STANFORD CANCER CENTER
Stanford University Medical Center
A National Cancer Institute-designated Cancer Center

Cellular Therapy Today and Tomorrow

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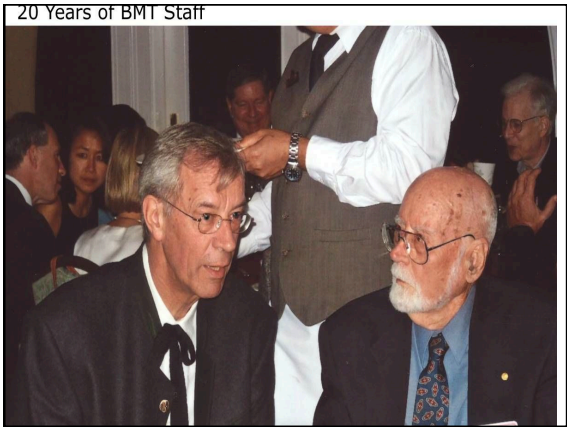
20 Years of BMT Staff

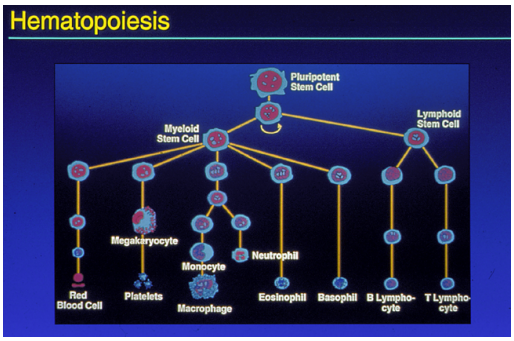


Cellular Therapy in Clinical Medicine

- Established
  - Hematopoietic cell or bone marrow transplantation
- Under investigation
  - Immune cell therapy for cancer and infectious diseases
  - Stem cell therapy for heart attacks and stroke
  - Mesenchymal stromal cell therapy in orthopedics
  - Islet cell transplantation for diabetes
- Future prospects
  - Stem cell therapies for broad range of disorders

Cellular Therapy in HCT

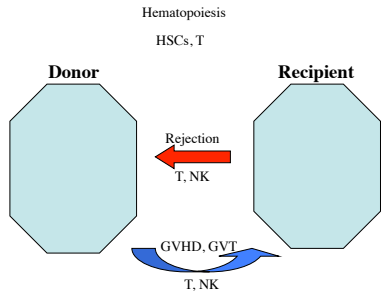
- Hematopoietic cell transplantation
- Disease control
  - Cytotoxic T cells
  - NK cells
  - CIK cells
  - Memory T cells
  - Dendritic cells
- Treatment of infections
  - CTLs
- Acceleration of immune reconstitution
  - CLP, CMP
- Treatment and prevention of GVHD
  - MSCs
  - Treg



Stem Cell Sources

- Bone Marrow
- Mobilized Peripheral Blood
  - G-CSF mobilized
  - Chemotherapy plus G-CSF
  - Other cytokines
- Cord Blood

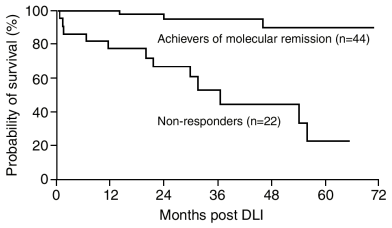
Theoretical Considerations



Challenges

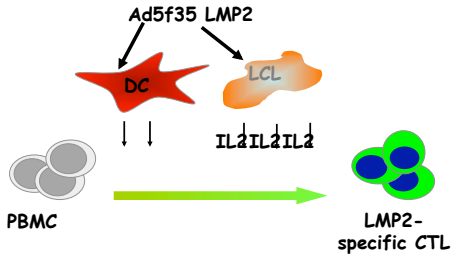
- Biological
  - Do the principles apply?
  - How different are the preclinical models from the clinical situation?
- Technical
  - Cell separation techniques (columns, FACS, cell expansion)
  - Availability of reagents
  - Laboratory requirements
- Regulatory
- Imaging
  - Do the cells traffic to the appropriate locations?
  - How long do they survive?
- Cost

Response to Donor Leukocyte Infusions in CML Patients

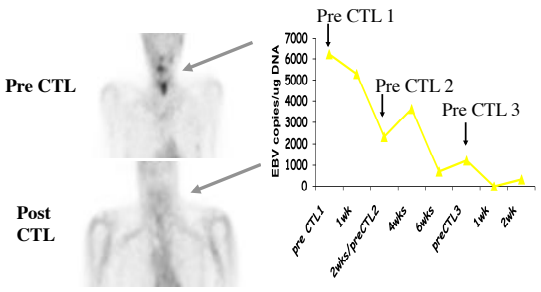


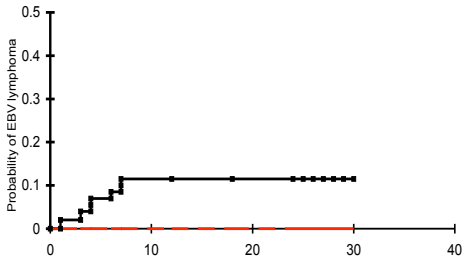
Dazzi et al. Blood 96:2712, 2000

Generation of LMP2-specific CTL with LMP2-transduced DC & LCL Stimulations

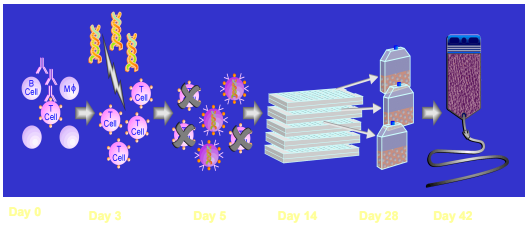


Complete Radiological Response EBV+ve NK-T NHL





Expansion of CTLs



Potential Targets

- Tumor specific
  - Bcr/abl
  - Wt-1
- Lineage specific
  - Minor HAG
  - CD19, CD20
  - PR1
  - Idiotype
- Viral
  - EBV

Virus Specific T Cells Products

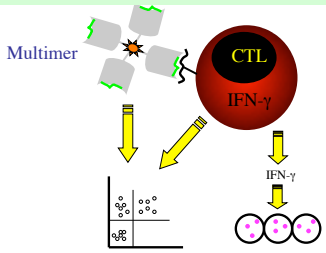
Donor Specific

- Direct-selection strategies in licensing trials in Europe – companies will likely lead US studies
- Rapid donor specific T cells using peptides and 7-10 day culture in studies in several centers

Third Party

- Studies with EBV, CMV and multivirus
- Greatest need probably CMV

Direct Selection T Cells

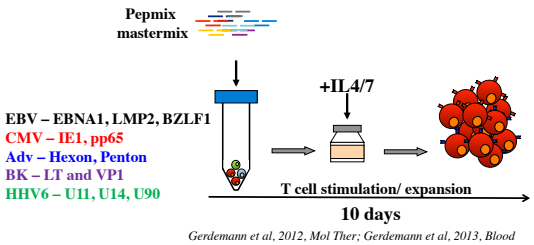


Multimer selection

Gamma interferon selection

Rapid CTLs

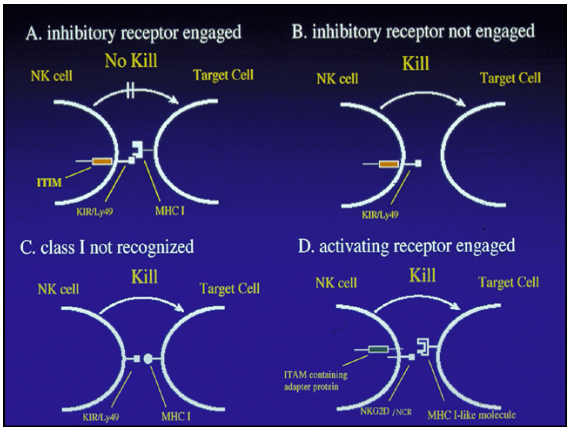
Administration of Rapidly Generated Multivirus-Specific Cytotoxic T-Lymphocytes for the Prophylaxis and Treatment of EBV, CMV, Adenovirus, HHV6, and BK virus Infections post Allogeneic Stem Cell Transplant



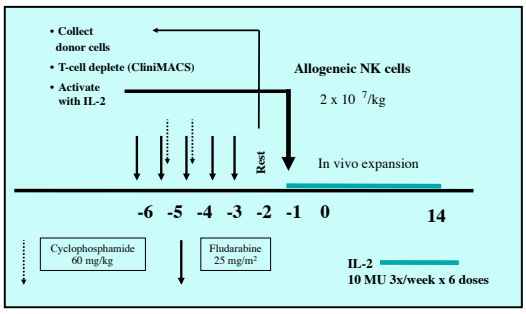
Gerdemann et al, 2012, Mol Ther; Gerdemann et al, 2013, Blood

Natural Killer Cells

- 5-10% of peripheral blood lymphocytes.
- Large granulated cells morphologically.
- Capable of killing virally infected and tumor cells without prior activation.
- Can be further activated with cytokines such as IL-2, IL-12 and IL-15.



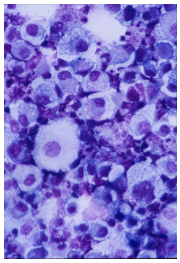
Haploidentical NK cell therapy in a non-transplant setting



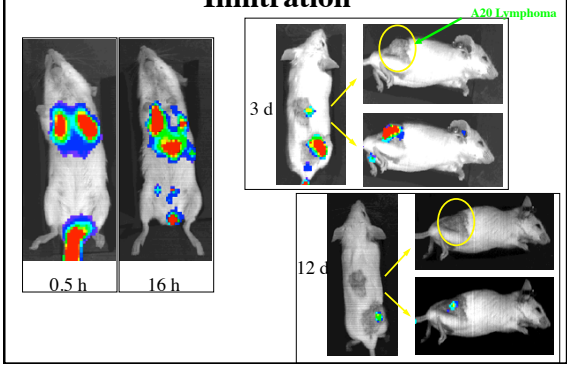
JS Miller et al, Blood 105:3051, 2005

Cytokine Induced Killer Cells (CIK Cells)

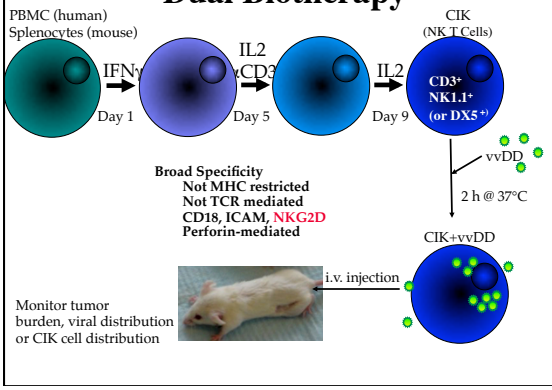
- Day 0: IFN-g
- Day 1: a-CD3 and IL-2
- Day 21: Expansion of T cells which co-express NK cell markers
- In vitro and in vivo anti-tumor cytotoxicity



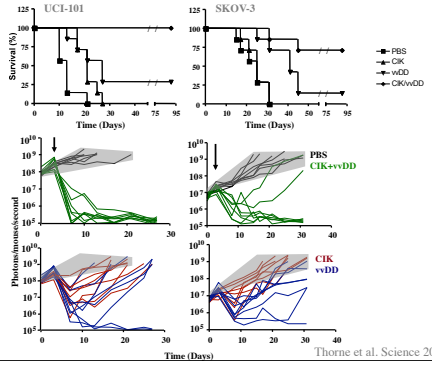
CIK Cell Trafficking and Tumor Infiltration



Dual Biotherapy

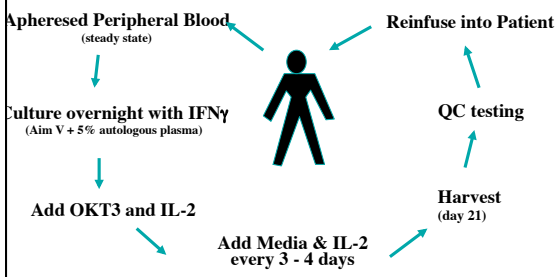


Dual Viral-Cell Therapy

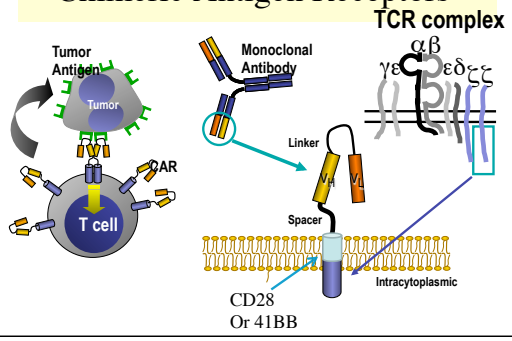


Thorne et al. Science 2006

CIK Process



Chimeric Antigen Receptors



CD19 as a Target

- Present on B lineage cells from the pro-B cell stage to mature B cells
- High expression most B lineage lymphoma and leukemia
- NOT expressed on hematopoietic stem cells (or other tissues)
  - Should not be myelosuppressive effects
  - Should not be other organ toxicities
- Will cause depletion normal B cells

CD19 CARs Strengths

- Multiple studies
- Promising results from some published results in CLL and NHL
- Recent reports of activity in relapsed ALL pre or post transplant - area where unmet need

Published Studies

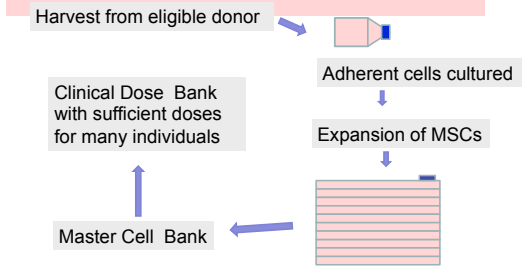
Reference	Targeted disease	Construct	Cell Activation	Auxiliary Therapy	n	Responses
Brentjens et al.	CLL	Retrovirus CD3ζ,CD28ζ	OKT3/ CD28	CTX	8	3 SD
Jensen et al.	DLCL or NHL	Plasmid CD3ζ	OKT3	Fludarabine IL-2	4	
Kalos et al.	CLL	Lentivirus CD3ζ,4-1BB	OKT3/ CD28	Bendamustine or Pentostatin/CTX	3	2 CR, 1 PR
Kochenderfer et al.	CLL or Follicular Lymphoma	Retrovirus CD3ζ,CD28ζ	OKT3	CTX/Fludarabine IL-2	9	1CR, 6 PR, 1SD,
Savoldo et al.	NHL	Retrovirus CD3ζ, CD28ζ	OKT3/ CD28	None or post auto SCT	8	4 SD

Total treated = 32
CR 3
PR 7
SD 8

Mesenchymal Stromal Cells (MSCs)

- Adherent cells that can differentiate into a variety of cell types, secrete cytokines and have immunomodulatory activities
- Treatment of
  - Acute GVHD
  - Organ damage (liver, pulmonary)
  - Poor marrow function
- 3rd party banks can be used

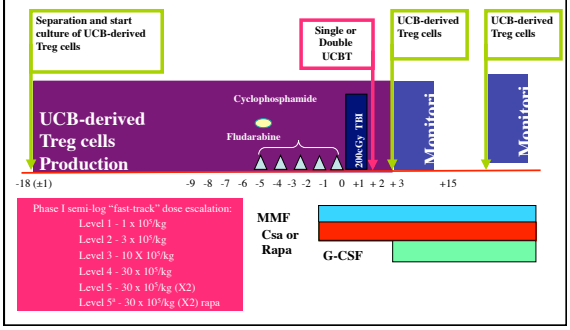
MSC Banks



Regulatory T Cells

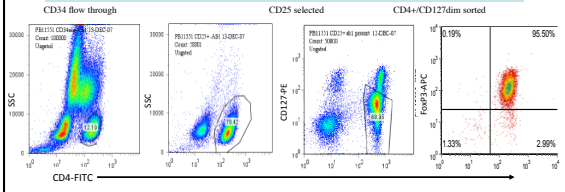
- Several populations of T cells that regulate immune responses termed regulatory T cells have been identified.
- Phenotype CD4+CD25+FoxP3+
- Can suppress GVHD
- Many investigators testing these cells in the clinic
  - Natural T regs
  - Expanded T regs
  - Enhanced T regs
  - Inducible T regs

Phase I Treatment Plan for Umbilical Cord Blood-derived Regulatory T Cells



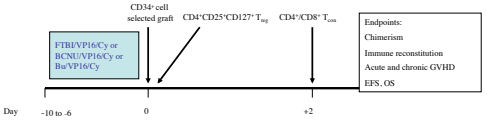
nTreg Unit Expansion and Activation Culture in Minnesota

Isolation of High Purity T_{reg} on a Clinical Scale at Stanford



Percent $CD4^+FoxP3^+$	94 (91–96)
Yield	1 x 10^8 (0.5–1.9 x 10^8)
Recovery	69% (51–88%)

Purified nT_{reg} + T_{con} Trial



Clinical Trials of Adoptive Transfer of Regulatory T Cells

T_{reg} + T_{con} in Haploidentical transplantation (U. of Perugia) Di Ianni et al. Blood 2011

Expanded UBC T_{reg} for GVHD prevention (U. Minnesota) Brunstein et al. Blood 2011

T_{reg} + T_{con} in MRD transplantation (Stanford)

T_{reg} for treatment of GVHD (planned)

Conclusions

- Cellular Therapy is a reality in clinical medicine
- Many different concepts
- Dissemination beyond small single institution clinical trials a major challenge
- Potential in many different fields beyond HCT