Understanding the Senolytic and Senomorphic Effects of Zoledronate in an Ageing Mouse Model

Eleanor Platt[1], Lucy Frost[1], James Edwards[2], Jinsen Lu[2], Graeme Wilkinson[1] & Gayle Marshall[1]

[1] Medicines Discovery Catapult, Block 35, Alderley Park, Cheshire, SK10 4ZF, UK.
[2] Nuffield Dept. of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Headington, Oxford, OX3 7LD

md.catapult.org.uk

1. Background

Bisphosphonates (BPs) have been used to treat patients with osteoporosis and osteopenia for over a quarter of a century. BP treatment has been shown to:

- Reduce skeletal re-modelling rate
- Strengthen bones
- Reduce fracture rates

There are two classes of BPs; non-nitrogenous such as Clodronate and nitrogenous such as Zoledronate.

Bisphosphonates work by inhibiting farnesyl pyrophosphate synthase, leading to osteoclast apoptosis.

Longitudinal studies have shown non-skeletal effects of BP treatment.

- 28% reduction in death[1]
- 67% reduction in cardiovascular mortality[2]
- 45% reduction in myocardial infarction[2]
- 39% reduction in colon cancer deaths[3]
- 59% reduction in mortality in ICU patients[4]

The aims of this study were to investigate changes in gene expression throughout the body triggered by Zoledronate treatment and to elucidate the mechanisms underlying this wide range of non-skeletal effects.

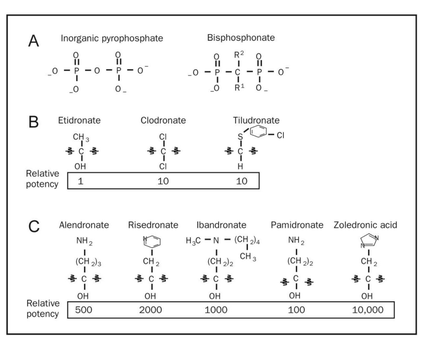


Figure 1: Bisphosphonate structures and relative potencies for osteoclast inhibition.[5]

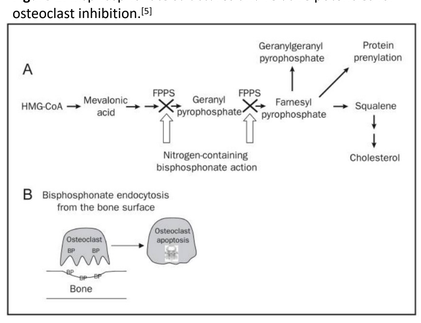


Figure 2: A. Nitrogen-containing BPs selectively inhibit farnesyl pyrophosphate synthase (FPPS) within osteoclasts. B. Osteoclast endocytosis of BPs from the bone surface leads to FPPS inhibition and osteoclast apoptosis.[5]

2. Mouse Model

Female C57BL/6N mice were grown to twenty-two months old before treatment with either:

- Zoledronate-treated (n=6)
- Vehicle-treated (n=6)

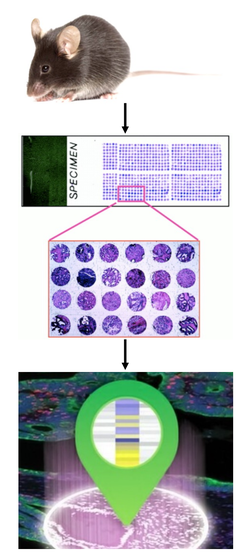
2-month-old, untreated mice were used as a control (n=6)

Zoledronate dosage administered to the mice was equivalent to that given to osteoporosis patients, adjusted for body weight of the mice.

Twelve tissues were harvested from each of the mice.

The tissues were formalin-fixed, paraffin embedded and tissue microarrays (TMAs) were generated using cores taken from 3-5 organs of each mouse.

Spatial transcriptomic analysis was performed on the Nanostring GeoMx Digital Spatial Profiler to detect changes in gene expression throughout the body in ageing and following Zoledronate treatment.



Age	Treatment	Number of mice	Tissues
2 month	Untreated	6	Brain, fat, quadricep, gastrocnemius muscle, heart, lung, kidney, spleen, liver, pancreas, small intestine and skin
24 month	Vehicle-treated	6	
24 month	Zoledronate	6	

3. Tissue Preparation and Regions of Interest (ROI) Selection

2 month Untreated (n=6) | 24 month Vehicle-treated (n=6) | 24 month Zoledronate-treated (n=6)

↓

12 tissues per mouse harvested and frozen at -80°C

↓

Tissues thawed and formalin fixed, paraffin embedded (Total = 216 tissues)

↓

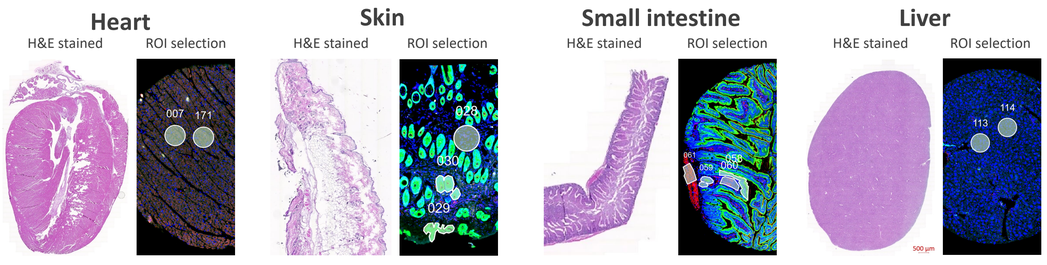
TMAs prepared and tested using DSP (Total = 504 ROIs)

↓

Whole transcriptome analysis = ~20,000 genes / ROI (~10 million data points)

Organ	Region within Organ			
Heart	Heart muscle			
Skin	Epithelial layer	Stroma	Hair follicle	
Small Intestine	Differentiated epithelial	Smooth muscle	Crypts	Stroma
Liver	Central vein region	Periportal region		
Fat	Fat			
Pancreas	Ducts	Acinar	Islets	
Kidney	Collecting duct & proximal tubules	Distal tubules & loop of Henle	Glomerulus	
Lungs	Bronchi	Alveoli	Stroma	
Spleen	Peri-arteriolar lymphoid sheet	Mantle zone	Red pulp	
Quadricep muscle	Muscle fibres			
Gastrocnemius muscle	Muscle fibres			
Brain	Pre-frontal cortex	Hippocampus	Cerebral cortex	

A small pilot study was performed using tissue frozen at -80°C to ensure the RNA within the tissue was of sufficient quality for whole transcriptome analysis.



4. Digital Spatial Profiler

Whole transcriptome analysis was performed on each TMA using the Nanostring GeoMx Digital Spatial Profiler (DSP).

Two serial sections were taken from each TMA and mounted.

- H&E stained
- Syto13 (nuclear), PanCK (epithelial) and CD45 (immune) stained

The DSP uses target complementary sequences tagged with a photocleavable sequencing barcodes to identify each protein-coding gene. Sequencing barcodes are photocleaved and collected from each ROI, before the libraries are prepared, pooled and sequenced.

Single cell deconvolution was performed by comparing previously published gene sets enriched within different cell types to the whole transcriptome data collected from each ROI.

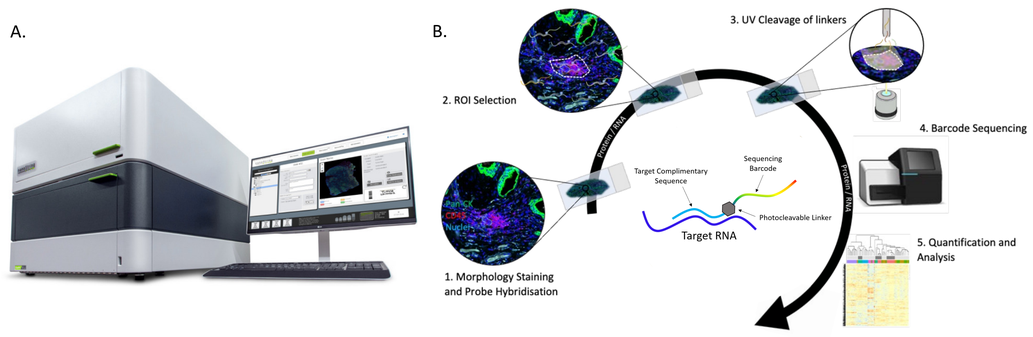
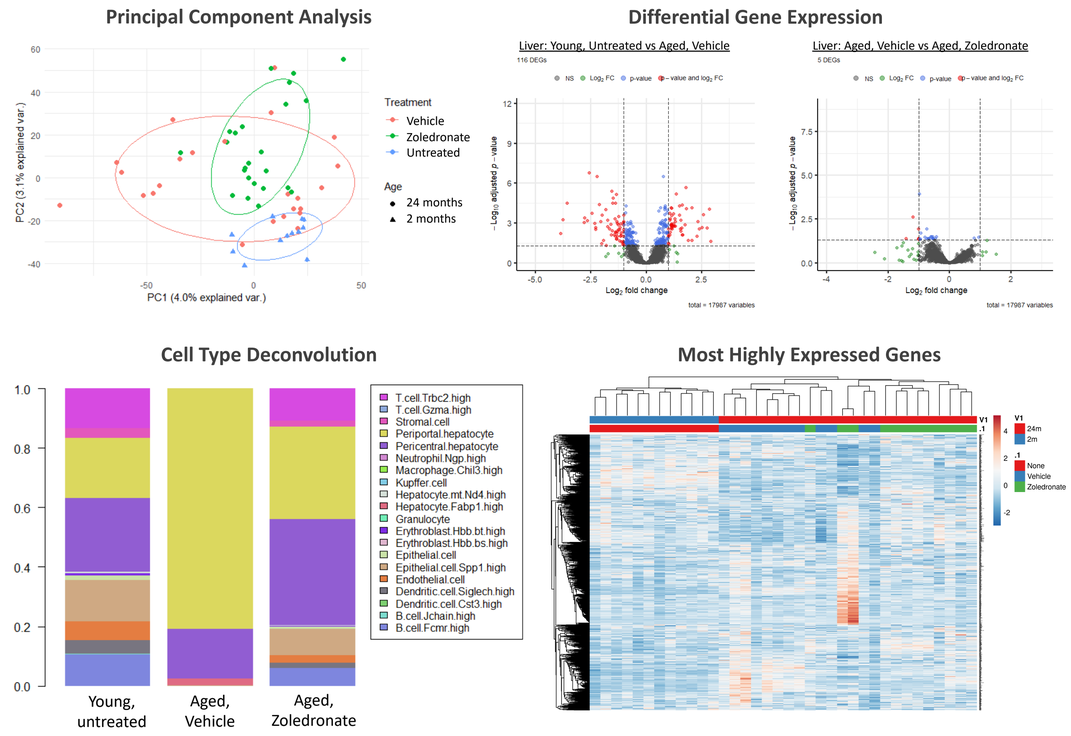


Figure 3: A. Nanostring GeoMx Digital Spatial Profiler (DSP). B. Workflow summary of testing FFPE tissue on the Nanostring GeoMx DSP.

5. Preliminary Results - Liver



6. Next Steps

Following completion of whole transcriptome analysis on all tissues, bioinformatic analysis will be performed, including:

- Differential gene expression
- Single cell deconvolution
- Gene set variation analysis
- Pathway analysis

The outputs from this study will be used to determine the effect of Zoledronate treatment across different tissues and better understand the ageing mouse model for this and future therapeutic studies.

Ultimately it is hoped that understanding the mechanisms underlying the wide-ranging effects of bisphosphonate treatment will allow us to identify potential therapeutics for age-related diseases.

References

1. Lyles KW, Colón-Emeric CS, Magaziner JS, Adachi JD, Pieper CF, Mautalen C, Hyldstrup L, Recknor C, Nordsletten L, Moore KA, Lavecchia C, Zhang J, Mesenbrink P, Hodgson PK, Abrams K, Orloff JJ, Horowitz Z, Eriksen EF, Boonen S; HORIZON Recurrent Fracture Trial. Zoledronic acid and clinical fractures and mortality after hip fracture. N Engl J Med. 2007 Nov 1;357(18):1799-809. doi: 10.1056/NEJMoa074941. Epub 2007 Sep 17. PMID: 17878149; PMCID: PMC2324066.
2. Sing CW, Wong AY, Kiel DP, Cheung EY, Lam JK, Cheung TT, Chan EW, Kung AW, Wong IC, Cheung CL. Association of Alendronate and Risk of Cardiovascular Events in Patients With Hip Fracture. J Bone Miner Res. 2018 Aug;33(8):1422-1434. doi: 10.1002/jbmr.3448. Epub 2018 May 9. PMID: 29744914.
3. Pazianas M, Abrahamsen B, Eiken PA, Eastell R, Russell RG. Reduced colon cancer incidence and mortality in postmenopausal women treated with an oral bisphosphonate--Danish National Register Based Cohort Study. Osteoporos Int. 2012 Nov;23(11):2693-701. doi: 10.1007/s00198-012-1902-4. Epub 2012 Mar 6. PMID: 22392160.
4. Lee P, Ng C, Slattery A, Nair P, Eisman JA, Center JR. Preadmission Bisphosphonate and Mortality in Critically Ill Patients. J Clin Endocrinol Metab. 2016 May;101(5):1945-53. doi: 10.1210/jc.2015-3467. Epub 2016 Jan 18. PMID: 26780569.
5. Drake MT, Clarke BL, Khosla S. Bisphosphonates: mechanism of action and role in clinical practice. Mayo Clin Proc. 2008 Sep;83(9):1032-45. doi: 10.4065/83.9.1032. PMID: 18775204; PMCID: PMC2667901.
6. NanoString: Expanding the power of spatial biology

UKRI Innovate UK