Adoptive T-Cell Immunotherapy

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Adoptive T-Cell Therapy: Concept and Principles

• Identifying and transferring tumor-reactive T-cells activated and expanded in vitro is a direct way to achieve the same goal as vaccines, cytokines, etc.

• This permits the use of reagents and methods perhaps not tolerated in vivo.

• This also allows independent manipulation of the recipient to optimize conditions for the infused T-cells.
T-Cell Adoptive Therapy: Concept and Principles

- Components of adoptive cellular immunotherapy:
  - T-cell repertoire recognizing tumor associated antigens
  - Host immunosuppression
  - Cytokine support
  - Antagonists of immune inhibition in the tumor microenvironment
T-Cell Adoptive Therapy: Concept and Principles

- Components of adoptive cellular immunotherapy:
  - T-cell repertoire recognizing tumor associated antigens
  - Host immunosuppression
  - Cytokine support
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Melanoma Tumor Infiltrating Lymphocytes (TIL)

- Human melanomas frequently contain resident T-cells that can recognize the autologous tumor

- These can be expanded in vitro using IL-2 and anti-CD3

- This consistent source of tumor-reactive T-cells allowed the development of the field of adoptive cell transfer
Melanoma TIL
(Tumor Infiltrating Lymphocytes)

Fresh digest          One week            Two weeks
Cyclophosphamide + Fludarabine
Non-Myeloablative Chemotherapy
TIL for Metastatic Melanoma

- Between 2000 and 2007, 93 patients with measurable metastatic melanoma were treated with a preparative lymphodepleting regimen followed by TIL and IL-2
- 86% had visceral metastases
- 83% had prior IL-2
- Only 2 patients were treated twice and there was one patient death during treatment due to sepsis
Survival of Patients with Metastatic Melanoma Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2

Overall RR = 56%
PR = 34%
CR = 22% (19 of 20 sustained >5yr)

(median potential follow-up 89 months)
Survival of Patients with Metastatic Melanoma Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2

Nearly all completely responding patients appear cured.
Impact of prior therapy on the response to ACT using selected TIL
New TIL Protocol

• Beginning March 2011, 101 patients were randomized to receive TIL and IL-2 after either Cy-Flu or Cy-Flu-TBI

• No treatment related mortality

• Long-term results pending and responses still evolving

• Interim overall RR for all pts is 54% with 17% CR
Tumor Associated Antigens

• Using tumor-reactive T-cells and expression cloning, over 100 tumor-associated antigens recognized by T-cells have been identified by multiple investigators.
Tumor-Associated Antigens Recognized by T-Cells

- **Melanocyte/melanoma differentiation antigens** (MDA; MART1, gp100, tyrosinase, TRP 1/2)
- **Tumor testis antigens** (NY-ESO1, MAGE, PRAME, SSX families)
- **Overexpressed normal proteins** (TERT, mesothelin)
- **Viral oncoproteins** (E6/E7, Merkel's)
- **Proteins containing tumor specific mutations** (B-catenin, PPP1R3B)
Responses to TCR-Engineered PBL Targeting MDA

• Two protocols targeting MART-1 and gp100 with TCR-transduced PBL showed response rates of 30% and 19% respectively.

• 81% of patients had rashes with melanocyte destruction, 42% developed uveitis and 42% experienced impaired hearing, indicative of MDA-mediated autoimmunity.
Targeting Melanocytic Proteins:
Anti-MART1 TCR-Engineered PBL
PBL with TCR Targeting CEA

Effective attack on normal self-antigens may cause unacceptable autoimmunity
Can Other Defined Melanoma Antigens Mediate Complete Regressions?

- 22 patients with metastatic melanoma were given PBL transduced with a TCR recognizing NY-ESO-1 (with Cy-Flu conditioning and IL2)

- The overall RR was 50% with:
  - 4 CRs: durations 51+, 41+, 26+, 25mo
  - 7 PRs: durations 22+, 10, 8, 5+, 5, 4, 3mo

- No autoimmune toxicities were seen
Gene Therapy with Anti-NY ESO1 TCR (Melanoma)
Targeting CD19

• Autoimmune B-cell destruction may be an acceptable toxicity when treating B-cell malignancies

• Anti-CD19 chimeric antigen receptor (CAR) used with preparative Cy-Flu but no IL-2

• Eight evaluable pts with heavily pre-treated DLBCL or PMBCL

• 4 CR and 2 PR

Kochenderfer, JCO 2014
Primary Mediastinal B-Cell Lymphoma

Pre-Treatment

22 Months
Future Targets for Receptor Gene Therapy

- **Chimeric Antigen Receptors**
  - Mesothelin
  - EGFR vIII
  - VEGFR2
  - GD2
  - Other CD Ags for hematological CA

- **T-Cell Receptors**
  - WT-1
  - MAGE family
  - Thyroglobulin
  - ???
Clinical Observations on Melanoma Patients Responding to TIL

- The vast majority of melanoma TIL contain anti-MDA reactivity

- Of the 93 patients given TIL and followed long term, there were 52 objective responders and 20 of these responses were ongoing at 5 years

- Only one of these patients developed uveitis and auditory symptoms indicative of MDA-mediated autoimmunity
What are the Cogent Tumor-Associated Antigens Recognized by TIL?

- Melanocyte/melanoma differentiation antigens (MDA; MART1, gp100, tyrosinase, TRP 1/2)
- Tumor testis antigens (NY-ESO1, MAGE, PRAME, SSX families)
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What are the Cogent Tumor-Associated Antigens Recognized by TIL?

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- Overexpressed normal proteins (TERT)
- Proteins containing tumor specific mutations (B-catenin, PPP1R3B)
Somatic mutation frequencies observed in exomes from 3,083 tumour-normal pairs.

Strategy:

Grow reactive TIL, identify MHC restriction

Whole exome sequence autochthonous tumor

Identify all potential 9-mers and 10-mers that could contain each mutated AA-residue

Rank for predicted peptide-MHC binding by algorithm

Synthesize top binding candidate peptides

Screen for recognition by TIL

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Limitations of Peptide Prediction Algorithms

- Need to know presenting MHC allele
- Many candidate peptides are not processed by the proteasome
- Labor intensive to synthesize and test all possible candidate peptides
- (Insertion and deletions not considered)
Tandem minigene (TMG):
String of minigenes encoding the mutated AA flanked by 12 AA

All potential mutated epitopes (up to 13-mers) and all MHC alleles are tested for processing, presentation and recognition
43 yo F with cholangiocarcinoma refractory to chemotherapy and metastatic to lungs and liver
Treated with bulk TIL empirically with minimal response
Tumor WES showed 26 non-synonymous mutations
Screened TIL against tandem minigenes and found a mutation (mut-ERBB2IP) reactive T-cell culture
Retrospectively, first TIL culture contained 10 billion of these T-cells (+ 30 billion unreactive T-cells)
New culture with 120 billion of these CD4 cells (95% pure) grown and given
Tandem minigene (TMG): String of minigenes encoding the mutated AA flanked by 12 AA

- Clone TMG into plasmid
- Transfect into APC
- Co-culture with T cells

75 nucl Minigene

• Tandem minigene (variable # of minigenes)

• Three Tandem Mini-Genes (TMGs) generated for Pt. MB

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• Only TMG-1 induces IFN-g secretion and upregulation of the CD4+ T-cell activation marker OX40

• Co-culture TIL + TMG-APC: IFN-g ELISPOT assay

• Flow cytometry

• OX40
• CD4
- Only the mutated minigene ERBB2IP is recognized by Pt. MB infusion TIL

IFN-γ ELISPOT assay: Minigenes in TMG individually ‘back-mutated’ to w.t.
Treatment #1: Liver

Pre-Treatment

7 Months
Treatment #2: Liver

Pre-Treatment

8 months
Treatment #1: Lungs
Treatment #2: Lungs
TIL From Colon Cancer

- Metastatic colon cancer (liver, lung, spleen, peritoneum)
- Prior Tx: FOLFOX + bevacizumab, FOLFIRI, FOLFOX + panitumumab, RFA, regorafenib
- Whole genome sequencing of liver lesion: 119 non-synonymous mutations

1. 24 fragment cultures of TIL grown
2. Nine TMGs synthesized encoding mutations

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<tr>
<th>TMG</th>
<th># minigenes</th>
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Multiple TIL Cultures Display Reactivity Against TMG-1

Co-culture of TIL fragments with RNA transfected DCs

IFN-γ ELISPOT

P1W1
P1W2
P1W3
P1W4
P1W5
P1W6
P3W1
P3W2
P3W3
P3W4
P3W5
P3W6
P2W1
P2W2
P2W3
P2W4
P2W5
P2W6
P4W1
P4W2
P4W3
P4W4
P4W5
P4W6

Eric Tran
• Mutation-reactive T cells can be detected in patients with metastatic gastrointestinal cancers

<table>
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<tr>
<th>Patient</th>
<th>Cancer</th>
<th># of mutations assessed</th>
<th>Mutation Reactive T cells detected?</th>
<th>Mutated gene recognized</th>
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Number of mutated T cell antigens identified in patients with Melanoma by whole exome sequencing

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Lung Cancer TIL

- TIL grown from intramuscular metastasis
  - 24 fragment-derived cultures

- WES showed approximately 270 non-synonymous mutations

- 18 TMGs synthesized and expressed in autologous DCs

- TIL cultures vs TMG assay performed
IFN-g Secretion

IFN-g (pg/ml)

TIL Fragment Cultures

TMG6
TMG8
TMG9
TMG10
TMG11
GFP

TIL F11c  TIL F2  TIL F5  TIL F6  TIL F11  TIL F20
Isolation of Reactive T-Cells by Sorting for Activation Markers

- 4-1BB (CD137) is a short-lived marker for recent T-cell activation

- Separation of fresh TIL by 4-1BB sorting can enrich for tumor reactivity
  
  (Gros, J Clin Invest 2014)

- FACS for clinical use have become available
4-1BB+ TIL3992 appear to be enriched in tumor-reactive cells

Coculture (TIL3992)
1e4 effectors:1e5 Target cells

Target = Established Tumor Line 3992

4-1BB+ TIL3992
(91%CD3+CD8+)

4-1BB- TIL3992
(19%CD3+CD8+)

Bulk TIL3992
(54%CD3+CD8+)

A. Gros
Ongoing clinical study evaluating the efficacy of 4-1BB+ selected TIL in melanoma patients

Total FrTu cells sorted: ~7e6 cells
4-1BB+ cell yield: 2e5 cells
Viability Post-sort: 95.3%
Rapid expansion protocol (15 days) → 13e9 infused cells
Schematic for Rapid Generation of Mutation-Specific TIL by Cell Sorting

1. **Establish initial TIL culture from fragments (2-4 weeks)**
2. **Sort 4-1BB+ T-cells**
3. **ELISA vs TMG to detect reactive wells**
4. **Rapid ExPansion with anti-CD3 and feeder cells**
   - **Co-culture with TMG-DC**
   - **2 Weeks**
   - **Cell Infusion**

**Input**
- Tumor
- (WES & TMGs)

**Outputs**
- Bulk TIL Culture
- Sorted T-cells
- Reactive wells
- Expanded T-cells
- Infused cells

**Steps**
- Establish initial TIL culture
- Sort T-cells
- ELISA vs TMG detection
- Rapid expansion
- Co-culture with feeder cells
- Cell infusion
Conclusions

• T-cell transfers can cure some patients of widespread metastatic melanoma

• Peptide and minigene approaches have both shown that many of these tumor-reactive TIL are recognizing tumor-specific mutations

• This finding may allow not only improvements in melanoma T-cell therapy, but may lead to adoptive cell therapy for nearly any cancer

• New TIL protocols, selecting for mutation reactivity, are open for lung, ovarian, breast, bladder and GI cancers
Acknowledgements:

Steven A. Rosenberg, Chief, NCI Surgery Branch

- Rob Somerville
  - TIL Lab
- Paul Robbins
- Udai Kammula
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- Stephanie Goff
- John Wunderlich
- Steve Feldman

- Ken-ichi Hanada
- Qiong Wang
- Yong-Chen Lu
- Eric Tran
- Alena Gros
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