Histological Correlation with MRI findings to monitor Gene Therapy in an "In Vivo" Equine Model

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Objective: Using MRI as a serial non-invasive technique to evaluate healing of surgically created large osteochondral defects in a weight-bearing femoral condyle in response to direct injection of cartilage and bone regeneration gene therapy (human bone morphogenetic proteins (BMP) 2 or -6) [1-2].

Methods: Four osteochondral defects were drilled (n=20 defects). Direct injection of gene therapy (BMP's), qMRI[3] (3T MRI (Achieva, Philips, Cleveland, Ohio, U.S.A.) and computed tomography (CT) were serially performed at 12, 24, and 52 weeks. At 52 weeks, histomorphometry and microtomographic analyses were performed to assess final subchondral bone and cartilage repair tissue quality.

Results: *DCE-MRI:* Amplitude in the lesion was significantly greater at 12 weeks than 24 and 52 weeks, decreased at 24 weeks, and was negligible by 52 weeks (P<0.05). Adjacent un-injured cartilage had negligible values at 12, 24 and 52 weeks (Fig 2). *dGEMRIC:* Lesions treated with Ad-BMP-6 had a greater $T1_{Gd}$ at 12 weeks (P<0.05) than GBSS treatment (Fig 4). The adjacent non-injured cartilage had a significant lower $T1_{Gd}$ value than lesion repair tissue for all time points, 12, 24 and 52 weeks. At 52 weeks $T1_{Gd}$ values were lower in both the lesion and the adjacent cartilage than at 24 weeks (P<0.05)(Fig 2). T2 mapping: T2 relaxation time was significantly greater in the lesion than adjacent cartilage (P<0.05) without differences among groups or time (Fig2). Histology at 12 weeks demonstrated positive safranin-O staining within the surface repair tissue of the Ad-BMP-6 group and not present in other groups (Fig 3G).

Conclusions: Serial qMRI of gene therapy (BMP's) to large weight-bearing osteochondral defects provided evidence of support to cartilage and subchondral bone regeneration. Furthermore, MRI findings correlated with Histology and CT results in this pony model.

Figure 1: Live pony (250lbs) under general anesthesia undergoing MRI.

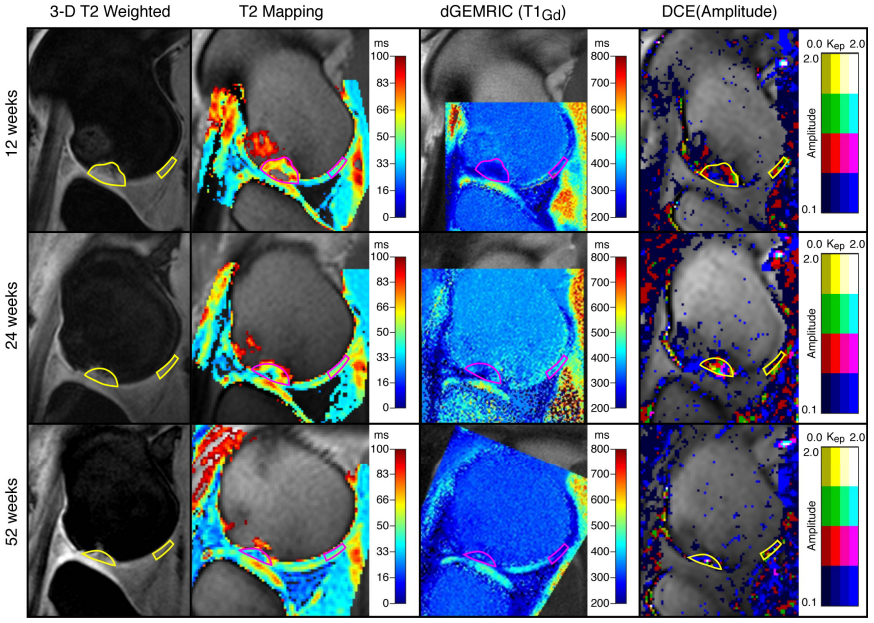

Figure 2 (top left): A. Representative quantitative MRI maps of sagittal sections through the osteochondral defect, showing a lesion treated with Ad-BMP-6 across time using T2 mapping, dGEMRIC and DCE-MRI.

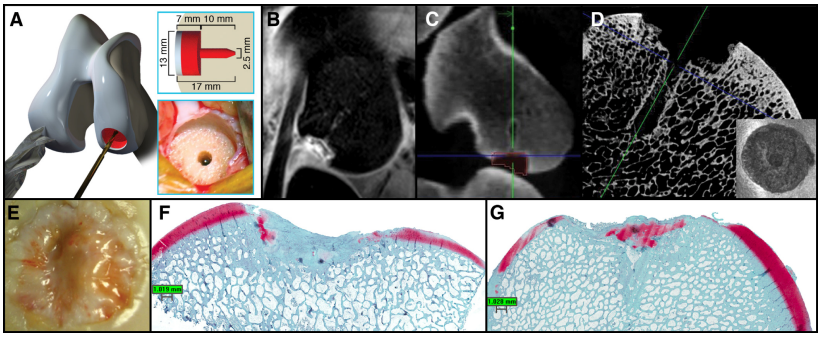

Figure 3: Images of osteochondral femoral defects in the pony model: Initially at surgery (A) and at 12 weeks in sagittal section in 3D T2 weighted MRI (B), live pony clinical CT (C), micro-CT (D), gross photograph (E) and Safranin-O histochemistry of Ad-GFP (F) and Ad-BMP6 (G). Positive safranin-O staining in the defect on histology of the Ad-BMP-6 treated defect supported the MRI findings in Figure 2 of greater $T1_{Gd}$ in the BMP-6 treated lesion (Fig. 4).

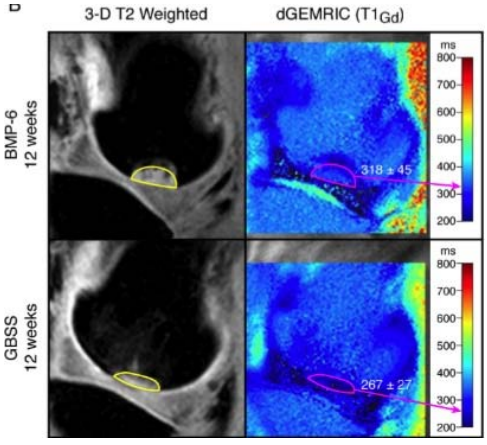

Figure 4: At 12 weeks, dGEMRIC shows greater $T1_{Gd}$ in the BMP-6 treated lesion than GBSS ($p < 0.05$).

References: 1. *Zachos T et al.* Mol Ther 2007;15:1543-1550. 2. *Ishihara A. et al.* J Orthop Res 2008;26:764-771. 3. *Clark D et al.* ISMRM proc 2010.