Adoptive T Cell Therapy
Metastatic Melanoma

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Adoptive T Cell Therapy with TILs

- Tumor removed by surgeon
- Tumor cut into small fragments
- Initial TIL expansion (2-4 weeks)
- Anti-CD3 Feeder-IL2
- Rapid expansion of TIL (REP) (14 days)
- TIL rapidly expanded in static or dynamic conditions
- Optional cryopreservation

- 30-45 days for TIL production
- ~100 x 10^9 cells
<table>
<thead>
<tr>
<th>Reference</th>
<th>Histology</th>
<th>Patients (n)</th>
<th>OR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosenberg et al, 1988</td>
<td>Melanoma</td>
<td>20</td>
<td>11 (55%)</td>
<td>1 (5%)</td>
<td>10 (50%)</td>
<td>First in human trial (TILs + IL-2)</td>
</tr>
<tr>
<td>Dudley et al, 2005</td>
<td>Melanoma</td>
<td>43</td>
<td>21 (49%)</td>
<td>5 (12%)</td>
<td>16 (37%)</td>
<td>Pre-conditioning regimen to improve TIL engraftment using “modern-era” lymphodepletion (NMA) and high dose interleukin-2 (IL-2)</td>
</tr>
<tr>
<td>Dudley et al, 2008</td>
<td>Melanoma</td>
<td>25 (2 Gy TBI)</td>
<td>13 (52%)</td>
<td>5 (20%)</td>
<td>8 (32%)</td>
<td>In sequential trials, response rate directly proportion to depth of pre-conditioning lymphodepletion, prompting evaluation in a randomized trial.</td>
</tr>
<tr>
<td>Rosenberg et al, 2011</td>
<td>Melanoma</td>
<td>25 (12 Gy TBI)</td>
<td>18 (72%)</td>
<td>10 (40%)</td>
<td>8 (32%)</td>
<td></td>
</tr>
<tr>
<td>Dudley et al, 2010</td>
<td>Melanoma</td>
<td>33 (NMA)</td>
<td>19 (58%)</td>
<td>3 (9%)</td>
<td>16 (49%)</td>
<td>Minimally cultured CD8-enriched TILs can mediate effective tumor regression.</td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>23 (6 Gy TBI)</td>
<td>11 (58%)</td>
<td>2 (9%)</td>
<td>9 (39%)</td>
<td></td>
</tr>
<tr>
<td>Itzhaki et al, 2011</td>
<td>Melanoma</td>
<td>31</td>
<td>15 (48%)</td>
<td>4 (13%)</td>
<td>11 (35%)</td>
<td>Minimally cultured bulk TILs can mediate effective tumor regression.</td>
</tr>
<tr>
<td>Pilon-Thomas et al, 2012</td>
<td>Melanoma</td>
<td>13</td>
<td>5 (38%)</td>
<td>2 (15%)</td>
<td>3 (23%)</td>
<td>Bulk TIL screened for IFN-γ secretion can mediate durable tumor regression.</td>
</tr>
<tr>
<td>Radvanyi et al, 2012</td>
<td>Melanoma</td>
<td>31</td>
<td>13 (42%)</td>
<td>2 (6%)</td>
<td>11 (35%)</td>
<td>TIL, particularly differentiated effector cells (CD8+/BTLA+) can mediate durable tumor regression.</td>
</tr>
<tr>
<td>Ellebaek et al, 2012</td>
<td>Melanoma</td>
<td>6</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Besser et al, 2013</td>
<td>Melanoma</td>
<td>80</td>
<td>23 (29%)</td>
<td>5 (6%)</td>
<td>18 (23%)</td>
<td>Complete and durable responses were induced after TIL treatment using NMA in combination with low-dose IL-2.</td>
</tr>
<tr>
<td>Tran et al, 2014</td>
<td>Cholangiocarcinoma</td>
<td>1</td>
<td>1 (100%)</td>
<td>-</td>
<td>1 (100%)</td>
<td>First successful treatment of a solid epithelial cancer using TILs targeting a mutated antigen.</td>
</tr>
</tbody>
</table>

* As measured by WHO or RECIST criteria
a An additional 6 patients resected, but not treated
b An additional 5 patients resected, but not treated
c 23 (29%) patients enrolled, but not treated
TIL in combination with lymfodepleting chemotherapy

3-step treatment:

Lymfodepleting chemotherapy

2 days: cyclophosphamide (60 mg/kg)
5 days: Fludarabin (25 mg/m²)

Intravenous infusion of in vitro expanded TILs

Day 7: reinfusion of 20–140×10⁹ TILs

High dose IL-2

720,000 IU/kg iv every 8 hours until limiting toxicity

Severe grade 3 and 4 toxicities associated with high dose bolus IL-2

Rosenberg et al, PNAS 2004
Adoptive TIL therapy and IL-2 dosing

Interleukin-2

- NCI studies → High dose (720,000 IU/kg i.v. every 8 hour)
- DK pilot study → low dose (2 MIU s.c. daily for 14 days)
- DK phase II → Intermediate dose (iv decrescendo schedule)

Low toxicity of low dose, manageable toxicity with intermediate IL-2 regimen
Pilot TIL study

Adoptive cell therapy with autologous tumor infiltrating lymphocytes and low-dose Interleukin-2 in metastatic melanoma patients

6 patients
2 CR, both patient had late solitary relapses (1y and 4y) but have ongoing NED (2y and 3y) after surgical removal
Low IL-2 toxicity
Phase II TIL study design

- 25 patients with progressive treatment-refractory metastatic melanoma

- 3-step treatment: HD chemotherapy, TIL infusion, IL-2
  - Young TILs
  - Wave bioreactor
  - Intermediate dose IL-2 (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
Long-lasting complete responses in patients with treatment refractory metastatic melanoma after adoptive cell transfer therapy with tumor-infiltrating lymphocytes and an attenuated continuous interleukin-2 regimen

Rikke Andersen, Marco Donia Eva Ellebaek, Troels Holz Borch, Per Kongsted, Trine Zeeberg Iversen, Lisbet Rosenkrantz Hölmich Helle Westergren Hendel, Özcan Met, Mads Hald Andersen, Per thor Straten, Inge Marie Svane.

25 patients treated

– Last follow-up: February 5, 2015
– Median follow-up time 19.8 months*
– 96% success rate for TIL-production
– 1 patient died 4 days after TIL infusion (intratumoral haemorrhage in brain metastasis)

RECIST 1.0 Responses

<table>
<thead>
<tr>
<th>No of patients</th>
<th>Responders (42%)</th>
<th>Non-responders (58%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CR</td>
<td>PR</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>24+</td>
<td>24+</td>
<td>22, 20+</td>
</tr>
<tr>
<td>14+</td>
<td>20,19+</td>
<td>9+, 6, 5, 5, 5, 4, 3</td>
</tr>
<tr>
<td>14+</td>
<td>9+</td>
<td>5, 5, 4, 3</td>
</tr>
</tbody>
</table>

Overall survival (%)

Time since TIL infusion (months)

n = 25
Median OS: 21.8 months

*Reverse Kaplan Meier Method
Best Change from Baseline in Target Lesion size (%)

Partial response patients with 100% change have non-target lesions present
Changes Over Time in Target Lesion Size (%)
## Baseline Patient and TIL Characteristics

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Responders OR, (n = 10)</th>
<th>Non-Responders OR, (n = 14)</th>
<th>P-value OR vs NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>56 (40;68)</td>
<td>51 (25;63)</td>
<td>0.20</td>
</tr>
<tr>
<td>Sex (% male)**</td>
<td>5 (50)</td>
<td>5 (36)</td>
<td>0.68</td>
</tr>
<tr>
<td>Primary tumor origin (% skin)**</td>
<td>8 (80)</td>
<td>10 (71)</td>
<td>1.00</td>
</tr>
<tr>
<td>AJCC Stage (% M1c)**</td>
<td>8 (80)</td>
<td>12 (86)</td>
<td>1.00</td>
</tr>
<tr>
<td>BRAF status (% wt)**</td>
<td>5 (50)</td>
<td>6 (43)</td>
<td>1.00</td>
</tr>
<tr>
<td>HLA-A2 (% HLA-A2)**</td>
<td>2 (20)</td>
<td>7 (50)</td>
<td>0.21</td>
</tr>
<tr>
<td>Tumor burden (cm)*</td>
<td>12.5 (1.9;34.2)</td>
<td>12.9 (5.5;21.2)</td>
<td>0.88</td>
</tr>
<tr>
<td>LDH level (% elevated)**</td>
<td>6 (60)</td>
<td>9 (60)</td>
<td>1.00</td>
</tr>
<tr>
<td>Metastatic sites*</td>
<td>2 (1;6)</td>
<td>4 (1;7)</td>
<td>0.30</td>
</tr>
<tr>
<td>Previous treatments*</td>
<td>2 (1;4)</td>
<td>2 (2;4)</td>
<td>0.63</td>
</tr>
<tr>
<td>Prior response to IL-2**</td>
<td>2 (22.2)</td>
<td>4 (26.7)</td>
<td>0.42</td>
</tr>
<tr>
<td>Prior response to Ipilimumab**</td>
<td>1 (12.5)</td>
<td>1 (7.7)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TIL characteristics</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TIL culture generation (days)*</td>
<td>22 (14;34)</td>
<td>21 (13;36)</td>
<td>0.60</td>
</tr>
<tr>
<td>TILs cryo before REP (n, %)**</td>
<td>5 (50)</td>
<td>6 (43)</td>
<td>1.00</td>
</tr>
<tr>
<td>Fold expansion during REP*</td>
<td>5690 (4100;7125)</td>
<td>4314 (2856;9975)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Infused Cells</strong></td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Total (x10^3)*</td>
<td>114 (82;143)</td>
<td>92 (61;200)</td>
<td>0.14</td>
</tr>
<tr>
<td>CD8 %*</td>
<td>53 (8;93)</td>
<td>42 (6;92)</td>
<td>0.56</td>
</tr>
<tr>
<td>CD4 %*</td>
<td>46 (5;91)</td>
<td>41 (4;93)</td>
<td>0.98</td>
</tr>
<tr>
<td>γδ %*</td>
<td>0.2 (0;11)</td>
<td>0.7 (0.2;51)</td>
<td>0.08</td>
</tr>
<tr>
<td>CD8 (x10^3)*</td>
<td>62 (7;122)</td>
<td>30 (6;172)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*) Median (range), Mann-Whitney
**) n (%), Fisher’s Exact test
# Toxicity

<table>
<thead>
<tr>
<th>Treatment characteristics (median, range)</th>
<th>All patients (n = 25)</th>
<th>Responders OR (n = 10)</th>
<th>Nonresponders NR (n = 14)</th>
<th>P-value OR vs NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days in hospital(^1)</td>
<td>19 (15;36)</td>
<td>17.5 (15-36)</td>
<td>20 (15-27)</td>
<td>0.12</td>
</tr>
<tr>
<td>Units RBC transfusion</td>
<td>5 (1;25)</td>
<td>5 (2;14)</td>
<td>5 (1;25)</td>
<td>0.52</td>
</tr>
<tr>
<td>Units PLT transfusion</td>
<td>7 (3;17)</td>
<td>7 (3;14)</td>
<td>7 (3;17)</td>
<td>0.81</td>
</tr>
<tr>
<td>Days with neutrophils &lt; 0.5 x10(^9)/l</td>
<td>8 (4;13)</td>
<td>8 (5;12)</td>
<td>9 (4;13)</td>
<td>0.79</td>
</tr>
<tr>
<td>Dose IL-2 administered, MIU(^2)</td>
<td>112 (50;135)</td>
<td>107 (58;135)</td>
<td>112 (50;135)</td>
<td>0.75</td>
</tr>
<tr>
<td>% IL-2 administered(^3)</td>
<td>95 (60;100)</td>
<td>93 (60;100)</td>
<td>96 (60;100)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

### Severe adverse events\(^4\) (n)

<table>
<thead>
<tr>
<th>Event</th>
<th>All patients</th>
<th>Responders</th>
<th>Nonresponders</th>
<th>P-value OR vs NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Febrile neutropenia</td>
<td>24</td>
<td>10</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Infections, verified(^5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary tract</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia(^6)</td>
<td>2</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Aspiration pneumonia</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central venous catheter</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal failure</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoperitonium</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Petechia</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Venous thromboembolism</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Delirium</td>
<td>2</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Mortality (grade 5)</td>
<td>1</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

### Autoimmune reactions, any grade (n)

<table>
<thead>
<tr>
<th>Event</th>
<th>All patients</th>
<th>Responders</th>
<th>Nonresponders</th>
<th>P-value OR vs NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitiligo</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uveitis</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vasculitis</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>1</td>
<td>1</td>
<td></td>
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</tr>
</tbody>
</table>
Anti-tumor Responses of Infusion Products

Tumor reactive CD8+ TILs (x10^6)/kg

- No significant Tumor regression
- Best reduction in target lesion size from baseline > 20%

n = 24
P = 0.043

vs autologous tumor
vs allogeneic tumors
Induction and persistence of antitumor responses in peripheral blood

- Tested against autologous tumor
- Tested against allogeneic tumors

Color code:
- Complete response
- Partial response
- Stable disease
- Progressive disease

No significant Tumor regression
Best reduction in target lesion size from baseline > 20%

n = 24
P = 0.0034
Induction of antitumor responses in the blood

Peripheral Memory

CR (+38 months)
Conclusions

• Adoptive cell therapy using lymphodepleting chemotherapy and IL-2 is feasible and safe to use at a European cancer center.

• Toxicity is manageable in a normal department of oncology.

• Complete and long-lasting responses can be obtained using intermediate dose IL-2 and complete responses can develop slowly over months.

• Clinical response correlates to tumor-reactivity in the T cell infusion product and to induction and persistence tumor-reactive T cells in the peripheral blood.
ACT clinical Trials at CCIT


  - Andersen et al, In submission, 2015

- **2014** - (MM1409): Phase III (168 pts): TIL + HD bolus IL-2 vs Ipilimumab

- **2014** - (MM1413): pilot study (12 pts): TIL + decrescendo IL-2 + IFN-α

- **2014** - (MM1414): pilot study (12 pts): TIL + decrescendo IL-2 + Vemurafenib

- **2015** - (GY1508): pilot study (6 pts): TIL + decrescendo IL-2 (C. Ovarii)

- **2015/16**: pilot study (6 pts): TIL + decrescendo IL-2 (Renal Cell Carcinoma)
ACT in combination with IFN-α

7 of 12 patients treated
ACT in combination with BRAF-inhibitor

5 of 12 patients treated
Patient cases
Patient MM909.26

- 47 y/o man
- **2006:** surgery for primary melanoma in the face, level IV, Breslow thickness 5 mm. Neck lymph node exairesis due to pos. sentinel node.
- Resection of several local recurrences
- **2012, January:** Resection of metastasis in the left lung
- **2012, April:** PET/CT pos. metastases in the small intestines + m. lattisimus dorsi
  - un-resectable
- **2012, May:** Interleukin-2 + Interferon-α2b
  - After first Interferon-α2b → discontinuation due to ileus and small bowel-obstruction → acute surgery with tumor- and bowel-resection
Patient MM909.26

• **2012, Juli:** Ipilimumab 3 vs 10 (4 courses)
  – Pseudo-progression, decreasing LDH

• **2012, October:** tumor-resection and TIL production (cryopreservation of TIL).

• **2013, January:** ileus and bowel-obstruction (several metastatic lesions in the intestines → resection of metastasis and small bowel → PD.

• **2013, January:** palliative radiotherapy (5 Gy x 5): tumor in the upper right thigh.
  • The weeks before T cell therapy: weight loss, several blood transfusions, pneumonia (i.v. Antibiotics), diarrhea, painful swollen leg
Patient MM909.26

- **2013, February**: ACT
  - Day -7 to -1: chemotherapy
  - Day 0: infusion of $131 \times 10^9$ TILs
  - Day 0-2: decrescendo IL-2 - (71 MIU, 60%)
- **Adverse events**
  - Day 2:
    - Capillary leak syndrome/septic shock,
    - Hypotension (hemodynamic unstable)
    - Respiratory distress
    - Decreasing diuresis
    - No beds in ICU $\rightarrow$ i.v. methylprednisolone 40 mg

Baseline PET/CT January 16, 2013

Palliative radiotherapy
Patient MM909.26

- Day 6: invagination and bowel obstruction + aspiration to the lungs and respiratory arrest after CT scan
  - Intubation, ICU, iv antibiotics
  - Resection of tumor and bowel (multiple metastasis in the intestines)

- Recovers completely
- Prolonged hospital stay (39 days), drug fever
Baseline

8 weeks: PR

CR according to RECIST after 13 months

CR ongoing 30 months after TIL

CR verified with MRI

16-01-2013

04-04-2013
Patient MM0909.17

- 49 y/o man
- 1996: resection of primary melanoma (SSMM) right shoulder blade, Clarks level 4
- 2006, July: recurrence.
  - Right axillary lymph node exairesis, 7/20 lymph node metastases.
  - PET/CT: 5 small liver metastases and 1 lymph node metastasis (right neck).
- 2006, November: Interleukin-2 + Interferon-α2b (2 courses)
  - Progression of liver metastases
- 2007, January: ipilimumab (4 courses)
  - Grade 2 diarrhea
  - Progression of liver metastases + new lesion and 1 liver portal lymph node metastasis.
Patient MM0909.17

- 2007, April: re-induction with Interleukin-2 + Interferon-α2b (4 courses)
  - Partial response (regression of liver metastases)
- 2008, March: progression of liver portal lymph node metastasis
  - Removed by surgery. Continued regression of liver metastases.
- 2008, June: progression
  - Lymph node metastases on the left and right side of the neck
- 2008, July: ipilimumab re-induction (4 courses)
  - Grade III diarrhea (pancolitis), hospitalized, CRP 2000, treated with Methylprednisolone + Remicade (infliximab)
  - SD
Patient MM0909.17

- **2009, May**: Suspicious lymph nodes on the neck.
  - Right neck lymph node exairesis with metastases in 1 of 23 lymph nodes.
  - Biopsies from left side neck lymph nodes → no malignant cells.
  - CT: No malignant disease → continues control

- **2011, April**: bowel invagination, acute surgery → melanoma metastasis in the bowel (8 x 5 x 5 cm)
  - CT: No malignant disease .

- **2011, August**: Recurrence
  - PET/CT: PET-pos. lymph node on the left side of the neck, mesenteric metastasis + new liver portal lymph node metastasis, suspicion of 1 liver metastasis (small).

- **2011, August**: Interleukin-2 + Interferon-α2b re-induction (2 courses)
  - Progression of mesenteric metastasis (4 x 2.5 cm) and lymph node in liver hilus (1.6 cm)

- BRAF-wt
Patient MM0909.17

- 2011, November: referred to us for ACT
- Surgery (TIL production): Mesenteric lesion (4 x 2.5 cm)
- Baseline PET/CT: liver hilus lymph node metastasis (3.7 cm)

Baseline PET/CT (January 24, 2012)
Patient MM0909.17

• **2012, January**: Admitted for ACT, PS 0, no comorbidity, no brain mets.
  – Day -7 to -1: cyclophosphamide + fludarabine
  – Day 0: infusion of $143 \times 10^9$ TILs
  – Day 0-5: decrescendo IL-2 (131,4 MIU, 100%)

• **Adverse events**
  – Chemotherapy: Fatigue, nausea, hyponatriemia grade 3
  – Grade 4 leucopenia, neutropenia, lymphopenia, thrombopenia → blood transfusion and prophylactic antibiotics - tazocin (piperacillin/tazobactam) + gentamycin
  – TIL: fever
  – Central line infection, Klebsiella Pneumonia and Staphylococcus Aureus (blood culture neg) → removal of central line, followed by oral Dicloxacillin (14 days)
Patient MM0909.17

Baseline PET/CT (January 24, 2012)

Before TILs

Tumor resected 7 month after ACT

6 months (August 9, 2012): confirmed PR but slightly increase in PET-activity

NED 43 months after ACT
Acknowledgements

From CCIT, Herlev Hospital, Denmark

**Inge Marie Svane**
Marco Donia
Eva Ellebæk
Troels Holz Borch
Trine Zeeberg Iversen
Per Kongsted
Julie Westerlin Kjeldsen
Per thor Straten
Mads Hald Andersen
Özcan Met
Tobias Wirenfeldt Klausen
Laboratory of Hematology, Herlev Hospital, DK

**Others**
Doctors and nurses at Dept. of Oncology, Herlev Hospital, DK
Lisbeth Hölmich Dept. of Plastic Surgery, Herlev Hospital, DK
Helle Hendel, Dept. of Clinical Physiology, Herlev Hospital, DK
Lars Bastholt, Dept. of Oncology, Odense Hospital, DK
Henrik Schmidt, Dept. of Oncology, Aarhus Hospital, DK