Challenges with Preclinical Models
Adoptive Cell Therapies
Helen Heslop
Disclosure

• Licensing agreement with Cell Medica for EBV-specific T cells in NHL and nasopharyngeal cancer

• Collaborative Research Agreement with Celgene for genetically modified T cells

• Founder Virocyte - third party monovirus T cells
Challenges with Preclinical Models

• In vitro studies not always predictive
• Murine models
  – Differences in immune systems
  – Differences in target antigens
• Immunodeficient mice
  – Can engraft human tumors but lack all immune components
  – Not predictive for off-target effects
Adoptive cell therapy against EBV-related malignancies: a survey of clinical results
Merlo et al, Expert Opinion 2008

“It is somehow surprising, for example, that the clinical transfer of anti-EBV adoptive immunotherapy has advanced very rapidly, bypassing a rigorous animal preclinical evaluation.”
Viral Infections Post Transplant

- Major cause morbidity and mortality
- Pharmacologic therapy not available for all viruses and expensive
- Recurrences when therapy stopped
- Clearly related to lack of virus specific T cell response
Generating Virus Specific T Cells

- Repeated stimulation with viral antigens expressed on antigen presenting cell
- Expand viral-antigen specific T-cells
- T cells with specificities for other antigens will not survive
EBV Lymphoma post BMT

- Incidence 1-25% following mismatched or unrelated donor BMT
- Predisposing factors:
EBV-specific T-cell Generation

1. LCL generation (4-6 weeks)

2. CTL expansion (4-6 weeks)

3. QC/QA (1-2 weeks)

Antitumor activity in immunodeficient mouse model

Activity of transplanted CD8+ versus CD4+ cytotoxic T cells against Epstein-Barr virus-immortalized B cell tumors in SCID mice.

Rencher SD, Slobod KS, Smith FS, Hurwitz JL

Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101.

PMID: 7916506 [PubMed - indexed for MEDLINE]

No mouse model for GVHD or other toxicity
EBV Specific T Cell IND 1993

- No toxicity models of EBV infection/cancers
- Submitted with human preclinical data
- FDA concern re risk of alloreactivity
  - Requested data on risk
  - Assay for alloreactivity as release criteria
Risk of Alloreactivity

• VSTs manufactured from transplant donor
• Donor chosen by transplant team as best available match
• Dose escalation study
  – Initial dose less than used in donor lymphocyte infusion
• Culture conditions should select against alloreactive cells
Assay for Alloreactivity

• No validated assay
• Elected to manufacture PHA blasts from recipient and use as target in cytotoxicity assay
• Release criteria <10% cytotoxicity
Assay for Alloreactivity

• Used in over 100 lines
• One line failed to meet criteria with cytotoxicity >50%
• Limitation
  – Cannot manufacture PHA blasts from SCID patients
  – Used parent cells
EBV T Cells Post HSCT

Small numbers $(10^4 - 10^5 / \text{kg})$
- Restore virus-specific immunity
- Reduce virus load
- Cure disease in over 80%
- Long-lasting protection
- Low toxicity
Trivirus-Specific T Cells
EBV, CMV and Adenoviruses

- 3 most common viral complications after HSCT
- Most donors immune
- Have detectable levels of T cells
Generation Of Multivirus-specific VSTs Using Ad5f35 Vectors

EBV-B95-8 → Ad5f35pp65 → B cell → EBV-LCLs → PBMCs → Weekly antigen (x2-4) → 4 weeks → Tri-VSTs
• Immunodeficient mouse models for EBV lymphoma
  – Can model antitumor activity (but already had clinical data)
  – Cannot model GVHD
• No models for multiple viral infections post transplant
In vitro expanded donor-derived virus-specific T cells targeting Adv, EBV, CMV
– Reconstituted antiviral immunity for EBV, CMV and Adv
– Effective in clearing disease

Clinical Outcome of Trivirus T Cells

Leen et al, Nat Med 2006
3rd Party VST Therapy

Bank of VSTs

Cryopreservation

G-Rex 10

Infected Patients

HLA - A

HLA - B

HLA - DR

HLA - A
Most Closely HLA Matched Allogeneic Virus Specific T-Lymphocytes to Treat Persistent Reactivation or Infection with Adenovirus, CMV and EBV after Hemopoietic Stem Cell Transplantation

**CAGT**
- Helen Heslop
- Ann Leen
- Clio Rooney
- Cath Bollard
- Malcolm Brenner
- Adrian Gee

**Other Sites**

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<td>MDACC</td>
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Third Party VSTs 2008

- More theoretical risk of alloreactivity
- Treating refractory disease
- PHA blasts not feasible
  - Patients post transplant
  - Manufacturing time
- No release criteria for alloreactivity
At day 42: Overall
74.0 (95% CI: 58.5-89.5)

At day 42:
CMV  73.9 (95% CI: 51.2-96.6)
EBV  66.7 (95% CI: 36.9-96.5)
AdV  77.8 (95% CI: 53.7-100)

Leen et al Blood 2013
Alloreactivity of Virus Specific T Cell Lines

- Over 90% lines and 45% of virus-specific T-cell clones cross-react against allo-HLA molecules as measured by gamma interferon production.
- T-cell receptor (TCR) gene transfer confirmed that allo-HLA reactivity and virus specificity were mediated via the same TCR.

Amir et al Blood 2010
Are Our VSTs Alloreactive?

- Panel of 44 T-APCs
- Stimulated with unlabeled T-APCs
- Responder cells that produced both TNF and GIFN
- Virus-specific CD4\(^+\) and CD8\(^+\) T cells displayed moderate reactivity with 1 to 5 T-APCs expressing the recipient's HLA allele

Melenhorst et al Blood 2010
Was There Alloreactivity In vivo

- 153 donor-derived lines
  - 28 haploidentical
  - 43 unrelated donors mismatched at one or more antigens
- No denovo GVHD
- Grade 1-2 GVHD reactivations
  - 13/153 overall
  - 6/71 mismatched

Melenhorst et al Blood 2010
Alloreactivity

- In vitro assays do not predict in vivo reactivity
- Preclinical studies are not always predictive
- Serendipity in choice of release assay
CRS after VST

- Rare compared with CAR Therapy
  - 2 out of 166 cases
- Correlates with bulky disease
18 year old post 9/10 URD
Developed EBV PTLD
• 2 weeks later, fever and hypotension requiring 2 inotropes
• Rapid resolution after steroids/Entanercept
Clinical Response

Pre VSTs

6 weeks post VSTs
Inflammation During Response - BKV

**Viral load**
- **Blood**: Decrease from wk-3 to wk4, then increase to wk6.
- **Urine**: Increase from wk-3 to wk4, then decrease to wk6.

**pVSTs**
- **Blood**: Increase from wk-3 to wk4, then decrease to wk6.
- **Urine**: Decrease from wk-3 to wk4, then increase to wk6.

**BKV copies/ml**
- **Blood**: Peaks at wk2 and wk3.
- **Urine**: Peaks at wk1 and wk2.

**T cells**
- **Bladder-derived T cells**: Increase from wk-3 to wk4, then decrease to wk6.

**Graphs**
- **SFC/5x10^5**
- **wk5**
Published Studies with Donor-derived VSTs

- Over 450 patients in over 30 studies
- 58 with GVHD mostly Grade I or II
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<td>Tzannou et al Tandem BMT 2016</td>
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Adoptive cell therapy against EBV-related malignancies: a survey of clinical results
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“It is somehow surprising, for example, that the clinical transfer of anti-EBV adoptive immunotherapy has advanced very rapidly, bypassing a rigorous animal preclinical evaluation.”
T Cell Therapy For Lymphoma

Heterogeneous tumor

PRAME
MAGEA4
SSX2
Survivin
NYESO1

MultiTAA
T cells
Tumor Antigen Specific T Cells
2011

Cytotoxic T Lymphocytes Simultaneously Targeting Multiple Tumor-associated Antigens to Treat EBV Negative Lymphoma

Ulrike Gerdemann¹, Usha Katari¹, Anne S Christin¹, Conrad R Cruz¹, Tamara Tripic¹, Alexandra Rousseau¹, Stephen M Gottschalk¹, Barbara Savoldo¹, Juan F Vera¹, Helen E Heslop¹, Malcolm K Brenner¹, Catherine M Bollard¹, Cliona M Rooney¹ and Ann M Leen¹

¹Center for Cell and Gene Therapy, Baylor College of Medicine, The Methodist Hospital, Texas Children’s Hospital, Houston, Texas, USA

• Risk of cross reactivity
  – Analysis for homology of target antigens and other proteins


Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy.

Antigen Escalation Phase = fixed dose 5x10^6/m^2 - 2 pts/stage:
Day 0: PRAME-specific T cells
Day 28: PRAME and SSX-specific T cells

Stage Two:
Day 0: PRAME and SSX-specific T cells
Day 28: PRAME/SSX/MAGE-specific T cells

Stage Three:
Day 0: PRAME/SSX/MAGE-specific T cells
Day 28: PRAME/SSX/MAGE/NYESO1-specific T cells

Stage Four:
Day 0: PRAME/SSX/MAGE/NYESO1-specific T cells
Day 28: PRAME/SSX/MAGE/NYESO1/Survivin-specific T cells
Conclusions

• With VST and TAA studies limited preclinical models to assess alloreactivity and other risks
• Some preclinical models did not correlate with in vivo effects
• Need clinical testing
Strategies to Reduce Risk

• Start with low doses
• Antigen escalation
• Intervals between patients
• Ability to ablate cells (or neutralize cytokines) if adverse effects ensue
  – Steroids
  – Suicide gene
  – Tociluzimab
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