Regenerative
Medicine

Recombinant human FGF-2 for the treatment of early-stage osteonecrosis of the femoral head: TRION, a single-arm, multicenter, Phase II trial

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Aim: This study aimed to evaluate the 2-year outcomes from a clinical trial of recombinant human FGF-2 (rhFGF-2) for osteonecrosis of the femoral head (ONFH). **Patients & methods:** Sixty-four patients with non-traumatic, precollapse and large ONFHs were percutaneously administered with 800 µg rhFGF-2 contained in gelatin hydrogel. Setting the end point of radiological collapse, we analyzed the joint preservation period of the historical control. Changes in two validated clinical scores, bone regeneration and safety were evaluated. **Results:** Radiological joint preservation time was significantly higher in the rhFGF-2 group than in the control group. The ONFHs tended to improve to smaller ONFHs. The postoperative clinical scores significantly improved. Thirteen serious adverse events showed recovery. **Conclusion:** rhFGF-2 treatment increases joint preservation time with clinical efficacy, radiological bone regeneration and safety.

Lay abstract: Osteonecrosis of the femoral head is a disease that causes pain in the hip joint, making it impossible to walk. The causes of the disease are the use of corticosteroids and the drinking of alcohol. As the disease progresses, the hip joint needs to be replaced with an artificial joint. Risks with hip replacement surgery can include infection, implant dislocation, implant fracture and implant wear. The goal of this trial was to treat the disease with simple surgery using a drug called FGF. The surgical wound was 1 cm and the surgery took only 5 min. The results in 64 patients were better than in those without treatment. FGF treatment can be a therapeutic option for osteonecrosis of the femoral head.

Future
Medicine

Osteonecrosis of the femoral head (ONFH) is an uncommon, orphan joint disease [1,2] that often causes pain, femoral head collapse and gait disturbance, resulting from the cell death of bone tissue [2–4]. The pathogenesis of ONFH is characterized by empty lacunae in osteocytes and is considered a temporary critical loss of vascular supply due to increased intraosseous pressure in the femoral head. However, the etiology of ONFH has not been fully elucidated [2,3,5]. Several risk factors have been identified, including corticosteroid use, high alcohol consumption, blood coagulation disorders (e.g., sickle cell disease), smoking and injuries (femoral neck fracture and hip dislocation, called traumatic ONFH). Systemic corticosteroid therapy and excessive alcohol intake are well-known representative risk factors for nontraumatic ONFH [2–4], with a reported incidence of 1.4–3.0/100,000 inhabitants in the UK [2] and 1.2/100,000 inhabitants in Japan [1]. An estimated 8.12 million patients with ONFH exist in China due to systemic corticosteroid use for SARS in 2002–2003 [5]. To date, the COVID-19 outbreak which began in 2019 has necessitated systemic corticosteroid therapy for 18% of COVID-19 patients in China [6]. As the incidence of corticosteroid-associated ONFH after SARS was reported to be 24%, the incidence of this condition might increase rapidly after the COVID-19 pandemic [7,8].

The most important pathogenic complication of ONFH is femoral head collapse. The lesion size and location of ONFH are considered the major causes of collapse. In clinical practice, 70–80% of patients with ONFH experience femoral head collapse and most require total hip arthroplasty (THA) [2–4,9]. Although THA is a currently successful orthopedic procedure, the main issue with ONFH is that it affects patients as young as 20 years of age; ONFH is the main reason for THA in this young population. Younger patients are hesitant to undergo artificial joint surgery due to its restrictions on sports activity, the lifelong risks of dislocation and infection, high medical costs and potential for further surgery [2–4]. Therefore, the ultimate goal of ONFH treatment is to preserve the hip joint, prevent collapse and avoid THA – a major clinical challenge for orthopedic surgeons.

Historically, joint-preserving surgeries included core decompression (CD) and bone grafts in the West and osteotomy in Japan [9–11]. Recently, surgical trends have shifted to early diagnosis and intervention using the least damaging CD technique [2–4]. MRI is useful for early diagnosis [3]. Thanks to the widespread use of MRI and the cautious use of corticosteroids, the early diagnosis of ONFH is increasing. Several studies have examined the efficacy of combining CD with cell or growth factors [12–16]. The biological augmentation of CD has shown promising results; however, the clinical results varied and no consensus exists on the strategy for joint-preserving surgery in the current ONFH guidelines published in several countries [2,4,5,11]. We previously reported a pilot trial for evaluating the safety of recombinant human FGF-2 (rhFGF-2) for ten patients with precollapse ONFH [17,18]. rhFGF-2 is a pleiotropic regulator of cell proliferation, migration and differentiation which exerts anabolic effects on osteogenesis [19], and its clinical application may be useful for bone regeneration. However, these trials had several limitations regarding efficacy, including patients with good prognosis who do not require surgery, a small sample size due to the rarity of the condition, and inadequate organization of the control data. Further high-quality studies with validated organized control data are needed for the regenerative procedure to be applied to the general clinical setting [2–4,13,17,18].

We conducted a Phase II clinical trial for ONFH with a 24-month follow-up to assess the efficacy and safety of rhFGF-2 treatment. The primary objective was to determine whether the actual collapse rate at 24 months was below the preset expected collapse rate of <35% (joint preservation rate ≥65%) and to compare the rates between the rhFGF-2 treatment group and a nonsurgically treated historical control group. The secondary objectives were to evaluate treatment safety and the effects of rhFGF-2 on two clinical scores and radiological bone regeneration. This was the first clinical trial to evaluate the efficacy of rhFGF-2 for ONFH as the primary outcome.

Patients & methods

Study design

This study on rhFGF-2 to prevent femoral head collapse (TRION) was an investigator-initiated, multicenter, prospective, open-label, single-arm, historical control, Phase II trial conducted at four university hospitals in Japan (The University of Tokyo, Gifu University, Osaka University and Kyoto University). The study was funded by the Japan Agency for Medical Research and Development, registered in the University Hospital Medical Information Network clinical trials registry (number UMIN000020340) and conducted in accordance with the latest regulatory requirements, including the Declaration of Helsinki and the International Council for Harmonisation's Good Clinical Practice regulations. The trial registered 65 patients, 64 of whom underwent rhFGF-2 treatment between
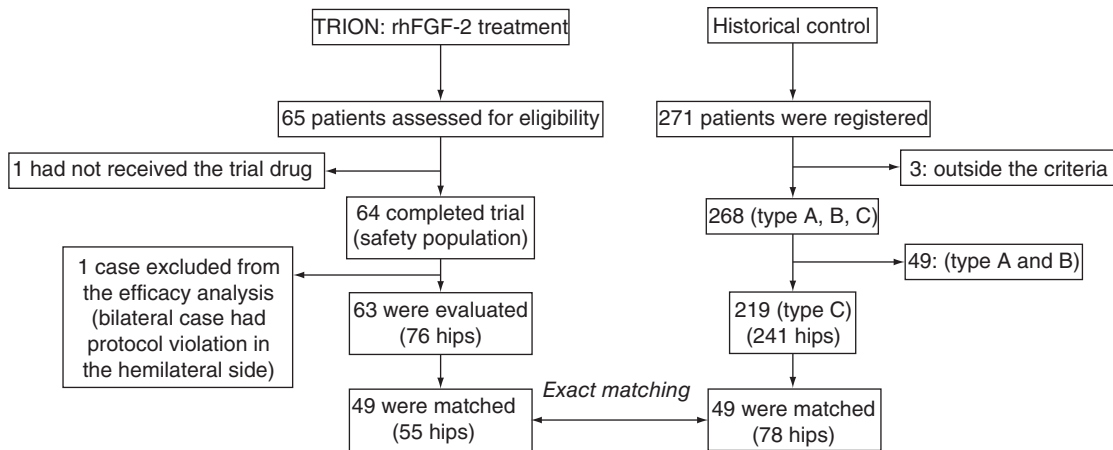
Figure 1.   One-to-one exact matching between the clinical trial participants and historical control registered data. Of the 65 participants enrolled in TRION, one did not receive the investigational drug, 64 were recruited into the safety analysis population and 63 were recruited into the full analysis set (one did not meet the eligibility criteria of a central post-enrollment determination group). Ultimately, 49 patients were matched.

17 February and 7 December 2016, at the four university hospitals. Of the 64 patients, one was excluded from the efficacy analysis due to having stage 3 disease. The protocol was published in December 2015, and data analysis was completed in November 2019. The study protocols were designed by the Institute for Advancement of Clinical and Translational Research of Kyoto University (iACT) and Gifu University and were approved by the institutional review board or ethics committee of each hospital (approval number K023). All patients in TRION provided written informed consent. An independent data and safety monitoring group performed multiple safety reviews. All radiological evaluations were ultimately approved by three specialists in the central image review committee. All data analyses were independently performed at the iACT data center. TRION evaluated efficacy and safety on an individual basis, not a joint basis, during the consultation by the Japanese regulatory authority (Pharmaceuticals and Medical Devices Agency [PMDA]), and suitable protocols were developed.

The historical control study was a multicenter, retrospective, observational study conducted at ten university hospitals (Hokkaido, Yamagata, Tokyo, Yokohama, Kanazawa, Gifu, Kyoto, Osaka, Okayama and Kagoshima) across Japan in accordance with the ethical guidelines for medical and health research involving human participants in Japan. The historical control consisted of 268 patients with nonsurgically treated ONFH who were analyzed radiologically during the joint preservation period, with the end point of radiological collapse of the femoral head. The protocol was first approved at Gifu University in December 2016, and data analysis was completed in July 2018. The methodology for the present study using a historical control was discussed and agreed upon with PMDA.

We compared the joint preservation duration with that of an exactly matched historical control (1:1) by using radiological collapse as the end point (Figure 1).

Participants

On 27 January 2016, TRION enrollment started at the four university hospitals, selecting patients aged 15–75 years with nontraumatic precollapse ONFH (stage 1 or 2) type C (type C1 or C2) diagnosed by MRI in accordance with the 2001 Japanese Investigation Committee (JIC) guidelines [20]. The JIC classification comprises JIC type (four types: from smaller size to larger size, A, B, C1, C2) and JIC stage (five stages: from early to secondary osteoarthritis via the femoral head collapse, 1, 2, 3A, 3B, 4). Stage 1 is defined as normal findings on radiographs but presence of ONFH on MRI. Stage 2 is defined as the presence of demarcating sclerosis without femoral head collapse. Stage 3 is subdivided into two stages: stage 3A (progression of femoral head collapse <3 mm) and stage 3B (progression of femoral head collapse ≥3 mm). Stage 4 is defined as secondary osteoarthritic changes following femoral head collapse. Type A is defined as the smallest osteonecrotic lesions of the four types (present only in the medial one-third or less). Type B involves osteonecrotic lesions present in the medial two-thirds or less. Type C lesions occupy more than the medial two-thirds of the weight-bearing surface. Type C2 lesions involve the largest osteonecrotic zone and exceed the acetabular edge, whereas type C1 lesions do not. The patients underwent bidirectional x-rays of the bilateral hip joint, CT scans and MRI at registration. The key exclusion criteria were
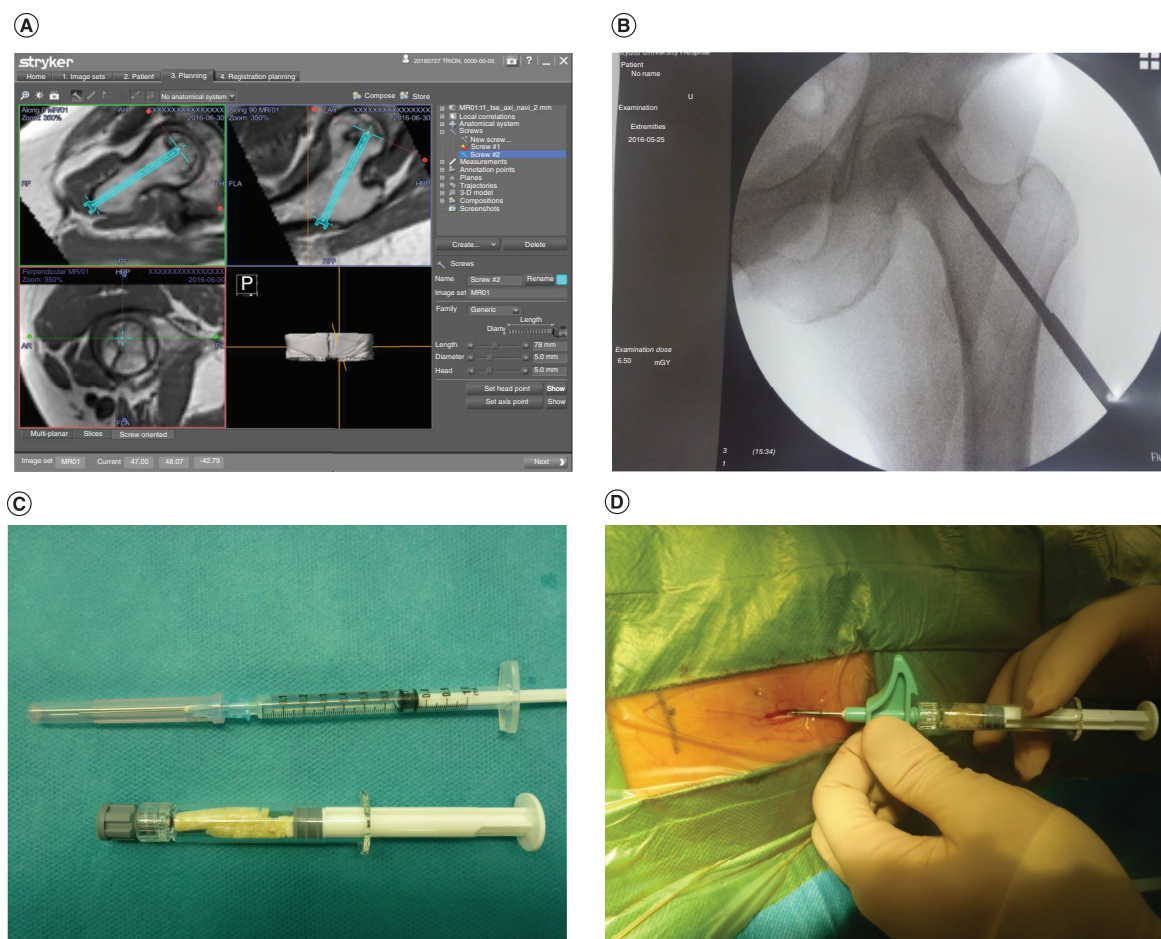
Figure 2.   Representative preoperative planning and intraoperative photographs. (A) A screenshot of the preoperative planning using navigation software. The surgeon planned the precise route of core decompression. **(B)** An intraoperative fluoroscopic image reaching the target site. **(C)** Preparation of rhFGF-2 solution (upper) and the freeze-dried gelatin (lower). A gel form was made by mixing the solution and the gelatin. **(D)** Injection of the gelatin hydrogel containing rhFGF-2. A syringe with long needle is available for percutaneous administration of gel forms.

postcollapse ONFH (stages 3 and 4), traumatic ONFH and lesions of type A or B, which have a low risk of collapse and a better prognosis. Other exclusion criteria included previous surgical treatment, bone mass index >30, malignancy, gelatin allergy and taking ≥15 mg of prednisolone. If the ONFH met the eligibility criteria bilaterally, rhFGF-2 was simultaneously administered to each femoral head.

Procedures
TRION

rhFGF-2 was provided by Kaken Pharmaceutical Co. (Tokyo, Japan). For a controlled release, a biodegradable gelatin hydrogel was constructed using glutaraldehyde cross-linking of acidic gelatin purified from natural bovine bone, as reported previously [17]. Freeze-dried cross-linked gelatin and 800 μg of rhFGF-2 were prepared by mixing in an injectable syringe with an aqueous solution 30 min prior to administration. Preoperative planning for the precise drilling was made using navigation software (OrthoMap software; Stryker, NJ, USA) [17]. With the patient under anesthesia in the supine position on a traction bed, we performed CD (percutaneous drilling). Under x-ray fluoroscopy guidance, a 1-cm skin incision was created, a 2.4-mm Kirschner wire was carefully inserted into the target ONFH lesion (referring to the preoperative planning) and a 4.8-mm diameter cannulated drill was inserted via the guide. Using a long-needle syringe, we injected the rhFGF-2 gelatin hydrogel into the femoral head (Figure 2). Weight bearing was prohibited on the operation day, but walking with full weight bearing was allowed the following day under the guidance of physical therapists. Before treatment, all participants underwent assessment of walking

capability, including passive hip motion, by the physical therapists. The Harris Hip Score (HHS), consisting of ten subcategories (ranging from 0 to 100.025 points, with higher scores indicating better outcomes) [21], and the University of California, Los Angeles (UCLA) activity rating scale (scores from 1 [no physical activity] to 10 [regular participation in impact sports]) [22] were evaluated at baseline and at 3, 6, 12, 18 and 24 months post-treatment. The HHS is based on pain (44 points), limp (11 points), support (11 points), distance walked (11 points), sitting (5 points), stairs (4 points), putting on shoes and socks (4 points), absence of deformity (4 points) and range of motion (5.025 points). Changes in JIC stage (1, 2, 3A, 3B and 4) and JIC type (four types, from smaller size to larger size: A, B, C1 and C2) were evaluated by x-rays and MRI. The period from diagnosis to radiological collapse in bilateral cases is defined as the period to the first collapsed side.

Historical control study

We conducted a study using data collected from the electronic medical records of ten university hospitals. The condition of the patients with ONFH was comparable to that of the patients in TRION who were diagnosed by the JIC classification, presenting with stage 1 or 2 nontraumatic ONFH. The patients were followed for more than 24 months and were diagnosed after 2002 using $\geq$1.5 Tesla MRI. We analyzed the collapse rate to determine the duration from diagnosis to radiological collapse; this period was defined similarly to the definition in TRION. For bilateral ONFH cases, the period from diagnosis to radiological collapse is defined as the first collapsed side. We analyzed the historical control data prior to the data analysis of TRION in July 2018.

Outcomes

The primary outcome was a preset expected collapse rate <35% (joint preservation rate $\geq$65%) at 24 months after administering rhFGF-2. The expected collapse rate for the primary outcome was established based on a prior pilot study with 10 patients [17] and a partial data analysis showing the natural course of ONFH [23]. Considering the 1-year collapse rate of 10% in the previous pilot study, the expected collapse rate at 24 months in this study was set at 15%. Moreover, the type C collapse rate was >38% at 2 years, with the end point of THA. The joint preservation time with the end point of THA included the period from collapse to THA and was considered to be much longer. Taking this difference into account, the target threshold collapse rate was set at 35% (joint preservation rate $\geq$65%) at 2 years to determine the significance of the treatment effect. With these conditions and assuming a type I error of 2.5% (one-sided) and a power of 90%, the required sample size based on a binomial distribution would be 56. Considering cases excluded from the analysis, the final sample size in this study was 64. Further, we compared the joint preservation duration determined using Kaplan–Meier analysis (with radiological collapse as an end point) with that of the 1:1 exact matched historical control. Exact matching was performed by adjusting for sex, type, background factors and lesion side. Joint preservation was defined as JIC stages 1 and 2 ONFH without radiological collapse. Radiological collapse was first assessed at each institution and ultimately approved by three members of the central image review committee. As subanalyses, we evaluated the 24-month collapse rate after rhFGF-2 treatment categorized according to patient background factors (corticosteroid use, alcohol abuse, idiopathic, corticosteroid use plus alcohol abuse) and type (C1 or C2). Secondary outcomes included changes in the type during observation (assessment of imaging for bone regeneration) and changes in the two clinical scores: HHS and UCLA activity rating scale before and after surgery (at baseline and at 3, 6, 12, 18 and 24 months post-treatment). Safety was assessed from the reports of adverse events (AEs) by investigators or trial personnel at each trial visit by laboratory testing and physical examination. An AE was defined as any sign of worsening of the participant's condition after this treatment, and AEs were classified as either serious or nonserious. AEs were reported and assessed by an external monitoring committee, with the severity classified as mild, moderate or severe [24]. Causal relationships between the clinical trial and resolution of each AE were judged and evaluated. The incidences of AEs and side effects in the safety analysis population were tabulated. We disaggregated the data by severity and causal relationship.

Statistical analysis

In the primary outcome, we calculated the two-sided 95% CI for the collapse rate in the full analysis set at 24 months after treatment. Additionally, we characterized the time to radiological femoral head collapse from initial diagnosis using Kaplan–Meier plots, employing log-rank tests and Cox regression analysis to compare the joint preservation times between groups. For the HHS and UCLA activity rating scale, we analyzed the scores at baseline and at 3, 6, 12, 18 and 24 months post-treatment using a mixed-effects model to fit the regression line to time [25]. Data for each clinical score after THA were excluded. In the safety analysis, we calculated the AE occurrence rate and

Table 1. Baseline characteristics of the enrolled patients and distribution of the four adjustment factors before and after exact matching.

Characteristic		Pre-matching			After exact matching		
		rhFGF-2 treatment	Control	p-value[§]	rhFGF-2 treatment	Control	p-value[§]
Patients (n)		63	268		49	49	
Age (years)	Mean ± standard deviation Median (range)	43.0 ± 14.3	43.8 ± 14.4	–	42.5 ± 14.8	43.8 ± 15.3	–
Sex, n (%)[†]	Male	39 (61.9)	133 (49.6)	0.079	26 (53.1)	26 (53.1)	1.00
	Female	24 (38.1)	135 (50.4)		23 (46.9)	23 (46.9)	
Stage, n (%)	Stage 1	24 (38.1)	77 (38.7)	–	17 (34.7)	15 (30.6)	–
	Stage 2	39 (61.9)	122 (61.3)		32 (65.3)	34 (69.4)	
Type, n (%)[†,‡]	Type A	0 (0.0)	19 (7.1)	< 0.0001	0 (0.0)	0 (0.0)	1.00
	Type B	0 (0.0)	50 (18.7)		0 (0.0)	0 (0.0)	
	Type C	63 (100.0)	199 (100.0)		49 (100.0)	49 (100.0)	
	Type C1	17 (27.0)[‡]	98 (36.6)		18 (36.7)	14 (28.6)	
	Type C2	46 (73.0)[‡]	101 (37.7)		31 (63.3)	35 (71.4)	
Side, n (%)[†]	Hemilateral/right	5 (7.9)	106 (39.6)	< 0.0001	5 (10.2)	5 (10.2)	1.00
	Hemilateral/left	6 (9.5)	84 (31.3)		5 (10.2)	5 (10.2)	
	Bilateral	52 (82.5)	78 (29.1)		39 (79.6)	39 (79.6)	
Background factors, n (%)[†]	Corticosteroid use	40 (63.5)	194 (72.4)	0.068[‡,§]	36 (73.5)	36 (73.5)	1.00[§]
	Alcohol abuse	20 (31.7)	48 (17.9)		11 (22.4)	11 (22.4)	
	Idiopathic	2 (3.2)	22 (8.2)		1 (2.0)	1 (2.0)	
	Corticosteroid use plus alcohol abuse	1 (1.6)	4 (1.5)		1 (2.0)	1 (2.0)	

One-to-one exact matching on the basis of the baseline demographics.
[†]Adjustment factors used in matching. For each adjustment factor, we compared the distribution before and after and matched by the frequency calculation and Chi-squared test. We employed Student's t test to calculate the p-value for age.
[‡]More severe type of the two affected sides in patients with both sides affected. Note that the type used for the subgroup analyses in this study is different from this type and is defined as the more severe type of the two 'trial sides' for the patients whose trial target hip was both sides.
[§]It is possible that the results of the Chi-squared test are not plausible.

its two-tailed 95% CI. We calculated 95% CIs for the proportion as Clopper–Pearson confidence limits and performed the statistical analyses using SAS software, version 9.4 (SAS Institute, NC, USA). All p-values <0.05 were considered significant.

Results

From 17 February to 7 December 2016, 64 patients underwent rhFGF-2 treatment. Of these, 63 patients were eligible for the study. Figure 1 shows the participant information, registered data and analysis targets, and Table 1 shows the enrolled patients' characteristics at baseline and the exact matching. The participants were 39 men and 24 women (mean age: 43.0 years) with precollapse ONFH (type C1: 31 joints; type C2: 45 joints) who were locally administered rhFGF-2. Thirteen patients (20.6%) underwent bilateral rhFGF-2 treatment. Seven patients discontinued participation in TRION and were analyzed as worsening cases. One patient underwent bone graft surgery and six patients underwent THA during the observational period. The historical control study registered 271 cases, three of which were ineligible and 268 of which were analyzed. Among them, there were 199 cases of type C (C1: 98; C2: 101). Table 2 shows the collapse rate of the historical control study calculated using the Kaplan–Meier analysis with the end point of radiological collapse. The 24-month collapse rates in the historical control were 40.8 and 70.3% for types C1 and C2, respectively. In the exactly matched control group, the 24-month collapse rate of type C was 73.5% (36/49; 95% CI: 58.9–85.1).

The 24-month collapse rate was 42.9% (27/63; 95% CI: 30.5–56.0) in the original rhFGF-2 treatment group, which was above the expected collapse rate (35%) in the sensitivity analysis (H0: p = 0.35; H1: p < 0.35; p = 0.92). In the exactly matched pair analysis, the median joint preservation time was 45.8 months (95% CI: 27.3 to undetermined) for the rhFGF-2 treatment group and 11.1 months (95% CI: 5.5–20.1) for the historical control group (log-rank test; p = 0.0003) (Figure 3A). A Cox regression analysis showed that the joint preservation rate was higher in the rhFGF-2 treatment group than in the historical control group (p = 0.0004), with a 2.6-fold

Table 2. Collapse rate of the historical control study calculated by the Kaplan–Meier analysis with the end point as the radiological collapse of the femoral head.

Type	Cases (n)	Time (months)	Collapse rate (95% CI)
Type A	19	12	0.0% (0.0–0.0)
		24	0.0% (0.0–0.0)
		36	0.0% (0.0–0.0)
Type B	50	12	0.0% (0.0–0.0)
		24	2.0% (0.3–13.4)
		36	10.8% (4.6–24.1)
Type C1	98	12	25.5% (18.0–35.4)
		24	40.8% (31.8–51.2)
		36	48.5% (38.8–59.1)
Type C2	101	12	57.4% (48.1–67.2)
		24	70.3% (61.3–78.9)
		36	76.7% (68.0–84.5)

Collapse rates at 12, 24 and 36 months were determined by the central image committee. Collapse rate and its 95% CI were calculated using the Kaplan–Meier method.

Table 3. Changes in the clinical outcomes of HHS and UCLA activity rating scale scores.

Score (range)	Baseline	3 months	6 months	12 months	18 months	24 months	p-value
HHS (0–100)	86.5 ± 11.3 83.9–89.1 89.0 (39.0–100.0)	90.7 ± 9.9 88.4–92.9 93.4 (44.0–100.0)	92.0 ± 9.1 89.9–94.1 94.0 (47.0–100.0)	91.6 ± 11.4 88.9–94.3 95.0 (44.7–100.0)	92.4 ± 12.2 89.5–95.3 97.4 (36.0–100.0)	93.0 ± 10.9 90.4–95.6 96.0 (39.0–100.0)	0.008[†]
UCLA score (1–10)	4.6 ± 1.6 4.2–5.0 4 (2–9)	4.9 ± 1.6 4.5–5.4 5 (2–9)	5.2 ± 1.4 4.8–5.6 5 (2–8)	5.5 ± 1.4 5.1–5.9 5 (2–8)	6.0 ± 1.4 5.6–6.3 6 (2–9)	6.2 ± 1.5 5.8–6.7 7 (3–10)	< 0.0001[‡]

Data in the upper row are expressed as mean ± standard deviation. Data in the middle row are 95% CIs. Data in the bottom row are the median and range. The p-value was calculated for the effect of time in a repeated measures linear mixed-effect model with a one-tailed test (significance at p < 0.025). The evaluation included all patients but excluded those who discontinued. The HHS ranged from 0 to 100.0, with higher positive scores indicating a more functional hip joint and lower negative scores indicating a less functional hip joint. The UCLA rating scale scores ranged from 1 to 10, with 10 indicating 'regularly participates in impact sports' and 1 indicating 'entirely inactive, dependent on others and cannot leave residence'.
[†]A mixed-effects model was employed to fit the regression line on time and showed a statistically significant increase in HHS over time (increase of 0.15/month; 95% CI: 0.03–0.29; p = 0.008).
[‡]A mixed-effects model was employed to fit the regression line on time and showed a statistically significant increase in the UCLA Activity Rating Scale score over time (increase of 0.06/month; 95% CI: 0.04–0.08; p < 0.0001).
HHS: Harris hip score; UCLA: University of California Los Angeles.

hazard ratio (95% CI: 1.5–4.5). In the type C1 matched pair analysis, the median joint preservation time was not significantly longer for the rhFGF-2 treatment group than the historical control group (log-rank test; p = 0.29) (Figure 3B). In the type C2 matched pair analysis, the median joint preservation time was 30.8 months (95% CI: 24.4–50.5) for the rhFGF-2 treatment group and 6.7 months (95% CI: 4.0–10.2) for the historical control group (log-rank test; p = 0.0004). A Cox regression analysis showed that the joint preservation rate was higher in the type C2 rhFGF-2 treatment group than in the type C2 historical control group (p = 0.0007), with a 2.9-fold hazard ratio (95% CI: 1.6–5.3) (Figure 3C). Among the background factors, the 24-month rate for femoral head collapse after rhFGF-2 treatment was higher for alcohol intake (66.7%; 95% CI: 29.9–92.5) than for corticosteroid use (27.3%; 95% CI: 13.3–45.5). Both the pretreatment and 24-month post-treatment HHS and UCLA scores showed a gradual increase and were analyzed using a mixed-effects model (Table 3). The postoperative clinical scores significantly improved, with an increase of 0.15/month for HHS (95% CI: 0.03–0.29; p = 0.008) and 0.06/month for the UCLA score (95% CI: 0.04–0.08; p < 0.0001). MRI at 24 months showed a decrease in osteonecrosis size due to bone regeneration after rhFGF-2 treatment (Figure 4), with a decreasing trend in type C2 and an increase in types A, B and C1 (Table 4). Thirteen serious AEs occurred in 18.8% of the patients. There were no complications related to the surgical technique. The severity of the AEs was mild or moderate (Table 5). There were no cases of discontinuation due to AEs and no clinically significant abnormalities associated with the clinical laboratory values. Participants had recovered from all AEs during the final follow-up.
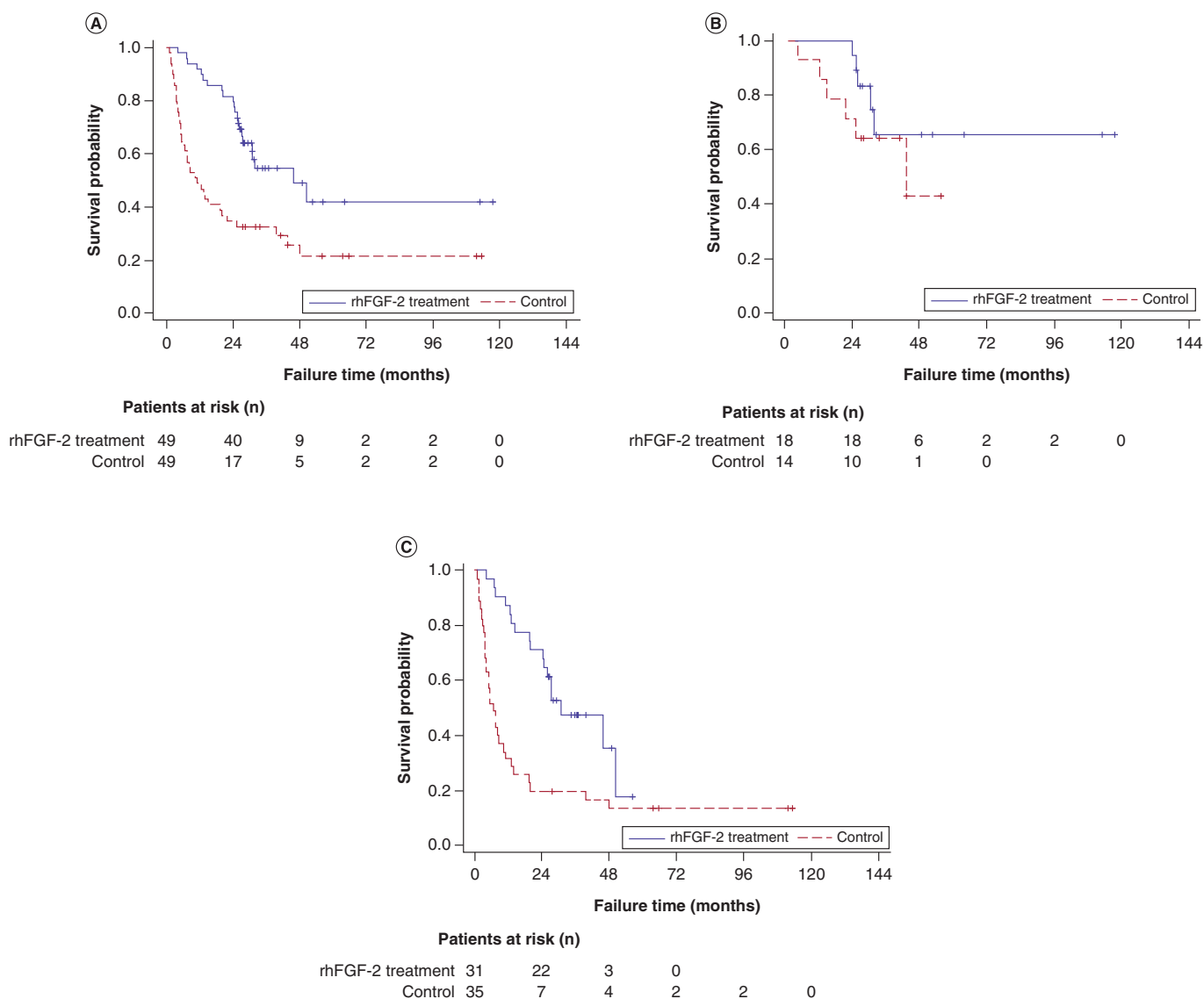
Figure 3.   Radiological joint preservation of the femoral head determined by Kaplan–Meier analysis between the rhFGF-2–treated and exact matched historical control groups. (A) Kaplan–Meier estimates comparing the radiological femoral head collapse as the end point between the overall rhFGF-2 treatment group and exact matched historical control group. The cumulative rates of 24-month survival indicate that the collapse rate in the patients with ONFH after rhFGF-2 treatment was more gradual than that in the control participants. There was a significant difference in terms of duration from the diagnosis to radiological collapse of the femoral head (hazard ratio: 2.6; p = 0.0003; Cox regression analysis). **(B)** Kaplan–Meier estimates for type C1 participants between the rhFGF-2 treatment group and exact matched historical control group. **(C)** Kaplan–Meier estimates for type C2 participants between the rhFGF-2 treatment group and exact matched historical control group.
ONFH: Osteonecrosis of the femoral head.

Table 4. Change of type at 24 months after rhFGF-2 treatment.

	n (hips)	Type A		Type B		Type C1		Type C2	
		n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Baseline	76	0 (0.0)	0.0–4.7	0 (0.0)	0.0–4.7	33 (43.4)	32.1–55.3	43 (56.6)	44.7–67.9
24 months	69	3 (4.3)	0.9–12.2	3 (4.3)	0.9–12.2	33 (47.8)	35.6–60.2	30 (43.5)	31.6–56.0

The evaluation included all patients; however, seven patients who discontinued were excluded. The types are categorized into four, from the smallest osteonecrotic area to the largest in size: types A, B, C1 and C2. Types A and B with a good prognosis were excluded from this trial and therefore are not present in the baseline. The preoperative largest size of osteonecrosis, type C2, tended to improve to smaller osteonecrosis of types A, B and C1 at 24 months postoperatively. This result was judged by the decrease in low signal area in the T1-weighted MRI, which denotes bone regeneration in the osteonecrotic area after rhFGF-2 treatment.
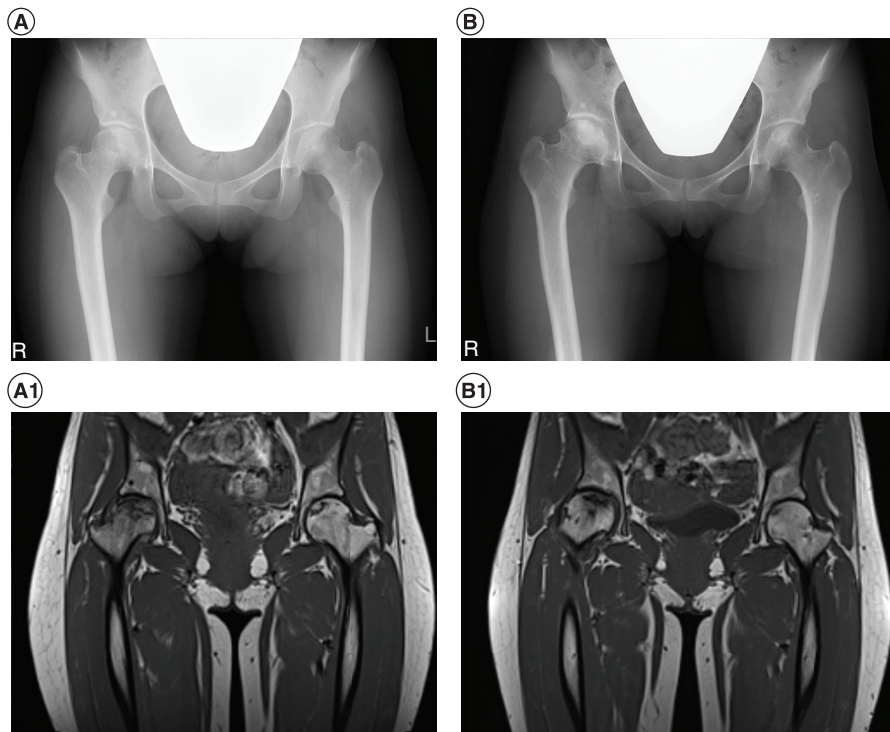
Figure 4.   Representative radiographs and magnetic resonance images. (A) A preoperative anterior–posterior pelvic radiograph of a 23-year-old woman who had bilateral precollapse ONFH due to systemic corticosteroid therapy for sudden deafness. **(B)** A 2-year postoperative radiograph showing radiological collapse on the right side and no progression on the left side. **(C)** Preoperative coronal T1-weighted MRI showing type C2 ONFH occupying more than the medial two-thirds of the weight-bearing portion and extending laterally to the acetabular edge (type C2) on the right side and not extending to the acetabular edge (type C1) on the left side. The patient was enrolled in the clinical trial and received bilateral rhFGF-2 treatment. **(D)** MRI of the same femoral neck and intertrochanteric region at 2 years postoperatively, showing collapse of the femoral head on the right side (stage 3A, type C1) and smaller type of ONFH (stage 1, type A) on the left side. In this case, we judged the radiological collapse and concluded it to be a worsened case.
ONFH: Osteonecrosis of the femoral head.

Table 5. Details of serious adverse events (safety population).					
Serious adverse events	n (%)	95% CI	Level of severity	Causal relationship with investigational drug	Outcome
	12 (18.8)	10.1–30.5			
Thrombotic thrombocytopenic purpura	1 (1.6)	0.0–8.4	Moderate	Unrelated	Resolved
Chest pain	1 (1.6)	0.0–8.4	Mild	Unrelated	Resolving
Herpes zoster	1 (1.6)	0.0–8.4	Moderate	Unrelated	Resolved
Pyelonephritis	1 (1.6)	0.0–8.4	Moderate	Unrelated	Resolved
Osteonecrosis	6 (9.4)	3.5–19.3	Mild	Unrelated	Resolved
Prostate cancer	1 (1.6)	0.0–8.4	Moderate	Unlikely[†]	Resolving
Renal cell carcinoma	1 (1.6)	0.0–8.4	Moderate	Unlikely[†]	Resolving
Loss of consciousness	1 (1.6)	0.0–8.4	Mild	Unrelated	Resolved

Determination of the causal relationship and outcomes of serious adverse events with the investigational drug.
[†]The cases of cancer (two cases) were 'unlikely' to be related to the intervention. The causal relationship was 'unrelated' for all other events, and all outcomes were judged to be resolved or resolving.

Discussion

ONFH is an orphan, refractory joint disease that causes femoral head collapse, pain and gait disturbance, requiring THA in young patients. Joint preservation has been the ultimate therapeutic goal for ONFH. Despite the generally accepted principle of early intervention with the least damaging technique to preserve the femoral head, the THA

rate for ONFH surgeries in the USA during 2009–15 was >94% [9]. For the younger generation, THA is not ideal due to its potential for dislocation, infection, activity limitation and later revisions [2–4]. Primary THA and revisions also pose a huge financial burden [9]. Consequently, joint-preserving regenerative therapies have been considered for preventing femoral head collapse. In this Phase II trial, rhFGF-2 treatment prevented ONFH progression and improved clinical scores. Based on the results of this historical control study, the collapse rate increased with an increase in the osteonecrotic lesion size. Precollapse type C2 ONFH had a 70.3% collapse rate 2 years after the initial diagnosis. In the rhFGF-2 treatment group, the overall collapse rate of 42.9% was above the expected threshold collapse rate of 35%. Seven patients underwent further operations, including six THAs in the 24 months. However, joint preservation was significantly higher (2.6-fold according to the Cox regression analysis; p = 0.0004) and the time to collapse was significantly longer in the rhFGF-2 group (45.8 months) than in the historical control group (11.1 months, log-rank test; p = 0.0003). In the type C1 matched pair analysis, the median joint preservation time was not significantly longer (log-rank test; p = 0.29), but in the type C2 matched pair analysis, the median joint preservation time was significantly longer for the rhFGF-2 treatment group than for the historical control group (log-rank test; p = 0.0004), with a 2.9-fold hazard ratio (Cox regression analysis; p = 0.0007). Bone regeneration with rhFGF-2 treatment tended to improve the ONFHs to smaller types, as demonstrated by postoperative MRI. This result was important in that bone regeneration was confirmed by objective image analysis. HHS and UCLA scores increased significantly over time. Interestingly, the outcomes in the subanalysis were worse for those with heavy alcohol consumption than for those who took corticosteroids, suggesting that the etiology of alcohol-related ONFH might differ from that of corticosteroid-associated ONFH [2,3,14,17]. We previously reported that patients using corticosteroids have been widely considered as at potentially higher risk for ONFH and can be easily diagnosed compared with patients who are not using corticosteroids [23]. Therefore regenerative treatments might be more suitable for patients who take corticosteroids and are at risk of ONFH. In the safety analysis, although 18.8% of the patients experienced serious AEs, they recovered without issues.

TRION showed the efficacy of rhFGF-2; however, the main drawback was the lack of a vehicle control. The gold standard for clinical trials is to employ randomization to generate a concurrent control group [26]. For certain rare diseases and pediatric clinical trials, however, regulatory agencies now recognize single-arm trials with historical controls for drug approval [27,28]. In the comparative study of cell therapies for ONFH, numerous studies have reported very poor outcomes in the controls, with high collapse rates (>70%) reported in control groups with CD alone [12–15]. These results suggested that the collapse rate of patients with CD alone is higher than that of nonsurgically treated patients; thus CD is rarely performed for ONFH in Japan. Setting up a control group was therefore judged (in discussions with PMDA) to be unfair to patients with this rare disease. To reduce the participant burden, we compared a single arm consisting of just a treatment group versus an exactly matched historical control. TRION was designed to minimize potential biases of the historical control and conducted appropriate selection for inclusion in the analysis. We carefully confirmed the acceptability criteria for historical controls by Pocock [28], similarities between historical and current population studies, prior medication and similar end points based on the same ONFH classification, region and contemporaneity. From the viewpoint of diagnostic correctness, when applying the status for an intractable disease, diagnoses according to the JIC classification are required annually in Japan. The JIC classification is widely used and has reported high reliability [29,30]. There were no differences in the preoperative treatment between the treatment and control groups because preventive surgery, such as CD for asymptomatic ONFH, was not recommended in Japan [9]. In addition, types A and B with good prognosis were excluded. We previously reported that the 5-year collapse rates in 212 precollapse hips were found to be 0, 7.9, 36.6 and 84.8% (joint preservation rate: 100, 92.1, 63.4 and 15.2%) for types A, B, C1 and C2, respectively [23]. Based on these considerations, we concluded that early treatment for asymptomatic type C disease, especially type C2, is justified. We believe that TRION has a high external validity that is as close as possible to actual clinical practice. The historically controlled TRION trial might provide a platform for evaluating the efficacy of future ONFH studies, because ONFH meets the six acceptability criteria for historical controls [28]. However, there are other limitations to this trial. The long-term results and safety after 2 years have not been considered. From our pilot study, 5-year outcomes appear to indicate no safety issues [18]; however, the long-term results should be clarified. There were no complications related to the surgical procedure, but the outflow of FGF-2 may cause inflammatory reactions and osteophyte formation in the hip joint. It is also unclear whether rhFGF-2 treatment is superior to cell therapies.

Since approximately the year 2000, regenerative therapies have emerged, combining CD with cells, bone substitutes and growth factors [12–18]. Treatment guidelines and reviews are frequently updated worldwide [2–5].

Cell therapies using autologous cells have been performed in numerous countries given the established safety of autologous tissue transplants. The beneficial effects of cell therapy have been suggested [12–16]; however, a recent concept review from the USA stated that the biological augmentation of CD has shown promising results in providing symptomatic relief and slowing the natural progression of ONFH, but more study is necessary to establish its efficacy [4]. We researched PubMed and Medline (20 January 2021) for clinical trials evaluating growth factor therapies to prevent femoral head collapse. We used the terms 'osteonecrosis', 'femoral head', 'growth factor' and 'trial'; however, we found no clinical studies other than our previous single-arm pilot study [17,18]. Our pilot trial suggested that rhFGF-2 treatment is a therapeutic solution for early-stage ONFH based on its medium-term safety, surgical simplicity and effectiveness, shown both radiologically and clinically [18]. Apart from FGF-2, recombinant human bone morphogenic proteins rhBMP-2 [31] and rhBMP-7 [32,33] have been employed in addition to bone grafting procedures but the trials were for off-label use and not for the purpose of drug approval [34]. The present clinical study was based on results from the preclinical research using an experimental rabbit model of ONFH [35].

Two rhFGF-2 topical products were recently launched for periodontitis [36] (Regrowth®; Kaken Pharmaceuticals Co., Ltd., Tokyo, Japan, 2016) and tympanic membrane perforation [37] (Retympa®; Nobelpharma Co., Ltd., Tokyo, Japan, 2019), which are supported by translational research showing that FGF-2 caused potent angiogenesis and cell proliferation *in vitro* and *in vivo* [19]. These products were approved in Japan as a combined gel carrier/growth factor kit. Growth factor is an inexpensive genetically modified protein with stable quality. In addition, gel carriers are less expensive, suitable for local injection through minimally invasive surgery, and have a longer action time on FGF-2 [38,39]. Clinical application of injectable growth factors could be useful because of its simple preparation technique, requiring no cell collection. As with the SARS outbreak, attention needs to be paid to a possible rapid increase in corticosteroid-associated ONFH after the COVID-19 pandemic [7,8]. Type C ONFH is a serious disease that presents with femoral head collapse, necessitating THA even in young patients, who will probably require further revision surgeries and face a huge financial burden over their lifetime. Therefore the primary concern, for both patients and surgeons, is joint preservation to prevent femoral head collapse. Our findings show that the clinical application of rhFGF-2 reduced ONFH progression, prevented femoral head collapse and resulted in significant improvements in two clinical outcome measures. Postoperative MRI at 24 months showed a trend toward reduction in the size of the ONFH due to bone regeneration after rhFGF-2 treatment. Patients recovered from serious AEs without problems. By excluding types A and B with good prognoses, TRION was limited to patients with type C ONFH who needed surgical intervention for joint preservation. TRION is considered a clinical trial with high external validity that is as close as possible to actual clinical practice. This new treatment for ONFH might have a major impact on patient outcomes for daily activities, healthcare burden and costs, which would likely influence future clinical guidelines. After COVID-19, joint-preserving regenerative surgery for corticosteroid-associated ONFH has reached a significant turning point; approval of novel regenerative therapies for ONFH by the relevant authorities is expected.

In conclusion, the 24-month joint preservation rate for early-stage ONFH was higher after rhFGF-2 treatment than for the historical control. This clinical trial suggests that rhFGF-2 treatment is a therapeutic solution for precollapse ONFH because of its clinical efficacy, promising bone regeneration, reduced physical and financial burden on patients, and safety.

Conclusion

In the TRION trial, 64 patients with ONFH at four hospitals in Japan were percutaneously administered 800 µg rhFGF-2-impregnated gelatin hydrogel. Our study suggests that rhFGF-2 treatment safely increases the joint preservation time with clinical efficacy and radiological bone regeneration. rhFGF-2 treatment may be one of the viable therapeutic options for early-stage ONFH.

Future perspective

ONFH is an orphan, refractory joint disease that causes femoral head collapse, pain and gait disturbance, requiring THA in young patients. Joint preservation has been the ultimate therapeutic goal for ONFH. Since approximately the year 2000, regenerative therapies have emerged, combining CD with cells, bone substitutes and growth factors. Numerous physicians have begun investigating various cell therapies based on CD surgery, but growth factors for ONFH are rarely reported. The present investigator-initiated, multicenter historical control trial (TRION) is the first study to show short-term efficacy with growth factor alone, leading to increased joint preservation time compared with exactly matched historical controls. However, further long-term studies are essential. Regenerative

therapy using growth factor is a simple method and is expected to be applied clinically for patients with ONFH in future. This new treatment might have a major impact on patient outcomes for daily activities, healthcare burden and costs, which would likely influence future clinical guidelines.

Summary points

- Osteonecrosis of the femoral head (ONFH) is an orphan, refractory joint disease that causes femoral head collapse, pain and gait disturbance.
- ONFH is a serious disease that presents with femoral head collapse, requiring total hip arthroplasty. The patients will probably require further revision surgery and face a huge financial burden over their lifetime.
- Regenerative medicine is considered the next treatment of interest to prevent collapse.
- The key inclusion criterion was nontraumatic, precollapse, larger ONFH (types C1 and C2), given that patients with types A and B need no further surgery and have good prognoses regardless of the intervention.
- We percutaneously administered gelatin hydrogel containing 800 μg recombinant human FGF-2 (rhFGF-2) to 64 patients with nontraumatic, precollapse and large ONFHs (types C1 and C2).
- Under the exact matching analyses (1:1), with radiological collapse as the end point, median joint preservation time was significantly higher in the rhFGF-2 group (45.8 months) than in the control group (11.1 months), with a hazard ratio of 2.6 (95% CI: 1.5–4.5; p = 0.0003).
- The postoperative clinical scores significantly improved, with an increase of 0.15/month for the Harris hip score (95% CI: 0.03–0.29; p = 0.008) and 0.06/month for the University of California Los Angeles activity rating scale (95% CI: 0.04–0.08; p < 0.0001).
- This clinical trial suggests that rhFGF-2 treatment can be a therapeutic option for precollapse ONFH because of its clinical efficacy, promising bone regeneration, reduction in physical and financial burden on patients, and safety.

Collaborators

TRION trial collaborators: Tsuyoshi Asano, Juji Ito, Hirofumi Oshima, Taizo Kaneko, Yoko Fukasawa, Hyonmin Choe, Shusuke Ueda, Kayoko Enomoto, Harue Tada, Akiko Kuroda, Yasuhiko Tabata, Yaichiro Okuzu, Toshiyuki Kawai, Koji Goto, Manabu Nankaku, Masaki Takao, Wataru Ando, Kazuki Yamada, Michio Yamamoto, Takashi Sakai, Yasuhiro Ishidou, Shigeru Yamada, Hidetsugu Ohara, Hiroshi Fujita, Hirotsugu Ohashi, Hirokazu Iida & Takashi Nakamura

Author contributions

Y Kuroda wrote the first draft of the manuscript, and all authors participated in writing the subsequent drafts and agreed to submit this manuscript for publication. Y Kuroda, H Abe, T Ito-Ihara, R Asada, S Morita, S Matsuda and H Akiyama were responsible for study conception, trial design, obtaining grant funding and trial management. T Tanaka, T Miyagawa, H Hamada, Y Fujimoto, D Takahashi, T Tetsunaga, A Kaneuji, M Takagi, Y Inaba, N Sugano and S Tanaka were responsible for data acquisition. H Abe and S Morita were responsible for the statistical analysis. All authors were responsible for the data interpretation and for drafting and approving the final submitted manuscript. List of the TRION collaborators: Tsuyoshi Asano, Juji Ito, Hirofumi Oshima, Taizo Kaneko, Yoko Fukasawa, Hyonmin Choe, Shusuke Ueda, Kayoko Enomoto, Harue Tada, Akiko Kuroda, Yasuhiko Tabata, Yaichiro Okuzu, Toshiyuki Kawai, Koji Goto, Manabu Nankaku, Masaki Takao, Wataru Ando, Kazuki Yamada, Michio Yamamoto, Takashi Sakai, Yasuhiro Ishidou, Shigeru Yamada, Hidetsugu Ohara, Hiroshi Fujita, Hirotsugu Ohashi, Hirokazu Iida, Takashi Nakamura.

Ethical conduct of research

The study protocols were designed by the Institute for Advancement of Clinical and Translational Research of Kyoto University (iACT) and Gifu University, and were approved by the Institutional Review Board or Ethics Committee of each hospital (Approval no. K023) in December 2015. The study was registered in the University Hospital Medical Information Network clinical trials registry (no. UMIN000020340), and was conducted in accordance with the latest regulatory requirements, including the Declaration of Helsinki and the International Council for Harmonisation – Good Clinical Practice regulations.

Data sharing statement

All data requests should be submitted to the corresponding author for consideration. Access to anonymized data may be granted following review.

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References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Ikeuchi K, Hasegawa Y, Seki T, Takegami Y, Amano T, Ishiguro N. Epidemiology of nontraumatic osteonecrosis of the femoral head in Japan. *Mod. Rheumatol.* 25(2), 278–281 (2015).

2. Petek D, Hannouche D, Suva D. Osteonecrosis of the femoral head: pathophysiology and current concepts of treatment. *EFORT Open. Rev.* 4(3), 85–97 (2019).

•   **A current concept review of nontraumatic osteonecrosis of the femoral head from Europe.**

3. Larson E, Jones LC, Goodman SB, Koo KH, Cui Q. Early-stage osteonecrosis of the femoral head: where are we and where are we going in year 2018. *Int. Orthop.* 42(7), 1723–1728 (2018).

4. Mont MA, Salem HS, Piuzzi NS, Goodman SB, Jones LC. Nontraumatic osteonecrosis of the femoral head: where do we stand today? A 5-year update. *J. Bone Joint Surg. Am.* 102(19), 1084–1099 (2020).

•• **A recent concept review of nontraumatic osteonecrosis of the femoral head from the USA.**

5. Zhao D, Zhang F, Wang B *et al.* Guidelines for clinical diagnosis and treatment of osteonecrosis of the femoral head in adults (2019 version). *J. Orthop. Translat.* 21, 100–110 (2020).

•   **A current guideline for osteonecrosis of the femoral head in China.**

6. Guan WJ, Ni ZY, Hu Y *et al.* Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* 382(18), 1708–1720 (2020).

7. Tang C, Wang Y, Lv H, Guan Z, Gu J. Caution against corticosteroid-based COVID-19 treatment. *Lancet* 395(10239), 1759–1760 (2020).

8. Zhang B, Zhang S. Corticosteroid-induced osteonecrosis in COVID-19: a call for caution. *J. Bone Miner. Res.* 35, 1828–1829 (2020).

9. Sodhi N, Acuna A, Etcheson J *et al.* Management of osteonecrosis of the femoral head. *Bone. Joint. J.* 102-B, 122–128 (2020).

10. Kuroda Y, Okuzu Y, Kawai T, Goto K, Matsuda S. Difference in therapeutic strategies for joint-preserving surgery for non-traumatic osteonecrosis of the femoral head between the United States and Japan: a review of the literature. *Orthop Surg.* 13(3), 742–748 (2021).

11. Ando W, Sakai T, Fukushima W *et al.* Japanese Orthopaedic Association 2019 Guidelines for osteonecrosis of the femoral head. *J. Orthop. Sci.* 26, 46–68 (2021).

•   **A recent concept review of nontraumatic osteonecrosis of the femoral head from Japan.**

12. Xu Y, Jiang Y, Xia C, Wang Y, Zhao Z, Li T. Stem cell therapy for osteonecrosis of femoral head: opportunities and challenges. *Regen. Ther.* 15, 295–304 (2020).

13. Maruyama M, Lin T, Pan CC *et al.* Cell-based and scaffold-based therapies for joint preservation in early-stage osteonecrosis of the femoral head: a review of basic research. *JBJS Rev.* 7, e5 (2019).

14. Hua KC, Yang XG, Feng JT *et al.* The efficacy and safety of core decompression for the treatment of femoral head necrosis: a systematic review and meta-analysis. *J. Orthop. Surg. Res.* 14, 306 (2019).

15. Villa JC, Husain S, van der List JP, Gianakos A, Lane JM. Treatment of pre-collapse stages of osteonecrosis of the femoral head: a systematic review of randomized control trials. *HSS J.* 12, 261–271 (2016).

16. Cao H, Guan H, Lai Y, Qin L, Wang X. Review of various treatment options and potential therapies for osteonecrosis of the femoral head. *J. Orthop. Translat.* 4, 57–70 (2015).

17. Kuroda Y, Asada R, So K *et al.* A pilot study of regenerative therapy using controlled release of rhFGF-2 for patients with pre-collapse osteonecrosis of the femoral head. *Int. Orthop.* 40, 1747–1754 (2016).

18. Kuroda Y, Ito-Ihara T, Abe H *et al.* Recombinant human FGF-2 therapy for osteonecrosis of the femoral head: 5-year follow-up. *Regen. Med.* 15, 2261–2271 (2020).

•• **The previous pilot trial to evaluate the safety of rhFGF-2 for osteonecrosis of the femoral head.**

19. Marie PJ, Miraoui H, Sévère N. FGF/FGFR signaling in bone formation: progress and perspectives. *Growth Factors* 30, 117–123 (2012).

20. Sugano N, Atsumi T, Ohzono K, Kubo T, Hotokebuchi T, Takaoka K. The 2001 revised criteria for diagnosis, classification, and staging of idiopathic osteonecrosis of the femoral head. *J. Orthop. Sci.* 601–605 (2002).

21. Harris WH. Traumatic arthritis of the hip after dislocation and acetabular fractures: treatment by mold arthroplasty. An end-result study using a new method of result evaluation. *J. Bone Joint Surg. Am.* 51-A, 737–755 (1969).

22. Amstutz HC, Thomas BJ, Jinnah R, Kim W, Grogan T, Yale C. Treatment of primary osteoarthritis of the hip. A comparison of total joint and surface replacement arthroplasty. *J. Bone Joint Surg. Am.* 66, 228–641 (1984).

23. Kuroda Y, Tanaka T, Miyagawa T *et al.* Classification of osteonecrosis of the femoral head: who should have surgery? *Bone Joint Res.* 8, 451–458 (2019).

• **Provided the collapse rate of the femoral head in the patients with osteonecrosis of the femoral head.**

24. Edwards IR, Biriell C. Harmonisation in pharmacovigilance. *Drug Saf.* 10, 93–102 (1994).

25. Lo S, Andrews S. To transform or not to transform: using generalized linear mixed models to analyse reaction time data. *Front. Psychol.* 6, 1171 (2015).

26. Beard DJ, Campbell MK, Blazeby JM *et al.* Considerations and methods for placebo controls in surgical trials (ASPIRE guidelines). *Lancet* 395, 828–838 (2020).

27. Lim J, Wang L, Best N *et al.* Reducing patient burden in clinical trials through the use of historical controls: appropriate selection of historical data to minimize risk of bias. *Ther. Innov. Regul. Sci.* 54, 850–860 (2020).

28. Pocock SJ. The combination of randomized and historical controls in clinical trials. *J. Chronic Dis.* 29, 175–188 (1976).

29. Takashima K, Sakai T, Hamada H, Takao M, Sugano N. Which classification system is most useful for classifying osteonecrosis of the femoral head? *Clin. Orthop. Relat. Res.* 476, 1240–1249 (2018).

30. Sultan AA, Mohamed N, Samuel LT *et al.* Classification systems of hip osteonecrosis: an updated review. *Int. Orthop.* 43, 1089–1095 (2019).

31. Lieberman JR, Conduah A, Urist MR. Treatment of osteonecrosis of the femoral head with core decompression and human bone morphogenetic protein. *Clin. Orthop. Relat. Res.* 429, 139–145 (2004).

32. Seyler TM, Marker DR, Ulrich SD, Fatscher T, Mont MA. Nonvascularized bone grafting defers joint arthroplasty in hip osteonecrosis. *Clin. Orthop. Relat. Res.* 466(5), 1125–1132 (2008).

33. Papanagiotou M, Malizos KN, Vlychou M, Dailiana ZH. Autologous (non-vascularised) fibular grafting with recombinant bone morphogenetic protein-7 for the treatment of femoral head osteonecrosis: preliminary report. *Bone Joint J.* 96-B(1), 31–35 (2014).

34. Kuroda Y, Kawai T, Goto K. Matsuda S. Clinical application of injectable growth factor for bone regeneration: a systematic review. *Inflamm. Regener.* 39, 20 (2019).

35. Kuroda Y, Akiyama H, Kawanabe K, Tabata Y, Nakamura T. Treatment of experimental osteonecrosis of the hip in adult rabbits with a single local injection of recombinant human FGF-2 microspheres. *J. Bone. Miner. Metab.* 28(6), 608–616 (2010).

36. Kitamura M, Akamatsu M, Machigashira M *et al.* FGF-2 stimulates periodontal regeneration: results of a multi-center randomized clinical trial. *J. Dent. Res.* 90, 35–40 (2011).

37. Omae K, Kanemaru SI, Nakatani E *et al.* Regenerative treatment for tympanic membrane perforation using gelatin sponge with basic fibroblast growth factor. *Auris Nasus Larynx* 44, 664–671 (2017).

38. Tabata Y, Nagano A, Ikada Y. Biodegradation of hydrogel carrier incorporating fibroblast growth factor. *Tissue Eng.* 5, 127–1247 (1999).

39. Tabata Y, Miyao M, Yamamoto M, Ikada Y. Vascularization into a porous sponge by sustained release of basic fibroblast growth factor. *J. Biomater. Sci. Polym. Ed.* 10(9), 957–968 (1999).