Clinical Translation of Immunotherapy using WT1 and CMV specific TCR Gene Transfer

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TCR Gene Transfer

• ‘Designer T cells’ with re-directed antigen specificity.

• Adoptively transfer T cells with known antigen specificity and known (high) functional avidity.

• Potential to manipulate homing ability, etc.
Retroviral TCR gene transfer

Antigen-specific CD8 T cell

Insert TCR into retro-viral vector

Clone DNA of antigen-specific TCR

Before

After

CD8

Antigen-specific multimer
CMV TCR Gene Transfer

Shao-An Xue, Liquan Gao, Emma Nicholson
CMV Disease Post Allogeneic Haematopoietic Stem Cell Transplantation

CMV Pneumonitis

CMV Inclusion Body

CMV Retinitis

CMV Colitis
CMV Reactivation Post Allogeneic HSCT

Recipient CMV seropositive

HSC Donor CMV seropositive

Infuse CMV-specific CTL

Donor engraftment and T cell expansion
Potential for expansion of CMV-specific T cells

CMV reactivation

Time

Day 0

Day 28

Pre- and post transplant immune suppression

Recipient CMV seropositive

HSC Donor CMV seronegative

CMV reactivation

Time

Day 0

Day 28

Pre- and post transplant immune suppression

Conditioning

Conditioning

Infuse CMV-specific CTL

Donor engraftment and T cell expansion
NO CMV-Specific T Cells
Generation of Cys1-hybrid CMV TCR construct

![Diagram of TCR constructs]

**Wild-type TCR**
- α, β
- SS

**Cys-1 TCR**
- α, β
- SS

**Hybrid TCR**
- α, β
- SS

**Cys-1 Hybrid TCR**
- α, β
- SS

![Flow cytometry plots]

- **Mock Td**
  - 0% (CD8+ mCβ)

- **CMV-TCR Td**
  - 41% (CD8+ mCβ)

- **Mock Td**
  - 0.1% (pNLV-Tetramer)

- **CMV-TCR Td**
  - 18% (pNLV-Tetramer)
  - 14% (pNLV-Tetramer)
Are MHC class I restricted CMV-specific CD4+ T cells functional? Do they function in the same way as a classical class II restricted CD4+ T cells?
Ag-specific function of CMV TCR-Td T cells

**CD4+ CMV-TCR Td**

- Irrelevant Peptide (CLG)
  - TNF: 5.8%
  - IFNγ: 0.2%
  - IL2: 0.4%

- Relevant Peptide (NLV)
  - TNF: 14%
  - IFNγ: 14.7%
  - IL2: 14%

**CD8+ CMV-TCR Td**

- Irrelevant Peptide (CLG)
  - TNF: 0.6%
  - IFNγ: 0.3%
  - IL2: 0.9%

- Relevant Peptide (NLV)
  - TNF: 2.7%
  - IFNγ: 11.8%
  - IL2: 7.6%
CMV TCR-Td T cells recognise endogenously processed antigen
Functional avidity of CMV-TCR Td CD8+ and CD4+ T cells

IFN\textsubscript{\textgamma} secretion

IL-2 secretion

CMV-TCR Td CD8+ T cells

CMV-TCR Td CD4+ T cells

CMV-TCR Td CD8+ T cells

CMV-TCR Td CD4+ T cells
CMV-TCR Td CD8+ and CD4+ T cells reject CMV pp65-expressing tumours in vivo

2x10⁶ K562A2-pp65-Luc tumor cells
Clinical GMP Grade CMV-TCR Td CD8+ T cells
A Phase I Safety, Toxicity and Feasibility Study of Adoptive Immunotherapy with CMV TCR-td donor-derived T cells for Recipients of Sibling Allo HSCT (CMV TCR-001)

Exclusion criteria:
aGVHD grades II-IV
Neuts < 0.5 x 10⁹/l

CMV TCR-td bulk donor-derived T cells
10⁴ - 10⁵/kg recipient weight

1st positive PCR (Pre-emptive Rx)

Start anti-viral Rx if:
↑↑Copy number at 1 wk
CMV PCR pos at + 2 wks
CMV disease

Immunology Ix:
Vβ13, tetramer staining
Ag-specific proliferation
Intracellular CK secretion
Cytotoxicity
TCR repertoire
PCR for Vβ13/TCR fragments

Clinical Assessment for GVHD
T cell subsets & phenotype
Class I restricted CD4+ T cell

Class I restricted CD4+ T cell + additional CD8

Virally infected cell or tumour cell

Class I MHC

TCR

CD4

CD8

Virally infected cell or tumour cell

Class I MHC

TCR

CD4
Co-transfer of CD8 co-receptor into CMV TCR-Td CD4+ T cells improves function
Co-transduction with additional CD8 co-receptor into CMV-TCR Td CD4+ T cells may improve in vivo function.

CMV-TCR Td CD4+ T cells

CMV-TCR Td CD8+ T cells

CMV-TCR and CD8 Td CD4+ T cells

d5  d10  d15  d20  d25
Improving function of TCR-td T cells

Maryam Ahmadi, Judy King
Co-transduction of TCR and CD3 into T cells

- Increased surface expression of TCR leads to increased functional avidity of the TCR
- CD3 rate limiting for expression of introduced TCR
- Can surface expression of TCR be increased by introduction of additional CD3?
- Does this lead to increased functional avidity of the T cell expressing a class I restricted TCR?
CD3 is rate limiting for TCR expression

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<th>F5 TCR</th>
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<td>Vβ2.1</td>
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BW cells
CD3 is rate limiting in primary T cells

Murine splenocytes
CD3 improves antigen-specific IL2 production

Sorted CD8+ murine T cells
CD3 improves antigen-specific proliferation

Vβ11

GFP

F5
F5+CD3

IFNγ pg/ml

NP conc, uM

CPM

10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1}

10 1000

F5-TCR
F5-TCR+CD3

NP conc, uM

CPM/1000

pNP peptide conc (uM)

Vβ11 improves antigen-specific proliferation
In vivo function of TCR+CD3 transduced T cells

EL4-NP tumour cells (luciferase)

TCR+ CD3 td CD8+ T cells (luciferase)

Monitor tumour clearance and T cell memory

d0

d1

> 8 weeks
CD3 limits tumour growth in vivo

**Graphs:**

1. **Tumour volume**
   - Mock
   - TCR
   - TCR+CD3
   - Days post tumor injection

2. **Phontons/sec x 10^10**
   - Mock
   - TCR
   - TCR+CD3
   - Days post tumor injection

**Images:**

- D5, D8, D11, D13, D17, D20, D33, D50
- TCR
- TCR + CD3
TCR+CD3 enhances T cell accumulation in tumours
TCR+CD3 enhances T cell accumulation in tumours
TCR+CD3 T cell persist in larger numbers than TCR-only T cells

Spleen

Lymph nodes
TCR+CD3 T cells dominate central and effector memory development
TCR+CD3 T cell mount an effective memory recall response
WT1 TCR Gene Transfer

Shao-An Xue, Liquan Gao, Sharyn Thomas, Irma Martinez-Davila
WT1 TCR-td T cells display in vitro function and eliminate autologous 1° leukaemia cells in NOD/SCID mice.

![Bar graph showing IFN-γ levels in Normal, AML, and CML T cells](image)


Clinical Scale Production of GMP grade WT1 TCR-td T cells

Lymphocyte Gate

Isotype Control

qRT-PCR of PBMC to determine persistence of GMP grade WT1 TCR-td T cells

Mock-Transduced

Post 1 Round

Diluted in 10⁶ PBMC

Vβ2

Vβ2

Ladder

0.4x10⁶ 4x10⁷ 4x10⁸ 400 40 4

PBMC

C(−)
UK Multi Centre, non-randomised, dose-escalation, Phase I/II pilot study
Infusion of WT1 TCR-td Autologous T cells into HLA-A2 + adult AML or CML patients

Cohort 1 (n=6): \( \leq 2 \times 10^7/kg \)
Cohort 2 (n=12): \( \leq 1 \times 10^8/kg \)

*Provision to escalate conditioning intensity if required
WT1 TCR Gene Therapy: Time Lines

Pre-clinical development: Ongoing
Including vector development

1st TCR expression in murine model

- Vector development (ongoing)
- In vitro expts with Td-T cells
- NOD/SCID In vivo expts (ongoing)

CRUK/LRF funding

LRF funding

DOH funding

Vector production commenced

Clin scale tests

MHRA CTA/EAG

Legal issues

EU ATMP Regs

- ? Opening End 2010

- STAuss/Morris
- In vivo data
- Blood/PNAS

- Rosenberg et al
- Science Phase 1 Trial

GTAC applic

Prov GTAC approval

Full GTAC approval

2001 2002 2003 2004 2005 2006 2007 2008 2009
Acknowledgments

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