MEK inhibitors in orthopaedics: applications in NF1

THE UNIVERSITY OF SYDNEY

Little DG[1,2], El-Hoss J[1,2],Deo N[1,2], Mikulec K[1,2], McDonald M[1,2], Schindeler A[1,2]

kids research Institute the children's hospital at Westmead

[1] *Orthopaedic Research & Biotechnology, The Kids Research Institute at The Children's Hospital at Westmead*

[2] *Discipline of Paediatrics and Child Health, The University of Sydney, Sydney, Australia*

• SUMMARY

- Congenital tibial dysplasia (CTD) is often associated to the genetic disorder Type 1 Neurofibromatosis (NF1). Patients suffering from CTD display bowing of the tibiae, and are prone to fracture and progression to pseudarthrosis (non-union).
- To date, therapies for CTD are very limited, and amputation at a young age is not an uncommon outcome for this condition. Recent genetic data suggests that local double-inactivation of the *NF1* gene may be implicated in CTD pathobiology, resulting in over-activation of the Ras-MAPK pathway.
- Using a published NF1 pseudarthrosis model, we hypothesized that use of a MEK inhibitor (PD0325901, Pfizer) would promote normal fracture repair in a NF1 pseudarthrosis fracture.
- We initially tested the impact of PD0325901 at 10mg/kg/day in normal fracture healing. Mice underwent a closed tibial fracture, and were assessed histologically at 10 and 21 days post fracture.
- We then tested PD0325901 at 10mg/kg/day, alone or in combination with local rhBMP2 in our NF1 fracture model.

MEK inhibitor treatment during fracture healing promoted cartilage formation and delayed resorption.

Combination treatment of PD0325901 and rhBMP2 synergistically promoted bone formation and fibrous tissue accumulation, but was no more effective that rhBMP2 at healing a pseudarthrosis.

• Background

Ras-MAPK and NF1 fracture healing

Type I Neurofibromatosis is a complex disorder that impacts the nervous and musculoskeletal tissues. It is associated with mutations in the *NF1* gene, resulting in over-activation of the Ras-MAPK pathway. PD0325901 is a new MEK inhibitor capable of limiting Ras-MAPK activation, and has been trialed in NF1 mouse models to treat neural lesions and leukemia (1,2). A sister compound (AZD6244, Selumetinib, AstraZeneca), is also under clinical trials in a pediatric NF1 population.

Congenital Tibial Dysplasia (CTD) is a severe complication that is associated to NF1, and results in a persistent non-union. In severe cases, a pseudarthrosis can develop with the accumulation of a highly fibrotic fracture callus. We recently developed a mouse model of NF1 pseudarthrosis that allows preclinical testing of novel compounds (3).

In this study, we hypothesized that the MEK inhibitor PD0325901 could rescue the fibrosis in our fracture model. However, the impacts of MEK inhibitors PD0325901 or AZD6244 have never been described in fracture healing. Therefore, **we performed an experiment to determine the impact of PD0325901 and AZD6244 during fracture healing. We then performed a second experiment to determine if PD0325901 in combination with rhBMP2 could prevent fibrosis in our NF1 fracture model**.

Impact of PD0325901 and AZD6244 in wild-type mice

To determine the impact of PD0325901 and AZD6244 during fracture healing, C57Bl6 mice underwent a closed tibial fracture. Mice were treated during the different stages of fracture healing as described in Figure 1.

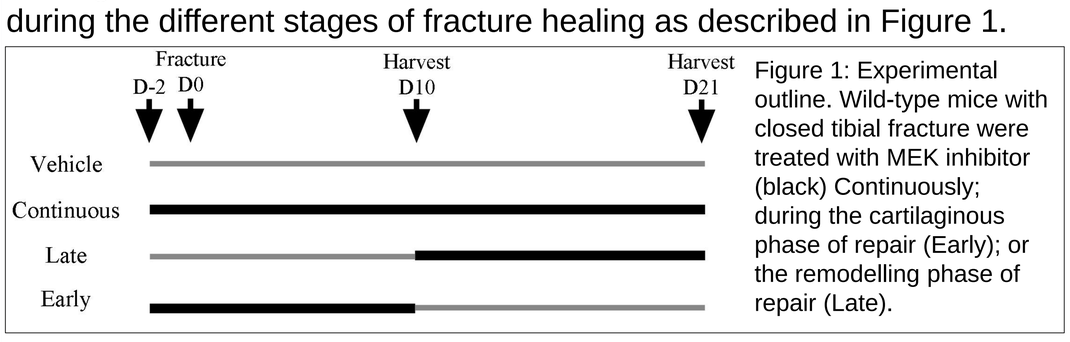

Figure 1: Experimental outline. Wild-type mice with closed tibial fracture were treated with MEK inhibitor (black) Continuously; during the cartilaginous phase of repair (Early); or the remodelling phase of repair (Late).

We found that at day 10, **PD0325901 significantly promoted cartilage deposition,** while AZD6244 only showed a trend (Figure 1A, B). If treatment was stopped at day 10, the cartilage was remodeled by day 21, and we saw no change in osteoclasts (Figure 1, Early group). **If treatment was started at day 10, cartilage resorption was delayed, and osteoclast surface was also reduced at day 21** (Figure 1, Late group). Taken together, these data suggest that **MEK inhibitor treatment can promote cartilage formation, delay cartilage resorption, and reduce osteoclast surface in fracture healing**.

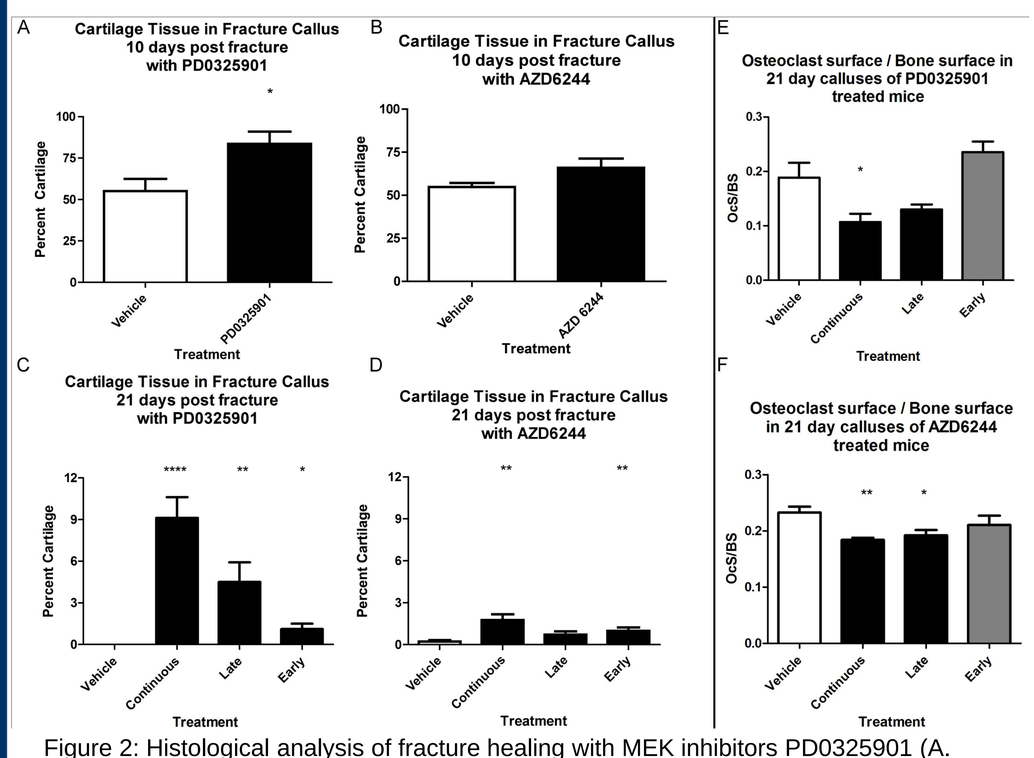

Figure 2: Histological analysis of fracture healing with MEK inhibitors PD0325901 (A. C, E) or AZD6244 (B, D, F) treatment at day 10 (A, B) or day 21 (C, D, E, F).

PD0325901 in NF1 pseudarthrosis

We used a previously published NF1 fracture model to determine if PD0325901 could be used to treat or prevent a NF1 pseudarthrosis (3). *Nf1*$^{flox/flox}$ mice were treated with a Cre expressing adenovirus at the site of an open fracture to induce a pseudarthrosis. PD025901 was administered alone at 10 mg/kg/day for the first 10 days of healing or in combination with local rhBMP2 (10ug). Mice were harvested at day 21 to assess fracture union rates, histology, and bone volume.

	Unions	Partial unions	Non-unions	Total N	P value
Vehicle	0	5 (36%)	9 (64%)	14	-
PD0325901 (10mg/kg)	1 (8%)	4 (31 %)	8 (61%)	13	1
rhBMP2 (local)	9 (69%)	2 (15%)	2 (15%)	13	0.0003*
PD0325901 + rhBMP2	12 (80%)	2 (13%)	1 (7%)	15	0.0001*

Table 1 – Union rates for treatment of NF1 mice with a pseudarthrosis

We found that treatment with PD0325901 did not result in a significant change in union rate in our NF1 mouse model of a pseudarthrosis (Table 1). **rhBMP2 and rhBMP2+PD0325901 both resulted in a significant increases in union rates**.

MicroCT analysis showed that rhBMP2 treatment significantly increased bone volume compared to vehicle by 3-fold (Figure 3). **Combination rhBMP2 + PD0325901 acted synergistically to promote bone formation in NF1 pseudarthrosis**: it promoted a 6-fold increase in bone formation compared to vehicle, and 2-fold over rhBMP2 alone. While we observed a change in bone volume, **there was also an increase in callus volume** resulting in a decrease in bone volume over tissue volume in the rhBMP2 and combination treatment (data not shown). Furthermore, **no treatment resulted in a change in fibrous tissue accumulation this model of NF1 pseudarthrosis** (Figure 3B).

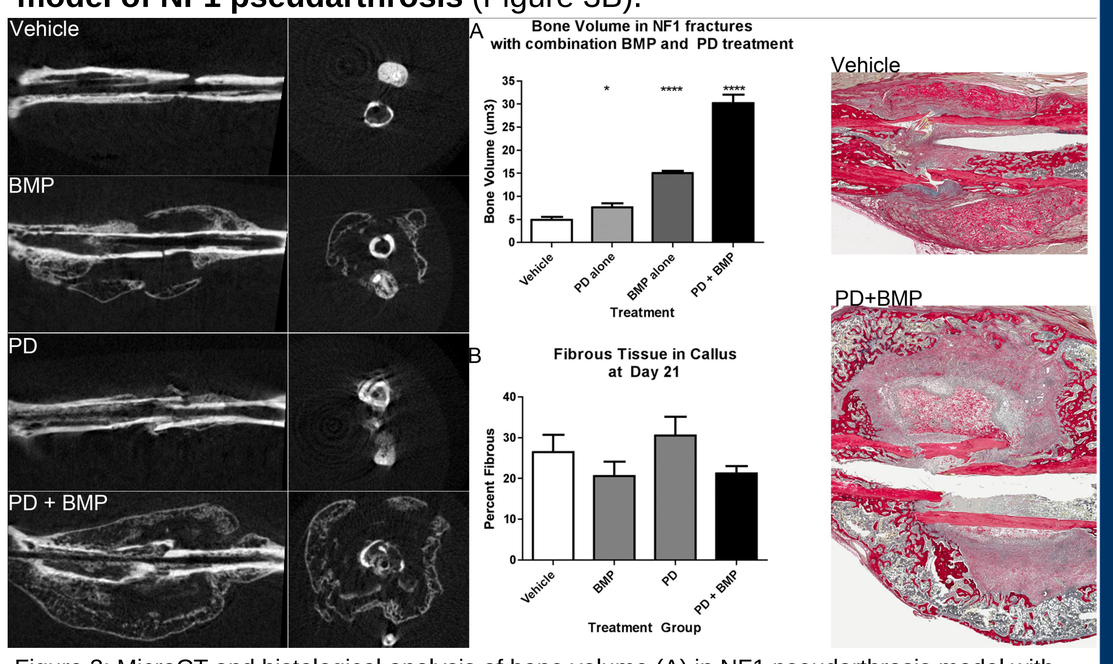

Figure 3: MicroCT and histological analysis of bone volume (A) in NF1 pseudarthrosis model with combination rhBMP2 and PD0325901 treatment. We observed a synergistic increase in bone volume with combination treatment. Large holes in the callus are filled with undifferentiated fibrous tissue (B).

• Results

• Discussion

PD0325901 and AZD6244 are a growing class of compounds to treat cancer. Yet, little information is available regarding their impacts on the skeleton or during fracture healing. In this study, we showed that MEK inhibitor treatment during fracture healing can promote cartilage deposition in the early phase of fracture healing, and delay cartilage resorption. We also found that osteoclasts surface was reduced, yet this was reversible.

In NF1 pseudarthrosis, treatment with PD0325901 did not increase union rate, suggesting that other pathways are involved. **Combination treatment with rhBMP2 synergistically increased bone volume and fibrous tissue in an NF1 callus**.

(1) Jessen, W. J. *et al. MEK inhibition exhibits efficacy in human and mouse neurofibromatosis tumors. The Journal of Clinical Investigation 123, 340-347*

(2) Chang, T. *et al. Sustained MEK inhibition abrogates myeloproliferative disease in Nf1 mutant mice. The Journal of Clinical Investigation 123, 335-339*

(3) El-Hoss, J. *et al. A murine model of neurofibromatosis type 1 tibial pseudarthrosis featuring proliferative fibrous tissue and osteoclast-like cells. Journal of Bone and Mineral Research 27, 68-78*