Centre for Advanced Medical Products

CAMP
Guiding ATMPs to patients

Work Package 1: Process development

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SWElife

VINNOVA

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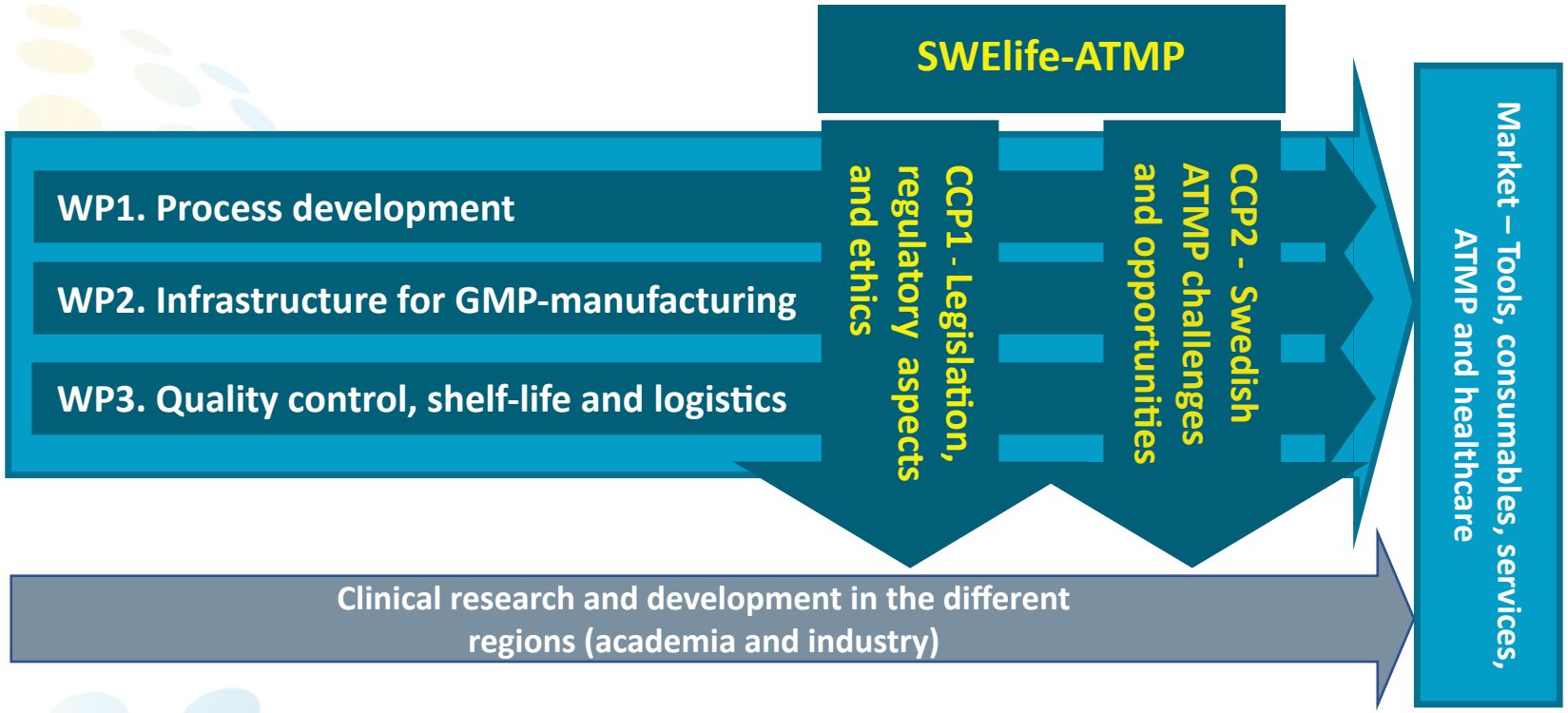
Dnr: 2017-02130

Research and development program

CAMP
Guiding ATMPs to patients

Work packages (WP1-3): Technologies and infrastructure to support and accelerate the translation of new treatments based on ATMPs to clinical phase

Cross cutting priorities (CCP1-2): Systems aspects, Swedish challenges and opportunities (operated within SWElife-ATMP project)



WP1 process development – Aims and Structure

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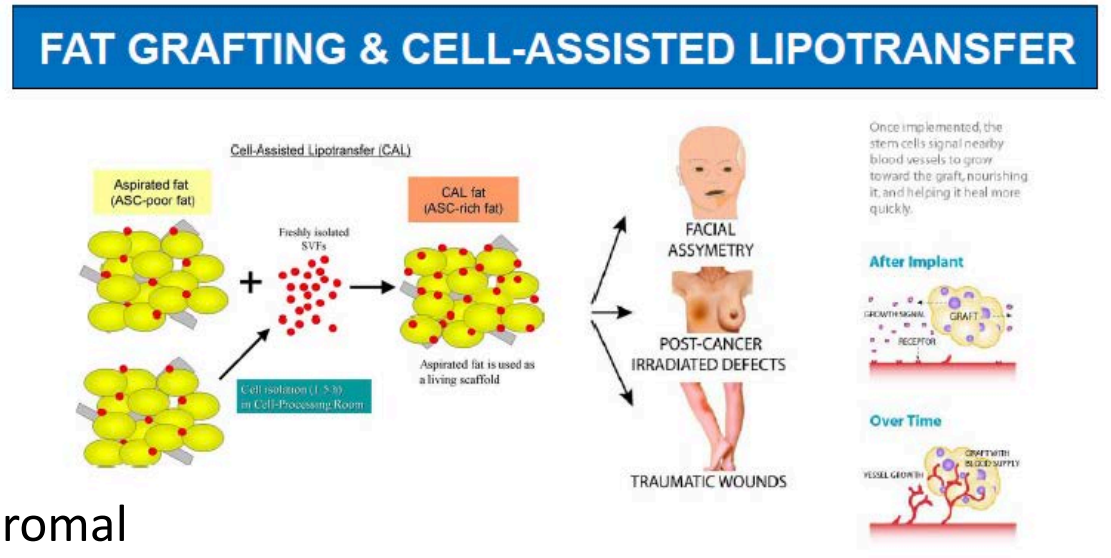
- Develop process development principles and methods for effective transfer from preclinical to clinical, GMP-compatible production.
- Generate a theoretical and practical knowledge base, available Sweden-wide (e.g. shared SOPs, practical training packages).

- Objectives are addressed in projects.
- Project work is conducted in close cooperation between partners.
- Current projects address:
  - Cell processing and cell preparation technologies
  - De- and re-cellullarisation techniques
  - Cell propagation/cell culture technologies
  - Extracellular vesicle technologies
  - Novel cell therapies

Label-free sorting of stromal cell preparations for clinical use

Background/Aims:

- Stromal (stem) cell preparations
  - High potential for regenerative, immune and gene therapies
  - Hetergeneous starting cell preparation and inhomogeneous products after culture
  - Aim: develop label-free sorting of (better) defined stromal cell products



Methods/technologies:

- Stromal cell isolation from human fat or bone marrow
- MNC enrichment (Sepax) & acoustic sorting (AcouSort)
- Propagation of stroma cells in culture, MSC characterization (phenotype, function)

Partners:

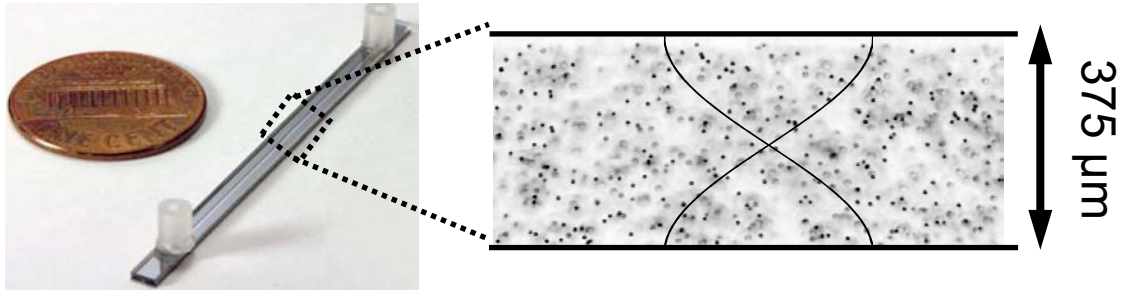
REGION SKÅNE · GE · AstraZeneca · AcouSort · Umeå Universitet · Västerbottens Läns Landsting

Label-free sorting of stromal cell preparations for clinical use

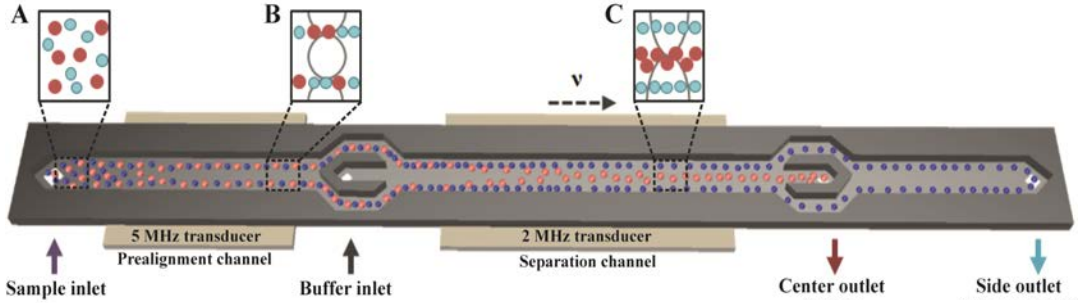
Results/Achievements:

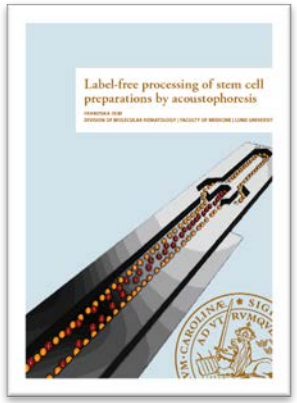
- Functionally different bone marrow stromal cell populations could be sorted without labelling (proliferation, clonogenicity)
- Primary colony-forming bone marrow cells could be enriched by acoustic sorting
- Overlap of phenotypically-defined SVF cell populations (marker expression, acoustic properties)

-> basis for future clinical applications



5 µm beads, acoustic resonance at ~2 MHz



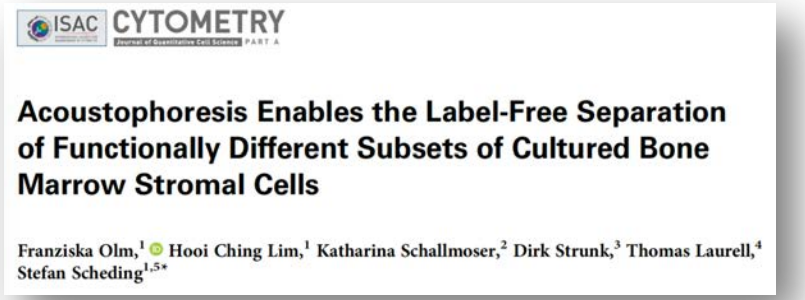



Microfluidics and Nanofluidics (2020) 24:64
https://doi.org/10.1007/s10404-020-02360-4

RESEARCH PAPER

Statistic estimation of cell compressibility based on acoustophoretic separation data

Fabio Garofalo[1] · Andreas Lenshof[1] · Anke Urbansky[1] · Franziska Olm[2] · Alexander C. Bonestroo[3] · Lars Ekblad[4] · Stefan Scheding[2] · Thomas Laurell[1]


ISAC CYTOMETRY

Acoustophoresis Enables the Label-Free Separation of Functionally Different Subsets of Cultured Bone Marrow Stromal Cells

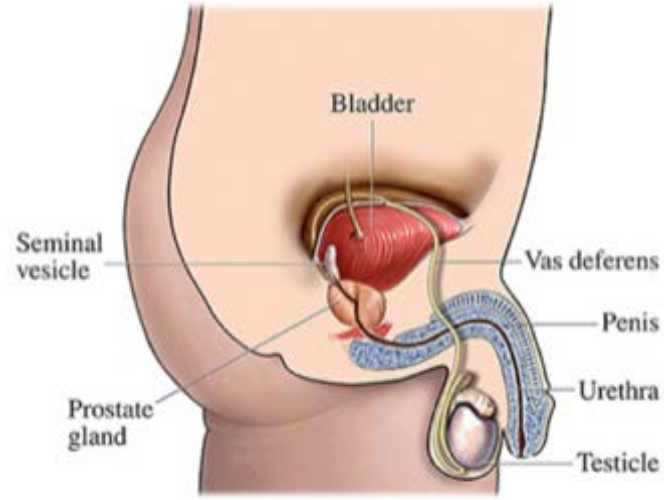
Franziska Olm,[1] Hooi Ching Lim,[1] Katharina Schallmoser,[2] Dirk Strunk,[3] Thomas Laurell,[4] Stefan Scheding[1,5*]

ATMP for urethra reconstruction with de- and recellularization technique

Background/Aim:

- Patients with urethral stricture, hypospadias, gender dysphoria -> unable to micturate
- Current open urethroplasty surgery techniques not sufficient

Aim: To develop a tissue-engineered urethra with autologous cells for implantation using de- and re-cellularization techniques.



Methods/technologies:

- Sheep model
- Buccal mucosa for epithelial cell culture -> Recellularization of allogeneic decellularized urethra
- Implantation of recellularized urethra into mucosa cell donor sheep

Partners:

VERIGRAFT Personalized Transplants

RI. SE

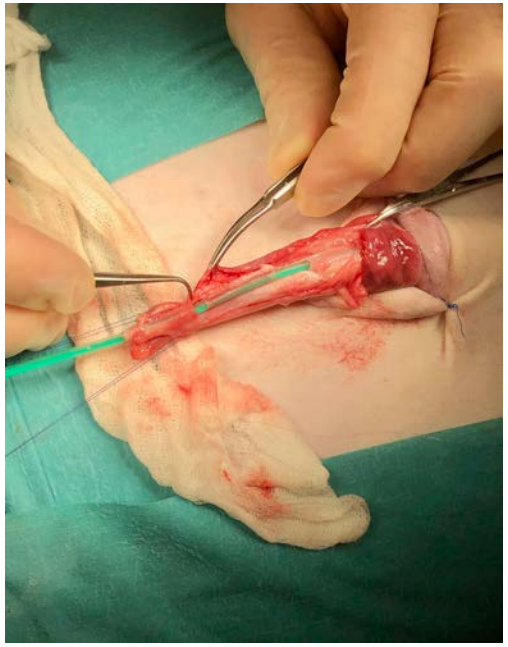
VÄSTRA GÖTALANDSREGIONEN SAHLGRENSKA UNIVERSITETSSJUKHUSET

GÖTEBORGS UNIVERSITET SAHLGRENSKA AKADEMIN

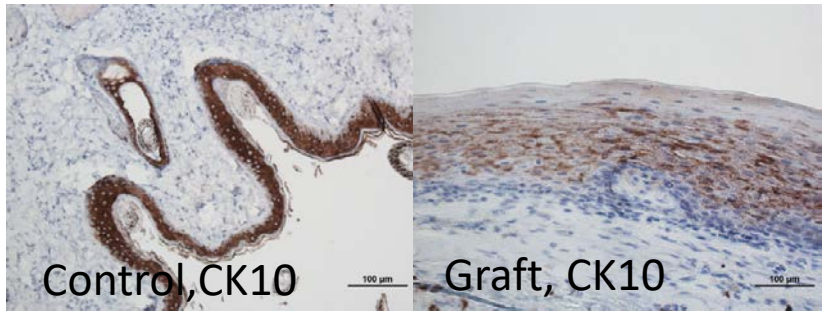
ATMP for urethra reconstruction with de- and recellularization technique

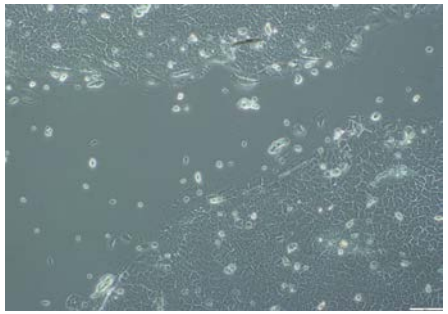
Results/Achievements:

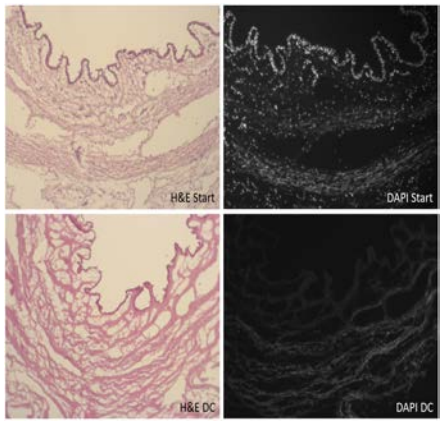
- Tissue collection, cell culture, de- and recellularization established
- Implantation of recellularized urethra
  - Epithelial cell engraftment (antibody staining)
  - Normal urethra anatomy post-op (X-ray)
  - Surgical complications in 1 animal



-> proof-of-concept achieved, base for clinical study



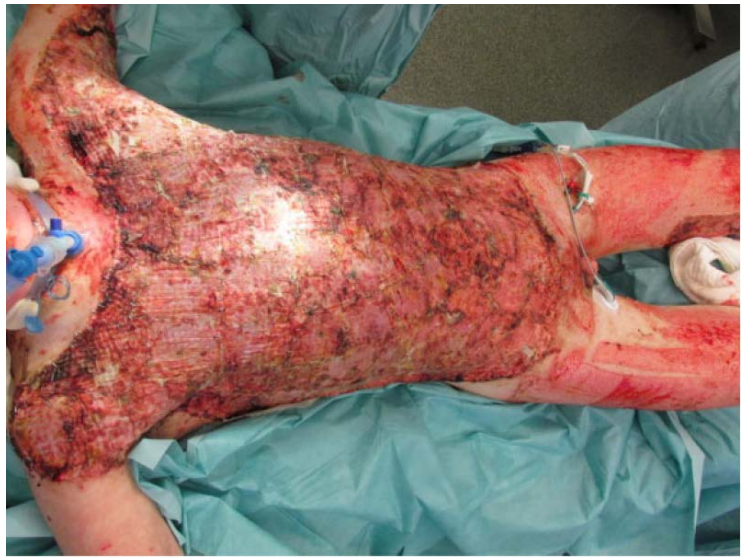




Development of Multilayered Tissue-Engineered Skin Construct

Background/Aim:

- Burn victims and chronic wound patients need a solution that provide faster recovery as well as physiological and cosmetically characteristics
- Aim: synthesize autologous cell-based personalized human skin for burn patients with extensive skin loss.



Houschyar et al. Plastic Surgery 2018

Methods/technologies:

1. Keratinocyte, fibroblast and adipogenic-derived stem cell (ADSC) isolation, expansion and differentiation in xenofree conditions.
2. Combination of cells with biomaterials
3. Bioprinting

- *In-vitro assays*
- *Ex-vivo standardized human skin burn model*
- *In vivo porcine surgical wound model*

Partners:

Region Östergötland

LINKÖPINGS UNIVERSITET

Karolinska Institutet

UMEÅ UNIVERSITET

conlega
biobusiness development and management

Development of Multilayered Tissue-Engineered Skin Construct

Results:

-> Workflow validation in autologous keratinocytes based model
-> Establishing optimum clinical protocol for stem cell differentiation
-> Apply approved workflow for stem cell based model


OPEN Human serum albumin as a clinically accepted cell carrier solution for skin regenerative application

Hady Shahin[1,2,3], Moustafa Elmasry[1,2], Ingrid Steinvall[1,2], Katrin Markland[4], Pontus Blomberg[4,5], Folke Sjöberg[1,2] & Ahmed T. El-Serafi[1,2,6]

SCIENTIFIC REPORTS | (2020) 10:14486

Established **combined extraction** of **keratinocyte, fibroblast and ADSC** from human and pig **skin** and human lipoaspirate, with acceptable yield, viability and characterization	**Keratinocyte isolation and expansion** in **xenofree** conditions established	To do: **ADCS differentiation** into **keratinocytes** under **xeno-free** conditions	Detailed **protocol** for **direct application** of **keratinocytes** for wound repair established
	MPA application for autologous keratinocyte application to burn patients ▼ Validation study in Vecura	**Direct application** of **stem cells** with/without biomaterials enhanced the **wound healing *in vivo*** and human **burn skin model** *ex-vivo*	Feasibility of **multi-layered construct (**fibroblasts + keratinocytes) was achieved as well as simple constructs of one layer of cells + biomaterials

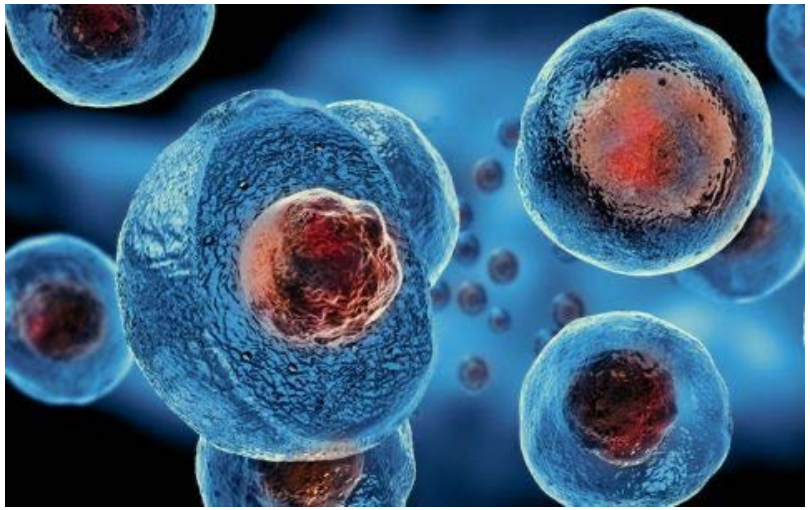
-> Basis for clinical keratinocyte trial + optimizing ADSC differentiation (epigenetic modifyers, biomaterials) and cell multilayer construct

GMP production of pluripotent stem cells and its derivatives

Background/Aim:

- To establish pluripotent stem cells (iPS and/or hES cells) for derivation of GMP approved cells for clinical studies.

- Aim: Seed bank and derivation of neuroprogenitors and retina cells (KI) and chondrocytes (GU).



https://biologydictionary.net/embryonic-stem-cell/

Methods/technologies:

- Workshops, discussions withs MPA.
- Cell culture technologies and methodology for determining pluripotency and end stage products.

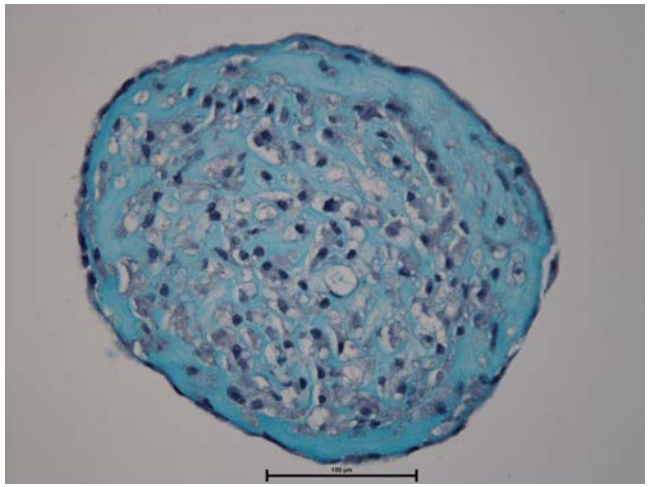
Partners: Gothenburg University, AstraZeneca, Vecura, KI, Uppsala Universitet, Takara Bio

GMP production of pluripotent stem cells and its derivatives

Results/Achievements:

- **KI cell-line registered in EU stem cell registry (https://hpscreg.eu)**
- **KI cell- lines transferred to Gothenburg University**
- **Chondrocyte differentiation protocol optimized together with TAKARA celline CHPS22.**
- **Celline transfer to Univ. of Leyden for MSC differentiation.**

iPS derived cartilage



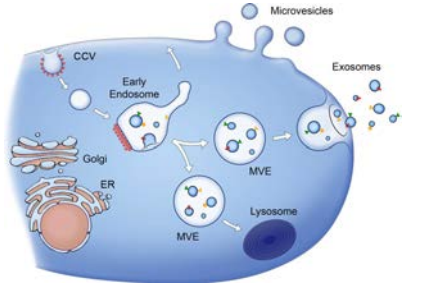
-> test of chondrocyte differentiated PSC in animal models

-> preparations for MPA discussions

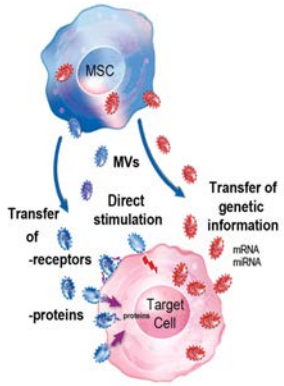
CAMP Extracellular Vesicles Projects – mRNA delivery and heart failure prevention

- EVs contain a number of molecules (proteins, lipids, miRNA, ect.)
- Central role in cell-cell communication, intercellular signaling, ...
- EVs transfer information to cells and affect cellular processes

-> potential cell-free therapeutics



Raposo and Stoorvogel, JCB 2013



Biancone, Nephrol Dial Transpl 2012

Development of producer lines for delivering therapeutic mRNAs through Extracellular Vesicles

Karolinska Institutet – A. Hussain, E. Alici, A. Pasetto
Vecura – P. Blomberg
Vycellix - Douglas Calder
CellProtect - Lena Wikingsson
AstraZeneca - Marcello Maresca

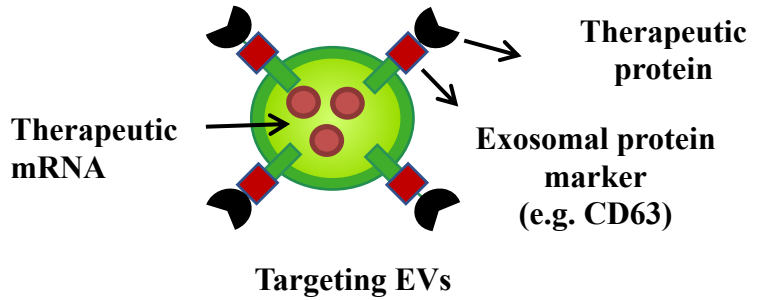
Extracellular vesicles (CARMEV) for prevention of heart failure

Akademiska University Hospital – K.H. Grinnemo
Uppsala University – J. Hilborn
AstraZeneca - L-M. Gan
IsletOne

Producer lines for delivering therapeutic mRNAs through Extracellular Vesicles

Aim:

- Tageted engineering of EVs for therapy



Results/Achievements:

- Cloning of Therapeutic Gene/s achieved
- Small scale EVs production established
- Therapeutic protein delivery realized
- PCR optimization/Antibody optimization for WB performed
- Stable Producer lines generated

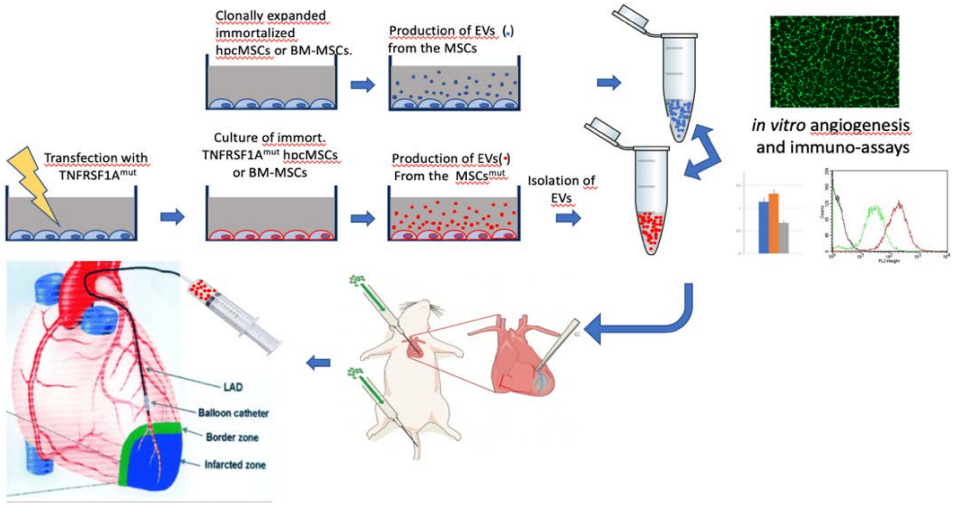
-> proceed with large scale production and testing, mRNA delivery, …

Extracellular vesicles (CARMEV) for prevention of heart failure

Background/Aim:

- Balloon dilatation (PCI) of the infarct-related coronary artery induces an ischemia-reperfusion injury (I/R) -> heart failure

- Aim: Develop extracellular vesicles (EVs) from bone marrow MSCs to inhibit I/R injury after MI -> heart failure prevention

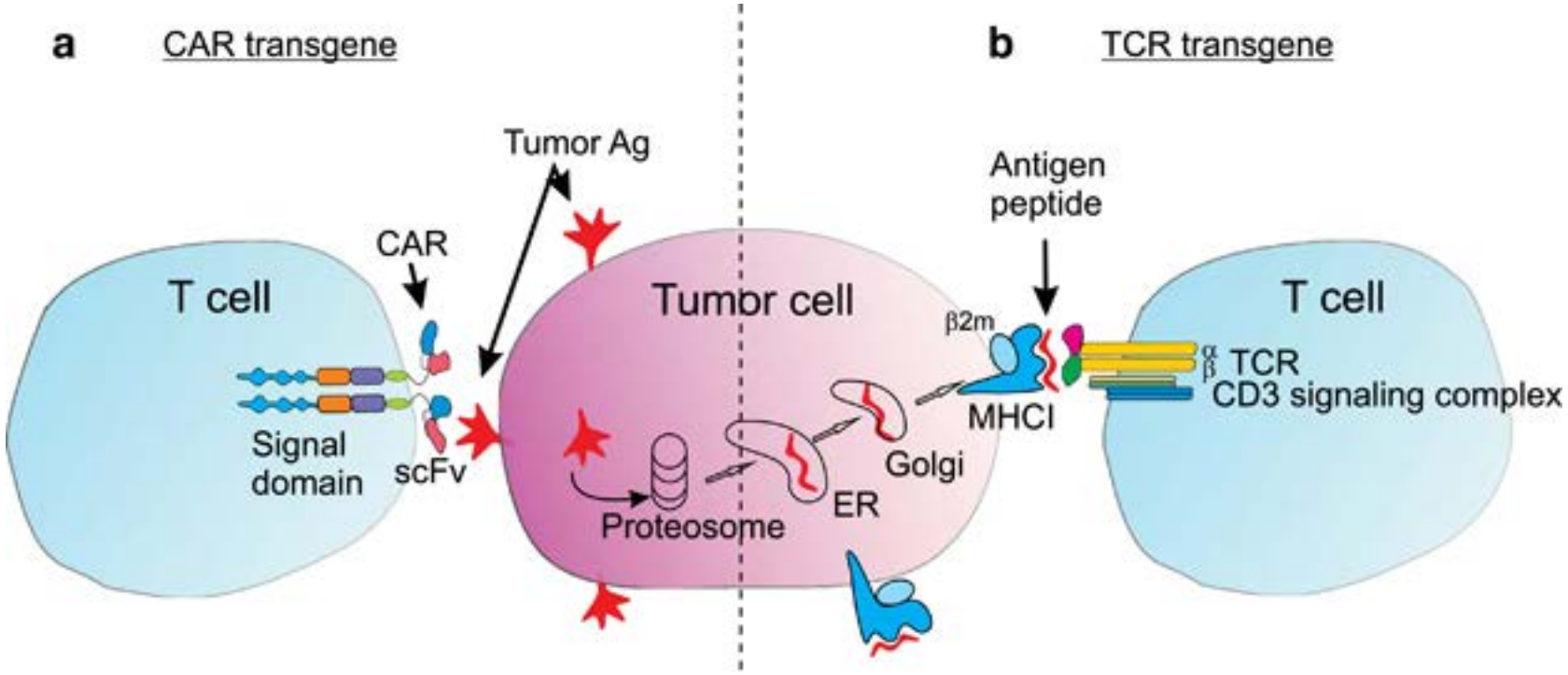


Results/Achievements:

- Fully GMP-adapted protocol for reproducible generation of EVs from BM-MSCs developed
- Patented MSC culture
- EV isolation optimized TFF (Tangetial Flow Filtration)
- Animal experiments ongoing

CAR-T cell therapy for malignant melanoma of the skin and the eye

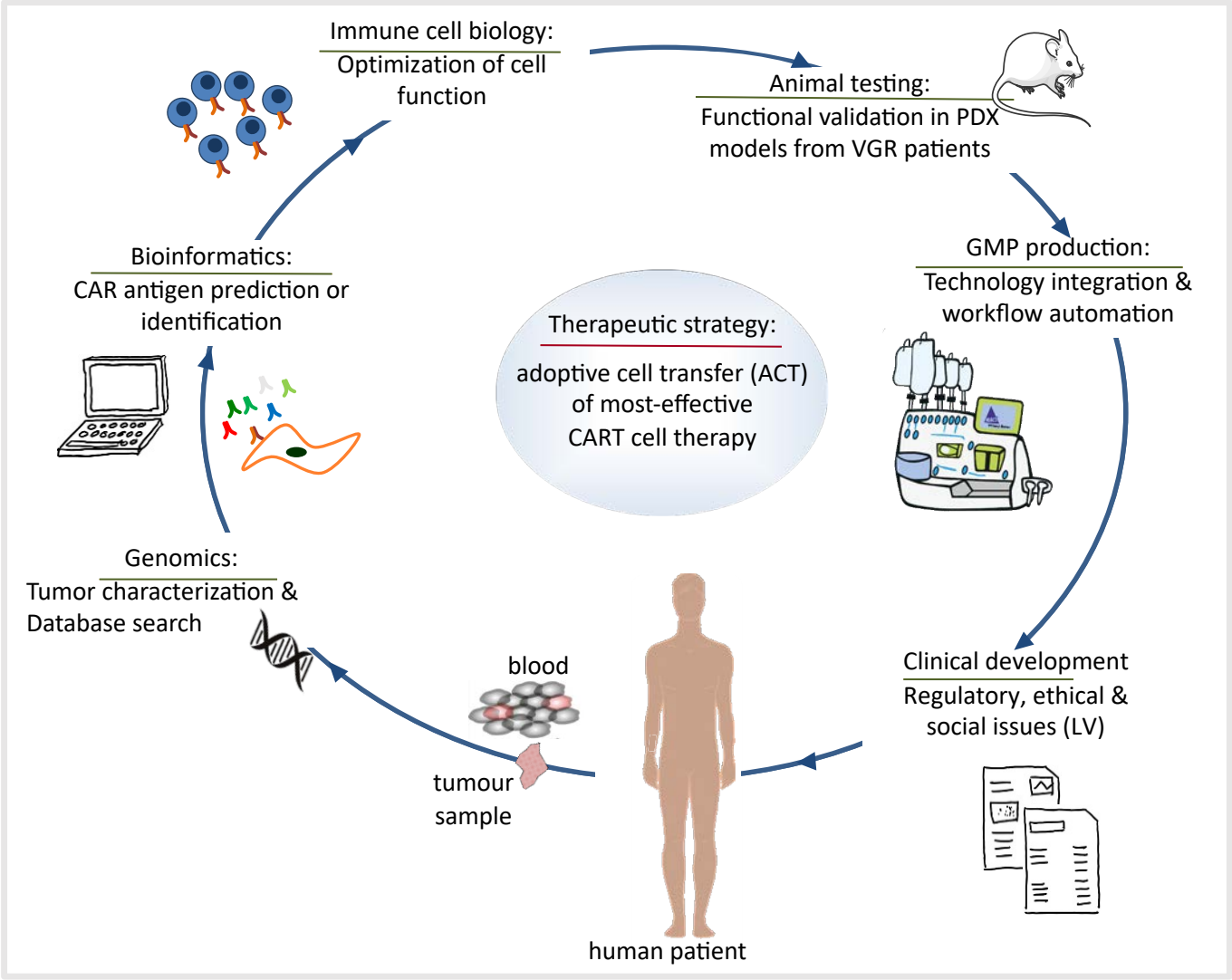
CAR-T cells can elicit tumor killing independent of MHC/HLA class 1



Signaling domain=
Eg CD3, CD28-CD3 or CD28-4BB1-CD3

CAR-T therapy against B-cell malignancies are approved but what about solid tumors?

CAR-T cell therapy for malignant melanoma of the skin and the eye



HER2 CAR-T Cells Eradicate Uveal Melanoma and T-cell Therapy–resistant Human Melanoma in Interleukin-2 (IL2) Transgenic NOD/SCID IL2 Receptor Knockout Mice

Cancer Research

Elin M.V. Forsberg[1,2], Mattias F. Lindberg[1,2], Henrik Jespersen[1,3], Samuel Alsén[1,2], Roger Olofsson Bagge[1,2], Marco Donia[4], Inge Marie Svane[4], Ola Nilsson[5], Lars Ny[1,3], Lisa M. Nilsson[1,2], and Jonas A. Nilsson[1,2]

University of Gothenburg – J. Nilsson, L. Nilsson
Sahlgrenska U Hospital – L. Ny, R. Olofsson A. Lindahl, A. Nelson
AstraZeneca – H. Gabrani