RNA-pulsed Dendritic Cells for Immunotherapy of Cancer

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DC biology: multiple signals for lymphocytes

**GM-CSF**

**Mono** → **IDC** → **Maturation Cocktail** → **mDC** → **Tumor Immunity**

**signal 1**
- RNA-encoding tumor antigens

**signal 2**
- positive costimulation
- negative costimulation

**signal 3**
- IL-12\(^{high}\)
- IL-10\(^{low}\)

**Th1 / CTL**

**NK**
Dendritic cell vaccines

Third generation dendritic cell vaccines: fast production (3d) and TLR activation
Generation of mature DCs in three days

**7-day protocol**

- **d0**: Monocytes
  - IL-4 GM-CSF
- **d1**: iDC
  - IL-4 GM-CSF
- **d2**: 3d-mDC
  - Maturation Cocktail
- **d3**: iDC
  - Maturation Cocktail
- **d4**: 7d-mDC

**3-day protocol**

- **d0**: Monocytes
  - IL-4 GM-CSF
Dendritic cell maturation cocktails utilizing TLR3 and TLR7/8 ligands

<table>
<thead>
<tr>
<th>Cocktail</th>
<th>Inflammatory cytokines interferons/additives</th>
<th>TLR ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>4C</td>
<td>TNF, IL-1β, IL-6, PGE2</td>
<td>none</td>
</tr>
<tr>
<td>Jonuleit</td>
<td>TNF, IL-1β, IFN-γ, PGE2</td>
<td>poly(I:C)</td>
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<tr>
<td>5C</td>
<td>TNF, IL-1β, IFN-γ, PGE2</td>
<td>TLR 3</td>
</tr>
<tr>
<td>5C + R848</td>
<td>TNF, IL-1β, IFN-γ, PGE2</td>
<td>poly(I:C)</td>
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<td>R848</td>
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<tr>
<td>5C + CL075</td>
<td>TNF, IL-1β, IFN-γ, PGE2</td>
<td>poly(I:C)</td>
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<td>CL075</td>
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<td></td>
<td></td>
<td>TLR 3</td>
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<td>TLR 7/8</td>
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Comparable phenotypes of 3d versus 7d mDC
Expression of costimulatory molecules: impact of time and maturation cocktails
Antigen expression is strong in 3d mDC after electroporation of ivt-RNA.
3d mDC migrate towards CCR7 signals

Chemokine directed migration

![Diagram showing mDC migrating towards CCL19 signals after 2 hours.](image)
IL-12(p70) secretion from 3d mDC upon CD40 ligation: signal 3 assay

24 h

cytokine response

mDC + murine fibroblasts

IL-12p70 [pg/ml]

IL-10 [pg/ml]

4C
5C
4C
5C
5C
5C

R848
CL075

n.s.

p=0.014
p=0.008

10^0
10^1
10^2
10^3
10^4
10^5

HelmholtzZentrum münchen
German Research Center for Environmental Health
Innate immunity: TLR 7/8 signals improve NK cell activation

activated NK cells (CD3-) CD56+/CD69+

DC-NK Cocultures

HelmholtzZentrum münchen
German Research Center for Environmental Health
Innate immunity: TLR 7/8 signals improve NK cell function

1) Measure IFN\(_\gamma\) in 24 h medium

2) Measure killing of K562 targets
Adaptive immunity: Th1/Tc1 polarization of allogeneic lymphocytes using TLR-activated 3d mDC

Generate 3d-mDC

7d coculture mDC + allo-T cells

ICS phenotype of T cells (5 h PMA/ionomycin)

IL-4

unstim. 4C

5C + R848 5C + CL075

IFN-γ

% IFN-γ positive T cells

% CD4 IFN-γ % CD8 IFN-γ

p < 0.01

w/o 4C 5C+ R848 5C+ CL075

* **

Helmholtz Zentrum München
German Research Center for Environmental Health
Adaptive immunity: Superior induction of antigen-specific T cells using TLR-activated 3d mDC

Melan-A pulsed mDC

7d coculture of mDC and autologous T cells

CD8

unstim.

21.2

5C

14.5

5C + R848

21.4

5C + CL075

22.8

% specific lysis

E:T

0 20 40 60 80 100

10

5C

4C

w/o DC

4C

5C

5C+R848

5C+CL075

w/o DC

Mel 624.38

Mel A375
TLR-activated 3d mDC display characteristics important for anti-tumor vaccines

- Fast production of mDC (3 days)
- High expression of antigens (ivt-RNA)
- Stable phenotype after maturation
- High IL-12p70 and low IL-10 production
- Superior activation NK cells
- Superior activation of antigen-specific T cells
- Polarization of Th1/Tc1 responses
Planned Clinical Phase I/II Trial:

**High risk AML patients with minimal residual disease**

Supported by SFB-455, BayImmuNet and HGF Alliance for Immunotherapy of Cancer

RNA-encoding tumor antigens
Survival in relation to karyotype

Haferlach et al. JCO 2003  

p < 0.0001
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