Clinical trial results:
Prospective, randomised, open label, multicentre Phase-III clinical trial to compare the efficacy and safety of the treatment with the autologous chondrocyte transplantation product co.don chondrosphere® (ACT3D-CS) with microfracture in subjects with cartilage defects of the knee with a defect size between 1 and 4 cm2

Summary

EudraCT number	2009-016466-82
Trial protocol	DE PL
Global end of trial date	09 September 2020

Results information

Result version number	v1 (current)
This version publication date	11 March 2021
First version publication date	11 March 2021

Trial information

Trial identification

Sponsor protocol code	cod16HS13

Additional study identifiers

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	NCT01222559
WHO universal trial number (UTN)	-

Notes:

Sponsors

Sponsor organisation name	CO.DON AG
Sponsor organisation address	Warthestraße 21, Teltow, Germany, 14513
Public contact	CO.DON AG, CO.DON AG, +49 341 99190 200, klifo@codon.de
Scientific contact	CO.DON AG, CO.DON AG, +49 341 99190 200, klifo@codon.de

Notes:

Paediatric regulatory details

Is trial part of an agreed paediatric investigation plan (PIP)	No
Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial?	No
Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial?	No

Notes:

Results analysis stage

Analysis stage	Final
Date of interim/final analysis	28 February 2020
Is this the analysis of the primary completion data?	Yes
Primary completion date	28 February 2020
Global end of trial reached?	Yes
Global end of trial date	09 September 2020
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

General Objectives:
- Assessment of the long-term efficacy and safety of the three-dimensional autologous chondrocyte transplantation product ACT3D-CS in comparison to microfracture (MF) for the treatment of cartilage defects of knee joints.

Primary Objectives:
- Change of overall KOOS (Knee Injury and Osteoarthritis Outcome Score) from Day 0 (baseline for both treatment groups=day before arthroscopy) to final assessment at 24 months after the end of the respective treatment, compared between ACT3D-CS and MF.

Secondary objectives:
- Assessment of several efficacy variables for all follow-up time points until 60 months: Change of KOOS and Change of KOOS subscores, MOCART MRI score, Change of modified Lysholm Score, Histological scores for cartilage repair 24 months after the respective treatment (ICRS II Histological Score, Bern Score, International Cartilage Regeneration & Joint Preservation Society (ICRS) Visual Histological Assessment Score)
- Safety variables

Protection of trial subjects:

Compliance: As the application of the test product was performed once only, by the investigator, compliance issues were not anticipated. Compliance with the study treatment was good; only two patients received below-range doses of chondrosphere because of inadequate cell growth. Other protocol deviations were mostly the use of pain medication on days when this was not allowed, incomplete questionnaires or missing visits. All patients took part in, and were compliant with, the post-operative rehabilitation programme.
Rescue medication: In case of pain, patients agreed only to use paracetamol in a mono- (maximum 4 g/day) or combination preparation and oral and/or topical NSAIDs during the trial, and to discontinue the use of these one week before each visit (whereby the use of paracetamol mono-preparation, maximum 4 g/day was allowed). However, in the morning of the visit day, no pain medication at all was allowed. Other pain medications were allowed during the surgical procedure and could be taken for a period not exceeding 4 weeks after surgery.
Precautionary and prohibited medications and procedures: For Group A (ACT3D-CS) the following rule was instituted: If propofol was used for anaesthesia on the day of the first arthroscopy, the taking of patient's own blood for use in cultivation of the chondrocytes in the patient's serum was to take place at least 4 hours after the end of propofol application. This was done because serum containing propofol is not suitable for the cultivation of chondrocytes.
Patient follow-up after treatment: Follow-up visits were performed at close intervals to monitor efficacy and safety after treatment.
Monitoring/auditing: Regular on-site monitoring was performed (as described in detail in the Monitoring Plan of this study) as well as quality-assurance audits by CO.DON AG or its designees. The clinical monitor and auditor reviewed all eCRFs and written Informed Consent forms.

Background therapy: -

Evidence for comparator:

Comparator used: Microfracture is a marrow-stimulating method based on the penetration of the subchondral bone plate at the bottom of the cartilage defect. Different instruments such as the bent awls used in microfracturing create persisting holes in the bone plate. The outflowing bone-marrow blood contains the pluripotent stem cells (hMSC) which are able to differentiate mainly into fibrochondrocytes, resulting in fibrocartilage repair.
Microfracture has been one of the comparators favoured by the Committee for Medicinal Products for

Human Use (CHMP) during the Scientific Advice Procedure. Despite its restriction to small defects in clinical practice, microfracture is selected as the most reasonable comparator to date.

Actual start date of recruitment	01 October 2010
Long term follow-up planned	No
Independent data monitoring committee (IDMC) involvement?	No

Notes:

Population of trial subjects

Subjects enrolled per country

Country: Number of subjects enrolled	Poland: 12
Country: Number of subjects enrolled	Germany: 90
Worldwide total number of subjects	102
EEA total number of subjects	102

Notes:

Subjects enrolled per age group

In utero	0
Preterm newborn - gestational age < 37 wk	0
Newborns (0-27 days)	0
Infants and toddlers (28 days-23 months)	0
Children (2-11 years)	0
Adolescents (12-17 years)	0
Adults (18-64 years)	102
From 65 to 84 years	0
85 years and over	0

Subject disposition

Recruitment

Recruitment details:

Patients were recruited at 11 sites from October 2010 to December 2014 (9 German sites, 3 Polish sites).

Pre-assignment

Screening details:

All patients with cartilage defects consulting the investigator during the recruitment phase of this clinical trial were informed of the trial. Patients who were interested in study participation, and had carefully read the Patient Information and Consent Form and had signed and dated it, were screened for eligibility (163 patients screened).

Period 1

Period 1 title	Day 0 (before first arthroscopy)
Is this the baseline period?	Yes
Allocation method	Randomised - controlled
Blinding used	Not blinded

Arms

Are arms mutually exclusive?	Yes
Arm title	ACT3D-CS

Arm description:

Autologous chondrocyte implantation (ACI) is based on the arthroscopic harvesting of the patient's own chondrocytes isolated from healthy cartilage, their culture in vitro to develop 3-dimensional spheroids (ACT3D-CS), and the subsequent implantation of these into the cartilage defect, resulting in hyaline cartilage repair.

Arm type	Experimental
Investigational medicinal product name	Spherox 10-70 spheroids/cm2 implantation suspension
Investigational medicinal product code	M09AX02 (ACT code)
Other name	The product was originally named co.don chondrosphere® in the study protocol and the relevant study documents.
Pharmaceutical forms	Solution and suspension for suspension for injection in pre-filled syringe
Routes of administration	Intraarticular use

Dosage and administration details:

Harvesting of a cartilage biopsy under arthroscopy and arthroscopic administration of the product by implantation at a dose level of 10–70 spheroids/cm2.

Arm title	Microfracture

Arm description:

Microfracture is a marrow-stimulating method based on the penetration of the subchondral bone plate at the bottom of the cartilage defect. Different instruments such as the bent awls used in microfracturing create persisting holes in the bone plate. The outflowing bone-marrow blood contains the pluripotent stem cells (hMSC) which are able to differentiate mainly into fibrochondrocytes, resulting in fibrocartilage repair.

Arm type	comparator surgical treatment

No investigational medicinal product assigned in this arm

Number of subjects in period 1	ACT3D-CS	Microfracture
Started	52	50
Completed	52	50

Period 2	
Period 2 title	main study period (0-60 months)
Is this the baseline period?	No
Allocation method	Randomised - controlled
Blinding used	Not blinded

Arms	
Are arms mutually exclusive?	Yes
Arm title	ACT3D-CS

Arm description:

Autologous chondrocyte implantation (ACI) is based on the arthroscopic harvesting of the patient's own chondrocytes isolated from healthy cartilage, their culture in vitro to develop 3-dimensional spheroids (ACT3D-CS), and the subsequent implantation of these into the cartilage defect, resulting in hyaline cartilage repair.

Arm type	Experimental
Investigational medicinal product name	Spherox 10-70 spheroids/cm2 implantation suspension
Investigational medicinal product code	M09AX02 (ACT code)
Other name	The product was originally named co.don chondrosphere® in the study protocol and the relevant study documents.
Pharmaceutical forms	Solution and suspension for suspension for injection in pre-filled syringe
Routes of administration	Intraarticular use

Dosage and administration details:

Harvesting of a cartilage biopsy under arthroscopy and arthroscopic administration of the product by implantation at a dose level of 10–70 spheroids/cm2.

Arm title	Microfracture

Arm description:

Microfracture is a marrow-stimulating method based on the penetration of the subchondral bone plate at the bottom of the cartilage defect. Different instruments such as the bent awls used in microfracturing create persisting holes in the bone plate. The outflowing bone-marrow blood contains the pluripotent stem cells (hMSC) which are able to differentiate mainly into fibrochondrocytes, resulting in fibrocartilage repair.

Arm type	comparator surgical treatment

No investigational medicinal product assigned in this arm

Number of subjects in period 2	ACT3D-CS	Microfracture
Started	52	50
Completed	23	27
Not completed	29	23
Microfracture not performed	-	1
No baseline value	3	-
Inadequate spheroid growth	1	-
Protocol deviation	25	22

Baseline characteristics

Reporting groups	
Reporting group title	ACT3D-CS
Reporting group description:	
Autologous chondrocyte implantation (ACI) is based on the arthroscopic harvesting of the patient's own chondrocytes isolated from healthy cartilage, their culture in vitro to develop 3-dimensional spheroids (ACT3D-CS), and the subsequent implantation of these into the cartilage defect, resulting in hyaline cartilage repair.	
Reporting group title	Microfracture
Reporting group description:	
Microfracture is a marrow-stimulating method based on the penetration of the subchondral bone plate at the bottom of the cartilage defect. Different instruments such as the bent awls used in microfracturing create persisting holes in the bone plate. The outflowing bone-marrow blood contains the pluripotent stem cells (hMSC) which are able to differentiate mainly into fibrochondrocytes, resulting in fibrocartilage repair.	

Reporting group values	ACT3D-CS	Microfracture	Total
Number of subjects	52	50	102
Age categorical Units: Subjects			
In utero	0	0	0
Preterm newborn infants (gestational age < 37 wks)	0	0	0
Newborns (0-27 days)	0	0	0
Infants and toddlers (28 days-23 months)	0	0	0
Children (2-11 years)	0	0	0
Adolescents (12-17 years)	0	0	0
Adults (18-64 years)	0	0	0
From 65-84 years	0	0	0
85 years and over	0	0	0
18–34 years inclusive	19	18	37
35–50 years inclusive	33	32	65
Age continuous Units: years			
arithmetic mean	36	37	
standard deviation	± 10	± 9	-
Gender categorical Units: Subjects			
Female	19	22	41
Male	33	28	61

Subject analysis sets	
Subject analysis set title	Safety
Subject analysis set type	Safety analysis
Subject analysis set description:	
All patients who signed an informed consent and were successfully randomised. ACT3D-CS: 52 patients Microfracture: 50 patients	
Subject analysis set title	ITT1

Subject analysis set type	Intention-to-treat

Subject analysis set description:

All patients who (i) were successfully randomised, (ii) received either ACT3D-CS on the day of implantation or microfracture on the day of arthroscopy, and (iii) completed the KOOS questionnaire at baseline and/or Day 0'.
ACT3D-CS: 48 patients
Microfracture: 49 patients

Subject analysis set title	ITT2
Subject analysis set type	Modified intention-to-treat

Subject analysis set description:

All patients who (i) were successfully randomised, (ii) for whom an arthroscopy was performed and (iii) completed the KOOS questionnaire at baseline. (Thus, if a patient in the ACT3D-CS group left the study between arthroscopy and implantation, the patient was to be included in the ITT2 but not in the ITT1 population.)
ACT3D-CS: 49 patients
Microfracture: 50 patients

Subject analysis set title	PP
Subject analysis set type	Per protocol

Subject analysis set description:

All patients of the ITT1 population without major protocol violations (according to the statistical analysis plan: Visit missing (for non-inclusion in the PP set, this was only treated as a major protocol violation for the analysis at the missed visit in question; for subsequent visits attended by the patient it was treated as a minor violation), early discontinuation (before visit after 6 weeks), Inclusion/Exclusion criteria violation at screening or baseline, randomised patients for ACT3D-CS not treated by ACT3D-CS, treatment failure, manufacture of ACT3D-CS not possible, any major violation of GCP by participants of the trial (except patients), withdrawal of informed consent by the patient was treated as a major protocol violation for the purposes of statistical analysis, although the withdrawal as such was explicitly permitted by the study protocol in line with GCP.
ACT3D-CS: 23 patients
Microfracture: 27 patients

Reporting group values	Safety	ITT1	ITT2
Number of subjects	102	97	99
Age categorical Units: Subjects			
In utero	0	0	0
Preterm newborn infants (gestational age < 37 wks)	0	0	0
Newborns (0-27 days)	0	0	0
Infants and toddlers (28 days-23 months)	0	0	0
Children (2-11 years)	0	0	0
Adolescents (12-17 years)	0	0	0
Adults (18-64 years)	0	0	0
From 65-84 years	0	0	0
85 years and over	0	0	0
18–34 years inclusive	37	36	36
35–50 years inclusive	65	61	63
Age continuous Units: years			
arithmetic mean	37	37	37
standard deviation	± 9	± 9	± 9
Gender categorical Units: Subjects			
Female	41	40	40
Male	61	57	59

Reporting group values	PP		
Number of subjects	50		
Age categorical Units: Subjects			
In utero	0		
Preterm newborn infants (gestational age < 37 wks)	0		
Newborns (0-27 days)	0		
Infants and toddlers (28 days-23 months)	0		
Children (2-11 years)	0		
Adolescents (12-17 years)	0		
Adults (18-64 years)	0		
From 65-84 years	0		
85 years and over	0		
18–34 years inclusive	18		
35–50 years inclusive	32		
Age continuous Units: years			
arithmetic mean	36		
standard deviation	± 10		
Gender categorical Units: Subjects			
Female	18		
Male	32		

End points

End points reporting groups

Reporting group title	ACT3D-CS

Reporting group description:

Autologous chondrocyte implantation (ACI) is based on the arthroscopic harvesting of the patient's own chondrocytes isolated from healthy cartilage, their culture in vitro to develop 3-dimensional spheroids (ACT3D-CS), and the subsequent implantation of these into the cartilage defect, resulting in hyaline cartilage repair.

Reporting group title	Microfracture

Reporting group description:

Microfracture is a marrow-stimulating method based on the penetration of the subchondral bone plate at the bottom of the cartilage defect. Different instruments such as the bent awls used in microfracturing create persisting holes in the bone plate. The outflowing bone-marrow blood contains the pluripotent stem cells (hMSC) which are able to differentiate mainly into fibrochondrocytes, resulting in fibrocartilage repair.

Reporting group title	ACT3D-CS

Reporting group description:

Autologous chondrocyte implantation (ACI) is based on the arthroscopic harvesting of the patient's own chondrocytes isolated from healthy cartilage, their culture in vitro to develop 3-dimensional spheroids (ACT3D-CS), and the subsequent implantation of these into the cartilage defect, resulting in hyaline cartilage repair.

Reporting group title	Microfracture

Reporting group description:

Microfracture is a marrow-stimulating method based on the penetration of the subchondral bone plate at the bottom of the cartilage defect. Different instruments such as the bent awls used in microfracturing create persisting holes in the bone plate. The outflowing bone-marrow blood contains the pluripotent stem cells (hMSC) which are able to differentiate mainly into fibrochondrocytes, resulting in fibrocartilage repair.

Subject analysis set title	Safety
Subject analysis set type	Safety analysis

Subject analysis set description:

All patients who signed an informed consent and were successfully randomised.
ACT3D-CS: 52 patients
Microfracture: 50 patients

Subject analysis set title	ITT1
Subject analysis set type	Intention-to-treat

Subject analysis set description:

All patients who (i) were successfully randomised, (ii) received either ACT3D-CS on the day of implantation or microfracture on the day of arthroscopy, and (iii) completed the KOOS questionnaire at baseline and/or Day 0'.
ACT3D-CS: 48 patients
Microfracture: 49 patients

Subject analysis set title	ITT2
Subject analysis set type	Modified intention-to-treat

Subject analysis set description:

All patients who (i) were successfully randomised, (ii) for whom an arthroscopy was performed and (iii) completed the KOOS questionnaire at baseline. (Thus, if a patient in the ACT3D-CS group left the study between arthroscopy and implantation, the patient was to be included in the ITT2 but not in the ITT1 population.)
ACT3D-CS: 49 patients
Microfracture: 50 patients

Subject analysis set title	PP
Subject analysis set type	Per protocol

Subject analysis set description:

All patients of the ITT1 population without major protocol violations (according to the statistical analysis plan: Visit missing (for non-inclusion in the PP set, this was only treated as a major protocol violation for the analysis at the missed visit in question; for subsequent visits attended by the patient it was treated

as a minor violation), early discontinuation (before visit after 6 weeks), Inclusion/Exclusion criteria violation at screening or baseline, randomised patients for ACT3D-CS not treated by ACT3D-CS, treatment failure, manufacture of ACT3D-CS not possible, any major violation of GCP by participants of the trial (except patients), withdrawal of informed consent by the patient was treated as a major protocol violation for the purposes of statistical analysis, although the withdrawal as such was explicitly permitted by the study protocol in line with GCP.
ACT3D-CS: 23 patients
Microfracture: 27 patients

Primary: Change of Overall KOOS Day0 - 24 months (ITT1)

End point title	Change of Overall KOOS Day0 - 24 months (ITT1)

End point description:

Knee Injury and Osteoarthritis Outcome Score (42-item, self-administered, self-explanatory questionnaire)

End point type	Primary

End point timeframe:

Change of overall KOOS from Day 0 (baseline for both treatment groups = pre-arthroscopy assessment) to final assessment 24 months after the end of the respective treatment, compared between the two study treatment groups (ACT3D-CS and microfracture).

End point values	ACT3D-CS	Microfracture		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	48	49		
Units: 0-100				
arithmetic mean (standard deviation)	24.9 (± 17.4)	21.5 (± 15.7)		

Attachments (see zip file)	Repeated measure ANCOVA 24 months non-

Statistical analyses

Statistical analysis title	Non-inferiority analysis 24 month[1]

Statistical analysis description:

Repeated-measures ANCOVA testing for non-inferiority of the change in overall KOOS (Day0 - 24 months) of ACT3D-CS vis-à-vis microfracture at 24 months (least-square mean difference from baseline for ACT3D-CS minus mean difference from baseline for microfracture).

Comparison groups	ACT3D-CS v Microfracture
Number of subjects included in analysis	97
Analysis specification	Pre-specified
Analysis type	non-inferiority
P-value	< 0.0001
Method	ANCOVA
Parameter estimate	least-square mean difference
Point estimate	5.5
Confidence interval	
level	95 %
sides	2-sided
lower limit	-0.8

Notes:

[1] - A low or upper value for the confidence interval may be missing. Values for both the lower and upper limit are expected to be provided with a 2-sided confidence interval.
Justification: Only the lower confidence interval is relevant for interpreting the result in terms of non-inferiority/superiority compared to the comparator surgical treatment.

Secondary: Change of Overall KOOS Day0 - 60 months (ITT1)

End point title	Change of Overall KOOS Day0 - 60 months (ITT1)

End point description:

Knee Injury and Osteoarthritis Outcome Score (42-item, self-administered, self-explanatory questionnaire).
Data for 60 months follow-up is reported here. Data for other follow-up time points is provided in attachment (V1 - 6 weeks, V2 - 3 months, V3 - 6 months, V4 - 12 month, V5 - 18 months, V6 - 24 months, V7 - 36 months, V8 - 48 month, V9 - 60 month).

End point type	Secondary

End point timeframe:

Change of overall KOOS from baseline (Day 0) to 12 months (interim analysis), 24 months (final analysis) and 36, 48 and 60 months follow-up analyses were compared between ACT3D-CS and microfracture.

End point values	ACT3D-CS	Microfracture		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	48	49		
Units: 1-100				
arithmetic mean (standard deviation)	28.0 (± 18.4)	23.7 (± 19.9)		

Attachments (see zip file)	Mean Difference of overall KOOS to

Statistical analyses

No statistical analyses for this end point

Secondary: Change of Overall KOOS Day0/Day0` - 60 months (ITT1)

End point title	Change of Overall KOOS Day0/Day0` - 60 months (ITT1)

End point description:

Knee Injury and Osteoarthritis Outcome Score (42-item, self-administered, self-explanatory questionnaire).
Data for 60 months follow-up is reported here. Data for other follow-up time points is provided in attachment (V1 - 6 weeks, V2 - 3 months, V3 - 6 months, V4 - 12 month, V5 - 18 months, V6 - 24 months, V7 - 36 months, V8 - 48 month, V9 - 60 month).

End point type	Secondary

End point timeframe:

Change of overall KOOS from Day 0 for microfracture (day before arthroscopy) or from Day 0' (day before implantation) for ACT3D-CS to 12, 24, 36, 48 and 60 months after the end of respective treatment.

End point values	ACT3D-CS	Microfracture		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	48	49		
Units: 0-100				
arithmetic mean (standard deviation)	23.4 (± 17.7)	23.7 (± 19.9)		

Attachments (see zip file)	Mean Difference of KOOS to day 0 (MF)/0'(ACT3D-CS)

Statistical analyses

No statistical analyses for this end point

Secondary: Change of KOOS subscores Day0 - 60 months (ITT1)

End point title	Change of KOOS subscores Day0 - 60 months (ITT1)

End point description:

Knee Injury and Osteoarthritis Outcome Score (42-item, self-administered, self-explanatory questionnaire). Data for subscore "pain" 60 months follow-up is reported here. Data for all Subscores and follow-up time points is provided in the attachment (V1 - 6 weeks, V2 - 3 months, V3 - 6 months, V4 - 12 month, V5 - 18 months, V6 - 24 months, V7 - 36 months, V8 - 48 month, V9 - 60 month).

End point type	Secondary

End point timeframe:

Change of the 5 subscores of the KOOS (pain, other symptoms, function in daily living (activities of daily living), function in sport and recreation, knee-related quality of life) from baseline (Day 0) to 12, 24, 36, 48 and 60 months.

End point values	ACT3D-CS	Microfracture		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	48	49		
Units: 1-100				
arithmetic mean (standard deviation)	87.2 (± 19.5)	80.0 (± 18.4)		

Attachments (see zip file)	KOOS subscores/20200812_Figures_Final_change KOOS subscores non-

Statistical analyses

No statistical analyses for this end point

Secondary: MOCART Score Day0 - 60 months (ITT1)

End point title	MOCART Score Day0 - 60 months (ITT1)

End point description:

MOCART - Magnetic Resonance Observation of Cartilage Repair Tissue (including 9 subscores). Number of subjects analyzed varies between follow-up time points (see subject numbers in the attachment).

Data for 60 months follow-up is reported here. Data for other follow-up time points is provided in the attachment (V1 - 6 weeks, V2 - 3 months, V3 - 6 months, V4 - 12 month, V5 - 18 months, V6 - 24 months, V7 - 36 months, V8 - 48 month, V9 - 60 month).

End point type	Secondary

End point timeframe:

MOCART (MRI Score) 12, 24, 36, 48 and 60 months after implantation or microfracture compared between the treatment groups.

End point values	ACT3D-CS	Microfracture		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	47[2]	43[3]		
Units: 1-100				
arithmetic mean (standard deviation)	71.4 (± 14.8)	76.7 (± 14.9)		

Notes:

[2] - out of ITT1 population (difference from total ITT1 population size ia due to missing results)

[3] - out of ITT1 population (difference from total ITT1 population size ia due to missing results)

Attachments (see zip file)	MOCART/MOCART Table.pdf

Statistical analyses

No statistical analyses for this end point

Secondary: Cartilage Repair Assessment (ITT1)

End point title	Cartilage Repair Assessment (ITT1)

End point description:

Assessment of cartilage repair according to ICRS compared between the treatment groups. This was only assessed for those patients who had consented to the additional arthroscopy as a "second-look" arthroscopy to be performed during Visit 6 (24 months follow-up). Here we only state data for number of patients with "normal" ICRS grade I cartilage as assessed during "second-look" arthroscopy. The full data for all ICRS grades is presented in the attachment in % of the number of patients assessed in the respective treatment arm.

End point type	Secondary

End point timeframe:

Arthroscopy and biopsy 24 months after implantation/microfracture.

End point values	ACT3D-CS	Microfracture		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	9[4]	7[5]		
Units: Numbers of patients	2	1		

Notes:

[4] - out of ITT1 population

[5] - out of ITT1 population

Attachments (see zip file)	ICRS cartilage repair 24 months/20200812_Figures_Final_cart

Statistical analyses

No statistical analyses for this end point

Secondary: ICRS Visual Histological Assessment Score (Safety)

End point title	ICRS Visual Histological Assessment Score (Safety)

End point description:

This scoring system allows one to assess the quality of the regenerated tissue and the extent to which its characteristics resemble those of native hyaline cartilage. The highest score of 3 reflects an ideal repair result of the hyaline cartilage, whereas the lowest score of 0 reflects the poorest repair result. Analyzed was the number of patients reaching a certain result in a certain criterion. Here we present only the number of patients for the criterion "surface" with the result "Smooth/continuous". All other data can be found in the attachment.

End point type	Secondary

End point timeframe:

at final assessment (24 months) compared between the treatment groups

End point values	ACT3D-CS	Microfracture		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	9[6]	7[7]		
Units: number of patients	2	3		

Notes:

[6] - out of Safety population

[7] - out of Safety population

Attachments (see zip file)	ICRS assessment/20200812_Figures_Final_ICRS.pdf

Statistical analyses

No statistical analyses for this end point

Secondary: Bern Score (ITT1)

End point title	Bern Score (ITT1)

End point description:

The Bern scoring system was applied to assess the formation of repair tissue in the R-biopsies (taken at "second-look"arthroscopy). The scoring includes the uniformity and intensity of the extracellular matrix (ECM) staining by Safranin O, which stains the proteoglycans in cartilage ECM (maximum score of 3). In addition, it assesses the amount of matrix present between the cells, as mature hyaline cartilage displays a low cell density with large amounts of matrix (maximum score of 3). The last category evaluates cellular morphology (maximum score of 3). A maximum score of 9 can be obtained, which would reflect mature hyaline cartilage.
Markers stained: collagen type II, aggrecan, collagen type I, CEP-68 (CRTAC1), EBF3, COMP, osteocalcin, LPL, S100B, NRN1, DLK1/Pref-1 and CD13 collagen type I, II, aggrecan, CEP-68 (CRTAC1), COMP and S100B.

End point type	Secondary

End point timeframe:

at final assessment (24 months) compared between the treatment groups.

End point values	ACT3D-CS	Microfracture		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	8[8]	7[9]		
Units: 0-9				
arithmetic mean (standard deviation)	6.1 (± 1.6)	5.9 (± 2.5)		

Notes:

[8] - out of ITT1 population

[9] - out of ITT1 population

Attachments (see zip file)	Bern Score/20200812_Figures_Final_Bern.pdf

Statistical analyses

No statistical analyses for this end point

Secondary: ICRS II Histological Score (ITT1)

End point title	ICRS II Histological Score (ITT1)

End point description:

ICRS II was developed comprising 14 criteria to assess parameters related to chondrocyte phenotype and tissue structure based on immunohistochemical staining. A visual analogue scale (0-100 mm) is used for scoring.

End point type	Secondary

End point timeframe:

at final assessment (24 months) compared between the treatment groups

End point values	ACT3D-CS	Microfracture		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	9[10]	7[11]		
Units: 1-100				
arithmetic mean (standard deviation)	58.8 (± 17.3)	42.9 (± 22.1)		

Notes:

[10] - out of ITT1 population

[11] - out of ITT1 population

Attachments (see zip file)	ICRS II/20200812_Figures_Final_ICRSII.pdf

Statistical analyses

No statistical analyses for this end point

Secondary: Change of modified Lysholm Score (ITT1)

End point title	Change of modified Lysholm Score (ITT1)

End point description:

The Lysholm Score (Lysholm Knee Scale) is an 8-item questionnaire that was originally designed as an outcome measure for ligament reconstruction but is commonly used as a measure for knee chondral damage. By removing the swelling item and using unweighted scores, a modified version of the Lysholm Knee Scale according to Smith et al. (2008) was used as an outcome measure for knee chondral damage.
Data for 60 months follow-up is reported here. Data for other follow-up time points is provided in attachment (V1 - 6 weeks, V2 - 3 months, V3 - 6 months, V4 - 12 month, V5 - 18 months, V6 - 24

months, V7 - 36 months, V8 - 48 month, V9 - 60 month).

End point type	Secondary

End point timeframe:

Change of modified Lysholm Score from baseline (Day 0) to 12, 24, 36, 48 and 60 months after the end of the respective treatment, compared between the treatment groups

End point values	ACT3D-CS	Microfracture		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	48	49		
Units: 0-24				
arithmetic mean (standard deviation)	5.7 (± 4.2)	4.8 (± 3.9)		

Attachments (see zip file)	Mean change of modified Lysholm

Statistical analyses

No statistical analyses for this end point

Secondary: IKDC Score Knee Examination Form – overall assessment (ITT1)

End point title	IKDC Score Knee Examination Form – overall assessment (ITT1)

End point description:

With the IKDC Subjective Knee Evaluation Form of the ICRS Cartilage Injury Evaluation Package (2000), the patient assesses his/her subjective symptoms and functioning of the knee during the past 4 weeks. The IKDC Subjective Knee Evaluation Form is scored by summing the scores for the individual items and then transforming the score to a scale that ranges from 0 to 100. The overall IKDC score was assessed in terms of the grades A–D, where 'A' represents a good rating and 'D' a poor one.
Here we only state the number of patients with grade A at 60 months follow-up in the respective treatment arms. Data for all follow-up time points and grades is provided in the attachment in % of the number of patients (V1 - 6 weeks, V2 - 3 months, V3 - 6 months, V4 - 12 month, V5 - 18 months, V6 - 24 months, V7 - 36 months, V8 - 48 month, V9 - 60 month).

End point type	Secondary

End point timeframe:

from baseline (Day 0) to 12, 24, 36, 48 and 60 months after the end of the respective treatment, compared between the treatment groups

End point values	ACT3D-CS	Microfracture		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	48	49		
Units: Number of patients	45	39		

Attachments (see zip file)	IKDC Score/20200812_Figures_Final_IKDC.pdf

Statistical analyses

No statistical analyses for this end point

Adverse events

Adverse events information

Timeframe for reporting adverse events:

From baseline to 60-month follow-up.
The start day of adverse-event reporting was the date on which the Informed Consent form was signed by the patient.

Adverse event reporting additional description:

Please note: The frequency threshold for reporting non-serious adverse events was ≥3 patients over all reporting groups.

Assessment type	Systematic

Dictionary used

Dictionary name	MedDRA
Dictionary version	15.1

Reporting groups

Reporting group title	ACT3D-CS

Reporting group description:

Autologous chondrocyte implantation (ACI) is based on the arthroscopic harvesting of the patient's own chondrocytes isolated from healthy cartilage, their culture in vitro to develop 3-dimensional spheroids (ACT3D-CS), and the subsequent implantation of these into the cartilage defect, resulting in hyaline cartilage repair.

Reporting group title	Microfracture

Reporting group description:

Microfracture is a marrow-stimulating method based on the penetration of the subchondral bone plate at the bottom of the cartilage defect. Different instruments such as the bent awls used in microfracturing create persisting holes in the bone plate. The outflowing bone-marrow blood contains the pluripotent stem cells (hMSC) which are able to differentiate mainly into fibrochondrocytes, resulting in fibrocartilage repair.

Serious adverse events	ACT3D-CS	Microfracture	
Total subjects affected by serious adverse events			
subjects affected / exposed	7 / 52 (13.46%)	8 / 50 (16.00%)	
number of deaths (all causes)	0	1	
number of deaths resulting from adverse events		0	
Neoplasms benign, malignant and unspecified (incl cysts and polyps)			
Abdominal neoplasm			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Adrenocortical carcinoma			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Breast cancer female			

subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Hodgkin's disease			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Injury, poisoning and procedural complications			
Cartilage injury			
subjects affected / exposed	0 / 52 (0.00%)	2 / 50 (4.00%)	
occurrences causally related to treatment / all	0 / 0	1 / 2	
deaths causally related to treatment / all	0 / 0	0 / 0	
Meniscus lesion			
subjects affected / exposed	0 / 52 (0.00%)	1 / 50 (2.00%)	
occurrences causally related to treatment / all	0 / 0	2 / 2	
deaths causally related to treatment / all	0 / 0	0 / 0	
Ankle fracture			
subjects affected / exposed	0 / 52 (0.00%)	1 / 50 (2.00%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Vascular disorders			
Deep vein thrombosis			
subjects affected / exposed	0 / 52 (0.00%)	1 / 50 (2.00%)	
occurrences causally related to treatment / all	0 / 0	1 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Cardiac disorders			
Cardiac arrest			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Sinus tachycardia			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	

Nervous system disorders			
Headache			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Eye disorders			
Visual impairment			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Gastrointestinal disorders			
Inguinal hernia			
subjects affected / exposed	0 / 52 (0.00%)	1 / 50 (2.00%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Hepatobiliary disorders			
Biliary colic			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Renal and urinary disorders			
Renal failure acute			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 2	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Psychiatric disorders			
Completed suicide			
subjects affected / exposed	0 / 52 (0.00%)	1 / 50 (2.00%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 1	
Musculoskeletal and connective tissue disorders			
Arthralgia			
subjects affected / exposed	0 / 52 (0.00%)	3 / 50 (6.00%)	
occurrences causally related to treatment / all	0 / 0	2 / 3	
deaths causally related to treatment / all	0 / 0	0 / 0	
Back pain			

subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Bone marrow oedema			
subjects affected / exposed	0 / 52 (0.00%)	1 / 50 (2.00%)	
occurrences causally related to treatment / all	0 / 0	1 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Chondromalacia			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Facet joint syndrome			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Intervertebral disc protrusion			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Joint adhesion			
subjects affected / exposed	0 / 52 (0.00%)	1 / 50 (2.00%)	
occurrences causally related to treatment / all	0 / 0	1 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Joint swelling			
subjects affected / exposed	0 / 52 (0.00%)	1 / 50 (2.00%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Osteochondrosis			
subjects affected / exposed	0 / 52 (0.00%)	1 / 50 (2.00%)	
occurrences causally related to treatment / all	0 / 0	1 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Rotator cuff syndrome			

subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Infections and infestations			
Cellulitis			
subjects affected / exposed	0 / 52 (0.00%)	1 / 50 (2.00%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Cystitis			
subjects affected / exposed	1 / 52 (1.92%)	0 / 50 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	

Frequency threshold for reporting non-serious adverse events: 0 %

Non-serious adverse events	ACT3D-CS	Microfracture	
Total subjects affected by non-serious adverse events			
subjects affected / exposed	40 / 52 (76.92%)	40 / 50 (80.00%)	
Injury, poisoning and procedural complications			
Ligament sprain			
subjects affected / exposed	4 / 52 (7.69%)	6 / 50 (12.00%)	
occurrences (all)	8	6	
Contusion			
subjects affected / exposed	6 / 52 (11.54%)	3 / 50 (6.00%)	
occurrences (all)	6	3	
Ligament rupture			
subjects affected / exposed	3 / 52 (5.77%)	2 / 50 (4.00%)	
occurrences (all)	3	2	
Hand fracture			
subjects affected / exposed	1 / 52 (1.92%)	2 / 50 (4.00%)	
occurrences (all)	1	2	
Vascular disorders			
Hypertension			
subjects affected / exposed	5 / 52 (9.62%)	3 / 50 (6.00%)	
occurrences (all)	5	3	

Nervous system disorders			
Headache			
subjects affected / exposed	2 / 52 (3.85%)	2 / 50 (4.00%)	
occurrences (all)	3	4	
Sciatica			
subjects affected / exposed	0 / 52 (0.00%)	2 / 50 (4.00%)	
occurrences (all)	0	2	
General disorders and administration site conditions			
Gait disturbance			
subjects affected / exposed	1 / 52 (1.92%)	1 / 50 (2.00%)	
occurrences (all)	3	1	
Gastrointestinal disorders			
Gastrooesophageal reflux disease			
subjects affected / exposed	1 / 52 (1.92%)	2 / 50 (4.00%)	
occurrences (all)	1	2	
Musculoskeletal and connective tissue disorders			
Arthralgia			
subjects affected / exposed	22 / 52 (42.31%)	23 / 50 (46.00%)	
occurrences (all)	29	32	
Joint effusion			
subjects affected / exposed	18 / 52 (34.62%)	17 / 50 (34.00%)	
occurrences (all)	24	23	
Joint swelling			
subjects affected / exposed	11 / 52 (21.15%)	14 / 50 (28.00%)	
occurrences (all)	13	16	
Back pain			
subjects affected / exposed	6 / 52 (11.54%)	4 / 50 (8.00%)	
occurrences (all)	8	4	
Chondromalacia			
subjects affected / exposed	4 / 52 (7.69%)	5 / 50 (10.00%)	
occurrences (all)	4	6	
Bone marrow oedema			
subjects affected / exposed	1 / 52 (1.92%)	4 / 50 (8.00%)	
occurrences (all)	1	4	
Intervertebral disc protrusion			

subjects affected / exposed	2 / 52 (3.85%)	1 / 50 (2.00%)	
occurrences (all)	2	1	
Tendonitis			
subjects affected / exposed	4 / 52 (7.69%)	0 / 50 (0.00%)	
occurrences (all)	4	0	
Osteoarthritis			
subjects affected / exposed	2 / 52 (3.85%)	1 / 50 (2.00%)	
occurrences (all)	2	1	
Cartilage injury			
subjects affected / exposed	2 / 52 (3.85%)	1 / 50 (2.00%)	
occurrences (all)	2	1	
Infections and infestations			
Nasopharyngitis			
subjects affected / exposed	7 / 52 (13.46%)	2 / 50 (4.00%)	
occurrences (all)	13	2	
Bronchitis			
subjects affected / exposed	3 / 52 (5.77%)	1 / 50 (2.00%)	
occurrences (all)	4	2	
Metabolism and nutrition disorders			
Hypertriglyceridaemia			
subjects affected / exposed	2 / 52 (3.85%)	1 / 50 (2.00%)	
occurrences (all)	2	1	

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

Date	Amendment
07 March 2012	extension of recruitment period; changes of in- and exclusion criteria to reflect general practice, for procedural clarifications, and to widen patient population; IMPD and IB update
31 January 2013	Adapted timelines to achieve the study recruitment target; adapted statistical details concerning the sample size.
21 March 2014	Expansion of the recruitment sites to Poland; adding a further score for histological assessment; adaption of time windows for the visits V3 to V9 due to the experience of the study sites; addition of a definition and a form to document patients who are lost to follow up; more detailed information for AE documentation; adapted timelines to achieve the study recruitment target; version update operating and product information on co.don chondrosphere
01 June 2015	Updated timelines and number of the final sample size and number of participating sites due to achievement of the target sample site; further back-up markers for histology; update to release-relevant analyzes; correction of incorrect approach with pregnant patients; version update operating and product information on co.don chondrosphere
07 November 2017	Update of markers for histology; clarification of name change of the IMP

Notes:

Interruptions (globally)

Were there any global interruptions to the trial? No

Limitations and caveats

Limitations of the trial such as small numbers of subjects analysed or technical problems leading to unreliable data.

None reported

Notes:

Online references

http://www.ncbi.nlm.nih.gov/pubmed/31317047

http://www.ncbi.nlm.nih.gov/pubmed/31893951