**Introduction**

Prostaglandin E1 (PGE) is known to cause bone formation with its activation of the EP receptor being primarily responsible for its anabolic actions (Pagkalos et al., J. Mol. Pharmacol., 2012). Current bone anabolic therapies for local application are typically protein-based and have relatively short shelf lives. In addition to being expensive, safety concerns are raised by the morphogenic activity associated with some of these therapies, limiting their potential use in orthopedic and other local applications. We have developed a series of novel difluorocarbam EP receptor agonists in order to alleviate these issues in a potential therapeutic agent (Barrett et al., J. Med. Chem., 2019). The work presented here is part of the ongoing characterization of our lead compound, KMN-159, which shows high selectivity and excellent potency for EP agonism and activation, potent stimulation of osteoblastic differentiation, and minimal activity on vasodilatation and on other EP receptor subtypes.

**Methods and Results**

### KMN-159 Has a Favorable Genotoxicity Profile and a Short in vivo Half-Life Following i.v. Dosing in Rats

KMN-159 was dissolved and loaded onto the indicated matrices as described above. Each matrix was incubated with 7.1 x 10⁶ primary rat bone marrow cells in 100 ml MEMα + 10% FCS + 100U/ml penicillin in a 37°C/5% CO₂ incubator. At the indicated times, 4.5 ml was removed and plated in a 24-well dish (0.5 ml/well; n = 8 wells). On day 4, cells were fed with plating medium supplemented with 3 x 10⁻⁸ M dexamethasone (0.25 ml/well). On day 9, cultures were lysed, and ALP activity measured.

For all matrices tested, a sufficient amount of KMN-159 was released by 15 minutes to achieve a concentration greater than that necessary for the maximal stimulation of ALP activity in the primary rat bone marrow culture system (1 μM). The similar activity profiles achieved with all three matrices was also an indication of the stability of KMN-159 loaded on a matrix (10-10 weeks for MCM and CPC).

### KMN-159 Released from Matrices Induces Osteogenic Differentiation in Rat Bone Marrow Cells

KMN-159 was dissolved and loaded onto a matrix and incubated with 7.1 x 10⁶ primary rat bone marrow cells in 100 ml MEMα + 10% FCS + 100U/ml penicillin in a 37°C/5% CO₂ incubator. At the indicated times, 4.5 ml was removed and plated in a 24-well dish (0.5 ml/well; n = 8 wells). On day 4, cells were fed with plating medium supplemented with 3 x 10⁻⁸ M dexamethasone (0.25 ml/well) and an extra 50 μg/ml ascorbic acid. On day 9, cultures were lysed, and ALP activity measured.

### Helistat Sponges Support ALP (+) and Mineralizing Colonies

Helistat sponges were loaded with either vehicle or KMN-159 (1 mg) as described above and dried overnight. Primary rat bone marrow cells (1 x 10⁷ cells in 0.25 ml MEMα + 10% FCS) were then added to each sponge, which was then placed into a T25 flask standing on end with 8 ml media. Cells were fed on day 4 as above and then fed every 3 days with 3 x 10⁻⁸ M dexamethasone. On the indicated days, sponges were fixed in 2% paraformaldehyde and stained for either ALP activity or von Kossa-stained for mineralization.

By day 9, ALP-positive colonies were seen in the sponges, by day 12, ALP activity spread throughout the sponges, and by day 20, mineral deposition was observed. These were not quantitative assays, it appears that sponges loaded with KMN-159 had increased ALP activity and mineralization.

**Conclusions**

- KMN-159 has favorable drug-like properties and has passed initial toxicity testing, and has a short in vivo half-life.
- KMN-159 is rapidly released from collagen and MCM matrices, and the released drug induces rat BMC osteoblastic differentiation.
- Helistat collagen sponges support rat BMC osteoblastic differentiation with KMN-159 appearing to increase differentiation in the sponges.
- KMN-159 does not induce ectopic bone formation in the rat muscle pouch model.
- The combination of KMN-159 plus matrix gives a cost-effective and chemically stable alternative to protein-based therapeutics.

**Acknowledgments**

We would like to thank the research group of Dr. Stefan Zwingenberger (University Medicine Carl Gustav Carus, TU Dresden, Dresden, Germany) for providing the MCM and CPC scaffolds loaded with KMN-159 as well as Ting Zhao in the Bioanalytical Chemistry group at Cayman Chemical for measuring the levels of KMN-159 in the cell culture medium samples. This work was supported by Cayman Chemical internal funds and NIAIM grant award R44AR076882.