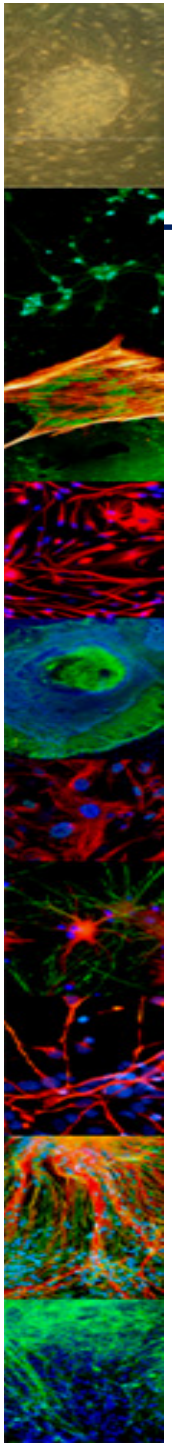

Victor Babes
University of Medicine
and Pharmacy
Timisoara

County Clinical
Emergency Hospital
Timisoara

Centre for Gene and Cellular Therapies in the Treatment of Cancer (ONCOGEN) Timisoara - A Step towards the Multidisciplinary Research in Eastern Europe

2014

CIFBT

A DECADE OF RESEARCH DEVELOPMENT

2001-2011

RESEARCH

INFRASTRUCTURE

PROFESSIONAL DEVELOPMENT

IMPACT

NATIONAL PROJECTS
FP6
FP7

POSDRU
(EU FUNDING)

CNCSIS
VIASAN
MATNANTECH
CEEX
SANCO
GENOSTEM (FP6)
CASCADE (FP7)
REBORNE (FP7)

SCIENTIFIC PAPERS
MONOGRAPHS
PATENTS
PROTOCOLS
PROCEDURES
CELL LINES
ANIMAL MODELS

ONCOGEN
(2010-2012)

CANCER RESEARCH CENTER
2013-2015

SECTORIAL OPERATIONAL PROGRAMME FOR HUMAN RESOURCES DEVELOPMENT
(2010-2013)

POSTDOC
PHD
MASTER
CURRICULAR DEVELOPMENT
MOBILITIES

HUMAN RESOURCES

10 new work places

15 maintained work places

Recruitment of young researchers

INFRASTRUCTURE

- 15 laboratories
  GMP facility
  BSL-3 facility
- Animal facility

AREAS OF RESEARCH

- Stem cells -fundamental studies
  - Mesenchymal stem cells
  - Hematopoietic stem cells
- Tissue engineering
- Reconstruction & regeneration
- Cancer research

R&D DIRECTIONS

STEM CELLS

Basic research Applied research

Pluripotent and Adult stem cells

CANCER RESEARCH

Basic research Applied research

Advanced immunotherapies with:

- dendritic cells
- NK cells
- cytotoxic cells

The Center for Immunophysiology and Biotechnologies Timisoara (CIFBT), oriented towards cellular therapies and regenerative medicine, belongs to the University of Medicine and Pharmacy Victor Babes Timisoara and was accredited as CNCSIS research center in 2004.
The research activity of CIFBT is coordinated by its director, Prof. Dr. Virgil Paunescu. Our multidisciplinary team comprises 22 members, specialized in cellular and molecular biology, biochemistry, immunology, biotechnology, clinical studies.

cifb timisoara
CENTER FOR IMMUNOPHYS. AND BIOTECHNOLOGIES TIMISOARA
UMFVBT

Birth of the Centre. The Seeds

EU policy in the field of research and technological development aims to establish the European Union as a leading knowledge-based economy

The ERA is the unified research area in which researchers, scientific knowledge and technology circulate freely, leading to:

- Better cooperation at every level,
- Better coordination of EU/national policies,
- Expansion of structural capacities,
- Increased networking

Birth of the Centre. The Root

EU funding opportunity for Romania: The Sectoral Operational Programme “Increase of Economic Competitiveness” (SOP IEC) is one of the 7 instruments for achieving the priorities of the National Development Plan 2007 – 2013, aiming:

- to strengthen the strategic focus of the Economic and Social Cohesion policies across Romania,
- to make the correct and appropriate linkages to the European policies and the Lisbon Strategy

Birth of the Centre. The Stem

The ONCOGEN Centre Timisoara

- is the result of structural funding granted through SOP IEC Romania, aimed to facilitate the translation of research results to advanced therapies applied in regenerative medicine and cancer
- is the only centre in this field in Western Romania

Facts:

- a total value of ~ 12 million EUR
- implementation over 44 months (2010 - 2014)
- a management team of 5 persons
- a support team of over 20 persons working in the background





Birth of the Centre. The Leafs

- **Centre for Gene and Cellular Therapies in the Treatment of Cancer → 2010-2014**
  - GMP Facility
  - Bio-bank
  - Stem Cell Department
  - Stem Cell Registry
  - HLA Typing Department
  - Immuno-phenotyping
  - Genomics, proteomics
  - Toxicology
  - Animal house facility (small/medium)

Birth of the Centre. The Leafs

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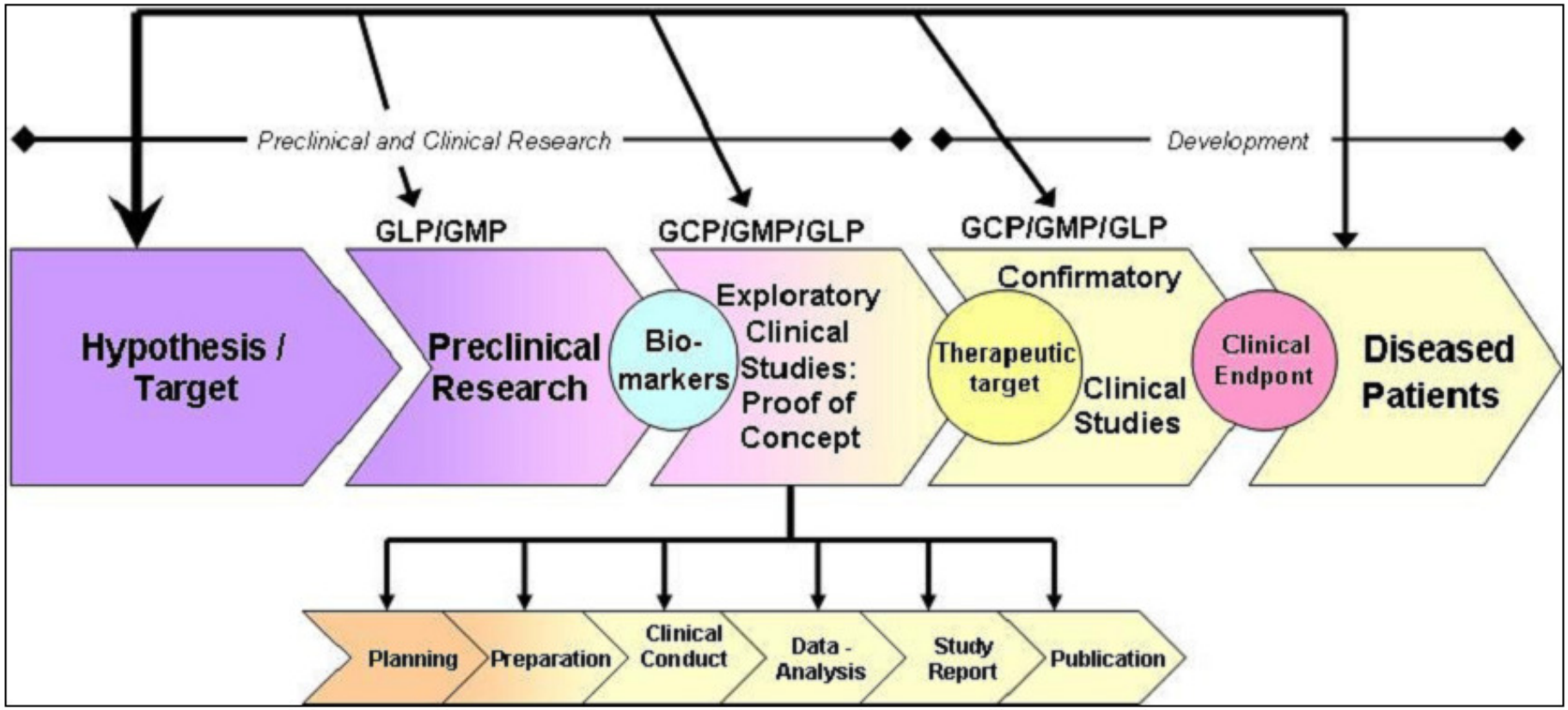
  - Stem Cell Registry
  - HLA Typing Department
  - Immuno-phenotyping
  - Genomics, proteomics
  - Toxicology
  - Animal house facility (small/medium)

Research areas

1. Adult stem cells differentiation potential → regenerative medicine
2. Tumor cell biology → advanced therapies
3. Health and environmental factors



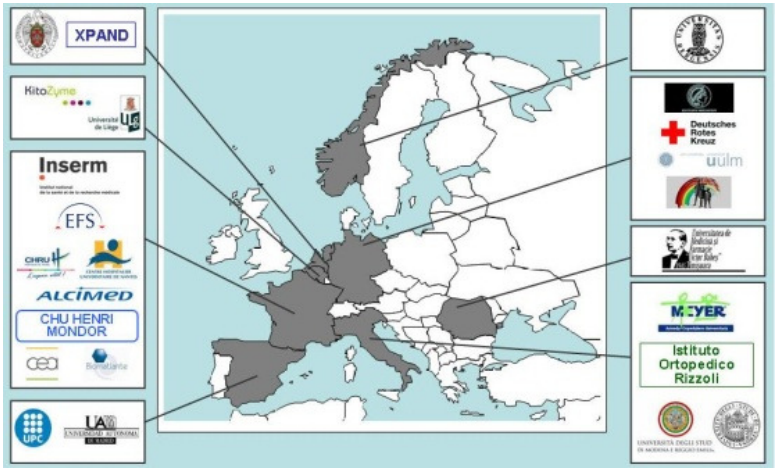
Fields of interest

1. Interdisciplinary integration of knowledge in:
    - immunology
    - molecular biology
    - biochemistry
    - imaging techniques
    - bioinformatics
    - genetic engineering
2. Application of worldwide progresses by using advanced therapies in the field of regenerative medicine and oncology
3. Development of research funding programmes to train highly skilled researchers in the field

Top feature

- The GMP facility (class A in class B, surface 150 m^2), designed for:
  1. Regenerative medicine → bone and soft tissue regeneration, based on the previous experience within FP7 projects (CASCADE, REBORNE)
  2. Anti-tumour immunotherapy → selection of antigen specific CTLs for autologous transplantation in patients with oncologic disorders
  3. Innovative therapies in immune diseases

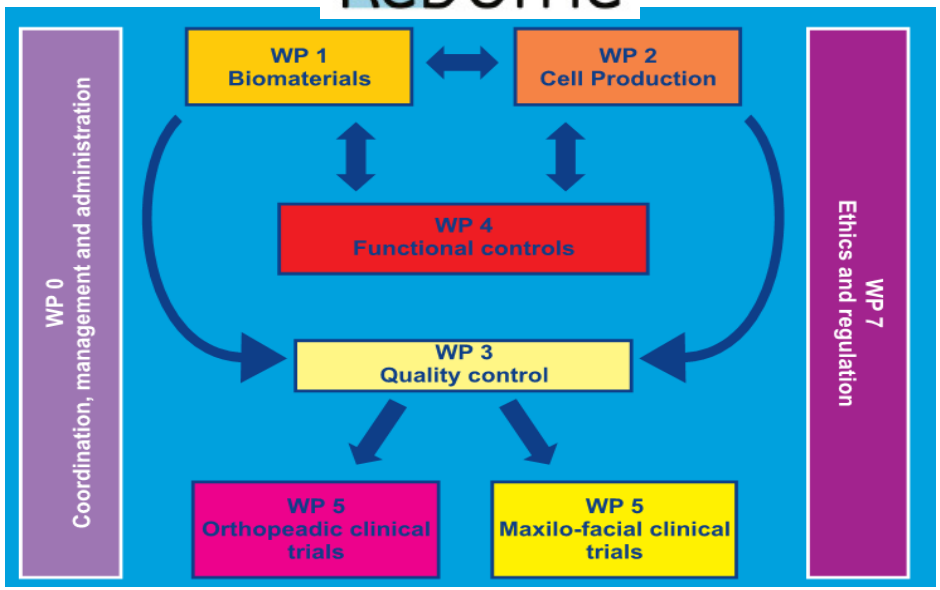
CASCADE



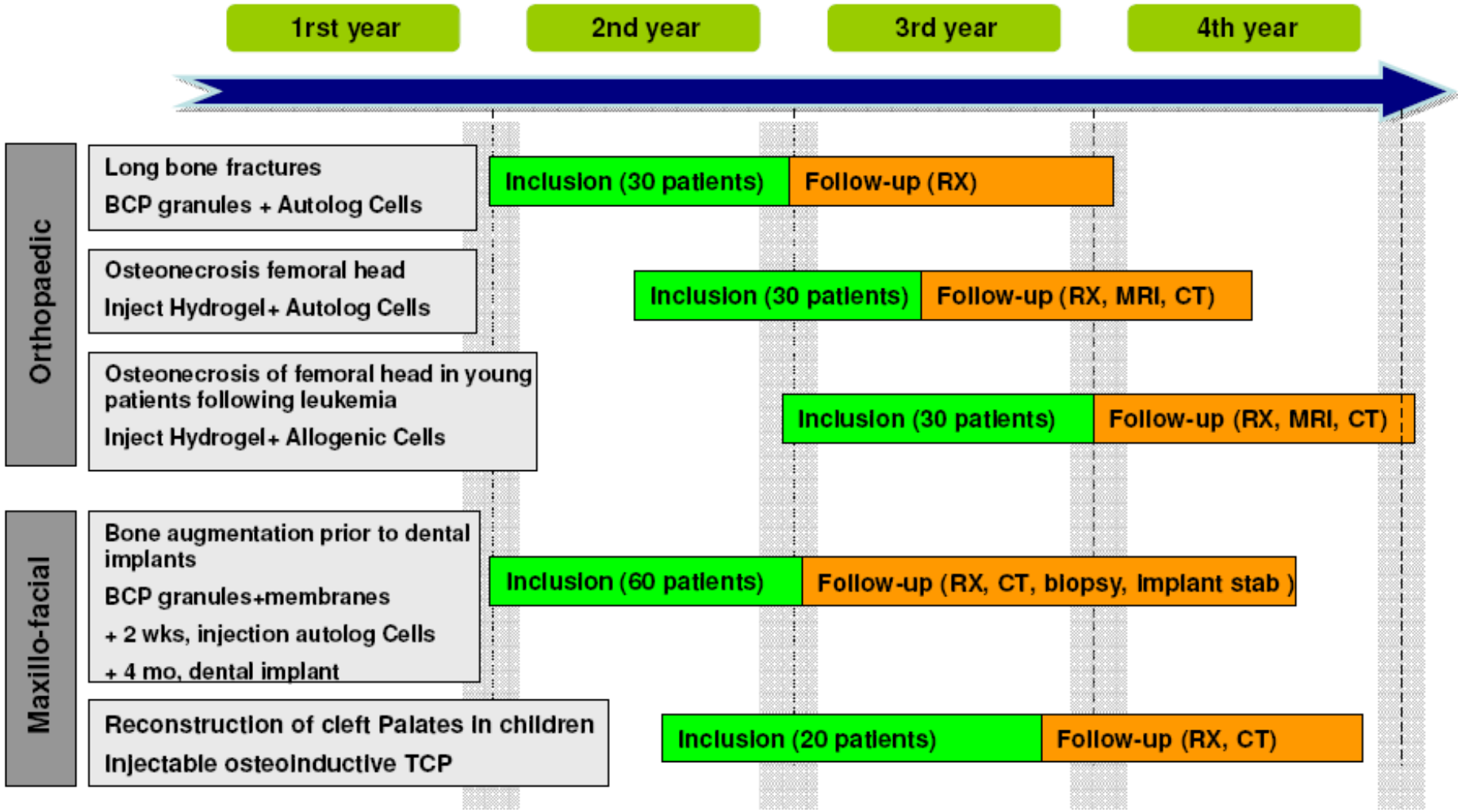
REBORNE

1. Regenerative medicine - REBORNE

- **Regenerating Bone defects using New biomedical Engineering approaches**, FP7 Large-scale integrating project (241879/2010-2014)
- Project coordinators: Luc Sensebe & Pierre Layrolle (France)
- Participants: 7 European countries
- Objective: To perform **clinical trials** using standard GMP cultivated MSCs and advanced biomaterials for triggering bone healing



1. Regenerative medicine - REBORNE



1. Regenerative medicine – ORTHO2

Reborne

Clinical staff

- Patient inclusion
- Serology checking
- Bone marrow harvesting

GMP facility

- CEU reception
- Culture
- Cell harvesting (D21) & Controls
- Packaging

Surgeon

- Cell injection
- Patient follow up

2. Anti-tumour immunotherapy

Current strategies

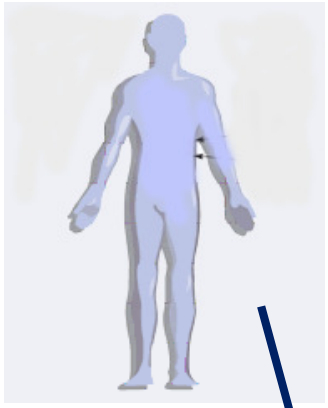
- Present anti-tumour strategies (chemotherapy, radiotherapy) target tumour cells or biological processes supporting tumour development
- Immunotherapy implies
  - the use of potent molecules blocking selected targets (monoclonal antibodies)
  - activation of self immune cells, functionally active against tumour cells (NKs, CTLs)

Our strategy

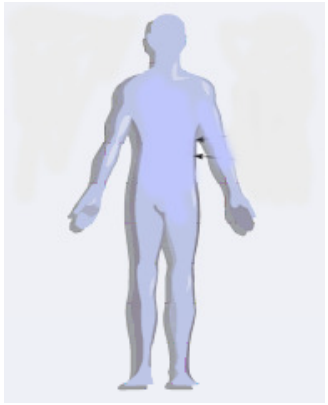
- Selection and expansion of autologous CTLs, functionally active against tumour microenvironment cells → tumour-associated fibroblasts (TAFs), known for their key role in supporting tumour development (Paunescu et al., *JCMM*, 2011)

Current strategies in generation of anti-tumour CTLs

Donor



Recipient



CTLs

Expansion of anti-tumour specific T cells in culture

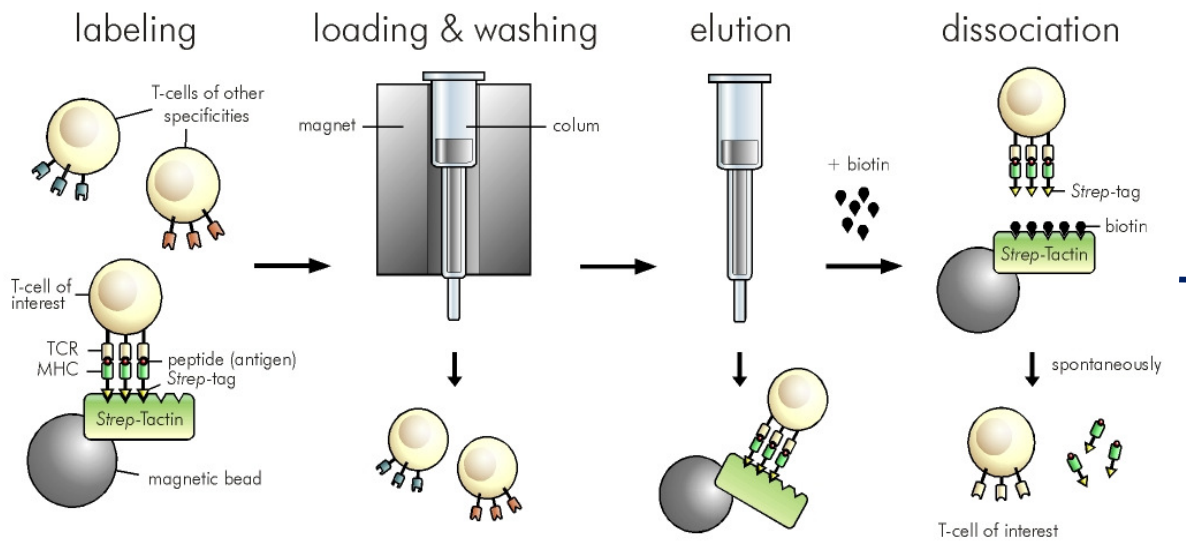
Adoptive transfer

Disadvantages
- compatible donor
- time consuming (weeks)
- large cell numbers required

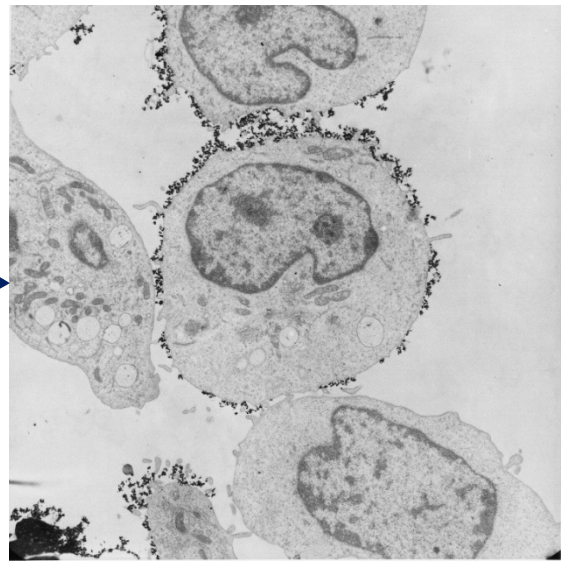
Our strategy in generation of anti-tumour CTLs

- Identification of antigens specific for tumour microenvironment cells → specifically TAFs
- Isolation of CTLs against TAFs specific antigens using streptamer technology → already identified are fibroblasts activation protein (FAP) and fibroblasts specific protein (FSP)
- In present: proof of concept

Streptamer technology

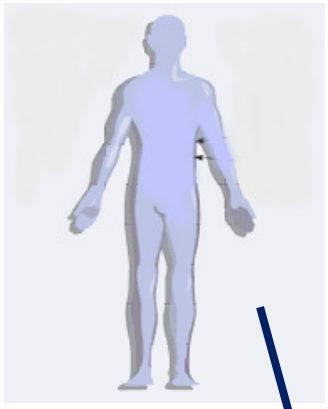


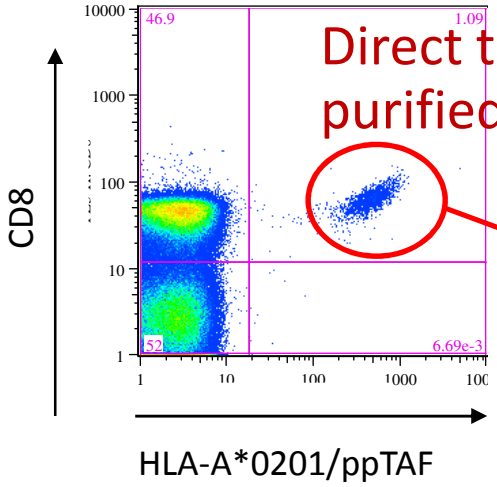
Specific CTLs



Our strategy in generation of anti-tumour CTLs

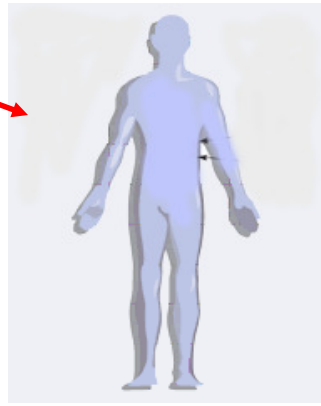
Donor

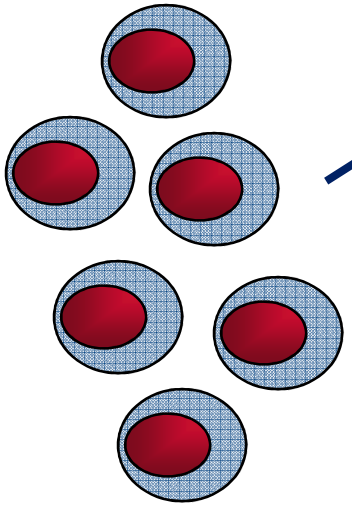




Direct transfer of Streptamer-purified T cells

Recipient





CTLs

Isolation of TAF-specific CTLs

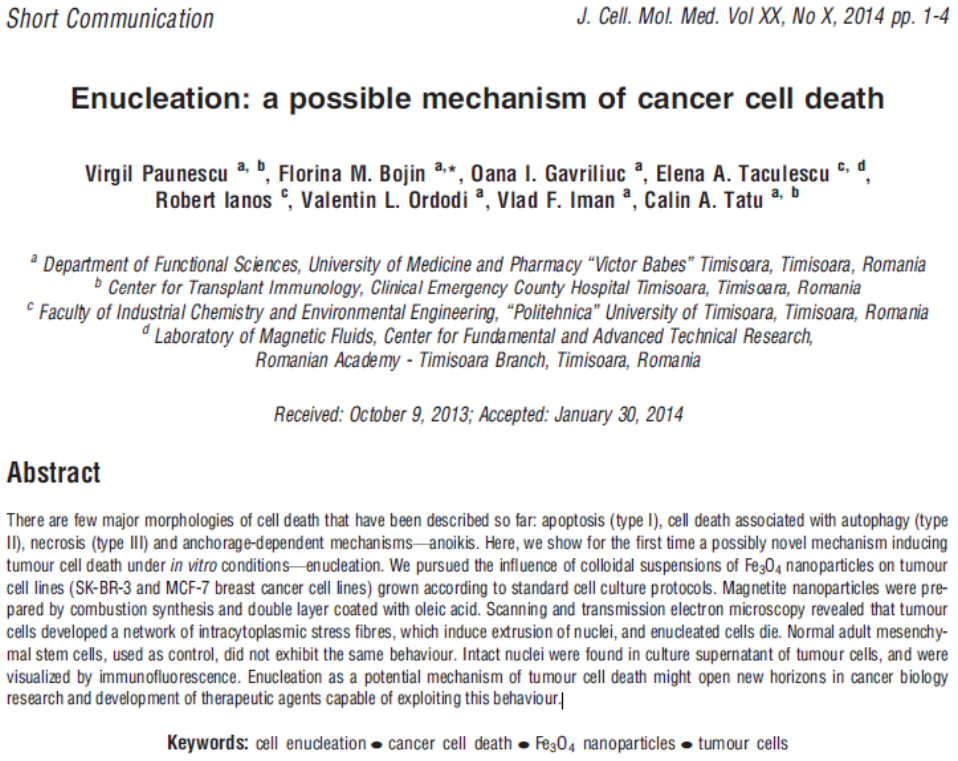
Autologous transfer

Advantages
- fast
- minimally manipulated
- GMP conditions
- viability, functionality

2. Anti-tumour immunotherapy

New concept - Enucleation: a possible mechanism of cancer cell death

- The major morphologies of cell death:
  - apoptosis (type I)
  - autophagy (type II)
  - necrosis (type III)
  - anchorage-dependent mechanisms – anoikis
- Enucleation was shown for the first time as a possibly novel mechanism inducing tumour cell death under *in vitro* conditions



Short Communication *J. Cell. Mol. Med. Vol XX, No X, 2014 pp. 1-4*

Enucleation: a possible mechanism of cancer cell death

Virgil Paunescu [a, b], Florina M. Bojin [a,*], Oana I. Gavriliuc [a], Elena A. Taculescu [c, d], Robert Ianos [c], Valentin L. Ordodi [a], Vlad F. Iman [a], Calin A. Tatu [a, b]

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[b] *Center for Transplant Immunology, Clinical Emergency County Hospital Timisoara, Timisoara, Romania*
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Abstract

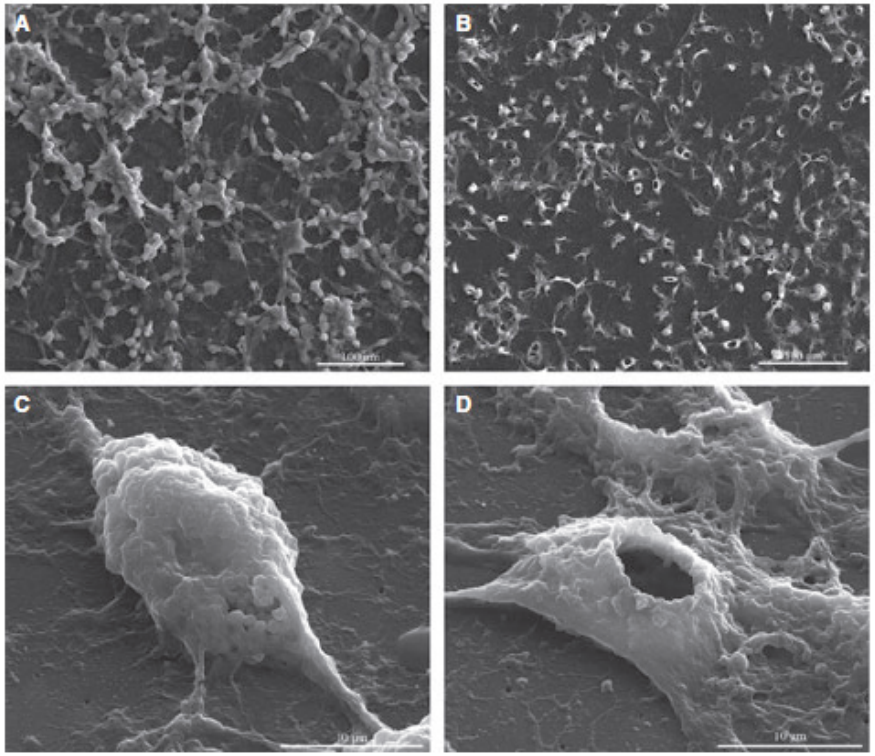
There are few major morphologies of cell death that have been described so far: apoptosis (type I), cell death associated with autophagy (type II), necrosis (type III) and anchorage-dependent mechanisms—anoikis. Here, we show for the first time a possibly novel mechanism inducing tumour cell death under *in vitro* conditions—enucleation. We pursued the influence of colloidal suspensions of Fe_3O_4 nanoparticles on tumour cell lines (SK-BR-3 and MCF-7 breast cancer cell lines) grown according to standard cell culture protocols. Magnetite nanoparticles were prepared by combustion synthesis and double layer coated with oleic acid. Scanning and transmission electron microscopy revealed that tumour cells developed a network of intracytoplasmic stress fibres, which induce extrusion of nuclei, and enucleated cells die. Normal adult mesenchymal stem cells, used as control, did not exhibit the same behaviour. Intact nuclei were found in culture supernatant of tumour cells, and were visualized by immunofluorescence. Enucleation as a potential mechanism of tumour cell death might open new horizons in cancer biology research and development of therapeutic agents capable of exploiting this behaviour.

Keywords: cell enucleation • cancer cell death • Fe_3O_4 nanoparticles • tumour cells

(Paunescu et al., *JCMM*, 2014)

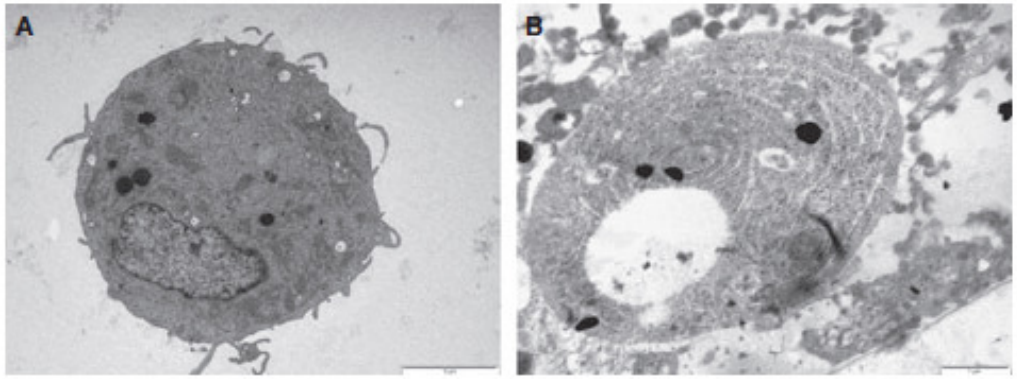
Enucleation: a possible mechanism of cancer cell death

- Aim: to evaluate *in vitro* the influence of colloidal suspension of Fe3O4 nanoparticles on tumour cell lines
  - SK-BR-3 and MCF-7 breast cancer cell lines grown according to standard cell culture protocols
  - Magnetite nanoparticles (MNP) were prepared by combustion synthesis and double layer coated with oleic acid
- After treatment with MPN suspension for 48 hrs: tumour cells developed a network of intracytoplasmic stress fibres, which induce extrusion of nuclei, and enucleated cells die

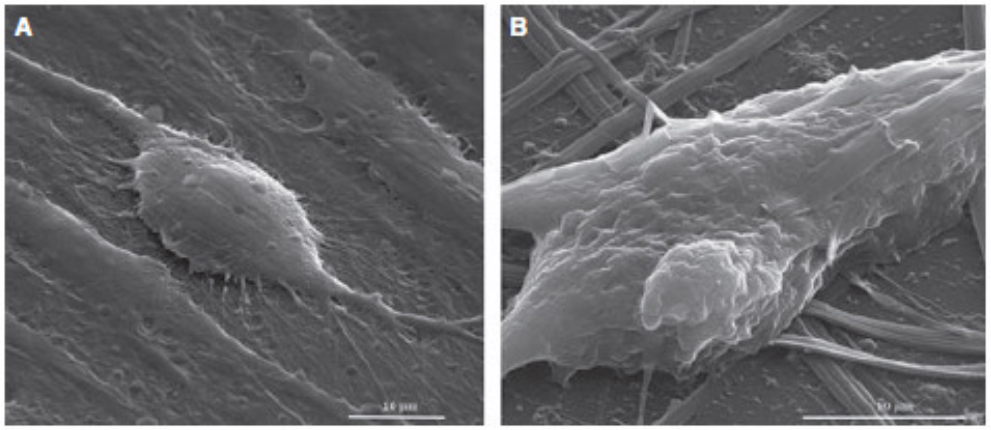


Scanning electron microscopy images of SK-BR-3 cells (A and C) control (untreated) SK-BR-3 cells; (B and D) SK-BR-3 cells treated with MNP suspension for 48 hrs

Enucleation: a possible mechanism of cancer cell death



Transmission electron microscopy of SK-BR-3 cells before treatment (A) and 48hrs after MNPs exposure (B)

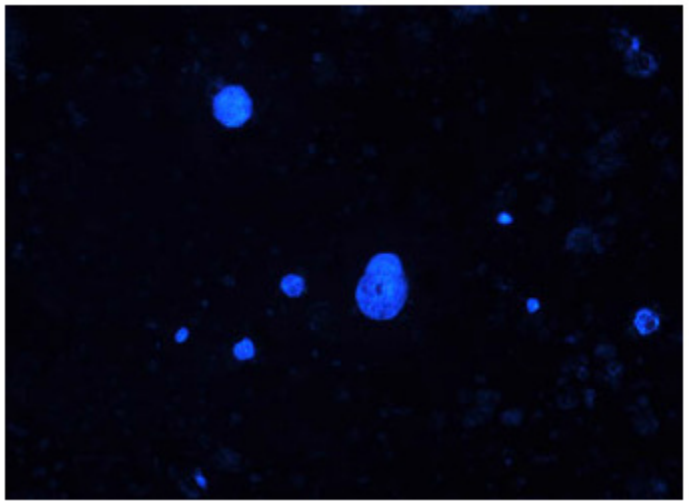


Scanning electron microscopy images of MSCs (used as control): (A) control (untreated) MSCs (3000x), (B) MSCs treated with MNPs suspension (5000x)

Enucleation: a possible mechanism of cancer cell death

- Intact nuclei were found in culture supernatant of tumour cells, and were visualized by immuno-fluorescence

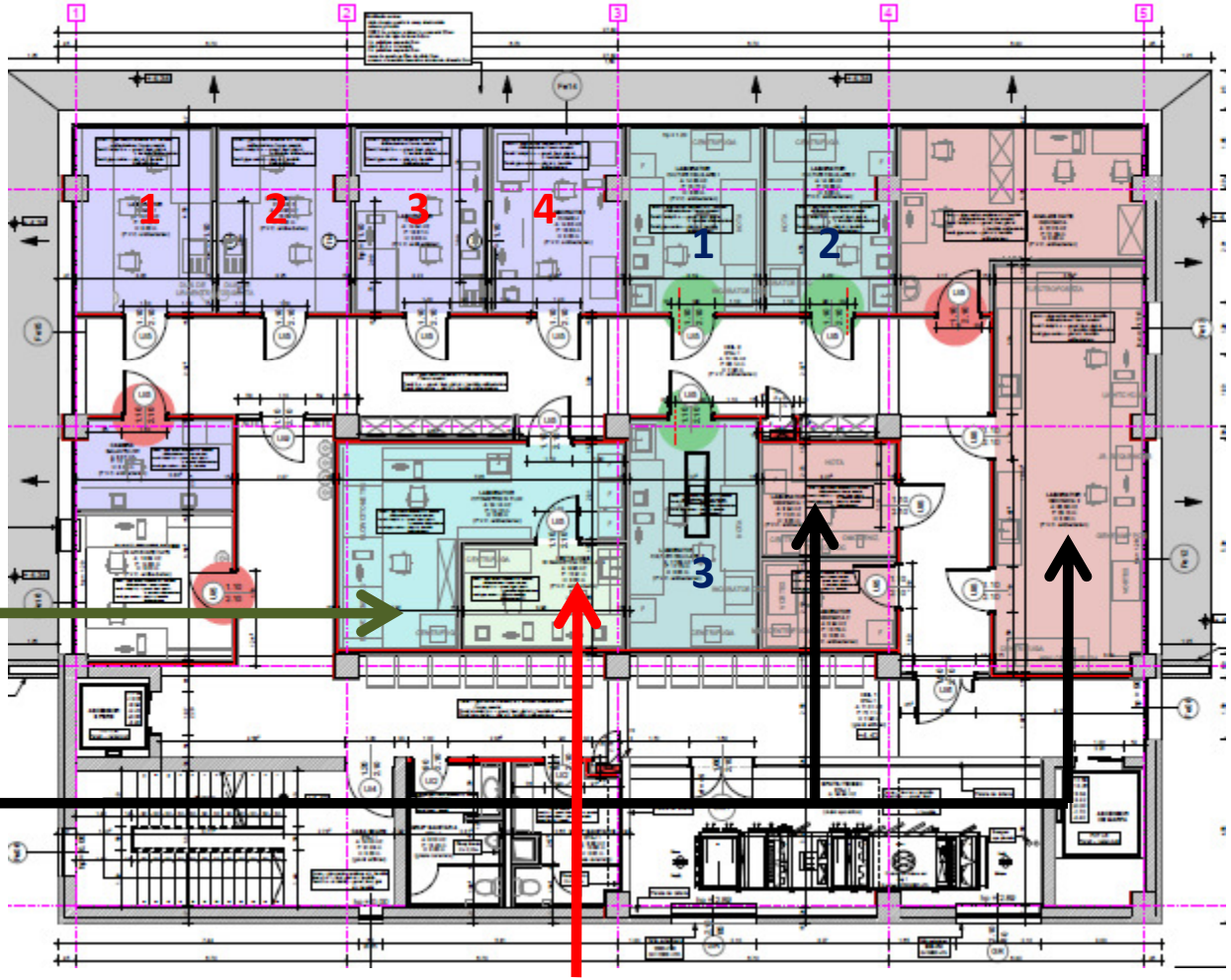
- Enucleation as a potential mechanism of tumour cell death might open new horizons in cancer biology research and development of therapeutic agents capable of exploiting this behaviour.



Round shape intact nuclei from culture supernatant of SK-BR-3 cells suffering the enucleation phenomenon (200x)

Research facilities

- 4 Laboratories for Biochemical and Toxicology Research
- 3 Cell Culture Facilities
- 1 Flowcytometry Laboratory
- Molecular Biology



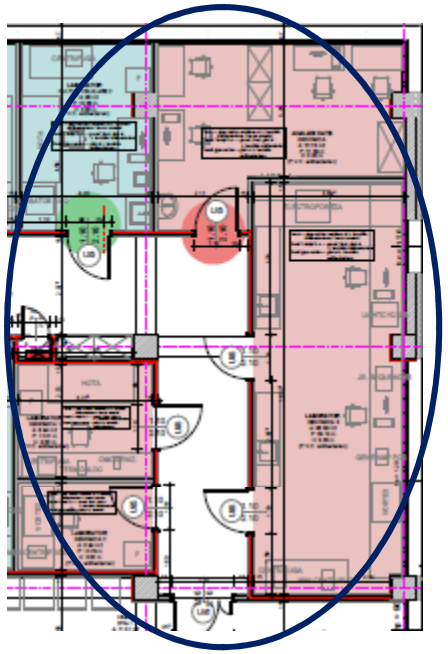
- Confocal Microscopy

Research/Clinical Level
Genomics and proteomics

- Reagents
- Equipment
- Manipulation
- Handling
- Measurements
- Data records

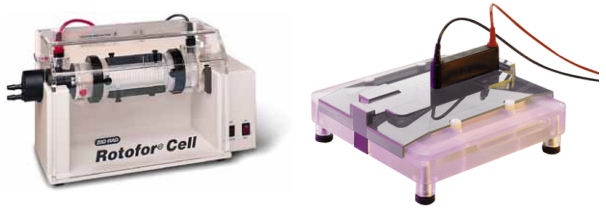
PCR hood

qRT-PCR



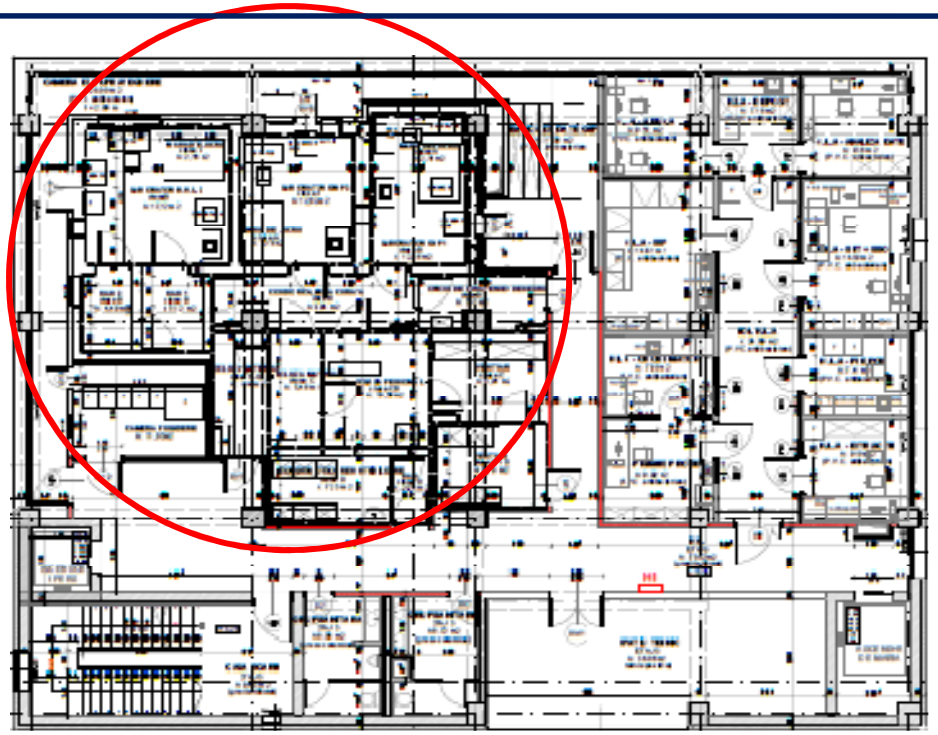
High-throughput data generation and analysis

Microarray and gene sequencing



Protein synthesis and validation

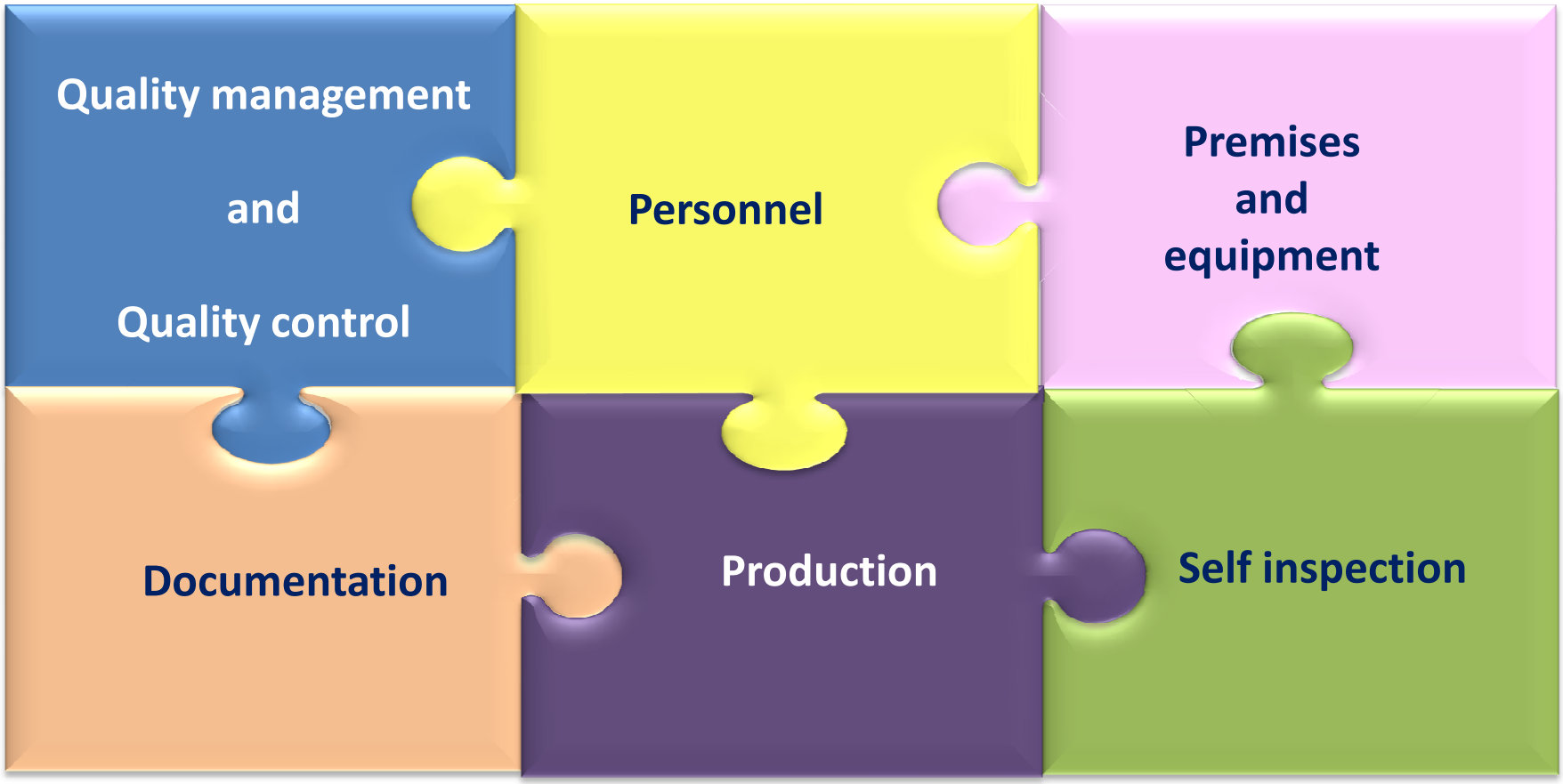
GMP Clean Room



- 3 Class A working spaces in Class B areas
- HEPA-filtered air supply
- Temperature and humidity controls
- Environmental monitoring system
- Cleaning and sanitization system
- Equipment maintenance system

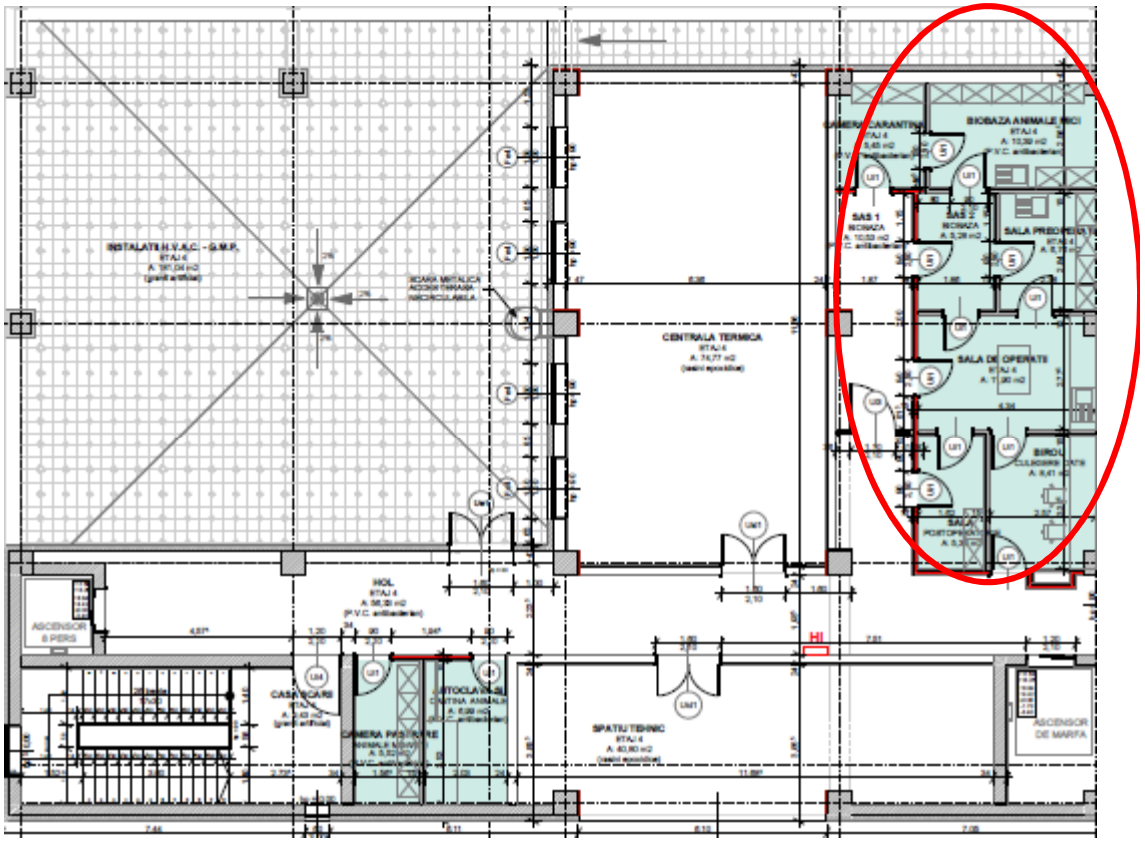
GMP Clean Room

- Our center is designed according to EudraLex - Volume 4 Good manufacturing practice (GMP) Guidelines, Part I - Basic Requirements for Medicinal Products:



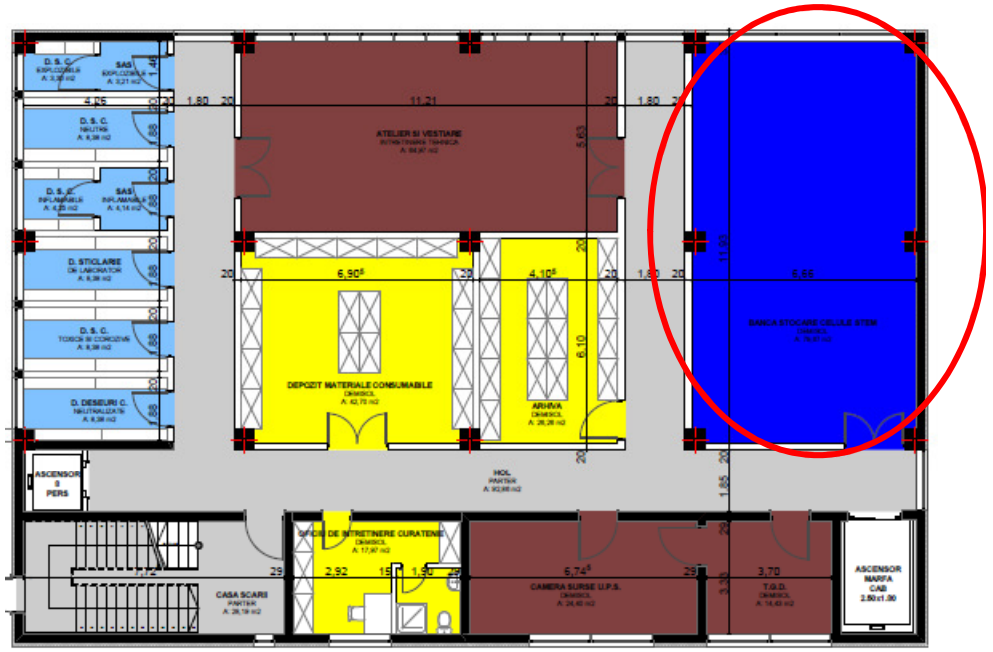
Animal Facility

In vivo imaging system
Hamamatsu Aequoria System



Anesthesia, surgery
Animal models
Small/medium animals

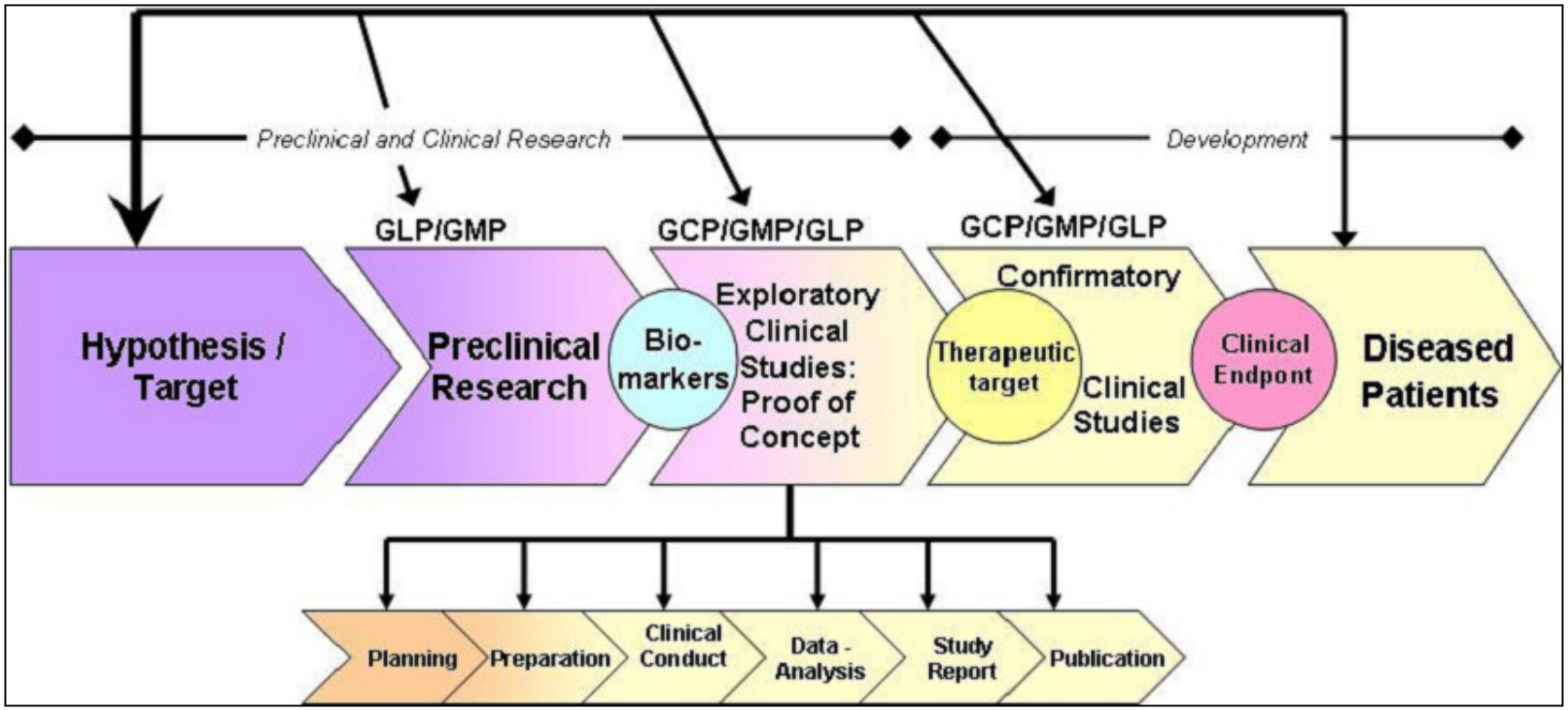
Cryostorage Department



- 76 m^2 for liquid nitrogen preservation of cells and other biological products
- Cryostorage for tubes and bags
- Controlled freezing temperatures
- Public and private long term preservation of cells
- Cell banks of tumor and normal cells

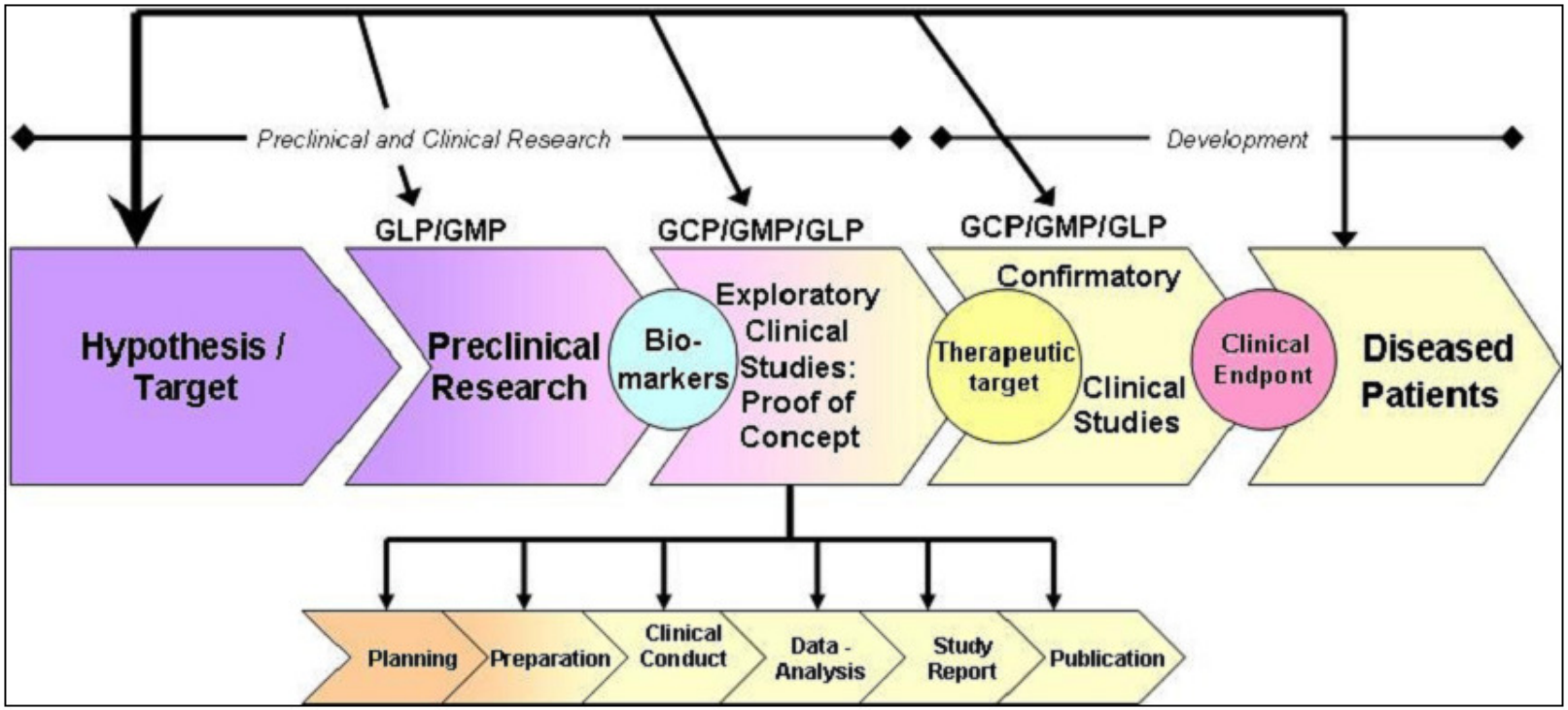
The Strategy of the Centre

1. To obtain the accreditation of the center in 2015
2. To network with similar research institutions involved in the field of:
   - Regenerative medicine → Adult stem cells study
   - Advanced therapies → Tumor cell biology
   - Health and environmental factors



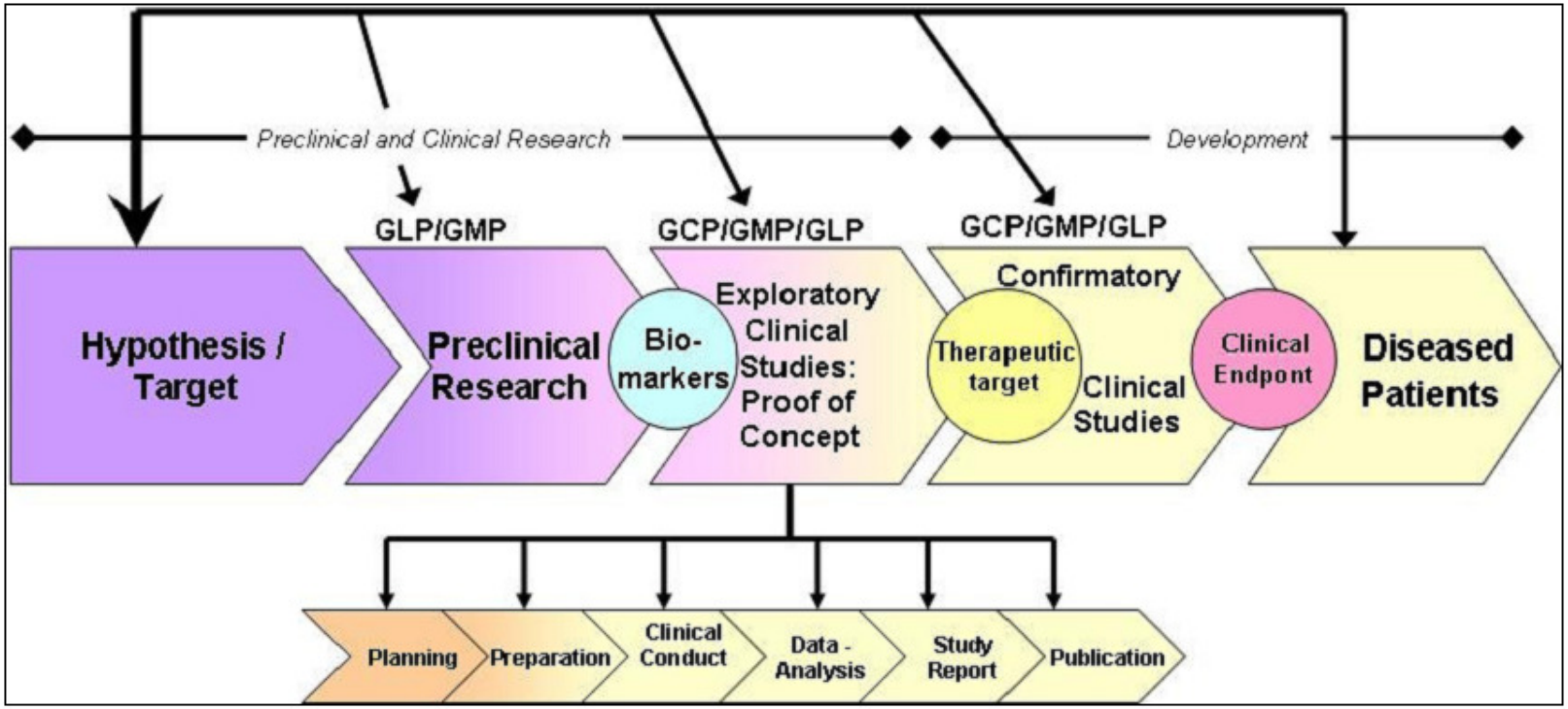
The Strategy of the Centre

3. To establish new collaborations in projects targeting the participation in the EU initiative HORIZON 2020
4. To open our research facilities to the entities interested to transfer a part of their specific activities in case of overloading or to develop new tests or clinical trials



The Strategy of the Centre

5. To carry on scientific research activities required by the private companies/SMEs in the field
6. To offer training for PhD and master students
7. To offer positions for PhD students and post Docs



Reborne

ONCOGEN

Deutsches Rotes Kreuz
DRK-Blutspendedienst
Baden-Württemberg – Hessen
gemeinnützige GmbH

Deutsches Rotes Kreuz
DRK-Blutspendedienste

EFS
ÉTABLISSEMENT FRANÇAIS DU SANG

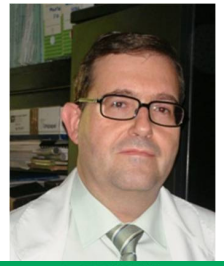
UNIVERSITÉ DE NANTES
Inserm

MEDICAL UNIVERSITY OF VIENNA

MEDICAL UNIVERSITY OF VIENNA

UNIMORE
UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

CELL FACTORY



UAM
UNIVERSIDAD AUTONOMA

ulm university
universität
uulm

“You must try to climb very high, if you want to see very far ...”

Constantin Brancusi (1876-1957)

The Endless Column (1938)