Characterization of a Novel EP_4-Selective Agonist for Dental Implant Osseointegration

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KEY FINDING

KMN-159, a novel EP_4 agonist, enhances expression of osteogenic genes *in vitro* and increases bone formation in an *in vivo* rat osseointegration model.

INTRODUCTION

- Cayman Drug Discovery designed, synthetized, and characterized more than 300 novel prostaglandin E_2 receptor 4 (EP_4) agonists.
- Agonists were screened for selectivity, potency, and drug-like properties.
- KMN-159 was selected as a candidate for further testing *in vivo*:
  - High selectivity towards the human EP4 receptor.
  - High potency in primary cell-based osteogenesis assays.
  - Favorable *in vitro* DMPK and safety profiles.
- Can KMN-159 enhance the osseointegration of dental implants?

Currently, there are no small molecules drugs available to increase dental osseointegration, and rhBMP2 is the only therapeutic used clinically. To test KMN-159 *in vitro*, a novel 3D model of osseointegration was developed to characterize the differentiation of bone marrow mesenchymal stem cells towards osteoblasts by studying markers of bone formation. KMN-159 was also tested *in vivo* in a rat pre-clinical model using maxillary molar extraction to study dental implant osseointegration.

Methods

Cell Culture and *in vitro* Osseointegration

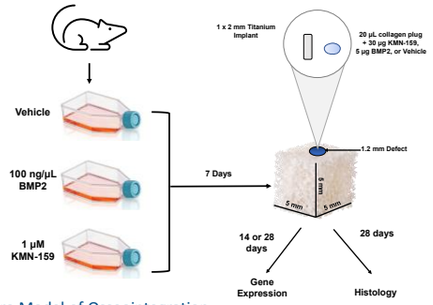

Figure 1- 3D *in vitro* Model of Osseointegration
Rat bone marrow cells were harvested from long bones and plated in culture flasks with KMN-159 (1 µM), rhBMP2 (100 ng/mL), or vehicle for 7d, after which they were seeded onto 5x5x5 mm deproteinized bovine bone blocks. A defect was made and a collagen plug and implant inserted. Blocks were then cultured to endpoints.

In vivo Maxillary Molar Extraction Model

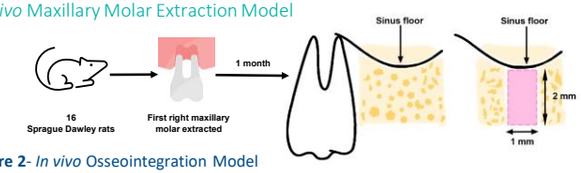

Figure 2- *In vivo* Osseointegration Model
Right maxillary molars were extracted from 16 Sprague-Dawley rats. After 1 month, a titanium dental implant (1x2 mm cylinder) was inserted into the sinus floor along with KMN-159 (1.2, 6, or 30 µg) or BMP2 (5 µg), and the implants were allowed to osseointegrate for up to 4 weeks.

In vitro Differentiation and Osseointegration

Model Validation

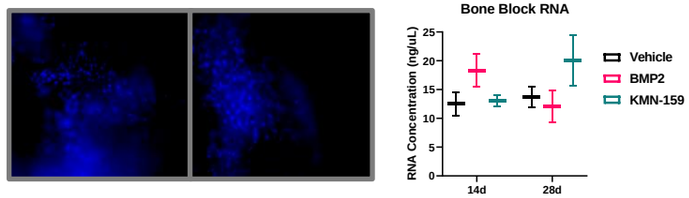

Figure 3- Validation of bone block system using rat bone marrow cells
Bone cells attach to bone block and survive for up to 28d in culture. Representative images of slices of bone blocks stained for nuclei show cells within the trabeculae of the bone block. These bone blocks yielded mRNA suitable for downstream analysis following homogenization and RNA isolation.

KMN-159 Increases Activity and Expression of Osteogenic Genes *in vitro*

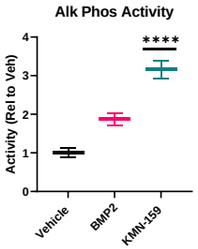

Figure 4- Alkaline phosphatase activity
Activity of alkaline phosphatase at 14d of culture was significantly elevated compared to vehicle in bone marrow cells treated with 1 µM KMN-159.
****= p<0.0001 compared to vehicle

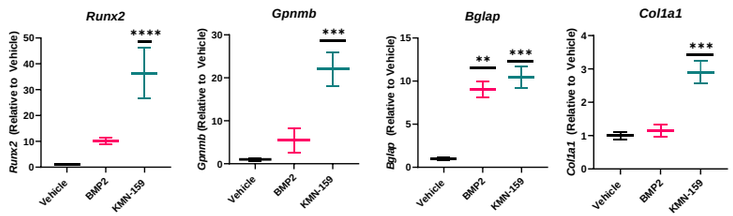

Figure 5- Expression of osteoblast differentiation factors and bone remodeling genes
KMN-159 increases the relative expression levels of osteoblast differentiation factors *Runx2* and Osteoactivin/*Gpnmb* at 14d in the 3D osseointegration *in vitro* system. After 28d in culture, KMN-159 led to elevated expression of osteocalcin/*Bglap* and type 1 collagen A1/*Col1a1*.
**=p<0.01, ***=p<0.001 compared to vehicle

In vivo Efficacy and Safety

KMN-159 Increases Bone Volume Fraction at 4-weeks Post-Implantation

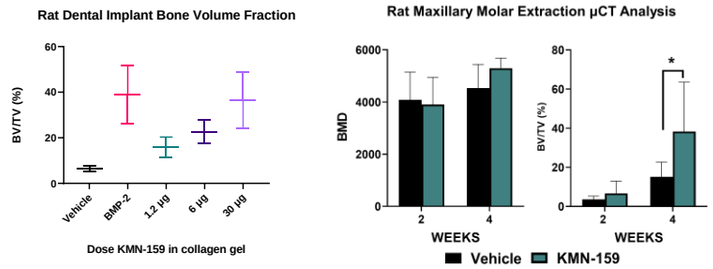

Figure 6- µCT analysis of rat implantation
At 21d post-implantation, KMN-159 displayed a dose-response relationship with respect to bone volume fraction. At 28d, there was significantly higher bone volume fraction compared to vehicle when treated with 30 µg of KMN-159 in a collagen plug. No safety events were observed during the 28d of observation.
*= p<0.05 compared to vehicle

CONCLUSIONS

- Cayman Drug Discovery has synthesized and tested a library of EP_4-selective agonists, leading to the selection of KMN-159 as a lead compound.
- To test KMN-159 *in vitro*, a novel osseointegration model was developed and demonstrated elevated gene expression and activity of osteogenic genes.
- KMN-159 increased bone volume fraction in a rat *in vivo* model of dental implant osseointegration and no safety or toxicity issues were observed after 28d.
- Future studies to demonstrate efficacy in larger animal models are ongoing.

REFERENCES

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