Clinical Trial Protocol

Regenerative Medicine

Efficacy and safety of autologous cell therapies for knee cartilage defects (autologous stem cells, chondrocytes or the two): randomized controlled trial design

Aim: The main aim of this trial is to test the safety and efficacy of autologous stromal/stem cells, chondrocytes or the two combined in the treatment of knee cartilage defects. **Patients & methods:** Patients with symptomatic chondral/osteochondral defects will be randomized to cell therapy treatment with one of three cell populations (1:1:1). The primary efficacy outcome is a functional knee score (Lysholm) at 15 months post-treatment and the primary safety outcome is the incidence of adverse events. Secondary objectives are to analyze repair tissues, quality of life and cost-utility assessments. Exploratory objectives are to identify predictors for success/potency and dose–response relationships. **Results & conclusion:** This trial has been carefully designed so that valuable scientific and clinical information can be gathered throughout and in the final analysis.

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Cell therapy has been used clinically in the treatment of cartilage injury for over 20 years [1]. There are now many variations of autologous chondrocyte implantation (ACI), but the principles for all are the same. A small sample of cartilage is removed from a minor load bearing region of a patient's damaged joint; chondrocytes are isolated by mechanical and enzymatic digestion and grown in monolayer culture *in vitro*. When the cell number has been amplified sufficiently, cells are implanted into the symptomatic defect in a second planned operation. The cell suspension is retained by either a patch of periosteum or a commercially available collagen membrane, sutured to the edges of the defect and sealed with fibrin. Our center has used ACI in approximately 500 procedures and we and others have shown that it has the potential to improve functional outcome [2–4] and generate repair tissue that offers a durable surface and is well integrated with the surrounding cartilage [5–8]. In our audits of ACI, 80% of patients improve by 15 months, and this improvement is sustained for 9–16 years [3,9]. Some randomized controlled trials reported a significant benefit of ACI or matrix-applied characterized ACI over microfracture [10] whereas others found no evidence for a difference between outcomes of these interventions in a multicenter randomized controlled trial [11].

A recent systematic review has suggested that ACI is cost-effective compared with microfracture across a range of scenarios [12]. These authors, as well as others showing the clear potential when comparing ACI with other treatments, have drawn the conclusion that a need exists for further large randomized trials with a low risk of bias and adequately long follow-up to inform us of the best way to treat cartilage defects [13–16]. Our ongoing multicenter randomized controlled ACTIVE trial (Autologous chondrocyte

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transplantation/implantation vs existing treatments) recruited 390 patients and will compare efficacy and cost–effectiveness data for ACI and the 'best alternative' treatment over a 10-year follow-up period. The first planned analysis of intermediate data at 5-year follow-up is currently taking place.

Others have favored the use of autologous bone marrow derived mesenchymal stromal/stem cells (BM-MSCs) in a similar procedure to ACI, where they were transplanted into cartilage defects beneath a periosteal flap [17–19]. One study compared the outcome of ACI with BM-MSC treatments for cartilage injury in an observational study of 36 gender and age matched patients in each group [20]. They found no evidence for a difference between the groups in terms of functional outcome, but noted that men fared better than women. Drawing inferences from small observational studies has many disadvantages, and the authors recommended a randomized trial to compare the two types of cell therapy.

The principal outcome in all clinical studies was a validated knee score that measures pain and knee function. The Lysholm knee scale is a long-established commonly used score. We have validated an improved weighting of this scale for patients with cartilage defects [21]. Secondary measures have mostly been diagnostic arthroscopy and histology of a biopsy of the repair tissue at 1 or 2 years. The biopsy has the disadvantage of only sampling a small area of each treated region [22,23]. In contrast, arthroscopic assessment can provide a recorded image of the macroscopic appearance of the whole surface of the defect and in particular an assessment of the lateral integration, whereas MRI provides an assessment of the whole joint including the underlying bone [22,23].

Data described in several laboratory-based studies indicate that a combination of culture-expanded chondrocytes and BM-MSCs produce a better quality of cartilage matrix than BM-MSCs alone [24–26]. Further, articular chondrocytes have been shown to initiate and drive the osteo-chondrogenic differentiation of BM-MSCs in *in vitro* coculture systems [27–29]. In addition, BM-MSCs have been suggested an appropriate choice of cell for treating larger, osteoarthritic defects [30] as they have both osteogenic and chondrogenic differentiation capability [31–33] and have shown encouraging results in generating both tissue types in large animal models of osteochondral injury [34]. There is general agreement that BM-MSCs can contribute to the niche environment [35,36] and influence other cells' behavior via their paracrine effect, in addition to having immunomodulatory effects [37]. One group is using chondrocytes immediately extracted from cartilage and combined with allogenic cultured BM-MSC in the clinic; this study is entitled 'Instant Allogeneic MSC Product accompanying Autologous Chondron Transplantation' or 'IMPACT'. Although, the number of chondrocytes is necessarily low, the early reports of using this technique in patients are encouraging [38]. The IMPACT study differs from ASCOT in both the origin and application of the cell populations used. In ASCOT, both cell populations are autologous, whereas in the IMPACT trial the BM-MSCs are allogeneic and derived from young individuals. Additionally, cell populations in ASCOT are culture-expanded, whereas in the IMPACT trial, the chondrocytes were obtained at the time of surgery and isolated as 'chondrons' (i.e., with their capsule of matrix surrounding them).

Our approach is to build on the demonstrated efficacy and safety of autologous culture-expanded cells. The question that needs addressing is which type of cell, autologous BM-MSCs or chondrocytes or indeed, their combination, gives the most efficacious and safest treatment of cartilage patients, particularly those with more severe degenerative lesions. These are lesions that expose or extend into subchondral bone (International Cartilage Repair Society; ICRS classification III or IV) and are possibly indicative of early osteoarthritis [39]. We currently treat these patients with ACI, but the above evidence suggests that autologous BM-MSCs or a combination of autologous chondrocytes with autologous BM-MSCs might be better. To date there has been no reported randomized clinical trial which has investigated the combined use clinically of culture expanded populations or to have directly compared ACI and BM-MSC applications for the treatment of chondral or osteochondral injury. The objective of the randomized controlled Phase II clinical trial presented here is to test the efficacy and safety of culture expanded bone marrow derived autologous stromal/stem cells, chondrocytes or a combined population of both of these for treating chondral or osteochondral defects in the knee.

Study design

Autologous stem cells, chondrocytes or the two (ASCOT) is a single-center, single-blinded, randomized controlled trial designed to determine which of three cell therapy strategies is best for treating chondral or osteochondral defects of the knee. The two specific research hypotheses addressed by the trial are: using autologous BM-MSCs to repair chondral or osteochondral defects in the knee gives a different functional outcome from using autologous chondrocytes. Using a combination of autologous BM-MSCs and autologous chondrocytes to repair chondral or osteochondral cartilage defects in the knee gives a different outcome from using either cell

type alone. Subjects will be randomly allocated, at a ratio of 1:1:1, to autologous BM-MSCs, autologous chondrocytes or a combination of the two. The target is to recruit 114 patients (38 per trial arm).

Patients & methods

Participants & outcomes

Subjects will be enrolled at a single specialist orthopedic hospital in the UK; the inclusion and exclusion criteria for the study are summarized in Box 1. Patients aged between 18 and 80 years of age with a symptomatic defect of the knee that exposes or extends into the subchondral bone (ICRS classification III or IV) will be eligible to take part in this study. Treatment with ACI must be appropriate for the patient, and prior surgical treatment to the defect may or may not have been performed. Patients will be excluded from the study if they are unable to provide written informed consent to participate, do not adequately understand verbal explanations or written information given in English, have a low probability of compliance with rehabilitation, a defect of greater than 20 cm^2, are shown to be positive for serology tests required by the cell provider, are pregnant or lactating. Patients will also be excluded if they show contraindications to autologous cell therapy, for example, inflammatory arthritis, current malignant tumor, therapy with steroids or methotrexate, opioid or anticoagulant medication that cannot be stopped prior to stage I surgery, bleeding tendency or known anaphylaxis to any product used in the chondrocyte preparation.

Interventions

Participants are assigned to one of three treatment arms: autologous stem cell implantation (ASI); ACI; or ASCI. All participants undergo an arthroscopy at stage I to debride the defect. Participants allocated to the ASI and ASCI treatment arms also undergo an iliac crest bone marrow biopsy; the aim of this is to harvest two 10 ml samples of bone marrow aspirate (obtained via two separate entry points) into a sterile tube containing heparin from which BM-MSCs are isolated. Participants allocated to the ACI or ASCI arms of the study have a hyaline cartilage biopsy taken during this arthroscopy; generally the central trochlea is used and the aim is to obtain 200 mg from which chondrocytes are isolated. Cell expansion takes place in a medicines and healthcare products Regulatory Agency licensed laboratory (John Charnley Laboratory, Cert No: UK MIA [IMP] 21276) based at the RJAH Orthopedic Hospital. The principal requirements for a satisfactory expansion are a cell count of 1–20 million cells with a viability of at least 90% (as assessed by trypan blue exclusion) and no reported infection. One flask of cells from each culture is used for cell characterization studies as described below. The remaining cells obtained as a result of the expansion process are resuspended on the day of surgery in the prescribed volume of excipient autologous serum and packaged for delivery to the operating theaters. Implantation of both the BM-MSC and chondrocyte products to repair the chondral/osteochondral defects is carried out during arthrotomy under sterile conditions and requires both preparation of the defect bed and attachment of a membrane to secure

Box 1. The inclusion and exclusion criteria for the ASCOT trial.

ASCOT inclusion criteria

- A symptomatic defect of the knee that exposes, or extends to or into, the subchondral bone (International Cartilage Repair Society; ICRS classification III or IV)
- The patient is aged between 18 and 80 years at the time of surgery
- Treatment with autologous chondrocyte implantation must be appropriate for the patient
- Surgical treatment (eg., debridement, abrasion, drilling, microfracture) may have been performed on the same defect at least 6 months previously and failed to relieve symptoms
- The patient is able to provide written informed consent to participate in the trial

ASCOT exclusion criteria

- Inability to understand verbal explanations or written information (in English) or having special communication needs
- Likely to show contraindications to autologous cell therapy: inflammatory arthritis, previous or current malignant tumor, therapy with steroids or methotrexate, opioid medication that cannot be stopped prior to stage I (harvest) surgery or anticoagulant medication use that cannot be stopped prior to surgery, bleeding tendency or known anaphylaxis to any product used in chondrocyte preparation
- Low probability of compliance with physiotherapy or follow-up, including a major life-threatening condition, as assessed by the research team
- A defect of greater than 20 cm^2 in total area
- The patient is shown to be positive for serology tests required by the cell provider. This includes HIV, hepatitis B and C, syphilis and human T-cell lymphotrophic virus I & II
- Pregnancy or lactation

the implanted cells. A commercially available porcine collagen membrane, Chondro-Gide® (Geistlich, Wolhusen, Switzerland), will be used and is both stitched and glued (with fibrin) into place. Chondro-Gide is the patch of choice in our center as we have previously shown that significantly better histological repair tissue was formed with regards to tissue and cell morphology, surface architecture and collagen type II production, when ACI was carried out with a Chondro-Gide patch rather than an autologous periosteal patch [8]. However, if the patient has any reason to avoid this (e.g., religious grounds) or if the membrane is not available, then an autologous periosteal patch will be used.

For single cell type therapies (ASI and ACI), one syringe containing autologous BM-MSCs or chondrocytes is implanted, containing between 1 and 20 million cells, depending on the cell growth kinetics for each individual patient. For ASCI, two syringes are supplied, one containing BM-MSCs the other autologous chondrocytes, each containing between 1 and 20 million cells, again depending on the growth kinetics of the individual patient.

Clinical outcomes

The primary outcome measure of the ASCOT trial is the patient-reported knee function (Lysholm score) at 15 months post-treatment, taking into account the preoperative score as a covariate [3]. The Lysholm score is a validated, long-established eight-item questionnaire of knee function. The scale was originally designed to assess patients following knee ligament surgery with a special emphasis on symptoms of giving way, and this is reflected in the weighted scoring system. However, we have validated an improved weighting of the Lysholm knee score for patients with cartilage defects [21] and this is the score that is used in this study.

The secondary outcome measures are to obtain a complete picture of benefit of one regenerative cell therapy over another, through a range of assessments, including histology, imaging and further measures of function and pain. The following parameters will be recorded: Incidence of adverse events; Quality of the repair tissue between 12 and 14 months after cell implantation will be assessed macroscopically (using both the Oswestry Arthroscopy Score and the ICRS arthroscopic cartilage repair assessment [40]) and microscopically (via needle biopsy and histological assessment using the OsScore and the ICRS II histology scoring system [41]). In addition, MRI and computerized tomography (CT) scans will also be obtained and scored at baseline and between 12 and 14 months after cell implantation. Images are analyzed using the Magnetic resonance imaging (MRI) Observation of Cartilage Repair Tissue Score [42] and Whole Organ MRI Score (WORMS [43]); Health-related quality of life, pain, knee function, activity levels and general mood of the patient is assessed using the Veterans RAND 12-item Health Survey, the Intermittent and Constant Osteoarthritis Pain score, Knee injury and Osteoarthritis Outcome Score (KOOS) physical function short form (KOOS-PS), the modified Lysholm knee score, Human Activity Profile and the International Positive and Negative Affect Schedule Short Form scores. Each of these is collected at outpatient appointments on two separate occasions prior to ACI, and 2 months (±2 weeks), 12 months (±4 weeks) and 15 months (± 4 weeks) postsurgery. Annual postal follow-ups will continue for up to 20 years postoperatively; Number of years free from further surgery; Patterns of rehabilitation and compliance to physiotherapy schedules are collected using participant diaries throughout the rehabilitation program (up to 15 months postoperatively); Unit costs per treatment are also recorded in order to perform a final cost-utility analysis.

Exploratory measures aim to find correlations within this study which could help to predict treatment success. For example, we aim to determine if there is a dose–response relationship and if so, whether there is a correlation between the dose (i.e., number of cells) and the area or volume of the defect being treated.

Cell characterization & biomarker studies

We are maximizing the potential to learn as much about the biology of treatment success and failure by studying characteristics of the cells being implanted and tissues collected throughout the procedure. For example, fat pad, synovial tissue and fluid and blood are being collected and analyzed for the presence of biomarkers which could provide additional information on the health of the joint prior to cell implantation or predict patients who will respond well to cell therapy [44]. In addition, we aim to gain a better understanding of the healing response in the joint and how this differs between individuals by studying biopsy tissue from chondrocyte harvest sites, where the initial 'injury' was created under controlled conditions. Finally, we are assessing the characteristics of both cell types for predictors of potency; such analyses will include cell growth kinetics and morphological analyses as well as RNA sequencing and immunoprofiling by flow cytometry, using similar profile analyses to those we have previously published [45]. The actual markers analyzed for both techniques are constantly being updated as the study progresses to keep up to date with current literature and available technologies,

but regardless of this, all cells are assessed for their adherence to the International Society for Cellular Therapy profile for MSCs (i.e., positive for CD73, CD90 and CD105 and negative for CD14, CD34, CD45, CD19 and HLA-DR).

Sample size

A sample size of 38 patients per trial arm (i.e., 114 patients in total) is sufficient to test the two hypotheses with 80% power at p = 0.05, based on detecting the minimal clinically important difference in Lysholm knee scores of 13 points between the groups: an effect size of 0.7-times the standard deviation of 18.5 in baseline Lysholm scores [3,46,47]. The chosen sample size allows a 10% loss to follow-up for various reasons in each arm, a number based on our multicenter trial of ACI versus alternative treatments (ACTIVE).

Assignment of interventions

Treatment is allocated by stratified randomization using the minimization method [48]. Stratification is based on the following known predictors of functional outcome: preoperative knee score (Lysholm), defect location (femoral or trochlear defect vs others), gender, age and the occurrence of previous marrow stimulation techniques. Randomization is carried out using dedicated computer software (StratOs®, Orthopaedic Institute Ltd, Oswestry, UK) and should not take place more than 3 months prior to the anticipated treatment date as delaying randomization will minimize pretreatment drop-out after randomization. Participants are blinded to the treatment they have been allocated. In order to maintain blinding to the treatment group, those participants allocated to the ACI arm are given a 5-mm incision in the area where a bone marrow biopsy would have taken place if they had been allocated to either ASI or ASCI. Histological and imaging scoring for cartilage repair is performed by blinded assessors.

Statistical analysis

Our primary efficacy outcome is the Lysholm knee score at 15 months. This score will be used to test the two research hypotheses, namely: using autologous BM-MSCs gives a different functional outcome from using autologous chondrocytes; and using a combination of BM-MSCs and chondrocytes gives a different outcome from using either cell type alone. Statistical analysis of the Lysholm score will be performed as analysis of covariance (ANCOVA) with the treatment group as an independent factor, adjusting for baseline score, age, gender, location of defect and the occurrence of a previous surgical treatment. Treatment groups will be tested at the two-sided 5% significance level. The analysis will be performed as two separate contrast analyses, testing each of the two hypotheses separately. ANCOVA is the preferred method of analyzing randomized trials with baseline and post-treatment measures, with no demonstrated disadvantage compared with nonparametric methods [49]. The results from the ANCOVA analysis will be used to obtain estimates of the size of the differences between the groups as specified by the contrasts, their 95% confidence intervals and their p-values. The data will be analyzed on an 'intention to treat' basis as well as according to actual treatment ('per protocol'). No subgroup analyses are planned other than the pre-specified subgroups defined by the stratification variables, namely preoperative functional knee score, age, gender, location of defect (femoral or trochlea vs others) and previous cartilage surgery. MRI, histology, arthroscopy and other outcome scores will be compared using one-way analysis of variance, if available using preoperative values as covariates. For the continuous outcome measures that are collected repeatedly over time (such as the Lysholm knee score), repeated measures analyses will be performed on the change from the baseline score, using standard multilevel modeling techniques [3]. Such analyses have the advantages of being able to combine results from different time-points to maximize power, and also to investigate the precise form of any benefit (whether, for example, any treatment benefit, should one exist, increases or decreases with time). Multilevel modeling also allows for suitably stratified analyses to be performed. Sensitivity analysis will be performed to determine whether any benefit of treatment is short-lived or persists for the duration of the study. Any re-intervention will be recorded and taken into account in the analysis. A health economic analysis (incorporating the Veterans RAND 12-item Health Survey) will estimate the cost per quality adjusted life year and number of years free from further surgery.

Ethical conduct & regulatory compliance

The study group received a positive ethical opinion from NRES Committee West Midlands – Coventry and Warwickshire on 29 July 2011 (11/WM/0175) and a Notice of Acceptance for a Clinical trial Authorization on 5 April 2013 (EudraCT number: 2010–022072–31). The John Charnley Laboratory is a Good Manufacturing Practice approved facility with a manufacturers authorization for investigational medicinal products MIA (IMP). Interim analyses of major endpoints and safety data will be supplied on a regular basis to an independent data monitoring and ethics committee who will make recommendations to

the trial steering committee in the event of any safety or ethical concerns. The study will be conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki. Written Informed consent will be sought from all participants prior to any study related procedures.

Discussion

There are several published and ongoing clinical trials comparing cell-based therapies for the treatment of cartilage defects to other surgical techniques (such as microfracture) [10,16]. However, to-date, no randomized trials have been performed determining whether there is a difference in functional knee score post-treatment with chondrocytes versus BM-MSCs (the first research hypothesis of the present study). In addition, the third arm, which combines *in vitro* culture expanded autologous chondrocytes and BM-MSCs, represents a first-in-man clinical intervention using a combination of these cell populations for the treatment of cartilage injury (the second research hypothesis). Furthermore, we have purposefully proposed a wide range of number of cells (i.e., doses) due to the fact that although a wide range of cell numbers are used in ACI and equivalent procedures, there is no clear scientific evidence for the optimal dose [50]. Hence, we included a range covering both that examined in the reviewed literature (0.5 to >10 million cells/cm^2 of defect area) [50] and the maximum range used in our center, hoping that the results from this trial could give an indication as to whether more or less cells produced a better clinical outcome or quality of repair tissue.

This Phase II randomized controlled trial has been carefully designed by a team of scientists and healthcare professionals in order to compare the safety and efficacy of the three autologous cell populations for the treatment of cartilage injury in the knee. The inclusion criteria for patients were chosen to reflect patients who typically present in clinics in a secondary or tertiary referral orthopedic center. To this effect they may, by some definitions, be suffering with early osteoarthritis (which we consider as a spectrum of disease [39] and the age range is wider than in many other trials in this area [51].

Short- and long-term outcome measures will include Lysholm score at 15 months post-treatment, the occurrence of adverse events, analysis of repair tissues, as well as, quality of life and cost-utility assessments. In addition, we aim to maximize the opportunity for reverse translational research, so that we can begin to understand more fully the biology of treatment success or failure and so that valuable long-term clinical information can be gathered throughout and in the final analysis.

Conclusion & future perspective

Results from this trial should enable us to understand more about the biology of cell-based treatment success and failure by analyzing in detail the characteristics of the transplanted cell populations and the joint tissues throughout the treatment process. It is hoped that the comprehensive characterization of the implanted cell population that will accompany the clinical trial will lead to a 'signature' of markers which can be used to define cell potency, a feature which is badly needed for the pharmaceutical label which now applies to Advanced Therapy Medicinal Product .

The establishment of reliable potency indicators, such as we hope this trial will enable, should be of great use to the cell therapy field and its governance. For example, they should facilitate the development of additional cell therapies, whether allogeneic or autologous, more rapidly by focussing on the most potent population.

Financial & competing interests disclosure

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Clinical trial registration number

ISRCTN registry - ISRCTN98997175

Executive summary

- There is a need for large randomized trials with a low-risk of bias and long follow-up to indicate the best treatment for cartilage defects.
- Advancements to autologous chondrocyte implantation (ACI) include using autologous bone marrow derived mesenchymal stromal/stem cells (BM-MSCs).
- The objective of this Phase II randomized controlled trial is to compare the safety and efficacy of three autologous cell populations for the treatment of cartilage injury in the knee.

Study design

- Autologous stem cells, chondrocytes, or the two is a single-center, single-blinded, randomized controlled trial designed to determine which of three cell therapy strategies is best for treating chondral or osteochondral defects of the knee.
- Hypothesis 1: using autologous BM-MSCs to repair chondral or osteochondral defects in the knee gives a different functional outcome from using autologous chondrocytes.
- Hypothesis 2: using a combination of autologous BM-MSCs and autologous chondrocytes to repair chondral or osteochondral cartilage defects in the knee gives a different outcome from using either cell type alone.
- Subjects will be randomly allocated, at a ratio of 1:1:1, to autologous BM-MSCs, autologous chondrocytes or a combination of the two. Recruitment target is 114 patients (38 per trial arm).
- Main inclusion criteria are patients aged 18–80 years old, with a symptomatic International Cartilage Repair Society (ICRS) grade III or IV defect, for which ACI is deemed appropriate.
- Main exclusion criteria are a defect of over 20cm^2, positive serology tests, pregnancy/lactation, inflammatory arthritis, current malignancy, therapy with steroids or methotrexate, opioid or anticoagulant medication that cannot be stopped prior to stage surgery, bleeding tendency or known anaphylaxis to any products used.
- Treatment is allocated by stratified randomization using the minimization method no more than three months prior to the anticipated treatment date (participants are blinded to treatment allocation).

Clinical outcomes

- The primary outcome measure is the patient-reported knee function (Lysholm score) at 15 months post-treatment.
- The secondary outcome measures include histology, imaging and further measures of function and pain.

Cell characterization & biomarker studies

- To understand the biology of treatment success/failure, we will study the characteristics of the cells being implanted and tissues collected throughout the procedure (e.g., fat pad, synovial tissue/fluid and blood).
- Presence/absence of putative biomarkers will be analyzed to try and predict patient responses to cell therapy.
- Cell characteristics of the implanted population include cell growth kinetics and morphological analyses as well as RNA sequencing and immunoprofiling by flow cytometry.

Discussion

- To-date, no randomized trials have been performed determining whether there is a difference in functional knee score post-treatment with chondrocytes versus BM-MSCs.
- The combination of *in vitro* culture expanded autologous chondrocytes and BM-MSCs, represents a 'first-in-man' clinical intervention for the treatment of cartilage injury.
- This cell therapy trial has been carefully designed so that an abundance of diverse clinical and scientific data is gathered throughout. In the final analysis, we hope to uncover biological mechanisms of failure and success so that future trials and treatments can be better designed in their next iteration.

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