Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

Growth factor (single or combination)	Animal model (species/strain, location and defect)	Human clinical trial and defect	Time	Delivery system	Dose/ Conc.	Analysis (read outs, efficacy and methodology)	Reference
1. Animal Models for direct BMP-2 delivery 1.1. Large Animal Models							
Dog							
rhBMP-2	Mongrel Dog, humerus segmental defect – 3 mm		4 weeks	HA/TCP, implant	100 to 800 µg	Significant bone ingrowth in lower dose groups with the greatest effect at 100 µg showing a 3.5 fold increase over controls. Backscatter SEM, histology and radiography.	(Sumner *et al.*, 2004)
rhBMP-2	Beagle Dog, resection between femoral head and medial half of the femoral end – 25mm		6 months	PLA-DX-PEG, implant	100 to 1,000 µg	New bone formation was observed in a dose dependent manner. New bone mass showed evidence of remodelling to normal cortical bone at 6 months. Histology and radiography.	(Murakami *et al.*, 2003)
rhBMP-2	Mongrel Dog, bilateral radial segmental defect - 25mm		12 to 48 weeks	Collagen sponge, implant	150 to 2,400 µg	Implants treated with rhBMP-2 achieved union and gross stability. Lower rhBMP-2 doses resulted in better biomechanical strength. Early signs of bone remodelling were observed. Biomechanical testing, histology and radiography.	(Sciadini and Johnson, 2000)
rhBMP-2	Foxhound Dog, cleft defect – 10 mm		2 and 4 months	PLGA + autogenous blood, implant	200 µg	Response to the rhBMP-2 may have been suboptimal either because the dose was too low or because the PLGA-autogenous blood delivery system did not temporally maintain and spatially position rhBMP-2 at the recipient bed. However, new bone area increased 2.4 fold with treatment compared to a 1.2 fold increase in untreated groups. Histology and radiography.	(Mayer *et al.*, 1996)
rhBMP-2	Hound Labrador Mongrel Dog, bilateral supra-alveolar defect – 5mm		3 to 8 weeks	Titanium porous oxide scaffold, implant	300, 600 and 1,200 µg	Lower rhBMP-2 doses promoted local bone formation, alveolar ridge augmentation and osseointegration. Bone height and area were increased a maximum 5.5 and 10.5 fold respectively, within treated groups compared to controls over all dosages. Histology, fluorescence microscopy and radiography.	(Wikesjo *et al.*, 2008)
rhBMP-2	Male Beagle Dog, cranial defect – 2.5 x 4cm		16 weeks	Collagen and HA/TCP, implant	310 µg to 2,150 µg	Implants induced bone formation within large cranial defects. Bone values increased from 25 % to 49 % with increasing rhBMP-2. Histology, histomorphometry and radiography.	(He *et al.*, 2009)
rhBMP-2	Female Mix-Breed Dog, femur segmental defect – 60 mm		24 weeks	Absorbable collagen sponge wrapped around allograft, implant	460 to 920 µg	Significantly greater new bone formation leading to improved bone union, a 2.5 fold increase in callus area and larger osteon radii. Histology and histomorphometry.	(Zabka *et al.*, 2001)
rhBMP-2	Dog, tibial segmental diaphyseal defect – 25mm		32 to 104 weeks	PLGA/ gelatin sponge, implant	800 µg	Bone union was observed with restored biomechanical strength and significantly increased torsional stiffness by 2.6 fold of that of intact tibia. Biomechanical testing, histology and radiography.	(Kokubo *et al.*, 2004)
rhBMP-2	Dog, mandible defect - 35mm		12 weeks	Collagen sponge, implant	1,600 µg	Enhanced bone regeneration was observed upon immediate BMP-2 delivery. Bone morphometry, immunofluorescence and qPCR.	(Hussein *et al.*, 2012)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

rhBMP-2	Beagle Dog, Postlateral interbody lumbar fusion		3 and 8 months	Open cell PLA, implant	2,300 µg	Osseous bridging of postlateral gutters occurred in the rhBMP-2 implanted sites after 2 months. CT and radiography.	(Sandhu *et al.*, 1995)
BMP-2 (Canine)	Adult Mongrel Dog, radial segmental defect – 3 mm		12 weeks	Demineralised bone or PLA enriched with BMP-2, implant	15,000 µg	A significant increase in new bone formation was observed when PLA containing 15 mg of BMP-2 was implanted. Histomorphometry and radiography.	(Heckman *et al.*, 1991)
BMP-2 (Bovine)	Adult Mongrel Dog, ulna segmental defect – 25 mm		1 to 3 months	Gelatin capsules, implant	100,000 µg	Calcified callus formation was observed early. By 12 weeks the defect was completely bridged by new bone. Bone area increased 4.5 fold compared to controls. Histology and radiography.	(Nilsson *et al.*, 1986)
rhBMP-2	Beagle Dog, ulna segmental defect – 20mm		16 weeks	PLGA/gelatin sponge, implant	1.6 µg/mL	Addition of rhBMP-2 significantly enhanced bone union through new bone formation after only 12 weeks. After 16 weeks, bone mineral content at the defect site increased approx. 6 fold. Analysis showed the appearance of cortical bone and marrow cells. Histology and radiography.	(Itoh *et al.*, 1998)
rhBMP-2	Male Beagle Dog, calvarial defect – 5 x 5cm		8 to 24 weeks	Collagen sponge, implant	200 µg/mL	Bone healing was observed alongside ectopic bone formation. Radiopacity reached 100 % after 8 weeks compared to controls reaching 32.7 % after 24 weeks. Spatial control of rhBMP-2 release is required for further improved healing. Histology and radiography.	(Kinsella *et al.*, 2011)
rhBMP-2	Beagle Dog, mandibular supra-alveolar premolar tooth defects - 5mm		8 weeks	Bioerodable particles + autologous blood, implant	200 µg /mL	Cementum regeneration was observed in all defects exhibiting 21 fold increase in bone area. Small amount of root resorption was seen in rhBMP-2 defects compared to controls. Histomorphometry.	(Sigurdsson *et al.*, 1995)
rhBMP-2	Beagle Dog, periodontal defect		8 weeks	DBM, bovine deorganified crystalline bone matrix (Bio-Oss), absorbable collagen sponge, PLGA or PLA granules (Drilac), implant	200 µg/mL	Substantial bone regeneration was observed in all defects implanted with rhBMP-2. DBM and Bio-Oss performed well as carriers for rhBMP-2-driven periodontal regeneration, although other impediments to their clinical use exist. Histology and histomorphometry.	(Sigurdsson *et al.*, 1996)
rhBMP-2	Mongrel Dog, zygomatic arch osteotomy – 8 to 10 mm		4 to 12 weeks	Absorbable collagen sponge, implant	400 µg/mL	Significant bone formation was observed in all rhBMP-2 grafted sites as early as 4 weeks and radiopacity of bone continued to increase over time. Histology and radiography.	(Yudell and Block, 2000)
rhBMP-2	Beagle Dog, mandibular premolar defect – 5mm		16 weeks	Collagen sponge, implant	430 µg/mL	Bone regeneration (height) and osseointegration within the defect were enhanced by 8.4 and 2.3 fold, respectively compared to controls. Histomorphometry and radiography.	(Sigurdsson *et al.*, 1997)
rhBMP-2	Adult Female Dog, bilateral tibial osteotomy – 10 mm		4 and 8 weeks	Rapidly resorbable calcium phosphate paste, injection	700 µg/mL	Treatment with rhBMP-2 significantly increased bone union through greater callus formation exhibiting elevated bending and torsion stiffness. Biomechanical testing and radiography.	(Edwards *et al.*, 2004)
Goat							
rhBMP-2	Ovariectomized Goat, osteoporosis model, vertebral defect, 5mm x 10mm		0 to 140 d	Loaded gelatin microspheres (GM) in CPC, implant	20 µg	rhBMP-2/GM/CPC composite provides ideal bone substitute capable of inducing accelerated bone healing within an osteoporotic model. Mineralisation rate increased approx. 2 fold. Biomechanical testing, histology, micro CT and radiography.	(Li *et al.*, 2010b)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

rhBMP-2	Adult Goat, bilateral closed tibial fracture		6 weeks	Absorbable collagen sponge, implant	860 µg	Total callus volume was significantly increased up to 2 fold, exhibiting greater torsional stiffness. Biomechanical testing, histology and radiography.	(Welch *et al.*, 1998)
Horse							
rhBMP-2	Horse, metacarpal bone drill defect, 4.5mm x 27mm		1 dy to 12 weeks	Gelatin-β-TCP sponge, implant	3 µg	BMP-2 treatment within the defect site accelerated bone regeneration. Histology and radiography.	(Tsuzuki *et al.*, 2012)
Monkey							
rhBMP-2	Rhesus Monkey, mandibular defect – 20 x 15 mm		6 and 12 weeks	PLGA coated gelatin sponge, implant	280 µg	Resected bone defects regenerated completely exhibiting signs of remodelling. Bone area and mineral content increased 3.1 and 3.3 fold respectively, compared to controls. CT, histology and radiography.	(Marukawa *et al.*, 2001)
rhBMP-2	Adult Rhesus Monkey, Percutaneous vertebroplasty – 3mm drill defect		2 and 6 months	CPC, injection	900 µg	New bone and vessel formation after 2 months. Fully replaced cement with mature bone by 6 months. Histology and radiography.	(Bai *et al.*, 2009)
rhBMP-2	Rhesus Monkey, lumbar interbody fusion		24 weeks	Collagen sponge and biphasic ceramic phosphate granules, implant	3,000 µg	Spine fusion was observed in all rhBMP-2 treated groups confined to the area in or adjacent to the carrier matrix with no ectopic bone formation. CT, histology and radiography.	(Akamaru *et al.*, 2003)
rhBMP-2	Adult Rhesus Macaque Monkey, posterolateral arthrodesis		24 weeks	HA/TCP (15%/85%) with collagen, or plain collagen sponge wrapped around HA/TCP, implant	6,000 µg (low dose) 10,000 µg (high dose)	High dose rhBMP-2 achieved lumbar fusion where lose dose did not. However, combination of low dose rhBMP-2 on collagen wrapped around the HA/TCP bulking agent did achieve lumbar fusion (3 fold lower dose). Histology, micro CT and radiography.	(Barnes *et al.*, 2005)
rhBMP-2	Rhesus Monkey, mandibular defect – 30 mm		15 and 30 weeks	PLGA coated gelatin sponge, implant	9,000 µg	Resected mandible completely regenerated with the addition of rhBMP-2. Excellent remodelling and consolidation of new bone was observed after loading. Histology and radiography.	(Marukawa *et al.*, 2002)
rhBMP-2	Rhesus Monkey, maxillary defect – 8 mm		3 months	Collagen sponge, implant	430 µg /mL	Cancellous bone formation was observed with increasing trabecular thickness. Fluorescence microscopy, histology and histomorphometry.	(Boyne *et al.*, 1998)
rhBMP-2	Adult Male Cynomolgus Monkey, fibular osteotomy – 1 mm blade		8 to 14 weeks	Calcium phosphate paste, hyaluronan gel, hyaluronan paste, and gelatin foam, implant	1,000 µg/mL	Complete bone bridging of defect osteotomies was observed exhibiting greater torsional stiffness compared to controls. Mean callus area, torsional stiffness and maximum torque was 1.9, 1.7 and 1.7 fold greater than controls. Histology and radiography.	(Seeherman *et al.*, 2004)
rhBMP-2	Female Rhesus Monkey, calvarial defect – 25 mm, craniotomy – 25 x 40 mm		6 months	Absorbable collagen sponge or bone flap, implant	1,500 µg/mL	Bone flaps treated with rhBMP-2 showed enhanced osseointegration through bone bridge formation leading to 71 % closure compared with 28 % closure in controls. CT, histology and MRI.	(Sheehan *et al.*, 2003)
Pig							
rhBMP-2	Yorkshire Pig, anterior lumbar interbody fusion		9 months	PCL/TCP, implant	600 µg	Implants demonstrated bone ingrowth and solid fusion of good mechanical strength. Fusion mass bone volume increased approx. 5 fold compared to scaffold alone. Histology, micro CT and radiography.	(Abbah *et al.*, 2011)
rhBMP-2	Adolescent Yorkshire Pig, mandibular defect (subperiosteal osteotomy)		3 months	Heliostat absorbable collagen sponge, implant	1,500 µg/mL	Host cell migration into the defect site and osteogenesis was observed alongside extensive vascular invasion. Histology and radiography.	(Carstens *et al.*, 2005)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

Sheep							
rhBMP-2	Sheep, lumbar interbody defect – 12mm depth and 7mm diameter		12 months	CPC/Silk Fibroin, injection	5 µg	Composites exhibited enhanced osteo-conduction/induction/genesis, allowing for less invasive spinal fusion surgery. Fusion rates were 3 fold higher with rhBMP-2. Biomechanical testing, CT, histology, histomorphometry and radiography.	(Gu *et al.*, 2011)
rhBMP-2	Sheep, trepanation defect within femoral epiphysis		3 months	TCP, implant	200 µg	Faster remodelling was observed with bone formation comparable to native bone. Bone and total mineral content increased approx. 3 and 4 fold respectively. Histology, histomorphometry and microradiography.	(Maus *et al.*, 2008)
rhBMP-2	Sheep, sinus floor augmentation		12 weeks	PLGA and gelatin sponge, implant	800 µg	Implants demonstrated higher bone density (44 % vs 30.9 %) and bone-implant contact (15.4 % vs 7.7 %) compared to controls. Histology and histomorphometry.	(Gutwald *et al.*, 2010)
rhBMP-2	Mature Female Crossbred Sheep, femur diaphyseal segmental defect – 25 mm		12 months	PLGA with autologous blood, implant	2,000 to 4,000 µg	Recanalization of the medullary cavity approached completion at 52 weeks followed by complete woven and lamellar bone bridging of the defect site. Histology and radiography.	(Kirker-Head *et al.*, 1998)
rhBMP-2	Sheep, femur segmental defect – 25 mm		3 months	Inactivate bone matrix, implant	unknown	All defects treated with rhBMP-2 exhibited bone regeneration with increased mean bending strength; 1.9 fold of that of contralateral femur, compared with 1.1 fold in the inactive matrix control group. Radiography.	(Gerhart *et al.*, 1993)
1.2. Small Animal Models							
Mouse							
BMP-2	ICR Mouse, cranial defect (parietal bone) – 4.6mm		4 weeks	CHPA/Hydrogel, implant	0.1 to 2 µg	Implants revealed novel bone formation through stimulation of endogenous osteoblast activity. Histology, micro CT and radiographic.	(Hayashi *et al.*, 2009)
rhBMP-2	Male CD-1 Nude Mouse, calvarial defect – 4mm		1 to 8 weeks	Human adipose stem cells on PLGA scaffold, intravenously injected rhBMP-2	0.5 µg daily (3 days post-operatively)	BMP-2 signalling beneficially modulates cell-mediated bone repair increasing approx. 2.6 fold. Histology, histomorphometry, in situ hybridization, micro CT and qPCR.	(Levi *et al.*, 2010)
rhBMP-2	Female ddY Mouse, subcutaneous implant		7 weeks	Gelatin hydrogel	3 µg	BMP-2 release enhanced cell accumulation for *de novo* generation of bone tissue. Histology, immunofluorescence and radiography.	(Kimura *et al.*, 2010)
rhBMP-2	Nude Mouse, tibial defect		6 weeks	Fibrous PLGA/HA Scaffold, implant	5 µg	Implants demonstrated improved bone healing as a result of sustained BMP-2 release. Biomechanical testing, histology, immunohistochemistry and radiography.	(Fu *et al.*, 2008)
rhBMP-2	Male ddy Mouse, ectopic implantation - left dorsal muscle pouch		3 weeks	PLA-PEG , implant	10 µg	New bone formation with trabeculae structures was observed. Bone mineral content and density were increased approx. 2 and 1.5 fold compared with collagen control. Histology and radiography.	(Saito *et al.*, 2003)
rhBMP-2	Male C57B6 Mouse, tibial fracture defect		7 and 14 d	Direct injection or collagen sponge implant	10 µg	BMP-2 treatment increased bone callus (approx. 2.3 fold) and cartilage formation (approx. 4 fold). Specifically, BMP-2 modulated periosteum cell fate and differentiation. Histology, histomorphometry and immunohistochemistry.	(Yu *et al.*, 2010b)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

rhBMP-2	CB17 SCID Mouse, subcutaneous implant		10 weeks	PLGA microspheres in coralline HA, implant	20 µg	Prolonged *in vivo* bone formation was observed up to 10 weeks. 17.61 % of the implant surface was covered with bone-like tissue. Histology, histomorphometry, immunohistochemistry, micro CT and SEM analysis.	(Fu *et al.*, 2010)
rhBMP-2	Male Mouse, ectopic intramuscular implant		3 d to 3 weeks	Type I collagen sponge, implant	30 µg	Induced β-catenin mediated signalling through Wnt ligands leading to both osteogenesis and chondrogenesis. Histology and immunohistochemistry.	(Chen *et al.*, 2007)
rhBMP-2	Male CD-1 Mouse, ectopic muscle pouch implantation		28 d	Infuse (BMP-2) and OP-1 (BMP-7) in collagen sponge within a gelatin capsule, implant	52.5 µg	Treatment with rhBMP-7 increased bone volume approx. 1.9 fold compared to rhBMP-2 treatment. Histology and micro CT.	(Barr *et al.*, 2010)
rhBMP-2	Sprague-Dawley Mouse, ectopic injection – quadriceps muscle		3 weeks	Chitosan and hyaluronan hydrogels, injection	150 µg	Induced bone formation at ectopic injection sites. Mineralised bone formation was 2 to 3 fold higher in hyaluronan hydrogels compared to chitosan hydrogels, though less mature. Differences between hydrogels were down to degradation rates. Histology and micro CT.	(Luca *et al.*, 2010b)
Rabbit							
rhBMP-2	Japanese White Rabbit, patella drill defect – 5mm depth, 5mm diameter		2 to 8 weeks	Type I Collagen, implant	1.18 µg	BMP-2 acts in a spatiotemporally stringent manner recruiting endogenous MSCs and driving osteochondral differentiation. Histology and immunohistochemistry.	(Mimura *et al.*, 2011)
rhBMP-2	Male New Zealand Rabbit, posterolateral lumbar fusion		16 weeks	Alginate and MSCs, implant	2.5 µg	Addition of MSCs allowed for low dose rhBMP-2-induced bone fusion mass formation. Torsional strength was increased approx. 17 % compared to samples without rhBMP-2. Histology, micro CT and radiography.	(Fu *et al.*, 2009)
rhBMP-2	Adult Male Japanese White Rabbit, condylar defect		2 to 24 weeks	PLGA and gelatin sponge, implant	5 µg	Bone and cartilage-like tissue growth was observed in all implants (with and without rhBMP-2) but no growth was observed in the control group. Histology.	(Ueki *et al.*, 2003)
rhBMP-2	New Zealand White Rabbit, calvarial defect – 6mm		12 weeks	HA/TCP, implant	5 µg	Implants revealed enhanced bone formation bridging defect sites. Approx. 1.3 fold increase in bone area was observed upon treatment with rhBMP-2. Histology.	(Schopper *et al.*, 2008)
rhBMP-2	New Zealand White Rabbit, cranial defect – 6mm long x 1mm deep		12 weeks	Deproteinized bovine bone/porcine collagen, (DBBB) or cortico-cancellous human bone block (CHBB), implant	6 µg (DBBB) and 5 µg (CHBB)	rhBMP-2 and DBBB exhibited vertical bone augmentation within cranial defects. Percentage area of new bone increased approx. 3.4 fold. Histology and histomorphometry.	(Kim *et al.*, 2010c)
rhBMP-2	Rabbit, radial segmental defect – 15 mm		24 weeks	PLGA capsule, implant	12 µg	Bone union was observed in bone implanted with rhBMP-2/PLGA. Bone area and mineral content increased approx. 4.25 and 5 fold, respectively. Histology and radiography.	(Mori *et al.*, 2000)
rhBMP-2	New Zealand White Rabbit, radial segmental defect – 15 mm		4 and 8 weeks	Interconnected-porous calcium hydroxyapatite and PLA-PEG co-polymer, implant	5 and 20 µg	All bone defects treated rhBMP-2 were completely repaired. After 8 weeks bone mineral density was increased 1.7 fold with rhBMP-2 treatment. Biomechanical testing, CT, histology and radiography.	(Kaito *et al.*, 2005)
rhBMP-2	New Zealand White Rabbit, parietal bone elevation		8 weeks	PEG and HA/TCP. implant	10 and 30µg	Enhanced bone regeneration and ingrowth within titanium cylinders was observed. Newly formed bone increased 2 fold compared to controls. Histology and histomorphometry.	(Jung *et al.*, 2008)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

BMP-2	New Zealand White Rabbit, alveolar cleft (drill defect – 6mm)		4 weeks	Gelatin hydrogel, implant	17 µg	Bone volume but not osteoid matrix was increased 1.5 fold compared to untreated controls, showing bone regeneration. Histology, histomorphometry and radiology.	(Sawada *et al.*, 2009)
rhBMP-2	Rabbit, bilateral mid-ulnar osteotomies - 0.5 to 1 mm		2 to 6 wks	Absorbable collagen sponge, implant	40 µg	Mineralised callus area was significantly increased 1.2 to 1.6 fold through addition of rhBMP-2 and exhibited enhanced mechanical strength (1.8 fold greater), both compared to controls. Biomechanical testing, histology and radiography.	(Bouxsein *et al.*, 2001)
rhBMP-2	New Zealand White Rabbit, distal ulna defect – 15mm		8 weeks	PCL, implant	75 µg	Osteoinductive rhBMP-2 loaded onto PCL scaffold acted in synergy to recruit, differentiate, and guide host bone ingrowth. Histology, micro CT and radiography.	(Bae *et al.*, 2011)
rhBMP-2	Rabbit, humerus segmental defect – 15 mm		6 weeks	PLA-PEG co-polymer incorporated into titanium fiber-mesh cylinders, implant	60 and 120 µg	New bone formed throughout the implant cylinders bridging the defect. All defects treated with the higher rhBMP-2 dose were repaired completely. Histology and radiography.	(Murakami *et al.*, 2002)
rhBMP-2	New Zealand White Rabbit, radial defect – 15mm		2 to 8 weeks	Chitosan hydrogel with TCP granules, injection	150 µg	Evidence of partial defect bridging with new woven and lamellar bone was observed. TCP addition increased mineralised bone formation 4.7 fold. Histology, micro CT and radiography.	(Luca *et al.*, 2010a)
rhBMP-2	Adult New Zealand White Rabbit, bilateral posterolateral arthrodesis		8 weeks	Type 1 collagen sponge wrapped around HA/TCP (15%/85%), implant	1,075 µg	100 % of rabbits treated with rhBMP-2 achieved lumbar fusion whereas without treatment no rabbits achieved lumbar fusion. Biomechanical testing, histology, micro CT and radiography	(Kraiwattanapong *et al.*, 2005)
rhBMP-2	New Zealand White Rabbit, forearm segmental defect – 15mm		12 weeks	Collagen and chitosan microspheres, implant	2500 µg	The composite scaffold accelerated bone repair in a large radial defect. Bone mineral content was 1.3 fold greater than in controls. Biomechanical testing, histology, micro CT and radiography.	(Hou *et al.*, 2012)
rhBMP-2	New Zealand White Rabbit, lumbar spinal fusion		8 weeks	Porous β-TCP granules, implant	5,000 to 150,000 µg	Implants revealed enhanced lumbar fusion in a dose-dependent manner. Biomechanical testing, CT and histology.	(Dohzono *et al.*, 2009)
rhBMP-2	New Zealand White Rabbit, calvarial defect - 7.9 mm		6 weeks	PLGA microspheres in aqueous sodium carboxymethylcellulose, implant	2 µg/mL	Significant bone growth was observed in all rhBMP-2 treated defects while defects without treatment or with rhBMP-2 free implants showed minimal bone healing. Prolonged and immediate rhBMP-2 release regenerated 75-79 % and 45 % of the defect area. Histology and histomorphometry.	(Woo *et al.*, 2001)
rhBMP-2	New Zealand White Rabbit, interfrontal suture expansion		33 days	Absorbable collagen sponge, implant	10 to 400 µg/mL	Stimulation with low dose rhBMP-2 formed new bone at suture edges during expansion, whilst higher doses stimulated suture fusion. Fluorescence microscopy, micro CT and radiography.	(Liu *et al.*, 2013)
rhBMP-2	Rabbit, bilateral mid-ulna osteotomies - 0.5 to 1 mm		4 weeks	Calcium phosphate paste, implant	670 µg/mL	Complete bone bridging was observed with greater mineralised callus formation (1.6 fold) in rhBMP-2 treated groups. Torsional stiffness and strength were also significantly increased by 1.6 and 2 fold respectively. Biomechanical testing, CT, histology and radiography.	(Li *et al.*, 2003)
rhBMP-2	Male New Zealand White Rabbit, tibial fracture - 0.5 mm		4 to 28 d	PLGA particles in dehydrated collagen gel, implant	4,000 µg/mL	Particles prevented migration of cells hindering rhBMP-2-induced bone callus formation, however, rhBMP-2 treatment increased callus formation by approx. 1.35 fold compared to controls. Histology.	(Bax *et al.*, 1999)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

rhBMP-2	Female Ovariectomized Wistar Rat, tibial segmental defect – 4mm		8 weeks	Collagen sponge, implant	1 µM	Implants demonstrated increased bone strength, callus formation and calcification. Histology and radiography.	(Sarban *et al.*, 2009)
rhBMP-2	Rabbit, calvarial defect (critical size)		6 weeks	PCL fumarate with PVA and HA scaffolds, implant	unknown	Delivery of biologically active rhBMP-2 promoted bone healing within critical size defects	(Kim *et al.*, 2012)
Rat							
rhBMP-2	Sprague-Dawley Rat, cranial defect – 5mm		8 weeks	PCL/PEG scaffold, implant	0.135 µg	New bone formation was observed bridging the cranial defect. Micro CT.	(Srouji *et al.*, 2011)
BMP-2-derived peptide	Wistar Rat, ectopic implantation in calf muscle		3 to 8 weeks	Alginate hydrogel, implant	0.2 µg	Ectopic bone formation was observed in alginate hydrogel linked with BMP-2-derived peptide. Histology and immunohistochemistry.	(Suzuki *et al.*, 2000)
rhBMP-2	Sprague-Dawley Rat, femur segmental defect – 5mm		9 weeks	Demineralised rat bone, implant	1.4 and 11 µg	rhBMP-2 induced endochondral bone formation within defects in a dose-dependent manner. High dose rhBMP-2 resulted in bone union. Biomechanical testing, histology and radiography.	(Yasko *et al.*, 1992)
rhBMP-2	Wistar Rat, cranial defect – 6mm		2 weeks	Monoolein and Poloxamer 407	1 to 7 µg	Analysis showed an approx. 1.4 fold increase in collagen fiber deposition with 3 µg BMP-2 compared to with lower doses. Histology.	(Issa *et al.*, 2009)
rhBMP-2	Sprague-Dawley Rat, femur segmental defect – 4 × 5 mm		3 to 9 weeks	β-TCP and monocalcium phosphate monohydrate - implant	1.26 and 6.28 µg	High dose rhBMP-2 generated a large bone shell around the defect, resulting in osseous union within 3 weeks. Failure torque and bone stiffness recovered 2 and 2.4 fold after 9 weeks compared with intact contralateral femur. Biomechanical testing, histology and radiography.	(Ohura *et al.*, 1998)
rhBMP-2	Male Sprague-Dawley Rat, femur segmental defect – 6mm		4 and 8 weeks	Biodegradable PUR, implant	2 µg	PUR scaffolds delivered sustained BMP-2 release inducing 1.5 fold more bone formation. Histology and micro CT.	(Brown *et al.*, 2011)
rhBMP-2	Long-Evans Rat, calvarial defect		7 to 21 d	Insoluble collagenous bone matrix, implant	2.2 and 6.5 µg	Mineralized bone formation was observed, maturing over 21 days. Histology, histomorphometry and radiography.	(Marden *et al*., 1994)
rhBMP-2	Sprague-Dawley Rat, calvarial defect – 8 mm		4 weeks	Fibrin or heparin-functionalised nanoparticle-fibrin gel, implant	4 µg	rhBMP-2 induced new bone formation which exhibited greater maturity and mineralisation. Defect closure increased from 24 % to 70 % with rhBMP-2 addition. Histology, immunohistochemistry and radiography.	(Chung *et al.*, 2007)
rhBMP-2	Female Sprague-Dawley Rat, bilateral femur defect – 6mm		2 to 12 weeks	Alginate & electrospun PCL, implant	5 µg	Defect sites exhibited effective bone healing with new bone formation. Mechanical loading further enhanced bone formation. Biomechanical testing, faxitron, histology and micro CT.	(Boerckel *et al.*, 2012)
rhBMP-2	Wistar Rat, cranial defect – 5mm		2 weeks	Monoolein	5 µg	rhBMP-2 within monoolein exhibited potent osteoinductive action generating 2 fold more new bone compared to monoolein alone. Histology and immunohistochemistry.	(Iyomasa *et al.*, 2012)
rhBMP-2	Male Wistar Rat, cranial defect – 6mm		4 weeks	Monoolein	5 and 10 µg	Analysis revealed a max. 3.8 fold increase in novel bone formation. Histology, histomorphometry and immunohistochemistry.	(Issa *et al.*, 2012)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

rhBMP-2	Female Sasco Sprague-Dawley Rat, bilateral segmental femur defects – 8mm		2 to 12 weeks	Alginate hydrogel within PCL nanofiber tube, implant	5 µg	Spatiotemporal protein delivery through alginate and PCL constructs showed evidence of enhanced bone regeneration with a 2.25 fold increase in total bone volume compared to collagen control gels. Biomechanical testing, histology, micro CT and radiography.	(Kolambkar *et al.*, 2011)
rhBMP-2	Male Wistar Rat, cranial defect – 6mm		4 weeks	Monoolein	5 µg	Addition of pure rhBMP-2 increased the bone trabeculae volume fraction 1.08 fold without collagen and 1.12 fold with collagen, compared to critical defect control. Histology and histomorphometry.	(Issa *et al.*, 2010)
rhBMP-2	Sprague-Dawley Rat, calvarial defect – 8mm		8 weeks	Apatite-coated PLGA/HA particulates within fibrin gel, implant	5 µg	Implants demonstrated virtually complete surface bone healing of the critical-sized calvarial defect. Approx. 50 fold increase in new bone was observed. Histology and radiography.	(Kim *et al.*, 2008)
rhBMP-2	Lewis Rat, femoral defect – 5mm		12 weeks	PLGA microparticles in PPF scaffold, implant	8 µg	New bone formation was observed but no defect bridging due to the barrier action of PPF. Torsional stiffness increased a max.3.6 fold compared to controls. Biomechanical testing, histology, micro CT and radiography.	(Henslee *et al.*, 2011)
rhBMP-2	Long-Evans Rat, calvarial defect – 8 mm		21 d	PLGA, implant	10 and 30 µg	Radiopacity and normal calvarial contouring restoration of inner and outer bone was observed. Histology, histomorphometry and radiography.	(Kenley *et al.*, 1994)
rhBMP-2	Long-Evans Rat, femur segmental defect – 5 mm		6 and 15 weeks	PPF/TCP and dicalcium phosphate dehydrate used as a carrier, implant	10 µg	Scaffolds with rhBMP maintained bone length and allowed for bone bridging exhibiting restored mechanical strength. Histology, micro CT and radiology.	(Chu *et al.*, 2007)
rhBMP-2	Male Sprague-Dawley Rat, femur segmental defect – 8mm		6 weeks	Collagen sponge, implant	30 µg	Implants showed significant healing with approx. 15.9 fold increase in new bone formation exhibiting 6.3 and 18 fold increase in breaking load and stiffness, respectively. Biomechanical testing, histology and qCT.	(Tolli *et al.*, 2011)
rhBMP-2	Sprague-Dawley Rat, closed tibial fracture		28 and 42 d	PLA, implant	50 µg	Callus volume was not affected however callus mineralisation was significantly increased approx. 17.1 and 36.9 fold following 28 and 42 d. Significantly elevated maximum load (2.1 fold) and torsional stiffness (2.3 fold) were also observed after 42 days. Biomechanical testing, histology and radiography.	(Schmidmaier *et al.*, 2002)
rhBMP-2	Female Sprague-Dawley Rat,		2 to 6 weeks	Collagen sponge, implant	50 µg	Mechanical loading further enhanced BMP-2-induced bone regeneration and defect bridging. Histology, histomorphometry and micro CT.	(Schwarz *et al.*, 2012)
rhBMP-2	Rat, ectopic subcutaneous injection and carrier implant		5 to 21 d	Demineralised matrix carrier, implant	12 to 115 µg	Ectopic bone formation was observed following mineralisation of hypertrophic cartilage. Histology.	(Wang *et al.*, 1990)
rhBMP-2	Male Wistar Rat, cranial defect – 8mm		4 and 12 weeks	CPC/PLGA microparticle injection	55 and 275 µg	Novel incomplete bone bridging of critical size cranial defects was observed at 12 weeks following slow sustained BMP-2 release. After 4 weeks, bone volume increased approx. 1.5 fold compared to plain controls. Histology, histomorphometry and micro CT.	(Bodde *et al.*, 2008)
rhBMP-2	Sprague-Dawley Rat, mid-diaphyseal femur defect – 6mm		1 to 4 weeks	Type I bovine collagen sponge, implant	200 µg	Defect sites inoculated with bacteria (*S. aureus*) and BMP-2 upregulated BMP-2 receptor expression; overall faster bone healing. qPCR.	(Brick *et al.*, 2009)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

rhBMP-2	Rat, ectopic intramuscular implant		21 d	PLG scaffold, implant	500 µg	BMP-2 treatment increased radiopacity approx. 2.3 fold, bone volume approx. 17.7 fold, and osteoid volume approx. 3.6 fold. Histology, histomorphometry and radiography.	(Whang *et al.*, 1998)
rhBMP-2	Rat, calvarial defect – 5 mm		unknown	PLGA scaffold, implant	0.03 to 0.24 µg/mL	Increased bone regeneration was observed in a dose dependant manner in rhBMP-2-treated mice, with higher dose of BMP-2 inducing greater bone area, volume and density. Micro CT.	(Cowan *et al.*, 2007)
rhBMP-2	Male Sprague-Dawley Rat, calvarial defect – 8mm		2 and 8 weeks	Absorbable collagen sponge, implant	25 µg/mL	Treatment enhanced local bone formation. Histology, histomorphometry and immunohistochemistry.	(Hyun *et al.*, 2005)
rhBMP-2	Long-Evans Rat, mandibular defect – 5 mm		4 weeks	PLGA/gelatin sponge, implant	100 µg/mL	Bone defects were completely filled with new bone and enhanced within rhBMP-2 groups. Histology and histomorphometry.	(Higuchi *et al.*, 1999)
rhBMP-2	Male Fisher Rat, cranial defect – 5mm		8 weeks	CDHA, implant	200 µg/mL	Sulfated chitosan coating modified BMP-2 release from CDHA implants promoting new bone formation. Histology, histomorphometry and micro CT.	(Zhao *et al.*, 2012)
rhBMP-2	Fischer Rat, subcutaneous implant		14 and 28 d	PLGA/ gelatin sponge, implant	500 µg/mL	Implanted sponge volume decreased 28 d and became filled with induced bone. CT, histology and radiography.	(Yokota *et al.*, 2001)
rhBMP-2	Male Long-Evans Rat, ectopic subcutaneous implantation in calf muscle		9 weeks	PLGA mixed with blood clot, implant	1,000 µg/mL	Bone formation was observed by 4 weeks, maturing by 9 weeks. Histology and radiography.	(Alpaslan *et al.*, 1996)
2. Animal Models for indirect BMP-2 delivery 2.1. Large Animal Models							
Dog							
hBMP-2 (adenovirus)	Beagle Dog, orbital defect – 12mm		12 and 24 weeks	Transduced BMSCs on biocoral composite, implant	2 x 10^7 cells	Enhanced repair of critical sized defects with almost complete bone bridging. Transduced cells generated bone covering 27.4 % more defect area compared to non-transduced cells (1.8 fold increase). Histology, histomorphometry and radiography.	(Xiao *et al.*, 2010)
Horse							
BMP-2 (adenovirus)	Pony, femoral condyle drill defect – 13mm		12 to 52 weeks	Direct injections	4 x 10^9 infectious units	Both chondrocyte cloning and subchondral bone mineral density were significantly enhanced upon BMP-2 treatment compared to controls. CT, histology, histomorphometry and quantitative MRI.	(Menendez *et al.*, 2011)
rhBMP-2 (adenovirus)	Horse, metatarsal osteotomy (1mm) and ostectomy (1cm)		6 weeks	E1 defective adenovirus-5 with cytomegalovirus promoter, injection	5 x 10^{11} particles	Large strong bone callus formation within defect sites was observed. Treated osteotomy and ostectomy defects showed a 1.8 and 2.8 fold increase in bone volume and a 1.8 and 1.3 fold increase in mineral density, respectively, compared to controls. Biomechanical testing, histology, qCT and radiography.	(Ishihara *et al.*, 2008)
BMP-2 (adenovirus)	Horse, metacarpal and metatarsal osteotomies – 1mm		6 weeks	Transduced dermal fibroblasts, injection	5 x 10^7 cells	Bone healing within defect sites through endochondral ossification and mineralisation. Bone volume within defect sites increased approx. 3.2 fold compared with controls. Biomechanical testing, histology, qCT and radiography.	(Ishihara *et al.*, 2010)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

Pig							
rhBMP-2 (liposome vector)	Adult Pig, calvarial defect – 10 mm diameter, 7 mm depth		4 weeks	Autologous bone graft with liposome vector, implant	12 µg	At 4 weeks, BMP-producing cells were present which significantly enhanced new bone formation, compared with control collagen groups. Immunohistochemistry and microradiography.	(Park *et al.*, 2007)
Sheep							
BMP-2 (adenovirus)	White Mountain Sheep, Monocortical iliac crest defect – 5 mm diameter and 20mm depth		8 weeks	Single local injection	1 x 10^{11} particles	Callus formations were significantly reduced following direct application of adenovirus showing a systemic retardation of bone formation contrary to data obtained from small animal models. Biomechanical testing, histology, micro CT and radiography.	(Egermann *et al.*, 2006)
2.2. Small Animal Models							
Minipig							
BMP-2 (adenovirus)	Miniature Swine, maxillary defect – 30 x 12 mm		3 months	Type I collagen gel, implant	5 x 10^7 cells/mL	Solid bone mass exhibiting good mineralisation and woven bone structure was observed (approx. 1.5 fold increase). Biomechanical strength was similar to native bone. Biomechanical testing, CT and histology.	(Chang *et al.*, 2003)
Mouse							
rhBMP-2 (adenovirus)	C3H/HeN Mouse, radial segmental defect – 25 mm		4 to 8 weeks	Collagen sponge, implant	3 µg	Analysis of new bone formation revealed genetically engineered cell-mediated segmental defect repair. Histology, histomorphometry and *in vivo* imaging.	(Gazit *et al.*, 1999)
hBMP-2 (plasmid construct)	Male ICR Mouse, gastrocnemius muscle injection		7 to 21 d	Plasmid injection with microbubbles and sonoporation	75,000 µg (plasmid)	Non-surgical method for BMP-2 induced bone formation heterotopically. Histology, immunohistochemistry and radiography.	(Osawa *et al.*, 2009)
rhBMP-2 (adenovirus)	SCID Mouse, ectopic intramuscular injection		2 to 5 weeks	Bilateral injection	1.5 x 10^7 particles	Radiodense bone containing mature bone marrow elements was observed. Histology and radiography.	(Musgrave *et al.*, 1999)
rhBMP-2 (retroviral vector)	Female Athymic Nude Mouse, bilateral segmental defect – 8mm		12 weeks	Transfected hMSCs on type I collagen coated PCL scaffold, implant	5 x 10^8 to 5 x 10^9 particles	Bone bridge formation was enhanced showing an approx. 1.3 and 1.5 fold increase in bone mineral volume after 6 and 12 weeks respectively, compared to control groups. Biomechanical testing, micro CT and radiography.	(Dupont *et al.*, 2012)
BMP-2 (retroviral vector)	Female C57BL/6 Mouse, femoral segmental defect – 4mm		6 weeks	Viral coated femoral allograft, implant	1 x 10^7 to 1 x 10^{10} particles	Callus formation was observed exhibiting a 3.8, 10.9 and 2.7 fold increase in bone volume, torsional rigidity and max. torque compared to plain allograft when treated with 10^{10} particles. Biomechanical testing, histology, histomorphometry and micro CT.	(Yazici *et al.*, 2011)
rhBMP-2 (adenovirus)	SCID Mouse, calvarial defect – 5 mm		4 weeks	Transduced primary muscle-derived cells on collagen sponge, implant	5 x 10^5 cells	Bone healing was observed within mice transplanted with genetically engineered muscle cells. Treated groups showed 95 % to 100 % defect closure after 4 weeks compared to 30 % to 40 % closure observed in controls. Histology and immunohistochemistry.	(Lee *et al.*, 2001a)
BMP-2 (lentivirus)	Balb/c/nu Mouse, femur fracture defect		4 weeks	Lentiviral transduced human MSCs in Matrigel, injection	5 x 10^5 cells	BMP-2-MSCs generated mature chondrocytes following injection, and correlate with extensive bone marrow formation. Histology and qPCR.	(Choi *et al.*, 2011)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

rhBMP-2 (retroviral vector)	Female C3H/HeN Mouse, radial bone segmental defect – 2.5mm, posterior spinal fusion		2 weeks (radial) 3 and 6 weeks (spine)	Transfected hMSCs within PFTBA enriched fibrin hydrogel, implant	1 x 10^6 cells (radial), 3 x 10^6 cells (spine)	Both defect sites displayed increased bone regeneration, and exhibited enhanced bone volume and quality (1.4 fold increase). New bone (2.5 fold increase) was also observed within ectopic implant sites subcutaneously and intramuscularly. Histology and micro CT.	(Kimelman-Bleich *et al.*, 2009)
BMP-2 (plasmid vector)	Female C3H/HeN Mouse – spinal fusion model		4 weeks	Genetically engineered MSCs, implant	2 x 10^6 cells	Newly formed bone fused the spine through active osteogenesis. Histology and micro CT.	(Hasharoni *et al.*, 2005)
rhBMP-2 (plasmid vector)	Female C3H/HeN Mouse – radial segmental defect – 2.5 mm		10 and 35 weeks	Genetically engineered MSCs on a collagen sponge, implant	2 x 10^6 cells	Graft sites showed significantly increased bone formation (approx. 4 fold) exhibiting enhanced biomechanical strength (approx. 2 fold) after 10 weeks. Biomechanical testing, histology and micro CT.	(Kallai *et al.*, 2010)
BMP-2 (adenovirus)	NOD/SCID Mouse, heterotopic quadriceps injection		2 weeks	Transduced ovine cells – PEG diacrylate micro-encapsulated, injection	3 x 10^6 cells	Heterotopic bone formation was observed within muscle tissue. Micro CT and radiography.	(Mumaw *et al.*, 2012)
rhBMP-2 (adenovirus)	SCID Mouse, ectopic implant in hindlimb muscle pouch		2 weeks	Transduced cells on collagen sponge, implant	5 x 10^6 cells	Orthotropic bone formation was shown 2 weeks following transplantation of transfected rat bone marrow MSCs. Histology and radiography.	(Abe *et al.*, 2002)
BMP-2 (adenovirus)	NOD/SCID Mouse, mandibular defect – 1mm		8 weeks	Collagen sponge, implant	5 x 10^6 cells	Significant enhancement of bone regeneration was observed within defects. Histology and micro CT.	(Steinhardt *et al.*, 2008)
BMP-2 (adenovirus)	NOD/SCID Mouse, heterotopic quadriceps injection		2 weeks	Transduced human MRC5 cells – PEG diacrylate micro-encapsulated, injection	5 x 10^6 cells	Microencapsulation aided prolonged cell function and BMP-2 induced bone formation heterotopically (approx. 1.8 fold increase in bone volume). Micro CT and radiography.	(Olabisi *et al.*, 2010)
BMP-2 and hMSCs	Mouse, critical sized cranial defect – 4 mm		5 weeks	Cells on silk fibroin, implant	50 µg/mL	Scaffolds loaded with BMP-2 and seeded with hMSCs resulted in significant bone ingrowth and formation (approx. 5.6 fold increase compared to scaffold alone). Histology and histomorphometry.	(Karageorgiou *et al.*, 2006)
rhBMP-2 (adenovirus)	Female CD Nude Mouse, radial segmental defect – 25 mm		45 d	Transduced human MSCs on collagen sponge, implant	unknown	Transduced human MSCs regenerated and bridged the defect site with both new cartilage and trabecular/cortical bone. Histology, histomorphometry and micro CT.	(Turgeman *et al.*, 2001)
Rabbit							
BMP-2 (adenovirus)	New Zealand White Rabbit, femur segmental defect – 13 mm		8 to 12 weeks	Injection into the defect site	2 x 10^{10} particles	Ossification across the defect site was observed within all treated groups, exhibiting enhanced biomechanical strength and bending stiffness (2.4 and 1.7 fold increase, respectively). Biomechanical testing, histology and radiography.	(Baltzer *et al.*, 2000)
BMP-2 (adenovirus)	Female New Zealand White Rabbit, femur defect – 10 mm		2 to 4 weeks	Injection into the defect site	7 x 10^{10} particles	Bridging-callus formation resulted in higher ossification within BMP-treated groups. Histology and radiography.	(Southwood *et al.*, 2004)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

BMP-2 (retroviral vector)	Female New Zealand White Rabbit, patella osteochondral defect – 3.6mm diameter x 3mm depth		4 and 12 weeks	Transfected chondrocytes within fibrinogen clot, implant	1 x 10^5 cells	BMP-2 increased bone tissue repair 2 fold, but failed to induce complete defect healing despite efficient transduction and stable expression. Histology, histomorphometry, immunohistochemistry and qPCR.	(Vogt *et al.*, 2009)
Rat							
BMP-2 (adenovirus)	Male Sprague-Dawley Rat, femur segmental defect – 5 mm		8 weeks	Percutaneous injection	4 x 10^8 particles	Osseous union was observed with increased defect bridging by lamellar and trabecular bone exhibiting high mineral density similar to intact bone. Mean bone volume was increased 5 fold compared to control groups. Histology, histomorphometry and radiography.	(Betz *et al.*, 2006)
BMP-2 (adenovirus)	Sprague-Dawley Rat, femur defect		8 weeks	Single injection	4 × 10^8 particles	Union incidence was markedly increased when administration of adenovirus was delayed. Greater bone mineral content within the defect site and improved average mechanical strength of the healed bone was observed. Biomechanical testing, DXA, micro CT and radiography.	(Betz *et al.*, 2007a)
BMP-2 (adenovirus)	Sprague-Dawley Rat, femur defect		8 weeks	Percutaneous injection	2.7 x 10^7 to 2.7 x 10^9 particles	Bone mineral content and bone volume of the defect receiving high dose adenovirus were significantly higher than those receiving lower dose, exhibiting trabecular bone and small amounts of cartilage. DXA, histology, micro CT and radiography.	(Betz *et al.*, 2007b)
hBMP-2 (adenovirus)	Fischer F344 Male Rat, calvarial defect – 8mm		8 weeks	Transduced autograft muscle	1 x 10^{10} viral particles (8mm x 2mm graft)	Transduced muscle underwent osteogenic differentiation forming new bone (approx. 2 fold increase in mineralised tissue). Rapid cartilage formation was observed which may have undergone endochondral ossification. DXA, histology and micro CT.	(Liu *et al.*, 2012)
rhBMP-2 (adenovirus)	Athymic RNU Nude Rat, fibula segmental defect – 2/4mm		1 to 12 weeks	Transduced human fibroblasts (MRC5), injected	5 x 10^4 to 5 x 10^7 cells	The system delivers 130x less BMP-2 and shows equivalent bone formation and regeneration to other studies. Bone volume increased 10 fold between high and low cell numbers. Histology, micro CT and radiography.	(Lazard *et al.*, 2011)
hBMP-2 (adenovirus, retrovirus and cationic lipid vectors)	Male Fischer Rat, calvarial defect – 8 mm		30 d	Transduced/transfected cells seeded on titanium mesh, implant	4 x 10^5 cells	Enhanced osteogenic capacity of transduced cells mediated bone healing. Immunohistochemistry, histology and histomorphometry.	(Blum *et al.*, 2003)
rhBMP-2 (adenovirus)	Wistar Rat, mandibular defect – 6 mm		1 to 8 weeks	Transduced cells on collagen sponge, implant	1 x 10^6 cells	Critical size defects were completely healed. Histology, in situ hybridisation and radiography.	(Park *et al.*, 2003)
hBMP-2 (adenovirus)	Rat, femur defect – 8 mm		2 months	Transduced cells on DBM, implant	5 x 10^6 cells	Bone healing with coarse trabecular structure was observed. Bone area increased approx. 7.5 fold compared to scaffold alone. Biomechanical testing, histology, histomorphometry and radiography.	(Lieberman *et al.*, 1999)
BMP-2 (adenovirus)	Female NIH rnu Athymic Nude Rat, Distal femoral osteotomy		2 weeks	Injection of transduced cells within alginate beads, or direct injection	5 x 10^6 cells	Direct injection showed completely healed defects. Bone density was 2 fold higher in treated groups compared to saline controls. However, bone healing within alginate groups was impeded by development of a chondroid mass. Histology and micro CT.	(Zachos *et al.*, 2007)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

BMP-2 (lentivirus)	Lewis Rat, vertebral lumbar fusion		4 to 8 weeks	Transduced rat bone marrow cells	5 x 10^6 cells	Sustained BMP-2 delivery induced increased bone formation and spinal fusion. Histochemistry, histology, micro CT and radiography.	(Miyazaki *et al.*, 2008)
BMP-2 (plasmid construct)	Sprague-Dawley Rat, calvarial defect – 8mm		4 to 16 weeks	Transfected rat cells in alginate gel, implant	1 x 10^7 cells	Complete repair of cranial defects was observed through new bone formation. Histology and RT-PCR.	(Lin *et al.*, 2008)
rhBMP-2 (retroviral vector)	Fischer and Sprague-Dawley Rat, calvarial defect – 5mm		4 to 10 weeks	Transfected dermal fibroblasts on collagen sponge, implant	2 x 10^7 cells	Treatment promoted bone formation in defect sites, increasing calcified tissue area approx. 9 fold compared to control groups. Histology, immunohistochemistry and radiography.	(Wang *et al.*, 2009)
3. Animal Models for combinational direct and indirect BMP-2 delivery 3.1. Large Animal Models							
Dog							
BMP-2 and VEGF	Beagle Dog, ectopic paraspinal muscle implant and ulna critical size defect		9 weeks	Gelatin (fast release) or PLGA microparticle (sustained release) loaded biphasic calcium phosphate scaffold, implant	12 µg BMP-2 0.4 µg VEGF (ectopic site) 120 µg BMP-2 4 µg VEGF (ulnar defect)	Ectopic BMP-2 fast release showed significantly more bone compared to sustained release, independent of the VEGF profile. Significant enhancement of bone formation was observed within all orthotopic groups compared to controls independent of growth factor release profile or combination. Histology, histomorphometry and immunohistochemistry.	(Geuze *et al.*, 2012)
BMP-2, bFGF, PDGF and TGF-β	Adult Hound, mandibular premolar implant osteotomies		12 weeks	Titanium implants with non-HA calcium phosphate cement	unknown	Growth factor combination increased bone formation compared to controls. Histology and histomorphometry.	(Meraw *et al.*, 2000)
Horse							
BMP-2 and BMP-7 (adenovirus)	Horse, metacarpal IV ostectomy – 15mm		2 to 16 weeks	Direct injection	2 x 10^{11} virus particles	Bone defect regeneration was not enhanced further than controls when treated with BMP-2 and BMP-7 viral particles, possibly due to incorrect dosing. DXA, histology and radiography.	(Southwood *et al.*, 2012)
Pig							
rhBMP-2 and VEGF	Female Domestic Pig, cranial drill defect – 4.2mm		1 to 4 weeks	Titanium plugs with adsorbed octacalcium phosphate, implant	5.26 µg BMP-2, 1.32 µg VEGF	Implants revealed enhanced bone volume density (approx. 1.6 fold increase), but not osseointegration. Histology, histomorphometry and micro-radiography.	(Ramazanoglu *et al.*, 2011)
3.2. Small Animal Models							
Minipig							
BMP-2 and BMP-7	Minipig, calvarial defect – 8mm		2 to 6 weeks	Collagen sponge, implant	5 µg	Combination treatment increased bone formation 2.1, 1.4 and 1.6 fold compared to controls, BMP-2 alone, and BMP7 alone. Histology, histomorphometry, immunohistochemistry,	(Sun *et al.*, 2012b)
BMP-2 and BMP-7	Guangxi Bama Minipig, calvarial defect – 8mm diameter x 4mm depth		2 to 6 weeks	Collagen sponge, implant	5 µg (30 ng/mm^3)	Low dose BMP-2/7 heterodimer facilitated more rapid bone regeneration (approx. 1.5 fold increase in bone volume compared to either factor alone) of improved quality. Micro CT.	(Wang *et al.*, 2012)
Mouse							
rhBMP-2, VEGF-A and FGF-2	CD-1 Mouse, calvarial defect – 2 mm		12 weeks	Collagen sponge, implant	0.2 µg	BMP-2 and VEGF-A showed enhanced healing capacities compared to FGF-2, however no significant differences between VEGF-A and BMP-2 were observed. Immunohistochemistry and micro CT.	(Behr *et al.*, 2012)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

rhBMP-2 and hMSCs	BALB/c Mouse, subcutaneous injection		2 to 7 weeks	pH/thermosensitive polymer hydrogel, injection	0.5 µg rhBMP-2 and 1 x 10^5 cells	Ectopic mineralisation and calcium deposition was observed. Histology and immunohistochemistry.	(Kim *et al.*, 2009)
rhBMP-2 and Epo	C57BL/6 Mouse, cranial defect – 5mm		5 to 42 d	Collagen sponge, implant	1 µg rhBMP-2 and 1,000 U/mL Epo	Epo enhanced BMP-2-induced bone formation. Histology, histomorphometry and micro CT.	(Sun *et al.*, 2012a)
rhBMP-2 and Bisphosphonate (zoledronic acid)	NF1deficient Mouse, neurofibromatosis model – fracture		3 weeks	CMC carrier, implant	10 µg	BMP-2 and bisphosphonate administration demonstrated enhanced bone union, decreasing non-unions by 50% and increased bone volume by 31%. Histology and radiography.	(Schindeler *et al.*, 2011)
rhBMP-2 and VEGF	Male MF1 nu/nu Mouse, femur segmental defect – 5mm		4 weeks	Alginate and PLA, implant	20 µg	Enhanced bone repair (approx. 3.2 fold increase) was observed within the defect environment. Histology, immunohistochemistry and micro CT.	(Kanczler *et al.*, 2010)
rhBMP-2 and BMP binding protein (BBP)	Male Lewis Rat, ectopic subcutaneous and intramuscular implantation		2, 4 and 7 d	Collagen sponge, implant	20 µg rhBMP-2 500 µg BBP	Addition of BBP reduced rhBMP-2 related inflammation area a max. 1.4 and 1.3 fold subcutaneously and intramuscularly, respectively. Histology, histomorphometry and MRI.	(Lee *et al.*, 2011)
BMP-2/VEGF (recombinant adeno-associated virus 6)	Athymic Nude Mouse, tibiae segmental defect – 2/3mm		5 weeks	Transduced male mouse MSCs, intravenous injection	1 x 10^6 cells: 5 x (2 x 10^5 cells per d)	Enhanced bone formation was observed within defect sites compared to control groups. Bone volume, peak load and stiffness increased approx. 6, 4.5 and 4 fold respectively, compared to control groups. DXA, histology, immunohistochemistry and micro CT.	(Kumar *et al.*, 2010b)
BMP-2 and Runx2 (plasmid vector)	Female Athymic Mouse (BALB/c-nu), subcutaneous implantation		6 weeks	Transfected adipose derived stem cells on PLGA scaffold, implant	1 x 10^6 cells	Histological analysis revealed enhanced bone formation (approx. 4 fold increase in bone area) through promoted osteogenic activity. Biochemistry, histology, RT-PCR and western blot.	(Lee *et al.*, 2010)
rhBMP-2 and Mouse α4 integrin (recombinant adeno-associated virus 2)	Female OVX C57BL/6 Mouse (osteopenia model)		1 to 15 weeks	Transduced male mouse MSCs, intravenous injection	2 x 10^6 cells: 5 x (4 x 10^5 cells) – 1 per day	Transduced MSCs were found to restore bone growth by both direct incorporation and indirect pro-osteoblastic activity. Bone mineral density increased 1.26 fold compared to non-transduced controls at 5 weeks. DXA, histology, immunohistochemistry and micro CT.	(Kumar *et al.*, 2010a)
BMP-2 and VEGF (plasmid vector)	BALB/c nu/ nu Nude Mouse, ectopic intramuscular implant		4 and 8 weeks	Transfected cells on β-TCP scaffold, implant	2.5 x 10^6 cells/mL	Co-expression showed significant bone formation compared to BMP-2 alone at 4 weeks. Bone area increased 10 fold by 8 weeks. VEGF may enhance BMP-2 induced bone formation through angiogenesis modulation. Histology, histomorphometry, immunohistochemistry and *in situ* hybridization.	(Samee *et al.*, 2008)
BMP-2 and BMP7 (adenovirus)	C57BL6 Mouse, critical sized calvarial defect – 7mm		4 weeks	Transduced mouse BLK cells in gelatin sponge, implant	4 x 10^6 cells	Implants revealed robust osteogenic activity with complete bone coverage of defects. Bone volume increased 2 and 1.7 fold compared to BMP-2 and BMP-7 alone, respectively. Histology, micro CT and radiography.	(Koh *et al.*, 2008)
Rabbit							
BMP-2 and VEGF	Male New Zealand Rabbit, intramedullary femur defect.		2 to 12 weeks	Growth factor loaded-PLGA microspheres within porous PLGA scaffolds, implant	3.5 or 17.5 µg BMP-2 0.35 or 1.75 µg VEGF	Co-expression exhibited a temporal dose-dependent positive synergistic effect on bone formation, with new bone after 2 weeks and neovascularisation after 4 weeks. Histology, histomorphometry and immunohistochemistry.	(Hernandez *et al.*, 2012)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

BMP-2 and TGF-β2	New Zealand White Rabbit, femoral condyle defect – 3mm		28 d	Copolymer coated titanium cylinders, implant	12.5 µg BMP-2 0.625 µg TGF-β2	Dual growth factor delivery induced 13.5% more bone formation compared to controls. Interestingly, delivery of BMP-2 alone increased bone formation by 15.4%. Biomechanical testing and micro CT.	(Thorey *et al.*, 2011)
BMP-2 and $rhVEGF_{165}$	New Zealand Rabbit, sinus floor elevation		4 and 12 weeks	Sonication-induced silk hydrogel, implant	30 µg BMP-2 20 µg $VEGF_{165}$	VEGF promoted higher tissue infiltration and accelerated gel degradation. Co-expression induced significantly larger bone area than single factor groups and the silk control (1.4 to 4.9 fold more bone). Histology, histomorphometry, micro CT and radiography.	(Zhang *et al.*, 2011a)
rhBMP-2 and MSCs (rabbit)	New Zealand White Rabbit, cranial defect – 15 mm		8 and 16 weeks	Coral scaffold, implant	200 µg rhBMP-2 1 x 10^7 MSCs	Generated new bone comparable to auto-bone-graft repair. Regenerated bone defect area was increased approx. 3.9 fold compared to scaffold alone. Histology, immunohistochemistry and radiography.	(Hou *et al.*, 2007)
BMP-2 and VEGF (baculovirus)	New Zealand White Rabbit, femoral segmental defect – 10mm		8 weeks	Transduced rabbit MSCs in PLGA scaffold, implant	3 x 10^6 cells	Increased bone formation of improved quality was observed. Torsional stiffness and maximum torque were increased 208 and 26.5 fold respectively, compared to scaffold alone. Biomechanical testing, histology, immunohistochemistry, micro CT, micro PET and radiography.	(Lin *et al.*, 2010)
BMP-2, VEGF and FLP (baculovirus)	Female New Zealand White Rabbit, calvarial defect – 8mm		4 and 12 weeks	Transduced rabbit MSCs in PLGA scaffold, implant	3 x 10^6 cells	FLP episome formation prolonged BMP-2/VEGF expression and augmented bone repair from rabbit MSCs. Bone regeneration area increased 3.4 fold compared to scaffold alone. Histology, micro CT, micro-PET and radiography	(Lin *et al.*, 2012a)
BMP-2 and VEGF	Female New Zealand White Rabbit, bilateral orbital defect – 12mm		4 to 16 weeks	Transduced BMSCs on biocoral composite, implant	4.8 x 10^6 cells BMP-2 1.2 x 10^6 cells VEGF	New bone deposition and formation was observed, indicating mimicking of natural bone development. Bone volume increased approx. 20 fold after 4 weeks, 3 fold after 8 weeks and 2 fold after 16 weeks, compared to controls. Histology, histomorphometry, immunocytochemistry, micro CT and radiology.	(Xiao *et al.*, 2011)
Rat							
BMP-2 and rMSCs	Male Sprague-Dawley Rat, calvarial defect – 8mm		1 to 9 weeks	PEG-DA and PEG-MMP degradable, implant	0.0025 µg (PEG-DA), 0.0075 µg (PEG-MMP) and 1 x 10^7 cells	PEG-MMP alone improved bone regrowth. Addition of BMP-2 and MSCs did not show further enhancement of bone regeneration. Histology and micro CT.	(Terella *et al.*, 2010)
BMP-2 and PDGF-BB	Male Sprague-Dawley Albino Rat, calvarial defect - 6 mm		4 weeks	Fibrinogen scaffold, implant	0.05 µg	Fibronectin and GFs improved bone healing compared to growth factors alone. Flow cytometry and micro CT.	(Martino *et al.*, 2011)
BMP-2 and VEGF	Male Fischer Rat, cranial defect – 8mm		12 weeks	Loaded PPF/gelatin microparticle composite, implant	0.5 to 2µg BMP-2 6 to 12µg VEGF	Dose-dependent increase in bone formation was observed with increased BMP-2 (approx. 5.3 fold). VEGF showed no further augmentation, however increased defect bridging was observed. Histology and micro CT.	(Young *et al.*, 2009)
rhBMP-2 and Zoledronic acid	Rat, femoral fracture model		6 weeks	Collagen sponge, implant	1 µg rhBMP-2	Effective fracture repair was observed with significantly enhanced bone fusion. Bone volume and stiffness increased approx. 1.5 and 8.3 fold respectively, compared to scaffold alone. Biomechanical testing, histology, micro CT and radiography.	(Doi *et al.*, 2011)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

BMP-2 and VEGF	Syngeneic Fischer-344 Rat, calvarial defect – 8 mm		4 and 12 weeks	Gelatin microparticles entrapped within PPFscaffolds, implant	2 µg BMP-2 12 µg VEGF	VEGF increased blood vessel formation and acted synergistically with BMP-2 to enhance bone formation. Bone volume was increased approx. 5 fold. Histology and micro CT.	(Patel *et al.*, 2008)
BMP-2 and rMSCs	Male Sprague-Dawley Rat, calvarial defect – 8mm		4 and 8 weeks	Chitosan gel, injection	2 µg BMP-2 3 x 10^5 cells	Significant bone regeneration was observed within defect sites showing increased bone volume (approx. 3.4 fold) and osseointegration. Histology, immunohistochemistry and micro CT.	(Stephan *et al.*, 2010)
BMP-2 and FGF-2	Rat, femur defect		4, 8 and 12 weeks	Nanostructured colloidal gelatin within porous titanium scaffold, implant	3 µg BMP-2 0.6 µg FGF-2	Bone formation was accelerated; observed earlier than controls. Increased bone volume was also observed exhibiting superior bone-implant integrity. Biomechanical testing, histology and micro CT.	(van der Stok *et al.*, 2013)
BMP-2 and FGF-2	Wistar Rat, ectopic intramuscular implantation		3 weeks	Type I collagen carrier, implant	5 µg BMP-2 0.0016 to 50 µg FGF-2	Combination treatment with 0.08 µg FGF-2 exhibited the greatest increase in bone area compared to controls (3.3 and 2.1 fold, assessed by radiopacity and histology, respectively), and BMP-2 alone (1.7 and 1.07 fold). Biochemistry, histology, histomorphometry and radiography.	(Fujimura *et al.*, 2002)
BMP-2 and FGF-2	Male ddY Mouse, subfascial implantation		1, 2 and 3 weeks	Porous collagen disc, implant	5 µg BMP-2 0.001 to 5 µg FGF-2	Combination treatment with lose dose FGF-2 (0.001 µg) increased mineral density, radiopacity and bone formation approx. 1.2, 2 and 1.5 fold, respectively after 3 weeks. DXA, histology, histomorphometry and radiography	(Nakamura *et al.*, 2005)
BMP-2 and PTH (1-34)	Harlan Sprague-Dawley Rat, mid-diaphyseal femoral defect – 5mm		8 weeks	PLGA microspheres in gelatin hydrogel, implant Subcutaneous injection (PTH (1-34))	6.5 µg BMP-2 10 µg/kg PTH	PTH did not induce bone formation in empty control defects. BMP-2 scaffolds with PTH treatment increased bone volume by approx. 4.1 and 1.7 fold compared to control defects and BMP-2 scaffold only, respectively. DXA, histology and micro CT.	(Kempen *et al.*, 2010)
rhBMP-2 and VEGF	Harlan Sprague-Dawley Rat, ectopic subcutaneous implant and femoral defect – 5mm		4 and 6 weeks	PLGA in poly-PPF (rhBMP-2) within gelatin hydrogel (VEGF), implant	6.5 µg rhBMP-2 and 2 µg VEGF	Microangiography showed an approx. 2 fold increase in vascularization within subcutaneous implants. Bone formation within orthotopic defects also demonstrated an approx. 2 fold increase compared to controls. Histology, histomorphometry and micro CT.	(Kempen *et al.*, 2009)
rhBMP-2 and Tobramycin	Sprague-Dawley Rat, femur segmental defect – 5mm		8 weeks	Collagen sponge, implant	11 µg rhBMP-2 and tobramycin	Enhanced bone formation was observed upon dual application of rhBMP-2 and tobramycin. Bone area was increased approx. 1.2 fold compared to controls. Biomechanical testing, DXA, histology, micro CT and radiography.	(Glatt *et al.*, 2009)
rhBMP-2 and rhBMP-7 (plasmids)	Male Wistar Rat, ectopic intramuscular injection		1 to 10 d	Direct injection and *in vivo* electroporation	12.5 µg	Enhanced bone formation and calcification was observed compared to single modalities, with upregulation of BMP-4. Histology, immunohistochemistry, radiography and rtPCR.	(Kawai *et al.*, 2006)
rhBMP-2 and rhBMP-7 (plasmid)	Male Wistar Rat, ectopic intramuscular injection		10 d	Direct injection of double cassette plasmid and *in vivo* electroporation	50 µg	Enhanced bone formation and calcification was observed compared to single modalities. Histology and radiography.	(Kawai *et al.*, 2009)
rhBMP-2 and bFGF	Rat (WKY, Lewis, Fisher), irradiated mandible		7 weeks	Liquid injection	100 µg	Irradiation reduced bone apposition, BMP-2 or bFGF alone generated new bone, but together they did not show bone maintenance. Fluorescence microscopy, histology and micro CT.	(Springer *et al.*, 2008)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

BMP-2 and BMP7 (adenovirus)	Sprague-Dawley Rat, bilateral single level posterolateral spine fusion		8 weeks	Allograft bone and CMC hematostatic foam soaked with adenovirus, implant	0.75 x 10^8 to 0.75 x 10^{11} particles	Co-administration of Ad.BMP-2 and Ad.BMP7 resulted in significantly greater numbers of mechanically stable fusions and also 2 fold higher mineralisation rate of bone volume in the fusion mass. Histology and radiography.	(Zhu *et al.*, 2004)
BMP-2 and rhPDGF-BB (adenovirus)	Male Sprague-Dawley Rat, calvarial defect – 8mm		2 to 4 weeks	rhPDGF protein and BMSCs expressing BMP-2, implant	1 x 10^6 cells	Increased bone regeneration by delivery of autologous AdBMP2-transfected BMSCs and rhPDGF-BB in both the amount of new bone formed and bone mineral density. The bone growth was greater than those observed in the group treated with AdBMP2-transfected BMSCs alone.	(Park *et al.*, 2013)
BMP-2 and BMP-7 (lentivirus)	Sprague-Dawley Rat, calvarial defect – 2mm		2 to 6 weeks	Transduced cells in β-TCP scaffold, implant	1 x 10^6 cells	New bone formation was observed after 4 weeks in all treated defects. Only dual factor treated defects exhibited complete defect regeneration after 6 weeks. Biochemistry, cytochemistry, histology, qPCR and radiography.	(Qing *et al.*, 2012)
4. Human Trials							
Facial Reconstruction and Bone Augmentation							
rhBMP-2		Human, maxillary sinus floor augmentation	16 weeks	Absorbable collagen sponge, implant	1.77 to 3.4 mg	Significant bone growth was observed increasing maxillary sinus floor height. Biochemistry, CT and histology.	(Boyne *et al.*, 1997)
rhBMP-2		Human, mandible lesion	3 to 18 months	Collagen sponge, implant	4 to 8 mg	Osseous restoration of the edentulous area was observed with new bone formation. Radiography.	(Herford and Boyne, 2008)
rhBMP-2		Human, maxillary sinus floor augmentation	4 and 6 months	Absorbable collagen sponge, implant	0.75 to 1.5 mg/mL	Induced adequate bone for the placement and functional loading of endosseous dental implants. 76 % of patients with treated dental implants remained functional after 36 months compared to 62 % of patients who received bone graft alone. CT.	(Boyne *et al.*, 2005)
rhBMP-2		Human, alveolar ridge augmentation	unknown	Absorbable collagen sponge, implant	0.75 to 1.5 mg/mL	New bone formation was observed similar to native bone. Bone adequacy for dental implant was approx. 2 fold greater than no treatment or placebo. CT and histology.	(Fiorellini *et al.*, 2005)
rhBMP-2		Human, mandible defect	22 months	Collagen sponge, implant	1.5 mg/mL	Mandibular defects can be successfully reconstructed with BMP-2 soaked collagen scaffolds. Radiography.	(Carter *et al.*, 2008)
rhBMP-2		Human, cleft alveolar defect	1 year	Collagen sponge within gelatin sponge, implant	1.5 mg/mL	Improved bone healing and reduced morbidity, exhibiting higher bone volume and mineralisation. Treated bone defects exhibited 95 % bone filling compared to 63 % within control iliac graft defects. CT and radiography.	(Dickinson *et al.*, 2008)
rhBMP-2		Human, maxillary sinus floor augmentation	6 to 24 months	Collagen sponge, implant	1.5 mg/mL	Significant bone formation comparable to native bone in structure and density was observed. Approx. 83 % of treated patients retained their implants, fully integrated and functional at 6 months. CT and radiography.	(Triplett *et al.*, 2009)
rhBMP-2		Human, mandible resection	9 months	Collagen sponge within titanium mesh, implant	unknown	Appreciable bone regeneration was observed bridging the maxillofacial defect. Radiography.	(Cicciu *et al.*, 2012)
Long Bone Fracture and Non-Union							
rhBMP-2		Human, long bone non-union	20 months	Collagen gel and cancellous allograft bone chips, implant	12 mg	Implant analysis demonstrated reduced operative time (approx. 35 %) and intraoperative blood loss (approx. 40 %), suggesting rhBMP-2 be used as an alternative to iliac crest autograft. Radiography.	(Tressler *et al.*, 2011)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

hBMP-2 and iNCP		Human, long bone non-union	9.3 to 48.9 months	Ultra-thin gelatin capsules or PLA/PGA, implant	50 to 100 mg	Bone union was observed in most patients, with new bone formation. Radiography.	(Johnson *et al.*, 1988a)
hBMP-2 and iNCP		Human, tibial segmental defect – 3 to 17 cm	18 months	PLA/PGA, implant	50 to 100 mg	Tibial defects within five of six patients healed without further surgical treatment. Radiography.	(Johnson *et al.*, 1988b)
rhBMP-2		Human, open tibial fracture	12 months	Absorbable collagen sponge, implant	0.75 to 1.5 mg/mL	High dose rhBMP-2 resulted in reduced risk of failure (approx. 44 %), significantly fewer invasive interventions and faster wound-healing (83 % compared to 65 % in control patients). Radiography.	(Govender *et al.*, 2002)
rhBMP-2		Human, open tibial fracture	12 months	Absorbable collagen sponge, implant	1.5 mg/mL	Implants were well tolerated and yielded a healing rate equivalent to that of autogenous bone-grafting. Radiography.	(Jones *et al.*, 2006)
rhBMP-2		Human, open tibial fracture	12 months	Absorbable collagen sponge, implant	1.5 mg/mL	Bone healing was observed, reducing the frequency of autogenous bone grafts, secondary interventions and lowering the rate of infection. Radiography.	(Swiontkowski *et al.*, 2006)
rhBMP-2		Human, open tibial fracture	unknown	Collagen sponge, implant	unknown	Implantation of rhBMP-2, combined with an absorbable collagen sponge, was found to be feasible and safe. Radiography.	(Riedel and Valentin-Opran, 1999)
Lumbar Fusion							
rhBMP-2		Human, anterior cervical discectomy and fusion (ACDF)	6 to 52 weeks	PEEK, implant	1.1 mg to 2.1 mg	Bone fusion was observed between vertebrae where PEEK spacers had been implanted. Bone formation was also observed beyond the PEEK spacer confines. Radiography.	(Klimo and Peelle, 2009)
BMP		Human, anterior cervical disectomy	2 to 3 years	Fibular allograft, implant	0.9 to 2.7 mg	Patients demonstrated spinal fusion with new bone formation comparable to traditional iliac crest bone graft. Radiography.	(Buttermann, 2008)
rhBMP-2		Human, lumbar disc disease	6 years	Collagen sponge within lumbar tapered fusion cage, implant	4.2 to 8 mg	Implants showed anterior intervertebral spinal fusion with new bone formation in 98 % of patients. Disability index scores were reduced for back and leg pain showing an increase in patients returning to work (63 %) at 6 months than were in work pre-operatively (52 %), and this was sustained at 6 years (68 %). Radiography.	(Burkus *et al.*, 2009)
rhBMP-2		Human, paediatric spinal fusion	11 to 18 months	Autologous iliac crest bone graft	12 mg	Spinal fusion was achieved in children with compromised bone healing due to congenital, local and systemic conditions. Radiography.	(Mladenov *et al.*, 2010)
rhBMP-2		Human, postero-lateral arthrodesis	2 years	Collagen sponge/HA-TCP ceramic bulking agent, implant	12 mg	Implants demonstrated trabecular bone bridging and intervertebral fusion within 95 % of treated patients compared to 70 % of control patients at 24 months. CT and radiography.	(Dawson *et al.*, 2009)
rhBMP-2		Human, spinal lumbar fusion	0.5, 1 and 6 years	PLGA, implant	15 mg	Bone fusion progressed for 2 years without further improvement up to 6 years. Bone union rate within treated patients was not statistically different to that in control patients, 82 % and 91 %, respectively. Radiography.	(Katayama *et al.*, 2009)
rhBMP-2		Human, postero-lateral arthrodesis	2 years	Type I Bovine Collagen sponge/HA-TCP particles, implant	20 mg	Significant enhanced bilateral bridging bone formation and intervertebral disc fusion was observed in 96 % of treated patients compared with 89 % of control patients. CT and radiography.	(Dimar *et al.*, 2009)

Supplementary Table 1. Bone tissue engineering *in vivo* utilising BMP-2 within animal models and human trials.

rhBMP-2		Human, anterior lumbar interbody fusion	6 to 24 weeks	Bovine absorbable collagen sponge, implant	1.5 mg/mL	All patients who received rhBMP-2 showed radiographic evidence of osteoinduction and interbody fusion. New bone formation was observed outside cage implants in 82 % of treated patients after 6 months, compared with 50 % of control patients after 24 months. CT and radiography.	(Burkus *et al*., 2003a)
rhBMP-2		Human, cervical discectomy and interbody fusion	3 to 24 months	Absorbable collagen sponge, implant	1.5 mg/mL	Interbody fusion was observed through formation of new bone bridging. Treated patients exhibited improved neck and arm pain scores. CT and radiography.	(Baskin *et al.*, 2003)
rhBMP-2		Human, posterior lumbar interbody fusion	2 years	Absorbable collagen sponge, implant	1.5 mg/mL	Fusion rate was higher within treated groups (92.3 %) compared with autograft control groups (77.8 %) at 24 months. Post-surgery pain scores were also improved. CT and radiography.	(Haid *et al.*, 2004)
rhBMP-2		Human, postero-lateral lumbar fusion	2 years	Absorbable collagen sponge, implant	1.5 mg/mL	rhBMP-2 treated patients exhibited significantly increased lumbar fusion. Treated patients exhibited a 97 % fusion rate compared to 77 % in control patients. CT.	(Singh *et al*., 2006)
rhBMP-2		Human, postero-lateral spine fusion	2 years	Absorbable collagen sponge, implant	1.5 mg/mL	Enhanced spinal fusion was observed within rhBMP-2 treated patients. Non-union rate within treated patients was only 4.2 % compared to 11.4 % in control patients. CT.	(Glassman *et al*., 2007)
rhBMP-2		Human, vertebral osteomyelitis	40 months	Collagen sponge, implant	1.5mg/mL	Spinal fusion was observed in all patients receiving rhBMP-2. Infection rate was significantly reduced. CT and radiography.	(O'Shaughnessy *et al*., 2008)
rhBMP-2		Human, anterior lumbar fusion	6 to 24 months	Absorbable collagen sponge, implant	unknown	Spinal fusion was successful in most patients with rhBMP-2 treatment. Operative time, blood loss and hospital stay were all benefited by rhBMP-2 addition on collagen sponge. Radiography.	(Burkus *et al*., 2003b)
rhBMP-2		Human, postero-lateral lumbar fusion	0.5, 1 and 2 years	Bone autograft, allograft and collagen sponge, implant	unknown	rhBMP-2 delivery exhibited bone healing and fusion comparable to autograft controls. CT and radiography.	(Taghavi *et al.*, 2010)

Abbreviations: BMP-2 *(bone morphogenetic protein 2)*, **BMSC** *(bone marrow stromal cell)*, **CDHA** *(calcium deficient hydroxyapatite)*, **CHBB** *(cortico-cancellous human bone block)*, **CHPA** *(cholesterol-bearing pullulan nanogel with acrylol residue)*, **CMC** *(calcium phosphate cement)*, **CPC** *(calcium phosphate cement)*, **CT** *(computer tomography)*, **DBBB** *(deproteinized bovine bone/porcine collagen)*, **DBM** *(demineralised bone matrix)*, **DX** *(para-dioxanone)*, **DXA** *(dual energy X-ray absorptiometry)*, **Epo** *(erythropoietin)*, **FGF** *(fibroblast growth factor)*, **GM** *(gelatin microspheres)*, **HA** *(hydroxyapatite)*, **hASC** *(human adult stem cell)*, **iNCP** *(bone matrix water insoluble non-collagenous proteins)*, **MMP** *(metalloprotease)*, **MRI** *(magnetic resonance imaging)*, **MSC** *(mesenchymal stem cell)*, **PCL** *(poly-caprolactone)*, **PEEK** *(polyetheretherketone)*, **PEG** *(poly-ethylene glycol)*, **PEG-DA** *(poly-ethylene glycol-diacrylate)*, **PET** *(positron emission tomography)*, **PLA** *(poly-lactic acid)*, **PFTBA** *(perfluorotributylamine)*, **PLG** *(poly(D,L-lactide-co-glycolide))*, **PLGA** *(poly-lactic co-glycolic acid)*, **PPF** *(poly-propylene fumarate)*, **PUR** *(polyurethane)*, **PVA** *(poly(vinyl alcohol))*, **qCT** *(quantitative computer tomography)*, **qPCR** *(quantitative polymerase chain reaction)*, **rhBMP-2** *(recombinant human BMP-2)*, **rtPCR** (*reverse transcription PCR*), **RT-PCR** (*real time PCR*), **TCP** *(tricalcium phosphate)*, **SEM** *(scanning electron microscopy)* and **VEGF** *(vascular endothelial growth factor)*.

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

Growth factor (single or combination)	Animal model (species/strain, location and defect)	Human clinical trial and defect	Time	Delivery system	Dose/ Conc.	Analysis (read outs, efficacy and methodology)	Reference
1. Animal Models for direct BMP-7/OP-1 delivery 1.1. Large Animal Models							
Baboon							
OP-1 (BMP-7)	Adult Male Chacma Baboon, calvarial defect		30 to 365 d	Porous HA, implant	100 and 500 μg	Treatment repaired calvarial defects. Histology and histomorphometry.	(Ripamonti *et al.*, 2001a)
rhOP-1	Adult Male Chacma Baboon, calvarial defect – 25mm		90 to 365 d	Irradiated bovine collagen matrix, implant	100, 500 and 2,500 μg	High dose treatment increased bone and osteoid volumes 2.3 and 1.6 fold after just 90 d. Low and medium dose also exhibited bone augmentation. Histology and histomorphometry.	(Ripamonti *et al.*, 2000)
rhOP-1	Adult Male Chacma Baboon, calvarial defect – 25mm		15 to 365 d	Collagen matrix, implant	100, 500 and 2,500 μg	Lower doses OP-1 induced complete regeneration of calvarial bone defect. Histology and histomorphometry.	(Ripamonti *et al.*, 1996)
Dog							
OP-1	Labrador Dog, femoral condyle defect, implant fixation		3 weeks	Bovine type I collagen carrier, implant	300 μg	Treatment increased the volume fraction of woven bone close to the implant 1.7 fold. Histology and histomorphometry.	(Jensen *et al.*, 2002)
OP-1	Dog, femoral condyle defect - 6mm (titanium implant with 0.75mm gap)		4 weeks	Allograft, implant	400 and 800 μg	Generation of mineralised tissue which improved implant fixation was increased 1.8 and 1.5 fold following revision surgery with low and high dose treatment, respectively. Biomechanical testing, histology and histomorphometry.	(Soballe *et al.*, 2004)
rhOP-1	Adult Male Dog, ulna segmental defect – 25mm		8 to16 weeks	Collagen carrier in demineralized, guanidine-extracted, insoluble bovine bone matrix, implant	1,200 μg	Treatment resulted in formation of new cortical and cancellous bone exhibiting mechanical strength between 1.7 and 1.9 fold that of controls. Biomechanical testing, histology and radiography.	(Cook *et al.*, 1994a)
rhOP-1	Adult Male Mongrel Dog, bilateral femur segmental defect – 60mm		2 to 12 weeks	Bovine type I collagen putty and allogenic cortical graft, implant	3,500 μg	Treatment increased mineralised area 1.9, 2.7 and 2.4 fold at 2, 4 and 6 weeks. Torsional stiffness and strength were increased 2.3 and 2.2 fold. Biomechanical testing, histology and microradiography.	(Fukuroku *et al.*, 2007)
OP-1	Mature Dog, humerus condyle defect – 4mm (HA coated titanium implant with 3mm gap)		6 weeks	Bovine type I collagen carrier, implant	0.65 and 3.25 x 10^5 μg	High dose had no effect on bone formation. Low dose had a moderate effect on bone healing but no effect on implant fixation. Biomechanical testing, histology and histomorphometry.	(Lind *et al.*, 2001)
OP-1	Adult Male Mongrel Dog, bilateral ulna segmental defect – 25mm		2 to 12 weeks	Bovine type-1 collagen carrier in autograft or allograft, implant	2.5, 5 and 7.5 x 10^5 μg	Histological and radiological analysis revealed a max. 1.6 and 1.9 fold increase (autografts) and 8.3 and 2.9 fold increase (allografts) in bone-graft incorporation and bone formation, respectively. Biomechanical testing histology and radiography.	(Salkeld *et al.*, 2001)
rhOP-1	Adult Hound-type Dog, femur segmental defect - 40 mm		2 to 12 weeks	Canine bone allograft, implant	1,000 μg/mL	Periosteal callus area was increased 1.7 and 1.3 fold after 2 and 12 weeks compared to controls. Bone porosity was increased 2.7 fold compared to controls. Biomechanical testing, histology, histomorphometry and radiography.	(Cullinane *et al.*, 2002)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

rhBMP-7	Hound Labrador Mongrel Dog, alveolar ridge defect – 5mm		4 and 8 weeks	Titanium porous-oxide, implant	1,500 and 3,000 µg/mL	Implants exhibited robust bone formation with no differences between doses. Histology and radiography.	(Leknes *et al.*, 2008)
OP-1	Mature Mongrel Dog, femur condyle defect – 20mm		4 months	Bone graft, implant	250 µg per µg bone	Defect healing was observed following allograft treatment with and without OP-1. Biomechanical testing, histology and radiography.	(Mont *et al.*, 2001)
rhOP-1	Adult Male Dog, ulna segmental defect – 25mm		12 weeks	Particulate collagen carrier and CMC, implant	1,750 and 3,500 µg per g carrier	Radiographic analysis revealed a 1.06 and 1.2 fold increase in defect regeneration score after 12 weeks with full and half dose OP-1. Biomechanical testing, histology and radiography.	(Cook *et al.*, 2005)
OP-1	Dog, ulna segmental defect – 25mm		12 weeks	Collagen, implant	unknown	Treatment with OP-1 resulted in 89 % of defects exhibited complete healing with mechanical strength 1.65 fold that of intact ulna. Biomechanical testing, histology and radiography.	(Cook *et al.*, 1998)
Goat							
rhOP-1	Adult Female Goat, closed tibial fracture		2 and 4 weeks	Collagen matrix, injection	1,000 µg	Local delivery of OP-1 increased callus volume 2.3 fold compared to untreated controls. OP-1 treatment with collagen matrix increased callus volume 2.5 and 1.4 fold compared to collagen matrix alone and untreated controls, respectively. Biomechanical testing, CT, DXA and histology.	(den Boer *et al.*, 2002)
rhOP-1	Adult Female Goat, closed tibial fracture		2 and 4 weeks	Bovine type I collagen matrix, injection	1,000 µg	Treatment with OP-1 increased histological scoring of woven bone volume 1.8 fold compared to untreated controls. Addition of collagen increased woven bone volume 1.4 and 1.25 fold compared untreated controls and collagen alone, respectively. Biomechanical testing and histology.	(Blokhuis *et al.*, 2001)
Monkey							
rhOP-1	African Green Monkey, tibia/ulna segmental defect – 20mm		20 weeks	Bovine type I collagen carrier, implant	250 to 2,000 µg (tibia) 1,000 µg (ulna)	Complete healing was observed within both treated defects at 6 to 8 weeks with defect bridging as early as 4 weeks. New cortices with areas of woven and lamellar bone were observed at 20 weeks. Histology and radiography.	(Cook *et al.*, 1995)
OP-1	African Green Monkey, ulna segmental defect – 20mm		1 to 20 weeks	Bovine type I collagen carrier, implant	1,000 µg	Lamellar and woven bone found at 12 weeks, with remodelling and revascularization found at 20 weeks. CT, histology, MRI and radiography.	(Cook *et al.*, 2002)
Sheep							
OP-1	OVX Female Sheep, mid-vertebral defect – 8mm		6 months	PLGA microspheres with CMC carrier, implant	370 µg	Treatment with OP-1 increased bone volume 1.4 fold, and torsional stiffness 3.3 fold, both compared to carrier alone. Biomechanical testing, DXA, histology and histomorphometry.	(Phillips *et al.*, 2006)
OP-1	Female Merino Sheep, lumbar interbody fusion		8 weeks	PLGA microbeads with HA, injection	1,600 µg	Radiographic scoring of defect bridging was increased 3.1 and 3.9 fold compared to HA alone and autograft control. Histology and radiography.	(Blattert *et al.*, 2002)
rhBMP-7	Merino Sheep, tibia segmental defect – 30mm		3 months	PCL with TCP scaffold, implant	1,750 and 3,500 µg	Treatment with rhBMP-7 enhanced bone formation approx. 8 and 4 fold compared to control scaffolds when given a 1.75 and 3.5 mg dose respectively. Biomechanical testing, histology, micro CT and radiography.	(Cipitria *et al.*, 2013)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

rhOP-1	Sheep, tibia segmental defect – 30mm		12 weeks	Granular porous HA, implant	2,500 µg	Treatment with OP-1 on HA increased torsional strength and stiffness approx. 3 fold compared to HA alone. Biomechanical testing, histology and radiography.	(den Boer *et al.*, 2003)
hOP-1	Female Merino Crossbreed Sheep, lumbar interbody fusion		1 to 6 months	Bovine hydroxylapatite (Bio-Oss),	2,500 µg	After 6 months bone formation was increased 2.1 and 1.3 fold (CT), and 2 and 1.3 fold (MRI) compared to HA alone and autograft alone. Biomechanical testing, CT, histology and MRI.	(Magin and Delling, 2001)
rhBMP-7	Merino Sheep, tibia segmental defect – 30mm		3 to 12 months	PCL with TCP scaffold, implant	3,500 µg	Bone volume was increased 3.3 and 1.9 fold after 3 and 12 months compared to scaffold alone. Biomechanical testing, histology, histomorphometry, micro CT and radiography.	(Reichert *et al.*, 2012)
rhOP-1	Sheep, mandible defect – 60mm		1 to 8 weeks	Bovine type I collagen matrix or allograft, implant	3,500 µg	Treatment with OP-1 increased bone formation approx. 1.5 fold, and addition of allograft further enhanced bone formation approx. 1.8 fold, both compared to control defects. Histology, immunohistochemistry and radiography.	(Forriol *et al.*, 2009)
rhBMP-7	Alpine Sheep, metatarsal defect – 30mm		4, 8 and 16 weeks	Bovine type I collagen, implant	3,500 µg	Increased callus formation and bone remodelling were observed at 8 and 16 weeks, respectively. Biomechanical testing, histology, histomorphometry and radiography.	(Donati *et al.*, 2008)
OP-1	Mature Merino Sheep, cemented hemiarthroplasty		6, 18 and 26 weeks	Type I collagen in allograft, implant	60 to 89 µg per g allograft	Extensive periosteal bone formation and remodelling of cortical and trabecular bone was observed. Histology, histomorphometry and radiography.	(McGee *et al.*, 2004)
BMP-7	Adult Sheep, mandible defect - 35mm		3 months	Type I collagen carrier, implant	1,000 µg/cm^3	Bone appositional rate was close to that of humans (1.98 µm/d), but bone volume was lower than native bone with an approx. 1.3 fold increase in trabecular spacing. Histology and histomorphometry	(Abu-Serriah *et al.*, 2004)
1.2. Small Animal Models							
Minipig							
rhOP-1	Adult Female Minipig, mandible defect – 45mm		12 weeks	Xenogenic bone mineral, implant	600 µg	Quantification by CT revealed a 2 fold increase in defect reconstruction. CT and histology.	(Terheyden *et al.*, 2001)
rhBMP-7	Infant Female Minipig, mandible replacement implantation – 180mm		6 weeks	Polylactide or titanium mesh, implant	3,500 µg	Compression resistance was statistically equivalent to natural porcine mandibular bone. Biomechanical testing, CT and histology.	(Warnke *et al.*, 2006)
Mouse							
BMP-7	C57BL/6 Mouse, ectopic intramuscular implantation		5 to 20 d	Collagen fleece, implant	5 µg	Ectopic bone nodule was observed at the implant site. Diclofenac treatment reduced trabecular number and increased spacing. Histology, histomorphometry, micro CT and radiography.	(Spiro *et al.*, 2010)
Bone-forming peptide 1 (BMP-7 derivative)	ICR Mouse, calvarial defect – 4mm		8 weeks	Immobilised on electrospun PLGA fibers, implant	200 µg/mL	Bone formation was increased 2.9 and 2.5 fold compared to control defects and PLGA fibers alone, respectively. Biochemistry, histology, micro CT, radiography and SEM.	(Lee *et al.*, 2013)
Rabbit							
OP-1	New Zealand White Rabbit, distraction osteotomy		5 weeks	Nanoparticle injection (Liposome core with alginate and chitosan layered shell)	0.5, 1 and 5 µg	Relative bone volume was increased 1.5, 1.7 and 1.8 fold following treatment with 0.5, 1 and 5 µg OP-1. Histology, histomorphometry, immunohistochemistry, micro CT and radiography.	(Haidar *et al.*, 2010b)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

rhOP-1	Rabbit, ulna non-union		8 or 12 weeks	Demineralised, guanidine-extracted, insoluble rabbit bone matrix, implant	3.13 to 400 µg	OP-1 treatment induced complete radiographic osseous union within 8 weeks, except for defects which received 3.13 µg. Defects were primarily regenerated with lamellar bone. Torsional strength was comparable to intact bone. Histology and radiography.	(Cook *et al*., 1994b)
rhOP-1	Rabbit, knee prosthesis		3 and 6 weeks	Bone allograft, implant	26.5 µg	Fusion rate was 82 % in treated defects compared to 10% in non-treated controls, and 42% in autograft controls. Histology and histomorphometry.	(Tagil *et al.*, 2003)
OP-1	New Zealand White Rabbit, femur condyle defect – 5mm(Lee *et al.*, 2013)		6 weeks	Periapatite-coated titanium implants (5mm x 9mm), implant	60 µg	Bone ingrowth and implant bone coverage were increased 2.9 and 2.6 fold respective, compared to controls. Biomechanical testing, histology, histomorphometry and radiography.	(Zhang *et al.*, 2004)
OP-1	Male New Zealand White Rabbit, tibia osteotomy and distraction osteogenesis – 0.25mm per 12 hours		3 weeks	Acetate buffer, injection	80, 800 and 2,000 µg	Low dose OP-1 increased bone regeneration volume 1.2 and 1.1 fold respective, compared to sham and carrier alone defects. High dose OP-1 increased both bone mineral content and density approx. 1.2 fold compared to controls. Biomechanical testing, DXA, histology, histomorphometry and immunohistochemistry.	(Hamdy *et al.*, 2003)
rhOP-1	Female New Zealand White Rabbit, distal tibia wedge osteotomy – 4mm		1 to 4 weeks	β-TCP particles and CMC, implant	200 µg	Bone mineral content was increased 1.2 fold compared to normal healing following OP-1 treatment. Maximum torque and torsional stiffness were increased 1.4 and 1.3 fold respective, compared to normal healing. Biomechanical testing, CT and histology.	(Tsiridis *et al.*, 2007b)
rhBMP-7	New Zealand White Rabbit, mandible osteotomy and distraction		7 weeks	Lactate buffer, injection	200 µg	Bone density was increased, but not significantly. Radiography.	(Zakhary *et al.*, 2005)
BMP-7	New Zealand White Rabbit, ulna defect – 15mm		2 to 10 weeks	Homologous compressed cancellous bone , implant	300 µg	Osteoid thickness within the regenerated defect area was increased approx. 1.4 fold compared to control defects. Histology, histomorphometry and radiography.	(Djapic *et al.*, 2003)
OP-1	New Zealand White Rabbit, tibial osteotomy distraction – 15mm		10 weeks	Bone filler, implant	800 µg	Bone density and length were not increased with treatment. Computerized axial tomography, histology and radiography.	(Lammens et al., 2009)
OP-1	New Zealand White Rabbit, posterolateral lumbar fusion in a pseudarthrosis model		5 and 10 weeks	Bovine type I collagen matrix and CMC, implant	1,200 µg	After 5 weeks OP-1 induced solid intertransverse process fusion in a rabbit model. CT, histology, histomorphometry and radiography.	(Grauer et al., 2004)
Rat							
rhOP-1	Rat, ectopic subcutaneous implantation		5 to 21 d	Rat collagen matrix, implant	0.025 to 50 µg	Cartilage calcification was observed after 9 d. Higher doses resulted in remodelling at day 9. At day 21 the carrier was almost completely replaced with new ossicle formation. Biochemistry, histology, immunohistochemistry and radioimmunoassay.	(Sampath *et al.*, 1992)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

OP-1	Wistar Rat, ectopic intramuscular injection		28 and 70 d	Nanoparticle injection (Liposome core with alginate and chitosan layered shell)	0.5 and 1 µg	Nanoparticles enabled localised delivery without health complications, and enhanced *de novo* bone formation. Biochemistry, histology and immunohistochemistry.	(Haidar *et al.*, 2010a)
rhBMP-7	Male Sprague-Dawley Rat, ectopic subcutaneous implantation		3 and 6 weeks	Encapsulated within PLGA nanospheres on PLLA scaffold, implant	5 µg	Radiopacity was increased approx. 2 and 1.9 fold compared to controls after 3 and 6 weeks respective. Histology and radiography.	(Wei *et al.*, 2007)
rhOP-1	Sprague-Dawley Rat, 6 mm segmental femur defect		2, 4 and 9 weeks	Lyophilized bovine type I collagen (carrier for the OP-1)	11 or 50 µg	OP-1 maintains its osteoinductive capability in a contaminated segmental bone defects Histology and quantitative radiography.	(Chen *et al.*, 2002)
rhOP-1	Sprague-Dawley Rat, *Staph. aureus*-infected femur segmental defect – 6mm		2 to 12 weeks	Lyophilised collagen carrier. Groups with and without antibiotic (ceftriaxone), implant	20 and 200 µg	Low dose: Mineralised callus formation was increased, 1.1 (4 weeks) to 4.4 (2 weeks) fold, and 1.4 (12 weeks) to 7.8 (2 weeks) fold with and without antibiotics respectively, compared to untreated controls. High dose: Mineralised callus formation was increased 6.1 (8 weeks) to 96 (2 weeks) fold, and 4.5 (8 weeks) to 21 (2 weeks) fold with and without antibiotics respectively, compared to untreated controls. Biomechanical testing, histology, micro CT and quantitative radiography.	(Chen *et al.*, 2006)
rhBMP-7	Long-Evans Rat, femur osteotomy and distraction 0.25mm every 12 hours - 10mm		2 and 4 weeks	Aqueous solvent, injection	20 µg	BMD was increased 4 fold compared to controls. Maximum torque and stiffness were increased 3.9 and 11.6 fold compared to controls. Biomechanical testing and radiography.	(Mizumoto *et al.*, 2003)
rhOP-1	Male Sprague-Dawley Diabetic Rat, femoral closed fracture		2 and 4 weeks	Bovine type I collagen carrier, implant	25 µg	Fracture callus area was increased 17, 29.5 and 9.8 fold (2 weeks), and 5.2, 4.3 and 2.6 fold (4 weeks) compared to carrier alone, sham surgery and sham surgery in non-diabetic mice respective. Biomechanical testing, histology and radiography.	(Kidder *et al.*, 2009)
rhBMP-7	Fisher 344 Rat, femoral non-union - atrophic		2 to 8 weeks	Rat tail tendon collagen buffer, implant	50 µg	63 % of treated defects had healed by 4 weeks, and 100 % by 8 weeks, compared to no healing in control defects. Maximum torque to failure and stiffness had increased 6.1 and 6.8 fold after 4 weeks and 5.9 and 11.7 fold after 8 weeks, respective. Biomechanical testing, histology and radiography.	(Makino *et al*., 2005)
rhBMP-7	Fisher 344 Rat (3 and18 month old), femoral closed fracture		3 and 6 weeks	Rat tail tendon collagen, implant	100 µg	Defect regeneration was more rapid within younger rats: 100 % healed at 3 weeks, compared to 56 % healed in older rats. Maximum torque and stiffness were increased 15.9 and 31 fold, and 8.6 and 29.8 fold compared to controls, in young and old rats, respectively after 3 weeks. Maximum torque and stiffness were increased 14.9 and 26.1 fold, and 6.8 and 7.5 fold compared to controls, in young and old rats, respectively after 6 weeks. Biomechanical testing, histology and radiography.	(Hak *et al.*, 2006)
rhBMP-7	Male Sprague-Dawley Rat, calvarial defect – 8mm		2 and 8 weeks	Absorbable collagen sponge, implant	25 µg/mL	Treatment enhanced local bone formation. Histology, histomorphometry and immunohistochemistry.	(Hyun *et al.*, 2005)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

rhBMP-7	Wistar Rat, mandible augmentation, and ectopic intramuscular implantation		50 d	Acetate buffer carrier on either anorganic bovine bone or autograft, implant	20 µL (mandible) 10 µL (ectopic)	Area of 'bone tissue of total core' increased 10.3 and 1.3 fold compared to controls with bovine bone and autograft respective. Area of newly mineralised bone increased 3.6 fold compared to control, but only with bovine bone. Ectopic implantation also demonstrated new bone formation. Histology, histomorphometry and microradiography.	(Roldan *et al.*, 2004)
2. Animal Models for indirect BMP-7/OP-1 delivery 2.1. Large Animal Models							
Dog							
rhBMP-7 (adenovirus)	Adult Hybrid Dog, mandible defect – 5mm		4 to 12 weeks	Chitosan- collagen scaffold, implant	2 x 10^{10} particles	Bone formation within the defect site increased approx. 2 fold compared to carrier alone. Histology and histomorphometry.	(Zhang *et al.*, 2007)
rhBMP-7 (adenovirus)	Beagle Dog, intervertebral disc transplantation		12 and 24 weeks	Transduced nucleus pulposus cells within allogenic intervertebral discs, implants	1 x 10^5 cells	Treatment with rhBMP-7 maintained allograft integrity, compared to controls which exhibited a 2.1 and 1.5 fold increase in disc degeneration at 12 and 24 weeks respectively. Biomechanical testing, histology, MRI and radiography.	(Chaofeng *et al.*, 2013)
rhBMP-7 and rhPDGF-B (adenovirus)	Adult Hybrid Dog, mandible defect – 6mm		4, 8 and 12 weeks	Chitosan/collagen scaffolds, implant	2 × 10^{10} particles/mL	New bone formation was increased approx. 2.3 and 1.6 fold following combination treatment after 4 and 8 weeks. Percentage defect fill and new bone implant contact were increased approx. 1.5 and 1.4 fold compared to controls after 12 weeks. Histology and histomorphometry.	(Zhang *et al.*, 2009b)
Goat							
BMP-7 (adenovirus)	Adult Goat, segmental femur defect – 25mm Nude Mouse, ectopic intramuscular injection		3 to 8 months (goat) 3 weeks (mouse)	Transduced cell/coral constructs, implant (goat) Direct injection (mouse)	5 x 10^7 cells (goat) 4 x 10^6 cells (mouse)	New bone formation was radiographically visible after 3 weeks within nude mice. Bone mass increased approx. 2.5 fold maximum after 3 months compared to an approx. 1.5 fold increase with non-transfected BMSCs. Histology, histomorphometry and radiography.	(Zhu *et al.*, 2010)
2.2. Small Animal Models							
Mouse							
BMP-7 (adenovirus)	BALB/c Mouse, calvarial defect – 3mm		1, 2 and 4 weeks	Silk fibroin, implant	1.8 x 10^{11} PFU/mL	An approx. 5 fold increase in bone volume within the defect site was observed compared to blank control, and 2 fold compared to scaffold alone. Biochemistry, cytochemistry, histology, micro CT, RT-PCR and SEM.	(Zhang *et al.*, 2012b)
BMP-7 (adenovirus)	SCID Mouse, calvarial defect – 3mm		4 weeks	Silk fibroin, implant	1 x 10^5 cells	No histological evidence of inflammation. New bone formation compared to little or no bone in negative controls. histology, immunocytochemistry, RT-PCR and SEM.	(Zhang *et al.*, 2010c)
BMP-7 and adLMP3 (adenovirus)	NIH-III Nude Mouse, ectopic subcutaneous implantation		3 weeks	Transduced periodontal ligament cells in type I collagen scaffold, implant	1 x 10^6 cells	BMP-7 alone induced radiographically visible bone formation after 3 weeks. Combination treatment induced approx. 4 fold increased bone formation after 4 weeks. Histology, micro CT and RT-PCR.	(Lin *et al.*, 2012c)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

BMP-7 (adenovirus)	N:NIH-bg-nu-xid Mouse, ectopic subcutaneous implantation		4 and 8 weeks	Transduced human gingival fibroblasts on PCL scaffold, implant	1.5 x 10^6 cells	New bone formation was observed in all groups after 8 weeks. Larger scaffold pore size resulted in early bone formation at 4 weeks (approx. 2 fold more bone with high pore size compared to low pore size). Biomechanical testing, histology and micro CT.	(Roosa *et al.*, 2009)
BMP-7 (plasmid vector)	BALB/c Nude Mouse, ectopic subcutaneous implantation		4 weeks	Transfected human dental pulp cells in chitosan/collagen composite, implant	1 x 10^6 cells/mL (1mL per 6 scaffolds)	No hard tissue was observed within treated groups compared to controls. Histology and immunohistochemistry.	(Yang *et al.*, 2012)
Rabbit							
BMP-7 (adenovirus)	New Zealand White Rabbit, bilateral mandibular defect - 12 x 8mm		4, 8 and 16 weeks	Transduced rabbit MSCs on nano-HA/PA scaffold, implant	2 x 10^6 cells	New bone volume within BMP-7 treated groups was increased 1.6, 1.3 and 1.03 fold compared to scaffold alone groups, at 4, 8 and 16 weeks, respectively. Biomechanical testing, histology, histomorphometry and radiography.	(Li *et al.*, 2010a)
Rat							
OP-1 (recombinant protein or plasmid vectors)	Adult Sprague-Dawley Rat, single-level posterior lumbar arthrodesis		2 and 4 weeks	With and without rat tail type I collagen carrier, injection	30 to 40 µg 25 and 250 µg (plasmid)	Bone formation was observed after delivery of 25 µg plasmid (75 %). Single-level spine fusion was only achieved with 30 µg recombinant protein in collagen carrier or 40 µg alone. Histology and radiography.	(Bright *et al.*, 2006)
rhBMP-7 (adenovirus)	Sprague-Dawley Rat, alveolar ridge osteotomy – 2mm		3 to 28 d	Collagen matrix, implant	2.5 × 10^{11} particles	Treatment enhanced alveolar bone defect fill, coronal new bone formation, and new bone-to-implant contact 1.5, 2 and 1.5 fold respective, compared to controls. Histology, histomorphometry and SEM.	(Dunn *et al.*, 2005)
BMP-7 (adenovirus)	Rat, spinal fusion		8 weeks	Transduced cells on allograft, implant	1.5x10^6 cells	New bone formation was increased 21 and 14 fold compared to saline and adenovirus controls. Biomechanical testing, histology and radiography.	(Hidaka *et al.*, 2003)
rhBMP-7 (adenovirus)	Lewis Rat, ectopic subcutaneous implantation		1, 2 and 4 weeks	Type I collagen matrix, implant	1 x 10^6 cells	Transduced cells initiated endochondral ossification with new trabeculae by 4 weeks. No new bone was observed in implants treated with non-transduced cells. Histology.	(Yang *et al.*, 2005)
BMP-7 (adenovirus)	Fisher Rat, calvarial defect – 9mm		4 weeks	Transduced cells in collagen carrier on gelatin sponge, implnat	2 x 10^6 cells	BMP-7 ex vivo gene therapy increased bone regeneration in rat calvarial defects even after a therapeutic dose of radiation. Histology, histomorphometry and radiography.	(Nussenbaum *et al.*, 2003)
3. Animal Models for combinational direct and indirect BMP-7/OP-1 delivery 3.1. Large Animal Models							
Baboon							
OP-1 and TGF-β1	Adult Male Chacma Baboon, ectopic intramuscular implantation Adult Male Chacma Baboon, bilateral calvarial defect – 25mm		30 to 90 d 1 to 12 months	Collagen matrix or HA discs, implant	Collagen: 5 to 125 µg OP-1 0.5 to 5 µg TGF-β1 HA: 20 to 2,500 µg OP-1 5 to 15 µg TGF-β1	OP-1within collagen matrix induced ectopic endochondral bone formation, and extensive defect regeneration. Combination treatment within collagen matrix induced large ossicles. Implantation of HA with OP-1 induced extensive bone formation by 30 days and bone remodelling by days 90 to 365. Histology.	(Ripamonti *et al.*, 2001b)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

rhOP-1 and TGF-β1	Chacma Baboon, ectopic intramuscular implantation and calvarial defect		14 to 90 d	Collagenous bone matrix, implant	25 to 100 µg OP-1 5 and 15 µg TGF-β1	OP-1 induced ectopic ossicle formation by day 30, where TGF-β1 did not. Combination treatment induced endochondral bone formation within the defect site with exuberant tissue formation and osteoid production compared to either factor individually. Histology and RT-PCR.	(Duneas *et al.*, 1998)
rhOP-1 and TGF-β3	Adult Chacma Baboon, ectopic intramuscular implantation		90 d	HA/calcium carbonate macroporous scaffold, implant	125 µg	Bone volume within implants was increased 4.2 fold after OP-1 treatment, compared to control implants. Combination with TGF-β3 further enhanced bone volume compared to controls (5.3 fold increase). Histology, histomorphometry, RT-PCR and radiography.	(Ripamonti *et al.*, 2010)
rhOP-1 and TGF-β1	Adult Male Chacma Baboon, ectopic intramuscular implantation		15 and 30 d	Insoluble collagen matrix, implant	5,000, 25,000 and 125,000 µg OP-1 0.5, 1.5, and 5 µg TGF-β1 (both per 100,000 µg matrix)	Combination treatment resulted in a 2 to 3 fold increase in mineralised bone and osteoid formation compared to OP-1 alone after 15 days. High dose OP-1 and TGF-β1 increased mineralised bone and osteoid formation approx. 2 fold. Biochemistry, histology and histomorphometry.	(Ripamonti *et al.*, 1997)
Dog							
hOP-1 and bone marrow or blood	Male Mongrel Dog, proximal and distal femur defect – 10mm		4 weeks	Bovine type I collagen and CMC, implant	3,500 µg OP-1 3.5 mL bone marrow or blood	Defects which received OP-1 and bone marrow exhibited an increase of 1.5 fold and decrease of 1.4 fold in bone volume, compared to those receiving OP-1 and blood for proximal and distal defects respectively. Histology and micro CT.	(Takigami *et al.*, 2007)
rhBMP-7 and rhPDGF-B (adenovirus)	Adult Hybrid Dog, mandible defect – 6mm		4, 8 and 12 weeks	Chitosan-collagen scaffold, implants	2 x 10^{10} particles/mL	New bone formation was increased approx. 2.3 and 1.6 fold following combination treatment after 4 and 8 weeks. Percentage defect fill and new bone implant contact were increased approx. 1.5 and 1.4 fold compared to controls after 12 weeks. Histology and histomorphometry.	(Zhang *et al.*, 2009b)
Horse							
BMP-7 and BMP-2 (adenovirus)	Horse, metacarpal IV ostectomy – 15mm		2 to 16 weeks	Direct injection	2 x 10^{11} virus particles	Bone defect regeneration was not enhanced further than controls when treated with BMP-2 and BMP-7 viral particles, possibly due to incorrect dosing. DXA, histology and radiography.	(Southwood *et al.*, 2012)
3.2. Small Animal Models							
Minipig							
BMP-7 and BMP-2	Minipig, calvarial defect – 8mm		2 to 6 weeks	Collagen sponge, implant	5 µg	Combination treatment increased bone formation 2.1, 1.4 and 1.6 fold compared to controls, BMP-2 alone, and BMP7 alone. Histology, histomorphometry, immunohistochemistry,	(Sun *et al.*, 2012b)
BMP-7 and BMP-2	Guangxi Bama Minipig, calvarial defect – 8mm diameter x 4mm depth		2 to 6 weeks	Collagen sponge, implant	5 µg (30 ng/mm^3)	Low dose BMP-2/7 heterodimer facilitated more rapid bone regeneration (approx. 1.5 fold increase in bone volume compared to either factor alone) of improved quality. Micro CT.	(Wang *et al.*, 2012)
OP-1 and rhTSP-1	Female Adult Minipig, femoral trochlea defect – 5mm, with 1mm x 3mm microfracture		6 and 26 weeks	Type I collagen, implant	35 µg OP-1 5 µg rhTSP-1	Combination treatment enhanced cartilage mineralisation approx. 1.3 and 1.75 fold compared to control defects. Histology, histomorphometry and immunohistochemistry.	(Gelse *et al.*, 2011)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

Mouse							
rhBMP-7 and MSCs or osteoblasts	NOD/SCID Mouse, ectopic subcutaneous implantation		8 weeks	PCL-TCP scaffold, implant	5 µg 2.5 x 10^5 cells	Bone volume fraction was significantly increased approx. 8.8 fold following treatment with BMP-7 and osteoblasts compared to scaffold controls. Biomechanical testing, biochemistry, histology, histomorphometry, micro CT and SEM	(Reichert *et al.*, 2011)
BMP-7 and VEGF	Black C57/B16 Mouse, ectopic subcutaneous implant		12 weeks	Injection within biphasic calcium phosphate ceramics, implant	5 µg BMP-7 2 µg VEGF	Woven bone formation was observed on scaffold surface and within pores. However, combination of VEGF and BMP-7 did not enhance bone formation significantly. Histology and SEM.	(Roldan *et al.*, 2010)
rhBMP-7 and BBP	Male Lewis Rat, ectopic subcutaneous and intramuscular implantation		2, 4 and 7 d	Collagen sponge, implant	20 µg rhBMP-7 500 µg BBP	Addition of BBP reduced rhBMP-7 related inflammation a max. 1.5 fold when administered subcutaneously and intramuscularly. Histology, histomorphometry and MRI.	(Lee *et al.*, 2011)
rhBMP-7 and pamidronate (bisphosphonate)	Female C57BL6/J Mouse, ectopic implantation in hind limbs		3 to 8 weeks	PDLLA scaffold, implant	25µg rhBMP-7 0.02 to 2 mg pamidronate	Localised delivery of rhBMP-7 and low dose pamidronate (0.02 mg) resulted in approx. 2 fold increased bone volume, whereas delivery with high dose pamidronate (2 mg) resulted in approx. 2 fold decreased bone volume. Histology, micro CT and radiography.	(Yu *et al.*, 2010a)
BMP-7 (adenovirus) and BMSCs	SCID Mouse, calvarial defect – 3mm		4 weeks	Silk fibroin porous scaffold implant	1.8 x 10^{11} PFU/mL 1 x 10^5 cells	Combination treatment increased bone volume approx. 5.5 fold compared to scaffold only control. Histology, immunohistochemistry and micro CT.	(Zhang *et al.*, 2011b)
rhBMP-7 and IGF-1 (adenovirus)	BALB/c-nu Mouse, ectopic subcutaneous implantation		8 weeks	Porous chitosan/collagen scaffold, implant	1 x 10^6 cells	Bone formation was 1.17 fold higher than controls, and 1.22 fold higher upon combination with IGF-1. Histology, histomorphometry and immunohistochemistry.	(Yang *et al.*, 2010)
BMP-7 and BMP2 (adenovirus)	C57BL6 Mouse, critical sized calvarial defect – 7mm		4 weeks	Transduced mouse BLK cells in gelatin sponge, implant	4 x 10^6 cells	Implants revealed robust osteogenic activity with complete bone coverage of defects. Bone volume increased 2 and 1.7 fold compared to BMP-2 and BMP-7 alone, respectively. Histology, micro CT and radiography.	(Koh *et al.*, 2008)
Rabbit							
rhBMP-7 and PTH (1-34)	Female New Zealand White Rabbit, tibial wedge osteotomy - 4 x 10mm		4 weeks	β-TCP particles and CMC, implant (rhBMP-7) Subcutaneous injection (PTH (1-34))	200 µg rhBMP-7 10 µg/kg/d PTH (1-34)	Micro CT analysis revealed approx. 1.6 and 1.4 fold increased bone volume and BMC compared to controls. Histological analysis revealed approx. 1.3 fold increased bone area compared to controls. Torsional rigidity and compressive strength increased approx. 1.4 and 1.2 fold compared to controls. Biomechanical testing, histology, immunohistochemistry and micro CT.	(Morgan *et al.*, 2008)
rhOP-1 and rhFGF-2	New Zealand White Rabbit, tibial bone harvest and drug test chamber		48 weeks	Acetate buffer (rhOP-1) or distilled water (rhFGF-2), infusion	0.1 µg/d rhOP-1 0.05 µg/d rhFGF-2	OP-1 and FGF-2 infusion increased percentage *de novo* bone formation approx. 1.3 and 1.2 fold respective. Combination treatment did not further enhance bone formation. Histology, histomorphometry and immunohistochemistry.	(Ma *et al.*, 2007)
BMP-7 and $VEGF_{165}$ (recombinant adeno-associated virus)	Male New Zealand Rabbit, ectopic intramuscular injection (hindlimb ischemia model)		2 to 8 weeks	Direct intramuscular injection	5.5 x 10^{11} particles	Co-expression stimulated angiogenesis and bone regeneration. Capillary density and mean blood flow were both increased approx. 2 fold. Biochemical testing, histology and radiography.	(Zhang *et al.*, 2010a)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

Rat							
rhOP-1 and MSCs	Male Wistar Rat, ectopic intramuscular implant		28 d	Insoluble collagen matrix on demineralised bone or allograft, implant	2 μg	OP-1 improved cell proliferation/differentiation and bone formation. Biochemistry, histology and SEM.	(Tsiridis *et al.*, 2007a)
rhBMP-7 and rhBMP-2 (plasmids)	Male Wistar Rat, ectopic intramuscular injection		1 to 10 d	Direct injection and *in vivo* electroporation	12.5 μg	Enhanced bone formation and calcification was observed compared to single modalities, with upregulation of BMP-4. Histology, immunohistochemistry, radiography and rtPCR.	(Kawai *et al.*, 2006)
rhBMP-7 and rhBMP-2 (plasmid)	Male Wistar Rat, ectopic intramuscular injection		10 d	Direct injection of double cassette plasmid and *in vivo* electroporation	50 μg	Enhanced bone formation and calcification was observed compared to single modalities. Histology and radiography.	(Kawai *et al.*, 2009)
BMP-7 and BMP2 (adenovirus)	Sprague-Dawley Rat, bilateral single level posterolateral spine fusion		8 weeks	Allograft bone and CMC hematostatic foam soaked with adenovirus, implant	0.75×10^8 to 0.75×10^{11} particles	Co-administration of Ad.BMP-2 and Ad.BMP7 resulted in significantly greater numbers of mechanically stable fusions and also 2 fold higher mineralisation rate of bone volume in the fusion mass. Histology and radiography.	(Zhu *et al.*, 2004)
BMP-7 and BMP-2 (lentivirus)	Sprague-Dawley Rat, calvarial defect – 2mm		2 to 6 weeks	Transduced cells in β-TCP scaffold, implant	1×10^6 cells	New bone formation was observed after 4 weeks in all treated defects. Only dual factor treated defects exhibited complete defect regeneration after 6 weeks. Biochemistry, cytochemistry, histology, qPCR and radiography.	(Qing *et al.*, 2012)
adBMP-7 and adPDGF-b (adenovirus)	OVX Female Wistar Rat, femur defect, drill 2mm diameter		2 and 4 weeks	Mesoporous bioglass/silk fibroin, implant	unknown	Dual combination resulted in approx. 12 fold increased bone volume compared to blank defect, 2 fold compared to blank scaffold, and 1.5 fold compared BMP-7 alone at 2 weeks. 15 fold compared to blank defect, approx. 1.9 fold compared to blank scaffold, 1.2 fold compared to BMP7 alone at 4 weeks. Histology, immunohistochemistry, micro CT and SEM.	(Zhang *et al.*, 2012a)
4. Human Trials for BMP-7/OP-1 delivery							
Lumbar Fusion							
rhBMP-7		Human, lumbar spinal fusion	6 weeks to 36 months	Bovine bone collagen and CMC, implant	7 mg (3.5 mg either side of posterolateral fusion)	Treatment induced an immune response in 25.6 % of patients, peaking between 6 weeks and 3 months, and tailed off after 24 months. Immune response did not correlate with fusion efficacy. Biochemistry and radiography.	(Hwang *et al.*, 2010)
rhOP-1		Human, posterolateral lumbar fusion	1 year	Collagen matrix, implant	7 mg (3.5 mg per side)	rhOP-1 reliably induced new bone formation even without autograft. CT, histology and radiography.	(Kanayama *et al.*, 2006)
rhOP-1		Human, intertransverse lumbar fusion	6 weeks and 3 to 24 months	Bone type I collagen and CMC within iliac crest autograft, implant	7 mg (3.5 mg per side)	Treated patients exhibited 20 % improvement in Oswestry score. Radiographic fusion was observed within 50 % of treated patients. 70 % exhibited bone bridging between the transverse processes. Clinical assessment and radiography.	(Vaccaro *et al.*, 2005)
rhOP-1		Human, posterolateral lumbar arthrodesis	1 year	Collagen matrix, implant	7 mg (3.5 mg per side)	Successful radiographic fusion was obtained using OP-1 Putty at a rate that was similar to autograft. Importantly, no adverse effects were observed. Clinical assessment and radiography.	(Vaccaro *et al.*, 2004)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

rhOP-1		Human, intertransverse process fusion	6 weeks and 3 to 12 months	Bone type I collagen and CMC within iliac crest autograft, implant	7 mg (3.5 mg per side)	75 % of patients exhibited improved Oswestry scores, whilst 55 % exhibited radiographic fusion and 91 % had bone bridging. Minimal adverse effects were observed indicating that OP-1 is safe to use for spinal fusions. Physical examination and radiography.	(Vaccaro *et al.*, 2003)
rhOP-1		Human, lumbar fusion	1 year	Bone type 1 collagen and saline putty, implant	7 mg (3.5 mg per side)	Treatment demonstrated equivalent lumbar fusion to that of autograft from the iliac crest. Radiography and radiostereometric analysis.	(Johnsson *et al.*, 2002)
OP-1		Human, anterior and posterior lumbar interbody fusion	3 and 10 months	Bovine bone collagen and CMC mixed with autograft, implant	17.5 mg (5 vials, 3.5 mg each)	OP-1 treatment demonstrated equivalent bone fusion compared to autograft alone providing a viable alternative. However, excessive dosage resulted in ectopic bone formation along the surgical track. CT, histology and radiography.	(Kim *et al.*, 2010b)
rhBMP-7		Human, posterolateral fusion and posterior fixation	3 to 18 months	Collagen carrier, implant	unknown	OP-1 treatment did not stimulate sufficient early structural bone support, and indications of enhanced bone resorption were observed. Clinical assessment, CT and radiography.	(Laursen *et al.*, 1999)
rhOP-1		Human, atlanto-axial posterior fusion	2 to 10 months	Collagen carrier, implant	unknown	Bridging of the bone only occurred in 1out 4 rheumatoid patients at 6 months. Radiography.	(Jeppsson *et al.*, 1999)
Mandible Reconstruction							
rBMP-7 and MSCs		Human, mandible reconstruction, tumor resection – 60mm	9 to 12 months	Demineralised bovine xenograft on titanium mesh, implant	2000 mg (rBMP-7) 5mL conc. (human bone marrow aspirate)	New bone formation was observed around the xenograft particles within the defect site after 9 months, and osseous implants remained stable after 1 year. Histology, histomorphometry and radiography.	(Hernandez-Alfaro *et al.*, 2012)
Maxillary Sinus Augmentation							
rhBMP-7		Human, maxillary sinus floor elevation	6 months	Collagen carrier, implant	2.5 mg	Three patient study. OP-1 initiated bone formation but remains insufficiently predictable for clinical translation as an alternative to bone graft. Averaged patient data revealed approx. 1.2 fold increased osteoid formation compared to autograft controls. Histology and radiography.	(van den Bergh *et al.*, 2000)
rhOP-1		Human, maxillary sinus floor elevation	6 months	Collagen carrier, implant	2.5 mg	Three patient study. Treatment induced bone formation inconsistently. Averaged patient data revealed approx. 2.2, 1.2 and 9.7 fold increased osteoid formation within OP-1 treated patients compared to DBM, autograft and no graft controls, respectively. Histology, histomorphometry and radiography.	(Groeneveld *et al.*, 1999)
Non-Union Fracture							
rhBMP-7		Human, upper and lower limb resistant non-union	5.6 months	TCP crystals and bovine bone collagen, implant	3.5 mg	21.5 months following initial injury, 52 patients with non-union fractures received rhBMP-7 treatment. Radiological union was achieved in 94 % after 5.6 months. Clinical assessment and radiography.	(Papanna *et al.*, 2012)
rhBMP-7		Human, tibial non-union	1 year	Bovine type I collagen mixed with saline or blood, implant	3.5 mg	BMP-7 groups showed significantly higher success rates. 78 % of patients who received autologous bone graft only exhibited successful union after 4 months. Comparatively, 92 % of patients with rhBMP-7 implantation displayed bony consolidation after 4 months. Clinical assessment and radiography.	(Zimmermann *et al.*, 2009)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

rhBMP-7		Human, aseptic atrophic femur non-union	12 to 68 months	Bovine type I collagen, implant	3.5 mg	86.7 % of non-union patients (26 cases) exhibited successful healing following BMP-7 treatment, in a median time of 6 months (range 4 to 10 months). Clinical assessment and radiography.	(Kanakaris *et al.*, 2009)
rhBMP-7		Human, atrophic upper or lower long bone fracture non-union	1 to 18 months	Bovine type I collagen mixed with autograft, implant	3.5 mg	100 % of treated patients (45 cases) displayed fracture union within 3 to16 months. Clinical assessment and radiography.	(Giannoudis *et al.*, 2009)
rhOP-1		Human (children), persistent non-union	4 to 56 months	Bovine type I collagen, implant	3.5 mg	Successful healing occurred in 17 out of 23 sites (74 %). Clinical assessment and radiography.	(Dohin *et al.*, 2009)
rhBMP-7		Human, aseptic tibia non-union	12 to 30 months	Bovine type I collagen, implant	3.5 mg	Non-union healing was achieved in 89.7 % of BMP-7 treated patients in a median period of 6.5 months (range 3 to 15 months). Clinical assessment and radiography.	(Kanakaris *et al.*, 2008)
rhBMP-7		Human, long bone non-union (femur, humerus, tibia, radius, ulna)	1 to 12 months	Bovine type I collagen, implant	3.5 mg	rhBMP-7 treatment resulted in successful fracture healing within 86.7 % of patients compared to 68.3 % of patients treated with platelet rich plasma. Healing time was also reduced following rhBMP-7 treatment. Clinical assessment and radiography.	(Calori *et al.*, 2008)
rhBMP-7		Human, distal tibia fracture	1 to 54 months	Bovine type I collagen, implant	3.5 mg	Healed fractures were significantly increased 3 and 3.2 fold compared to controls after 16 and 20 weeks. Mean time to union, duration of absence from work, and time for which external fixation was required were each significantly reduced 1.5, 1.4 and 1.4 fold respectively, following BMP-7 treatment. Clinical assessment and radiography.	(Ristiniemi *et al.*, 2007)
rhBMP-7		Human, pelvic girdle non-union	12 to 27 months	Bovine type I collagen, implant	3.5 mg	Fusion was achieved in 89 % of patients. 78 % of patients reported excellent or good subjective functional results after a mean 12 months. Clinical assessment and radiography.	(Giannoudis *et al.*, 2007)
rhBMP-7		Human, persistent fracture non-union (clavicle, femur, humerus, patella, tibia, ulna)	12 to 27 months	Bovine type I collagen with and without iliac crest autograft, implant	3.5 mg	Mean number of procedures per fracture was reduced 3.5 fold following BMP-7 treatment. Mean hospital stay and cost of treatment per fracture were reduced 3.4 and 1.9 fold respective following BMP-7 treatment. Clinical assessment and radiography.	(Dahabreh *et al.*, 2007)
rhBMP-7		Human, long bone non-union	3 to 29.2 months	Osigraft®; alone, with autograft, or with an osteoconductive agent.	3.5 mg	83.8 % of non-unions healed treated groups, with an average healing time of 7.9 months. Clinical assessment and radiography.	(Ronga *et al.*, 2006)
rhOP-1		Human, symptomatic proximal pole scaphoid non-union	1 to 24 months	Collagen carrier combined with autograft or allograft, implant	3.5 mg	Treatment with OP-1 and allograft increased sclerotic bone formation 1.4 and 1.7 fold compared to OP-1 with autograft after 3 and 9 months respective. Significant sclerotic bone resorption was observed after 24 months. Improved functional measurements were recorded at 4 months and implants exhibited accelerated vascular ingrowth. CT, radiography and scintigraphy.	(Bilic *et al.*, 2006)
rhBMP-7		Human, upper and lower limb non-union (clavicle, femur, humerus, patella, tibia, ulna)	6 to 27 months	Collagen carrier and bone autograft, implant	3.5 mg	Clinical and radiological union occurred in 92.3 % of cases. Clinical assessment and radiography.	(Dimitriou *et al.*, 2005)

Supplementary Table 2. Bone tissue engineering *in vivo* utilising BMP-7/OP-1 within animal models and human trials.

rhOP-1		Human, tibial non-union	2 years	Type I collagen carrier , implant	3.5 mg	OP-1 was safe and effective treatment for tibial non-unions. Clinically and radiographically assessed fracture healing success was observed in 81 % and 75 % of patients, respectively. This was comparable to autograft controls where 85 % and 84 % of patients exhibited successful fracture healing. Clinical assessment and radiography.	(Friedlaender *et al.*, 2001)
Osteotomy							
rhOP-1		Human, high tibial osteotomy (24 patients)	1 year	Type I collagen carrier, implant	2.5 mg	Following OP-1 treatment all patients except one showed new bone formation as early as 6 weeks. Clinical assessment, DXA and radiography.	(Geesink *et al.*, 1999)
rhBMP-7		Human, corrective radius osteotomy with internal fixation	2 to 52 weeks	Collagen paste, implant	3.5 mg	Patients treated with rhBMP-7 healed at a slower rate than patients treated with autograft. rhBMP-7 did not confer the same stability as autograft, reducing healing capacity and potentially causing osteolysis. Clinical assessment and radiography.	(Ekrol *et al.*, 2008)
BMP-7		Human, tibia osteotomy and distraction – 70mm	0.5 to 2.5 years	Collagen carrier mixed with autograft, implant	unknown	The defect was successfully filled and exhibited radiological evidence of remodeling. Tibia length was 10 mm short of target. BMP-7 treatment enhanced bone formation leading to successful bone union. Clinical assessment and radiography.	(Burkhart and Rommens, 2008)
Pseudarthrosis							
OP-1		Human, tibia and fibula pseudarthrosis with associated neurofibromatosis and bone resection	28 months	Collagen matrix, implant	3.5 mg	Complete healing of the resected defects was observed at 28 months with a 50 mm difference in length between limbs. The limb was load bearing and pain free. Clinical assessment and radiography.	(Fabeck *et al.*, 2006)
OP-1		Human, congenital tibia pseudarthrosis	1 to 12 months	Collagen matrix mixed with autograft, implant	3.5 mg (7 mg following revision surgery in 1 case)	New bone formation around the pseudarthrosis site within all 5 patients was not observed. However, no side effects were observed either. Clinical assessment, radiography and scintigraphy.	(Lee *et al.*, 2006)
BMP-7		Human (11 year old), congenital tibia pseudarthrosis and sclerotic tibia segment resection	0.5 to 45 months	Collagen carrier, implant	unknown	Tibia was load bearing after 2 weeks, and exhibited new bone formation after 4 weeks. 5 months later, non-union healing was observed, leading to equal limb length between legs at 8 months. At 45 months thicker cortical bone was observed and the limb was fully weight bearing. Clinical assessment, histology and radiography.	(Anticevic *et al.*, 2006)

Abbreviations: BBP *(BMP binding protein)*, **BMC** *(bone mineral content)*, **BMD** *(bone mineral density)*, **BMP-7** *(bone morphogenetic protein 7)*, **BMSC** *(bone marrow stromal cell)*, **CMC** *(carboxymethylcellulose)*, **CT** *(computerised tomography)*, **DBM** *(demineralised bone matrix)*, **DXA** *(dual energy x-ray absorbiometry)*, **FGF-2** *(fibroblast growth factor 2)*, **HA** *(hydroxyapatite)*, **IGF-1** *(insulin-like growth factor 1)*, **LMP3** *(LIM domain protein-3)*, **MRI** *(magnetic resonance imaging)*, **MSC** *(mesenchymal stem cell)*, **OP-1** *(osteogenic protein 1)*, **OVX** *(ovariectomised)*, **PA** *(polyamide)*, **PCL** *(poly(caprolactone))*, **PDGF-b** *(platelet derived growth factor b)*, **PDLLA** *(poly (D,L-lactic acid))*, **PFU** *(plaque forming units)*, **PLGA** *(poly(lactic-co-glycolic acid))*, **PLLA** *(poly(L-lactic acid))*, **rhBMP-7/OP-1** *(recombinant human BMP-7/OP-1)*, **RT-PCR** *(real time polymerase chain reaction)*, **SCID** *(severe combined immuno-deficient)*, **SEM** *(scanning electron microscopy)*, **TCP** *(tricalcium phosphate)*, **TGF-β1/3** *(transforming growth factor beta 1/3)*, **TSP-1** *(thrombospondin 1)*.

Supplementary Table 3. Bone tissue engineering *in vivo* utilising FGF singularly and in combination.

Growth factor (single or combination)	Animal model (species/strain, location and defect)	Human clinical trial and defect	Time	Delivery system	Dose/ Conc.	Analysis (read outs, efficacy and methodology)	Reference
1. Animal Models for direct FGF delivery 1.1. Large Animal Models							
Dog							
FGF-2	Adult Male Beagle Dog, alveolar bone defect – 5mm		8 weeks	Collagen minipellets, implant	0.15 µg	Bone area was significantly increased approx. 2.7 fold compared to controls. Histology, histomorphometry and radiography.	(Hosokawa *et al.*, 2000)
bFGF	Beagle Dog, furcation class II defect – 3mm x 4mm		6 weeks	Gelatin carrier, implant	30 µg	New bone, trabecular and cementum formation rates were increased 2.4, 2.7 and 2.6 fold compared to controls. Histology and histomorphometry.	(Murakami *et al.*, 2003)
bFGF	Beagle Dog, tibial fracture		2 to 32 weeks	Direct injection	200 µg	Callus area increased 3 and 2 fold after 4 and 8 weeks respective, compared with controls. BMC was also increased 2 fold after 8 weeks. Histology, (Hirata *et al.*, 2013)histomorphometry and radiography.	(Nakamura *et al.*, 1998)
bFGF	Male Beagle Dog, mandible defect – 5mm		2 to 8 weeks	Hydroxypropyl cellulose gel, implant	300 µg/mL	Treatment significantly increased bone formation 1.3 fold compared to control defects. Histology, histomorphometry and radiography.	(Shirakata *et al.*, 2010)
Primate							
FGF-2	Non-Human Primate, inflamed furcation defect		8 weeks	Gelatinous carrier, topical application	100 to 400 µg/mL	Significant periodontal regeneration was observed with treatment. No instances of epithelial down-growth, ankylosis or root resorption were observed. Unknown.	(Takayama *et al.*, 2001)
1.2. Small Animal Models							
Mouse							
FGF-1 or FGF-2	Male Swiss ICR White Mouse, subcutaneous injection over calvaria		3 to 35 d	Direct injection	0.002 to 2 µg/d (4 doses/d over 3 d)	High dose treatment (2 µg/d) increased bone width approx. 7 and 3 fold compared to low dose treatment (0.002 µg/d) with FGF-2 and FGF-1, respectively. Addition of heparin resulted in a respective approx. 5 and 3.5 fold increase in bone width between high and low dose treatment. Histology and histomorphometry.	(Dunstan *et al.*, 1999)
FGF-1	OVX Female Sprague Dawley Rat, osteoporosis model		28 to 56 days (6months after OVX)	Intravenous injection	200 µg/kg/d	Bone area was increased approx. 10 fold following continuous treatment for 28 d, and approx. 8 fold after 56 days, both compared to non-treated OVX controls. Histology and histomorphometry.	
FGF-18	FGFR3$^{-/-}$ Mouse (impaired bone formation model), femur segmental defect – 5mm, intramedullary injection		6 weeks	Titanium coated nylon rods, implant	0.5 µg	FGF-18 stimulation resulted in an approx. 5 fold increase in percentage bone volume compared to PBS treated controls. Histology and micro CT.	(Carli *et al.*, 2012)
rhFGF-2	Diabetic Mouse, calvarial defect		14 d	Polyglycolate/polylactide membrane, implant	5 µg	Treatment reversed bone healing inhibition observed within diabetic mice. Histology and histomorphometry	(Santana and Trackman, 2006)

Supplementary Table 3. Bone tissue engineering *in vivo* utilising FGF singularly and in combination.

rhFGF-2	Female ddY Mouse, implantation beneath maxillary periosteum		7, 14 and 28 d	Gelatin hydrogel, implant	20 μg	Bony maxilla was augmented 1.6 fold compared to original volume. Histology, histomorphometry and immunohistochemistry.	(Kodama *et al.*, 2009)
Rabbit							
FGF-2	Male Japanese White Rabbit, femur full thickness circular osteochondral defect – 5mm		3 to 24 weeks	HA/Collagen nanocomposite fibers, implant	0.5 and 5 μg	Low/high dose FGF-2 increased subchondral bone score a max. 4.8 fold and 2.4 fold (6 weeks) compared to control defects. Histology, histomorphometry, immunohistochemistry and micro CT.	(Maehara *et al.*, 2009)
bFGF	Rabbit, femur segmental defect – 10mm		6 weeks	Collagen minipellet, implant	1.4 to 2 μg	Treatment increased callus filling of the defect 6 fold reaching 90 % bridging. However, the callus did not change into mature bone. Histology, histomorphometry and radiography.	(Inui *et al.*, 1998)
FGF-1 or FGF-2	Rabbit, tibial fracture		10 d	Direct injection	3 μg	Neither FGF treatment accelerated fracture healing. However, calluses were more mature when treated with FGF-2. Histology and histomorphometry.	(Bland *et al.*, 1995)
FGF-2	Japanese White Rabbit, femoral condyle full thickness defect – 5mm		2 and 4 weeks	Interconnected porous calcium HA scaffold, implant	100 μg	After 4 weeks, control scaffolds exhibited 1.5 fold increased lamellar bone formation compared to FGF-2 scaffolds. However, treated scaffolds exhibited significantly greater vascularisation and rapid osseointegration. Histology and histomorphometry.	(Nakasa *et al.*, 2008)
FGF-2	Japanese White Rabbit, ectopic subcutaneous implantation		4 weeks	Interconnected porous calcium HA scaffold, implant	100 μg	Extensive new osteoid deposition was observed within FGF-2 implanted scaffolds, where only fibrous tissue was observed within control scaffolds. Histology and histomorphometry.	(Nakasa *et al.*, 2005)
FGF-2	Female New Zealand Rabbit, tibia segmental defect – 5mm		2 to 12 weeks	Collagen and β-TCP, implant	200 μg	Treated defects were completely filled with new bone tissue where implant alone and no implant controls exhibited 32 % and 14 % defect filling. Maximum load increased 2.1 fold compared to non-treated controls. Biomechanical testing, micro CT and radiography.	(Komaki *et al.*, 2006)
FGF-2	New Zealand White Rabbit, tibial metaphysis implant		3 weeks	Titanium drug test chamber, phagocytosable polyethylene particles, continuous infusion	0.0005 to 0.5 μg/day	Treatment with FGF-2 at 0.05 μg/d increased bone formation 1.3 fold. Addition of low dose particles and FGF-2 at 0.05 μg/d only increased bone formation 1.14 fold. Histology, histomorphometry and immunohistochemistry.	(Goodman *et al.*, 2003)
Rat							
rhFGF-2	Adult Male Sprague Dawley Rat, mandibular defect – 5mm		12 and 24 d	Type I collagen sponge, implant	0.01, 0.1 and 1 μg	Medium and high dose treatment increased bone defect bridging 2.3 and 1.9 fold respectively after 12 d, and 1.3 fold after 24 d, compared to carrier alone. Histology, histomorphometry and radiography.	(Zellin and Linde, 2000)
bFGF	OVX Sprague Dawley Rat, tibia condyle defect		3 months	Matrigel within titanium cylinder (1mm x 10mm), implant	5 μg	Treatment increased bone formation and mechanical stability 2 and 4 fold respective. Biomechanical testing, histology and micro CT.	(Gao *et al.*, 2009)
FGF-1	Male Sprague Dawley Rat, ectopic intramuscular implantation		2 and 4 weeks	Fibrinogen-deproteinated bovine HA, implant	7 μg	Treatment increased blood vessel formation approx. 2.6 and 2 fold compared to untreated controls after 2 and 4 weeks. Treatment also stimulated osteogenesis. FTIR, histology, histomorphometry and SEM.	(Kelpke *et al.*, 2004)

Supplementary Table 3. Bone tissue engineering *in vivo* utilising FGF singularly and in combination.

FGF-2	Male Wistar Rat, calvarial defect – 5mm		2, 4 and 8 weeks	HA ceramic button, implant	0.5 (direct application), 8 and 20 μg (precipitated)	Low dose (8 μg) precipitated HA ceramic button implants exhibited the greatest increase in bone extension into the defect site and cranial thickness, approx. 5 and 1.7 fold respectively. Biochemistry, histology and RT-PCR	(Tsurushima *et al.*, 2010)
bFGF	OVX Female Sprague Dawley Rat, severe osteopenia model		120 d	Subcutaneous injection	1,000 μg/kg (5days per week)	Treatment with bFGF increased trabecular bone volume 2.7 (micro CT) and 2 fold (histology) compared to OVX controls. Biomechanical testing, biochemistry, histology, histomorphometry and micro CT.	(Lane *et al.*, 2003b)
bFGF	Rat, skeletal growth and development		7 to 21 d	Intravenous injection	100 to 300 μg/kg/d (first 7 days)	Growth plate width of the proximal tibia was increased 3 and 1.6 fold after 7 and 21 d compared to controls when treated with high dose bFGF. Histology, histomorphometry and radiography.	(Nagai *et al.*, 1995)
FGF-2	Rat, skeletal growth and development		2 weeks	Intravenous injection	100 μg/kg/d	Treatment increased osteoblast proliferation and new bone formation. Calcified bone area of the tibia increased approx. 1.2 fold. Histology, histomorphometry and radiography.	(Mayahara *et al.*, 1993)
FGF-2	Male Sprague Dawley Rat, calvarial defect – 5mm		3 weeks	Electrospun bioactive glass nanofibers and collagen fibrils, implant	10, 50 and 100 μg/mL	Bone formation was increased approx. 1.3, 1.9 and 16.4 fold compared to composite alone, collagen alone, and blank control, resulting in an approx. 1.3, 1.9 and 4.2 fold increase in defect closure, respectively. Histology and histomorphometry.	(Hong *et al.*, 2010)
FGF-2	Wistar Rat, implantation within the parietal bone/periosteum pocket		2 weeks	Covalent conjugate with carbon nanotubes in collagen sponge, implant	10 μg/mL	Delivery system exhibited favourable biocompatibility and FGF-2 addition accelerated new bone formation. Histology and SEM.	(Hirata *et al.*, 2013)
2. Animal Models for indirect FGF delivery 2.1. Large Animal Models							
Dog							
bFGF (plasmid)	Beagle Dog, root furcation		6 weeks	Transfected cell implantation	unknown	Accelerated regeneration of periodontal bone tissue was observed following implantation of transfected cell compared to non-transfected controls. Clinical examination, histology, micro CT and radiography.	(Tan *et al.*, 2009)
2.2. Small Animal Models							
Mouse							
modified FGF-2, β-globulin promoter (plasmid vector)	Irradiated C57BL/6 Mouse, intravenous injection		6 to 11 weeks	Transfected cells intravenously injected	6.25×10^4 to 5×10^5 cells	FGF-2 expression was restricted to the bone marrow cavity where a 5 fold increase was observed compared to controls. Trabecular density increased approx. 2.7 fold. Osteogenic markers increased a max. 7 fold (Runx2). Biochemistry, pQCT, histology, RT-PCR	(Meng *et al.*, 2012)
FGF-2 (plasmid vector)	Adult Nude CD-1 Mouse, calvarial defect – 4mm		1 to 20 weeks	Transfected cells on HA/PLGA scaffold, implant	5×10^4 cells	Chemically controlled FGF-2 release with Shield-1 induced an approx. 2 fold increase in bone volume compared to controls. CT, radiography, histology	(Kwan *et al.*, 2011)
modified FGF-2 (plasmid vector)	W41/W41 Mouse, hematopoietic deficient model, intravenous injection		10 to 14 weeks	Transfected cells intravenously injected	Average peripheral blood serum conc. 2.5μg/mL (0.077 to 6.4 μg/mL)	Histomorphometry analysis revealed a 53.5 fold increase in percentage cancellous bone, whilst CT analysis revealed a 2.5 fold increase in trabecular BMD both within the femur. Biochemistry, histology, histomorphometry and pQCT.	(Hall *et al.*, 2007)

Supplementary Table 3. Bone tissue engineering *in vivo* utilising FGF singularly and in combination.

Rabbit							
bFGF (plasmid vector)	New Zealand White Rabbit, radius segmental defect – 15mm		2 to 12 weeks	Transfected cells on porous β-TCP scaffold, implant	5 x 10^6 cells	Examination revealed capillary vasculature at 2 weeks, vascularised bone at 4 weeks, and large regenerated defect areas with new bone formation. Histology, immunohistochemistry, radiography, SEM.	(Guo *et al.*, 2006)
Rat							
bFGF (plasmid vector)	Sprague Dawley Rat, calvarial defect – 8mm		4, 8 and 12 weeks	Transfected cells on HA/PA66 scaffold, implant	2 x 10^5 cells	Addition of transfected cells increased new bone volume approx. 2 fold compared to controls with non-transfected cells. Histology, immunohistochemistry, histomorphometry, DXA, radiography, micro CT, PCR.	(Qu *et al.*, 2011)
3. Animal Models for combinational direct and indirect FGF delivery 3.1. Small Animal Models							
Dog							
bFGF, BMP-2, PDGF and TGF-β	Adult Hound, mandibular premolar implant osteotomies		12 weeks	Titanium implants with non-HA calcium phosphate cement	unknown	Growth factor combination increased bone formation compared to controls. Histology and histomorphometry.	(Meraw *et al.*, 2000)
3.2. Small Animal Models							
Mouse							
FGF-2, rhBMP-2 and VEGF-A	CD-1 Mouse, calvarial defect – 2 mm		12 weeks	Collagen sponge, implant	0.2 μg	BMP-2 and VEGF-A showed enhanced healing capacities compared to FGF-2, however no significant differences between VEGF-A and BMP-2 were observed. Immunohistochemistry and micro CT.	(Behr *et al.*, 2012)
Rabbit							
rhFGF-2 and rhOP-1	New Zealand White Rabbit, tibial bone harvest and drug test chamber		48 weeks	Distilled water (rhFGF-2) or acetate buffer (rhOP-1), infusion	0.05 μg/d rhFGF-2 0.1 μg/d rhOP-1	OP-1 and FGF-2 infusion increased percentage *de novo* bone formation approx. 1.3 and 1.2 fold respective. Combination treatment did not further enhance bone formation. Histology, histomorphometry and immunohistochemistry.	(Ma *et al.*, 2007)
FGF-2 and IGF-1 (plasmid vector)	Female Rabbit, full thickness femoral condyle osteochondral defect – 3.2 x 4mm		3 weeks	Transfected cells encapsulated within gelatin, implant	4 x 10^6 cells/mL (FGF-2 conc. 0.0061 μg/mL IGF-1 conc. 0.2548 μg/mL)	Combination treatment increased subchondral bone score approx. 2.6 fold compared to control implants. Biochemistry, histology and immunohistochemistry	(Madry *et al.*, 2010)
Rat							
FGF-2 and BMP-2	Wistar Rat, ectopic intramuscular implantation		3 weeks	Type I collagen carrier, implant	0.0016 to 50 μg FGF-2 5 μg BMP-2	Treatment with 0.08 μg FGF-2 exhibited the greatest increase in bone area compared to controls (3.3 and 2.1 fold, assessed by radiopacity and histology, respectively), and BMP-2 alone (1.7 and 1.07 fold). Biochemistry, histology, histomorphometry and radiography.	(Fujimura *et al.*, 2002)
FGF-2 and BMP-2	Male ddY Mouse, subfascial implantation		1, 2 and 3 weeks	Porous collagen disc, implant	0.001 to 5 μg FGF-2 5 μg BMP-2	Combination treatment with lose dose FGF-2 (0.001 μg) increased mineral density, radiopacity and bone formation approx. 1.2, 2 and 1.5 fold, respectively after 3 weeks. DXA, histology, histomorphometry and radiography	(Nakamura *et al.*, 2005)

Supplementary Table 3. Bone tissue engineering *in vivo* utilising FGF singularly and in combination.

FGF-2 and BMP-2	Rat, femur defect		4, 8 and 12 weeks	Nanostructured colloidal gelatin within porous titanium scaffold, implant	0.6 µg FGF-2 3 µg BMP-2	Bone formation was accelerated; observed earlier than controls. Increased bone volume was also observed exhibiting superior bone-implant integrity. Biomechanical testing, histology and micro CT.	(van der Stok *et al.*, 2013)
bFGF and rhBMP-2	Rat (WKY, Lewis, Fisher), irradiated mandible		7 weeks	Liquid injection	100 µg	Irradiation reduced bone apposition, BMP-2 or bFGF alone generated new bone, but together they did not show bone maintenance. Histology, immunohistochemistry and micro CT.	(Springer *et al.*, 2008)
FGF-2 and melatonin	Female Wistar Rat, tibia condyle defect – 2mm		4 weeks	Titanium implant (2mm x 4mm) Intraperitoneal injection	10 µg FGF-2 100 mg/kg melatonin (4 weeks)	Bone–implant contact and bone density were increased 1.7, 1.5 and 2.8 fold, and 1.2, 1.1 and 1.8 fold compared to FGF-2 alone, Matrigel alone and control defects, respectively. Histology and histomorphometry.	(Takechi *et al.*, 2008)
bFGF and estrogen	OVX Female Sprague Dawley Rat, severe osteopenia model		3 weeks	Subcutaneous injection	1,000 µg/kg/d bFGF 10 µg/kg estrogen (4 d per week)	Bone formation rate, osteoblast surface and osteoid surface were increased 1.8, 4 and 8 fold respectively, following combination treatment compared to OVX controls. Biochemistry, histology and histomorphometry.	(Iwaniec *et al.*, 2003)
bFGF and 17β-estradiol or PTH (1-34)	OVX Female Sprague Dawley Rat, severe osteopenia model		60 to110 d	Intravenous injection Subcutaneous injection	200 µg/kg/d for 15 d bFGF (starting on day 60) 10 µg/kg/3x per week 17β-estradiol 80 µg/kg/5x per week PTH (1-34) (both starting on day 82)	Percentage bone volume and connectivity were increased 1.6 and 2.5 fold when treated with bFGF and PTH, and 1.1 and 1.9 fold when treated with bFGF and 17β-estradiol, both compared to OVX controls after 110 days. Biochemistry and XTM.	(Lane *et al.*, 2003a)

Abbreviations: BMC *(bone mineral content)*, **BMD** *(bone mineral density)*, **CT** *(computerised tomography)*, **DXA** *(dual energy X-ray absorpiometry)*, **FGF** *(fibroblast growth factor)*, **FTIR** *(fourier transform infrared spectroscopy)*, **HA** *(hydroxyapatite)*, **IGF-1** *(insulin-like growth factor 1)*, **OVX** *(ovariectomised)*, **PA66** *(polyamide 66)*, **PCR** *(polymerase chain reaction)*, **PLGA** *(poly(lactic-co-glycolic acid))*, **pQCT** *(peripheral quantitative CT)*, **PTH** *(parathyroid hormone)*, **RT-PCR** *(real time PCR)*, **SEM** *(scanning electron microscopy)*, **TCP** *(tri-calcium phosphate)* and **XTM** *(X-ray tomographic microscopy)*.

Supplementary Table 4. Bone tissue engineering *in vivo* utilising PDGF within animal models and human trials.

Growth factor (single or combination)	Animal model (species/strain, location and defect)	Human clinical trial and defect	Time	Delivery system	Dose/ Conc.	Analysis (read outs, efficacy and methodology)	Reference
1. Animal Models for direct PDGF delivery Small Animal Models							
Minipig							
PDGF	Minipig, bilateral mandibular alveolar defect		3 months	Collagen (Mucograft) on titanium mesh, implant	750 µg	PDGF and collagen matrix appeared to accelerate soft tissue healing and promote bone formation (1.5 fold). Histology and histomorphometry.	(Herford *et al.*, 2012)
PDGF	Minipig, bilateral mandibular defect - 30 mm x 20 mm		3 months	Collagen (Mucograft) on titanium mesh, implant	750 µg	New bone formation within the defect site was increased approx. 1.45 fold compared to controls. Titanium mesh exposure was reduced 50% following PDGF treatment. Histology, histomorphometry and radiography.	(Herford and Cicciu, 2012)
Mouse							
PDGF-BB	(nu/nu) Mouse, ectopic intramuscular implantation		8 weeks	DBM, implant	0.01, 0.1, and 1 µg/mg	PDGF inhibits intramuscular osteoinduction by demineralized bone matrix in immuno-compromised mice. High dose PDGF reduced the area of new bone and bone marrow at 28 and 56 d. Histology and histomorphometry	(Ranly *et al.*, 2005)
Rabbit							
PDGF-BB	New Zealand White Mature Male Rabbit, calvarial defect - 8 mm		4 weeks	Porous PLLA/TCP membranes, implant	0.5 µg	PDGF-BB releasing molded PLLA-TCP membrane improved bone defect regeneration. Histology and histomorphometry.	(Lee *et al.*, 2001b)
PDGF	Male New Zealand Rabbit, femoral condyle defect - 15 mm		4 weeks	HA, PLGA microspheres and Pluronic®, implant	0.6 and 1.2 µg	PDGF enhanced bone formation compared to the non-treated bone defect. Histology and histomorphometry.	(Delgado *et al.*, 2012)
PDGF BB	Rabbit, unilateral tibial osteotomy		4 weeks	Collagen, implant	80 µg	PDGF stimulated bone fracture healing however mechanical strength was not affected compared to non-operated contralateral bones. Biomechanical testing, histology, histomorphometry and radiography.	(Nash *et al.*, 1994)
Rat							
PDGF-BB	Sprague Dawley Rat, craniotomy - 8 mm		4 weeks	Chitosan sponge, implant	0.1, 0.2 and 0.4 µg	PDGF treatment increased bone volume approx. 10 fold and 1.5 fold compared to untreated and chitosan only treated defects, respectively. Histomorphometry and histological analysis.	(Park *et al.*, 2000)
PDGF-BB	Sprague Dawley Rat, craniotomy - 8 mm		4 weeks	PLLA/chitosan emulsion, implant	0.2 µg	Controlled release of PDGF-BB from chitosan-based scaffolds significantly promoted bone growth compared to controls. Biochemistry, histology and SEM.	(Lee *et al.*, 2002)
rhPDGF-BB	Diabetic Wistar Rat, femur fracture		6, 8, and 12 weeks	β-TCP/type I bovine collagen matrix, implant	22 and 75 µg	High dose treatment increased percentage bone area within the callus approx. 1.5 fold compared to control. Conversely, low dose treatment increased maximum torque to failure approx. 1.5 fold. Biomechanical testing, histology and histomorphometry.	(Al-Zube *et al.*, 2009)

Supplementary Table 4. Bone tissue engineering *in vivo* utilising PDGF within animal models and human trials.

PDGF	Male Sprague-Dawley Rat, closed tibial fracture		10 d	PDLLA-coated titanium, implant	50 µg	Local application of PDGF from biodegradable PDLLA-coated implants significantly accelerated fracture healing compared to control groups. Histology, immunohistochemsitry and radiography.	(Bordei, 2011)
PDGF-BB, VEGF or BMP-2	Rat, femoral segmental defect - 3.8 mm		8 weeks	Fibrin matrix, implant	1 µg/mL	PDGF-BB and VEGF failed to increase bone healing in the atrophic non-union model. BMP-2 was the only growth factor to enhance bone formation (2 fold). Histology and radiography.	(Kaipel *et al.*, 2012)
rhPDGF-BB	Male Sprague-Dawley Rat, distraction model and osteogenesis, unilateral mid-diaphyseal lengthening - 7 mm		35 to 63 d	Bovine collagen dissolved in sodium acetate buffer, injection	100, 300 and 1,000 µg/mL/week (4 weeks)	Union rate was increased 9 fold following PDGF treatment. Medium dose injections increased bone volume approx. 1.9 fold (histology) and approx. 2.4 fold (radiography). Histology, micro CT and radiography.	(Moore *et al.*, 2009)
PDGF-BB	Rat, calvarial defect		2 weeks	PLLA-coated PGA mesh, implant	unknown	After 2 weeks complete reunion of the bone defect was achieved. Biochemistry, histology, histomorphometry and SEM.	(Park *et al.*, 1998)
PDGF-BB	Rat, craniotomy - 8 mm		2 and 4 weeks	TCP/chitosan sponge, implant	unknown	PDGF-BB increased bone formation in the cranial defect above control groups. Histology and histomorphometry.	(Lee *et al.*, 2000)
2. Animal Models for indirect PDGF delivery Small Animal Models							
Mouse							
PDGF-A (adenovirus)	SCID Mouse, ectopic subcutaneous implantation		3 and 6 weeks	Transduced cells on PLGA scaffold, implant	1×10^6 cells	PDGF-A treatment exhibited reduced implant size and mineralisation at 3 weeks, but increased mineral formation at 6 weeks. Histology, histomorphometry and northern blot.	(Anusaksathien *et al.*, 2004)
Rat							
PDGF-B (plasmid vector)	Male Sprague Dawley Rat, alveolar ridge defect - 1 mm		10 to 21 d	Type 1 collagen matrix, implant	5.5×10^8 to 5×10^9 PFU/mL 300 µg/mL	PDGF treatment increased defect site fill approx. 1.8 fold (histology) and bone area fraction within the defect site approx. 1.7 fold (backscatter SEM) after 10 d. Biochemistry, biomechanical testing, histology, micro CT and backscatter SEM.	(Chang *et al.*, 2010)
PDGF-B (adenovirus)	Sprague-Dawley Rat, alveolar bone defect - 3 mm		5 weeks	Collagen matrix, implant	5.5×10^8 to 5.5 x 10^9 PFU/mL	At 35 d, bridging of the defect was complete. Animals receiving high-dose PDGF-B demonstrated greater evidence of cementogenesis along the tooth root. Biochemistry, histology and RT-PCR.	(Chang *et al.*, 2009)
PDGF-B and BMP-7 (adenovirus)	OVX Rat, femoral defect - 2 mm		2 and 4 weeks	Mesoporous-glass/silk scaffold, implant	unknown	Combination treatment increased new bone volume approx. 2 fold compared to scaffold alone after 4 weeks. Histology, immunohistochemistry and micro CT.	(Zhang *et al.*, 2012a)
3. Animal Models for combinational direct and indirect PDGF delivery 3.1 Large Animal Models							
Dog							
PDGF and IGF-1	Dog, mandibular premolar implant osteotomies - 8.5 mm x 3.75 mm		8 weeks	Implants with methylcellulose gel	5 µg/mL	Combination treatment increased bone formation compared to controls. Histology and histomorphometry.	(Nociti Junior *et al.*, 2000)

Supplementary Table 4. Bone tissue engineering *in vivo* utilising PDGF within animal models and human trials.

rhPDGF-B and rhBMP-7 (adenovirus)	Adult Hybrid Dog, mandible defect - 6mm		4, 8 and 12 weeks	Chitosan/collagen scaffolds, implant	2×10^{10} particles/mL	New bone formation was increased approx. 2.3 and 1.6 fold following combination treatment after 4 and 8 weeks. Percentage defect fill and new bone implant contact were increased approx. 1.5 and 1.4 fold compared to controls after 12 weeks. Histology and histomorphometry.	(Zhang *et al.*, 2009b)
PDGF, bFGF, BMP-2 and TGF-β	Adult Hound, mandibular premolar implant osteotomies		12 weeks	Titanium implants with non-HA calcium phosphate cement	unknown	Growth factor combination increased bone formation compared to controls. Histology and histomorphometry.	(Meraw *et al.*, 2000)
PDGF and IGF-1	Dog, extraction sockets with large buccal dehiscences		18 weeks	ePTFE membranes, implant	unkown	Implant to bone contact, area of bone adjacent to implant and total length of the implant surface were increase approx. 2 fold compared to controls. Histology and histomorphometry.	(Becker *et al.*, 1992)
3.2 Small Animal Models							
Rabbit							
PDGF, TGF-β1and VEGF	Male New Zealand Rabbit, intramedullary femur defect - 6 mm (1.5 to 2 cm depth)		4 weeks	Brushite cement, implant	0.25 µg PDGF 0.1 µg TGF-β1 (liquid phase) 0.35 µg VEGF (PLGA encapsulated microspheres)	Triple combination increased new bone formation approx. 10 fold compared to controls where individual factor treatment increased new bone formation maximum 5 fold. Histology and histomorphometry.	(Reyes *et al.*, 2012)
PDGF-BB, VEGF and MSCs	Mouse, ectopic implantation Rabbit, segmental bone defect		unknown	PRP membrane, implant	unknown	In an ectopic mouse model as well a rabbit segmental bone defect model the platelet-rich plasma-based membrane & PDGF was able to biomimic a periosteal response *in vivo* enhancing bone regeneration.	(El Backly *et al.*, 2013)
Rat							
PDGF-BB and BMP-2	Male Sprague-Dawley Albino Rat, calvarial defect - 6 mm		4 weeks	Fibrinogen scaffold, implant	0.05 µg	Fibronectin and growth factors improved bone healing compared to growth factors alone. Flow cytometry and micro CT.	(Martino *et al.*, 2011)
PDGF-BB and BMSCs	Fisher Rat, calvarial defect - 5 mm		8 weeks	β-TCP scaffold, implant	0.001 and 0.05 µg/mL 2×10^7 cells	Combination treatment increased percentage new bone area approx. 2.5 fold compared to controls. Histology, histomorphometry and micro CT.	(Xu *et al.*, 2012)
rhPDGF-BB and osteogenin	Rat, craniotomy (8 mm)		4 weeks	Rat collagen, matrix	20, 60 and 200 µg PDGF-BB 30 and 150 µg osteogenin	Osteogenin alone increased radiopacity dose dependently; maximum 5 fold increase at high dose compared to controls. Combination with PDGF did not further enhance new bone tissue formation (approx. 5.5 fold with osteogenin alone and approx. 4.5 fold with combination treatment). Histology and histomorphometry.	(Marden *et al.*, 1993)
rhPDGF-BB and BMP-2 (adenovirus)	Male Sprague-Dawley Rat, calvarial defect – 8mm		2 to 4 weeks	rhPDGF protein and BMSCs expressing BMP-2, implant	1×10^6 cells	Increased bone regeneration by delivery of autologous AdBMP2-transfected BMSCs and rhPDGF-BB in both the amount of new bone formed and bone mineral density. The bone growth was greater than those observed in the group treated with AdBMP2-transfected BMSCs alone.	(Park *et al.*, 2013)

Supplementary Table 4. Bone tissue engineering *in vivo* utilising PDGF within animal models and human trials.

4. Human Trials for PDGF delivery							
Periodontitis							
rhPDGF-BB		Human, advanced periodontitis and interproximal intrabony and/or molar Class II furcation defects	9 months	Bone allograft, implant	0.5, 1 and 5 mg/mL	Vertical probing depth was reduced 1.12 fold and clinical attachment level was increased 1.17 fold, both following treatment compared to control anorganic bovine bone in collagen. Clinical assessment, histology and radiography.	(Nevins *et al.*, 2003)

Abbreviations: bFGF *(basic fibroblast growth factor)*, **BMSC** ***(b****one marrow stromal cell)*, **BMP-2** *(bone morphogenetic protein 2)*, **BMP-7** *(bone morphogenetic protein 7)*, **CT** *(computer tomography)*, **DBM** *(demineralised bone matrix)*, **HA** *(hydroxyapatite)*, **IGF-1**, *(insulin growth factor-1)*, **OVX** *(ovariectomised)*, **PCL** *(poly(epsilon-caprolactone)*, **PDGF** ***(****platelet-derived growth factor)*, **PDLLA** *(poly (D, L lactic acid))*, **PFU** *(plaque forming units)*, **PGA** *(polyglycolic acid)*, **PLGA** *(poly(lactic-co-glycolic) acid)*, **PLLA** *(poly(l-lactide)*, **PRP** *(platelet-rich plasma)*, **PTFE** *(polytetrafluoroethylene)*, **RT-PCR** *(real time polymerase chain reaction)*, **SEM** *(scanning electron microscopy)*, **TCP** *(tricalcium phosphate)*, **TGF- β1** *(transforming growth factor-beta 1)* and **VEGF** *(vascular endothelial growth factor)*.

Supplementary Table 5. Bone tissue engineering *in vivo* utilising PTH within animal models and human trials.

Growth factor (single or combination)	Animal model (species/strain, location and defect)	Human clinical trial and defect	Time	Delivery system	Dose/ Conc.	Analysis (read outs, efficacy and methodology)	Reference
1. Animal Models for direct PTH delivery 1.1. Large Animal Models							
Dog							
PTH (1-34)	American Hound Dog, proximal tibial defect		4 weeks	Plasma-sprayed titanium, implant Subcutaneous injection	5 μg/kg/d	Surface fraction of woven bone (but not lamellar bone) at the implant interface was increased by 1.4 fold in the PTH group. No significant difference in bone or fibrous tissue was observed in the circumferential regions around the implant and there were no differences in mechanical parameters. Biomechanical testing and histomorphometry.	(Daugaard *et al.*, 2012)
PTH (1-34)	American Hound Dog, proximal tibial defect - 1mm		4 weeks	Plasma-sprayed titanium, implant Subcutaneous injection	5 μg/kg/d	PTH increased bone formation in the outer gap (2.7 fold) and inner gap (2.5 fold), implant interface (1.6 fold), shear stiffness (2 fold) and energy absorption (1.7 fold). Biomechanical testing and histomorphometry.	(Daugaard *et al.*, 2011)
PTH (1-34)	American Foxhound Dog, mandibular defect - 1.5 x 4mm		4 and 12 weeks	RGD-modified PEG hydrogel, implant	20 μg/mL hydrogel	All implants osseointegrated. PTH increased the area of newly formed bone 1.4 and 1.3 fold, at 4 and 12 weeks respectively, and was comparable to the autologous bone graft gold standard. Histology and histomorphometry.	(Jung *et al.*, 2007a)
Monkey							
PTH (1-34)	Cynomolgus Monkey, femoral transverse osteotomy fracture		26 weeks	Subcutaneous injection	0.75 or 7.5 μg/kg/d	Complete union occurred in all groups, although callus was largest in control groups, and smallest in 7.5 μg PTH-treated groups (1.4 fold less). PTH (7.5 μg) increased ultimate stress 1.6 fold and elastic modulus 1.5 fold. Percentage bone area and osteoclast number were reduced with both 0.75 and 7.5 μg PTH. Biomechanical testing, histology, micro CT and radiography.	(Manabe *et al.*, 2007)
Teriparatide (PTH (1-34))	OVX Cynomolgus Monkey		1.5 to 4.5 years	Subcutaneous injection	5 μg/kg/d	Teriparatide increased vertebral and femoral BMD (1.3 and 1.1 fold), BMC (1.4 and 1.3 fold), ultimate load (1.5 and 1.3 fold) and stiffness (1.5 and 1.4 fold). Withdrawal of PTH treatment for 3 years reduced these differences in the vertebra, but less so in the femur. Bone neoplasia was not detected in any groups. Biomechanical testing, histology and radiography.	(Vahle *et al.*, 2008)
Sheep							
PTH (1-34)	Sheep, femoral and humeral cylindrical defects - 8 x 13mm		5 and 8 weeks	Transglutaminase / PTH fusion protein incorporated fibrin gels, implant	50, 100, 400, or 1,000 μg/mL	PTH fusion protein increased percentage bone 2.2 fold (100 μg/mL), 2.1 fold (400 μg/mL), and 3.4 fold (1,000 μg/mL) compared to fibrin only gels. Histology and micro CT.	(Arrighi *et al.*, 2009)

Supplementary Table 5. Bone tissue engineering *in vivo* utilising PTH within animal models and human trials.

1.2. Small Animal Models							
Mouse							
PTH (1-84), PTH (1-34), PTH (28-48) or PTH (53-84)	ICR Neonatal Mouse		17 d	Subcutaneous injection	0.05 or 0.2 μg/g/d	Tibial DNA content was increased with low dose PTH (1-84) and (1-34) (approx. 5.9 and 6.3 fold), low and high dose PTH (28-48) (approx. 6.3 fold), and was not affected by PT13.1H (53-84). Mandibular condyle DNA content was dose-dependently increased with PTH (1-84), (1-34) and (28-48) but was unaffected by PTH (53-84). Low dose PTH (28-48) increased cellular proliferation 3 fold, and IGF expression 1.3 fold. Biochemistry, *in situ* hybridisation and radiography (auto).	(Rihani-Bisharat *et al.*, 1998)
Teriparatide	C57BL/6 Mouse, femoral diaphyseal defect - 4mm		2, 4 and 9 weeks	Subcutaneous injection	0.4, 4, or 40 μg/kg/d	Significant effects were only observed with 40 μg PTH and with either immediate or 1 week post-op treatment. PTH treatment increased percentage union ratio (approx. 2 fold), osteoclast number (between 3 and 4 fold) and markers of bone formation (collagen type I, osteocalcin). Biomechanical testing, histology, histomorphometry, micro CT and RT-PCR.	(Takahata *et al.*, 2012)
PTH (1-34)	CD1 Mouse, tibial defect - 0.5mm		4 weeks	Calcium phosphate coated titanium implants	10 or 100 μg	The highest dose of PTH improved osseointegration of implants, increasing bone-implant contact by 1.6 fold compared to controls. Histology, histomorphometry, micro CT, radiography and SEM.	(Yu *et al.*, 2012b)
PTH (1-34)	C57BL/6 Mouse, closed femoral fracture		2 to 28 d	Subcutaneous injection	30 μg/kg/d	PTH increased callus formation, with a 3 fold increase in chondrogenesis over osteogenesis, and increased expression of Wnts 4, 5a, 5b, and 10b. Histology, histomorphometry, PCR, radiography and western blot.	(Kakar *et al.*, 2007)
Teriparatide	C57BL/6 Mouse, femoral segmental defect - 2 x 4mm		6 and 9 weeks	PLA or PLA/β-TCP scaffold, implant Subcutaneous injection	40 μg/kg/d	Teriparatide increased new bone volume in PLA and PLA/β-TCP scaffolds at 6 weeks (3 fold and 2.1 fold) and in PLA/β-TCP scaffolds at 9 weeks (1.8 fold). 30% of teriparatide-treated defects demonstrated a bridging union. No union was observed in control animals. Biomechanical testing, histology and micro CT.	(Jacobson *et al.*, 2011)
Teriparatide	C57BL/6 Mouse, femoral defect - 4mm		4 and 6 weeks	Devitalised femoral allograft, implant Subcutaneous injection,	40 μg/kg/d	Teriparatide induced prolonged cartilage formation at 4 weeks, leading to enhanced trabeculated bone callus formation and graft-host integration at 6 weeks. Teriparatide increased normalized callus volume (2 fold), union ratio (2 fold), torsional rigidity (3.8 fold), and yield torque (1.5 fold). Biomechanical testing, histology and micro CT.	(Reynolds *et al.*, 2011)
Teriparatide	C57BL/6 Mouse, femoral fracture		7 to 21 d	Subcutaneous injection	40 μg/kg/d	Teriparatide treatment significantly increased the fracture callus volume at 7, 10 and 14 d (approx. 1.4 fold, 4 fold, and 2.1 fold, respectively), with enhanced cartilage formation at day 10 and bone formation at day 14 and upregulation of Osterix expression. Histology, histomorphometry, immunohistochemistry and radiography.	(Kaback *et al.*, 2008)
PTH (1-34), PTH (2-34) or, PTH (1-31)	Swiss/Webster Mouse		15 d	Subcutaneous injection	40 to 800 μg/kg/d	PTH (1-34) significantly increased serum OCN, bone ALP and bone TRAP (maximum 1.7 fold, 1.8 fold, and 1.7 fold increase after 10 days respectively) as well as bone density (1.2 fold). PTH (2-34) and PTH (1-31) were less potent. Biochemistry, biomechanical testing and QCT.	(Mohan *et al.*, 2000)

Supplementary Table 5. Bone tissue engineering *in vivo* utilising PTH within animal models and human trials.

PTH (1-34)	C57BL/6x129/Sv OVX *Ampka1*$^{+/+}$ and *Ampka1*$^{-/-}$ Mouse		6 weeks	Subcutaneous injection	80 µg/kg/d	PTH treatment increased BV/TV (as well as other trabecular and cortical bone indexes) by approx. 4 fold in OVX *Ampkα1*$^{+/+}$ mice, but only by approx. 2.2 fold in OVX *Ampkα1*$^{-/-}$ mice. By contrast, non-OVX *Ampkα1*$^{-/-}$ mice responded better to PTH treatment than non-OVX *Ampkα1*$^{+/+}$ mice (approx. 2.2 fold compared to 1.3 fold increase in BV/TV respectively). Histology, hitomorphometry, micro CT, RT-PCR and western blot.	(Jeyabalan *et al.*, 2012)
PTH (1-34)	Wild-type and *Pth*$^{-/-}$ Mouse, mid-diaphyseal femoral fracture		2 and 4 weeks	Subcutaneous injection	80 µg/kg/d	Bone healing was impaired in *Pth*$^{-/-}$ sham mice. Addition of PTH enhanced fracture healing at 4 weeks in wild-type and *Pth*$^{-/-}$ mice, increasing calcified callus formation (approx. 1.3 and 1.8 fold), maximum load (approx. 1.4 and 1.3 fold), maximum stress (1.5 fold), and energy to failure (approx. 2.5 and 2 fold), although healing was better in wild-type compared to *Pth*$^{-/-}$ mice. Biomechanical testing, histology, immunohistochemistry, micro CT, radiography, RT-PCR and western blot.	(Ren *et al.*, 2011)
PTH (1-34)	Mouse, femoral fracture non-union - 1.7mm		6 weeks	Subcutaneous injection	30 µg/kg	6.3 fold increase in rate of bony union and 3.9 fold reduction in mean gap size in PTH groups compared to controls. Histology and radiography.	(Lin *et al.*, 2012b)
Black bear PTH (1-84)	C57BL/10ScSn/DMD-*mdx* Mouse		10 weeks	Subcutaneous injection	28 nmol/kg	Black bear PTH increased bone volume fraction, with a greater response in mdx mice than wild type (7 fold and 2 fold increase, respectively). Black bear PTH also increased trabecular number and osteoblast area, and decreased osteoclast area, in mdx mice only. Biomechanical testing, histology and micro CT.	(Gray *et al.*, 2012)
Rabbit							
Teriparatide	New Zealand White Rabbit, posterolateral arthrodesis		8 weeks	Autologous iliac crest, implant Subcutaneous injection	10 µg/kg/d	PTH treatment increased fusion rates1.7 fold and improved fusion quality by Emery grading. Biomechanical testing, histology, manual palpation and radiography.	(Lehman *et al.*, 2010)
PTH (1-34)	New Zealand White Rabbit, posterolateral spinal fusion		6 weeks	Subcutaneous injection	10 µg/kg/d	PTH increased fusion (2.7 fold), percentage bone (2.1 fold), percentage cartilage (9.9 fold), osteoblast numbers (2.1 fold) and osteoclast numbers (2.5 fold). Biomechanical testing, faxitron radiography, histology, histomorphometry and micro CT.	(O'Loughlin *et al.*, 2009)
PTH (1-34)	New Zealand White Rabbit, tibial wedge osteotomy - 4 x 10mm		4 weeks	β-TCP / CMC scaffold, implant Subcutaneous injection	10 or 40 µg/kg/d PTH	Low dose PTH increased BMC 1.3 fold. Biomechanical testing, histology, QCT and radiography.	(Tsiridis *et al.*, 2007b)
PTH (1-34)	New Zealand White Rabbit, tibial mid-diaphyseal osteotomy and distraction osteogenesis		5, 14 and 28 d	Subcutaneous injection	25 µg/kg/d	PTH increased BMC (1.5 fold), BMD (1.3 fold), ultimate load (1.3 fold) and work to failure (2 fold). Biomechanical testing, DXA and micro CT.	(Aleksyniene *et al.*, 2009)
PTH (1-34)	Rabbit, calvarial defect		8 weeks	Titanium implants filled with PEG matrix and HA/TCP granules, implant	20 or 100 µg/mL matrix	PTH increased new bone formation; 1.7 fold (20 µg) and 1.9 fold (100 µg) increase in percentage mineralised bone, and 2.2 fold (20 µg) and 2.3 fold (100 µg) increase in regenerated bone area, both compared to empty defects. Histology and histomorphometry.	(Jung *et al.*, 2007b)

Supplementary Table 5. Bone tissue engineering *in vivo* utilising PTH within animal models and human trials.

Rat							
PTH (1-38)	PX Sprague-Dawley Rat, calcium-free diet		1 to 24 h	Subcutaneous infusion	0.4 to 800 μg/kg/d	Continuous PTH infusion stimulated bone resorption, resulting in a dose response increase in serum-ionized calcium and osteoclast numbers (2.1 fold and 3 fold increase over vehicle control at 24 h respectively), as well as a dose-dependent decrease in OPG mRNA (3-fold decrease after 6 h, 7.5 fold decrease after 24 h) and reciprocal increase in RANKL mRNA (27-fold increase after 6 h, 5.5 fold increase after 24 h). Markers of bone formation were significantly reduced. Biochemistry, histology, immunohistochemistry and northern blot.	(Ma *et al.*, 2001)
PTH (1-34)	OVX Sprague-Dawley Rat, femoral fracture		35 d	Subcutaneous injection	3, 10 or 30 μg/kg/d	PTH dose-dependently increased BMD (approx.1.1 fold, 1.2 fold and 1.5 fold) as well as BMC, cortical area and cortical thickness. Woven bone was established within the defects and intramedullary spaces, but not the periosteum. Systemic effects of PTH were seen at vertebral non-fracture sites. Biochemistry, biomechanical testing, histology, histomorphometry, and QCT.	(Komatsu *et al.*, 2009)
PTH (1-34)	Fischer 344 Rat, bone neoplasms		24 months	Subcutaneous injection	5, 30 or 75 μg/kg/d	Incidence of bone tumour increased exponentially, but only in the 30 and 75 μg PTH doses, and only after 500 to 600 d of treatment. Bone mass increased with all PTH treatments, but reduced after treatment withdrawal. Histology and QCT.	(Vahle *et al.*, 2004)
Rat PTH (1-34) or bovine PTH (1-34)	OVX Sprague-Dawley Rat		4 and 8 weeks	Subcutaneous injection	5, 25 or 50 μg/kg/d	All doses of rat and bovine PTH improved bone in OVX rats, increasing BMC (1 to 2.6 fold - rat PTH, 1.3 to 2.7 fold - bovine PTH), BMD (1.2 to 2.1 fold - rat PTH, 1.3 to 2.5 fold - bovine PTH) and BV/TV (5.4 to10 fold - rat PTH, 7.3 to 13.1 fold - bovine PTH). Bovine PTH was 4 to 6 fold more potent than rat PTH. Histology, histomorphometry and radiography.	(Li *et al.*, 2001)
PTH (1-34)	Sprague-Dawley Rat, unilateral mandibular fracture		7 and 21 d	Subcutaneous injection	10 μg/kg/d	PTH increased callus formation and bone density at 7 days, and new bone formation at 21 d. Histology and radiography.	(Rowshan *et al.*, 2010)
PTH (1-34)	Sprague-Dawley Rat, mid-diaphyseal femoral fracture		42 d	Subcutaneous injection	10 μg/kg/d	PTH induced callus formation and remodelling, increasing ultimate load 2.2 fold, BMC 1.5 fold and BMD 1.3 fold, as well as proliferation, osteoclast number and bone markers (collagen I, osteonectin, osteocalcin and serum calcium). Biochemistry, biomechanical testing, DXA, histology, *in situ* hybridisation and northern blot.	(Nakajima *et al.*, 2002)
PTH (1-34)	OVX Wistar Rat, proximal tibial cancellous bone osteotomy		4 weeks	Subcutaneous injection	14.3 μg/kg/d	PTH increased cancellous bone volume 1.8 fold and osteoid surface 1.8 fold and 1.6 fold, in both sham and OVX animals respectively, and repressed bone resorption parameters including eroded surface (1.8 fold), osteoclast surface (1.4 fold) and osteoclast number (5.3 fold), within OVX animals only. PTH also increased cellular proliferation and percentage bone union (1.5 fold in sham and 1.6 fold in OVX animals). Histology and histomorphometry.	(Nozaka *et al.*, 2008)

Supplementary Table 5. Bone tissue engineering *in vivo* utilising PTH within animal models and human trials.

Rat PTH (1-34)	Sprague-Dawley Rat, calvarial defect - 8mm		4 and 8 weeks	β-TCP scaffold, implant Subcutaneous injection,	15 μg/kg/d	At 4 weeks PTH increased percentage bone fill 2 fold, which was further increased with β-TCP (3.1 fold). At 8 weeks PTH increased percentage bone fill 1.3 fold, and 1.6 fold with β-TCP. Histology, histomorphometry and radiography.	(Yun *et al.*, 2010)
Rat PTH (1-34)	Sprague-Dawley Rat, mechanical vertebral loading		1, 2 and 4 weeks	Subcutaneous injection	15 μg/kg/d	PTH and mechanical treatment alone increased trabecular bone formation and mineralisation. Combination of treatments enhanced the effect. Histology, histomorphometry and micro CT.	(Kim *et al.*, 2003)
PTH (1-84)	OVX Sprague-Dawley Rat, bilateral tibial fracture		30 d	Subcutaneous injection	15 or 150 μg/kg/d	Low and high dose increased callus formation, with 1.3 and 1.5 fold increases in percenatge trabecular bone, and improved mechanical strength with 1.6 and 2 fold increases in ultimate load, 1.5 and 1.8 fold increases in ultimate stiffness, and 1.2 and 1.9 fold increases in ultimate stress. Biomechanical testing, histology and histomorphometry.	(Kim and Jahng, 1999)
PTH (1-34)	OVX Sprague-Dawley Rat, posterolateral spinal fusion		4 and 6 weeks	Subcutaneous injection	30 μg/kg/d	PTH induced 2.5 fold (4 weeks) and 1.6 fold change (6 weeks) in fusion rate, approx. 2 fold increase in OCN and 1.2 fold increase in collagen type I, and increased micro CT assessed bone parameters with increased trabecular bone formation. Biochemistry, histology, manual palpation, micro CT and radiography.	(Qiu *et al.*, 2013)
PTH (1-34)	Sprague-Dawley Rat, posterolateral spinal fusion		6 weeks	Subcutaneous injection	30 μg/kg/d	PTH increased fusion rate 1.5 fold and serum osteocalcin 1.5 fold. Biochemistry, histology and manual palpation,	(Lawrence *et al.*, 2006)
PTH (1-34)	Sprague-Dawley Rat, ulna stress fracture		2, 4 and 8 weeks	Subcutaneous injection	40 μg/kg/d	After 8 weeks PTH treatment increased BMD (1.1 fold), BMC (1.1 fold) percentage crack repair (2.5 fold), ultimate force (1.2 fold) and stiffness (1.1 fold). Biomechanical testing, DXA, histology and histomorphometry.	(Sloan *et al.*, 2010)
PTH (1-34)	Fisher 344 Rat, mid-shaft femoral bone marrow ablation		3 months	Dorsal neck subcutaneous injection	40 μg/kg/d	Treatment with PTH for 3 months enhanced endosteal and periosteal bone formation, with a 1.3 fold increase in cortical thickness. Biomechanical testing, CT, histology and radiography.	(Zhang *et al.*, 2010b)
PTH (1-34)	Fisher 344 Rat, femoral bone marrow ablation		21 d	Dorsal neck subcutaneous injection	40 μg/kg/d	PTH promoted formation of lamellar bone after marrow ablation, increasing osteocalcin serum levels approx. 1.7 fold and reducing serum TRAP levels approx. 1.2 fold, as well as increasing the maximum load 1.3 fold and stiffness 1.2 fold. Biochemsitry, biomechanical testing, histology, micro CT, QCT and radiography.	(Zhang *et al.*, 2008)
PTH (1-34)	Sprague-Dawley Rat, spinal arthrodesis		2 to 42 d	Autologous iliac bone graft Subcutaneous injection	40 μg/kg/d	100 % of PTH-treated animals showed spinal fusion at day 28 and 42, a 1.2 fold increase compared to controls. BV/TV increased 1.4 fold after 42 d of PTH treatment, as well as trabecular thickness (1.1 fold) and number (1.2 fold). PTH also increased a number of osteoclast and osteoclast-related genes. Biochemistry, histology, manual palpation, micro CT, PCR and radiography.	(Abe *et al.*, 2007)

Supplementary Table 5. Bone tissue engineering *in vivo* utilising PTH within animal models and human trials.

PTH (1-34)	Wistar Rat, femoral open or closed fracture		6 weeks	Subcutaneous injection	50 μg/kg/d	100 % of closed fractures healed regardless of treatment, while only 52 % of open fractures healed in control groups with no significant increase after PTH treatment (58%). However PTH did increase other parameters in closed and open fractures; callus volume (1.5 fold / 1.7 fold), BMC (1.3 fold / 1.4 fold), stiffness (1.4 fold / 1.5 fold) and callus trabecular BV/TV (1.6 fold/ 1.3 fold). Biomechanical testing, histology, QCT and radiography.	(Tagil *et al.*, 2010)
PTH (1-34)	Wistar Rat, calvarial defect - 5mm		35 d	Subcutaneous injection, PTFE membrane covering defect site	60 μg/kg/d	PTH increased dry weight (1.5 fold), ash weight (1.5 fold) and ultimate stiffness (1.9 fold). Biomechanical testing.	(Andreassen and Cacciafesta, 2004)
PTH (1-34)	Wistar Rat, tibial closed fracture		20 and 40 d	Subcutaneous injection	60 and 200 μg/kg/d	High dose PTH increased ultimate load (1.8 fold) and callus volume (2 fold) after 20 d. 60 and 200 μg PTH increased ultimate load (2.3 and 2.8 fold), ultimate stiffness (3.1 and 3.5 fold) and callus volume (1.4 and 1.7) after 40 d. Biomechanical testing, DXA and histology.	(Andreassen *et al.*, 1999)
PTH (1-34)	Sprague-Dawley Rat		1 to 24 h	Subcutaneous injection	80 μg/kg/d	PTH induced rapid and transient induction of *c-fos* (8.4 fold increase at 1 h, returning to basal levels by 6 h), *c-jun* (2.8 fold increase at 1 hour, returning to basal levels by 3 h) and *c-myc* (2.1 fold increase at 1 h, returning to basal levels by 3 h) mRNA. PTH reduced proliferation within 12-24 h, and induced IL-6 mRNA at 1 h only. Northern blot and radiolabelling.	(Onyia *et al.*, 1995)
PTH (1-34)	Sprague-Dawley Rat, femoral transverse osteotomy		3, 6 and 12 weeks	Subcutaneous injection	10 or 30 μg/kg	High dose PTH pre-treatment and 10 and 30 μg PTH post-treatment increased BMD 1.1 fold 12 weeks after fracture. Ultimate load was significantly increased (1.5 fold) after 30 μg PTH post-treatment 12 weeks after fracture, as well as percentage bone area. Biomechanical testing, histology, histomorphometry and QCT.	(Komatsubara *et al.*, 2005)
PTH (1-34)	OVX Sprague-Dawley Rat, proximal tibial osteotomy		1 to 4 weeks	Subcutaneous injection	30 μg/kg	PTH treatment induced a 1.2 fold increase in tibial BMD at 4 weeks compared to control, as well as increases in bone volume and osteoid surface. Enhanced bone union was observed at 2 weeks (but not at 4 weeks). Histology, histomorphometry and immunohistochemistry.	(Tsuchie *et al.*, 2013)
PTH (1-34)	ORX Sprague-Dawley Rat, bilateral metaphyseal tibial osteotomy		12 weeks	Subcutaneous injection	40 μg/kg	PTH increased OCN serum levels in both sham and ORX animals (3.1 fold). Osseous bridging was increased 1.5 fold in sham animals and 1.1 fold in ORX animals treated with PTH. Biochemistry, histology and CT.	(Komrakova *et al.*, 2011)
PTH (1-34)	OVX Wistar Rat, periodontal disease model		4 weeks	Subcutaneous injection	40 μg/kg	PTH reduced periodontal bone loss by 2.3 fold, and increased optical density by 1.1 fold. Histology, histomorphometry, radiography and SEM.	(Marques *et al.*, 2005)
PTH (1-34)	OVX Female Sprague Dawley Rat, severe osteopenia model		120 d	Subcutaneous injection	40 μg/kg	Treatment with PTH (1-34) increased trabecular bone volume 2.9 (micro CT) and 1.9 fold (histology) compared to OVX controls. Biomechanical testing, biochemistry, histology, histomorphometry and micro CT.	(Lane *et al.*, 2003b)

Supplementary Table 5. Bone tissue engineering *in vivo* utilising PTH within animal models and human trials.

PTH (1-34)	Sprague-Dawley Rat, mechanical tibial loading (4-point bending)		4 weeks	Subcutaneous injection	50 μg/kg	PTH treatment and mechanical bending induced bone formation. Combination increased this anabolic effect. Finite element analysis and histology.	(Roberts *et al.*, 2009)
PTH (1-34)	Wistar Rat, mandibular distraction osteogenesis		10 d, 1 and 3 weeks	Subcutaneous injection	60 μg/kg	PTH increased bone volume at 10 days (1.6 fold), 1 week (1.7 fold) and 3 weeks (1.7 fold). Histology, immunohistochemistry and micro CT.	(Ali *et al.*, 2012)
PTH (1-34)	OVX Wistar Rat		14 weeks	Subcutaneous injection	60 μg/kg	PTH restored metaphyseal bone loss following OVX after 2 weeks of treatment, with maximum increases after 6 weeks; BV/TV increased approx. 2.5 fold, trabecular number approx. 2.1 fold, and trabecular thickness approx. 1.6 fold. PTH also increased epiphyseal bone parameters but to a lesser degree. Biomechanical testing and micro CT.	(Brouwers *et al.*, 2009)
PTH (1-34), PTH (1-31) or monocyclic PTH (1-31)	Wistar Rat, tibial closed fracture		8 and 16 weeks	Subcutaneous injection	15 nmol/kg	PTH increased ultimate load 1.6 fold (PTH (1-34)) and 1.7 fold (PTH(1-31), monocyclic PTH(1-31)), ultimate stiffness 1.6 fold (PTH (1-34), PTH (1-31)) and 1.5 fold (monocyclic PTH (1-31)), and diaphyseal BMC 1.2 fold (PTH (1-34)), 1.3 fold (PTH (1-31)), and 1.2 fold (monocyclic PTH (1-31)), after 8 weeks of treatment. No differences observed between groups after removal of PTH treatment, although mechanical quality and strength continued to increase. Biomechanical testing and DXA.	(Andreassen *et al.*, 2004)
2. Animal Models for combinational direct and indirect PTH delivery 2.1 Large Animal Models							
Sheep							
PTH (1-34) and tiludronate	Romanov-Limousin Sheep		3 months	Subcutaneous injection	500 IU/d PTH 1 mg/kg/d tiludronate	Bone turnover was increased with PTH treatment and reduced with tiludronate or combined PTH/tiludronate treatment. PTH alone increased serum OCN 2 to 4 fold, whereas tiludronate or combined therapy did not alter OCN levels. PTH alone increased osteoclast number (approx. 4.3 fold) which was reduced with tiludronate or combined therapy (approx. 6.3 fold). Biochemistry, histology and histomorphometry.	(Delmas *et al.*, 1995)
2.2 Small Animal Models							
Mouse							
PTH (1-34) and rapamycin	C57BL/6J Mouse		44 d	Rapamycin pellet, implant Subcutaneous injection	10, 30, or 90 μg/kg/d PTH 3 mg/kg/d rapamycin	PTH alone increased BMD 1.2 fold (10 μg) and 2 fold (30 and 90 μg), BMC 1.4 fold (10 μg), 2.2 fold (30 μg) and 2.9 fold (90 μg), as well as bone / mineral CT parameters. Addition of rapamycin decreased BMC and bone / mineral CT parameters, especially at higher PTH doses. Biochemistry, DXA, histomorphometry, micro CT and QCT.	(Niziolek *et al.*, 2009)
PTH (1-34) and human PDL cells	CD-1 Nude Mouse, subcutaneous implantation - 3.5mm		4 weeks	Gelatin sponge, implant Subcutaneous injection	40 μg/kg/d 3 x 10^6 cells	PTH increased expression of bone markers ALP (1.7 fold), OPN (1.2 fold) and OCN (1.3 fold), mineralisation (1.9 fold), and blood serum OCN levels (3 fold). Biochemistry, histology and immunohistochemistry.	(Wolf *et al.*, 2012)

Supplementary Table 5. Bone tissue engineering *in vivo* utilising PTH within animal models and human trials.

PTH (1-34) and BMSCs	NIH III Nude Mouse, subcutaneous implantation - 3.5 x 5mm		15 weeks	Gelatin sponge, implant Subcutaneous injection	40 µg/kg/d 2 to 3 x 10^6 cells	PTH increased the number of BMSC-derived ossicles 1.9 fold, percentage bone volume 2.5 fold, BMD 2.3 fold, and calcium content 1.9 fold when administered for 3 weeks. Biochemistry, histology, histomorphometry, micro CT, northern blot, radiography and raman spectroscopy.	(Pettway *et al.*, 2005)
PTH (1-34), BMSCs and zoledronic acid	NIH III Nude Mouse, subcutaneous implantation		3 weeks	Gelatin sponges, implant Intraperitoneal injection (zoledronic acid) Subcutaneous injection (PTH)	40 µg/kg/d PTH 3 µg/mouse/d zoledronic acid	PTH alone administered 1 d or 1, 2, or 3 weeks after BMSC implantation increased bone formation approx. 3 fold, 4.3 fold, 2 fold and 2 fold, respectively. PTH alone increased osteoblast numbers (administered 1 d, 1 week or 2 weeks after BMSC implantation) or osteoclast numbers (administered 3 weeks after BMSC implantation). Combined PTH and zoledronic acid reduced cell proliferation and marker expression but did not reduce PTH-induced bone formation. Biochemistry, histology, histomorphometry, micro CT and RT-PCR.	(Pettway *et al.*, 2008)
PTH (1-34) and GFP-labelled MSCs	C57BL/6J Mouse, and C57BL/6J/*Rag2*$^{-/-}$ Mouse, intramedullary injection		1 week	Subcutaneous injection	40 µg/kg 5 x 10^5 cells	1.5 to 2 fold increase in osteoblast Smad phosphorylation in C57BL/6J mice treated with PTH, and a 6 fold increase in Smad phosphorylation in bone marrow transplanted GFP-labelled MSCs in C57BL/6J/*Rag2*$^{-/-}$ mice treated with PTH. 1.8 fold increase in bone marrow transplanted Osterix^{+} GFP-labelled MSCs in C57BL/6J/*Rag2*$^{-/-}$ mice after PTH treatment, indicating stimulation of osteoblast differentiation. FACS and immunocytochemistry.	(Yu *et al.*, 2012a)
PTH and PTHrP (1-84) peptide	Wildtype and PTHrP knockout Mouse		1 month	Subcutaneous injection	0.2 µg/d	Increased endochondral long bone formation was observed in both trabecular (approx. 3 fold) and cortical bone (approx. 2 fold). Augmented growth plate chondrocyte proliferation, differentiation and cartilage matrix mineralisation. Faxitron analysis, histology, immunohistochemistry, micro CT, radiography, RT-PCR and western blot analysis.	(Xue *et al.*, 2005)
Rabbit							
PTH (1-34) and rhBMP-7	New Zealand White Rabbit, tibial wedge osteotomy - 4 x 10mm		4 weeks	β-TCP particles and CMC, implant (rhBMP-7) Subcutaneous injection (PTH (1-34))	10 µg/kg/d PTH 200 µg rhBMP-7	Micro CT analysis revealed approx. 1.6 and 1.4 fold increased bone volume and BMC compared to controls. Histological analysis revealed approx. 1.3 fold increased bone area compared to controls. Torsional rigidity and compressive strength increased approx. 1.4 and 1.2 fold compared to controls. Biomechanical testing, histology, immunohistochemistry and micro CT.	(Morgan *et al.*, 2008)
Rat							
PTH (1-34), PTH (28-48) with IL-6 and IL-6 soluble receptor	Wistar Rat, tibial fracture		1 to 6 weeks	Subcutaneous injection	1 µg PTH 40 ng IL-6 100ng IL-6 soluble receptor	At 14 days PTH (1-34) alone increased tibia volume compared to non-fractured bones. Combination of PTH and IL-6/IL-6 soluble receptor significantly increased tibia volume approx. 2.4 fold (PTH (1-34)) and 3 fold (PTH (28-48)), compared to fractured vehicle-treated control tibia. At 5 weeks PTH treatment enlarged callus formation, whereas PTH with IL-6/IL-6 soluble receptor led to full healing, and increased the power to failure approx. 3.1 fold (PTH (1-34)) and 2.3 fold (PTH (28-48)). Biomechanical testing, histology and radiography.	(Rozen *et al.*, 2007)

Supplementary Table 5. Bone tissue engineering *in vivo* utilising PTH within animal models and human trials.

PTH (1-34) and ibandronate	OVX Sprague-Dawley Rat		8 and 12 weeks	Subcutaneous injection	10 µg/kg PTH 7 µg/kg ibandronate	PTH and ibandronate alone or combined reversed ovariectomy induced deteriorations in trabecular and cortical bone. Combined PTH/ibandronate restored bone microarchitectural indices earlier than that PTH alone, preserving mean BMD. Micro CT, nanoindentation testing, QCT and SEM.	(Yang *et al.*, 2013)
PTH (1-34) and BMP-2	Harlan Sprague-Dawley Rat, mid-diaphyseal femoral defect - 5mm		8 weeks	BMP2-incorporated PLGA microspheres in gelatin hydrogel, implant Subcutaneous injection	10 µg/kg PTH 6.5 µg BMP-2	PTH did not induce bone formation in empty control defects. BMP-2 scaffolds with PTH treatment increased bone volume by approx. 4.1 and 1.7 fold compared to control defects and BMP-2 scaffold only, respectively. DXA, histology and micro CT.	(Kempen *et al.*, 2010)
PTH (1-34) and alendronate	OVX Wistar Rat		14 weeks	Subcutaneous injection	40 µg/kg PTH 15 µg/kg alendronate	PTH alone and combined with alendronate increased BV/TV (approx. 2.4 and 2.9 fold increase respectively at 14 weeks), cortical thickness (approx. 1.3 and 1.4 fold increase respectively at 14 weeks) trabecular thickness (approx. 1.5 fold increase at 14 weeks) and stiffness (approx. 1.3 and 1.5 fold increase at 14 weeks) over the treatment period. Micro CT.	(Campbell *et al.*, 2011)
PTH (1-34) and zoledronic acid	OVX Sprague-Dawley Rat, proximal tibial defect -1mm		12 weeks	HA-coated titanium, implant Subcutaneous injection	60 µg/kg PTH 1 mg/mL zoledronic acid	Combination treatment increased BV/TV by 2.2 fold, trabecular number by 1.3 fold, percentage osseointegration by 1.6 fold, bone to implant contact by 1.8 fold and percent bone area by 2.1 fold, compared to controls. Monotherapy with PTH or zoledronic acid showed no difference when compared to each other. Biomechanical testing, histology and micro CT.	(Li *et al.*, 2013)
PTH (1-34) and bFGF	OVX Female Sprague Dawley Rat, severe osteopenia model		60 to 100 d	Subcutaneous injection Intravenous injection	80 µg/kg/5x per week PTH (1-34) (starting on day 82) 200 µg/kg/d for 15 d bFGF (starting on day 60)	Percentage bone volume and connectivity were increased 1.6 and 2.5 fold when treated with PTH and bFGF compared to OVX controls after 110 days. Biochemistry and XTM.	(Lane *et al.*, 2003a)
PTH (1-34), pamidronate and ibandronate	Sprague-Dawley Rat, tibial defect - 1.7 x 3mm		2 weeks	Bisphosphonates bound to stainless steel screws, implant Subcutaneous injection	Unknown (PTH) 300 ng/cm^2 pamidronate 340 ng/cm^2 ibandronate	PTH alone, and bisphosphonate alone increased pull-out force (1.9 fold and 1.5 fold) and pull-out energy (2.3 fold and 1.7 fold), compared to controls. Combining PTH with bisphosphonate further increased the pull-out force (2.1 fold) and pull-out energy (3 fold), compared to both controls and individual treatment. Biomechanical testing.	(Aspenberg *et al.*, 2008)
3. Human Trials for PTH delivery							
Healthy Adults							
PTH (1-34)		Human, healthy young adults	7 d	Continuous infusion pump	2 to 4 pmol/kg/h	PTH treatment increased bone resorption markers and decreased bone formation markers. Higher doses induced hypercalcemia. Biochemistry.	(Horwitz *et al.*, 2011)

Supplementary Table 5. Bone tissue engineering *in vivo* utilising PTH within animal models and human trials.

Low Bone Mineral Density							
Teriparatide		Human, institutionalized with low BMD	1 year	Subcutaneous injection	20 μg/d	No adverse effects were observed with teriparatide treatment, and calcium levels remained unchanged. Teriparatide rapidly increased the P1NP marker of bone formation and the C-telopeptide marker of bone resorption, which remained elevated at 12 months (2.1 and 2.7 fold increase respectively). Biochemistry.	(Ryder *et al.*, 2010)
Mandibular Repair							
Teriparatide		Human, bisphosphonate-related osteonecrosis of the mandible	2 and 3 months	Subcutaneous injection	20 μg/d	PTH increased osteocalcin 5.4 to 5.7 fold, and C-telopeptide 2.4 to 3.2 fold. Biochemistry.	(Kwon *et al.*, 2012)
Teriparatide		Human, edentulous lower jaw with titanium mandibular implant	9 weeks	Subcutaneous injection	20 μg/d	Teriparatide increased new bone volume in the periosteal, cortical and medullary compartments (1.1, 1.5 and 1.6 fold respectively), and new bone to implant contact in the periosteal and medullary compartments (1.2 and 4.7 fold respectively) but reduced bone to implant contact in the cortical compartment. Histology and histomorphometry.	(Kuchler *et al.*, 2011)
Teriparatide		Human, periodontal osseous defect	12 months	Subcutaneous injection	20 μg/d	Teriparatide resulted in significant linear resolution of periodontal defects, with increased bone gain (11.6 fold) and attachment (3.7 fold), and reduction in probing depth (1.8 fold) at 12 months. Biochemistry, clinical assessment and radiography.	(Bashutski *et al.*, 2010)
Postmenopausal Women							
Teriparatide		Human, postmenopausal women	6 and 18 months	Subcutaneous injection	20 μg/d	Teriparatide increased trabecular volumetric BMD by 1.8 % and 4.6 % at 6 and 18 months respectively. At 18 months teriparatide also increased strength-density ratio by 4 %, hip area BMD by 2.8 %, and femoral neck area BMD by 3.7 %. DXA and QCT.	(Keaveny *et al.*, 2012)
Teriparatide		Human, postmenopausal women with distal radial fracture		Subcutaneous injection	20 or 40 μg/d	Low dose teriparatide decreased time to complete cortical bridging from 9.1 weeks to 7.4 weeks (1.3 fold) while high dose teriparatide did not significantly reduce healing time (8.8 weeks). Clinical assessment, CT and radiography.	(Aspenberg *et al.*, 2010)
PTH (1-34)		Human, postmenopausal women with atraumatic vertebral fracture	24 months	Subcutaneous injection	20 or 40 μg/d	PTH reduced the risk of one or more new vertebral fracture by 7 fold, reduced the number of fractures by 2.8 fold (20 μg) and 4.5 fold (40 μg) and increased BMD dose-dependently. Biochemistry and radiography.	(Neer *et al.*, 2001)
PTH (1-84) with ibandronate		Human, postmenopausal women with low bone mass	24 months	Oral tablet (ibandronate) Subcutaneous abdominal injection (PTH)	100 μg/d PTH 150 mg/month ibandronate	Trabecular BMD increased at both radius (2.26 %) and tibia (3.22 %). Cortical thickness and BMD decreased at the radius (1.9 % and 0.76 %). Cortical porosity increased at the tibia (9.43 %). Biochemistry, DXA and QCT.	(Schafer *et al.*, 2013)
PTH (1-34)		Human, postmenopausal women with pelvic fracture		Subcutaneous injection	100 μg/d	PTH treatment reduced fracture healing time (1.6 fold) and increased healing rate (11 fold). CT and radiography.	(Peichl *et al.*, 2011)

Supplementary Table 5. Bone tissue engineering *in vivo* utilising PTH within animal models and human trials.

PTH (1-84)		Human, postmenopausal women with osteoporosis	4 to 24 weeks	Subcutaneous injection	100 μg/d	PTH treatment increased bone formation markers P1NP (446.1 %) and bone specific ALP (129.6 %) compared to baseline and improved quality of life parameters. Biochemistry and quality of life analysis.	(Quesada-Gomez *et al.*, 2011)
PTH (1-84) with alendronate		Human, postmenopausal women with low BMD	1 and 2 years	Oral tablet (alendronate) Subcutaneous injection (PTH) Patients received different treatment regimes in year 1 and year 2	100 μg/d PTH 10 mg/d alendronate	All treatment groups apart from PTH (year 1) / placebo (year 2) increased femoral strength after 2 years (PTH (year 1) / alendronate (year 2) 7.7 fold, PTH and alendronate (year 1) / alendronate (year 2) 4.2 fold, alendronate (year 1) / alendronate (year 2) 4.8 fold), with corresponding increases in trabecular density. DXA and QCT.	(Keaveny *et al.*, 2008)
PTH (1-34)		Human, postmenopausal women with osteoporosis	36 months	Subcutaneous injection	400 U/d	PTH increased Haversian system area by 1.1 fold, lamellar thickness by 1.2 fold, and percentage osteons by 2 to 4 fold. Birefringence, radiography, SEM and TEM.	(Ascenzi *et al.*, 2012)
Vertebral Fracture							
Teriparatide		Human, prevalent vertebral fracture	72 weeks	Subcutaneous injection	56.5 μg/week	Teriparatide treatment reduced the occurrence of new vertebral fracture by 4.7 fold and significantly increased BMD (by 6.4, 3 and 2.3 fold at the lumbar spine, hip, and femoral neck respectively). Biochemistry, DXA and radiography.	(Nakamura *et al.*, 2012)
Abbreviations: ALP *(alkaline phosphatase)*, **BMC** (*bone mineral content)*, **BMD** *(bone mineral density)*, **BMSC** (*bone marrow stromal cell*), **BV/TV** *(bone volume/tissue volume)*, **CMC** *(carboxymethyl cellulose)*, **CT** (*computerised tomography)*, **DXA** (d*ual-energy X-ray absorptiometry)*, **FACS** *(fluorescence-activated cell sorting)*, **GFP** *(green fluorescent protein)*, **HA** *(hydroxyapatite)*, **IGF** *(insulin-like growth factor)*, **IL-6** *(interleukin-6)*, **OCN** *(osteocalcin)*, **OPG** *(osteoprotegrin)*, **ORX** *(orchiectomised)*, **OVX** *(ovariectomised)*, **P1NP** *(procollagen type 1 N propeptide)*, **PDL** *(periodontal ligament cells)*, **PEG** *(polyethylene glycol)*, **PLA** *(poly lactic acid)*, **PLGA** *(poly(lactic-co-glycolic) acid)*, **PTFE** *(polytetrafluoroethylene)*, **PX** *(parathyroidectomized)*, **QCT** (*quantitative CT)*, **RANKL** *(receptor activator of nuclear factor kappa-B ligand)*, **RGD** *(arginylglycylaspartic acid)*, **RT-PCR** *(real time polymerase chain reaction)*, **SEM** (*scanning electron microscopy)*, **TCP** *(tricalcium phosphate)*, **TEM** (*transmission electron microscopy)*, **TRAP** *(tartrate resistant acid phosphatase)* and **XTM** *(X-ray tomographic microscopy)*.							

Supplementary Table 6. Bone tissue engineering *in vivo* utilising PTHrP within animal models and human trials.

Growth factor (single or combination)	Animal model (species/strain, location and defect)	Human clinical trial and defect	Time	Delivery system	Dose/ Conc.	Analysis (read outs, efficacy and methodology)	Reference
1. Animal Models for direct PTHrP delivery Small Animal Models							
Mouse							
C-terminal PTHrP (107-139) peptide	Male ARC Swiss-Webster Mouse		12 d	Periosteal calvarial injection	0.00035 to 13.8 µg daily	Substantial anti-osteoclastic effects were observed, with a 70 % decrease in osteoclast number. Mineralised bone area increased 1.1 fold at the highest dose. Histology and histomorphometry.	(Cornish *et al.*, 1997)
C-terminal PTHrP (107-139) peptide	Male CD-1 Diabetic Mouse		14 d	Subcutaneous injection	100 µg/kg every other day	PTHrP was highly osteogenic reversing diabetic-induced decreases in bone mass, structure, and gene expression. PTHrP also exhibited angiogenic effects (via VEGF stimulation). Histology, immunohistochemistry, micro CT, RT-PCR and western blot analysis.	(Lozano *et al.*, 2010)
N-terminal PTHrP (1-36) peptide	Male CD-1 Diabetic Mouse		13 d	Subcutaneous injection	100 µg/kg every other day	PTHrP reversed deleterious diabetic effects on bone cell number and function. DXA, histology and micro CT.	(Lozano *et al.*, 2009)
Rabbit							
PTHrP analogue (RS-66271)	New Zealand White Rabbit, Modified segmental ulnar defect – 1 mm		6 to 10 weeks	Subcutaneous daily injection	10 µg/kg/d	PTHrP enhanced healing, union, and biomechanical strength. Radiographic intensity was increased 1.2 to 1.4 fold, and callus area increased 2.1 fold anteroposteriorly and 4.2 fold laterally. Torque and stiffness were increased 1.9 and 1.6 fold, respectively. Biomechanical testing and radiography.	(Bostrom *et al.*, 2000)
C-terminal PTHrP epiptope (107-111) - osteostatin	Female New Zealand Rabbit, lateral and medial femoral epiphyseal defect – 5 x 4/5mm		4 to 8 weeks	Osteostatin-loaded silica-based mesoporous SBA15 material scaffold, implant	100 nM	PTHrP (osteostatin) favoured bone regeneration, induced revascularisation via VEGF, and decreased bone resorption. Bone volume was increased approx. 2 to 10 fold dependent on conditions. Histology, immunohistochemistry and micro CT.	(Trejo *et al.*, 2010)
Rat							
N-terminal PTHrP (1-36) peptide	Female Sprague-Dawley Rat, osteopenia model following ovariectomy		6 months	Subcutaneous injection	40 µg/kg/d	PTHrP increased bone formation rate, mass, and biomechanical properties. Ultimate load was increased up to 3 fold. Biochemical and biomechanical testing, DXA and histomorphometry.	(Stewart *et al.*, 2000)
C-terminally substituted PTHrP analogue (RS-66271)	Rat, osteopenia model following ovariectomy		unknown	Subcutaneous injection	80 µg/kg/d	PTHrP analogue reversed trabecular and cortical bone loss, and increased surface area covered by osteoblasts. Electron microscopy and histomorphometry.	(Vickery *et al.*, 1996)
C-terminal PTHrP (1-34) peptide.	Male Sprague-Dawley Rat		12 or 26 d	Subcutaneous injection	10 to 320 µg/kg/d	Increased cortical bone mass was observed, alongside an increase of 1.3 to 1.4 fold in trabecular bone (higher dose only). Biochemical testing and histomorphometry.	(Hock *et al.*, 1989)
Synthetic PTHrP (1-74)	Male Sprague-Dawley Rat		12 days	Subcutaneous injection	0.01 or 0.02 µmol/kg/d	Synthetic PTHrP increased bone weight and calcium / hydroxyproline content at low-dosage levels for prolonged periods. Biochemical testing.	(Weir *et al.*, 1992)

Supplementary Table 6. Bone tissue engineering *in vivo* utilising PTHrP within animal models and human trials.

2. Animal Models for combinational direct and indirect PTHrP delivery Small Animal Models							
Mouse							
PTHrP (1-86) peptide and PTH	Wildtype and PTHrP knockout Mouse		1 month	Subcutaneous injection	0.2 µg/d	Increased endochondral long bone formation was observed in both trabecular (approx. 3 fold) and cortical bone (approx. 2 fold). Augmented growth plate chondrocyte proliferation, differentiation and cartilage matrix mineralisation. Faxitron analysis, histology, immunohistochemistry, micro CT, radiography, RT-PCR and western blot analysis.	(Xue *et al.*, 2005)
N-terminal PTHrP (1-36) peptide and C-terminal PTHrP (107-139) peptide	OVX Female Charles River C57BL/6J Mouse, osteopenia model		4 or 8 weeks	Sub-cutaneous daily injection	80 µg/kg/d (5 days/week)	PTHrP (1-36) exerted anabolic bone effects. PTHrP (107-139) exerted anabolic bone effects and significantly reduced bone resorption. Mineral density (1.7 to 2.2 fold) and total volume (1.5 fold) were significantly increased. Biochemical testing, histology, immunohistochemistry, micro CT and RT-PCR.	(de Castro *et al.*, 2011)
N-terminal PTHrP (1-36) peptide and C-terminal PTHrP (107-139) peptide	Male CD1 type I Diabetic Mouse,		4 weeks	Subcutaneous injection	100 µg/kg every other day	PTHrP reversed diabetes-induced loss of bone mineral density (approx. 1.1 fold) and content (approx. 1.4 fold). Biochemical testing, DXA, immunohistochemistry and RT-PCR.	(Portal-Nunez *et al.*, 2010)
3. Human Trials for PTHrP delivery							
Healthy Adults							
N-terminal PTHrP (1-36) peptide		Human, healthy postmenopausal estrogen deficient females	2 weeks	Subcutaneous abdominal injection	1.36 to 6.56 µg/kg/d	Activated bone formation with increased alkaline phosphatase and osteocalcin expression observed alongside reduced bone resorption (approx. 1.3 to 1.45 fold decrease in resorption markers). Biochemical testing.	(Plotkin *et al.*, 1998)
N-terminal PTHrP (1-36)		Human, healthy adult - systemic infusion	7 d	Continuous intravenous infusion	2 followed by 4 pmol/kg/h	Bone resorption increased rapidly, but was reversed following cessation of PTHrP infusions. Bone formation was suppressed by 1.3 to 1.4 fold, but recovered after PTHrP withdrawal. Biochemical testing.	(Horwitz *et al.*, 2011)
N-terminal PTHrP (1-36) peptide		Human, healthy adult - systemic infusion	2 or 4 d	Continuous intravenous infusion	8 to 28 pmol/kg/h	Profoundly suppressed bone formation following multiple doses over time. Biochemical testing.	(Horwitz *et al.*, 2005)
N-terminal PTHrP (1-34) peptide		Human, healthy adult - systemic infusion	3 x 12 h infusions	Intermittent intravenous infusion	8 or 80 pmol/kg/h	Significantly increased serum total ionized calcium, urinary phosphate and serum VitD3. Biochemical testing.	(Fraher *et al.*, 1992)
Abbreviations: PTHrP *(parathyroid hormone-related peptide)*, **CT** *(computerised tomography)*, **DXA** *(dual energy X-ray absorptiometry)*, **VEGF** *(vascular endothelial growth factor)*, **RT-PCR** *(real time polymerase chain reaction)* and **VitD3** *(1,25-dihydroxyvitamin D3).*							

Supplementary Table 7. Bone tissue engineering *in vivo* utilising TGF-β3 singularly and combination.

Growth factor (single or combination)	Animal model (species/strain, location and defect)	Human clinical trial and defect	Time	Delivery system	Dose/ Conc.	Analysis (read outs, efficacy and methodology)	Reference
1. Animal Models for direct TGFβ-3 delivery 1.1. Large Animal Models							
Baboon							
TGF-β3	Adult Chacma Baboon, maxillary and mandibular furcation – 10 to 12 mm		60 d	Matrigel, implant	75 µg	Significantly increased periodontal tissue regeneration was observed (approx. 75 %) which could be further enhanced with the addition of inducible minced muscle tissue. Histology and histomorphometry.	(Teare *et al.*, 2008)
TGF-β3	Adult Chacma Baboon, maxillary and mandibular furcation – 10 to 12 mm		60 d	Matrigel, implant	75 and 125 µg	Enhanced alveolar bone regeneration (approx. 3 fold), coronal extension and cementogenesis (approx. 1.6 fold) was observed at lower dose TGF-β3 in Matrigel with the addition of minced muscle tissue. Histology and histomorphometry.	(Ripamonti *et al.*, 2009b)
TGF-β3	Adult Chacma Baboon, ectopic intramuscular injection Calvarial defect – 25 mm		30 and 90 d	Collagenous matrix, implant	5, 25, 125 and 250 µg/mg	Significant ectopic endochondral bone formation with large mineralized and corticalized ossicles was observed with minced autogenous fragments of the rectus abdominis muscle which may have provided inducible stem cells (approx. 1.9 fold increase). Histology, histomorphometry, PCR and western blotting.	(Ripamonti *et al.*, 2008)
1.2. Small Animal Models							
Mouse							
TGF-β3	Male Sprague Dawley Rat, acute transection of the rotator cuff		2 and 4 weeks	Calcium phosphate matrix, implant	2.75 µg	Newly formed bone exhibiting. After 2 weeks approx. 5.4 % more bone and 23.3 % more cartilage was observed exhibiting significantly greater ultimate stress at 4 weeks. Biomechanical testing, histomorphometry, immunohistochemistry and micro CT.	(Kovacevic *et al.*, 2011)
Rat							
TGF-β3	Sprague Dawley Rat, postnatal day 9 posterior interfrontal suture		14 d	Collagen gel, implant	0.003 and 0.03 µg	High dose maintained the suture site in an unossified state. Immunohistochemistry.	(Opperman *et al.*, 2002)
2. Animal Models for combinational direct and indirect TGFβ-3 delivery Small Animal Model							
Mouse							
TGF-β3 (recombinant adeno-associated virus)	Nude mouse, ectopic subcutaneous implant		30 d	PLA/PEG scaffold, implant	1 x 10^6 cells	Sizeable 3D cartilage constructs were observed exhibiting 18.8 and 30.3 fold increase in collagen type II and Sox9 expression. Histology, immunohistochemistry and western blot.	(Rizk and Rabie, 2013)
3. Animal Models for combinational direct and indirect TGFβ-3 delivery 3.1. Large Animal Models							

Supplementary Table 7. Bone tissue engineering *in vivo* utilising TGF-β3 singularly and combination.

Baboon							
TGF-β3 and OP-1	Adult Chacma Baboon, ectopic intramuscular implant		90 d	HA/calcium carbonate macroporous scaffold, implant	125 µg	Co-expression induced enhanced bone formation substantially greater than individual expression (max. 1.7 fold) and controls (max. 5.3 fold). Formation of large mineralized and corticalized ossicles provides a therapeutic strategy for human osteoinduction. Histology, histomorphometry and rt-PCR.	(Ripamonti *et al.*, 2010)
Sheep							
TGF-β3 and ovine MSCs (sheep)	Adult Sheep, patella punch defect – 4 mm		9 weeks	Chitosan/fibrin clot, implant	0.05 µg TGF-β3 1 x 10^7 MSCs	Partial thickness defect was filled with chondrocyte-like cells and hyaline-like cartilaginous extracellular matrix demonstrating neocartilage repair. Chondrogenesis was observed after 3 weeks. TGF-β3 enhanced cell proliferation and favoured integration of the neotissue within the host cartilage. Histology, immunohistochemistry and RT-PCR.	(Mrugala *et al.*, 2008)
3.2. Small Animal Models							
Mouse							
TGF-β3, BMP-2 and MSCs	CB-17 SCID Mouse, ectopic subcutaneous implant		3 to 22 weeks	Alginate gel, implant	0.02 µg TGF-β3 0.2 µg BMP-2 1 x 10^6 cells	Combinational treatment induced significant implanted MSC-mediated bone formation. After 3 weeks cartilaginous tissue was observed with interspersed bone. By 15 weeks extensive bone was observed (approx. 13 fold increase in bone area). Histology and histomorphometry.	(Simmons *et al.*, 2004)
TGF-β3 and Chondrocytes (rabbit)	Female BALB/c Mouse, ectopic subcutaneous injection		1 to 8 weeks	pNIPAm-co-AAc hydrogel scaffold, injection	0.1 µg/mL TGF-β3 5 x 10^6 cells	Enhanced chonrogenesis with significant cartilage specific extracellular matrix production including collagen (approx. 1.6 fold) and GAG (approx. 4 fold) after 8 weeks. Histology and immunohistochemistry.	(Park *et al.*, 2009)
TGF-β3 and Chondrocytes (rabbit)	NOD Mouse, ectopic subcutaneous implant		6 weeks	Chondroitin sulphate bound TGF-β3 within PEG-PCL hydrogel, implant	0.1 µg/mL TGF-β3 5 x 10^6 cells	Significant chondrogenesis was observed within constructs demonstrating suitability for cell-based cartilage tissue engineering approaches. GAG and collagen were increased approx. 3 and 1.7 fold, respectively. Histology and immunohistochemistry.	(Park *et al.*, 2010b)
TGF-β3 and hMSCs	Female BALB/c Nude Mouse, ectopic subcutaneous injection New Zealand White Rabbit, full thickness condyle defect – 3 mm		1 and 7 weeks (Mouse) 1 and 6 weeks (Rabbit)	hMSC loaded pNIPAm-co-AAc hydrogel scaffold, injection	0.1 µg/mL TGF-β3 1 x 10^6 hMSCs	Significant chondrogenesis at ectopic sites, and enhanced hyaline cartilage tissue regeneration of condyle defects was observed with 2 fold increase in GAG content. Biochemical testing, histology, immunohistochemistry and rt-PCR.	(Park *et al.*, 2010a)
TGF-β3 and hMSCs	Female BALB/c Nude Mouse, ectopic subcutaneous injection New Zealand White Rabbit, full thickness condyle defect – 3 mm		1 to 4 weeks (Mouse) 1 to 6 weeks (Rabbit)	hMSC and heparinized PLL nanoparticle loaded hydrogel scaffold, injection	0.1 µg/mL TGF-β3 1 x 10^6 hMSCs	TGF-β3 enhanced hMSC survival and proliferation within ectopic implants, inducing expression of chondrocyte-specific extracellualr matrix genes and their proteins. TGF-β3 and hMSCs exhibited enhanced regeneration of hyaline articular cartilage within defect sites. Histology and immunohistochemistry.	(Park *et al.*, 2011)

Supplementary Table 7. Bone tissue engineering *in vivo* utilising TGF-β3 singularly and combination.

TGF-β3, hMSCs and polyplexed Sox9 protein	Female BALB/c Mouse, ectopic subcutaneous injection		1 to 8 weeks	hMSCs with Sox9/heparinized TGF-β3 coated dexamethasone loaded-PLGA microparticles, injection	0.1 µg/mL TGF-β3 2 x 10^6 hMSCs 1,000 µg/mL Sox9	Enhanced chondrogenesis with increased cartilage extracellular matrix GAG production (2.5 fold). Provides a tool for simultaneous delivery of genes and growth factors preventing dedifferentiation of transfected cells. Histology, immunohistochemistry and rt-PCR.	(Park *et al.*, 2012)
Rabbit							
TGF-β3 and Chondrogenic MSCs	New Zealand White Rabbit, femoral condyle defect		12 weeks	PLGA scaffold and fibrin glue, implant	0.01 µg/mL TGF-β3 unknown, MSC number	Good cartilage formation was observed with improved mechanical strength compared to controls (30-60 % in mechanical stiffness). Biomechanical testing and histology.	(Lee *et al.*, 2004)
TGF-β3 and Chondrocytes (rabbit)	Nude Mouse, ectopic subcutaneous injection		8 weeks	Hydrogel scaffold, injection	0.1 µg/mL TGF-β3 5 x 10^6 cells	Organised chondrogenesis and increased extracellular matrix production within implanted cell loaded scaffolds. Collagen and GAG were observed within lacunae structures. Type II collagen production was increased 22 fold. Biochemical testing, histology, immunohistochemistry and rt-PCR.	(Na *et al.*, 2006)
TGF-β3 and Chondrocytes (rabbit)	Nude Mouse, ectopic subcutaneous injection		1 to 8 weeks	Hydrogel scaffold, injection	0.1 µg/mL TGF-β3 1 x 10^6 cells	Enhanced cartilage and related extracellular matrix production containing collagen (3.8 fold) and sulfated GAG (2.8 fold). May provide a rapid and clinically effective approach to cartilage regeneration. Biochemical testing, histology and immunohistochemistry.	(Choi *et al.*, 2007)
TGF-β3 and MSCs (rabbit)	New Zealand White Rabbit, femoral condyle defect – 6 x 3 mm		12 weeks	PLGA scaffold, implant	0.01 µg/mL TGF-β3 2 x 10^6 cells	Cartilage regeneration similar to native tissues, showing good integration and void filling. Demonstrated approx. 80 % Young's modulus of native cartilage. Collagen and GAG content increased approx. 10 and 8 fold, respectively. Biomechanical testing, histology and immunohistochemistry.	(Han *et al.*, 2008)
Rat							
TGF-β3 and BMP-2	Female Sasco Sprague Dawley Rat - femoral segmental defect – 8 mm		4 to 16 weeks	PLDL scaffolds, implant	0.02 µg TGF-β3 0.2 µg BMP2	Increased mineralization with a higher but not consistent rate of bone bridging across the defect. A non-significant torsional strength improvement was achieved. 12.8 fold increased bone formation was observed compared to empty defects. Biomechanical testing, histology and micro CT.	(Oest *et al.*, 2007)
TGF-β3 and TGF-β1	Male Sprague Dawley Rat, proximal humerus defect – 0.5 mm drill defect		1 to 4 weeks	Osmotic pump, continuous infusion	0.005µg/d	TGF-β isoforms did not improve supraspinatus tendon-to-bone healing. This may have been due to a suboptimal delivery method or suboptimal timing/duration of growth factor administration. Defect sites were predominantly filled with scar tissue. Biomechanical testing, histology and immunohistochemistry.	(Kim *et al.*, 2010a)

Abbreviations: BMP-2 *(bone morphogenetic protein 2)*, **CT** *(computer tomography)*, **GAG** *(Glycos-Amino Glycan)*, **HA** *(hydroxyapatite)*, **MSC** *(mesenchymal stem cell)*, **OP-1** *(osteogenic protein 1)*, **PCL** *(poly-caprolactone)*, **PCR** *(polymerase chain reaction)*, **PEG** *(poly-ethylene glycol)*, **PLDL** *(poly(L-lactide-co-D,L-lactide))*, **PLA** *(poly-lactic acid)*, **PLGA** *(poly(lactic co-glycolic acid))*, **PLL** *(poly-L-lysine)*, **pNIPAm-co-AAc** *(poly(N-isopropylacrylamide-co-acrylic acid))*, **RT-PCR** *(real time PCR)*, **rt-PCR** *(reverse transciption PCR)* and **TGF-β3** *(transforming growth factor beta 3)*.

Supplementary Table 8. Bone tissue engineering *in vivo* utilising VEGF singularly and combination.

Growth factor (single or combination)	Animal model (species/strain, location and defect)	Human clinical trial and defect	Time	Delivery system	Dose/ Conc.	Analysis (read outs, efficacy and methodology)	Reference
1. Animal Models for direct VEGF delivery Small Animal Models							
Mouse							
$rhVEGF_{165}$	Male MF1 nu/nu Mouse, femur segmental defect – 5 mm		4 weeks	PLA scaffold and hBMSCs, implant	1.7 µg	Significant osteogenic growth and repair was observed. VEGF/hBMSCs increased blood vessel formation compared to control. Bone volume was increased approx. 1.65 fold. Histology, immunohistochemistry, micro CT and radiography.	(Kanczler *et al.*, 2008)
rhVEGF	Male BALB/c Mouse, calvarial defect – 4 mm		4 weeks	Biphasic calcium phosphate ceramics, implant	5 µg/mL	Promoted biomaterial vascularization but had no distinct impact on bone formation. Histology, histomorphometry and intravital microscopy.	(Wernike *et al.*, 2010)
2. Animal Models for indirect VEGF delivery Small Animal Models							
Mouse							
$VEGF_{165}$ (plasmid vector)	Male C57BL/6 Mouse, ectopic subcutaneous implant and intra-femoral defect – 1 x 8 mm		30 d	Collagen/calcium phosphate scaffold, implant	0.35 µg/mm^3 (plasmid DNA to scaffold approx. 20 µg)	Increased bone formation (2 fold) was observed, however blood vessel formation was not significantly different to controls. Histology, histomorphometry and rt-PCR.	(Keeney *et al.*, 2010)
Rabbit							
$VEGF_{165}$ (plasmid vector)	New Zealand White Rabbit, radial segmental defect – 15 mm		16 weeks	Transfected cells on Biocoral (calcium carbonate) scaffold, implant	5 x 10^6 cells	Significantly enhanced vascularization and increased bone formation by 1.6 fold compared to controls. Histology, histomorphometry, micro CT and radiography.	(Geiger *et al.*, 2007)
VEGF (plasmid vector)	Adult Rabbit, tibial segmental defect – 10 mm		12 weeks	Transfected cells delivered via impregnated gelfoam, implant	5 x 10^6 cells	Newly formed trabecular bone, callus formation and ossification across the entire defect were observed. Bone volume doubled. Histology, immunohistochemistry, micro CT and radiography.	(Li *et al.*, 2009b)
3. Animal Models for combinational direct and indirect VEGF delivery 3.1. Large Animal Models							
Dog							
VEGF and BMP-2	Beagle Dog, ectopic paraspinal muscle implant, ulna critical size defect		9 weeks	Gelatin (fast release) or PLGA microparticle (sustained release) loaded biphasic calcium phosphate scaffold, implant	0.4 µg VEGF 12 µg BMP-2 (ectopic site) 4 µg VEGF 120 µg BMP-2 (ulna defect)	Ectopic BMP-2 fast release showed significantly more bone compared to sustained release, independent of the VEGF profile. Significant enhancement of bone formation was observed within all orthotopic groups compared to controls independent of growth factor release profile or combination. Histology, histomorphometry and immunohistochemistry.	(Geuze *et al.*, 2012)
Pig							
VEGF and rhBMP-2	Female Domestic Pig, cranial drill defect – 4.2mm		1 to 4 weeks	Titanium plugs with adsorbed octacalcium phosphate, implant	1.32 µg VEGF 5.26 µg BMP-2,	Implants revealed enhanced bone volume density (approx. 1.6 fold increase), but not osseointegration. Histology, histomorphometry and micro-radiography.	(Ramazanoglu *et al.*, 2011)

Supplementary Table 8. Bone tissue engineering *in vivo* utilising VEGF singularly and combination.

3.2. Small Animal Models							
Mouse							
VEGF-A , FGF-2 and rhBMP-2	CD-1 Mouse, calvarial defect – 2 mm		12 weeks	Collagen sponge, implant	0.2 µg	VEGF-A and BMP-2 showed enhanced healing capacities compared to FGF-2, however no significant differences between VEGF-A and BMP-2 were observed. Immunohistochemistry and micro CT.	(Behr *et al.*, 2012)
VEGF and BMP-7	Black C57/B16 Mouse, ectopic subcutaneous implant		12 weeks	Injection within biphasic calcium phosphate ceramics, implant	2 µg VEGF 5 µg BMP-7	Woven bone formation was observed on scaffold surface and within pores. However, combination of VEGF and BMP-7 did not enhance bone formation significantly. Histology and SEM.	(Roldan *et al.*, 2010)
VEGF and rhBMP-2	Male MF1 nu/nu Mouse, femur segmental defect – 5mm		4 weeks	Alginate and PLA, implant	20 µg	Significantly increased new endochondral bone matrix formation (approx. 3.2 fold). Histology, immunohistochemistry and micro CT.	(Kanczler *et al.*, 2010)
VEGF and BMP-4 (retrovirus)	SCID Mouse, ectopic intramuscular implant		7 to 35 d	Transduced cells on gelatin sponge, implant	2 x 10^5 cells	Co-expression of BMP-4 and VEGF impaired ectopic endochondral bone formation at high VEGF ratio. When VEGF release was low and sustained, any negative effects were no longer observed. Biochemical testing, histology and radiography.	(Li *et al.*, 2009a)
VEGF/BMP-2 (recombinant adeno-associated virus 6)	Athymic Nude Mouse, tibiae segmental defect – 2/3mm		5 weeks	Transduced male mouse MSCs, intravenous injection	1 x 10^6 cells: 5 x (2 x 10^5 cells) – 1 per day	Enhanced bone formation was observed within defect sites compared to control groups. Bone volume, peak load and stiffness increased approx. 6, 4.5 and 4 fold respectively, compared to control group. DXA, histology, immunohistochemistry and micro CT	(Kumar *et al.*, 2010b)
VEGF and BMP-2 (plasmid vector)	BALB/c nu/ nu Nude Mouse, ectopic intramuscular implant		4 and 8 weeks	Transfected cells on β-TCP scaffold, implant	2.5 x 10^6 cells/mL	Co-expression showed significant bone formation compared to BMP-2 alone at 4 weeks. Bone area increased 10 fold by 8 weeks. VEGF may enhance BMP-2 induced bone formation through angiogenesis modulation. Histology, histomorphometry, immunohistochemistry and *in situ* hybridization	(Samee *et al.*, 2008)
Rabbit							
VEGF and BMP-2	Male New Zealand Rabbit, intramedullary femur defect.		2 to 12 weeks	Growth factor loaded-PLGA microspheres within porous PLGA scaffolds, implant	0.35 or 1.75 µg VEGF 3.5 or 17.5 µg BMP-2	Co-expression exhibited a temporal dose-dependent positive synergistic effect on bone formation, with new bone after 2 weeks and neovascularisation after 4 weeks. Histology, histomorphometry and immunohistochemistry.	(Hernandez *et al.*, 2012)
VEGF, PDGF and TGF-β1	Male New Zealand Rabbit, intramedullary femur defect - 6 mm (1.5 to 2 cm depth)		4 weeks	Brushite cement, implant	0.35 µg VEGF (PLGA encapsulated microspheres) 0.25 µg PDGF 0.1 µg TGF-β1 (liquid phase)	Triple combination increased new bone formation approx. 10 fold compared to controls where individual factor treatment increased new bone formation maximum 5 fold. Histology and histomorphometry.	(Reyes *et al.*, 2012)
$rhVEGF_{165}$ and BMP-2	New Zealand Rabbit, sinus floor elevation		4 and 12 weeks	Sonication-induced silk hydrogel, implant	20 µg $VEGF_{165}$ 30 µg BMP-2	VEGF promoted higher tissue infiltration and accelerated gel degradation. Co-expression induced significantly larger bone area than single factor groups and the silk control (1.4 to 4.9 fold more bone). Histology, histomorphometry, micro CT and radiography	(Zhang *et al.*, 2011a)

Supplementary Table 8. Bone tissue engineering *in vivo* utilising VEGF singularly and combination.

VEGF and BMP-2 (adenovirus)	Female New Zealand White Rabbit, bilateral orbital defect – 12mm		4 to 16 weeks	Transduced BMSCs on biocoral composite, implant	1.2 x 10^6 cells VEGF 6 x 10^6 cells BMP-2	New bone deposition and formation was observed, mimicking natural bone development. Bone volume increased approx. 20 fold after 4 weeks, 3 fold after 8 weeks and 2 fold after 16 weeks, compared to controls. Histology, histomorphometry, immunocytochemistry, micro CT and radiology.	(Xiao *et al.*, 2011)
$VEGF_{165}$ and BMP-7 (recombinant adeno-associated virus)	Male New Zealand Rabbit, ectopic intramuscular injection (hindlimb ischemia model)		2 to 8 weeks	Direct intramuscular injection	5.5 x 10^{11} particles	Co-expression stimulated angiogenesis and bone regeneration. Capillary density and mean blood flow were both increased approx. 2 fold. Biochemical testing, histology and radiography.	(Zhang *et al.*, 2010a)
VEGF and BMP-2 (baculovirus)	New Zealand White Rabbit, femoral segmental defect – 10mm		8 weeks	Transduced rabbit MSCs in PLGA scaffold, implant	3 x 10^6 cells	Increased bone formation of improved quality was observed. Torsional stiffness and maximum torque were increased 208 and 26.5 fold respectively, compared to scaffold alone. Biomechanical testing, histology, immunohistochemistry, micro CT, micro-PET and radiography.	(Lin *et al.*, 2010)
VEGF, BMP-2 and FLP (baculovirus)	Female New Zealand White Rabbit, calvarial defect – 8mm		4 and 12 weeks	Transduced rabbit MSCs in PLGA scaffold, implant	3 x 10^6 cells	FLP episome formation prolonged BMP-2/VEGF expression and augmented bone repair from rabbit MSCs. Bone regeneration area increased 3.4 fold compared to scaffold alone. Histology, micro CT, micro-PET and radiography.	(Lin *et al.*, 2012a)
VEGF, PDGF-BB and MSCs	Rabbit, segmental bone defect Mouse, ectopic implantation		unknown	PRP membrane, implant	unknown	In an ectopic mouse model as well a rabbit segmental bone defect model the platelet-rich plasma-based membrane & PDGF was able to biomimic a periosteal response in vivo enhancing bone regeneration.	(El Backly *et al.*, 2013)
Rat							
VEGF and rhBMP-2	Harlan Sprague-Dawley Rat, femoral defect – 5mm		4 and 6 weeks	PLGA in PPF (rhBMP-2) within gelatin hydrogel (VEGF), implant	2 µg VEGF 6.5 µg rhBMP-2	Microangiography showed sufficient vascularization. Implants demonstrated bone formation within orthotopic defects. Vessel volume doubled, and bone volume increased approx. 3 fold, compared to control scaffolds. Histology, histomorphometry, microangiography, micro CT and radio-labelling.	(Kempen *et al.*, 2009)
VEGF and BMP-2	Male Fischer Rat, cranial defect – 8mm		12 weeks	Loaded PPF/gelatin microparticle composite, implant	6 to 12µg VEGF 0.5 to 2µg BMP-2	Dose-dependent increase in bone formation was observed with increased BMP-2 (approx. 5.3 fold). VEGF showed no further augmentation, however increased defect bridging was observed. Histology and micro CT.	(Young *et al.*, 2009)
VEGF and BMP-2	Syngeneic Fischer-344 Rat, calvarial defect – 8 mm		4 and 12 weeks	Gelatin microparticles entrapped within PPF scaffolds, implant	12 µg VEGF 2 µg BMP-2	VEGF increased blood vessel formation and acted synergistically with BMP-2 to enhance bone formation. Bone volume was increased approx. 5 fold. Histology and micro CT.	(Patel *et al.*, 2008)

Abbreviations: BMP-2 *(bone morphogenetic protein 2)*, **CT** *(computer tomography)*, **DXA** *(dual energy X-ray absorptiometry)*, **FGF-2** *(fibroblast growth factor 2)*, **hBMSC** *(human bone marrow stromal cell)*, **PET** *(positron emission tomography)*, **PLGA** *(poly(lactic co-glycolic acid))*, **PPF** *(poly-propylene fumarate)*, **rhVEGF** *(recombinant human VEGF)*, **rt-PCR** *(reverse transcription polymerase chain reaction)* and **VEGF** *(vascular endothelial growth factor)*.

Supplementary Table 9. Bone tissue engineering *in vivo* utilising select Wnt proteins.

Growth factor (single or combination)	Animal model (species/strain, location and defect)	Human clinical trial and defect	Time	Delivery system	Dose/ Conc.	Analysis (read outs, efficacy and methodology)	Reference
1. Animal Models for direct Wnt delivery Small Animal Models							
Mouse							
Wnt 3A	Col2.3-11βHSD2 transgenic mice (Subcutaneous calvarial injection) – disrupted glucocorticoid signalling (delayed skeletal development)		3 d	Direct defect injection	100 ng	Parietal bone volume and mineralisation were significantly increased. Suture areas were significantly reduced after 3 d (approx. 1.4 fold). Biochemical testing, histology, immunohistochemistry, *in situ* hybridisation, micro CT and RT-PCR.	(Zhou *et al.*, 2009)
Wnt 3A	Axin2 LacZ/LacZ Mouse, tibial defect – 1 mm		28 d	Liposomal vesicles, injection	0.5 µg/mL	Increased osteogenesis resulting in accelerated mineralisation and osteoid deposition. Stimulated skeletal progenitor cell proliferation and osteoblastic differentiation. Histology.	(Minear *et al.*, 2010)
2. Animal Models for indirect Wnt delivery Small Animal Models							
Chick							
Wnt 6 (plasmid vector)	Chick embryo, forelimb bud injection		4 d	Transfected CHO cells, injection	unknown	Inhibition of chondrogenesis was observed within central limb mesenchyme, and promotion of myogenesis within peripheral limb mesenchyme. *In situ* hybridisation.	(Geetha-Loganathan *et al.*, 2010)
Mouse							
Wnt 1 (lentivirus)	Female Immuno-deficient Mouse, subcutaneous injection		12 weeks	Transduced cells on HA/TCP, implant	1 x 10^6 cells	Enhanced bone formation up to 1.3 fold at injection site (dependant on cell dose). Histology and immunohistochemistry.	(Liu *et al.*, 2009)
Wnt 3A (plasmid vector)	SCID Mouse, 1. direct injection into implanted human bone 2. subcutaneous injection 3. subcutaneous injection with implanted human multiple myeloma cells		1. 11 weeks 2. 9 weeks 3. 4 weeks	Transfected H929 cells, injection (1 and 2) Recombinant Wnt 3A, injection (3)	1. 5 x 10^5 cells 2. 5 x 10^6 cells 3. 16 µg	No effect was observed outside of implanted human bone. Within implanted human bone, tumour growth was attenuated and mineral density elevated through activation of osteogenesis leading to the increased presence of osteocalcin-expressing cells. 1. Bone preservation was observed with only 18 % loss compared to 56 % loss in untreated hosts. 2. BMD increased by 1.12 fold. 3. Inhibited multiple myeloma cell progression. Histology, immunohistochemistry and radiography.	(Qiang *et al.*, 2008)

Supplementary Table 9. Bone tissue engineering *in vivo* utilising select Wnt proteins.

Wnt 4 (retrovirus)	Beige nu/nu Mouse, subcutaneous implant 1. Nude Rat, alveolar defect – 2 x 3 mm 2. SCID Mouse – calvarial defect – 5 mm		1. 8 weeks (Mouse) 2. 5 weeks (Nude Rat and SCID Mouse)	Transduced MSCs in HA/TCP or PLGA scaffolds, implant	4 x 10^6 cells (Mouse) 2.5 x 10^5 cells (Nude Rat) 5 x 10^5 cells (SCID Mouse)	Enhanced and integrated new mineralised bone tissue was observed in greater abundance compared to controls. Alveolar bone observed within the rat model was increased 3 to 5 fold. Histology and micro CT.	(Chang *et al.*, 2007)
Wnt 3A (plasmid vector)	Female CD-1 nu/nu Mouse, intramuscular injection		2 weeks	Transfected L-cells co-injected with porcine articular chondrocytes	5 x 10^6 cells (12 injections)	Wnt-3A induced proliferation and de-differentiation of articular chondrocytes when co-injected in vivo. After 2 weeks a 1.5 fold increase in cartilage was observed. Histology.	(Nalesso *et al.*, 2011)
Wnt 5A (plasmid vector)	FVB/N Mouse, transfected embryo		New-born mouse	Plasmid injection directly into mouse embryo	N/A (transgenic animal)	Ectopic Wnt 5A expression results in a variety of developmental defects including reduced endochondral and intramembranous bone formation. However, controlled spatiotemporal expression did exhibit increased calvarial ossification. Histology, histomorphometry and immunohistochemistry.	(van Amerongen *et al.*, 2012)
Wnt 10B (plasmid vector)	C57BL/6 Mouse, transfected embryo		4 weeks (ovariectomy study) 23 months (ageing study)	Plasmid injection directly into mouse embryo	N/A (transgenic animal)	Transfected mice exhibited increased bone volume (approx. 3 fold). Wnt 10B maintained femoral architecture including bone volume, mineral density and trabeculae number, following either ovariectomy (bone loss model) or ageing. Bone volume increased 12 fold by 23 months. Biomechanical testing, micro CT and radiography.	(Bennett *et al*., 2005)
Wnt 10B (plasmid vector)	C57BL/6 Mouse, transfected embryo		3 months	Plasmid injection directly into mouse embryo	N/A (transgenic animal)	Increased osteoblastogenesis was observed leading to increased bone formation through elevated mineral density and higher trabeculae number. Overall, 1.8 fold increase in bone formation was observed. Histomorphometry, micro CT and radiography.	(Bennett *et al*., 2007)
Abbreviations: CT *(computer tomography)*, **HA** *(hydroxyapatite)*, **MSC** *(mesenchymal stem cell)*, **PLGA** *(poly(lactic co-glycolic acid))*, **RT-PCR** *(real time polymerase chain reaction)*, **SCID** *(severe combined immuno-deficient)*, **TCP** *(tri-calcium phosphate)* and **Wnt** *(wingless-type MMTV integration site family)*.							