Perspectives and new process developments in cell therapy



Prof.Frank Emmrich

© Fraunhofer IZI

Fraunhofer IZI

The Fraunhofer-Gesellschaft (FhG) –
Europe's Largest Organisation for Applied Research



Headquarters in Munich

- FhG is one of the "big four" German non-University research organisations
  - **> 60 Institutes in Germany (> 80 locations)**
  - **> 23,000 employees**
  - **> 2 billions € total annual financing**
- Focused on applied research projects with industry partners
  - Fraunhofer supports development efforts of its partners by providing them with new technologies, product candidates, problem solutions etc. with wide benefit for the society
  - Main part of the research revenue is derived from contracts with industry and from publicly funded research projects together with the industry

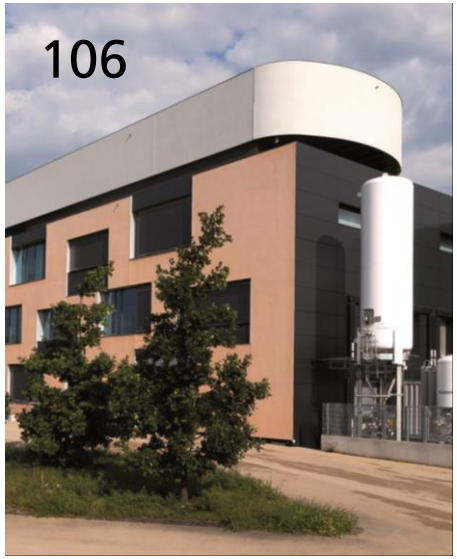
email : frank.emmrich@medizin.uni-leipzig.de
© Fraunhofer IZI
Fraunhofer IZI

Fraunhofer IZI - sites

Leipzig



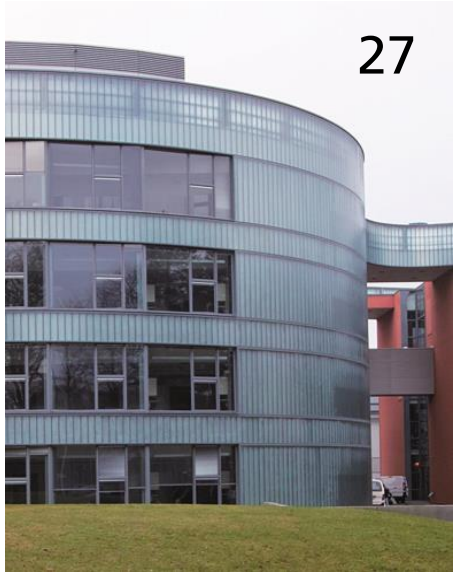
Potsdam/Golm



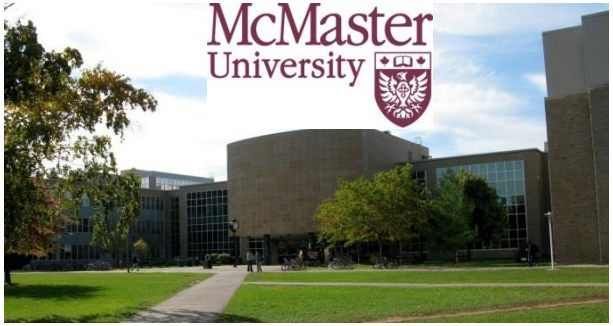
Halle



Rostock



Hamilton
Ontario
Canada





Hwasun
Gwangju
Korea

Fraunhofer Project Center BEAM JLCI Joint Laboratory CNUHH

Employees January 2016: 551

© Fraunhofer IZI

Fraunhofer IZI

The Fraunhofer IZI: Connected core competencies with innovation potential

Cell therapies for regenerative medicine	Immunological products for control of diseases	Drug discovery and preclinical development	Diagnostic products, biomarkers, biobanking, imaging
Cell and stem cell technologies, tissue engineering, manufacturing (GMP)	Vaccine technologies and antibody development	Ischemic diseases, oncology, inflammatory and autoimmune diseases	Biomarker validation, assay development, cryopreservation technologies



Email : frank.emmrich@izi.fraunhofer.de

Fraunhofer IZI

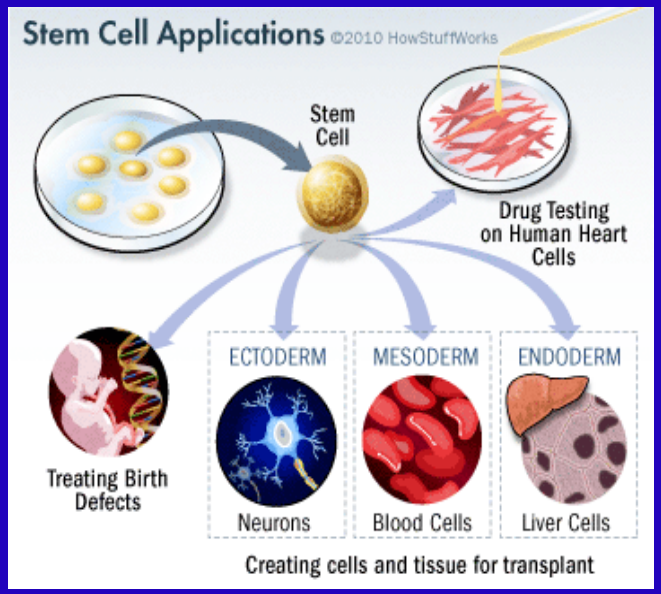
Technological Challenge

Combining various technologies

Automated skin tissue engineering „Tissue Factory“

Four Fraunhofer institutes





Storage

Cell Extraction

Cell Expansion

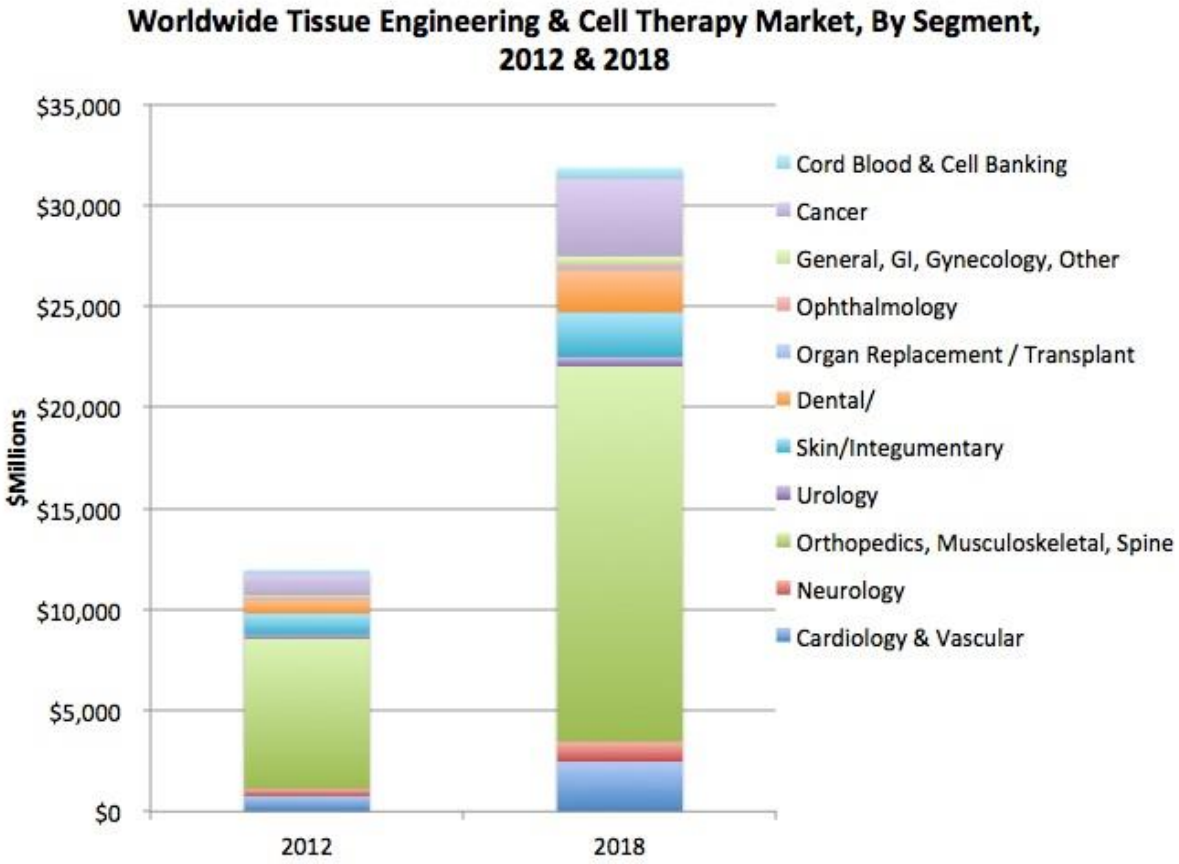
Tissue Remodeling

Robot

Human Skin-Products

Cosmetics Industry
Pharmaceutical Industry

„Tissue Factory“ has been funded by the Fraunhofer Future Foundation

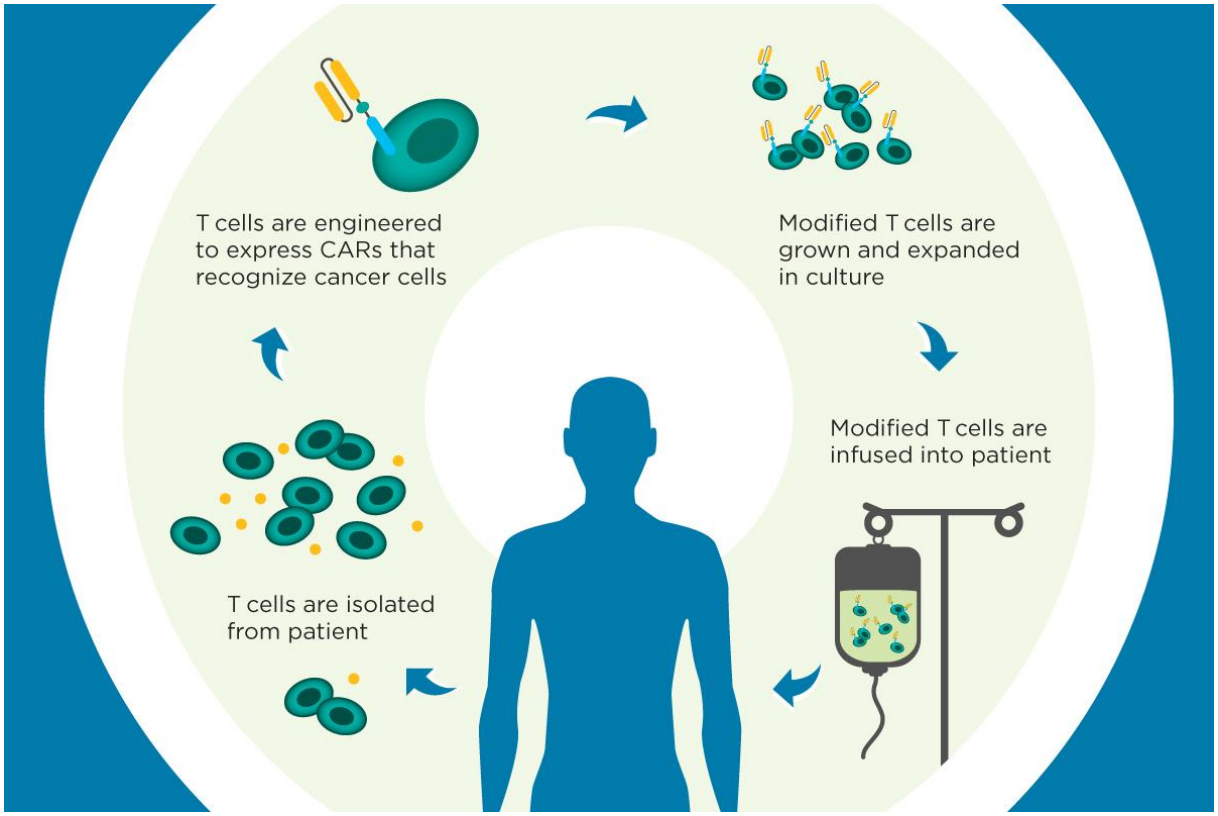


Quelle: http://www.healthworkscollective.com/patrickdriscoll/164911/tissue-engineering-and-cell-therapy-market-outlook

Mechanistical Challenge

Understanding and manipulating
genes and molecules and complex systems
– like the immune system

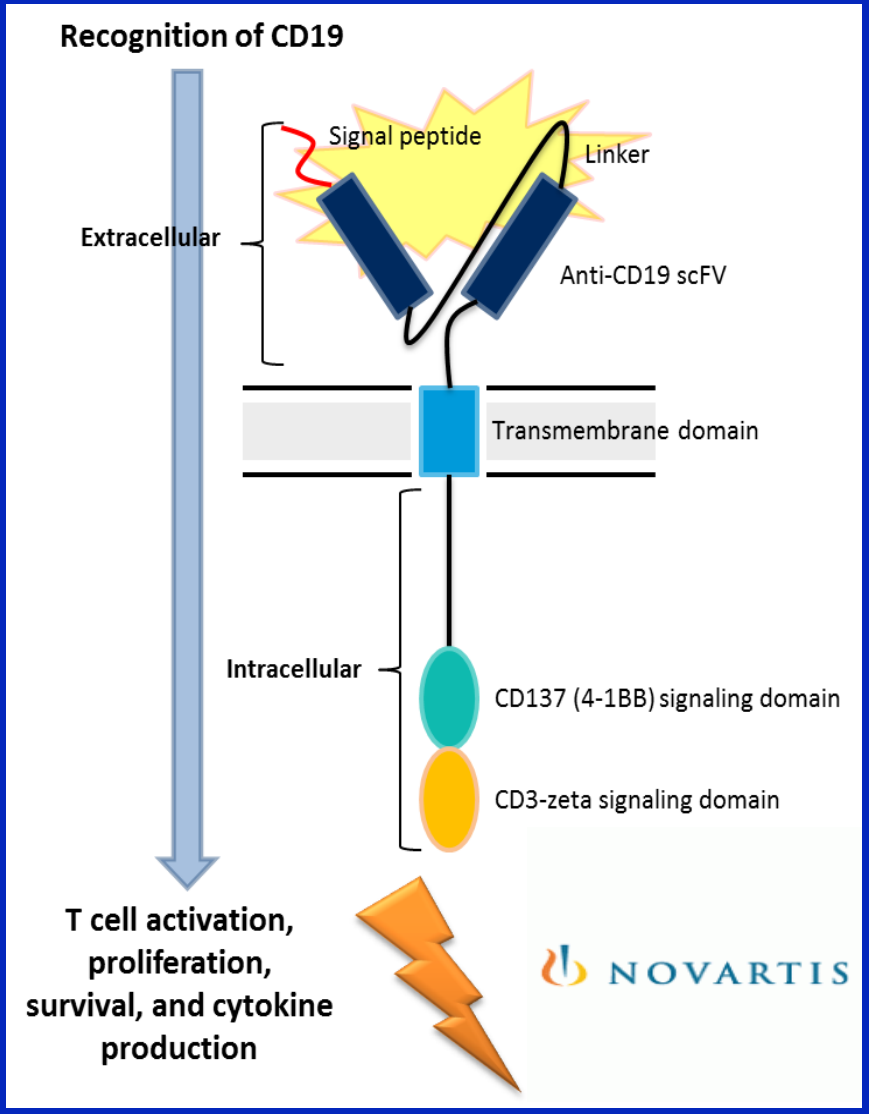
CAR T-Cells



Quelle: https://www.mskcc.org/blog/car-t-cell-therapy-growing-area-research

CAR T Cells for Cancer Therapy

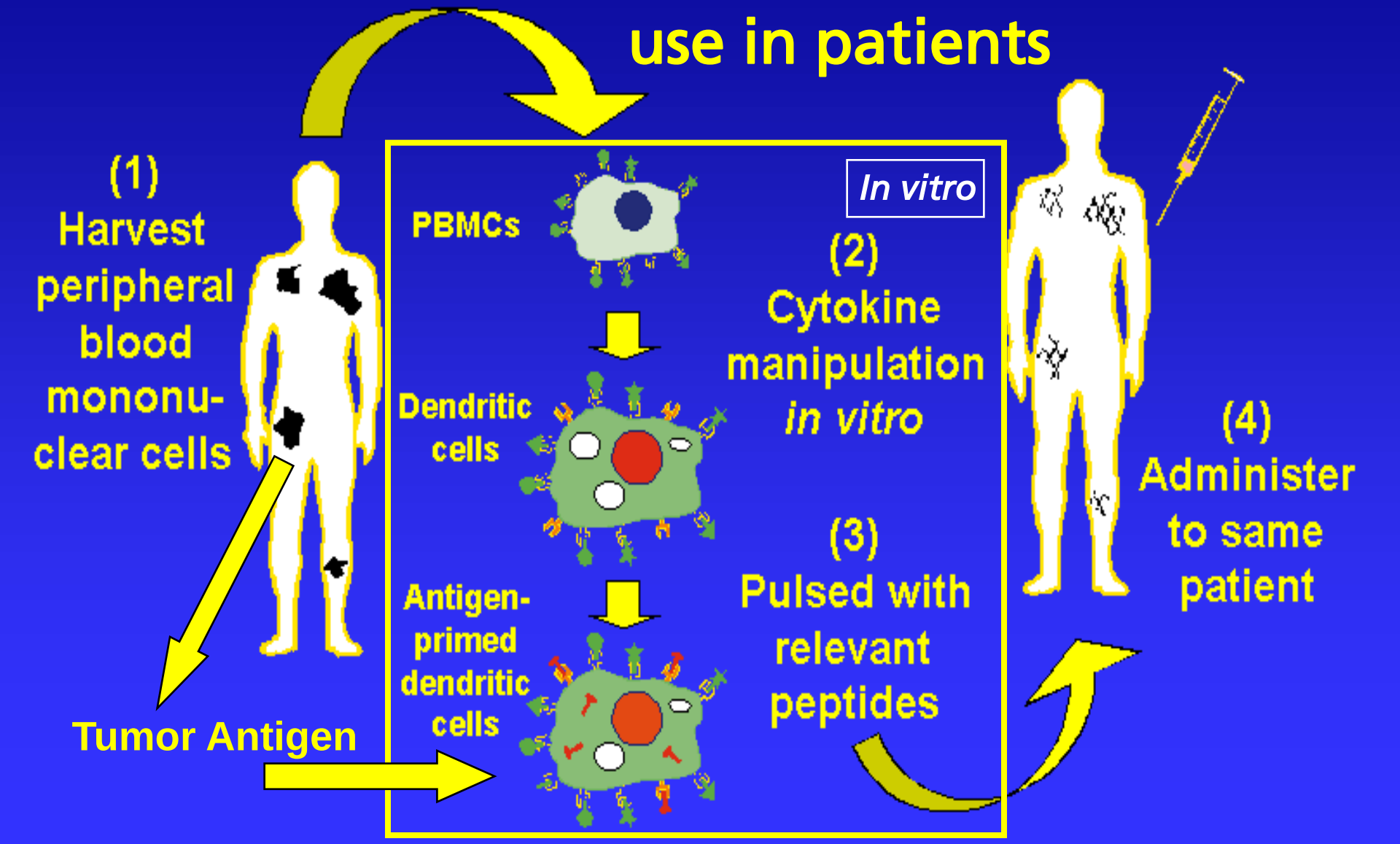
Creation of autologous T cells with genetically engineered artificial antigen receptors for CD19 and intracellular components for cell activation



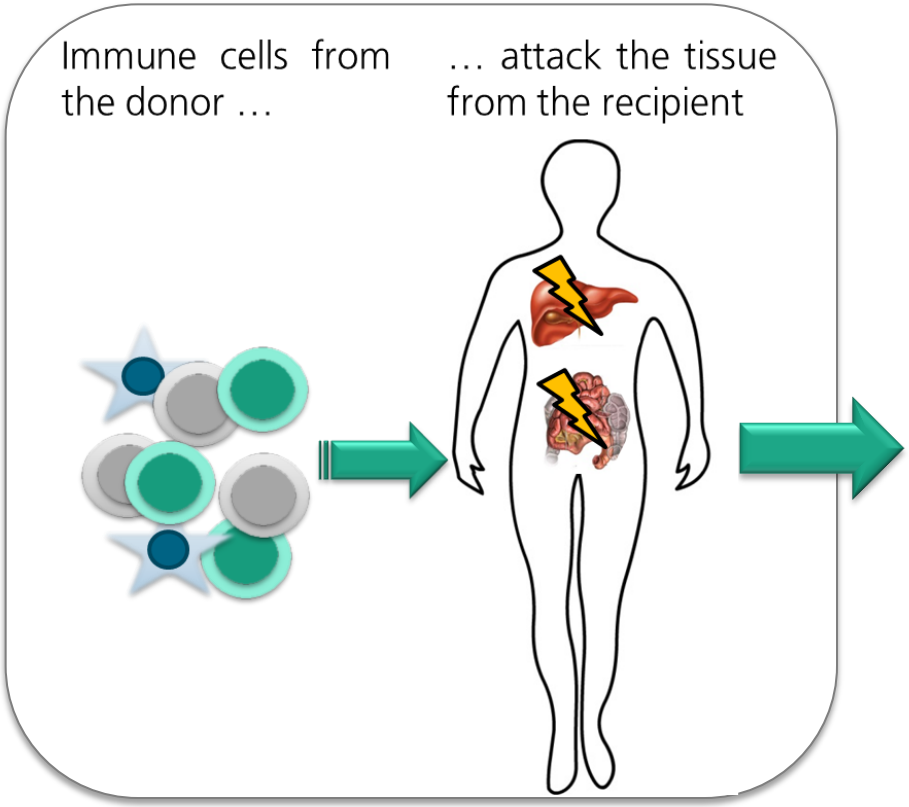
HSC = Human Stem Cells Tx = Transplantation BM = Bone Marrow

CAR = Chimeric Antigen Receptor CD = Cluster of Differentiation

Dendritic Cell Vaccines



Graft-versus-Host Disease (GvHD)



Overall survival of 30% after 6 months[5]

Current treatment options: many side effects, infections, tumor recurrence

Limits the stem and immune cell applications in other diseases that can be cured (e.g. autoimmune)

Global treatment costs between **645 Million – 2 Billion €**

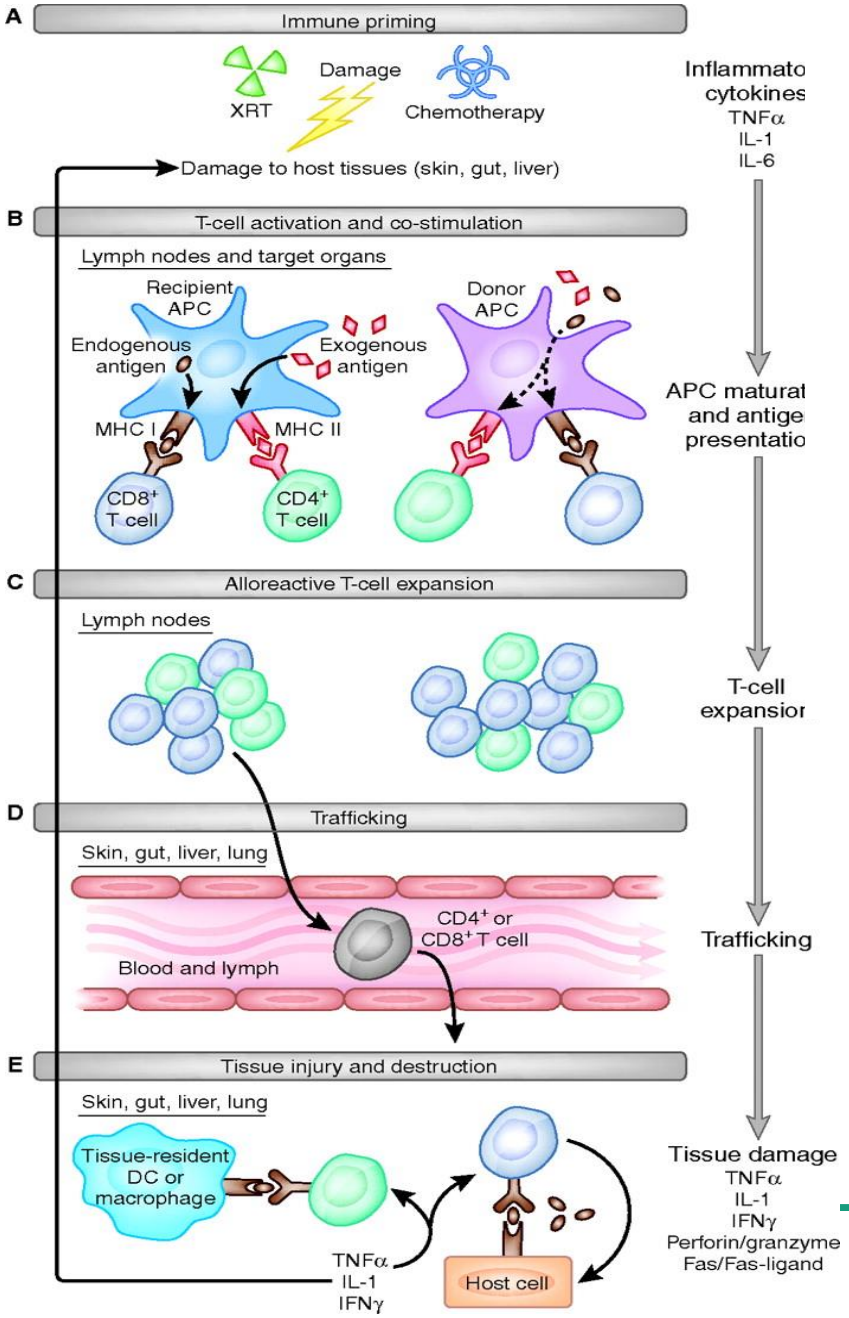
5 Wolff D et al. Dtsch Arztebl Int 2011; 108(43): 732–40. DOI: 10.3238/arztebl.2011.0732

© Fraunhofer IZI

Fraunhofer IZI

Pathophysiology of GVHD

- Immune-competent T cells transplanted into immune-compromised host.
- Host cannot reject the graft due to decreased immunity. But graft T cells perceive the recipients tissue as **foreign** and react against it.
- This leads to activation of CD4 and CD8 T cells ultimately causing inflammation and killing of host cells.





immunologyclinic.com

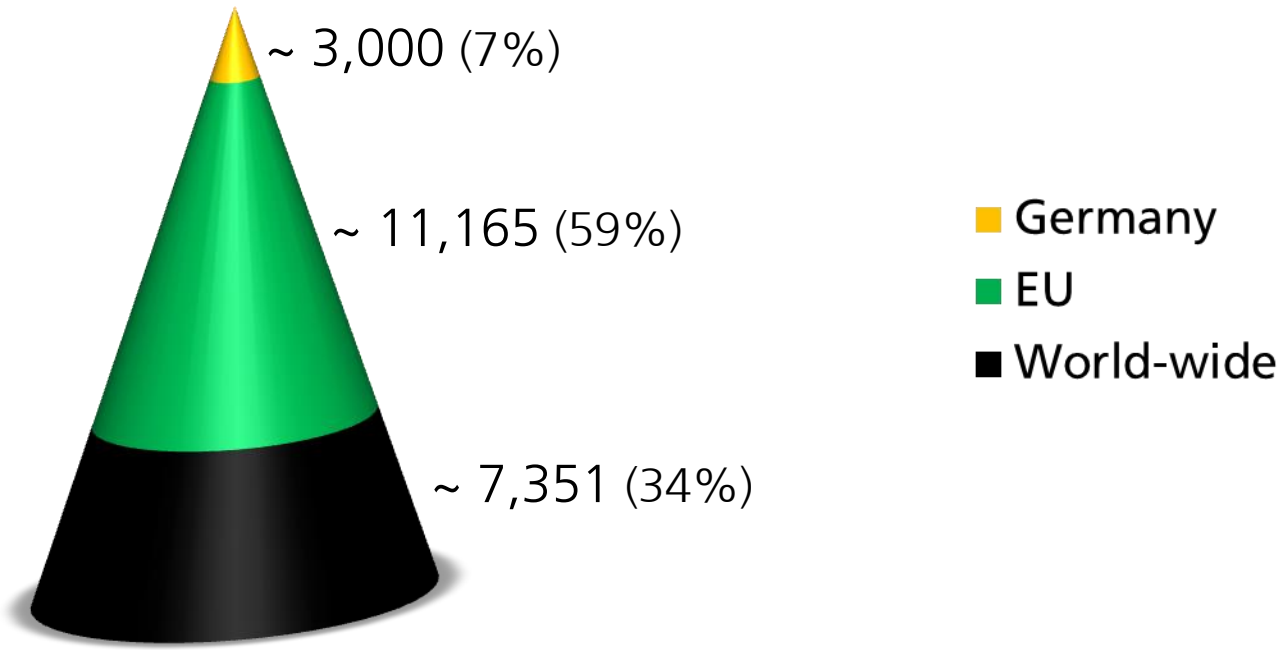


emedicine.medscape.com

Fraunhofer IZI

Hematopoietic Stem Cell Transplantation

- World-wide: >21,000 Hematopoietic Stem Cell Transplantations[1-4]



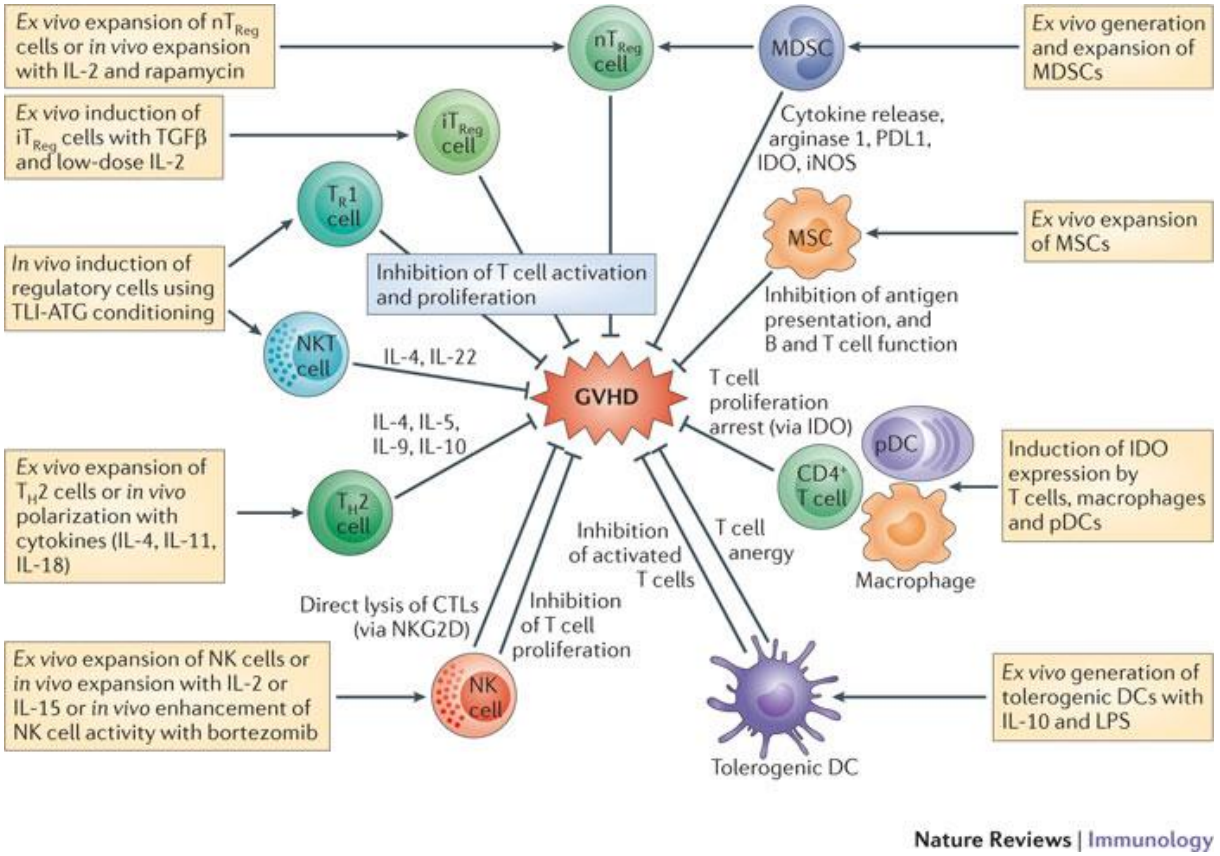
© Fraunhofer IZI

1 Gratwohl et al. JAMA. 2010;303(16):1617-1624. doi:10.1001/jama.2010.491
2 Passweg et al. Bone Marrow Transplantation (2014) 49, 744–750; doi:10.1038/bmt.2014.55
3 DAG-KBT 2015
4 Niederwieser et al. Blood (2013); 122 (21):2133

Fraunhofer IZI

Multitude of therapeutic options

Potential targets for cellular immunotherapies in GVHD.

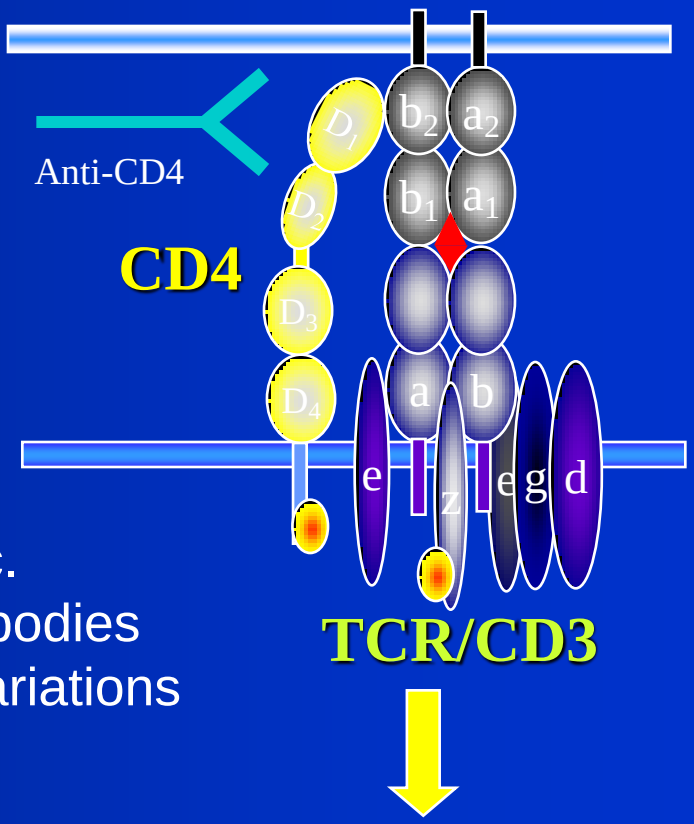


Quelle: http://www.nature.com/nri/journal/v12/n6/fig_tab/nri3212_F5.html

Anti-CD4 antibodies are helpful tools for induction of immunological tolerance

H.Waldmann et al.

(1) interference with the functional molecular complex of the T cell receptor during antigen recognition
(2) Initiation of a negative signal for subsequent or parallel triggering of the T cell receptor complex.

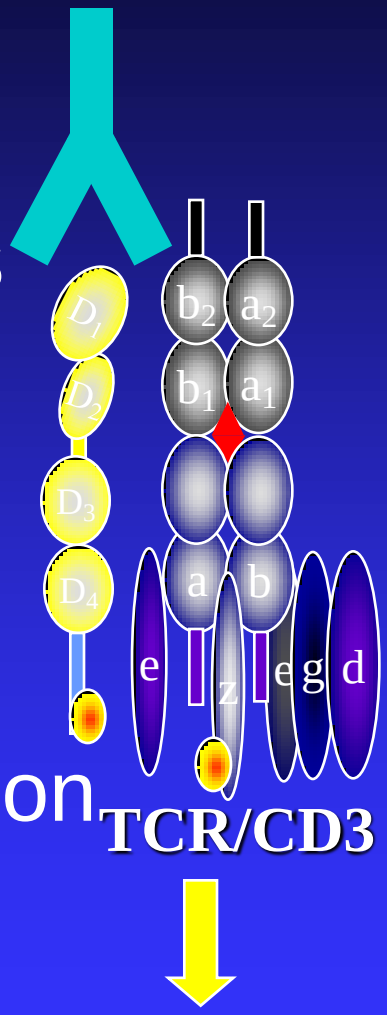


Monoclonal antibodies are species-specific. Moreover, the therapeutic potential of antibodies to a certain antigen differs greatly due to variations in epitope and affinity.

Therapeutic antibodies to a murine system $\neq$ Therapeutic antibodies to a human system

1

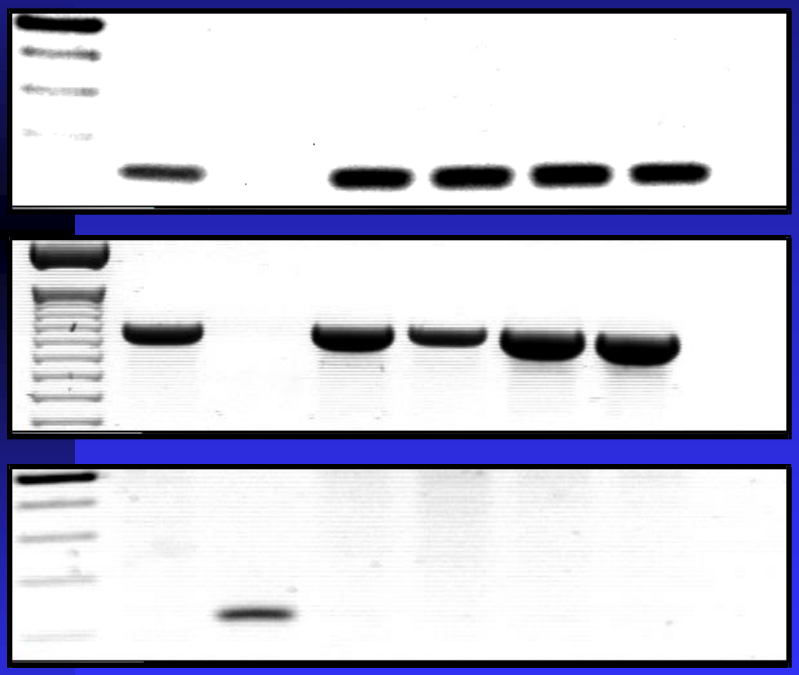
Select appropriate anti-human CD4 antibodies for tolerance induction

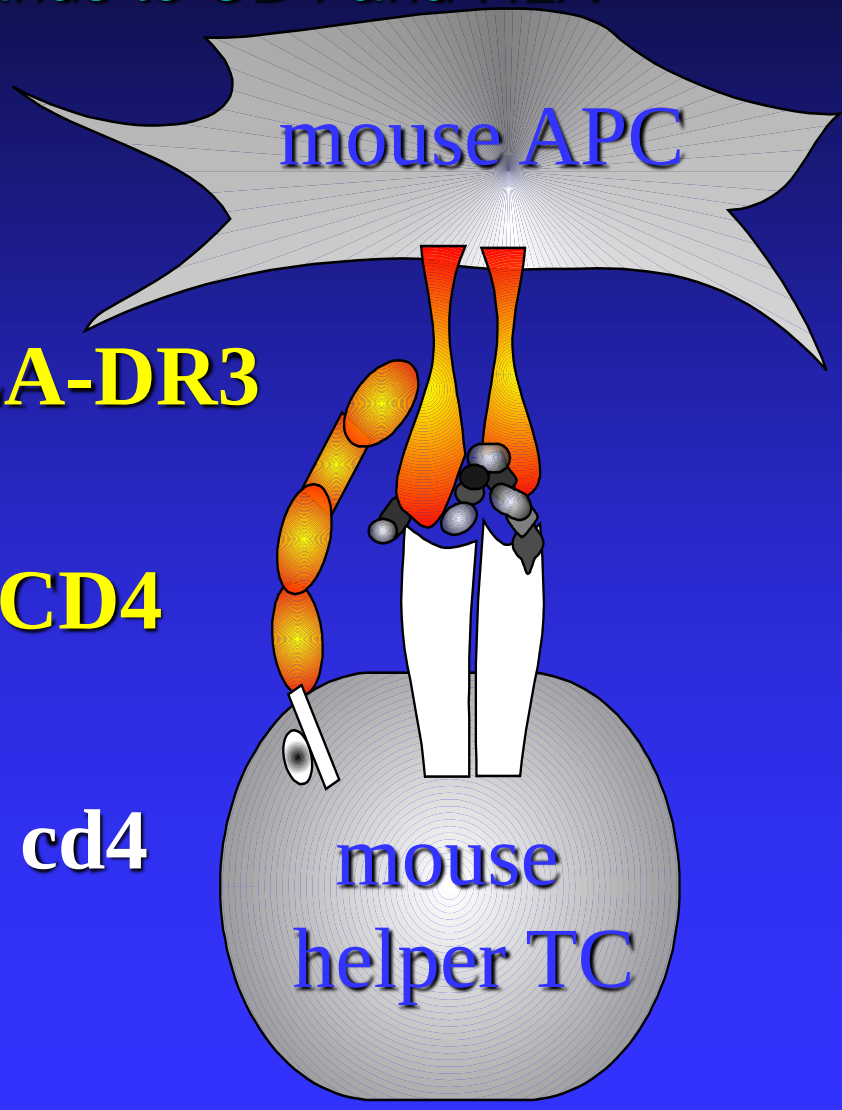


Requires experimental simulation of a complex situation !

CD4/DR3 mouse

a transgenic animal model for the functional investigation of molecular ligands to CD4 and HLA-DR3





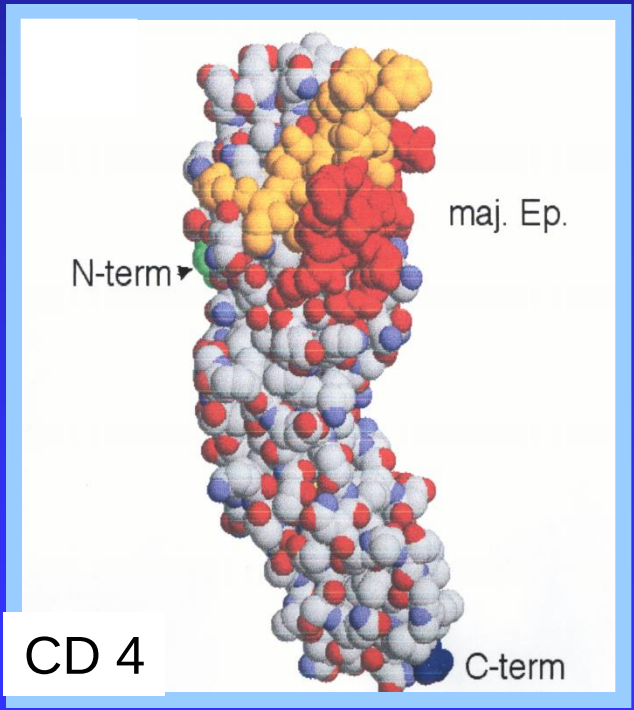
Selection of appropriate monoclonal anti-CD4 antibodies

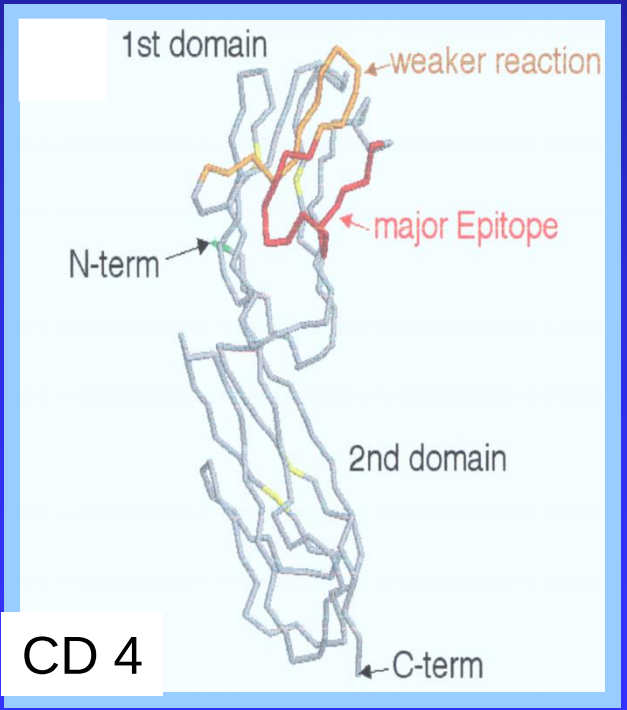
Screening for high affinity

Selection of appropriate isotype

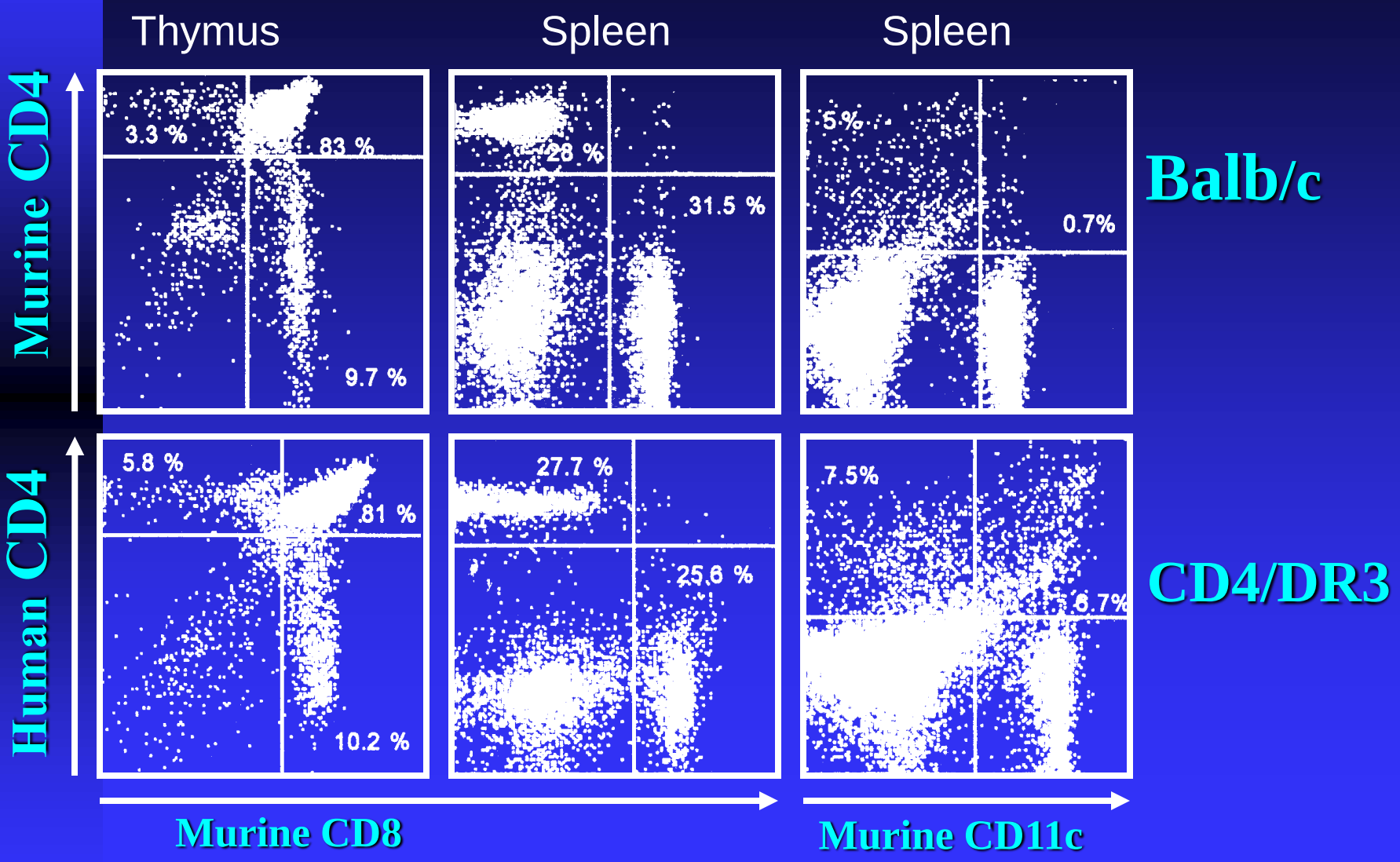
Selection of appropriate epitope

Functional screening for tolerance induction





CD4 and CD11c expression in transgenic mice



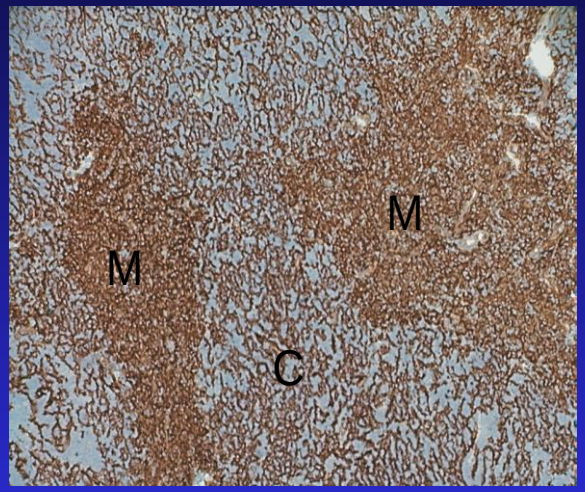
CD4 and MHC II immunohistology

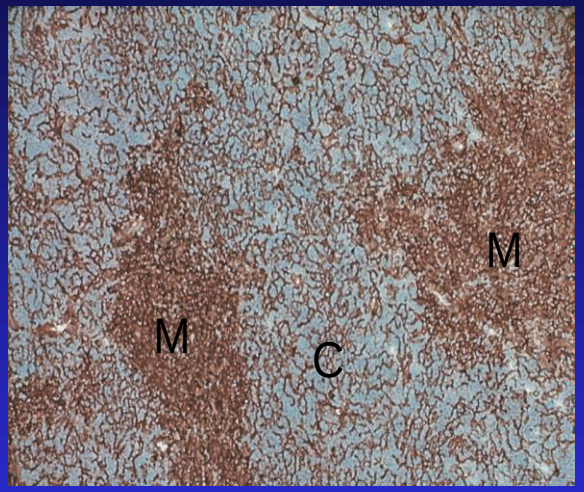
Wild type

CD4/DR3 transgenics

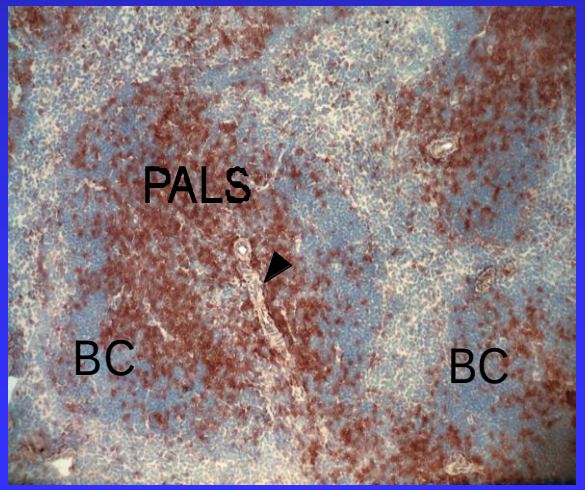
I-A / I-E

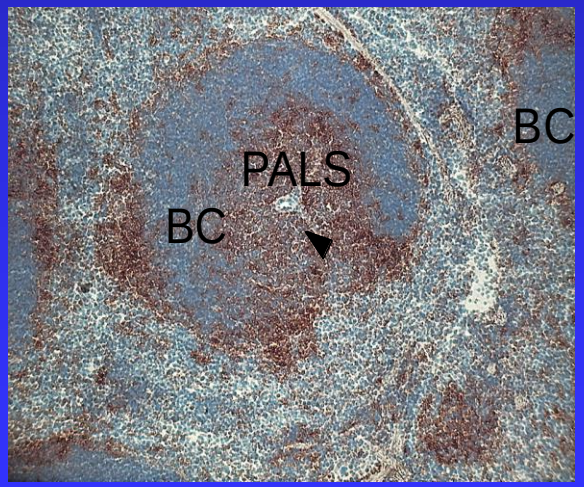
HLA-DR





Thymus





Spleen

Mouse CD4

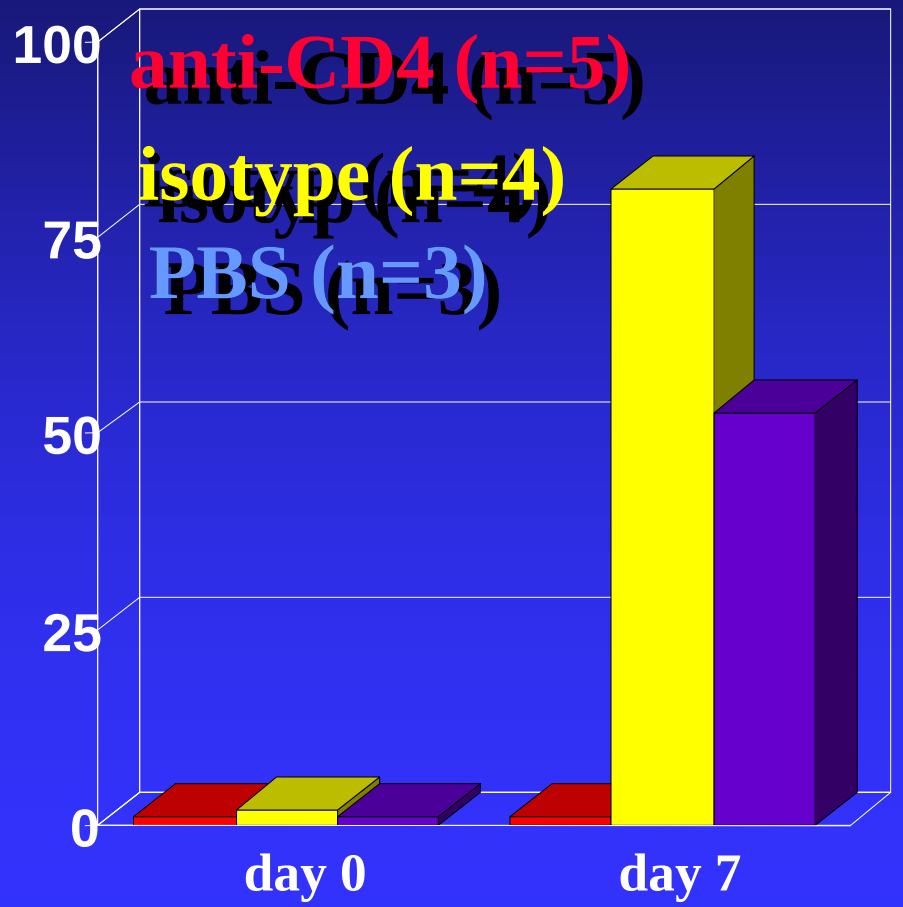
Human CD4

Suppression of the primary immune respo to Tetanustoxoid (TT) in CD4/DR3 mice by anti-human CD4 antibody (MAX.16H5)

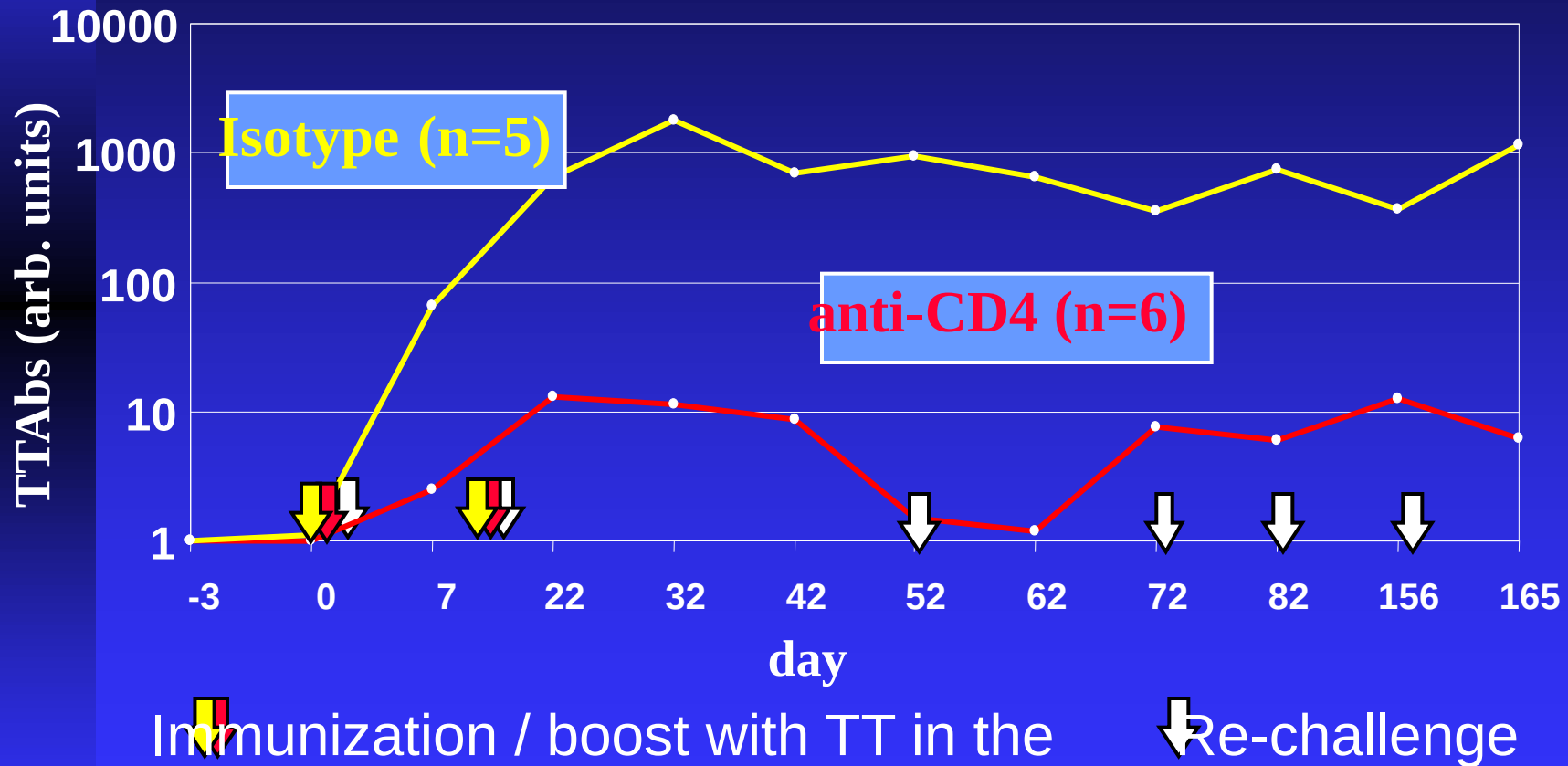
- TT was given at days

  -2 -1 0

- TT antibodies (% ELISA units) were assessed at days 0 7



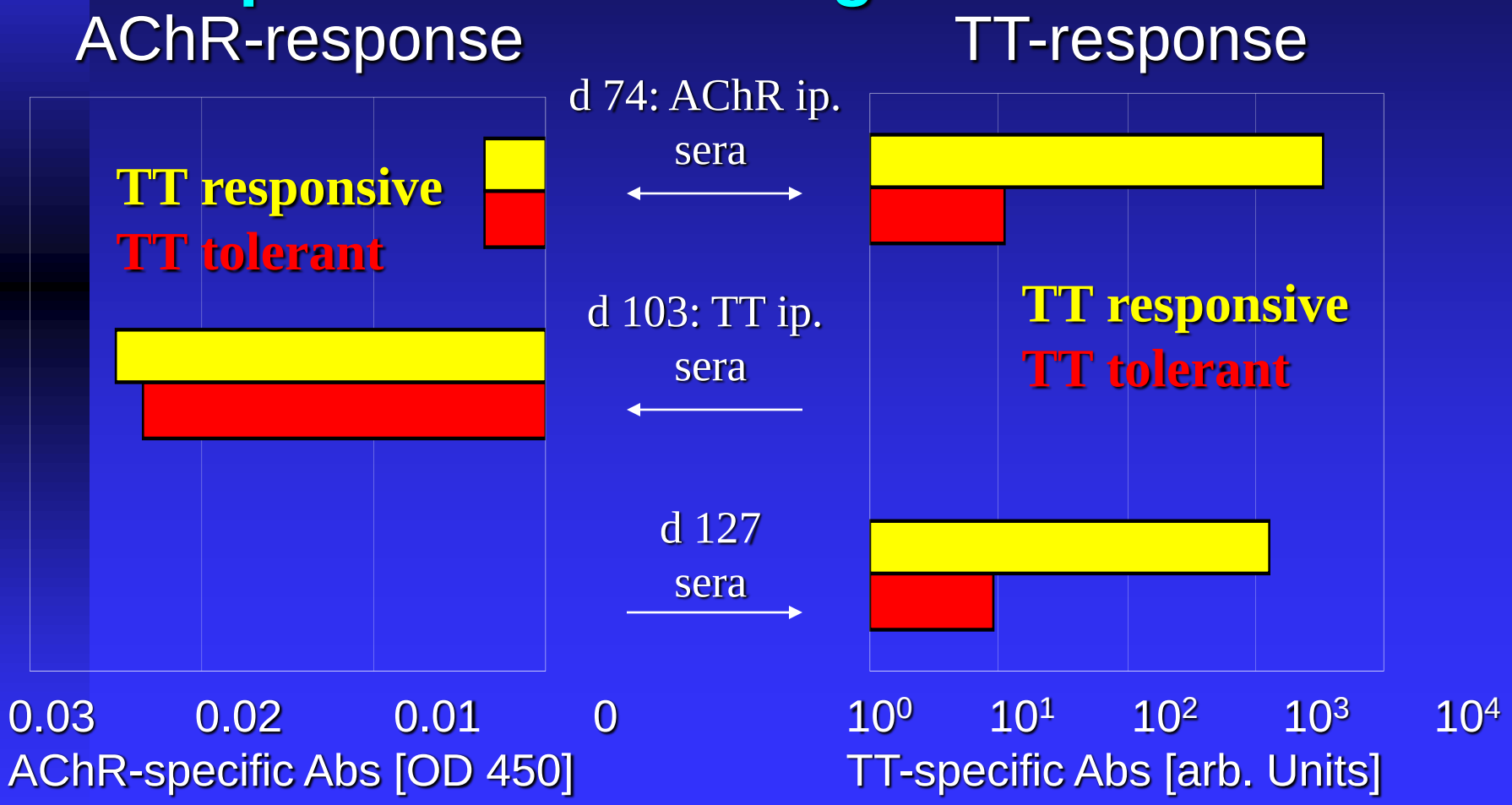
Application of anti-human CD4 + antigen induces long-term unresponsiveness to T in CD4/DR3 mice



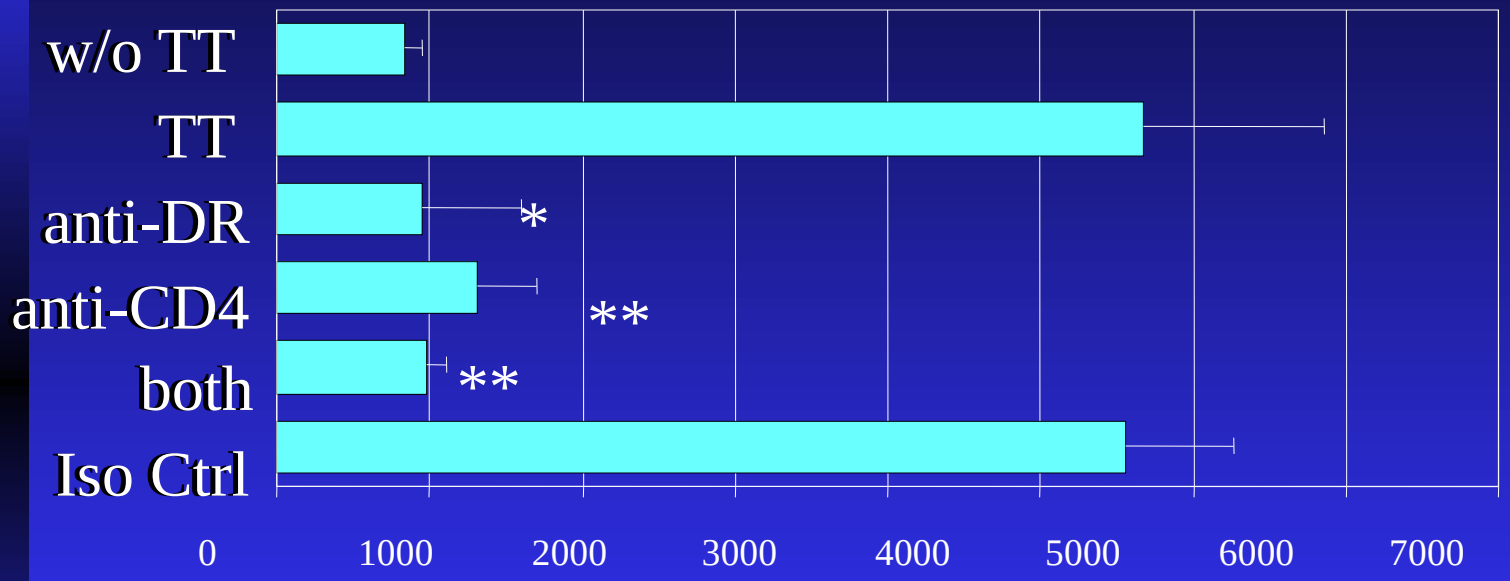
Immunization / boost with TT in the presence of either anti-CD4 or isotype control

Re-challenge with TT alone

Anti-human CD4 induced unresponsiveness reveals characteristics of specific immunological tolerance



Transgenic CD4 and DR3 molecules are engaged in specific recognition and funct

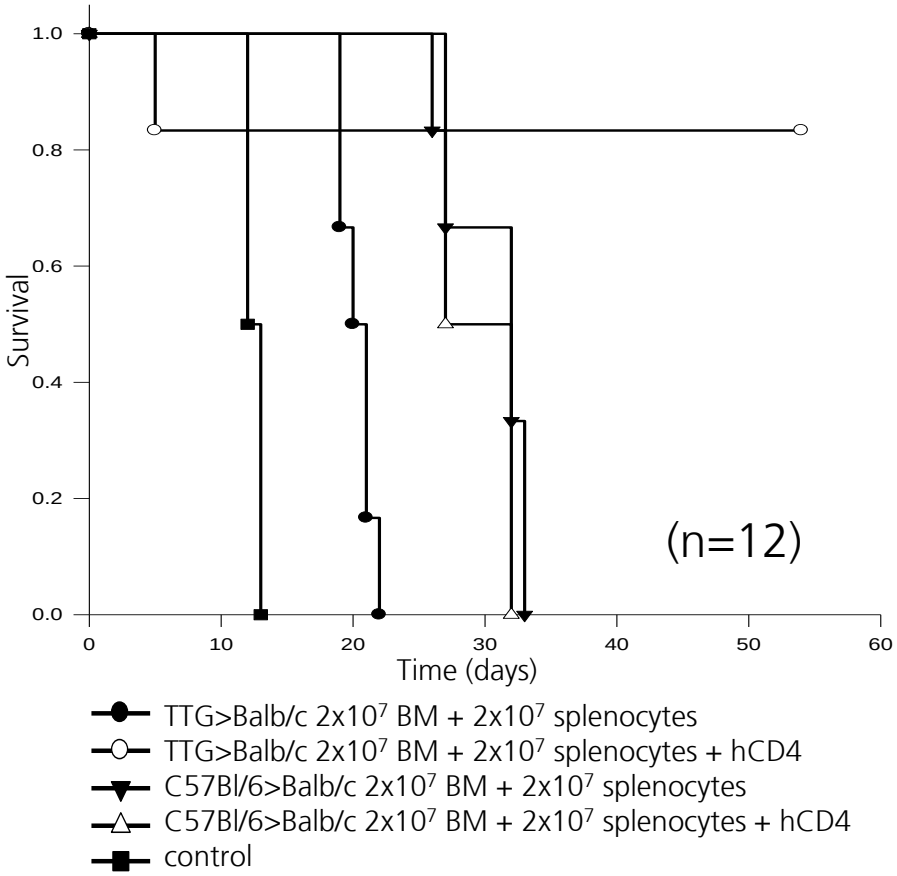


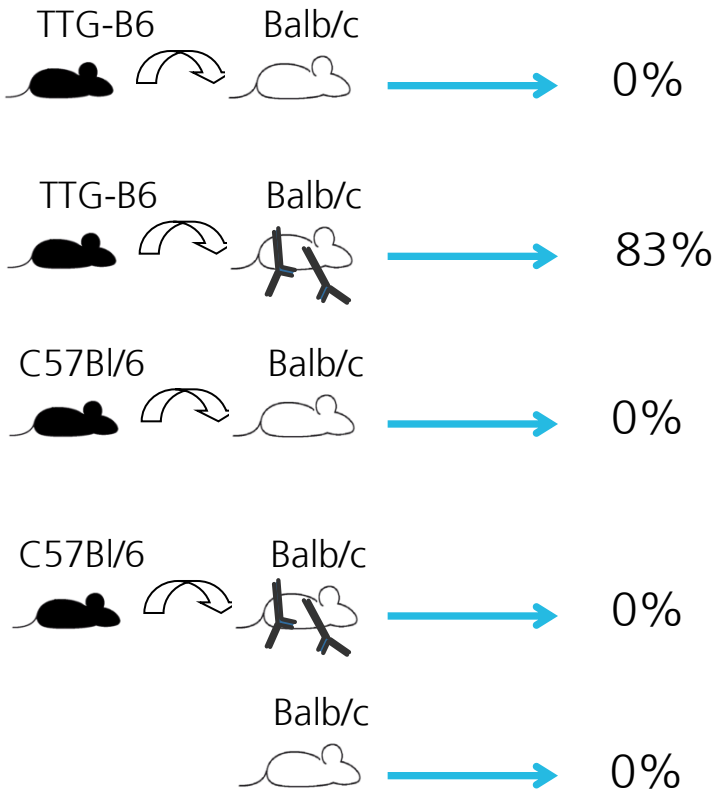
Spleen cell suspensions of TT immunized CD4/DR3 mice were restimulated
n vitro with 20 µg/ml TT. Mabs to HLA, CD4, or an control Ab of the respective
sotype were added. Cultures were terminated after a 16h pulse with 1µCi ^{3}H-TdR

Results are expressed as mean differences to isotype supplemented cultures

are significant for $p < 0.05$ (*) and $p < 0.01$ (**)

Results: Survival after transplantation of TTG in Balb/c ± anti-CD4





- Survival rate significantly increased in antibody treated mice transplanted with TTG donor cells
- Specific for transplantation TTG to Balb/c mice

© Fraunhofer IZI

Fricke et al. World Immune Regulation Meeting-IV/V, 2010/2011, Davos, Switzerland
Fricke et al. Annual Meeting German Society of Immunology, Leipzig, Germany

Fraunhofer IZI

Implications for clinical use: Translation of treatment strategy

- Anti-CD4 antibodies effectively suppress GvHD development after murine HSCT in a full MHC mismatch model (TTG-C57Bl/6 mice I

  Balb/c mice) and did not affect GvL effect negatively.

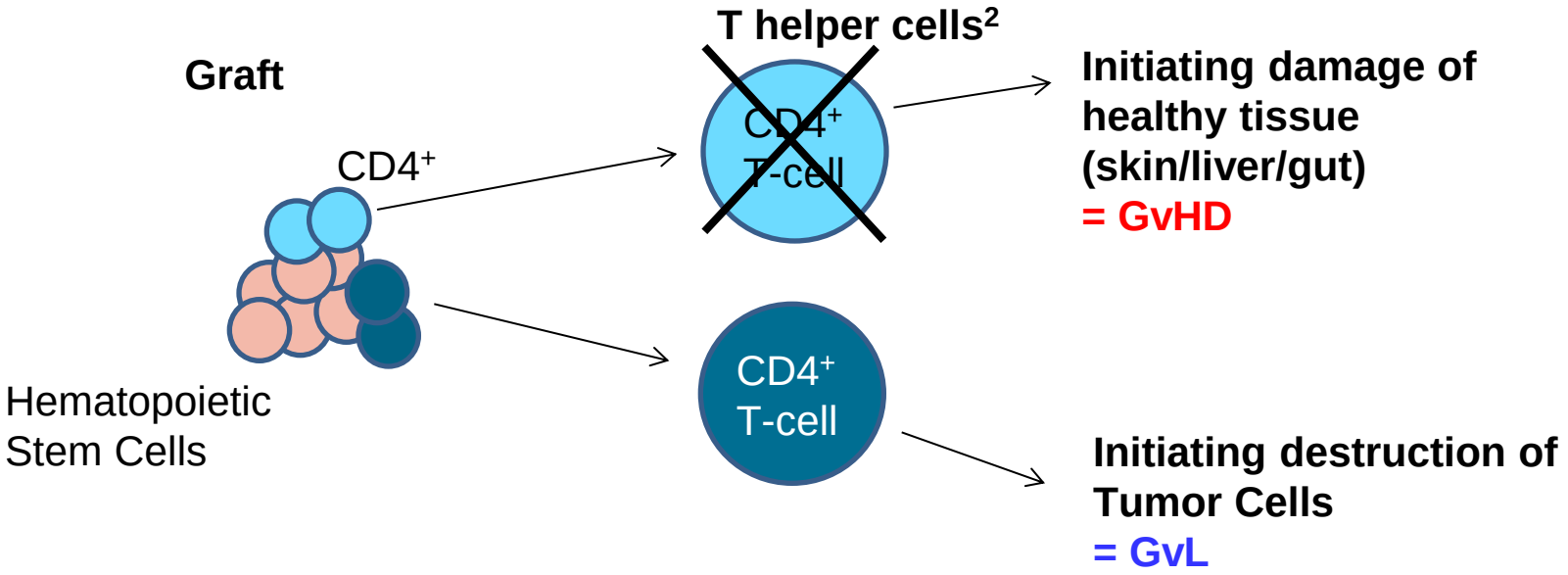
- No additional treatment with conventionally immunosuppressive

  drugs was necessary→ Induction of immune tolerance in allografts

© Fraunhofer IZI

GvHD (Graft-versus-Host-Disease) - the beneficial GvL effect

- Incidence depending on risk factors between 10%-80%, significant 40%[1]
- Conventional immunosuppressive drugs: toxic, refractory courses
- Donor T cells attack healthy tissue of the recipients
- Additional treatment costs for GvHD between 75.000 und 225.000 Euro



1 Devergie HSCT Revised Edition EBMT and ESH 2008.
2 Trapani et al. Nat. Rev. Immu. 2, 735-747, doi:10.1038/nri911.

© Fraunhofer IZI

Fraunhofer IZI

Prevent GvHD from the beginning

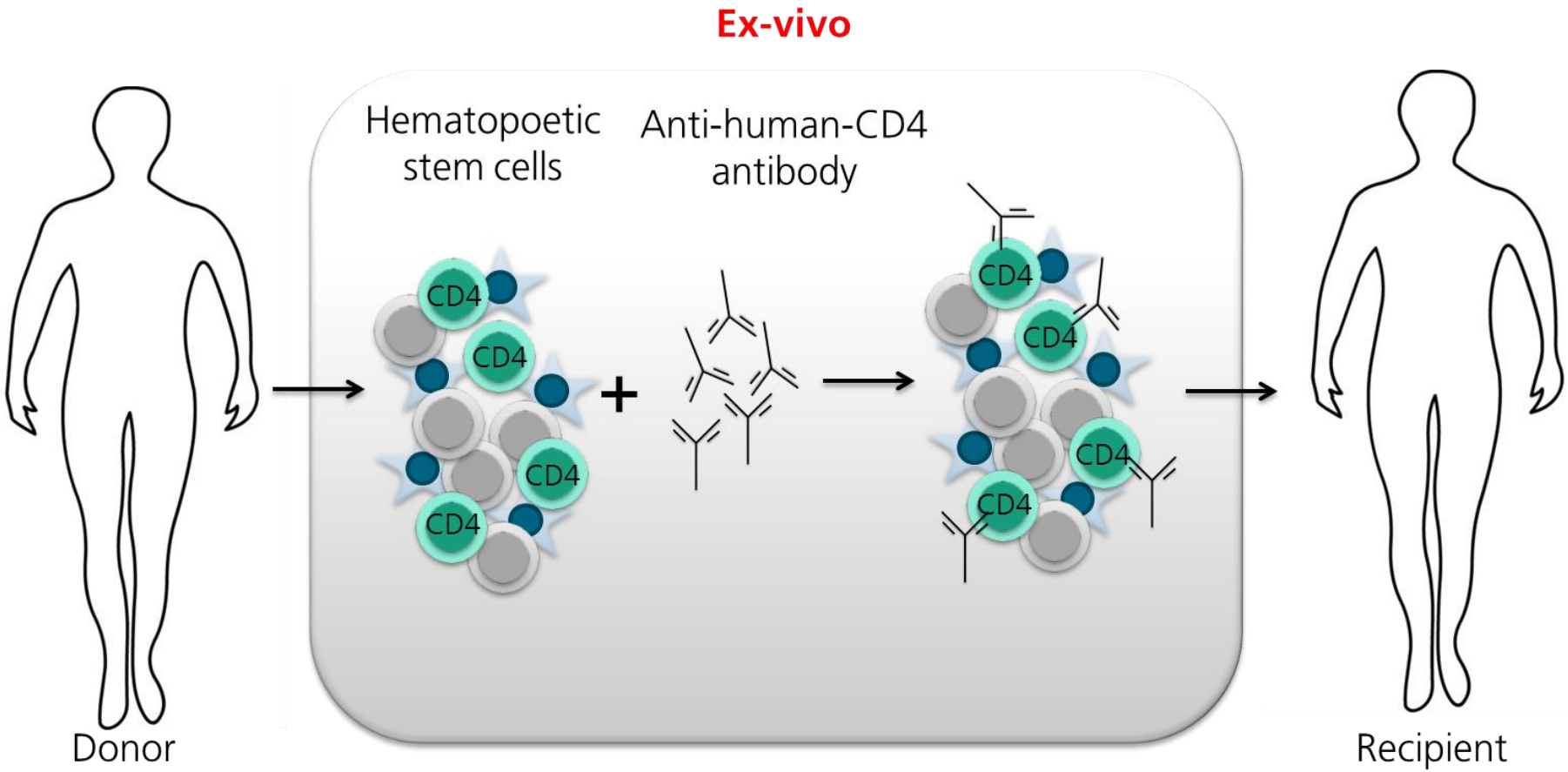
BUT

- **Obtain the Anti-Tumor-Effect (GvL)**
- **Turn off the toxicity caused by conventional immunosuppressive drugs**
- **Develop a clinically applicable, gentle therapy method**

→ Reach an improved survival after transplantation

© Fraunhofer IZI

New therapeutic strategy: model/method[6-9]



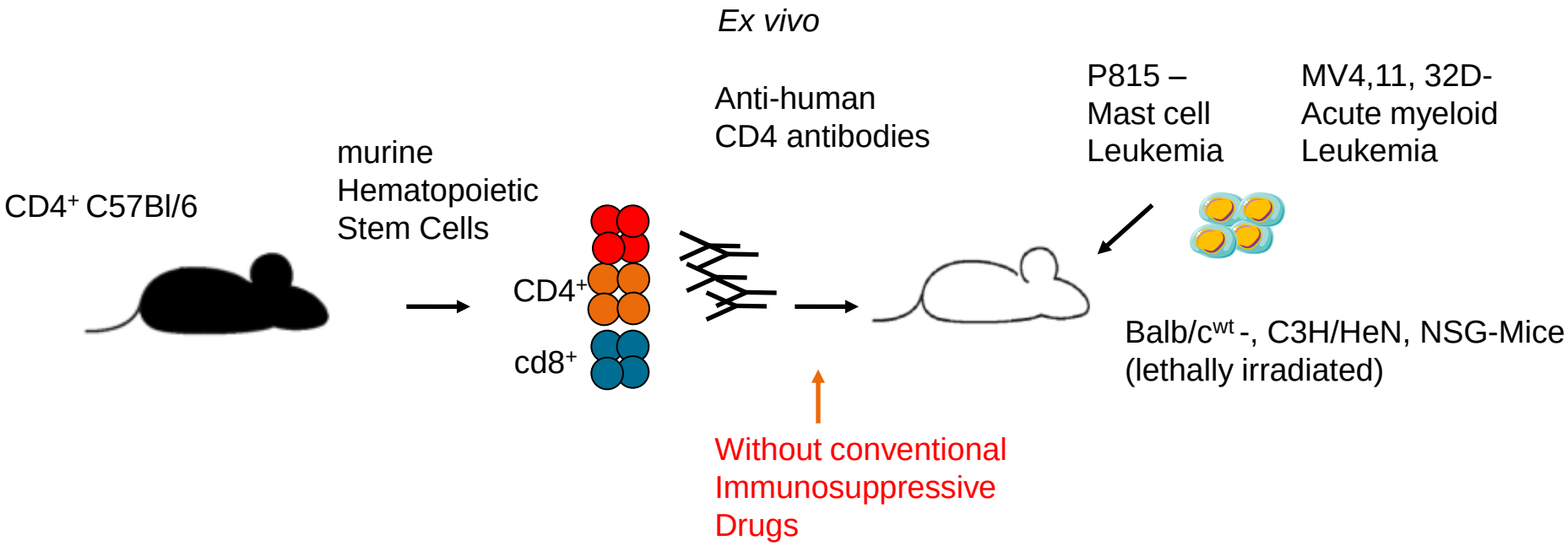
Without current immunosuppressants

© Fraunhofer IZI

6 Fricke et al. Cell Mol Life Sci. 2010 Dec;67(23):4095-106. doi: 10.1007/s00018-010-0427-2.
7 Fricke et al. Cytometry A. 2012 Jun;81(6):476-88. doi: 10.1002/cyto.a.22061.(C2015).
8 Fricke et al. Cell Mol Life Sci. 2014 Jun;71(11):2135-48. doi: 10.1007/s00018-013-1476-0.
9 Schmidt et al. Cytometry A. 2015 Apr;87(4):334-45. doi: 10.1002/cyto.a.22619.

Fraunhofer IZI

Anti-human CD4 antibodies: Models/Methods[1-4]

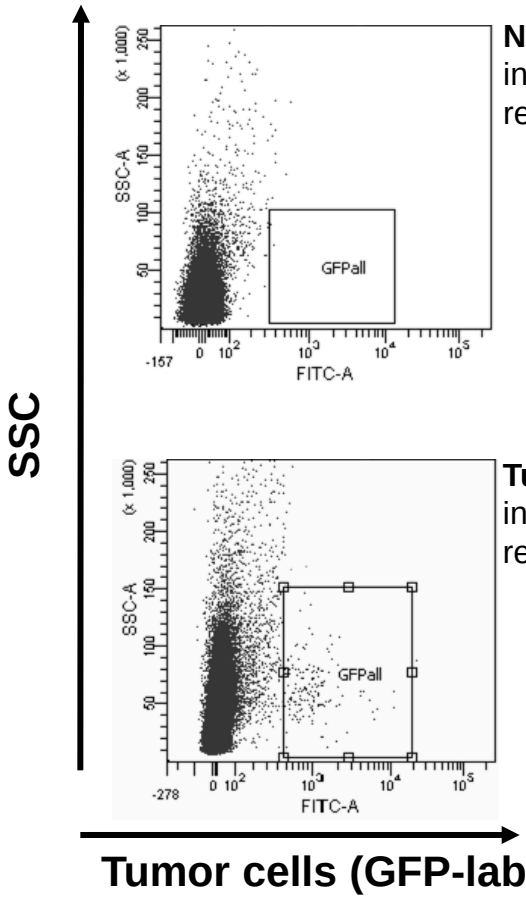


© Fraunhofer IZI

1 Fricke et al. PLoS One. 2009 Jul 7;4(7):e6157. doi: 10.1371/journal.pone.0006157 (C2015).
2 Fricke et al. Cell Mol Life Sci. 2010 Dec;67(23):4095-106. doi: 10.1007/s00018-010-0427-2.
3 Fricke et al. Cytometry A. 2012 Jun;81(6):476-88. doi: 10.1002/cyto.a.22061.(C2015).
4 Fricke at al. Cell Mol Life Sci. 2014 Jun;71(11):2135-48. doi: 10.1007/s00018-013-1476-0.

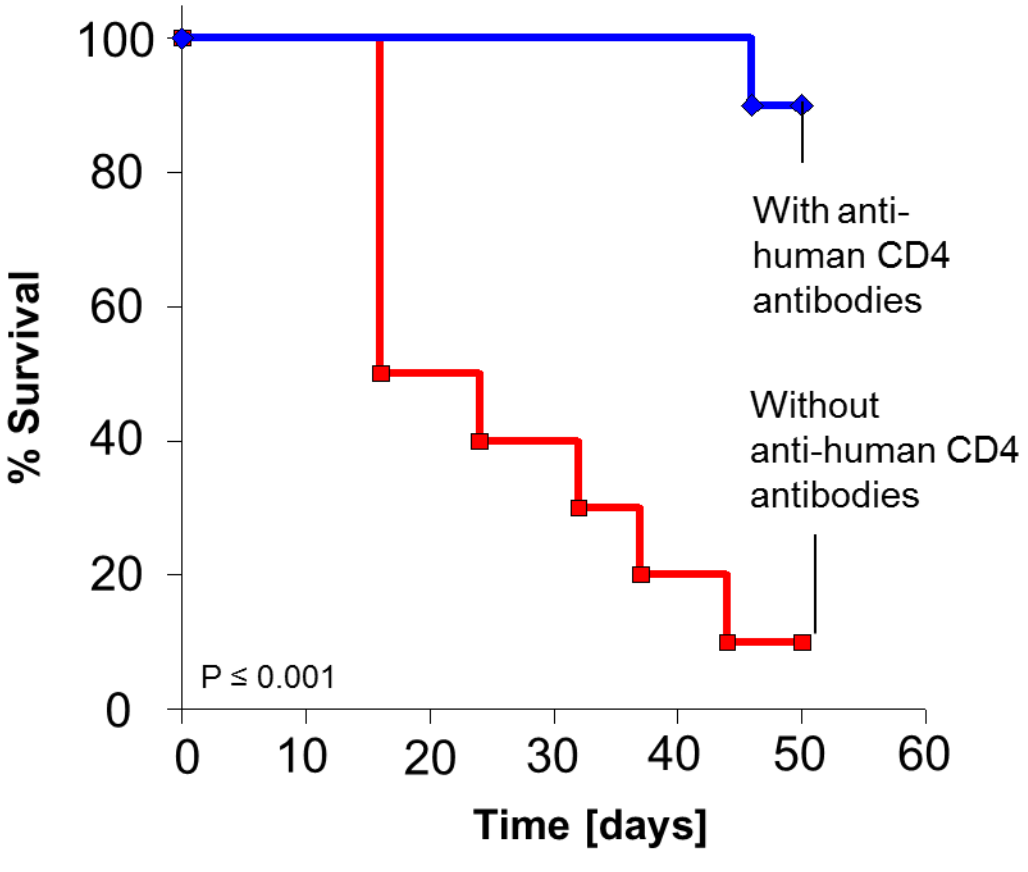
Fraunhofer IZI

Results: Anti-Tumor-Effect (GvL) without GvHD



No tumor cells in transplanted recipients

Tumor cells in transplanted recipients



Transplantation without antibodies + Mast Cell Leukemia (n=10)

Transplantation with antibodies + Mast Cell Leukemia (n=10)

© Fraunhofer IZI

Fricke at al. Cell Mol Life Sci. 2014 Jun;71(11):2135-48. doi: 10.1007/s00018-013-1476-0.
Schmidt et al. Cytometry A. 2015 Apr;87(4):334-45. doi: 10.1002/cyto.a.22619.

Fraunhofer IZI

Summary: Anti-human CD4 antibodies for GvHD Prevention

- Ex-vivo incubation of a hematopoetic graft by anti-CD4 antibodies or treatment of recipients at day of transplantation prevents or reduces GvHD significantly (full MHC-mismatch transplantation model).
- In leukemic mice receiving anti-human CD4 antibodies, survival was significantly higher as compared to the controls.
- Deterioration of the Graft-versus-Leukemia effect (GvL) by anti-CD4 antibodies could not be observed (Mast cell leukemia, AML).
- Freedom of GvHD was transferrable to third party recipients.
- Using human immune cell grafts, anti-CD4 antibodies prolongate GvHD development significantly compared to controls.

© Fraunhofer IZI

Conclusion

GvHD ↓

- **Anti-Tumor-Effect preserved (GvL)[1]**
- **Suppression of GvHD without conventional immunosuppressive drugs, even at full MHC-Mismatch[2]**
- **Advantage of the methodology: Ex vivo modification of hematopoietic grafts**

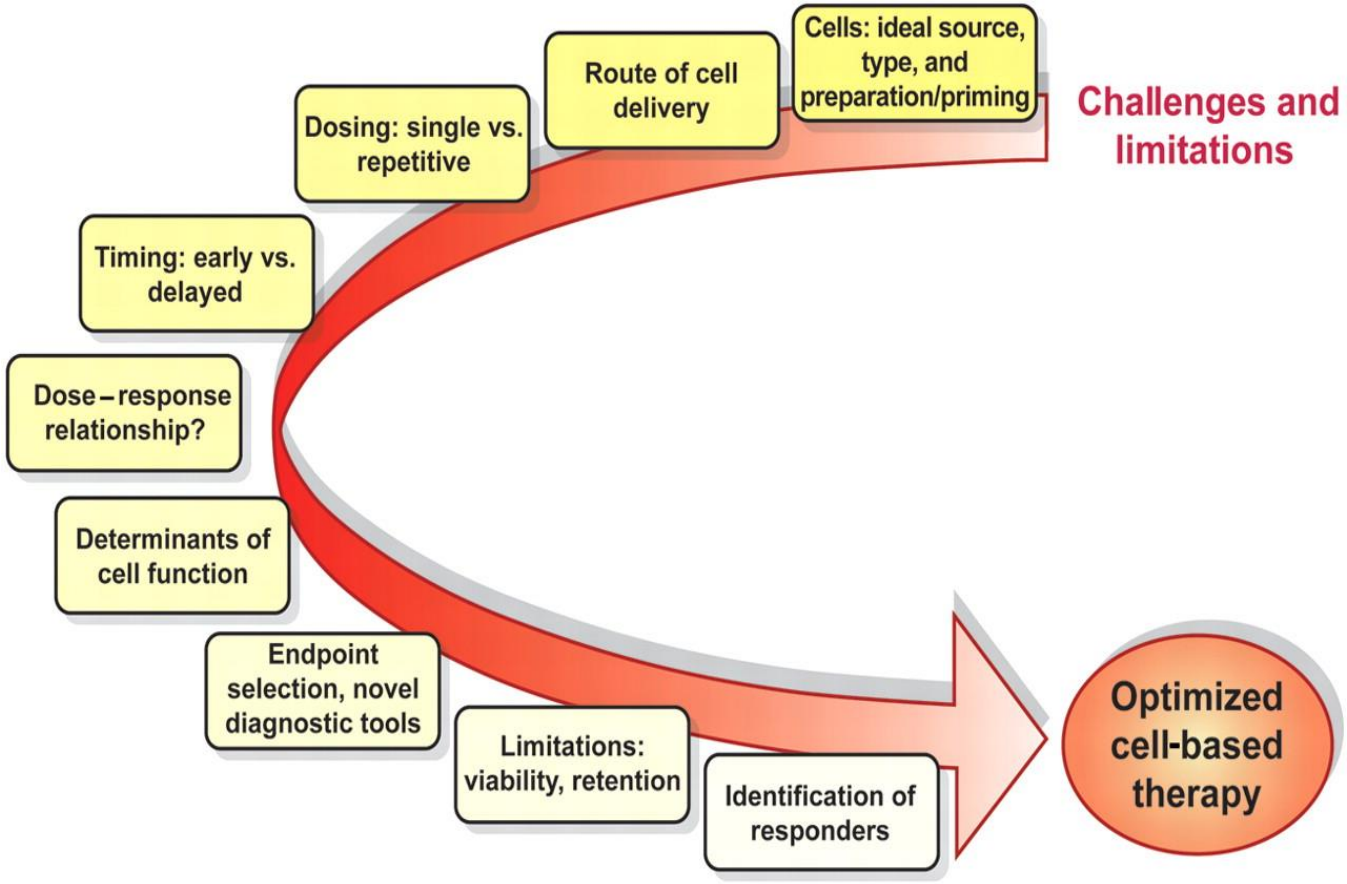
→ **Separation of GvHD from the Anti-Tumor-Effect (GvL)[3] with anti human CD4 antibodies is possible**

→ **Improved survival after transplantation**

1 The most important requirement for allogeneic HSCT: Chao N. Blood. 2012 Feb 2;119(5):1102-3. doi: 10.1182/blood-2011-12-395905.
2 The most important requirement for Transplantation Immunology: Scott et al. Clin Immunol. 2010 Aug;136(2):161.
3 Future of allogeneic hematopoietic stem cell transplantation: Storb R., Keynote Lecture DAG-KBT, 26.02.2015, Hamburg, FHCRC, Seattle, USA.

Challenge of novel therapeutic agents

CELLS



Quelle: Quelle: http://eurheartj.oxfordjournals.org/content/32/10/1197



PD Dr. Stephan Fricke
Specialist Internal Medicine
Immunologist



Nadja Hilger, Dipl.-Biol.
Biologist (Stem Cell Transplantation in mice)



Anne-Marie Müller, M.Sc.
Biotechnologist (Histology)

Increase security and applicability of Stem Cell Therapies for human beings

Christoph Halbich &

Jakob Glaser
Medical PhD students



Natalia Shurawel
Biologist (FACS)



Ellen Svanidze
Biologist (*in-vitro* models)



Claudia Müller, M.Sc.
Toxicologist (Humanized Mouse Models)

© Fraunhofer IZI

Fraunhofer IZI

Fraunhofer



Gefördert aus Mitteln der Europäischen Union

Europa fördert Sachsen.
EFRE
Europäischer Fonds für regionale Entwicklung

Bundesministerium für Bildung und Forschung

Freistaat SACHSEN

LEIPZIG

© Fraunhofer

Fraunhofer