Gene edited CAR-T cell therapy

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Waseem Qasim, UCL
Disclosures

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- Research funding from Miltenyi Biotec, Bellicum, Cellmedica, Cell therapy Catapult, Calimmune, Autolus.

- Consultancy/stock/royalty with Autolus Ltd, Catapult-TCR & Orchard Therapeutics
Institute of Child Health/UCL

Great Ormond St. Hospital, London

>100 procedures/year
Transplant

TALEN

T cells

Clinical

Gene Editing

CRISPR
Stem cells

1-2 months
- RBCs
- Neutrophils
- Plts

6-24 months
- T-cells
- B-cells
- Recovery of Immunity

T-cell

- Anti-rejection
- Anti-virus
- Anti-leukaemia
- GVHD
Host Preparation: conditioning intensity

Minimal intensity (MIC)
- None

Reduced intensity (RIC)
- MMF/CYA
- 2Gy TBI +/- Flu
- Cyclo +/- 3Gy TBI
- Flu/Cyclo
- Flu or Cyclo / Bu(8)
- Flu/Melphalan
- Melphalan/Cyclo

Myelo-ablative (MAC)
- Bu(14-16)/Cy(120-200)
- Bu/Flu
- TBI/Cy(120)
- Flu/TBI/Cy

Alemtuzumab or ATG
Graft manipulation to remove T cells & prevent GVHD

In vivo

Alemtuzumab (Campath-1H)
Humanised anti-CD52

Antithymocyte Globulin
Rabbit, Fresnius

Cyclophosphamide

Ex-vivo

CD34 selection

CD3/CD19 depletion

$\alpha\beta$ TCR depletion
Re-directing T cells with recombinant receptors

TCR

CD28

CD3

ε δ γ ε

ζ ζ

1st 2nd 3rd Generation

CAR

VH V\_L

scFv

ζ

1 costim. domain e.g. CD28 or 4-1BB

ζ

2 costim. domains e.g. CD28 + 4-1BB
### Table 1. CD19 CAR Therapy for Acute Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th>References</th>
<th>Disease</th>
<th>CAR</th>
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Ad, adult; allo, allogeneic T cells; auto, autologous T cells; CR, complete remission; gRV, \(\gamma\)-retroviral vector; LV, lentiviral vector; Ped, pediatric; V, vector type; 1:1 CD4/CD8 ratio.

\(^a\)With TALEN-mediated TCR deletion.
Suicide gene therapy strategy

Gene Transfer Vector

T cells

Single donor

Multiple Recipients

Gene Editing
Boosting T Cell responses

T cell

TCR-CD3 complex

HLA

CD52

Viral Vector (LV)

X

X

Leukaemia

CD19+

CD3 41BB

CAR
Tools for gene editing

Double strand break

Strand invasion by homology arms

Targeted insertion
Manufacture of UCART19

**Viral vector inserts**

- CAR19
- TCR gene knockout
- CD52 gene knockout

**TALEN mRNA**

Diagram showing TCR gene knockout and CD52 gene knockout.
NGS of TRAC, CD52 and 15 candidate sites for off target TALEN (Illumina MiSeq) shows background level off target effects

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Elimination of human leukemia in immunodeficient mice

Control

UCART19
Event Free survival following 2\textsuperscript{nd} BMT for leukaemia relapsing after first BMT.

IBFM data courtesy of Thomas Klingebiel

- L 11 months old
- High risk infant ALL aged 3 months
- Marrow relapse 2 months after fully conditioned BMT (8/10)
- No response to Blinatumomab 70% leukaemia blasts in marrow
- Treated June 2015 under MHRA specials licence

\textbf{Special Licence Therapy - first applications}

Event Free survival following 2\textsuperscript{nd} BMT for leukaemia relapsing after first BMT.

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<th>Time [Months]</th>
<th>&lt;200 d: n=19; events 16; censured 3</th>
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<tr>
<td>&lt;200 d</td>
<td>&gt;200 d: n=87; events 57; censured 30</td>
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\textit{p}=0.04
UCART19 ‘bridge’ to transplantation

- Lymphodepletion?
- CAR19 cell dose?
- Residual TCR+ dose?
- Time to allo-SCT?
- Conditioning for allo-SCT?
S1: No CRS following UCART19 infusion
S2

- H 16 months old
- High risk infant ALL aged 4 weeks
- Marrow relapse 9 months after fully conditioned BMT (10/10). Skin GvHD
- No response to Blinatumomab
  - 80% leukaemia blasts in marrow
- Treated December 2015
CD3

CAR

CD52

VCN

Chimerism

Recipieent
UCART19
Donor

MRD

UCART19

Ailo-SCT

MLL
Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells

Waseem Qasim,1,2* Hong Zhan,1 Sujith Samarasinghe,2 Stuart Adams,2 Persis Amrolia,1,2
Sian Stafford,1 Katie Butler,1 Christine Rivat,1 Gary Wright,2 Kathy Somana,2 Sara Ghorashian,1
Danielle Pinner,2 Gul Ahsan,2 Kimberly Gilmour,2 Giovanna Lucchini,2 Sarah Inglott,2
William Mifsud,2 Robert Chiesa,2 Karl S. Peggs,3 Lucas Chan,4 Farzin Farzeneh,4
Adrian J. Thrasher,1 Ajay Vora,5 Martin Pule,3 Paul Veys1

Autologous T cells engineered to express chimeric antigen receptor against the B cell antigen CD19 (CAR19) are achieving marked leukemic remissions in early-phase trials but can be difficult to manufacture, especially in infants or heavily treated patients. We generated universal CAR19 (UCART19) T cells by lentiviral transduction of non–human leukocyte antigen–matched donor cells and simultaneous transcription activator-like effector nuclease (TALEN)–mediated gene editing of T cell receptor α chain and CD52 gene loci. Two infants with relapsed refractory CD19+ B cell acute lymphoblastic leukemia received lymphodepleting chemotherapy and anti-CD52 serotherapy, followed by a single-dose infusion of UCART19 cells. Molecular remissions were achieved within 28 days in both infants, and UCART19 cells persisted until conditioning ahead of successful allogeneic stem cell transplantation. This bridge-to-transplantation strategy demonstrates the therapeutic potential of gene-editing technology.
**Document title**  CLINICAL STUDY PROTOCOL

**Study title**  A phase 1, open label, non-comparative, monocenter study to evaluate the safety and the ability of UCART19 to induce molecular remission in paediatric patients with relapsed/refractory B acute lymphoblastic leukaemia

**Test drug**  UCART19

**Indication**  Paediatric relapsed/refractory B-ALL

**Development phase**  Phase I

**Protocol code**  UCART19_02

**EudraCT Number**  2015-004293-15

Dose Escalation Study to Evaluate the Safety, Tolerability and Biological Activity of a Single Dose of UCART19 in Patients With Relapsed / Refractory (R/R) B-cell Acute Lymphoblastic Leukaemia (ALL) and Chronic Lymphocytic Leukaemia (CLL) (CALM)
UCART19-PALL overview

• First in man, phase 1, open-label, non-comparative study
• Great Ormond Street Hospital, London
• 10 children with relapsed or refractory CD19-B-ALL

✔ between 6 months and <18 years
✔ relapsed or refractory CD19+ B-ALL
✔ morphologically confirmed
  – MRD load of $\geq 1 \times 10^{-3}$
  – exhausted investigational/non-investigational treatment options
✔ Estimated life expectancy $\geq 12$ weeks
✔ Fit for allo-SCT & donor available
✔ Eastern Cooperative Oncology Group (ECOG) performance status $< 2$

• 3m post UCART +12m post-SCT study period
• Long-term follow-up study, 15 years
Study design:

- **Start lymphodepletion**
- **Start cytoreduction (if applicable)**
- **Screening period**
- **Signed ICFs (general + optional)**
- **Safety**

**Key Events:**
- **D-7 to D0:**
  - BMA at D-1
  - Early BMA (optional)
  - Disease evaluation (BMA)

**Treatment Period:**
- **from D0 to D84 or until allo-HSCT conditioning regimen initiation**
- **duration of hospitalization might be adapted upon medical judgment**

**Follow-up Period:**
- **12-month follow-up period starting at D84 for non-responder patients**

**Additional Notes:**
- **Cyto reduction Treatment period (if applicable)**
- **Lymphodepletion treatment period (inpatient hospitalization)**
- **12-month follow-up period of 12 months starting after allo-HSCT date**
UCART19 dosage in children:

- Dose banding
- Based on 30-40% CAR+, >99% TCR KO, > 40% CD52 KO
- 1.1-2.3x10^6/kg CD19CAR+/RQR8^+TCRαβ^-T-cells
- <5.05x10^4/kg of TCRαβ^+ cells

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<th>Vials per Pt</th>
<th>Max TCR+ dose (x10^4 per Kg)</th>
<th>Min CAR19 dose (x10^6 per Kg)</th>
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CRISPR/Cas9 engineered T cells

Sichuan University, China; CRISPR–Cas9-modified autologous T-cells for metastatic lung Ca. NCT02793856

UPENN; rTCR engineered cells with knockout of T-cell receptor and PD-1

GOSH/ICH; CRISPR-CAR knockout TCR, B2m, PD1
b) Vector genome RNA

Reverse Transcription and Genomic Integration:
LTR duplication (3'U3/CRISPR cassette is duplicated in the 5'LTR)

5'LTR

ΔU3 PIII gRNA scaffold R U5 Ψ cPPT hPGK CAR19 WPRE ΔU3 PIII gRNA scaffold R U5

3'LTR

Proviral DNA

d) Coupled sgRNA expression from vector
+ Cas9 mRNA by electroporation
(Transient expression of Cas9 protein)
PBMC UT
Terminal CD19-CAR
Pre-TCRαβ Depletion
Post TCRαβ Depletion
Control 1
Control 2
Final Product
96.9% CAR
<1% TCR
CRISPR-Cas9

- On and off target landscape being characterised
- Multiplexing with MHC, PD1, CD52 targets
- In translation to clinic for T cell applications
SUMMARY

- Demonstration of ‘universal’ concept in paediatric ALL using TALEN gene editing now being assessed in Phase 1 trials
- Somatic, differentiated cells undergoing ex-vivo engineering, and used in a time-limited in vivo application
- Further targets in development, CD3, CD20, CD22, CD123
- CRISPR/Cas9 applications in translation
Paul Veys, Oana Cicarlie, Hong Zhan, Christos Geordiadis, Ulrike Mocke, Lauren Nickolay, Roland Preece, Jane, Rasiyaah, Persis Amrolia, Sujith Samarasinghe, Sian Stafford, Stuart Adams, Katie Butler, Gary Wright, Karthika Somana, Sara Ghorashian, Danielle Pinner Gul Ahsan, Kimberly Gilmour, Giovanna Lucchini, Sarah Inglott, William Mifsud, Robert Chiesa, Anna-Sophie Gautron, Laurent Poirot, Philippe Duchateau, Julianne Smith, Sophie Derniame Aymeric Ducler, Adrian Thrasher, Martin Pule, Ajay Vora, Lucas Chan, Farzin Farzenah, Nursing and Laboratory teams GOSH, Plasmid Factory, Bioreliance.