Targeting Tumor Antigens by Redirecting T cells using Bispecific Antibodies

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Clinical Problem

- Patients with metastatic breast cancer, hormone refractory prostate cancer, and other cancers have limited clinical options.

- Chemotherapy, irradiation, or high dose chemotherapy have become dose-limiting.

- New non-toxic strategies are needed to provide an anti-tumor effect without enhancing treatment toxicities.
Perspective

Earlier Talks
- **Humoral Immunity** and Antibodies – Paul Sondel
- **Monoclonal Antibodies** in Cancer Therapy – Ralph Schwall

Later Talks
- **Cytokines** for Cancer Therapy – Jan Dutcher
- **Cellular** Therapies – Robert Dillman
- Critical Factors that Limit Success – Soldano Ferrone
Activated T Cells (ATC)

Signal 1
Binding of OKT3
Activates the T cells

Signal 2
IL-2 or Anti-CD28
Keeps T cells alive

Grow and Divide

Produce Cytokines/Chemokines

Directly Kill Tumor Cells
A Balancing Act for Anti-tumor Effects

TH0

TH1

Anti-tumor effects

IL-1
IL-2
TNFα
IFNγ

TH2

Suppressive effects

IL-4
IL-5
IL-10
IL-13
Definitions

**ATC:** Activated T cells produced by anti-CD3 activation and culture in low dose IL-2 for 6-14 days.

**BiAb:** Consists of two mAbs produced by chemical, genetic, or hybridoma technology with 2 specificities (could be single chain fragment variable regions, scFVs).

**Armed ATC:** ATC with a BiAb that binds to CD3 on T cells and to a TAA on the tumor (artificial TCR).
Combination of Cellular and Humoral Therapeutic Strategy

- The specificity of monoclonal antibodies

\textbf{AND}

- Non MHC restricted cytotoxicity mediated by T cells, NK cells, or other effector cells
# Preclinical Studies Using BiAbs

<table>
<thead>
<tr>
<th>mAb</th>
<th>Target</th>
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</thead>
<tbody>
<tr>
<td>Anti-Tenascin</td>
<td>Glioma</td>
</tr>
<tr>
<td>Anti-Glioma</td>
<td>Human Glioma</td>
</tr>
<tr>
<td>Anti-CD13</td>
<td>AML</td>
</tr>
<tr>
<td>Anti-MUC1</td>
<td>Bile Duct CA</td>
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<tr>
<td>Anti-EpCAM</td>
<td>Epithelial Cell Adhesion on AdenoCA</td>
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<tr>
<td>OC/TR</td>
<td>Folate Receptor on ovarian CA</td>
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<tr>
<td>Anti-kDal K29</td>
<td>Renal cell carcinoma</td>
</tr>
<tr>
<td>Anti-G250</td>
<td>Renal cell carcinoma</td>
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<tr>
<td>OKT9</td>
<td>Anti-transferrin receptor</td>
</tr>
<tr>
<td>Anti-AMOC-31</td>
<td>40 kDa membrane glycoprotein on carcinomas</td>
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<tr>
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<tbody>
<tr>
<td>Anti-idiotype</td>
<td>BCL1 lymphoma</td>
</tr>
<tr>
<td>Anti-CD19</td>
<td>Leukemic B cells</td>
</tr>
<tr>
<td>Anti-tumor (Fab)2</td>
<td>Retargeting TIL</td>
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<tr>
<td>Anti-CEA</td>
<td>Carcinoembryonic antigen</td>
</tr>
<tr>
<td>Anti-Her2</td>
<td>Her2 on RCC, colon, breast</td>
</tr>
<tr>
<td>Anti-CD20</td>
<td>NHL</td>
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<tr>
<td>Anti-PSA</td>
<td>Prostate specific antigen</td>
</tr>
<tr>
<td>Anti-CA19-9</td>
<td>Carcinomas</td>
</tr>
<tr>
<td>Anti-HLA-DR beta chains</td>
<td>B cells</td>
</tr>
<tr>
<td>Anti-EGFR</td>
<td>Glioma, neuroblastoma, colon, pancreatic, lung</td>
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BiAb Trials

- **SHR-1**: Anti-CD3 x **anti-CD19** quadroma for NHL; 10 mcg - 5 mg. No adverse effects except thrombocytopenia.

- **BIS-1**: anti-CD3 x **anti-EGP-2** for epithelial carcinoma-associated transmembrane glycoprotein; MTD of 5 mcg/kg; induced high levels of TNF and IFN; dyspnea, vasoconstriction and fever without anti-tumor effect.

- **2B1**: anti-CD16 x **anti-HER2** quadroma for Her2+ tumors; DLT were fever, chills, N/V, and leucopenia; HAMA in 14 of 15; MTD was 2.5 mg/m².
BiAb Trials

- **HRS-3/A9**: anti-FCRIII x **anti-CD20** for HD of B cell malignancy; MTD not reached at 64 mg/m²/dose

- **MDX-H210**: anti-CD64 x **anti-Her2** for breast, ovarian, prostate CA; doses ranged from 1-40 mg/m² without DLT

- **MDX-447**: anti-CD64 x **anti-EGFR** for renal and head and neck cancer; Hypotension DLT, doses up to 40 mg/m²

- **H22x Ki-4**: anti-CD64 x **anti-CD30** for Hodgkin's Disease, doses up to 20 mg/m²

- **Common thread**: deletion of Fc portions improved toxicity profiles
Trials Using BiAb Armed T cells

- Nitta, 1990, anti-CD3 x anti-glioma armed lymphocytes; 4 of 10 pts had tumor regression.

- Lamers, 1992, ATC armed with anti-CD3 x anti-Mov28 were used to treat ovarian Ca.

- Canevari, 1995, ATC with anti-CD3 x anti-folate receptor given intraperitoneal with IL-2 resulted in tumor regression in advanced ovarian; 7 of 26 (4 CRs, 3 PRs).

- These early studies provided the impetus to develop engineered T-bodies and molecular engineering of BiAbs for targeting TAA.
Begin with the “End” In Mind

STRATEGY: Make T Cells better killers by redirecting or focusing their non-MHC restricted cytotoxicity on TAAs

MEANS: Arm T cells with BiAbs directed at TAAs

GOALS:
1. Improve tumor lysis
2. Immunize the patient by inducing specific CTL and humoral anti-tumor responses.
3. Induce remissions with persistent anti-tumor immunity.
Phase I/II Studies of Ex vivo Expanded T cells

1. Phase I up to 40 billion ATC given on days 1, 4, 7, and 11 for a total of 160 billion ATC after Cytoxan/TBI and PBSCT for hematologic malignancies without adverse effects.

2. Phase I up to 80 billion anti-CD3/anti-CD28 coactivated T cells given in 8 doses to patients with solid tumors with IL-2. Safe with no dose limiting toxicities (J Immunotherapy 5:408, 2001) as well as with low dose Cytoxan.

3. Phase II 210 –310 billion ATC. 10 billion ATC given 3 times/wk for 3 weeks and then 20 billion/week for 6 weeks after PBSCT for stage IV breast cancer. 70% OS and 50% PFS at 32 months without regimen or cell-based adverse effects. (Autologous Blood and Marrow Transplant 10th Proc, pp95, 2001).
Development of Bispecific Antibodies

- Produced by chemical heteroconjugation of existing mAbs, recombinant DNA technology, or a combination thereof.

- A variety of design formats allow for: 1) different sizes to allow tissue penetration; 2) enhanced specificity; 3) increased affinity to effector cells.

- Applications: targeting effector cells, targeting toxins, drugs, prodrugs, enzymes, DNA, anti-vascular agents, gene therapy vectors, radionuclides, and others (use your imagination)
BiAb Production by Chemical Heterconjugation

Anti-CD3

Traut’s reagent

Sulpho - SMCC

Anti-TAA

1

2

3

Anti-CD3 x Anti-TAA
Targeted Killing by T cells with BiAbs

\[
\text{Anti-CD3} + \text{Anti-TAA} = \text{Anti-CD3 x Anti-TAA}
\]

Armed T Cell

T Cell

Tumor Lysis
Production of Armed T cells

PBMC from Pheresis

OKT3 (20 ng/ml) + 100 IU/ml of IL-2

ATC are split every other day

Harvest, Arm with BiAb and Cryopreserve after 10-14d

Quality Control (Bacteria, fungal, and Mycoplasma stain) 7 days

Testing for cytotoxicity and cytokine production
Characteristics of Armed ATC

1. Exhibit non-MHC restricted cytotoxicity ("promiscuous killers").

2. Secrete IL-2, IFNγ, TNFα, GM-CSF, MIP-1, and RANTES after antibody receptor binding.

3. >95% CD3+ cells, 60-80% CD8 cells, 20-40% CD4 cells, and <5% CD56+ cells.

4. Patient CD3 cells expand up to 30 fold in 14 days.
Preclinical Questions

- How long will the BiAb remain on ATC?
- How long will armed ATC kill?
- Will binding to tumor trigger cytokine secretion?
- How many times will armed ATC kill?
- How long can armed ATC be detected in patients?
Targeting and Killing

Unarmed

Armed
Killing of MCF-7 Cells by Armed T Cells

% Specific Killing vs. E:T ratio for different Ab concentrations:
- No Ab
- 0.5 ng Her2Bi
- 5 ng Her2Bi
- 50 ng HerBi
Cytokine Production by Armed T Cells Exposed to SK-BR3

Normal

Patient

No Ab
OKT3
OKT3xRIT
Her2Bi 50 ng
Her2Bi 100 ng

IFN
IFN+T
TNF
TNF+T
GM-CSF
GM-CSF+T

PG/10^6 ATC/24 hrs

pg/10^6 ATC/24 hrs

0
500
1000
1500
2000
2500
3000
3500

0
500
1000
1500
2000
2500
3000
3500

0
500
1000
1500
2000
2500
3000
3500

Cytotoxicity Directed at Prostate Cancer Lines

**PC-3**

- % Specific Cytotoxicity
- E/T: 0 5 10 15 20 25
- ATC
- Her2Bi 50 ng

**DU-145**

- % Specific Cytotoxicity
- E/T: 0 5 10 15 20 25
- ATC
- Her2Bi 50 ng
IFNγ Secretion upon Repeated SK-BR-3 Restimulation

-48 0 48 96 144 192 240 288 336 384

pg IFNγ/10⁶ cells

Hours

Unarmed ATC
Armed ATC
Prevention of Prostate Cancer

COINJECTION OF PC-3 PROSTATE CANCER CELLS + ACTIVATED T CELLS
KAPLAN-MEIER PLOTS

PROPORTION REMAINING ALIVE

DAYS AFTER COINJECTION

0 10 20 30 40 50 60 70 80 90 100 110

PC-3 alone [control]
PC-3 + unarmed ATC
PC-3 + 10^7 armed ATC
PC-3 + 2x10^7 armed ATC

P = 0.00005

7/7 6/12 3/13 0/12
PC-3 tumor cells ($10^7$) were implanted SC in flanks of SCID-Beige mice. 7 days later when tumors were ~0.05 cc, treatments were started once/week x 4 weeks and tumor growth monitored. Results are from 2 experiments (n=10 mice/group).
Trafficking of Her2Bi Armed ATC in Beige/SCID

A RPMI/ IL-2 IV; C) IL-2 + armed ATC (2 x 10^8 cells) IV; D) IL-2 + armed-ATC (4 x 10^7 cells) IV; or E) IL-2 (3000 IU) + armed ATC (2 x 10^8 cells) IT. Tumors were excised 18 hr after treatment, formalin fixed, paraffin embedded, sectioned, and stained for human CD3+ cells.
# Immunotherapy Approaches

<table>
<thead>
<tr>
<th>Cells</th>
<th>CMV CTL to Prevent Infection</th>
<th>EBV for LPD</th>
<th>MM Specific CTL after Chemo</th>
<th>DLI after Allo-BMT</th>
<th>ATC after HDC+PBSCT for BrCa</th>
<th>Armed ATC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
<td>Specific</td>
<td>Specific</td>
<td>Specific</td>
<td>Polyclonal</td>
<td>Polyclonal</td>
<td>Specific</td>
</tr>
<tr>
<td><strong>Dose</strong></td>
<td>$10^6$/kg</td>
<td>$\sim 5 \times 10^9$/kg</td>
<td>$10^9$/kg</td>
<td>$10^8$/kg</td>
<td>$3 \times 10^9$/kg</td>
<td>$4 \times 10^9$/kg (0.5x $10^9$/kg)</td>
</tr>
<tr>
<td><strong>Effect</strong></td>
<td>Prevents CMV Pneumonia</td>
<td>Treatment of LPD</td>
<td>Induce CR in MM</td>
<td>Induce CRs in CML&gt;A ML&gt;ALL</td>
<td>Improve PFS?</td>
<td>Decrease Bone Pain, PSA, CA 27-29</td>
</tr>
<tr>
<td><strong>Target</strong></td>
<td>Viral</td>
<td>EBV LPD</td>
<td>Solid tumor</td>
<td>Liquid tumors</td>
<td>Solid tumor</td>
<td>Solid tumors</td>
</tr>
</tbody>
</table>

- CMV: Cytomegalovirus
- LPD: Lymphoproliferative Disease
- MM: Multiple Myeloma
- DLI: Donor Lymphocyte Infusion
- ATC: Adoptive Transfer Cell Therapy
- BrCa: Breast Cancer
- Allo-BMT: Allogeneic Bone Marrow Transplant
- HDC+PBSCT: High-Dose Chemotherapy and Peripheral Blood Stem Cell Transplant

**Effect:**
- Prevents CMV Pneumonia
- Treatment of LPD
- Induce CR in MM
- Induce CRs in CML>A ML>ALL
- Improve PFS?
- Decrease Bone Pain, PSA, CA 27-29

**Dose:**
- $10^6$/kg
- $\sim 5 \times 10^9$/kg
- $10^9$/kg
- $10^8$/kg
- $3 \times 10^9$/kg
- $4 \times 10^9$/kg (0.5x $10^9$/kg)
Protocols: FDA and IRB Approved

Breast Cancer
- RWH #356-46: TAC + Armed ATC for Stage II-III BrCa
- RWH #351-46: Armed ATC for Stage IV BrCa (NCI-R01 funded)

Hormone Refractory Prostate Cancer
- RWH #355-46: Armed ATC for HRPC

Eligibility:
- Metastatic, measurable, or evaluable sites
- Phase I: Her2/neu positive or negative
- Phase II: Her2/neu positive
- No active cardiac disease, ECOG PS 0-2, life expectancy >3 months

Lymphoma
- RWH #394-46: Armed ATC targeting CD20 lymphomas after PBSCT
  FDA approved 11/02/04 and (Leukemia & Lymphoma Society funded)
Treatment Schema for Breast and Prostate

GM-CSF  250 ug/m²/dose

Wk1 Wk2 Wk3 Wk4 Wk8

ATC Expansion
ATC Infusions

Screening
Leukopheresis

Tumor Evaluation
Immune Evaluation

3 Wks

IL-2  300,000 IU/m²/day
Detection of Armed ATC in Blood

Days after Initiation

% Cells

0 5 10 15 20 25 30

0 10 20 30 40 50 60 70

IgG2a Pre
IgG2a Post
T cells Pre
T cells post

Detection of Armed ATC in Blood
Pre and Post EliSpots

IFN-γ EliSpots with PBMC isolated from a patient before and after the 4th infusion of Her2Bi-armed ATC (5 x 10⁹). Her2/neu-specific IFN-γ secretion by T cells was measured by exposing 10⁵ PBMC to SK-BR-3 cells at an E:T of 10 for 2 h at 37°C and then the PBMC to an EliSpot plate coated with anti-IFN-γ.
EliSpots from Stage IV BrCa Pt

![Graph showing IFNγ EliSpots/10^6 Cell plated over time with unstimulated and stimulated conditions.](image)

- **Pre Treament**
- **Pre Inf 5**
- **1 hr Post Inf 5**

- **Y-axis:** IFNγ EliSpots/10^6 Cell plated
- **X-axis:** Time points (Pre Treatment, Pre Inf 5, 1 hr Post Inf 5)

Legend:
- Red: unstimulated
- Orange: stimulated
Overall Survival in Phase I Trials with Her2Bi-armed ATC

- Stage II/III BrCa \( (n = 6) \)
- Stage IV BrCa \( (n = 8) \)
- HRPC \( (n = 7) \)

Percent Surviving vs. Months
RWH# 04-394-46 Infusion of ATC Armed With CD20Bi for CD20+ NHL

Leukopheresis → ATC Expansion → Cryopreservation → PBSCT

CTC Preparative Regimen

G-CSF Priming 14 days

Wk1 Wk2 Wk3 Wk4 Wk5 Wk6 Wk7 Wk8 Wk9

IL-2 (300,000 IU/m2/day)

Dose Level 1: 5 billion/infusion Total 75 billion
Dose Level 2: 10 billion/infusion Total 150 billion
Dose Level 3: 15 billion/infusion Total 225 billion
Dose Level 4: 20 billion/infusion Total 300 billion
Armed ATC

- Grow and divide after engaging and killing the tumor
- Secrete chemokines and cytokines multiple times
- Bind and kill tumors cells multiple times
- Survive in vivo > 3 weeks
- Develop into Ag-specific CTL over 2 weeks in culture
- Patients infused with armed ATC develop levels of cytokines during their infusion
- PBMC from patients develop cytotoxicity that persists up to a month after the last infusion
1. Armed ATC kill multiple times.

2. Armed ATC proliferate after engaging tumor and do not undergo apoptosis via Fas/FasL or ACID.

3. Large numbers (320+ billion) of armed ATC can be produced in 2 weeks whereas cloned CTL are time consuming requiring a customized effort.
4. Armed ATC may develop into Ag-specific CTL directed at other TAA AND induce endogenous T cells to become cytotoxic.

5. “Multiple infusional vaccinations” may immunize patients to their autologous tumor.

6. Significant amounts of cytokines are found in patient serum during and after infusions with a Th1 profile.

7. There is a strong suggestion that overall survival for metastatic breast and HRPC patients is improved even with small numbers of patients.
OKT3 + Anti-Her2/neu

Breast
Prostate

OKT3 + Anti-CD20

Lymphoma
ALL?
CLL?

OKT3 + Anti-EGFR

Colon
Pancreatic
Lung
Glioblastoma
Neuroblastoma

Eight infusions of armed ATC
PBSCT + Armed ATC
**Concepts/Principles**

- Bispecific antibodies can be used to target effector cells to tumors.

- The non-MHC restricted cytotoxicity can be redirected with BiAbs.

- The targeted cell therapy can be used in combination with cytokine, chemotherapy, or stem cell transplant strategies that make immune space and reduce tumor burdens.

- The platform allows flexibility for targeting different TAAs by switching BiAbs.
Immunotherapy Team (A Rhode Island Consortium)

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Facilities:
1. Specialized ultra-clean rooms that are FDA approved for producing T cells and bispecific antibodies for clinical trials.
2. Seamless clinical coordination between institutions/practices
3. Immunotherapy clinic that is staffed with experienced nurses.