Is autologous chondrocyte implantation (ACI) an adequate treatment option for repair of cartilage defects in paediatric patients?

Marietta Kaszkin-Bettag

Head Preclinical & Medical Affairs, PharmaLex GmbH, Joseph-Meyer-Straße 13-15, 68167 Mannheim, Germany

Cartilage lesions in the knee of juvenile patients require an effective repair to regain life-long functional activity of the joint. Autologous chondrocyte implantation (ACI) is discussed to be advantageous over other methods for cartilage repair regarding long-term outcome. ACI has successfully been applied in juvenile patients, although currently recommended for patients ≥18 years of age. Only few controlled clinical trials present evidence of efficacy and safety of ACI in adolescent patients. ACI products have to undergo the process of a marketing authorisation application, including the submission of a paediatric investigation plan (PIP). Data from prospective clinical studies or retrospective collection of long-term data in paediatric patients should be submitted for risk–benefit evaluation by the Paediatric Committee (PDCO).

Introduction

For establishing autologous chondrocyte implantation (ACI) as an adequate and potentially first-line treatment option for repair of cartilage defects in paediatric patients, several aspects for treatment and data collection have to be considered. These aspects are crucial for obtaining a marketing authorisation for a specific cartilage-derived, tissue-engineered product as a paediatric investigation plan is mandatory. Tissue-engineered products fall under the Regulation 1394/2007/EC for Advanced Therapy Medicinal Products (ATMP) and require the procedure of a marketing authorisation application. Moreover, the demonstration of the benefit for paediatric patients over other currently used therapies is important for reimbursement negotiations and development of therapy recommendations by medical societies.

Articular cartilage injury is a common orthopaedic problem affecting many people including children and adolescents. The main cause for cartilage defects in the knee includes acute traumatic injuries. The exact incidence of symptomatic high-grade chondral injuries is poorly defined. It has been reported that between 5% and 10% of young, active patients below the age of 40, who present with a haemarthrosis of the knee after a specific traumatic event, will have a focal chondral injury [1].

Corresponding author: Kaszkin-Bettag, M. (marietta.kaszkin-bettag@pharmalex.com)

Another form of joint injury is osteochondritis dissecans (OCD), which is an acquired, potentially reversible idiopathic lesion of subchondral bone resulting in delamination and sequestration with or without articular cartilage involvement and instability [2,3]. OCD of the knee is a relatively rare lesion among the population as a whole, with a prevalence of between 0.01% and 0.06% [4], however, it rather often occurs in children and adolescent patients in connection with competitive sports activities [5].

Unrecognised or untreated defects may increase the risk of osteoarthritis, which is one of the most common disabling disorders affecting more than 10% of the Western population. For example, approximately 175,000 total knee replacement operations are performed annually in Germany as a consequence of osteoarthritis [6] (see: http://www.krankenhaus-report-online.de/). According to the OECD Health Data 2011, knee replacement rates nearly doubled since 2000 in the United States. In Denmark, the knee replacement rate almost tripled between 2000 and 2009. Two hundred and thirteen knee replacement surgeries per 100,000 population per year were performed in Germany in 2009 and the United States in 2008, thereby being on top of the OECD countries [7]. The growing volume of knee replacement is contributing to health expenditure growth as these are expensive interventions. This underscores the need to recognise and treat cartilage defects as early as possible.

 1359-6446/06/$ - see front matter http://dx.doi.org/10.1016/j.drudis.2013.04.007

When is no treatment required?

In paediatric patients, non-operative measures, that is, physical measures and pharmacologic treatment (non-steroidal anti-inflammatory drugs (NSAIDs), nutriceuticals), are a reasonable treatment option providing symptomatic relief for a limited period, usually measured in months. It has to be considered that children that are still growing and have open epiphyseal growth plates have a higher self-healing capacity of smaller lesions (type of lesion classified as ICRS (International Cartilage Repair Society) or Outerbridge grades I or II [8]) compared to adults. This is because of a higher bone marrow stem cell concentration compared to adults and this regeneration can be stimulated by physiotherapy [9].

As soon as the epiphyseal growth plates are closed, adolescents are considered as skeletally mature and are 'biologically young adults' regarding their skeletal system and joint cartilage [10]. The age of 14 in male and the age of 13 in female adolescents are mentioned as starting age for this transition. A complete closure of the epiphyseal growth plate in femur and tibia is achieved at ages up to 21, such that skeletal maturity varies widely.

However, it has only rarely been reported whether the patients were radiologically diagnosed with a closed or an open epiphyseal growth plate before treatment. It must be assumed that the decision for the intervention with ACI was made by the orthopaedic independently of this diagnosis, but was rather driven by the size of the lesion.

For the treatment of cartilage defects, the classification of the paediatric age groups according to the ICH E11 guideline [11] is artificial and medically not justified. A 15-year-old adolescent with closed epiphyseal growth plate is more similar to a 20-year old than the 20-year old to an older patient regarding cartilage structure and regeneration capacity. This was confirmed by two recent studies on biomarkers for cartilage repair investigating collagen type II, aggrecan and CD44 positive cells. It could be demonstrated that the quality of chondrocytes after in vitro expansion seems to strongly depend on the age of the patients [11]. Chondrocytes from patients between 15 and 20 years of age showed significantly higher expression rates of hyaline cartilage-specific markers when compared to chondrocytes from older patients between 20 and 50 years of age. In a retrospective consecutive case series based on a review of a prospective database (2006–2010), 267 patients including 19 children and adolescents $\leq$18 years of age with ACI in the knee were analysed [12]. A correlation analysis in all patients $<$30 years revealed statistically significant associations between age and aggrecan or collagen type II expression. From a cluster analysis, an age-dependent expression of these markers was proposed separating groups with an average age of 18.1 ± 2.3 and 23.6 ± 4.2 years, respectively ($p < 0.02$) and the age border between adults and juveniles was suggested to be at about 20 years (Table 1).

When should treatment be considered?

A lesion $>1\,cm^2$ with an ICRS (International Cartilage Repair Society) grade III and IV in an adolescent approaching skeletal maturity might require surgical intervention. In addition, cartilage injuries in children may initially remain undiagnosed, resulting in progression of the chondral defect, which at a later time point has no other options than to be treated surgically. Thus, a proper diagnosis of the cartilage lesion is important. Operative treatment should also be considered in those adolescent patients approaching epiphyseal closure whose lesions have been unresponsive to nonoperative management [2].

As initial investigation, the method of first choice is radiography for children with acute knee trauma or OCD to evaluate malalignment and degenerative changes of the knee joint if the diagnosis cannot be done by visual assessment [13]. In addition, radiological verification of epiphyseal closure is recommended as threshold for lowest level of age of intervention (expected for patients between 12 and 18 years of age). Epiphyseal closure defines skeletally mature adolescents as 'adults'. Magnetic resonance imaging (MRI) can also be applied, however, this technique is less established in children and thus difficult to interprete and validate, in addition it is expensive.

Arthroscopy is an invasive method and should be used when radiography and MRI are not sufficient and comorbidities, such as the condition of the opposing articular surface, ligament and meniscus status in the knee, and other unsuspected cartilage defects need to be examined.

What are the consequences for the Paediatric Investigation Plan (PIP) for a newly developed intervention for cartilage repair?

According to the ICH E11 definition of the paediatric population, a PIP for ACI products can be waived for preterm infants up to children of 11 years. On the other hands, a PIP may apply to adolescents 12–18 years of age under the condition of a diagnosed closed epiphyseal growth plate. As an alternative, a lower age limit may be defined, for example, 15 years of age.

This may represent a challenge for the Applicant as clinical data have to be provided demonstrating that the treatment of adolescents (15 to $<$18 years) with a closed growth plate results in a similar clinical outcome as the treatment of, for example, 'young adults' (i.e. 18–21 years old). A regulatory strategy might be that data from clinical studies with ACI in young adults being 18–21 years old can be extrapolated to adolescents and no additional clinical studies for this particular paediatric age group need to be conducted. This strategy could be supported by identifying publications describing treatments of cartilage defects in patient populations including patients below the age of 18 years (which are few so far). In addition, data from the manufacturer's records and patients' cards at the clinics, particularly with a long-term follow-up of several years, could provide information about the clinical efficacy and safety of a particular treatment and could be collected in form of a case series.

What is the ultimate ambition of the treatment of cartilage lesions?

Generally, clinical efficacy of any repair method should demonstrate the improvement of clinical symptoms together with a structural repair as surrogate parameters for the prevention of osteoarthritis. The filling of the defect with hyaline-like repair cartilage tissue and the integration into native healthy tissue is proposed to be an essential factor to provide long-term durability and a 'normal' knee joint as described in the reflection paper on in vitro cultured chondrocyte containing products for cartilage repair of the knee [14]. An improvement of articular functionality and consequently, of quality of life, ability to work or to follow daily

TABLE 1

Outcome of ACI for cartilage repair in clinical studies involving adolescents

Author	Number of subjects/ age range	Methods	Defect type/ defect size	Outcome measurement/ follow up (FU) period	Results	Conclusions
Bentley *et al.* [36] **prospective, randomised study**	100/16–49 yr (mean 31.3)	Fifty-eight patients with ACI, 42 patients with OATS	Majority of lesions post-traumatic, mean defect size 4.66 cm^2.	Modified Cincinnati, Stanmore scores, objective clinical assessment. Mean FU 19 months.	88% of patients with good or excellent results after ACI, 69% with good or excellent results after OATS; all patellar mosaicplasties failed.	ACI is significantly superior over OATS to repair articular defects of the knee.
Dai and Cai [39] **prospective pilot study**	7/14–19 (mean 16.6 ± 1.5)	MACI on scaffold	Mean defect size 7.1 cm^2 (range 4–12 cm^2), at medial femoral condyle, lateral femoral condyle, and medial femoral condyle extending to trochlea glove, classified as ICRS grades III and IV.	KOOS, IKDC, Lysholm, ICRS. FU 1–3 years.	One year after ACI reduced knee pain and swelling, significant improvements in various scores ($p < 0.05$). After 1 year, none of the subjects had moderate to severe limitation in daily activities. All scores showed a statistically significant improvement ($P < 0.05$).	Overall, subjective and objective improvements were seen in all patients.
Krishnan *et al.* [40] **prospective cohort study**	37/15–36 yr (mean 23.8 yr) with juvenile-onset and 40 yr (36–44) with adult-onset OCD	ACI, coverage with Chondro-Gide® (porcine type I/III collagen membrane)	Comparison of the outcome after ACI-C between patients with juvenile- and those with adult-onset OCD.	Modified Cincinnati rating system, Stanmore functional rating system and a visual analogue pain score. FU 2–7 yr.	Clinical examination showed that 27 (72.3%) of patients had excellent or good results. Their modified Cincinnati rating system improved from a mean pre-operative value of 46.2–68.0 ($p = 0.001$). The Stanmore functional rating and visual analogue scores improved from a mean preoperative score of 2.85 and 5.3 to 1.51 and 2.88, respectively ($p < 0.05$). Of the 23 biopsies taken at 1 year, 11 (47.8%) showed either hyaline-like or a mixture of hyaline-like and fibrocartilage, and 12 demonstrated fibrocartilage (52.2%). Excellent and good clinical results were seen in 82.1% of those with juvenile-onset OCD but in only 44.4% of those with adult-onset disease.	Among juvenile-onset cases the results suggest that age rather than the state of the physis was more important in determining the outcome. A larger defect (>600 mm^2) was related to an inferior clinical outcome for adult-onset disease, whereas a similar size in juvenile disease did not influence the outcome. This suggests a benefit of this ACI method for the treatment of juvenile OCD.
Macmull *et al.* [41] **case report**	1 male 14 yr	ACI (chondrocytes, covered with porcine derived type I/III collagen membranes)	Patient with osteomyelitis and subsequent septic arthritis of the knee at the age of 3 yr. At the age of 14 yr old, he presented at the hospital with a gradual onset of pain in the right knee, duration 24 months. Diagnosis: full-thickness medial femoral condyle articular osteochondral defect measuring 4 cm × 3 cm and a full-thickness patellar lesion measuring 4 cm × 2.5 cm.	Bentley, Modified Cincinnati and VAS scores were taken at 6, 12 and 24 months postoperatively. FU 9 yr.	Bentley scores were 2, 1 and 1, respectively. At corresponding time points, Modified Cincinnati scores were 72, 82 and 88 and VAS were 3.5, 2 and 1.5. Clinical assessment at 9 years postoperatively revealed a Bentley score of 0 and the modified Cincinnati rating system score of 86. Pain measurement on the VAS was 0 out of 10. Radiographic assessment at 9 years postoperatively revealed preservation of the medial joint space on plain X-ray. Arthroscopy was performed at 3 years postoperatively after the patient complained of some mild discomfort. This patient had collagen type I/III patches to cover the defects. The patches underwent marked hypertrophy and required debridement back to a smooth articulating surface.	In this case report, the usual indications for ACI have been extended. At a review after 9 years the alignment and the stability of the knee were normal and the tibial articular surface intact. Restoration of the femoral articular surface has given the knee the optimal biological and mechanical situation compared with the alternatives of osteotomy or arthroplasty.
Micheli *et al.* [5] **registry-based multicentre observational prospective cohort**	37/11–17 yr (mean 15.5 ± 1.6 yr)	ACI with Carticel®, fixation of the periosteal patch over the defect with fibrin glue	Cartilage repair registry patients or other cartilage repair procedures less than 18 years old at the time of ACI, at least one treated full thickness lesion implantation performed. Fourteen patients had OCD. The majority of patients ($n = 32$) had tibial-femoral alignment between 5° and 10° and normal patella tracking ($n = 31$). The others had tibial-femoral alignment or lateral patella tracking. Pre-treatment procedures were MF in 11 patients and debridement in 20 patients.	Modified Cincinnati Evaluation Protocol, patients evaluated preoperatively at the index arthroscopy and implantation. Postoperative assessments obtained at various follow-up time points. Mean FU 4.3 yr, min. 2 yr.	Patients reported a mean improvement in the Cincinnati overall condition score of 3.8 points, pain score of 4.1 points, and swelling score of 3.4 points, encouraging findings given that 72% (23/32) of patients had at least 1 prior cartilage repair procedure before cartilage harvest. Total defect area, baseline overall condition score, concurrent procedures, and sex did not have a significant effect on patient outcomes. One patient had an implantation that failed.	Results suggest that ACI may be an effective option for children and adolescents with large symptomatic chondral lesions of the distal femur.
Mithoefer *et al.* [37] **case series**	20/12–18 yr	ACI	Full-thickness articular cartilage lesions of the knee, who had failed prior surgical and nonsurgical treatment and had at least one surgical procedure before ACI.	Subjective patient outcome rating, knee activity scores (Tegner, Lysholm) and level of athletic participation. FU 2 yr.	96% of patients were rated with good or excellent results; 60% of patients returned to an athletic level equal or higher than that before knee injury.	Treatment of full thickness articular injuries of the knee in adolescent athletes with ACI yields a high rate of functional success at a mean follow-up of 47 months.

Moseley *et al.* [42] retrospective case series	72 (44 male, 28 female)/14–53 yr (mean 37 yr)	ACI with Carticel®	Cartilage Repair Registry patients with full-thickness distal femur lesions, ACI-treated full-thickness (Outerbridge grade 3 or 4), lesions on the distal femur (medial or lateral femoral condyle, or trochlea), 4 patients with OCD, mean total defect surface area was 5.2 ± 4.15 cm^2.	Mean overall condition, pain and swelling, scores at baseline, 1–5 years of follow-up, and 6–10 years of follow-up for all patients and improved patients. FU 6–10 yr.	96% of patients were rated with good or excellent results; 60% of patients returned to an athletic level equal or higher than that before knee injury. Mean improvement in overall condition, pain, and swelling scores from baseline occurred regardless of gender, workers' compensation status, diagnosis of osteochondritis dissecans, lesion size, BMI, age, history of microfracture within the 5 years before the index arthroscopy, or performance of concurrent procedures at the time of chondrocyte implantation. Eighteen patients had subsequent operations, and 12 patients had treatment failures (total knee replacements, diagnosis of ACI failure with no subsequent treatment, osteochondral autograft, repeated ACI, and abrasion arthroplasty.	Chondrocyte implantation for large, symptomatic, full-thickness lesions of the distal femur can result in early improvement that is sustained at longer follow-up (up to 10 years) in the majority of patients and that is independent of age.
Pascual-Garrido *et al.* [43] prospective case series	62/15.8–49.4 yr (mean 31.8 yr)	ACI with Carticel®	The mean defect size was 4.2 cm^2. Patellofemoral defects.	Lysholm, IKDC, KOOS; includes the 5 categories of Pain, Symptoms, Activities of Daily Living, Sport, and Quality of Life, Tegner, Cincinnati, and Short Form-12. FU 4 yr (2–7 yr).	Significant improvements in the preoperative to postoperative scores, with the exception of the Short Form-12 Mental. Patients reported the overall condition of their knee as excellent, very good, or good in 71% of the cases. Patients undergoing anteromedialization tended toward better outcomes than those without realignment. Forty-four percent of patients needed a subsequent procedure. There were 4 clinical failures (7.7%), which were defined as progression to arthroplasty or conversion to osteochondral allograft transplantation. Subgroup analysis revealed no differences in patient age at implantation, gender, or defect size.	Autologous chondrocyte implantation is a viable treatment option for chondral defects of the patellofemoral joint. Combined autologous chondrocyte implantation with anteromedialization improves outcomes more than autologous chondrocyte implantation alone. Patients with failed prior cartilage procedures can also expect sustained and clinically meaningful improvement.
Peterson *et al.* [44] prospective	58/14–52 yr (mean 26.4)	ACI	Patients with radiographically documented OCD of the knee.	Tegner-Walgren, Lysholm, Brittberg-Peterson, VAS, microscopic quality of graft integrity. FU 5.6 yr.	91% of patients had good to excellent overall rating on the basis of clinical evaluation. Ninety-three per cent of patients had improvement on a patient self-assessment questionnaire.	Treatment of OCD of the knee with ACI produced an integrated repair tissue with a successful clinical result in $>$90% of patients.
Rogers *et al.* [45] prospective single-centre cohort study	57 (31 male, 26 female)/15–51 yr (mean 31.6)	ACI with porcine type I/III collagen membrane cover	Primary surgical indication was persisting pain and/or mechanical symptoms resulting from trauma, OCD, or chondromalacia patella at medial femoral condyle with a mean area of 3.14 cm^2 (range 1.0–7.0 cm^2).	Modified Cincinnati, VAS, Bentley functional rating score, Lysholm & Gillquist Score, Patient functional outcome, Brittberg, Patient rating. All patients were assessed annually. FU 6 yr.	All scores were significantly improved compared to the respective mean preoperative scores and showed continued sequential improvement up to 6 years postsurgery. Twenty-four patients underwent a second look arthroscopy with a fair to excellent grade of repair. Three cases were biopsied showing graft hypertrophy. There were no graft failures.	Results demonstrate a statistically significant functional improvement over 6 years compared to preoperative scores in patients undergoing ACI.
Schmal *et al.* [12] retrospective consecutive case series based on a review of a prospective database (2006–2010)	Nineteen patients $\leq$18 yr out of 267 in total; Paediatric: 11 male, 8 female 11–18 yr (mean 16.7 ± 2.0 yr) Adults: 20–58 yr (mean 36.6 ± 8.5 yr)	ACI	Cartilage lesions caused by OCD and trauma at lateral or medial femoral condyle, patella or trochlea. Mean defect size 3.9 ± 2.0 cm^2. Open epiphysis in 6 cases, closure was seen in all patients older than 15 years.	Analysis of cartilage-specific markers for age association, KOSS for MRI assessment.	Statistically significant correlation between collagen type II expression and age. Age border between adults and juveniles at 20 years. MRI assessed by KOSS at baseline 4.8 ± 2.3 points, declined significantly to 3.3 ± 2.3 points at 6 months ($p = 0.025$) and 3.3 ± 2.9 at 12 months after surgery.	Age-related expression of cartilage-specific markers allows a reliable discrimination between juveniles and adults. Skeletal maturity defined by the closure of epiphyses with about 15 years does not allow a conclusion about cartilage maturity and healing potential.

ACI: autologous chondrocyte implantation; FU: follow-up; ICRS: International Cartilage Repair Society; IKDC: International Knee Documentation Committee; KOOS: Knee Osteoarthritis Outcome Score; KOSS: MRI assessment of knee osteoarthritis; MF: microfracture; MRI: magnetic resonance imaging; OATS: osteoarticular transfer system; OCD: osteochondritis dissecans; VAS: visual analogue scale; yr: years.

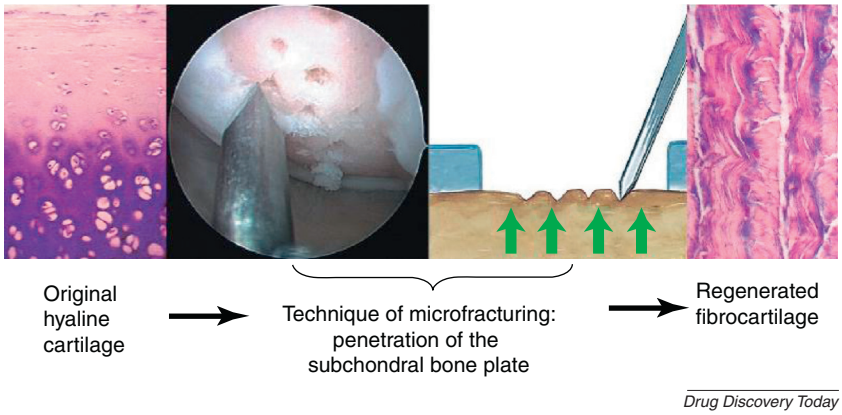

FIGURE 1

Microfracturing and regenerated fibrocartilage. Tiny holes are made in the bone near the damaged cartilage. These microfractures release the mesenchymal stem cells in bones that build new cartilage to replace damaged cartilage. Picture modified from: http://www.westpfalz-klinikum.de/e15882/e15878/e14230/e25640/e18607/e18633/index_ger.html.

and sports activity is expected particularly by a physically active population as adolescents and young adults mostly are.

Overview of cartilage repair procedures

While several surgical approaches have been described, it remains difficult to compare the efficacy of these techniques because of the lack of a sufficient number of well-designed controlled trials in the literature.

Marrow stimulation procedures such as microfracture (Fig. 1) are the first line therapy for small lesions <2–4 cm^2 [8,15–18].

Osteochondral allograft transplantation (OATS) is established as second line therapy for small lesions [1,19].

Since 1994, ACI has been established using the patient's own cartilage as source for regenerating chondrocytes and retransplanting them into the defect area in the knee or other joints (Fig. 2 [20]). During the last years, scaffolds were developed consisting of hyaluron or collagen, on which the autologous chondrocytes are seeded, and which are implanted into the joint to cover the defect area (e.g. [21,22]). A further, more recent approach is the use of mesenchymal stem cells differentiating into chondrocytes and contributing to cartilage repair [23].

Treatment recommendations for each technique are based on differences in outcomes with respect to the size of the defect and the relative advantages and disadvantages of each. In particular, the size of the cartilage defect(s) is the predominant factor that guides surgical management and technique selection.

Since 1994, several autologous chondrocyte-derived products are on the market. However, only one cell suspension product, ChondroCelect®, has been approved so far under the new legislation for tissue-engineered products for adult patients with ICRS grade III or IV defects on the femoral condyle [24]. Two further products are currently under the assessment of the Committee for Medicinal Products for Human Use (CHMP). Recently, the marketing authorisation application for Hyalograft® C was withdrawn [25].

Other products currently on the market according to transitional national rules in Germany are, for example,

- BioSeed®-C,
- chondrosphere®,
- Novocart®/Novocart 3D®.

ACI is a two-step procedure. From an arthroscopic biopsy of healthy hyaline cartilage, chondrocytes are isolated and further cultivated in vitro by the manufacturer, and the resulting chondrocytes or chondrocyte-derived products such as matrix-associated chondrocytes (e.g. three-dimensional spheroids) are then re-implanted into the cartilage defect (Fig. 2 [20,35,46]).

1. Step:
   - During endoscopy, removal of cartilage from a lesser weight bearing area of the affected joint.
   - Enzymatical isolation and subsequent cultivation of chondrocytes for cell expansion which is in second and third generation products followed by formation of three-dimensional spheroids respective ingrowth in scaffolds at the manufacturer's site under Good Manufacturing Practice (GMP) conditions.
2. Step
   - Transplantation of chondrocytes or three-dimensional spheroids respective chondrocytes planted in three-dimensional scaffolds into the defect area of the joint by arthrotomy or mini-arthroscopy. Cell suspensions need to be covered with a periosteal flap taken from the proximal tibia, or collagen membrane to prevent floating of cells to other areas; three-dimensional spheroids do not need a cover but adhere with their own chondrocyte-derived extracellular matrix [26,27].

The advantage of this technique is that ACI products are eligible also for large defects >4 cm^2. It was reported that even defects >10 cm^2 were successfully treated [27]. It is suggested that the hyaline repair tissue built by the chondrocyte is similar to the native surrounding cartilage and has a higher long-term stability compared to fibrocartilage produced by bone-marrow stimulating techniques (e.g. microfracture). The disadvantage of ACI is that 2 interventions by endoscopy are needed within 2 months: one for biopsy-taking, one for transplantation.

Currently, the first-line method for children and adolescents with small defects 2 up to 4 cm^2 is microfracture [28,29]. By

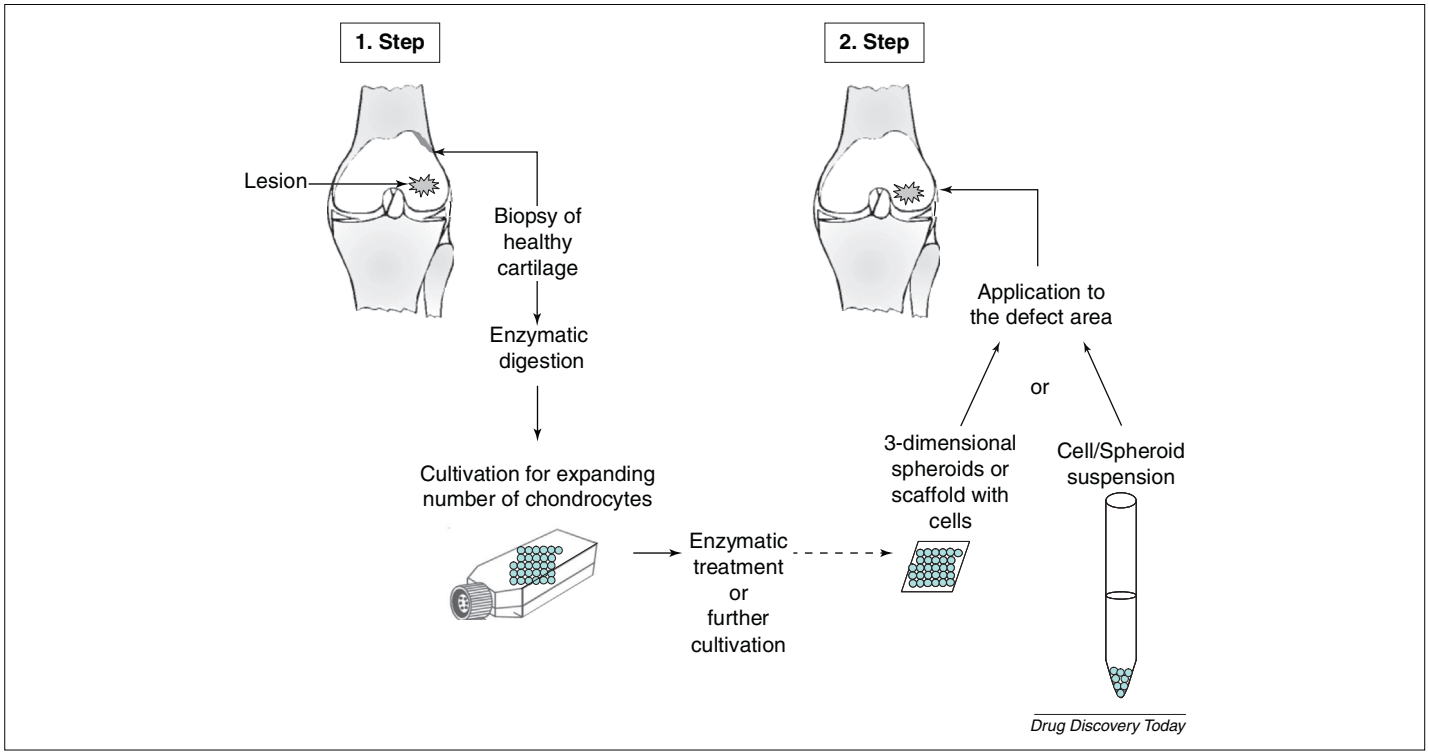

FIG. 2

Diagram of chondrocyte transplantation in the right femoral condyle. ACI consists of a two-step procedure. First step: during arthroscopy, a small cartilage biopsy is taken from a lesser weight bearing area of the affected joint. Cartilage material is harvested from the joint and sent to a GMP-certified manufacturer, where the chondrocytes are enzymatically separated from the cartilage tissue and brought in monolayer culture to expand the number of chondrocytes (proliferation step). Then further enzymatic treatment follows and depending on the product either a cell suspension is prepared or further manufacturing steps are necessary for establishing either spheroids or cells on a scaffold. In the second step, samples are applied to the defect area by arthrotomy or miniarthroscopy. Single cells need a cover by a periosteal flap taken from the medial tibia or by a collagen membrane [35,46]. Spheroids do not need fixation but adhere to the defect area with their own extracellular matrix. Chondrocytes seeded on allogenic scaffolds usually need fixation with fibrin glue [34].

penetrating the subchondral bone in the defect area, the bone marrow is stimulated and mesenchymal stem cells infiltrate and produce fibrocartilage ([46], Fig. 1). The advantage of microfracture is that it is a one-step surgical procedure. The disadvantage is that it is recommended only for small defects up to max 4 cm^2 and that the long-term stability of repair fibrocartilage is discussed to be inferior to that of hyaline cartilage [1]. Poor fill of defects after MF was indeed reported for 20–50% of cases, with formation of intra-lesional osteophytes in the defect in 25–50% of MF procedures [17]. Particularly in a long-term perspective, the repair tissue seems to develop mechanical properties inferior to those of hyaline cartilage as it has been shown to degenerate over time because of shear forces [30–34]. This point has to be considered particularly for younger, physically active patients with large cartilage lesions. However, owing to the lack of structural data from clinical studies in adolescent patients with any of the methods described above, it is not conclusively known if the fibrocartilage tissue built in the joints of young patients is inferior to hyaline cartilage.

The use of ACI in general, and in paediatric patients in particular, is widely accepted by orthopaedics as is demonstrated by inclusion of such patients in the clinical investigations of ACI presented in Table 1. The general problem is that no guidelines based on comparative trials exist for the treatment of articular cartilage defects for *any* patient population. No randomised controlled clinical trials were available at the time of the development of first recommendations on cartilage repair. The German Societies for Traumatology (DGU) and Orthopaedic Surgery (DGOOC) from 2004 [18] or the working group of the Belgian Orthopaedics Societies on cartilage repair in the knee from 2007 [35] recommended for the use of ACI:

- Chondral defects ICRS grade III or IV diagnosed by arthroscopy/MRI.
- Defect size approx. 3–10 cm^2 [18] or 2–12 cm^2 [35].
- Defect location: femoral condyle, trochlea, patella, talus.
- Patient age: 18–50 years [18], or from radiological closure of the epiphysis up to 50 years [35].

More recently, a guideline for the design and conduct of clinical studies in knee articular cartilage repair was developed by Mithoefer *et al*. in 2011 [8]. Here, it is confirmed that peer-reviewed studies have demonstrated the efficacy of ACI in patients under the age of 18 years [5,37].

Other case reports, case series or prospective clinical studies with ACI including paediatric patients are presented in Table 1 and demonstrate the clinical efficacy of this procedure in a broad age range of patients (11–53 years of age), although only few studies [5,37] were prospectively designed for the paediatric patient population.

The challenges of a PIP for ACI

For any chondrocyte-derived product, for which a marketing authorisation application is planned, the submission of a PIP is obligatory according to the Regulation 1394/2007/EC for ATMP.

However, no incentives in form of a Paediatric Use Marketing Authorisation (PUMA) or Supplementary Protection Certificate (SPC) are available for ACI manufacturers because of the nature of their products and indications to develop a PIP for their product and to conduct clinical trials in paediatric patients. As a medical need for ACI in juvenile patients with large cartilage lesions has been identified, different options can be taken into account when preparing a PIP.

A deferral of the PIP in case that at the time point of marketing authorisation application (MAA), no or not enough clinical data are available. An approval may be granted under the condition of a post-marketing clinical study in the paediatric population.

However, a crucial aspect was given in the guideline from Mithoefer *et al*. [8] regarding the conduct of a controlled clinical trial in paediatric patients. It was stated that although cell-based cartilage repair has been shown to be effective in patients <18 years, inclusion of patients younger than 18 years in controlled trials is not routinely recommended because of the legal and practical implications related to the consent process and the ethical treatment of minors. In this guideline, patient's age between 18 and 60 years is proposed as appropriate for inclusion in cartilage repair studies. Therefore, the following alternative approaches may be considered and should be discussed with the Paediatric Committee (PDCO).

A retrospective analysis of clinical data from daily clinical practice could be performed for those products, which have already been marketed, before Regulation 1394/2007/EC for ATMPs came into force, and for which, a considerable amount of source data from the patients' charts at the clinics are available. Collection of these data by a trained clinical monitor could be performed to assess retrospectively the efficacy and safety of a particular product in form of a case series or single case reports performing statistical analyses of the available data.

Another source could be data from published clinical studies with a specific product in the scientific literature. However, the majority of clinical studies were conducted in adult patients, and only few prospective or retrospective studies included juvenile patients (Table 1). Single case studies show a positive clinical outcome of ACI in adolescents demonstrating that they were able to return to their pre-injury activity level. Overall, the quality of reporting the data from clinical studies including paediatric patients is in need of improvement as essential information about the juvenile patients is lacking. Adolescents were described as part of the total patient pool, and no separate information about number, demographic data, baseline characteristics, and most importantly, no separate analysis of the clinical outcome were reported. Thus, an interpretation of study results with respect to a specific paediatric age group is impossible.

Examples: In a prospective, randomised study comparing ACI with OATS, 100 patients with an age range of 16–49 years were included. It was not indicated, how many patients were below the age of 18. No analysis of the results for the patients <18 years was performed [36].

An observational registry-based study specifically analysing data from 37 adolescent patients 11–17 years of age was conducted by Micheli *et al*. [5]. Here, a matrix-associated chondrocyte product (Carticel®) was implanted. During the follow-up period of up to 4.3 years, a significant clinical improvement was observed, and only one patient had a treatment failure. However, no information about the progress of defect filling was given.

Another clinical study by Mithoefer *et al*. [37] has reported the outcomes of 20 adolescent athletes (12–18 years of age, mean lesion size 6.4 cm^2), who were treated with ACI and followed up for 47 months in average. Results from this study suggest that ACI yielded a high rate of functional success using established knee scoring systems that included the Lysholm score and Tegner activity score. Nineteen patients rated their results as good or excellent, and only one patient reported a fair outcome. In this study, patients with open as well as closed growth plate were included, and the outcome did not reveal a statistically significant difference between both subgroups, however, the limited number of patients in this study may not have been sufficient to demonstrate a statistically significant effect of epiphyseal status on articular cartilage repair in adolescents.

Questionnaires for adolescents — a special challenge

In clinical studies as well as in daily clinical practice, patients are asked questions regarding pain and functional impairment of the knee and impairment of daily life activities using validated questionnaires such as the ICRS, which includes among others the IKDC (International Knee Dokumentation Committee) Current Health Assessment Form and the IKDC Subjective Knee Evaluation Form-2000 for patient-reported outcome. Often, physicians are confronted with the disagreement of adolescent patients to give their consent for participating in a clinical study and to fill in questionnaires. In addition, in certain cases there may be an intellectual problem to understand the meaning of the questions. Thus, developers of questionnaires should take this into account when designing forms to be filled in by juvenile patients for assessing patient-reported clinical outcomes. Additional value may be provided by the use of telephone surveys to assess quality of life in paediatric patients as this type of questioning was reported to be less burdensome procedure to the patient and to minimise the risk of missing important clinical data [38].

Summary and conclusions

ACI may be an effective option for adolescents with chondral lesions needing surgical treatment. Retrospective analyses of meaningful data collected from patient's charts can support a PIP for a chondrocyte-derived product, for which a MAA is planned. A proper reporting of data is crucial to convince the PDCO about the efficacy and safety of a particular ACI product. The PDCO, however, needs to consider that regarding cartilage repair, adolescents with a closed epiphyseal growth plate should be classified as biological ''young adults'', thus suggesting that an extrapolation of clinical data obtained in clinical studies with young adults above the age of 18 may be possible. In addition, adolescents with an open epiphyseal growth plate, but with a larger cartilage effect, which at the discretion of the orthopaedic may probably not repair by self-healing, may benefit from the long-term repair capacity of the ACI technique.

Conflicting interests

The author declared the following potential conflicts of interest with respect to the authorship and/or publication of this article:

M. Kaszkin-Bettag is a consultant for the co.don AG (Teltow, Germany). The author received no financial support for the preparation and authorship of this article.

Acknowledgement

The author thanks Dr. Zaklina Buljovcic for her scientific and regulatory support in preparing this article.

References

1 Alford, J.W. and Cole, B.J. (2005) Cartilage restoration. Part 1. Basic science, historical perspective, patient evaluation, and treatment options. *Am. J. Sports Med.* 33, 295–306
2 Kocher, M.S. *et al.* (2006) Management of osteochondritis dissecans of the knee: current concepts review. *Am. J. Sports Med.* 34, 1181–1191
3 Schenck, R.C., Jr and Goodnight, J.M. (1996) Osteochondritis dissecans. *J. Bone Joint Surg. Am.* 78, 439–456
4 Fonseca, F. and Balaco, I. (2009) Fixation with autogenous osteochondral grafts for the treatment of osteochondritis dissecans (stages III and IV). *Int. Orthop.* 33, 139–144
5 Micheli, L.J. *et al.* (2006) Articular cartilage defects of the distal femur in children and adolescents: treatment with autologous chondrocyte implantation. *J. Pediatr. Orthop.* 26, 455–460
6 Statistisches_Bundesamt, (2010) *Krankenhausreport 2010.*
7 OECDiLibrary: http://www.oecd-ilibrary.org/sites/health_glance-2011-en/04/07/index.html;jsessionid=3dln9sdrovedm.delta?contentType=&itemId=/content/chapter/health_glance-2011-35-en&containerItemId=/content/serial/19991312&accessItemIds=/content/book/health_glance-2011-e
8 Mithoefer, K. *et al.* (2011) Guidelines for the design and conduct of clinical studies in knee articular cartilage repair. International Cartilage Repair Society recommendations based on current scientific evidence and standards of clinical care. *Cartilage* 2, 100–121
9 Steinwachs, M.R. (2006) Cartilage repair – autologous chondrocyte transplantation and autologous matrix-induced chondrogenesis. *Eur. Musculoskelet. Rev.* 65–68
10 Martin, D.D. *et al.* (2011) The use of bone age in clinical practice. Part 1. *Horm. Res. Paediatr.* 76, 1–9
11 ICH Topic E11, (2001) *Clinical Investigation of Medicinal Products in the Paediatric Population. CPMP/ICH/2711/99.*
12 Schmal, H. *et al.* (2013) Autologous chondrocyte implantation in children and adolescents. *Knee Surg. Sports Traumatol. Arthrosc.* 21 (3), 671–677
13 Gomoll, A.H. *et al.* (2010) Collagen membrane as cover for autologous chondrocyte implantation. Letter to the editor. *Am. J. Sports Med.* 38, NP4
14 European_Medicines_Agency_Committee_for_Advanced_Therapies, (2009) *Reflection Paper on In-vitro Cultured Chondrocyte Containing Products for Cartilage Repair of the Knee. EMA/CAT/CPWP/568181/2009..*
15 Williams, R.J., III and Harnly, H.W. (2007) Microfracture: indications, technique, and results. *Instr. Course Lect.* 56, 419–428
16 Mithoefer, K. *et al.* (2006) Chondral resurfacing of articular cartilage defects in the knee with the microfracture technique. Surgical technique. *J. Bone Joint Surg. Am.* 88 (Suppl 1 Pt 2), 294–304
17 Mithoefer, K. *et al.* (2009) Clinical efficacy of the microfracture technique for articular cartilage repair in the knee: an evidence-based systematic analysis. *Am. J. Sports Med.* 37, 2053–2063
18 Behrens, P. *et al.* (2004) Indications and implementation of recommendations of the working group Tissue Regeneration and Tissue Substitutes for Autologous Chondrocyte Transplantation (ACT). *Z. Orthop. Ihre Grenzgeb.* 142, 529–539
19 Gudas, R. *et al.* (2009) A prospective, randomized clinical study of osteochondral autologous transplantation versus microfracture for the treatment of osteochondritis dissecans in the knee joint in children. *J. Pediatr. Orthop.* 29, 741–748
20 Brittberg, M. *et al.* (1994) Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N. Engl. J. Med.* 331, 889–895
21 Brun, P. *et al.* (2008) Characteristics of repair tissue in second-look and third-look biopsies from patients treated with engineered cartilage: relationship to symptomatology and time after implantation. *Arthritis Res. Ther.* 10, R132
22 Rodriguez-Merchan, E.C. (2013) Regeneration of articular cartilage of the knee. *Rheumatol Int* 33 (4), 837–845
23 Huselstein, C. *et al.* (2012) Mesenchymal stem cells for cartilage engineering. *Biomed. Mater. Eng.* 22, 69–80
24 European_Parliament_and_Council, (2007) *Regulation (EC) No. 1394/2007 of the European Parliament and of the Council of 13 November 2007 on Advanced Therapy Medicinal Products and Amending Directive 2001/83/EC and Regulation (EC) No. 726/2004.*
25 EMA/CAT/25123/2013. CAT monthly report of application procedures, guidelines and related documents on advanced therapies. January 2013 meeting
26 Rössing, (2007) Zur Diskussion – Neue Technik zur arthroskopischen, autologen Chondrozytentransplantation mittels Chondrosphere. *Z. Orthop. Unfall.* 145, 276–277
27 Fickert, S. *et al.* (2011) One-year clinical and radiological results of a prospective, investigator-initiated trial examining a novel, purely autologous 3-dimensional autologous chondrocyte transplantation product in the knee. *Cartilage* http://dx.doi.org/10.1177/1947603511417616
28 Steadman, J.R. *et al.* (2003) Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. *Arthroscopy* 19, 477–484
29 Salzmann, G.M. *et al.* (2012) Microfracture for treatment of knee cartilage defects in children and adolescents. *Pediatr. Rep.* 4, e21 Epub June 19, 2012
30 Lindahl, A. *et al.* (2003) Cartilage repair with chondrocytes: clinical and cellular aspects. *Novartis Found. Symp.* 249, 175–186
31 Manfredini, M. *et al.* (2007) Autologous chondrocyte implantation: a comparison between an open periosteal-covered and an arthroscopic matrix-guided technique. *Acta Orthop. Belg.* 73, 207–218
32 Kon, E. *et al.* (2009) Arthroscopic second-generation autologous chondrocyte implantation compared with microfracture for chondral lesions of the knee: prospective nonrandomized study at 5 years. *Am. J. Sports Med.* 37, 33–41
33 Harris, J.D. *et al.* (2010) Autologous chondrocyte implantation: a systematic review. *J. Bone Joint Surg. Am.* 92, 2220–2233
34 Bedi, A. *et al.* (2010) Management of articular cartilage defects of the knee. *J. Bone Joint Surg. Am.* 92, 994–1009
35 Vanlauwe, J. *et al.* (2007) Repair of symptomatic cartilage lesions of the knee: the place of autologous chondrocyte implantation. *Acta Orthop. Belg.* 73, 145–158
36 Bentley, G. *et al.* (2003) A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. *J. Bone Joint Surg. Br.* 85, 223–230
37 Mithofer, K. (2005) Functional outcome of knee articular cartilage repair in adolescent athletes. *Am. J. Sports Med.* 33, 1147–1153
38 Dunaway, S. *et al.* (2010) Reliability of telephone administration of the PedsQL Generic Quality of Life Inventory and neuromuscular module in Spinal Muscular Atrophy (SMA). *Neuromuscul. Disord.* 20, 162–165
39 Dai, X.S. and Cai, Y.Z. (2012) Matrix-induced autologous chondrocyte implantation addressing focal chondral defect in adolescent knee. *Chin. Med. J. (Engl.)* 125, 4130–4133
40 Krishnan, S.P. *et al.* (2006) Who is the ideal candidate for autologous chondrocyte implantation? *J. Bone Joint Surg. Br.* 88, 61–64
41 Macmull, S. *et al.* (2010) Treating articular cartilage injuries of the knee in young people. *Br. Med. J.* 340, 587–592
42 Moseley, J.B., Jr *et al.* (2010) Long-term durability of autologous chondrocyte implantation: a multicenter, observational study in US patients. *Am. J. Sports Med.* 38, 238–246
43 Pascual-Garrido, C. *et al.* (2009) Recommendations and treatment outcomes for patellofemoral articular cartilage defects with autologous chondrocyte implantation: prospective evaluation at average 4-year follow-up. *Am. J. Sports Med.* 37 (Suppl 1), 33S–41S
44 Peterson, L. *et al.* (2003) Treatment of osteochondritis dissecans of the knee with autologous chondrocyte transplantation: results at two to ten years. *J. Bone Joint Surg. Am.* 85-A (Suppl 2), 17–24
45 Rogers, B.A. *et al.* (2010) Sequential outcome following autologous chondrocyte implantation of the knee: a six-year follow-up. *Int. Orthop.* 34, 959–964
46 Moyad, T.F. (2011) Cartilage injuries in the adult knee: evaluation and management. *Cartilage* 2, 226–236