A first-in-human, double-blind, randomised, placebo-controlled, dose ascending study of intra-articular rhFGF18 (sprifermin) in patients with advanced knee osteoarthritis

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Abstract

Objective

To evaluate the safety of intra-articular sprifermin (primary), and to evaluate systemic exposure, biomarkers, histology, and other cartilage parameters in patients with advanced osteoarthritis (OA).

Methods

This was a first-in-human, double-blind, randomised, placebo-controlled trial of single and multiple ascending doses of sprifermin from 3–300 µg in knee OA patients scheduled for total knee replacement. Patients were randomised 3:1 to sprifermin or placebo, injected into the target knee once or once weekly for 3 weeks, and followed-up for 24 weeks.

Results

Fifty-five patients were treated with sprifermin, 25 with single and 30 with multiple doses, 18 received placebo. There was no clear difference between the active and placebo groups in incidence, severity, and nature of reported treatment emergent adverse events. Acute inflammatory reactions were slightly more common with sprifermin 300 µg, but none led to discontinuation. No clear difference was seen between placebo and sprifermin in physician-assessed local tolerability, pain, or swelling in the knee. No meaningful changes over time, or differences between treatment groups, were observed for safety laboratory parameters or ECG. Although individual abnormalities were observed, no patterns were evident suggesting a relation to treatment or potential safety concern. No systemic sprifermin exposure, anti-FGF18 antibodies, or clear-cut effects on systemic biomarkers were detected.

Conclusion

This first clinical trial of sprifermin revealed no serious safety concerns, although larger studies are needed. The possibility of positive effects of intra-articular sprifermin on histological and other cartilage parameters in knee OA also warrant further investigation.

Key words

fibroblast growth factor 18, sprifermin, knee osteoarthritis

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Introduction

Osteoarthritis (OA) may cause severe pain and functional impairment. Pharmacological OA treatments temporarily relieve pain, but provide no structural or long-term benefit (1, 2). Accordingly, there is an unmet need for disease-modifying OA drugs (DMOADs) that slow, halt, or reverse the chronically progressive course of disease (1, 3, 4).

Endogenous human fibroblast growth factor 18 (FGF18) is a 20 kD protein expressed and secreted by chondrocytes and osteoblasts (5, 6). FGF18 is not a general growth mediator, but acts in the adult organism as a specific signal for chondrocyte proliferation, osteoblast differentiation, and production of cartilage matrix (7-9). Sprifermin is a recombinant, truncated, non-glycosylated form of human FGF18, investigated as a potential DMOAD.

This first-in-human study was designed to assess local and systemic safety following intra-articular (*i.a.*) sprifermin injection into knee joints of OA patients. Patients scheduled for total knee replacement (TKR) were included to facilitate the analysis of knee tissues directly exposed to sprifermin. Given the exploratory nature of this trial, small sample size, short observation time and no planned inferential statistical analysis, the primary objective was to evaluate local and systemic safety of *i.a.* sprifermin after single and multiple dose regimens. Secondary objectives were to explore cartilage parameters and systemic exposure. Pre-specified exploratory analyses investigated potential treatment effects on structure assessed by magnetic resonance imaging (MRI), x-ray, histology, biomechanical testing, and OA symptoms.

Methods

Study design and treatment

This multicentre, double-blind, randomised, placebo-controlled study evaluated single ascending dose (SAD) and multiple ascending dose (MAD) regimens of *i.a.* sprifermin in consecutive patient cohorts. Following evaluation of local and systemic safety of a single dose level by an independent Safety Review Board (SRB), multiple administrations of the same dose were tested in another patient cohort, before proceeding to the next higher single dose level. For the lowest dose (3 μg), only single administration was tested. For each dose cohort, the SRB reviewed data from at least 2 weeks of follow-up after last injection. The first two cohorts (SAD 3 μg and 10 μg) included four patients each, and subsequent cohorts eight patients each. The highest tolerated MAD level was repeated in a second cohort of eight patients. Dose escalation was limited at 300 μg, based on observations in the trial and stepwise evaluations of the dose levels (Suppl. Fig. 1). The 3 μg dose was not expected to be effective, because although effective in rats, it was only partially effective in dogs. Doses of 10, 30, 100, and 300 μg in humans are closer to those that provided efficacy in dogs.

For safety and PK evaluation, patients were hospitalised for 24 hours following first injection (and up to 4 hours following each subsequent injection in MAD cohorts). Only one of the first four patients in each dose cohort received study-drug in one day.

Within each cohort, patients were sequentially randomised 3:1 to receive sprifermin or placebo according to a central randomisation list, using an interactive voice response system. In SAD cohorts, patients received a single sprifermin injection into the target knee; in MAD cohorts, one dose per week was injected into the target knee for 3 consecutive weeks. Follow-up visits, including measurement of symptoms and biomarkers, were scheduled at 3, 4, 8, 12, and 24 weeks after first injection. For patients who had TKR within the trial period, cartilage samples were collected during surgery for immunohistochemical (10) and biomechanical analyses (11). MRI and x-ray assessments of the target knee were completed at baseline and before surgery or at termination visit in Week 24 (x-ray only if ≥8 weeks from first injecion). X-ray and MRI data were read centrally.

During and within 3 months preceding the trial, treatment with *i.a.* corticosteroids or hyaluronic acid derivatives was not allowed. Symptomatic treatment, *e.g.* stable treatment with nonsteroidal

anti-inflammatory drugs, was allowed during the trial. Acetaminophen (paracetamol) was used as rescue medication.

This study (NCT00911469) was conducted in compliance with Good Clinical Practices; the Declaration of Helsinki; EMA guidance (12); local regulations and Ethics Committee(s).

Patients

Males and females ≥40 years of age diagnosed with primary femorotibial knee OA for ≥6 months, based on clinical and radiological criteria of the American College of Rheumatology (13), were enrolled. Patients were scheduled for TKR of the target knee according to the National Institutes of Health consensus statement (14), at least 2 weeks after anticipated last injection of trial medication. Although most forms of secondary OA were excluded, patients with knee OA risk factors, obesity and post-meniscectomy status were enrolled. Patients with history of malignancy within the past 5 years were excluded except adequately treated basal and squamous cell carcinoma of the skin. For females, postmenopausal status or surgical sterilisation was required; men had to use contraception. All patients provided informed consent.

Endpoints

Primary endpoints were i) nature, incidence, and severity of treatment-emergent adverse events (TEAEs), defined as any AE starting on or after Day 1 of treatment until 30 days after last treatment, or AE related to treatment up to 30 days after study termination; ii) incidence of self-reported acute inflammatory reactions (AIRs); a similar definition based on a 30 mm increase visual analog scale (VAS) pain and observation of local swelling was used successfully in an OA trial involving *i.a.* injections of interleukin-1 receptor antagonist (IL-1ra; anakinra (15)); iii) local tolerability based on investigators' examination of the target knee at study visits and patients' diary cards; and iv) laboratory safety parameters (including blood chemistry, haematology, and urinalysis) and 12-lead ECG. Investigators classified each reported AE as local (concerning only the knee treated) or systemic.

Secondary endpoints (Suppl. Table I) were change over time in levels of markers of cartilage and bone formation and degradation, and changes in serum levels of cytokines related to inflammation. Blood levels of FGF18 and anti-FGF antibody formation were also assessed.

Tertiary endpoints (Suppl. Table II) included changes in knee cartilage thickness and volume (16) and in semi-quantitative structural cartilage and bone parameters (17), joint space width (JSW), immunohistochemistry (18), chondrocyte proliferation (19) and pain. At some sites, MRI measurements were a logistical obstacle, but as they were not the primary focus of this trial, missing MRI was considered a minor protocol violation. Exploratory endpoints (Suppl. Table II) were thickness and biomechanical properties (Young's modulus (20)) of cartilage samples from the anterior lateral femoral condyle.

Statistical analysis

In this first-in-human study evaluating the safety of sequentially increasing sprifermin doses and frequencies, there were small sample sizes in each corresponding cohort. No formal sample size calculation or statistical testing was conducted and results were presented using descriptive statistics. All patients receiving at least one sprifermin dose were included in the intention-to-treat (ITT) and safety populations. Immunohistological and biomechanical analyses of cartilage samples were performed for the TKR population, comprising all patients in the ITT population undergoing TKR during trial participation (on or before termination visit date).

Table I. Patient disposition and patient baseline characteristics.

	Sprifermin (n=55)	Placebo (n=18)	All (n=73)
Disposition of randomised patients (ITT population), n randomised (%)			
SAD cohorts	25 (45.5)	8 (44.4)	33 (45.2)
MAD cohorts	30 (54.5)	10 (55.6)	40 (54.8)
Major protocol violations	3 (5.5)	1 (5.6)	4 (5.5)
Treatment discontinued	0	0	0
Study withdrawal	1 (1.8)	2 (11.1)	3 (4.1)
TKR during the trial	40 (72.7)	16 (88.9)	56 (76.7)
MRI follow-up	22 (40.0)	8 (44.4)	30 (41.1)
X-ray follow-up	36 (65.5)	12 (66.7)	48 (65.8)
Baseline patient characteristics (ITT population)			
Age (years), mean (range)	65.4 (48.5–87.1)	70.1 (59.7–77.8)	66.6 (48.5–87.1)
Women, n (%)	30 (54.5)	9 (50.0)	39 (53.4)
BMI (kg/m^2), mean (range)	29.5 (21.1–46.7)	29.8 (22.4–46.8)	29.6 (21.1–46.8)
Kellgren-Lawrence, n (%)			
4	29 (52.7)	14 (77.8)	43 (58.9)
3	17 (30.9)	2 (11.1)	19 (26.0)
≤2	3 (5.5)*	0 (0)	3 (4.1)
No central reading available†	6 (10.9)	2 (11.1)	8 (11.0)

BMI: body mass index; ITT: intent-to-treat; MAD: multiple ascending dose; MRI: magnetic resonance imaging; SAD: single ascending dose; TKR: total knee replacement.
*Baseline grades of 2, 1, and 0 were each seen in one patient. However, note that the study did not exclude TKR candidates with severe femoro-patellar OA who did not have femorotibial OA; †Trial inclusion criteria did not specify a minimum Kellgren-Lawrence grade for the femorotibial joint; patients may, therefore, have had relatively well-preserved femorotibial joints despite advanced OA elsewhere in the knee that required joint replacement.

Results

Patient population

Of 85 patients screened, 73 were included in the ITT population. All cohorts were completed and all randomised patients completed treatment. Changes from baseline in quantitative MRI measures were available for 30 patients and post-treatment x-ray for 48 patients (Table I). TKR was performed a median of 10.2 weeks (range 4.1–30.4) from first treatment. Treated knee cartilage samples were obtained post-operatively from 56 patients in

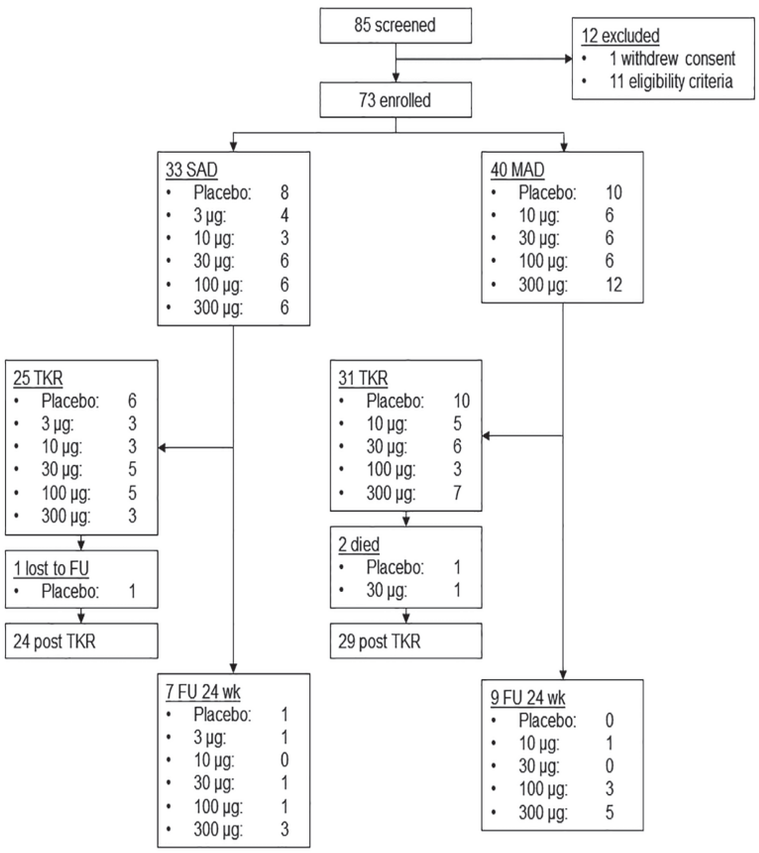

Fig. 1. Patient flow chart.
FU: follow-up; MAD: multiple ascending dose; SAD: single ascending dose; TKR: total knee replacement; wk: weeks.

the ITT population (76.7%). For ethical reasons, there was no pre-operative baseline sampling of cartilage, so only post-treatment data are available. One patient in the MAD 30 µg cohort (sprifermin) and one in the MAD 100 µg cohort (placebo) died during the trial and one patient in the SAD 300 µg cohort (placebo) was lost to follow-up. There were no other withdrawals or premature study discontinuations (Fig. 1). Major protocol deviations were observed in four patients in MAD cohorts: one patient (10 µg sprifermin) had a history of colon cancer, and three patients (10 µg, 100 µg sprifermin, and placebo) missed pain assessments or self-assessed target knee swelling.

Baseline characteristics were typical for an advanced OA patient population. There were 25% more patients in the placebo group *versus* sprifermin with Kellgren-Lawrence (K-L) grade 4 (Table I). Baseline K-L grades in the femorotibial joint of ≤2 were seen in three patients (5.5%; inclusion criteria did not specify minimum K-L grade for femorotibial joint). Accordingly, few patients may have had relatively well-preserved femorotibial joints but advanced painful OA in the femoro-patellar joint requiring joint replacement (Table I).

Safety

The overall proportion of patients experiencing at least one TEAE was not increased in the sprifermin group versus placebo, at both single and multiple doses (Table II). The most common TEAEs, coded using Medical Dictionary for Regulatory Activities (MedDRA) version 13.0 and tabulated by System Organ Class (SOC) and Preferred Term (PT), in SAD and MAD were musculoskeletal/connective tissue disorders (arthralgia and joint stiffness) (9 (36.0%) *vs*. 2 (25.0%) in SAD, and 10 (33.3%) *vs*. 3 (30.0%) patients in MAD in sprifermin and placebo groups, respectively), and nervous system disorders (headache (2 (8.0%) *vs*. 3 (37.5%) in SAD, and 8 (26.7%) *vs*. 4 (40.0%) patients in MAD in sprifermin and placebo groups, respectively). In the SAD cohorts, events graded as moderate in severity were more frequent in patients treated with sprifermin (26.5%) than placebo (16.7%), while the frequency of events graded as severe was similar between the placebo (11.1%) and combined sprifermin group (11.8%). In the MAD cohorts, events graded as moderate were slightly more frequent in the placebo (44.0%) than the combined sprifermin group (40.6%), while events graded as severe were reported only in the sprifermin group (4.3%).Three out of 18 (16.7%) *vs*. 9 out of 34 (26.5%) TEAEs in SAD, and 2 out of 25 (8%) *vs*. 8 out of 69 (11.6%) TEAEs in MAD cohorts were classified as local in placebo and sprifermin groups, respectively. Fifteen out of 18 (83.3%) *vs*. 25 out of 34 (73.5%) TEAEs in SAD, and 23 out of 25 (92%) *vs*. 61 out of 69 (88.4%) TEAEs in MAD cohorts were classified as systemic in placebo and sprifermin groups, respectively (Table II).

One out of 18 (5.5%) *vs*. 7 out of 55 (12.7%) patients experienced a total of one *vs*. eight AIRs in the placebo and sprifermin groups, respectively, of which four occurred in the 300 µg cohort (one SAD and three MAD; one MAD patient experienced two reactions) (Table III). No patient withdrew from treatment because of an AIR. No clear difference was seen between placebo and sprifermin in investigator-assessed local tolerability parameters (stiffness, itching, pain, swelling, redness, or other symptoms) or in self-reporting of pain or swelling in the target knee (Suppl. Table III). No meaningful changes over time or differences between treatment groups were observed for safety laboratory parameters and ECG recordings.

Two deaths unrelated to drug treatment were reported: a 65-year-old man, who received multiple 30 µg doses of sprifermin, died from pulmonary embolism 2 days after TKR surgery and 23 days after last dose of sprifermin. A 76-year-old man with history of ischaemic heart disease, who received multiple placebo injections, died from myocardial infarction 91 days after last treatment (event not qualified as TEAE).

Table II. Incidence and severity of all, local and systemic TEAEs by treatment group (safety population).

		All TEAEs		Local TEAEs		Systemic TEAEs	
		SAD	MAD	SAD	MAD	SAD	MAD
Placebo	Patients with event, n (%)	6 (75.0%)	9 (90.0%)	3 (37.5%)	1 (10.0%)	5 (62.5%)	9 (90.0%)
SAD=8 MAD=10	Events, n (mild/moderate/severe)	18 (13/3/2)	25 (14/11/0)	3 (2/0/1)	2 (0/2/0)	15 (11/3/1)	23 (14/9/0)
Sprifermin, total	Patients with event, n (%)	14 (56.0%)	23 (76.7%)	6 (24.0%)	8 (26.7%)	13 (52.0%)	21 (70.0%)
SAD=25 MAD=30	Events, n (mild/moderate/severe)	34 (21/9/4)	69 (38/28/3)	9 (2/4/3)	8 (3/3/2)	25 (19/5/1)	61 (35/25/1)
3 µg	Patients with event, n (%)	3 (75.0%)	-	1 (25.0%)	-	2 (50.0%)	-
SAD=4*	Events, n (mild/moderate/severe)	9 (7/2/0)	-	1 (1/0/0)	-	8 (6/2/0)	-
10 µg	Patients with event, n (%)	2 (66.7%)	5 (83.3%)	1 (33.3%)	1 (16.7%)	2 (66.7%)	5 (83.3%)
SAD=3 MAD=6	Events, n (mild/moderate/severe)	3 (2/0/1)	19 (16/3/0)	1 (0/0/1)	1 (0/1/0)	2 (2/0/0)	18 (16/2/0)
30 µg	Patients with event, n (%)	4 (66.7%)	6 (100%)	3 (50.0%)	2 (33.3%)	4 (66.7%)	5 (83.3%)
SAD=6 MAD=6	Events, n (mild/moderate/severe)	14 (8/5/1)	16 (12/3/1)	4 (1/2/1)	2 (2/0/0)	10 (7/3/0)	14 (10/3/1)
100 µg	Patients with event, n (%)	3 (50.0%)	4 (66.7%)	0	2 (33.3%)	3 (50.0%)	4 (66.7%)
SAD=6 MAD=6	Events, n (mild/moderate/severe)	3 (3/0/0)	15 (2/12/1)	0	2 (0/1/1)	3 (3/0/0)	13 (2/11/0)
300 µg	Patients with event, n (%)	2 (33.3%)	8 (66.7%)	1 (16.7%)	3 (25.0%)	2 (33.3%)	7 (58.3%)
SAD=6 MAD=12	Events, n (mild/moderate/severe)	5 (1/2/2)	19 (8/10/1)	3 (0/2/1)	3 (1/1/1)	2 (1/0/1)	16 (7/9/0)

MAD: multiple ascending dose; SAD: single ascending dose; TEAEs: treatment emergent adverse events.
*Protocol amendment 2 limited the numbers of patients to be enrolled into the SAD 3 mcg and 10 mcg cohorts to 4; however, 5 patients had already been enrolled into the SAD 3 mcg cohort when the amendment took effect.

Table III. Incidence of AIR by treatment group (safety population).

		AIRs	
		SAD	MAD
Placebo	Patients with event, n (%)	1 (12.5%)	0
SAD=8 MAD=10	Events, n	1	0
Sprifermin,	Patients with event, n (%)	2 (8.0%)	5 (16.7%)
total SAD=25 MAD=30	Events, n	2	6
3 µg	Patients with event, n (%)	0	-
SAD=4	Events, n	0	-
10 µg	Patients with event, n (%)	0	0
SAD=3 MAD=6	Events, n	0	0
30 µg	Patients with event, n (%)	1 (16.7%)	0
SAD=6 MAD=6	Events, n	1	0
100 µg	Patients with event, n (%)	0	2 (33.3%)
SAD=6 MAD=6	Events, n	0	2
300 µg	Patients with event, n (%)	1 (16.7%)	3 (25.0%)
SAD=6 MAD=12	Events, n	1	4

MAD: multiple ascending dose; SAD: single ascending dose; AIRs: acute inflammatory reactions.

Eight other serious adverse events (SAEs) were reported. Three SAEs (gastrointestinal haemorrhage, gouty arthritis, and post-procedural infection) were classified as TEAEs, all of which affected placebo patients. Five remaining SAEs (second-degree atrioventricular block (pre-treatment), joint contracture (Day 112), angioedema (Day 131), decreased mobility (Day 122), and spinal OA (Day 74)) did not satisfy the definition of TEAE; they concerned one placebo and four sprifermin patients, who received a single 10 µg dose or multiple doses of 30, 100, and 300 µg, respectively. No pattern among these SAEs was recognised and none was considered related to treatment by the investigator. No AE led to withdrawal or study discontinuation.

• *Systemic exposure*

Antibodies against FGF18 or FGF18 were not detected in serum. Systemic biomarkers of cartilage and bone metabolism or inflammation did not indicate a systemic effect of sprifermin (data not shown). Changes over time showed no relationship to treatment or dose over 3 consecutive weeks until 24 weeks after first dose.

Cartilage parameters

• *Histology*

In the SAD and MAD cohorts, Mankin scores were similar in sprifermin and placebo groups (except for 3 µg in the SAD) (Table IV). Although this study was not designed to test for Mankin score differences between sprifermin and placebo, in the SAD and MAD groups, the average Mankin scores appeared lowest in patients receiving sprifermin 100 µg (Fig. 2).

• *Biomechanical properties of cartilage*

Young's modulus values appeared highest on sprifermin 100 µg and 300 µg in the SAD and 30 µg and 100 µg in the MAD cohorts (Table IV).

• *Immunohistochemistry*

The histological analyses raised no safety concerns. (Fig. 2; Table IV). Scores appeared to be lower with sprifermin than placebo in the SAD group,

Table IV. Results of the histological examination of cartilage samples taken during TKR (TKR population).

		Modified Mankin score*		Young›s modulus (MPa)		% positive PCNA staining†	
	n	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)
SAD							
Placebo	6	2.3 ± 1.4	2.3 (1.6–3.0)	0.8 ± 0.4	1.0 (0.3–1.2)	52.4 ± 10.5	50.0 (45.8–56.3)
3 µg	3	2.6 ± 0.5	2.9 (2.0–2.9)	0.4 ± 0.3	0.4 (0.2–0.7)	44.0 ± 6.3	44.4 (41.0–47.2)
10 µg	3	2.4 ± 0.8	2.3 (1.6–3.2)	0.7 ± 0.4	0.8 (0.2–0.9)	45.8 ± 8.3	45.8 (41.7–50.0)
30 µg	5	1.9 ± 0.9	2.0 (1.9–2.2)	0.8 ± 0.2	0.7 (0.6–0.8)	48.6 ± 24.2	54.2 (37.5–66.7)
100 µg	3	1.7 ± 0.8	1.3 (1.1–2.6)	1.5 ± 0.5	1.4 (1.1–2.1)	52.5 ± 15.2	45.8 (45.8–50.0)
300 µg	3	1.8 ± 1.0	2.1 (0.7–2.6)	1.0 ± 0.3	1.1 (0.8–1.2)	39.6 ± 30.5	35.4 (20.8–54.2)
MAD							
Placebo	7	3.0 ± 1.7	2.6 (2.0–4.1)	0.7 ± 0.5	0.6 (0.5–0.8)	51.8 ± 14.4	54.2 (50.0–57.6)
10 µg	4	2.6 ± 2.2	2.5 (1.1–4.2)	0.7 ± 0.4	0.6 (0.3–1.1)	49.2 ± 16.5	45.8 (37.5–54.2)
30 µg	5	2.0 ± 0.7	2.0 (2.0–2.0)	1.3 ± 0.3	1.5 (1.3–1.5)	59.7 ± 17.8	58.3 (43.8–72.9)
100 µg	3	1.4 ± 1.0	1.8 (0.3–2.1)	1.2 ± 0.4	1.2 (0.8–1.6)	73.6 ± 10.5	75.0 (68.8–79.2)
300 µg	7	2.2 ± 0.5	2.2 (1.8–2.7)	0.7 ± 0.1	0.7 (0.7–0.8)	63.2 ± 14.5	66.7 (54.2–72.9)

IQR: interquartile range; MAD: multiple ascending dose; MPa: megapascals; PCNA: proliferating cell nuclear antigen (a marker specific for the S-phase of the cell cycle); SAD: single ascending dose; SD: standard deviation; TKR: total knee replacement.
*As samples without subchondral bone were analysed, only the cartilage integrity was evaluated. Therefore the Modified Mankin score covers range between 0 and 13; †PCNA-stained sections of cartilage were obtained during TKR and scored by three experienced operators.

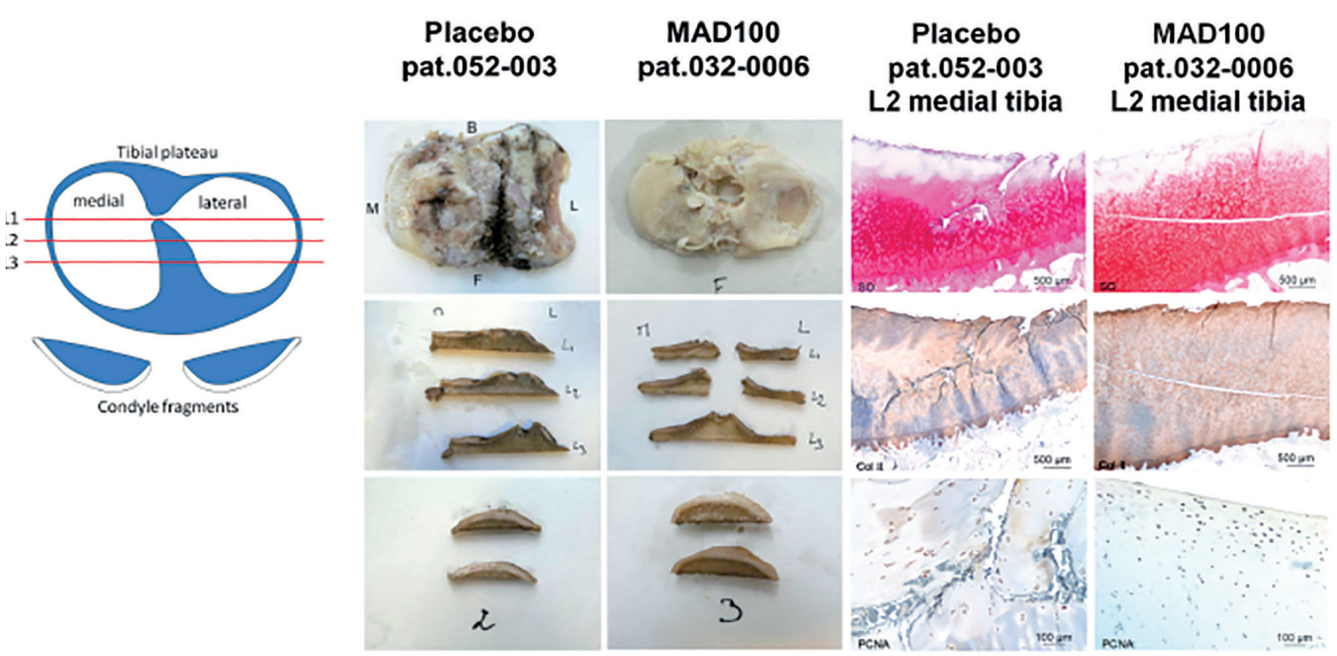

Fig. 2. Histology and immunohistochemistry of weight bearing joint areas. MAD: multiple ascending dose; TKR: total knee replacement.
A) Schematic representation of the location of the cuttings for histology obtained from TKR: L1 before lesion; L2 through the lesion; L3 behind the lesion; condyle fragments. L, level. **B**) Macroscopic examples of the obtained material. **C**) Histological examples (evaluated also by Mankin score, see Table IV).

but higher with sprifermin than placebo in the MAD group.

Clinical

• *Imaging*

Changes from baseline in quantitative MRI measures were available for 30 patients overall. Semi-quantitative MRI assessment revealed no meaningful changes over time or differences between treatment groups in WORMS parameters (bone marrow oedema, synovitis, and effusion). Some observations in quantitative MRI variables, particularly in the medial femorotibial compartment, might be compatible with anabolic effect on joint cartilage; however, group numbers were small and the relatively short follow-up duration varied among patients precluding comparative analysis. On x-rays, no consistent differences in JSW over time up to Week 24 or between treatment groups were observed.

• *Symptoms*

No effect on clinical symptoms was found using the KOOS questionnaire, although the trial was not designed to show such effects.

Discussion

Pharmacological interventions to modify the course of OA have raised considerable interest over recent years. However, DMOAD development continues to face challenges (1, 21, 22).

Safety of OA drugs is critical, due to the high prevalence of OA and its chronicity, plus the high prevalence of other disease comorbidities. For sprifermin, *i.a.* treatment stimulated repair of cartilage damage in animal models of OA (9, 21, 23), with no measurable systemic effects (23).

We describe the first clinical trial of *i.a.* sprifermin in patients with advanced knee OA. The study population enabled examination of human knee cartilage after sprifermin exposure *in vivo*. Ascending dose design further enabled (a) evaluation of local and systemic safety over a broad dose range, and (b) first evaluation of treatment effect on cartilage. The optimally efficacious and safe dose of sprifermin remains to be determined in larger clinical trials of longer duration. A 1-year proof-of-concept (PoC) trial examining multiple dose regimens up to 100 μg in 192 patients with less severe OA has recently been completed (24).

The safety profile during the present trial over the entire dose range of single and repeated doses was acceptable. Incidence, severity, and nature of reported TEAEs raised no local or systemic safety concerns for doses up to 300 μg. The nature of TEAEs was similar between active and placebo treatment and as expected for a population of patients with advanced OA undergoing TKR surgery. The presence/absence of osteophytes following sprifermin exposure was not examined in this study. However, in the PoC trial, no meaningful changes in osteophytes on WORMS analyses were observed after sprifermin exposure (24).

A trend towards increased incidence of AIRs was observed with sprifermin, which may be linked to mechanism of action; however, further data from future studies with more patients are required to clarify this effect. AIRs were identified based on patient-reported measures rather than physician-assessed local symptoms. At the 300 μg dose, AIRs were observed in one SAD and three MAD patients, with one MAD patient experiencing two reactions. AIRs occurred within the first 24 hours after injection, and none led to discontinuation or special treatment. The PoC study confirmed similar incidences of SAEs, TEAEs and AIRs between sprifermin dosed up to 100 μg and placebo (24). In line with animal data (23, 25), neither study revealed any detectable systemic exposure or effect elicited by *i.a.* doses.

No significant changes of MRI or JSW could be expected in this small study of relatively short duration. In a larger study of one year in duration, anabolic response of less damaged OA cartilage to sprifermin was demonstrated through significant dose-dependent reductions in loss of total and lateral femorotibial cartilage thickness and volume on MRI, and in JSW narrowing in the lateral femorotibial compartment on x-ray (24).

This trial did not show effect on clinical symptoms, however it was not designed or powered to demonstrate this. For ethical reasons it was not feasible to sample cartilage biopsies at inclusion of our study. Still, it is encouraging that a considerable number of observations from independent immunohistochemical and biomechanical analyses of tissue samples obtained at TKR showed sprifermin-treated tissues were no worse than tissues from placebo-treated patients. On the contrary, patients who received sprifermin showed a tendency towards lower average Mankin scores, with higher values for Young's modulus, in higher multiple dose groups *versus* lower doses and placebo. Additional investigation will be needed to demonstrate significant beneficial effect of sprifermin on immunohistological and biomechanical properties of human knee cartilage.

In summary, the results of this first-in-human study did not raise safety concerns following single or multiple *i.a.* administration of sprifermin at doses up to 300 μg. However, further clinical trials with longer observation periods and larger sample sizes will be required to more fully establish safety and optimal dose regimen of *i.a.* sprifermin in the treatment of OA. Verification of the possible anabolic effect of sprifermin on joint cartilage will also require further evaluation in follow-up studies optimally designed and powered to show whether such effects would lead to durable structural changes in the joint detectable by MRI or x-ray. The results presented here are nonetheless encouraging and support further clinical development of *i.a.* sprifermin for the treatment of OA.

Conclusion

Although further clinical trials with longer observation periods and larger sample sizes are needed to more fully establish its safety and efficacy, in this trial sprifermin did not result in any measurable systemic effects and revealed no serious safety concerns.

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