CAR T CELLS: CHIMERA OR REALITY?

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Science

Breakthrough of the Year

Cancer Immunotherapy

T cells on the attack
From antibodies to adoptive cell therapy

1. **Cancer patient**
   - **Excise tumor mass**
   - **Harvest PBMCs by apheresis**

2. **T cell activation**
   - **Transduction**

3. **TIL cell isolation**

4. **TIL cell expansion**
   - **Infusion**
   - **Host condition chemotherapy**

5. **CART cells**
6. **TCR T cells**
7. **Lymphodepleted cancer patient**
Adoptive T cell therapy projects at OPBG

- Evaluation of potential tumor-specific antigens;
- Cloning of specific T Cell Receptors (TCR, HLA-restricted);
- Cloning of specific Chimeric Antigen Receptors (CAR, HLA-unrestricted);
- Production of clinical grade products;
- Conduction of Clinical Trials.
Chimeric Antigen Receptor
mAbs vs CARs

Transient effect
Limited tissue bio-distribution
Requirement for high expression of the target molecule

Persistence for the prolonged lifetime of the cell
Active penetration of solid tissues
Ability to recognize tumor cell subsets with low antigen density
Multiple lytic activities following target recognition
Toxicity

• Cytokine Release Syndrome (CRS)
  – Severity related to disease burden
  – Reversed with anti-IL6 therapy
  – Severe CRS mirrors HLH/MAS

• Tumor Lysis Syndrome
  – Not a prominent feature, but may be with high WBC

• Neurotoxicity
  – Seen in several CD19 immunotherapy trials: NCI, CHOP/UPENN, MSKCC, Blinatumomab
  – Fatal events have been recently reported

• Chronic B cell aplasia requiring IgG replacement
Example of approach to CAR T cell production

Ab, antibody; PBMC, peripheral blood mononuclear cells

NEXT CHALLENGES: CAR T cell in solid tumors
Neuroblastoma

• Third most common paediatric malignancy
• 10.2 cases per million of children
• More than 90% of the diagnosed cases are children aged ≤ 5 years

Localized Neuroblastoma

Disseminated Neuroblastoma

Period of diagnosis
1985-91
1999-05
1979-84
1992-98

Cumulative Proportion Surviving

Follow-Up (years)

P < .001

P < .001

88.5% (95% CI; 80.7 to 93.3)
67.3% (95% CI; 56.0 to 76.3)
62.9% (95% CI; 52.1 to 71.9)
33.3% (95% CI; 21.8 to 45.2)
29.3% (95% CI; 22.3 to 36.5)
23.5% (95% CI; 18.1 to 29.3)
26.0% (95% CI; 20.6 to 31.8)
6.7% (95% CI; 3.5 to 11.4)


Abbreviations: GN, ganglioneuroma; GNB, ganglioneuroblastoma.

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Mechanism of GD2 antibody-targeted destruction of neuroblastoma by CDC and ADCC

A  CDC: Complement dependent cytotoxicity

C’1–9 Cascade  
Tumor cell

Complement activation

Membrane attack complex

Tumor cell

Cell lysis

B  ADCC: Antibody-dependent cell-mediated cytotoxicity

IL-2  
NK cell

GM-CSF  
Killer cell

FcrR

Perforin granzyme fas ligand

Monocyte Macrophage Granulocytes

Tumor cell

Necrosis & apoptosis

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CCR Focus
ANBL0032: A COG Study
13-cis-Retinoic Acid ± Ch14.18 with cytokines post Stem Cell Transplant

Ch14.18 + GM-CSF
Ch14.18 + IL-2
Ch14.18 + GM-CSF
Ch14.18 + IL-2
Ch14.18 + GM-CSF

Randomize

RA RA RA RA RA RA RA

0 24 56 80 112 122 150
Baylor College of Medicine (BCM) Phase I trial (NCT00085930) – Study design

• 19 pts with High-Risk Neuroblastoma, relapsed/refractory or after initial therapy
• Partial in vivo lymphodepletion (unconjugated rat anti-hCD45)
• First generation CAR-ATCs + CAR-CTLs administered at 3 dose levels:
  • $1.2 \times 10^7$ cells/m$^2$ ($0.4 \times 10^6$ cells/Kg)
  • $5 \times 10^7$ cells/m$^2$ ($1.7 \times 10^6$ cells/Kg)
  • $1 \times 10^8$ cells/m$^2$ ($3.3 \times 10^6$ cells/Kg)

Safety data

No severe or dose-limiting toxicities have been identified. Three patients had grade 1 to 3 localized pain (2 at a site of biopsy-proven tumor necrosis and 1 in her lower leg at a site with no evidence of active disease).

Louis C. et al, Blood 2001
<table>
<thead>
<tr>
<th>CAGT no.</th>
<th>Age, y</th>
<th>Sex</th>
<th>Stage at diagnosis</th>
<th>Dose level</th>
<th>Disease burden at CTL infusion</th>
<th>Response at 6 weeks</th>
<th>Best response</th>
<th>GD2 T cells last detected, weeks after infusion</th>
<th>Clinical outcome</th>
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<tr>
<td>1662</td>
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<td>PD</td>
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<td>DOD 4 mo after infusion</td>
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<td>CR 1 yr 9 mo after infusion</td>
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<td>SD</td>
<td>PR</td>
<td>72</td>
<td>AWD 2 y 8 mo after infusion</td>
</tr>
</tbody>
</table>

Louis C. et al, Blood 2001
BCM Phase I trial (NCT00085930) – Results (2)

→ Improving CAR T cells persistence is mandatory:
  • Lymphodepletion
  • 2nd and 3rd generation CAR constructs

Louis C. et al, Blood 2001
Third Generation CARs Targeting GD2 (14.G2a)

Which is the optimal co-stimulation for adoptive T cell therapy in neuroblastoma?
iC9 suicide gene

iCasp9 = FKBP12v36 + ΔCaspase9

*All human sequence

BP-004 Study
Clinical trial started on September 2014 in OPBG

Phase I/II Study of BPX-501 T Cells from an HLA-partially Matched Family Donor After Negative Selection of TCR αβ+T Cells in Pediatric Patients With Hematological (malignant and non-malignant) Disorders

ClinicalTrials.gov identifier: NCT02065869
EUDRACT number: 2014-000584-41
Safety profile

- Cytokine-dependent expansion
- Vector copy Number Analysis
- Polyclonality
- Cytogenetic Analysis
- Molecular Cytogenetic Analysis (CGH Array)
- Telomer elongation
2016 timeline for starting treatment of patient

Jan  Feb  Mar  Apr  May  Jun  Jul  Aug  Sept  Oct  Nov  Dec

- Beginning of GMP production of MCB & WCB
- Collection in GMP of the supernatant for the GLP study
- Collection of GMP supernatant
- Tests for the product release
- Beginning of scale-up for the ATMP (CAR-T) production
- Validation runs of the procedure in GMP
- GLP preclinical study
- IMPD submission
- Start of the trial
## Ongoing clinical trials

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Cancers</th>
<th>Gene transfer</th>
<th>CAR construct</th>
<th>Lymphodepletion</th>
<th>Dose levels</th>
<th>Phase/ID</th>
<th>Sponsor</th>
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<tbody>
<tr>
<td>GD2</td>
<td>Sarcoma</td>
<td>Retrovirus</td>
<td>3rd generation + iC9</td>
<td>Cyclo (1800 mg/m²/day x 2 days)</td>
<td>DL1: $1 \times 10^5$/Kg</td>
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<tr>
<td></td>
<td>Neuroblastoma</td>
<td>Retrovirus</td>
<td>3rd generation + iC9</td>
<td>Flu (25 mg/m² days -4, -3, -2) + Cyclo (300 mg/m², days -4, -3, -2)</td>
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<td>GD2</td>
<td>GD2-positive sarcoma</td>
<td>Retrovirus</td>
<td>3rd generation + iC9 (VZV-Tcells)</td>
<td>None (VZV vaccine boosting)</td>
<td>DL1: $1 \times 10^6$/m²</td>
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<tr>
<td>GD2</td>
<td>Neuroblastoma</td>
<td>Retrovirus</td>
<td>3rd generation + iC9</td>
<td>Cyclo (500 mg/m² x 2 days) + Flu (30 mg/m² x 3 days) + Pembrolizumab (2 mg/kg on days -1 and +21)</td>
<td>DL1: $1 \times 10^8$/m²</td>
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<td>Not reported</td>
<td>Cohort II: Cyclo (300 mg/m², days -4 to -1)</td>
<td>DL1: $1 \times 10^7$/m²</td>
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<td>Cohort III: Cyclo (300 mg/m², days -4 to -1) + Flu (25 mg/m² days -5 to -1)</td>
<td>DL1: $3 \times 10^6$/m²</td>
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<tr>
<td>GD2</td>
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<td>Retrovirus</td>
<td>3rd generation + iC9 (NK T cells)</td>
<td>Cyclo (500 mg/m² x 2 days) + Flu (30 mg/m² x 3 days)</td>
<td>DL1: $1 \times 10^6$/m²</td>
<td>I/NCT02 107963</td>
<td>NCI</td>
</tr>
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</table>
How can we improve patients outcome?

- One of the characteristics of the ideal tumor-specific T cell is the ability to infiltrate tumor environment.

- To invade tumor environment T cells must digest:
  - Sub-endothelial basement membrane (SBM)
  - Extracellular matrix (ECM)

- Major components of SBM and ECM are **Heparan sulphate proteoglycans (HSPGs)**

- T cells must release the enzyme **heparanase** to degrade HSPGs

Caruana I, et al. Nature Medicine, April 2015
HPSE and CAR-GD2 co-expression improve overall survival in NB xenograft mouse models (I)

Tumor free at day 40:
- CAR: 6/22
- CAR(I)HPSE: 18/26

** p<0.0001
* p<0.007
p=0.008
Engineered CAR T Cells Targeting the Cancer-Associated Tn-Glycoform of the Membrane Mucin MUC1 Control Adenocarcinoma
Engineered CAR T Cells Targeting the Cancer-Associated Tn-Glycoform of the Membrane Mucin MUC1 Control Adenocarcinoma
Factors influencing CAR T-cell activity
ACKNOWLEDGEMENTS

Dipartimento di Oncoematologia e Terapia Trasfusionale
Onco-Haematology Clinical Staff

Unità di Immunoterapia dei Tumori
Concetta Quintarelli
Biagio De Angelis
Ignazio Caruana
Francesca Del Bufalo
Domenico Orlando
Iole Boffa
Marika Guercio
Vinicia Polito
Beatrice Conti
Rosaria Cristantielli
Tamascia Belardinilli
Valeria Caposotto

Officina Farmaceutica
Marco Dieci
Andrea La Sala
Carla Paganin

Bambino Gesù OSPEDALE PEDIATRICO

Baylor College of Medicine
Bellicum PHARMACEUTICALS
Now, this is not the end. It is not even the beginning of the end, but it is, perhaps, the end of the beginning......