Examples of Substance Registration according to ISO-IDMP 11238 Substance Standard and EU Regulation, Structurally Diverse Substance

Cell-based medicinal products

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Advanced Therapy Medicinal Product (ATMP)

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Outline of the presentation

- Regulatory basis and definitions in accordance with the EU Directive and Guidelines;
- Description of the Cell-based products in view of the IDMP ISO-11238 Standard (Structurally Diverse Substances, source material human cells;
- Important data elements for unique identification of the cell products;
- Examples, different type of cell-based medicinal products:
  - 3 chondrocyte products
  - Viable epithelial cell-sheet
  - Active cellular immunotherapy product

Advance Therapy Medicinal Products:
1) Directive 2009/120/EC: Part IV

Somatic cell therapy medicinal product:

Somatic cell therapy medicinal product means a biological medicinal product which has the following characteristics:

a) Contains or consists of cells or tissues that have been subject to substantial manipulation so that biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered, or of cells or tissues that are *not* intended to be used for the same essential function(s) in the recipient and the donor;

b) Is presented as having properties for, or is used in or administered to human being with a view to treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its cells or tissues.

- The active substance shall be composed of the engineered cells and/or tissues
- Additional substances (e.g. scaffolds, matrices, etc) which are combined with manipulated cells of which they form an integral part shall be considered as starting materials, even if not of biological origin.

HUMAN CELL-BASED MEDICINAL PRODUCTS

Cells are complex biological systems

- Cells respond to (subtle) external triggers.
- Cells are heterogenic (intra- & inter-batch)
- Quality parameters determined by:
  - Origin and history of starting material
  - Each manufacturing step
  - Dissociation procedure of cells (mechanical/enzymatic/transport conditions)
  - Culture conditions (cytokines, media components, cell-cell contact, etc.)
  - Cell doubling level (de-differentiation)
- QC only limited part of Quality parameters

Risk-based approach

The following general risk criteria (non-exhaustive) can be used in the estimation of the overall risk of the product:

- Origin (autologous-allogeneic)
- Ability to proliferate and/or differentiate
- Ability to initiate an immune response (as target or effector)
- Level of cell manipulation (*in vitro/ex vivo* expansion/activation/ differentiation /genetic manipulation/ cryo-conservation)
- mode of administration (e.g. ex vivo perfusion, local or systemic surgery)
- Duration of exposure or culture (short to permanent) or life span of cell;
- Combination product (cells and bioactive molecules or structural materials)

HUMAN CELL-BASED MEDICINAL PRODUCTS

e.g.: **ChondroCelect; Hyalograft; MACI; Oranera; Provenge**

ChondroCelect

Autologous expanded cartilage-derived chondrocytes

Hyalograft

Autologous expanded cartilage-derived chondrocytes grown on hyaluronan scaffold

MACI

Autologous expanded cartilage-derived chondrocytes attached to a collagen membrane

Same Active substance?

Oranera (CAOMECS);

Multilayer cell-sheet of autologous expanded oral mucosal epithelial cells

Provenge: cellular immunotherapy product

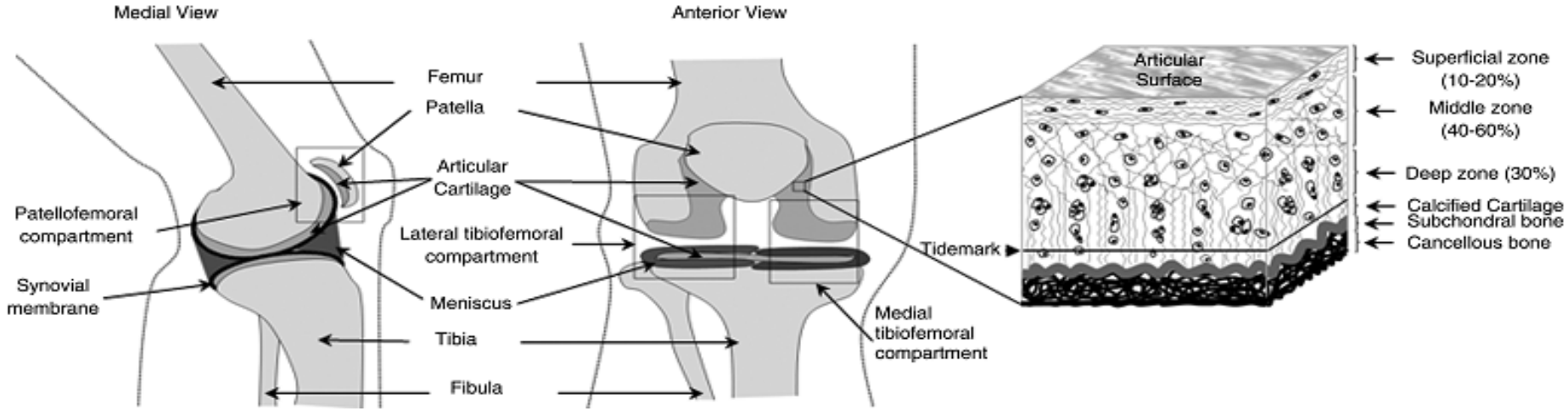
Autologous Peripheral Blood Mononuclear Cells activated with PAP-GM-CSF (PBMCs with rDNA fusion protein)

Advanced Therapy Medicinal Product: **Treatment strategies**

Strategies to promote the formation of hyaline-like cartilage by transplanted autologous chondrocytes, as hyaline cartilage has superior mechanistic properties:

- **Autologous Osteochondral-graft Transplantation (AOT) techniques, where fragments of healthy cartilage, including the chondrocytes, are harvested from non-bearing site and directly transplanted into the lesion.** *(Applicable for small lesions only)*
- **Autologous Chondrocyte Implantation (ACI), in which autologous chondrocytes are extra-corporally multiplied by cultivation.**
  After several weeks, the cells are back-transplanted in the lesions. *(In larger lesions.)*
  With ACI, implanted chondrocytes are kept in situ by a cover, consisting of either a **periosteal flap (first-generation ACI technique, ACI-P)** or a **collagen membrane (second generation technique, ACI-C)**
  The periosteal flap or collagen-membrane covers are applied to prevent cartilage hypertrophy.
  (ChondroCelect)
- ***Hyalograft and MACI*** (matrix-induced autologous chondrocyte implantation) are a spin-off of ACI-C.
  The chondrocytes are multiplied and seeded on the collagen /hyaluronan based scaffold prior to transplantation.
  The aim of developing MACI was to shorten and facilitate the ACI transplantation procedure, and to obtain a more equalized distribution of cells and cartilage forming. With ACI-C or ACI–P, cells are injected under the cover, which might cause uneven distribution or higher risk for leakage.

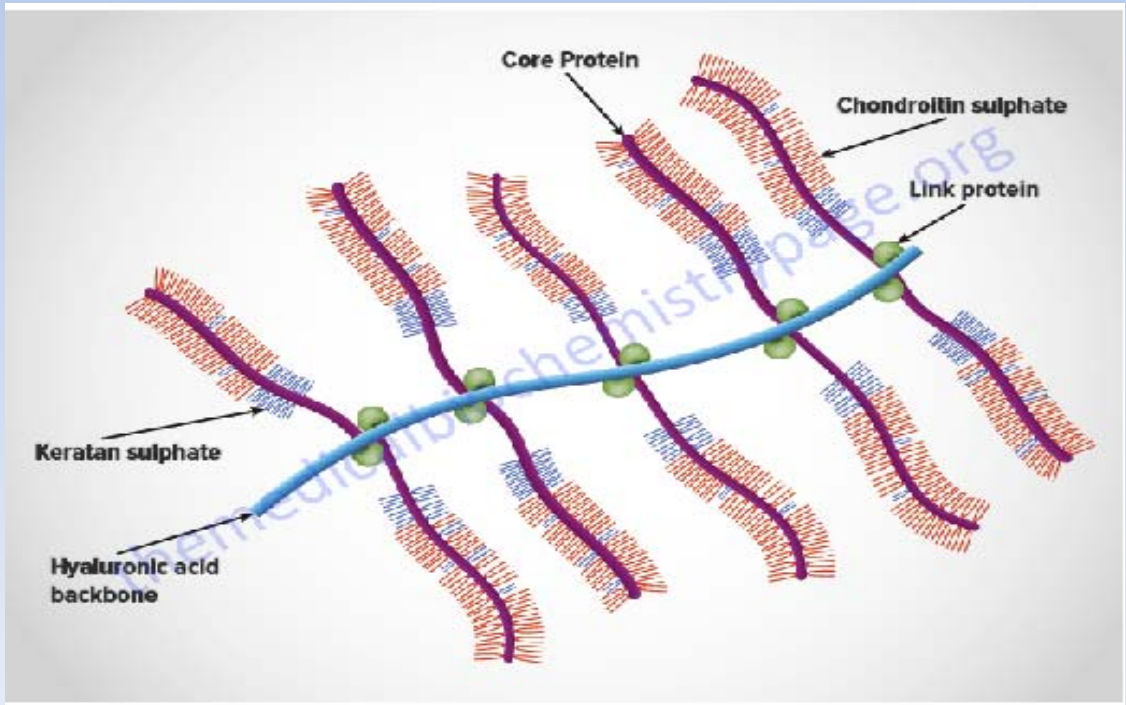
Figure 1.1.2 The structural and compartmental organization of the knee joint.



Advanced Therapy: Articular Cartilage

Proteoglycans (Aggrecan), Chondrocyte Marker

Aggrecan also known as **cartilage-specific proteoglycan core protein** (CSPCP) or **chondroitin sulfate proteoglycan 1. It** is **a protein that in humans is encoded by the ACAN gene. The encoded protein is an integral part of the** extracellular matrix in cartilagenous tissue and it withstands compression in cartilage.



Structure of an aggrecan-type complex proteoglycan

Aggrecan is a Proteoglycan (protein modified with large number of carbohydrates) and **the human form of the core protein has 2316 Amino acids** and has 4 domains: **(N-G1-G2-CS-G3-C).**

Mol. Mass > 2.500 kDa.

The core protein (210 -250 kDa) has 100 –150 Glyco-aminonoglycan (GAG) chains attached to it.

Advanced Therapy Medicinal Product:
General Information; Two Products: Hyalocraft C and MACI

The drug substance in Hyalograft C autograft is defined as characterized viable autologous chondrocytes expanded *in vitro* seeded and cultured on a *hyaluronan based scaffold.*

It is prepared by isolation of autologous chondrocytes obtained from patient's cartilage biopsy. Then the chondrocytes are expanded on monolayer, seeded and cultured on 3D-hyaluronan based scaffold (Hyalograft C).

The scaffold is a non-woven pad composed of a hyaluronic acid benzyl ester polymer and is a class III Medical Device CE marked by a Notified Body.

The Product is indicated for surgical repair of symptomatic cartilage defects of the femoral condyle caused by acute or repetitive trauma.

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The drug substance in MACI (Matrix-induced autologous chondrocyte implantation) is defined as a suspension of human viable autologous chondrocytes expanded *ex vivo* from autologous cartilage tissue with the expression of specific marker genes, seeded onto *Type I/ III Collagen membrane* at a density of 0.5 – 1.0 x 10^6 cells/ cm^2.

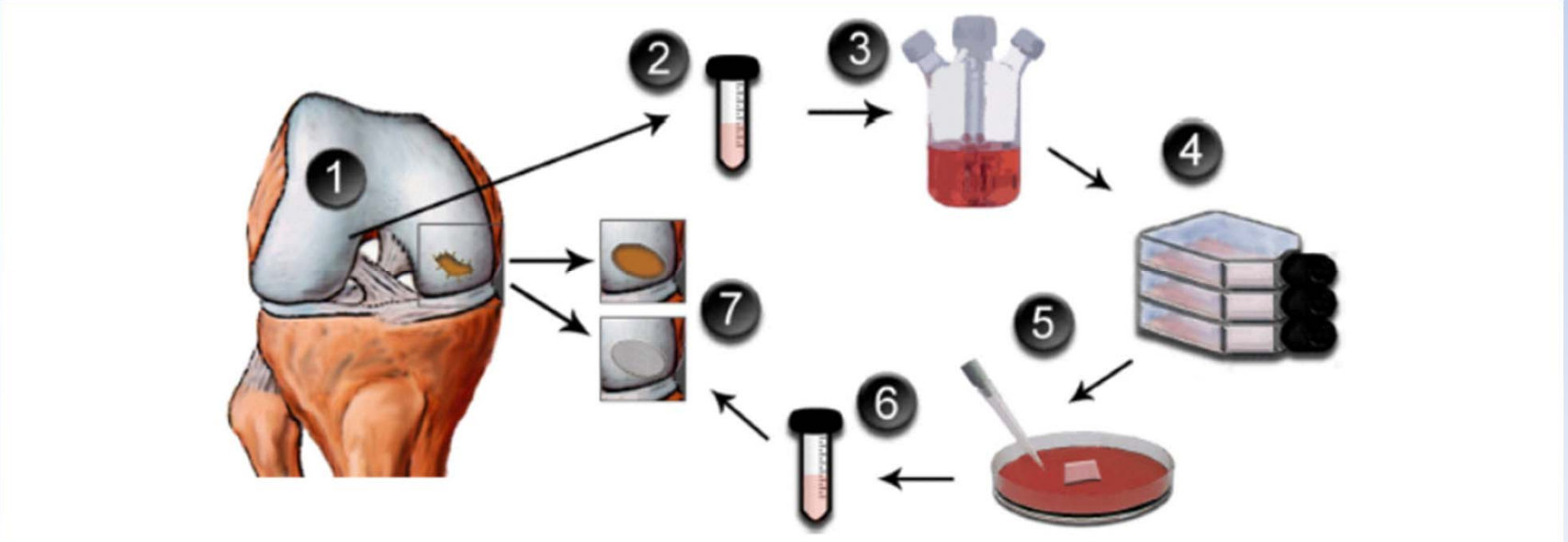
The membrane is a purified resorbable porcine peritonium collagen scaffold and is trimmed to the correct size and shape of the cartilage defect and held in place onto the lesion with a fibrin sealant. The matrix is a CE marked Class III device in Europe.

The Product is indicated to be used in skeletally mature patients for the repair of symptomatic cartilage defects of the knee (grade III and IV of the arthroscopic staging of osteochondrial lesions.

Advanced Therapy Medicinal Product:
Manufacturing Process, Identification, Characterization

The manufacturing process of the Active Substance consists of the following steps:

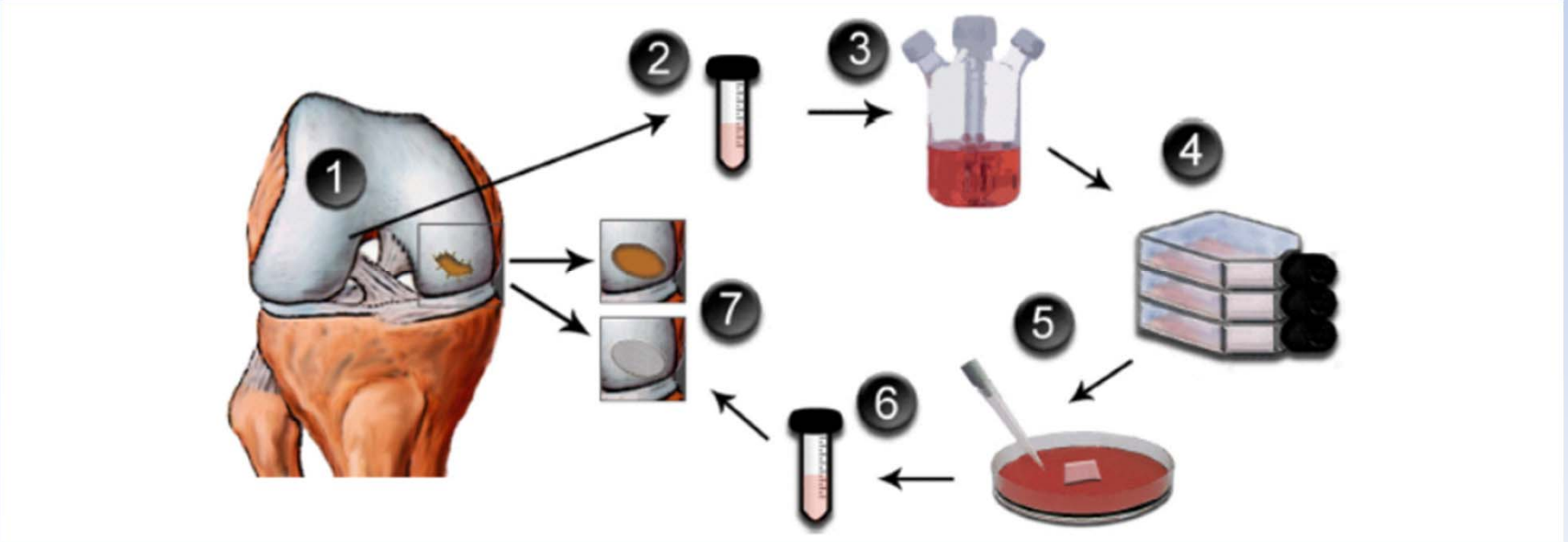
- **Biopsy digestion (mechanical/collagenase)**
- **Expansion culture (growth factors, cell doubling level)**
- **Cell culture harvest (trypsine) and wash**
- **Pooled cell suspension is seeded onto a membrane / scaffold**
- **Incubation period on membrane**



Advanced Therapy Medicinal Product:
Manufacturing Process, Identification, Characterization

Critical Steps:

- Total Cell count and viability after biopsy processing/ collagenase treatment;
- Cell yield, viability, days in culture and viability for the total expansion step;
- Adhesion of cells to the membrane
- Critical quality specifications measured during expansion and growth on the scaffold and specifications for morphology assessment



Identification, Charaterization, Potency

- Identification of essential features needed to form hyaline cartilage
- Molecular markers (at both the phenotypic and epigenetic levels), which provide measures of degree of differentiation towards chondrocytic or fibroblastic lineage
- No truly chondrocyte-specific genes have been found: expression of gene markers normally abundant in chondrocytes decreases greatly in culture

IDENTITY TEST

An assay technique was developed that will distinguish cultured human chondrocytes from human synovial fibroblasts (synoviocytes), the cell type most likely to contaminate an articular cartilage biopsy. HAPLN1 is over expressed in chondrocytes, while MFAP5 was consistently over expressed in synoviocytes.

A qPCR assay was developed that measures HAPLN1 and MFAP5 mRNA expression in a MACI implant drug product sample following standard techniques.

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Identification, Charaterization, Potency and Results

POTENCY TEST

- The MACI potency assay measures the gene expression of aggrecan (ACAN) using the same PCR technology described for the identity assay, and is performed with RNA isolated from the same 0.5 cm2 sample.
- **Aggrecan expression** is normalized to housekeeping gene GAPDH.

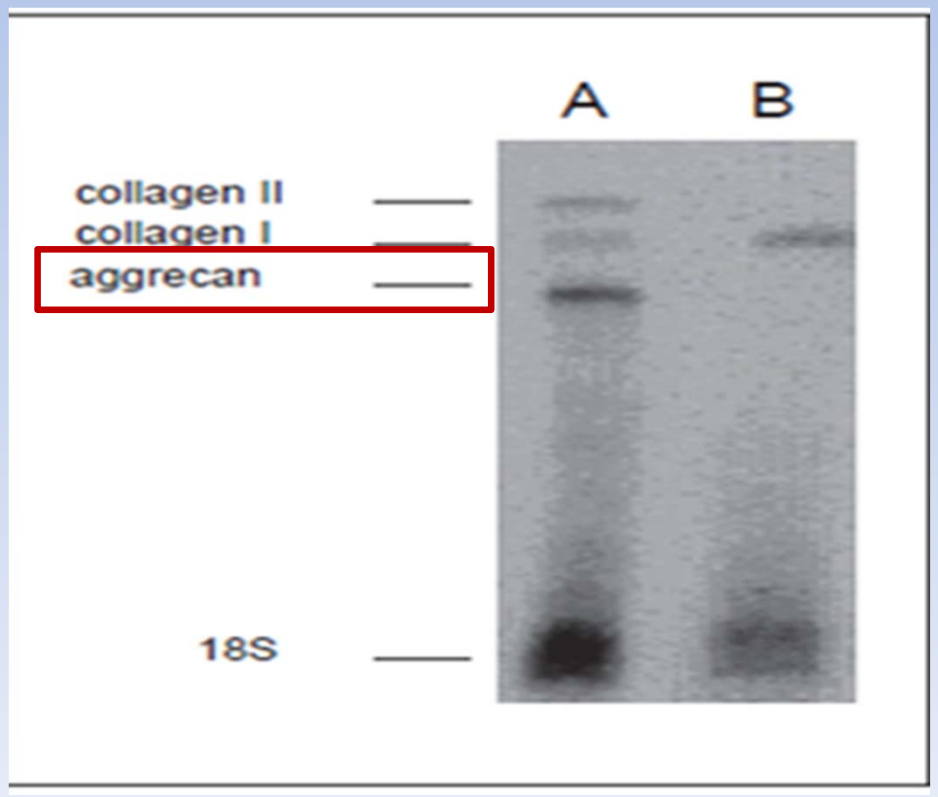


Fig.: Expression of Collagen II, Collagen I and Aggrecan in Chondrocytes (lane A) or Dermatan Fibroblasts (lan B) recovered from MACI Assemblies and Cultured in Alginate.

Advanced Therapy Medicinal Product:
Potency, Identification, Characterization

Potency

- ChondroCelect: Cellular expression patterns of genes relevant for cartilage and chondrocyte biology
- MACI: Aggrecan mRNA expression by real-time PCR.
- Hyalograft C: Measurement of COMP protein; Cartilage Oligomeric Matrix Protein (COMP): non-collagenous glycoprotein of articular extracellular matrix.

Identity

- MACI: Quantitative RT-PCR assay based on expression analysis of ratio of two markers, the chondrocytic marker HAPLN1 and the synovial/fibroblastic marker MFAP5. (synoviocytes/fibroblasts are impurities)
- Hyalograft C: RT-PCR assay of Aggrecan mRNA against a house-keeping gene in Chondrocytes vs. Fibroblast cells

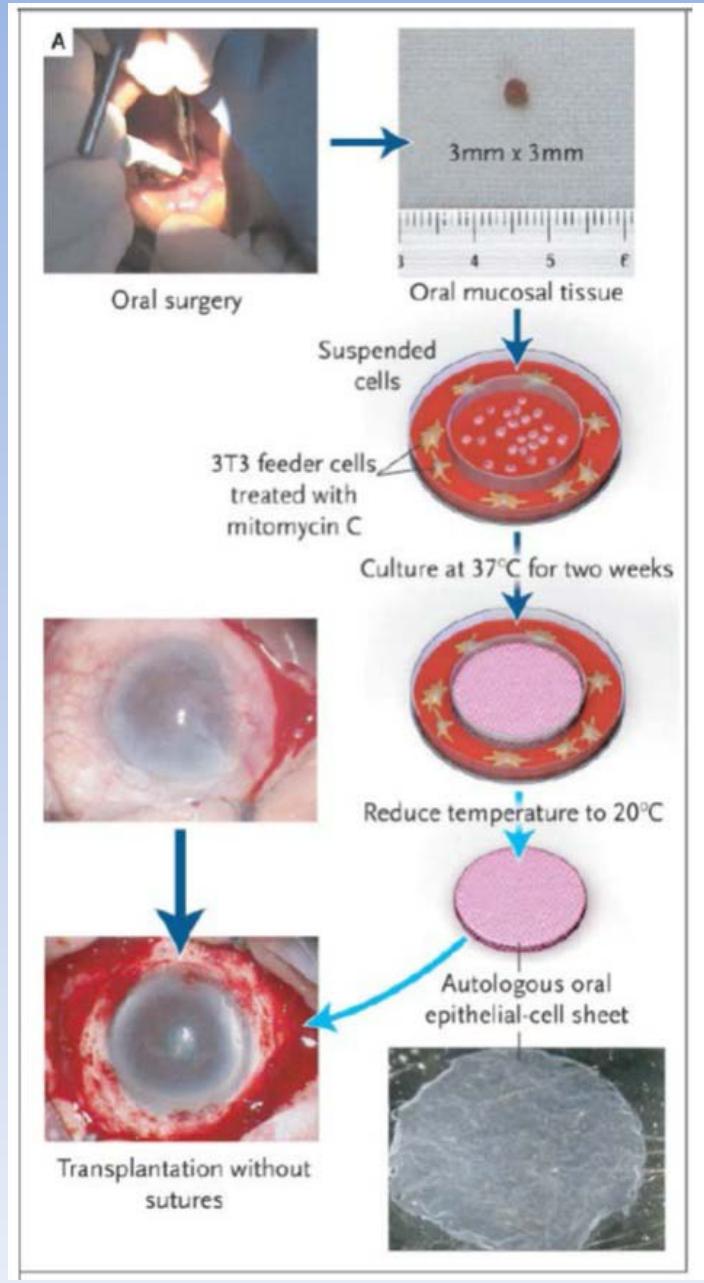
Cellular Impurities: Synoviocytes/fibroblasts

Advanced Therapy Medicinal Products

Oranera (previously known as CAOMECS; withdrawn)

- Tissue graft to replace damaged corneal epithelium in patients with limbal stem cell deficiency (LSCD)

- Cultured Autologous Oral Mucosal Epithelial Cell-Sheet (CAOMECS) is a multilayer cell-sheet produced by culturing of oral mucosal epithelial cells obtained from buccal mucosa)



Advanced Therapy Medicinal Products
Tissue-engineered product: OraNera

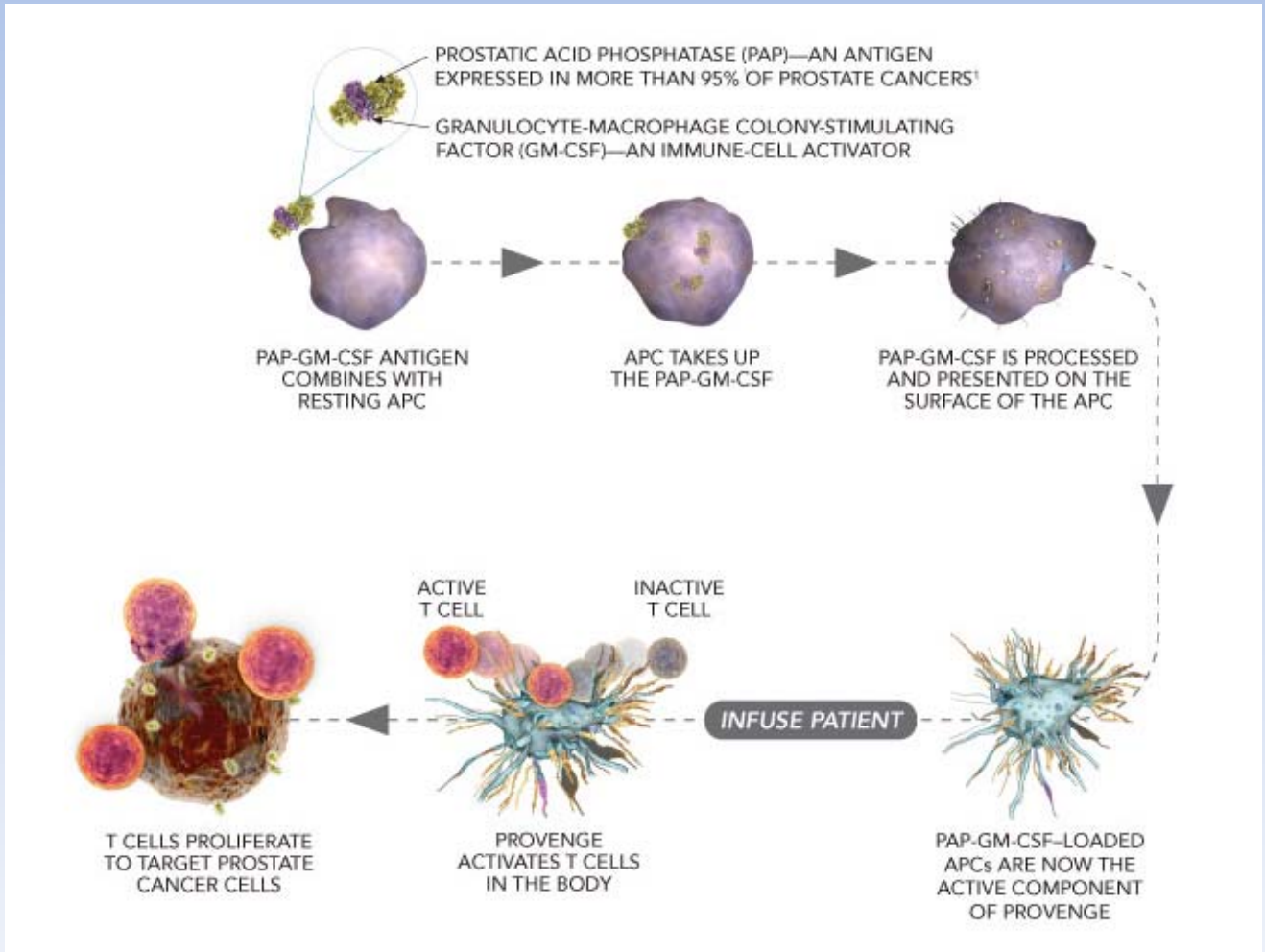
Manufacturing steps:

- Biopsy & Transportation
- Preparation of single mucosal cells by dispase and trypsin treatment/ Cell culturing in the presence of feeder cells
- Packaging the multi-layered cell sheet (Drug Substance)
- Transportation to the hospital where the sheet is harvested and grafted (Drug Product)
- Specific Cell Culture medium components: feeder cells treated with Mitomycin C; foetal calf serum and penicillin

- Active substance: Viable epithelial cell-sheet
- Defined as: Tissue-engineered product
- Potency: Epithelial stem cell marker p63 (insufficient)
- Identity: Mucosal epithelial cell and epithelial stem cell markers

Advanced Therapy Medicinal Products
Cellular immunotherapy product: Provenge

INN: Autologous peripheral-blood mononuclear cells activated with prostatic acid phosphatase granulocyte-macrophage colony-stimulating factor (sipuleucel-T)



Cellular immunotherapy product: Provenge
Active substance

- The active substance (also referred to as Sipuleucel-T) consists of autologous PBMCs, including APCs that have been activated ex vivo with the recombinant fusion protein PA2024.
- The fusion protein is composed of prostatic acid phosphatase (PAP) fused to granulocyte-macrophage colony-stimulating factor (GM-CSF).
- PAP uptake into APCs is followed by intracellular processing and presentation of PAP-derived peptides on Major Histocompatibility Complex (MHC) molecules to T cells.

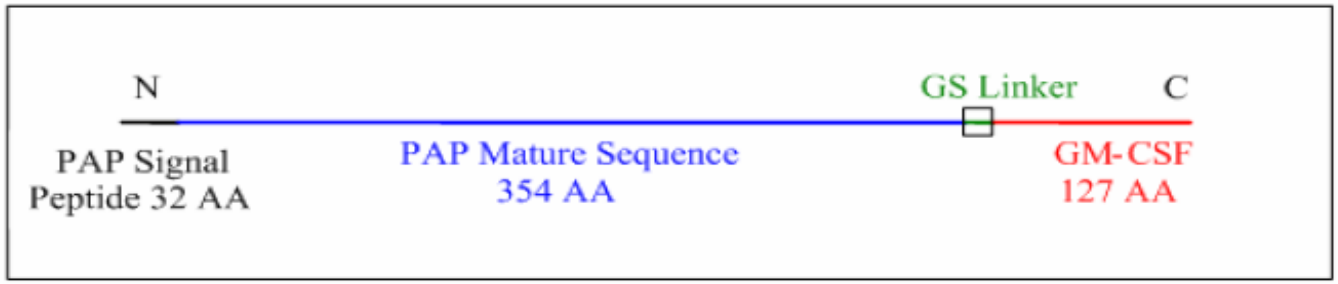
- **Other mononuclear cell types found in the product include: T cells, B cells, Natural Killer (NK) cells, and APCs (including monocytes and dendritic cells (DCs)). Activated APCs are contained within the CD54+ cell population, which includes monocyte-derived APCs and DCs.**
- **DCs (the most effective APCs) represent a small percentage of all cells.**
- **CD54 expression is used to assess product potency of Provenge by measuring the number of CD54+ cells, and CD54 upregulation during ex vivo culture.**

Cellular immunotherapy product: Provenge
Fusion Protein

- The recombinant fusion glycoprotein is a starting material used in the manufacture of sipuleucel-T consisting of a prostatic acid phosphatase (354 AA) linked to granulocyte-macrophage colony-stimulating factor (127 AA) by a glycine-serine dipeptide.
- It has 5 potential N-glycosylation sites and 1 potential O-glycosylation site.
- The molecular mass of the PA2024 is appr. 132 kDa because it exists in solution as a dimer.

- PA2024 consists of the following:
- **PAP signal peptide** - 32 amino acids, proteolytically removed during post-translational processing
- **PAP mature protein** - 354 amino acids with three potential N-glycosylation sites (two amino acid substitutions: valine to isoleucine at residue 264 and serine to arginine at residue 384)
- **Glycine/serine dipeptide synthetic linker**

Figure 1: Schematic of Precursor Form of PA2024 Fusion Protein



Cellular immunotherapy product: Provenge
Fusion Protein (*continued*)

Figure 2: PA2024 Amino Acid Sequence (Precursor Protein)

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  1 MRAAPLLLAR AASLSLGFLF LLFFWLDRSV LAKELKFVTL VFRHGDRSPI  50
 51 DTFPTDPIKE SSWPQGFGQL TQLGMEQHYE LGEYIRKRYR KFLNESYKHE 100
101 QVYIRSTDVD RTLMSAMTNL AALFPPEGVS IWNPILLWQP IPVHTVPLSE 150
151 DQLLYLPFRN CPRFQELESE TLKSEEFQKR LHPYKDFIAT LGKLSGLHGQ 200
201 DLFGIWSKVY DPLYCESVHN FTLPSWATED TMTKLRELSE LSLLSLYGIH 250
251 KQKEKSRLQG GVLINEILNH MKRATQIPSY KKLIMYSAHD TTVSGLQMAL 300
301 DVYNGLLPPY ASCHLTELYF EKGEYFVEMY YRNETQHEPY PLMLPGCSPS 350
351 CPLERFAELV GPVIPQDWST ECMTTNSHQG TEDRTDGSAP ARSPSPSTQP 400
401 WEHVNAIQEA RRLLNLSRDT AAEMNETVEV ISEMFDLQEP TCLQTRLELY 450
451 KQGLRGSLTK LKGPLTMMAS HYKQHCPPTP ETSCATQIIT FESFKENLKD 500
501 FLLVIPFDCW EPVQE                                      515
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∇—Potential glycosylation sites
Cyan—PAP signal peptide, 32 amino acids
Dark blue—PAP mature protein, 354 amino acids
Orange—Amino acid substitutions
Green—Glycine/serine dipeptide synthetic linker
Pink—GM-CSF mature protein127 amino acids

The mature PAP amino acid composition is presented in fig.2

Biological activity of fusion protein has determined by measuring the bioactivity of GM-CSF using an in vitro TF-1 cell-proliferation based assay.

Advanced Therapy Medicinal Products
Complexity is still to come

Cell products in development /conceivable :

- Cells transduced with viral vector
- Fused cells
- Hepatocytes
- Stem cells
- Stem-cell derived hepatocytes
- Stem-cell derived myocard cells
- Cells with transplanted organelles (e.g mitochondria)
- Complete organs