Melanoma Therapy using Adoptive Transfer of Expanded Tumor Infiltrating T cells; Prospects and Pitfalls

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First things first

Nothing to declare
## Outline of talk....

### The CCIT experience

* Why initiate TIL therapy in melanoma?
* Sum-up of our TIL trial incl clinical data
* Biological monitoring
* Next steps
Outline of talk....

The CCIT experience

* Why initiate TIL therapy in melanoma?
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A case story

* Complete response and yet.....
The CCIT experience

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A case story

* Complete response and yet.....

Some more monitoring:

* A glance at CD4 T cells among TIL
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From CCIT, Herlev Hospital, Denmark

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The CCIT experience

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* Biological monitoring
* Next steps
Why initiate TIL therapy in melanoma

Cancer Regression and Autoimmunity in Patients After Clonal Repopulation with Antitumor Lymphocytes

Mark E. Dudley, John R. Wunderlich, Paul F. Robbins, James C. Yang, Patrick Hwu, Douglas J. Schwartzentruber, Suzanne L. Topalian, Richard Sherry, Nicholas P. Restifo, Amy M. Hubicki, Michael R. Robinson, Mark Raffeld, Paul Duray, Claudia A. Seipp, Linda Rogers-Freezer, Kathleen E. Morton, Sharon A. Mavroukakis, Donald E. White, Steven A. Rosenberg

We report here the adoptive transfer, to patients with metastatic melanoma, of highly selected tumor-reactive T cells directed against overexpressed self-derived differentiation antigens after a nonmyeloablative conditioning regimen. This approach resulted in the persistent clonal repopulation of T cells in those cancer patients, with the transferred cells proliferating in vivo, displaying functional activity, and trafficking to tumor sites. This led to regression of the patients’ metastatic melanoma as well as to the onset of autoimmune melanocyte destruction. This approach presents new possibilities for the treatment of patients with cancer as well as patients with human immunodeficiency virus–related acquired immunodeficiency syndrome and other infectious diseases.

9) and may depend on the destruction of regulatory cells, disruption of homeostatic T cell regulation, or abrogation of other normal tolerogenic mechanisms.

To determine whether prior lymphodepletion might improve the persistence and function of adoptively transferred cells, 13 HLA-A2+ patients with metastatic melanoma received immunodepleting chemotherapy with cyclophosphamide and fludarabine for 7 days before the adoptive transfer of highly selected tumor-reactive T cells and high-dose interleukin-2 (IL-2) therapy (10) (Table 1). These patients all had progressive disease refractory to standard therapies, including high-dose IL-2, and eight patients also had progressive disease despite aggressive chemotherapies. The patients received an average of 7.8 × 10^10 cells (range, 2.3 × 10^10 to 13.7 × 10^10) and an average of nine doses of IL-2 (range, 5 to 12 doses). The T cells used for treatment were derived from tumor-infiltrating lymphocytes (TILs) and were rapidly expanded in vitro (11). All cultures were highly reactive when stimulated with an HLA-A2+ melanoma or an autologous melanoma cell line (Table 1 and table S1).

Six of the 13 patients had objective clinical responses to treatment and four others demonstrated mixed responses, with significant shrinkage of one or more metastatic...
Why initiate TIL therapy in melanoma

Survival of patients with metastatic melanoma treated with autologous TILs and IL-2
(median follow-up 62 mo)
Why initiate TIL therapy in melanoma

Complete lasting responses
Why initiate TIL therapy in melanoma

Rosenberg et al., CCR, 2011, 17: 4550

Complete lasting responses

Most of these 20+ % would have died from disease !!!!!
TIL therapy: the short version

1. Excise tumor mass
2. TIL isolation
   - T cell in vitro activation and expansion
3. Chemotherapy and/or radiotherapy
4. TIL transfer
   - ± cytokines
TIL therapy: the short version

TILs are mainly CD8 biased T-cell cultures
TIL therapy: the short version

TILs are mainly CD8 biased T-cell cultures

TIL therapy: the short version

TILs are mainly CD8 biased T-cell cultures

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The CCIT experience

Interleukin-2

- **USA** → **High dose** (720,000 IU/kg i.v. every 8 hour)
- **CCIT Pilot study** (6 patients) → **low dose** (2 MIU s.c. daily for 14 days)
- **CCIT Amendment phase II** (recruiting – 25 treated) → **Intermediate dose** (iv decrescendo regimen: 18 MIU/m² over 6 h, 12 h and 24 h, 4.5 MIU/m² over 24 h for 3 days)*


Clinical trials.gov ID: NCT00937625
Clinical Response (RECIST 1.0)

31 patients treated
- 92% success rate for TIL-production
- 1 patient dead (CNS haemorrhage in brain metastasis)
- 2 patients evaluation pending

28 patients evaluated
- **4 CR** (49 (NED), 13 (NED), +33, +16 months)
- **7 PR** (+31 (NED), 12, +23 (NED), 12, +14, 8, +6 months)
- **12 SD** (4-6 months)
- **5 PD**

NED = No evidence of disease
Implementation of Lower Doses of IL-2

- Reduced Toxicity
- Similar Clinical Results

Clinical Response

n=23

CR
PR
SD
PD

△ New lesion

Partial response patients with 100% change have non-target lesions present.
Patient MM0909.20 – PR/PMR (12 months)
Patient MM0909.26 – PR/PMR (+9 months)
Patient MM0909.31 – PR/PMR (+8 months)

Baseline  2 months  4 months  7 months
ACT using TIL in melanoma

Although phase III data are needed to make firm conclusions it seems that TIL therapy.....

* When it works it can eradicate huge tumor masses....
ACT using TIL in melanoma

Although phase III data are needed to make firm conclusions it seems that TIL therapy.....

* When it works it can eradicate huge tumor masses....(also with lower dose of IL-2)
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Immune reactivity in TIL cultures

Monitoring of TIL cultures:

* Which antigens are recognized in the TIL lines?
* Can they be followed over time upon treatment?
* Correspondence between TAA recognition and clinical course?

• What have we done??
Immune reactivity in TIL cultures

Monitoring of TIL cultures:

* Which antigens are recognized in the TIL lines?
* Can they be followed over time upon treatment?
* Correspondence between TAA recognition and clinical course?

• What have we done??

• Short answer – all we could !!!
Immune reactivity in TIL cultures

This is where I skip some 40 slides (except a few...) based on published data and go to the conclusion.....
Peptide specific T cells among TIL...

Which peptide specificities to look for…?

Aim ; To look for all known peptides restricted by HLA-A1, -A2, -A3, -A11, (A24), and -B7

Antigens; All published peptide antigens searched in
* Cancer Immunity database (van den Eynde & van der Bruggen),
* Cancer-testis antigen database (CTpedia) (Almeida et al.),
* published antigen list (Novellino et al, CII)
* pubmed search)

That gave us 174 peptides to study using tetramers
(145/A2, 10/A1, 11/A3, 3/A11, and 5/B7)
Examples of T-cell responses detected:

**Differentiation antigens:**
- gp100
  - HLA-B7
  - HLA-A3
  - HLA-A1
- Tyrosinase
  - HLA-A1
- MAGE-A1
  - HLA-A1
- Cancer - testis antigens:
  - TAG
    - HLA-A3
- Overexpressed antigens:
  - GNt-V
    - HLA-A2
  - AIM-2
    - HLA-A1
- Mutation antigens:
  - N-ras
    - HLA-A1
Challenges for immune monitoring

ACT based on in vitro expanded TIL (as judged by tetramer analyses...) leads to...

* Quite low-frequency responses in TIL cultures (even...) prior to administration

Andersen, R.S, Cancer Res. 72, 2012
Kvistborg, P., Oncoimmunology, 2, 2013
Andersen, R.S., Oncoimmunology, 7, 2013
Ellebaek, E., J. Translational Medicine, 10, 2012
Challenges for immune monitoring

ACT based on in vitro expanded TIL (as judged by tetramer analyses...) leads to....

* Quite low-frequency responses in TIL cultures (even...) prior to administration

Could we be looking at the wrong peptides ??
"Total" versus tetramer reactivity

% cytokine producing cells of CD8+ cells

<table>
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<tr>
<th>T2</th>
<th>Tumor</th>
<th>T2</th>
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<th>Tumor</th>
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<td>MM131207 M3</td>
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<td>MM131207 REPM3</td>
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<td>MM909.06 M5</td>
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<td>MM909.09 Y REP</td>
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<tr>
<td>T2</td>
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Ag-spec. TILs: ~1.5%

MAGE-A1

Mart-1

MLA

gp100

IMD

KMW

IFN-γ

TNF-α

IL-2

IFN-γ+TNF-α

IFN-γ+TNF-α+IL-2
Challenges for immune monitoring

ACT based on in vitro expanded TIL (as judged by tetramer analyses…) leads to….

* Quite low-frequency responses in TIL cultures (even…) prior to administration

So… TIL cultures (seem) not to be dominated by the presence of cancer specific T cells – rather to the contrary…..
Challenges for immune monitoring

ACT based on in vitro expanded TIL (as judged by tetramer analyses...) leads to...

* Quite low-frequency responses in TIL cultures (even...) prior to administration....

Also when considering global T-cell reactivity against autologous tumor cell lines

Andersen, R.S, Cancer Res. 72, 2012
Kvistborg, P., Oncoimmunology, 2, 2013
Andersen, R.S., Oncoimmunology, 7, 2013
Ellebaek, E., J. Translational Medicine, 10, 2012
Challenges for immune monitoring

ACT based on in vitro expanded TIL (as judged by tetramer analyses…) leads to….

* Quite low-frequency responses in TIL cultures (even…) prior to administration

* Tetramer based monitoring after administration probably not feasible (in most cases…….)
Challenges for immune monitoring

ACT based on in vitro expanded TIL (as judged by tetramer analyses…) leads to….

* Quite low-frequency responses in TIL cultures (even…) prior to administration

* Tetramer based monitoring after administration probably not feasible (in most cases…….)

So – we (i.e., Marco Donia) have done some old fashioned low-tech cellular monitoring instead!!
Anticancer responses of TIL infusion products using a cellular assay

Donia M et al., J Invest Dermatol 2013
Donia M et al., Unpublished
Anticancer responses of TIL infusion products using a cellular assay

Tumor reactive CD8 T cell/10^6

Donia M et al., J Invest Dermatol 2013
Donia M et al., Unpublished
Anticancer Responses in Peripheral Blood

% Tumor reactive of CD8+ PBLs

Color Code:
- Complete Response
- Decrease >20%
- No significant tumor regression

Censored (alive)

n=21

Donia M et al., Unpublished
TIL characteristics which corresponds with clinical response

- (CD8 T) Cell numbers infused
- Tumor reactivity – in the culture and among PMBC
- Persistence in the patient
- “Young” T cells better than “old” (telomere/CD27)
TIL characteristics which corresponds with clinical response

- (CD8 T) Cell numbers infused
- Tumor reactivity – in the culture and among PMBC
- Persistence in the patient
- “Young” T cells better than “old” (telomere/CD27)

Lack of markers that would allow selection of patients before or even after treatment
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Next steps for TIL based ACT

- Randomized phase III trial
  - Generate robust efficacy data
  - Approval of TIL therapy as standard treatment
    (Inge Marie Svane, PI, J. Haanen, Netherlands Cancer Institute, R. Hawkins, University of Manchester)

(Phase III; 162 patients, ippi against TIL, High dose IL-2, expected time: 2 years)
Next steps for TIL based ACT

- Randomized phase III trial
  - Generate robust efficacy data
  - Approval of TIL therapy as standard treatment
    (Inge Marie Svane, PI, J. Haanen, Netherlands Cancer Institute, R. Hawkins, University of Manchester)

The hope is to establish the efficacy of TIL based ACT – and establish this treatment as a standard treatment of malignant melanoma in Europe!

(Phase III; 162 patients, ippi against TIL, High dose IL-2, expected time: 2 years)
The CCIT experience; conclusions

- TIL therapy can eradicate huge tumor masses (even with lower dose IL-2)
- Reactivity against auto (and allo) melanoma cells corresponds with clinical response
- Phase III study initiated (please join)

(Phase III; 162 patients, ippi against TIL, High dose IL-2, expected time: 2 years)
Outline of talk....

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A case story

* Complete response and yet.....

Some more monitoring:

* A glance at CD4 T cells among TIL
Case story; Complete responder

- 42-year old male
- Good performance status

- Previous treatments:
  - IL-2/interferon
  - Ipilimumab
  - DC-vaccination
  - Resection of large metastases; left side of the neck and right cheek
Administered TILs July 2011
Clinical Response - CR/CMR

Patient no.909.11
Case story; immune reactivity against autologous tumor

Patient no.909.11

Control

Autologous tumour

Infusion product (TILs)

Baseline (PBMC)

After 4 weeks (PBMC)
Relapse in August 2012 – surgically resected -> NED+

+ 13 months: Disease recurrence

Patient no.909.11
Relapse in August 2012 – surgically resected -> NED+

+ 13 months: Disease recurrence

+13 months: PBMC reactivity

Patient no.909.11
TIL reactivity against first and recurrent autologous tumor lines

Patient no. 909.11

Old Tumor

New Tumor

TIL reactivity against first and recurrent autologous tumor lines

Patient no. 909.11

Old Tumor

New Tumor
Reactivity in PBMC against first and recurrent autologous tumor lines

Patient no. 909.11

PBLs+13mo vs OLD Tumor

PBLs+13mo vs NEW Tumor
What T cell recognition rely on...
What T cell recognition rely on...
Down regulation of APM components the in recurrent tumor
Down regulation of APM components in recurrent tumor

Decrease in the expression of APM components in relapse cancer cell line !!!
Case story conclusion

With more powerful responses we will see more frequent immune escape by cancer cells !!!
Case story conclusion

With more powerful responses we will see more frequent immune escape by cancer cells!!!

In turn underscoring the need to study escape mechanisms and ways to counteract escape!!
Partially restored TIL recognition upon IFN-g upregulation of HLA.....

So Marco looked at whether down expression of APM could be addressed
Partially restored TIL recognition upon IFN-γ upregulation of HLA.....

So Marco looked at whether down expression of APM could be addressed

New TIL vs new tumor

Old TIL vs new tumor

No IFN treatment

IFN treatment

In patient no.909.11
Partially restored TIL recognition upon IFN-g upregulation of HLA.....a frequent phenomenon

Up-regulation of HLA molecules by IFN-g for increased recognition by autologous CD8 TILs
Partially restored TIL recognition upon IFN-g upregulation of HLA.....a frequent phenomenon

Up-regulation of HLA molecules by IFN-g for increased recognition by autologous CD8 TILs
TIL in combination with IFN-α

Counteracting Immune Escape
• Combination with Interferons

Principal Investigator: Rikke Andersen, M.D.
soon on clinicaltrials.gov
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Up-regulation of HLA molecules.....

Cytotoxic CD8 T cells secrete IFN-γ which in turn lead to up-regulation of class I and II molecules.

Dudley et al. Science, 298, 2002
MHC Class II in Melanoma

Fig. 2  Distribution of HLA class II phenotypes in 42 human melanoma cell lines (ESTDAB). Phenotypes 1–4 representing various patterns of HLA class II expression on the studied melanoma cell lines are presented. Surface expression of HLA class II molecules was determined by flow cytometry (mean fluorescence intensity, MFI) using a panel of HLA class II specific antibodies. To analyse induction with IFN-gamma the melanoma cells were treated with 800 U/ml for 48 h.
Tumor Reactive CD4+ T cells infiltrates melanoma

Most TIL cultures are CD8 biased... but contain CD4 T cells as well...

% of T cells responding to autologous tumor cultures
Tumor Reactive CD4+ T cells infiltrates melanoma

Most TIL cultures are CD8 biased..... but contain CD4 T cells as well...

% of T cells responding to autologous tumor cultures

<table>
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<tr>
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<th>With Response</th>
<th>No Response</th>
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<tr>
<td>CD4</td>
<td>n=18</td>
<td>n=16</td>
</tr>
<tr>
<td>CD8</td>
<td>n=30</td>
<td>n=4</td>
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50 %
90 %

CCIT, Confidential
MHC Class II attracts Inflammatory CD4$^+$ T cells

CD4 T cells are most prominently present if cancer cells express class II molecules

\[
\text{% responding of CD4}^+ \text{ TILs} \\
\begin{array}{c}
\text{Constitutive Class II -} \\
\text{Constitutive Class II +}
\end{array}
\]

\[p = 0.003\]
MHC Class II attracts Inflammatory CD4$^+$ T cells

CD4 T cells are most prominently present if cancer cells Express class II molecules

What do they do ??
Cytokine profiles of CD4+ and CD8+ T cells

% responding of CD4+ TILs

p = 0.003

CD4

CD8

p = 0.0027

TNF
IFN-γ
CD107a
IL-2
IL-17A
MIP-1α
MIP-1β

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IFN increase recognition by CD8\(^+\) TILs

% Tumor Reactivity of autologous CD8 TILs

![Graph showing tumor reactivity comparison between Control and + IFN-\(\gamma\)]
IFN increase recognition by CD8⁺ TILs

% Tumor Reactivity of autologous CD8 TILs

[Graph showing the comparison of tumor reactivity under control conditions, with IFN-γ, and with TNF.]
CD4^+ -derived TNF inhibits recognition by CD8^+ TILs

% Tumor Reactivity of autologous CD8 TILs

![Graph showing the effect of TNF and IFN-γ on the % Tumor Reactivity of CD8 TILs. The graph compares different conditions: Control, + IFN-γ, + TNF, and + IFN-γ + TNF. The data points indicate a decrease in reactivity with the addition of TNF and IFN-γ, with significant differences marked by * and **.](image-url)
CD4⁺-derived TNF inhibits recognition by CD8⁺ TILs

IFN increase CD8 recognition - addition of TNF abolish the effect of IFN
CD4\(^+\)-derived TNF inhibits recognition by CD8\(^+\) TILs

IFN increase CD8 recognition - addition of TNF abolish the effect of IFN

IFN from CD8 cells at the tumor site may increase MHC expression
But CD4 derived TNF may inhibit the increase in immune recognition
CD4\(^+\)-derived TNF inhibits recognition by CD8\(^+\) TILs

Increase in recognition by IFN

Back to baseline with added TNF
A glance at CD4 T cells: Conclusions

- Constitutive MHC class II$^+$ melanomas attract tumor reactive CD4$^+$ T cells
A glance at CD4 T cells: Conclusions

- Constitutive MHC class II$^+$ melanomas attract tumor reactive CD4$^+$ T cells
- Tumor reactive CD4$^+$ T cells show a marked inflammatory phenotype
Constitutive MHC class II$^+$ melanomas attract tumor reactive CD4$^+$ T cells

Tumor reactive CD4$^+$ T cells show a marked inflammatory phenotype

Tumor reactive CD4$^+$ T cells may dampen CD8$^+$ T cell recognition of melanoma cells
Final Conclusions

- TIL based ACT in melanoma – highly efficient in a significant fraction of patients – even with lower dose of IL-2

- These more powerful anti-cancer responses are likely to lead to more frequent escape of cancer cells from immune recognition

- CD4 T cells need further study – but may under certain conditions not be supportive of the cytotoxic response mediated by CD8 T cells
Thank you for your attention.....!!

Questions !!
Genetic (whole-exome) Sequencing

- Confirms the same origin of the tumors

- Single Mutational events do not explain biological differences

Dr. Göran Jönsson
Melanoma Genomics Unit
University of Lund, Sweden
A) Tumor 1 Tumor 2
   TAG
   18s RNA

B) Graph showing PD-L1 expression over time.

C) Western blot analysis:
   Tumor 1 Tumor 2
   TAP1 TAP2 β2m Tapasin LMP2 HC10 LMP10 GAPDH PSZ

D) Graph showing tumor size over days after inoculation:
   Tumor 1 Tumor 2
   Days after inoculation
   Tumor Size (mm²)