EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Human Use (CHMP)

Assessment report

MACI

Common name: matrix applied characterised autologous cultured chondrocytes

Procedure No. EMEA/H/C/002522/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Executive Summary

Cartilage defects of the knee occur along a spectrum of disease and severity. Larger, more chronic lesions are often symptomatic, may contribute to joint misalignment and can cause disabling symptoms such as pain, catching, locking, and swelling. Focal chondral lesions that are left untreated may progress to debilitating joint pain, dysfunction and degenerative arthritis. The exact incidence of symptomatic chondral knee lesions in the overall population is difficult to determine. In some large-scale epidemiological studies, cartilage injuries were seen in 5-11% of diagnostic knee arthroscopies in predominantly young adult populations with knee pain.

A number of surgical procedures have been developed to treat patients with focal chondral defects in the knee. Repair techniques such as microfracture aim at marrow stimulation and induce the formation of fibrocartilage repair tissue. Single-stage restoration techniques such as osteochondral autograft, mosaicplasty, and osteochondral allograft attempt to replace the cartilage defect with host or donor articular cartilage. Another restoration technique, autologous chondrocyte implantation (ACI), was first reported in 1994 and attempts to generate hyaline or hyaline-like cartilage. ACI requires two surgical procedures, first to harvest autologous chondrocytes, which are then grown extra-corporeally, and then to transplant the cultivated cells back into the lesions. The benefit of ACI over other restoration techniques is that larger lesions can be treated.

In April 2013, the European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP), based on an opinion of the Committee for Advanced Therapies (CAT), recommended the authorisation of MACI (matrix applied characterised autologous cultured chondrocytes) for the repair of symptomatic, full-thickness cartilage defects of the knee[1] of 3-20 cm^2 in skeletally mature adult patients. MACI is a third generation ACI product where autologous chondrocytes are seeded onto a collagen membrane of porcine origin, which is secured into the lesion with fibrin glue. At implantation, the membrane is trimmed to the correct size and shape, and implanted cell-side down into the base of the defect; the implant is secured in place using fibrin sealant. The recommended dose of MACI implant is 500,000 to 1 million cells per cm^2 of defect. The dose is the same for all patients, regardless of age.

In the main clinical study supporting the authorisation (SUMMIT[2]), MACI was superior to microfracture treatment for symptomatic cartilage defects of the knee with a range of defect sizes from 3 to 20 cm^2. In this prospective, randomised, open-label parallel-group trial, 72 patients received MACI and 72 were treated by microfracture. A clinically and statistically significant difference in the improvement from baseline to Week 104 was seen for the co-primary endpoint of KOOS (Knee Injury and Osteoarthritis Outcome Score) for Pain and Function in patients treated with MACI over the comparator ($p=0.001$). Significantly more patients treated with MACI (87.50%) met the responder analysis criteria than patients treated with microfracture (68.06%), which is considered clinically relevant. The primary efficacy endpoint was corroborated by several other patient reported outcome measures and a responder analysis of the primary efficacy measures demonstrated superior clinical efficacy for patients treated with MACI compared to microfracture.

In view of the comparator used the potential effect of lesion size was considered important by the Committees. In a subgroup analysis of the group with larger lesions (> 4 cm^2) in the pivotal study, MACI was superior to microfracture (KOOS response rates 97% vs. 77%), while a positive trend was seen for the individual components of the co-primary efficacy parameter for both pain and function. However, in the group with smaller lesions (< 4 cm^2), where microfracture is considered the treatment

[1] Defined as grade III and IV of the Modified Outerbridge Scale
[2] Study code MACI00206

of choice, there was also a benefit for MACI (KOOS response rates (78% vs. 61%). Overall, the Committees concluded that the benefit of MACI is not restricted to a particular size of lesion and can be used for lesions from 3 to 20 cm^2.

It was noted by the Committees that MACI and microfracture treatment did not show statistically significant differences with regard to the structural endpoints, infill of defects as assessed by MRI and the quality of repair tissue as assessed by the histology score. The hypothesis that MACI leads to superior quality of hyaline cartilage repair compared to non-transplantation techniques like microfracture has thus not been established. There is generally no consensus on whether structural repair as measured by MRI or histology scoring systems is able to distinguish the true functional repair of cartilage defects, and hence be a meaningful surrogate for clinical outcomes. Consequently, in the view of the Committees improvements in the clinical outcomes of pain and function, as observed in the study, remain the most clinically valid endpoints in cartilage repair studies.

Two types of important unfavourable effects were identified, those related to the MACI implant and those related to peri-operative complications. In the main clinical study, fewer patients treated with MACI reported treatment-emergent (serious) adverse events than those treated with microfracture, despite MACI requiring two surgical procedures. The difference in incidence of treatment-emergent serious adverse events was mainly due to more serious cases of treatment failure, cartilage injury and arthralgia in the microfracture group compared with MACI. The results of the SUMMIT study are consistent with the known safety profile for MACI, including the safety information reported in the published literature. Overall, based on the exposure of more than 6,000 patients to MACI, the two main risks related to MACI are symptomatic graft hypertrophy and graft delamination (complete or partial, possibly leading to loose bodies in the joint or graft failure). No case of graft hypertrophy was seen with MACI in this study while one case of delamination was observed. The risks related to peri-operative complications of surgical intervention of the knee are haemarthrosis, arthrofibrosis, localised surgical site inflammation, localised surgical site infection or thromboembolic events. The SmPC provides appropriate information on these, and educational material for healthcare professionals involved in the surgical treatment or follow-up of patients treated with MACI will detail how to recognise the signs and symptoms of important known and potential risks of the product.

Based on the robust clinical data from a prospective study showing clinically relevant effects and confirming an acceptable and manageable safety profile, the Committees concluded that the benefit/risk balance of MACI for the repair of symptomatic, full-thickness cartilage defects of the knee is positive. The clinical study data was further supported by information from published literature as MACI has been available in some European countries since 1998 in accordance with national legislation before coming under the new legal framework for advanced therapies. MACI has now been recommended for licensing as the first advanced-therapy medicine to be combined with a medical device. Advanced therapy medicines are those regulated under new legislation specifically aimed at authorisation, supervision and monitoring of medicines that are made from genes and cells to ensure that they are safe and effective. Combined advanced-therapy medicines contain one or more medical devices as an integral part of the medicine, as in this case where the cells are embedded in a biodegradable matrix or scaffold. As part of the ongoing monitoring of MACI, the Agency requested the 5-year follow-up data from the main clinical study, which will provide information on the sustainability of the cartilage repair and maintenance of effect of MACI compared to microfracture over time, as well as the long-term safety of the medicine.

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List of abbreviations

ACI	Autologous Chondrocyte Implantation
ACL	Anterior Cruciate Ligament
ACT	Autologous Chondrocyte Transplant
ADL	Activities of Daily Living
ADR	Adverse Drug Reaction
AE	Adverse Event
ANOVA	ANalysis Of VAriance
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
CAT	Committee for Advanced Therapies
CBMP	Cell Based Medicinal Product
CHMP	Committee for Medicinal Products for Human Use
CRF	Case Report Form
CSP	Concurrent Surgical Procedure
eCRF	electronic Case Report Form
EMA	European Medicines Agency
EQ-5D	European Quality of Life (EuroQOL) 5 dimensions questionnaire
FC	Femoral condyle
GAG	Glycosaminoglycan
GCP	Good Clinical Practice
GLP	Good Laboratories Practices
GRE	Gradient Echo
ICF	Informed Consent Form
ICRS	International Cartilage Repair Society
IEC	Independent Ethics Committee
IKDC	International Knee Documentation Committee
KOOS	Knee Injury and Osteoarthritis Outcome Score
LFC	Lateral Femoral Condyle
LOCF	Last Observation Carried Forward
MANOVA	Multivariate Analysis of Variance

MedDRA	Medical Dictionary for Regulatory Activities
MF	MicroFracture
MFC	Medial Femoral Condyle
MI	Multiple Imputation
MRI	Magnetic Resonance Imaging
MOCART	Magnetic Resonance Observation of Cartilage repair tissue
MRI	Magnetic Resonance Imaging
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OA	Osteoarthritis
OATS	Osteochondral Autograft Transfer System
OCD	Osteochondritis Dissecans
PGE2	Prostaglandin E2
PP	Per Protocol
PRO	Patient-Reported Outcome
PT	Preferred Term
PVD	Peripheral Vascular Disease
QOL	Knee-Related Quality of Life
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SF-12 12-Item	Short-Form Health Survey
SF-36 36-Item	Short-Form Health Survey
SOC	System Organ Class
SRA	Sports and Recreational Activities
SSP	Subsequent Surgical Procedure
VAS	Visual Analogue Scale

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Genzyme Europe B.V. submitted on 1 September 2011 an application for Marketing Authorisation to the European Medicines Agency (EMA) for MACI, 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 24 March 2011.

The applicant applied for the following indication: MACI is to be used in skeletally mature patients for the repair of symptomatic cartilage defects of the knee (grade III and IV of the arthroscopic staging of osteochondral lesions as described by the Modified Outerbridge Scale).

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application.

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

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/111/2011 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/111/2011 was not yet completed as some measures were deferred.

The PDCO issued an opinion on compliance for the PIP EMEA-00979-PIP01-10.

Applicant's request for consideration

New active Substance status

The applicant requested the active substance matrix applied characterised autologous cultured chondrocytes contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

Scientific Advice

The applicant received Scientific Advice from the CAT/CHMP on 19 July 2007 and 18 March 2010. The Scientific Advice pertained to quality, non-clinical and clinical of the dossier.

Licensing status

MACI has been on the market in the following countries: Austria, Belgium, Denmark, Germany, Greece, Ireland, Italy, The Netherlands, Norway, Portugal, Spain, and the United Kingdom in line with national legislations.

1.2. Manufacturers

Manufacturer of the active substance

Genzyme Biosurgery ApS
Oliefabriksvej 45
DK - 2770 Kastrup
Denmark

An inspection of this manufacturing site was carried out by the Danish Health and Medicines Authority. The findings of the inspection are in compliance with the EU Good Manufacturing Practice requirements.

Manufacturer responsible for batch release

Genzyme Biosurgery ApS
Oliefabriksvej 45
DK - 2770 Kastrup
Denmark

1.3. Steps taken for the assessment of the product

The CAT (Co)-Rapporteurs, the CHMP Coordinator and the PRAC (Co)-Rapporteurs appointed by the CHMP were:

Rapporteur: Dr Narayanan Gopalan/ Dr Bridget Heelan Co-Rapporteur: Dr Johannes Ovelgönne

- The application was received by the EMA on 01 September 2011.
- The procedure started on 21 September 2011.
- The Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 9 December 2011. The Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 9 December 2011.
- During the meeting on 19 January 2012, 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 19 January 2012.
- The applicant submitted the responses to the CAT consolidated List of Questions on 15 October 2012.
- The summary report of the inspection carried out at the following site: Genzyme Biosurgery ApS on 17-18 April 2012 was issued on 11 September 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 19 November 2012.
- During the CAT meetings on 7 December 2012 and 15 February 2013, the CAT agreed on 2 lists of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CAT Lists of Outstanding Issues on 21 January 2013 and 25 March 2013, respectively.

- During the meeting on 19 April 2013, 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 MACI.
- During the meeting on 25 April 2013, 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 MACI.

2. Scientific discussion

2.1. Introduction

Problem statement

Articular (hyaline) cartilage in the knee distributes the load between the femur and tibia to avoid high point stresses on load-bearing areas within the knee and to provide a low friction-bearing surface, assisted by the synovial fluid. After an individual reaches skeletal maturity, their articular cartilage does not spontaneously heal or regenerate if damaged, for example, by acute trauma or through progressive mechanical degeneration. Hyaline cartilage does not have a blood, nerve, or lymphatic supply which could induce or help mediate an intrinsic repair response. However, prior to skeletal maturity, open femoral growth plates suggest an intrinsic articular cartilage repair response not seen in adults.

Cartilage defects of the knee occur along a spectrum of disease and severity. At one end of this spectrum are small, acute lesions that are often diagnosed incidentally at the time of knee arthroscopy, and are not necessarily initially symptomatic. At the other end are larger, more chronic lesions that are often symptomatic and may contribute to joint mal-alignment. These lesions can cause disabling symptoms such as pain, catching, locking, and swelling. Focal chondral lesions that are left untreated may progress to debilitating joint pain, dysfunction and degenerative arthritis.

The chondral lesion may be due to trauma or diseases like osteochondritis dissecans (OCD). Some rare hereditary forms of OCD have been described, but in most cases no familial component is present. Chondromalacia patellae (CMP) is a specific diagnosis in adolescents and young adults, where the chondral internal layer of the patella may become swollen and softened, often causing significant pain. The exact etiology of CMP is unknown.

The exact incidence of symptomatic chondral knee lesions in the overall population is unknown. In some large-scaled epidemiological studies, cartilage injuries were observed 5-11% of diagnostic knee arthroscopies in predominantly young adult populations with knee pain. However, focal chondral lesions are often co-incident findings to other abnormalities of the knee such as meniscus or ACR-lesions. It can be a challenge to determine whether pain or malfunction of the knee is related to the chondral lesions (which might be asymptomatic), or other orthopedic disorders of the knee-joint such as malalignment of the joint components.

There are a number of surgical procedures which have been developed to treat patients with focal chondral defects in the knee. The repair techniques include marrow stimulation such as abrasion arthroplasty, drilling, and microfracture, which penetrate the subchondral bone and induce the formation of fibrocartilage repair tissue. While the short-term clinical outcomes which have been demonstrated after marrow stimulation show good results, the clinical durability of marrow stimulated repair tissue has shown a functional decline with further follow-up.

Restoration techniques such as osteochondral autograft, mosaicplasty, and osteochondral allograft attempt to replace the cartilage defect with host or donor articular cartilage in a single stage. Studies in the literature indicate that clinical outcomes after osteochondral autograft have shown good results after as long as seventeen years of follow-up in patients with defects measuring up to 5 cm. Restoration with autologous chondrocyte implantation requires two surgical procedures and attempts to generate hyaline or hyaline-like cartilage. The literature suggests that long-term follow-up for up to 20 years after autologous chondrocyte implantation has shown satisfaction in the majority of patients, with sustained improvement in clinical outcomes and magnetic resonance imaging findings.

Autologous chondrocyte implantation (ACI) was first reported in 1994. With ACI, autologous chondrocytes are extra-corporally multiplied by cultivation. After several weeks, the cells are transplanted back into the lesions. The benefit of ACI techniques over osteochondral autografts and mosaicplasty is that larger lesions could be treated.

First generation ACI required the harvesting of an autologous periosteal patch, which was used to secure the chondrocytes in situ (ACI-P). This requirement led to complications such as intra-articular adhesions, periosteal hypertrophy and delamination of the defect. Second generation ACI techniques replaced the need for a periosteal patch with a resorbable collagen membrane (ACI-C). Matrix-induced autologous chondrocyte implantation (MACI) is a third generation ACI product. The autologous chondrocytes are supplied seeded onto a type I/III collagen scaffold, which is secured into the lesion with fibrin glue.

There are no internationally accepted treatment guidelines on how and when to treat cartilage lesions. A recent survey under 242 European orthopaedic surgeons revealed that there is consensus that debridement and/or MF is first-choice treatment for full-thickness cartilage lesions up to 3 cm^2. Controversy existed for treatment of lesions exceeding 3 cm^2; in this survey, (M)ACI was preferred by 33.5% of the experts, followed by MF (19.0%), debridement (15%) and with lesser frequency osteochondral plug transplantation (9.5%), and several other techniques such as abrasio (Salzmann *et al.* 2011).

About the product

MACI (Matrix applied characterised autologous cultured chondrocytes) consists of autologous chondrocytes, seeded on a collagen membrane of porcine origin (type I/III ACI-Maix). MACI is an Advanced Therapy Medicinal Product (ATMP) defined as a combined tissue-engineering product (TEP). The drug substance is designated as the human autologous cartilage-derived cultured chondrocytes combined with a CE marked purified, resorbable porcine-derived, collagen type I/III membrane. . For the manufacture of MACI, autologous chondrocytes are derived from the patient's own cartilage cells. The cultured autologous chondrocytes are then subsequently seeded onto ACI-Maix™ membrane. At implantation, the membrane is trimmed to the correct size and shape of the cartilage defect, and implanted cell-side down into the defect base; the implant is secured in place using fibrin sealant (please see Figure 1 below). The recommended dose of MACI implant is 500,000 to 1 million cells/cm2 of defect. The autologous cultured chondrocyte dose is the same for all patients, regardless of age.

Figure 1 Schematic overview of MACI (Source Jacobi *et al.* Sports Medicine, arthroscopy, rehabilitation, therapy & technology 2011, 3:10)

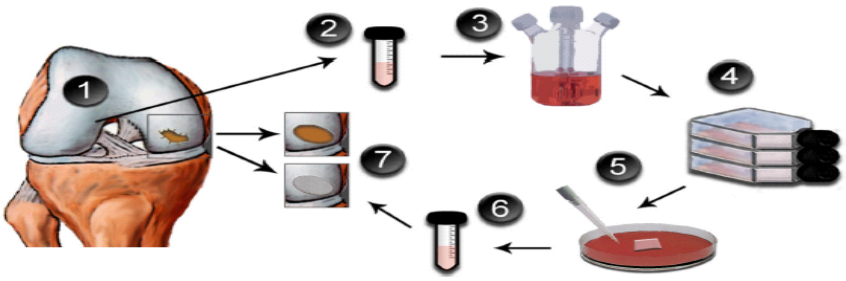


Figure 1 The MACI procedure. (1) Initial arthroscopy with evaluation of the injured cartilage and harvest of a full-thickness cartilage biopsy; (2) the biopsy is sent in a sterile and cooled container to the cell culture laboratory; (3) the cartilage is enzymatically digested; (4) expansion of the chondrocytes in monolayer culture for about four weeks; (5) the cells are seeded onto the scaffold a few days before implantation; (6) the engineered implant is sent back to the surgeon in a sterile container; (7) definitive surgery with debridement of the injured cartilage followed by implantation of the MACI-implant, which is trimmed to fit the defect size and glued with a thin layer of fibrin glue.

The applicant has sought the following indication: "*MACI is to be used in skeletally mature patients for the repair of symptomatic cartilage defects of the knee (grade III and IV of the arthroscopic staging of osteochondral lesions as described by the Modified Outerbridge Scale).*" A defect of III/IV of the modified Outerbridge Scale means a full-thickness lesion of the cartilage with a diameter of 0.5 inch (1.27 cm) or more, fissuring down to the bone. The underlying bone is still intact.

Type of Application and aspects on development

The applicant has submitted an application under Article 8 (3) of Directive 2001/83/EC. Prior to the introduction of the (EC) Regulation No 1394/2007 and during the ATMP transitional period, MACI was available in certain European countries (i.e. Austria, Belgium, Denmark, Germany, Greece, Ireland, Italy, The Netherlands, Norway, Portugal, Spain and the United Kingdom), in accordance with national legislations. As such, MACI has been available in some European markets since 1998, as well as in Australia and parts of Asia. In 2005, Genzyme acquired the Verigen Corporation and the MACI implant technology. Until that time, approximately 4,000 patients in Europe and Australia had been treated with MACI.

Scientific advice was given by the CHMP on two occasions. Quality, non-clinical and clinical data were discussed in the first set of scientific advice, in 2007 (EMA/H/SA/901/1/2007/III). In the follow up advice procedure (EMA/H/SA/901/1/FU/1/2010/ADT/II) in 2010, further advice was given for the quality and non-clinical development.

The following guidelines are applicable for this application: Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPMP/568181/2009) and the Guideline on human cell-based medicinal products (EMEA/CHMP/41069/2006). In general, the chosen endpoints and active treatment comparator are also suggested in these guidelines.

The non-clinical development programme for MACI included in vivo primary pharmacodynamic studies, in vitro secondary pharmacodynamic studies, a long-term equine study (safety pharmacology, biodistribution, local tolerance), single-dose toxicity studies in horses, and local tolerance studies (rat, rabbit, horse). Single-dose toxicity in mice, genotoxicity, local tolerance, cytotoxicity and sensitisation studies were performed with the ACI-Maix™ membrane only. In addition, publications are also presented as further supportive evidence of the use of MACI or a similar collagen membrane-based delivery of chondrocytes for repair of focal cartilage defects.

With regards to the clinical dossier, a company-sponsored, multi-centre, randomised, open trial (SUMMIT: Superiority of MACI Versus Microfracture Treatment" (MACI00206)) in 144 subjects was performed. The aim of this trial was to demonstrate the superiority of MACI implant versus arthroscopic microfracture for the treatment of symptomatic articular cartilage defects of the femoral

condyle, including the trochlea. The Company sought advice at the SAWP regarding the design. In addition, studies from the literature and internal study reports from Academia were provided.

2.2. Quality aspects

2.2.1. Introduction

The MACI product consists of autologous cultured chondrocytes on a CE marked purified, resorbable porcine derived collagen type I/III membrane (ACI-Maix™). The production starts with autologous cartilage from which chondrocytes are cultured in a limited number of passages. The membrane with autologous chondrocytes is implanted using fibrin sealant.

2.2.2. Active Substance / final drug product

General information

The drug substance is a cell suspension of autologous cartilage-derived cultured chondrocytes expanded from autologous cartilage tissue attached to a CE marked purified, resorbable porcine derived collagen type I/III membrane (ACI-Maix™). The cells are incubated on the membrane for a couple of days. The starting biopsy material is removed from full thickness articular cartilage from non-weight bearing areas of the knee. The drug substance is defined as cultured autologous human chondrocytes combined with the purified porcine-derived collagen type I/III membrane. Drug substance and final drug product manufacturing are seen as a continuous process.

Manufacture

Genzyme has designed a training program which is mandatory for surgeons interested in treating patients with MACI. The training includes details for collection of a biopsy sample including guidance on location and size of sample to collect.

The manufacture of the drug substance is patient-specific and uses a make-to-order methodology. The process is limited with set parameters and specific ranges to control the manufacture of the chondrocytes while allowing adjustments for total days in culture and expansions required by each unique lot. The process is designed to minimise the total population doublings of the cells while still yielding a sufficient cell number to manufacture drug product to have it available for surgery on the date requested. All drug substance lots will go through the biopsy collection, biopsy processing and primary culture flows. Depending on the yield from the primary culture, the final cell suspension for membrane loading (key intermediate) may go directly to the final passage flow or require additional expansion and go to the expansion flow prior to the final passage flow and there may also be cryopreservation steps.

Manufacturer

MACI is currently manufactured at 3 Genzyme facilities: however only the Kastrup, Denmark facility will manufacture for the European Union (EU).

Table 1 Drug substance manufacturer

Manufacturer	Responsibilities
Manufacturer of Drug Substance Genzyme Biosurgery ApS Oliefabriksvej 45 DK-2770 Kastrup, Denmark	Biopsy acceptance for manufacture* Drug substance manufacture Drug substance QC testing Drug substance release testing
Qualified Testing Laboratory	**Responsibilities**
Biological Testing Lab for Patient Blood Samples: MVZ synlab pharma institute GmbH (formerly known as Gemeinschaftspraxis Für Laboratoriumsmedizin GbR) Turmstraße 21, 10559 Berlin, Germany	Biological testing of blood from autologous tissue donors

* Release of biopsy into manufacture as required by EU Directives 2004/23/EC and 2006/17/EC
QC=quality control

Manufacturer of the ACI-Maix™ membrane:

Matricel GmbH

Kaiserstrasse 100

52134 Herzogenrath, Germany

Biopsy collection and transport

Prior to the harvest of the biopsy, a transport kit is sent to the surgeon. The Cartilage Biopsy Transport Kit is an integrated packaging system designed to ensure biopsy viability during transport by protecting it from excessive physical and thermal variation or shock(s) and preventing ingress of contaminants and adventitious agents during shipping. The kit will also transport blood samples to the manufacturing facility prior to transfer to the clinical testing lab. The transport kits have been validated to maintain an internal temperature between 2°C and 37°C for at least 3 days from the point of biopsy packaging to the point of receipt at the manufacturing site. This temperature will sustain both the tissue and blood sample prior to further processing.

When a physician determines MACI is the best treatment option for the patient, the surgeon removes approximately 200 mg of healthy articular cartilage by arthroscopy from a non-weight-bearing area of the knee. To ensure the procured biopsy sample is of optimal quality, aseptic techniques for biopsy harvesting are utilised in accordance with standard medical practice and in accordance with EU Directive 2006/17/EC at an appropriately accredited, designated and authorised facility. In accordance with EU Directive 2006/17/EC, blood samples taken at the time of biopsy are tested for biological infectious agents including HIV-1, HIV-2, Hepatitis B, Hepatitis C and Treponema palladium in a qualified laboratory authorised by a competent authority.

The biopsy is transported from the hospital to the manufacturing site in a packaging system to ensure biopsy viability during transport.

Upon receipt at the manufacturing site, each biopsy kit is individually accessioned and inspected for proper packaging and completion of all documentation. A unique lot number is assigned as a sequential

five digit number tracked with demographic information on the patient such as country, hospital and the patient's name/initials. A sub-lot indicator is added during accessioning to designate the lot as first passage. Each kit is inspected for outside integrity and proper components. The integrity of the biopsy container and the presence of biopsy tissue are confirmed.

Description of manufacturing process and process controls

The manufacturing process consists of the following steps:

1. Biopsy processing
2. Primary culture
3. Cryopreservation and thawing if required
4. Expansion culture if required
5. Final passage
6. Loading of cells onto membrane
7. Final drug product manufacturing steps

1. Biopsy processing

The manufacturing process starts with digestion of the extracellular matrix of the biopsy cartilage to release chondrocytes in order to start the culturing process.

2. Primary culture

The cells are cultured in growth media containing gentamicin. There were concerns about the use of gentamicin in the manufacture of MACI, the applicant has agreed to remove it as post-authorisation measure (i.e. recommendation). After the primary expansion there is a decision point with respect to total cell yield, the immediate requirement for the product or if the implantation will be later. In the latter case the cells are cryopreserved, or the cells are put in secondary expansion or final passage culture, depending on total cell yield from primary culture.

3. Cryopreservation and thawing

In order to control the number of population doublings cryopreservation is introduced as a facultative step in the manufacture of MACI.

4. Expansion culture

If primary cell yield is below the minimum cryopreservation number or insufficient to seed the membrane order, a secondary (expansion) culture is started prior to initiation of a final passage culture. Expansion cultures are 2nd passage cells, initiated from freshly trypsinised or thawed primary cells.

5. Final passage

Final passage cultures can be either second or third passage cultures. When the order is received, the number of cells required for final passage flask inoculation is dependent on the number of membranes requested.

After the final passage the cells are counted and pelleted by centrifugation. .

The specifications for final cell suspension for membrane loading (key intermediate) include a sterility test following EP: 2.6.27.

6. Loading of cells onto membrane

The membrane and the prepared cell suspension are incubated for a few days.

7. Final drug product manufacturing process steps

The membrane is rinsed with serum free, antibiotic free, phenol red free transport media. A strip is cut from the membrane for release testing. Each membrane is packaged in 1 dish consisting of a bottom, lid with O-ring and a holding ring. The final drug product is confirmed to be intact prior to aseptically transferring cell side up in to the primary transport container, where it is covered with transport media. The drug product is shipped backed to the hospital for implant.

Control of materials

The starting material for the MACI drug substance is a patient's biopsy. Surgeons are trained to remove approximately 200 mg of cartilage tissue from a non-weight-bearing area of the knee. In accordance with EU Directive 2006/17/EC, the patient's blood is drawn around the time of biopsy collection and tested for biological infectious agents including HIV-1, HIV-2, Hepatitis B, Hepatitis C and *Treponema pallidum* in a qualified laboratory authorised by a Competent Authority.

The ACI-Maix ™ membrane starts as a dried 4 cm x 5 cm purified porcine-derived collagen type I/III membrane (from porcine peritoneum) and is a CE marked Class III device in Europe. Certificate of Analysis includes: appearance, colour, size, pH, denaturation temperature, weight, endotoxin, and sterility. Virus inactivation by NaOH, Acetone and Gamma irradiation has been validated. The company test each batch in-house by preparing 'mock' drug product and ensuring compliance with pre-set specifications.

Other raw material components used during the transport of the biopsy and the expansion of the cells has been adequately controlled.

Control of critical steps and intermediates

In general the company has sufficiently justified the control strategy.

Pharmaceutical development and process validation

Incremental changes have taken place in the drug product manufacture between 1998 and the present day to enhance product characterisation (by inclusion of additional specifications) manufacturing robustness (by inclusion of process changes) and compliance with CPMP/BWP/1793/02. Prior to introduction of the ATMP Regulation, MACI was available in certain European countries in accordance with national laws. The MACI process between 1998 and 2008 is referred to as Process 1. This process was improved (Process 2) including limiting cell culture days, passage number and membrane seeding density. Additional improvements were made in response to Regulatory Agency feedback. These improvements were made to reduce any potential risks from animal-derived materials used in the manufacturing process and deliver a homogeneous product. These improvements have been validated for the current process referred to in this submission as Process 3, with manufacture in Kalstrup Denmark

Characterisation of the final drug product

Identity

The identity assay is a quantitative reverse transcriptase polymerase chain reaction (RTPCR) assay based on the gene expression analysis of two markers, the chondrocytic marker HAPLN1 and the synovial/fibroblastic marker MFAP5.

Potency

Potency testing is based on the measurement of aggrecan mRNA expression by real-time PCR.

It has been requested that this newly developed potency assay be further validated against ability to form functional cartilage. . Until the new potency and identity assays are validated the applicant should monitor new patients for safety and efficacy which could be linked with lack of validation of these parameters. The MAH should present updates in special section of the PSUR and RMP.

Finished Medicinal Product

The finished product is manufactured, routinely controlled and batch released by Genzyme Biosurgery ApS in Denmark. Operations are in compliance with Good Manufacturing Practice (GMP).

MACI finished product (for composition see table below) is shipped in an isotonic medium (excipient) and does not require any thawing, reconstitution, dilution, re-suspension or rinsing steps prior to use. The size of the final implant is 14.5 cm^2 (starting implant is approximately 20 cm^2 (4 cm x 5 cm); final product testing requires 5.5 cm^2 membrane/cell product prior to packaging.) Prior to implant the drug product is cut to fit the shape and size of the cartilage defect by the treating surgeon. In approximately 5% of cases patients require 2 implants.

Table 3 Net batch formulation for 1 drug product Unit

Components	Amount per Unit	Function	Reference Standard
Autologous chondrocyte cells attached to a collagen type I/III membrane	5 x10^5 to 1 x10^6 cells/cm^2 on a 14.5 cm^2 membrane	Active substance consisting of chondrocyte cells to supply long-term regenerative function and the collagen type I/III membrane to assure correct localisation of the cells within the defect	N/A
DMEM phenol red free	18mL added to primary container. 61 µL/cm^2 absorbed (0.9 mL per unit)	Provides physiological osmolality and pH	N/A

Figure: 1. * batch formula for a 2 membrane order is 2x each component

Product Specification

The finished product release specifications were appropriate and included the following tests: visual inspection, viability, minimum cell number detection, identity, potency, sterility of the final product, endotoxin and mycoplasma.

Sterility, mycoplasma and endotoxin testing is sufficiently validated.

Batch analysis data

Batch analysis data demonstrate that the company is able to consistently manufacture drug product with process 3 based on the presented release criteria.

Reference standards

MACI is manufactured from an autologous biopsy sample and each patient's order is considered 1 unique lot. There are no industry-established reference standards for this type of cell therapy.

Container closure system

The MACI implant drug product is shipped in a clear polystyrene container with a green polycarbonate cover that is sealed using an o-ring and helical lock closure.

Stability of the product

The shelf life is 6 days when held in the shipping box.

Adventitious agents

MACI implant is an aseptic product, free of detectable adventitious agents at shipment. Specifications for drug product release require a negative mycoplasma result, an endotoxin result of 1 ≤ EU/mL and all sterility samples to be negative or negative to date. The manufacture of the drug product lots is conducted within an Annex I cGMP Grade A biosafety cabinet (BSC) housed within a Grade B clean room. Continuous non-viable partial monitoring is installed and active in each BSC and used during all steps of manufacturing. Viable air samples are collected during all process steps and surface samples are collected during all critical process steps and reviewed prior to lot release.

Regional information

Medical Device

ACI-Maix ™ membrane is a CE marked medical device. The ACI-Maix ™ Notified Body assessment report, declaration of conformity, EC design examination certificate, certificate of quality assurance approval, instructions for use, and full table of contents from the ACI-Maix CE dossier were provided. Documents related to the ACI-Maix ™ manufacturing process and manufacturing controls, TSE and viral safety risk evaluation, and stability studies were also provided.

Certificates of Suitability

Certificates of Suitability issued by the European Directorate for the Quality of Medicine (EDQM) have been obtained by the suppliers for all bovine derived materials used in the development and manufacture of MACI®.

Medicinal Products Containing or Using in the Manufacturing Materials of Animal and/or Human Origin

Donor testing and Biopsy procurement/testing are suitably controlled according to EU Directive 2004/23/EC and subsequent implementing Directives 2006/17/EC and 2006/86/EC. The Company is in compliance with the current version of the EC Note for Guidance 'Guideline for Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Medicinal Products (EMEA/410/01)'. The suppliers of bovine material source from BSE-free countries and use controlled/monitored herds. All FBS used is gamma irradiated with a validated process at a dose of 30 and 45 kGy. Each lot of FBS is qualified for use in manufacturing by the Company. Trypsin and ACI-Maix ™ membrane are of porcine origin (no known cases of TSE) and both are suitably gamma irradiated using a validated process, including demonstrated inactivation of porcine parvovirus.

2.2.3. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of MACI drug substance and finished product has been presented in a satisfactory manner. The results of the tests carried out indicate satisfactory consistency and uniformity of the manufacturing process and finished product.

2.2.4. Conclusions on the chemical, pharmaceutical and biological aspects

The CAT has identified the following RMP measure necessary to address the identified quality developments issues that may have a potential impact on the safe and effective use of the medicinal product.

The applicant should validate the newly developed potency assay for MACI. Until the new potency and identity assays are validated the applicant should monitor new patients for safety and efficacy which could be linked with lack of validation of these parameters. The MAH should present updates in special section of the PSUR and RMP

The CHMP endorse the CAT assessment regarding the conclusions on the chemical, pharmaceutical and biological aspects as described above.

2.2.5. 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 recommends the following points for investigation:

Description of Recommendations
1. The applicant is recommended to collect identity and potency data for final cell suspension for loading on the membrane and provide report with proposed IPC acceptance criteria.
2. The applicant is recommended to remove Gentamicin from the manufacturing process, preferably at the biopsy processing stage, but no later than the final passage trypsinisation step.
3. The applicant is recommended to introduce of an additional sterility step for the final DP

Description of Recommendations
(taken from product in final primary container).
4. The applicant is recommended to develop an alternative quantitation assay for cell number on the membrane.

The CHMP endorse the CAT assessment regarding the recommendations for future quality development as described above.

2.3. Non-clinical aspects

2.3.1. Introduction

The non-clinical data consists of studies conducted by the applicant supported by literature references. MACI consists of autologous cultured chondrocytes delivered on a purified, resorbable, porcine-derived collagenous membrane (ACI-Maix ™ membrane) class III CE marked for use with human chondrocytes which is held in place with fibrin glue. For the non-clinical studies presented, autologous chondrocytes were derived from the animal in the study and used in the implant. Some of the non-clinical studies did not use MACI i.e. autologous chondrocytes were seeded onto a similar but not identical collagen membrane as used in MACI (referred to as MACI-like in the report). The non-clinical data included in vivo primary pharmacodynamic studies (rabbit, sheep and horse), in vitro secondary pharmacodynamic studies, a long-term equine study (safety pharmacology, biodistribution and local tolerance), single-dose toxicity studies, local tolerance studies (rat, rabbit and horse). Single-dose toxicity in mice, genotoxicity, local tolerance, cytotoxicity and sensitisation studies were performed with the ACI-Maix ™ membrane only.

Scientific advice was given by the CHMP on two occasions. Non-clinical data were discussed in both sets of scientific advice, in 2007 (EMA/H/SA/901/1/2007/III) and a follow up scientific advice procedure (EMA/H/SA/901/1/FU/1/2010/ADT/II) in 2010.

All studies with ACI-Maix ™ membrane alone were performed in accordance with the GLP. The pharmacology studies and the biodistribution study were not conducted in compliance with GLP. Toxicology studies in the equine model were not performed in compliance with GLP. The applicant showed that no GLP compliant facility was available at the time of studies initiation but that GLP principles were followed and documented throughout the conduct of the studies. Overall, taking the above into account, the CHMP considered that the provision of data generated in a non-compliant facility was acceptable.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Rabbit model

The Willers study (Willers *et al.* 2005) examined the ability of MACI to repair femoral cartilage defects in a rabbit model. Two separate studies are reported: a time course experiment evaluating extent of repair at 6- and 12-week time points and a dose-response experiment evaluating the effect of various cell densities at an 8-week time point. In the time course study, osteochondral defects (3 mm diameter x 3 mm deep) were created in the patellofemoral groove of 16 rabbits and either treated with MACI or

left untreated. There was no membrane-alone treatment arm. Repair was evaluated by semi-quantitative histological scoring using a modified International Cartilage Repair Society (ICRS) scoring system at 6 and 12 weeks postoperatively. In the dose-response study, treatment of similar osteochondral defects was performed with the ACI-Maix membrane seeded with 0, low (10^4 cells/cm^2), medium (10^5 cells/cm^2) or high (10^6 cells/cm^2, same density as MACI) cell densities. Repair of these groups was compared by histology to untreated osteochondral defects at 8 weeks post-implantation. In the time course study, 2 of the 4 MACI-treated animals displayed a very good restoration of osteochondral architecture and were morphologically similar to healthy rabbit articular cartilage. Another animal showed very good osteochondral regeneration but lacked perfect restoration of osteochondral architecture and had hyaline-like cartilage. Failure of repair in one animal of four treated in each group was reported as a recognised complication of quadruped models of cartilage repair. MACI treatments led to notable repair tissue growth (mostly fibrocartilaginous) within 6 weeks, with decreases in cellularity and cartilage thickness by 12 weeks. In untreated defects, the repair tissue was characterised as "fibrous tissue and fibrocartilage," which poorly integrated to the surrounding tissues at both the 6- and 12-week time points. In the dose-response study, among membranes containing cells, there were no detectable differences in histologic scoring of repair tissue within this cell-density range (10^4 to 10^6 cells/cm^2). However, defects treated with cell-seeded membranes were significantly better histologically (regardless of seeding density) than either cell-free membranes ($p<0.05$) or untreated defects ($p<0.01$) after 8 weeks.

Study GENZ 06-0147 was conducted to confirm and extend the results of previous rabbit studies examining efficacy of MACI, but using a more clinically relevant (i.e. shallower) defect in a rabbit trochlea. Two shallow osteochondral defects (3 mm diameter x 1 mm depth) were created in the femoral trochlea of 20 New Zealand white rabbits. In each rabbit, 1 defect was treated with MACI (seeded with approximately $1x10^6$ cells/cm^2), while the other was treated with a cell-free ACI-Maix membrane. The addition of microfracture (MF) was also assessed in this study. MF is a cartilage repair surgical technique that works by creating tiny fractures in the underlying bone. This causes new cartilage to develop. At sacrifice, each defect was scored histologically for inflammation and cartilage repair following the method of Sellers *et al.* 2000. Histological scoring indicated that defects treated with MACI in a non-MF setting at the 24-week timepoint had significantly lower (i.e. better) scores compared to the same treatment group at the 12-week timepoint ($p<0.05$), indicating a progression of cartilage repair with time. Elastin, a component of the ACI-Maix membrane, was noted to be present at both timepoints, but in decreased amounts at 24 weeks versus 12 weeks, indicating an active degradation/resorption process. When elastin was present, these remnants were often associated with a minimal to mild chronic histiocytic inflammation within the defect that subsided over time. Minimal to moderate granulomatous and eosinophilic inflammation was also present in the subchondral marrow immediately beneath the defects in all treatment groups. This inflammatory response also tended to subside over time, but remains at detectable levels (minimal to mild) even at the 24-week timepoint. No other significant differences were reported among groups.

Sheep model

The objective of Dorotka *et al.* 2005 was to examine the repair of femoral chondral defects in sheep, using a MACI-like product where autologous chondrocytes were seeded onto a collagen type I/II/III membrane. These studies were conducted with or without additional marrow stimulation (MF), at 4- and 12-month timepoints. Two defects were created for each of 27 sheep and were divided into 4 groups: empty defect with no MF, empty defect with MF, cell-free membrane with MF and chondrocyte seeded collagen membrane. Chondral defects were scored according to the O'Driscoll and Pineda scoring systems. At 4 months post-implantation, the percent of total defect fill in Groups 1, 2, 3 and 4 was 22%, 54%, 43% and 65%, respectively. The highest amount of reparative tissue was found in the

animals treated with collagen membrane seeded with autologous chondrocytes in combination with MF (Group 4) at both timepoints (p=0.02). Furthermore, the animals in Group 4 exhibited more hyaline-like tissue (p=0.03) than the untreated group (Group 1), or groups treated with either MF alone (Group 2) or with cell-free membrane with MF (Group 3) at 4 and 12 months post-implantation. Histologic scores for defect repair in Group 4 were significantly better (p=0.03) than those in the untreated and the cell-free membrane groups at 12 months post-implantation.

In a second ovine study the objective of Jones *et al.* 2008 was to establish a model of articular cartilage injury repair and to examine the efficacy of non-destructive techniques for assessing cartilage regeneration by MACI. Defects were created on the trochlea and medial femoral condyle of 21 sheep randomised into 1 of 3 groups: untreated controls, treatment with MACI (approximately $5x10^6$ cells per membrane) or treatment with membrane only. Each group was divided into 8-, 10- and 12-week time points. Repair outcomes were examined using confocal arthroscopy, MRI, histology assessed using the ICRS scoring system and biomechanical compression analysis. Analysis of the trochlear cartilage repair tissue revealed a significant improvement between the MACI and membrane-only treatment groups at 10 weeks post-implantation (p<0.05). MRI assessment showed that MACI-treated defects was significantly improved (p<0.05) compared to both the untreated and cell-free membrane groups at all time points. MACI-treated defects were characterised by a mixed repair of hyaline-like cartilage and fibrocartilage with good infill and integration, pericellular specific proteoglycan staining and weak type II collagen staining. Trochlea histology scores demonstrated significant improvement in tissue regeneration after MACI-treatment from 8 to 12 weeks post-implantation. MACI samples showed a significant degree of decreased stiffness in comparison to both the untreated and native tissue groups (p<0.05).

Horse

Four studies have been presented in a large animal (horse) to mimic the clinical situation in humans in line with the CAT reflection paper on *in-vitro* cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009). Two studies were reported from the literature (Frisbie et al 2004 & 2008) and used a porcine intestine derived collagen membrane. The two others, conducted by the applicant used MACI.

The objective of Frisbie *et al.* 2004 was to evaluate the effect of cartilage harvest in a low-weight-bearing region of the knee. The investigators also compared the performance in an equine model of repair tissue obtained following a MACI-like implantation of autologous chondrocytes seeded onto collagen type I/III small intestine submucosa (SIS) membrane, to membrane alone and to defects left empty. Fifteen skeletally mature horses were included in this study. Cartilage defects (15 mm diameter) were created in the medial trochlear ridge and were either treated with a MACI-like product, membrane only or were left empty. Twelve horses were sacrificed following the 3-month arthroscopies. The remaining 3 horses were sacrificed following arthroscopy at 12 months post-implantation. Arthroscopic outcome parameters were subjectively graded and repair tissues were assessed histologically using a modified O'Driscoll scale. Cartilage harvest to obtain autologous chondrocytes did not result in differences in lameness, pain response to joint manipulation, range-of-motion or joint effusion compared with normal joints. No statistically significant differences were noted in defect repair among the treatment groups based on arthroscopic evaluation at 3 months, though defects treated with cell-seeded membrane or membrane-only were graded higher (i.e. better) than the empty defects. Overall histological scores were significantly better (p<0.05) at 3 months post-implantation for defects receiving the collagen membrane seeded with autologous chondrocytes compared to membrane-alone and empty defect groups. At 12 months post-implantation, the quality of repair in defects treated with membrane-only had deteriorated significantly compared to the other treatment

groups. Arthroscopic repair tissue quality score was significantly better ($p<0.05$) in defects receiving collagen membrane seeded with chondrocytes compared to the membrane-only or empty defect treatment groups.

In the second study from Frisbie *et al.* 2008 the efficacy of femoral cartilage defect repair using a MACI-like implant was examined, autologous chondrocytes were seeded onto a collagen type I/III SIS membrane which were secured to defects using bioresorbable staples. Approximately 300 mg of articular cartilage was harvested from the proximal aspect of the lateral trochlear ridge of the femur before isolation of chondrocytes for culture and subsequent seeding ($1x10^6$ cells/cm^2) onto SIS membranes. The study was performed in 15 horses with arthroscopic evaluation at 3-, 6- and 12-months post-implantation. Cartilage defects (15 mm diameter, not penetrating the subchondral bone, 2 defects per horse) were created in the femoral trochlear ridge and these were either treated with membranes seeded with autologous chondrocytes, with membrane-only or were left empty. Nine horses were sacrificed at 12 months post-implantation and 6 defects were analysed per treatment group. The remaining 6 horses were sacrificed at 18 months post-implantation and 4 defects per treatment group were analysed. Defect repair was evaluated by histological analysis using a modified O'Driscoll scoring system. Analysis of overall raw histological scores demonstrated a significant improvement with the chondrocyte-seeded membrane compared with the cell-free membrane or untreated defects. The nature of the repair tissue was graded as being more hyaline-like in the defects treated with the chondrocyte-seeded membrane. Review at 18 months post-implantation showed that repair tissue had better integration to the surrounding cartilage compared to that seen in the empty defects.

The applicant performed study GENZ 06-0239 to confirm the equine data generated from the previous Frisbie *et al.* study using MACI in a small, shorter-term pilot study. Two defects were created in 1 hind limb of each animal (total of 6 animals) in the study: 1 defect was treated with a MACI implant (seeded with 0.9 to $1.1x10^6$ cells) and the other was left untreated. At 3 months post-treatment, animals were subjected to a physical examination, second-look arthroscopy with scoring of the defect areas, blood characterisation and synovial tissue biopsy. At 6 months post-treatment, animals were sacrificed and repair was analysed by gross observation, histology, immunohistochemistry (type II collagen) and biochemical analysis. Histological assessment of cartilage repair tissue was based on percent defect fill, chondrocyte predominance, perilesional chondrocyte cloning, subchondral attachment, perimeter attachment, surface fibrillation and matrix staining (collagen type II and proteoglycan). At 3 months, second-look arthroscopy results showed an overall score (composed of percent filling, surface smoothness, integration peripherally and basally, tissue colour, softness, and pannus formation) in 4 of the 6 horses that was superior for MACI implants, while in 1 horse MACI-treated and untreated defects were equal and in the remaining horse, control treatment was superior to MACI treatment. At the 6-month endpoint, total gross observation scores for MACI-treated (4.7 ± 3.1) and control (7.0 ± 3.8) defects were not different ($p=0.065$), despite the strong trend ($p<0.100$). Comparison of histologic scores revealed significantly better total composite scores for MACI-treated defects (17.9 ± 4.2) compared to empty defect controls (21.5 ± 2.2; $p=0.036$), and scores were superior for MACI-treated defects in all 9 subcategories except perimeter attachment and presence of a tidemark. Biochemical composition of the repair tissue was also assessed at 6 months post-implantation. Type II collagen was found in both MACI-implanted and untreated defects. In the MACI-treated defects, type II collagen was sporadically deposited throughout the repair tissue, with a lower density adjacent to the implanted membrane. In the empty defects, type II collagen was only observed as a thin rim at the base of the defect. In all animals, the glycosaminoglycans (GAG) content of the MACI repair tissue was higher than that in the untreated defects, but lower than normal equine tissue, and cell content of repair tissue in MACI-treated defects was higher than that in untreated control defects.

The objective of study GENZ 09-4417 was to investigate the longer-term performance of MACI in a 53-week equine study. In this study, 27 horses were included with 2 defects per knee. Animals in Group 1 (n=12) received MACI in 1 defect and cell-free membrane in the other defect ; animals in Group 2 (n=12) were treated with MACI in 1 defect and the other defect was left empty (no treatment). Animals in Group 3 (n=3) did not receive treatment in either defect and served as untreated controls. At 3 months post-treatment, animals were subjected to a physical examination, second-look arthroscopy with scoring of the defect areas, blood characterisation and synovial tissue biopsy. All animals were sacrificed at 53 weeks post-implantation and repair was analysed by gross observation, histology, immunohistochemistry (type II collagen), biochemical analysis and mechanical testing (aggregate modulus and hydraulic permeability, depth dependence of the mechanical properties of repair cartilage, frictional properties of repair cartilage and the strength of the interface between repaired tissue and surrounding cartilage). Histological assessment of cartilage repair was similarly conducted as in GENZ 06-0239. Results indicated that surgical adhesion with fibrin sealant successfully secured membranes in the defects with no evidence of displacement based upon the interim arthroscopic evaluation and final necropsy. MACI implanted defects had better attachment to subchondral bone than ACI-Maix membrane alone, which occasionally delaminated. The 12 week second-look arthroscopy results showed an overall score (composed of percent filling, surface smoothness, repair tissue integration, tissue colour and pannus formation) in defects in Groups 1 and 2 were all significantly improved compared to the defects in Group 3 (empty/empty). There were no differences between MACI and cell-free ACI-Maix membrane in the individual parameters analysed or in the total score. At the 53-week endpoint, total gross observation scores for MACI-treated were significantly better than cell-free ACI-Maix membrane and empty defects. Histologic, histochemical and immunohistochemical scoring revealed significantly better total composite scores for MACI-treated defects (13.88 ± 0.93) compared to cell-free ACI-Maix membrane (21.08 ± 1.36) and empty defect controls (20.25 ± 0.96 single empty defect; 22.83 ± 1.05 empty/empty defect). The scores were significantly superior for MACI-treated defects in chondrocyte predominance, toluidine blue reaction and collagen type II formation compared to cell-free ACI-Maix membrane and empty defects. Type II collagen was more abundant throughout the deeper and middle zones of the MACI implanted defects. In the empty defects, narrow zones of collagen type II formation adjacent to the subchondral bone attachment were observed. Cartilage glycosaminoglycan (GAG) and DNA were analysed in the defect repair tissue and opposite joints previously associated with adjacent cartilage biopsy harvest. Defects treated with MACI had a trend toward increased cartilage GAG content compared to cell-free ACI-Maix membrane and empty defects, but was lower than in normal equine tissue. Cartilage DNA content from defects treated with MACI indicated the defects were less cellular than empty defects but generally more cellular than normal cartilage. Confocal strain mapping to measure the depth dependent shear properties of repaired cartilage and the frictional properties of repaired cartilage was also assessed. MACI grafts showed properties in confined compression that were not statistically different than native tissue, while ACI-Maix membrane alone and empty defects were significantly different. There were no statistically significant differences between MACI, ACI-Maix membrane alone and empty defects in shear modulus. Boundary mode friction coefficient was similar in all groups and similar to control cartilage.

Secondary pharmacodynamic studies

Secondary pharmacodynamic effects are not expected for MACI due to the local application of the product. Two *in vitro* studies were presented in support of cell viability and cell-membrane interactions between chondrocytes and the ACI-Maix membrane.

The first study was performed to examine cell viability of human chondrocytes attached to the ACI-Maix membrane following 3 or 14 days in culture (Study V101201). The relative strength of adhesion

of the chondrocytes to the membrane was also tested by vortexing the cell-seeded membrane and determining the amount of cells in the supernatant compared to the amount of cells from the membrane. The results were also compared to those obtained with another membrane, Chondro-Gide. Cell viability following 3 and 14 days incubation was 91 to 98% in all instances of culture period and membrane used. Adhesion to the ACI-Maix membrane following vortexing was high, only 1.1% and 1.4% was seen in supernatant. In contrast 20.4% of cells detached with the Chondro-Gide membrane.

In the second study (GENZ 06GSTR018) rabbit chondrocytes were seeded onto ACI-Maix membrane and were assessed for cell viability prior to seeding, post-seeding at 60 minutes, 4 and 7 days. Interactions of the chondrocytes to the membrane were also examined; the distribution of cells on the surface of the membranes and any changes in collagen fibre formation in membranes seeded with or without cells. Attachment of chondrocytes to the ACI-Maix membrane showed continued attachment to the collagen matrix up to the 7 days of the study. Viability of the chondrocytes was consistent, ranging from 96.2% to 100% for each time-point. Owing to the structure of the ACI-Maix membrane, the surface of the membrane which is seeded is porous and allowed attachment of cells, whereas the opposite surface was relatively non-porous and allows for the confinement of the chondrocytes to only the porous side of the membrane and therefore in the cartilage defect. This design prevents fibrotic cells from migrating into the defect due to the non-porous nature of the outer surface of the ACI-Maix membrane.

Safety pharmacology programme

Based on the mode of action of MACI and given that it is implanted locally to a chondral lesion site in the knee, it is not expected that there should be effects of its use on other organ systems. As part of the 53 week equine study (GENZ 09-4417), clinical parameters, haematology/serum chemistry and synovial fluid were analysed to assess safety-related pharmacology due to the MACI implant. Haematological analysis at study termination showed no significant differences between groups. Results of serum chemistry panels on all horses showed few values outside of the reference range. Synovial fluid analysis at 53 weeks post-implantation revealed few abnormalities. Although total protein was slightly elevated in 1 animal from each group, these elevations were not associated with cytological abnormalities. There was no evidence of induction of any clinical adverse effect following implantation with MACI in this long term equine model. In addition, there were no effects on the major physiological systems as noted in the toxicity studies (see section 2.3.4).

Pharmacodynamic drug interactions

Given the local nature of this product, it is not expected that there should be effects due to interaction with medicinal products utilised pre-, peri- or post-operatively as part of the intended clinical use of MACI. As such, no pharmacodynamic drug interaction study has been conducted. This is in accordance with the CHMP guideline on human cell-based medicinal products (EMA/CHMP/410869/2006).

2.3.3. Pharmacokinetics

Conventional ADME studies have not been performed for MACI in line with the CHMP guideline on cell-based medicinal products (EMA/CHMP/410869/2006).

Absorption

MACI is implanted *in vivo* to the site of action. Conventional absorption studies are therefore not considered relevant for MACI.

Migration

MACI is predicated on the delivery of autologous chondrocyte cell suspension which must remain in the implantation site in order to repair the lesion by generation of a neotissue. At implantation, a uniform layer of chondrocytes are stably bound to a matrix that is implanted, delivering the cells to the defect. In addition, the MACI implant is held in place with fibrin glue, which presents another barrier to cell migration out of the defect and into the joint space. As part of the 53-week equine study (GENZ 09-4417) an analysis of migration (biodistribution) of chondrocytes from the implantation site to other sites in the body was performed. In particular, the popliteal and inguinal lymph nodes were examined. At 53 weeks post-implantation and tissues were collected for histological evaluation. Histological findings indicate that no lymph nodes had chondrocytes present or pericellular matrix suggestive of collagen type II deposition. No ectopic cartilage tissue was identified in lymph nodes or other organs examined. Occasional lymph node secondary follicle development was evident in nodes from both the treated and untreated limbs. The right ileofemoral node from the implanted limb in 1 animal in Group 1 had type I collagen deposition.

Metabolism

Conventional metabolism studies are not considered to be relevant for cell-based medicinal products. MACI consists entirely of biological components (cells, collagen I and II and elastin). The metabolism of these products is expected to follow the normal metabolic pathways for proteins and cells.

Excretion

Collagen and elastin from the membrane naturally degrade or are resorbed following the metabolic pathways for proteins. It is unlikely that there is any formal excretion of the intact collagen product or degradation products.

Pharmacokinetics drug interactions

Given that MACI is implanted locally to a chondral lesion site in the knee and does not enter into the blood circulation, it is not expected that there should be effects due to interaction with medicinal products utilised pre-, peri- or post-operatively. As such, no pharmacodynamic drug interaction data have been collected.

2.3.4. Toxicology

Single dose toxicity

In the single dose toxicity study in mice (GENZ 06008) acute systemic toxicity was evaluated via intravenous injection of sodium chloride extract and intraperitoneal injection of cotton seed oil (CSO) extracts of the ACI-Maix collagen membrane; both at a concentration of 50 mL/kg. The test article was administered to 2 treatment groups of 5 male mice. Two additional groups of 5 male animals each served as the controls and received the corresponding extraction vehicle. Clinical observations were conducted immediately, at 4, 24, 48 and 72 hours post-dose. Animals were then euthanised and necropsy evaluations were conducted of the administration site, heart, lung, gastrointestinal tract, liver, spleen, kidney and reproductive organs. Results showed that extracts of the ACI-Maix collagen membrane did not produce toxicity when administered to mice over a 72 hour in-life period, when compared with administration of vehicle. All animals survived for the full course of the study and at no time point did any animal exhibit reportable signs of toxicity. No macroscopic abnormalities were found in any of the animals.

Single dose toxicity of the MACI implant was evaluated in the 2 horse studies: GENZ 06-0239 and GENZ 09-4417.

In the 6 month pharmacodynamic study GENZ 06-0239, six horses were selected to investigate the 24-week efficacy of MACI following single administration. For each horse, a biopsy was taken from either the right or left limb, with a proximal and distal defect created in the other limb. Chondrocytes isolated from biopsy specimens were expanded in culture and seeded at a density of approximately $1x10^6$ cells/cm^2 on the ACI-Maix membrane, then implanted in 1 of 2 full-thickness cartilage defects (15 mm diameter) in the lateral trochlear ridge. The second defect served as a non-treated control. The assignment of a defect to receive MACI or act as control was randomly decided.

At implantation, histologic evaluation of MACI grafts showed that they were populated over the entire rough surface by live cells. Surgical adhesion with fibrin sealant successfully secured membranes in the defects with no evidence of displacement based upon the interim arthroscopic evaluation and final necropsy. No pannus (inflammatory exudate overlying synovial cells) was present. Synovial fluid from the grafted femoropatellar joints and from the previously biopsied femoropatellar joints was normal for total protein (< 2.5 g/dL), with the exception of 1 (control) joint (2.9 g/dL). Complete blood counts and chemistry panels for all animals were mostly normal and none were clinically significant. Finally, the biopsied synovial membrane from 5 of 6 MACI-treated joints showed mildly increased inflammatory cell infiltrate (perivascular cuffing, p=0.02) and total score (p=0.03) and a trend towards higher sub-intimal fibrosis (p=0.07) compared to controls. At 3 months, prostaglandin E2 (PGE2) concentrations (a marker of inflammation) were significantly higher in the MACI-treated joints compared to the contralateral control joints (p=0.03). Although PGE2 concentration in synovial fluid was significantly higher at 6 months compared to 3 months post-implantation for both MACI-treated and control joints (p=0.03 and p=0.01, respectively), the difference between MACI-treated and control defects at 6 months post-implantation was not significant (p=0.0503). The 3-month arthroscopic evaluation of MACI–treated defects showed a minor synovial inflammatory response when compared with empty defect controls. At the 6-month endpoint, analysis of synovial fluid revealed some inflammation in the MACI-treated joints of 4 out of 6 horses. However, inflammation was also observed in the control joints of 3 out of 6 animals. Despite observations of slight inflammation, all categories yielded no statistically significant difference between the control (untreated, empty defect) and the MACI-treated implant at 6 months post-implantation.

In the 53 week study GENZ 09-4417, 27 horses were included with 2 defects per knee. Animals in Group 1 (n=12) received MACI in 1 defect and cell-free membrane in the other defect ; animals in Group 2 (n=12) were treated with MACI in 1 defect and the other defect was left empty (no treatment). Animals in Group 3 (n=3) did not receive treatment in either defect and served as untreated controls. At 3 months post-treatment, animals were subjected to a physical examination, second-look arthroscopy with scoring of the defect areas, blood characterisation and synovial tissue biopsy. Horses were euthanised at week 53 after implantation and assessed for potential systemic effects of the treatment on major organ systems. At implantation, surplus MACI was analysed to assess the numbers of live and dead chondrocytes populating the surface of the membrane. The results indicate that no MACI implants were identified as having less than 70% viability. Surgical adhesion with fibrin sealant successfully secured membranes in the defects with no evidence of displacement based upon the interim arthroscopic evaluation and final necropsy at 53 weeks post-implantation. No pannus formation was observed at the 12-week second look arthroscopy or at termination. Haematological analysis at study termination showed no significant differences between groups. Results of serum chemistry panels on all horses showed few values outside of the reference range. Complete blood counts showed no significant differences between the groups. Chemistry panels for all animals were within normal limits, except for 2 horses in Group 1, 1 in Group 2 and 1 in Group 3; aspartate transaminase was slightly above the reference range. Creatinine phosphokinase was

slightly elevated in 1 horse in Group 1, consistent with mild muscle bruising. These slight elevations were not considered clinically significant. Synovial fluid analysis at the 53-week endpoint revealed few abnormalities. Slight elevations in white blood count and total protein were evident in several implanted and biopsied limbs from all groups. However, no cytologic abnormalities were recognised. Synovial fluid collected at pre-operative baseline and at 12-week second look arthroscopy showed no statistically significant differences in PGE2 levels between Group 1, Group 2, or Group 3. Although PGE2 concentration in the synovial fluid was higher at 12 weeks for all groups, PGE2 levels had returned to pre-operative baseline levels at 53 weeks for Groups 1 and 2. Both defects left empty (Group 3) appeared to stimulate a PGE2 response at 12 weeks which persisted and did not return to baseline levels at 53 weeks. Synovial membrane histology at 53 weeks revealed few differences between joints receiving MACI and the various controls. Villous architecture, subintimal fibrosis, intimal thickness and vascularity were all similar in joints from Groups 1, 2 and 3 and in the previously biopsied opposite joints and in the unoperated normal joints. Significant differences were only evident where occasional lymphocytic accumulations resulted in minor perivascular cuffing. Selected screening of organs for toxicity showed no macroscopic abnormalities in any of the animals. The presence of lymphocytes in selected organs was noted from all groups, however, these findings were not of clinical significance. No ectopic cartilage tissue was identified in lymph nodes or other organs examined.

Repeat dose toxicity

MACI is intended for a single implantation into the knee. Therefore, in line with the guideline on human CBMP (EMA/CHMP/410869/2006), repeat dose toxicity studies are considered not relevant.

Genotoxicity

Genotoxicity studies were not performed because these are not required for CBMP in accordance with the CHMP guideline on human CBMP (EMA/CHMP/410869/2006) unless the nature of any expressed product indicates an interaction directly with DNA or other chromosomal material; which is not the case for MACI. Two studies were conducted to examine the mutagenic potential of saline and dimethylsulfoxide (DMSO) extracts from the ACI-Maix collagen membrane. They were found to be non-mutagenic to *S. typhimurium* strains TA98, TA 100, TA1535 and TA1537, and to *E. coli* strain WP2uvrA. Due to the lack of effect in the mutagenicity studies, no *in vivo* tests were conducted.

Carcinogenicity

In compliance with the guidance issued in ISO 10993-1 (2010), conventional carcinogenicity testing was not conducted on the ACI-Maix collagen membrane (CE marked Class III device). A genotoxicity study was conducted. Based on the negative genotoxicity results coupled with the knowledge of this device and similar collagen-based devices it was concluded that a long-term animal carcinogenicity study was not required. The risk of transformation of the biopsied cells was studied in a chromosomal stability study (see other toxicity).

Reproduction Toxicity

In line with the guideline on human CBMP (EMA/CHMP/410869/2006) reproductive and developmental toxicity studies were not performed. Based on the local nature of delivery and the mechanism of action, MACI product will not affect fertility or pre- or post-natal development. No studies have been conducted in juvenile animals with the ACI-Maix collagen membrane. The cartilage in juvenile animals is still developing and any lesion is subject to spontaneous repair, as such it would not be appropriate to investigate the effects of ACI-Maix membrane.

Local Tolerance

Nine studies conducted in rat, rabbit and horse addressed the local tolerance of MACI or the ACI-Maix collagen membrane.

Two studies assessed local tolerance of the ACI-Maix membrane following muscle implantation at 4- and 13-weeks in rats (GENZ 05013 and GENZ 05014 respectively). At 4- and 13- weeks post implantation, the macroscopic reaction was not significant as compared to the negative control implant material and the control material. Although the microscopic reaction at 4 weeks was classified as a slight irritant, at 13 weeks, the ACI-Maix membrane was considered a non-irritant when compared to the negative control. A rabbit muscle implantation study (GENZ 06015) compared ACI-Maix with a comparative and negative control. Microscopically, compared to the comparative controls, the ACI-Maix membrane was classified as a non-irritant; compared to the negative control, the ACI-Maix membrane was classified as a moderate irritant. Elastin staining of the ACI-Maix membrane and the comparative controls was similar morphologically and consisted of positive fibrillar elongate material at each region of implantation. There were no significant differences in the elastin staining intensity between ACI-Maix and comparative controls.

To determine if the local tolerance subsided over time, a study was conducted to assess the potential local toxic effects of ACI-Maix following 12- and 26- weeks post-implantation in rat subcutaneous tissue (UWAOrthop004). The histological appearances were indistinguishable between ACI-Maix and control membranes at 12- and 26-weeks post-implantation. Both membranes were partially degraded after 12 weeks. In some areas, the ACI-Maix collagen fibres were degraded but the elastin fibres remained. At 26-weeks post-implantation, the infiltrated mesenchymal tissue was well integrated with the implanted membranes. There was neither inflammatory response nor foreign body multinuclear giant cells observed. No necrosis, haemorrhage and generation of fibro debris or fibrosis were observed. Intracutaneous testing (Study 02-1975-G2) was conducted to assess the potential of ACI-Maix membrane extracts to produce irritation following a single intradermal injection in rabbits. Based upon the Primary Irritation Index, both ACI-Maix membrane extracts (NaCl and CSO) were considered a negligible irritant as the test sites did not show a significantly greater biological reaction than the sites injected with the control articles.

Willers *et al.* 2005, as reported in the section “primary pharmacodynamic studies”, showed that although the majority of the MACI-treated rabbits (3 of the 4) displayed some indication of osteochondral repair, 1 animal was cited with graft failure likely due to an infection at 6 weeks post-implantation. It remains unclear if the infection was due to the MACI implant. In study GENZ 06-0147 in rabbit, as described previously, it was noted that the presence of (remnants of) elastin (a component of the ACI-Maix membrane) was often associated with a minimal to mild chronic histiocytic inflammation within the defect. As the amounts of elastin decreased over time (24 weeks versus 12 weeks) the inflammation subsided over time but remained at detectable levels (minimal to mild) even at the 24 week time point.

Studies GENZ 06-0239 and GENZ 09-4417, as previously detailed, have evaluated local tolerance in an equine model. In GENZ 06-0239, although mild lymphoid accumulation developed in synovial tissues from several horses biopsied at 3 months, no synovial tissue showed lymphoid cell accumulation at 6 months. In GENZ 09-4417, the results indicate that MACI had no signs of significant inflammatory synovial fluid changes at 12-week second look arthroscopy or at termination. There were no significant abnormal complete blood counts or chemistry panel changes.

Other toxicity studies

Two *in vitro* studies were conducted to determine the cytotoxic effects of the ACI-Maix membrane (GENZ 06006 and GENZ GT-361-TX-1). These tests showed that the ACI-Maix membrane extract showed no evidence of causing cell lysis or reactivity or cytotoxicity. An ISO Maximisation Sensitisation study (GENZ 05040) was performed to evaluate the potential of saline and sesame oil extracts of the ACI-Maix collagen membrane to induce dermal sensitisation. Under the conditions of the study, both extracts of the ACI-Maix membrane were classified as a weak sensitiser (grade 0).

Chromosomal stability testing was conducted on human chondrocytes expanded for autologous chondrocyte transplantation for MACI (GENZ RR07030). The karyotype of chondrocytes was evaluated at various stages of culture. In summary, the results indicated that cultured chondrocytes are likely to maintain a normal karyotype for at least 10 passages, or 46 population doublings. This data suggests that chondrocytes generated for MACI maintain stable karyotypes for extended periods in culture. The culture process used for the equine studies and for the pivotal clinical trial ensured that cells were not cultured beyond 3 passages, ensuring that all cultured chondrocytes employed in the production of MACI retain their normal karyotype.

The toxicity of several commercially available fibrin glues (Tisseel, Evicel, CoSeal and FloSeal) towards human cultured chondrocytes ($3x10^5$ or $6x10^5$ per 6 cm^2 culture dish) was tested (GENZ RR08028). These tests were conducted in conjunction with BioGlue, which contains formaldehyde and has been shown to be toxic to chondrocytes. No toxicity was observed for chondrocytes with Evicel, CoSeal, FloSeal or Tisseel at 24 and 72 hours. By contrast, BioGlue, which contains formaldehyde, was toxic to chondrocytes at both timepoints and cell densities.

2.3.5. Ecotoxicity / environmental risk assessment

A justification for not performing an environmental risk assessment (ERA) was provided in line with guideline on the environmental risk assessment of the medicinal products for human use (EMEA/CHMP/SWP/4447/00). MACI is composed of autologous cartilage-derived cultured chondrocytes (between $4x10^5$ and $8x10^5$ cells/ml) in Dulbecco's Modified Eagle Medium (DMEM) containing 45 µg/ml gentamycin and 8.9 ± 0.2% irradiated fetal bovine serum. The cells administered to the patient have been shown to remain in the implantation site and are not released to the environment. Incidental cell leakage results in metabolism or degradation, as is the case for natural release of cells within the body. Therefore, due to its nature, the use of MACI is not expected to pose a risk for the environment.

2.3.6. Discussion on non-clinical aspects

Pharmacology

MACI consists of autologous cultured chondrocytes delivered on a purified, resorbable, porcine-derived collagenous membrane (ACI-Maix membrane) CE marked for use with human chondrocytes which is held in place with fibrin glue. For the non-clinical studies presented, autologous chondrocytes were derived from the animal in the study and used in the implant. The pharmacological action of MACI is based on the presence of expanded autologous chondrocytes in an articular cartilage defect, where they generate a repair tissue that fills the defect and restores joint function. A series of studies were carried out to investigate the efficacy of MACI on cartilage lesion repair in a variety of animal models (rabbit, sheep and horse) and to evaluate the pharmacologic actions of the cells after implantation by analysis of the extracellular matrix they produced. In these studies, with time points extending up to 53 weeks post-implantation, repair was assessed at various time points by observation (arthroscopic and at necropsy), histological evaluation and mechanical testing. Histological assessment has been

used in 2 rabbit, 2 sheep and 4 equine studies utilising MACI or MACI-like implants (i.e. autologous chondrocytes seeded onto a similar but not identical collagen membrane as used in MACI). The MACI implants were prepared using autologous cells from the animal subjects and seeded onto collagenous membranes in a manner substantially similar to that used for preparation of the human clinical product. In small animal models, the very thin cartilage means it is not possible to recreate the surgical environment of a chondral defect without subchondral penetration. As such, MACI was implanted in osteochondral defects in rabbits. In larger animals (horse), MACI was applied to chondral defects, representative of the human clinical situation.

The effect of cell seeding density and implantation period was examined during the course of a published study in rabbits (Willers et al, 2005). The study suggested that the impact of cell density was limited as no differences were seen between cell densities of 10^4-10^6 cells/cm^2. Due to some limitations in this study the applicant was requested to further justify the dose of 10^6 cells/cm^2 used for the non-clinical and clinical studies. It was clarified that the dose selection was based on a combination of animal studies published in the literature and conducted by the applicant as well as experience gained with use in humans. In order to maximise treatment efficacy, non-clinical studies were conducted at the high dose level (10^6 cells/cm^2). The pilot equine study (GENZ 06-0239) demonstrated that MACI was generally well tolerated based on clinical parameters and synovial fluid analysis. The pivotal equine study (GENZ 09-4417) demonstrated that MACI did not induce toxicity based on clinical parameters, synovial fluid analysis and toxicity screening of selected organs. Therefore, given that there was no significant safety issue in animals treated with MACI at 10^6 cells/cm^2, the dose level selected of 500,000 to 1 million cells per cm^2 can be considered justified and supportive of the clinical use of this dose/density.

Two publications (Dorotka *et al*. 2005 & Jones *et al*. 2008) were presented in sheep. These have shown increased chondral defect repair following treatment with MACI or MACI-like implants although repair with MACI was characterised by a mixed repair of hyaline-like cartilage and fibrocartilage with good integration with the surrounding tissue.

Four studies have been presented in a larger animal, the horse, to mimic the clinical situation in humans as much as possible and in line with the reflection paper on *in-vitro* cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009). The first two studies were reported from the literature (Frisbie *et al.* 2004 & 2008) using a MACI-like membrane. The two publications contain limited detail on experimental procedures, reproducibility and control samples. Results showed that there was improved arthroscopic repair in ACI-treated animals compared to membrane only or untreated articular defects. Evaluation showed significant improvement up to 18 months post-implantation in horses that received the MACI-like implant. As the collagen membrane used to seed the harvested chondrocytes differ from that used for MACI the findings of these studies are of limited value. However, these results indicates that, in an equine model, delivery of autologous cells on a collagen scaffold to a chondral defect leads to more durable articular cartilage defect repair than either cell-free membranes or leaving the defect unfilled.

As a pilot study the aim of study GENZ 06-0239 was to confirm whether the articular cartilage repair effects observed in the studies performed by Frisbie *et al.* could be replicated with MACI in an equine model. Two defects were created in 1 hind limb of each animal in the study: 1 defect was treated with a MACI implant and the other was left empty or untreated. At 3 months, there was significant improved scoring of defects treated with MACI (membrane seeded with 1x10^6 cells/cm^2) compared to empty defects in 4 of the 6 horses. In 1 horse the overall score was equal and in the remaining horse, the untreated was superior to MACI. These results indicated that MACI contributes to cartilage repair in a large animal model.

To follow up this pilot equine study, a larger (n=27), 53-week duration equine study (GENZ 09-4417) was conducted. Twelve animals received MACI in 1 defect and the other defect was left empty (no treatment), 12 animals were treated with MACI in 1 defect and cell-free membrane (ACI-Maix) in the other defect and 3 animals did not receive treatment in either defect and served as controls. The final study report was provided during the procedure. At 53 weeks, MACI was significantly better than ACI-Maix membrane alone in every cartilage healing gross category, including percent smooth white (hyaline-like) tissue, filling of defect, integration to surrounding cartilage and overall tissue colour. Furthermore, histological examination showed that MACI significantly improved the total healing score and several individual histologic parameters compared to empty defects, including defect fill, chondrocyte predominance, toluidine reaction and collagen type II formation. Chondrocyte predominance, toluidine reactive zones and collagen type II formation were also improved with MACI compared to the cell-free ACI-Maix membrane. These results indicate that MACI generated a reparative response superior to that observed in cell-free ACI-Maix, or single or dual untreated defects but no “good-as-new” cartilage was formed. During the procedure further information on the characterisation of the chondrocytes following implantation confirmed that the presence of cells resulted in an increased proteoglycan and collagen II production, indicating that the cells display some chondrogenic properties. Data from the residual membrane showed also that the viability of the cells on the membrane was good. Results indicated that the cells do not fully migrate into the membrane rather they remain at the cell seeded side of the membrane. There is also no evidence to suggest that there is an over-proliferation of the transplanted cells in the defect. Also, as requested by the CHMP in order to better describe the characteristics of the manufactured MACI implant for these equine studies, the applicant has provided some further information on the number of cells obtained from the biopsy, duration of cell culture, passage number and differentiation potential. Taken together, the equine and human batches were manufactured according to the same procedures and underwent the same quality checks, except for those parameters for which the assay was species specific. The available data does not indicate that there are large species differences between the human and equine batches.

Both equine studies were not conducted in full GLP compliance. Equine surgical studies are not typically GLP compliant. Generally, surgeries, post-operative care and long term rehabilitation are completely or partially conducted in shared clinical premises that are non GLP compliant facilities. Overall taking into account that no suitable GLP compliant facility was available and that the applicant showed that GLP principles were followed and documented the CHMP considered that the provision of data generated in a non-compliant facility was acceptable.

Given the local application of this product, no secondary pharmacodynamics data were collected. Two studies have been presented to demonstrate the cell viability and cell-membrane interactions between chondrocytes and the ACI-Maix membrane. Both human and rabbit chondrocytes were shown to have high adhesion to ACI-Maix membranes and demonstrate high cell viability up to 14 days in culture. Given the structure of the ACI-Maix membrane chondrocytes would be confined to the defect and the non-porous nature of the outer surface of the membrane prevents fibrotic cells from migrating to the defect. Overall, there is no concern on the secondary pharmacodynamic effects for MACI implant.

According to the guideline on cell based medicinal products, safety pharmacology studies should be considered depending on the characteristics of the product. Based on the mode of action of MACI and given that it is implanted locally to a chondral lesion site in the knee, it is not expected that there should be effects of its use on other organ systems. There were no effects on the major physiological systems noted in the toxicity studies. The lack of signals observed in the associated studies provides sufficient support for not conducting dedicated safety pharmacology studies.

Given the local nature of this product, pharmacodynamic drug interactions are not to be expected for this autologous cell based product.

Pharmacokinetics

Conventional ADME studies have not been performed and are not considered to be relevant for cell-based medicinal products. This is consistent with the CHMP guideline on cell-based medicinal products (EMA/CHMP/410869/2006). Pharmacokinetic studies are limited to the biodistribution of MACI (Study GENZ 09-4417) to examine the potential of the implanted autologous cells to migrate from the implantation site to other sites in the body. The goal of Study GENZ 09-4417 was to assess the efficacy and toxicity of the MACI implant over 53-weeks but it has been used to further determine potential migration of the implanted chondrocytes to other sites in the body, in particular to the popliteal and ileofemoral lymph nodes. Potential biodistribution of the implanted chondrocytes has been assessed by gross and histological examination of several tissues, including popliteal and ileofemoral lymph nodes. Distribution data from this study indicates that there is no migration (and subsequent proliferation) from the site of application to the major organs or to the lymph nodes. There were also no reports of distribution in the heart, lung, liver, spleen, adrenal glands, kidneys or popliteal lymph nodes. One MACI-treated horse showed evidence that in the right deep iliofemoral node subcapsular sinuses were filled with a lightly eosinophilic, fibrillary material (collagen). Further examination of this confirmed this to be type I collagen, and not the result of type II collagen deposition. There was no evidence to suggest migration of chondrocytes on examination of the lymph nodes or in the synovial fluid. Overall results from Study GENZ 09-4417 provide sufficient evidence of the retention of MACI implant to the implantation site for up to 53 weeks.

No data on pharmacokinetic interactions were provided. MACI does not enter into the blood circulation therefore it is not expected that there should be effects due to interaction with medicinal products.

Toxicology

Three single dose toxicity studies in mice and horses, 2 *in vitro* genotoxicity studies and 9 local tolerance studies in rats, rabbits and horses were performed to evaluate the safety of MACI. Furthermore, other toxicity studies were conducted to assess the cytotoxicity, sensitisation, chromosomal stability of the chondrocytes of MACI and the toxicity of the fibrin glue on the ACI.

In a single dose toxicity study in mice, extracts of AC-Maix were administered either via IV injection for saline extracts or IP injection for cotton seed oil extracts. All animals survived during full 72 hour length of study with no reported signs of toxicity that can be attributed to the ACI-Maix collagen membrane.

Single dose toxicity of the MACI implant was evaluated in 2 horse studies over the course of 6 months and 53-weeks. The pilot 6 month study was performed in six horses implanted with approximately $1x10^6$ cells/cm^2 autologous cultured chondrocytes seeded on to the ACI-Maix membrane. MACI was generally well tolerated based on clinical parameters and synovial fluid analysis and although mild lymphoid accumulation developed in synovial tissues from several horses biopsied at 3 months, no synovial tissue showed lymphoid cell accumulation at 6 months. One of the six MACI-implanted defects showed mild lymphoid reaction at 6 months. This animal showed a number of minor abnormalities at 3 months in haematology tests (reduced Hb, RBC, MPV and total bilirubin; increased level of glucose); however these changes were not apparent during analysis at necroscopy after 6 months.

In the 53-week equine study, a total 27 horses were included, divided into 3 groups, Group 1 (MACI in 1 defect and ACI-Maix cell-free membrane in the other defect); Group 2 (MACI in 1 defect and 1 empty defect), Group 3 (2 empty defects to serve as untreated controls). There were increased WBCs and

total protein observed in a number of implanted limbs and biopsied joints, affecting all groups. At the 3 month arthroscopy 10 horses (6 in Group 1, 3 in Group 2 and 1 in Group 3) a displayed sign of mild lameness and this was predominantly in the implant limb (2 of 10 from the biopsy limb). At the end of the study no horse displayed signs of lameness. Arthroscopy at 3 months post-implantation showed no significant improvement in defect repair scoring between control and treated defects. By the end of the study there was improved smooth hyaline like tissue, colour and fill of defects and better integration to surrounding tissues (bone and cartilage) for the MACI-treated defects compared to ACI-Maix membrane only defects. This was also seen for MACI-treated defects compared to control (empty) defects in the same animal. Overall, the results from the 53-week study indicate that no pannus formation was observed at the 12-week second look arthroscopy or at termination and synovial fluid, complete blood counts and serum chemistry panels at the 53-week endpoint revealed few non-significant abnormalities. Selected screening of organs for toxicity showed no macroscopic abnormalities in any of the animals. These results indicated that there are no significant safety concerns.

There have been no studies conducted to assess the repeat-dose toxicity as the clinical use of MACI is based upon a single implantation. No reproductive and developmental toxicity studies were conducted as MACI is based on the local delivery and based on the mechanism of action there is no evidence that MACI will affect fertility or pre- or post-natal development. No genotoxicity studies were conducted for autologous cultured chondrocytes in line with the CHMP guideline on human CBMP (EMA/CHMP/410869/2006). Carcinogenicity testing of the ACI-Maix collagen membrane was not conducted considering the negative genotoxicity results with the ACI-Maix collagen membrane and as implantation data suggest that the membrane will degrade over time.

The local effects of ACI-Maix membrane were adequately examined and discussed in a number of dedicated local tolerance studies. It has been shown to be a non-sensitising, non-irritant with no reactivity following short and long term implantation. Observations from the pivotal 53-week study indicated that MACI is well tolerated long-term.

The cytotoxicity of the ACI-Maix membrane was studied in two *in vitro* studies. These tests showed that the ACI-Maix membrane extract showed no evidence of causing cell lysis or reactivity or cytotoxicity. The ACI-Maix membrane extracted in NaCl or sesame oil showed no evidence of causing delayed dermal contact sensitisation in the guinea pig and is considered to be a weak sensitizer.

Furthermore, chromosomal stability testing was conducted on human chondrocytes expanded for autologous chondrocyte transplantation for MACI. As part of the assessment, the karyotype of chondrocytes was evaluated at various stages of culture. In summary, these results indicated that cultured chondrocytes are likely to maintain a normal karyotype for at least 10 passages, or 46 population doublings. Dedicated tumourigenesis studies have not been performed, as the culture process for the MACI drug substance is limited to three passages for clinical batches and this is considered to be well within any concerns for transformation or senescence of biopsied chondrocytes. As cell transformation is related to the number of DNA replications (=cell doubling), three passages of cell culture corresponds to approximately 16 cell doublings whereas chromosomal abnormalities were first observed in cells entering passage 20 (73-94 cell doubling). The results from this study do provide evidence that immortalisation of human chondrocytes would not occur during the limited time of exposure to *in vitro* culture conditions. Chromosome stability has also been established and would imply that the risk of tumourigenic growth is limited. Overall, the applicant has argued that combination of the study to examine chromosome stability and the lack of tumourigenic findings observed from the long-term equine studies demonstrated that the risk of tumourigenic growth is limited. The Committee considered this conclusion to be acceptable.

During implantation procedure the MACI implant is secured in the defect using Tisseel fibrin glue. Toxicity of various fibrin glues has been investigated with cultured human chondrocytes and no toxicity with fibrin sealant which does not contain formaldehyde was observed. This suggests that any fibrin sealant which does not contain formaldehyde can be used during the MACI implantation procedure. The SmPC includes guidance that fibrin sealants containing formaldehyde should not be used.

On the basis of the ERA discussions and the nature of the product, the CHMP concluded that MACI is not expected to pose a risk for the environment.

The CHMP endorse the CAT discussion on the non-clinical aspects as described above.

2.3.7. Conclusion on the non-clinical aspects

MACI was evaluated in small (rabbit) and large (sheep, horse) animal models of focal cartilage lesion repair with timepoints extending up to 53 weeks post-implantation. In these studies, cartilage repair was assessed at various timepoints by observation (arthroscopic and at necropsy), histological evaluation and mechanical testing. Across species, MACI-treated joints generated a cartilage repair response that was more effective than defects in joints that were left untreated or treated with ACI-Maix cell free membranes. Histological analysis in horse indicates that the repair tissue in MACI-treated defects is more hyaline-like in structure and composition than that of untreated defects, with higher levels of both proteoglycan and type II collagen, indicating that the cells display some chondrogenic properties. Data from the residual membrane showed that the viability of the cells on the membrane is good. There is also no evidence that there is continuous proliferation of the transplanted cells in the defect that could have caused overfill of the defect. Overall, the non-clinical evidence suggests that delivery of autologous chondrocytes on the ACI-Maix membrane promotes proliferation and re-differentiation of seeded cells, and may result in synthesis of hyaline-like cartilage repair tissue.

No significant safety issue in animals treated with MACI at 10^6 cells/cm² have been observed, the dose level selected of 500,000 to 1 million cells per cm^2 can be considered justified and supportive of the clinical use of this dose/density.

Potential biodistribution of the implanted chondrocytes has been assessed by gross and histological examination of several tissues, including popliteal and ileofemoral lymph nodes in the long-term equine study. There were no reports of distribution in the heart, lung, liver, spleen, adrenal glands, kidneys or popliteal lymph nodes or in the synovial fluid. Overall the results provide sufficient evidence that implanted chondrocytes remain at the implantation site. As a process of the implantation technique, retention of the MACI product and the implanted chondrocytes is ensured by use of fibrin sealant and use of the membrane itself.

Non-clinical data based on implantation of MACI did not reveal any special hazard for humans based on studies of safety pharmacology, single dose toxicity, genotoxicity and local tolerance. Nonclinical *in vitro* investigations have shown that the ACI-Maix collagen membrane is non-cytotoxic, non-mutagenic, non-reactive (short- and long-term implantation), non-sensitising, a negligible irritant, and non-toxic (acute systemic). Toxicity results of various fibrin glues tested showed that fibrin sealants containing formaldehyde should not be used with MACI. Chromosomal stability testing showed that cultured chondrocytes maintain a normal karyotype for at least 10 passages (46 population doublings). Chondrocytes generated for MACI maintain stable karyotypes as the culture process ensured that cells were not cultured beyond 3 passages.

Results from studies in horses showed that MACI was generally well tolerated and did not induce toxicity based on clinical parameters, synovial fluid, membrane analysis and toxicity screening of selected organs. Overall, implantation of MACI appeared to lead to a minor inflammatory response in rabbits and horses, which was slightly elevated at early timepoints but tended to decrease over time.

Conventional reproductive and developmental toxicity studies were not considered relevant, given the nature and the intended clinical use of the product and given the local nature of the product, adverse effects of MACI on pregnancy and on nursing infants are not anticipated. However, as MACI will be implanted using invasive surgical techniques, it is not recommended during pregnancy and breast-feeding may be discontinued taking into account the benefits of treatment for the woman and the risk to the infant. There are no data on possible effects of MACI treatment on fertility.

Overall, the non-clinical aspects of MACI have been adequately documented and meet the requirements to support this application.

The CHMP endorse the CAT conclusions on the non-clinical aspects as described above.

2.4. Clinical aspects

2.4.1. Introduction

The clinical data consist of the pivotal trial "SUMMIT" (MACI00206) supported by several clinical studies reported from the literature.

SUMMIT ("Superiority of MACI Versus Microfracture Treatment") is a multi-centre, randomised, open trial in 144 subjects. The aim of this trial was to demonstrate the superiority of MACI implant versus arthroscopic microfracture for the treatment of symptomatic articular cartilage defects of the femoral condyle, including the trochlea. The applicant sought advice from the EMA/CHMP regarding the design. The applicant implemented the advices regarding the choice of the comparative treatment (Microfracture (MF), the primary endpoint (KOOS, a functional score), secondary endpoints (MRI and biopsies), blinding of MRI and histology assessments and standardisation of pain-medication. The study was run in 7 European countries (Czech Republic, France, The Netherlands, Norway, Poland, Sweden, and the United Kingdom). Patients in the pivotal clinical study will be followed for a total of 5 years post-study treatment: 2 years already in the MACI00206 study and an additional 3 years in the study's on-going extension trial (MACI00809, EudraCT: 2009-016970-33). The extension (MACI00809) commenced in December 2010 and a final study report is expected in 2015.

In addition, studies from the literature and internal study reports from Academia were provided. The Academia studies were small-scaled, non-randomised prospective studies including 18-21 subjects.

GCP

The clinical trial SUMMIT (MACI00206) was performed in accordance with GCP as claimed by the applicant.

- Tabular overview of clinical studies

Study ID§ (number of centres, location)	Study Treat-ment	Subjects entered/ completed	Follow-up (y)	Gender M/F Mean age (y)	Mean lesion diameter at baseline (cm)	Endpoints
Randomised, parallel, active controlled, non-blinded studies						
#SUMMIT 7 (EU)	MACI MF	144/137	2	93/51, 34	>3^	*KOOS, MRI, histology, functional: mCRS, IKDC,

Study ID§ (number of centres, location)	Study Treat-ment	Subjects entered/ completed	Follow-up (y)	Gender M/F Mean age (y)	Mean lesion diameter at baseline (cm)	Endpoints
						SF-12
Basad, 2010 (1, DE)	MACI MF	40/39 20/17	2	42/18, 34.2	4-10^	T-L, (ICRS),
Bartlett, 2005a (1, UK)	MACI ACI-C	47 44	1	54/37, 33.7	6.1 6.0	Functional: mCRS, VAS-pain, Stanmore score; ICRS; histology
Bachmann, 2004 (1, DE),	MACI MF	27 7	2	18/9, 33	2.9 3.1	MRI, Functional : L-G
Non-Randomised, active controlled, non-blinded studies						
Salzmann, 2009 (1, DE)	MACI AOT	9 9	2-6	16/2, 33	3-12[a](range) 0.9-2.6[b]	T-L, MCRS, VAS-pain, SF-36
Gikas, 2009 (1, UK)	MACI ACI	231 101	1-9	176/156, 33.4	4.2 3.5	L-G, MCRS, VAS-pain, Bentley functional score, PaGA, Brittberg, histology
Non-Randomised, non-controlled observational studies						
Wood, 2006 (1, AUS) Winalski, 2007	MACI	18/18	2	11/7, 39.2	3.16	KOOS*, MRI, PaGA MRI
Ebert,2011a(1, AUS)	MACI	41/35	5	21/20, 38.5	3.0	KOOS, 6 m walk-test, ROM, SF-36, PaGA, MRI
Marlovits, 2006, 2010 (1, AUT)	MACI	21/21	2-5	18/3	5.1	KOOS, IKDC, L-G, T-L, Brittberg-rating, Noyes, MRI
Anders, 2008 (1, DE)	MACI	50	2	26/24,30.3 (range 14-44)	4.1	Lysholm, DGKKT, MRI, VAS-pain
Behrens, 2006 (2, DE)	MACI	38/25	5	19/19, 35	4.1	Mayer-score, T-L, L-G, ICRS, IKDC, histology
Cherubino, 2003 (1, IT)	MACI	13/13	0.5	9/4, 35	3.5	ICRS, MCRS, Lysholm, Tegner, MRI
D'Anchise, 2005 (2, IT)	MACI	35	1-2	23/12, 33.1	4.0	VAS-pain, ICRS/IKDC, Lysholm, tegner, histology
Ebert, 2008, 2010, 2011b (1, AUS)$	MACI	70	2-5	45/25, 38	3.3	KOOS, SF-36, VAS pain, 6-m walk test, ROM, MRI

§: First author is mentioned if Study number is not available, # studies considered as pivotal by the CAT/CHMP, *primary endpoint, $: randomisation for post-operative rehabilitation program (intense or conventional), ^inclusion criteria, a: ICRS grade III (cartilage lesion till subchondreal bone), b=ICRS Grade IVa/Ivb ; subchondreal bone lesion; AOT was applied in smaller but deeper lesions, and MACI in more superficial but larger lesions. AC= acive controlled, DGKKT: German Society of Autologous Cartilage and Bone Cell transplantation, ICRS: International Cartilage Repair Society, IKDC=International Knee Documentation Committee knee examination form, L-G= Lysholm-Gillquist score, m=minutes, mCRS=Modified Cincinnati Rating Score, MRI= magnetic resonance imaging, Noyes= Noyes Sports Activity score, NR=not reported, OBS=observational, PaGA=Patient Global Assessment, Ran=randomised, T-L= Tegner-Lysholm Activity score, SF: Short-Form QOL scale, y=years

2.4.2. Pharmacokinetics

No clinical pharmacokinetic studies (absorption, distribution, metabolism and excretion) have been performed during the development program of MACI. Conventional studies of absorption, distribution, metabolism and excretion are not considered relevant in accordance with the CHMP guideline on human cell-based medicinal products (EMA/CHMP/410869/2006).

2.4.3. Pharmacodynamics

Mechanism of action

Following cartilage damage, scar tissue often forms which is, for the most part, fibrocartilage; due to the inferior biological and mechanical properties of fibro-cartilage, as compared with hyaline cartilage, scar tissue tends to break down leading to functional loss and increased symptoms. The marrow stimulating techniques, of which micro-fracture (MF) is most commonly used, penetrate the

subchondral bone and cause release of marrow components into the defect site. The reparative response produced from these procedures is one that may generate primarily fibro-cartilage, which is not as effective in maintaining joint function as hyaline cartilage. In concept, the MACI implant would contribute to the repair of articular cartilage defects through proliferation of seeded chondrocytes, resulting in synthesis of hyaline-like repair tissue.

Primary pharmacodynamics

Histological and MRI assessments of repair tissue provide information on the extent of defect fill and hyaline or hyaline-like cartilage regeneration resulting from MACI treatment. This is line with the CAT reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009), assessment of repair tissue by histology and magnetic resonance imaging (MRI) are "considered adequate tools for the pharmacodynamic assessment of autologous chondrocytes containing products". In the randomised pivotal SUMMIT trial, biopsies were obtained from 116 of the 144 included subjects (60 after MACI and 56 after MF), after 2 years of follow-up. MRI was performed in 139 subjects (70 in the MACI group and 69 in the MF group). The mean International Cartilage Repair Society (ICRS) II Overall Assessment score was comparable for the MACI and MF groups and there was no significant difference (p=0.717) between the treatment groups. The results of the full analysis set were confirmed by the PP set.

Table 5 Histology - microscopic ICRS II overall assessment at Week 104: full analysis set

	MACI N=72	Microfracture N=72
n	60	56
Mean (SD)	64.3 (22.34)	64.5 (22.78)
Median	75.0	70.8
Min, Max	0, 95	7, 97
LS Means	63.82	62.31
Difference (LS Means)	1.52	
P-Values		
Treatment	0.717	
Centre	0.108	

Overall Assessment refers to the overall quality of the repair tissue on a scale from 0 (fibrous tissue) to 100 (articular cartilage). ICRS=International Cartilage Repair Society; LS=least-squares; SD=standard deviation.

Of the 144 randomised patients, 134 underwent MRI evaluation at Week 52 and 139 at Week 104. An overview of the results for MRI degree of defect fill is presented in the table below. At Week 104, improvement since study treatment in defect fill was evident for patients in both treatment groups; the defects were filled to more than 50% for the majority of patients and the proportion of patients with >75% defect fill was comparable between patients treated with MACI or MF. There was no significant difference between the treatment groups in MRI degree of defect fill at Week 52 or Week 104. The results of the full analysis set were confirmed by the PP set.

Table 6 MRI Degree of defect fill: Full Analysis Set

n (%)	MACI N=72	Microfracture N=72	P-Value [a]
Visit 8 (Week 52)			
76 to 100%	35 (48.6)	40 (55.6)	0.744
51 to 75%	20 (27.8)	11 (15.3)	
26 to 50%	7 (9.7)	5 (6.9)	
0 to 25%	7 (9.7)	9 (12.5)	
Measure of agreement	Weighted kappa	0.604	
	95% CI	0.459, 0.748	
Visit 10 (Week 104)			
76 to 100%	35 (48.6)	41 (56.9)	0.920
51 to 75%	23 (31.9)	12 (16.7)	
26 to 50%	4 (5.6)	7 (9.7)	
0 to 25%	8 (11.1)	9 (12.5)	
Measure of agreement	Weighted kappa	0.571	
	95% CI	0.421, 0.722	

[a]P-value: calculated for MRI degree of defect fill intervals, using a CMH $\chi 2$ Test: Row Means Score Differ (α=0.05) to compare between treatment groups. Note: MRI as assessed by the independent blinded evaluators by means of consensus. Degree of Defect Fill is a measure of the completeness of defect repair produced by the graft.
CI=confidence interval; CMH=Cochran-Mantel-Haenszel; MRI=magnetic resonance imaging.

In 7 other studies from the literature that were included in the dossier, biopsies were taken to evaluate cartilage forming after MACI transplantation in patients. From the 248 subjects enrolled, biopsies and histology data were obtained from 42 subjects (17%). In about 52% of the biopsies obtained, hyaline or mixed hyaline-fibro-cartilage was detected. In 40% of the cases, fibro-cartilage was formed. In the remaining cases, fibrous tissue or undifferentiated cartilage was found.

Evidence that the estimate of 50% of hyaline forming is fair could be obtained from a randomised cohort study by Gikas et al. 2009, where biopsies were taken at 12 months in every subject. Biopsies were taken from 248 patients either randomised to ACI-C (=102) or MACI (n=146). To be noted, ACI is an autologous chondrocyte transplantation method where the cultivated chondrocytes are injected under a prior applied collagen-cover. In this study, hyaline forming was observed in 50.8% of the grafts (23.8% hyaline-like and 27.0% mixed), fibro-cartilage in 45.6% and fibrous tissue in 3.6% of the cases in this study (pooled analyses ACI-C and MACI).

2.4.4. Discussion on clinical pharmacology

The lack of clinical PK studies is considered acceptable in line with the CAT reflection paper on *in-vitro* cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009) and the CHMP guideline on human cell- based medicinal products (EMA/CHMP/410869/2006). In the SUMMIT study, histological and MRI assessments were completed as secondary endpoint assessments in addition to the co-primary clinical endpoint assessments. Both the MACI and MF groups performed well according to the pre-defined structural endpoints with good infill of defects as assessed by MRI and good quality repair tissue as assessed by the ICRS II overall assessment histology score. There were no statistically significant differences between the treatment groups in the structural endpoint evaluations. Nevertheless, improvement of pain and function was superior for MACI in this study (see efficacy section). The finding that no statistical difference was observed between MACI and MF on the structural endpoint assessments was unexpected, especially considering the robust differences in clinical endpoints favouring MACI as discussed in the efficacy section. Assessment of the association between structural endpoints (ICRS II Overall Assessment and MRI Degree of Defect Fill) and clinical efficacy (change from Baseline in KOOS Pain and Function [SRA]) at Week 104 showed a lack of correlation between the tissue characteristics and clinical improvement. The lack of correlation was consistent across both the MACI and MF treatment groups. Some patients with poor structural outcome scores demonstrated significant improvement in pain and

function whereas the opposite was true for some patients. This lack of correlation helps to explain why no differences between the MACI and MF treatment groups were observed on the structural endpoints in the MACI00206 study.

A systematic review of controlled ACI studies which evaluated clinical, histological and MRI assessment results reported mixed findings for the association between clinical and structural outcomes (Vavken *et al.* 2010). When comparing ACI with MF, Knutsen *et al.* reported a lack of association between histology scores and clinical outcome at 2 and 5 years post-treatment (Knutsen *et al.* 2004, Knutsen *et al.* 2007). In another study comparing ACI to MF, Saris *et al.* 2008 reported no statistically significant difference in clinical outcome despite better histological scores for patients treated with ACI after 1-year follow-up. After 36 months of follow-up from the same study, Saris *et al.* found no statistically significant difference between ACI and MF treatment groups in structural assessment of the repair using MRI, but a difference in clinical outcome emerged for patients treated with ACI (Saris *et al.* 2009). Another systematic review and meta-analysis of morphological MRI and its ability to predict clinical outcome following repair of articular cartilage has been completed (de Windt *et al.* 2013), comprising 32 studies (total number of patient 1019). A majority (81%) were case studies or cohort studies utilising similar standardised MRI techniques. Six (6) studies had a Coleman score ranging from 78 to 96, whilst the remaining 26 studies had a Coleman score ranging from 24 to 72. According to the authors, the most important finding was the lack of conclusive evidence to ascertain whether morphological MRI can reliably predict clinical outcome following cartilage repair as for the majority of MRI parameters, limited or no correlation was found. Nine studies (28%) found a correlation between clinical outcome and the composite magnetic resonance observation of cartilage repair tissue (MOCART) or Henderson score. Of the 6 high level studies reviewed, none reported correlation between MRI and clinical outcomes in their primary objectives.

It is possible that the capability to characterise the overall functional quality of the cartilage repair (which influences clinical outcomes) is not adequate in the methodologies used in the SUMMIT study and may have been a factor in the uncorrelated results between clinical and structural outcomes. Also one limitation of histological evaluation is the inability to ensure that the biopsy is representative of the total cartilage repair tissue, due to its inherent inhomogeneity. Biopsies only represent a small fragment of the repair tissue. In the studies, samples were taken after different time-points after surgery, hampering the analyses of pooled datasets. Nevertheless, hyaline forming has been observed after MACI, although in a limited number of patients.

Overall, based on the available data in the field of cartilage repair, there is no clear consensus on whether structural repair as measured by MRI or histology scoring systems is able to distinguish the true functional repair of cartilage defects. As stated in the ICRS Guidance (Mithoefer *et al.* 2011), “Despite recent advances, further scientific data are still required to establish a valid correlation between structural and clinical study endpoints.” Consequently, improvements in the clinical outcomes of pain and function remain at this time the most important and clinically valid endpoints in cartilage repair studies.

2.4.5. Conclusions on clinical pharmacology

Clinical pharmacology studies have not been conducted on MACI. This is acceptable and in line with the current guideline in force EMA/CAT/CPWP/568181/2009 and EMA/CHMP/410869/2006. Histology and MRI scores were similar between MACI and MF. The hypothesis that MACI leads to superior quality of hyaline cartilage repair compared to non-transplantation techniques like MF has not been established. The cartilage repair histology scores were similar between both treatments and there was no relationship between clinical outcomes of pain and function and histology as described in the efficacy section of this report. Data from the literature also indicated a lack of correlation between structural

endpoints (histology, MRI) and clinical efficacy. The Committee considered more important that a significant degree of clinical efficacy for MACI has been demonstrated in clinical setting, rather than the demonstration of MACI as a superior healing process compared to the MF. Overall, current clinical and non-clinical evidence suggests that delivery of autologous chondrocytes on the ACI-Maix membrane promotes proliferation and re-differentiation of seeded cells, and may result in synthesis of hyaline-like cartilage repair tissue.

The CHMP endorse the CAT assessment regarding the conclusions on the Clinical pharmacology as described above.

2.5. Clinical efficacy

2.5.1. Dose response study

No clinical dose-finding studies have been performed. In line with the CHMP guideline on human cell-based medicinal products (EMA/CHMP/410869/2006), the recommended dose is based on findings during non-clinical development (see non-clinical aspect).

2.5.2. Main study

Study MACI00206: A prospective, randomized, open-label, parallel-group, multicentre study to demonstrate the superiority of matrix-induced autologous chondrocyte implantation (MACI implant) versus arthroscopic microfracture for the treatment of symptomatic articular cartilage defects of the femoral condyle including the trochlea.

Methods

Study Participants

The planned patient population consisted of male and female patients between the ages of 18 and 55 years (inclusive), with at least 1 symptomatic outerbridge grade III or IV focal cartilage defect on the medial femoral condyle (MFC), lateral femoral condyle (LFC) and/or trochlea (defect size equal to or greater than 3.0 cm^2 irrespective of location). Patients with osteochondritis dissecans were also eligible for inclusion providing a bone graft was not required. Patients with osteoarthritis in the target knee joint (Kellgren-Lawrence Grade 3 or 4) were excluded.

Table 7 Modified Outerbridge and Kellgren-Lawrence grading scales

2.5.3. Conclusions on clinical pharmacology

Modified Outerbridge Grades (Noyes, 1989, *Am J Sports Med*)	
Grade I	Softening and swelling of the cartilage
Grade II	Fragmentation and fissuring in an area ≤1.27 cm (≤half an inch) in diameter
Grade III	Fragmentation and fissuring in an area >1.27 cm (>half an inch) in diameter
Grade IV	Erosion of cartilage to the bone
Kellgren-Lawrence Grading Scale	
Grade 0 (None)	Normal
Grade 1 (Doubtful)	Doubtful narrowing of joint space and possible osteophytic lipping
Grade 2 (Minimal)	Definite osteophytes, possible narrowing of joint space
Grade 3 (Moderate)	Moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour
Grade 4 (Severe)	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour

Main inclusion criteria

- At screening

1. Symptomatic focal cartilage defects as defined by Knee Injury and Osteoarthritis Outcome Score (KOOS) Pain score <55.
2. Aged ≥18 and ≤55 years.

- During arthroscopy

1. Modified Outerbridge Grade III or IV focal cartilage defect(s) located on the femoral condyles, including the trochlea, that allowed treatment with the same surgical procedure as determined at randomisation.
2. Cartilage lesions determined by arthroscopy prior to randomisation and treatment with at least 1 defect size ≥3.0 cm^2 on the femoral condyles and/or the trochlea (including osteochondritis dissecans lesions that did not require a bone graft).
3. Stable knee (i.e. anterior and posterior cruciate ligaments should be free of laxity as well as stable and intact). Ligament repair or reconstruction procedures were allowed prior to or concurrent with arthroscopy and/or arthrotomy.
4. Intact meniscus or partial meniscus (≥50% of functional meniscus remaining). Meniscal repair or resection might be performed either staged or concurrent with the cartilage repair procedure provided that the surgeon was able to confirm that ≥50% of functional meniscus would remain after the corrective meniscal treatment.

Main exclusion criteria

- At screening

1. Any surgery on the knee joint within 6 months prior to screening (excluding diagnostic arthroscopy).
2. Symptomatic musculoskeletal conditions in the lower limbs that could impede measurement of efficacy for the target knee joint.

3. In the target knee joint, patient required or had a history of a total meniscectomy or meniscal allograft or had a bucket handle tear or displaced tear that required a meniscectomy removing >50% of the meniscus.
4. Malalignment requiring an osteotomy to correct tibial-femoral or patella-femoral alignment. Retinaculum releases were allowed if indicated to correct patella maltracking.
5. History of osteoarthritis (Kellgren-Lawrence Grade 3 or 4) in the target knee joint as diagnosed by clinically appropriate X-rays obtained at the Screening visit or within the previous 12 weeks.
6. Concomitant inflammatory disease or other condition that affected the joints (e.g. rheumatoid arthritis, metabolic bone disease, psoriasis, gout, symptomatic Chondrocalcinosis)
    - During arthroscopy

1. Modified Outerbridge Grade III or IV defect(s) located on the patella or tibia.

Treatments

During the initial index arthroscopy, eligible patients were randomised to 1 of the 2 following treatment groups:

- MACI
- Microfracture (MF)

All Outerbridge Grade III and IV cartilage defects that were to be treated in the target knee had to be treated with the same procedure. Grade I or II cartilage defects were either left untreated or could be treated with debridement only. An index arthroscopy was performed within approximately 8 weeks of the screening visit to assess the chondral defect(s) and the condition of surrounding cartilage. During the index arthroscopy, all patients who met the eligibility criteria and were considered suitable for treatment in the study by the surgeon had a cartilage biopsy taken prior to randomisation to study treatment.

MACI: Patients randomised to MACI treatment returned to the study site within approximately 4 to 8 weeks of the index arthroscopy for implantation of the MACI product via arthrotomy. MACI consisted of autologous cultured chondrocytes seeded onto a CE marked purified resorbable porcine-derived collagen type I/III membrane (ACI-Maix). The final MACI product started as a 20 cm^2 (5 x 4 cm) type I/III collagen membrane seeded with autologous cultured chondrocytes at a density of 500,000 to 1 million cells per cm^2. At implantation, the membrane was trimmed to the correct size and shape of the cartilage defect and implanted cell-side down into the debrided base of the defect; the implant was secured in place using fibrin sealant in a thin layer on the base. A description of any other permitted concurrent surgical procedures (CSPs) performed during the arthrotomy had to be recorded.

Microfracture: Patients randomised to treatment with MF underwent the study procedure during the index arthroscopy. The size of the chondral defect(s), both in the unprepared state and after the treatment procedure, was recorded.

Rehabilitation programmes: All patients followed a recommended post-operative rehabilitation programme. Details were provided in the Rehabilitation Guidelines. Patients were monitored for compliance with the rehabilitation schedule and achievement of rehabilitation goals. The rehabilitation programme was the same for patients in both treatment groups.

Objectives

The objective of this study was to demonstrate superior efficacy and evaluate the safety of MACI compared with arthroscopic MF in the treatment of patients (aged 18 to 55 years) with symptomatic articular cartilage defects of the femoral condyle, including the trochlea.

Outcomes/endpoints

Efficacy:

Co-primary efficacy variable:

- Change from baseline to Week 104 for the patient's Knee injury and Osteoarthritis Outcome Score (KOOS) pain and function (Sports and Recreational Activities [SRA]) scores.

Patients completed the KOOS at screening and at each post-treatment assessment from Week 24 through the Week 104 (i.e. final) study visit. The KOOS is a validated knee-specific instrument developed to assess the patients' opinion of their knee and associated problems. The KOOS included the following 5 separately scored subscales which in total addressed 42 items:

- Pain (9 items)
- Function (SRA) (5 items)
- Function in Activities of Daily Living (ADL; 17 items)
- Knee-Related Quality of Life (QOL; 4 items)
- Other Symptoms (e.g. swelling, restricted range-of-motion [7 items])

A 5-point Likert scale was used to record the response to each item ranging from 0 (no problems) to 4 (extreme problems). Within each subscale, items were added up and normalised to a value between 0 (extreme problems) and 100 (no problems).

Secondary efficacy variables:

- Histological evaluation of structural repair of evaluable biopsies harvested from the core of the index lesion during arthroscopy at Week 104. Evaluation of histological data was performed by independent central review blinded to the patient's treatment. An appropriate histological evaluation score was used to assess the structural repair. The microscopic International Cartilage Repair Society (ICRS) II variable "Overall Assessment" was to be regarded as the most important histological assessment variable addressing the related histology efficacy endpoint.
- MRI assessments of structural repair parameters at baseline and at Weeks 52 and 104 including:
  - degree of defect fill based on the thickness of repair tissue,
  - degree of integration of the repair tissue with adjacent native cartilage,
  - signal intensity of the repair tissue relative to adjacent native cartilage.

  Evaluation of MRI data was performed by independent central review blinded to the patient's treatment. Appropriate MRI sequences were used to image cartilage repair tissue to allow assessment of parameters. The variable "degree of defect fill" was to be regarded as the most important MRI assessment variable addressing the related MRI efficacy endpoint.

- Response rate based on KOOS Pain and Function (SRA) scores: the proportion of patients who responded to treatment at Week 104. A responder was defined as a patient with at least a 10-point improvement in both the KOOS Pain and Function (SRA) scores from Baseline.
- Treatment failure rate: the proportion of patients in each treatment group assessed as treatment failures at Week 104 (Note: this analysis was planned but not completed as the low number of treatment failures made this not evaluable).
- Change from baseline at Week 104 in the remaining 3 subscales of the KOOS instrument (i.e. other Symptoms, Knee-Related Quality of Life [QOL], Activities of Daily Living [ADL])

Tertiary efficacy variables

- Change from baseline at Weeks 24, 36, 52 and 78 in all 5 subscales of the KOOS instrument (i.e. Pain, other Symptoms, QOL, ADL, Function [SRA]).
- Response rate based on KOOS Pain and Function (SRA) scores: the proportion of patients who responded to treatment at Weeks 24, 36, 52 and 78.
- Treatment failure rate: the proportion of patients in each treatment group assessed as treatment failures atWeeks 24, 36, 52 and 78 (Note: this analysis was planned but not completed as the low number of treatment failures made this not evaluable).
- Average time to treatment failure: the time to treatment failure was based on the date that the surgeon decided that surgical re-treatment of the original index lesion was required relative to the date of the original study surgery (i.e. arthroscopy for MF and arthrotomy for MACI implant). Treatment failure was only determined in relation to the original treated defect(s) (Note: this analysis was planned but not completed as the low number of treatment failures made this not evaluable)
- Change from baseline at Weeks 52 and 104 in the patient's evaluation of overall knee condition using the Modified Cincinnati Knee Rating System
- Change from baseline at Weeks 52 and 104 in the patient's evaluation of overall knee condition using the International Knee Documentation Committee (IKDC) Subjective Knee Evaluation Form
- Change from baseline at Weeks 52 and 104 in the 12-Item Short-Form Health Survey (SF-12) Acute Version 2.0 for the 8 subscales (physical functioning, role-physical, bodily pain, general health, vitality, social function, role-emotional, mental health) and the physical and mental summary components
- Change from baseline at Weeks 52 and 104 in the European Quality of Life (EuroQOL) 5 dimensions (EQ-5D) health state
- Macroscopic ICRS "Cartilage Repair Assessment" score during arthroscopy at Week 104 in patients undergoing arthroscopy for harvesting of biopsy of the index lesion.

Safety:

- Rate of treatment-emergent adverse events (AEs).
- Rate of treatment-emergent serious adverse events (SAEs).
- Rate of subsequent surgical procedures (SSPs).
- Physical examination and knee examination findings.

Exploratory:

The relationship between MRI and the co-primary variables at Weeks 52 and 104 was explored by means of canonical correlation analyses and visually with the aid of scatterplots. The relationship between MRI and histology at Week 104 was similarly investigated.

Sample size

The sample size calculation was based on the bivariate co-primary efficacy parameters of change from baseline to Week 104 in KOOS Pain score and Function (SRA). The test was performed at α=0.05. The power was chosen to be 85%. Assuming an improvement difference between groups at Week 104 of 12 points in KOOS Pain and 12 points in Function (SRA), standard deviations (SDs) of 20 for KOOS Pain and 30 for KOOS Function (SRA), as well as a correlation coefficient between the change from Baseline at Week 104 between KOOS Pain and Function (SRA) of 0.56, 62 patients per treatment group (124 patients in total) would be needed to have 85% power. In order to account for possible early discontinuations from the study, an additional 20 patients (15%) were to be randomised and treated, resulting in 72 patients per treatment group (144 patients in total).

Randomisation

All patients were randomly assigned to the MACI or MF groups during the index arthroscopy using an interactive voice response system. Patients were allocated to treatment according to a computer-generated randomisation schedule provided by the applicant.

Blinding (masking)

This was an open-label study and blinding procedures were not required.

Statistical methods

Analysis Sets:

Three analysis populations were defined for this study:

- The Full Analysis set, consisting of all randomised patients who received study treatment (i.e. MF during the index arthroscopy or MACI implant during arthrotomy). The Full Analysis set was used to analyse efficacy.
- The Per Protocol (PP) set, defined as those patients in the Full Analysis set without any significant criteria violation that could possibly influence the efficacy analyses. This PP set was used for sensitivity analyses of primary and secondary efficacy variables.
- The Safety set, consisting of all randomised patients who underwent arthroscopy at Visit 2. The Safety set was used for analysis of safety variables.

Efficacy:

The co-primary efficacy parameter, change from baseline to Week 104 in KOOS Pain and Function (SRA) scores, was analysed with a multivariate analysis of variance (MANOVA) model. The analysis was conducted at the significance level of α=0.05.

Analyses of KOOS data included specification of a last observation carried forward (LOCF) method for handling of missing data. Missing or incalculable KOOS subscale scores were also imputed using a 2-step multiple imputation (MI) scheme.

Safety:

The number (%) of patients with treatment-emergent AEs (TEAEs) and treatment-related AEs was presented for each treatment group by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC) and Preferred Term (PT). The incidence rate of treatment-emergent SAEs was also presented for each treatment group.

The incidence rate of treatment-emergent AEs was compared between treatment groups overall and in 2 time periods following surgery: the early post-operative period (up to and including 12 weeks after study treatment) and late post-operative period (more than 12 weeks after study treatment). Any SAEs reported between the Screening visit and prior to the index arthroscopy were listed. For patients in the MACI group, AEs starting after biopsy but before implantation were listed. Additional listings of AEs leading to discontinuation were generated. The number (%) of patients with SSPs was presented by treatment group. The frequency of SSP 0, ≥1 (the number of different dates at which surgical repair occurred, not the number at a specified date) was analysed using a logistic regression model with treatment, age, gender and total surface area of all lesions as covariates in the model.

Exploratory:

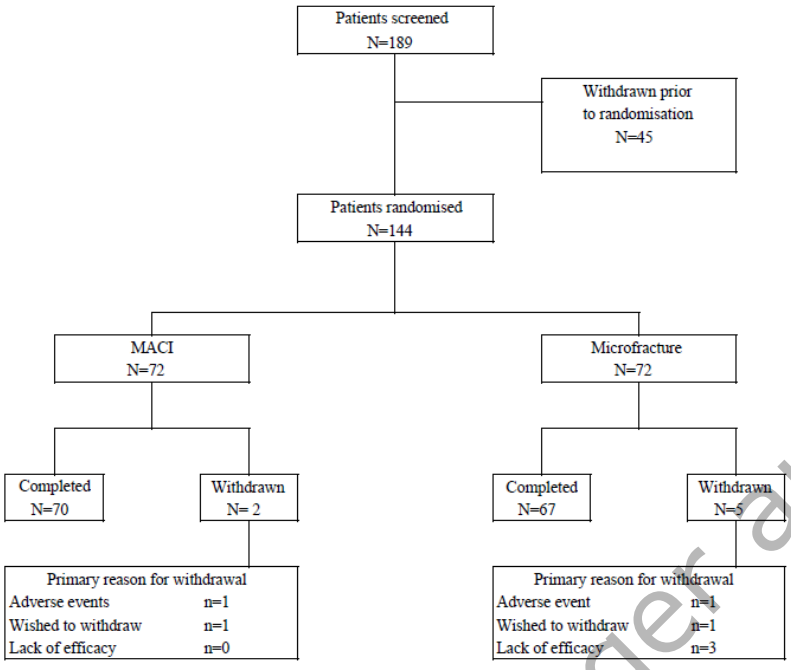
The relationship between MRI and the co-primary variables at Weeks 52 and 104 was explored by means of canonical correlation analyses and visually with the aid of scatterplots. The relationship between MRI and histology at Week 104 was similarly investigated. As a post hoc exploration, the association of change from Baseline in KOOS Pain and KOOS Function (SRA) at Week 104 by ICRS II Overall Assessment was graphically presented with scatterplots.

Results

Participant flow

The disposition for all patients is illustrated in Figure 2. There were 189 patients screened. In total, 144 patients were randomised; 72 patients were randomised to the MACI group and 72 patients were randomised to the MF group. Seven patients prematurely discontinued the study after randomisation: 2 patients in the MACI group and 5 patients in the MF group. Of these 7 patients, 3 patients from the MF group discontinued due to lack of efficacy. No patients from the MACI group discontinued the study due to lack of efficacy. One patient in each treatment group withdrew from the study due to an AE.

Figure 2 Patient disposition



Recruitment

The first patient was recruited on 21 May 2008 and the last patient on 20 March 2012. A total of 16 investigators at 16 study sites, across 7 countries in Europe, enrolled patients. Three sites in 3 countries were closed because no patients were enrolled.

Conduct of the study

There were 2 amendments to the original protocol bringing clarifications and corrections throughout the protocol. The CHMP considered that Amendment 1 (dated 28 January 2008) and Amendment 2 dated 20 December 2010) have not impacted the outcome of the study. Changes to the planned analyses were as follows:

1. Where appropriate, Fisher's exact test was to be replaced by the Cochran-Mantel-Haenszel χ^2 test, unless it was concluded that the normal approximation to binomial distribution was not valid. The Cochran-Mantel-Haenszel χ^2 test allows for stratification and analyses incorporating ordinality of data.
2. With respect to MRI assessment, degree of defect fill was to be regarded as the principle MRI indicator of response to treatment. Changes from Baseline results were not presented or used for statistical analyses since degree of defect fill was presented as ordinal data.
3. Statistical analyses of treatment failure rates at Weeks 24, 36, 52, 78 and 104, were planned to be replaced by providing the product limit estimates at Weeks 24, 36, 52, 78 and 104 in order to adjust for patient drop-out during the study.

The finalisation of the SAP Version 2.0 (dated 11 February 2008) occurred prior to data entry. SAP Version 3.0 (dated 10 July 2008) included updates regarding histological parameters that were incorporated prior to the first histology assessment for the first patient at or after Visit 6 (Week 24). Subsequent additional analyses were finalised in an addendum which involved:

1. Additional KOOS analyses with respect to handling of missing data (using a 2-step MI scheme) and covariates for quantitative analyses.
2. Stratification for KOOS response rate analyses only for treatment group.
3. All Patients Randomised set for primary and secondary analyses.
4. Additional subgroup analyses for analyses of co-primary and secondary endpoints.
5. Multiplicity in secondary analyses.

Additionally, to explore the association between KOOS Pain and Function (SRA) and ICRS II overall assessment histology scores at Week 104, post hoc visualisation was done with the aid of scatterplots.

Protocol Deviations

In the Full Analysis set, the number of patients with at least 1 major protocol deviation was higher in the MF group than in the MACI group (see Table 8). For both treatment groups, incomplete or missed study procedure or assessment was the most commonly reported major protocol deviation: 15 patients (20.8%) in the MACI group and 21 patients (29.2%) in the MF group.

Table 8 Major protocol deviations – Full Analysis Set

n (%)	MACI N=72	Microfracture N=72
Any Major Protocol Deviation	30 (41.7)	35 (48.6)
Inclusion, exclusion criteria	7 (9.7)	2 (2.8)
Other	4 (5.6)	4 (5.6)
Study procedure or assessment	15 (20.8)	21 (29.2)
Subject information or informed consent	6 (8.3)	10 (13.9)
Visit completion or timing	4 (5.6)	8 (11.1)

Baseline data

Demographic Characteristics

In both treatment groups, the majority of patients were male and the median age was 34 to 35 years. The mean Body Mass Index (BMI) was approximately 26 for both treatment groups, which is classified as overweight according to the WHO Global Database on BMI.

Table 9 Demographic characteristics

	MACI N=72	Microfracture N=72
Age (years)		
n	72	72
Mean (SD)	34.8 (9.16)	32.9 (8.78)
Median	35.0	34.0
Min, Max	18, 54	18, 54
Sex, n (%)		
Male	45 (62.5)	48 (66.7)
Female	27 (37.5)	24 (33.3)
Race and Ethnicity, n (%)		
White	72 (100%)	72 (100%)
Hispanic or Latino	0	0
Weight (kg)		
n	72	71
Mean (SD)	82.61 (15.07)	84.20 (15.64)
Median	83.00	84.00
Min, Max	52.0, 129.0	54.0, 134.0
Height (cm)		
n	71	69
Mean (SD)	177.65 (10.35)	178.26 (8.75)
Median	180.00	178.00
Min, Max	150.0, 198.0	160.0, 199.0
BMI (kg/m^2)		
n	71	69
Mean (SD)	26.21 (4.34)	26.44 (4.03)
Median	25.70	26.10
Min, Max	18.9, 45.2	18.9, 36.3

Baseline disease characteristics

Medical/surgical history

A summary of medical/surgical history reported at baseline by >5% of patients in any treatment group was presented. More patients in the MACI group (58 patients [80.6%]) reported a medical/surgical history than patients in the MF group (50 patients [69.4%]). In both treatment groups, patients most frequently reported current allergic conditions and current and non-current musculoskeletal conditions.

Target knee history

Target knee history reported at baseline is summarised in Table 4. All patients in the study had an index lesion in 1 target knee; this was the right knee for 44 patients (61.1%) in the MACI group and 33 patients (45.8%) in the MF group. For both treatment groups, acute trauma was the most common underlying aetiology of the index lesion (33 patients [45.8%] in the MACI group and 45 patients [62.5%] in the MF group). Chronic degenerative defects were twice as common in the MACI group (18 patients [25.0%]) compared to the MF group (9 patients [12.5%]). Defects due to osteochondritis dissecans were present in 8 patients (11.1%) in the MACI group and 12 patients (16.7%) in the MF group. The duration since the onset of symptoms was longer in the MACI group compared to the MF group (median 1142 versus 736 days, respectively). The level of sports activity with physical strain on the knee prior to the onset of symptoms was higher for patients in the MF group as compared to the MACI group. Sports activity was rated as highly competitive in 17 patients (23.6%) in the MACI group and 27 patients (37.5%) in the MF group; as recreational in 40 patients (55.6%) in the MACI group and 32 patients (44.4%) in the MF group; and as minimal in 10 patients (13.9%) in the MACI group and 8 patients (11.1%) in the MF group.

Table 10 Baseline target knee history

n (%)	MACI N=72	Microfracture N=72
Target Knee		
Left knee	28 (38.9)	39 (54.2)
Right knee	44 (61.1)	33 (45.8)
Aetiology		
Acute trauma	33 (45.8)	45 (62.5)
Chronic degeneration	18 (25.0)	9 (12.5)
Osteochondritis dissecans	8 (11.1)	12 (16.7)
Unknown	9 (12.5)	6 (8.3)
Other [a]	4 (5.6)	0
Duration Since Onset of Symptoms (days)		
n	71	72
Mean	2134.9	1345.0
Median	1142.0	736.0
Min, Max	20.0, 10204.0	38.0, 5632.0
Level of Sports Activity [b]		
Elite competitive	1 (1.4)	1 (1.4)
Highly competitive	17 (23.6)	27 (37.5)
Recreational	40 (55.6)	32 (44.4)
Minimal	10 (13.9)	8 (11.1)
None	4 (5.6)	4 (5.6)

[a] Included: degeneration after trauma 22 years ago; subchondral bone cyst; after anterior cruciate ligament reparation; pain in right knee during walking December 2007 and mechanical complaints. [b] Refers to sports activity with physical strain on the knee prior to onset of symptoms.

Radiograph and knee examination of target knee

Radiographs of the target knee, including a routine standing anterior/posterior view of the knee, a patellofemoral view with the knee flexed at 30º to 45º and a full-length X-ray of the leg, were similar between the MACI and MF groups. For both treatment groups, patella and tibiofemoral alignment abnormalities were uncommon (incidences ≤10%). However, more patients in the MF group (39 patients [54.2%]) were rated as normal on the Kellgren-Lawrence Grading Scale compared with patients in the MACI group (32 patients [44.4%]).

Knee examinations did not reveal any clinically meaningful differences at screening between the treatment groups. In both groups, approximately one-third of patients had mild effusion in the target knee and slightly over half of patients had no effusion in the target knee. Tibiofemoral joint pain in the target knee was reported at similar frequencies across treatment groups, both medially and laterally. An equal proportion (29.2%) of patients in both treatment groups experienced pain upon palpitation in the patellofemoral joint of the target knee. In approximately half of these patients, the pain was located in the medial compartment. The majority of patients were rated as normal for patella alignment and stability of the ACL, PCL, MCL and LCL and the proportions of patients with mild or gross laxity in the PCL, MCL and LCL were low (between 0% and 2.8%). However, gross laxity of the ACL was reported more frequently for patients in the MACI group than in the MF group.

Defects of the target knee

The target defects were similar between the 2 treatment groups at baseline. For both treatment groups, the index lesion was most frequently located in the MFC, next most frequently in the LFC, followed by the trochlea. No index lesions were located at the patella or tibia (as per protocol). Prior to treatment, the median size of the index lesion and the median total defect size surface area were the same for both treatment groups (4.0 cm^2 and 4.5 cm^2, respectively); some patients also received treatment for lesions in addition to the index lesion. The majority of patients in both treatment groups had an index lesion that was completely contained. As per the inclusion criteria, all patients in the

study had a Modified Outerbridge Grade III or Grade IV index lesion. However, the index lesion was classified as Grade IV for more patients in the MF group compared to the MACI group.

Twenty-one percent (15/72) of the patients treated with MF had a lesion size > 6 cm^2. Of these, 93% (14/15) had a lesion size between 6 and 10 cm^2 (see Table 11).

Table 11: Baseline index lesion defect size (cm^2): Full Analysis Set

Baseline index lesion defect size (cm^2)	Statistic	MACI N=72	Mfx N=72	Total N=144
3 to ≤ 6 cm^2	n (%)	63 (87.5)	57 (79.2)	120 (83.3)
> 6 to ≤ 10 cm^2	n (%)	6 (8.3)	14 (19.4)	20 (13.9)
> 10 to ≤ 15 cm^2	n (%)	1 (1.4)	1 (1.4)	2 (1.4)
> 15 to ≤ 20 cm^2	n (%)	2 (2.8)	0	2 (1.4)
	n	72	72	144
	Mean (SD)	4.9 (2.8)	4.7 (1.8)	4.8 (2.3)
	Median	4.0	4.0	4.0
	Min, Max	3.00, 20.00	3.00, 11.25	3.00, 20.00

Max=maximum, Min=minimum, MFX=microfracture

Prior and concomitant therapy

Prior orthopaedic knee surgeries

An overview of prior orthopaedic knee surgeries reported in patients who have undergone at least 1 previous knee surgery is presented in Table 12. The proportion of patients with at least 1 prior orthopaedic knee surgery (target or non-target knee) was comparable for the 2 treatment groups, however, the median days since the last surgery for patients in the MACI group was more than twice that for patients in the MF group.

The most common (>5% of patients in any treatment group) prior surgical procedures in the target knee were (anterior cruciate ligament) ACL repair, debridement of cartilage lesion, diagnostic arthroscopy, fixation of osteochondritis dissecans (OCD) fragment, hardware removal, lavage, loose body removal, MF, partial meniscectomy (lateral), partial meniscectomy (medial), shaving, subchondral drilling, synovectomy/synovial excision and other. Of these, the procedures performed more frequently in the MF group included MF, partial meniscectomy (lateral) and shaving.

Table 12 Prior orthopaedic knee surgeries in patients with at least 1 previous knee surgery

n (%)	MACI N=72	Microfracture N=72
Any Prior Orthopaedic Knee Surgery	65 (90.3)	60 (83.3)
Days since last surgery		
n	65	60
Mean (SD)	2407.0 (2553.01)	1410.1 (1710.00)
Median	1476.0	685.5
Min, Max	9, 12582	2, 7370

Prior Surgical Procedure		
Abrasion arthroplasty	2 (3.1)	0
ACL repair	9 (13.8)	5 (8.3)
Biopsy harvest for ACI	1 (1.5)	0
Bone graft	1 (1.5)	0
Debridement of cartilage lesion	20 (30.8)	13 (21.7)
Diagnostic arthroscopy	35 (53.8)	28 (46.7)
Fixation of OCD fragment	4 (6.2)	2 (3.3)
Hardware removal	4 (6.2)	3 (5.0)
Lateral meniscal repair	1 (1.5)	0
Lateral release of patella retinaculum	2 (3.1)	2 (3.3)
Lavage	7 (10.8)	1 (1.7)
Loose body removal	16 (24.6)	13 (21.7)
Medial collateral ligament repair	0	1 (1.7)
Medial meniscal repair	0	1 (1.7)
Microfracture	12 (18.5)	18 (30.0)
Osteochondral autograft	2 (3.1)	0
Partial meniscectomy, lateral	4 (6.2)	7 (11.7)
Partial meniscectomy, medial	17 (26.2)	7 (11.7)
Patella tracking	0	1 (1.7)
Shaving	12 (18.5)	12 (20.0)
Subchondral drilling	7 (10.8)	4 (6.7)
Synovectomy/synovial plica excision	5 (7.7)	4 (6.7)
Tibial osteotomy	1 (1.5)	0
Other	11 (16.9)	10 (16.7)

Procedures are listed in alphabetical order. Percentages are based on the number of patients that had a previous knee surgery. ACI=autologous chondrocyte implantation; ACL=anterior cruciate ligament; OCD=osteochondritis dissecans

Concurrent surgical procedures (CSPs)

For patients in the MACI group, CSPs include those occurring during either the Visit 2 (arthroscopy and cartilage biopsy) or the Visit 3 surgeries (MACI implantation) or at the Week 104 (i.e. final) visit core biopsy, while for patients in the MF group, CSPs include those occurring during the Visit 2 surgery (arthroscopy, cartilage biopsy and MF treatment) or at the Week 104 (i.e. final) visit core biopsy.

An overview is presented in Table 6 of CSPs reported in patients for whom a biopsy was done during the Visit 2 or 3 surgery or during the Week 104 (i.e. final) visit core biopsy. Overall, the type and frequency of CSPs were comparable for the 2 treatment groups. During the cartilage biopsy/implantation, CSPs were performed in approximately one-third of patients in both treatment groups, whilst during the core biopsy at Week 104, CSPs were performed in approximately one-fourth of patients in both treatment groups. For both treatment groups, loose body removal was the most frequently performed CSP during the cartilage biopsy/implantation and the core biopsy at Week 104. In addition, for approximately two-thirds of the patients who had a CSP performed during the core biopsy at Week 104, the CSP was classified as 'other'.

Table 13 Concurrent surgical procedures in patients during biopsy or treatment

n (%)	MACI N=72	Microfracture N=72
Cartilage Biopsy/Implantation		
Any Concurrent Procedure	26 (36.1)	22 (30.6)
Medial meniscus procedure		
Partial meniscectomy	6 (23.1)	4 (18.2)
Lateral meniscus procedure		
Partial meniscectomy	2 (7.7)	4 (18.2)
Ligament procedure ACL		
Graft reconstruction	4 (15.4)	6 (27.3)
Other		
Loose body removal	7 (26.9)	9 (40.9)
Synovectomy/synovial plica excision	6 (23.1)	3 (13.6)
Lateral Release of Patella Retinaculum	0	1 (4.5)
Other	4 (15.4)	3 (13.6)
Core Biopsy at Week 104		
Any Concurrent Procedure	19 (26.4)	17 (23.6)
Medial meniscus procedure		
Partial meniscectomy	1 (5.3)	0
Lateral meniscus procedure		
Partial meniscectomy	0	1 (5.9)
Ligament procedure ACL		
Graft reconstruction	0	0
Other		
Loose body removal	6 (31.6)	5 (29.4)
Synovectomy/synovial plica excision	2 (10.5)	3 (17.6)
Lateral Release of Patella Retinaculum	0	0
Other	13 (68.4)	13 (76.5)

Percentages are based on the number of patients for whom a concurrent surgical procedure was done at the particular visit. ACL=anterior cruciate ligament.

Concomitant medications

Concomitant medications include those that were started at or after Visit 1 (Screening) or that were started prior to Visit 1 but were continued during the study. For the 2 treatment groups, the use of concomitant medications was generally comparable. Approximately three-fourths of patients in both treatment groups had any concomitant medication. The most frequently used class of concomitant medication for both treatment groups was non-steroidal anti-inflammatory/anti-rheumatic drugs; half of the patients in each group used this medication which was most commonly ibuprofen. For both treatment groups, the next most frequently used class of concomitant medication was analgesics and antipyretics; approximately 42% of patients in each group used this medication which was most commonly paracetamol.

Concomitant pain medications

Concomitant pain medications include those that were taken within 4 weeks prior to Visit 6 (Week 24), Visit 7 (Week 36), Visit 8 (Week 52), Visit 9 (Week 78) and Visit 10 (Week 104). Overall, the use of concomitant pain medication was comparable for the 2 treatment groups (45% to 49% of the patients in each group) and the use of concomitant pain medication decreased over time in both treatment groups from Visit 6 to Visit 10. For both the MACI and MF groups, the most frequently reported concomitant pain medications included the non-steroidal anti-inflammatory/anti-rheumatic drug ibuprofen and the analgesic paracetamol. Statistical comparison (χ^2 test) of the response categories

'Pain Medication' versus 'No Pain Medication' between the MACI and MF groups did not reveal significant differences in concomitant pain medication usage at any of the visits or overall.

Treatment Compliance

All 144 randomised patients received study treatment: 72 patients with MACI and 72 patients with MF. Patients underwent arthroscopy at Visit 2 and were randomised during the arthroscopy. All 144 randomised patients had a biopsy completed during Visit 2; 1 screen failure patient had a biopsy taken for commercial treatment as the patient was found not eligible for study randomisation – the investigator concluded at the time of the arthroscopy that the patient should not be randomised to treatment and instead should be treated with commercially-available MACI. The 72 patients randomised to MF treatment underwent the procedure during Visit 2. All 72 patients randomised to MACI treatment underwent the implantation procedure during Visit 3.

Numbers analysed

A total of 189 patients were included in the All Patients Screened set; no patients were excluded from the All Patients Screened set. The All Patients Randomised set included 144 patients; 45 patients were excluded from the All Patients Randomised set since they had not been randomised to study treatment. The Full Analysis set (for both the MI and LOCF approaches) included 144 patients and was the primary population used for the analysis of efficacy. No patients were excluded from the Full Analysis set.

The Per Protocol (PP) set was used for sensitivity analysis of primary and secondary efficacy endpoints. The PP set for the MI approach included 127 patients and the PP set for the LOCF approach included 126 patients. For 23 patients, at least some data were excluded from the PP set for reasons including major eligibility criteria violation, failure to adequately complete the rehabilitation programme, concomitant pain medication taken during the 4 weeks prior to a KOOS assessment visit, medical condition/surgical history, or prior orthopaedic knee surgery within 6 months prior to Screening. The Safety set included 144 patients and was the population used for the analysis of safety; no patients were excluded from the Safety set.

Table 14 Data sets analysed

	Total
All Patients Screened Set, n	189
All Patients Randomised Set, n (%)	144/189 (76.2)
Safety Set, n (%)	144/144 (100.0)
Full Analysis Set - MI, n (%)	144/144 (100.0)
Full Analysis Set – LOCF, n (%)	144/144 (100.0)
Per Protocol Set - MI, n (%)	127/144 (88.19)
Per Protocol Set - LOCF, n (%)	126/144 (87.54)

LOCF=last observation carried forward; MI=multiple imputation

Outcomes and estimation

Primary efficacy endpoint

An overview of the co-primary efficacy results (Full Analysis set, LOCF analysis) is presented in Table 8. From Baseline to Week 104, an improvement in KOOS Pain and Function (SRA) ratings was reported for patients in both treatment groups, however the mean improvement was significantly greater (p=0.001, LOCF) for patients treated with MACI compared to those treated with MF. The additional improvement of MACI over MF in change from baseline at Week 104 was >10 points for both

Pain and Function (SRA). The above-mentioned Full Analysis set results of the LOCF analysis were confirmed by the MI analysis results. The Full Analysis set results for both the LOCF and MI analyses were furthermore also confirmed by the PP set results.

Table 15 Co-primary efficacy parameter - LOCF: Full Analysis Set

		MACI N=72		Microfracture N=72	
		Pain	Function (SRA)	Pain	Function (SRA)
Baseline	n; Mean (SD)	72; 37.00 (13.52)	72; 14.86 (14.68)	71; 35.45 (12.09)	71; 12.57 (16.67)
Visit 10 (Week 104)	n; Mean (SD)	72; 82.45 (16.18)	72; 60.90 (27.84)	70; 70.85 (24.22)	70; 48.71 (30.33)
Change From Baseline to Week 104	n; Mean (SD)	72; 45.45 (21.08)	72; 46.04 (28.35)	69; 35.23 (23.91)	69; 35.83 (31.63)
Final Model (Reduced)					
LS Means		44.13	46.05	32.37	34.64
Difference (LS Means)		11.76	11.41		
P-Values					
Treatment		0.001			
Centre		0.002			
Baseline KOOS Pain		<0.001			
Baseline KOOS Function (SRA)		<0.001			
Sample Covariance Matrix					
Pain		10.067	11.009		
Function (SRA)		11.009	21.612		

Baseline: the last non-missing value collected prior to study treatment at Day 1. P-value (Final Model): multivariate analysis of variance conducted with treatment and centre as fixed effects and statistically significant covariates from the initial model, conducted at α=0.05 level of significance using the Wilks' Lambda test statistic. LOCF=last observation carried forward; LS=least-squares; KOOS=Knee Injury and Osteoarthritis Outcome Score; SD=standard deviation; SRA=Sports and Recreational Activities

Secondary efficacy endpoints

Histological evaluation of structural repair

Of the 144 randomised patients, 116 underwent a second-look arthroscopy and biopsy at Week 104. There were no apparent differences between the groups in nonparticipation in second-look arthroscopy and biopsy as the 116 patients included 60 MACI group patients and 56 MF group patients. An overview of the results for the ICRS II Overall Assessment score at Week 104 is presented in Table 8. The mean ICRS II Overall Assessment score was comparable for the MACI and MF groups and there was no significant difference (p=0.717) between the treatment groups. The results of the Full Analysis set were confirmed by the PP set. A greater Overall Assessment score at Week 104, in favour of patients treated with MF, was seen for subgroups with the lesion located at the trochlea, lesion aetiology of osteochondritis dissecans and no prior cartilage repair surgery. A greater Overall Assessment score, in favour of patients treated with MACI, was seen for subgroups with >1 prior cartilage repair surgery.

Table 16 Histology – Microscopic ICRS II overall assessment at Week 104: Full Analysis Set

	MACI N=72	Microfracture N=72
n	60	56
Mean (SD)	64.3 (22.34)	64.5 (22.78)
Median	75.0	70.8
Min, Max	0, 95	7, 97
LS Means	63.82	62.31
Difference (LS Means)	1.52	
P-Values		
Treatment	0.717	
Centre	0.108	

Overall assessment refers to the overall quality of the repair tissue on a scale from 0 (fibrous tissue) to 100 (articular cartilage). ICRS=International Cartilage Repair Society; LS=least-squares; Max=maximum; Min=minimum; SD=standard deviation.

Imaging evaluation of structural repair

Of the 144 randomised patients, 134 underwent MRI evaluation at Week 52 and 139 at Week 104. There were no apparent differences between the groups in nonparticipation in MRI evaluation at Weeks 52 or 104; the 134 patients at Week 52 included 69 MACI group patients and 65 MF group patients, whilst the 139 patients at Week 104 included 70 MACI group patients and 69 MF group patients. An overview of the results for MRI Degree of Defect Fill is presented in Table 9. Inferential analyses were not completed for MRI parameters other than Degree of Defect Fill. At Week 104, improvement since study treatment in defect fill was evident for patients in both treatment groups; the defects were filled to more than 50% for the majority of patients and the proportion of patients with >75% defect fill was comparable between patients treated with MACI or MF. There was no significant difference between the treatment groups in MRI Degree of Defect Fill at Week 52 or Week 104. The results of the Full Analysis set were confirmed by the PP set.

Pre-specified exploratory analyses for MRI Degree of Defect Fill by total defect size (>4 cm^2 and >5 cm^2), index lesion size (>4 cm^2, >5 cm^2), lesion location, aetiology, prior surgical history of target knee, prior cartilage repair surgical history, site and gender and other MRI Qualitative Parameters did not show any notable differences in treatment groups.

Table 17 MRI Degree of Defect Fill: Full Analysis Set

n (%)	MACI N=72	Microfracture N=72	P-Value [a]
Visit 8 (Week 52)			
76 to 100%	35 (48.6)	40 (55.6)	0.744
51 to 75%	20 (27.8)	11 (15.3)	
26 to 50%	7 (9.7)	5 (6.9)	
0 to 25%	7 (9.7)	9 (12.5)	
Measure of agreement	Weighted kappa	0.604	
	95% CI	0.459, 0.748	
Visit 10 (Week 104)			
76 to 100%	35 (48.6)	41 (56.9)	0.920
51 to 75%	23 (31.9)	12 (16.7)	
26 to 50%	4 (5.6)	7 (9.7)	
0 to 25%	8 (11.1)	9 (12.5)	
Measure of agreement	Weighted kappa	0.571	
	95% CI	0.421, 0.722	

[a] P-value: calculated for MRI degree of defect fill intervals, using a CMH χ^2 Test: Row Means Score Differ

(α=0.05) to compare between treatment groups. Note: MRI as assessed by the independent blinded evaluators by means of consensus. Degree of Defect Fill is a measure of the completeness of defect repair produced by the graft. CI=confidence interval; CMH=Cochran-Mantel-Haenszel.

Response rate based on KOOS Pain and Function scores

An overview of the KOOS Pain and Function (SRA) response rate results is presented in Table 10. The percentage of patients who responded to treatment at Week 104 (had at least a 10-point improvement from Baseline in both Pain and Function [SRA]) was significantly greater (p=0.016) for patients in the MACI group compared to the MF group. The results of the Full Analysis set were confirmed by the PP set. The results were also confirmed by the unstratified analysis (p=0.011). Exploratory analyses for Pain and Function (SRA) response rates by total defect size (>4 cm^2 and >5 cm^2), index lesion size (>4 cm^2, >5 cm^2), lesion location, aetiology, prior surgical history of target knee, prior cartilage repair surgical history and gender were also evaluated. A greater response rate, in favour of patients treated with MACI, was seen for subgroups with >1 prior cartilage repair surgery.

Table 18 KOOS response rate: Full Analysis Set

n (%)	MACI N=72	Microfracture N=72	p-value [a]
Visit 10 (Week 104) Stratified by centre			
Responded	63 (87.50)	49 (68.06)	0.016
Not Responded	9 (12.50)	20 (27.78)	
Missing	0	3 (4.17)	
Visit 10 (Week 104) Unstratified			
Responded	62 (86.11)	48 (66.67)	0.011
Not Responded	7 (9.72)	18 (25.00)	
Missing	3 (4.17)	6 (8.33)	

a P-value: calculated for response categories 'Responded' and 'Not responded' using a CMH χ^2 Test (α=0.05) to compare between treatment groups.
KOOS Response Rate: a patient is regarded as a responder for KOOS if a 10-point improvement in both KOOS Pain and Function (SRA) scores were achieved with respect to Baseline. Otherwise, the patient is regarded as a non-responder. CMH=Cochran-Mantel-Haenszel; KOOS=Knee Injury and Osteoarthritis Outcome Score

Treatment failure rate at Week 104

The planned analyses concerning treatment failure rates and treatment group differences were not possible due to the small number of per protocol treatment failure cases. Five patients were referred as having a treatment failure. Of the 5 patients, 4 were from the MF group and 1 was from the MACI group. Two of the 4 patients from the MF group were considered to be a per protocol treatment failures. The 1 patient from the MACI group was not considered to be a per protocol treatment failure. No patients treated with MACI prematurely discontinued study participation due to lack of efficacy, while this occurred for 3 patients treated with MF.

Change from baseline at Week 104 in remaining subscales of KOOS

From baseline to Week 104, improvement in ratings of ADL, QOL and Other Symptoms was reported for patients in both treatment groups, while the improvements for all 3 KOOS subscales were significantly greater for patients in the MACI group compared to the MF group (Table 11). Across all 3 subscales, the change from baseline to Week 104 was >25 points for both treatment groups, however the changes in ADL (p<0.001), QOL (p=0.029) and Other Symptoms (p<0.001) were superior for patients treated with MACI. The results of the Full Analysis set were confirmed by the PP set for ADL and Other Symptoms, whilst a trend towards confirmation was shown for QOL (p=0.074). The results of the LOCF analysis (Full Analysis and PP sets) were confirmed by the MI analysis results.

Exploratory analyses for ADL, QOL and Other symptoms by total defect size (>4 cm^2 and >5 cm^2), index lesion size (>4 cm^2, >5 cm^2), lesion location, aetiology, prior surgical history of target knee, prior cartilage repair surgical history, site and gender was also evaluated. A greater change from baseline at Week 104 in ADL, in favour of patients treated with MACI, was seen for subgroups with the lesion located at the trochlea or LFC. A greater change from baseline at Week 104 in ADL and QOL and Other Symptoms, in favour of patients treated with MACI, was seen for subgroups with >1 prior cartilage repair surgery.

Table 19 Other KOOS subscales - LOCF: Full Analysis Set

	MACI N=72	Microfracture N=72
Activities of Daily Living		
Baseline (n)	72	72
Mean (SD)	43.51 (18.15)	42.57 (19.55)
Visit 10 (Week 104) (n)	72	71
Mean (SD)	87.21 (16.47)	75.75 (24.21)
Change From Baseline to Week 104 (n)	72	71
Mean (SD)	43.70 (24.52)	32.76 (26.78)
LS Means	42.39	30.38
Difference (LS Means)	12.01	
P-Values		
Treatment	<0.001	
Centre	0.011	
Baseline Value	<0.001	
Quality of Life		
Baseline (n)	72	72
Mean (SD)	18.75 (14.65)	17.19 (14.06)
Visit 10 (Week 104) (n)	72	71
Mean (SD)	56.16 (23.91)	47.27 (26.99)
Change From Baseline to Week 104 (n)	72	71
Mean (SD)	37.41 (27.24)	29.93 (28.11)
LS Means	38.00	29.03
Difference (LS Means)	8.98	
P-Values		
Treatment	0.029	
Centre	0.024	
Baseline Value	<0.001	
Other Symptoms		
Baseline (n)	72	72
Mean (SD)	48.26 (16.85)	44.39 (18.58)
Visit 10 (Week 104) (n)	72	71
Mean (SD)	83.73 (13.98)	72.23 (19.47)
Change From Baseline to Week 104 (n)	72	71
Mean (SD)	35.47 (20.83)	27.31 (24.59)
LS Means	34.37	22.76
Difference (LS Means)	11.61	
P-Values		
Treatment	<0.001	
Centre	0.002	
Baseline Value	<0.001	

Baseline: the last non-missing value collected prior to study treatment at Day 1.
P-value: analysis of covariance conducted with treatment and centre as fixed effects and Baseline subscale as covariate, conducted at α=0.05 level of significance.
LOCF=last observation carried forward; LS=least-squares; KOOS=Knee Injury and Osteoarthritis Outcome Score; SD=standard deviation

Tertiary efficacy endpoints

Tertiary KOOS endpoints

Change from baseline in all subscales of KOOS

For patients in both treatment groups, improvement from baseline was seen by Week 24 and through Week 78. At Week 24, change from baseline was significantly greater (p=0.031) for patients in the MACI group compared to the MF group in Other Symptoms and a trend towards significantly greater (p=0.078) improvement from baseline was seen for ADL. There was no significant difference between treatment groups for Pain and Function (SRA) or QOL. At Week 36, change from baseline was significantly greater (p<0.030) for patients in the MACI group compared to the MF group in Pain and Function (SRA), ADL and Other Symptoms; there was no significant difference between treatment groups for QOL. At Weeks 52 and 78, change from baseline was significantly greater (p<0.025) across all KOOS subscales for patients in the MACI group compared to the MF group. The Full Analysis set results for Pain and Function (SRA) were confirmed by the PP set; PP set analysis was not performed for the other KOOS subscales. The LOCF analysis results for all subscales were confirmed by the MI analysis results.

Response rate based on KOOS Pain and Function scores

Response rates were not significantly different between the treatment groups at Weeks 24 to 78. A trend towards significantly better (p=0.098) response rates favouring patients treated with MACI (n=63, 87.50%) compared with MF (n=53, 73.61%) was evident at Week 78. These results were confirmed by the results of the analysis not stratified by centre.

Tertiary treatment failure endpoints

The planned analyses concerning treatment failure rates and treatment group differences were not relevant due to the small number of treatment failure cases.

Tertiary patient-reported outcome endpoints

An overview of the tertiary endpoints associated with the Modified Cincinnati Knee Rating System, IKDC Subjective Knee Evaluation Form, SF-12 Acute Version 2.0 and EQ-5D VAS is shown in Table 12.

Table 20: Tertiary patient-reported outcome measure parameters: Full Analysis Set

	MACI N=72		Microfracture N=72	
Modified Cincinnati Knee Rating System	**n**	**Mean (SD)**	**n**	**Mean (SD)**
Baseline	72	3.0 (1.2)	72	3.0 (1.2)
Visit 10 (Week 104)	72	6.4 (2.1)	71	5.4 (2.2)
Change From Baseline to Week 104	72	3.4 (2.4)	71	2.4 (2.3)
LS Means		3.41		2.36
Difference (LS Means)		1.05		
P-Values [a]				
Treatment		0.002		
Centre		0.005		
Baseline Value		<0.001		
IKDC Subjective Knee Evaluation	**n**	**Mean (SD)**	**n**	**Mean (SD)**
Baseline	71	32.87 (13.28)	72	29.29 (13.43)
Visit 10 (Week 104)	72	65.74 (18.52)	71	58.84 (22.28)
Change From Baseline to Week 104	71	32.94 (21.87)	71	29.31 (23.81)
LS Means		32.65		26.71
Difference (LS Means)		5.94		
P-Values [a]				
Treatment		0.069		
Centre		0.004		
Baseline Value		<0.001		
SF-12 Physical Component Score	**n**	**Mean (SD)**	**n**	**Mean (SD)**
Baseline	72	-1.77 (0.86)	69	-1.93 (0.82)
Visit 10 (Week 104)	72	-0.32 (0.89)	71	-0.82 (1.12)
Change From Baseline to Week 104	72	1.45 (1.20)	68	1.06 (1.21)
LS Means		1.53		1.02
Difference (LS Means)		0.51		
P-Values [a]				
Treatment		0.001		
Centre		<0.001		
Baseline Value		<0.001		
SF-12 Mental Component Score	**n**	**Mean (SD)**	**n**	**Mean (SD)**
Baseline	72	0.04 (1.16)	69	-0.17 (1.30)
Visit 10 (Week 104)	72	0.45 (0.87)	71	0.49 (1.00)
Change From Baseline to Week 104	72	0.41 (1.06)	68	0.63 (1.39)
LS Means		0.44		0.53
Difference (LS Means)		-0.09		
P-Values [a]				
Treatment		0.523		
Centre		0.275		
Baseline Value		<0.001		
EQ-5D Visual Analogue Scale Score	**n**	**Mean (SD)**	**n**	**Mean (SD)**
Baseline	72	60.8 (20.9)	72	56.2 (22.1)
Visit 10 (Week 104)	72	77.5 (15.3)	70	73.4 (18.4)
Change From Baseline to Week 104	72	16.7 (22.0)	70	16.7 (25.3)
LS Means		17.94		14.18
Difference (LS Means)		3.75		
P-Values [a]				
Treatment		0.148		
Centre		0.002		
Baseline Value		<0.001		

[a] $p<0.05$ using ANOVA conducted with treatment and centre as fixed effects and Baseline as covariate
ANOVA=analysis of variance; LS=least-squares; SD=standard deviation

Modified Cincinnati Knee rating system

From baseline to Week 104, an increase in ratings was reported for patients in both treatment groups, however, the mean improvement in knee function from baseline to Week 104 was significantly greater (p=0.002) for patients in the MACI group compared with the MF group. There was also a significant difference (p=0.018) between the treatment groups in favour of the MACI group for change from baseline at Week 52.

International Knee Documentation Committee Subjective Knee Evaluation

From baseline to Week 104, patients in both treatment groups had increases in their IKDC scores. A trend towards a significantly greater (p=0.069) mean improvement in knee function from baseline to Week 104 was shown for patients in the MACI group compared with the MF group. There was a significant difference (p=0.009) between the treatment groups in favour of the MACI group for change from baseline at Week 52.

12-Item Short-Form Health Survey

From baseline to Week 104, patients in both treatment groups had increases in their SF-12 physical and mental health scores. The mean improvement in physical health from baseline to Week 104 was significantly greater (p=0.001) for patients in the MACI group compared with the MF group. There was no difference (p=0.523) in improvement in mental health between the treatment groups from baseline to Week 104. At Week 52, there was also a significant difference (p=0.029) between the treatment groups in favour of the MACI group for change from baseline in physical health, but not for mental health (p=0.209).

EuroQOL 5-Dimensions Questionnaire

From baseline to Week 104, patients treated with MACI or MF had comparable increases in their EQ-5D VAS scores. No significant difference between the treatment groups for mean improvement from baseline in overall health status was seen at Week 104 (p=0.148) or Week 52 (p=0.335).

Macroscopic ICRS Cartilage Repair

An overview of the ICRS Cartilage Repair Assessment scores is presented in Table 13. The Macroscopic ICRS Cartilage Repair scores were comparable for patients treated with MACI or MF. There were no significant differences between the treatment groups at Week 104 for overall repair assessment, degree of defect repair, graft integration to border zones or macroscopic appearance. For the majority of patients in both treatment groups, overall repair assessment was Grade II and the defect repair was in line with surrounding native cartilage. More patients treated with MACI compared to MF were assessed as having the defect repair completely integrated to border zones and with an intact smooth surface, however the differences were not statistically significant.

Table 21 Macroscopic ICRS cartilage repair assessment scores: Full Analysis Set

n (%)	MACI N=72	Microfracture N=72	p-value [a]
Overall Repair Assessment			0.145
Grade I (Normal)	14 (19.44)	8 (11.11)	
Grade II (Nearly normal)	41 (56.94)	35 (48.61)	
Grade III (Abnormal)	4 (5.56)	12 (16.67)	
Grade IV (Severely abnormal)	5 (6.94)	4 (5.56)	
Missing	8 (11.11)	13 (18.06)	
Degree of Defect Repair			0.430
In Line With Surrounding Cartilage	45 (62.50)	38 (52.78)	
75% Repair of Defect Depth	10 (13.89)	9 (12.50)	
50% Repair of Defect Depth	4 (5.56)	7 (9.72)	
25% Repair of Defect Depth	4 (5.56)	3 (4.17)	
0% Repair of Defect Depth	1 (1.39)	2 (2.78)	
Missing	8 (11.11)	13 (18.06)	
Graft Integration to Border Zones			0.519
Complete Integration	21 (29.17)	15 (20.83)	
Demarcating Border <1 mm	20 (27.78)	20 (27.78)	
¾ Integrated, ¼ With Border >1 mm	14 (19.44)	13 (18.06)	
½ Integrated, ½ With Border >1 mm	3 (4.17)	7 (9.72)	
No Contact to ¼ Integrated	6 (8.33)	4 (5.56)	
Missing	8 (11.11)	13 (18.06)	
Macroscopic Appearance			0.164
Intact Smooth Surface	25 (34.72)	16 (22.22)	
Fibrillated Surface	21 (29.17)	22 (30.56)	
Small, Scattered Fissures	13 (18.06)	13 (18.06)	
Several Small or Few but Large Fissures	3 (4.17)	5 (6.94)	
Total Degeneration of Grafted Area	2 (2.78)	3 (4.17)	
Missing	8 (11.11)	13 (18.06)	

[a] P-value: calculated for subscale categories using a CMH $\chi 2$ Test: Row Means Score Differ ($p<0.05$) to compare between treatment groups CMH=Cochran-Mantel-Haenszel; ICRS=International Cartilage Repair Society

Exploratory Findings

At both Week 52 and Week 104, there were no significant associations found between the MRI and KOOS data. At Week 104, there were no significant associations found between the MRI and microscopic ICRS II histology data. At Week 104, a lack of association between the pain and function outcomes and the overall histology assessment score was observed.

Ancillary analyses

Subgroup analyses were performed for lesion defect size, as MF may be considered the treatment of choice for smaller lesions < 3-4 cm^2, whereas it is less optimal for larger lesions. The analyses indicated a trend that MACI was superior to MF, for both smaller and large lesions. See Tables 14 below.

Furthermore, concurrent knee surgery of the meniscus and anterior cruciate ligament may bias the primary outcomes of pain and function of the knee. This kind of surgery was performed in 1/3 of the study population. Subgroup analyses excluding these subjects with other knee surgery confirmed the overall conclusions i.e. that MACI was superior to MF regarding improvement in pain and function. See Table 15 below.

Age was not a significant factor in the pivotal study.

Table 22 Co-primary efficacy parameter by index lesion defect size - LOCF: Full Analysis Set

		MACI N=37		Microfracture N=41	
		Pain	Function (SRA)	Pain	Function (SRA)
Index Lesion Defect Size ≤ 4 cm²					
Baseline	n; Mean (SD)	37; 37.99 (14.58)	37; 16.22 (15.02)	40; 36.18 (10.81)	40; 13.25 (14.96)
Visit 10 (Week 104)	n; Mean (SD)	37; 80.48 (19.54)	37; 57.30 (29.41)	39; 66.56 (23.70)	39; 44.87 (30.77)
Change From Baseline to Week 104	n; Mean (SD)	37; 42.49 (22.42)	37; 41.08 (27.94)	38; 29.90 (21.31)	38; 30.92 (29.15)
Analysis Model					
LS Means		41.20	41.16	26.11	31.14
Difference (LS Means)		15.09	10.01		
P-Value					
Treatment		0.005			
Index Lesion Defect Size > 4 cm²					
		MACI N=35		Microfracture N=31	
		Pain	Function (SRA)	Pain	Function (SRA)
Baseline	n; Mean (SD)	35; 35.95 (12.42)	35; 13.43 (14.39)	31; 34.50 (13.70)	31; 11.69 (18.88)
Visit 10 (Week 104)	n; Mean (SD)	35; 84.52 (11.56)	35; 64.71 (25.95)	31; 76.25 (24.15)	31; 53.55 (29.56)
Change From Baseline to Week 104	n; Mean (SD)	35; 48.57 (19.39)	35; 51.29 (28.22)	31; 41.76 (25.61)	31; 41.85 (33.93)
Analysis Model					
LS Means		49.60	55.35	40.25	42.83
Difference (LS Means)		9.35	12.52		
P-Value					
Treatment		0.161			

Baseline: the last non-missing value collected prior to study treatment at Day 1.
P-value: multivariate analysis of covariance conducted with covariate adjustment for centre, age, baseline KOOS Pain, Function (SRA), conducted at α=0.05 level of significance using the Wilks'Lambda test statistic. LOCF=last observation carried forward; LS=least-squares; KOOS=Knee Injury and Osteoarthritis OutcomeScore; SD=standard deviation; SRA=Sports and Recreational Activities
CMH=Cochran-Mantel-Haenszel; KOOS=Knee Injury and Osteoarthritis Outcome Score

Table 23 KOOS response rates at Week 104 excluding patients with concurrent knee surgery: Full Analysis Set

n (%)	MACI N=46	Microfracture N=50	p-value [a]
Visit 10 (Week 104)			
Responded	41 (89.13)	29 (58.00)	0.002
Not Responded	5 (10.87)	18 (36.00)	
Missing	0	3 (6.00)	

[a] P-value: calculated for response categories 'Responded' and 'Not responded' using a CMH χ^2 Test (α=0.05) to compare between treatment groups. KOOS Response Rate: a patient is regarded as a responder for KOOS if a 10-point improvement in both KOOS Pain and Function (SRA) scores was achieved with respect to Baseline. Otherwise, the patient is regarded as a non-responder.
CMH=Cochran-Mantel-Haenszel; KOOS=Knee Injury and Osteoarthritis Outcome Score

Summary of main study

The following tables summarise the efficacy results from the main study supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 24 Summary of Efficacy for trial MACI 00206

Title: A prospective, randomized, open-label, parallel-group, multicenter study to demonstrate the superiority of matrix-induced autologous chondrocyte implantation (MACI implant) versus arthroscopic microfracture (MF) for the treatment of symptomatic articular cartilage defects of the femoral condyle including the trochlea			
Study identifier	MACI 00206 (SUMMIT)		
Design	Prospective, randomized, open-label, parallel-group, multicenter study		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		2 years not applicable 3 years
Hypothesis	Superiority		
Treatments groups	Test product		MACI, 2 years post treatment FU, 72 patients
	Active comparator		Microfracture , 2 years post treatment FU , 72 patients
Endpoints and definitions	Co-Primary endpoint	KOOS Pain and Function (SRA)	Change from Baseline to Week 104 for the patient's KOOS Pain and Function (Sports and Recreational Activities [SRA]) scores
	Secondary endpoint	Histology	Histological evaluation of structural repair of evaluable biopsies harvested from the core of the index lesion during arthroscopy at Week 104.
	Secondary endpoint	MRI	MRI assessments of structural repair parameters at Baseline, and at Weeks 52 and 104
Database lock	20-3-2012 completed		
Results and Analysis			

Analysis description	**Primary Analysis**		
Analysis population and time point description	Full analysis set 2years		
Descriptive statistics and estimate variability	Treatment group	MACI	MF
	Number of subject	72	72
	Co-Primary endpoint - Pain - Function Means At week 104	 82.45 60.9	 70.85 48.71
	SD	16.18 27.84	24.22 30.33

	Histology Overall score Week 104	64.3	64.5
	SD	22.34	22.78
	MRI Degree of defect fill 76-100% Week 104	35	41
	SD	48.6	58.9
Effect estimate per comparison	Co-Primary endpoint	Comparison groups	MACI vs MF
		Difference LS means - Pain - Function	 11.76 11.41
		P-value	0.001
	Secondary endpoint: Histology	Comparison groups	MACI vs MF
		Difference LS means	1.52
		P-value	0.717
	Secondary endpoint: MRI	Comparison groups	MACI vs MF
		Weighted kappa	0.571
		95% CI	0.421, 0.722
		P-value	0.920

Clinical studies in special populations

In current clinical practice with patients ≥ 65, MACI treatment is not commonly used. Disorders of the knee in patients ≥ 65 years are often associated with osteoarthritis and degenerative joint disease where use of MACI is not applicable. In the phase III SUMMIT trial, data for patients ≥ 65 years is not available. Only 7 patients in the age group 50-55 received MACI. Comprehensive data specific to elderly patients are not available in the supportive publications submitted. Overall, in the absence of data, the use of MACI in elderly ≥ 65 years with generalised degeneration of the cartilage or osteoarthritis is not recommended.

No data related to MACI treatment in skeletally mature patients younger than 18 years is currently available. MACI should not be used in adolescents/children. The applicant will conduct a retrospective investigation of safety and prospective investigation of safety and efficacy data in paediatric patients treated for cartilage defects with MACI. The study shall be completed by December 2017.

Supportive studies

In support of the pivotal SUMMIT trial the applicant submitted studies reported from the literature and few internal unpublished reports from academic centres. In total 19 articles from the literature and reports were submitted. In all studies patients with lesions of Outerbridge III/IV were selected for MACI. Initially, pending completion of the SUMMIT trial, the applicant submitted some of these reports as pivotal data to demonstrate the efficacy and safety of MACI. However, considering the methodological limitations of these studies (e.g. single-centre, small sample size) and uncertainties

regarding protocol compliance and monitoring, they can only be regarded as supportive of the pivotal SUMMIT trial.

Basad et al. *2010*

In this randomised, controlled single-centre study, 40 patients treated with MACI and 20 patients treated with MF were followed for 2 years. The study was conducted between 2000 and 2005 and included patients from 18 to 50 years of age with a single, symptomatic chondral defect of the knee. A greater proportion of study patients were males (71%) than females (29%); the difference in proportion of males was larger for the MF group than for the MACI group. The mean age for patients in the MACI group (33.00) was lower than for those in the MF group (37.50), although the difference was not statistically significant. The majority of patients in both groups had defects localised on the condyles (MACI: n=29 [73%], MF: n=16 [80%]). The mean size of treated lesions was not provided, however, per the study inclusion criteria, a single defect of 4 to 10 cm^2 was required. Patients randomised to the MF group were treated using an arthroscopic procedure. Patients in the MACI group were treated using the Genzyme type I/III collagen membrane. Two different manufacturing sites provided membranes used in this study which reflects a difference in manufacturing processes, but does not reflect a difference in the membrane. Mini-arthrotomy was performed for all implantations. Concomitant treatment was reported for an ACL lesion in 1 MF patient and smaller meniscal lesions in 2 MACI patients and 3 MF patients. All patients were required to follow a standardised post-operative rehabilitation programme that was specific to their study treatment.

The authors concluded that MACI treatment is superior to MF treatment for repair of symptomatic cartilage defects larger than 4 cm^2. Clinical outcome was assessed using the Lysholm, Tegner and ICRS scores. Patients in both treatment groups showed significant clinical improvement over time ($p<0.0001$) as assessed by all 3 outcome measures. However, MACI treatment was significantly more effective over time than MF treatment based on Lysholm ($p=0.005$), Tegner ($p=0.04$) and ICRS ($p=0.03$) scores.

The outcomes of the most relevant functional score is summarised below. Optimal response of Lysholm scores was observed at 12 months. From that point, the response was maintained for MACI till 24 months, but declined for MF. MACI was significantly more effective over time than MF ($p = 0.005$).

Table 25 Basad study - results of the main functional score

	Basad (Lysholm)		
	MACI	MF	p-value#
baseline	Mean 52 (SD26, median: 58) (n=39)	Mean 55(SD25, median 56) (n=17)	
12 m	Mean 92 (SD 11, median 95) (n=38)	Mean 82 SD22, median 90) (n=17)	
24 m	Mean 92 (SD 9m median 94) (n=33)	Mean 69 SD26, median 70) (n=15)	
Change (12 m)	40*	27	
Change (24 m)	40	14	p=0.005

Wood et al. *2006, Winalski et al. 2007 and Ebert* et al. *2011 (University of Western Australia)*

These are reporting results from an observational, non-controlled, open, single center study on 18 subjects. The results come from 2 reports (Wood *et al.* 2006, Winalski *et al.* 2007) and 1 publication (Ebert *et al.* 2011). The 2 reports present the results of a 2-year follow-up for the 18 patients treated between December 2002 and March 2004; one reporting the clinical data (Wood), the other the MRI-

data (Winalski). The publication (Ebert et al 2011) reports the total 5-year follow-up for some of these patients and for additional ones (at least 23) treated between August 2001 and November 2004.

In this study there were 18 patients (20 knees, 25 defects) who received MACI with the ACI-Maix membrane (with arthrotomy approach) for full-thickness chondral defects of the knee from December 2002 through March 2004 (Wood *et al.* 2006). Patients were predominantly male (61%) and had a mean age of 39 years at time of implant. Twenty-five defects were treated with the majority located in the condyles; a single lesion was treated in each of 15 knees and 2 lesions were treated in each of 5 knees. About half the patients had received a prior procedure on the treated knee and a majority had no concomitant procedures performed at the time of MACI implant. The treated lesions had a mean surface of 3.16 cm^2 (SD=3.77), with a range in size from 0.60 to 15.00 cm^2. The authors found that patients treated with MACI experienced significant clinical improvement including significant improvement in pain and knee function. The MRI data in this patient series demonstrated that the MACI implant technique resulted in growth of some amount of repair tissue (complete or incomplete) for 24 of the 25 graft sites (96%). The graft repair tissue completely covered the repair site for most grafts (80%) at the final available timepoint of 12 months (n=2) or 24 months (n=18). The volume of the repair tissue remained stable or increased in 15 of the 16 defects (94%) with more than 1 post-operative MRI timepoint. The independent review (Winalski *et al.* 2007) of the imaging data collected for the 18 patients (20 knees, 25 defects) to assess structural outcome concluded that the magnetic resonance images of the group of patients treated with MACI implant have shown growth and maintenance of repair tissue within the femoral articular cartilage defects that is comparable to that observed with Carticel. The pattern and incidence of graft failure appear similar as well. However, the occurrence of complications such as repair tissue hypertrophy and intra-articular adhesions appeared to be much less common.

Ebert et al reported the results of 5-year follow-up of 41 patients (44 knees, 53 defects) treated with MACI. The results reported are for the 35 patients with complete 5-year follow-up data. There was some overlap of patients from the 2-year Wood study. The majority of the defects were localised on the medial (n=22) or lateral (n=11) femoral condyles, while the remaining defects were localised on the patella (n=11) or trochlea (n=9). The treated lesions had a mean surface of 3.0 cm^2 with a range in size from 1.0 to 9.0 cm^2. The authors reported that the 5-year follow-up study demonstrated that MACI treatment resulted in functional tissue infill to 24 months that was maintained to 5 years. Patients treated with MACI reported improved clinical outcome over the 5 years following MACI treatment. Most improvement in knee pain, symptoms and ADL took place in the first year following surgery and most improvement in knee-related quality of life and sport and recreational activities took place in the first 2 years; improvement was maintained through 5 years post-treatment. Positive evidence of structural repair was demonstrated by MRI at 5 years post-treatment.

Marlovits et al. 2006 and 2010

It is an observational, non-controlled, open, single center study in 21 subjects. This includes one internal Investigator reports by Marlovits, of 2 years' follow-up data (2006) and an abstract from a presentation of a congress by the same author, briefly discussing of 5 years' follow-up data of these subjects (2010).

In this study, the patient population (n=21) was composed of 86% males (n=18) and 14% females (n=3) with a mean age of 35.2 years (SD=7.37; minimum=20.1; maximum=47.8). A total of 18 patients were affected by chondral defects of the knee caused by trauma, while in 3 patients the lesion was not directly correlated by the patient to a specific traumatic event. Sixteen (76%) patients had undergone previous surgical procedures to the affected knee, such as meniscal and/or ligament surgery, patellofemoral surgery and osteotomy. Five (23%) of these patients had already been subjected to procedures aimed at cartilage repair, including debridement and bone marrow stimulation

techniques, which proved unsuccessful. The vast majority of patients had defects localised to the femoral condyles (76%, n=16), while the remaining 24% (n=5) patients had defects localised to the patella. The treated lesions (n=24) had a mean surface area of 5.1 cm^2 (SD=2.1). Patients in this study were treated with the MACI implant using either the Chondro-Gide or ACI-Maix membrane. The authors concluded that MACI is a successful treatment option for patients with cartilage lesions of the knee based on improved clinical and structural outcomes observed and the absence of any related adverse events reported. Clinical outcome measures included in this study were the IKDC, Lysholm-Gillquist, a VAS of knee pain (scale from 0 to 10 with 0 representing no pain and 10 severest pain), KOOS, Brittberg, Tegner-Lysholm and Noyes Sports Activity. Overall, knee ratings significantly improved up to 2 years following treatment based on the IKDC ($p<0.001$) and Lysholm-Gillquist ($p<0.001$) measures. Significant improvement in knee pain over time was reported using the VAS ($p<0.001$). All 5 subscales of the KOOS significantly improved over the 2-year follow-up period ($p\leq 0.005$). Knee symptoms also significantly improved over time as measured by the IKDC ($p<0.001$). Patient rating of knee function, as assessed by the Brittberg score, showed significant improvement over the study period ($p<0.001$); 18 patients (86%) reported improved condition at 2 years compared to baseline. Participation in sports activity was assessed using the Tegner-Lysholm and Noyes score. Significant improvement in mean activity level over time was reported using the Tegner-Lysholm ($p<0.001$); assessment of sports activity level as assessed by the Noyes score provided consistent results ($p\leq 0.002$). Positive evidence of structural repair was demonstrated by MRI at 2 years post-treatment.

In the 5 years follow-up of 21 patients (poster 2010) the authors reported that MACI treatment resulted in clinically and statistically significant improvement through 5 years post-treatment. The clinical outcome measures included in this study were the IKDC, Lysholm-Gillquist and the KOOS. Significant clinical improvement ($p<0.05$) over time was seen as assessed by each of the measures. MRI assessment at 5 years showed complete filling for 83% of defects grafted. Scoring of the MRI data revealed a MOCART score of 75.7 (SD=18.0) on a scale with a maximum MOCART score of 100.

The following additional supportive studies have been provided:

Three comparative, randomised studies with 1- to 9-year follow-up

Bachmann et al. 2004

This randomised, controlled, single-centre study included 34 patients (MACI=27, MF=7) 18 to 50 years of age treated for a circumscribed cartilage defect of the knee (34 grafts in total) and followed for 2 years post-treatment. The most frequent defect location treated in both groups was the medial condyle. The average defect size was similar for the 2 treatment groups. The patients randomised to MF were treated using an arthroscopic procedure. Patients in the MACI group were treated using a type I/III porcine collagen membrane. Based on the MRI results the authors concluded that MACI is superior to MF in terms of defect fill and signal intensity of repair tissue compared to surrounding cartilage. Per the clinical results the authors concluded that MACI showed better outcome compared to MF. For those treated with MACI, the assessment of defect fill revealed an increase in the thickness of regenerative cartilage tissue over time to 24 months post-treatment and equalisation with the surrounding cartilage level. The defect fill seen following MF treatment was variable.

Bartlett et al. 2005

A randomised, controlled, single-centre study was carried out to compare MACI treatment versus CACI treatment (similar to ACI technique but uses a type I/III collagen membrane rather than a periosteal

flap to cover the defect) for repair of isolated osteochondral defects of the knee, larger than 1 cm^2, in patients 15 to 50 years of age. Ninety one patients had been randomised to MACI (n=47) or CACI (n=44) treatment. The study results presented include 1-year follow-up; no end date for the randomised component of the study was provided. There were 53 grafts provided to patients in the MACI group and 59 grafts provided to patients in the CACI group. Mean defect size was 6.1 (range: 1.0 to 22.0) cm^2 for the MACI group and 6.0 (range: 1.5 to 16.0) cm^2 for the CACI group. The authors reported that both MACI and CACI treatment led to significant improvements in clinical and functional outcome and second-look arthroscopy and histology findings were similar between the treatment groups. Clinical outcome was assessed using the modified Cincinnati knee score, the Stanmore rating scale, VAS (scale from 0 to 10 with lower scores representing better function), second-look arthroscopy and repair tissue histology. Significant improvements in pain and function from pre-treatment to 1 year post-treatment were seen for patients in both treatment groups; there were no significant differences in clinical improvement between the 2 groups. Arthroscopy was routinely scheduled for all patients at 1 year post-treatment and was completed for 18 patients in the MACI group and 24 patients in the CACI group; no information was provided to explain why biopsies were not completed for all patients. Of these patients, 67% of patients in the MACI group and 79% of patients in the CACI group had grafts macroscopically rated as excellent or good; the difference between the groups was not statistically significant. Diagnostic histology assessment at 1 year post-treatment was completed for 11 patients in the MACI group and 14 patients in the CACI group; no information was provided to explain why diagnostic histology was not completed for all patient biopsies. Hyaline-like cartilage or mixed hyaline-like and fibrocartilage was seen in 36% of MACI patients and 43% of CACI patients; the difference between the treatment groups was not significant.

Gikas et al. 2009

This was a single-centre, non-randomised cohort study. There were 231 patients treated with MACI and 101 patients treated with ACI/CACI. Grafting of dual defects was provided for 9 MACI patients and 3 ACI/CACI patients. The mean defect size for patients in the MACI group (4.2 cm^2, range: 1.0 to 12.2) was larger than for patients treated with ACI/CACI (3.5 cm^2, range: 1.0 to 7.0), but the difference was not statistically significant. Principal reasons for treatment were trauma, OCD and chondromalacia patellae. The majority of patients in both groups had a history of previous surgical treatment. An arthrotomy approach was used for all grafting procedures; a type I/III collagen membrane was used for the CACI and MACI grafts. All patients followed a structured rehabilitation programme after treatment. According to the authors, both MACI and ACI/CACI showed significant clinical improvement following treatment that was maintained over time. For some measures of clinical outcome, ACI/CACI was significantly better than MACI at 1 year post-treatment, but there were no significant differences between the techniques in subsequent years. Histological assessment provided evidence of hyaline-like or mixed hyaline and fibrocartilage in almost half of the biopsies and, consistent with the idea that repair quality improves over time, later biopsies were more likely to show hyaline-like tissue. Clinical outcome was assessed using the modified Cincinnati knee score, VAS (scale from 0 to 10 with lower scores representing less pain), the Bentley score, patient functional outcome score, Lysholm-Gillquist score, the Brittberg score and histology. Significant clinical improvements over time ($p<0.0001$) following MACI and ACI/CACI treatment were demonstrated by the scores for all the clinical outcome measures.

One comparative, non-randomised study with mean follow-up of more than 3 years:

Salzmann et al. 2009

This non-randomised, single-centre study included 18 patients (MACI=9, osteochondral autologous transplantation [OAT]=7) treated for symptomatic cartilage lesions of the knee. The average defect

size was larger in the MACI group (6.3 cm^2; range: 3.0 to 12.0 cm^2) compared to the OAT group (2.3 cm^2; range: 0.9 to 2.6 cm^2). The patients treated with MACI had defects classified as ICRS Grade III or IV and received MACI by an open approach using a type I/III porcine collagen membrane (MACI Verigen). Patients treated with OAT had defects classified as ICRS IVa or IVb and received treatment by an open approach. Based on the MRI results the authors concluded that MACI and OAT result in different ultrastructural outcome. Per the clinical results the authors concluded that MACI showed consistently better outcome compared to OAT. Structural outcome was assessed using MRI. A comparison of the T2 values of healthy femoral cartilage was similar between the groups, but the T2 values of the repair tissue following MACI (46.8 ms, SD=8.6) was significantly lower (p=0.048) compared to that after OAT (55.5 ms, SD=6.7). For those treated with MACI, the repair tissue values were significantly lower (p=0.046) compared to healthy cartilage (52.5, SD=7.9) while for those treated with OAT, the repair tissue was significantly higher (p=0.041) compared to healthy cartilage (49.9, SD=5.1). There were no significant differences between the groups in terms of the MOCART score. Clinical outcome was consistently higher following MACI compared with OAT. Statistical significance was only reached for the Lysholm-Gillquist measure (p=0.04).

Eight non-comparative studies with up to 5 years of patient follow-up:

Anders et al. 2008

In this study, 50 patients (58 defects) were treated with the MACI implant for Grade III or IV focal cartilage lesions of the knee and followed for an average of 24 (range: 21 to 29) months. The majority of the defects were located on the medial condyle and the average defect size was 4.1 (range: 1.6 to 6.1) cm^2. The MACI implant, using a type I/III porcine collagen membrane (MACI-Genzyme), was performed by arthrotomy. The authors concluded that MACI leads to a significant increase in function, excellent pain reduction and high levels of patient satisfaction. For patients overall, significant clinical improvement (p<0.01) was observed with all outcome measures: Lysholm-Gillquist score improved from 57.3 to 87.4, DGKKT improved from 55.3 to 85.5, VAS assessment of pain improved from 5.5 to 2.1 and VAS assessment of function improved from 4.5 to 7.6. An arthroscopic assessment of 11 patients (12 grafts) was completed at 3 to 38 months post-treatment due to patient symptoms. Biopsies of the 12 grafts revealed primarily hyaline tissue for 2 grafts, mixed hyaline/fibrocartilaginous tissue in 4 grafts, primarily fibrocartilaginous tissue in 5 grafts and fibrocartilaginous tissue in 1 graft. Assessment of defect fill by MRI at an average of 19.8 (range: 16 to 28) months post-treatment indicated that defect restoration of at least 75% was present in 14 of 21 (67%) patients with degenerative cartilage damage, 13 of 19 (68%) patients with traumatic cartilage damage and 8 of 10 (80%) patients with OCD.

Behrens et al. 2006

In this study 38 patients were treated with MACI for localised Grade III or IV cartilage defects and then followed up to 5 years post-treatment. The mean follow-up was 34.5 (range: 6 to 60) months. The mean age was 35 (range: 18 to 58) years. Defects were most frequently located on the medial condyle (n=16, 42%); 9 (24%) patients had multiple defects. The average defect size was 4.08 (range: 0.64 to 17.75) cm^2; for the majority of patients the cause of the defect was unknown. Of the 38 patients, 25 (66%) had a history of previous surgical treatment for the knee. All defects were treated with MACI using the Chondro-Gide membrane and an arthrotomy approach. No description of post-operative rehabilitation was provided. The 5-year follow-up results reported below are for 11 of the 38 patients.

Clinical outcome was assessed using patient subjective rating of knee function, the Meyer score, the Lysholm-Gillquist score, the Tegner-Lysholm score, the ICRS score and the IKDC score. Second-look arthroscopy for diagnostic reasons was completed for 6 patients; a biopsy for histological assessment

was obtained from 4 of the patients. At 5 years post-treatment, 8 of 11 patients rated their knee function as better or much better compared to before MACI treatment. Significant clinical improvement from pre-treatment to 5 years post-treatment was shown by the Meyer (p=0.007), Lysholm-Gillquist (p=0.04) and ICRS (p=0.03) scores. The Tegner-Lysholm score was improved at the 5-year follow-up, but the difference from pre-treatment level was not statistically significant (p=0.41). Analyses were completed to assess whether the improvements seen were influenced by age, sex, defect location, defect size and history of previous operation on the knee; the analyses demonstrated that the clinical improvements observed were independent of these factors. Clinical improvement of the treated knee was also demonstrated by the shift in patient IKDC ratings from pre-treatment (normal=41%, nearly normal=29%) to 5-year follow-up (normal=73%, nearly normal=9%). The ICRS Histological Assessment Scale was used to evaluate the 4 patient biopsies which were collected at least 1 year following treatment. None of the biopsies showed evidence of hyaline cartilage. The regenerative tissue appeared smooth and continuous for 3 of the biopsies and irregular and discontinuous in 1 biopsy. The authors stated that there appeared to be no correlation between the histology and clinical results for the 4 patients.

Cherubino et al. 2003

In this study, there were 13 patients treated with the MACI implant for deep cartilage defects; 11 patients were treated for a knee lesion while 2 patients were treated for an ankle lesion. The mean age of the patients was 35 (range: 18 to 49) years; there were 9 male patients and 4 female patients. The location of the defects was on the medial femoral condyle for 8 patients, the lateral femoral condyle for 2 patients, the femoral trochlea for 1 patient and the talar dome for 2 patients. The average defect size was 3.5 (range: 2.0 to 4.5) cm^2. For 6 of the patients the lesion was due to OCD and for the remaining 7 patients the lesion was due to trauma. Of the 13 patients, 5 had a history of previous surgical treatment of the defect. The MACI implantation procedures used an arthrotomy approach and the implant included a type I/III collagen bilayer membrane (MACI Verigen). Clinical outcome measures included in this study were ICRS score, modified Cincinnati knee score, Lysholm-Gillquist score and Tegner-Lysholm score. Structural outcome was assessed with MRI. Clinical improvement was demonstrated using the ICRS evaluation form. Pre-treatment assessment showed 1 patient as abnormal and the remaining 5 patients as severely abnormal. At the last patient follow-up, 2 patients were rated as nearly normal and 4 patients were rated as normal. Clinical improvements were also shown by the change in score from pre- to post-treatment for the other clinical outcome measures. No results of statistical analysis of the clinical outcome scores were reported. At 6 and 12 months post-treatment, MRI assessment was completed. For all patients, cartilage restoration of the defects with evidence of hyaline-like tissue was shown.

D'Anchise et al. 2005

Starting in September 2000, 56 consecutive patients with chondral defects of the knee were treated with MACI. Of these patients, 35 patients (36 knees, 43 defects) with an average age of 33.1 (range: 18 to 51) years had at least 6-month follow-up data and were followed up to 24 months post-treatment. The average follow-up of the 35 patients was 22 (range: 6 to 39) months. The average defect size was 4.0 cm^2, although some defects were greater than 12 cm^2. The majority of defects were located on the medial femoral condyle and were caused by trauma. All MACI implants included a type I/III collagen membrane (MACI Verigen) and were placed into the defect using an arthrotomy approach. The measures of clinical outcome included VAS pain (scale from 0 to 10 with lower scores representing less pain), well-being, functional state, symptoms in activity, IKDC, Lysholm-Gillquist and Tegner-Lysholm. Biopsies for histological assessment were available for 3 patients who underwent second-look arthroscopies; the second-look arthroscopies were not completed due to clinical or functional reasons. Significant improvements in clinical outcome from baseline were shown by the VAS

pain (by 1 month post-treatment, p<0.0001), well-being (by 3 months post-treatment, p<0.0001), functional state (by 3 months post-treatment, p<0.0001), IKDC (by 6 months post-treatment, p<0.0001) and Lysholm-Gillquist (by 6 months post-treatment, p<0.0001). The histology assessment of the 3 biopsies (1 at 1 year post-treatment and the other 2 at 2 years post-treatment) showed regeneration of hyaline cartilage.

Ebert et al. 2008

This study included 62 patients treated with MACI for localised, full-thickness cartilage defects of the medial or lateral femoral condyle of the knee; the patients were randomised to either a traditional or an accelerated post-treatment rehabilitation programme. The patients were followed for 3 months post-treatment in this study; results of 2-year follow-up post-treatment for this cohort were reported in 2 additional publications: structural outcome in Ebert, 2010, Cartilage and clinical outcome in Ebert, 2011, Cartilage. Of the 62 patients in this study, 40 were male and 22 were female; patients were between 16 and 62 years of age. Defects ranged in size from 0.65 to 10.00 cm2. All patients were treated with a MACI implant that included an ACI-Maix membrane. No information was provided about the surgical procedure used for MACI implantation. Measures of clinical outcome included KOOS, SF-36, VAS (pain frequency and severity on a scale from 0 to 10 with lower scores representing worse pain), knee flexion and extension, 3-repetition straight leg raise, 6-minute walk test and patient activity level. Patients overall showed significant clinical improvement (p<0.05) from pre-treatment to 3 months post-treatment for KOOS Other Symptoms, VAS pain frequency and pain severity and SF-36 mental component score. Significant decline (p<0.05) was seen for KOOS Function in Sports and Recreation and SF-36 physical component score. Patients Ebert, 2010, *Cartilage* (this publication reports 2-year clinical outcome follow-up) in the accelerated group only showed significant improvement over time (p<0.05) in KOOS Pain, 6-minute walk test and activity level.

Ebert et al. 2011

Structural outcome at 2 years post-treatment was reported for the 62 patients described above and an additional 8 patients. As described previously, all patients had been treated with MACI using the ACI-Maix membrane and randomised to 1 of 2 possible post-treatment rehabilitation programmes. Defects ranged in size from 0.65 to 10.00 cm^2. The results showed that the MRI composite score for patients overall significantly (p<0.0001) improved over time from 3 to 24 months post-treatment; there was no significant group or interaction effect observed for the MRI composite score over time. A significant change over time was seen for signal intensity, graft infill, subchondral lamina, subchondral bone and effusion; no significant group or interaction effects were observed.

Marlovits et al. 2004

The 24-month follow-up results of a pilot study to assess the use of MACI treatment for Grade III or IV (ICRS criteria) cartilage defects of the medial (n=10) or lateral (n=6) femoral condyle were reported for 16 patients (male=15, female=1). The average age of patients was 33.1 (range: 20.1 to 44.3) years. Defects ranged from 2.6 to 10.9 (mean=4.7) cm^2. All patients received the MACI implant that included a type I/III collagen bilayer membrane (MACI Verigen) and using an arthrotomy approach. Patients followed a standard post-operative rehabilitation protocol. Assessment of clinical outcome included KOOS, IKDC, Lysholm-Gillquist, Marshall, ICRS and Cincinnati scores. Results of statistical analysis of changes in scores over time were not reported. Pain and crepitus decreased from pre-treatment to 6 months post-treatment. Although a return to pre-injury sports level was not complete, all patients showed improvement in most clinical outcome scores at 12 months post-operative. The Lysholm-Gillquist score rose from 49.8 (SD=3.34) at pre-treatment to 94.0 (SD=2.23) at the 12-month follow-up. Improvements in KOOS (0 to 100 possible for each subscale) from pre-treatment to the 24-month follow-up were as follows: Function in Sports and Recreation (11.5 [SD=13.1] to 71.2

[SD=31.7]), QOL (31.6 [SD=13.9] to 76.2 [SD=3.1]), Pain (38.4 [SD=11.2] to 88.5 [SD=8.2]), Other Symptoms (53.4 [SD=13.1] to 87.3 [SD=9.4]) and Activities of Daily Living (52.6 [SD=13.4] to 92.0 [SD=6.7]).

Zhang et al. 2006

Over the period from December 2004 to August 2005, 3 patients were treated with the MACI implant for deep cartilage lesions of the knee due to OCD; the specific locations of the defects in the knee were not provided. Of the 3 patients, 2 had a history of previous surgical treatment of the knee. The patients, all males, ranged from 15 to 32 (mean=24) years of age and the defect size ranged from 6.0 to 10.5 (mean=8.0) cm^2. The MACI implant used for the 3 patients included a type I/III collagen membrane (MACI Verigen). An arthrotomy approach was used for MACI implantation. A structured rehabilitation programme was provided to the patients. Clinical outcome was assessed using the IKDC knee score, second-look arthroscopy and histology. Structural repair was evaluated using MRI. The follow-up assessment with MRI and arthroscopy were routinely scheduled at approximately 6 months post-treatment. Clinical and functional improvement was shown as the mean IKDC knee score (0 to 100 possible) rose from 36.0 pre-treatment to 86.2 at 10 months post-treatment; results of statistical analysis of score change over time were not reported. Second-look arthroscopy revealed well-integrated and close to full restoration of defects with cartilage comparable to healthy cartilage in colour, lustre and hardness. Evidence of hyaline-like tissue was demonstrated by MRI at 6 months post-operative. Biopsy of 1 patient was completed approximately 18 months post-operative for histological assessment (not due to clinical or functional reasons) and showed evidence of hyaline-like cartilage.

Zheng et al. 2007

In this study, 56 patients were treated for a Grade IV (Outerbridge) focal cartilage defect with MACI; the results presented pertain to the 11 patients who consented to biopsy of their repair cartilage and underwent biopsy of the graft for histological assessment of the repair tissue. There were 6 female patients and 5 male patients; the average age was 39.9 (range: 20 to 51) years. The defects had an average size of 5.32 (range: 1 to 14) cm2 and were all located on the medial femoral condyle except for a single defect on the patella. All patients had a history of 3 or more previous surgical knee treatments. The patients' MACI implant included the ACI-Maix membrane and implantation was completed using an arthrotomy approach. All patients followed a post-operative graduated rehabilitation programme. Clinical outcome of MACI treatment was assessed by histological evaluation. Cartilage biopsies were completed from 48 hours to 24 months post-operative. The reasons for the biopsy procedures were as follows: 'recall of the procedure' (48 hours post-operative), 'post-operative infection' (21 days post-operative), 'worker's compensation patient who complained of no improvement' (6 months post-operative), assessment to 'acquire justification on the performance of maximum physical activity' (8 and 12 months post-operative), 'other surgical procedures to the joint were required' (12 and 18 months post-operative), 'harvested from a patient who died unexpectedly by unrelated means' (18 months post-operative) and due to 'second MACI procedure was introduced for other defects' (24 months post-operative). Cartilage-like tissue was seen at 21 days post-operative and hyaline-like cartilage was evident from 6 months post-treatment except for 2 of 4 biopsies at 12 months post-treatment that showed fibrocartilage or mixed hyaline-like and fibrocartilage. This study demonstrated 75% hyaline-like cartilage regeneration at 6 months post-treatment.

Two single-patient case reports, 1 patient followed for 1 year and 1 patient followed for 2 years:

Ronga et al. 2004

In this report, treatment and outcome information were described for a male aged 25 years with a chondral defect of the posterior portion of the lateral tibial plateau of the knee due to direct trauma. Due to the location of the 2 cm2 lesion, an arthroscopic approach was used for MACI implantation; the MACI implant included the Chondro-Gide membrane. The patient followed a post-operative rehabilitation programme. Clinical assessment was completed using the modified Cincinnati, Lysholm-Gillquist, Tegner-Lysholm and IKDC measures. At 12 months post-treatment, the patient had full range-of-motion, no longer reported knee pain and had returned to performing all activities as prior to the traumatic injury. The knee was rated as normal (ICRS scoring). Clinical improvement was shown on all clinical outcome measures; results of statistical analysis of changes in scores over time were not reported.

Ronga et al. 2006

In this report, treatment and outcome information were described for a male aged 40 years with a complex injury of the knee due to trauma suffered while participating in sports activity. Imaging assessment (MRI) revealed a tear of the medial meniscus, rupture of the ACL and a 5 cm^2 lesion on the medial femoral condyle. The patient underwent arthroscopic repair of the meniscus (partial meniscectomy and collagen meniscus implant [CMI]) and reconstruction of the ACL with bone-patellar tendon-bone graft followed by a structured rehabilitation programme. The patient was able to return to normal activities after 6 months but continued to report knee pain when using stairs. Second-look arthroscopy at this time showed that the CMI/ACL treatment had been successful and a cartilage biopsy was performed for harvesting of cells for MACI treatment. After 5 weeks, MACI implantation was performed using an arthrotomy approach and the implant included a type I/III collagen membrane (MACI Verigen). The patient followed a post-operative rehabilitation programme. Clinicalassessment was completed using the modified Cincinnati, Lysholm-Gillquist,Tegner-Lysholm and IKDC measures At 24 months following the CMI/ACL surgery (approximately 17 months after MACI treatment), the patient had full range-of-motion and the knee was rated as nearly normal (ICRS scoring). Clinical improvement was shown on all clinical outcome measures; results of statistical analysis of changes in scores over time were not reported. Assessments at 12 and 24 months following the CMI/ACL surgery (approximately 5 and 17 months after MACI treatment) using MRI indicated that the graft had provided good restoration of the articular surface with hyaline-like tissue.

2.5.4. Discussion on clinical efficacy

Design and conduct of the clinical study

In support of this application the applicant conducted a pivotal 2-year Phase 3 study (MACI00206 – "SUMMIT" Study). This was a prospective, randomised, open-label, parallel-group, multicentre study to demonstrate the superiority of MACI versus arthroscopic MF for the treatment of symptomatic articular cartilage defects of the femoral condyle, including the trochlea. Patients were aged between 18—55 years and had at least 1 symptomatic focal cartilage defect as defined by KOOS Pain score <55. A cartilage biopsy was taken prior to randomisation to study treatment. Cells harvested from patients randomised to the MACI treatment group are used in the preparation of the MACI implant. Patients randomised to treatment with MACI return within approximately 4 to 8 weeks of the index arthroscopy to undergo the chondrocyte implantation procedure via arthrotomy. Patients randomised to MF undergo the procedure during the index arthroscopy.

The applicant sought advice from the EMA/CHMP regarding the comparator choice. MF was recognised as an acceptable reference therapy for a superiority trial (see also additional analyses below).

The study's co-primary efficacy variables are the change from baseline to Week 104 for the patient's KOOS Pain and Function in sports and recreation (Function) scores. The co-primary endpoint included only pain and function (SRA) from the 5 components of the full KOOS instrument which is a patient related outcome. However, as the remaining 3 components (Activities of Daily Living, Quality of Life and Other Symptoms) were included as secondary endpoints and additionally structural endpoints including histology and MRI were also included in the secondary endpoints, the CHMP considered that this was acceptable. Patient-reported outcome data is acceptable as primary endpoint in the pivotal study, given the current lack of other outcome measures that are both sensitive and objective. For patient-reported outcomes, validated methods to assess improvement of function and pain should be used (e.g. Knee injury and Osteoarthritis Outcome Score (KOOS)) as described in the EMA/CAT/CPWP/568181/2009 reflection paper. While the histology evaluation used the overall score as the most important histology variable, the MRI evaluation focused on defect fill being the most important outcome variable. The tertiary and exploratory endpoints were considered appropriate. The open label nature of Study MACI00206 is also acceptable in line with the above-mentioned reflection paper.

In total, 144 patients were randomised; 72 patients to the MACI group and 72 to the MF group. From the baseline population characteristics patients' age, sex, race, weight, height and BMI were similar across the treatment groups. For both treatment groups, acute trauma was the most common underlying aetiology of the index lesion. Although the patient demographics appeared to be balanced between the groups, the MACI group included twice as more patients with chronic degenerative defects, less highly competitive sports participants, less patients rated as normal on Kellgren-Lawrence grading and a longer duration since last orthopaedic knee surgery. These are fairly well established risk factors that may adversely impact outcomes after cartilage repair surgery. Overall the target defects were similar between the 2 treatment groups at baseline; the index lesion was most frequently located in the MFC. Prior to treatment, the median size of the index lesion and the median total defect size surface area were the same for both groups (4.0 cm^2 and 4.5 cm^2, respectively).The proportion of patients with at least 1 prior orthopaedic knee surgery (target or non-target knee) was high but comparable for the 2 treatment groups (90.3% vs 83.3%) in the MACI and MF groups respectively, however the median days since the last surgery for patients in the MACI group was more than twice that for patients in the MF group. The type and frequency of concurrent surgical procedures (CSPs) and use of concomitant medications (including pain medication) were comparable for both groups.

Efficacy data and additional analyses

Efficacy data

From baseline to Week 104, an improvement in Pain and Function (SRA) ratings were reported for patients in both treatment groups. However, the mean improvement in both Pain and Function (SRA) was significantly greater (p=0.001) for patients treated with MACI compared to those treated with MF. The additional improvement of MACI over MF in change from baseline at Week 104 was >10 points for both Pain and Function (SRA). The results of the co-primary endpoint clearly indicate that treatment outcome was superior with MACI when compared with MF to a high degree of statistical significance. Results also appeared to be consistent with all 3 data set analyses.

The responder analysis showed significantly superior results with MACI when compared to MF with proportion of patients who had not responded being twice as high in the MF group. The percentage of patients who responded to treatment at Week 104 (had at least a 10-point improvement in both Pain

and Function [SRA] from Baseline) was significantly greater (p=0.016) for patients in the MACI group (87.50%) compared to the MF group (68.06%). Improvements from Baseline to Week 104 for all 3 KOOS subscales were significantly greater for patients in the MACI group compared to the MF group. Across all 3 subscales, the change from baseline to Week 104 was >25 points for both treatment groups, however the changes in ADL (p<0.001), QOL (p=0.029), and Other Symptoms (p<0.001) were superior for patients treated with MACI. The mean ICRS II Overall Assessment score at Week 104 was comparable for the MACI and MF groups and there was no significant difference between the treatment groups (p=0.717). Therefore contrary to expectations the results of the histology analysis did not show any superiority of MACI despite the apparent clinical superiority in the clinical co-primary endpoint. However, a greater Overall Assessment score at Week 104, in favour of patients treated with MF, was seen for subgroups with the lesion located at the trochlea, lesion aetiology of osteochondritis dissecans and no prior cartilage repair surgery. A greater Overall Assessment score, in favour of patients treated with MACI, was seen for subgroups with >1 prior cartilage repair surgery, which is an unexpected finding since this can negatively impact on the outcome. There was no significant difference between the treatment groups in MRI Degree of Defect Fill at Week 52 or Week 104. Improvement in defect fill was evident for patients in both treatment groups. The Macroscopic ICRS Cartilage Repair scores were comparable for patients treated with MACI or MF. Therefore, similar to the histology observations, the MRI evaluation did not show any superiority of MACI despite the demonstrated clinical superiority in the clinical co-primary endpoint. A higher proportion of patients underwent MRI examination as compared to histology assessment 134/144 vs 116/144 respectively. Unlike histology evaluations for some sub-groups, pre-specified exploratory analyses for MRI Degree of Defect Fill by total defect size (>4 cm^2 and >5 cm^2), index lesion size (>4 cm^2, >5 cm^2), lesion location, aetiology, prior surgical history of target knee, prior cartilage repair surgical history, site and gender did not show any notable differences in treatment groups. Overall the data showed a lack of correlation between tissue characteristics and clinical improvements, regardless of the treatment group. As discussed in the section Discussion on clinical pharmacology, improvements in clinical outcomes of pain and function remain the most important and clinically valid endpoint in cartilage repair studies.

The results of the tertiary KOOS endpoints including all subscales and response rates at all pre-specified time points showed generally consistent results with co-primary and secondary endpoints. Indeed, additional support for improved clinical efficacy with MACI treatment was evident in other patient-reported outcome measures included in the study. Improvement on the Modified Cincinnati Knee Rating System and the SF-12 physical health score from Baseline to Weeks 52 and 104 was significantly greater for patients in the MACI group. For the IKDC Subjective Knee Evaluation, there was a significant difference between the treatment groups in favour of the MACI group for change from baseline at Week 52 and a trend towards a significantly greater mean improvement at Week 104. Comparable improvement was seen for both treatment groups on the EQ-5D and SF-12 mental component score.

The planned analyses concerning treatment failure rates and treatment group differences were not relevant due to the small number of treatment failures. Only 1 patient on MACI (versus 4 on MF) experienced a treatment failure. Furthermore, no patient withdrew from the study on MACI compared with 3 on MF.

<u>Additional analyses concerning the comparator and lesions size</u>

MF has been used as the active comparator for MACI. However, MF is not indicated for cartilage lesions greater than 4.0 cm^2 as per the EMA reflection paper on chondrocyte products (EMA/CAT/CPWP/568181/2009). Therefore, MF may be considered inappropriate as the choice of

active control since there was no upper limit of the lesion size in the inclusion criteria. Subjects were included with lesion size of 3-20 cm^2 (median 4 cm^2). The Committee rose that the treatment effect of MACI compared to MF may have been overestimated, as both subjects with small and larger lesions were included in the pivotal trial, whereas MF may not be an optimal treatment option for larger lesions. Upon the CHMP request, to further support the benefit of MACI in small and larger lesions, subgroup analyses were provided for primary outcomes and KOOS responder rates with a cut off at 4 cm^2.

A clinically significant difference (p=0.005) in the improvement from baseline to 2 years post-treatment was seen for the co-primary efficacy endpoint in patients with lesion size ≤4 cm^2 treated with MACI compared to MF (LS Means Difference - Pain: 15.09, Function [SRA]: 10.01. In patients with lesion size > 4 cm^2, a larger improvement for both Pain and Function (SRA) from baseline to 2 years post-treatment was seen for patients treated with MACI compared to MF (Pain: 9.35, Function [SRA]: 12.52). The results of these additional subgroup analyses were consistent with the full group results which demonstrated the superiority of MACI treatment over MF.

The percentage of patients who responded to treatment at Week 104 (had at least a 10-point improvement from baseline in both Pain and Function [SRA]) was greater in the MACI treatment group compared with MF for both subgroups ≤ 4 cm^2 (78.4% vs. 61%) and > 4 cm^2 (97.1% vs 77.4%). The results of these additional subgroup analyses were also consistent with the full group results which demonstrated the superiority of MACI treatment over MF. Subgroup analyses for KOOS response rate (those with at least a 10-point improvement in both Pain and Function [SRA]) at Week 104 were also presented for patients < 40 years and patients < 40 years with a lesion size ≤ 4 cm^2. For the KOOS responder rate at Week 104 by age < 40 years, a greater proportion of patients treated with MACI versus MF were responders (90% versus 69%, respectively), indicating that MACI performed better in the younger (< 40 years) age group. For the KOOS responder rate at Week 104 by age < 40 years and lesion size ≤ 4 cm^2, the percentage of patients who responded to MACI treatment at Week 104 was greater than the percentage of patients who responded to MF (83% versus 65%, respectively). Overall, the results of these analyses demonstrated that the majority of patients in both treatment groups met predefined responder criteria if they were aged 40 years or younger and/or had a lesion size ≤ 4 cm^2. Overall, all sub-group analyses of KOOS response rate favoured MACI over MF.

To further clarify the benefit of MACI, subgroup analyses were presented for the co-primary efficacy endpoint at Week 104 for lesion sizes of 3 to 6 cm^2 and 6 to 10 cm^2. The vast majority of patients were in the 3 to 6 cm^2 subgroup. Further breakdown into subgroups with lesion size >10 cm^2 was not possible as there were only 3 patients in the MACI group and 1 patient in the MF group. Although the size of this subgroup was rather small, a clinically significant difference in the improvement from baseline to 2 years post-treatment was seen for the co-primary efficacy endpoint in patients with lesion size 3 to ≤ 6 cm^2 treated with MACI compared to MF (Pain: 12.89, Function [SRA]: 12.56. In the > 6 to ≤ 10 cm^2 subgroup, the improvement from baseline to 2 years post-treatment was greater for patients treated with MACI compared to MF (Pain: 18.59, Function [SRA]: 18.27). The percentage of patients who responded to treatment at Week 104 (had at least a 10-point improvement from baseline in both Pain and Function [SRA]) was greater for patients in the MACI group compared to the MF group for the subgroup of patients with lesion size 3 to ≤ 6 cm^2 (86% versus 63%, respectively). The response rates were also greater for patients in the MACI group (100%) compared to MF (86%) for the subgroup of patients with lesion size > 6 to ≤ 10 cm^2.

The overall conclusion from the lesion size subgroup analyses confirms the treatment effect of MACI over MF across all lesion sizes. There is no suggestion that the benefit of MACI should be limited to a particular size of lesion. The study results have shown the clinical superiority of MACI over MF

treatment for symptomatic cartilage defects of the knee with a range of defect sizes from 3.0 to 20.0 cm^2.

Furthermore, the applicant also clarified that the use of MF with larger lesions and as a comparator treatment in studies of cartilage repair is supported by current clinical practice and the literature. The publication by Steadman describes the MF technique as indicated for full-thickness lesions of the femur, tibia, or patella and is without any lesion size limitation (Steadman *et al.* 1997). Orthopaedic and sports surgeons use MF as a first-line treatment for chondral lesions > 4 cm^2 in routine clinical practice, in part as a result of limitations of other ethically appropriate alternative treatments (e.g. limited allograft supply). The EMA reflection paper states that "for patients with lesions > 4 cm^2, no standard therapy has unequivocal efficacy, therefore superiority against best standard of care is currently the reasonable option". Overall the use of MF as reference therapy for the trial MACI00206 is acceptable.

To allow a full evaluation of the benefit in each of the primary KOOS subscales separately, distinct analyses for change from baseline to Week 104 in KOOS Pain and KOOS Function (SRA) were completed using ANCOVA with centre, age and baseline score as covariates and with missing data imputed using the LOCF method. The results for change from baseline to Week 104 in KOOS Pain demonstrated a clinically and statistically significant difference in the improvement from baseline to 2 years post-treatment in patients treated with MACI compared to MF (LS Means Difference: 11.73 [95% confidence interval (CI): 5.42, 18.05], p<0.001). The results for change from baseline to Week 104 in KOOS Function (SRA) demonstrated a clinically and statistically significant difference in the improvement from baseline to 2 years post-treatment in patients treated with MACI compared to MF (LS Means Difference: 11.47 [95% CI: 2.17, 20.77], p=0.016). Overall, a statistically significant benefit for MACI compared to MF was seen for both endpoints; pain (p<0.001) and function (p=0.016). This shows that the combined significant result was not driven entirely by one of the components.

Additional subgroup analyses for ICRS II Overall Assessment score and MRI Degree of Defect Fill at Week 104 were presented for lesion size cut-offs of 4 cm^2 and 6 cm^2. The results the analyses showed that the Overall Assessment scores for histological assessment were similar between the treatment groups across the subgroups, except that the subgroup with lesion size > 6 to ≤ 10 cm^2 showed a larger difference between the subgroups, favouring the MF group. This result must be interpreted with caution due to the very low sample size in the subgroup with lesion size > 6 to ≤ 10 cm^2 (MACI: n=6, MF: n=14). Concerning MRI assessment, the distribution of scores for Degree of Defect Fill was similar between the treatment groups across the subgroups. Additional subgroup analyses for histology and MRI have been completed for age <40 years and age <40 years with a lesion size ≤4 cm^2. The results of the analyses for both ICRS II Overall Assessment score and MRI Degree of Defect Fill were similar between the MACI and MF treatment groups across the subgroups.

Concurrent knee surgery, like meniscus surgery or anterior cruciate ligament reconstruction, may bias the primary outcomes of pain and knee function. Therefore, to allow further evaluation of the robustness and relevance of the effect seen in the co-primary endpoint and KOOS response rate analyses, additional analyses were completed excluding patients with concurrent knee surgery and using a more stringent cut-off for KOOS response rate (those with at least a 20-point improvement in both Pain and Function [SRA]) at Week 104.

Subgroup analyses excluding patients with concurrent knee surgery

The results for change from baseline to Week 104 in KOOS Pain and Function demonstrated a clinically significant difference in the improvement from baseline to 2 years post-treatment in patients with no concurrent knee surgery treated with MACI compared to MF. A larger difference between the treatment

groups in KOOS Pain and Function (SRA) was seen in the analysis excluding patients with concurrent surgery compared to the analysis including all patients (Pain: 14.32 versus 11.76, respectively, Function [SRA]: 16.73 versus 11.41, respectively). The percentage of patients with no concurrent knee surgery who responded to treatment at Week 104 (had at least a 10-point improvement from baseline in both Pain and Function [SRA]) was greater for patients in the MACI group compared to the MF group. A larger difference between the treatment groups in response rate was seen in the analysis excluding patients with concurrent surgery compared to the analysis including all patients (31% versus 20%, respectively).

Additional sensitivity analyses for KOOS responder rate at Week 104

The results of the sensitivity analyses for KOOS response, overall and with subgroups with 4 cm^2 and 6 cm^2 cut-offs, using a more stringent 20-point improvement cut-off, favoured MACI and were consistent with the results from the predefined 10-point responder analyses.

The study was not initially planned and powered for subgroup analyses therefore results should be interpreted with caution. However the consistency of observed results across the various subgroup analyses reinforce the robustness of the results from the primary analysis showing superiority of MACI over MF for the repair from 0 to 20 cm^2.

Supportive studies

In the 19 studies presented, approximately 800 patients were treated with MACI. The majority of implant procedures have been performed by arthrotomy. Variability within and between studies was present for patient characteristics such as age, treatment history, defect size, location and aetiology. Due to the heterogeneity of the studied populations demographic characteristics cannot be fully assessed. For some of the non-comparative studies, it was difficult to determine whether the study was conducted prospectively or retrospectively. Details concerning the number of patients, knees and defects treated per study were not presented consistently across the studies. Moreover, there is likely some overlaps of patients between some studies (e.g. between the Basad, 2010 and Bachmann, 2004, Radiologe). The extent of these overlaps remains unclear. The membrane used in the studies was not systematically the ACI-MAIX™ membrane which is the proposed commercial product. Also considering the methodological limitations of these studies (e.g. single-centre, small sample size, non-comparative for some of them) and uncertainties regarding protocol compliance and monitoring, these studies can only be regarded as supportive of the pivotal SUMMIT trial.

In patients for whom MACI treatment has been provided and efficacy evaluated, rather consistent findings of improvement in patient function, pain and quality of life as well as in graft repair have been reported. Outcomes, sometimes only briefly discussed, were assessed at different time points (or not specified sometimes) and with different statistical analysis methods (sometimes none were reported). Therefore, it limits the comparability of data between studies. Overall though, study results across the studies are generally consistent regarding the efficacy of MACI treatment. The studies have reported evidence of clinical improvement, including pain and function, with MACI treatment for the repair of cartilage defects. Clinical improvements were seen within 3 weeks to 3 months following surgery and improvements in clinical outcome over time have been documented up to 5 years post-treatment. Several studies have reported repair tissue assessment with biopsy or MRI. Due to a lack of information in the literature it is not possible to determine the exact number of patients assessed by each measure, but approximately more than 50 patients treated with MACI were assessed with a biopsy and more than 150 were assessed with MRI. Based on biopsy data, cartilage regeneration of mixed or hyaline-like tissue has been observed although there is some variability in results among the

studies; MRI data have shown that a majority of cartilage repair grafts provided good fill and integration of the defects.

2.5.5. Conclusions on the clinical efficacy

In the randomised trial in 144 patients MACI was superior compared to standard care of MF with symptomatic cartilage defects of the knee with a range of defect sizes from 3.0 to 20.0 cm^2 (grade III and IV Modified Outerbridge Scale), regarding mean improvement of pain and function. A clinically and statistically significant difference in the improvement from baseline to Week 104 was seen for the co-primary endpoint of KOOS Pain and Function (SRA) in patients treated with MACI compared to MF.

The primary efficacy endpoint was corroborated by several other PRO measures and a responder analysis of the primary efficacy measures. Superior clinical efficacy was demonstrated for patients treated with MACI compared to MF on the remaining 3 KOOS subscales (ADL, QOL, and Other Symptoms) and other validated PRO measures included in the study (Modified Cincinnati Knee Rating System, SF-12 physical health score, and IKDC Subjective Knee Evaluation). The treatment effect is considered clinically relevant: significantly more patients treated with MACI (87.50%) met the responder analysis criteria (defined as improvement from baseline to Week 104 of at least 10 points in both KOOS Pain and Function [SRA]) than patients treated with MF (68.06%).

While MACI has shown benefit in the overall treated population, the Committee considered important to see a comparison of MACI with MF specifically in smaller lesions, to ensure that the observed advantage of MACI was not restricted to the group where MF could be suboptimal i.e. larger lesions (> 4 cm^2). Statistically significant benefit over MF ($p=0.005$) was seen in the group with lesion size $\leq$ 4 cm^2 showing that even in the group with smaller lesions where MF is considered optimal there is benefit for MACI. Benefit was seen for both pain (difference 15.09) and function (difference 10.01). There was also a positive trend in KOOS response rates (78% vs. 61%). For the larger lesions (> 4 cm^2) there was a statistically significant difference in KOOS response rates (97% vs. 77%) in favour of MACI, while a positive trend was seen for the co-primary efficacy parameter for both pain (difference 9.35) and function (difference 12.52). The analysis using a cut-off of 6 cm^2 was less informative as almost all the patients were on the small side of this cut-off, but all the comparisons again favoured MACI. Overall the results of these analyses are consistent with the full group results which demonstrated superiority of MACI treatment over MF. Results showed that the benefit of MACI is not restricted to a particular size of lesion and that the treatment effect was not related to lesion size.

Pain and function reviewed in separate analyses, as opposed to the combined analysis used for the primary endpoint showed a statistically significant benefit for MACI compared to MF was seen for both endpoints; pain ($p<0.001$) and function ($p=0.016$). This shows that the combined significant result was not driven entirely by one of the components.

Concurrent knee surgery, like meniscus surgery or anterior cruciate ligament reconstruction, may bias the primary outcomes of pain and knee function. The subgroup analyses in subjects without concurrent knee-surgery (about 67% of the overall study population, equally distributed over the two study arms) support the overall study outcome, i.e. that MACI is superior to MF regarding reduction in pain and improvement of function. The robustness of the overall study outcomes was further supported by sensitivity analyses using a more stringent outcome, i.e. the KOOS-20 responder rates (defined as an improvement of 20 points of more from a KOOS scale of 100). Although statistical significance was not achieved, the point estimates indicate the same trend as for the overall analyses and primary endpoints.

Both the MACI and MF groups performed well according to the structural endpoints with good infill of defects as assessed by MRI and good quality repair tissue as assessed by the ICRS II Overall

Assessment histology score; however, there were no statistically significant differences between the treatment groups in the structural endpoint. Assessment of the association between structural endpoints (histology and MRI) and clinical efficacy (change from Baseline in KOOS Pain and Function [SRA]) at Week 104 showed a lack of correlation between the tissue characteristics and clinical improvement. The lack of correlation was consistent across both the MACI and MF treatment groups. Overall, there is no clear consensus on whether structural repair as measured by MRI or histology scoring systems is able to distinguish the true functional repair of cartilage defects, and hence be a meaningful surrogate for clinical outcomes. Improvements in clinical outcomes of pain and function, as observed in the study, remain the most important and clinically valid endpoints in cartilage repair studies. The pivotal SUMMIT study is extended to 5 years. This will provide further data on the sustainability of the chondral repair and maintenance of effect of MACI compared to MF over time.

The efficacy of MACI demonstrated in the pivotal study is supported by data from the literature. Notwithstanding several methodological deficiencies of these studies, they consistently reported a relevant improvement of pain and function of the knee that is persistent over the years.

The CHMP endorse the CAT conclusion on clinical efficacy as described above.

2.6. Clinical safety

Patient exposure

All 144 randomised patients received a study treatment: 72 patients with MACI and 72 patients with MF. The median study duration was between 733 and 739 days and the median rehabilitation duration was between 358 and 363 days. Patients' rehabilitation status from Visit 4 (Week 6) to Visit 7 (Week 36) progressed without major differences between the 2 groups. At the time of Visit 8 (Week 52), compared to the MF group, there were less patients in the MACI group who had completed rehabilitation (MACI: 45 [62.5%], MF: 51 [70.8%]) and more patients who had terminated rehabilitation early (MACI: 9 [12.5%], MF: 6 [8.3%]). Overall, patients in both treatment groups took a similar number of days to complete the study and to complete rehabilitation. Almost all patients in both treatment groups had progressed to full weight-bearing (>95%) and to full active range-of-motion (>90%) at Week 52.

Adverse events (AEs)

A brief summary of all reported AEs for the 2 treatment groups is presented in the table below.

Table 26 Summary of adverse events - Safety Set

n (%)	MACI N=72	Microfracture N=72
At Least 1 AE	57 (79.2)	61 (84.7)
At Least 1 SAE	11 (15.3)	19 (26.4)
At Least 1 TESAE	11 (15.3)	19 (26.4)
Any Death	0	0
Discontinued Study Due to TEAE	1 (1.4)	1 (1.4)
At Least 1 TEAE	55 (76.4)	60 (83.3)
At Least 1 Related TEAE	25 (34.7)	28 (38.9)
At Least 1 Severe TEAE	7 (9.7)	10 (13.9)

Treatment-emergent: defined as an AE with a start date beyond or equal to that of study treatment at Day 1.
TEAEs leading to study discontinuation: obtained using the 'Primary reason for discontinuation' in the 'Completion/ Discontinuation' CRF panel and the 'Other action taken' in the 'Adverse Event' CRF panel. Related: relationship to study treatment reported as 'Definite', 'Probable', 'Possible', or missing. Severe: severity reported as 'Severe' or missing.
TESAE=treatment-emergent serious adverse event.

An overview of treatment-emergent AEs (TEAES) reported in >5% of patients in any treatment group, regardless of severity and relationship to study treatment, is provided in the table below. TEAEs were most frequently reported within the musculoskeletal and connective tissue disorders system organ class (SOC) for both treatment groups (48 patients [66.7%] in the MACI group and 52 patients [72.2%] in the MF group). The most common TEAEs were arthralgia (51.4% in the MACI group versus 63.9% in the MF group), headache (18.1% versus 29.2%), nasopharyngitis (13.9% versus 9.7%), back pain (11.1% versus 9.7%) and cartilage injury (4.2% versus 12.5%).

Table 27 Treatment-emergent adverse events per system organ class and preferred term reported in >5% of patients in any treatment group –Safety Set

n (%)	MACI N=72	Microfracture N=72
Any TEAE	55 (76.4)	60 (83.3)
Gastrointestinal Disorders	6 (8.3)	7 (9.7)
Abdominal pain	0 (0.0)	5 (6.9)
General Disorders and Administration Site Conditions	10 (13.9)	10 (13.9)
Pyrexia	4 (5.6)	2 (2.8)
Treatment failure	1 (1.4)	4 (5.6)
Infections and Infestations	23 (31.9)	17 (23.6)
Influenza	4 (5.6)	5 (6.9)
Nasopharyngitis	10 (13.9)	7 (9.7)
Injury, Poisoning and Procedural Complications	19 (26.4)	20 (27.8)
Cartilage injury	3 (4.2)	9 (12.5)
Procedural pain	3 (4.2)	4 (5.6)
Musculoskeletal and Connective Tissue Disorders	48 (66.7)	52 (72.2)
Arthralgia	37 (51.4)	46 (63.9)
Back pain	8 (11.1)	7 (9.7)
Joint effusion	5 (6.9)	4 (5.6)
Joint swelling	7 (9.7)	4 (5.6)
Ligament sprain	2 (2.8)	4 (5.6)
Nervous System Disorders	16 (22.2)	24 (33.3)
Headache	13 (18.1)	21 (29.2)
Respiratory, Thoracic and Mediastinal Disorders	5 (6.9)	5 (6.9)

System Organ Class and Preferred Term are ordered alphabetically. AEs were coded using MedDRA Version 14.1.
Treatment-emergent adverse event (TEAE): defined as an AE with a start date beyond or equal to that of study treatment At Day 1. If a patient experienced more than 1 AE with the same Preferred Term or Primary System Organ Class, each patient was counted at most once within each Preferred Term or Primary System Organ Class. A cut-off point of 5% was applied to the incidence of Preferred Terms. MedDRA=Medical Dictionary for Regulatory Activities, TEAE=treatment-emergent adverse event.

Thirteen patients (18.1%) in the MACI group had at least 1 AE during the time between arthroscopy and MACI implantation. Among these 13 patients, arthralgia (4 patients, 5.6%) and haemarthrosis (2 patients, 2.8%) were the only AEs reported in more than 1 patient.

An overview of TEAEs by maximum severity that were reported in >5% of patients in any treatment group is provided in Table 20. In both treatment groups, the majority of TEAEs were of mild or moderate intensity. The proportion of patients with at least 1 TEAE of severe intensity was 9.7% in the MACI group and 13.9% in the MF group. The only TEAE with maximum of severe intensity reported in >5% of patients in any treatment group was arthralgia (2 patients [2.8%] in the MACI group and 5 patients [6.9%] in the MF group). The TEAEs with maximum of moderate intensity reported in >5% of patients in any treatment group were cartilage injury (1 patient [1.4%] in the MACI group and 6 patients [8.3%] in the MF group) and arthralgia (12 patients [16.7%] in the MACI group and 16 patients [22.2%] in the MF group).

Table 28 Treatment-emergent adverse events per system organ class and preferred term reported in >5% of patients in Any Treatment Group by severity – Safety Set

n (%)	MACI N=72			Microfracture N=72		
	Severe	Moderate	Mild	Severe	Moderate	Mild
Any TEAE	7 (9.7)	23 (31.9)	25 (34.7)	10 (13.9)	24 (33.3)	26 (36.1)
Gastrointestinal Disorders	0 (0.0)	1 (1.4)	5 (6.9)	1 (1.4)	2 (2.8)	4 (5.6)
General Disorders and Administration Site Conditions	1 (1.4)	4 (5.6)	5 (6.9)	3 (4.2)	1 (1.4)	6 (8.3)
Infections and Infestations	0 (0.0)	6 (8.3)	17 (23.6)	0 (0.0)	2 (2.8)	15 (20.8)
Influenza	0 (0.0)	0 (0.0)	4 (5.6)	0 (0.0)	0 (0.0)	5 (6.9)
Nasopharyngitis	0 (0.0)	1 (1.4)	9 (12.5)	0 (0.0)	0 (0.0)	7 (9.7)
Injury, Poisoning and Procedural Complications	1 (1.4)	6 (8.3)	12 (16.7)	1 (1.4)	9 (12.5)	10 (13.9)
Cartilage injury	0 (0.0)	1 (1.4)	2 (2.8)	0 (0.0)	6 (8.3)	3 (4.2)
Musculoskeletal and Connective Tissue Disorders	3 (4.2)	20 (27.8)	25 (34.7)	5 (6.9)	21 (29.2)	26 (36.1)
Arthralgia	2 (2.8)	12 (16.7)	23 (31.9)	5 (6.9)	16 (22.2)	25 (34.7)
Back pain	1 (1.4)	2 (2.8)	5 (6.9)	0 (0.0)	2 (2.8)	5 (6.9)
Joint swelling	0 (0.0)	3 (4.2)	4 (5.6)	0 (0.0)	1 (1.4)	3 (4.2)
Nervous System Disorders	2 (2.8)	2 (2.8)	12 (16.7)	2 (2.8)	4 (5.6)	18 (25.0)
Headache	1 (1.4)	2 (2.8)	10 (13.9)	1 (1.4)	3 (4.2)	17 (23.6)
Respiratory, Thoracic and Mediastinal Disorders	0 (0.0)	2 (2.8)	3 (4.2)	1 (1.4)	0 (0.0)	4 (5.6)

System Organ Class and Preferred Term are ordered alphabetically. AEs were coded using MedDRA Version 14.1.
Treatment-emergent: defined as an AE with a start date beyond or equal to that of study treatment at Day 1.
If a patient experienced more than 1 AE with the same Preferred Term or Primary System Organ Class, the AE with Maximum severity was counted for that Preferred Term or Primary System Organ Class. (TE)AE=(treatment-emergent) adverse event, MedDRA=Medical Dictionary for Regulatory Activities

An overview of TEAEs considered related to the study treatment by the investigator with an incidence >5% in any treatment group is provided in Table 21. The incidence of TEAEs was comparable for the 2 treatment groups (25 patients [34.7%] in the MACI group and 28 patients [38.9%] in the MF group). Related TEAEs with an incidence >5% in any treatment group were treatment failure (1 patient [1.4%] in the MACI group and 4 patients [5.6%] in the MF group), arthralgia (19 [26.4%] in the MACI group and 23 [31.9%] in the MF group) and joint swelling (5 [6.9%] in the MACI group and 2 [2.8%] in the MF group).

Table 29 Treatment-emergent adverse events considered related to study treatment by the investigator per system organ class and preferred term with an incidence >5% in Any Treatment Group – Safety Set

n (%)	MACI N=72		Microfracture N=72	
	Related	Unrelated	Related	Unrelated
Total TEAEs	25 (34.7)	30 (41.7)	28 (38.9)	32 (44.4)
General Disorders and Administration Site Conditions	2 (2.8)	8 (11.1)	4 (5.6)	6 (8.3)
Treatment failure	1 (1.4)	0 (0.0)	4 (5.6)	0 (0.0)
Musculoskeletal and Connective Tissue Disorders	25 (34.7)	23 (31.9)	27 (37.5)	25 (34.7)
Arthralgia	19 (26.4)	18 (25.0)	23 (31.9)	23 (31.9)
Joint swelling	5 (6.9)	2 (2.8)	2 (2.8)	2 (2.8)

System Organ Class and Preferred Term are ordered alphabetically. AEs were coded using MedDRA Version 14.1. Treatment-emergent: defined as an AE with a start date beyond or equal to that of study treatment at Day 1. Related: relationship to study treatment reported as 'Definite', 'Probable', 'Possible' or missing. Unrelated: relationship to study treatment reported as 'Remote/Unlikely' or 'Not related'.

Serious adverse event / deaths / other significant events

No deaths occurred in study MACI00206.

An overview of treatment-emergent serious adverse events (TESAEs), regardless of severity and relationship to study treatment, is provided in Table 22. TESAEs were reported more frequently in the MF group (26.4%) than in the MACI group (15.3%). The difference in incidence rates was mainly due to more serious cases of treatment failure, cartilage injury and arthralgia in the MF group compared to the MACI group. TESAEs reported in more than 1 patient within any treatment group were treatment failure (1 patient [1.4%] in the MACI group and 4 patients [5.6%] in the MF group), cartilage injury (2 patients [2.8%] in the MACI group and 6 patients [8.3%] in the MF group), meniscus lesion (2 patients [2.8%] in the MACI group and no patients in the MF group) and arthralgia (no patients in the MACI group and 3 patients [4.2%] in the MF group).

Table 30 Treatment-emergent serious adverse events per system organ class and preferred term - Safety Set

n (%)	MACI N=72	Microfracture N=72
Any TESAE	11 (15.3)	19 (26.4)
Cardiac Disorders	1 (1.4)	0 (0.0)
Arrhythmia	1 (1.4)	0 (0.0)
Gastrointestinal Disorders	0 (0.0)	1 (1.4)
Abdominal pain	0 (0.0)	1 (1.4)
General Disorders and Administration Site Conditions	2 (2.8)	4 (5.6)
Impaired healing	1 (1.4) [a]	1 (1.4) [a]
Treatment failure	1 (1.4) [a]	4 (5.6) [a]
Infections and Infestations	1 (1.4)	1 (1.4)
Pneumonia	1 (1.4)	0 (0.0)
Post-operative wound infection	0 (0.0)	1 (1.4)
Wound infection staphylococcal	0 (0.0)	1 (1.4)

Injury, Poisoning and Procedural Complications	5 (6.9)	7 (9.7)
Cartilage injury	2 (2.8)	6 (8.3)
Graft delamination	1 (1.4) [a]	0 (0.0)
Head injury	0 (0.0)	1 (1.4)
Meniscus lesion	2 (2.8)	0 (0.0)
Transplant failure	1 (1.4) [a]	0 (0.0)
Traumatic fracture	0 (0.0)	1 (1.4)
Musculoskeletal and Connective Tissue Disorders	1 (1.4)	7 (9.7)
Arthralgia	0 (0.0)	3 (4.2) [a]
Arthritis	0 (0.0)	1 (1.4)
Joint lock	0 (0.0)	1 (1.4) [a]
Knee deformity	1 (1.4)	0 (0.0)
Loose body in joint	0 (0.0)	1 (1.4)
Osteochondrosis	0 (0.0)	1 (1.4)
Pain in extremity	0 (0.0)	1 (1.4)
Patellofemoral pain syndrome	0 (0.0)	1 (1.4)
Neoplasms Benign, Malignant and Unspecified (Incl Cysts and Polyps)	1 (1.4)	0 (0.0)
Prostate cancer	1 (1.4)	0 (0.0)
Nervous System Disorders	0 (0.0)	1 (1.4)
Multiple sclerosis	0 (0.0)	1 (1.4)
Pregnancy, Puerperium and Perinatal Conditions	1 (1.4)	1 (1.4)
Abortion spontaneous	1 (1.4)	1 (1.4)
Renal and Urinary Disorders	1 (1.4)	0 (0.0)
Urinary retention	1 (1.4)	0 (0.0)
Respiratory, Thoracic and Mediastinal Disorders	1 (1.4)	1 (1.4)
Pulmonary embolism	1 (1.4)	1 (1.4) [a]
Vascular Disorders	1 (1.4)	0 (0.0)
Thrombosis	1 (1.4)	0 (0.0)

System Organ Class and Preferred Term are ordered alphabetically. AEs were coded using MedDRA Version 14.1.
`Treatment-emergent: defined as a SAE with a start date beyond or equal to that of study treatment at Day 1.
MedDRA=Medical Dictionary for Regulatory Activities, (TE)(S)AE=(treatment-emergent)(serious)adverse event
a SAE considered at least possibly related to study treatment by the Investigator.

Adverse events of interest, based on previous clinical experience with MACI, include potential peri-operative complications related to arthroscopy or arthrotomy (haemarthrosis, haematomas at surgical site, intra-articular adhesions, arthrofibrosis, localised surgical site inflammation, localised surgical site infection, thromboembolic events) and potential complications related to MACI (symptomatic graft hypertrophy and graft delamination [complete or partial, possibly leading to loose bodies in the joint or graft failure]. Graft delamination refers to a loosening, either partial or total, of the graft from the subchondral bone and from the surrounding cartilage. A total graft delamination is a serious complication and the patient may experience locking, pain and swelling after an acute distortion of the knee.

An overview of all AEs of interest is provided in Table 23. The proportion of patients with at least 1 AE of interest was 9.7% in the MACI group and 4.2% in the MF group. Haemarthrosis was the only AE of interest reported in more than 1 patient in any treatment group (2 patients [2.8%] in the MACI group and 1 patient [1.4%] in the MF group).

Table 31 Adverse events of interest per system organ class and preferred term – Safety Set

n (%)	MACI N=72	Microfracture N=72
Any AE of Interest	7 (9.7)	3 (4.2)
Infections and Infestations	1 (1.4)	0 (0.0)
Post-operative wound infection	1 (1.4)	0 (0.0)
Injury, Poisoning and Procedural Complications	1 (1.4)	0 (0.0)
Graft Delamination	1 (1.4)	0 (0.0)
Musculoskeletal and Connective Tissue Disorders	3 (4.2)	2 (2.8)
Arthritis	0 (0.0)	1 (1.4)
Arthrofibrosis	1 (1.4)	0 (0.0)
Haemarthrosis	2 (2.8)	1 (1.4)
Respiratory, Thoracic and Mediastinal Disorders	1 (1.4)	1 (1.4)
Pulmonary embolism	1 (1.4)	1 (1.4)
Vascular Disorders	2 (2.8)	0 (0.0)
Deep vein thrombosis	1 (1.4)	0 (0.0)
Thrombosis	1 (1.4)	0 (0.0)

System Organ Class and Preferred Term are ordered alphabetically. AEs were coded using MedDRA Version 14.1. AEs of Interest: include potential peri-operative complications related to arthroscopy/arthrotomy and potential complications related to MACI implant. If a patient experienced more than 1 AE of interest with the same Preferred Term or Primary System Organ Class, each patient was counted at most once within each Preferred Term or Primary System Organ Class. AE=adverse event, MedDRA=Medical Dictionary for Regulatory Activities

An overview of the number of patients with subsequent surgical procedures (SSPs) was provided. The proportion of patients with at least 1 SSP was comparable for the 2 treatment groups (8.3% in the MACI group and 9.7% in the MF group). The difference between the 2 treatment groups was not significant (p=0.427). A significant effect for age (odds ratio=0.926, p=0.038) was shown such that having at least 1 SSP decreased in likelihood with increasing age. An effect for gender was close to significant (odds ratio=0.307, p=0.056) where having at least 1 SSP was less likely for females.

Table 32 Overview of subsequent surgical procedures – Safety Set

n (%)	MACI N=72	Microfracture N=72
Any SSP	6 (8.3)	7 (9.7)
1 SSP	6 (8.3)	5 (6.9)
2 SSPs	0	2 (2.8)

Frequency of SSPs per patient is calculated relative to the number of SSP hospitalisations per patient. SSP=subsequent surgical procedure

Laboratory findings

Clinical laboratory tests were not part of the safety assessments in this study.

Safety in special populations

Three patients were reported with pregnancy-related events. One patient in the MACI group and one patient in the MF group were reported with spontaneous abortion. These events were classified as serious, remote and unlikely related or not related to the study drug or the surgery. The third patient in the MF treatment group was reported with a non-serious event of gestational hypertension that was considered by the investigator as mild and not related to study treatment or to the surgery; the patient was treated with medication and the event resolved.

Safety related to drug-drug interactions and other interactions

No safety issue related drug-drug interaction was observed in this study. Given the nature and the intended local use of MACI no interaction with other medicinal products, food or other substance is expected.

Discontinuation due to adverse events

In both treatment groups, 1 patient (1.4%) prematurely discontinued the study due to TEAEs. In the MACI group, the patient was reported with impaired healing, arthralgia and headache. The patient discontinued due to event of impaired healing of the target knee; the event was considered by the investigator to be moderate, possibly related to study treatment and remote/unlikely related to overall surgery. In the MF group, the patient was reported with head injury, traumatic fracture, arthralgia and headache. The patient discontinued due to events of traumatic fracture and head injury; both events were considered by the investigator to be severe and not related to study treatment or to overall surgery.

Safety reported from the literature

The studies presented in support of the pivotal study, cover safety data obtained in approximately 500 patients treated with MACI for a defect located on the femoral condyles, trochlea, or patella with defect size varying between approximately 0.6 cm^2 and 22.0 cm^2. The duration of MACI post-treatment follow-up of safety varied between 4 weeks and 5 years. The majority of studies followed patients for at least 1 or 2 years after surgery.

In the 2-year follow-up study (Wood *et al.* 2006), involving 18 patients (20 knees, 25 defects), 5 adverse events (all serious) were reported in 4 patients. All 4 patients experienced knee-related serious adverse events; 1 patient experienced 2 serious adverse events (graft delamination and osteoarthritis) in the contralateral knee. The 3 remaining patients had MACI implanted on the patella. One serious adverse event (tendonitis) was considered by the investigator to be probably related to both the MACI procedure and the modified arthrotomy procedure; the other events (post-procedural haematoma and chondrolysis) were considered not related or unlikely related to the MACI implant product or arthrotomy. One patient died in a biking accident considered unrelated to MACI implantation.

The publication presenting 5-year patient data in 41 patients (44 knees, 53 defects) from the University of Western Australia (Ebert *et al.* 2011) indicated that 2 patients developed deep vein thrombosis in the early post-operative stage and 1 patient had post-operative haematoma. The events were treated and the patients recovered without sequelae. The patient with post-operative haematoma is the same patient as the one with serious post-operative haematoma in the 2-year follow-up study report. Data were available for 53 grafts (44 knees) at 3 months, for 50 grafts (41 knees) at 12 and 24 months, and for 46 grafts (38 knees) at 5 years. Hypertrophy was reported in 1/53 grafts (2%) at 3 months after surgery, 6/50 grafts (12%) at 12 months after surgery, 8/50 grafts (16%) at 24 months after surgery, and 6/46 grafts (13%) cases at 5 years after surgery. Four of the 6 hypertrophy cases at 5-year follow-up exhibited mechanical symptoms and subsequent knee pain and have undergone arthroscopic graft debridement, resulting in a decrease in knee pain and relief of symptoms. It is not clear to what extent these hypertrophic cases overlap with those in the 2-year follow-up study. There was 1 case of graft failure, despite encouraging MRI results at 3 months after surgery. It is not clear whether this patient is the same patient as the one with complete detachment during the 2-year follow-up.

In the 2-year follow-up study (Winalski *et al.* 2007, 20 knees, 25 defects), oedema-like marrow signal among recipients of femoral grafts was assessed for 10 grafts pre-operatively, 16 grafts at 3 months, 17 grafts at 12 months, 16 grafts at 24 months and 2 grafts at 36 months. The pre-operative incidence of oedema was 70% (7/10 grafts). The peak incidence was observed at 3 months after MACI implant surgery (88%, 14/16 grafts). After the third month, the incidence gradually declined to 44% (7/16 grafts) at 24 months. The majority of the oedema cases were mild or moderate. At 24 months, 11/16 (69%) grafts showed no or mild oedema-like marrow signal, whereas 5/16 (31%) grafts showed moderate or severe oedema-like marrow signal. Oedema-like marrow signal among recipients of patellar grafts was assessed for 4 grafts pre-operatively and for 5 grafts at the later timepoints. The pre-operative incidence of oedema was 50% (2/4 grafts). At 3 months after MACI implant surgery, 4/5 (80%) grafts demonstrated severe oedema and 1/5 (20%) grafts showed mild oedema. Four of the 5 grafts had at least 1 follow-up MRI assessment after Month 3; in all 4, the oedema-like marrow signal resolved completely (timing of assessment of outcome was not reported). Subchondral bone cysts were observed with femoral grafts only. Seven (35%, 7/20) femoral grafts showed subchondral cysts on the pre-operative MRI scan and the same percentage of grafts showed cysts on at least 1 post-operative MRI scan. Five femoral grafts (25%, 5/20) showed subchondral cysts at the last post-operative scan. All cysts were small and occurred at a low (1 or 2) number. Thin adhesions were observed in 2 knees with patellar grafts; in both cases, the patient had also undergone anterior cruciate ligament (ACL) reconstruction. Additionally, both knees showed focal areas of fibrosis anterior to the ACL grafts and it may be that the minor adhesive bands were part of this process. Both adhesions resolved by the final timepoint. None of the knees without ACL reconstructions showed any signs of capsular fibrosis. For these 18 knees, the infra-patellar fat pads, the most sensitive region for observing arthrofibrosis, appeared entirely normal. No adhesions were seen in patients with femoral grafts. Hypertrophy was observed with 2 femoral grafts in 2 patients. One of these patients had also undergone ACL reconstruction. In both patients, the hypertrophy was observed both at the 12-month and 24-month MRI scan and was not symptomatic. At the last MRI observation, 3/20 (15%) femoral grafts and 3/5 (60%) patellar grafts were partially absent and 1/20 (5%) femoral grafts and 0 patellar grafts were completely absent.

No adverse events were reported by the investigator during the 2-year follow-up study involving 21 patients (Marlovits *et al.* 2006). Post-operative swelling and effusion resolved in all patients within the first 4 weeks after surgery and were not rated as product-specific adverse events, because the events were considered part of the normal post-operative course. No post-operative infections were recorded. No patient had to be re-admitted to the hospital and no re-operation was necessary. No abnormal pathological values were observed in any of the 21 patients after surgery. No extensive blood loss (decreased red blood cells, haemoglobin, or haematocrit) or signs of acute inflammation (elevated white blood cells, thrombocytes, or C-reactive protein) were reported. Six patients had elevations of post-operative levels of C-reactive protein, which was considered normal in the context of the surgical procedure after arthrotomy. Data on subchondral bone oedema rates were only provided for femoral grafts and were available for 16 grafts pre-operatively, 15 grafts at 1 month, 14 grafts at 3 months, 16 grafts at 6 months and 17 grafts at 12 and 24 months. The pre-operative rate of oedema in recipients of femoral grafts was 31% (5/16 grafts). The peak incidence was observed at 3 months after surgery (71%, 10/14 grafts). After the third month, the incidence gradually declined to 24% (4/17 grafts) at 24 months. The majority of oedema cases were mild or moderate. There were 8 instances of severe oedema, occurring at baseline (pre-operative, 1 graft) and at 1 month (4 grafts), 6 months (1 graft), 12 months (1 graft) and 24 months (1 graft) after surgery, respectively. The severe cases from Month 6 onwards were in the same patient. Subchondral bone cysts were observed with femoral grafts only (1/17, 6%). Five defects showed subchondral cysts pre-operatively and all subchondral cysts resolved following surgery. In 1 of the 5 cases, the subchondral cyst re-occurred at 24 months after surgery. Adhesions were observed with 2/7 (29%) patellar grafts, including 1 patient with severe osteoarthritis

on the 1-month MRI scan. Both adhesions were present throughout most of the post-operative timeframe. No adhesions occurred in patients with femoral grafts. Hypertrophy was only observed in patients with femoral grafts (2/17, 12%; both cases resolved). At the end of the 2-year follow-up, 2/17 (12%) femoral grafts were partially absent. For 1 of the grafts, a late deterioration was observed from completely in position/present to partially absent. Data on the presence/absence of patellar grafts were not provided. At the end of the 5-year follow-up, 2 of the 21 patients in the study had been re-operated on with alternative treatments due to graft failure (Marlovits, 2010, AAOS Annual Meeting).

In a 2-year randomised study by Basad *et al.* 2010, which compared MACI (40 patients with a single defect) with MF (20 patients with a single defect), 1 patient in the MF group was reported with treatment failure and 1 patient in the MACI group had persistent pain after 12 months; a second-look arthroscopy revealed an even and firm regenerated tissue surface with good bonding to the surrounding tissue. Persistent subchondral oedema led to retrograde bone grafting, which relieved the pain. “Some” patients (treatment not specified) experienced slight swelling and inflammation of the knee after partial weight-bearing. Rest, local cryotherapy and non-steroidal anti-inflammatory drugs generally reduced the symptoms within a few days. There were no serious adverse events.

In the 2-year randomised study by Bachmann *et al.* 2004, which compared MACI (27 patients with a single defect) with MF (7 patients with a single defect), excessive growth beyond the level of surrounding cartilage was observed in 1 case of osteochondritis dissecans in a 14 year-old female patient in the MACI group. The binding of the regenerated material to adjacent bone or cartilage was good, with no cleft observed between implant and bone. A gap between the implant and adjacent cartilage was observed immediately post-surgery in 6/27 patients and in subsequent follow-ups in 4/27 patients. Marked articular effusions were seen relatively frequently immediately post-surgery (15/27 cases) and receded in all but 1 patient. Subchondral oedema in the bone bed under the regenerated tissue occurred immediately post-surgery in 3/27 patients and in subsequent follow-up in 5/27 patients. At 3 months after MF, strong-signal oedema or sclerotic channels in the bone bed were discernible in 3/7 patients. At 6 to 12 months post-surgery, the subchondral bone showed a normal signal and the bony bordering lamella was displayed as a sharp line.

In a 1-year randomised study by Bartlett *et al.* 2005, which compared MACI (47 patients, 53 defects) with CACI (44 patients, 59 defects), the rate of graft hypertrophy was 6% (3/47) in the MACI group compared to 9% (4/44) in the CACI group. In the MACI group, 1 patient developed symptomatic graft hypertrophy after 6 months which was treated by arthroscopic debridement. A further 2 patients developed hypertrophy of the graft which was debrided at 1-year arthroscopy. In the CACI group, 1 patient developed symptoms of painful catching at 9 months after surgery, secondary to graft hypertrophy and 3 cases were observed at 1-year arthroscopy. Two of the 53 grafts in the MACI group had failed at 1-year arthroscopy (1 at the medial femoral condyle and 1 at the patella). One patient in the MACI group developed superficial wound infection. Three patients in each treatment group required manipulation of the knee under anaesthesia. The authors noted that there were no significant general complications in any of their patients.

In the following publications, reporting on a total of 113 patients who received MACI implant, no adverse events or complications occurred (Cherubino *et al.* 2003, D’Anchise *et al.* 2005, Ebert *et al.* 2008, Zhang *et al.* 2006). In the non-comparative study by Anders *et al.* 2008, including 50 patients (58 defects), there were 11 cases of mild or moderate effusion and 8 arthroscopic revisions (in 8 patients) that were considered associated with MACI. Of these 8 arthroscopic revisions, 1 was due to hypertrophy of a femoral and a patellar graft in a single patient (requiring shaving), 1 was due to partial implant failure, 1 was due to partial implant detachment, 1 was due to implant failure/interposition implant and 2 were due to debridement. The reasons for the remaining 2 arthroscopic revisions were not provided.

Other reported complications and adverse events in non-comparative studies included 3 cases of partial or complete graft detachment in 2 studies in a total of 86 patients (85 analysed) (Ebert *et al.* 2010, Ebert *et al.* 2011, Marlovits *et al.* 2005) and 4 cases of graft failure in 3 studies in a total of 164 patients (150 analysed) (Behrens *et al.* 2006, Ebert *et al.* 2010, Ebert *et al.* 2011, Zheng *et al.* 2007).

In the Ebert publication (Ebert *et al.* 2011) with data for 70 patients (69 analysed), graft hypertrophy was reported in 3 (4%) patients at 3 months after surgery, 11 (16%) patients at 12 months after surgery and 19 (27%) patients at 24 months after surgery. It is not clear to what extent these cases involve the same patients. All hypertrophic cases remained asymptomatic. In the retrospective study by Zheng *et al.* 2007 in a cohort of 56 patients, 1 case of localised infection was reported. One patient in this study was reported to have died by unrelated means at 18 months after surgery. This patient was also reported in the 2-year follow-up of the pivotal investigator-initiated study Wood, 2006, Investigator Report.

Deaths

Three deaths were reported in the studies reported. In the investigator-initiated study (Wood, 2006, Investigator Report), a 56 year-old male patient died from closed head injury in a biking accident considered unrelated to the MACI implantation. The same patient was also reported in the retrospective supportive study publication by Zheng *et al.* 2007. In the 2-year prospective study by Bachmann *et al.* 2004, 1 patient died. No safety narrative is available for this patient so no further details were provided. In the 24-month prospective study series by Ebert *et al.* (Ebert *et al.* 2010, Ebert *et al.* 2011), 1 patient died in a motor vehicle accident. The timing of death is not clear from the publications: the 2010 publication reports that the patient died at 3 months after surgery, while the 2011 publication states that this was at 7 months after surgery. The patient was excluded from the study analyses. No safety narrative is available for this patient so no further details were provided.

AEs of interest

In general, the adverse events of interest considered to be associated with MACI (symptomatic graft hypertrophy and graft delamination) have not frequently been reported in the studies but the incidence of graft hypertrophy appeared to increase over time. AEs of interest considered to be associated with peri-operative complications (haemarthrosis, haematoma at surgical site, arthrofibrosis, localised surgical site inflammation, localised surgical site infection, thromboembolic events and knee pain [arthralgia]) were not frequently reported. Haematoma at the surgical site was reported in the investigator-initiated study (Wood *et al.* 2006) (1/18 patients in the 2-year follow-up). In a publication presenting 5-year patient data from Ebert *et al.* 2011, the same patient was also reported (1/35 patients). The event was reported as serious. In the pivotal study by Basad *et al.* 2010, “some” patients (treatment not specified) were noted with swelling and inflammation after partial weight-bearing. Details on the number of patients were not provided. The events were reported as non-serious. In several of the studies presented, cases of oedema (Wood *et al.* 2006, Marlovits *et al.* 2006, Bachmann *et al.* 2004, Ronga *et al.* 2004) or cases of swelling or effusion (Marlovits *et al.* 2006, Bachmann *et al.* 2004, Anders *et al.* 2008) were reported. For most cases, symptoms were mild or moderate and decreased with time. There was one case of superficial wound infection, corresponding to an incidence of 2% (1/47 patients) (Bartlett *et al.* 2005) and 1 case of localised infection (1/56 patients) (Zheng *et al.* 2007). No further details were provided for these 2 patients. In the publication presenting 5-year patient data (Ebert *et al.* 2011), deep vein thrombosis occurred in 2 patients in the early post-operative stages (2/35 patients). The patients were treated and recovered without sequelae. In the publication presenting 5-year patient data (Ebert *et al.* 2011), 4/6 hypertrophy cases after 5 years of follow-up were reported as ‘exhibiting mechanical symptoms and subsequent knee pain’ (4/35

patients). In the study by Basad *et al.* 2010, 1 patient (1/40 patients) was reported with "persistent pain and subchondral oedema" following MACI surgery.

Post-marketing experience

Prior to the introduction of the Advanced Therapy Medicinal Products (ATMP) Regulations, MACI was available in certain European countries since 1998 (i.e. Austria, Belgium, Denmark, Germany, Greece, Ireland, Italy, The Netherlands, Norway, Portugal, Spain and the United Kingdom) in accordance with national legislations. MACI has been available also in Australia and parts of Asia. In 2005, the Applicant acquired the Verigen Corporation and the MACI implant technology. Until that time, approximately 4,000 patients in Europe and Australia had been treated with MACI. Prior to 2005 there was no formal safety reporting process available for MACI or required. As a result, there was only anecdotal information and no formal documentation. More than 5,000 patients worldwide have been treated with MACI since the applicant acquired MACI in 2005. Upon acquisition in 2005, the Applicant introduced a safety reporting system for MACI. All safety events discussed are those reported to the Genzyme Global Safety Database from 2005 to the cut-off date for this submission (17 March 2011).

As of 17 March 2011, a total of 43 post-marketing case reports including a total of 72 adverse events in association with MACI have been reported. These reports included 28 case reports (48 adverse events) from Europe and 15 case reports (24 adverse events) from Australia. Of the 72 adverse events in the 43 case reports, 40 events were serious and 32 were non-serious. Of the 40 serious adverse events, 23 were reported for 18 patients in the EU; 6 of the serious adverse events were considered by the reporter as possibly or probably related (possible: C-reactive protein increased and joint effusion; probable: synovitis, arthralgia, graft complication and implant site oedema).

The adverse events for which a potential relationship with MACI cannot be excluded, in decreasing order of frequency reported from post-marketing data, include graft delamination or complications, local complaints of pain in the grafted knee, hypertrophy, swelling, effusion, infection, synovitis, joint crepitation and oedema. Other post-marketing case reports that have been received by the applicant describe a cerebrovascular accident with sudden vision loss (reporter causality: unrelated), a cerebrovascular accident with hemiplegia (reporter causality: unrelated), a fatal pulmonary embolism (reporter causality: unassessable), a deep vein thrombosis (reporter causality: remotely/unlikely) and a deep vein thrombosis with pulmonary embolism (reporter causality: remotely/unlikely).

Deaths

A post-marketing event of pulmonary embolism causing death was reported for 36 year-old male patient. The patient had died approximately 5 weeks after implant. The surgeon did not provide information regarding the relationship between the AE of pulmonary embolism and MACI treatment. The causality was reported as not assessable.

2.6.1. Discussion on clinical safety

Safety reported from the SUMMIT study (MACI00206)

In the SUMMIT trial the proportion of patients with at least 1 TEAE was lower in the MACI group (76.4%) than in the MF group and (83.3%). The most common TEAEs were arthralgia (51.4% in the MACI group versus 63.9% in the MF group), headache (18.1% versus 29.2%), nasopharyngitis (13.9% versus 9.7%), back pain (11.1% versus 9.7%) and cartilage injury (4.2% versus 12.5%). The incidence of TEAEs considered related to study treatment was comparable between the 2 treatment groups (34.7% in the MACI group and 38.9% in the MF group). The most common related event was

arthralgia (26.4% in the MACI group and 31.9% in the MF group). In both treatment groups, most TEAEs were of moderate or mild intensity. The proportion of patients with at least 1 TEAE of severe intensity was lower in the MACI group (9.7%) than in the MF group (13.9%). One patient (1.4%) in each treatment group discontinued the study prematurely due to TEAEs. Treatment-emergent SAEs were reported more frequently in the MF group (26.4%) than in the MACI group (15.3%). The difference in incidence rates was mainly due to more serious cases of treatment failure, cartilage injury and arthralgia in the MF group compared with the MACI group. No deaths occurred in the study. The proportion of patients with at least 1 AE of interest was 9.7% in the MACI group and 4.2% in the MF group. These events include potential peri-operative complications related to arthroscopy or arthrotomy and potential complications related to the product. Haemarthrosis was the only AE of interest reported in more than 1 patient in any treatment group (2.8% in the MACI group and 1.4% in the MF group). The other events of interest reported in the MACI group were postoperative wound infections (1), graft delamination (1), arthrofibrosis (1), pulmonary embolism (1) and thrombosis (2). The proportion of patients with at least 1 SSP was comparable for the 2 treatment groups (8.3% in the MACI group and 9.7% in the MF group).

During the procedure the applicant submitted safety results from the SUMMIT Extension study (data cut-point: 15 August 2012). In the extension study, the number of patients with TEAEs reported beyond 2 years post-treatment was not substantially increased from that reported in the SUMMIT study. The proportion of patients with at least 1 TEAE remained lower for patients in the MACI group (77.8%) than in the MF group (84.7%). When considering the cumulative safety data from the core study and the its extension, the most common (>10% of patients in any treatment group) TEAEs were the same as that in the core study: arthralgia (52.8% in the MACI group versus 65.3% in the MF group), headache (19.4% versus 29.2%), nasopharyngitis (13.9% versus 9.7%), back pain (11.1% versus 9.7%), and cartilage injury (5.6% versus 15.3%). In the SUMMIT Extension study, there have been no cases of graft hypertrophy/overgrowth reported and no additional cases of graft delamination or arthrofibrosis have been reported for patients treated with MACI. The severity of TEAEs was consistent from that reported in the SUMMIT study. No deaths occurred as part of the Extension study and the TESAEs were reported more frequently in the MF group (30.6%) than in the MACI group (22.2%). The difference in incidence rates was still mainly due to more serious cases of treatment failure, cartilage injury and arthralgia in the MF group compared with the MACI group. When considering the cumulative safety data from the SUMMIT study and the SUMMIT Extension study a favourable safety profile for MACI continues to be shown beyond 2 years post-treatment. It is still apparent that, compared with MF, fewer patients treated with MACI reported TEAEs and TESAEs than those treated with MF. For both treatment groups, no additional patients have discontinued the study due to a TEAE and there has not been a substantial increase in TEAEs and TESAEs reported. The results thus far from the SUMMIT Extension study are consistent with those of the SUMMIT study.

Per the data in the applicant global safety database (patient exposure of 6,020 patients), the most frequently reported AEs of interest were graft delamination at 17 cases (reported frequency of 0.30%) and symptomatic graft hypertrophy at 13 cases (reported frequency of 0.22%); the remaining AEs of interest were reported at a frequency of 0.10% or less. Although this seems considerably lower than has been reported it is unlikely that all AEs in patients treated with MACI in routine clinical use have been reported to the applicant's Global Safety Database, and as such the reported frequencies may represent an underestimation of the actual frequencies. Nevertheless it is reassuring to note that neither the pivotal SUMMIT study nor the SUMMIT Extension study has shown any case of graft hypertrophy.

In the pivotal trial, two cases of haemarthrosis were reported for MACI out of 72 patients (2.7%). Antithrombotic agents were used in 11% of the patients' trial and NSAIDs in more than 50% of the subjects. It is uncertain whether these cases could have been related to the use of anticoagulants or

NSAIDs. The use of thrombolytic prophylaxis may be difficult to avoid in post-operative clinical practice. Cases of thrombosis and embolus have also been reported following MACI procedure. Overall, Post-operative haemarthrosis occurs mainly in patients with a predisposition to haemorrhage or poor surgical haemorrhage control. It is therefore recommend that, as reflected in section 4.4 of the SmPC, the patient's haemostatic functions should be screened prior to surgery and that thromboprophylaxis should be administered according to local guidelines. Haemarthrosis may be a result of bleeding from the subchondral plate during the surgery. Bleeding through the subchondral plate should be avoided, but if it occurs, it must be controlled as recommended in the SmPC.

Infections were commonly reported in the overall data presented: local treatment guidelines regarding the use of antibiotic prophylaxis around orthopaedic surgery should therefore be followed as recommended in the SmPC.

No data of re-treatment after failure are currently available. Study MACI00206 allows re-treatment and follow-up of such patients. The study results concerning patient re-treatment will be available at a later stage as part of the final 5-year clinical study report for the extension study.

Many of the safety risk observed with MACI are related to the quality and accuracy of the surgical techniques used in administering MACI to the patients and to the aftercare and rehabilitation of patients. A controlled physiotherapy, including early mobilisation, range-of-motion exercises, and partial weight-bearing is recommended as soon as possible to promote graft maturation and to reduce the risk of post-operative thromboembolic events and joint stiffness. Following implantation, the patient should follow an appropriately controlled, phased rehabilitation programme as recommended by the treating physician based on the MACI rehabilitation manual as described in the RMP.

Safety reported from the literature

The studies submitted in support of the clinical safety of MACI covers approximately 500 patients with a defect size varying between approximately 0.6 and 22.0 cm^2 located on the femoral condyles, trochlea or patella. The exact number of patients could not be provided due to overlapping populations across publications. The exact range of defect sizes could not be derived, because some publications only provided the mean value. The duration of MACI post-treatment follow-up varied between 4 weeks and 5 years. The imaging safety measures included MRI and other radiographic assessments, such as graft hypertrophy, graft delamination, adhesions, oedema, synovitis and cysts. However, it appeared that imaging may have been carried out in response to a patient complaint and not systematically or the finding could have been incidental. The CHMP also noted that certain adverse events, such as oedema, effusion, swelling or pain could have been under-reported because these are considered expected findings following arthrotomy and not relayed specifically to the MACI implant. Also most studies presented did not provide data on AEs by seriousness. The study populations, surgical techniques, rehabilitation programmes, treatment of concomitant lesions, safety assessment (including definitions of AEs) and timing and duration of post-operative follow-up differed across studies. Details on prophylactic operative and peri-operative medications that are dependent on local practices at the treating centre (e.g. antibiotics), as well as details on the use of other minor concomitant treatments generally were not reported. Direct comparison between studies is also not possible. Overall, adverse events have not been evaluated in a systematic manner as would be in a controlled clinical trial. As a results an under reporting of AEs is likely and the safety observations reported are therefore regarded only as supportive of the pivotal trials results.

Review from the literature showed that generally AEs related to MACI treatment (graft delamination, graft hypertrophy; graft failure) were not frequently reported in the studies. The events reported were not indicative of a new safety signal and generally in line with events reported in the pivotal study (e.g.

synovitis, arthralgia). General complications related to surgical intervention (e.g. localised inflammation and oedema, localised infection, arthrofibrosis, haemarthrosis and thromboembolic events) were also reported. There were 3 deaths reported, however, none were reported related to MACI.

Safety reported from post-marketing experience

As of 2005 to the cut-off date 17 March 2011, a total of 43 post-marketing case reports including a total of 72 adverse events (40 serious, 32 non serious) in association with MACI have been reported. Of the 40 serious events reported in 30 patients, 6 were considered possibly or probably related (C-reactive protein increased, joint effusion, synovitis, arthralgia, graft complication and implant site oedema). One death (pulmonary embolism - causality: unassessable) occurred 5 weeks after treatment was reported. Graft delamination or complications, local complaints in the grafted knee of pain, hypertrophy, swelling, effusion, infection, synovitis, joint crepitation and oedema were identified as AEs for which a potential relationship with MACI cannot be excluded (decreasing order of frequency). Overall, the events reported were consistent with those reported in the SUMMIT trial in term of frequency and seriousness. The data did not indicate a new safety signal.

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

2.6.2. Conclusions on the clinical safety

The safety profile of MACI is supported by the results from the SUMMIT and SUMMIT extension studies in addition to those presented from the literature and the applicant safety database. In the SUMMIT study, the most frequently reported AE for patients treated with MACI or MF was arthralgia and the rate was lower for patients treated with MACI (51.4%) compared to MF (63.9%). Overall, the frequency of other reported common AEs, severe AEs and SAEs was low. In addition, the rate of treatment failure was low and was favourable for MACI treatment (1.4%) compared to MF treatment (5.6%). The results from the on-going SUMMIT Extension study (cut-off date 15 August 2012) are consistent with those of the SUMMIT study. Although it is unlikely that all AEs in patients treated with MACI have been reported in the published studies and as such the actual frequencies may be underestimated, the rates are reinforced by the low frequency at which AEs, including severe and serious AEs, were reported in the randomised, controlled SUMMIT study. The events reported from the post-marketing experience were consistent with those reported in the SUMMIT trial in term of frequency and seriousness. The data did not indicate a new safety signal.

Overall, the results of the SUMMIT study showed a favourable safety profile for MACI relative to MF. Despite MACI requiring 2 surgeries, compared with MF which requires 1, fewer patients treated with MACI reported TEAES and TESAEs than those treated with MF. The difference in rates of TESAEs was mainly due to more serious cases of treatment failure, cartilage injury and arthralgia in the MF group compared with MACI. The treatment groups were comparable regarding experience of related TEAEs and occurrence of at least 1 subsequent surgical procedure. Most treatment-emergent AEs were of moderate or mild intensity. Based on the exposure of more than 6000 patients to MACI treatment in the knee, complications may be related to the arthrotomy procedure, general complications related to surgical intervention, other knee pathology (such as ligamentous or meniscal pathology) or the biopsy. Other complications have been identified as causally related to MACI. Overall, the following important risks have been identified related to either MACI: symptomatic graft hypertrophy or graft delamination (complete or partial, possibly leading to loose bodies in the joint or graft failure); or peri-operative complications related to surgical intervention of the knee: haemarthrosis, arthrofibrosis, localised surgical site inflammation, localised surgical site infection or thromboembolic events.

Many of the risk observed with MACI are more related to the quality and accuracy of the surgical techniques used in administering MACI and to the quality of the rehabilitation of the patients. Following implantation, the patient should follow an appropriately controlled, phased rehabilitation programme based on the MACI rehabilitation manual as described in the RMP.

Due to the surgical nature of the underlying procedure, implantation with MACI has a major influence on the ability to drive and use machines. During the rehabilitation period that follows MACI treatment patients should refer to their treating physician and follow their advice.

Further data will become available to further characterise the long term safety of MACI in the treatment of from the 5 years extension study MACI00809. The extension study will provide 5-year follow-up for participants in the MACI00206 study. The final study report is expected in 2015 as detailed in the RMP. Data on re-treatment after failure may also become available.

The CHMP endorse the CAT conclusion on clinical safety as described above.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CAT considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

2.8. Risk Management Plan

The CAT received the following PRAC Advice on the submitted Risk Management Plan:

PRAC Advice

Based on the PRAC review of the Risk Management Plan version 5, the PRAC considers by consensus that the risk management system for autologous chondrocytes (MACI) in the treatment of for repair of articular cartilage defects in symptomatic patients, is acceptable. This advice is based on the following content of the Risk Management Plan:

Safety concerns

Table 33 Summary of the safety concerns

Summary of safety concerns	
Important identified risks	Related to MACI • Symptomatic graft hypertrophy • Graft delamination (complete or partial possibly leading to loose bodies in the joint or graft failure) Related to peri-operative complications • Haemarthrosis • Arthrofibrosis • Localised surgical site inflammation • Localised surgical site infection • Thromboembolic events
Important potential risks	• Intra-articular adhesions • Deep infection • Medication error
Important missing information	• Long term safety and efficacy • Safety in children • Safety in the elderly • Safety in pregnancy and lactation • Use in patients with osteochondritis dissecans (OCD), chondromalacia patellae (CMP), immunosuppressive agents • Use in patients re-treated with MACI or after treatment failure

Pharmacovigilance plans

Table 34: Ongoing and planned measures in the PhV development plan

Activity / Study title (type of activity, study title [if known] category 1-3)*	**Objectives**	**Safety concerns addressed**	**Status** **Planned, started,**	**Date for submission of interim or final reports (planned or actual)**
MACI00809 (SUMMIT extension study) Category 3	to examine the 5-year efficacy and safety of MACI, compared with arthroscopic microfracture, in patients who received study treatment in the pivotal Genzyme sponsored study (MACI00206) for treatment of symptomatic articular cartilage defects of the femoral condyle, including the trochlea.	Long term safety and efficacy	Started	31/12/2015
Retrospective investigation of	To establish the safety profile of MACI in children	Safety on children (PIP)	Planned (start date	In accordance with the PIP

safety and prospective investigation of safety and efficacy data in paediatric patients treated for cartilage defects with MACI (PIP) Category 3	with a closed femoral growth plate.		May 2013)	decision, study Last Patient Last Visit by 31/12/2017

*Category 1 are imposed activities considered key to the benefit risk of the product.
Category 2 are specific obligations
Category 3 are required additional PhV measures (to address specific safety concerns or to measure effectiveness of risk minimisation measures)

The PRAC, having considered the data submitted, was of the opinion that the proposed pharmacovigilance measures are sufficient to identify and characterise the risks of the product.

The PRAC also considered that the studies in the post-authorisation development plan are sufficient to monitor the effectiveness of the risk minimisation measures.

Risk minimisation measures

Table 35: Summary table of Risk Minimisation Measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Important Identified risks related to MACI implant		
Symptomatic graft Hypertrophy Graft delamination (complete or partial, possibly leading to loose bodies in the joint or graft failure)	Events added to section 4.8 of proposed SmPC	1. Limitation of the use of the product to adequately trained and experienced clinicians only, including a controlled distribution system to specialised (accredited) centres only. Selection and accreditation of centres by marketing authorisation holder and/or member states authorities. 2. Specific risk communication: The educational programs; informed consent forms; protocols, mechanisms and signs& symptoms of important identified or potential adverse reactions, ensuring that any patient who have received treatment prior to the age of consent or in need of information at a later stage will receive risk communication. 3. Training of healthcare professionals includes proper procurement, storage, handling, administration and indicative symptoms of important identified or potential adverse reactions, clinical follow-up (a rehabilitation protocol). Training involves in-service training by Genzyme using official documentation and accompanying resources (e.g., training slide presentation). Official sign-off of training is required before a biopsy is processed. 4. Use of traceability data for surveillance purposes (e.g. an established registry of batches of products distributed to a particular centre and its record linkage to the Pharmacovigilance database of reports received from that centre.
Important identified risks related to peri-operative complications		
Safety concern	**Routine risk minimisation measures**	**Additional risk minimisation measures**
Haemarthrosis Arthrofibrosis Localised surgical site inflammation Localised surgical site infection Thromboembolic events	SmPC section 4.4 (Special warnings and precautions for use) states that: Local treatment guidelines regarding the use of antibiotic prophylaxis around orthopaedic surgery should be followed. Events added to section 4.8 of proposed SmPC	Measures 1-4 as above, plus 5. The rehabilitation will minimize the likelihood of arthofibrosis
Important potential risks		
Safety concern	**Routine risk minimisation measures**	**Additional risk minimisation measures**
Intra-articular	SmPC section 4.4 (Special	Measures 1-4 as above, plus

adhesions Deep infection Medication errors	warnings and precautions for use) states that: Local treatment guidelines regarding the use of antibiotic prophylaxis around orthopaedic surgery should be followed.	5. The rehabilitation will minimize the likelihood of arthrofibrosis (as a result of intra-articular adhesions)
Important missing information		
Safety concern	**Routine risk minimisation measures**	**Additional risk minimisation measures**
Long-term safety and efficacy	Not applicable	Safety and efficacy profile is adequately presented in the proposed product label. Risk minimization activities to be re-evaluated when additional data becomes available.
Use in children	Section 4.2 of proposed SmPC states: The safety and efficacy of MACI in children less than 18 years of age have not been established.	None
Use in the elderly	Section 4.2 of proposed SmPC states: 'Elderly (over 65 years of age): The use of MACI in this age group has not been studied. The use of MACI in elderly with generalised degeneration of the cartilage or osteoarthritis is not recommended.'	None
Safety in pregnancy	Section 4.6 of proposed SmPC states: Pregnancy: Limited clinical data on exposed pregnancies are available. Conventional reproductive and developmental toxicity studies are not considered relevant, given the nature and the intended clinical use of the product. Given the local nature of the product, adverse effects of MACI on pregnancy are not anticipated. However as MACI will be implanted using invasive surgical techniques, implantation it is not recommended during pregnancy. Lactation: There are no data on the use of MACI during lactation. Given the local nature of the product, adverse effects of MACI on the nursing infant	None

	are not anticipated. However as MACI will be implanted using invasive surgical techniques, a decision must be made whether to discontinue breast-feeding taking into account the benefits of treatment for the woman and the risk to the infant. Fertility: There are no data on possible effects of MACI treatment on fertility.	
Use in patients with osteochondritis dissecans; chondromalacia patellae; using immunosuppressive agents	None	None
Use in patients retreated with MACI or re-treatment after graft failure	None	None

The PRAC, having considered the data submitted, was of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication.

In addition, the PRAC requested an updated RMP to be submitted within a year from the granting of the MA. The RMP is expected to reflect long term safety data for MACI from on-going SUMMIT extension study and the post-marketing experience.

In addition to the above RMP measure, the CAT has agreed to the addition of the following RMP measure:

Safety concerns

Table 36 Summary of the safety concerns

Summary of safety concerns	
Important potential risks	• Suboptimal biological activity of the chondrocytes implanted

Pharmacovigilance plans

Table 37: Ongoing and planned measures in the PhV development plan

Activity / Study title (type of activity, study title [if known] category 1-3)*	**Objectives**	**Safety concerns addressed**	**Status** **Planned, started,**	**Date for submission of interim or final reports (planned or actual)**
Study to validate a	Validate the newly	Suboptimal	Planned	31 December

newly developed potency assay Category 3	developed potency assay. Until the new potency and identity assays are validated the applicant should monitor new patients for safety and efficacy which could be linked with lack of validation of these parameters. The MAH should present updates in special section of the PSUR and RMP	biological activity of the chondrocytes implanted	(start date April 2013)	2016 (with periodic progress reports in line with PSUR timelines)

*Category 1 are imposed activities considered key to the benefit risk of the product.
Category 2 are specific obligations
Category 3 are required additional PhV measures (to address specific safety concerns or to measure effectiveness of risk minimisation measures)

The CHMP endorse the PRAC and CAT advice on the RMP.

2.9. 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.*

3. Benefit-Risk Balance

Benefits

Beneficial effects

MACI has been investigated in a prospective, randomised, open-label parallel-group trial (MACI00206) in 144 patients with Outerbridge Grade III or IV focal cartilage defects of the knee of 3-20 cm^2. Seventy-two patients received MACI and 72 were treated by MF technique. Results showed that after 2 years MACI was superior to MF treatment for symptomatic cartilage defect of the knee regarding the improvement of pain and function according to the KOOS scale (Knee Injury and Osteoarthritis Outcome Score). A clinically and statistically significant difference in the improvement from baseline to Week 104 was seen for the co-primary endpoint of KOOS Pain and Function (SRA) in patients treated with MACI compared to MF. The additional improvement of MACI over MF in change from baseline at Week 104 was >10 points for both Pain and Function (SRA).

The primary efficacy endpoint was corroborated by several other PRO measures and a responder analysis of the primary efficacy measures. Superior clinical efficacy was demonstrated for patients treated with MACI compared to MF on the remaining 3 KOOS subscales (ADL, QOL, and Other Symptoms) and other validated PRO measures included in the study (Modified Cincinnati Knee Rating System, SF-12 physical health score, and IKDC Subjective Knee Evaluation). The treatment effect observed is considered clinically relevant: significantly more patients treated with MACI (87.50%) met the responder analysis criteria (defined as improvement from baseline to Week 104 of at least 10 points in both KOOS Pain and Function [SRA]) than patients treated with MF (68.06%). In this study, one subject in the MACI arm was adjudicated as treatment failure, versus four in the MF arm, thereby confirming efficacy.

In a subgroup analyses of the pivotal study for the larger lesions (> 4 cm^2), MACI was superior to MF (KOOS response rates 97% vs. 77%), while a positive trend was seen for the co-primary efficacy parameter for both pain and function. Furthermore, also in the group with smaller lesions (< 4 cm^2), where MF is considered the treatment of choice, there was a benefit for MACI (KOOS response rates (78% vs. 61%). Overall, the benefit of MACI is not restricted to a particular size of lesion and can be used for lesions from 3 to 20 cm^2 as studied in the SUMITT trial (MACI00206).

Concurrent knee surgery, like meniscus surgery or anterior cruciate ligament reconstruction, may bias the primary outcomes of pain and knee function. The subgroup analyses in subjects without concurrent knee-surgery (about 67% of the overall study population, equally distributed over the two study arms) supported the robustness of the overall study outcome, i.e. that MACI is superior to MF regarding reduction in pain and improvement of function. The robustness of the overall study outcomes was also supported by a sensitivity analysis using a more stringent outcome, i.e. the KOOS-20 responder rates (defined as an improvement of 20 points of more from a KOOS scale of 100). Although statistical significance was not achieved, the point estimates indicate the same positive trend as for the overall analyses and primary endpoints.

The efficacy of MACI demonstrated in the pivotal study was generally supported by data reported from the literature. Notwithstanding methodological deficiencies of these published studies, they consistently reported a relevant improvement of pain and function of the knee that is persistent over the years.

Uncertainty in the knowledge about the beneficial effects

Both the MACI and MF groups have performed well according to the structural endpoints with good infill of defects as assessed by MRI and good quality repair tissue as assessed by the histology score but the difference was not statistically significant. Thus, the concept of superior cartilage repair by MACI over MF has not been confirmed by MRI and histology assessment. The evaluation of the association between structural endpoints (histology and MRI) and clinical efficacy (change from Baseline in KOOS Pain and Function [SRA]) at Week 104 showed a lack of correlation between the tissue characteristics and clinical improvement. There is no clear consensus on whether structural repair as measured by MRI or histology scoring systems is able to distinguish the true functional repair of cartilage defects, and hence be a meaningful surrogate for clinical outcomes. Improvements in clinical outcomes of pain and function, as observed in the study, remain the most important and clinically valid endpoints in cartilage repair studies.

As MACI consists in 2 surgical procedures while the comparative MF procedure consists in one surgical procedure, treatments could not be blinded in the pivotal study. Biases in pain and function scores cannot therefore be fully excluded. However, this study design (open label) and PRO measures, as reliable method to assess efficacy, are in line with the EMA guidance document EMA/CAT/CPWP/568181/2009. In addition, the treatment effect observed was considered as sufficiently robust.

The pivotal study is extended to 5 years (SUMMIT Extension study MACI00809). This will provide further data on the sustainability of the cartilage repair and maintenance of effect of MACI compared to MF over time. Long-term follow-up efficacy analyses will be completed at 3, 4, and 5 years post-treatment. A final study report is expected by 31.12.2015 as detailed in the RMP.

Risks

Unfavourable effects

The safety profile of MACI was supported by the results from the SUMMIT and SUMMIT extension studies in addition to those presented from the literature and the applicant's safety database. In the SUMMIT study, the most frequently reported AE with MACI or MF was arthralgia and the rate was lower for patients treated with MACI (51.4%) compared to MF (63.9%). Overall, the frequency of other reported common AEs, severe AEs and serious AEs was low. Fewer patients treated with MACI reported TEAES and TESAEs than those treated with MF. The difference in rates of TESAEs was mainly due to more serious cases of treatment failure, cartilage injury and arthralgia in the MF group. The treatment groups were comparable regarding experience of related TEAEs and occurrence of at least 1 subsequent surgical procedure. Most TEAEs were of moderate or mild intensity. In addition, the rate of treatment failure was low and was favourable for MACI treatment compared to MF treatment. Overall, the results of the SUMMIT study showed a favourable safety profile for MACI relative to MF. The results from the on-going SUMMIT Extension study (cut-off date 15 August 2012) were consistent with those of the SUMMIT study. The events reported from the post-marketing experience and the literature (in mind the methodological limitations) were also consistent with those reported in the SUMMIT trial in term of events, frequency and seriousness. The data did not indicate a new safety signal and were generally consistent with the safety profile of ACI.

Overall, based on the cumulated exposure of approximately 6000 patients to MACI treatment, complications may be related to the arthrotomy procedure, general complications related to surgical intervention, other knee pathology (such as ligamentous or meniscal pathology) or the biopsy. Other complications have been identified as causally related to MACI. The following important risks have been identified related to either MACI as reflected in the SmPC and RMP: symptomatic graft hypertrophy or graft delamination (complete or partial, possibly leading to loose bodies in the joint or graft failure); or peri-operative complications related to surgical intervention of the knee: haemarthrosis, arthrofibrosis, localised surgical site inflammation, localised surgical site infection or thromboembolic events.

Many of the risk observed with MACI are more related to the quality and accuracy of the surgical techniques used in administering MACI and to the quality of the rehabilitation of the patients. Following implantation, the patient should follow an appropriately controlled, phased rehabilitation programme based on the MACI rehabilitation manual as described in the RMP in order to promote graft maturation and to reduce the risk of post-operative thromboembolic events and joint stiffness.

Uncertainty in the knowledge about the unfavourable effects

Additional data will become available to further characterise the long term safety of MACI in the repair of symptomatic cartilage defects of the knee from the 5 years extension study MACI00809. The extension study will provide 5-year follow-up for participants in the MACI00206 study. The final study report is expected by 31.12.2015 as detailed in the RMP. Therefore, in agreement with the PRAC an updated RMP will be submitted within a year from the granting of the MA to reflect long term safety data from this on-going extension study and the post-marketing experience.

The applicant has proposed a potency assay specification which is consistent with the data used in the pivotal clinical trial. However, to further ensure the quality of batches manufactured, the applicant will develop a new potency assay validated against the ability to form stable cartilage as detailed in the RMP.

Benefit-risk balance

Importance of favourable and unfavourable effects

Superiority in pain and knee function was shown compared to MF technique in the randomised, controlled SUMMIT study and there were less graft failures in the MACI arm. The treatment effect observed is considered clinically relevant: significantly more patients treated with MACI (87.50%) met the responder analysis criteria (defined as improvement from baseline to Week 104 of at least 10 points in both KOOS Pain and Function [SRA]) than patients treated with MF (68.06%).

Two types of important unfavourable effects were identified. Those related to MACI implant: graft hypertrophy and graft delamination and those related to peri-operative complications: haemarthrosis, arthrofibrosis, localised surgical site inflammation, localised surgical site infection or thromboembolic events. The routine and additional pharmacovigilance and risk minimisation activities as described in the RMP are considered adequate to manage these risks. Additional data will become available to further characterise the long term safety of MACI in the treatment of from the 5 years extension study MACI00809. Data on re-treatment after failure may also become available.

Discussion on the benefit-risk balance

ACI techniques including MACI were already established in clinical practice, before the new regulation of advanced therapies became into force. Prior to the introduction of the ATMP Regulations, MACI was available in several European countries since 1998 in accordance with national legislation (i.e. Austria, Belgium, Denmark, Germany, Greece, Ireland, Italy, The Netherlands, Norway, Portugal, Spain and the United Kingdom).

In a randomised controlled study with 2-year follow-up, designed in accordance with the current guidelines in forces (EMA/CAT/CPWP/568181/2009) for the conduct of studies in knee articular cartilage repair, MACI has showed superiority over MF treatment for symptomatic cartilage defects of the knee with a range of defect sizes from 3.0 to 20.0 cm^2. A clinically and statistically significant difference in the improvement from baseline to Week 104 was demonstrated for the co-primary endpoint of KOOS Pain and Function (SRA) in patients treated with MACI compared to MF. The effect of MACI was not impaired for larger chondral lesions size (>4 cm^2) and not biased by concurrent knee surgery, according to subgroup analyses. The subjects were non-blinded by necessity, as the surgery techniques of MF and MACI are different. One limitation of the open label design is the potential bias introduced to PROs. Histology and MRI scores were similar between MACI and MF and the hypothesis that MACI leads to superior quality of hyaline cartilage repair compared to non-transplantation techniques like MF, has not been established. In the absence consensus on whether structural repair as measured by MRI or histology scoring systems is able to distinguish the true functional repair of cartilage defects, improvements in clinical outcomes of pain and function, as observed in the study, remain the most clinically valid endpoints in cartilage repair studies. It remains also more important that fewer subjects dropped-out because of treatment failure in the MACI arm, thereby confirming efficacy. Studies reported from the literature generally supported the results observed from the controlled pivotal trial. Notwithstanding methodological deficiencies they consistently reported a relevant improvement in pain and function of the knee that is persistent over the years.

Despite MACI requiring 2 surgeries, fewer patients treated with MACI reported treatment-emergent AEs and SAEs than those treated with MF. The difference in incidence of treatment-emergent SAEs was mainly due to more serious cases of treatment failure, cartilage injury and arthralgia in the MF group compared with MACI. The treatment groups were comparable regarding experience of related treatment-emergent AEs and at least 1 SSP. The results of this study are consistent with the known safety profile for MACI, including the safety information reported in the published literature. Overall,

based on the exposure of more than 6,000 patients to MACI, the 2 main risks related to MACI are symptomatic graft hypertrophy and graft delamination (complete or partial, possibly leading to loose bodies in the joint or graft failure). No case of graft hypertrophy was seen with MACI in this study while one case of delamination was observed. Other risks observed were peri-operative complications related to surgical intervention of the knee such as haemarthrosis and local infection. The routine (information in the SmPC) and additional (educational programs) risk minimisation activities as described in the RMP are considered adequate to manage these risks.

The results of this study are consistent with the known safety profile for MACI, including the safety information reported in the published literature. In conclusion, the safety data so far do not appear to raise significant safety concerns and are also consistent with the general safety profile of autologous chondrocyte implantation. Additional data will become available to further characterise the long term safety of MACI in the treatment of from the 5 years extension study MACI00809.

Overall, based on the efficacy and safety data presented for MACI, the benefit/risk balance of MACI is considered positive for the repair of symptomatic, full-thickness cartilage defects of the knee (grade III and IV of the Modified Outerbridge Scale) of 3-20 cm^2 in skeletally mature adult patients.

The CHMP endorse the CAT conclusion on Benefit Risk balance as described above.

4. Recommendations

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CAT considers by consensus that the risk-benefit balance of MACI in the treatment of the repair of symptomatic, full-thickness cartilage defects of the knee (grade III and IV of the Modified Outerbridge Scale) of 3-20 cm^2 in skeletally mature adult patients is favourable and therefore recommends the granting of the marketing authorisation.

Based on the draft CHMP opinion adopted by the CAT and the review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of MACI in the treatment of the repair of symptomatic, full-thickness cartilage defects of the knee (grade III and IV of the Modified Outerbridge Scale) of 3-20 cm^2 in skeletally mature adult patients is favourable and therefore recommends the granting of the marketing authorisation is and therefore recommends the granting of the marketing authorisation.

Conditions or restrictions regarding supply and use

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

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation. Subsequently, the marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of

Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

• 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.

If the dates for submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

• Additional risk minimisation measures

The Marketing Authorisation Holder (MAH) shall agree the content and the delivery of the Educational Program with the National Compentent Authority prior to the launch of MACI in that Member State. The MAH shall ensure, prior to the distribution of the product to a particular Healthcare Establishment, that all surgeons and other healthcare professionals involved in the handling and administration of MACI or its components, as well as those involved in follow-up of patients treated with MACI in the Healthcare Establishment, receive the educational pack.

The MAH shall ensure the traceability of each implant by using unique identification numbers assigned to each biopsy (Biopsy ID number), membrane and final MACI product (MAH ID number), as described in the Risk Management Plan.

The educational pack for healthcare professionals shall contain the following components:

- Summary of Product Characteristics
- Educational material on the surgical procedures
- Educational material on appropriate follow-up

The educational material for surgeons and other healthcare professionals involved in the surgical treatment of patients receiving MACI shall include the following key messages:

- Guidance on the selection of suitable patients for MACI treatment and the importance of using MACI only in the approved indication
- The importance of explaining to the patients:
    - The risks asociated with the surgical procedures and MACI
    - The need for clinical follow-up
    - The need for rehabilitation following articular cartilage repair

- The need to screen donors using patient questionnaires and laboratory tests for hepatitis C, hepatitis B, HIV and syphilis
- Details on biopsy procurement, and storage and handling of the biopsy harvest
- That MACI is an autologous product and should only be administered to the patient that the biopsy was taken from. Details on the receipt, storage and handling of MACI and its preparation for implantation, including cross checks of patient details and Biopsy ID and MACI product ID numbers
- Details of the implantation procedure
- Details of appropriate disposal of MACI implant trimmings or unused MACI implants
- Details on how to recognise the signs and symptoms of important identified or potential risks of the product
- Clinical follow up details

The training materials for healthcare professionals involved in the follow-up of patients treated with MACI shall include the following key messages:

- The need for rehabilitation following articular cartilage repair
- Details on how to recognise the signs and symptoms of important identified or potential risks of the product
- Details of the rehabilitation program

The CHMP endorse the CAT conclusion on the additional risk minimisation activities.

- **Obligation to conduct post-authorisation measures**

Not applicable.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

New Active Substance Status

The active substance is defined as the cultured engineered cells combined with a CE marked purified, resorbable porcine-derived, collagen type I/III membrane, as such MACI (cultured autologous human chondrocytes attached to the purified porcine-derived collagen type I/III membrane and confirmed positive for the expression of chondrocyte-specific marker genes) is considered an Advanced Therapy Medicinal Product (ATMP) not previously authorised as a medicinal product in the European Union therefore the new active substance claim made by the applicant can be supported.

Therefore, based on the CAT review of data on the quality properties of the active substance, the CAT considers that autologous chondrocytes seeded on to a collagen membrane is qualified as a new active substance.

The CHMP endorse the CAT conclusion on the new active substance status claim.