Interventional study with investigational medicinal product (IMP)

Clinical Study Protocol

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

Short Title: Nose to Knee II

Study Type:	Clinical trial phase II with Investigational Medicinal Product (IMP)
Study Categorization:	C
Study Registration:	ClinicalTrial.Gov Registration number: NCT02673905 EudraCT Registration number: **2015-005162-34**
Study Identifier:	n.a.
Sponsor, Sponsor-Investigator or Principal Investigator:	Prof. Dr. med. Marcel Jakob Department of Traumatology, University Hospital Basel Spitalstrasse 21, CH-4031 Basel, Switzerland email: Marcel.Jakob@usb.ch Tel.: +41 61 328 72 40
Investigational Product:	N-TEC (nasal chondrocyte tissue engineered cartilage) N-CAM (nasal chondrocyte cell activated matrix)
Protocol Version and Date:	Version number 07, 10.04.2018

Signature Page(s)

Study number ClinicalTrial.Gov Registration number: NCT02673905

EudraCT Registration number: **2015-005162-34**

Study Title 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-therapies (N-CAM)

The following Investigators have approved the protocol version 07 (dated 10.04.2018), and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm if applicable and the local legally applicable requirements.

Sponsor-Investigator / Principal Investigator:

Prof. Dr. Marcel Jakob

Basel 13.4.18 _____

Place/Date Signature

Principal Investigator / Study coordinator:

Dr. Marcus Mumme

Basel 13.4.18 _____

Place/Date Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site	Medical Center - University of Freiburg, Germany Department of Orthopedics and Trauma Surgery, Hugstetter Strasse 49, 79106 Freiburg, Germany
Principal investigator	Dr. Tayfun Yilmaz

Freiburg 12.04.2018

Place/Date

Signature

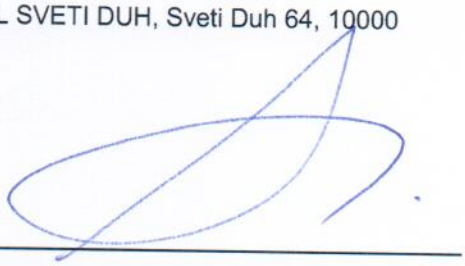
Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site	UNIVERSITY HOSPITAL SVETI DUH, Sveti Duh 64, 10000 Zagreb, Croatia)
Principal investigator	Prof. Dr. Alan Ivkovic

ZAGREB, 12.4.2018.

Place/Date



Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site	Istituto Ortopedico Galeazzi (IOG), Via Galeazzi 4, 20161 Milano, Italy
Principal investigator	Prof. Dr. Giuseppe Peretti

11.04.2018
Place/Date

Signature

Local Principal Investigator at study site:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site	Orthopädische Klinik König-Ludwig-Haus Lehrstuhl für Orthopädie der Universität Würzburg Brettreichstrasse 11 97074 Würzburg Germany
Principal investigator	Dr. Lothar Seefried

Würzburg, 11.04.18

Place/Date

Signature

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STUDY SYNOPSIS

Sponsor / Sponsor-Investigator	University Hospital Basel / Prof. Dr. med. Marcel Jakob
Study Title:	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
Short Title / Study ID:	Nose to Knee II
Protocol Version and Date:	Version 07, 10.04.2018
Trial registration:	EudraCT Registration-Number: **2015-005162-34** . Date: 12.11.15. ClinicalTrials.Gov Registration-Number: NCT02673905. Date: 1.2.2016
Study category and Rationale	This study is an investigator initiated, interventional phase II trial with two IMPs considered “Advanced Therapy Medicinal Product (ATMP)”, intended to evaluate the efficacy and compare different maturation stages of a tissue engineered product. Since the IMPs have no market authorization in any country this trial is considered category C.
Clinical Phase:	Clinical trial phase II
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 articular chondrocytes (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.

Objective(s):	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 actived 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.
Outcome(s):	Primary endpoint: • KOOS score: The primary endpoint is the KOOS subjective score at the 24-month visit. The difference in the KOOS-score will be compared between the two groups (Comparison of Efficacy of treatment) Secondary endpoints: • MOCART and 3D MOCART Scores (MRI): The MRI will be performed at 3, 12, 24 months follow-up visits and MOCART and 3D MOCART scores calculated. (Assessment of stability and integration) • dGEMRIC evaluation (MRI): 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. (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.

Study 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 2) Expansion of cells (2 weeks) and seeding into the collagen scaffold (Chondro-Gide ®), followed by 2 days of *in vitro* culture 3) 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 multi-center 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.

Inclusion / Exclusion criteria:	Patients between 18 - 65 years old with symptomatic full-thickness cartilage lesions on the femoral condyle and/or trochlea and/or patella of the knee will be enrolled in this trial. **Key inclusion criteria:** • Patient has one or two symptomatic cartilage defect of grade III or IV (according to the ICRS classification) on the femoral condyle and/or the trochlea and/or patella of the knee. • Patient has a maximum baseline score of ≤75/100 on the KOOS subjective knee evaluation. • Total size of cartilage defects is ≥2cm^2 and ≤ 8 cm^2 (assessed by MRI) **Key exclusion criteria:** • Presence of > 2 symptomatic cartilage defects Grade III/IV (according to the ICRS classification) • Prior surgical treatment of the target knee within 12 months using mosaicplasty and/or microfracture (Note: prior diagnostic arthroscopy with debridement and lavage are acceptable within 12 months). • Instability of the knee or axis deviation of ≥5° (Note: Anterior cruciate ligament repair and/or realignement surgery is accepted if performed within 6 weeks of the planned cartilage treatment.) • Patient has radiologically apparent degenerative joint disease in the target knee as determined by Kellgren and Lawrence grade >2.
Measurements and procedures:	After written informed consent to the study, the patient is checked for eligibility according to the inclusion and exclusion criteria defined. In order to acquire baseline data, standard MRI will be performed before intervention, if not already done during diagnosis by an external radiology department. The patient will be given the relevant questionnaires in order to collect the baseline data for the clinical scores and assigned to a treatment group by randomization. 3-5 weeks before the surgery, depending on the treatment group, a nasal cartilage biopsy and autologous blood (72ml), needed for the manufacturing, are harvested under local anesthesia. After 3-5 weeks of manufacturing the graft or cell-seeded scaffold is implanted. Clinical follow-up will be done at 6 weeks as well as at 3, 12 and 24 months to assess recovery. After 3, 12 and 24 months an MRI will be performed in addition to the clinical assessment, and questionnaires filled out by the patient (12 und 24 months) to collect the clinical data.
Study Product / Intervention:	The active components of the tissue engineered cartilage graft (**N-TEC**) are expanded **human autologous nasal chondrocytes and cartilage matrix proteins** produced by the cells. The N-TEC is generated using autologous nasal chondrocytes isolated by enzymatic digestion from a 6mm diameter biopsy of the nasal septum and expanded for 2 weeks in monolayer. After expansion, 50 million cells are seeded on a 30 x 40 mm collagen membrane (Chondro-Gide®) and cultured for two additional weeks to allow for extracellular matrix deposition by the cells. The graft is cut and shaped by the surgeons according to the defect size and implanted in the knee using suturing. The graft is expected to heal in the defect and integrate with the adjacent tissue.

Secondary treatment arm:	The active component for the cell-seeded scaffold (N-CAM) is expanded **human autologous nasal chondrocytes**. The product for the secondary treatment arm is generated in the same way as the study product, but only cultured for 2 days after cell seeding onto the scaffold. Therefore the cells are attached to the scaffold, but no extracellular matrix produced. The graft is cut and shaped by the surgeons according to the defect size and implanted in the knee using suturing. The graft is expected to heal in the defect and integrate with the adjacent tissue.
Number of Participants with Rationale:	The total number of Patients is 108 trial participants considering an overall drop-out rate of 10%. Each group will consist of 54 patients. The patients will be recruited and treated by five centers in Switzerland, Germany, Croatia and Italy to achieve a defined number of patients per center. This sample size is estimated to be able to show the superiority of N-TEC to N-CAM regarding the primary endpoint and is based on the study of Saris et al., 2008.
Study Duration:	Estimated duration is 4 years for the main investigational plan (i.e. from start of screening of first participant to last participant processed and finishing the study)
Study Schedule:	01/2017: Treatment of first patient 10/2019: Treatment of last patient 01/2019: 24 months (final) follow-up of first patient 10/2021: 24 months (final) follow-up of last patient

Investigator(s):	
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Principal Investigator (I)	Prof. Dr. Giuseppe Peretti Istituto Ortopedico Galeazzi (IOG) Via Galeazzi 4, 20161 Milano, Italy Tel: +39 02 50319967 Email: gperetti@iol.it
Principal Investigator (D)	Dr.Lothar Seefried Orthopedic Clinic König-Ludwig-Haus Lehrstuhl für Orthopädie der Universität Würzburg Brettreichstrasse 11, 97074 Würzburg, Germany Tel: +49 931 803 3575 Fax: +49 931 803 1598 Email: l-seefried.klh@uni-wuerzburg.de

Other investigators	Dr. Sebastian Müller (CH) PD Dr. Kaywan Izadpanah (D) Dr. Damir Hudetz (HR) Dr. Laura Mangiavini (I) Dr. Franca Genest (D)
Study Centre(s):	5 Centers: University Hospital Basel (Basel, Switzerland) University Hospital Freiburg (Freiburg, Germany) University Hospital Sveti Duh (Zagreb, Croatia) Istituto Ortopedico Galeazzi (IOG) (Milano, Italy) Orthopedic Clinic König-Ludwig-Haus (Würzburg, Germany)
Statistical Considerations:	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 (H0 : $KOOS_{N-TEC} - KOOS_{N-CAM} = 0$). The alternative hypothesis is that N-TEC and N-CAM differ significantly in terms of the primary endpoint (HA : $KOOS_{N-TEC} - KOOS_{N-CAM} = \square\square 0$). Absolute differences ($\square$) of 10 score points or more are considered clinically relevant. Sample size was estimated to be able to show the superiority of N-TEC to N-CAM regarding the primary endpoint. Assumptions for sample size calculation were based on the study of Saris and colleagues (Saris et al., 2008), who reported 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. We have assumed that the effect size after 24 months will be at least as large. Sample size was calculated using a resampling method. Each sample size (n_i=1,...,21 = 40, ..., 160) was evaluated by sampling R = 999 times, $n_i/2$ KOOS scores from a normal distribution with $\square$ = 75 and $\square$ = 17 for the N-CAM group, and $n_i/2$ KOOS scores from a normal distribution with $\square$ = 75 + $\square$ and $\square$ = 17 for the N-TEC group. The size of $\square$ was varied between 5 and 15. Values that exceeded 100 were set to 100. N-CAM and N-TEC will be tested for a difference in KOOS score using a two-sided t-test. Superiority of N-TEC to N-CAM is declared when the test shows a significant result. Sample size was set to ensure at least 80% power (1 - $\square$ = 0.8), at a significance level of $\square$ = 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%.
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP or ISO EN 14155 (as far as applicable) as well as all national legal and regulatory requirements

STUDY SUMMARY IN LOCAL LANGUAGE

Obwohl Gelenkknorpelschäden häufig bei älteren Menschen als Folge langjähriger Abnutzung entstehen, treten sie auch regelmässig bei jüngeren Menschen als Folge von Unfällen auf. Insbesondere bei grösseren Knorpeldefekten erfolgt so gut wie nie eine spontane Selbstheilung. Wenn diese Defekte unbehandelt bleiben, ist das Risiko für die Entstehung einer Arthrose in späteren Jahren deutlich erhöht. Allerdings erfordern die heutigen Strategien zur Heilung dieser Defekte komplizierte Operationstechniken, langwierige Rehabilitationsmassnahmen und sind limitiert bezüglich der Anwendung bei sehr grossen Verletzungen und der Verfügbarkeit/Qualität des Spendergewebes. Zudem führen sie nach wie vor oft zu unbefriedigenden klinischen Ergebnissen aufgrund der geringeren Qualität des Reparaturgewebes. Häufig bleiben dauerhafte Schmerzen und eine eingeschränkte Beweglichkeit bzw. Funktion des Gelenkes bestehen. Selbst die Anwendung der neueren Zelltherapien konnte noch keine auf Dauer befriedigenden Resultate zeigen. Ein innovativer, vielversprechender Ansatz ist die Gewebezüchtung, bei der im Labor ein Stück Knorpel aus körpereigenen Zellen hergestellt wird. Erste Resultate einer klinischen Studie Phase I zeigen, dass der Einsatz von gezüchtetem Nasenknorpel für die Regeneration von Gelenkknorpel sicher und machbar ist. Zudem sind auch die ersten klinischen Resultate bezüglich der Wirksamkeit vielversprechend.

Das Ziel dieser klinischen Phase II Studie ist, die Wirksamkeit eines reifen Gewebes (mit knorpelspezifischen Proteinen) mit jener eines unreifen Gewebes (wenig bis keine knorpelspezifischen Proteine) zu vergleichen. Dazu werden 108 Patienten in die Studie aufgenommen und in zwei Gruppen unterteilt, von denen die eine das unreife und die andere das reife Gewebe erhält. Die Patienten müssen eine oder zwei symptomatische, isolierte Knorpelläsion der Stufe III-IV (nach Einstufung durch die International Cartilage Repair Society (ICRS)) von 2 bis zu 8cm^2 an der Kniegelenksfläche vom Oberschenkelknochen und/oder der Trochlea und/oder der Kniescheibe aufweisen, unter 65 Jahren sein sowie mündlich und schriftlich ihr Einverständnis erklärt haben, um in die Studie aufgenommen zu werden. Nach Erhalt der schriftlichen Einverständniserklärung, werden die Patienten getestet, ob sie alle weiteren Ein- und Ausschlusskriterien erfüllen. Anschließend wird dem Patienten Blut (72ml) sowie eine Knorpelbiopsie (Gewebeprobe) aus der Nasenscheidewand entnommen. Die Knorpelzellen (Chondrozyten) werden aus dem Gewebe isoliert, 2 Wochen vermehrt und dann auf eine Kollagenmatrix aufgebracht. Für das unreife Gewebe wird das entstandene Konstrukt weitere 2 Tage kultiviert, damit sich die Zellen an das Gewebe anheften können. Für das reife Gewebe wird weitere 2 Wochen kultiviert, damit die Zellen ein Knorpelgewebe bilden können. Nach Prüfung der Qualität des Implantates durch die Herstellung erfolgt die Freigabe basierend auf der Sterilität, Zellviabilität und im Falle des reifen Gewebes der Matrixbildung. Im Anschluss wird das Konstrukt in das Knie implantiert. 6 Wochen, sowie 3, 12 und 24 Monate nach der Operation wird eine Nachsorge durchgeführt. Hierbei werden bei den 3,12 und 24 Monatskontrollen MRTs durchgeführt, sowie bei den 12 und 24 Monatskontrollen Fragebögen (KOOS, EQ-5d) durch den Patienten ausgefüllt.

Während die Fragebögen (insbesondere der Knee injury and Osteoarthritis Outcome Score (KOOS-Score)) einen Aufschluss über die Wirksamkeit der Behandlung aus der subjektiven Sicht des Patienten geben, ist mit Hilfe der MRTs eine Aussage über die Integration des Implantates in den Defekt sowie die Qualität des Reparaturgewebes möglich. Im Nachhinein werden die Daten zudem im Zusammenhang mit dem Status des Defektes zum Zeitpunkt der Behandlung (akut (Symptome seit weniger als 2 Jahren) oder chronisch (Symptome seit mehr als zwei Jahren)) analysiert. Dieses gibt einen Aufschluss darüber, ob eine bestimmte Behandlung (unreifes oder reifes Gewebe) für eine bestimmte Indikation (akut oder chronisch) effektiver ist.

English version

Although cartilage damages in the joint develop mostly in older people due to degeneration of the cartilage, they also occur regularly in young people due to accidents. Especially in large cartilage defects there is no spontaneous self-healing. If these defects are left untreated, the risk of the development of osteoarthritis later on is significantly increased. However, the current treatment options for these defects involve difficult operation techniques, require tedious rehabilitation and are limited in the application for large injuries and the availability/quality of the donor material. Furthermore, they often lead to not entirely satisfactory clinical results due to the low quality of the repair tissue. In many cases permanent pain and restricted mobility persist. Even the use of the new cell therapies has not led to satisfactory results in the long term. An innovative promising approach is tissue engineering,

where cartilage is manufactured in the laboratory using the body's own cells. First results of a clinical phase I study show that the use of engineered nasal cartilage for the regeneration of articular cartilage (knee joint) is feasible and safe. In addition the preliminary clinical results regarding the efficacy are also promising.

The goal of this phase II clinical study is to compare the efficacy of a mature graft (with cartilage specific proteins) with the one of an immature graft (little to no cartilage specific proteins). In order to achieve this we will enroll 108 patients in the study and divide them in two groups, one receiving the immature graft and the other the mature graft. Patients must display a symptomatic, isolated cartilage lesion grade III-IV (according to the grading by the International Cartilage Repair Society (ICRS)) from 2 to 8 cm^2 on the femoral condyle and/or the trochlea and/or patella, have to be between 18-65 years old and must consent in oral and written manner in order to be enrolled in the study. After written informed consent has been obtained, the patients will be tested to see if they comply with all other inclusion and exclusion criteria. Subsequently blood (72ml) and a cartilage biopsy (tissue sample) from the nasal septum of the patient will be taken. The cartilage cells (Chondrocytes) are isolated from the tissue, expanded for 2 weeks and placed on a collagen matrix. For the immature graft the resulting construct will be cultured for 2 more days to allow the cells to adhere to the matrix. For the mature graft the construct will be cultured for 2 more weeks, to allow the cells to form cartilage tissue. After performing the quality tests the implant will be released by the manufacturer based on the sterility, cell viability and in case of the mature graft the deposition of matrix. Subsequently, the construct will be implanted in the knee. At 6 weeks as well as 3, 12 and 24 months after the operation follow-ups will be performed. During the follow-ups at 12 and 24 months questionnaires (KOOS, EQ-5d) will be filled out by the patient and MRIs will be performed at 3,12 and 24 months.

While the questionnaires (especially the Knee injury and Osteoarthritis Outcome Score KOOS-Score)) provide subjective information about the efficacy of the treatment, the MRIs will shed light on the integration of the implant in the defect and give information about the quality of the repair tissue. Retrospectively the data will be analyzed in correlation to the status of the defect at time of treatment: acute (symptoms since less than 2 years) or chronic (symptoms since more than 2 years). This will give an indication whether one treatment (immature or mature graft) is more effective for a defined indication (acute or chronic) than the other.

[illegible]

I questionari, specialmente il KOOS-Score (Knee injury and Osteoarthritis Outcome Score), forniranno informazioni soggettive riguardo l'efficacia del trattamento, mentre la RMN consentirà di valutare il grado di integrazione dell'impianto nella lesione e darà informazioni sulla qualità del tessuto di riparazione. In retrospettiva, i dati saranno analizzati in correlazione allo stato della lesione, definito come acuto (sintomi presenti da meno di due anni) o cronico (sintomi presenti da più di due anni), al momento del trattamento. Tutto ciò chiarirà quale trattamento (innesto più immaturo o di alto livello di maturazione) sia più efficace per una specifica situazione (acuta o cronica).

Croatian version

Iako se oštećenja zglobne hrskavice razvijaju prvenstveno kod starijih ljudi uslijed degenerativnih procesa, također se redovito pojavljuju i kod mlađih ljudi kao posljedica akutne traume. Pogotovo kod velikih oštećenja hrskavice ne postoji spontano zacijeljivanje. Ukoliko se ovakva oštećenja ne liječe značajno je povećan rizik od kasnijeg razvoja osteoartitisa. Međutim, trenutne mogućnosti za liječenje ovih oštećenja uključuju komplicirane kirurške tehnike, zahtijevaju dugotrajn rehabilitaciju, a postoje i značajna ograničenja u dostupnosti / kvaliteti materijala donora. Nadalje, ovakvi postupci često ne rezultiraju zadovoljavajućim kliničkim rezultatom zbog relativno loše kvalitete repariranog tkiva. U mnogim slučajevima bol i ograničena pokretljivost ostaju kao trajne posljedice. Čak i primjena novih staničnih terapija ne dovodi do dugoročnih zadovoljavajućih rezultata. Tkivni inženjering predstavlja inovativni obećavajući pristup, gdje se hrskavica proizvodi u laboratoriju uz pomoć vlastitih stanica. Prvi rezultati faze I kliničke istraživanja pokazuju da je korištenje nosne hrskavice (potrebom tkivnog inženjeringa) za regeneraciju hrskavice (zgloba koljena) izvedivo i sigurno. Osim toga preliminarni klinički rezultati u pogledu učinkovitosti su također vrlo obećavajući.

Cilj faze II kliničke studije jest usporedba učinkovitosti-liječenja zrelim presatkom (koji sadrži proteine specifične za zglobnu hrskavicu) i nezrelim presatkom (koji sadrži malo ili nimalo proteina specifičnih za zglobnu hrskavicu). Kako bi postigli taj cilj u studiju će biti uključeno 108 pacijenata koji će biti podijeljeni u dvije skupine, jedna skupina će biti liječena nezrelim presatkom a druga zrelim presatkom. Potencijalni pacijenti za studiju moraju imati simptomatsku, izoliranu hrskavičnu lezija stupnja III-IV (prema ocjenskoj ljestvici Međunarodnog društva za popravak hrskavice (ICRS)), veličine od 2 do 8 cm^2 na kondilu i / ili trohleji i /ili pateli femura, moraju biti između 18-65 godina i moraju u pismenom i usmenom obliku dati pristanak za sudjelovanje u studiji. Nakon što je dobiven pismeni informirani pristanak, pacijenti će biti obrađeni kako bi se utvrdilo da li su u skladu sa svim drugim kriterijima uključivanja i isključivanja. Potom će im se uzeti uzorak krvi (72 ml) i hrskavice (uzorak tkiva) iz nosnog septuma. Stanice hrskavice (hondrociti) izoliraju se iz tkiva, umnažaju tijekom 2 tjedna te nasađuju na kolagenski nosač. U slučaju nezrelog presatka stanica će biti uzgajane još 2 dana kako bi stanice prianjale na nosač. Kod zrelog presatka on će se uzgajati još 2 tjedna, kako bi se omogućilo da stanice stvore hrskavično tkivo. Nakon provedenog ispitivanja kvalitete presadak će biti isporučen od strane proizvođača na temelju sterilnosti, vijabilnosti stanica, a u slučaju tkivne terapije i proizvodnji izvanstaničnog matriksa. Nakon toga, presadak se ugrađuje u koljeno. Nakon 6 tjedana, kao i nako 3, 12 i 24 mjeseci nakon operacije provoditi će se praćenje pacijenata. Tijekom vremena praćenja od 12 i 24 mjeseca upitnici (KOOS, EQ-5d) će biti ispunjeni od strane pacijenta a MR slikovna obrada će biti napravljen nakon 3, 12 i 24 mjeseca.

I dok nam upitnici (osobito KOOS-Score) pružaju subjektivnu informacije o učinkovitosti liječenja, magnetska rezonancija će rasvijetliti kvalitetu integracije implantata u oštećenje i dati informacije o kvaliteti repariranog tkiva. Retrospektivno podaci će se analizirati u korelaciji sa statusum oštećenja u trenutku operacije: akutna (simptomi traju kraće od 2 godine) ili kroničnih simptoma (već više od 2 godine). To će nam ponuditi objašnjenje da li je jedna ovih opcija (zreli i nezreli presadak), učinkovitija kod različitih indikacija (akutni ili kronični).

ABBREVIATIONS

AC	Articular Chondrocytes
ACI	Autologous Chondrocyte Implantation
AE	Adverse Event
ASR	Annual Safety Report
ATMP	Advanced Therapy Medicinal Product
CA	Competent Authority (e.g. Swissmedic)
CEC	Competent Ethics Committee
CRF	Case Report Form
CTU	Clinical Trial Unit
CRO	Contract Research Organization
ClinO	Ordinance on Clinical Trials in Human Research *(in German: KlinV, in French: OClin)*
eCRF	Electronic Case Report Form
ENT	Ear-Nose-Throat
FACS	Fluorescence Activated Cell Sorter
CTCAE	Common terminology criteria for adverse events
DSUR	Development safety update report
DSMC	Data Safety Monitoring Committee
dGEMRIC	delayed Gadolinium Enhanced Magnetic Resonance Imaging of Cartilage
GAG	Glycosaminoglycan
GCP	Good Clinical Practice
GDP	Good Distribution Practice
GMP	Good Manufacturing Practice
GFP	Green Fluorescent Protein
IB	Investigator's Brochure
Ho	Null hypothesis
H1	Alternative hypothesis
HFG	Humanforschungsgesetz (Law on human research)
HMG	Heilmittelgesetz
HRA	Federal Act on Research involving Human Beings
ICRS	International Cartilage Repair Society
IMP	Investigational Medicinal Product
IIT	Investigator-initiated Trial
ISO	International Organisation for Standardisation
ITT	Intention To Treat
KlinV	Verordnung über klinische Versuche in der Humanforschung *(in English: ClinO, in French OClin)*

KOOS	Knee injury and Osteoarthritis Outcome Score (unless mentioned otherwise, the KOOS will be a mean of all 5 subscores)
LPTh	Loi sur les produits thérapeutiques
LRH	Loi fédérale relative à la recherche sur l'être humain
MACI	Matrix Assisted Chondrocyte Implantation
MD	Medical Device
MRI	Magnetic Resonance Imaging
MOCART	Magnetic resonance Observation of CARTilage repair tissue
NC	Nasal Chondrocytes
N-TEC	Nasal chondrocyte - Tissue Engineered Cartilage
N-CAM	Nasal chondrocyte – Cell Activated Matrix
OClin	Ordonnance sur les essais cliniques dans le cadre de la recherche sur l'être humain *(in German : KlinV, in English : ClinO)*
Patient ID	Patient Identification number
PEI	Paul-Ehrlich-Institute, Federal Competent authority, Germany
PI	Principal Investigator
QP	Qualified Person
SAE	Serious Adverse Event
SADR	Serious Adverse Drug Reaction
SDV	Source Data Verification
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
IL-1□	Interleukin-1□
3D	Three Dimensional
Mature tissue	This term is used in the protocol for the IMP with a prolonged culture time allowing the autologous cells to produce extracellular matrix. The term refers to the active ingredient: cells and extracellular matrix.
Immature tissue	This term is used in the protocol for the IMP with a short culture time allowing the autologous cells to adhere. The term refers to the active ingredient: cells. Therefore the final form of the IMP is a cell-seeded scaffold with little to no extracellular matrix.

STUDY SCHEDULE

Study Periods	Screening		Treatment		Follow-up			
Visit	1	2	3	4	5	6	7	8
Time (hour, day, week)	□8m	□2m	- 3-4.3 w	Day 0	6 w	3m	12m	24m
Day	□240	□60	- 19-30	0	42	90	360	720
Acceptable time delay			0d	1d	2w	2w	6w	8w
Patient Information and Informed Consent	x							
Medical History	x							
In- /Exclusion Criteria	x					GFR	GFR	GFR
Laboratory Tests (Serology)	x		x					
Pregnancy Test	x							
Randomisation		x						
MRI	x	x*****				x	x	x
Questionnaires	x	x****					x	x
Harvesting of biopsy and blood			x					
Surgery (implantation)				x				
Clinical examination	x	x	x	x	x	x	x	x
Physiotherapy*					x	x	x***	
Adverse Events**	x	x	x	x	x	x	x	x

[illegible]

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor, Sponsor-Investigator

Prof. Dr. med. Marcel Jakob

Department of Traumatology, University Hospital Basel

Spitalstrasse 21, CH-4031 Basel, Switzerland

Tel.: +41 61 328 72 40

Fax : +41 61 265 7322

email: Marcel.Jakob@usb.ch

The Sponsor-Investigator will assume all the responsibilities of the Sponsor, University Hospital Basel in accordance with the ClinO and the ICH-guideline E6 (R1). Furthermore, the Sponsor-Investigator will act as Principal Investigator and also assume those responsibilities as indicated in chapter 1.2. He has been involved in the design of the study and will perform the implantation during the course of the study. He will also take part in the interpretation of the data and writing of the report.

1.2 Principal Investigator(s)

The Principal Investigators are responsible for the conduct of the clinical trial in accordance with GCP-regulations and ICH-guideline E6 (R1). They are involved in the design and planning of the clinical trial and are responsible for all trial related medical decisions at their site. Furthermore they have the responsibility of training all further staff involved in the performance of the clinical trial at their site and reporting of all SAEs to the Sponsor-Investigator.

Dr. Marcus Mumme

Department of Traumatology, University Hospital Basel

Spitalstrasse 21, CH-4031 Basel, Switzerland

Tel.: +41 61 704 2808

Fax.: +41 61 265 3990

email: Marcus.Mumme@usb.ch

In addition to the other responsibilities, Dr. Mumme, as PI of University Hospital Basel, will be in charge of coordinating the clinical trial among partners and ensure harmonization of the procedures.

Dr. Tayfun Yilmaz

Medical Center - University of Freiburg, Germany
Department of Orthopedics and Trauma Surgery

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Brettreichstrasse 11, 97074 Würzburg, Germany
Tel: +49 931 803 3575
Fax: +49 931 803 1598
Email: l-seefried.klh@uni-wuerzburg.de

1.3 Statistician ("Biostatistician")

Power analysis has been performed by the CTU Basel.

[illegible]

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Department klinische Forschung, Clinical Trial Unit
Schanzenstrasse 55, 4031 Basel, Switzerland
Tel.: +41 61 556 52 04
Email: deborah.vogt@usb.ch

1.4 Laboratory

Test on serology will be performed by the laboratories of the dedicated hospitals at the clinical sites.

Switzerland:	Klinische Mikrobiologie
First serology	Universitätsspital Basel
	Petersgraben 4, 4031 Basel
	Schweiz
Second serology:	Institut für Virologie und Immunologie
(day of harvesting)	Universität Würzburg,
	Versbacher Strasse 7, 97078 Würzburg
	Germany
Germany:	Institute of Virology
(Freiburg)	Hermann-Herder-Str. 11
	79104 Freiburg
	Germany
Germany:	MVZ für Laboratoriumsmedizin und Mikrobiologie Würzburg
(Würzburg)	Grombühlstr. 12
	97080 Würzburg
	Germany

Italy: Laboratorio Analisi
IRCCS Istituto Ortopedico Galeazzi
Via R. Galeazzi 4, 20161 Milano,
Italia

Croatia: Department of clinical microbiology
University Hospital for Infectious Diseases
Mirogojska 8, 10000 Zagreb
Croatia

1.5 Monitoring institution

Monitoring will be performed by the CTU at the clinical sites in Basel (Switzerland), Freiburg and Würzburg (Germany) and a CRO in Milano (Italy) and Zagreb (Croatia).

Switzerland: Universitätsspital Basel
Department Klinische Forschung, Clinical Trial Unit
Schanzenstrasse 55, 4031 Basel, Switzerland

Germany: Medical Center - University of Freiburg, Germany
Clinical Trials Unit
Elsaesser Str. 2
79110 Freiburg
Germany

Italy: Consorzio Italiano per la Ricerca in Medicina (CIRM),
Viale Zara, 81, 20159 Milano, Italy

Croatia: Smart Medico d.o.o.,
Županova 5, 10000 Zagreb, Croatia

1.6 Data Safety Monitoring Committee

The data safety monitoring committee is an independent group of experts which monitor patient's safety and treatment efficacy data while a clinical trial is ongoing. They can assess at certain intervals the progress of a clinical trial, the safety data and the critical efficacy endpoints and recommend to the sponsor whether to continue, modify or stop a trial. The sponsor of this multicenter clinical trial, has assessed the need for a data safety monitoring committee according to the guidelines (EMEA/CHMP/EWP/5872/03 Corr) taking into consideration the following parameters: the characteristics of this clinical study, clinical indication, study end points and study duration, as well as study population.

After examination, the set-up of a DSMC was not considered necessary due to the following aspects:

- The clinical study is not double blind
- Follow up of 2 years
- Clinical indication: not a life threatening disease, no prior knowledge or suspicion that the IMPs have the potential to harm patients
- Study population: does not involve vulnerable population such as a paediatric population or mentally disabled patients.
- Biostatistical expertise provided by the CTU (clinical trial units)

In addition, although the final responsibility for the conduct of a clinical trial lies with the study sponsor and the investigators, different groups will oversee various aspects of the clinical trial:

- Ethical committees from 4 countries (D: Ethical committee of the Albert-Ludwigs-University Freiburg; Ethical committee of the medical faculty of the University Würzburg, I: San Raffaele Hospital Ethical Committee; Hr: Središnje etičko povjerenstvo; CH: Ethikkommission Nordwest- und Zentralschweiz (EKNZ))

- Ethics and clinical regulatory committee: established within the context of an Horizon 2020 European grant, this committee will be chaired by ECRIN-ERIC (European Clinical Research Infrastructure Network), highly experienced in the conduct of clinical trials according to GCP regulations as well as the interactions with EC and regulatory authorities in different countries through the ECRIN-ERIC partners.

1.7 Any other relevant Committee, Person, Organisation, Institution

Data management will be carried out by:

Medacta international SA
Dario Bergadano
Medical Affairs Director
Strada Regina, 6874 Castel San Pietro, Switzerland

2. ETHICAL AND REGULATORY ASPECTS

The study will be carried out in four countries. The decision of the respective ethical committees and competent authorities concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study. The clinical study in the respective country can only begin once approval from all required authorities has been received.

2.1 Study registration

The study will be registered at EudraCT, Swiss Federal Complementary Database and ClinicalTrials.gov. Results of the study will be posted in the database as well, if possible.

2.2 Categorization of study

According to the „Ordinance on Clinical Trials in Human Research (ClinO)“ of September, 20th 2013, Article 19: “Categorisation of clinical trials of medicinal products” and Article 21: “Clinical trials of transplant products” this trial is category C since the medicinal products are not authorised in Switzerland.

2.3 Competent Ethics Committee (CEC)

The responsible investigator/PI at each site ensures that consenting vote from the respective Competent Ethics Committee (CEC) (EKNZ (CH), San Raffaele Hospital Ethical Committee (I), Ethical committee of the Albert-Ludwigs-University Freiburg and Ethical committee of the medical faculty of the University Würzburg, (D), Central Ethics Committee (HR)) is obtained for the clinical study.

It is the duty of the PI to report all changes in the research activity and all unanticipated problems involving risks to humans; including the planned or premature study end and the final report. No changes will be made to the protocol without prior Sponsor/investigator and CEC approval, except where necessary to eliminate apparent immediate hazards to study participants.

Premature study end or interruption of the study shall be reported within 15 days. The regular end of the study will be reported to the CEC within 90 days, the final study report shall be submitted within one year after study end. Amendments are reported according to chapter 2.10.

2.4 Competent Authorities (CA)

As CA approval is necessary for all studies in category B or C, the Sponsor-Investigator and the PI of each site will obtain approval from the respective competent authority (Swissmedic (CH), Agenzia Italiana del Farmaco (AIFA) (I), Paul-Ehrlich-Institut (NCA) and Regierungspräsidium Freiburg and Regierungspräsidium Oberfranken (Würzburg) (D) (Competent local authority) and Croatian Ministry of Health (HR)) before the start of the clinical trial.

All adverse drug reactions that are both serious and unexpected are subject to expedited reporting. They will be reported to the local authority of the country where they occurred by the PI of the site as well as by the sponsor to Swissmedic, as authority of the Sponsor’s country. All other events from all sites will be reported in the annual safety report.

For events occurring in Switzerland reporting will be made to the local regulatory authority (Swissmedic). The description and the severity of the event, anticipated or not, causal association and dosing variable will be included in the reporting. Fatal or life-threatening unexpected adverse drug reactions will be reported as soon as possible but no later than 7 calendar days, all others no later than 15 calendar days.

Events in Germany will be reported to PEI and the ethical committee as well as to local authorities, if applicable, within the same time limits as in Switzerland. Croatia also applies the same time limits for reporting to Croatian Ministry of Health and the ethical committee. Italy also applies the same time limits for reporting.

The regular end of the study will be reported to the authorities within 90 days (CH) and the final study report will be submitted within one year after study end. Amendments will be reported according to chapter 2.10.

All SUSARs or SADR’s or lethal SAEs occurring in all centers will be reported to Swissmedic within the respective time lines.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance with the protocol and the principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, the Swiss Law and Swiss regulatory authority's requirements. The CEC and regulatory authorities will receive annual safety and interim reports and be informed about study stop/end in agreement with local requirements.

Additionally this study will be carried out in accordance to the German Arzneimittelgesetz and Transplantationsgesetz.

For the manufacturing of the IMPs the guidelines of GMP and GDP will be adhered to.

2.6 Declaration of interest

The investigators have no conflict of interest.

2.7 Patient Information and Informed Consent

The principal investigators at each site will explain to each patient the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail in an oral and written way (Patient Information). Each patient will be informed that its participation in the study is voluntary and that he/she may withdraw from the study at any time, and such withdrawal of consent will not affect his/her subsequent medical assistance and treatment. His/Her data will be anonymized upon withdrawal of informed consent. The patient will also be informed about alternative treatments. Patients will not be coerced to participate.

The patient must be informed that his/her medical records may be examined by authorized individuals other than their treating physician.

All participants for the study will be provided with the patient information and a consent form describing the study and providing sufficient information for the patient to make an informed decision about their participation in the study. Patients will be given the opportunity to discuss the treatment options and study participation with relatives or other close persons. Each participant will be given a minimum of 24 hours to decide whether to participate or not.

The patient information sheet and the consent form will be submitted to the respective CEC and to the competent authority to be reviewed and approved. Patient information and consent form will be written in the local language of the respective country. The formal consent of a participant, using the approved consent form, must be obtained before the participant is submitted to any study procedure.

The participant should read and consider the statement before signing and dating the informed consent form, and will be given a copy of the signed document. The consent form must also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

2.8 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilizing subject identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorised representatives of the Sponsor (-Investigator), a competent authority (Swissmedic (CH), Agenzia Italiana del Farmaco (AIFA) (I), Paul-Ehrlich-Institut (NCA) and Regierungspräsidium Freiburg and Regierungspräsidium Oberfranken (Würzburg) (Competent local authority) (D) and Croatian Ministry of Health (HR)), or an ethics committee (EKNZ (CH), San Raffaele Hospital Ethical Committee (I), Ethical committee of the Albert-Ludwigs-University Freiburg and Ethical committee of the medical faculty of the University Würzburg, (D), Središnje eti ko povjerenstvo (HR)) may require direct access to parts of the medical records relevant to the study, including participants' medical history.

2.9 Early termination of the study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances, for example:

- ethical concerns,
- insufficient participant recruitment,
- if the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention

2.10 Protocol amendments

The Sponsor-investigator (Prof. M. Jakob) and the Principal investigator/study coordinator (Dr. M. Mumme) are allowed to amend the protocol. All Principal investigators from each side are allowed to provide suggestions for a protocol amendment.

Important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) will be communicated (within 5 days) to relevant parties (e.g., investigators, CEC, competent authorities, trial participants, trial registries, journals, regulators).

Substantial amendments are only implemented after approval of the CEC and CA respectively.

Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the CEC/CA. Such deviations shall be documented and reported to the sponsor and the CEC/CA as soon as possible.

All non-substantial amendments are communicated to the CA as soon as possible if applicable and to the CEC within the Development Safety Update Report (DSUR).

3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

Articular cartilage injuries remain a clinical challenge, and are associated with pain, disturbed function and disability. The number of articular cartilage defects diagnosed each year in the EU and USA reaches about 2 million (Hambly et al., 2009). When not properly treated, such lesions predispose to osteoarthritis and may finally result in total replacement of the joint, with massive costs for the healthcare system. 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, there is still no universally accepted and successful treatment approach for articular cartilage defects. As a first step conservative treatment with physical therapy in conjunction with activity modification and weight loss, if necessary, is applied. Surgical intervention should be considered if a full-thickness cartilage defect grade III or IV is diagnosed and if conservative treatment has not provided acceptable pain relief. The ultimate goal in the treatment is to achieve the regeneration of organized functional hyaline cartilage. However, current therapeutic options such as arthroscopic debridement, microfracture, 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 graft material (Gomoll et al, 2010). Even the more advanced cell-based therapies, in addition to involving technically challenging operations associated with donor-site morbidity and highly variable outcome, do not provide entirely satisfactory treatment. Moreover, due to the use of Articular Chondrocytes (AC) in these therapies, their applicability for older patients is limited, due to the age-dependent properties of articular chondrocytes. Such techniques, although improving symptoms in short-term follow-up, cannot offer predictable and reproducible restoration of cartilage structure and function and have yet to prove cost effectiveness.

However, a tissue-therapy based on the use of autologous Nasal Chondrocytes (NC) could overcome these drawbacks and lead to a measurable benefit for the patient. A phase-I study using an engineered nasal cartilage tissue performed to evaluate the safety and feasibility of the procedure, showed promising clinical results (see Chapter 3.4). Despite the known advantages and disadvantages (Table 3.1.1) a direct comparison of the efficacy of a mature graft (active ingredient is cells and matrix) vs an immature graft (active ingredient only cells) is difficult due to the paucity of well-designed randomized and controlled trials, especially for the more recently developed techniques such as ACI, MACI and Tissue Engineering. Thus in this trial we aim at carrying out a phase II clinical trial for cartilage repair, introducing the following two main innovations: 1) the use of autologous nasal chondrocytes (NC) as cell source superior to articular chondrocytes (AC) (see chapter 3.2) and 2) the delivery of a mature graft as opposed to an immature graft (see chapter 3.2).

The main objective is the comparison of a mature versus an immature graft in order to determine the impact of graft maturation on the clinical outcome:

> => Determine whether implantation of a more mature graft is beneficial for the quality and durability of the repair tissue and the clinical outcome, measured by a superiority of at least 10 points in the main primary outcome (self-assessed KOOS score)

In addition, since the integration with the surrounding cartilage might be less efficient for a mature graft than for an immature graft (cell-seeded scaffold) as described *in vitro* by Obradovic et al, 2001, we want to assess this integration using the non-invasive MRI technique. Further we want to evaluate the quality of the repair tissue in order to determine its influence on the clinical outcome as experienced by the patient.

> => Determine the potential of the mature graft to integrate with the adjacent cartilage (MOCART) and the formation of hyaline cartilage as repair tissue as assessed by dGEMRIC analysis

Recent studies have indicated that ‘acute’ cartilage lesions have a more favorable prognosis following cellular therapy than those defined as ‘chronic’. Although a threshold time to distinguish between acute and chronic defects after onset of the traumatic event has not yet been defined, there is increasing consensus on the fact that 2-3 years after the trauma the joint starts displaying homeostatic changes and possible traits of early degeneration. On one hand, a more mature cartilage graft, due to the higher mechanical stability and superior cell protection, could play a critical role in the repair of chronic cartilage lesions. On the other hand, an immature graft could be sufficient to trigger anabolic regenerative processes in the case of acute defects. Therefore, it is necessary to compare efficacy of N-TEC and N-CAM in the clinical settings of acute (defined as <1 years) vs chronic (defined as >1 and

< 5 years) cartilage lesions. This analysis will be performed retrospectively.

=> determine whether the efficacy of each treatment has a correlation to the characteristics of the defect (e.g. “acute” versus “chronic” setting) possibly allowing the most promising treatment in relation to the time after the initial cartilage injury (onset of symptoms) to be selected

	Therapy	Grafted material	Advantages	Disadvantages
Existing tissue engineered products	Spherox®	Spheroids of human autologous matrix-associated chondrocytes for implantation suspended in isotonic sodium chloride solution.	Only autologous cells implanted (no scaffold) Arthroscopic implantation possible	- Variability of ACs - Cells directly exposed to inflammatory and mechanical assaults (self-synthesizing extracellular matrix) - Issues linked to cell retention in the defects - Long rehabilitation protocol
	*MACI® (Matrix-induced autologous chondrocyte implantation)	autologous AC seeded in collagen type I/III membrane	Better retention of cells in the defect Initial mechanical stability Partial chondrogenic re-differentiation	- Rather fibroblastic cell phenotype in repair tissue - Variability of ACs - Possible foreign body reaction to the scaffold - Cells directly exposed to inflammatory and mechanical assaults (no newly formed extra-cellular matrix) - Long rehabilitation protocol
Nose to Knee II immature graft	N-CAM	autologous NC seeded in collagen type I/III matrix	Same as MACI® More potent and more reproducible cell source (NC vs AC) Higher cell density as compared to MACI	- Limited chondrogenicity - Cells directly exposed to inflammatory and mechanical assaults (no newly formed extra-cellular matrix) - Long rehabilitation protocol
Nose to Knee II mature graft	N-TEC	tissue engineered cartilage from autologous expanded NC cultured in collagen type I/III matrix	Good cell protection (presence of ECM) More reproducible tissue quality (NC vs AC) Mechanical stability, easier implantation/ fixation	- Possible problem of integration with the surrounding cartilage - Longer production time - Higher production costs

Table 3.1.1 Comparison of existing tissue engineered products (with market authorization) with the approach used in the Nose to Knee II study.

*The marketing authorisation for MACI has been suspended at the recommendation of the Agency's Committee for Medicinal Products for Human Use since 17.12.2014

3.2 Investigational Product (treatment, device) and Indication

The investigational medicinal product is considered a combined ATMP (Tissue engineered product). The active components of the tissue engineered cartilage graft (**N-TEC**) are expanded **human autologous nasal chondrocytes and cartilage matrix proteins** produced by the cells. The N-TEC

is generated using autologous nasal chondrocytes isolated by enzymatic digestion from a 6mm diameter biopsy of the nasal septum and expanded for 2 weeks in monolayer. After expansion, 50 million cells are seeded on a 30 x 40 mm collagen membrane (Chondro-Gide®) and cultured for two additional weeks to allow for production of extracellular matrix by the cells. The graft is cut and shaped by the surgeons according to the defect and implanted in the knee using suturing. The graft is expected to heal the defect and integrate with the adjacent tissue maturing further in the process.

Suitability of cell source

In contrast to articular cartilage, harvesting of nasal cartilage, also characterized as hyaline cartilage, is less invasive and can be performed as an outpatient procedure under local anaesthesia. This procedure leads to minimal donor site morbidity due to the fact that the donor site is more easily accessible and not subjected to high levels of physical force. The number of nasal chondrocytes which can be collected from a small biopsy of 6mm diameter is sufficient for the generation of an autologous cartilage graft of clinically relevant size (e.g. 12 cm^2) (Tay et al, 2004; Fulco et al, 2014). Moreover this donor site can also be used in older patients (>70 years) or patients with limited availability of healthy articular cartilage.

In addition, the properties of NC are less age dependent (Rotter et al, 2002). Importantly, the NC not only proliferate faster than articular chondrocytes (AC) but have a higher and more reproducible chondrogenic capacity (Kafienah et al, 2002). Isolated chondrocytes from nasal cartilage tissue have been used to successfully engineer *in vitro* and/or *in vivo* 3D cartilaginous tissues (Pelltari K, 2014; Fulco I, 2014; Scotti C, 2014; Candrian C, 2008; Farhadi J, 2006; Miot S, 2005; Kafienah W, 2002). As shown in figure 3.2.1, the contents of GAG and Collagen type II and, consequently, the mechanical properties are superior in tissues engineered from NC as compared to those from AC.

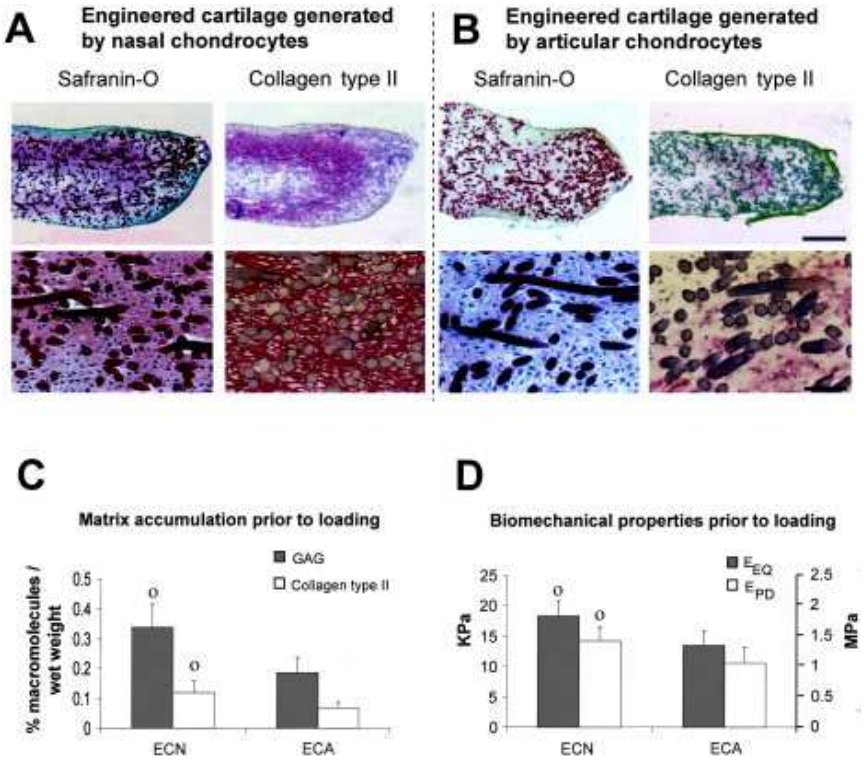

Fig. 3.2.1. Properties of engineered cartilage tissue generated by nasal chondrocytes (ECN) or articular chondrocytes (ECA) prior to mechanical loading. **A** and **B,** Safranin O and type II collagen immunohistochemical staining of representative ECN or ECA after 2 weeks of culture in chondrogenic medium. Top rows (bar = 500 □m) show lower-magnification views of bottom rows (bar = 100 □m). **C,** Glycosaminoglycan (GAG) and type II collagen content of ECN and ECA. **D,** Equilibrium modulus (E_{EQ}, left y-axis) and pulsatile dynamic modulus (E_{PD}, right y-axis) of ECN and ECA. Values are the mean and SEM results from 5 independent experiments. o = significant difference versus ECA.

Despite the numerous advantages of NC over AC in the generation of cartilage grafts, the use of NC to treat articular lesions has raised questions regarding the compatibility of these cells with the joint environment. Therefore *in vitro* experiments have been conducted to study the response of NC to experimental conditions mimicking the mechanical and inflammatory joint environment. Although NC

are not subjected to high mechanical forces in their native environment, they are able to respond to physical forces and surface motion resembling joint loading similarly to articular chondrocytes, and can up-regulate molecules typically involved in joint lubrication (Candrian et al., 2008).

Tissues generated from NC also have a higher capacity to recover after a short exposure to IL-1β (simulating inflammation following surgery) as compared to tissues generated from AC (Scotti et al., 2012).

Finally, the capacity of NC (1) to acquire the molecular identity of AC following implantation in experimental articular cartilage defects in an autologous setting and (2) to contribute to the repair of the damaged cartilage has been shown in a large animal study in goats (Pelttari K, 2014).

Biological properties

Analysis of the biological properties of grafts previously manufactured according to these protocols display abundant cartilaginous matrix embedding well differentiated chondrocytes (Fulco et al, 2014) (figure 3.2.2), indicating that re-differentiation of the chondrocytes after expansion has been achieved.

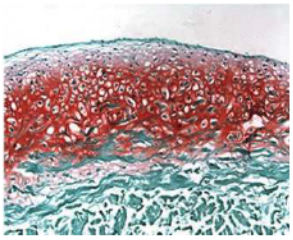

Fig. 3.2.2 Histological image of a tissue engineered cartilage graft. Cell morphology and matrix deposition are shown in histological Safranin O staining.

Hematoxylin and Eosin (H&E staining, figure 3.2.3) showed the presence of living cells. Elastica von Gieson (figure 3.2.3) revealed the presence of collagen and elastic fibers in the cartilage graft. Mucopolysaccarides, a characteristic of cartilage, could be detected by alcian blue-PAS (figure 3.2.3). Furthermore immunohistochemistry staining indicated that the engineered nasal cartilage grafts were positive for collagen type II, pro-collagen type I, S100, podoplanin and Sox-9, but negative for p53 indicating a healthy status of the cells within the engineered tissue (figure 3.2.3, see investigator's brochure for details). This broad characterization confirmed that the NC-engineered tissue exhibits typical characteristics of native hyaline cartilage.

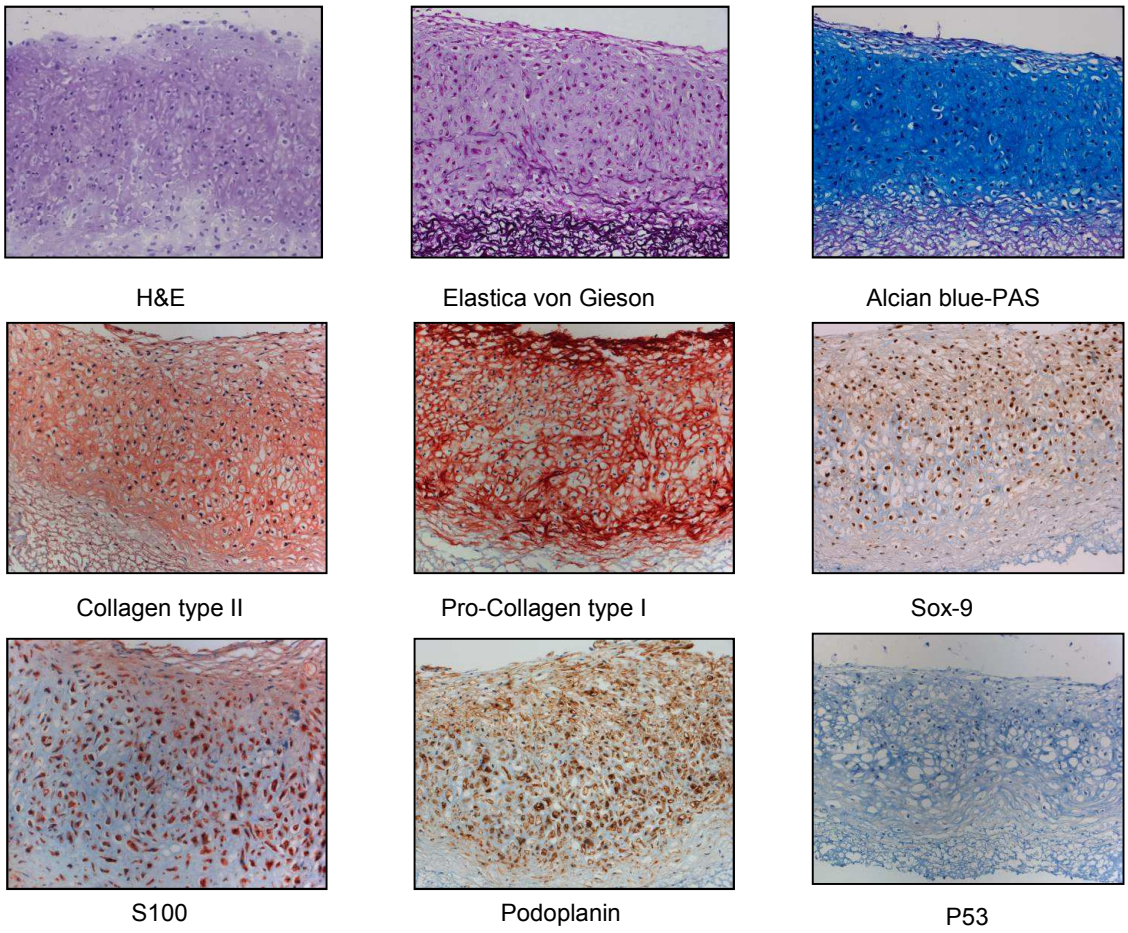

Fig. 3.2.3 Histological and Immunohistochemical characterization of N-TEC

Mechanical properties

In order to be used in a clinical setting for the repair of articular cartilage defects, sufficient mechanical properties at the time of implantation (stable enough for suturing, while still flexible enough to be fitted to the curvature of the site) could be a possible advantage of engineered cartilage grafts. By the time that fixation is dissolved, mechanical stability is required to resist contraction by scar tissue formation and exposure to local or external forces in the recipient bed (i.e., compression, tensile and bending forces).

Preclinical investigations support this theory having shown *in vitro* (Farhadi et al, 2006) as well as *in vivo* in an orthotopic large animal model (Miot et al, 2012; Pelttari et al, 2014) that pre-cultivation of engineered human nasal cartilage enhances the mechanical properties relevant for use in a clinical setting.

3.3 Preclinical Evidence

Several *in vitro* experiments have shown that nasal chondrocytes are a suitable cell source for the production of tissue engineered cartilage grafts to treat articular cartilage injuries in the knee. This is not only due to their advantageous properties as compared to articular chondrocytes, but also due to the facts that nasal chondrocytes can adapt to the environment of the knee regarding weight loading and their resistance to inflammatory responses (see chapter 3.2).

Furthermore several animal studies have been performed to investigate the behavior of tissue engineered nasal cartilage in the environment of the joint.

Migration study (see IB for details)

To answer the question whether implanted nasal chondrocytes are still detectable in the repair tissue and/or migrating in surrounding articular tissues, we performed two experiments in a goat animal model where autologous articular chondrocytes, labelled using GFP lentivirus for tracking purposes, were cultured in Chondro-Gide® and implanted in a joint defect.

After 4 weeks, 3 months and 6 months respectively, animals were sacrificed and immunohistochemical analysis of reparative tissue showed that GFP positive chondrocytes were still detected in the tissue, indicating that the implanted chondrocytes remained at the site of implantation. In addition, the reparative tissue was cartilaginous, as demonstrated by positive Safranin-O staining specific for cartilage extracellular matrix. After 3 months chondrocytes were exhibiting typical shape within lacuna but were not displaying a columnar organization.

No GPF-positive chondrocytes could be detected by FACS analyses in any of the tissues surrounding the joint/implantation site (fat pad, patella, meniscus, cruciate ligaments, and synovium; n = 3 goats). Therefore, it could be concluded that the implanted chondrocytes, either from articular or from nasal origin, did not migrate into the tissues surrounding the joint up to 3 months after implantation (Mumme et al, 2016).

Tumorigenicity (see IB for details)

Different groups have reported that chondrocytes from different cell sources showed no signs of malignant transformation after cell expansion in 2D, assessed by different techniques (Kamil et al, 2002 and 2003, Brandl et al, 2010, Trimborn et al, 2011).

The N-TEC, composed of autologous nasal chondrocytes and the Chondro-Gide® membrane, has been used in a previous clinical trial “Tissue engineered nasal cartilage for reconstruction of the alar lobule” (TpP-I-2010-002) for reconstructive purposes following resection of a non-melanoma skin cancer and thus implanted in a tumor site. Second look biopsies were analyzed 6 months after implantation and histological analysis performed by a pathologist. There was no evidence of tumor formation. The one year follow-up also showed no indication of tumor formation in any of the patients.

The same nasal cartilage graft was used in a second clinical trial “Tissue engineered nasal cartilage for the regeneration of articular cartilage in the knee after traumatic injury- phase I clinical trial” (TpP-I-2012-001). There was no indication of local tumor formation detected in the MRI of the 1 or 2 year follow-up for the first patients.

In addition an animal study (mouse) was performed to assess the tumor potential of constructs generated with nasal chondrocytes from 3 patients of the previous trial expanded in 2D in the presence of growth factors. After implantation none of the operated mice showed weight loss or abnormalities with palpation indicating absence of tumor at the implantation site. After 6 months constructs were explanted and assessed histologically. In addition, five different organs (lung, liver, the kidneys, spleen, and local lymph nodes) were harvested from the same mice, No signs of tumor formation could be found in any of the explanted tissues, Therefore it can be concluded that the expanded nasal chondrocytes do not have any tumor potential when used for cell based constructs.

Capacity for cartilage repair (see IB for details)

A long-term study in goats was performed to obtain preclinical evidence of the suitability of NC for the repair of articular cartilage defects. Tissue-engineered constructs were generated using autologous NC (and AC to serve as controls) and implanted into experimental defects created at a clinically relevant location, namely load-bearing sites of the articular condyle. The results of this study (Pelttari et al., 2014) show, per the semi quantitative O’Driscoll scoring system, that the quality of the repair tissue significantly improved from 3 to 6 months after implantation only when using NCs, such that at 6 months the repair quality achieved by NCs was statistically superior to AC controls. The improved quality of the repair tissue using NC compared with AC was confirmed histologically by a stronger and more uniform staining for glycosaminoglycans at 6 months (figure 3.3.1).

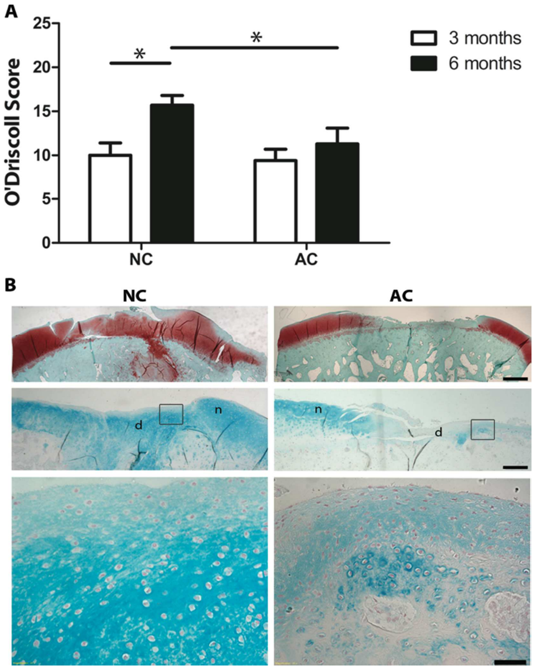

Fig. 3.3.1 Goat nasal chondrocytes (NC) in articular cartilage repair. (**A**) O'Driscoll scores of the repair quality of the NC- or AC-treated goat articular defects at 3 and 6 months (n = 3 animals per time point) after implantation. * = p<0.05. (**B**) Safranin O and Alcian blue staining of representative repair tissues at the defect site (d) and of adjacent native articular cartilage (n) 6 months after implantation of NC or AC. Lower Alcian blue images show higher magnification (scale bar, 50 µm) of the regions framed in the respective upper panels (scale bar, 1mm)

In summary, the results of these animal studies support the compatibility and efficacy of nasal chondrocytes for articular cartilage repair.

3.4 Clinical Evidence to Date

The two IMPs have been used in a number of clinical trials. A summary of all clinical trials is given in the table below.

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Table 3.4.1 Summary of clinical trial to date with N-TEC and N-CAM respectively

One of the proposed IMPs (N-TEC) has been used in phase I trials for two clinical indications, the reconstruction of the alar lobule after tumor excision and the regeneration of articular cartilage lesions in the knee after traumatic injury.

Clinical trial Phase I: Nose to Nose (TpP-I-2010-002)

N-TEC was first used in a prospective single center phase I clinical trial for the reconstruction of the alar lobule after tumor excision (Clinical-trials.gov (NCT 01242618), Swissmedic TpP-I-2010-002) and the results were recently published in "The Lancet" (Fulco et al, 2014). Five patients (76-88 years) with two-layer defects from non-melanoma skin cancer in the alar lobule accepted the novel procedure. The engineered cartilage grafts (25 x 25 x 2 mm) were shaped intra-operatively and implanted after tumor excision under paramedian forehead or nasolabial flaps, as in the standard reconstruction using native cartilage. After six months, during flap refinement, second look biopsies of repair tissues were harvested and histologically analyzed. At least one year after implantation, when reconstruction is typically stabilized, patients were assessed for safety, aesthetic (figure 3.4.1) and functional outcomes. Monofilament and rhinomanometry tests were used to quantify alar cutaneous sensibility, mechanical stability and respiratory flow rate. All engineered grafts contained a mixed hyaline-fibrous cartilage matrix. Six months after implantation, reconstructed tissues displayed fibro-muscular-fatty structures typical of the alar lobule. After one year, all patients were satisfied with the aesthetic and functional outcome and no adverse events were recorded. The procedure resulted in sensibility and structural stability of the reconstructed area, with adequate respiratory function and no donor site morbidity.

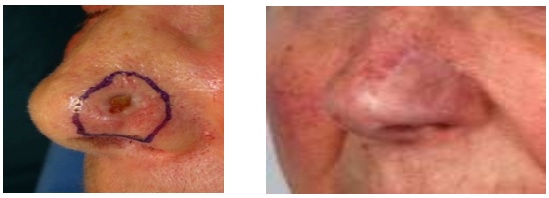


Fig. 3.4.1 Photographs of the affected alar lobule (patient Nr.1) before (left: skin tumor with margins contouring) and 1 year after reconstruction (right)

These results indicate that engineered autologous nasal cartilage tissues can be safely used in clinical applications such as reconstruction in replacement of native cartilage grafts.

Clinical trial Phase I: Nose to Knee (TpP-I-2012-001)

A similar nasal cartilage graft, larger in size (30 x 40mm), is currently used in a prospective phase I clinical trial. In this study (Clinical-trials.gov: NCT01605201, Swissmedic TpP-I-2012-001) a total of 25 patients (initially 10, extended to 25) between 18 and 55 years suffering from traumatic articular cartilage injuries in the knee are being treated with N-TEC. The goal is to test the safety and feasibility of this approach for the regeneration of articular cartilage. Patients are enrolled if they display a maximum of two defects with a total size of 2-8cm^2, Grade III-IV according to the ICRS scale on the femoral condyle and/or trochlea femoris. Corresponding lesions or advanced osteoarthritis are currently exclusion criteria. The primary endpoint (safety) is assessed by the number of (serious) Adverse Reactions. In addition, data are collected on efficacy based on the biochemical composition of the repair tissue (GAG-content as assessed by delayed Gadolinium enhanced MRI of Cartilage

(dGEMRIC), radiological outcome (structure and integration of the cartilage as assessed by MOCART scoring of MRI), clinical scores (KOOS, LYSHOLM & IKDC) and questionnaires of patient satisfaction. The results from the first 10 patients have been published in "The Lancet" (Mumme et al, 2016)

Safety and stability

So far 18 patients (15 having completed the 2 year follow-up) have been treated with no occurrence of serious adverse reactions. Two patients were excluded due to multiple accidents (after 23 months) and intraoperative findings (defect size too large). The graft was stable in situ in all patients but one, where the transplant was destroyed due to another unrelated accident occurring during sports exercise (patient excluded due to multiple accidents). Adverse events reported so far are summarized in the table below.

Adverse Events/reactions Patients (n=18)	**No. events**	**Patient #**	**Time point after implantation**
Serious Adverse Reactions	**0**		
Adverse Reaction	**3**		
- Hematoma due to surgery	1	#17	0 month
- Partial graft delamination (no intervention necessary)	1	#15	12 months
- saphenous nerve irritation	1	#17	13 months
Serious Adverse Events	**5**		
Additional injuries with hospitalization & surgery (same knee)	2		
- new cartilage lesions in the afflicted knee at other location with hospitalization and re-surgery (deterioration)	1	#2	12 months
- knee instability due to ACL insufficiency	1	#17	7 months
Additional injuries with hospitalization & surgery (other location)	3		
- new sports injury in the contralateral knee	1	#9	17 months
- distortion of the contralateral knee (accident)	1	#14	1 month
- acute appendicitis	1	#18	0 month
Adverse Events	4		
- meniscus lesion of contralateral knee	1	#8	11 months
- ankle distorsion (sports injury)	1	#9	11 months
- new sports injury of the afflicted knee without hospitalization or surgery	2	#7 & #9	20 months (#7) 11 months (#9)

Table 3.4.1: Summary of adverse events reported so far for 16 patients (two patients excluded)

Preliminary clinical efficacy

The early clinical observations indicate not only safety and feasibility of the procedure, but together with Magnetic Resonance Imaging (MRI) data also show promising results for efficacy of the treatment (15 patients have completed the 24 months follow-up, 2 are excluded, 1 has not yet reached the 24 months follow-up). Clinical scores (average and range have been summarized in the table below (Table 3.4.1).

Table 3.4.2 Preliminary clinical scores of the phase I study

An improvement of more than 10 points in the KOOS sub-scores is considered a clinically relevant improvement. The average improvement of all scores is always above 10 points, but with large standard deviations since results vary from one patient to another. On an individual basis, more than half of the patients (15 evaluated so far) benefited from the treatment (see below).

Table 3.4.3: Improvement of clinical scores: Average and individual basis

The radiological evaluation of N-TEC treatment is promising (figure 3.4.2). The quantification of glycosaminoglycan by dGEMRIC indicates hyaline cartilage tissue formation. More precisely, by comparison of the repair tissue with native healthy cartilage from radiological measurements, the relative □R1 has been calculated (1.0 indicating no difference detectable between repair tissue and native healthy cartilage). With the resulting relative □R1 of 1.9 (0.89 – 4.13) after 6 months, 1.5 (0.78 – 2.17) after 12 months and 1.20 (0.71 - 1.51) after 24 months, a progressive additional maturation of the tissue over time can be observed. This is further supported by histological data (figure 3.4.3). When compared to literature, N-TEC treatment might result in even more hyaline tissue compared to MACI (relative □R1 2.18, Trattnig et al 2008) or ACT (2.40, Trattnig et al 2007) or microfracture (3.39, Trattnig et al 2008). The morphological MOCART score after 6 months was 61 (25-80), after 12 months 56 (25-80) and after 24 months 48 (10-80). This might be due to occasional cleft formation at the interface between native and repair cartilage.

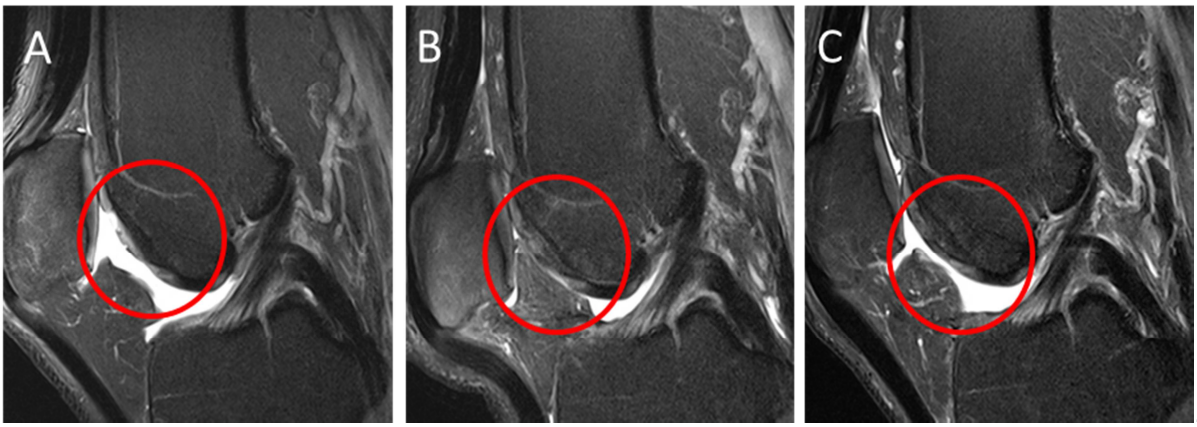


Fig. 3.4.2 Sagittal MRI of the knee before surgery (A) indicating the cartilage defect (red circle). Maturation of the repair tissue after 6 months (B) and 12 months (C), demonstrating the repair tissue in situ.

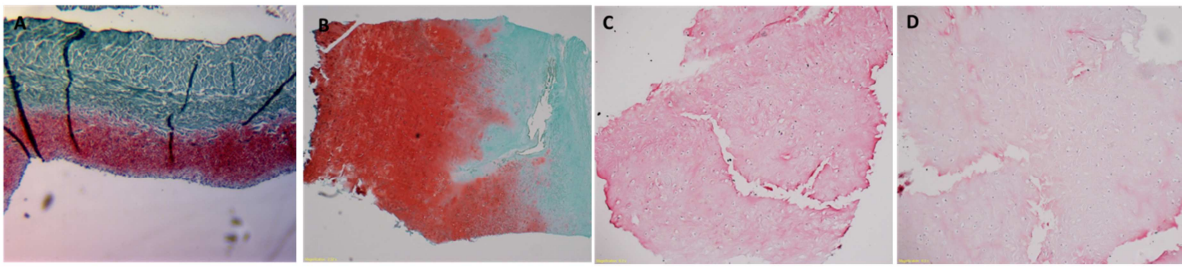


Fig. 3.4.3 Histological analysis indicates chondrogenic differentiation of the graft at time of implantation (A). 18 months after surgery, the repair tissue is more mature with abundant deposition of glycosaminoglycans (B) and with positive collagen type II (C) and widely negative collagen type I immunohistochemistry (D) typical for hyaline cartilage.

Clinical trial Phase II: Nose to Knee II (2016TpP2004, EudraCT: 2015-005162-34)

The current clinical trial "Nose to Knee II" described in this study protocol is a phase II multicenter clinical trial based on the phase I study described above, which focuses on efficacy and the comparison of grafts in different maturation stages and therefore requires higher patient numbers to be statistically significant. Patients, between 18 and 65 years old, suffering from articular cartilage lesions not related to an inflammatory state, are treated in four different countries: Croatia, Germany, Italy and Switzerland and will be followed up for 2 years. At 6 weeks as well as 3, 12 and 24 months after the operation, follow-ups are performed. During the follow-ups at 12 and 24 months questionnaires (KOOS, EQ-5d) are filled out by the patient and MRIs will be performed at 3,12 and 24 months.

3.4.1 Safety

So far, 23 grafts have been produced at two manufacturing sites, 12 N-TEC (3 in Basel, 9 in Wurzburg) and 11 N-CAM (2 in Basel, 9 in Wurzburg). All batches produced passed the release criteria and were within specifications (see IMPD). The first IMP manufactured was a N-TEC graft produced in Basel and implanted the 09.01.2017. No adverse reactions have been recorded up to now. One AE was observed for patient USB01197001 (plate removal after osteotomy) and one SAE for patient USB08197702 (scrotal atheroma). For regular updates and further information, please consult the Investigator's Brochure (IB).

3.4.2 Efficacy

Nineteen patients had the three months MRI showing all grafts to be in place and integrated within the surrounding cartilage. Two patients have so far reached the one year follow-up. KOOS sub-scores were improved for both patients (one N-TEC and one N-CAM). For regular updates and further information, please consult the Investigator's Brochure (IB).

Patient	USB01197001 (N-TEC)			USB03198803 (N-CAM)		
KOOS	preop	12 months	Change from baseline	preop	12 months	Change from baseline
Symptoms	42.9	100	57.1	53.6	89.3	35.7
Pain	44.4	100	55.6	47.2	91.7	44.5
ADL	57.4	98.5	41.2	67.7	100.0	32.4
Sport	5.0	90.0	85.0	25.0	85.0	60
QoL	12.5	75.0	62.5	25.0	62.5	37.5

Table 3.4.5: Clinical scores at baseline (preop), after 12 months and change from baseline. (ADL: Activities of daily life, QoL: Quality of Life). All scores are from 0-100, with 100 being the best score.

In summary, the clinical data indicate that engineered nasal cartilage grafts are safe for use in clinical applications for reconstructive as well as orthopedic purposes.

Taking into account all scientific and clinical data, the use of the engineered nasal cartilage graft is feasible and safe.

3.5 Dose Rationale / Medical Device: Rationale for the intended purpose in study (pre-market MD)

Previous studies have shown that the cell seeding density has a significant impact on the generation of cartilaginous constructs in terms of GAG content and collagen type II expression (Francioli et al. 2010). High seeding densities (8.3 Million/cm$^{2)}$ lead to an increase in GAG and collagen type II expression and thus a cartilaginous tissue of higher quality as compared to low seeding densities (2.1 Million/cm^2). However, the amount of cells that can be isolated from a reasonable sized biopsy has also to be taken in consideration, since large biopsies could be unavailable and/or increase donor site morbidity. Therefore a seeding density of 50 Million cells per construct (size 30 x 40 mm, seeding density of 4.2 Million cells/cm^2) has been selected for preclinical animal studies (Pelttari et al. 2014, see IB for details) and the previous and ongoing clinical trials (Fulco et al, 2014, Nose to Knee (TpP-I-2012-001) see above). The chosen cell seeding density leads to cartilaginous tissues suitable for regenerative medicine.

3.6 Explanation for choice of comparator (or placebo)

As the most promising approach currently, and reported to provide clinical improvement to young patients with focal cartilage defects in mid-term follow-up (Kon et al, 2009), we have selected cell-therapy techniques as the most appropriate comparator. The established cell-based technique MACI® has been used in clinical applications since 1998 and is a standard product with marketing authorization in Europe since 2013 (currently suspended). In order to eliminate differences due to the cell source and allow a more appropriate evaluation of the impact of graft maturation on the clinical outcome, nasal chondrocytes instead of articular chondrocytes will be used (figure. 3.6.1), therefore exploiting the concept of cellular therapies, not using a licensed product. Since nasal chondrocytes have been proven *in vitro* to have superior properties as compared to articular chondrocytes, results at least comparable to the standard MACI® procedure can be expected.

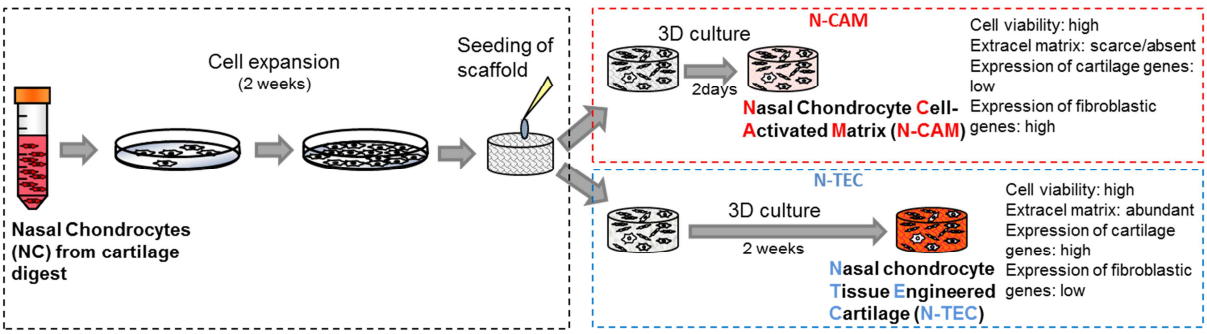


Fig. 3.6.1 Illustration of the procedures to generate the NC-based IMPs and of their main characteristics

We believe that in this setting, where a mature and immature graft is manufactured in a similar way using nasal chondrocytes, the superiority of the mature graft in comparison to the immature graft can be shown.

3.7 Risks / Benefits

The spontaneous healing capacity of cartilage is poor and untreated defects predispose to osteoarthritis. Common current strategies such as arthroscopic debridement, microfracture, autologous osteochondral grafting, cartilage allografts and autologous chondrocytes implantation (ACI/MACI) still have drawbacks or produce unsatisfactory long term results. These drawbacks could be overcome using a tissue based therapy.

Potential risks and benefits

During this trial all patients will be treated according to current hospital standards. Nasal cartilage biopsies will be harvested by an experienced plastic or ENT-surgeon. The manufacturing of the graft will be performed in a GMP-facility with given manufacturing authorization from Regierung von Oberfranken, Ansbach, Germany in consultation with PEI (NCA) or from Swissmedic, Switzerland depending on the manufacturing site. Manufacturing will be performed in compliance with GMP-guidelines and according to verified protocols. In-Process controls will be conducted at defined points during the process to ensure absence of microbiological, endotoxin or mycoplasma contamination. The quality of the graft will be tested before implantation according to verified protocols. Experienced orthopedic surgeons will implant the graft into the knee and perform the follow-up. Technically the operation is no more challenging than standard procedures as ACI, and the follow-up includes all standard procedures with the addition of an MRI analysis. Since autologous cells are implanted and autologous serum is used instead of bovine serum, the risk of immune reaction or of disease transmission is minimal. Furthermore, *in vitro* and *in vivo* studies have already proven that nasal chondrocytes can survive in the joint environment and respond to mechanical loading similar to articular chondrocytes. The certified Chondro-Gide® membrane is already in clinical use. The combination of nasal chondrocytes cultured in this matrix has already successfully been used in two other clinical trials, for the reconstruction of the alar lobule after tumor resection and for the treatment of cartilage defects in the knee.

From standard therapies it is known that localized defects on the patella on average have higher complication rates and clinically inferior outcomes as compared to defects on the femoral condyle or trochlea. For N-CAM and N-TEC treatment, there are no clinical data available yet concerning the outcome of treatment for patella defects.

Taking into account the pre-clinical results and safety measures, the risk for the patient is not increased as compared to the standard procedures.

However, several benefits can be expected from the implantation of cartilage grafts into focal cartilage defects as compared to state-of-the-art surgical treatments:

1. Shorter rehabilitation times

Implantation of a more mature tissue should lead to shorter rehabilitation times, earlier postoperative joint loading and a faster return of the patient to daily life activities and sports.

2. Possibility to treat larger defects

This technique can be used for cartilage defects up to 8 cm^2 in size.

3. More durable regeneration

We assume that in the long term the repair tissue will be hyaline cartilage instead of fibrocartilage and will therefore have better durability and stability as compared to the outcome of current methods.

4. Reduced donor-site morbidity

When AC are used for cartilage repair currently, harvesting of autologous cartilage plugs or biopsies from a low weight bearing site generate an additional defect in the joint, which has been reported to be detrimental to the surrounding healthy articular cartilage. Biopsies taken from the nasal septum lead to negligible donor-site morbidity. Safety of harvesting procedure was previously reported in a first trial in which similar nasal cartilage biopsies were taken to generate tissue engineered grafts for the reconstruction of alar lobule after tumor resection (Clinicaltrials.gov: NCT01242618, Fulco et al, Lancet 2014).

5. Superior graft quality

Articular cartilage has to be harvested from a healthy, low weight bearing area, therefore limiting the

availability of material. In case of some diseases harvesting of healthy cartilage might not even be possible. Furthermore, it has been shown that properties of articular chondrocytes are dependent on age, while those of nasal chondrocytes are less age dependent and show greater proliferation and differentiation capacity. This might allow this technique to be applied to older patients or those with insufficient amounts of healthy articular cartilage.

Overall the risk for the patient is not considered to be higher as compared to standard procedures, but the benefits might have a significant impact especially for large defects.

Expected Adverse Reactions

Based on the experience of the phase I study, no major risks are expected. Expected adverse reactions are mostly related to the tissue withdrawal and implantation:

Expected Adverse Reaction related to nose cartilage withdrawal

- Local hematoma
- Local bleeding
- Local pain

Expected Adverse Reaction related to tissue implantation

Local:

- Pain
- Swelling and/or hematoma
- Plate removal in case of necessary osteotomy

Systemic:

- Post-anesthetic nausea
- vomiting
- fever

No adverse reactions are expected to follow-up procedures such as MRI/dGEMRIC. These expected adverse reactions are common for the standard treatment and therefore will only be classified according to chapter 10.1.1 and recorded in the CRF. They are not subject to reporting.

Risk assessment

Risks could be related to the manufacturing, the quality of the product itself or the implantation of the product. Due to the use of a certified, clinically used matrix with autologous cells, the adherence to GMP- and GCP-regulation and a close follow-up schedule, the overall risk for the patient is expected to be low. Possible risks have been identified, evaluated and appropriate measures to reduce the risk described (Table 3.7.1). For residual risk a contingency plan is available. If, during the process, further risks are identified or other measures have to be taken, the plan will be adapted. Microbiological, Endotoxin and Mycoplasma testing will be carried out as in process controls. In case of serious adverse events related to the IMP, the respective ethical commissions and national authorities will be informed. Along with the patient information, the patient receives the contact details of the responsible doctor along with the instruction to contact the doctor at any time for any ambiguities, emergencies, unexpected or adverse events during or after the study.

In summary, this procedure contains no expected additional risks as compared to the standard procedure for these cases.

Major	High chance of occurrence, critical for the trial
Medium	Medium chance of occurrence, critical for the trial
Minor	Low chance of occurrence, may be critical for the trial
Acceptable	No contingency plan required

Table 3.7.1: Risk factors in the use of a tissue engineered products for cartilage regeneration.

Risk		Initial risk	Risk Control/Mitigation	Residual Risk	Contingency Plan
Construct	Contamination of biopsy	medium	• Biopsy harvesting according to Good Clinical Practice • Check for microbiological contamination in the supernatant of the biopsy after transport • Training of plastic surgeons • Addition of antibiotics in the transport medium and first week of expansion	acceptable	
	Contamination during media change	medium	• Check for microbiological & endotoxin contamination and mycoplasma in the media after expansion and pre-culture phase • Use of clean room • Work according to SOPs	minor	abort of the transplant generation if there are positive results for contamination
	Contamination during transport	minor	• Packaging according to the norm for sterile packing • Work according to SOP	acceptable	
	Failure to generate a graft	medium	• if the standard surgical method has to be used, there is no disadvantage	acceptable	
	Cell number for seeding not reached	medium	• working according to SOPs • correct biopsy size	minor	In case of <50M and >38M cells, ≥95% viability and adequate morphology seeding of a smaller scaffold
	Contamination of the construct is a critical risk. Since most cultures show signs of contamination within 48h, the sample of the last media change is taken 48 - 72h prior to implantation. The last result will be retrospective regarding microbiological contamination.				

Risk		Initial risk	Risk Control/Mitigation	Residual Risk	Contingency Plan
Safety for patient	Infection during surgical intervention at the biopsy site	minor	• standard preparation of patient for operation • additional treatment of the biopsy site to avoid infection • routine precautions as in any operation	acceptable	
	Infection through transplant	minor	• no positive tests regarding mycoplasma, endotoxin or microbiological contamination (last result retrospective)	acceptable	
	Infection after operation	minor	• same as for standard operation	acceptable	

			• close follow up of patient		
	Complications after implantation	minor	• same as for standard operation • arthroscopy to determine cause and further treatment	acceptable	
	Loss of implant	minor	• same as for standard operation	acceptable	
	Rejections and immune reaction	minor	• autologous cells and serum are used	acceptable	
	Contrast agent (Dotarem®)associated with nephrogenic systemic fibrosis	minor	• exclusion of patients with renal impairment	acceptable	
	Allergic or hypersensitive reactions to contrast agent (Dotarem®)	minor	• patients with known allergies or asthma will receive anti-allergy medication before operation	acceptable	
	Allergic reaction to antibiotics used partially during production	minor	• exclusion of patients with known allergies	acceptable	
	Unforeseen side effects	minor	• immediate contact with study doctor for further investigation and treatment	acceptable	
Risk		Initial risk	Risk Control/Mitigation	Residual Risk	Contingency Plan
Patient's rights	Insufficient patient information	minor	• no participation of patient without signed informed consents	acceptable	
	Protection of personal rights	minor	• no personal data besides those relevant for the study (age and sex) given to people involved in the production • data treated according to usual hospital practice	acceptable	
	Incidental findings during screening or follow-up	medium	• patient will be asked as part of informed consent whether he/she would like to be informed of these	acceptable	

Potential threats to the study

Beyond our studies, no engineered cartilage has been used in patients for cartilage regeneration. Even though several products have been claimed as engineered cartilage implants, histological analyses have shown limited matrix deposition. (figure 3.7.1).

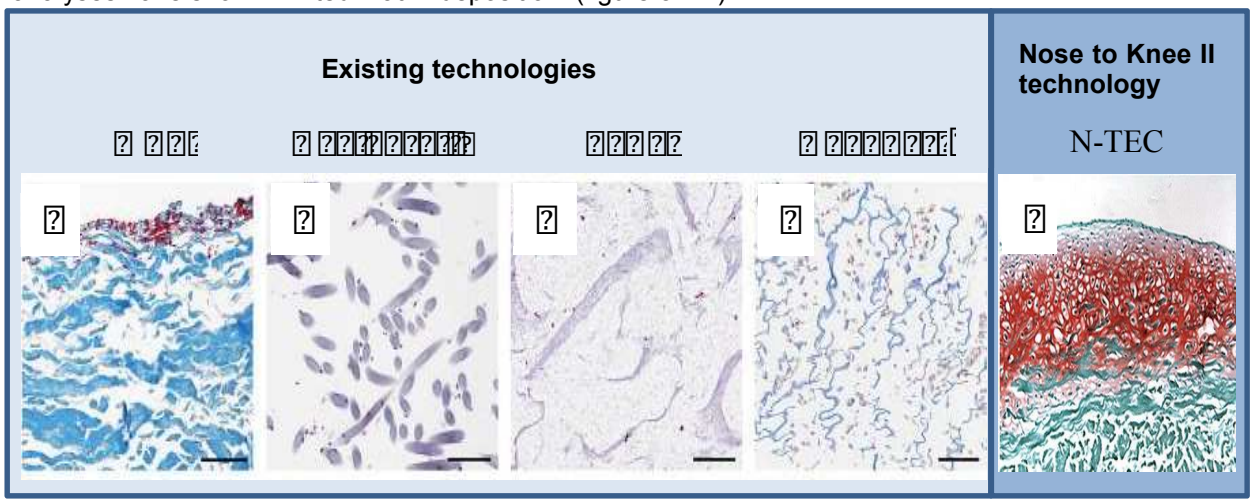


Fig. 3.7.1 Histological images of the four different scaffolds types. Cell morphology and matrix deposition are shown in histological alcian blue stainings (A, C, D), hematoxylin and eosin staining (B), or in Safranin-O staining (E). Note the presence of chondrocytes but no matrix deposited. (A-D) From Albrecht C et al, 2011.

Post-trial care

Patients will be followed-up on a voluntary basis up to 5 years after implantation and can contact study doctors at any time during or after the study.

3.8 Justification of choice of study population

Patients between 18 - 65 years of age with one or two symptomatic full-thickness cartilage lesions from 2 cm^2 to 8 cm^2 (per lesion, however, not exceeding a total size of 8 cm^2 for all lesions) on the femoral condyle and/or trochlea and/or patella of the knee due to a traumatic injury will be enrolled in this trial. The patients cannot display signs of advanced osteoarthritis, since this would lead to an inhomogeneous patient group: several not yet fully understood factors influence the clinical outcome and would therefore render the clinical results difficult to interpret. The proposed inclusion and exclusion criteria however, will lead to a patient cohort conform with the clinical indications of the standard therapies.

Patients will be selected from those attending normal consultations in the 5 clinical centers.

4. STUDY OBJECTIVES

4.1 Overall Objective

The study aims at the comparison of a therapy with a mature graft versus a therapy with an immature graft for the regeneration of articular cartilage lesions in the knee after injury. Two main innovations are introduced: 1) the use of autologous nasal chondrocytes (NC) as cell source superior to articular chondrocytes (AC) and 2) the delivery of a mature graft as opposed to an immature graft. Data will also be analyzed retrospectively to identify the possibility of treatment selection in relation to the time after the initial cartilage injury (acute vs chronic cartilage lesions as defined by onset of symptoms).

4.2 Primary Objective

This proposed phase II trial seeks to primarily define whether a mature graft will improve the clinical efficacy for the patient, leading to an increase of at least 10 points in the main primary outcome (self-assessed score KOOS) after 24 months as compared to the group receiving the immature graft. Comparison between groups will allow assessment of whether the mature graft is superior to the immature graft.

4.3 Secondary Objectives

The secondary objective is to assess the stability and integration of the graft with the adjacent tissues as well as the remodeling of the implanted graft towards native cartilage.

Furthermore another questionnaire (EQ-5d) and an additional time point (12 month) for the 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.

Additionally the number of treatment failure at 24 months will be analyzed. Treatment failure is defined as objective pathological clinical findings by the investigator directly correlated with subjective patients complaints resulting in a deterioration of the subjective clinical outcome assessed by KOOS and the 5 KOOS subscores. In case of treatment failure, a root cause analysis will be performed. A clinical deterioration is defined as reduction in the KOOS score of > 10 compared to baseline. A non-responding to treatment is defined as improvement of < 10 in the KOOS score.

4.4 Safety Objectives

Safety of the procedure will be assessed by the number of SADRs and SUSARs within the 2 year time-frame of the trial related to the implants. Reoperations due to new accidents or incidents at other sites than the operated site will be recorded, but not counted as related to the implant.

5. STUDY OUTCOMES

5.1 Primary Outcome

The KOOS score will be used to measure the primary outcome. The primary endpoint is the difference in the KOOS at 24 months between the two techniques (comparison of the efficacy of the technique). The KOOS score, covering the fields of Symptoms, Pain, Activities of daily life, Sport activities and Quality of life, is suitable for assessing the improvement for the patient. This validated questionnaire is widely used to assess efficacy of cartilage repair therapies.

5.2 Secondary Outcomes

The stability and integration as well as the morphological properties of the graft will be assessed by the MOCART and 3D MOCART Scores (magnetic resonance observation of cartilage repair tissue) derived from the MRI. The secondary endpoint will be the 24-month assessment. MRI will be performed at 3, 12, 24 months follow-up.

The remodeling of the tissue after implantation towards native cartilage will be assessed by dGEMRIC evaluation (MRI) from the 24-month assessment of the relative R1. The dGEMRIC evaluation will be recorded at 3, 12, 24 months follow-up.

The KOOS will be recorded for patients at baseline visit 1 or 2 and at the 12- and 24-month follow-up assessments. Variations over time will be recorded through completion of the questionnaires at enrolment and at each follow-up visit (12 and 24 months after treatment). An increase of at least 10 points after 2 years in the KOOS is considered a relevant improvement for the patient, thus proving the efficacy of the treatment.

5.3 Other Outcomes of Interest

Retrospectively data will be analyzed to identify the possibility of treatment selection (mature graft vs. immature graft) in relation to the time after onset of symptoms (acute vs chronic cartilage lesions) in order to determine if one treatment is more beneficial than the other (e.g. higher stability, better integration etc.).

In addition a subgroup analysis to compare the outcome with regard to the localization of the defect (patella vs. femoral condyle/trochlea) will be performed after the study.

5.4 Safety Outcomes

The study will evaluate the safety of the implantation of an immature or a mature graft by the number of SADRs or SUSARs from baseline assessment up to 60-months follow-up assessment.

Adverse events/adverse drug reaction will be graded according to severity, expectedness, and relationship to trial treatment and reported according to the regulations.

No adverse events are expected except for those associated with any operation (see chapter 3.7)

6. STUDY DESIGN

6.1 General study design and justification of design

This study is an interventional phase II study comparing the efficacy of a mature graft vs. an immature graft in cartilage repair after traumatic injury. 108 patients will be enrolled in 5 clinical centers in order to determine the possible benefits and superiority of a mature graft as compared to an immature graft. Patients will be randomized to ensure equal numbers in each treatment group at each center, but there will be no blinding in this study due to the fact that the manufacturing processes for the two IMPs have a different duration. The implantation date, thus the time after biopsy harvesting required for manufacturing of the graft, will disclose to both patient and surgeons to which treatment group the patient was assigned.

After the initial screening procedures, including MRI and blood tests, the patient is asked to fill out the baseline questionnaires and a biopsy is taken from the nasal septum. Either about three or five weeks later, depending on the treatment group, the IMP is implanted in the cartilage defect in the knee. The follow-ups at week 6 include only clinical issues such as wound healing. After 3, 12 and 24 months patients are additionally assessed by MRI and given validated questionnaires (12 and 24 month) to self-assess their condition. Patients are enrolled in the trial for 2 years, but will be asked to volunteer for further assessment by questionnaires for up to 5 years (see study schedule in summary).

In addition to the follow-ups, the patients will receive continuous physiotherapy starting shortly after implantation and lasting up to 1 year post-operatively if necessary.

A potential limitation in this trial is the fact that surgeons and patients are aware which IMP is implanted in the defect due to the properties and appearance of the IMP (surgeons) and the time between biopsy and implantation date (patient). However, although the assessment for the primary outcome is subjectively performed by the patient using validated questionnaires, the secondary outcome is assessed by independent medical personnel.

6.2 Methods of minimizing bias

6.2.1 Randomization

A permuted-block (2, 4 and 6 size) randomisation method will be performed. This method will prevent confounding of the treatment effect with "disease duration". For each centre there will be a randomisation list. The list will be generate by Sealed Envelope online software application (https://www.sealedenvelope.com/) and provided to database manager in order to implement and allow the automatic group assignation.

6.2.2 Blinding procedures

The study will be unblinded. Manufacturing of immature grafts is performed in a shorter time than for the mature grafts and therefore the operation date is at an earlier time point, thus disclosing the type of IMP implanted. In addition the physical properties of the two IPMs are different in terms of handling and appearance and thus can be easily identified by the surgeons. Therefore, it is not feasible to blind the study.

6.2.3 Other methods of minimizing bias

Patients will fill out validated questionnaires, widely used in clinical assessment for the efficacy of cartilage repair treatment.

All MRIs of all clinical centers will be analyzed at the University Hospital Basel by medical personnel with special focus on cartilage MRI. They will be blinded to assess for MOCART and 3D MOCART scores and dGEMRIC evaluation.

6.3 Unblinding Procedures (Code break)

Not applicable

7. STUDY POPULATION

Patients presenting at hospitals will be treated in five clinical centers (Switzerland, Germany, Italy and Croatia) by the respective PIs as mentioned in Chapter 1.2. Harvesting of the starting material (nasal cartilage biopsy and blood) will be performed as an outpatient procedure, while the implantation will be performed as an in-patient procedure in the respective hospital. At each clinical center a defined number of patients should be enrolled (half for therapy with an immature graft, half for therapy with a mature graft). In case that less patients are enrolled in one of the centers, another center would have the possibility to enroll more patients than initially planned.

7.1 Eligibility criteria

Participants fulfilling all of the following inclusion criteria are eligible for the study:

- Patient is □18 and □65 years old at time of screening.
- Patient has a localized articular cartilage defect of the femoral condyle and/or the trochlea and/or patella of the knee. 2 localized cartilage defects are accepted if the total defect size is □ 8 cm^2, both cartilage defects are located at the femoral condyle and/or the trochlea and/or patella and both cartilage defects are to be treated with N-CAM or N-TEC.
- Patient has a defect of grade 3 or 4 according to the ICRS classification.
- Patient has a defect size □2 and □8 cm^2 as assessed by MRI/arthroscopy.
- Patient has an opposite intact (□ICRS Grade 2) articulating joint surface (no "kissing lesions").
- Patient has an intact meniscus (maximum 1/2-resection).
- Patient has a stable knee joint or sufficiently reconstructed ligaments. If not, ligament repair has to be done during the operation or within 6 weeks of the planned cartilage treatment.
- Patient has a maximum baseline score of 75/100 in the KOOS subjective knee evaluation.
- Patient is willing and able to give written informed consent to participate in the study and to comply with all study requirements, including attending all follow-up visits and assessments and to complete postoperative rehabilitation regimen.

The presence of any one of the following exclusion criteria will lead to exclusion of the participant:

- Patient is the investigator or any sub-investigator, research assistant, pharmacist, study coordinator, other staff or relative thereof directly involved in the conduct of the protocol or in a dependency or employment with the sponsor.
- Patient is unable to understand the patient information
- Patient is unable to undergo magnetic resonance imaging (MRI) or is sensitive to gadolinium
- Patient has had prior surgical treatment of the target knee within 12 months using mosaicplasty and/or microfracture (Note: prior diagnostic arthroscopy with debridement and lavage are acceptable within 12 months). Anterior cruciate ligament repair is accepted, if the target knee is stable or a primary ACL reconstruction is performed within 6 weeks of the planned cartilage treatment.
- Patient has free range of motion of the affected knee joint or □10° of extension and flexion loss.
- Patient has a relevant meniscus tear. Partial meniscal removal allowed, if not exceeding 1/2. Suture of meniscocapsular separation is allowed. Suture of meniscus tear is allowed if the same compartment is not afflicted by symptomatic cartilage injury, and the graft is planned for trochlea and /or patella and /or contralateral compartment. If the same compartment is afflicted, suture is not allowed in parallel, but if successful, cartilage treatment might be added 12 months later.
- Patient has radiologically apparent degenerative joint disease in the target knee as determined by Kellgren and Lawrence grade >2.
- Patient has evidence of joint disease e.g. chronic inflammatory arthritis, and/or infectious arthritis.
- Patient has an unstable knee joint or insufficiently reconstructed ligaments. If ligament repair is necessary, the repair has to be performed during the operation or within 6 weeks of the planned cartilage treatment.
- Patient has malalignment (no valgus- or varus-deformity) in the target knee □5°. In suspected cases, the mechanical axis must be established radiographically through complete leg imaging during standing and in a.p. or rather p.a. projection. If alignment surgery is necessary, surgery has to be performed within 6 weeks of the planned cartilage treatment.

- Patient has an osteochondral defect which cannot be reconstructed (bony substance defect of >3mm depth need to be reconstructed with autologous bone graft from tibia or iliac crest). Bone marrow edema is allowed.
- Any concomitant painful or disabling disease of the spine, hips, or lower limbs that would interfere with evaluation of the afflicted knee.
- Patient has a known systemic connective tissue disease.
- Patient has a known autoimmune disease.
- Patient has a known immunological suppressive disorder or is taking immunosuppressives.
- Patient is currently systemically or intra-articularly taking steroids and/or has used steroids within the 30 days prior to the planned treatment.
- The patient has a known history of HIV/AIDS. (Protection of staff)
- The patient has a known history of Treponema pallidum (syphilis). (Protection of staff)
- The patient has an active hepatitis B or C infection with verified antigens. Patients with a cured hepatitis B or C infection and/or verified antibodies are not excluded. (Protection of staff)
- The patient has at the site of surgery an active systemic or local microbial infection, eczematization or inflammable skin alterations (including Protozoonosis: Babesiosis, Trypanosomiasis (e.g. Chagas-Disease), Leishmaniasis, persistent bacterial infections, such as Brucellosis, spotted and typhus fever, other Rickettsiosis, Leprosy, Recurrent Fever, Melioidosis or Tularaemia).
- Patient has a known history of cancer.
- Patient has a known history of primary hyperparathyroidism, hyperthyroidism, reduced kidney function (GFR < 80 ml/min), or prior pathological fractures, independent of the genesis.
- Patient has any degenerative muscular, vascular or neurological condition that would interfere with evaluation of outcome measures including but not limited to Parkinson's disease, amyotrophic lateral sclerosis (ALS), or multiple sclerosis (MS).
- Patient has a body mass index (BMI) >35 kg/m^2.
- Patient is pregnant, lactating or anticipates becoming pregnant within 24 months after surgery.
- Patient is currently participating, or has participated in any other clinical study within 3 months prior to the screening visit.
- Patient has known current or recent history of illicit drug or alcohol abuse or dependence defined as the continued use of alcohol or drugs despite the development of social, legal or health problems.
- Patient has psychiatric or cognitive impairment that, in the opinion of the investigator, would interfere with the patient's ability to comply with the study requirements, e.g., Alzheimer's disease.
- Patient has any other condition, which, in the opinion of the investigator, would make the patient unsuitable for the study.
- Patient is unable to tolerate local anesthesia
- Any known allergies, especially for porcine collagen, penicillin or streptomycin
- Patient is unwilling and/or unable to give written informed consent to participate in the study and to comply with all study requirements, including attending all follow-up visits and assessments and to complete postoperative rehabilitation regimen.

Intraoperative Exclusion Criteria:

- Patient has a total defect size <2 or defect size extends graft size and could therefore not be treated in total.
- Patient has >2 independent cartilage lesions
- Patient has symptomatic full-thickness (ICRS Grade 3 or 4) of tibial plateau.

7.2 Recruitment and screening

There is no advertisement for recruitment, since no healthy volunteers are eligible for the trial. No compensation or payment will be given to the participants. Patients attending for normal consultation at the 5 clinical centers will be screened for participation. If diagnosed with one or two symptomatic, cartilage lesions on the femoral condyle and/or trochlea and/or patella, patients will be informed of the trial and asked to participate. Before enrollment in the study the patient will receive information in an oral and written manner by the PI of the respective center and will be given time to discuss the treatment options and study participation with relatives or close persons. Patients will not be coerced into participating and will not suffer any disadvantage in treatment by declining to participate: they will be treated according to current standards. On signing the informed consent, the patient will be

investigated, to see if they are eligible for the clinical trial and meet the inclusion and exclusion criteria defined in the study protocol. A baseline MRI will be performed, the questionnaires filled out by the patient and screening for pregnancy, if applicable, as well as for viruses and other parameters listed in the exclusion criteria carried out. If the patient complies with the eligibility criteria, they will be assigned to a treatment group, noted in the enrolment log and given a Patient-ID.

7.3 Assignment to study groups

The study group is assigned in phase of patient acceptance to the trial by the MyClinical Data database. Once the investigator creates the patient schedule and confirm that the patient is eligible for the study, according to inclusion and exclusion criteria and following consent form signed by the patient, the software will attribute to study group based on random algorithm.

7.4 Criteria for withdrawal / discontinuation of participants

Patients can be excluded from the study by the respective PIs at any point for the following reasons:

- Medical reasons where a continuation of the trial would jeopardize the health of the patient
- Withdrawal of informed consent (final medical follow-up mandatory for patient safety)
- Non-compliance with the required procedures as stated in the patient information and informed consent or refusal of the follow-up examinations which are necessary to assess the safety and efficacy of the treatment
- Abortion of the clinical study
- Contamination of the IMP during manufacturing
- IMP manufacturing not successful
- Patient fulfills intraoperative exclusion criteria (section 7.1)

In case patients are excluded from the study, the PI will inform the patient and discuss a final medical follow-up date with them. The treatment cannot be terminated, since the intervention is an implantation. In case patients are excluded before implantation due to manufacturing issues or intraoperative exclusion criteria the surgeon will decide for a suitable treatment. The patient will be marked as excluded/study aborted in the enrolment log signed by the PI.

Patients excluded from the study after implantation will not be replaced, since a drop-out rate of 10% has been included in the power-calculation.

8. STUDY INTERVENTION

8.1 Identity of Investigational Products (treatment / medical device)

8.1.1 Experimental Intervention (treatment / medical device)

Name: N-TEC

Sources: GMP production unit at Fraunhofer Institute, Germany (QP: PD Dr. Oliver Pullig) or DBM clean room at University Hospital Basel, Switzerland (QP: PD Dr. Werner Krenger)

Composition: Chondro-Gide® combined with autologous nasal chondrocytes and extracellular matrix containing cartilage specific proteins produced by the cells during preculture

Appearance: glossy-white (cartilaginous)

Size: 30x40 mm

Dose: 50 million cells

Route: Implantation in the cartilage defect of the knee and fixation by sutures

No commercial product available on the market.

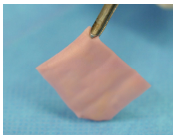
8.1.2 Secondary study arm (treatment / medical device)

Name: N-CAM

Sources: GMP production unit at Fraunhofer Institute, Germany (QP: PD Dr. Oliver Pullig) or DBM clean room at University Hospital Basel, Switzerland (QP: PD Dr. Werner Krenger)

Composition: Chondro-Gide® combined with autologous nasal chondrocytes and minimal to no extracellular matrix deposited

Appearance: comparable to appearance of the Chondro-Gide® membrane alone



Size: 30x40 mm

Dose: 50 million cells

Route: Implantation in the cartilage defect of the knee and fixation by sutures

The commercial product comparable to the IMP is MACI®. This is also based on a matrix of porcine collagen Type I/III combined with autologous cells such as Chondro-Gide®. The difference is that for MACI®, articular chondrocytes are used, while here nasal chondrocytes will be seeded in order to eliminate differences related to the cell source.

8.1.3 Packaging, Labelling and Supply (re-supply)

Labels for both products will be designed in accordance with the EU-GMP-guidelines, Volume 4 Annex 13. The labels will be in the local language of the country where the IMP will be used. The IMPs will be packed individually for each patient in a primary container (tube) within a secondary transport container and shipped by overnight courier to the respective clinic.

8.1.4 Storage Conditions

The product will be used immediately on receipt for implantation. Therefore no storage is intended.

8.2 Administration of experimental and control interventions

8.2.1 Experimental Intervention

After manufacturing, the engineered tissue will be sent to the respective clinic, cut and shaped according to the defect size by the surgeon and immediately implanted using sutures for fixation until the graft heals in. The sutures are bioresorbable and dissolve with time. No additional preparations are necessary. Any unused part of the generated graft will be sent to the research lab (Tissue Engineering) in Basel involved in the clinical trial for research provided the patient has given prior consent.

8.2.2 Control Intervention

After manufacturing, the cell-seeded graft will be sent to the respective clinic, cut and shaped according to the defect by the surgeon and immediately implanted using sutures for fixation until the graft heals in. The sutures are bioresorbable and dissolve with time. No additional preparations are necessary. Any unused part of the generated graft will be sent to the research lab (Tissue Engineering) in Basel involved in the clinical trial for research provided the patient has given prior consent.

8.3 Dose / Device modifications

Not applicable.

8.4 Compliance with study intervention

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.

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.

8.5 Data Collection and Follow-up for withdrawn participants

Data will be collected until the time of withdrawal using the questionnaires and MRIs at the time of follow-up. No data will be collected afterwards. A final medical visit is recommended for the safety of the patient after withdrawal (minimum 1 year after treatment).

8.6 Trial specific preventive measures

There are no specific preventive measures for this trial apart from the ones taken for standard operations. Pregnancy tests will be performed at time of enrolment. Contraception using a reliable method such as hormonal in combination with a mechanical contraception or double mechanical contraception such as diaphragm in combination with condom is advised during study participation.

Immunosuppressive medication is not allowed during study participation, as it could increase infection rate and/or mask signs of inflammation/infection.

Steroidal medication is not allowed during study participation as it could compromise repair tissue formation.

8.7 Concomitant Interventions (treatments)

Physiotherapy will be given to the patient after implantation to restore mobility and muscle formation. This is part of the study protocol. Patients need to follow a rehabilitation program to ensure proper reintegration into daily activities, work and sports and leisure. This process is be accompanied by physiotherapists.

The rehabilitation program includes restriction of weight-bearing and range of motion. Critical limits in weight bearing and range of motion are defined as follows:

- Partial weight bearing (15kg) with use of crutches for 6 weeks. Afterwards stepwise increase as tolerated till total weight bearing.

- Immobilization of the knee in extension for the first week. Limitation of range of motion Ext/Flex

0/0/30° for the second week, Ext/Flex 0/0/60° for the third and fourth week, Ext/Flex 0/0/90° for the fifth and sixth week. Afterwards free range of motion.

The rehabilitation program is complemented with passive continuous motion application and amongst others with strength and proprioceptive training. Due to the different possibilities for postsurgical physiotherapy in the different countries with different health insurance coverage, a supporting web-based rehabilitation platform will be integrated (VideoReha.com). Patients and physiotherapists have the possibility to follow instructive videos suggesting appropriate exercises in the different phases of rehabilitation. This is an optional offer for patients and physiotherapists.

8.8 Study Drug / Medical Device Accountability

The immature and mature grafts will be prepared, produced, tested, packed and shipped to the respective clinic according to GMP and GDP standards and reviewed by the National Competent Authorities (D, CH). The IMPs will be implanted immediately on arrival according to the study protocol reviewed and approved by the competent authorities of the respective countries and will not be stored. Each IMP will be individually produced for the patient: therefore the batch size is one and the batch number will equal the patient ID. The remains of the IMPs will not be used further in the clinic, but will be sent to the Tissue Engineering Lab, University Hospital Basel, for further scientific research purposes, provided the patient has given prior consent, and will be destroyed in this process.

8.9 Return or Destruction of Study Drug / Medical Device

Remains of the IMP after implantation as well as excess cells from production will be sent to the Tissue Engineering Lab, University Hospital Basel, 4031 Basel, Switzerland for research purposes if the patient has given prior consent. Remains of the IMPs will be destroyed during analysis. If the patient does not consent to donating samples for research, they will be discarded according to the individual hospital's policies.

9. STUDY ASSESSMENTS

9.1 Study flow chart(s) / table of study procedures and assessments

Study Periods	Screening		Treatment		Follow-up			
Visit	1	2	3	4	5	6	7	8
Time (hour, day, week)	□8m	□2m	- 3-4.3 w	Day 0	6 w	3m	12m	24m
Day	□240	□60	- 19-30	0	42	90	360	720
Acceptable time delay			0d	1d	2w	2w	6w	8w
Patient Information and Informed Consent	x							
Medical History	x							
In- /Exclusion Criteria	x					GFR	GFR	GFR
Laboratory Tests (Serology)	x		x					
Pregnancy Test	x							
Randomisation		x						
MRI	x	x*****				x	x	x
Questionnaires	x	x****					x	x
Harvesting of biopsy and blood			x					
Surgery (implantation)				x				
Clinical examination	x	x	x	x	x	x	x	x
Physiotherapy*					x	x	x***	
Adverse Events**	x	x	x	x	x	x	x	x

[illegible]

9.2 Assessments of outcomes

9.2.1 Assessment of primary outcome

The primary outcome is the KOOS subjective score at 24-month follow-up visit and it will be assessed by the KOOS questionnaire. The KOOS questionnaire is a self-assessment by the patient and covers the areas of symptoms, pain, activities of daily life, sport activities and quality of life. The patient will complete the questionnaire online during each visit, i.e. at time of enrolment (baseline) as well as at 24 month follow-up visit. Additional questionnaires can be filled out on a voluntary basis at month 60. The score is automatically calculated according to the equation defined in the questionnaire. This validated questionnaire is widely used for clinical assessment of cartilage repair and therefore will also provide a high comparability to other studies.

9.2.2 Assessment of secondary outcomes

The secondary outcome is related to the stability, integration and remodeling of the graft to assess the quality of the repair tissue in order to determine whether there is a correlation to the primary outcome. Stability and integration are assessed using MRI, where the pictures will be interpreted by experienced and independent medical personnel. The MOCART (magnetic resonance observation of cartilage repair tissue) score will be used to assess the integration, stability and morphological properties of the graft at 24 month follow-up. The score parameters include: filling of the defect, integration of the border zone to the adjacent cartilage, intactness of the subchondral lamina, intactness of the subchondral bone, relative signal intensities of the repair tissue compared to the adjacent native cartilage, and others.

Since the harvesting of second look biopsies is not planned as standard for ethical reasons, dGEMRIC is chosen as an alternative non-invasive method to assess the quality of the repair tissue. This method will give a quantification of glycosaminoglycan content of the repair tissue in relation to the healthy native cartilage.

The KOOS Score is also collected after 12 month to evaluate the development of the patient's health status. An additional self-assessment questionnaire used is the EQ-5d questionnaire. These questionnaires provide additional information for comparability with other studies.

9.2.3 Assessment of other outcomes of interest

Retrospectively the data collected in the primary and secondary outcome will be used for analysis with regard to the onset of symptoms. The patients will be classified regarding the onset of symptoms as acute (onset < 2 years) or chronic (onset > 2 years) and differences between the treatment groups as well as within the treatment groups will be analyzed in order to identify possible correlation between the efficacy of the IMP treatment and the time of onset of symptoms.

In addition a subgroup analysis to compare the outcome with regard to the localization of the defect (patella vs. femoral condyle/trochlea) will be performed in order to evaluate, how the outcome of patella defects, which are known to have an inferior clinical outcome in standard therapies, is in case of treatment with N-TEC and N-CAM.

9.2.4 Definition of treatment failure

Treatment failure is defined as: "Objective pathological clinical findings by the investigator, which are directly correlated with subjective patients complaints resulting in a deterioration of the subjective clinical outcome assessed by KOOS and the 5 KOOS subscores."

Clinical deterioration is defined as reduction in the KOOS score of > 10 compared to baseline. A non-responding to treatment is defined as improvement of < 10 in the KOOS score.

Furthermore, revision surgery rate independent of cause and deterioration of the subjective clinical outcome assessed by KOOS and the 5 KOOS subscores independent of correlation with IMPs will be assessed and analyzed.

9.2.5 Assessment of safety outcomes

9.2.5.1 Adverse events

All AEs (see Chapter 10 for definition) will be recorded in the eCRF. Among the expected adverse events are those associated with the application of the IMP (anesthesia & operation) as described in Chapter 3.7 under "Risks & Benefits". Other adverse events can be associated with surgical and medical procedures, arthralgia, joint effusion, decreased joint range of motion, and infections (joint, wound) or related to the IMP itself. These parameters will be checked at each follow-up visit or if the patient reports any kind of indisposition in between the visits.

All SAEs will be reported to the Sponsor-investigator or his designee according to the SOP of the Sponsor. Information in the reporting form includes: reporting center, Patient-ID, Information on surgery, other treatments already performed and details of the adverse event (affected site, symptoms, time of onset/end, time course, diagnosis and expected outcome (if possible)). The PI of the respective clinic as well as the Sponsor-investigator or his designee will perform an assessment on causality and decide on the necessary reporting.

All SAEs resulting in death will be reported to the ethical committee and the national competent authority as well as to Swissmedic within 7 days.

All SUSARs and SADRs will be reported by the Sponsor-Investigator to Swissmedic, the respective national competent authorities and the local ethical committee within 7 days in case of death; otherwise within 15 days, as well as to all investigators involved in the trial. Reports to local ethical committees will be performed by the PI of the respective site.

Any occurring adverse event will be documented and treated during and after the trial. The patients will be followed until side effects are resolved or stable for at least 3 months.

9.2.5.2 Laboratory parameters

Not applicable

9.2.5.3 Vital signs

Not applicable

9.2.6 Assessments in participants who prematurely stop the study

Depending on the time of withdrawal, participants prematurely leaving the study might enter the normal follow-up schedule for patients after knee operations performed with the standard procedure or will be scheduled for a final medical consultation for their own safety.

9.3 Procedures at each visit

9.3.1 Visit 1: Screening visit (-240 days or less)

The screening visit will take place after the informed consent has been signed. Blood will be taken from the patient and sent to the respective laboratories for analysis of the parameters listed in the inclusion and exclusion criteria. MRI can be performed if not available from a recent visit to an external physician. Questionnaires will be filled to see, if the patient fulfills inclusion and exclusion criteria. The visit should take place maximal 8 months before the treatment start (Biopsy harvesting). If all inclusion and exclusion criteria are met, the patient can be randomized.

9.3.2 Visit 2: Screening visit (Day -60 or less)

The baseline data for the questionnaires regarding the clinical scores (KOOS, EQ-5d) and an MRI will be acquired, if not already available. A physical examination of the knee joint will be performed according to the ICRS knee examination form. Provided that the patient fulfills all inclusion/exclusion criteria, the patient is accepted for the clinical trial and randomly assigned to a treatment group. The elapsed time between visit one and two should be a maximum of 6 months. Questionnaires must not be older than 6 months before start of treatment (biopsy harvesting). Otherwise they must be repeated. Screening visit 1 and 2 can be combined.

9.3.3 Visit 3: Harvesting of nasal cartilage biopsy and blood (Day -14 or -28)

The nasal cartilage and the blood will be harvested during an out-patient procedure, with the patient returning for a check-up and removal of the tamponade the next day. Additional serology testing (HIV 1 and 2, Hepatitis B, Hepatitis C, Syphilis) have to be performed.

9.3.4 Visit 4: Implantation (Day 0-5)

The patient will be admitted to the hospital on the day of implantation and will stay in hospital for an average of 5 days after their operation. During this time follow-up will be performed according to the standard post-operative procedures and results will be controlled and recorded in the patient's dossier. All AE will be recorded in the eCRF and graded.

9.3.5 Visit 5: Clinical follow up (day 42 (6w))

The recovery and clinical course will be checked and the patient will be asked about any AEs. The threads will be removed after 2 weeks. In addition, the patient will be asked about any consequences from the harvesting procedure of the nasal cartilage. Furthermore the patient will be asked about the ongoing physiotherapy.

9.3.6 Visit 6: Clinical follow-up and efficacy assessment (Day 90 (3m))

The wound healing will be checked and the patient asked about any AEs. In addition the patient will be asked about any consequences from the harvesting procedure of the nasal cartilage and the ongoing or completed physiotherapy. Furthermore an MRI/dGEMRIC will be performed to assess the stability

and remodeling of the graft. The renal function will be assessed before the contrast agent (Dotarem®) is given.

9.3.7 Visit 7-8: Clinical follow-up and efficacy assessment (Day 360 (12m), Day 720 (24m))

The wound healing will be checked and the patient asked about any AEs. In addition the patient will be asked about any consequences from the harvesting procedure of the nasal cartilage and the ongoing or completed physiotherapy. Furthermore the patient will be asked to fill out the questionnaires regarding the clinical scores (KOOS, EQ-5d) and an MRI/dGEMRIC will be performed to assess the stability and remodeling of the graft. The renal function will be assessed before the contrast agent (Dotarem ®) is given.

10. SAFETY

10.1 Drug studies

The Sponsor's SOPs provide more detail on safety reporting.

During the entire duration of the study (category C), all adverse events (AE) and all serious adverse events (SAEs) are collected, fully investigated and documented in source documents (patient's records) and case report forms (CRFs). Study duration encompasses the time from when the participant signs the informed consent until the last protocol-specific procedure has been completed, and includes a safety follow-up period of up to 2 years and post-trial up to 5 years.

10.1.1 Definition and assessment of (serious) adverse events and other safety related events

An **Adverse Event (AE)** is any untoward medical occurrence in a patient or a clinical investigation participant administered a pharmaceutical product and which does not necessarily have a causal relationship with the study procedure. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

A **Serious Adverse Event (SAE)** is classified as any untoward medical occurrence that:

- results in death,
- is life-threatening,
- requires in-patient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalization, but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious. [ICH E2A]

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasia or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

SAEs should be followed until resolution or stabilization. Participants with ongoing SAEs at study termination (including safety visit) will be further followed up until recovery or until stabilization of the disease after termination.

Assessment of Causality

Both Investigator and Sponsor-investigator make a causality assessment of the event in relation to the IMP. The causality is based on the question, whether there is a "reasonable possibility" or "no reasonable possibility" that the study treatment caused the event.

Unexpected Adverse Drug Reaction

An "unexpected" adverse drug reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for drugs that are not yet approved and Product Information for approved drugs, respectively).

Suspected Unexpected Serious Adverse Reactions (SUSARs)

The Sponsor-Investigator evaluates any SAE that has been reported regarding seriousness, causality and expectedness. If the event is related to the investigational product and is both serious and unexpected, it is classified as a SUSAR.

Assessment of Severity

All AEs will be classified according to the National Cancer Institute **Common Terminology Criteria for Adverse Events** (CTCAE) Version 4.03 (2010) as Grade 1 mild, 2 moderate, 3 severe, 4 life threatening.

10.1.2 Reporting of serious adverse events (SAE) and other safety related events

Reporting of SAEs

All SAEs must be reported immediately and within a maximum of 24 hours to the Sponsor-Investigator of the study or his substitute. The Sponsor-Investigator will re-evaluate the SAE.

SAEs resulting in death are reported to the local Ethics Committee (via local Investigator) within 7 days.

The other Ethics Committees involved in the trial as well as Swissmedic receive SAEs resulting in death via Sponsor-Investigator within 7 days.

Reporting of SUSARs

A SUSAR needs to be reported to the local Ethics Committee (local event via local Investigator) and to Swissmedic for category B and C studies (via Sponsor-Investigator) within 7 days, if the event is fatal, or within 15 days (all other events).

The Sponsor-Investigator must inform all Investigators participating in the clinical study of the occurrence of a SUSAR. All Ethics Committees involved in the trial will be informed about SUSARs in Switzerland via Sponsor-Investigator according to the same timelines.

All SAEs having a suspected link to the IMP administered (**S**erious **a**dverse **d**rug **r**eaction (SADR)) must be reported in the same way as SUSARs.

Reporting of Safety Signals

All suspected new risks and relevant new aspects of known adverse reactions that require safety-related measures, i.e. so called safety signals, must be reported to the Sponsor-Investigator within 24 hours. The Sponsor-Investigator must report the safety signals within 7 days to the local Ethics Committee (local event via local Investigator) and to Swissmedic in case of a category B or C study.

The Sponsor-Investigator must immediately inform all participating Investigators about all safety signals. The other Ethics Committees involved in the trial will be informed about safety signals via the Sponsor-Investigator.

Reporting and Handling of Pregnancies

Pregnant participants will be followed-up in the normal way, but will not be subjected to the dGEMRIC MRI due to the need to apply a contrast agent (Dotarem®). Further follow-up after the end of the study and the outcome of the pregnancy is not necessary.

Periodic reporting of safety

A development safety update report (DSUR) is submitted once a year to the local Ethics Committees and to the national authorities via Sponsor-Investigator.

For multicenter studies the DSUR contains information from all sites including information from sites outside Switzerland. The Sponsor-Investigator prepares it, and then submits it to the National Competent Authorities and participating Investigators.

10.1.3 Follow up of (Serious) Adverse Events

Any SAE or AE initiated within the study period (ongoing, completed or withdrawn) shall be followed until considered as resolved by the investigator. All efforts shall be made to gather laboratory and clinical data which will be reported in the CRF and the SAE narrative.

11. STATISTICAL METHODS

11.1 Hypothesis

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 (H0 : $KOOS_{N-TEC} - KOOS_{N-CAM} = 0$). The alternative hypothesis is that N-TEC and N-CAM differ significantly in terms of the primary endpoint (HA : $KOOS_{N-TEC} - KOOS_{N-CAM} = \square \square 0$). An absolute difference ($\square$) 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.

11.2 Determination of Sample Size

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 (Saris et al., 2008), 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. 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,...,21 = 40, ..., 160) was evaluated by sampling R = 999 times, $n_i/2$ KOOS scores from a normal distribution with $\square$ = 75 and $\square$ = 17 for the N-CAM group, and $n_i/2$ KOOS scores from a normal distribution with $\square$ = 75 + $\square$ and $\square$ = 17 for the N-TEC group. The size of $\square$ 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 - $\square$ = 0.8), at a significance level of $\square$ = 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% (Figure 11.2.1).

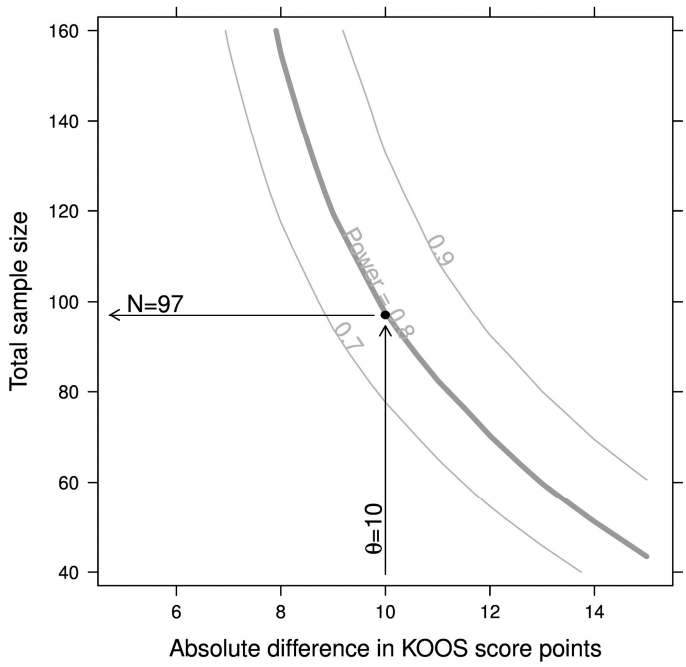


Fig. 11.2.1: Sensitivity of the sample size with regard to absolute difference in KOOS after 24 months (N-TEC vs. N-CAM), assuming a mean KOOS of 75 for N-CAM. An example is given based on an expected absolute difference of 10 score points (Curves are smoothed and are for illustrative purpose only)

11.3 Statistical criteria for termination of trial

Not applicable

11.4 Planned Analyses

11.4.1 Datasets to be analysed, analysis populations

The **full analysis set (FAS)** consists of all patients who were randomized, regardless of treatment conclusion or loss of follow-up (Intention To Treat principle – ITT).

The **per protocol set (PPS)** consists of all patients who have at least one follow-up measure. Patients in the PPS will be analyzed according to the treatment received without randomization.

The FAS will be used for the primary analysis and the secondary analyses as described. The PPS will be used for sensitivity analyses of the primary analysis and the secondary analyses as described.

Demographics and relevant baseline variables will be summarized for the FAS. Categorical data will be presented as frequencies and percentages. For continuous variables, the lower and upper quartile, the median, the mean and the standard deviation will be presented.

11.4.2 Primary Analysis

The 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.

Sensitivity analyses: 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.

11.4.3 Secondary Analyses

The following secondary endpoints will be analyzed:

• KOOS score at 12 months after surgery

• relative GAG-content (rel. □R1) 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.

11.4.4 Interim analyses

Adjustments are not necessary in this trial, since the application is a one-time procedure and there are no stopping guidelines foreseen for this trial. It is not expected, that the implant will have a negative systemic effect.

11.4.5 Safety analysis

All SARs and SUSARs are recorded. The annual safety report summarizes all SAEs, SARs and SUSARs. No other safety analysis is planned.

11.4.6 Deviation(s) from the original statistical plan

If substantial deviations of the analysis as outlined in this chapter are needed for whatever reason the protocol will be amended. All deviations of the analysis from the protocol or from the detailed analysis plan will be listed and justified in a separate chapter of the final statistical report.

11.5 Handling of missing data and drop-outs

Careful trial planning and conduct should minimize the occurrence of missing data as far as possible. We will consider bootstrap multiple imputation procedures for the analyses of the FAS. No imputation of missing values is planned for the PPS. Thus, if there are patients lost to follow-up visits, they will not be included in analyses of endpoints measured at later follow ups.

A drop-out rate of 10% was considered for sample size estimation.

All primary and secondary analyses described above will be repeated with the PPS and included as sensitivity analyses.

12. QUALITY ASSURANCE AND CONTROL

The Sponsor-PI or his designee is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs and working instructions at all sites of the multicenter study. All SOPs and working instructions will be written in English to ensure understanding and harmonized procedures for treatment, rehabilitation and follow-up at all sites. The PI of each site is responsible for proper training of all involved study personnel at his site.

The KOOS-questionnaire as a measure for the primary outcome will be included in the electronic CRF to be filled out by the patient directly at the site.

All investigators directly involved in the patient's information, study intervention and follow-up will have to provide the certificate of GCP-training and/or follow additional national and local requirements on investigator qualification.

12.1 Data handling and record keeping / archiving

Data will be collected only from patients who have given their written consent to our study. Their medical records will be handled confidentially. Only members of the study team, monitors and the respective authorities (ethical committees, competent authorities) will have access to the data.

An enrolment log will be kept at each site as well as a code list to link the patient's name with their patient ID. Documents will be stored according to the hospital regulations, but for a minimum of 30 years.

12.1.1 Case Report Forms

All data collected during this study will be directly recorded in an eCRF specific for the patient. This applies to follow-up data as well as to the questionnaires completed by the patient. Data that need to be entered after a follow-up visit (e.g. lab results from screening) will be entered in the eCRF by single data entry. All MRI data will be entered as a double data entry by Basel. Each physician of the trial will access the eCRF via the Internet using his own login and thereby be identified by the system. Only authorized personnel will receive a login. The eCRF will contain the following information:

1. Baseline characteristics
    - Patient ID and birthdate
    - History of injury
    - Patient data (age, BMI etc.)
    - Cartilage defect classification
    - Medical history (inclusion/exclusion criteria)
    - Laboratory results
    - Scores from questionnaires

2. Intervention variables
    - Data for harvesting of the nasal biopsy and blood
    - Data for implantation (assessment of graft, cartilage defect classification)
    - Follow-up regarding clinical examination

3. Outcome variables
    a) Clinical efficacy
        - Questionnaires (e.g. KOOS) at months 12, 24

    b) Graft stability and remodeling
        - MRI and dGEMRIC data at months 12, 24

    c) Safety
        - Adverse events/adverse drug reactions: graded according to severity (CTCAE Version 4.03/2010 as grade 1 mild, 2 moderate, 3 severe, 4 life threatening, 5 death), expectedness, and relationship to trial treatment

12.1.2 Specification of source documents

Source data will be available at all sites to document the existence of the study participants on each site. Source data will include the following documents:

- medical records of each patient (although for the most of them not part of the eCRF, these records are used to record all data routinely associated with the patient's treatment). These can be paper documents or electronic.
- informed consents of the patient for the study and any regular informed consents
- laboratory results (entered in eCRF after a visit)
- MRI results (entered in eCRF after a visit)
- Data directly recorded in the eCRF (e.g. questionnaires, patient data, intervention data). These can also be recorded on paper and entered in the eCRF later by single entry.

Location of Source data:

Switzerland:

Source data in paper form will be kept in the office of the PI under lock and key. Source data will also be stored electronically in the hospital system.

Germany:

Source Data will be kept in the office of the Study Coordinator, under lock and key. Source data will also be stored electronically in the hospital system. (Freiburg)

Source Data will be kept in the office of the Study Coordinator, under lock and key (Würzburg).

Italy:

Source data in paper form, as informed consent, will be kept in the Project Manager office, under lock and key. The medical records of patients will be stored in the hospital paper archive (located outside the hospital). Source data will also be stored electronically in the hospital system.

Croatia:

Study specific source documents will be stored at PI's office with restricted access.

12.1.3 Record keeping / archiving

Switzerland:

All study data must be archived for a minimum of 20 years after study termination or premature termination of the clinical trial. Archiving will be carried out according to hospital regulations.

Germany:

All Study Data must be archived for a minimum of 30 years (regarding transplantation act) after study termination or premature termination of the clinical trial. Archiving of study related documents will be performed in the hospitals archive.

Italy:

All study data must be archived for a minimum of 10 years after study termination or premature termination of the clinical trial. Archiving will be carried out according to hospital regulations.

Croatia:

All study data must be archived for a minimum of 20 years after study termination or premature termination of the clinical trial. Archiving will be carried out according to hospital regulations.

12.2 Data management

Data collected during the study, according to the study flowchart, will be stored in an electronic database "MyClinicalData" provided by the sponsor, generated and administered by Medacta International SA (Switzerland). Data entries are exposed to the restricted users through a web application. Each investigator will have access to the database through his/her personal page. The database let the investigator to create patient case schedule and fill out the eCRF previewed by the study protocol.

All the client interface data consists in HTML pages, previously generated by armoured server side programming levels. This architecture allows shielding the database access operations.

Due to the sensitivity of health issues, exchanging and collecting information brings along several concerns regarding privacy and confidentiality.

According to the Swiss Federal Law 235.1, about Protection of Personal Data, Medacta International SA has taken every measure possible to guarantee that the information, with which we are entrusted, is not misused or accessed, by accident or by malice, by any unauthorized persons. Medacta International has the right to treat only clinical data, following a written consent by the person involved and properly stored by the investigator(s) in charge. For details about the security access see ref chapter 12.2.2.

Several error preventing actions are implemented at each level of the software, increasing the data quality along with all the technical choices, which are oriented to provide a lean, topical and easily accessible tool. Further validation information is listed in chapter 12.2.4.

12.2.1 Data Management System

The system is used to collect clinical data with the purpose of monitoring medical devices, medicinal products, in compliance with European Directives. Medacta is responsible of the development and the maintenance of the software through internal developer and administrator profiles, along with the management of the internal functionality operations through internal clinical data operator profiles. Regarding the database system administration the responsibility is assigned to the external hosting provider SecurityLab. The software will be functionally checked by Medacta staff on an isolated environment during a test phase that previews the verification of all interface functionality for each user profile, analysing the tracing logs.

For hosting details ref. chapter 12.2.3.

12.2.2 Data security, access and back-up

The access to the data is strictly channelled through a limited set of user groups. Users can access the system data through a user-password authentication. Medacta staff manages the user creation and generates one-time codes to let the user complete his/her registration. Since the registration operation has been completed, the user login is active and can access the web application. The user can any time modify his/her personal password by accessing the web application.

Whenever a study person leaves the study, the study surgeon must notify it to the Medacta staff in order to block the related login and blinding all the data.

The physical and network security of the web service is left up to a hosting entity (SecurityLab) (see E Security Procedure 2015-05-28).

All the communication with the My Clinical data is encrypted (https). The users of the web application have to identify themselves to the system before accessing the data, several rules have been set up to limit the data that each user can view, to the strict necessary.

The web application is regularly checked for security flaws by a specialized company and multiple backup files that are treated by the security measures of the hosting company.

The physical and network security of My Clinical Data web service is maintained by SecurityLab (Switzerland). The server is physically housed in a datacenter in Ticino (Switzerland) in a dedicated rack. The provided service has carrier redundancy with the major national providers to guarantee continuity of service. The physical access to the server room is limited by badge. A video surveillance of the room is present. An alarm system with central monitoring 24/7 against intrusion, fire (RAS) and flooding is also present.

The network is protected by a firewall and a web application firewall which analyses the traffic against intrusions.

The firewall only allows web access to the server from outside via port 443 (https).

Web security is controlled by Symantec VeriSign certified SSL web server certificate with 256-bit encryption on the server.

The logs of the server are continuously monitored for suspicious traffic.

Web application maintenance access is restricted to the Medacta network.

12.2.3 Analysis and archiving

The entered data can be extracted any time by the users in electronic format attuo for statistic and planning purpose. In particular, the user can extract the calendar sheet with patient code list and related visit plan, and the data related to the eCRF previously filled out. The Administrator, Medacta International, may on specific demand provide a full data extraction of the study.

The data are stored in the online database internally developed by Medacta. The type and frequency of clinical data collection is in accordance with the relative study protocols approved by competent authorities. The clinical data of the patients are stored in a Medacta dedicated virtual server hosted and maintained by SecurityLab. The stored data are referenced using pseudo anonymous codes. The clinical data for an IMP product has to be stored for 30 years.

The clinical data could be eliminated from the database in advance, according to a subject specific request. The investigator will inform Medacta International of case code clinical data to be removed by the specific form. The clinical data of a specific case code or of the clinical study will be immediately not usable on the database but the clinical data removal procedure needs a technical delay of 30 days due to the complete elimination of all backups.

In case of damage of physical support, the hosting entity (Security Lab) follows an own procedure for physical destruction of the support.

12.2.4 Electronic and central data validation

The data submitted to the My Clinical Data is systematically verified for its consistency and if an error occurs no insertion will be made. Also the output data are checked for their consistency, to limit erroneous data retrieval. Duplications, data type incongruence, mandatory field check etc are preventively avoided by client side vehiculant fields, check controls, interface messages, data relational constraints and a robust programming core

12.3 Monitoring

Monitoring is the act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, Standard Operating Procedures (SOPs), Good Clinical Practice (GCP), Good Manufacturing Practice (GMP) and the applicable regulatory requirements.

The purpose is to verify that:

- The rights and well-being of the human subjects are protected
- The reported trial data are accurate, complete and verifiable from source documents
- The conduct of the trial is in compliance with the currently approved protocol/amendments, GCP, GMP and regulatory requirements
- The ongoing quality of the trial is continuously verified.

The monitoring will be performed by the respective CTUs (Clinical Trial Units) at the sites or by local CROs under the coordination and supervision of ECRIN-ERIC, with the CTU Basel as the lead CTU.

A monitoring plan as well as SOPs, describing in detail the documents and data to be monitored, will be established to guarantee a harmonized monitoring.

An initiation visit before the start of the trial, interim visits and a close-out visit at the end of the trial are foreseen for each site. Regular telephone conferences between the CTUs/CROs shall ensure harmonization of the procedures.

All source data and documents will be accessible to monitors and questions arising from monitoring can be answered by any participant.

12.4 Audits and Inspections

The responsible ethics-committees, authorities and the sponsor have the right to and may inspect the study and/or manufacturing site at any time prior to, during or after the clinical conduct. There will be no audits in addition to the monitoring and inspections. Study documentation and the source data/documents are accessible to auditors/inspectors (also CEC and CA) and questions will be answered during inspections. All involved parties must keep the participant data strictly confidential.

12.5 Confidentiality, Data Protection

Direct access to source documents will be granted for purposes of monitoring (12.3), audits and inspections (12.4) (ICHE6, 6.10) to the CTU and Swissmedic who will also have access to protocol, dataset, statistical code, etc. during and after the study. Other persons with access to source documents will be the treating physicians. Source documents will not be used for dissemination and publications and no patient data allowing the identification of the patient will be published.

12.6 Storage of biological material and related health data

Consent of the patient has to be obtained for the use of excessive cells and remains of the implants. Remains of IMPs (implants) after transplantation will be used for research if the patient has consented to this and will therefore be destroyed during analysis. Excessive cells not used for the generation of the implant will be transferred to Basel and used for research to study this and similar diseases. All biological materials given to the lab will be encoded. Only the Principal Investigators have access to the code. No connection is made between the biological material and patient data.

13. PUBLICATION AND DISSEMINATION POLICY

Information on the study will be disseminated through the public website of the project.

The publication of the results is planned to be by the submission of papers to peer-reviewed journals. In addition the results will be communicated at national and international conferences and seminars as well as through presentations for lay persons.

The results will also be published on educational platforms such as VUMEDI for clinical personnel.

Study results will be published in the registered databases.

14. FUNDING AND SUPPORT

14.1 Funding

The clinical study will be performed within the framework of the EU-H2020 Project BIO-CHIP (No. 681103) and mainly funded through the project. Partners in Switzerland will be funded through the contribution by SERI (State Secretariat for Education, Research and Innovation). There will be no additional costs for the patient and insurance companies.

14.2 Other Support

Personnel are in addition funded by the respective hospitals. Facilities and equipment are also partly funded by the respective hospitals.

15. INSURANCE

Insurance will be provided by the Sponsor. A copy of the certificate is filed in each investigator site file and the trial master file.

16. REFERENCES

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17. APPENDICES

1. IMP: IB
2. Case Report Form (CRF)
3. Patient Information and informed consent
4. Questionnaires if relevant for outcome (KOOS)

18. HISTORY OF CHANGES

Changes from the accepted version (V03) to the study protocol include the following:

page	old	new
Page 3/ Page 14 / Page 22/ Page 24	Universitätsklinikum Freiburg Clinic for Orthopedic and Trauma Surgery	Medical Center - University of Freiburg, Germany Department of Orthopedics and Trauma Surgery
Page 23 (laboratory)	Institut für Klinische Chemie und Laboratoriumsmedizin Universitätsklinikum Freiburg Hugstetter Str. 49 79106 Freiburg, Germany	Institute of Virology Hermann-Herder-Str. 11 79104 Freiburg Germany
Page 24 (Monitoring)	Universitätsklinikum Freiburg Studienzentrum, Projektkoordination Klinische Studien Elsässer Strasse 2, 79110 Freiburg, Germany	Medical Center - University of Freiburg, Germany Clinical Trials Unit Elsaesser Str. 2 79110 Freiburg Germany
Throughout the document pages 25-26	Landesbehörde	Competent local authority
Page 25	Bundesoberhörde	National Competent Authority
Page 35 (results of 10 first patients published)		The results from the first 10 patients are accepted for publication in “The Lancet” (Mumme et al, 2016)
Page 37 (inclusion of 2nd authority)	manufacturing authorization from Regierung von Oberfranken, Ansbach, Germany in consultation with PEI (Bundesoberbehörde)	manufacturing authorization from Regierung von Oberfranken, Ansbach, Germany in consultation with PEI (Bundesoberbehörde) or from Swissmedic, Switzerland depending on the manufacturing site.
Page 49 (inclusion of 2nd manufacturing site)	GMP production unit at Fraunhofer Institute, Germany (QP: Dr. Oliver Pullig)	GMP production unit at Fraunhofer Institute, Germany (QP: Dr. Oliver Pullig) or DBM clean room at University Hospital Basel, Switzerland (QP: PD Dr. Werner Krenger)
Page 50/51	Not existing	Include paragraph on Rehabilitation
Page 51 (NCA in Switzerland)	reviewed by the National Competent Authorities (GER)	reviewed by the National Competent Authorities (GER, Switzerland)

page	old	new
Page 53: 9.2.4.1 Adverse events		Adapted to SOP
Page 55: Assessment of causality	Table with causality grading deleted and causality assessment simplified	The causality is based on the question, whether there is a “reasonable possibility” or “no reasonable possibility” that the study treatment caused the event
Page 56	An annual safety report is submitted…	An annual safety report or development safety update report (DSUR) is submitted…
Page 61 (Data handling and record keeping, archiving)	Minimum of 20 years	Minimum of 30 years
Page 67-68		Inclusion of a new reference Mumme et al 2016

Changes from the accepted version (V04) to the study protocol include the following:

Due to both products (N-TEC and N-CAM) being classified as Tissue engineered products from a regulatory point of view the terms “tissue therapy” and “cell therapy” have been replace throughout the protocol with the terms reflecting the maturation stage of the cartilaginous tissue: “immature” (N-CAM) and “mature” (N-TEC).

page	old	new
3/14/22	Prof. Dr. Philipp Niemeyer	Dr. Tayfun Yilmaz
10	MOCART Score (MRI): The MRI will be performed at 3, 12, 24 months follow-up visits and MOCART scores calculated.	MOCART and 3D MOCART Scores (MRI): The MRI will be performed at 3, 12, 24 months follow-up visits and MOCART and 3D MOCART scores calculated.
10		• Additional secondary endpoint is the number of treatment failure at 24 months. The difference will be compared between the two groups.
40/54/55/57		Dotarem®
43		Additionally the number of treatment failure at 24 months will be analyzed. Treatment failure is defined as objective pathological clinical findings by the investigator directly correlated with subjective patients complaints resulting in a deterioration of the subjective clinical outcome assessed by KOOS and the 5 KOOS subscores. In case of treatment failure, a root cause analysis

page	old	new
		will be performed. A clinical deterioration is defined as reduction in the KOOS score of > 10 compared to baseline. A non-responding to treatment is defined as improvement of < 10 in the KOOS score.
44/45	…the MOCART Score…	…the MOCART and 3D MOCART Scores…
46	- Patient has an opposite intact (ICRS Grade 1) articulating joint surface (no "kissing lesions"). - Patient has an intact meniscus (maximum 1/3-resection). - Patient has an onset of symptoms of < 5 years	- Patient has an opposite intact (ICRS Grade 2) articulating joint surface (no "kissing lesions"). - Patient has an intact meniscus (maximum 1/2-resection). - deleted - Patient has free range of motion of the affected knee joint or 10° of extension and flexion loss
47	Patient has a body mass index (BMI) >30 kg/m^2	Patient has a body mass index (BMI) >35 kg/m^2
53		**9.2.4 Definition of treatment failure** Treatment failure is defined as: "Objective pathological clinical findings by the investigator, which are directly correlated with subjective patients complaints resulting in a deterioration of the subjective clinical outcome assessed by KOOS and the 5 KOOS subscores." Clinical deterioration is defined as reduction in the KOOS score of > 10 compared to baseline. A non-responding to treatment is defined as improvement of < 10 in the KOOS score. Furthermore, revision surgery rate independent of cause and deterioration of the subjective clinical outcome assessed by KOOS and the 5 KOOS subscores independent of correlation with IMPs will be assessed and analyzed.
54	…(KOOS, EQ-5d) and an MRI/dGEMRIC will be acquired, if not already…	…(KOOS, EQ-5d) and an MRI will be acquired, if not already…
69	In press. The lancet 2016	. Lancet 388:1985-94 (2016).

Changes from the accepted version (V05) to the study protocol include the following:

page	old	new
6/12/14/15/16/24/25/27/45/48/50 (the new center/ethic committee has been added throughout the protocol)		Clinical center Orthopädische Klinik König-Ludwig-Haus Julius-Maximilians-Universität Würzburg Brettreichstrasse 11 97074 Würzburg Germany Ethics committee: Ethical committee of the medical faculty of the university of Würzburg
14	01/2019: Treatment of last patient 01/2021: 24 months (final) follow-up of last patient	10/2019: Treatment of last patient 10/2021: 24 months (final) follow-up of last patient
16	Amir Steinitz	Sebastian Müller
22/55	First visit: 8 months (2w delay) Second visit: 2 months (2w delay) needs to be filled at visit 2, if not done, but minimal 2 months before implantation	First visit: □8 months Second visit: □2 months needs to be filled at visit 2, if not done, but not older than 6 months before implantation
24 (labs added for serology for Switzerland (second serology) and Germany (Würzburg)		Switzerland: Second serology: (day of harvesting) Institut für Virologie und Immunologie, Universität Würzburg, Versbacher Strasse 7, 97078 Würzburg Germany (Würzburg): Institut für Virologie und Immunologie, Universität Würzburg, Versbacher Strasse 7, 97078 Würzburg Germany
29	…within the Annual Safety Report (ASR).	…within the Development Safety updated report (DSUR).
31	Chondro-Celect ®	Spherox®
37/38		Results phase I updated (Clinical and radiological scores, addition of Table with Adverse events)
41 (added)		Plate removal in case of

page	old	new
		necessary osteotomy
42	Risk control/mitigation: • Check for microbiological and endotoxin contamination and mycoplasma in the supernatant of the biopsy after transport Risk: minor Mitigation: abort of the transplant generation	Risk control/mitigation: • Check for microbiological contamination in the supernatant of the biopsy after transport • Addition of antibiotics in the transport medium and first week of expansion Risk: acceptable Mitigation: -
57 (added)	Visit 1: Screening visit (-240 days) 1.1.1 Visit 2: Screening visit (Day -60)	Visit 1: Screening visit (-240 days or less) Questionnaires will be filled to see, if the patient fulfills inclusion and exclusion criteria. The visit should take place maximal 8 months before the treatment start (Biopsy harvesting). If all inclusion and exclusion criteria are met, the patient can be randomized. Visit 2: Screening visit (Day -60 or less) The elapsed time between visit one and two should be a maximum of 6 months. Questionnaires must not be older than 6 months before start of treatment (biopsy harvesting). Otherwise they must be repeated. Screening visit 1 and 2 can be combined.
60	An annual safety report or development safety update report (DSUR) is submitted once a year to the local Ethics Committee via local Investigator and to Swissmedic in case of a category B or C study via Sponsor-Investigator. For multicenter studies the annual safety report contains information from all sites including information from sites outside Switzerland. The Sponsor-Investigator prepares it, and then submits it to the National Competent Authorities and participating Investigators who will submit it to their local committees.	A development safety update report (DSUR) is submitted once a year to the local Ethics Committees and to the national authorities via Sponsor-Investigator. For multicenter studies the DSUR contains information from all sites including information from sites outside Switzerland. The Sponsor-Investigator prepares it, and then submits it to the National Competent Authorities and participating Investigators.

page	old	new
65	medical records of each patient … • Data directly recorded in the eCRF (Questionnaires, patient data, intervention data).	• medical records of each patient … These can be paper documents or electronic. • Data directly recorded in the eCRF (e.g. questionnaires, patient data, intervention data). These can also be recorded on paper and entered in the eCRF later by single entry.
72 (added)		Mumme M, Steinitz A, Nuss KM, Klein K, Feliciano S, Kronen P, Jakon M, Von Rechenberg B, Martin I, Barbero A, Pelttari K. Regenerative potential of tissue engineered nasal chondrocytes in goat articular cartilage defects. Tissue Eng-A 22:1286-1295 (2016)

Changes from the accepted version (V06) to the study protocol-V07 include the following:

page	old	new
13/17/18/19/45/49/50	cartilage lesions on the femoral condyle and/or trochlea of the knee	cartilage lesions on the femoral condyle and/or trochlea and/or patella of the knee
11/56		Subgroup analysis to compare the outcome with regard to the localization of the defect (patella vs. femoral condyle/trochlea)
40		From standard therapies it is known that localized defects on the patella on average have higher complication rates and clinically inferior outcomes as compared to defects on the femoral condyle or trochlea. For N-CAM and N-TEC treatment, there are no clinical data available yet concerning the outcome of treatment for patella defects.
36/37		Inclusion of table summarizing all clinical studies with N-TEC and N-CAM
40/41		Inclusion of preliminary results from current phase II study and

page	old	new
		reference to IB
49	Patient has a relevant meniscus tear. Partial meniscal removal allowed, if not exceeding 1/2. Suture is not allowed in parallel, but if successful, cartilage treatment might be added 12 months later.	Suture of meniscocapsular separation is allowed. Suture of meniscus tear is allowed if the same compartment is not afflicted by symptomatic cartilage injury, and the graft is planned for trochlea and /or patella and /or contralateral compartment. If the same compartment is afflicted,
50	Patient has an osteochondral defect (bony substance defect of >3mm depth). Bone marrow edema is allowed.	Patient has an osteochondral defect which cannot be reconstructed (bony substance defect of >3mm depth need to be reconstructed with autologous bone graft from tibia or iliac crest). Bone marrow edema is allowed.