

## DOCTORAL THESIS

# Therapeutic Aptamer Targeting Sclerostin Loop3 for Promoting Bone Formation Without Increasing Cardiovascular Risk in Osteogenesis Imperfecta Mice

WANG, Luyao

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## Abstract

Sclerostin inhibition demonstrated bone anabolic potential in osteogenesis imperfecta (OI) mice, whereas humanized therapeutic sclerostin antibody romosozumab for postmenopausal osteoporosis imposed clinically severe cardiac ischemic events. Cardiovascular abnormalities are associated secondary features of OI patients, implying high cardiovascular risk for OI patients during sclerostin antibody treatment. Therefore, it is desirable to develop a next generation of sclerostin inhibitors to promote bone formation without increasing cardiovascular risk for OI.

Cardiac ischemic events were contributed by chronic progressive inflammatory diseases such as aortic aneurysm (AA) and atherosclerosis. The central residues of sclerostin form three loops (loop1, loop2 and loop3). Notably, our *in vivo* data showed that sclerostin antibody, which targeted both sclerostin loop2 and loop3, elevated serum levels of inflammatory cytokines and chemokines, and aggravated AA and atherosclerosis in *Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup>* mice with AngII infusion. Both our *in vitro* and *in vivo* data indicated that transgenic introduction of human sclerostin suppressed inflammatory cytokines and chemokines expression, prevented AA and atherosclerosis progression in *Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup>* mice with AngII infusion. Either loop2&3 deficiency by genetic truncation or loop2&3 inhibition by pharmacologic sclerostin antibody attenuated the suppressive effects of sclerostin on the expression of inflammatory cytokines and chemokines in primary macrophages and aortic vascular smooth muscle cells (VSMCs) from *Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup>* mice *in vitro*, whereas loop3 deficiency by genetic truncation maintained the above suppressive effects of sclerostin. Consistently, transgenic introduction of loop3 deficient sclerostin and full-length sclerostin showed similar suppressive effect on expression of inflammatory cytokines and chemokines, and

progression of AA and atherosclerosis in *Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup>* mice with AngII infusion *in vivo*. It indicated that the protective effect of sclerostin on cardiovascular system was independent of loop3. Moreover, after normalized by bone formation in *Col1a2<sup>+G610C</sup>* mice, the relative bone formation in *Col1a2<sup>+G610C</sup>* mice with transgenic introduction of loop3 deficient sclerostin was significantly higher than that in *Col1a2<sup>+G610C</sup>* mice with transgenic introduction of full-length sclerostin, suggesting the important role of loop3 in sclerostin's antagonistic effect on bone formation. Taken together, inhibitors specifically targeting sclerostin loop3 are worthy of investigation on the bone anabolic efficacy and the cardiovascular risk in OI mice.

A sclerostin loop3-specific aptamer aptscl56 was tailored selected by our lab. In this study, it was further validated that aptscl56 could bind to both recombinant sclerostin and sclerostin in the serum of the selected OI patients and healthy controls, via targeting loop3. The *in vitro* data demonstrated that aptscl56 could inhibit sclerostin's antagonistic effect on Wnt signaling and promote osteogenic potential in osteoblasts from *Col1a2<sup>+G610C</sup>* mice, whereas had no influence on sclerostin's suppressive effect on the expression of inflammatory cytokines and chemokines in primary macrophages and aortic VSMCs from *Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup>* mice with AngII treatment. After PEG40k conjugation, subcutaneously administrated PEG40k-aptscl56 (Apc001PE) showed 72 times longer elimination half-life ( $T_{1/2}=57.798h$ ) than the free aptscl56 ( $T_{1/2}=0.8h$ ) in *Col1a2<sup>+G610C</sup>* mice. In cardiovascular safety studies, unlike therapeutic sclerostin antibody, Apc001PE had no effect on inflammatory cytokines and chemokines expression, AA and atherosclerosis progression in *Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup>* mice with AngII infusion. Thereinto, macrophages infiltration, VSMCs contractile phenotype loss and cell apoptosis in suprarenal aortas and aortic roots were not altered by Apc001PE administration. The aggravation of AA and atherosclerosis induced by therapeutic sclerostin antibody in *Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup>* mice were attenuated by supplement of exogenous loop2m (loop2 mutant which failed to

suppress the expression of inflammatory cytokine but remained binding to therapeutic sclerostin antibody), further validating that the cardiovascular protective effect of sclerostin was independent of loop3 in *Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup>* mice. Further, Apc001PE had no influence on the suppressive effects of transgenically introduced sclerostin on inflammatory response, AA and atherosclerosis progression in *hSOST<sup>Tg</sup>.Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup>* mice with AngII infusion. In bone pharmacodynamic studies, Apc001PE demonstrated the efficiency in promoting bone formation, increasing bone mass and improving bone microarchitecture integrity in *Col1a2<sup>+G610C</sup>* mice via targeting loop3, which were evidenced by comparing the difference in the bone anabolic potential of Apc001PE between with and without pretreatment of loop3m (loop3 mutant which failed to inhibit Wnt signaling but remained binding to aptscl56). Further, Apc001PE inhibited the antagonistic effect of transgenically introduced sclerostin on bone formation in *hSOST<sup>Tg</sup>.Col1a2<sup>+G610C</sup>* mice via targeting loop3, whereas Apc001PEm (PEG40K-conjugated aptscl56 mutant which could not bind to sclerostin) had no bone anabolic effect. In toxicity studies, the serum levels of liver and kidney function indexes and hematologic parameters were not altered by a single or multiple administration(s) of Apc001PE in healthy C57BL/6 mice. Microscopic examination revealed normal cell structure, no lesion or pathological changes in vital organs, especially brain/cerebellum/cerebral vessels and heart/aortic root, of healthy SD rats in the aptamer groups, even at ultrahigh dose.

Apc001PE for OI was granted orphan drug designation by US FDA in 2019 (DRU-2019-6966). This work facilitated the development of the next generation of sclerostin inhibitors specifically targeting sclerostin loop3 to promote bone formation without increasing cardiovascular risk in OI.

# Table of Contents

DECLARATION .....	i
Abstract.....	ii
Acknowledgements .....	v
Table of Contents .....	vi
Abbreviations .....	x
Chapter 1. Background.....	1
Chapter 2. Materials and Methods .....	6
2.1 Mice and Genotyping .....	6
2.2 Mouse models of aortic aneurysm and atherosclerosis .....	7
2.3 Assessment of aortic aneurysm .....	8
2.4 Assessment of atherosclerosis .....	8
2.5 Immunohistochemistry (IHC).....	9
2.6 Enzyme-linked immunosorbent assay (ELISA) .....	9
2.7 Primary peritoneal macrophages isolation from <i>Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup></i> mice .....	10
2.8 Aortic VSMCs isolation from <i>Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup></i> mice .....	10
2.9 Osteoblasts isolation from <i>Col1a2<sup>+G610C</sup></i> mice.....	11
2.10 Quantitative real-time PCR .....	11
2.11 TOP-Wnt-induced luciferase reporter assay .....	12
2.12 Micro-CT analysis.....	12
2.13 Bone histomorphometric analysis .....	13
2.14 Bone mechanical test.....	14
2.15 Statistical analysis.....	15
Chapter 3. The role of sclerostin and its loops in regulating bone formation and cardiovascular events progression.....	16
3.1 Aim.....	16
3.2 Experimental design.....	16
3.2.1 Experimental design for specific aim (1).....	16
3.2.2 Experimental design for specific aim (2).....	17
3.2.3 Experimental design for specific aim (3).....	17
3.2.4 Experimental design for specific aim (4).....	17
3.2.5 Experimental design for specific aim (5).....	18
3.3 Results.....	21

3.3.1 Therapeutic sclerostin antibody elevated serum levels of inflammatory cytokines and chemokines, and aggravated AA and atherosclerosis in <i>Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup></i> mice with AngII infusion.....	21
3.3.2 Either loop2&3 deficiency or inhibition attenuated sclerostin's suppressive effects on expression of inflammatory cytokines and chemokines <i>in vitro</i> , whereas loop3 deficiency maintained the above suppressive effects of sclerostin .....	24
3.3.3 Loop2 and/or loop3 were critical for sclerostin's antagonistic effect on Wnt signaling pathway and osteogenic potential <i>in vitro</i> .....	25
3.3.4 The protective effect of sclerostin on cardiovascular system in <i>Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup></i> mice was independent of loop3 <i>in vivo</i> .....	28
3.3.5 Loop3 played an important role in sclerostin's antagonistic effect on bone formation in <i>Col1a2<sup>+G610C</sup></i> mice <i>in vivo</i> .....	31
3.4 Conclusion.....	35
Chapter 4. Binding ability of aptscl56 to the sclerostin in the serum from the selected OI patients and healthy controls.....	36
4.1 Aim.....	36
4.2 Experimental design.....	36
4.2.1 Experimental design for specific aim (1).....	36
4.2.2 Experimental design for specific aim (2).....	36
4.3 Results.....	37
4.3.1 Aptscl56 could bind to recombinant sclerostin via targeting loop3.....	37
4.3.2 Aptscl56 could bind to sclerostin in the serum from the selected OI patients and healthy controls via targeting loop3.....	38
4.4 Conclusion.....	41
Chapter 5. The effect of aptscl56 on osteogenic potential and on expression of inflammatory cytokines and chemokines <i>in vitro</i> .....	42
5.1 Aim.....	42
5.2 Experimental design.....	42
5.2.1 Experimental design for specific aim (1).....	42
5.2.2 Experimental design for specific aim (2).....	42
5.3 Results.....	43
5.3.1 Aptscl56 inhibited sclerostin's antagonistic effect on Wnt signaling and osteogenic potential in primary osteoblasts isolated from <i>Col1a2<sup>+G610C</sup></i> mice via targeting loop3 <i>in vitro</i> .....	43
5.3.2 Aptscl56 had no influence on sclerostin's suppressive effect on expression	

of inflammatory cytokines and chemokines in primary macrophages and aortic VSMCs isolated from <i>Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup></i> mice <i>in vitro</i> .....	43
5.4 Conclusion.....	47
Chapter 6. Chemical modifications and pharmacokinetic analysis.....	48
6.1 Aim.....	48
6.2 Experimental design.....	48
6.2.1 Experimental design for specific aim (1).....	48
6.2.2 Experimental design for specific aim (2).....	49
6.3 Results.....	49
6.3.1 PEG40k conjugation extended the elimination half-life of aptscl56 in <i>Col1a2<sup>+G610C</sup></i> mice. ....	49
6.4 Conclusion.....	52
Chapter 7. Cardiovascular safety studies <i>in vivo</i> .....	53
7.1 Aim.....	53
7.2 Experimental design.....	53
7.2.1 Experimental design for specific aim (1).....	53
7.2.2 Experimental design for specific aim (2).....	53
7.3 Results.....	54
7.3.1 Apc001PE had no effect on inflammatory cytokines and chemokines expression, AA and atherosclerosis progression in <i>Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup></i> mice with AngII infusion .....	54
7.3.2 Apc001PE had no influence on the suppressive effects of transgenically introduced sclerostin on inflammatory response, AA and atherosclerosis progression in <i>hSOST<sup>Tg</sup>.Col1a2<sup>+G610C</sup>.ApoE<sup>-/-</sup></i> mice with AngII infusion .....	63
7.4 Conclusion.....	66
Chapter 8. Bone pharmacodynamic studies <i>in vivo</i> .....	67
8.1 Aim.....	67
8.2 Experimental design.....	67
8.2.1 Experimental design for the specific aim (1) .....	67
8.2.2 Experimental design for the specific aim (2) .....	68
8.3 Results.....	69
8.3.1 Apc001PE promoted bone formation, increased bone mass and improved bone microarchitecture integrity in <i>Col1a2<sup>+G610C</sup></i> mice via targeting sclerostin loop3.....	69
8.3.2 Apc001PE inhibited the antagonistic effect of transgenically introduced human sclerostin on bone formation in <i>hSOST<sup>Tg</sup>.Col1a2<sup>+G610C</sup></i> mice via targeting	

sclerostin loop3 .....	87
8.4 Conclusion.....	93
Chapter 9. Toxicity evaluation.....	94
9.1 Aim.....	94
9.2 Experimental design.....	94
9.2.1 Histopathology for toxicity evaluation .....	94
9.2.2 Biochemistry and hematology assays for toxicity evaluation .....	94
9.3 Results.....	95
9.4 Conclusion.....	100
Chapter 10. Discussion, conclusion and future impact.....	101
Reference .....	107
CURRICULUM VITAE .....	114