06 November 2025
EMA/CAT/216988/2025
Committee for Advanced Therapies (CAT)

Withdrawal assessment report

Jelrix

International non-proprietary name: Autologous cartilage-derived articular chondrocytes, in-vitro expanded

Procedure No. EMEA/H/C/004594/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

ACI	Autologous chondrocyte implantation
ACI-C	Collagen membrane covered ACI
ACI-P	Periosteal ACI
ACL	Anterior cruciate ligament
ACT	Autologous chondrocyte transplantation
ADME	Absorption, distribution, metabolism, and excretion
AE	Adverse event
AESI	Adverse event of special interest
ALP	Alkaline phosphatase
AMIC	Autologous matrix-induced chondrogenesis
AR	Adverse reaction
ATMP	Advanced therapy investigational medicinal product
BMI	Body mass index
BOCF	Baseline observation carried forward
BTPEG	α,ω-bisthio-polyethylene glycol
CfB	Change from baseline
CI	Confidence interval
cm^2	Square centimetre
CSP	Clinical study protocol
CSR	Clinical study report
C_t	The Ct or threshold cycle value is the cycle number at which the fluorescence generated within a reaction crosses the fluorescence threshold, a fluorescent signal significantly above the background fluorescence. At the threshold cycle, amplified product is first visible in the data. For visualisation of PCR products, the number of product molecules must exceed the detection limit of the reaction (at Ct, approximately 1011 to 1012 product molecules are present in the reaction). The threshold cycle is inversely proportional to the original relative expression level of the gene of interest.
DGOU	German Society for Orthopaedics and Traumatology (Deutsche Gesellschaft für Orthopädie und Unfallchirurgie)
DLP	Data lock point
DMEM	Dulbecco's modified Eagle's medium

DMSO	Dimethyl sulphoxide
DVT	Deep vein thrombosis
EQ VAS	Visual analogue scale provided by EuroQoL group
EQ-5D-5L	EuroQoL Group descriptive system of health-related quality of life states consisting of 5 dimensions with each 5 responses/levels of severity
FAS	Full analysis set
FSE	Fast spin echo
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GCP	Good clinical practice
GLCM	Gray-level co-occurrence matrix
ICH	International Council on Harmonization
ICRS	International Cartilage Regeneration & Joint Preservation Society
iHOT	International hip outcome tool
IKDC	International Knee Documentation Committee
IMP	Investigational medicinal product
IPC	In-process control
ITT	Intent to treat
IUDR	Imputation using drop-out reason
HA	Hyaluronan
KOOS	Knee injury and osteoarthritis outcome score
LAL	Limulus amoebocyte lysate
LS	Least squares
LOCF	Last observation carried forward
M-ACI	Matrix-associated autologous chondrocyte implantation
MACT	Matrix-associated autologous chondrocyte transplantation
MAHSA	Maleimido human serum albumin
MFx	Microfracture technique (according to Steadman)
Mio.	Million
ml	Millilitre
mm	Millimetre
MMRM	Mixed effect model for repeated measurements
MOCART	Magnetic resonance observation of cartilage repair tissue

MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
ms	Milliseconds
n	Number of observations/ frequency count
N.a.	Not applicable
NIS	Non-interventional study
NSAID	Non-steroidal anti-inflammatory drug
OA	Osteoarthritis
OATS	Osteochondral autograft transfer system
OCD	Osteochondritis dissecans
OCT	Osteochondral transplantation
OR	Odds ratio
PDCO	Pediatric Committee
PEI	Paul Ehrlich Institute
Ph.Eur.	Pharmacopoeia Europaea
PIP	Pediatric investigational plan
PP	Per-protocol
PRO	Patient reported outcome
PSM	Propensity score matching
PSUR	Periodic safety update report
PT	Preferred term
Q1, Q2, Q3, Q4	First, second, third, fourth quarter
QoL	Quality of life
RCT	Randomized controlled trial
Rec	Recreation
RT-qPCR	Quantitative real time polymerase chain reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SD	Standard deviation
SmPC	Summary of product characteristics
SOC	System organ class

T2	Transverse relaxation time
TEAE	Treatment-emergent adverse event
TESAE	Treatment-emergent serious adverse event
VAS	Visual Analogue Scale

1. Background information on the procedure

1.1. Submission of the dossier

The applicant TETEC Tissue Engineering Technologies AG submitted on 21 November 2023 an application for marketing authorisation to the European Medicines Agency (EMA) for Jelrix, through the centralised procedure falling under Article 3(1) - Indent 1a - Advanced Therapy Medicinal Product as defined in Article 2 of Regulation (EC) No 1394/2007.

The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 21 July 2022

The applicant applied for the following indication:

Repair of symptomatic, localised, full-thickness cartilage defects of the knee joint (International Cartilage Regeneration & Joint Preservation Society (ICRS) grade III or IV) with defect sizes up to 12 cm2 in adults and adolescents with closed epiphyseal growth plate in the affected joint.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8(3) of Directive 2001/83/EC - complete and independent application. The applicant indicated that Autologous cartilage-derived articular chondrocytes, in-vitro expanded was considered to be a new active substance.

The application submitted is

composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies)

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0315/2021 on the agreement of the granting of a (product-specific) waiver.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's request(s) for consideration

1.5.1. New active Substance status

The applicant requested the active substance Autologous cartilage-derived articular chondrocytes, in-vitro expanded contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.5.2. Scientific recommendation on Classification

The applicant TETEC Tissue Engineering Technologies AG submitted on 21 November 2023 an application for Scientific recommendation on Classification to the European Medicines Agency (EMA) for Jelrix, which was designated as an Advanced Therapy Medicinal Product on 21 July 2022.

1.6. Scientific advice

The applicant received the following Scientific Advice on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
25 June 2016	EMEA/H/SA/3345/1/2016/ADT/II EMEA_H_SA_3345_1_2016_ADT_II.doc	Ferran Torres, Olli Tenhunen
17 October 2019	EMEA/H/SA/3345/2/2019/ADT/II NOVOCART Inject_FAL_3345-2-2019.pdf	Markku Pasanen, Karl-Heinz Huemer

The Scientific Advice pertained to the following quality, non-clinical and clinical aspects:

EMEA/H/SA/3345/1/2016/ADT/II - Clinical development

- The proposed plan for an uncontrolled, open-label design; primary and secondary endpoints; assessment of the primary endpoint at 18 months; statistical plan; efficacy and safety data package for MAA; safety monitoring; the proposed inclusion of paediatric patients with closed epiphysis.

EMEA/H/SA/3345/2/2019/ADT/II – Quality and Non-clinical development

- Whether data and knowledge from the development of the comparable product NOVOCART 3D are also relevant for the active substance (AS) of NOVOCART Inject; agreement to on manufacturing aspects and risk minimisation measures with regard to the use of human serum agreement that data from manufacturing of NOVOCART Inject for the Germany market are suitable for manufacturing process validation for centralised MAA; agreement that human serum albumin represents an appropriate starting material for maleimido human serum albumin (MAHSA) manufacturing; agreement that NOVOCART Inject should not be considered as a medicinal product derived from human blood or human plasma; whether the components of the hydrogel can be

considered as excipients for NOVOCART Inject manufacturing and relevant information should therefore be provided in CTD-Module 3.2.P.4; agreement that the mixture of hyaluronic acid and MAHSA can be prepared in batch size and that use of the batch items for NOVOCART Inject manufacturing replaces preparation of the reference product for release testing.

- Agreement that no additional animal study is required to demonstrate the pharmacological effect of NOVOCART Inject; agreement that the tumourigenicity study performed during NOVOCART 3D together with toxicity assessment according to DIN EN ISO 10993 is appropriate for non-clinical toxicity evaluation for NOVOCART Inject.

1.7. Steps taken for the assessment of the product

The CAT Rapporteur and Co-Rapporteur appointed by the CHMP were:

CAT Rapporteur: Maria Luttgen CAT Co-Rapporteur: Berendina Maria van den Hoorn

The application was received by the EMA on	21 November 2023
The procedure started on	28 December 2023
The CAT Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	19 March 2024
The CAT Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	02 April 2024
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	27 March 2024
The PRAC agreed on the PRAC Assessment Overview and Advice to CAT during the meeting on	11 April 2024
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	19 April 2024
The applicant submitted the responses to the CAT consolidated List of Questions on	16 January 2025
The CAT Rapporteur circulated the Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	25 February 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	13 March 2025
The CAT agreed on a list of outstanding issues <in writing and/or in an oral explanation> to be sent to the applicant on	21 March 2025
The applicant submitted the responses to the CAT List of Outstanding Issues on	13 May 2025
The CAT Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT and CHMP members on	28 May 2025

The outstanding issues were addressed by the applicant during an oral explanation before the CAT during the meeting on	12 June 2025
The CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a marketing authorisation to Jelrix on	18 July 2025
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a marketing authorisation to Jelrix on The detailed explanations of the scientific grounds for the differences between the draft opinion adopted by the CAT and the CHMP opinion adopted by the CHMP are annexed to the CHMP opinion.	24 July 2025
Furthermore, the CAT and CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	24 July 2025

1.8. Steps taken for the re-examination procedure

The CAT Rapporteur and Co-Rapporteur appointed by the CHMP were:

CAT Rapporteur: Sol Ruiz CAT Co-Rapporteur: Jan Mueller Berghaus

The applicant submitted written notice to the EMA, to request a re-examination of Jelrix CHMP opinion of 24 July 2025, on	04 August 2025
The CHMP appointed Sol Ruiz as CAT Rapporteur and Jan Mueller-Berghaus as CAT Co-Rapporteur on	18 September 2025
The applicant submitted the detailed grounds for the re-examination on	24 September 2025
The re-examination procedure started on	25 September 2025
The CAT Rapporteur's re-examination assessment report was circulated to all CAT and CHMP members on	07 October 2025
The CAT Co-Rapporteur's assessment report was circulated to all CHMP members on	07 October 2025
The CAT adopted the AR and the list of questions to AHEG on	13 October 2025
The CAT Rapporteur circulated the Joint Assessment Report on the detailed grounds for re-examination to all CHMP members on	14 October 2025
The CHMP agreed on the CAT Assessment Report and list of questions to the AHEG on	16 October 2025
AHEG was convened to address questions raised by the CAT and CHMP on	31 October 2025

The CAT considered the views of the AHEG as presented in the minutes of this meeting	6 November 2025
The detailed grounds for re-examination were presented by the applicant during an oral explanation before the CAT on	6 November 2025
The CAT, in the light of the scientific data available and the scientific discussion within the Committee, re-examined its initial opinion and in its final opinion concluded that the application did not satisfy the criteria for authorisation and did not recommend the granting of the marketing authorisation on	6 November 2025
The application was withdrawn on	11 November 2025

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Cartilage defects and degenerative joint disorders are very common. Damage to articular cartilage tissue can be caused by trauma, degeneration or the separation of an articular cartilage subchondral bone segment from the remaining articular surface. Symptoms include pain, swelling, effusions, stiffness and joint locking, depending on the amount of load carried by the injured area. This may cause significant functional impairment, often limiting one's ability to work, play sports, and perform activities of daily living. Furthermore, progressing focal cartilage lesions of the knee are associated with the development of osteoarthritis (OA) over time. The risk for the development of OA increases with the degree and chronicity of the initial cartilage lesion. Adequate repair of cartilage defects may prevent further cartilage loss, early onset of knee OA and to delay the need for total knee replacement.

The proposed indication is: Repair of symptomatic, localised, full-thickness cartilage defects of the knee joint (International Cartilage Regeneration & Joint Preservation Society (ICRS) grade III or IV) with defect sizes up to 12 cm^2 in adults and adolescents with closed epiphyseal growth plate in the affected joint.

2.1.2. Epidemiology

Articular cartilage defects of the knee are a very common orthopaedic problem. Traumatically caused chondral lesions are one of the main reasons for osteoarthritis, which is one of the major disabling disorders and affects more than 10% of the Western population.

2.1.3. Aetiology and pathogenesis

Articular cartilage provides a low-friction gliding surface with underlying shock-absorbing and force-guiding layers promoting smooth, frictionless movements of joints. Healthy articular cartilage contains up to 80% of water and only few chondrocytes. The ability of articular cartilage to regenerate is very limited, especially in

adults (Gaissmaier et al., 2006; Niemeyer et al., 2016a). In the knee, articular cartilage covers the ends of the bones and the inner surface of the patella.

Damage to articular cartilage tissue (by trauma, degeneration or the separation of an articular cartilage subchondral bone segment from the remaining articular surface [osteochondritis dissecans, OCD]) can cause irreversible structural degeneration. Since cartilage defects usually do not heal, they often progress to higher grade lesions. Progressing focal cartilage lesions of the knee are associated with the development of osteoarthritis (OA) over time. The risk for the development of OA increases with the degree and chronicity of the initial cartilage lesion (Angele et al., 2022; Dell'Accio and Vincent, 2010; Gaissmaier et al., 2006; Niemeyer et al., 2016a; Pettit et al., 2021).

2.1.4. Clinical presentation, diagnosis

Articular cartilage defects of the knee can lead to chronic joint complaints such as pain, swelling, effusions, stiffness and joint locking. Symptoms may cause significant functional impairment, often limiting one's ability to work, play sports, and perform activities of daily living.

Diagnosis is made through magnetic resonance imaging (MRI) and/or arthroscopy.

2.1.5. Management

Treatment options for cartilage defects depend on the symptoms, defect size and amount of defect sites as well as patient's age and activity level. Conservative treatment includes analgesics and physical therapy. As a next step, arthroscopic debridement and lavage are often performed. If symptoms (e.g. pain) persist and become more problematic, surgery is typically recommended. The currently available surgical options for the treatment of symptomatic localised, full-thickness cartilage lesions can be divided into reparative (e.g., bone marrow stimulation [BMS] techniques) and restorative procedures (e.g., osteochondral transfer and chondrocyte implantation). Reparative cartilage procedures, such as microfracture, stimulate fibrocartilaginous repair, whereas restorative procedures, aim to replace the defective articular surface with cartilage of hyaline properties. Bone marrow stimulation techniques are only recommended for the treatment of smaller defects (<4 cm^2).

Different autologous chondrocyte implantation (ACI) methods have been developed since 1987. A few ACI products have been approved via the centralised procedure. Some had been approved, but were withdrawn. Currently, the only M-ACI product approved via the centralised procedure in the EU is Spherox® which received marketing authorisation in July 2017 (https://www.ema.europa.eu/en/medicines/human/EPAR/spherox).

2.2. About the product

Jelrix is in vitro expanded autologous human articular chondrocytes embedded in a hyaluronan-containing cross-linking albumin hydrogel. Two surgical interventions are necessary for treatment with Jelrix: a tissue procurement (autologous cartilage biopsy) as well as the actual implantation performed via a minimally invasive approach (arthroscopy or mini-arthrotomy). The amount of Jelrix to be administered depends on the size of the cartilage defect that has to be filled.

The proposed indication is:

Repair of symptomatic, localised, full-thickness cartilage defects of the knee joint (International Cartilage Regeneration & Joint Preservation Society (ICRS) grade III or IV) with defect sizes up to 12 cm^2 in adults and adolescents with closed epiphyseal growth plate in the affected joint.

Jelrix meets the definition of tissue engineered products as laid down in article 2(1b) of Regulation (EC) No 1394/2007 and thus meets the criteria of an Advanced Therapy Medicinal Product (ATMP) as laid down in article 2(1a) of said regulation. Moreover, classification as a tissue-engineered product according to article 2(1b) of Regulation 1394/2007 has been confirmed by the European Medicines Agency (EMA) (EMA/CAT/635656/2015). Therefore, the applicant applies for marketing authorisation via the centralized procedure on the legal basis of Article 8(3) of Directive 2001/83/EC and according to Article 3(1) of Regulation (EC) No. 726/2004.

2.3. Type of Application and aspects on development

2.4. Quality aspects

2.4.1. Introduction

The finished product (FP) is an advanced therapy, tissue engineered product presented as dispersion and solution for implantation gel containing 8 – 32 x 10^6 *in vitro* expanded autologous human articular chondrocytes as active substance (AS).

Jelrix finished product (FP) comprises two components: the cellular component (Component A) and the 'cross-linking agent' α,ω-bis-thio-polyethylene glycol (BTPEG) (Component B) that forms the implantation gel *in situ* at the site of administration.

Other ingredients are:

Component A:

Dulbecco's Modified Eagles Medium (DMEM)/Ham's F12 medium

gamma irradiated human AB serum

- sodium caprylate

- N-acetyltryptophanate

maleimido human serum albumin (MAHSA)

Ostenil plus:

- sodium hyaluronate, mannitol, sodium chloride, disodium phosphate and sodium dihydrogen phosphate

Component B:

α,ω-bis-thio-polyethylene glycol (BTPEG)

HCl (for pH-adjustment)

sodium chloride

water for injections

The product is shipped in a protective polystyrene container which holds two glass vials: Vial A (cellular component) and Vial B (BTPEG cross-linking agent). Both vials are closed with rubber injection stoppers. The closed vial is crimped with a flip-off seal system manufactured from polypropylene homopolymer and aluminium strips for pharma caps.

2.4.2. Active Substance

2.4.2.1. General Information

The active substance (AS) of Jelrix is *in vitro* expanded autologous chondrocytes. The cells are isolated from cartilage tissue biopsies harvested from the knee joint of the patient for whom the corresponding final product is intended.

Because of the nature of the active substance - neither a structural formula nor a description of general physico-chemical properties is applicable. However, comprehensive general information on the biological properties of chondrocytes is provided. Background information relevant for the proposed mechanism of action is provided.

Phenotypic appearance as presented in the dossier shows the typical chondrocyte morphology when cultured under adherent monolayer conditions. Cell size of chondrocytes is not specified. However, as chondrocyte size in native cartilage tissue varies from 10 to 30 µm, this is considered acceptable. In addition, the properties of different phenotypes of chondrocytes and the selection of markers for the potency testing are described.

2.4.2.2. Manufacture, process controls and characterisation

Manufacturer(s)

The complete active substance manufacturing and all analytical testing is taking place at one single site:

TETEC Tissue Engineering Technologies AG, Aspenhaustrasse 18, 72770 Reutlingen, Germany.

The applicant provided valid EudraGMDP references for the MIA/GMP certificates and QP declaration.

Procurement of autologous cartilage biopsies is performed and coordinated by TETEC AG by quality agreements with cooperating hospitals in EU to ensure compliance with 2004/23/EC, 2006/17/EC, 2006/86/EC, and 2015/565. This is endorsed. TETEC is also stated to hold a Tissue Establishment licence issued by the German Medicinal Products Act (AMG). In cases where TETEC is not the responsible tissue establishment, a qualified institution is contracted to act on TETEC's behalf. Only authorized qualified tissue establishment as laid down in 2004/23/EC are contracted. National legal requirements of each country in accordance with health authorities is also be followed.

Description of manufacturing process and process controls

Overall, the descriptions and charts are generally sufficiently detailed to describe the manufacturing and sampling points for process controls (in-process control (IPC) and AS release) and to allow for assessment.

The standard manufacturing procedure is a continuous process without intermediates. The starting material consists of autologous cartilage biopsies. Upon arrival of this material at the manufacturing site, the cartilage biopsies (and an accompanying blood sample for serology testing) undergo an incoming inspection including packaging, labelling, and integrity checks before a batch number is set. TETEC also state that transport time and temperature is verified.

Overall, the manufacturing process steps have been sufficiently described. In Process Controls (IPCs) (parameters and tests) are included in the detailed flow charts.

A table with consumables used in the process has been included with a statement of the material and sterility.

In addition to the standard manufacturing process of Jelrix, the dossier also includes an optional parallel process which introduces a cryopreservation step. This alterative process step is described and included in the flow charts in S.2.2. This optional freezing step is justified by the short shelf-life of the product and reserved for unexpected cases when surgery/administration has to be postponed which would make treatment in due time impossible. It is stated to be used in unpredictable circumstances (e.g. due to patient illness) to adapt the timing of the finished product manufacturing and thereby avoid product loss.

In support of cryopreservation, the applicant has appended an R&D report "Effect of cryopreservation and storage of chondrocytes in liquid nitrogen on the quality of test samples representing NOVOCART Inject plus implantation matrix". This report includes a side-by-side comparison with split material from 7 donors. To further support the feasibility of the cryopreservation step, the applicant initially provided information on 401 historical batches that had been produced with a frozen intermediate step. The data show that 395/401 fulfilled the specification that were in place at the time of the initial application and that no common root cause to the OOS were detected. The applicant was then asked to also retrospectively compare the 401 cryopreserved batches with the Jelrix phase III batches and discussed data it in relation to current updated new specification limits, the dot-plots show that the cryopreserved batch results generally overlap with phase III clinical batches, but that some differences also can be observed. The differences have been discussed and justified from a comparability perspective. The applicant is asked to comment how the batches were numbered/grouped and if any material changes were introduced between these two groups (n <250 vs n>250) that could explain the trends seen and that would be relevant to be discussed in relation to the commercial process (**OC**).

In general, parallel manufacturing processes are not endorsed due to the risks of introducing product variability, however, considering the risk of having to waste autologous patient material in combination with the invasive nature of acquiring new starting material, the justification for an optional freezing step may be deemed acceptable from an ethical perspective if used in exceptional cases. Still, considering that cryopreservation increases the risk of out of specification results, the applicant is asked to explain how it is ensured that cryopreservation will only be used as a last option (where batches otherwise would have been discarded) and not as part of a more flexible/routine option (**OC**). The applicant's proposition to further investigate, develop, and re-validate the optional cryopreservation step is endorsed and should be submitted when completed.

IPCs and process parameters related to the cryopreservation steps have been adequately described. A visual inspection of the cell culture is performed, and cell counts/viability are controlled before freezing and after thaw with adequate limits.

In support of the storage time of the cryopreserved intermediate (maximum 6 month), the applicant has compared cell counts and viability of the 401 produced cryopreserved batches. Although this is not a trend

following study, no negative tendencies could be observed between batches that had been stored for different time periods up to >300 days and the proposed storage can be considered justified.

Control of materials

The donors of the autologous starting material are screened and tested in accordance with 2004/23/EC together with the implementing and amending Directives 2006/17/EC, 2006/86/EC, and 2015/565/EC. Any sero-positive batches are quarantined and manufactured in a dedicated quarantine incubator and with decontamination programs of the isolator in place to exclude cross-contamination risks.

The procurement of the cylinders of articular cartilage is performed by trained personnel at the clinic. The biopsy is placed in a PET bottle and sealed with an HDPE closure. The tissue is transported in an insulation box with TIC system cooling elements. A maximum time between procurement of the cartilage-bone cylinders and start of its processing has been set to 7 days, maximum transport time is 72h, and transport/storage temperature is 2-8 C.

A list of raw materials used in the process has been provided, including their origin and the attributes that are controlled either by in-house testing or tests included on the certificate of analysis (CoA). Representative certificates for the reagents used for the active substance manufacturing has been appended. The quality grade of all non-compendial raw materials is indicated and use of any research grade materials is adequately described. Source (supplier/vendor) for each raw material is included. The raw materials are generally regarded to be sufficiently justified.

Information on the control of stock solutions () and of cell culture solutions (culture medium, formulation medium, cryopreservation medium, and TrypLE select) are provided. The in-house sterile filtration of stock solutions has been detailed and is generally considered adequately controlled.

Materials of bacterial/cellular/animal/human origin

TSE-EDQM certficates and CoAs/COOs have been provided.For assessment of TSE risk and viral safety, information can be found in adventitious agents safety evaluation section.

The A and AB serum used as raw materials and excipient are described. Information on the plasma collection sites (including inspection status), donor screening, manufacturing steps, and a description of the virus control strategy has been adequately provided. CoAs have been included showing that the testing covers an acceptable panel of both NAT and antibody testing. It should however be confirmed that CE-marked test kits are used for serology testing of A and AB serum or alternatively it should be justified that equivalent validated tests are applied. As an additional viral risk reduction, gamma-irradiation of the human A and AB serum is performed. Literature references supporting the effectivity of gamma-irradiation to reduce virus contaminants have been provided. Certificates of Irradiation of the serum are attached. The applicant has confirmed that a traceability system from donors of material used for the preparation of A and AB serum to the finished product exist and that records will be kept for 30 years.

The use of A serum in the manufacturing has been briefly discussed and evaluated. It is acknowledged that A and AB serum has been used in the clinic since 2013 to produce batches both under the hospital exemption and in the pivotal phase III clinical trial supporting Jelrix. The A serum as an impurity is addressed.

Control of critical steps and intermediates

The applicant has provided an overview table together with brief text descriptions which summarises the process control strategy of the active substance manufacturing. Critical steps include processing of cartilage-bone cylinders, enzymatic digestion of cartilage tissue, cell cultivation, cell harvesting, and the optional

cryopreservation/thawing step. They are supported by pharmaceutical development, based on the manufacturer's experience and could be considered in general acceptable.

Processing of cartilage-bone cylinders include a visual inspection to prevent bony or calcified tissue containing osteogenic cells from entering the manufacturing process. Although it is unclear exactly how efficient this control is, it appears as a sound initial approach to minimise osteogenic contaminations and is thus supported. In addition, there is also a visual test after digestion to control matrix dissociation.

The cell seeding and initiation of culturing includes controls of microscopic visual control of digestion to single cells or small cluster of cells, cell count, cell viability and microbial tests. Overall, these tests are deemed suitable at this step.

Cell culturing and cell harvest process controls include microbial examinations, visual controls, gene expression, population doubling viability and cell count.

Process validation and/or evaluation

For the process validation, the applicant has included 25 batches and a strategy with three different run scenarios: a) low amounts cells throughout cultivation and at cell harvest, b) low number of viable cells when seeding isolated from the starting material, and c) high cell count throughout cultivation of active substance. The outcome of the active substance process validation showed that all three scenarios displayed variability but provided batches that fulfilled the current set of specifications.

Based on the presented data, the standard manufacturing process appears to produce AS batches within the current specification limits in a robust manner.

It is noted that cryopreserved cells were used to increase the number of active products for the Jelrix process validation. The cryopreservation is further assessed and discussed in Description of Manufacturing Process and Process Controls.

Validation of the final FP formulation step with cells and MAHSA-HA of FP is satisfactory.

Manufacturing process development

As the product was initially produced and provided under the hospital exemption, process updates during clinical development have not been documented through clinical phases. Instead, several manufacturing changes appear to have been implemented individually over the years without formal comparability between specific versions of the process. In the dossier, changes that have been introduced from October 2016 onwards are indicated and described. A table clarifying which batches have been used in the different preclinical studies and for supporting clinical data that are used to demonstrate product safety, including information when and how they were produced and a justification of their representativeness for Jelrix has also been provided.

In summary, although some differences were identified in the included development studies/reports for each update, it generally provides some support for the feasibility of introducing these changes and to initiate a more holistic comparison of the IMP phase III material with the Jelrix process data.

Thus, in addition to the development studies, a comparison between Jelrix PPQ batches and batches from the IMP of the pivotal Phase III study was performed. Results from both Jelrix and the IMP fulfilled the specifications. The applicant has performed a retrospective analysis and plotted the PPQ batches against phase III batch data.. In general, data from Jelrix PPQ batches are shown to be within the historical range of

clinical batches with few exceptions. Overall, the data supports comparability between the PPQ batches (including changes introduced after pivotal study) and the phase III clinical batches.

Characterisation

Capacity of in vitro expanded human articular chondrocytes to synthesise components of articular cartilage.

Critical attributes for the intended biological function in vivo are cell viability and the capacity to produce articular hyalin-cartilage associated ECM components. The capacity of in vitro expanded human articular chondrocytes to synthesize components of articular cartilage has been investigated in several studies using related products: NOVOCART 3D, NOVOCART Inject and NOVOCART Inject plus samples. It is stated that "the chondrocytes were isolated and cultured according to the corresponding experimental set-up" which included some "deviations". A brief description of differences in the manufacturing process of the samples used for characterization as compared to the commercial process are provided and include differences in the anatomical location of the starting material (i.e. cartilage flakes versus cartilage-bone explants in the commercial manufacture), serum irradiation, cryo-preservation of chondrocytes after isolation which is not allowed in the commercial process, number of culture flasks used during expansion, different cell expansion periods and differences in the analysis of data obtained in the gene expression experiments. These deviations have been discussed, and their possible impact on critical quality attributes of the cell-based product have been risk assessed. The methods used for the analysis performed in the characterization studies have been sufficiently described.

The stability of the hydrogel components was evaluated with respect to different concentrations of the reactive maleimide groups used for preparation of the MAHSA and to different storage times (0, 3 and 4 days) after preparation of the component A. All these studies were performed in the absence of cells in the A component. The effect of storage of the component A item on gel properties and gel formation at higher maleimide concentrations has been sufficiently addressed.

The stability of chondrocytes during storage of component A and the influence of crosslinking on cell viability and gene expression was investigated with respect to viability and markers initially proposed as relevant for the purity, impurity, potency of the cellular component of Jelrix. Additional markers were also investigated. Following the agency request, the marker used for determination of chondrocytes identity has been replaced during the evaluation process with. The relevance of these markers for the characterisation and control of Jelrix are discussed below. Studies were performed on component A material stored for 3 or 4 days before crosslinking, directly after crosslinking and after 24 hours of culture within the crosslinked hydrogel. Material obtained from twenty-three donors has been included in this analysis. Different test groups included test samples prepared from cells of different donors (not necessarily same donors included in all groups) and data were analysed on mean values, which resulted in significant variability. Additional marker analysis provided for some biomarkers where mRNA or protein expression was reported relative to the results obtained for harvested AS did not provide additional conclusive information with respect to the possible effects of the investigated processing steps on cell quality.

Immunohistochemistry studies were performed to detect extracellular aggrecan and collagen type II within the hydrogels. Exemplary pictures have been provided for different test groups with different cell numbers, maleimide concentrations and different storage periods for component A. Although no differences can be readily identified between groups, it can be agreed that extracellular matrix is secreted during *in vitro* culture of the hydrogels containing Jelrix cells.

Impact of population doublings on the matrix formation capacity of in vitro expanded chondrocytes.

Studies were conducted to investigate whether the dedifferentiation of the chondrocytes in culture after isolation is always reversible or if redifferentiation of the cells may become impossible after a certain number of population doublings. These studies were performed using material cultured *in vitro* in both collagen scaffolds (NOVOCART) and the NOVOCART® Inject/Jelrix implantation hydrogel.

Possible impact of in vitro expansion on genomic stability of human articular chondrocytes.

Studies were performed to investigate the genomic stability of in vitro expanded chondrocytes and array comparative genomic hybridization (aCGH) analyses were used for this purpose. In general, the method is considered relevant for genomic stability studies.

Impurities

Nonviable chondrocytes and cell types other than chondrocytes have been indicated as product-related impurities. Nonviable chondrocytes are controlled at the level of active substance and component A finished product with established specifications.

Other cell types identified as possible impurities are endothelial cells, osteoblasts and synoviocytes. While synoviocytes are more extensively discussed and studied, the possible presence of endothelial cells and osteoblasts has been sufficiently addressed.

Several characterisation studies were carried out to identify suitable markers for identification of chondrocytes, synoviocytes and for determination of a surrogate marker for potency determination. These different technical approaches included analysis of gene expression, flow cytometry techniques, Raman spectroscopy and proteomic studies using mass spectrometry. For these studies populations of chondrocytes and synoviocytes established from human donor tissue purchased from the U.S where expanded. The relevance of these materials for Jelrix cells is considered sufficiently justified.

Two proteomic studies using mass spectrometry were performed to identify relevant markers for discrimination between chondrocytes and synoviocytes. The studies are provided, and the methods are sufficiently described and considered relevant. Additional markers have been investigated during the evaluation process, showing differential mRNA expression between chondrocytes and synoviocytes. Considering the overlapping pattern of expression for all these markers in chondrocytes and synoviocytes, the applicant is requested to implement a robust panel of markers for determination of Jelrix identity and purity (**MO**). Furthermore, validation exercises should be performed, and a summary of the results should be provided (**MO**).

Analysis of the correlation of potency marker mRNA expression levels in AS and the clinical outcome measured as Knee injury and osteoarthritis outcome score (KOOS) has also been provided The outcome of the clinical study is inherently related to the Critical Quality Attributes (CQA), e.g. potency assay (**MO**). Additional characterisation studies have been provided suggesting negative correlations between markers. The applicant should further scientifically justify, ideally supported by data, the capability of the proposed potency test to identify samples with insufficient quality and successfully define relevant release and stability limits. (**MO**).

Immunohistochemistry data are provided to investigate the synthesis of cartilage matrix molecules using in vitro 3D cultures .

Molecular impurities:

Process-related impurities are currently identified. Data confirmed a stepwise depletion with possible concentrations in the final product at levels below the detection limit of the assay system.

For some impurities , possible final concentrations are provided resulting from theoretical calculations. Descriptions of these calculations have been provided. The dossier is stating the intent of using A serum instead of AB serum for the manufacturing of the commercial product. AB serum is also used as an excipient and discussed in the relevant section. An evaluation of the maximal possible levels in the final product has been provided and the comparability between materials manufactured using A serum or only AB serum is discussed.

2.4.2.3. Specification

The proposed active substance release specification includes tests for content (total cell number and cell viability), cell population doublings, potency, Identity, impurity and microbial purity (microbial contamination and mycoplasma). Expression of all markers included in the quality control strategy is detected both in chondrocyte- and synviocyte-populations. As discussed in the characterization section, the currently provided data do not yet support the control strategy as proposed and additional markers, as identified by the applicant, are considered needed. (MO). Limited characterization studies are presented to demonstrate a link between the proposed potency marker and a relevant biologic activity related to the intended MoA of Jelrix.

The methods used for the control of active substance are briefly described but in general considered sufficient. Additional data on the validation of the method(s) used for determination of the newly introduced markers remain to be provided (MO). The sampling strategy for gene expression analysis, , is sufficiently justified.

AS batch data have been provided for 25 PPQ batches manufactured using the proposed commercial manufacturing process and for 100 batches used in the clinical trial. All batches complied with the currently proposed specifications.

A container closure system for AS is not needed since the cells are used immediately for manufacturing/formulation of the final product. This approach is considered acceptable.

Additionally, a specific reference standard is not required.

2.4.2.4. Stability

Not applicable, as the cells are used immediately for manufacturing/formulation of the final product.

2.4.3. Finished Medicinal Product

2.4.3.1. Component A

2.4.3.1.1. Description of the product and Pharmaceutical Development

The 'component A' of the final product contains a dispersion of in vitro expanded autologous human articular chondrocytes as active substance together with the (hydro) gel component, the MAHSA-HA solution, containing maleimido human serum albumin (MAHSA) and hyaluronan (HA). At the site of administration, the cells are embedded in a cross-linked hydrogel as implantation gel. This hydrogel forms in situ during final

product administration using a double chamber syringe (Tetec application system) mixing component A and component B during injection.

The composition/description of the finished product is provided and considered sufficiently detailed. The finished product component A consists of the in vitro expanded autologous chondrocytes in formulation medium (DMEM/F12) that are mixed with a solution of maleimido human serum albumin (MAHSA), hyaluronic acid (HA) and DMEM/F12 medium.

Pharmaceutical development

Components of the Finished Product (component A, suspension for implantation matrix)

The applicant has provided a brief description of the constituents of the component A finished product. The *in vitro* expanded autologous human articular chondrocytes are suspended in formulation media (DMEM/F12) supplemented with gamma-irradiated human AB serum. For the formulation of the component A finished product, the suspension of the *in vitro* expanded autologous human articular chondrocytes is combined with the excipient MAHSA-HA solution.

Component A formulation as well as effects of mixing of both A and B components were studied (TETEC R&D Department; Project No. 100). This report is discussed and evaluated in the characterisation section.

The inclusion of formulation media and gamma-irradiated human AB serum as excipients are justified by their roles to promote cell viability and cell function. Formulation development and comparability studies for the *in vitro* expanded chondrocytes active substance are described in the manufacturing process development chapter.

MAHSA-HA (together with component B) provides a three-dimensional matrix environment to aid the chondrocyte cells to exert the intended pharmacological activity. To support that *in vitro* expanded human articular chondrocytes require an appropriate three-dimensional environment, the applicant references study report project No. 98 (Comparison of two different implantation matrices that contain in vitro expanded autologous chondrocytes regarding support of chondrogenic phenotype and re-differentiation).

MAHSA-HA should be considered as a novel excipient since it is the first time it is being used as excipient in a finished product. For novel excipients, full details of manufacture, process development, characterisation, controls and stability with cross references to supporting safety data should be provided according to "Guideline on excipients in the dossier for application for marketing authorisation of a medicinal product (EMEA/CHMP/QWP/396951/2006).

Overall, the description of the constituents of Component A in conjugation with information provided and assessed elsewhere in the dossier is generally considered acceptable.

A nitrosamine risk assessment has been provided /updated during the procedure and is satisfactory.

Formulation development

Formulation development related to the *in vitro* expanded autologous human articular chondrocytes is also provided and assessed in manufacturing process development section S.2.6. A discussion on formulation changes, an overview of all formulation comparability studies, and a risk assessment taking potential comparability issues between commercial formulation and clinical trial formulations into account and effects on quality attributes is provided.

For MAHSA, a development study with three different maleimide concentrations, were used to prepare equivalents of the component A (TETEC R&D Report).

Manufacturing Process Development

A single component A manufacturing development has been outlined which aims to standardise MAHSA-HA preparations.. Standardization of the manufacturing is in general considered positive.

Overage(s)

N/A

Container closure system

This section accurately describes the container closure design in sufficient details. The vials are made from borosilicate glass and has, according to the description, been selected based on its suitability for the storage of sensitive pharmaceuticals due to its chemical resistance, leak tightness and solidity. The brown glass offers additional light protection to the product. Injection stoppers and the flip-off seal system are made for packaging of medicinal products.

Compatibility

The dual chamber syringe and the mixing device has been specifically designed for application of the hydrogel and holds a CE-mark. The application system is not co-packed with Jelrix, but corresponds to a 'Referenced Device' in the product information of Jelrix.

A CE-certificate has been provided and it is clarified that the TETEC application system is placed on the market as "legacy device" according to Article 120 of Regulation (EU) 2017/745 and certified as medical device according to Directive 93/42/EEC (Registration No.: 51460-17-01) valid until December 31st, 2027.

The device is CE-marked for its intended purpose and has been placed on the market since 2008 as a certified medical device. The applicant has provided a general description of the certification process by the notified body (DEKRA Certification GmbH, Stuttgart, Germany), which included biocompatibility studies, bench testing, usability and risk analysis, as well as their incoming goods inspection and standard rheology testing.

Microbiological control strategy

The microbiological control strategy has been satisfactorily addressed.

***Control of Excipients and novel excipient**:*

DMEM/F12, human serum albumin 20%, maleimido human serum albumin (MAHSA), hyaluronic acid (supplied as Ostenil plus) and gamma-irradiated human AB serum are listed as excipients of Jelrix.

MAHSA is considered a novel excipient and sufficient details of manufacture, characterisation, and controls with cross references to supporting safety data have been provided in line with EMEA/CHMP/QWP/396951/2006 "Guideline on excipients in the dossier for application for marketing authorisation of a medicinal product - Revision 2".

MAHSA is produced by TETEC in house from a pharmaceutical grade human albumin solution. Adequate and justified specifications are set for each testing parameter. Endotoxin and sterility testing are performed according to the Ph. Eur. 2.6.14 and 2.6.1, respectively. The other testing methods are adequately described and validated.

Adequate information on the control of starting materials, including human serum albumin solution has been provided.

The control of all listed excipients is briefly described in module 3.2.P.4.1. The specifications and justification of specification are acceptable for all non-novel excipients.

Detailed method descriptions, validation summaries as well as complete validation reports have been provided for key methods (size exclusion chromatography, rheometry, sterility testing and endotoxin testing). The performed method validations are acceptable.

Representative certificates of analysis for human serum albumin 20%, hyaluronic acid (Ostenil plus) and DMEM/F12 culture medium are provided.

Human serum albumin 20%, which is used as a raw material for the generation of MAHSA, is an authorised medicinal product (Albiomin 20% (200 g/l) solution for infusion), Germany, MA-number: PEI.H.03574.01.1. The information provided is considered adequate.

2.4.3.1.2. Manufacture of the product and process controls

The name addresses and responsibilities of the finished product manufacturer and testing sites have been declared. The finished product manufacturer, TETEC AG, is the same as the manufacturing site as for the active substance. Additionally, testing of endotoxin at the level of finished product is performed by a contract laboratory. Valid eudraGMDP references for the MIA's and/or GMP certificates for these 2 sites are provided.

The batch formula has been provided in P.3.2. Since Jelrix is an autologous product, batch size is n=1. Cartilage tissue from a single patient is used for manufacture of one batch of the active substance and this batch is used for one batch of the cellular component (component A) of the final product (8 to 32 x 10^6 cells in 0.8 mL formulation medium (DMEM/F12 and gamma-irradiated human serum) and 3.2 m of excipient solution (MAHSA-HA and DMEM/F12).

The manufacturing of component A is described as a continuous process. Harvested chondrocytes, representing the final active substance, are directly processed for the finished product manufacturing without any holding step. The manufacturing has been briefly described and a flow chart is also presented. The component A vial is closed using a fresh stopper and crimped.

The final product is intended for autologous use and the traceability system (DIS) from procurement to the final product is explained in the dossier. The applicant states that as soon as the Member States will have implemented a product code according to Article 8 (2) of Directive 2004/23/EC of the European Parliament and the Council, this product code will replace the DIS and will appear on the primary package as required by Article 12 of Regulation (EC) No 1394/2007.

The applicant has identified three critical risks of the finished product manufacturing. The identified risks are addressed via the testing at the final finished product specification (cell viability, cell count, endotoxin, and microbial control) which is further discussed in P.5.

As there is no active substance storage or intermediate, the applicant refers to the process verification study already presented in the active substance part which covers the whole manufacturing (See S.2.5). The 25 batches of active substance that were manufactured according to certain scenarios were immediately used for finished product formulation and included in the validation of finished product manufacturing. The scenarios represent identified worst-case conditions.

The Aseptic process validation (APV) has been described and the results from the latest validations have been provided.

2.4.3.1.3. Product specification, analytical procedures, batch analysis

Specifications of the active substance relevant for finished product release

The proposed release finished product specification includes test for total cell number, cell viability, microbial determination and endotoxins. Tests for differentiation (cell population doublings), identity, impurity and potency are included in the component A finished product release but are performed at the level of active substance. The proposed strategy is considered sufficiently justified. The assays performed for determination of population doubling, identity, purity and potency have been discussed at the AS level. All other methods included in the control of component A are compendial methods, briefly described in the dossier.

Validation summaries as well as complete validation reports have been provided for each method.

Batch data are provided for 25 process validation batches, although with incomplete values for cell count and cell viability, and for 100 Phase III clinical batches, although with no data provided for cell count and cell viability.

It is noted that in the batch analysis, values for the storage (G') and loss (G'') modulus describing the gelation process are included although storage modulus is only presented in the specifications for component B finished product. Some studies were performed to investigate the impact of gelation on the quality of the cellular component of Jelrix, further discussed in the characterization section. The data provided are not supporting the proposed specification limits for G' as proposed in the release strategy for component B **(OC)**.

Container closure system

The container closure system for the primary packaging is a standard borosilicate glass injection vial with a rubber stopper and a polypropylene/aluminium flip-of seal. Quality certificates for each of the 3 components have been provided. The primary packaging is sterilized at Eurofins inpac Medizintechnik GmbH by a validated γ-irradiation procedure. A validation report and radiation certificate for this procedure has been provided. A container closure integrity test that covers two different use scenarios has been performed and the test report has been provided.

The vials of component A and B are placed protective transfer device and in turn an outer transport container as secondary packaging. No further claims for the function of the secondary packaging are made and therefore no further documentation is provided. This is considered acceptable.

A qualification report of the supplier for the outer transport container (Thermoversandbox 72) is provided. Summer/winter ambient temperature profiles were taken into account. Extreme summer (43°C) /winter (-5,1°C) conditions were also tested. In conclusion the system has been qualified for "ambient temperature shipments" to keep the inner temperature specification of +2.0° C to +8.0° C for ⩾ 72 hrs.

2.4.3.1.4. Stability of the product

The stability studies cover up to 126 h storage at 2-8 °C. The containers used in the stability studies are the same as those proposed for routine storage. The test specifications were met for all parameters at all timepoints up to 102 h.

The maximum shelf life is found acceptable.

No stability studies were performed under stressed conditions because it was deemed unsuitable to conduct stress-testing studies for a cell-based product. The justification for the omission of stressed studies was found acceptable.

The stability data package in total is acceptable and the claimed maximum shelf life of 102 h stored at 2-8 °C is supported.

2.4.3.1.5. Adventitious agents

The applicant has declared that the following materials are derived from animal or human origin: cartilage-bone cylinder starting material, gamma-irradiated A and AB serum, MAHSA, Collagenase, neutral Protease, FGF-2, and Trypsin.

Starting material

Human autologous chondrocytes are obtained from cartilage-bone cylinders. Donor testing is conducted in accordance with Directive 2006/17/EC and includes HIVp24-Ag, Anti-HIVp24-antibody against HIV-1 and HIV-2, hepatitis B surface antigen (HBsAg), HBsAg confirmatory assay, anti-hepatitis B core antibodies (anti-HBc), anti-hepatitis C virus antibodies (anti-HCV), and Treponema pallidum antibody. All tests are stated to be CE-marked. The applicant explains that positive test results will not necessarily prevent the manufacture of the active substance and the final product. In such cases the biopsy tissue and the isolated cells are physically separated to eliminate the risk of cross-contamination. Any sero-positive batches are quarantined and manufactured in a dedicated quarantine incubator and with decontamination programs of the isolator in place to exclude cross-contamination risks.

A and AB serum

Details on A and AB serum are provided. Information on the plasma collection sites (including inspection status), donor screening, manufacturing steps, and a description of the virus control strategy has been adequately provided. Serum donors are screened and tested in accordance with Directive 2002/98/EC and 2004/33/EC. CoAs has been provided, covering an acceptable panel of extraneous agent NAT and Ab tests.

As an additional measure of safety, a gamma-irradiation step of the human A and AB serum is performed. The applicant has shown in S.2.3 that the irradiated serum does not adversely affect the growth of chondrocytes. The CoI of the sera are provided. The applicant has confirmed that a traceability system from donors of material used for the preparation of A and AB serum to the finished product exist and that records will be kept for 30 years.

HSA

20% human serum albumin (HSA) solution is a medicinal product provided by Biotest Pharma GmbH, Dreieich, Germany (MA number: PEI.H.03574.01.1, PMF-number: EMEA/H/PMF/000009/05).

Collagenase and normal protease

Collagenase and neutral protease is declared to have been produced using culture media with bovine peptone. TSE safety for this material is assured by an EDQM certificate of suitability. A summary of the validation of the viral inactivation of the media by autoclaving has also been included.

FGF-2

FGF-2 is expressed in E. coli. The product does not contain any materials from animal or human source. However, lactose-monohydrate originating from Germany and derived from milk is used during fermentation. In accordance with the "Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3)", it is agreed that bovine milk is unlikely to present any risk of TSE contamination.

TrypLE select

TrypLE Select is deemed safe from the viral point of view, as no animal derived components are utilized in its manufacturing process.

Non-viral microbial risks

The applicant has provided an adequate overall summary of the aseptic manufacturing and non-viral microbial risks.

2.4.3.2. Component B

2.4.3.2.1. Description of the product and Pharmaceutical Development

Component B, solution for implantation gel, contains the bisthio-polyethylene glycol solution (BTPEG solution) of the medicinal product Jelrix. There is no active substance in Component B. Composition: BTPEG, NaCl, HCl 10, Water for Injections.

Pharmaceutical development

The information on Component B components, formulation development, physicochemical and biological properties, manufacturing process development, container closure system, and microbiological attributes are discussed in the dossier.

Novel excipient - BTPEG

BTPEG is considered to be a novel excipient from a regulatory perspective, although the applicant did not recognise it as such in the initial application. The applicant has amended the description during the procedure. BTPEG is considered to be a novel excipient, remaining issues were resolved . The remaining deficiencies concern information on impurity control, Ph. Eur. compliance, and information on the primary container. The manufacturing process is briefly described, with a schematic presentation of structures as expected. The structural characterization of BTPEG has been acceptably described. The proposed limits for residual solvents are agreed to be compliant with ICH Q3C and no further information is expected. The general evaluation of elemental impurities and ICH Class 1 solvents are accepted with reference to ICH Q3C and Q3D.

While issues concerning the limits for impurity control in the excipient, it is agreed that the BTPEG specification covers the basic, expected parameters. Certificates of suitability are presented to support tests not performed in house, which is acceptable for an excipient.

The analytical methods used to control BTPEG are described in sufficient detail and have been acceptably validated, except for the impurity method (OC).

Information on reference standards and stability is available.

The primary container closure system for the BTPEG powder consists of a polyethylene terephthalate glycol-modified (PETG) bottle and a high-density polyethylene (HDPE) closure cap. Compliance with relevant regulations has been demonstrated, but there is an open question regarding specifications (OC).

A nitrosamine risk assessment has been provided /updated during the procedure and is satisfactory.

2.4.3.2.2. Manufacture of the product and process controls

Manufacturer: TETEC Tissue Engineering Technologies AG, Aspenhaustr. 18, 72770 Reutlingen, Germany. Satisfactory GMP compliance has been demonstrated – see active substance.

The manufacturing process has been described. A large number of deficiencies were initially identified and not all have been acceptably resolved. A Major Objection raised on the aseptic processing remains to be resolved. Hold times and process times are not sufficiently described and justified and more information is necessary. (**MO**).

No design spaces are claimed.

Process validation has been performed on three batches with the aim to demonstrate that the BTPEG solution can be manufactured at the intended batch size with expected quality. All questions are not yet resolved, and the lack of supportive validation data is part of the remaining Major Objection. Validation of the filtration process is currently ongoing, and this data should be provided (MO).

2.4.3.2.3. Product specification, analytical procedures, batch analysis

Separate release and shelf-life specifications have been provided. The proposed specifications are not yet acceptable. Revision and/or further information and justifications are requested (OC).

Additional data should also be provided on how gelation was evaluated during stability testing (OC).

Real time release testing is not proposed.

The analytical procedures have been described in relevant detail and the method validations/verifications are acceptable. Batch analysis (three batches, manufactured for process validation) with all results complying with the currently proposed specification limits are presented.

Considering the nature of the tests proposed for control of Component B, no objections are made to the statement made by the applicant that no reference standards exist.

Container closure system

The applicant refers to Component A for detailed documentation on the container-closure parts. It is stated that the primary container-closure system parts comply with Ph. Eur. standards for packaging materials. No specific monograph reference is given for the glass, the statement is however understood to refer to 3.2.1 Glass containers for pharmaceutical use.

2.4.3.2.4. Stability of the product

After manufacture and release of a BTPEG solution batch, the filled vials are stored at -80 °C. On the day component A of Jelrix is released, one item from a batch of BTPEG solution is taken to represent component B of the corresponding batch of Jelrix. Both components are shipped and stored at 2°C to 8°C until use. Therefore, two stability studies have been initiated for the BTPEG solution; the first to prove the declared shelf life for long-term storage at -80°C, and the second study to demonstrate the shelf life of the final product at 2–8 °C.

15 months' data for four batches have been submitted for long-term storage at -80 °C of Component B alone. The applicant refers to Component A studies for storage of the complete finished product (i.e., components A and B together) at 2–8 °C. No fundamental objections are made to this overall strategy, but the applicant should ensure that the worst-case scenario is addressed by using Component B batches at the end of the long-term shelf-life in the Component A+B stability studies. (OC)

Photostability of the finished product has been demonstrated.

The applicant claims a shelf-life of 15 months for Component B stored at 2–8 °C. No deviations from the proposed release and shelf-life specifications have been observed. However, there are open questions regarding the specifications. (OC).

Since the shelf-life of Component A is shorted, the claimed shelf life for the combined Finished product corresponds to that of Component A (102 hours).

2.4.3.2.5. Adventitious agents

Component B does not included substances with potential for adventitious agents risk.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Component A

Component A consists of 8 to 32 x 10^6 *in vitro* expanded autologous chondrocytes (active substance) formulated in 0.8 ml formulation medium (DMEM/F12 and gamma-irradiated human AB serum) and 3.2 ml of excipient solution (MAHSA-HA and DMEM/F12).

The quality assessment of component A has identified several issues and concerns which are detailed and explained throughout the assessment report. A Major Objection was raised regarding the product quality control strategy, where it is questioned how potency, identity and purity markers and their corresponding limits have been derived. The characterisation data provided, and the additional analysis of correlation with clinical outcome are not considered sufficient to support the control strategy. The information provided for the manufacturing and control of MAHSA-HA as a novel excipient is considered in general adequate.

The standard manufacturing procedure is a continuous process without intermediates. It should however be noted that an optional freezing step is proposed. While parallel processes are generally not endorsed, from an ethical perspective it could be acceptable. However, based on that cryopreservation now is reported to potentially increase the risk of OOS-batches, the applicant is asked to explain how it is ensured that cryopreservation will only be used as a last option (where batches otherwise would have been discarded) and not seen as part of a more flexible/routine option.

In conclusion, a revision of the control strategy and a rework of the overall information provided in the dossier with new additional content added is required, therefore Jelrix Component A is currently not approvable from a quality perspective.

Component B

Component B is an aqueous solution of the gel-forming excipient α,ω-bistiopolyetylenglykol (BTPEG), NaCl and HCl. There is no active substance in component B.

While the function of BTPEG in the gel formation process taking place once Components A and B are mixed are adequately discussed, critical deficiencies in the description of Component B as a finished product remain. Compliance with key guidelines has not been fully demonstrated and most prominently, the description of the current aseptic processing lacks fundamental information. As a consequence, a Major Objection with several sub-issues is raised.

Jelrix Component B is currently not approvable from a quality perspective.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

At the time of the CHMP opinion, Major Objections (**MO**) on quality remain (as detailed in the D180 List of Outstanding Issues and CAT/CHMP Grounds for Refusal), relating to:

1) the proposed strategy to control purity, impurity and potency of the active substance and of Component A in Jelrix. These quality attributes may impact clinical efficacy.

2) The sterilisation process for Component B.

Other Concerns (OC) are highlighted above and are also detailed in the D180 List of Outstanding Issues.

2.4.6. Recommendation(s) for future quality development

Should the benefit/risk assessment for Jelrix conclude positive in the context of a future application, in the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommended points for investigation.

2.5. Non-clinical aspects

2.5.1. Introduction

The non-clinical development program of the presented ATMP must be seen in the context of the historical development of NOVOCART® Inject and the regulations effective at the time. There is a range of different preparations that are mentioned in the nonclinical dossier, and for the assessment of this product it is advantageous to distinguish between these products.

Jelrix is related to TETEC AG's product NOVOCART® Inject, in clinical use since 2008, and under the name 'NOVOCART® Inject plus', used in the pivotal phase III clinical trial that started in 2017. All these three chondrocyte-containing preparations are delivered using a cross-linked hydrogel. For information, there is also another similar product called NOVOCART® 3D for which approval was applied for under the name

Artobend (EMEA/H/C/004598). This differs somewhat more as the chondrocyte presentation was made with the help of a collagen scaffold.

2.5.2. Pharmacology

No dedicated non-clinical (pharmacology) studies have been conducted with Jelrix. However, four non-clinical in vitro and in vivo primary pharmacology studies conducted with NOVOCART® inject or NOVOCART®3D were submitted to support the pharmacological activity of the presented ATMP.

NOVOCART® Inject is the most similar product to Jelrix in that it presents chondrocytes in the same hydrogel, but not identical as there are differences in the manufacturing processes.

No secondary pharmacodynamic nor safety pharmacology studies have been conducted, which has been justified. The justification is considered acceptable.

2.5.2.1. Primary pharmacodynamic studies

Jelrix constitutes of in vitro expanded autologous human articular chondrocytes embedded in a hyaluronan-containing cross-linking albumin hydrogel.

In vivo study in Göttingen minipigs

In an in vivo pharmacodynamic study in Göttingen minipigs, NOVOCART Inject, a product related to Jelrix, was administered into the knee joints of minipigs. The selection of minipigs followed guidelines outlined in the "Reflection paper on in vitro cultured chondrocyte containing products for cartilage repair of the knee" (EMA/CAT/CPWP/568181/2009). Cartilage damage was induced, and the effects of hydrogel (with or without chondrocytes) and fibrin-based gel (with or without chondrocytes) were compared. However, quantitative data of the cell-free formulations were not included in the report and the rationale for choosing fibrin gel as a comparator lacks which limits the value of this study.

While hydrogel and fibrin gel containing chondrocytes exhibit positive staining for extracellular matrix markers (e.g., collagen type II and proteoglycan), uncertainties arise because both hydrogel and fibrin without chondrocytes also show strong positive staining for these markers. Notably, hydrogel without chondrocytes shows stronger staining for collagen type II compared to hydrogel with chondrocytes, which is further reflected in the histological scoring results (modified O'Driscoll score) presented by the applicant. The reason for this difference is not discussed further by the applicant. As mentioned, there are no adequate control animals to confirm effect of the hydrogel or fibrin gel without chondrocytes. Due to these limitations, it is not agreed that concept for the product has been supported in vivo. However, it is acknowledged that due to cross-species differences that may affect the human chondrocytes' ability to thrive optimally, further non-clinical proof-of concept studies are not warranted.

In addition, infiltration of mononuclear cells and signs of inflammatory responses were observed after injection of both chondrocyte formulations. However, the applicant only mentions in their report that in cell-free gels also influx of leukocytes was observed, but this was not shown in a graph or quantified. Thus, it is unclear whether it is the induced injury itself, the chondrocytes or the gel that triggers immune responses making the assessment on the noted similarities between NOVOCART Inject and fibrin uncertain.

In vitro studies

Primary pharmacodynamic activities of in vitro expanded human articular chondrocytes were evaluated in two in vitro studies (Study 98 and 100). Therein, the effectiveness of both NOVOCART Inject implantation matrix and the biphasic collagen scaffold like NOVOCART 3D in promoting chondrocyte differentiation, as evidenced by increased collagen type II expression and decreased collagen type I expression, was evaluated. The ratio of collagen type II to collagen type I mRNA levels over four weeks indicates sustained differentiation. This is important as there is a possibility that chondrocytes lose their (re)differentiation ability under/after in vitro expansion. Additionally, biochemical analysis and immunohistochemistry confirmed the deposition of glycosaminoglycan, aggrecan, and collagen type II, supporting the potential of both matrices for cartilage regeneration applications. Thus, it is agreed that the redifferentiation capacity and chondrocytic phenotype of the chondrocytes seems to be maintained when presented in the hydrogel, and by assessing these endpoints, show similar results compared with the collagen scaffold used in NOVOCART 3D.

The similarity between redifferentiation capacity and matrix formation between NOVOCART Inject and NOVOCART 3D can also be seen as a bridging study to the fourth primary pharmacology study performed with in vitro expanded human chondrocytes seeded on the biphasic scaffold (called Matricart at that time) and referred to as NOVOCART 3D herein. Therein, ectopic cartilage formation both in vitro and in vivo (following subcutaneous implantation in SCID mice for 69-84 days) was confirmed. In this in vivo study SCID mice were implanted s.c. with 10 million cells per cm2 test item (biphasic scaffold). Although the in vivo PD study in SCID mice may be considered to have some supporting potential to Jelrix, it is deemed to be of limited regulatory value at this point. This study is further limited by the fact that the SCID mouse subcutaneous implantation model does not recreate the native chondrocyte environment of the articulating joint.

A clarification on the selected cell concentration was provided regarding the non-clinical PD studies. The cell doses were consistent with those used in previous studies (NOVOCART 3D and NOVOCART Inject). For NOVOCART Inject, a lower cell dose was used in a smaller volume, resulting in a comparable cell concentration. In the minipig study, a higher cell dose than intended for Jelrix was used, which was attributed to the study being conducted independently.

In addition, the applicant has provided insight in the MoA of cartilage implants based on literature data, which suggest that chondrocytes in Jelrix would have more functionalities than only production of ECM components, resulting in a complex interaction between different factors and tissues in vivo to regenerate the cartilage.

In summary, there are no strong non-clinical data supporting effectiveness of the product. Regarding the impact of induced cartilage damage in minipigs, there's no evident difference in efficacy between the hydrogel with or without chondrocytes. Therefore, the concept is not considered proven in vivo. The need for additional studies to investigate the effect of the product has been considered, but owing to the limitations of animal models, the best approach is to investigate this in a clinical setting.

2.5.2.2. Secondary pharmacodynamic studies

No secondary pharmacodynamic studies have been conducted.

2.5.2.3. Safety pharmacology programme

No safety pharmacology studies have been conducted.

2.5.2.4. Pharmacodynamic drug interactions

No pharmacodynamic drug interactions studies have been conducted.

2.5.3. Pharmacokinetics

Typical pharmacokinetic studies on absorption, distribution, metabolism, and excretion (ADME studies), nor biodistribution, have not been performed for this product. Conventional ADME studies are usually not relevant for human cell-based medicinal products as stated in the Guideline on Human Cell- based Medicinal Products (EMEA / CHMP / 410869 / 2006).

The absence of biodistribution studies is in accordance with the 'Reflection paper on in vitro cultured chondrocyte containing products for cartilage repair of the knee' (EMA/CAT/CPWP/568181/2009).

The applicant has presented data from minipig and SCID mouse studies, as well as histological analyses from patients treated with NOVOCART® Inject. These studies showed that chondrocytes remained viable within the hydrogel for at least 4 weeks and contributed to the formation of regenerative cartilage in vitro. Histological analyses of patient biopsies demonstrated the presence of chondrocytes in the formed cartilage even 18 months post-treatment. Additional support for the persistence and lifespan of chondrocytes is provided by in vitro studies (Benz et al., 2010; Weitkamp et al., 2023; Niemietz et al., 2014), which showed a high percentage of viable cells after 28–42 days in culture, as well as minipig studies where the hydrogel was sufficient to fill defects without the need for a covering membrane. Metabolism and excretion of both excipients BTPEG and MAHSA have been predicted based on the available information of related compounds polyethylene glycol and human serum albumin; they are expected to be degraded and excreted predominantly via urine. In addition, two in vitro metabolism studies have been conducted for the new excipients BTPEG and MAHSA. It was found that BTPEG was not metabolized in either microsomes or hepatocytes. MAHSA was only partially metabolized in microsomes, and data regarding its metabolism in hepatocytes are lacking.

2.5.4. Toxicology

No dedicated non-clinical Toxicology studies have been conducted with the presented ATMP. This is considered acceptable due to the nature of the product. The applicant has conducted a tumorigenicity study evaluating the tumour forming potential of chondrocytes isolated from donors. The applicant has also performed a series of studies according to standards relevant to medical device assessment evaluating different aspects of toxicology of the cell free hydrogel matrix. These studies were compiled in one report and presented in the local tolerance section of Module 4. Finally, in vitro cytotoxicity testing of different PEG crosslinkers according to EN IS0 10993-5 were performed.

The applicant has submitted a GLP compliant, 20 week, tumorigenicity study using a collagen scaffold developed for the company product NOVOCART® 3D. Here postmortem derived chondrocytes were isolated from 3 donors. Three different culture conditions were used differing mainly in the amount FGF-2 supplemented to the cell culture medium. The human fibrosarcoma cell line HT1080 or rat chondrosarcoma

(RCS) cells were used as positive controls. The collagen scaffolds were seeded with 560 000 cells and then implanted subcutaneously SCID mice. None of the animals implanted with chondrocytes developed tumours, whereas tumour formation was observed in both positive control groups.

Biocompatibility of the hydrogel was addressed according to standards used for assessment of implantable medical devices. A wide range of toxicological evaluations were performed according to ISO 10993 ranging from in vitro and in vivo tests, as well as chemical analysis of the hydrogel. According to the applicant, the presented data and conclusion of the studies lend support for the non-toxic profile of the hydrogel. The results of test methodology and results of the studies are briefly summarised. No data on quality control of the test item provided.

BTPEG was evaluated in an in vitro fluorometric assay designed for detecting cytotoxic effects using two study approaches: in the first approach, extracts from hydrogels (maleimido albumin gels) prepared with the different PEG cross linkers, and in the second the diluted PEG crosslinkers were tested directly in an in vitro cytotoxicity assay employing L929 cells. In the fluorometric assay evaluating hydrogel extracts no cytotoxicity (measured as metabolic activity) was detected in any of the tested extract dilutions with any of the PEG crosslinker batches. Further, microscopic evaluation of cell morphology did not detect any aberrations in the stimulated cells. In the in vitro toxicity assay evaluating BTPEG crosslinkers, cytotoxicity was detected at a concentration of 7,5 mM, but not at the intermediate concentration of 3 mM and lowest concentration of 2 mM. It is noted however that the margins between these two concentrations is low. Additional in vitro cytotoxicity assays have been submitted by the applicant indicating that MAHSA and BTPEG has low cytotoxic potential, although a concentration dependent trend towards cytotoxicity was noted for BTPEG. One of the three batches of BTPEG showed cytotoxicity at the highest tested concentration of 5 mM. No discussion on safety margins of BTPEG is provided by the applicant.

Further safety aspects of BTEPG and MAHSA are presented in two expert opinion safety assessments for each of the two excipients.

The applicant has evaluated the genotoxic potential of the MAHSA and BTPEG and has submitted two GLP compliant AMES assays for each of the excipients and one Mouse Lymphoma Assay for evaluating the genotoxic potential of BTPEG. The studies indicated that MAHSA and BTPEG are non-genotoxic.

2.5.5. Ecotoxicity/environmental risk assessment

Due to the nature of the product, no environmental risk is expected from this product.

2.5.6. Discussion on non-clinical aspects

Jelrix is composed of in vitro expanded autologous human articular chondrocytes embedded in a hyaluronan-containing cross-linking albumin hydrogel. The non-clinical development programme consists of pharmacology studies (in vivo and in vitro) as well as toxicology studies. For the development approach of this product, the applicant refers to the EMA reflection paper on 'In vitro cultured chondrocytes containing products for cartilage repair of the knee' (EMA/CAT/CPWP/568181/2009) and to the 'Guideline on Human Cell-based Medicinal Products' (EMEA/CHMP/410869/2006). These two guidance documents are considered relevant for the development of Jelrix. The submitted proof of principle studies performed with the scaffold matrix are in line with EMA/CAT/CPWP/568181/2009. However, Jelrix contains a different matrix (hydrogel) with likely different PK characteristics (i.e. with respect to barrier function for chondrocyte migration). No dedicated single and multiple dose toxicology studies have been performed for the chondrocytes, which is in line with

EMEA/CHMP/410869/2006. Due to their autologous origin, the chondrocytes are not expected to elicit an immunological reaction.

Pharmacology:

A 'proof-of-concept' study conducted in minipigs to compare the regenerative capacity and potential inflammatory responses of chondrocytes presented in a hydrogel with a fibrin-based gel formulation. While signs of regeneration of cartilage based on positive staining for proteoglycans and collagen type II was observed after treatment with chondrocytes in the hydrogel formulation, no significant difference to groups treated with chondrocyte-free hydrogel (or the fibrin-gel formulation) could be observed. Thus, this study did not provide support that in vitro expanded chondrocytes in hydrogel improve healing of cartilage damage. This is also the case for the in vitro study no 100. In this study, viability and gene or protein expression has not been analysed in (stored) chondrocytes that had been incubated for 24 hours (part 3) or cultivated for several weeks (part 4) without hydrogel. It is therefore not clear whether the incubation/cultivation period, the hydrogel or the combination of both has led to the changes observed in the gene expression profile and extracellular matrix synthesis. Moreover, considering 1) the absence of in vivo data regarding stability of the hydrogel and retention of the chondrocytes and synthesized cartilage components in the cartilage defect (see discussion below under Pharmacokinetics), and 2) the uncertainty regarding nutrient supply of the matrixed chondrocytes in vivo (compared to in vitro culture), it is not clear to which extent the data from study no. 100 is clinically relevant. The efficacy of the hydrogel-chondrocyte combination to regenerate hyalin cartilage and repair the chondrocyte defect in vivo has been evaluated and assessed clinically (see clinical section of this report).

With regards to potential proinflammatory effects, similar increases in infiltration of CD45 positive cells and increased expression of proinflammatory cytokines was observed after treatment with chondrocytes presented in hydrogel compared to chondrocytes in fibrin-based gel. In addition, the applicant mentioned in their report that in cell-free gels also influx of leukocytes was observed, but this was not shown in a graph or quantified. It is thus unclear whether it is the induced injury itself, the chondrocytes or the gel that triggers immune responses.

There were more limitations to the minipig study. There was considerable individual variation in the regeneration assessment observed. Moreover, the actual quality of the cartilage (e.g. production of different cartilage components, integration in native tissue, congruence of cartilage surface) was not separately investigated, although some factors were part of the total histological scoring. Likely, the 4-week time point is too short to assess the full quality of the regenerate.

Taken together, no final conclusions regarding efficacy –or safety- can be drawn from the study in mini-pigs. It is not agreed with the applicant that this study provide non-clinical support for proof-of-concept. However, considering the inherent limitations of animal models for studying human cell products (e.g. cross-species differences may affect the human chondrocytes' ability to thrive optimally), further non-clinical in vivo proof-of concept studies are not warranted.

It is agreed with the applicant that the in vitro redifferentiation capacity and chondrocytic phenotype of the chondrocytes is maintained when presented in the hydrogel, and by assessing these endpoints, show similar results compared with the collagen scaffold used in NOVOCART 3D. The visual results of an additional in vitro study, comparable to non-clinical study no. 100, showed that the chondrocytes (almost $3.2x10^7$ chondrocytes (i.e. maximum amount of cells within Jelrix component A) in component A solutions or in hydrogels produced with 50.6 mM maleimide) were still alive after 4 weeks.

Results from in vitro studies 98 and 100 could be used as argument that non-clinical studies conducted with NOVOCART 3D (scaffold matrix) can be used as supportive data for this application. But there are some differences between the matrices that could have an impact on the in vivo efficacy and retention of the cells (and synthesised extracellular matrix components) within the matrix. First, the hydrogel volume is several folds higher than the scaffold, resulting in a lower cell density. Second, cells and synthesised cartilage-related molecules are better retained in the scaffold as the hydrogel does not have a retention barrier (primarily consists of water and has less dense material). The applicant did not conduct an in vivo comparison between both products and only the scaffold (NOVOCART 3D) has been tested in SCID mice.

The study on NOVOCART 3D in SCID mice adds little/no regulatory value at this point because the SCID mouse subcutaneous implantation model does not recreate the native chondrocyte environment of the articulating joint. In addition, the applicant did not use chondrocytes without a matrix as additional control in vitro or in vivo (in SCID mice). Considering that the matrix serves to retain chondrocytes at the implant site, the feasibility and value of such control in vivo may be limited. Nevertheless, it is not clear whether the matrix may also serve other goals. As such, chondrocytes culturing without a matrix could have been a relevant in vitro control to determine the functions of the matrix may also serve other goals. In addition, chondrocytes were expanded in lower concentrations of human serum and a 10-fold higher amount of FGF-2 (with different durations/media change time points) when compared to the product used for clinical application. These differences in culture conditions may result in different functionality/viability of the cells, but this was not further discussed or evaluated by the applicant. Also taking into account the use of another matrix with a different cell density and barrier function compared to hydrogel, the clinical relevance of this study is not clear, and not considered pivotal to show the proof-of-concept of the currently proposed product. For a more clinically relevant in vivo study with a more suitable chondrogenic environment and the possibility to evaluate integration into surrounding tissue (including fill up of the cartilage defect), the applicant could have considered to cultivate their cells (in matrix) on native human cartilage explants for implantation in SCID mice, although it would still remain an ectopic animal model. An orthotopic cartilage model (i.e. large animal) could have even been more appropriate. The applicant has provided rationale for the lack of studies with orthotopic large animal models. Considering the extensive clinical experience with NOVOCART products (including data on defect repair and durability of the newly formed cartilage) and in line with the Scientific Advice for NOVOCART 3D (EMEA/H/SA/3755/1/2018/ADT/II), this absence of a large animal model (apart from the minipig) can be endorsed.

Based on the numerous limitations of the studies, it is not possible to conclude that proof of concept with respect to regenerative potential in vivo has been shown, although the primary pharmacology studies indicate that in vitro expanded human chondrocytes cultured or implanted with a matrix can survive (at least for several weeks) and produce cartilage-related extracellular matrix components. Therefore, the non-clinical data presented here can only be considered as support for the chondrocyte´s ability to synthesize extracellular matrix components. In addition, data addressing quality of the newly formed cartilage tissue (including mechanical performance), integration thereof in the native tissue and long-term durability of this cartilage after implantation in a joint (orthotopic location, e.g. larger animal model) is lacking. However, it is acknowledged that owed to cross-species differences further non-clinical proof-of concept studies are not warranted. The most appropriate way to demonstrate the actual efficacy of the chondrocytes in hydrogel, including structural/histological evaluation of cartilage quality and durability is in the clinic (see clinical AR/Overview) and are not within the remit of the current non-clinical studies.

Based on the non-clinical studies provided, the focus of the non-clinical pharmacology program is to demonstrate that chondrocytes can produce cartilage components, which has been confirmed. Given the

limitations of animal models and the available data, the most appropriate approach is to investigate the effect in a clinical setting.

Pharmacokinetics:

Hydrogel

The evaluation of the hydrogel and its components focuses on their pharmacokinetics, metabolism, and potential safety concerns. The analysis considers available non-clinical data, in vitro metabolism studies, and expert opinions to assess their behavior in vivo. This discussion will review the fate of the hydrogel and its key components—BTPEG, MAHSA, and HA—along with relevant safety and efficacy considerations.

In the pharmacodynamic minipig study, it was determined that the use of hydrogel as matrix did not require additional covering with a collagen membrane (as is needed for a fibrin-based gel) to avoid leakage of the gel from the defect area. In vitro and in vivo data suggest hydrogel persistence and integration with cartilage. No ectopic cartilage formation was observed, and patient biopsies indicate full hydrogel remodeling within months. However, the clinical biopsies are few and have not been systematically evaluated, raising questions about their reliability. Moreover, concerns about premature gel disruption due to excessive swelling or inflammatory responses have not been thoroughly addressed. Therefore, potential premature hydrogel rupture in the case of early treatment failure should be considered. Some data has been provided on the new excipients BTPEG and MAHSA. BTPEG, a high molecular weight PEG (approx. 10 kDa), is expected to primarily be excreted renally, based theoretically on excretion for other HMW PEGs. While it does not interact with negatively charged cartilage, its bis-thiol groups introduce uncertainties regarding aggregation or altered excretion. Studies indicate limited metabolism, reducing concerns about reactive metabolites. Based on the provided literature, an immunological response is generally unlikely for PEGs. In vitro studies show limited first-pass metabolism, yet hepatocyte studies were inconclusive, leaving its systemic fate uncertain. HA has a longer half-life in joints (up to 3 weeks), degrading locally before systemic clearance in the liver. Based on the provided information, the risk for local or systemic toxicity with (reactive) MAHSA and BTPEG is considered low.

Chondrocytes

The applicant presented data showing that chondrocytes in the hydrogel are functional and produce ECM components, confirming proof-of-concept. However, chondrocyte viability was not evaluated during these 4 weeks in the initial studies, so further in vitro studies were conducted. These showed that some cells survived after 4 weeks, though no quantitative data comparing initial versus 4-week viability were provided. Considering the available in vivo data (minipig and patients), suggesting that sufficient viable chondrocytes remain to produce regenerative cartilage tissue in situ, no additional in vitro quantitative viability data will be requested.

As previously mentioned, the minipig study indicated that the hydrogel, without a covering membrane, could retain cells for 4 weeks, resulting in defect filling, but the persistence of all human cells and ECM components within the hydrogel was unclear. Long-term persistence was not evaluated due to immunogenicity concerns, and the specific roles of chondrocytes and hydrogel (versus spontaneous healing) in the observed effects was uncertain. Clinical biopsies from treated patients indicate production and maturation of cartilage components by chondrocytes that can persist for a long-term in the regenerating tissue, but the data were limited and not systematically evaluated.

The applicant also clarified that chondrocytes in the hydrogel cannot migrate easily, though they may adhere to or interact with subchondral bone, potentially contributing to tissue integration. The hydrogel is designed

to trap cells by its mesh size, HA presence and cartilage production, thus preventing cell migration and of loss of ECM components. Although these data do not provide proof that some chondrocytes or their synthesized ECM components can migrate/diffuse out of the hydrogel, sufficient chondrocytes and synthesized substances seem to be retained within the hydrogel to produce cartilage and the presence of (small numbers of) chondrocytes in synovial fluid or even blood will likely not be a clinically relevant safety issue considering the autologous nature of these cells.

Overall, the provided data suggest that chondrocytes remain viable for at least 4 weeks, can form cartilage and can persist in the formed cartilage for months. The potential migration of chondrocytes and ECM components out of the hydrogel is unlikely to be clinically significant. Long-term clinical outcomes will determine whether chondrocyte loss or ECM component loss impacts efficacy. In patients with graft failure, the loss of (too many) chondrocytes and/or synthesized ECM components should be considered.

Application system

The application system provides approximately 2.6 mL of chondrocyte-containing hydrogel after accounting for preparation and administration losses. This volume is sufficient to fill the largest anticipated cartilage defect (2.4 mL), eliminating the need for a second syringe.

Following successful hydrogel formation testing, the material remains sufficiently soft for injection during the first minutes after mixing, although some additional pressure may be required. A spare mixing unit and needle are provided in case of clogging. Even if replacement is needed, the remaining volume is expected to be sufficient to fill the largest cartilage defects.

Toxicology:

No conventional toxicology studies have been performed for Jelrix. This is considered acceptable due to the nature of the active ingredient of the product (human autologous chondrocytes). The applicant has conducted a tumorigenicity study evaluating the tumour forming potential of chondrocytes isolated from donors. The applicant has also submitted summaries of studies on the hydrogel matrix performed to originally support biocompatibility of the hydrogel for use as a medical device and thus conducted according to ISO standards. These studies were compiled in one report and presented in the local tolerance section of Module 4. Finally, in vitro cytotoxicity testing of BTPEG and MAHSA according to EN IS0 10993-5 were performed. Finally, the genotoxic potential of BTPEG and MAHSA has been evaluated.

Tumorigenic potential

The submitted tumorigenicity study was performed using a collagen scaffold developed for the company product NOVOCART® 3D and thus bears no relevant resemblance to the present product that uses a hydrogel matrix with crosslinked albumin. Chondrocytes were isolated from three postmortem donors and expanded in vitro. Different cell culture conditions were tested and the cells are harvested after 17 days before being seeded in a biphasic matrix composed of two collagenous compartments, namely, a dense membrane prepared from bovine pericard and a sponge prepared from bovine fibre meshwork of the subcutis, before being subcutaneous implanted into SCID mice. There is however no quality data on the cells engrafted into the SCID mice, and thus the purity of the cell with respect to presence of other cell types cannot be ascertained. In addition, the collagen matrix differs from the hydrogel matrix used for Jelrix with respect to biochemical composition and gel density. It cannot be ruled out that this might lead to different distribution pattern of the cells as well as effects on cell survival, enabling cells embedded in the hydrogel used for Jelrix to have a different distribution pattern persistence. As such, there is the possibility that the biodistribution of

cells may not be equivalent between Jelrix and NOVOCART® 3D. The different compositions might also have different impact on long-term survival of the chondrocytes once administered.

Tumourigenic potential of the extracted, and cultured cells was evaluated in animals in which with 5.6x10e5 cells human cells, grown for 17 days, were seeded on 0.28 cm^2 Novocart Basic (NZ collagen) scaffolds, representing a seeding density of 2x10e6 cells/cm^2. Clinically a density of 0.75-4 x$10e^6$ cells/cm^2 are used that have been cultured for a period of maximum 25 days. In the tumorigenicity study, the applicant did not test up to maximum growth period (25 days) and the maximum applied cell density (4x10e6 cells/cm^2) used in the clinic, that might potentially result in underestimating the tumorigenic risk of chondrocytes due to the culture procedure with this test. The applicant was asked to discuss these points and how it affects the sensitivity of the study. In their reply, the applicant explained that the applied cell density in the tumorigenicity study was 2x10^6 cells/cm^2 correlating to 8.7x10^6 cells/cm^3 and 6.6x10^6 cells/cm^3 when the thickness of the collagen scaffold (in NOVOCART® 3D) of 2.3 – 3.0 mm (0.23 – 0.3 cm) respectively would be considered. The applied cell density with Jelrix is between 1.6 – 6.4x10^6 cells /mL, which is just below the dose used in the tumorigenicity study. For a tumorigenicity study, this margin is considered very minor.

Cells were expanded for 17 days corresponding to a population doubling of 5, lower than used in the clinic. However, the applicant mentions that internal studies confirmed that cultured chondrocytes at passage 2 with a cumulated population doublings of 21.6 did not result in any chromosomal aberrations and therefore the applicant does not expect that these would have performed differently in the tumorigenicity study. This is considered a slightly weak and indirect support for choosing a lower culturing time of the cells for the tumorigenicity study.

However, as the chondrocytes are autologous in nature and manipulated to a minimal level, the tumorigenic safety will not be regarded a problem. This is also confirmed by a study the from Zscharnak and colleagues that did not observe tumour formation for an autologous chondrocyte product cultured up to passage 2 and 3. Furthermore, the safety is supported by clinical data over a period of 30 years, where not a single case of tumour formation related to ACI, including malignant cartilaginous tumours, has been reported.

No tumour formation was observed with chondrocytes whereas tumour formation was observed with the two tumour cell lines used as positive controls. It is acknowledged that the risk for tumor development is considered low for this type of product, but there are limitations with this animal model. Although, the difference regarding tumorigenic potential between animals injected with test article and the positive controls are clear-cut, it is questionable to which degree this study has a clinically relevant predictive potential given the species barriers which makes it difficult to ascertain that the cells receive optimal survival stimuli. It is also worth noting that immunocompromised animals lack functioning tumour surveillance so even an indication of tumour development in this model would be difficult to extrapolate to the clinical situation. Reassuringly, in vitro characterisation of tumourigenic potential of cultured chondrocytes performed as part of the quality evaluation of NOVOCART® Inject plus did not identify a concern indicated and all together the risk for tumour development is considered low.

The studies on genomic integrity submitted as part of the nonclinical studies is evaluated by the Rapporteur as part of the quality studies. The Co-rapporteur has provided some additional comments and a question in section 3.1 of this report.

Genomic integrity analysis

To evaluate the effect of the substantial manipulation on the genomic integrity of the chondrocytes, The applicant used aCGH as detection method, which provides an average backbone resolution less than 1 MB

and an enhanced resolution in regions that are known for higher chromosomal instability (microdeletion and microduplication regions) as well as in centromeric and sub-telomeric regions.

In a primary analysis, balanced structural chromosome aberrations as well as low level mosaics were not detectable by aCGH. Besides, the use of growth factors did not induce a gain or loss of DNA in the autosomal DNA. Additionally, no sex chromosome anomalies were detected in the female donors. In contrast, all male donors showed a loss of Y chromosome sequences to various degrees. In a secondary analysis, in 3 of 5 male and 2 of 2 female donors, there was no evidence of chromosomal changes in terms of a gain or a loss in genomic material within the limits of the resolution of the array CGH platform used (around 50 kb). However, in two other male donors (donor 64 years and donor 52 years), there was a loss of the Y chromosome in a small proportion of the analysed cells (around 15% in donor 1, around 5% in donor 3). It is assumed that the loss of the Y chromosome in some of the cultivated cells of these donors occurred during the initial cultivation (P0 passage) and was not increased by the subsequent cultivation of the cells (P2 passage) or the cryopreservation (P0 chondrocytes after cryopreservation).

The applicant described that the findings from this analysis reveal the loss of Y-chromosomes in all expanded male samples of higher age (> 50 years). No discussion on the loss Y chromosomes has been provided. The applicant was asked to discuss the potential implications of the loss of the Y chromosome in all expanded chondrocytes of male donors > 50 years of age, identified following aCGH analysis, especially in a context of no implemented control of the stability of the karyotype for the release of the in vitro expanded autologous human articular chondrocytes seeded into the hydrogel. It was explained that balanced structural chromosome aberrations and low-level mosaics were not found by aCGH on expanded chondrocytes and that use of growth factors did not induce a gain or loss of DNA in the autosomal DNA. Furthermore, sex chromosome anomalies were not detected in the female donors. Further, in the first study, Y chromosome loss was already present in the freshly isolated cells as detected by single cell analysis as published by Stumm and colleagues, suggesting it already had occurred in cartilage tissue. Also in the second study, the loss was already present in the freshly isolated chondrocyte isolation. No chromosome loss seems to be induced by culturing.

Local tolerance testing

The chondrocytes are administered in a hydrogel component A that can form a matrix with the crosslinker component B that are mixed upon administration. As hydrogel component A the applicant has used ALBUGEL, with the composition activated human albumin (containing the maleimide addition), medium, hyaluronic acid. Component A was tested for biocompatibility The applicant has submitted studies testing the biocompatibility of (ALBUGEL). These studies cover different toxicological aspects and were performed in accordance with ISO 10993 under GLP compliance. The implantation matrix passed all tests, and the cell-free material was considered 'biocompatible'. The data in the main document is only summarized; raw data and statistical analysis are not present. Additional lab reports of the summarised studies are in German and an assessment cannot be performed by the Rapporteur. It is not clear whether the test were performed with MAHSA and a crosslinking agent, thus on gel (extracts) which is suggested on page 7 of the PDF submitted 23-11-2023 (3833787), or whether the experiments were conducted without the crosslinking agent, as is suggested from the list of materials specified for each of the separate tests (p 18, 22, 27, 32, 26, 29, 51, 54, 56, 61 and 69). In the separate PDFs that were submitted with the second round, the crosslinking agent is not named, however in the emails is referred to a 'vernetzer', that might suggest use of crosslinking agent. The applicant was asked to clarify whether a crosslinking agent is added to the activated albumin and hyaluronic acid and thus a gel was tested, or whether only activated albumin with hyaluronic acid was tested for biocompatibility studies. The applicant commented that the biocompatibility studies were conducted with a crosslinked gel,

although this was not clearly stated at each separate experiment. Therefore, no information on MAHSA itself is present, apart from the in vitro genotoxicity study. When this in vitro genotoxicity assay can be agreed, the risk assessment on BTPEG and MAHSA based on literature data in combination with the biocompatibility data on the gel can be agreed, as it is highly likely that the material that will enter the knee is crosslinked hydrogel including the cells. This issue considered resolved, but a follow-up question is filed as the assessment has as further impact on SmPC. The applicant is asked to amend section 5.3 **(SmPC).**

Notably, two excipients have been identified not previously toxicologically qualified, namely maleimido human serum albumin (MAHSA) and α,ω-bisthio-polyethylene glycol (BTPEG). The applicant has received scientific advice (EMEA/H/SA/3345/2/2019/ADT/II) on the non-clinical development and herein received support for the applicant's proposal not to conduct additional nonclinical tumourigenicity or safety studies, however, it was stated that the toxicological qualification of the cross-linking agent BTPEG needs to be addressed. No in vivo testing with component B, crosslinker Bis-Thio-PEG (BTPEG) is presented in the nonclinical part of the dossier. The applicant has, however submitted a study presented in the quality part of the dossier aimed to study in vitro cytotoxicity of different batches of PEG crosslinkers (study 50372). Two approaches were used for assessing cytotoxicity: in the first approach, extracts from polymerised maleimido albumin gels prepared with the different PEG cross linkers tested in an in vitro cytotoxicity assay employing L929 cells. In the second cytotoxicity study, diluted PEG crosslinkers were tested directly in the same cytotoxicity assay. The extracts from the polymerised gels did not exhibit cytotoxicity. In the study evaluating of BTPEG as a single agent, cytotoxicity was noted at the highest concentration tested, but not at the intermediate concentration and the lowest concentration.. It is noted however that the margins between the highest and the intermediate concentration is low and therefore not considered satisfactory from a safety point of view. There was also an observed trend towards cytotoxicity in the intermediate concentration group compared to the lowest concentration group.

A new GLP compliant in vitro cytotoxicity assay conducted in accordance with the DIN EN ISO 10993-5 (study number STUGC23AA1830-2) aiming at evaluating the cytotoxic potential of BTPEG showed cytotoxicity at the highest concentration of 5mM for one of the three tested lots. Although there is a clear concentration dependent trend towards toxicity and the margin with the clinical used concentration of BTPEG (3mM) is not large, it can be agreed that gelling occurs within seconds to minutes leaving the time of reactive BTPEG 'meeting' towards the cells limited. As soon as gel formation has occurred, the safety of the cells is not considered further at risk. Therefore, the intermediate concentration present in the injection unit can be considered as a worst-case scenario for the joint and therefore this study can be considered to support the safety of BTPEG for the cells.

A new GLP compliant in vitro cytotoxicity assay was also conducted for MAHSA. MAHSA did not have a cytotoxic effect at concentrations up to 7.5 mM, which exceeds the concentration in the final mix to be administered (). Although the margin is considered limited, it is not expected that the concentration MAHSA in Jelrix will be exceeded to that extent () and therefore the margin can be considered acceptable. It should be noted that the DP (MAHSA plus cells) has a shelf life of 102 hours, but that viability of the cells is slightly affected although within the acceptance criterium.

In addition, there is no data of crosslinking of the active forms of BTPEG of MAHSA to proteins and cells at the injection site. The applicant has provided a justification based on that it can be considered that free amounts of the excipients are low, and that the redox environment of the synovial fluid is inactivating, and thus limiting the potential for severe local toxicity (e.g. collateral damage) due to inappropriate crosslinking but also systemic exposures. In addition, the gel may act as a barrier for diffusion of unreacted excipients. Furthermore, it is expected that crosslinking to proteins at the injection site may elicit an immune rection.

Although this might be difficult to detect in in vivo model no immunogenic reactions have been reported in the clinical setting. This in conjunction that there are no safety concerns in the clinic for the excipients, it can be assumed that the risk of systemic exposure of BTPEG and MAHSA is considered negligible. However, due to the lack of data of local effects of the excipients there is an uncertainty regarding leakage of small quantities and local effects in the joint. This insecurity concerning local reactions must be reflected in the risk/benefit discussion.

No in vivo local toxicity or systemic toxicity studies have been performed for the excipients. For BTPEG the applicant has relied on data of in vivo studies with similar products. For MAHSA immunogenicity effects when injecting human serum albumin into animals may interfere with proper interpretation of toxicity effects.

Besides the available cytotoxicity studies, the toxicity profile and lack of data on local toxicity of each of the excipients BTPEG and MAHSA is considered unsatisfactory. It is therefore considered important to evaluate safety aspects together with quality data. The applicant has previously submitted an ISO 10993 biological testing including evaluation of biocompatibility, cytotoxicity, sensitisation, irritation, pyrogenicity, systemic toxicity and genotoxicity of the hydrogel. This has been included in 5.3 in the SmPC. It should be noted that these tests were performed on gel extracts, and such do and not evaluate each of the excipients.

In addition, the applicant was asked to summarize all safety data obtained in the pharmacology studies (especially the data from the study in which the hydrogel was used) and in the tumorigenicity study with regard to the cells and the hydrogel (and component A and B) and provide a conclusion on the (absence of) toxicity findings observed in clinical trials. In the minipig study no visible inflammation was visible and published studies in SCID mice, dogs, and sheep did not show any signs of inflammation or toxicological reactions. Although this is agreed, these are non-GLP pharmacology studies that do not pre-specify any toxicological endpoints whether systemic or local, there might be a risk we are missing out on toxic effects.

In addition, the *in vivo* minipig study (Pharmacology section), the use of porcine-based hydrogel with or without human chondrocytes in the joint induced an influx of leukocytes and hence an immunogenic response. The applicant has addressed this issue referring to both the minipig study and evaluated a ricks and risk mitigation. The offered wight if evidence, although weak, concludes that Jelrix is not expected to be immunogenic, and that Patients with chronic inflammation in the knee should not be treated with Jelrix.

Further, the risk of clinical issues with dedifferentiation/aberrant redifferentiation of chondocytes is considered low.

Finally, 2 GLP compliant AMES tests using the plate incorporation method for MAHSA (report 4137512) and BTPEG (report 4137511), respectively, evaluating the mutagenic potential of the excipients. These studies were performed at ICCR-Roßdorf GmbH, Germany. The applicant has also initiated a mammalian in vitro genotoxicity study (mouse lymphoma assay) to evaluate the potential genotoxicity of BTPEG. The AMES tests, and mouse lymphoma assay indicate that BTPEG and MAHSA does not have a mutagenic potential.

Assessment of paediatric data on non-clinical aspects

N/A

2.5.7. Conclusion on non-clinical aspects

Based on the numerous limitations of the primary in vivo pharmacology studies, it is not possible to conclude that proof of concept has been shown. However, it is acknowledged that owed to cross-species differences

further non-clinical proof-of concept studies are not warranted. The most appropriate way to demonstrate the effects of Jelrix is in the clinic. Although the proof-of-concept (i.e. redifferentiation of chondrocytes and potential to synthesized ECM components that may form regenerative tissue in a cartilage defect in humans) has been shown in vitro, the non-clinical PD studies have considerable limitations.

Traditional non-clinical pharmacokinetic studies have not been conducted and this is generally acceptable. Based on the provided information, the risk for local or systemic toxicity with the new excipients MAHSA and BTPEG is considered low.

No formal toxicology studies have been performed for Jelrix. The applicant has conducted a tumorigenicity study evaluating the tumour forming potential of chondrocytes showing that chondrocytes does not have tumorigenic potential.

Biocompatibility was assessed for the hydrogel covering different aspects of toxicology according to ISO 10933. The findings of the studies are considered of limited value for the total toxicological evaluation of the hydrogel as a whole and for its constituent new excipients, namely MAHSA and BTPEG. The applicant has however submitted in vitro cytotoxicity studies, and importantly negative AMES test for BTPEG and MAHSA, and negative mouse lymphoma assay for BTPEG.

The safety of the hydrogel and the excipients BTPEG and MAHSA are considered to be sufficiently substantiated when contained in the gel. However, as there are no in vivo local toxicity data nor data on systemic toxicity on BTPEG and MAHSA, toxicity of the single substances remains not fully characterised and thus a toxicity potential cannot be definitively excluded.

The submitted toxicological documentation is considered limited. Provided that the applicant addresses the outstanding issue in a satisfactory manner, the product is considered approvable from a non-clinical point of view.

2.6. Clinical aspects

2.6.1. Introduction

Table 1. Tabular overview of clinical studies

Type of study (control) Location of study centres	Reference / study ID / Report	Product/ Patients treated with NOVOCART® Inject (plus), N	Localisation of defect	Follow-up time (months)	Status
I. Clinical data on NOVOCART® Inject plus (IMP) in the knee					
Pivotal Phase III trial Phase III prospective, single-arm, MRI assessor blinded, multi-centre trial* 5 European countries	Study ID: AAG-G-H-1624 EUDRA-CT No 2016-002817-22 Report on primary analysis 2020 Report addendum on *ad hoc* analysis of 36 months data	**NOVOCART® Inject plus (IMP**) 100	Knee	Primary analysis at 24 months (all patients) Overall follow-up: 60 months	Ongoing Primary analysis report 24 months available Report addendum on *ad hoc* analysis of 36 months data available Long-term Follow-up (> 24 months post-treatment) ongoing
Analysis from pivotal Phase III trial Propensity score matched pair analysis NOVOCART® Inject plus versus MFx§	Report on matched pair analysis	**NOVOCART® Inject plus** (from study AAG-G-H-1624) 72 Microfracture (MFx) (from study AAG-G-H-1202) 72	Knee	Analysis at 24 months	Completed Report available

Type of study (control) Location of study centres	Reference / study ID / Report	Product/ Patients treated with NOVOCART® Inject (plus), N	Locali-sation of defect	Follow-up time (months)	Status
II. Clinical data on NOVOCART® Inject (marketed): in the knee - supportive					
Sponsored by Applicant					
Retrospective, non-interventional, multi-centre study Germany	RENOVO Study ID: AAG-O-H-1521 PEI: NIS330 ClinicalTrials.gov: NCT02941120 Final report	**NOVOCART® Inject** 245	Knee	Mean follow-up 20.3 months (range 1.5 months to 57 months)	Completed Report available
Prospective non-interventional, multicentre study Germany	NINJA Study ID: AAG-O-H-2123 PEI: NIS653 ClinicalTrials.gov: NCT05391841	**NOVOCART® Inject** 30 planned Study will not start before Q3 2023)	Knee	Primary analysis at 24 months (all patients) Overall follow-up: 60 months	No data available yet
Investigator initiated (published literature and not yet published report from the German cartilage registry)					
Multi-centre, nationwide data collection from German Cartilage Registry Germany	Report from the German Cartilage Registry by Niemeyer et al., November 2021	**NOVOCART® Inject** 93 (and 90 NOVOCART® 3D and 154 co.don chondrosphere®)	Knee	36 months	Completed
Retrospective consecutive case series ǂ Germany	(Schlumberger et al., 2019)	**NOVOCART® Inject** 15	Knee	Mean follow-up 4.3 years (4.0–4.8 years)	Completed

Type of study (control) Location of study centres	Reference / study ID / Report	Product/ Patients treated with NOVOCART® Inject (plus), N	Locali-sation of defect	Follow-up time (months)	Status
Retrospective consecutive case series ǂ Germany	(Schlumberger et al., 2020)	**NOVOCART® Inject** 62	Knee	Mean follow-up 31,0 ± 14,8 months (range 12.5 – 61.4 months)	Completed
Retrospective consecutive case series Germany	(Blanke et al., 2021)	**NOVOCART® Inject** 29	Knee	Mean follow-up 24.9 ± 1.1 months (range 24-27 months)	Completed
Matched pair analysis comparing hydrogel-based and scaffold-based ACI Consecutive case series Germany	(Niethammer et al., 2022)	**NOVOCART® Inject** 25 (and 25 with scaffold-based ACI NOVOCART® 3D)	Knee	2 years	Completed
Retrospective case series Germany	(Kayaalp et al., 2021)	**NOVOCART® Inject** 5	Knee	28 ± 7months (20 – 40 months)	Completed
III. Clinical data on NOVOCART® Inject (marketed): in the hip - supportive					
Sponsored by Applicant					
Prospective non-interventional, multi-centre study # Germany	HIPACTION Study ID: AAG-O-H-1304 PEI: NIS 252 ClinicalTrials.gov: NCT02179346 Final report	**NOVOCART® Inject** 21	hip	Follow-up 24 months	Completed Report available
Investigator initiated (published literature)					
Prospective case series ** Germany	(Thier et al., 2018)	**NOVOCART® Inject**	Hip	Mean follow-up 12 months	Completed

Type of study (control) Location of study centres	Reference / study ID / Report	Product/ Patients treated with NOVOCART® Inject (plus), N	Locali-sation of defect	Follow-up time (months)	Status
		13		(range 6 to 24 months)	
Prospective case series †, ** Germany	(Thier et al., 2017)	**NOVOCART® Inject** 19 (and 10 patients with Chondrosphere®)	Hip	Mean follow-up 19 months (range 6 to 24 months)	Completed
IV. Clinical safety data on NOVOCART® Inject (marketed) from post-marketing pharmacovigilance: in the knee and the hip - supportive					
Ongoing routine pharmacovigilance including signal detection assessment and benefit-risk assessment	Signal detection reviews, PSURs (see Module 2.7.4, Section 2.7.4.6)	**NOVOCART® Inject (plus)** Over 6,000 (status 05/2022)	Mainly knee, but also hip	(Various)	Ongoing activities

PSUR: Periodic Safety Update Report.

*The 24 months results of the Phase III study (AAG-G-H-1624) were also published by (Niemeyer et al., 2022b)

§ The analysis included data from the phase III study (AAG-G-H-1624) on patients treated with NOVOCART® Inject plus and data on patients treated with microfracture from a previous phase III study for the M-ACI product NOVOCART® 3D plus in the same indication treatment of cartilage defects of the knee (study AAG-G-H-1202).

ǂ Populations may be overlapping in publications by (Schlumberger et al., 2019) and (Schlumberger et al., 2020)

12 month results of the HIPACTION study (AAG-O-H-1304) were published by (Bretschneider et al., 2019).

† In this study a total of 29 patients were treated with arthroscopic M-ACI. 19 patients were treated with NOVOCART® Inject and 10 patients with Chondrosphere®. No information on the rationale for allocating patients to one or the other M-ACI product is given.

** Populations may be overlapping in publications by (Thier et al., 2017) and (Thier et al., 2018)

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Conventional absorption, distribution, metabolism, and excretion (ADME studies) have not been performed.

2.6.2.2. Pharmacodynamics

Mechanism of action

The primary mode of action of Jelrix is the biological reconstruction of damaged cartilage by implanted viable human autologous articular chondrocytes in order to repair cartilage lesions.

In the pivotal Phase III trial (AAG-G-H-1624) , assessment of pharmacodynamics is mainly performed by MRI. The respective images are assessed by an observer blinded for patients' medical conditions (except defect localisation, number of defects and defect size) and clinical outcome. In addition, MRI images in a subgroup of patients are available from a propensity score matched pair analysis investigating efficacy of NOVOCART® Inject plus based on patients treated with NOVOCART® Inject plus from the Phase III study (Study AAG-G-H-1624) compared to patients treated with microfracture from a previous Phase III study with another M-ACI product of the applicant (NOVOCART® 3D plus) in the same indication, i.e., treatment of cartilage defects of the knee (study AAG-G-H-1202).

Post-transplantation MRI examinations are also available from the HIPACTION study (AAG-O-H-1304) and the studies by Blanke et al. (Blanke et al., 2021) and Kayaalp et al. (Kayaalp et al., 2021). Furthermore, additional information from MRI assessments is expected from the ongoing non-interventional NINJA study (AAG-O-H-2123). Some publications provide results of second-look arthroscopies or histological analyses, however this concerns only few patients.

Primary and Secondary pharmacology

Primary pharmacodynamics were predominantly investigated *in vitro*. According to the applicant, two studies have demonstrated the capability of the drug substance to form cartilage tissue, i.e. to redifferentiate and synthesise relevant extracellular matrix molecules when embedded in the hydrogel representing the implantation matrix of the presented ATMP. The first study, 'Persistence, biocompatibility and early influence of NOVOCART Inject on the regeneration of full-thickness cartilage defects in the minipig knee' (Research Centre for Experimental Orthopaedics at the Heidelberg University Hospital) showed that NOVOCART® Inject remained in the defect over the observation period of 4 weeks and that a membrane cover did not offer an advantage regarding persistence of the implanted hydrogel.

Furthermore, since the presented ATMP contains the same drug substance as TETEC AG's first matrix-associated chondrocyte implantation (M-ACI) product 'NOVOCART® 3D', a primary pharmacodynamics study from the preclinical development of NOVOCART® 3D has been considered to provide supportive data for the presented ATMP. Although the used SCID mouse model was not entirely optimal to recreate the native chondrocyte environment of an articulating joint, it did allow for an assessment of potency as measured by ectopic cartilage formation. As a result, the biochemical (glycosaminoglycan (GAG) quantification) and histological examination (Alcian blue staining for GAG and immunostaining using antibodies against collagen type II and aggrecan) suggests a rapid deposition of extracellular matrix, in its essence being cartilage-like, both by composition and by microscopic appearance.

No secondary pharmacodynamics studies have been performed.

2.6.3. Discussion on clinical pharmacology

PK studies were not performed as this is considered not relevant for the current application (*EMEA/CHMP/410869/2006*).

No clinical studies with a primary PD objective have been performed. However, in the pivotal trial 1624, MRI assessments were performed in a predefined subset of patients, which were used for pharmacodynamic evaluation in accordance with the *"Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee"* (EMA/CAT/CPWP/568181/2009). Within this context, the Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score was calculated and T2 mapping was performed. Further, histological and immunohistochemical assessments (biopsy of regenerated cartilage with ICRS II scoring) in case of treatment failure requiring re-surgery were planned.

MOCART scores and T2 mapping were available from 25 patients with 31 cartilage lesions, at months 12 and 24. MOCART sum scores improved from 70 (sd 22.4, median 75) at 12 months to 80 (sd 56.7, median 90) at 24 months. The main sub score 'defect filling' did not change between 12 and 24 months and was complete in about 60% of the patients; integration of graft tissue to border zone improved from 73% to 82% at 24 months. T2 mapping parameter 'relaxation time in repair tissue' was slightly smaller at 24 months compared to 12 months with a numerically decreased standard deviation, which might suggest maturation of the graft. T2 mapping parameters 'global ratio' and 'zonal ratio' were approximating normal (i.e. 1) at 12 months and remained stable up to 24 months. No relation was found between KOOS scores and MRI data at 24 months or 60 months. Notably, three patients had empty defects and were diagnosed with osteoarthritis, but these were all KOOS responders (see efficacy section). Also, no relation was found between KOOS and transplant cell density.

This absence of correlations between clinical data and structural data appears in line with literature, including reports on Spherox (Niemeyer et al., 2020b). Therefore, MOCART -score cannot be considered as a reliable surrogate biomarker for clinical efficacy. In addition, the small sample size (n = 25) in the current development limits any conclusions.

No formal dose-response studies were performed prior to the pivotal trial; the recommended dose from the German NOVOCART Inject SmPC (authorized since 2016) was used in the pivotal study. This dose has been applied in clinical practice since 2008, when NOVOCART Inject was introduced on the German market. However, no rationale for this posology was provided in the dossier.

2.6.4. Conclusions on clinical pharmacology

No pharmacokinetics data is available, which is acceptable.

Formal PD studies were not performed either, but as an alternative MRI data were analysed (MOCART score and T2 mapping) which indicated graft maturation between 12 months and 24. Graft filling at months 12 and 24 was complete in a stable 60% of patients while 3 grafts were found empty at 24 months (12%). No associations were reported between MRI and KOOS, nor with cell-density. The small sample may account for this.

2.6.5. Clinical efficacy

Table 2: Clinical studies

Uncontrolled studies

Study ID	Enrolment status Start date Total enrolment/ enrolment goal	Design Control type	Study & control drugs Dose, route of administration and duration Regimen	Population Main inclusion/ exclusion criteria
Study ID: AAG-G-H-1624 EudraCT 2016-002817-22 ClinicalTrials.gov: NCT03319797	Ongoing since April 2017, recruitment and treatment completed, (long-term) follow- up period of treated patients ongoing Primary analysis report (24 months results) available. Clinical study report addendum on ad hoc analysis of 36 months data available. Number of subjects: 100	Prospective, multicentre, single-arm phase III trial in 5 European countries	NOVOCART® Inject plus surgically implanted using arthroscopy or mini-arthrotomy of the knee.	Adults and adolescents aged 14 - 65 (adolescents with closed epiphyseal growth plates) with focal articular cartilage defects (ICRS Grade III or IV) of the femoral condyle (medial or lateral), the trochlea, the patella or the tibial plateau (medial or lateral) and a defect size of ≥ 4 and ≤ 12 cm2 post debridement. If the patient has two grade III or IV cartilage defects the total defect size is ≥ 4 and ≤ 12 cm2 post debridement. The defects must be located on different, knee joint areas and both cartilage defects are to be treated with NOVOCART® Inject plus.
'RENOVO' Study ID: AAG-O-H-1521 PEI: NIS330	Completed, Final Report available.	Retrospective, non-interventional,	NOVOCART® Inject surgically implanted using arthroscopy or	In accordance with the SmPC: Adult and paediatric patients with closed

ClinicalTrials.gov: NCT02941120	Number of subjects: 245	multi-centre study	mini-arthrotomy of the knee	epiphyseal growth plate with focal articular cartilage defects of the knee. Defect size ≥2.5 and ≤10 cm2. Defect grade of III or IV ICRS
'HIPACTION' Study ID: AAG-O-H-1304 PEI: NIS 252 ClinicalTrials.gov: NCT02179346	Completed, Final Report available. Number of subjects: 21	Prospective, non-interventional, multi-centre clinical study	NOVOCART® Inject surgically implanted using arthroscopy of the hip	In accordance with the SmPC: Adult patients with focal articular cartilage defects of the hip. Defect size ≥1.5 and ≤8.0 cm2. Defect grade of III or IV ICRS.

Reports of analyses of data from more than one study

Study ID	**Enrolment status** **Start date** **Total enrolment/ enrolment goal**	**Design** **Control type**	**Study & control drugs** **Dose, route of administration and duration** **Regimen**	**Population** **Main inclusion/ exclusion criteria**
N./A: propensity score matched-pair analysis (NOVOCART® Inject plus patients derived from study AAG-G-H-1624 and microfracture [MFx] patients from study AAG-G-H-1202)	Completed Propensity Score Matched-Pair Analysis Report available. Number of subjects: 144 (NOVOCART® Inject plus: 72 Microfracture: 72)	Matched-Pair Analysis (NOVOCART® Inject plus patients from Phase III trial vs. MFx patients from NOVOCART® 3D plus Phase III trial).	NOVOCART® Inject plus surgically implanted using arthroscopy or mini-arthrotomy of the knee. Control group: Microfracture	Adult and adolescents aged 14 - 65 (adolescents with closed epiphyseal growth plates) with focal articular cartilage defects of the knee, no prior cartilage repair

Other study reports

Study ID	**Enrolment status** **Start date**	**Design** **Control type**	**Study & control drugs**	**Population** **Main inclusion/ exclusion criteria**

	Total enrolment/ enrolment goal		**Dose, route of administration and duration Regimen**	
Report from the German Cartilage Registry by Niemeyer et al., November 2021	Completed. Report available. NOVOCART® Inject: n= 93 NOVOCART ® 3D: 90 chondrosphere /Spherox®: n=154	Comparison of three different M-ACI products based on data from the German cartilage registry.	NOVOCART® Inject NOVOCART ® 3D: 90 chondrosphere /Spherox®	Patients with isolated cartilage defects in the knee, with an intact corresponding joint surface, intact or less than 1/3 resected meniscus, with a defect size of >2.5 cm2 and no concomitant surgery
Study ID: AAG-G-H-1202, EudraCT 2011-05798-22	Primary analysis report (24 months results) available. The end of the study as a whole (i.e., when the last patient on study has completed his/her 5-year visit) was in Q1/2023 but the study report is not available yet. Patient exposure: NOVOCART® 3D plus: 176 Microfracture: 86	Prospective, randomised, unmasked, multicentre. Included in the propensity score matched-pair analysis described under “Reports of analyses of data from more than one study”	NOVOCART® 3D plus’ surgically implanted using mini-arthrotomy of the knee Comparator: microfracture	Adults and adolescents aged 14 - 65 (adolescents with closed epiphyseal growth plates) with articular cartilage defects (ICRS Grade III or IV) of the femoral condyle or the trochlea of the knee of 2 - 6 cm2 post-debridement. Two defects are acceptable, if the total defect size is ≤ 6 cm2 and the size of each individual lesion is ≥ 2 cm2

Published literature

Reference study ID/Report	**Status** **Follow-up time (months)**	**Design**	**Product** **Location of defect**

(Schlumberger et al., 2019)	Completed. N= 62 Mean follow-up 4.3 years (4.0 - 4.8 years)	Retrospective consecutive case series Germany	NOVOCART® Inject Knee
(Schlumberger et al., 2020)	Completed N=62 Mean follow-up 31,0 ± 14,8 months (range 12.5 - 61.4 months)	Retrospective consecutive case series Germany	NOVOCART® Inject Knee
(Blanke et al., 2021)	Completed N=29 Mean follow-up 24.9 ± 1.1 months (range 24 - 27 months)	Retrospective consecutive case series Germany	NOVOCART® Inject Knee
(Niethammer et al., 2022)	Completed N=25 2 years follow-up	Matched-pair analysis comparing hydrogel-based and scaffold-based ACI Consecutive case series Germany	NOVOCART® Inject Knee
(Kayaalp et al., 2021)	Completed N=5 28 ± 7months (20 - 40 months)	Retrospective case series Germany	NOVOCART® Inject Knee
(Janacova et al., 2022)†	Completed N=20 24 Months	Independent subgroup analysis of Phase III study AAG-G-H-1624	NOVOCART® Inject plus Knee
(Richter et al., 2016)#	Completed N=2 9 and 11 months	Case report Germany	NOVOCART® Inject Knee

(Thier et al., 2018)	Completed N=13 Mean follow-up 12 months (range 6 to 24 months)	Prospective case series ** Germany	NOVOCART® Inject Hip
(Thier et al., 2017)	Completed N=19 (10 patients with co.don chondrosphere/ Spherox®) Mean follow-up 19 months (range 6 to 24 months	Prospective case series comparing NOVOCART® Inject and co.don chondrosphere/ Spherox® §, ** Germany	NOVOCART® Inject Hip

Populations may be overlapping in publications by (Schlumberger et al., 2019) and (Schlumberger et al., 2020) † This publication reports on additional MRI analyses on 20 patients from the MRI population of the Phase III study (AAG-G-H-1624) which was done independently of the phase III trial
This publication reports on arthroscopic second look (1 patient) and immunohistological/ histological analysis (1 patient) to assess repair tissue § No information on the rationale for allocating patients to one or the other M-ACI product is given.
** Populations may be overlapping in publications by (Thier et al., 2017) and (Thier et al., 2018

2.6.5.1. Dose-response studies

NOVOCART® Inject plus contains 2 - 8 Mio. cells per ml cell suspension (ca. 8 - 32 Mio. cells per 4 ml cell suspension). The total volume of NOVOCART® Inject plus is 5 ml (4 ml cell suspension and 1 ml cross-linker). The volume to be administered depends on the size of the prepared defect. At an application height of 2.5 mm, the dosage of NOVOCART® Inject plus is 0.4 to 1.6 Mio. cells per cm2 defect area.

No dose-response study was performed since based on the experience from clinical use of the product in clinical practice the existing dosing from the SmPC was applied.

Nevertheless, potential dose-response relationship was addressed in the pivotal Phase III trial AAG-G-H-1624. The variable "transplant cell density" (millions of cells per cm2 defect area) was included as an independent variable in the covariate analyses to assess its impact on KOOS response at Month 24, KOOS mean change from baseline over time, MOCART subscore 1 (defect filling) at Month 24, and MOCART sum score at Month 24. Generally, the potential impact of cell density was not nominally significant in any of these analyses based on the decision threshold of $p \leq 0.05$.

2.6.5.2. Main study AAG-G-H-1624

Title of Study

Study AAG-G-H-1624: "Prospective, multicenter, single-arm Phase III clinical trial to evaluate the efficacy and safety of NOVOCART® Inject plus in the treatment of cartilage defects of the knee"

Methods

This is a prospective, multicenter, single-arm Phase III clinical trial to evaluate the efficacy and safety of NOVOCART® Inject plus in the treatment of cartilage defects of the knee in adult patients aged between 18 and 65 years and pediatric patients (14 to 17 years) with closed epiphysis.

The study consists of 3 periods (with a maximum individual study duration of about 5 years and 4 months):
- Screening phase (Visit 1; within 3 months prior to Visit 2).
- Treatment phase (tissue harvest at Visit 2 after arthroscopic confirmation of eligibility criteria; ACI 3 to 4 weeks later at Visit 3).
- Follow-up phase (hospital discharge at Visit 4, follow-up at Months 3, 6, 12, 18, 24, 36, 48, and 60 [Visits 5 to 12]).

Study Participants

Inclusion criteria

Pre-operative inclusion criteria (Visit 1)

1. Patient is ≥ 18 and ≤ 65 years old at screening. Pediatric patients (14 to 17 years) with closed epiphysis may be included in selected countries. Closure of the distal femoral epiphysis of the target knee needs to be confirmed by MRI or x-ray.
2. Patients with focal articular cartilage defect(s) of the femoral condyle (medial or lateral), the trochlea, the patella or the tibial plateau (medial or lateral) of the knee.
3. Patient has one or two defects in the target knee with a defect-grade of III or IV according to the International Cartilage Repair Society (ICRS) classification or Grade III or IV ICRS classification of osteochondritis dissecans lesions.
4. If the patient has only one Grade III or IV defect, the size of the defect is ≥4 and ≤12 cm2 post-debridement. If the patient has two Grade III or IV cartilage defects the total defect size is ≥4 and ≤12 cm2 post-debridement. The defects must be located on different knee joint areas and both cartilage defects are to be treated with NOVOCART® Inject plus.
    a: Knee joint areas were defined as lateral or medial condyle, trochlea, patella, lateral or medial tibial plateau.
5. Patient has an intact, well-contained chondral structure surrounding the defect (i.e., no diffusely thinned-out cartilage surface) and an intact articulating joint surface opposite to the defect(s) to be treated (≤ Grade I ICRS or ICRS-OCD classification).
6. Patient has intact menisci in the target knee; a maximum of 50% resection per meniscus is allowed. Completely healed meniscal transplants are accepted.
7. Patient has a stable knee joint or sufficiently reconstructed ligaments and no patella malalignment or a sufficiently corrected patella malalignment. Ligament repair is accepted, if performed before, during or within 6 weeks after NOVOCART® Inject plus implantation. Correction of patella malalignment must be performed before or during NOVOCART® Inject plus implantation.
8. Patient has free range of motion (ROM) of the affected knee joint or ≤10° of active extension and flexion loss (compared to the other knee).
9. Patient has a baseline score of <65/100 in the overall KOOS (KOOS5).
10. Only for female patients of childbearing potential (sexually mature, pre-menopausal and not surgically sterile): The patient is willing to use a medically accepted method of contraception until the day of NOVOCART® Inject plus implantation.

11. Patient is willing and able to give written informed consent to participate in the study and to comply with all follow-up visits and assessments and the postoperative rehabilitation program.

Intra-operative inclusion criteria (Visit 2)

1. Patient is not pregnant as confirmed by urine pregnancy test before arthroscopy.
2. Patients with focal articular cartilage defect(s) of the femoral condyle (medial or lateral), the trochlea, the patella or the tibial plateau (medial or lateral) of the knee.
3. Patient has one or two defects in the target knee with a defect-grade of III or IV according to the International Cartilage Repair Society (ICRS) classification or grade III or IV ICRS classification of osteochondritis dissecans lesions (ICRS-OCD).
4. If the patient has only one Grade III or IV defect, the size of the defect is ≥4 and ≤12 cm2 post-debridement. If the patient has two Grade III or IV cartilage defects the total defect size is ≥4 and ≤12 cm2 post-debridement. The defects must be located on different knee joint areas and both cartilage defects are to be treated with NOVOCART® Inject plus.
    a: Knee joint areas were defined as lateral or medial condyle, trochlea, patella, lateral or medial tibial plateau.
5. Patient has an intact, well-contained chondral structure surrounding the defect (i.e., no diffusely thinned-out cartilage surface) and an intact articulating joint surface opposite to the defect(s) to be treated (≤ Grade I ICRS or ICRS-OCD classification).
6. Patient has intact menisci in the target knee; a maximum of 50% resection per meniscus is allowed. Completely healed meniscal transplants are accepted.

Exclusion criteria

Pre-operative exclusion criteria (Visit 1)

1. Patient is unable to undergo magnetic resonance imaging (MRI).
2. Patient has Grade II cartilage defects according to the ICRS or ICRS-OCD classification in the target knee.
3. Patient has more than 2 Grade III or IV cartilage defects according to the ICRS or ICRS-OCD classification in the target knee.
4. The cartilage defects (ICRS or ICRS-OCD Grade I or III or IV) affect more than 3 out of 6 joint areas of the target knee.
a: Knee joint areas were defined as lateral or medial condyle, trochlea, patella, lateral or medial tibial plateau.
5. Patient had a prior biologic reconstructive procedure (e.g., microfracture, mosaicplasty, chondrocyte implantation) in the target knee at a location different from the defect location to be treated in the study. Prior biologic reconstructive procedures are accepted, if the previously treated defect is the same defect that is planned to be treated with NOVOCART® Inject plus (i.e. the prior method has failed) and these procedures were performed ≥24 months prior to screening Visit 1.

6. Patient had other prior surgical interventions interfering with the assessment of the NOVOCART® Inject plus treatment. Prior diagnostic arthroscopies with debridement and lavage are acceptable.
7. Patients with subchondral bone defects more than 2 mm deep (after resection of affected bone) unless adjuvant defect filling will be performed before or during NOVOCART® Inject plus implantation.
8. Patient has degenerative joint disease in the target knee as determined by Kellgren and Lawrence Grade >2.
9. Patient has chronic inflammatory arthritis and/or infectious arthritis.
10. Patient has joint space narrowing >1/3 in the target knee when compared to the other knee or <3 mm

joint space.
11. Patient has malalignment (valgus- or varus-deformity) in the target knee. Note: In suspected cases, the mechanical axis must be established radiographically by complete leg imaging in standing position and in anteriorposterior (a.p.) or rather posterioranterior (p.a.) projection. The Mikulicz line is not allowed to deviate more than 5 mm of the eminentia intercondylaris. If alignment is necessary, surgery has to be performed before, during or within 6 weeks after NOVOCART® Inject plus implantation.
12. Patient has arthrofibrosis in the target knee.
13. Patient has diffuse chondromalacia (Grade 1 "softening or swelling of cartilage" according to Outerbridge allowed).
14. Patient has metabolic arthropathies (e.g., gout, pseudo-gout).
15. Patient has bilateral lower limb pain or low back pain.
16. Patient has a known systemic connective tissue disease.
17. Patient has a current uncontrolled diabetes.
18. Patient has a known history of autoimmune disease.
19. Patient has a known history of immunological suppressive disorder or is taking immunosuppressants.
20. Patient is currently systemically or intra-articularly taking steroids and/or has used steroids within the last 30 days prior to screening Visit 1.

21. The patient has a history of Human Immunodeficiency Virus (HIV)/ Acquired Immune Deficiency Syndrome (AIDS), Human T-cell lymphotropic virus (HTLV), syphilis (Treponema pallidum) or active hepatitis B or C (HCV) infection with verified antigens. Patients with a cured hepatitis B or C infection and/or verified antibodies are not excluded.
22. The patient has a history of borreliosis.
23. The patient has an active systemic or local (at the site of surgery) infection, eczematization or inflammable skin alterations.
24. Patient has a known history of cancer within the past 5 years.
25. Patient has a known history of osteoporosis, uncontrolled primary hyperparathyroidism or hyperthyroidism, chronic renal failure or prior pathological fractures independent of the genesis.
26. Patient has any degenerative muscular 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).
27. Patient has a body mass index (BMI) >35 kg/m2.
28. Patient is a woman who is pregnant or lactating.
29. Patient is currently participating, or has participated, in any other clinical study within 3 months prior to screening Visit 1.
30. Patient has known current or recent history of illicit drug or alcohol abuse or dependence.
31. 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.
32. Patient has a known intolerance to any constituents of NOVOCART® Inject plus.
33. Patient has comorbidities preventing the patient from undergoing surgery.
34. Patient has any other condition, which, in the opinion of the investigator, would make the patient unsuitable for the study.

Intra-operative exclusion criteria (Visit 2)

1. Patient has Grade II cartilage defects according to the ICRS or ICRS-OCD classification in the target knee.
2. Patient has more than 2 Grade III or IV cartilage defects according to the ICRS or ICRS-OCD classification in the target knee.

3. The cartilage defects (ICRS or ICRS-OCD Grade I or III or IV) affect more than 3 out of 6 joint areas of the target knee.
    a: Knee joint areas were defined as lateral or medial condyle, trochlea, patella, lateral or medial tibial plateau.
4. Patients with subchondral bone defects more than 2 mm deep (after resection of affected bone) unless adjuvant defect filling will be performed before or during NOVOCART® Inject plus implantation.
5. Patient has arthrofibrosis in the target knee.
6. Patient has diffuse chondromalacia (Grade 1 "softening or swelling of cartilage" according to Outerbridge allowed).

Treatments

The experimental treatment (Advanced Therapy Investigational Medicinal Product; ATIMP) in this study is NOVOCART® Inject plus. In addition, NOVOCART® Inject plus was applied in conjunction with a rehabilitation program according to the rehabilitation regimen defined in the clinical study protocol. NOVOCART® Inject plus is a 3rd generation matrix-based autologous chondrocyte implantation system to treat full-thickness cartilage defects. The injectable nature of NOVOCART® Inject plus allows to treat difficult-to-reach locations (for example at the patella or tibia), because the hydrogel seeps into small defect niches after application. Due to the adhesive properties of NOVOCART® Inject plus which solidifies within 1 to 3 minutes after application into the defect, no additional fixation of the carrier material (suturing or fixation with fibrin glue) or any cover (like a periosteal flap) is required.

The treatment with NOVOCART® Inject plus required 2 surgeries. During the first surgery (performed at Visit 2), autologous chondrocytes for transplant production were arthroscopically harvested. About 3 to 4 weeks later (Visit 3), the finished product NOVOCART® Inject plus (consisting of the expanded autologous chondrocytes carried in a human albumin/hyaluronan solution and a cross-linker solution) was transplanted during a second session. In general, NOVOCART® Inject plus is designed to be transplanted arthroscopically, but (mini)-arthrotomy might be indicated depending on corrective surgeries concomitantly performed to NOVOCART® Inject plus implantation. In each case, the final product was administered using a double chamber syringe supplied with the final product in order to allow mixing of the 2 components and subsequent cross-linking of the cell- carrying hydrogel at the site of administration. NOVOCART® Inject plus contains 2 - 8 million cells per mL cell suspension (about 8 - 32 million cells per 4 mL cell suspension). The total volume of NOVOCART® Inject plus is 5 mL (4 mL cell suspension and 1 mL cross-linker). The volume to be administered depends on the size of the prepared defect. At an application height of 2.5 mm, the dosage of NOVOCART® Inject plus is 0.4 to 1.6 million cells per cm2 defect area. In addition to the double-chamber syringe needed for product administration, surgical instruments (harvesting trephines and instruments required for debridement) were provided by the Sponsor to ensure similar procedural preconditions among the participating study site.

Rehabilitation measures

The primary goals of rehabilitation measures after chondral restorative procedures of the knee are return to full weight-bearing, the restitution of preoperative range of motion, the restoration of muscle strength, and the recovery of neuromuscular control. Thus, all study patients were to undergo post-treatment rehabilitation (based on [Hirschmüller et al. 2011]) starting on the day of implantation as outlined in the CSP. Rehabilitation (pre- or post-treatment) was to be reported in the eCRF and the patient’s medical records.

Concomitant and rescue therapies

Permitted concomitant medications

Outside the time windows mentioned (wash-out, tissue harvest until 2 weeks post NOVOCART® Inject plus implantation) normal standard of care should be followed for pain management, DVT prophylaxis, and for prophylactic antibiotics (per os [p.o.] or intravenous [i.v.]).
In general, topical treatments (ointments, creams) were allowed during the study with the exception of topic use of steroids nearby the target knee during the time windows mentioned below.

Permitted concomitant surgical procedures

Patients with the following conditions were allowed to be included in the study, provided that these conditions were corrected within the below-specified timeframes:
- In patients with instable knee joint, ligament repair or alignment of valgus- or varus-deformities had to be performed before, during or within 6 weeks after NOVOCART® Inject plus implantation.
- In patients with patella malalignment, correction of patella malalignment had to be performed before or during NOVOCART® Inject plus implantation.
- In patients with subchondral bone defects more than 2 mm deep (after resection of affected bone) adjuvant defect filling had to be performed before or during NOVOCART® Inject plus implantation. When bone grafting was done, NOVOCART® Inject plus application had to be done by mini-arthrotomy (not by arthroscopy). Depending on the depth of the defect, treatment with cancellous bone grafting or bone block augmentation (cylinder type cancellous bone) should be done. A tourniquet was indicated. The surgeon was to take care that after tourniquet release, the NOVOCART® Inject plus graft had stayed in place. Artificial bone substitutes or allogenic bone grafts were not recommended.
Concomitant surgeries performed at Visit 3 (NOVOCART® Inject plus implantation) were to be completed prior to NOVOCART® Inject plus injection into the defect. Subsequent surgical procedures, if required, were permitted throughout the study and should preferably be performed by the investigator who had performed the initial procedure. Otherwise, the relevant medical information had to be provided (via a medical records release) to the investigator for study reporting. Patients undergoing subsequent surgeries were not necessarily to be withdrawn from the study.

Prohibited concomitant medications

<u>Wash-out period</u>

Steroids were prohibited from 30 days prior to screening (Visit 1) until 2 weeks post NOVOCART® Inject implantation). The local use of steroids (e.g., topic or intra-nasal application to treat allergies) was allowed, if no application was done nearby the target knee.
The following medications were to be stopped a minimum of 7 days prior to arthroscopy/randomization (Visit 2):
- Indomethacin,
- Salicylates (e.g., Aspirin). Cardiovascular prophylaxis with low-dose
- aminosalicylic acid (ASA; ≤150 mg/day) was allowed to be continued

<u>Prohibited medications through 2 weeks post-surgery</u>

Excluded therapies and medications from tissue harvest at Visit 2 through 2 weeks post NOVOCART® Inject plus implantation at Visit 3 were:
☐ Intra-articular analgesics;
☐ Intra-articular antibiotic wash;

- Hyaluronic acid or platelet rich plasma (PRP);
- Chondroplasty using thermal probes or lasers;
- Indomethacin;
- Salicylates (e.g., Aspirin). Cardiovascular prophylaxis with low-dose ASA (≤150 mg/day) could be continued.
- Corticosteroids. Local use of corticosteroids (e.g., topic or intra-nasal application to treat allergies) was allowed, if no application was is done nearby the target knee.

For post-operative pain management (until 2 weeks after NOVOCART® Inject plus implantation), it was recommended to use non-NSAIDs (non-steroidal anti-inflammatory drugs) such as:
- Metamizole,
- Paracetamol/acetaminophen,
- Opioid derivatives such as Tilidin®.

If post-operative prophylaxis of DVT was indicated, it was recommended to use low-molecular weight heparins.

Objectives

Outcomes/endpoints

Primary objective

The primary objective of this study was to demonstrate efficacy of the NOVOCART® Inject plus for the treatment of articular cartilage defects of the knee based on the Knee Injury and Osteoarthritis Outcome Score [KOOS] total responder rate 24 months after implantation. The primary objective was to show that the KOOS responder rate (percentage of patients who improved their total KOOS by at least 10 points compared to the preoperative value) is over 40% 24 months after treatment.

Primary endpoint

The primary efficacy endpoint of the study was the responder rate based on the total KOOS (calculated as the average of the 5 subscale scores [ensuring identical weight from all subscales]) after 24 months of treatment.

Secondary objectives
- to assess the patients' evaluation of efficacy of NOVOCART® Inject plus in terms of symptoms, function, quality of life and satisfaction with NOVOCART® Inject plus implantation,
- to assess the physicians' evaluation of the functional efficacy of NOVOCART® Inject plus,
- to evaluate cartilage (tissue-structure) regenerative effects of NOVOCART® Inject plus in a subgroup of 48 patients,
- to assess the treatment failure rate and time to treatment failure,
- to characterize the safe use of NOVOCART® Inject plus and the related surgical procedures.

<u>Other objectives of the study were:</u>
- Durability of the treatment effect,
- Long-term safety,
- Health economics analyses (see Section 9.7.9.1),
- Correlations between biological activity and quality and potency measurements.

Secondary endpoints
1) Change in the total KOOS from baseline to the 24-month assessment.

2) Change in the 5 individual subscores of the KOOS from baseline to the 24-month assessment.
3) Change from baseline in the International Knee Documentation Committee (IKDC) subjective score from baseline to the 24- month assessment.
4) IKDC subjective score responder rate, defined as the proportion of patients with >20.5-point improvement in the IKDC subjective score from baseline to the 24-month visit.
5) Change from baseline to the 24-month visit in the EQ-5D-5L questionnaire.
6) Change from baseline to the 24-month visit in activity level /functional status.
7) Patient satisfaction with treatment 24 months after NOVOCART® Inject plus implantation.
8) Change from baseline to the 24-month visit in the grading according to the IKDC objective.
9) In vivo performance measured by the 24-month morphological assessment of the Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score and biochemical MR assessment by T2 mapping in a subgroup of 48 patients.
10) Treatment failure rate, defined as all surgical re-interventions affecting the closed surface of the transplant area (the surface was not closed when the defect area was Grade 3 or 4 ICRS) and/or required additional cartilage repair modalities on the target defect.
11) Time to treatment failure.
12) Treatment-related adverse events (TEAEs) from baseline to the 24-month assessment.

Other endpoints of the study were:
1) Variables assessed for the primary and secondary endpoints at other scheduled time points.
2) Continuous assessment of adverse events (AEs).
3) Health economics variables related to NOVOCART® Inject plus treatment:
   a) Work/school status
   b) Days of work/school missed due to knee pain/function issues in the target knee
   c) Days of hospitalization due to knee pain/function issues in the target knee
   d) Number of postoperative doctor visits (except scheduled study visits) for knee pain/function issues in the target knee
   e) Number of postoperative physical therapy visits in the context of NOVOCART® Inject plus implantation.
4) Biomarker analyses: Clinical data collected in this study were used to establish correlations between biological activity and quality and potency measurements. To allow for such correlations, samples for the analysis of marker molecules on protein or mRNA level were collected in this study. Cell culture supernatants were available during the cell manufacturing process and mRNA samples were routinely acquired for quality control of the product and were used for additional analyses.
5) Histological evaluation of structural repair based on the ICRS II score in patients undergoing subsequent surgeries due to treatment failure (actually not done as there were no cases to be analyzed).

Description of the efficacy variables

KOOS

The KOOS has been developed as an instrument to assess the patients' opinion about their knee and associated problems. The KOOS total score (KOOS5) consists of the 5 subscales pain, other symptoms, activities of daily living (ADL), function in sport and recreation (sport/rec) and knee-related QoL. The last week is taken into consideration when answering the questions. Standardized answer options are given (5 Likert boxes) and each question gets a score from 0 to 4. A normalized score (100 indicating no symptoms and 0 indicating extreme symptoms) is calculated for each subscale. The result can be plotted as an outcome

profile. In addition, the KOOS total score, defined as the average of the 5 subscale scores (ensuring equal weighting of all subscales), can be calculated. The KOOS is patient-
administered and takes about 10 minutes to fill out.

IKDC 2000 subjective

The IKDC subjective score is an established, knee-specific, patient-reported outcome measure. The questionnaire covers 3 separate categories: "symptoms" (7 questions), "sports activity" (2 questions), and "current knee function" (1 question). The IKDC subjective total transformed score has a span from 0 to 100, with higher values indicating higher levels of function and lower levels of symptoms.

IKDC objective score

The IKDC objective score is performed by the investigator to evaluate a variety of knee conditions including ligament, meniscal, articular cartilage, arthritis, and patellofemoral injuries. The assessment consists of a functional assessment of the knee (range of motion, rotation, crepitation), as well as instrumental and/or imaging-based evaluation of the different compartments.
The form (2000 IKDC knee examination form) contains items that fall into one of 7 measurement domains. The 7 domains assessed by the knee examination form are:

1. Effusion
2. Passive Motion Deficit
3. Ligament Examination
4. Compartment Findings
5. Harvest Site Pathology
6. x-ray Findings
7. Functional Test

Each item is evaluated using a 4-grade classification into "A: normal", "B: nearly normal", "C: abnormal", and "D: severely abnormal".

EQ-5D-5L

The EQ-5D is a standardized measure of health status developed by the EuroQol Group in order to provide a simple, generic measure of health for clinical and economic appraisal [EuroQoL-Group 1990]. This measure originally consisted of 5 dimensions (mobility [1], self- care [2], usual activities [3], pain/discomfort [4], and anxiety/depression [5]) with each 3 response levels (no problems, some problems, extreme problems). In order to improve the instrument's sensitivity and to reduce ceiling effects, 5 levels of severity were included later on in each of the existing 5 EQ-5D dimensions (1=no problems, 2=slight problems, 3=moderate problems, 4=severe problems, and 5=extreme problems/performance inability), and this updated measure is called EQ-5D-5L (i.e., Euro-QoL with 5 dimensions and 5 levels) [Herdman et al. 2011].

Using the 5 dimensions as digits (see aforementioned sequence) and the 5 levels as values, an individual health state can be expressed as a 5-digit code, ranging from 11111 (no problems in none of the 5 dimensions) to 55555 (extreme problems in all 5 dimensions). This coding allows the expression of 3,125 (55) different health states. The EQ-5D-5L health states can be converted to a single index value. The index values, presented in country-specific value sets, are a major feature of the EQ-5D instrument, facilitating the calculation of quality-adjusted life years (QALYs) that are used to perform
economic evaluations of health care interventions. In addition to the assessment of the 5 dimensions, the EQ also includes a 20 cm vertical visual analogue scale (EQ-VAS), where the endpoints are labelled "best imaginable health state" (value 100) and "worst imaginable health state" (value 0). The respondent is asked to record his/her current health condition by ticking a value on the 0 - 100 scale, and this information can be used as a quantitative measure of health outcome as judged by the individual respondents.

Overall, the data collected using EQ-5D-5L can be presented in various ways, basically by presenting results from the EQ-5D-5L descriptive system as a health profile, from the VAS as an overall self-rated health status (range: 0=worst imaginable health state to 100=best imaginable health state), or from the EQ-5D-5L index value. The index value is formally anchored from 0=death to 1=perfect health but, depending on the crosswalk value set for a specific country, concrete index values might be slightly lower than 0 or slightly higher than 1. Importantly, higher values indicate better health conditions. The self-reported health status captured by EQ-5D-5L relates to the respondent's situation at the time of completion; thus, no recall period is stipulated.

In the current study, EQ results were provided as EQ-5D-5L index value and EQ-VAS score. The hypothetical range of the index value is from -0.661 to 1 based on the validated national value set for Germany published in 2018 [Ludwig et al. 2018].

Analysis of activity level and functional status

On Visits 1 and 5-12/EOS, the patients' activity level and functional status was documented as ordinal results of 1 to 4, with 1 representing the highest activity level or functional status and 4 the lowest.

The activity levels were labelled as follows: "(1) high competitive sportsman/woman", "(2) well-trained and frequently sporting", "(3) sporting sometimes", and "(4) non-sporting".

The functional levels were classified as follows: "(1) I can do everything that I want to do with my joint", "(2) can do nearly everything that I want to do with my joint", "(3) I am restricted and a lot of things that I want to do with my joint are not possible", and " (4) I am very restricted and I can do almost nothing with my joint without severe pain and disability".

Activity level and functional status data were summarized using frequency tabulations for each scheduled visit when data were collected. Statistical significance of the change from baseline in activity level/functional status was tested at Month 24 using the Wilcoxon signed-rank test. Only subjects with non-missing change from baseline values were considered for the signed-rank test.

Analysis of patient satisfaction with treatment

For the patient-reported evaluation of satisfaction with treatment, the patients were answered 4 questions:

1. Compared with before joint surgery, how would you rate your operated joint now? (better, about the same, worse)
2. What is your overall satisfaction level with the joint surgery? (satisfied, neutral, dissatisfied)
3. If you could go back in time and make decision again, would you again choose to have your joint surgery? (yes, uncertain, no)
4. How would you rate the results of your joint surgery? (good/excellent, fair, poor)

The following tables were generated for the analysis of patient satisfaction with treatment (ITT and PPS, overall and in Subgroups A and B):

- Summary of patient satisfaction with treatment
- Summary of KOOS total change from baseline by categories of treatment satisfaction

MOCART scoring and T2 mapping

Within the follow-up study period, a subgroup of patients is scheduled to undergo 3 additional MRI examinations (at Months 12, 24, and 60) to acquire specific imaging data on cartilage repair tissue morphology and ultrastructural composition under NOVOCART® Inject plus treatment. Only study sites that fulfil the technical requirements are participating in the MRI sub-study.

The Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score is used to assess in vivo performance of cartilage repair. The MOCART scoring system is an evaluation method for repaired cartilage useful for long-term follow-up. It has been shown to be reliable and reproducible [Marlovits et al. 2006b, Trattnig et al. 2007] with low inter- observer variability [Marlovits et al. 2006a]. The MOCART score is defined by 10 ordinal and dichotomous subscales: degree of defect repair and filling, integration to border zone, surface of the repair tissue, structure of the repair tissue, signal intensity of the repair tissue (dual FSE and 3D GRE), subchondral lamina, subchondral bone, adhesion, and effusion.

Table 3. MOCART scoring system

Assessed variable	Finding	Score allocation
1) Filling of defect	Subchondral bone exposed	0
	Incomplete: <50% of adjacent cartilage	5
	Incomplete: >50% of adjacent cartilage	10
	Hypertrophy	15
	Complete [a]	20
2) Integration to border zone (Cartilage interface)	Defect visible: >50% of length	0
	Defect visible: <50% of length	5
	Incomplete: Demarcating border visible	10
	Complete	15
3) Surface	Surface damaged >50% of depth	0
	Surface damaged <50% of depth	5
	Surface (lamina splendens) intact	10
4) Structure	Inhomogeneous or cleft formation	0
	Homogenous	5
5) Signal intensity of repair tissue (Dual T2-FSE)	Markedly hyperintense	0
	Moderately hyperintense	5
	Normal (isointense)	15
6) Signal intensity of repair tissue (3D GRE)	Markedly hypointense	0
	Moderately hypointense	5
	Normal (isointense)	15
7) Subchondral lamina	Not intact	0
	Intact	5
8) Subchondral bone	Not intact	0
	Intact	5
9) Adhesions	Yes	0
	No	5
10) Effusion	Yes	0
	No	5
	Total points (maximum)	100

a: In accordance with MOCART Version 2.0, the category "complete" includes minor hypertrophy (up to 150% filling of the total defect volume).

T2 relaxation time measurement is a marker for cartilage water and collagen content as well as collagen matrix organization that reflects the integrity and vitality of cartilage and cartilage regenerated tissue [Domayer et al. 2008, Kurkijarvi et al. 2007, Welsch et al. 2008, Welsch et al. 2009a, Welsch et al. 2009b, Welsch et al. 2009c]. T2 mapping offers insight into the collagen fibre network organization as well as the water content of the repair tissue on a quantitative basis by calculation of the T2 relaxation times in repair tissue with decreasing values for T2 relaxation time (up to a certain extent) and smaller T2 standard

deviation indicating a higher structural organization of the regenerate tissue. Moreover, the T2 relaxation time measured in the repair tissue can be set in relation to the T2 relaxation time measured in the surrounding healthy cartilage tissue, thereby resulting in
a "global" T2 ratio (if only the full-thickness tissue areas are measured) and in a "zonal" T2 ratio (if, in addition, the differences in T2 relaxation times in the superficial and deep zones of the cartilage areas are considered).

All post-treatment MRI images of patients participating in the MRI-substudy are sent to the imaging core laboratory (HFMRC Vienna) for blinded review (i.e., reviewers are blinded with regard to patient details [i.e., name, birth date, etc.], patients' medical conditions [except defect localization, number of defects and defect size], and clinical outcomes).

No baseline status was documented, because both the MOCART score and the T2 mapping characteristics are post-operative assessments that mainly refer to the cartilage repair tissue.

Treatment failure

Patients with symptoms suspicious of treatment failure were to be examined by the investigator via MRI or arthroscopy before a revision surgery was performed. In case of doubt regarding the need for revision surgery, it was recommended that the investigator consults the international coordinating investigator. Intraoperatively diagnosed treatment failures were defined as all surgical re-interventions affecting the closed surface of the transplant area (the surface is not closed when the defect area is Grade 3 or 4 ICRS) and/or required additional cartilage repair modalities on the target defect.

Explanatory notes:
- Removal of hypertrophic tissue was not to be regarded as treatment failure unless the closed graft surface is affected.
- Correction of insufficient graft integration, e.g., by microfracture the gap between graft and surrounding cartilage of the originally treated defect, was to be regarded as treatment failure.
- Treatment of adjacent defects to the originally treated defect was not to be regarded as a treatment failure.
- Surgical lysis of adhesions was not to be regarded as a treatment failure

The patient classification as "treatment failure" was performed by a DMC.

In addition, as by clinical study protocol (CSP), in patients who experience treatment failure and need to undergo re-surgery, biopsies will be taken from the transplant area for histological evaluation of structural repair. Structural repair will be assessed based on the ICRS II scoring system (Mainil-Varlet et al., 2010) to gain a better understanding of treatment failure. The ICRS II score will be assessed centrally.

Sample size

Based on the primary efficacy endpoint, an overall response rate of 55% was considered to be achievable taking into account a dropout rate of up to 15%.

The primary objective of the study was to show that the response rate was above a pre-defined no-effect level of 40%.

Using a one-sided exact binomial test with significance level 0.0232 (initially adjusted for one interim analysis that was never performed), a power of 80% and a response rate of 55% the required number of patients was estimated to be 96.

Randomisation and blinding (masking)

Randomisation: not applicable. Patients were treated in sequential order of their eligibility confirmation at each study site.

Blinding: not applicable.

Statistical methods

The primary analysis was based on the Intent-to-treat population (ITT) which included all patients who had received the NOVOCART® Inject plus implantation.

The primary efficacy endpoint was the responder rate defined as the proportion of subjects with a ≥10 points improvement in the total KOOS (/KOOS 5) from baseline to Month 24 post-transplantation.

In estimating the sample size, an overall KOOS5 response rate of 55% had been considered. Using a one-sided exact binomial test with significance level 0.0232, initially adjusted for one interim analysis that was never performed, and a power of 80%, the required number of patients was estimated to be 96.

The primary objective of the study was to show that the endpoint rate was above a pre-defined no-effect level and hence, the threshold for efficacy in KOOS5 was a response rate of 40%.

The primary hypothesis was tested by a one-sided exact binomial test. The finally used significance level was 0.025 one-sided. The response rate was presented with a two-sided 95% Clopper-Pearson (exact) binomial confidence interval.

For the primary efficacy endpoint analysis, patients with missing endpoint assessments and patients classified as treatment failures before or at month 24 were handled as "non-responders".

The inferential part of the study was defined to include the primary endpoint alone.

The analyses of secondary efficacy endpoints were to be interpreted as exploratory.

For the analyses of continuous secondary efficacy variables, a linear mixed effect model for repeated measurements (MMRM) was used assuming MAR (missing at random).

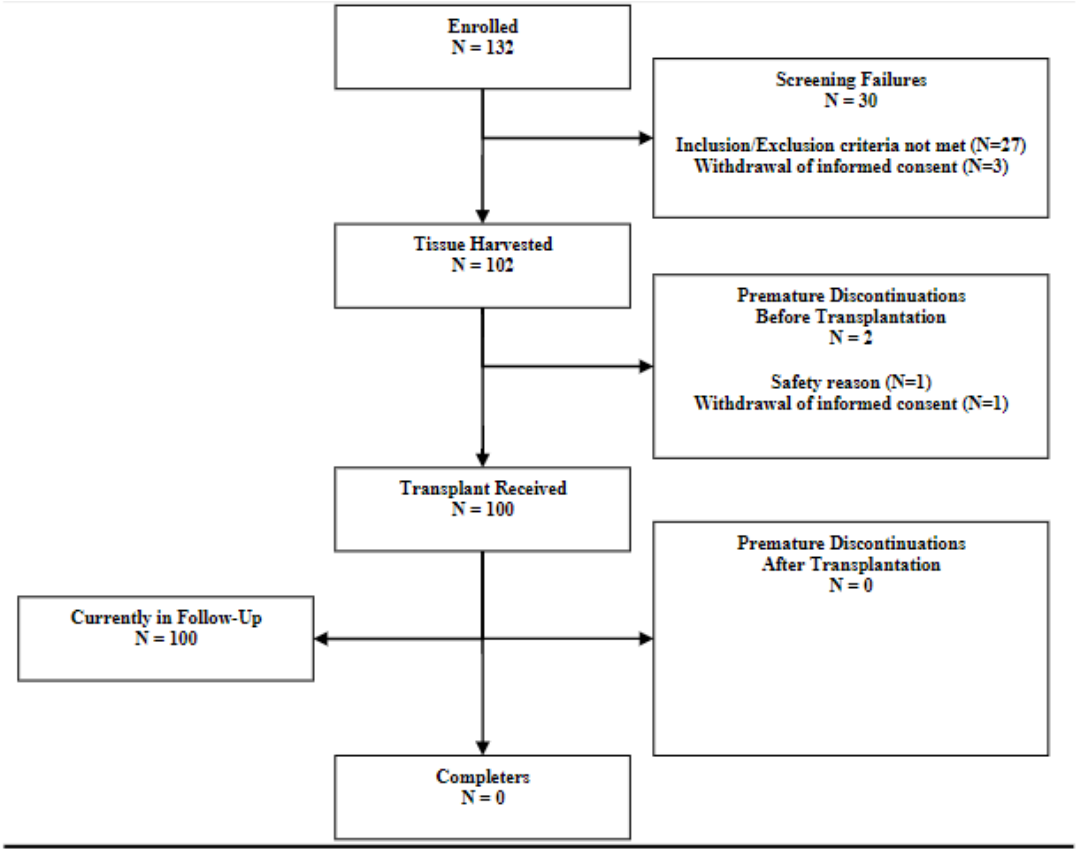
An interim analysis was initially planned according to a group-sequential analysis design but was never performed (deleted within protocol amendment 4).

Results

Participant flow

A total of 132 patients were initially enrolled in the study, and 102 patients thereof were actually treated (i.e., completed Visit 2). The primary reasons for the screening failure among the 30 patients who remained untreated were non-fulfilment of eligibility criteria (27 patients) and withdrawal of informed consent (3 patients). 102 study patients were treated (i.e., those with executed tissue harvest at Visit 2, thereby constituting the SAF). A total of 100 patients are still on study follow-up, while 2 patients had discontinued the study participation before the implantation at Visit 3, but after cell sampling at Visit 2. No specific reason was provided from patient No. 6-01-04 who withdrew his consent for further study participation; the specified safety reason in patient No. 6-02-06 was "patient did not stop using ticagrelor 5 days before surgery". The duration of follow-up among the 100 ITT patients through the cut-off date for the analysis on 22-Feb-2021 was 727.4 ± 17.81 days (median: 729.0 days, range: 686-778 days).

Figure 1. Patient disposition after 24 months of study



Source: Section 15, Post-text figure 15.1.1

Recruitment

The first patient was included in the study on 10-Oct-2017. Per country, the enrolment was highest with 33 patients in the Czech Republic, followed by 32 patients in Lithuania, 24 patients in Hungary, 10 patients in Germany, and 1 patient in Switzerland.

Conduct of the study

The study conduct was in line with the declaration of Helsinki and GCP guidelines. One site audit was performed and another was planned to be performed in 2020; from both, findings were not reported by the applicant.

Changes to the analysis as laid down in the protocol and amendments were described in both SAP final version 1.0 and SAP final version 2.0 as follows:

The categorical effect "region" referenced in the protocol section 14.3.3 and used in efficacy analyses is replaced with the more precise categorical effect "country".

As described in the CSR, the following *post hoc* analyses were performed:

Following the identification of the fixed factor "country" as an influencing variable, country-wise descriptive analyses for the changes from baseline were provided for KOOS total, IKDC subjective, EQ-5D-5L index score, and EQ-VAS.

KOOS responder rates and IKDC responder rates were statistically analysed by country. Moreover, mean changes from baseline in KOOS total score and IKDC subjective score were analysed in total and by country using an MMRM, in which an interaction term "country*visit" was introduced in addition to the standard factors/covariates' "country", "visit", and "baseline KOOS/IKDC value".

MOCART sum scores were additionally calculated based on a data set in which the 3 patients with missing repair tissue had been excluded (i.e., 22 instead of 25 MRI-ITT patients).

ACI characteristics were additionally calculated for the subgroup of ITT patients without any concomitant surgeries at Visit 2 and/or Visit 3 (N=78)

Baseline data

Table 4. General demographic data in total and by age subgroups (ITT)

Characteristic		All patients N=100	Age subgroups ≤25 years N=13	Age subgroups >25 years N=87
Age (years)	n	100	13	87
	Mean ± SD	39.8 ± 11.51	20.5 ± 3.20	42.7 ± 9.31
	Median	41.0	21.0	43.0
	Range	15-62	15-25	26-62
Age subgroups A and B (years), n (%)	≥14 – ≤25	13 (13.0)	13 (100.0)	0
	>25 – ≤65	87 (87.0)	0	87 (100.0)
Age categories (years), n (%)	≥14 – <18	2 (2.0)	2 (15.4)	0
	≥18 – <65	98 (98.0)	11 (84.6)	87 (100.0)
Sex, n (%)	Male	63 (63.0)	11 (84.6)	52 (59.8)
	Female	37 (37.0)	2 (15.4)	35 (40.2)
Childbearing potential, n (% of females)	Yes	27 (73.0)	2 (100.0)	25 (71.4)
	No	10 (27.0)	0	10 (28.6)
Ethnicity, n (%)	Not Hispanic	100 (100.0)	13 (100.0)	87 (100.0)
Race, n (%)	White	99 (99.0)	13 (100.0)	86 (98.9)
	Unknown	1 (1.0)	0	1 (1.1)
Smoking status, n (%)	Yes	25 (25.0)	3 (23.1)	22 (25.3)
	No	75 (75.0)	10 (76.9)	65 (74.7)
Cigarettes per day	n	25	3	22
	Mean ± SD	13.0 ± 7.59	12.0 ± 7.21	13.1 ± 7.79
	Median	10.0	10.0	10.0
	Range	1-30	6-20	1-30
Body weight (kg)	n	100	13	87
	Mean ± SD	84.4 ± 15.14	83.8 ± 14.33	84.5 ± 15.33
	Median	85.0	79.0	86.0
	Range	53-120	68-115	53-120
Body height (cm)	n	100	13	87
	Mean ± SD	176.6 ± 10.17	181.2 ± 12.06	175.9 ± 9.75
	Median	176.5	185.0	175.0
	Range	154-199	159-197	154-199
Body mass index (kg/m²)	n	100	13	87
	Mean ± SD	27.03 ± 4.095	25.65 ± 4.249	27.24 ± 4.056
	Median	26.85	24.40	27.10
	Range	20.2-34.4	20.4-33.6	20.2-34.4
	Q1/Q3	23.45/30.10	22.20/28.50	23.90/30.90

Q1=First quartile; Q3=Third quartile; SD=Standard deviation
Source: Section 15, Post-text tables 15.1.6.1.1, 15.1.6.2.1, and 15.1.6.3.1

Table 5. Prior medical conditions on the target knee (ITT)

MedDRA SOC MedDRA preferred term	All patients (N=100) n (%)
At least one prior medical condition	**39 (39.0)**
Injury, poisoning and procedural complications	**35 (35.0)**
Meniscus injury	28 (28.0)
Ligament rupture	15 (15.0)
Joint dislocation	2 (2.0)
Cartilage injury	1 (1.0)
Ligament sprain	1 (1.0)
Musculoskeletal and connective tissue disorders	**10 (10.0)**
Chondropathy	2 (2.0)
Synovitis	2 (2.0)
Arthralgia	1 (1.0)
Arthrofibrosis	1 (1.0)
Joint stiffness	1 (1.0)
Osteochondrosis	1 (1.0)
Plica syndrome	1 (1.0)
Synovial cyst	1 (1.0)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	**1 (1.0)**
Lipoma	1 (1.0)

Source: Section 15, Post-text table 15.1.7.1.1.1

Table 6. Surgical history – prior surgeries on the target knee (ITT)

Table 11-5: Surgical history – prior surgeries on the target knee (ITT)

MedDRA SOC MedDRA preferred term	All patients (N=100) n (%)
Patients with at least one prior surgery	**63 (63.0) [a] / 52 (52.0) [b]**
Surgical and medical procedures	**51 (51.0)**
Meniscus removal	27 (27.0)
Joint debridement	16 (16.0)
Ligament operation	15 (15.0)
Chondroplasty	7 (7.0)
Meniscus operation	3 (3.0)
Removal of foreign body from joint	3 (3.0)
Synovectomy	3 (3.0)
Osteochondral autograft	2 (2.0)
Arthrolysis	1 (1.0)
Baker's cyst excision	1 (1.0)
Cartilage graft	1 (1.0)
Chondrectomy	1 (1.0)
Joint dislocation reduction	1 (1.0)
Lipoma excision	1 (1.0)
Tenoplasty	1 (1.0)
Investigations	**17 (17.0) [a] / 2 (2.0) [b]**
Arthroscopy	17 (17.0) [a] / 2 (2.0) [b]

a: Data considering all arthroscopies.
b: Data excluding the solely diagnostic arthroscopies.
Source: Section 15, Post-text tables 15.1.7.3.1.1 and 15.1.7.4.1.1

From the patients summarized in Table 6, eight patients had previously failed prior cartilage repair on the target defect (chondroplasty n=8, osteochondral autograft n=2, cartilage graft n=1). Two patients had 2 failed cartilage repairs.

Table 7. Protocol AAG-G-H-1624 (Primary Analysis) – Concomitant surgeries (Target knee) – Intent-to-Treat

Subgroup: Age **System Organ Class** **Preferred Term**	**Total** **(N=100)** **n (%)**	**Age ≤ 25** **(N=13)** **n (%)**	**Age > 25** **(N=87)** **n (%)**
Number of subjects with at least one concomitant surgery on the target knee	22 (22.0)	5 (38.5)	17 (19.5)
Surgical and medical procedures	22 (22.0)	5 (38.5)	17 (19.5)
Ligament operation	11 (11.0)	3 (23.1)	8 (9.2)
Osteotomy	5 (5.0)	0	5 (5.7)
Meniscus removal	3 (3.0)	0	3 (3.4)
Tenoplasty	3 (3.0)	2 (15.4)	1 (1.1)
Chondroplasty	1 (1.0)	0	1 (1.1)
Meniscus operation	1 (1.0)	0	1 (1.1)

Table 8. Protocol AAG-G-H-1624 (Primary Analysis) – Subsequent surgical interventions (Target knee) – Intent-to-Treat

Subgroup: Age **System Organ Class** **Preferred Term**	**Total** **(N=100)** **n (%)**	**Age ≤ 25** **(N=13)** **n (%)**	**Age > 25** **(N=87)** **n (%)**
Number of subjects with at least one subsequent surgical intervention on the target knee	12 (12.0)	2 (15.4)	10 (11.5)
Surgical and medical procedures	12 (12.0)	2 (15.4)	10 (11.5)
Removal of internal fixation	5 (5.0)	0	5 (5.7)
Meniscus removal	3 (3.0)	1 (7.7)	2 (2.3)
Chondroplasty	2 (2.0)	0	2 (2.3)
Arthrolysis	1 (1.0)	0	1 (1.1)
Joint dislocation reduction	1 (1.0)	0	1 (1.1)
Meniscus operation	1 (1.0)	1 (7.7)	0
Osteosynthesis	1 (1.0)	0	1 (1.1)
Osteotomy	1 (1.0)	0	1 (1.1)

Table 9. Protocol AAG-G-H-1624 (Primary Analysis) – Summary of knee rehabilitation – Intent-to-Treat

Subgroup: Age					
Parameter	**Category**	**Statistic**	**Total (N=100)**	**Age ≤ 25 (N=13)**	**Age > 25 (N=87)**
Participation in rehabilitation	Yes, according to protocol	n (%)	98 (98.0)	13 (100.0)	85 (97.7)
	Yes, other program	n (%)	0	0	0
	No	n (%)	0	0	0
	Unknown	n (%)	2 (2.0)	0	2 (2.3)
CPM device used	Yes	n (%)	98 (98.0)	13 (100.0)	85 (97.7)
	No	n (%)	0	0	0
	Missing	n (%)	2 (2.0)	0	2 (2.3)
Rehabilitation duration (days)		n	98	13	85
		nmiss	2	0	2
		Mean	106.0	104.5	106.2
		SD	44.48	22.37	47.04
		Min	42	71	42
		Q1	80.0	91.0	79.0
		Median	93.0	98.0	91.0
		Q3	118.0	120.0	115.0
		Max	308	147	308
Number of physiotherapy visits		n	98	13	85
		nmiss	2	0	2
		Mean	22.9	22.3	23.0
		SD	8.68	5.20	9.11
		Min	6	10	6
		Q1	20.0	24.0	19.0
		Median	24.0	24.0	24.0
		Q3	24.0	24.0	24.0
		Max	58	30	58

Table 10. Protocol AAG-G-H-1624 (Primary Analysis) – Post-surgery non-drug knee therapies – Intent-to-Treat

System Organ Class **Preferred Term**	**Total (N=100) n (%)**
Number of subjects with at least one post-surgery non-drug knee therapy	65 (65.0)
Surgical and medical procedures	65 (65.0)
Manual lymphatic drainage	31 (31.0)
Cooling therapy	30 (30.0)
Joint fluid drainage	22 (22.0)
Muscle electrostimulation therapy	14 (14.0)
Physiotherapy	10 (10.0)
Kinesitherapy	8 (8.0)
Hydrotherapy	3 (3.0)
Laser therapy	2 (2.0)
Magnetic therapy	2 (2.0)
Compression garment application	1 (1.0)
Cryotherapy	1 (1.0)
Ergotherapy	1 (1.0)
Joint stabilisation	1 (1.0)
Peripheral nerve neurostimulation	1 (1.0)
Rehabilitation therapy	1 (1.0)
Social circumstances	4 (4.0)
Orthosis user	4 (4.0)

Table 11. Ongoing medical conditions on the target knee (ITT)

MedDRA SOC MedDRA preferred term	All patients (N=100) n (%)
Patients with at least one ongoing medical condition	**23 (23.0)**
Injury, poisoning and procedural complications	**17 (17.0)**
Ligament rupture	8 (8.0)
Joint dislocation	5 (5.0)
Meniscus injury	4 (4.0)
Ligament injury	1 (1.0)
Musculoskeletal and connective tissue disorders	**6 (6.0)**
Knee deformity	6 (6.0)
Bone cyst	1 (1.0)

Source: Section 15, Post-text table 15.1.7.2.1.1

About half of the study patients (51.%) reported ongoing medical conditions that were unrelated to the target knee. Ongoing conditions that occurred in more than one patient (1.0%) at the PT level were "obesity" (23.0%), "hypertension" (10.0%), "asthma" (6.0%), "hypothyroidism" (5.0%), "hypercholesterolaemia", "drug hypersensitivity", and "multiple allergies" (3.0% each), and "allergy to animal", "depression", "essential hypertension", "food allergy" (2.0% each).

Table 12. Time since start of first symptoms and diagnosis (ITT)

		All patients	Time since first knee symptoms	
			≤10.77 months	>10.77 months
Time period		N=100	N=50	N=50
Time since start of first symptoms (months)	Mean	22.691	4.547	40.836
	SD	26.763	2.456	27.735
	Median	10.770	4.175	30.670
	Min	0.49	0.49	10.95
	Max	126.56	10.59	126.56
Time since diagnosis (months)	Mean	11.292	4.328	18.255
	SD	22.855	15.134	26.970
	Median	2.270	1.730	5.425
	Min	0.07	0.20	0.07
	Max	114.56	108.35	114.56

SD=Standard deviation.
Source: Section 15, Post-text table 15.1.7.5.1

Table 13. Summary of cartilage defects – all treated lesions (ITT)

Characteristic		All patients (N=100) n (%)
Number of treated defects	N	130
Defect side	Right, n (%)	58 (44.6)
	Left, n (%)	72 (55.4)
Defect location	Lateral femoral condyle, n (%)	15 (11.5)
	Medial femoral condyle, n (%)	65 (50.0)
	Trochlea, n (%)	29 (22.3)
	Patella, n (%)	16 (12.3)
	Lateral tibial plateau, n (%)	4 (3.1)
	Medial tibial plateau, n (%)	1 (0.8)
Defect location (pooled)	Femoral condyle, n (%)	80 (61.5)
	Patellofemoral, n (%)	45 (34.6)
	Tibial, n (%)	5 (3.8)
ICRS grade [a]	3 , n (%)	90 (69.2)
	4 , n (%)	34 (26.2)
ICRS-OCD grade [b]	3 , n (%)	3 (2.3)
	4 , n (%)	3 (2.3)
Diagnosis	Traumatic cartilage lesion, n (%)	78 (60.0)
	Osteochondritis dissecans, n (%)	6 (4.6)
	Focal degenerative lesion, n (%)	46 (35.4)
Defect size at V3 (cm^2), post-debridement	N	130
	Mean ± SD	4.82 ± 1.856
	Median (range)	4.50 (1.0-10.4)
Total defect size (cm^2) [c]	N	100
	Mean ± SD	6.27 ± 2.080
	Median (range)	6.00 (4.0-12.5)
Larger defect size (cm^2) [d]	N	100
	Mean ± SD	5.43 ± 1.552
	Median (range)	5.00 (3.0-10.4)
Smaller defect size (cm^2) [d]	N	30
	Mean ± SD	2.80 ± 1.261
	Median (range)	2.50 (1.0-6.0)

SD=Standard deviation; V=Visit

Note: Apart from total defect size, all percentages are calculated based on the number of treated lesions (N=130). Defect sizes are post-debridement.

a: 124 lesions with assessable ICRS grade; missing values not shown.

b: Applicable only for patients with osteochondritis dissecans (n=6).

c: The mean total defect size is for the number of patients (N=100).

d: Lesions were classified into larger and smaller lesions, i.e., in patients with 2 lesions, the classification was based on the size of the respective lesions, while in patients with 1 lesion only, this lesion was classified as the larger lesion.

Source: Section 15, Post-text table 15.1.9.4.1

Table 14. Summary of treatment surgeries – tissue harvest and ACI (ITT)

Procedure type		All patients (N=100) n (%)
Tissue harvest		
Access mode	Arthroscopy, n (%)	98 (98.0)
	Mini-arthrotomy, n (%)	2 (2.0)
Duration (minutes)	Mean ± SD	26.9 ± 14.24
	Median (range)	25.0 (7-80)
Biopsy taken?	Yes, n (%)	100 (100.0)
No. of explants taken	4, n (%)	1 (1.0)
	3, n (%)	96 (96.0)
	2, n (%)	3 (3.0)
Implantation (all patients)		
Access mode	Arthroscopy, n (%)	46 (46.0)
	Mini-arthrotomy, n (%)	49 (49.0)
	Open knee surgery, n (%)	5 (5.0)
Duration (minutes)	Mean ± SD	46.2 ± 26.15
	Median (range)	40.0 (10-161)
Total volume of transplant administered (mL)	Mean ± SD	2.6 ± 1.19
	Median (range)	2.1 (1-5)
Implantation (only patients without concomitant surgeries; N=78)		
Access mode	N	78
	Arthroscopy, n (%)	40 (51.3)
	Mini-arthrotomy, n (%)	36 (46.2)
	Open knee surgery, n (%)	2 (2.6)
Duration (minutes)	n obs.	78
	Mean ± SD	39.6 ± 19.80
	Median (range)	35.0 (10-161)
Total volume of transplant administered (mL)	n obs.	78
	Mean ± SD	2.7 ± 1.19
	Median (range)	2.2 (1-5)

n obs.=Number of observations; SD=Standard deviation
Source: Section 15, Post-text tables 15.1.9.1.1 and 15.4.3

MRI sub-population

In the main analysis of the pivotal Phase III clinical trial at 24 months, a total of 25 patients with 30 cartilage defects treated with NOVOCART® Inject from the ITT population had evaluable MRI data and constituted the MRI sub-population as planned by protocol. This accounts for 25% of the ITT population. Only study sites that fulfil the technical requirements are participating in the MRI sub-study. All patients in the ITT with MRIs evaluated in the MRI core laboratory are included.

The mean total defect size among the 25 study patients (included in the MRI analyses (with 30 treated lesions) was overall similar to the total ITT population (MRI population: mean 6.60 ± 2.3 cm2, range: 4.0 - 12.5 cm2; ITT population: 6.27 ± 2.1 cm2, range: 4.0 - 12.5 cm2) while the individual defect size was slightly larger in the MRI population (MRI population: mean 5.50 ± 1.9 cm2, range: 1.0 – 9.0 cm2; ITT population: 4.82 ± 1.9 cm2, range: 1.0 – 10.4 cm2).

Table 15. Protocol AAG-G-H-1624 (Primary Analysis) – Demographics – Intent-to-Treat (MRI subset)

Protocol AAG-G-H-1624 (Primary Analysis)
Table 15.1.6.1.3 Demographics – Intent-to-Treat (MRI Subset)

Subgroup: Time Since Target Knee Symptoms (months)					
Parameter	Category	Statistic	Total (N=25)	≤ 10.77 (N=13)	> 10.77 (N=12)
Age (years)		n	25	13	12
		nmiss	0	0	0
		Mean	39.6	39.7	39.6
		SD	13.48	14.35	13.12
		Min	15	15	17
		Q1	28.0	27.0	28.5
		Median	41.0	42.0	41.0
		Q3	50.0	50.0	49.0
		Max	60	58	60
Age (years)	≥14-≤25	n (%)	4 (16.0)	2 (15.4)	2 (16.7)
	>25-≤65	n (%)	21 (84.0)	11 (84.6)	10 (83.3)
	≥14-<18	n (%)	2 (8.0)	1 (7.7)	1 (8.3)
	≥18-<65	n (%)	23 (92.0)	12 (92.3)	11 (91.7)
	≥65-<85	n (%)	0	0	0
Gender	Male	n (%)	17 (68.0)	8 (61.5)	9 (75.0)
	Female	n (%)	8 (32.0)	5 (38.5)	3 (25.0)
Ethnic group	Hispanic	n (%)	0	0	0
	Not Hispanic	n (%)	25 (100.0)	13 (100.0)	12 (100.0)
Race	White	n (%)	25 (100.0)	13 (100.0)	12 (100.0)
	American Indian	n (%)	0	0	0
	Asian	n (%)	0	0	0
	Native Hawaiian	n (%)	0	0	0
	Black or African	n (%)	0	0	0
	Unknown	n (%)	0	0	0
Childbearing potential	Yes	n (%)	5 (62.5)	3 (60.0)	2 (66.7)
	No	n (%)	3 (37.5)	2 (40.0)	1 (33.3)

Numbers analysed

One hundred subjects were included in the analysis of primary- and secondary endpoints (ITT). Twenty-five subjects were included in the MRI subgroup for analyses of structural endpoints.

Outcomes and estimation

Primary efficacy endpoint (KOOS responder rate)

The primary efficacy endpoint was the response based on the KOOS total score, defined as an improvement (i.e., increase) of at least 10 points from baseline at Month 24.
Table 16 below displays results up to final analysis at month 60.

Table 16. Summary of responder rates based on the changes in overall KOOS score (≥ 10 points from baseline) over time through Month 60 (ITT)

Time point			All patients (N=100) n (%)
Month 3	Responder category	n obs.	100
		Responder	71 (71.0)
		Non-responder	29 (29.0)
		Worsening by ≥10 points [a]	3 (3.0)
Month 6	Responder category	n obs.	100
		Responder	87 (87.0)
		Non-responder	13 (13.0)
		Worsening by ≥10 points [a]	1 (1.0)
Month 12	Responder category	n obs.	100
		Responder	95 (95.0)
		Non-responder	5 (5.0)
		Worsening by ≥10 points [a]	1 (1.0)
Month 18	Responder category	n obs.	100
		Responder	92 (92.0)
		Non-responder	8 (8.0)
		Worsening by ≥10 points [a]	3 (3.0)
Month 24	Responder category	n obs.	100
		Responder	93 (93.0)
		Non-responder	7 (7.0)
		Worsening by ≥10 points [a]	3 (3.0)
Month 36	Responder category	n obs.	99
		Responder	93 (93.9)
		Non-responder	6 (6.1)
		Worsening by ≥10 points [a]	2 (2.0)
Month 48	Responder category	n obs.	98
		Responder	91 (92.9)
		Non-responder	7 (7.1)
		Worsening by ≥10 points [a]	2 (2.0)
Month 60	Responder category	n obs.	97
		Responder	90 (92.8)
		Non-responder	7 (7.2)
		Worsening by ≥10 points [a]	2 (2.1)

n obs.=Number of patients with observation

Note: Patients with a missing endpoint assessment up to Month 24 or treatment failures at any time were counted as non-responder.

a: This category was created to capture the proportion of patients with an extent of worsening that is diametral to the definition of response.

Source: Section 15, Post-text table 15.2.1.2.1

Secondary efficacy endpoints (up to month 60, main evaluation month 24)

KOOS total score changes from baseline over time through Month 60

Table 17. Summary of mean overall KOOS and changes from baseline over time through Month 60 (ITT)

		All patients (N=100)	
Time point		Absolute value	Change from baseline
Baseline	n obs.	100	-
	Mean ± SD	39.77 ± 14.321	-
	Median	40.10	-
	Range	12.1-63.9	-
Month 3	n obs.	94	94
	Mean ± SD	66.28 ± 15.802	26.42 ± 21.015
	Median	67.30	25.75
	Range	25.8-97.8	(-23.8)-78.4
Month 6	n obs.	99	99
	Mean ± SD	74.02 ± 15.868	34.28 ± 18.945
	Median	77.10	34.30
	Range	33.0-100.0	(-11.0)-77.8
Month 12	n obs.	99	99
	Mean ± SD	78.80 ± 15.042	38.96 ± 19.528
	Median	82.40	38.90
	Range	30.9-100.0	(-17.9)-84.1
Month 18	n obs.	99	99
	Mean ± SD	81.00 ± 17.378	41.06 ± 21.463
	Median	86.30	41.40
	Range	19.4-100.0	(-16.9)-86.8
Month 24	n obs.	99	99
	Mean ± SD	82.35 ± 16.439	42.55 ± 20.574
	Median	87.00	41.90
	Range	28.6-100.0	(-14.9)-77.2
Month 36	n obs.	98	98
	Mean ± SD	83.38 ± 15.686	43.30 ± 21.076
	Median	89.25	41.60
	Range	30.6-100.0	(-18.7)-80.7
Month 48	n obs.	97	97
	Mean ± SD	84.04 ± 15.869	44.20 ± 21.085
	Median	89.90	44.60
	Range	24.9-100.0	(-18.1)-80.4
Month 60	n obs.	96	96
	Mean ± SD	84.71 ± 17.069	44.86 ± 22.258
	Median	90.70	45.20
	Range	26.7-100.0	(-30.2)-85.1

n obs.=Number of patients with observation; SD=Standard deviation
Source: Section 15, Post-text table 15.2.1.1.1

KOOS subscore changes from baseline over time through Month 24

Table 18. Summary of mean changes from baseline in KOOS subscores at months 24 and 60 (ITT)

Subscore			All patients (N=100) Absolute value	Change from baseline
Pain	Baseline	n obs.	100	-
		Mean ± SD	50.08 ± 17.134	-
		Median (range)	48.60 (8.3-88.9)	-
	Month 24 (original)	n obs.	99	99
		Mean ± SD	88.58 ± 13.533	38.51 ± 20.734
		Median (range)	94.40 (36.1-100.0)	38.90 ((-25.0)-83.3)
	Month 24 (adjusted) [a]	n obs.	99	99
		Mean ± SD	88.39 ± 13.709	38.31 ± 21.045
		Median (range)	94.40 (36.1-100.0)	38.90 ((-25.0)-83.3)
	Month 60 (original)	n obs.	96	96
		Mean ± SD	89.89 ± 14.290	39.49 ± 22.245
		Median (range)	94.40 (33.3-100.0)	38.90 ((-30.6)-88.9)
	Month 60 (adjusted) [a]	n obs.	96	96
		Mean ± SD	89.77 ± 14.433	39.37 ± 22.422
		Median (range)	94.40 (33.3-100.0)	38.90 ((-30.6)-88.9)
Symptoms	Baseline	n obs.	100	-
		Mean ± SD	52.73 ± 17.571	-
		Median (range)	53.60 (14.3-85.7)	-
	Month 24	n obs.	99	99
		Mean ± SD	86.36 ± 14.668	33.72 ± 21.908
		Median (range)	92.90 (28.6-100.0)	32.10 ((-32.1)-85.7)
	Month 60	n obs.	96	96
		Mean ± SD	88.28 ± 15.755	35.67 ± 23.290
		Median (range)	92.90 (32.1-100.0)	35.70 ((-42.9)-85.7)
ADL	Baseline	n obs.	100	-
		Mean ± SD	52.53 ± 19.007	-
		Median (range)	51.50 (10.3-91.2)	-
	Month 24	n obs.	99	99
		Mean ± SD	91.34 ± 13.433	38.66 ± 22.336
		Median (range)	97.10 (38.2-100.0)	39.70 ((-20.6)-86.8)
	Month 60	n obs.	96	96
		Mean ± SD	92.50 ± 13.865	39.74 ± 23.965
		Median (range)	98.50 (39.7-100.0)	41.20 ((-35.3)-88.2)
Sport/Rec	Baseline	n obs.	100	-
		Mean ± SD	21.00 ± 16.422	-
		Median (range)	20.00 (0.0-65.0)	-
	Month 24	n obs.	99	99
		Mean ± SD	75.71 ± 22.790	54.65 ± 26.169
		Median (range)	80.00 (10.0-100.0)	60.00 ((-20.0)-100.0)
	Month 60	n obs.	96	96
		Mean ± SD	79.15 ± 22.945	58.16 ± 28.250
		Median (range)	85.00 (5.0-100.0)	60.00 ((-30.0)-100.0)
QoL	Baseline	n obs.	100	-
		Mean ± SD	22.53 ± 13.993	-
		Median (range)	18.80 (0.0-56.3)	-
	Month 24	n obs.	99	99
		Mean ± SD	69.78 ± 24.118	47.24 ± 25.683
		Median (range)	75.00 (0.0-100.0)	50.00 ((-12.5)-100.0)
	Month 60	n obs.	96	96
		Mean ± SD	73.72 ± 24.679	51.26 ± 25.997
		Median (range)	75.00 (6.3-100.0)	56.30 ((-25.0-100.0)

ADL=Activities of daily living; BL=Baseline; n obs.=Number of patients with observation; QoL=Quality of life; SD=Standard deviation; Sport/rec=Sport and recreation

Note: Corresponding data for the remaining evaluation time points are provided in the source table.

a: In patients with significantly higher analgesic use during the week before and at the time of the post-baseline KOOS assessment, the respective pain score was replaced with the baseline pain score (unless the assessed pain score was lower/equal the baseline pain score).

Source: Section 15, Post-text table 15.2.2.5.1

The differences between the non-adjusted an adjusted mean pain subscore changes from baseline at Month 24 (and at all other assessment time points) were small (38.51 points in the regular analysis and 38.31 points in the adjusted analysis at Month 24).

Mean change from baseline in IKDC subjective score

Table 19. Summary of mean IKDC subjective score and changes from baseline over time through Month 60 (ITT)

		All patients (N=100)	
Time point		Absolute value	Change from baseline
Baseline	n obs.	100	-
	Mean ± SD	34.34 ± 12.809	-
	Median (range)	35.60 (5.7-65.5)	-
Month 3	n obs.	96	96
	Mean ± SD	55.69 ± 15.609	21.66 ± 19.498
	Median (range)	56.30 (21.8-89.7)	19.50 ((-14.9)-71.3)
Month 6	n obs.	98	98
	Mean ± SD	65.54 ± 16.855	31.50 ± 17.804
	Median (range)	65.50 (27.6-100.0)	29.85 ((-1.1)-81.6)
Month 12	n obs.	100	100
	Mean ± SD	70.84 ± 16.547	36.48 ± 19.190
	Median (range)	71.30 (26.4-100.0)	36.80 ((-12.6)-88.5)
Month 18	n obs.	99	99
	Mean ± SD	73.85 ± 18.965	39.39 ± 21.689
	Median (range)	78.20 (24.1-100.0)	41.40 ((-28.7)-82.8)
Month 24	n obs.	99	99
	Mean ± SD	75.75 ± 17.509	41.24 ± 20.315
	Median (range)	80.50 (29.9-100.0)	42.50 ((-12.6)-83.9)
Month 36	n obs.	98	98
	Mean ± SD	76.96 ± 17.585	42.24 ± 22.277
	Median (range)	81.60 (32.2-)100.0	43.70 ((-12.6)-92.0)
Month 48	n obs.	97	97
	Mean ± SD	78.03 ± 17.913	43.48 ± 21.145
	Median (range)	81.60 (25.3-100.0)	46.00 ((-13.8)-92.0)
Month 60	n obs.	96	96
	Mean ± SD	79.43 ± 18.213	44.86 ± 22.264
	Median (range)	85.10 (24.1-100.0)	47.10 ((-21.8)-92.0)

n obs.=Number of patients with observation; SD=Standard deviation
Source: Section 15, Post-text table 15.2.3.1.1

IKCD objective physician score

Table 20. Summary of IKDC objective score (ITT)

Time point		All patients (N=100) n (%)
Baseline	n	100
	Normal	52 (52.0)
	Nearly normal	33 (33.0)
	Abnormal	13 (13.0)
	Severely abnormal	2 (2.0)
Month 12	n	97
	Normal	81 (83.5)
	Nearly normal	12 (12.4)
	Abnormal	4 (4.1)
	Severely abnormal	0
	Missing	3
Month 24	n	93
	Normal	85 (91.4)
	Nearly normal	5 (5.4)
	Abnormal	3 (3.2)
	Severely abnormal	0
	Missing	7
	p-value [a]	<0.0001
Month 36	n	95
	Normal	86 (90.5)
	Nearly normal	7 (7.4)
	Abnormal	2 (2.1)
	Severely abnormal	0
	Missing	4
	p-value [a]	<0.0001
Month 48	n	94
	Normal	85 (90.4)
	Nearly normal	7 (7.4)
	Abnormal	2 (2.1)
	Severely abnormal	0
	Missing	4
	p-value [a]	<0.0001
Month 60	n	93
	Normal	88 (94.6)
	Nearly normal	4 (4.3)
	Abnormal	1 (1.1)
	Severely abnormal	0
	Missing	4
	p-value [a]	<0.0001

Note: Corresponding data for the remaining time points (Months 3, 6, and 18) are provided in the source table.
a: P-value from the Wilcoxon signed-rank test for the change from baseline.
Source: Section 15, Post-text table 15.2.7.1

Mean change from baseline in EQ-5D-5L index

Table 21. Summary of mean EQ-5D-5L index values and changes from baseline over time through Month 60 (ITT)

Table 11-25: Summary of mean EQ-5D-5L index values and changes from baseline over time through Month 60 (ITT)

		All patients (N=100)	
Time point		Absolute value	Change from baseline
Baseline	n obs.	100	-
	Mean ± SD	0.605 ± 0.281	-
	Median (range)	0.720 ((-0.292)-1.000)	-
Month 3	n obs.	98	98
	Mean ± SD	0.880 ± 0.105	0.277 ± 0.290
	Median (range)	0.901 (0.411-1.000)	0.185 ((-0.251)-1.173)
Month 6	n obs.	99	99
	Mean ± SD	0.916 ± 0.102	0.308 ± 0.284
	Median (range)	0.943 (0.478-1.000)	0.210 ((-0.240)-1.173)
Month 12	n obs.	100	100
	Mean ± SD	0.923 ± 0.123	0.317 ± 0.287
	Median (range)	0.943 (0.211-1.000)	0.201 ((-0.236)-1.149)
Month 18	n obs.	99	99
	Mean ± SD	0.925 ± 0.128	0.320 ± 0.294
	Median (range)	0.964 (0.211-1.000)	0.227 ((-0.201)-1.179)
Month 24	n obs.	100	100
	Mean ± SD	0.933 ± 0.112	0.327 ± 0.292
	Median (range)	1.000 (0.500-1.000)	0.230 ((-0.263)-1.209)
Month 36	n obs.	98	98
	Mean ± SD	0.931 ± 0.132	0.319 ± 0.311
	Median (range)	1.000 (0.205-1.000)	0.232 ((-0.515)-1.209)
Month 48	n obs.	97	97
	Mean ± SD	0.944 ± 0.113	0.335 ± 0.303
	Median (range)	1.000 (0.205-1.000)	0.238 ((-0.515-1.292)
Month 60	n obs.	96	96
	Mean ± SD	0.936 ± 0.140	0.327 ± 0.321
	Median (range)	1.000 (0.205-1.000)	0.227 ((-0.603)-1.292)

n obs.=Number of patients with observation; SD=Standard deviation
Source: Section 15, Post-text table 15.2.4.2

Proportion of patients with treatment failure

One of the 100 ITT patients (1.0%) experienced treatment failure through Month 24. This patient underwent microfracture on the target knee due to complete graft delamination (). The surgery was performed on treatment Day 603 (i.e., in the period between 18 and 24 months).

Analysis of secondary endpoints related to MRI investigations

MOCART subscores at Months 12 and 24

Table 22. Proportions of patients by separate MOCART subscore assessments at months 12 and 24 – all lesions (MRI-ITT)

		All patients (N=25)	
Subscore	Assessment criterion	Month 12 n (%)	Month 24 n (%)
1) Filling of defect	n patients / n lesions	25 / 30	24 / 28
	Complete [a]	18 (60.0)	17 (60.7)
	Hypertrophy	5 (16.7)	5 (17.9)
	Incomplete: >50% of adjacent cartilage	4 (13.3)	3 (10.7)
	Incomplete: <50% of adjacent cartilage	0	0
	Incomplete (subchondral bone exposed) [b]	3 (10.0)	3 (10.7)
	Missing lesion data	0	2
2) Integration to border zone	n patients / n lesions	25 / 30	24 / 28
	Complete	22 (73.3)	23 (82.1)
	Incomplete: Demarcating border visible	5 (16.7)	2 (7.1)
	Defect visible: <50% of length	0	0
	Defect visible: >50% of length	0	0
	Missing tissue	3 (10.0)	3 (10.7)
	Missing lesion data		2
3) Surface of repair tissue	n patients / n lesions	25 / 30	24 / 28
	Surface (lamina splendens) intact	16 (53.3)	24 (85.7)
	Surface damaged <50% of depth	10 (33.3)	1 (3.6)
	Surface damaged >50% of depth or total degeneration	1 (3.3)	0
	Missing tissue	3 (10.0)	3 (10.7)
	Missing lesion data	0	2
4) Structure of repair tissue	n patients / n lesions	25 / 30	24 / 28
	Homogenous	16 (53.3)	19 (67.9)
	Inhomogeneous or cleft formation	11 (36.7)	6 (21.4)
	Missing tissue	3 (10.0)	3 (10.7)
	Missing data	0	2
5) Signal intensity of repair tissue (Dual T2-FSE)	n patients / n lesions	25 / 30	24 / 28
	Normal (iso-intense)	15 (50.0)	22 (78.6)
	Moderately hyperintense	12 (40.0)	3 (10.7)
	Markedly hyperintense	0	0
	Missing tissue	3 (10.0)	3 (10.7)
	Missing lesion data	0	2
6) Signal intensity of repair tissue (3D GRE)	n patients / n lesions	25 / 30	24 / 28
	Normal (iso-intense)	16 (53.3)	23 (82.1)
	Moderately hyperintense	11 (36.7)	2 (7.1)
	Markedly hyperintense	0	0
	Missing tissue [b]	3 (10.0)	3 (10.7)
	Missing lesion data	0	2
7) Subchondral lamina	n patients / n lesions	25 / 30	24 / 28
	Intact	22 (73.3)	20 (71.4)
	Not intact	8 (26.7)	8 (28.6)
	Missing lesion data	0	2
8) Subchondral bone	n patients / n lesions	25 / 30	24 / 28
	Intact	9 (30.0)	10 (35.7)
	Not intact	21 (70.0)	18 (64.3)
	Missing lesion data	0	2

Subscore	Assessment criterion	All patients (N=25) Month 12 n (%)	Month 24 n (%)
9) Adhesions	n patients / n lesions	25 / 30	24 / 28
	No	28 (93.3)	27 (96.4)
	Yes	2 (6.7)	1 (3.6)
	Missing lesion data	0	2
10) Effusion	n patients / n lesions	25 / 30	24 / 28
	No	16 (53.3)	21 (75.0)
	Yes	14 (46.7)	7 (25.0)
	Missing lesion data	0	2

Note: Percentages are based on the number of lesions. Corresponding data for the larger lesion and the smaller lesion (if applicable) can be found in the source table.

a: The category "complete" includes minor hypertrophy (up to 150% filling of the total defect volume) according to MOCART version 2.0, see [Schreiner *et al.* 2019].

b: For the subscore filling of the defect, 3 patients with missing tissue (in 3 lesions) were included into the category "Incomplete (subchondral bone exposed)". Please note that, according to the central review, these 3 patients (~~2-09-02, 2-11-08, and 4-07-01~~) had "advanced stage of osteoarthritis" (see Post-text listing 16.2.6.6), which might explain the lack of defect filling in these patients.

Source: Section 15, Post-text table 15.2.8.1

The proportion of lesions with complete defect filling decreased to 46.4% at Month 60. Increases from Month 12 to Month 24 were observed for the proportions of lesions with complete integration of the graft tissue to the border zone, intact surface of repair tissue, normal signal intensity and absence of subchondral changes. These proportions have then decreased at Month 60 (64.3% for complete integration, 64.3% for intact surface and 25.0% for normal signal intensity), except for the absence of subchondral changes to 35.7%.

Decreasing proportions from Month 12 to Month 24 to Month 60 were seen for lesions with homogeneous structure (from 66.7 to 53.6% to 42.9%), and absence of bony defects or overgrowth (from 50.0% to 21.4% to 3.6%). In summary, the shifts across categories over time suggested generally stable graft conditions from Month 12 to Month 24, but slight regression at Month 60.

Table 23. Summary of mean T2 mapping parameters at months 12, 24, and 60 – both lesions (MRI-ITT)

Time point		All patients (N=25) Lesion tissue	Reference tissue
T2 relaxation time (ms)			
Month 12	n patients / n lesions	21 / 25	21 / 25
	Mean ± SD	56.156 ± 7.149	52.482 ± 7.376
	95%-CI	[53.205; 59.108]	[49.437; 55.527]
	Median	55.580	52.420
	Range	41.05-68.51	37.12-63.34
Month 24	n patients / n lesions	20 / 23	20 / 23
	Mean ± SD	50.770 ± 5.821	53.386 ± 7.377
	95%-CI	[48.253; 53.288]	[50.196; 56.576]
	Median	50.250	54.030
	Range	42.01-70.37	36.62-67.22
Month 60	n patients / n lesions	20 / 23	20 / 23
	Mean ± SD	56.193 ± 8.995	55.277 ± 9.678
	95%-CI	[52.303; 60.082]	[51.092; 59.462]
	Median	54.020	54.740
	Range	40.94-74.24	38.38-85.73
T2 standard deviation (ms)			
Month 12	n patients / n lesions	21 / 25	21 / 25
	Mean ± SD	13.974 ± 5.346	10.343 ± 3.288
	95%-CI	[11.768; 16.181]	[8.986; 11.700]
	Median	12.150	9.020
	Range	6.22-24.89	5.71-18.15
Month 24	n patients / n lesions	20 / 23	20 / 23
	Mean ± SD	12.919 ± 3.961	11.193 ± 5.269
	95%-CI	[11.206; 14.632]	[8.915; 13.472]
	Median	12.780	8.770
	Range	6.99-21.75	6.31-25.00
Month 60	n patients / n lesions	20 / 23	20 / 23
	Mean ± SD	16.057 ± 8.033	10.356 ± 6.764
	95%-CI	[12.583; 19.531]	[7.431; 13.281]
	Median	13.090	8.540
	Range	6.55-38.17	5.09-38.22

CI=Confidence interval; SD=Standard deviation

Note: Mean values are based on the number of lesions. Corresponding data for the larger lesion and the smaller lesion (if applicable) can be found in the source table.

Source: Section 15, Post-text table 15.2.8.3

Association between MRI parameters and clinical outcomes

In spite of improvements in clinical outcomes and progressing graft maturation and cartilage reorganisation, no meaningful associations between certain clinical outcomes and certain MRI findings were observed based on the data of the current analysis.

2.6.5.3. Study #2 AAG-G-H-1202

This study is briefly presented below since its control group microfracture (MFx) has been used in the matched pair analysis together with study AAG-G-H-1624, please see section 3.3.4.7 Analysis performed across trials.

Study title: A Clinical Study to Evaluate the Safety and Effectiveness of NOVOCART® 3D plus Compared to Microfracture in the Treatment of Articular Cartilage Defects of the Knee

Background

NOVOCART® 3D plus is a matrix-based autologous chondrocyte transplantation system for the treatment of localized full-thickness cartilage defects of the knee joint. It is composed of ex vivo-expanded autologous chondrocytes seeded on a bioresorbable biphasic collagen scaffold of bovine origin. Autologous cartilage, which was sampled during the arthroscopy at Visit 2, was the source tissue for chondrocyte isolation and culturing. Three to 4 weeks after the sampling at Visit 2, transplantation of the chondrocyte-seeded scaffold was performed at Visit 3 after mini-arthrotomy of the knee.

The control treatment was microfracture. The reference (control) treatment in this study consisted of microfracture performed according to the method developed by Steadman. Generally, microfracture surgery is a minimally invasive articular cartilage repair surgical technique that creates multiple perforations (or microfractures) in the subchondral bone, causing the release of mesenchymal stem cells from the bone marrow that can differentiate into appropriate articular cartilage-like cell lines.

Study design

This was a prospective, multi-centre, randomized, controlled, open- label, clinical study with 2 parallel treatment arms to compare the efficacy and safety of autologous chondrocyte transplantation using NOVOCART® 3D plus vs. microfracture (according to Steadman) in patients with cartilage defects of the knee. The study consisted of a screening phase (Visit 1), a treatment phase (Visit 2 [arthroscopy and subsequent tissue harvest or microfracture depending on randomization] and treatment Visit 3 [only for patients randomized to NOVOCART® 3D plus transplantation]), a core study phase through Month 24 after the study treatment (i.e., transplantation or microfracture), and a long-term follow-up phase through Month 60 after treatment.

The primary objective of this study was to demonstrate that the efficacy of the NOVOCART® 3D plus autologous chondrocyte transplantation system is superior to microfracture for the treatment of articular cartilage defects of the knee.

The primary endpoint was improvement from baseline in the 2000 IKDC subjective knee score to the score measured at the 24-month follow-up assessment visit.

Secondary endpoints comprised for instance changes in KOOS total score, SF-36, MOCART score and treatment failure rate.

Main inclusion criteria:

The study population consisted of adult and (in selected countries only) paediatric patients (i.e., 14 to 17 years old) with closed epiphyseal growth plate with focal articular cartilage defects of the knee. Eligibility was assessed in 2 steps, i.e., at Visit 1 (pre-operative screening) and Visit 2 (initial arthroscopy), since the final determination of study eligibility could not be made until the patients had undergone arthroscopy.

Main pre-operative inclusion criteria at Visit 1 (based on medical history and/or MRI) were:

1. Patient is ≥18 and ≤65 years old at screening or (in selected countries only) is a pediatric patient (14 to 17 years old) with closed epiphyseal growth plate (confirmation of closure of the epiphyseal growth plate of the index knee by x-ray or MRI required).
2. Localized articular cartilage defect of the femoral condyle or the trochlea of the knee. Two localized cartilage defects are accepted, if the total defect size is ≤6 cm2 and the size of each individual lesion is ≥2

cm2, both cartilage defects are located at the femoral condyle and/or the trochlea, and both cartilage defects are to be treated with NOVOCART® 3D plus or microfracture.
3. Defect size ≥2 and ≤6 cm2.
4. Intact articulating joint surface (Grade ≤2 of International Cartilage Repair Society [ICRS] classification, no "kissing lesions").
5. Intact meniscus (a maximum of 50% resection was allowed).
6. Patient has a stable knee joint or sufficiently reconstructed ligaments (if not, ligament repair was to be done before, during or within 6 weeks after cartilage treatment with autologous chondrocyte transplantation [ACT] / microfracture).
7. Free range of motion of the affected knee joint or ≤10° of extension and flexion loss.
8. Defect grade of III or IV according to the ICRS classification.
9. Baseline score of ≤60/100 on the 2000 IKDC subjective knee evaluation

Main intra-operative inclusion criteria at Visit 2 (no specific exclusion criteria were defined for that time point) were the exclusion of pregnancy and the verification of the aforementioned pre-operative inclusion criteria Nos. 2, 3, 4, 5, 6, and 8. Patients who were still eligible at that time were then intraoperatively randomized to either treatment with N3D or MFx.

Study population and data sets

Overall, the following data sets were analysed for the main study analysis:
• Safety analysis set (SAF): 177 patients in the N3D group and 86 patients in the MFx group (263 patients overall);
• Full analysis set (FAS): 177 patients in the N3D group and 85 patients in the MFx group (262 patients overall);
• Per-protocol set (PPS): 162 patients in the N3D group and 81 patients in the MFx group (243 patients overall);
• Magnetic resonance imaging (MRI) substudy analyses (MRI- FAS): 75 FAS patients in the N3D group and 35 FAS patients in the MFx group (110 patients overall).

The FAS (N=262) consisted of 189 males (72.1%) and 73 females (27.9%). The mean age of the patients was 39.9 ± 10.6 years and ranged from 17 to 64 years (2 paediatric patients were included in the main analysis). The mean age was similar between treatment groups, whereas the proportion of patients at the age of 14 to 25 years was numerically higher (18.8% vs. 7.9%) and, vice versa, the proportion of patients at the age of 26 to 50 years slightly smaller (62.4% vs. 76.3%) in the MFx group compared with the N3D group. No other imbalances were observed between the treatment groups in terms of the general demographic characteristics or the general medical history. Prior surgeries on the target knee were performed in 60.0% of patients in the MFx group and59.3% of patients in the N3D group.

"Meniscus removal" was more frequently done in the MFx group than in the N3D group (32.9% vs. 21.5%), while "arthroscopy" was performed more frequently in the N3D group than in the MFx group (26.0% vs. 17.6%). In terms of the onset of first knee symptoms, both the mean and median time elapsed since the occurrence of the first symptoms (as well as the time since establishing the definite diagnosis) were longer in the N3D group compared with the MFx group: The median time since the occurrence of the first symptoms was 9.0 months in the N3D group vs. 7.0 months in the MFx group, and the median time since diagnosis was 9.0 months in the N3D group vs. 5.5 months in the MFx group. The impact of these numerical imbalances between treatment groups (age groups, previous target knee surgeries, symptom duration) remains unclear.

Lesion characteristics:

Most of the study patients (233 FAS patients, 88.9%) had one single lesion, while 29 patients (11.1%) had 2 lesions (i.e., 291 lesions in 262 patients). All reported lesions had an ICRS grade of 3 (51.5% of lesions) or 4 (48.5% of lesions). Most of the cartilage lesions in either treatment group were of traumatic origin (77.4% of the lesions in the MFx group and 78.3% of lesions in the N3D group); the remaining lesions were osteoarthritic lesions (MFx: 7.5%; N3D: 2.5%) or had another etiology (MFx: 15.1%; N3D: 19.2%). Both the total lesion size (MFx group: 3.6 ± 1.3 cm2; N3D group: 3.8 ± 1.4 cm2) and the mean larger lesion size (MFx group: 3.5 ± 1.2 cm2; N3D group: 3.6 ± 1.2 cm2) were similar in the 2 treatment groups.

Concomitant surgeries

A total of 176 N3D patients (FAS) underwent ACT treatment of the larger lesion; 20 patients additionally underwent treatment of the smaller lesion. Two cartilage biopsies were taken in most N3D patients (90.4%), while 3 biopsies were taken from 16 patients (9.0%). Sole suturing of the larger lesion was done in 62.7% of N3D patients; the additional use of pins and glue was documented in 17.5% and 5.6%, respectively (1 additional patient had suturing with both pins and glue). Pins only or glue only were used in 11.9% and 1.1% of patients, respectively. The mean length of incision required for the ACT was 6.3 ± 2.7 cm (range: 3.0 to 19.5 cm), and the mean duration of the subsequent hospitalization was 3.7 ± 1.8 days (range: 1 to 11 days). The mean time required for the arthroscopy for tissue harvest at Visit 2 in the N3D group was 31.3 ± 17.2 minutes (range: 9 to 120 minutes), and the mean time required for ACT at Visit 3 was 63.5 ± 28.6 minutes (range: 21 to 180 minutes). Concomitant surgeries on the target knee (during study procedures or planned surgeries up to 6 weeks after study procedure) were performed in 8 MFx patients (9.4%) and 29 N3D patients (16.4%). Most of these concomitant surgeries in both treatment groups were related to ligament surgery (19 patients [10.7%] in the N3D group and 7 patients [8.2%] in the MFx group).

Outcomes

Table 24. Synopsis Table A: overview of main efficacy results (excluding imagining variables) observed at month 24 in the main analyses (FAS)

	MFx group (N=85)	N3D group (N=177)
IKDC subjective score [a]		
n obs.	81	172
LS mean change from BL	29.92	30.93
95.48%-CI for change	[25.57; 34.26]	[27.91; 33.96]
Contrast [95.48%-CI] / p-value [b]	1.02 [-4.17; 6.21] / p=0.6929	
IKDC response rates		
Response I (>20.5), n/N (%)	50/74 (67.6)	118/167 (70.7)
OR [95%-CI] / CMH test [c]	1.17 [0.65; 2.11] / p=0.6112	
Response II (>11.5), n/N (%)	61/74 (82.4)	133/167 (79.6)
OR [95%-CI] / CMH test [c]	0.84 [0.41; 1.70] / p=0.6262	
IKDC objective score, modified		
n obs.	77	164
LS mean change from BL	-1.06	-1.05
95.48%-CI for change	[-1.23, -0.89]	[-1.17; -0.93]
Contrast [95.48%-CI] / p-value [b]	0.01 [-0.19; 0.21] / p=0.9374	
KOOS total score		
n obs.	81	172
LS mean change from BL	25.61	28.21
95.48%-CI for change	[21.48; 29.74]	[25.31; 31.12]
Contrast [95.48%-CI] / p-value [b]	2.60 [-2.32; 7.53] / p=0.2885	
KOOS total response rate		
Response, n/N (%)	56/74 (75.7)	140/167 (83.8)
OR [95%-CI] / CMH test [c]	1.67 [0.85; 3.26] / p=0.1337	
Treatment failure rate [d]		
Definition 1 [e], n/N (%)	0	5/168 (3.0)
Definition 2 [f], n/N (%)	0	2/168 (1.2)
CMH test [c], Definition 1/2	p=0.1380 / p=0.3541	
SF-36 physical component score		
n obs.	78	169
LS mean change from BL	10.83	11.92
95.48%-CI for change	[8.89; 12.76]	10.58; 13.27]
Contrast [95.48%-CI] / p-value [b]	1.10 [-1.18; 3.37] / p=0.3319	
SF-36 mental component score		
n obs.	78	169
LS mean change	2.29	4.68
95.48%-CI for change	[0.13; 4.45]	[3.19; 6.18]
Contrast [95.48%-CI] / p-value [b]	2.39 [-0.14; 4.92] / p=0.0581	
Duration of surgical procedure [g]		
n obs.	85	176
Mean ± SD in minutes	38.1 ± 25.42	63.5 ± 28.60
Median (range) in minutes	30.0 (10-135)	55.0 (21-180)
P-value from van Elteren test	p<0.0001	
Rate of unplanned surgeries on the target knee		
n/N (%)	3/74 (4.1)	16/168 (9.5)
OR [95%-CI] / CMH test c	2.40 [0.69; 8.37] / p=0.1555	

BL=Baseline; CMH= Cochran-Mantel-Haenszel; CI=Confidence interval; LS=Least square; OR=Odds ratio

Note: Efficacy variables are not arranged in order of the hierarchical testing procedure. The term "rate" used in this table refers to the number of patients with a given outcome.

a: Primary efficacy analysis.

b: LS mean change from baseline at Month 24 from main analysis model (IUDR); the treatment contrast is calculated as "value in the N3D group minus value in the MFx group", i.e., a positive contrast (IKDC objective: score: negative contrast) is in favor of the N3D group, and *vice versa*.

c: Cochran-Mantel-Haenszel test stratified by region. P-value of general association statistic.

d: No Odds ratios estimable, as no events occurred in the MFx group.

e: All graft/microfracture-related conditions requiring surgical re-intervention.

f: All conditions that require surgical re-interventions affecting the closed surface of the transplant/microfracture area and/or require additional cartilage repair modalities on the target defect.

g: Mean duration of microfracture at Visit 2 in the MFx group or transplantation at Visit 3 in the N3D group.

Table 25. Synopsis Table B: overview of main imaging results (MOCART sum score and standard T2 mapping) observed at month 24 in the main analysis (MRI-FAS)

	MFx group (N=35)	N3D group (N=75)
MOCART sum score		
n obs.	25	61
Absolute LS mean at Month 24	73.68	77.13
95%-CI for LS mean	[66.10; 81.27]	(71.95; 82.32]
Contrast [95%-CI] / p-value a	3.45 [-5.40; 12.30] / p=0.4409	
T2 relaxation time (ms)		
n obs.	20	51
Absolute LS mean at Month 24	49.41	52.60
95%-CI for LS mean	[43.17; 55.66]	[48.36; 56.84]
Contrast [95%-CI] / p-value b	3.18 [-4.07; 10.43] / p=0.3812	
T2 standard deviation		
n obs.	20	51
Absolute LS mean at Month 24	10.32	11.89
95%-CI for LS mean	[7.19; 13.45]	[9.73; 14.06]
Contrast [95%-CI] / p-value b	1.57 [-2.02; 5.17] / p=0.3839	
T2 global ratio °		
Pts. within range 0.8-1.2, n/N (%)	15/20 (75.0)	29/51 (56.9)
OR [95%-CI] / CMH test d	0.45 [0.14; 1.41] / p=0.1705	
T2 zonal ratio °		
Pts. within range 0.8-1.2, n/N (%)	13/20 (65.0)	41/51 (80.4)
OR [95%-CI] / CMH test d	2.20 [0.70; 6.87] / p=0.1723	

BL=Baseline; CMH= Cochran-Mantel-Haenszel; CI=Confidence interval; LS=Least square; OR=Odds ratio

Note: The MRI results provided in this table refer to the larger lesion. However, the corresponding analyses of all lesions yielded similar results.

a: The treatment contrast is calculated "value in the N3D group minus value in the MFx group", i.e., a positive contrast is in favor of the N3D group, and *vice versa*.

b: The treatment contrast is calculated "value in the N3D group minus value in the MFx group", i.e., a negative contrast is in favor of the N3D group, and *vice versa*.

c: The "normal range" for these ratios (comparing regenerate tissue with normal tissue) is 0.8 to 1.2 (ideal ratio: 1.0).

d: Cochran-Mantel-Haenszel test stratified by region. P-value of general association statistic.

Table 26. Synopsis Table C: overview of treatment-emergent adverse events (SAF)

Patients with:	MFx group N=86 n (%)	N3D group N=177 n (%)	Total N=263 n (%)
TEAEs	82 (95.3)	169 (95.5)	251 (95.4)
Serious TEAE	10 (11.6)	31 (17.5)	41 (15.6)
TEAEs related to N3D	n.a.	9 (5.1)	9 (3.4)
TEAEs related to ACT [a]	n.a.	153 (86.4)	153 (58.2)
TEAEs related to tissue harvest/biopsy	n.a.	99 (55.9)	99 (37.6)
TEAEs related to transplantation	n.a.	147 (83.1)	147 (55.9)
TEAEs related to MFx	71 (82.6)	n.a.	71 (27.0)
Serious TEAEs (TESAEs) related to N3D (product/procedure) or MFx	0	11 (6.2)	11 (4.2)
Severe TEAEs	7 (8.1)	24 (13.6)	31 (11.8)
TEAEs leading to subsequent surgical interventions on the TK	3 (3.5)	17 (9.6)	20 (7.6)
Related [b] TEAE leading to subsequent surgical interventions on the target knee	0	7 (4.0)	7 (2.7)
Treatment failure (Def. No. 1) [c]	0	5 (2.8)	5 (1.9)
Treatment failure (Def. No. 2) [d]	0	2 (1.1)	2 (0.8)
TEAEs leading to death	0	1 (0.6)	1 (0.4)

n.a.=Not applicable; TEAE=Treatment-emergent adverse event
Note: All data included in this in-text table are based on number of patients.
a: Events related to tissue/harvest biopsy and/or transplantation procedure.
b: Events related to product or ACT procedures in the N3D group; related to microfracture in the MFx group.
c: All graft/microfracture-related conditions requiring surgical re-intervention.
d: All conditions that require surgical re-interventions affecting the closed surface of the transplant/microfracture area and/or require additional cartilage repair modalities on the target defect.

2.6.5.4. Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 27. Summary of efficacy for trial AAG-G-H-1624

Title: Prospective, multicenter, single-arm Phase III clinical trial to evaluate the efficacy and safety of NOVOCART® Inject plus in the treatment of cartilage defects of the knee.		
Study identifier	Study ID: AAG-G-H-1624 EUDRA-CT No 2016-002817-22 CTgov. No.: NCT03319797	
Design	The present study was a prospective, multicenter, single-arm phase III clinical trial to evaluate the efficacy and safety of NOVOCART® Inject plus in the treatment of patients with focal cartilage defects of the knee (medial or lateral femoral condyle or tibial plateau, trochlea or patella).	
	Duration of main phase:	24 months
	Duration of Run-in phase:	Not applicable
	Duration of Extension phase:	36 months
Hypothesis	To show that at 24 months the overall KOOS responder rate (R) defined as the proportion of patients with a ≥10-points improvement from baseline is higher than the no-effect level of 40%. The hypotheses to be tested are:	

	H0: R ≤ 40% vs. H1: R > 40% The hypotheses will be tested using a one-sided exact binomial test at a significance level $\alpha = 0.025$. Equivalently, a rejection of the null-hypothesis will occur if the lower bound of the two-sided 95% confidence interval for the response rate R is >40%.		
Treatments groups	NOVOCART® Inject plus (matrix-associated autologous chondrocyte implantation) → 'NInject plus'		102 patients were eligible for the study and underwent cartilage biopsy (included in the safety set, SAF) and 100 thereof were treated with NInject plus (intent-to-treat set, ITT). Two SAF patients were not included in the ITT set, because they had discontinued the study prior to implantation. Duration of treatment: n/a (one-time application)
Endpoints and definitions	Primary endpoint	KOOS responder rate	Responder rate based on the overall Knee injury and Osteoarthritis Outcome Score (KOOS) defined as the average of the 5 subscale scores (ensuring identical weight from all subscales) at the 24 months follow-up assessment. The overall KOOS responder rate is defined as the proportion of patients with ≥ 10 points improvement in the overall KOOS from baseline to the 24 months visit.
	Secondary endpoint	Overall KOOS change from Baseline (CfB)	Change in the overall KOOS from baseline to the 24 months assessment
	Secondary endpoint	KOOS subscore CfB	Change in the 5 individual subscores of the KOOS from baseline to the 24 months assessment
	Secondary endpoint	IKDC CfB	Change from baseline in the International Knee Documentation Committee (IKDC) subjective score from baseline to the 24 months assessment
	Secondary endpoint	IKDC responder rate	IKDC subjective score responder rate, defined as the proportion of patients with > 20.5 points improvement in the IKDC subjective score from baseline to the 24 months visit
	Secondary endpoint	EQ-5D-5L CfB	Change from baseline to the 24 months visit in the EQ-5D-5L questionnaire
	Secondary endpoint	IKDC objective	Change from baseline to the 24 months visit in the grading according to the IKDC surgeons part (IKDC objective)
	Secondary endpoint	MOCART and T2 mapping	*In vivo* performance measured by the 24 months morphological assessment of the Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score and biochemical MR assessment by T2 mapping in a subgroup of patients
	Secondary endpoint	Treatment failure rate	Treatment failure rate: intraoperatively diagnosed treatment failures are defined as all surgical re-interventions affecting the closed surface of the transplant area (the surface is not closed when the defect area is grade 3 or 4 ICRS) and/or require additional cartilage repair modalities on the target defect.
Database lock	22-Feb-2021		

Results and Analysis

Results of the main analysis based on the 24-month efficacy data of all 100 subjects treated with NOVOCART® Inject plus in this study.

Analysis description	**Primary Analysis**
Analysis population and time point description	Analysis populations • The intent-to-treat population (ITT) includes all patients who have received the NOVOCART® Inject plus transplantation.

- The per-protocol set (PP) includes all patients in the ITT without major protocol violations.
- The safety analysis set (SAF) includes all patients with tissue harvested for NOVOCART® Inject plus transplantation.
- The MRI subset includes all patients in the ITT with MRIs evaluated in the MRI core lab.

All efficacy analyses were based on the ITT population (100 patients) and all safety analyses on the safety population (SAF, 102 patients).
Due to the congruency of the ITT and PP populations (N=100 and N=98, respectively), the study results observed in the 2 analysis populations were almost identical. Therefore, the description of efficacy data is focused on the ITT population.

Primary and secondary endpoints were analysed after 24 months post transplantation.

Descriptive statistics and estimate variability

Summary of responder rates based on the changes in KOOS total score (≥10 points from baseline) over time through Month 24 (ITT)

Time point			All patients (N=100) n (%)
Month 3	Responder category	n obs.	100
		Responder	71 (71.0)
		Non-responder	29 (29.0)
		Worsening by ≥10 points [a]	3 (3.0)
Month 6	Responder category	n obs.	100
		Responder	87 (87.0)
		Non-responder	13 (13.0)
		Worsening by ≥10 points [a]	1 (1.0)
Month 12	Responder category	n obs.	100
		Responder	95 (95.0)
		Non-responder	5 (5.0)
		Worsening by ≥10 points [a]	1 (1.0)
Month 18	Responder category	n obs.	100
		Responder	92 (92.0)
		Non-responder	8 (8.0)
		Worsening by ≥10 points [a]	3 (3.0)
Month 24	Responder category	n obs.	100
		Responder	93 (93.0)
		Non-responder	7 (7.0)
		Worsening by ≥10 points [a]	3 (3.0)

n obs.=Number of patients with observation
Note: For the primary analysis, patients with a missing endpoint assessment were counted as non-responder.
a: This category was created to capture the proportion of patients with an extent of worsening that is diametral to the definition of response.
Source: CSR AAG-G-H-1624, Table 11-10

Effect estimate per comparison

Primary analysis: KOOS total responder rates over time through Month 24 (ITT)

Time point			All patients (N=100)
Month 3	Test statistic	Responder rate (%)	71.0
		95%-CI [a]	[61.1; 79.6]
		p-value [b]	<0.0001
Month 6	Test statistic	Responder rate (%)	87.0
		95%-CI [a]	[78.8; 92.9]
		p-value [b]	<0.0001
Month 12	Test statistic	Responder rate (%)	95.0
		95%-CI [a]	[88.7; 98.4]
		p-value [b]	<0.0001

	Month 18	Test statistic	Responder rate (%)	92.0
			95%-CI [a]	[84.8; 96.5]
			p-value [b]	<0.0001
	Month 24 [c]	Test statistic	Responder rate (%)	93.0
			95%-CI [a]	[86.1; 97.1]
			p-value [b]	<0.0001

CI=Confidence interval; n obs.=Number of patients with observation
Note: Patients with missing endpoint assessments and patients classified as treatment failures before or during the respective slotted visit were handled as non-responders, regardless of the KOOS total response.
a: Clopper-Pearson (exact) binomial confidence interval.
b: From one-sided exact binomial test of hypotheses H_0: Rate ≤40% vs. H1: Rate >40%.
c: Designated time point for the confirmatory primary efficacy analysis.
Source: CSR AAG-G-H-1624, Table 11-11

Analysis description

Secondary endpoint analysis (prespecified)

Descriptive statistics and estimate variability

Summary of mean KOOS total score and changes from baseline over time through Month 24 (ITT)

		All patients (N=100)	
Time point		**Absolute value**	**Change from baseline**
Baseline	n obs.	100	-
	Mean ± SD	39.77 ± 14.321	-
	Median	40.10	-
	Range	12.1-63.9	-
Month 3	n obs.	94	94
	Mean ± SD	66.28 ± 15.802	26.42 ± 21.015
	Median	67.30	25.75
	Range	25.8-97.8	(-23.8)-78.4
Month 6	n obs.	99	99
	Mean ± SD	74.02 ± 15.868	34.28 ± 18.945
	Median	77.10	34.30
	Range	33.0-100.0	(-11.0)-77.8
Month 12	n obs.	99	99
	Mean ± SD	78.80 ± 15.042	38.96 ± 19.528
	Median	82.40	38.90
	Range	30.9-100.0	(-17.9)-84.1
Month 18	n obs.	99	99
	Mean ± SD	81.00 ± 17.378	41.06 ± 21.463
	Median	86.30	41.40
	Range	19.4-100.0	(-16.9)-86.8
Month 24	n obs.	99	99
	Mean ± SD	82.35 ± 16.439	42.55 ± 20.574
	Median	87.00	41.90
	Range	28.6-100.0	(-14.9)-77.2

n obs.=Number of patients with observation; SD=Standard deviation
Source: CSR AAG-G-H-1624, Table 11-13

Summary of mean changes from baseline in KOOS subscores at Month 24 (ITT)

Subscore			All patients (N=100) Absolute value	All patients (N=100) Change from baseline
Pain	Baseline	n obs.	100	-
		Mean ± SD	50.08 ± 17.134	-
		Median (range)	48.60 (8.3-88.9)	-
	Month 24 (original)	n obs.	99	99
		Mean ± SD	88.58 ± 13.533	38.51 ± 20.734
		Median (range)	94.40 (36.1-100.0)	38.90 ((-25.0)-83.3)
	Month 24 (adjusted) [a]	n obs.	99	99
		Mean ± SD	88.39 ± 13.709	38.31 ± 21.045
		Median (range)	94.40 (36.1-100.0)	38.90 ((-25.0)-83.3)
Symptoms	Baseline	n obs.	100	-
		Mean ± SD	52.73 ± 17.571	-
		Median (range)	53.60 (14.3-85.7)	-
	Month 24	n obs.	99	99
		Mean ± SD	86.36 ± 14.668	33.72 ± 21.908
		Median (range)	92.90 (28.6-100.0)	32.10 ((-32.1)-85.7)
ADL	Baseline	n obs.	100	-
		Mean ± SD	52.53 ± 19.007	-
		Median (range)	51.50 (10.3-91.2)	-
	Month 24	n obs.	99	99
		Mean ± SD	91.34 ± 13.433	38.66 ± 22.336
		Median (range)	97.10 (38.2-100.0)	39.70 ((-20.6)-86.8)
Sport/ Rec	Baseline	n obs.	100	-
		Mean ± SD	21.00 ± 16.422	-
		Median (range)	20.00 (0.0-65.0)	-
	Month 24	n obs.	99	99
		Mean ± SD	75.71 ± 22.790	54.65 ± 26.169
		Median (range)	80.00 (10.0-100.0)	60.00 ((-20.0)-100.0)
QoL	Baseline	n obs.	100	-
		Mean ± SD	22.53 ± 13.993	-
		Median (range)	18.80 (0.0-56.3)	-
	Month 24	n obs.	99	99
		Mean ± SD	69.78 ± 24.118	47.24 ± 25.683
		Median (range)	75.00 (0.0-100.0)	50.00 ((-12.5)-100.0)

ADL=Activities of daily living; BL=Baseline; n obs.=Number of patients with observation; QoL=Quality of life; SD=Standard deviation; Sport/rec=Sport and recreation

Note: Corresponding data for the evaluation time points prior to Month 24 are provided in the source table.

a: In patients with significantly higher analgesic use during the week before and at the time of the post-baseline KOOS assessment, the respective pain score was replaced with the baseline pain score (unless the assessed pain score was lower/equal the baseline pain score).

Source: CSR AAG-G-H-1624, Table 11-17

Summary of mean IKDC subjective score and changes from baseline over time through Month 24 (ITT)

Time point		All patients (N=100)	
		Absolute value	Change from baseline
Baseline	n obs.	100	-
	Mean ± SD	34.34 ± 12.809	-
	Median	35.60	-
	Range	5.7-65.5	-
Month 3	n obs.	96	96
	Mean ± SD	55.69 ± 15.609	21.66 ± 19.498
	Median	56.30	19.50
	Range	21.8-89.7	(-14.9)-71.3
Month 6	n obs.	98	98
	Mean ± SD	65.54 ± 16.855	31.50 ± 17.804
	Median	65.50	29.85
	Range	27.6-100.0	(-1.1)-81.6
Month 12	n obs.	100	100
	Mean ± SD	70.84 ± 16.547	36.48 ± 19.190
	Median	71.30	36.80
	Range	26.4-100.0	(-12.6)-88.5
Month 18	n obs.	99	99
	Mean ± SD	73.85 ± 18.965	39.39 ± 21.689
	Median	78.20	41.40
	Range	24.1-100.0	(-28.7)-82.8
Month 24	n obs.	99	99
	Mean ± SD	75.75 ± 17.509	41.24 ± 20.315
	Median	80.50	42.50
	Range	29.9-100.0	(-12.6)-83.9

n obs.=Number of patients with observation; SD=Standard deviation
Source: CSR AAG-G-H-1624, Table 11-19

Main analysis of responder rates based on the changes in IKDC subjective score over time through Month 24 (ITT)

Time point		All patients (N=100)	
		Responder I (>20.5)	Responder II (≥11.5)
Month 3	Responder, n (%)	44 (44.0)	64 (64.0)
	95%-CI for responder rate [a]	[34.1; 54.3]	[53.8; 73.4]
	Non-responder, n (%)	56 (56.0)	36 (36.0)
	Worsening by ≥11.5 points, n (%) [b]	2 (2.0)	
Month 6	Responder, n (%)	70 (70.0)	81 (81.0)
	95%-CI for responder rate [a]	[60.0; 78.8]	[71.9; 88.2]
	Non-responder, n (%)	30 (30.0)	19 (19.0)
	Worsening by ≥11.5 points, n (%) [b]	0	
Month 12	Responder, n (%)	82 (82.0)	92 (92.0)
	95%-CI for responder rate [a]	[73.1; 89.0]	[84.8; 96.5]
	Non-responder, n (%)	18 (18.0)	8 (8.0)
	Worsening by ≥11.5 points, n (%) [b]	1 (1.0)	

	Month 18	Responder, n (%)	84 (84.0)	87 (87.0)
		95%-CI for responder rate [a]	[75.3; 90.6]	[78.8; 92.9]
		Non-responder, n (%)	16 (16.0)	13 (13.0)
		Worsening by ≥11.5 points, n (%) [b]		1 (1.0)
	Month 24	Responder, n (%)	84 (84.0)	89 (89.0)
		95%-CI for responder rate [a]	[75.3; 90.6]	[81.2; 94.4]
		Non-responder, n (%)	16 (16.0)	11 (11.0)
		Worsening by ≥11.5 points, n (%) [b]		1 (1.0)

CI=Confidence interval

Note: Patients with missing endpoint assessments and patients classified as treatment failures before or during the respective slotted visit were regarded as non-responders, regardless of the IKDC subjective response.

a: Clopper-Pearson (exact) binomial confidence interval.

b: Data are given as n (%). This category was created to capture the proportion of patients with an extent of worsening that is diametral to the definition of Response II; i.e., by ≥11.5 points.

Source: CSR AAG-G-H-1624, Table 11-21

Summary of mean MOCART sum scores at Months 12 and 24 – all lesions (MRI-ITT)

Time point		All patients (N=25)
Month 12	n patients / n lesions	25 / 30
	Mean ± SD	70.0 ± 22.44
	95%-CI	[61.6; 78.4]
	Median (range)	75.0 (10-100)
Month 24	n patients / n lesions	24 / 28
	Mean ± SD	80.0 ± 25.68
	95%-CI	[70.0; 90.0]
	Median (range)	90.0 (10-100)

CI=Confidence interval; SD=Standard deviation

Source: CSR AAG-G-H-1624, Table 11-29

Effect estimate per comparison

Overview of main efficacy results observed at Month 24 in the main analysis (ITT)

Efficacy variable at Month 24		All patients (N=100)
Overall KOOS	LS Mean (SE)	42.04 (1.827)
Mean change from BL	95%-CI	[38.41; 45.66]
	p-value [c]	<0.0001
KOOS Pain	LS Mean (SE)	37.94 (1.504)
Mean change from BL	95%-CI	[34.96, 40.93]
	p-value [c]	<0.0001
KOOS Symptoms	LS Mean (SE)	32.17 (1.613)
Mean change from BL	95%-CI	[28.97, 35.37]
	p-value [c]	<0.0001
KOOS ADL	LS Mean (SE)	38.19 (1.531)
Mean change from BL	95%-CI	[35.15, 41.23]
	p-value [c]	<0.0001
KOOS Sports/rec	LS Mean (SE)	54.61 (2.485)

	Mean change from BL	95%-CI	[49.68, 59.54]
		p-value [c]	<0.0001
	KOOS QoL	LS Mean (SE)	47.25 (2.595)
	Mean change from BL	95%-CI	[42.10, 52.40]
		p-value [c]	<0.0001
	IKDC subjective	Responder rate (%)	84.0
	Responder rate I (>20.5 points CfB)	95%-CI [a]	[75.3; 90.6]
	IKDC subjective	Responder rate (%)	89.0
	Responder rate II (≥11.5 points CfB)	95%-CI [a]	[81.2; 94.4]
	IKDC subjective	LS Mean (SE)	41.63 (1.878)
	Mean change from baseline	95%-CI	[37.91; 45.36]
		p-value [c]	<0.0001
	IKDC objective	*Normal, n (%) [d]*	*52 (52.0)*
	Categorical analysis	*Nearly normal, n (%) [d]*	*33 (33.0)*
		Abnormal, n (%) [d]	*13 (13.0)*
		Severely abnormal, n (%) [d]	*2 (2.0)*
		Normal, n (%)	85 (91.4)
		Nearly normal, n (%)	5 (5.4)
		Abnormal, n (%)	3 (3.2)
		Severely abnormal, n (%)	0
		Missing, n	7
		p-value [e]	<0.0001
	EQ-5D-5L index value	LS Mean (SE)	0.326 (0.012)
	Mean change from baseline	95%-CI	[0.303; 0.349]
		p-value [c]	<0.0001
	EQ-VAS	LS Mean (SE)	25.03 (1.618)
	Mean change from baseline	95%-CI	[21.82; 28.23]
		p-value [c]	<0.0001
	MOCART	n patients / n lesions	24 / 28
		Mean ± SD	80.0 ± 25.68
		95%-CI	[70.0; 90.0]
		Median (range)	90.0 (10-100)
	Treatment failure	Failure rate (%)	1 (1.0)

CfB=Change from baseline; CI=Confidence interval; LS=Least squares; SE=Standard error; VAS=Visual analogue scale

a: Clopper-Pearson (exact) binomial confidence interval.

b: From one-sided exact binomial test of hypotheses H0: Rate ≤40% vs. H1: Rate >40%.

c: From linear MMRM with "country" and "visit" as fixed factors, and "baseline value" as covariate.

d: Baseline values (provided for descriptive comparison).

e: P-value from the Wilcoxon signed-rank test for the change from baseline.

2.6.5.5. Analysis performed across trials (pooled analyses and meta-analysis)

The main study AAG-G-H-1624 was a single-arm study without a control group. In this section, a comparative analysis with matched external data from study AAG-G-H-1202 is presented.

Study title:

A propensity score matched pair analysis of two pivotal studies investigating the safety and efficacy of NOVOCART® 3D plus compared to microfracture and the efficacy and safety of NOVOCART® Inject plus in the treatment of cartilage defects of the knee (NOVOCART® Inject plus Study (NInject) and NOVOCART® 3D plus Study (N3D)).

The applicant acknowledge that the lack of a control group is a limitation of the NOVOCART® Inject plus study. Therefore, in order to mimic a control group, efficacy data of the NOVOCART® Inject plus study (NInject trial) were compared with the microfracture control group of the NOVOCART® 3D plus phase III study (N3D trial) by propensity score matching.

Study objectives

The objective of this matched pair analysis was to investigate whether NOVOCART® Inject plus treatment in patients with larger cartilage defects (4 – 12 cm2) is non-inferior or even superior compared with microfracture treatment in patients with smaller cartilage defects in the knee (2 – 6 cm2) after a follow-up of 24 months.

Data for NOVOCART® Inject plus were derived from the single arm phase III study AAG-G-H-1624 (NInject trial), and data for microfracture treatment originate from the control group in the randomized phase III study AAG-G-H-1202 comparing NOVOCART® 3D plus and microfracture (N3D trial).

Assessment was based on two patient-reported outcome measures, the Knee Injury and Osteoarthritis Outcome Score (KOOS) and the International Knee Documentation Committee (IKDC) score, which were collected in both studies preoperatively and at the same timepoints following ACI transplantation.

Primary and secondary endpoints

The primary efficacy endpoint of this analysis was the change in the overall KOOS from baseline to the 24 months assessment. The overall KOOS score is an unweighted average of the 5 subscale scores pain, symptoms, ADL, sport/rec, and QoL.

The primary efficacy endpoint was descriptively analysed using two testing procedures:
1a. Non-inferiority of NInject to MFx in overall KOOS change from baseline at 24 months.
1b. Superiority of NInject to MFx in overall KOOS change from baseline at 24 months.

Non-inferiority was descriptively shown, if the lower bound of the 95%-confidence interval for the difference between the NInject and microfracture groups was greater than -8.5 points.
Superiority of NInject versus MFx was suggested, if the lower bound of the 95%-confidence interval was greater than 0. The descriptive non-inferiority margin was based on a previous study design reported by Niemeyer and colleagues (Niemeyer et al., 2019).

The secondary efficacy endpoints of this analysis were:
1. Overall KOOS response rate, defined as the proportion of patients with ≥10 points improvement in the overall KOOS, at the 24 months assessment.
2. Change in the 5 individual subscores of the KOOS from baseline to the 24 months visit.
3. Change in the IKDC subjective score from baseline to the 24 months assessment.
4. IKDC subjective score responder rate, defined as the proportion of patients with >20.5 points improvement in the IKDC subjective score from baseline to the 24 months assessment.

Both the primary and secondary efficacy endpoints were analysed descriptively.

Methods
This was a propensity score matched pair analysis of two pivotal studies investigating the safety and efficacy of NOVOCART® 3D plus compared to microfracture (study AAG-G-H-1202) and the efficacy and safety of NOVOCART® Inject plus (study AAG-G-H-1624) in the treatment of cartilage defects of the knee.

Study design AAG-G-H-1624 (please also see section 3.3.4.2):

This is a prospective, multicenter, single-arm Phase III clinical trial to evaluate the efficacy and safety of NOVOCART® Inject plus in the treatment of cartilage defects of the knee in adult patients aged between 18 and 65 years and pediatric patients (14 to 17 years) with closed epiphysis. The primary efficacy variable was the KOOS total score, which was completed at baseline (Visit 1) and from Visit 5 onwards at each scheduled visit through month 24. Secondary endpoints were for example change in from baseline by month 24 in IKDC, EQ-5D-5L, MOCART scores and treatment failure rate.

Study design AAG-G-H-1202 (please also see section 3.3.4.3):

The study AAG-G-H-1202 "A Clinical Study to Evaluate the Safety and Effectiveness of NOVOCART® 3D plus Compared to Microfracture in the Treatment of Articular Cartilage Defects of the Knee" was a prospective, randomised, active-controlled, open label (MRI assessor blinded), multi-centre study. The duration of the main phase was 24 months. The primary endpoint was improvement from baseline in the 2000 IKDC subjective knee score to the score measured at the 24-month follow-up assessment visit. Secondary endpoints comprised for instance changes in KOOS total score, SF-36, MOCART score and treatment failure rate.

Propensity score matching procedure

A logistic regression model was used for the two treatment groups to estimate the propensity score. The assignment to the therapy group represented the target variable. The following variables expected to have an impact on the therapy effect were included in the model:

1. Baseline overall KOOS,
2. time since first symptoms,
3. prior target knee surgery (yes, no),
4. age,
5. sex.

These variables were selected based on their statistical significance in the primary models as well as models of covariate effects for the KOOS score in the N3D trial.

Patients from the NInject trial with prior failed cartilage repair on the target knee were excluded from this analysis. This was an exclusion criterion in study 1202.

The propensity score was determined from the estimated group membership probabilities based on a logistic regression model. In the PSM procedure, each patient in the treatment group was provided with one patient from the comparison group (1:1 matching). The assignment of a matching partner was based on the propensity score: The matching partners were study participants from different treatment groups with identical or with only minimally different propensity score. Finally, the study participants paired with the propensity score yielded the collective used for further analysis to assess the therapy effect. Patients without matching partners were excluded from further evaluation.

A greedy algorithm was used for matching patients from the NInject and N3D trials. The width required for matching patients was set at 0.2 times the pooled standard deviation estimate of the logit of the propensity score.

Inclusion-/exclusion criteria compared between trials

Differences in inclusion/exclusion criteria between the two studies is described in Table 28 below.

Table 28. Differences in inclusion/exclusion criteria of the Phase III clinical trials AAG-G-H-1624 and AAG-G-H-1202 considered potentially relevant for propensity score matching

Clinical trial and treatments **----------** **Differences in inclusion/ exclusion criteria**	**AAG-G-H-1624** **NOVOCART® Inject plus**	**AAG-G-H-1202** • **NOVOCART® 3D plus** • **MFx**	**Discussion of potential relevance and consequences for PSM**
Defect size	4 to 12 cm^2	2 to 6 cm^2	Initial aim: use overlapping defect sizes across both studies (4 – 6 cm^2) for matching Preliminary analysis: only 30 patients treated with MFx from trial AAG-G-H-1202 had defects ≥ 4 cm^2 ⇒ sample size too small for planned statistical analysis ⇒ defect size was not included as a variable in the model to estimate the propensity score*
Defect localisation	Femoral condyle, trochlea, patella or tibial plateau	Restricted to femoral condyle or trochlea	Results RENOVO study (AAG-O-H-1521): no significant impact of defect localisation on KOOS or IKDC (Results AAG-G-H-1624: no significant impact of defect localisation on KOOS or IKDC- *covariate analysis performed after PSM*) ⇒ defect localisation not included as a variable in the model to estimate the propensity score
Prior cartilage defect treatment on the target knee	Accepted, *if the previously treated defect is the same defect that is planned to be treated with NOVOCART® Inject plus* and cartilage repair procedures were performed ≥ 24 months prior to screening. Excluded at a location *different*	Excluded	Worse outcome after secondary ACI/M-ACI (ACI/M-ACI after other cartilage repair methods, especially MFx) compared to primary ACI/M-ACI ⇒ Patients from the trial AAG-G-H-1624 with prior failed cartilage repair on the target knee (8 patients) were excluded from the matched pair analysis

	from the defect location to be treated		

PSM : propensity score matching, MFx : microfracture
* This may represent a bias in favour of the microfracture group (that included patients with smaller defects).

Statistical method

The absolute change in KOOS5 from baseline to 24 months was analysed using a linear mixed effect model for repeated measurements (MMRM) assuming MAR. The model was to include the fixed categorical effects of treatment, region, slotted visits (Month 3 to Month 24), and treatment-by-visit interaction, as well as the continuous fixed covariate of the baseline KOOS5 score. All longitudinal observations of the KOOS5 up to and including Month 24 were to be used to estimate the treatment and covariate effects.

The least squares mean estimates for the change from baseline to endpoint in KOOS5 was used to estimate the treatment effect.

Observations obtained after the date of treatment failure were to be set to missing.

Results

Population

A total of 72 matched pairs (total of 144 patients) were selected from the eligible population (92 in the NOVOCART® Inject plus group and 85 in the MFx group).

Both treatment groups in the matched paired population used for the analysis were comparable regarding baseline KOOS, gender, age, BMI, duration of symptoms, smoking status and previous knee surgeries (please see Table 6, 8). The mean KOOS at baseline was 41.71 ± 13.089 points in the NInject group and 43.14 ± 14.467 points in the MFx group.

There were differences in defect characteristics between treatment groups in terms of defect location (no patellar or tibial defects in the MFx group), number of defects (more patients with more than one defect in the NOVOCART® Inject plus group) and defect etiology (more patients with degenerative lesions in the NOVOCART® Inject plus group; no OCD lesions in the MFx group). A further difference between both treatment groups concerned defect size which was clearly larger in the NOVOCART® Inject plus group compared to the MFx group (total defect size: 6.4 cm2 vs. 3.7 cm2). More concomitant- and subsequent surgical procedures were performed in the NOVOCART Inject plus treatment arm (Table 1-2). There were differences in defect characteristics and surgical interventions between the two groups are discussed below.

Population characteristics, all eligible subjects

Table 29. Matching procedure variable summary

Variable			NInject group N=92	MFx group N=85
Baseline Overall KOOS	All	n obs.	92	85
		Mean ± SD	39.4141 ± 14.3601	47.2188 ± 16.7811
		Range	12.1-63.9	6.8-83.5
		Mean diff.	-7.8047	
	Matched	n obs.	72	72
		Mean ± SD	41.7097 ± 13.0887	43.1361 ± 14.4673
		Range	13.2-63.9	6.8-70.5
		Mean diff.	-1.4264	
Time since symptoms (months)	All	n obs.	92	85
		Mean ± SD	18.6304 ± 21.7901	14.7647 ± 18.6213
		Range	0-100	0-98
		Mean diff.	3.8657	
	Matched	n obs.	72	72
		Mean ± SD	18.4861 ± 20.1362	16.3333 ± 19.4994
		Range	1-84	0-98
		Mean diff.	2.1528	
Prior surgeries on target knee	All	n obs.	92	85
		Mean ± SD	0.4022 ± 0.4903	0.4000 ± 0.4899
		Mean diff.	0.0022	
	Matched	n obs.	72	72
		Mean ± SD	0.3611 ± 0.4803	0.3750 ± 0.4841
		Mean diff.	-0.0139	
Age	All	n obs.	92	85
		Mean ± SD	39.5870 ± 11.6375	39.4471 ± 11.6859
		Range	15-62	18-61
		Mean diff.	0.1399	
	Matched	n obs.	72	72
		Mean ± SD	39.3333 ± 12.0480	39.2778 ± 11.8859
		Range	15-62	18-61
		Mean diff.	0.0556	
Gender	All	n obs.	92	85
		Mean ± SD	0.3587 ± 0.4796	0.2824 ± 0.4501
		Mean diff.	0.0763	
	Matched	n obs.	72	72
		Mean ± SD	0.2917 ± 0.4545	0.2917 ± 0.4545
Logit propensity score	All	n obs.	92	85
		Mean ± SD	0.2356 ± 0.5430	-0.0741 ± 0.5730
		Range	-0.8011-1.4915	-1.2823-1.4048
		Mean diff.	0.3098	
	Matched	n obs.	72	72
		Mean ± SD	0.1419 ± 0.4974	0.0735 ± 0.4822
		Range	-0.8011-1.4915	-0.8131-1.4048
		Mean diff.	0.0684	

N=Number of eligible patients; n obs.=Number of patients with observation; SD=Standard deviation

Note: The means for "prior surgeries on target knee" and "gender" correspond to the proportions of patients without a prior surgery and females in the sample, respectively.

Source: Post-text table 1.6.2

Population characteristics, matched pair analysis

Table 30. Demographic and general patient characteristics

			NInject (N=72)	MFx (N=72)	Total (N=144)
Age (years)		Mean (SD)	39.3 ± 12.05	39.3 ± 11.89	39.3 ± 11.93
		Median	41.0	40.0	40.5
		Range	15 - 62	18 - 61	15 - 62
Gender	Male	n (%)	51 (70.8)	51 (70.8)	102 (70.8)
	Female	n (%)	21 (29.2)	21 (29.2)	42 (29.2)
Race	White	n (%)	71 (98.6)	68 (94.4)	139 (96.5)
	Black	n (%)	0	3 (4.2)	3 (2.1)
	Other	n (%)	0	1 (1.4)	1 (0.7)
	Unknown	n (%)	1 (1.4)	0	1 (0.7)
Tobacco use	Yes	n (%)	18 (25.0)	18 (25.0)	36 (25.0)
	No	n (%)	54 (75.0)	54 (75.0)	108 (75.0)
BMI (kg/m^2)		Mean (SD)	27.05 ± 4.083	27.42 ± 3.894	27.24 ± 3.980
		Median	26.75	27.20	26.80
		Range	20.4 - 34.4	20.1 - 34.9	20.1 - 34.9
Time since first symptoms (mo)		Mean (SD)	18.5 ± 20.14	16.3 ± 19.50	17.4 ± 19.78
		Median	9.0	8.0	8.5
		Range	1 - 84	0 - 98	0 - 98

SD=Standard deviation; mo=months; BMI=body-mass index
Source: Post-text table 1.1, Post-text table 1.3

Table 31. Most frequent ongoing medical conditions

System organ class Preferred term	NInject N=72 n (%)	MFx N=72 n (%)	Total N=144 n (%)
Patients with at least one ongoing medical condition	**44 (61.1)**	**72 (100.0)**	**116 (80.6)**
Musculoskeletal and connective tissue disorders	**7 (9.7)**	**72 (100.0)**	**79 (54.9)**
Chondropathy	1 (1.4)	70 (97.2)	71 (49.3)
Knee deformity	4 (5.6)	1 (1.4)	5 (3.5)
Chondromalacia	0	3 (4.2)	3 (2.1)
Osteoarthritis	0	2 (2.8)	2 (1.4)
Osteochondrosis	1 (1.4)	1 (1.4)	2 (1.4)
Spinal pain	1 (1.4)	1 (1.4)	2 (1.4)
Synovial cyst	0	2 (2.8)	2 (1.4)
Metabolism and nutrition disorders	**17 (23.6)**	**21 (29.2)**	**38 (26.4)**
Obesity	15 (20.8)	18 (25.0)	33 (22.9)
Hypercholesterolaemia	3 (4.2)	2 (2.8)	5 (3.5)
Gout	0	2 (2.8)	2 (1.4)
Immune system disorders	**11 (15.3)**	**12 (16.7)**	**23 (16.0)**
Seasonal allergy	0	6 (8.3)	6 (4.2)
Drug hypersensitivity	2 (2.8)	3 (4.2)	5 (3.5)
Allergy to animal	2 (2.8)	2 (2.8)	4 (2.8)
Food allergy	2 (2.8)	2 (2.8)	4 (2.8)
Allergy to arthropod sting	1 (1.4)	1 (1.4)	2 (1.4)
Multiple allergies	1 (1.4)	1 (1.4)	2 (1.4)
House dust allergy	0	2 (2.8)	2 (1.4)
Vascular disorders	**9 (12.5)**	**13 (18.1)**	**22 (15.3)**
Hypertension	6 (8.3)	13 (18.1)	19 (13.2)
Essential hypertension	2 (2.8)	0	2 (1.4)
Peripheral venous disease	1 (1.4)	1 (1.4)	2 (1.4)
Injury, poisoning and procedural complications	**12 (16.7)**	**6 (8.3)**	**18 (12.5)**
Ligament rupture	6 (8.3)	4 (5.6)	10 (6.9)
Meniscus injury	3 (4.2)	2 (2.8)	5 (3.5)
Joint dislocation	3 (4.2)	0	3 (2.1)
Ligament injury	1 (1.4)	1 (1.4)	2 (1.4)
Respiratory, thoracic and mediastinal disorders	**6 (8.3)**	**5 (6.9)**	**11 (7.6)**
Asthma	4 (5.6)	5 (6.9)	9 (6.3)

Note: Selected are concomitant medical conditions with a prevalence of at least 5.0% at the SOC level and, subsequently within the selected SOCs, with a prevalence of at least 1.0% at the preferred term level.
Source: Post-text table 1.2.3

Concomitant medical conditions pertaining to the SOC "musculoskeletal and connective tissue disorders" were reported in all study patients in the MFx group but only 9.7% in the NInject group. This is explained by the fact that in the N3D trial the target defect to be treated in the study was to be reported in the eCRF as an ongoing medical condition (mainly coded as PT “chondropathy”), whereas in the NInject trial the target defect had to be documented separately.

Table 32. Prior surgeries on the target knee

System Organ Class Preferred Term	NInject (N=72) n (%)	MFx (N=72) n (%)	Total (N=144) n (%)
Patients with at least one target knee surgical history event	**46 (63.9)**	**45 (62.5)**	**91 (63.2)**
Surgical and medical procedures	**36 (50.0)**	**38 (52.8)**	**74 (51.4)**
Meniscus removal	20 (27.8)	26 (36.1)	46 (31.9)
Ligament operation	12 (16.7)	11 (15.3)	23 (16.0)
Joint debridement	10 (13.9)	11 (15.3)	21 (14.6)
Removal of foreign body from joint	3 (4.2)	3 (4.2)	6 (4.2)
Meniscus operation	2 (2.8)	3 (4.2)	5 (3.5)
Synovectomy	2 (2.8)	2 (2.8)	4 (2.8)
Arthrolysis	1 (1.4)	2 (2.8)	3 (2.1)
Joint irrigation	0	2 (2.8)	2 (1.4)
Baker's cyst excision	1 (1.4)	0	1 (0.7)
Bone debridement	0	1 (1.4)	1 (0.7)
Chondrectomy	1 (1.4)	0	1 (0.7)
Chondroplasty	0	1 (1.4)	1 (0.7)
Knee operation	0	1 (1.4)	1 (0.7)
Lipoma excision	1 (1.4)	0	1 (0.7)
Osteotomy	0	1 (1.4)	1 (0.7)
Tenoplasty	1 (1.4)	0	1 (0.7)
Investigations	14 (19.4)	14 (19.4)	28 (19.4)
Arthroscopy	14 (19.4)	14 (19.4)	28 (19.4)

Source: Post-text table 1.2.1

Table 33. Summary of defect characteristics – all treated defects

			NInject (N=72)	MFx (N=72)	Total (N=144)
Number of defects		n	96	79	175
Defect side	Left	n (%)	54 (56.3)	36 (45.6)	90 (51.4)
	Right	n (%)	42 (43.8)	43 (54.4)	85 (48.6)
Defect location	Femur	n (%)	82 (85.4)	79 (100.0)	161 (92.0)
	Tibia	n (%)	4 (4.2)	0	4 (2.3)
	Patella	n (%)	10 (10.4)	0	10 (5.7)
Defect grade	1	n (%)	0	0	0
	2	n (%)	0	0	0
	3	n (%)	72 (75.0)	41 (51.9)	113 (64.6)
	4	n (%)	24 (25.0)	38 (48.1)	62 (35.4)
Diagnosis	Traumatic	n (%)	58 (60.4)	62 (78.5)	120 (68.6)
	OCD	n (%)	6 (6.3)	0	6 (3.4)
	Osteoarthritis	n (%)	0	5 (6.3)	5 (2.9)
	Focal degenerative	n (%)	32 (33.3)	0	32 (18.3)
	Avascular necrosis	n (%)	0	0	0
	Other	n (%)	0	12 (15.2)	12 (6.9)

OCD=osteochondritis dissecans

Note: Corresponding data separated by larger (n=144 defects) / smaller defects (n=31 defects) can be found in the source table. All percentages provided in this table are based on the number of defects.

Source: Post-text table 1.4.1

Table 34. Summary of defect sizes - all treated defects

Parameter	Statistic	NInject (N=72)	MFx (N=72)	Total (N=144)
Patients with 2 defects	n (%)	24 (33.3)	7 (9.7)	31 (21.5)
Patients with 1 defect	n (%)	48 (66.7)	65 (90.3)	113 (78.5)
Any defect size (cm^2)	n	96	79	175
	Mean (SD)	4.78 ± 1.714	3.37 ± 1.277	4.14 ± 1.682
	Median	4.50	3.00	4.00
	Range	1.0 - 10.4	0.5 - 6.0	0.5 - 10.4
Larger defect size [a] (cm^2)	n	72	72	144
	Mean (SD)	5.35 ± 1.433	3.52 ±1.214	4.44 ± 1.611
	Median	5.00	3.04	4.00
	Range	4.0 - 10.4	2.0 - 6.0	2.0 - 10.4
Smaller defect size (cm^2)	n	24	7	31
	Mean (SD)	3.05 ± 1.276	1.79 ± 0.765	2.76 ± 1.284
	Median	3.00	2.00	2.50
	Range	1.0 - 6.0	0.5 - 2.7	0.5 - 6.0
Total defect size [b] (cm^2)	n	72	72	144
	Mean (SD)	6.37 ± 2.116	3.70 ± 1.284	5.03 ± 2.200
	Median	6.00	3.45	4.50
	Range	4.0 - 12.5	2.0 - 6.0	2.0 - 12.5

SD=Standard deviation

a: In patients with 2 defects, defects were classified into larger defect and smaller defect based on the size of the defects. In patients with 1 defect only, this defect was classified as larger defect.

b: Sum of larger and smaller defect.

Source: Post-text table 1.4.2

Table 35. Concomitant surgeries on the target knee

System Organ Class Preferred Term	NInject (N=72) n (%)	MFx (N=72) n (%)	Total (N=144) n (%)
Patients with at least one concomitant surgery on the target knee	**15 (20.8)**	**7 (9.7)**	**22 (15.3)**
Surgical and medical procedures	**15 (20.8)**	**7 (9.7)**	**22 (15.3)**
Ligament operation	9 (12.5)	6 (8.3)	15 (10.4)
Meniscus operation	1 (1.4)	2 (2.8)	3 (2.1)
Osteotomy	3 (4.2)	0	3 (2.1)
Meniscus removal	2 (2.8)	0	2 (1.4)
Chondroplasty	1 (1.4)	0	1 (0.7)
Tenoplasty	1 (1.4)	0	1 (0.7)

Source: Post-text table 1.5.1

Table 36. Subsequent surgical interventions on the target knee

System Organ Class Preferred Term	NInject (N=72) n (%)	MFx (N=72) n (%)	Total (N=144) n (%)
Patients with at least one subsequent surgical intervention on the target knee	**9 (12.5)**	**3 (4.2)**	**12 (8.3)**
Surgical and medical procedures	**9 (12.5)**	**3 (4.2)**	**12 (8.3)**
Meniscus removal	2 (2.8)	2 (2.8)	4 (2.8)
Removal of internal fixation [a]	3 (4.2)	0	3 (2.1)
Joint dislocation reduction	1 (1.4)	1 (1.4)	2 (1.4)
Adhesiolysis	0	1 (1.4)	1 (0.7)
Arthrolysis	1 (1.4)	0	1 (0.7)
Chondroplasty	1 (1.4)	0	1 (0.7)
Ligament operation	0	1 (1.4)	1 (0.7)
Meniscus operation	1 (1.4)	0	1 (0.7)
Osteosynthesis	1 (1.4)	0	1 (0.7)
Osteotomy	1 (1.4)	0	1 (0.7)
Patients with at least one unplanned subsequent surgical intervention on the target knee [b]	6 (8.3)	3 (4.2)	9 (6.3)

a: Hardware removal after osteotomy
b: Calculated by author based on Post-text listing 1.5.
Source: Post-text table 1.5.3

Analysis of primary efficacy endpoint

The primary efficacy endpoint was descriptively analysed using two testing procedures:
1a. Non-inferiority of NInject to MFx in overall KOOS change from baseline at 24 months.
1b. Superiority of NInject to MFx in overall KOOS change from baseline at 24 months.

Non-inferiority was descriptively shown, if the lower bound of the 95%-confidence interval for the difference between the NInject and microfracture groups was greater than -8.5 points.

Superiority of NInject versus MFx was suggested, if the lower bound of the 95%-confidence interval was greater than 0.

Table 37. LS mean overall KOOS changes from baseline and treatment contrasts at time points month 3 through month 24 (no imputations)

		NInject (N=72)	MFx (N=72)
Changes from BL at **Month 3**	n obs.	69	69
	LS Mean (SE)	22.58 (2.34)	15.80 (2.06)
	95%-CI	[17.96; 27.21]	[11.74; 19.87]
Treatment contrast [a]	LS Mean Difference (SE)	6.78 (2.78)	
	95%-CI	[1.28; 12.28]	
	P-value	0.0161	
Changes from BL at **Month 6**	n obs.	72	66
	LS Mean (SE)	29.98 (2.36)	22.28 (2.14)
	95%-CI	[25.32; 34.64]	[18.05; 26.51]
Treatment contrast [a]	LS Mean Difference (SE)	7.70 (2.86)	
	95%-CI	[2.04; 13.36]	
	P-value	0.0080	
Changes from BL at **Month 12**	n obs.	71	64
	LS Mean (SE)	34.60 (2.35)	25.90 (2.13)
	95%-CI	[29.95; 39.24]	[21.69; 30.10]
Treatment contrast [a]	LS Mean Difference (SE)	8.70 (2.84)	
	95%-CI	[3.07; 14.32]	
	P-value	0.0027	
Changes from BL at **Month 18**	n obs.	72	58
	LS Mean (SE)	36.76 (2.35)	28.35 (2.18)
	95%-CI	[32.10; 41.41]	[24.04; 32.65]
Treatment contrast [a]	LS Mean Difference (SE)	8.41 (2.88)	
	95%-CI	[2.71; 14.12]	
	P-value	0.0042	
Changes from BL at **Month 24**	n obs.	72	60
	LS Mean (SE)	36.93 (2.57)	26.91 (2.45)
	95%-CI	[31.85; 42.00]	[22.07; 31.74]
Treatment contrast [a]	LS Mean Difference (SE)	10.02 (3.26)	
	95%-CI	[3.57; 16.46]	
	P-value	0.0026	
Significance of effects over time (p-value)	Treatment	0.0012	
	Region	0.0539	
	Visit	<0.0001	
	Treatment*Visit	0.8288	
	Baseline	<0.0001	

BL=Baseline; CI=Confidence interval; N=Number of patients in the matched pairs set; n obs.=Number of patients with observation; SE=Standard error; LS=Least-squares

a: The treatment contrast is calculated as "value in the NInject group minus value in the MFx group", i.e., a positive contrast is in favor of the NInject group, and *vice versa*.

Source: Post-text table 2.1.3.

Analysis of secondary efficacy endpoints

Changes from baseline to Month 24 in KOOS sub scores

Table 38. Summary of mean changes from baseline in KOOS subscores at month 24

KOOS subscore			NInject group N=72	MFx group N=72
Pain	Abs. value at BL	n obs.	72	72
		Mean ± SD	51.85 ± 16.315	52.31 ± 15.461
	Abs. value at Month 24	n obs.	72	60
		Mean ± SD	88.24 ± 13.468	80.32 ± 18.526
	Change from BL	*Mean ± SD*	*36.39 ± 20.072*	*28.47 ± 23.325*
Symptoms	Abs. value at BL	n obs.	72	72
		Mean ± SD	55.32 ± 16.792	55.41 ± 21.228
	Abs. value at Month 24	n obs.	72	61
		Mean ± SD	85.96 ± 14.625	78.57 ± 20.780
	Change from BL	*Mean ± SD*	*30.63 ± 19.916*	*24.01 ± 29.343*
ADL	Abs. value at BL	n obs.	72	72
		Mean ± SD	55.13 ± 16.815	58.48 ± 18.434
	Abs. value at Month 24	n obs.	72	61
		Mean ± SD	90.92 ± 13.612	85.36 ± 18.370
	Change from BL	*Mean ± SD*	*35.79 ± 20.469*	*27.38 ± 27.032*
Sport/Rec	Abs. value at BL	n obs.	72	72
		Mean ± SD	22.64 ± 15.744	23.54 ± 17.370
	Abs. value at Month 24	n obs.	72	61
		Mean ± SD	75.00 ± 23.721	61.89 ± 28.478
	Change from BL	*Mean ± SD*	*52.36 ± 26.122*	*38.20 ± 32.635*
QoL	Abs. value at BL	n obs.	72	72
		Mean ± SD	23.64 ± 14.125	25.99 ± 15.479
	Abs. value at Month 24	n obs.	72	61
		Mean ± SD	68.94 ± 24.889	59.04 ± 27.176
	Change from BL	*Mean ± SD*	*45.33 ± 26.162*	*33.11 ± 30.511*

Abs.=Absolute; ADL= Activities of daily living; BL=Baseline; n obs.=Number of patients with observation; N=Number of patients in the matched pairs set; QoL=Quality of life; SD=Standard deviation; Sport/Rec=Sports and recreation

Note: Corresponding data for the time points prior to Month 24 and for the total population are provided in the source table.

Source: Post-text table 2.1.2.

Changes from baseline to month 24 in IKDC subjective score

Table 39. LS mean IKDC subjective score changes from baseline and treatment contrasts at time points month 3 through month 24 (no imputations)

Table 21 LS mean IKDC subjective score changes from baseline and treatment contrasts at time points Month 3 through Month 24 (no imputations)

		NInject (N=72)	MFx (N=72)
Changes from BL at Month 3	n obs.	69	70
	LS Mean (SE)	19.20 (2.32)	16.03 (2.02)
	95%-CI	[14.61; 23.78]	[12.04; 20.02]
Treatment contrast [a]	LS Mean Difference (SE)	3.17 (2.73)	
	95%-CI	[-2.24; 8.57]	
	P-value	0.2490	
Changes from BL at Month 6	n obs.	71	67
	LS Mean (SE)	28.55 (2.44)	22.62 (2.20)
	95%-CI	[23.74; 33.36]	[18.27; 26.98]
Treatment contrast [a]	LS Mean Difference (SE)	5.93 (2.96)	
	95%-CI	[0.07; 11.78]	
	P-value	0.0474	
Changes from BL at Month 12	n obs.	72	65
	LS Mean (SE)	34.25 (2.49)	27.08 (2.28)
	95%-CI	[29.33; 39.18]	[22.57; 31.59]
Treatment contrast [a]	LS Mean Difference (SE)	7.17 (3.07)	
	95%-CI	[1.10; 13.24]	
	P-value	0.0209	
Changes from BL at Month 18	n obs.	72	59
	LS Mean (SE)	36.15 (2.58)	30.01 (2.44)
	95%-CI	[31.05; 41.24]	[25.18; 34.84]
Treatment contrast [a]	LS Mean Difference (SE)	6.14 (3.25)	
	95%-CI	[-0.30, 12.57]	
	P-value	0.0614	
Changes from BL at Month 24	n obs.	72	61
	LS Mean (SE)	37.80 (2.69)	30.43 (2.57)
	95%-CI	[32.50; 43.11]	[25.36; 35.51]
Treatment contrast [a]	LS Mean Difference (SE)	7.37 (3.43)	
	95%-CI	[0.59; 14.15]	
	P-value	0.0334	
Significance of effects over time (p-value)	Treatment	0.0244	
	Region	0.2509	
	Visit	<0.0001	
	Treatment*Visit	0.5462	
	Baseline	<0.0001	

BL=Baseline; CI=Confidence interval; N=Number of patients in the matched pairs set; n obs.=Number of patients with observation; SE=Standard error; LS=Least-squares

a: The treatment contrast is calculated as "value in the NInject group minus value in the MFx group", i.e., a positive contrast is in favor of the NInject group, and *vice versa*.

Source: Post-text table 2.2.2.

MOCART scores

The MRI subpopulation consisted of 21 patients with 25 lesions in the NInject group and 28 patients with 31 lesions in the MFx group. Mean lesion size of the MRI subpopulation was 5.33 ± 1.672 cm2 (range: 1.0 – 9.0 cm2) in the NInject group and 3.31 ± 1.075 cm2 (range: 2.0 – 6.0 cm2) in the MFx group. Mean total defect size in the MRI subpopulation (i.e., all lesions per patient added to one single value) was 6.34 ± 2.386 cm2

(range: 4.0 – 12.5 cm2) in the NInject group and 3.66 ± 1.178 cm2 (range: 2.0 – 6.0 cm2) in the MFx group.

Table 40. Statistical analysis of MOCART sum score through month 24

		NInject group N=21	MFx group N=28
Month 12			
	n obs.	21	27
Absolute LS mean values	LS mean	74.29	63.85
	95%-CI	[64.01; 84.56]	[54.92; 72.78]
Treatment contrast [a]	LS mean difference (SE)	10.44 (6.76)	
	95%-CI	[-3.18; 24.05]	
	p-value	0.1296	
Month 24			
	n obs.	20	21
Absolute LS mean values	LS mean	86.89	69.08
	95%-CI	[76.99; 96.78]	[60.28; 77.88]
Treatment contrast [a]	LS mean difference (SE)	17.81 (6.56)	
	95%-CI	[4.57; 31.05]	
	p-value	**0.0096**	
Significance of effects through Month 24			
Global p-value	Treatment	0.0338	
	Visit	<0.0001	
	Treatment*Visit	0.0313	

CI=Confidence interval; n obs.=Number of patients with observation; SE=Standard error; LS=Least-squares

a: The treatment contrast is calculated as "value in the NInject group minus value in the MFx group", i.e., a positive contrast is in favor of the NInject group, and *vice versa*.

Source: Post-text MRI tables 2.2.2 and 2.2.3

Correlation analyses between MRI parameters and clinical outcomes

No significant correlation between specific MOCART parameters and KOOS change from baseline were found in the matched pair analysis.

2.6.5.6. Supportive study(ies)

Supportive clinical efficacy data with the marketed product NOVOCART® Inject

As NOVOCART® Inject has been continuously marketed in Germany since 2008, data derive from studies conducted in Germany. The applicant has sponsored non-interventional studies to assess the use of NOVOCART® Inject in clinical practice: one retrospective non-interventional study on treatment of cartilage defects in the knee joint (RENOVO, AAG-O-H-1521) and one prospective, non-interventional study on treatment of cartilage defects in the hip joint (HIPACTION, AAG-O-H-1304). One prospective, non-interventional, multicentre study exclusively in paediatric patients (NINJA, AAG-O-H-2123) is planned by the applicant. However, this study will not start before Q3 2023 (no data available yet). These data are complemented by clinical studies and case series initiated by investigators and published in the literature and data from the German Cartilage Registry (KnorpelRegister DGOU) (Report from the German Cartilage Registry by Niemeyer et al., November 2021).

Study RENOVO, AAG-O-H-1521

Study design

This was a retrospective, multi-centre, non-interventional study performed in 2015. All medical centres having treated at least 10 patients with NOVOCART® Inject (18 sites) were invited for the study, thereof 7 sites participated. In principle all treated patients should be contacted. Based on a discussion with the "Paediatric Committee" (PDCO) of the EMA, at least 10 paediatric patients were to be included.

Objectives

The primary study objective was the retrospective assessment of clinical data to evaluate the safety of NOVOCART® Inject utilised according to the label in a routine care setting. This assessment was based on the assessment of adverse reactions (ARs) that occurred from tissue harvest through the end of the observation period.

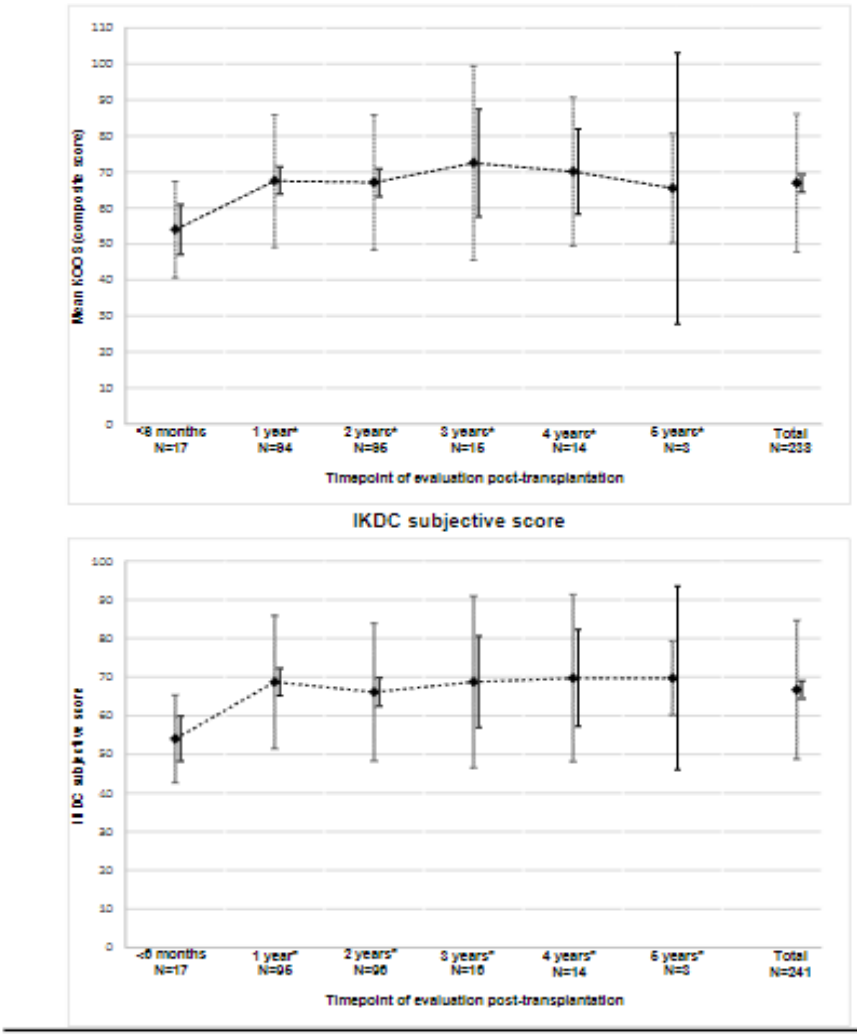
The secondary study objective was the evaluation of knee symptoms and function after the application of NOVOCART® Inject at the end of the observation period. These assessments were performed using standardised, patient-reported questionnaires (IKDC subjective; KOOS).

Study population

Out of the 515 patients treated at these sites, up to the final analysis in 2018 only 245 could be reached and were willing to give written informed consent for collection of safety and efficacy relevant information related to their NOVOCART® Inject treatment from their medical records. Slightly more men than women were enrolled of the 245 patients enrolled in the study (57.6% vs. 42.4%). The patients' mean age was 36.7 ± 11.7 years (range: 12 to 69 years); 11 patients (4.5%) were minors (i.e., <18 years of age at the time of arthroscopy). The mean BMI was 26.2 ± 4.2 kg/m2 (range: 16.7 to 37.6 kg/m2), and 57 patients (24.4%) reported current tobacco use. The mean duration of follow-up (i.e., the period from transplantation to the completion of the follow-up questionnaire) was about 20 months and ranged from 1.5 months to 57 months. The majority of patients (212 patients, 86.5%) had a follow-up period of 2.5 years at maximum, whilst only 33 patients (13.5%) had a follow-up period of longer than 2.5 years. In 17 patients (6.9%), the follow-up period was shorter than 6 months.

Results of efficacy variables

Figure 2. Follow-up outcomes in KOOS composite score and IKDC subjective score



*: ± 6 months of follow-up
Legend: Provided are the arithmetic mean score values over time and in total (i.e., pooled over all time points). The small dotted vertical errors bars indicate the standard deviation, while the slightly right-shifted, solid bold vertical error bars visualize the corresponding 95%-CI of the mean.
Note: Both scores are transformed to range from 0 to 100 scores points; higher score values are associated with better clinical outcomes. Confidence limits may be outside the score range from 0 to 100 due to the assumption of normal distribution for the calculation of CIs.
Source: Post-text tables 2.1-1 and 2.1-9

A final assessment of the effectiveness of NOVOCART® Inject based on the data of this study is not possible because of the lack of corresponding baseline data, which precludes the calculation of pre-post changes for KOOS and IKDC scores as well as the assessment of corresponding response rates (i.e., proportions of patients achieving a minimal clinically important improvement from baseline).

Study HIPACTION (AAG-O-H-1304)

Until December 2022, the approved indication of the German label also allowed the treatment of cartilage defects of the hip.

Use of NOVOCART® Inject in defects of the hip in clinical practice was investigated in an observational study called HIPACTION (AAG-O-H-1304). A total of 21 patients were enrolled in 3 study centers across Germany. The study was conducted from 09-Dec-2014 (first patient's first visit) to 30-Jan-2018 (last patient's last visit). The observation period following the NOVOCART® Inject transplantation was 24 months, during which the disease-specific, patient-reported outcome measure iHOT33 and the patient-reported QoL measures EQ-5D-5L index value and EQ VAS were documented at baseline and subsequently at Months 6, 12, and 24. In addition, MRIs of the pelvis were collected 6, 12, and 24 months after transplantation and the radiological MOCART score was assessed centrally.

Results

Population

The majority were male (81.0%), 23.8% were current smokers, the mean age was 32.3 ± 10.0 years (range: 20 to 53 years), and the median BMI was 25.5 ± 3.6 kg/m2 (range: 20.0 to 34.7 kg/m2). A total of 3 patients (14.3%) had undergone previous hip surgery prior to study enrolment (within 8 to 14 months; see Table 5-4) for head/neck offset correction. Non-drug-treatment (physiotherapy etc.) and drug treatment within the past 14 days was applied in 5 patients (23.8%) and 6 patients (28.6%), respectively. Two separate cartilage lesions were observed in 2 patients (9.5%), in whom both the anterior cranial femur head and the acetabulum were involved. Pure acetabular lesions were seen in the remaining 19 patients (90.5%). The mean total lesion size was 3.0 ± 1.4 cm2 and ranged from 1.5 to 8.0 cm2. All patients had additional surgical procedures performed on the index hip during Visit 2 (tissue harvest) and/or Visit 3 (transplantation). A total of 20 patients (95.2%) underwent a "head-neck offset correction" at Visit 2 or Visit 3. Labrum repair was performed in 13 patients (61.9%). Most of the interventions were performed during the initial arthroscopy at Visit 2. An additional surgical intervention on the contralateral hip after the treatment with NOVOCART® Inject was documented for 6 patients (28.6%).

Results of efficacy variables

Table 41. Course of mean iHOT33 over time, changes from baseline, and response rates.

iHOT		Visit 1 (Baseline)	Visit 5 (Month 6)	Visit 6 (Month 12)	Visit 7 (Month 24)
Score values per visit	N obs.	20	21	20	16
	Mean ± SD	52.9 ± 21.1	76.8 ± 19.8	82.0 ± 21.9	85.8 ± 14.8
	Median	50.8	81.6	92.0	93.5
	Range	14.2 to 92.8	18.7 to 99.2	16.7 to 100.0	50.9 to 99.6
Changes from baseline [a]	N obs.		20	19	16
	Mean ± SD		23.3 ± 26.3	29.2 ± 30.3	33.6 ± 25.3
	Median	na	20.0	32.6	32.3
	Range		-26.4 to 82.0	-28.7 to 81.0	-8.1 to 81.9
	p-value [b]		0.0008	0.0005	<0.0001
Patients with response [c]	N		21	21	21
	Missing, [n (%)]		1 (4.8)	2 (9.5)	5 (23.8)
	No, [n (%)]	na	5 (23.8)	4 (19.1)	2 (9.5)
	Yes, [n (%)]		15 (71.4)	15 (71.4)	14 (66.7)
	95%-CI for Yes		[49.8; 86.4]	[49.8; 86.4]	[45.2; 83.0]
Patients with response (adjusted) [c, d]	N		20	19	16
	No, [n (%)]	na	5 (25.0)	4 (21.1)	2 (12.5)
	Yes, [n (%)]		15 (75.0)	15 (78.9)	14 (87.5)

CI=Confidence interval; na=Not applicable; N obs.=Number of observations; SD=Standard deviation
Note: The iHOT33 total score is a score ranging from 0 to 100, higher values indicate lower levels of problems/symptoms.
a: Mean/median changes were calculated as <visit value> minus <baseline value>, i.e., negative changes indicate deterioration and, vice versa, positive changes indicate improvement from baseline.
b: For change from baseline; derived from paired t-test.
c: Defined as an improvement by at least 6 score points compared to baseline (MCID).
d: Percentages adjusted to N patients with available data (calculated by author).

Table 42. Patient-based patterns of single MOCART categories over time.

Variable	Finding	Patients [n (%)] with finding at: Month 6 N=20	Month 12 N=20	Month 24 N=14
Filling of defect	Subchondral bone exposed	0	0	0
	Incomplete <50%	2 (10.0)	3 (15.0)	1 (7.1)
	Incomplete >50%	2 (10.0)	3 (15.0)	1 (7.1)
	Hypertrophy	4 (20.0)	2 (10.0)	1 (7.1)
	Complete	11 (55.0)	11 (55.0)	11 (78.6)
	Not evaluable	1 (5.0)	1 (5.0)	0
Integration to border zone	Defect visible: >50%	0	0	0
	Defect visible: <50%	3 (15.0)	1 (5.0)	0
	Demarcating border visible	2 (10.0)	2 (10.0)	0
	Complete	14 (70.0)	16 (80.0)	14 (100.0)
	Not evaluable	1 (5.0)	1 (5.0)	0
Surface	Surface damaged >50%	0	0	0
	Surface damaged <50%	6 (30.0)	4 (20.0)	3 (21.4)
	Surface intact	13 (65.0)	15 (75.0)	11 (78.6)
	evaluable	1 (5.0)	1 (5.0)	0
Structure	Inhomogeneous / cleft	11 (55.0)	8 (40.0)	4 (28.6)
	Homogenous	8 (40.0)	11 (55.0)	10 (71.4)
	Not evaluable	1 (5.0)	1 (5.0)	0
Signal intensity (Dual T2-F SE)	Markedly hyperintense	0	2 (10.0)	1 (7.1)
	Moderately hyperintense	10 (50.0)	10 (50.0)	5 (35.7)
	Normal (iso-intense)	9 (45.0)	7 (35.0)	8 (57.1)
	Not evaluable	1 (5.0)	1 (5.0)	0
Subchondral lamina	Not intact	3 (15.0)	2 (10.0)	1 (7.1)
	Intact	17 (85.0)	18 (90.0)	13 (92.9)
Subchondral bone	Not intact	9 (45.0)	7 (35.0)	5 (35.7)
	Intact	11 (55.0)	13 (65.0)	9 (64.3)
Adhesions	Yes	1 (5.0)	0	0
	No	19 (95.0)	20 (100.0)	14 (100.0)
Effusion	Yes	15 (75.0)	9 (45.0)	2 (14.3)
	No	5 (25.0)	11 (55.0)	12 (85.7)

Note: The allocation of score points is described in Table 4-1.

Literature

Table 43. Supportive literature: investigator initiated (published literature)

Type of study (control) Location of study centres	Reference / study ID / Report	Product Patients treated, N	Localisation of defect	Follow-up time (months)	Status
Retrospective consecutive case series □ Germany	(Schlumberger et al., 2019)	**NOVOCART® Inject** 15	Knee	Mean follow-up 4.3 years (4.0 - 4.8 years)	Completed
Retrospective consecutive case series □ Germany	(Schlumberger et al., 2020)	**NOVOCART® Inject** 62	Knee	Mean follow-up 31,0 + 14,8 months (range 12.5 - 61.4 months)	Completed
Retrospective consecutive case series Germany	(Blanke et al., 2021)	**NOVOCART® Inject** 29	Knee	Mean follow-up 24.9 + 1.1 months (range 24 - 27 months)	Completed
Matched-pair analysis comparing hydrogel-based and scaffold-based ACI Consecutive case series Germany	(Niethammer et al., 2022)	**NOVOCART® Inject** 25 (25 with NOVOCART® 3D)	Knee	2 years	Completed
Retrospective case series Germany	(Kayaalp et al., 2021)	**NOVOCART® Inject** 5	Knee	28 + 7months (20 - 40 months)	Completed
Independent subgroup analysis of Phase III study AAG-G-H-1624	(Janacova et al., 2022)†	**NOVOCART® Inject plus** 20	Knee	24 months	Completed
Case report Germany	(Richter et al., 2016)#	**NOVOCART® Inject** 2	Knee	9 and 11 months	Completed
Prospective case series ** Germany	(Thier et al., 2018)	**NOVOCART® Inject** 13	Hip	Mean follow-up 12 months (range 6 to 24 months)	Completed
Prospective case series comparing NOVOCART® Inject and co.don chondrosphere/ Spherox®§, ** Germany	(Thier et al., 2017)	**NOVOCART® Inject** 19 (10 patients with co.don chondrosphere/ Spherox®)	Hip	Mean follow-up 19 months (range 6 to 24 months)	Completed

□ Populations may be overlapping in publications by (Schlumberger et al., 2019) and (Schlumberger et al., 2020)
† This publication reports on additional MRI analyses on 20 patients from the MRI population of the Phase III study (AAG-G-H-1624) which was done independently of the phase III trial
# This publication reports on arthroscopic second look (1 patient) and immunohistological/ histological analysis (1 patient) to assess repair tissue
§ No information on the rationale for allocating patients to one or the other M-ACI product is given.
** Populations may be overlapping in publications by (Thier et al., 2017) and (Thier et al., 2018)

Table 44. Overall KOOS and IKDC responder rates after transplantation with NOVOCART® Inject (plus) in the knee across studies.

		Overall KOOS responder rate (%)[a] at time point of follow-up (months)			IKDC responder rate (%)[b] at time point of follow-up (months)		
Study		3	12	24	3	12	24
Pivotal clinical investigations with NOVOCART® Inject plus							
Phase III clinical trial AAG-G-H-1624		71.0 95% CI [61.1; 79.6] P < 0.0001 [c]	95.0 95% CI [88.7; 98.4] P < 0.0001 [c]	93.0 95% CI [86.1; 97.1] P < 0.0001 [c]	44 95% CI [34.1; 54.3]	82 95% CI [73.1; 89.0]	84 95% CI [75.3; 90.6]
Supportive clinical investigations with NOVOCART® Inject (plus)							
Matched pair analysis NOVOCART® Inject plus (AAG-G-H-1624) versus MFx (AAG-G-H-1202) Propensity score matched pair analysis report	NInject plus	73.6 cc: 76.8	94.4 cc: 95.8	94.4 cc: 94.4	43.1 cc: 44.9	80.6 cc: 80.6	83.3 cc: 83.3
	MFx	65.3 cc: 68.1	72.2 cc: 81.3	65.3 cc: 78.3	38.9 cc: 40.0	61.1 cc: 67.7	61.1 cc: 72.1
Retrospective, non-interventional, multi-centre study RENOVO AAG-O-H-1521		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Report from the German cartilage registry by Niemeyer et al., November 2021	NInject	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	N3D	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	Chondro.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
(Schlumberger et al., 2019)		n.r.	n.r.	n.r.	n.r.	Mean 9.6 mo (0.4 – 1.3 years) **66.7**	Mean 4.3 years (4 – 4.8) **66.7**
(Schlumberger et al., 2020)		n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
(Blanke et al., 2021)		n.r.	n.r.	n.r.	n.r.	**62.1**[d]	n.r.
(Niethammer et al., 2022)	NInject	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
	N3D	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
(Kayaalp et al., 2021)		n.r.	n.r.	n.r.	n.r.	n.r.	n.r.

KOOS: Knee Injury and Osteoarthritis Outcome Score; IKDC: International Knee Documentation Committee; NInject: NOVOCART® Inject; N3D: NOVOCART® 3D; Chondro.: chondrosphere®; MFx: Microfracture; n.r.: not reported; n.a.: not applicable; CI: confidence interval: CC: complete case

a: KOOS responder rate is defined as ≥10 score points change from baseline

b: IKDC responder rate is defined as >20.5 score points from baseline

c: From one-sided exact binomial test of hypotheses H0: Rate ≤40% vs. H1: Rate >40%. # At a mean follow-up of 9.6 months (range 0.4 to 1.3 years)

d: Minimally clinically important difference is 16.7 score points in this publication

Niemeyer et al has performed a comparison of clinical outcomes in patients with cartilage defects of the knee receiving co.don chondrophere (renamed SPHEROX since EMA approval), NOVOCART 3D or NOVOCART Inject based on data from the German Cartilage Registry (KnorpelRegister DGOU). Of the 5363 patients screened for eligibility 337 met the inclusion criteria (isolated defect, with an intact corresponding joint surface, intact or less than 1/3 resected meniscus, with a defect size of>250 mm2, no concomitant surgery). At the endpoint of the study at 36 months, an increase of more than 20 points by the means of the KOOS score was observed for all three products. No significant differences were found for the improvement over time comparing KOOS after 36 months to preoperative KOOS values. Without statistically significance slightly

higher values have been analysed for Novocart Inject®, followed by co.don chondrosphere® and Novocart 3D®.

In the article by Schlumberger et al 2019 15 patients undergoing ACI with NOVOCART Inject were included. Improvements were seen in the subjective IKDC score (pre-operative 44.5 ± 15.9, first follow-up 71.1 ± 15.9, $p < 0.001$, second follow-up 72.6 ± 17.3, $p < 0.001$). There were no significant differences in pre-injury and follow-up Tegner activity scales. Two patients required re-operation in the index knee, but unrelated to the ACI procedure. Axis deviations, meniscal injury or ligamentous instability were treated with corrective osteotomy, meniscal surgery or ligament reconstruction, respectively.

In a retrospective consecutive case series performed by Schlumberger et al (2020), 62 patients with a mean age of 34 ± 11 years (range 15 to 56 years) and a mean follow up of 31.0 ± 14.8 months (range 12.5 - 61.4 months) after transplantation with NOVOCART® Inject in the knee were evaluated. In 18 patients (29%) multifocal cartilage damage was treated Concomitant with ACT (cell harvesting) additional procedures were performed in 15 patients (18%) - specifically, 7 ACL reconstructions (5 of which involved meniscal refixation), 3 realignment osteotomies, and 7 MPFL reconstructions. The average size of the treated cartilage defects was 4.7± 2.8 cm2. The subjective IKDC and total KOOS at follow-up were 66± 10 and 73± 19 points (pain: 81± 17, symptoms 76± 17, activities of daily living: 87± 14, function in sports and recreation: 64± 26, quality of life: 54± 27).

In the retrospective study performed by Blanke et al in 2021, 29 patients had received NOVOCART Inject. The following concomitant surgeries were performed: plica resection (n=14) and removal of free joint bodies (n=6). All patients were subject to a postoperative rehabilitation program. Improvements were seen in all clinical outcome scales (VAS, Tegner score, IKDC) from baseline to follow-up (mean 24.9 months) and the values were in line with previous studies, MCID values were only reached in 48% to 69%. Ten patients (34.5%) showed complete filling of the defect, graft hypertrophy was detected in 6 patients (20.7%) and complete integration into adjacent cartilage was achieved in 24 patients (82.8%).

Niethammer et. al. performed a matched pair analysis in 2022 comparing NOVOCART Inject with NOVOCART 3D in a total of 50 patients. The median IKDC score improved comparable after 2 years from 41.4 to 66.7 points in the Hydrogel-ACI group and from 36.5 to 67.8 points in the scaffold-ACI group ($p < 0.001$). Improvements were seen after 6 months in the NOVOCART Inject, while the first improvements found in the NOVOCART 3D group were after 12 months. In the NOVOCART Inject group, the median VAS score improved from 4.0 preoperatively to a score of 2.0 after 2 years, while in the NOVOCART 3D group it improved from a level of 7.0 preoperatively to 2.0 ($p < 0.001$).

Kayaalp et al published a retrospective case series in 5 patients in 2021. The mean age was 23 ± 6 (range 14–30) years; mean follow- up time after surgery 28 ± 7 (range 20–40) months. All patients returned to full activity without residual knee ROM restriction. Clinical examination at the latest follow- up revealed a Kujala score of 90 ± 12 points and Lysholm score of 95 ± 5 points. MRI showed filled cartilage defects in all patients. Mocart score was 63 ± 7 points. Cartilage was inhomogeneous and hyperintense at the repaired site.

In 2022 Janacova et al published additional MRI analyses from 20 patients in the MRI subgroup in the pivotal phase III trial AAG-G-H-1624. The aim of the study was to assess the texture of repair tissue and tissue adjacent to the repair site after matrix-associated chondrocyte transplantation (MACT) of the knee using gray-level co-occurrence matrix (GLCM) texture analysis of T2 quantitative maps. The patients underwent examination on a 3 T MR scanner, including a T2 mapping sequence 12 and 24 months after MACT. Changes between the time points in mean T2 values and 20 GLCM features were assessed for repair tissue, adjacent

tissue, and reference cartilage. Differences in T2 values and selected GLCM features between the three cartilage sites at two time points were analyzed using linear mixed-effect models. A decrease in T2 values after MACT, between time points, was observed only in repair cartilage ($p < 0.001$). Models showed differences in GLCM features between repair tissue and reference cartilage, namely, autocorrelation ($p < 0.001$), correlation ($p = 0.015$), homogeneity ($p = 0.002$), contrast ($p < 0.001$), and difference entropy ($p = 0.047$). Values in repair and adjacent tissue became similar to reference tissue over time. The authors conclude that GLCM is a useful add-on to T2 mapping in the evaluation of knee cartilage after MACT by increasing the sensitivity to changes in cartilage structure. Furthermore, the authors concluded that the results suggest that cartilage tissue adjacent to the repair site heals along with the cartilage implant.

2.6.6. Discussion on clinical efficacy

Data on clinical efficacy of Jelrix was derived from a single pivotal trial (AAG-G-H-1624). Supportive data was provided by propensity score matched pair analyses from the pivotal trial with the MFx arm of study AAG-G-H-1202. Additional study data were provided by the applicant, of which paediatric efficacy data and data on the impact of defect size on efficacy outcomes after NOVOCART Inject treatment from the RENOVO study only were considered relevant and included in the assessment.

Pivotal phase III trial AAG-G-H-1624

Design and Objectives

The **single pivotal trial** was a prospective, 60-month, single-arm, multicentre phase III study to investigate the efficacy and safety of NOVOCART Inject plus in patients with focal cartilage defects of the knee between 4-12 m^2 post-debridement. The main analysis was performed 24 months post-transplantation, which is in line with previous Scientific Advice (EMA/CHMP/SAWP/400714/2016). Sixty-months data was included in the 120 LoQ responses, which is considered an adequate follow-up duration.

According to EMA reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair (EMA/CAT/CPWP/568181/2009) randomized controlled trials are expected in this medical field. However, the **single arm** study design has been discussed in a previous Scientific Advice for NOVOCART Inject (EMA/CHMP/SAWP/ 400714/2016); a blinded placebo group was considered unfeasible, and an active control arm was considered difficult because 1) these usually address smaller defect sizes than NOVOCART Inject plus (e.g., MFx for defects up to 2-2.5 cm^2); 2) these have previously shown smaller efficacy compared to NOVOCART Inject (e.g. mosaicplasty); and 3) authorised ACI / M-ACI products at start of the trial in 2016 were not available. As such, a single arm trial was reluctantly deemed acceptable, although it was concluded that '*…it appears that both the uncontrolled single pivotal clinical study and the intended supportive clinical data of the applicant's proposed phase III clinical development program have their inherent uncertainties that ultimately will be at the risk of the applicant in the case of an MAA. At this moment, it is uncertain that a single uncontrolled pivotal clinical trial without a previous compelling clinical development will be sufficient to provide consistent, sound and compelling evidence on the efficacy and safety profile of the product. Notwithstanding, the appropriateness of the quality and quantity of the information submitted will be ultimately evaluated at the time of the submission of the MAA.'* At present there is no golden standard surgical treatment for cartilage damages but the inclusion of a treatment-as-usual / standard-of-care control group (e.g. arthroscopy, debridement + rehabilitation) would have been an appropriate alternative to an active control arm. Instead, the applicant presented a propensity score matched pair analysis (discussed below). The single arm design inherently introduces substantial bias which hampers adequate assessment of efficacy of NOVOCART Inject plus. Furthermore, this is a single pivotal study and therefore an especially

robust methodology would have been expected in combination with very compelling results both statistically and clinically (please refer to EMA guideline on single pivotal studies CPMP/EWP/2330/99).

Patient population

Adult patients between 18 and 65 years of age, and paediatric patients 14 to 17 years of age with closed epiphyseal growth plates, with one or two symptomatic cartilage defects of the knee, were included. Defect size was set limited to 4-12 cm^2 with ICRS Grade III/IV, with defects located at the femoral condyle, trochlea, patella and / or tibial plateau. Baseline overall KOOS was set at < 65/100. Majority of the patients received study treatment as first line.

Treatment

Treatment in this single-arm study was NOVOCART Inject plus combined with a rehabilitation program. NOVOCART Inject plus required two surgeries; 1) an arthroscopic harvest of autologous chondrocytes for transplant production, and 2) transplantation of the expanded autologous chondrocytes in a cross-linker solution 3-4 weeks later, via mini-arthrotomy or arthroscopy depending on (per protocol) required concomitant corrective surgeries (i.e., ligament repair, alignment of varus / valgus deformities, correction of patella alignment, filling of subchondral bone defects > 2 mm). The transplantation was administered using a double chamber syringe. About 8 - 32 million cells per 4 mL cell suspension were transplanted; the volume depended on the size of the prepared defect. At an application height of 2.5 mm, the dosage is 0.4 to 1.6 million cells per cm^2 defect area. In section 4.2 of the SmPC, it is included that the product must only be administered in the context of a Matrix-assisted Autologous Chondrocyte Implantation (M-ACI) by an appropriately qualified orthopaedic surgeon which is considered acceptable by CAT/CHMP.

As from the first day of surgery, patients had to participate in a rehabilitation program until the end of study. A total of 98% of patients adhered to this program.

Outcome and endpoints

The primary endpoint was the subjective overall KOOS responder rate (proportion of patients ≥ 10 points improvement) based on the average of the 5 subscales (Pain, other Symptoms, Function in daily living (ADL), Function in Sport and Recreation (Sport/Rec), and knee-related Quality of Life) from baseline to 24 months follow-up. KOOS is a commonly used, validated scale and the definition of the endpoint is in line with the Scientific Advice (EMA/CHMP/SAWP/400714/2016), and as such can be accepted. The 10 points cut-off, although arbitrary, is supported by the study of Roos and Lohmander (2003). This study, however, was conducted in patients undergoing an ACL reconstruction at a follow-up of 6 months and it remained unclear how this relates to the population included in the current application. The assessment at 24 months follow-up is considered an adequate timeframe in which beneficial effects of ACI can be demonstrated (EMA/CHMP/SAWP/400714/2016).

Key secondary endpoint was change in overall KOOS from baseline to 24 months. Other secondary endpoints were the separate KOOS variables, IKDC (International Knee Documentation Committee; subjective score changes compared to baseline, responder rates with validated cut-offs of ≥ 11.5 and > 20.5), health-related quality of life measures (EQ-5D-5L), patient satisfaction, health economic variables, time to / proportion of patients with treatment failure, and structural outcome (MRI; Magnetic Resonance Observation of Cartilage Repair Tissue [MOCART] score and transverse relaxation time [T2] mapping parameters). The focus was on evaluation at 24 months.

Overall, the set of endpoints is considered acceptable as it reflects relevant, both subjective and objective, validated endpoints for the target population and is in line with the Scientific Advice

(EMA/CHMP/SAWP/400714/2016). However, the uncontrolled study design makes the interpretation of results of subjective endpoints especially difficult since such endpoints are inevitably affected by patient expectations and thus uncertainties in the observed effect are introduced as also highlighted in the scientific advice.

Sample size calculation

In estimating the sample size, a one-sided exact binomial test was used with significance level 0.0232 (initially adjusted for one interim analysis that was never performed). A drop-out rate of up to 15% was anticipated. With a power of 80% and an assumed response rate of at least 55%, the required number of patients was 96. This approach was generally accepted, and the 15% drop-out rate was in line with the > 10% as enclosed in the Scientific Advice (EMA/CHMP/SAWP/400714/2016).

Statistical methods

The absence of a randomised concurrent control group implies that the pivotal evidence relies on baseline comparisons alone. This offers no sound basis for statistical inference. The risk that estimates are biased cannot be ignored. Potential biases may have arisen from e.g., patient selection, endpoint assessments, and importantly, as discussed above, other concomitant interventions.

The primary endpoint in study AAG-G-H-1624 was a patient-administered instrument assessing five outcomes including for example pain and quality of life. Without a randomised control group, it is not possible to discriminate patient outcomes caused by the test treatment from outcomes caused by other factors. Further, the impact of patient's expectations cannot be ignored.

Pre-planning is essential for all confirmatory trials. In the setting of a single arm study the standard needs to be set even higher implying for example that the statistical analysis plan needs to be finalised before trial initiation (EMA/CHMP/564424/2021). There was one SAP version drafted before start of patient enrolment which contained the primary endpoint hypothesis testing, but the SAP was not finalised until almost a year after start of patient enrolment. The final version 1.0 (dated 25-Sep-2018) was preceded by 7 drafts. Besides final version 1.0, there exists a final version 2.0 (dated 18-May-2021), also preceded by several draft versions.

It is noted that the changes made commonly have been explained by either "updated according to the Sponsor's comments" or "updated after sponsor's review". The most notable changes have been listed. The impact of the changes made within these SAP amendments are difficult to assess. However, amendments cannot be considered anything else but potentially data driven.

For the primary analysis, the ITT population was initially defined to include "all patients with tissue harvested for NOVOCART® Inject plus transplantation" (CSP version 3.0). Later, the ITT definition was changed to include all patients who had received NOVOCART® Inject plus transplantation (CSP version 4.0). This is the analysis set used for the primary efficacy analysis.

Although these two sets differed by only two patients, it had been preferred if the primary population used for analysis had not been subject to any change after study initiation. This to avoid concerns over inclusion/exclusion of patients in the analysis set being based on observed individual outcomes. In analogy with the preferred "all subjects randomised" (in a randomised study), the primary analysis set should rather have included all subjects having entered the study The requested additional analysis of the primary endpoint including all subjects who underwent tissue harvesting (i.e. the safety set) showed that the adding of two non-responders had an insignificant impact on the primary outcome estimate compared with the primary ITT analysis.

The inferential part of study AAG-G-H-1624 was defined to comprise the primary endpoint alone. There was one secondary endpoint denoted as "key". The analyses of secondary efficacy endpoints were all to be seen as exploratory. While p-values from formal hypothesis testing are conceptionally of subordinate relevance compared to estimation, the general principles for randomised clinical trials apply also for single-arm trials (EMA/CHMP/564424/2021). Hence, it had been expected that multiplicity would had been addressed considering at least some non-exploratory secondary endpoints.

The threshold for efficacy in KOOS5 was a response rate of 40%. Testing was performed using a one-sided exact binomial test at a final significance level of 0.025. Efficacy was to be concluded if the lower bound of the two-sided 95% CI for the response rate according to Clopper-Pearson was >40%.

Secondary endpoints were analysed with 2-sided tests with significance levels of 0.05, without hierarchical testing. As type I error rate was not controlled across these endpoints, they are considered as supportive analyses only.

The existence and a priori definition of a success criterion for the primary endpoint is endorsed. However, the threshold for the response rate for when to conclude that the study was successful was not justified. Given the study design, it would have been expected that the clinical validity of the success criterion in the therapeutic context had been justified based on e.g., knowledge about the knee joints defects in question, uncertainties around the variability of the primary outcome and treatment effect estimates (EMA/CHMP/564424/2021). This hampers the reliance and robustness on the clinical outcome.

In summary, none of the outcomes are considered statistically and clinically compelling.

Results

The study conduct was in line with the declaration of Helsinki and GCP guidelines.

There were 4 protocol amendments since September 2016. Most changes were minor and unlikely to affect outcome, but the impact of changes in the eligibility criteria with regard to time since prior biologic reconstructive procedures and broadening of the defect aetiology, as well as the change in the definition of treatment failure into a less rigid one were clarified.. The primary population for analysis preferably would not have been subject to any change after study initiation.

A total of 132 patients was screened and 100 patients were ultimately treated with NOVOCART Inject plus (ITT population), including 2 adolescents. The per protocol (PP) population comprised 98 patients, thus excluding 2 patients due to major protocol deviations (unrelated AE's). From 25 patients, MRI data were collected (MRI-ITT analysis set). Patient flow was well reported and there were no dropouts from follow-up at 24 months. The majority of patients was male, aged median 40 years (15 – 62), with mean symptom duration of about 2 years (median 10.8 months). Patients > 65 years of age were not included; this can be accepted as knee osteoarthritis (Kellgren and Lawrence Grade > 2) is very common in elderly. Consequently, this is listed as a contra-indication in section 4.3 of the SmPC. Mean baseline KOOS was 39.8 (sd 14.3; range 12.1 – 63.9). With regard to cartilage defect characteristics, trauma was the aetiology of the cartilage defects in 60% of the subjects, 4.6% of subjects suffered from osteochondritis dissecans and 35.4% had degenerative lesions. Approximately half of the participants had normal knee status according to IKDC objective score at baseline. Defect location was mainly at the femur condyle (62%) and the patella-femoral joint (35%); most with ICRS-grade III (69%). Median defect size post debridement was 4.50 cm^2 (range 1.0-10.4 cm^2).

Adolescent data
Only 2 adolescents with closed epiphyseal growth plates were included in the pivotal study instead of 10 as planned in the PIP. A systematic review on the use of ACI in the adolescent knee from other studies demonstrated improvement in clinical outcome scores consistent with adults in skeletally mature individuals <18 years of age. In contrast, skeletally immature patients, i.e. patients with open epiphyseal growth plates, may have a certain self-healing capacity for cartilage lesions due to a higher bone marrow stem cell concentration compared to adults. Findings from adult population could in principle be extrapolated to adolescents with closed epiphyseal growth plates, despite the fact that only a few adolescents were included in the pivotal study.

Efficacy data
the efficacy of NOVOCART Inject plus in defects 4-12 cm^2 is not considered demonstrated. Addtionall analysis were submitted based on data from the German Cartilage Registry (KnorpelRegister DGOU) and a subgroup analysis from the non-interventional retrospective RENOVO study (AAG-O-H-1521) based on defect size (<4 cm^2 vs. 4-6 cm^2 vs. >6 cm^2).
The CAT/CHMP considers that thenew registry analyses with NOVOCART® Inject product regarding defect sizes below 4 cm2 cannot be accepted as pivotal efficacy demonstration in light of the previous failed randomised controlled study with the similar NOVOCART® 3D plus product, assessed in the previous procedure EMEA/H/C/004598.

Patient population
Only three conditions (i.e., trauma, osteochondritis dissecans, and focal degenerative) were included in the pivotal trial. It remains unclear whether the efficacy of NOVOCART Inject plus is similar in each of these three groups and whether this can be extrapolated to cartilage defects from other aetiologies due to the small sample size hampering the interpretation of the subgroup analysis according to different aetiologies. . Of note, the same methodological concerns apply to difficulties to interpret efficacy in a subgroup analysis of a single arm trial or retrospective observational trials as for the whole population. The data (from studies 1624 and 1521 (RENOVO) presented by the applicant stratified by aetiology subgroup, indicated smaller changes in efficacy endpoints (partly due to higher baseline values) compared to baseline, together with higher frequencies of AE's, in patients with degenerative lesions compared to the other aetiologies. In addition, 4 of the 5 transplant failures (RENOVO study) were observed in patients with degenerative defects. The smaller benefit of (M)ACI in degenerative disease compared to other aetiologies aligns with current scientific knowledge (degeneration > grade II is a contra-indication for treatment with NOVOCART Inject plus). Overall, the B/R seems less favourable for degenerative defects compared to defects from other origins

First part of the treatment with NOVOCART Inject plus (harvesting chondrocytes) was performed by arthroscopy in 98% of the patients (2 had mini-arthrotomy). The second part (transplantation) was performed by arthroscopy in 46% of the cases while 49% underwent mini-arthrotomy. A total of 22 patients underwent a concomitant intervention during the first or second surgery, and another 12 patients subsequent surgery and all patients had damaged tissue removed (if any) before injection. Considering "concomitant surgeries" the applicant did not list debridement/chondroplasty (contouring and removal of devitalised or detached cartilage fragments, joint lavage, irrigation). The nature of the concomitant/subsequent surgical interventions were such that an influence on the primary endpoint is likely (for example ligament operation, meniscus removal, joint dislocation reduction). The majority (65%) of study participants also underwent post-surgery non-drug knee therapies (for example joint fluid drainage, cooling therapy, physiotherapy) and in principle all participants underwent a rehabilitation program as specified in the study protocol.

Consequently, the interpretation of the results derived from Study AAG-G-H-1624 is considered severely complicated by the uncertainty what regards whether observed individual outcomes are undoubtedly caused by the treatment with NOVOCART Inject plus alone. The effect of all concomitant procedures and active rehabilitation needs to be considered when interpreting results from a single arm trial Given the nature of treatment, compliance to NOVOCART Inject plus was no issue. Compliance to the post-transplantation rehabilitation was almost complete (98%).

The primary endpoint overall KOOS responder rate at 24 months was reached in 93% of the patients (CI 86.1 – 97.1; $p < 0.0001$). The 93% exceeded the pre-specified margin for clinical relevance of 40%, but as the treatment effect of NOVOCART Inject plus cannot be determined, neither can clinical relevance be concluded. Sensitivity analyses, including correction for the use of analgesic medication prior to and during KOOS assessment, were in line with the primary analysis. Mean change in overall KOOS at 24 months compared to baseline (key secondary endpoint) was 42.0 points (SE 1.83; CI 38.4 – 45.7) which was nominally statistically significant and clinically relevant. Results for the secondary endpoints were in support of the primary and key secondary endpoints. In particular, IKDC subjective scores improved from 34.3 (sd 12.8) at baseline to 75.8 (sd 17.5) at 24 months. Overall, onset of effect in terms of KOOS responder rate was seen as from 3 months post-transplantation, gradually increased until 12 months and remained rather stable up to 24-60 months.

MRI is one of the accepted methods to assess pharmacodynamic parameters related to cartilage repair after administration of chondrocyte containing products and an alternative to histology (EMA reflection paper EMA/CAT/CPWP/568181/2009). Structural MRI endpoints MOCART and T2 mapping parameters, were obtained at 12 and 24 months in 25 patients representing 30 lesions. MOCART sum scores changed from 70 (sd 22.4) at 12 months to 80 (sd 56.7) at 24 months. The main sub score *defect filling* did not change between 12 and 24 months: Complete defect filling was observed in 61% of the lesions. Numerical improvements were observed for the MOCART sub scores *integration of graft tissue to border zone* (complete in 73% at 12 months and 82% at 24 months), *surface of repair tissue* (intact in 53% and 86% resp.), *structure of repair tissue* (homogeneous in 53% and 68%), and *signal intensity of repair tissue* (normalisation in appr. 50% and 80%). T2 relaxation time in repair tissue was slightly smaller at 24 months compared to 12 months, and the T2 standard deviation numerically decreased, suggesting maturation of the graft. Global and zonal ratios were approximating normal (i.e., 1) at 12 months already and remained stable at 24 months. The data were obtained in only a subset of the patients (25%) and the (clinical) relevance of findings from MRI assessments is unclear.

Treatment failure was defined by the applicant as 'all surgical re-interventions affecting the closed surface of the transplant area (the surface is not closed when the defect area is grade 3 or 4 ICRS) and / or require additional cartilage repair modalities in the target defect' (3rd protocol amendment). Such failure was observed in 1 patient and occurred between 18 and 24 months in one of the two defects treated (a 2 cm^2 defect at the medial condyle), probably due to a meniscal tear resulting in unphysiological load on the graft. Overall, data suggest an absent relation between functional and structural outcome (see PD discussion above). Altogether, this increases the uncertainty of the clinical efficacy of NOVOCART Inject plus.

Pre-planned subgroup analyses for KOOS responder rate at 24 months showed no clear impact of age (≤ 25 years and > 25 years) (92% versus 93%). Post-hoc univariate subgroup analyses indicated among others a larger overall change in KOOS in patients with concomitant surgery on target knee (53.8 (46.6 – 61.0) versus 39.3 (34.6 – 44.0) with mean baseline KOOS 36.7 versus 40.6 resp.). Numerically larger improvements were seen in patients with traumatic cartilage lesions (mean 45.8, CI 40.5 – 51.1) compared

to those with osteochondritis dissecans (mean 40.3, CI 23.8 -56.8) and focal degenerative lesions (mean 37.5, CI 30.7 – 44.4).

Paediatric data

Two adolescents were included in the pivotal trial, 15 and 17 years old, with OCD and a traumatic lesion, both ICRS grade III. Both were KOOS and IKDC subjects I/II responders, MOCART scores were 80 and 90 points at 24 months. No additional surgeries were performed in these patients.

Supportive data

Propensity score matched pair analysis

Propensity score matched pair analyses were performed to investigate whether NOVOCART Inject plus treatment was non-inferior or even superior to microfracture (MFx) treated patients at 24 months follow-up. For this purpose, patients from the pivotal study 1624 (discussed above) were matched with microfracture (MFx)-treated patients from study AAG-H-O-**1202**.

Study 1202 (N.B. the pivotal study of the application for Artrobend; withdrawn by the applicant due to MO's on efficacy and safety) was originally designed as a prospective, multi-centre, randomised, active controlled, open-label study comparing efficacy and safety of NOVOCART 3D plus versus microfracture (MFx) in patients with cartilage defects of the knee. Eligible patients had 1-2 focal cartilage defects of the femur condyle or trochlea of the knee with (total) defect size 2-6 cm^2 and ICRS grade III or IV. Primary endpoint was the change in IKDC subjective score from baseline to 24 months; secondary endpoints included a.o. KOOS change from baseline to 24 months and the MOCART score.

Matching procedure and data analyses

Eligibility criteria between studies 1202 and 1624 differed on several aspects, most noteworthy were defect size (2-6 cm^2 in study 1202 versus 4-12 cm^2 in study 1624) and defect localisation (restricted to femur condyle or trochlea in study 1202 versus femoral, condyle, trochlea, patella, or tibial plateau in study 1624). Patients were 1:1 matched for overall KOOS at baseline, time since first symptoms, prior target knee surgery, age, and -gender. The applicant selected these variables based on statistical significant associations demonstrated in previous studies.

The primary endpoint of the propensity score matched pair analysis was the change in overall KOOS from baseline to 24 months, and not KOOS response rate as in the pivotal study. The non-inferiority margin for NOVOCART Inject plus versus MFx was set at -8.5 points, which was based on literature, and superiority was set at a lower bound of the 95% CI > 0. KOOS (≥ 10 points improvement) and IKDC (> 20.5 points improvement) responder rates at 24 months, as well as MOCART sum scores were compared. The statistical analysis plan was developed prior to data analysis of the pivotal trial 1624, except for the MRI analyses.

Results

A total of 144 patients was matched; 72 in both treatment groups. Final 24 months data was available in 72 (NOVOCART Inject plus) and 64 (MFx) patients, respectively. The total population consisted of mostly male patients (about 70%) of white race, with a mean age of 39 years and a mean BMI of 27 kg/m^2. Baseline KOOS values were 41.7 (sd 13.1) in NOVOCART Inject plus and 43.1 (sd 14.5) in MFx. Noteworthy differences (after matching) were: a) More frequent concomitant surgery on the target knee in NOVOCART Inject plus compared to MFx (21% versus 9.7%), b) Larger total defect size in NOVOCART Inject plus compared to MFx (6.4 cm^2 (sd 2.1) versus 3.7 cm^2 (sd 1.3)), and c) Frequency of defect aetiologies: in NOVOCART Inject plus trauma, osteochondritis dissecans, and focal degenerative lesions (60%, 6%, and

34% resp.), versus trauma, osteoarthritis, and other causes in MFx (79%, 6%, and 15%). Patients treated with NOVOCART Inject plus also more often had 2 defects and grade IV defects compared to patients treated with MFx (33% versus 9.7%, and 48% versus 25% respectively). Overall, the differences between both patient populations affect the validity of the comparison.

The primary endpoint change in overall KOOS at 24 months compared to baseline was larger for NOVOCART Inject plus compared to MFx (LS mean difference 10.02 (se 3.26), 95% CI 3.57 – 16.46). Sensitivity analyses yielded comparable results. KOOS responder rate was higher in NOVOCART Inject plus compared to MFx (94% versus 65%; OR 7.9 (95% CI 2.54 – 24.38)), as was the IKDC responder rate (83% versus 61%; OR 2.71 (95% CI 1.22 – 6.01)). Sensitivity analyses indicated that the findings were not robust. Structural data were available for 21 patients treated with NOVOCART Inject plus and 28 patients treated with MFx. MOCART sum scores at 24 months were higher for NOVOCART Inject plus compared to MFx (LS mean difference 17.8 (SE 6.56; 95% CI 4.57 – 31.05). A tendency was observed for higher MOCART sub scores in NOVOCART Inject plus versus MFx at 24 months, but not for defect filling, subchondral lamina or bone, and adhesions. There was no correlation between MOCART scores and KOOS changes from baseline at 24 months. Treatment failure in terms of unplanned surgeries of the target knee was reported for 6 patients (8.3%) in NOVOCART Inject plus and 3 patients (4.2%) in MFx.

In conclusion, the propensity score matched pair analyses suggest a more favourable outcome for NOVOCART Inject plus compared to MFx. However, the comparison is methodologically flawed by relevant differences between both patient populations and is further complicated by the absence of data on the rehabilitation program in MFx. In summary, the currently presented matched pair analysis using external historical data cannot be considered valid as pivotal evidence for regulatory approval

RENOVO study

The RENOVO study (study AAG-O-H-**1521**) is a completed, retrospective, multi-centre, non-interventional study in German adult and paediatric patients to evaluate safety and efficacy of NOVOCART Inject (N.B. not NOVOCART Inject plus) in a routine care setting. All medical centres having treated at least 10 patients with NOVOCART® Inject (18 sites) were invited for the study, thereof 7 sites participated. The added value of this study is enclosed in a) efficacy data in adolescents (< 18 years) and young adults (18 – 25 years), and b) data on unplanned re-surgeries and treatment / transplant failure. Relevant efficacy endpoints were mean overall KOOS scores and the IKDC subjective score; these were completed at the end of follow-up at a variable interval (median follow-up 19.1 months, sd 1.5 – 57.0 months) and baseline values were not available.

Out of the 515 patients treated up to the final analysis only 245 could be reached and were willing to give written informed consent for collection of safety and efficacy relevant information related to their NOVOCART® Inject treatment from their medical records. The study population thus consisted of 245 patients, including 11 adolescents and 36 patients 18 - 25 years of age, with a full-thickness cartilage defect ICRS Grade II/IV, with size 2.5 – 10 cm^2. Data from 10/11 adolescent patients were reported, but the 11^{th} patient dropped out. Median age was 36 (range 12 – 69) years. About 30% of the patients had a traumatic lesion, another 30% had degeneration, 10% osteochondritis dissecans and 10% chondromalacia; the remainder had other / unclear aetiologies. Mean defect size was 5.9 (sd 3.6) cm^2, which is smaller than in pivotal study 1624. Over 80% of the patients underwent concomitant additional surgery. Demographic and disease characteristics were not separately presented for patients < 18 and 18-25 years of age).

a) Efficacy data in adolescents and young adults: At the end of the follow-up period, overall KOOS was numerically higher in patients < 18 years (n = 10) compared to those 18-25 years (n = 29), and those > 25

years of age (n = 153) (78.3, sd 11.2; 71.9, sd 17.2; and 66.8, sd 19.5 resp.). The IKDC subjective score was also numerically higher in patients < 18 years compared to those 18-25 years, and those > 25 years of age (76.8, sd 16.4; 72.3, sd 15.3; 66.3, sd 18.2 resp.).

b) Data on unplanned re-surgeries and treatment / transplant failure: Unplanned re-surgeries were performed in 18 (7.3%) patients, not stratified by age nor reason for re-surgery . Five patients (2%) had surgery directly on the transplanted defect (chondroplasty, knee arthroplasty). Five patients, all > 25 years of age, had transplant failure. Two of them underwent MFx to treat (residual) cartilage defects, the other 3 ultimately received a prosthesis > 6 months after transplantation.

In conclusion, data from the RENOVO study might suggest a larger treatment response in adolescents and young adults compared to adults; transplant failure was not observed in these patients treated with NOVOCART Inject. However, the added value of the RENOVO study is considered minimal due to its weak methodological quality (including its retrospective, uncontrolled design, absence of baseline assessment, variable follow-up). Although additional clarification on several issues (patient characteristics by age group, the reason for drop-out of one adolescent and reasons for re-surgery) would be informative, these issues are not pursued as the eventually provided data are considered of insufficient value to contribute to the B/R of NOVOCART Inject plus. Although the RENOVO study was performed with NOVOCART Inject and not NOVOCART Inject plus, this unlikely affected outcome.

Summary of clinical efficacy in adolescents

Paediatric data originated from the pivotal study 1624 (n = 2) and the RENOVO study (n = 11). Additional data is expected from the NINJA study (AAG-O-H-2123; also with NOVOCART Inject) which started Q3 2023 (no data available yet).

Paediatric data from the pivotal trial was available from 2 patients, 15 and 17 years old, with OCD and a traumatic lesion, both ICRS grade III. Both were KOOS and IKDC subjects I/II responders, MOCART scores were 80 and 90 points at 24 months. No additional surgeries according to the applicants definition were performed in these patients (see Table 11-42 CSR).

Paediatric data from the retrospective, uncontrolled RENOVO study is described above. In short, overall KOOS composite scores at follow-up were numerically higher in patients < 18 years (n = 10) compared to those 18-25 years (n = 29), and those > 25 years of age (n = 153). For IKDC subjective scores comparable results were reported. Treatment failure did not occur in paediatric patients.

Altogether, the very limited evidence emerging from the clinical development program of NOVOCART Inject (plus) for adolescents, does not raise new safety concerns. Efficacy data is scarce but likely comparable to what is observed in the overall study populations. Finally, the fact that adolescents with closed epiphyseal growth plates can be considered skeletally mature, provides further support for inclusion of adolescents in the indication if the overall B/R supports approval.

2.6.7. Conclusions on clinical efficacy

The primary- and secondary endpoints were met but the single arm design of the single pivotal study prohibit proper evaluation of the effect of Jelrix and its clinical relevance. This is a major drawback for the efficacy assessment.: At least part of the overall clinical effect is likely ascribed to concomitant (surgical) interventions, as well as the intensive rehabilitation program. The propensity score matched pair analyses could not solve these issues due to the inherent non interventional design. Further, the patient populations

were considered different with respect to several critical characteristics, including differences in lesion characteristics, numbers of patients with concomitant surgery, and rehabilitation programs. The uncertainty in establishing the effect of Jelrix is further increased by the absence of a relation between functional and structural endpoints.

Patients with defect sizes < 4 cm2 were not included in the pivotal trial. The benefits of two surgical interventions for these smaller defects cannot be extrapolated from data on larger defects.

For smaller defects, there are several treatment options. In addition the natural course and effects of rehabilitation may play an important role in the overall observed effect. It is also noted that in these analyses, the B/R seems less favourable for degenerative defects compared to defects from other origins. In conclusion the additional registry analyses provided during assessment regarding defect sizes below 4 cm2 cannot be accepted as pivotal efficacy demonstration for Jelrix**.**

The clinical efficacy of Jelrix has not been demonstrated.

2.6.8. Clinical safety

The clinical safety assessment is based on

1. one single pivotal uncontrolled phase 3 study where all patients were treated with NOVOCART® Inject plus,
2. a propensity score matched pair analysis comparing patients treated with NOVOCART® Inject plus in the pivotal phase 3 study with control data from a previous study (AAG-G-H-1202) which compared Novocart 3D with microfracture, and
3. supportive data from non-interventional studies using an NOVOCART® Inject and available data from the literature, (Table 50).

Table 45. Overview of clinical studies supporting the safety of NOVOCART® Inject plus

Type of study (control) Location of study centres	Reference / study ID / Report	Product/ Patients treated with NOVOCART® Inject (plus), N	Localisation of defect	Follow-up time (months)	Status
NOVOCART® Inject plus					
Phase III prospective, single-arm, multi-centre trial 5 European countries	Study ID: AAG-G-H-1624 EUDRA-CT No 2016-002817-22 ClinicalTrials.gov: NCT03319797 Report on main analysis 2021	**NOVOCART® Inject plus (IMP**) 100	Knee	Main analysis at 24 months (all patients) Overall follow-up: 60 months	Main analysis report available Long-term follow-up (up to 60 months post-treatment) ongoing
Propensity score matched pair analysis NOVOCART® Inject plus versus MFx	Propensity score matched pair analysis report	**NOVOCART® Inject plus** (from study AAG-G-H-1624) 72 Microfracture (MFx) (from study AAG-G-H-1202) 72	Knee	Analysis at 24 months	Completed
NOVOCART® Inject					
Supportive: sponsored by Applicant					
Retrospective, non-interventional, multi-centre study Germany	RENOVO Study ID: AAG-O-H-1521 PEI: NIS330 ClinicalTrials.gov: NCT02941120 Final report	245	Knee	Mean follow-up 20.3 months (range 1.5 months to 57 months)	Completed

Type of study (control) Location of study centres	Reference / study ID / Report	Product/ Patients treated with NOVOCART® Inject (plus), N	Locali-sation of defect	Follow-up time (months)	Status
Prospective non-interventional, multicentre study Germany study	NINJA Study ID: AAG-O-H-2123 PEI: NIS653 ClinicalTrials.gov NCT05391841	30 planned Study will not start before Q3 2023	Knee	Main analysis at 24 months (all patients) Overall follow-up: 60 months	No data available yet
Prospective non-interventional, multi-centre study Germany	HIPACTION[#] Study ID: AAG-O-H-1304 PEI: NIS 252 ClinicalTrials.gov: NCT02179346 Final report	21	Hip	Follow-up 24 months	Completed
Supportive: Investigator initiated (published literature)					
Retrospective consecutive case series [‡] Germany	(Schlumberger et al., 2019)	15	Knee	Mean follow-up 4.3 years (4.0–4.8 years)	Completed
Retrospective consecutive case series [‡] Germany	(Schlumberger et al., 2020)	62	Knee	Mean follow-up 31,0 ± 14,8 months (range 12.5 – 61.4 months)	Completed
Retrospective consecutive case series Germany	(Blanke et al., 2021)	29	Knee	Mean follow-up 24.9 ± 1.1 months (range 24-27 months)	Completed

Type of study (control) Location of study centres	Reference / study ID / Report	Product/ Patients treated with NOVOCART® Inject (plus), N	Locali-sation of defect	Follow-up time (months)	Status
Prospective case series ** Germany	(Thier et al., 2018)	13	Hip	Mean follow-up 12 months (range 6 months to 24 months)	Completed
Prospective case series §, ** Germany	(Thier et al., 2017)	19 (and 10 patients with Chondrosphere®)	Hip	Mean follow-up 19 months (range 6 months to 24 months)	Completed

12 month results of the HIPACTION study (AAG-O-H-1304) were published by (Bretschneider et al., 2019).
§ In this study a total of 29 patients were treated with arthroscopic M-ACI. 19 patients were treated with NOVOCART® Inject and 10 patients with Chondrosphere®. No information on the rationale for allocating patients to one or the other M-ACI product is given.
⸸ Populations may be overlapping in publications by (Schlumberger et al., 2019) and (Schlumberger et al., 2020)
** Populations may be overlapping in publications by (Thier et al., 2017) and (Thier et al., 2018)

2.6.8.1. Patient exposure

The overall exposure is summarised below (Table 51).

Table 46. Overall exposure to NOVOCART® Inject (plus)

Study	Patients treated with NOVOCART® Inject (plus)♦	Paediatric patients	Defect location	Follow-up
Phase III clinical trial AAG-G-H-1624	100	2	Knee	Planned 60 months Main analysis: 24 months
Matched pair analysis	(72)[a]	0	Knee	24 months
Retrospective non-interventional, multicentre study RENOVO (AAG-O-H-1521)	245	11	Knee	Mean follow-up 20.3 months (range 1.5 months to 57 months)
Prospective non-interventional, multicentre study NINJA (AAG-O-H-2123)	0[b]	Planned 30[b]	Knee	Study will not start before Q3 2023 Planned: 60 months Main analysis: 24 months
Prospective non-interventional, multi-centre study HIPACTION (AAG-O-H-1304)	21	0	Hip	24 months
Published literature	Approx. 106[c]	Included but no accurate number given; range 13-18	Knee	Mean follow-up varied between 24.9 and 51.6 months (range varied between 12 and 61.4 months)
Published literature	32[c]	0	Hip	Mean follow-up 12-19 months (range 6-24 months)
Post-marketing	6,160[d, e]	230	Knee, Hip, Talus	n.a.
	5,940	226	Knee	n.a.
	218	3	Hip	n.a.
	2	1	Talus	n.a.

n.a.: not applicable. approx.: approximately
♦ Include all treated patients of all ages and subgroups
a: Patients from the Phase III clinical trial AAG-G-H-1624.
b: This study is exclusively in paediatric patients. The study will not start before Q3 2023 and therefore no data are available yet.
c: Populations may be overlapping. The number given here is the sum of number of patients provided by publications in Module 2.7.4, Table 1.
d: Current Periodic Safety Update Report (PSUR) (No. 6, DLP 31-05-2022): including also the 245 patients from the non-interventional study RENOVO as well as the 21 patients from the non-interventional study HIPACTION as described in the PSUR.
e: Current status of treated/exposed patients based on the number of products sold between 17-12-2008 and 31-05-2022

2.6.8.2. Adverse events

Phase III clinical trial, AAG-G-H-1624

About 70% of patients had experienced at least one treatment-emergent adverse event (TEAE), i.e. an AE that started at or after the first therapeutic intervention (i.e., autologous cartilage biopsy) during the 24 months period after transplantation. An overview of the TEAEs through Month 24 among the 102 patients enrolled in the current main analysis is provided below (Table 52).

Table 47. Overall summary of treatment-emergent adverse events (SAF) (Phase III clinical trial, AAG-G-H-1624)

	All patients (N=102)	
AE characteristics - Patients with:	n events [a]	n (%) patients
at least one TEAE	267	72 (70.6)
at least one TESAE	22	14 (13.7)
at least one TEAE related to treatment surgery	75	39 (38.2)
at least one TEAE related to tissue harvest	11	9 (8.8)
at least one TEAE related to implantation	65	37 (36.3)
at least one TEAE related to NOVOCART® Inject plus	7	4 (3.9)
at least one related TEAE [b]	75	39 (38.2)
at least one related TESAE	2	2 (2.0)
at least one severe TEAE	12	7 (6.9)
at least one TEAE leading to subsequent target knee surgery	10	7 (6.9)
at least one TEAE leading to discontinuation	0	0
at least one TEAE leading to death	0	0

SAF: Safety analysis set

a: Please note that the subsequent safety tables are all patient-based and do not show the number of events. This information can be taken from the referenced source tables.

b: TEAEs related to treatment surgery (tissue harvest and/or implantation) and/or to the NOVOCART® Inject plus product.

Source: AAG-G-H-1624, CSR, Table 12-11

Adverse events by SOC

Table 48 summarizes the incidence of all TEAEs at the SOC level.

Table 48. Incidence of all TEAEs at the SOC level

Primary SOC	All patients (N=102) n (%)
Patients with any TEAE	72 (70.6)
Musculoskeletal and connective tissue disorders	62 (60.8)
Injury, poisoning and procedural complications	21 (20.6)
Infections and infestations	11 (10.8)
Nervous system disorders	5 (4.9)
Vascular disorders	5 (4.9)
General disorders and administration site conditions	4 (3.9)
Psychiatric disorders	4 (3.9)
Blood and lymphatic system disorders	3 (2.9)
Gastrointestinal disorders	3 (2.9)
Metabolism and nutrition disorders	3 (2.9)
Cardiac disorders	2 (2.0)
Respiratory, thoracic and mediastinal disorders	2 (2.0)
Ear and labyrinth disorders	1 (1.0)
Immune system disorders	1 (1.0)
Investigations	1 (1.0)
Renal and urinary disorders	1 (1.0)
Reproductive system and breast disorders	1 (1.0)
Skin and subcutaneous tissue disorders	1 (1.0)

Note: All data included in this in-text table are based on number of patients. SOCs are sorted in descending frequency in the total population. Absolute numbers of events and preferred terms within SOCs can be found in the source table.

Source: Section 15, Post-text table 15.3.1.2.1

The most frequent TEAEs at the PT level is summarised below (Table 49).

Table 49. TEAEs occurring in at least 2 patients (≥2.0%) at the PT level

Preferred term	All patients (N=102) n (%)
Arthralgia	40 (39.2)
Joint effusion	18 (17.6)
Joint swelling	18 (17.6)
Joint crepitation	7 (6.9)
Meniscus injury	7 (6.9)
Chondropathy	5 (4.9)
Procedural pain	5 (4.0)
Back pain	3 (2.9)
Influenza	3 (2.9)
Joint dislocation	3 (2.9)
Joint stiffness	3 (2.9)
Ligament rupture	3 (2.9)
Osteochondrosis	3 (2.9)
Viral upper respiratory tract infection	3 (2.9)
Bone marrow oedema	2 (2.0)
Corona virus infection	2 (2.0)
Deep vein thrombosis	2 (2.0)
Depression	2 (2.0)
Implant site pain	2 (2.0)
Insomnia	2 (2.0)
Intervertebral disc protrusion	2 (2.0)
Joint adhesion	2 (2.0)
Joint lock	2 (2.0)
Muscle atrophy	2 (2.0)
Pain in extremity	2 (2.0)
Radius fracture	2 (2.0)
Synovitis	2 (2.0)
Tibia fracture	2 (2.0)
Urinary tract infection	2 (2.0)

Note: All data included in this in-text table are based on number of patients. Absolute numbers of events can be found in the source table.
Source: Section 15, Post-text table 15.3.1.2.1

2.6.8.3. Serious adverse events, deaths, and other significant events

Serious adverse events

The TESAEs that occurred in the study through Month 24 are summarised below (Table 50).

Table 50. Incidence of all serious TEAEs (SAF), (Phase III clinical trial, AAG-G-H-1624)

SOC Preferred term	All patients (N=102) n (%)
Any TESAE	**14 (13.7)**
Musculoskeletal and connective tissue disorders	**5 (4.9)**
Arthralgia	1 (1.0)
Chondropathy	1 (1.0)
Intervertebral disc protrusion	1 (1.0)
Joint adhesion	1 (1.0)
Joint instability	1 (1.0)
Knee deformity	1 (1.0)
Lateral patellar compression syndrome	1 (1.0)
Injury, poisoning and procedural complications	**3 (2.9)**
Ligament rupture	1 (1.0)
Meniscus injury	1 (1.0)
Multiple injuries	1 (1.0)
Pancreatic contusion	1 (1.0)
Splenic rupture	1 (1.0)
Transplant failure	1 (1.0)
Cardiac disorders	**2 (2.0)**
Acute myocardial infarction	1 (1.0)
Myocardial infarction	1 (1.0)
Vascular disorders	**2 (2.0)**
Deep vein thrombosis	1 (1.0)
Shock haemorrhagic	1 (1.0)
Gastrointestinal disorders	**1 (1.0)**
Haematemesis	1 (1.0)
Infections and infestations	**1 (1.0)**
Rectal abscess	1 (1.0)
Nervous system disorders	**1 (1.0)**
Multiple sclerosis	1 (1.0)
Reproductive system and breast disorders	**1 (1.0)**
Acquired hydrocele	1 (1.0)
Respiratory, thoracic and mediastinal disorders	**1 (1.0)**
Vocal cord cyst	1 (1.0)

Note: All data included in this in-text table are based on number of patients. Absolute numbers of events can be found in the source table.
Source: AAG-G-H-1624, CSR Table 12-20

Deaths

Until now, no deaths, life-threatening conditions or persistent physical disabilities related (or unrelated) to ACI with NOVOCART® Inject (plus) were reported from the phase III trial (AAG- G-H-1624) or the non-interventional studies sponsored by the applicant (RENOVO [AAG-O-H- 1521], HIPACTION [AAG-O-H-1304]) or from publications on NOVOCART® Inject (Schlumberger et al., 2019; Schlumberger et al., 2020; Blanke et al., 2021; Thier et al., 2017; Their et al., 2018).

Adverse events of special interest

The following were classified as adverse events of special interest:

- Treatment related AEs
- Treatment related SAEs
- AEs leading to subsequent surgical interventions
- AEs leading to treatment failure

A summary of treatment-related AEs is shown in Table 52. TEAEs by severity is summarised below (Table 51). Treatment-related TESAEs were reported in 2 patients (2.0%). These included transplant failure and lateral patellar compression syndrome.

Table 51. Incidence of all related TEAEs by severity (SAF), (Phase III clinical trial, AAG-G-H-1624)

	All patients (N=102)	
	Severity:	
SOC Preferred term	**Moderate** **n (%)**	**Mild** **n (%)**
Any event within column	**8 (7.8)**	**36 (35.3)**
Musculoskeletal and connective tissue disorders	**6 (5.9)**	**35 (34.3)**
Arthralgia	1 (1.0)	18 (17.6)
Joint effusion	1 (1.0)	17 (16.7)
Joint swelling	4 (3.9)	8 (7.8)
Joint crepitation	-	5 (4.9)
Muscle atrophy	-	2 (2.0)
Joint lock	-	1 (1.0)
Joint stiffness	-	1 (1.0)
Lateral patellar compression syndrome	1 (1.0)	-
Muscular weakness	1 (1.0)	-
Injury, poisoning and procedural complications	**2 (2.0)**	**1 (1.0)**
Post procedural haematoma	-	1 (1.0)
Procedural hypotension	1 (1.0)	-
Transplant failure	1 (1.0)	-
Blood and lymphatic system disorders	**-**	**1 (1.0)**
Bone marrow oedema	-	1 (1.0)
Skin and subcutaneous tissue disorders	**-**	**1 (1.0)**
Scar pain	-	1 (1.0)

Note: All data included in this in-text table are based on number of patients. Absolute numbers of events can be found in the source table.
Source: AAG-G-H-1624, CSR, Table 12-19

Subsequent surgical interventions (SSIs) on the target knee were performed in 12 study patients overall (12.0%; see Table 52).

Table 52. Subsequent surgical interventions on the target knee (ITT), (Phase III clinical trial, AAG-G-H-1624)

SOC Preferred term	Planned N=100 n (%)	Unplanned N=100 n (%)	Total N=100 n (%)
Patients with any subsequent surgery on the target knee	**5 (5.0)**	**7 (7.0)**	**12 (12.0)**
Surgical and medical procedures	**5 (5.0)**	**7 (7.0)**	**12 (12.0)**
Removal of internal fixation	5 (5.0)	0	5 (5.0)
Meniscus removal	0	3 (3.0)	3 (3.0)
Chondroplasty [a]	0	2 (2.0)	2 (2.0)
Arthrolysis	0	1 (1.0)	1 (1.0)
Joint dislocation reduction	0	1 (1.0)	1 (1.0)
Meniscus operation	0	1 (1.0)	1 (1.0)
Osteosynthesis	0	1 (1.0)	1 (1.0)
Osteotomy	0	1 (1.0)	1 (1.0)

Note: Only surgeries occurring after treatment Day 43 are included.
a: Both patients underwent MFx; in one case the event was considered a treatment failure, because the MFx was due to transplant failure with complete graft delamination.
Source: AAG-G-H-1624, CSR Table 12-8

One case of treatment failure was reported through Month 24 (formal incidence: 1.0%).

2.6.8.4. Laboratory findings

Safety laboratory data were not systematically monitored in the phase 3 study as no effect on blood or urine parameters is expected due to the nature of the investigational product (local administration). However, in patients where laboratory parameters have been assessed, clinically relevant laboratory measurements were to be documented as AEs. The review of TEAEs showed that TEAEs directly associated with abnormal laboratory values (mainly to be found within the SOC "investigations", "blood and lymphatic system disorders", and "metabolism and nutrition disorders") were generally infrequent (each PT experienced by one patient only), and that none of these few TEAEs were assessed as related to treatment with NOVOCART© Inject/transplantation.

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable.

2.6.8.6. Safety in special populations

Paediatric patients

A total of two paediatric patients were treated with the NOVOCART® Inject plus in the Phase III clinical trial, both experiencing non-serious adverse events (joint effusion and arthralgia in both patients).

Pregnancy and lactation

No safety data related to NOVOCART® Inject (plus) treatment during pregnancy or breastfeeding period are available. According to the applicant, pregnancy and lactation are contraindications for NOVOCART® Inject as by the currently approved SmPC as in general surgery is not recommended in pregnant or lactating women.

Elderly

No specific data in elderly were presented. Predefined subgroup analyses according to age were performed in the safety analysis set: subgroup A (patients ≤ 25 years) compared to subgroup B (patients > 25 years). Generally, incidence differences between the 2 age groups should be interpreted cautiously because of the small sample size in subgroup A (13 patients).

Age >65 years was an exclusion criterion in the NOVOCART Inject/Jelrix phase III study. The oldest patient enrolled in the NOVOCART Inject/Jelrix phase III trial was 62 years old. Table 58 below lists the safety information for the following age groups: all patients (<65 years), patients <18 years, patients 18 - ≤25 years and patients >25 years.

Table 53. Percentage of patients in the safety population of the NOVOCART® Inject/Jelrix phase III trial (102 patients) with adverse events up to the 60 months timepoint (irrespective of relationship to treatment)

MedDRA Terms	All Patients Age <65 number (percentage)	Age <18 number (percentage)		Age 18 - ≤25 number (percentage)	Age >25 number (percentage)
Total AEs	80 (78.4)	2 (100)		7 (63.6)	71 (79.8)
Serious AEs – Total	18 (17.6)	0		2 (18.2)	16 (18.0)
- Fatal	0	0		0	0
- Hospitalization/prolong existing hospitalization	16 (15.7)	0		2 (18.2)	14 (15.7)
- Life-threatening	1 (1.0) [a]	0		0	1 (1.1)
- Disability/incapacity	0	0		0	0
- Other (medically significant)	1 (1.0)	0		0	1 (1.1)
AE leading to drop-out	0	0		0	0
Psychiatric disorders	6 (5.9)	0		1 (9.0)	5 (5.6)
Nervous system disorders	6 (5.9)	0		0	6 (6.7)
Accidents and injuries	24 (23.5)[b]	0		4 (36.4)	20 (22.5)
Cardiac disorders	3 (2.9)	0		0	3 (3.4)
Vascular disorders	6 (5.9)	0		0	6 (6.7)
Cerebrovascular disorders	0	0		0	0
Infections and infestations	30 (29.4) [c]	1 (50)		3 (27.3)	26 (29.2)
Anticholinergic syndrome	0	0		0	0
Quality of life decreased [d]	3 (3.0)	0		0	3 (3.4)
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	5 (4.9) [e]	0		0	5 (5.6)
<other AE appearing more frequently in older patients>	0	0		0	0

[a] Unrelated to treatment (myocardial infarction)
[b] All unrelated to treatment. Terms included in the search: accident, injuries, contusion, fracture, rupture
[c] All unrelated to treatment and not on the target knee. 20 out of the 30 patients had corona virus infection.
[d] Patients with decrease in EQ-5D-5L index and EQ-5D-5L VAS
[e] Fractures in 3 patients (unrelated to treatment), fall in 1 patient (fall by bike, unrelated), procedural hypotension in 1 patient (related to surgery)

Descriptively, numerically higher incidences for patients > 25 years of age vs. patients ≤ 25 years of age were observed for all TEAEs (71.9% vs. 61.5%).

2.6.8.7. Immunological events

Not applicable since the product is intended for use in the knee only.

2.6.8.8. Safety related to drug-drug interactions and other interactions

No interaction studies have been performed. There is no anticipated pharmacological interaction with other drugs.

2.6.8.9. Discontinuation due to adverse events

Not applicable since Jelrix is intended for a single treatment.

2.6.8.10. Post marketing experience

Until 31-05-2022 (data lock point of PSUR No. 6), more than 6,000 patients have been treated with NOVOCART® Inject.

Table 54. Cumulative patient exposure# from marketing experience by gender and age group in the marketing period 17-12-2008 to 31-05-2022

Gender	Age range								
	< 18			18-50			> 50		
	Knee	Hip	Talus	Knee	Hip	Talus	Knee	Hip	Talus
Male	113	2	0	3,350	178	0	264	4	0
Female	113	1	1	1,864	30	1	215	2	0
Total	**226**	**3**	**1**	**5,214**	**208**	**1**	**479**	**6**	**0**

For 22 patients (21x knee joint, 1x hip joint), who were treated between 17-12-2008 and 31-05-2022, not all of the relevant information was provided by the hospital/ medical centre.
Includes all post marketing sources including the non-interventional studies RENOVO (AAG-O-H-1521) and HIP-ACTION (AAG-O-H-1304).
Source: PSUR (No. 6, DLP 31-05-2022)

A summary tabulation of (S)ARs related to NOVOCART® Inject treatment in the knee that have been reported from different post-marketing sources from 2008 up to the DLP of PSUR No. 6 is presented below (Table 55).

Table 55. Cumulative* summary tabulation of (S)ARs related to the NOVOCART® Inject transplant itself and/ or the surgical procedure of transplantation, knee joint

pSOC	PT	No of related SARs cumulatively*	No of related non-serious ARs cumulatively*
Bone and lymphatic system disorders	Bone marrow oedema	0	4[2]
Cardiac disorders	Bradycardia[3]	0	1[2]
General disorders and administration site conditions	Impaired healing	1[2]	0
Infections and infestations	Arthritis bacterial	1[1]	0
	Postoperative wound infection	0	1[2]
Injury, poisoning and procedural complications	Deep vein thrombosis postoperative	1[2]	0
	Graft complication (i.e. graft hypertrophy)	1[1]	0
	Graft delamination	1[1]	0
	Meniscus injury	1[2]	0
	Transplant failure	2[1] + 5[2]	0
Investigations	C-reactive protein increased	0	1[1]
Musculoskeletal and connective tissue disorders	Arthralgia	1[2]	1[1] + 3[2]
	Arthrofibrosis	3[2]	1[1]
	Bone cyst	0	1[1]
	Chondropathy (i.e. ulceration of cartilage)	0	1[2]
	Joint adhesion	1[1]+ 1[2]	0
	Joint effusion	1[1]+ 1[2]	0
	Joint lock	1[2]	0
	Joint noise (i.e. joint crepitation)	0	1[2]
	Joint swelling	0	1[2]
	Joint range of motion decreased	0	1[2]
	Loose body in joint	1[1]	0
	Synovitis	1[1]	0
Nervous system disorders	Complex regional pain syndrome	2[2]	0
Total		**26**	**17**

* from 2008 until the DLP of PSUR No. 6 (31-05-2022). Individual patients may have experienced more than one (S)AR. 1 = (S)ARs from clinical use outside the non-interventional studies; 2 = (S)ARs from the non-interventional study RENOVO; 3 = non-serious AR related to anaesthesia
Source: PSUR (No. 6, 31.05.2022)

2.6.9. Discussion on clinical safety

As stated in the EMA "Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee", autologous chondrocytes-containing products have been used for more than 15 years in clinical practice and the experience for this class of products is relevant and has to be considered. For products for which clinical data has been gathered before entry into force of Reg No. (EC) 1394/2007, the acceptability of safety data will depend on the quality of the data and their collection over the years.

The clinical safety assessment is based on

1. one single pivotal uncontrolled phase 3 study NOVOCART® Inject plus,

2. a propensity score matched pair analysis comparing patients treated with NOVOCART® Inject plus in the pivotal phase 3 study with control data from a previous study (AAG-G-H-1202) which compared Novocart 3D with microfracture, and
3. supportive data from non-interventional studies using an NOVOCART® Inject) and available data from the literature.

An important limitation for the assessment of safety are the limited comparative data leading to uncertainties and bias as previously explained in the EMA scientfici advice. The collection of safety data differed between trials 1621 and trial 1202, therefore the applicant only made a comparison for re-surgery of the target knee (see Efficacy section). The applicant was asked to present a comparative analysis of AEs and SAEs after 4 weeks in the propensity score matched groups of studies 1621 (Jelrix) and 1202 (MFx). Descriptively, AEs were overall more frequent in the MFx group (78%) as compared to the Jelrix-treated group (64%). The most common AEs (arthralgia, joint swelling, joint effusion) occurred less in the Jelrix group as compared to MFx, except for joint crepitation (8.3% *versus* 4.2%). Also, procedural pain (5.6% *versus* 0) and pain in extremity (2.8% *versus* 0) were more frequent in the Jelrix group. However, as these were not SAEs and the frequency was low, it is not considered that these ADRs impact negatively on B/R. It was also clarified that the treatment-emergent AEs of chondropathy (5.6% versus 2.8%) and osteochondrosis (4.2% versus 0) did not concern the filled lesion. Similarly, in the Jelrix-treated group, the SAEs of chondropathy, joint adhesion, arthralgia, were not of the target lesion casu quo the target knee. Also,all the SAEs were considered to be unrelated by the investigator.

The respective frequencies of AEs for specific preferred terms were comparable at 24 and 60 months. At Month 60, there were cumulatively a total 18 patients with a total of 31 SAEs reported, as compared to 14 patients with 22 SAEs reported at Month 24. Almost none of the reported events were considered (possibly) related to either the biopsy procedure, surgery, or the investigational product. Two events with a possible causal relationship (lateral patellar compression syndrome and transplant failure) were previously reported in the interim CSR. Overall, no new safety signals were identified from this safety data update.

Exposure

The overall safety database includes 100 patients exposed to NOVOCART Inject plus in the pivotal phase 3 trial AAG-G-H-1624, 266 patients exposed to NOVOCART Inject in non-interventional trials (knee: 245, hip:21), 138 cases from published literature (knee: 106, hip: 32), and 6160 cases post-marketing (knee: 5940, hip: 218, talus: 2).

The safety database and length of follow-up is considered sufficient to make a proper safety assessment.

Adverse events were reported in 72/102 patients (70.6%), and serious adverse events were reported in 14/102 patients (13.7%). Most of the adverse events were related to implantation (37/102 patients, 36.3%) and only 4/102 (3.9%) were considered directly related to NOVOCART Inject plus (please find details below).

Most AEs were reported within the SOCs "musculoskeletal and connective tissue disorders", "injury, poisoning and procedural complications" and "infections and infestations". The most commonly reported AEs were arthralgia, joint effusion and joint swelling.

As previously discussed, the following were not recorded as adverse events in the study:

- Postoperative wound pain within 2 weeks post tissue harvest or transplantation
- Joint swelling/local oedema within 4 weeks post tissue harvest or transplantation

- Joint effusion within 4 weeks post tissue harvest or transplantation (exception: if puncture is necessary, also joint effusion within 4 weeks post tissue harvest or transplantation has to be documented as AE and the puncture has to be documented as concomitant non-drug knee therapy).

Nonetheless, arthralgia, joint effusion and joint swelling were the most commonly reported adverse events. The applicant was asked to clarify whether these events include those reported at later time points. It was explained that knee pain, swelling and effusion were exempted from AE documentation within the first 4 post-treatment weeks of the study, unless they were above the level expected in the context of treatment surgery regarding their severity and/or duration. This is a critical issue that hampers the characterisation of such AEs during the first weeks after the procedure. It may explain also why the frequencies reported for arthralgia, joint effusion and joint swelling in the Phase III clinical trial AAG-G-H-1624 appear to be relatively low.

Treatment-related AEs were observed in 4 patients (3.9%): "arthralgia" (2 patients), "joint crepitation" (2 patients), "joint swelling" (1 patient), "transplant failure" (1 patient), and "bone marrow oedema" (1 patient). Treatment-related serious adverse events were reported in 2 patients (2.0%). These included transplant failure and lateral patellar compression syndrome. Subsequent surgical interventions (SSIs) on the target knee were performed in 12 study patients overall (12.0%). There was one case of treatment failure reported through Month 24 (1.0%).

According to the applicant, no deaths, life-threatening conditions or persistent physical disabilities related (or unrelated) to ACI with NOVOCART® Inject (plus) were reported from the phase III trial (AAG- G-H-1624) or the non-interventional studies sponsored by the applicant (RENOVO [AAG-O-H- 1521], HIPACTION [AAG-O-H-1304]) or from publications on NOVOCART® Inject.

Overall, it appears that adverse events are restricted to the target joint, are mild to moderate and transient. Arthralgia, joint effusion and joint swelling but also joint crepitation were most common, and can be expected with this kind of surgery. SAEs were infrequent, but there were cases of graft failure and treatment failure, and cases of arthrofibrosis and complex regional pain syndrome, that should be considered for section 4.8 of the SmPC. Graft hypertrophy and bone marrow oedema are included in the SmPC as ADRs, which is acceptable. The pattern of AEs that was found in pivotal study 1624 and in supportive study 1521 was confirmed by the post-marketing data. In the post-marketing data, there were few instances of graft loss/treatment failure, that were partly reappearances (double counts) of cases in the two clinical studies.

Graft loss and treatment failure were relatively uncommon. In the pivotal trial, there were three patients with a graft loss (no defect filling) after 24 months but only one was re-operated upon due to complaints; the applicant considers only one case as treatment failure. However, in the retrospective study, treatment failure occurred in 5 patients and only in 1 patient this seems not to be associated with graft failure. In conclusion, transplant failure, with a frequency 'common' as is proposed as ADR in section 4.8 is accepted Graft hypertrophy on MRI occurred in 18% of patients after 24 months, but no surgical re-interventions due to graft hypertrophy had to be performed thus far.

'Kissing lesions' (i.e., lesions on corresponding articular surfaces) were a contra-indication in the retrospective study, but not in the pivotal study. Kissing lesions are often considered a contra-indication, due to a risk of graft failure, and to aid to a favourable environment for healing and avoid graft loss. The applicant argued that patients with BLs benefit from cartilage repair, although inferior clinical improvement may be expected compared to patients with unipolar lesions. However, the observed differences do not justify excluding patients with BLs from cartilage repair.

It is agreed there is some evidence from mainly 2 studies (Bumberger 2023, Vasiliadis 2011) that patients with bipolar lesions can undergo Autologous Chondrocyte Implantation successfully, although with an increased failure risk as compared to patients without bipolar lesions. In the literature, there is no clear evidence for performing alternative procedures for these opposite lesions, although ACI does not appear to be the worst option. To increase the probability of a good clinical outcome, it could be an option to postpone the filling of the opposing defect. In that case however, the risk for treatment failure must be individually weighed against the risks and inconvenience of waiting time and performing a second surgery. Jelrix is for single use and unused medicinal product must not be stored for later applications (SmPC section 6.6), which means that postponing the second filling needs the use of the cryopreserved cells from the parallel process (see Quality Section of the Overview) or needs new harvesting that leads to 2 consecutive surgeries.

Cases of arthrofibrosis did not occur in the pivotal trial but occurred in the supportive retrospective study. These are serious events and influence B/R, because they are debilitating and require subsequent surgical interventions. Apparently, in the scientific literature, arthrofibrosis is discussed as one of the most frequently occurring postoperative clinical complications following (matrix-associated) ACT, independent of the (1st, 2nd, 3rd) generation and the used product (Harris et al., 2011, Ebert et al., 2017). Accordingly, arthrofibrosis is/was included as ADR in the SmPC's of all other chondrocyte transplant products (e.g., Spherox). The post-marketing data did not contribute new cases, other than those already described for study 1521. Therefore, arthrofibrosis was considered as ADR (of surgery) and included in section 4.8 of the SmPC.

Cases of complex regional pain syndrome did not occur in the pivotal trial but occurred in the supportive retrospective study. Accordingly, complex regional pain syndrome was included as ADR (of surgery) in section 4.8 of the SmPC.

Safety in special populations

Only limited data on safety in paediatric patients are available.

In the pivotal clinical trial (1624) 2 paediatric patients were included and in the retrospective study (1521) 11 paediatric patients were included. No specific safety issues are apparent in adolescents (with closed epiphyseal growth plates) or young adults<25 as compared to adults.

In trial 1624 both paediatric patients each experienced 2 non-serious and mild or moderate TEAEs (joint effusion, arthralgia and joint swelling), of which only mild joint effusion (1 patient) was considered related to implantation surgery. The other events were considered unrelated to the study treatment. No transplant failures, re-surgeries or SAEs occurred in these two patients. In study 1521, no related adverse reactions (including transplant failures or reoperations) were reported in the 11 paediatric patients. Subgroup analyses were performed in both studies (Summary of Clinical Safety, Tables 31 and 32 on pages 68-69, pages 69 and 70). Accordingly, the safety profile in the respective age groups (≤25 years versus >25 years) appears similar with only slight numerical differences and no consistent difference/pattern between age groups could be identified.

The number of elderly in studies 1624 and 1521 is limited; the eldest patient in pivotal study 1624 was 62 years of age and in retrospective study 1521 69 years of age. This is in line with the indication, as traumatic cartilage defects are much more common in adolescents and young adults, as compared to elderly, who also may have advanced osteoarthritis (included as contraindication) as unfavourable comorbidity or consequence.

Subgroup analyses for BMI strata (< 30, ≥30) were provided upon request. The cut-off off for the strata was acceptable given the BMI range of 20.2 – 34.4. No clear differences in outcome on KOOS at months 24 and 60 were seen.

Safety related to drug-drug interactions and other interactions

No interaction studies have been performed; the applicant considered that there is no anticipated pharmacological interaction with other drugs (see Pharmacokinetics discussion). According to the SmPC, the implant must not be brought in direct contact with disinfecting or cytotoxic solutions or compounds, since this may harm or destroy the human articular chondrocytes contained in the product. Thus, intra-articular application of analgesics is also not recommended. Therefore, it is acceptable that these interactions are *not* considered as important potential risk in the RMP (it is included in the RMP for Spherox).

Pregnancy and lactation

According to the SmPC, no adequate data exist regarding the application of autologous chondrocytes in pregnant women and in breast-feeding women. Given the local nature of the medicinal product, adverse reactions on pregnancy or on the nursing infant are not anticipated. General recommendations have been provided, which are considered acceptable.

Supportive safety data

In the retrospective non-interventional knee study RENOVO (AAG-O-H-1521), **AEs were reported in 35 (14.3%) out of the 245 study patients. Among the reported events there were 4 cases of bone marrow oedema, one case of postoperative wound infection and one case of deep vein thrombosis.** The thrombosis occurred 9 days after the transplantion and required treatment with an anticoagulant during a hospital stay. The event was considered related to the surgical procedure. The RENOVO report included a second SAE of thrombosis, described as abdominal thrombosis, which occurred 7 months after the NOVOCART transplantion. This case was not considered related to either the surgery or to the implant.

According to the applicant, a total of 4 cases of (deep vein) thrombosis postoperative have been reported from post-marketing experience so far; 3 non-serious events after autologous cartilage biopsy of the knee, all based on spontaneous case reporting, and 1 serious case post-implantation (serious), reported from the above non-interventional study RENOVO. No case of embolism has been reported so far according to the applicant. Postoperative (deep vein) thrombosis is included in section 4.8 as an ADR related to the surgical procedure with frequency 'not known'. This is considered acceptable.

In the propensity score matched pair analysis for the pivotal Phase III trial, only surgical interventions after M-ACI implantation or microfracture were assessed in both studies and safety data from the propensity score matched pair analysis are limited to this outcome. Subsequent surgeries on the target knee were reported in 9/72 patients (12.5%) in the NOVOCART® Inject plus group and 3/72 patients (4.2%) in the MFx group. Although of some concern that subsequent surgical interventions were more frequent in the NOVOCART® Inject plus group, the number of patients is small and there is no specific pattern observed with regards to surgical procedures. Since this was an inter-study comparison, no firm safety conclusions can be made from this analysis although the presented data do not provide supportive safety data in favour of NOVOCART® Inject plus.

In the prospective non-interventional hip study HIPACTION, AEs were reported in 4/21 patients (19%). Serious AEs were reported in 3 patients (arthralgia, bacterial arthritis and hip surgery). Of these, 2 SARs (arthritis bacterial and arthralgia) were assessed as related to the surgical procedure. None of the reported

events was assessed as related to the NOVOCART® Inject transplant itself. The third SAE ("hip surgery") concerned the contralateral (untreated) hip joint and is considered related to the underlying disease. Bacterial arthritis has been included as an ADR related to the surgical procedure with frequency 'not known', which is considered acceptable.

The applicant has summarised available data from the literature, including 106 patients treated with NOVOCART® Inject in the knee. Most of the reported events are expected in the context of surgery, and no specific safety concerns related to the ACI treatment were observed.

Post-marketing data

NOVOCART Inject has been on the market in Germany since 2008. The applicant performs continuous routine pharmacovigilance activities in accordance with current legal requirements. Also, the applicant has submitted PSURs to the concerned authority (PEI) in Germany.

From 2008 until 31-05-2022 (data lock point of PSUR No. 6), more than 6,000 patients have been treated with NOVOCART® Inject. The applicant presented a cumulative summary tabulation of adverse reactions related to the NOVOCART® Inject transplant itself and/ or the surgical procedure of transplantation (limited to knee injections). A total of 26 serious adverse reactions and 17 non-serious adverse reactions were reported. These include 1 case of septic arthritis, 2 cases of complex regional pain syndrome, 7 cases of transplant failure, and 4 cases of arthrofibrosis. Overall, most of these are expected in patients undergoing arthroscopy and no specific safety signals are observed.

Feed-back from healthcare professionals (**EFORT, the European Federation of National Associations of Orthopaedics and Traumatology)**

The treatment of symptomatic, localised, full-thickness cartilage defects of the knee joint, can be considered controversial. All techniques currently in use sustain limitations and issues which prevent the orthopaedic community to recommend any of those as a universal therapy. In this context, the expectations from future treatments include: a predictable attachment of the medicament to the subchondral bone (limiting the risk of delamination and migration, even if implanted with arthroscopy); a predictable cartilage regeneration, with a dose depending on the size and location of the solution, also healing the gaps with the normal cartilage; a known timing and evolution of the medicament to produce the cartilage regeneration (to allow for motion, weight-bearing and function as soon as possible).

In the particular case of autologous, in vitro expanded chondrocytes, associated risks may include:

(1) chondral tissue hypertrophy;

(2) potential dedifferentiation of the cells;

(3) complications with seeding and maintaining the cells into the defect;

(4) in case a combined therapy (cells plus a biomaterial, whether as a membrane or matrix), risks associated to the biomaterial cleavage, integration with subchondral bone, integration with surrounding cartilage, are to be taken into consideration.

While it is agreed that new therapies are of significant interest to the orthopaedic community, given the unmet needs due to the complexity of the lesion and repair, it is emphasised that clinical trials proving feasibility, safety and efficacy are of course required before a final recommendation can be placed.

In the **prospective non-interventional hip study HIPACTION,** cases of septic/bacterial arthritis occurred. This has been included as an adverse reaction in section 4.8.

2.6.10. Conclusions on clinical safety

The single-arm study design and its limited safety data collection impairs the possibility to make a thorough safety assessment of Jelrix.

Some uncertainties with potential impact on safety are raised in quality and pre-clinical parts of this report. These include for example harvest of cells other than chondrocytes. potential use of a different variant of serum (A) in the final product than what was studied in the clinical trials (AB), and insufficient characterisation of the hydrogel components in preclinical studies.

Overall, the safety profile observed in the pivotal clinical study is overall expected for a chondrocyte-containing product and its associated surgical proceduresi, and the risks are considered possible to handle through adequate warnings/information in the SmPC and educational materials. However, there are some important deficiencies that needs to be considered in the safety data presented for the current product. These include the lack of a comparator in the pivotal study, as well as the exclusion of certain adverse events from reporting. The most important deficiency, however, is that no robust efficacy of the product has been shown. Since implantation of autologous chondrocytes requires an additional surgical intervention under regional or general anaesthesia that per se are associated with certain risks, the absence of a demonstrated efficacy cannot outweigh the risks with this product.

2.7. Risk Management Plan

As the benefit/risk balance has been found to be negative, RMP cannot be agreed at this stage.

The CHMP endorses the CAT conclusion on the RMP as described above.

2.8. Product information

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Cartilage defects and degenerative joint disorders are very common. Damage to articular cartilage tissue can be caused by trauma, degeneration or the separation of an articular cartilage subchondral bone segment from the remaining articular surface.

Symptoms include pain, swelling, effusions, stiffness and joint locking, depending on the amount of load carried by the injured area. This may cause significant functional impairment, often limiting one’s ability to work, play sports, and perform activities of daily living. Furthermore, progressing focal cartilage lesions of the knee are associated with the development of osteoarthritis (OA) over time. The risk for the development of

OA increases with the degree and chronicity of the initial cartilage lesion. Adequate repair of cartilage defects may prevent further cartilage loss, early onset of knee OA and to delay the need for total knee replacement.

The proposed indication is: Repair of symptomatic, localised, full-thickness cartilage defects of the knee joint (International Cartilage Regeneration & Joint Preservation Society (ICRS) grade III or IV) with defect sizes up to 12 cm^2 in adults and adolescents with closed epiphyseal growth plate in the affected joint.

The treatment with Jelrix requires 2 surgeries. During an initial arthroscopy (autologous cartilage biopsy), osteochondral tissue samples are taken from a non-weight bearing area of the knee which are sent for production of the Jelrix transplant. The product is administered during a second surgery of the knee (an arthroscopy or mini-arthrotomy).

3.1.2. Available therapies and unmet medical need

Treatment options for cartilage defects depend on the symptoms, defect size and amount of defect sites as well as patient's age and activity level. Conservative treatment includes analgesics and physical therapy. As a next step, arthroscopic debridement and lavage are often performed. If symptoms (e.g. pain) persist and become more problematic, surgery is typically recommended. The currently available surgical options for the treatment of symptomatic localised, full-thickness cartilage lesions can be divided into reparative (e.g., bone marrow stimulation [BMS] techniques) and restorative procedures (e.g., osteochondral transfer and chondrocyte implantation). Reparative cartilage procedures, such as microfracture, stimulate fibrocartilaginous repair, whereas restorative procedures, aim to replace the defective articular surface with cartilage of hyaline properties. Bone marrow stimulation techniques are only recommended for the treatment of smaller
defects (<4 cm^2).

Different autologous chondrocyte implantation (ACI) methods have been developed since 1987. A few ACI products have been approved via the centralised procedure so far. Some had been approved, but were withdrawn. Currently, the only M-ACI product approved via the centralised procedure in the EU is Spherox® which received marketing authorisation in July 2017 (https://www.ema.europa.eu/en/medicines/human/EPAR/spherox).

"NOVOCART® Inject" has been used in Germany since 2008. At that time, no marketing authorisation was required for this type of product that could be provided through the hospital exemption. However, it meets the definition of tissue engineered products as laid down in article 2(1b) of Regulation (EC) No 1394/2007 and thus meets the criteria of an Advanced Therapy Medicinal Product (ATMP) as laid down in article 2(1a) of said regulation. Moreover, classification as a tissue-engineered product according to article 2(1b) of Regulation 1394/2007 has been confirmed by the European Medicines Agency (EMA) (EMA/CAT/635656/2015). Therefore, the applicant applies for marketing authorisation via the centralized procedure on the legal basis of Directive 2001/83/EC Article 8(3) and according to Article 3 (1) of Regulation (EC) No. 726/2004.

Jelrix has been developed from the products NOVOCART® 3D, NOVOCART® 3D plus, NOVOCART® Inject and NOVOCART® Inject plus.

NOVOCART® 3D plus was subject to MAA under the name of Artobend in 2020 (EMEA/H/C/004598) but the product failed to meet its primary endpoint which was superiority or non-inferiority vs microfracture (pivotal study AAG-G-H-1202) and the application was later withdrawn.

NOVOCART® Inject was developed to facilitate product administration. To achieve this goal, the scaffold in NOVOCART® 3D (plus) was replaced by a cross-linked matrix that solidifies in situ during product administration to form a biodegradable hydrogel. The hydrogel is proposed to offer ideal conditions for cartilage regeneration. The initial low viscosity supports a homogeneous cell distribution as well as good ingrowth of the regenerated tissue into the defect bed. Due to the injectable nature of the hydrogel it can be administered via mini-arthrotomy or arthroscopically without jeopardizing cell viability.

NOVOCART® Inject plus is the investigational medicinal product that was used in the pivotal study in the present MAA for Jelrix. It is noted that NOVOCART Inject is comparable but not identical to the applied product NOVOCART Inject plus; differences. These differences, however, are unlikely to affect efficacy (see Quality discussion).

For the commercial manufacturing of Jelrix, additional process changes have been introduced compared to NOVOCART® Inject plus..

3.1.3. Main clinical studies

The applicant has submitted a pivotal single-armed phase III trial with NOVOCART® Inject plus (AAG-G-H-1624) and a propensity score matched pair analysis comparing subjects in this trial with the control group receiving microfracture in the previous phase III trial (1202). Supportive studies are also presented. No dose-finding study was performed. The proposed posology was aligned with the posology of the marketed product in Germany.

The pivotal study AAG-G-H-1624 was a prospective, multicenter, single-arm Phase III clinical trial to evaluate the efficacy and safety of NOVOCART® Inject plus in the treatment of cartilage defects of the knee. The aetiology of cartilage damages included degenerative lesions as well as traumatic lesions. A total of 132 patients were initially enrolled in the study, tissue was arthroscopically harvested from 102 patients and transplant received by 100 patients i.e. actually treated with expanded autologous chondrocytes carried in a human albumin/hyaluronan solution and a cross-linker solution. Standard of care concomitant medications and rehabilitation measures were applied. Concomitant surgical procedures included ligament repair or alignment of valgus- or varus-deformities, correction of patella malalignment, filling of subchondral bone defects. All patients were followed 24 months after implantation for the primary outcomes. The final CSR including 60-month data was submitted as a part of Day 120 LoQ responses of this procedure. .

The applicant also submitted supportive clinical data, including a propensity scored matched pair analysis comparing patients treated with Jelrix in the pivotal study with patients treated with microfracture in a previous study (i.e. historical controls).

Additional data comes from the RENOVO study (study AAG-OH-H-1521). This is a completed, retrospective, multi-centre, non-interventional study in 245 German adult and paediatric patients to evaluate safety and efficacy of NOVOCART Inject treatment of cartilage defects of the knee in a routine care setting. In this study, 11 adolescents < 18 years and a total of 36 patients 18 - 25 years of age, with a full-thickness cartilage defect ICRS Grade II-IV, with size 2.5 – 10 cm2, were included. Primary endpoint was the overall KOOS at follow-up which was of variable duration (median 19.1; range 1.5 – 57.0 months); no baseline KOOS was available.

3.2. Favourable effects

The primary endpoint was KOOS responder rate (improvement of at least 10 points in KOOS total) by month 24 and this rate was 93% (95% CI 86.1; 97.1). The main secondary endpoints included change in mean KOOS total from baseline to month 24 and change in IKDC subjective- and objective scores. LS mean for KOOS total from baseline to month 24 was 42.04 (SE 1.827). IKDC subjective LS mean change from baseline to month 24 was 41.63 (SE 1.878) by month 24. With regards to IKDC objective scores, 52% of the subjects were assessed as having normal scores at baseline, a number which increased to 85% by month 24. For KOOS and IKDC endpoints, numerical improvements were seen as from 3 months after transplantation.

The overall KOOS among the 100 patients was 40 ± 14 points at baseline and increased to 82 ± 16 points at Month 24 (LS mean change from baseline 42, 95%-CI: 38 - 45). The overall KOOS among the 97 patients available for the final study analyses at Month 60 was 85 ± 17 points (LS mean change 44 points, 95%-CI: 40 – 48). The MOCART score was assessed in a subgroup of 25 subjects. Numerical improvements in MOCART sum and several sub scores were seen (complete integration of graft tissue to border zone, intact surface of repair tissue, homogeneous structure of repair tissue, and normalisation of signal density of repair). The MOCART sum score did not substantially change between month 12, 24 and 60.

There were only 2 paediatric subjects in the pivotal study AAG-G-H-1624. The primary endpoint was met in both subjects. In the retrospective RENOVO study, overall KOOS composite scores at follow-up were numerically higher in patients < 18 years (n = 10) compared to those 18-25 years (n = 29), and those > 25 years of age (n = 153). For IKDC subjective scores comparable results were reported. Treatment failure did not occur in paediatric patients.

A propensity score matched pair analysis was performed using the subjects in the pivotal study (AAG-G-H-1624) and the control group undergoing microfracture in a previous study with NOVOCART Inject 3D plus (study AAG-G-H-1202). The primary efficacy endpoint of this analysis was the change in the overall KOOS from baseline to the 24 months assessment. The LS mean difference between the two groups in overall KOOS from baseline to month 24 was 10.02 (SE 3.26). According to the applicant non-inferiority and superiority for NOVOCART Inject plus vs. microfracture was thereby descriptively shown.

3.3. Uncertainties and limitations about favourable effects

The treatment effect and its clinical relevance

Major uncertainty concerns the treatment effect of Jelrix; this is due to the single pivotal study that had a single treatment arm that cannot isolate the effect of Jelrix on the clinical outcomes. As the treatment effect cannot be defined, neither can it be determined whether the effect is clinically relevant. Essential elements that will have impacted positively the outcome at 24 months after treatment, are the rehabilitation program and concomitant interventions during initial surgery. Subgroup analyses indicated substantially larger improvements in overall KOOS at 24 months in patients who underwent concomitant surgery (22/100) compared to those who did not. Considering "concomitant surgeries" the applicant did not mention specifically debridement/chondroplasty (contouring and removal of devitalised or detached cartilage fragments, joint lavage, irrigation). It is likely that the vast majority had these types of standard interventions concomitantly with the Jelrix related interventions in trial AAG-G-H-1624.

The added value of the propensity score matched pair analysis, to reduce uncertainty on the treatment effect of Jelrix by the introduction of an external control arm, is considered limited. Several patient and defect characteristics remained different between the groups after matching, including defect size, which hampers

unequivocal interpretation of data. Further concomitant treatment was more frequent in NOVOCART Inject plus compared to MFx, whichh in result, may have exaggerated the observed treatment difference between NOVOCART Inject plus and MFx.

Another uncertainty related to the efficacy is the absence of a dose-response relation, combined with unclarities in different manifestations of treatment failure (e.g., empty defect and other MOCART scores, KOOS deterioration, unplanned surgical re-interventions) and their mutual correlation. MRI data were only obtained in a small sample of patients, n = 25, which may doubt the robustness of the findings.

Adolescents: Only 2 adolescents with closed epiphysial growth plates were included in the pivotal study, while a minimum of 10 patients was initially required according to the PIP, although ultimately a waiver was provided (P/0315/2021). The supportive data presented by the applicant from 10 paediatric patients is considered of limited value to assess efficacy, mainly due to variable follow-up duration and the absence of baseline (KOOS) values. The extrapolation of data to adolescents and inclusion of this subgroup in the indication thus requires further justification. Safety data (from the pivotal and RENOVO trials) should be presented and discussed stratified for patients ≤ 18 years and > 18 years of age (instead of 25 years) and weighed against the efficacy findings in the subgroups.

Cartilage defect sizes < 4 cm2: Patients with defect sizes < 4 cm2 were only to a limited extent represented in the single pivotal study, and it remains uncertain whether data from defect sizes 4-12 cm2 studied in the pivotal trial can be extrapolated to defects < 4 cm2, in particular in terms of B/R. Several national and international clinical associations recommend (M-)ACI as standard procedures for treatment of moderately large to large cartilage defects of the knee only (> 2-4 cm2) (e.g. NICE 2017, Niemeyer et al., 2022). However,the registry analyses regarding defect sizes below 4 cm2 cannot be accepted as pivotal efficacy demonstration for Jelrix for the >2-4 cm2 defects, also in light of the previous failed study that was assessed in procedure EMEA/H/C/004598. The issue regarding efficacy and thus the B/R for small defects was not resolved.

Underlying aetiology of cartilage defects: Trauma, osteochondritis dissecans (OCD), and later also focal degenerative lesions were the included aetiologies of cartilage defects. Although forest plots did not unravel large differences at the overall change in KOOS at 24 months, traumatic lesions may benefit most from treatment, but the numbers were small, introducing uncertainty on the robustness of effects in the subgroups, especially for OCD. Furthermore, it remains uncertain whether data can be extrapolated to cartilage defects of other origin. The applicant considers the B/R of NOVOCART Inject plus to be positive for all **defect aetiologies** and proposes to keep them in the indication. However, the data (from studies 1624 and 1521 (RENOVO)) presented by the applicant stratified by aetiology subgroup, indicated smaller changes in efficacy endpoints (partly due to higher baseline values) compared to baseline, together with higher frequencies of AE's, in patients with degenerative lesions compared to the other aetiologies. Interestingly, 4 of the 5 transplant failures (RENOVO study) were observed in patients with degenerative defects. The smaller benefit of (M)ACI in degenerative disease compared to other aetiologies aligns with current scientific knowledge (degeneration > grade II is a contra-indication for treatment with NOVOCART Inject plus). Overall, the B/R seems less favourable for degenerative defects compared to defects from other origins.

2nd look arthroscopy and cylinder biopsy were not systematically performed in the clinical studies. A few biopsy result descriptions are available from literature and a single post-marketing case that received NOVOCART® Inject was described by the applicant. It is not possible to draw any firm conclusion on anticipated efficacy of Jelrix on a histological level from the few cases presented.

3.4. Unfavourable effects

In the pivotal clinical trial AAG-G-H-1624 (24-month data), adverse events were reported in 72/102 patients (70.6%), and serious adverse events were reported in 14/102 patients (13.7%). The incidence of related SAEs was 2.0% (one patient with transplant failure and another patient with lateral patellar compression syndrome as a consequence of surgery). No patients discontinued the study due to TEAEs. There were no deaths in the study. Most of the adverse events were related to implantation (37/102 patients, 36.3%) and only 4/102 (3.9%) were considered directly related to NOVOCART Inject plus.

Most AEs were reported within the SOCs "musculoskeletal and connective tissue disorders", "injury, poisoning and procedural complications" and "infections and infestations". The applicant stated in the scientific advice that adverse events (AEs) were to be collected throughout the study. However, symptoms expected in the context of tissue harvest/transplantation surgery (postoperative wound pain, joint swelling, joint effusion) were not documented as AEs within the first 4 post-treatment weeks of the study, unless they were above the level expected in the context of treatment surgery regarding their severity and/or duration. Nonetheless, arthralgia, joint effusion and joint swelling were the most commonly reported adverse events.

Treatment-related AEs (i.e. an AE that started at or after the autologous cartilage biopsy or during the 24 months period after transplantation), were observed in 4 patients (3.9%): "arthralgia" (2 patients), "joint crepitation" (2 patients), "joint swelling" (1 patient), "transplant failure" (1 patient), and "bone marrow oedema" (1 patient). Related TEAEs were usually mild or moderate.

Transplant failure, including unplanned surgical re-intervention of the target lesion and incomplete defect filling (on MRI), may not be seen as adverse drug reactions, but they are regarded as unfavourable effects in the context of Benefit/Risk. In the pivotal trial, there were three patients with **graft loss** (no defect filling) after 24 months, but only one was re-operated upon due to complaints; the applicant considers only one case as **treatment failure**. However, in the retrospective study, treatment failure occurred in 5 patients and only in 1 patient this seems not to be associated with graft failure. Accordingly, **unplanned surgical re-intervention** of the target knee due to transplant failure occurred in 1 patient in the pivotal trial and in 4 patients in the observational study.

Cases of **arthrofibrosis** and of **complex regional pain syndrome** did not occur in the pivotal trial, but did occur in the supportive retrospective study. According to the case descriptions, the three cases of arthrofibrosis and the two cases of complex regional pain syndrome were serious and related to the surgery.

Graft hypertrophy on MRI occurred in 18% of patients after 24 months, but no surgical re-interventions due to graft hypertrophy were performed. **Bone marrow oedema** occurred in single cases in the pivotal trial and the retrospective study, with no apparent clinical consequences.

No specific safety issues are apparent in **adolescents** (with closed epiphyseal growth plates) or young adults<25 as compared to adults.

The applicant provided the final report (final 5-year follow-up) for study AAG-G-H-1624 and a brief update of the safety data. Cumulatively, a total of 31 SAEs were reported in a total of 18 patients. Almost none of the reported events were considered (possibly) related to either the biopsy procedure, surgery, or the investigational product. Two events with a possible causal relationship (lateral patellar compression syndrome and transplant failure) had already been reported (24-month data). No new safety signals were identified.

In the retrospective non-interventional knee study RENOVO (AAG-O-H-1521), patients were treated with NOVOCART© Inject. AEs were reported in 35 (14.3%) out of the 245 study patients. Among the reported

events there were 4 cases of bone marrow oedema, one case of postoperative wound infection and one case of deep vein thrombosis.

In the propensity score matched pair analysis for the pivotal Phase III trial, only surgical interventions after M-ACI implantation or microfracture were assessed in both studies and safety data from the propensity score matched pair analysis are limited to this outcome. Subsequent surgeries on the target knee were reported in 9/72 patients (12.5%) in the NOVOCART® Inject plus group and 3/72 patients (4.2%) in the MFx group.

3.5. Uncertainties and limitations about unfavourable effects

The major uncertainties from a safety perspective pertains to the limited availability of comparative data. As such, additional safety data were asked for the non-randomized (propensity score-matched) comparison with MFx. Studies in patients with application to the hip joint, and t several small case series published in the public domain were submitted as supportive studies, However, they are not considered to have a significant contribution to the safety assessment in the current application. In the pivotal study, several signs and symptoms that were a priori considered as normal consequences of the surgical procedure were not standardly registered, by protocol.

In the pivotal clinical trial 1621, only two **paediatric** patients (2 males, 15 and 17 years old) with radiologically proven closed epiphyseal growth plates were enrolled and treated. In the retrospective study 1521, a total of 11 paediatric patients were enrolled and treated. According to the post-marketing data up to 2022 (PSUR No. 6), a total of 179 paediatric patients have been treated with NOVOCART Inject in the knee joint, including the patients enrolled in retrospective study 1521. The need for acquiring additional data in paediatric patients is included in the **RMP**.

The number of elderly is limited; the eldest patient in pivotal study 1624 was 62 years of age and in retrospective study 1521 69 years of age. This is covered in the SmPC.

Some uncertainties with potential impact on safety are raised in quality and pre-clinical parts of this report. These include for example harvest of cells other than chondrocytes, potential use of a different variant of serum (A) in the final product than what was studied in the clinical trials (AB), and insufficient characterisation of the hydrogel components in preclinical studies.

3.6. Effects Table

Table 56. Effects table

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
KOOS total responder rate m24	Responder: increase in KOOS total with at least 10 points from baseline to m24	%	93% (95% CI 86.1; 97.1)	N/A	Single pivotal trial. n=100 (ITT). Single-armed. Several concomitant- and subsequent interventions other than study drug.	Study AAG-G-H-1624 Table 13-1
m60			93% [95% CI 85.7; 97.0]		N=97	Post-text table 15.2.1.4.1
KOOS mean change from baseline to m24		Points	LS Mean (SE) 42.04 (1.827)	N/A	Single pivotal trial. n=100 (ITT). Single-armed.	Study AAG-G-H-1624 Table 13-1
m60			44.13 (1.875)		N=96	Post-text table 15.2.2.1.1
IKDC subjective mean change from baseline to m24		Points	LS Mean (SE) 41.63 (1.878)	N/A	Single pivotal trial. n=100 (ITT). Single-armed.	Study AAG-G-H-1624 Table 13-1
m60			44.55 (1.990)		N=96	Post-text table 15.2.3.2.1
IKDC objective number of participants with normal score		%	Baseline: 52 m24: 85 m60: 88	N/A	Single pivotal trial. n=100 (ITT). Single-armed.	Study AAG-G-H-1624 Table 13-1 Post-text table 15.2.7.1Page 151 of 1135
MRI derived structural data	MOCART sum score m12 and m24	Points	m12: 73.5 ± 23.60 m24: 72.3 ± 20.97 m60: 64.6 ± 21.77	N/A	Single pivotal trial. Single-armed. Subgroup analysis, n=25. Representability of substudy population uncertain.	Post-text tables 15.2.8.2.1 and 15.2.8.2.2

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
MRI derived structural data	Proportion of patients with T2 global ratio in normal range m12 and m24	%	m12: 60.0 m24: 73.9 m60: 69.8	N/A	Single pivotal trial. Single-armed. Subgroup analysis,based on 25 lesions at m12, and 23 leaions ar m60. Representability of substudy population uncertain.	Post-text tables 15.2.8.3 and 15.2.8.4Page 168 of 1135
Treatment failure	ITT population	N (%)	1/100 (1%)	N/A		Summary of clinical safety table 26

Unfavourable Effects (up to m60)						
Arthralgia	Joint pain in in safety analysis set	N (%)	43/102 (42.2%)	N/A	Postoperative wound pain within 2 weeks post tissue harvest or transplantation was not recorded as AE.	Summary of clinical safety table 15
Joint effusion Joint swelling	>4 weeks after treatment, safety analysis set	N (%)	19/102 (18.6%) 20/102 (19.6%)	N/A	Joint swelling/local oedema within 4 weeks post tissue harvest or transplantation was not recorded as AE.	Summary of clinical safety table 15
TEAEs that required a surgical intervention	Safety analysis set (n=102)	N (%)	7/102 (6.9%)			Summary of clinical safety table 14

Long-lasting debilitating complications after knee surgery	Arthrofibrosis or complex regional pain syndrome	%	0	--	Known consequence of surgery, with n=5 (2.0%) cases in the observational study.	Study 1624, study 1521

Abbreviations: KOOS Knee injury and osteoarthritis outcome score. IKDC International Knee Documentation Committee. MRI Magnetic resonance imaging. MOCART Magnetic resonance observation of cartilage repair tissue.
Notes:* an *ad hoc* analysis was performed on the key efficacy parameters after 36 months follow-up . Cut-off date for this analysis was 21-Oct-2022.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Quality

Component A

The quality assessment of component A has identified several critical deficiencies as detailed and explained throughout the assessment report.

The currently proposed quality control strategy was not substantiated to demonstrate that the potency, identity and purity markers and their corresponding limits are sufficient to ensure a good quality of Jelrix.

Futher justifications for the proposed control strategy and additional information for the completeness of the dossier needs to be provided before Jelrix Component A can be accepted from a quality perspective.

Component B

There are critical deficiencies in the description of Component B as a drug product. Media fill, process validation and filter data, supporting the suitability of the proposed commercial manufacturing process are missing.

Favourable effects

In this single pivotal study, the primary endpoint, KOOS responder rate at 24 months compared to baseline, was met; the percentage responders using a pre-defined cut-off for improvement of 10 points was 93%. This was supported by results of the (key) secondary endpoints (KOOS change from baseline, KOOS sub scores, IKDC responder rate, IKDC change from baseline, and EQ-5D-5L), although these were not corrected for multiplicity. Structural changes were also observed using MRI data obtained in only a subset of patients (n=25), but only around 60% showed complete defect filling. The (clinical) relevance and robustness of the observed changes remains unclear.

An important shortcoming, however, is that no internal control group was enclosed in the study, and that data were not replicated because only one pivotal trial was performed with NOVOCART Inject plus. According to the EMA "*Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee*", the study design of a confirmatory clinical trial in this medical field should follow a randomised, controlled approach with appropriate comparator. A control arm in which the subjects underwent all surgical- and nonsurgical interventions except receiving the study drug (in other words standard of care) would have

been adequate. The present study design introduced substantial uncertainties prohibiting conclusions to be drawn regarding the efficacy with NOVOCART Inject Plus.

The propensity score matched pair analyses, providing comparison with MFx as an external active control arm suggested larger treatment effects for NOVOCART Inject plus compared to MFx. However, such conclusions cannot be drawn mainly due to differences in defect characteristics between both groups. The single arm AAG-G-H-1624 study cannot isolate the effect of NOVOCART Inject plus. It is likely that standard surgical procedures such as debridement and removal of loose cartilage fragments followed by rehabilitation would result in significant symptomatic improvements. The observed individual outcomes may thus have occurred without treatment with NOVOCART Inject plus. Comparisons with external/historical control group is not appropriate as pivotal evidence due to deficient control of potential biases. This does not meet regulatory standards for a new product in this disorder. There are several randomized controlled trials in the literature have compared autologous chondrocyte implantation with other cartilage procedures but there is insufficient evidence on superiority of any method. Further controlled studies would be feasible.

Finally, the absence of a clear exposure-response relation combined with unclarities in different manifestations of treatment failure (e.g., empty defect and other MOCART scores, KOOS deterioration, unplanned surgical re-interventions) and their mutual relation further contributes to the uncertainty of the effect of Jelrix.

Only 2 adolescents were treated with NOVOCART Inject plus. From a pathophysiological perspective, comparable (short-term) efficacy in this population might be expected, as was seen for another M-ACI product, Spherox, based on data from 60 adolescent patients. Even if (long-term) differences cannot be ruled out, altogether, the very limited evidence emerging from the clinical development program of NOVOCART Inject (plus) for adolescents, does not raise new safety concerns. Efficacy data is scarce but likely comparable to what is observed in the overall study populations. Finally, the fact that adolescents with closed epiphyseal growth plates can be considered skeletally mature, provides further support for inclusion of adolescents in the indication.

Further, treatment benefit was not demonstrated in cartilage defects < 4 cm2, and these smaller defects are also not commonly included in clinical practice recommendations. Including this population to the indication was not agreed. Registry analyses regarding defect sizes below 4 cm2 cannot be accepted as pivotal efficacy demonstration for Jelrix for these defects, also in light of the previous failed study that was assessed in procedure EMEA/H/C/004598.

Finally, the unspecified aetiology of cartilage defects in the proposed indication was not agreed.

Unfavourable effects

As stated in the EMA "Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee", autologous chondrocytes-containing products have been used for more than 15 years in clinical practice and the experience for this class of products is relevant and has to be considered. For products for which clinical data has been gathered before entry into force of Reg No. (EC) 1394/2007, the acceptability of safety data will depend on the quality of the data and their collection over the years.

Despite the limitations in sample size (N=100) the safety data may potentially generalise to the intended target population, with limitations regarding lesion size (no data <4 cm2 in the pivotal study), and the number of paediatrics and elderly. These latter two limitations are covered by the SmPC (paediatrics and elderly) and RMP (paediatrics). The applicant provided the final report (final 5-year follow-up) for the pivotal study AAG-G-H-1624 and a brief update of the safety data. No new safety signals were identified. The

question however remains whether B/R in the population of the pivotal study with lesions between 4 cm2 and 12 cm2 generalises to the population with defect sizes <4 cm2, according to the indication as proposed by the applicant. In principle, the safety data itself may extrapolate to patients with smaller lesions.

Overall, it appears that adverse events are restricted to the target joint, are mild to moderate and transient. Arthralgia, joint effusion and joint swelling but also joint crepitation were most common AEs and can be expected with this kind of surgery. Arthralgia, joint effusion and joint swelling, etcetera, were not assessed in the first weeks after transplantation. This is not a main limitation, as these events can be expected in nearly all patients. These expected transient and mild events do not negatively weigh in the B/R, given the indication. For application with Novocart Inject plus (Jelrix), surgical techniques can be minimally invasive, as compared to earlier MACI techniques using a solid matrix that has to be cut and sewn and sometimes covered. Transmission of infectious agents could have an impact on B/R but did not occur. The risk is covered in the RMP for follow-up, which is agreed.

SAEs were infrequent but are of relevance for the B/R weighing. Graft loss and treatment failure are serious events but were relatively uncommon. Notably, not all cases of graft loss led to clinical consequences and re-surgery, but in principle, graft loss would be expected to have an unfavourable short- and/or long-term outcome. Arthrofibrosis and complex regional pain syndrome were infrequent but are well-known as seriously debilitating complications of (knee) surgery.

Overall, the safety profile observed in the pivotal clinical study is overall expected for a chondrocyte-containing product and its associated surgical procedures, and the risks are considered possible to handle through adequate warnings/information in the SmPC and educational materials. However, there are some important deficiencies that needs to be considered in the safety data presented for the current product. These include the lack of a comparator in the pivotal study, as well as the exclusion of certain adverse events from reporting. The most important deficiency, however, is that no robust efficacy of the product has been shown. Since implantation of autologous chondrocytes requires an additional surgical intervention under regional or general anaesthesia that per se are associated with certain risks, this impacts the B/R of the product.

3.7.2. Balance of benefits and risks

The benefit/risk balance is **negative**. There are important deficiencies in the quality documentation of the product.

Further, there are severe methodological deficiencies in the pivotal, single-armed trial. Since this is a medical field with a history of failed trials a robust methodology would have been expected in combination with compelling results both statistically and clinically. It is not possible to isolate the potential drug effect from the expected improvement that would have occurred with solely the concomitant standard surgical interventions and procedures together with rehabilitation programme that was given concomitantly in the study. In addition to the pivotal study a comparison of study AAG-G-H-1624 data to an external and historical control group using the microfracture (MFx) control group from the study AAG-G-H-1202 was performed. This propensity score matched pair analysis is not considered valid as pivotal evidence due to major deficiencies in particular related to the control of potential bias.

The CHMP considered that the remaining uncertainties should also be weighed against the severity of the applied indication, in particular that cartilage defects, although disabling, are neither rare nor life-threatening.

3.7.3. Additional considerations on the benefit-risk balance

Input from patient and healthcare provider engagement

3.7.3.1. Patient representatives

The **Osteoarthritis Foundation International** has been invited to share patients' perspectives. A summary of their response is provided below.

Osteochondral defects (OCDs) are areas of damage to the cartilage and underlying bone in a joint. These defects can be difficult to identify early on due to their gradual onset, often caused by wear and tear, repeated stress, or trauma. Knees, being weight-bearing joints, are particularly susceptible to OCDs. OCDs can cause significant pain, limiting mobility and autonomy, potentially impacting mental health. Daily activities may become challenging due to these limitations. Over time, damaged cartilage can progress to osteoarthritis (OA). OA causes pain and directly affects the mobility and autonomy of those people who suffer from this disease, accelerating the decline in mental health. Daily activities can pose a serious obstacle because of the associated limitations of the disease.

There are currently no treatments capable of delaying the progression of the disease, most of them focus on relieving pain and improving joint function using analgesics (anti-inflammatories and opioid derivatives). Concerning surgical options, patients experience frustration due to the long waiting lists and frequently fail to meet eligibility criteria. For instance, younger individuals may find healthcare professionals inclined to delay surgery, while older individuals might be deemed unsuitable candidates. Furthermore, it is important to highlight that surgical replacement does not guarantee the cessation of symptoms, since between 10-20% of patients who undergo total knee replacement continue to suffer from pain.

Addressing the therapeutic and unmet medical needs of OA patients requires a comprehensive approach that encompasses pain management, disease modification, precision medicine, comorbidity management, patient education, and access to equitable care.

Despite limited familiarity with cellular therapy and regenerative medicine, OA patients are drawn to these emerging technologies due to the glimmer of hope they offer for a cure, pain relief, and personalised treatment options. The promise of regaining control over their health further fuels their optimism toward these innovative therapies. The willingness to participate in innovative treatments and the desire to contribute to research underlines the importance of active patient involvement in the development and implementation of new therapies.

Nonetheless, we observed that knowledge about cell therapies and regenerative medicine is very scarce, becoming non-existent in some cases. However, once it was explained, patients expressed their concerns about tumorigenesis, genetic alterations, and the possible immune reaction to the new therapy, as well as possible interactions with their other conditions (comorbidities). Other concerns are related to accessibility due to age issues, the significant economic burden and the requirement for multiple surgical procedures are noteworthy factors. In addition to these concerns, it is imperative to avoid viral infection, ensure maximum sterility conditions, and ensure that there is no rejection, which might result in the development of fibrosis within the cartilage.

Despite concerns, patients are not afraid but rather motivated to participate in the various clinical phases, aiming for the approval of these therapies to benefit the entire OA community. In fact, OA patients also point out concerns about age limitations for undergoing these therapies, as some studies exclude individuals over 65 years old. Finally, there is concern if the access to these therapies will require significant economic resources excluding most patients to be treated.

Conclusion

OAFInoted that there is a great need to improve the portfolio of treatments that are effective at the symptomatic level, that is, capable of reducing pain and improving function, but also capable of slowing, reversing or reducing the progression of the disease, and that are safe and affordable for patients. To achieve this, validated protocols and techniques are required to quantify the disease-modifying effect (DMOAD effect) in clinical trials. OAFI also note the need to make it mandatory to carry out questionnaires on quality of life during the different clinical phases, since the information in this matter is quite limited.

3.7.3.2. Healthcare representatives

The **European Federation of National Associations of Orthopaedics and Traumatology (EFORT)** has been invited to share perspectives on behalf of healthcare professionals' organisations and their response is cited below:

EFORT, as the European Federation of National Associations of Orthopaedics and Traumatology, and allied to European subspecialty societies, has developed an internal consultation with experts, including knee surgeons from various countries and organizations, and specifically involving also experts from ESSKA, the European Society of Sports traumatology, Knee surgery and Arthroscopy.

The treatment of *symptomatic, localised, full-thickness cartilage defects of the knee joint (International Cartilage Regeneration & Joint Preservation Society (ICRS) grade III or IV) with defect sizes up to 12 cm2 in adults and adolescents with closed epiphyseal growth plate in the affected joint*, can be considered controversial. The largest the lesion, the more difficult to heal with available, standard-of-care treatments for chondral lesions which include: nano and microfractures (for small size lesions), even associated with matrix (autologous matrix-induced chondrogenesis [AMIC], for larger size up to medium size defects); mosaicplasty or osteochondral autograft transfer (OAT) (for full thickness lesions with sufficient bone to anchor the plugs, up to medium size defects); allograft osteochondral transplant (for osteochondral lesions even if larger size); autologous chondrocyte implantation (ACI), or membrane- or matrix-assisted chondrocyte implantation (MACI, 1st and 2nd generation) for small to large defects.

All of these techniques, currently in use, sustain limitations and issues which prevent the orthopaedic community to recommend any of those as a universal therapy for these chondral lesions. Furthermore, the problems increase in adult patients when the localized chondral lesion may progress towards joint degeneration, where the indication is no longer the cartilage repair. Similarly, if subchondral bone is affected, the chondral defect may evolve to osteochondral defect and the cartilage repair alone may not be sufficient. Therefore, chondral lesions can receive different treatments depending on the size: in small size lesions (under 2.5 cm^2), frequently microfractures are used with reasonable success, or OAT if high demand; medium size lesions (2.5 to 5 cm^2) frequently receive OAT, ACI/MACI, but also AMIC, with variable results; those of largest size (above 5 cm^2), although managed depending on the location, are treated in high demand patients with OAT, ACI/MACI, AMIC, or osteochondral transplant, but frequently represent a challenge for surgeons and patients, and an unmet need for the society.

Current techniques raise issues such as the formation of fibrocartilage instead of hyaline cartilage, after microfracture or surrounding the grafts in mosaicplasty, frequently not healing the gaps towards normal cartilage; the eventual delamination of patches covering the lesion such as in AMIC or ACI; or the occasional cartilage overgrowth (hyaline-like or fibrous) in ACI or MACI solutions. Furthermore, the timing to regain full motion, full weight bearing and full function, particularly in high demand patients, is unclear and may impact in the complications and final outcome.

Depending on the size and location of the lesion, the functional mid-term results with mosaicplasty, ACI and MACI, may be satisfactory in 75% of the cases, although a deterioration in the long-term up to 50% has been detected with ACI.

In this context, the expectations from future treatments include: a predictable attachment of the medicament to the subchondral bone (limiting the risk of delamination and migration, even if implanted with arthroscopy); a predictable cartilage regeneration, with a dose depending on the size and location of the solution, also healing the gaps with the normal cartilage; a known timing and evolution of the medicament to produce the cartilage regeneration (to allow for motion, weight-bearing and function as soon as possible).

The clinical endpoints to show clinical benefit may include: absence of complications such as effusion or stiffness; timing to recover full range-of-motion and weight-bearing; improvement in pain during motion or weight-bearing at certain timing (6 weeks for full motion, 9-12 weeks for full weight-bearing); imaging proofs (quantitative MRI) of filling the defect and decrease/disappearance of subchondral oedema. Histologic proofs of hyaline cartilage regeneration would also be appropriate.

In the particular case of autologous, in vitro expanded chondrocytes, associated risks may include:

(1) chondral tissue hypertrophy;

(2) potential dedifferentiation of the cells;

(3) complications with seeding and maintaining the cells into the defect;

(4) in case a combined therapy (cells plus a biomaterial, whether as a membrane or matrix), risks associated to the biomaterial cleavage, integration with subchondral bone, integration with surrounding cartilage, are to be taken into consideration.

Of course, all these risks should be assessed and clarified in the preclinical studies.

While this kind of proposal may hold significant interest to the orthopaedic community, given the unmet needs due to the complexity of the lesion and repair, clinical trials proving feasibility, safety and efficacy are of course required before a final recommendation can be placed.

3.8. Conclusions

The single arm AAG-G-H-1624 study cannot isolate the effect of Jelrix and does therefore not meet regulatory standards for a new product in this common, rather benign disorder.

It is possible that surgical procedures such as debridement, removal of loose cartilage fragments and the subsequent rehabilitation could result in significant symptomatic improvements. The observed individual outcomes may thus have occurred without the specific component Jelrix.

The main risks of the treatment are related to required additional surgical procedures and anesthesia itself, risks for transplant loss or treatment failure (lack of effect), and risks for debilitating painful consequences,

such as arthrofibrosis or complex regional pain syndrome. The occurrence of these risks are low, however, only be acceptable if the efficacy of the product can be robustly shown.

It is concluded that the overall benefit /risk balance of Jelrix is negative.

4. Recommendations

Outcome

Based on the CAT review of data on quality, safety and efficacy for Jelrix in the treatment of cartilage defects, the CHMP considers by majority decision that the quality and efficacy of the above-mentioned medicinal product is not sufficiently demonstrated, and therefore recommends the refusal of the granting of the marketing authorisation for the above-mentioned medicinal product. The CHMP considers that:

- The suitability of the currently proposed strategy to efficiently control purity, impurity and potency of Component A is not sufficiently demonstrated. These relate to specifications, identity and purity markers, validation and discriminatory nature of the potency assay.
- Media fill, process validation and filter data, supporting the suitability of the proposed commercial manufacturing process are missing, impacting sterility assurance of Component B.
- The efficacy of Jelrix has not been demonstrated as the single-arm trial AAG-G-H-1624 was not able to isolate the treatment effect.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, pharmacovigilance system, risk management plan to address other concerns as outlined in the list of outstanding issues cannot be agreed at this stage.

Furthermore, following review of the available data in the context of the applicant's claim of new active substance status, the CAT position at the time of this report is reflected in the appendix. However, in light of the negative recommendation, the CAT is of the opinion that it is not appropriate to conclude on the new active substance status at this time.

Divergent position(s)

CAT Divergent position to the majority recommendation is appended to this report.

CHMP Divergent position to the majority recommendation is appended to this report.

5. Re-examination of the CHMP opinion of 24 July 2025

Following the CAT and CHMP conclusion that Jelrix was not approvable due to major objections raised on quality and clinical aspects of the application, the applicant submitted detailed grounds for the re-examination of the grounds for refusal on 23 September 2025.

5.1. Detailed grounds for re-examination submitted by the applicant

5.1.1. Ground 1 (quality)

The suitability of the currently proposed strategy to efficiently control purity, impurity and potency of Component A is not sufficiently demonstrated. These relate to specifications, identity and purity markers, validation and discriminatory nature of the potency assay.

With this additional information, the applicant believes that the revised control strategy aligns with ICH Q11 guidance and is able to bridge the previously identified gaps that were grounds for refusal. The amended control strategy is able to show the following:

1) The identity, purity, and potency of Jelrix is sufficiently controlled by using a marker panel as suggested by D195 JAR with 5 relevant and validated markers. The trends identified whilst assessing the additional markers support the trends already observed with the originally proposed release markers and serve as additional proof for identity, purity of populations, and potency of the product.

The applicant therefore considers that the proposed control strategy is suitable and can identify both chondrocyte and synoviocyte populations to an acceptable level and is therefore also able to identify and discriminate sub potent products. Additional release specifications for the drug substance as set out in Table b-4 such as cell number and cell viability further ensure product potency.

- Annex_1_R&D Report 085.6_final
- Annex_2_RD Report 085.7_version2_final
- Annex_3_Validation report_VA147_3.0_Report_Final_OCR
- Annex_4_GEN-797-2-1.0 Establishment of Specifications (part 1) – signed
- Annex_5_R&D Report 137 Potential osteoblast impurities
- Annex_6_R&D Report 139 Correlation Analysis of ACAN mRNA to biological Activity

5.1.2. Ground 2 (quality)

Ground 2

Media fill, process validation and filter data, supporting the suitability of the proposed commercial manufacturing process are missing, impacting sterility assurance of Component B.

The comprehensive data package provided demonstrates that the sterility assurance of Component B is robust and in alignment with EMA guidelines. All critical elements – including validated sterile filtration, bioburden control strategies, aseptic process simulations, and end-to-end process validation – have been addressed and finalized or are on track for completion within the specified timelines. The multi-layer contamination control strategy, supported by process design, historical sterility testing and clinical safety data, confirms the reliability and compliance of the manufacturing process. Furthermore, the absence of microbiology-related safety concerns in both clinical routine and trial settings reinforces the effectiveness of the applied sterility assurance measures. Importantly, the use of a 30 ml bioburden sample volume, although deviating from EMA/CHMP/CVMP/QWP/850374/ 2015, is scientifically justified. This volume achieves a 95% sensitivity for

detecting ≥ 1 CFU at a contamination level of 0.1 CFU/ml and reflects practical limitations in testing larger volumes. Therefore, the applicant considers the concerns raised in the re-examination to be fully resolved.

- Annex_7_Risk_027_1.0_Pre-Use Integrity Test (PUPSIT) for Sterile Single-Use Merck KGaA SLGP033RB Filters (TTC-70059)
- Annex_8_R-24-0003494-RINS Rinsing Bubble Point Determination Test Report
- Annex_9_VA_168_1.0_Report Report SartoCheck 5 Bubble Point Test Program and Post-Use Filter Integrity Testing
- Annex_10_VA_181_1.0_Report_Filter integrity Testing and holding time with Sartocheck 5
- Annex_11_R-24-0003494-BIND Active Pharmaceutical Ingredient Binding Test Report
- Annex_12_R-24-0003494-COMCFIL Filter Compatibility Certification
- Annex_13_R-24-0003494-BRTC Bacterial Retention Study Report
- Annex_14_R-24-0003494-EXTTCH-SLGP Extractables Validation Report
- Annex_15_REP-16925_01_ Jelrix Extractable study for process filter MILLEX-GP Report
- Annex_16_VA_165_2.0_Report_Validation-Report_Sterile-Filtration_BTPEG
- Annex_17_VA178_and VA017_Validation_MediaFill
- Annex_18_VA_138_3.0_Report_Validation-report-BTPEG

5.1.3. Ground 3 (efficacy)

The efficacy of Jelrix has not been demonstrated as the single-arm trial AAG-G-H-1624 was not able to isolate the treatment effect.

Cartilage defects are a serious medical condition that can cause chronic pain and functional impairment, significantly impacting a patient's quality of life. These defects do not heal on their own and can lead to early osteoarthritis (OA) and eventually require a total knee replacement - an irreversible, highly invasive procedure with risks for serious complications and the need for future revision surgeries.

For focal, symptomatic, full-thickness cartilage defects (≥2 cm^2), ACI is the recommended standard of care, as it provides durable, long-term results and can prevent or delay the progression to OA.

However, there is still a high unmet medical need for ACI treatment in Europe, as cartilage lesions are common, and the availability of M-ACI therapies is limited, leaving many patients without the recommended standard of care.

Jelrix (marketed as NOVOCART® Inject in Germany) - based on the clinical evidence provided in the following sections - has the potential to address this need and provide a guideline-compliant option for patients with cartilage defects of the knee.

Jelrix PHASE III TRIAL

The pivotal study AAG-G-H-1624 is a prospective, multicenter, international, single-arm Phase III clinical trial to evaluate the efficacy and safety of Jelrix in the treatment of patients with large (≥4 cm^2 to 12 cm^2) focal cartilage defects of the knee. This pivotal trial started in 2017. Data from the primary analysis (follow-up 24

months) and long-term follow-up (60 months) are available. The methodological aspects (main inclusion criteria, assessment criteria and statistical analysis) are summarized in Appendix II.

Given that the Phase III trial investigated patients with cartilage defects of 4 to 12 cm² in size, the applicant proposes to adjust the minimum defect size for Jelrix treatment from 2 cm² to 4 cm². This leads to the following proposed indication:

"Repair of symptomatic, localized, full-thickness cartilage defects of the knee joint (International Cartilage Regeneration & Joint Preservation Society (ICRS) grade III or IV) with a single defect size of 4 to 12 cm^2 in patients with closed epiphyseal growth plate in the affected joint."

JUSTIFICATION OF SINGLE ARM DESIGN

The primary objective of the Jelrix phase III trial was to develop a treatment specifically for patients with larger cartilage defects, addressing a clear and persistent unmet medical need in this population.

However, while various cartilage repair methods exist, each has inherent limitations that restrict its suitability as a comparator in an RCT investigating ACI in this patient population (see Section "Treatment approaches for cartilage defects").

Regarding MFx, the 'Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee' (EMA/CAT/CPWP/568181/2009) stated that MFx may only be an appropriate comparator for smaller cartilage lesions less than 4 cm^2.

The hesitance of investigators to treat patients with MFx for larger defects was apparent in the applicant´s previous Phase III trial with the NOVOCART® 3D plus product (AAG-G-H-1202) in patients with defects of 2 – 6 cm^2. Patient recruitment in this trial was slow because investigators considered MFx to be an inadequate comparator in defects of that size, a sentiment that also led to the trial's rejection by the Dutch competent authority and caused similar recruitment delays for other companies.

The use of OAT as comparator for ACI is even more limited, with recommendations primarily focused on treating osteochondral defects of the femoral condyles, typically not exceeding 2–3 cm² in size.[3,58-60] Currently, the recommended limit for defect size as by the guideline for treatment of focal cartilage defects of the knee joint by the DGOU is 1 cm^2.[51]

As a procedure with a palliative approach, debridement/chondroplasty is not recommended in any treatment guideline as a standalone option for younger to middle-aged, physically active patients with focal, full-thickness, and clinically symptomatic cartilage defects of the knee.[28,355] Therefore, debridement/chondroplasty would have entirely lacked a scientific and clinical basis to be accepted as a comparator treatment in an RCT against ACI in larger cartilage defects.

While from a theoretical, scientific point of view a comparison with another ACI product might be desirable, a controlled trial with another commercially available ACI product is generally not feasible. ACI products differ from other medicinal products by their complex manufacturing process and individual character as they are produced for each individual patient by a highly specialized process. A clinical trial with a commercially available ACI product as comparator, would require a competitor to accept the use of its authorized product in a study designed to support the approval of a rival therapy — an arrangement that is unrealistic. Moreover, relying on a competitor's product would mean being entirely dependent on their marketing strategy, including availability in specific countries and clinical centers. There is also no guarantee that the competitor will maintain a long-term presence in the EU market. For example, ChondroCelect® was withdrawn from the market by its marketing authorization holder, TiGenix, in July 2016 for commercial

reasons. As a result, by the time the pivotal Phase III trial AAG-G-H-1624 was initiated, ChondroCelect® was no longer available as a comparator.

In summary, at the time when the pivotal Phase III trial AAG-G-H-1624 was planned and initiated, no scientifically acceptable and feasible comparator was available for patients with cartilage defect sizes ranging from 4 to 12 cm². This was acknowledged by both the Paul-Ehrlich-Institute (PEI) and the EMA during the respective scientific advice meeting/in the official correspondence (EMA/CHMP/SAWP/400714/2016; Meeting Minutes, 28 July 2015).

Of note, an RCT comparing a treatment for large focal cartilage defects such as Jelrix against "standard of care (SoC)" or "investigator's choice", as suggested by the EMA, cannot provide a more robust or less biased measure of the treatment effect (see Section "The Jelrix Phase III Study Meets the Regulatory Standards for Single Arm Trials" below) and presents severe ethical, operational and statistical challenges (see Appendix III).

RESULTS

Study Population

The patient population of the Phase III trial AAG-G-H-1624 mirrors the patient characteristics typically observed in external datasets (e.g., registries, historical trials, observational studies) and is summarized in Table 57.

Table 57: Patient demographic and baseline characteristics

	All patients (N=100)
Sex, n (%)	
Male	63 (63.0)
Female	37 (37.0)
Age (years), mean ± SD (range)	39.8 ± 11.5 (15-62)
Body mass index (kg/m^2), mean ± SD (range)	27.0 ± 4.1 (20.2-34.4)
Smoking status, n (%)	
Yes	25 (25.0)
No	75 (75.0)
Patients with at least one prior surgery [*], n (%)	52 (52.0)
Meniscus removal	27 (27.0)
Joint debridement	16 (16.0)
Ligament operation	15 (15.0)
Chondroplasty	7 (7.0)
Time since first symptoms (months), mean ± SD	22.7 ± 26.8 (0.5-126.6)
Concomitant surgeries [†], n (%)	22 (22.0)
Ligament operation	11 (11.0)
Osteotomy	5 (5.0)
Meniscus removal	3 (3.0)
Tenoplasty	3 (3.0)
Number of defects per patient, n (%)	
One defect	70 (70)
Two defects	30 (30)
Defect location, n defects (%)	
Femoral condyle	80 (61.5)

Patellofemoral	45 (34.6)
Tibial plateau	5 (3.8)
ICRS grade, n defects (%)	
3	93 (71.5)
4	37 (28.5)
Lesion etiology, n defects (%)	
Traumatic	78 (60.0)
OCD	6 (4.6)
Focal degenerative	46 (35.4)
Defect size (cm^2), mean ± SD (range)	
All lesions	4.8 ± 1.9 (1.0-10.4)
Larger lesion ‡	5.4 ± 1.6 (3.0-10.4)
Total §	6.3 ± 2.1 (4.0-12.5)
Operative access, n (%)	
Arthroscopy	46 (46.0)
Mini-arthrotomy	49 (49.0)
Open knee surgery	5 (5.0)
Duration of implantation (minutes), mean ± SD (range)	
All patients	46.2 ± 26.2 (10-161)
Patients without concomitant surgery	39.6 ± 19.8 (10-161)

n, number of patients; n defects, number of defects; SD, standard deviation; ICRS, International Cartilage Regeneration & Joint Preservation Society; OCD, osteochondritis dissecans

*Only surgeries performed in more than 3 patients are given

†Performed concomitantly to tissue harvest or product implantation. Only surgeries performed in more than 1 patient are given

KOOS Results

The primary outcome variable is the Knee injury and Osteoarthritis Outcome Score (KOOS), a validated patient reported outcome (PRO), consisting on 5 subscores (pain, symptoms, activities of daily living [ADL], sports and recreation [sports/rec.], and knee-related quality of life [QoL]).

The use of the KOOS as primary outcome variable in cartilage repair studies is in agreement with the reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009) as well as with the recommendations by the International Cartilage Repair Society[61]. Accordingly, the KOOS (which ranges from 0 indicating extreme symptoms to 100 indicating no symptoms) is one of the most widely used PRO instruments used in clinical studies to assess treatment for patients with articular cartilage defects.

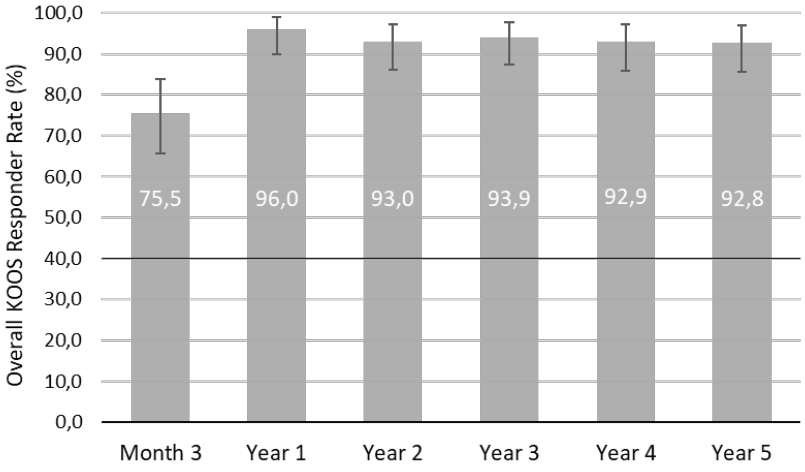
The primary endpoint in this trial is the responder rate based on the overall KOOS defined as the average of the 5 subscale scores (ensuring equal weighting of all subscales) at the 24 months follow-up assessment. The overall KOOS responder rate is defined as the proportion of patients with ≥ 10 points improvement from baseline to the 24 months visit.

Overall KOOS Response Rate (Primary Outcome)

Two years after treatment with Jelrix, 93% of patients were KOOS responders, i.e. with scores improved by ≥10 points compared to their pre-operative level. The study met its primary efficacy endpoint as the 95% confidence interval (CI) was between 86.1% and 97.1% and thus the lower 95%-CI limit was significantly higher than the prespecified level of clinical relevance of 40% ($p<0.0001$). The KOOS responder rate observed at Year 2 was maintained out to Year 5, where the rate was 92.8% (95%-CI: 85.7 - 97.0,

p<0.0001). The analysis of the KOOS responder rates over time up to Year 5 indicated statistically significant rates (i.e., KOOS responder rates of >40%) at all timepoints from as early as Month 3 (75.5%, 95%-CI: 65.6 – 83.8, p<0.0001) (Figure 3). Thus, the primary study endpoint (i.e., a KOOS responder rate of >40%) was descriptively met at each assessment time point from Month 3 to Year 5. The sensitivity analyses (based on adjustment for increased pain medication given within 7 days prior to the assessment time point [prespecified] and for patients without concomitant and/or subsequent surgeries [post-hoc, see response to MO154 in the Day 120 List of Questions for details]), showed results that were almost identical to the main analysis and thus supported its validity.

Figure 3: Overall KOOS responder rates (≥10 points improvement) over time



Vertical error bars indicate the 95%-confidence intervals. The horizontal line indicates the 40% threshold for clinical relevance. KOOS, Knee Injury and Osteoarthritis Outcome Score

KOOS Change from Baseline

The overall KOOS among the 100 ITT patients was 39.8 points at baseline and continuously increased to 82.4 ± 16.4 at Year 2 (least squares [LS] mean increase 41.8 points, 95%-CI: 38.2 - 45.4, p<0.0001) and 84.7 points at Year 5 (LS mean increase 44.1 points, 95%-CI: 40.4 - 47.9, p<0.0001). The largest improvement occurred within the first year after treatment (Table 58). Accordingly, stable or slightly higher values between Year 1 and Year 5 were seen in all KOOS subscores. The mean changes from baseline were significant in the overall KOOS and in all 5 KOOS subscores at all timepoints measured from Month 3 to Year 5 (p<0.0001). The greatest improvements in KOOS subscores at Year 5 (i.e., an increase of more than 50 points) were found for sports/rec. (LS mean increase: 58.2, 95% CI: 53.2 - 63.2) and QoL (LS mean increase: 50.5; 95%, CI: 45.2 - 55.8) (Table 58).

Table 58: Patient-Reported Outcome Scores and Changes from Baseline over time

Score [a]	Mean ± SD	LS mean change from baseline [95%-CI] [b]
KOOS overall		
Baseline	39.8 ± 14.3	
Month 3	66.3 ± 15.8	26.0 [22.6; 29.3]

Year 1	78.8 ± 15.0	38.6 [35.3; 41.8]
Year 2	82.4 ± 16.4	41.8 [38.2; 45.4]
Year 5	84.7 ± 17.1	44.1 [40.4; 47.9]
KOOS pain		
Baseline	50.1 ± 17.1	
Month 3	77.9 ± 14.3	27.2 [24.3, 30.1]
Year 1	86.2 ± 13.0	35.7 [32.9, 38.4]
Year 2	88.6 ± 13.5	37.6 [34.7; 40.6]
Year 5	89.6 ± 14.3	38.9 [35.9; 42.0]
KOOS symptoms		
Baseline	52.7 ± 17.6	
Month 3	76.2 ± 15.8	22.2 [18.9, 25.4]
Year 1	84.3 ± 12.7	30.6 [28.0, 33.3]
Year 2	86.4 ± 14.7	32.2 [29.0; 35.3]
Year 5	88.3 ± 15.8	34.1 [30.8; 37.5]
KOOS ADL		
Baseline	52.5 ± 19.0	
Month 3	81.1 ± 13.5	28.1 [25.3, 30.9]
Year 1	89.9 ± 13.1	37.0 [34.2, 39.7]
Year 2	91.3 ± 13.4	37.9 [34.9; 41.0]
Year 5	92.5 ± 13.9	39.0 [35.9; 42.1]
KOOS sports/rec.		
Baseline	21.0 ± 16.4	
Month 3	49.0 ± 24.7	27.9 [22.6, 33.2]
Year 1	68.9 ± 22.1	48.0 [43.2, 52.8]
Year 2	75.7 ± 22.8	54.6 [49.7; 59.6]
Year 5	79.2 ± 22.9	58.2 [53.2; 63.2]
KOOS QoL		
Baseline	22.5 ± 14.0	
Month 3	47.2 ± 20.8	24.5 [20.0, 28.9]
Year 1	63.8 ± 23.5	41.2 [36.3, 46.2]
Year 2	69.8 ± 24.1	46.8 [41.6; 51.9]
Year 5	73.7 ± 24.7	50.5 [45.2; 55.8]
IKDC subjective		
Baseline	34.3 ± 12.8	
Month 3	55.7 ± 15.6	21.6 [18.3, 24.9]
Year 1	70.8 ± 16.5	36.8 [33.3, 40.3]
Year 2	75.8 ± 17.5	41.3 [37.6; 45.1]
Year 5	79.4 ± 18.2	44.6 [40.6; 48.5]
EQ-5D-5L index		
Baseline	0.61 ± 0.28	
Month 3	0.88 ± 0.11	0.27 [0.25, 0.29]
Year 1	0.92 ± 0.12	0.31 [0.29, 0.34]
Year 2	0.93 ± 0.11	0.32 [0.30; 0.35]
Year 5	0.94 ± 0.14	0.32 [0.29; 0.35]
EQ-5D-5L VAS		
Baseline	59.5 ± 19.2	
Month 3	75.5 ± 13.2	15.8 [13.1, 18.5]
Year 1	81.3 ± 14.4	21.9 [18.9, 24.9]
Year 2	84.1 ± 15.5	24.7 [21.5; 27.9]
Year 5	87.4 ± 15.7	27.7 [24.4; 30.9]

KOOS, Knee injury and Osteoarthritis Outcome Score; SD, standard deviation; ADL, Activities of Daily Living; Sports/rec., Sports and recreation; QoL, Quality of Life; IKDC, International Knee Documentation Committee score; EQ-5D-5L, EuroQoL – 5 dimensions – 5 levels; VAS, Visual analogue scale; LS, Least squares
a: All scores range from 0 (worst) to 100 points (best score) except for EQ-5D-5L index (-0.661 to 1 points)
b: Changes from baseline were analyzed via a linear mixed model for repeated measures, with country and visit as fixed factors, and baseline value as a covariate. All patients with post-baseline data were included. Changes from baseline were highly significant for all outcome measures at all assessment time points (p<0.0001).

Other Clinical Efficacy Results

The IKDC subjective score and the EQ-5D-5L (index value and VAS) showed highly consistent outcomes in line with the KOOS outcomes, i.e., early, increasing, and nominally significant improvements from (including) Month 3 onwards until Year 2 and sustained or even further slightly increasing improvements through Year 5 (Table 59).

After 5 years, 94.6% of patients were rated as "normal" (91.4% at Year 2) and 4.3% as "nearly normal" in the IKDC objective knee examination (Table 59). Only one patient was assessed as "abnormal" at the 5-year follow-up (3 patients at Year 2) and none of the patients as "severely abnormal".

Table 59: IKDC objective score observed at Months 24 and 60 in the main analysis (ITT)

		All patients	
		Month 24 (N=100)	**Month 60 (N=97)**
IKDC objective Categorical analysis	*Normal, n (%) [a]*	*52 (52.0)*	
	Nearly normal, n (%) [a]	*33 (33.0)*	
	Abnormal, n (%) [a]	*13 (13.0)*	
	Severely abnormal, n (%) [a]	*2 (2.0)*	
	n	93	93
	Normal, n (%)	85 (91.4)	88 (94.6)
	Nearly normal, n (%)	5 (5.4)	4 (4.3)
	Abnormal, n (%)	3 (3.2)	1 (1.1)
	Severely abnormal, n (%)	0	0
	Missing, n	7	4
	p-value [b]	<0.0001	<0.0001

a: Baseline values (provided for descriptive comparison).
b: P-value from the Wilcoxon signed-rank test for the change from baseline.

In summary, patients treated with Jelrix experienced consistent, clinically meaningful and durable improvements across all clinical endpoints up to 5 years (KOOS responder rate 92.8%, KOOS LS mean change from baseline 44.1 points, 95% CI: 40.4 - 47.9, p<0.0001).

Nominally significant mean improvements from the pre-operative status were already noticed 3 months after treatment (first measurement time point), and primary study endpoint (i.e., a KOOS responder rate of >40%) was met at each assessment time point from Month 3 onwards through Year 5.

Structural Outcomes

MRI assessments using MOCART 2.0 showed predominately unchanged favorable morphological conditions at 12, 24, and 60 months after surgery, with a trend towards a slight numerical deterioration of questionable relevance at Month 60. T2 mapping indicated increasing tissue maturation towards healthy (hyaline-like)

cartilage up to 24 months with stable results through at least 60 months, consistent with the course of the clinical variables.

For more details, please refer to the responses to MO 154d and OC 157 in the Day 120 List of Questions.

Safety Results

The most common treatment-related AEs occurring in >5% of patients up to Year 5 were arthralgia (18 patients, 17.6%), joint effusion (18 patients, 17.6%) and joint swelling (10 patients, 9.8%). None of the related AEs were severe; 8 patients (7.8%) experienced moderate and 36 patients (35.3%) mild related AEs.

Most related AEs occurred within the first-year post treatment, while no related AEs on the target knee were documented between 2 and 5 years.

The treatment failure rate (defined as proportion of patients requiring surgical re-interventions affecting the closed surface of the transplant area) was 1% (one patient with graft failure).

Of note, since 2008, more than 6,000 patients have been treated with NOVOCART® Inject (the trade name under which Jelrix is marketed in Germany), with the vast majority being for knee defects. Continuous safety monitoring and reporting are conducted, with data compiled in Periodic Safety Update Reports (PSURs).

Overall, the safety profile of Jelrix/NOVOCART® Inject as available from the phase III trial and marketing experience is in agreement with the general safety profile of the class of ACI/M-ACI products. No new or unexpected safety events or other issues have been identified from the Phase III trial (AAG-G-H-1624), the supportive studies and routine pharmacovigilance activities.

More detail on safety is given in Module 2.7.4 and Module 2.5.

CLINICAL SIGNIFICANCE OF TREATMENT EFFECT

Jelrix Exceeds Primary Endpoint with a Substantial Margin

The pre-specified primary efficacy endpoint required a lower 95%-CI boundary of the KOOS overall responder rate to exceed 40%. This threshold was considered conservative, given that large, symptomatic cartilage lesions are unlikely to heal spontaneously without intervention[53] and a substantial rehabilitation effect is not expected by 24 months (see below Section "Addressing Potentially Confounding Factors in the Jelrix Study"). Jelrix significantly surpassed this criterion, achieving a KOOS response rate of 93.0% at 24 months and 92.8% at 60 months (based on a ≥10 points improvement threshold), with a lower 95%-CI limit of 86.1% at 24 months and 85.7% at 60 months. This indicates a substantial margin of efficacy, as the observed response rates would have still surpassed even a theoretical threshold more than twice as high (e.g., up to 86%), validating the strong and sustained treatment effect.

Tipping point analysis

As requested by EMA, a tipping point analysis was performed in order to determine the threshold for response definition (i.e., change from baseline), above which the requirement of a lower two-sided 95%-CI boundary of >40% for the responder rate would no longer be fulfilled. The corresponding analyses are provided Table 60 and are additionally illustrated in Figure 4 and Figure 5.

For Month 24, the tipping point lies between a change from baseline of ≥41 points (95%-CI: 40.8 - 61.6) where the 40% threshold is still surpassed, and a change from baseline of ≥42 points (95%-CI: 38.9 - 59.2) where the 40% threshold is no longer surpassed.

For Month 60, the tipping point lies between a change from baseline of at least 44 points (95%-CI: 41.2 - 61.8) where the 40% threshold is still surpassed, and a change from baseline of at least 45 points (95%-CI: 39.2 - 59.8) where the 40% threshold is no longer surpassed.

Of note, the responder threshold of ≥41 points improvement at which the threshold for clinical relevance of 40% was still surpassed at 24 months is considerably higher than any of the responder thresholds reported in other studies (see Section "Benchmarking Jelrix response rates against those of other ACI/cartilage repair techniques" below).

In summary, both tipping point analyses at both timepoints indicate that a substantial majority of patients achieved a clinically meaningful benefit from treatment with Jelrix, even considering responder thresholds much higher than used for primary analysis of the Jelrix trial.

Table 60: Tipping Point Analysis of Overall KOOS Responder Rate

Timepoint	Responder Threshold		Total (N=100)
Month 24*	KOOS Change ≥ 10	n	100
		Responder r (%)	93 (93.0)
		95% CI	[86.1; 97.1
		P-value	**<.0001**
	KOOS Change ≥ 20	n	100
		Responder r (%)	87 (87.0)
		95% CI	[78.8; 92.9]
		P-value	**<.0001**
	KOOS Change ≥ 30	n	100
		Responder r (%)	74 (74.0)
		95% CI	[64.3; 82.3]
		P-value	**<.0001**
	KOOS Change ≥ 40	n	100
		Responder r (%)	54 (54.0)
		95% CI	[43.7;64.0]
		P-value	**0.0032**
	KOOS Change ≥ 41	n	100
		Responder r (%)	51 (51.0)
		95% CI	[40.8;61.1]
		P-value	**0.0168**
	KOOS Change ≥ 42	n	100
		Responder r (%)	49 (49.0)
		95% CI Lower	[38.9;59.2]
		P-value	0.0423
Month 60**	KOOS Change ≥ 10	n	97
		Responder r (%)	90 (92.8)
		95% CI	[85.7; 97.0]
		P-value	**<.0001**
	KOOS Change ≥ 20	n	97
		Responder r (%)	85 (87.6)
		95% CI	[79.4;93.4]
		P-value	**<.0001**
	KOOS Change ≥ 30	n	97
		Responder r (%)	76 (78.4)

	95% CI	[68.8;86.1]
	P-value	**<.0001**
KOOS Change ≥ 40	n	97
	Responder r (%)	59 (60.8)
	95% CI	[50.4;70.6]
	P-value	**<.0001**
KOOS Change ≥ 44	n	97
	Responder r (%)	50 (51.5)
	95% CI Lower	[41.2;61.8]
	P-value	**0.0140**
KOOS Change ≥ 45	n	97
	Responder r (%)	48 (49.5)
	95% CI	[39.2;59.8]
	P-value	0.0366

N: Number of subjects in the intent-to-treat population
n: Number of subjects with data available at specified slotted visit; r: Number of responders; CI: Confidence interval
Responder rate is calculated using r as the numerator and n as the denominator.
Clopper-Pearson (exact) binomial confidence intervals are displayed.
P-value of one-sided exact binomial test of hypotheses H0: Rate ≤ 40% vs. H1: Rate > 40% is shown. The hypothesis is tested using a one-sided exact binomial test at a significance level **α=0.025**.
*Patients with missing KOOS assessment at a certain timepoint were considered non-responders
**Follow-up analysis was based on a complete case approach, i.e., only non-missing KOOS scores were analyzed. Patients classified as treatment failures before or during the respective slotted visit were handled as non-responders, regardless of the KOOS response.

Figure 4: Tipping Point Analysis of overall KOOS Responder Rate – Month 24

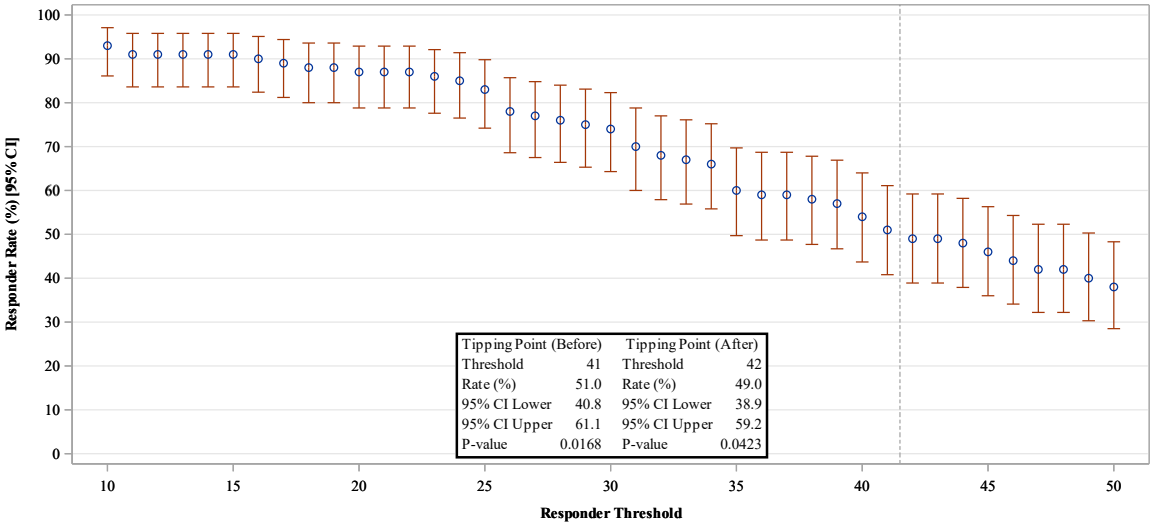
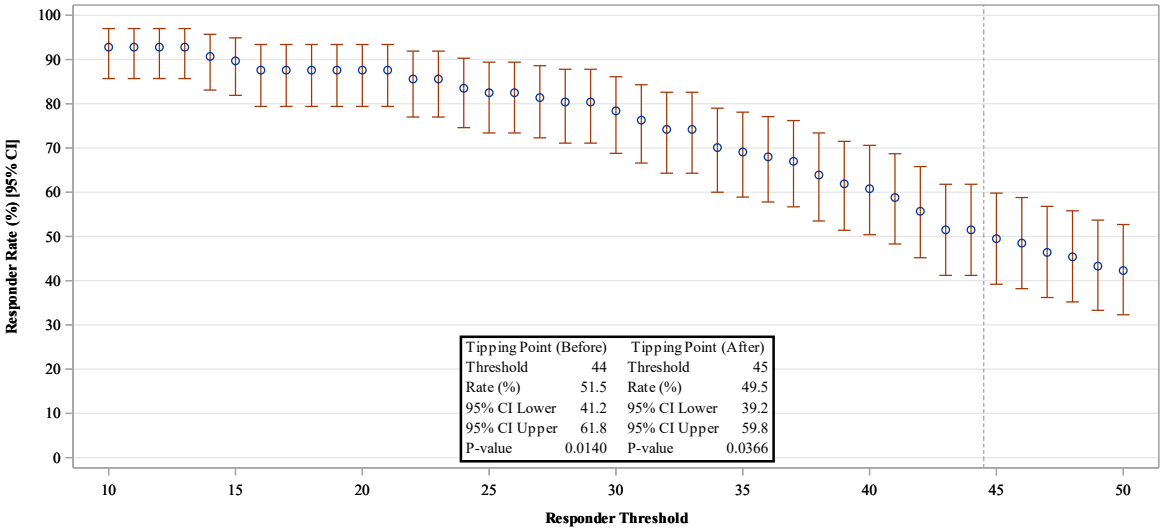


Figure 5: Tipping Point Analysis of overall KOOS Responder Rate – Month 60



Benchmarking Jelrix response rates against those of other ACI/cartilage repair techniques

Generally the minimally clinically important difference (MCID) in KOOS is reported as 8-10 points.[62] Also, Engelhart et al. suggest a minimal change of approximately 10 points as meaningful.[63] In the study by Ibarra et al. the MCIDs obtained for KOOS pain and sports/rec. were 9.8 and 15.4 points at 2 years, respectively.[64]

From this perspective, an improvement in overall KOOS of at least 10 units was considered appropriate for the responder analysis by the applicant for the Phase III trial. This was also agreed on by the EMA during the scientific advice meeting. This choice was based on the above-cited published definitions and evaluations and is in accordance with many clinical studies with ACI products. In these studies MCID responders were defined based on an overall KOOS improvement of ≥10 points.[42,65-68]

However, the definitions of KOOS responders in studies with ACI products were heterogenous and not always oriented to the MCID. Even smaller cut-offs were used in clinical studies as responder criterion for the overall KOOS, for example cut-offs of 8 points[69] and 7 points[70] in clinical studies for the previously EMA approved product Spherox®.

To put the Jelrix Phase III trial results into perspective, the applicant compared results achieved with Jelrix with those from other clinical studies. Where necessary, the Jelrix trial data were re-analyzed using the different cut-offs for MCID or other response definitions like the substantial clinical benefit (SCB) as used in the comparative trials from the published literature.

In a randomized, controlled trial with ChondroCelect® (TiGenix NV, Leuven, Belgium), patients with an increase from baseline in overall KOOS (excluding the subscore sports/rec.) of at least 10 percentage points were considered responders.[68] In this study, which included patients with smaller cartilage defects of the knee (mean 2.6 cm^2), the response rate at 36 months was 83% in the ChondroCelect® group and 62% in the microfracture (MFx) group.

In comparison, the response rate in the overall KOOS (including the subscore sports/rec.) in the Jelrix trial with clearly larger defects treated (mean defect size: 4.8 cm^2; mean total defect size: 6.3 cm^2) was 93.9% at 36 months, i.e., higher than the ACI and MFx responder rates in the ChondroCelect® study.

For the product MACI™ a cut-off of 10 points was also chosen in a randomized phase III trial versus MFx with a responder defined as having at least a 10-point improvement in both the KOOS pain and function (sports/rec.) subscales.[71] The responder rate in the MACI™ group was 86% after 2 years and 78% after 5 years (68% and 73% in the MFx group) in patients with a mean defect size of 5.1 cm^2 (4.9 cm^2 in the MFx group).[72] When the same responder criterion was applied to the Jelrix trial (i.e., ≥10 points improvement in KOOS pain and sports/rec.), the responder rates were 90.0% and 89.7% after 2 and 5 years, respectively, which was higher than for the MFx and the ACI group of the MACI™ study at both timepoints. For this comparison it needs to be considered that baseline values for KOOS pain and sports/rec. were higher in the NOVOCART® Inject /Jelrix trial (KOOS pain: 50.8 points, KOOS sports/rec. 21.0 points), thereby leaving less room for improvement than in the MACI™ trial (KOOS pain: 37.1 points, KOOS sports/rec. 15.4 points).

Please note that in both the ChondroCelect® and the MACI™ trials, patients received concomitant surgeries and underwent a defined rehabilitation program. Concomitant procedures in the MACI™ trial were performed in 36.1% of patients in the ACI group and 30.6% in the MFx group, with the predominant procedures being loose body removal, meniscectomy, ligament reconstruction and synovectomy.[71] In the ChondroCelect® trial, overall 13% of patients had concomitant lesions treated.[68]

Ogura et al. investigated the SCB in terms of KOOS subscores, i.e. an improvement in an individual KOOS subscore that is needed for a patient to feel substantially better, in 92 patients who had received ACI (either collagen membrane covered ACI or matrix-associated ACI) for the treatment of knee cartilage defects after a minimum follow-up of 2 years (mean 2.3 years). SCBs were defined as ≥27.7 points improvement compared with the preoperative level for pain, ≥14.28 points for symptoms, ≥29.4 points for ADL, ≥30.0 points for sports/rec. and ≥37.5 points for QoL.[73]

The SCB rates according to the definition by Ogura in the total population of the Phase III trial (AAG-G-H-1624) at 24 months were 72.0% for KOOS pain, 84.0% for symptoms, 62.0% for ADL, 84.0% for sports/rec., and 73.0% for QoL. These SCB rates were maintained at the same level up to 5 years (Table 61). Notably, the lower limits of the 95%-CIs at 2 years and 5 years were all well above the assumed threshold for clinical relevance of 40% defined for the overall KOOS in the Phase III trial. Overall, the proportion of patients achieving SCB in each subscore in the Jelrix trial at 2 years (and 5 years) was clearly higher than reported in the study by Ogura et al. at a similar mean follow-up of 2.3 years (Table 62).[73] Please, note that the patient population of Ogura et al. was comparable to that in the Jelrix trial in terms of defect size (total defect area: 6.9 cm^2 vs. 6.3 cm^2) and number of defects (1.6 vs. 1.3), but patients were younger (mean age 31.4 vs. 39.8 years), concomitant procedures were performed more frequently (67% vs. 22.0%) and more defects were located at the patella (43.4% vs. 12.3%).

Table 61: Substantial clinical benefit responder rates in the Phase III trial AAG-G-H-1624 at Year 2 and Year 5

		Jelrix SCB Response rate % [95%-CI]			Ogura SCB Response rate %
KOOS Subscore	SCB (points)	Baseline Score ± SD	Year 2	Year 5	Year 2
Pain	≥27.7	50.1 ± 17.1	72.0 [62.1 - 80.5]	70.1 [60.0 – 79.0]	45.8
Symptoms	≥14.28	52.7 ± 17.6	84.0 [75.3 - 90.6]	83.5 [74.6 – 90.3]	37.6

ADL	≥29.4	52.5 ± 19.0	62.0 [51.7 - 71.5]	61.9 [51.4 – 71.5]	37.3
Sports/Rec.	≥30	21.0 ± 16.4	84.0 [75.3 - 90.6]	83.5 [74.6 – 90.3]	58.3
QoL	≥37.5	22.5 ± 14.0	73.0 [63.2 - 81.4]	74.2 [64.3 – 82.6]	50.6

SCB, Substantial clinical benefit; SD, Standard deviation; ADL= Activities of daily living; QoL=Quality of life; Sport/Rec.=Sports and recreation; KOOS, Knee Injury and Osteoarthritis Outcome Score

One study investigated an FDA approved aragonite-based acellular implant (Agili-C) versus surgical SoC (MFx or debridement) in patients with cartilage defects of the knee (1 to 7 cm^2).[74] Here, a responder was defined as having an overall KOOS improvement of ≥30 points. Also in this study, concomitant procedures were performed (surgery on meniscus in 69 out of 251 patients overall) and patients had to follow a defined rehabilitation protocol (including initial partial weightbearing, CPM use, strength training and prohibition of high impact activities during the first year). At 2 years, the responder rate was 77.8% in the implant group as opposed to 33.6% in the surgical SoC group (p<0.0001). Applying the same responder criterion to the Jelrix study, the responder rate at 24 months was 74.0%, and thus comparable to the implant group of the Agili-C study, with the lower limit of the 95% CI of 64.3 (see above tipping point analysis,Table 61) being clearly higher than the responder rate of 33.6% in the surgical SoC group of the Agili-C study. Please, note that while baseline KOOS values were comparable (Agili-C 41.3 vs. Jelrix 39.8), defect sizes in the Agili-C study were smaller (total defect size Agili-C: 3.9 cm^2, SoC: 3.4 cm^2 https://www.accessdata.fda.gov/cdrh_docs/pdf21/ P210034B.pdf) than in the Jelrix Phase III trial (total defect size: 6.3 cm^2).

In summary, Jelrix compares favorably to other (M)-ACI products and especially to the control groups used in the cited randomized trials (including the control group of the Agili-C phase III trial), regardless of the responder criteria used. Of note, in all these trials, patients followed a defined rehabilitation program after cartilage repair treatment, and a proportion of those patients also received concomitant surgeries.

Benchmarking KOOS outcomes of Jelrix to population-based reference data

The overall KOOS (with possible values from 0 [worst] to 100 [best]) among the Jelrix Phase III study patients was 39.8 points at baseline and continuously increased to 84.7 points at Year 5 (LS mean increase 44.1 points, 95%-CI: 40.4 - 47.9, p<0.0001). To provide context for these findings, the study's results were compared to population-based reference data (Table 62).

The reference data, with overall KOOS scores ranging between 80.6 and 89.3 points, indicates that a perfect score of 100 is not a clinically realistic benchmark, as it is not achieved even in representative samples of the general population. The 5-year KOOS score of 84.7 points falls well within this expected range for the general population, thereby underscoring the significant treatment effect of Jelrix.

Table 62: KOOS Scores of Jelrix patients 5 years post-treatment compared to population-based reference data

Score	**Jelrix (mean age 39.8 years**)		Larsen et al.[75] Age ≥ 18 years [a]	Paradowski et al.[76] Age 18–34[b]		Paradowski et al.[76] Age 35–54[b]	
	Baseline	**5 years**		Male	Female	Male	Female
KOOS overall							
Mean	39.8	**84.7**	80.6 [c]	88.8 [c]	89.3 [c]	83.3 [c]	85.9 [c]

KOOS pain							
Mean	50.1	**89.6**	85.3	92.2	92.1	87.4	88.8
KOOS symptoms							
Mean	52.7	**88.3**	85.1	87.2	89.1	86.5	89.5
KOOS ADL							
Mean	52.5	**92.5**	86.7	94.2	95.2	89.1	88.6
KOOS sports/rec.							
Mean	21.0	**79.2**	70.9	85.1	86.4	76.0	79.3
KOOS QoL							
Mean	22.5	**73.7**	74.9	85.3	83.6	77.7	83.4

a: Larsen et al.: A national record-based representative sample of adult citizens derived from the Danish Civil Registration System (about 2800 patients)
b: Paradowski et al.: Population-based cohort randomly retrieved from the National Population Records for the region Skåne of Southern Sweden (134 patients 18 – 34 years, about 160 patients aged 35 – 54 years)
c: Calculated by author based on KOOS subscores

ADDRESSING POTENTIALLY CONFOUNDING FACTORS IN THE JELRIX STUDY

Debridement

Debridement is an integral component of all ACI procedures and also microfracture.

While debridement as a palliative procedure can offer short-term symptomatic relief for knee cartilage damage, clinical trials are limited and provide insufficient evidence to support its efficacy for the treatment of isolated defects, particularly for larger, higher-grade lesions, and, in comparison to ACI, which delivers superior outcomes over extended follow-up periods. *Please, refer to Appendix I for details.*

Moreover, pure debridement leaves the underlying problem, i.e. the cartilage defect, untreated. Untreated cartilage defects, however, even in an otherwise stable joint, tend to increase in size[17] and have been observed to be associated with a significant risk to develop OA.[11,48,77-81] Consequently, debridement ultimately requires the patient to undergo additional surgeries to achieve a long-term, durable repair with potential disease-modifying activity.

This has been impressively demonstrated by a large retrospective cohort study comparing data from 157,730 patients with a history of arthroscopic chondroplasty of the knee to general population data from patients without such a history. Overall, following chondroplasty, 5.9% of patients underwent knee arthroplasty within 1 year, 9.4% within 2 years, 14.2% within 5 years and 17.6% within 8 years. The study found that patients who underwent chondroplasty (including debridement and abrasion using mechanical 'shavers' or thermal or radiofrequency techniques) had a 17-fold increased risk of receiving a knee arthroplasty compared to the general population. The relative risk of undergoing arthroplasty at a young age was particularly elevated, reaching 171 times the general population rate for those aged 30 to 39.[82]

Consequently, and in contrast to ACI, debridement/chondroplasty is not recommended in treatment guidelines as a standalone option for young to middle-aged, active patients with larger, focal, full-thickness, symptomatic knee cartilage defects.[49,51,83-86]

Furthermore, the applicant is not aware of any study that investigated structural repair after debridement based on imaging data. In contrast, M-ACI is reported to repair cartilage defects with hyaline-like cartilage tissue.[87] Similarly, Jelrix has shown to enable the generation of high-quality, hyaline-like cartilage, as confirmed by 2- and 5-year T2 zonal data, a key indicator of hyaline-like cartilage structure[88,89], which showed that up to 78.3% of repaired lesions fell within the ideal range of healthy cartilage. This is supported

by arthroscopic and histological findings (refer to the response to OC 167 in the Response to the Day 120 List of Questions). Furthermore, Janacova et al. demonstrated by gray-level co-occurrence matrix (GLCM) texture analysis of quantitative T2 maps that not only the defect area but also the damaged perilesional cartilage benefited from Jelrix treatment in terms of tissue structure approximation to healthy cartilage.[4]

Rehabilitation

Based on the scientific literature, cartilage repair surgery in general, is always associated with a rehabilitation program with the main goal of protecting the fragile new tissue and promoting its maturation and integration through controlled and progressive activity, and it can be assumed that those programs share common features (for details refer to the response to MO 154 "rehabilitation" in the Response to the Day 120 List of Questions).

It is generally accepted that rehabilitation after knee surgery including ACI or another cartilage repair procedure is a crucial aspect of successful recovery. However, rehabilitation only is not recommended in treatment guidelines as a standalone option for patients with larger, focal, full-thickness, symptomatic knee cartilage defects.[49,51,83-86]

Regarding the extent and duration of the rehabilitation effect, several systematic reviews comparing physiotherapy exercises with no intervention after knee arthroplasty found small to medium effects on physical function, pain, range of motion and quality of life in the short term (3 - 4 months). However, these beneficial effects of physiotherapy exercises were no longer recognizable after 12 months.[90-92] In a systematic review of randomized controlled trials comparing therapeutic exercise with non-exercise controls in patients with knee and/or hip OA, only a small, positive overall effect of therapeutic exercise on pain and physical function was found. However, this effect was considered of questionable clinical importance by the authors, particularly after medium (6 months) and longer intervals (12 months).

Wondrasch et al. evaluated the feasibility and effects of an active rehabilitation program over 3 months (similar length as in the Jelrix trial) for patients with full-thickness cartilage lesions of the knee who had not yet undergone cartilage repair surgery.[94] For this purpose, forty-eight patients (mean age ± SD: 34.1 ± 13.4 years) with full-thickness cartilage lesions on the femoral condyles (mean defect size: 2.9 ± 1.3 cm^2) and a Lysholm score below 75 participated in the rehabilitation program consisting of knee and hip progressive resistance training, cardiovascular training and neuromuscular training. Patients were tested before and after completing the rehabilitation program by using different knee-specific patient-reported outcome measures including the IKDC and the KOOS sore with its 5 different subscales. The average adherence rate to the rehabilitation program was 83%. Four patients (9%) showed adverse events, as they could not perform the exercises due to pain and effusion.

Table 63 shows the KOOS and IKDC data reported by Wondrasch et al. compared to the respective 3-months data from the applicant´s phase III trial AAG-G-H-1624 (excluding patients who had received concomitant and/or subsequent surgery). It's notable that the patients in the Jelrix trial were older (39.8 ± 11.5 years) and had significantly larger defects (6.3 ± 2.1 cm^2) compared to the Wondrasch study's patients (34.1 ± 13.4 years and 2.9 ± 1.3 cm^2). Since older age and larger defect size could negatively impact efficacy, especially in the short term, this comparison might be expected to disfavor Jelrix.

Despite this, Jelrix demonstrated consistently superior results. After 3 months, statistically significant improvements were seen for all KOOS and IKDC scores ($p<0.0001$). In contrast, in the rehabilitation-only group from the Wondrasch study, improvements were only statistically significant for KOOS QoL and IKDC scores, with the other scores being non-significant.

The magnitude of improvement was also considerably higher for Jelrix for all scores: overall KOOS: 24.8 vs. 9.5 points; KOOS pain: 25.9 vs. 6.9; KOOS symptoms: 20.7 vs. 7.6; KOOS ADL: 28.2 vs. 5.6; KOOS sports/rec.: 28.1 vs. 16.1; KOOS QoL: 24.1 vs. 12.2 and IKDC: 21.8 vs. 10.6. Importantly, the lower confidence intervals for the Jelrix scores were consistently higher than the values reported by Wondrasch et al., further demonstrating Jelrix's treatment effect (Table 63).

In light of these findings and the substantial, durable long-term improvements seen with Jelrix — with mean KOOS scores continuing to improve up to 24 months (41.8 points) and remaining stable through 60 months (44.1 points) — the effect of rehabilitation is highly unlikely to be the primary driver of these results.

Furthermore, no significant associations between KOOS change from baseline and the number of physiotherapy visits or the overall duration of rehabilitation were observed in the Jelrix Phase III trial (AAG-G-H-1624) at 24 and 60 months which supports this conclusion.

Table 63: Comparison of clinical outcome in patients having received pre-treatment rehabilitation for 3 months in the study by Wondrasch et al. and 3-months data of the applicant´s phase III trial AAG-G-H-1624 in patients without concomitant and/or subsequent surgeries

	Wondrasch et al.	**Jelrix phase-III trial AAG-G-H-1624**
Baseline /demographic		
Number of patients	48	72
mean age ± SD, years	34.1 ± 13.4	39.8 ± 11.5*
Mean defect size, cm^2	2.9 ± 1.3	6.3 ± 2.1*
Treatment	*No treatment* (yet) No surgery	*Jelrix* only, No CSI / no SSI
Rehabilitation program	**Yes**	**Yes**
Efficacy Results as Change from baseline	Mean change from baseline At 3 months	LS mean change from baseline At 3 months
KOOS overall	9.5	24.8 (95% CI: 20.0-29.5, p<0.0001)
KOOS pain	5.9 ± 16.4 (n.s.)	25.9 (95% CI: 21.8-30.0, p<0.0001)
KOOS symptoms	7.6 ± 15.4 (n.s.)	20.7 (95% CI: 16.2-25.1, p<0.0001)
KOOS ADL	5.6 ± 16.4 (n.s.)	28.2 (95% CI: 24.2-32.2, p<0.0001)
KOOS sports/rec.	16.1 ± 24.5 (n.s.)	28.1 (95% CI: 20.8-35.4, p<0.0001)

KOOS QoL	12.2 ± 16.4 (p<0.05)	24.1 (95% CI: 18.0-30.1, p<0.0001)
IKDC	10.6 ± 15.8 (p<0.05)	21.8 (95% CI: 17.1-26.5, p<0.0001)

n.s.; not significant; CSI: concomitant surgical interventions, SSI: subsequent surgical interventions at the target knee

*This refers to the total population (N=100)

Comparative studies: Demonstrating Jelrix's Efficacy Beyond Debridement and Rehabilitation

In comparative analyses where both patient groups underwent the foundational procedures of debridement and rehabilitation (i.e., these have contributed to both groups equally), the Jelrix/NOVOCART® Inject group demonstrated superior clinical (and MRI) outcomes. This compelling evidence validates the efficacy of Jelrix, proving its therapeutic value extends beyond the benefits conferred by debridement and post-operative care alone:

In the matched-pair analysis conducted by the applicant (see Appendix IV), Jelrix has shown KOOS and IKDC outcomes in large cartilage defects of the knee (4-12 cm^2) that are superior to the outcomes of MFx in smaller defects (2-6 cm^2) through 24 months of follow-up. Of note, this superiority in clinical outcome was supported by structural data showing a significantly higher MOCART 1.0 score compared with MFx. Groups were balanced in terms of key parameters such as sex, age, BMI, proportion of patients with prior surgeries on the target knee and symptom duration. Post-hoc subgroup analyses revealed **no evidence suggesting bias** in favor of Jelrix due to imbalances in defect characteristics or concomitant/subsequent surgeries. Interestingly, the Jelrix group included more patients with multiple defects, as well as larger defect sizes - factors typically considered disadvantageous for clinical outcomes.

Based a second, registry-based matched pair analysis in patients with large knee cartilage defects and no concomitant surgeries (see Appendix V), NOVOCART® Inject has shown to be clinically effective and provides a clinically meaningful benefit with better results for patient reported outcomes (KOOS, IKDC, patient satisfaction) between one and two years compared to Spherox®. As all patients were treated in Germany suggesting similar rehabilitation standards following ACI, the study is not expected to be biased in this regard.

This principle similarly holds true for other comparative studies, such as the MACI™ trial[71], where a superiority over microfracture could not have been demonstrated if the primary benefit were derived from debridement and rehabilitation.

The observed advantage for Jelrix, therefore confirms that its unique cellular components embedded in the hydrogel provide a distinct therapeutic effect.

THE JELRIX PHASE III STUDY MEETS THE REGULATORY STANDARDS FOR SINGLE ARM TRIALS

The regulatory standards for single arm trials are set out in the "Reflection paper on establishing efficacy based on single-arm trials submitted as pivotal evidence in a marketing authorization application" (EMA/CHMP/458061/2024). Although the Jelrix Phase III trial commenced in 2017, well before the publication of this reflection paper (drafted in January 2023, finalized in September 2024), it broadly aligns with the principles outlined in this guidance.

Most importantly, the results of the trial provided strong evidence that the single-arm design was appropriate to isolate the treatment effect in this indication:

- **Clinical improvements are substantial:**
    - Jelrix demonstrated significant and clinically meaningful improvement in patient outcomes with responder rates of 93% at 2- and 5-years post-treatment.
    - The study's success criterion (40% responder rate) was surpassed with a high degree of confidence (95%-CI at 24 mo: 86.1% - 97.1%).
    - The magnitude of the treatment effect was further illustrated by a tipping point analysis showing that this threshold was still surpassed with a responder definition of up to ≥41 points improvement.
    - All secondary outcomes, including IKDC subjective and objective measures, EQ-5D-5L and MRI, consistently supported the primary outcome through 5 years of follow-up.
    - Jelrix compares favorably to other (M)-ACI products and especially to the control groups used in the respective randomized trials.
- **The findings were robust, with no significant bias detected:**

  To account for potential sources of bias, the trial employed rigorous sensitivity and subgroup analyses. These methods, along with matched-pair analyses, confirmed that the results were directly attributable to the treatment.
    - Debridement and rehabilitation: In matched pair analyses (vs. MFx and Spherox, respectively) where both patient groups underwent debridement and rehabilitation, the Jelrix/NOVOCART® Inject group demonstrated superior clinical (and MRI) outcomes, validating its efficacy beyond these foundational procedures.
    - Concomitant/subsequent surgery and pain medication: subgroup analyses/sensitivity analyses showed that the effect of Jelrix is not compromised by these procedures.
    - Patient drop-out/invalid measurements: these events were addressed with a conservative approach, whereby patients lacking a valid measurement at the primary assessment time point were classified as non-responders.

Furthermore, an RCT comparing Jelrix versus standard of care (SoC) as suggested by the EMA (apart from the additional ethical, operational and statistical challenges outlined in Appendix III) would not provide a more robust or less biased measure of the treatment effect and, therefore, does not have additional value compared to the single-arm design in this indication:

- Blinding is impossible due to the fundamentally different surgical methods used for Jelrix and various SoC and therefore the risk of assessment bias remains.
- To control for any rehabilitation effect, a uniform post-operative rehabilitation protocol would be required for both Jelrix and the SoC group. However, different cartilage repair procedures have distinct rehabilitation requirements.

  For example, debridement does not necessitate the strict initial weight-bearing restrictions required for ACI or microfracture. Therefore, imposing a single scheme would introduce a significant trial bias by deviating from established clinical practice for several SoCs.

- Even in a randomized controlled trial, intercurrent event bias (e.g., differences in analgesic use or subsequent surgeries) cannot be adequately controlled. This confounding factor becomes a greater concern when comparing a single treatment to a diverse range of SoC procedures.

- The variability in SoC, influenced by the availability of and preferences for specific treatments in different countries and sites, would result in highly heterogeneous control arms and could lead to center effect bias and limited generalizability.

OVERALL CONCLUSION

In conclusion, the Jelrix Phase III trial demonstrated that the treatment is safe and effective and provides significant and robust clinical benefit for patients with large cartilage defects, with high and sustained responder rates (93%) exceeding the pre-specified 40% threshold for efficacy in both the short- and long-term (up to 5 years) - of note, even a hypothetical threshold of 80% would have been surpassed. The magnitude of the effect was supported by a tipping point analysis and favorable comparisons to other M-ACI products and their controls.

The considerable magnitude of the clinical improvement, combined with the comprehensive bias mitigation strategies - including rigorous sensitivity and subgroup analyses, along with matched-pair analyses comparing Jelrix/ NOVOCART® Inject with MFx and Spherox® - confirm that the treatment effect was successfully isolated and directly attributable to Jelrix in this single-arm trial.

5.2. Rapporteurs' assessment

5.2.1. Ground 1 (quality)

The suitability of the currently proposed strategy to efficiently control purity, impurity and potency of Component A is not sufficiently demonstrated. These relate to specifications, identity and purity markers, validation and discriminatory nature of the potency assay.

5.2.1.1. Re-examination Rapporteur´s assessment

After the D195 report the applicant (TETEC Tissue Engineering Technologies AG) requested a re-examination of Jelrix marketing authorisation application. In the aforementioned request it is addressed that more than one marker is necessary to ensure Jelrix identity and purity due to the difficulty of finding a marker that completely differentiates synoviocytes and chondrocytes. The applicant carried out a screening selecting genes that mostly present preferential expression in chondrocytes except that are mainly expressed in synoviocytes.

Gene expression is selected as a surrogate marker of potency.The applicant provided a correlation matrix to further evaluate mRNA expression of different chondrocyte markers in 2D and 3D culture settings. mRNA expression in 2D cultures negatively correlates between markers: vs expression in 2D cultures also negatively correlates with the mentioned markers and positively with. The data supports mRNA expression as a surrogate marker for potency determination by protein expression analysis for markers relevant for the proposed MoA.

The acceptance ranges for potency, identity and impurity parameters were set calculating a tolerance interval with a confidence interval of 95% with 99% content of all responder data (93 out of 100 patients treated with

clinical batches). The applicant indicates that discrimination of subpotent batches with insufficient quality is achieved by measuring the presence of cellular contamination through the amplified panel (). Although the current panel improves the quality control of Component A, as the patients used to set specification limits are considered responders it cannot be demonstrated that the proposed potency assay is able to identify batches with insufficient biologic activity. In the oral explanation in June, the applicant proposed to further investigate the specification limits of, by comparing and correlating *in vitro* 3D culture data including GAG production, gene expression, and quantitative specific protein expression (). The study is expected to be completed by the end of 2025 which exceeds the time frame of this re-examination. This approach may better support specification limits.

In the D195 JAR report it was recommended to use ddPCR-based methods as they are considered more precise and more sensitive than the currently used qPCR-based method to measure markers expression. This recommendation is still supported. **(REC)**

The final validation report for gene expression via RT-PCR is supplied in Annex 3. However, it cannot be assessed because it was completed after D195, so it cannot be considered for the purpose of this application.

As there is still not enough evidence to demonstrate that the drug substance potency test is able to discriminate subpotent batches and the validation was completed after the oral explanation, the current ground for refusal remains: 'The suitability of the currently proposed strategy to efficiently control purity, impurity and potency of the active substance and Component A finished product is not sufficiently demonstrated. These relate to specifications, identity and purity markers, validation and discriminatory nature of the potency assay'. **(MO)**

From the quality perspective it would be recommended to establish a stronger relationship between the current gene expression panel and the clinical practise. In order to ensure that the control of potency, identity and impurity is robust enough to identify subpotent batches.

5.2.1.2. Re-examination Co-Rapporteur´s assessment

Introduction

In summary, the intended biological function of Jelrix relies on the capacity of the chondrocytes to synthesize functional relevant articular cartilage extracellular matrix components (ECM), including collagen type II, glycosaminoglycans (GAGs), and proteoglycan (PG) core proteins such as in aggrecan (ACAN). The initial quality control (QC) strategy proposed for commercial Jelrix product was primarily based on that established for Novocart Inject Plus, reflecting the manufacturing process used for the pivotal clinical batches, covering parameters such as identity/purity/impurities, and potency (see below). Control is based on qRT-PCR analysis of marker gene expression relative to GAPDH, performed on drug substance samples and taken forward as specification for drug product component A release.

Product-related impurities such as endothelial cells and osteoblasts raised no concern and are thus not specifically addressed by the QC, as these cell types are neither naturally occurring in the sourced tissue, present at low levels, or lack functional relevance or safety risk in the final product. In contrast, FLS (fibroblast-like synoviocytes) impurities and analytic distinction from chondrocytes raised major concerns from the agency throughout assessment.

While the applicant has implemented several risk mitigation steps to avoid FLS introduction via the biopsy starting material (Refer to background information in section 2 of this report), these cannot fully exclude introduction of FLS from synovial fluid into the cell culture and should therefore be controlled for product release

The agency's concerns raised lead to several proposals by the applicant for revision of phenotypic release marker set and/or acceptance ranges, as further discussed in this section. As a result, the purity, impurity and potency release specifications for the intended commercial process as proposed during the MAA evaluation with the d195 2nd LoI, differ substantially from those used for the process applied during the clinical pivotal trial, which is the basis for the clinical evaluation for this MAA (see summary of the development below):

Conclusion of re-assessment grounds for re-examination #1

Taken together, in conclusion of the re-assessment the concerns on the informative value of the ACAN potency marker are shared. Although mRNA expression is not fully exclusive for chondrocytes, in combination with a suitable specification, indicating a high Aggrecan expression, the selection of Aggrecan as relevant biologic activity marker with gene expression as a potency marker is regarded well justified according state of knowledge. Subpotent batches originate from FLS impurities, if cell viability is in specification. Control on FLS is achieved by the finally proposed matrix control strategy, where acceptance range demand ≤ 10% FLS.

With regard to purity/impurity control, as described in section 2, FLS may be co-harvested with the cartilage biopsy. For risk mitigation, the applicant introduced several steps which are regarded important to avoid potential incoming burden. However, investigations targeted on the effectiveness of these measures were not found. In any case, the risk mitigations cannot fully exclude a potential introduction of FLS, e.g by transferred synovial fluid. Moreover, cell culture conditions also enable growth of FLS. As worst-case scenario, this may lead to a predominance of synovial cells over time, even if initially only a small number is introduced. During the 2D adherent cell culture process, morphological control is deemed not to effectual distinguish FLS and chondrocytes.

Thus, the agency's requests for a robust quality control strategy regarding purity/impurities throughout the procedure is supported. According to literature a universally agreed "FLS cut-off margin" was not found, while a max of 5-10% in ACI appears clinically acceptable and has been used for ACI products.

Furthermore, one should be aware that alternative cartilage treatment techniques carry an inherent risk of fibrocartilage formation. A respective question to AHEG is recommend to confirm according current knowledge to which extend FLS impurities should not be accepted from a clinical perspective.

In view of re-assessment, the extended matrix release testing after d195 2nd LoOI, proposing with tightened acceptance ranges, supplemented with the additional marker can contribute to a more robust product control as it has been partially supported by the agency during the final evaluation phase. The re-examination Co-Rapp supports this approach. The revised QC-panel contributes to prevent high FLS impurities at the drug substance level, which is the main relevant concern for functionality of the product.

Due to dependence of the proposed QC setting on the acceptability of the clinical data, the re-examination concludes that quality Mo1c and Mo1a may not be raised as reasons for rejection, provided that the clinical MO can be resolved.

However, the limited data basis and the pending method validation data for the new markers at end of procedure, and the high number of other OCs remain valid remaining concerns to justify the grounds of refusal as raised by the agencies final report. Additional information of the applicant has meanwhile been submitted, but if analysed correctly this was not in due time of the procedure. Thus, to resolve the concerns an additional round of RfSI and post-approval measures would be needed.

In conclusion of the re-assessment, the concerns on the informative value of the potency marker, although mRNA expression is not fully exclusive for chondrocytes, the selection of Aggrecan as relevant biologic activity

marker with gene expression as surrogate, is regarded well justified according state of knowledge. Subpotent batches originate from FLS impurities, if cell viability is in specification. Control on FLS is achieved by the proposed matrix control strategy, where acceptance range demand ≤ 10% FLS.

Mo1c: is regarded as not perfect, but still relevant and suitable biologic activity marker, reflecting current knowledge. The correlation of a quality potency marker with a positive clinical outcome is not routinely required for the approval of ATMPs and typically does not show a statistically significant correlation, even when clinical efficacy of an ATMP has been demonstrated. Subpotent batches originate from FLS impurities. Control is achieved by the proposed matrix control strategy, where FLS acceptance range is ≤ 10%. A recommendation should be made to further investigate potency data in clinical batches and RWD on efficacy (responders/non-responders).

MO1b: PCR Validation report for RT-PCR on new proposed control markers was announced in Oral explanation, while not submitted at that stage and not shown on slide deck. Therefore, MO1b remained unsolved at end of procedure. Provided that the clinical MO and second quality MO can be resolved, it may be envisioned to downgrade as OC for 3rd LoOI and/or condition, if possible. The final decision should consider the high number of other remaining OCs.

MO1a: in conjunction, with proposed tightened acceptance ranges are, despite some limitations, regarded overall acceptable for QC of identity and purity. The introduction of the proposed new identity marker adds additional value and is acceptable. The introduction of the proposed identity marker is regarded supportive. The introduction of the proposed impurity marker may be regarded supportive, but appears of more limited additional value. By literature in the field, and as concluded on review of the applicant's data, the overlapping expression pattern of markers for chondrocytes and FLS remains a limitation. Of note, the initial MO did suggest, but not explicitly request a multiple marker approach. Nevertheless, the now proposed overall test matrix broadens the quality control of the cell product to a level which could be accepted. However, the presented data base available during the procedure for (protein expression, analysis in stability study) is very limited. Moreover, the new markers have not yet been evaluated in clinical batches. It may be envisioned to downgrade as OC and to raise a condition or recommendation to strengthen the data base, provided that the clinical MO can be resolved.

Points potentially resolvable are as follows:

A clinical positive risk-benefit conclusion is considered a pre-requisite for resolving the quality MO, regarding the request to correlate the potency release test with clinical outcome. Only if acceptable clinical data are available can this correlation be downgraded to an OC.

are the markers that are currently in place. ACAN in combination with ACE are considered sufficiently qualified and validated for use in product release testing.

Additional data are requested for, which are intended to further support the marker matrix and were not applied during clinical development. Data for these markers may be requested post-approval as commitment.

5.2.1.3. BWP discussion

Following discussion of the re-examination case presented by the Rapporteur and Co-Rapporteur, the BWP agreed that ad interim that both Quality Grounds for Refusal (Ground !) remained negative and not resolved.

Although the BWP had previously acknowledged certain justifications, and even indicated a willingness to accept some data post-MA, the overall conclusion at this stage remains negative.

5.2.1.4. CAT discussion- Ground 1

The BWP both Quality Grounds for Refusal remained negative and not resolved.

The CAT in majority was of the view that the Ground 1 remain unsolved and should be addressed in an OE

The Agency would like to highlight the applicant that

- No new data produced after the opinion of the CHMP should be introduced in a re-examination procedure. Therefore any new data not submitted before the opinion would be considered out of scope of the procedure. (OC)

It is considered that annex 3 represent new data not submitted in the previous round. Therefore these data cannot be assessed. The applicant is asked to clarify. (OC)

- New analysis of data previously assessed are acceptable in a scope of a re-examination.

CHMP :

The CHMP agreed with the CAT.

5.2.2. Ground 2 (quality)

Media fill, process validation and filter data, supporting the suitability of the proposed commercial manufacturing process are missing, impacting sterility assurance of Component B.

5.2.2.1. Re-examination Rapporteur´s assessment

Filter's general information and validation are in compliance with EMA/CHMP/CVMP/QWP/850374/2015 (Annex 7-16), although validation of the filter hold time (Annex 10), validation of extractables and leachables substances from the filter (Annex 14) and validation of bioburden control & time limits (Annex 16) cannot be considered in this assessment because the data was obtained outside the time frame for this request. The information contained in Annex 17 and Annex 18 related to aseptic process simulation cannot be assessed for the same reason. Although some of the required data to solve this ground for refusal was provided, it cannot be evaluated. In consequence, the second ground of refusal remains: 'Media fill, process validation and filter data, supporting the suitability of the proposed commercial manufacturing process are missing, impacting sterility assurance of Component B'. **(MO)**.

5.2.2.2. Re-examination co-Rapporteur´s assessment

The individual aspects of sub-items MO2a to d are discussed separately in the following sections:

MO2a: With MO2a, a maximum filtration time should be defined and supported by filter retention data, demonstrating a capacity of minimum 10e7 CFU/cm2.

In response to D180 Quality Question 2 c, a study on bacterial retention conducted by Solvias with component B was presented (Q02_Annex 11). However, due to growth limitations, the filters could not be challenged with the required ≥ 10e7 CFU/cm2 in all replicates and therefore only resulted in a log reduction value of 6.3 – 6.7. With the same response it was also referred to a filter retention study initiated by Merck (P-24-0003494-BRTC, Q02_Annex 12), but the required data from the study were not fully available for the D195 second report.

In the oral explanation, the applicant indicated on the slide deck a filtration time of ≤ 3 min (flow rate of 25 –

50 mL/min) and also referred to the bacterial retention study R-24-0003494-BRTC, which has been completed in the meantime and in which a maximum retention capacity of 7.8·10e7 CFU/cm2 was achieved at maximum contact times of 40 minutes.

The maximum filtration time was thus defined, however, the data from the bacterial retention study R-24-0003494-BRTC dated July 4, 2025, were only provided as part of the request for re-examination and therefore cannot be considered here. Therefore, the re-assessment agrees with the agencies that MO2a remained unsolved at the end of the procedure.

Accordingly, the re-examination concludes that MO2a is still considered unresolved, but that new data is already available that may solve the underlying concern. As the clinical MO is considered resolved, it may be envisioned to downgrade as OC, to be resolved via commitment to strengthen the data base.

MO2b: MO2b requested that unless thoroughly scientifically justified, the sample volume should be increased, and the limit tightened for the bioburden sampling prior to sterilising filtration.

The re-examination does not fully support this MO with regard to the acceptance criterion for pre-filtration bioburden testing and thus the sampling volume. This is also reflected in the comments from Germany on the D150 and D195 JARs.

It is agreed that the proposed former bioburden acceptance limit with sampling of 2 mL is not in line with the EMA sterilization guideline (≤ 10 CFU/100 mL). However, it is noted that other guidance documents such as Ph. Eur. 5.1.1 and EudraLex Vol. 4 Annex I do not define a specific acceptance criterion, but refer to the efficiency of the sterilization method as determined during validation of sterile filtration (i.e. sufficient safety margins in context of the filter retention capacity and total volume filtered).

It seems reasonable to ask why the possibility of increasing the batch size was not considered, as noted by the agencies, but this does not appear to be an MO, provided that an acceptable justification is available for the current batch size and a sufficient safety margin in context of the filter retention capacity and total volume filtered is demonstrated as part of filter validation.

During the question rounds and on the slide deck in the oral explanation, the applicant stated that the current capacity limit per batch of component B is approximately 60 vials (i.e. maximum 60 mL), since this is a manual filling and capping process. The justification for the current batch size and thus, that sampling of 100 mL is physically impossible, therefore appears in general acceptable. However, during the oral explanation, the applicant has even proposed a change in the manufacturing process, which includes both a significantly higher sample volume of 30 mL (previously 2 mL) and a tighter limit of ≤ 3 CFU/30 mL (previously ≤ 1 CFU/1 mL) for the pre-filtration bioburden testing.

The requirements of the MO (increase in sample volume and tightening of the bioburden limit) are thus met.

Therefore, the re-examination concludes that MO2b is considered solved.

Point resolved

MO2c: With MO2c, a summary and conclusion of three consecutive media fill runs should be presented, including information on the evaluated batch size, fill time and aseptic hold times. Worst case conditions should be covered. Media fills should be in compliance with cGMP.

The re-examination agrees with the agencies that respective documents were not submitted in the request rounds and that the applicant has indicated the corresponding studies as ongoing during the assessment phases (e.g. D150 and D180 JAR).

In the oral explanation, the applicant referred to a batch size of 60 vials on the slide deck as well as general

process parameters such as a filling time for the vials of 1.5 hours and a duration of sterile filtration of 3 minutes. However, he pointed out that the requested data of the 3 consecutive media fill runs will be finalized by August 2025. Therefore, the re-assessment supports the agencies position that MO2c remained unsolved at the end of the procedure.

As announced, data from the media fill runs (VA_017_14.0_Report), were provided as part of the request for re-examination. However, these data cannot be considered here.

Accordingly, the re-examination concludes that MO2c is still considered unresolved, but that new data is already available that may solve the underlying concern. As the clinical MO is considered resolved, it may be envisioned to downgrade as OC, to be resolved via commitment to strengthen the data base.

MO2d: With OC 2d process validation data covering proposed maximum process times and hold times should be provided. The maximum time at room temperature should be defined and supported by data. Manufacturing and analytical testing should be performed in line with procedures and limits described in the dossier and response documents.

As the validation report for the manufacturing and filling of Component B has been provided after the oral explanation / opinion on the MAA, those cannot be considered for evaluation during the re-examination procedure. This is not considered a refusal concern, but could be downgraded to an OC and raise a condition for submission of the data in frame of a variation to a potential marketing authorization.

Conclusion of re-assessment MO2

Not fully resolved with the data available at time of CHMP opinion of initial MA assessment. However, new data are already available. Therefore, it may be envisioned to downgrade as OC, to be solved via commitments.

5.2.2.3. BWP discussion

Following discussion of the re-examination case presented by the Rapporteur and Co-Rapporteur, the BWP agreed that ad interim that both Quality Grounds for Refusal (Ground 1 and Ground 2) remained negative and not resolved.

Although the BWP had previously acknowledged certain justifications, and even indicated a willingness to accept some data post-MA, the overall conclusion at this stage remains negative.

5.2.2.4. CAT discussion- Ground 2

The CAT in majority was of the view that the Ground 2 remain unsolved and should be addressed in an OE

The Agency would like to highlight the applicant that

- No new data produced after the opinion of the CHMP should be introduced in a re-examination procedure. Therefore any new data not submitted before the opinion would be considered out of scope of the procedure and cannot be taken into account for assessment. (OC)

- New analysis of data previously assessed are acceptable in a scope of a re-examination.

In conclusion, the following data related to ground 2 are out of scope of the procedure

- Validation of the filter hold time

- Validation of extractables and leachables substances from the filter

- Validation of bioburden control & time limits

- Validation of filling

5.2.2.5. CHMP conclusion

The CHMP agreed with the CAT recommendations.

5.2.3. Ground 3 (efficacy)

The efficacy of Jelrix has not been demonstrated as the single-arm trial AAG-G-H-1624 was not able to isolate the treatment effect.

5.2.3.1. Re-examination Rapporteur´s assessment

The primary source of uncertainty in the assessment of the Marketing Authorisation Application (MAA) for Jelrix arises from the single-arm design of the pivotal study (AAG-G-H-1624), which precluded the isolation of the treatment effect. To support their position that the efficacy of Jelrix has been adequately demonstrated the applicant has presented several arguments that are outlined below.

Firstly, the applicant justifies the choice of a single arm design due to the lack of scientifically acceptable and feasible comparator for patients with cartilage defects of 4 to 12 cm^2. They argue that available treatments such as microfracture or osteochondral autografting techniques are typically indicated for smaller defects, while debridement/chondroplasty is not recommended as a standalone treatment in this patient population. Furthermore, comparison with another ACI product is not deemed feasible. In this context, an RCT comparing Jelrix against best SoC appears a possible option. In this regard, the applicant pointed out that this strategy presents high risk of bias and barriers to practical implementation due to the variability in treatment approaches, ethical concerns, recruitment problems, complexity of trial design and extended duration.

The difficulties in conducting an RCT in this setting can be acknowledged, but they do not preclude the feasibility of an RCT. In fact, given the subjective nature of the primary endpoint it appears essential. According to the *Reflection paper on in-vitro cultured chondrocyte containing products for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009),* "the study design should follow a randomised, controlled approach with appropriate comparator." Moreover, for patients with lesions of more than 4 cm^2 superiority against best SoC is proposed as a reasonable option.

It is also important to note that this application is based on a single-pivotal trial, with no replication. Moreover, the existence of a failed study (study AAG-G-H-1202) involving a similar product (NOVOCART 3D plus) adds, to some extent, additional uncertainty regarding the efficacy of Jelrix in treating cartilage defects. The limitations of a MAA based on one single pivotal trial were also highlighted in the SA (EMA/CHMP/SAWP/400714/2016): "[..] *it is uncertain that a single uncontrolled pivotal clinical trial without a previous compelling clinical development will be sufficient to provide consistent, sound and compelling evidence on the efficacy and safety profile of the product".*

Secondly, a justification on the clinical significance of treatment effect is provided. The primary endpoint of the study was the responder rate based on the total KOOS (calculated as the average of the 5 subscale scores) after 24 months of treatment. KOOS response rate was defined as the proportion of patients with ≥10 points improvement from baseline to the 24 months visit. A threshold of 40% for the 95% CI boundary of the KOOS overall responder rate was established to declare statistical significance. Based on these

assumptions, the study met the primary endpoint with KOOS response rate after 24 months of treatment of 93% (95% CI 86.1; 97.1) and 93% (95% CI 85.7; 97.0) at month 60.

To further substantiate the response threshold, a tipping point analysis was provided. According to this analysis, the point beyond which a threshold of 40% would not be reached is 41 at month 24 and 44 at month 60. This provide some reassurance that even considering a higher difference, the objective of the study would have been met. Nevertheless, it does not solve the uncertainties derived from the lack of a control arm.

In addition, the definition of responder appears to be in line with the literature and the one used in other clinical trials. The applicant claims that the results obtained with Jelrix appear comparable to those observed with other ACI products and the control groups used in the clinical trials, regardless of the responder criterion used. While the choice of a ≥10% improvement in KOOS can be considered justified, the main uncertainty regarding the treatment effect - stemming from the lack of a control arm - still remains.

Third, a justification is provided on the potentially confounding factors in the study, such as debridement and rehabilitation. It is understood that patients in the study AAG-G-H-1624 underwent (or may have undergone) debridement prior to Novocart Inject plus treatment and subsequent surgery was also allowed. Moreover, all patients received post-treatment rehabilitation.

The applicant argues that while debridement can offer short-term symptomatic relief it does not treat the underlying cartilage defect and therefore it is not recommended as a standalone option. On the contrary, they claim that Jelrix has shown the ability to repair the cartilage defect with high-quality hyaline-like cartilage. However, this affirmation is based on a limited number of cases. Graft status information is available for only 5 of the 7 patients from the phase 3 pivotal trial who underwent a second arthroscopy. Notably, one of these patients experienced a serious AE of "transplant failure". A few additional cases have been reported in patients treated with NOVOCART outside the pivotal trial, of which only 3 had biopsies. Thus, although these findings are promising, the limited number of data precludes drawing definitive conclusions.

While it is acknowledged that debridement may provide short-term symptom relief, the possibility that it may have contributed, to some extent, to the observed effect cannot be excluded. A similar consideration applies to the rehabilitation program. In the absence of a control group, it is not possible to determine the extent to which these interventions may have influenced the outcomes and therefore the actual effect of Jelrix cannot be firmly established.

In this context, the applicant presented a propensity score matched-pair analysis comparing Jelrix data from study AAG-G-H—1624 to an external control (microfracture) derived from a separate Phase III study conducted by the applicant (AAG-G-H-1202) in which both treatment groups underwent debridement and rehabilitation. A total of 144 patients were included in the matched pair set (72 patients per arm). Jelrix/Novocart® Inject showed superior efficacy in terms of KOOS change from baseline to the 24-month and the overall KOOS responder rates, among other endpoints. Notwithstanding, in addition to the intrinsic methodological limitations associated with this type of analysis/comparisons, substantial differences in baseline characteristics between the respective patient populations significantly constrains the interpretability of these results. Furthermore, such analyses are not deemed pivotal for regulatory decision-making and may only be regarded as informative.

Reference is also made to a publication of a retrospective registry-based matched-pair analysis comparing Novocart® Inject versus Spherox®.

Fourth, justification that the pivotal study meets regulatory standards for single arm trials. The applicant claims that the study broadly aligns with the principles outlined in the "*Reflection paper on establishing efficacy based on single-arm trials submitted as pivotal evidence in a marketing authorisation application*" (EMA/CHMP/458061/2024). However, one the principles of the guideline is that "*From a regulatory standpoint the primary endpoint of a SAT must also be objectively measurable and able to isolate treatment effects".* This is considered a key aspect for accepting a single-arm trial which is not fulfilled by the study AAG-G-H-1624.

Proposed indication

The initially claimed indication for Jelrix was: Repair of symptomatic, localised, full-thickness cartilage defects of the knee joint (International Cartilage Regeneration & Joint Preservation Society (ICRS) grade III or IV) with defect sizes up to 12 cm^2 in adults and adolescents with closed epiphyseal growth plate in the affected joint.

At the time of the CHMP opinion, the indication as proposed by the applicant was: Repair of symptomatic, localised, full-thickness cartilage defects of the knee joint (International Cartilage Regeneration & Joint Preservation Society (ICRS) grade III or IV) with defect sizes from 2 cm^2 to 12 cm^2 in patients with closed epiphyseal growth plate in the affected joint. Patient selection should be based on the clinical judgment, considering the individual patient's condition and the clinical data provided in section 5.1.

The applicant is now proposing a revised indication limited to defect sizes ranging from 4 to 12 cm^2, in line with the patient population enrolled in study AAG-G-H-1624. The indication as proposed by the applicant is as follows:

"Repair of symptomatic, localized, full-thickness cartilage defects of the knee joint (International Cartilage Regeneration & Joint Preservation Society (ICRS) grade III or IV) with a single defect size of 4 to 12 cm^2 in patients with closed epiphyseal growth plate in the affected joint"

The proposed indication could be considered acceptable, provided that the efficacy is convincingly demonstrated.

In **conclusion**, Jelrix has shown positive outcomes in terms of the KOOS responder rate, primary endpoint of the study, and several secondary endpoints. However, the open-label design of the single pivotal trial hampers the ability to isolate the treatment effect.

Further, MRI data were available for a limited number of subjects, with no baseline comparisons possible. Besides, no correlation was found between clinical and structural endpoints.

Finally, graft status information was sparse, preventing definitive conclusions.

As a result, the Ground for refusal 3 is not considered solved.

5.2.3.2. Co-Rapporteur's assessment

A single pivotal, single-arm trial with the patient-reported outcome PRO KOOS as primary endpoint, accompanied by secondary endpoints that are mainly subjective scores and a structural endpoint measured in only 25% of the ITT population was submitted by the applicant. It is agreed that it is considered difficult to isolate the treatment effect based on these data despite the (set of) endpoints was considered acceptable as it reflects relevant, both subjective and objective, validated endpoints for the target population and is in line with the Scientific Advice and the primary- and secondary endpoints having been met.

The primary endpoint overall KOOS responder rate at 24 months was reached in 93% of the patients (CI 86.1 – 97.1; p < 0.0001). The 93% exceeded the pre-specified margin for clinical relevance of 40%.

In that regard, it is acknowledged that some improvement of the KOOS in the observed timeframe might be achieved by cartilage debridement alone (DOI: 10.1016/j.arthro.2016.01.055, DOI: 10.2106/JBJS.23.00568). However, the observed magnitude of KOOS response seems rather unlikely to be based on debridement only. It is also acknowledged there is no proven correlation of MRI findings and KOOS, with a KOOS non-responder having a complete defect filling with intact surface, homogeneous repair tissue and a MOCART score of 95/100 based on MRI (listings 16.2.6.1 and 16.2.6.7).

However, the totality of evidence for Jelrix is not limited to the single pivotal trial with limited follow-up, which is not suitable for evaluating the true clinical benefits of the treatment, i.e. preventing osteoarthritis and delaying or preventing early knee arthroplasty.

Complete defect filling, as demonstrated in 18/25 patients at month 12 and 17/24 patients at month 24 in the pivotal trial is not expected in the natural course of focal, full-thickness cartilage defects of the knee joint if left untreated. However, complete defect filling is expected to reduce premature degenerative changes compared to the natural course and thus delay early knee arthroplasty. It is acknowledged that the number of patients who underwent MRI (n = 25) is limited and a rather small proportion of all enrolled subjects. Bias might be induced by a selection of patients. However, as the MRI substudy was planned a priori only in study sites that fulfilled the technical requirements, and MRI data is also available for a KOOS non-responder, the limited sample size is unfortunate but is not considered to introduce unacceptable bias. Despite the shortcomings, the MRI results are overall considered suitable to isolate the treatment effect.

Thus, efficacy as demonstrated by meeting the predefined endpoint in the KOOS after Jelrix treatment is complemented by confirmation of structural repair that can only be attributed to Jelrix, and that itself is associated with a reasonable expectation of long-term clinical benefit.

During the initial MAA, it was concluded that the safety profile of the Jelrix procedure and product is in line with what can be expected from autologous chondrocyte transplantation, with uncertainties remaining due to limitations in the safety dataset.

With the re-examination, the applicant proposed to amend the sought indication, now being restricted to 4-12 cm^2, which reflects the pivotal trial. Thus, no extrapolation of B/R consideration to smaller defects than included in the pivotal trial is needed.

Considering the totality of evidence, the Co-Rapporteur reaches a favourable benefit/risk conclusion, acknowledging that uncertainties in particular regarding the clinical benefit may need to be addressed post-marketing, e.g. through a registry-based study evaluating treatment failure rates after Jelrix.

The applicant pointed out that Spherox is currently the only authorised ACI on the European market with only one manufacturing site, and was unable to meet the broader demand across the European Union. One might agree that the high number of ACIs with the Jelrix equivalent under hospital exemption may indicate a medical need that cannot be fully met by Spherox alone. As the product to be marketed in Europe under the name Jelrix has been on the national market since 2008 and has been administered to thousands of patients, an approval with the aforementioned registry-based study as imposed measure might be in the patients' and physicians' interest.

5.2.3.3. CAT discussion on Ground 3

Based on presentation of both rapporteurs' views, at present the CAT by majority supports the Rapporteur view (Ground 3 not solved) but will reach a final opinion after consultation of the AHEG.

5.2.3.4. CHMP conclusion

The CHMP agreed with the CAT recommendation.

5.3. Ad Hoc Expert Group (AHEG) consultation

The applicant requested an Ad-Hoc Expert Group consultation during the re-examination procedure. The CAT adopted the list of questions to the Ad-Hoc Expert Group. The questions and answers provided during the AHEG meeting are listed below :

5.3.1. LoQ to the AHEG

The Experts are invited to discuss the following questions:

1. Do the experts consider that the efficacy of Jelrix in the treatment of cartilage defects 4-12 cm2 can be considered demonstrated based on the results of the single-arm pivotal study AAG-G-H-1624? Aspects related to the lack of control and possibility to isolate the effect of Jelrix from concomitant surgical procedures and rehabilitation should be considered in the expert discussions.

2. How do the experts value and interpret the results of the MRI-substudy from a clinical perspective? In 25 patients with 31 cartilage lesions, MOCART sum scores improved from 70 (sd 22.4, median 75) at 12 months to 80 (sd 56.7, median 90) at 24 months. The main sub score 'defect filling' did not change between 12 and 24 months and was complete in about 60% of the patients; integration of graft tissue to border zone improved from 73% to 82% at 24 months.

3. Would a Randomised Clinical Trial of ACI in full-thickness focal cartilage defects ≥4cm² have been feasible? If yes, what would have been the comparator? Which clinical and structural endpoints are considered clinically relevant? Which endpoint(s) could be addressing the development of osteoarthritis?

5.3.2. AHEG meeting

An ad-hoc expert group meeting (AHEG) was convened on 31st October 2025 in the context of the re-examination procedure.

The minutes are provided below:

Question 1

Do the experts consider that the efficacy of Jelrix in the treatment of cartilage defects 4-12 cm2 can be considered demonstrated based on the results of the single-arm pivotal study AAG-G-H-1624? Aspects related to the lack of control and possibility to isolate the effect of Jelrix from concomitant surgical procedures and rehabilitation should be considered in the expert discussions.

The experts considered that study AAG-G-H-1624 demonstrated the efficacy of Jelrix for the treatment of cartilage defects of 4-10 cm2 for individual defects and up to 12 cm2 for multiple defects. The lack of control was unavoidable at the time when the study was designed. Mosaicoplasty surgery is only indicated in lesions below 2cm, chondrocyte autographs or other autologous chondrocyte implantation (ACI) products could be a comparator but were not available in the EU at the time of initiation of the study. ACI is only applied in very specific settings and not in a harmonized way in Europe. Debridement procedure only, as comparator, would not be possible as it is not indicated in large lesions, and it may worsen the cartilage defect. Also, rehabilitation would not be a good comparator as this is not a surgical procedure with intention to modify the structure. Furthermore, debridement is part of all surgical procedures and similarly occurs with rehabilitation. In conclusion no comparator was clearly considered feasible, and the single arm trial was a reasonable option. It was noted that this was in accordance with the scientific advice.

The Experts discussed the results of the study and considered overall that the positive effects observed on the KOOS score and MRI are indicative of an effect, while acknowledging that due to the lack of control, the isolation of effects from the surgical procedure and rehabilitation is not possible.

The patient representative raised a concern about the lack of control as a limitation of this study.

Question 2

How do the experts value and interpret the results of the MRI-substudy from a clinical perspective? In 25 patients with 31 cartilage lesions, MOCART sum scores improved from 70 (sd 22.4, median 75) at 12 months to 80 (sd 56.7, median 90) at 24 months. The main sub score 'defect filling' did not change between 12 and 24 months and was complete in about 60% of the patients; integration of graft tissue to border zone improved from 73% to 82% at 24 months.

The experts agree that the quality of the MRI sub-study is very good and the MOCART scores results at 12 and 24 months and T2 mapping show favourable outcomes. The improvement observed after one year on MOCART score from 70 to 80 was considered positive, suggestive of progressive graft maturation in this population, where worsening can occur. The Experts discussed the observed large standard deviation at 24 months and agreed that it may be due to heterogeneity of the population, the low number of participants, and may derive mainly from 3 patients, who would have deserved more attention. A more detailed sub-group analysis of the clinical outcomes from the 25 patients would have been useful to get further insight but was not considered crucial for the outcome of the discussion. Biopsy is not recommended by any societies for this type of study due to the need of a second surgery in the patients and may not be considered due to ethical reasons.

Overall, all experts agreed that, although an MRI was not available for the 3/4 of the studied population, which would have been useful even if not as sophisticated as what was performed, the provided MRI results observed that the MOCART score supports structural improvement and should be seen as supportive to the KOOS/clinical outcome data. All experts agreed that a correlation with clinical outcome is usually not

observed in the literature which is a rather expected result in this heterogenous patient population. It was discussed that a demonstration of clinical correlation with structural endpoints would require a much higher sample size together with more objective outcome measures.

While agreeing on the positive supportive results, the Experts highlighted their limitation due to the limited sample size of 25 evaluated patients.

Question 3

Would a Randomised Clinical Trial of ACI in full-thickness focal cartilage defects ≥4cm² have been feasible? If yes, what would have been the comparator? Which clinical and structural endpoints are considered clinically relevant? Which endpoint(s) could be addressing the development of osteoarthritis?

RCT:

The AHEG agreed that a RCT with ACI was not feasible at the time of development of study. Nowadays, it might have been feasible but not in all countries.

Comparator:

The experts acknowledged that having a comparator would be the ideal approach. However, in a surgical trial, randomisation is limited by not having a good comparator that could be claimed as the standard of care.

Debridement is used in practice, however debridement is not a recommended treatment/SoC, especially for larger defects, as it can give worse results. Mosaicplasty is indicated for lesions under 2 cm^2, hence could not be a control in the current setting of larger lesions. The use of less acceptable sham/control treatment as control poses ethical problems. Similarly, rehabilitation only used as control may not be acceptable because the goals would not be structural, such as in a surgical procedure. In summary it was not possible to find appropriate control even if the chondrocytes alone or the gel alone would be the preferred control (in line with SA letters).

Clinical and structural endpoints:

The experts discussed that clinical endpoints should be the primary endpoints and structural endpoints supportive of clinical outcomes measures. KOOS is universally accepted as primary endpoint among the scientific community. IKDC, WOMAC could also be considered as additional measurements. QoL, EQ5D-5L can be also used for secondary endpoints.

The AHEG considered that a 60-month follow-up is necessary, but this would be done in post-marketing setting. It should include PROs, KOOS at 5 years, but also standard MRI in every patient, at least to have insight on structural remodelling on MRI for the whole patient population.

Prevention of osteoarthritis/endpoint:

The AHEG commented that at present there are no early measurements able to detect/present osteoarthritis at early stage. Standing X-rays is the most frequently used imaging in practice to evaluate knee osteoarthritis, but preferably in advanced stages. MRI could help as being the more sensitive. Ultrasound was also raised for degeneration, defects before being visible on X-rays. Recent initiatives are currently being developed for early osteoarthritis diagnosis and more instruments could be available in the future. In

summary, standing X-rays, MRI and PROMs are currently available, and other measurements are being defined that could be considered in future studies.

The patient representative mentioned that improvement in pain and function overtime plus durable cartilage repair preventing joint replacement would be desirable.

5.4. Discussion on the Benefit-risk (CAT discussion)

The CAT discussed the application following the AHEG meeting. The different views were presented as follows:

5.4.1. Rapporteur´s assessment

Quality

The current 5-gene panel is not validated, then it cannot ensure the potency, purity and identity of component A. Component B sterile filtration validation was also not validated at the time of the final decision. In conclusion quality grounds for refusal remain unsolved.

Clinical

Jelrix has shown positive outcomes in terms of the KOOS responder rate, primary endpoint of the study, and several secondary endpoints. However, the open-label design of the pivotal trial hampers the ability to isolate the treatment effect. Moreover, the application is based on a single pivotal trial.

Propensity score matched-pair analysis comparing Jelrix data to an external control (microfracture), from other Phase III study conducted by the applicant (AAG-G-H-1202) does not solve the current uncertainties due to the lack of controlled data. Such analyses are not deemed pivotal for regulatory decision-making and may only be regarded as informative.

Further, MRI data were available, but for a limited number of subjects, with no baseline comparisons possible, and no correlation found between clinical and structural endpoints.

In addition, graft status information was sparse, preventing definitive conclusions.

The safety profile appears consistent with what is expected for ACI products. Arthralgia, joint effusion and joint swelling were most common AEs. SAEs were infrequent.

The limited data available in adolescents does not raise new safety concerns. Thus, an indication in this subgroup of patients, as proposed by the applicant, could be supported, provided that the efficacy of the product is convincingly demonstrated.

The opinion of the Rapporteur did not change after the AHEG.

5.4.2. Co-Rapporteur´s assessment

Quality

are the markers that are currently in place. in combination with ACE are considered sufficiently qualified and validated for use in product release testing.

Additional data are requested for, and particularly for, which are intended to further support the marker matrix and were not applied during clinical development. Data for these markers may be requested post-approval.

As far as the proposed potency test was requested to demonstrate ability to identify samples with insufficient quality, the Co-Rapporteur understood this to be due to the concluded not demonstrated clinical efficacy, which is resolved from a Co-Rapporteurs point of view.

Regarding aseptic process validation and process validation for component B, new data are already available. Therefore, it may be envisioned to downgrade as OC and to solve via commitment.

Clinical

The main goals of focal cartilage lesion treatment are pain reduction, improvement of joint function, restoration of the damaged joint surface, and prevention or delay of the onset of osteoarthritis, thus ultimately prevention or delay of early knee arthroplasty.

Following Jelrix administration, pain reduction and improvement of joint function was demonstrated by KOOS improvement and restoration of the damaged joint surface was demonstrated in MRI imaging.

Overall, the safety profile observed in the pivotal clinical study is expected for a chondrocyte-containing product and its associated surgical procedures, and the risks are considered possible to handle through adequate warnings/information in the SmPC and educational materials.

After the AHEG, the opinion of the Co-Rapp remains unchanged and is further substantiated/supported by the positive outcomes of the AHEG discussion.

5.4.3. BWP outcome

Quality

The suitability of the currently proposed strategy to efficiently control purity, impurity and potency of Component A is not sufficiently demonstrated. These relate to specifications, identity and purity markers, validation and discriminatory nature of the potency assay.

The applicant provided a 5-gene panel in order to measure potency, purity and identity: are considered sufficiently qualified and validated for use in product release testing. were selected to improve the panel and were not applied during clinical development. The suitability of the new three markers is acknowledged. The complementing markers have been qualified, however, the validation of these could not be completely assessed due to missing validation data during the procedure.

It is agreed that the current panel could potentially have greater capacity to measure potency, identity and impurities, but further experimental support is still needed to sustain the proposed specifications, e.g. regarding detection of subpotent batches.

Media fill, process validation and filter data, supporting the suitability of the proposed commercial manufacturing process are missing, impacting sterility assurance of Component B.

Regarding aseptic process validation and process validation for component B, new data were made available that could not be assessed in the context of this re-examination procedure because it was generated after the issuing of the negative opinion.

The BWP position on the quality grounds for refusal was that they remained unsolved.

5.4.4. CAT discussion and conclusion

Overall, the CAT concluded that the benefit/risk balance of Jelrix is negative and that the grounds for refusal were negative.

The applicant was invited to an OE to discuss the outstanding grounds for refusal

5.4.5. Withdrawal of the application by the applicant

The applicant, taking into account the CAT AR, decided to withdraw its application before the CHMP discussion.

The Application was withdrawn on 11 November 2025