Adoptive T-cell Therapy for Melanoma: Trials and Tribulations in the Quest for FDA Approval

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Disclosures

• Genesis BioPharma - SAB
Presence of tumor-reactive T-cells in metastatic melanomas (TIL)

Gall bladder-associated metastasis

M. Ross, L. Radvanyi
Adoptive T-cell therapy (ACT): Increasing the tumor-specific T-cell army

Directly infuse high numbers of expanded Tumor-infiltrating Lymphocytes (TIL)

IL-2

Blockade

Tumor
TIL versus single target Ag approaches (TCR/CAR transduction)

- Mutanome (mutatopotes)
- Differentiation and self Ags
- Recognized by T cells
Current state-of-the-art “selected” TIL ACT protocol for melanoma

Pre-REP stage (30-100 x 10^6)
- Tumor line / cryo tumor cells
- Selection of tumor-specific (↑ IFN-γ) fragments for REP

REP stage (20-150 x 10^9)
- OKT3 + IL-2 + feeders

HD IL-2
- Lymphodepleted patient (Cy + Flud)

3-5 wks  ↔  2 wks
Timing of preconditioning and TIL+IL-2 therapy: MDACC

Lymphodepletion

cytoxan fludarabine

Day

-7 -5 -1

TIL infusion

High-dose IL-2

High-dose IL-2

0 1 5 21 22 26
Responses to TIL therapy

Patient #2150/2153

before therapy
1 month
18 months

Patient #2054/2256

responses to TIL therapy
Response to TIL therapy

Pre-treatment 1-2 months Post-treatment
Response of brain metastasis to TIL
Waterfall plot of tumor regression in first 31 treated patients: MDACC

48% response rate (>50% decrease in tumor burden)
### Clinical Response data from MDACC (as of July 10, 2012)

**Best overall response:**

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>CR*</th>
<th>PR*</th>
<th>Total</th>
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<tbody>
<tr>
<td>51</td>
<td>2 (4%)</td>
<td>21 (41%)</td>
<td>23 (45%)</td>
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*Some patients are still undergoing clinical response*

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**Poster #1: Bernatchez et al.**

Adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients: Role of specific lymphocyte subsets
Kaplan-Meier curves of overall survival at MDACC (N=31)

Overall survival (from time of TIL infusion)

Landmark analysis (from 3 month post-TIL)

- PD/SD
- CR/PR

$p=0.033$
Growing Network of TIL Therapy Centers

- Durable clinical responses in 40-50% of metastatic melanoma patients (NMA preparative regimen)
- Collectively, over 300 patients have been treated over last 10 years with autologous TIL+IL-2 (NMA)
Roadblocks to TIL Commercialization

- No reliable biomarkers of TIL product potency: Need biomarkers in TIL

- Practical “off-the-shelf” APCs for TIL expansion Needed (feeder problem)

- Open culture systems not amenable to automation

- Selection of tumor-reactive TIL needed for best efficacy:
  - Need tumor targets
  - Unreliable *in vitro* assays

- No predictive biomarkers for patient selection up-front:
  - Tumor microenvironment
  - Systemic/genetic elements

- Labor-intensive
- Long process (5-7 wks)
**Problem**

- No reliable biomarkers of infused TIL potency predictive of response
- No predictive biomarkers for patient selection for therapy:
  - clinical response
  - initial TIL outgrowth
  - expanded TIL phenotypes
- No method to isolate tumor-specific TIL up-front for expansion (save time)
- PBMC feeder problem and availability (costs)
- Open systems → too much manual handling (labor/cost)

**Solution**

- Phenotypic biomarker analysis:
  - T-cell EM subsets (CD8)
  - Novel markers
  - Function (Ag-specific / polyclonal)
- Predictive tests before tumor resection for TIL outgrowth:
  - IHC biomarkers in tumors
  - gene expression in tumors
  - systemic/genetic markers (blood)
- Selection from fresh TIL isolates using activation markers?
- Off-the-shelf APCs with defined stimulatory molecules
- Automated closed systems for selection and expansion
Total TIL infused and CD8+ T cells are critical parameters

- Total TILs infused (x 10^9)
  - CR/PR
  - PD/SD

  - p = 0.0002

- % CD8+ T-cell in TIL
  - CR/PR
  - PD/SD

  - p = 0.0009
Total TIL and % CD8 TIL Infused and Clinical Response: Sheba and MDACC Data

Sheba (N= 49)

Sheba + MDACC combined (N= 78)

MDACC (N= 29)
Analysis of CD8+ T-cell differentiation status in infused TIL

- **T\text{CM/MSC}**
  - CD45RA-
  - CD62L+
  - CD27+
  - CD28+

- **T\text{EM}**
  - CD45RA-
  - CD62L-
  - CD27+
  - CD28+
  - GB+
  - Perf-

- **T\text{EFF}**
  - CD45RA-
  - CD62L-
  - CD27-
  - CD28+
  - GB++
  - Perf+

- **T\text{TDE}**
  - CD45RA-/+ 
  - CD62L-
  - CD27-
  - CD28-
  - CD57+
  - GB++
  - Perf++

PD-1
BTLA
TIM-3
PD-1 and BTLA expression on CD8+ TIL

Post-REP TIL (treated)

Analysis of all patients (N=31)

P < 0.05
BTLA+ TIL correlated with positive clinical response and not PD-1+ or TIM3+
BTLA: “B- and T- Lymphocyte Attenuator”

- Ig family member (monomer like PD-1).
- Negative costimulatory molecule binding HVEM.
- May be a novel CD8+ T-cell differentiation marker.

Poster #8: Haymaker et al.
BTLA: New marker for a highly proliferative CD8+ TIL subset associated with melanoma regression during adoptive cell therapy
Persistence of CD8+BTLA+ TIL clones

Infused TIL Vβ genes
BTLA+

BTLA-

Day 0

Persisting Vβ genes in blood
BTLA+

BTLA-

Day 56
Can biomarkers or signatures be identified as predictors of TIL efficacy?

- **Tumor**
  - IHC
  - Gene expression
  - RPPA
    - T cell subsets
    - DCs
    - iNOS
    - NT
    - MDSCs
    - Chemokine signatures

- **TIL**
  - Phenotype (subsets)
  - Function
  - Senescence issues
  - Gene expression
  - Ag specificity
    - CD8 subsets
    - CTL activity
    - Gene expression
    - Telomere length
    - Methylome
    - Mutanome and neo-epitopes?

- **Inborn host factors**
  - "Immune phenotype" of patient
  - Tumor progression factors
    - SNPs (GWAS)
    - Inflammatory cytokines (serum)
    - Tumor markers (serum)
Predictive biomarkers in TIL therapy

- Surgery
  - Initial TIL expansion (Pre-REP phase)
    - 4-5 weeks
      - PBMC, Serum/plasma, FFPE/frozen tissue

- TIL rapid expansion (REP phase)
  - 2 weeks
    - Sample of pre-REP TIL, Sample of post-REP TIL

- TIL infusion + HD IL-2
  - 10-12 weeks
    - PBMC, Serum/plasma
    - PBMC and serum (day 14, 21, 40-42, 70 post TIL infusion)

IHC predictive markers?
Predictive biomarkers by IHC in original tumors used to grow TIL

1. **T cells:**
   - CD3, CD8, CD4 (peri-tumoral / intra-tumoral)
   - Foxp3
   - PD-1 / BTLA
   - TNF-R family (4-1BB / OX40)

2. **Negative and positive markers of inflammation:**
   - protein nitrotyrosination (NT) → peroxynitrite
   - iNOS
   - CXCL10/IP-10

3. **Macrophages/myeloid cells/DCs:**
   - CD163
   - S100A9
   - CD66b
   - DC-LAMP

4. **Other immunosuppressive factors:**
   - pSTAT3
   - IDO
CD8 in original tumor used to grow TIL (peritumoral near invasive margin versus intratumoral)
Association between CD8 by IHC and CD8 % in expanded TIL (N= 42 pts)

Total CD8

\[ P = 0.049, r^2 = 0.305 \]

Peritumoral / invasive margin

\[ P = 0.046, r^2 = 0.310 \]
Association between CD8 expression by IHC in tumor and TIL clinical response

Peritumoral / invasive margin

\[ P = 0.08 \quad P = 0.0072 \quad P = 0.0273 \]

Intratumoral

\[ P = 0.75 \quad P = 0.17 \quad P = 0.01 \]

Total CD8

\[ P = 0.23 \quad P = 0.0064 \quad P = 0.0073 \]
Association between higher CD8 % at invasive margin tumor and survival

Log Rank $P=0.04$
Relevance of TIL at the invasive margin (peri-tumoral)

Tumor

*in vivo*
Relevance of TIL at the invasive margin (peri-tumoral)

Tumor fragment
ex vivo

IL-2
Can we select up-front tumor-specific TIL for expansion?

Pre-REP stage

Selection of tumor-specific (↑ IFN-γ) fragments for REP

REP stage

OKT3 + IL-2 + feeders

HD IL-2

Lymphodepleted patient (Cy + Flud)
Can we select up-front tumor-specific TIL for expansion?

Pre-REP stage

REP stage

Selection

OKT3 + IL-2 + feeders

HD IL-2

Lymphodepleted patient (Cy + Flud)

3-5 wks

2 wks
TCR activation induced costimulatory molecules (Ig and TNF-R families)

- Ig superfamily: ICOS, CTLA4, PD-1
- TNF-R family: 4-1BB, OX40

4-1BB and PD-1 induction

- No tumor cells
- Plus tumor cells (24 h)
Presence of recently activated 4-1BB+ and OX40+ T cells in tumor isolates

4-1BB staining by IHC
Selection of 4-1BB+ CD8+ T cells highly enriches tumor Ag-specific cells.

- TIL isolation from tumors
- Anti-41BB Ab bead selection
- REP
- ELISPOT

Handling before REP:
- 41BB selected
- 1146
- Unselected
- 72
- 13
- Control
- Ag-specific
- 47
Addition of agonistic anti-4-1BB Abs during TIL production

- IL-2
- HD IL-2
- Anti-4-1BB
- Lymphodepleted recipient (NMA)
- 30-100 x 10^6
- 1000-2000 X
- 20-150 billion
Anti-4-1BB Ab increases CD8+ T-cell yield and preserves CD28 expression

TIL 2014

TIL 2354

Pre-REP

IL-2+4-1BB

IL-2
Anti-41BB during TIL expansion increases GB and Perforin expression

Perforin changes

Pre-REP       REP       REP+ α41BB

P<0.05

CD8+ Perf+ (%)

TIL 2292

Post-REP

Post-REP+4-1BB

Caspase 3-cleavage (%)

Effector:Target

T 3:1  1:1  1:3

T 2473+OKT3  2473 4-1BB REP+OKT3  2478+OKT3  2481+OKT3

IFN-γ (pg/ml)

P<0.05

0  1000  2000  3000  4000  5000
“Off-the-shelf” APCs for TIL REP: Engineered K562 cells

Generation #1 aAPC (master cell bank made)
**Problem**

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What the future TIL expansion protocol will look like (21-day process)?

- Tumor
- Cell suspension
- Clinical-grade cell sorting
- Closed dual bioreactor
- Pre-REP (7 days)
  - IL-2/15/21
- REP (14 days)
  - TIL+aAPC+ IL-2/15/21

Days:
- 1 day
- 21 days

Cells:
- 4-1BB+
- ICOS+
- PD-1+
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