Chimeric Antigen Receptor Immunotherapy for neuroblastoma

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Types of Immunotherapy

- Antibodies
- Vaccines
- Cell therapies
  - NK cells
  - T cells
Antibody

- CH1
- CH2
- CH3

- VH
- VL

NBL cell

- GD2
- Antigen X

Killer cell
- macrophage
- NK cell
- Other

GM-CSF

Activation

IL-2
Chimeric Antigen Receptors (CARs)

Antibody against Tumour Surface Ag

First generation CAR

Linker

scFv

VH

VL

Hinge or Spacer

T Cell Receptor

Activation signal to the cell
Chimeric Antigen Receptors (CARs)

Antibody against Tumour Surface Ag

T Cell Receptor

First generation CAR

Second generation CAR

Activation signal to the cell
Classical Adoptive Immunotherapy

Blood sample → T-cells extracted → T-cells selected → Administered back to patient

Modified T-cells recognize and kill tumour

Preparative Chemotherapy before T-cells

Melanoma

Morgan RA et al; Science. 2006; 314:126-9
Lymphodepletion enhances efficacy of T-cell therapy

Survival of Patients with Metastatic Melanoma Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2

Proportion Surviving vs. Survival Time in Months

- Chemotherapy n=43
- TBI 1200: n=25
- TBI 200: n=25
- NMA: n=43
- (No lymphodepleting regimen: n=86)

Kindly provided by Mark Dudley Surgery Branch NCI
Development of viruses as delivery systems for genes
Packaging sequence

gag/pol coding region

Envelope coding region

New gene coding region

gag/pol protein

Envelope protein

Natural Virus

Viral Vector

Cytoplasm

Nucleus
Gene-therapy: Integrating Vectors

Mitosis

Cell division

T cell

Two diploid cells
Immunotherapy with engineered T-cells

- Blood sample extracted
- T-cells engineered
- Modified T-cells recognize and kill tumour
- Administered back to patient

Chimeric Antigen Receptor: Grafting desired specificity onto a T-cell

- Monoclonal antibody
  - scFv
  - VH
  - VL
  - Linker
  - Spacer
  - Transmembrane
  - Intracytoplasmic

- αβ: TcR complex
  - γε
  - εδ
  - ζζ

- Antigen
  - Tumor

Adapted from: Eshhar Z PNAS 1993
T-cells

- Homing
- Recognition
- Direct Killing
- Proliferation
- Releases payloads
- Recruits other cells
CAR Therapy – Recent Successes Targeting CD19

Porter DL et al.;

Kochenderfer JN et al;
Blood. 2010 Nov 18;116(20):4099-102
NCARGD2

A phase I study of 2\textsuperscript{nd} generation GD2 CAR in relapsed / refractory Neuroblastoma

**UCL/GOSH**
- Talia Gileadi
- Simon Thomas
- Eva Kokalaki
- Brian Philip
- Karin Straathof
- Martin Pule
- Waseem Qasim
- Adrian Thrasher
- John Anderson

**CRUK (DDO team)**
- David Edwards
- Nigel Westwood
- Stephen Nabarro
- Claire Barton
Clinical responses: first and second generation CAR studies

Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia

David L. Porter, M.D., Bruce L. Levine, Ph.D., Michael Kalos, Ph.D., Adam Bagg, M.D., and Carl H. June, M.D.
**Optimization of GD2 CAR design**

- **Chimeric antigen receptor**
  - **Ectodomain**
  - **V<sub>H</sub>**
  - **V<sub>L</sub>**
  - **scFv**
  - **Signal**
  - **Linker**
  - **Spacer**
  - **Transmembrane**
  - **Intracytoplasmic**

- **Humanization**
- **FcR binding sites removed**
- **2<sup>nd</sup> generation**

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**Retroviral Cassette**

- **LTR**
- **ψ**
- **2A**
- **RQR8**
- **aGD2-CAR**
- **SAR**
- **LTR**
Costimulatory signals in CAR: Proliferation and cytokine release

![Diagram of CAR structure with labels for Signal, Linker, scFv, v, Spacer, Transmembrane, and Signal Transmembrane Space]

![Graph showing fold expansion for NT_SupT1, GD2_SupT1, and LAN-1]
Co-expression of RQR8 safety switch
Trial Design

- Phase I dose escalation; 3 cohorts (between 9 and 15 patients)
- Fixed number of infused T cells, escalation of conditioning regime
- Single centre for phase I
Clinical trial progress timeline

- 2006: Neuroblastoma Society Grant for second generation vector engineering and testing
- 2010: Completion of preclinical study
- Feb 2011: Application for trial funding
- June 2011: Adoption of trial onto Cancer Research UK Drug Development Office trials portfolio
- Nov 2012: Completion of final vector testing
- Jan 2013: Generation of a high titre producer clone
- Feb 2013: Approval of trial protocol by CRUK sponsor
- April 2013: Start of Clinical grade vector manufacture (Eufets)
- Dec 2013: Clinical vector delivery
- Feb 2014: Submission of trial and GMP regulatory application based on scale up data
- April 2014: earliest possible trial opening
Neuroblastoma Research projects at UCL/GOSH
(CRUK, Children with Cancer, Wellcome, GOHCC, Leukaemia and Lymphoma Research, NIHR)

• Making new antibodies to target neuroblastoma; development of new CAR
  – ALK
  – B7H3
• Combining anti-neuroblastoma antibodies with gamma delta T cells
• Making Bi-specific antibodies (BiTES)