

The Effect of Clodronate on Gene Expression in the Horse

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Background

- Currently two FDA-approved bisphosphonate (BP) drugs (Osphos® and Tildren®) for use in horses > 4 y of age to treat navicular syndrome
- Growing concern over BP use in young exercising horses due to their analgesic and adverse effects
- Hypothesized that BPs prevent proper healing of microcracks in bone, which may lead to catastrophic injury in racehorses
- BPs have a very short detection window in blood, thus the reliability of current drug tests is questionable

Hypothesis

- There are one or more differentially expressed genes detectable in the peripheral blood of horses following the administration of clodronate
- These differentially expressed genes will be involved in bone growth, bone remodeling, or analgesia
- This gene list will provide subsequent biomarkers for drug testing

Aim

Using transcriptomics, determine differentially expressed genes following the administration of the bisphosphonate, clodronate (Osphos®), to healthy exercising Thoroughbred horses

Methods

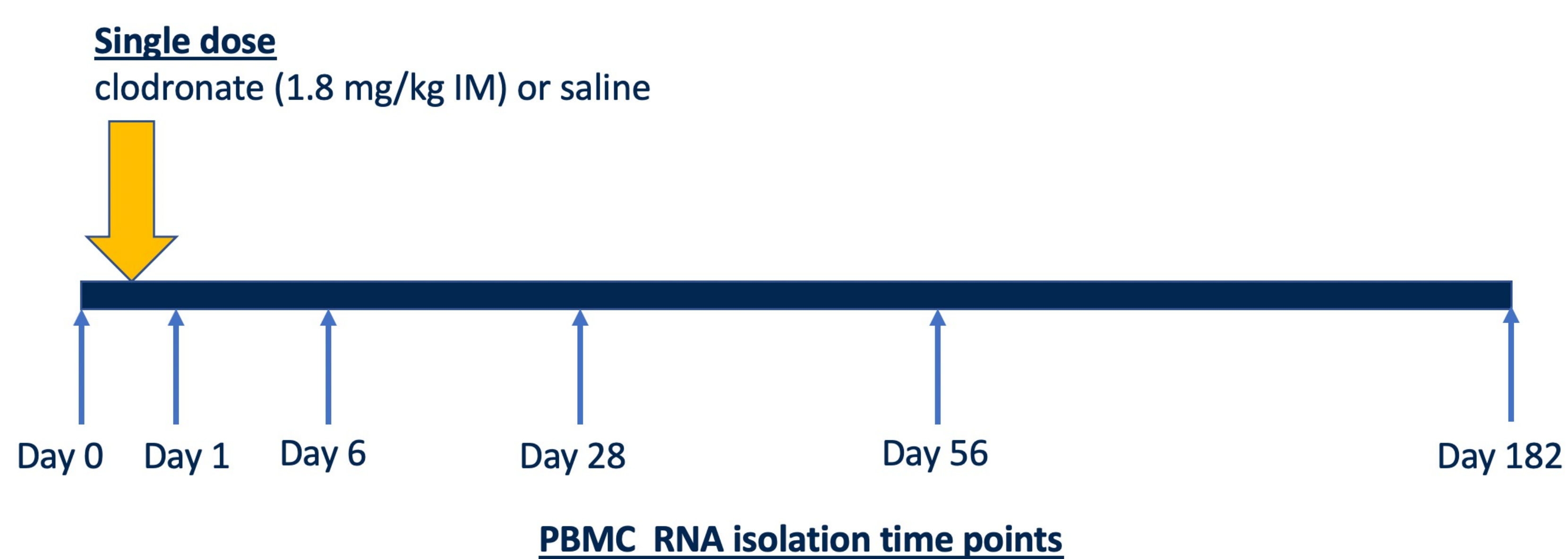


Figure 1: Experimental design for sample collection. Eleven healthy exercising Thoroughbred horses were used in this study. Seven were administered clodronate and four were administered saline as controls. RNA was isolated from peripheral blood mononuclear cells (PBMCs) immediately before the single dose and then on days 1, 6, 28, 56, and 182 post-dose. RNA was then sequenced.

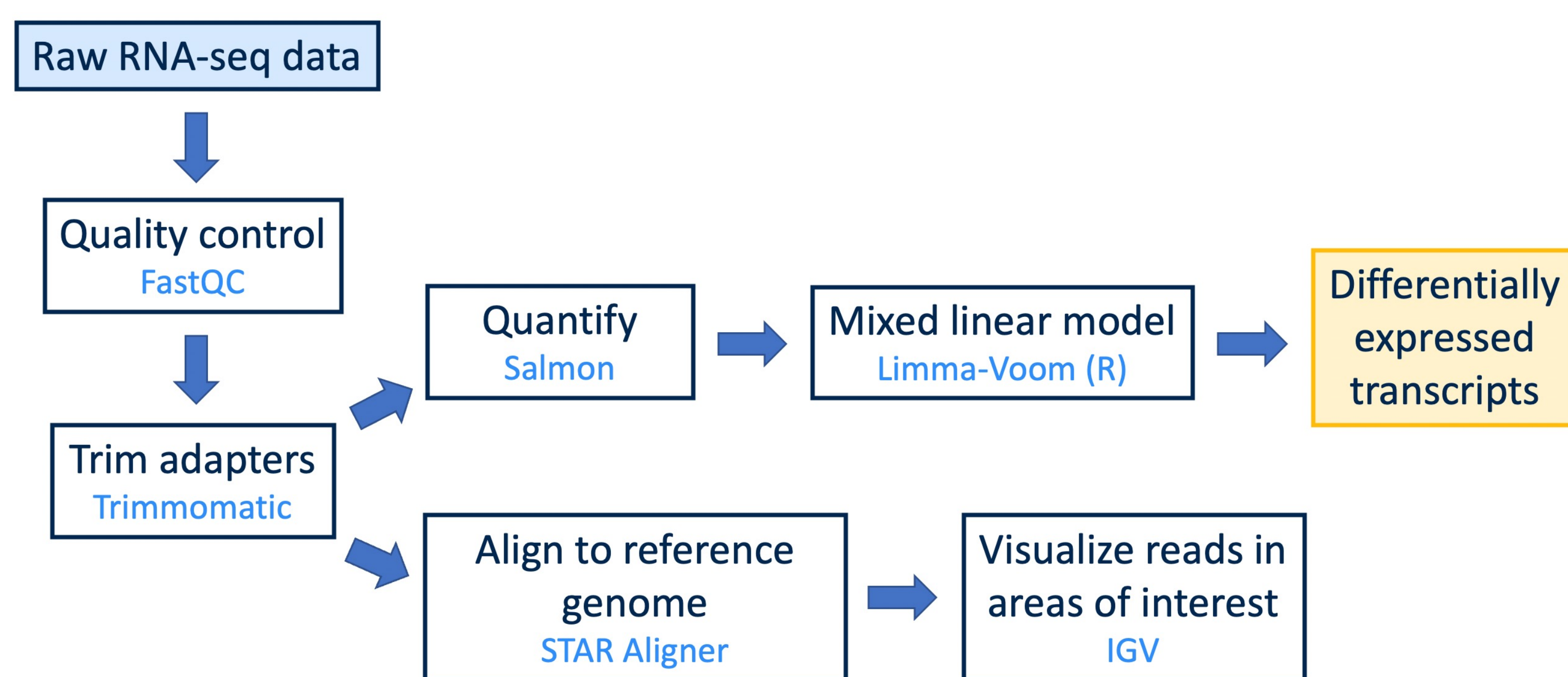


Figure 2: Graphical representation of RNA-seq analysis pipeline. The algorithm used is represented in light blue. In the mixed linear model, fixed effects include sex, treatment, and time, and interactions of these variables and random effect was horse. For each annotated gene, the mixed linear model gives \log_2FC , average expression, P value, and Benjamini-Hochberg adjusted P value.

Results

RNA-seq Analysis: No transcripts were differentially expressed with a Benjamini-Hochberg FDR adjusted $P < 0.05$

Pathway Analysis:

Pathway	Dysregulation	Raw P Value	FDR P Value
p38 MAPK	↑	0.0005	0.04
Ras	↑	0.0003	0.04
Cadherin	↓	0.0003	0.02

Discussion

Limitations

- Clodronate does not act directly on PBMCs, thus it may not cause differential gene expression that is detectable with a single-gene method
- Unbalanced sample design, with almost twice as many individuals in the treatment group compared to the control group
- A single, minimum dose for effect on navicular syndrome was used. A higher dose or multiple doses may affect gene expression differently.

Pathways

- Clodronate is detectable in the blood for approximately 24 hours, which follows with the acute dysregulation of cellular pathways seen only on day 1 post-dose
- p38 MAPK and Ras pathways are important for osteoclast differentiation
- Osteoclast apoptosis resulting from clodronate use may trigger an upregulation of osteoclast differentiation pathways to compensate for loss

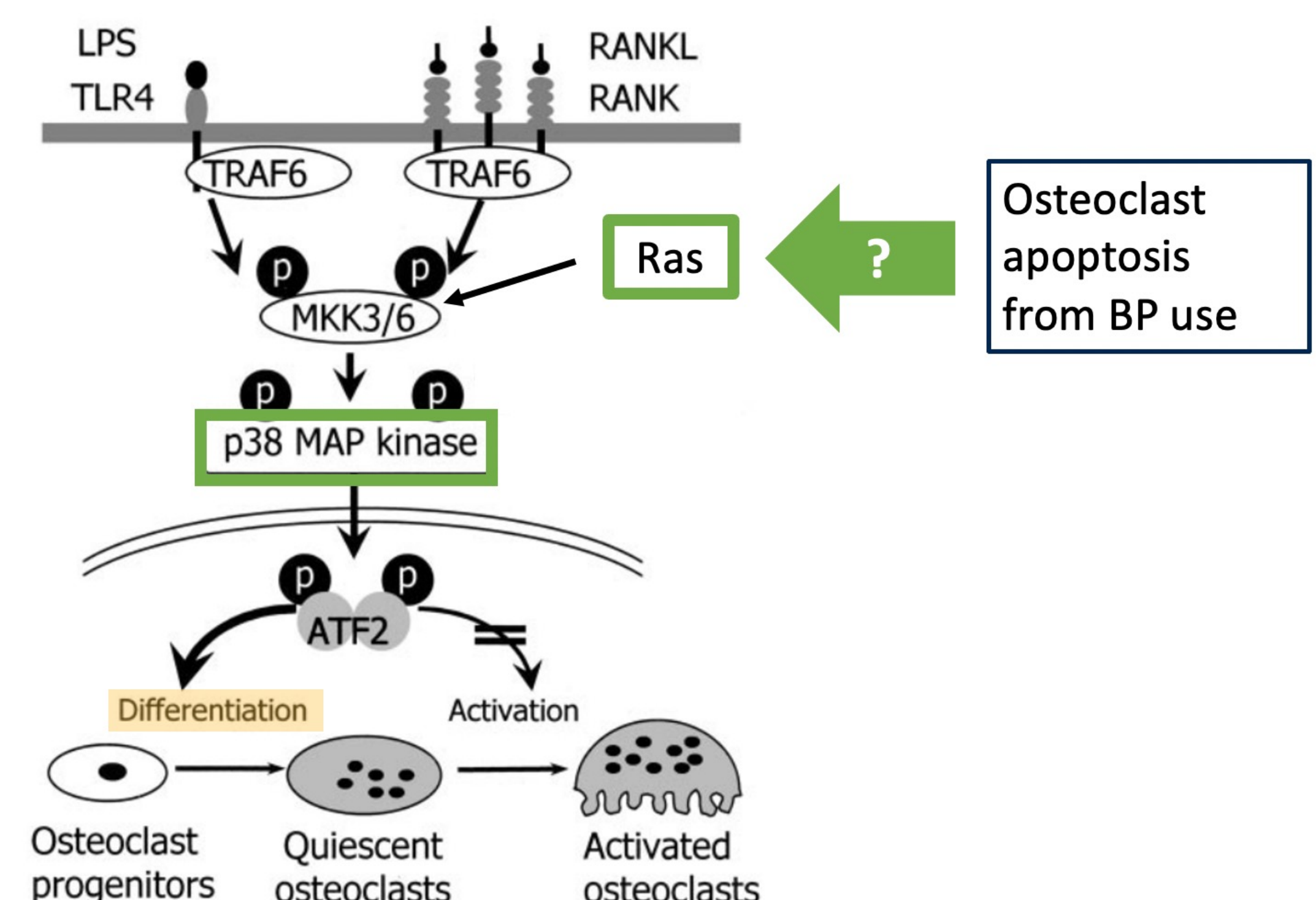


Figure 3: Graphical representation of Ras and p38 MAPK roles in osteoclast differentiation. Adapted from Li et al., *Endocrinology*, 2002.

Conclusion

- No detectable differentially expressed transcripts in peripheral blood of horses following administration of clodronate (Osphos®)
- With acute clodronate use, p38 MAPK and Ras pathways are upregulated and cadherin pathway is downregulated on day 1 post-dose
- Transcriptomics using peripheral blood may not be a reliable way to test for clodronate use over an extended period of time

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