

## TOXICOLOGY WRITTEN SUMMARY

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**ABBREVIATIONS**

ANOVA	One-way analysis of variance
β-TCP	Beta-tricalcium phosphate
CNA A	Complete new attachment apparatus
DFDBA	Demineralised freeze-dried bone allograft
DMSO	Dimethyl sulphoxide
HDPE	High density polyethylene
INN	International non-proprietary name
MEM	Minimum essential medium
PDGF	Platelet-derived growth factor
PETG	Glycol-modified polyethylene terephthalate
PII	Primary irritation index
PMA	Premarket Approval Application
rhPDGF-BB	Recombinant human platelet-derived growth factor BB homodimer
SBA	Summary basis of approval (FDA)
U.S. FDA	United States Food And Drug Administration

## 2.6 NONCLINICAL SUMMARY

### 2.6.6 Toxicology

#### 2.6.6.1 Brief Summary

The extensive history of use of  $\beta$ -TCP as a bone void filler and bone cement has demonstrated that this material is biocompatible and nontoxic. Implantation of  $\beta$ -TCP in animal bone repair models is not associated with any detectable increases in serum calcium, or serum phosphate, nor any calcification of the kidneys arising from resorption of the implant (for example, Cameron *et al.*, 1977; reviewed in Rawlings, 1993). The conclusion that  $\beta$ -TCP lacks significant toxicity is supported by over 25 years of safe use in the clinic for dental, maxillofacial and orthopaedic indications. Study reports are provided in Appendix 2.6.6.1-1

rhPDGF-BB based products have been on the market for over 10 years, initially in Regranex<sup>®</sup>, a product for the treatment of diabetic foot ulcers (marketed in the US and in the EU) and GEM 21S, a product for the treatment of periodontal defects (marketed in the US and Canada). Augment has been used to treat approximately 471 patients in both pilot and pivotal clinical studies with no report of any serious adverse event. Over 80,000 units of GEM 21S have been distributed over the past three years with no reported serious adverse events resulting from the use of the product, including no known cases of cancer. Regranex has been used in over 750,000 cases to date.

BioMimetic has worked in collaboration with the FDA to develop a program of preclinical safety and toxicology studies that are intended to provide additional data to support the safety of Augment. These studies and others already conducted by the company to evaluate the safety of rhPDGF-BB alone or in combination with  $\beta$ -TCP matrix are described in this section. The results of these studies demonstrate that Augment is biocompatible and nontoxic. The results of pharmacokinetics analyses demonstrate that rhPDGF-BB administration leads to brief and minimal systemic exposure.

Single and repeated dose toxicity of rhPDGF-BB has been characterized in intravenous and intramuscular studies in rats. rhPDGF-BB has also been evaluated in one reproductive toxicity study in rats and in one *in vitro* genotoxicity study.

## 2.6.6.2 Single-Dose Toxicity

### 2.6.6.2.1 Acute Toxicity of rhPDGF-BB Following Intravenous Administration in Rats

The purpose of the study was to evaluate the acute toxicity of rhPDGF-BB when administered intravenously in a rat model. The study was conducted in compliance with GLP guidelines.

#### **Study Procedure**

The study design included 60 Sprague-Dawley rats that were randomised into 3 dose groups based upon pre-dose body weights. Intravenous administration of rhPDGF-BB was chosen because it mimics the route of absorption of the protein in humans when Augment is administered to bleeding bone lesions, and represented a worst-case scenario for systemic exposure to rhPDGF-BB. Each dose group included 5 males and 5 females, and the endpoints for the study were at 2 and 15 days after administration, as shown in Table 2.6.6-1. Dose groups included a control (vehicle only) that received 20 mM sodium acetate, pH 6.0, and two treatment groups injected with rhPDGF-BB in 20 mM sodium acetate, pH 6.0 at concentrations of either 0.15 mg/mL or 3.0 mg/mL. Toxicological endpoints included clinical observations, body weight measurements, urinalysis, haematology, serum chemistry, blood coagulation parameters and gross and microscopic histopathology at termination.

Group Number	No. of Animals / Timepoint <sup>a</sup>		Sample	rhPDGF-BB Concentration	Dose (mg/kg)	Dosage Volume (mL/kg) <sup>b</sup>
	Males	Females				
1	5	5	Buffer	0.00 mg/mL	0.0	1.33
2	5	5	rhPDGF-BB	0.15 mg/mL <sup>c</sup>	0.2	1.33
3	5	5	rhPDGF-BB	3.00 mg/mL	4.0	1.33

<sup>a</sup> Necropsy time points were Days 2 and 15

<sup>b</sup> Route of administration = IV

<sup>c</sup> 0.3 mg/mL rhPDGF-BB diluted 1:1 with 20 mM sodium acetate, pH 6.0 at WuXi Apptec to yield 0.15 mg/mL rhPDGF-BB for administration

Dose levels were selected based on the maximum allowable human clinical dose per kg body weight. The maximum clinical exposure expected for human subjects treated with Augment is approximately 2700 µg (approximately 39 µg rhPDGF-BB /kg, assuming an average patient

weight of 70 kg). The maximum dose used in the acute toxicity study was approximately 1000 µg rhPDGF-BB (0.333 mL of a 3 mg/mL solution of rhPDGF-BB) which is 4000 µg/kg, based on rats weighing 250 g. Therefore, the total maximum dose administered in this study was approximately 100 times the maximum expected human dose, while the low dose (approximately 200 µg/kg) was approximately 5 times the maximum expected human dose.

### **Results**

There were no unscheduled deaths during the study. Based upon clinical observations, all animals terminated on either Day 2 or Day 15 were normal until the time of euthanasia. There were no remarkable changes in body weight related to rhPDGF-BB administration. Analysis of haematological and coagulation parameters, revealed no signs of acute toxicity related to rhPDGF-BB administration at either of the dose levels. Sporadic elevations in fibrinogen levels were within the historical range of normal values and observed across all the study groups (including the control group) and sexes, and were, therefore, not attributed to the test article. Minor sporadic changes in serum chemistry parameters, such as chloride (Cl<sup>-</sup>), blood urea nitrogen (BUN), albumin (ALB), alkaline phosphatase (ALP), and alanine transaminase (ALT) were observed, but were not attributed to the test article because they also occurred in both control and rhPDGF-BB-treated groups. Urinalysis parameters revealed no signs of toxicity attributable to rhPDGF-BB. Neither gross necropsy observations nor the results of a blinded, microscopic, histopathological assessment of the tissues obtained from the animals following necropsy revealed any signs of acute toxicity associated with rhPDGF-BB.

### **Conclusions**

Under the conditions of this study, it was concluded that intravenous administration of rhPDGF-BB at either low (0.15 mg/mL) or high (3.0 mg/mL) dose did not elicit any significant toxicological effects in rats. These results suggest a high margin of safety for rhPDGF-BB in a worst-case scenario where it was administered intravenously directly into the circulation with broad systemic exposure. The high dose for this study, 0.333 mL of 3.0 mg/mL rhPDGF-BB administered to a 250 g rat (4000 µg/kg body weight), was approximately 100 times the maximum clinical dose (39 µg/kg body weight based on a 70 kg patient) permitted for use in human patients receiving Augment.

#### **2.6.6.2.2 Evaluation of the chronic toxicity and carcinogenicity of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) mixed with $\beta$ -tricalcium phosphate ( $\beta$ -TCP) matrix (Augment™ Bone Graft) implanted in a rat model (Study Number 1524-003)**

In 2008, BMTI was in communication with the FDA regarding a request from the Agency to assess the long-term toxicological/carcinogenicity potential of rhPDGF-BB. The FDA and BMTI agreed on a study design in a rat implantation model to be conducted over 12 months under GLP. BMTI has conducted the GLP study to evaluate the long-term chronic toxicity and carcinogenic potential of rhPDGF-BB combined with Augment  $\beta$ -TCP matrix following surgical implantation adjacent to the femur, in a rat model.

#### **Study Procedure**

Three hundred (150 male/150 female) CrI:CD(Sprague-Dawley) rats were randomly distributed into 3 groups (Table 2.6.6-2). Mean body weight for male and female rats was approximately 190.0 g and 161.5 g, respectively. A volume of 0.1 mL of either 0.3 mg/mL rhPDGF-BB in 20 mM sodium acetate, pH 6.0 (test article) or 20 mM sodium acetate, pH 6.0 (control article), was combined with 0.1 cc of  $\beta$ -TCP. Two hundred (200) microliter aliquots of control or test article were implanted adjacent to the femur and underneath the overlying muscle. This is a total dose of 30  $\mu$ g rhPDGF-BB and 0  $\mu$ g rhPDGF-BB, respectively. The test article group received an average dose of, approximately, 158  $\mu$ g rhPDGF-BB /kg for the male rats and 186  $\mu$ g rhPDGF-BB /kg for the female rats. These dose levels are, approximately, 4 times the maximum clinical dose (39  $\mu$ g/kg body weight based on a 70 kg patient) permitted for use in human patients receiving Augment.

<b>Table 2.6.6-2: Group Assignments</b>			
<b>Group Number</b>	<b>Treatment</b>	<b>Number of Animals<sup>a,b</sup></b>	
		<b>Male</b>	<b>Female</b>
1	Control Article	50	50
2	Test Article	50	50
3	Sham Surgery	50	50

<sup>a</sup> 10 animals/sex/group were allowed to recover for 30 ( $\pm 2$ ) and 180 ( $\pm 3$ ) days, and the remaining 30 animals/sex/group were allowed to recover for 365 ( $\pm 2$ ) days.

<sup>b</sup> An additional 15 animals/sex were assigned to study for use as sentinel animals strictly for serology.

All animals were treated on Day 0 and were euthanized after 30, 180 or 365 days. Both macroscopic and microscopic evaluations were performed to evaluate toxicity and tumour incidence. Serum was collected for haematology, coagulation and clinical chemistry determinations. Bone marrow was also collected from all animals at all time points. Additionally, anti-PDGF-BB antibody formation was determined using ELISA.

### **Results:**

Clinical Observations: No treatment-related mortality or effects on the clinical condition of the rats were observed. No remarkable article-related changes in body weight or body weight gain were observed. All animals observed for clinical signs and general health prior to dosing were found to be normal and remained normal until necropsy. At Days 180 and 365, occasional individual animals in both the control article and test article groups (both sexes) had mild to moderate increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and/or sorbitol dehydrogenase. On occasion, similar alterations of lesser magnitude were observed in the sham surgery group. Statistical significance was not generally reached as these individuals were noticeable outliers within the respective groups. Changes of this magnitude and pattern are often incidental and were unlikely to be test article-related in this case. No significant changes in urinalysis parameters across treatment groups and gender were observed. Similarly, no significant changes in bone marrow parameters across treatment groups and gender were observed. In general, minor changes in clinical chemistry were observed, but due to the sporadic

changes across treatment groups, gender and time point, the changes were not attributed to treatment with rhPDGF-BB.

Pathology: There were no test article-related microscopic findings on Days 30, 180 or 365 of the study. On Day 30, minimal foreign body granulomas were present at the implant site within the skeletal muscle across groups (control article, test article, and sham surgery) in the majority of animals examined. These granulomas contained material consistent with surgical sutures and were considered a result of the operative procedure. These foreign body granulomas were not present on Day 180. Minimal to mild granulation tissue was noted at the implant site in animals from the control article and the test article groups (no animals from the sham surgery group displayed this change) on Day 30, Day 180 and Day 365. This finding was likely related to the  $\beta$ -TCP matrix and was not considered rhPDGF-BB-related.

The granulation tissue was present either along the periosteum surface of the femur or within the adjacent skeletal muscle and, on Day 30, was characterized by a cavitating space surrounded by a loose fibrovascular tissue that contained occasional mononuclear cells and rare giant cells; on Day 180, the fibrovascular tissue was denser and more organized and on Day 365 had somewhat reduced in size. Mild hyperostosis was present on the periosteum surface of the femur where the granulation tissue was present at days 180 and 365 but not at day 30. It consisted of well differentiated bone that was undistinguishable from the normal femoral bone. This hyperostosis was considered secondary to a local inflammatory/irritating effect of the  $\beta$ -TCP matrix on the periosteum surface of the femur.

Carcinogenicity: An adenocarcinoma of the mammary gland was noted in one of the 10 females of the test article (rhPDGF-BB) group on Day 180. This finding was considered incidental based on its unique occurrence and the absence of any hyperplastic changes noted in this group. There were no test article-related neoplastic microscopic observations noted in either sex on Day 365. Benign or malignant tumours present in the study were of the type commonly seen in rats of this age and strain and were considered incidental and unrelated to administration of rhPDGF-BB.

Anti-PDGF Antibody Formation: Serum test article antibody analysis of 590 samples found one sample to be positive for anti-rhPDGF-BB antibodies. The presence of the anti-rhPDGF-BB

antibodies was noted in one animal at Day 30. This animal was treated with the control article. Further testing of the positive sample was not possible due to insufficient serum volume. None of the animals treated with the test article were positive for anti-PDGF-BB antibodies.

### **Conclusions:**

The results of this study demonstrated that implantation of the test article was not associated with any unexpected mortality, clinical findings, or changes in body weight or food consumption. In addition, implantation of the test article was not associated with any treatment related changes in haematology, coagulation, clinical chemistry, or bone marrow parameters. It should be noted that statistically significant changes in red blood cell mass and coagulation parameters were noted. These changes, while present, were within historical ranges for rats of this age and strain and were not considered to be treatment related.

Upon necropsy and histopathologic evaluation, no differences were noted in tissue response between the sham or control treated animals and the test article implanted animals. Serum test article antibody analysis of 590 samples found one sample to be positive for anti-rhPDGF-BB antibodies. The presence of the anti-PDGF-BB antibodies was noted in one animal treated with the control article; none of the animals treated with the test article were positive for anti-PDGF-BB antibodies. The results of this study demonstrated that implantation of the test article did not result in any toxicity or tumourgenicity, and that the test article was biocompatible.

## **2.6.6.3 Repeat-Dose Toxicity**

### **2.6.6.3.1 Bone Response to Intramuscular Injections of rhPDGF-BB**

The purpose of this study was to evaluate the test substance, rhPDGF-BB, for its short- and long-term effects on muscle and bone tissues when injected multiple times in rats.

### **Study Procedure**

The test substance, rhPDGF-BB 1.0 mg/mL, was diluted and administered at 3 different concentrations, 10, 30, and 100 µg/mL in 20 mM sodium acetate, pH 6.0, with 20 mM sodium acetate serving as the control. The test animals were Fischer rats (40 females and 40 males) with weight ranges of 77.8 to 183.7g. The animals received intramuscular injections every other day

for two weeks (7 injection days). Two injections were made on each injection day (100  $\mu$ L for each individual injection); both in the right leg, one at the lateral side of the femur adjacent to the mid-point of the femur and a second injection over the proximal aspect of first metatarsus of each animal. Each treatment group consisted of twenty animals. Ten animals per group were euthanized at the 2 week time point, 24 hours after the final injection. The remaining ten animals were euthanized at the 8 week time point, 42 days after the final injection. The injection sites were tattooed, photographed, and radiographed prior to the first injection. At both the 2 and 8 week endpoints, the injection sites were again photographed, and the excised sites evaluated by a veterinary pathologist.

The animals in the high dose (100  $\mu$ g/mL) group received a total dose of 20  $\mu$ g/day as a result of two 100  $\mu$ L injections. Based upon a rat weighing approximately 125 grams on average, this corresponds to a dose of 160  $\mu$ g rhPDGF-BB /kg/day. This is approximately 4 times the maximum dose being evaluated in the US clinical study using Augment in foot and ankle fusions. In this study a patient can receive up to three (3) kits of Augment, with each kit containing 3 mL of rhPDGF-BB at 0.3 mg/mL in 20 mM sodium acetate, pH 6.0. The maximum dose that a patient could receive in this trial is 9 mL of 0.3 mg/mL rhPDGF-BB for a total of 2.7 mg of the protein. Assuming an average patient weight of 70 kg, this maximum dose level corresponds to a total dose of 39  $\mu$ g rhPDGF-BB /kg body weight. The combined dose for the rats in the high dose (100  $\mu$ g/mL) group over the course of the study, with seven injection days, was 140  $\mu$ g or 1120  $\mu$ g/kg. This is approximately 28 times the maximum clinical dose.

## **Results**

Detailed results of the repeat dose toxicity study are presented in Tables 2.6.6-3, 2.6.6-4, and 2.6.6-5.

Table 2.6.6-3: Repeat dose toxicity study - results						
Bioreactivity ratings (difference between test scores and control scores)						
Injection Site		Dose Level	2 weeks		8 weeks	
Metatarsus	Soft Tissue	100 µg/mL	2.2	Mild Reaction	0.0	No Reaction
		30 µg/mL	1.5	No Reaction	0.0	No Reaction
		10 µg/mL	1.5	No Reaction	0.0	No Reaction
	Bone	100 µg/mL	2.9	Mild Reaction	0.0	No Reaction
		30 µg/mL	1.4	No Reaction	0.0	No Reaction
		10 µg/mL	0.7	No Reaction	0.0	No Reaction
Femur	Soft Tissue	100 µg/mL	0.9	No Reaction	0.1	No Reaction
		30 µg/mL	0.7	No Reaction	0.0	No Reaction
		10 µg/mL	1.1	No Reaction	0.0	No Reaction
	Bone	100 µg/mL	2.6	Mild Reaction	0.0	No Reaction
		30 µg/mL	1.2	No Reaction	0.0	No Reaction
		10 µg/mL	0.9	No Reaction	0.0	No Reaction

Gross observations at the injection sites at 2 weeks were limited to swelling at the metatarsal site for animals receiving the test article which returned to normal by the 8-week time point. The femoral sites in most animals were normal except for 4 animals (2 in the 10 µg/mL group and 2 in the 30 µg/mL group) that had diffuse haemorrhaging on the muscle near the femoral injection site 2 weeks after receiving the test article. The muscle at the femur was normal in all animals examined at 8 weeks.

Representative sections from each injection site from each animal were examined by light microscopy for changes (bioreactivity) in response to the injection of the test or control substance. The muscle and bone were evaluated separately.

Table 2.6.6-4: Repeat dose toxicity study - results				
Pathology Findings for 2 and 8 weeks				
Injection Site		Dose Level	2 weeks	8 weeks
Metatarsus	Soft Tissue	100 µg/mL	3.3	0.9
		30 µg/mL	2.6	1.0
		10 µg/mL	2.6	1.0
		Control	1.1	1.1
	Bone	100 µg/mL	4.3	0.0
		30 µg/mL	2.8	0.0
		10 µg/mL	2.1	0.0
		Control	1.5	0.1
Femur	Soft Tissue	100 µg/mL	1.9	0.8
		30 µg/mL	1.8	0.7
		10 µg/mL	2.1	0.6
		Control	1.1	0.7
	Bone	100 µg/mL	3.9	0.0
		30 µg/mL	2.5	0.0
		10 µg/mL	2.2	0.0
		Control	1.3	0.1

Histologically, there were no significant differences between the soft tissue reactions for the test and control sites at the 2 and 8 week time points, except at the 2-week time point at the metatarsal injection site in the high dose (100 µg/mL) group, where there was a low-grade cellular reaction.

<b>Table 2.6.6-5: Repeat dose toxicity study - results</b>								
<b>Summary comparison of bone pathology finding at metatarsus and femur</b>								
<b>Metatarsus Site Average Bone Scores</b>	<b>10 µg/mL</b>		<b>30 µg/mL</b>		<b>100 µg/mL</b>		<b>Control</b>	
	<b>2 week</b>	<b>8 week</b>	<b>2 week</b>	<b>8 week</b>	<b>2 week</b>	<b>8 week</b>	<b>2 week</b>	<b>8 week</b>
Inflammatory cell infiltrate	0.9	0.0	0.9	0.0	1.1	0.0	0.5	0.0
Bone resorption	0.1	0.0	0.3	0.0	0.6	0.0	0.0	0.0
Osteolysis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hyperplasia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Osteogenesis	0.6	0.0	0.8	0.0	1.3	0.0	0.5	0.0
Fibroplasia	0.6	0.0	0.9	0.0	1.4	0.0	0.5	0.1
Exostosis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<b>Femur Site Average Bone Scores</b>	<b>10 µg/mL</b>		<b>30 µg/mL</b>		<b>100 µg/mL</b>		<b>Control</b>	
	<b>2 week</b>	<b>8 week</b>	<b>2 week</b>	<b>8 week</b>	<b>2 week</b>	<b>8 week</b>	<b>2 week</b>	<b>8 week</b>
Inflammatory cell infiltrate	0.8	0.0	0.7	0.0	0.9	0.0	0.5	0.0
Bone resorption	0.2	0.0	0.3	0.0	0.6	0.0	0.0	0.0
Osteolysis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hyperplasia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Osteogenesis	0.6	0.0	0.8	0.0	1.3	0.0	0.3	0.0
Fibroplasia	0.7	0.0	0.8	0.0	1.2	0.0	0.5	0.1
Exostosis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Based on microscopic observations, there were no significant differences in reaction at the bone for the test and control sites at the 2- and 8-week time points, with one exception: the high dose (100 µg/mL) group exhibited a mild bone reaction characterised by increased osteogenesis and fibroplasia at the outer (cortical) layers of the bone at the 2-week time point in both injection sites. This increase was an expected observation given the known biological activity of rhPDGF-BB, which has both chemotactic and mitogenic activity for cells that would be present at the injection site. This type of response is also the effect what would be expected and desired in fracture/fusion site treated with rhPDGF-BB and highlights the role that rhPDGF-BB plays in tissue repair. The enhanced cellular activity was observed only at the 2-week time point and not at the 8-week time point, demonstrating that cellular activities stimulated by rhPDGF-BB are

transient, and that following stoppage of the injections the tissues remodelled back to normal. No ectopic bone formation was observed at either injection site at either time point for any of the doses administered. Additionally, there were no signs of local or systemic toxicity due to locally administered rhPDGF-BB.

### **Conclusions**

Based on the results of the study, the test substance rhPDGF-BB can be considered mildly reactive at a dose of 100 µg/mL in both soft tissue and bone when injected in close proximity to bone tissues at volumes of 0.1 mL every other day for 2 weeks. Although mildly reactive within 24 hours of the final dose this reaction is transient and is not present in either the soft tissue or the bone 6 weeks after the final dose.

These results were similar to those obtained by Knight *et al.*, 1998 in that rats receiving multiple injections of rhPDGF-BB showed signs of accelerated bone remodelling, including bone resorption and cortical osteogenesis, at the injection sites one day following cessation of the injections. This study demonstrates that the mild acute effects of rhPDGF-BB, on bone remodelling and soft tissues, are completely reversed 6 weeks after the last dose.

#### **2.6.6.4 Genotoxicity**

##### **2.6.6.4.1 Bacterial Mutagenicity Test - AMES Assay**

The mutagenic potential of rhPDGF-BB (or its metabolites) was assessed in an *in vitro* test system.

### **Study Procedure**

The AMES assay evaluated the potential of rhPDGF-BB to induce histidine (His) reversion in *S. typhimurium* (His<sup>-</sup> to His<sup>+</sup>) or tryptophan reversion in *E. coli* (Trp<sup>-</sup> to Trp<sup>+</sup>). The plate incorporation assay was conducted with four strains of *Salmonella typhimurium* (TA97a, TA98, TA100 and TA1535) and one strain of *Escherichia coli* (WP2-uvrA<sup>-</sup>). The stock 10.0 mg/mL rhPDGF-BB was provided as a colourless liquid and represented the maximum characterized concentration of rhPDGF-BB tested. rhPDGF-BB was tested in triplicate at six dose levels with the top dose tested at 10 mg/mL (1.0 mg/plate). Lower doses were prepared as half-log dilutions

in 20 mM sodium acetate and were equivalent to 0.316, 0.100, 0.0317, 0.0100 and 0.0032 mg/plate.

The assay was conducted in the presence and absence of an exogenous mammalian activation system (S9). A dose range finding study consisting of 1.0, 0.316, 0.100, 0.0317, 0.0100 and 0.0032 mg/plate was conducted. No substantial toxicity was observed for any of the dose levels tested. Therefore, the top 3 doses (1.0, 0.316 and 0.100 mg/plate) were used in a definitive assay. All doses were administered in 0.1 mL volume. Positive and negative controls (saline  $\pm$  S9 activator) were included in the assay.

### **Results**

The 1.0 mg/plate dose induced a slight toxicity for strain TA100 (w/S9) as observed by a thinning of the microcolony background lawn and a slight reduction in colony counts, although the colony counts were within normal range. The 0.316 and 0.100 mg/plate doses did not induce toxicity for any strain, background lawns were normal. All negative controls were within acceptable ranges and all positive controls exhibited increased reversion rates which validated the assay.

### **Conclusion**

10 mg/mL (1.0 mg/plate) rhPDGF-BB was non-mutagenic based on the criteria of the assay.

#### **2.6.6.5 Carcinogenicity**

##### **2.6.6.5.1 Evaluation of the chronic toxicity and carcinogenicity of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) mixed with $\beta$ -tricalcium phosphate ( $\beta$ -TCP) matrix (Augment™ Bone Graft) implanted in a rat model**

In 2008, BMTI was in communication with the FDA regarding a request from the Agency to assess the long-term toxicological/carcinogenicity potential of rhPDGF-BB. The FDA and BMTI agreed on a study design in a rat implantation model to be conducted over 12 months under GLP. BMTI has conducted the GLP study to evaluate the long-term chronic toxicity and carcinogenic potential of rhPDGF-BB combined with Augment  $\beta$ -TCP matrix following surgical implantation adjacent to the femur, in a rat model.

### **Study Procedure**

Three hundred (150 male/150 female) Crl:CD (Sprague-Dawley) rats were randomly distributed into 3 groups (Table 2.6.6-2). Mean body weight for male and female rats was approximately 190.0 g and 161.5 g, respectively. A volume of 0.1 mL of either 0.3 mg/mL rhPDGF-BB in 20 mM sodium acetate, pH 6.0 (test article) or 20 mM sodium acetate, pH 6.0 (control article), was combined with 0.1 cc of  $\beta$ -TCP. Two hundred (200) microliter aliquots of control or test article were implanted adjacent to the femur and underneath the overlying muscle. This is a total dose of 30  $\mu$ g rhPDGF-BB and 0  $\mu$ g rhPDGF-BB, respectively. The test article group received an average dose of, approximately, 158  $\mu$ g rhPDGF-BB /kg for the male rats and 186  $\mu$ g rhPDGF-BB /kg for the female rats. These dose levels are approximately 4 times the maximum clinical dose (39  $\mu$ g/kg body weight based on a 70 kg patient) permitted for use in human patients receiving Augment.

<b>Table 2.6.6-6: Group Assignments</b>			
<b>Group Number</b>	<b>Treatment</b>	<b>Number of Animals<sup>a,b</sup></b>	
		<b>Male</b>	<b>Female</b>
1	Control Article	50	50
2	Test Article	50	50
3	Sham Surgery	50	50

<sup>a</sup> 10 animals/sex/group were allowed to recover for 30 ( $\pm$ 2) and 180 ( $\pm$ 3) days, and the remaining 30 animals/sex/group were allowed to recover for 365 ( $\pm$ 2) days.

<sup>b</sup> An additional 15 animals/sex were assigned to study for use as sentinel animals strictly for serology.

All animals were treated on Day 0 and were euthanized after 30, 180 or 365 days. Both macroscopic and microscopic evaluations were performed to evaluate toxicity and tumour incidence. Serum was collected for haematology, coagulation and clinical chemistry determinations. Bone marrow was also collected from all animals at all time points. Additionally, anti-PDGF-BB antibody formation was determined using ELISA.

### **Results:**

Clinical Observations: No treatment-related mortality or effects on the clinical condition of the rats were observed. No remarkable article-related changes in body weight or body weight gain

were observed. All animals observed for clinical signs and general health prior to dosing were found to be normal and remained normal until necropsy. At Days 180 and 365, occasional individual animals in both the control article and test article groups (both sexes) had mild to moderate increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and/or sorbitol dehydrogenase. On occasion, similar alterations of lesser magnitude were observed in the sham surgery group.

Statistical significance was not generally reached as these individuals were noticeable outliers within the respective groups. Changes of this magnitude and pattern are often incidental and were unlikely to be test article-related in this case. No significant changes in urinalysis parameters across treatment groups and gender were observed. Similarly, no significant changes in bone marrow parameters across treatment groups and gender were observed. In general, minor changes in clinical chemistry were observed, but due to the sporadic changes across treatment groups, gender and time point, the changes were not attributed to treatment with rhPDGF-BB.

Pathology: There were no test article-related microscopic findings on Days 30, 180 or 365 of the study. On Day 30, minimal foreign body granulomas were present at the implant site within the skeletal muscle across groups (control article, test article, and sham surgery) in the majority of animals examined. These granulomas contained material consistent with surgical sutures and were considered a result of the operative procedure. These foreign body granulomas were not present on Day 180. Minimal to mild granulation tissue was noted at the implant site in animals from the control article and the test article groups (no animals from the sham surgery group displayed this change) on Day 30, Day 180 and Day 365. This finding was likely related to the  $\beta$ -TCP matrix and was not considered rhPDGF-BB-related.

The granulation tissue was present either along the periosteum surface of the femur or within the adjacent skeletal muscle and, on Day 30, was characterized by a cavitating space surrounded by a loose fibrovascular tissue that contained occasional mononuclear cells and rare giant cells; on Day 180, the fibrovascular tissue was denser and more organized and on Day 365 had somewhat reduced in size. Mild hyperostosis was present on the periosteum surface of the femur where the granulation tissue was present at days 180 and 365 but not at day 30. It consisted of well

differentiated bone that was undistinguishable from the normal femoral bone. This hyperostosis was considered secondary to a local inflammatory/irritating effect of the  $\beta$ -TCP matrix on the periosteum surface of the femur.

Carcinogenicity: An adenocarcinoma of the mammary gland was noted in one of the 10 females of the test article (rhPDGF-BB) group on Day 180. This finding was considered incidental based on its unique occurrence and the absence of any hyperplastic changes noted in this group. There were no test article-related neoplastic microscopic observations noted in either sex on Day 365. Benign or malignant tumours present in the study were of the type commonly seen in rats of this age and strain and were considered incidental and unrelated to administration of rhPDGF-BB .

Anti-PDGF Antibody Formation: Serum test article antibody analysis of 590 samples found one sample to be positive for anti-rhPDGF-BB antibodies. The presence of the anti-rhPDGF-BB antibodies was noted in one animal at Day 30. This animal was treated with the control article. Further testing of the positive sample was not possible due to insufficient serum volume. None of the animals treated with the test article were positive for anti-PDGF-BB antibodies.

### **Conclusions:**

The results of this study demonstrated that implantation of the test article was not associated with any unexpected mortality, clinical findings, or changes in body weight or food consumption. In addition, implantation of the test article was not associated with any treatment related changes in haematology, coagulation, clinical chemistry, or bone marrow parameters. It should be noted that statistically significant changes in red blood cell mass and coagulation parameters were noted. These changes, while present, were within historical ranges for rats of this age and strain and were not considered to be treatment related.

Upon necropsy and histopathologic evaluation, no differences were noted in tissue response between the sham or control treated animals and the test article implanted animals. Serum test article antibody analysis of 590 samples found one sample to be positive for anti-rhPDGF-BB antibodies. The presence of the anti-PDGF-BB antibodies was noted in one animal treated with the control article; none of the animals treated with the test article were positive for anti-PDGF-BB antibodies. The results of this study demonstrated that implantation of the test

article did not result in any toxicity or tumourgenicity, and that the test article was biocompatible.

## **2.6.6.6 Reproductive and Developmental Toxicity**

### **2.6.6.6.1 Recombinant Human Platelet-derived Growth Factor-BB (rhPDGF-BB): an Intravenous Injection Teratology Study in the Rat**

The reproductive toxicity of rhPDGF-BB was studied by daily intravenous administration of rhPDGF-BB in gravid rats over 21 days of gestation. This study entitled Recombinant Human Platelet-derived Growth Factor-BB (rhPDGF-BB): an Intravenous Injection Teratology Study in the Rat (Charles River Lab Study No. 901703) is summarised below.

#### **Study Procedure**

Female Sprague-Dawley rats were mated for 4 days and positive identification of spermatozoa, through vaginal lavage, was termed Day 0 of gestation. Three groups consisting of 22 female rats/group (approximately 11 weeks old; 232 - 292 g) received single daily doses of vehicle control (20 mM sodium acetate, pH 6.0) or rhPDGF-BB at a dose of either 40 or 400 µg/kg/day of via intravenous injection for 20 days. The target injection volume was 1 mL/kg/day.

Clinical signs were evaluated twice daily. Physical examinations, measurements of body weight and food consumption were performed on gestation Days 0, 3, 6, 9, 12, 15, 18 and 21.

On Day 21 of gestation, all rats were euthanized and a gross pathological examination was performed for all animals. The reproductive tract of each dam was dissected out, the ovaries removed and the corpora lutea counted. The gravid uterus was weighed, the uterine contents (including the placentas) were examined and the number and position of live and dead foetuses, including early, middle and late resorptions were recorded. Each foetus was weighed, given a detailed external examination and the external sex recorded. No histopathological assessment was conducted on the dams or foetuses.

Additional satellite groups consisting of 6 female rats/group were included for the purpose of toxicokinetic analysis at pre-dose and day 21 of gestation. Vehicle control satellite group consisted of 3 female rats. Maternal blood (approximately 1 mL) samples were collected in

sodium heparin tubes on days 0 (Pre-Dose) and 24 hours post-terminal dose (day 21 of gestation). Foetal blood sampling was performed on Day 21 of gestation. rhPDGF-BB concentration was determined by ELISA. Antibody formation against rhPDGF-BB was determined by an ELISA developed to identify rat antibodies against rhPDGF-BB.

## **Results**

No treatment related mortality or significant clinical effects were observed in any treatment group. Effects on body weight, corrected body weight gains and food consumption were unremarkable in all treatment groups throughout the study.

The gross pathological assessment was unremarkable in all treatment groups. Miscellaneous gross observations seen in various organs and tissues were considered to be agonal or incidental in origin.

The uterine parameters assessed (*i.e.*, pregnancy rate, number of corpora lutea, implantation sites, live and dead fetuses, sex ratio, resorptions and pre- and post-implantation losses) were unaffected in all treatment groups. Foetal weights were unaffected by treatment.

The incidence of litters and fetuses with major malformations were unaffected by treatment in all treatment groups. One foetus (Animal No. 2518/5) at 40 µg/kg/day had multiple malformations including absent lower jaw (agnathia), proboscis, absent mouth (astomia), absence of one eye (anophthalmia) with the other eye small (microphthalmia), single naris and reduced pinnae (microtia). A second foetus (Animal No. 3516/6) in the 400 µg/kg/day group had situs inversus of the thoracic and abdominal organs. Due to the low incidence, these findings were considered to be spontaneous in origin.

The incidence of minor external and visceral anomalies were unaffected by rhPDGF-BB. One control foetus (Animal No. 1506/16) had a reduced accessory lung lobe that was not related to treatment. In the 400 µg/kg/day treatment groups, a reduction in the incidence of fetuses and litters with incomplete ossification of the interparietal and hyoid bones was observed. Additionally, an increase in the incidence of fetuses and litters with rudimentary 14<sup>th</sup> rib(s) was observed for the 400 µg/kg/day treatment group. The values were within the historic control

data ranges (for total minor skeletal anomalies - 10.7 to 42.2% of foetuses affected) and therefore, these observations were not considered treatment related.

Rat plasma samples were assayed for rhPDGF-BB using an ELISA. The plasma levels of rhPDGF-BB in all dams and all foetuses was below the level of detection (<0.625 mg/mL). Rat plasma samples were tested in an anti-drug-antibody ELISA for the presence of anti-PDGF-BB antibodies. No antibodies were detected except in a pre-treatment sample of one dam that tested positive for anti-rhPDGF-BB antibodies. Immunodetection with rhPDGF-BB showed the antibodies to be non-specific for the test article.

### **Conclusions**

The administration of rhPDGF-BB at 40 and 400 µg/kg by intravenous injection, to female rats from Gestation Days 0 to 20 resulted in neither maternal toxicity nor adverse effects on embryo-foetal development in this study. Toxicokinetic analysis of plasma at Day 21 gestation, indicate no detectable rhPDGF-BB and no detectable neutralizing antibodies against rhPDGF-BB. Based on these results, the NOAEL for maternal toxicity and the NOAEL for embryo foetal development is 400 µg/kg/day, the highest dose tested in this study.

#### **2.6.6.7 Local Tolerance**

##### **2.6.6.7.1 Biocompatibility of Augment**

The Company has conducted a panel of medical device biocompatibility/toxicology studies in compliance with ISO 10993 and USP guidelines. Studies are presented that evaluated Augment β-TCP alone or in combination with rhPDGF-BB and on β-TCP with or without rhPDGF-BB. An abbreviated panel of tests were conducted on rhPDGF-BB samples stored under non-standard storage conditions at 30°C and 5°C.

The toxicology/biocompatibility data and studies described herein were designed to evaluate the rhPDGF-BB material and its use in conjunction with β-TCP. Testing was conducted in compliance with EP 3.1.9 testing and was performed by Toxikon Corporation (Bedford, MA). The toxicology/biocompatibility analysis included the following tests:

- Systemic Injection
- Intracutaneous Injection
- Muscle Implantation
- Cytotoxicity - Agar Diffusion
- Cytotoxicity - L929 MEM Elution

Results of the toxicology/biocompatibility testing for Augment are provided in Table 2.6.6-7.

<b>Table 2.6.6-7: Summary of Augment biocompatibility testing</b>			
<b>Report No.</b>	<b>Type of Study</b>	<b>Study Description</b>	<b>Result</b>
05-6012-G3	Class VI Test - USP	Systemic Injection	None of the test or control animals exhibited overt signs of toxicity at any of the observation points. None of the animals injected with the test article extracts showed a significantly greater biological reaction compared to animals treated with control article extracts.
		Intracutaneous Injection	There were no significant signs of erythema or edema observed at any of the test or control article sites. The difference between the test and control article mean reaction scores (erythema/edema) was less than 1.0 for all extracts.
		Muscle Implantation	There were no overt signs of toxicity noted in either animal. Macroscopic evaluation of the test and control article implant sites showed no significant infection, encapsulation, haemorrhage, necrosis or discoloration. The difference between the average scores for all of the categories of biological reaction for the test and control article implant sites did not exceed 1.0.
05-6012-G2	<i>in vitro</i> Cytotoxicity	Agar Diffusion Test	No biological reactivity (Grade 0) was observed in the L929 mammalian cells at 48 hours, post exposure to the test article extracts. The observed cellular response obtained from the positive control article (Grade 3) and negative control article (Grade 0) confirmed the suitability of the test system. The test article, $\beta$ -Tricalcium Phosphate with rhPDGF-BB (1 mg/mL), was considered non-cytotoxic and meets the requirements of the Agar Diffusion Test, ISO 10993-5, 1999.
05-6012-G1	<i>in vitro</i> Cytotoxicity	L929 MEM Elution Test	No biological reactivity (Grade 0) was observed in the L929 mammalian cells at 48 hours, post exposure to the test article extracts. The observed cellular response obtained from the positive control article (Grade 4) and negative control article (Grade 0) confirmed the suitability of the test system. The test article, $\beta$ -Tricalcium Phosphate with rhPDGF-BB (1 mg/mL), was considered non-cytotoxic and meets the requirements of the Elution Test, ISO 10993-5, 1999.

Note: All testing was done at Toxikon in compliance with the U.S. FDA GLP Regulation, 21 CFR Part 58, and requirements of the U.S. Pharmacopeia.

## 2.6.6.8 Other Studies

### 2.6.6.8.1 Biocompatibility of rhPDGF-BB following Prolonged Storage at Recommended and Stressed Conditions

As part of its development efforts, BioMimetic conducted modified cytotoxicity and systemic toxicity testing on stability samples of rhPDGF-BB that had been stored for one year at either 5°C (recommended storage condition) or at 30°C (stress conditions). Prior to biocompatibility testing, the stressed materials (held at 30°C) were assessed and shown by RP-HPLC analysis to contain known degradation products that would have caused the rhPDGF-BB preparation to fail release and stability specifications. The purpose of the evaluation was to determine if there were potential differences in toxicology/biocompatibility testing between materials that had been degraded via temperature stressing causing them to fall outside of stability specifications, as compared to stable material, maintained at the label claim of 2-8°C. Results of this evaluation are provided in Table 2.6.6-8.

<b>Table 2.6.6-8: Summary of toxicology/biocompatibility testing of rhPDGF-BB stability studies</b>		
<b>rhPDGF-BB Lot # PD03089 Stability Sample Tested</b>	<b>Study (Modification)</b>	<b>Conclusion</b>
12 mo. at 30°C	USP and ISO Modified Systemic Toxicity Study - Solution (Modified to increase observations from 72 hrs to 7 days; dose solution instead of extract)	Passed. Non-toxic
12 mo. at 5°C	USP and ISO Modified Systemic Toxicity Study - Solution (Modified to increase observations from 72 hrs to 7 days; dose solution instead of extract) Genotoxicity: Bacterial Reverse Mutation Study (Saline Extract)	Passed. Non-toxic
12 mo. at 30°C	ISO Modified Intracutaneous Study Solution (Modified for a chemical solution)	Passed. Slight irritant
12 mo. at 5°C	ISO Modified Intracutaneous Study Solution (Modified for a chemical solution)	Passed. Slight irritant
12 mo. at 30°C	Cytotoxicity Study Using the Agarose Overlay Method (Liquid)	Passed. <Grade 2 (mild reactivity)
12 mo. at 5°C	Cytotoxicity Study Using the Agarose Overlay Method (Liquid)	Passed. <Grade 2 (mild reactivity)

#### **2.6.6.8.2 Cytotoxicity**

Tests for cytotoxicity were performed using an *in vitro* mouse fibroblast cell system. rhPDGF-BB material stored for at least 12 months at 5 or 30°C was used as test article (the material stored at 30°C has shown degradation at the 12 month time point). The test article was applied to a filter disc. Sodium chloride applied to a filter disc served as filter disc control, HDPE as negative control, and latex as positive control. Wells containing confluent fibroblast cell monolayers were prepared as described for the first series, with the exception that the growth medium was replaced with a MEM-agarose mixture supplemented with neutral red and allowed to solidify over the cells. Test and control articles were placed on the solidified agarose surface and incubated at 37°C for 24 hours. The cultures were then examined macro- and microscopically. The positive control had produced a zone of lysis (reactivity “moderate”) thus confirming the validity of the test system. Neither test article nor disc or negative control showed evidence of causing cell lysis (reactivity “none”).

#### **2.6.6.8.3 Immunogenicity**

Augment is being developed for single local administration at the site of foot and ankle fusion. As a result of the treatment plan, significant systemic exposure is unlikely. In addition, once PDGF is in the circulatory system it is rapidly cleared through the kidney and liver, with circulating alpha phase ( $t_{\alpha}$ ) half-life reported to be 2.31 minutes and terminal phase ( $t_{1/2}$ ) half-life to be 7.46 hours. Local tolerance and sensitisation studies with rhPDGF-BB administration have been performed and are presented in the appropriate sections in this filing (Section 2.6). . Immunogenicity testing was performed for both the long term carcinogenicity and the teratology studies. No formation of antibodies was detected. Additionally, immunogenicity testing in humans to monitor for both total anti-rhPDGF-BB antibody levels and neutralizing antibody levels is a component of the Augment foot and ankle clinical study; the human data will be presented as part of Module 4.

### 2.6.6.9 Discussion and Conclusions

The results from these studies provide additional data supporting the safety of rhPDGF-BB administered either locally or intravenously in animal models. The results of the biocompatibility testing described above demonstrate that:

- Augment has a satisfactory toxicology/biocompatibility profile;
- rhPDGF-BB when combined with  $\beta$ -TCP matrices from various sources has a consistent toxicology/biocompatibility profile; and
- rhPDGF-BB stored for twelve months at elevated temperature, which led to the formation of increased degradation products, did not raise any toxicology issues.

The sum of the data from the biocompatibility studies demonstrates that rhPDGF-BB combined with  $\beta$ -TCP from a variety of sources, including  $\beta$ -TCP, is non-toxic and biocompatible, which supports the safety profile for the Augment product. In addition, the results from a repeat-dose toxicity study to evaluate bone tissue responses to rhPDGF-BB in rats, and an acute toxicity study to evaluate systemic toxicity following intravenous administration of rhPDGF-BB in rats were presented.