ADOPTIVE T CELL THERAPY FOR CANCER

ESMO Preceptorship on Immuno-Oncology - Madrid, 2-3 February 2017
CANCER IMMUNOTHERAPY

Recent developments have demonstrated that immunotherapies are capable of achieving durable antitumor responses in patients with metastatic cancer.

Two major developments that have led to this success have been:
- demonstration that many cancer patients have a T cell repertoire capable of recognizing their cancer
- realization that the tumor microenvironment is inhibitory to the function of this repertoire.
ADOPTIVE T CELL THERAPY FOR CANCER (ACT)

✓ isolation and reinfusion of T lymphocytes into patients to treat cancer

Generation and expansion of tumor-reactive T cells *ex vivo*

Expansion of **endogenous** tumor-reactive T cell repertoires

Generation of **artificial** tumor-reactive T cell

Park *et al.* Trends Biotechnol (2011)
T CELL RECOGNITION

The structure of the T cell receptor (TCR) and its ligand, the peptide-MHC complex.
ADOPTIVE T CELL THERAPY FOR CANCER (ACT) USING TUMOR-INFILTRATING LYMPHOCYTES (TILS): ACT-TILS

✓ Successful use of IL-2 in the treatment of metastatic melanoma and RCC suggested that a manipulation of the host immune system could provoke an endogenous reaction capable of mediating cancer regression.

✓ Early efforts to identify the cells that could mediate tumor regression: lymphokine-activated killer (LAK) cells.

✓ Isolation and ex vivo expansion of naturally occurring lymphocytes from tumor biopsies (TILs).

Steven A. Rosenberg
Surgery Branch, National Cancer Institute
Bethesda (USA)
ACT-TILS: PROTOCOL

First in human trial with TILs was performed in 1988 at the Surgery Branch, NCI by Steven A. Rosenberg.

Studies of gene-marked TIL showed that *in vivo* survival was very short with most patients having no TIL detectable by RT-PCR a month later.

Lymphodepletion (nonmyeloablative chemotherapy regimen) was introduced just prior to cell infusion. This substantially increases TIL persistence and the incidence and duration of clinical responses.
When all of these principles are employed an objective response rate >50% was seen with 20% of melanoma patients maintaining durable CRs after 5–8 years of follow-up.

54-year-old male with metastatic melanoma (lungs and liver) treated with autologous TILs plus IL-2 following a NMA regimen in December 2003. The patient underwent a CR and remains disease-free 10 y later.
ACT–MINIMALLY CULTURED “YOUNG” TILS

- Studies evaluating the characteristics of TILs from responders and non-responders determined that longer telomere length correlated with in vivo persistence and tumor regression.
- Decreasing the number of days required to culture TILs to treatment levels and consequently reducing in vitro IL-2 exposure.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Histology</th>
<th>Patients (n)</th>
<th>OR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Rosenberg et al, 1988</td>
<td>Melanoma</td>
<td>20</td>
<td>11 (55%)</td>
<td>1 (5%)</td>
<td>10 (50%)</td>
<td>First in human trial (TILs + IL-2)</td>
</tr>
<tr>
<td>Dudley et al, 2005</td>
<td>Melanoma</td>
<td>43</td>
<td>21 (49%)</td>
<td>5 (12%)</td>
<td>16 (37%)</td>
<td>Pre-conditioning regimen to improve TIL engraftment using &quot;modern-era&quot; lymphodepletion (NMA) and high dose interleukin-2 (IL-2)</td>
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<td>Dudley et al, 2008</td>
<td>Melanoma</td>
<td>25 (2 Gy TBI)</td>
<td>13 (52%)</td>
<td>5 (20%)</td>
<td>8 (32%)</td>
<td>In sequential trials, response rate directly proportion to depth of pre-conditioning lymphodepletion, prompting evaluation in a randomized trial.</td>
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<td>Rosenberg et al, 2011</td>
<td>Melanoma</td>
<td>25 (12 Gy TBI)</td>
<td>18 (72%)</td>
<td>10 (40%)</td>
<td>8 (32%)</td>
<td>Minimally cultured CD8-enriched TILs can mediate effective tumor regression.</td>
</tr>
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<td>Dudley et al, 2010</td>
<td>Melanoma</td>
<td>33 (NMA)</td>
<td>19 (58%)</td>
<td>3 (9%)</td>
<td>16 (49%)</td>
<td>Minimally cultured bulk TILs can mediate effective tumor regression.</td>
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<tr>
<td>Itzhaki et al, 2011</td>
<td>Melanoma</td>
<td>23 (6 Gy TBI)</td>
<td>11 (48%)</td>
<td>2 (9%)</td>
<td>9 (39%)</td>
<td>Bulk TIL screened for IFN-γ secretion can mediate durable tumor regression.</td>
</tr>
<tr>
<td>Pilon-Thomas et al, 2012</td>
<td>Melanoma</td>
<td>13^a</td>
<td>5 (38%)</td>
<td>2 (15%)</td>
<td>3 (23%)</td>
<td>TIL, particularly differentiated effector cells (CD8+/BTLA+) can mediate durable tumor regression.</td>
</tr>
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<td>Radvanyi et al, 2012</td>
<td>Melanoma</td>
<td>31</td>
<td>13 (42%)</td>
<td>2 (6%)</td>
<td>11 (35%)</td>
<td>Complete and durable responses were induced after TIL treatment using NMA in combination with low-dose IL-2</td>
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<td>Ellebaek et al, 2012</td>
<td>Melanoma</td>
<td>6^b</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
<td>0</td>
<td>Complete and durable responses with NMA TIL. First intent-to-treat analysis, demonstrating a 29% dropout rate, mainly due to disease progression.</td>
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<td>Besser et al, 2013</td>
<td>Melanoma</td>
<td>80^c</td>
<td>23 (29%)</td>
<td>5 (6%)</td>
<td>18 (23%)</td>
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ACT–YOUNG TILS

✔ The technically challenging task of developing autologous tumor lines used for testing the tumor reactivity of TILs may be clinically unnecessary, thereby reducing the time from resection to treatment for patients with advanced disease.
ACT–TILS LIMITATIONS

• Requirement for surgery to isolate the tumor. In some cases, a biopsy is not possible.

• In about 20% cases it is not possible to obtain tumor-reactive autologous TILs.

• The ability to generate consistently TILs with antitumor activity: it has only been possible to obtain TIL with therapeutic capacity from melanoma samples. Mutation rate higher than in other cancers. Extrapolated to lung cancer associated with smoking?
Genetic engineering of peripheral blood lymphocytes provides to introduce tumor-reactive TCRs for effective ACT therapy.
ACT-ENGINEERING “ARTIFICIAL” T CELLS: TCR GENE TRANSFER

High-affinity TCRs can be cloned from naturally occurring “rare” tumor-reactive T cells.

Transgenic mice for a given MHC allele immunized with AAT to isolate the TCR genes from the reactive T cells.

Phage display – in vitro selection
### ACT-ENGINEERING “ARTIFICIAL” T CELLS: TCR GENE TRANSFER

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<th>Reference</th>
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<th>Antigen (epitope)</th>
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<td>15</td>
<td>2(13%)</td>
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<td>First demonstration of use of TCR-engineered T cells to mediate tumor regression. No treatment-related toxicities.</td>
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<td>Demonstrated importance of clonal selection. Grade 3 ototoxicity in 8 patients.</td>
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<td>Melanoma</td>
<td>Gp100 (aa 154-162)</td>
<td>A*02</td>
<td>16</td>
<td>3(19%)</td>
<td>1(6%)</td>
<td>2(13%)</td>
<td>Demonstrated use of TCR with murine constant regions. Grade 3 ototoxicity in 1 patient.</td>
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<td>Parkhurst et al, 2011</td>
<td>Colon</td>
<td>CEA (aa 691-699)</td>
<td>A*02</td>
<td>3</td>
<td>1(33%)</td>
<td>0</td>
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<td>Recognition of CEA in normal colonic mucosa resulted in 3 patients with severe colitis.</td>
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<td>Robbins et al, 2011</td>
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<td>17</td>
<td>9(53%)</td>
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<td>First report using of TCR-engineered T cells targeting a cancer germline antigen to mediate tumor regressions. Modification of CDR2 of TCR alpha chain to increase TCR avidity without altering antigen specificity. No major toxicities observed.</td>
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<td>Melanoma</td>
<td>MAGE-A3 (aa 112-120)</td>
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<td>9</td>
<td>5(56%)</td>
<td>1(11%)</td>
<td>4(44%)</td>
<td>Previously undescribed MAGE-A12 expression in brain tissue resulted in 3 patients with severe neurologic toxicity, including 2 TRM.</td>
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<td>Linette et al, 2013</td>
<td>Melanoma, myeloma</td>
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<td>0</td>
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<td>Off-target activity against myocardial protein titin resulted in 2 TRM secondary to cardiogenic shock.</td>
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ACT-ENGINEERING “ARTIFICIAL” T CELLS: TCR GENE TRANSFER

### Table 2. ACT Clinical Trials Employing T Lymphocytes Engineered to Express Specific T-Cell Receptors for the Treatment of Solid Cancers

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AU

AARHUS UNIVERSITET

LUIZ ÁLVAREZ-VALLINA

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ACT-ENGINEERING “ARTIFICIAL” T CELLS: TCR GENE TRANSFER

Risks:
✓ Generation of self-reactive αβ TCR due to recombination with endogenous chains

Disadvantages:
✓ Recognition restricted to certain MHC allelic variants (non-generalizable therapy)
✓ Frequent downregulation of MHC molecules in tumors
✓ Difficulty in isolating high-affinity TCR

Thomas et al. Immunology (2010)
ACT-ENGINEERING “ARTIFICIAL” T CELLS: CHIMERIC ANTIGEN RECEPTORS (CARS)

✓ The “CAR” concept: hot-wiring T cells against tumors
ACT-ENGINEERING “ARTIFICIAL” T CELLS: CHIMERIC ANTIGEN RECEPTORS (CARS)

- CARs recognize intact cell surface proteins and therefore elude MHC restriction

- Applicable to all patients irrespective of their MHC haplotypes

- Overcome MHC downregulation by tumors, which deprives T cells of a ligand for their endogenous TCRs
CAR “MECHANICS”
GENERATION OF THERAPEUTIC CAR-T CELLS

CAR-T CELLS FOR HEMATOLOGIC MALIGNANCIES

ACT with CAR-T cells has been remarkably successful in hematological malignancies. CR is achieved in a substantial fraction of patients in multiple studies using CAR-T 19 cells.
Although the first CAR-T 19 clinical reports focused on non-hodgkin lymphoma (NHL) and chronic lymphocytic leukaemia (CLL), the most dramatic results have been obtained in ALL.

Three centres made seminal contributions to these clinical studies: the Memorial Sloan Kettering Cancer Center for adult ALL, and the Children’s Hospital of Philadelphia and the National Cancer Institute for childhood ALL.

All reported the same two main toxicities: B cell aplasia and cytokine responses, sometimes causing severe CRS.
Recently in a phase 2 study conducted by Juno Therapeutics with its anti-CD19 CAR T-cell therapy (JCAR015) in adults with relapsed or refractory B-cell ALL several deaths have been reported due to severe neurotoxicity (cerebral edema).
To date, there has been limited success using CAR-T cells for the treatment of solid cancers
Solid tumors present additional challenges to CAR-T cell therapies, given that the tumor environment is strongly immunosuppressive, and the vast majority of known TAAs that could be targeted by CAR-T cells are also expressed at low levels on normal tissues (on-target/off-tumor toxicities).

The T cell-mediated destruction of normal tissue is a major limiting factor in the clinical use of CAR-T cell therapies. One case has been reported in which low-level ErbB2 (Her2/neu) expression on lung epithelia may have led to fatal toxicity in a patient infused with CAR-T cells.
STRATEGIES TO CIRCUMVENT CAR-T CELL-ASSOCIATED TOXICITIES IN SOLID TUMORS

DUAL-RECEPTOR STRATEGY

a

T cell

No activation

CD3ε

4-1BB

CD28

CAR

CCR

A+

B-

Normal tissue 1

PSCA: prostate stem cell antigen

b

Normal tissue 2

A-, B+

No activation


c

Tumor

A+, B+

Activation

PSMA: prostate-specific membrane antigen
STRATEGIES TO CIRCUMVENT CAR-T CELL-ASSOCIATED TOXICITIES IN SOLID TUMORS

THE FUTURE OF ACT

Engineered T cells secreting bispecific antibodies

- Prolonged \textit{in situ} secretion of bsAbs would provide time for tumor-infiltrating T lymphocytes to proliferate and attack the cancer cells.

- Recruitment is not restricted to the gene-modified T cells, as in the CAR approach.
THE FUTURE OF ACT ENGINEERED ARTIFICIAL T CELLS SECRETING BSABS

✓ anti-CEA\textsuperscript{x}anti-CD3 bsAbs secreted by gene-modified human cells, has the potential to cure CEA\textsuperscript{+} malignancies

Compte \textit{et al.} Stem Cells (2009)
Compte \textit{et al.} Oncoimmunology (2014)
Mølgaard \textit{et al.} Gene Ther (2017)
ENGINEERED T CELLS SECRETING BISPECIFIC ANTIBODIES

ORIGINAL ARTICLE

Improved anti-leukemia activities of adoptively transferred T cells expressing bispecific T-cell engager in mice

X Liu¹, DM Barrett², S Jiang¹, C Fang¹, M Kalos¹,³,⁴, SA Grupp², CH June¹,³ and Y Zhao¹,³

Despite the impressive clinical efficacy of T cells engineered to express chimeric antigen receptors (CAR-Ts), the current applications of CAR-T cell therapy are limited by major treatment-related toxicity. Thus, safer yet effective alternative approaches must be developed. In this study, we compared CD19 bispecific T-cell engager (BiTE)-transferred T cells that had been transfected by RNA electroporation with CD19 CAR RNA-transferred T cells both in vitro and in an aggressive Nalm6 leukemia mouse model. BiTEs were secreted from the transferred T cells and enabled both the transferred and bystander T cells to specifically recognize CD19⁺ cell lines, with increased tumor killing ability, prolonged functional persistence, increased cytokine production and potent proliferation compared with the CAR-T cells. More interestingly, in comparison with CD3/CD28 bead-stimulated T cells, T cells that were expanded by a rapid T-cell expansion protocol (REP) showed enhanced anti-tumor activities for both CAR and BiTE RNA-electroporated T cells both in vitro and in a Nalm6 mouse model (P < 0.01). Furthermore, the REP T cells with BiTE RNAs showed greater efficacy in the Nalm6 leukemia model compared with REP T cells with CAR RNA (P < 0.05) and resulted in complete leukemia remission.

Blood Cancer Journal (2016) 6, e430; doi:10.1038/bcj.2016.38; published online 3 June 2016
ENGINEERED T CELLS SECRETING BISPECIFIC ANTIBODIES
ENGINEERED T CELLS SECRETING BISPECIFIC ANTIBODIES

✓ anti-CD19xanti-CD3 bsAbs produced and delivered by gene-modified T cells, has the potential to cure CD19+ malignancies with controllable toxicities and without long-term B-cell aplasia
ACKNOWLEDGEMENTS

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