EMA/167433/2013
Committee for Advanced Therapies (CAT)

Withdrawal assessment report

Hyalograft C autograft

(Characterised viable autologous chondrocytes expanded in vitro, seeded and cultured on a hyaluronan-based scaffold)

EMEA/H/C/002657

Note

Day 120 Assessment Report as adopted by the CAT with all information of a commercially confidential nature deleted.

This should be read in conjunction with the "Question and Answer" document on the withdrawal of the application: the Assessment Report may not include all available information on the product if the CHMP assessment of the latest submitted information was still on-going at the time of the withdrawal of the application.

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu
An agency of the European Union

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
ACI	Autologous Chondrocyte Implantation
AE	Adverse Event
CCI	Characterized Chondrocyte Implantation
CI	Confidence Interval
CMH	Cochran-Mantel-Haenszel
FU	Follow-Up
HC	Hyalograft® C autograft
ICRS	International Cartilage Repair Society
IKDC	International Knee Documentation Committee
KOOS	Knee Injury and Osteoarthritis Outcome Score
LFC	Lateral Femoral Condyle
MFC	Medial Femoral Condyle
MF	Microfracture
MOCART	Magnetic Resonance Observation of Cartilage Repair Tissue
MRI	Magnetic Resonance Imaging
RCT	Randomized Controlled Trial
RR	Relative Risk
SAP	Statistical Analysis Plan
SE	Standard Error
SD	Standard Deviation
TLFs	Tables, Listings and Figures
WMD	Weighted Mean Difference

1. RECOMMENDATION

Based on the review of the data on quality, safety and efficacy, the Rapporteurs consider that the application for Hyalograft C autograft in the treatment of surgical repair of symptomatic cartilage defects of the femoral condyle (medial, lateral or trochlea), caused by acute or repetitive trauma, is not approvable since "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time.

The major objections precluding a recommendation of marketing authorisation, pertain to the following principal deficiencies are summarised as follows:

Multidisciplinary

Quality, non-clinical and clinical

1. Comparability of the commercial product and product batches used in clinical studies cannot be confirmed due to lack of quality detail (manufacturing process/IPC specifications/release specifications) for batches used in non-clinical and clinical studies and the comparability exercise that was conducted did not demonstrate comparability.
2. The impact of the growth factors and on the cell characteristics and functionality has not been properly addressed and there are safety concerns over the quality of the reagents.

Quality

1. Process validation: the ability to manufacture a product of consistent quality is not demonstrated.
2. Control of growth factors: adventitious agent safety and biological activity/functionality.
3. Characterisation.
4. Control of key intermediate, drug substance and drug product.
5. The potency assay is not sufficiently correlated to biological activity of the product and the specification limits are not appropriately justified.

Non-Clinical

See multidisciplinary question above**.**

Clinical

1. There are concerns with respect to the pivotal prospective cohort studies by Kon et al (2009 and 2011)
2. The lack of Randomised Controlled Trial (RCT) should be justified and additional information / data is required for the key clinical studies by Kon et al.
3. The proposed indication is not justified by data and further information is therefore requested.

4. In the absence of conclusive efficacy, lack of safety data is a major concern. Further information on non-serious events and narratives for Serious Adverse Events (SAEs) are requested.

Proposal for questions to be posed to additional experts

Proposal for inspection

GMP inspection(s)

The GMP status of the current production premises is satisfactory; the Applicant has provided the GMP certificate and full descriptions of the manufacturing site. However, the GMP status of manufacturing of the product lots used in the published studies (characterisation, non-clinical and clinical studies) is unknown and should be clarified. No GMP inspection is foreseen at this stage.

GCP inspection(s)

NA.

New active substance status

Based on the review of the data it is considered that the active substance: characterized viable autologous chondrocytes expanded in vitro, seeded and cultured on a hyaluronan based scaffold contained in the medicinal product Hyalograft C Autograph is to be qualified as a new active substance in itself, although this is not based on an assessment by the applicant. However, the applicant is asked to justify the status of Hyalograft C Autograft as a new active substance (see List of other concerns).

2. EXECUTIVE SUMMARY

2.1. Problem statement

Articular cartilage is highly organized avascular tissue composed of chondrocytes embedded within an extracellular matrix of collagens, proteoglycans and noncollagenous proteins which allows for painless low friction movement of the synovial joints. The avascular nature of cartilage limits the number of cells able to respond to trauma to existing chrondrocytes, thereby reducing the ability of cartilage to repair itself after trauma. Cartilage produced as part of the repair process tends to be more fibrocartilage than hyaline cartilage. The fibrocartilage has inferior biological and biomechanical properties compared with hyaline cartilage and may undergo changes consistent with extensive osteoarthritis if another supraphysiological blow is experienced.

Several options have become available to treat cartilage lesions, and the appropriate surgical repair technique is based upon clinical symptoms, patient characteristics and expectations, as well as the size and depth of the cartilage lesions.

The Autologous Chondrocytes Implantation (ACI) procedure is a recent technique first developed by Brittberg et al. in 1987. Articular cartilage is biopsied or taken from the patient's knee during arthroscopic surgery. The chondrocytes from the cartilage are then isolated and grown over a 6-8 week period. After a sufficient number of chondrocytes has been cultured, they are injected back into

the defect of the knee, where a periosteal flap is surgically affixed to cover the defect. The harvesting of the periosteal flap and its fixation is a complex, time consuming procedure entailing significant morbidity to the patient.

According to the Applicant, chondrocytes can be easily isolated from small biopsies and expanded *in vitro*, however when cultured in two-dimensional substrate they lose their characteristic phenotype, namely collagen type II and expression and the maintenance of a differentiate phenotype is improved by the use of a three dimensional scaffold.

2.2. About the product

Hyalograft C autograft is an Advanced Therapy Medicinal Product (ATMP) defined as a tissue-engineering product (TEP). Hyalograft C autograft is composed of expanded autologous chondrocytes harvested from patient healthy femoral articular cartilage and grown on the Hyalograft C scaffold. The scaffold is registered as class III medical device and is a non-woven pad composed of Hyaff11, a hyaluronic acid benzyl ester polymer. The product is supplied as square inserts (2 x 2 cm each) containing 4 million cells aseptically processed and buffered in RPMI (Rockefeller Park Memorial Institute) medium. The product is intended for the surgical repair of symptomatic cartilage defects of the femoral condyle (medial, lateral) or trochlea, caused by acute or repetitive trauma in adults and a subset of the paediatric population (≥ 16 years old with a closed growth plate).

Treatment with Hyalograft C autograft is a two-step surgical procedure. In the first step a cartilage biopsy is obtained arthroscopically from healthy articular cartilage of the patient's knee. Chondrocytes are isolated from the biopsy, expanded *in vitro* prior to being seeded onto the Hyalograft C scaffold. Hyalograft C autograft is then implanted via intra-articular arthroscopy or arthrotomy under sterile conditions. Hyalograft C autograft is available as implants of 2x2 cm^2, each one containing $4x10^6$ viable, autologous chondrocytes.

2.3. The development programme/compliance with CHMP guidance/scientific advice

The non-clinical development programme for Hyalograft C consisted of a series of *in vitro* and *in vivo* studies to investigate the scaffold itself, Hyaff11 (a hyaluronan derivative, the component of Hyalograft C scaffold) and Hyalograft C autograft (cells and scaffold). A total of 5 *in vitro* studies were performed to evaluate chondrocytes behaviour once cultured onto the Hyalograft C scaffold. An *ex vivo* experiment was also conducted to assess the functional integration of Hyalograft C cultured with chondrocytes with the surrounding tissue. The *in vivo* chondrogenic potential of Hyalograft C autograft was evaluated in six animal studies. In 4 studies an ectopic cartilage forming assay (ECFA) model in the nude mouse was used, while 2 studies were conducted in orthotopic animal models. In the ECFA models, cultured expanded human chondrocytes cultured on the Hyalograft C scaffold were then subcutaneously implanted in immune-compromised mice for up to three months.

The clinical program consists of data available from investigator-initiated studies and literature reports. To support this data the applicant has planned a pivotal Phase 3 randomised controlled study in Europe, designed following EMA Scientific Advice, entitled "A randomized active treatment-controlled, evaluator-blind multicenter study of a Second-Generation Autologous Chondrocyte Implantation (Hyalograft C autograft) to provide Treatment of Symptomatic Articular Cartilage Defects of the Femoral Condyle or the Trochlea"

This will be a multi-center study, involving up to 20 centers in Europe and the United States comparing Hylograft C (Group A) to microfracture (Group B) in a group of patients with articular cartilage defects 2-4 cm2 after debridement, classified as ICRS grade III or IV. Additionally a third open label arm will be included with defects larger than 4 cm2 after debridement, classified as ICRS grade III or IV.

The proposed timelines include start of enrollment from Q3 2012 to Q3 2014, interim analysis at Q4 2016 (24 months), submission of interim report to EMA Q1 2017 and submission of final report in Q1 2018.

The applicant has obtained scientific advice on the design of the planned clinical phase III study including choice of comparator and endpoints which is generally consistent with the CHMP recommendations.

2.4. General comments on compliance with GMP, GLP, GCP

GMP

Compliance with GMP is demonstrated.

GLP

The primary pharmacodynamics data for Hyalograft C autograft is based mostly on non-GLP *in vitro* studies and small animal models (rabbit), with some evidence from large animal models (ovine). These studies were non-GLP which is not in conformity with the pharmaceutical standards. However, these deficiencies were considered to be acceptable given the specificity of the development programme for this product. One study evaluating Hyalograft C implants in nude mice after 1, 2 and 3 months was conducted in compliance with the GLP regulations. The toxicology program for the Hyaff 11 biomaterial, a class III medical device, was conducted in accordance with the directive 93/42/EEC. These studies were performed in compliance with the GLP regulations.

GCP

The applicant has stated that this section is not applicable since there were no clinical trials conducted outside the European Union. However, it is difficult to assess the GCP status of the studies from the publications and the study reports provided and no conclusion can be drawn.

2.5. Type of application and other comments on the submitted dossier

- Legal basis

This is an application for a Marketing Authorisation to the European Medicines Agency (EMA) for Hyalograft C autograft, submitted by the applicant Anika Therapeutics through the centralised procedure falling within Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.
The legal basis for this application refers to Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application

- Accelerated procedure

The Applicant has applied for accelerated assessment according to Article 14(9) of Regulation 726/2004. The request for accelerated procedure was declined by the CAT in February 2012.

- Conditional approval

The applicant has applied for conditional approval on the basis for a positive benefit risk ratio supported by the likelihood of provision of comprehensive clinical data, unmet medical needs and benefits from immediate availability outweighing risks. (Please refer to the full Benefit/Risk evaluation in the Overview). The decision on the conditional approval can only be made after a positive B/R profile has been achieved.

- Exceptional circumstances

N/A

- Biosimilar application

N/A

- 1 year data exclusivity

N/A

- Significance of paediatric studies

Hyalograft C autograft is not considered an appropriate therapeutic option in children prior to skeletal maturity since an open growth plate enables an intrinsic articular cartilage repair not seen in in adults.

In accordance with Articles 7, 8, and 30 of Regulation (EC) No 1901/2006 and Article 23 of Regulation (EC) No 1901/2006 , the PIP Decision consists of a waiver and a deferred clinical study (PIP # EMEA-000736-PIP01-09).

The waiver applies to the paediatric population from birth to less than 16 years of age, for implant use, on the grounds that the specific medicinal product does not represent a significant therapeutic benefit over existing treatments.

The included paediatric population age group will range from 16 to less than 18 years of age.

At least 30 patients from 16 to less than 18 years with a closed growth plate will be included as part of the proposed clinical trial referred to above.

The date of completion will be by December 2017 and the completion of this study is deferred.

3. SCIENTIFIC OVERVIEW AND DISCUSSION

3.1. Introduction

3.2. Quality aspects

The drug substance manufacturing process essentially consists of extraction of chondrocytes from the patient biopsy followed by several round of cell culture expansion

The drug product is composed of two implant pieces of the active substance packed in transport medium on a thermo sealed tray, sealed with a Mylar film.

The applicant describes the manufacture as a continuous process from drug substance to drug product,. Validation of the washing steps have been conducted through validation of the removal of process-related impurities. No further validation is considered necessary. Sterilisation data for the primary packaging material is missing, as well as some other minor issues relating to transport validation. The transport medium used as an excipient is widely used in cell culture and should not pose any problems for the final product. The specifications for the excipient are acceptable.

The starting material for the drug substance is patient biopsy obtained by the surgeon at the clinical site and then shipped to the applicant's manufacturing site using a kit and instructions as provided by the applicant. The patient is screened for serological tests according to Directive 2006/17/EC before release of the cartilage biopsy for production. The Hyalograft C biodegradable scaffold is a white non-woven pad composed of hyaff11, a benzyl ester of hyaluronic acid, also manufactured by the applicant. It is available as 2x2cm pad packaged in a petri dish contained in a heat-sealed peel-pouch (supplied in a 1 or 2 pad presentation). This is a class III medical device and as such is certified for use by a Notified Body ('CE' mark). The scaffold is supplied sterile and according to specifications; a copy of the CE certificate has been provided.

The description of the manufacturing process for both the drug substance and drug product is considered limited and the available control measures suggest an uncontrolled process. There is insufficient detail of the operating conditions for each step (volumes used etc, with working ranges) which suggests poor process control. It is like that there are suitable operating conditions/SOP's in-house so the applicant is requested to provide assurance that standardised operating conditions are in-place.

Raw materials are not qualified for human use and the applicant should either provide information on the manufacturing and quality of those materials or change them into materials that are approvable for human use. In the latter case, additional comparability studies would be needed. In addition, both quality and non-clinical information show that the cells are continuously proliferating on the scaffold and neovascularisation of the cartilage tissue was found. Therefore, safety concerns related to the potential for uncontrolled growth should be addressed. As a result, a major objection is raised regarding growth factors (multidisciplinary quality/non-clinical/clinical).

A major objection is raised concerning the control strategy for the key intermediate (cell suspension) the drug substance and the drug product. Of particular concern is the lack of control on the cell expansion phase Details of the manufacturing process are considered commercially confidential according to Annex 1, point I.3 of EMEA/45422/2006. The approach taken by the company for process validation and comparability testing is unclear

This lack of control of the DS manufacturing process is also mirrored by the lack of control at the level of drug substance and drug product. Specifications for release testing should include identity, purity, potency, impurities, sterility, cell viability and cell number, unless suitably justified. The applicant should incorporate suitable testing into either the DS or DP steps and validate surrogate testing, although at this point in the products history it is advised to conduct identity/purity and potency on the DP.

The deficiencies in process control are a significant factor in the major objection raised for DS/DP process validation. It is considered the lack of suitable pre-set specifications and controls precludes a proper process validation exercise. More significantly in terms of process validation, the applicant has not performed an appropriate validation exercise, i.e. the manufacture of 3 consecutive full-scale 'commercial' batches (maximum foreseen batch size). Instead the applicant has processed 5 biopsies,

divided each in half and processed each half at the maximum or minimum operating condition. This is a development study, not a validation study. In any event there are highly significant differences in the potency and viability values for min/max treatment for the 5 batches tested which is not discussed. The applicant must set appropriate specifications/validate each step during development and prior to appropriate validation. Of particular note is the lack of appropriate validation of the high population doubling level and the applicant is advised to define the PDL by looking at the effect on key quality parameters, not only the karyotype. In general, the applicant should consider conducting process validation using non-patient material where patient material is limited.

There are significant deficiencies in the characterisation of the drug substance and therefore a major objection is raised. In terms of characterisation of cells on the scaffold, the analysis is restricted to analysis of chemical degradation products of the scaffold and homogeneity of cell distribution on the scaffold so the combination product is poorly characterised in terms of key quality attributes. The applicant appears to have conducted limited characterisation studies (few reported in the non-clinical part) to identify the critical quality attributes on the actual key intermediate (cell suspension) DS or DP. Identity/purity is determined using a RT-PCR relative quantification method In short the identity/purity assay does not appear to be a suitable measure of chondrogenic potential (major objection). The assay methodology also requires further demonstration of validity. There are also issues regarding the proliferation/differentiation status of cells on the scaffold: cell viability increases over time with no apparent change in cell number which is unclear; according to the non-clinical section cells proliferate on the scaffold for up to 60 days and the CoII/CoIII ratios suggest the cells not to be differentiated in the 3D environment. In the non-clinical section, published information on the cell number: matrix relationship has been provided and the culture medium containing growth factors and higher cell density on the scaffold have been associated with a better outcome. However, the impact of the growth factors and on cell phenotype/genotype and functionality has not been addressed.

Apart from fibroblast contamination the applicant has examined additional cellular impurities. The reason to exclude the others is supported; however the requirement to include a specification for osteoblasts has not been clarified. The measurement and specification limits for protein purity should be further justified.. The assay should be replaced by some other method, if removal of process-related impurities cannot be demonstrated through process validation. Furthermore, information on the assays used for testing of residual growth factors after washings should be provided and as most of the impurities are biologically active, recombinant human proteins, the applicant should demonstrate the reliability of the assays for the intended use.

Even though the product is based on autologous cells, combined with a matrix, the company should consider establishing a cell bank of healthy chondrocytes, which could be grown in a small satellite matrix piece along with the DS manufacturing. This is seen important, as the production involves many raw materials. . All these molecules are part of signalling cascades in human cells and the very few tools the applicant has to control the complex product are not sufficient without any reference material.

A major objection is raised regarding the potency assay. The company has not studied the level of expression needed to ensure proper cartilage forming capacity of the product. The specification for potency () was not justified in terms of the batch data or for values from batches tested in clinical trials (the applicant provides no information on quality data for batches used in clinical trials) so this potency assay has not been correlated with pre-clinical or clinical efficacy.

A major objection is raised concerning comparability. Comparability of the commercial product and product batches used in clinical studies cannot be confirmed due to lack of quality detail (manufacturing process/IPC specifications/release specifications) for batches used in non-clinical and clinical studies. In addition, an appropriate comparability exercise has not been conducted. Several

changes have been made during manufacture.. A comparability exercise was conducted comparing a "minimum" process versus a "maximum" process, where the incubation/culture times and number of certain steps varied. In these experiments potency was considerably decreased for all batches produced by the new process () although viability was significantly increased for all batches (). The applicant does not discuss these differences, stating the product is the same and no further clinical testing is required. This is not supported and further information/data is required to demonstrate comparability of the clinical materials and the commercial product.

The applicant claims a shelf-life however this is not supported by the limited evidence supplied. The quality indicating parameters used to assess stability were viability and potency. The data for viability show considerable variability, however the more significant issue is with the potency assay. These issues are not discussed by the applicant. Even though the potency values are within specification, the specification is not justified. The applicant is advised to test a further range of stability indicating parameters.

The virus safety of Hyalograft C autograft depends entirely on testing of source material and reagents derived from human and animal materials used in the manufacturing process. Production steps with virus reducing capacity are not included, although the Applicant considers that washing steps might contribute some viral removal. The safety of the product in respect of potential adventitious agent contamination is controlled at several levels, namely the control on biopsy, raw and starting materials, including the FMEA risk analysis procedure for each raw material used and details of the scaffold, the auditing and control of the relative suppliers, a focus on a description of the materials of animal origin the adventitious agent testing procedure applied throughout the manufacture process, including a media fill validation study and a validation study for the absence of viruses and testing on the DS/DP.

Directive 2004/23/EC and the implementing directives 2006/17/EC and 2006/86/EC stipulate the minimum requirements for biopsy collection and testing to ensure compliance with current adventitious agent safety standards. It is considered the applicant has sufficiently demonstrated compliance. In-house QC testing of biopsy tissue consists of mycoplasma and microbial burden testing.

In terms of raw materials, the applicant states that material sourcing and control procedures are in accordance with GMP, with supplier qualifications established using a risk-based approach and a scoring method. As a result of the FMEA analysis all reagents were deemed to have an 'acceptable risk'. The overall approach for the control of raw materials appears suitable although the applicant is asked to provide the full risk-assessment report as the conclusion of 'acceptable risk' is not clearly demonstrated. Not all examples of CoO, CoA are provided and for some reagents it is not clear how sterility is assured as sterilisation/ testing appears not to take place. For the fetal bovine serum and the trypsin viral safety could be considered satisfactory provided that the Applicant can submit detailed description of gamma irradiation procedure and the virus validation for it.

The TSE safety of Hyalograft C autograft is not sufficiently demonstrated, since only for FBS a EDQM certificate is submitted. The Applicant should discuss the TSE safety for all animal derived material.

During manufacture, adventitious agent testing (mycoplasma, endotoxin and sterility unless stated otherwise) is performed Mycoplasma, endotoxin and sterility testing are carried out. However further information is required to demonstrate the validity of these methods, including the method for endotoxin testing. The final sterility results are not available at release, although the full QP release procedure has not been fully described and it is not clear if the sterility data are available before implantation. The adventitious agent testing regime appears acceptable, provided the assays are validated against standard Ph.Eur. methods and the applicant has shown that testing on media is a suitable surrogate for testing on scaffold, and that testing on transport media in contact with DP is suitable.

The Applicant has performed the validation for the aseptic process and for the manufacturing process for the absence of viruses.

In summary, provided the applicant can provide the additional information requested, the applicants control strategy for manufacture of the DP in terms of adventitious agent safety assurance to current standards appears acceptable.

Regarding the new active substance status, it is considered that based on the review of the data the active substance characterized viable autologous chondrocytes expanded in vitro, seeded and cultured on a hyaluronan based scaffold contained in the medicinal product Hyalograft C Autograft is considered a new active substance in itself, although this is not based on an assessment by the applicant. However, the applicant is asked to justify the status of Hyalograft C Autograft as a new active substance, a corresponding question has been added to Other Concerns.

Conclusions on the chemical, pharmaceutical and biological aspects

The raw materials used in the manufacturing process, particularly the growth factors, comparability, process validation, characterisation, quality control of the cell suspension DS/DP, validation of analytical tools and potency raise several concerns and 6 major objections are raised on quality grounds (including 2 multidisciplinary with non-clinical and clinical). The shortcomings hamper all conclusions on quality aspects and the overall control strategy is considered inadequate.

3.3. Non clinical aspects

Pharmacology

Pharmacological characterisation of Hyalograft C autograft consisted of in vitro functional assessment of human articular chondrocytes grown on Hyaff 11 scaffold, in vivo evaluation of Hyalograft C autograft implanted into nude mice, and evaluation of the ability of autologous chondrocytes grown on Hyaff 11 scaffold to repair cartilage defects in orthotopic rabbit, sheep and goat models.

In a series of in vitro characterisation tests it was shown that human articular chondrocytes expanded in vitro and seeded on Hyaff 11 scaffold first dedifferentiate in monolayer culture and lose their characteristic chondrocyte gene expression signature. After seeding onto the 3-dimensional Hyaff scaffold it is claimed that the cells maintain their proliferation capacity on the at least up to the longest observation time point 60 days. After 21 days on Hyaff scaffolds, the cells re-differentiate and start expressing collagen II, a marker for differentiated chondrocytes and markers of extracellular matrix while the expression of collagen I, a marker for dedifferentiated chondrocytes reduced. It is not known for how long the re-differentiated human chondrocytes maintain their proliferative capacity. This may be of concern as it is generally assumed that differentiated chondrocytes have a very low or absent proliferative capacity. If cell proliferation continues in vivo it may lead to hypertrophic growth of cartilage tissue, or even to uncontrolled growth of the transplanted cells. The Applicant is asked to provide data to show how long human chondrocytes cultured on Hyaff 11 scaffold continue proliferating. This is raised as part of the multidisciplinary major objection.

Different biomaterials were tested for their capability of supporting cell proliferation and re-differentiation into chondrocytes. Hyaff 11 appeared superior to its sulphate derivative Hyaff 11-S, porcine collagen scaffold and gelatin gel matrix in its ability to support re-differentiation of cells cultured in monolayer as determined by electron microscopy evaluation of cell phenotype and the

differentiation index. The ratio of collagen II/I was ~1 and remained stable during the observation period.

Proof of concept was demonstrated in orthotopic animal models in rabbits, sheep and goat using autologous chondrocytes seeded on Hyaff 11 scaffold. Most of the data collected come from a large cohort of rabbits, treated with various combinations of Hyaff11- based scaffolds and autologous cells. In a study with 18 rabbits autologous Hyalograft C scaffolds were implanted in the defects created in the medial femoral condyles. At 24 weeks after implantation, hyaline-like cartilage displaying a well-organized columnar architecture and synthesis of extracellular matrix was detected in the animals treated with Hyalograft C while lesions were covered with connective tissue and the biomaterial was completely degraded in the animals treated with scaffold alone. In the sheep model, different techniques such as fibrin glue, sutures and woven biomaterial to cover the implant were used. Spontaneous repair was observed in untreated control animals both at 3 and 6 months. Therefore, the ability of autologous chondrocytes to repair cartilage defect in sheep remains elusive. The data from the orthotopic goat model did not allow conclusions on the efficacy of the treatment in repairing cartilage defects.

The Applicant acknowledges the limitations of these animal studies and discusses that they cannot be considered truly representative to the clinical application, however given the time at which these studies were conducted they were the best available option.

There is a lack of detail regarding experimental methods used especially in relation to the culture conditions used to prepare the chondrocytes following biopsy and seeding onto the scaffold for most of the studies presented in the summary and the final reports. The Applicant is asked to provide details of the experimental methods, culture conditions, passage number, characterisation of the chondrocytes and determination of chondrocytes numbers in all of the in vivo studies conducted with Hyalograft C autograft. This is raised as a other concern.

Studies on secondary pharmacodynamics, safety pharmacology or pharmacodynamics drug interactions were not conducted

Pharmacokinetics

In line with the guideline on human cell based medicinal products (EMA/CHMP/410869/2006) conventional pharmacokinetics studies to investigate absorption, distribution, metabolism or excretion were not conducted for Hyalograft C autograft.

The Applicant argues that in autologous chondrocyte implantation products chondrocytes are maintained in the defect area by a with fibrin glue. Cells are physically retrained by adherence of cells on the scaffold. Chondrocytes on Hyalograft C autograft are terminally differentiated cells, with high affinity to the Hyalograft C scaffold. In addition the scaffold is contained in the joint and as the articular cavity has poor vascularisation and limited fluid exchange with the rest of the body and so there is very little possibility of these cells moving and distribute themselves in other tissue or organs.

Lack of biodistribution data might be acceptable as chondrocytes on Hyalograft C are differentiated cells grown and attached onto the Hyaff 11 scaffold. The likelihood that cells from the Hyalograft C autograft implant could migrate or distribute from the site of implantation is small as it is placed into the articular cavity which is a confined space with poor vascularisation. However, the currently available quality and nonclinical data raise a concern which might necessitate further elaboration. Nonclinical data indicates that the cells are continuously proliferating in vitro on the Hyaff 11 scaffold at least up to the longest observation time point while they are expressing markers for differentiated chondrocytes and ECM. It is unclear what these proliferating cells are and how long they are

proliferating, and do these proliferating cells have a migratory capacity. The Applicant is asked for further clarification and discussion on potential risk of hypertrophic growth of the implant in vivo or a risk of uncontrolled growth related to cell proliferation. See multidisciplinary major objection.

Toxicology

The Applicant uses data from the pharmacology studies to address the toxicological profile of Hyalograft C scaffold seeded with cells, which is acceptable given the specific nature of this therapy.

Assessment of safety of Hyalograft C implants manufactured according to the current manufacturing process was performed in nude mice in a GLP compliant study. Immunocompromised nude mice were implanted (subcutaneous tissue on the animals back) with Hyalograft C scaffold (non-woven HYAFF 11). Human chondrocytes were isolated from articular cartilaginous tissue biopsies, expanded in cultures and then seeded inside non-woven matrices of HYAFF11, the benzyl ester of hyaluronic acid. 1x106 chondrocytes were seeded onto each scaffold. The dose implanted was approximately 170 fold higher than that intended for use in humans (average weight of 70 kg) in terms of scaffold and approximately 800 fold higher in terms of cell number. There were no test article-related deaths during the study. Histological findings demonstrated a limited tissue response to the treatment in all the animals and at each end point (1, 2 and 3 months). In the implants at 2 or 3 months post implantation, cartilage maturation appeared to stop or even regress due to neovascularisation of the tissue and the presence of macrophages associated with reabsorption of the material. The Applicant is required to fully discuss this finding and the relevance to man

Data from orthotopic rabbit, sheep and goat models using autologous chondrocytes is limited to a small number of animals. The length of these studies ranged between 3 days and 24 weeks. In some of the studies animals were monitored daily swelling and redness of the operated joints and pain or hypersensitivity of the surgical area. No inflammatory response or any other adverse events correlated to the scaffold were observed. Histological examination of the implantation site showed that the Hyaff 11 scaffolds were present in the defects up to 4 weeks or less from implantation and had completely reabsorbed after 12 weeks.

No repeat application studies were conducted with Hyalograft C as autologous chondrocyte transplantation is indicated for single application only.

No genotoxicity studies were conducted with Hyalograft C. As stated in the guideline on human cell based medicinal products (EMEA/CHMP/410869/2006), genotoxicity, reproductive and developmental toxicity studies are not required for human cell based medicinal products.

The expansion of cells involves a combination of growth factors which can promote cell proliferation beyond the normal limits. Both quality and nonclinical in vitro data indicate that cells are continuously proliferating on Hyaff 11 scaffold at least up to the longest observation time point while they are expressing markers for differentiated chondrocytes and ECM. It is unclear what these proliferating cells are and how long they are proliferating, and do these proliferating cells have a migratory capacity. Continuous proliferation capacity may pose a risk of hypertrophic growth of the implant in vivo or a risk of uncontrolled growth. Moreover, quality characterisation of the cells is limited to karyotype analysis by G banding. Therefore, further clarification and discussion on potential risks is required. See multidisciplinary major objection.

The whole toxicological package was discussed with EMA during a Scientific Advice procedure (EMEA/H/SA/1233/1/2009/ADT/III) and in-line with this advice, it is agreed that conventional carcinogenicity studies are not feasible with Hyalograft C and that data from existing pharmacology studies could be used to address the issue of carcinogenicity. The Applicant should further discuss the

persistence of the cells after implantation in terms of tumourgenisty and carcinogenicity taking into account all non-clinical studies conducted.

No local effects were noted in rabbits, sheet or goats treated orthotopically with Hyalograft C.

A complete GLP compliant toxicological dossier was submitted on Hyaff11. The Hyalograft scaffold based on hyaluronic acid has been evaluated according to the ISO 10993 series standards for both short term and long term biocompatibility. No effects were seen in a single dose rat study (5000 mg/kg Hyaff11) or in a topical rat study at 2000 mg/kg.

Hyaff11 was tested in a number of genotoxicity studies. Over all Hyaff11 was considered non-genotoxic. Hyaff11 was considered a non-irritant in an acute rat eye irritation study, rabbit irritancy studies and in a guinea pig skin sensitisation and delayed dermal sensitisation study.

In a rat tissue response study tissue response following muscle implantation of HYAFF 11 in rats were examined for up to 5 months post-implantation. A macrophage-mediated response was seen. A mild inflammatory reaction was seen during the acute phase. Active phagocytosis of the dissolved material was observed. This inflammatory response, characterised by the presence of macrophages, was still persistent 5 months after the implantation. These were expected findings. These data along with the clinical data suitably support the use of HYAFF 11 for this therapy.

The immunogenicity of Hyaluronic acid and Hyaff11 was examined by inducing IgG and IgM response in mice following 4 injections. Hyaluronic acid did not provoke a humoral response, while HYAFF 11 showed slight immunogenic activity. No cross-reactivity of HYAFF 11 response to Hyaluronic acid was observed. In a complement activation study conducted with human plasma. Hyaff11 unable to trigger one of tree complement activation pathways (Bb. iC3b and SC5b-). This data along with the clinical data suitably support this Application.

Ecotoxicity / environmental risk assessment

Assessment of environmental risk from the degradation products hyaluronic acid and hippuric acid that could be released after implantation of Hyalograft C autograft was presented. The amounts of hyaluronic acid and hippuric acid that could be released were considered extremely limited, and therefore, it was concluded that Hyalograft C is not expected to pose a risk to the environment.

Discussion on non-clinical aspects

Non-clinical data provide a rationale for the Hyalograft combination product in terms of composition and ex vivo culture of the implant. Proof of concept for Hyalograft C in repairing of cartilage defects of femoral condyle was demonstrated in the orthotopic rabbit model in which hyaline-like cartilage displaying a well-organized columnar architecture and synthesis of extracellular matrix was detected in the cartilage defects after 24 weeks of implantation. Safety data with human articular chondrocytes is limited to nude mice implanted with Hyalograft C and evaluated up to 3 months. Implantation sites exhibited infiltration of macrophages involved in reabsorption of the biomaterial, and neovascularisation. Orthotopic rabbit, sheep and goat models using autologous chondrocytes provided supporting evidence of tolerability of the treatment.

The data indicating that cells are continuously proliferating on the 3-dimensional Hyaff 11 scaffold is concerning as differentiated chondrocytes are assumed to have very low or absent proliferative capacity. As a mixture of growth factors are used in the ex vivo expansion and culture on the scaffold,

the concern of abnormal cell behaviour should be addressed to exclude potential risks of hypertrophic growth of the implant or uncontrolled growth of the cells.

Additional information is required on experimental methods used especially in relation to the culture conditions used to prepare the chondrocytes following biopsy and seeding onto the scaffold for most of the in vivo studies. In addition persistence of the cells after implantation in terms of tumourgenisty and carcinogenicity requires discussion. In particular the histological data from all of the in vivo studies should be provided and discussed in terms of tumourgenisty/carcinogenicity.

Conclusion on non-clinical aspects

The non-clinical data package is considered limited and additional data on proliferative capacity of cells on the scaffold is requested to exclude potential risks of hypertrophic growth of the implant or uncontrolled growth of the cells (see multidisciplinary major objection).

3.4. Clinical aspects

Tabular overview of clinical studies

The clinical studies are summarised below in the section on efficacy

Pharmacokinetics

For the clinical development of cell-based ATMP such as Hyalograft C, conventional studies of absorption, distribution, metabolism, and excretion are not considered relevant in accordance with the EMA Guideline on Human Cell-Based Medicinal Products (EMA/CHMP/410869/2006). In addition, the EMA Reflection Paper on In-Vitro Cultured Chondrocyte Containing Products for Cartilage Repair of the Knee (EMA/CAT/CPWP/568181/2009) indicates that 'there is no clear common agreement for clinical kinetic data needed to be analysed in clinical setting'. The lack of clinical PK studies is therefore considered acceptable.

Pharmacodynamics

The applicant has submitted data for this application in the form of investigator reports and published literature with respect to efficacy which includes pharmacodynamic data in the form of histology, ICRS score and MRI studies.

Assessment of repair tissue by histology and magnetic resonance imaging (MRI) is considered appropriate for the pharmacodynamic assessment of autologous chondrocytes containing products as outlined in the EMA Reflection Paper on In-Vitro Cultured Chondrocyte Containing Products for Cartilage Repair of the Knee (EMA/CAT/CPWP/568181/2009). Histological and MRI assessments of repair tissue provide information on the extent of defect fill and hyaline or hyaline-like cartilage regeneration resulting from treatment. Volume of defect fill is a measure of the completeness of defect repair produced by the graft. Regeneration of hyaline or hyaline-like tissue is determined by histological analysis of biopsied tissue, using stains specific for collagens and proteoglycans. Another measure of hyaline or hyaline-like tissue development is MRI signal intensity, using specifically designed scan sequences or contrast media. Both histology and MRI assessments can provide information on other characteristics of the repair tissue such as the extent of repair tissue integration with surrounding native cartilage as well as a view of the surface of the repair tissue. Defect repair completeness is also demonstrated by graft integration with the underlying bone.

In contrast to first generation ACI products, second generation ACI products seed the chondrocytes on a bioresorbable matrix, such as hyaluronic acid or collagen, following which the ACI can be implanted at the defect site. In this respect the applicant has developed a scaffold delivery vehicle of hyaluronic acid, to facilitate implantation of chondrocytes without the need for an open surgical procedure. The intrinsic mucoadhesive properties of Hyalograft C 3D scaffold allow for differentiated chondrocytes of the Hyalograft C autograft to be retained at the lesion site. Hyalograft C autograft hermetically seals the seeded cells eliminating the requirement for a periosteal flap

Primary pharmacodynamics studies assessed the ability of implanted autologous chondrocytes to survive and maintain the phenotype for the cartilage repair. The Hyalograft C scaffold permits the maintenance of a differentiated phenotype which allows chondrocyte cells to express specific gene markers for hyaline articular cartilage, with an increase production in collagen type II mRNA expression. The results demonstrated that Hyalograft C scaffolds allow chondrocyte attachment and also provide adequate support to permit the expression of the differentiated chondrocytic phenotype.This finding was confirmed using cultured expanded human chondrocytes cultivated on Hyalograft C scaffold and then subcutaneously implanted in athymic mice for up to three months. Explants examination after implantation showed formation of hyaline-like cartilage.

Histology data

Cartilage repair tissue type results were reported in 6 studies after a follow-up time of slightly more than 12 month [Hollander et al, 2006, Podskuba et al, 2006, Marcacci et al, 2007, Marcacci et al, ICRS 2007 Ferruzzi et al, 2008, Gobbi et al, 2009]. Hyaline-like tissue was observed in 36/68 patients (52.9%) while fibrocartilage was observed in 17/68 patients (25.0%), and mixed-type was observed in 15/68 patients (22.1%), respectively.

The applicant lists 6 studies for histology data, but another study describing histology data for a substantial number of patients was also included (Brun P et al 2008), however, there is patient overlap with the other studies and therefore this is not included but is briefly outlined below.

The results are summarised in Table 1 below

Table 1: Histology outcomes after Hyalograft C

Short Ref	Surgical Technique	Time (months)	Number of Patients with Biopsies	Hyaline-like	Mixed	Fibrocartilage
Ferruzzi et al., 2008	arthroscopic ACI Hyalograft® C autograft	12	10	10 (100.00%)		
Gobbi et al., 2009	Hyalograft® C autograft	15.67	3	2 (66.67%)	1 (33.33%)	
Hollander et al., 2006	Hyalograft® C autograft	16	23	10 (43.48%)	3 (13.04%)	10 (43.48%)
Marcacci et al., 2007	Hyalograft® C autograft	13.5	2	1 (50.00%)		1 (50.00%)
Marcacci et al., ICRS, 2007	Hyalograft® C autograft	15.2	22	12 (54.55%)	6 (27.27%)	4 (18.18%)
Podskubka et al., 2006	Hyalograft® C autograft	10.5	8	1 (12.50%)	7 (87.50%)	

In the study by Marcacci et al (2007 ICRS) it is notable that the proportion of hyaline-like samples was found to be increased in the group of biopsies harvested after a minimum of 18 months from implantation than in those harvested at earlier times (83.3% and 43.8% respectively).

Furthermore, none of the biopsies harvested after 18 months were classified histologically as fibrocartilage. Tissue regeneration was found even when implants were placed in joints that had

already progressed to osteoarthrosis. It is also notable that evaluation was done in a blinded manner by 2 investigators.

In the second study by Marcacci (2007) histological evaluation was done after a short follow up of 12 months in only 2 patients and is therefore difficult to evaluate in such small numbers . Additionally it is not clear from the publication if evaluation was done in a blinded manner

It is particularly notable in the study by Hollander et al (2006) that the repair tissue was mature hyaline cartilage in 36% of biopsies taken from non-arthritic joints and 67% of biopsies from the osteoarthritic knees. Furthermore, 3 patients had advanced osteoarthritis (Ahlback score of IV on the V-point scale), and in 2 of th ese cases, the cartilage repair tissue was clearly hyaline. The authors also comment that, some patients can have a good clinical outcome despite generating fibrocartilage at the repair site, and the reasons for this discrepancy are not clear. It should also be noted that the evaluation was done in a blinded manner by 2 investigators.

A striking feature of the study by Ferruzi et al (2008) which compared ACI to Hyalograft C, appears to be the greatly increased proportion of patients showing hyaline cartilage in 89% of the samples from the open series and in 100% of the samples from the arthroscopic series. This means that all patients receiving hyalograft C showed hyaline cartilage at 12 months which seems to be at odds with most other studies. In this respect it is unclear if evaluation was carried out in a blinded manner.

In contrast, it should be noted that only in one case, mature cartilage of the hyaline type, was observed in the study by Podskuba et al (2006). The authors commented that the period of one year may be too short to allow complete rebuilding of the newly formed immature cartilage tissue into hyaline cartilage. Additionally, it was noted that the degradation of the carrier material is largely completed within approximately 10 months in the vast majority of patients. Furthermore, there is no indication that evaluation was blinded.

Only 3 patients were evaluated for histology at a mean of 14.75 months in the study by Gobbi et al (2009). Hyaline-like cartilage was noted in 2 of these, while the third patient showed mixed histology. It is difficult to evaluate the histology in such small numbers. Additionally it is not clear from the publication if evaluation was done in a blinded manner.

As mentioned earlier the study by Brun et al 2008 included an overlap of patients from other studies. However, histological analysis was carried out by 2-3 investigators who were blind to the treatment. It is particularly notable that the percentage of hyaline regenerated tissues was significantly greater in biopsies obtained after, versus before, 18 months of implantation (45.4% hyaline cartilage in the biopsies taken after 18 months compared to 23.7% hyaline cartilage in subjects with biopsies taken before 18 months). This proportion appears to increase with increasing time since implantation. Additionally, persistence of symptoms appeared to reflect the presence of a nonhyaline cartilage repair tissue. A notable feature of the histology studies appears to be the wide variation in the percentage of hyaline cartilage in the biopsies (12.5%-100%). It is not entirely clear whether this may have arisen as a result of the variation in cell viability and potency which has been observed over a period of time see Quality assessor's report).This also raises the issue whether the dose used is optimal since no dose finding studies were carried out during non-clinical and clinical development. Nevertheless, there appears to be some evidence of clnical correlation in that symptoms appear to reflect the presence of fibrocartlage. Although no biopsies were done for microfracture, the comparative study with first generation liquid culture ACI showed slightly better results for Hyalograft C. Nevertheless, it is not entirely clear how many patients were entered into the histology studies and those who actually had a biopsy which could introduce selection bias. It is also not entirely clear whether any of the histology assessments were done according to the ICRS histology evaluation scoring system.

MRI Data

MRI MOCART results were reported in 8 studies [Gobbi et al,2006, Kon et al 2010], Marlovits et al 2006, Trattnig et al, 2006, Domayer et al 2007, Ferruzzi et al 2008, Gobbi et al, 2009, Filardo et al, 2011] (Table 2).

Although Kon et al 2006 has been quoted by the applicant as providing MRI data in the summary of efficacy, this reference has not been provided in the list of literature references. However, an additional publication by Welsch GH. et al, 2010, not included by the applicant in the studies mentioned above is included below.

Table 2 MRI MOCART Individual Scores by Study

Short Ref	Surgical Technique	Time Group	Patients with MRI	Complete defect repair and filling	Complete integration to border zone	Intact surface of the repair tissue	Homogenous structure of the repair tissue	Isointense Dual T2-FSE signal intensity of the repair tissue
Domayer et al., ICRS, 2007	Hyalograft® C autograft	60 M	9	7 (77.78%)	9 (100.00%)	6 (66.67%)	6 (66.67%)	
Ferruzzi et al., 2008	arthroscopic ACI Hyalograft® C autograft	24 M	50		46 (92.00%)			
Ferruzzi et al., 2008	arthroscopic ACI Hyalograft® C autograft	60 M	50		47 (94.00%)			
Filardo et al., 2011	Hyalograft® C autograft	60 M	42	24 (57.14%)	26 (61.90%)	21 (50.00%)	18 (42.86%)	18 (42.86%)
Gobbi et al., 2006	Hyalograft® C autograft	24 M	32	23 (71.88%)				
Gobbi et al., 2009	Hyalograft® C autograft	60 M	24	17 (70.83%)				
Kon et al., 2010b	Hyalograft® C autograft	60 M	40	26 (65.00%)	26 (65.00%)	24 (60.00%)	22 (55.00%)	24 (60.00%)
Marlovits et al., 2006	Hyalograft® C autograft	24 M	13	8 (61.54%)	10 (76.92%)	9 (69.23%)	11 (84.62%)	12 (92.31%)
Trattnig et al., 2006	Hyalograft® C autograft	24 M	23	15 (65.22%)	18 (78.26%)			23 (100.00%)

Short Ref	Surgical Technique	Time Group	Patients with MRI	Isointense 3D-GE-FE signal intensity of the repair tissue	Subchondral lamina intact	Subchondral bone changes	Adhesions	Effusion
Domayer et al., ICRS, 2007	Hyalograft® C autograft	60 M	9			3 (33.33%)		
Ferruzzi et al., 2008	arthroscopic ACI Hyalograft® C autograft	24 M	50					
Ferruzzi et al., 2008	arthroscopic ACI Hyalograft® C autograft	60 M	50					
Filardo et al., 2011	Hyalograft® C autograft	60 M	42	20 (47.62%)	19 (45.24%)	26 (61.90%)	2 (4.76%)	6 (14.29%)
Gobbi et al., 2006	Hyalograft® C autograft	24 M	32			5 (15.63%)		3 (9.38%)
Gobbi et al., 2009	Hyalograft® C autograft	60 M	24			5 (20.83%)		7 (29.17%)
Kon et al., 2010b	Hyalograft® C autograft	60 M	40	23 (57.50%)	18 (45.00%)	20 (50.00%)	1 (2.50%)	15 (37.50%)
Marlovits et al., 2006	Hyalograft® C autograft	24 M	13	12 (92.31%)	11 (84.62%)	5 (38.46%)	1 (7.69%)	5 (38.46%)
Trattnig et al., 2006	Hyalograft® C autograft	24 M	23	23 (100.00%)	14 (60.87%)	9 (39.13%)		

In the 8 studies specified, data were reported for 118 patients at 24 month and 165 patients at 60 months, although not all patients were assessed for all endpoints, (see Table 3) . In this analysis 46 patients (67.7%) had a complete defect repair at 24 month and 74 patients (64.4%) at 60 month and a complete integration to the border zone was seen in 74 (86.1%) at 24 month and 108 (76.6%) at 60 month. Adhesions were observed in 1 (7.7%) at 24 month and 3 (3.7%) at 60 month and effusions in 8 (17. 8%) at 24 month and 28 (26.4%) at 60 month, (Table 4).

At the last follow up 233 patients underwent an MRI. In subjects with MOCART results, 120 (65.6%) had a complete defect repair at last FU and 136 (76.8%) had a complete integration to the border zone. Adhesions were observed in 4 (4.2%) and effusions in 36 (23.8%).

Table 3: MRI MOCART Individual Scores: Overall.

Time Group	Patients with MRI	Complete defect repair and filling	Complete integration to border zone	Intact surface of the repair tissue	Homogenous structure of the repair tissue	Isointense Dual T2-FSE signal intensity of the repair tissue	Isointense 3D-GE-FE signal intensity of the repair tissue	Subchondral lamina intact
24 M	118	46 (67.65%)	74 (86.05%)	9 (69.23%)	11 (84.62%)	35 (97.22%)	35 (97.22%)	25 (69.44%)
60 M	165	74 (64.35%)	108 (76.60%)	51 (56.04%)	46 (50.55%)	42 (51.22%)	43 (52.44%)	37 (45.12%)
Last Follow-up Overall	233	120 (65.57%)	136 (76.84%)	60 (57.69%)	57 (54.81%)	77 (65.25%)	78 (66.10%)	62 (52.54%)

Table 4: MRI MOCART and Defect Repair.

Time Group	Patients with MRI	Subchondral bone changes	Adhesions	Effusion
24 M	118	19 (27.94%)	1 (7.69%)	8 (17.78%)
60 M	165	54 (46.96%)	3 (3.66%)	28 (26.42%)
Last Follow-up overall	233	73 (39.89%)	4 (4.21%)	36 (23.84%)

Discussion on clinical pharmacology

The MRI data which have been provided related mainly to publications with respect to case series.

No MRI data have been provided from the pivotal cohort studies in comparison to microfracture. However, it should be noted that the full MOCART scoring system with all endpoints was used only in 3 studies which included Marlovits 2006, Kon et al 2010 and Welsch 2010, whereas the Henderson scoring system was used in the 2 studies by Gobbi et al 2006 and 2009. Only a proportion of patients who were entered into the studies went on to have MRI evaluation. Some studies provided image acquisition data, and few included assessment of the results by independent reviewers (1-3) who were in some cases blinded.

Four studies provided data showing clinical correlation with the MRI findings (Kon et al 2010, Marlovits 2006, Trattnig et al 2006 and Gobbi et al 2006).

In the study by Marlovits et al 2006, a statistically significant correlation was observed at 2 years between the clinical outcome and some of the radiological variables, including the filling of the defect, the structure of the repair tissue, changes in the subchondral bone and the signal intensities.

Kon et al 2010 reported that the total MOCART score was statistically correlated to the IKDC subjective evaluation A significant correlation between the signal intensity (3D-GE-FS) of the repair tissue and the IKDC subjective score values was also found, whereas other specific MOCART parameters did not show a statistically significant correlation with the clinical scores.

The publication by Gobbi et al did not specify the clinical correlations with MRI findings but importantly noted that pain was still present to a clinically important level (55 points on visual analog scale) in a clinically significant percentage (47%) of patients.

Filling of the defect on MRI and clinical outcome showed a good correlation in 21 patients and a poor correlation in only two patients was reported byTrattnig et al..

Notably, the publications by Marlovits et al and Kon et al did not specify that the independent reviewers were blinded; however, one independent observer who was blinded evaluated the MRI data in the publication by Gobbi et al.

From the limited MRI data which is available, there is therefore some evidence of clinical correlation, however, due to the limitations in the studies, it is not possible to draw firm conclusions, as with the histology results, it is not clear how many patients who entered the study actually had MRI and thus selection bias cannot be excluded.

Conclusions on clinical pharmacology

The pharmacodynamic data which have been submitted are consistent with the requirements as outlined in the CAT Reflection Paper on Chondrocyte products. These have included histology, MRI and macroscopic ICRS visual scores which together have provided data on the structural endpoints reported in some of the publications.

With respect to histology, 6 studies have been submitted with a total of 375 subjects of which 68 (18%) patients had biopsies for histological evaluation. Although the overall percentage of biopsies which showed the presence of hyaline cartilage was 53%, there was wide variation in the individual studies which ranged from 12.5% to 100 %. Mixed hyaline cartilage and fibrocartilage were observed in 25% and 22 % of subjects respectively. Since less than 20% of patients had biopsies there is the possibility of selection bias impacting on the results. Additionally, samples were obtained at varying time points. Furthermore, only 3 studies indicated that evaluation was done in a blinded manner. Nevertheless, treatment with Hyalograft C does show some evidence of efficacy with the presence of hyaline cartilage, which appears to increase with increasing duration of follow up, since the proportion of hyaline-like cartilage was found to be increased from the group of biopsies taken after a minimum of 18 months than those taken at earlier times (83.3% and 43.8 %) respectively (Marcacci et al 2007 ICRS). However, this is in stark contrast to the study by Ferruzi et al in which 100% of the patients showed hyaline cartilage at 12 months. It is not entirely clear whether this may be related to the wide variation in cell viability and potency over a period of time. Additionally, the large study by Brun et al showed that persistence of symptoms appeared to reflect the presence of non-hyaline cartilage. Thus there is some evidence to suggest that the quality of repair has clinical correlation.

With respect to MRI, data were reported for 118 patients at 24 months and 165 patients at 60 months.

It should be noted that the full MOCART scoring system with all endpoints was used only in 3 studies, and only a proportion of patients who were entered into the studies went on to have MRI evaluation.

However, using MOCART parameters the analysis showed that 46 patients, (67.7%) had a complete defect repair at 24 months and 74 patients (64.4%) at 60 months and a complete integration to the border zone was seen in 74 (86.1%) at 24 months and 108 (76.6%) at 60 months.

At the last follow up 233 patients underwent an MRI. In subjects with MOCART results, 120 (65.6%) had a complete defect repair at last FU and 136 (76.8%) had a complete integration to the border zone. Adhesions were observed in 4 (4.2%) and effusions in 36 (23.8%).

Some studies provided image acquisition data, and few included assessment of the results by independent reviewers (1-3) who were in some cases blinded.

Four studies provided data showing clinical correlation with some MRI findings (Kon et al 2010, Marlovits 2006, Trattnig et al 2006 and Gobbi et al 2006).

In the study by Marlovits et al 2006, a statistically significant correlation was observed at 2 years between the clinical outcome and some of the radiological variables, including the filling of the defect, the structure of the repair tissue, changes in the subchondral bone and the signal intensities

Kon et al 2010 reported that the total MOCART score was statistically correlated to the IKDC subjective evaluation A significant correlation between the signal intensity (3D-GE-FS) of the repair tissue and the IKDC subjective score values was also found, whereas other specific MOCART parameters did not show a statistically significant correlation with the clinical scores.

However, it is notable that although the publication by Gobbi et al did not specify the clinical correlations with MRI findings, pain was still present to a clinically important level (55 points on visual analog scale) in a clinically significant percentage (47%) of patients.

Filling of the defect on MRI and clinical outcome showed a good correlation in 21 patients and a poor correlation in only two patients (Trattnig et al).

From the limited MRI data which is available, there is therefore some evidence of clinical correlation, however, due to the limitations in the studies, it is not possible to draw firm conclusions.

The ICRS visual score was evaluated in only 3 studies. In two of these studies only a small proportion of the total patients were evaluated and thus there is a possibility of selection bias. Although the grade of repair does support efficacy in the very limited data provided, the results need to be interpreted with caution. The data do not show a correlation between the grade of repair and the histological cartilage type.

It is a highly unfortunate that the 2 prospective controlled observation studies did not provide any structural endpoint data either with respect to histology, MRI or macroscopic changes.

Nevertheless, taken together, the pharmacodynamic parameters including histology, MRI and macroscopic changes, provide some evidence of efficacy. However, due to a lack of stringency in the methodology of the studies, the results need to be interpreted with caution due to limitations in the studies as previously outlined which is also borne out to some extent from the wide variation in the results.

Clinical efficacy

The data for clinical efficacy is entirely based upon publications and investigator reports. No actual data from completed or ongoing randomised clinical trials have been provided, however, the applicant has

planned a comparative RCT comparing Hyalograft with microfracture for which CHMP scientific advice has been obtained and which is due to be completed in 2018 .The current dossier includes two prospective cohort studies which are considered as “pivotal” studies by the applicant. In addition, 26 other publications including investigator reports are provided as supportive data (see Table 6 below).

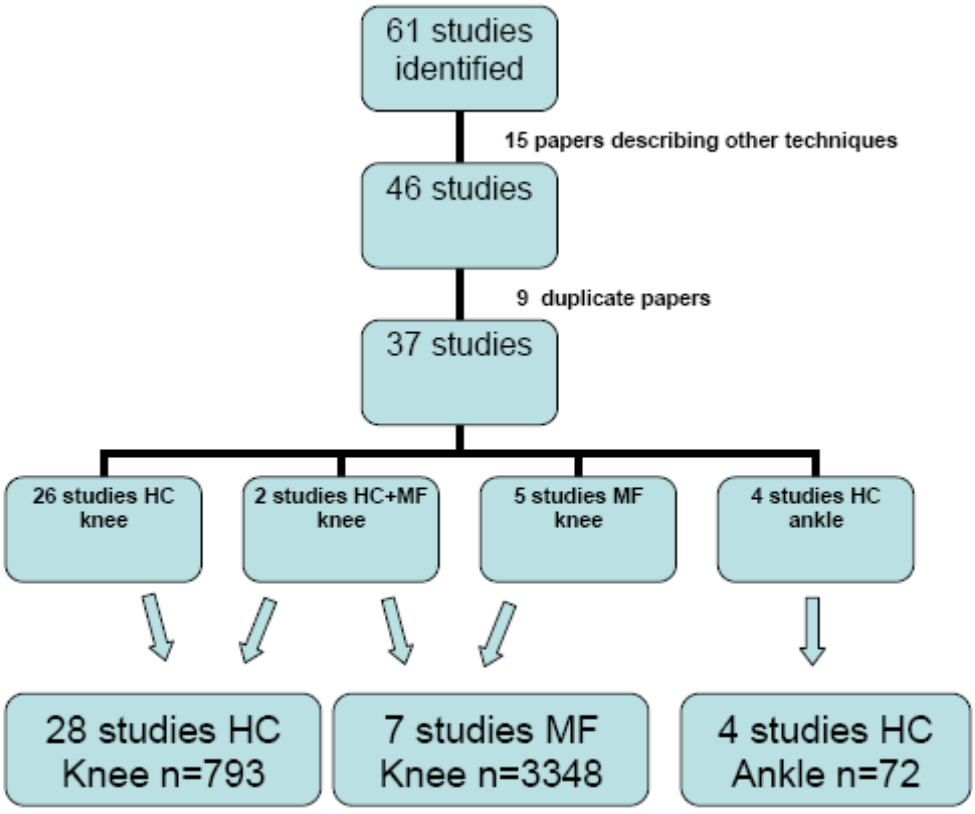
The applicant has carried out a systematic review and meta-analysis of these studies. A literature search was conducted in Pubmed to find all available Hyalograft® C autograft literature:

An evidence-based systematic analysis had been conducted by Mithoefer et al. in 2009 to assess the clinical efficacy of the microfracture technique for articular cartilage repair and long-term improvement of knee function. Twenty-eight studies describing 3122 patients were included in the review. Since Mithoefer et al utilized a search period from January 1, 1966 to October 31, 2007, references since this time period were included if not already included within the Mithoefer meta-analysis.

The clinical studies identified from literature search or available study reports were evaluated by two independent reviewers. Of these, only studies involving subjects treated with Hyalograft® C autograft and/or microfracture were included. All prospective cohort studies and prospective or retrospective observational studies with or without control groups were included. The studies including duplicate data were excluded (e.g. a publication of the same study at an earlier time point was excluded, if the same data was represented in the subsequent publication). However, the studies with common subjects, but reported at different follow-up time points were included, in which the subjects are counted once at a specific time point in the statistical analysis.

A total of 61 relevant studies were identified as full publications, abstracts and study reports (see Figure 1. Of these 26 articles reported results for Hyalograft® C autograft in the knee, 5 articles reported results for microfracture, 2 articles reported results from prospective cohorts studies comparing both Hyalograft® C autograft and microfracture and 4 articles in the ankle. Figure 1 presents the classification of studies. The 7 articles presenting results of studies for microfracture include a large evidence-based systematic analysis by Mithoefer et al. in 2009, in which results from 28 studies published between January of 1966 and October 31, 2007 (the cut-off used by Mithoefer et al were analyzed. Included in the microfracture analysis are 6 articles with data on microfracture which were published after October 31, 2007 and up to the cut-off of October 15, 2011.

Figure 1: Identification of studies



HC - Hyalograft® C autograft
MF- microfracture

Most of the studies were single arm observational studies evaluating the treatment effect over time. For Hyalograft C autograft no randomized controlled studies were conducted and reported. Two prospective comparative cohort studies were conducted by Kon et al., 2009 and Kon et al., 2011. These two studies provide the strongest evidence for the comparison of the treatment effect of Hyalograft C autograft versus microfracture. The conclusion of the comparison based on these two studies is supported by the indirect comparison of the results presented in separate single arm cohort studies in which the two studies were included as well for a complete analysis of all Hyalograft® C autograft and microfracture studies.

Patient Populations

In the Hyalograft C autograft studies in the knee, 793 patients were included with a mean age of 32 years compared to microfracture studies with 3348 patients included and a mean age of 38 years .The Hyalograft C autograft studies were conducted in Austria, Italy, and San Marino while the studies with microfracture were conducted in Belgium, Germany, Turkey, Italy, and Netherlands; the large evidence-based systematic review did not provide the information on the location of the studies.

In most studies a mixed population was enrolled regarding the cause of defect, the majority of the patient was traumatic, but also degenerative or osteochondritis dissecans patients were treated. Defect locations were mostly medial femoral condyle, lateral femoral condyle, patella and trochlea.

Efficacy Variables

The following efficacy variables have been initially identified as commonly used for evaluation of clinical outcome of cartilage repair. Since the systematic review and meta-analysis are data-driven processes, it is expected that the actual variables included in the final analysis could be different from the list below. Additional efficacy variables which are available in the publications were extracted and presented as appropriate.

The efficacy variables include the following:

- International Knee Documentation Committee (IKDC) subjective score
- IKDC objective score
- Tegner activity score
- Lysholm-Gillquist score
- Magnetic resonance observation of cartilage repair tissue (MOCART)
- Macroscopic and microscopic repair cartilage evaluation
- International cartilage repair score (ICRS)

It should be noted that the aetiology of the defects was described in some of the studies; often the cause of the lesion was not known or reported, while for some patients lesions could be linked with trauma, degenerative damage, or osteochondritis dissecans (OCD). The duration of symptoms prior to Hyalograft C treatment, previous surgical treatment, and the manner in which previous treatment was described was not consistent; the extent of patient experience of previous treatment varied within and between studies. Additionally, the timing of MRI and histology assessment and parameters assessed was not consistent across studies. Furthermore, the duration of follow-up varied across studies, although most included 1 or 2 year follow-up assessments.

The studies are summarised below in Table 6

Table 6 Details of all Studies for evaluation of Efficacy (Publications, Abstracts and Investigator Reports)

Type of the study	Study identifier	Location of Study Report	Level of evidence	Objective of the study	Study design and type of Control	Test product; Dosage; Type of surgical application	Number of Subjects	Diagnosis	Follow-up Duration	Study status; Type of Report
Efficacy	Kon et al. 2009[1]	5.3.5.1.1	2	To compare clinical outcome of HC with MF at 5 yrs fup	Multicenter (n=2), cohort prospective study	Hyalograft C autograft; arthroscopy (HC); Microfracture	80 40 HC 40 MF	Symptomatic chondral lesions of the femoral condyle or trochlea	Min 5 yrs	Published
Efficacy and safety	Kon MFX et al. 2011	5.3.5.1.1	2	To evaluate whether HC allows highly demanding athletes a better functional recovery compared with the bone marrow stimulation approach	Multicenter (n=3), cohort prospective study	Hyalograft C autograft (HC); arthroscopy; Microfracture	41 21 HC 20 MF	Symptomatic chondral lesions of the femoral condyle or trochlea	Mean 7.5 years (range 2-11 years)	Published
Efficacy and safety	Manfredini et al. 2007	5.3.5.1.2	4	To compare clinical outcome of HC with Carticel	Monocenter cohort study	Hyalograft C autograft; miniarthrotomy/arthroscopy; Carticel- arthrotomy	32 15 HC 17 Carticel	Chondral lesions of the femoral condyle, patella or trochlea	mean follow-up 48.5 months in the Carticel group and 13.5 months in the HC group	Published
Efficacy and safety	Ferruzzi et al. 2008	5.3.5.1.2	4	To compare clinical outcome of HC with Carticel	Monocenter prospective cohort study	Hyalograft C autograft; arthroscopy; Carticel arthrotomy	98 50 HC 48 Carticel	Posttraumatic chondral lesions of the femoral condyle or OCD	5 years	Published
Efficacy and safety	Kon MACI et al. 2011	5.3.5.1.3	4	To compare clinical outcome of HC and MACI in pts older than 40 yrs	Multicenter (n=2) prospective case series	Hyalograft C autograft; arthroscopy; MACI procedure (arthrotomy)	61 22 HC 39 MACI	Symptomatic chondral lesions of the femoral condyle	4.9 ± 0.9 years	Published
Efficacy and safety	DeWindt et al. 2012	5.3.5.1.3	4	to report on the clinical outcome of	Prospective clinical case series,	Hyalograft C Microfracture	166 Microfracture	Cartilage lesions on the femur, trochlea and patella	MF: 38 ± 5 months,	Published

Type of the study	Study identifier	Location of Study Report	Level of evidence	Objective of the study	Study design and type of Control	Test product; Dosage; Type of surgical application	Number of Subjects	Diagnosis	Follow-up Duration	Study status; Type of Report
				a large heterogenic cartilage repair population		Carbon-fiber Scaffolds	(n=65) Hyalograft (n=54) carbon-fiber scaffolds n=47		HC=36 ± 8 months Carbon fiber scaffolds 36 ± 6 months, respectively	
Efficacy and safety	Marcacci et al 2005[2,3,4, 5, 6]	5.3.5.2	3	To investigate the subjective symptomatic, functional and heath-related quality of life outcomes of patients treated with Hyalograft C.	Multicenter (n=11),observational, prospective investigator initiated	Hyalograft C autograft; arthrotomy/arthroscopy	192	Cartilage lesions caused by trauma/microtrauma/OCD	38 months	Published
Efficacy and safety	Marcacci et al 2007, ICRS[3],	5.3.5.2	3	To analyze clinical outcomes of HC at 4 years	Prospective	Hyalograft C autograft arthroscopy	192	Defects was localized on femoral condyles, trochlea	minimum 24 months follow up (47 of these patients achieved minimum 36 months follow-up and 21 patients minimum 48 months follow-up)	Abstract
Efficacy and safety	Pavesio et al, 2003[2,10]	5.3.5.2	4	To report on preliminary clinical findings	Multicenter (n=11),observational, prospective investigator initiated	Hyalograft C autograft; arthrotomy/arthroscopy	67	Femoral condyle, patella, tibial plateau	17.5 months	Published

Type of the study	Study identifier	Location of Study Report	Level of evidence	Objective of the study	Study design and type of Control	Test product; Dosage; Type of surgical application	Number of Subjects	Diagnosis	Follow-up Duration	Study status; Type of Report
Efficacy and safety	Nehrer et al, 2007[9]	5.3.5.2	4	To report on clinical results of HC at 5 years follow-up	Monocenter, prospective observational	Hyalograft C	51	Defects of the femoral condyles, patellar,	5 years	Abstract
Safety	Study CT/222/97-02 "Scapinelli report	5.3.5.2	4	Tolerability and applicability of HC	Monocenter, prospective, uncontrolled, pilot clinical trial	Hyalograft C autograft; arthrotomy	6	Traumatic cartilage lesions or secondary to OCD	6-18 months	Concluded, Final Report
Safety	Study CT/222/98-02 "Zorzi report	5.3.5.2	4	Tolerability and applicability of HC	Monocenter, prospective, uncontrolled, pilot clinical trial	Hyalograft C autograft; arthrotomy	16	Traumatic cartilage lesions or OCD	12 months	Concluded, Final Report
Safety	Study CT/222/98-03 –Fantini report	5.3.5.2	2	Tolerability and applicability of HC	Monocenter, prospective, uncontrolled, pilot clinical trial	Hyalograft C autograft; arthrotomy	12 HC-14 Control	Traumatic or chronic inflammatory rheumatism in the initial phase or in remission from inflammation defect situated in the femoral condyle, or trochlea	12 months	Concluded, Final Report
Efficacy, Safety, PD	Report Nehrer et Marlovits, 2004[9]	5.3.5.2	4	Evaluation of clinical outcomes and safety	Multicenter (n=2), observational, prospective, investigator initiated	Hyalograft C autograft; arthrotomy	24	Symptomatic cartilage lesions of the femoral condyles and patella	2 years	Concluded, Final Report
Efficacy, Safety, PD	Report Marcacci 2006[4,10]	5.3.5.2	4	Long term evaluation of the subjective symptomatic, functional and health-related quality of life outcomes and safety	Multicenter (n=11),observational, prospective investigator initiated	Hyalograft C autograft; arthrotomy/arthroscopy	206	Cartilage lesions caused by trauma/microtrauma/OCD	5 years	Concluded ; Final report
Efficacy and safety	Nehrer et al. 2006[9]	5.3.5.2	4	Evaluation of the mid-term efficacy and safety of HC	Monocenter, prospective observational	Hyalograft C autograft; arthrotomy/arthroscopy	36	Traumatic cartilage lesions or OCD with persistent symptoms in the femoral condyles and patella (3 salvage patients)	3 years	Published

Type of the study	Study identifier	Location of Study Report	Level of evidence	Objective of the study	Study design and type of Control	Test product; Dosage; Type of surgical application	Number of Subjects	Diagnosis	Follow-up Duration	Study status; Type of Report
PD	Hollander et al. 2006[2]	5.3.5.2	4	To observe the maturation of HC once implanted into humans	Biopsies from different centers, analyzed in two centers	Hyalograft C autograft	23	Chondral lesions of the knee	Mean 16 months (6-30 mths)	Published
PD	Brun P. et al. 2008	5.3.5.2	4	To study the characteristics of repair tissue	Biopsies from different centers, analyzed in two centers	Hyalograft C autograft	63 (70 biopsies)	Chondral lesions of the knee	Mean 14.1 mths (range 5-33 months)	Published
PD	Marlovits S. et al. 2006	5.3.5.2	4	To analyze with high resolution (HR) MRI the cartilage repair	HR-MRI	Hyalograft C autograft	13	Chondral lesions of the knee	2 years	Published
PD	Trattnig S et al. 2006	5.3.5.2	4	To perform HR-MRI to follow-up patients after HC implant for 2 years	HR-MRI	Hyalograft C autograft	23	Chondral lesions of the knee	2 years (18-30 mths)	Published
Efficacy, Safety, PD	Kon E. MRI et al. 2011	5.3.5.2	4	To evaluate the clinical outcome of arthroscopic HC implant at a minimum of 5 yrs and correlate it with MRI	prospective endpoint MRI	Hyalograft C autograft; arthroscopy	50 (40 MRI)	Chondral lesions of the femoral condyles or trochlea from 1.0 to 5.0cm^2	5 years (5-7 years)	Published
Efficacy, safety	Gobbi A et al. 2006[6]	5.3.5.2	4	To analyse the efficacy of HC in patellofemoral lesions	Multicenter (n=3), prospective case series	Hyalograft C autograft; arthroscopy/miniarthrotomy	32	Patellofemoral chondral lesions of the knee	2 years	Published
Efficacy,safety	Podskubka A et al. 2006	5.3.5.2	4	To evaluate efficacy of HC in knee deep chondral lesions	Monocenter, prospective case series	Hyalograft C autograft; miniarthrotomy	8	Femoral condyle lesions	(mean 10 months)	Published
Efficacy, safety	Marcacci M et al 2007[5]	5.3.5.2	4	To analyze outcome of pts treated by arthroscopic HC with a minimum of 2 years fup	Monocenter, prospective observational study	Hyalograft C autograft; arthroscopy	70	Medial/lateral Femoral condyle and trochlea	Min 2 yrs 47pt-3yrs 21pt-4yrs	Published

Type of the study	Study identifier	Location of Study Report	Level of evidence	Objective of the study	Study design and type of Control	Test product; Dosage; Type of surgical application	Number of Subjects	Diagnosis	Follow-up Duration	Study status; Type of Report
Efficacy, safety	Gobbi A et al. 2009[4]	5.3.5.2	4	To evaluate outcome of HC in patellofemoral lesions	Multicenter (n=3), prospective case series	Hyalograft C autograft; arthroscopy/miniarthrotomy	34	Patella, trochlea, lateral femoral condyle	mean period of 75.5 months (range, 60-105 months)	Published
Efficacy, safety	Nehrer S et al., 2009[8]	5.3.5.2	4	To evaluate improvement of HC in full-thickness chondral lesions	Monocenter, prospective case series	Hyalograft C autograft; arthroscopy/miniarthrotomy	53 (42 primary indication and 11 secondary)	23 Simple defects (femoral condyle, <4 cm^2), 22 complex (>4 cm^2, FC, trochlea patella, or multifocal) and 8 salvage cases (kissing and early osteoarthritic).	2-7 yrs	Published
Efficacy, safety	Domayer et al, 2007[9]	5.3.5.2	4	Aim to improve MACI as reported by Brittberg	Monocenter, prospective case series	Hyalograft C autograft; arthroscopy	53	Defects on the femoral condyles, tibial, patellar	Not specified	Abstract-
Efficacy , safety	Filardo G. et al 2011[1]	5.3.5.2	4	To analyze clinical outcomes of HC up to 7 years	Monocenter, prospective case series	Hyalograft C autograft; arthroscopy	62	Traumatic, / microtraumatic/degenerative , OCD femoral condyle lesions	7 yrs	Published
Efficacy, safety	Kon et al, ICRS, 2007[3]	5.3.5.2	4	To analyze clinical outcomes of HC at 4 years	Prospective case series	Hyalograft C autograft; arthroscopy	54	focal chondral defects involving femoral condyles and trochlea	4 years	Abstract
Efficacy, safety	Giannini, S. al., 2010[7]	5.3.5.2	4	To describe evolution in cartilage repair methods in repair of ankle lesions	Cohort study	open autologous chondrocyte implantation – Carticel/ Hyalograft C autograft/BMDC transplantation	81 Carticel (n=10) HC (n=46) BMDC (n=25)	Focal talar dome osteochondral lesions (ankle)	36 months minimum to 130 months	Published

Type of the study	Study identifier	Location of Study Report	Level of evidence	Objective of the study	Study design and type of Control	Test product; Dosage; Type of surgical application	Number of Subjects	Diagnosis	Follow-up Duration	Study status; Type of Report
Efficacy	Rossner, 2011 -Report Meta-analysis 205699	5.3.5.3	2-Evidence based systematic meta-analysis	To assess the efficacy and safety of Hyalograft C autograft implantation for articular cartilage repair and meta-analysis on all identified studies	Meta-analysis Review	Hyalograft C autograft; arthroscopy Microfracture (MF)	4141 overall 793 HC 3348 MF	Chondral lesions of the knee	(0.5-7.8)	Concluded Final report
Efficacy, safety	Giannini S. al., 2008[7]	5.3.5.4	4	To evaluate efficacy of HC in ankle lesions	Monocenter prospective case series	Hyalograft C autograft; arthroscopy	46	Focal talar dome osteochondral lesions (ankle)	3 yrs	Published
Efficacy, safety	Thermann H et al., 2008	5.3.5.4	4	To evaluate efficacy of HC and Arthrocell3D in ankle lesions	Bicenter prospective case series	Hyalograft C autograft; arthroscopy	9	Ankle lesions (talus)	3.7 yrs (2.6-4.8)	Published
Efficacy , safety	Nehrer S. et al. 2011	5.3.5.4	4	To evaluate efficacy of HC and ACI in ankle lesions	Monocenter prospective case series	Hyalograft C autograft; miniarthrotomy	17 13 HC 4 Carticel	Chronic symptomatic talar lesions (trauma, OCD, 1 hemangioma)	5 yrs (2-11 yrs)	Published
Efficacy, PD	Welsch GH et al.2010	5.3.5.4	3	To compare cartilage repair tissue after HC to CaReS, a collagen-based scaffold, with MRI imaging and-T2 mapping.	Monocenter prospective controlled vs CaRes cohort study	Hyalograft C autograft, /CaRes; miniarthrotomy	20 (10 HC, 10 CaRes)	Femoral chondral lesions	2 years	Published
Efficacy, safety	Della Villa S et al. 2010	5.3.5.4	3	To assess the influence of an intensive rehabilitation program and sport activity on HC results.	Bicenter prospective cohort study	Hyalograft C autograft; arthroscopy	65 HC (31 athletes 34 non athl.)	Cartilaginous lesions of the femoral condyle or trochlea	mean 5 years minimum 3 years	Published

Type of the study	Study identifier	Location of Study Report	Level of evidence	Objective of the study	Study design and type of Control	Test product; Dosage; Type of surgical application	Number of Subjects	Diagnosis	Follow-up Duration	Study status; Type of Report
Efficacy, safety	Filardo G et al.2011-OCD	5.3.5.4	4	To analyze results of HC associated with bone grafting in OCD	Monocenter prospective case series	Hyalograft C autograft; miniarthrotomy	32 (34 knees)	Symptomatic OCD grade III or IV ICRS	6 yrs	Published
Safety	Marlovits et al, 2004	5.3.5.4	5	Report herein the first known incidence of the emergence of borrelial arthritis following HC implant for repair of a cartilage defect.	Case Report	Hyalograft C autograft	1	Medial femoral condyle	Not specified	Published

[1]Patient population in Kon et al 2009 overlaps with Filardo et al 2011
[2]Hollander et al., 2006 reports biopsy results from various multicenter studies which include patients from the paper by Pavesio et al. 2003 and Marcacci et al, 2005
[3] The paper by Marcacci et al., ICRS, 2007 had an overlap in study population with Marcacci et al., 2005.
[4]The study report by Marcacci 2006 reported similar patients as presented in other papers by Marcacci et al., 2005
[5] The data presented in Kon et al., ICRS, 2007 are part of Marcacci et al., 2007.
[6]The paper by Gobbi et al., 2006 reported results which were later included in an update by Gobbi et al., 2009
[7] Patient population in Giannini et al 2008 overlaps with Giannini et al 2010
[8]. The first cohort in the paper by Nehrer et al., 2009 were already presented in the paper by Nehrer et al., 2006. and the Nehrer et al report 2004 overlaps with Nehrer et al 2006 and Nehrer et al 2009
[9] The Nehrer et al ICRS, 2007 were already presented in the paper by Domayer et al, ICRS, 2007
[10]Pavesio et al, 2003 is part of Marcacci report, 2006

Planned Randomized Control Study - Study Protocol Hyalograft C- 01-02

In addition to the executed studies submitted, the applicant is also planning a randomized controlled trial entitled "A randomized active treatment-controlled, evaluator-blind multicenter study of a Second-Generation Autologous Chondrocyte Implantation (Hyalograft C autograft) to provide Treatment of Symptomatic Articular Cartilage Defects of the Femoral Condyle or the Trochlea" (Protocol Hyalograft C- 01-02).

This will be a multi-centre study, involving up to 20 centres in Europe and the United States comparing Hylograft C (Group A) to microfracture (Group B) in a group of patients with articular cartilage defects 2-4 cm2 after debridement, classified as ICRS grade III or IV. The sample size in Group A and B will be 200 first line treatment subjects to complete 172 (86 per group). Additionally 35 patients to complete 28 subjects are planned for a third open label arm with defects larger than 4 cm2 after debridement, classified as ICRS grade III or IV.

The study is intended to establish superiority of Hyalograft C autograft over microfracture in Group A and Group B using the Knee Injury and Osteoarthritis Outcome (KOOS) score at 36 months from surgery. For the sample size calculation it is assumed that the treatment difference between Hyalograft C autograft and microfracture (Group A and B) at 36 months will be 10 points with a standard

deviation of 20 corresponding to an effect size of 0.5 which is considered a clinically relevant difference.

Secondary endpoints will include:

a. MOCART Score

b. EuroQol

c. Tegner activity score

d. Evaluator Global Assessment

e. Individual KOOS subscales

i. Pain

ii. Symptoms

iii. Function in daily living (ADL)

iv. Function in Sport and Recreation (Sport/Rec)

v. Knee related quality of life (QOL)

f. Physical evaluation of index knee (swelling, redness, effusion)

Dose-response studies and main clinical studies

Dose response study(ies)

No clinical dose-finding studies have been performed in line with the EMA Guideline on Human Cell-Based Medicinal Products (EMA/CHMP/410869/2006) and the Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee EMA/CAT/CPWP/568181/2009. The recommended dose is also not based on any data during non-clinical data development.

The EMA CAT Reflection Paper recommends the use of exploratory endpoints to address pharmacodynamics, dose evaluation and safety. None of the studies submitted formally studied optimal dose and thus it is unclear if the dose which will be studied in the proposed phase III trial and for marketing authorisation is optimal with respect to efficacy and safety

Main Studies

Kon et al (2009)

The purpose of this study was to compare the clinical outcome of patients treated with second-generation autologous chondrocyte implantation implants with those treated with the microfracture repair technique at 5-year follow-up.

This was the larger one of the only two cohort studies which were judged by the applicant to have a grading of 2 with respect to level of evidence and considered pivotal and is therefore assessed first, as the main study.

Methods

- **Study participants**

Active patients with grade III to IV cartilaginous lesions on the weightbearing surface of the medial or lateral femoral condyle or trochlea were enrolled in the study between 2000 and 2002. Forty patients

treated with Hyalograft C second-generation ACI and 40 with the microfracture technique were prospectively evaluated and followed for a minimum of 5 years.

Inclusion criteria

1. Patients between 16 and 60 years who had clinical symptoms such as knee pain or swelling with grade III to IV chondral lesions of the femoral condyles or trochlea from 1.0 to 5.0 cm2.

Exclusion criteria

1. Chondral lesions greater than 5.0 cm_2 or less than 1.0 cm_2.

2. Tibial plateau chondral lesions, diffused arthritis or bipolar ("kissing") lesions, noncorrected knee instability, or axial deviation.

3. Patients with infective, tumor, metabolic, and inflammatory pathologic changes

The inclusion criteria appear to restrict the lesion size from 1-5 cm while the indication in the SPC does not specify any lesion size but refers to the grade of the lesion with respect to the modified Outerbridge scale (III &IV). This implies that lesions both less and greater than 5.0 cm are indicated. However, the study design does not allow for such a comparison since microfracture is not a treatment option in lesions larger than 4.0 cm and lesions outside 1-5cm were not studied here.

- **Treatments**

Definitive diagnosis of chondral lesion and sizing was performed during the arthroscopic procedure. The patients with ACL lesions underwent an associated surgical procedure for ACL reconstruction during the same surgical session with cartilage harvesting or microfracture, so all knee instabilities were corrected during the treatment. All patients gave their consent to participate, complied with the required postoperative rehabilitation regimen, and were consecutively treated and prospectively evaluated.

Hyalograft C Surgical Technique

The treatment consisted of 2 arthroscopic steps. The first procedure consisted of a biopsy of healthy cartilage for autologous chondrocyte cell culture. A 150- to 200-mg cartilage biopsy specimen was taken from a nonweightbearing site on the articular surface (intercondylar notch) and sent to the processing center in a serum-free nutritional medium. Chondrocytes were seeded on a hyaluronic acid–based scaffold (Hyaff 11) to obtain the bioengineered tissue Hyalograft C.

After 6 weeks, the second step was performed. All patients in this group were treated arthroscopically.

According to the technique developed, 19 a variable diameter (6.5-8.5 mm) delivery device with a sharp edge was used to evaluate the size of the defect to ensure complete coverage of the defect. A circular area with regular margins for graft implantation was prepared with a specially designed cannulated low-profile drill. The delivery device was then filled with a hyaluronic acid patch, which was transported and positioned in the prepared area. The graft was pushed out of the delivery device and precisely positioned within the defect where it remained tightly adhered to the subchondral bone. Because of the physical adhesive characteristics of the graft, no fibrin glue or sutures were used to fix

the implant. Under arthroscopic control, the stability of implanted stamps was evaluated during the cyclic bending of the knee.

Microfracture Surgical Technique

An initial thorough diagnostic examination of the knee was performed. After identifying the full-thickness chondral lesion, the unstable cartilage was removed, including cartilage loosely attached to the surrounding rim, using a shaver and/or a handheld angled curette . When present, the calcified layer of cartilage was also removed using a curette. Once the exposed subchondral bone plate was thoroughly debrided, multiple holes were made using a Steadman arthroscopic pin. The holes were placed perpendicular to the joint surface, approximately 3 to 4 mm apart and about 2 to 4 mm deep, with care taken not to damage the subchondral plate between the holes. Once the holes were completed, the irrigation fluid pump pressure was lowered to visualize the release of fat droplets and blood from the microfracture holes into the knee. All instruments were then removed. Routine use of an intra-articular drain was not uitilised.

Rehabilitation Protocol

The same rehabilitation protocol was used for both treatment groups.

In the early stage (0-6 weeks), the rehabilitation strategies focus on controlling pain, effusion, loss of motion, and muscle atrophy, and the main goal of the treatment was to protect the treated zone by preventing weightbearing for about 4 weeks. Management of postoperative pain allows for early mobilization, which contributes to faster resolution of swelling, promotes defect healing and joint nutrition, and prevents the development of adhesions. On the second postoperative day, self-assisted mobilization of the knee or continued passive motion 6 hours daily with 1 cycle per minute was recommended until 90° of flexion was attained. Controlled mobilization exercises with reduced range of motion, early isometric and isotonic exercises, and controlled mechanical compression were performed. In the third or fourth week, weight touchdown with crutches was allowed and was usually completed within 6 to 8 weeks after surgery. Most of the patients achieved full weightbearing by 6 weeks. Gait training in a swimming pool facilitated the recovery of normal gait. At the beginning of the ninth week, active functional training was started. Symptoms of overloading (pain, effusion, tenderness) noticed by the surgeon or physical therapist were signals to retard the rehabilitation protocol. At 11 to 32 weeks after surgery, the rehabilitation goal was to return to a correct running pathway by proprioceptive, strength, and endurance exercises and aerobic training. When rehabilitation progresses without complication, this stage may end within 30 to 32 weeks after surgery. At the end of this time, the patient should be able to go up and down stairs and run forward at midspeed without symptoms. The remainder of rehabilitation was dedicated to the return to previous sport activity. Return to sports was allowed no earlier than 10 to 12 months after surgery.

- **Objectives**

To compare the clinical outcome of patients treated with second-generation autologous chondrocyte implantation implants with those treated with the microfracture repair technique at 5-year follow-up.

- **Outcomes / endpoints**

All patients were evaluated preoperatively and at 2- and 5-year follow-up.

1. The clinical outcome of all patients was analyzed using the cartilage standard evaluation form as proposed by the International Cartilage Repair Society. A knee function test was performed by the surgeon (not an independent reviewer) according to the International Knee Documentation Committee (IKDC) Knee Examination Form. The lowest ratings in effusion, passive motion deficit, and ligament examination were used to determine the final functional grade of the knee (normal, nearly normal, abnormal, or severely abnormal).
2. A return to sporting activities was also recorded at 2- and 5-year follow-up, evaluated with the Tegner score, and compared with pre-operative and pre-injury levels.

IKDC subjective knee evaluation form

In this questionnaire, a higher score indicates higher levels of function and lower levels of symptoms. Accordingly, a score of 100 indicates no limitations of activities of daily living or sports and the absence of symptoms.

IKDC Objective knee examination form

A knee functional test is performed by the surgeon and rated for effusion, passive motion deficit and ligament examination to determine the final functional grade of the knee (normal, nearly normal, abnormal and severely abnormal).

Tegner Activity

The activity scale indicates level of sports activity and labour ranging from 0 to 10, from sick leave /pension due to knee problems to competitive sports (soccer, football, rugby - national elite) respectively.

Follow-up Evaluation

The operation was considered a failure if the patient needed a reoperation because of symptoms due to primary defects. For failed patients, the last clinical evaluation before reoperation was considered.

Although KOOS is the most common outcome measure used, the outcomes utilised in the study are acceptable. However, it is notable that the outcomes were limited mainly to patient reported outcomes (PRO). The recommendation in the CAT Reflection Paper is to combine both PRO and a structural endpoint either as a co-primary endpoint or keep the structural endpoints as secondary. It should also be noted that treatment outcome evaluation was not carried out by an independent reviewer.

- ***Sample size***

A sample-size estimation showed that 40 patients in each group would be required to demonstrate a difference with IKDC subjective score of the 2 groups of at least 0.5 standard deviation from the mean (according to Cohen's conventions for a medium effect size) with α level of .05 and a β level of 80%.

- **Randomisation**

This was a non-randomised study, however, the choice of the treatment procedures was determined by health and insurance policy of the institutions; all Hyalograft C procedures were performed in 1 institution and all microfractures in another.

- **Blinding (masking)**

This was an open trial for obvious reasons.

While it is acceptable to carry out open trials in such a situation, it would nevertheless be considered necessary to carry out the evaluation in a blinded manner.

- **Statistical methods**

All continuous variables were expressed in terms of mean ± standard deviation of the mean. The IKDC objective scores are expressed in terms of median and 25th and 75th percentiles. If the Kolmogorov-Smirnov test showed most continuous variables were not normal, a nonparametric test was performed. The Wilcoxon test was performed to test the hypotheses about continuous data differences between preinjury, preoperative, and follow-up evaluations.

The Mann-Whitney test and Kruskal-Wallis test were used to test the hypotheses about continuous data differences between 2 treatments and among 3 different lesion size groups, respectively. The Pearson's $\chi 2$ test was performed to investigate the difference in frequency distribution between 2 treatments. The Spearman rank correlation analysis was performed to investigate relationships between 2 continuous variables. The τ-b Kendall correlation analysis was performed to investigate relationships between IKDC and continuous variables. For all tests, $P < .05$ was considered significant.

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software version 14.1 (SPSS Inc, Chicago, Illinois).

Results

- **Participant flow**

Full details are not provided in the publication, however, from the limited data available forty patients treated with Hyalograft C second-generation ACI and 40 with the microfracture technique were prospectively evaluated and followed for a minimum of 5 years. During this period, 43 patients were treated with Hyalograft C and 42 patients with microfracture, but 2 and 3 patients were not evaluated at 5-year follow-up in the microfracture and Hyalograft C groups, respectively.

- **Recruitment**

The study was carried out at two different centres situated in Bologna and Milan in Italy which only utilised a single surgical procedure of either microfracture or ACI.

- **Conduct of the study**

Details not available

- **Baseline data**

The data are summaried below in Table 7

The male:female ratio was 60:20. The mean age at surgery was 29.8 years. The most common cause of the defect was trauma (56.25% of the cases) followed by microtraumatic-degenerative (38.75% of the cases) and osteochondritis dissecans (5% of the cases). Overall, 67.5% of chondral lesions were situated on the medial condyle, 27.5% on the lateral femoral condyle, and in 4 cases the lesion was located on the trochlea. The mean size of the defects was 2.4 cm2 (1.4-4.4 cm2). Eighty-four percent of the patients were well-trained athletes, and 12.5% played sports at a highly competitive level (Table7).

Table 7 Demographic data

	Microfracture Group	Hyalograft C Group	Significance
Gender, male/female, n	27/13	33/7	NS
Age, y	30.6	29.0	NS
Sport activity, %			
Well trained	82.5	85.0	NS
Competitive	12.5	12.5	NS
Etiology, n			Pearson's χ^2 test, $P = .031$
Traumatic	27	18	
Microtraumatic/degenerative	13	18	
Osteochondritis dissecans	0	4	
Associated surgery, n	28	23	NS
ACL	20	16	NS
Previous surgery, n	10	18	Pearson's χ^2 test, $P = .036$
ACL	2	8	
Debridement	3	6	
Mosaicplasty	—	2	
Defect size (SD), cm^2	2.5 (0.79)	2.2 (0.75)	NS
Location, n			NS
Medial femoral condyle	28	26	
Lateral femoral condyle	10	12	
Trochlea	2	2	

[a]Statistical analysis showed more traumatic cases and less presence of previous surgery in the group treated with microfractures. NS, not significant; ACL, anterior cruciate ligament; SD, standard deviation.

There appears to be a statistically significant imbalance between the groups with respect to lesion aetiology as well as previous surgery. There were more traumatic cases (Pearson's χ2 test, P = .031) and lower incidence of previous surgery (Pearson's χ2 test, P = .036) in the group treated with microfracture: 47.5% of the patients in the group treated with Hyalograft C underwent previous surgery, including 20% of patients previously treated for cartilage lesions, whereas 25% of the patients in the group treated with microfracture underwent previous surgery, including 7.5% of patients treated for cartilage lesions. No data on BMI has been provided.

- **Numbers analysed**

Forty three patients were treated with Hyalograft C and 42 patients with microfracture, but 2 and 3 patients were not evaluated at 5-year follow-up in the microfracture and Hyalograft C groups, respectively. No further details are available.

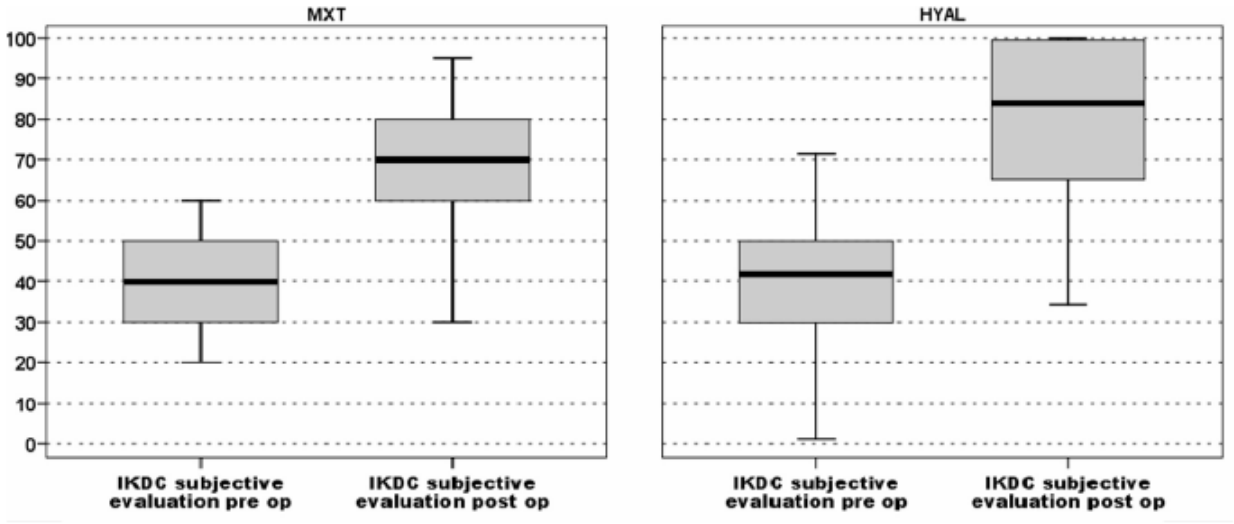
- **Outcomes and estimation**

IKDC subjective score:

The subjective IKDC score in the Hyalograft C autograft group, increased from 40.5±15.2 to 80.2 ±19.1 at 5 year follow-up (p<0.001) and the subjective IKDC score in the MFX group, increased from 41.1±12.3 preoperatively to 70.2±14.7 at 5 year follow-up (p<0.001).

Figure 2 shows the IKDC subjective score achieved at 5-year follow-up by both groups of patients. The values are expressed in median and 25th and 75th percentiles. The MFX group improved from 40 (30-50) to 70 (60-80), and the Hyalograft C autograft group improved from 42 (29-50) to 84 (63.5-99.5). When comparing the two groups results, a higher improvement in IKDC subjective (p=0.003) score was observed in the Hyalograft C autograft group compared with the MFX group at 5-year follow-up.

Figure 2. Comparison of the International Knee Documentation Committee (IKDC) subjective score achieved at 5-year follow-up by both groups of patients.



The values are expressed in median and 25th and 75th percentiles. The microfracture (MXT) group improved from 40 (30-50) to 70 (60-80), and the Hyalograft C (HYAL) group improved from 42 (29-50) to 84 (63.5-99.5). Post-op, post-operatively; pre-op, preoperatively.

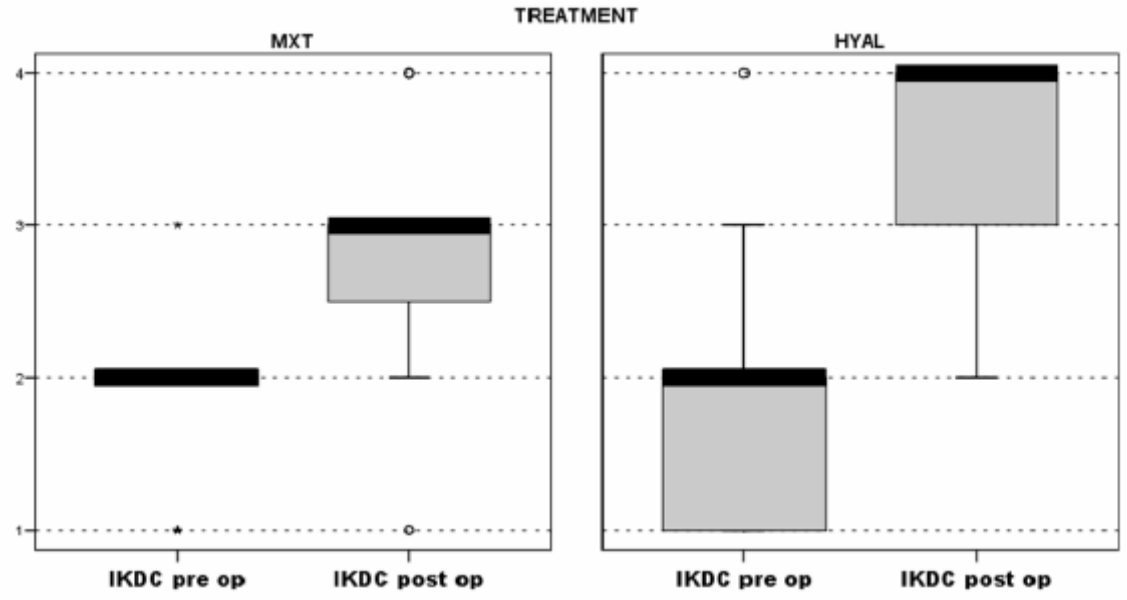
IKDC objective score:

The Hyalograft C autograft group had 90% normal or nearly normal knees (and 29 A, 6 B, and 5 C at 5-year follow-up) at 5-year follow-up (p<0.001) compared to 15% normal or nearly normal knees preoperatively (1 A, 4 B, 15 C, and 19 D preoperatively) (see Figure 3).

In the MFX group the IKDC objective score increased from 2.5% normal and nearly normal knees before the operation (1 B, 32 C, and 7 D) to 75% normal and nearly normal knees (6 A, 24 B,7 C, and 3 D) at 5-year follow-up and showed a statistically significant improvement (Wilcoxon test, p < .001).

Figure 3 shows the comparison of the IKDC objective scores achieved at 5-year follow-up by both groups of patients. The values are expressed in median and 25th and 75th percentiles. The microfracture group improved from 2 (2-2) to 3 (2.5-3), and the Hyalograft C group improved from 2 (1-2) to 4 (3-4). When comparing the two groups results, a higher improvement in IKDC objective ($p<0.001$) score was observed in the Hyalograft C autograft group compared with the microfracture group at 5-year follow-up.

Figure 3. Comparison of the International Knee Documentation Committee (IKDC) objective scores achieved at 5-year follow-up by both groups of patients. The values are expressed in median and 25th and 75th percentiles.



The microfracture (MXT) group improved from 2 (2-2) to 3 (2.5-3), and the Hyalograft C (HYAL) group improved from 2 (1-2) to 4 (3-4). Post op, postoperatively;
pre op, preoperatively; 4, A (normal knee); 3, B (nearly normal); 2, C (abnormal); 1, D (severely abnormal).

Tegner score:

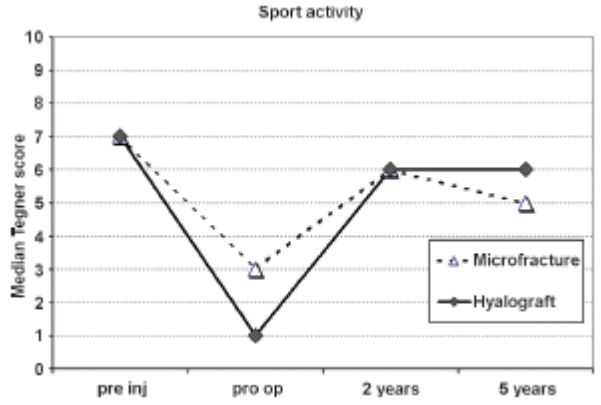
The Hyalograft C autograft group showed a statistically significant improvement in Tegner scores ($p<0.001$) from preoperative level to 2- and 5-year follow-up.

The MFX group showed a statistically significant improvement ($p<0.001$) from preoperative level to 2- and 5-year follow-up; however, a decrease in sport activity from 2- to 5-year follow-up was documented ($p<0.001$).

Figure 4 shows the trend of values of Tegner score in both groups of patients. In the MFX group, scores passed from 7(6-8) preinjury to 3 (3-4) preoperatively and improved to 6 (6-7) at 2-year follow-up and to 5 (4-6) at 5-year follow-up, whereas the Hyalograft C group passed from 7 (6-7) preinjury to 1 (1-3) preoperatively, with an improvement to 6 (3-7) at 2 years maintained at 5-year follow-up.

Therefore the return to sports activity (Tegner) in the first 2 years was similar in both groups and remained stable after 5 years in the Hyalograft C autograft group, it worsened in the microfracture group.

Figure 4. Trend of values of Tegner score in both groups of patients.



In the group of patients treated with microfracture, 20 patients achieved the preinjury activity level at 2 years after surgery, but only 7 of the patients remained at the same level after 5 years. In the group of patients treated with Hyalograft C, 18 patients achieved the preinjury activity level at 2 years after surgery, and 18 of the patients were practicing sports at the same level after 5 years. In both groups, patient age was significantly correlated to sport activity resumption: older patients experienced greater difficulty in attempting to return to preinjury sport activity levels (Spearman rank correlation, P = .05). However, patient age did not influence the clinical outcome evaluated with IKDC objective and subjective scores.

Other parameters such as size (Table 8) and mechanism of cartilage lesions and associated and previous surgery did not influence significantly the clinical outcome in both groups.

TABLE 8 Analysis of the Clinical Outcome Related to the Defect Size[a]

		International Knee Documentation Committee		
Size of Lesion, cm^2	No. of Patients	Objective: 5-Year Median (25th-75th)	Subjective: 5-Year Mean (SD)	Tegner: 5-Year Median (25th-75th)
Overall				
<2	24	3.5 (3-4)	74.4 (15.8)	5.5 (4-6)
≥ 2 < 3	36	4 (3-4)	76.9 (19.5)	6 (4-7)
≥3	20	3 (3-4)	73.2 (17.0)	5 (3-5.5)
Kruskal-Wallis test		NS	NS	NS
Microfractures				
<2	11	3 (3-3)	73.6 (11.2)	5 (5-6)
≥2 <3	18	3 (2-3)	68.9 (17.3)	5 (4-6)
≥3	11	3 (2,5-3)	69.1 (13.9)	5 (3.5-5.5)
Kruskal Wallis test		NS	NS	NS
Hyalograft C				
<2	13	4 (4-4)	75.0 (19.3)	6 (4-6)
≥ 2 < 3	18	4 (4-4)	84.9 (18.6)	6.5 (3-7)
≥3	9	4 (3-4)	78.3 (19.9)	4 (3-5)
Kruskal-Wallis test		NS	NS	NS

[a]No statistically significant difference was found between groups. SD, standard deviation; NS, not significant.

A separate analysis of clinical outcome of the patients with associated ACL reconstruction was performed. No statistically significant differences were observed at 5- year follow-up in both treatment groups (Table 9).

TABLE 9 Separate Analysis of the Clinical Outcome of the Patients Who Underwent ACL Reconstruction[a]

Associated ACL Reconstruction	No. of Patients	International Knee Documentation Committee		Tegner: 5-Year Mean (SD)
		Objective: 5-Year Median (25th-75th)	Subjective: 5-Year Mean (SD)	
Overall				
Yes	36	3 (3-4)	75.7 (16.3)	5.7 (2.2)
No	44	3 (3-4)	74.9 (18.9)	4.8 (2.0)
Mann-Whitney test		NS	NS	NS
Microfractures				
Yes	20	3 (3-3)	70.8 (11.6)	5.6 (1.9)
No	20	3 (2-3)	69.8 (17.6)	5.0 (1.7)
Mann-Whitney test		NS	NS	NS
Hyalograft C				
Yes	16	4 (4-4)	81.8 (19.3)	5.8 (2.6)
No	24	4 (4-4)	79.1 (11.6)	4.7 (2.1)
Mann-Whitney test		NS	NS	NS

[a]No statistically significant difference was found between groups. SD, standard deviation; NS, not significant.

One case that had graft failure at the early stage after microfracture (around 18 months of follow-up) was treated with autologous chondrocyte transplantation.

The results from this prospective cohort study show that both groups achieved statistically significant improvement with respect to clinical outcomes. However, it would appear that the clinical results assessed with objective and subjective IKDC scores at medium-term follow-up were found to be somewhat better in the group treated with arthroscopic autologous chondrocyte transplantation. In addition, there was no decrease in the resumption of sports activity from 2 to 5 years in patients treated with autologous chondrocyte transplantation, whereas a decrease in sports activity was detected in the group treated with microfracture from 2- to 5-year follow-up.

Nevertheless, the results need to be interpreted with caution due to several limitations in the study. Firstly the absence of randomisation is a concern despite the choice of treatment being dictated by the health and insurance policy of the individual institution.

Secondly, in an open trial, blinded evaluation would be considered important for the assessment of efficacy, however, the IKDC knee function objective test was performed by the surgeon and not by an independent reviewer.

Thirdly, there is no evidence provided from structural data either from histology or MRI which would have supported the clinical data.

Fourthly there is presence of imbalance between the groups with respect to the aetiology of the lesion as well as previous surgery. The high incidence of associated surgery also represents an important source of bias in the study. This makes it difficult to evaluate whether the improved clinical results are actually related to the implant or to other surgical procedures. However, the applicant has additionally analysed factors such as lesion size and previous surgery and the results do not suggest that these have impacted on efficacy. Nevertheless, these results appear to conflict with the results seen in De Windt et al 2012, in which patients on Hyalograft showed reduced improvement in those with prior ACL reconstruction compared to those who did not.

Fifthly, there is no data provided on the use of pain killers and BMI since this could have impacted on the interpretation of the results.

Additionally, previous sporting activity appears to be positively correlated with better outcome especially in young active sportsmen. Therefore the results in both groups are not entirely unexpected.

Finally, the results would be difficult to extrapolate to the age range in the trial since most of the patients in the study were active and young, and all cartilaginous lesions were contained. Consequently, the results cannot be applied to older patients with large, uncontained degenerative lesions which would need to be reflected in the SPC. There is evidence to suggest that Hyalograft may be less effective in patients over 40 years.

However, the lesion size in the inclusion criteria from 1-5cm2 does not allow for a valid comparison since microfracture is not indicated for lesions larger than 4.0 cm.

Kon et al 2011

This study was done to evaluate whether the regenerative cell-based approach allows highly demanding soccer athletes a better functional recovery compared with the bone marrow stimulation approach (microfracture).

This is the second prospective cohort study which has been graded by the applicant as being level 2 evidence and is comparatively smaller than the first cohort study (2009). It is not entirely clear whether there is any overlap of patients from the first study or whether this is merely an extension study with a group of selected patients from the first study who are active soccer athletes.

Methods

- **Study participants**

Forty-one professional or semi-professional male soccer players were treated from 2000 to 2006 and evaluated prospectively at 2 years and at a final 7.5-year mean follow-up (minimum, 4 years). Twenty-one patients were treated with arthroscopic second-generation autologous chondrocyte implantation (Hyalograft C) and 20 with the microfracture technique. The clinical outcome of all patients was analyzed using the cartilage standard International Cartilage Repair Society (ICRS) evaluation package. The sport activity level was evaluated with the Tegner score, and the recovery time was also recorded.

The patients who presented with anterior cruciate ligament (ACL) lesions underwent a combined surgical procedure of ACL reconstruction during the same surgical session with cartilage harvesting or microfracture

Inclusion criteria

Male athletes complaining of clinical symptoms, such as knee pain or swelling with grade III to IV chondral lesions of the femoral condyles or trochlea greater than 1 cm2, as assessed by arthroscopic evaluation.

Exclusion criteria

1. Patella or tibial plateau chondral lesions
2. Diffuse arthritis or bipolar (‘‘kissing’’) lesions
3. Untreated tibiofemoral or patellofemoral misalignment, and knee instability.

As mentioned earlier for the previous cohort study, the inclusion criteria appear to restrict the lesion size to > 1cm while the indication in the SPC does not specify any lesion size but refers to the grade of the lesion with respect to the modified Outerbridge scale (III &IV). This implies that all lesions greater than 1.0 cm are indicated. However, the study design does not allow for such a comparison since microfracture is not a treatment option in lesions larger than 4.0 cm and although the mean lesion size appears to be < 4.0 cm, the range of lesion size has not been provided.

Treatments

ACI with Hyalograft C

The procedure consisted of 2 arthroscopic surgical steps. The first step involved taking a 150- to 200-mg healthy cartilage biopsy specimen from a nonweightbearing site of the articular surface (intercondylar notch) for autologous chondrocyte cell culture and subsequent seeding onto the hyaluronic acid–based scaffold Hyaff 11 (Fidia Advanced Biopolymers, Padova, Italy). The second step consisted of implanting the bioengineered tissue Hyalograft C (Fidia Advanced Biopolymers) according to the surgical technique described by Marcacci et al.27 A variable diameter delivery device with a sharp edge was used to evaluate the size of the defect. A circular area with regular margins for graft implantation was prepared with a specially designed cannulated low profile drill. The delivery device was then filled with a hyaluronic acid patch, which was transported and placed in the prepared area. The graft was pushed out of the delivery device and positioned precisely within the defect, where it remained tightly fixed to the subchondral bone. Under arthroscopic control, the stability of implanted stamps was evaluated also during cyclic bending of the knee. Dislodgement of the implanted patch was not observed in this study.

Microfracture Technique

After identification of the chondral lesion, the unstable cartilage was removed including cartilage loosely attached to the surrounding rim using a shaver and/or a hand-held angled curette. When present, the calcified layer of cartilage was also removed. Then, multiple holes were made using a Steadman arthroscopic pin. The holes were placed perpendicular to the joint surface approximately 3 to 4 mm apart and about 2 to 4 mm deep, with care taken not to damage the subchondral plate between the holes. Once the holes were completed, the irrigation fluid pump pressure was lowered to visualize the release of fat droplets and blood from the microfracture holes into the knee.

Rehabilitation Protocol

The same step-based rehabilitation approach was used for both treatment groups. The rehabilitation protocol included 4 stages. The transition from one stage to the next was allowed when specific goals were reached concerning functional recovery and clinical outcome.

In the early stage (0-6 weeks), the rehabilitation strategies focused on controlling pain, effusion, loss of motion, and muscle atrophy, and the main goal of the treatment was to protect the initial healing phases by preventing weight bearing for about 4 weeks. Management of postoperative pain allowed for early mobilization, which contributed to faster resolution of swelling, promoted defect healing and joint nutrition, and prevented the development of adhesions. On the second postoperative day, self-assisted mobilization of the knee or continued passive motion 6 hours daily with 1 cycle per minute was recommended until 90_ of flexion was attained. Controlled mobilization exercises with reduced range

of motion (ROM), early isometric and isotonic exercises, and controlled mechanical compression were performed. By the third or fourth week, weight touchdown with crutches was allowed and was usually completed within 6 to 8 weeks after surgery. Gait training in a swimming pool facilitated the recovery of normal gait.

The transition to the second stage was allowed after swelling had resolved and when full knee extension, at least 120_ of knee flexion, and a correct gait cycle with full weight bearing were achieved. The main goal of the second stage was to return to a normal running mode. The strategy of this stage focused on strengthening exercises in the open and closed kinetic chain, selecting a pain-free ROM, proprioceptive exercises, and aerobic training. Treadmill running was allowed and increased according to the clinical progress. Criteria for progression to the next stage were no pain or swelling after 8 to 10 km/h of running for 15 minutes, good strength recovery compared with the contralateral limb evaluated by clinical examination, and single-legged hop test\20% compared with the contralateral limb.

The main goal of the third stage was to recover sport specific skills. The strategy focused on eccentric strengthening exercises, advanced proprioceptive exercises, and a sport-specific reconditioning program. When there was no pain or effusion during sport-specific drills and a complete endurance recovery, the final goal was to reintroduce the athlete to competition (phase 4), with specific exercises and drills performed on a playing field first, and then strength exercises for muscular groups of both the injured and the non-injured limbs and stretching of the posterior muscle chain of the lower limbs to maintain physical fitness and prevent the risk of re-injuries.

Follow-up Evaluation

The operation was deemed to have failed if the patient needed to repeat surgery because of symptoms due to primary defects. For failed patients, the last clinical evaluation before repeat surgery was considered.

Objectives

Outcomes/endpoints

All patients were evaluated preoperatively, at 2 years, and at a final 7.5-year mean follow-up.

1. The clinical outcome of all patients was analyzed using the cartilage standard evaluation form as proposed by the International Cartilage Repair Society (ICRS). A functional knee test was performed according to the International Knee Documentation Committee (IKDC) knee examination form. The lowest ratings in effusion, passive motion deficit, and ligament examination were used to determine the final functional status of the knee (normal, nearly normal, abnormal, or severely abnormal).

2. Patients were asked to evaluate their functional level using the EQ-VAS Score (Visual analogue scale).

3. Returning to sports was evaluated with the Tegner score relatively to preoperative and preinjury levels, and time to recover was also recorded.

Sample size

No details provided.

Randomisation

This was a non-randomised study, however, three orthopaedic centres participated in this study and each centre performed only 1 treatment, and so the patient treatment allocation was according to the centre the patients went to (all the second-generation ACI treatments were performed at 1 centre, whereas 2 groups of 7 and 13 patients, respectively, received micro fracture in the other 2 centres).

Blinding (masking)

This was an open trial for obvious reasons.

Statistical methods

All continuous data were expressed in terms of the mean and the standard deviation of the mean. One-way analysis of variance was performed to assess differences among groups when the Levene test for homogeneity of variances was not significant ($P < .05$); otherwise, the Mann-Whitney test was used. The general linear model for repeated measures with the Bonferroni correction for multiple comparisons was performed to test differences of the scores at different follow-up times. The influence of grouping variables on scores at different follow-up times was investigated by the general linear model for repeated measures, with the grouping variable as a fixed effect. The Pearson nonparametric x2 test (contingency tables with more than 2 groups) and Fisher nonparametric exact test (contingency tables with 2 groups) were performed to investigate the relationships between grouping variables. The Pearson correlation was used to assess the correlation between continuous variables. The Friedman test was used to test differences among different follow-up times of the objective IKDC. The life table survival analysis with the Wilcoxon- Gehan statistic was used to compare cumulative rates of returning to competitive sports in the 2 groups. For all tests, $P<.05$ was considered significant. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS 15.0, SPSS Inc, an IBM company, Armonk, New York).

Results

Participant flow

Full details are not available, however, 21 patients were treated with arthroscopic second-generation ACI and 20 with the microfracture technique. Every centre performed only 1 treatment, and so the patient treatment allocation was according to the centre the patients went to (all the second-generation ACI treatments were performed at 1 centre, whereas 2 groups of 7 and 13 patients, respectively, received microfracture in the other 2 centres).

Recruitment

The study was carried out at three orthopaedic centres in Milan, Bologna and Pavia in Italy. Each centre performed only 1 treatment, and so the patient treatment allocation was carried out according to the centre the patients went to (all the second-generation ACI treatments were performed at 1

centre, whereas 2 groups of 7 and 13 patients, respectively, received microfracture in the other 2 centres).

Conduct of the study

Details not available

Baseline data

The 2 groups of patients were homogeneous for age, defect size, location, previous and combined surgery, and follow-up, as reported in detail in Table 10.

Table 10 Comparison of the Characteristics of the 2 Groups of Soccer Players Treated With Microfracture Technique and Second-Generation Autologous Chondrocyte Implantation (ACI), Respectively[a]

	Microfracture	Hyalograft C	Significance
Athletes, n	20	21	
Level	Tegner 10: 5 Tegner 9: 15	Tegner 10: 6 Tegner 9: 15	NS
Age, mean ± SD (range), y	26.5 ± 4.5 (18-35)	23.7 ± 5.7 (16-37)	NS
Final follow-up, mean ± SD (range), mo	89 ± 25 (48-132)	94 ± 14 (60-120)	NS
Defect size, mean ± SD, cm^2	1.9 ± 0.6	2.1 ± 0.5	NS
Associated surgery	10 (50%) (4 ACL, 1 MCL, 1 tibial osteotomy, 1 loose body removal, 1 calcification removal, 3 meniscectomy, 2 patellar debridement)	12 (57%) (10 ACL, 10 meniscectomy, 2 meniscal suture, 1 loose body removal)	NS
Previous surgery	6 (30%) (7 meniscectomy, 1 shaving, 1 ACL, 1 MCL)	8 (38%) (2 meniscectomy, 2 ACL, 2 microfracture, 1 debridement, 2 loose body removal, 2 shaving, 1 mosaicplasty, 1 patellar realignment)	NS
Location	12 MFC (60%) 4 LFC (20%) 3 trochlea (15%) 1 MFC, LFC (5%)	13 MFC (62%) 4 LFC (19%) 2 trochlea (9%) 1 MFC, trochlea (5%) 1 LFC, trochlea (5%)	NS

[a]Statistical analysis shows the homogeneity of the 2 groups. ACL, anterior cruciate ligament; MCL, medial collateral ligament; MFC, medial femoral condyle; LFC, lateral femoral condyle; NS, not significant.

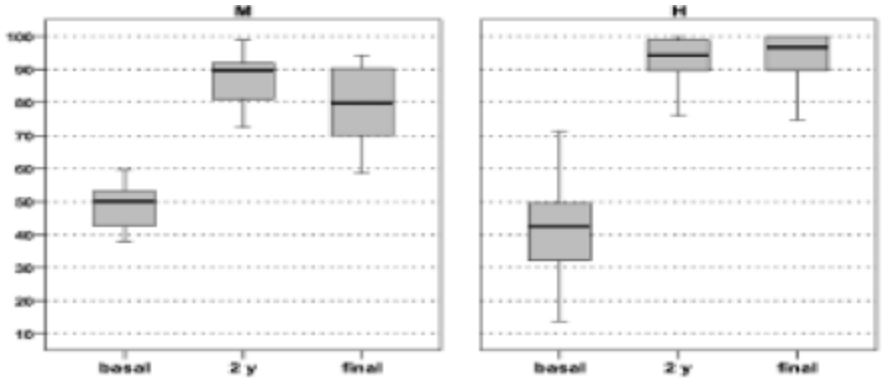
Although the two groups appear to be generally balanced with respect to baseline parameters and the minor differences are not statistically significant, it should be noted that the percentage of patients with normal and near normal knees was higher in the Hyalograft group compared with the microfracture group (19% vs 5% respectively).

Results

IKDC subjective score: The Hyalograft C autograft group showed a significant improvement from preoperative score (43.2±13.7) to 2 years' follow-up (90.5±12.8) ($p<0.0005$); then, results remained stable until the final follow-up evaluation (91.0±13.9) ($p<0.0005$). The MFX group showed significant improvement from preoperative (47.3±8.5) to 2 years' follow-up (86.8±9.7) ($p<0.0005$), followed by

a significant deterioration of the results at the final follow-up to 79.0±11.6, (p<0.0005) compared with the previous evaluation at 2 years (Figure 5). Therefore Hyalograft C autograft showed a more durable outcome with significantly better results at the final evaluation (p=0.005).

Figure 5: Hyalograft C autograft (HC) compared to Microfracture (MFX) by IKDC Subjective Score.



IKDC objective score:

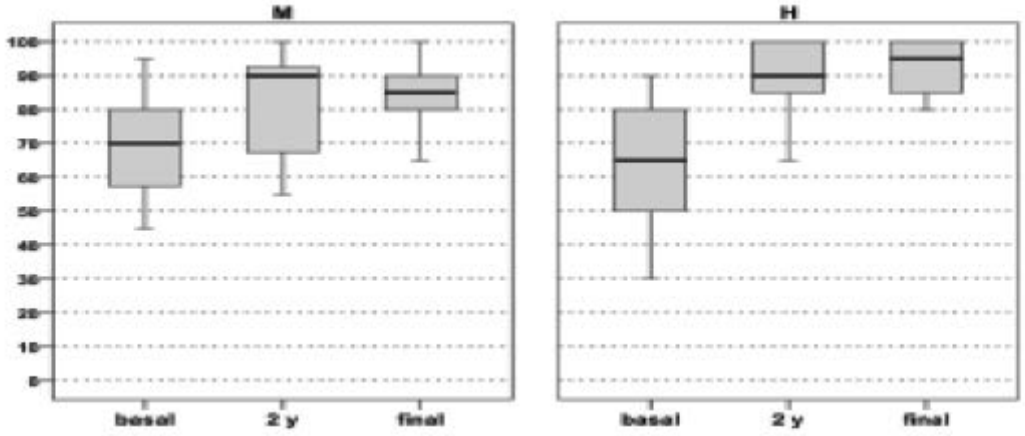
In the Hyalograft C autograft group the IKDC objective increased significantly from 19% normal and nearly normal knees before the operation to 95% at 2 years' follow-up (p<0.0005); then, results remained stable with 95% normal and nearly normal knees at the final follow-up evaluation (Table 11). In the MFX group there were 5% normal and nearly normal knees before the operation to 90% at 2 years' follow-up (p<0.0005); then, results remained stable with 90% normal and nearly normal knees at the final follow-up evaluation. No differences between groups were found in the IKDC objective score.

Table 11 Objective International Knee Documentation Committee (IKDC) Score: Improvement from the Preoperative Level to 2 Years' and Final Follow-up in the 2 Treatment Groups

	Time	A	B	C	D
Microfracture	Basal	0	1	14	5
	2-year follow-up	13	5	2	0
	Final follow-up	13	5	2	0
Hyalograft C	Basal	2	4	9	6
	2-year follow-up	16	4	1	0
	Final follow-up	15	5	1	0

EQ-VAS: The EQ-VAS increased from 64.1±17.2 to 90.5±9.3 at 2 years (p<0.0005) and 91.2±10.2 at the final follow-up (p=0.0005) in the HC autograft group and in the MFX group from 70.0±14.7 to 81.8±15.0 at 2 years (p=0.07) and to 84.0±10.8 at the final follow-up (p=0.001) as can be seen in Figure 6. The HC group gave better results than MFX at the final evaluation (p=0.035).

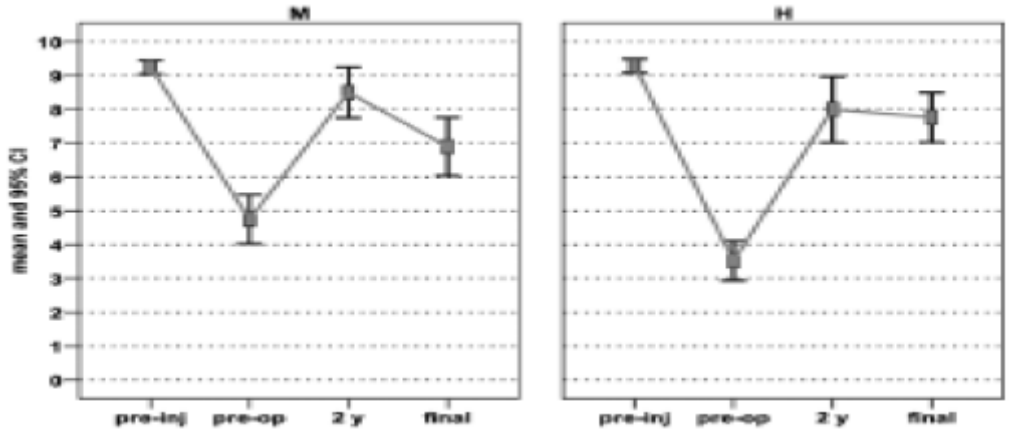
Figure 6: Comparison of EQ-VAS between Hyalograft C autograft (H) and Microfracture (M).



Tegner Score:

In the Hyalograft C autograft group the Tegner score in the Hyalograft C autograft group showed a significant improvement from preoperative level (3.5±1.3) to 2 years (8.0±2.1) (p<0.0005) and final follow- up (7.8±1.6) (p<0.0005). Patients returned to pre-injury activity level (9.3±0.5) at 2 years. In the MFX group the Tegner score showed a significant improvement from preoperative level (4.7±1.6) to 2 years (8.5±1.6) (p<0.0005) and final follow-up (6.9±1.8) (p=0.003) (Figure 6). Patients returned to pre-injury activity level (9.2±0.4) at 2 years; however, a decrease in sport activity from 2 years to final follow-up was found (p=0.001, Figure7).

Figure 7: Tegner Score in Microfracture (M) treated patients versus Hyalograft C autograft (H).



Return to sports:

In the MFX group 80% of patients returned to sports with 75% at their previous level, and in the Hyalograft C autograft group 86% returned to sports with 67% at their previous level. There was no difference in the length of time played at the same level after recovery: 3.5±2.4 years in the MFX group and 3.0±2.9 years in the Hyalograft C autograft group. Patients treated with MFX needed a median of 8 months before playing their first official soccer game, whereas the Hyalograft C autograft group required a median time of 12.5 months (p=0.009). A marked difference was found in the time needed to return to training with the team, with a median of 6.5 months in the MFX versus 10.2

months in the Hyalograft C autograft group (p=0.01), and to return to competitive sport activity level (Figures 8 and 9).

Figure 8. Time needed to return to training with the team in the microfracture and Hyalograft C group, respectively.

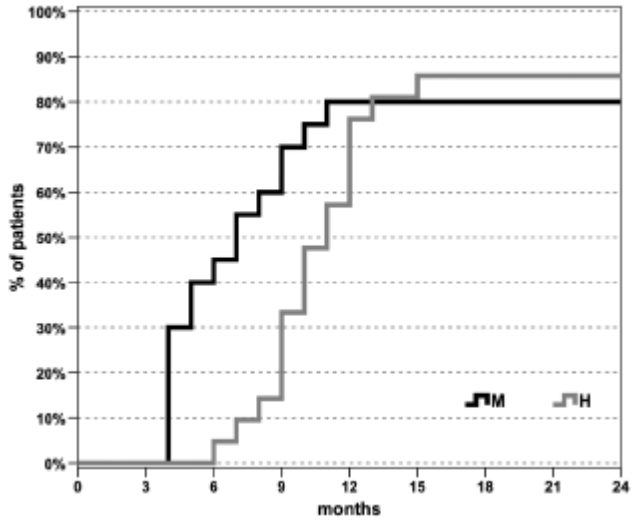
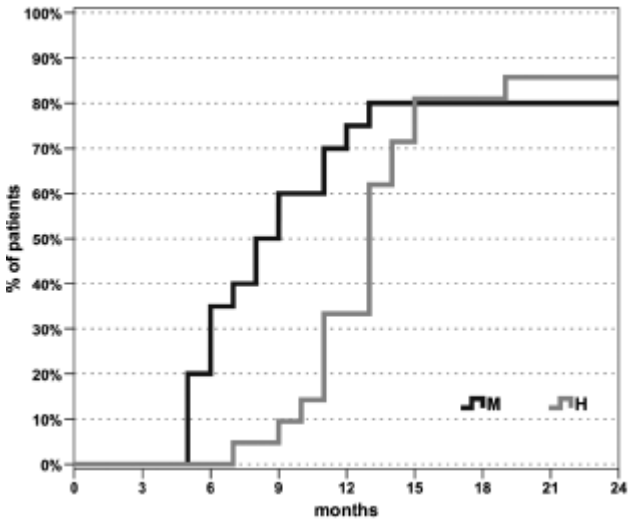


Figure 9. Time needed to return to competitive sport activity level (first official soccer game) in the microfracture and Hyalograft C group, respectively.



The results from this comparatively smaller prospective cohort study demonstrated that both groups showed statistically significant improvement with respect to clinical outcomes. However, with respect to the IKDC subjective score, the MFX group showed significant improvement from preoperative to 2 years' follow-up, followed by a significant deterioration of the results at the final follow-up compared with the previous evaluation at 2 years. It would therefore appear that the clinical outcome with Hyalograft C is more durable with significantly better results at the final evaluation. Similar significant outcomes were noted with the objective IKDC score at both 2 and 7.5 years with results being stable, and no significant differences between treatments were noted. Nevertheless, it is notable that the percentage of patients with normal and near normal knees was higher in the Hyalograft group compared with the microfracture group (19% vs 5% respectively) at baseline which increased to 95% and 90% at 2 years and remained stable at the final follow up at 7.5 years.

There is some inconsistency between these two results in that while the subjective score shows superiority of Hyalograft, this is not confirmed by the more robust objective IKDC score despite the substantial imbalance in the percentage of patients with normal or near normal knees in the Hyalograft versus microfracture groups at baseline (19% vs 5% respectively).

With respect to the EQ-VAS score, although both groups showed a significant final outcome, the results appear to be marginally better with the Hyalograft group, particularly at the earlier time point of 2 years.

Similar results were seen for the Tegner score, however a significant decrease in sporting activity was noted between 2 and 7.5 years in the microfracture group.

The real difference in both treatments was apparent in the return to sports activities since the microfracture group required a median of 8 months before playing the first official soccer game while this was 12.5 months in the Hyalograft group. A similar difference was noted in the time to return to training (6.5 vs 10.2 months respectively). However, despite the slower recovery in the Hyalograft group the 2 groups appear to show a similar success rate in returning to the previous sport activity.

Nevertheless, the results need to be interpreted with caution due to limitations in the study similar to the first cohort study. These involve the absence of randomisation which remains a concern despite the choice of treatment being dictated by each centre which performed only one treatment

Additionally, in an open trial, blinded evaluation would be considered important for the assessment of efficacy, however, there is no information regarding this in the publication.

There is also no evidence provided from structural data either from histology or MRI which would have supported the clinical data. Furthermore, there is presence of imbalance between the groups with respect to normal/near normal IKDC objective score at the baseline .

Finally, all of the patients in the study were active and young, and all cartilaginous lesions were contained. Additionally as outlined earlier, previous sporting activity appears to be positively correlated with better outcome especially in young active sportsmen. Therefore the results in both groups are not entirely unexpected. Consequently, the results cannot be applied to older patients with large, uncontained degenerative lesions which is not reflected in the SPC and there is evidence to suggest that Hyalograft C may be less effective in patients older than 40 years.

In addition, the lesion size in the inclusion criteria from >1 cm2 with no upper limit does not allow for a valid comparison since microfracture is not indicated for lesions larger than 4.0 cm.

Clinical studies in special populations

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

The applicant has carried out a meta-analysis of the submitted publications and investigator reports.

This essentially focuses on the clinical outcomes, where available, involving IKDC subjective, objective, Tegner and Lysholm-Gillquist scores.

In addition, the applicant has also included in the analysis data from microfracture studies and has made an indirect comparison between the results with Hyalograft C and microfracture.

IKDC subjective scores

For the 24 month follow-up, results from 330 patients from 9 studies were reported and showed an improvement from 40.3±15.3 to 78.2±19.7, while for the 60 month follow-up results from 242 patients from 7 studies were reported and showed an improvement from 40.9±16.0 to 74.9±20.6. It should be noted that the aforementioned 60 month follow-up results per the Statistical Analysis include longer FU outcomes if reported (e.g., the last follow-up outcome of the studies was used for the 60 month FU. For example, if a study reported outcomes at 60 month, 72 month, and 84 month FU, the 84 month follow-up was used). When only patients who had assessments both at 24 month and 60 month are considered (189 patients), the IKDC subjective scores remained at the same level during this long-term follow up with 77.9±19.4 at 24 month and 77.4±21.1 at 60 month. The results indicated that the improvement achieved at 24 month follow-up was largely maintained at 60 month, demonstrating durable treatment effects throughout the follow-up of 5 years. These results are also consistent with the analysis when all patients are analysed by comparing pre-operative and post-operative (last follow-up) values. In a total of 515 patients with pre- and postoperative values at the last follow-up, the IKDC subjective mean scores improved from 39.7±14.1 to 77.3±19.8 at the last follow-up assessment (Table 9). For the evaluation of the treatment effect on an annual basis all data for each year of follow up were used in the analysis. As the number of patients available for the assessment each year varied over time, a strict comparison of the treatment effects over time is limited but is presented here for completeness. However, the results from this analysis were consistent with the trends seen in the analysis at 24 and 60 month, specifically the effects achieved early on were maintained throughout the follow up even up to 84 month (Table 15).

It should be noted that the number of patients available for the assessment each year appears to be very variable over time, and therefore a critical comparison of the treatment effects over time is necessarily limited. Nevertheless, the results appear to be consistent with the trends seen in the analysis at 24 and 60 months, with respect to durability of efficacy.

Table 15 IKDC subjective score – annual results

Time Group	N	Weighted Mean	SD
Pre-operative	515	39.7	14.1
12	149	74.5	17.6
24	330	78.2	19.7
36	65	82.9	18.1
48	22	69.1	20.6
60	158	74.8	20.2
84	62	77.3	21.5

IKDC objective scores

For the 24 month follow-up, results from 385 patients were reported and showed a significant improvement in 90.1% of the patients reached a rating of normal or nearly normal, while for the 60 month follow-up results from 241 patients were reported and showed an improvement of 91.7% in the same categories (Table 16). These results indicate that the improvement achieved at 24 month was maintained through 60 month, demonstrating durable treatment effects throughout the follow-up of 5 years. These results are consistent with the analysis when all patients are analyzed with pre-operative

and post-operative (last follow-up) values. In a total of 422 patients with pre- and postoperative values at the last follow-up, the rating in the IKDC objective score of normal or nearly normal reached 92.18% at the last follow-up assessment

Table 16 IKDC Objective Score: Overall

Time Group	N	Normal	Nearly Normal	Abnormal	Severely Abnormal
Pre-operative	387	23 (5.94%)	70 (18.09%)	154 (39.79%)	140 (36.18%)
24 M	385	222 (57.66%)	125 (32.47%)	30 (7.79%)	8 (2.08%)
60 M	241	143 (59.34%)	78 (32.37%)	18 (7.47%)	2 (0.83%)

Although the number of patients is considerably lower at 60 months, the proportion of normal and near normal knees appears to be stable at both time points, thus indicating durability of effect.

Tegner Score

The Tegner score was reported in 6 studies. For the 24 month follow-up, results from 190 patients were reported while for the 60 month follow-up results from 133 patients were reported. The pre-operative values ranged from 1.7-3.5 with an average of 2.0±1.4 and were similar or the same in the cohorts who had the 24 or 60 month follow-up data (2.2±1.3). The Tegner scores improved significantly at 24 and 60 monthmonth follow-up compared to pre-operative values. The results indicated that the improvement achieved at 24 month follow-up (5.6±2.3) was maintained at 60 month (5.6±2.5) demonstrating durable treatment effects throughout the follow-up of 5 years (Table 17). This is also evident if only patients who had assessments both at 24 month and 60 month are considered. In this group of 71 patients, the Tegner scores remained at the same level during this long-term follow up with 6.0±2.5 at 24 month and 5.8±2.4 at 60 month (Statistical Appendix Table 2.2.1). In a total of 252 patients with pre- and postoperative values at the last follow-up, the Tegner mean scores improved from 2.0±1.4 to 5.5±2.4 at the last follow-up assessment (Statistical Appendix Table 2.6).

Data up to 84 month follow-up were available for an analysis of the annual treatment effects of HC. A similar pattern as for the IKDC subjective score was observed for the Tegner score (Table 18), in that after a significant initial improvement the score remained largely at the same level throughout the follow-up to 84 month.

Table 17 Tegner Activity Score: Overall

Time Group	N	Mean	SD	95% LCI	95% UCI
Pre-operative	286	2.0	1.4	1.8	2.2
24 M	190	5.6	2.3	5.3	5.9
60 M	133	5.6	2.5	5.2	6.0

Table 18 Tegner score – annual results

Time Group	N	Weighted Mean	SD
Pre-operative	286	2.0	1.4
24	190	5.6	2.3
36	65	5.7	1.9
48	54	4.9	2.7
60	71	5.8	2.4
84	62	5.3	2.6

Although the Tegner score shows similar results to IKDC subjective and objective scores, the annual changes do not appear to be consistent and show a degree of variability at 48 months with a lower score, nevertheless, the trend does appear to be somewhat stable.

Lysholm-Gillquist Score

The Lysholm-Gillquist score was reported in 2 studies. For the 24 month follow-up, results from 36 patients were reported while for the 60 month follow-up results from 53 patients were reported. The pre-operative values of 57.6±15.8 improved significantly at 24 and 60 month follow-up. The results showed that the improvement achieved at 24 month follow-up (72.4±22.5) were maintained at 60 month (85.0±19.4) demonstrating durable treatment effects throughout the follow-up of 5 years (Table 19). In a total of 89 patients with pre- and postoperative values at the last follow-up, the Lysholm-Gillquist scores improved from 57.6±15.8 to 83.3±19.4 at the last follow-up assessment. In the analysis of the annual scores of HC, data were available up to the 60 month follow up. A similar pattern as for the IKDC subjective score was observed for the Lysholm-Gillquist score (Table 20), that after a significant initial improvement the score steadily improved until the follow-up at 60 month.

Table 19 Lysholm-Gillquist Score by Study

Short Ref	Surgical Technique	Time Group	N	Mean	SD
Domayer et al., ICRS, 2007	Hyalograft® C autograft	Pre-operative	53	57.6	16.9
Domayer et al., ICRS, 2007	Hyalograft® C autograft	60 M	53	85.0	19.4
Nehrer et al., 2006	Hyalograft® C autograft	Pre-operative	36	57.5	14.0
Nehrer et al., 2006	Hyalograft® C autograft	24 M	36	72.4	22.5

Table 20 Lysholm-Gillquist score – annual results

Time Group	N	Weighted Mean	SD
Pre-operative	97	57.6	15.8
12	36	69.7	25.3
24	36	72.4	22.5
36	36	80.8	19.3
60	53	85.0	19.4

Although only 2 studies reported on the Lysholm-Gillquist score, the results appear to be consistent over time.

Subgroup analyses

The most comprehensive data presentation with extensive subgroup analyses was performed by Marcacci 2006 in a multicenter observational study. The study evaluated the outcome of cartilage lesions of the knee treated with Hyalograft® C autograft. A total of 206 patients were enrolled and 179 patients were evaluated with a minimum of 2 years follow-up and an average of 47.2 month follow-up.

There were 12 graft failures among the 206 patients (5.8%), which occurred primarily in higher risk patients. The vast majority of the patients benefited from the Hyalograft® C autograft procedure. The average improvement on the IKDC subjective scores in the overall population was 39.9±24.3 points. This improvement was similar to the effects achieved in the comparative cohort studies by Kon. The standardized effect size (ES) can be calculated as mean/SD for the change from pre-operative to the final assessment scores and reached large values in the overall population, ES=1.64.

The results in this study indicated a very consistent improvement across various subgroups suggesting that a similar treatment benefit was achieved across subgroups and allowing one to generalize the treatment effects achieved in the overall population to all patients in this study with various background characteristics. In univariate and multivariate analyses only 3 factors were identified ($p<0.05$) to represent potentially prognostic factors, pre-operative IKDC subjective scores (no specific results were presented in the report), the duration of symptoms and osteoarthritis and Ahlback degree. As expected the improvement was smaller in the higher risk groups but still achieved a large effect size of more than 0.8.

Table 21 summarises the results from this study. The treatment benefits were consistent across a variety of baseline characteristics, e.g. age, duration of symptoms, previous surgery, observation period, location of lesion and aetiology, with an effect size around 1.64 observed in the overall population as presented by Marcacci 2006. Improvement was largest in patients with ≤ 20 years of age with an effect size of 2.83, in a small subset of patients (n=19) with Osteochondritis dissecans with an effect size of 2.74 and in subjects with a duration of symptoms of less than 1 year with an effect size of 2.4. Patients with duration of symptoms of 1 year or more still had an effect size of 1.05. Patients with osteoarthritis and Ahlback degree III-IV had a smaller but still substantial improvement with an effect size of 0.87.

Table 21 IKDC subjective score - change from baseline in all patients and subgroups

	Number of patients	Mean IKDC change from baseline	SD	Effect size
All patients	178	39.9	24.3	1.64
Age				
≤20 yrs	21	45.3	16.0	2.83
21-40 yrs	88	38.8	23.5	1.65
≥41 yrs	68	39.7	27.5	1.44
Duration of symptoms				
≤1 yr	91	47.9	20.0	2.4
>1 yrs	47	28.8	27.4	1.05
Previous surgery				
No	120	41.7	23.1	1.81
Yes	58	36.1	26.6	1.36
Observation time in months				
≥24 and <36	35	36.7	19.1	1.92
≥36 and <48	40	34.2	24.1	1.42
≥48 and <60	81	41.1	26.5	1.55
≥60	22	50.8	21.1	2.41
Lesion type/location				
Single condyle	123	40.6	24.6	1.65
Single trochlea or patella	13	36.5	20.0	1.83
Multiple with condyle	38	40.2	22.9	1.76
Aetiology				
Trauma	55	43.1	22.4	1.92
Osteochondritis dissecans	19	43.3	15.8	2.74
Microtrauma degenerative	102	37.6	26.6	1.41
Osteoarthritis				
No	123	44.2	20.5	2.16
Ahlback degree I-II	26	29.1	29.7	0.98
Ahlback degree III-IV	21	26.4	30.4	0.87

In addition to the subgroup analyses by patient characteristics, Marcacci also presented an analysis in the subgroup for patients where complete FU data were available at various time points up to an average of 62 month. The results of 37 patients were presented in the report and demonstrated an improvement from 37.4±15.0 at pre-surgery to 80.7±19.4 at a mean of 21 month follow-up, 82.7±20.2 at 49 months FU and 84.3±21.2 at 62 months FU ($p < 0.0001$).

The study by Marcacci et al 2006 which collectively analysed a small number of studies included the largest series of patients. The sub-group analysis would appear to support the efficacy noted with Hyalograft C in the various sub-groups analysed. However, for reasons elaborated earlier the results need to be interpreted with caution due to the limitations in the studies which were mainly case observation studies and especially since the above analysis involves only the subjective score.

However, it should be noted that Hyalograft®C was applied to the defect without the use of any fixation method in the 54.2% of cases. Fibrin glue was used either alone (29.2% of defects) or in

combination with sutures (5.5%), while only sutures were chosen for Hyalograft®C fixation in the remaining 11.0% of defects. Sutures were mainly used for large defects in any location, including patella, where however in most cases the defect area averaged 2 cm2 and thus no fixation systems were used

Failure rates

Failures rates were reported in 13 studies with Hyalograft® C autograft in the knee. The overall failure rate was 51/551 patients (9.3%) and was based on 10 studies since 3 studies were excluded from the summary of overall failure rates since their population was covered by other references. In the 2 comparative trials of Kon et al 2009 and Kon et al 2011, 1 case in each treatment group was reported as failure. In the studies with microfracture excluding the meta-analysis by Mithoefer a total of 10/62 patients (16.1%) reported failure of the procedure. In the large evidence-based systematic analysis by Mithoefer a failure rate of 2.5% at 2 years was reported which increased to 23%-31% between 2-5 years follow-up.

Based on these data the failure rate seems lower with Hyalograft® C autograft compared to microfracture.

Efficacy in microfracture studies

Efficacy results for microfracture are largely based on the evidence-based systematic review by Mithoefer 2009 and the additional 6 studies reported after the cut-off for the Mithoefer analysis. In the systematic review Mithoefer reported the following results:

Results: Twenty-eight studies describing 3122 patients were included in the review. The average follow-up was 41 months, with only 5 studies reporting follow-up of 5 years or more. Six studies were randomized controlled trials and the mean Coleman Methodology

Score was 58 (range, 22-97). Microfracture effectively improved knee function in all studies during the first 24 month after microfracture, but the reports on durability of the initial functional improvement were conflicting. Several factors were identified that affected clinical outcome. Defect fill on magnetic resonance imaging was highly variable and correlated with functional outcome. Macroscopic repair cartilage quality positively affected long-term failure rate, while the influence of histologic repair tissue quality remained inconclusive.

The systematic analysis shows that microfracture provides effective short-term functional improvement of knee function but insufficient data are available on its long-term results. Shortcomings of the technique include limited hyaline repair tissue, variable repair cartilage volume, and possible functional deterioration. The quality of the currently available data on microfracture is still limited by the variability of results and study designs. Further well-designed studies are needed to determine the long-term efficacy of microfracture and to define its specific clinical indications compared to other cartilage repair techniques.

The two efficacy outcome variables which were presented in the systematic review and subsequent studies with microfracture were Tegner score and Lysholm-Gillquist score at the end of follow-up, which was about 41 month on average.

The Tegner score at the last follow-up assessment with MF was 4.9±0.9 in 1320 patients The Lysholm-Gillquist score at the last follow-up assessment with MF was 80.9±6.7 in 1315 patients.

It should be noted that the systematic review by Mithoefer et al included studies with a widely varying Coleman Index which determines the quality of the studies. In this respect some studies were particularly notable in having a Coleman index of 22 which is considered very low and thus the results need to be interpreted with caution.

Comparison of Hyalograft® C autograft with microfracture

Although there are limitations in the indirect comparisons of study results which are not based on direct head-to-head comparisons, the indirect comparison coupled with the conclusions from direct comparisons are complimentary and are supportive for the efficacy conclusion on Hyalograft® C autograft in the context of other surgical techniques.

Based on the two efficacy outcome variables (Tegner score and Lysholm-Gillquist score) assessed at the last post-operative follow-up, which were presented in the systematic review, and subsequent studies with microfracture as well as in the systematic review of all studies with Hyalograft® C autograft in this report, a statistically significant difference was observed for the Tegner score $p<0.0001$ and the Lysholm-Gillquist score $p=0.0035$ (Table 22).

Table 22 Overall Comparison of Hyalograft® C autograft and Microfracture

	HC		MF		
	N	Mean (SD) at last follow-up	N	Mean (SD) at last follow-up	p-value
Tegner	252	5.5 (2.4)	1320	4.9 (0.9)	<0.0001
Lysholm-Gillquist	89	83.3 (19.4)	1315	80.9 (6.7)	0.0035

Given the limitations of indirect comparison, the results appear to show a significant difference between Hyalograft and Microfracture, nevertheless this seems to be inconsistent from the actual difference between means which appears to be minimal and it is arguable whether the difference is clinically relevant.Accordingly the findings need to be interpreted with caution.

Supportive study(ies)

The supportive studies provided data on comparison with other ACI products, histology and MRI data and apart from the 2 pivotal cohort studies , the majority of the studies were single arm case observation studies classed as being level 4 evidence as well as investigator reports.

The studies which provided histology, MRI and ICRS visual score data have been very briefly outlined in section on pharmacodynamics. All studies were analysed in the meta-analysis section.

The individual study efficacy data, where available are summarised below. The collective data are presented in the meta-analysis.

Active comparator studies

Manfredini et al, 2007: Level 4 evidence prospective cohort study, with 13.5 month year mean follow-up for Hyalograft C autograft group and 48.5 months in the Carticel group.

Study Design

In this cohort study, clinical outcomes of the Carticel method (n=17) (cells in suspension and use of a periostal flap as coverage) was compared with the Hyalograft C autograft technique using miniarthrotomy or arthroscopy (n=15). Lesions Outerbridge grades III or IV, ranges 2 and 9 cm2

(Carticel) and 1.5-6 cm2 (Hyalograft C autograft). The mean follow-up time was 48.5 months in the Carticel group and 13.5 months in the Hyalograft C autograft group.

Study Results

HSS (Hospital for Special Surgery) and ICRS (International Cartilage Repair Society) scores: A statistically significant improvement was observed in both groups at six months ($p<0.001$ for Carticel and $p=0.002$ for Hyalograft C autograft) and at twelve months after surgery ($p<0.001$ both for Carticel and Hyalograft C autograft) with no statistically significant differences between the two groups.

Ferruzzi A. et al, 2008: Level 4 evidence prospective cohort study with 5 years mean follow-up

Study Design

Ninety-eight patients with ICRS grade 3 or 4 posttraumatic or OCD chondral lesions, localized into the femoral condyles were selected for the study. Forty-eight patients with cartilage lesions of mean size of 6.4 cm2 were treated with Carticel implantation (open ACI) and fifty patients with cartilage lesions of mean size of 5.9 cm2 were treated with Hyalograft C autograft (arthroscopic ACI). All patients underwent clinical assessment before surgery, then at 6, 12, 18, 24 and yearly up at least 5 years.

Study Results

IKDC subjective:

Statistical analysis of the IKDC subjective scores showed a significant difference between the results obtained at the final follow-up and the preoperative score, in both groups ($p<0.0005$). The open ACI group (Carticel) showed progressive improvement until twenty-four months; whereas, in the arthroscopic ACI group (Hyalograft C autograft), the improvement was more rapid and remained stable after eighteen months showing a significant difference in the results obtained at twelve months ($p=0.007$) in favour of the arthroscopic ACI series (Hyalograft C autograft)

IKDC Functional Scores:

In both groups the IKDC functional score showed a significant difference in the results obtained at the final compared to the preoperative score ($p<0.0005$). The arthroscopic ACI group showed a more rapid and stable increase at 18 months while the open ACI group showed progressive improvement until 24 months.

IKDC Objective Scores: Both, the Carticel and Hyalograft C autograft group showed better objective IKDC scores at final follow-up compared with the preoperative scores ($p<0.0005$). Subjects in the Carticel continued to improve for twenty-four months, whereas subjects in the Hyalograft C autograft group showed a more rapid improvement that remained stable after eighteen months. The comparison

of open surgery vs. arthroscopic Hyalograft C autograft implantation showed a significant difference at twelve months in favour of Hyalograft C autograft (p=0.0023)

Additional surgeries: The arthroscopic Hyalograft C autograft group had statistically fewer additional surgeries compared to the open series (p=0.036).

MRI and histology data have been described earlier in section 2.2, Pharmacodynamics

Kon E. et al. 2011- MACI: level of evidence 4

Study Design

Kon et al, 2011 conducted a study in 61 patients with grade III to IV cartilaginous lesions to analyze the clinical outcome of cartilage lesions of the femoral condyles treatment using second-generation autologous chondrocyte implantation (ACI) techniques (Hyalograft C autograft and Chondro-Gide MACI). Subjects had no clear signs of osteoarthritis and were a minimum age of 40 years and were prospectively evaluated at 5 years' follow-up. Twenty-two patients were treated with arthroscopic Hyalograft C autograft implantation, and 39 underwent the open Chondro-Gide MACI procedure. The mean age was at surgery 45.5 ± 4.9 years (range 40-62). The mean lesion defect site (after debridment) was 2.6± 1.2 cm2 for Hyalograft C autograft and 3.1±1.1 for MACI.

Study Results

IKDC subjective:

The subjective IKDC score improved markedly from the baseline evaluation 36.8 ± 8.4 to the different follow-ups (p < .0005). The improvement achieved at 12 months, 60.1 ± 22.4 was further increased at the 24-month evaluation 66.5 ±21.8 (p = 0.008), whereas results were stable from 2 years to the final midterm evaluation 68.1 ±21.8 When comparing the Hyalograft C autograft and MACI groups, similar scores were found at the baseline level (39.2 ± 10.7 Hyalograft C autograft vs 35.5 ± 6.6 MACI). Scores were favorable for Hyalograft C autograft at the 24-month follow-up (69.7± 21.4 vs MACI 64.7 ± 22.2) and at the final evaluation (Hyalograft C autograft 69.1 ± 20.6 vs MACI 67.6 ± 22.8).

Hyalograft C autograft showed a faster improvement at 12 months with higher subjective IKDC scores 67.4 ± 21.5 than the group of patients treated with MACI implant (55.9 ± 22.1) (p = 0.049)

IKDC objective score:

The objective IKDC score increased from 20% of normal (A) and nearly normal (B) knees before the treatment to 54% at 12 months, 79% at 24 months, and 80% at the final follow-up, showing a statistically significant improvement (P<.0005) at all the follow-up times with respect to the baseline level. The improvement achieved at 12 months was further increased at the 24-month evaluation (P = 001), whereas results were stable from 2 years to the final midterm evaluation.

DeWindt TS et al. 2012: level of evidence 4

Study Design

DeWindt et al conducted a study to report on the clinical outcome of a large heterogenic group of patients treated with Hyalograft C autograft, microfracture or carbon fiber procedures by a single surgeon. Between 2006 and 2008, 216 patients suitable for focal cartilage repair were prospectively followed. The patients had traumatic or degenerative symptomatic full thickness grade III to IV ICRS lesions on the femur, trochlea and patella. Three separate cohorts were suitable for analysis: MF

(n=65) mean age 40±12, Hyalograft C autograft (n=54) mean age 37±9 and carbon-fiber scaffolds (n=47) mean age 47±9. The average follow-up time for MF, Hyalograft C and carbon-fiber scaffolds was 38±5 months, 36±8 months and 36±6 months, respectively. In the MF group the mean lesion size was 1.8±1.5 and 35% were second line treatment and in the Hyalograft C autograft group the mean lesion size was 3.9±2.3 and 41% were second line treatment, Carbon Fiber group mean lesion size was 2.7±1.9 and 72% were second line treatment. Microfracture was used for first and second line treatment of smaller defects (≤2.5 cm2). Hyalograft C autograft was used for the first and second line treatment of larger defects (>2.5 cm2), while carbon-fiber scaffold implantation was used as a salvage procedure for medium to large (≥1.5 cm2) early osteoarthritic (ICRS grade III–IV) defects. The

Hyalograft C autograft technique was preferred over MF for patients with high (sport related) physical demands with lesions 1.5–2 cm2 as well as patients with more than two previous cartilage repair procedures. Hyalograft C autograft technique was preferred over MF for patients with high (sport related) physical demands with lesions 1.5–2 cm2 as well as patients with more than two previous cartilage repair procedures.

Three years after surgery, statistically significant improvement was seen for all scores (Brittberg-Peterson VAS, KOOS, Lysholm) in both the Hyalograft C autograft and MF groups ($p<0.001$). The improvement from baseline was higher for both the MF and Hyalograft C autograft cohorts, compared to carbon-fiber scaffolds and statistically significant for the KOOS sports and QoL subscales ($p<0.05$).

Covariates influencing patient outcome

Linear regression analysis indicated no treatment by covariate interaction and patient age, BMI, defect cause and defect size did not seem to influence clinical outcome. Furthermore, defect location did not have an influence on clinical outcome since patellar and medial lesions did not significantly differ in outcome scores compared to other defect locations for any of the treatment groups. For Hyalograft C autograft, patients with prior ACL reconstruction had reduced improvement from baseline compared to patients without prior ACL reconstruction (VAS B -20.6, overall KOOS B -13.4, Lysholm B -19.8, $p=0.006–0.042$). In all patients, single defects scored 13.0, 8.4 and 8.1 points higher on KOOS Sports and QoL and Lysholm scale respectively when compared to multiple defects ($p<0.05$).

Summary of all individual study results where available

Results for IKDC subjective objective and Tegner activity scales for individual studies are shown in Tables 23-25

Table 23 IKDC Subjective Score by Study for Pre-operative vs Last Follow Up

Short Ref	Surgical Technique	Time	N	Mean	SD
Della Villa et al., 2010	Hyalograft® C autograft in Athlete group	0	31	44.4	2.9
Della Villa et al., 2010	Hyalograft® C autograft in Athlete group	36	31	90.7	11.7
Della Villa et al., 2010	Hyalograft® C autograft in Control group	0	34	34.3	14.2
Della Villa et al., 2010	Hyalograft® C autograft in Control group	36	34	75.7	22.4
Domayer et al., ICRS, 2007	Hyalograft® C autograft	0	53	40.5	19.2
Domayer et al., ICRS, 2007	Hyalograft® C autograft	60	53	66.1	18.9
Ferruzzi et al., 2008	arthroscopic ACI Hyalograft® C autograft	0	50	45.5	.
Ferruzzi et al., 2008	arthroscopic ACI Hyalograft® C autograft	60	50	87.9	.
Filardo et al., 2011	Hyalograft® C autograft	0	62	39.6	15.0
Filardo et al., 2011	Hyalograft® C autograft	84	62	77.3	21.5
Gobbi et al., 2009	Hyalograft® C autograft	0	34	46.1	19.3
Gobbi et al., 2009	Hyalograft® C autograft	60	34	70.4	21.4
Kon et al., 2010b	Hyalograft® C autograft	0	50	39.0	13.8
Kon et al., 2010b	Hyalograft® C autograft	60	50	80.1	22.8
Kon et al., 2011a	Hyalograft® C autograft	0	21	43.2	13.7
Kon et al., 2011a	Hyalograft® C autograft	60	21	91.0	13.9
Kon et al., 2011b	Hyalograft® C autograft	0	22	39.2	10.7
Kon et al., 2011b	Hyalograft® C autograft	48	22	69.1	20.6
Marcacci et al., 2005	Hyalograft® C autograft	0	141	39.3	14.0
Marcacci et al., 2005	Hyalograft® C autograft	24	141	78.6	20.2
Marcacci et al., 2007	Hyalograft® C autograft	0	70	41.5	.
Marcacci et al., 2007	Hyalograft® C autograft	48	21	78.5	.
Nehrer et al., 2009	Hyalograft® C autograft as primary indication	0	42	41.4	.
Nehrer et al., 2009	Hyalograft® C autograft as primary indication	84	18	69.0	.
Nehrer et al., 2009	Hyalograft® C autograft as secondary indication	0	11	31.0	.
Nehrer et al., 2009	Hyalograft® C autograft as secondary indication	60	6	31.0	.
Pavesio et al., 2003	Hyalograft® C autograft	0	67	37.0	9.2
Pavesio et al., 2003	Hyalograft® C autograft	18	67	78.1	17.7
Podskubka et al., 2006	Hyalograft® C autograft	0	8	46.0	.
Podskubka et al., 2006	Hyalograft® C autograft	9	8	74.0	.

Table 24 IKDC Objective Score by Study for Pre-operative vs Last Follow Up

Short Ref	Surgical Technique	Time Group	Time	N	Normal	Nearly Normal	Abnormal	Severely Abnormal
Della Villa et al., 2010	Hyalograft® C autograft in Athlete group	Pre-operative	0	31	1 (3.23%)	4 (12.90%)	18 (58.06%)	8 (25.81%)
Della Villa et al., 2010	Hyalograft® C autograft in Athlete group	Follow-up	36	31	27 (87.10%)	3 (9.68%)	1 (3.23%)	
Della Villa et al., 2010	Hyalograft® C autograft in Control group	Pre-operative	0	34	4 (11.76%)	9 (26.47%)	9 (26.47%)	12 (35.29%)
Della Villa et al., 2010	Hyalograft® C autograft in Control group	Follow-up	36	34	24 (70.59%)	8 (23.53%)	2 (5.88%)	
Ferruzzi et al., 2008	arthroscopic ACI Hyalograft® C autograft	Pre-operative	0	50			14 (28.00%)	36 (72.00%)
Ferruzzi et al., 2008	arthroscopic ACI Hyalograft® C autograft	Follow-up	60	50	16 (32.00%)	34 (68.00%)		
Filardo et al., 2011	Hyalograft® C autograft	Pre-operative	0	62	1 (1.61%)	12 (19.35%)	29 (46.77%)	20 (32.26%)
Filardo et al., 2011	Hyalograft® C autograft	Follow-up	84	62	45 (72.58%)	12 (19.35%)	4 (6.45%)	1 (1.61%)
Gobbi et al., 2009	Hyalograft® C autograft	Pre-operative	0	34		8 (23.53%)	14 (41.18%)	12 (35.29%)
Gobbi et al., 2009	Hyalograft® C autograft	Follow-up	60	34	15 (44.12%)	16 (47.06%)	2 (5.88%)	1 (2.94%)
Kon et al., 2010b	Hyalograft® C autograft	Pre-operative	0	50	1 (2.00%)	6 (12.00%)	27 (54.00%)	16 (32.00%)
Kon et al., 2010b	Hyalograft® C autograft	Follow-up	60	50	40 (80.00%)	5 (10.00%)	5 (10.00%)	
Kon et al., 2011a	Hyalograft® C autograft	Pre-operative	0	21	2 (9.52%)	4 (19.05%)	9 (42.86%)	6 (28.57%)
Kon et al., 2011a	Hyalograft® C autograft	Follow-up	60	21	15 (71.43%)	5 (23.81%)	1 (4.76%)	
Marcacci et al., 2005	Hyalograft® C autograft	Pre-operative	0	46	7 (15.22%)	15 (32.61%)	20 (43.48%)	4 (8.70%)
Marcacci et al., 2005	Hyalograft® C autograft	Follow-up	24	46	22 (47.83%)	22 (47.83%)	1 (2.17%)	1 (2.17%)
Marcacci et al., 2007	Hyalograft® C autograft	Pre-operative	0	70	4 (5.71%)	10 (14.29%)	29 (41.43%)	27 (38.57%)
Marcacci et al., 2007	Hyalograft® C autograft	Follow-up	24	70	47 (67.14%)	15 (21.43%)	7 (10.00%)	1 (1.43%)
Nehrer et al., 2009	Hyalograft® C autograft as primary indication	Pre-operative	0	42	6 (14.29%)	13 (30.95%)	10 (23.81%)	13 (30.95%)
Nehrer et al., 2009	Hyalograft® C autograft as primary indication	Follow-up	84	18	12 (66.67%)	6 (33.33%)		
Nehrer et al., 2009	Hyalograft® C autograft as secondary indication	Pre-operative	0	12	2 (16.67%)	2 (16.67%)	2 (16.67%)	6 (50.00%)
Nehrer et al., 2009	Hyalograft® C autograft as secondary indication	Follow-up	60	6			6 (100.00%)	

Table 25 Tegner Activity Score by Study for Pre-operative vs Last Follow Up

Short Ref	Surgical Technique	Time	N	Mean	SD
Della Villa et al., 2010	Hyalograft® C autograft in Athlete group	0	31	2.1	1.8
Della Villa et al., 2010	Hyalograft® C autograft in Athlete group	36	31	7.3	1.6
Della Villa et al., 2010	Hyalograft® C autograft in Control group	0	34	1.8	1.3
Della Villa et al., 2010	Hyalograft® C autograft in Control group	36	34	4.3	2.1
Filardo et al., 2011	Hyalograft® C autograft	0	62	1.7	1.3
Filardo et al., 2011	Hyalograft® C autograft	84	62	5.3	2.6
Gobbi et al., 2009	Hyalograft® C autograft	0	34	2.6	1.5
Gobbi et al., 2009	Hyalograft® C autograft	60	34	4.7	.
Kon et al., 2010b	Hyalograft® C autograft	0	50	1.7	1.3
Kon et al., 2010b	Hyalograft® C autograft	60	50	5.0	2.7
Kon et al., 2011a	Hyalograft® C autograft	0	21	3.5	1.3
Kon et al., 2011a	Hyalograft® C autograft	60	21	7.8	1.6
Kon et al., ICRS, 2007	Hyalograft® C autograft	0	54	1.7	1.1
Kon et al., ICRS, 2007	Hyalograft® C autograft	48	54	4.9	2.7

Efficacy results of Hyalograft® C autograft for articular cartilage repair in the ankle

Four studies were identified which reported results from studies with Hyalograft® C autograft for the articular cartilage repair in the ankle. Two of these studies reported the result from the same population and are counted only once in the analysis. These studies were prospective cohort studies, performed in Austria, Germany and Italy. A total of 72 patients were enrolled in these studies. The age range was from 15-50 years with a mean age of 30 years. Minimum follow-up in 2 studies by Giannini was 36 months and in the study by Nehrer et al 2011, 24 months. The mean follow-up was reported as 57.5 month in the study by Giannini et al 2010, 61.2 months in the study by Nehrer et al 2011 and 44.4 months in the study by Thermann et al.

The functional scores showed a significant improvement in the studies and the MRI assessment showed a high rate of success regarding complete integration of the regenerated tissue with the surrounding cartilage

Given the limitations of the low level of evidence of the active comparator studies due to lack of stringency especially with respect to inclusion criteria, lesion size and follow up periods ,the results with Hyalograft C would appear to be approximately comparable to the other ACI products, both first and second generation.

The results from the individual studies appear to be generally consistent with the results from the meta-analysis with respect to the improvement in functionality as well as duration of effect .However, it should be noted that the data were available from only a minor proportion of the studies and therefore should be treated with caution. The ankle joint efficacy data are not relevant to this application and have been provided for completeness of information.

Discussion on clinical efficacy

Autologous chondrocyte implantation (ACI) was first reported in 1994, and more than 12,000 patients have since undergone treatment. First generation ACI required the harvesting of an autologous periosteal patch, which was used to secure the liquid culture chondrocytes in situ. 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 variety of scaffolds. Hyalograft C has been shown to deliver comparative clinical results to traditional ACI as reported in the literature, but appears to offer several advantages including reduced operative time, reduced tourniquet time and the ability to perform the implantation via minimally invasive methods such as mini-arthrotomy or arthroscopy. In addition pre-implantation chondrocyte phenotype manipulation also has shown impressive outcomes.

Some recent studies which compared ACI and MF, showed conflicting evidence which complicates the formation of evidence-based decisions. However, compared to the higher invasiveness of the first generation cell-based techniques, MF is an effective procedure for smaller (< 4.0 cm2) cartilage defects.

Nevertheless it is still not entirely clear which cartilage repair or restoration technique provides the best long-term clinical outcome since the use of various surgical techniques and availability of different products including a variety of study designs complicate firm conclusions being drawn due to inconsistent results.

Design and conduct of clinical studies

Due to the different chondrocyte seeded scaffolds available on the market, and the variability in their manufacturing processes and materials used, there is confounding in the objective comparison of clinical studies using different products. This is further compounded by the fact that, cartilage repair studies are of heterogeneous design and differ widely in their inclusion criteria, patient demographics and history, consistency of lesion size and location for comparison, clinical scores, follow-up periods and multiple centres with different surgeons, which complicates evaluation and prevents firm conclusion being drawn.

In this respect, comparing different cartilage repair publications shows that relatively small, heterogeneous study populations, study centre experience and the effects of different rehabilitation protocols may confound results.

It should particularly be noted that, of all 28 studies which have been submitted in the dossier, there does not appear to be a single study which can satisfy the criteria for a pivotal phase III study carried out to GCP standards. However, the applicant has submitted data from prospective cohort studies which can be considered the next best alternative to a randomised trial. Although these studies provide evidence of some efficacy there are numerous limitations which complicate the full evaluation of efficacy. In addition the choice of control is questionable and would appear to be invalid, since MF is only indicated for lesions smaller than 4.0cm.

Other studies have varied widely in the design and methodology, the majority being non-randomised observational studies and although being open label, single arm, single centre, did not involve blinded evaluation of the outcomes in the majority.

Additionally, it is not very clear if any studies deviated from the protocol and in a substantial proportion the protocol has not been available to confirm whether the conduct of the study differed.

Accordingly, the substantial variability within and between studies with respect to patient characteristics such as age treatment history, defect size, location and aetiology, complicates a collective evaluation of efficacy, to draw firm conclusions in the absence of a phase III trial carried out to GCP standards.

Efficacy data and additional analyses

The evidence for efficacy from the studies considered to be pivotal appears to have several limitations. In the comparative cohort studies by Kon et al (2009 and 2011), the study design does not allow for a valid comparison, because microfracture is not considered a treatment option in lesions larger than 4.0 cm as defined in the EMA CAT Reflection Paper. It is also unclear whether there was blinded evaluation of the outcomes. Furthermore there were no secondary structural endpoints included. Additionally, data on pain medication were not available, which could have impacted on the patient reported outcomes. It is also unclear, whether the dose utilised has been optimal since no clinical dose finding studies were carried out.

Nevertheless, the efficacy of Hyalograft in the group of young semi-professional highly athletic patients cannot be ignored, especially with respect to superiority over microfracture as far as durability of effect is concerned.

There are also concerns regarding Quality with respect to cell potency and viability which have varied considerably and thus comparability is an issue which impacts on both efficacy and safety as seen in the wide variation with the histology results with respect to the percentage of biopsies showing hyaline cartilage regeneration.

Nevertheless, approximately 800 patients were treated with Hyalograft C and the clinical studies appear to provide a degree of evidence of clinical improvement, including pain and function that appears to be consistent and which, in a limited number of patients, appears to be sustained up to 7.5 years. Given the low level of evidence of the supportive active comparator studies, Hyalograft shows approximately similar results to other ACI products and microfracture.

With respect to histology evaluation, 6 studies have been submitted with a total of 375 subjects of which 68 (18%) patients had biopsies for histological evaluation. Although the overall percentage of biopsies which showed the presence of hyaline cartilage was 53%, there was wide variation in the individual studies which ranged from 12.5% to 100 %. Mixed hyaline cartilage and fibrocartilage were observed in 25% and 22 % of subjects respectively. Since less than 20% of patients had biopsies there is the possibility of selection bias impacting on the results. Additionally, samples were obtained at varying time points. Furthermore, only 3 studies indicated that evaluation was done in a blinded manner. Furthermore, it would appear that none of the studies used the ICRS scoring system. Nevertheless, treatment with Hyalograft C does show some evidence of efficacy with the presence of hyaline cartilage, which appears to increase with increasing duration of follow up, since the proportion of hyaline-like cartilage was found to be increased from the group of biopsies taken after a minimum of 18 months than those taken at earlier times (83.3% and 43.8 %) respectively. Additionally, there was some evidence of clinical correlation in that there was persistence of symptoms with the presence of fibrocartilage.

MRI data have shown that of 8 studies a proportion of cartilage repair grafts provided good fill and integration of the defects. Nevertheless the full MOCART scoring system was used in only 3/8 studies and only a few studies evaluated the results in a blinded manner.

Data were reported for 118 patients at 24 months and 165 patients at 60 months.

It should be noted that the full MOCART scoring system with all endpoints was used only in 3 studies, and only a proportion of patients who were entered into the studies went on to have MRI evaluation.

However, using MOCART parameters the analysis showed that 46 patients, (67.7%) had a complete defect repair at 24 months and 74 patients (64.4%) at 60 months and a complete integration to the border zone was seen in 74 (86.1%) at 24 months and 108 (76.6%) at 60 months. Four studies provided data showing clinical correlation with some MRI parameters.

Nevertheless, a number of patients in the studies did not appear to have either biopsies or MRI and thus it is difficult to quantitate responses to treatment. This is particularly true for the 2 “pivotal” prospective cohort studies.

From a clinical perspective, although a definitive phase III pivotal study to GCP standard has been planned which will not be fully completed for a further five years, the available evidence of efficacy in a very focused population of young athletic sportmen cannot be ignored, nevertheless, a full evaluation of the benefit risk is currently not possible from the available data, concerns regarding which need to be satisfactorily addressed before a marketing authorisation can be considered.

Conclusions on clinical efficacy

Studies which aim to evaluate cartilage repair techniques can be confounded by the subjective nature of pain reported by patients, including individual responses to medication, physical therapies and surgical interventions and accordingly, objective outcomes like arthroscopy and MRI are considered necessary, as opposed to depending on endpoints measured solely by patient reported outcomes. Some studies in the literature consider that, ACI is superior to MF and also improves upon the first and

second generation chondrocyte-based cartilage repair techniques because it appears to show reproducibility, safety, less operative time, surgical simplicity and reduced invasiveness. Nevertheless, conclusive evidence of this needs to be demonstrated in a randomised controlled trial carried out to GCP standard for regulatory requirements.

The totality of evidence would appear to suggest that Hyalograft shows limited, but generally consistent evidence of efficacy, both clinical and structural from the pivotal and supportive data, especially in a focused population of young athletic sportsmen, which however appear to have substantial limitations that currently preclude firm conclusions from being drawn.

Nevertheless, from a regulatory standpoint, there are a number of efficacy concerns including Quality concerns regarding comparability with respect to cell viability and potency which could impact on the evaluation of efficacy to allow a full benefit risk evaluation, before a marketing authorisation can be considered in the absence of data from the planned phase III clinical trial.

Clinical safety

Safety evidence is constituted by all publications/reports on Hyalograft C autograft and by the Company's post-marketing surveillance activity since 1999.
The evaluation of safety was performed as part of the meta-analysis and the clinical safety data are based on the same studies which have been tabulated in the section on Clinical Efficacy.
In 12 studies for Hyalograft® C autograft in the knee no information was provided on adverse events, 8 studies confirmed that no severe or serious adverse events occurred. Eight studies reported adverse events. From the seven studies on microfracture, only 3 studies reported adverse events.

Safety data from completed studies both pivotal and supportive, were obtained by evaluation of the individual investigator reports and literature publications and are also included from the post-marketing experience.

However, it would appear that the safety data have been provided as described in the respective reports and publications and analyses other than those conducted by the investigators or authors of the reviewed studies and described in the reports and publications themselves do not appear to have been performed. Furthermore, there appears to be considerable overlap of patients between studies.

It should also be taken into consideration that there is under-reporting of the adverse event data in published literature.

Patient exposure

From the inception of marketing in the European community through September 30, 2011, a total of 5,348 patients have been exposed to Hyalograft C. The numbers of patients in the publications and investigator reports included a total of 793 patients. However, the numbers of patients in the 8 studies which reported adverse events included 460 patients.

Adverse events

Analysis of Adverse reactions

One-hundred and ninety-four adverse events occurred in a total of 5,348 patients (3.6%), and 83 (1.6%) were serious adverse events.

The only important identified and potential risk is pyrexia in the postoperative period, moderate, non-serious, not higher than 38°C which resolved with appropriate treatment and without consequences.

Other observed adverse events were normal postsurgical adverse events as effusion, pain, swelling, arthrosynovitis and edema, non-serious adverse events and expected to be present due to the surgical intervention. Limited hypertrophy of the grafts was present, because there is no need of the periosteal flap which is one of the main causes of hypertrophic events after the common ACI technique.

Common Adverse reactions

The most common adverse reaction was graft failure reported in 62 patients. Post-operative pyrexia is the next common reported adverse reaction, in 36 patients (Table 27). In addition expected post-operative adverse events were observed in low frequency including, effusion, synovitis, pain, swelling and hypertrophy. Post-operative events were transient.

From the seven studies on micro fracture, only 3 studies (Table 26) reported adverse events which were 9/62 patients with articular effusions, 13/22 patients with knee pain, 10/22 patients joint swelling and 4/22 patients with symptomatic knee joint crepitation. In the systematic review of 28 studies with micro fracture by Mithoefer it was reported that in one of these 28 studies adverse effects were reported such as arthralgia (57%), effusion (5%), and crepitation (1.6%), with serious procedure-related adverse effects in 13%. Local septic complications and deep vein thrombosis were observed in up to 2%.

Since the application includes published studies, it needs to be taken into account that literature studies generally reflect an interest in specific safety issues. In contrast to company-sponsored trials, they do not provide safety information based upon explicit definitions or criteria, nor do they collect such information within a specified timeframe. Therefore, the term ‘common adverse events’ may refer to adverse events as reported in the reviewed studies, rather than adverse events occurring at a certain frequency.

Table 26 presents the individual studies and the adverse events which reported in these studies.

Table 26 Studies reporting Adverse events

Study Reference	N of Patients	Follow-up Time (Month)		Adverse Events
		Mean (SD)	Minimum	
Surgical Technique: **Hyalograft® C autograft**; Location: **Knee**				
Nehrer et al., 2006 [53]	36		36	3 (8.3%) pts with moderate fever, 2 of which had concomitant effusion.
Nehrer et al., 2009 [49]	42		24	2 moderate effusion
Marlovits et al., 2004 [66]	1			Report the first known incidence of the emergence of borrelial arthritis following ACI for repair of a cartilage defect. Five weeks after transplantation, the patient presented with diffuse swelling of the operated knee, moderate pain, and a body temperature of 37.2°C (mainly at night).
Marcacci et al., 2005 [47]	141	38 (9)		9 patients, 5 not related to Hyalograft® C autograft; 3 moderate fever postoperation, 1 arthrosynovitis
Fantini report, 2004 [67]	12			3 SAE of Fever; 5 none serious AEs: 1 Renal colic, 1 hyperpyrexia, 1 fever up to 38°C, 1 synovitis left knee with fever, and one Tumefaction with mild effusion. No AE is drug related.
Marcacci report, 2006 [17]	206	47.2 (11.0)		26 AEs in 25 patients: 18 are knee or surgery-related, among these 6 were judged possibly related to Hyalograft® C autograft treatment or with no clear: 1. Fever from 1 to 5 day (post grafting), 2. constant, persistent pain; 3. Arthrosynovitis (associated to fever), 4. Fever (37.5-38°C) after 15 days post-grafting, 5. Fever after 15 days post-grafting, and 6. Knee swelling (arthrocentesis) (this is the one without clear cause). There was one death after 51 months of follow-up due to bladder cancer, which is considered not treatment related
Scapinelli report, 2004 [68]	6		18	4 AEs in 4 pts: 1 case of slight temperature (37.5-37.7°C) 2 weeks after graft, 1 serious AE of raised temperature (mean 37.8°C) with persistent knee pain for 35 days after graft, 1 joint effusion (haemarthrosis), 1 slight temperature 2 weeks after graft (37-37.5°C)
Zorzi report, 2004 [69]	16	12	12	2 adverse events affecting 2 different patients, 1 case of swelling with fever max 37.8 °C (at visit 1) and 1 case of joint stiffness (at visit 1)
Surgical Technique: **Microfracture**; Location: **Knee**				
Asik et al., 2008 [10]	90	62	24	9 patients recurrent painless articular effusions
Mithoefer et al., 2009 [9]	3122	41 (5)	12	Generally rare. One study reported adverse effects such as arthralgia (57%), effusion (5%), and crepitation (1.6%), with serious procedure-related adverse effects in 13%. Local septic complications and deep vein thrombosis were observed in up to 2%.
Gudas et al, 2009 [12]	22	50.4*	36	13 (59%) patients with knee pain, 10 (45%) patients with joint swelling between 14 and 34 days after operations, 4 (18%) patients with symptomatic knee joint crepitation.

.

Analysis of Adverse reactions by Organ System or Syndrome

Table 27 below lists all adverse reactions by Organ system. The most prevalent reactions were graft failure (n=62), post-operative fever (n=36), and joint effusion (n=14). The majority of adverse reactions were reported for the musculoskeletal system sequelae of cartilage defect repair procedure or progression of cartilage degeneration.

Table 27. Frequency of Adverse Reactions by Organ System

SOC	Coded AE	Verbatim	N	Study reference (n AEs within that study)
Gastrointestinal disorders	Blockage, intestinal	Intestinal blockage	1	Report Marcacci 2006 (1)
Gastrointestinal disorders	Pancreatitis	pancreatitis	1	Report Marcacci 2006 (1)
General Disorders And Administration Site Conditions	Hematoma	Hematoma	1	Report Nehrer et Marlovits, 2004 (1)
General disorders and administration site conditions	Pyrexia	Post-operative fever	36	Study CT/222/97-02 –Scapinelli report (3), Study CT/222/98-02 – Zorzi report (1),Study CT/222/98-03 –Fantini report (6),Report Nehrer et Marlovits, 20049 (9) Report Marcacci 2006 (3) Nehrer et al. 2006 (3), Podskubka A. et al., 2006 (2) Postmarketing Surveillance (9)
Immune system disorders	Rheumatoid arthritis, articular	Acute articular rheumatism	1	Report Marcacci 2006 (1)
Infections and infestations	Infection, lyme disease	Borreliosis	1	Report Nehrer et Marlovits, 2004 (1)
Injury, Poisoning and Procedural complications	Wound dehiscence	Wound dehiscence	1	Report Marcacci 2006 (1)
	Fall	Trauma (fall)	1	Report Nehrer et Marlovits, 2004 (1)
	Adhesions, intra-articular	Adhesions of. kissing lesions	1	Report Nehrer et Marlovits, 2004 (1)

SOC	Coded AE	Verbatim	N	Study reference (n AEs within that study)
	Adhesion	Adhesion	1	Postmarketing Surveillance (1)
	Delaminations	Delaminations	5	Nehrer et al, ICRS 2007 (2), Filardo et al OCD 2011 (3)
	Edema	Subchondral edema	2	Filardo et al OCD 2011 (2)
Musculoskeletal and connective tissue disorders	Arthrosynovitis	Arthrosynovitis	6	Report Marcacci 2006 (1), Postmarkting Surveillance (2), Study CT/222/98-03 –Fantini report (1) Podskubka A. et al., 2006 (2)
	Arthritis	Arthritis at the opposite knee	1	Report Marcacci 2006 (1)
	Arthrofibrosis	Arthrofibrosis	4	Report Marcacci 2006 (2)/ Post Marketing Surveillance (2)
	Arthralgia	Knee pain	1	Study CT/222/97-02 ¯Scapinelli report (1)
	Arthrosis, hip	Hip trouble (arthrosis)	1	Report Marcacci (1)
	Fracture	Displaced fracture	1	Report Marcacci 2006 (1)
	Hypertrophy	Hypertrophy	6	Nehrer S. et al. 2011 (1) Postmarketing Surveillance (2) Ferruzzi et al, 2008 (2), Gobbi A et al. 2009 (1)
	Ineffective therapeutic product	Graft failure	62	Kon MFX et al. 2011 (1), Kon MACI et al. 2011 (4), de Windt et al 2012 (8), Report Nehrer et Marlovits, 2004 (1) Kon E. MRI et al. 2011 (2), Gobbi A et al. 2009 (1), Nehrer S et al., (12), Filardo G. et al 2011 (7), Marcacci et al., ICRS, 2007 (19) Filardo G et al.2011 OCD (4), Della Villa et al., 2010 (3)
	Joint Stiffness due to intra-articular adhesions	Joint Stiffness due to intra-articular adhesions	1	Study CT/222/98-02 ¯Zorzi report (1)

SOC	Coded AE	Verbatim	N	Study reference (n AEs within that study)
	Swelling	Swelling	4	Study CT/222/98-02 ¯Zorzi report (1), Postmarketing Surveillance (1) Report Nehrer et Marlovits, 2004 (1) Gobbi A et al. 2009 (1)
	leg length discrepancy	Leg problems (legs of unequal length)	1	Report Marcacci 2006 (1)
	Osteonecrosis	Osteonecrosis under the graft	1	Report Marcacci 2006 (1)
	Haemarthrosis	Haemarthrosis	1	Study CT/222/97-02 –Scapinelli report (1)
	Effusion	Joint effusion	14	Manfredini et al 2007 (1), Study CT/222/98-03 –Fantini report (1), Report Nehrer et Marlovits, 20049 (2), Nehrer et al. 2006 (2) Nehrer S et al., 2009 (2) Postmarketing Surveillance (6)
	Spinal disk, herniated	Slipped disk	1	Report Marcacci 2006 (1)
	Osteoarthritis	Total knee replacement due to progressing osteoarthritis	8	Nehrer et al, ICRS 2007 (8)
	Tumefaction	Tumefaction	1	Study CT/222/98-03 –Fantini report (1)
	Fibrosis	Fibrosis	1	Gobbi A et al. 2006 (1)
Neoplasm, benign, malignant and unspecified	Carcinoma, ovarian	Ovarian carcinoma	1	Report Marcacci 2006(1)
	Death, bladder cancer	Death (bladder cancer)	1	Report Marcacci 2006(1)

SOC	Coded AE	Verbatim	N	Study reference (n AEs within that study)
Nervous System Disorders	Pain	Constant, persistent pain, Pain at the external part of the knee, Pain,	8	Report Nehrer et Marlovits, 2004 (1), Report Marcacci 2006 (2) Postmarketing Surveillance (2) Giannini, et al., 2010 (2),Gobbi A et al. 2009 (1)
	Pain, knee	Pain at the knee anterior part	1	Report Marcacci 2006 (1)
Pregnancy, puerperium and perinatal conditions	Childbirth, ceaserian section	Caesarian section	1	Report Marcacci 2006(1)
Renal and urinary disorders	Renal Colic	Renal Colic	1	Study CT/222/98-03 –Fantini report (1)
Reproductive system and breast disorders	Tumor, breast	Mammary tumor	1	Report Marcacci 2006 (1)
Surgical and medical procedures	Knee, arthrocentesis	Knee swelling (arthrocentesis)	1	Report Marcacci 2006(1)
	Reconstruction, cruciate ligament	Reconstruction of the cruciate ligament after a fall	1	Report Marcacci 2006 (1)
	Surgery, thyroid cancer	Surgery for thyroid cancer	1	Report Marcacci 2006 (1)
	Surgery, tibia	Removal of a ventral osteophyte of the distal tibia	1	Nehrer S. et al. 2011 (1)
	Total knee arthroplasty	Total knee replacement due to progressing osteoarthritis	8	Nehrer et al, ICRS 2007 (8)
Vascular disorders	Death, embolism	Death due to embolism.	1	Postmarketing Surveillance (1)

Related Adverse events

Report Marcacci, 2006

The study report by Marcacci 2006 reported similar patients as presented in other papers by Marcacci et al., 2005 Pavesio et al, 2003 is part of Marcacci report, 2006 and is the largest series of patients.

A total number of 26 AEs in 25 patients were recorded among the total 206 patients included. Among the 6 events where the relationship was judged possible or not clear, the most common finding was the occurrence of a moderate post-operative fever (3 events), in one case associated to arthrosynovitis, all resolved without consequences after appropriate treatment. There was one osteonecrosis under the graft site, which however was probably present at the time of Hyalograft C autograft implantation. The serious adverse reactions reported include an ovarian carcinoma, surgery thyroid cancer, reconstruction of the cruciate ligament, a childbirth caesarean section and a death to bladder cancer which were all deemed not to be related to Hyalograft C autograft. The adverse reactions are listed in Table 28 below.

There were 12 graft failure (5.8%) in the entire population included (n=206). Out of 12, 11 occurred in salvage/complex cases and tibio-femoral kissing lesion, in an attempt to delay arthroplasty, while the last was a detachment due to osteochondritis dissecans. The 12 graft failures are included amongst the 19 graft failures reported in Marcacci et al, 2007 and are not listed in the table below.

Table 28. Adverse reactions from Marcacci 2006

System Organ Class	Coded AE	Serious event	Relationship to product
Gastrointestinal disorders	Blockage, intestinal	No	Non-related
	Pancreatitis	No	Non-related
General disorders and administration site conditions	Pyrexia	No	Possible
	Pyrexia	No	Possible
	Pyrexia	No	Possible
Immune system disorders	Rheumatoid arthritis, articular	No	Non-related
Injury, Poisoning and Procedural complications	Wound dehiscence	No	Non-related
Musculoskeletal and connective tissue disorders	Osteonecrosis	No	Non-Clear
	Arthrofibrosis	No	Non-related
	Arthritis	No	Non-related
	Arthrofibrosis	No	Non-related
	Fracture	No	Non-related
	Leg length discrepancy	No	Non-related
	Arthrosynovitis	No	Possible
	Spinal disk, herniated	No	Non-related
	Arthrosis, hip	No	Non-related
Neoplasm, benign, malignant and unspecified	Carcinoma, ovarian	Yes	Non-related
	Death, bladder cancer	Yes	Non-related
Nervous System Disorders	Pain	No	Possible
	Pain	No	Non-related
	Pain, knee	No	Non-related
Pregnancy, puerperium and perinatal conditions	Childbirth, ceaserian section	YES	Non-related
Reproductive system and breast disorders	Tumor, breast	No	Non-related
Surgical and medical procedures	Knee, arthocentesis	No	Non-indicated
	Surgery, thyroid cancer	Yes	Non-related
	Reconstruction, cruciate ligament	Yes	Non-related

Overall, the most common adverse events appeared to be graft failure, pyrexia and effusion. From the report by Marcacci 2006 which included the largest series of patients (206), frequently related events appear to be pyrexia and pain. These are consistent with previously reported events with other ACI products.

However, from the safety data which is available, it would appear that there seems to be under-reporting of events as a substantial proportion of studies reported no adverse events. It is also not entirely clear if particular adverse events, such as oedema, effusion, swelling, or pain, have been under-reported because these may be considered expected findings following arthrotomy and not related specifically to the Hyalograft implant.

It should also be noted that the study populations, surgical techniques, rehabilitation programmes, treatment of concomitant lesions, safety assessments (including definitions of adverse events) and timing, and duration of post-operative follow-up do not appear to have been uniformly similar across

studies. Additionally, details on prophylactic operative and peri-operative medications that are dependent on local practices at the treating centre, as well as details on the use of other minor concomitant treatments do not appear to have been generally reported.

Serious adverse events and deaths

Not all studies provided data on serious adverse events.There were 5 SAEs reported in the study report by Marcacci et al 2006 but considered unrelated. In the study report by Nehrer and Marlovits 2004, 4 SAEs were reported, 3 events of pyrexia (2 unclear, 1 possibly related) and 1 event of intra-articular adhesions which was considered definitely related. In the study report by Scapinelli, 2004, 2 SAEs of pyrexia and arthralgia were considered possibly related. Three SAEs of pyrexia occurred in the study report by Fantini et al in which the relationship was unclear.

No additional serious adverse reactions were reported in the publications and study reports received by the Company.

The incidence of SAEs cannot be properly evaluated due to the fact that most literature studies presented did not provide data on adverse events by seriousness.

Other Significant Adverse Events

Graft Failure

Graft failure was described in 51 (9.3%) of patients from 10 studies which included 551 patients in total, range 0-20.8% (81.8% in secondary indication including complex lesions and salvage procedures).

Delamination

Delamination was reported in 5 patients from two studies.

Graft Hypertrophy

Overall, 6 cases of graft hypertrophy were reported in 3 studies (4 patients) and 2 cases from post-marketing surveillance.

No additional significant adverse reactions were reported in the publications and study reports received by the Company.

The incidence of graft failure and graft hypertrophy need to be interpreted with caution since data reporting could be incomplete. Nevertheless, graft failure appears to be commonly reported for Hyalograft C from 0% - 20.8%. Graft hypertrophy is more common in first generation ACI using periosteal flap and therefore the comparatively low incidence is consistent with expected figures. The 5 cases of delamination also included 3 cases in a study of OCD patients in whom ACI was used in association with bone grafting. Full data is not available for the other two cases in the ICRS abstracts.

Deaths

There were two cases of death reported. Limited information is available regarding these events. One death occurred 51 months post-treatment due to bladder cancer [Marcacci, 2006]. This death was considered unrelated to the Hyalograft C autograft implantation. No additional information is available.

From Post-Market surveillance of the product, one death was reported in 2004, due to embolism. The complainant reported that it was not related to the drug product. No additional information is available.

Laboratory findings

No clinical laboratory evaluations were routinely performed during the studies described in the meta-analysis.

Safety in special populations

Intrinsic Factors

The populations below have not been studied or have only been studied to a limited degree:

- Children – The safety and efficacy in children and adolescents (age less than 18 years old) have not yet been established. Due to the nature of the procedure, Hyalograft C autograft is not recommended for use in children and adolescents. The subset of paediatric population 16 to 18 years is included in planned randomized controlled clinical trial and the indications for this paediatric group will be re-evaluated upon completion of the study.

- Elderly – Limited data are available on adult patients older than 55 years. The product is not intended to be used in geriatric population not due to safety, but due to efficacy considerations.

- Osteoarthritis population – Limited data are available on osteoarthritis patients. The product is not intended to be used in case of osteoarthritis, especially not in case of advanced osteoarthritis of the knee.

- Pregnant or lactating women – There are no data available on the use of Hyalograft C autograft during pregnancy. The product is not intended to be used in pregnant and lactating women.

- Patients with relevant co-morbidity such as clinically significant renal, hepatic or cardiac impairment – the product is not intended to be used in patients with clinically significant renal, hepatic or cardiac impairment.

Extrinsic Factors

The applicant states that since the drug product is an autologous local implant into the knee joint; there is no need to individualise therapy or patient management based on *extrinsic ethnic factors* in ICH E5.

Immunological events

No specific data for immunological events has been provided or discussed by the applicant.However, immune reaction to autologous cells is unlikely to occur.

Safety related to drug-drug interactions and other interactions

No formal pharmacodynamics drug interaction studies have been performed. Hyalograft C autograft is an autologous cellular product indicated for the surgical repair of symptomatic cartilage defects of the femoral condyle. Hyalograft C autograft implantation is intended to be performed via intra-articular arthroscopy or arthrotomy under sterile conditions after debridement. Considering both the intended clinical use and the applied surgical procedure, there are no major potential concerns regarding pharmacodynamics interactions with pre-, peri- or post-operatively commonly administered medicinal products. However, the Applicant is requested to discuss the potential interactions with at least warfarin, other anti-coagulants, intra-articular or systemic corticosteroids, other immunosuppressants, and certain antibiotics. Fibrin glue has been used sometimes to fixate the grafts.

Clinical experience reported up-to-date indicate that fibrin glue is the system most frequently used if Hyalograft C autograft fixation is necessary. In this case, fibrin glue is applied on top of the graft edges. There are not potential concerns regarding the concomitant use of fibrin glue in clinical practice, as it did not reveal any safety signals, except viral transmission concern that was addressed during time with the replacement of bovine thrombin with virally-inactivated human thrombin, with careful donor screening and ultimately with new technique which uses the patient's own blood to make purified fibrin I without the need for exogenous thrombin.

Discontinuation due to AES

N/A

Discussion on clinical safety

The studies submitted for the clinical safety data included a total of 5,348 patients who have been exposed to Hyalograft C including post-marketing data. The numbers of patients in the publications and investigator reports included a total of 793 patients. However, 8 studies reported adverse events. It should be noted also that there were overlapping populations across publications.

The defect size varied between approximately <1.0 and 19 cm2 and was located on the femoral condyles, trochlea, or patella. In addition, the exact range of defect sizes could not be derived, because some publications only provided the mean value. The duration of Hyalograft C post-treatment follow-up varied between few months and 7.5 years.

The safety issues with ACI products are mainly those arising directly as a result of the implant like graft hypertrophy and delamination and those related to the orthopaedic surgery such as haemarthrosis, wound infection and serious AEs such as thrombosis.

In addition the imaging safety measures included MRI and other radiographic assessments of some parameters, such as graft hypertrophy, graft delamination, adhesions, oedema, synovitis and cysts, however, it would appear that imaging may have been carried out in response to a patient complaint or the finding could have been incidental.

It should also be 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 related specifically to the Hyalograft C implant.

Furthermore, the study populations, surgical techniques, rehabilitation programmes, treatment of concomitant lesions, safety assessments 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. Accordingly, direct comparisons between studies are not possible.

In the quality and non-clinical sections the use of growth factors is connected to high proliferative growth of the cells, even when seeded onto the matrix. Non-clinical study on single dose toxicity (FB607) reported of neovascularisation of the newly formed cartilage tissue, which is a phenomenon not foreseen for a normally avascular tissue. The use of growth factors in production of the implant during clinical studies has not been discussed in relation to these putative safety signals.

Unfortunately, literature studies generally reflect an interest in specific safety issues and unlike company-sponsored trials, do not provide safety information based upon explicit definitions or criteria, nor do they collect such information within a specified timeframe. Therefore, the term 'common adverse events' refers to adverse events as reported in the reviewed studies, rather than adverse events occurring at a certain frequency.

Importantly, there is no information on concomitant drug therapy including anti-inflammatory drugs which may have been used and could complicate the evaluation of safety as well as efficacy.

Most literature studies did not provide data on adverse events by seriousness. Therefore, a summary of serious adverse events for these studies cannot be provided which is a significant deficiency in the safety data.

None of the studies reported data on clinical laboratory evaluations

Conclusions on clinical safety

A full assessment of safety from the publications and study reports cannot be made since there are several limitations in the data which precludes firm conclusions from being drawn. Unfortunately no pivotal clinical studies are ongoing to provide robust data. From the available data however, there do not seem to be major safety issues or those which appear to be unexpected The adverse event noted directly with the implant has usually been graft hypertrophy which is much more common with liquid culture first generation implants than with second generation ACI products. Additionally, the fact that the procedure can be achieved via arthroscopy as opposed to an open procedure using periosteal flap for first generation implants generally implies fewer adverse events due to the surgical procedure. Nevertheless, different generation ACI techniques utilising a variety of scaffolds and other excipients may modify the safety profile and therefore the data submitted is not considered adequate for the purposes of regulatory requirements. Furthermore, the use of growth factors and safety concerns arising from the non-clinical findings (monocyte infiltration, neovascularisation) request further evaluation of the safety findings. Accordingly, the full safety data would need to be provided.

Pharmacovigilance system

The Rapporteurs consider that the Pharmacovigilance system as described by the applicant has several deficiencies, necessitating further information to be provided as follows:

The detailed description of the pharmacovigilance system DDPS should be dated and authorised. As version 2 is provided, a revision history should be included.

The name and contact details of the contracted medical advisor together with a CV detailing experience in pharmacovigilance should be provided. Confirmation should be provided that the person is available 24/7.

The mobile phone number and location for the deputy QPPV together with a CV detailing experience in pharmacovigilance is requested.

The organisation chart should show the direct reporting line (i.e. position which the person reports to) for the QPPV, deputy QPPV and the contracted medical advisor.

Interfaces with other departments involved with pharmacovigilance activities (e.g. regulatory affairs, marketing, quality assurance) should be identified and a brief summary of pharmacovigilance activities undertaken by each unit should be provided.

A description of the following activities should be provided: the processing of SUSARs, PSURs, ASRs, cumulative review (signal detection), the frequency and the specific roles of the parties involved and risk benefit analysis.

A separate flow chart for the processing of PSURs should be provided. The flow chart indicating the flow of safety reports from various sources and of different types from the receipt of the information to the final stage of reporting to the regulatory authority should include internal and external timelines for the major processing steps and should show receipt by distributors and the timeline for receipt by Anika. The processing steps should include duplicates and medical review as well as follow –up.

A copy of the registration document of the QPPV with the Eudravigilance system should be provided.

The Applicant should provide the name, address and contact details of any third party providers e.g. distributors together with an outline of the agreements. A detailed list of the pharmacovigilance activities performed by the applicant and as listed in the contractual agreement should be provided. Details of any product specific arrangements should also be provided in an appendix.

Detail as to who is overall responsible for pharmacovigilance training both at Anika and at distributors, the methodology of the training provided and the period of review for ongoing training should be provided. The applicant should confirm that training in the handling of suspected adverse reaction reports is provided to any staff who might receive such a report including sale/medical representatives, CROs and reception staff

The Applicant is asked to confirm that pharmacovigilance documentation will be retained indefinitely in accordance with legislation and that written procedures will be amended to reflect this.

The applicant should provide a statement on access control to the archiving facilities and the fire/water safety provisions.

The Applicant should state what the basis is and the frequency for ongoing internal and external audits and who performs them. The name of the group overall responsible for CAPAs should be provided.

Information on quality control management e.g. control of compliance with 15 day report and PSUR submission dates should be provided.

In conclusion, the applicant must ensure that the system of pharmacovigilance is in place and functioning before the product is placed on the market.

Risk management plan

The Applicant has provided a risk management plan for Hyalograft C autograft that is deficient in several areas. There are substantial omissions from the RMP considering the product's status as an ATMP, and the RMP will require extensive revision before it can be considered acceptable.

Regarding non-clinical data, no carcinogenicity studies have been conducted and the Applicant is requested to discuss in the RMP the persistence of the cells after implantation in terms of tumourgenicity and carcinogenicity taking into account all non-clinical studies conducted. Furthermore, the findings concerning monocyte infiltration and neovascularisation should be taken into account in the RMP, as well as the use of several growth factors in production of the implant.

The Applicant has only identified one important safety concern (identified risk); post-operative fever. This is not acceptable. The Applicant is asked to reconsider the observed adverse events from safety experience with both Hyalograft C autograft and the surgical procedure for implanting this product in the patient to assess whether these are important identified risks. Furthermore other safety concerns for both the product (based on the properties of the product) and procedure (based on events associated with knee surgery) should be assessed for inclusion as important potential risks. The Applicant also has not identified any categories of missing information.

The Applicant has not provided a discussion on the specific risks of advanced therapy medicinal products under the section 'Additional EU Requirements' (for example: flow chart of the logistics of therapy, risks to patients in relation to quality characteristics, storage and distribution of the product, risks related to administration procedures etc)

Routine Pharmacovigilance and routine risk minimisation activities are proposed for the only safety concern identified by the Applicant (postoperative fever). Despite the complicated nature of propagation and transplantation of Hyalograft C autograft, the Applicant also has not identified medication errors as a potential risk.

No proposed safety/efficacy studies are discussed in the RMP.

The Applicant does not consider a risk minimization plan to be necessary and also has not discussed the need for an efficacy follow up plan (as per the CHMP Guideline on Safety and Efficacy Follow up – Risk Management of ATMPs).

The Applicant provided the following summary tables of proposed pharmacovigilance activities and proposed risk minimisation activities for the only safety concern identified:

Summary of Important Safety Concerns and Planned Pharmacovigilance Actions (table 10)

Safety Concern	**Planned Action(s)**
Important identified risks	
Pyrexia (intermittent fever in the post-operative period 38°C)	Routine pharmacovigilance: • Preparation of reports for regulatory authorities: Expedited adverse drug reaction (ADR) reports; Periodic Safety Update Reports (PSURs). • The SOP define the processes of continuous monitoring of the safety profile of Hyalograft C autograft including signal detection, issue evaluation, updating of labelling, and liaison with regulatory authorities.
Important potential risks	
none	
Important missing information	
none	

Summary of Planned Actions for Each Safety Concern (table 12)

Safety concern	Routine risk minimisation activities sufficient?	Description of routine activity and justification
Identified risks		
Pyrexia (≤38°C)	Yes	Routine pharmacovigilance will be conducted for Hyalograft C autograft regardless of whether or not additional actions will be found appropriate. This routine Pharmacovigilance will include the following: Systems and processes outlined in the Standard Operating Procedures that ensure that information about all suspected adverse reactions that are reported to the personnel of the company are collected and collated in an accessible manner; The preparation of reports for regulatory authorities; Expedited adverse drug reaction (ADR) reports; Periodic Safety Update Reports (PSURs); The SOP define the processes of continuous monitoring of the safety profile of Hyalograft C autograft including signal detection, issue evaluation, updating of labeling, and liaison with regulatory authorities; and Other requirements, as defined by local regulations.
Important potential risks		
none		
Important missing information		
none		

The Applicant should clarify how the correct (safe and efficacious) use of Hyalograft C will be ensured, for example, whether surgeons are required to undergo any training prior to use of the product. Use of training materials for physicians to advise on indications for use and correct administration technique, and also for patients to ensure safe and effective rehabilitation post implantation should be produced.

The Applicant should discuss the feasibility of conducting active surveillance such as a post authorisation study, to gather safety information on Hyalograft C autograft treatment in the post-marketing setting. The need for efficacy follow up should also be discussed.

Considering the complexity of the Hyalograft C autograft procedure and the autologous nature of the finished product, further discussion on the potential for medication errors is required. Further detail on the Applicant's traceability system should also be included in the RMP.

4. ORPHAN MEDICINAL PRODUCTS

N/A

5. BENEFIT RISK ASSESSMENT

Anika Therapeutics has filed a marketing authorisation application for Hyalograft C Autograft in the following indication:

"*surgical repair of symptomatic cartilage defects of the femoral condyle (medial, lateral) or trochlea, caused by acute or repetitive trauma (Outerbridge Grade III-IV) in adults."*

Majority of the data (product characterisation, non-clinical and clinical) is provided as scientific publications without actual raw data, study protocols or study reports. In these publications the actual manufacturing process and quality of the products used have not been defined, making firm conclusions extremely difficult.

Benefits

The clinical experience on Hyalograft C Autograft is based on over 5300 patients treated to date. However, data from 793 patients treated with Hyalograft C Autograft has been provided in this submission.

The Applicant has submitted in total 37 publications / study reports, of which seven studies were with surgical comparator and one meta-analysis. Of these only two Kon et al. studies (2009 and 2011a) had microfracture as a comparator and can be considered to meet the criteria of being prospective trials with acceptable comparator groups. The rest five of the seven prospective comparative cohort studies as well as the submitted meta-analysis and the study reports can only provide marginal supportive evidence for efficacy. However, all these data together bring valuable/some substantiation into the totality of evidence.

The two main studies provided demonstrated similar efficacy of the Hyalograft C Autograft to microfracture in active young patients based on primary objective IKDC score. In addition, a trend of more stable result in a long-term follow-up compared to the microfracture group was seen.

In these studies the confirmatory structural or histological analysis as recommended in the guidelines was not performed. However, the study by Marlowitz et al. provides supportive evidence of structural repair in the Hyalograft C Autograft treated patients. Two years after implantation, high-resolution MRI was used to analyze the repair tissue with nine pertinent variables. A complete filling of the defect was found in 61.5%, and a complete integration of the border zone to the adjacent cartilage in 76.9%. An intact subchondral lamina was present in 84.6% and an intact subchondral bone was present in 61.5%. Isointense signal intensities of the repair tissue compared to the adjacent native cartilage were seen in 92.3%. The clinical outcome after 2 years was evaluated using the knee injury and osteoarthritis outcome score (KOOS). The clinical scores were correlated with the MRI variables. A statistically significant correlation was found for the variables "filling of the defect," "structure of the

repair tissue," "changes in the subchondral bone," and "signal intensities of the repair issue". In a relatively large multicenter uncontrolled observational study Marcacci et al (2005) present interim results on consecutive elective patients in a natural clinical setting. Efficacy analysis performed on 141 patients at 24 months with adequate outcome instruments showed significant and sustained efficacy. Second look arthrosopy data was available for 55 patients, of which only 22 provided biopsy data. Also this data supports structural and clinical improvement of the lesions, but further information is needed to demonstrate validity of the results. In conclusion, the data from the clinical studies suggest that Hyalograft C Autograft has a significant treatment effect, but for the conclusive demonstration of efficacy and safety, a properly controlled randomized study(ies) is needed. The Applicant has provided a study protocol for an RCT, which is proposed to be conducted post-marketing. This could be approvable only in situation, where positive benefit/risk profile can be demonstrated at the time of MA approval. Thus, further data is needed to demonstrate appropriate quality of the published data and the Applicant is requested to provide study protocols, study reports and raw data, if available, for those studies that are considered crucial for B/R evaluation (Kon et al., 2009, Kon et al., 2011, Marlowitz et al., 2006, Marcacci et al., 2005).

Beneficial effects

Autologous chondrocyte implantation (ACI) was first reported in 1994, and more than 12,000 patients have since undergone treatment. First generation ACI required the harvesting of an autologous periosteal patch, which was used to secure the chondrocytes in situ. 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 variety of scaffolds. Hyalograft C autograft, is a second generation ACI product. The chondrocytes are supplied seeded onto a 3D Hyaluronan scaffold, which is simply secured into the lesion.

Hyalograft C autograft has been shown to deliver comparative clinical results to traditional ACI as reported in the literature, but appears to offer several advantages including reduced operative time, reduced tourniquet time and the ability to perform the implantation via minimally invasive methods such as mini-arthrotomy or arthroscopy

The beneficial effects can be translated into improvement both in pain and functionality which are considered to be clinically meaningful outcomes.

One of the problems has been that chondrocytes from articular biopsies have low potential for proliferation and after repeated passaging the number of cell divisions decrease and eventually the cells dedifferentiate. *In vitro* experiments have shown that three-dimensional biodegradable polymer scaffolds have maintained the ability of chondrocytes to differentiate and enhance the expression of the differentiated phenotype. In theory, this could be an advantage in the 2nd generation ACI techniques in which the cells are seeded on three-dimensional scaffold. However, several growth factors are used during the manufacturing process to support the cell growth and proliferation and the matrix is coated with to ensure better attachment of the cells. These issues raise questions about the biocompatibility of the cells and the matrix and the advantage of the combination needs further justification.

Uncertainty in the knowledge about the beneficial effects

Unknown quality and manufacturing processes of the products used in the non-clinical and clinical studies remains one of the key issues including concerns regarding cell potency and viability which have varied considerably and thus comparability is an issue which could impact on both efficacy and safety and thus prevents any firm conclusions to be made.

In addition, quality of the clinical data cannot be verified, as study protocols, study reports and individual patient listings are missing from the submission.

The evidence for efficacy from the studies considered to be pivotal appears to have several limitations. No study was carried out to a GCP standard. In the comparative cohort studies by Kon et al, the study design does not allow for a valid comparison, because microfracture is not considered a treatment option in lesions larger than 4.0 cm as defined in the EMA CAT Reflection Paper.

It is also unclear whether there was blinded evaluation of the outcomes. Furthermore there were no secondary structural endpoints included in the Kon et al. studies, although structural data was provided elsewhere. Additionally, data on pain medication were not available, which could have impacted on the patient reported outcomes. It is also unclear, whether the dose utilised has been optimal since no clinical dose finding studies were carried out. Furthermore, previous sporting activity appears to be positively correlated with better outcome especially in young active sportsmen. Therefore the results in both groups are not entirely unexpected. Also the observations on long-term efficacy are inconclusive since the baseline severity of the lesions on subjective scale was not comparable between the groups.

Other studies have varied widely in the design and methodology, the majority being non-randomised case observational studies and although being open label, single arm, single centre, did not involve blinded evaluation of the outcomes in a substantial number of studies.

Variability within and between studies was present for patient characteristics such as age, treatment history, and defect size, location, and aetiology. Furthermore, it should be noted that outcomes were assessed at different time points which therefore limits comparability of data between studies.

Risks

From the currently available data, no major safety concerns can be identified, although it must be noted that reporting on the adverse events is limited, as well as identification of possible risks. The most common adverse reaction was graft failure reported in 62 patients (incidence 7.4 %). Post-operative pyrexia is the next common reported adverse reaction, in 36 patients. Two cases of death have been reported: one due to ovarian carcinoma 51 months after implantation and one due to thromboembolism, timing is not known. Whether the deaths were product-related, is unknown.

However, according to the dossier, several growth factors are used in the manufacturing process of the product and the matrix is coated with to enhance cell adhesion on the matrix. Information on whether the same growth factors and coating have been used in production of cells of the studies forming the major safety database is missing. In the quality and non-clinical sections the use of growth factors is connected to high proliferative growth of the cells, even when seeded onto the matrix. This is of concern, as the cells on the matrix should be re-differentiating and forming extracellular matrix (ECM), not dividing. Whether the high growth rate of the cells can lead e.g. to hypertrophic growth or even uncontrolled growth of the cells in vivo, is unknown. Non-clinical study on single dose toxicity (FB607)

reported of neovascularisation of the newly formed cartilage tissue, which is a phenomenon not foreseen for a normally avascular tissue. Furthermore, based on the characterisation studies, the applicant seems to be most worried about the outgrowth of cellular impurities, which without growth factors normally do not pose difficulties for chondrocyte culturing. These issues have not been addressed in the safety evaluation or RMP.

Unfavourable effects

Based on earlier clinical studies and the commercial experience with Hyalograft C autograft, potential complications of treatment have been identified, including surgical procedure complications associated with the surgical exposure required for Hyalograft C implantation. These 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 procurement. The following adverse events and complications are already identified with Hyalograft C autograft implant. Firstly, adverse events related to the Hyalograft C autograft implant: symptomatic graft hypertrophy or graft delamination, possibly leading to graft failure. Secondly, peri-operative complications: intra-articular adhesions, wound dehiscence, haemarthrosis, effusion, arthrofibrosis, infection and knee pain (arthralgia).

Uncertainty in the knowledge about the unfavourable effects

Literature studies generally reflect an interest in specific safety issues and unlike company-sponsored trials, do not provide safety information based upon explicit definitions or criteria, nor do they collect such information within a specified timeframe. For example, the term ‘common adverse events’ refers to adverse events as reported in the reviewed studies, rather than adverse events occurring at a certain frequency. The number of AE:s, ADR:s, and SAE:s seem strongly underestimated.

Certain adverse events, such as oedema, effusion, swelling, or pain, could have been under-reported because these are considered expected findings following arthrotomy/ arthroscopy and not related specifically to the Hyalograft implant. Due to extensive double-reporting and cross-reporting, the exact number of exposed patients is impossible to elucidate from the study reports. Cases of thromboembolic events have also been described with similar products.

Relevant concomitant morbidity has not been properly considered and discussed in detail and mmunological events such as post-operative fever need to be discussed.

Furthermore, the study populations, surgical techniques, rehabilitation programmes, treatment of concomitant lesions, safety assessments and timing, and duration of post-operative follow-up were not similar across studies and complicate direct comparisons between studies.

Balance

Importance of favourable and unfavourable effects

Improvements in pain and function as well as in structural outcomes are considered to be relevant and sensitive clinical favourable outcomes.

Graft hypertrophy, delamination and failure, together with perioperative complications as well as delayed complications including thrombosis and pulmonary embolism are important unfavourable effects.

The efficacy data gathered from different studies propose sustainable efficacy for Hyalograft C Autograft. However, published data without study protocols, study reports and individual patient listings cannot be considered conclusive. The putative risks related to the use of several growth factors and coating material of the matrix have not been addressed at all, although high proliferative growth of the cells and even neovascularisation of the regenerated cartilage tissue are reported in non-clinical studies. Also the quite high rates of graft failures and treatment-related complications (pyrexia, delamination, hypertrophy) are seen important risks for the patients. In the absence of conclusive demonstration of efficacy, the lack of systematic safety reporting and data are considered a major concern.

Benefit-risk balance

Studies which aim to evaluate cartilage repair techniques can be confounded by the subjective nature of pain reported by patients, including individual responses to medication, physical therapies and surgical interventions and accordingly, objective outcomes like arthroscopy and MRI are considered necessary, as opposed to depending on endpoints measured solely by patient related outcomes. Some studies in the literature consider that, second generation ACI is superior to MF and also improves upon the first generation chondrocyte-based cartilage repair techniques because it appears to show reproducibility, safety, operative time, surgical simplicity and reduced invasiveness. Nevertheless, conclusive evidence of this needs to be demonstrated in a randomised controlled trial carried out to GCP standard for regulatory requirements.

While the totality of evidence would appear to suggest that Hyalograft C shows evidence of efficacy which cannot be ignored, especially in a focused population of young athletic sportmen, the promising results apparent from both the pivotal and supportive data appear to have limitations.

With respect to safety, the available data have significant deficiencies due to incomplete and under-reporting and it has not been possible to obtain a full safety profile to draw firm conclusions.

Although there seems to be benefit from the treatment to the patients, the uncertainties related to the provided data together with the identified risks and poor safety reporting outweigh the positive signals of efficacy. Therefore, no definite conclusion on efficacy can be made at this time and the overall B/R of Hyalograft C Autograft is currently considered negative.

Conditional approval

The Applicant has applied for conditional approval according to Article 14(7) of Regulation (EC) No 726/2004.). The decision on the conditional approval can only be made after a positive B/R profile for the product is achieved.

5.1 Conclusions

The overall B/R of Hyalograft C autograft is negative, as there are several major objections with respect to the current dataset with respect to Quality, non-clinical and clinical issues, precluding a marketing authorisation.