18 May 2017
EMA/349863/2017
Committee for Medicinal Products for Human Use (CHMP)

CHMP assessment report

Spherox

Common name: spheroids of human autologous matrix-associated chondrocytes

Procedure No. EMEA/H/C/002736/0000

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Administrative information

Name of the medicinal product:	Spherox
Applicant:	CO.DON AG Warthestr. 21 14513 Teltow GERMANY
Active substance:	Spheroids of human autologous matrix-associated chondrocytes
Common Name:	Spheroids of human autologous matrix-associated chondrocytes
Pharmaco-therapeutic group (ATC Code):	Other drugs for disorders of the musculo-skeletal system (M09AX02)
Therapeutic indication:	Repair of symptomatic articular cartilage defects of the femoral condyle and the patella of the knee (International Cartilage Repair Society [ICRS] grade III or IV) with defect sizes up to 10 cm2 in adults.
Pharmaceutical form:	Implantation suspension
Strength:	10–70 spheroids/cm²
Route of administration:	Intraarticular use
Packaging:	Applicator Pre-filled syringe
Package sizes:	1 to10 sterile tubes with up to 2 applicators

	each + 1 syringe per applicator and 1 to 10 sterile tubes with 1 pre-filled syringe each +1 indwelling cannula or 1 filter stem per pre-filled syringe

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List of abbreviations

2D	Two-dimensional
3D	Three-dimensional
ACI	Autologous chondrocyte implantation
ACL	anterius cruciatum Ligamentum
ACT	Autologous chondrocyte transplantation
ACT3D	Autologous chondrocyte transplantation with a 3-dimensional chondrocyte product
ACT3D-CS	Spherox
ADME	Absorption, distribution, metabolism, excretion
ADR	Adverse Drug Reaction
AG	Corporation (Aktiengesellschaft)
AE	Adverse Event
ALP	Alcaline phosphatase
AMG	Arzneimittelgesetz (German Medicines Act)
approx.	Approximately
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
ATMP	Advanced Therapy Medicinal Product
BL	Baseline
BMI	Body Mass Index
BMP-2/4	Bone morphogenic protein 2/4
BrdU	Bromdesoxyuridin
BVOT	Belgische Vereniging voor Orthopedie en Traumatologie (Belgian Society of Orthopaedics and Traumatology)
CAT	Committee for Advanced Therapies (at the EMA)
CBMP	Cell-Based Medicinal Products
CCI	Characterised chondrocyte implantation
CE	Conformitee European
CEP	Chondrocyte Expressed Protein
CHMP	Committee for Medicinal Products for Human Use
CI Biopsy	Biopsy out of native cartilage for cell isolation
CINHAL	Cumulative Index to Nursing and Allied Health Literature
CPM	continuous passive motions
CPWP	Cell Products Working Party
CRF/eCRF	Case Report Form / electronic Case Report Form
CRO	Contract Research Organisation

CTD	Common Technical Document
CTGTC	Cellular, Tissue, and Gene Therapies Advisory Committee
CTR	Clinical Trial Report
dGEMRIC	delayed gadolinium-enhanced MRI of cartilage
DGKKT	German Society for Bone and Cartilage Transplantation
DGOOC	Deutsche Gesellschaft für Orthopädie und Orthopädische Chirurgie (German Society for Orthopaedics and Orthopaedic Surgery)
DGU	Deutsche Gesellschaft für Unfallchirurgie (German Society for Traumatology)
DNA	Deoxyribonucleic Acid
EBM	Evidenced Based Medicine
EBV	Epstein Barr Virus
EC	European Commission
ECG	Electrocardiogram
ect.	etcetera
e.g.	for example (Exempli gratia)
EGF	Epidermal growth factor
EMA (formerly EMEA)	European Medicines Agency
EMBASE	Excerpta Medica Database
EQ-ZERT	Europäisches Institut zur Zertifizierung von Managementsystemen und Personal (European institute for certification of management systems and personnel)
FA	Final Assessment
FDA	Food and Drug Administration
FCS	Fetal Bovine Serum
FU	Follow-Up
bFGF2	Fibroblast growth factor-2
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
h	hour
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human immunodeficiency Virus
HS	Human Study
HSS	Hospital for Special Surgery Score
IA	Interims Analysis
IB	Investigator's Brochure
ICH	International Conference on Harmonisation

ICRS	International Cartilage Repair Society
IDE	Investigational Device Exemption
IDN	Identification Number
IFE	Institute for Research and Developemt (now: Pierrel Research Group)
IGF-1/2	Insulin-like growth factor 1/2
IIT	Integrated Isolator Technology
IKDC	International Knee Documentation Committee
IL-1β	Interleukin 1 β
IMPD	Investigational Medicinal Product Dossier
IND	Investigational New Drug
IPC	In-Process Control
KOOS	Knee injury and Osteoarthritis Outcome Score
LIMS	Laboratory Information Management System
MA	Marketing Authorisation
MACI	Matrix-associated chondrocyte implantation
M-ACT	Matrix-associated chondrocyte transplantation
max.	maximum
MC	Modified Cincinnati
MEDLINE	Medical Literature Analysis and Retrieval System Online
MF	Microfracture
min	Minutes
mm	Millimeter
MMP	Matrix Metalloproteinase
MOCART	Magnetic Resonance Observation of Cartilage Repair Tissue
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
n	number
NaCl	Sodium chloride
NAHS	Non-arthritic hip score
n.i.	not indicated
Nm	Nanometer
NRG	(Invented) Name Review Group
n.s.	not significant
OA	Osteoarthritis
OATS	Osteochondral Autograft Transfer System (sometimes used synonymously with mosaicplasty)
OCD	Osteochondritis dissecans

OP	Operation
PBS	Phosphate Buffered Saline
PDGF	Platelet derived growth factor
PEI	Paul-Ehrlich-Institute
PIL	Patient Information Leaflet
QoL	Quality of Life
RBC	Red Blood Cell
RCT	Randomised Controlled Trial
ROM	Range of Motion
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
SAE	Serious Adverse Event
SAWP	Scientific Advice Working Party
SCID	Severe Combined Immunodeficiency
SD	Standard deviation
SF-36	Short form health survey
SmPC	Summary of Product Characteristics
SOBCOT	Société Belge de Chirurgie Orthopédique et de Traumatologie (Belgian Society of Orthopaedics and Traumatology)
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TGF-β	Tumour growth factor
TIMP	Tissue Inhibitors of Matrix Metalloproteinases
TNF	Tumour necrosis factor
USA or US	United States of America
VAM	Swiss Drug Regulation
VAS	Visual Analogue Scale
WBC	White Blood Cell
WOMAC-D-Osteoarthritis Index	Western Ontario and Mc Master Universities Arthritis Index
ZKBS	Zentrale Kommission für Biologische Sicherheit, central commission for biological safety
γ-GT	Gamma glutamyltransferase

1. Background information on the procedure

1.1. Submission of the dossier

The applicant CO.DON AG submitted on 3 December 2012 an application for marketing authorisation to the European Medicines Agency (EMA) for Spherox, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 19 April 2012.

The applicant applied for the following indication:

Treatment of isolated acute or chronic chondral or osteochondral articular cartilage defects of traumatic genesis or unknown etiology (e.g. Osteochondritis dissecans). Spherox is applicable for defect sizes up to 10 cm^2 (International Cartilage Repair Society [ICRS] grade III or IV). Treatment is eligible for single as well as multiple adjacent defects. The medicinal product is indicated for adults and adolescents with closed epiphyseal growth plate.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that spheroids of human autologous matrix-associated chondrocytes was considered to be a new active substance.

The application is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0253/2012 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0253/2012 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

The applicant requested the active substance, spheroids of human autologous matrix-associated chondrocytes, contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Scientific Advice

The applicant received Scientific Advice from the CHMP on 30/01/2009 and 02/09/2009. The Scientific Advice pertained to quality, non-clinical and clinical aspects of the dossier.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

CAT Rapporteur: Lennart Åkerblom CAT Co-Rapporteur: Paula Salmikangas

CHMP Coordinator (Rapporteur): Kristina Dunder

CHMP Coordinator (Co-Rapporteur): Tuomo Lapveteläinen

- The application was received by the EMA on 3 December 2012.
- The procedure started on 27 December 2012.
- The CAT Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 15 March 2013. The CAT Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 15 March 2013.
- The PRAC Rapporteur's Assessment Report was circulated to all PRAC, CAT and CHMP members on 5th April 2013.
- During the meeting on 8-11 April 2013, the PRAC agreed on the PRAC Assessment Overview and Advice to CAT/CHMP. The PRAC Assessment Overview and Advice was sent to the applicant on 12 April 2013.
- During the meeting on 18-19 April 2013, the CAT agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 25 April 2013.
- The applicant submitted the responses to the CAT consolidated List of Questions on 15 December 2016.
- A GMP inspection has taken place at the site responsible for the manufacture of the medicinal product in Germany between 11 and 12 September 2013. The outcome of the inspection was issued on 27.09.2013.
- The Rapporteurs (CAT & PRAC) circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CAT and CHMP members on 31 January 2017.
- During the PRAC meeting on 6 to 9 February the PRAC agreed on a PRAC Assessment Overview and Advice to CAT/CHMP. The PRAC assessment Overview and Advice was sent to the applicant on 17 February 2017.

- During the meeting on 15-17 February 2017, the CAT agreed on a List of Outstanding Issues to be sent to the applicant. The List of Outstanding Issues was updated following the CHMP meeting that took place from 20 to 23 February and was adopted by CAT via written procedure on 27 February 2017. The final List of Outstanding issues was sent to the applicant on 28 February 2017.
- The applicant submitted the responses to the CAT List of Outstanding Issues on 12 March 2017.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CAT and CHMP members on 29 March 2017.
- During the CAT meeting on 11th April 2017, outstanding issues were addressed by the applicant during an oral explanation before the CAT.
- During the meeting on 10 to12 April 2017, the CAT agreed on a 2nd List of Outstanding Issues to be sent to the applicant. The List of Outstanding issues was sent to the applicant on 12 April 2017.
- The applicant submitted the responses to the CAT 2nd List of Outstanding Issues on 21 April 2017.
- The CAT Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the 2nd List of Outstanding Issues to all CAT and CHMP members on 3 May 2017.
- During the meeting on 10 to 12 May 2017, the CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Spherox on 12th May 2017.
- During the meeting on 15 to 18 May 2017, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Spherox on 18th May 2017.

2. Scientific discussion

2.1. Problem statement

Articular cartilage is a complex structure that has an important function in load-bearing joints and joint mobility. These characteristics are determined mainly on the composition of cartilage and particularly its extracellular matrix whose production the chondrocytes are involved in. The integrity of the extracellular matrix is essential for the mechanical and structural capacity of the cartilage. Due to the lack of blood or lymphatic vessels in the cartilage, cell infiltration does not occur and the capacity of the defect to heal after trauma is reduced. Without a surgical intervention the risk for the development of arthrosis is present. A cartilage lesion can reduce the joint function, cause pain and swelling of the joint.

Current methods to treat cartilage defects include autologous chondrocyte implantation (ACI), marrow stimulation techniques (e.g. microfracture) and mosaicplasty. Small lesions, especially in young patients, are known to heal without treatment. On the other hand, for large lesions there are only limited treatment options still available, as the surgical treatments are not suitable for such purpose.

On the 30th December 2008, Regulation 1394/2007/EC for advanced therapies came into force. The product Spherox and all other chondrocyte products on the market fall within the definition of a tissue-engineered

product as defined in Article 2(1)(b) of Regulation 1394/2007/EC and therewith became subject to the centralised authorisation procedure.

For the initial MAA filed for Spherox on 20 December 2012, the indication for Spherox as proposed by the applicant was as follows:

"Treatment of isolated acute or chronic chondral or osteochondral articular cartilage defects of traumatic genesis or unknown etiology (e.g. Osteochondritis dissecans). Spherox is applicable for defect sizes up to 10 cm² (International Cartilage Repair Society [ICRS] grade III or IV). Treatment is eligible for single as well as multiple adjacent defects. The medicinal product is indicated for adults and adolescents with closed epiphyseal growth plate."

Following review of the response to the list of questions and list of outstanding issues during the procedure, the proposed indication has been revised as follows:

"Repair of symptomatic articular cartilage defects of the femoral condyle and the patella of the knee (International Cartilage Repair Society [ICRS] grade III or IV) with defect sizes up to 10 cm2 in adults."

2.1.1. Disease or condition

Three factors are used to assess the initial cartilage lesion of the knee: the patient's clinical status and the lesion's size and type. The indication for management is based on the deterioration of the functional status measured by pain and functional limitation; these criteria are validated by a number of clinical scores. The lesion size can be measured in different ways. The standard radiological work-up can be used to estimate the width on the AP view of the knee and the length on the lateral knee image. However, the CT arthrogram, MRI, and arthro-MRI provide a more precise appreciation of the width on AP slices and length on sagittal slices; this measurement makes it possible to calculate the surfaces. Arthroscopy directly measures the size of the lesion using a probe, cylindrical gauges, or the measurement of the lesion arc. Directly visualizing the lesion, arthroscopy can also appreciate the depth of the lesion using the ICRS grades:

- Grade 1: nearly normal (superficial lesions): softening, fibrillations, lacerations, fissures;
- Grade 2: abnormal (less than 50% of cartilage depth);
- Grade 3: severely abnormal (cartilage defects extending down to more than 50% of cartilage depth);
- Grade 4: severely abnormal (lesion extending past subchondral plate, bone exposed)

Articular cartilage injuries affect around 285,600 people in Germany (number of arthroscopic operations of cartilage defects and meniscal operations from 2008; *Deutsches Statistisches Bundesamt*) and are therefore a common orthopaedic problem. Consequences of cartilage defects are reduced function of the joint and pain. Traumatically caused chondral lesions are accepted as one of the main reasons for osteoarthritis, which is one of the major disabling disorders and affects more than 10% of the Western population. This applies to cartilage defects in the knee as well as in other joints, e.g. the hip.

2.1.1. Clinical presentation, diagnosis

Articular cartilage damage can occur to the articular cartilage on its own as an isolated condition, or in conjunction with other injuries. Anterior cruciate ligament injuries of the knee are commonly associated with

damage to the medial or inner and lateral or outer surfaces of the femur and tibia. In the instance of an ACL tear during a twisting movement of the knee, the articulating surfaces of the femur and tibia become damaged. Symptoms include recurrent pain and swelling in the joint. There may be locking of the knee due to loose bodies floating within the joint. The patient may also experience audible clunks and clicking noises when moving the knee.

2.1.2. Management

The therapeutic tools aim to fill the cartilage loss so as to restore joint congruence, if possible to induce hyaline healing and thus prevent long-term osteoarthritic degeneration. The currently available surgical options for the treatment of cartilage defects can be divided into transplant procedures and bone marrow stimulation techniques. While autologous chondrocyte implantation and osteochondral transplantation (OCT, OATS, mosaicplasty) represent the group of transplant procedures; microfracture, abrasion arthroplasty and drilling procedures are among the techniques used for bone marrow stimulation. On the basis of the available literature, arthroscopic microfracture (MF) is the procedure with the best evidence within the bone marrow stimulation techniques. However, factors associated with an unfavourable prognosis include a large size of the defect and age over 40 years. ACT is an established procedure for the treatment of localized full-thickness cartilage defects of the knee. According to the German Society of Orthopaedics and Traumatology (DGOU), ACI is indicated for symptomatic cartilage defects starting from defect sizes of more than 3-4 cm^2; in the case of young patients or those active in sports at 2.5 cm^2. Advanced degenerative joint disease is the most important contraindication. For the hip, numerous joint-preserving treatments exist for chondral and osteochondral lesions, mirroring the treatment options in the knee. These include total hip arthroplasty, MF, articular cartilage repair, ACT, mosaicplasty, and osteochondral allograft transplantation. There is limited clinical information on the use of ACT in the hip.

About the product

Spherox is an advanced therapy medicinal product, composed of spheroids, which are spherical aggregates of ex vivo expanded chondrocytes with self-synthesized extracellular matrix. The drug product can be described as application unit containing individual number of spheroids in physiological saline solution. The active compound of Spherox consists of spheroids of human autologous matrix-associated chondrocytes, dispensed in a 0.9% NaCl solution. The product is used to treat and repair cartilage defects in the knee. It is applied in a re-transplantation procedure using a 3-dimensional autologous chondrocyte transplantation (ACT3D) in the donor's cartilage defect. The number of spheroids to be implanted is calculated individually by the physician depending on the estimated defect size (10-70 spheroids/cm^2) at the time of biopsy procurement. The implants are delivered either in a syringe or an application system, so called co.fix with a stem length 150 mm containing 60 spheroids. The drug substance is prepared by isolation of autologous chondrocytes from patient's cartilage biopsy and the expansion of cells in a monolayer culture. After the expansion step, chondrocytes are transferred to coated culture plates, where they cannot adhere to the surface, but start to form cell aggregates, spheroids. For harvesting, the spheroids are washed and transferred into 0.9% NaCl solution.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as implantation suspension equivalent containing 10-70 spheroids/cm^2 spheroids of human autologous matrix-associated chondrocytes as active substance.

Another ingredient is Sodium chloride.

The product is available in a pre-filled syringe or in an applicator (co.fix 150) as primary packaging unit.

The applicator (stem length 150 mm (co.fix 150)) is packed in a sterile tube and additionally surrounded by an extra bag. A tube may contain a maximum of two co.fix 150. The catheter of the applicator is made of thermoplastic polyurethane, the sealing plug on one side of acrylonitrile butadiene styrene and a silicone stopper on the other side. The applicator is delivered with an application device (sterile injection syringe).

The pre-filled syringe consists of a luer lock, a sealing ring and a cover cap. It is packed in a sterile tube with a screw-type cap and additionally surrounded by an extra bag. All parts of the pre-filled syringe are made of polypropylene, the sealing ring of isoprene. Silicone oil serves as lubricant. The pre-filled syringe is delivered with an application device (indwelling cannula or filter stem).

The number of primary packaging units delivered depends on the type of the primary packaging unit and the number of spheroids necessary for the specific defect size (10-70 spheroids/cm^2). One applicator has a maximum capacity of 60 spheroids in a volume of up to 200 microlitre isotonic sodium chloride solution. One pre-filled syringe has a maximum capacity of 100 spheroids in a volume of up to 1000 microlitre isotonic sodium chloride solution.

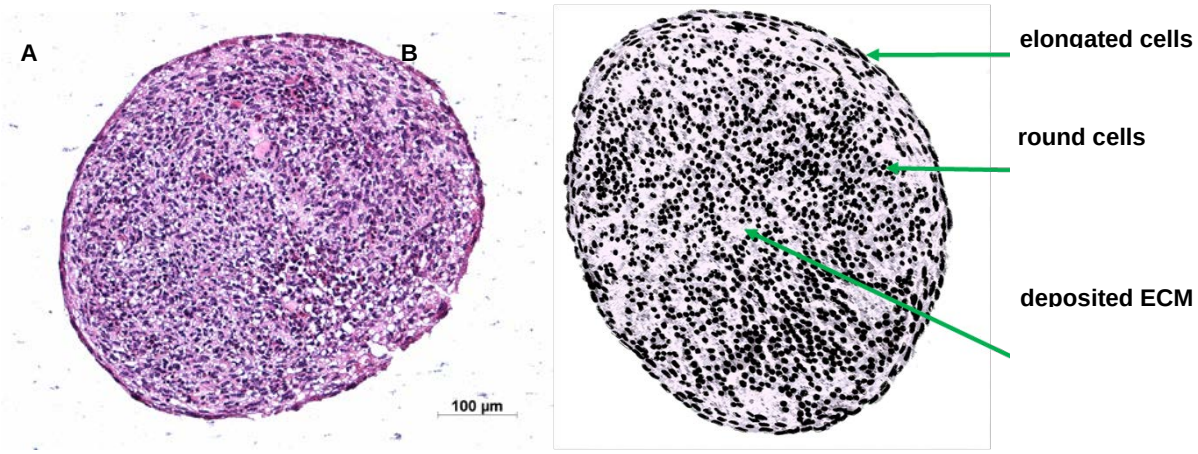
2.2.2. Active substance

General information

Spherox is a 3-dimensional autologous chondrocyte product that consists of matrix-associated chondrocytes in a form of a spheroid.

A spheroid is an aggregate of articular cartilage cells that have formed and differentiated into a 3-dimensional round multicellular structure. The cells within the spheroids display mainly a round cell shape and are surrounded by a self-synthesised extracellular matrix (ECM). In contrast, the cells present at the rim of the spheroids have an elongated shape (see Figure 1).

Figure 1 histological appearance of chondrocytes in a spheroid



(A) Haematoxylin-eosin (HE) staining showing the histology and the appearance of chondrocytes in a spheroid (Spherox). The cell nuclei are stained blue and the cytoplasm and matrix pink. A scale bar is indicated in the panel. (B) Schematic representation of a section of a spheroid illustrating the histological features of a spheroid. Note the elongated cells at the rim of the spheroid, and the round cells in the interior of the spheroid, surrounded by deposited ECM (Spherox after two weeks cultivation; refer to non-clinical study report no. cod 16/60, Module 4.2.1.2.1. The size of the spheroid depicted in the figure is approximately 500 µm).

The formation of the spheroids coincides with the synthesis and deposition of an ECM by chondrocytes. This results in an encapsulation of the chondrocytes within this self-synthesized ECM. The ECM in spheroids has a hyaline-like character and the ECM components CEP-68, aggrecan, glycosaminoglycans (GAGs), collagen type II as well as the early ECM protein collagen type I are present in spheroids. This indicates a chondrogenic phenotype of chondrocytes within spheroids and their potential to synthesise and deposit cartilage specific ECM components.

Safranin O positive staining affirmed the presence of hyaline cartilage specific GAGs. Collagen type I, which is an early, structurally important protein, was shown to be produced especially near the surface of the spheroids. Moreover, spheroids also express different chondrogenic growth factors, including TGFbeta, BMP2/4, IGF-1, and PDGF.

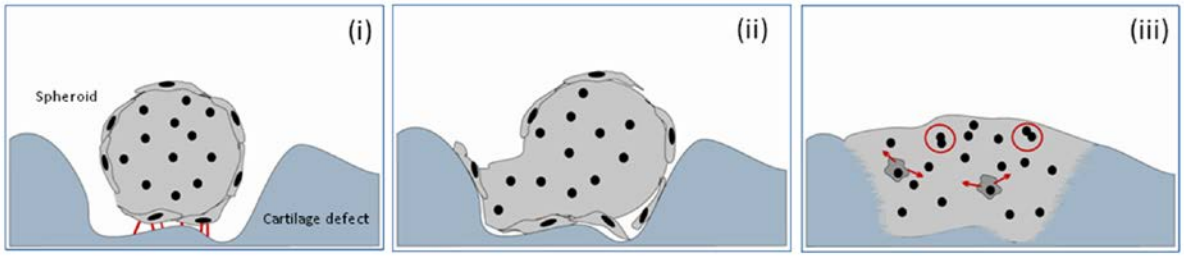
The observed protein expression underlines that the Spherox system is generating cells that are able to support and to promote the function necessary to foster growth of hyaline-like cartilage and subsequent healing of a cartilage defect.

The size of the spheroids varies depending on the cultivation time of the spheroids. With prolonged cultivation time, the size of the spheroids does not appear to change any further.

The structural and functional integration of transplants into defects is essential for their long-term functionality. The spheroid's adhesion and subsequent remodelling are crucial steps in its structural and functional integration into native cartilage. This adhesion and integration process of in vitro formed autologous spheroids consist of three phases: (i) initial fixation of spheroids by cell mediated adhesion to host tissue; (ii) widening of the spheroids and integration into the adhesion area by migration of surface chondrocytes along the irregular surface (e.g. fissures) of the host tissue, resulting in spheroid remodelling

(iii), synthesis and secretion of cartilage-specific proteins into the defect cavity that have structural and regulating function. This final step leads to gap filling between single spheroids and host tissue, as well as their biochemical integration into the surrounding host tissue (see Figure 2).

Figure 2 Schematic representation of the adhesion and integration of spheroid to the defect



Schematic representation of the adhesion (i) of a cartilage cell spheroid to the defect ground through adhesion points (indicated by the red lines). This is followed by (ii) widening of the spheroids and its integration into the adhesion area by migration of surface chondrocytes along the irregular surface of the defect ground. In the last remodelling step (iii) the spheroid is completely integrated into the cartilage defect. Red arrows indicate synthesis and secretion of cartilage-specific proteins into the defect cavity with structural and regulating function. Red circles indicate a putative cell proliferation, which leads to a formation of new chondrocytes within the defect.

Manufacture, process control and characterisation

The manufacturing process of Spherox is a continuous process including blood and biopsy procurement manufacture of the active substance and formulation of the finished product and no holding step or storage of the active substance occurs. The manufacture of Spherox active substance and finished product takes place at CO.DON. AG, Warthestr. 21, 14513 Teltow, Germany under GMP conditions.

Chondrocytes are isolated from a cartilage biopsy taken from the patient's own healthy cartilage area of the respective joint. The donation and procurement of the biopsy material is performed in compliance with the Directives 2004/23/EC and 2006/17/EC.

Transportation of the biopsy occurs within 48 hours at 5 °C to 25 °C inside the transport box to the production facility. This step has been shown to have an impact on the process and the product and thus the applicant has demonstrated the suitability of the transport conditions.

The manufacturing process consists of the following steps: starting material is acquired from a cartilage biopsy of the patient's healthy cartilage area, autologous serum is separated for further production, cells are isolated from the biopsy and expanded in monolayer cell culture, cells are then cultured on coated wells to form three dimensional spheroids. The obtained spheroids are harvested, formulated in saline and packaged.

The process flow is clearly described in the dossier and the main steps are standardized.

Production of the spheroids is based on coated culture plates, where the cells cannot adhere to the surface, but result in large chondrocyte aggregates, spheroids. The optimal size of the spheroids has been defined based on the requirements during the surgical application. The number of cells per spheroid varies however the applicant has been able to address this issue by presenting data in support of the limits set.

No reprocessing steps are proposed for manufacture of the spheroids.

The manufacturing process of Spherox is a continuous process starting from blood and biopsy procurement to active substance and to finished product formulation and no holding step or storage of the active substance occurs therefore no primary packaging of the active substance is described.

Control of materials

All raw materials are of adequate quality and suitability for the intended use. Donor testing is conducted according to Dir. 2004/23/EC and all test kits used for this purpose are CE certified. The quality of the biopsies and patient blood (serum) has been assessed and there are in-process tests / controls in place to ensure adequate quality of the materials before production. The laboratory where the biopsy is taken has been confirmed to be approved and inspected by the responsible Competent Authority.

The applicant has paid particular attention to the starting materials and has excluded any animal derived materials and external growth supplements. The main starting material is comprised of the patient´s own chondrocytes, isolated from a cartilage biopsy. The cells are propagated in cell culture media that contain only the patient´s own serum as a growth supporting agent.

Control of critical steps and intermediates

A comprehensive overview of critical in-process controls and critical in-process tests performed throughout manufacture of Spherox has been provided. Acceptable information has been provided on the control system in place to monitor and control the active substance and finished product manufacturing process.

Previously, major issues were raised in relation to the validation of the manufacturing process as regards characterization of operational parameters and overall robustness of the process. Furthermore issues were raised regarding the suitability of in-process control (IPC) to maintain the differentiation potential during monolayer culture and the genotypic/phenotypic profile of chondrocytes during spheroid cultivation and the overall design of the manufacturing process was questioned.

During the procedure the applicant has included new tests for identity to be applied during monolayer as well as spheroid stages. Two identity tests for chondrocytes introduced were as follows: one IPC at the end of monolayer cultivation and one as a release test for the finished product. These tests are based on quantitative polymerase chain reaction (qPCR) analysis of the marker gene expression and followed the approach to set acceptance criteria according to the ICH guidelines Q6B.

The applicant was able to demonstrate improved control during monolayer culture and spheroid cultivation. The provided validation data include IPCs and release parameters for 30 batches used for implantation which also include data from new tests (identity, potency and impurity). Consistency of the manufacturing process has been demonstrated by comparing process validation data with data from Phase II/ III clinical trials. No efficacy data could be presented for these batches; however a comparison of ranges of operational control (e.g. cell culture medium change, splitting conditions etc.) has been performed. In addition, quality parameter like cellular impurity by synoviocytes and cellularity of the finished product have been compared Obtained data demonstrate that the new tests applied are adequate to maintain the differentiation potential in the monolayer as well as the genetic/phenotypic stability, including culture of spheroids. Based on clinical trial data, operational ranges for ML cultivation time (P0 and total ML cultivation time) as well as spheroid cultivation time and total cultivation time (ML+3D) have been adjusted/tightened such that the efficacy of Spherox was not negatively affected by the parameter.

The IPCs applied are considered adequate for the purpose of maintaining the differentiation potential in monolayer culture whilst also maintaining the genetic/phenotypic stability, including culture of spheroids. A

marker for selection of the potential cellular impurity (synoviocytes) has also been implemented and the chosen marker has been found acceptable. The applicant has also adjusted the specifications in line with batch data and set a limit for synoviocytes.

Data provided suggests that markers representative for chondrogenic potential, potency and impurities (e.g. synoviocytes) do not vary significantly from time of testing at day 14 up to the maximal allowed 6 week cultivation time. Furthermore, analysis of clinical data does not suggest any influence from cultivation time when comparing responder vs non–responder after ranges and limits have been adjusted.

To confirm the new and adjusted process ranges and limits, the applicant is requested in an Annex II condition to conduct a new, prospective process validation as a post manufacturing measure using batches manufactured under the revised and more tightly controlled process parameters. The applicant is also requested to review the results from batches manufactured with the newly defined process parameters (as will be validated in a prospective process validation). Based on these results, more tightly defined operational ranges and limits in the manufacturing process will be considered. The timeline for fulfilling this condition is by April 2019.

Process validation

As regards consistency in manufacturing, the presented data from batches produced deviate from set parameters of cultivation time (P0), cellular impurity and cellularity (cells per spheroid). Indeed such deviations during process validations would normally not be accepted. However, this is not a traditional development and no dedicated process validation programme has been performed. Rather process data consists of a collection of data from batches produced in the past, which can be considered to be clinically justified.

Therefore, the CAT/CHMP has requested the Applicant to conduct a prospective process validation post-marketing using batches manufactured with the agreed and well-controlled process as detailed above.

Manufacturing process development

In 1994, Peterson and Brittberg (Brittberg et al, 1994) published one of the first instructions for successful cell-cultivation of chondrocytes in the New England Journal. In the following year, CO.DON AG began to cultivate chondrocytes as described by Peterson and Brittberg. Subsequently, CO.DON AG developed the method further by using other combinations of media without growth factors and additives. In 1997, CO.DON AG obtained the permission for manufacturing cell products by the federal competent authority in Germany and started to manufacture autologous cell transplants of high quality standard at that time. Cell cultivation was performed with patients' own material (cells and blood) using a standard laminar air flow box and standard methods for cultivation. Since June 2000 CO.DON AG produces the transplants in a so called Integrated Isolator Technology (IIT).

From 1997 until January 2009, more than 1,200 patients were treated with co.don chondrotransplant. Co.don chondrotransplant has been applied as cell suspension first using a periosteal flap to close the defect and to keep cells in place. The goal of CO.DON AG was to develop a self-adhesive transplant product, i.e. without the need of suturing a periosteal flap. In 2004, the product Spherox was introduced into the market, an evolution of the first product co.don chondrotransplant. The cells are cultivated in a 3-dimensional environment and produce own extra-cellular matrix which results in the spheroid form. From 2004 till September 2012, more than 3,600 patients have been treated with Spherox.

The product has undergone substantial changes since 1997, therefore early version of the product have not been taken into consideration for the assessment as they are considered unrelated. Only changes after 2004 are considered related and relevant for this application.

Since 2004, the active substance manufacturing process has been changed to introduce several changes including introduction of protease/collagenase enzyme solution for dissolution of a spheroid to count the cell number within spheroids, adjustment of the seeding range of monolayer-cultivated cells on well plates, removal of trypsin for enzyme digestion of the biopsy, monolayer and spheroid culture times, changes in in-process parameters and evaluation including in process monitoring, in process tests and in process controls.

In addition, changes to the serological testing of the patient's blood were also introduced in order to bring the test panel in line with serological testing in accordance with 2004/23/EC.

Changes to the quality control methods introduced were made for sterility testing, control of bacterial endotoxins test of coated material used for the spheroid culture, analysis of chondrogenic marker expression for testing of identity and potency, mycoplasma testing and regular bacterial endotoxin testing.

In relation to comparability between the proposed commercial process and earlier versions of the product a major objection was raised during the procedure. During the procedure the applicant was able to resolve these concerns by performing a retrospective comparative analysis of batches including the updated tests for impurities, identity and potency. In the context of the comparability exercise data presented showed that the process validation lots are comparable with lots from Phase II and Phase III trials. Deviations were found when these lots were compared with lots routinely manufactured under the hospital exemption (2013-2015), however this was considered acceptable. The commercial process is built on the process validation from 2015 and 2016 which was found to correlate with data from lots used in the Phase II and Phase III clinical trials. As mentioned above process validation will be confirmed post-marketing using batches from a well-controlled process using material from clinical trials.

Characterisation

For characterisation of the spheroids the applicant provided data from non-clinical studies. The applicant has further presented non-clinical studies in support of the newly introduced identity and potency markers. The chosen markers are deemed acceptable, yet there remains a request to reconsider the proposed specification limits once the process is revalidated.

The main focus in the product characterisation studies has been to provide evidence for non-clinical proof of concept and the studies performed are concentrated on Spherox in vitro/in vivo functionality, aggregation, integration and remodelling. Basic quality characterization studies have not been conducted, although some information can be extracted from the non-clinical studies.

The optimal culture period for the spheroids to achieve optimal size is claimed to be less than 2 weeks. Results from spheroid characterisation, however, show constant expression of ColI, whereas chondrogenic markers ColII, Aggrecan and S100 are upregulated only after longer 3D culture periods. Only cells that were derived directly from monolayer culture at P0 showed expression of cartilage markers after 3 weeks.

The applicant has demonstrated aggregation, integration and remodelling capacity of the spheroids in vitro, shown in cells and spheroids cultured for 14 days. Spheroids have been also subjected to immunohistochemical analysis, which demonstrated high expression of cartilage proteins, when the spheroids were co-cultured with human cartilage explants. These results suggest that the chondrocytes in the spheroids studied are able to form good quality hyaline cartilage, when the 2D and 3D culture times are properly controlled. Furthermore, it seems that the cells in the spheroids after controlled culture are able to re-

differentiate in an appropriate environment. For spheroid size, efficacy is demonstrated in the clinical trial batches for spheroids with a diameter between 200 and 850 µm. This is found acceptable.

Impurities:

In general, the data are supportive of the fact that IPCs applied are adequate for the purpose of maintaining the differentiation potential in ML whilst also maintaining the genetic/phenotypic stability, including culture of spheroids. These studies also highlight the need for consistent control of maximal PDL in the ML stage.

A marker for selection of the potential cellular impurity (synoviocytes) has also been implemented and the chosen marker has been found acceptable. The applicant has now adjusted the specifications in line with batch data and set the requested limit for synoviocytes (see above).

Reference standards

No reference material is available for the active substance or finished product. However, several reference materials are used in the analytical methods. These have been adequately described and are considered acceptable for their intended use.

Specification

The manufacturing process of Spherox is a continuous process; there is no intermediate holding step for the active substance and therefore no specification is provided for the active substance. The information for the finished product release and shelf life specifications as well as analytical methods is described under finished product.

Stability

The manufacturing process of Spherox is a continuous process starting from blood and biopsy procurement to active substance and to finished product formulation and no holding step or storage of the active substance occurs. Therefore, stability of the active substance is not separately analysed.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The finished product is presented as implantation suspension equivalent containing 10-70 spheroids/cm^2 spheroids of human autologous matrix-associated chondrocytes as active substance for intraarticular use during a surgical procedure.

The active substance, spheroids of human autologous matrix-associated chondrocytes, is formulated in 0.9% sodium chloride solution and is delivered using three primary packing units for application, depending on the preferences of the surgeon and number of spheroids require, which corresponds to the defect area. These include a syringe of max 100 spheroids or applicator (co.fix 150) of 150 mm containing a maximum of 60. The composition of the finished product is presented in Table 4.

Table 4. Composition of the finished product

Ingredient	Reference	150 mm applicator	syringe	Function
Spheroids of human autologous matrix-associated chondrocytes	In-house	Maximum of 60 spheroids	Maximum of 100 spheroids	Active substance
NaCl solution	Ph. Eur.	Maximum of 200 µl	Maximum of 1000 µl	Excipient

The applicator (co.fix 150) is placed into a sterile tube and additionally surrounded by an extra bag. One tube may contain a maximum of two co.fix 150. The applicator is made of thermoplastic polyurethane, the sealing plug on one side of acrylonitrile butadiene styrene and a silicone stopper on the other side. The applicator is delivered with an application device (sterile injection syringe).

The pre-filled syringe consists of a luer lock, a sealing ring and a cover cap. The filled syringe is packed into a sterile tube with a screw-type cap and additionally surrounded by an extra bag. All parts of the pre-filled syringe are made of polypropylene, the sealing ring of isoprene. Silicone oil serves as lubricant. The pre-filled syringe is delivered with an application device (indwelling cannula or filter stem).

The number of primary packaging units delivered depends on the type of the primary packaging unit and the number of spheroids necessary for the specific defect size (10-70 spheroids/cm²). One applicator has a maximum capacity of 60 spheroids in a volume of up to 200 microlitre isotonic sodium chloride solution. One pre-filled syringe has a maximum capacity of 100 spheroids in a volume of up to 1,000 microlitre isotonic sodium chloride solution.

The chosen material for primary packaging complies with Ph.Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

The formulation contains no overage or overfill. The intended commercial formulation is the same as that used during the clinical studies.

Pharmaceutical development

The applicant has described the development of the finished product formulation, which has been based on NaCl/PBS solutions.

Physiological saline solution (0.9% NaCl) is the only excipient used for Spherox. The purpose of saline is mimicking of the usual cellular environment and transport in 0.9% NaCl solution avoids that the spheroids dry up and stores them in a physiological environment.

Physiological saline solution (0.9% NaCl) is a well-known pharmaceutical excipient and its quality is compliant with Ph.Eur. requirements. There are no novel excipients used in the finished product formulation

The development of the product has been focused to achieve the following requirements:

1. Three-dimensional cell transplant containing chondrocytes in a terminated proliferation stage and initiated differentiation stage
2. Avoiding suturing of periosteal flap and any other additional fixation step and therefore avoiding artificial matrix structures
3. Short operation time

4. Mini-arthrotomic or arthroscopic procedure
5. Simple surgical procedure
6. Maintaining autologous nature of the product and excluding animal derived materials

To improve handling by the surgeon, in 2010 the applicant developed and introduced an applicator system (co.fix), which is formed in the shape of a catheter, in addition to the existing syringe formulation. The applicator allows placing spheroid by spheroid into the defect using a less invasive operation-method. The applicator system (co.fix) is made out of polyurethane which was selected on the basis of its low adhesion of the spheroids during the transportation and application procedure.

Validation of the co.fix applicator system demonstrated tightness of the system and product stability. In conclusion, both primary packaging systems (syringe or co.fix applicator) have been evaluated for their suitability and adequacy for the intended use.

Manufacture of the product and process controls

The manufacturing process of Spherox is a continuous process from biopsy procurement to finished product formulation and no holding step is foreseen for the active substance. The manufacturer of the product is CO.DON AG, Warthestr. 21, 14513 Teltow, Germany.

Manufacture of the finished product is performed by washing of the spheroids and addition of 0.9% NaCl solution and filling into the primary packaging units (i.e. pre-filled syringe and applicator with a stem length of 150 mm (co.fix 150)).

One batch includes all spheroids which originate from human chondrocytes obtained from the biopsy of one patient taken during one surgery.

The product may be delivered in more than one of the above mentioned primary packaging units. The number of primary packaging units delivered depends on the type of primary packaging unit and the number of spheroids necessary for the specific defect size (10–70 spheroids/cm²). The quantity of spheroids formulated per batch for the two presentations is depicted in Table 5.

Table 5. Quantity of spheroids formulated per batch for the three presentations

Volume of syringe/ applicator	Maximal Volume of drug product (spheroids and 0.9% NaCl solution)	Origin	Spheroids applied/cm^2 defect	Spheroids per primary packaging unit
Syringe 3 ml	Up to 1,000 µl	1 or more biopsies	10–70	Up to 100 spheroids
Application system 150 mm, 200 µl	Up to 200 µl	1 or more biopsies	10–70	Up to 60 spheroids

Primary packaging units (i.e. pre-filled syringe and applicator with a stem length of 150 mm (co.fix 150)) have been evaluated for tightness using microbial medium and by simulating the transplant storage conditions. Transport validation was carried out.

For labelling, the primary packaging unit(s), the sequential order number (batch number) and the number of contained spheroids are marked. The secondary packaging unit and the final packaging unit are marked with the patient key. The packaging unit (pouch) is then labelled with the product label which is printed on a sticker. Additional labelling information specific to a Member State is provided on the label in a boxed area (blue box), as required. The patient identification code includes an abbreviation of name and birth date of the patient.

Process validation

As this is a continuous process from biopsy collection to finished product formulation process validation is discussed under active substance above.

Process controls

As this is a continuous process from biopsy collection to finished product formulation process controls are detailed under active substance above.

Characterisation

Characterisation of the spheroids is covered under active substance.

For finished product characterisation, the applicant has identified relevant process related impurities (i.e. DMSO, papain, gentamycin and amphotericin) and theoretical concentrations of these substances in the finished product are presented. It has been concluded that the remaining levels of these process related impurities are toxicologically irrelevant. As regards product related impurities such as contaminating cell types, the applicant discussed the potential risk of other cell types derived from collection and purification of the biopsy and in particular the presence of contaminating cells from the synovium, in the spheroids. A suitable marker for selection of the potential cellular impurity (synoviocytes) has been implemented. Data support that based on levels in historical batches, the need for testing of other cellular impurities from bone and fat tissue is not required. A new limit for synoviocytes is set.

Product specification

The specification of finished product includes tests to specify the following quality attributes: Number of viable cells per spheroid, percent of cell viability, expression level of chondrogenic marker (identity), expression level of potency marker), synoviocyte content (determined by the expression level of a synoviocyte marker)and tests for mycoplasma, bacterial endotoxins and sterility.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH Q2 (R1).

The previously used identity markers were reconsidered and novel markers were identified using a genome wide screening method by gene array analyses as described in the non-clinical study report (cod 55). Using this method, a marker was identified and validated as a novel identity marker for in-process control of the Monolayer and at a lower level in the 3D cultivation of Spherox.

For potency expression levels are determined by qPCR. The reasoning behind the introduction of a marker for potency has been accepted. The target concentration in each sample is calculated relative to a non-regulated reference gene (i.e. housekeeping gene) and the result is expressed as a target/reference ratio (T/R), thus normalizing the expression levels of the target gene. The expression of potency as a T/R, a relative value for potency, has been considered to be acceptable.

The applicant has also addressed the issue concerning the relevance of the limit the potency marker As no efficacy data are present for the validation lots from 2015 these lots have been compared with data from lots used in clinical studies. The process validation batches and batches from the clinical trials were addressed by comparing the operational ranges of process parameters used during clinical trials and the process validation. The data presented are in support of comparable process performance between lots manufactured for the clinical trials and lots manufactured after the process validation. It can be concluded that the results indicate that the manufacturing process has not influenced the quality and gives a comparable finished product.

The results for cell number and viability testing demonstrated the assays to be robust, accurate and reliable.

The provided process validation data include IPCs and release parameters for 30 consecutive batches used for implantation. According to the applicant, all batches were released onto the German market, which in this case means under the hospital exemption. Thus, the clinical relevance of batches used for validation was unclear. The applicant has now justified why the data from the process validation are comparable to those used in the Phase II and Phase III studies. Still, the applicant is expected to conduct a prospective process validation post MA using batches manufactured with a well-controlled process. Also, the applicant is recommended to further explore those parameters that do not correlate with efficacy to verify that proper limits have been set. Furthermore, quality data post MA should be collected from sufficient number of batches to demonstrate consistency, proper quality and genetic stability of the cells in the FP and to alleviate the risks of high numbers of non-responders in the future.

The sterility test is evaluated after release of finished product. The number of cells per spheroid is performed on one spheroid only.

Batch analysis

The provided validation data include IPCs and release parameters for 30 batches used for implantation which also include data from new tests (identity, potency and impurity). The clinical relevance of batches used for validation has been clarified and a comparability comparison was done with batches from clinical trials.

As a post-authorisation commitment, the applicant has agreed to further confirm process validation using batches from a well-controlled process post-marketing (see above).

Reference materials

No reference material is available for the active substance or finished product.

Stability of the product

The proposed shelf life for Spherox is 72 hours. The product should be stored at temperatures between 1 °C and 10 °C, not frozen and not irradiated. In addition the outer packaging should not be opened before use to prevent microbial contamination.

Stability studies were performed on 3 batches of each primary package. Tests were performed for chondrogenic markers, cells per spheroid, viability and sterility.

Based on the available stability data, the shelf life and storage conditions as stated in the SPC are acceptable. The presented data are conclusive and also supportive of the stability of the product. The updated viability specification is deemed as sufficient in view of available data and clinical efficacy.

Adventitious agents

The raw materials used in the cultivation process are with exception of collagenase not of animal origin. For collagenase the valid EDQM TSE certificate is provided.

Autologous material (blood and tissue) is used. Blood testing according to 2006/17/EC is performed. Viral contamination of autologous donor at time of treatment cannot be excluded, however this is considered an acceptable risk because it represents no new infection and it is not transplanted to critical tissue with respect to possible damage.

Viral safety of the process has been checked in a risk analysis through FMEA-method (Failure Mode and Effects Analysis) comparing likelihood and effect (available on request). All raw and starting materials have been found to be uncritical.

Regarding microbiological safety, sterilisation by heat or UV of the finished product or sterile filtration is not possible for the product consisting of the viable chondrocytes. Microbial control during manufacture is based on a process design which focuses on risk mitigation. This is achieved by the use of an integrated isolator (IIT®) system under regular microbiological monitoring (comprising air and surface sampling) for the entire production process.

Sterility is tested at several steps of the manufacturing process including the monolayer culture and spheroid culture An automated system is used, which performs microbiological control of cellular products according to Ph. Eur. 2.6.27 instead of the currently used sterility testing method according to Ph. Eur. 2.6.1. An action plan is in place if positive results are seen in the sterility testing post treatment. The applicant's strategy to control sterility in the manufacturing process is considered to be acceptable.

Bacterial endotoxin testing according to Ph.Eur. 2.6.14 is performed on all material coming into direct contact with the product and as part of finished product release testing.

Mycoplasma testing is performed during manufacture and as part of finished product release using quantitative PCR method in accordance with Ph. Eur. 2.6.7.

In conclusion the adventitious agent safety evaluation and controls implemented during manufacture and control of Spherox are considered acceptable.

GMO

Not applicable.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The application for Spherox was received by the EMA on 3 December 2012. The product has been legally on the market in Germany since 2004, and has also been available in Belgium, Greece, Italy, Spain and Austria. During this period changes have been introduced to the manufacturing process to take account of scientific development (e.g. elimination of animal derived materials) and to improve the manufacturing process.

During assessment, several major objections have been raised on the quality side pertaining to the validation of the manufacturing process and operational ranges applied. In particular maximal culture times for the critical intermediate (cells from 2D culture) and for the active substance (spheroids) were questioned and

alternate steps during manufacture were considered not to be supported by relevant data. With respect to the control strategy for contaminating cell types a test for purity was requested and the chosen potency assay was considered insufficiently developed and justified.

There was also a concern on the comparability of the product batches used in clinical trials versus those to be released for commercial use should be demonstrated.

Regarding microbiological control the applicant was asked to introduce a rapid test as a release test and/or justify his strategy for control on microbiological safety in order to guarantee sterility of the product.

During the procedure the applicant has been able to resolve all major objections and other concerns.

The applicant was able to address the concern on process validation and demonstrate that the final agreed specifications are clinically justified. With respect to comparability the applicant presented a comparison between clinical trial batches and batches in the process validation which was considered to be acceptable to confirm the comparability between the clinical and commercial batches. Furthermore, the culture times for the 2D and 3D cell cultures have been compared with clinical data and the culture ranges and limits have been tightened accordingly. Thus, as such the design of the manufacturing process and its control is considered acceptable.

As regards consistency in manufacturing, the presented data from batches produced show some deviations according to the set parameters of cultivation time (P0), cellular impurity and cellularity (cells per spheroid). Indeed such deviations during process validations would not be found acceptable. However, this is not a traditional dedicated process validation programme that has been performed. It is instead a collection of data from batches produced in the past, which can be considered to be clinically justified.

Therefore, the applicant will conduct a prospective process validation post marketing authorisation using batches manufactured with a well-controlled process. Also, quality data will be collected post marketing from a sufficient number of batches to demonstrate consistency, quality and genetic stability of the cells in the finished product (see Annex II condition number 1).

The applicant is further requested to review the results from batches manufactured with the newly defined process parameters (as will be validated in a prospective process validation). Based on these results, more tightly defined operational ranges and limits in the manufacturing process will be considered. This approach is considered to be acceptable.

The applicant is also requested to validate the potency assay post marketing and monitor its correlation with the efficacy outcome (see Annex II condition number 2).

The applicant has presented a three pillar approach as a strategy for microbiological control of the manufacturing process starting from testing of biopsy to the release of product. The introduction of a rapid method for release has been accepted. Also, the action plan in place if positive results are seen in the sterility testing post treatment is found acceptable.

The question on the appropriate standard term to describe the pharmaceutical form was discussed during the procedure. The applicant's proposal for 'living tissue equivalent' could not be supported by the CAT/CHMP. The CAT/CHMP considered that 'implantation suspension' would more appropriately describe the finished product presentation for Spherox. The Committee felt that the term 'implantation suspension' would better describe the structural appearance of Spherox as the product is to be administered using a syringe rather than tweezers thus resembling rather a suspension than a solid product (e.g. a matrix or a tissue).

From a quality point of view, the marketing authorisation application for Spherox is considered approvable.

However, the CAT/CHMP has identified the two conditions as detailed in section 2.2.5. to further address the quality development issues that may have a potential impact on the safe and effective use of the medicinal product.

The classification of these post-authorisation commitments as Annex II conditions (see 2.2.5) is justified on the basis that the process validation and the validation of the potency assay are both essential to confirm a consistent manufacturing process and to obtain a coherent assay for potency determination of the finished product. A consistent manufacturing process and a validated potency assay are necessary to deliver in a reliable manner the finished product with the demonstrated safety and efficacy.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The CAT/CHMP has identified the following measures necessary to address the identified quality developments issues that may have a potential impact on the safe and effective use of the medicinal product:

1. To conduct a prospective process validation study post marketing using batches manufactured with a well-controlled process and to collect quality data from a sufficient number of batches to demonstrate consistency, quality and genetic stability of the cells in the finished product. On the basis of the process validation study, in process controls should be reviewed and the acceptance criteria tightened accordingly for the manufacturing process for P0 culture time, total ML culture time, spheroid culture time and amount of synovial impurities. **Timeline:** April 2019

2. To re-validate the potency assay post marketing and to monitor its correlation with the efficacy outcome. **Timeline:** March 2018

2.2.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT/CHMP recommends the following points for investigation:

None.

2.3. Non-clinical aspects

2.3.1. Introduction

The treatment of articulate cartilage defects with Spherox is intended to generate cartilage repair tissue which fills and integrates well within the defect and which is of comparable quality as the original hyaline articulate cartilage. Accordingly, the nonclinical programme consisting of in vitro and in vivo pharmacology studies was conducted to evaluate the characteristics of the cells in the spheroids and the capacity of the spheroids to regenerate hyaline cartilage. In addition, nonclinical safety tests were also conducted.

2.3.2. Pharmacology

Primary and secondary pharmacodynamic studies

A combination of *in vitro* studies and *in vivo* studies was conducted to characterise the functionality of the spheroids and to show proof-of-concept.

Initial *in vitro* studies were conducted to show that human chondrocytes from different donors were capable of generating stable three-dimensional aggregates of cells (spheroids) and to evaluate the optimal culturing conditions to generate chondrophere of a consistent quality. The size of a spheroid was shown to be dependent on the seeding density of monolayer chondrocytes used for its formation as well as on the cultivation time. Viability of human spheroids was addressed by evaluating apoptosis and necrosis up to 8 weeks of cultivation *in vitro*. A small number of single apoptotic and necrotic cells were detected evenly distributed in the spheroids indicating that the cells in the interior of the spheroids are viable and not subjected to low nutrient or oxygen supply.

Comparative analyses of chondrocytes from different species showed that only a limited number of animals including Göttinger mini-pig and Merino sheep were capable of producing stable spheroids. These species were then selected for proof-of-concept studies.

Different aspects, critical for spheroids to regenerate cartilage (proof of principle), were investigated in 3-dimentional cell cultures, in co-cultures of spheroids and cartilage explants and subsequently in *in vivo* proof of concept studies. This approach included evaluation of morphology of chondrocytes within the spheroids and the investigations showed that cells within the spheroids are round in shape, whereas cells at the surface are elongated similarly to native cartilage where elongated chondrocytes are observed at the cartilage surface. Initially, the cell density was high in the spheroid but that decreased with time in parallel with increased production of extracellular matrix (ECM).

Analysis of markers for chondrocyte differentiation and production of hyaline extra-cellular matrix: Human chondrocytes cultivated in the 3D system for at least 2 weeks formed spheroids which expressed extracellular matrix components, hyaline-cartilage specific markers and chondrogenic growth factors *in vitro* indicating the potential to form hyaline-like cartilage tissue *in vivo* after implantation. Aggrecan was expressed at a constant level during the time span of 12 weeks. Similarly, collagen type I was consistently expressed throughout the observation period. Hyaline-specific glycosaminoglycans (GAGs) could be detected only after 6 weeks of culture. However, there was marked inter-individual variability in expression of hyaline-specific marker in spheroids, in particular in expression of collagen type II. The expression of hyaline-specific markers was in general lower in *in vitro* cultured spheroids compared to native cartilage. Expression of hyaline-specific markers such as S100, collagen type II and aggrecan was confirmed in this study. However, in the experimental set up, a range of monolayer passages, number of cells for initiating spheroid culture and different cultivation times on the spheroids was tested. It was not reported whether such production variations had any influence on the functionality of the spheroids. During the initial assessment the uncertainties regarding the above stated variations, experimental set up, cultivation times, number of cells etc. were questioned. In the updated dossier and upon submission of responses, the applicant performed several *in vitro* studies which aimed to further characterise the cells and to establish a more reliable production of the spheroids. The vast majority of the generated data is considered to be related to the actual quality of the products and hence the data is presented in section 2.2.

The cultivation time in monolayer culture had a strong influence on the capacity of chondrocytes to re-differentiate to a chondrocytic phenotype capable of producing hyaline ECM. Already at monolayer passage 3,

there was a loss of collagen type II expression in some patient samples, which could not be regained in spheroid cultures. The marked variability in chondrocytic expression markers is a concern and no reliable marker for predicting efficacy *in vivo* has been identified. According to the applicant, a systemic analysis of markers in spheroids and correlation to biopsies is included in ongoing phase III studies. Considering that the clinical data can be correlated to quality specifications of the product, the lack of solid non-clinical data in this area is acceptable.

Investigations on adhesion, fusion of spheroids and integration of spheroids to host tissue surface at implantation in vitro: Spheroids were able to adhere firmly to femur condyle cartilage explants within a 20-30 min of co-culture. Rapid adhesion was also observed in the *in vivo* biodistribution study conducted in merino sheep. In an experiment where spheroids and cartilage explants were co-cultured for up to 11 weeks, the complete process from adhesion, fusion and integration onto the explants was demonstrated. The process involved firm adhesion to the surface of the explants, cell-cell/matrix contacts, and migration of chondrocytes on the surface of spheroids and along the surface of native cartilage (remodelling). This was followed by fusion of spheroids into larger aggregates where gaps between single spheroids were filled with newly synthesised ECM. Eventually new repair tissue covered the explants. Chondrocytes were observed to migrate into existing fissures and grooves of the explants. The migratory capacity appears beneficial for the effect i.e. cells migrate to fill-in cartilage tissue. However, migration might also be a potential safety issue if the cells should have a capacity to infiltrate e.g. subchondral bone when implanted in full thickness cartilage defects or if the subchondral bone is denuded upon debridement of the defect. In addition, it is noted that the applicant hypothesizes that the mechanism of action of the chondrocyte spheroids may include proliferation of chondrocytes within the defect.

Upon request from the CAT/CHMP, the applicant accounted for the extent of chondrocytes proliferation within the spheroids and the risk for potential migration/infiltration. Both, the *in vitro* and *in vivo* data show a low level of proliferation both before transplantation as well as after transplantation. The applicant summarised the non-clinical and clinical data available on migratory/infiltration and claims that there are no non-clinical data, no clinical evidence and/or clinical relevance for biodistribution and/or infiltration of chondrocytes into the subchondral bone. In general it is very unfortunate that the pivotal efficacy and safety study of spheroids in merino sheep failed to show efficacy due to loss of the implant since relevant data from this study should have added additional important high level data on the issue at hand. Nevertheless, despite the uncertainties which accompany the lack of pivotal data, it is the view of the CAT/CHMP that the risk for migration/infiltration is low and that additional non-clinical biodistribution studies are not called for.

In vivo proof-of-concept for regeneration of hyaline cartilage from implantation of spheroids: In vivo proof-of-concept studies were conducted in immunodeficient mice and in two large animal models: a pilot study in minipig and a pivotal 6-month study in Merino sheep. In these studies human Spheroxs or autologous spheroids produced in an analogous way were used. Dose levels were comparable to, or slightly higher (up to 2.3 times) than the maximum intended clinical dose of 70 spheroids/cm^2.

The mode of action of human spheroids was demonstrated in immunocompromised SCID mice. The fused human spheroids cultivated on native human cartilage explants were subcutaneously implanted into the SCID mice. The implanted spheroids integrated into the surrounding tissue, remodelled, migrated and filled up the cartilage defect with *de novo* synthesised repair tissue showing characteristics of chondrogenic hyaline-like ECM. These data demonstrate that the spheroids are capable of producing hyaline cartilage tissue when implanted into a chondrogenic environment. However, this model did not allow for an evaluation of the biomechanical properties of the repair tissue as this was an ectopic model. Subsequently, proof of concept was investigated in a pilot study in minipigs (large animal orthotopic model) using porcine spheroids

manufactured in an analogous way to Spherox. Defects reflecting the average size of human cartilage defects were created in the minipig knee joints (trochlear groove) and treated with porcine spheroids in three animals. The dose of 10–70 spheroids/cm^2 defect was used. Two animals with untreated defects served as controls. Two months after implantation the spheroids had filled and integrated well in the defect. In contrast, non-treated defects displayed either overspreading of scar tissue or incomplete filling. The spheroid induced repair tissue displayed several histological/immunohistological properties typical for hyaline cartilage. The biomechanical properties were similar to native cartilage; however the limited study period of two months is too short for drawing any conclusions regarding long term effects.

Long term efficacy was intended to be evaluated in the orthotopic large animal model, Merino sheep, after 6 and 9 months post-implantation using autologous ovine spheroids manufactured in an analogous way to Spheroxs. However, the data showed no difference between spheroid treated and non-treated defects at 9 months post-implantation as repair tissue was not detected. Evidence points to an early loss of the implanted spheroids, possibly due to the use of a bilateral model which prevented immobilisation and therefore healing of the joint.

There are no concerns related to secondary pharmacodynamics.

Safety pharmacology programme

Safety pharmacology was not evaluated with Spherox. This is acceptable given that the product consists of autologous chondrocytes that are implanted locally - into their natural environment and that these cells do not secrete any substances that would have an effect on vital organ functions. Similarly, there are no concerns related to secondary pharmacodynamics.

Pharmacodynamic drug interactions

No studies addressing pharmacodynamic drug interactions were performed and the Committee did not consider these necessary due to the nature of the medicinal product.

2.3.3. Pharmacokinetics

Conventional studies evaluating the ADME are not considered feasible or relevant for cell based medicinal product. Instead, the applicant followed the guideline on human cell based medicinal products (EMEA/CHMP/410869/2006) and reflection paper on in vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009) and evaluated the potential for biodistribution of the product in two *in vivo* studies.

In a GLP compliant study using immunodeficient NSG mice, biodistribution of cells out from human Spheroxs implanted subcutaneously into the back was investigated after 4 weeks. There were no indications of significant systemic distribution of the cells, or were there any indications of tumour or ectopic tissue formation after 6 months in the tumourigenicity part of this study. This supports the notion of the low biodistribution capacity of the cells of the Spherox product. However, skin biopsies taken in the immediate vicinity of the implantation site were occasionally HLA positive (marker for human cells). These were most likely related to the technical challenges i.e. fragmentation of the Spherox implants, rather than an indication of actively migrating cells from Spherox implant. The selection of organs collected for PCR analysis:

liver, lymph nodes, lung, spleen and kidneys represent the most likely tissues to which cells could distribute if they would escape from the implant and lack of data from other organs is thus acceptable.

Although the data regarding the lack of potential for biodistribution of human Spheroxs seems convincing, this information should be interpreted with caution given that this ectopic model and the spheroids were implanted subcutaneously: while the use of immunodeficient mice assures that the implanted human chondrocytes are not rejected, the local environment in the mouse skin may not provide the optimal physiological condition for promoting survival and function of human chondrocytes.

Biodistribution was also evaluated in an orthotopic setting in the Merino sheep. In this short-term study three Merino sheep were implanted in one joint with unlabelled spheroid to evaluate adhesion and injected with fluorescently labelled spheroids in the contra-lateral joint to evaluate biodistribution. This study indicated that spheroids are retained within the joint. However, this study has several flaws which limit its value: initial experiment could not exclude that PLK26 labelling did not have an effect on the quality of spheroids, only a local visual inspection was conducted to trace the spheroids that had been injected into the joint (i.e. systemic biodistribution of spheroids or cells was not conducted). Lastly, only a small number of animals was treated in this study and allogenic cells were used. It should be noted that poplietal lymph nodes (lymph nodes draining the knee joint) of two of the animals were enlarged. This could be due to the surgical procedure, but it cannot be excluded that an immune response towards the allogeneic cells was induced. If so, the allogeneic immune response could have limited any potential for biodistribution of cells of the spheroids escaping the joint. Conventional metabolism and excretion studies are not considered relevant for cell-based medicinal products and have not been performed with Spherox. Given the nature of the product, there is no potential for pharmacokinetic drug interactions. Omission of such studies is agreed.

2.3.4. Toxicology

No conventionally designed toxicity studies on Spherox have been performed; given that the product is autologous and that manufacturing takes place without any growth factors or other external stimuli that requires safety testing, such studies are not considered necessary. However, in line with the Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009), safety endpoints have been incorporated into the five different proof of concept and biodistribution/tumourigenicity studies in immunodeficient mice, minipigs and the Merino sheep.

In these studies, either human Spheroxs or autologous spheroids produced analogously to human Spheroxs were used. Safety of Spherox was also addressed as a primary objective in two *in vitro* studies assessing cell proliferation and senescence. In the *in vivo* studies, a single dose of spheroids was either injected or implanted into a knee joint or administered subcutaneously in the back of the animal. Except for one study in the minipig, in which up to 3.2 fold the maximum clinical dose was applied, all studies used doses similar to the intended clinical dose. Addressed safety aspects included potential single dose toxicity, local toxicity/tolerance, biodistribution and tumourigenicity. No genotoxicity, conventional carcinogenicity and reproductive- and developmental toxicity studies were performed which is in line with the guideline on cell-based medicinal products (EMEA/CHMP/410869/2006).

Local tolerance and systemic toxicity after implantation of autologous spheroids in the joint: One pivotal GLP-compliant efficacy and safety study Merino sheep was designed to evaluate local and systemic toxicity during 6 months of implantation of autologous spheroids in a femoral condyle defect. There were no indications of treatment related adverse effects and the implant was well tolerated. Nevertheless, this study failed to show an effect of the product due to loss of Spherox implant. The use of a bilateral model appears to be the main

contributing factor to the loss of transplant. In the updated dossier the applicant has submitted the finalized study report (9 months bilateral sheep study, study number 41/02-B). The complete report do not indicate any changes as to the data generated after 6 months, i.e. “no negative effect of the treatment of cartilage defects with ACT3D-S on the general health or the healing of the operated joint was observed”. However, since several lines of evidence point towards a loss of the test item from the implantation site the value of safety data collected in this study can in general be regarded as low

Due to the failure of the GLP-study in the Merino sheep, the nonclinical safety data with Spherox and analogously produced spheroids needed to be supported by studies conducted in immunocompromised mice and in minipigs. There were some deficiencies in the experimental design which hamper the evaluation of safety of the individual studies, e.g. low numbers of treated animals, lack of GLP-compliance and limited study duration to allow long term safety assessment, but collectively they provided good support that implanted chondrocyte spheroids are well tolerated. There were no obvious signs of general or local toxicity observed in any of the studies. However, *in vitro* co-culture studies have indicated that Spheroxs have capacity to migrate into grooves and fissures of cartilage explants. This is primarily seen as beneficial for the effect of the product but may also pose a risk for infiltration into subchondral bone.

The potential for biodistribution and tumour development was tested as recommended in the reflection paper on *in vitro* cultured chondrocyte containing products and the guideline on cell-based medicinal products. There was no indication of hypertrophy or ectopic cartilage formation or other clear signs of active biodistribution including migration of the implanted cells from Spherox into the surrounding tissues were observed in any studies carried out, including the 6 months GLP study in immunodeficient mice which support the absence of the tumourigenic potential.

The notion that in clinical situation, the Spherox implant will not be in open tissue surroundings such as in subcutaneous mice model, and the notion that no ectopic cartilage formation was observed in the mouse GLP study related to the Spherox product (in which two spontaneous tumours, not of human origin were observed) or in any other *in vivo* studies conducted supports the low biodistribution and tumourigenic potential of the Spherox product. In the GLP mice biodistribution study, occasional HLA-positive cells outside the larger Spherox / HLA positive implant area on the mice was observed, but these were most likely related to the fragmentation of the Spherox implant.

Clarification was sought by the CAT/CHMP on the quality and fate of the Spherox implants *in vivo*, i.e. the applicant was asked to provide a discussion concerning if the Spherox fragmentation and difficulties on identifying the Spheroxs in mouse GLP study, and the study failure (GLP bilateral sheep model) could be related to possible biological reason or the varied consistency of the Spherox product or solely related to the technical reasons (implantation or detection technique) and the choice of the animal model. The applicant suggests that the spheroid fragmentations, as observed in both animal studies, are solely due to technical reasons and that no data suggests that the study failure were related to any biological properties (apoptosis, necrosis or degeneration) of the transplants. The CAT/CHMP agreed.

To investigate whether the manufacturing of Spheroxs increases the risk for cell transformation of chondrocytes, the effect of extended monolayer culture (up to passage 10 for the senescence study) on the proliferation rate and senescence of chondrocytes in the extended cultures was characterised. Data from these studies suggest that cells from five tested samples reached senescence. However, there are also indications about the variability between donors in this regard. Data also indicate a risk for genetic instability which increases with higher number of passages. Also, there seems to be variability between patients in this regard. Thus, the risk for genetic alterations cannot be completely excluded by keeping the number of

passages to a minimum. Consequently, the risks associated with genetic instability will be addressed on a-patient-basis by product quality parameters.

Reproductive and developmental toxicity studies were not conducted. In line with guidelines on cell-based medicinal products (EMEA/CHMP/410869/2006), the applicant has provided an acceptable justification for omitting these studies: Spherox contains autologous cells with a local mechanism of action, and the risk for biodistribution of cells is limited.

2.3.5. Ecotoxicity / environmental risk assessment

Spherox does not contain GMOs. It is composed of spheroids, spherical aggregates of *ex vivo* expanded human autologous cells with self-synthesized extracellular matrix. It belongs to the group of biological medicinal products which according to the EMA guideline EMEA/CHMP/SWP/4447/00, is exempted from the obligation to perform an environmental risk assessment. The excipient, isotonic sodium chloride solution, an electrolyte, is exempted as well.

The product is provided as individual treatment dose (10–70 spheroids/cm² defect) and applied by intraarticular injection. The spheroids administered to the patient are likely to remain in the implantation site. No biodistribution or excretion is expected. Therefore, the product is not released to the environment. The use of Spherox is unlikely to result in any risk to the environment.

2.3.6. Discussion on the non-clinical aspects

The *in vitro* and *in vivo* characterisation of Spherox demonstrated that human chondrocytes are capable of producing three dimensional spheroids that upon implantation into a chondrogenic environment either *in vitro* or *in vivo,* fuse together, remodel, migrate, fill up the space between the spheroids and the cracks and fissures in the cartilage defect with *de novo* hyaline like cartilage and extracellular matrix.

The mode of action was convincingly demonstrated in *in vitro* cartilage explants and *in vivo* in subcutaneously implanted cartilage explants. This was further corroborated with the data from a large animal model, minipig, demonstrating formation of hyaline-like cartilage repair tissue in the defects after two months post-implantation. Long term evaluation of efficacy and safety in the orthotopic femoral condyle cartilage defect model in the Merino sheep failed to show any evidence of repair tissue at six and nine months post-implantation due to loss of transplant.

There was no indication on significant distribution of Spheroxs/chondrocytes to major organs based on data from a GLP-compliant biodistribution study of human Spheroxs implanted subcutaneously into immunodeficient mice. For the evaluation of the potential for biodistribution, it should also be considered that the site where the product is applied – the cartilage- is avascular. Thus for biodistribution to occur the implanted cells would have to actively migrate through tissues out to the capsule of the joint which contains blood and lymph vessels. Despite the deficiencies of the biodistribution studies the *in vitro* and *in vivo* studies have shown that spheroid show rapid adherence to the defect surface and do not shown tendency for ectopic cartilage formation. There are no indications of invasive behaviour of the cells and if the cells are correctly implanted and that the integrity of the joint capsule is not damaged (e.g. by surgical procedures) after the implantation, biodistribution does not seem likely. In general it is very unfortunate that the pivotal efficacy and safety study of spheroids in merino sheep failed to show efficacy due to loss of the implant since relevant data from this study should have added additional important high level data on the issue at hand. Nevertheless, despite the uncertainties which accompany the lack of pivotal data, it is the view of the

CAT/CHMP that the risk for migration/infiltration is low and that additional non-clinical biodistribution studies are not called for.

Spherox appeared to be well tolerated, and no significant safety concerns were identified in any of the studies.

The long term effects of the Spherox product cannot be fully evaluated from the *in vivo* data as the GLP compliant sheep study of 9 months duration failed due to loss of Spherox implants. Despite the deficiencies of the pivotal safety study, when taking all other available data into account:

- Lack or notable local and systemic toxicity of human spheroids in a 6-months *in vivo* pharmacodynamic study in SCID mice and in a 6-months GLP-compliant biodistribution/tumourigenicity study in immunodeficient mice.
- Lack of notable local and systemic toxicity in a 2-month study in the minipig implanted with up to 230 spheroids/cm^2 of autologous porcine spheroids into the knee joint.
- Lack of significant biodistribution in SCID mice implanted subcutaneously with human Spheroxs.
- Safety data will be collected from the obligatory phase III clinical studies, which is expected to override non-clinical data.

The lack of non-clinical data on local and general toxicity after long-term exposure to autologous spheroids is considered acceptable. In addition, there have been no unexpected findings arise in the ongoing clinical studies of autologous spheroids, see section 2.6.

2.3.7. Conclusion on the non-clinical aspects

In summary, the CAT/CHMP concluded that the application is approvable from the non-clinical point of view.

2.4. Clinical aspects

2.4.1. Introduction

Pivotal results are available from 2 clinical trials, namely a Phase II study (cod 16 HS 14) and a confirmatory Phase III study (cod 16 HS 13). For the Phase II clinical trial (cod 16 HS14) results are available from interim analysis (final assessment – 1 year after treatment) as well as 2-year follow-up (24 month) and 3-year follow-up (36 month) data. For the Phase III clinical trial (cod 16 HS13) results are available from an interim analysis – 1-year (12 months) after treatment.

- Study cod 16 HS 14 is a prospective, randomised, open-label (blinded radiologist), uncontrolled, multicentre trial to investigate the efficacy and safety of the treatment of large defects (4-10 cm2) with three different doses of Spherox in subjects with cartilage defects of the knee. The study is conducted in Germany. Patients will be followed for 60 months. The current submission contains 3-year follow-up data for this study.
- Study cod 16 HS 13 is a prospective, randomised, open-label (central reading by blinded radiologist) active-controlled, multicentre trial to compare the efficacy and safety of treatment with Spherox to microfracture in subjects with cartilage defects of the knee with a defect size between 1 and 4 cm^2. The

study is conducted in Germany and Poland. Patients will be followed for 60 months. The current submission includes the results from a 12-month interim analysis for regulatory purposes. The formal evaluation of superiority is to be performed 24 months after the last patient had received study treatment.

The clinical dossier also consists of data made available from investigator-initiated studies and published literature reports. This included 3 retrospective and 8 prospective studies, mostly non-GCP compliant except for one prospective uncontrolled investigator-initiated study stated to be GCP compliant by the Applicant (Fickert et al, 2011). During the procedure, additional supportive efficacy and safety data from patients treated in clinical routine practice between 2004 and 2012 were submitted. This included four monocentric (Germany), retrospective, non-interventional data collections, cod RS1 SR (2015); cod RS2 RS (2015); cod RS3 TS (2016); and cod RS4 WZ (2014). In addition, the applicant submitted an additional prospective uncontrolled investigator-initiated study on knee cartilage defects (Siebold et al, 2015) as well as a retrospective study (Fickert et al, 2014) and a prospective uncontrolled investigator-initiated follow-up study by Körsmeier et al (2014) to support the treatment with Spherox of cartilage defects in the hip.

GCP

The pivotal clinical trials were performed in accordance with GCP as claimed by the applicant

Tabular overview of clinical studies

Summary of submitted studies is provided in the tables below.

Listing of clinical studies with Spherox for knee cartilage lesions

Reference	Clinical investigation details					
	Pro-spective	Retro-spective	Controlled	Number of patients treated with chondro-sphere	Number of patients treated by other methods	Follow-up time (months)
Pivotal clinical investigations						
Phase II clinical trial cod16HS14	X			75		12-month final assessment 24- and 36-month follow-up reports
Phase III clinical trial cod16HS13	X		X	52	50 (MF)	12
Supportive clinical investigations						
Retrospective data collection cod RS1 SR (2015)		X		19		12
Retrospective data collection cod RS2 RS (2015)		X		20		Mean 35 (24–62)
Retrospective data collection cod RS3 TS (2016)		X		34	10 (chondro-transplant+ with periosteal flap)	chondrotransplant: 98 (48–144) chondrosphere: 22 (2–96)

Reference	Clinical investigation details					
	Pro-spective	Retro-spective	Controlled	Number of patients treated with chondro-sphere	Number of patients treated by other methods	Follow-up time (months)
Retrospective data collection cod RS4 WZ (2014)		X		36		12
Siebold et al (2015)	X			41		Mean 34.5 (6–72)
Maiotti et al (2012)	X			23		Mean 39 (36–41)
Fickert et al (2011)	X			37		12 and 24
Rössing et al (2010)	X			42		12 (MRI) 24 (clinical)
Ruhnau (2008)		X		36		Mean 14 (6–29)
Baum (2008)		X		10		Mean 13 (7–24)
Alevrogiannis et al (2008)	X			16		Minimum 12
Schreyer et al (2006)/Schreyer (2010)	X			30	40 (chondro-transplant* with periosteal flap)	48
Paediatric investigations						
co.don Retrospective Data Collection 2012 (cod 16 HS 16)		X		29		Up to 57
cod 16 HS 17 paed (2016) (Interim Analysis)	x	x		29		Mean 63 (42–96) (interim analysis)
Total number of studies / patients	**9**	**8**	**1**	**529***	**100**	

* Potential overlap between various supportive studies was identified. co.don® AG has confirmed that at least 418 individuals were treated with chondrosphere in all investigations.
+ chondrotransplant = chondrocyte suspension (ACT product) manufactured by co.don® AG until 2004. During transplantation into defect chondrocyte suspension was covered with perioseatl flap.

Note: the initial submission included an additional ongoing prospective study in 7 patients with defects of the talus (Thermann et al. 2008), which has not been included in the current table of studies by the applicant.

Listing of clinical studies with Spherox for hip cartilage lesions

Reference	Clinical investigation details					
	Pro-spective	Retro-spective	Controlled	Number of patients treated with chondrosphere	Number of patients treated by other methods	Follow-up time (months)
Fickert et al (2014)		X		6		Mean 11.2
Körsmeier et al (2012); Follow-up: Körsmeier et al (2014)	X			18 (follow-up:16)		Mean 16.09
Total number of studies / patients	**1**	**1**	**0**	**24**	**0**	

2.4.2. Pharmacokinetics

The applicant did not perform conventional pharmacokinetic studies to investigate absorption, distribution and excretion, and this is in line with CHMP guideline on cell based medicinal products (EMEA/CHMP/410869/2006), which states that the conventional ADME studies are not relevant for human cell-based products. The EMA Reflection Paper on In Vitro Cultured Chondrocyte Containing Products for Cartilage Repair of the Knee (EMA/CAT/CPWP/568181/2009) states that there is no clear common agreement for clinical kinetic data needed to be analysed in the clinical setting. Migration of the cells might be a risk and the applicant has conducted several nonclinical *in vivo* studies to evaluate possible biodistribution of spheroids, tumourigenicity and ectopic formation of cartilage after application of Spherox. All of the non-clinical results suggest low biodistribution and tumorigenic potential of the Spherox product. In addition, the mode of action does not indicate the capacity of the chondrocytes to migrate through tissues or blood vessels. According to the applicant, no toxic substances are used during the manufacturing of Spherox.

2.4.3. Pharmacodynamics

No clinical studies with a primary pharmacodynamic objective have been performed. However, in clinical trials Phase II (cod 16HS14) and Phase III (cod 16HS13) macroscopic, histological, immunohistochemical as well as MRI assessment were performed. In accordance with “Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee” (CAT, 2010), a pharmacodynamic evaluation was performed with focus on

• Macroscopic assessment – second-look arthroscopy

• Histological, immunohistochemical assessment – biopsy of regenerated cartilage

• MRI assessment – Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) evaluation.

Histological and immunohistochemical staining of biopsies allow for assessment of chondrogenesis, repair tissue formation and contaminating cell types.

In the Phase II study cod 16HS14, MRI together with the MOCART scoring was performed 13 weeks (3 months) and 12 months after implantation followed (or to be followed) by additional such assessments after 18, 24, 36, 48 and 60 months. Twelve months after implantation, an arthroscopy was conducted and a

biopsy (R-Biopsy) and histological analyses of the regenerated cartilage tissue was taken from the subset of patients who consented to this, in order to evaluate cartilage formation and repair as well as to investigate the expression of markers for chondrocyte differentiation. In addition, potential markers for different cell types (synoviocytes, bone, fat) were investigated to determine the purity of the regenerated tissue. Second-look arthroscopy with R-biopsy was optional, and R-biopsies from 8 patients were obtained. Assessment of second-look arthroscopy was performed by the treating physician. Assessment of R-biopsies was performed by an independent, blinded pathologist.

In the Phase III study, similar to the Phase II study, MOCART (MRI Score) was (and is to be) obtained at 3, 12, 24, 36, 48 and 60 months after implantation or microfracture and also includes the obtaining of ICRS visual histological assessment scores at 24 months (following a second-look arthroscopy and biopsy taking in consenting patients), Bern score, and additional histological assessment scores. At current, pharmacodynamic assessments by MRI and MOCART scoring have been performed in the Phase II clinical trial (cod 16 HS 14) for up to 3 years after Spherox implantation, and in the Phase III clinical trial (cod 16 HS 13) for up to 1 year.

Supportive post-marketing experience from control arthroscopy has become available from a prospective case series with 41 patients (Siebold et al, 2015), a prospective case series with 23 patients (Maiotti et al, 2012) and a retrospective analysis of 10 patients (Baum, 2008). Histological and/or immunohistochemical analyses were performed in the supportive study Maiotti et al (2012). Supporting post-marketing experience with MRI assessment is available from 4 retrospective studies initiated by the Applicant (cod 16 HS 16; cod 16 HS 17 paed; cod RS1 SR; cod RS4 WZ) and several publications, Alevrogiannis et al (2008); Baum (2008); Fickert et al (2011); Maiotti et al (2012); Rössing et al (2010); Siebold et al (2015).

MRI assessment with MOCART scoring: In the Phase II and Phase III clinical trials, an improvement in the MOCART score from 3 to 12 months was observed. In the Phase II study, at 12 months a plateau was reached, at a MOCART score of about 70–80 points (out of 100), and this has been sustained until the most recent follow-up visit 36 months after implantation. At 12, 18, 24 and 36 months no difference between any of the doses was observed. In the Phase III clinical trial, the MOCART score was respectively 67 and 62 for the ACT3D-CS and MF groups, 3 months after implantation, improving to 81 and 77 after 12 months. MRI evaluation with MOCART scoring of commercially available Spherox (cod RS1 SR [2015]; cod RS4 WZ [2014]) showed similar results compared to the clinical trials.

In the investigator- initiated study by Fickert et al (2011) an improvement in MOCART score from 3 months to 12 months was reported.

In adolescent patients (14 to 17 years of age) two investigations were performed; cod 16 HS 16 (2012) and cod 16 HS 17 paed (2016). The MOCART score up to 57 months after implantation (mean between 40 and 60 points at all visits) in adolescent patients in the study cod 16 HS 16 (2012) was relatively low. This have been the result of an incomplete assessment of individual score items by the physicians, according to the applicant.

The mean MOCART score derived from the MRI results of the study cod 16 HS 17 paed (2016) was 74.7 ± 12.0 in the follow-up period of 3.5 to 8 years (mean 63.3 months).

Macroscopic assessment (second-look arthroscopy): Second-look arthroscopies in the pivotal Phase II clinical trial revealed in 7 out of 8 samples at least ‘nearly normal’ cartilage with ICRS-CRA.

In the study by Siebold et al (2015), in 52 defects (91%) a ‘normal’ or ‘nearly normal’ result according to ICRS CRA was found in second-look arthroscopy.

A normal appearance of regenerated tissue and good integration was also found in studies by Maiotti et al (2012) (N = 3) and Baum (2008) (N = 9).

Histological and immunohistochemical assessment: Histological and immunohistochemical analyses in the Phase II clinical trial revealed hyaline cartilage characteristics (N = 7 patients). The overall mean Bern score was 6.4 ± 2.8 (on a scale from 0-9), which indicates successful growth and development of cartilage cells from the implant and production of hyaline-like ECM. The presence of most of the hyaline-cartilage-specific proteins was observed in all repair tissues, with varying expression levels however. In 5 patients collagen type II were found. Proteoglycans were present in all samples, as shown by the Alcian Blue staining and by the presence of aggrecan. However, none of the samples showed strong expression of all markers. This suggested that all repair tissues displayed hyaline cartilage characteristics to some extent, but that no sample displayed a true hyaline cartilage phenotype.

Maiotti et al (2012) reported hyaline cartilage in all cases (*N = 3*). However, details of the type of analyses and the assessments for this finding are not available from the authors.

The results from these assessments are discussed in details by study in section 2.5.

2.4.4. Discussion on clinical pharmacology

The applicant discussed the relevance of the pharmacodynamic parameters evaluated in their studies initiated and those described in publications related to Spherox.

A second-look arthroscopy allows a macroscopic assessment of defect repair and integration of the autograft into adjacent cartilage. However, since arthroscopy and procurement of biopsy are of invasive nature, magnetic resonance imaging (MRI) has become the most important tool for the follow up of patients with chondral autografts. MRI is currently the standard method for cartilage evaluation, as it allows morphological assessment of the cartilage surface, thickness, volume and subchondral bone. MRI is therefore ideal for the evaluation of the morphological status of cartilage defects and the repair tissue throughout the post-operative period. The MOCART scoring system was designed to record systematically only those observations that can be most accurately and reproducibly determined. The MOCART scale runs from 0 (worst possible case) to 100 points (normal).

There are nine criteria that are evaluated in the MOCART rating system and result in a maximum score of 100 points for a theoretical perfect repair tissue equivalent to native cartilage:

1. Degree of defect repair and filling of defect: Thickness of repair tissue (0–20 points)

2. Extent of integration to border zone: Visibility of defect zone resp. graft margins (0–15 points)

3. Surface of repair tissue: Amount of damages, e.g. fibrillations, fissures, ulcerations (0–10 points)

4. Structure of repair tissue: Homogeneity (0–5 points)

5. Signal intensity of repair tissue: Intensity of Dual T2-FSE and 3D-GE-FS (0–15 points each)

6. Condition of subchondral lamina: Intactness (0–5 points)

7. Condition of subchondral bone: Intactness, alterations (0–5 points)

8. Presence of adhesions (0–5 points)

9. Presence of effusions (0–5 points)

The MOCART scoring system is considered a valid and reliable tool assessing cartilage repair although the level of correlation between MOCART score and clinical outcome has not been fully clarified with a defined need for more high-quality prospective studies of imaging and clinical outcomes (de Windt TS, et al. Am J Sports Med. 2013;,41:1695-1702, and comment by Blackman AJ, et all [letter to the Editor, Am J Sports Med. 2013;41: NP49-50]).

To specifically evaluate the quality of repair tissue in patient biopsies, the Histological Endpoint Committee of the International Cartilage Repair Society (ICRS) developed a Visual Assessment Histological Scale in 2003. It is used to assess the quality of the regenerated tissue and the extent to which its characteristics resemble those of native hyaline cartilage. It assesses 6 components of repair, including surface architecture, matrix, cell distribution, cell population viability, subchondral bone, and abnormal cartilage mineralization. The ICRS I system uses discrete integer scores (0-3) to describe a continuum of tissue features in heterogeneous tissues and has shown low interreader agreement (Hoemann et al, Cartilage. 2011;2[2]:153-72).

The ICRS II scoring system addresses various aspects of cartilage repair and has been developed and validated by the Histology Working Group of the ICRS. ICRS II uses a continuous visual analog scale (VAS) and 14 criteria to assess parameters related to chondrocyte phenotype and tissue structure. It was first applied clinically in a large prospective randomized trial in which the clinical and histological results of microfracture and ACI were compared (Saris DB et al, Am J Sports Med. 2008;36:235-46). In a recent review, it was concluded that, of the various scores available for analysis of in vivo repaired cartilage, the ICRS II score seems (the most) suitable, being validated and comprehensive (Rutgers M, et al. Osteoarthritis Cartilage. 2010;18[1]:12-23).

In addition, the Bern scoring system was applied to assess the formation of repair tissue. This scoring system utilizes 3 categories of scoring, namely uniformity and darkness of cartilage ECM staining by Safranin O which stains proteoglycans (maximum score of 3); the amount of matrix present between the cells (maximum score of 3); and cellular morphology (maximum score of 3). A maximum score of 9 can be obtained which would reflect mature hyaline cartilage. The score has been validated for assessment of pellet-cultured (in vitro generated) neocartilage and showed good correlation of cartilage quality with glycosaminoglycan content (Grogan SP et al, Tissue Eng. 2006;12:2141-9) and can be used for evaluation of in vitro engineered cartilage (Osteoarthritis Cartilage. 2010;18(1):12-23).

Altogether, the pharmacodynamics assessments obtained and planned for the Phase II and III studies are consistent with the recommendations in the CAT/CHMP Reflection Paper.

2.4.5. Conclusions on clinical pharmacology

Phase II (cod 16 HS 14) and Phase III (cod 16 HS 13) clinical studies provided some evidence on hyaline forming or hyaline regeneration as well as defect fill based on histology. In addition, MRI data have been provided that suggest chondral repair as assessed by MOCART scoring up to 36 months after treatment. In the Phase III clinical trial no notable differences in MRI-based outcome were found between ACT and microfracture up to 12 months follow-up. Results from second-look arthroscopy and histological and immunohistochemical assessment of R-biopsies (24-month follow-up) are not available yet, but will provided in post-marketing reporting.

2.5. Clinical efficacy

The pivotal programme comprises of two clinical trials, namely Phase II (cod 16 HS 14) and Phase III (cod 16 HS 13). These studies were conducted in parallel, with mutually exclusive study populations based on defect size.

Study No.	Phase	Design	Title
Phase III clinical trial cod 16 HS 13, EudraCT-No. 2009-016 466-82 resub	III pivotal	Prospective, randomised, open-label, multicentre Phase III, controlled with microfracture	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 cm^2.
Phase II clinical trial cod 16 HS 14, EudraCT-No. 2009-016 816-20	II pivotal	Prospective, randomised, open-label, multicentre Phase II with 3 different doses of co.don chondrosphere	Prospective, randomised, open label, multicentre Phase-II clinical trial to investigate the efficacy and safety of the treatment of large defects (4-10 cm^2) with three different doses of the autologous chondrocyte transplantation product co.don chondrosphere (ACT3D-CS) in subjects with cartilage defects of the knee.

Supportive data for the knee trial results comprise of four retrospective data collections initiated by CO.DONAG (cod RS1 SR; cod RS2 RS; cod RS3 TS; cod RS4 WZ), six prospective investigator-initiated studies by Maiotti et al (2012), Fickert et al (2011), Rössing et al (2010), Alevrogiannis et al (2008), Schreyer et al (2006) (follow-up: Schreyer (2010) and two retrospective data collections, Ruhnau (2008), Baum (2008). Furthermore, two clinical investigations in adolescent patients have been conducted (cod 16 HS 16; cod 16 HS 17 paed). Supportive data for the hip were published by Körsmeier et al (2012) and follow-up: Körsmeier et al (2014) and Fickert et al (2014).

2.5.1. Dose response studies

The aim is to engraft on average 30 spheroids per cm^2 defect (acceptable range 10 - 70 spheroids per cm^2 defect). According to the applicant, the dose is calculated by the following steps until final estimation of the dose (defined as spheroids per cm^2 defect):

1. Estimation and documentation of the defect size during surgery for biopsy acquisition;
2. Calculation of the minimal spheroid number necessary and assessment of actual producible spheroids/cm^2 defect at the time of seeding of cells for spheroid formation;
3. Final assessment of spheroids production and calculation of dose (between 10–70/cm^2 defect) at release.

Dose response was evaluated in the phase II study, which was the pivotal trial for defects of the 4-10 cm^2 size. The trial is described in detail in the section 2.5.2. Thus, the release specification for the finished product Spherox applies to the number of spheroids per cm^2 defect and not to a specified number of cells per cm^2 defect. The dose used in clinical practice since market entry (2004) is 10–70 spheroids/cm^2 defect.

2.5.2. Main studies

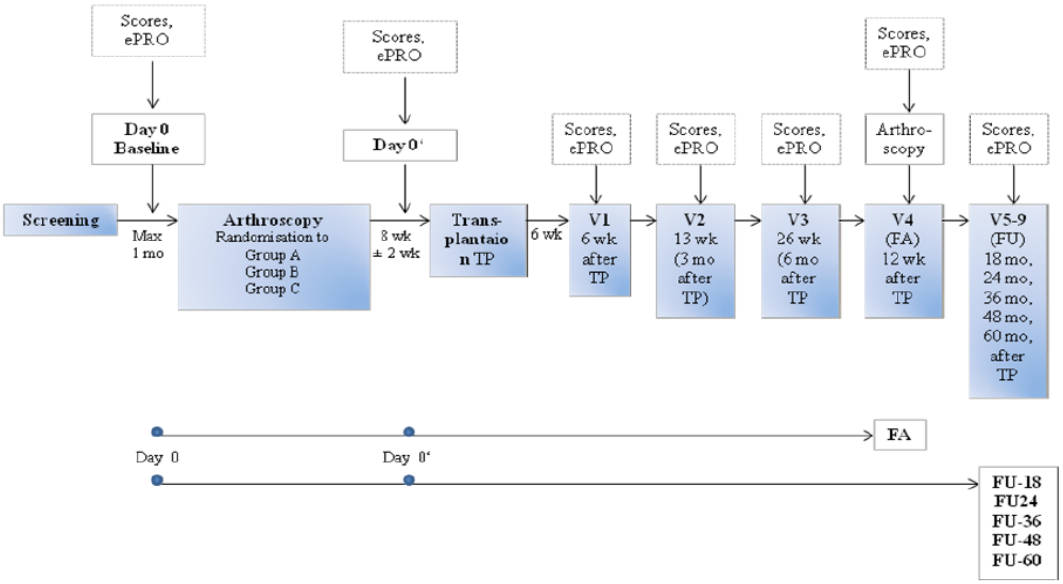
Phase II study Cod 16 HS 14

Study cod 16 HS14 is an ongoing prospective, randomised, open label, multicentre phase II clinical trial to investigate the efficacy and safety of the treatment of large cartilage defects (defect sizes between 4 and 10 cm^2) with 3 different doses of the ACT product Spherox in subjects with cartilage defects of the knee.

Methods

After implantation surgery, patients were recalled for assessments after 6 weeks, 13 weeks (3 months), 26 weeks (6 months), with final assessment after 12 months. Additional follow-up assessments have taken place after 18 months, 24 months and 36 months (the cut-off point for the present response submission). Future follow-up assessments will take place after 48 and 60 months, respectively.

Flowchart study cod 16 HS14



Study Participants

The patient population consisted of male or female patients between 18 and 50 years of age; with isolated ICRS grade III or IV single defect on medial or lateral femoral condyle, trochlea, tibia and retropatellar defect, also osteochondritis dissecans; defect size: 4 to 10 cm^2 after debridement to healthy cartilage, up to 6 mm in depth (assessment with MRI at screening and by estimation during arthroscopy before randomisation); nearly intact surrounding chondral structure around the defect as well as corresponding joint area. To qualify for inclusion, patients had to agree to adhere to a strict rehabilitation protocol and follow-up programme and agree to adhere to rules regarding pain medication.

The main exclusion criteria were:

- Defects in both knees at the same time;
- Radiological signs of osteoarthritis;
- Any signs of knee instability;

- Valgus or varus malalignment (more than 5° over the mechanical axis);
- Clinically relevant second cartilage lesion on the same knee;
- More than 50% resection of a meniscus in the affected knee or incomplete meniscal rim;
- Rheumatoid arthritis, parainfectious or infectious arthritis, and condition after these diseases;
- Pregnancy and planned pregnancy (no MRI possible);
- Obesity (body mass index > 30 kg/m2);
- Uncontrolled diabetes mellitus;
- Serious illness or poor general health as judged by the physician;
- Previous treatment with ACT in the affected knee;
- Microfracture performed less than one year before screening in the affected knee;
- Meniscal implant or mosaicplasty (Osteoarticular Implant System) in the affected knee;
- Meniscal suture (in the affected knee) three months prior to baseline;
- Having received hyaluronic acid intra-articular injections in the affected knee within the last 3 months of baseline;
- Taking specific osteoarthritis drugs such as chondroïtin sulphate, diacerein, N-glucosamine, piascledine, capsaicin within two weeks of baseline;
- Corticosteroid treatment by intra-articular route within the last month of baseline or systemic (all routes) corticosteroids within the last two weeks before baseline;
- Chronic use of anticoagulants;
- Any concomitant painful or disabling disease of the spine, hips or lower limbs that would interfere with evaluation of the afflicted knee;
- Any clinically significant or symptomatic vascular or neurological disorder of lower extremities;
- Any evidence of the following diseases in the affected knee: septic arthritis, inflammatory joint disease, recurrent episodes of pseudogout, Paget's disease of bone, ochronosis, acromegaly, haemochromatosis, Wilson's disease, primary osteochondromatosis, heritable disorders, collagen gene mutation

The inclusion and exclusion criteria are considered acceptable. There was no grading of KOOS (the primary outcome variable) at screening. This might have been useful to further characterize the study population.

Treatments

The principle of the ACT3D is based on the arthroscopic harvesting of the patient's own chondrocytes isolated from healthy cartilage which are cultivated *in vitro* to develop 3-dimensional spheroids (ACT3D-CS) that are subsequently transplanted into the cartilage defect. The following doses were administered: low-dose group: 3–7 spheroids/cm^2; medium-dose group: 10–30 spheroids/cm^2; high-dose group: 40–70 spheroids/cm^2. The dose used in clinical practice since market entry (2004) is 10–70 spheroids/cm^2 defect. Therefore, one patient group received 10–30 spheroids/cm^2 and the second group 40–70 spheroids/cm^2 defect.

The applicant was requested to justify the choice of the lowest proposed dose of 3-7 spheroids/cm^2. It was based on ethical considerations to choose a potential 'minimum effective dose' of 3–7 spheroids/cm^2 as suboptimal levels may lead to incomplete defect filling with risk of progression to osteoarthritis. Furthermore, according to the applicant, the use of the gaps between the different dose-groups should ensure discrimination of the clinical outcome between the different dose groups. After surgery, the patient underwent a strict rehabilitation programme that already started during his/her stay in the clinic, and which continued at home for up to three months after the intervention. On the day of discharge from hospital, at Visit 1 (after 6 weeks) and at Visit 2 (after 13 weeks), the investigator assessed the conduct of the rehabilitation programme up to the respective time point and recorded his/ her assessment in the eCRF.

Patients were to agree to use only paracetamol mono- (max 4 g/day) or combination preparations and oral and/or topic NSAIDs during the trial and to discontinue the use of oral and/or topic NSAIDs and/or paracetamol combination preparation 1 week before each visit whereas the use of paracetamol mono-preparation (max 4 g/day) is allowed. However, in the morning of the visit day, no pain medication was allowed. Other pain medications were allowed during the surgical procedure and could be taken for a period not exceeding 4 weeks after surgery. Patients were to record all prescribed medication in their patient's diary.

Objectives

The overall objective of the study was to confirm the clinical efficacy of the Spherox treatment, using the patient reported KOOS [and other scores for functionality, and its structural outcome (measured with MRI and structural scores) using a dose range of 10–70 spheroids/cm^2.

Outcomes / endpoints

Primary efficacy variable: Change of overall KOOS from baseline (Day 0) to final assessment at Visit 4 (12 months after implantation), determined for each dose group and between the dose groups (ITT population). KOOS is a 42-item, self-administered, self-explanatory questionnaire that covers five patient-relevant dimensions: Pain, Other symptoms, Function in daily living (ADL), Function in sport and recreation (Sport/Rec), and Knee-related quality of life (QoL). Scores are then transferred to a 0-100 scale (100 indicating no symptoms and 0 indicating extreme symptoms) which is then calculated for each subscale. The overall KOOS score is determined by averaging transformed subscores. As a patient-reported outcome measure, KOOS is considered an appropriate and validated scoring system to assess improvement of function and pain in patients with cartilaginous defects and in principle an acceptable primary endpoint to show improvement of symptoms.

Secondary endpoints:

- Change of overall KOOS from baseline (Day 0) to 24, 36, 48 and 60 months (follow-up, FU) after implantation, determined for each dose group.
- Change of overall KOOS from baseline (Day 0) to 24, 36, 48 and 60 months (follow-up, FU) after implantation, compared between the dose groups.
- Change of overall KOOS from Day 0' (pre-implantation day) to 12, 24, 36, 48 and 60 months (follow-up, FU) after implantation, determined for each dose group and compared between the dose groups.
- Change of the five subscores of the KOOS (from baseline (Day 0, the pre-arthroscopy day) as well as from Day 0' (pre-implantation day) to 12, 24, 36, 48 and 60 months (follow-up, FU) after implantation, determined for each dose group and between the dose groups.

- MOCART (MRI Score) 12, 24, 36, 48 and 60 months after implantation compared between the dose groups.
- Arthroscopy and biopsy 12 months after implantation, assessment of cartilage repair after ACT3D to be compared for each dose group and between the dose groups.
- ICRS Visual Histological Assessment Score at final assessment (FA, 12 months) determined for each dose group and compared between the dose groups.
- ICRS II Histological Score at final assessment (12 months) determined for each dosage group and compared between the dosage groups (R-Biopsy).
- Bern Score and additional histological assessment scores at final assessment (12 months) determined for each dose group and compared between the dose groups.
- Change of ICRS/IKDC from baseline (Day 0) as well as from Day 0 to 12, 24, 36, 48 and 60 months after implantation determined for each dose group and compared between the dose groups.
- Change of modified Lysholm Score from baseline (Day 0) as well as from Day 0' to 12, 24, 36, 48 and 60 months after implantation determined for each dose group and compared between the dose groups.
- Days of absence from work (employment) and/or days of inability to pursue usual activities

Sample size

The sample size calculation was based on showing differences between 12 months and baseline (within treatment group). No comparisons between treatment groups were taken into account. 12.5 is considered a clinically relevant difference in standardised KOOS score. 75 patients were planned to be included, 25 per treatment group.

Randomisation and blinding

Randomisation was performed via a central IVRS. Patients were stratified prospectively by defect size into two classes (4 cm^2 up to but not including 7 cm^2 and 7–10 cm^2). As the study was open-label, the investigators were not blinded. However, the patients were blinded to their spheroid dose level. The blinding of the patients was maintained up to the final assessment and during the remaining follow-up period.

All clinical assessments were performed by independent evaluators at each site. An independent central radiologist ("blinded reader") assessed all MRI pictures after study intervention without knowledge of which dose had been applied or of the time point (of patient participation in the trial) at which the image was obtained. An independent central, blinded pathologist assessed the cartilage biopsies, taken from patients of all three groups after 12 months, for the evaluation of (i) the extent of cartilage repair, (ii) any indication of migration of the implanted spheroids, and (iii) the protein expression of chondrocyte-specific markers. The sections were evaluated, at the same time, after the last biopsy had been taken. The pathologist was to perform his assessments without knowledge of which dose level had been administered to the individual patient. The original study protocol and protocol amendment 1.7 state that there were to be 2 pathologists; each was to make an independent assessment of the same sections. However, this approach of using 2 pathologists was abandoned due to difficulties in identifying two independent expert pathologists from independent institutes. Nevertheless, preparation of histological specimen and staining of sections were performed by a separate independent institute of pathology and sections were transferred to the expert pathologist.

Statistical methods

For sample size estimation, a sample size of at least 25 patients was planned. The principle of testing ordered hypotheses was applied with respect to KOOS. Prospective evaluation of superiority comprised two steps, in series:

- Test for superiority against baseline for the three dose groups (first the highest, then the second highest, then the lowest), using the paired t test procedure; and
- Comparison of the three dose groups with one another.

No adjustment for multiple testing was made. The final evaluation of superiority was performed after 12 months (FA) – that is, 12 months after the last patient had received study treatment. Exploratory follow-up statistical evaluations will be carried out additionally after 24, 36, 48 and 60 months. Thus, while differences between treatment groups were evaluated, they were not the basis for sample size calculations.

Results

Participant flow

This trial is conducted at 10 sites (orthopaedic clinics) in Germany. A total of 163 patients were screened. Of these, 75 patients were eligible for inclusion in this study. Twenty-five patients each were randomized to the low dose group (3–7 spheroids/cm^2): the medium dose group (10–30 spheroids/cm^2); and the high dose group (40–70 spheroids/cm^2), respectively.

The ITT population included 73 patients. Two patients did not receive a transplant and were included in the safety population only. Seven patients withdrew, or were withdrawn prematurely from the study during the first 12 months: 2 in the low-dose group, none in the medium-dose group and 5 in the high-dose group. Up to the present 3-year follow-up window, 16 patients withdrew, or were withdrawn prematurely: 6 in the low-dose group, 1 in the medium-dose group and 9 in the high-dose group. In total, major protocol violations led to exclusion of 22 patients from the PP population during the initial year of the study (12 months after transplantation). The majority of these patients (14 of the 22) were high-dose patients. In 11 of these cases, the high dose was not fully achieved as planned. On the other hand, 6 patients received a higher than 'low' dose (see table below). In each case, the reason was either the fact that the chondrocyte culture did not generate the required number of spheroids or that the assessment of the actual defect size (measured at the time of implantation) had not been sufficiently accurate. A further patient (low-dose group) was wrongly included (defect too small) and subsequently received too high a dose. The issue of the deviation from the randomised treatment was approached by performing an ancillary efficacy analysis based upon re-grouping of the patients according to the actual dose administered.

			Dose group			
			Low	Medium	High	Total
Actual dose (spheroids/cm²)	*Below 'low'*	(<3)	–	–	–	–
	Low	(3–7)	18	–	–	18
	'Low' to 'medium'	(>7, <10)	4	2	–	6
	Medium	(10–30)	2	22	5	29
	'Medium' to 'high'	(>30, <40)	–	1	6	7
	High	(40–70)	–	–	13	13
	Above 'high'	(>70)	–	–	–	–
	Total		24	25	24	73

Doses at the planned level for the respective group are emphasized by shading.

Recruitment

The study included patients from orthopaedic clinics in Germany (75 patients).

Conduct of the study

The original protocol was issued on 8th July 2010. Four protocol amendments were implemented. An updated Statistical Analysis Plan (SAP) was issued on 23rd November 2015. Most of the protocol changes were of practical and/or administrative nature with no significant impact on study outcome. Also, additional analysis of subgroups and covariates was introduced: primary and secondary efficacy endpoints were to be investigated for the following prospectively defined subgroups and covariates in the analysis; the subgroup: patients with compliance in terms of postoperative rehabilitation; the covariates: age of patients, orthopaedic clinics. Amendment 3 introduced a change in definitions for documenting AEs which appears to have pertained to a restriction of what events would be reported as an AE. However, this change was never implemented, i.e. AEs were reported as appropriate.

Baseline data

The analysis population (safety population) comprised 75 patients (22 women, 53 men) aged 34 ± 9 years (19 to 48 years). All but two of the study patients were Caucasian (73 patients, 97%); There was an imbalance with respect of smoking, with roughly equal numbers of smokers and nonsmokers in the low-dose group but a minority of smokers (less than 1 in 4) in the two other dose groups. Thirteen of 25 patients in the low-dose group, 18/25 in the medium-dose group and 15/25 in the high-dose group were recorded as having concomitant illnesses at screening. The distribution did not substantially differ between the groups.

Medication before the study was recorded for 36 (48%) of the patients overall and with slightly different frequencies in the three dose groups (low dose, 60%; medium dose 36%; high dose 48%; numbers of medications taken were unremarkable and were approximately balanced across the treatment groups. Defect sizes ranged from 2 to 10 cm^2. The average defect size at arthroscopy was 5.0 ± 1.9 cm across all dose groups. The average defect size as seen at the time of implantation was 5.6 ± 1.6 cm. The waiting times between the pre-arthroscopy day and the pre-implantation day were similar between the dose groups. For all groups combined, this was 53 ± 10 days. Primary defects located on patella or femur were treated; the tibia was not represented. Type and grading of defect are summarised in the tables below. Primary (study) defects were mostly (47/75; 63%) of the patella, the rest (28/75; 37%) being of the femur; no primary defect was of the tibia. ICRS grades were mostly III C or IV A, and were fairly evenly distributed between the groups.

ICRS grade of knee defects on day of arthroscopy (ITT population)

Primary defect location:	Femur			Patella		
Dose group: ICRS grade	Low $N=9$	Medium $N=10$	High $N=9$	Low $N=15$	Medium $N=15$	High $N=15$
III	–	–	–	–	–	–
III A	–	1	–	–	1	–
III B	1	–	1	–	2	1
III C	3	1	3	4	3	2
IV	1	–	–	–	2	3
IV A	4	7	5	11	7	9
IV B	–	1	–	–	–	–
IV C	–	–		–	–	–
All	9	10	9	15	15	15

Numbers of patients are given.

Type of knee defect on day of arthroscopy (ITT population)

Dose group: Diagnosis	Low $N=12$	Medium $N=14$	High $N=13$	All patients $N=75$
Traumatic cartilage lesion	14	14	9	37
Osteochondritis dissecans	1	2	3	6
Osteoarthritis	2	2	3	7
Other	8	7	10	25

Numbers of patients are given.

Numbers analysed

The study allowed a wider range of primary defect locations. The primary defect was either the patella (47/75 patients, 63%), medial condyle (24/75 patients, 32%) or lateral condyle (4/75 patients, 5%). Tibia plateau lesions were not included. The cause or reason for developing a cartilage defect was in most cases 'trauma' followed by 'other'. In the study, of the 75 patients, 37 had a traumatic cartilage lesion and 24 had 'other', as compared to 7 with OD and 7 with OA, respectively. The applicant was requested to further specify the diagnosis subgroup 'other' to obtain more clarity on the origin of the defect. The rationale for using the category 'other' was to stay in line with the validated forms of the International Cartilage Repair Society (ICRS) Cartilage Injury Evaluation Package. In few cases, 'overloading' was assumed by investigators but in most cases, the reasons were unknown and no further specifics were captured. No bias is assumed to have been caused by the use of this subgroup however.

Outcomes and estimation

Primary endpoints: The primary variable was the overall KOOS, calculated for the ITT population by averaging the normalised sub-scores. The primary analysis was that of the primary variable 12 months after implantation; the hierarchical statistical testing yielded the following results:

- Step 1, comparison between Visit 4 and baseline for the high-dose group: $p = 0.0005$
- Step 2, comparison between Visit 4 and baseline for the medium-dose group: $p < 0.0001$
- Step 3, comparison between Visit 4 and baseline for the low-dose group: $p = 0.0002$
- Step 4, between-group comparison:

High vs. medium dose: $p = 0.7160$

Medium vs. low dose: $p = 0.9693$

High vs. low dose: $p = 0.6933$

Overall KOOS up to 12 months after transplantation (ITT population)

Visit	Dose group:	Low $N = 24$*	Medium $N = 25$	High $N = 24$	All $N = 73$*
Values at each visit					
Pre-arthroscopy (baseline)	mean ± SD	60.4 ± 13.6	59.6 ± 15.4	51.1 ± 15.4	57.0 ± 15.2
	median	62	62	52	58
Pre-implantation (Day 0')	mean ± SD	62.1 ± 12.9	62.4 ± 15.5	51.5 ± 18.2	58.7 ± 16.3
	median	64	60	53	59
Visit 1 (6 weeks)	mean ± SD	52.6 ± 18.9	49.3 ± 12.5	46.9 ± 14.2	49.6 ± 15.3
	median	49	47	47	48
Visit 2 (3 months)	mean ± SD	65.7 ± 15.6	63.5 ± 10.6	58.0 ± 62.4	62.4 ± 15.1
	median	65	62	60	62
Visit 3 (6 months)	mean ± SD	75.2 ± 14.0	73.4 ± 8.9	61.8 ± 19.7	70.2 ± 15.8
	median	80	72	61	72
Visit 4 (12 months)	mean ± SD	77.8 ± 14.1	76.3 ± 11.5	65.3 ± 23.2	73.2 ± 17.6
	median	78	77	60	74
Changes from baseline					
Pre-implantation (Day 0')	mean ± SD	1.8 ± 9.5	2.8 ± 8.4	0.4 ± 11.5	1.7 ± 9.8
	median	3	5	1	3
Visit 1 (6 weeks)	mean ± SD	–8.3 ± 19.9	–10.3 ± 15.0	–4.1 ± 16.9	–7.6 ± 17.2
	median	–6	–10	–1	–7
Visit 2 (3 months)	mean ± SD	4.3 ± 16.8	3.9 ± 16.0	7.0 ± 15.5	5.0 ± 15.9
	median	3	4	6	4
Visit 3 (6 months)	mean ± SD	14.2 ± 17.5	13.8 ± 16.2	10.7 ± 17.9	12.9 ± 17.0
	median	17	11	11	15
Visit 4 (12 months)	mean ± SD	16.9 ± 18.5	16.7 ± 17.9	14.2 ± 18.5	15.9 ± 18.1
	median	18	17	15	17

Baseline = Day 0. [* The baseline value for the overall KOOS of one patient in the low-dose group was missing, because of incomplete response to the ePRO questions. Therefore, $N = 23$ for this group (and $n = 72$ total) at baseline and in the

The time course of the overall KOOS changes was similar for the 3 dose groups although the high-dose group showed numerically the smallest improvement from baseline. Overall, the data showed a clinically relevant and sustained improvement in KOOS. Since all patients underwent a strict rehabilitation after transplantation

and given the absence of dose-response, as well as the absence of a comparator arm in this study, the applicant was requested to further discuss the impact of the rehabilitation program on the primary outcome. The standardised rehabilitation period lasted only 3 months with additional instructions beyond this time period left at the discretion of the investigator. A review of the current literature indicates a lack of studies with regard to later stages of rehabilitation (after the early postoperative period), how this should be optimized, and the need to investigate the effect of an ongoing or midterm (1-2 years) rehabilitation intervention. It could be considered that a longer standardised rehabilitation and the use of a control arm (e.g. rehabilitation with allowance of pain medication for a limited period of time) in the Phase II study would have been desirable from a methodological perspective to better contextualize the effects of rehabilitation and pain medication although this should be weighed against other concerns e.g. in terms of postponing surgical intervention in patients with large cartilage lesions.

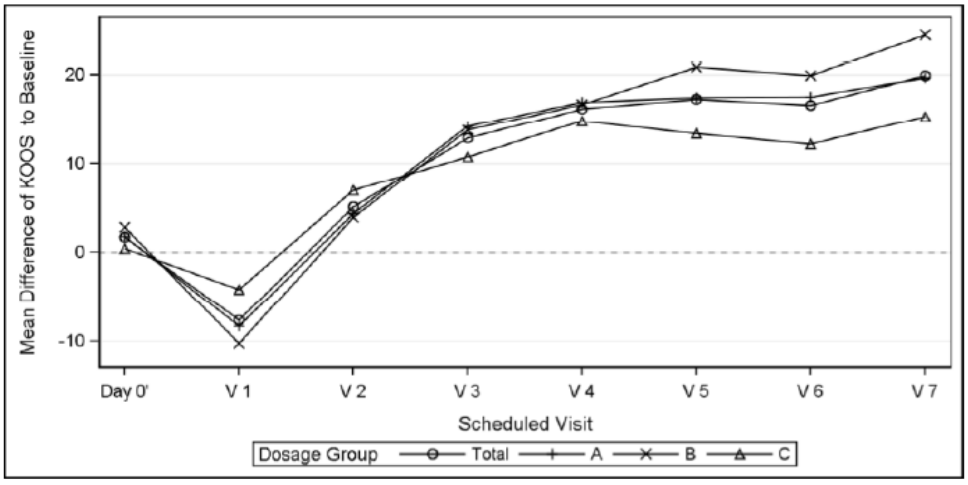
Secondary endpoints: Following the primary analysis, in the next 12 months the improvement in the primary variable 'overall KOOS' continued in the low-dose and medium-dose groups, while levelling off slightly in the high-dose group. The assessment of the primary variable 'overall KOOS' for the ITT population showed a statistically significant improvement, as compared with baseline, in all 3 within-group analyses (for the high-, medium- and low-dose group respectively) one year after study treatment (see above under primary analysis), similarly: *p* 0.0002, <0.0001 and 0.0054 respectively, after two years and without further notable change after three years (*p*<0.0001, <0.0001, and 0.0003).

Overall KOOS up to 36 months after transplantation (ITT population)

Visit	Dose group:	Low $N = 24$*	Medium $N = 25$	High $N = 24$	All $N = 73$*
Values at each visit					
Pre-arthroscopy (baseline)	mean ± SD	60.4 ± 13.6	59.6 ± 15.4	51.1 ± 15.4	57.0 ± 15.2
	median	62	62	52	58
Visit 4 (12 months)	mean ± SD	77.8 ± 14.1	76.3 ± 11.5	65.9 ± 22.6	73.4 ± 17.3
	median	78	77	60	74
Visit 5 (18 months)	mean ± SD	78.6 ± 13.2	80.4 ± 10.6	64.5 ± 22.8	74.6 ± 17.6
	median	78	82	60	77
Visit 6 (24 months)	mean ± SD	78.5 ± 15.0	79.5 ± 14.6	63.3 ± 20.9	73.8 ± 18.4
	median	80	82	58	79
Visit 7 (36 months)	mean ± SD	80.2 ± 13.2	84.1 ± 12.0	66.4 ± 22.0	77.0 ± 17.8
	median	82	85	62	81
Changes from baseline					
Visit 4 (12 months)	mean ± SD	16.9 ± 18.5	16.7 ± 17.9	14.8 ± 18.0	16.1 ± 17.9
	median	18	17	15	17
Visit 5 (18 months)	mean ± SD	17.4 ± 17.4	20.8 ± 13.8	13.4 ± 19.7	17.3 ± 17.1
	median	20	20	16	18
Visit 6 (24 months)	mean ± SD	17.5 ± 18.8	19.9 ± 14.8	12.2 ± 19.5	16.6 ± 17.8
	median	21	19	11	18
Visit 7 (36 months)	mean ± SD	19.7 ± 15.6	24.5 ± 15.2	15.3 ± 17.5	19.9 ± 16.3
	median	21	23	12	22

Baseline = Day 0. [* The baseline value for the overall KOOS of one patient in the low-dose group was missing, because of incomplete response to the ePRO questions. Therefore, $N = 23$ for this group (and $n = 72$ total) at baseline and in the entire lower block.]

Overall KOOS up to 36 months – changes from baseline (ITT population)



KOOS subscores: All KOOS subscores showed some improvement; these improvements ranged from approximately 10 percentage points to approximately 25 percentage points. This was observed about equally for all dose groups, without a discernible relationship between dose and effect. The strongest mean improvements compared with baseline (ITT overall) were seen for 'knee-related quality of life' (22.3 percentage points) and 'function in sport and recreation' (17.0 percentage points). The significance testing performed for the primary analysis was repeated for each of the KOOS subscores for each visit. All of the within-group comparisons (Visit 4, Visit 6 or Visit 7 against baseline) gave p values below 0.05, with the single exception of 'sport and recreation' for the high-dose group at Visit 6 only (p = 0.1285, t test). In contrast, all of the between-group comparisons gave p values that were above 0.05.

Change in KOOS from Day 0' (pre-implantation day): Using a different reference point as 'baseline', namely the day prior to implantation (Day 0'), changes in overall KOOS with respect to Day 0' did not differ to any relevant extent with respect to baseline. The significance testing performed for the primary analysis was repeated for each of the KOOS subscores. All of the within-group comparisons (Visit 4 against baseline, or Visit 4 against Day 0') gave p values below 0.05.

MOCART scores: The mean MOCART total scores – on a scale from 0 (worst) to 100 (best) – at Visit 2 were 59.8, 64.5 and 64.7 for the low-dose, medium-dose and high- dose group respectively, and 62.9 for 'all patients'; at Visit 4 these were 74.1, 74.5 and 68.8 for the respective dose groups and 72.4 for 'all patients. At Visits 5, 6 and 7 (18, 24 and 36 months after study treatment) the improvement was maintained. Modest improvements in MOCART score from Visit 2 onward were observed with no evidence of a dose-response. Also, the absence of a baseline measurement precluded a within-group comparison from baseline. In a between-group ANOVA for Visits 4 (12 months), 6 (24 months) and 7 (36 months), no statistically significant results were obtained (i.e. all p values were >0.05).

In the study report, it was stated: 'the baseline score can be regarded as having been the worst possible or close to it' (i.e. close to zero). Further justification was requested for this statement as no assessment of MOCART was actually obtained before the start of the Phase II study (or the Phase III study). In the literature, for example, in a recent study using MACI in 21 patients with symptomatic, traumatic chondral defects (Marlovits et al, Am J Sports Med. 2012), the quality of repair tissue was assessed by magnetic resonance imaging using the MOCART score at baseline as well as at months 3 and 6 and years 1, 2, and 5. In this case series, the mean defect size was 5.1 cm2 (range, 2.4-9.9 cm2) and the baseline MOCART was

52.9 ± 12.5. It was sufficiently clarified that no comparisons to baseline were made and could be made; the statistical analysis were solely performed using the available MOCART scores after treatment, with the starting point at 3 months up to 36 months follow-up. Some overall MOCART scores were missing as exemplified by the table below. This related to the fact that the overall score is a composition based on 9 items and the overall value could only be obtained in patients for whom all items were evaluated, which was not always the case. The missing scores were caused to large extent by the missing item 5b, as the required MRI sequence for this item was not conducted by some radiologists. However, the Applicant provided a rationale for the missing data, as well as a comparative assessment of the scores for the 8/9 items for the patients not included due to missing item 5b as compared to the patients with complete data which did not demonstrate a selection bias. Additional MOCART data will be forthcoming with the submission of future follow-up data.

The correlation between KOOS and MOCART results was investigated by calculating Spearman's correlation coefficient for all patients for whom both KOOS and MOCART results were available. A dose-dependence of a better subjective or functional outcome in correlation with an increase in the structural parameters of the MOCART score could not be observed. This lack of correlation between clinical and radiological outcomes after surgical interventions for cartilage is consistent with the published literature. The reasons for this are likely multifactorial but are currently insufficiently understood.

MOCART scores by visit (ITT population)

	Visit 2 (3 months)				Visit 4 (12 months)			
	Dose group				Dose group			
	Low N = 20	Medium N = 19	High N = 19	All N = 58	Low N = 22	Medium N = 19	High N = 21	All N = 62
Mean ± SD	59.8 ± 10.9	64.5 ± 10.3	64.7 ± 9.4	62.9 ± 10.3	74.1 ± 13.0	74.5 ± 14.5	68.8 ± 11.4	72.4 ± 13.0
Minimum	45	50	45	45	55	50	45	45
Lower quartile	53	60	60	60	60	60	65	60
Median	60	60	65	60	75	75	70	70
Upper quartile	68	75	70	70	85	90	75	80
Maximum	85	85	90	90	95	100	100	100
	Visit 5 (18 months)				Visit 6 (24 months)			
	Low N = 23	Medium N = 22	High N = 21	All N = 66	Low N = 23	Medium N = 25	High N = 21	All N = 69
Mean ± SD	72.2 ± 11.7	72.0 ± 17.0	69.0 ± 13.4	71.1 ± 14.0	72.2 ± 11.5	76.0 ± 14.2	71.2 ± 9.7	73.3 ± 12.1
Minimum	50	20	50	20	35	55	55	35
Lower quartile	65	65	60	65	70	65	65	65
Median	70	73	65	70	75	70	70	70
Upper quartile	80	85	75	80	80	90	75	80
Maximum	95	95	100	100	85	100	90	100
	Visit 7 (36 months)							
	Low N = 23	Medium N = 25	High N = 21	All N = 69				
Mean ± SD	72.4 ± 11.8	79.6 ± 13.1	72.1 ± 13.9	74.9 ± 13.2				
Minimum	50	50	30	30				
Lower quartile	65	70	65	70				
Median	70	80	70	75				
Upper quartile	85	90	80	85				
Maximum	90	100	95	100				

Differences in *N* from total population sizes are due to missing results.

*Arthroscopic assessment of cartilage repair (according to ICRS):*_Visual assessment of repair according to ICRS was done at Visit 4 for those patients (8) who consented to the additional arthroscopy and regenerated tissue biopsy. For two patients (high-dose group), their cartilage was assessed as 'normal', five (medium- and low-dose groups) as 'nearly normal' and one (low-dose group) as 'abnormal'. None were rated as 'severely abnormal'.

ICRS visual histological assessment: Eight patients (3 with femoral condyle lesions, 5 with retropatellar lesions) consented to a biopsy of the regenerated tissue (R-biopsy) in conjunction with a second-look arthroscopy. The ICRS visual histological assessment scale allows the assessment of the quality of the regenerated tissue and the extent to which its characteristics resemble those of native hyaline cartilage. This scoring system assesses 6 components of repair in histology sections (surface, matrix, cell distribution, cell population viability, subchondral bone, and cartilage mineralisation) (no assessment of subchondral bone could be performed in the current study). Biopsy specimens could be analysed for 7 patients (see table below). The repair tissue in almost all R-biopsies showed a normal presence of predominantly viable cells (6 of 7 patients) and a normal cartilage mineralization (5 of 6 patients), obtaining the maximum score for these 2 features. For the other features describing the histological appearance of the repair tissue, a larger variability was observed between the different patients. The majority of the R-biopsies (3 of 5 patients) showed a smooth cartilage surface, whereas the remaining 2 patients showed an irregular surface. For the remaining features, cellular distribution and type of extracellular matrix (ECM), the patients were divided in 3 groups, of approximately 1/3 showing a high score, 1/3 a middle score and 1/3 a low score.

Individual ICRS histological assessments

Patient no.	Dose group	Feature				
		I. Surface	II. Matrix	III. Cell distribution	IV. Cell viability	VI. Cartilage mineralisation
1702	low	Smooth/ continuous	Fibrocartilage	Individual cells/ disorganized	Predominantly viable	Abnormal/inappropriate location
1803	medium	Discontinuities/ irregularities	Fibrous tissue	Individual cells/ disorganized	Partially viable	(*)
2220	low	Smooth/ continuous	Fibrocartilage	Clusters	Predominantly viable	Normal
2221	high	(*)	Hyaline	Columnar	Predominantly viable	Normal
2235	low	Discontinuities/ irregularities	Fibrous tissue	Individual cells/ disorganized	Predominantly viable	Normal
2259	low	(*)	Hyaline	Columnar	Predominantly viable	Normal
2301	medium	Smooth/ continuous	Fibrocartilage	Individual cells/ disorganized	Predominantly viable	Normal

For details see earlier report version. (*) No assessment recorded.

The overall results indicate that the cell transplant resulted in the generation of a cartilage repair tissue that varied from good to low quality but the number of biopsies was very limited. The biopsy samples were mainly from retropatellar site and only three samples were from weight-bearing joint. Overall, the number of samples for histology is too small and exposed to selection bias to draw firm conclusions on cartilage quality.

Bern Score and histological and immunohistochemical staining of chondrogenic markers: The Bern score was originally developed as a histological grading system for assessment of neocartilage generated in vitro, based on Safranin O staining. A maximum score of 9 can be obtained which would reflect mature hyaline cartilage.

The overall mean Bern score (based on 7 biopsies) was 6.4 ± 2.8. The value of 6.4 (on a scale from 0 to 9), indicates successful growth and development of cartilage cells from the implant and production of hyaline-like ECM, resulting in the formation of repair tissue displaying hyaline cartilage characteristics. As expected from the very small numbers of patients, between-group statistical tests did not reveal any trend. A large variation in the different patient samples was observed, with the repair tissues showing varying characteristics. However, the presence of most of the hyaline cartilage-specific proteins was observed in all repair tissues, with varying expression levels. Proteoglycans were present in all samples, as shown by the Alcian Blue staining and the presence of aggrecan. In addition, the chondrogenic marker proteins COMP, S100B and DLK1/PREF-1 were present in all samples analyzed, whereas CRTAC1 was absent in only one sample. However, none of the samples showed strong expression of all markers. This suggested that all repair tissues displayed hyaline cartilage characteristics to some extent, but that no sample displayed a true hyaline cartilage phenotype.

ICRS II histological scoring: Assessment was performed according to the ICRS II histological scoring. Overall, the overall ICRS histological score at Visit 4 was 46 ± 29 (out of maximum of 100 for healthy cartilage), with a median value of 60 and a range from 10 to 70. The number of patients available for this was limited to 7, and limited further due to missing subscores.

*ICRS/IKDC Knee examination form (surgeon's part) – overall assessment:*_The overall IKDC score was assessed in terms of the grades A–D, defined in "2000 IKDC Knee Examination Form" , where 'A' represents a good rating and 'D' a poor one. Baseline distributions between the dose groups were well comparable, as shown by a Kruskal–Wallace test (p = 0.3459).

IKDC Knee Examination Form – overall assessment (ITT population)

Dose group:	Low dose				Medium dose				High dose				All patients			
IKDC Grade:	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Pre-arthroscopy day	15	5	3	1	10	12	2	1	9	11	3	1	34	28	8	3
Pre-implantation day	10	11	2	1	7	11	7	–	6	8	7	3	23	30	16	4
Visit 1 (6 weeks)	2	4	4	14	1	6	6	12	3	4	3	14	6	14	13	40
Visit 2 (3 months)	11	8	4	1	7	12	4	2	10	6	7	1	28	26	15	4
Visit 3 (6 months)	16	6	2	–	17	6	2	–	13	9	1	1	46	21	5	1
Visit 4 (12 months)	21	3	–	–	21	3	1	–	18	4	2	–	60	10	3	–
Visit 5 (18 months)	22	2	–	–	24	1	–	–	16	6	2	–	62	9	2	–
Visit 6 (24 months)	23	1	–	–	24	1	–	–	15	6	3	–	62	8	3	–
Visit 7 (36 months)	22	1	1	–	24	1	–	–	19	4	1	–	65	6	2	–

Numbers of patients with the respective grade at each visit are shown.

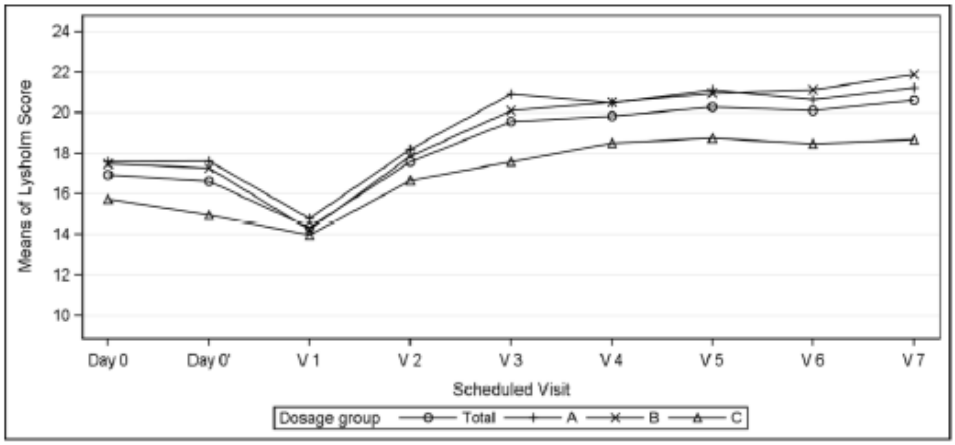
Within-group comparisons using Wilcoxon's signed-rank test gave statistically significant results, for the low-, medium- and high-dose groups respectively for almost all comparisons (from Day 0 to Visits 4, 6, and 7 for each dosage group, respectively and also from Day 0' to Visits 4, 6, and 7 for each dosage group).

IKDC Subjective Knee Evaluation Form (patient's part): The 2000 IKDC Subjective Knee Evaluation Form is used to detect improvement or deterioration in symptoms, function, and sports activities due to knee impairment. The possible score range 0–100, where 100 = no limitation with daily or sporting activities and the absence of symptoms. Results were similar for as for the physical IKDC knee examination, i.e.

statistically significant changes from baseline (both with respect to Day 0 baseline and Day 0' baseline) for all dose groups with a lack of difference between the groups.

Modified Lysholm Score: The Modified Lysholm Score (used as a self-complete measure in surgical studies involving patients with chondral damage) allows a maximum of 24 points (the higher the score, the better the patient's knee condition). Similar to the IKDC Subjective Knee Evaluation Form, there were clear potentially significant within-group differences ($p < 0.05$, descriptive level), but no systematic between-group effects. An MCID has not been defined for the modified Lysholm scale.

Modified Lysholm Score – mean values (ITT population)



Ancillary analyses

Subgroup analyses: The primary analysis (overall KOOS score) was repeated for the patient strata 'defect size 4–6.99 cm2' in 63 ITT patients and '7–10 cm2' in the remaining 10 ITT patients. Statistical significance for overall KOOS score was reached for the larger stratum (4–6.99 cm2), but not for the smaller one with larger lesions (7– 10 cm2), as could be expected on the basis of the much smaller patient numbers (only 10) in the latter category.

KOOS overall score was also analysed by the following categories:

-Diagnosis: Traumatic cartilage lesion, osteochondritis dissecans, osteoarthritis (but without radiological signs of osteoarthritis, as these constituted an exclusion criterion), avascular necrosis (AVN) or other diagnoses

-Defect location: Femur, tibia or patella (however, no patients with primary tibia lesions were enrolled)

-Patient's age category: 18–34 years or 35–50 years

-Patient's sex: Male / female

In general, no systematic trends that might have reflected underlying differences between the results of treatment at the follow-up visits (12, 24 and 36 months after implantation) were found. The applicant was requested to further discuss the relevance of prior treatment of the cartilage defect on the outcome of second line ACI. For example, autologous chondrocyte implantation after failed microfracturing appears to be associated with a significantly higher failure rate and inferior clinical outcome when compared with ACI as a first-line treatment (Pestka JM, et al. Am J Sports Med. 2012:40[2]:325-31). However, it was confirmed that none of the patients in the Phase II study was subjected to prior treatment with microfracture. A comparison of patients with previous knee surgeries showed a lower KOOS value at baseline than patients without previous surgeries. However, the 36 month follow up (Visit 7) for Phase II revealed a clear improvement by

20.6 KOOS points from baseline, similar to the 22.84 KOOS points in the group of patients without previous knee surgery. The applicant was also requested to discuss the treatment benefit in the femoral cartilage lesion subgroup over the whole spectrum of the studied lesion size. In addition, in the high dose group, there were fewer patients with traumatic lesion and more patients with OD, OA or other diagnosis included. A descriptive subgroup analysis was provided, which showed numerically better results in femur lesions compared to patellar lesions and better response in traumatic lesions when compared to the overall response in femur lesions. The mean improvement in KOOS score was similar between lesion size strata ≥4-<7 cm^2 and ≥7-10 cm^2 in the ITT population.

Supportive analyses of the primary endpoint: The primary analysis was repeated on an 'observed cases' basis, i.e., with no imputation in the case of missing data. The results of the 'observed cases' analysis were consistent with, and thus supported, those of the primary analysis. Significance levels were, as is to be expected, somewhat weaker (greater p values) than in the primary analysis, but no p values exceeded 0.05 that had not done so in the primary analysis. The within-group and between-group PP comparisons were performed as for the ITT population. For the PP analysis of the overall KOOS score – analogous to the primary ITT efficacy analysis – the following results were obtained:

Step 1, comparison between Visit 4 and baseline for the high-dose group: p = 0.6174

Step 2, comparison between Visit 4 and baseline for the medium-dose group: p = 0.0015

Step 3, comparison between Visit 4 and baseline for the low-dose group: p = 0.0033

Step 4, between-group comparison, high vs. medium dose: p = 0.2568

medium vs. low dose: p = 0.6178

high vs. low dose: p = 0.1560

Thus, had the PP analysis been a formal hierarchical analysis, then it would have been terminated after the first comparison (step 1) and continued, if at all, at a descriptive level. The second and third comparisons (steps 2 and 3) gave p values below the significance threshold. As already found for the ITT analysis, the difference between treatments (step 4) was not statistically significant. As mentioned early, major protocol violations affected in particular the patient numbers in the high-dose group based on a spheroid dose being given below the required range. Based on this, the applicant conducted an analysis of the primary efficacy variable by dosage group ('as treated') (using 3-8.5 spheroids/cm2 [low dose], 8.6-35 spheroids/cm2 [median dose] and 35.1-70 speroids/cm2 [high dose] dose levels (see cod 16 HS 14, 36-month follow up CSR):

Low dose:	3–8.5 spheroids/cm²	$N = 20$
Medium dose:	8.6–35 spheroids/cm²	$N = 38$
High dose:	35.1–70 spheroids/cm²	$N = 15$

This 'as treated' analysis yielded similar results as the primary ITT analysis (ie significant differences from baseline at Visit 4 for all three dose groups with no differences between the groups). The applicant was asked to further evaluate possible tendencies towards a dose response relationship on a continuous scale if possible, for example, using actual given dose for each patient and change from baseline to Month 12 in KOOS for each patient as the response. The applicant provided this analysis both for the ITT and the PP population. No evidence of a dose response relationship was present.

Phase III study Cod 16HS 13

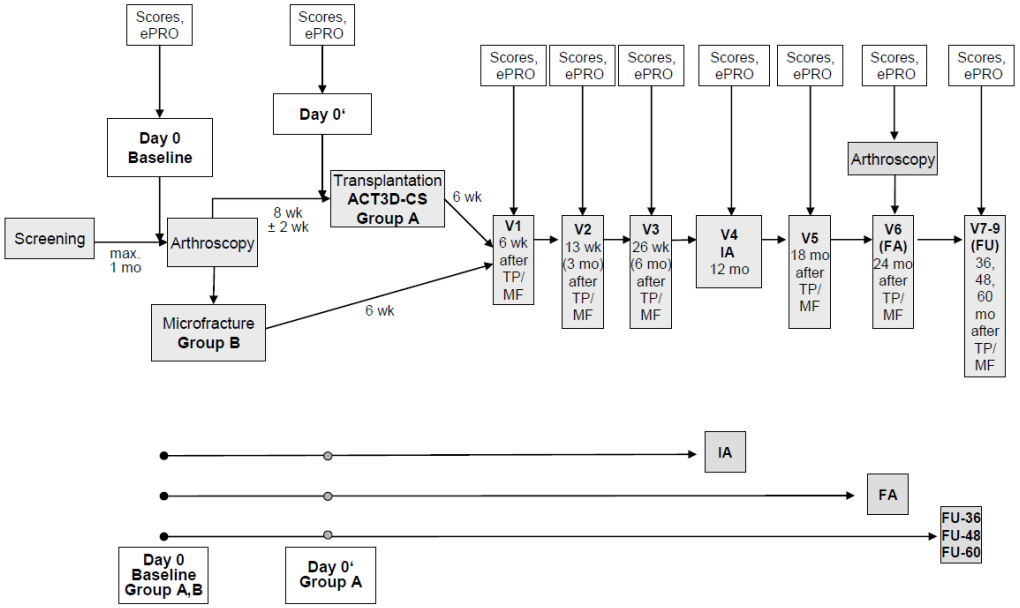
Study cod 16 HS 13 is an ongoing prospective, randomised, open label, multicentre Phase III clinical trial to compare the efficacy and safety of treatment with the autologous chondrocyte transplantation product Spherox (ACT3D-CS) with microfracture (MF) in subjects with cartilage defects of the knee with a defect size between 1 and 4 cm^2.

Methods

After transplantation of ACT3D-CS (Group A) or microfracture (Group B), post-intervention observational phases of 6 weeks, 3, 6, 12 months (interim analysis (IA)), 18 months, 24 months (final assessment), 36, 48 and 60 months (follow-up assessments) are planned for patients in both groups.

Results are available from the Interim Analysis Report (12 months after treatment).

Flowchart study cod 16 HS13



wk = weeks, mo = months, ePRO = electronic Patient Reported Outcome, IA=Interim Analysis, V = visit, TP = transplantation (group A), MF = microfracture (group B). For definitions of Day 0 and Day 0', see Appendix 1

The choice of comparator i.e. microfracture for this range of defect size has been agreed upon in a previous central advice procedure (EMEA/H/SA/1146/1/FU/2009/SME/ADT/II) and is also suggested as an option in the EMA reflection paper on chondrocytes products (EMA/CAT/CPWP/568181/2009). Follow-up times were calculated from Day 0 for the microfracture group and Day 0' for the ACT3D-CS group. This implicates that time from randomisation differed between the two treatment groups in this study.

Study participants

The included patient population consisted of male or female patients between 18 and 50 years of age; with isolated ICRS grade III or IV single defect on femoral condyle; defect size: 1 to < 4 cm^2 after debridement to healthy cartilage, up to 6 mm in depth; nearly intact surrounding chondral structure around the defect as well as corresponding joint area. To qualify for inclusion, patients had to agree to adhere to strict rehabilitation protocol and follow-up programme and agree to adhere to rules regarding pain medication.

In contrast to the Phase II study cod 16 HS 14, patients with primary defect of the patella, tibia or of the trochlea alone were not included. The same exclusion criteria were used as in the Phase II study cod 16 HS 14 except that patients with osteochondritis dissecans (OCD) were excluded in study cod 16 HS 13.

Treatments

Group A: ACT3D-CS. The dose was allowed to range from 10-70 spheroids/cm^2.

Group B: Microfracture (MF)

The technique of microfracture was performed according to Steadman et al (2003). 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.

Post-treatment rehabilitation programme

Patients treated with ACT3D-CS: After surgery, the patient underwent a strict rehabilitation programme that already started during his/her stay in the clinic, and which continued at home for up to three months after the intervention.

The physical therapists were instructed by the investigators to follow strictly the rehabilitation programme. On the day of his/her discharge from hospital, the patient received instructions for continuing with regular physical exercise. On the day of discharge from hospital, at Visit 1 (after 6 weeks) and at Visit 2 (after 13 weeks), the investigator assessed the conduct of the rehabilitation programme up to the respective time point and recorded his/ her assessment in the eCRF.

Patients treated with microfracture: Lesions on the weight-bearing surfaces on the condyles were to be treated immediately after the operation, beginning in the recovery room, with a continuous passive motions (CPM) machine, according to the following instructions: “The initial range of motion is 30° to 70° and this is increased as tolerated by 10° to 20° until full range of motion is obtained. The rate of the machine is usually one cycle per minute, but the rate varies based on patient performance and comfort, the patients use the CPM machine for 6 to 8 hours per 24 hours. If patients are unable to use the CPM machine, then they receive instructions for passive flexion and extension of the knee with 500 repetitions 3 times a day. Patients are encouraged to gain full passive range of motion of the injured knee as soon as possible after the surgical procedure.”

Cold therapy was used for all patients for 1 to 7 days after the operation.

Thus, after surgery all patients underwent a strict rehabilitation programme for three months. This programme is likely to affect functional outcomes and hence the effect measured in this trial is the effect of the combination of the intervention under study and the rehabilitation programme. As the rehabilitation programmes appeared to differ between the two treatment arms and Amendment 2 of the Phase III study mentioned that improvements to the rehabilitation programme were made, further discussion was requested as to whether this could have favoured one of the treatment groups. However, the Applicant provided sufficient justification that the components of the rehabilitation program over the first 3 months were very similar between the groups and any minor differences did not affect the outcome of the study.

It was also clarified that although there are no head-to-head studies that compare different rehabilitation programs in terms of clinical outcome using different methods/products, it is most likely that the recovery period after ACI depends not only by the surgical procedure but also the individual patients’ condition (e.g.

degree of sportiness, weight, and concomitant illnesses). The Spherox rehabilitation program allows for full load of the operated knee after 8 - 12 weeks which is in line with other matrix-assisted chondrocyte implantation procedures. However, as mentioned earlier, the current literature indicates a need for more studies on the influence of intermediate and longer-term phases of rehabilitation on the final outcome.

Concomitant medication

In case of pain, patient agreed only to use paracetamol mono- (maximum 4 g/day) or a combination preparation and oral and/or topical NSAIDs during the trial and to discontinue the use of oral and/or topical NSAIDs and/or paracetamol combination preparation 1 week before each visit (i.e. only the use of paracetamol mono-preparation, maximum 4 g/day was allowed). However, in the morning of the visit day, no pain medication was allowed. Other pain medications were allowed during the surgical procedure and could be taken for a period not exceeding 4 weeks after surgery.

Objectives

The objectives of this study is the assessment of the long term efficacy and safety of the three dimensional autologous chondrocyte transplantation product ACT3D-CS in comparison to microfracture in the treatment of cartilage defects of knee joints.

Outcomes / endpoints

Primary efficacy variable: Change of overall KOOS from Day 0 (baseline for both treatment groups = pre-arthroscopy assessment) to final assessment (FA) 24 months after the end of the respective treatment, compared between the two study treatment groups (ACT3DCS and microfracture).

Secondary endpoints:

- Change of overall KOOS from baseline (Day 0) to 12 months (interim analysis, IA) and 36, 48 and 60 months (follow-up, FU) after the end of the respective treatment, compared between the treatment groups.
- Change of overall KOOS from Day 0 for microfracture or from Day 0' for ACT3D-CS to 12, 24, 36, 48 and 60 months after the end of respective treatment.
- Change of the 5 subscores of the KOOS (pain, other symptoms, function in daily living (activities of daily living, ADL), function in sport and recreation (sport/rec), knee-related quality of life (QoL) from baseline (Day 0 = pre-arthroscopy day) to 12, 24, 36, 48 and 60 months after the end of the respective treatment, compared between the treatment groups.
- MOCART (MRI Score) 12, 24, 36, 48 and 60 months after implantation or microfracture compared between the treatment groups.
- Arthroscopy and biopsy 24 months after implantation/microfracture, assessment of cartilage repair to be compared between the treatment groups.
- ICRS Visual Histological Assessment Score at final assessment (24 months) compared between the treatment groups.
- ICRS II Histological Score at final assessment (24 months) compared between the treatment groups (this comparison was therefore not performed for the present interim report).
- Bern Score and additional histological assessment scores at final assessment (24 months) compared between the treatment groups.

- Change of ICRS/IKDC from baseline (Day 0) to 12, 24, 36, 48 and 60 months after the end of the respective treatment, compared between the treatment groups.
- 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.
- Days of absence from work (employment) and/or days of inability to pursue usual activities during the last year or since the last study visit, respectively, and time point when patient was back at work and/or to could pursue usual activities.

Sample size

The sample size calculation was based on showing non-inferiority of ACT3D-CS compared to microfracture after 24 months taking an interim analysis after 12 months into account. A non-inferiority margin of 8.5 in the standardised KOOS score was used. Per treatment group a total of 67 patients (without drop outs) are recommended to proof non-inferiority of ACT3D-CS in comparison to microfracture after 24 months. A dropout rate of about 4 % of patients is expected who cannot be transplanted at Day 0'. Consequently 67 patients in the microfracture group and 73 patients in the ACT3D-CS group are recommended to proof non-inferiority.

Randomisation and blinding

Randomisation was performed via a central IVRS. Patients were stratified into two age groups: 18–34 years inclusive and >35 years. As the study was open-label. However, an independent radiologist (central reading by "blinded reader") assessed all MRI pictures after study intervention without knowledge of the applied procedure and time point of patient participation in the trial. An independent central, blinded pathologist assessed the cartilage biopsies, taken from patients of both groups after 24 months, for the evaluation of the extent of cartilage repair, of any indication of migration of the implanted spheroids, and of the protein expression of chondrocyte-specific markers. The sections were evaluated at the same time after the last biopsy had been taken. The pathologist had to make his assessments without knowledge of which treatment had been given to the individual patient.

Statistical methods

The statistical hypotheses were tested hierarchically:

First, relevant clinical improvement of ACT3D-CS versus baseline (Day 0) is tested. If the lower bound of the one-sided 97.5% confidence interval of the change in overall KOOS at 24 months (in the interim analysis: 12 months) versus baseline (Day 0) *is greater than 10 percentage points*, then relevant clinical improvement are to be considered to have been shown, and the next step (noninferiority test of ACT3D-CS in comparison with microfracture) is performed.

The difference between the improvement in overall KOOS after ACT3D-CS minus the improvement in overall KOOS after microfracture is tested, with a hypothesised value of zero and a non-inferiority margin of –8.5 points. Thus, non-inferiority is considered proven if the lower bound of the confidence interval for the difference is greater than –8.5.

The same test is used to assess superiority, with superiority considered proven if the lower bound of the confidence interval for the difference is greater than zero.

In the present interim analysis the 12-month data are used. In the original protocol an O'Brien-Fleming alpha-spending function was planned to be used for the interim analysis. Since the reason for the interim

analysis is for submission to authorities and not to stop or change the trial in any way this was changed in an amendment . This is considered acceptable.

Results

Participants flow

The following definitions were used for the analysis populations:

- The Safety population: all patients who signed an informed consent and were successfully randomized
- ITT1 population: all patients who (i) were successfully randomized, (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'.
- ITT2 population: all patients who (i) were successfully randomized, and (ii) completed the KOOS questionnaire at baseline and/or Day 0'.
- PP population: all patients of the ITT1 population without major protocol violations.

Study populations at 12 months after treatment

Treatment group:	ACT3D-CS	Microfracture	All patients
Safety population	52	50	102
ITT2 population	49	50	99
ITT1 population	48	49	97
PP population	39	41	80

Numbers of patients are given.

The principal efficacy assessment was based on the ITT1 population, with supporting ITT2 and PP Analyses. The ITT1 population included 97 patients. Five patients either had lacking baseline values (3) or did not receive their respective ACT3D-CS (1) or MF (1) treatment. The three patients who did not produce a baseline KOOS assessment were therefore excluded from the ITT2 population. Two of the ITT2 population patients did not receive treatment and were therefore excluded from the ITT1 population. The three patients excluded from the ITT population due to missing baseline values were all randomised to the ACT3D-CS group, representing 6% of this group. However, there is no indication that the exclusion of these patients altered the results in a significant way. Major protocol violations led to exclusion of 22 patients from the PP population, 13 in the ACT3D-CS group and 9 in the MF group.

Recruitment

This trial is being conducted at 11 sites (orthopaedic clinics) in Germany (10) and Poland (1). This interim analysis included all the patients who attended for their 12-month visit (i.e., excluding any patients prematurely withdrawn from the study).

Conduct of the study

The original protocol was issued on 8th July 2010. Five protocol amendments were issued: Amendment no. 1 (7th March 2012), amendment no. 2 (31th January 2013), amendment no. 3 (21th March 2014), amendment

no. 3.1 (25th June 2014), amendment no. 4 (1st June 2015). Most changes were of practical and/or administrative nature.

Baseline data

The study population comprised 41 women (40%) and 61 men (60%). All but two of the study patients were Caucasian (100 patients, 98%); the other two, both in the ACT3D-CS group, were recorded as respectively Asian and Black. The two treatment groups were well balanced in respect of age, sex, height, weight, BMI and alcohol consumption. There was a slight imbalance in respect of smoking, with approximately equal numbers of smokers and non-smokers in the microfracture group but a minority of smokers (14/52, 27%) in the ACT3D-CS group. Clearly, more patients were smokers in the microfracture group, which is unfavourable for the comparator. The applicant provided a descriptive subgroup analyses by smoking/non-smoking and age. The overall results indicate a numerically slightly lower change from baseline in KOOS in smokers treated with MF as compared to non-smokers. This was not the case for Spherox. No firm conclusions can be drawn given the limited patient numbers in the respective subgroups and hence, no further follow up is required.

Primary (study) defects were almost all of femur, with only one being in the patella and femur (this patient's inclusion was a protocol violation.

Demographic data and baseline characteristics (Safety population)

Treatment group:	ACT3D-CS $N = 52$	Microfracture $N = 50$	All patients $N = 102$
Sex: female	19	22	41
male	33	28	61
Age [years]	36 ± 10	37 ± 9	37 ± 9
Height [cm] *	177 ± 9	175 ± 9	176 ± 9
Weight [kg] *	81.3 ± 15.4	79.7 ± 13.5	80.5 ± 14.4
BMI [kg/m^2] *	25.7 ± 3.3	25.8 ± 3.0	25.8 ± 3.1
range	18.8 – 31.2	18.2 – 30.0	18.2 – 31.2
Patient smoked: yes	14	20	34
no	38	30	68
Patient drank alcohol: yes	30	24	54
no	22	26	48
Pre-debridement defect size [cm^2]	2.2 ± 0.7	2.0 ± 0.8	2.1 ± 0.8
range †	0.5 – 3.5	0.8 – 4.0	0.5 – 4.0
Post-debridement defect size [cm^2]	2.7 ± 0.8	not applicable	not applicable
range †	1.4 – 5.0		
Defect location (primary) Femur	52	49	101
Tibia ‡	–	–	–
Patella	–	–	–
Femur and patella	–	1 §	1
Defect location (further defects <ICRS grade 3) Femur	–	–	–
Tibia	2	3	5
Patella	10	10	20

* Numbers of patients or mean ± SD, or where appropriate the range (minimum–maximum), are given. † All values were within the allowed range (1–4 cm2), despite one protocol deviation. ‡ This would have represented a violation of inclusion criterion no. 2 § Violation of inclusion criterion no. 2

Type and grading of the defect are summarized in the tables below. ICRS grades were mostly III B or IV A, and were evenly balanced between the treatment groups.

Type of knee defect on day of arthroscopy (Safety population)

Treatment group: Diagnosis	ACT3D-CS N = 52	Microfracture N = 50	All patients N = 102
Traumatic cartilage lesion	19	24	43
Osteoarthritis *	1	4	5
Osteochondritis dissecans	–	1	1
Avascular necrosis	–	–	–
Other	32	21	53

Numbers of patients are given. * Osteoarthritis, but without radiological signs of osteoarthritis, as these constituted an exclusion criterion. This error has not yet been removed from the study data base; it is thus still in the listing and in some tables including the source table mentioned.

Use of medication before screening was reported by 48 (47%) of the patients overall, with slightly imbalanced frequencies in the two treatment groups: 21 patients (40%) in the ACT3D-CS group and 27 patients (54%) in the microfracture group. The most frequently used prior medications were enoxaparin and midazolam.

Concomitant medications and rehabilitation: All the patients were informed about the rehabilitation and follow-up programme and all took part in this programme after treatment. Frequently prescribed concomitant medications were pantoprazole and anticoagulants. Concomitant pain medication use was collected as a safety variable only. The numbers of patients who took pain medication are summarized in the table below. Overall, numbers of patients taking pain medication increased initially after treatment and then decreased between Visits 1 and 2.

Use of concomitant pain medication (safety population)

	Treatment group: Visit	ACT3D-CS N = 52	Microfracture N = 50
Any pain medication	Screening	21 (40%)	26 (52%)
	Pre-arthroscopy day	9 (17%)	4 (8%)
	Pre-implantation day	26 (51%)	*n.a.*
	Visit 1	36 (72%)	32 (67%)
	Visit 2	10 (20%)	15 (31%)
	Visit 3	10 (20%)	9 (19%)
	Visit 4	7 (15%)	11 (24%)
NSAID medication	Pre-arthroscopy day	6 (12%)	5 (10%)
	Pre-implantation day	2 (4%)	*n.a.*
	Visit 1	7 (14%)	5 (10%)
	Visit 2	2 (4%)	6 (13%)
	Visit 3	2 (4%)	2 (4%)
	Visit 4	2 (4%)	4 (9%)

Numbers of patients are given.

The pattern of pain medication use showed no notable differences between the treatment groups. In both groups, there was an overall trend showing a reduced patient incidence of pain medication use. This could be viewed as supportive evidence to the decrease in KOOS subscore 'pain'. Not unexpectedly, patients who took pain medication had lower KOOS values than patients who did not. However, in the microfracture group, the improvement (change from baseline) at 12 months follow-up compared to baseline was comparable in patients taking pain medication (improvement of 16.1 KOOS points) and patients taking no pain medication (improvement of 16.9 KOOS points). In the Spherox group however, the improvement at 12 months follow-up in patients taking pain medication (improvement of 12.1 KOOS) was lower than in patients taking no pain medication (improvement of 24.2 KOOS points). Why the MF arm was not similarly affected is not entirely clear but this discrepancy might be related to the small numbers of patients using pain medication at Visit 4 and beyond.

Numbers analysed

The cause or reason for developing a cartilage defect was in most cases 'trauma' followed by 'other'. In the Phase III study, 43 of the 102 patients had a traumatic cartilage lesion and 53 had 'other'. There were 5 patients with OA, 1 with OD and no patients with avascular necrosis. The applicant was requested to further specify the diagnosis subgroup 'other' to obtain more clarity on the origin of the defect. The rationale for using the category 'other' was to stay in line with the validated forms of the International Cartilage Repair Society (ICRS) Cartilage Injury Evaluation Package. In few cases, 'overloading' was assumed by investigators but in most cases, the reasons were unknown and no further specifics were captured. Thirty-five of the 52 patients in the ACT3D-CS group (67%) and 36/50 patients in the microfracture group (72%) were recorded as having concomitant illnesses at screening. Overall, the treatment groups were well balanced in respect of their medical history.

Outcomes and estimation

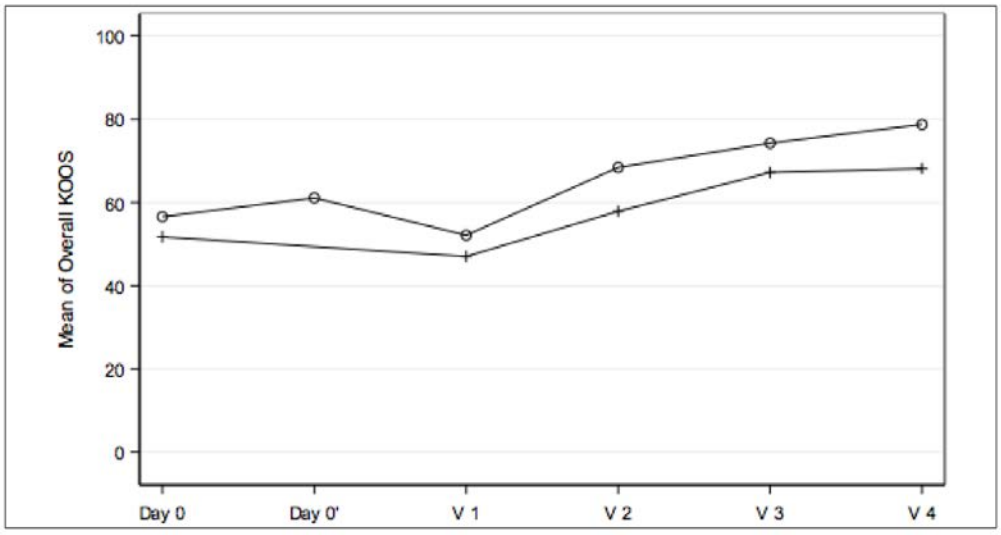
Interim analysis results of the primary efficacy variable

Complete descriptive statistics for overall KOOS at each study visit and the change at each visit from baseline is provided in the table below. An initial decrease, with a minimum at Visit 1, is seen for all groups; this is interpreted as being associated with the surgical procedure.

Overall KOOS by visit (ITT1 population)

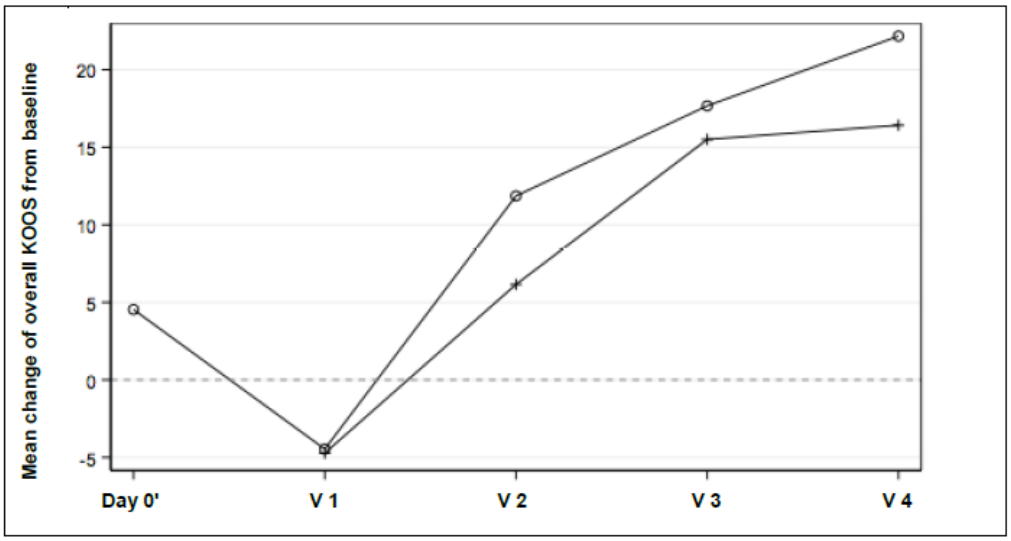
Visit	Treatment group:	ACT3D-CS *N* = 48	Microfracture *N* = 49
Values at each visit			
Pre-arthroscopy (baseline)	mean ± SD median	56.6 ± 15.4 53	51.7 ± 16.5 51
Pre-implantation (Day 0')	mean ± SD median	61.1 ± 18.2 58	–
Visit 1 (6 weeks)	mean ± SD median	52.1 ± 17.4 56	47.0 ± 16.1 48
Visit 2 (3 months)	mean ± SD median	68.4 ± 17.6 71	57.9 ± 16.9 56
Visit 3 (6 months)	mean ± SD median	74.2 ± 18.0 77	67.2 ± 16.7 69
Visit 4 (12 months)	mean ± SD median	78.7 ± 18.6 84	68.1 ± 18.6 70
Changes from baseline			
Pre-implantation (Day 0')	mean ± SD median	4.5 ± 13.3 1	–
Visit 1 (6 weeks)	mean ± SD median	–4.5 ± 18.2 –4	–4.7 ± 16.0 –4
Visit 2 (3 months)	mean ± SD median	11.9 ± 19.9 9	6.2 ± 17.8 6
Visit 3 (6 months)	mean ± SD median	17.7 ± 18.3 14	15.5 ± 14.7 15
Visit 4 (12 months)	mean ± SD median	22.2 ± 18.3 20	16.4 ± 15.1 15

Overall KOOS at each visit – mean values (ITT1 population)



ITT1 population. ○, ACT3D-CS group; +, microfracture group. Day 0 = baseline. Day 0' = day before implantation. y-axis maximum range 0–100. Visits V1–V4 took place 6 weeks and 3, 6 and 12 months after implantation.

Overall KOOS at each visit – changes from baseline Day 0 (ITT1 population)



The hierarchical statistical testing procedure for the ITT1 yielded the following results:

Step 1: Test for relevant clinical improvement from baseline to Visit 4 for the ACT3D-CS group:

Difference = 22.2 (CI 16.9–27.5), p < 0.0001 [For comparison, though not part of Step 1: Difference for microfracture = 16.4 (CI 12.1–20.8), p < 0.0001.]

Step 2: Test for non-inferiority of ACT3D-CS vis-à-vis microfracture (mean difference from baseline for ACT3D-CS minus mean difference from baseline for microfracture):

Difference = 5.7 with lower bound of CI equal to –1.0. Since this lower bound has a value greater than –8.5, non-inferiority is formally demonstrated.

Step 3: Test for superiority of ACT3D-CS vis-à-vis microfracture (mean difference from baseline for ACT3D-CS minus mean difference from baseline for microfracture):

Difference as for Step 2. Since the lower bound has a value smaller than zero, superiority is not formally demonstrated.

Analysis of variance (ANOVA) yielded the following least-squares means with corresponding standard errors and 95% confidence intervals:

ACT3D-CS: 23.1 ± 2.3 (18.5–27.6)

Microfracture: 15.5 ± 2.3 (11.0–20.0)

The hierarchical statistical testing procedure for the PP population yielded the following results:

Step 1: Test for relevant clinical improvement from baseline to Visit 4 for the ACT3D-CS group:
Difference = 21.9 (CI 16.2–27.7), p < 0.0001 [For comparison, though not part of Step 1: Difference for microfracture = 16.1 (CI 11.1–21.1), p < 0.0001.]

Step 2: Test for non-inferiority of ACT3D-CS vis-à-vis microfracture (mean difference from baseline for ACT3D-CS minus mean difference from baseline for microfracture):

Difference = 5.9 (p = 0.0001) with lower bound of CI equal to –1.6. Since this lower bound has a value greater than –8.5, non-inferiority is demonstrated at the descriptive level.

Step 3: Test for superiority of ACT3D-CS vis-à-vis microfracture (mean difference from baseline for ACT3D-CS minus mean difference from baseline for microfracture):

Difference as for Step 2. Since the lower bound has a value smaller than zero, superiority is not demonstrated.

Analysis of variance (ANOVA) yielded the following least-squares means with corresponding standard errors and 95% confidence intervals:

ACT3D-CS: 22.2 ± 2.5 (17.2–27.1)

Microfracture: 15.9 ± 2.4 (11.0–20.7)

Of note, the formal primary efficacy analysis is change of overall KOOS from Day 0 to final assessment (FA) 24 months after the end of the respective treatment, compared between the two study treatment groups (ACT3DCS and microfracture). The result of this interim analysis is that the ACT3D-CS treatment is not inferior to microfracture, but superiority was not demonstrated. Overall KOOS at 12 months was however numerically in favour of ACT3D-CS.

Secondary endpoints

Analysis of KOOS without imputation (observed cases)

Step 1: Test for relevant clinical improvement from baseline to Visit 4 for the ACT3D-CS group:
Difference = 23.6 (CI 18.3–28.8), p < 0.0001 [For comparison: Difference for microfracture = 16.7 (CI 11.8–21.5), p < 0.0001.]

Step 2: Test for non-inferiority of ACT3D-CS vis-à-vis microfracture:

Difference = 6.9 (p < 0.0001) with lower bound of CI equal to –0.1. Since this lower bound has a value greater than –8.5, non-inferiority is demonstrated

Step 3: Test for superiority of ACT3D-CS vis-à-vis microfracture:

Difference as for Step 2. The lower bound is <0, so superiority is not demonstrated.

Analysis of variance (ANOVA) yielded the following least-squares means with corresponding standard errors and 95% confidence intervals:

ACT3D-CS: 24.7 ± 2.3 (20.2–29.3)

Microfracture: 15.5 ± 2.3 (11.0–20.1)

Analysis of KOOS using the ITT2 population

Step 1: Test for relevant clinical improvement from baseline to Visit 4 for the ACT3D-CS group:

Difference = 21.7 (CI 16.4–27.0), p < 0.0001

[For comparison: Difference for microfracture = 16.1 (CI 11.8–20.4), p < 0.0001.]

• Step 2: Test for non-inferiority of ACT3D-CS vis-à-vis microfracture:

Difference = 5.6 (p < 0.0001) with lower bound of CI equal to –1.1.

Since this lower bound has a value greater than –8.5, non-inferiority is demonstrated.

• Step 3: Test for superiority of ACT3D-CS vis-à-vis microfracture:

Difference as for Step 2. The lower bound is <0, so superiority is not demonstrated.

ANOVA yielded the following least-squares means with corresponding standard errors and 95% confidence intervals:

ACT3D-CS: 22.5 ± 2.3 (17.9–27.0)

Microfracture: 15.4 ± 2.3 (10.9–19.9)

Analysis of KOOS using Day 0' for the ACT3D-CS group

Analyses were repeated using Day 0' for the ACT3D-CS group in the ITT1 population, OC, and the PP population. The results were consistent with the primary analysis.

Analysis of KOOS subscores

The significance testing performed for the primary analysis was repeated for each of the KOOS-related secondary variables.

Descriptive statistics for the PP population is shown in the table below.

	Treatment group: Variable	ACT3D-CS N = 39	Microfracture N = 41	Source table
Value at baseline	Overall KOOS	56.2 ± 14.9	55.2 ± 14.9	14.2.4.1
	KOOS 'symptoms'	72.0 ± 15.4	71.2 ± 19.0	14.2.5.3.1
	KOOS 'pain'	62.7 ± 18.0	61.8 ± 16.7	14.2.5.2.1
	KOOS 'function in daily living'	71.2 ± 19.6	72.2 ± 17.1	14.2.5.4.1
	KOOS 'function in sport and recreation'	42.8 ± 25.3	40.1 ± 24.7	14.2.5.5.1
	KOOS 'knee-related quality of life'	32.4 ± 14.1	30.9 ± 16.7	14.2.5.6.1
Value at Day 0'	Overall KOOS	60.2 ± 17.2	–	14.2.4.1
	KOOS 'symptoms'	77.6 ± 14.5	–	14.2.5.3.1
	KOOS 'pain'	68.9 ± 20.4	–	14.2.5.2.1
	KOOS 'function in daily living'	75.2 ± 19.0	–	14.2.5.4.1
	KOOS 'function in sport and recreation'	43.2 ± 26.6	–	14.2.5.5.1
	KOOS 'knee-related quality of life'	35.9 ± 17.4	–	14.2.5.6.1
Value at Visit 2	Overall KOOS	66.6 ± 17.9	60.2 ± 16.7	14.2.4.1
	KOOS 'symptoms'	80.0 ± 16.0	76.9 ± 16.3	14.2.5.3.1
	KOOS 'pain'	75.7 ± 18.5	69.0 ± 18.7	14.2.5.2.1
	KOOS 'function in daily living'	80.7 ± 18.0	76.5 ± 18.1	14.2.5.4.1
	KOOS 'function in sport and recreation'	48.6 ± 28.6	39.9 ± 25.1	14.2.5.5.1
	KOOS 'knee-related quality of life'	48.1 ± 22.8	38.9 ± 17.9	14.2.5.6.1
Value at Visit 4	Overall KOOS	78.2 ± 18.3	71.3 ± 17.2	14.2.4.1
	KOOS 'symptoms'	85.5 ± 16.7	80.6 ± 16.3	14.2.5.3.1
	KOOS 'pain'	83.5 ± 16.8	78.2 ± 19.0	14.2.5.2.1
	KOOS 'function in daily living'	90.2 ± 16.1	85.0 ± 15.7	14.2.5.4.1
	KOOS 'function in sport and recreation'	70.5 ± 27.4	61.2 ± 25.6	14.2.5.5.1
	KOOS 'knee-related quality of life'	61.1 ± 24.5	51.5 ± 20.3	14.2.5.6.1

	Treatment group: Variable	ACT3D-CS N = 39	Microfracture N = 41	Source table
Change from baseline	Overall KOOS	21.9 ± 17.6	16.1 ± 15.9	14.2.4.1
	KOOS 'symptoms'	13.6 ± 21.2	9.4 ± 16.2	14.2.5.3.1
	KOOS 'pain'	20.8 ± 16.3	16.4 ± 18.1	14.2.5.2.1
	KOOS 'function in daily living'	19.0 ± 17.7	12.8 ± 16.6	14.2.5.4.1
	KOOS 'function in sport and recreation'	27.7 ± 30.2	21.1 ± 23.4	14.2.5.5.1
	KOOS 'knee-related quality of life'	28.7 ± 20.7	20.6 ± 24.7	14.2.5.6.1

Mean values ± SD are shown throughout.

Analysis of KOOS subscores: comparison between treatment groups baseline and Visit 4 by the *t* test (PP population)

	Treatment group Variable	**Difference** N = 39	**Lower CL** N= 41	Source table
Change from	Overall KOOS	6.8	-0.8	Table 14.2.4.6
	KOOS 'symptoms'	4.1	-4.3	Table 14.2.5.3.3

	KOOS 'pain'	4.4	-3.3	Table 14.2.5.2.3
	KOOS 'function in daily living'	6.2	-1.5	Table 14.2.5.4.3
	KOOS 'function in sport and recreation'	6.6	-5.5	Table 14.2.5.5.3
	KOOS 'knee-related quality of life'	8.1	-2.0	Table 14.2.5.6.3

Subscore analyses gave the same qualitative result as the full-KOOS analysis: non-inferiority (lower CI >–8.5) was demonstrated at the descriptive level, and superiority (for which lower CI >0) was not.

Analysis of MOCART scores

MOCART scores at Visits 2 and 4 (ITT1 population)

	Visit 2 (3 months)		Visit 4 (12 months)	
	Treatment group		Treatment group	
	ACT3D-CS *N* = 27	Microfracture *N* = 29	ACT3D-CS *N* = 30	Microfracture *N* = 34
Mean ± SD	67 ± 16	62 ± 11	81 ± 13	77 ± 13
Minimum	30	45	60	45
Lower quartile	55	55	70	65
Median	70	60	85	75
Upper quartile	75	65	90	85
Maximum	95	95	100	100

Differences from total population sizes are due to missing results.

MOCART was not assessed prior to study treatment. A comparison between absolute values for the two treatment groups at the 3-month and 12-month visits yielded the following differences (score in the ACT3D-CS group minus score in the microfracture group (ITT1 population):

Visit 2: 5.1, with confidence interval –2.3 to +12.5

Visit 4: 4.1, with confidence interval –2.4 to +10.6

MOCART scores at Visits 2 and 4 (PP population)

	Visit 2		Visit 4	
	Treatment group		Treatment group	
	ACT3D-CS *N* = 23	Microfracture *N* = 24	ACT3D-CS *N* = 25	Microfracture *N* = 28
Mean ± SD	65.7 ± 16.0	60.4 ± 9.5	82.4 ± 12.8	76.6 ± 11.0
Minimum	30	45	60	60
Lower quartile	55	53	70	68
Median	70	60	85	75
Upper quartile	75	65	90	85
Maximum	95	80	100	95

Differences from total population sizes are due to missing results.

Results were similar for the PP population (score in the ACT3D-CS group minus score in the microfracture group): Visit 2: 5.2, with confidence interval –2.6 to +13.1; Visit 4: 5.8, with confidence interval –0.8 to +12.4.

The MOCART analysis for the PP population came close to showing a (descriptive) statistically significant difference between the treatment groups at Visit 4 (p=0.0845). Thus, overall, modest improvements in MOCART score from Visit 2 to Visit 4 (12 months) can be observed, similar between the treatment groups. The absence of a baseline measurement precluded a within-group comparison from baseline. In addition, a substantial proportion of overall MOCART scores were missing with results for the overall MOCART score available for 27 of 48 patients in the ACT3D-CS group and 29 of 48 patients in the MF group. This was a consequence of missing assessments for item 5b (Signal intensity of the repair tissue - 3DGE- FS) so that the overall value could not be assessed. This item 5b requires a MRI conducted, among others, with a fat sat sequence, which was not conducted by some radiologists. When this was recognized, the Sponsor reminded the participating radiologists to conduct all required sequences. All others 8 items (except item 5b) were assessed for a minimum of 41 patients (ACT3D-CS group) and 44 patients (MF group). Individual items were reported in the CSR. Also, the applicant provided a comparative assessment of the scores for the 8/9 items for the patients not included due to missing item 5b as compared to the patients with complete data which did not demonstrate a selection bias.

Correlation between KOOS and MOCART scores

The correlation between KOOS and MOCART results was investigated by calculating Spearman's correlation coefficient for all patients for whom both KOOS and MOCART results were available. A better subjective or functional outcome in correlation with an increase in the structural parameters of the MOCART score could not be observed. This is consistent with the published literature and the reasons for this are not fully understood.

IKDC Knee Examination Form – overall assessment

In the IKDC Knee Examination Form overall score, at Visit 4 (12 months) 20 patients in the ACT3D-CS group and 17 in the MF group showed improvement, while 3 in each group showed worsening (24 and 29 showed no change). A Kruskal–Wallace test to compare the changes in the groups did not shown significance (p = 0.7208).

IKDC Knee Examination Form – overall assessment (ITT1 population)

Treatment group:	ACT3D-CS group				Microfracture group			
IKDC Grade:	A	B	C	D	A	B	C	D
Pre-arthroscopy day	26	12	9	–	25	18	4	2
Pre-implantation day	26	18	4	–	*n.a.*			
Visit 1	14	12	7	15	13	16	10	10
Visit 2	26	14	4	4	21	22	5	1
Visit 3	34	10	3	1	31	15	3	–
Visit 4	41	6	1	–	34	14	1	–

Numbers of patients with the respective grade at each visit are shown.

IKDC Subjective Knee Evaluation Form

Baseline values were somewhat higher in the ACT3D-CS group than in the microfracture group; for the ACT3D-CS group. In both treatment groups they were lower (reflecting a poorer subjective assessment of the patients' knee condition) at Visit 1. Thereafter the patients' assessment improved from visit to visit. The improvement was numerically slightly greater in the ACT3D-CS group but without statistical significance.

IKDC Subjective Knee Evaluation Form (ITT1 population)

Treatment group: Visit	ACT3D-CS group *N* = 48	Microfracture group *N* = 49
Values at each visit		
Pre-arthroscopy (baseline)	54.5 ± 15.9	47.8 ± 14.6
Pre-implantation (Day 0')	56.4 ± 16.6	*n.a.*
Visit 1 (6 weeks)	46.4 ± 15.2	40.0 ± 14.4
Visit 2 (3 months)	63.0 ± 17.1	52.6 ± 15.4
Visit 3 (6 months)	69.3 ± 17.6	60.5 ± 15.4
Visit 4 (12 months)	75.3 ± 18.5	64.6 ± 19.9
Changes from baseline		
Pre-implantation (Day 0')	1.9 ± 10.8	*n.a.*
Visit 1 (6 weeks)	–8.1 ± 19.2	–7.8 ± 14.5
Visit 2 (3 months)	8.5 ± 19.3	4.8 ± 13.9
Visit 3 (6 months)	14.8 ± 17.7	12.7 ± 14.0
Visit 4 (12 months)	20.8 ± 18.6	16.8 ± 15.6

Mean values ± SD are shown throughout. Baseline = Day 0.

Modified Lysholm score

Values were similar between the treatment groups at baseline (Day 0); a small improvement was seen in the ACT3D-CS group between Day 0 and Day 0' for the ACT3D-CS group. Scores decreased immediately after the operation and thereafter improved in both treatment groups. Both treatment groups showed significant improvement between baseline and Visit 4 (12 months after study treatment).

Modified Lysholm score (ITT1 population)

Treatment group: Visit	ACT3D-CS N = 48	Microfracture N = 49
Values at each visit		
Pre-arthroscopy (baseline)	16.8 ± 4.0	16.0 ± 3.3
Pre-implantation (Day 0')	18.5 ± 3.7	*n.a.*
Visit 1 (6 weeks)	15.4 ± 2.7	14.2 ± 3.7
Visit 2 (3 months)	19.1 ± 3.4	17.7 ± 3.8
Visit 3 (6 months)	20.4 ± 3.0	19.4 ± 3.4
Visit 4 (12 months)	21.1 ± 3.0	19.8 ± 3.2
Changes from baseline		
Pre-implantation (Day 0')	1.7 ± 3.3	*n.a.*
Visit 1 (6 weeks)	–1.4 ± 3.9	–1.8 ± 3.3
Visit 2 (3 months)	2.3 ± 4.7	1.7 ± 3.6
Visit 3 (6 months)	3.6 ± 4.0	3.4 ± 3.2
Visit 4 (12 months)	4.3 ± 4.3	3.8 ± 3.0

Mean values ± SD are shown throughout.

Both treatment groups showed significant improvement between baseline and Visit 4 (12 months after study treatment) ($p<0.0001$ both groups) with no significant difference between the groups. Results were consistent between the ITT1 and the PP population.

Ancillary analyses

The following subgroup analyses were conducted for the overall KOOS and MOCART:

Age: Patients were stratified prospectively by age into two classes by randomisation procedure (18 - 34 and ≥ 35 - 50 years). The age category '18–34 years' was represented by 19 ITT patients in the ACT3DCS group and 18 patients in the microfracture group. The age category '35–50 years' comprised 33 patients treated with ACT3D-CS and 32 patients treated with microfracture. Both age groups showed an increase in overall KOOS from baseline to Visit 4. KOOS overall values at Visit 4 were 86.5 for ACT3D-CS ($p < 0.0001$) and 68.1 for microfracture ($p = 0.0015$) for the younger patients aged 18 – 34 years. For the older group an overall KOOS of 74.1 for ACT3D-CS and 68.2 for microfracture was assessed (both p values <0.0001).

Results (mean differences with respective 95% confidence intervals) were as follows (ITT1 population):

- 18–34 years: ACT3D-CS 21.3 [13.4–29.2] microfracture 12.9 [5.7–20.0]
- ≥ 35 - 50 years: ACT3D-CS 22.7 [15.3–30.1] microfracture 18.5 [12.9–24.1]

Results for the PP population were:

- 18–34 years: ACT3D-CS 21.4 [12.5–30.3] microfracture 12.9 [4.9–21.0]
- ≥ 35 - 50 years: ACT3D-CS 22.2 [14.4–30.1] microfracture 17.9 [11.2–24.5]

Diagnosis: Only the categories 'traumatic cartilage lesion' (ACT3D-CS, N = 19; MF, N = 24) and 'others' (ACT3D-CS, N = 32; MF, N = 21) comprised sufficient numbers of patients to allow meaningful comparison between the subgroups of the ITT1 patients (there were no AVN patients at all). For 'traumatic cartilage

lesion' the mean overall KOOS rose in the first year after ACT3D-CS from 61.2 to 80.2 and for MF from 49.9 to 62.4. For 'others' the KOOS score increased from 54.0 at baseline to 77.5 after 12 months for ACT3D-CS and for MF from 50.7 to 70.4. All these comparisons between baseline and the 12-month visit gave $p < 0.05$. The results (mean differences of KOOS with respective 95% confidence intervals) were as follows (ITT1 population): Traumatic cartilage lesion: ACT3D-CS 19.1 [10.5–27.6] MF 12.5 [6.1–18.9]; Others: ACT3D-CS 23.4 [16.2–30.7] MF 19.7 [12.7–26.7].

Gender: No trends related to gender were observed.

Duration of knee symptoms: Duration of knee symptoms before screening was recorded for the ACT3D-CS-treated patients only. The results for KOOS score were as follows:

- < 1 year: mean ± SD, 87.6 ± 9.1; median 89; range 60–100
- 1 year: mean ± SD, 76.8 ± 17.6; median 78; range 34–100

A weak negative correlation was found between KOOS score at the 12-month visit and the previous duration of knee symptoms ($\rho = -0.228$ for N = 39 ITT1 patients; –0.209 for N = 30 PP patients). N values are small, as many patients did not provide information about the duration of symptoms. Nevertheless, the sign of Spearman's ρ suggests that a longer pre-treatment period with symptoms is associated with a poorer condition after treatment.

BMI: No correlation was found between KOOS score at Visit 4 (12 months after treatment) and BMI (Spearman's $\rho = 0.066$ for N = 97 ITT1 patients; 0.108 for N = 80 PP patients).

Overall, the subgroup analyses showed results that were generally consistent with the overall results. The applicant was requested to discuss the fact that duration of knee symptoms appeared to have been recorded only for a subgroup of 39 ACT3D-CS-treated patients, which showed a numerically lower KOOS outcome at 12 months for those patients with longer disease duration. According to a recent review (Mithoefer et al, Cartilage. 2011;2[2]:100-21) several studies have demonstrated that 'age of the defect', that is, time since onset of the cartilage injury of more than 12 to 36 months, had a clear negative effect on both MF and cellular therapy outcomes. Thus, an imbalance between the treatment group would be potentially relevant in this regard. It was clarified that duration of symptoms was reported by 39 patients in the ACT3D-CS group and by 40 patients in the microfracture (MF) group. The other patients of the treatment groups did not report and/or did not recall the duration of symptoms during screening. The duration of symptoms for these patients is unknown. Descriptive statistics for the outcome KOOS overall was provided by duration of symptoms for the subgroup category ≤ 1 year und > 1 year. This showed no major difference in KOOS outcome between the symptom duration subgroups in either treatment arm. Higher preoperative activity rates (Tegner score > 4) have been shown to influence the function and activity outcomes of microfracture (Gracitelli GC, et al. Cochrane Database Syst Rev. 2016;3[9]). While the Tegner activity score was not recorded in the Phase III study other outcomes related to patient activity levels such as the KOOS subscore 'function in sport and recreation' appeared to be balanced at baseline.

The applicant also provided additional subgroup analyses to study the influence of recorded meniscal, ligament and the other surgeries on clinical outcome. These subgroup analyses indicated similar benefit in terms of KOOS change from baseline in both subgroups (surgery yes/no). In addition, to further understand the external validity of the trial, the applicant was requested clarify whether there was a difference in primary outcome based on region (e.g. comparing sites in Germany to those in Poland). The number of patients enrolled outside Germany was very low however, limiting interpretability. The applicant performed an ANOVA analysis to verify that no centre effect was present and to show that criteria for the recruitment were

constant and balanced between centres. In the analysis all Spherox-treated patients (72 patients in Phase II and 48 in the Phase III trial) with baseline assessment and Visit 4 results, were included. No significant differences were seen between the study centres, whether small or large, based on KOOS response. This implies that similar criteria in recruitment and similar treatment practices between centres were used.

The product has been legally on the market in Germany since 2004, and has also been available in Belgium, Greece, Italy, Spain and Austria. According to the applicant, already since that time, all surgeons and other healthcare professionals involved in the handling and administration of Spherox as well as those involved in follow-up of patients treated with the product, receive training through a training programme prior to the distribution of Sperox. This is done to avoid differences in treatment and rehabilitation after ACT3D with Spherox or MF.

Analysis performed across trials (pooled analyses and meta-analysis)

A pooled analysis of Spherox-treated patients from both clinical trials Phase II (cod 16 HS 14) and Phase III (cod 16 HS 13) was performed to increase the overall sample size and to compare the main endpoints in subgroups by defect size at implantation (1 to < 4 cm^2, ≥ 4 to 10 cm^2), sex (male, female), age (18–34 years, 35–50 years), location (femur, patella), diagnosis (traumatic cartilage lesion, osteoarthritis, osteochondritis dissecans, and others) and femoral condyle location (medial, lateral). The study was a pooled analysis of the 12-month data from both studies. A statistically significant increase in overall KOOS was seen in all subgroups investigated, with the exception of the diagnosis subgroups 'OA' and 'OD' which contained few patients (8 and 6, respectively). Excluding the latter subgroups, the mean improvements in the overall KOOS between baseline and 12-months visit were between 16.2 and 22.0 in the subgroups. The results below reflect the pooled ITT population.

Defect size: At Visit 4 (12 months after implantation), the mean and median increase with respect to baseline was somewhat lower in the group with larger defect sizes (n=73) (mean change, 16.2 ± 18.2; p = 0.0050) than in the 'small defect size' group (n=48) (mean change, 22.0 ± 17.8; p<0.0001), but the improvements were statistically significant in both subgroups.

Gender: The improvements from baseline to Visit 4 were statistically significant in both gender subgroups: for women (n=40) the mean change was 20.4 ± 17.2 (p = 0.0005) and for men (n=81) it was 17.7 ± 18.7 (p = 0.0004).

Age: The mean and median overall KOOS values at baseline were numerically slightly lower in the age group '35–50 years' (n=61) than in the age group '18–34 years' (n=60). Hence, the Product is not recommended for use in the patients of 50 The absolute mean and median values of the overall KOOS remained lower for the older patients than for the younger ones at all visits. However, the mean and median improvements were numerically only slightly smaller in the older patient subgroup, and the improvements between baseline and Visit 4 were statistically significant in both subgroups: for '18–34 years' the mean change was 18.7 ± 14.8 (p<0.0001) and for '35–50 years' it was 18.4 ± 21.1 (p = 0.0029). Of note, in the Phase II study (cod 16 HS 14), a statistically significant improvement was seen in the younger age group (18-34 years) at the 12 month visit in all dose levels, while in the older age group (35–50 years), a statistical difference was not seen. However, in the Phase III study, a clear improvement in mean KOOS score at 1-year interim analysis was seen in both age groups.

Defect location: Baseline mean and median overall KOOS values were lower in the patella subgroup (n=45) than in the femur subgroup (n=76). The absolute mean and median KOOS values were numerically slightly lower in the patella than in the femur subgroup at all post-implantation visits. The improvements in the

overall KOOS from baseline to Visit 4 were statistically significant in the femur subgroup (mean change, 18.7 ± 19.3; *p* = 0.0002) and patella subgroup (mean change, 18.2 ± 16.5; *p* = 0.0019).

Femoral condyle location: Overall KOOS values (mean and median) were lower in the 'lateral femoral condyle' subgroup (n=56) than in the 'medial femoral condyle' subgroup (n=18) at all visits. The improvements in the overall KOOS from baseline to Visit 4 were statistically significant in both subgroups: the mean change was 21.1 ± 15.8 (*p* = 0.0086) in the 'medial femoral condyle' subgroup and 17.6 ± 20.1 (*p* = 0.0068) in the 'lateral femoral condyle' subgroup.

Diagnosis: At baseline, the mean and median overall KOOS values were distinctly higher in the patients with OD than in all other diagnosis subgroups. The improvements between baseline and Visit 4 were statistically significant in the diagnosis subgroups 'traumatic cartilage lesion' (mean change, 18.2 ± 14.6; *p* = 0.0001) and 'others' (mean change, 20.3 ± 19.3; *p* = 0.0004), but not in the OD and OA subgroups (see table below).

Overall KOOS at each visit by diagnosis (ITT); pooled analysis

Visit / Subgroup:		Traumatic *N = 54*	OD *N = 6*	OA *N = 8*	Others *N = 53*
Values at each visit					
Pre-arthroscopy (baseline)	mean ± SD	58.3 ± 15.4	65.8 ± 13.1	55.4 ± 19.2	54.5 ± 14.5
	median	59	68	54	52
Visit 4 (12 months)	mean ± SD	76.6 ± 15.8	67.9 ± 16.0	77.4 ± 13.1	75.0 ± 20.8
	median	80	64	81	80
Changes from baseline					
Patients with change from baseline		54	6	8	52
Visit 4 (12 months)	mean ± SD	18.2 ± 14.6	2.1 ± 16.3	22.0 ± 29.2	20.3 ± 19.3
	median	18	5	27	20
	98% CI	(14.2, 22.2)	(-15.1, 19.2)	(-2.4, 46.4)	(14.9, 25.6)
	p	0.0001	0.2889	0.2832	0.0004

Overall, these pooled analyses point towards consistency of efficacy between the variously defined subgroups.

Clinical studies in special populations

No studies have been performed in patients over 50 years of age.

Study cod 16 HS 17paed

Study cod 16 HS 17paed is a prospective, non-interventional investigation to evaluate the long-term safety and linked efficacy of the three-dimensional autologous chondrocyte implantation product in paediatric patients from closure of the epiphyses to less than 18 years of age treated with the product until December 2011. The completion of this study was deferred to June 2020. The current submission includes an interim report, dated 15 September 2016 using a data cut-off date of 31 January 2016. Study cod 16 HS 17paed is an ongoing non-interventional, open-label, multicentre surveillance study. The objective is to assess the long-term safety and linked efficacy of Spherox in paediatric patients from 15 to less than 18 years of age at the time of implantation. It is planned to include at least 80 patients from approximately 40 centres with data collected by the physicians based on medical records. Physical examination data based on a physical

examination visit are to be collected in a subpopulation of at least 30 patients. A MRI examination within a time window of ± 3 month of the physical examination can be performed in a subpopulation for MOCART analysis. Patients are asked to complete web-based questionnaires at home once during the study.

At the interim cut-off date (31 January 2016), data were available for 33 patients. Four patients were not included in the full analysis set (FAS), on which data analysis was based, because either the implantation date was missing or an inclusion criterion was not met. The FAS comprised 29 patients for the interim analysis. The Physical Examination (PE) subpopulation comprised 23 patients (79% of the 29 FAS patients). The time between documentation of the data as part of this study and the ACTD3D with Spherox ranged between approximately 3.5 and 8 years (mean: 63.3 months).

Study population: The FAS consisted of 15 boys (52%) and 14 girls (48%) between 15 and 17 years of age (mean age, 16.0 ± 0.9 years). The status of the epiphyseal plate was 'closed' in 12 patients (41%), 'open' in 8 patients (28%), and was not documented in 9 patients (31%). Diagnoses of cartilage lesion were trauma (48%), OD (48%), and 'other' (3%). The mean duration of symptoms (i.e. duration of pain until implantation) was 20.9 ± 24.6 months. Thirty-two defects in 29 patients were found. Locations of defects were the femur (22 defects), tibia (1 defect) and patella (9 defects). The sizes of the cartilage lesions ranged between 2.2 and 12.0 cm2 (mean defect area size, 4.6 ± 2.4 cm2). The mean Spherox dose was 33.8 ± 15.3 spheroids/cm^2.

Demographic characteristics (cod 16 HS 17 paed)

		FAS (*N* = 29)
Sex [*n* (%)]	Male	15 (52%)
	Female	14 (48%)
Age [years] at implantation	*N*	29
	Mean (SD)	16.0 (0.9)
	Median (Min, Max)	16 (15.17)
Epiphyseal closure at implantation [*n* (%)]	Open	8 (28%)
	Closed	12 (41%)
	Not documented	9 (31%)
Follow-up time [months]	*N*	29
	Mean (SD)	63.3 (14.5)
	Median (Min, Max)	58 (41.95)
Defect size at the time of implantation [cm^2]	*N*	29
	Mean (SD)	4.6 (2.4)
	Median (Min, Max)	4.0 (2.2, 12.0)
Number of defects [*n* (%)]	Patients with 1 defect	26 (90%)
	Patients with > 1 defect	3 (10%)
Location of cartilage lesion [*n* (%)][a]	Femur	22 (69%)
	Tibia	1 (3%)
	Patella	9 (28%)
Diagnosis [*n* (%)]	Cartilage lesion caused by	
	Trauma	14 (48%)
	Osteochondritis dissecans (OD)	14 (48%)
	Osteoarthritis	0
	Avascular necrosis	0
	n.a.	1 (4%)

[a] Percentages are based on the total number of defects (*N*=32) in the FAS.

Treatment failure rate (FAS): Treatment failure was the primary endpoint (as defined in the PIP). This was considered to have occurred when a patient required additional surgical treatment after ACT3D treatment and the required procedure involved 'extensive debridement for lesion expansion' or 'violation of the subchondral bone' or 'ACI'. Five (17% of 29) patients had additional surgery at the affected knee after ACT3D. One of the patients met the criteria for treatment failure; thus, the treatment failure rate in the overall set was 0.034. As this one patient with treatment failure had a closed epiphyseal growth plate at the time of transplantation; the treatment failure rate in the subgroup 'closed' was 0.083. The time to treatment failure was 34.3 months.

Physical examination: Physical examination findings were available for 23 patients and were normal in the majority of the patients: the muscles examined were equal-sized on both sides in 83% of the PE subpopulation, none of the patients had knee effusion, and few patients had patella/meniscus signs and/or pressure pain. The mean passive and active motion ranges of the affected knees were normal.

MRI examination: MRI was obtained in the physical examination (PE) subpopulation which comprised 23 patients. The defect repair and filling was complete in 8 patients (53% of 15 patients) and incomplete (but nonetheless >50% of adjacent cartilage) in 2 patients (13%); hypertrophy was seen in 5 patients (33%). The integration to border zone was complete in 9 patients (60%) and incomplete in 6 patients with the demarcating border visible in 3 patients (20%) and defect visible <50% of the length of RT in the other 3 patients (20%). The surface of the RT was intact in 7 patients (47%) and damaged (<50% of the RT depth) in 8 patients (53%). The structure of the RT was homogenous in 10 patients (67%) and inhomogeneous in 5 patients (33%).

MRI examination and MOCART score (cod 16 HS 17 paed)

		FAS Subpopulation 'PE' (*N = 23*)
MRI performed during the last 2 months [*N* (%)][a]	Yes	15 (65%)
	No	2 (9%)
	Missing data	6 (26%)
Degree of defect repair and defect-filling [*N* (%)][b]	Complete	8 (53%)
	Hypertrophy	5 (33%)
	Incomplete	2 (13%)
Integration to border zone [*N* (%)][b]	Complete	9 (60%)
	Incomplete	6 (40%)
Surface of the RT [*N* (%)][b]	Intact	7 (47%)
	Damaged	8 (53%)
Structure of the RT [*N* (%)][b]	Homogeneous	10 (67%)
	Inhomogeneous or cleft formation	5 (33%)
MOCART score	*N*	*15*
	Mean (SD)	74.7 (12)
	Median (Min, Max)	80 (61)
	Q1, Q3	61

[a] Percentages are based on the total number of patients in the subpopulation. [b] Percentages are based on the total number of patients with MRI examination. MOCART score range: 0–100 (with higher scores indicating better quality of the RT).

The mean MOCART score was 74.7 ± 12.0 (*N* = 15) at the follow-up examination 63.3 months after treatment, indicating, on average, good repair tissue growth.

Knee Injury and Osteoarthritis Outcome Score (KOOS): The mean overall KOOS score was 82.6 ± 11.6 (*N* = 22), indicating very mild problems with the knee injury up to 95 (mean, 63.6) months after treatment. For the subscales, mean scores were: 94.9 ± 7.4 (ADL), 88.5 ± 10.4 (pain), 83.1 ± 16.1 (other symptoms), 78.6 ± 20.2 (function in sports and recreation), and 67.6 ± 17.2 (knee-related QoL). The mean overall KOOS scores in the subgroups by epiphyseal plate status were: 86.7 ± 7.1 (open, *N* = 6), 82.0 ± 11.3 (closed, *N* = 8), and 80.0 ± 14.7 (not documented, *N* = 8). The subscale scores were roughly similar between the patients with 'closed' and 'not documented' epiphysis status. In the subgroup with 'open' epiphysis, the subscale 'Sport/Rec.' was not as strongly affected as in the other subgroups: for this subscore the mean scores were 90.8 ± 6.7 (open), 76.3 ± 21.2 (closed), and 71.9 ± 23.6 (not documented).

IKDC Current Health Assessment Form (SF-36): Seven patients (24.1%) of the 29 did not complete the IKDC Current Health Assessment Form. The mean Physical Component Summary (PCS) summary score in the overall set of 22 patients was 52.3 ± 7.0 and the mean Mental Health Component (MCS) score was 53.9 ± 6.3, indicating physical and mental health above the average in the U.S. general population norm for up to 95 (mean: 63.6) months after treatment. The subgroup with undocumented status of the epiphysis showed the lowest mean PCS but the highest mean MCS. The mean PCS scores were: 56.4 ± 4.0 (open, *N* = 6), 52.5 ± 4.2 (closed, *N* = 8), and 49.1 ± 9.6 (not documented, *N* = 8). The mean MCS scores were: 52.1 ± 6.7 (open, *N* = 6), 52.0 ± 7.3 (closed, *N* = 8), and 57.2 ± 3.9 (not documented, *N* = 8).

IKDC Subjective Knee Evaluation Form: The mean IKDC score was 81.1 ± 12.7 (*N* = 22), indicating on average mild limitations in respect of 'activities of daily living', 'sports activities' and 'symptoms' for up to 95 (mean, 63.6) months after treatment.

Modified Lysholm Knee Scoring: The mean total score was 21.0 ± 2.4, indicating that the knee problems on average did not affect, or affected only marginally, the patients' ability to manage activities of daily living (the best possible score of 24 indicates no knee problems) for up to 95 (mean, 63.6) months after treatment. The mean total scores in the epiphysis subgroups were 22.0 ± 1.1 (open, *N* = 6), 20.1 ± 3.3 (closed, *N* = 8), and 21.0 ± 1.9 (not documented, *N* = 8).

Overall, this study provides interim descriptive data on symptoms and structural repair during the treatment follow-up. The outcome scores after the follow-up period of on average 63 months reached levels comparable to those observed for adult patients treated with Spherox after 3 years.

Study Cod 16 HS 16

The applicant has conducted a non-interventional, multicentre (8 centres in Germany), retrospective data collection from 29 children/adolescent patients having knee joint cartilage defect who were followed up to seven years from the Spherox transplantation. The data were collected from former patients treated with ACT3D for cartilage defects. The study planned to cover a period of up to 84 months comprising up to 7 visits. Data were obtained for up to 57 months after the implantation. The analysis of efficacy and safety variables took account of subgroups as defined by the status of the epiphyseal plate (open / closed / no information). A total of 29 adolescent patients were enrolled and analysed in this study, of whom 7 patients (26%) presented with an open and 6 (21%) with a closed epiphyseal plate. The mean MOCART score tended to increase (see table below), which may point to an average increase in cartilage repair tissue over time, but the very low number of patients available for data collection at the majority of the pre-selected time intervals has to be taken into account.

Available MOCART data from cod 16 HS 16 for adolescent patients

Time interval when MRT where performed	No of patient data (MOCARTs) available	MOCART Mean (range)
1 (7 to <13 weeks)	0	N/A
2 (13 to <26 weeks)	2	40 (35-45)
3 (26 to <52 weeks)	5	44 (20-60)
4 (52 to <78 weeks)	9	53.9 (40-70)
5 (78 to <156 weeks)	1	55
6 (156 to <234 weeks)	2	50 (45-55)
7 (≥234 weeks)	1	60

N/A = not available

The mean VAS pain score, as self-assessed by the patients, decreased from 46.0 mm at baseline to 39, 25, 8 and 20 mm at the 2nd to 5th time interval, respectively.

Available VAS data from cod 16 HS 16 for adolescent patients

Time interval	Statistic	VAS Pain	SF36 Physical Functioning	SF36 Mental Health
Baseline	N	8	5	5
	Mean (Range)	46.0 (0.0 - 80.0)	42.1(30.2 - 53.6)	54.7 (51.8 - 58.4)
1st time interval	N	1	0	0
(7 to <13 weeks)	Mean	55.0	N/A	N/A
2nd time interval	N	4	3	3
(13 to <26 weeks)	Mean	38.5 (10.0 -80.0)	46.8 (31.1 - 59.9)	48.8 (32.3 - 61.0)
3rd time interval	N	6	6	6
(26 to <52 weeks)	Mean (Range)	25.0 (0.0 - 80.0)	51.3 (39.3 - 61.5)	54.3 (50.2 - 59.7)
4th time interval	N	6	4	4
(52 to <78 weeks)	Mean	8.0 (0.0 - 18.0)	56.4 (53.3 - 60.3)	56.0 (53.0 - 57.2)
5th time interval	N	3	3	3
(78 to <156 weeks)**	Mean	20.0 (10.0 to 30.0)	57.0 (52.7 - 60.29	48.2 (36.2- 57.19

** no data are available for the 6th and 7th time interval

N/A = not available

For all IKDC assessments, there were improvements from baseline to almost all postoperative time intervals, which were most pronounced for the effusion, compartment findings, X-ray findings and functional test (subgroup data not shown).

IKDC final* evaluation by subgroups trauma / OCD / other and all patients enrolled (N=29)

Characteristic	Category	Statistic	All	Subgroups		
				Trauma	OCD	Other
Baseline	Valid N	N	27	9	12	6
	A	n (%)	11 (40.7%)	4 (44.4%)	5 (41.7%)	2 (33.3%)
	B	n (%)	6 (22.2%)	1 (11.1%)	4 (33.3%)	1 (16.7%)
	C	n (%)	5 (18.5%)	0 (0.0%)	3 (25.0%)	2 (33.3%)
	D	n (%)	5 (18.5%)	4 (44.4%)	0 (0.0%)	1 (16.7%)
1st time interval (7 to <13 weeks)	Valid N	N	5	1	1	3
	A	n (%)	1 (20.0%)	1 (100.0%)	0	0
	B	n (%)	0	0	0	0
	C	n (%)	0	0	0	0
	D	n (%)	4 (80.0%)	0	1 (100.0%)	3 (100.0%)
2nd time interval (13 to <26 weeks)	Valid N	N	12	3	6	3
	A	n (%)	7 (58.3%)	1 (33.3%)	3 (50.0%)	3 (100.0%)
	B	n (%)	5 (41.7%)	2 (66.7%)	3 (50.0%)	0
	C	n (%)	0	0	0	0
	D	n (%)	0	0	0	0
3rd time interval (26 to <52 weeks)	Valid N	N	12	4	5	3
	A	n (%)	12 (100.0%)	4 (100.0%)	5 (100.0%)	3 (100.0%)
	B	n (%)	0	0	0	0
	C	n (%)	0	0	0	0
	D	n (%)	0	0	0	0
4th time interval (52 to <78 weeks)	Valid N	N	9	4	4	1
	A	n (%)	8 (88.9%)	4 (100.0%)	3 (75.0%)	1 (100.0%)
	B	n (%)	1 (11.1%)	0	1 (25.0%)	0
	C	n (%)	0	0	0	0
	D	n (%)	0	0	0	0
5th time interval (78 to <156 weeks)	Valid N	N	7	1	6	0
	A	n (%)	6 (85.7%)	1 (100.0%)	5 (83.3%)	0
	B	n (%)	0	0	0	0
	C	n (%)	1 (14.3%)	0	1 (16.7%)	0
	D	n (%)	0	0	0	0
6th time interval (156 to <234 weeks)**	Valid N	N	1	0	1	0
	A	n (%)	1 (100.0%)	0	1 (100.0%)	0
	B	n (%)	0	0	0	0
	C	n (%)	0	0	0	0
	D	n (%)	0	0	0	0

*Note that the term "final" evaluation represents an overall evaluation without a relation to time point of assessment.
**no data for 7th time interval available
N = Number of available patient data; n (%) = Number of patient data (% = n/N*100)
A: "normal", B: "nearly normal", C: "abnormal", D: "severely abnormal"

The Applicant reported an overlap of 12 patients between cod 16 HS 16 and cod 16 HS17 paed. Thus, the additional informative value of cod 16 HS 16 is rather limited.

Supportive studies

The applicant submitted 16 supportive studies/publications with efficacy information.

Retrospective study: cod RS1 SR (2015)

This retrospective study cod RS1 SR (2015) included patients with cartilage defects of the knee, treated with Spherox in routine clinical practice by Dr. Rössing at his centre in Mannheim (Germany) between October 2008 and November 2011. All patients treated in this period were contacted; of these 18 patients consented (out of 26) and were analysed with a 12 month follow-up period. The range of patients' age (15–51 years), body mass index (19.1–31.1 kg/m^2) and sex (8 female, 11 male) can be regarded as representative. The dose was between 6 and 52 spheroids/cm2. Defect sizes ranged from 3 to 10 cm^2. Primary defects located on the patella, femur and tibia were treated. The longest follow-up period was 12 months after implantation. Efficacy was measured by five independent assessments at baseline (before implantation) and different points in time after implantation. Efficacy variables included Lysholm Score (12 month post-OP), Tegner Activity Scale (12 month post-OP), Kellgren and Lawrence Classification (12 month post-OP) and WOMAC-D Score (6 weeks and 3 months post-OP). The increase in Kellgren Lawrence grade represents a very slight

worsening – though not clinically relevant. The improvements in Lysholm (from 52 to 81 points (out of 100) and Tegner (from 2.4 to 4.7 points [out of 10]) scores indicate an improvement in the patient's clinical outcome. Changes in WOMAC-D score were not significant. For MOCART assessment (MRI Score) the 12-month post-OP value was 79 ± 20.5 points (out of 100). 14 patients showed complete filling of the defect, 13 of these showed a homogeneous repair-tissue structure, 12 showed complete integration with the adjacent cartilage and 12 an intact repair-tissue surface.

Efficacy scores (cod RS1 SR)

	WOMAC_D TOTAL	Lysholm	Tegner	Kellgren Lawrence
Baseline	*N = 13*	*N = 14*	*N = 19*	*N = 19*
Mean ± SD	33.67 ± 23.24	51.96 ± 23.52	2.4 ± 0.8	0.3 ± 0.6
follow-up	3 months	12 months	12 months	12 months
post-OP	*N = 10*	*N = 15*	*N = 19*	*N = 19*
Mean ± SD	22.15 ± 13.44	80.57 ± 18.63	4.7 ± 1.8	0.7 ± 0.7
Change from Baseline	*N = 10*	*N = 14*	*N = 19*	*N = 19*
Mean ± SD	–13.22 ± 23.81	27.57 ± 28.21	2.3 ± 1.9	0.4 ± 0.6
p	*p* = 0.1131	*p* = 0.0029	*p* < 0.0001	*p* = 0.0073

Note: One of the 18 consenting patient was operated twice and therefore counted twice.

As addressed in response to the question from the CAT/CHMP, potential overlap of this study was found with the Rössing 2010 study and also with the paediatric investigation cod 16 HS 17 paed. Because of the high degree of overlap (and without the availability of sufficient detail to assess this precisely), the applicant believes that cod RS1 SR includes at least 17 patients not discussed elsewhere.

Retrospective study: cod RS2 RS (2015)

Retrospective study cod RS2 RS (2015) included patients with cartilage defects of the knee, treated with Spherox in routine clinical practice by Dr. Siebold at the ATOS Clinic in Heidelberg (Germany) between April 2006 and August 2010. Twenty patients gave their consent for analysis of their medical records. As clarified by the applicant, this study fully overlaps with the Siebold 2015 study (see below). The applicant concludes cod RS2 SR does not include patients not also discussed in the Siebold study.

Retrospective study: cod RS3 TS (2016)

Retrospective study cod RS3 TS (2016) included patients with cartilage defects of the knee, treated with Spherox in routine clinical practice by Dr. Schreyer in Darmstadt (Germany) between 1998 and 2012. Forty-four patients (out of 90) gave their consent for analysis of their medical records. The range of patient age was 17–56 years and included 17 females, 27 males. Thirty-four patients were treated with Spherox and ten patients with chondrotransplant (chondrocyte suspension covered with a periosteal flap – classical ACI). The knee cartilage defect was located on the femur for all 10 chondrotransplant patients. For Spherox in 29 patients the defect was located on the femur, 3 patients had a defect on the patella, and the remaining 2 patients had a defect on the tibia. The defect size ranged from 0.7 to 6.3 cm^2 among the chondrotransplant patients, with a mean of 4.0 ± 2.0 cm^2. Among the Spherox-treated patients, the defects were sized between 1.5 and 11.0 cm^2, with a mean of 5.77 ± 2.64 cm^2. The following defect aetiologies in patients treated with chondrotransplant were observed: 6 trauma, 1 ostechondroitis dissecans (OD), and 3

degenerative. The aetiologies of the defects in patients treated with Spherox were: 14 trauma, 3 OD, and 15 degenerative. The amounts of spheroids implanted led to a mean dose of 27 ± 25 spheroids/cm² (range, 2–139 spheroids/cm²). The mean follow-up time was: 98 months (range, 48–144 months) for chondrotransplant and: 22 months (range, 2–96 months) for Spherox. Efficacy was measured by six independent assessments at baseline (before implantation) and at different points in time after implantation and included Lysholm Gillquist Score, Tegner Lysholm Score, HSS Score, DGKKT Score, Tegner Score, and Cincinnati Score.

Efficacy scores (cod RS3 TS)

Product	Efficacy score (maximum range)		Pre-implantation (baseline)	Follow-up visit (months after implantation)			
				6	12	24	60
chondrosphere	Lysholm–Gillquist (0–100)	Mean ± SD (CfB *p* value)	*N = 31* 55.4 ± 22.3	*N = 19* 87.5 ± 10.5 *(<0.0001)*	*N = 18* 90.5 ± 11.5 *(<0.0001)*	*N = 10* 92.0 ± 9.9 *(<0.0001)*	*N = 3* 93.3 ± 11.6 *(0.0156)*
	Tegner–Lysholm (0–100)	Mean ± SD (CfB *p* value)	*N = 22* 63.1 ± 20.5	*N = 15* 87.7 ± 7.9 *(0.0005)*	*N = 16* 90.9 ±10.2 *(0.0053)*	*N = 8* 91.4 ± 12.9 *(0.1362)*	*N = 3* 95 ± 8.7 –
	HSS (0–100)	Mean ± SD (CfB *p* value)	*N = 31* 74.3 ± 16.6	*N = 19* 95.3 ± 4.8 *(<0.0001)*	*N = 18* 95.6 ± 7.0 *(<0.0001)*	*N = 10* 99.8 ± 0.6 *(0.0004)*	*N = 3* 98.3 ± 2.9 *(0.2039)*
	DGKKT (0–100)	Mean ± SD (CfB *p* value)	*N = 31* 46.9 ± 20.3	*N = 19* 79.3 ± 13.1 *(<0.0001)*	*N = 18* 85.0 ± 14.4 *(<0.0001)*	*N = 10* 89.7 ± 10.7 *(<0.0001)*	*N = 3* 90.7 ± 16.2 *(0.0086)*
	Tegner Activity (0–10)	Mean ± SD (CfB *p* value)	*N= 31* 2.5 ± 1.3	*N= 19* 3.8 ± 0.9 *(0.0013)*	*N= 18* 4.1 ± 1.2 *(0.0064)*	*N= 10* 4.2 ± 1.0 *(0.0010)*	*N= 3* 6.0 ± 2.7 *(0.0742)*
	Cincinnati	Proportion of patients with good or excel- lent outcome	*N = 31* 6%	*N= 19* 21%	*N= 18* 55%	*N= 10* 80%	*N= 3* 67%

CfB = Change from Baseline. Means ± SD are given. *p* values are for comparisons between the follow-up and baseline values.

The assessment of the efficacy scores (i.e. Lysholm Gillquist, Tegner Lysholm, HSS, DGKKT, Tegner Activity, and Cincinnati) revealed a consistent significant (p value at descriptive level) improvement of the mean scores from pre-operation examination (baseline) to the follow-up visits 12 and 24 months post-OP. The amount of data for time frames later than 24 months post-OP the amount of available patient data is very low (≤ 4 patients). The chondrotransplant results are not shown here but can be found in the report. No substantial differences between the two ACI products were found. As clarified by the applicant, this study has high degree of overlap with the previously submitted Schreyer et al (2006), follow-up: Schreyer (2010) studies but no detailed information of patients from Schreyer et al., 2006 and Schreyer, 2010 are available to identify the exact extend of overlap.

Retrospective study: cod RS4 WZ (2014)

The retrospective study cod RS4 WZ (2014) includes patients with cartilage defects of the knee, treated with Spherox in routine clinical practice by Dr. Zinser at the St. Vinzenz Hospital, Dinslaken (Germany) between 2009 (introduction of the standard questionnaire) and the end of 2012. All the 90 patients treated in this period were contacted; of these, 36 patients consented to analysis of their medical files. The range of patient age was 18–54 years, 16 females, 20 males. Defect sizes ranged from 0.5 to 10.8 cm2. Primary defects

located on the patella or the femur were treated. The dose was between 8 and 94 spheroids/cm². The follow-up examinations were conducted 3, 6, 12 months after implantation. The primary analysis of the IKDC Subjective Knee Evaluation Score, 12 months after implantation, revealed an improvement from initially 38.8 ± 14.9 points (out of 100) to 61.0 ± 17.7 points ($p < 0.0001$, at the descriptive level). IKDC Current Health Assessment scores showed improvement for 'physical component summary' ($p < 0.0001$; change from baseline 13 points), but not 'mental component summary' ($p = 0.2461$; change from baseline 3 points). For the 20 patients for whom a MOCART assessment was available, the 12-month result was 70 (out of 100). This study has an overlap with Study 16 (cod 16HS17 paed) concerning one patient.

Prospective study by Siebold et al (2015)

In the publication by Siebold et al (2015), a total of 41 patients (31 men, 10 women) with 57 full-thickness articular cartilage lesions of the knee underwent a second-look arthroscopy at the ATOS Klinik (Heidelberg, Germany) after treatment with Spherox between 2009 and 2014. Chondral defects were located at the femur (47 defects), patella (8 defects) and tibia (2 defects). The mean defect size was 4.3 ± 3.4 cm² (range 0.5–20 cm2). Assessment of clinical follow-up took place 6–72 months after treatment (mean, 34.5 months). At follow-up, evaluation of KOOS showed an average mean of 81.0 ± 12.9 for 'pain', 76.8 ± 16.6 for 'symptoms', 85.1 ± 14.9 for 'ADL', 55.3 ± 27.7 for 'sport and recreation' and 50.6 ± 23.8 for 'QoL'. The IKDC score was 63.0 ± 18.8, the Lysholm score was 79.0 ± 18.0, and the Tegner score was 4 (range 1–6). Subjective assessment according to the VAS scale was on average 7.4 ± 2.1 for overall satisfaction and 6.7 ± 2.5 satisfaction for the operated knee. Second-look arthroscopies were performed 13.8 ± 13.2 months (range: 6–72 months) after treatment. Arthroscopic assessment was performed according to ICRS-CRA. The cartilage status of the 57 lesions was rated as "normal" (CRA I) in 12 lesions (21%), "nearly normal" (CRA II) in 40 (70%) and "abnormal" (CRA III) in 5 (9%). None of the treated defects were rated as "severely abnormal" (CRA IV). Regarding the degree of defect repair, 40 lesions (70%) were completely filled to the level of the surrounding cartilage shoulder and 9 lesions (16%) reached only 75% of the height of the surrounding cartilage shoulder. In terms of integration into the adjacent cartilage, 46 of 57 lesions (81%) showed a complete integration into the surrounding cartilage. The macroscopic appearance was an "intact smooth surface" or a (superficially) "fibrillated surface" in 43 lesions (75%). The potential predictors BMI, size of defect, location of defect and follow-up time did not have a significant impact on the CRA. A possibly significant correlation ($p = 0.04$) was found only between age and CRA. Prior to surgery, patients were clinically and radiologically assessed for tibiofemoral and patellofemoral malalignment. When pathologic, this was addressed simultaneously with, e.g. a high tibial osteotomy (HTO) or a medial tibial tubercle transfer, to offload the involved cartilage lesion and to optimise cartilage healing. Anterior cruciate ligament reconstruction and meniscal surgery were performed, too. The publication reports that concomitant surgery was necessary in 27 (65.8 %) of 41 patients which limits the interpretability of the study.

Körsmeier et al (2014)

Initial results from this study (Körsmeier et al [2012] were reported at the 29th AGA conference in 2012 in Zürich. During the period from March 2011 to August 2012, 18 patients (3 female, 15 male), aged 20–47 years (mean, 32.6 years) with proven cartilage defects caused exclusively by cam-impingement were biopsied, and received Spherox implants arthroscopically six weeks later. In this follow up study, the outcome of 16 of these patients with an average of 16.09 (SD, 5.3; range, 9.5–28.8) months follow-up after treatment with Spherox was described. The mean age of the two female and fourteen male patients was 31.75 years, ranging from 20 to 47. At each visit, the nonarthritic hip score (NAHS) and the Western Ontario and McMaster Universities Arthritis Index (WOMAC) were determined. In addition, patient satisfaction at the last follow-up examination was evaluated by means of a questionnaire completed by the patients. The mean

area of the chondral defects, as determined during arthroscopy, was 4.52 cm2, ranging from 3 to 6 cm2 (SD 1.13 cm2). Six weeks after the arthroscopy, the WOMAC and NAHS improved significantly ($p < 0.001$). Six weeks after the implantation procedure (12-week monitoring), a further significant enhancement of both scores was observed ($p < 0.001$). At the last follow-up, the WOMAC showed no further increase, while the NAHS further improved slightly and significantly in comparison with the 12-week monitoring. This exploratory study showed short-term improvement in the clinical scores of patients undergoing ACT 3D during impingement surgery. As no biopsies were taken in this study, there are no data on the quality of the reparative tissue. As is concluded in this publication, further prospective-randomized studies with a larger cohort of patients and longer follow-up periods are needed to clarify the comparative value of ACT 3D in comparison with other treatment options for chondral defects of the hip.

Fickert et al (2014)

This was a retrospective evaluation of outcome of the treatment with Spherox administered to patients with cartilage defects of the hip. A total of six patients (five men, one woman) aged between 25 and 45 years were included in this investigation of the treatment with ACT3D. All patients completed follow-up examinations. The average time of follow-up was 11.2 months. According to the ICRS classification all patients were diagnosed with a full-thickness chondral defect of the hip (grade IIIa–IIId). Three defects were located on the anterolateral-anteromedial acetabulum, one on the anterolateral acetabulum, one on the anteromedial acetabulum and one on the anterosuperior femoral head. The average defect size was 3.5 cm2. All acetabular defects were caused by cam impingement, one of the most common indications for hip arthroscopy. The cause of the femoral lesion was trauma. The duration of symptoms (pain, impairment in general health, and daily life functions) varied between 5 and 17 months. None of the patients had undergone surgical treatment of the affected hip before. During index arthroscopy, five patients received additional treatment in the affected hip: offset reconstruction was performed in five patients, three received additional labral repair and two partial resection of the labrum. Primary endpoint was the subjective improvement of symptoms and functionality displayed in the MHHS, NAHS and SF36 at 12 months after ACT3D compared to baseline (day before transplantation). Patients who underwent Spherox of the hip showed an overall improvement according to the Non-Arthritic-Hip score when comparing to baseline [median score at baseline: 67.5 % (SD 22.2); 12 months after Spherox: 95.6 % (SD 4.4)]. 6 weeks after surgery no improvement was monitored. 3 months after surgery improved scoring results were noted. At 12 months after Spherox significant improvement has been noticed (p = 0.02). Patients also showed significant improvement in in the Modified-Harris-Hip Score compared to baseline [p = 0.04; median score at baseline: 74.5 points (SD 17.2); 12 months after Spherox: 98.0 points (SD 2.8)]. Interpretability of this study is limited given the very small number of patients and lack of a control group. No second-look arthroscopies with chondral biopsies for histomorphological assessment or MRI-scans have been performed.

Fickert et al (2011)

This study is an uncontrolled, investigator-initiated, single-centre, prospective clinical trial to evaluate the subjective and functional outcome in the treatment of full-thickness cartilage defects of the knee during the 1-year follow-up period. According to the Applicant, this study has been conducted according to Good Clinical Practice (GCP). Thirty-six patients with chondral lesions of knee with defect size between 1.0 – 12.0 cm^2 and one patient in whom the knee joint defect size was not assessed were enrolled to receive Spherox treatment. The location of the lesion was either patella-femoral (43.2%) or femoral condyle (48.6%), three of the patients (8.1%) having both defect types. Over half of the patients (54.1%) enrolled had had previous surgical operation in the affected knee.

The MOCART score was assessed by two independent readers in 20 patients at three months and 14 patients at 12 months post-transplantation (Table 4). The similar assessment was received from both readers, the results after 12 months being improved compared to the results at three months from transplantation. However, the overall improvement (approximately 70 of 100 points at 12 month time point) between three and 12 months was not statistically significant. The filling of the defect was assessed to be approximately 15 of 20 points and the integration 12.3 of 15 at 12 months post-transplantation. After one year, bone marrow oedema was still present in eight patients. The subchondral lamina was not intact in 6 patients and was intact in 8 patients.

Assessment of defect repair with the MOCART score at 3 and 12 months after transplantation of spheroids

	MOCART score			
Subdomains (points)	3 months after transplantation: R1	3 months after transplantation: R2	12 months after transplantation: R1	12 months after transplantation: R2
1. Degree of defect repair and filling (0-20)	12.25 ± 6.38	13.25 ± 7.3	15.35 ± 6.03	14.66 ± 7.18
2. Integration to border zones (0-15)	12.25 ± 3.79	11.75 ± 4.06	12.14 ± 4.25	12.5 ± 3.25
3. Surface (0-10)	6 ± 3.47	6.31 ± 3.66	7.85 ± 3.23	8.57 ± 2.34*
4. Structure of the repair tissue (0-5)	1.75 ± 2.44	1.25 ± 2.22	2.85 ± 2.56	3.21 ± 2.48*
5. Signal intensity of the repair tissue:				
Dual T2-FSE (0-15)	7.22 ± 4.60	7.22 ± 4.91	9.16 ± 6.33	9.58 ± 5.82
3D-GRE-FSE (0-15)	7.05 ± 4.69	8.23 ± 4.65	9.16 ± 6.68	10.41 ± 5.82
6. Subchondral lamina (0-5)	3.94 ± 2.09	3.25 ± 2.44	3.21 ± 2.48	3.21 ± 2.48
7. Subchondral bone (0-5)	1.75 ± 2.44	1.25 ± 2.22	1.78 ± 2.48	1.42 ± 2.34
8. Adhesions (0-5)	4.5 ± 1.53	4.25 ± 1.83	5 ± 0	4.64 ± 1.33
9. Synovitis (0-5)	4 ± 2.05	4 ± 2.05	5 ± 0*	4.64 ± 1.33
Total	58.75 ± 20.25	60 ± 22.99	68.92 ± 27.46	71.07 ± 25.20

Note: Values in parentheses represent minimal and maximal possible points. R1 = reader 1; R2 = reader 2.
*$P < 0.05$ (t test): significant difference to the 3-month assessment of reader 1.

Statistically significant increases in the IKDC score from 44.0 points at baseline to 64.0 points at 12 months after ACT3D were seen. The maximal improvement was reached after 6 months and remained constant up to 12 months. For seven patients no IKDC ratings were available. Tegner score improved from median 1 point (range 0 – 7 points) to 4 points at 12 months after transplantation. The improvement was statistically significant. The statistically significant improvement between baseline and 12 months was reached in additional three endpoints, Lysholm score, VAS for pain, and SF-36-physical. The fastest improvement and plateau was reached at six month time-point with only minor change seen thereafter. The deviation in the obtained scores was not seen between the readers. Given the structured follow up and outcome, the results from this study may support proof of concept.

Changes in self-administered assessment scores from baseline (day before transplantation) to post-transplantation visit at 12 months (Fickert et al, 2011)

Outcome Variables		baseline → 12 mo
Lysholm	n	35 / 30
	Median	48 → 82.5
	Wilcoxon-Test p-value	<0.0001
VAS	n	35 / 30
	Median	6 → 3
	Wilcoxon-Test p-value	0.001
IKDC	n	30 / 31
	Median	44 → 64
	Wilcoxon-Test p-value	<0.0001
Tegner	n	35 / 30
	Median	1 → 4
	Wilcoxon-Test p-value	<0.0001
SF-36 physical	n	27 / 31
	Median	44.75 → 72.75
	Wilcoxon-Test p-value	<0.0001
SF-36 mental	n	28 / 30
	Median	66.84 → 83.06
	Wilcoxon-Test p-value	<0.0001
SF-36 total	n	27 / 30
	Median	112.08 → 155.44
	Wilcoxon-Test p-value	<0.0001

n=number of patients; mo=months

Rössing et al (2010)

This was an uncontrolled, prospective investigator-initiated study. The study included 43 patients, 29 male and 14 female (The mean age of the patients was 42.5 years (range 18 – 50). Transplantation of Spherox was performed via full arthroscopic technique. Mean size of chondral lesion was 4.7 cm^2 (2.5-9.5) and the dose of spheroids was 6-116.8/cm^2. Pre and post-surgery evaluations were performed with WOMAC, Tegner score and Lysholm Gillqvist score. Mocart/MRI evaluation was performed after 12 months. The follow-up was 24 months. The study showed improvement in all assessment scores after one year and further slight improvement after 2 years. The Spherox dose varied greatly in the patients treated in the study (6-116). There was no mentioning of defect location or diagnosis. Outcome evaluations did include MRI but after relatively short follow-up time. The study was presented as a conference poster and has not been published.

Maiotti et al (2012)

This was an uncontrolled, prospective investigator-initiated study. The study included 23 patients18 male and 5 female. (The mean age was 29.2 ± 10.4). Patients received the product by arthroscopy. The dose of spheroids was not provided. The patient diagnosis was osteochondral lesions of the knee; 15 patients with lesions of the medial femoral condyle, 6 lateral, and 2 femoral trochlea lesions. Pre and post-surgery evaluations were performed with IKDC, VAS, and Lysholm score. An MRI evaluation was performed at 12 and 24 months. Median follow up was 39 months (range 36-41). MRI showed complete defect filling in 22 of 23 patients. Arthroscopy and biopsies showed good integration and hyaline cartilage. The value of this study is quite limited as the dose and defect size are unknown. It is also not known how many patients completed the study.

Schreyer et al (2010)

This was a prospective investigator initiated study without controls performed between 1998-2009. The study included 70 patients, 41 male and 29 female (mean age 38 years). Patients were selected to one of three

treatment groups; Group 1: 40 patients received the product with periosteal flap coverage Group 2: 14 patients received Spherox by an open operation procedure Group 3: 16 patients received Spherox by an arthroscopical procedure. Dose of spheroids was unknown. Pre and post-surgery evaluations performed with DGKKT score, HSS score, Tegner score and Lysholm score. No Mocart/MRI evaluation and histology were performed. Follow up was 48 months. All scores improved compared to baseline. MRI should complete defect fill in 6 months.

2.5.3. Discussion on clinical efficacy

The applicant submitted altogether 218 literature references mostly of the published studies with conventional ACT treatment and another ACT3D treatment Hyalograft. Eleven reports related to the efficacy of the ACT3D Spherox. These consisted of only one peer reviewed research paper, the rest being case studies, conference posters, and study reports without detailed information of the conducted study. Only in one study active comparator, co.don chondrotransplant cell suspension product (Applicant´s own investigational product), was used. These 11 studies/reports add up to 255 patients treated with Spherox. In addition, at the time of the initial submission, more than 3,600 patients had been treated in six EU countries; however, no data on these patients were provided.

The inclusion criterion in all controlled studies was grade III to IV ICKD or 3/4 in Outerbridge scale for the severity of the chondral lesion in all studies presented. The surgical technique was arthroscopic implantation, except in the study by Fickert et al, 2011 where open medial arthrotomy was used and in Schreyer et al, 2010 study where both open procedure and arthroscopic technique was used for the Spherox transplantation. Miniarthrothomy was applied also in three patients in Schreyer et al, 2006 study and in 36 patients in Ruhnau, 2008 study as well as in 10 patients reported by Baum, 2008. In total, arthroscopy was used in 125 patients, open miniarthrotomy in 86 and open procedure in 14 patients. In a retrospective cod 16 HS 16 study of 29 adolescent patients the surgical technique was not specified. The number of patients in the submitted published reports was relatively small ranging from 6 to 42 subjects per report. The lack of controlled studies was also considered a problem since spontaneous improvement and even repair of the smaller chondral defects has been described, which applies particularly to younger patients without degenerative changes. Also, the absence of study protocols with regard to all of the submitted studies limited the possibility to evaluate how e.g. the missing data was handled or how the patients were selected to the study. Only one study, Fickert et al, 2011, provided in the dossier was conducted in compliance with ICH GCP, according to the applicant. This study had two independent readers for the subjective and structural data. The results in this study were favourable for Spherox in functional and structural and pain endpoints, but the data of the long-term results, the follow-up period being only one year, or the quality of the repaired chondral tissue by histology, was not demonstrated. In addition, no study protocol or individual data listings are available to allow assessment of the validity of the study results. The number of associated surgical treatments both before and during the Spherox treatment and the lack of data regarding possible use of rescue pain medication hampered the interpretation of the study results.

MRI analysis showed complete filling in the majority of patients in four studies (Schreyer et al, 2006; Baum, 2008; Rössing et al, 2010, and Maiotti et al, 2012) where these results were reported, and adequate filling in Alevrogiannis et al, 2008 study, according to the authors. However, in Rössing et al, 2010 paper the integration to the border zone was observed only in 55% of patients after one year from the transplantation. Overall, the results of these studies should be considered cautiously since the reporting did not fulfil the

regulatory standards by lacking the protocol for the prospective study, the information of the consistency of the test product and comparative data. In Fickert et al, 2011 study the structural analysis was performed by MRI using MOCART score and favourable results were obtained in the degree of defect repair and filling as well as in the integration to border zones by two independent readers. Unfortunately, in this paper the number of patients reaching complete defect filling and the integration of the repaired tissue, was not given. In structural studies, the histology was described only from six patients and the quality and type of neocartilage remained unclear. It is known that the level of hyaline-type of tissue, resembling naïve cartilage, has an impact to the strength and functionality of the newly-formed cartilage, and predicts the persistence of the satisfactory joint function. Two of the reports dealt with the results obtained from the treatment of hip and talus lesions. The applicant had not restricted the use of Spherox by lesion site or medical history underlying the cartilage defect in the proposed indication, which was not considered acceptable. One of the studies was a report from the retrospectively collected data from the child/adolescent population treated for knee cartilage lesion. This study contained also patients with open epiphyseal plate not indicated for Spherox. It was considered that more data from the paediatric population would be needed to substantiate the use of Spherox in this patient population, hence the current indication is restricted to adults only.

Design and conduct of clinical studies

Study cod 16 HS14 is a prospective, randomised, open label, multicentre Phase II clinical trial to investigate the efficacy and safety of the treatment of large cartilage defects with 3 different doses of Spherox (ACT3D-CS) in 75 patients. Patients were to be aged between 18—50 years with a single symptomatic ICRS grade III or IV chondral lesion (including OCD) between 4 and 10 cm2 of the medial or lateral femoral condyle, trochlea, tibia, or a retropatellar defect. A cartilage biopsy was taken prior to randomisation to one of 3 dose groups for treatment with Spherox, 3–7 spheroids/cm2; 10–30 spheroids/cm2; and 40–70 spheroids/cm2. Patients were blinded to their dose. After implantation surgery, patients were recalled for assessments after 6 weeks, 3, and 6 months, with final assessment after 12 months. Additional follow-up assessments have taken place after 18, 24, and 36 months (the cut-off point for the present submission). Future assessments will take place after 48 and 60 months, respectively. MRI images were assessed centrally by a blinded reader and histological assessments performed centrally by a blinded pathologist. Primary defect locations were mostly the patella (47/75) or the femur (28/75); the tibia was not represented. Any additional defects were treated, and their results recorded, if they were at the same location. ICRS grades were mostly III C or IV A.

The study's primary efficacy variable was the change from baseline to Visit 4 (12 months after implantation for each dose group) in 'overall KOOS'. A test for superiority against baseline (Day before arthroscopy = Day 0) for the three dose groups (using a dose hierarchy) was performed.

Study cod 16 HS13 is a prospective, randomised, open-label, active-controlled, multicentre Phase III clinical trial to compare the efficacy and safety of the treatment with the autologous chondrocyte transplantation product ACT3D-CS with MF in 102 patients with smaller cartilage defects of the knee. Patients were to be aged between 18—50 years with a single symptomatic ICRS grade III or IV chondral lesion between 1 and <4cm2 of the medial or lateral femoral condyle. After implantation surgery or MF procedure, patients were recalled for assessments after 6 weeks, 3, 6, and 12 months with the final evaluation of superiority to be performed after 24 months (to be submitted). Additional follow-up assessments will take place after 36, 48 and 60 months, respectively. MRI images were assessed centrally by a blinded reader and histological assessments are performed centrally by a blinded pathologist. ICRS grades were mostly IV A, followed by IIIB and IIIA. The current submission reports the 12-month interim analysis of the primary variable 'overall KOOS'.

The designs of the studies are largely consistent with previously provided CHMP scientific advice, including the choice of active comparator in the Phase III trial. However, the company was recommended to demonstrate a dose-response in Phase II trial given the absence of a suitable comparator for studies in large lesions. This has been complicated by the narrow dose range selected for this study.

Non-interventional studies in paediatric patients (cod16HS16 and cod16HS17): Paediatric data include surveillance studies with retrospective data collection were conducted in a descriptive manner in adolescent patients (14 to 18-years and 15- to 18-years patients in cod16HS16 and cod16HS17 studies, respectively). A low number of subjects were available for the analysis (29 FAS subjects in each study) and only small number of subjects in each study had verified closed epiphyseal plate (6 and 12 subjects in cod16HS16 and cod16HS17 studies, respectively). The cod 16 HS16 study was submitted in the initial phase of the MAA. In response to the CAT/CHMP LoQ, the applicant provided interim data from the cod16HS17 study and the cut-off date for the patients recruited was until the end of January 2016. The follow-up time in the cod16HS16 study was up to seven years and in the cod16HS17 study the data was documented for mean 63.3 months (range 2.5 and 8 years). Wide variety in patient characteristics and disease history was involved in both studies. The lesions were classified as grade III and IV lesions in both studies. The lesions in the cod16HS17 study were located mainly in the femur (22), but patients with patellar lesions (6) and lesions in tibia (1) were also included. The lesions in cod16HS16 study were the cartilage defects in the knee joint. The mean lesion size was 6.8 cm2 (2.3-12 cm^2) and 4.6 cm2 (2.2 – 12 cm^2) and the average number of spheroids implanted per cm2 was 25.1 and 33.8 in cod16HS16 and cod16HS17 studies, respectively. Osteochondritis dissecans was the underlying condition in 13 and 14 patients in these studies, respectively. In the cod16HS17 study concurrent surgeries were performed during biopsy in 10 patients and during implantation in 11 patients. In the cod16 HS 16 study further interventions to ipsilateral graft were reported for 16 (55%) patients who received between 1 to 3 interventions per patient. The endpoints in cod16HS16 study were MOCART, VAS pain score, IKDC, and quality of life (SF-36). The primary analysis according to the PIP in cod16HS17 study was treatment failure rate and the secondary analyses were physical examination, IKDC and modified Lysholm scores.

Efficacy data and additional analyses

In Study cod 16 HS14, the assessment of the primary variable 'overall KOOS' for the ITT population showed a statistically significant improvement, compared with baseline, in all three within-group analyses (for the high-, medium- and low-dose group respectively p = 0.0010, 0.0001 and 0.0002 one year after study treatment, and maintained up to 3 years. For all dose groups combined, the mean overall KOOS rose in the first year after treatment from 57.0 to 73.4 on a scale from 0 (worst) to 100 (best) and continued to rise slightly, reaching 77.0 after 36 months. Changes within each dose group were of similar magnitude and between-group (pairwise) analyses did not reveal any statistically significant differences between the dose groups. At the 12-month assessment, KOOS subscores also generally showed within-group improvements – mostly with p <0.05 – while between-group comparisons did not show any significant difference. Between the 24-month and 36-month visits most KOOS values – overall and subscores showed a small increase, in the order of 2–3 percentage points.

Structural MRI evaluation by MOCART scoring was not assessed before study treatment but showed modest numeric improvement over time without notable differences between the dose groups. The mean MOCART total scores – on a scale from 0 (worst) to 100 (best) – after 3 months were 59.8, 64.5 and 64.7 for the low-dose, medium-dose and high-dose group respectively, and 62.9 for 'all patients'; after 12 months these were 74.1, 74.5 and 68.8 for the respective dose groups and 72.4 for 'all patients'. After 18, 24 and 36 months

after study treatment the scores were maintained: respective dose-group scores after 24 months were 72.2, 76.0 and 71.2, and 73.3 overall, and after 36 months these were 72.4, 79.6, 72.1 and 74.9 overall.

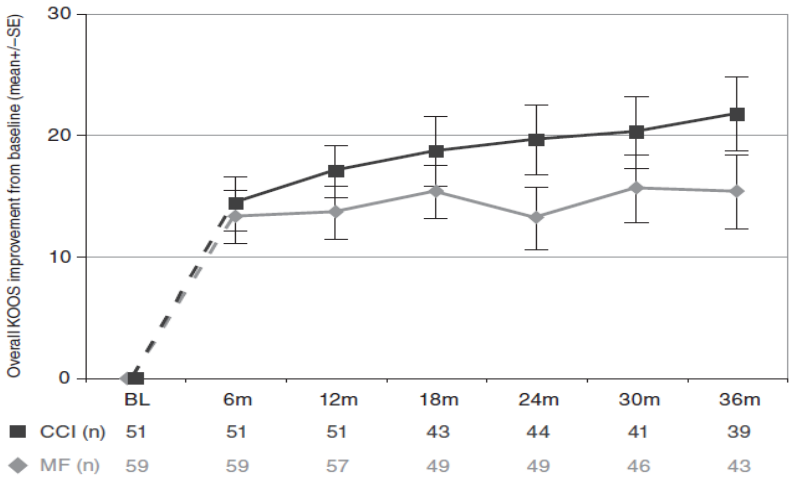
Arthroscopic assessment of cartilage repair (ICRS- cartilage repair assessment) after 12 months for a subset of 8 patients gave the result 'normal' or 'nearly normal' in seven cases and 'abnormal' in one. Results of the R-biopsy assessments after 12 months suggested that all repair tissues displayed hyaline cartilage characteristics to some extent, but that no sample displayed a true hyaline cartilage phenotype. Two out of 7 R-biopsies showed hyaline-like to hyaline cartilage repair tissue, 4 out 7 R-biopsies were fibrocartilage (mixed-type hyaline-fibrocartilage tissue), 1 out of 7 biopsies showed fibrous tissue and one R-biopsy could not be evaluated.

All patients in this study underwent a strict rehabilitation up through 3 months after transplantation. Thus, the total effect measured in this trial would be the combination of the intervention under study and the rehabilitation programme. Also, while KOOS was used as an patient reported outcome instrument of knee symptoms with a look-back period of one week, patients were allowed to use paracetamol monotherapy (max 4 g/day) up to the morning of the visit day as well as the use of oral and/or topic NSAIDs (or combinations of paracetamol and NSAIDs) during the trial up to a week before each visit. It was viewed that the use of these concomitant pain medications might have impacted the KOOS rating and the Applicant was requested to address this. In response, the Applicant conducted subgroup analyses comparing patients with and without pain medication. The change in KOOS from baseline was lower in the subgroup with pain medication but showed improvement in both groups, with a mean change from baseline in KOOS of 9.5 vs. 19.4 at Visit 4 (12 months) and 10.5 vs. 24.3 at Visit 7 (36 months).

Eleven patients still used pain medication at 36 months. The reasons are not fully clear but do not appear to be explainable by defect size. In addition, a review of the data listings did not indicate a preponderance of femur lesions; four of the patients had a primary cartilage defect located at the femur whereas the remainder had a patellar lesion. Overall, the majority of these patients tended to show a decrease in pain medication over time.

The Applicant was also requested to address the apparent absence of a dose-response e.g. by exploring a dose response relationship on a continuous scale. The Applicant provided the requested analysis both for the ITT and the PP population. The results were very similar and no evidence of a dose response relationship was present. The reasons for this are not clear. It seems possible that the lack of dose-response is partly due to the deviation from the randomised treatment that occurred in this study; in particular, in the high dose group there were a number of patients who did not receive their dose as planned due to the fact that the chondrocyte culture did not generate the required number of spheroids and/or that the estimation of actual defect size had been insufficiently accurate.

In Study cod 16 HS13, in the 12-month interim analysis of the primary variable 'overall KOOS' for the ITT1 population, both Spherox (ACT3D-CS) and the comparator MF showed a statistically significant improvement relative to baseline. For patients treated with ACT3D-CS the mean overall KOOS score rose from 56.6 ± 15.4 at baseline to 78.7 ± 18.6 at Visit 4 12 months later (change from baseline 22.2 ± 18.3), while for those treated by MF this score rose correspondingly from 51.7 ± 16.5 to 68.1 ± 18.6 (change from baseline 16.4 ± 15.1). These 12-month results for MF are consistent with those reported previously for MF in a similar population (e.g. Saris et al, 2009) as shown below.



Mean improvement from baseline in overall KOOS) and KOOS subdomains to month 36

CCI, characterized chondrocyte implantation using a cell therapy product; MF, microfracture

From: Saris et al. Characterized chondrocyte implantation results in better clinical outcome at 36 months in a randomized trial compared to microfracture. Am J Sports Med. 2009;37 (Supp 1):10S-19S).

According to the between-group comparison conducted in the 12-month interim analysis for study cod 16 HS 13, the ACT3D-CS treatment passed the test of significant non-inferiority compared with MF (point estimate of 5.7) but in spite of a numerically better outcome was not found to be statistically significantly superior. Results for the PP population were consistent with the primary analysis.

Structural MRI evaluation by MOCART scoring was assessed at Visits 2 (3 months) and 4 (12 months). The overall MOCART score showed a modest numerical improvement from Visit 2 to Visit 4 in both treatment groups, being 67 for the ACT3D-CS group and 62 for the MF group at Visit 2 (3 months), improving respectively to 81 and 77 at Visit 4 (12 months). Other structural assessments are currently not available as the second-look arthroscopy and R-biopsy procedures (in consenting patients) are planned for the 24 month after implantation/microfracture visit.

Similar to the Phase II trial, the impact of the rehabilitation programme and concomitant pain medication use needed to be further discussed for this study in order to facilitate the interpretation of the trial results. The Applicant was requested to address elements such as the potential for spontaneous improvement in KOOS symptoms over the course of time, the degree and sustenance of KOOS benefit that can be derived from a rehabilitation program such as the one used in the two pivotal studies, and the impact of concomitant pain medication. The Applicant has explained that cartilage in adult patients has limited self-healing capacity due to its avascular and aneural nature. There is a lack of systematic data in relation to the natural history of untreated primary cartilage lesions in particular for the first few years after the defect has occurred, as these early stages may not be associated with pain or other symptoms.

It was sufficiently clarified that the standardized rehabilitation program (up to 3 months) was very similar between the ACT3D-CS and MF treatment arms.

The Applicant conducted a subgroup analysis based on the use of concomitant pain medication that showed similar results for ACT3D-CS as compared to the Phase II study, namely that both subgroups improved, although with a numerically lower improvement at 12 months follow-up in patients taking pain medication (gain of 12.1 KOOS points) compared to patients taking no pain medication (24.2). This numerical difference

in KOOS outcome between the subgroups was not observed in the MF arm but it should be considered that the number of patients still using pain medication at Visit 4 was quite small.

Regarding structural outcome measures, while MOCART scores showed modest numeric improvements after treatment in both studies, there was no correlation between the KOOS and MOCART outcome measures. In addition, in study cod 16 HS 13, a substantial proportion of MOCART overall scores appeared to have been missing. This relates to missing assessments of some items that contributed to the overall MOCART score.

The lack of correlation between clinical and radiological outcomes after surgical interventions for cartilage in the current studies is consistent with previous findings and published literature. The reasons for this are likely multifactorial but currently insufficiently understood.

Results of the R-biopsy assessments after 12 months suggested that all repair tissues displayed hyaline cartilage but no sample displayed a true hyaline cartilage phenotype. Results from the Phase III study are not yet available and will be provided post-marketing.

In the Phase III study cod 16 HS 13, duration of knee symptoms was recorded only for a subgroup of patients, 39 patients of the ACT3D-CS group and 40 patients of the MF group. The other patients of the treatment groups did not report and/or did not recall the duration of symptoms during screening. The duration of symptoms for these patients was unknown. According to a recent review (Mithoefer et al, Cartilage. 2011;2[2]:100-21) several studies have demonstrated that 'age of the defect', that is, time since onset of the cartilage injury of more than 12 to 36 months, had a clear negative effect on both MF and cellular therapy outcomes. However, the Applicant provided an analysis of overall KOOS by duration of symptoms for the category ≤ 1 year und > 1 year which did not show any relevant differences between the subgroups.

Further justification was also requested regarding the defect location. No patients with primary tibia plateau or hip lesions were included in the pivotal trials. In particular for the hip, literature data on the use of ACT appeared to be limited (Körsmeier 2014) and this is reflected in the very limited data submitted by the Applicant. It would appear that prospective-randomized studies with a larger cohort of patients and longer follow-up periods would be needed to clarify the comparative value of ACT3D-CS in comparison with other treatment options for chondral defects of the hip. This has been agreed by the Applicant and tibia and hip lesions have been removed from the sought indication accordingly.

Autologous chondrocyte implantation after failed microfracturing appears to be associated with a significantly higher failure rate and inferior clinical outcome when compared with ACI as a first-line treatment (Pestka JM, et al. Am J Sports Med. 2012:40(2):325-31). Thus, the relevance of prior treatment of the cartilage defect on the outcome of second line ACI was to be further discussed. However, no patients failing prior treatment with MF were included in the pivotal trials. Subgroup analyses indicated similar benefit in terms of KOOS change from baseline in the subgroups previous surgery yes/no.

The proportions of patients not reaching clinically relevant improvement (i.e. treatment failure) of the overall KOOS score (>8 points improvement) in Spherox group was substantial both in Phase 2 and 3 trials, 36% and 31%, respectively. Furthermore, a number of subjects reached worse results in overall KOOS at 1-year time point compared to the baseline. This was further discussed by the Applicant, clarifying that 10 subjects from Phase II and 4 patients from Phase III reached worse results in overall KOOS at the 1-year follow-up compared to baseline but 5 of these 10 Phase II patients showed improvement in KOOS (as compared to baseline) when a longer follow-up period was used and 2 other patients were lost to follow-up. The Applicant was also requested to clarify the correlation between the cases of failures and the quality of the implant (cell growth rate, biomarker data), localization and size of the lesion (Phase 2), and baseline KOOS score level.

According to the applicant, 40 out of 120 patients (Phase II + Phase III) were non-responders at the 1-year follow-up but it is expected that the newly defined ranges and limits implemented in the manufacturing process will reduce the non-responder rate as 24 out of these 40 non-responders would not have passed the newly defined limits and ranges for process parameters, in-process controls or release parameters. The adjusted operational ranges and limits will be implemented in the manufacturing process and assessed by a new process validation, which is part of the condition required by the CAT/CHMP.

Spherox has been used in several European countries besides Germany, from 2004 up to the end of 2012 according to the transitional period as defined in Article 29 (2) of Regulation (EC) No 1394/2007. However, with regard to the pivotal studies, very few patients enrolled outside Germany. Thus, an analysis of differences in primary outcome based on country was not meaningful.

In addition to the pivotal Phase II and III studies, the applicant provided additional supportive data consisting of 4 retrospective studies and additional literature references in response to the Day 120 LoQ. These offered some supportive evidence in favour of the efficacy of Spherox.

The quality of the available literature on chondral injuries in the paediatric knee is poor, is usually limited to small case series and there is a lack of comparative trials.

In addition to the already submitted retrospective data collection cod 16 HS 17 paed, the applicant provided an interim report for paediatric investigation cod 16 HS 17 paed to assess the long-term safety and linked efficacy of Spherox in paediatric patients from 15 to less than 18 years of age at the time of implantation, in the context of a paediatric investigation plan.

The results in cod16HS16 suggested overall favourable result and sustained treatment effect in up to 7 patients by IKDC score, the increase in mean MOCART score in few patients having MRI results, decrease in VAS pain and increase in SF36 physical functioning, but decrease in SF36 mental health score. Altogether, these results were not informative due to very small number of patients (5-8 at baseline).

The interim results from the study cod16HS17 showed additional surgery overall in 5 patients (17.2%), concerning the affected knee in 2 patients and other surgery in 3 patients. Mean time period from treatment to surgery was 34.3 months. According to the Applicant the treatment failure was seen only in one patient with closed epiphysis. In the physical examination (23 patients) none of the patients showed knee effusion or Baker's cyst. Five patients experienced pressure pain and in one patient patellar motion was abnormal. In evaluated 22 patients the mean IKDC subjective knee evaluation score was 81.1, which represents a good IKDC grade in treatment outcome. The knee alignment was normal in all except one of 23 patients and patella position in all except one patient. In general, the physical examination produced normal result in 12 patients and nearly normal in 9 patients, while the result was missing in two patients. Mean modified Lysholm score was 21.0 in 22 patients. The results of this study are confounded by the missing scores in six to seven patients in different evaluations and high heterogeneity of the study population, having possibly an impact to the results.

Overall, these data provide a limited, descriptive indication that the patients are doing well after the procedure. Data regarding patients with open epiphysis are very limited, hence the indication cannot be granted for treatment of children or adolescents. Accordingly, the use of Spherox in patients < 18 years of age is no longer part of the refined indication.

It should be considered that a substantial part of the available long-term data originates from a patient population with patellar non-weight-bearing lesions. These patients constituted more than 60% of the patient population in the Phase II trial, whereas only 20 patients with femur lesions with defect size 4-6.99 cm^2 and

8 patients with larger defect sizes of 7-10 cm^2 were included. A subgroup analysis of the data showed a numerically lower mean change from baseline in KOOS for femur lesions compared to patella, regardless of lesion size stratum, but the improvements in both groups are however clinically relevant. Nevertheless, given the limited sample size of weight-bearing femur lesions for which long-term data is available, the 24-month data of the cod 16 HS 13 Phase III study in femur condyle lesions will provide valuable additional information on clinical as well as structural outcome measures.

Whereas the current data provide sufficient proof of efficacy in the approved indication, the applicant will conduct the long term follow up Phase III study, as requested by the CAT/CHMP. As these uncertainties are considered key to the benefit-risk of the product, the submission of the study data is made condition to this MA.

2.5.4. Conclusions on the clinical efficacy

The applicant has provided the available efficacy data from the ongoing Phase II and III trials as well as supportive data from retrospective studies and publications from the literature. These data are supportive of the clinical efficacy of Spherox and the benefit-risk is considered positive for the revised indication.

The CAT/CHMP considers the following measures necessary to address issues related to efficacy:

Post-authorisation efficacy study (PAES): 60-month follow-up data for study cod 16 HS 13.

In order to evaluate the long-term efficacy and safety of Spherox vs. microfracture in patients with cartilage defects of the knee with a defect size between 1 and < 4 cm^2, the MAH should conduct and submit the results of the ongoing prospective, randomised, open label, multicentre study.

2.6. Clinical safety

Clinical experience with Spherox derives from two pivotal clinical trials (phase II cod 16 HS14 and phase III cod 16 HS13), and sixteen supportive clinical investigations. Additionally, there is post-marketing data derived from an exposure of approximately 8 000 patients.

Patient exposure

General tabular overview of clinical investigations with Spherox in the knee and hip is included in section 2.4.1. The below tables outline the data on patient exposure to Spherox treatment.

Exposure to the test product: Dose administered (ITT population) Phase II clinical trial (cod 16 HS14).

Dose group:	**Low** *N* = 24	**Medium** *N* = 25	**High** *N* = 24	**All** *N* = 73
Spheroids/cm²				
Mean ± SD	10.8 ± 15.7	27.5 ± 13.0	41.7 ± 12.4	26.7 ± 18.5
Median	7.0	28.8	43.4	28.2
Range	6.8 – 84.0	11.3 – 83.1	11.6 – 68.3	6.8 – 84.0
Number of spheroids				
Mean ± SD	37.5 ± 10.8	125.6 ± 39.9	204.3 ± 51.4	122.5 ± 77.8
Median	35	120	209	120
Range	28 – 63	51 – 224	93 – 290	28 – 290

The ITT population is used here in order to exclude the two patients for whom no attempt was made to administer chondrosphere

Exposure to the test product: Dose administered (safety population) Phase III clinical trial (cod 16 HS13)

Measure of exposure		**ACT3D-CS group** *N* = 52	**Microfracture group** *N* = 50
Spheroids/cm² based on defect area as found by arthroscopy before debridement *	Mean ± SD	31.7 ± 22.5	*not applicable*
	Median	28.5	
	Range	10 – 130	
Spheroids/cm² based on defect area as found at implantation, after debridement *	Mean ± SD	24.9 ± 15.3	
	Median	21.1	
	Range	6 – 70	
Number of spheroids	Mean ± SD	64.9 ± 40.4	
	Median	60	
	Range	12 – 175	

* Area at arthroscopy was used for determination of dose (amount of chondrosphere®); area at implantation was post-debridement and therefore more accurate.

The applicant claims that there are in total 529 patients with cartilage defects of the knee treated with Spherox. However, 127 adult patients out of the 529 patients were from the two pivotal clinical trials (75 patients in the phase II and 52 patients in the phase III) and the other data were from supportive clinical investigations (published case series, retrospective and prospective cohort studies) of which only one study there were GCP compliant safety data (Fickert et al (2011)). Thus the number of patients with valid safety

data is 127 and 37, i.e. 164. This is a limited number. The follow up is also limited for these patients, although the follow-up in supportive studies reach 5 years.

Adverse events

Incidence of adverse events at 12 months by SOC and PT; pooled analysis of Phase II and Phase III clinical trial (adapted by the CAT/CHMP)

System Organ Class Preferred Term	Total N (%)
Total number of patients	127 (100%)
Total number of AEs (ne) and number of subjects with at least one AE (n)	93 (73.2%)
Musculoskeletal and connective tissue disorders	87 (68.5%)
Joint effusion	75 (59.1%)
Arthralgia	16 (12.6%)
Joint swelling	11 (8.7%)
Back pain	2 (1.6%)
Muscular weakness	1 (0.8%)
Bone cyst	1 (0.8%)
Bone pain	1 (0.8%)
Chondromalacia	1 (0.8%)
Joint crepitation	1 (0.8%)
Ligament disorder	1 (0.8%)
Muscle tightness	1 (0.8%)
Synovial cyst	1 (0.8%)
Tendon disorder	1 (0.8%)
Infections and infestations	16 (12.6%)
Nasopharyngitis	6 (4.7%)
Influenza	2 (1.6%)
Respiratory tract infection	2 (1.6%)
Bronchitis	1 (0.8%)
Conjunctivitis	1 (0.8%)
Gastrointestinal infection	1 (0.8%)
Onychomycosis	1 (0.8%)
Rhinitis	1 (0.8%)
Tooth infection	1 (0.8%)
Upper respiratory tract infection	1 (0.8%)
Injury, poisoning and procedural complications	15 (11.8%)
Ligament sprain	4 (3.1%)
Joint dislocation	2 (1.6%)
Contusion	1 (0.8%)
Fall	1 (0.8%)
Hand fracture	1 (0.8%)
Ligament rupture	1 (0.8%)
Periorbital haematoma	1 (0.8%)

System Organ Class Preferred Term	Total N (%)
Post-traumatic pain	1 (0.8%)
Rib fracture	1 (0.8%)
Road traffic accident	1 (0.8%)
Suture related complication	1 (0.8%)
Wound dehiscence	1 (0.8%)
Nervous system disorders	6 (4.7%)
Headache	3 (2.4%)
Convulsion	1 (0.8%)
Muscular weakness	1 (0.8%)
Paraesthesia	1 (0.8%)
General disorders and administration site conditions	4 (3.1%)
Pain	2 (1.6%)
Gait disturbance	1 (0.8%)
Adverse drug reaction	1 (0.8%)
Discomfort	1 (0.8%)
Tenderness	1 (0.8%)
Vascular disorders	7 (5.5%)
Hypertension	2 (1.6%)
Thrombophlebitis	2 (1.6%)
Deep vein thrombosis	1 (0.8%)
Haematoma	1 (0.8%)
Lymphoedema	1 (0.8%)
Gastrointestinal disorders	3 (2.4%)
Diarrhoea	2 (1.6%)
Dysphagia	1 (0.8%)
Skin and subcutaneous tissue disorders	2 (1.6%)
Alopecia	1 (0.8%)
Psoriasis	1 (0.8%)
Skin discolouration	1 (0.8%)
Immune system disorders	2 (1.6%)
Drug hypersensitivity	1 (0.8%)
Hypersensitivity	1 (0.8%)
Metabolism and nutrition disorders	2 (1.6%)
Hypothyroidism	1 (0.8%)
Vitamin D deficiency	1 (0.8%)
Blood and lymphatic system disorders	1 (0.8%)
Bone marrow oedema	1 (0.8%)
Investigations	1 (0.8%)
Gamma-glutamyltransferase increased	1 (0.8%)
Reproductive system and breast disorders	1 (0.8%)
Cervicitis cystic	1 (0.8%)
Respiratory, thoracic and mediastinal disorders	1 (0.8%)

System Organ Class Preferred Term	Total N (%)
Tonsillar inflammation	1 (0.8%)
Surgical and medical procedures	1 (0.8%)
Skin neoplasm excision	1 (0.8%)

Study cod 16 HS13 (Phase III), adverse events by MedDRA SOC and PT (MedDRA Version: 15.1; safety population), 12 months

Treatment group:	ACT3D-CS *N* = 52		Microfracture *N* = 50	
	n_P (%)	n_E	n_P (%)	n_E
Any SOC	29 (56%)	65	29 (58%)	81
Musculoskeletal and connective-tissue disorders	25 (48%)	37	21 (42%)	41
Joint effusion	17 (33%)	19	13 (26%)	15
Arthralgia	7 (13%)	7	12 (24%)	15
Joint swelling	7 (13%)	7	8 (16%)	9
Back pain	–	–	2 (4%)	2
Injury, poisoning and procedural complications	7 (13%)	7	6 (12%)	9
Ligament rupture	1 (2%)	1	2 (4%)	2
Post-traumatic pain	1 (2%)	1	1 (2%)	2
Hand fracture	1 (2%)	1	1 (2%)	1
Joint dislocation	2 (4%)	2	–	–
Infections and infestations	4 (8%)	6	6 (12%)	8
Nasopharyngitis	2 (4%)	3	1 (2%)	1
Cystitis	–	–	1 (2%)	2
General disorders and admin. site conditions	1 (2%)	6	1 (2%)	3
Pain	1 (2%)	3	1 (2%)	2
Gait disturbance	1 (2%)	2	1 (2%)	1
Gastrointestinal disorders	–	–	4 (8%)	7
Gastrooesophageal reflux disease	–	–	2 (4%)	2
Nausea	–	–	1 (2%)	2
Vascular disorders	3 (6%)	3	3 (6%)	3
Hypertension	1 (2%)	1	2 (4%)	2
Nervous system disorders	1 (2%)	1	3 (6%)	4
Headache	–	–	2 (4%)	3
Skin and subcutaneous disorders	1 (2%)	2	1 (2%)	1
Psychiatric disorders	–	–	2 (4%)	2
Reproductive system and breast disorders	1 (2%)	1	1 (2%)	1

Numbers of patients (n_P) and events (n_E) are given. Cut-off <2 patients overall.

The applicant presented pooled results from the two pivotal studies at 12 months follow-up. The most frequent AE is reported in SOC of Musculoskeletal and connective tissue disorders, which is expected after a

surgical procedure. There are several AE reported only ones or twice, without clustering. The limited number of events makes it difficult to draw firm conclusions. However, when comparing ACT3D-CS and microfracture there are no indication that there are relevant differences between the two treatments.

There were two AEs in SOC Immune system disorders in HS13 and one similarly in HS14 and there have been plausible causes for hypersensitivity reactions other than Spherox implantation. Furthermore, from the phase II (HS14) and the phase III (HS13) studies it can be estimated that the incidence of treatment related AEs (ADRs) after biopsy arthroscopy occur at rates 20/75 (26.6%) and 11/52 (21.1%) and after implantation arthroscopy at rates 28/75 (37.3%) and 20/52 (38.4%) in the studies HS14 and HS13, respectively. The applicant provided additional information demonstrating that treatment related adverse events were reported from 38.4% patients receiving Spheroxs and from 52.0% of patients undergoing microfracturing, up until week 6 (from transplantation or microfracturing). Thereby the post-operative safety profile of Spherox appears more favourable, compared to microfracturing; and this is even despite the need for two arthroscopies for Spherox.

Additionally, safety data from 37 patients being treated in the study by Fickert (2011) can be added, 7 patients experienced a total of 8 AEs during the 12-month follow-up. Two patients reported mild swelling and 2 patients reported mild knee effusion. The events lasted up to 3 months after transplantation. Two patients experienced moderate blocking of the knee joint, 1 patient at 6 months after transplantation and 1 patient throughout the follow-up period. At clinical examination, no movement restriction was noted and MRI examination did not reveal any loose particles. One of these patients also experienced superficial thrombophlebitis. One patient experienced 2 SAEs (deep vein thrombosis and pulmonary embolism).

The retrospective data, and literature references to prospective studies do not evoke new safety risks, nor does the post-marketing experiences from 8 000 treated subjects. Overall, there are few events reported in limited number of subjects treated within prospective clinical studies with proper follow-up of safety data, although the supportive data does not indicate different safety profile.

Serious adverse event / deaths / other significant events

Study cod16HS14

Serious adverse events (safety population); cod 16 HS 14, 3-year follow-up Report

Study period	Patient no.	Dose group	Adverse event	Severity	Relationship to treatment	Outcome
Months 1–24	1114	medium	Syncope	severe	unlikely	resolved
	1803	low	Convulsion	moderate	none	resolved
	2251	high	Arthralgia	moderate	none	resolved
	2253	high	Umbilical hernia	mild	none	resolved
	2301	low	Meniscus lesion	severe	none	resolved
	2411	high	Chondropathy Chondropathy	severe severe	probable probable	resolved resolved
Months 25–36	1113	low	Chondropathy	moderate	unlikely	resolved
	1118	medium	Chondropathy	moderate	none	not resolved
	1306	low	Arthralgia	moderate	probable	resolved
	2241	low	Uterine cyst	moderate	none	resolved
	2249	high	Arthralgia	moderate	none	resolved

There were two SAEs considered related to the study treatment of the Spherox in the phase II trial (two episodes of chondropathy in one patient and arthralgia in one patient). The applicant submitted additional information and cartilage graft hypertrophy was observed in one patient in the two controlled RCTs. Rest of the three "chondropathies" observed had plausible explanations for not being attributable to Spherox transplantation. Graft hypertrophy is listed as rare undesirable effect in 4.8 of the SmPC.

Study cod16HS13

Two serious adverse events were reported in the cod 16 HS 13 at 12 months follow up:

- Deep vein thrombosis (resolved, probably treatment-related)
- Meniscus lesion (resolved, unlikely to be treatment-related)

Both patients were in the microfracture group. No SAEs were reported in the phase III trial considered related to the study treatment of the Spherox.

Fickert et al (2011)

One 46-year-old female patient experienced 2 SAEs of severe intensity. Directly after surgery, she developed deep vein thrombosis, leading to pulmonary embolism. As stated in the article, the events were considered not related to Spherox by the investigator but rather to the relatively long duration of the surgical procedure (148 minutes). The patient recovered.

No deaths were reported during the phase II and phase III clinical studies, and none have been reported in connection with the commercial use of Spherox.

Immunological events: No data for immunological events were provided. It is agreed that there is low risk of activation of the immune system in a typical situation with absence of bleeding or damaged lineage cellular surfaces inside the joint. However, as 58 of 75 subjects reported joint effusion at 36 months follow-up and since joint effusion could be potentially considered as a marker of local inflammation, there is a possibility that immunologic competent cells are present. The compositions of joint effusions are not known, and thereby it is difficult to assess the risk for immunological events, although no intraarticular infection or bleedings were reported. The occurrence of joint effusion is mainly reported during the first year (90% of the joint effusions in study HS14) and thereby it is reasonable to claim that they are a result from the surgical procedure rather than late occurring complications.

Laboratory findings

Clinical laboratory evaluations were performed in the pivotal studies cod 16 HS13 and cod 16 HS14. Blood samples for measurement of standard laboratory values were taken in both trials at screening, three months after implantation and 12 months after implantation. Haematology for red-blood-cell count, white-blood-cell count and platelets showed mean and median values within the respective reference ranges. Individual values did not lie substantially outside these ranges. There was in no case any general shift between the three time points of measurement. Scatter plots and shift tables did not reveal any tendency towards a general migration of values. For serum chemistry of hepatic function all mean and median values were within the respective reference ranges (taking account of the slightly different ranges for males and females). Scatter plots and shift tables did not reveal any tendency towards a general migration of values. For serum chemistry of metabolism, all mean and median values for triglycerides were within the reference range; for cholesterol, mean and median values lay at the top of the reference range, and in some cases somewhat above it, throughout. In neither case were any general trends discernible and above-range lipid values are

frequently encountered. Scatter plots and shift tables did not reveal any tendency towards a general migration of values.

Safety in special populations

Summary of adverse events – overall and by subgroup (SAF); pooled analysis

	Any AE		Any treatment-related* AE		Any SAE	
Subgroup	n_e	n_p (%)	n_e	n_p (%)	n_e	n_p (%)
Total *N = 127*	207	93 (73%)	131	84 (66%)	2	2 (2%)
1 - 3.99 cm^2 *N = 51*	66	30 (59%)	45	25 (49%)	0	0
4 - 10 cm^2 *N = 73*	141	63 (86%)	86	59 (81%)	2	2 (3%)
Female *N = 41*	84	31 (76%)	40	26 (63%)	1	1 (2%)
Male *N = 86*	123	62 (72%)	91	58 (67%)	1	1 (1%)
18 – 34 y *N = 61*	86	42 (69%)	58	39 (64%)	0	0
35 – 50 y *N = 66*	121	51 (77%)	73	45 (68%)	2	2 (3%)
Femur *N = 79*	126	53 (67%)	76	46 (58%)	0	0
Patella *N = 45*	81	40 (89%)	55	38 (84%)	2	2 (4%)
Medial *N = 20*	32	13 (65%)	21	12 (60%)	0	0
Lateral *N = 57*	93	39 (68%)	54	33 (58%)	0	0
Traumatic *N = 56*	80	42 (75%)	58	40 (71%)	0	0
OD *N = 6*	16	5 (83%)	5	4 (67%)	0	0
OA *N = 8*	22	8 (100%)	8	6 (75%)	1	1 (13%)
Others *N = 57*	89	38 (67%)	60	34 (60%)	1	1 (2%)

ne, number of events; np, number of patients

The applicant was asked to discuss planned methods for evaluating the influence of intrinsic and extrinsic factors on safety outcomes for Spherox in the Phase II and III study programme. There were no differences according to implantation in medial or lateral knee, reason for cartilage damage, age (younger or older than 34 years) or gender. Patients with larger lesions, and lesion in patella were more often reporting AE and TAE. The applicant also discussed weight (dichotomised under and over 80 kg), smoking status and concomitant illness, and there are similar rates of AE reported for these groups.

Paediatric population: AEs reported in the prospective data collection from the study cod 16 HS17 paed a prospective non-interventional investigation to assess the long-term safety and linked efficacy of Spherox in

paediatric patients from 15 to less than 18 years of age treated with the product until December 2011. The full analysis set (FAS) comprised 29 patients for the interim analysis. The time between documentation of the data as part of this study and the ACT3D with Spherox ranged between approximately 3.5 and 8 years (mean, 63.3 months). All AEs/ADRs were reported for 1 patient each. Most AEs were in the SOC 'musculoskeletal and connective tissue disorders' (6 AEs; 6 patients, 21%) and the SOC 'injury, poisoning and procedural complications' (4 AEs; 3 patients, 10%). One AE in one patient (3%) was in the SOC 'general disorders and administration site conditions'.

In study cod 16 HS16, 2012 AEs were collected retrospectively from 2004 to 2011 at eight clinical sites in Germany. In total, 39 patients aged below 18 years were identified as having been treated with Spherox at the participating sites. Data from 29 patients were obtained.

Two TAE (symptom progression and no therapeutic response) were reported for 1 patient at the 1st visit after 9 months. In the same patient, additional 4 non-treatment related AEs were observed, which comprised undifferentiated connective tissue disease, arthropathy and skeletal injury during the 5th time interval (78 to <156 weeks) and arthropathy during the 6th time interval (156 to <234 weeks). The remaining 5 non-treatment related AEs in 5 patients comprised infection (13 to <26 weeks), ligament sprain (156 to <234 weeks), joint surgery (52 to <78 weeks) and 2 events of muscle atrophy (1 event at 13 to <26 weeks and 1 event at 7 to <13 weeks).

Safety related to drug-drug interactions and other interactions

The applicant discussed the risk of interactions with other pain medications. The number of AE decreases during time as expected post-operatively. Regarding the use of corticosteroids, this was an exclusion criterion before implantation and no subjects were treated with corticosteroids during the pivotal studies.

Discontinuation due to adverse events

No discontinuations due to AEs have been reported.

Post marketing experience

Supportive efficacy and safety data from patients treated nationally in routine clinical practice between 2004 and 2012 were provided, as described in section 2.5.

2.6.1. Discussion on the clinical safety

The proposed use of Spherox relates to the treatment of isolated acute or chronic chondral or osteochondral articular cartilage defects of traumatic genesis or unknown etiology in adults and adolescents with closed epiphyseal growth plate. In the EU, several autologous chondrocyte products including Spherox have been on the market under national legislations. Spherox has been on the market in Germany since 2004, and has also been available in Belgium, Greece, Italy, Spain and Austria. Spherox has been used in medicinal practice for the treatment of cartilage defects up to 10 cm2, classified as Outerbridge grade 3 or 4 or ICRS grade III or IV. From a clinical point of view it seems conceivable that Spherox could, potentially, offer safety advantages over conventional ACT application, based on the lower invasiveness of the surgical procedure and the lack of need for fixation through a periostal flap or use of membrane. Additional safety advantages of Spherox over

other matrix-associated ACT methods could include the absence of the need for allogenic fibrin glue and exogenous scaffolding materials.

The available safety results from 127 patients with cartilage defects of the knee from ongoing phase II and phase III clinical trials do not indicate unexpected major safety issues, and observed AEs/SAEs are mainly related to surgical complications. Additional data on safety we reported in literature from 37 patients (Fickert, 2011). However, safety data of Spherox collected and registered systemically from clinical trials are considered limited. The supportive clinical investigations included published case series, retrospective and prospective cohort studies. A full safety assessment from these supportive investigations cannot be made, due to some limitations including risk of underreporting. Nevertheless, the supportive information together with post-marketing experience does not reveal new safety concerns. Safety profile of Spherox has been adequately described in the Product Information and supportive data is expected from the post-authorisation studies.

For paediatric patients with knee cartilage defect, safety information of Spherox is limited and based on 29 subjects experiencing 10 AEs, with systemically monitored safety data from one controlled clinical trial (cod 16 HS17 paed) with mean follow-up 63.3 ± 14.5 months (range: 14-95 months). Although the follow-up is deemed adequate the number of patients is low and hence, it is not possible to draw firm conclusions. The reported AEs do not indicate a new safety signal in this subpopulation.

Based on the information from the applicant, it could be concluded that osteoarthritis is a localised condition, not a spectrum of systemic diseases. However, this is not the case and the concept of osteoarthritis has been further elaborated. The Applicant considers that in the biopsies taken from individuals with predisposition to osteoarthritis of genetic origin, the chondrocytes would still be able to produce viable Spheroxs. This would be possible due to removal of the “osteoarthritic environment” allowing the chondrocytes to produce normal hyaline cartilage matrix. This has been, however, only investigated in vitro and there is no clinical data to support the assumption of the Applicant. Moreover, the viable Spheroxs would, again, meet the “osteoarthritic environment” after transplantation. Therefore diagnosed primary (generalised) osteoarthritis is a contraindication.

Long term follow up of all treated patients in the ongoing clinical study cod 16 HS 13 has been requested by the CAT/CHMP in an Annex II condition and the applicant agreed to this.

From the safety database all the adverse reactions reported in clinical trials and post-marketing have been included in the Summary of Product Characteristics.

2.6.2. Conclusions on the clinical safety

Safety information is limited and based on 127+37 subjects (cartilage defects of the knee), with limited follow-up (12-36 months). The supporting studies and post-marketing experiences, even if possibly underreporting the AEs, do not provide any signal for additional safety concern and include follow-up exceeding more than 5 years. The most important adverse events that have been reported are those expected for post-operative situation with limited or single cases of venous thrombosis, pulmonary embolism, delayed wound healing, joint lock, joint effusion, and joint swelling.

The CAT/CHMP considers the following measures necessary to address issues related to safety:

Post-authorisation efficacy study (PAES): 60-month follow-up data for study cod 16 HS 13.

In order to evaluate the long-term efficacy and safety of Spherox vs. microfracture in patients with cartilage defects of the knee with a defect size between 1 and < 4 cm^2, the MAH should conduct and submit the results of the ongoing prospective, randomised, open label, multicentre study.

2.7. Risk Management Plan

The CAT/CHMP received the following PRAC Advice on the submitted Risk Management Plan (RMP):

The PRAC considered that the RMP version 5.0, dated 17 May 2017, is acceptable.

The PRAC also agreed that the Applicant should evaluate in the PSURs the data collected via the annual safety and efficacy follow-up questionnaire to health professionals.

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Important identified risks • Delamination of transplant • Hypertrophy of transplant • Lack of efficacy (result of delamination) Important identified risks • Medication errors • Local infection (due to surgical procedure) • Other surgery related events (e.g. pain, joint effusion, thrombosis, embolism) • Interaction of the transplant with antibiotics or disinfectants • Transmission of infective diseases • Procedure-related events Missing information • Long term safety and efficacy • Interacting substances	**Routine risk minimisation measures:** Section 4.2 of the SmPC: restriction of the indication to defects of the condyle and patella of the knee. Section 4.4 of the SmPC: recommendations to verify that the product is being administered to the correct patient. **Additional risk minimisation measures:** Training material for surgeons and surgical staff Training material for other health care professionals Controlled access system	**Routine pharmacovigilance activities** Annual safety and efficacy follow-up questionnaire to health professionals to be included in the PSUR **Additional pharmacovigilance activities** Study cod 16 HS 14 – EudraCT Nr. 2009-016816-20 - (phase II) Study cod 16 HS 13 - EudraCT No.: 2009-016466-82 – (phase III)

Safety concern	Risk minimisation measures	Pharmacovigilance activities
used e.g. pain relieving medication and corticosteroids		

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP and CAT considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.9. New Active Substance

The applicant declared that spheroids of human autologous matrix-associated chondrocytes has not been previously authorised in a medicinal product in the European Union.

The CAT/CHMP, based on the available data, considers Spheroids of human autologous matrix-associated chondrocytes to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Labelling exemptions

A request to omit certain particulars from the labelling as per Art.63.3 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

Only batch number and number of spheroids will be included in the immediate container (application system or syringe).

Both statements ‘Keep out of the sight and reach of children’ and ‘Read the package leaflet before use’ will be omitted from the outer packaging (pouch), as these are not considered to be relevant for the health care professionals.

The main arguments put forward by the company are:

1) The product is delivered to the clinics and applied by a qualified surgeon in a medical facility by

intraarticular implantation.

2) The specific manufacturing process is not allowing for a sticker to be applied to immediate containers

3) The very small size of the immediate containers

4) The outer packaging should not be opened before utilisation of the product to prevent microbiological contamination.

The particulars to be omitted as per the QRD Group decision described above will however be included in the Annexes published with the EPAR on EMA website, and translated in all languages but will appear in grey-shaded to show that they will not be included on the printed materials.

A request to omit certain particulars from the package leaflet as per Art.63.3 of Directive 2001/83/EC has been submitted by the applicant and has been found unacceptable by the QRD Group for the following reasons:

The Group rejected the proposal to exclude section 5 'How to Store X' from the leaflet because the leaflet could potentially be used by Health Care Professionals as a source of information.

2.10.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Spherox (spheroids of human autologous matrix-associated chondrocytes) is included in the additional monitoring list as it contains a new active substance.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Articular cartilage is a complex structure that has an important function in load-bearing joints and joint mobility. These characteristics are determined mainly on the composition of cartilage and particularly its extracellular matrix whose production the chondrocytes are involved in. The integrity of the extracellular matrix is essential for the mechanical and structural capacity of the cartilage. Due to the lack of blood or lymphatic vessels in the cartilage, cell infiltration does not occur and the capacity of the defect to heal after trauma is reduced. Without a surgical intervention the risk for the development of arthrosis is present. A cartilage lesion can reduce the joint function, cause pain and swelling of the joint.

The proposed indication of Spherox is:

"Repair of symptomatic articular cartilage defects of the femoral condyle and the patella of the knee (International Cartilage Repair Society [ICRS] grade III or IV) with defect sizes up to 10 cm^2 in adults."

3.1.2. Available therapies and unmet medical need

Cartilage lesions such as in the knee commonly occur, mostly due to trauma. Articular cartilage has limited capacity for intrinsic repair and may result in fibrous tissue or fibrocartilage, which has inferior properties compared to normal healthy hyaline cartilage. Deep lesions till the subchondral bone (Outerbridge scale III-IV) may lead to osteoarthritis at young age, if left untreated. Surgical approaches to repair damaged cartilage such as microfracture (MF) are limited by the production of fibrocartilaginous tissue, which is less resistant than natural cartilage. In addition, microfracture is less suitable for treating larger lesions (exceeding ~4 cm^2). ACI (autologous chondrocyte implantation) is an alternative treatment option whereby extra-corporal cultivated autologous chondrocytes are implanted in the chondral focal lesions with the aim to form hyaline cartilage. The first generation ACI products utilised autologous chondrocytes as a cell suspension, administered under periosteal flap or biodegradable membrane. One of the limitations of traditional ACI has been that the quality of the neocartilage has been mostly fibrocartilage type of tissue, which is considered to be less durable resulting in compromised treatment results in long-term. Furthermore, the use of a periosteal flap has turned out problematic, since it requires an additional surgical intervention and in some cases the use of a flap has induced hypertrophic growth of the treated cartilage. In an attempt to achieve repair tissue more similar to native articular cartilage, development of ACI has progressed to the third generation, in which autologous cells are cultured in a 3-dimensional (3D) matrix before implantation, such as Spherox.

3.1.3. Main clinical studies

3.2. Favourable effects

In comparison with the conventional cell suspension products, Spherox is able to adhere to the defect area and thus, exogenous fixation with a periosteal flap is not required. Since it can be applied in a minimal invasive arthroscopic procedure, operation time can be shortened. Traditionally, chondrocytes from articular biopsies have a low potential for proliferation and after repeated passaging the number of cell divisions decrease, and eventually the cells dedifferentiate. With Spherox, the cells are expanded first in 2D conditions, and then cultured in an environment that enhances 3D growth of the cells into aggregates and accumulation of cartilage-type ECM proteins into spheroids. It appears that these cells, while highly dedifferentiated after the 2D culture, still retain their re-differentiation capacity when taken into the proper environment. In addition, autologous serum is used in the cell culture. No other human or animal-derived exogenous material is used during the manufacturing process, which minimises the risk of cell transformation.

In nonclinical studies, the *in vitro* and *in vivo* characterisation of Spherox demonstrated that human chondrocytes are capable of producing three dimensional spheroids that upon implantation into a chondrogenic environment either in vitro or in vivo, fuse together, remodel, migrate, fill up the space between the spheroids and the cracks and fissures in the cartilage defect with de novo hyaline like cartilage and extracellular matrix. The mode of action was demonstrated in *in vitro* cartilage explants and *in vivo* in subcutaneously implanted cartilage explants. This was further corroborated with the data from a large animal model, minipig, demonstrating formation of hyaline-like cartilage repair tissue in the defects after two months post-implantation although long term evaluation of efficacy and safety in the orthotopic femoral condyle cartilage defect model in the Merino sheep failed to show any evidence of repair tissue at six months post-implantation.

Interim data from the pivotal Phase III study cod 16 HS13 (ACT3D-CS versus MF in patients with 1-<4 cm^2 cartilage defects of the femoral condyle) showed an improvement in patient symptoms and function in both treatment groups. In the 12-month interim analysis of the primary variable 'overall KOOS' for the ITT1 population, both Spherox (ACT3D-CS) and the comparator MF showed a statistically significant improvement relative to baseline. For patients treated with ACT3D-CS the mean overall KOOS score rose from 56.6 ± 15.4 at baseline to 78.7 ± 18.6 at Visit 4 12 months later (change from baseline 22.2 ± 18.3), while for those treated by MF this score rose correspondingly from 51.7 ± 16.5 to 68.1 ± 18.6 (change from baseline 16.4 ± 15.1) ($p<0.0001$ in both cases). The KOOS subscores yielded the same qualitative result as the full-KOOS analysis. Repetition of the analyses with the PP population was consistent with the primary analysis. Structural MRI evaluation by MOCART scoring was assessed at Visits 2 (3 months) and 4 (12 months). The overall MOCART score showed a modest numerical improvement from Visit 2 to Visit 4 in both treatment groups, being 67 for the ACT3D-CS group and 62 for the MF group at Visit 2 (3 months), improving respectively to 81 and 77 at Visit 4 (12 months). Other structural assessments are currently not available as the second-look arthroscopy and R-biopsy procedures (in consenting patients) are planned for the 24 month after implantation/microfracture visit, which will be followed in the post-authorisation study. Results from the IKDC assessments and from the modified Lysholm score were supportive, revealing overall improvements in both treatment groups between baseline and Visit 4.

Three-year data from the Phase II clinical trial cod 16 HS14 (dose-level comparison study in large cartilage defects (4–10 cm^2) at various locations in the knee (femur, patella)) showed a significant and sustained improvement in overall KOOS compared with baseline, in all three within-group analyses (for the high-, medium- and low-dose group respectively $p = 0.0010$, 0.0001 and 0.0002 one year after study treatment; 0.0005, <0.0001 and 0.0002 respectively after two years and without further change after three years). Changes within each dose group were of similar magnitude, and the three between-group (pairwise) analyses did not reveal any statistically significant differences between the dose groups. For all dose groups combined, the mean overall KOOS rose in the first year after treatment from 57.0 to 73.4 on a scale from 0 (worst) to 100 (best) and continued to rise slightly, reaching 77.0 after 36 months. At the 12-month assessment, KOOS subscores also generally showed strong within-group improvements – mostly with $p < 0.05$ – while between-group comparisons did not show any significant difference.

In both studies, subgroup analyses indicated a similar benefit in terms of KOOS change from baseline regardless of previous surgery. There was no relevant difference in KOOS outcome based on a subgroup analysis by duration of symptoms. No patients were enrolled who had previously failed treatment with MF for their lesion. Patients in both studies underwent a strict rehabilitation up through 3 months after transplantation. This programme is likely to have affected functional outcomes. Thus, the total effect measured in the respective studies should be viewed as the combination of the intervention under study and the rehabilitation programme, also implemented during the development. In both studies, there was a reduction in the use of concomitant pain medication following treatment. In the Phase II study, 15% of the patients treated with ACT3D were using concomitant pain medication at Visit 7 (36 months after treatment) as compared with 44% at screening. In the Phase III study, 15% of patients treated with ACT3D and 24% of the patients treated with MF were using concomitant pain medication at Visit 4 (12 months after treatment) as compared with 40% and 52%, respectively, at screening. In both studies, patients treated with ACT3D who used pain medication showed lower KOOS outcome (also in terms of change from baseline) as compared to those patients who did not but in both studies, both pain medication subgroups (yes/no) showed relevant gains in KOOS. This, together with the overall decrease in pain medication use over time, is considered to be supportive of efficacy.

3.3. Uncertainties and limitations about favourable effects

Regarding structural outcomes, while MOCART scores showed modest numeric improvements after treatment in both studies, there was no consistent correlation between the KOOS and MOCART outcome measures. This is consistent with published literature and the reason for this is not known.

Results of limited (n=7) R-biopsy assessments after 12 months suggested that repair tissues displayed hyaline cartilage but no sample displayed a true hyaline cartilage phenotype. The newly-formed chondral tissue showed variable quality and fibrous tissue or fibrocartilage was seen in most of the samples. The early time point of the histological sampling however did not allow for the analysis of mature cartilage and there were too few samples (only 3 samples taken from the weight-bearing cartilage) to be able to draw conclusions on the quality of the cartilage. This, amongst other requirements, will be addressed by future data from the Phase III study as it is planned for biopsies to be obtained at 24 months after treatment. Furthermore, considering that the majority of patients (47/75) in the Phase II trial had patellar lesions, i.e., a non-weight-bearing location. Longer-term (two- and three-year) active controlled data from the pivotal Phase III trial in femoral cartilage lesions will therefore be of value to confirm the durability and adequate quality of the newly-generated cartilage in weight-bearing joint lesions in full physical activity to guarantee normal joint functionality.

Structural MRI evaluation by MOCART scoring was not assessed before study treatment but showed a modest and sustained numeric improvement over time. The respective dose-group scores after 36 months were 72.4, 79.6, 72.1 and 74.9 overall. Arthroscopic assessment of cartilage repair (ICRS- cartilage repair assessment) after 12 months for a subset of 8 patients gave the result ‘normal’ or ‘nearly normal’ in seven cases and ‘abnormal’ in one. Results of the R-biopsy assessments after 12 months however showed that all repair tissues displayed hyaline cartilage characteristics to some extent, but that no sample displayed a true hyaline cartilage phenotype. Biopsy results from the Phase III study are not yet available. Thus, supportive information from histological assessments is currently quite limited.

On the quality side, there remain two obligations to be addressed post-authorisation. These are (1) to conduct a prospective process validation study and to review the in process controls and acceptance criteria accordingly for key parameters of the manufacturing process (P0 culture time, total monolayer culture time, spheroid culture time and amount of synovial impurities) and (2) to re-validate the potency assay and monitor its correlation with the efficacy outcome. These conditions are considered important to support the consistent manufacture of Spherox finished product with a coherent measure of potency.

3.4. Unfavourable effects

Clinical experience with Spherox derives from two pivotal clinical trials (phase II cod 16 HS14 and phase III cod 16 HS13), sixteen supportive clinical investigations, and post-marketing data from 8 000 patients. The available safety results from 127 patients with cartilage defects of the knee from ongoing phase II and phase III clinical trials do not indicate unexpected major safety issues, and observed adverse events/serious adverse events are mainly expected post-operatively. In the phase III study, comparison of Spherox and microfracture showed no differences in AE profile based on the available results from 1-year after treatment; and in the phase II study there was no sign of increased rate of AE over time up to 36 months. The supportive studies together with post marketing experience do not reveal new safety concerns and include follow-up exceeding 5 years.

3.5. Uncertainties and limitations about unfavourable effects

Safety data of Spherox collected/registered systemically from clinical trials is still considered limited basically based on the 127 and 37 patients with 12-36 months follow-up. The pivotal studies will continue and report longer follow-up, i.e. 60-months. The study will evaluate the long-term efficacy and safety of Spherox vs. microfracture in patients with cartilage defects of the knee with a defect size between 1 and < 4 cm^2.

The supportive clinical investigations included published case series, retrospective and prospective cohort studies but a full safety assessment from these supportive investigations cannot be made; due to their limitations including risk of underreporting and therefore more comparable with spontaneous reporting system.

For paediatric patients with knee cartilage defect, safety information of Spherox is limited and based on 29 subjects experiencing 10 AE, with systemically monitored safety data from one controlled clinical trial with mean follow-up 63.3 ± 14.5 months (range: 14-95 months). Although the follow-up is deemed adequate the number of patients is considered insufficient and the originally applied for indication, which included adolescent patients, was restricted to adults only.

For patients with hip cartilage defect, safety information of Spherox is limited and based on only 24 patients, with no systemically monitored safety data. Hence, the applicant in line with the CAT/CHMP recommendation agreed to restrict the indication to remove hip lesions from the applied indication.

3.6. Effects Table

Effects table for Spherox (ACT3D-CS) in the treatment of cartilage defects of the knee and hip

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Visit 4, 12 m 16.1 ± 17.9 Visit 6, 24 m 16.6 ± 17.8 Visit 7, 36 m 19.9 ± 16.3	Change from baseline in overall KOOS for all 3 doses overall		Three dose groups: 3-7; 10-30; and 40-70 spheroids/cm2	N/A	Uncontrolled study design but significant and clinically relevant improvement from baseline up to 3 years after treatment	**Pivotal Phase II study Cod 16 HS 14 (knee, defect size 4-10 cm2) N=75**
Visit 2, 3 m 62.9 Visit 4, 12 m 72.4 Visit 6, 24 m 73.3 Visit 7, 36 m 74.9	MOCART score over time for all 3 doses overall				Supportive Descriptive No baseline assessment	

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Visit 4, 12 m ACT3D-CS: 22.2 ± 18.3 Microfracture: 16.4 ± 15.1	Change from baseline in overall KOOS at 12 m after treatment		10-70 spheroids/cm2	MF	Δ from baseline ACT3D-CS: 22.2 (CI: 16.9–27.5), *p* <0.0001 Comparison to MF: Δ = 5.7 with lower bound of CI: –1.0 -Non-inferiority to MF demonstrated -Superiority not demonstrated (based on interim analysis at 12 m)	**Pivotal Phase III study Cod 16 HS 13 (femoral condyle, defect size 1-<4 cm2 N=102**
ACT3D-CS: Visit 2, 3 m 67 ± 16 Visit 4, 12 m 81 ± 13 Microfracture: Visit 2, 3 m 62 ± 11 Visit 4, 12 m 77 ± 13	MOCART score over time				Supportive Descriptive No baseline assessment	
Only 1 patient met criteria for treatment failure; Mean KOOS total score 82.6 ± 11.6 (n=22) Mean MOCART score 74.7 ± 12.0 (n=15);	Need for surgical re-treatment, KOOS MOCART (subpop)		Mean: 33.8 (15.3) spheroids/cm2		Limited evidence (supportive descriptive results, non-interventional surveillance study)	**Paediatric pts Cod 16 HS 17paed (knee, defect size 2.2-12 cm2) N=29**
Statistically significant improvement for NHS, mHHS, SF 36)	Subjective improvement: Non Arthritic Hip Score, Modified Harris Hip Score, SF-36		Not reported	N/A	Limited (retrospective evaluation, very few patients, no control-group, no second-look arthroscopy, no MRI assessment)	**Fickert et al. 2014 (hip, 5 acetabular, 1 femoral, defect size <2-6 cm2) N=6**

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
WOMAC: Baseline: 58 ± 12 FU: 91 ± 14 NAHS: Baseline: 51 ± 13 FU: 77 ± 8	Significant improvements in NAHS and WOMAC from baseline to follow-up (3 months)		Mean 135 (90-180) spheroids	N/A	Limited (prospective case series, few patients, no control-group , limited follow-up time of 3 months) Difficult to assign: Treatment effect is combined result of 2 surgeries: removal of cam deformities in first arthroscopy and treatment with Spherox in 2nd arthroscopy. Improvements started already after 1st surgery	**Körsmeier et al. 2014 (hip defects due to cam-impingement, defect size 3-6 cm2) N=16**
Unfavourable Effects						
Incidence of adverse events at 12 months by PT (> 5 subjects, Pooled analysis of Phase II and Phase III clinical trial, study Cod 16 HS 13, Cod 16 HS 14 and Control group (Microfracture) from Cod 16 HS 13)						
Total number of patients			127 (100%)	50 (100%)	Small number of subjects with short time of follow-up	
Joint effusion			75 (59.1%)	13 (26%)	Most commonly reported, expected AE postoperatively, although late occurring joint swelling can indicate adverse event from treatment.	
Arthralgia			16 (12.6%)	12 (24%)	Expected AE postoperatively.	
Joint swelling			11 (8.7%)	8 (16%)	Expected AE postoperatively, although late occurring joint swelling can indicate adverse event from treatment.	
Nasopharyngitis			6 (4.7%)	1 (2%)	Not related to treatment.	

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

In comparison with the conventional cell suspension products, Spherox is able to adhere to the defect area and thus, exogenous fixation with a periosteal flap is not required. Since it can be applied in a minimal invasive arthroscopic procedure, operation time can be shortened, thus offering the possibility of reducing surgical complications and optimising the safety profile of the product. In addition, autologous serum is used in cell culture. No other human or animal-derived exogenous material is used during the manufacturing

process, which minimizes the risk of cell transformation. This may represent a safety advantage.

One-year interim data from the Phase III clinical trial (ACT3D-CS versus MF) in patients with 1-<4 cm^2 lesions of the femoral condyle showed a statistically significant improvement in the patient symptoms and function as assessed by KOOS in both treatment groups. These improvements are considered clinically relevant and descriptive statistical testing concluded non-inferiority of ACT3D-CS to MF with regards to this outcome measure. The structural MRI evaluation by MOCART scoring showed improvement up to 12 months after treatment, with good results in both treatment groups with regard to defect repair. Overall, these results indicate that treatment with Spherox for improvement of patient symptoms and function is non-inferior to that of treatment with MF, and for some outcome parameters, numerically somewhat better.

In the uncontrolled Phase II clinical trial in patients with large cartilage lesions of the knee (femur, patella, 4-10 cm^2), maintenance of improved functionality and reduction of symptoms without marked deterioration was observed up to 3 years after treatment. This was accompanied by improvements in MOCART assessments. However, histological data on the quality of the regenerated tissue are limited, with biopsies showing hyaline-like to mixed-type repair tissue. There remains a significant need for chondrocyte products for treatment of symptomatic cartilage defects, particularly for larger defects, for which other methods such as MF are not recommended. However, there has also been significant utilization of ACI in smaller defects in clinical practice in recent years.

Supportive investigations from routine clinical practice in adults and adolescents were submitted, comprising a rather heterogeneous patient population. The ongoing PIP (EMEA-001264-PIP01-12) requires the data collection from 80 paediatric patients with closed knee epiphyseal growth plate not later than June 2020. Consequently, the current data available, 29 FAS patients with only 12 patients with verified closed epiphyseal growth plate in study cod16HS17 would not be adequate for granting the paediatric indication in the current MA procedure.

During the evaluation and on request of the CAT/CHMP, the applicant addressed the concerns with regards to the originally applied for broad indication. Cartilage lesions in the tibia, hip, as well as paediatric patients are no longer included due to the absence of reliable clinical trial data. This is endorsed by the CAT/CHMP.

The observed adverse events are expected mainly post-operatively. The procedure with Spherox includes one harvest and one implantation, rendering the patient to two sessions of surgery, and thereby, an increased number of surgeries could be viewed as a disadvantage. However, the surgery is regarded as less invasive in comparison to other described techniques. Thereby, the procedure in total could be regarded comparable when it comes to the adverse events. Safety information, based on 127 and 37 subjects with cartilage defects of the knee with follow-up 12-36 months, is limited. However, the available safety results from 127 patients from ongoing phase II and phase III clinical trials do not indicate unexpected major safety issues, and observed adverse events / serious adverse events are mainly expected post-operatively. In the phase III study, comparison of Spherox and microfracture showed no differences in AE profile based on the available results from 1-year after treatment; and in the phase II study there was no sign of increased rate of AE over time up to 36 months. Overall, safety data of Spherox collected/registered systemically from clinical trials is considered limited. The supporting studies and post-marketing experiences are suspected to be substantively underreporting AEs, nevertheless they do not provide any signal for additional safety concern. As a condition to the marketing authorisation, the applicant will follow up patients long term, i.e. 60 months.

The obligations to conduct post-authorisation measures include two quality-related obligations as follows (1) to conduct a prospective process validation study and to review the in process controls and acceptance criteria accordingly for key parameters of the manufacturing process (P0 culture time, total monolayer

culture time, spheroid culture time and amount of synovial impurities) and (2) to re-validate the potency assay and monitor its correlation with the efficacy outcome. These two obligations are justified on the basis that the process validation and the validation of the potency assay are both essential to confirm a consistent manufacturing process and to obtain a coherent assay for potency determination of the finished product. A consistent manufacturing process and a validated potency assay are necessary to deliver in a reliable manner the finished product with the demonstrated safety and efficacy.

3.7.2. Balance of benefits and risks

The benefit/risk balance of Spherox for the sought indication is positive. In the view of the remaining uncertainties, the CAT/CHMP requested conditions to be fulfilled in post-marketing phase, see section 4.

3.8. Conclusions

The overall B/R of Spherox is positive.

Divergent positions are appended to this report.

4. Recommendations

Outcome

Based on the CAT/CHMP review of data on quality, safety and efficacy, the CAT/CHMP considers by majority decision that the risk-benefit balance of Spherox is favourable in the following indication:

Repair of symptomatic articular cartilage defects of the femoral condyle and the patella of the knee (International Cartilage Repair Society [ICRS] grade III or IV) with defect sizes up to 10 cm^2 in adults.

The CAT/CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Additional risk minimisation measures

Prior to launch of Spherox in each Member State, the Marketing Authorisation Holder (MAH) must agree about the content and format of the training programme and the controlled distribution programme, including communication media, distribution modalities and any other aspects of the programme, with the National Competent Authority.

The main objectives of the educational programme are to provide training to surgeons and other health professionals on proper procurement, storage, handling and administration of Spherox.

The MAH shall ensure that in each Member State where Spherox is marketed, all surgeons and other health professionals who are expected to prescribe and administer the product have access to the educational materials including:

- The Summary of Product Characteristics
- Training materials for surgeons and training materials for other health professionals
- Prescriber checklist
- Forms for documentation

- **The training material for surgeons and surgical staff** shall contain the following key elements:
  - Information on Spherox, including the indication currently approved and legal basis
  - Detailed description of the biopsy harvest procedure and the administration procedure, the implantation by knee-joint arthrotomy and the follow-up protocol
  - Preparation of the patient for the procedure and subsequent monitoring
  - The need to officially confirm that training has been conducted prior to the biopsy.
  - The importance to complete the checklist
  - Recommendations on rehabilitation post biopsy and post transplantation

- **The training material for other health professionals** shall contain the following key elements:
  - Information on Spherox, including the indication currently approved and legal basis
  - The need to screen donors for hepatitis B, hepatitis C, HIV and syphilis
  - Detailed description of the handling of the biopsy harvest and of the product, elements on the preparation for the implantation, the schedule for the patient follow-up and recommended physiotherapy.
  - The need to officially confirm that training has been conducted prior to the biopsy.

- **The Prescriber checklist** shall contain the following key messages:
  - Corroboration that the patient receiving the product is the right patient receiving the appropriate product
  - Confirmation of the appropriate side of the implantation
  - A reference to the fact that the patient has been informed and understands the benefits and risks of the product and the associated procedures

The MAH shall ensure that in each Member State where Spherox is marketed, a system aimed to control access to the product beyond the level of control ensured by routine risk minimisation measures. The following requirements need to be fulfilled before the product is prescribed and dispensed:

- Specific testing and examination of the patient to ensure compliance with strictly defined clinical criteria
- The patient should document the receipt and understanding of the information on the product
- The product will only be available to surgeons certified to prescribe and administer Spherox
- Measures to ensure the traceability of the product and guarantee the identification of: patient data; diagnosis leading to the treatment; information on biopsy including date of the operation, adverse events reported during the procedure and quality of the biopsy; information on the implant, including all in-process controls and final product controls.

Obligation to conduct post-authorisation measures

The MAH shall complete, within the stated timeframe, the below measures:

Description	Due date
Post-authorisation efficacy study (PAES): 60-month follow-up data for study cod 16 HS 13. In order to evaluate the long-term efficacy and safety of Spherox vs. microfracture in patients with cartilage defects of the knee with a defect size between 1 and < 4 cm^2, the MAH should conduct and submit the results of the ongoing prospective, randomised, open label, multicentre study.	Interim reports: To be submitted annually Final study report: 01-Mar-2021
To conduct a prospective process validation study post marketing using batches manufactured with a well-controlled process and to collect quality data from a sufficient number of batches to demonstrate consistency, quality and genetic stability of the cells in the finished product. On the basis of the process validation study, in process controls should be reviewed and the acceptance criteria tightened accordingly for the manufacturing process for P0 culture time, total ML culture time, spheroid culture time and amount of synovial impurities.	April 2019
To re-validate the potency assay post marketing and to monitor its correlation with the efficacy outcome.	March 2018

New Active Substance Status

Based on the CAT/CHMP review of the available data, the CAT/CHMP considers that spheroids of human autologous matrix-associated chondrocytes is considered to be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

Divergent Positions - Spherox (EMEA/H/C/002736/0000)

The undersigned members of CAT did not agree with the CAT's opinion recommending the approval of the granting of a Marketing Authorisation for Spherox. Based on the review of the data and the applicant's response to the CAT and CHMP LoQ, LoI on quality, safety, efficacy and risk management plan, it is considered that the application for

Spherox, in the treatment *of repair of symptomatic articular cartilage defects of the femoral condyle and the patella of the knee (International Cartilage Repair Society [ICRS] grade III or IV) with defect sizes up to 10 cm^2 in adults*

is not approvable due to a negative benefit/risk profile.

The reasons for divergent opinion were as follows:

- Spherox has been studied in two clinical trials: Phase II study was an open label, non-controlled dose escalation study in large lesions and the Phase III study was a randomised controlled study for small lesions (<4cm^2), using microfracture (MF) as the comparator. The clinical outcome was assessed using a validated KOOS (Knee injury and Osteoarthritis Outcome Score) scoring, including subscales for Pain, other Symptoms, Function in daily living (ADL), Function in sport and recreation (Sport/Rec) and knee related Quality of life (QOL). According to EMA guideline on In-vitro cultured chondrocyte containing products for cartilage repair of the knee (CAT/CPWP/568181/2009) the clinical efficacy results in small lesions should demonstrate clinical superiority over MF or non-inferiority over MF, if structural superiority can be demonstrated at the time of MAA. Both clinical studies demonstrate some improvement in clinical data when mean results are compared with those of baseline, however, only clinical non-inferiority to MF could be demonstrated in the Phase III study. From a subgroup analysis superiority could be identified for the quality of life subscore, however, the patients were allowed to use maximum pain medication up to the day when the questionnaire was filled in and the use of pain medication was not systematically followed or documented for the entire patient populations.

  To complete the benefit risk assessment the pivotal 24 month data of the phase III study are needed.

- Efficacy based on comparative structural endpoints is not proven. Structural repair has been studied by MRI in both studies using MOCART scoring; however, the MOCART baseline results are missing for a large number of patients thus hampering the assessment of structural outcome. As part of Phase II study biopsies from 8 patients were taken for immunohistochemical analysis, 7 of them were actually studied. Out of the seven samples only one showed some level of hyaline cartilage formation, all the rest (6/7) containing mainly Collagen I positive mixed fibrous tissue. Thus superior structural repair was not demonstrated for Spherox. Additionally, the number of non-responders in both clinical studies was high (>30%) both for Spherox and MF. As Spherox treatment requires two interventions when compared to one with MF, the benefit for the patients is not demonstrated.

- A clear negative correlation was found for the cell and spheroids culture times and clinical efficacy. Also the number of fibroblast impurities (synoviocytes) showed a negative correlation with efficacy. However, the in process controls and process parameters have not been defined through a prospective process validation and thus the maximum culture times and impurity levels of the commercial process remain unjustified. As these quality parameters negatively correlate with efficacy

it cannot be ruled out that the high number of non-responders could be due to an uncontrolled process.

- Likewise, Absence of validation of the proposed potency assay based on an mRNA surrogate marker that can reflect the intended function is critical as potency remains the most relevant parameter to ensure a consistent and well controlled autologous product.

- Paula Salmikangas, (Finland)

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Asterios Tsifsoglou, (Greece)

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- Tarmo Tiido, (Estonia)

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Ivana Haunerová, Czech Republic

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- Hans Ovelgonne, (Netherlands)

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Una Riekstina, (Latvia)

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- Margarida Menezes-Ferreira, (Portugal)

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Paolo Gasparini , (Italy)

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- Romaldas Maciulaitis, (Lithuania)

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Simona Badoi, (Romania)

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Divergent Positions - Spherox (EMEA/H/C/002736/0000)

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- Bruno Sepodes, (Portugal)

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- Daniela Melchiorri, (Italy)

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- Johann Lodewijk Hillege, (Netherlands)

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- Ondrej Slanar, (Czech Republic)

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- Tuomo Lapveteläinen (Finland)

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- Romaldas Maciulaitis, (Lithuania)

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