Chapter 11
Characterized Chondrocyte Implantation Challenges Current Paradigms for the Treatment of Symptomatic Joint Surface Lesions

J. Vanlauwe, M. Jelic, M.J. Limbourg, and F.P. Luyten

Abstract The use of autologous cells derived from an articular cartilage biopsy to treat joint surface lesions was introduced in 1994 by Brittberg and Peterson. In a 2-step procedure, chondrocytes were harvested from a minor weight-bearing area of the knee joint during arthroscopy, expanded *ex vivo* and implanted during an arthrotomy 2–3 weeks later. However, throughout the *in vitro* expansion process, articular chondrocytes progressively lose their phenotypic traits and capacity to form stable cartilage tissue, thereby jeopardizing proper *in vivo* repair.

Data on dedifferentiation revealed that *in vivo* tissue formation of stable cartilage is governed by the interaction between environmental factors and inherent phenotypical characteristics. Characterized chondrocytes are an expanded population of cartilage cells that express a marker profile predictive for the formation of ectopic hyaline-like cartilage *in vivo* in a consistent and reproducible manner. A controlled and consistent manufacturing process was developed to maintain this phenotype stability. This involved optimisation of the biopsy procedures and mostly the culture process parameters. Characterized viable autologous cartilage cells expanded *ex vivo* expressing specific marker proteins were introduced in clinical practice in 2004.

JVL and MJ equally contributed to the manuscript

J. Vanlauwe
Department of Orthopedics and Traumatology,
University Hospital Brussels, Brussels, Belgium

M. Jelic
Department of Orthopaedic Surgery, Clinical Hospital Center Zagreb,
School of Medicine, University of Zagreb Salata 6, Zagreb, 10000, Croatia

M.J. Limbourg
Department of Medical and Scientific Affairs, TiGenix NV,
Romeinse Straat 12/2, Leuven 3001, Belgium

F.P. Luyten (✉)
Division of Rheumatology, University Hospitals KU Leuven,
Herestraat 49, Gasthuisberg, Leuven B 3000, Belgium
e-mail: frank.luyten@uzleuven.be

P.J. Emans, L. Peterson (eds.), *Developing Insights in Cartilage Repair*,
DOI 10.1007/978-1-4471-5385-6_11, © Springer-Verlag London 2014

A prospective randomized multicenter controlled trial compared characterized chondrocyte implantation (CCI) to microfracture in the treatment of symptomatic cartilage defects of the femoral condyles. The primary endpoint was successfully reached at 1 year, with CCI showing superior tissue regeneration. Clinical outcome at 12–18 months measured by the overall Knee injury and Osteoarthritis Outcome Score (KOOS) was comparable for both treatments. An extension at 3 and 5 years confirmed that a good clinical outcome was maintained over time for both treatments in the overall patient population. Strikingly, sub analysis of the long-term follow-up data revealed that early treatment by CCI resulted in statistically significant and most importantly clinically relevant better results when compared to microfracture, supporting a critical window of opportunity for genuine tissue regeneration. In addition, data from a large compassionate use program, whereby lesions were treated at diverse locations in the knee joint, corroborated the benefit of CCI found in the RCT. These data sets allow now to better define the treatment algorithms for symptomatic joint surface lesions of the knee in clinical practice.

Keywords Cartilage repair • Characterized chondrocyte implantation • Randomized controlled trial • Long term • Treatment algorithm

Key Points

- In order to minimize dedifferentiation during in vitro expansion, a standardized culture procedure for expansion of human articular chondrocytes was optimized resulting in a well characterized product,
- The development of a robust production process and the consistent results in structural benefit and patient outcomes together with an excellent safety profile have led to the approval of ChondroCelect as the first ATMP by EMA in 2009.
- Characterized Chondrocyte Implantation showed superior structural cartilage repair at 1 year as compared to microfracture.
- ChondroCelect treatment results in a significant and clinically relevant benefit compared to microfracture in patients with a symptom onset since less than 3 years.
- The development of cell based approaches in the field of cartilage repair has contributed substantially to regenerative medicine approaches in general.

11.1 Introduction

Cartilage lesions are common disorders of the knee joint and a frequent cause of knee pain and functional disturbances. Hjelle et al. found single, International Cartilage Repair Society (ICRS) grade III or IV defects of at least 1 square centimetre (cm^2), in 7.1 % of arthroscopies in patients under fifty [1]. Next to the negative impact on patients' activities and quality of daily life, functional limitations in this professionally active group cause a considerable societal burden [2].

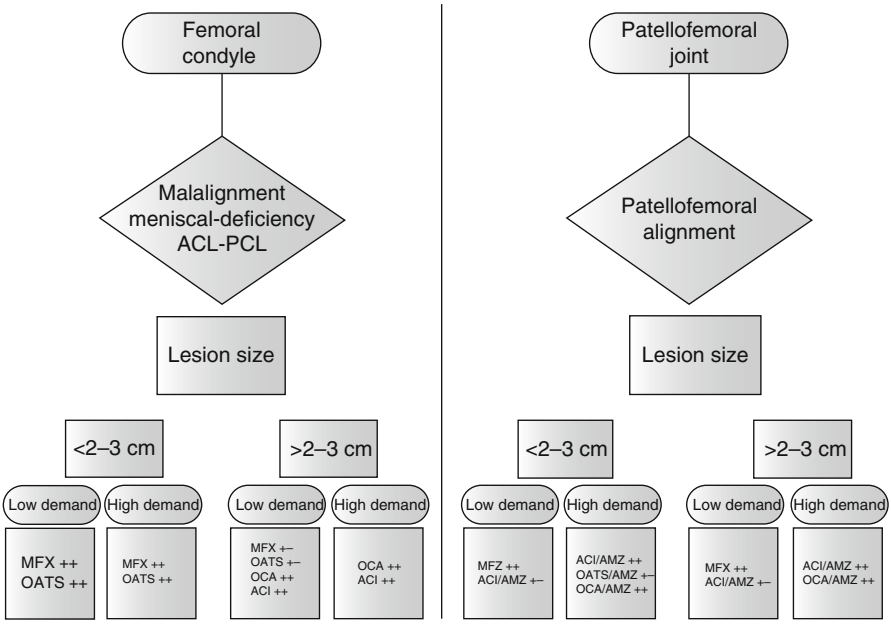

Fig. 11.1 Historical treatment paradigm: lesion size as first criterion in treatment selection. *MFX* microfracture, *OATS* osteoarticular transfer system, *OCA* osteochondral allograft, *ACI* autologous chondrocyte transplantation, *AMZ* anteromedialization (Reproduced with permission from Cole et al. [5])

The natural healing potential of articular cartilage is known to be limited and can predispose to the development of early and established osteoarthritis [3]. Therefore, it is clinically relevant and important to treat cartilage lesions adequately in an early stage, in particular for individuals at risk.

In general, surgical options for knee joint cartilage repair aim at restoring normal, pain-free motion. The procedures can be grouped into three categories: first symptomatic treatments, including arthroscopic debridement and lavage, next repair techniques such as bone marrow stimulation leading to clinical improvement and third restorative and regenerative procedures, including osteochondral grafting and autologous chondrocyte implantation (ACI) aiming to regenerate the joint surface with structural characteristics close to its original integrity [4]. (Fig. 11.1: treatment algorithm). Patient specific variables such as co-morbidities and underlying pathologies play a critical role in the choice and the scope of treatment options. Besides, location and size of the lesion, previous treatment must be taken into account when selecting the most suitable treatment for an individual. In the past, lesion size was applied as a major determinant for defining the best type of surgical technique, being it reparative or restorative, early on in the decision tree [5].

The aim of any surgical treatment is to restore normal, pain-free motion and ultimately postpone or prohibit the onset of osteoarthritis [6]. In the 80's, successful

repair of focal patellar defects were reported by transplantation of cultured autologous cells in the rabbit [7, 8]. In 1994 Brittberg and Peterson published a report on 23 patients in whom deep cartilage defects in the knee were treated by autologous cell implantation (ACI). During an arthroscopic procedure, a biopsy was taken from a minor weight-bearing area. The chondrocytes were cultured *ex vivo* and subsequently, after 14–21 days implanted under a periostal flap during an arthrotomy [9]. Despite overall favourable results, several variables related to the chondrocyte expansion have been identified which may interfere with the *in vivo* formation of stable cartilage. For instance, *in vitro* expansion of articular cartilage derived cells has been known to progressively dedifferentiate and lose their chondrogenic capacity [10, 11]. The loss of phenotypic traits has been shown to result in the formation of disorganized fibrocartilage possibly affecting clinical outcomes [12].

Dell'Accio et al. conducted basic research on mesenchymal cell populations' ability to form stable cartilage *in vivo* in the nude, i.e. immune suppressed, mouse, allowing to study human cell populations and their behaviour *in vivo*. The Ectopic Cartilage Formation Assay (ECFA) was developed, allowing to test and monitor potency and capacity to form a cartilage implant, resistant to vascular invasion and mineralisation, or replacement by bone or fibrous tissue *in vivo*. Furthermore, a set of molecular markers were identified, allowing to predict the outcome of the *in vivo* assay, irrespective of the donor age [13]. The experiments conducted by Dell'Accio et al. not only resulted in the identification of cell populations that retain their cartilage-forming capacity and phenotype *in vivo* but were also at the basis of the development of a standardized and reproducible culturing process aiming at preserving the chondrocyte phenotype capable of producing stable hyaline cartilage.

Based on this research, characterized chondrocyte implantation (CCI) has been developed with the goal to improve the clinical outcome of ACI [6, 14–16]. By definition, characterized chondrocytes are an *in vitro* expanded population of chondrocytes which express a marker profile predictive of the capacity to form hyaline-like cartilage *in vivo* through a standardized, consistent and reproducible process. The expansion procedure, originally optimized by means of the marker profile, was designed as such in order to preserve phenotypic traits and biological activity. As a result, CCI leads to improved potency of individual cell batches and homogeneity in the chondrogenic capacity (Tigenix, data on file). The medicinal product resulting from this manufacturing process, ChondroCelect®, has been granted market authorization by the European regulatory bodies in 2009 as the first centrally approved Advanced Therapy Medicinal Product (ATMP) [17].

A phase III, prospective, multicenter randomized controlled trial was conducted to compare efficacy and safety of CCI versus microfracture (MF) in the repair of single symptomatic cartilage lesions of the femoral condyle [15]. Patients aged between 18 and 50 years, with a single symptomatic cartilage lesion between 1 and 5 cm^2 of the femoral condyles were included. In the CCI arm, 51 patients were treated whereas 61 patients underwent microfracture. The primary endpoint of the trial was the demonstration of structural superiority at 12 months post treatment, both by histological and histomorphometrical assessment. The secondary endpoints related to the clinical outcome, assessed by the overall Knee injury and Osteoarthritis

Outcome Score (KOOS), for which the assumption was made that clinical outcome at 12 and 18 months after CCI should at least be as good as MF.

Histological examination of a biopsy of the repair tissue at 12 months showed superior structural repair in the CCI arm compared to the MF arm [15]. MRI data support the better quality of repair after CCI at 3 years [16]. There was consistent improvement up to 36 months in the clinical outcome as measured by the KOOS in both treatment arms. The estimated benefit at 36 months was larger in the CCI group. The finding that patients with less than 3 years since onset of symptoms (N = 27 in the CC arm and N = 32 in the MF arm) benefited most from CCI allowed to better identify suitable patient populations based on their medical history only [16]. Five year follow-up data on the study patients confirmed the outcomes: patients treated with CCI within 3 years of symptom onset presented with a statistically significantly better clinical outcome, and more importantly a clinically relevant difference versus microfracture [6]. Efficacy results were further corrobated in the compassionate use program in a larger and more varied patient population, presenting with lesions over 5 cm^2, patellar and multiple defects [18].

The safety profile of CCI does not show major differences from that of microfracture. The most commonly found adverse reactions are arthralgia, joint swelling, effusion and crepitation. The majority of observed safety signals in the CCI group relate to the use of the arthrotomy procedure and are present in the early postoperative period. At 60 months, most of the Adverse Events (AEs) had resolved [6].

The good safety profile, and the clinically relevant benefit of CCI over MF provide arguments in favour of revisiting current treatment paradigm, taking into account that time of symptom onset appears to be a crucial determinant of treatment selection. Moreover, basic research reveals more insights into the configuration and functioning of the subchondral region. It becomes clear that knee joint homeostasis as previously suggested [19], and consequently the durable clinical outcome of cartilage repair surgery, is largely defined by proper functioning of the tidemark, the transition zone between cartilage and bone [20].

11.2 From Autologous Chondrocyte Implantation (ACI) to Characterized Chondrocyte Implantation (CCI)

11.2.1 ACI Historically

In the original paper by Brittberg et al., autologous chondrocytes were expanded *in vitro* during a 2 to 3 week culturing process. The chondrogenic phenotype was assessed by microscopical evaluation of clonal growth and metachromatic staining in a small fraction of the isolated cells [9].

However, the loss of the articular cartilage phenotype during *in vitro* expansion culture has been recognized as a major hurdle for ACI [21]. For a long time, monitoring of phenotypic stability throughout the culturing process was based on two

surrogate markers. The expression of type II collagen, a key component of cartilage' extracellular matrix (ECM), basically reflects the differentiation state of the chondrocytes at the time the test is being done. The capacity to form colonies in anchorage-independent conditions moreover seems to be a feature of chondrogenic cells [10] to a large extent but is not a trustworthy variable to predict cartilage forming capacity *in vivo* [13].

11.2.2 Cell Characterization and Technology Development

Dell'Accio et al. [13] developed a nude mouse model resulting in a standardized and validated screening assay which allows to measure the potential of human chondrocytes to form stable cartilage *in vivo*, the Ectopic Cartilage Formation Assay (ECFA). The assay consists of the following steps: in a first step the freshly isolated chondrocytes are obtained from human donors, within 12 h post-mortem. After expansion of the cells in monolayer, four to five million viable cells are re-suspended in 50 μl of phosphate buffered saline and are injected intramuscular (IM) into the thigh of a nude mouse. Thereafter, the cells are allowed to grow in this "in vivo bioreactor" for a period of 3 weeks. Subsequently, the tissue generated at the injection site, is harvested for histological examination.

In all study animals, a distinct cartilage implant was retrieved a week after injection. Safranin O staining, reflecting the presence of sulphated proteoglycans, was nearly comparable to what is seen in normal articular cartilage, however the implants were hypercellular and did not show the typical cartilage architecture. Further histological and immune-staining did not reveal any vascular invasion, bone formation nor the presence of collagen bundles as in fibrocartilage. The properties of serially passaged chondrocytes were assessed, which showed that the cells lose their cartilage-forming potential after 2–3 passages *in vitro*. In an attempt to identify molecular markers with might be predictive of the *in vivo* cartilage forming capacity, the expression of molecules involved in formation and maintenance of chondrocytes' phenotype were monitored throughout the culturing process. The development of consistent and donor age independent parameters, predictive of the *in vivo* cartilage formation potential, have not only been used to identify cell populations but also to design and optimize a reproducible cell culturing process (Fig. 11.2: the mouse model).

The insights that lead to the development of the ECFA allowed to design an *in vitro* assay where the same cell populations tested in the ECFA were analyzed in a comparative micro-array analysis. Cellular expression patterns of genes relevant for cartilage and chondrocyte biology were studied, in an attempt to identify both positive and negative markers predictive of *in vivo* cartilage formation capacity. As such, 150 positive markers, genes that are highly expressed in the cells that produced a cartilage implant in the ECFA and are not or very weak in the cells without

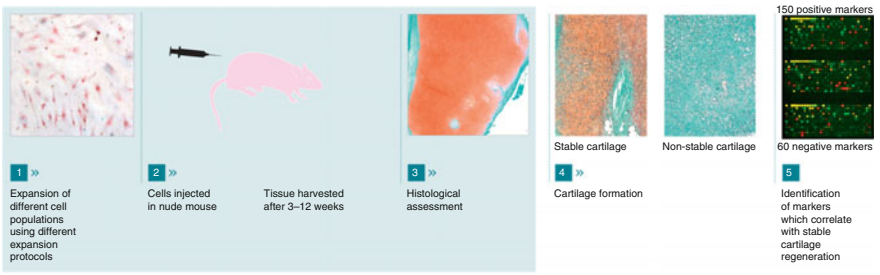

Fig. 11.2 Nude mouse model: comparison of cell populations that pass and fail proprietary in vivo assay on gene expression profiles (Reprinted with permission from Dell'Accio et al. [13])

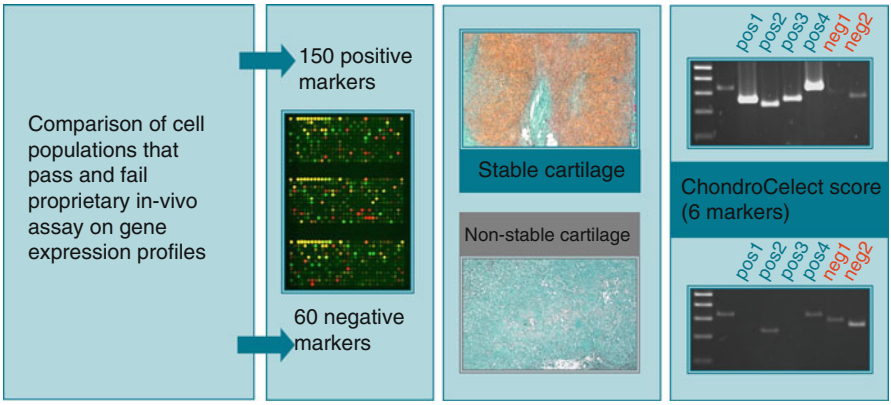

Fig. 11.3 Identification of molecular markers, resulting in ChondroCelect score

chondrogenic potential, and 60 negative markers, genes highly expressed in cells without cartilage formation potential in the ECFA, were identified.

Of these 210 markers, 4 positive and 2 negative markers were selected, based on their capacity to generate cartilage tissue in the ECFA. Each of the individual markers can be scored based on their overall expression level in the assay, adding up to the ChondroCelect score. The score, ranging from −6 to +6, is considered a potency assay for the cartilage forming capacity. A major advantage of the score is that it is compatible with a routine manufacturing setting, by means of Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) methodology (Fig. 11.3: ChondroCelect score).

A set of molecular markers reflecting *in vivo* cartilage formation also enabled to optimize the expansion process. Variables that were investigated include but are not limited to culture media, serum batches, enzymatic treatments, passaging methods, culture vessels and population doublings. These investigations formed the basis for the development of a more robust cell product consisting of a cell suspension of autologous articular cartilage derived cells capable of forming ectopically stable hyaline and not transient cartilage.

Fig. 11.4 Standardization and optimization starts at the time of biopsy: the ChondroCelect Harvester

11.2.3 ChondroCelect, Standardized Medicinal Product Based on Autologous Cells

The starting material for the production of ChondroCelect consists of 200–300 mg of healthy cartilage, arthroscopically harvested from a minor weight bearing area of the medial or lateral part of the trochlea or the intercondylar notch. To standardize the biopsy process, a specific device has been designed: the ChondroCelect Harvester (Fig. 11.4). According to the estimated size of the debrided lesion, one to three slivers with a length of 8 mm are required for the manufacturing process. Biopsy procurement boxes with sterile biopsy vials are stored in the orthopaedic units under temperature controlled conditions. Once the biopsies are harvested they are immediately sent to the cell expansion facilities (CEF) without interruption of the cold chain. Upon arrival at the CEF, only tissue from donors whose blood samples are negative for HIV type 1 and 2, hepatitis (HBV, HCV) and syphilis will be further processed. This measure of precaution is more stringent than existing regulation, but is primarily introduced to ensure absolute protection of the plant sterility zones.

The manufacturing process starts with the biopsy digestion: cartilage fragments are isolated and chondrocytes are released from the extracellular matrix, washed, counted and seeded in culture medium. Upon confluence of the cell cultures, they are detached from culture flasks and seeded onto new flasks, a process called a 'passage'. This step is being repeated until a sufficient number of cells are obtained, the optimum being one million cells to cover a 1 cm^2 defect, mostly based on the cell number found in corresponding mature articular cartilage . However, as has been demonstrated that cells lose their chondrogenic capacity after four to six passages, the maximum number of passages allowed in the ChondroCelect production process is three [13]. The expansion process is further optimized by comparing the molecular signature of cells with a preserved capacity to form hyaline cartilage to cells which develop inferior cartilage tissue prone to vascular invasion, calcification and bone formation (also in part described in reference [13]), In parallel, culture conditions are set in such a way as not to enrich for other cell lineage populations, e.g. fibroblasts. When the appropriate number of cells is reached, the cells are harvested from the wells, washed, counted and cell viability is checked. The culturing process is variable in time span, partly due to inherent cell characteristics and partly

because the cell yield relates to the defect size. Therefore, and also to offer flexibility for selection of the CCI timing, a cryopreservation step was introduced. The final product can be reconstituted for implantation between 9 and 13 weeks after the biopsy. The dosage required for implantation, 0.8–1 million cells per cm^2, is delivered in vials containing four million cells per 0.4 ml excipiens.

Historically, the technique for performing ACI, consisted of suturing of a periosteal flap (or later on a bio-membrane) over the debrided lesion followed by the injection of the cultured cells beneath the water-sealed membrane. In a recent publication by Steinwachs, a variation is presented: the chondrocyte suspension is applied onto the bio-membrane and after approximately 10 min time needed for the cells to adhere to the membrane, it is sutured into the debrided lesion [22]. No direct comparisons have been made between both techniques, in terms of ease of use or outcomes.

As illustrated above, CCI implies process design, from biopsy to implantation, optimized and standardized to maximally preserve the phenotypic traits and biology, reducing the variability of the final product, despite its autologous origin: for each of these steps the process has been optimized, specific devices have been developed and are being used, and all stakeholders have been trained to comply with preset quality criteria. Thus, a selection at the end of the culturing process itself has become obsolete due to the optimised and robust culturing process which enriches for the superior cartilage forming cells. . In order to investigate its clinical significance, a well designed prospective multicenter trial was initiated.

11.3 Bringing Research to the Bedside: The CCI Randomized Controlled Trial

In 2002, an international prospective randomized multicenter controlled trial was set up involving 13 orthopaedic centres. In consensus, microfracture was chosen as the control arm, because it was considered the existing treatment standard of femoral cartilage lesions, although quite controversial at that time [15].

11.3.1 Microfracture Technique

Microfracture is a surgical technique developed by Steadman to enhance chondral repair by making multiple microfractures in the subchondral bone plate. The mesenchymal stem cells, growth factors and other substances released from the marrow form a ‘super clot’ providing a suitable environment in which the stem cells are believed to differentiate into cartilaginous like tissue within the lesion [23]. The repair tissue consists predominantly of collagen type I and resembles fibrocartilage, thereby less resistant to shear and compression loads as compared to hyaline cartilage [24].

Microfracture is widely used, mainly because it is a one-step procedure and because it has a good potential for symptomatic improvement [25, 26]. In the first randomized controlled trial, published well after the start of the CCI trial, Knutsen compared ACI with MF and found no statistically significant difference with regard to structural outcome at 2 years post surgery [27] and clinical outcome up to 5 years [28]. It is of note that this study confirmed independently the international consensus on the proper clinically relevant comparator for cell based repair at that time being microfracture.

11.3.2 Study Population and Baseline Characteristics [15]

Eligible patients were aged between 18 and 50 years, and had a single symptomatic cartilage lesion (International Cartilage Repair Score III or IV) between 1 and 5 cm^2 of the femoral condyle. Patients with the presence of a clinically relevant patello-femoral cartilage lesion, osteochondritis dissecans (OCD), a lesion over 0.5 cm depth and microfracture performed less than a year before baseline were excluded. Randomized patients were treated with CCI, using periosteum to cover the defect, or MF. For each of the trial arms the same standardized surgical technique and rehabilitation protocol was enforced. Patients who entered the 12 month study were evaluated with 3-month intervals by an independent investigator not involved in the surgery, and were invited to participate in the extension program up 'till 5 years post surgery.

The sample size was determined based on the definition of a treatment success as the presence of hyaline cartilage characteristics of the repair tissue, and in contrast fibro-cartilage or non-cartilage as a failure. It was assumed that 30 % of patients would report with a successful result after MF, and that an improvement in success rate to 60 % with CCI would be a clinically relevant improvement.

A total of 118 patients were randomized to treatment, 57 to CCI and 61 to MF. Of the CCI patients, six subjects could not be treated because they fell out of specs for the CC score criterium which was enforced: they are included for analysis in the safety population but not in the efficacy analysis. The randomisation to CCI and MF was successful for age (mean age 33.9 years and 33.9 years, respectively), gender (61 and 67 % males), and weight (mean 78.1 and 80.6 kg). There was a slightly higher proportion of patients in the MF group with an acute onset of symptoms compared to the ChondroCelect arm. The median duration of time since onset of knee injury was slightly longer in the ChondroCelect group than in the MF group (2.0 years versus 1.6 years). The presence of concomitant cartilage lesions was comparable in both groups (30 % versus 25 %). Proportionally more patients in the CCI group had undergone previous knee surgery (88 % versus 77 %). The size of the lesion post-debridement was similar in both treatment groups (mean 2.64 and 2.44 respectively) and reflects what is typically encountered in the orthopaedic practice. This lesion size was expected to respond well to both techniques in order to avoid bias in favour of cell transplantation.

As for any of the cartilage restoration procedures, it was imperative that concomitant pathology such as mal-alignment or meniscus lesions were corrected prior to or at the time of index surgery.

11.3.3 Histology and Histomorphometry Outcomes After 12 Months [15]

The original primary objective of the study was to show an advantage of ChondroCelect over MF by demonstrating superiority on the structural repair as assessed by histology and histomorphometry. At 12 months post treatment, biopsy specimens were obtained arthroscopically from the centre of the repair tissue.

Staining with safranin O (a measure of proteoglycan) and anticollagen II antibody, reflective of good quality cartilage tissue, was performed and the staining was expressed as a ratio of the total surface by blinded pathologists. From the CCI and the MF group, 50 and 43 biopsy specimens were analysed respectively. The adjusted mean sum of ratios was significantly higher ($P = 0.003$) for the CCI group than for the patients treated with MF (Fig. 11.5a: Collagen type 2 staining above and SafraninO staining for the best samples of both groups, showing a clear morphological superiority of CCI over microfracture in homogeneity and collagen fibre organization).

Histopathologists scored the quality of cartilage repair by means of the Mean Overall Histology Assessment Score (ICRS II score) [29], assessing components related to chondrocyte phenotype, tissue structure and other possible negative characteristics of the repair tissue such as vascularisation or calcification. Each of the items was rated on a visual analogue scale. The adjusted mean overall histology assessment score was significantly higher ($P = 0.012$) for the CCI group. The adjusted mean scores for components of structural repair relating to chondrocyte phenotype and some components relating to tissue structure were also significantly higher in the CCI group (Fig. 11.5b: subscores reflecting chondrocyte phenotype and some scores reflecting tissue structure were significantly better for the CCI group).

Superiority of ChondroCelect over MF could be demonstrated for both efficacy measures for structural repair: the histomorphometric and the histological endpoint. This suggests that after CCI the regenerated tissue is indeed more hyaline-like and richer in chondrocytes and proteoglycan content of the ECM, which is a prerequisite for resistance to compressive strength.

11.3.4 Clinical Outcome as Measured by KOOS at 12 Months [15]

The second primary objective of the study was to demonstrate non-inferiority on the clinical endpoint, measured as change from baseline in Knee injury and Osteoarthritis Outcome Score (KOOS) for the average of the 12- to 18-months follow-up data. The KOOS questionnaire is patient-rated and consists of 42 items divided over 5 subscales: pain (9 items), other symptoms such as swelling (7 items), activities of daily life (17 items), function in sport and recreation (5 items) and knee-related Quality of Life (QoL) (4 items) [30]. Each of the items has to be scored taking into

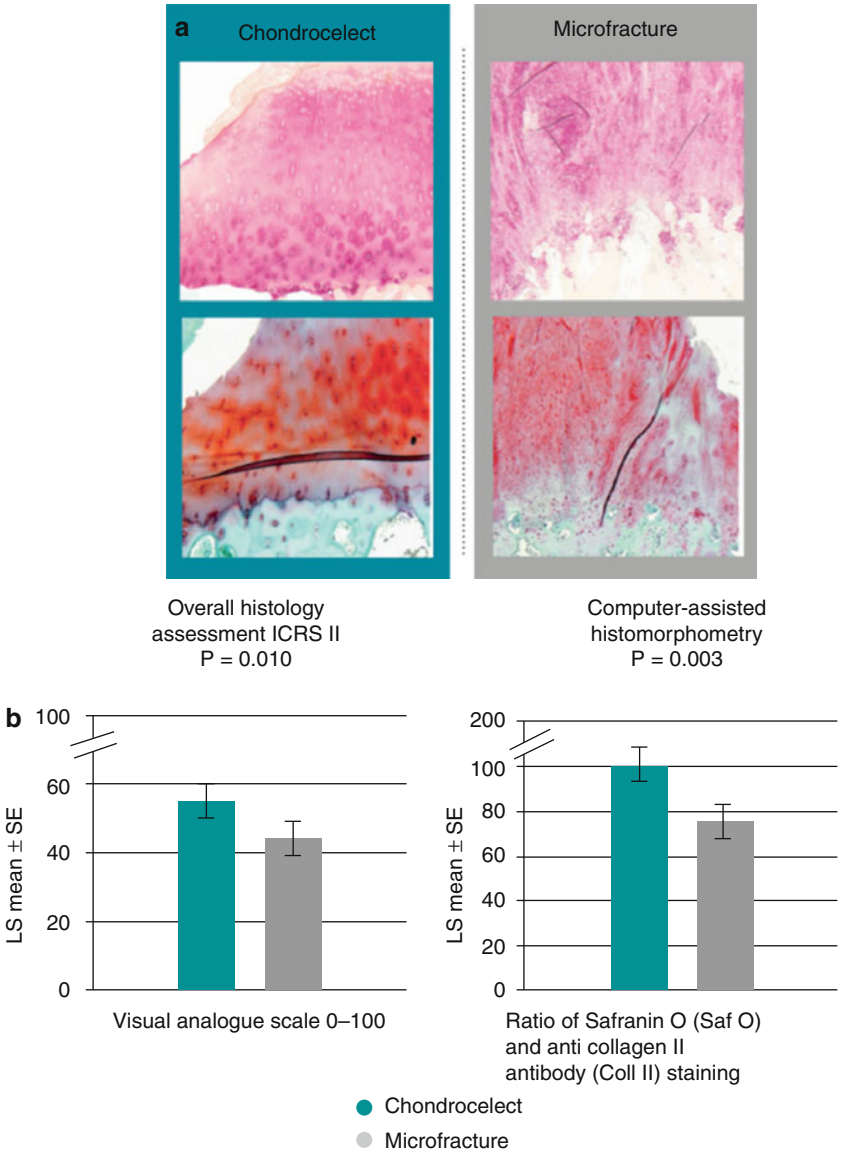

Fig. 11.5 (**a**, **b**) Structural outcome 12 months after CCI, as assessed by histology and histomorphometry

account the previous week. Standardized answer options are given in five Likert boxes leading to a score between 0 and 4. A normalized score whereby 100 meaning no symptoms and 0 indicates extreme symptoms, is calculated for each subscale. The result can be plotted as an outcome profile. KOOS has since been used in a large number of trials and is now considered one of the most meaningful clinical endpoints for knee pathologies to date.

At 18 months, 46 out of the 51 patients treated with CCI were still in the study, and 52 out of the 61 subjects in the MF arm.

The adjusted means for the change from baseline to the mean of 12–18 months in overall KOOS and the subdomains of pain, symptoms/stiffness, ADL and QoL were similar for both study arms. The results fulfill the predefined criteria for non-inferiority in this now co-primary clinical endpoint (as discussed and agreed upon with the regulatory bodies) and both changes are clinically relevant (≥10 points on a scale of 0–100) [31]. Although CCI requires an arthrotomy, which might enforce a slower recovery, this does not appear to affect 1 year outcomes as measured by the KOOS because the clinical improvement versus baseline is comparable in both treatment groups.

11.3.5 Maintenance of Effect in the Long Term [16, 18]

Both treatment groups experienced statistically significant improvements in overall KOOS. Scores continued to improve for 24 months in the CCI group, whereas the maximum for MF was reached approximately 12 months post treatment. In general, the improvement at 2 years was maintained throughout the follow-up period. The clinical benefit versus baseline at 60 months showed a positive trend for CCI versus MF, but no statistically significant differences were found in the overall population (P = 0.116) (Fig. 11.6: the clinical benefit of CCI and MF were maintained at 5 years).

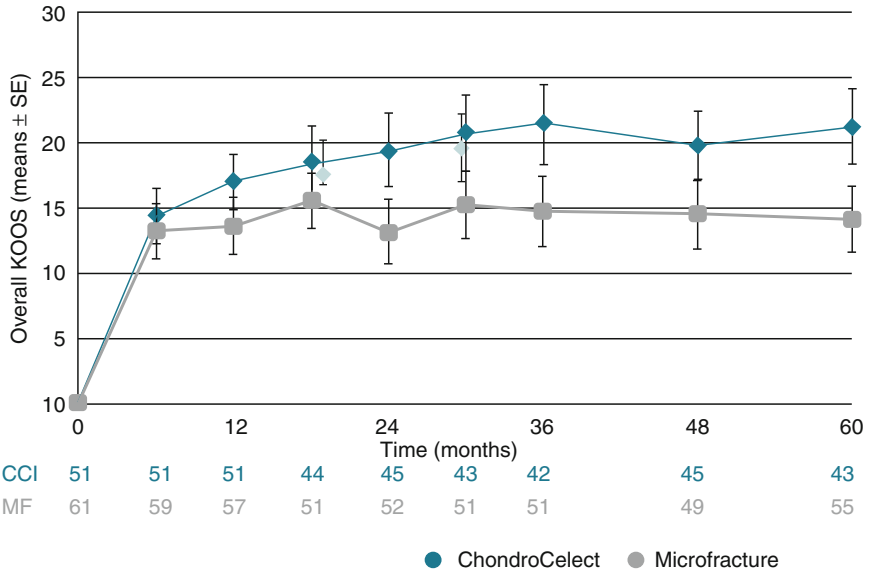

Fig. 11.6 Evolution of overall KOOS (average of all KOOS domains except Sports) throughout 60 month follow-up. Full Analysis Set, including all available data (long-term follow-up) and Last Observation Carried Forward for failures (Vanlauwe et al. [6])

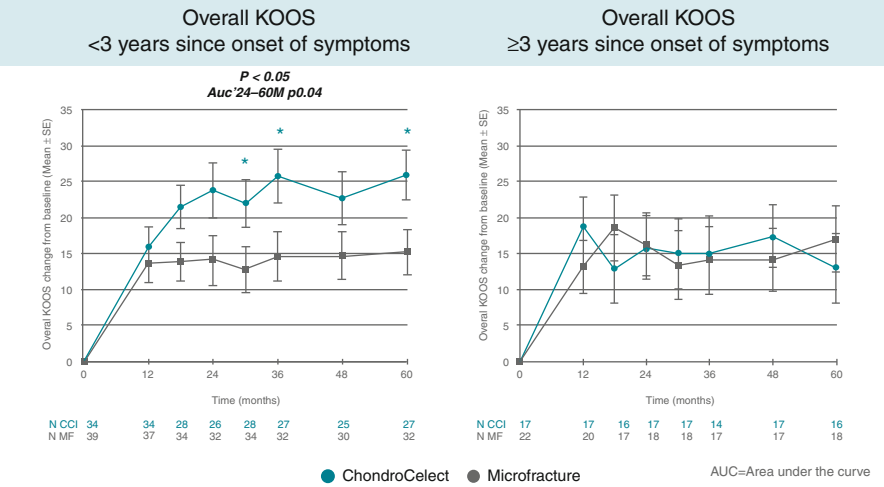

Fig. 11.7 Overall KOOS evolution in patient population with onset of symptoms below and above 3 years. Full Analysis Set and Last Observation Carried Forward for failures (Vanlauwe et al. [6])

Looking for parameters that might predict a favourable outcome, commonly defined as identification of responders to treatment, it was noted that patients with a symptom onset less than 3 years, not only showed a statistically significant difference in overall KOOS improvement compared to MF, but more important had a clinically relevant greater improvement (P = 0.026). Significant differences were also observed in the 'pain' and 'QoL' subscales (Fig. 11.7: Significant difference in overall KOOS in favour of CCI in the <3 years onset group, compared to the >3 years onset group).

Survival analysis did not show statistically significant differences between both treatment arms (Fig. 11.8: Kaplan-Meier survival curve for both treatment arms). However, treatment failures, defined as a re-intervention affecting more than 20 % of the index lesion, seem to occur mostly in the first 3 years post treatment for microfracture treated patients, earlier than in the CCI group. Defining failure has its limitations, in this case it typically relied on clinical symptoms and signs associated with an MRI and/or arthroscopic evaluation to assess whether the cause of failure is due to deterioration of the index lesions.

ACI often is performed in patients who failed traditional first-line treatments such as debridement, MF or osteochondral autograft techniques. However, recent evidence suggests that marrow stimulation techniques have a strong negative effect on subsequent cartilage repair and should be used judiciously in cartilage defects that are amenable to cell based regeneration. In a review of 329 patients, defects that had prior treatment affecting the subchondral bone (microfracture, abrasion chondroplasty and drilling) failed at a rate three times higher than that of non-treated defects [32]. Outcomes were classified as complete failure if more than 20 % of a graft had to be removed in later procedures due to persistent symptoms.

The results from this trial are somewhat different with the outcomes published by Knutsen et al., where no statistically significant differences in clinical outcome, as

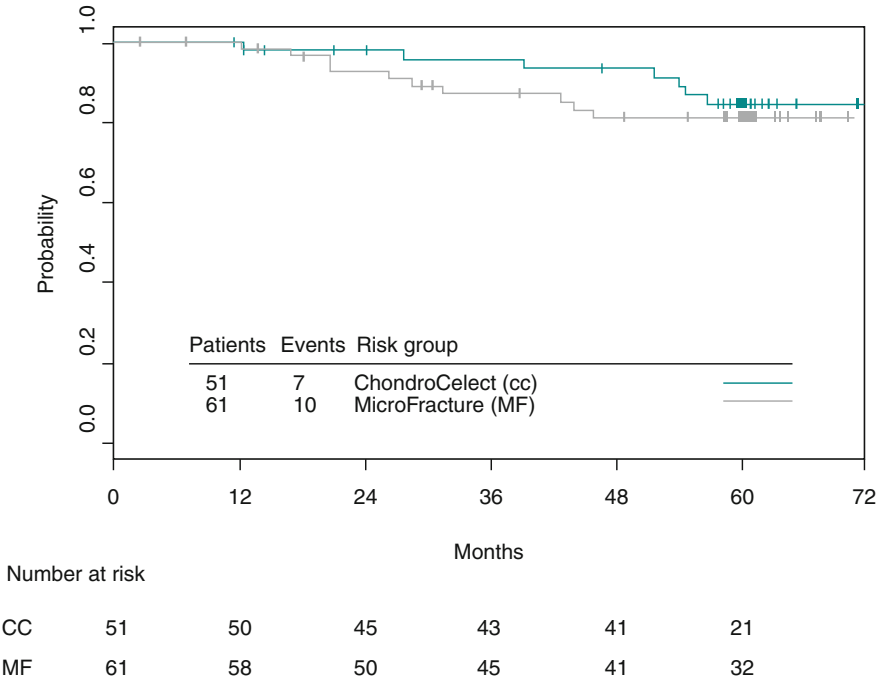

Fig. 11.8 Time to treatment failure (Kaplan Meier graph): Full Analysis Set population at 60 months post surgery (Vanlauwe et al. [6])

measured by the Tegner and International Knee Documentation Committee (IKDC) scores, between MF and ACI at 2 and 5 year follow-up were found [28]. Although osteoarthritis patients were excluded from participation, over a third of the study population in the Knutsen trial displayed radiographic signs of osteoarthritis after 5 years. In contrast, in the RCT comparing CCI to MF, less than 5 % of patients were found to display osteophytes after 5 years [33]. This, together with a higher proportion of chronic lesions in the Knutsen trial (median duration of symptoms 36 months), is reinforcing the hypothesis that cartilage lesions must be treated early on before impairment of the joint biology and loss of homeostasis have led to a 'point of no return'. Many other factors may be of relevance to the different outcomes of the trials including the improved product profile of the cells, a more rigorously controlled trial with respect to inclusion and exclusion criteria, tight control (with audit) of the data and better training of the surgeons.

11.3.6 Imaging Outcomes

Magnetic Resonance Imaging (MRI) scans were performed at baseline, 12, 24 and 36 months. The characteristics of the repair tissue were assessed by means of the Magnetic resonance Observation of Cartilage Repair Tissue (MOCART)

score and nine additional items. Filling of the defect, surface of the repair tissue, subchondral lamina and subchondral bone reaction were identified as the most important determinants of repair tissue quality. At 36 months after surgery, no statistically significant differences for these were observed between the groups, except for subchondral bone reaction which was more prominent in the MF group (P = 0.056). Progressive elevation of the subchondral bone plate was also more pronounced in the MF group than amongst CCI patients (12.1 % versus 8.3 % at baseline; 51.5 % versus 25.0 % at 36 months). In 49 patients, radiographic data were available at baseline and 60 months. No difference in radiographic changes between both treatment arms was observed [6]. In the failure analysis at 5 years in the CCI versus MF trial, the relation with item 5 from the ICRS2 score (subchondral bone changes) was nearly significant (.056) adding to the increasing importance given in literature to the function and the restoration of the subchondral plate as a hallmark in successful cartilage repair, but also in the resistance to development of osteoarthritis.

11.4 Safety Profile of Innovative Treatments: The CCI Experience

11.4.1 Patient Exposure in Clinical Trial and Real Life Setting

A total of 421 patients have been exposed to ChondroCelect in a clinical trial setting.

In the RCT, 61 subjects underwent MF treatment and 57 patients were randomized to CCI, of whom 51 actually underwent CCI. For the safety analysis, all AEs experienced by the patient from the time of the screening visit until the completion of the initial 12-month and extension studies were captured in the case report form (CRF). The retention rate throughout the extension program was high: Forty-three CCI patients (84 %) and 45 MF patients (74 %) provided data for the 5-year follow-up [6].

In the compassionate use program, safety data were available from 334 patients (90.3 %) at database lock. The average exposure, defined as the time between CCI and data capture, was 811 days, ranging between 160 and 1,512 days [18].

In both, the clinical trial and the compassionate use program (CUP), the absolute dose of ChondroCelect received was determined by the size of the lesion(s) treated: ranging between one and five million cells in the RCT, whereas in the CUP lesions up to 20 cm^2 were treated. Despite the relatively short existence of the therapy, patient exposure has thus been quite large both in terms of number of patients, heterogeneity of treated population and follow-up time.

11.4.2 Early AE Profile of CCI [15]

Direct comparison of AE types and frequencies should be evaluated bearing in mind that CCI is a two step procedure with a biopsy arthroscopically harvested, and subsequent implantation of the medicinal product during an arthrotomy. In the RCT, the implanted cells were sealed with a periostal membrane, which necessitated an infrapatellar incision. Thus, 'relatedness' of an AE in the CCI group refers to both, the surgical procedure and the medicinal product itself. In contrast, MF is a single step arthroscopic procedure conducted under local or general anaesthesia [23]. In the first 12-months of the RCT, similar proportions of patients reported treatment-emergent AEs, the majority having a mild or moderate intensity, in the CCI group (50/57, 88 %) versus the MF arm (50/61, 82 %). This was equally true for severe AEs, reported in 12 and 13 % of patients respectively. Relatedness of AEs to the study procedure was confirmed in 67 % of cases in the CCI group and 59 % of cases in the MF arm.

Arthralgia, was the most commonly reported AE in both treatment groups, present in 61 % of CCI subjects and 57 % of MF patients [15].

As CCI requires an arthrotomy, it is not surprising that joint swelling was more frequent in the CCI group versus MF arm. The reported frequency of joint swelling is higher after ChondroCelect (19 %) than after MF (13 %). Joint swelling, a sign of extravasation of fluid in and/or around the knee, is a known symptom after arthrotomy as a result of the inflammatory synovial reaction due to incision [34]. The majority of cases, 7 out of 11, in the CCI group occur in the first 4 weeks after surgery, compared to none in the MF group ($P = 0.003$). One month after surgery, there are no significant differences between both treatments. Apart from the temporary aspect, no cases of postoperative joint swelling were considered severe or serious.

Related AEs of joint crepitation were significantly more common in the CCI arm (12 %) versus MF (1.6 %). Joint crepitation is perceived as being of limited clinical significance and is common even in the normal population [35].

The use of a periosteal flap, conform the initial publication of the technique by Brittberg and Peterson, is known to trigger hypertrophy of the repair tissue, which may cause physical impairment and consequently necessitate arthroscopic shaving. The incidence of hypertrophy at 12 months was 25 % for the CCI group versus 13 % of MF patients ($P = 0.156$). All reported AEs of hypertrophy were mild or moderate in severity in both treatment groups. None was recorded as severe and none was reported as serious.

Overall, no patients were discontinued from the study due to AEs.

11.4.3 Safety and Tolerability in the Long Term [6, 18]

Throughout the whole follow-up period of the RCT, 98 % of patients in the CCI arm and 84 % of MF patients reported at least 1 treatment-emergent AE.

However, all AEs had resolved at 60 months, except for effusion in 3 and 1 cases of after CC and MF respectively, and joint crepitation present in 1 subject of each group.

The most common AE in the early phase, arthralgia, was at 36 months still present in 14 % of CCI cases versus 4 % in the MF group. Joint swelling was not reported in the CCI group beyond 36 months. Joint crepitation, was more frequent in the CCI group compared to MF, but markedly resolves over time: 12 % versus 2 % in the short term, 11 % versus 0 % between 18 and 36 months and 2 % for each treatment group at 60 months [6].

In the compassionate use program, frequencies of AEs are consistently lower as compared to the RCT. Relative underreporting of AEs is indeed one of the methodological limitations of this type of studies. However, with respect to the relative frequency of the AEs, a similar safety profile was observed despite the more heterogenous patient population [18].

In 62.0 % of cases, the reported AE was considered to be related to the surgical procedure. The most commonly reported AEs were knee pain (23.8 %), joint effusion (8.5 %), joint swelling (8.2 %), joint crepitation (6.1 %), muscle atrophy (6.1 %) and decreased joint range of motion (ROM) (5.7 %). The majority of cases (77.6 %) were rated mild to moderate in intensity and 74.4 % were considered unlikely related or unrelated to the medicinal product ChondroCelect.

From 334 patients, 24 serious AEs were received, of which 3 were judged to be possibly related to the product and surgery: 1 in which the ROM was decreased, and 2 cases in which it was judged that the therapeutic product was ineffective.

In contrast with the findings from the RCT (25 % for CCI and 13 % for MF), cartilage hypertrophy was reported overall in 6 of 334 patients (2.1 %). This is most likely explained by the use of a biological membrane, Chondro-Gide™, in the CUP. It is known from the literature that hypertrophy rates are lower in case a biological membrane is used as compared to periostal grafts [36]. Based on these insights, and in order to minimize morbidity, the use of a biomembrane to seal of the implanted chondrocytes has anno 2012 become the standard of CCI . In vitro biocompatibility data for the ChondroGide membrane in combination with ChondroCelect has been generated and approved by EMA.

Interestingly, safety data were collected from 84 patients treated for a patellar lesion. Thus, in the overall safety data, this particular subpopulation contributes for 25.1 %. The observations suggest that patients treated by CCI for a patellar lesion are more prone to developing arthrofibrosis (five patients out of the total of seven patients who developed arthrofibrosis), decreased ROM (8 patellar cases out of the 16 cases which developed decreased ROM) and joint crepitations (9 patellar cases out of the 18 cases which reported crepitations. The rehabilitation program after patellar treatment is indeed clearly different from the femoral protocol in order to prevent early shear and loosening of the graft. This might largely explain these findings.

Patients with lesions larger than 5 cm^2 (range 0.25–20.0 cm^2; median 3.0 cm^2, mean 3.5 cm^2) have been treated under compassionate use only. The safety data obtained in these patients do not indicate a particular safety concern.

In an early phase of the compassionate use program, 16 minors have been treated with ChondroCelect. No specific safety signal was detected in these patients. However, if a surgeon believes that the benefit/risk ratio justifies use of CCI in a particular patient (Marketing Authorisation of ChondroCelect is only granted for patients over 18) complete closure of the growth plate must be documented.

There were no patient deaths recorded during the study. No patients are recorded as being discontinued from the study due to SAEs.

11.5 Discussion and Conclusions

Regenerative Medicine (REGMED) approaches are widely investigated in many fields of Medicine including in musculoskeletal applications. Despite significant advances in the understanding of the processes of tissue repair, the clinical impact of REGMED approaches is still limited. However, some applications have made great progress including skin and cartilage repair. REGMED approaches aim at restoring tissue integrity thereby not leaving any trace behind of the repair process. Fracture healing is a nice example of perfect regeneration in the postnatal mammalian species. Joint surface healing and in particular healing of articular cartilage has been much more challenging as nature is not capable of achieving this. It is an ambition of REGMED to break these boundaries by trying to obtain improved healing of what nature may not always achieve. In view of this, we believe that from the biological perspective comparing microfracture techniques with cell implantation is comparing apples with oranges. Indeed, microfracture induces local fracture healing, ultimately not destined to regenerate an articular surface. The microenvironment may contribute to the maintenance of a cartilage intermediate (a callus type of repair tissue) in the endochondral bone healing process, but there is ample evidence that this is not leading to hyaline articular cartilage. Thus bone marrow stimulation techniques violate the tidemark and subchondral bone plate,and the repair tissue originates from other cellular compartments and go through bone fracture repair pathways [20].

In contrast, articular chondrocyte implantation attempts to preserve the cartilage-bone interface and the resulting regenerate appears to mimick more closely the original and surrounding tissue. Indeed, as soon as cells are implanted a communication is established between the grafted cells and the neighbouring tissue, a phenomenon deemed crucial for the success of the regeneration process. If the implanted or recruited cells are foreign to the articular tissue, this communication might be jeopardized. This is why the data from this CCI trial and other trials, including the failure analyses that have been done, should trigger the orthopaedic community to revisit the treatment algorithm of cartilage lesions. After correction of all surrounding variables such as alignment, ligaments and menisci, more specifically in a patient group which only developed their symptoms recently, the use of autologous chondrocytes should be regarded as a first line regenerative treatment for cartilage in any lesion larger than 2–3 cm^2.

The challenge to prove that REGMED approaches are also of clinical relevance is certainly quite difficult, since several variables are affecting clinical outcomes. They include not only the proper characterization and optimization of the cellular product towards optimal performance, but also factors as microenvironment are of crucial importance. Indeed, proper communication with the surrounding tissues is a key goal for successful regeneration, and we need to translate that into appropriate in- and exclusion criteria, optimized surgical procedures and rehabilitation strategies. Prevention of treatment-emergent side-effects is crucial for any ACI or other cartilage regeneration procedures. In the development of CCI, semi-customized rehabilitation schemes have been specified, which are available to the treating physiotherapist [37]. In addition, we may have to adapt and improve clinical outcome measurements to make them more sensitive to detect and discriminate the distinct mechanisms of repair.

The prospective, multicenter controlled RCT designed to evaluate the efficacy of CCI versus microfracture was the first of its kind. Despite the still somewhat limited number of patients treated when compared to clinical trials in other medical disciplines, there was a lack of evidence for cell based regenerative approaches from controlled trials [38]. This was particularly the case for cartilage repair techniques, including ACI.

It might be considered a shortcoming that CCI was here compared to MF instead of other cell-based technologies, whereas anno 2012 different cell products are available. Differences in efficacy amongst cell products have not been demonstrated in clinical trials. So far, ChondroCelect is the only regenerative cartilage therapy approved as ATMP, which means that efficacy, safety and pharmaceutical quality have strictly been monitored and investigated. For each of these determinants, ample information is available in the public field, which is not necessarily the case for traditional ACIs. In the systematic review by Harris et al., seven trial reports were mentioned in which CCI or ACI was compared to MF, no direct comparisons between ACIs or ACI and CCI do exist [39]. Van Wilder tries to overcome this lack by computing indirect treatment comparisons (ITCs) based on the individual study results of the ACI/CCI versus MF [41]. This methodology was established and validated by the Canadian Agency for Drugs and Technologies. He argues that two out of the seven trials identified by Harris refer to the same study population which was the subject of another publication. Furthermore, he excludes the study by Basad et al. because the large lesions present in this population were in favour of ACI rather than MF treatment [40]. From the four remaining trials, six ITCs were calculated of which four yielded a significant difference, by definition representing ‘large’ treatment effects [41]. Although in ITCs a number of assumptions are being made and one might argue about the validity of the methodology, it is interesting to see that cartilage repair cellular therapies do have different outcomes. It is indeed well know from the literature that minor deviations or variations in culturing conditions can have a huge impact of differentiation of stem cells and adult progenitor cells [42]. Besides divergence at the level of the product used for the treatment, patients presenting with cartilage lesions are a heterogeneous group which is not necessarily adequately addressed when defining inclusion criteria [33].

The lack of correlation between structural improvement from an early stage on and clinical benefit remains a topic for further research. Remodelling and maturation of the cartilage repair tissue after autologous-chondrocyte implantation is progressive and is believed to go on beyond 18 months [43]. It might well be that the advantageous effects resulting from superior quality tissue regeneration require follow-up beyond the time horizons of this trial [44].

The overall safety profile shows that the main difference in treatment related adverse events compared to microfracture is related to the arthrotomy. Many investigational products which can be applied by means of minimally invasive techniques are currently being tested. However, from a patients' perspective, long-term clinical outcomes should be the main driver for any further development. Ease of use and shortening of operation time are features which increase the short term comfort level for surgeon and patient but may not have any inherent long term value.

In conclusion, we believe that the use of cell based approaches in the field of cartilage repair has contributed substantially to REGMED approaches in general, and we and others are capitalizing on this experience to further achieve benefits for our patients. In addition, the lessons learnt from this impressive body of work has triggered new and improved approaches for the prevention and treatment of osteoarthritis, considered as the "holy grail" in the field of musculoskeletal disorders. We hope that young investigators see the ample opportunities to contribute to these major developments and that the field of musculoskeletal disorders and diseases will continue to attract the brightest minds out there!.

References

1. Hjelle K, Solheim E, Strand T, Muri R, Brittberg M. Articular cartilage defects in 1,000 knee arthroscopies. Arthroscopy. 2002;18:730–4.
2. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions. Bull World Health Organ. 2003;81:646–56.
3. Trattnig S, Domayer S, Welsch GW, Mosher T, Eckstein F. MR imaging of cartilage and its repair in the knee–a review. Eur Radiol. 2009;19:1582–94.
4. Cole BJ, Pascual-Garrido C, Grumet RC. Surgical management of articular cartilage defects in the knee. J Bone Joint Surg Am. 2009;91:1778–90.
5. Cole BJ, Pascual-Garrido C, Grumet RC. Surgical management of articular cartilage defects in the knee. Instr Course Lect. 2010;59:181–204.
6. Vanlauwe JJE, Saris DB, Victor J, Almqvist KF, Bellemans J, Luyten FP. Five year outcome of characterized chondrocyte implantation versus microfracture for symptomatic cartilage defects of the knee: early treatment matters. Am J Sports Med. 2011;39(12):2566–74.
7. Peterson L, Menche D, Grande D, et al. Chondrocyte transplantation - an experimental model in the rabbit. In: Transactions from the 30th Annual Orthopedic Research Society, Atlanta. 1984. Palantine: Orthopedic Research Society; 1984. p. 284.
8. Grande DA, Pitman MI, Peterson L, Menche D, Klein M. The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. J Orthop Res. 1989;7:208–18.
9. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med. 1994;331:889–95.

10. Benya PD, Shaffer JD. Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell. 1982;30:215–24.
11. Giovannini S, Diaz-Romero J, Aigner T, Mainil-Varlet P, Nesic D. Population doublings and percentage of S100-positive cells as predictors of in vitro chondrogenicity of expanded human articular chondrocytes. J Cell Physiol. 2010;222:411–20.
12. Jones DG, Peterson L. Autologous chondrocyte implantation. J Bone Joint Surg Am. 2006;88:2502–20.
13. Dell'accio F, De Bari C, Luyten FP. Molecular markers predictive of the capacity of expanded human articular chondrocytes to form stable cartilage in vivo. Arthritis Rheum. 2001;44:1608–19.
14. Vanlauwe J, Almqvist F, Bellemans J, Huskin JP, Verdonk R, Victor J. Repair of symptomatic cartilage lesions of the knee: the place of autologous chondrocyte implantation. Acta Orthop Belg. 2007;73:145–58.
15. Saris DB, Vanlauwe J, Victor J, Haspl M, Bohnsack M, Fortems Y, et al. Characterized chondrocyte implantation results in better structural repair when treating symptomatic cartilage defects of the knee in a randomized controlled trial versus microfracture. Am J Sports Med. 2008;36:235–46.
16. Saris DB, Vanlauwe J, Victor J, Almqvist KF, Verdonk R, Bellemans J, et al. Treatment of symptomatic cartilage defects of the knee: characterized chondrocyte implantation results in better clinical outcome at 36 months in a randomized trial compared to microfracture. Am J Sports Med. 2009;37 Suppl 1:10S–9.
17. European Medicines Agency, EMEA/724428/2009. 2009. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Product_Information/human/000878/WC500026031.pdf.
18. Vanlauwe J, Huylebroek J, Van der Bauwhede J. Clinical outcomes of characterized chondrocyte implantation. Cartilage. 2012;3(2):173–80.
19. Saris DB, Dhert WJ, Verbout AJ. Joint homeostasis. The discrepancy between old and fresh defects in cartilage repair. J Bone Joint Surg Br. 2003;85:1067–76.
20. Madry H, van Dijk CN, Mueller-Gerbl M. The basic science of the subchondral bone. Knee Surg Sports Traumatol Arthrosc. 2010;18:419–33.
21. Hoshiba T, Yamada T, Lu H, Kawazoe N, Chen G. Maintenance of cartilaginous gene expression on extracellular matrix derived from serially passaged chondrocytes during in vitro chondrocyte expansion. J Biomed Mater Res A. 2012;100:694–702.
22. Steinwachs M, Peterson L, Bobic V, Verdonk P, Niemeyer P. A consensus statement on surgical technique: cell-seeded collagen matrix-supported autologous chondrocyte transplantation (ACT-CS). Cartilage. 2012;3(1):5–12.
23. Steadman JR, Rodkey WG, Rodrigo JJ. Microfracture: surgical technique and rehabilitation to treat chondral defects. Clin Orthop Relat Res. 2001;(391 Suppl):S362–9.
24. Bedi A, Feeley BT, Williams III RJ. Management of articular cartilage defects of the knee. J Bone Joint Surg Am. 2010;92:994–1009.
25. Steadman JR, Briggs KK, Rodrigo JJ, Kocher MS, Gill TJ, Rodkey WG. Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. Arthroscopy. 2003;19:477–84.
26. Gobbi A, Nunag P, Malinowski K. Treatment of full thickness chondral lesions of the knee with microfracture in a group of athletes. Knee Surg Sports Traumatol Arthrosc. 2005;13:213–21.
27. Knutsen G, Engebretsen L, Ludvigsen TC, Drogset JO, Grontvedt T, Solheim E, et al. Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. J Bone Joint Surg Am. 2004;86-A:455–64.
28. Knutsen G, Drogset JO, Engebretsen L, Grontvedt T, Isaksen V, Ludvigsen TC, et al. A randomized trial comparing autologous chondrocyte implantation with microfracture. Findings at five years. J Bone Joint Surg Am. 2007;89:2105–12.
29. Mainil-Varlet P, Van Damme B, Nesic D, Knutsen G, Kandel R, Roberts S. A new histology scoring system for the assessment of the quality of human cartilage repair: ICRS II. Am J Sports Med. 2010;38:880–90.

30. Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynnon BD. Knee Injury and Osteoarthritis Outcome Score (KOOS)–development of a self-administered outcome measure. J Orthop Sports Phys Ther. 1998;28:88–96.
31. Roos EM, Lohmander LS. The Knee injury and Osteoarthritis Outcome Score (KOOS): from joint injury to osteoarthritis. Health Qual Life Outcomes. 2003;1:64.
32. Minas T, Gomoll AH, Rosenberger R, Royce RO, Bryant T. Increased failure rate of autologous chondrocyte implantation after previous treatment with marrow stimulation techniques. Am J Sports Med. 2009;37:902–8.
33. Luyten FP, Denti M, Filardo G, Kon E, Engebretsen L. Definition and classification of early osteoarthritis of the knee. Knee Surg Sports Traumatol Arthrosc. 2012;20:401–6.
34. Muckle DS. Open meniscectomy: enhanced recovery after synovial prostaglandin inhibition. J Bone Joint Surg Br. 1984;66:193–5.
35. Jiang CC, Liu YJ, Yip KM, Wu E. Physiological patellofemoral crepitus in knee joint disorders. Bull Hosp Jt Dis. 1993;53:22–6.
36. Gooding CR, Bartlett W, Bentley G, Skinner JA, Carrington R, Flanagan A. A prospective, randomised study comparing two techniques of autologous chondrocyte implantation for osteochondral defects in the knee: Periosteum covered versus type I/III collagen covered. Knee. 2006;13:203–10.
37. Van Assche D, Van Caspel D, Vanlauwe J, Bellemans J, Saris DB, Luyten FP, et al. Physical activity levels after characterized chondrocyte implantation versus microfracture in the knee and the relationship to objective functional outcome with 2-year follow-up. Am J Sports Med. 2009;37 Suppl 1:42S–9.
38. Hanzlik S, Mahabir RC, Baynosa RC, Khiabani KT. Levels of evidence in research published in The Journal of Bone and Joint Surgery (American Volume) over the last thirty years. J Bone Joint Surg Am. 2009;91:425–8.
39. Harris JD, Siston RA, Pan X, Flanigan DC. Autologous chondrocyte implantation: a systematic review. J Bone Joint Surg Am. 2010;92:2220–33.
40. Basad E, Ishaque B, Bachmann G, Sturz H, Steinmeyer J. Matrix-induced autologous chondrocyte implantation versus microfracture in the treatment of cartilage defects of the knee: a 2-year randomised study. Knee Surg Sports Traumatol Arthrosc. 2010;18:519–27.
41. Van Wilder P. Advanced therapy medicinal products and exemptions to the regulation 1394/2007: How confident can we be? An exploratory analysis. Front Pharmacol. 2012;3:12.
42. Roobrouck VD, Clavel C, Jacobs SA, Ulloa-Montoya F, Crippa S, Sohni A, et al. Differentiation potential of human postnatal mesenchymal stem cells, mesoangioblasts, and multipotent adult progenitor cells reflected in their transcriptome and partially influenced by the culture conditions. Stem Cells. 2011;29:871–82.
43. Roberts S, Hollander AP, Caterson B, Menage J, Richardson JB. Matrix turnover in human cartilage repair tissue in autologous chondrocyte implantation. Arthritis Rheum. 2001;44:2586–98.
44. Henderson I, Lavigne P, Valenzuela H, Oakes B. Autologous chondrocyte implantation: superior biologic properties of hyaline cartilage repairs. Clin Orthop Relat Res. 2007;455:253–61.