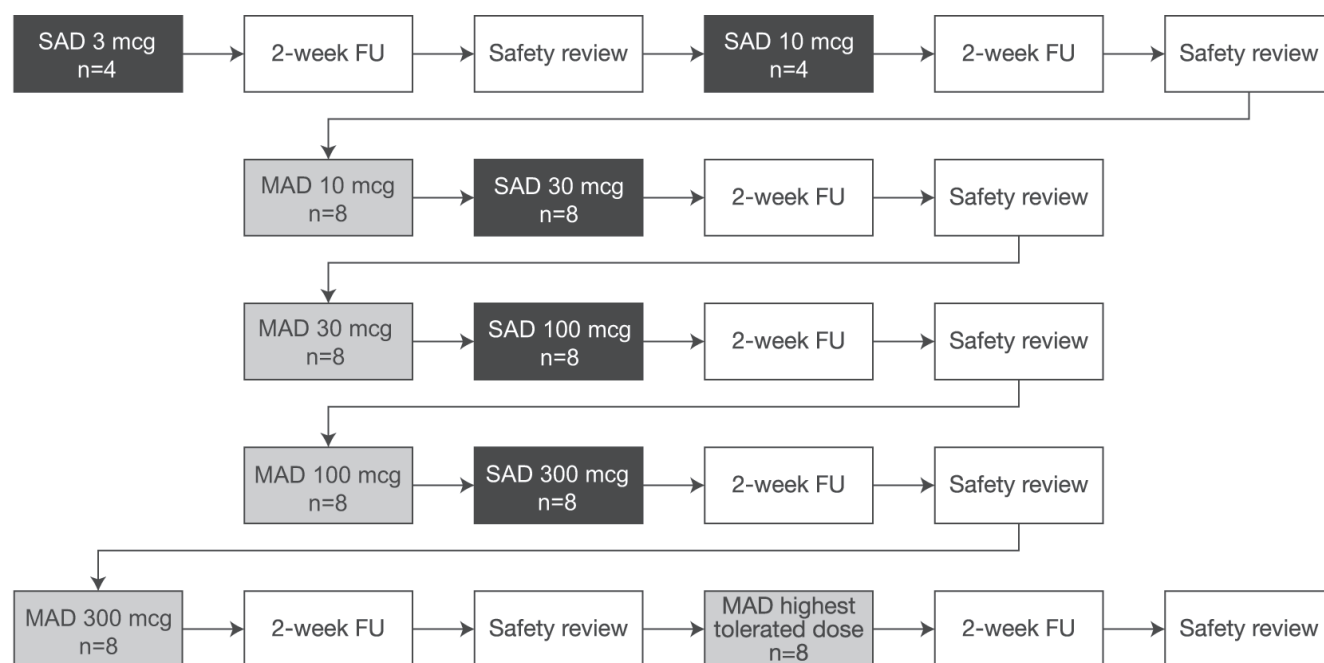


Supplementary material



Supplementary Fig. 1. Dose escalation scheme.

Supplementary Table I. Secondary endpoints.

Marker or endpoint	Abbreviation	Comments / assay details
Markers of cartilage formation		
Serum aggrecan chondroitin sulfate epitope 846	CS846	Synarc, Lyon, France
Serum IIA pro-peptide forms from N-terminus processed type II collagen	PIIANP	
Serum pro-peptides from C-terminus processed type II collagen	PIICP	
Markers of cartilage degradation		
Urine C-telopeptide cross-linking of type II collagen	CTX-II	Synarc, Lyon, France
Serum cartilage oligomeric matrix protein	COMP	
Markers of bone formation		
Serum bone-specific alkaline phosphatase	ALP	Synarc, Lyon, France
Markers of bone degradation		
Serum C-telopeptide cross-linking of type I collagen	CTX-I	Synarc, Lyon, France
Serum cytokines related to inflammation		
IL1 β ; IL6; IL8; TNF- α ; IFN γ	-	Synarc, Lyon, France
Other		
Blood FGF18	-	ELISA with LLQ 100 pg/ml; Merck Serono
Anti-FGF18 antibodies	-	ELISA; Merck Serono

ELISA: enzyme-linked immunosorbent assay; FGF18: human fibroblast growth factor 18; IFN: interferon; IL: interleukin; LLQ: lower limit of quantification; TNF: tumour necrosis factor.

Supplementary Table II. Tertiary and exploratory endpoints.

Marker or endpoint	Assessment method	Comments / details*
Tertiary		
Change in cartilage thickness and volume in target knee	Quantitative MRI (16)	Chondrometrics, Aining, Germany
Semi-quantitative structural parameters of cartilage and bone in target knee	MRI + WORMS (17)	Boston Imaging Core Lab, Boston, MA, USA
Change in joint space width	X-ray	Boston Imaging Core Lab, Boston, MA, USA
Joint cartilage immunohistochemistry after TKR	Mankin (18) scoring of safranin-O-stained histologic sections*	Structure (normal to complete disorganisation, 0–6 points) Cells (normal to hypocellularity, 0–3 points) Safranin-O staining (normal to no dye noted, 0–4 points) n.b. Cartilage scores only; no subchondral bone in samples used Novotec SARL, and Novotec, Lyon, France
Chondrocyte proliferation	PCNA†	Novotec SARL and Novotec, Lyon France
Knee symptoms and function	KOOS (19)	–
Pain in target joint	100 mm VAS	–
Exploratory		
Equilibrium Young’s modulus of full-thickness cylindrical (4 mm diameter) cartilage samples from the anterior lateral femoral condyle	Stress-relaxation testing in unconfined compression geometry (20)	Tissue taken at TKR Higher Young’s modulus values indicate higher compressive stiffness

KOOS: Knee Injury and Osteoarthritis Outcome Score; MRI: magnetic resonance imaging; PCNA: proliferating cell nuclear antigen staining; TKR: total knee replacement; VAS: visual analogue scale; WORMS: Whole-Organ Magnetic Resonance Imaging Score.

*Data acquisition for MRI and x-ray set up by VirtualScopics, Rochester, NY, USA; immunohistochemical analysis was carried out by Jukka S. Jurvelin, Kuopio, Finland, using a modified version of the Mankin score; †PCNA-stained sections of cartilage were obtained during TKR and scored by three experienced operators.

Supplementary Table III. Investigator examination: incidence and severity of stiffness, itching, and pain by treatment group (safety population).

	Stiffness		Itching		Pain	
	SAD	MAD	SAD	MAD	SAD	MAD
Placebo	(62.5/0/37.5/0)*	(33.3/44.4/22.2/0)*	(87.5/0/12.5/0)*	(100.0/0/0/0)*	(50.0/12.5/25.0/12.5)*	(11.1/55.6/33.3/0)*
SAD=8 MAD=10	(62.5/0/37.5/0)†	(22.2/55.6/22.2/0)†	(87.5/0/12.5/0)†	(100.0/0/0/0)†	(50.0/25.0/12.5/12.5)†	(22.2/44.4/33.3/0)†
	(71.4/28.6/0/0)‡	(33.3/50.0/16.7/0)‡	(100.0/0/0/0)‡	(100.0/0/0/0)‡	(42.9/42.9/14.3/0)‡	(66.7/33.3/0/0)‡
Sprifermin, total	(32.0/28.0/32.0/8.0)*	(53.6/17.9/14.3/14.3)*	(96.0/4.0/0/0)*	(85.7/14.3/0/0)*	(28.0/28.0/40.0/4.0)*	(32.1/17.9/39.3/10.7)*
SAD=25 MAD=30	(32.0/32.0/32.0/4.0)†	(53.6/17.9/17.9/10.7)†	(100.0/0/0/0)†	(89.3/10.7/0/0)†	(28.0/44.0/24.0/4.0)†	(35.7/25.0/28.6/10.7)†
3 µg	(41.7/33.3/16.7/8.3)‡	(36.4/59.1/4.5/0)‡	(95.8/4.2/0/0)‡	(95.5/4.5/0/0)‡	(58.3/25.0/12.5/4.2)‡	(50.0/36.4/9.1/4.5)‡
SAD=4	(50.0/25.0/25.0/0)*	-	(100.0/0/0/0)*	-	(25.0/50.0/25.0/0)*	-
	(50.0/25.0/25.0/0)†	-	(100.0/0/0/0)†	-	(25.0/50.0/25.0/0)†	-
	(50.0/25.0/25.0/0)‡	-	(100.0/0/0/0)‡	-	(50.0/50.0/0/0)‡	-
10 µg	(66.7/0/0/33.3)*	(50.0/33.3/0/16.7)*	(100.0/0/0/0)*	(83.3/16.7/0/0)*	(33.3/0/66.7/0)*	(33.3/16.7/33.3/16.7)*
SAD=3 MAD=6	(66.7/0/0/33.3)†	(66.7/16.7/0/16.7)†	(100.0/0/0/0)†	(100.0/0/0/0)†	(33.3/33.3/33.3/0)†	(33.3/16.7/33.3/16.7)†
	(33.3/66.7/0/0)‡	(25.0/75.0/0/0)‡	(100.0/0/0/0)‡	(100.0/0/0/0)‡	(66.7/33.3/0/0)‡	(75.0/0/25.0/0)‡
30 µg	(16.7/50.0/16.7/16.7)*	(66.7/0/33.3/0)*	(83.3/16.7/0/0)*	(66.7/33.3/0/0)*	(16.7/50.0/16.7/16.7)*	(50.0/16.7/33.3/0)*
SAD=6 MAD=6	(16.7/50.0/33.3/0)†	(66.7/0/33.3/0)†	(100.0/0/0/0)†	(66.7/33.3/0/0)†	(33.3/50.0/0/16.7)†	(50.0/33.3/16.7/0)†
	(40.0/60.0/0/0)‡	(100.0/0/0/0)‡	(80.0/20.0/0/0)‡	(100.0/0/0/0)‡	(60.0/20.0/20.0/0)‡	(75.0/25.0/0/0)‡
100 µg	(33.3/33.3/33.3/0)*	(50.0/33.3/16.7/0)*	(100.0/0/0/0)*	(100.0/0/0/0)*	(33.3/33.3/33.3/0)*	(16.7/50.0/33.3/0)*
SAD=6 MAD=6	(33.3/33.3/33.3/0)†	(33.3/50.0/16.7/0)†	(100.0/0/0/0)†	(100.0/0/0/0)†	(16.7/50.0/33.3/0)†	(33.3/33.3/33.3/0)†
	(66.7/33.3/0/0)‡	(50.0/50.0/0/0)‡	(100.0/0/0/0)‡	(100.0/0/0/0)‡	(100.0/0/0/0)‡	(25.0/25.0/25.0/25.0)‡
300 µg	(16.7/16.7/66.7/0)*	(50.0/10.0/10.0/30.0)*	(100.0/0/0/0)*	(90.0/10.0/0/0)*	(33.3/0/66.7/0)*	(30.0/0/50.0/20.0)*
SAD=6 MAD=12	(16.7/33.3/50.0/0)†	(50.0/10.0/20.0/20.0)†	(100.0/0/0/0)†	(90.0/10.0/0/0)†	(33.3/33.3/33.3/0)†	(30.0/20.0/30.0/20.0)†
	(16.7/0/50.0/33.3)‡	(10.0/80.0/10.0/0)‡	(100.0/0/0/0)‡	(90.0/10.0/0/0)‡	(16.7/33.3/33.3/16.7)‡	(40.0/60.0/0/0)‡

MAD: multiple ascending dose; SAD: single ascending dose; *Grade: % (none/mild/moderate/severe) Week 0: pre-dose; †Grade, % (none/mild/moderate/severe) Week 0: 4h post-injection; ‡Grade, % (none/mild/moderate/severe) Week 24/Termination.