Randomized, multi-center phase II clinical trial for the regeneration of cartilage lesions in the knee using nasal chondrocyte-based tissue (N-TEC) or nasal chondrocyte-based cell (N-CAM)-therapies

Statistical analyses plan

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Contents

Inhalt

1. Introduction

1.1. Background and rationale

Cartilage tissue has a limited capacity for self-repair due to its avascular and aneural nature. Articular cartilage injuries that are not properly treated are associated with pain and disability and are known to double the incidence of degenerative joint disorders in the elderly.
Cartilage repair treatments have the potential not only to relieve pain and improve the quality of life for younger patients, but also to slow down or eliminate the need for joint replacement in the elderly. However, current therapeutic options such as arthroscopic debridement, micro fracture, autologous osteochondral grafting and use of allografts suffer from major drawbacks, such as defect-size limitations, long and complex rehabilitation times, donor-site morbidity and limited availability of graft material. Even the more advanced cell-based therapies, in addition to involving technically challenging operations associated with donor-site morbidity and highly variable outcome, provide no fully satisfactory treatment, especially for elderly patients. Moreover, these therapies, comprising mainly the implantation of cells, lack the complex biological and mechanical signals which can be delivered via a more developed (mature) graft. In this trial we will introduce two innovations: 1) the use of autologous nasal chondrocytes (NC) as a cell source superior to articularchondrocytes (AC), thus exploiting the already proven higher and more reproducible properties of NCs regarding proliferation and differentiation capacity, which are less dependent on the age of the donor as compared to AC and 2) the delivery of a mature graft as opposed to an immature graft.

1.2. Objectives

The goal of this trial is to compare the clinical efficacy of a mature graft (i.e., nasal chondrocyte tissue engineered cartilage, N-TEC) with that of an immature graft (i.e., nasal chondrocyte cell activated matrix, N-CAM) for the treatment of cartilage lesions in the knee.
This proposed phase II trial will evaluate whether implantation of a mature cartilage graft improves the clinical efficacy, leading to an increase of at least 10 points in the main primary outcome (KOOS (mean of subscores)). In addition, the integration of the grafts with the surrounding tissues as well as the quality of the repair tissue will be assessed.

Retrospectively data will be analyzed to possibly identify the most suitable treatment (mature or immature graft) in relation to the onset of symptoms (acute vs chronic cartilage lesions). This will require enrolling a total of 108 patients in a multicenter, prospective study involving 5 clinical centers.

2. Study methods

2.1. Trial design

The study will be designed as an unblinded multicenter, randomized phase II study for the comparison of a therapy with a mature versus a therapy with an immature graft.

- mature graft (N-TEC):
  1) Nasal cartilage biopsy, performed by Ear-Nose-Throat (ENT)/Plastic surgeon
  2) Expansion of cells (2 weeks), seeding onto the collagen scaffold (Chondro-Gide®) , followed by 2 weeks of in vitro culture
  3) Implantation of the cartilage graft into the knee joint via arthrotomy/mini-arthrotomy, debridement down to subchondral lamella and stable cartilage rim, fixation by sutures (e.g. Monocryl 5-0), no drainage

- immature graft (N-CAM):
  1) Nasal cartilage biopsy, performed by ENT/Plastic surgeon Expansion of cells (2 weeks) and seeding into the collagen scaffold (Chondro-Gide®), followed by 2 days of in vitro culture
  2) Implantation of the cell-seeded scaffold into the knee joint via arthrotomy/mini-arthrotomy, debridement down to subchondral lamella and stable cartilage rim, fixation by sutures (e.g. Monocryl 5-0), no drainage

This phase II study will be performed as a prospective, randomized and unblinded study. Patients will be enrolled at 5 clinical centers in Basel (CH), Freiburg (D), Zagreb (HR), Milan (I) and Wuerzburg (D). The multicentre study, enrolling a total of 108 patients, is planned for the duration of 4 years including follow-up times of two years for each patient. The study will start with the signature of the informed consent by the first patient and end with the two-year follow up of the last patient. Patients may be asked to participate in a further follow-up up to 5 years based on questionnaires on a voluntary basis.

2.2. Randomisation

Upon enrolment in the study, patients will be given a patient ID and entered into the database myclinicaldata (Medacta) by the PI or designated persons of the respective center. Patients will be randomized to the N-TEC (mature tissue) or N-CAM (immature tissue) group.

A permuted-block (2, 4 and 6 size) randomization method will be performed. A randomization list will be associated to each center, guaranteeing an equal distribution of the two groups in all the centers (center stratification). The list is generated by Sealed Envelope online software application (https://www.sealedenvelope.com/).

After the screening phase, patients will be allocated to the randomization group through the electronic data capture (EDC) system, which automatically links the patient to the group correspondent to the free available slot of the randomization list, previously uploaded to the system.

The generation of the sequence from sealed envelope website and its upload into the EDC system, will be managed by the owner of platform, who is not involved in clinical center activities (patient selection, clinical visits, radiological evaluation). The clinical centers personal does not have access to the randomization sequences.

2.3. Sample size

The primary endpoint is the Knee injury and Osteoarthritis Outcome Score (KOOS) measured 24 months after surgery. The score ranges from 0–100; the higher the score, the better the outcome. The null hypothesis is that there is no difference in the primary endpoint between N-TEC and N-CAM ($H_0 : KOOS_{N-TEC} - KOOS_{N-CAM} = 0$). The alternative hypothesis is that N-TEC and N-CAM differ significantly in terms of the primary endpoint ($H_A : KOOS_{N-TEC} - KOOS_{N-CAM} = \delta \neq 0$). An absolute difference (δ) of 10 score points or more is considered clinically relevant. These hypotheses will

determine whether the primary objective, namely that therapy with a mature graft will improve the clinical efficacy for the patient, is met.

Sample size was estimated to be such that it would be able to show the superiority of N-TEC to N-CAM regarding the primary endpoint. Assumptions for sample size calculation were based on a study, in which an increase in a mean overall KOOS score after 18 months of 18 points to a final value of 74.73 with a standard deviation of 17.01 was reported[1]. We have assumed that the effect size after 24 months will be at least as big. Sample size was calculated using a resampling method. Each sample size (n_i=1, ... 160) was evaluated by sampling R = 999 times, n_i/2 KOOS scores from a normal distribution with $\mu = 75$ and $\sigma = 17$ for the N-CAM group, and n_i/2 KOOS scores from a normal distribution with $\mu = 75 + \theta$ and $\sigma = 17$ for the N-TEC group. The size of θ was varied between 5 and 15. Values that exceeded 100 were set to 100. N-CAM and N-TEC were tested for a difference in KOOS score using a two-sided t-test. Superiority of N-TEC to N-CAM was declared when the test showed a significant result. Sample size was set to ensure at least 80 % power ($1 - \beta = 0.8$), at a significance level of $\alpha = 5$ %. For this study, 108 patients should be recruited to ensure 97 evaluable patients at a power of 80%, considering an overall drop-out rate of 10%.

2.4. Framework

All endpoints will be analysed for the superiority of N-TEC compared to N-CAM.

2.5. Stratification

Unless explicitly mentioned, analyses will not be stratified.

2.6. Statistical interim analyses and stopping guidance

No interim analysis planned.

3. Data management

All data will be entered in case report forms (CRFs) at the local site. Data collected during the study will be stored in an electronic database “MyClinicalData” provided by the sponsor, generated and administered by Medacta International SA (Switzerland).

3.1. Data export

The entered data will be exported from the trial database to a statistical software package.

3.2. Data validation

Data submitted to the MyClinicalData is systematically verified for its consistency and if an error occurs no insertion will be made. Further data validation and cleaning will be conducted after completion of data entry but before database lock.

4. Statistical principles

4.1. General

All recorded and derived variables will be presented by treatment group (and visits, if appropriate) using descriptive summary tables. Continuous variables will be summarised by mean and standard deviation, or median and quartiles. Categorical variables will be summarised with absolute and relative frequencies. In all summaries, the treatment groups will be displayed in the following order: N-CAM, N-TEC. For all parameters, baseline is defined as the last available pre-treatment value (i.e. the last non-missing value available before randomisation).

4.2. Confidence intervals and p-values

The statistical testing will be two-sided with a significance level of 5%. All tests will be accompanied by an effect measure (N-TEC vs N-CAM) with a 95% confidence interval (95% CI).

4.3. Adherence and protocol deviations

Major protocol deviations are:

• Violation of inclusion or exclusion criteria

• no outcome assessment at 12 or 24 months postop, respectively.

These protocol deviations will be summarised by treatment group using absolute and relative frequencies.

Cross-overs to the other treatment arm are not possible in the setting of this study. Since the treatment itself (implantation) will be done by the surgeons and is a one-time application there is no risk of non-compliance. Surgical procedures will be harmonized by training and written SOPs. Violation of inclusion or exclusion criteria such as further lesions in the knee might become apparent only at the time of surgery, so after randomization. Patients will not be followed-up in that case. In case of missed follow-up appointments, the surgeons will contact the patients and reschedule the visit. Questionnaires will be filled in by the patient directly at the time of follow-up using an eCRF. Adherence to recommendations regarding sport activities cannot be monitored; performance at physiotherapy will be asked of the patient, but not monitored.

4.4. Analysis populations

The population for analysis will comprise all patients as randomized, hence the full analysis set (FAS). This corresponds to an analysis according to the intention-to-treat (ITT) principle. The per-protocol set (PPS) consists of all patients who received the treatment they had been assigned to and will be used for efficacy analyses. The safety population consists of all patients in the FAS and it will be used for the analysis of safety outcomes.

5. Trial Population

5.1. Screening data

Screening data is not collected in the eCRF but the number of screened patients will be calculated from screening logs.

5.2. Eligibility

Inclusion and exclusion criteria are defined in the study protocol. Data about reason for non-inclusion is not documented in the eCRF and will not be available.

5.3. Patient flow

A CONSORT patient flow diagram will be drawn following the CONSORT 2010 standards.[2]

5.4. Withdrawal/follow-up

All withdrawals and losses to follow-up will be listed with time points and reasons (if available).

5.5. Baseline patient characteristics

Evaluations of the baseline characteristics will be based on the PPS. They will be presented in a descriptive summary table by treatment group. Continuous variables will be shown as mean and

standard deviation or median and quartiles, categorical variables as absolute and relative frequencies. No statistical comparisons of patient characteristics at baseline will be performed.

6. Analysis based on the Study Protocol

6.1. Outcome definitions

Primary endpoint:

- KOOS score: The primary endpoint is the overall KOOS subjective score at the 24-month visit. The difference in the KOOS-score will be compared between the two groups to assess efficacy of N-TEC.

Secondary endpoints:

- MOCART and 3D MOCART Scores: Assessment of stability and integration
- dGEMRIC evaluation: Assessment of quality of the repair tissue
- A further questionnaire (EQ-5d) at 12 and 24 month and an additional time point (12 month) for KOOS will allow the more detailed analysis of the clinical development of the patient's recovery and elucidate changes in the perceived quality of life before and after treatment.
- Additional secondary endpoint is the number of treatment failure at 24 months. The difference will be compared between the two groups.

Other outcomes:

- Retrospective analysis of primary and secondary endpoint data with regard to the onset of symptoms to identify a possible selection of treatment of acute (onset < 1 years) or chronic (onset >1 years) lesions.
- Subgroup analysis to compare the outcome with regard to the localization of the defect (patella vs. femoral condyle/trochlea)

Safety:

- Any AE and SAEs will be recorded regarding event descriptions, onset, resolution dates and relationship to the IMP. All SADR or SUSAR will be reported to Basel as leading center and the respective authorities.

6.2. Outcome derivation

Primary outcome is the mean of all 5 sub-scores (symptoms, pain, activity of daily living, sport and quality of life) assessed at 24 months postoperatively.

Secondary outcomes MOCART and 3D MOCART scores at 3, 12, 24 months after surgery will be calculated from MRIs carried out at follow-up visits. The relative delta R1 will be evaluated by dGEMRIC and recorded at 3, 12, 24 months follow-up visits and referenced to the native cartilage of the treated knee based on MRI.

6.3. Analysis methods

6.3.1 Primary analysis

The primary analysis will be done on the PPS. Missing data will be handled according to section 6.4. Primary objective is to demonstrate in patients younger than 65 years with articular knee

cartilage injuries the superiority of N-TEC to N-CAM, assessing by KOOS measured 24 months after surgery.

The primary endpoint will be analyzed with a linear mixed effects model, including therapy (N-TEC vs. N-CAM; categorical variable) as single explanatory variable (fixed effect), and including study center as random effect variable to account for the multicenter study design.

As a sensitivity analysis, we will analyze the primary endpoint by adjusting for covariates and testing for interactions. The linear mixed-effects model will include therapy (N-TEC vs. N-CAM; categorical variable), onset of symptoms (stratified categorical: acute: <= 1 years; chronic: > 1 years) and the covariate baseline KOOS (continuous variable) as explanatory variables (fixed effects). In addition, the model will include the interaction between therapy and onset of symptoms. A significant interaction would indicate that the outcome of the therapy depends on how long the symptoms have been present. Further, study center will be included as a random effect variable in the model to account for the multicenter study design.

6.3.1.2. Analysis of secondary endpoints

The following secondary endpoints will be analyzed:

- KOOS score at 12 months after surgery
- relative GAG-content (rel. ΔR1) by delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) 3, 12 and 24 months after surgery
- MOCART score at 3, 12 and 24 months after surgery
- EQ-5d score at 12 and 24 months after surgery

All secondary endpoints will be analyzed with a linear mixed effects model as described for the sensitivity analysis of the primary endpoint (with the corresponding baseline scores as covariate). If necessary, endpoints will be transformed in order to fulfill assumption of a normal residual distribution, or, if no reasonable transformation can be found, generalized linear mixed-effects models will be applied.

6.4. Missing data

The number of non-missing observations will be presented for each endpoint. We will use multiple imputations in case primary outcome assessments are missing, based on baseline and outcome variables (see next paragraph). Since missing values in these variables are possible, chained equations will be used. Continuous variables will be imputed using linear regression and binary variables using logistic regression. We will construct and analyse 20 imputated data sets and combine results using Rubin's rules.[3]

Baseline patient characteristics and outcome variables (e.g. KOOS sub-scores) will be considered for multiple imputations. Variables with more than 50% missing values will not be used for the imputation model. Categorical variables with a frequency of 5% or less in one category will also be omitted. If two binary variables accord in more than 95% or less than 5% of patients, only one will be used.

6.5. Evaluation of safety parameters

Evaluation of safety parameters will be based on the safety population (FAS, section 4.4). They will be listed according to the treatment the patient actually received with the time points of onset. If many adverse events should be observed, we will compare frequency between treatment groups.

6.6. Statistical software

The statistical analysis will be performed by Surgical Outcome Research Center using the statistical software Stata 16.0 (StataCorp LLC, 4905 Lakeway Drive, College Station, Texas 77845).

7. Changes from the Study Protocol

7.1. Analysis set

Two randomized patients did not receive treatment and study outcome (KOOS score at 24 months after surgery) was not assessed in these patients. So conducting the analysis according to ITT was not possible. We hence analysed the PPS instead of the FAS.

7.2. Further secondary outcomes

As further secondary outcomes, we included all 5 KOOS sub-scores (symptoms, pain, activity of daily living, sport and quality of life) assessed at 12 and 24 months postoperatively. Indeed these sub-scores typically provide important information with respect a patients' benefit from either treatment.

7.3. Sensitivity analysis

We found that the distribution of KOOS at the time of outcome had the pattern of a ceiling effect in both treatment arms (Fig 1), even more pointed in the N-TEC group. Thus, the median (solid line) is a better summary measure than the mean (dashed line).

Figure 1. Distribution of primary outcome

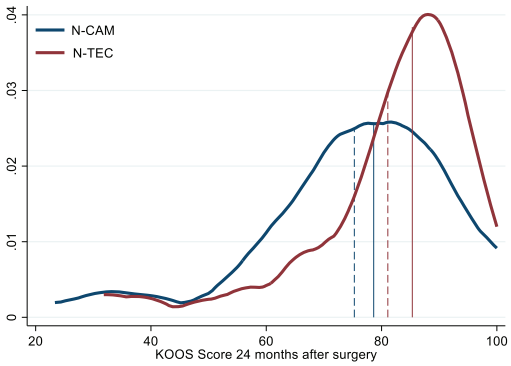

Figure 1. Distribution of primary outcome

Hence, we decided to use median regression models to derive treatment effect with standard errors (SE) estimated based on bootstrapping with 5000 repetitions. We chose bootstrapped SEs to avoid 95% CIs exceeding 100, since there are no data points over 100 to bootstrap from. We furthermore adjusted for baseline KOOS score to gain precision of the treatment effect, given that the outcome is derived from self-ratings and dropped center as random effect.

8. References

1. Saris DBF, Vanlauwe J, Victor 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. The American Journal of Sports Medicine. 2009;37(1_suppl):10-19. doi:10.1177/0363546509350694

2. http://www.consort-statement.org/consort-2010

3. Rubin DB. Multiple **imputation for nonresponse** in surveys. New York: John Wiley & Sons; 2004.

Dr. Brigitta Gahl, the 28th of November 2022