Dendritic cell therapy as cellular immunotherapy

Prof. Dr. Evelien Smits
1 March 2018
Acute myeloid leukemia (AML)… the facts

Data from: NCI SEER, 2005-2011 cohort

5-yr OS: 26.2%

high relapse rate
75% in CR1 relapse <2yr
probability CR2 only 25%

Relapse prevention is a major therapeutic goal

THE PROBLEM
Antitumor immune response

Degli-Esposti and Smyth, Nat Rev Immunol 2005
Dendritic cell vaccine against cancer

Cancer cell

Non-active killer cell

Active killer cell

Cancer cell

Transfer of cancer marker through gene transfer

Dendritic cell

‘license to kill’

ATTACK

Cell death

Dead cancer cell
Drug discovery

1. How to obtain enough dendritic cells?

2. How to load them with a tumor antigen?

3. Which tumor antigen?

4. Do these cells activate tumor-specific T cells?
Preparation of dendritic cells

- Buffy coats
- CD14+ cell isolation
- WT1 mRNA
- Electroporation

**WHITE BLOOD CELLS**

**MONOCYTES**

**MATURE DENDRITIC CELLS**

*In vitro culture*
6 days GM-CSF + IL-4
+ 2 days TNF-α/PGE2/KLH
mRNA electroporation as DC loading method

(Van Tendeloo et al, Blood 2001)

- Full-length antigen: polyepitope vaccination
- Highly efficient, clinically safe and transient modification method
- Can be used for every patient (every haplotype)
Rationale of WT1-targeted DC vaccine

Wilms’ tumor 1 protein as antigen of choice (Anguille et al, Leukemia 2012)

- Overexpressed in AML and in many solid tumors
- Indispensable for leukemogenesis process
- Proven immunogenicity in cancer patients

The Prioritization of Cancer Antigens: A National Cancer Institute Pilot Project for the Acceleration of Translational Research

Martin A. Cheever,1 James P. Allison,2 Andrea S. Ferris,3 Olivia J. Finn,4 Benjamin M. Hastings,3 Toby T. Hecht,5 Ira Mellman,7 Sheila A. Prindiville,6 Jaye L. Viner,6 Louis M. Weiner,6 and Lynn M. Matrisian6

Table 3. Cancer antigen pilot prioritization: ranking based on predefined and preweighted criteria

<table>
<thead>
<tr>
<th>Antigens (rank/reference number and name)</th>
<th>Cumulative score</th>
<th>Therapeutic function (0.32)</th>
<th>Immunogenicity (0.17)</th>
<th>Oncogenicity (0.15)</th>
<th>Specificity (0.15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. WT1</td>
<td>0.81</td>
<td>0.75 (fair)</td>
<td>1.0 (trials)</td>
<td>1.0 (oncogenic)</td>
<td>0.54 (oncofetal)</td>
</tr>
<tr>
<td>2. MUC1</td>
<td>0.79</td>
<td>0.75 (fair)</td>
<td>1.0 (trials)</td>
<td>1.0 (oncogenic)</td>
<td>0.23 (post-translational)</td>
</tr>
<tr>
<td>3. LMP2</td>
<td>0.78</td>
<td>0.75 (fair)</td>
<td>1.0 (trials)</td>
<td>0.34 (viral)</td>
<td>1.0 (absolute)</td>
</tr>
</tbody>
</table>
Preclinical proof of concept

WT1 cDNA isoform D (KTS+ exon 5+)

RNA transcription + 5’ capping + 3’ polyA

electroporation

WT1 expression in electroporated DC

Van Driessche et al, Leukemia 2005
Van Driessche et al, Cytotherapy 2009

© S. Anguille
Center for Cell Therapy and Regenerative Medicine (CCRG) @ UZA

- Multidisciplinary research center for innovative cell therapies
- Personalized cell therapy using dendritic cells (autologous) or limbal epithelial cells (autologous or allogeneic)
- First patient treated in 2005
- GMP accreditation: February 2015, as first Belgian academic cell therapy center
Vaccine preparation

**WHITE BLOOD CELLS**

- Apheresis

**WT1 mRNA**

- Electroporation

**MATURE DENDRITIC CELLS**

- In vitro culture
  - 6 days GM-CSF + IL-4
  - + 2 days TNF-α/PGE2/KLH

**MONOCYTES**

- CliniMACS
  - CD14+ cells

**Smits et al, Methods Mol Biol 2016**
Vaccination

Figuur: S. Anguille
Acute Myeloid Leukemia
Patients & Methods

<table>
<thead>
<tr>
<th>pre</th>
<th>w0</th>
<th>w2</th>
<th>w4</th>
<th>w6</th>
<th>post</th>
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<tbody>
<tr>
<td>IM (in vitro)</td>
<td>vaccine 1</td>
<td>vaccine 2</td>
<td>vaccine 3</td>
<td>vaccine 4</td>
<td>IM (in vitro)</td>
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<tr>
<td>BM biopsy</td>
<td>MRD</td>
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<td>MRD</td>
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- Evaluation of **clinical** responses
  - **Bone marrow (BM) biopsy**: monitor **hematological** remission
  - **Minimal residual disease (MRD)**: monitor **molecular** remission
    - analysis of WT1 mRNA expression levels by qRT-PCR
    - WT1 mRNA is a tumour marker in AML and an early predictor of relapse
WT1 transcript levels in blood is a sensitive MRD marker in AML

- Quantification of WT1 RNA copy number in PB by qRT-PCR
- Correlates with other molecular MRD markers, eg AML-ETO t(8;21) (Cilloni et al, 2002).
- **Absence of normalization** of WT1 levels following induction chemotherapy is always indicative of imminent **clinical relapse** (TTR 7 mo)
- A rise of WT1 mRNA copies after initial normalization (= molecular relapse) is absolute predictor of **clinical relapse** (TTR 12 mo)

(Cilloni et al, 2008 & 2009)
## Patients & Methods

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<td>IM (in vivo)</td>
<td>vaccine 6</td>
</tr>
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### Evaluation of **immunological** responses

- **Immune monitoring (IM) in vitro**: cytokine secretion, flow cytometry for lymphocyte subsets, determination of WT1-specific T-cells
- **Immune monitoring (IM) in vivo**: delayed-type hypersensitivity (DTH) skin test with immunohistochemistry of injection site biopsy
• Induction of **molecular remission** following DC vaccination (9/24)
  • ↓WT1 RNA expression levels in PB/BM under DC vaccination

Van Tendeloo et al, PNAS 2010
Transiently stable disease (SD) in AML patient under DC vaccination (4/30)
AML: Overall survival

Anguille et al, Blood 2017

A. All patients (n=30)

mOS = 41.8 m [81.1 m]

B. Patients < 65 yr (n=15)

mOS = n.r. [82.0 m]

C. Patients ≥ 65 yr (n=15)

mOS = 17.9 m [81.1 m]

D. Responders (n=13) vs. non-responders (n=16)

mOS = n.r. [82.0 m]
mOS = 12.4 m [65.0 m]

P = 0.0105
Immunological effects

Positive DTH skin test (induration ≥ 2 mm in all patients)

- Bottom left: KLH/WT1/DC
- Top left: KLH only
- Top middle: WT1/DC
- Bottom middle: NaCl
- Top right: DC only
Immunological results

Significant increase in WT1-specific IFN$_{\gamma}$ and TNF$_{\alpha}$, but not IL-5 producing CD8+ DIL, solely in the long-term survivor group.

Anguille et al, Blood 2017
Solid tumors
## Solid tumors: Study schedule

<table>
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<th>Study schedule</th>
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<td><strong>pre</strong></td>
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<td>vaccine 1 IM</td>
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<tr>
<td>Imaging TM</td>
<td>TM</td>
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<tr>
<td><strong>T0</strong></td>
<td>vaccine 2</td>
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<td></td>
<td>TM</td>
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<tr>
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<td>vaccine 5 IM</td>
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<tr>
<td></td>
<td>TM</td>
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<tr>
<td><strong>w8</strong></td>
<td>Vaccine 6 in vitro &amp; in vivo Imaging</td>
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<td>TM</td>
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<tr>
<td><strong>w10</strong></td>
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<td></td>
<td>TM</td>
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<tr>
<td><strong>w12</strong></td>
<td>Vaccine 8</td>
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<tr>
<td></td>
<td>TM</td>
</tr>
<tr>
<td><strong>w14</strong></td>
<td>T2</td>
</tr>
</tbody>
</table>
Glioblastoma multiforme

Overall survival from Dx (GBM patients, n=9)

100

75

50

25

14.7 months
Stupp et al.,
N Engl J Med 2005

Time (months)

6
12
18
24
30
36
42
48
54
60
Metastatic breast cancer

Median overall survival: 43.8 months

Overall survival from M1 (MBC patients, n=11)

21.7 months
Kiely et al., J Clin Oncol 2011
Malignant pleural mesothelioma (n=10)

- Median OS from start of chemotherapy: **35.7 m** vs. 22 months (Hillerdal et al, J Thoracic Oncology 2008)
Clinical use of dendritic cells for cancer therapy

Sébastien Anguille, Evelien L. Smits, Eva Lion, Viggo F van Tendeloo, Zwi N Berneman

Since the mid-1990s, dendritic cells have been used in clinical trials as cellular mediators for therapeutic vaccination of patients with cancer. Dendritic-cell-based immunotherapy is safe and can induce antitumour immunity, even in patients with advanced disease. However, clinical responses have been disappointing, with classic objective tumour response rates rarely exceeding 15%. Paradoxically, findings from emerging research indicate that dendritic cell-based vaccination might improve survival, advocating implementation of alternative endpoints to assess the true clinical potency of dendritic cell-based vaccination. We review the clinical effectiveness of dendritic cell-based vaccine therapy in melanoma, prostate cancer, malignant glioma, and renal cell carcinoma, and summarise the most important lessons from almost two decades of clinical studies of dendritic cell-based immunotherapy in these malignant disorders. We also address how the specialty is evolving, and which new therapeutic concepts are being translated into clinical trials to leverage the clinical effectiveness of dendritic cell-based cancer immunotherapy. Specifically, we discuss two main trends: the implementation of the next-generation dendritic cell vaccines that have improved immunogenicity, and the emerging theory of combination of dendritic cell vaccination with other cancer therapies.

Introduction

2013 marked the 40th anniversary of the discovery by Cohn and Steinman of a new type of immune cell:

Clinical efficacy of DC cancer therapies

- Clinical benefit in terms of **objective clinical responses** is real, but limited
- Where **overall survival** has been monitored, there is clear evidence that DC immunotherapy can have clinical benefit

Safety

The safety of dendritic cell-based immunotherapy has been well documented in many phase 1 clinical studies.
Clinical trials @ CCRG since 2005

- DC VACCINE FOR LEUKEMIA
  - PHASE I/II
  - 2005, 2009

- DC VACCINE FOR MESOTHELIOMA Glioblastoma
  - PHASE I/II
  - 2012

- DC VACCINE FOR MULTIPLE SCLEROSIS
  - PHASE I/II
  - 2016

- DC VACCINE FOR CMV HIV
  - PHASE I/II
  - 2007, 2012

- PHASE II MULTICENTRE
  - 2012
- To the bedside and back -

**Optimizing DC vaccines**

1. Multi-antigen approach

2. Immunostimulatory cytokines: IFN-α and IL-15
   Activate T cells, NK cells and γδ T cells

3. Silencing of programmed death ligands using silencing RNA
RHAMM – CD168

Willemen et al, Oncotarget 2016
Immunostimulatory cytokines and PD-L silencing:

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- Johan Van den Bergh
- Yannick Willemens
- Heleen Van Acker
- Maarten Versteven
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- Different recruiting centers in Belgium