Under anaesthesia



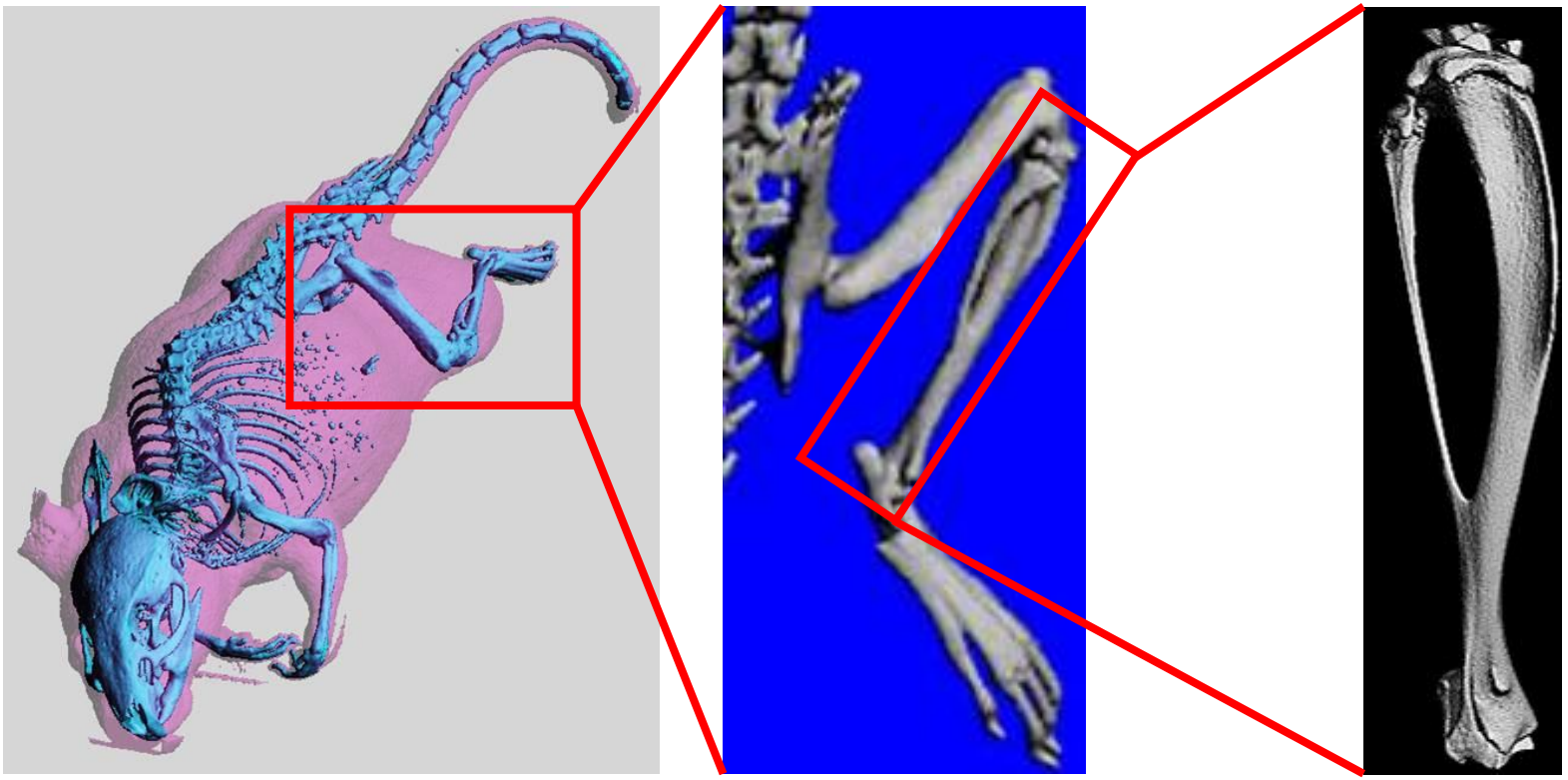

**In vivo scan of mouse tibia**

**VivaCT 80 scanner**

# Whole body or whole bone

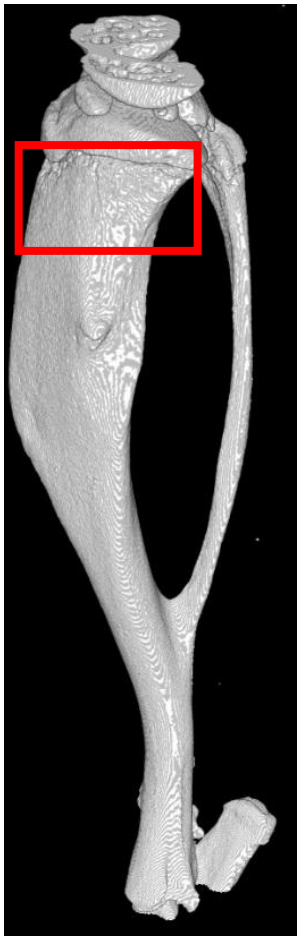
**WHOLE BODY IN VIVO SCAN (~70µm)**

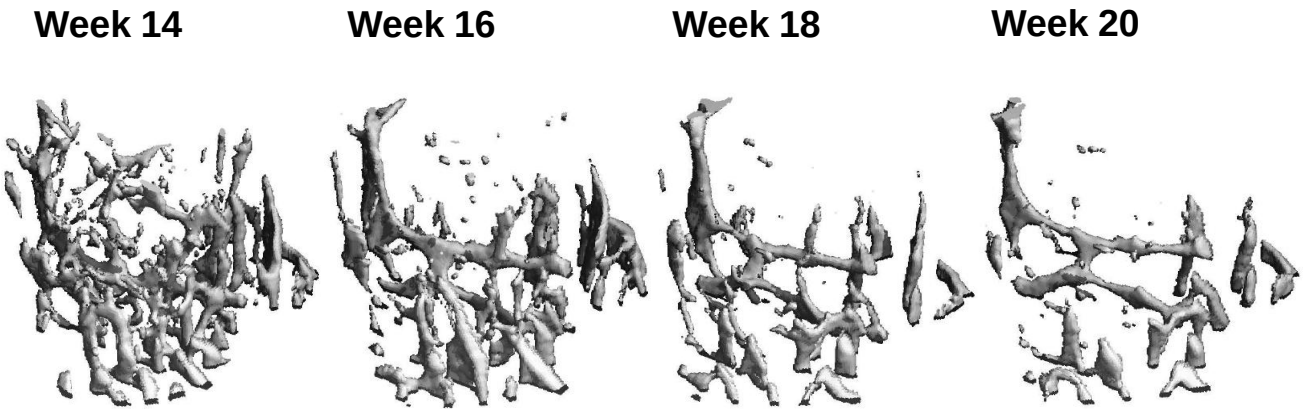
**TIBIA IN VIVO SCAN (~10µm)**



# In vivo MicroCT

**LONGITUDINAL MEASUREMENTS: Proximal Trabecular Bone**

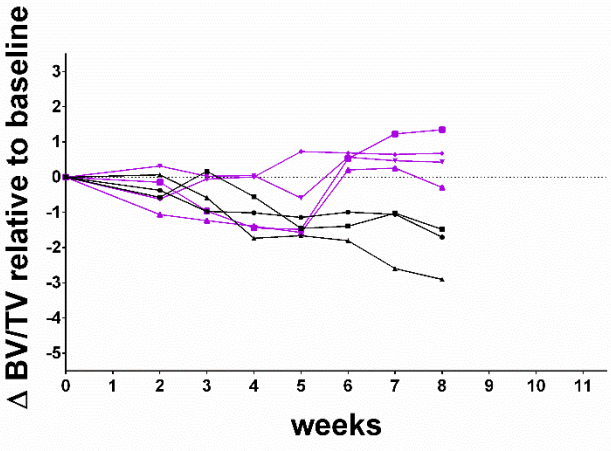




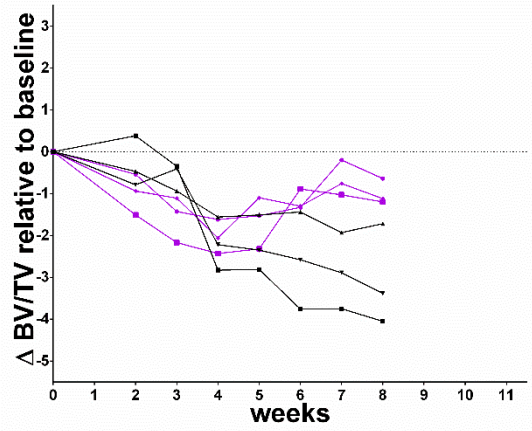

**Ovariectomized mice *(model of accelerated bone resorption)***

# Results – traditional method

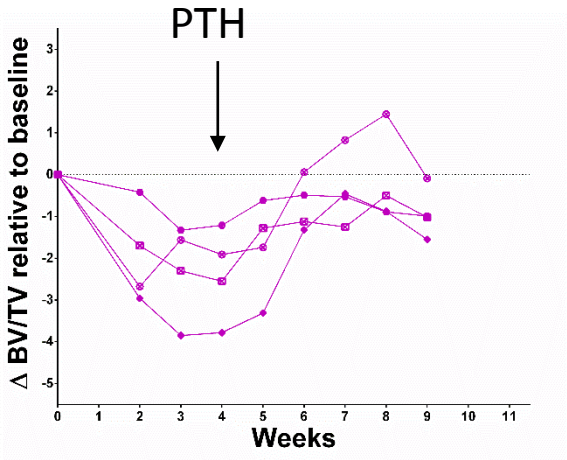
Sham



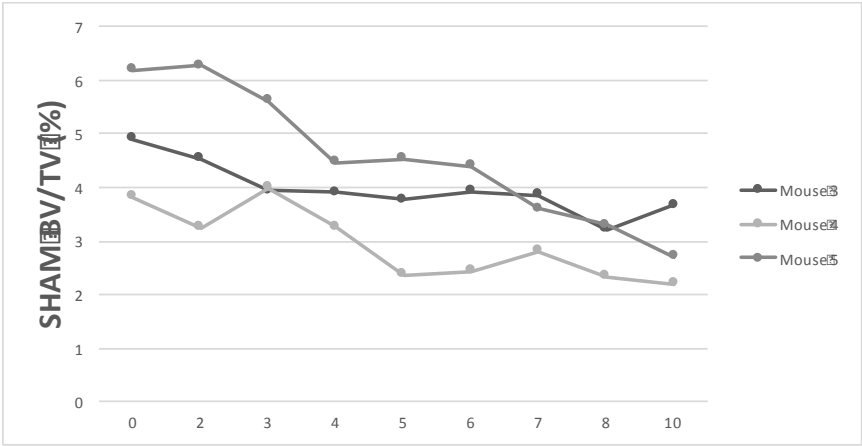

OVX




OVX + PTH

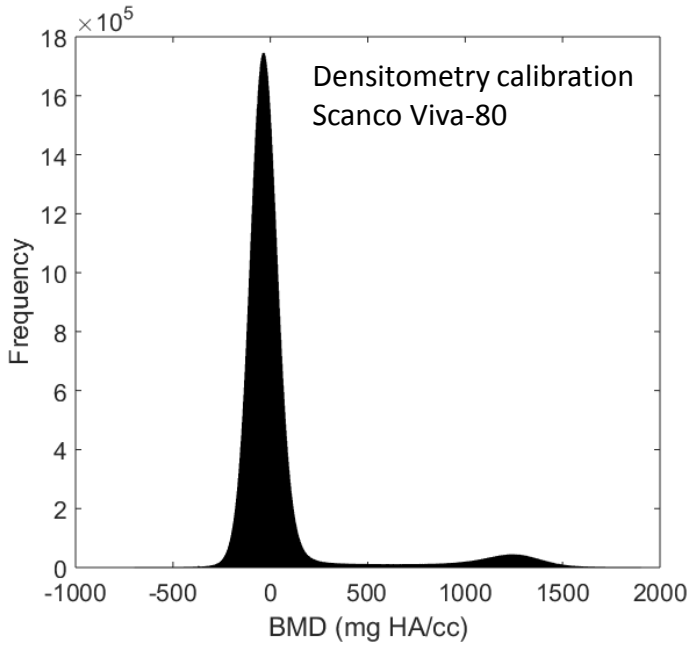


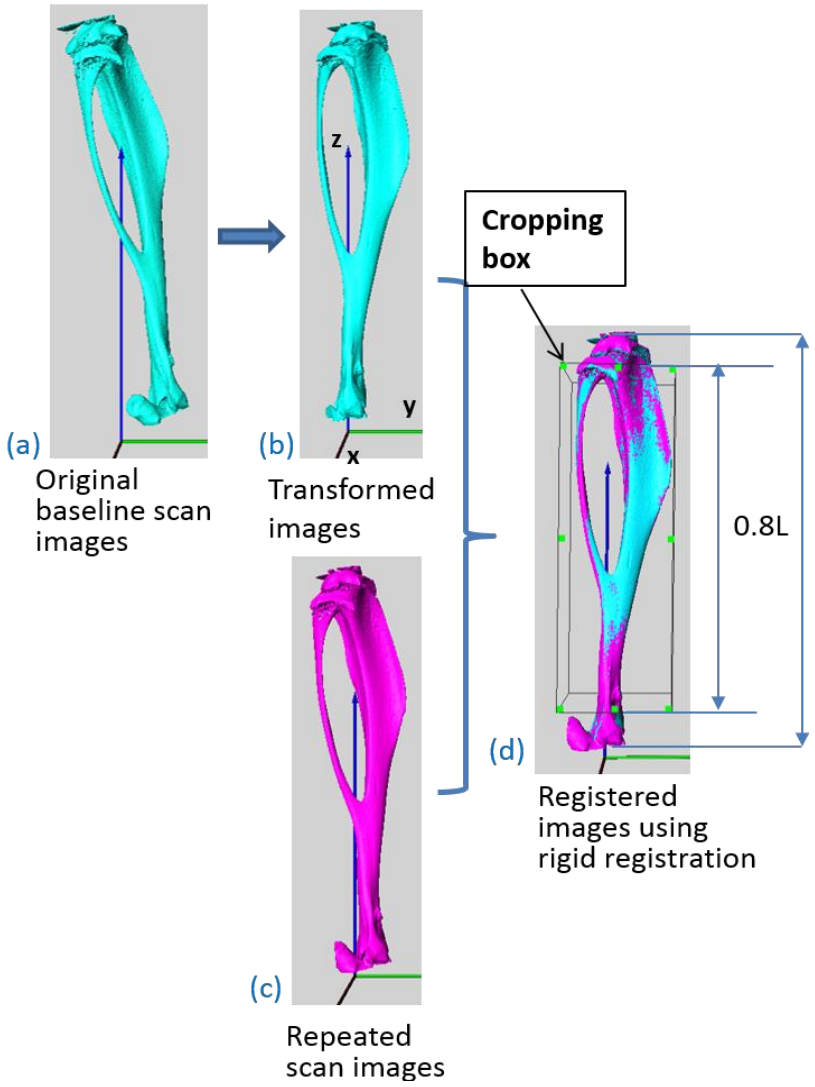

Longitudinal analysis:
34% reduction in the number of animals to discriminate BV/TV



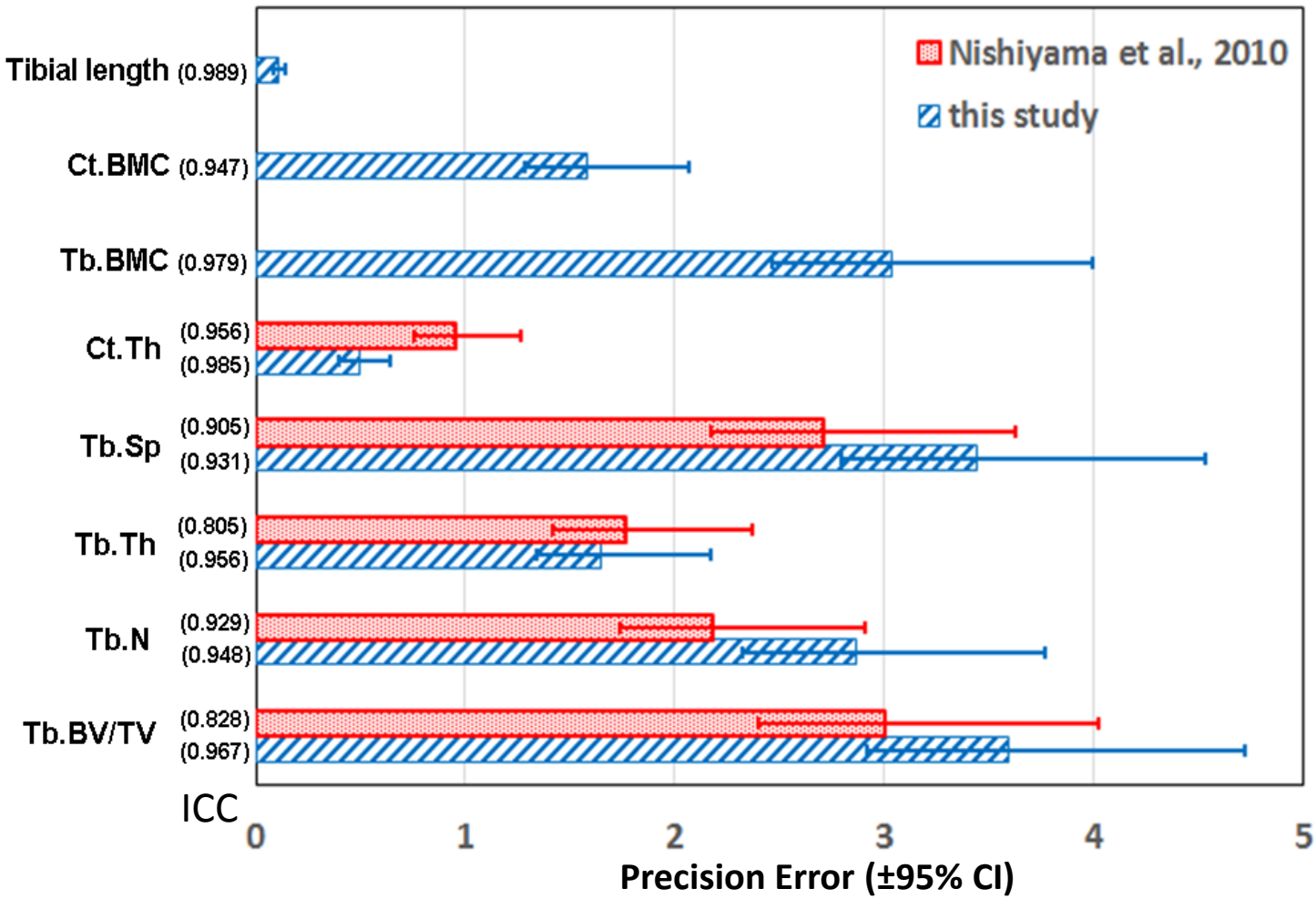
# Method: reproducibility

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



# Results - reproducibility

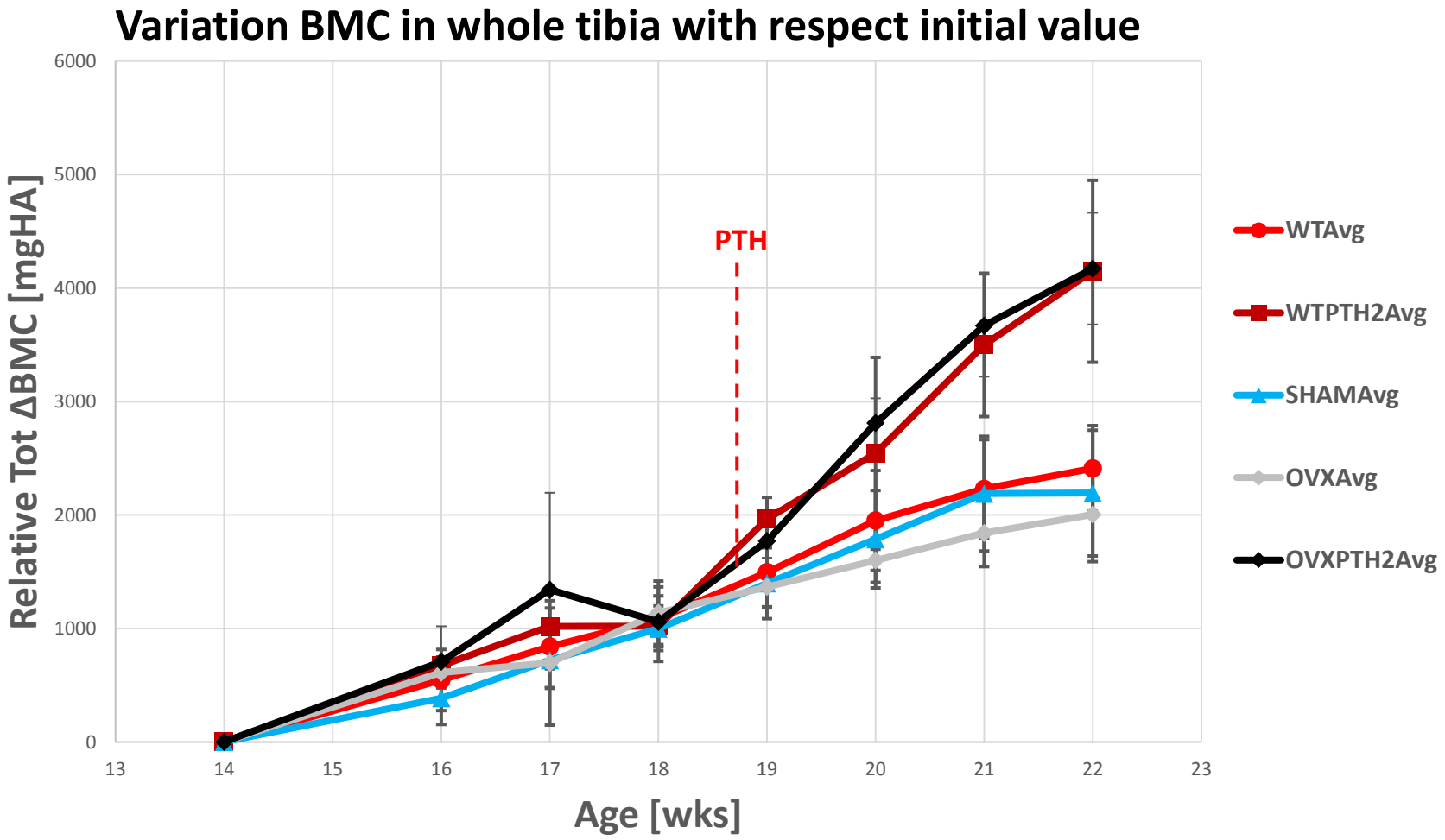



**Lu** Y, *et al.* J Biomech. 2016 Jul 5;49(10):2095-9.

# Results: sample size reduction

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

# Overall changes in BMC

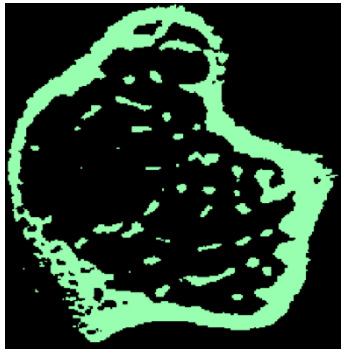


# Accurate quantification of bone adaptation question validity of current animal models

# Tissue adaptation – voxel-based

Rigid Registration plus Boolean operations

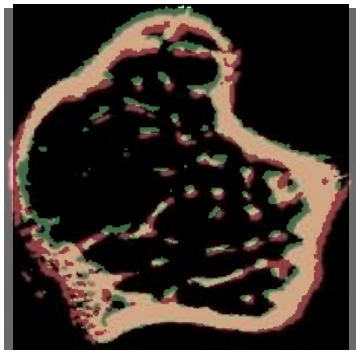
Scan1



ScanN

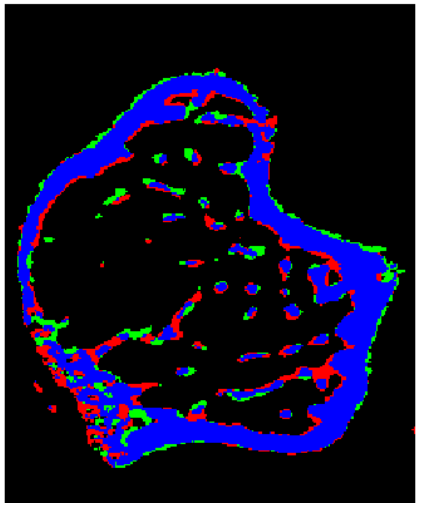
Rigid Registration

Overlapped images



Scan1 and ScanN

Boolean Operations



Only Scan1 = Resorbed bone

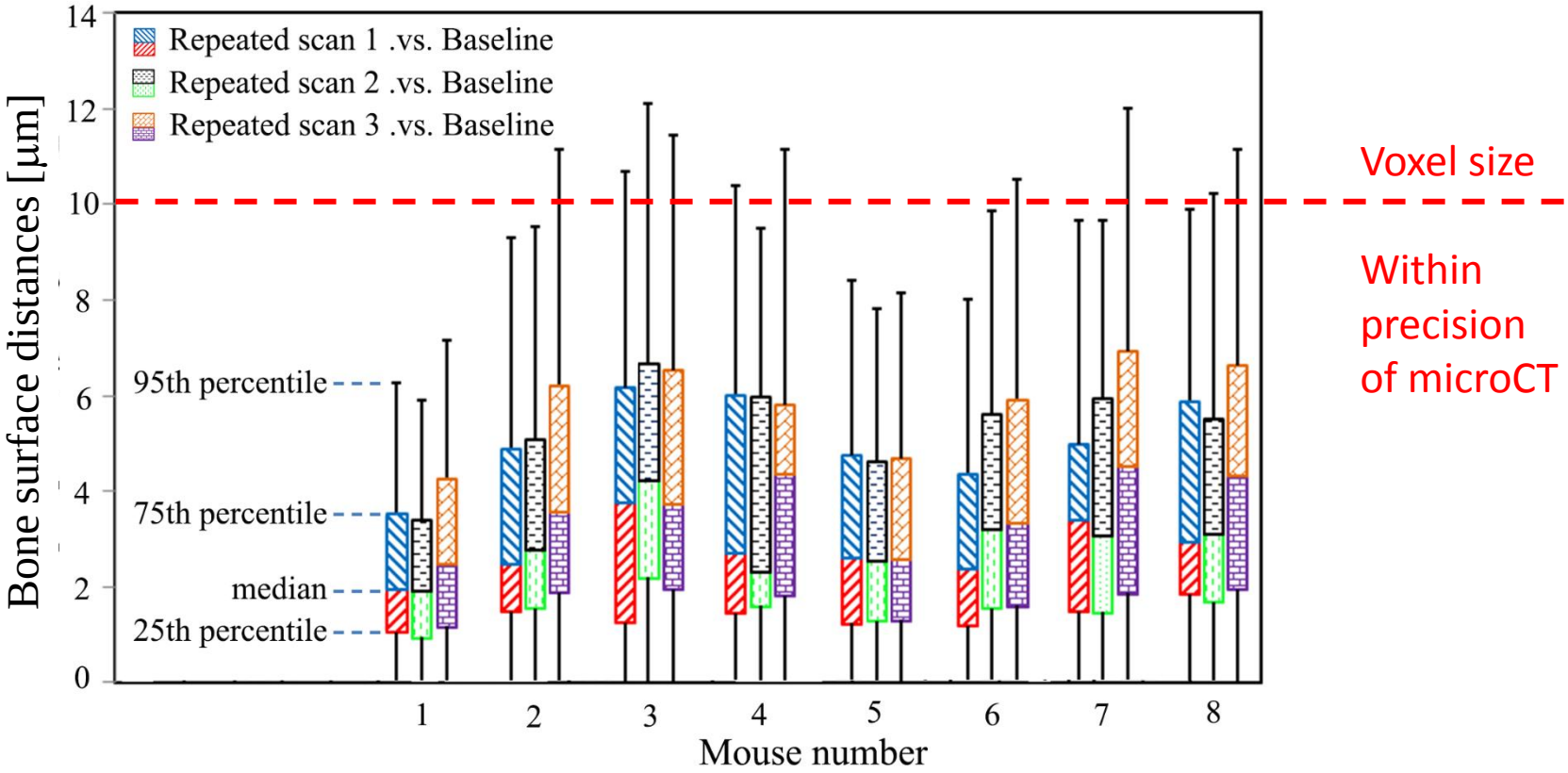
Only ScanN = Formed bone

Both Scans = Constant bone

**Schulte** *et al*, Bone, 2011

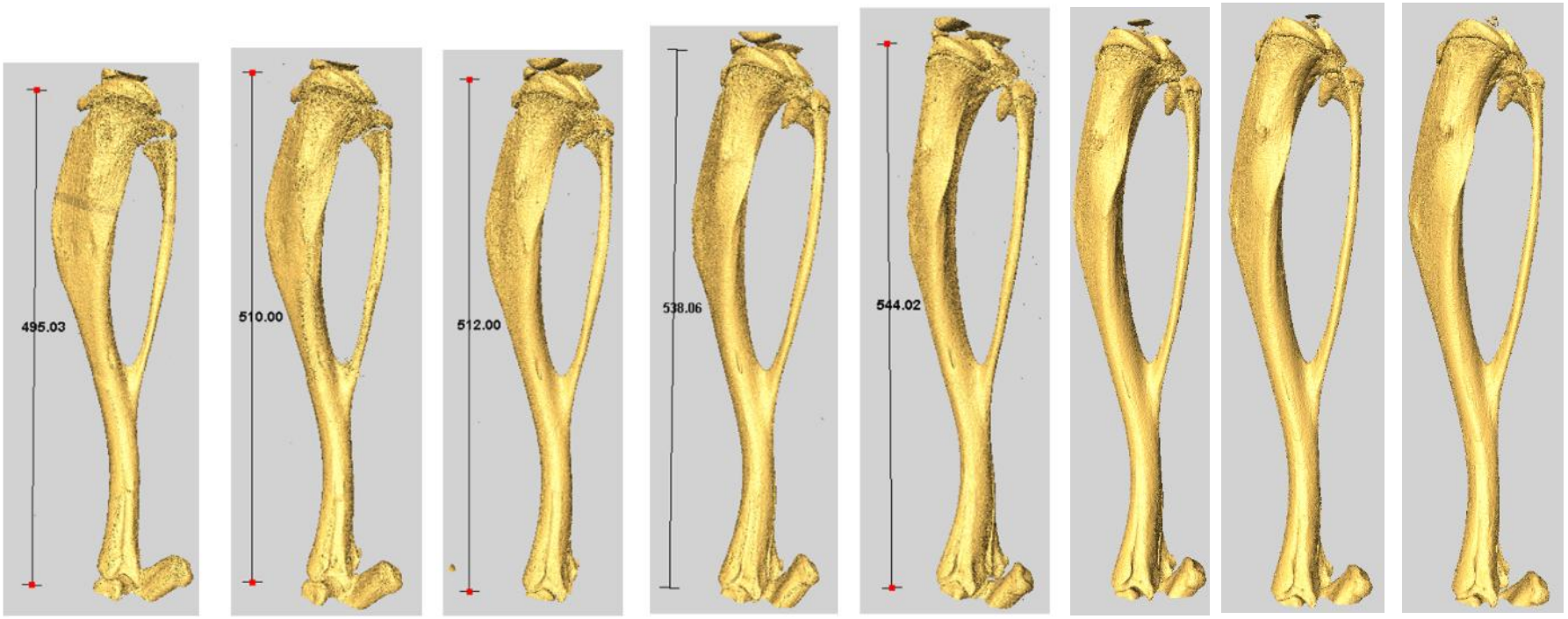
# Voxel-based method

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




**Lu** Y, *et al.* Med Eng Phys. 2015 Nov;37(11):1091-7.

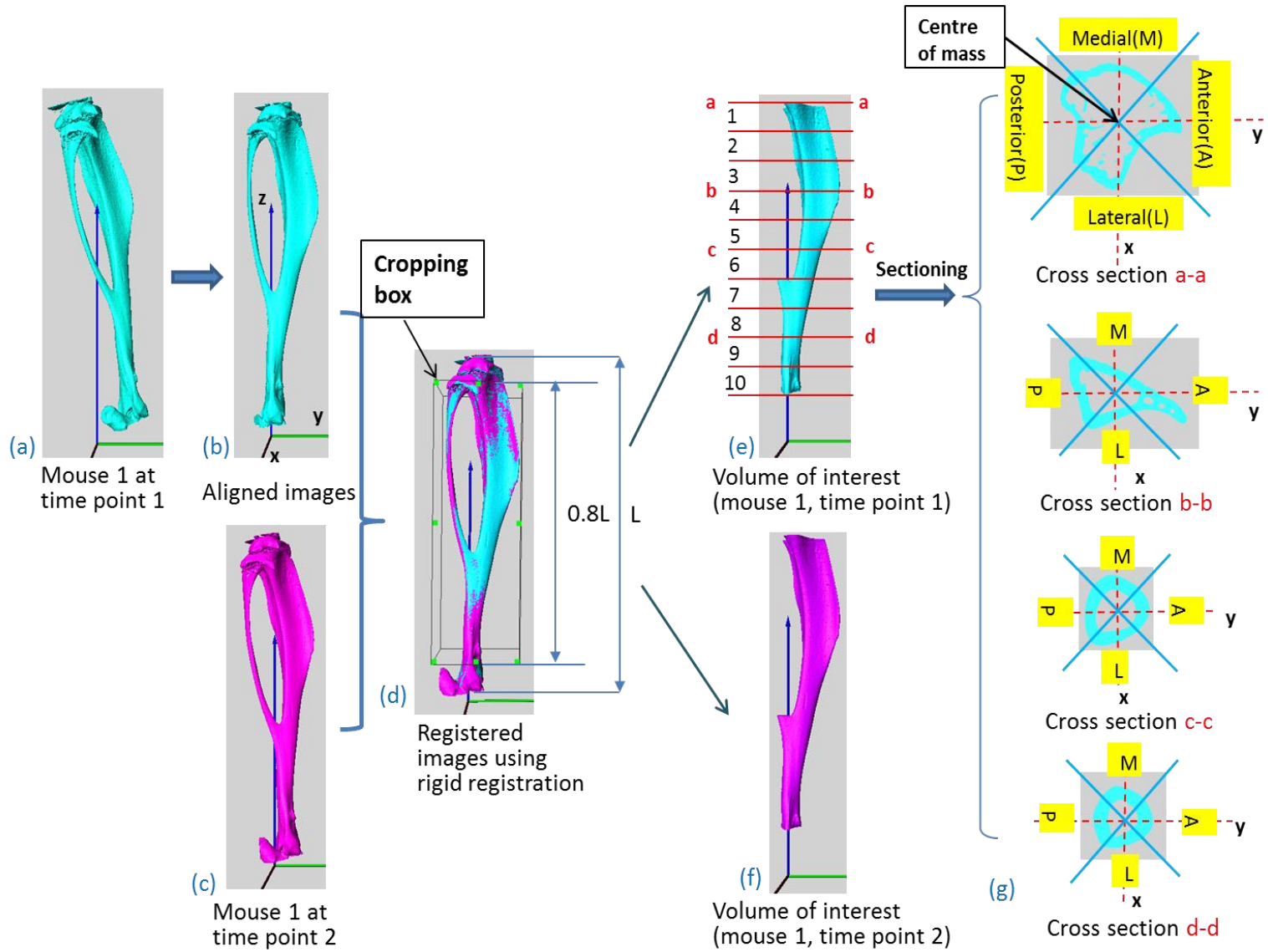
# 14 wks C57BL/6 tibia still growing



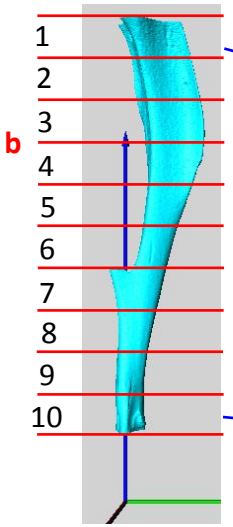

6 7 8 14 15 16 18 20

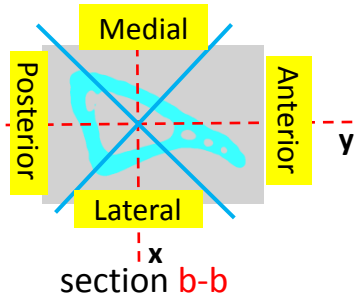
Mouse age [wks]

# Method: longitudinal study



# % Changes BMC: SHAM vs WT groups




-20 -10 -3 0 3 10 20

**Lateral**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | 5.05 | 6.28 | 7.09 | 6.31 | 3.53 | 6.63 | 5.29 |
| C01 | 5.13 | 7.01 | 7.64 | 6.38 | 3.36 | 6.31 | 4.90 |
| C02 | -1.56 | -1.29 | 0.05 | 0.25 | -1.11 | -0.04 | -1.67 |
| C03 | -1.44 | -1.08 | -1.37 | -1.08 | -2.34 | -0.44 | -2.25 |
| C04 | -1.25 | -1.87 | -2.36 | -2.66 | -2.53 | -0.10 | -2.32 |
| C05 | -3.09 | -3.37 | -4.23 | -4.41 | -3.47 | -0.70 | -2.48 |
| C06 | -2.75 | -3.30 | -3.76 | -3.70 | -4.07 | -1.22 | -2.52 |
| C07 | 5.95 | 3.29 | -1.63 | -3.15 | -4.27 | -1.76 | -4.01 |
| C08 | 3.25 | 1.82 | 0.30 | -1.98 | -2.25 | 0.09 | -2.11 |
| C09 | -4.08 | -3.00 | -1.97 | -3.17 | -3.27 | 0.13 | -4.20 |
| C10 | -3.42 | -1.55 | 0.15 | -1.19 | -1.03 | 0.22 | -4.84 |

**Medial**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | -3.35 | 0.44 | 2.10 | 3.84 | 1.59 | 4.16 | 0.29 |
| C01 | -3.62 | 0.87 | 2.65 | 3.96 | 1.37 | 3.79 | 0.22 |
| C02 | -3.90 | -3.95 | -3.93 | -3.61 | -5.03 | -1.83 | -5.14 |
| C03 | -3.48 | -4.22 | -2.96 | -1.40 | -0.50 | 3.11 | -0.41 |
| C04 | -7.05 | -6.33 | -4.51 | -3.11 | -1.83 | -1.20 | -2.35 |
| C05 | -4.07 | -3.61 | -0.84 | -0.49 | 0.07 | 2.38 | 1.54 |
| C06 | -5.18 | -4.44 | -3.03 | -1.16 | -1.66 | 0.16 | -2.07 |
| C07 | -2.48 | -2.91 | -2.68 | -2.36 | -3.44 | -0.89 | -2.00 |
| C08 | -1.46 | 1.31 | 0.92 | 0.09 | -1.90 | 1.21 | 1.70 |
| C09 | -1.44 | -0.33 | -0.77 | -0.66 | -3.14 | -1.68 | -4.27 |
| C10 | -1.36 | -0.53 | -0.18 | -1.03 | -2.70 | -1.44 | -4.18 |

**Anterior**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | -2.02 | -1.88 | 1.16 | 1.37 | 2.48 | 1.37 | -0.37 |
| C01 | -2.68 | -1.72 | 1.28 | 0.62 | 1.87 | 0.74 | -0.76 |
| C02 | -4.41 | -4.06 | -2.70 | -2.82 | -2.46 | -3.13 | -4.96 |
| C03 | -4.70 | -4.27 | -3.25 | -2.47 | -3.59 | -3.07 | -5.93 |
| C04 | -5.05 | -3.71 | -4.27 | -2.38 | -2.95 | -2.50 | -5.47 |
| C05 | -5.27 | -3.17 | -4.12 | -2.95 | -3.47 | -1.46 | -2.40 |
| C06 | -5.63 | -2.52 | -0.72 | -1.40 | -1.99 | -0.69 | -2.03 |
| C07 | -6.71 | -4.38 | -1.59 | -1.79 | -1.14 | 0.36 | -1.68 |
| C08 | -1.68 | 0.84 | 0.80 | 0.00 | -2.02 | -1.48 | -3.12 |
| C09 | -1.94 | -0.64 | -0.37 | -2.89 | -4.83 | -4.74 | -6.10 |
| C10 | -5.32 | -3.33 | -0.59 | -0.78 | -2.11 | -1.78 | -3.97 |

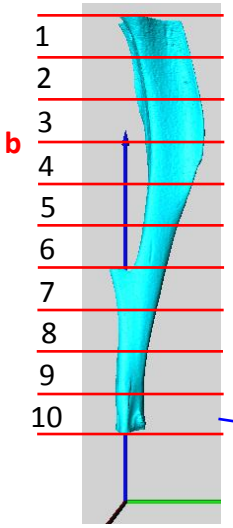
**Posterior**

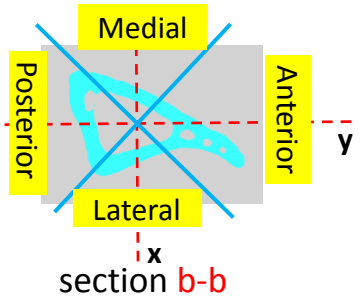
| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | 1.05 | 2.45 | 2.62 | 2.49 | 4.20 | 5.11 | 1.30 |
| C01 | 1.67 | 3.85 | 4.13 | 3.55 | 4.67 | 5.46 | 1.92 |
| C02 | -4.01 | -2.34 | -0.64 | -2.17 | -2.46 | -2.09 | -4.99 |
| C03 | -3.55 | -2.70 | -2.45 | -2.22 | -3.16 | -2.37 | -5.00 |
| C04 | -3.70 | -3.39 | -3.78 | -3.14 | -2.87 | -1.04 | -3.31 |
| C05 | -3.19 | -2.38 | -4.10 | -4.65 | -3.59 | -1.17 | -2.29 |
| C06 | -5.60 | -5.66 | -5.66 | -6.48 | -5.06 | -3.03 | -4.29 |
| C07 | -7.27 | -6.13 | -3.61 | -4.23 | -3.06 | -1.86 | -4.60 |
| C08 | -3.66 | -1.05 | -1.32 | -1.92 | -2.80 | -2.24 | -2.17 |
| C09 | -0.60 | 0.80 | -0.06 | -1.03 | -2.48 | -2.36 | -3.14 |
| C10 | 0.95 | 1.80 | 1.18 | 0.53 | 0.76 | 1.84 | -0.05 |

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





**Lateral**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | -2.70 | -9.96 ** | -7.01 * | -9.53 ** | -9.95 ** | -12.88 ** | -9.32 ** |
| C01 | -2.46 | -10.67 ** | -8.28 * | -10.63 ** | -10.69 ** | -13.52 ** | -9.71 ** |
| C02 | 5.00 | 0.96 | 4.79 | 1.97 | -1.00 | -3.53 | -1.36 |
| C03 | 1.79 | -0.77 | 4.34 | 1.18 | -0.06 | -2.52 | -0.98 |
| C04 | 1.72 | 0.49 | 5.64 | 1.41 | -0.10 | -3.43 | -1.27 |
| C05 | 2.55 | 1.77 | 5.05 | 2.63 | 0.26 | -2.97 | -1.75 |
| C06 | 3.12 | 3.12 | 5.08 | 3.64 | 2.51 | -0.84 | 0.25 |
| C07 | 1.66 | 5.41 | 12.93 ** | 6.09 | 4.03 | 0.84 | 3.74 |
| C08 | 4.02 | 7.13 * | 10.36 ** | 5.83 | 4.49 | 0.70 | 2.69 |
| C09 | 3.82 | 1.83 | 4.72 | 1.64 | 1.37 | -2.69 | 0.40 |
| C10 | 1.89 | -1.62 | 0.31 | -3.56 | -1.81 | -6.70 * | -3.81 |

**Medial**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | 0.04 | -8.68 ** | -9.03 ** | -10.27 ** | -11.13 ** | -11.70 ** | -11.85 ** |
| C01 | 0.42 | -8.76 ** | -9.59 ** | -10.69 ** | -11.25 ** | -11.99 ** | -11.97 ** |
| C02 | 4.42 | 2.27 | 4.15 | 3.45 | 2.01 | 0.89 | 2.73 |
| C03 | 2.78 | -2.03 | -0.69 | 0.29 | -0.07 | -3.22 | 0.85 |
| C04 | 5.63 | 1.49 | 1.03 | 1.83 | 0.75 | -1.44 | -1.66 |
| C05 | 1.98 | 0.10 | -1.43 | -1.14 | -2.31 | -4.46 | -4.90 |
| C06 | 3.01 | -0.50 | 0.61 | -1.89 | -2.48 | -5.51 * | -4.48 |
| C07 | 1.53 | 1.97 | 3.28 | 0.78 | -0.54 | -3.47 | -0.63 |
| C08 | 0.87 | -0.90 | 0.61 | 0.58 | -1.02 | -3.22 | -2.57 |
| C09 | 1.54 | -2.07 | -1.90 | -1.14 | -2.10 | -4.70 | -2.32 |
| C10 | 2.97 | -3.07 | -2.63 | -4.43 | -2.12 | -4.69 | -2.48 |

**Anterior**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | -2.09 | -7.86 ** | -8.49 ** | -11.50 ** | -12.63 ** | -12.90 ** | -11.32 ** |
| C01 | -2.00 | -8.48 ** | -10.04 ** | -12.91 ** | -13.73 ** | -14.37 ** | -12.81 ** |
| C02 | 4.45 | 1.52 | 1.74 | -1.01 | -3.80 | -4.53 | -3.66 |
| C03 | 3.20 | -0.61 | -0.34 | -2.06 | -2.45 | -3.28 | -1.19 |
| C04 | 1.78 | -3.46 | -2.54 | -3.52 | -4.17 | -5.02 | -3.24 |
| C05 | 5.12 | -1.13 | 2.35 | 3.16 | 1.52 | -0.23 | 0.43 |
| C06 | 4.54 | 0.87 | 3.28 | 4.55 | 3.08 | 2.22 | 3.25 |
| C07 | 3.16 | 0.38 | 2.58 | 2.41 | 0.48 | -1.43 | 0.05 |
| C08 | 4.03 | 1.73 | 3.99 | 3.68 | 2.01 | 1.97 | 2.29 |
| C09 | 2.75 | -0.47 | 0.89 | 1.06 | -0.11 | 0.00 | 1.34 |
| C10 | 2.53 | -2.60 | -2.23 | -3.71 | -1.62 | -4.21 | -3.27 |

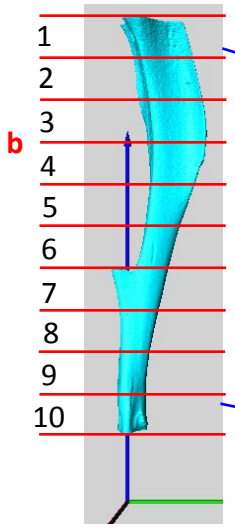
**Posterior**

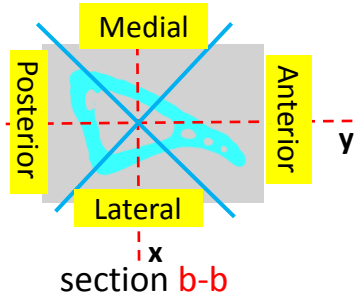
| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | -4.62 | -10.99 ** | -8.49 ** | -11.23 ** | -11.60 ** | -11.64 ** | -10.35 ** |
| C01 | -4.21 | -11.15 ** | -10.30 ** | -12.73 ** | -12.95 ** | -12.98 ** | -11.59 ** |
| C02 | 4.73 | 2.77 | 5.58 | 1.97 | 0.31 | -1.30 | -1.19 |
| C03 | 2.26 | -0.13 | 3.97 | 1.31 | 0.21 | -1.20 | -1.28 |
| C04 | 4.71 | 1.51 | 5.34 | 4.00 | 2.04 | 0.11 | 0.88 |
| C05 | 5.80 | 3.89 | 5.12 | 4.09 | 1.44 | 0.02 | 1.31 |
| C06 | 6.17 | 4.58 | 3.46 | 1.54 | -1.37 | -2.88 | -2.10 |
| C07 | 4.60 | 0.22 | -0.64 | 1.13 | -0.06 | -1.73 | -0.19 |
| C08 | 2.16 | 0.45 | -1.00 | 0.80 | -1.85 | -1.06 | -0.89 |
| C09 | 0.81 | 0.24 | 1.09 | 0.68 | -0.10 | -0.24 | 0.01 |
| C10 | 1.11 | -1.01 | 2.49 | 0.19 | 0.82 | -1.49 | -0.90 |

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




-20 -10 -3 0 3 10 20

**Lateral**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | -0.34 | 0.58 | -5.41 | 5.79 | 5.24 | 11.21** | 13.28** |
| C01 | -0.16 | 1.23 | -5.02 | 5.82 | 4.93 | 10.37** | 12.69** |
| C02 | 0.06 | 0.88 | -2.61 | 3.64 | 4.92 | 10.02** | 11.83** |
| C03 | -0.15 | 3.12 | -0.36 | 4.28 | 6.51 | 12.58** | 12.96** |
| C04 | -0.29 | 2.78 | -1.70 | 2.59 | 5.66 | 12.05** | 12.58* |
| C05 | 0.71 | 1.92 | -1.63 | 2.16 | 5.80 | 13.01** | 14.63** |
| C06 | 0.95 | 1.24 | -3.52 | 2.18 | 4.12 | 10.87* | 12.42** |
| C07 | 0.36 | 3.66 | -1.45 | 2.84 | 1.68 | 8.38 | 9.57** |
| C08 | 0.94 | 3.42 | -1.26 | 3.63 | 4.78 | 9.58* | 11.44** |
| C09 | -5.39 | -2.10 | -8.20 | 0.61 | 2.23 | 9.60* | 11.16** |
| C10 | -4.57 | -0.98 | -8.27 | -2.78 | -1.00 | 3.91 | 5.79 |

**Medial**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | 1.05 | 1.04 | -1.16 | 9.22* | 9.42* | 16.28** | 17.65** |
| C01 | 0.41 | 0.89 | -1.71 | 8.94* | 8.81* | 15.11** | 15.49** |
| C02 | 4.50 | 4.49 | 3.38 | 10.16 | 10.28 | 18.47** | 18.26* |
| C03 | 0.56 | -0.54 | -2.26 | 5.71 | 7.78 | 19.49** | 16.26** |
| C04 | -1.33 | -2.34 | -1.80 | 4.06 | 10.66* | 15.58** | 16.75** |
| C05 | 0.12 | 0.27 | 0.27 | 4.20 | 8.86* | 17.50** | 17.98** |
| C06 | -0.55 | 0.44 | -2.29 | 3.07 | 5.95 | 12.38** | 12.72** |
| C07 | 0.86 | 2.08 | -1.34 | 3.56 | 2.60 | 10.30** | 12.18** |
| C08 | 0.11 | 0.77 | -1.90 | 0.96 | -0.50 | 7.00 | 9.95* |
| C09 | -1.42 | 0.23 | -1.12 | 3.83 | 2.06 | 8.02 | 7.26* |
| C10 | -1.50 | 1.21 | -5.20 | 0.86 | 0.71 | 7.40 | 9.85** |

**Anterior**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | 3.69 | 3.33 | 0.02 | 6.42 | 11.84** | 15.95** | 24.31** |
| C01 | 2.37 | 2.90 | -0.81 | 5.33 | 11.37** | 14.16** | 20.20** |
| C02 | 0.60 | 1.05 | -2.40 | 2.54 | 8.38 | 11.46** | 16.85** |
| C03 | 0.46 | 0.66 | -0.76 | 4.80 | 7.09 | 8.30* | 11.24** |
| C04 | 1.16 | 1.24 | -2.38 | 4.04 | 5.93 | 10.31** | 11.34** |
| C05 | 1.00 | 1.26 | 0.13 | 4.49 | 4.40 | 10.75** | 11.53** |
| C06 | -0.02 | -0.10 | -0.55 | 3.02 | 4.61 | 10.14** | 11.82** |
| C07 | -0.98 | 0.09 | -0.64 | 4.34 | 5.75 | 11.48** | 14.23** |
| C08 | 1.78 | 1.30 | 1.70 | 4.01 | 3.82 | 9.04* | 11.62** |
| C09 | -1.08 | -1.04 | 1.06 | 0.27 | 0.27 | 5.69 | 9.47** |
| C10 | -2.61 | -2.11 | -2.43 | 2.28 | 6.76 | 9.18* | 11.64** |

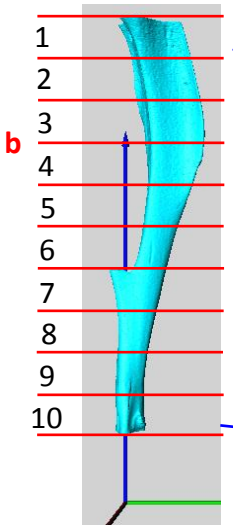
**Posterior**

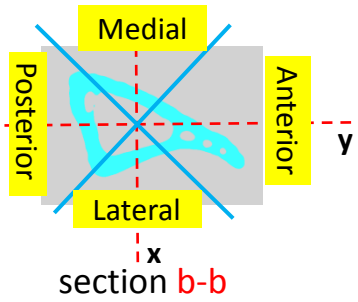
| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | 0.57 | 3.05 | -1.58 | 7.06 | 9.72* | 16.38** | 24.05** |
| C01 | 0.81 | 3.24 | -1.49 | 7.24* | 9.28* | 15.53** | 19.30** |
| C02 | -0.57 | 2.03 | -0.95 | 4.74 | 9.61** | 11.79** | 15.32** |
| C03 | -0.13 | 2.08 | -1.46 | 6.00 | 6.71 | 11.61* | 13.46** |
| C04 | -0.88 | -0.22 | -3.61 | 2.54 | 4.35 | 9.44* | 10.98** |
| C05 | 1.57 | 2.93 | -2.91 | 3.73 | 4.74 | 10.51** | 12.38** |
| C06 | 0.78 | 1.92 | -2.66 | 4.50 | 4.72 | 9.70* | 11.22** |
| C07 | -2.89 | -1.77 | -5.00 | 3.36 | 5.28 | 10.26** | 11.98** |
| C08 | 2.04 | 1.56 | 1.11 | 4.05 | 1.33 | 8.06* | 10.07** |
| C09 | 3.16 | 4.01 | 1.75 | 5.85 | 1.30 | 9.13** | 10.11** |
| C10 | -0.29 | 2.50 | -1.40 | 6.76 | 2.71 | 13.69** | 14.09** |

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

**(* p<0.05, ** p<0.01)**

# % Changes BMC: OVXPTH vs WTPTH groups




-20 -10 -3 0 3 10 20

**Lateral**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | 0.42 | -1.30 | -1.35 | -5.44 | -2.83 | -3.54 | -3.82 |
| C01 | 0.05 | -2.67 | -2.82 | -6.84 | -4.08 | -4.31 | -5.02 |
| C02 | 2.98 | 3.81 | 1.31 | -1.81 | -1.26 | -4.13 | -4.76 |
| C03 | 1.16 | 0.45 | -1.40 | -3.78 | -4.10 | -7.21 * | -7.05 * |
| C04 | 2.19 | 0.35 | -0.96 | -3.15 | -2.69 | -6.33 | -6.84 * |
| C05 | -0.72 | -0.06 | -1.97 | -3.64 | -2.13 | -5.45 | -5.81 |
| C06 | -1.11 | 0.59 | -1.52 | -4.74 | -1.56 | -3.48 | -2.79 |
| C07 | 6.84 * | 3.93 | 0.07 | -1.56 | 4.27 | 1.76 | 3.31 |
| C08 | 1.15 | 2.36 | 3.38 | -1.24 | 2.83 | 1.47 | 1.30 |
| C09 | -0.39 | 3.40 | 8.91 * | -1.64 | 1.84 | -0.24 | -1.13 |
| C10 | 0.32 | 2.41 | 9.35 * | -0.69 | 3.80 | -0.14 | 0.14 |

**Medial**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | -3.93 | -2.27 | -6.47 | -10.96 ** | -5.50 | -4.20 | -6.76 |
| C01 | -3.53 | -2.08 | -5.93 | -10.95 ** | -5.54 | -3.83 | -5.69 |
| C02 | -0.81 | -1.33 | -6.32 | -8.70 | -3.79 | -4.73 | -5.55 |
| C03 | -1.61 | -0.82 | -5.34 | -5.64 | -3.07 | -9.56 ** | -7.97 * |
| C04 | -1.86 | 1.46 | -2.57 | -2.84 | -3.48 | -6.87 | -6.73 |
| C05 | -4.53 | -3.33 | -6.29 | -5.10 | -4.52 | -8.83 ** | -8.29 * |
| C06 | -2.17 | -1.13 | -1.73 | -3.69 | -2.62 | -4.79 | -4.85 |
| C07 | -1.09 | -2.93 | -5.28 | -6.77 | -0.80 | -3.30 | -3.36 |
| C08 | -1.15 | -0.92 | -5.52 | -4.83 | 1.07 | -1.43 | -1.09 |
| C09 | -1.62 | -3.02 | -4.86 | -6.06 | -1.98 | -4.32 | -3.07 |
| C10 | 2.27 | 1.66 | 4.40 | -2.79 | 3.30 | -0.75 | 0.48 |

**Anterior**

| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | -9.38 * | -11.19 ** | -10.56 ** | -14.33 ** | -11.12 ** | -11.33 ** | -14.65 ** |
| C01 | -8.77 * | -11.36 ** | -10.73 ** | -15.06 ** | -12.24 ** | -11.60 ** | -13.43 ** |
| C02 | -2.77 | -1.95 | -0.71 | -4.74 | -4.13 | -5.86 | -7.22 |
| C03 | -1.82 | -0.60 | -4.89 | -8.39 | -5.64 | -2.29 | -4.05 |
| C04 | -4.47 | -2.71 | -6.27 | -8.53 | -4.22 | -3.28 | -5.43 |
| C05 | -3.64 | -0.66 | -4.40 | -5.17 | -1.16 | -2.57 | -3.83 |
| C06 | -2.26 | 1.42 | 3.07 | 1.06 | 2.95 | 0.82 | -0.09 |
| C07 | -3.59 | -1.60 | 2.50 | -1.75 | 2.67 | 1.43 | 0.19 |
| C08 | -2.95 | 0.13 | -1.14 | -2.65 | 1.83 | 0.59 | -1.09 |
| C09 | -1.58 | -1.29 | -1.82 | -2.74 | 1.55 | 0.41 | -0.40 |
| C10 | -1.08 | 0.78 | 1.68 | -4.02 | -1.98 | -2.23 | -2.78 |

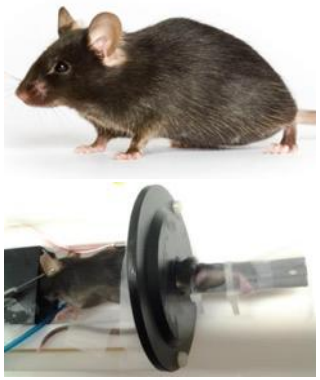
**Posterior**

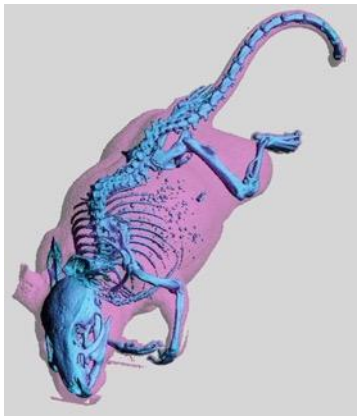
| Region \ Age [week] | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|
| C01Ct | -4.96 | -7.98 * | -8.10 * | -11.05 ** | -3.18 | -4.83 | -8.41 * |
| C01 | -5.43 | -8.69 * | -8.81 * | -11.53 ** | -3.79 | -5.34 | -6.24 * |
| C02 | -0.58 | 0.73 | 1.67 | -4.43 | -3.42 | -2.92 | -4.77 |
| C03 | -1.25 | -0.38 | 0.53 | -5.87 | -1.47 | -2.78 | -5.18 * |
| C04 | -0.45 | 1.47 | -0.58 | -4.05 | 1.18 | 0.86 | -1.22 |
| C05 | -0.36 | -0.08 | -1.56 | -6.05 | 0.21 | 0.46 | -0.61 |
| C06 | -1.80 | -1.99 | -3.52 | -8.57 | -3.30 | -3.98 | -4.71 |
| C07 | -2.85 | -0.05 | 4.88 | -4.12 | 0.33 | -0.22 | -1.07 |
| C08 | -3.58 | -0.64 | -3.17 | -3.83 | 2.09 | -1.55 | -1.87 |
| C09 | -2.29 | 0.99 | 0.15 | -2.18 | 4.09 | -0.88 | -0.65 |
| C10 | 5.63 | 4.28 | 4.50 | -3.62 | 5.23 | -2.12 | -1.98 |

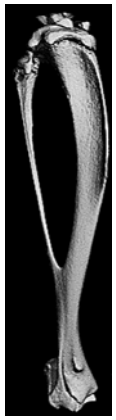
Mean relative percent difference [%] between OvxPth (N=7) and WTPth (N=5) groups

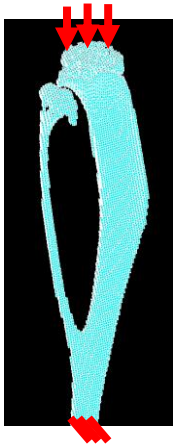
**(* p<0.05, ** p<0.01)**

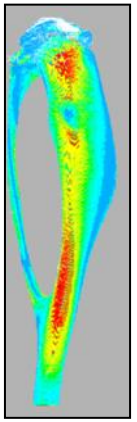
# VPM predictors are discriminating

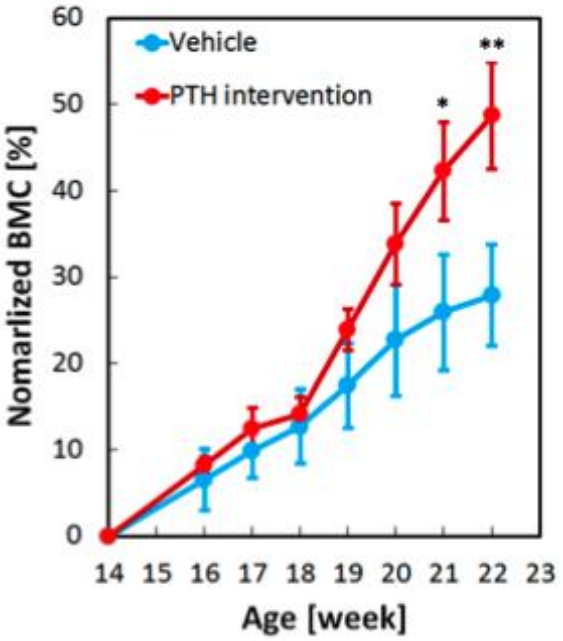


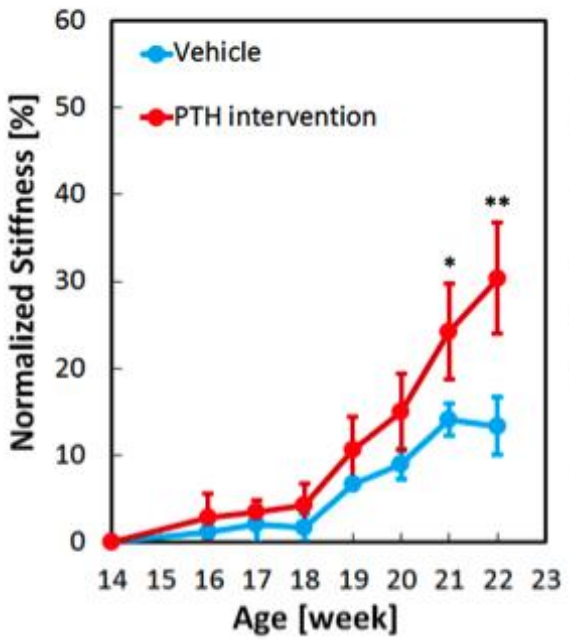


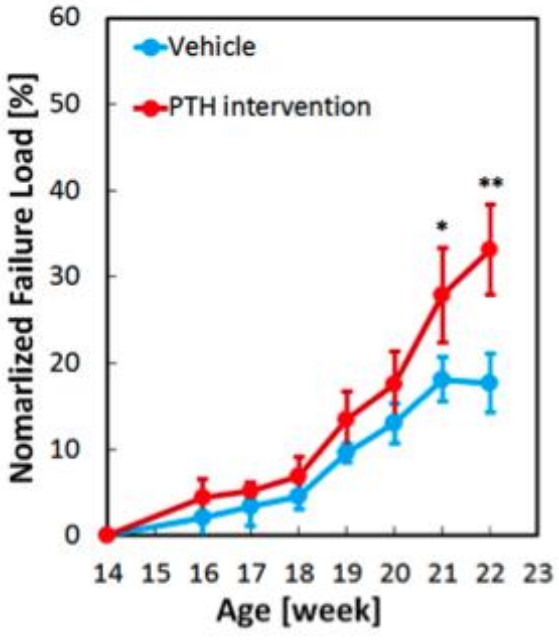






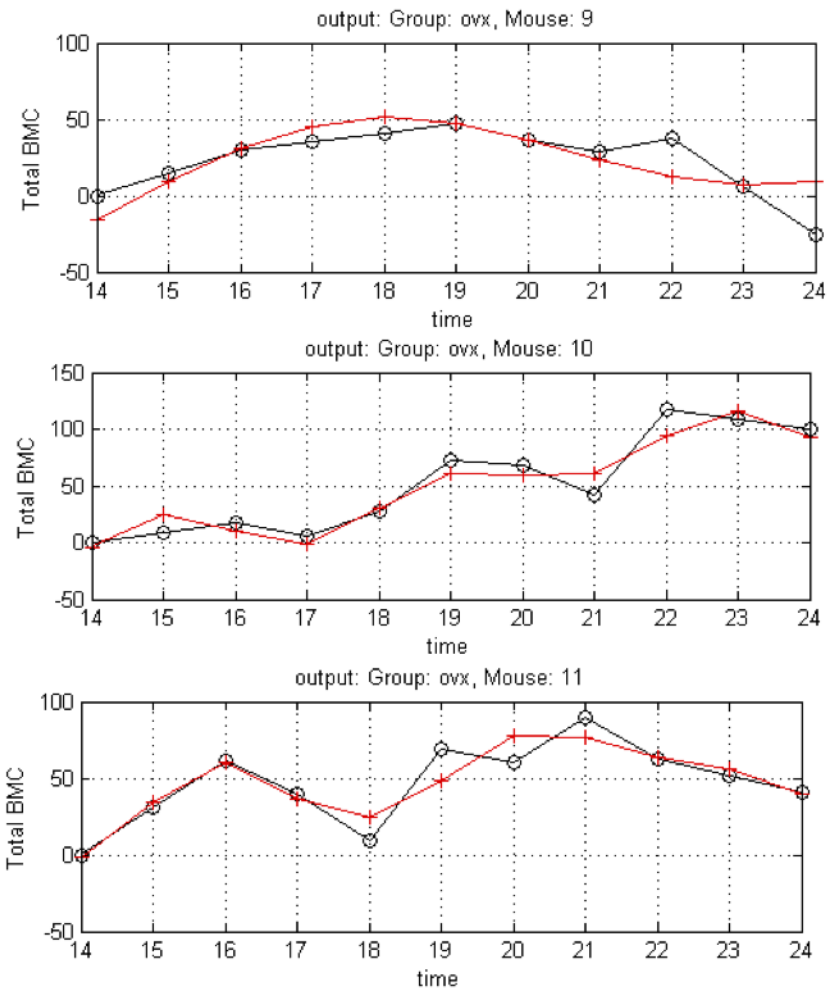


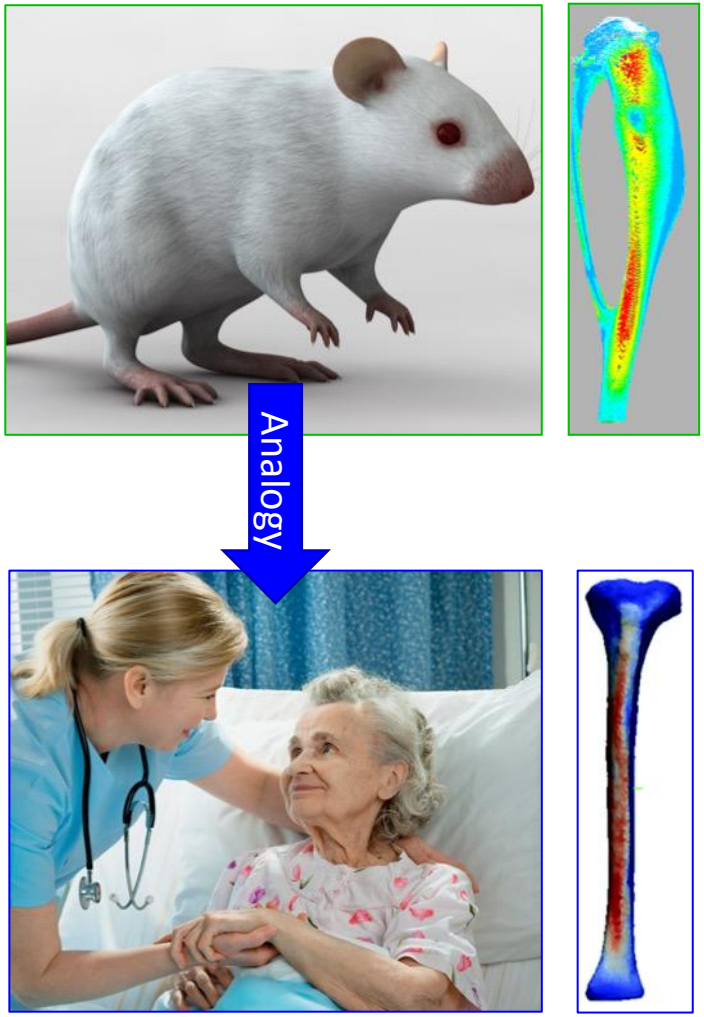



# The future

## VPM predictors




## VPM Scaling



# 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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