Adoptive cell therapy in the era of immune checkpoint blockade

John Haanen, MD PhD

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Disclosures

Advisory role: BMS, Pfizer, Roche, Novartis, Ipsen, MSD, Neon Therapeutics

Research grant: GSK, BMS, MSD
Aim of this presentation

- Basic aspects of ACT
- How does it work and how well does it work?
- Is there still a place for ACT in the era of checkpoint inhibitors?
What is ACT?

- Infusion of an immune cell product with the aim to induce or augment an anti-tumor immune response
Which cells are transferred?

• Mostly CD3+ T cells
  – Tumor-infiltrating lymphocytes
  – Antigen-receptor gene modified T cells (blood derived)
    • TCR gene modified T cells
    • Chimeric antigen receptor (CAR) gene modified T cells
  – T cell clones/lines (oligoclonal population) from blood

• Other cell types: NK cells, DC
How does ACT work?

How effective is ACT?
How effective is ACT?

- Infusion of peripheral blood derived T cells
- Infusion of TCR gene modified T cells
- Infusion of TIL
Isolation of melanoma-specific CD8 T cells from peripheral blood

Labarriere et al. Clin Dev Immunol 2013
Infusion of MART-1 specific T cells

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>KPS (%)</th>
<th>Prior Therapy</th>
<th>Disease Sites</th>
<th>Melan-A Expression*</th>
<th>No. of T-Cell Infusions</th>
<th>Adverse Effects</th>
<th>Eosinophilia (%)†</th>
<th>Clinical Course</th>
<th>Duration of Clinical Course (months)</th>
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Abbreviations: KPS, Karnofsky performance status; F, female; Chemo, chemotherapy; Immuno, immunotherapy; Sk, skin; Lu, lung; Fever I°, WHO grade I, < 38°C; PD, progressive disease; M, male; Li, liver; B, bone; LN, lymph node; Fever II°, WHO grade II, 38-40°C; PR, partial regression; SD, stable disease; MR, mixed response; IFN, interferon; CR, complete regression.

*Staining of tumor specimens was performed with an anti-Melan-A (A103; Novocastra, Newcastle, United Kingdom) monoclonal antibody; 2+, 50-75% of cells reactive; 3+ > 75% of cells reactive.

†Maximum peak eosinophil levels after T-cell transfer; eosinophils % of total leukocytes.
Infusion of MART-1 and gp100-specific T cell clones

Table 1. Patient demographics and clinical summary

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<tr>
<th>ID no.</th>
<th>Age</th>
<th>Sex</th>
<th>Previous Tx*</th>
<th>Disease sites†</th>
<th>Target antigen</th>
<th>No. of infusions</th>
<th>Toxicity‡</th>
<th>Type</th>
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Yee et al. PNAS 2002

Transferred melanoma-specific CD8+ T cells persist, mediate tumor regression, and acquire central memory phenotype


*Program in Immunology, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; 1General Oncology and Hematology, Seattle Cancer Care Alliance and University of Washington, Seattle, WA 98109; and Department of Laboratory Medicine, University of Washington, Seattle, WA 98195

Edited by Tak W. Mak, The Campbell Family Institute for Breast Cancer Research, Ontario Cancer Institute at Princess Margaret Hospital, University Health Network, Toronto, ON, Canada, and approved February 3, 2012 (received for review August 30, 2011)
Successful treatment of metastatic melanoma by adoptive transfer of blood-derived polyclonal tumor-specific CD4+ and CD8+ T cells in combination with low-dose interferon-alpha

Els M. E. Verdegaal · Marten Visser · Tamara H. Ramwadhdoebé · Caroline E. van der Minne · Jeanne A. Q. M. J. van Steijn · Ellen Kapiteijn · John B. A. G. Haanen · Sjoerd H. van der Burg · Johan W. R. Nortier · Susanne Osanto

The NEW ENGLAND JOURNAL of MEDICINE

Brief Report

Treatment of Metastatic Melanoma with Autologous CD4+ T Cells against NY-ESO-1

Naomi N. Hunder, M.D., Herschel Wallen, M.D., Jianhong Cao, Ph.D., Deborah W. Hendricks, B.Sc., John Z. Reilly, B.Sc., Rebecca Rodmyre, B.Sc., Achim Jungbluth, M.D., Sacha Gnatic, Ph.D., John A. Thompson, M.D., and Cassian Yee, M.D.
Conclusion

- Infusion of peripheral blood derived melanoma-specific T cells is feasible
- Time consuming (4-16 weeks)
- Few but sometimes lasting responses are seen
- How to improve?
  - Are we targeting the right antigens? (shared vs neo-ag)
  - Are we infusing the right T cells?
  - Combination therapy? (add ICI)
Infusion of gene-modified T cells

Kershaw et al. Nat Rev Cancer 2013
Genetically modified peripheral blood lymphocytes

CAR T cell concept

Further development of CARs

Success of CD19 CAR T cell therapy

<table>
<thead>
<tr>
<th>Study</th>
<th>CAR design</th>
<th>Trial design</th>
<th>Malignancy</th>
<th>Outcome*</th>
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<td>Brenthens et al. 2011</td>
<td>Murine CD19 scFv–CD28/CD3ζ</td>
<td>Pilot (nine patients)</td>
<td>CLL (n =8), acute lymphoblastic leukemia (n = 1)</td>
<td>One PR</td>
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<td>Kalos et al. 2011</td>
<td>Murine CD19 scFv–4-1BB/CD3ζ</td>
<td>Pilot (three patients)</td>
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<td>Kochenderfer et al. 2012</td>
<td>Murine CD19scFv–CD28/CD3ζ</td>
<td>Pilot (eight patients)</td>
<td>Non-Hodgkin lymphoma (n = 4) CLL (n = 4)</td>
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<td>Maude et al. 2014</td>
<td>Murine CD19 scFv–4-1BB/CD3ζ</td>
<td>Phase I/II (30 patients)</td>
<td>Acute lymphoblastic leukemia</td>
<td>CR 90%</td>
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<td>Lee et al. 2015</td>
<td>Murine CD19scFv–CD28/CD3ζ</td>
<td>Phase I (21 patients)</td>
<td>Acute lymphoblastic leukemia</td>
<td>CR 70%</td>
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<td>Kochenderfer et al. 2015</td>
<td>Murine CD19scFv–CD28/CD3ζ</td>
<td>Phase I (15 patients)</td>
<td>Non-Hodgkin lymphoma (n = 11) CLL (n = 4)</td>
<td>CR 53% PR 26%</td>
<td>[14]</td>
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</table>
Schedule of treatment of TCR gene therapy

Patient: 

- Informed consent + screening
- Leukapheresis
- Preparation of gene modified T cells
- Start chemotherapy: Cyclophosphamide + fludarabin for total of 7 days
- Infusion of transduced T cells
- High dose IL-2
- Monitoring response and survival

mnd 1, 2, 3, 6
Clinical experience with TCR gene therapy

• 2006: MART-1 TCR gene therapy
  – RR 13% (n=15)
    (Morgan et al., Science 2006)

• 2009: MART-1 and gp100 TCR gene therapy
  – RR 30% (MART-1 TCR; n=20)
  – RR 19% (murine gp100 TCR; n=16)
    (Johnson et al., Blood 2009)
DMF5 and gp100 specific TCR were highly expressed by transduced CD4 and CD8 T cells

Johnson et al., Blood 2009
Clinical activity of MART-1 and gp100-specific TCR gene therapy
Clinical experience with TCR gene therapy

• 2006-2016: MART-1 and gp100 TCR gene therapy

• 2011: NY-eso-1 TCR gene therapy in melanoma and synovial sarcoma
  – RR 45% (n=11) and 67% (n=6)
  (Robbins et al., J Clin Oncol 2011)
Patient characteristics and outcome

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Sites of Disease</th>
<th>Prior Treatment</th>
<th>No. of Cells (x10^6)</th>
<th>No. of IL-2 Doses</th>
<th>% of CD3</th>
<th>% of CD4</th>
<th>NY-ESO-1 Tetramer Positive</th>
<th>% of CD8</th>
<th>% of CD4</th>
<th>Vp13.1 Positive (%)</th>
<th>NY-ESO-1 Positive</th>
<th>NY-ESO-1 Negative</th>
<th>Response†</th>
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Robbins et al., J Clin Oncol 2011
### Patient characteristics and outcome

**Table 1. Characteristics of Patients and Administered T Cells**

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<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Sites of Disease</th>
<th>Prior Treatment</th>
<th>No. of Cells ($\times 10^3$)</th>
<th>No. of IL-2 Doses</th>
<th>% of CD3 Positive</th>
<th>% of CD8 Positive</th>
<th>% of CD4 Positive</th>
<th>NY-ESO-1 Tetramer Vp13.1 Positive (% of CD3)</th>
<th>NY-ESO-1 Positive</th>
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<td>&lt; 30 PD</td>
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</table>

Robbins et al., J Clin Oncol 2011
Cancer immunity cycle

1. Release of cancer cell antigens (cancer cell death)
2. Cancer antigen presentation (dendritic cells/ APCs)
3. Priming and activation (APCs & T cells)
4. Trafficking of T cells to tumors (CTLs)
5. Infiltration of T cells into tumors (CTLs, endothelial cells)
6. Recognition of cancer cells by T cells (CTLs, cancer cells)
7. Killing of cancer cells (Immune and cancer cells)

CTLA4
PD1/PDL1
IL-2

Adoptive T cell transfer

Chen & Mellman Immunity 2013
Role for T cells in cancer

Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer

Lin Zhang, M.D., Jose R. Conejo-Garcia, M.D., Ph.D., Dionyssios Katsanos, M.D., Ph.D., Phyllis A. Gimotty, Ph.D., Marco Massobrio, M.D., Giorgia Regnani, M.D., Antonis Makrigiannakis, M.D., Ph.D., Heidi Gray, M.D., Katia Schlienger, M.D., Ph.D., Michael N. Liebman, Ph.D., Stephen C. Rubin, M.D., and George Coukos, M.D., Ph.D.

The immune contexture in human tumours: impact on clinical outcome

Wolf Herman Fridman, Franck Pagès, Catherine Sautès-Fridman and Jérôme Galon

Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon, et al.
Science 313, 1960 (2006);
DOI: 10.1126/science.1129139

Cancer Research

Immunotype and Immunohistologic Characteristics of Tumor-Infiltrating Immune Cells Are Associated with Clinical Outcome in Metastatic Melanoma

Tumor infiltrating lymphocytes: TIL therapy in melanoma

Response rate 40-70%

From: Restifo et al., Nat Rev Immunol 2012
Overall survival of metastatic melanoma patients treated with TIL (ITT analysis)

1-year OS: 46%
2-year OS: 30%

Besser et al., Clin Canc Res 2013
Clinical data N10TIL003: ongoing complete response 4 years

Biopsy at wk 7 showed no viable tumor cells
Immunotherapy of melanoma: TIL therapy

TIL are grown from melanoma tumors

Rapid Expansion

A few million T cells

1x10^{11} T cells
The big unknown

- Which cytotoxic T cells mediate cancer regression?
- Could we specifically boost their numbers?

Tumor-infiltrating lymphocytes (TIL) are grown from melanoma tumors

Rapid Expansion

Infusion of TIL + IL-2

Patient pretreated with lymphodepleting chemotherapy
What could tumor-specific cytotoxic T cells detect on human cancer?

1. Self antigens (to which tolerance is incomplete) *Shared between patients*

2. ‘Neo-antigens’, epitopes that arise as a consequence of tumor-specific mutations *In large part patient-specific, hence generally ignored*
Melanoma associated epitope panel

HLA-A2 restricted peptide panel includes 145 epitopes

- Cancer/Testis antigens: e.g. MAGE family 27%
- Melanoma differentiation antigens: e.g. MART-1 18%
- Overexpressed antigens: e.g. Survivin 45%
- Mutated antigens: e.g. CDK4 4%
- Unclassified antigens: e.g. MG50 6%

Kvistborg et al., Oncoimmunology 2012
Visualizing the composition of TIL

T cell responses are very low magnitude

<table>
<thead>
<tr>
<th>Patient</th>
<th>Young CD8 enriched TIL (NIH)</th>
<th>Young TIL (Ella)</th>
<th>Selected TIL</th>
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</table>
TILs against shared tumor antigens

• In the majority of TILs T cells specific for shared antigens can be found
  – Melanocyte differentiation Ags (Mart-1, gp100, etc)
  – Cancer/Testis gene products (NY-eso-1, MAGE, SSX-2, etc)
  – Overexpressed Ags (Meloe etc.)
• Low frequency (mostly below 1%)
• No correlation with response

Kvistborg et al., Oncoimmunology 2012
TIL therapy broadens the tumor-reactive CD8\(^+\) T cell compartment in melanoma patients

Pia Kvistborg,\(^{1,+}\) Chengyi Jenny Shu,\(^{1,+}\) Bianca Heemskerk,\(^1\) Manuel Fankhauser,\(^1\) Charlotte Albaek Thrue,\(^2\) Mireille Toebes,\(^1\) Nienke van Rooij,\(^1\) Carsten Linnemann,\(^1\) Marit M. van Buuren,\(^1\) Jos H.M. Urbanus,\(^1\) Joost B. Beltman,\(^3\) Per thor Stratmans,\(^2\) Yong F. Li,\(^4\) Paul F. Robbins,\(^4\) Michal J. Besser,\(^5,6\) Jacob Schachter,\(^5\) Gemma G. Kenter,\(^7\) Mark E. Dudley,\(^4\) Steven A. Rosenberg,\(^4\) John B.A.G. Haanen,\(^1\) Sine Reker Hadrup\(^2\) and Ton N.M. Schumacher\(^1,+\)
What could tumor-specific cytotoxic T cells detect on human cancer?

1. Self antigens (to which tolerance is incomplete)
   *Shared between patients*

2. ‘Neo-antigens’, epitopes that arise as a consequence of tumor-specific mutations
   *In large part patient-specific, hence generally ignored*
Pt 004:

Resected tumor material

Isolate tumor cells

Isolate tumor-infiltrating T cells

Screen with MHC multimer technology

Identify tumor-specific mutations

Predict potential epitopes
Pt 004:

DNAH17_{H\rightarrow Y} (0.003%)  
VLFEDAVA_{H} > VLFEDAVAY

CDK4_{R\rightarrow L} (1.604%)  
ARDPHSGHFV > ALDPHSGHFV

GCN1L1_{L\rightarrow P} (0.407%)  
ALLETLSLL > ALLETPSLLL
Are neo-antigens superior cancer rejection antigens?
Are neo-antigens superior cancer rejection antigens?

**Pre-enrichment**

- **CDK4_{R>L}**
  - 2.2%

- **GCN1L1_{L>P}**
  - 0.59%

**Post-joint enrichment**

- **CDK4_{R>L}**
  - 72.2%

- **GCN1L1_{L>P}**
  - 20.2%

**Combined**

- 2.8%
- 92.4%
1) Inject human melanoma (NSG-mice)

2a) Inject autologous bulk T-cell product

2b) Inject autologous neo-Ag enriched T-cell product

3) Monitor tumor growth
Neo-antigen enriched TIL can mediate superior tumor control
1) Create human melanoma PDX model (NSG-mice)
1) Create human melanoma PDX model (NSG-mice)

2a) treat with T cells transduced with autologous C/T Ag specific TCRs

2b) treat with T cells transduced with autologous Neo Ag specific TCRs

Assess whether neo-antigen specific TCRs outperform C/G specific TCRs
Do neo-antigen specific TCRs* outperform C/G antigen specific TCRs**?

caution: n=1 expt, repeat ongoing

* 2 TCRs, against CDK4 and GCN1L1 neo-antigens
** 4 TCRs, against 3 MAGE-C2 epitopes, 1 MAGE-A10 epitope
What have we learned from TIL therapy?

- TIL contain oftentimes many melanoma-specific CD8 and CD4 T cell populations
  - Against shared antigens (MDA, C/T, overexpressed)
  - Against neo-antigens
- Upon infusion of TIL, the tumor-reactive CD8 and CD4 T cell compartment is broadened in melanoma patients
What have we learned from TIL therapy?

- Objective clinical response rates vary between 38% and 72% of treated melanoma patients in phase II clinical trials (mostly heavily pretreated pts)
- Median OS in this group 16 months
- Patients with CR upon TIL have an excellent prognosis
How does this compare to checkpoint inhibitors?

1-year OS results

- DTIC: 24%
- Ipilimumab: 41%
- TIL*: 50% ?
- Vemurafenib: 53%
- Young TIL*: 68% ?
- Dabrafenib + Trametinib: 74%
- Nivolumab/Pembrolizumab: 74%
- Ipi+Nivo*: 85% ?

* no phase 3 data

Comparison between TIL and checkpoint inhibitors

- TIL: one treatment
  - Complex GMP and patient specific production process
  - Drop-out rate up to 25% of pts
  - In hospital (2-3 weeks)
  - Predictable and manageable side-effects
  - High treatment costs

- Ipilimumab: 4 infusions
  - Anti-PD1: >>4 infusions
  - Off-the-shelf product
  - No ipilimumab in LDH > 2x ULN
  - Outpatient clinic
  - Unpredictable, but manageable side effects
  - Even higher treatment costs
How to further develop TIL therapy

1. Approval of TIL therapy as treatment option for MM
   - RCT
   - A large phase II trial in checkpoint inh failing pts

2. Enrichment for tumor-reactive TIL

3. Generate a personalized TIL product

4. Expand TIL therapy beyond melanoma
How to further develop TIL therapy

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Taking the next step for TIL based ACT

Randomized phase III study comparing TIL based ACT to standard ipilimumab treatment in metastatic melanoma

To obtain EMA approval of ‘classical’ TIL therapy as an ATMP

- **NL:**
  - John Haanen: NKI-AVL, Amsterdam, The Netherlands

- **DK:**
  - Inge Marie Svane: Herlev Hospital, Copenhagen,
Phase II trial in refractory MM patients

- Lion Biotechnology

**Pipeline**

<table>
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<th>Sponsor</th>
<th>Indication</th>
<th>Phase 1</th>
<th>Phase 2</th>
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<td>Phase 2 Complete</td>
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<td>Pilot trials in progress</td>
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</tr>
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PD1 identifies patient-specific tumor-reactive TIL
PD1 rather than TIM-3, LAG-3 or 4-1BB captures best tumor-reactive CD8 TIL

Gros et al., J Clin Invest 2015
How to further develop TIL therapy

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3. Generate a personalized TIL product

4. Expand TIL therapy beyond melanoma
Isolation of neoantigen-specific T cells from tumor and peripheral lymphocytes

Cyrille J. Cohen, Jared J. Gartner, Miryam Horovitz-Fried, Katerina Shamalov, Kasia Trebska-McGowan, Valery V. Bliskovsky, Maria R. Parkhurst, Chen Ankri, Todd. D. Prickett, Jessica S. Crystal, Yong F. Li, Mona El-Gamil, Steven A. Rosenberg, and Paul F. Robbins

1Laboratory of Tumor Immunology and Immunotherapy, Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel. 2Surgery Branch and 3Laboratory of Cancer Biology and Genetics, National Cancer Institute (NCI), NIH, Bethesda, Maryland, USA.

Prediction of potential neoantigen epitopes based on exome sequencing/RNA-seq

Generation of large panels of neo-epitopes/MHC tetramers

Analysis and sorting of reactive T cell from several sources (FTDs, TIL infusion bag, PBMCs)

TCR isolation

Expansion and functional assays

Cohen et al., J Clin Invest 2015
How to further develop TIL therapy

1. Approval of TIL therapy as treatment option for MM
   - RCT
   - A large phase II trial in checkpoint inh failing pts
2. Enrichment for tumor-reactive TIL
3. Generate a personalized TIL product
4. Expand TIL therapy beyond melanoma
Cancer Immunotherapy Based on Mutation-Specific CD4+ T Cells in a Patient with Epithelial Cancer

Eric Tran, Simon Turcotte, Alena Gros, Paul F. Robbins, Yong-Chen Lu, Mark E. Dudley, John R. Wunderlich, Robert P. Somerville, Katherine Hogan, Christian S. Hinrichs, Maria R. Parkhurst, James C. Yang, Steven A. Rosenberg

Complete Regression of Metastatic Cervical Cancer After Treatment With Human Papillomavirus–Targeted Tumor-Infiltrating T Cells

Sanja Stevanović, Lindsey M. Draper, Michelle M. Langhan, Tracy E. Campbell, Mei Li Kwong, John R. Wunderlich, Mark E. Dudley, James C. Yang, Richard M. Sherry, Udai S. Kammula, Nicholas P. Restifo, Steven A. Rosenberg, and Christian S. Hinrichs
Take home messages

• TIL research and therapy has contributed extensively to our understanding of cancer immunity
• TIL therapy and other ACT will be developed further to become a personalized drug treatment
• TIL therapy may be combined with other (non)-immunotherapies in the future
Cancer exome-guided immunomonitoring

Nienke van Rooij
Marit van Buuren
Daisy Philips
Mireille Toebes
Laura van Dijk
Pia Kvistborg

Ton Schumacher

PDX models
Kristel Kemper
Daniel Peeper

Sanger Institute
Sam Behjati
Mike Stratton

Utrecht University
Can Kesmir

MHC-based technologies
Chemical Biology
Boris Rodenko
Huib Ovaa
CCIT, Copenhagen
Sine Hadrup
STAGE Therapeutics
Lothar Germeroth

SB, NIH
Marc Dudley
Steven Rosenberg

ELLA institute, Israel
Mchal Besser
Jakob Schachter

Cancer Immunotherapy
Dream Team

Clinical translation
Bianca Heemskerk
Sander Kelderman
Raquel Gomez
Joost van den Berg
Samira Michels
Bastiaan Nuijen
Christian Blank
Hans van Thienen
Marnix Geukes
Henk Mallo