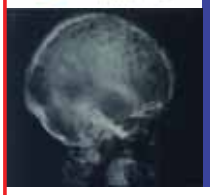

# A novel antagonist of the canonical Wnt-signalling pathway, SOSTDC1, is expressed in an experimental model of myeloma and suppresses bone formation

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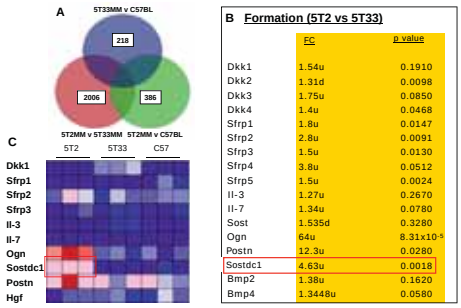
## Introduction

Patients with multiple myeloma (MM) commonly present with devastating bone disease mediated by increased bone resorption and suppressed bone formation. We have previously shown that blocking activity of the Wnt antagonist DKK1 promotes osteoblastogenesis and inhibits development of bone lesions in experimental models of MM[1]. In the 5T murine models of MM, tumour cells home to the bone marrow. Injection of 5T2MM cells into C57BLKaLwRij mice results in osteolytic bone disease whereas injection of 5T33MM cells does not[2]. Microarrays revealed that the BMP/Wnt antagonist, SOSTDC1, is significantly upregulated in 5T2MM-bearing animals (+4.6-fold, p<0.005), compared to 5T33MM-bearing mice (Figure 1). We hypothesise that elevated levels of secreted SOSTDC1 in the bone microenvironment reduce osteoblastogenesis and bone formation, and that this contributes to the bone disease associated with MM.




B Formation (5T2 vs 5T33)

| | FC | p value |
|---|---|---|
| Dkk1 | 1.54u | 0.1910 |
| Dkk2 | 1.31d | 0.0098 |
| Dkk3 | 1.75u | 0.0850 |
| Dkk4 | 1.4u | 0.0468 |
| Sfrp1 | 1.8u | 0.0147 |
| Sfrp2 | 2.8u | 0.0091 |
| Sfrp3 | 1.5u | 0.0130 |
| Sfrp4 | 3.8u | 0.0512 |
| Sfrp5 | 1.5u | 0.0024 |
| Il-3 | 1.27u | 0.2670 |
| Il-7 | 1.34u | 0.0780 |
| Sost | 1.535d | 0.3280 |
| Ogn | 64u | 8.31x10$^{-5}$ |
| Postn | 12.3u | 0.0280 |
| Sostdc1 | 4.63u | 0.0018 |
| Bmp2 | 1.38u | 0.1620 |
| Bmp4 | 1.3448u | 0.0580 |

Figure 1: **Genes implicated in studies of bone formation are upregulated in a murine model of osteolytic bone disease associated with MM.** Affymetrix® chip-generated expression data were analysed using Partek Genomic Suite™(A). Data were collated for significantly dysregulated genes implicated in studies of bone formation (B). A colorgram (generated using GenePattern™) displays corroborative qPCR data, generated using additional biological replicates (C). FC: fold-change, u: upregulated, d: downregulated.

## Materials & Methods

6-week old mice were injected subcutaneously, above the calvaria, with rhSOSTDC1 (30µg.kg.day$^{-1-1}$), or vehicle, and skulls were examined using µCT and histomorphometry. In a second study (Figure 2), 9-week old C57BLKaLwRij mice received intravenous rhSOSTDC1, or vehicle, and tibiae were examined using µCT and both static and dynamic histomorphometry. *in vitro* experiments were performed, using mouse primary OB, in order to investigate the effect of SOSTDC1 on Wnt- and BMP-induced OB differentiation. Cells were cultured in the presence or absence of Wnt3a (RnD Systems) and treated with SOSTDC1 or a positive control, DKK1.

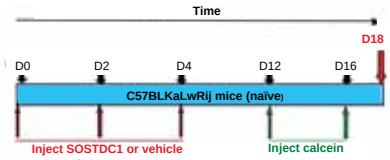



**Figure 2: Schematic of the model used for investigating the effect of elevated SOSTDC1 on bone formation in C57BLKaLwRij mice.** SOSTDC1 was injected via the tail vein. Bone volume (BV) was analysed using µCT and OB/OC numbers assessed via standard histomorphometry.

## Results

Mice treated subcutaneously with SOSTDC1 (above the calvaria) exhibited locally-reduced bone volume and significantly reduced OB number (Ob.N) and perimeter (Ob.Pm)(n=4 per group)(Figure 3).

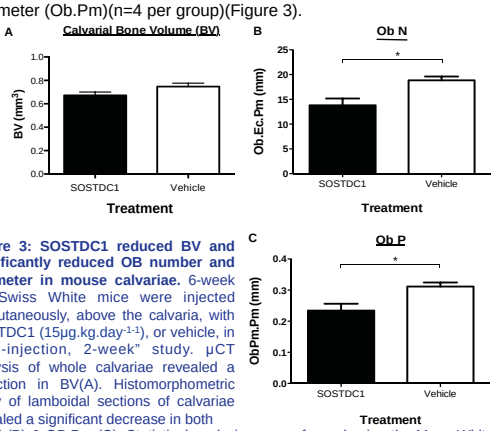



**Figure 3: SOSTDC1 reduced BV and significantly reduced OB number and perimeter in mouse calvariae.** 6-week old Swiss White mice were injected subcutaneously, above the calvaria, with SOSTDC1 (15µg.kg.day$^{-1-1}$), or vehicle, in a "3-injection, 2-week" study. µCT analysis of whole calvariae revealed a reduction in BV(A). Histomorphometric study of lamboidal sections of calvariae revealed a significant decrease in both OB.N (B) & OB.Pm (C). Statistical analysis was performed using the Mann-Whitney U test for unpaired non-parametric data.

In the second study, mice treated intravenously with SOSTDC1 (n=8 per group) had reduced bone volume. In addition, OB number and perimeter were significantly reduced on both cortico-endosteal and trabecular surface (data not shown), in the tibiae of mice treated with SOSTDC1 (Figure 4).

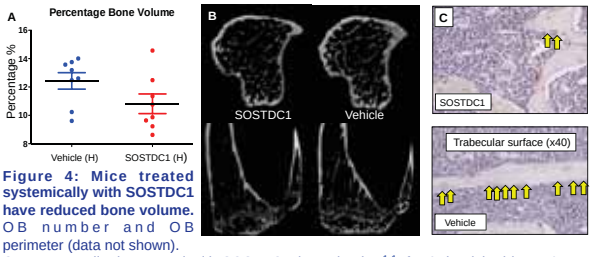



**Figure 4: Mice treated systemically with SOSTDC1 have reduced bone volume.** OB number and OB perimeter (data not shown).
C57BLKaLwRij mice treated with SOSTDC1 (75µg.kg.day$^{-1-1}$, for 6 days) had lower % BV than control (A). Transverse and longditudinal µCT scans show reduced trabecular bone in SOSTDC1-treated mice (B). Tartrate-resistant acid phosphatase (TRAP) stained tibial sections show a reduction in OB.N and OB.Pm in treated mice (C). These differences were significant (data not shown). H: high dose.

Analysis of calcein labelled contralateral tibiae, using standard dynamic histomorphometry, suggested that bone formation rate was reduced, and mineral apposition rate was significantly reduced, in SOSTDC1-treated mice compared to control (Figure 5).

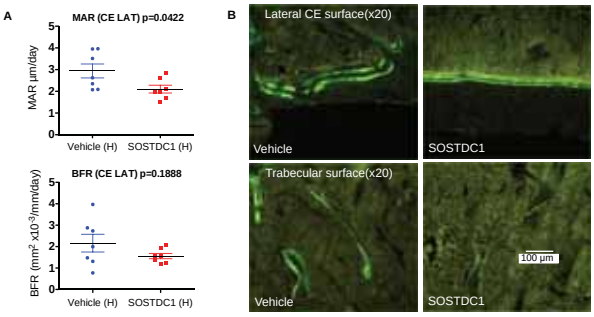
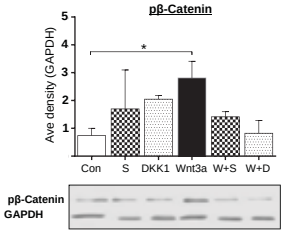



**Figure 5: Mineral apposition rate (MAR) was significantly reduced at the lateral cortico-endosteal (CE) surface of mouse tibiae following treatment with SOSTDC1.** Calcein labelled tibiae from C57BLKaLwRij mice treated with SOSTDC1 (as above) were analysed using standard dynamic histomorphometry. SOSTDC1-treated mice had significantly lower MAR than control (p=0.0422), as well as reduced bone formation rate (mineral apposition rate x mineralising surface)(A). Representative images of tibial CE and trabecular surface were recorded for qualitative assessment of bone formation (B). Statistical analysis was performed using Student's unpaired t test.

SOSTDC1 suppressed Wnt- and BMP-induced phosphorylated β-catenin levels in cultured mouse OB (Figure 6). Data obtained using BMP2/7 not shown.

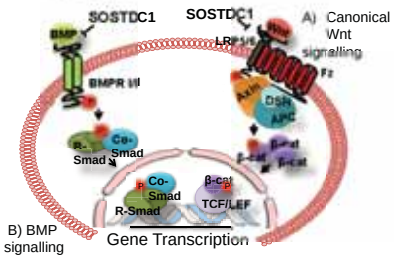
**Figure 6: SOSTDC1 suppressed Wnt-induced phosphorylated β-catenin levels in cultured mouse OB .** Freshly isolated mouse primary OB were treated with 50ng.ml$^{-1}$ Wnt3a in the presence or absence of SOSTDC1 (250ng.ml$^{-1}$) for 30 minutes. Phosphorylated β-catenin levels were measured using Western Blot, using GAPDH as loading control. Con: Control, W: Wnt, S: SOSTDC1, D: DKK1. For multiple comparisons, data were analysed using one-way Anova.




## Discussion

Our *in vivo* data suggest that SOSTDC1 is a significant inhibitor of OB activity. Taken together with the *in vitro* studies, which demonstrate that rhSOSTDC1 inhibits both Wnt- and BMP-induced OB differentiation (outlined in Figure 7), they suggest that blocking myeloma-derived/-induced SOSTDC1 may be of therapeutic value in patients with myeloma bone disease.

**Figure 7. Schematic diagram outlining regulation of A) activated β-catenin by canonical Wnt signalling and B) phosphorylated smads by BMP, in osteoblasts.** SOSTDC1 may block Wnt and BMP pathways by binding to the LRP5/6/Fz receptor complex or the BMP ligand, respectively.




## References

[1]Chantry, Buckle *et al* 2009 *J Bone Miner Res*. Mar; 24(3):425-36.

[2]Buckle *et al* 2012 *PLoS ONE*. 7(8):e41127.

**Conflict of interest – none declared by authors.**