Technical University Munich. January 30, 2013

FROM “BENCHTOP” TO “GMP”: MY "LESSON LEARNED" AFTER 15 YEARS IN THE FIELD OF CELLULAR THERAPIES"

ULSS 6 VICENZA

Giuseppe Astori
Technical Director
Laboratory of Advanced Cellular Therapies
Department of Hematology

FONDAZIONE PROGETTO EMATOLOGIA

A TRIBUTE

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

ABTEILUNG TUMORVIRUS-CHARAKTERISIERUNG

Prof. Dr. Harald zur Hausen Nobel Prize for Medicine, 2008 and E-M de Villers

SUMMARY

1992 -1996 AT THE BENCHTOP: DO WE REALLY NEED A QA SYSTEM?

1997-2002 TISSUE ENGINEERING IN ACADEMIA

2003-2006 CELLULAR THERAPY IN THE MEDICAL DEVICE COMPANY

2006-2010 REGENERATIVE MEDICINE IN A CLINIC

2011-PRESENT-CELLULAR THERAPIES IN HOSPITAL

WE REALLY NEED A QUALITY ASSURANCE SYSTEM?

1992-1997
Institute of Microbiology and Virology
University of Udine
Faculty of Medicine
PEOPLE: 30
TYPE: Public Institution
QUALITY ASSURANCE: NO
ACCREDITATIONS: NO

LESSON LEARNED

PRO

- You do not use your time to fill **G**reat **M**ountains of **P**aper (GMP)
- You can easily hide your mistakes
- You can make a change in you method simply changing the SOP

CONS

- The process «could» be out of control
- Difficult to trace a mistake

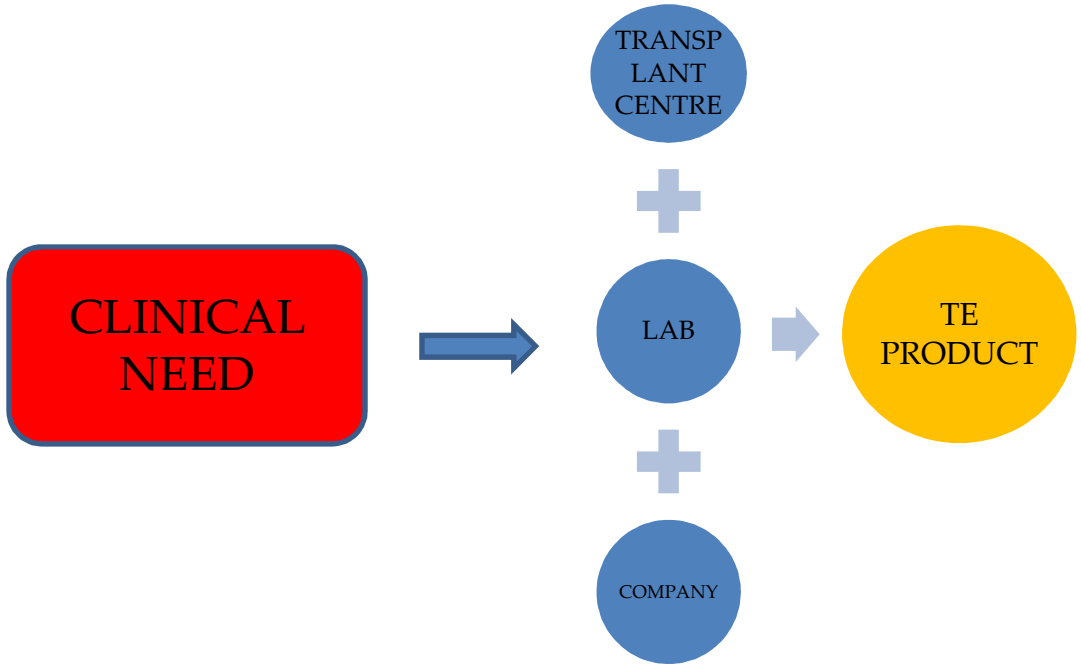
- A QA SYSTEM IS MORE THAN A COLLECTION OF SOPs IN A NOTEBOOK

- DO NOT FORGET TO MAINTAIN YOUR ISTRUMENTATION

TISSUE ENGINEERING

1998-2003
CO.RI.BI
University of Udine
EMPLOYEES: 10
TYPE: Public-Private Consortium
QUALITY ASSURANCE: ISO
ACCREDITATIONS: NO
AIM: tissue engineriing

PRODUCTS:
- Bioartificial liver
- Cell separation systems
- Closed systems for UCB banking



THE BIOARTIFICIAL LIVER

CELL SOURCE:
- Porcine hepatocyte from the abbattoir
- Cryopreserved human hepatocytes from deceased donors

QUALITY CONTROL:
- Cell viability
- Sterility
- PERV (porcine endogenous retrovirus)

- PRODUCTION: laminar flow hood in an unclassified background

SIX PATIENTS TREATED AS A BRIDGE TO LIVER TRANSPLANTATION

American Journal of Transplantation 2003; 4: 286–289
Blackwell Munksgaard

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doi: 10.1046/j.1600-6143.2003.00310.x

Case Report

First Report of Cryopreserved Human Hepatocytes Based Bioartificial Liver Successfully Used as a Bridge to Liver Transplantation

Umberto Baccarani [a,*], Annibale Donini[b], Andrea Sanna[b], Andrea Risaliti[a], Alessio Ciariani[b], Bruno Nardo[c], Antonino Cavallari[c], Gerardo Martinelli[d], Lorenza Ridolfi[e], Gianni Bellini[f], Mario Scalamogna[g] and Fabrizio Bresadola[a]

[a]*Department of Surgery & Transplantation University of Udine, Udine, Italy*
[b]*Department of Surgery, University of Ferrara, Ferrara, Italy*
[c]*Department of Surgery & Transplantation and*
[d]*Intensive Care Unit University of Bologna, Bologna, Italy*
[e]*AIRT Emilia Romagna, Bologna, Italy,* [f]*RanD S.r.l, Medolla (MO), Italy* [g]*Nord Italia Transplant program Milano, Italy*
[*]*Corresponding author: Umberto Baccarani, umberto.baccarani@uniud.it*

donor's organs that would be available on time for emergency transplantation (1). Research, in the past 10 years, has focused on the development of devices designed to stabilize the patient, avoid major neurological complication and finally gain time for the transplant in FHF cases listed for emergency transplantation. Pure artificial techniques such as plasmapheresis, charcoal hemoperfusion and ultrafiltration have not had a significant impact on temporary support of FHF patients (2). Thereafter, several authors developed and tested clinical trial of bioartificial devices, which are composed of artificial and biological components, represented by live liver cells (3–6). Results using bioartificial liver devices, although not homogenous and characterized by different perfusion design, variable type and amount of cell used, showed not only successful bridging to liver transplantation, but also the possibility of spontaneous recovery of liver function after adequate temporaneous support. However, great controversy still exists

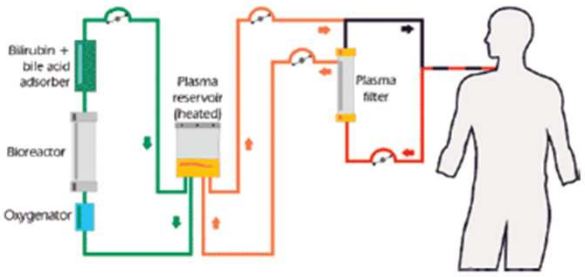


Figure 1: Configuration of the Performed® BAL device (courtesy of Rand, Medolla, Italy).

LESSON LEARNED

PRO

- MINIMAL REGULATORY REQUIREMENTS (EC APPROVAL ONLY)
- LOW-COST MAINTENANCE OF THE LABORATORY
- FAST AND EASY IN VIVO TRANSLATION

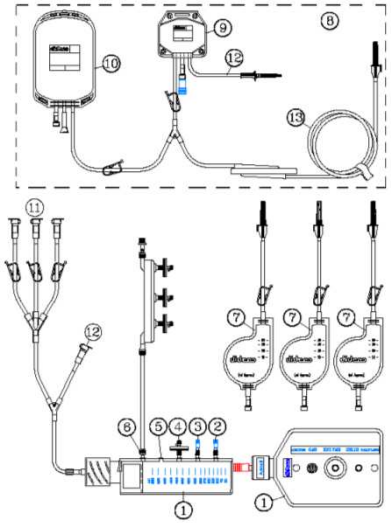
CONS

- UNCONTROLLED RISK LEVEL FOR PATIENTS AND OPERATORS

A LOW REGULATORY REQUIREMENT ALLOWS FOR A FAST IN VIVO TRANSLATION

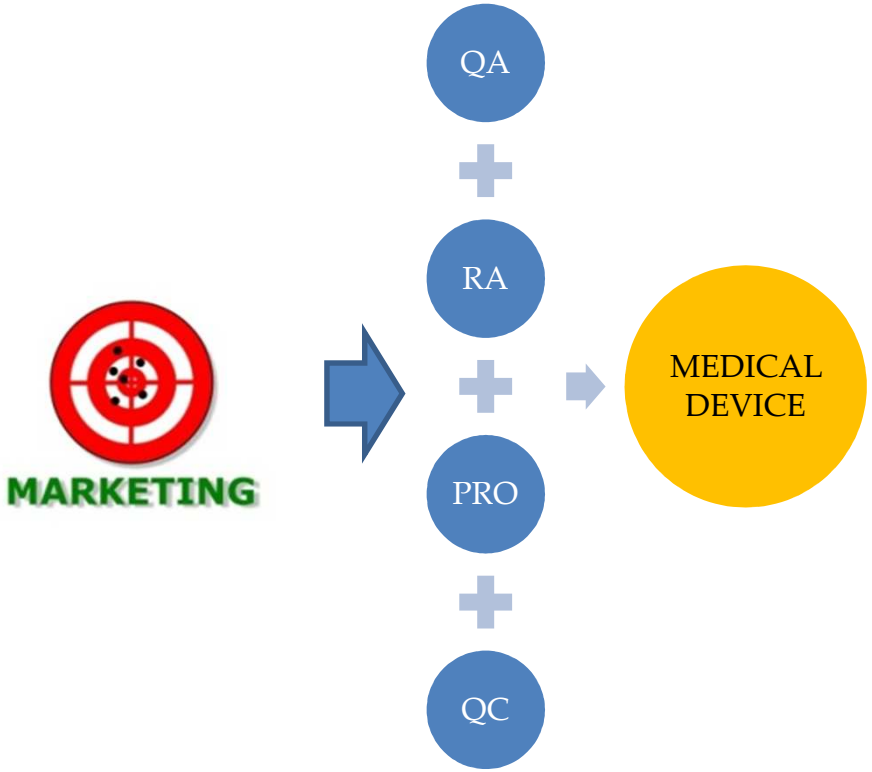
IN A GREAT MEDICAL DEVICE COMPANY

2003-2006
DIDECO (Mirandola, Modena)
EMPLOYEES: 800
IN R&D: 40
CLEAN ROOM: 5,000 m²
TYPE: Private
QUALITY ASSURANCE: ISO
PRODUCTS: cardiopulmonary, blood management, cellular therapies



COMPANY ORGANIZATION

- QA: A SOLID ISO SYSTEM WAS IN PLACE
- PRODUCTION SITE: FULLY COMPLIANT
- QC: FULLY COMPLIANT WITH MEDICAL DEVICE DIRECTIVE
- LOGISTIC

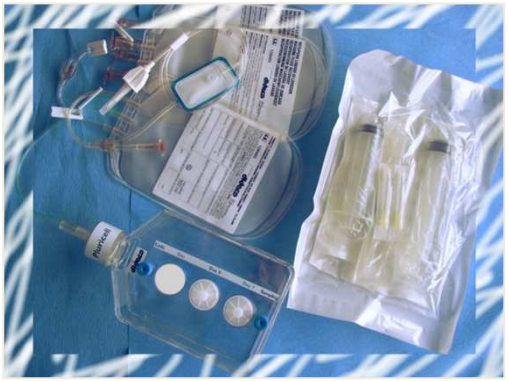


CELLULAR THERAPY: THE PLURICELL SYSTEM

EX-VIVO EXPANSION OF HEMATOPOIEIC PROGENITOR CELLS

DISPOSABLE KIT

Closed expansion chamber
- Washing and collection bags



REAGENTS KIT

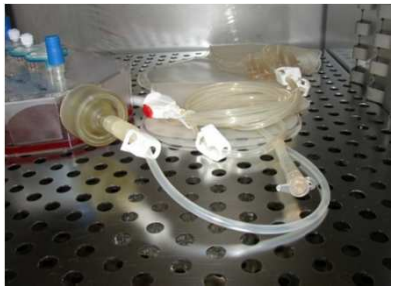
Ready to use cytokines
Ready to use growth media

PLURICELL PROCEDURE

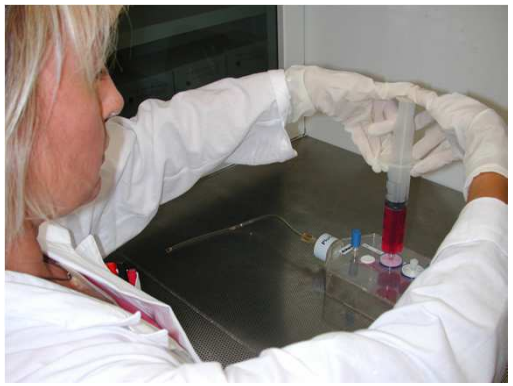
UCB FROM UNRELATED DONOR

CD34+ SELECTION (60 min.)

SEEDING IN PLURICELL (15 min.)



CELL INCUBATION



AFTER 1 WEEK ADD MEDIA (10 min.)

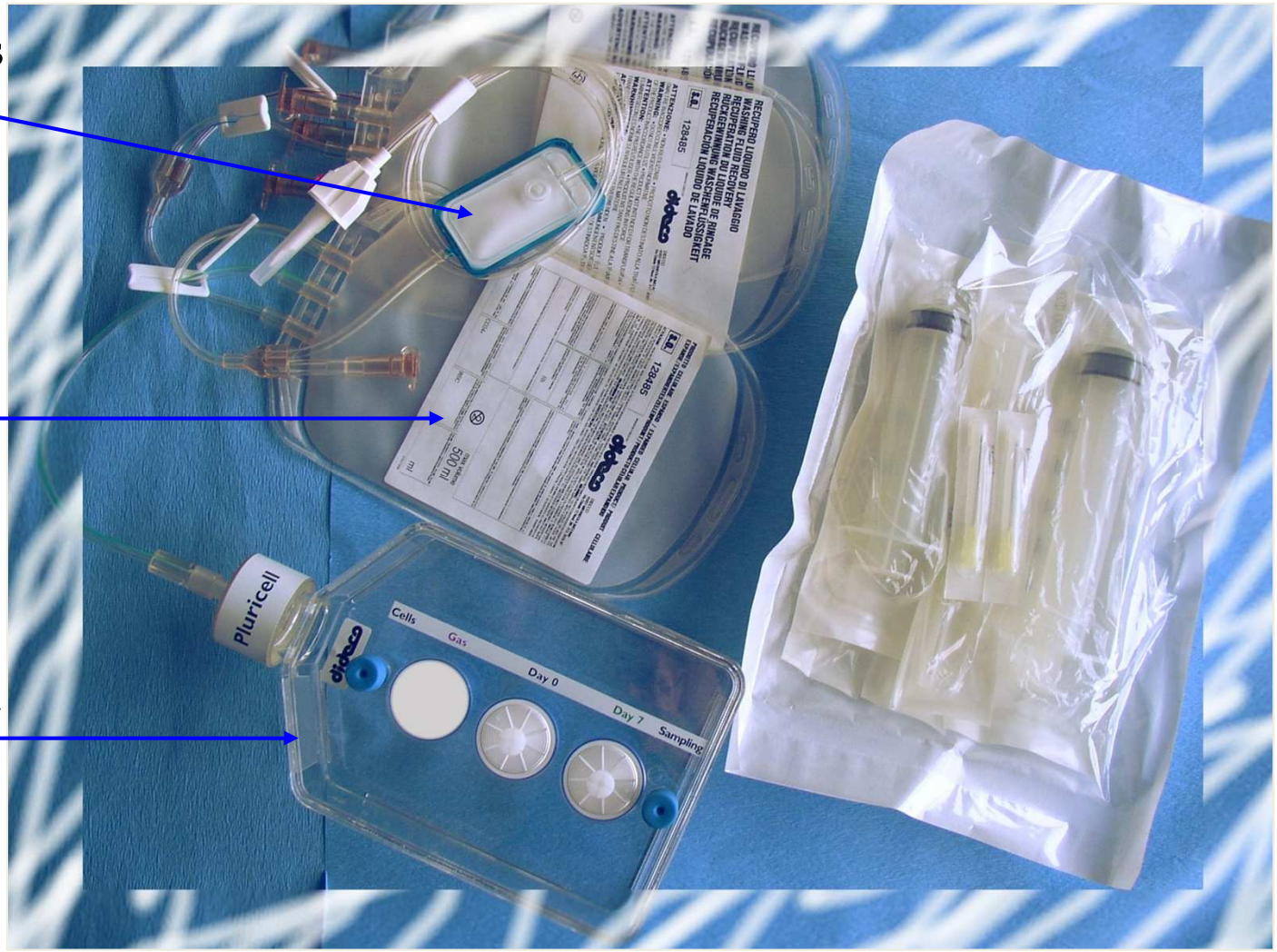
AFTER 12 DAYS HARVEST CELLS (30 min.)

KIT DISPOSABLE

Sterilizing filters

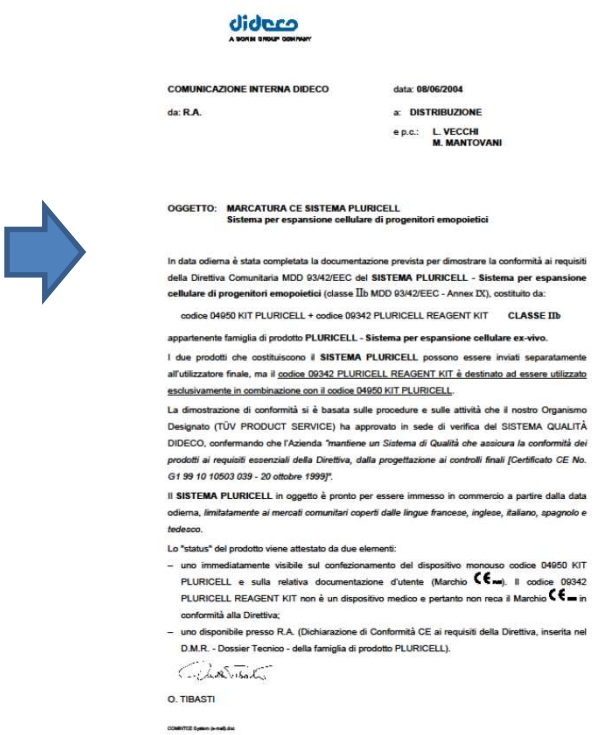
Washing and collection bags

Expansion chamber



PLURICELL HISTORY AND PARADOX

- DIDECO PLURICELL WAS A CLASS 2b MEDICAL DEVICE
- CLASS 2b: device intended for modifying the biological composition of blood and intended for infusion into the body
- NOTIFIED BODY: TÜV, Munich



didecо
A BIOMET GROUP COMPANY

COMUNICAZIONE INTERNA DIDECO data: **08/06/2004**

da: **R.A.** a: **DISTRIBUZIONE**

e p.c.: **L. VECCHI**
M. MANTOVANI

OGGETTO: MARCATURA CE SISTEMA PLURICELL
Sistema per espansione cellulare di progenitori emopoietici

In data odierna è stata completata la documentazione prevista per dimostrare la conformità ai requisiti della Direttiva Comunitaria MDD 93/42/EEC del **SISTEMA PLURICELL - Sistema per espansione cellulare di progenitori emopoietici** (classe IIb MDD 93/42/EEC - Annex IX), costituito da:

codice 04950 KIT PLURICELL + codice 09342 PLURICELL REAGENT KIT **CLASSE IIb**

appartenente famiglia di prodotto **PLURICELL - Sistema per espansione cellulare ex-vivo.**

I due prodotti che costituiscono il **SISTEMA PLURICELL** possono essere inviati separatamente all'utilizzatore finale, ma il codice 09342 PLURICELL REAGENT KIT è destinato ad essere utilizzato esclusivamente in combinazione con il codice 04950 KIT PLURICELL.

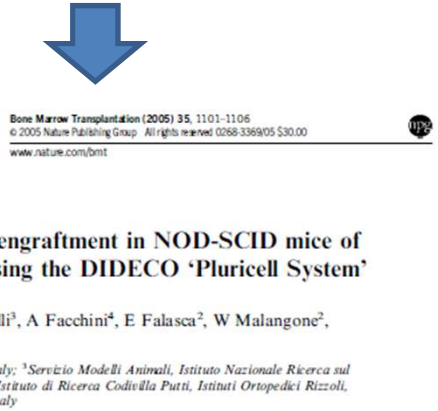
La dimostrazione di conformità si è basata sulle procedure e sulle attività che il nostro Organismo Designato (TÜV PRODUCT SERVICE) ha approvato in sede di verifica del SISTEMA QUALITÀ DIDECO, confermando che l'Azienda *"mantiene un Sistema di Qualità che assicura la conformità dei prodotti ai requisiti essenziali della Direttiva, dalla progettazione ai controlli finali [Certificato CE No. G1 99 10 10503 039 - 20 ottobre 1999]".*

Il **SISTEMA PLURICELL** in oggetto è pronto per essere immesso in commercio a partire dalla data odierna, *limitatamente ai mercati comunitari coperti dalle lingue francese, inglese, italiano, spagnolo e tedesco.*

Lo "status" del prodotto viene attestato da due elementi:

- uno immediatamente visibile sul confezionamento del dispositivo monouso codice 04950 KIT PLURICELL e sulla relativa documentazione d'utente (Marchio CE). Il codice 09342 PLURICELL REAGENT KIT non è un dispositivo medico e pertanto non reca il Marchio CE in conformità alla Direttiva;
- uno disponibile presso R.A. (Dichiarazione di Conformità CE ai requisiti della Direttiva, inserita nel D.M.R. - Dossier Tecnico - della famiglia di prodotto PLURICELL).

O. TIBASTI



Bone Marrow Transplantation (2005) 35, 1101–1106
© 2005 Nature Publishing Group All rights reserved 0268-3369/05 $30.00
www.nature.com/bmt

Pre-clinical studies

Evaluation of *ex vivo* expansion and engraftment in NOD-SCID mice of umbilical cord blood CD34+ cells using the DIDECO 'Pluricell System'

G Astori[1], V Adami[2], G Mambrini[1], L Bigi[1], M Cilli[3], A Facchini[4], E Falasca[2], W Malangone[2], I Panzani[1] and A Degrassi[5]

[1]*DIDECO srl, Mirandola, MO, Italy;* [2]*SB Technology, Udine, Italy;* [3]*Servizio Modelli Animali, Istituto Nazionale Ricerca sul Cancro, Genova, Italy;* [4]*Laboratorio di Immunologia e Genetica, Istituto di Ricerca Codivilla Putti, Istituti Ortopedici Rizzoli, Bologna, Italy; and* [5]*DPMSC, Universita' degli Studi di Udine, Italy*

DEVICE WAS ON THE MARKET BUT NEVER PURCHASED

LESSON LEARNED

PRO

- REGULATORY REQUIREMENTS FOR MEDICAL DEVICE WELL DEFINED
- COMPLETE COMPANY ORGANIZATION (MKT, QA, QC, PRO, REGULATORY, LOGISTIC
- FINANCIAL RESOURCES

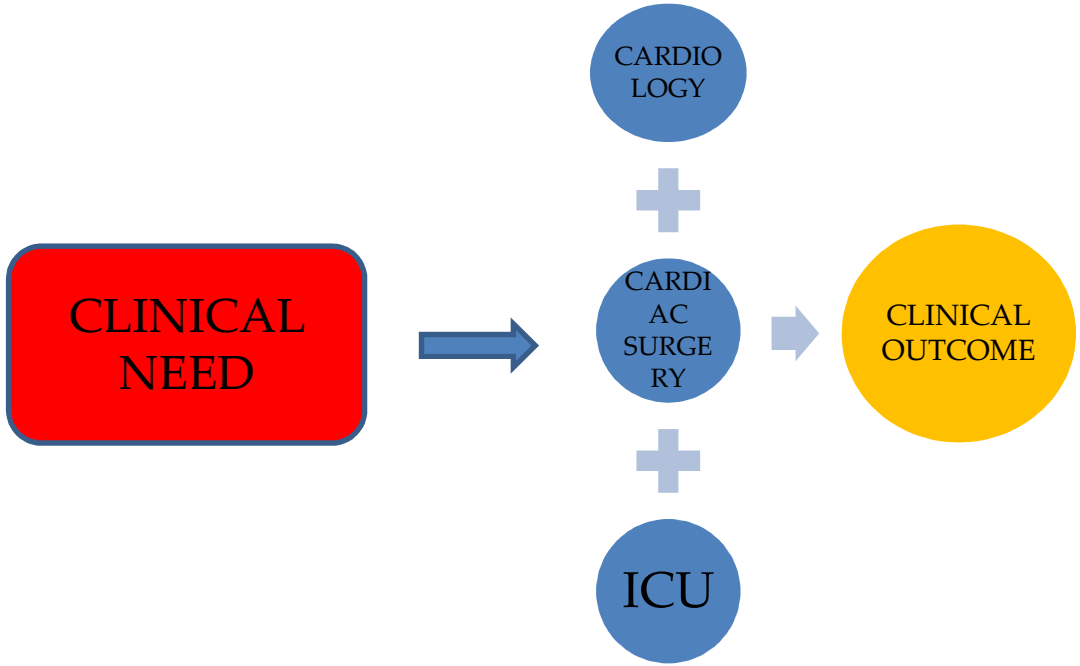
CONS

- REVENUES!
- MARKET NOT YET READY
- REGULATION ON ATMP NOT YET «IN PLACE»
- SLOW INTERACTION WITH HIGH MANAGEMENT

- MAKE A SOLID MARKET PLAN BEFORE STARTING
- WAIT FOR CLEAR REGULATORY REQUIREMENTS: INTERACT WITH YOUR REGULATORY AGENCY AS FAR THIS IS POSSIBLE

CARDIOCENTRO TICINO

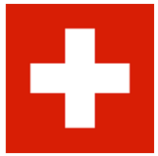
2006-2010
CARDIOCENTRO TICINO (Lugano, Switzerland)
EMPLOYEES: 280 people
SPECIALIZATION: cardiology, cardiosurgery,



CARDIOCENTROTICINO

REGULATIONS

Regulation on ATMPs 1394/2007
issued 10.12.2007



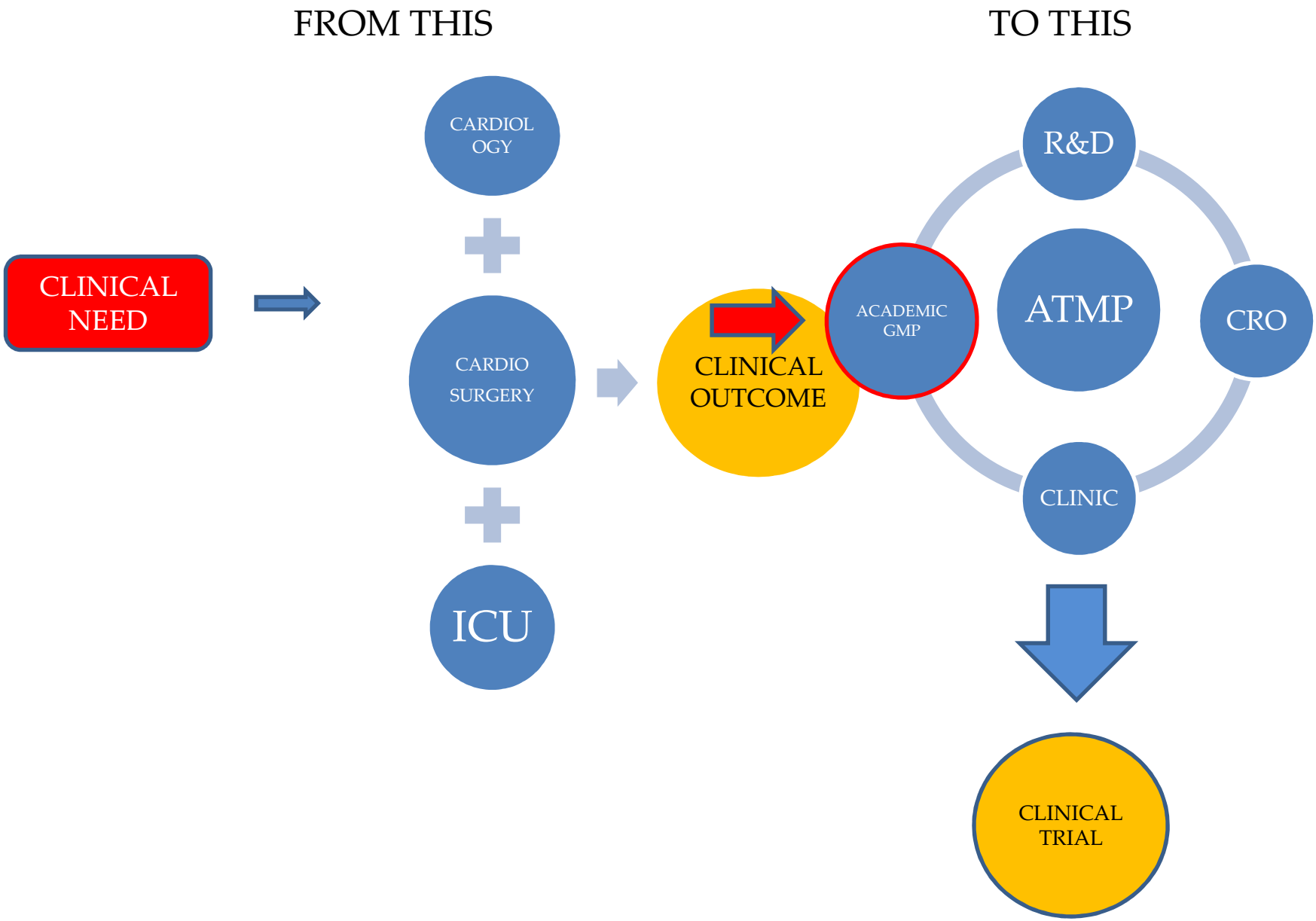
Federal law on organs, tissues and cell transplantation issued 01.07.2007

- entities manufacturing or distributing *transplant products* require an establishment licence from Swissmedic
- Swissmedic must be notified of clinical trials that involve *transplant products*.

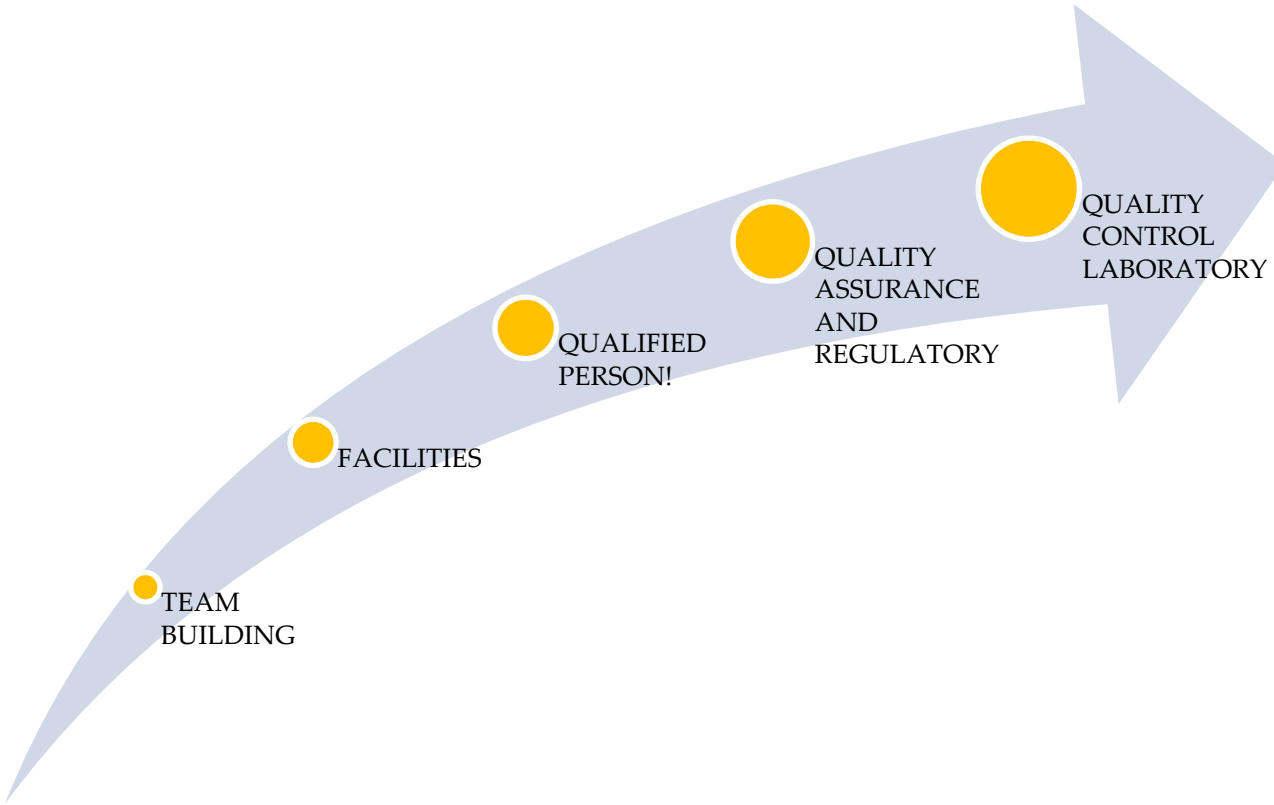
swissmedic

WHAT I FOUND «ON SITE»:	
FUNDINGS	YES
«GMP» PERSONNEL	NO
R&D	NO
CLEAN ROOM	NO

A NEW CLUSTER ORGANIZATION



PRIORITIES



GMP

TEAM BUILDING - DECISIONS

- SEARCH ONLY «GMP TRAINED» PEOPLE RECRUITED FROM «PHARMA»
- START WITH THE MINIMAL NUMBER OF PEOPLE REQUESTED BY GMPs

30 MONTS FROM «START» TO ACCREDITATION

04/2006
START

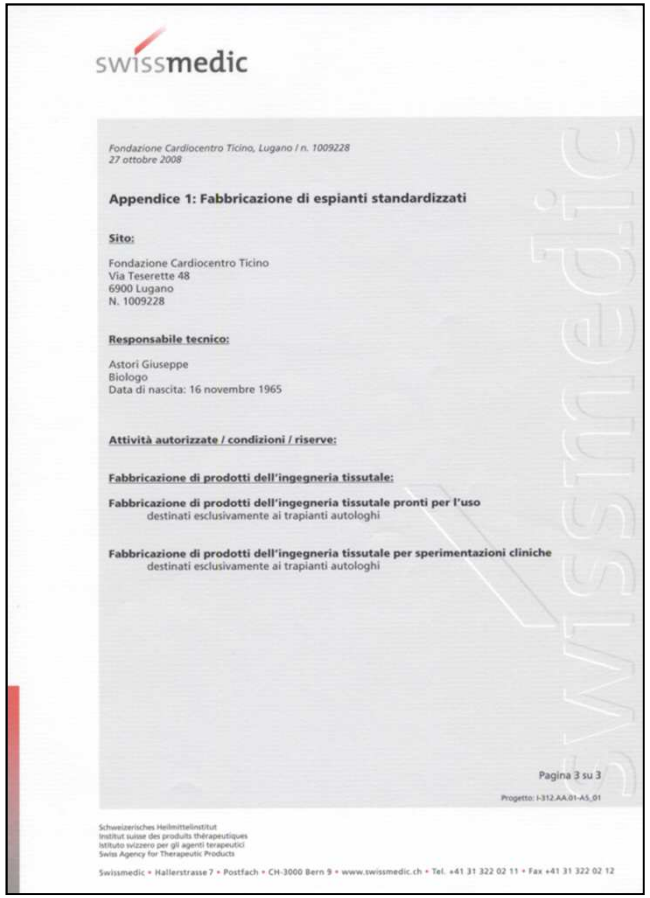
09/2006
CLEAN ROOM COMPLETED

2008
QUALITY SYSTEM COMPLETED

03/2008
START VALIDATION

09/2008
SWISSMEDIC INSPECTION

11/2008
GMP CERTIFICATION



swissmedic

Fondazione Cardiocentro Ticino, Lugano / n. 1009228
27 ottobre 2008

Appendice 1: Fabbricazione di espianti standardizzati

Sito:

Fondazione Cardiocentro Ticino
Via Teserette 48
6900 Lugano
N. 1009228

Responsabile tecnico:

Astori Giuseppe
Biologo
Data di nascita: 16 novembre 1965

Attività autorizzate / condizioni / riserve:

Fabbricazione di prodotti dell'ingegneria tissutale:

Fabbricazione di prodotti dell'ingegneria tissutale pronti per l'uso
destinati esclusivamente ai trapianti autologhi

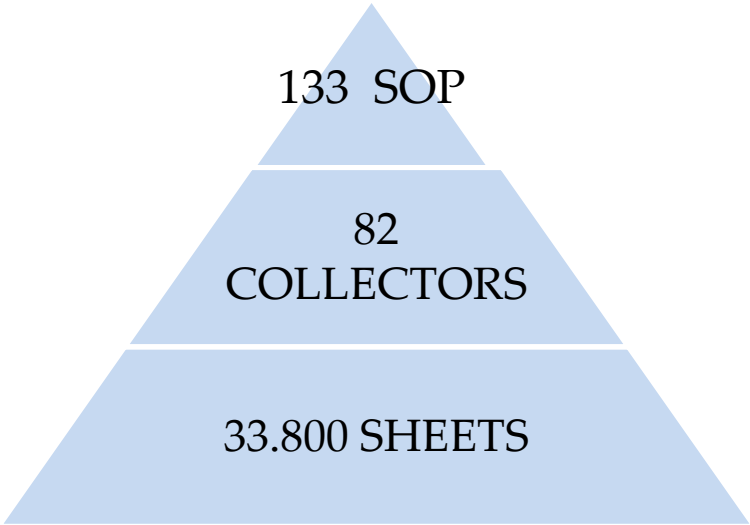
Fabbricazione di prodotti dell'ingegneria tissutale per sperimentazioni cliniche
destinati esclusivamente ai trapianti autologhi

Pagina 3 su 3

Progetto: I-312.AA.01-A5_01

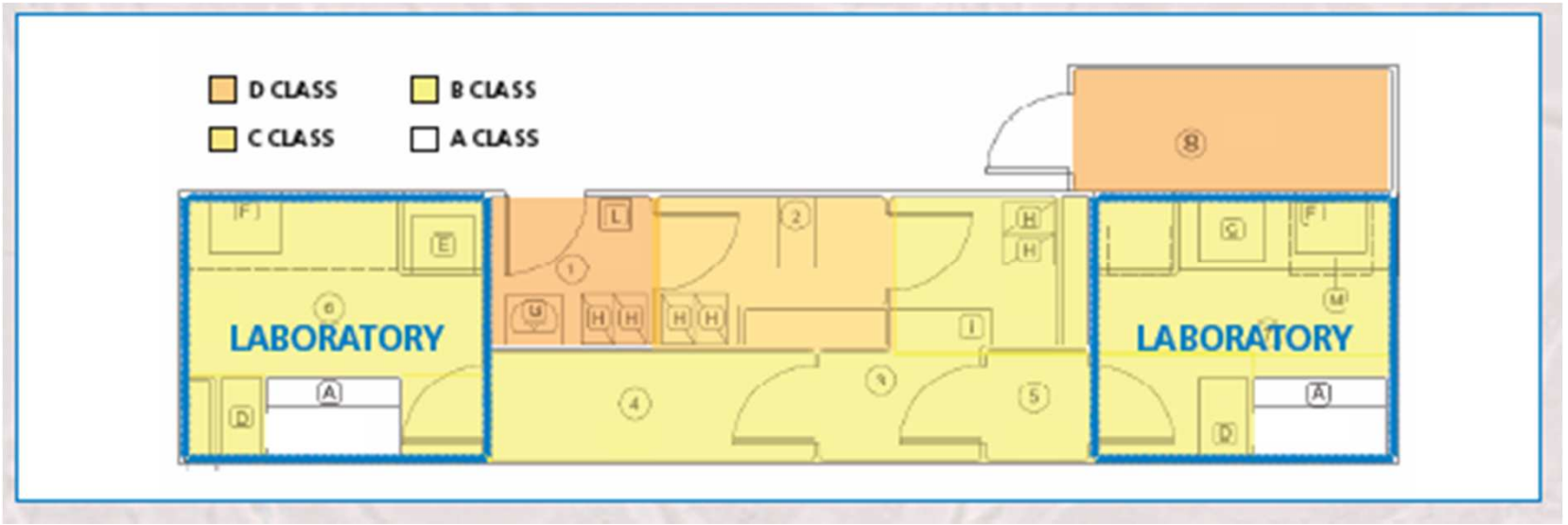
Schweizerisches Heilmittelinstitut
Institut suisse des produits thérapeutiques
Istituto svizzero per gli agenti terapeutici
Swiss Agency for Therapeutic Products

Swissmedic • Hallerstrasse 7 • Postfach • CH-3000 Bern 9 • www.swissmedic.ch • Tel. +41 31 322 02 11 • Fax +41 31 322 02 12



THE FACILITY

- TWO LABS IN POSITIVE ΔP
- MANUFACTURER: BACKGROUND WITH ACADEMIC GMPs
- CONSULTIANT: BACKGROUND WITH BIOLOGICS IN PHARMA



PRODUCTION AREA

AIR MONITORING

ACTIVE MICROBIOLOGICAL SAMPLING

8 FIXED POINTS IN A/B AREA

PORTABLE SAMPLING IN C/D AREA

THE QUALITY CONTROL LAB:

IN HOUSE OR OUTSOURCING?	
PRO	**CONS**
CHEAP?	INITIAL INVESTMENT (INSTRUMENTATION,) PREMISES,VALIDATION)
FAST	REQUIRES AUTHORIZATION
FLEXIBLE	SKILLED PERSONNEL

IIN HOUSE QUALITY CONTROL

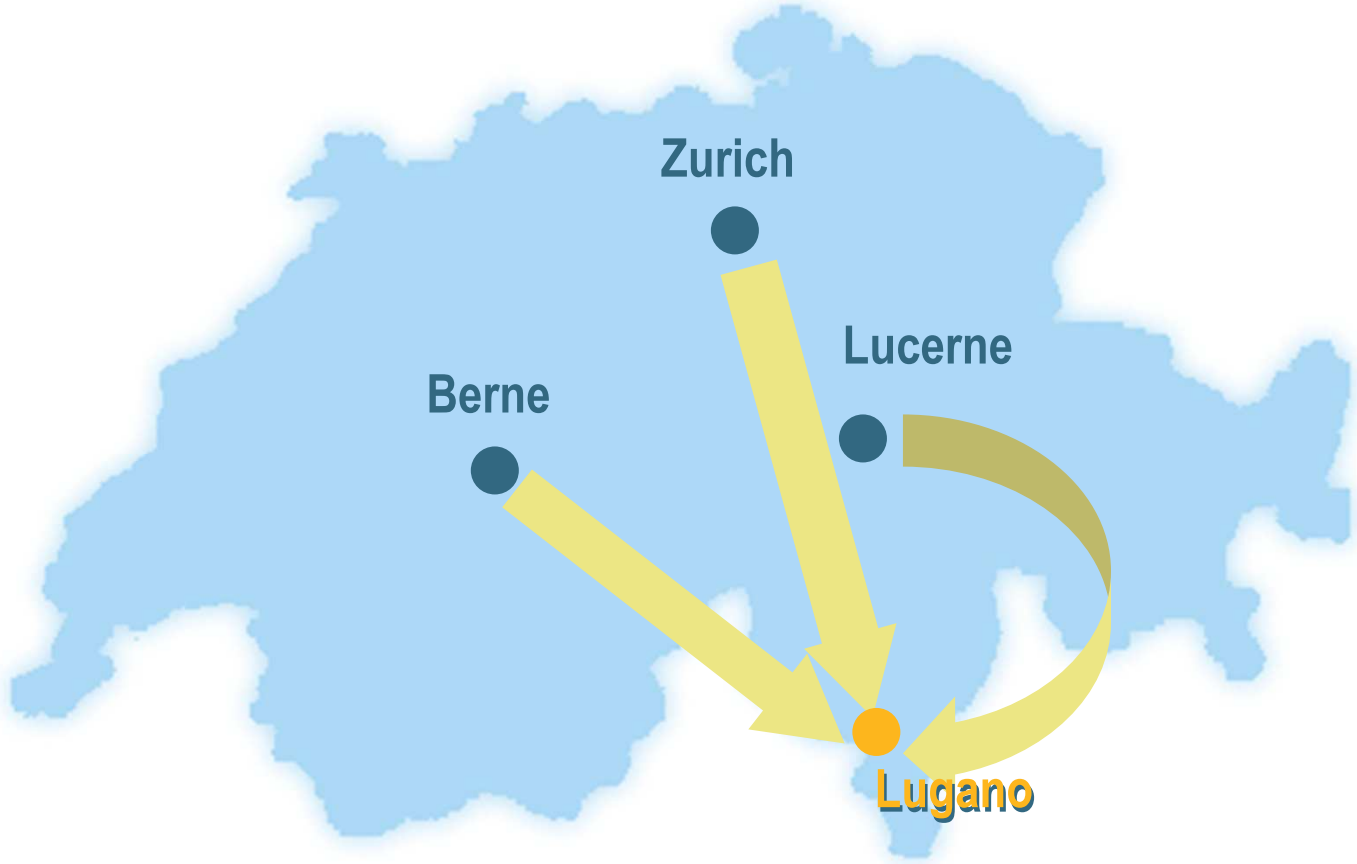
ANALYTICAL METHODS	
STERILITY	EuPh 2.6.1
MICROBIOLOGICAL CONTROL OF CELL SUSPENSIONS	EuPh 2.6.27
ENDOTOXINS	EuPh 2.6.14
TOTAL COUNT OF AEROBIC MICROORGANISMS (EUPH 2.6.12)	EuPh 2.6.12
CELL COUNT AND VITALITY ASSESSMENT VIA FLOW CYTOMETRIC ANALYSIS (EUPH 2.6.29)	EuPh 2.6.29
FLOW CYTOMETRIC ANALYSIS WITH CELL SURFACE MARKERS (EUPH 2.6.24)	EuPh 2.6.24

CLINICAL PROTOCOLS AT CARDIOCENTRO TICINO

Name	Patients	Study Type	Cells	Route of administration	Approval status
STIM	AMI	Monocentric Safety	BM-MNC	Intracoronaric s-top Flow Technique	Completed
SWISS AMI	AMI	Multicentric Randomized	BM-MNC	Intracoronaric s-top Flow Technique	Completed
METHOD	CHF	Monocentric Randomized	BM-MNC	Transendocardic Noga Guided	Ongoing
YNSTEM	AMI	Multicentric Double blind	CD133 + Selected cell	Intramiocardial During CABG	Ongoing
CIRCULATE	Critical limb ischemia	Monocentric Randomized	BM-MNC	Intra Arterial	Ongoing

THE SWISS-AMI CLINICAL TRIAL

Swiss multicenter Intracoronary Stem cells Study in Acute Myocardial Infarction

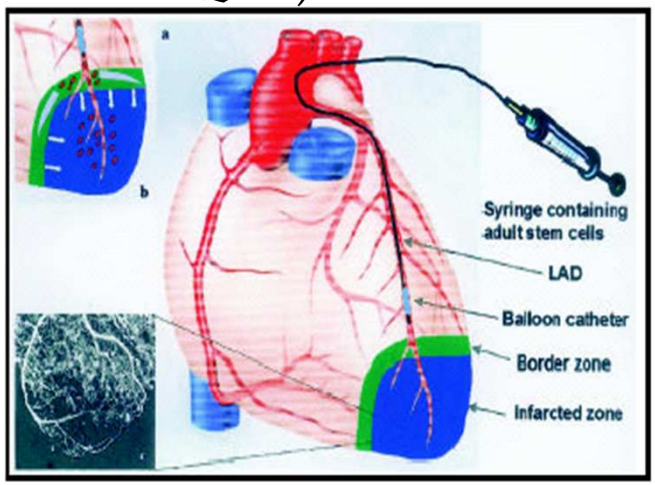


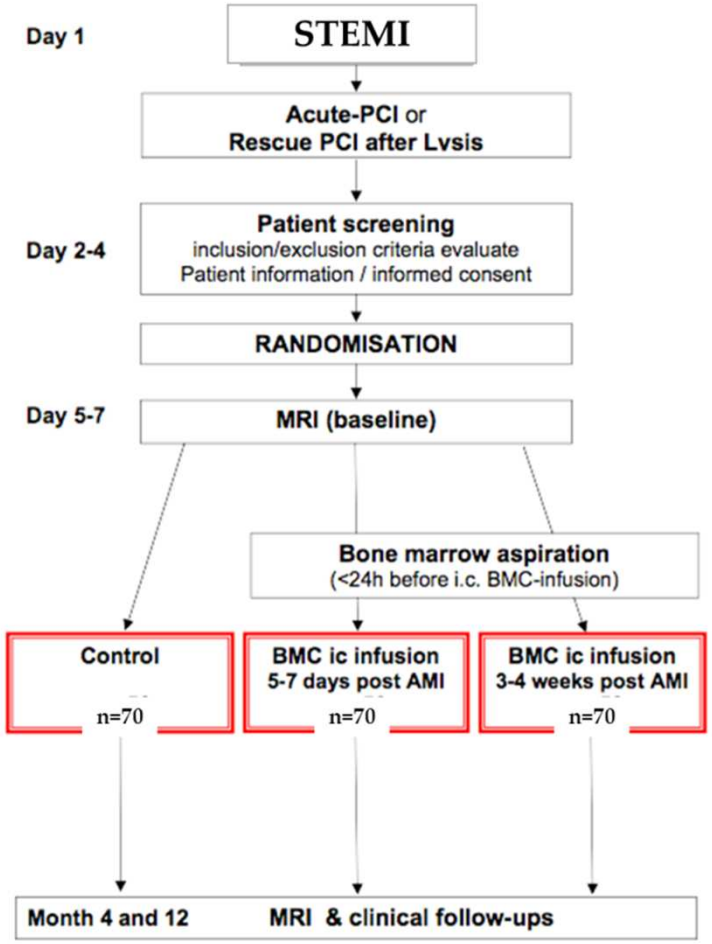
SWISS-AMI STUDY DESIGN

- 210 PATIENTS ENROLLED
- (the most extensive CT in cardiac regeneration)

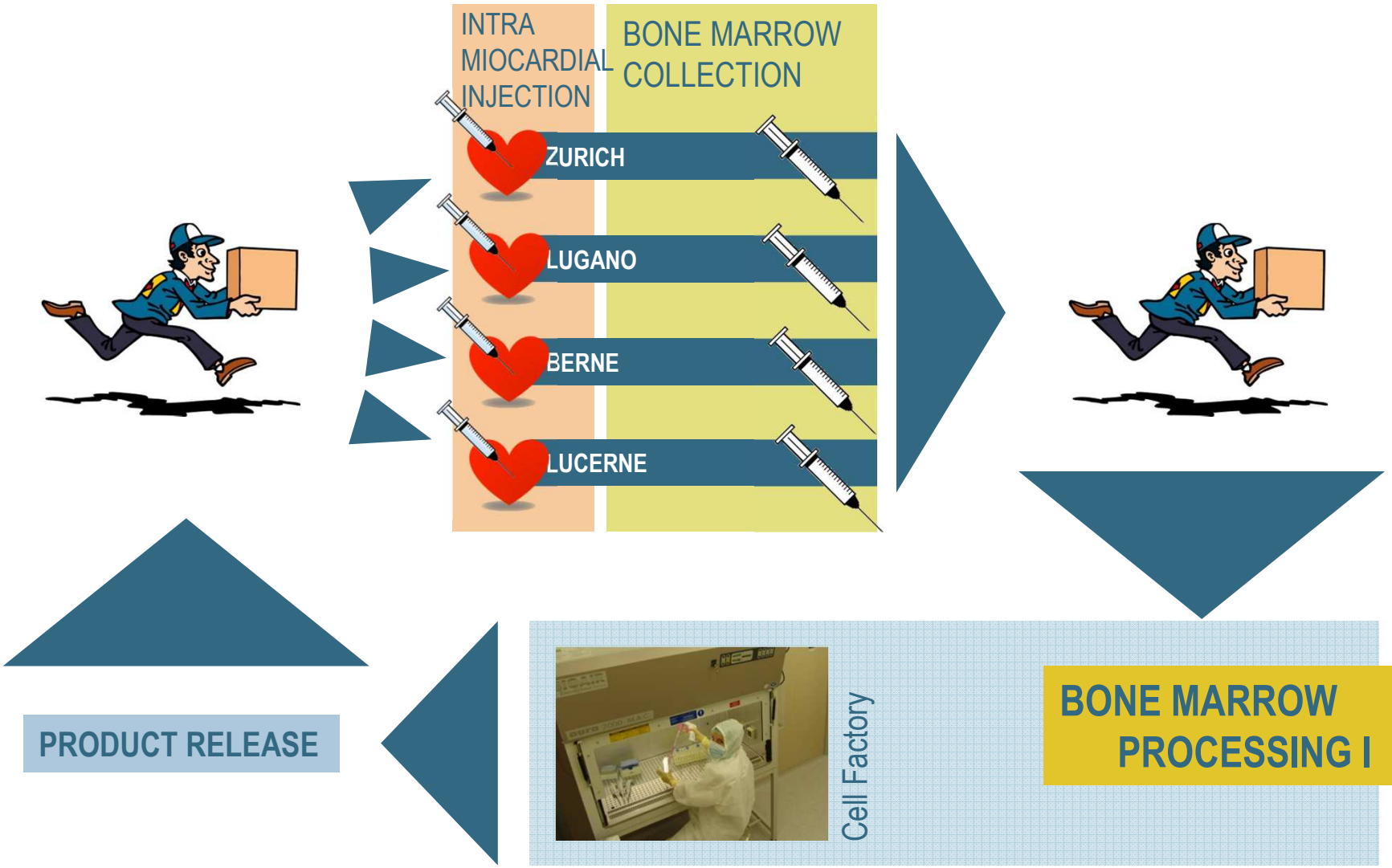
- PRIMARIY ENDPOINT: CHANGE IN GLOBAL **LVEF** BY CARDIAC MRI AT 4 MO

- CELL INFUSION: BY USING AN OVER THE WIRE BALLOON CATHETER (STOP FLOW TECHNIQUE)





THE SWISS-AMI CLINICAL TRIAL



THE SWISS-AMI CLINICAL TRIAL

Cell-based therapy for myocardial repair in patients with acute myocardial infarction: Rationale and study design of the SWiss multicenter Intracoronary Stem cells Study in Acute Myocardial Infarction (SWISS-AMI)

Daniel Sürder, MD,[a,b] Jürg Schwitter, MD,[a] Tiziano Moccetti, MD,[b] Giuseppe Astori, PhD,[b] Kaspar Rufibach, MD,[c] Sven Plein, MD,[d] Viviana Lo Cicero, PhD,[b] Sabrina Soncin, PhD,[b] Stephan Windecker, MD,[c] Aris Moschovitis, MD,[c] Andreas Wahl, MD,[c] Paul Erne, MD,[f] Peiman Jamshidi, MD,[f] Christoph Auf der Maur, MD,[f] Robert Manka, MD,[g] Gianni Soldati, PhD,[b] Ines Bühler, MD,[a] Christophe Wyss, MD,[a] Ulf Landmesser, MD,[a] Thomas F. Lüscher, MD,[a] and Roberto Corti, MD[a] *Zurich, Lugano, Bern, and Luzern, Switzerland; and Leeds, United Kingdom*

American Heart Association®
Learn and Live℠

Circulation

Circulation

Intracoronary injection of bone marrow derived mononuclear cells, early or late after acute myocardial infarction - effects on LV-function. <I>4 months results of the SWISS-AMI trial</I>

Daniel Sürder, Robert Manka, Viviana Lo Cicero, Tiziano Moccetti, Kaspar Rufibach, Sabrina Soncin, Lucia Turchetto, Marina Radrizzani, Giuseppe Astori, Jürg Schwitter, Paul Erne, Michel Zuber, Christoph Auf der Maur, Peiman Jamshidi, Stephan Windecker, Aris Moschovitis, Andreas Wahl, Ines Bühler, Christophe Wyss, Sebastian Kozerke, Ulf Landmesser, Thomas F Lüscher, and Roberto Corti

CIRCULATIONAHA/2012/145375

RESULTS AT 4 MONTHS

LVEF CONTROL: -0.4%

LVEF "EARLY": +1.8%

LVEF "LATE: +0.8%

STRATEGIES TO SURVIVE

FLOW CYTOMETRY

- Cell cycle
- Viability
- Apoptosis
- Cell phenotype

ELISA/ELISPOT

- Ab
- Cytokines
- Functional assays (cytotoxicity, MLR, proliferation)

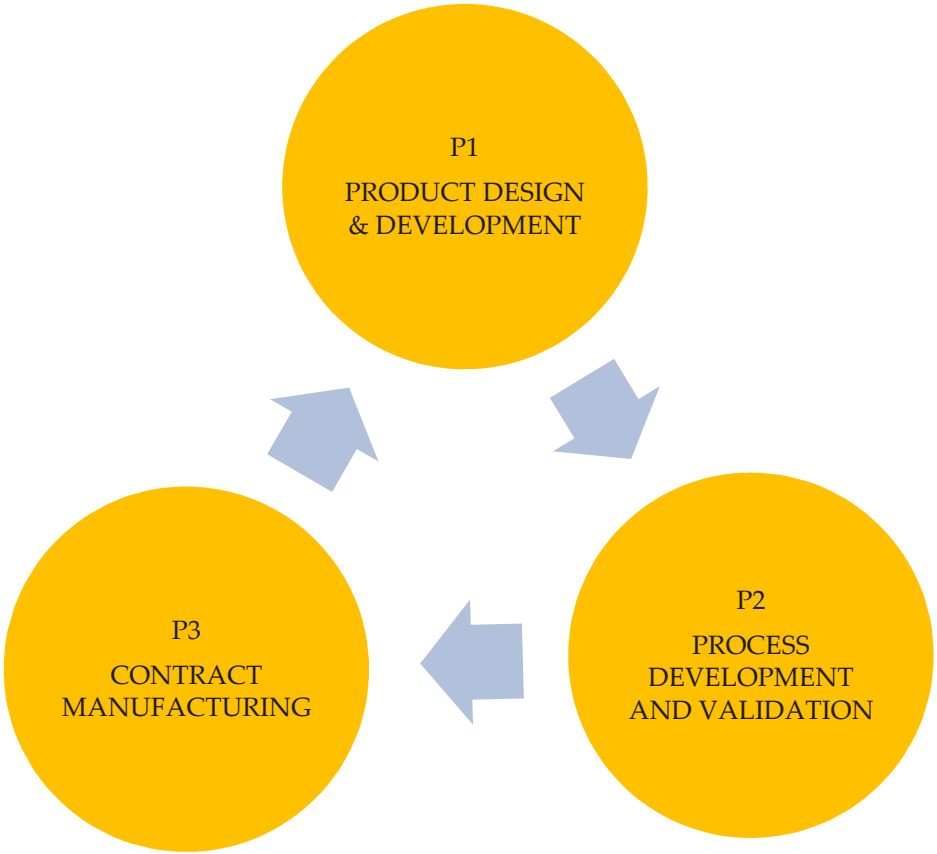
STERILITY

ENDOTOXINS

PCR

Gene expression

- Mycoplasma



LESSON LEARNED

PRO

- small organization, fast decision-making
- quick communication between the GMP team and the direction
- “reduced” bureaucracy allows communication between GMP team and regulatory agencies
- Good funding allows selection of qualified personnel

CONS

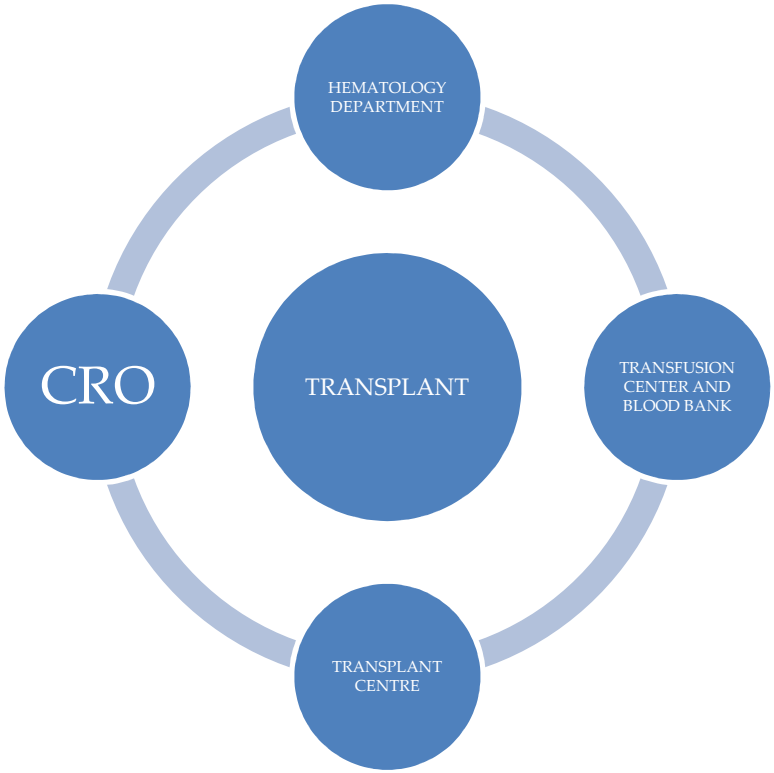
- Harmonization EU-CH
- Small territory (cases reduced)

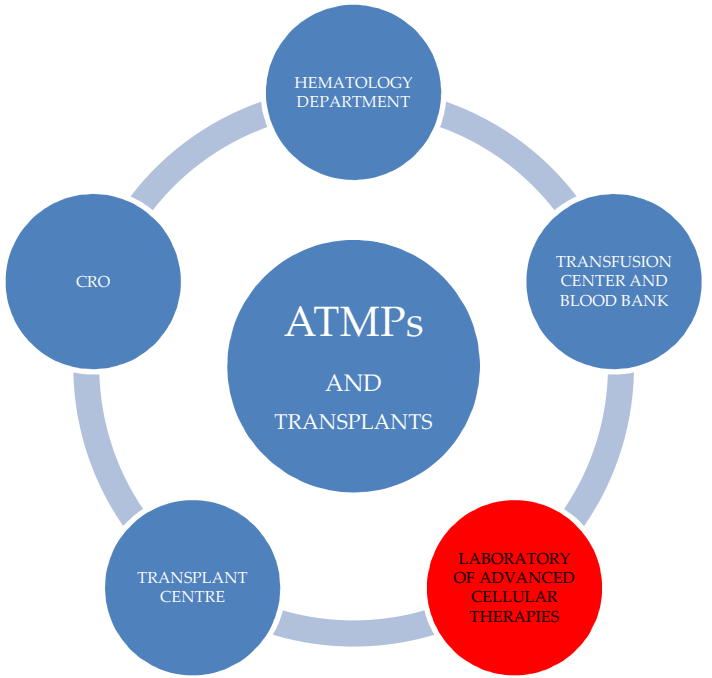
LESSON LEARNED: ACT AS ACADEMIA BUT THNK AS A COMPANY

LABORATORY OF ADVANCED CELLULAR THERAPIES (LTCA)

2011-PRESENT
VICENZA GENERAL HOSPITAL: 3889 EMPLOYEES
DEPARTMENT OF HEMATOLOGY AND CELLULAR THERAPIES: 95 EMPLOYEES

TRANSPLANT CENTRE:
ACCREDITATION: JACIE
AVERAGE NUMBER OF TRANSPLANTS /YEAR: 56
ALLOGENEIC: 23





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JACIE joint accreditation committee isct ebmt

THE ACADEMIC GMP NETWORK OF VENETO REGION

ABOUT 5 MILLION PEOPLE

1 VICENZA GENERAL HOSPITAL
LABORATORIO DI TERAPIE CELLULARI AVANZATE
Cellular therapy in Hematology.

2 VERONA UNIVERSITY HOSPITAL
Regenerative medicine (MSCs). Prof. M. Krampera

3 PADOVA UNIVERSITY HOSPITAL
Gene therapy. Prof. G. Palù

4 TREVISO REGIONAL HOSPITAL
Tissue Banking. Dott. A. Paolin

5 VENEZIA-MESTRE GENERAL HOSPITAL
EYE BANK FOUNDATION
Dott. D. Ponzin

CONSORZIO PER LA RICERCA SUL TRAPIANTO
DI ORGANI, TESSUTI, CELLULE E MEDICINA RIGENERATIVA
CONSORTIUM FOR RESEARCH IN ORGAN, TISSUE
AND CELL TRANSPLANTATION AND REGENERATIVE MEDICINE

CORIT

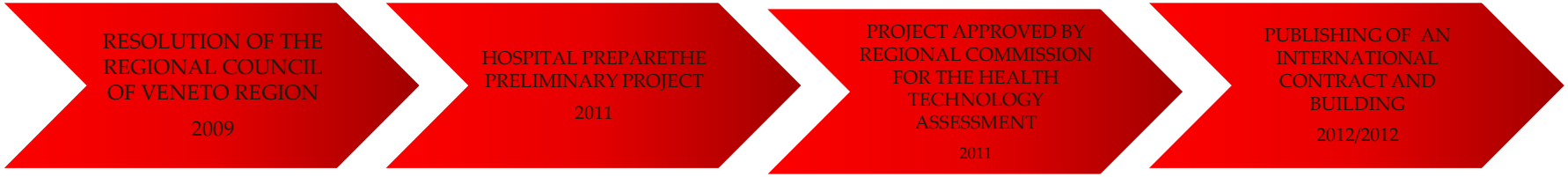
THE LOCATION: PALAZZO GIUSTINIANI-BAGGIO (YEAR 1655)

BEFORE

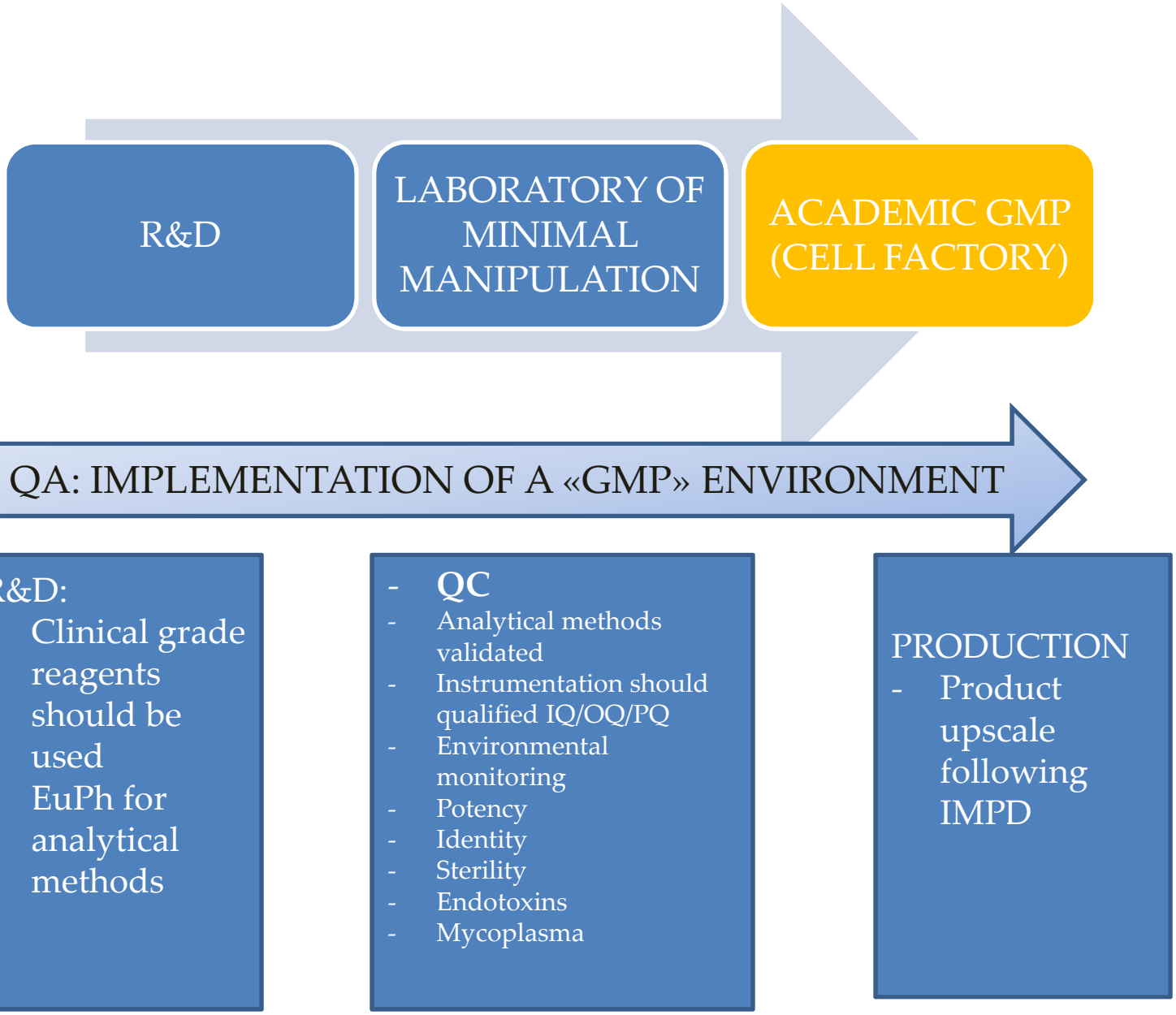
DURING RESTORATION

NOW

THE «GMP» PROCESS FOR APPROVAL



FROM BENCHTOP TO GMP



INVESTIGATIONAL MEDICINAL PRODUCT DOSSIER

…NON CLINICAL DATA SHOULD BE PRODUCED UNDER GLP…

ARE WE IN COMPLIANCE?

Reflection paper on stem cell-based medicinal products

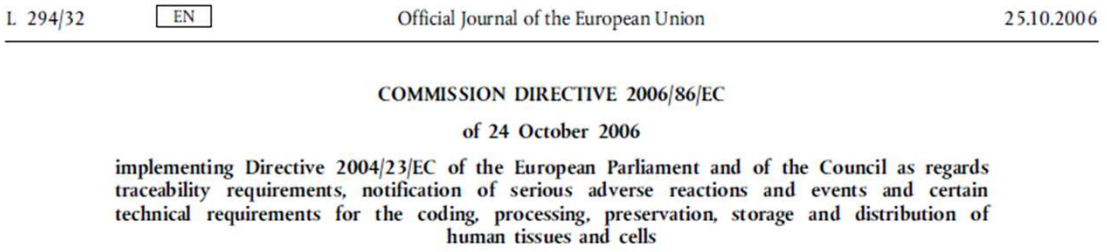
EMA/CAT/571134/2009

QUALITY	NON-CLINICAL	CLINICAL
• STARTING MATERIALS (safety) • MANUFACTURING PROCESS (design and validation) • identity • Purity • Potency • Tumorigenicity • Genomic stability	• Animal models • Biodistribution • Tumorigenicity • Differentiation “in vivo” • Immune rejection	• pharmacodynamic/ pharmacokinetic • dose finding • efficacy • Safety • Pharmacovigilance

MINIMAL AND SUBSTANTIAL MANIPULATION OF CELLS

MINIMAL MANIPULATION

- Cell banking
- Cell purging



L 294/32 EN Official Journal of the European Union 25.10.2006

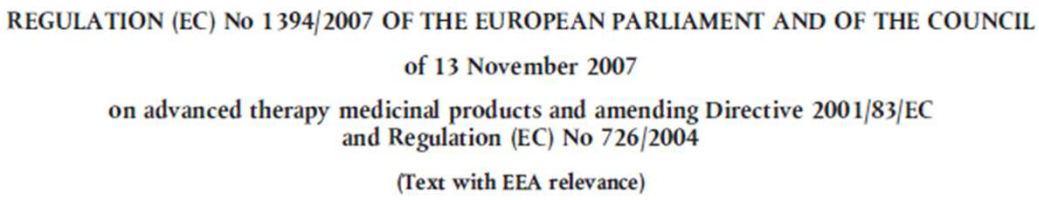
COMMISSION DIRECTIVE 2006/86/EC

of 24 October 2006

implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells

SUBSTANTIAL MANIPULATION (ATMP)

- Ex-vivo expansion
- Genetic modification
- Cellular activation



REGULATION (EC) No 1394/2007 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 13 November 2007

on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004

(Text with EEA relevance)

LABORATORY OF MINIMAL MANIPULATION

- LAMINAR FLOW HOOD IN «A» CLASS
- TWO ROOMS IN «D» CLASS
- CLINIMACS
- STERILE CONNECTON DEVICE
- CENTRIFUGE FOR BAGS

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CLINICAL STRATEGIES IN MINIMAL MANIPULATION

CMV-SPECIFIC CD8+ T CELL SELECTION AGAINST VIRAL REACTIVATION AFTER HSCT

- Prophylaxis for CMV reactivation in transplanted patients
- Reducing recurrent CMV reactivation

CD8-DEPLETED DONOR LYMPHOCYTES INFUSION (DLI) AFTER RELAPSE

- Effective in molecular relapse
- Prevent GvHD
- Quantitative monitoring of **WT1** expression in acute myeloid leukemia
- Early detection of relapse

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ATMPs

ISOLATION AND EXPANSION OF CYTOKINE INDUCED KILLER (CIK) CELLS FROM PB-MNC

TREATMENT OF PATIENTS RELAPSING AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

CIK are non-MHC-restricted T cell population CD3+CD56+with antitumor potential both in vitro and in vivo obtained from PBMC after stimulation with INF-ϒ anti CD3 Ab and IL2.

Lu and Negrin RS. J Immunol. 1994

ISOLATION AND EXPANSION OF CMV-SPECIFIC T-CELLS BASED EITHER ON IFN-γ PRODUCTION OR ACTIVATION-DEPENDENT EXPRESSION OF CD154

ADOPTIVE IMMUNOTHERAPY AGAINST CMV REACTIVATION AFTER HSCT

Feuchtinger et al., Blood, 2010

ISOLATION AND EXPANSION OF MESENCHYMAL STROMAL CELLS FROM BM, UCB, ADIPOSE TISSUE

TREATMENT OF ACUTE AND CHRONIC GVDH

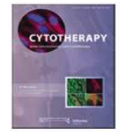
Le Blank K., Leukemia 2007

FAST PRODUCTION OF HUMAN PLATELET LYSATE BY USING ULTRASOUNDS

BACKGROUND:

- The use of FBS is discouraged by regulatory authorities
- Human platelet lysate has been proposed as a possible substitute of FBS.

Doucet et al., J Cell Physiol, 2005



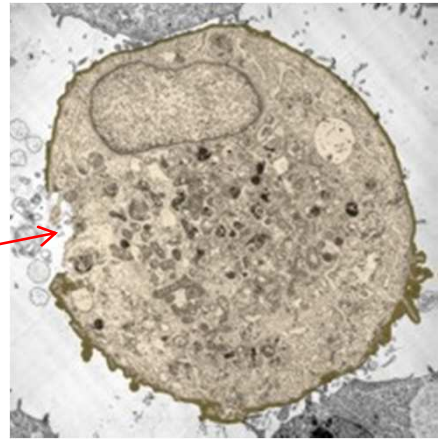
PRODUCTION OF HUMAN PLATELET LYSATE BY USING ULTRASOUNDS FOR THE EX-VIVO EXPANSION OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS

Martina Bernardi[1,2], Elena Albiero[1,2], Alberta Alghisi[3], Katia Chieregato[3], Chiara Lievore[3], Domenico Madeo[2], Francesco Rodeghiero[1,2] and Giuseppe Astori[3]

WE HAVE DEVELOPED A FAST PRODUCTION METHOD BASED ON THE USE OF ULTRASOUNDS

CAVITATION

- Occurs when ultrasound "hits" gas bubbles in fluids
- The compression and rarefaction of the fluid make bubbles contract and expand.
- If cells are present cavitation breaks cell membranes.



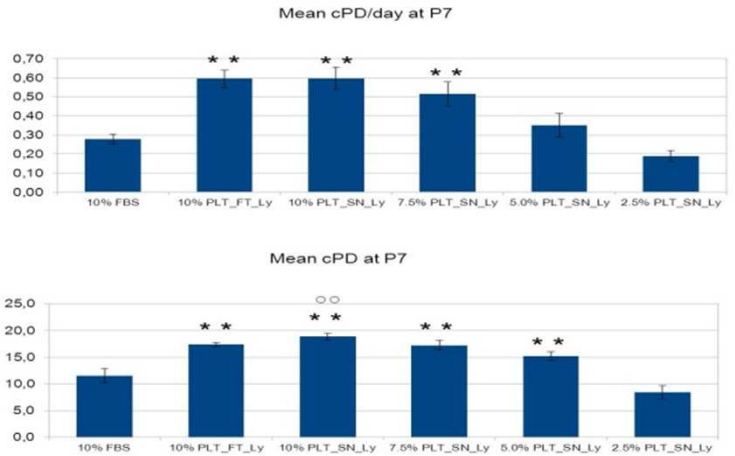
PLATELET LYSATE IS READY IN 30 MINUTES INSTEAD OF 24 HRS

"EFFICIENCY" OF PLATELET LYSATE IN THE EXPANSION OF BM –MSC

PDGF-AB release

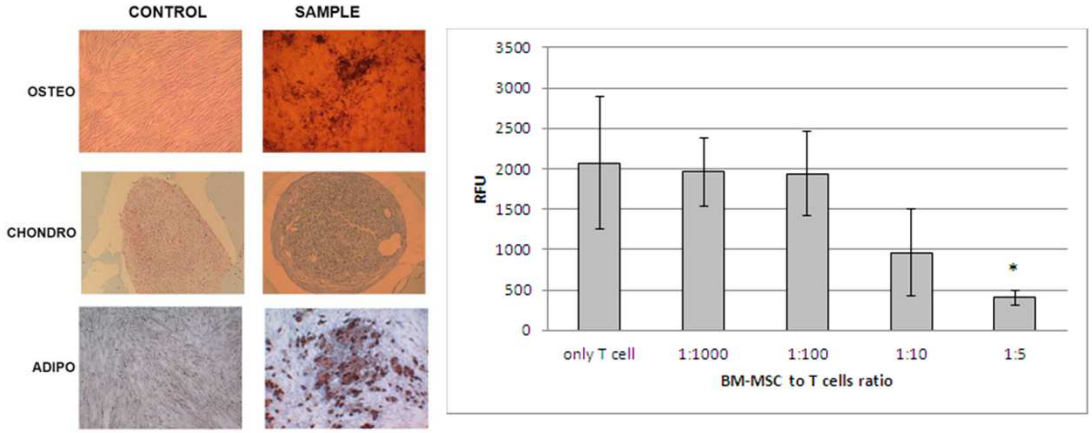
Platelet concentration in the PRP (109/ml)	Total PDGF-AB (pg/ml)	Concentration (pg/ml) and % of PDGF-AB in the supernatant after 30 min sonication	Concentration (pg/ml) and % of PDGF-AB in the supernatant after freezing/thawing
1.3x103 ±0.18	$1.2x10^5 \pm 2.6x10^4$	$8.7x10^4 \pm 3x10^4$ (74%)	$8.1x10^4 \pm 4.9x10^3$ (68%)

PLT LYSATE performed better in terms of doubling time than FBS



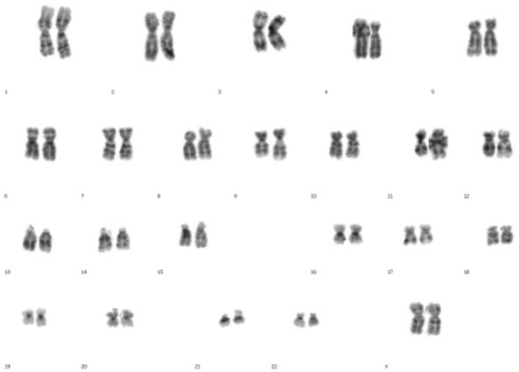
BM-MNC cultured in PLT LYSATE:

1. expressed MSC markers
2. differentiate into adipo osteo and chondro lineages
3. maintained immunosuppressive activity



"SAFETY" OF PLATELET LYSATE IN THE EXPANSION OF BM –MSC

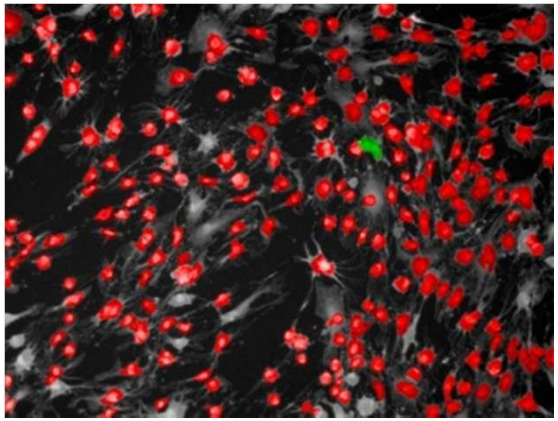
KARIOTYPE:
no genetic alterations at P=7

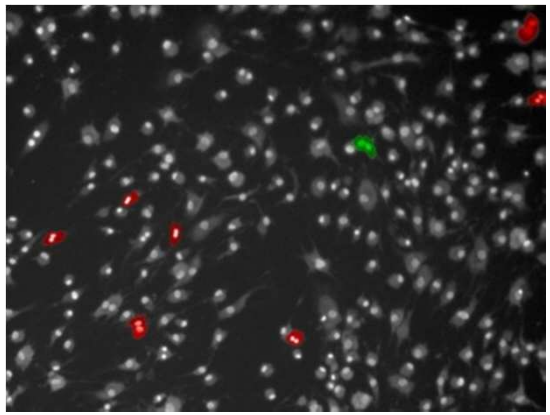


THE MICRONUCLEUS ASSAY FOR GENOTOXICITY (OECD GUDELINE 487, 22/08/2010)

Performed to evaluate the micronucleus frequency on CHO cells exposed to PLT LYSATE

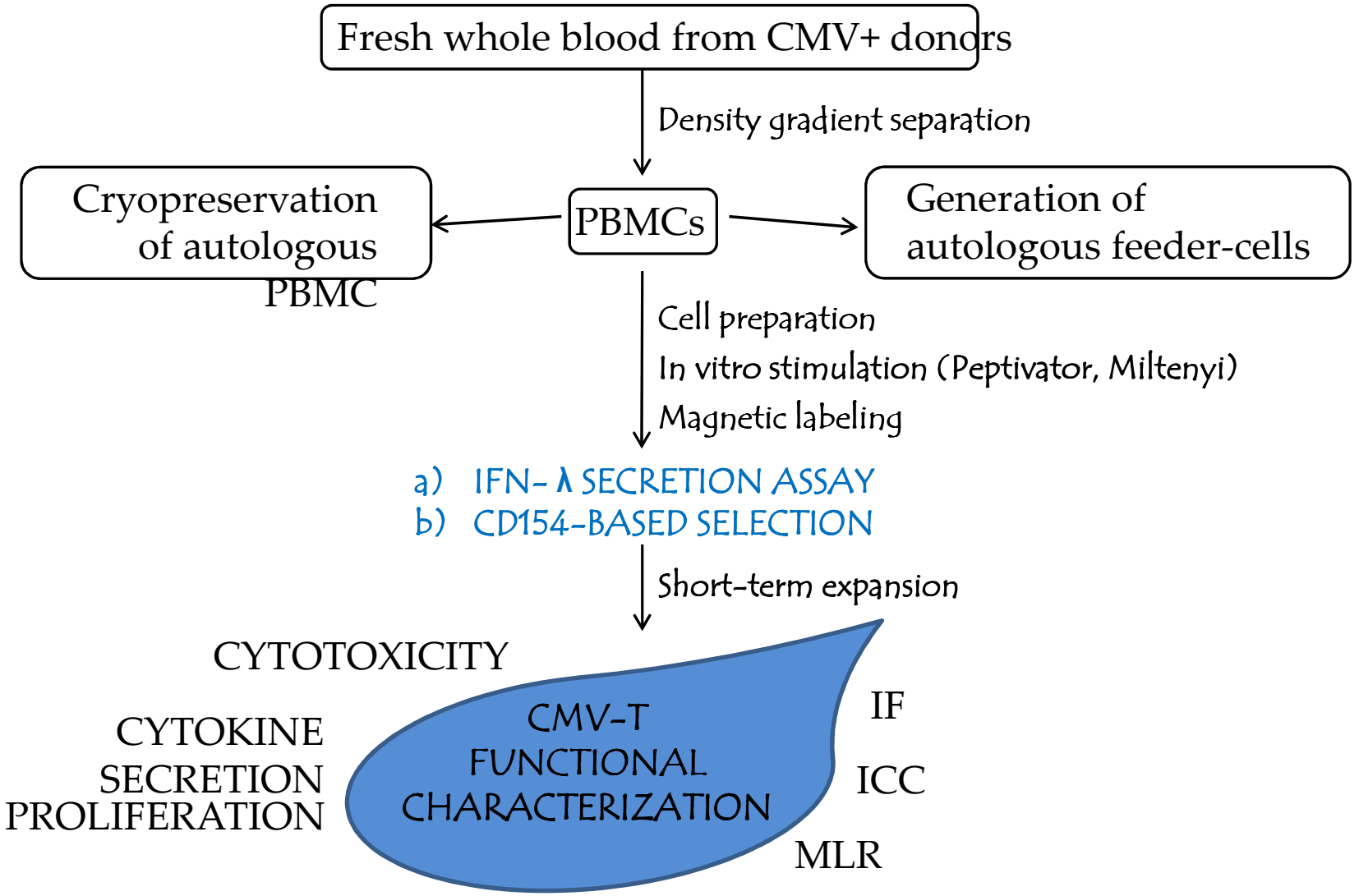
HIGH CONTENT IMAGING SYSTEM
OPERETTA, Perkin Elmer





ADOPTIVE IMMUNOTHERAPY AGAINST CMV REACTIVATION AFTER HSCT

Pre-clinical isolation and expansion of CMV-specific T-cells based either on IFN-γ production or activation-dependent expression of CD154



LESSON LEARNED

PRO

- Great hospital means great casistic
- Existence of a transplant centre (JACIE accredited)

CONS

- Great dimensions means slow communication between the GMP team and direction
- bureaucracy and administrative steps slow down the process

LESSON LEARNED: THINK AND ACT «GMP» AS SOON AS POSSIBLE

LESSON LEARNED

Don't be afraid to act for fear of making a mistake.
A man who never made a mistake, never made anything.

(Robert Baden-Powell)

LABORATORY OF ADVANCED CELLULAR THERAPIES

LTCA
- Francesco Rodeghiero
- Giuseppe Astori
- Lara Albania
- Elena Albiero
- Martina Bernardi
- Silvia Castegnaro
- Katia Chieregato
- Cristina Zanon
- Alfredo Amoroso
- Alberto Monastero
- Sabrina Sella

MICROBIOLOGY AND VIROLOGY
- Mario Rassu
- Francesca Furlan
- Michela Pascarella
- Martina Stevan

TRANSPLANT CENTER
- Roberto Raimondi
- Carlo Borghero
- Francesca Elice
- Carlo Visco

CIBIO UNIVERSITY OF TRENTO
- Alessandro Quattrone
- Valentina Adami

HUMAN GENETICS
- Annamaria Montaldi
- Paola Celli

IMMUNOHEMATOLOGY AND BLOOD BANK
- Alberta Alghisi
- Chiara Lievore
- CinziaTagliaferri