# Injectable SM-102 Lipid Nanoparticles encapsulating β-catenin mRNA to promote bone formation in fractures

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**INTRODUCTION:** The most frequent fracture amongst long bones is tibial shaft fractures, and despite surgical interventions upwards of 15-48% of patients with these type of fractures fail to heal.[1-4] Additionally, the risk of developing impaired fracture healing (non-union or delayed union) was found to be higher in patients with open tibial fractures and especially fractures caused by high-energy traumas, often requiring longer work leaves and increased hospital stays.[1-3] Canonical Wnt signaling has been well studied in its implications for promoting bone repair and thus the activation of canonical Wnt has frequently been explored as a therapeutic approach in fracture healing. However, delivering Wnt ligands has been hindered due to their poor solubility and alternatively inhibitors of the Wnt pathway have been targeted as a therapeutic approach to stimulate canonical Wnt.[5] Delivery of mRNA is an attractive strategy recently popularized by the novel coronavirus vaccine that delivers genetic material without genomic integration. We hypothesize that delivery of mRNA encoding β-catenin stimulates canonical Wnt pathway and accelerates fracture repair.

**METHODS:** MC3 and SM-102 LNP's were generated using a microfluidic device (NanoAssemblr) and characterized for surface charge and size by Zeta-sizer and zeta-potential. All *in vitro* studies were conducted using pre-chondrogenic cell line ATDC5 cells. All of *in vivo* procedures listed in this study were approved by our IACUC and ARRIVE guidelines will be followed in reporting results. Delivery platforms were tested *in vivo* using a murine tibial fracture model stabilized with an intermedullary rod and all mRNA platforms were injected into the fracture callus 6 days following fracture induction. IVIS imaging was used to locate and quantify firefly luciferase. Transfection kinetics and efficiency were studied using Firefly mRNA (TriLink Biotech.) as a reporter gene and mRNA was isolated at various timepoints following delivery, *n=5/group*. A non-destructive β-catenin mRNA was developed in the GMP-compliant Houston Methodist RNA Core Facility using modified nucleotides, CleanCap technology and run through a codon optimality tool.[5] Canonical Wnt activation was determined by using qRT-PCR (*axin2, runx2, osterix*) and Wnt reporter assay (TOPFLASH, Addgene). µCT was used to assess bone after delivery of β-catenin LNP's 2 and 3 weeks following fracture repair, *n=5-9/group*. Wnt ligand, rhWnt3a protein, was used as a positive control to show Wnt activation. One-way and two-way ANOVAs were used for statistical analyses followed by Tukey's HSD.

**RESULTS:** MC3 and SM-102 LNPs both had net neutral surface charge (-6.07 and -2.78 mV respectively) and were of similar size (97 and 87 nm respectively) post encapsulation with Firefly Luciferase (FLuc) mRNA. When delivering 10 µg FLuc mRNA/mouse locally within the fracture callus, MC3 first lost significance of luciferase expression over the PBS control day 6 post-injection and SM-102 lost significance at day 8 post-injection (**FIG 1A-B**). Additionally, RNA was isolated from the fracture callus and tested for firefly luciferase gene expression resulting in SM-102 trending higher in luciferase signal over MC3 LNPs (**FIG 1C**). A biodistribution study was conducted to determine if the LNPs distribute systemically following localized injections. *Ex-vivo* IVIS images taken 18 hours after mRNA injection revealed no significant accumulation in any tissue evaluated except the fracture callus (**FIG 2**). Following the generation of β-catenin mRNA, the sequence was first tested *in vitro* on chondrocytes showing activation of canonical Wnt pathway through qRT-PCR and Wnt reporter assay, TOPFLASH (**FIG 3A**). The β-catenin-SM-102 LNP's were then tested *in vivo* to determine if they stimulated bone formation using µCT revealing a higher trend in bone volume over tissue volume at week 2 following fracture over the PBS control (**FIG 3B-C**).

**DISCUSSION:** In this study, we aimed to generate an injectable platform to stimulate bone formation after fracture through activation of the canonical Wnt pathway. When testing various delivery platforms, SM-102 LNPs were found to prolong and enhance luciferase signal for 8 days after initial injections and showed minimal distribution when tested *in vivo*. Next, a non-destructive β-catenin mRNA sequence was developed and shown to successfully activate canonical Wnt. The generated β-catenin mRNA – SM-102 LNPs was tested in a murine fracture model and shown to increase bone over PBS control. Future directions of this study will focus on dosing of the mRNA to determine if increased doses result in more significant bone being promoted locally at the fracture site.

**SIGNIFICANCE/CLINICAL RELEVANCE:** In order to combat the precedence of non-union and delayed healing in tibia fractures, we are developing an mRNA platform which activates the canonical Wnt pathway to promote and enhance bone formation at the site of the fracture.

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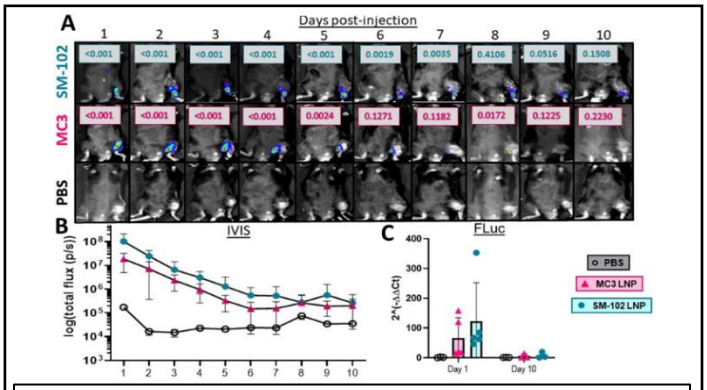


**Figure 1.** IVIS imaging was performed daily after injections of 2 LNP formulations (A) and the luciferase expression was quantified (B). RNA was isolated from the fracture and firefly luciferase gene was measured (C).

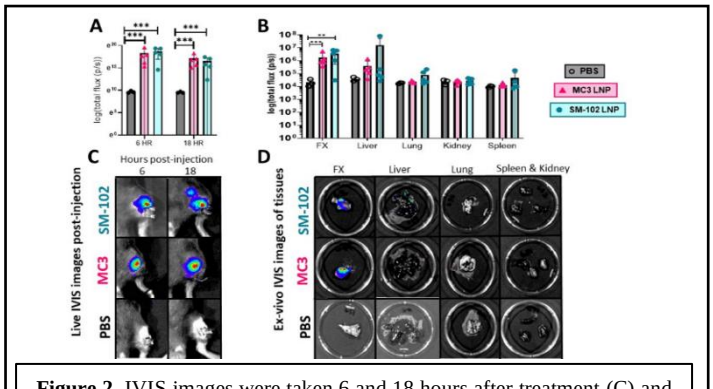


**Figure 2.** IVIS images were taken 6 and 18 hours after treatment (C) and quantified (A). A biodistribution study was performed by taking ex-vivo IVIS images of (D) fractured leg, liver, lung, spleen and kidney and the signal was then quantified (B).

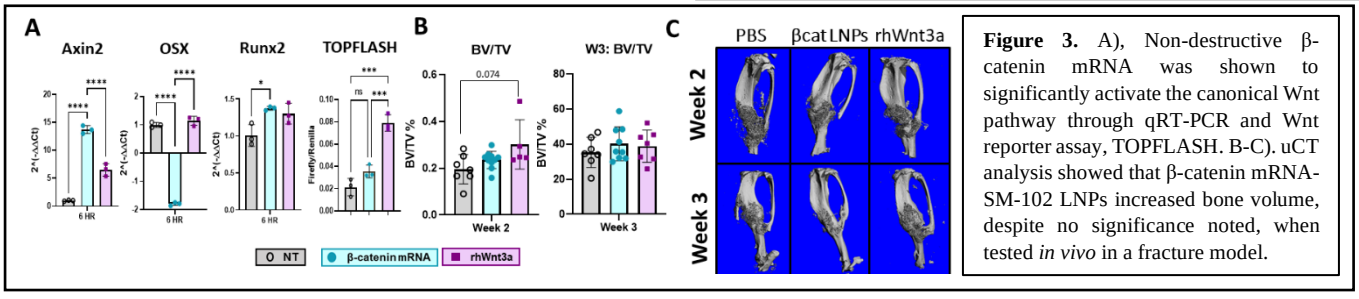


**Figure 3.** A), Non-destructive β-catenin mRNA was shown to significantly activate the canonical Wnt pathway through qRT-PCR and Wnt reporter assay, TOPFLASH. B-C). uCT analysis showed that β-catenin mRNA-SM-102 LNPs increased bone volume, despite no significance noted, when tested *in vivo* in a fracture model.