Dissecting therapy-induced T-cell responses in melanoma
Tumor-infiltrating lymphocyte (TIL) therapy of melanoma

Patient pretreated with lymphodepleting chemotherapy

TIL are grown from melanoma tumors

Rapid Expansion

Infusion of TIL + IL-2

- 50% response rate in trials in multiple centers (US, Israel)
- Clinical effect at least partially mediated by cytotoxic T cells
- Which cytotoxic T cells mediate cancer regression?
- Could we specifically boost their numbers?

Tumor-infiltrating lymphocyte (TIL) therapy of melanoma

TIL are grown from melanoma tumors

Rapid Expansion

Infusion of TIL + IL-2

Patient pretreated with lymphodepleting chemotherapy
- Survival benefit in metastatic disease
- Data from murine models suggest a role for cytotoxic T cells

Hodi et al. *NEJM* 2010
- Which cytotoxic T cells mediate cancer regression?
- Could we specifically boost their numbers?
- Could we distinguish responders and non-responders?
Detecting tumor-specific cytotoxic T cells

How a T cell sees a target cell

How an MHC tetramer sees a T cell

CD8+ T cell

TCR

MHC I

antigen presenting cell

T cell

T cell
How an MHC tetramer sees a T cell

Detecting tumor-specific cytotoxic T cells
Detecting tumor-specific cytotoxic T cells

How an MHC tetramer sees a T cell

Hundreds of different melanoma-associated T cell antigens have been described

Two problems:
1. Generation of very large collections of MHC tetramer reagents is not possible with standard technology
2. Even if you would have such a collection, the amount of biological material is limiting
Generation of pMHC multimers by UV-induced peptide exchange

$GILGFVF(o-NO_2)L$

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\begin{align*}
\text{H}_2\text{N} & \quad \text{O} \quad \text{N} \\
\text{H} & \quad \text{O} \\
\text{H} & \quad \text{O} \\
\text{N} & \quad \text{H} \\
\text{O} & \quad \text{O} \\
\text{N} & \quad \text{H} \\
\text{O} & \quad \text{O} \\
\text{N} & \quad \text{H} \\
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\text{N} & \quad \text{H} \\
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\end{align*}
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\begin{align*}
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\text{O} & \quad \text{O} \\
\text{N} & \quad \text{H} \\
\text{O} & \quad \text{O} \\
\text{N} & \quad \text{H} \\
\text{O} & \quad \text{O} \\
\text{N} & \quad \text{H} \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{O2N} & \quad \text{GILGFVF}(o-\text{NO}_2)\text{L} \\
\text{ON} & \quad \text{+H}_2\text{N} \\
\text{ON} & \quad \text{O} \\
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\end{align*}
\]

Toebes et al. Nat. Med. 2006
Bakker et al. PNAS 2008
Generation of pMHC multimers by UV-induced peptide exchange

Bakker et al. *PNAS* 2008
Generation of pMHC multimers by UV-induced peptide exchange

Toebes et al. Nat. Med. 2006
Bakker et al. PNAS 2008
Generation of pMHC multimers by UV-induced peptide exchange

![Diagram of peptide exchange process]

- **Peptide 1**
- **Peptide 2**
- **Peptide 3**

**Rescue Peptides**

**Disintegration**
In crystallo MHC ligand exchange

UV-sensitive ligand pre-cleavage
(KILGJ*VFJV)

UV-sensitive ligand post-cleavage
(visualization of the reaction intermediate)

In crystallo exchange with HIV RT\textsubscript{468-476}
(ILKEPVHG)

In crystallo exchange with HIV Env\textsubscript{120-128}
(KLTPLCVT)

Celie et al. JACS 2009
Generation of pMHC multimers by UV-induced peptide exchange

Allows the creation of very large MHC multimer collections for all prevalent HLA class I alleles

- HLA-A24, -B40, -B58 (G. Grotenbreg, NUS, Singapore)
How to monitor hundreds of T cell populations per patient?

Aim: Detection of low frequency T cell populations
~ 0.02% of CD8 T cells

Required: record >50,000 CD8 T cells ~ 2 ml fresh blood

For 100 potential antigens: ~ 200 ml blood

25 parallel measurements per sample: ~ 8 ml blood
What if you couple the same pMHC to multiple fluorophores & start combining?
Generate fluorochrome conjugated MHC multimers

Mix to create a collection of differentially encoded MHC multimers

Assembly of combinatorial codes on T cell surfaces

Analysis by flow cytometry

Hadrup et al. Nat. Methods 2009
Example of a combinatorial coding read-out

<table>
<thead>
<tr>
<th>APC</th>
<th>QD 605</th>
<th>QD 625</th>
<th>QD 655</th>
<th>QD 705</th>
<th>QD 800</th>
<th>PE-Cy7</th>
</tr>
</thead>
</table>

8 colors → 28 T cell reactivities
Selection of melanoma-associated epitope panel

Panel of 216 melanoma-associated peptides

- public databases
- literature analysis

Antigen class distribution

- Overexpressed: 44%
- Cancer-Germline: 31%
- Differentiation: 18%
- Mutation: 5%
- Not fully characterised: 2%
Panel of 216 melanoma-associated peptides

- public databases
- literature analysis

Antigen class distribution

- Overexpressed: 44%
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- Mutation: 5%
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Examples:
- e.g. MAGE family
- e.g. Meloe-I
- e.g. CDK4^{R24C}
- e.g. MART-I
Dissecting therapy-induced T cell responses in melanoma: POC

Patient pretreated with lymphodepleting chemotherapy
TIL are grown from melanoma tumors
Rapid Expansion
Infusion of TIL + IL-2

- Do we detect tumor-specific T cell responses in the cell product?
- If so, what do these T cells recognize?
- Does composition of the cell product predict post-treatment immune reactivity?
Identification of diverse T cell populations in HLA-A2+ TIL products

Numbers represent the % MHC tetramer+ T cells out of total CD8+ cells
Identification of diverse T cell populations in HLA-A2⁺ TIL products

Numbers represent the % MHC tetramer⁺ T cells out of total CD8⁺ cells
Identification of diverse T cell populations in HLA-A2+ TIL products

pt.LD

MART-1 (#78)
1.210%
ELAGIGILTV
Gp100 (#36)
1.897%
YLEPGPVTA
Gp100 (#39)
0.131%
VLYRGFSV
Gp100 (#56)
0.083%
IMDQVPFSV

pt.KS

MART-1 (#78)
0.633%
ELAGIGILTV
Meloe-1 (#80)
1.45%
TLNDECWPA

gp100 (#36)
0.453%
YLEPGPVTA
gp100 (#38)
0.376%
ITDQVPFSV
gp100 (#39)
0.010%
VLYRGFSV
gp100 (#42)
0.016%
AMLGHTMEV
gp100 (#44)
0.028%
KTGQYWQV
gp100 (#56)
0.42%
IMDQVPFSV
Summary of identified T cell responses in 15 T cell products

<table>
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<tr>
<th>Epitope</th>
<th>PSC</th>
<th>CZE</th>
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</table>

Frequency:
- **Green**: 0.01 - 0.99%
- **Orange**: 1.00 - 4.99%
- **Red**: > 4.99%
| Epitope                | PSC | CZE | RAV | RTE | MZU | LR | LN | KS | MV | CR | ER | MA | LD | SW | HE |
|-----------------------|-----|-----|-----|-----|-----|----|----|----|----|----|----|----|----|----|----|----|
| Mart-1<sub>ELA</sub> |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| gp100<sub>IMD</sub>  |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| gp100<sub>YLE</sub>  |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| gp100<sub>VLY</sub>  |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| gp100<sub>ITD</sub>  |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| gp100<sub>AML</sub>  |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| gp100<sub>KTW</sub>  |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| TRP2<sub>VYD</sub>   |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| TRP2<sub>SVY</sub>   |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| MAGE A10<sub>GLY</sub> |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| MAGE C2<sub>L1F</sub>|     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| MAGE C2<sub>ALK</sub>|     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| MAGE C2<sub>V1W</sub>|     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| SSX-2<sub>KAS</sub> |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| Meloe-1<sub>T LN</sub> |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| Telomerase<sub>RLF</sub> |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| CDK4<sub>ACD</sub>   |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |
| MG50<sub>RLG</sub>   |     |     |     |     |     |    |    |    |    |    |    |    |    |    |    |    |

- Total of 39 melanoma-reactive T cell responses in 15 T cell products
- Number of responses ranges from 0 to 8
- Predominant recognition of MDA and C/G antigens
- T cell reactivity is *highly* patient-specific
### Summary of identified T cell responses in 15 T cell products

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Patient</th>
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<td>MG50RLG</td>
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</table>

- Total of 39 melanoma-reactive T cell responses in 15 T cell products
- Number of responses ranges from 0 to 8
- Predominant recognition of MDA and C/G antigens
- T cell reactivity is *highly* patient-specific

- Are these T cell populations relevant for melanoma recognition?
- Does this analysis predict post-treatment immune reactivity?
Isolation of a melanoma-reactive T cell populations for functional validation

MAGE-A10
5.3%
GLYDGMEHL

MAGE-C2
0.2%
ALKDVEERV

MAGE-C2
0.9%
LLFGLALIEV

MAGE-C2
1.5%
VIWEVLNAV

MG-50
0.8%
RLGPTLMCL

CDK4 R24C
2.3%
ACDPHSGHFV

Numbers represent the % MHC tetramer+ T cells out of total CD8+ cells
Recognition of autologous melanoma by identified T cell populations

The diagram shows the percentage of CD8+ cells producing IFNγ in response to various peptides and autologous tumors. The x-axis represents different melanoma antigens, while the y-axis represents the percentage of CD8+ IFNγ+ cells. The bars indicate the response to T2 cells with and without peptides, and the autologous tumor response.
Detecting *post-therapy* T cell reactivity in patients treated with TIL

- Total of 19 post-infusion responses in 10 HLA-A2+ TIL-treated patients
- 79% of pre-infusion responses is seen back in post-infusion PBMNC
- 95% of T cell responses in PBMNC post-therapy is predicted by in vitro reactivity
Conclusions

- We have developed and validated technology for the high-throughout detection of therapy-induced melanoma-specific T cell reactivity

- T cell responses in TIL cell products are diverse, biased towards MDA and C/G antigens, and highly variable between patients

- In ‘young TIL’, individual T cell responses are generally of a (surprisingly) low magnitude

- T cell responses in TIL cell products predict immune reactivity post-therapy - No epitope spreading -

Is it possible to influence TIL cell product composition?
Individual tumor fragments yield distinct TIL products
Dissecting melanoma-specific T cell responses upon other immunotherapeutic treatments?

- Immune reactivity induced by anti-CTLA4 or anti-PD1 treatment?

- Immunological consequences of combination therapy? (e.g. BRAF inhibition plus anti-CTLA4)
Self-destructive MHC ligands & combinatorial coding

MHC-based monitoring
Jenny Shu
Manuel Fankhauser
Pia Kvistborg
Mireille Toebes
Arne Bakker
Carsten Linnemann

Chemical Biology
Boris Rodenko
Huib Ovaa

Clinical translation
Bianca Heemskerk
Annelies Jorritsma
Raquel Gomez
Nienke van Rooij
Bastiaan Nuijen
Christian Blank
John Haanen

CCIT, Copenhagen
Sine Hadrup
Charlotte Albæk Thrue

Surgery Branch, NIH
Steven Rosenberg
Mark Dudley
Individual tumor fragments yield distinct TIL products

CDK4\textsuperscript{R24C}

F1 0.016
F2 0.433
F4 0.005
F5
F7 0.212
F9 0.160

% CDK4\textsuperscript{R24C}-specific CD8$^+$ T cells
Self-assembling molecular codes

Hadrup et al. Nat. Methods 2009