Targeting Lewis Y-positive Multiple Myeloma and Acute Myeloid Leukaemia with Gene-modified T cells

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Rationale for T-cell Therapy

1) Treatable but Incurable Diseases

2) Role of Immune System

- Immunosuppression by disease
- Expansion of T-cell clones $\rightarrow OS \uparrow$
  
  (Brown et al., Leukemia 1997; Raitakari et al., Haematol. Oncol. 2003)

- Activation of T-cells against tumour cells
  
  (Noonan et al., Cancer Res. 2005; Teague et al., Nat. Med. 2006)

- GvM/L (Corradini et al., J Clin Oncol 1999; Bruno et al., N Engl J Med 2007)
Lewis Y Antigen as Target

- **Blood group-related Carbohydrate Antigen**
  (Watkins, Science 1966)

- **Associated with >70% of human epithelial cancers**
  (Hakomori, Annu Rev Immunol 1984; Sakamoto et al., Cancer Res 1986)

- **Restricted expression in normal tissues**
  (Hellstrom et al., Cancer Research 1990; Kitamura et al., Proc Natl Ac Sci USA 1994)

- **Phase I with humanised anti-LeY Mab showed safety**
  (Scott et al., Clin Cancer Res 2007)

- **Expression on haematological malignancies?**

  **Limited evidence** (Cao et al., Glycobiology 2001)
Lewis Y Antigen as Target

MM cell line RPMI-8226

Other MM cell lines:
OPM-2, NCI-H929, U266, LP-1: No / weak LeY-expression
Lewis Y Antigen as Target

AML cell line K562

Other AML cell lines:
KG-1 cells negative for LeY, HL-60 and CC-1 low, KG-1a moderate LeY expression
Lewis Y Antigen as Target

• **MM:** 25/48 BM samples LeY-positive (52%)
  - Median MFI PC: 9.98
  - Median MFI lymphs: 0.8

• **AML:** 15/33 BM samples LeY-positive (46%)
  - Median MFI blasts: 54.4
  - Median MFI lymphs: 2.2

• **Consistently Negative:**
  - Normal lymphocytes
  - Lymphoid malignancies
**LeY expression on primary MM cells**

**Antibody:** murine anti-LeY-FITC (isotype IgG3), clone 3S193
Le\textsuperscript{Y} expression on primary AML cells

Antibody: murine anti-LeY-FITC (isotype IgG3), clone 3S193
Structure of the anti-LeY chimeric receptor

TCR-ζ CD28 α-LeY antigen

signalling Molecules (CD3ζ + CD28)

CD8 a-LeY

T cell

Tumour cell
The Vector Construct

5’ LTR  SD  \(\psi\)  SA  3’ LTR

After Transduction: Day 5

Anti-LeY Anti-idiotypic

42% Anti-LeY

CD8  46%

CD4  51%
Targeting Lewis Y in vitro:
MM cell lines

\[ \% \text{max. IFN-\gamma secretion} \]

- RPMI 8226-13
- LP-1
- U266
- OPM-2
- NCI-H929

Legend:
- **anti LeY**
- **Control**
Targeting Lewis Y in vitro: AML cell lines

% max. IFN-g secretion

- K562
- KG1A
- KG1
- AMLCC1

- anti LeY
- Control
Anti-LeY T-cells

Untransduced T-cells
Anti-Lewis Y - Cytotoxicity *in vitro* - AML

**Anti-LeY T-cells**

**Untransduced T-cells**
Targeting Lewis Y \textit{in vivo}

RPMI 8226 in NOD/Scid

![Graph showing tumor-free percentage over days after tumor inoculation for Anti-LeY T cells and Control T cells. The graph indicates a significant advantage for Anti-LeY T cells in prolonging tumor-free status.]

- **Anti-LeY T cells**
- **Control T cells**

**DAYS AFTER TUMOR INOCULATION**

**TUMOR-FREE [%]**

- 100
- 80
- 60
- 40
- 20
- 0

- 0 20 40 60 80
Safety of anti-LeY-T-cells in vitro
Safety of anti-LeY-T-cells *in vivo*

**Group 1:** Balb/c CD45.2, n=15 → 2.5 Gy

**Group 2:** Balb/c CD45.2, n=30 → 2.5 Gy + 1x10⁷ CD45.1 anti-LeY-T-cells (Ptprrca) ≈ 5x10⁸/kg
Clinical Trial Phase I

Step 1: Isolate PBMC

Step 2: Insert anti-LeY receptor gene into T-cells

Step 3: Expand T-cells

Step 4: Transfusion
Production of anti-LeY-T-cells

- **Day -12**: Apheresis MNC
  - Activation $10^8$-$10^9$ MNC $\rightarrow$ Okt-3+IL2

- **Day -9**: Transduction 1

- **Day -8**: Transduction 2

- **Day 0**: $^{111}$In-Labeling and Infusion of $5 \times 10^8$-$4 \times 10^{10}$ T-cells
Clinical Trial

Apheresis MNC

Autograft/FCR

Restaging: Persisting Disease

Production Anti-LeY T-cells

Fludarabine-Conditioning

Transfusion Anti-LeY T-cells

Safety + Efficacy
Translation

• Optimize the conditions and techniques (LewisY: cell concentration, Retronectin concentration, transduction)
• Scaling up techniques (100mL----10000mL; 1x 10^6 ---1x 10^{10})
• Developing a closed system/New delivery system
• Developing SOPs (one page research protocol-100 pages Batch Record)
• Productions Parameters (Identify critical steps !)
• Validation (Process, Equipment)
• Stability
• QC/QA
• Training
• Dry/Engineering Runs
Part 1 - admin aspects (who, what, dose, where, QC by whom, suppliers)

- **APHERESIS**
  - CBCT
  - Peter MacCallum Cancer Centre

- **STORAGE IN LIQUID NITROGEN**
  - CBCT
  - Peter MacCallum Cancer Centre

- **PRODUCTION OF RTV supernatant**
  - EUFETS AG, Germany

- **AUTOLOGOUS INFUSION**
  - Peter MacCallum Cancer Centre

- **TRANSDUCTION of T-Cells**
  - CBCT
  - Peter MacCallum Cancer Centre

- **ACTIVATION OF T-Cells**
  - CBCT
  - Peter MacCallum Cancer Centre

- **EXPANSION OF T-Cells**
  - CBCT
  - Peter MacCallum Cancer Centre

- **STERILITY TESTING**
  - CBCT
  - Outsourced Laboratories

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**Parameter**

- **Acceptance criteria**
- **Volume**
  - 100 mL
- **Viability**
  - Viable ≥ 70%
- **Sterility assay**
  - Day -7
  - No bacterial or fungal contamination (after 7 days)
- **Sterility assay**
  - (Gram staining)
  - Final product
  - No bacterial contamination detected
- **Endotoxin Assay**
  - Day -2
  - < 0.25 EU/mL
- **TCR Gamma Gene Clonality Assay**
  - In process testing (Day -2)
- **Labelling of bag**
  - Complies with specifications
## Dose

<table>
<thead>
<tr>
<th>Name</th>
<th>Specification</th>
<th>Quantity/Dose unit</th>
<th>Unit</th>
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</thead>
<tbody>
<tr>
<td>T-cells</td>
<td>Autologous, viable T-lymphocytes that are transduced with a chimeric LewisY antigen receptor</td>
<td>A single dose unit will contain:</td>
<td>1 dose</td>
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<tr>
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<td>• minimum $5 \times 10^8$ and maximum of $4 \times 10^{10}$ viable T-cells,</td>
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<tr>
<td></td>
<td></td>
<td>• minimum $5 \times 10^7$ and maximum of $1 \times 10^9$ Indium-111 oxine labelled cells,</td>
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<td>• at least 5 % of T-cells are gene modified per unit</td>
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Quality control methods for CBCT manufactured constituents: All in process components as well- Apheresis, frozen MNCs, T cells etc

- **Morphology**: morphologically consistent with mononuclear white blood cells, by light microscopy

- **Phenotype**: 
  - ≥ 5% anti-LeY TCR positive
  - ≥ 80% CD3 positive T-cells
  - ≥ 80% CD8 or CD4 positive T-cells
  - ≥ 5% CD4 positive T-cells

- **Sterility**: no bacteria or fungal growth as determined by microbiology testing

- **Endotoxin**: no endotoxin contamination as determined by bacterial endotoxin testing

- **Viability**: ≥ 70% viability as assessed by trypan blue exclusion

- **Purity**: No evidence of clonal TCR gene re-arrangements using diagnostic molecular pathology methods
Absence of retroviral vector-mediated transformation of gene-modified T-cells after long-term engraftment in mice

Rationale for doses

Part 4- Clinical
Other adoptive immunotherapies
Adoptive transfer of gene modified T cells
T cells engineered to express ta specific receptors
Specific clinical and product steps (apheresis, retrovirus, PG13 cell line, LeY Ab

Potential risks
Apheresis, cell infusion, CD28 domain(!), radiolabels,
autoimmunity, murine retrovirus and RCR testing (before - EUFETS, during -batched & after- if clinically indicated), insertional mutagenesis

Evaluation of Risks v Benefits
Indication, alternative treatments, risk mitigation
Part 5- Documentation of fatal or life threatening SAEs
  Safety Monitoring and SAE reporting
  Study schedule
  Anticipated toxicities
  SMC

Part 6- Summary for HREC

Appendices
CoAs, IBs, EUFETS GMP licences, forms (production, PICF)
References

Typical issues
Production specifics
Fullscale validations- how and when
Procurement of proteins, vector and ancillary materials
Closed or not, automated or not
Pre clin data and tox- small animal vs non human primate, GLP or not
Indication...oncology vs others
Trial Manufacturing Issues

Constituents and specifications
Essential Processes
Product Specifications
Containers/Shelf life & storage precautions
Arrangements for Quality Control
In process specs and methods for QC
Test Methods
Validation/Qualification, Stability
Batch Release Testing

Regulatory Concerns Common to All Cellular Components
Product Safety/Impurities (i.e., FCS in final product)
Donor screening and testing
Product testing
Adventitious agents, tumorigenicity, pyrogenicity
Product Characterization
Identity, purity, potency, viability, stability
Manufacturing Process
Reproducibility/Consistency of Product batches
Development of in-process and lot release Specifications
Materials

GMP/ CLINICAL GRADE Reagents
FCS? (Used during generation of retroviral stock manufacture)
Human Serum (rather a short shelf-life 12-24 months)
Using “approved” AIM-V media containing the antibiotics
OKT 3 expensive/short shelf-life
Material that have been discontinued in the last 12 months:
Lifecell Culture Flasks
Consumables for Cytomate
Process 1

Apheresis of Peripheral Blood Mononuclear Cells (MNC/1-5X10^8 per bag)
↓
Evaluated/Tested/Patient screening
↓
Cryopreservation of apheresis product
(Bags and Test Vials)/Tested
Process 2

Generation of T-cell Product

Day 0
MNC product thawed
↓
Washed
↓
Activation AIM V media/Cytokine IL-2/OKT 3

Day 3&4
Transduction Round 1
The transduction procedure will be performed in the cell culture vessels which are pre-coated with clinical grade RetroNectin
Process 2

Transduction Round 2
Day 5-12
Expansion AIM V media/Human AB Serum/Cytokine IL-2
Day 12
Harvest
Cytomate Wash and
Volume Reduction
↓
Final Product Formulation
↓
Release Testing
Sampling and Testing Scheme in the Generation of *Ex vivo transduced T cells*

Apheresis collected MNC product

**Patient Screening/NAT/ Serology**

Frozen MNC product

**Sterility**-

- Initial (Prior to Processing)-

  **Cell Count/Viability**-Trypan blue exclusion

In-processing

**USP Sterility, LAL –Endotoxin, Cell Count/Viability**-Trypan blue exclusion
Final Product

- **BULK- Supernatant collected while reducing volume**
  - RCR-GalV (5%) (PG-4 S+L Cell Culture Assay)
  - Sterility
  - Mycoplasma

- **Formulated Product (Infused fresh)**
  - Release testing prior to infusion
  - T-cell Phenotype (Identity-anti-Idiotype %)
  - Purity
  - TCR Gamma Gene Clonality Assay
  - Sterility Gram Stain
  - Endotoxin-
  - Cell counts-Viability-Trypan blue exclusion
Post-release testing
Mycoplasma by PCR
Endotoxin
Sterility
RCR- Gal V (PG-4 S+L Cell Culture Assay 1% or 1x10^8 whichever is less)
RCR Testing Issues

FDA/CBER: Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors

Testing of Ex Vivo Transduced Cells
Requirements (5% of total supernatants; 1% of total pooled cells or $10^8$ cells (whichever is smaller))
RCR Testing Issues

Protocols:
the culture of supernatant or co-culture of cells with a permissive cell line, a minimum of five passages is carried out to amplify any potential RCR present.

Cost  AU$ 11,000-15,000 per sample

Our options: Testing cells only; cells and Supernatant; pool patient samples ..If positive –run individual
The Functional T-cell Phenotype

- CD3+8+
- Naive
- CM
- EM
- Effector

- FSC
- CD8
- CD3
- CCR7
- CD45RA

Event Counts:
- Ungated: 174995
- Event Count: 135388
- Event Count: 45268
- 130907_#3-d10- tube 2.fcs
- 130907_#3-d10- tube 2.fcs
- Event Count: 45268
Conclusions / Future Plan

✓ Lewis Y-Expression on MM, AML
✓ Efficacy *in vitro* and *in vivo*
✓ Safety *in vitro* and *in vivo*
✓ Scale-up in GMP-Facilities
✓ T-cell subset analysis:
  Promising Functional Phenotype
✓ OGTR- application – full approval
✓ CTX-application - conditional approval

Start Clinical Trial expected Q3 2009