Randomized Placebo-Controlled and Controlled Non-Inferiority Phase III Trials Comparing Trafermin, a Recombinant Human Fibroblast Growth Factor 2, and Enamel Matrix Derivative in Periodontal Regeneration in Intrabony Defects

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Additional Supporting Information may be found in the online version of this article.

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ABSTRACT
We investigated the efficacy, safety, and clinical significance of trafermin, a recombinant human fibroblast growth factor (rhFGF)-2, for periodontal regeneration in intrabony defects in Phase III trials. Study A, a multicenter, randomized, double-blind, placebo-controlled study, was conducted at 24 centers. Patients with periodontitis with 4-mm and 3-mm or deeper probing pocket depth and intrabony defects, respectively, were included. A total of 328 patients were randomly assigned (2:1) to receive 0.3% rhFGF-2 or placebo, and 323 patients received the assigned investigational drug during flap surgery. One of the co-primary endpoints, the percentage of bone fill at 36 weeks after drug administration, was significantly greater in the rhFGF-2 group at 37.131% (95% confidence interval [CI], 32.7502 to 41.5123; n = 208) than it was in the placebo group at 21.579% (95% CI, 16.3571 to 26.8011; n = 100; p < 0.001). The other endpoint, the clinical attachment level regained at 36 weeks, was not significantly different between groups. Study B, a multicenter, randomized, blinded (patients and evaluators of radiographs), and active-controlled study was conducted at 15 centers to clarify the clinical significance of rhFGF-2. Patients with 6-mm and 4-mm or deeper probing pocket depth and intrabony defects, respectively, were included. A total of 274 patients were randomly assigned (5:5:2) to receive rhFGF-2, enamel matrix derivative (EMD), or flap surgery alone. A total of 267 patients received the assigned treatment during flap surgery. The primary endpoint, the linear alveolar bone growth at 36 weeks, was 1.927 mm (95% CI, 1.6615 to 2.1920; n = 108) in the rhFGF-2 group and 1.359 mm (95% CI, 1.0683 to 1.6495; n = 109) in the EMD group, showing non-inferiority (a prespecified margin of 0.3 mm) and superiority of rhFGF-2 to EMD. Safety problems were not identified in either study. Therefore, trafermin is an effective and safe treatment for periodontal regeneration in intrabony defect, and its efficacy was superior in rhFGF-2 compared to EMD treatments. © 2015 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals, Inc. on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: CLINICAL TRIALS; CYTOKINES; CELL/TISSUE SIGNALING; ENDOCRINE PATHWAYS; DENTAL BIOLOGY

Introduction
Periodontitis progressively destroys periodontal tissues and can ultimately lead to the loss of the affected teeth.\(^1,\)\(^2\) Complete regeneration of periodontal tissue destroyed by periodontitis is the ideal therapeutic goal. Unfortunately, conventional surgical treatments, which mechanically remove the bacterial biofilm, rarely induce periodontal regeneration in the defective tissue. Several biologics such as recombinant human platelet-derived growth factor (rPDGF)\(^3\) and enamel matrix derivative (EMD)\(^4\) are currently available for clinical use. However, the efficacy of these agents requires further improvement. Fibroblast growth factor (FGF)-2 exhibits potent angiogenic and mitogenic activity in mesenchymal cells within the periodontal ligament, and has been reported to be effective in regenerating periodontal tissue in animal models.\(^5,\)\(^6\) Previously reported exploratory studies\(^9,\)\(^10\) found that trafermin, a recombinant human FGF-2 (rhFGF-2), significantly improved the percentage bone fill compared with the placebo, and the optimal dose of rhFGF-2 for periodontal regeneration was 0.3%. Here, we report the results of two recently conducted Phase III studies. The first (Study A) is a randomized placebo-controlled trial to confirm the efficacy and safety of rhFGF-2, and the second (Study B) is a non-inferiority trial to compare the efficacy of rhFGF-2 and EMD, and clarify the clinical significance of rhFGF-2.

Patients and Methods

Study A

Study design and participants
A multicenter, randomized, double-blind, and placebo-controlled study was conducted at 24 centers in 23 University Dental Hospitals in Japan (Supporting Information Appendix 8). Male and female adult patients (age ≥20 years with no upper limit) with one interproximal defect requiring surgical treatment were screened. The major inclusion criteria were the following: (1) a probing pocket depth of 4 mm or deeper after initial preparation and (2) a vertical intrabony defect of 3 mm or deeper determined by radiography. Patients were excluded based on the following major criteria: (1) history of a malignant tumor; (2) severe diabetes with a 6.9% or higher serum level of National Glycohemoglobin Standardisation Program, (NGSP) hemoglobin (Hb) A1c; (3) history of bisphosphonate use; (4) osteoporosis; (5) a consciousness disorder or severe disorders of the kidneys, liver, blood, or circulatory system; and (6) pregnancy or nursing. This trial was conducted in accordance with the Good Clinical Practice (GCP) Guidelines and the Declaration of Helsinki, and the protocol was reviewed and approved by the institutional review board (IRB) of each hospital. All participants provided informed written consent and the trial was registered with ClinicalTrials.gov (number NCT00734708).

Randomization and masking
Randomization was independently performed by the Registration Center (Adjust Co., Ltd., Sapporo, Japan). Patients were randomly assigned (2:1) to receive 0.3% rhFGF-2 solution or placebo (hydroxypropyl cellulose, vehicle of 0.3% rhFGF-2) with a block size of six. The Registration Center sealed and kept the allocation codes in confidence until the clinical trial was completed, and all data were locked.

Procedures
Each flap surgery was performed in accordance with the modified Widman procedure.\(^11\) The proposed surgical area was anesthetized using local anesthetic. Following intracrevicular incision, buccal and lingual full-thickness (mucoperiosteal) flaps were evaluated. Following reflection of the mucoperiosteal flap, all granulation tissue associated with the bone defect was removed. Soft and hard deposits on the root surface were removed by root planning. After that, 0.2 mL of the assigned formulation prepared with hydroxypropyl cellulose was administered to the intrabony defect. Clinical inspections were performed at baseline and 1, 2, and 4 weeks after administration. Serum anti-FGF-2 antibodies were measured at baseline as well as 2 and 4 weeks after administration. Clinical and radiographic data at the test site were obtained at baseline and 12, 24, and
36 weeks after administration. Medical findings for both the oral cavity and whole body were confirmed by interviews and visual inspection on day 1 and weeks 1, 2, 4, 12, 24, and 36 after administration. Any treatment with an impact on the clinical, radiographic, or both measurements (eg, oral surgery in the vicinity of the test site, a prosthesis, or root canal treatment at the test site) or the use of bisphosphonates was disallowed until after the week 36 study after drug administration was completed.

Outcomes

The prespecified co-primary endpoints were as follows: (1) percentage bone fill determined by radiography 36 weeks after drug administration and (2) clinical attachment level (CAL) regained 36 weeks after drug administration.

The geometrically standardized radiography used photographic indicators (Cone Indicator-II, Hanshin Technical Laboratory, Nishinomiya, Japan). Five doctors specializing in Dental Radiology at the Department of Oral Diagnosis at the Tohoku University Graduate School of Dentistry (Sendai, Japan) independently measured the percentage bone fill. The degree of change in the tooth axis heights between the cementoenamel junction (CEJ) and bottom of the bone defect was defined as linear alveolar bone growth, and the percentage bone fill was calculated by dividing the linear alveolar bone growth by the depth of the bone defect at baseline. If the CEJ was extinguished by the restorative treatment, the restoration was considered as a landmark. The detailed methods for the radiographic measurements have been described previously. The median of five measurements taken from the same image was selected for efficacy analysis.

The CAL was defined as the distance between the control point (the cement-enamel junction or margin of the restorative material) and the bottom of the gingival sulcus and was measured by investigators at each hospital. All examiners used constant force using TUCL probes (Shioda Dental Co., Ltd., Nasukarasuyama, Japan). The tip of the TUCL probe was regulated to buckle at the hinge of the handle following the application of a load greater than 30 g on the tip. Probing was performed in the deepest pocket of the test site determined by the walking stroke at baseline. An occlusal stall was used to improve repeatability. Intraexaminer and interexaminer calibrations were performed three times (before, during, and at the end of the trial), and protocol-specific training was conducted before each measurement. The safety endpoints were frequency and severity of adverse events including abnormal laboratory values, which exceeded the prespecified criteria.

Statistical analysis

Based on the previous study, it was assumed that the CAL regained 36 weeks after drug administration was 2.48 and 1.86 mm in the rhFGF-2 and placebo groups, respectively. A total of 200 and 100 patients would be needed in the rhFGF-2 and placebo groups, respectively, to establish the superiority of rhFGF-2 treatment with a CAL regained with 80% power at a 5% two-sided significance level under the assumption of about 10% dropout. Depending on the results of the F-test, the Student’s t test or Aspin-Welch test was used to analyze the primary endpoints. The significance level was set at 5% (two-sided), and two-sided p values were reported. The analyses population was determined by the prespecified rule described in the protocol. Efficacy analyses of the primary endpoints were performed in the full analyses set (FAS) population using last-observation-carried-forward (LOCF) method. FAS included all patients who were administered the investigational drug and had at least one postbaseline efficacy measurement. The robustness of the efficacy analyses was assessed in the per-protocol set (PPS) population including the patients who did not receive the disallowed medication and had efficacy measurements 36 weeks after drug administration. Safety analyses were performed in the safety population, defined as all patients who were administered the investigational drug. All analyses were performed using SAS, version 9.1.3 (SAS Institute, Inc., Cary, NC, USA).

Study B

Study design and participants

A multicenter, randomized, blinded (patients and evaluators of radiographs), and active-controlled study was conducted at 15 centers in 15 University Dental Hospitals in Japan (Supporting information Appendix 9). The major inclusion criteria were the following: (1) a probing pocket depth of 6 mm or deeper after initial preparation, (2) a vertical intrabony defect of 4 mm or deeper determined by radiography, and close adherence to requirements of studies determining the historical size of the EMD-induced effect. The major exclusion criteria were the same as those in Study A. This trial was conducted in accordance with the GCP Guidelines and the Declaration of Helsinki, and the protocol was reviewed and approved by domestic or external IRBs. All participants provided informed written consent and the trial was registered at the University Hospital Medical Information Network Clinical Trial Registry (number UMIN000008231).

Randomization and masking

The randomization was independently performed by the Registration Center (Adjust Co., Ltd., Sapporo, Japan). Patients were randomly assigned (5:5:2) to receive rhFGF-2, EMD, or flap surgery alone. The minimization method was applied, and the depth of the bone defect and type of the test tooth were used as adjustment factors. Additionally, Zelen’s method was used to balance the number of patients allocated to the three groups at each institution. The patients and evaluators of radiographs were blind to allocation codes until the clinical trial was completed, and all data were locked.

Procedures

During flap surgery, a sufficient volume of the assigned formulation, limited to 0.4 and 0.7 mL in rhFGF-2 and EMD groups, respectively, was administered to fill the intrabony defect. EMD made using the same process and equipment was purchased from Straumann Japan K.K. (Tokyo, Japan). The excess from the amount used for the test site could be administered to other affected areas. The schedule and disallowed medication were the same as those in Study A.

Outcomes

The primary endpoint was determined as linear alveolar bone growth 36 weeks after drug administration. The secondary endpoints were as follows: (1) percentage bone fill determined by radiography 36 weeks after drug administration and (2) CAL regained 36 weeks after drug administration. The method of radiographic assessment was the same as that in Study A. For the
CAL measurement, all examiners used standard PCP-UNC-15 periodontal probes (Hu-Friedy, Chicago, IL, USA). Intraexaminer and interexaminer calibrations were performed prior to the trial, and training to perform periodontal probing with a force of 30 g was performed before each measurement.

Statistical analysis
Based on the previous study,[12–15] it was assumed that the size of the EMD-induced effect on linear alveolar bone growth 36 weeks after administration was 0.81 mm. The estimated size of the rhFGF-2–induced effect from the pooled data in Study A and previous studies[9,10] was about 1.05 mm. A total of 100 patients each in the rhFGF-2 and EMD groups would be needed to determine the non-inferiority with 80% power at a margin of 0.3 mm (about 1/3 of 0.81 mm) under the assumption of about 10% dropout. A total of 40 patients would be needed in the flap surgery alone group to demonstrate the assay sensitivity of this trial. The unstratified design was used to calculate a two-sided 95% CI for the difference in linear alveolar bone growth 36 weeks after drug administration (rhFGF-2 minus EMD groups). Statistical analyses were also performed. The non-inferiority of rhFGF-2 to EMD was accepted if the lower limit of the 95% CI for the between-group difference was −0.3 mm or greater. When non-inferiority was established, the superiority of the rhFGF-2 treatment was concluded if the lower limit of the 95% CI was, more than 0 mm. The analyses population was determined by the pre-specified rule described in the protocol. The efficacy analyses of the primary endpoint was performed in the PPS population; other efficacy analyses were based on the FAS using LOCF and safety analyses were performed in the safety population. All analyses were performed using SAS (version 9.3).

Role of funding source
Both trials reported here were funded by Kaken Pharmaceutical Co., Ltd., (Tokyo, Japan). Kaken Pharmaceuticals designed the studies, and the academic authors participated in the development of the study designs and protocols. Kaken Pharmaceuticals also performed data collection and analysis. The corresponding author had full access to all the data in the studies and final responsibility for the decision to publish the manuscript.

Results

Study A
Between October 6, 2008, and April 30, 2009, 351 patients were screened, 328 of whom were randomly assigned to receive rhFGF-2 treatment or a placebo. The follow-up ended on February 9, 2010 (Fig. 1). A total of 323 patients received the
assigned administration and were included in the safety population (Fig. 1). A total of 322 patients completed the 36-week period following drug administration. Three patients in the safety population were excluded from the FAS (Fig. 1). The baseline characteristics of the two groups were essentially similar (Table 1). The percentage bone fill at 36 weeks was significantly greater in the rhFGF-2 group (37.13%; 95% CI, 32.7502% to 41.5123%) than it was in the placebo group (23.286%; 95% CI, 18.5839% to 28.9828%) was greater than it was in the EMD group (23.286%; 95% CI, 18.5839% to 27.9875%). The between-group difference at 12 weeks increased with time (Table 2). The average of the percentage bone fill at 36 weeks was consistently higher in the rhFGF-2 group than it was in the placebo group for the various patient subgroups (Supporting Information, Appendix 4). In the FAS population, the primary endpoint was achieved: the lower limit of the 95% CI for the difference in linear alveolar bone growth at 36 weeks after administration was greater than –0.3 mm, showing the non-inferiority of rhFGF-2 to EMD (Student’s t test, p < 0.001, Table 4). Furthermore, because the lower limit was greater than 0 mm in the FAS population, these results also show the superiority of rhFGF-2 to EMD (Student’s t test, p < 0.05, Table 4). The assay sensitivity of this trial was demonstrated in the PPS population. Specifically, the mean difference in linear alveolar bone growth at 36 weeks between the EMD and flap surgery alone groups was greater than the margin of 0.3 mm, and the lower limit of the 95% CI in the EMD group (1.0683 mm) was greater than the average in flap surgery alone group (0.676 mm). In the FAS population, the percentage bone fill 36 weeks following drug administration in the rhFGF-2 group (34.369%; 95% CI, 29.7549% to 38.9828%) was greater than it was in the EMD group (23.286%; 95% CI, 18.5839% to 27.9875%). The between-group difference at 12 weeks increased with time (Table 5). The average of the linear alveolar bone growth at 36 weeks was consistently higher in the rhFGF-2 group than it was in the EMD group for the various patient subgroups (Supporting Information, Appendix 4). In the FAS population, the CAL regained at 36 weeks was 2.7 (95% CI, 2.46 to 2.95), 2.3 (95% CI, 2.02 to 2.59), and 1.7 mm (95% CI, 1.27 to 2.13) in the rhFGF-2, EMD, and flap surgery alone groups, respectively (Table 5). The results of all efficacy endpoints in the FAS analyses population were consistent with those in the PPS population (data not shown). Other clinical data are shown in the Supporting Information, Appendix 2. Most adverse events were mild, and the frequency was not associated with any group (Supporting Information, Appendix 3). The production of anti-FGF-2 antibodies was not observed in any patient.

**Study B**

Between October 11, 2012, and July 31, 2013, 321 patients were screened, 274 of whom were randomly assigned to receive rhFGF-2, EMD, or flap surgery alone. The follow-up ended on May 19, 2014 (Fig. 3). A total of 267 patients received the assigned treatment and were included in the safety population (Fig. 3). A total of 263 patients completed the 36-week period following drug administration. Two patients in the safety population were excluded from the FAS (Fig. 3) and five in the FAS population were excluded from PPS (Fig. 3). The baseline characteristics of the groups were essentially similar (Table 3). In the PPS population, the primary endpoint was achieved: the lower limit of the 95% CI for the difference in linear alveolar bone growth at 36 weeks after administration was greater than –0.3 mm, showing the non-inferiority of rhFGF-2 to EMD (Student’s t test, p < 0.001, Table 4). Furthermore, because the lower limit was greater than 0 mm in the FAS population, these results also show the superiority of rhFGF-2 to EMD (Student’s t test, p < 0.05, Table 4). The assay sensitivity of this trial was demonstrated in the PPS population. Specifically, the mean difference in linear alveolar bone growth at 36 weeks between the EMD and flap surgery alone groups was greater than the margin of 0.3 mm, and the lower limit of the 95% CI in the EMD group (1.0683 mm) was greater than the average in flap surgery alone group (0.676 mm). In the FAS population, the percentage bone fill 36 weeks following drug administration in the rhFGF-2 group (34.369%; 95% CI, 29.7549% to 38.9828%) was greater than it was in the EMD group (23.286%; 95% CI, 18.5839% to 27.9875%). The between-group difference at 12 weeks increased with time (Table 5). The average of the linear alveolar bone growth at 36 weeks was consistently higher in the rhFGF-2 group than it was in the EMD group for the various patient subgroups (Supporting Information, Appendix 4). In the FAS population, the CAL regained at 36 weeks was 2.7 (95% CI, 2.46 to 2.95), 2.3 (95% CI, 2.02 to 2.59), and 1.7 mm (95% CI, 1.27 to 2.13) in the rhFGF-2, EMD, and flap surgery alone groups, respectively (Table 5). The results of all efficacy endpoints in the FAS analyses population were consistent with those in the PPS populations. Other clinical data are shown in the Supporting Information, Appendix 2. Most adverse events were mild, and the frequency was not associated with any group (Supporting Information, Appendix 3). The production of anti-FGF-2 antibodies was not observed in any patient.

**Discussion**

In previously reported exploratory studies, rhFGF-2 significantly improved the percentage bone fill compared with the placebo, and the optimal dose of rhFGF-2 for periodontal regeneration was found to be 0.3%. At the phase III trial stage, we conducted Study A to clarify the efficacy and safety of

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**Table 1. Baseline Characteristics (Study A)**

|                          | Placebo (n = 107) | rhFGF-2 (n = 213) |
|--------------------------|------------------|------------------|
| Age (years), mean ± SD   | 53.0 ± 11.77     | 54.2 ± 11.32     |
| Gender, n (%)            |                  |                  |
| Male                     | 33 (30.8)        | 92 (43.2)        |
| Female                   | 74 (69.2)        | 121 (56.8)       |
| Smoking habit, n (%)     |                  |                  |
| Yes                      | 20 (18.7)        | 48 (22.5)        |
| No                       | 87 (81.3)        | 165 (77.5)       |
| Bone defect on radiographs (mm), mean ± SD | 4.882 ± 1.9245a | 4.991 ± 1.7470a |
| Probing depth (mm), mean ± SD | 6.2 ± 1.44     | 6.1 ± 1.28b     |
| Bleeding on probing, n (%) |              |                  |
| –                        | 42 (39.3)        | 84 (39.4)        |
| +                        | 65 (60.7)        | 129 (60.6)       |
| Mobility of tooth, n (%) |                  |                  |
| 0                        | 77 (72.0)        | 135 (63.4)       |
| 1                        | 29 (27.1)        | 68 (31.9)        |
| 2                        | 1 (0.9)          | 10 (4.7)         |
| Plaque index, n (%)      |                  |                  |
| 0                        | 71 (66.4)        | 116 (54.5)       |
| 1                        | 30 (28.0)        | 83 (39.0)        |
| 2                        | 5 (4.7)          | 14 (6.6)         |
| 3                        | 1 (0.9)          | 0 (0.0)          |
| Classification of bone defect, n (%) |            |                  |
| 2-walled                 | 44 (41.1)        | 89 (41.8)        |
| 3-walled                 | 37 (34.6)        | 66 (31.0)        |
| 2/3-walled               | 21 (19.6)        | 46 (21.6)        |
| Other                    | 5 (4.7)          | 12 (5.6)         |
| Extirpation of pulp, n (%) |              |                  |
| Not done                 | 89 (83.2)        | 170 (79.8)       |
| Done                     | 18 (16.8)        | 43 (20.2)        |
| Test tooth, n (%)        |                  |                  |
| Anterior                 | 19 (17.8)        | 43 (20.2)        |
| Premolar                 | 48 (44.9)        | 99 (46.5)        |
| Molar                    | 40 (37.4)        | 71 (33.3)        |

Data are mean ± SD or n (%).

aSix patients (three each in rhFGF-2 and placebo groups) were not assessed because of inadequate radiographs.
bOne patient included here was not assessed.
rhFGF-2 in periodontal regeneration. Study A showed that the percentage bone fill at 36 weeks increased with rhFGF-2 treatment in the intrabony defect but the CAL regained was not significantly different between the groups. To clarify the clinical significance of rhFGF-2, the efficacy of FGF-2 was compared with that of a currently available biologic for periodontal regeneration in Study B. This study was a non-inferiority trial comparing rhFGF-2 and EMD, which are similar in shape and mode of application. The result of Study B revealed the non-inferiority and superiority of rhFGF-2 to EMD in bone growth.

Table 2. Efficacy outcomes (Study A)

|                  | Placebo | rhFGF-2 |
|------------------|---------|---------|
|                  | n      | Mean   | SD    | n      | Mean   | SD      | Difference (95% CI) | Test |
| Bone fill (%)    |        |        |       |        |        |        |                   |      |
| 12 weeks         | 100    | 5.746  | 17.4193 | 205    | 7.656  | 18.8780 | 1.910 (–2.5098 to 6.3299) | –    |
| 24 weeks         | 100    | 14.263 | 19.6299 | 205    | 23.756 | 25.9038 | 9.493 (3.7238 to 15.2617)  | –    |
| 36 weeks         | 100    | 21.579 | 26.3177 | 206    | 37.081 | 32.1937 | 15.502 (8.2097 to 22.7939)  | –    |
| 36 weeksa         | 100    | 21.579 | 26.3177 | 208    | 37.131 | 32.0493 | 15.552 (8.2935 to 22.8108)  | p < 0.001 |
| CAL regained (mm) |        |        |        |        |        |        |                   |      |
| 12 weeks         | 106    | 1.7    | 1.33   | 212    | 1.7    | 1.44   | 0.0 (–0.29 to 0.37) | –    |
| 24 weeks         | 106    | 1.8    | 1.51   | 213    | 1.9    | 1.50   | 0.0 (–0.30 to 0.40) | –    |
| 36 weeks         | 106    | 2.0    | 1.48   | 211    | 2.1    | 1.58   | 0.1 (–0.24 to 0.48) | –    |
| 36 weeksa         | 106    | 2.0    | 1.48   | 213    | 2.1    | 1.58   | 0.1 (–0.25 to 0.47) | p = 0.541 |

Data are mean ± SD; n, number of participants providing data.

rhFGF-2 = recombinant human fibroblast growth factor-2; CI = confidence interval; CAL = clinical attachment level; LOCF = last-observation-carried-forward.

*Missing data were imputed by LOCF based on a prespecified protocol.

Safety problems were not identified in either study. Most adverse events were mild and the frequency was not associated with any group while anti-FGF-2 antibody generation was not observed after rhFGF-2 administration. In previous studies(9,10) rhFGF-2 was undetectable in serum after administration, suggesting that rhFGF-2 administered locally to periodontal tissue defects, was seldom transported systemically to create adverse drug reactions.

A total of 600 patients, constituting the largest clinical study of periodontal regenerative therapy, were administered doses of rhFGF-2 in the course of our clinical trials. To the best of our knowledge, we are the first to directly compare the efficacy of different periodontal regenerative biologics in a multicenter, blinded, randomized, and active-controlled trial.

The selection criteria were almost the same as those for general periodontal surgery. Although Study A included patients with 4 and 3 mm or deeper pockets and bone defects, respectively, Study B included 6 and 4 mm or deeper pocket and bone defects, respectively. This is because the important details of study B adhered closely to those of previous studies determining the historical size of EMD-induced effects. The effect size of EMD in Study B, which was the difference in linear alveolar bone growth at 36 weeks after administration (EMD group minus flap surgery alone group), was about 0.7 mm. This result was consistent with the estimated historical effect size of EMD (0.81 mm). The superiority of the rhFGF-2 treatment to the EMD indicated in Study B was also supported by a previous case report. Some cases of good responses to rhFGF-2 have been reported(16,17) and Ninomiya and colleagues(18) compared the efficacies of rhFGF-2 and EMD using symmetrical lesions in the same patient and detected greater bone recovery with rhFGF-2 than with EMD.

Study A showed no significant difference between the rhFGF-2 and placebo groups in CAL regained, which is in agreement with our previous Phase II trials. The double-blinded, randomized trial on rhPDGF(3) also did not demonstrate significant efficacy for CAL regained at 24 weeks after drug administration. CAL regained is one of the clinical surrogate endpoints for the definitive histological evaluation of the attachment apparatus. However, CAL does not accurately measure the coronal level of connective tissue attachment to the root surface, and this endpoint could signify that resolution of tissue inflammation,

Fig. 2. Outcome of rhFGF-2 administration by dental radiographs (Study A). Radiographic outcome of a FGF-2–administered individual. A 0.3% FGF-2–administered 45-year-old man. The arrows indicate the remaining alveolar bone crest or the bottom of the bone defect. The depth of the intraosseous defect before administration was measured at 5.56 mm on the X-ray. The radiographs clearly show that the bone defect was filled with the newly generated alveolar bone at 36 weeks after administration. The percentage of bone fill at 36 weeks was 74.67%, with 3 mm CAL regained.
long junctional epithelial adhesions, or both occurred after conventional periodontal surgery. Histological studies in animal models revealed that rhFGF-2 increased the amount of new cementum with Sharpey’s fibers, new functionally oriented periodontal ligament fibers, and new alveolar bone more than the carrier-treated or nontreated did. The current periodontal regenerative therapies have their own demerits. These include autogenous bone grafting or cell transplantation, which causes inconvenience to the patients in obtaining materials harvested from their own bodies; EMD is extracted and semipurified from the tooth germs of fetal pigs. Furthermore, the source of EMD origin is a cause of concern to some patients and its mode of action on periodontal regeneration has not been fully elucidated. In addition, the outcome of guided tissue regeneration is known to be technique-sensitive. Although treatments using human recombinant growth factors such as rhFGF-2 and rhPDGF do not have those demerits, their mitogenic ability might raise a potential risk of tumor growth or induction of tumor metastasis. In animal models, a single injection of rhFGF-2 into the tumor inoculation site promoted tumor growth and metastasis. In contrast, repeated injections of rhFGF-2 at a remote site from the tumor were ineffective in promoting tumor growth and metastasis. In animal models, a single injection of rhFGF-2 into the tumor inoculation site promoted tumor growth and metastasis. In contrast, repeated injections of rhFGF-2 at a remote site from the tumor were ineffective in promoting tumor growth and metastasis. In the course of our clinical trials, malignancy-related adverse events were observed in four of the total of 600 rhFGF-2-treated patients. The details of the malignancy-related adverse events are shown in the Supporting Information, Appendix 7. All of them occurred outside the oral area and the causal relationships between these adverse events and rhFGF-2 were ruled out mainly because rhFGF-2 locally administered to the test site was seldom transported systemically and tumors were located far from the administrated area. These findings indicate that the local application of rhFGF-2 rarely activates malignant neoplasm but this is a potential risk of rhFGF-2 even though not observed in the study population. Therefore, rhFGF-2 should not be used in patients with oral tumors and a postmarketing surveillance study is planned to clarify the potential risk of rhFGF-2–induced tumor growth or induction of tumor metastasis. In addition, we are aware of the possibility that not all rare adverse effects were detected in our studies.

Although the present studies did not provide long-term data, the previous study found that the efficacy of rhFGF-2 for bone growth at 72 weeks retained the 36-week level and malignancy-related adverse events, an abnormal growth in alveolar bone or ankylosis did not occur after 36 weeks. Another limitation of our studies is that tooth survival after rhFGF-2 administration was not assessed. Kitamura and colleagues conducted an 8-year surveillance study of 79 patients in an exploratory phase II trial. This study suggested that 0.3% rhFGF-2 prolonged the time for reoccurrence of periodontitis compared with placebo, but tooth loss was rarely observed in all groups.

Although smoking has a negative effect on bone regeneration after periodontal treatment, rhFGF-2 displayed efficacy on bone growth in patients who smoked the present studies. Additionally, the efficacy of rhFGF-2 was not affected by differences in age, sex, type of tooth, vitality of dentin-pulp, and classification of bone defect. The postmarketing stage could clarify the more detailed factors contributing to the efficacy of rhFGF-2 treatment.

Disclosures

SM, EI, and MO received research grants from Kaken Pharmaceuticals. A university at which TF and TS are associated received institutional research grant funding from Kaken Pharmaceuticals.
MW, MA, and HK are employees and stockholders of Kaken Pharmaceuticals. All study hospitals at which MK, YF, TF, MM, KK, HS, YO, MY, TN, SS, KI, TO, YI, TA, KY, HY, MF, TN, TH, KM, MY, RS, YH, and KN are associated received institutional research grant funding from Kaken Pharmaceuticals to do this study.

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Table 3. Baseline Characteristics (Study B)

|                         | Flap surgery alone (n = 43) | EMD (n = 112) | rhFGF-2 (n = 110) |
|-------------------------|-----------------------------|---------------|-------------------|
| Age (years), mean ± SD  | 55.2 ± 12.28                | 54.8 ± 12.12  | 56.3 ± 12.31      |
| Gender, n (%)           |                             |               |                   |
| Male                    | 18 (41.9)                   | 40 (35.7)     | 40 (36.4)         |
| Female                  | 25 (58.1)                   | 72 (64.3)     | 70 (63.6)         |
| Smoking habit, n (%)    |                             |               |                   |
| Yes                     | 5 (11.6)                    | 15 (13.4)     | 17 (15.5)         |
| No                      | 38 (88.4)                   | 79 (86.6)     | 93 (84.5)         |
| Bone defect on
         radiographs (mm),
         n (%)                  | 5.380 (1.6697)*             | 5.752 (1.7662) | 5.729 (1.6050)    |
| Probing depth (mm),
         n (%)                  | 7.0 (1.08)                  | 7.0 (1.20)    | 6.8 (1.16)        |
| Bleeding on
         probing, n (%)         |                             |               |                   |
| −                       | 9 (20.9)                    | 37 (33.0)     | 40 (36.4)         |
| +                       | 34 (79.1)                   | 75 (67.0)     | 70 (63.6)         |
| Mobility of tooth,
         n (%)                  |                             |               |                   |
| 0                       | 34 (79.1)                   | 82 (73.2)     | 76 (69.1)         |
| 1                       | 7 (16.3)                    | 22 (19.6)     | 30 (27.3)         |
| 2                       | 2 (4.7)                     | 8 (7.1)       | 4 (3.6)           |
| Plaque index, n (%)     |                             |               |                   |
| 0                       | 28 (65.1)                   | 67 (59.8)     | 69 (62.7)         |
| 1                       | 13 (30.2)                   | 41 (36.6)     | 39 (35.5)         |
| 2                       | 2 (4.7)                     | 4 (3.6)       | 2 (1.8)           |
| 3                       | 0 (0.0)                     | 0 (0.0)       | 0 (0.0)           |
| Classification of
         bone defect, n (%)   |                             |               |                   |
| 2-walled                | 10 (23.3)                   | 39 (34.8)     | 29 (26.4)         |
| 3-walled                | 17 (39.5)                   | 34 (30.4)     | 40 (36.4)         |
| 2/3-walled              | 12 (27.9)                   | 22 (19.6)     | 38 (34.5)         |
| Other                   | 4 (9.3)                     | 17 (15.2)     | 3 (2.7)           |
| Vitality of dentin-
         pulp, n (%)          |                             |               |                   |
| Vital                   | 30 (69.8)                   | 87 (77.7)     | 93 (84.5)         |
| Non-vital               | 13 (30.2)                   | 25 (22.3)     | 17 (15.5)         |
| Unknown                 | 0 (0.0)                     | 0 (0.0)       | 0 (0.0)           |
| Test tooth, n (%)       |                             |               |                   |
| Anterior                | 8 (18.6)                    | 23 (20.5)     | 23 (20.9)         |
| Premolar                | 17 (39.5)                   | 42 (37.5)     | 41 (37.3)         |
| Molar                   | 18 (41.9)                   | 47 (42.0)     | 46 (41.8)         |

Data are mean ± SD or n (%).
EMD = enamel matrix derivative; rhFGF-2 = recombinant human fibroblast growth factor-2.
*One patient was not assessed because of inadequate radiographs.

MW, MA, and HK are employees and stockholders of Kaken Pharmaceuticals. All study hospitals at which MK, YF, TF, MM, KK, HS, YO, MY, TN, SS, KI, TO, YI, TA, KY, HY, MF, TN, ST, HK, TN, TH, KM, MY, RS, YH, and KN are associated received institutional research grant funding from Kaken Pharmaceuticals to do this study.

Table 4. Efficacy Outcomes (Study B)

|                       | Flap surgery alone | EMD | rhFGF-2 |
|-----------------------|--------------------|-----|---------|
| Linear bone growth (mm); PPS | 43 (0.676 1.0530)  | 109 (1.359 1.5144) | 108 (1.945 1.5111) |
| Linear bone growth (mm); FAS | 43 (0.676 1.0530)  | 112 (1.341 1.5111) | 110 (2.7 1.51)  |
| Bone fill (%); FAS    | 42 (1.330 1.390)   | 112 (2.3 1.39)    | 112 (2.3 1.39)   |

Data are mean ± SD; n, number of participants providing data.
EMD = enamel matrix derivative; rhFGF-2 = recombinant human fibroblast growth factor.-2. CI = confidence interval; PPS = per-protocol set; FAS = full analysis set; CAL = clinical attachment level.
participants in acquisition of the data, interpretation of the results. TF and TS participated in evaluation the X-ray film data and interpretation of the results. EI and MO participated in confirming safety of all patients through the period, interpretation of the data. MK, MA, HK, MW, and SM participated in the making the trial design, data analysis, interpretation of the results and writing the manuscript. All authors gave final approval of the version to be submitted and any revised version. The results of our study should be confirmed by other research groups to rule out potential bias.

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