Disclosure of Interest

Shigetaka Shimodaira

I declare no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
• Introduction
  • Dendritic cell (DC) and immune cells under cancer environment
  • Cancer mortality and incidence in Japan
• Manufacturing of a DC Vaccine and Wilms’ Tumor Gene 1 peptides-pulsed DC (WT1-DC) vaccination
• Immune monitoring with tetramer assay and enzyme immunosorbent (ELISPOT) assay
• DC vaccination technology
  • Granulocyte colony-stimulating factor (G-CSF) and DC vaccine and vaccination
  • Allogenic WT1-DC vaccination for acute leukemia
In the last few years of his life, Dr. Ralph Steinman made himself into an extraordinary human lab experiment, testing a series of unproven therapies - including some he helped to create - as he waged a very personal battle with pancreatic cancer.
Cancer-associated antigens

Cancer cells

Cytokines

Mφ-mediated tumor inhibition

Plasma cell

Effecter immune cells

Immune suppressive factors

TGF-β / IL-10 / VEGF / PGE2 / PD-L1

Immune suppressive cells

Tolerogenic DCs

Myeloid-derived suppressor cells (MDSC)

Cancer cells

Dendritic cells (DCs)

NK / NKT / γδT

FoxP3+ Treg

Regulatory T cells

PD-L1/L2

PD1

CTLA4

CD25

CD4

CD8+

CTL

PD1

PD-L1/L2

Peptides on HLA Class I

Peptides on HLA Class II

CTL/Th1 / Th2 / Th17

CD4+

Th

CD4

Immune suppressive factors

TGF-β / IL-10 / VEGF / PGE2 / PD-L1
An accelerator and brakes
Induction of the specific immunity against cancer to be memorized

Dendritic cells (DCs)

 CTL/Th1 / Th2 / Th17

 CTL

 Peptides on HLA Class I

 Peptides on HLA Class II

 CD4+

 Th

 CD8+

 NK / NKT / γδT

 NK

 NKT

 CTL

 CTL/Th1 / Th2 / Th17

 CTL

 CD4+

 Th

 Immune suppressive cells

 FoxP3+

 Treg

 CTLA4

 CD25

 CD4

 PD1

 PD-L1/L2

 Cancer cells

 Myeloid-derived suppressor cells (MDSC)

 Tolerogenic DCs

 Immune checkpoint inhibitor

 Immune suppressive factors

 TGF-β / IL-10 / VEGF / PGE2 / PD-L1

 Mφ-mediated tumor inhibition

 Plasma cell

 Mφ-mediated tumor inhibition
Immune-related response criteria identify survivors among 227 patients enrolled in phase II studies of ipilimumab 10 mg/kg monotherapy that would have had progressive disease according to modified World Health Organisation criteria.


Hoos A. Ann Oncol. 2012;23:viii47-viii52
A model scenario demonstrating implications of delayed separation of survival curves.

HR_E = 1
HR_D
HR_{overall}

HR_E = early hazard ratio (before separation)
HR_D = delayed hazard ratio (after separation)
HR_{overall} = hazard ratio for entire curve

median survival time
Harrington-Fleming test

Hoos A. Ann Oncol. 2012;23:viii47-viii52
Projection of cancer mortality and incidence in 2014

(1) Expected number of cancer deaths by site (2014)

Males, All sites 217,600

Females, All sites 149,500
Projection of cancer mortality and incidence in 2014

(2) Expected number of cancer incidence by site (2014)

Males, All sites 501,800

Females, All sites 380,400
Conventional mature IL-4-DC (mIL-4-DC)

Mature interleukin (IL)-4-DCs were generated using monocyte-rich fractions from leukapheresis with granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-4 using the adhesion method.

Differentiation into mDCs

using OK-432 and PGE2

CD14-CD11c+ immature DC

Day5

Ag presenting molecules (MHC class I/II)
Costimulatory molecules (CD80/86, CD40)
CD 83 (antigen presentation and lymphocyte activation)
CCR7 (CD197, migration into the lymph nodes)

CD14-CD11c+CCR7+ mDC

Day6

OK-432 (Picibanil): a mixture originating from group A Streptococcus pyogenes
The number of DCs dependent on the number of apheresed monocytes.

Analysis of the yield of DCs.

The number of DCs and viability before cryopreserved.

n=102
Mean ± S.D.  15.5 ± 8.3
95.7± 4.6
Single color-flow cytometric analysis of the yield of DCs

Quality control (Verification)

- Viability: ≥70%
- Purity: Percentage of HLA-DR^+CD86^+: ≥70%
- Bacterial test: Negative
- Endotoxin test: < 0.25 EU/mL
- Mycoplasma negation examination: Negative

WT1 (Wilms’ Tumor Gene 1) peptides: the highest priority in cancer associated antigens

- Isolated as a cause gene of infant nephroma Wilms tumor.
- WT1 protein is overexpressed in every malignant tumor, which would potentially be a target of the cancer vaccine.
- For HLA-A*24:02 molecule, the peptide (modified WT1 peptide) replaced as the second amino acid of methionine (M) with tyrosine (Y) induces WT1-specific CTL more strongly than that of wild type.

- Compatibility of the WT1 class I peptide in Japanese: HLA-A*24:02 (60%), A*02:01 (20%), A*02:06 (15%)
- Compatibility of the WT1 class II helper peptide: HLA-DRB1*08:03, DRB1*15:01, DRB1*15:02, DPB1*05:01, DPB1*09:01 (approximately 90% of Japanese)

WT1-126 peptide-9 amino acid & HLA-A:02:01 molecule

Real time PCR and WT1-monoclonal Ab.
WT1 expression using immunohistochemistry
Poorly differentiated adenocarcinoma of the colon expressing WT1++HLA-class I+ HLA-class II+EMA+

EMA, epithelial membrane antigen; HLA, human leukocyte antigen; WT1, Wilms’ tumor 1.
Tetramer assay and Enzyme immunosorbent (ELISpot) assay for the detection of Wilms’ tumor1-specific T cells induced by dendritic cell vaccination.
Correlation between the ELISpot assay and the Tetramer assay

ELISpot assay

(WT1 specific spots/1 × 10^6 PBMCs)

Tetramer assay

( % WT1 Tetramer positive cells in the CD3^+CD8^+ )
WT1-DC vaccine and vaccination

1. DC vaccine therapy approved under “Advanced Medical Care in Japan”

2. DC manufacturing protocol:
   2.1. HLA-DNA typing to determine WT1 compatibility
   2.2. Apheresis: 165 mL mononuclear cells collected from 4 L of blood (COM-TEC; Fresenius Kabi, Germany)
   2.3. Adhesive monocytes cultured with IL-4 and GM-CSF
   2.4. Mature DCs induced by OK-432 (streptococcal preparation) stored in the gas layer of liquid N₂ until clinical use.

3. DC vaccination: 1–3 × 10⁷ DC injected into the axillary and inguinal areas with OK-432 (1–2 KE/dose) at intervals of 2 weeks for 7 sessions (1 course) during individual chemotherapy and radiotherapy.
Safety of WT1-DC vaccination (N=168)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Session</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin reaction (Erythema &gt; 3cm)</td>
<td>1st session</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>7th session</td>
<td>38%</td>
</tr>
<tr>
<td>Fever (Grade 1 or 2)</td>
<td>1st session</td>
<td>62%</td>
</tr>
<tr>
<td></td>
<td>7th session</td>
<td>48%</td>
</tr>
<tr>
<td>Acute allergy*</td>
<td>Grade 2</td>
<td>2 / 168</td>
</tr>
<tr>
<td>Pneumonitis† (m-PLS)</td>
<td>Grade 3</td>
<td>1 / 168</td>
</tr>
</tbody>
</table>

*, Neck and anterior skin eruption; †, Chemotherapeutic drugs including CPM and MTX, other than DC vaccine.
100 patients with adenocarcinoma, including 22 with lung, 18 with breast, 9 with stomach, 25 with colorectal, and 26 with pancreatic adenocarcinoma

The presence of WT1-specific CTLs according to the following criteria

**Tetramer analysis**: 1) comprising at least 0.06% in the CD3\(^{+}\)CD8\(^{+}\) -subset of counted 50,000–100,000 lymphocytes, or 2) forming a clustered and not diffuse population.

**ELISPOT assays**: 1) at least 15 WT1-specific spots per \(1 \times 10^6\) PBMCs and 2) at least 50% more WT1-specific spots than negative peptide (HIV peptide) spots.
Induction of antigen-specific cytotoxic T lymphocytes by chemoradiotherapy in patients receiving WT1-targetted DC vaccinations for pancreatic cancer.

(A) WT1-peptide/HLA-A*24:02 tetramer analysis. Percentages represent the proportion of tetramer-positive cells in the total CD8$^+$ T cell population.
(B) Number of IFN$\gamma$ producing clones in ELISpot assays with WT1$^{235-243}$ peptide (HLA-A*24:02). Black square, WT1; white square, negative control.

Inclusion criteria ($N=354$)
- expected prognosis: over 4 months
- WBC > 2,500 cells/$\mu$L
- Hb > 7.0 g/dL
- 70,000 counts/$\mu$L

Post-operative recurrence? or inoperable?

Inoperable ($N=275$) → Combined chemotherapy during DC vaccine?

Yes $N=20$

Cases for analyses ($N=255$)

Administration of granulocyte colony-stimulating factor (G-CSF, 75 μg of filgrastim) 16–18 h prior to apheresis increase in the number of monocytes.

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Mean±S.D.</td>
<td>410±195</td>
<td>586±238</td>
</tr>
</tbody>
</table>

*p<0.0001*
mIL-4-DC vaccine primed with G-CSF.

Mean ± S.D.  

<table>
<thead>
<tr>
<th>Group</th>
<th>Total number of DC (×10⁷)</th>
<th>Mean</th>
<th>± S.D.</th>
<th>G-CSF(-)</th>
<th>24~96h</th>
<th>&lt;24h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14.4</td>
<td>±8.0</td>
<td>n=35</td>
<td>n=26</td>
<td>n=47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.7</td>
<td>±7.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.8</td>
<td>±8.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p<0.0001

<table>
<thead>
<tr>
<th>Group</th>
<th>DC/Monocyte-ratio (%)</th>
<th>Mean</th>
<th>± S.D.</th>
<th>G-CSF(-)</th>
<th>24~96h</th>
<th>&lt;24h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15.2</td>
<td>±6.9</td>
<td>n=35</td>
<td>n=26</td>
<td>n=47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.1</td>
<td>±7.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.0</td>
<td>±7.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p=0.002

p=0.004
mIL-4-DC vaccine primed with G-CSF.

<table>
<thead>
<tr>
<th></th>
<th>CD11c</th>
<th>CD14</th>
<th>CD80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-CSF(-)</td>
<td>98.9 ± 1.9</td>
<td>97.7 ± 6.2</td>
<td>47.1 ± 20.2</td>
</tr>
<tr>
<td>24-96h</td>
<td>99.9 ± 0.8</td>
<td>3.7 ± 8.1</td>
<td>46.5 ± 20.5</td>
</tr>
<tr>
<td>&lt;24h</td>
<td></td>
<td></td>
<td>57.6 ± 17.0</td>
</tr>
<tr>
<td>n=35</td>
<td>n=26</td>
<td>n=47</td>
<td>n=47</td>
</tr>
</tbody>
</table>

\[ p = 0.004 \]
\[ p = 0.040 \]
\[ p = 0.012 \]
\[ p = 0.015 \]
Administration of G-CSF (75 μg of filgrastim) 16–18 h prior to apheresis revealed up-regulation of matrix metalloproteinase (MMP)-9 expression in monocytes.

DNA array analysis on CD14+ monocytes between rhG-CSF prior to apheresis and post administration: RT² Profiler™PCR Array (QIAGEN)
The acquisition of WT1 antigen-specific cytotoxic T cells by mIL-4-DC vaccine harboring HLA class-A*24:02/WT1 peptide.

<table>
<thead>
<tr>
<th>G-CSF (75 μg of filgrastim) 16–18 h prior to apheresis</th>
<th>+</th>
<th>–</th>
<th>$p$ value¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (N=95)</td>
<td>70</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>The number of DCs (1 course)</td>
<td>$8.57\pm2.40\ (\times10^7)$</td>
<td>$7.98\pm2.55\ (\times10^7)$</td>
<td>$p=0.196$</td>
</tr>
<tr>
<td>Ratio of induction of WT1-CTL (tetramer⁺/CD8⁺T-cells ≥0.8%)</td>
<td>28 (40%)</td>
<td>5 (20%)</td>
<td>$p&lt;0.0001$</td>
</tr>
<tr>
<td>The median of WT1-CTL (%) (tetramer⁺/CD8⁺T-cells)</td>
<td>$0.060\ (0.01-7.11)$</td>
<td>$0.057\ (0.00-0.39)$</td>
<td>$p=0.471$</td>
</tr>
</tbody>
</table>

¶, Mann-Whitney $U$-test
Patient: 12 year-old-girl

Disease onset: March, 2005
Laboratory data:
<Peripheral Blood>  WBC  191,170/μl  (blast 82%)
<Bone marrow>  NCC  720.0 x 10^3/μl  (blast 85.8%)
  CD9 78%, CD10 95%, CD19 95%, CD20 35%, CD22 67%,
  CD24 89%, CD33 0%, CD34 95%, HLA-DR 97%, Cy-μ 1%,
Karyotype:  46,XX  [20]
  WT1 mRNA:  1.1 x 10^4 copies/μgRNA

Diagnosis: B-cell precursor acute lymphoblastic leukemia

Figure 2. Saito S, et al: *Cytotherapy*. **2015;17:330-5.**

**A**

Before vac.  
After vac. #7  
After vac. #14  
4th relapse

CD8 FITC

10^2 10^3 10^4

Tetramer PE

10^2 10^3 10^4

**B**

Spot-forming cells within 5 x 10^3 CD8+ T cells

WT1  
Control

Before vac.  
After vac. #7  
After vac. #14  
4th relapse
Supplementary Figure 1. Saito S, et al: Cytotherapy. 2015;17:330-5.
Conclusion

• DC-based immunotherapy targeting WT1 was indicated to be safe and feasible for the management of advanced cancers exhibiting ‘delayed separation’ curve in some patients.

• DC vaccine primed with G-CSF harboring HLA class-A*24:02/WT1 peptide exhibited a significant increase in the acquisition of WT1 antigen-specific cytotoxic T cells.

• Allogenic WT1-DC vaccination may be safe, tolerable, and even feasible for pediatric donors and patients with relapsed leukemia after hematopoietic stem cell transplantation (HSCT). This strategy would be relevant to the scope of the development of personalized therapy in HSCT.

• In future, the blockade of immune checkpoints in combination with DC-vaccination would be promising therapeutic strategies to activate therapeutic anti-tumor immunity for advanced cancers and hematological malignancies.