13.35-15.05	**Cell Processing and HLA Typing** **Chairs**: Mickey Koh (Singapore/UK) and Hind Alhumaidan (KSA)
13.35-13:50	**Minimal requirements for a cell processing laboratory** Mickey Koh (Singapore/UK)
13.50-14:05	**Minimal requirements for a HLA laboratory** Abulghani Tbakhi (Jordan)
14.05-14:20	**Stem cell processing: method and graft characterization** Paul Eldridge (USA)
1420-15.05	**Roundtable discussion: How to successfully establish a cell processing facility** **Moderator:** Mickey Koh (Singapore/UK) **Panellists:** Hind Alhumaidan (KSA) Miguel Abboud (Lebanon) Moheeb Alawwami (KSA) Basim Albeirouti (KSA) Usama Gergis (USA) Ibrahim Alghemlas (KSA) Nina Worel (Austria) Paul Eldridge (USA) Abulghani Tbakhi (Jordan)

WBMT Graft Processing and HLA Typing Workshop

Dr Mickey BC Koh
St George's Hospital, London, UK
Health Sciences Authority, Singapore

Mickey.koh@stgeorges.nhs.uk
Mickey_koh@hsa.gov.sg

Main functions:

-Overseeing the safe receipt/handling of donor stem cells
-Defining the product: its quality and characteristics----Paul Eldridge
-any manipulation required for the transplant---- Paul Eldridge

-Safe delivery back to the hospital/patient including infectious diseases

-quality assurance

Graft Processing

- Integral part of the transplant programme
- Specialised manpower and equipment: ?is cost factored into transplant calculation
- Minimal to advanced extensive processing
- Stem cell sources: BM vs PBSC vs Cord
- Essential parameter in determining engraftment; graft versus host disease; immune reconstitution; relapse

Scope of Talk

- Physical premises and considerations
- Equipment/reagents and personnel needed
- Range of Processing Services offered
- Guidance documents and resources

Key Considerations

- Minimally Manipulated Products in support of a transplant programme.
- improvements will be made as additional resources become available and as volume and scope of clinical transplant services increase
- Growth into more complex cell processing. Haplo-identical/ CD34 selection/T cell depletion
- Cell Therapy: Haematolo-oncology and regenerative medicine
- **Financial and Regulatory considerations for each stage**

Physical Considerations

- **Does every transplant programme require a processing lab?**
- **Does centralising reduce costs and make best use of manpower?**
- **How do you plan for growth of transplant numbers**
- **Number of centres; transplant numbers; distances from lab to centres**
- Hospital based vs involvement of the Transfusion Service
- Examples of processing labs in the UK and Singapore

Strengths of Transfusion Laboratories and Blood Banks

- Harvesting and handling of apheresis and cellular products
- Quality systems with a focus on “processes”
- Product safety focus including stringent donor testing
- Mulitidisciplinary: technologists, similar staff training; microbiologists
- Back-up power supplies

Scope of Talk

- Physical Layout and considerations
- **Equipment/reagents and personnel needed**
- Range of Processing Services offered
- Guidance documents and resources

Required Equipment:

Dedicated:

Biosafety Cabinet	Refrigerator	Balance (Scale)
Water bath	Centrifuge	Freezer (≤ -70^{o}C)
Hematology Analyzer	Tubing sealer	Personal computer
Plasma Extractor	SCD	
Pipette Aid	Hemostats	Tubing stripper
Cryo-transporter	Micropipettes	Label printer

Shared:

Hematology Analyzer	Flow Cytometer	Micro Lab
Microscope	LN_2 Freezer	Reference Thermometer

Equipment/Reagents

- Reliable Maintenance and Availability
- Qualification/validation and monitoring of equipment/reagents
- Back up/ Contingency:

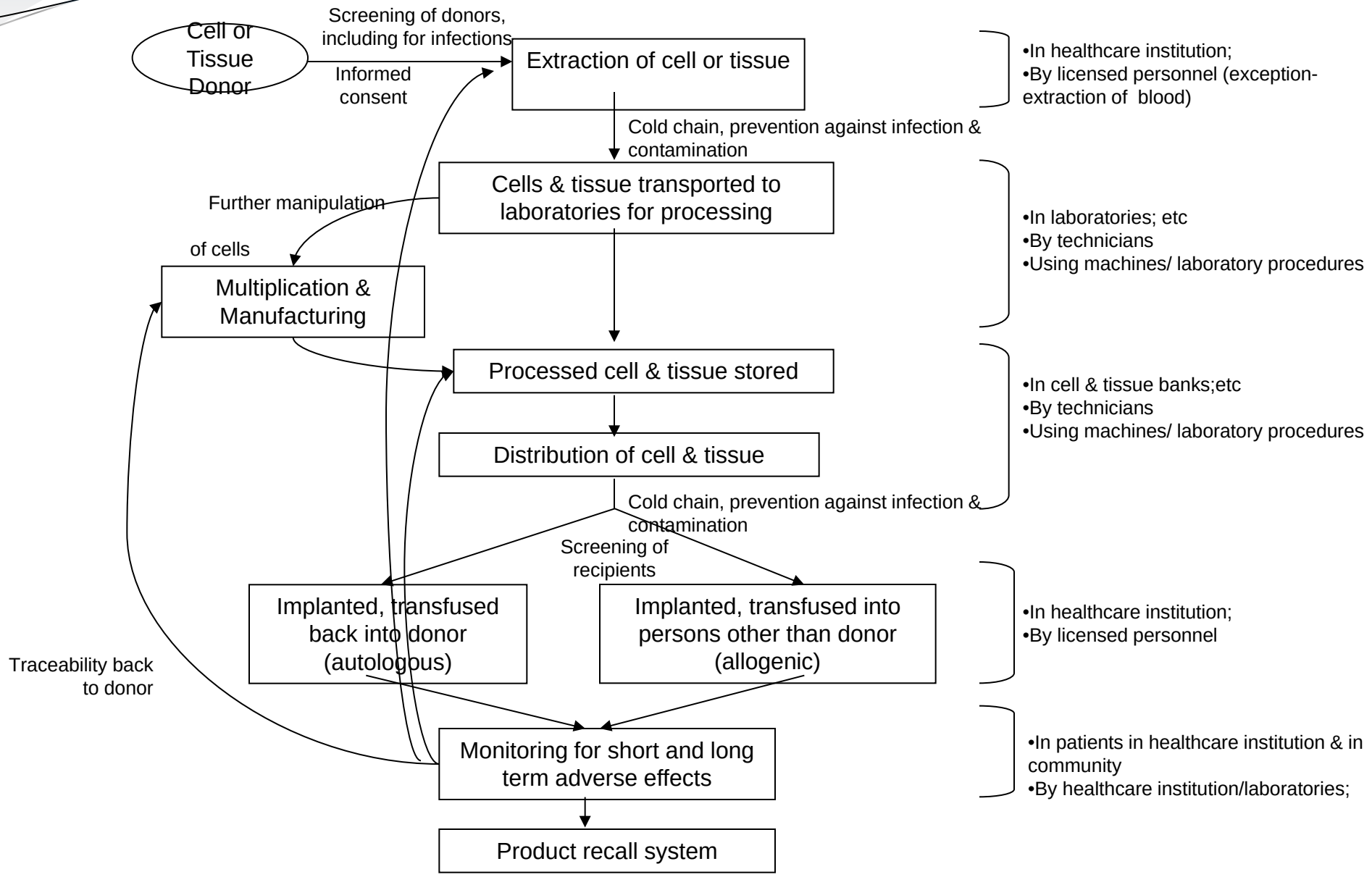
- **As Complexity and Volume increases:**
- Automation: cell washers
- Cell selection devices: CliniMACS
- Closed systems: Prodigy
- Bioreactors; Modular Systems

Important Considerations

- Qualified staff and Training programmes
- Quality systems
- Quarantine
- Non-conforming product
- Labelling and Cold Chain Transport
- Country Regulations.
- Traceability of Stem Cell donations
- Infectious Disease Testing

Annex A

General flowchart of cell & tissues used for therapy from source to final usage

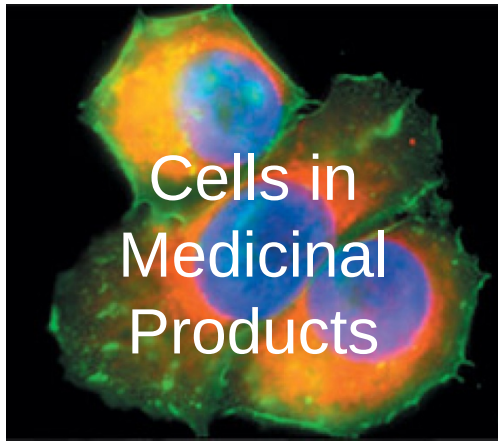


Regulatory Requirements concerning Advanced Therapy Medicinal Products (ATMP)

Challenges for Advanced Therapy Medicinal Products

Starting Material
Testing of Donors
Microbiological Safety of Procurement
Cell banking
Origin of Cell lines

Clinical Aspects
Safe initial dose
Indications
Reactions in humans
Follow-up



Transport to manufacturing site:
Cold Chain
Viability of Cells
Chemicals for Cryopreservation
Sterility

Non-Clinical Aspects
Toxicology
Pharmacovigilance
Biodistribution
Tumorigenicity
Immunogenicity

Quality Aspects
Identity
Purity
Sterility
Tumorigenicity

QC Testing

Attribute	Test Method	Specification
Donor Screening	Summary of Records; Donor Eligibility Form	Donor Eligible
Inf Disease Testing	Certified Laboratory	Negative (except CMV)
Infusion Volume	Measurement	≤20mL / Kg / Infusion
DMSO Volume	Calculation	≤ 1mL / Kg / Day
TNC Count	Cell Counter	As Measured
CD34+ Cell Count	Flow Cytometry	≥ 2×10^6 / kg
CD3+ Cell Count	Flow Cytometry	As measured
RBC Content	Cell Counter	≤25mL / Adult Infusion
Viability	Flow Cytometry	≥ 80% (pre-freeze)
Sterility	Bacterial Culture; Fungal Culture	No Growth

Clinical Focus

- Representation at Clinical Transplant Meetings
- Correlation with engraftment data and clinical outcomes (CD34; TNC; viability; microbiology)
- assurance that the clinical outcomes match the reliability of processing
- Apheresis /processing/ staff /equipment all contribute
- /Threshold of $2x10^6$ CD34/kg and a desirable $5x10^6$ CD34/kg

Scope of Talk

- Physical Layout and considerations
- Equipment/reagents and personnel needed
- **Range of Processing Services offered**
- Guidance documents

Auto vs Allo

- Autologous: freezing capacity and secure storage for the stem cell graft. Largely PBSC based with some mobilisation failure
- Allogeneic: red blood cell (RBC) and plasma depletion services. DLIs, Marrow and cord blood
- Is Autologous processing more challenging? **Non cryopreserved an alternative? No DMSO**
- Allo products if given fresh actually needs less doing than auto except for plasma/red cell depletion in ABO mismatched transplants. If PBSC-only plasma depletion and this often not mandatory

Key Partner Organizations:

AABB	www.aabb.org
ISCT	www.celltherapysociety.org
FACT/JACIE	www.factwebsite.org
ISBT	www.isbtweb.org
ICCBBA:	www.iccbba.org
WBMT:	www.wbmt.org
CTCLAG	
AHCTA	

World Health Organization

AIDE-MEMOIRE

for National Health Authorities*

Tissue and cell transplantation represent essential and rapidly developing therapies in modern healthcare. It is the responsibility of national health authorities to ensure that the needs of patients are met with a supply of safe tissues and cells of appropriate and consistent quality. A nationally supported legislative framework which defines consent requirements and supports donation and a regulatory system which authorises tissue and cell banks are prerequisites to achieving this goal. Donation and transplantation activities should be organised in a transparent way with the provision of adequate information and data to enable the public to make informed choices.

Tissue and cell transplantation carry risks of disease transmission. Viruses (including HIV, hepatitis B and C), bacteria, fungi, parasites

Access to Safe and Effective Cells and Tissues for Transplantation

☑ **Checklist**

National Oversight

- ❑ Legislative/Regulatory framework
- ❑ Appropriate national/international standards
- ❑ Inspection and authorisation of screening, testing, retrieval, processing, storage, distribution, import and export
- ❑ Surveillance and vigilance including transplantation transmitted disease
- ❑ Monitoring and reporting of donation, processing, distribution , import, export and transplantation activity data

Cell Processing Panel Discussion (40 min):

1. Where to set up such a facility?
   -hospitals vs blood banks (distance, infrastructure, expertise, lab testing)
2. Growing the facility: numbers and complexity
3. Cost of Building and Operating/Maintaining.
4. Capabilities, Staff expertise, Training across regions/countries. ?Twinning

Cell Processing Panel Discussion:

5. Clinical Interface between the Clinical Programme and the Processing Facility
6. Working within regulatory frameworks. What exists and what needs to be developed for each country? Engaging govt officials and regulators
7. What innovative or novel cell processing solutions have the panellists been involved? How has this affected the landscape of graft processing and transplants?

HLA Typing Discussion:

8. Experiences of setting up a stem cell processing lab. Widely variable region: constrained resources, instability to state of the art? Panellists to share their involvement in this

9. How should one start? Auto vs Allo. The cell processing perspective

10. Advanced cell processing and Regenerative medicine

11. HLA: cost vs volume vs expertise.

12. Low res vs high ?only low res needed for siblings? DNA based techniques: can we adapt from other labs?

Minimal Requirements and Essential Features for Setting up a Stem Cell Processing Laboratory.

- **Thomas Leemhuis, Douglas Padley, Carolyn Keever-Taylor, Dietger Niederwieser, Takanori Teshima, Francesco Lanza, Christian Chabannon, Paul Szabolcs, Ali Bazarbachi, Mickey BC Koh (chair)**

- On behalf of the Graft Processing Subcommittee of the Worldwide Network for Blood and Bone Marrow Transplantation (WBMT).

Regulation follows clinical practice

4 categories to consider

- **Standard Transplants: no ex vivo expansion**
  -international accreditation; reporting centres
- **Newer Cell Therapy Protocols**
- **Embryonic Stem cells; iPS cells, other stem cells.**
- **Regenerative medicine and “aesthetics”**

The Ideal Graft Engineered Transplant

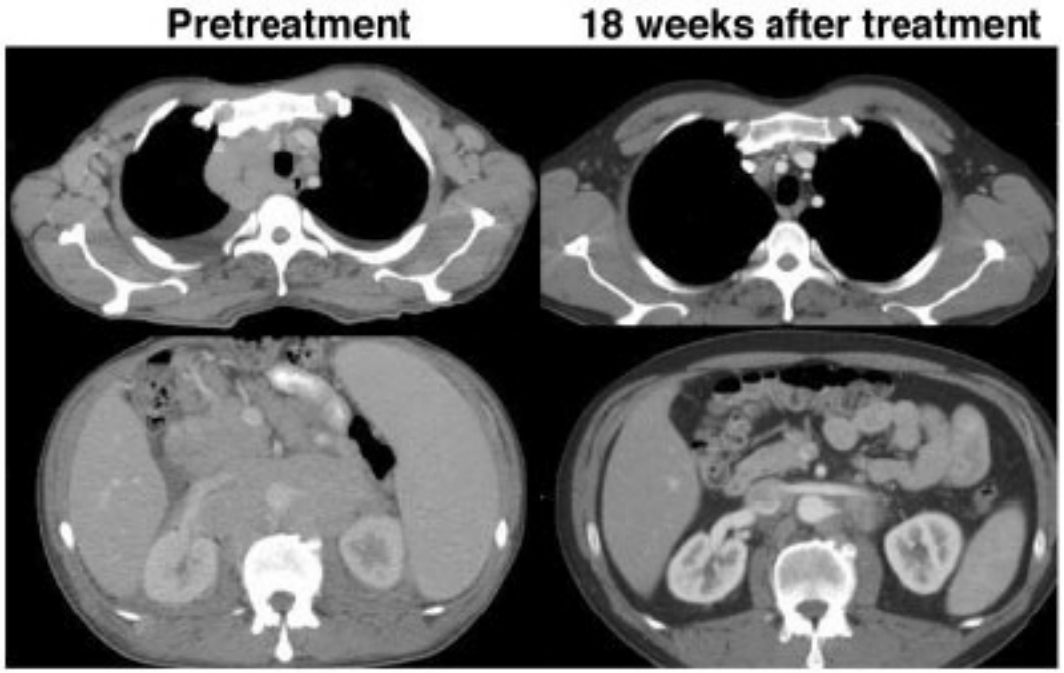
- Minimal Conditioning (cells to facilitate engraftment)
- Graft enriched for haematopoietic progenitor cells
- Addback of Immune effectors
  to maximise anti tumour activity
  promote broad immune reconstitution
  enhance anti viral immunity
  abrogate clinical GvHD

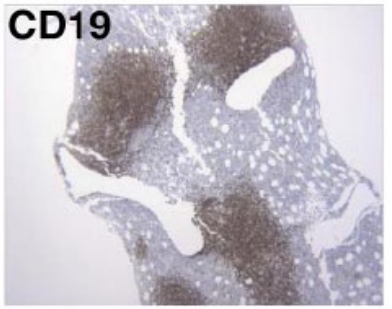
The NEW ENGLAND JOURNAL of MEDICINE

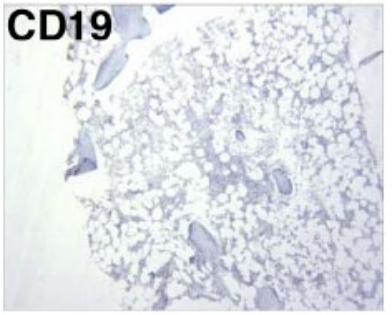
BRIEF REPORT

Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia

David L. Porter, M.D., Bruce L. Levine, Ph.D., Michael Kalos, Ph.D., Adam Bagg, M.D., and Carl H. June, M.D.







Europe/UK

- Minimally manipulated cells and tissues
  - Not a medicinal product
  - Regulated under the Tissue Directive (2004/23/EC) - donation, testing, procurement, processing, storage and distribution across EU
  - Tissue establishment authorisation by national drug regulatory authorities
- UK – Human Tissue Authority (HTA)
  - The scope of this Code includes all human tissues (including haemopoietic progenitor cells bone marrow, peripheral blood, cord/placental blood) used for therapeutic purposes including those used in clinical trials.

Europe/UK

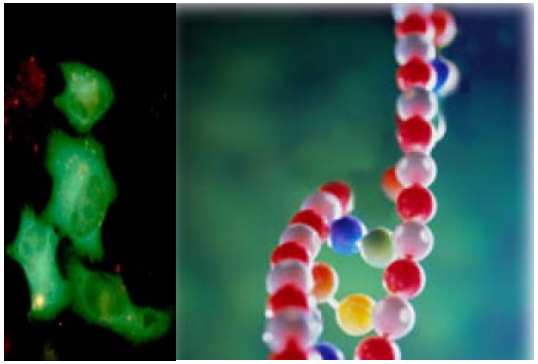
- Substantially manipulated cells / non-homologous use
  - Advanced therapy medicinal product (ATMP) – medicinal product
    - Somatic cell therapy medicinal product
    - Gene therapy medicinal product
    - Tissue engineered product
  - Comply with tissue regulations
  - Centralised approval procedure by European Medicines Agency (EMA) for marketing authorisation
  - Clinical trial authorisation by national drug regulatory authority

Advanced Therapy Medicinal Products (ATMP)

Advanced Therapy Medicinal Products

Gene Therapy

- gm cells and nucleic acids

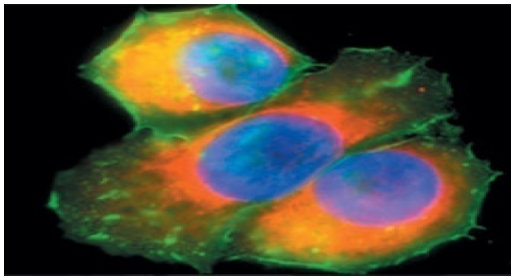


- recombinant nucleic acids in
  - viral or non-viral repl.-incomp. vectors,
  - DNA or RNA,
  - gm cells,
  - rec. replicating viruses/micro-org.

Cell-based MPs (CBMP)

Somatic Cell Therapy

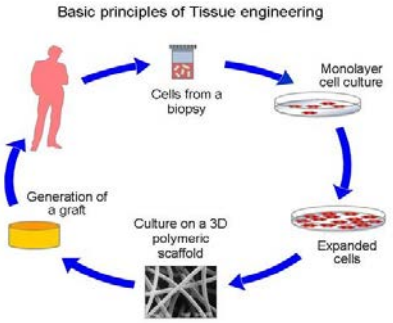
immunological SCTs



- **engineered** cells used for disease
  - -Treatment
  - -Prevention
  - -Diagnosis

Tissue Engineered Products

- ACT, stem cells for tissue repair



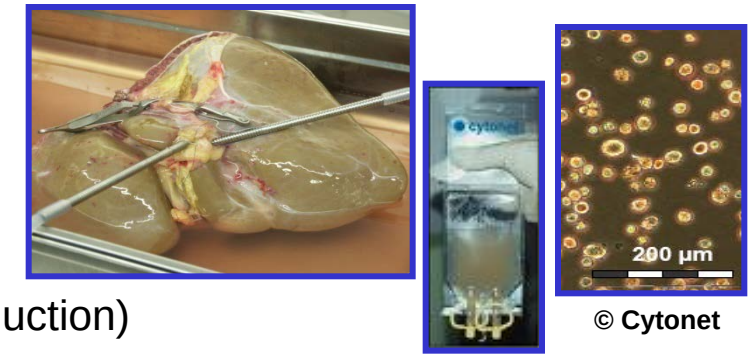
- **engineered** cells used for tissue
  - Regeneration,
  - Repair or
  - Replacement

Regulatory Requirements concerning Advanced Therapy Medicinal Products (ATMP)

Somatic Cell Therapy Medicinal Products

Allogenic liver cell suspensions

- Treatment of acute sepsis or inherited metabolic liver failure



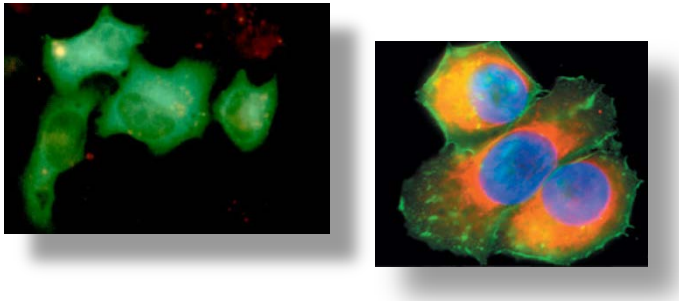
© Cytonet

Allogenic pancreatic islets

- Therapy of Type I Diabetes (restore insulin production)

Immunotherapeutics

- CTLs or NK cell transfer for adoptive immuntherapy



Cell-based Therapeutic Vaccines

- Peptide-loaded DC used as tumor vaccines
- Fused Tumor/DC hybrid cells

Adult stem cells (HSC, BM-MSC, ADSC, placental MSC, USSC)

- BM fractions for treatment of heart failure
- MSC for treatment of limb ischemia

Advanced Therapy Medicinal Products (ATMP)

Tissue Engineered Products

Autologous Chondrocyte Transplantation

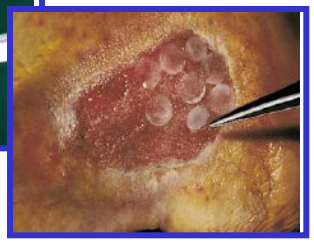
www.tetec-ag.de

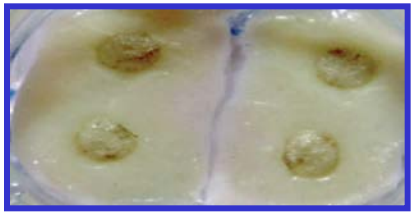
www.biotissue-tec.com

www.codon.de

Skin Replacement

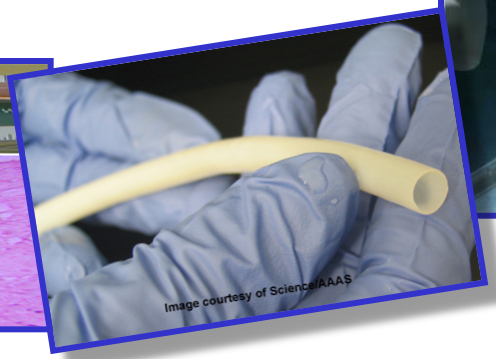
www.euroderm-biotech.com

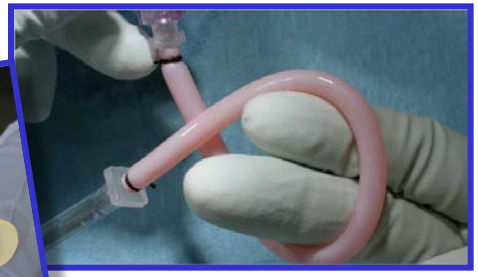




www.A-skin.nl

Vessel Replacement





www.cytograft.com

TEVGs- Decellularized tissue-engineered vascular grafts (Humancyte, USA)

Drug vs Cellular Product

	Biotechnology	*Cell Therapy*
Product	Cultured cells generate product	Living cells ***are*** product
Raw Material	Seed cell lines	Unique, primary tissue
Variability, Heterogeneity	Limited	Substantial
Product Definition	Well-defined, definable products	Product defined through trials Full definition likely unattainable
Process, Testing	Established early	Evolve through trials
Process Scale	Bulk processes predominate	Patient-specific products common

Cellular Therapy Course

- Multiple infusions along a period of time or single infusions, or implantation.
- Engraftment or not (depending on potency)
- Early toxicities
  - Infusion reactions
- Late toxicities:
  - Second malignancies (?)
  - "Miss-differentiation"
  - GVHD
  - Malignancy Relapse
- Milestones are not well defined for all cellular therapies.

iPS Cells

- Embryonic stem cell (ESCs): derived from embryos and is capable of long term self-renewal and differentiation into all cells and tissues in the human body.
- manipulation of transcription factor expression can change cell fate
- Yamanaka's Nobel Prize winning work: screened for factors within a pool of 24 pluripotency-associated candidate genes and identified that 4 transcription factors comprising Klf4, Sox2, c-Myc, and Oct4 was sufficient to produce induced pluripotent stem cells (iPSCs/iPS)
- adult human skin fibroblasts. Since then have demonstrated that iPSCs can be derived from other somatic cell populations using this cocktail of genes, including keratinocytes, neural cells, stomach and liver cells.

Clinical Applications and Concerns

- Tumorigenecity
- Premature senescence
- Japan: age-related macular degeneration (AMD) and is scheduled to commence 2014
- In vitro red cell generation
- Disease modelling: single gene vs multigenic disorders
- Drug testing

HIGH LEVEL RESOLUTION……less chance of a match……

1. low resolution (2 digit) - identifies broad families of alleles belonging to the same serotypic group (e.g. A*02)
2. intermediate resolution (allele string) - identifies alleles that have common sequence determinants and thus share hybridisation pattern (e.g. A*02:05/08/22)
3. high resolution (minimum 4 digit) - identifies single allele

The impact of HLA genotyping on survival following unrelated donor haematopoietic stem cell transplantation

Bronwen E. Shaw,[1,2] Rafael Arguello,[3] Christian A. Garcia-Sepulveda[3] and J. Alejandro Madrigal[1,4]

Table I. Relevant studies of the impact of HLA matching on unrelated donor transplant outcome.

	Number of patients	Disease	Conditioning	TCD	Overall survival dependant of HLA matching status
Flomenberg *et al* (2004)	1874	Mixed	MA	Minority	Worse survival HLA-A, -B, -C, -DRB1 mm
Lee *et al* (2007)	3857	Mixed	MA	22%	8/8 52% (1 year) 7/8 43% 6/8 33%
Arora *et al* (2009)	1052	CML CP1	MA	15%	8/8 52% (5 year) 7/8 40% multiple mm 21-34%
Petersdorf *et al* (2007)	4796	Mixed	MA	16%	Worse survival HLA-A, -B, -C mm
Morishima *et al* (2002)	1298	Mixed	MA (predominantly)	Minority	10/10 65% (3 year) 9/10 40% (HLA-A or -B mm)
Kawase *et al* (2007)	1790	Leukaemia	MA	No	Worse survival HLA-A, -B, -DQB1 mm
Shaw *et al* (2005)	144	Mixed	RIC	100%	10/10 = 9/10
Maris *et al* (2003)	89	Mixed	NMA	No	NS
Niederwieser *et al* (2003)	52	Mixed	NMA	No	NS
Ho *et al* (2006)	111	Mixed	NMA	No	10/10 51% 9/10 27% (HLA-C mm)
Tiercy *et al* (2004)	114	CML	MA	2/3 No 1/3 yes	10/10 > 9 or less/10 NS
Shaw *et al* (2010)	488	Leukaemia	MA/RIC	83%	10/10 40% (5-year) 9/0 38% (NS) <9/10 24%

TCD, T cell depletion; MA, myeloablative; RIC, reduced intensity conditioning; NMA, nonmyeloablative; CML, chronic myeloid leukemia; CP1, first chronic phase; mm, mismatch, 8/8 – HLA-A, -B, -C, -DRB1; 10/10 – (8/8 + HLA-DQB1); NS, not significant.

Summary of donor selection strategy (HLA matching)

1. Matched sibling is better than a matched unrelated donor but the difference is narrowing.
2. Recent available data from large studies suggest that an **8/8 (A,B,C,DR)** matched donor is the best choice. **HLA-DQ** may be considered and this constitutes a **10/10 match**. ?DP matching
3. In some studies the use of a 7/8 (9/10) matched donor has been associated with an outcome equally as good as an 8/8 (10/10) matched donor. The clinical situations where a single mismatch may be tolerated include: in the T-cell depleted setting, or in advanced stage disease. ***Transplant Risk versus Risk of Relapse***

Summary of donor selection strategy (HLA matching)

3. In certain circumstances (and particularly if more than one donor is available) typing for the HLA-DPB1 locus should be done, and the degree and type of matching considered in donor selection.
4. **How to select between HLA mismatched donors?** (i.e. which mismatched locus should be chosen in preference) remains incompletely answered. ?Significance related to different locus mismatches.. may be that certain mismatches are permissive