Therapeutic vaccination against HPV16-induced disease

Cornelis J.M. Melief MD, PhD
HPV infection
HPV infection cycle is linked to keratinocyte differentiation program

Normal viral life cycle

Viral protein expression

HPV virion
Episomal DNA
Early proteins
Late proteins

E1, E2, E5
E6, E7
L1, L2
E4
E1, E2, E5
Natural history of cell-mediated adaptive immune response to high risk HPV16

Immunity
- CD4+ Th1/Th2 immunity to E2, E6, E7 & L1
- CD8 immunity to E6 (E7?)
- T cells Circulate & Migrate

Immune failure
- No E6, E7 CD4+ immunity
- Impaired CD4+ T-cells
- Infrequent CD8+ T-cells
- Regulatory T-cells

>99% minority CD4+ Th1/Th2 immunity to E2, E6, E7 & L1

Impaired CD4+ T-cells

Infrequent CD8+ T-cells

Regulatory T-cells
Overview of different types of potential therapeutic vaccines

- **Viral vector based vaccines:** TA-HPV, MVA
tremendous problems with antigenic competition by vector sequences
- **DNA vaccines**
  Inefficient way to achieve long-lived antigen expression in DC
- **DC based vaccines**
laborious and expensive. Direct in vivo DC targeting of antigen more attractive
- **Protein vaccines:** TA-CIN, E6E7 Iscomatrix
  relatively inefficient CD8 CTL induction
- **Peptide vaccines:** Minimal HLA class I binding peptides
  exogenous loading of MHC class I molecules→ tolerance. Lack of proper CD8 memory responses due to lack of CD4 help

→ Synthetic Long Peptide vaccines
Long-peptide vaccine comprises both a HPV16 E7 CD8+ and a CD4+Th-epitope

Minimal CTL peptide epitope E7^{49-57} : RAHYNIVTF

Long peptide E7^{43-77} : GQAEPD{RAHYNIVTF}CCKCDSTLRLCVQSTHVDIR

Long peptide vaccine in HPV16 Mouse Tumour Model

GQAEPDRAHYNIVTFCCCKCDSTLRLCVQSTHVDIR

Zwaveling et al, J. Immunol., 2002
Antigen presentation after vaccination with extended peptides is predominantly focused onto DC in the draining lymph node

Bijker et al EJi, 2008

A), B) Cells presenting antigen to CFSE-labeled OT1 T cells 2 days after peptide injection
Comparison of SLP with intact protein vaccination for access to cross-presentation pathways for protective CD8+ and CD4+ T cells (Zhang et al. J. Biol. Chemistry 284, 9184, 2009)

- HIV-NEF-derived SLP are superior to intact NEF protein, because:
  1. SLP traffick not only to endosomes, but also to the cytosol
  2. SLP activate CD4+ and CD8+ cells. Intact protein activates mainly CD4+ cells
  3. SLP vaccination protects much better than protein against challenge with a lethal does of recombinant –Nef vaccinia virus
  4. SLP need to be properly adjuvanted
Efficiency of processing by mouse DC of long peptide versus intact protein

**Kinetics of MHC class I antigen presentation.** To determine the efficiency of MHC class I presentation of exogenously loaded antigen, cross-presentation, DC were cultured with soluble Ovalbumin protein or the derived synthetic long peptide (OVA-31) encoding the immunodominant MHC class I epitope, SIINFEKL presented in the context of Kb molecules. DC were pulsed for 0, 1, 2, 3, 4, 5, and 24 h with the antigens followed by extensive washing and mild paraformaldehyde fixation to inhibit further processing beyond above mentioned timepoints. DC were then co-cultured further O/N in the presence of the CD8 T cell hybridoma (B3Z) which produces IL-2 upon recognition of Kb/SIINFEKL.
License to Kill

TLR Ligands → CD40L → CD40 → iDC → activation → mDC

T-helper cells produce IL-2, which promotes costimulation and activation of T-killer cells.

Clinical grade HPV16 therapeutic vaccine consists of synthetic overlapping long peptides comprising all potential CTL and Th epitopes.
Phase I, end stage cervical cancer

Interferon γ Elispot assay

<table>
<thead>
<tr>
<th></th>
<th>Before vaccination</th>
<th>After vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E6-I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E6-II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E6-III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E6-IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E7-I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E7-II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Kenter, Clin Cancer Res, 2008
Vaccination against HPV-16 Oncoproteins for Vulvar Intraepithelial Neoplasia

Gemma G. Kenter, M.D., Ph.D., Marij J.P. Welters, Ph.D.,
A. Rob P.M. Valentijn, Ph.D., Margriet J.G. Lowik,
Dorien M.A. Berends-van der Meer, Annelies P.G. Vloan, Farah Essahsah,
Lorraine M. Fathers, Rienk Offringa, Ph.D., Jan Wouter Drijfhout, Ph.D.,
Amon R. Wifelman, Ph.D., Jaap Oostendorp, Ph.D., Gert Jan Fleuren, M.D., Ph.D.,
Sjoerd H. van der Burg, Ph.D., and Cornelis J.M. Melief, M.D., Ph.D.
Vaccination of 20 HPV16+ VIN3 patients with HPV16 SLP vaccine

Kenter et al. NEJM, 2009

HPV16-induced premalignant lesion of vulva

Non-specific symptoms: pain, itching, burning

Diagnosis: vulvoscopy, biopsies

Non-treated: can progress to cancer

Therapy: surgery, laser vaporization (mutilating)

Chronic disease: recurrence following standard treatment

Chronic disease: Only 1.3% resolves spontaneously
Trial Design, Phase II, HPV16+ Vulvar Intraepithelial Neoplasia (VIN III)

Endpoints

Immunology
- Proliferation assay
- IFNγ ELISPOT
- Cytokine analysis (CBA, ELISA)
- CD4/CD8 analyses (ICS)

On PBMC and Biopsies (VIN lesion, vaccination site)

Clinical responses
- Symptoms
- Change in lesion size
- Change in histology
- Change in HPV detection
Lymphocyte Proliferation Test (ex-vivo 6 days)

**pre-vac**

- cpm vs. medium, E6.1, E6.2, E6.3, E6.4, E7.1, E7.1, MRM

**post-vac**

- cpm vs. medium, E6.1, E6.2, E6.3, E6.4, E7.1, E7.1, MRM

**Cytokines (pg/ml)**

- IFNγ, TNFα, IL-10, IL-5, IL-4, IL-2

HPV16-SLP vaccination in VIN3
Clinical results at 24 months


Pre vax  Post vax

PR

CR

10 NR/PR,
9/9 CR

Average lesion size (square centimetre)

non-CR

CR

**

D
T-cell response after SLP® vaccination of VIN3 patients correlates with clinical outcome

Kenter et al, New England J. of Med, 2009
Peak of cytokine production after the first vaccination  Welters et al. PNAS 2010.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Large</th>
<th>Small</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-vac</td>
<td>0</td>
<td>0</td>
<td>0.52</td>
</tr>
<tr>
<td>1-vac</td>
<td>16.53</td>
<td>21.80</td>
<td>0.44</td>
</tr>
<tr>
<td>2-vac</td>
<td>9.24</td>
<td>23.53</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>3-vac</td>
<td>6.04</td>
<td>19.90</td>
<td>&lt;0.009</td>
</tr>
<tr>
<td>4-vac</td>
<td>13.34</td>
<td>28.25</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Tregs induced/boosted by vaccination only in patients with large VIN3 lesions

Welters et al. PNAS 2010.
HPV16-SLP vaccine induces:

- HPV16 specific T-cell proliferation in 20/20 VIN III patients.
- HPV16 specific IFNγ-producing CD4+ T-cells in 19/20 patients.
- IFNγ-producing CD8+ T-cells in 19/20 patients.
- Migration of HPV16 specific T-cells to vaccination site in 7/18 patients.
- Complete clearance of the VIN grade III lesion in:
  - 5/20 patients, 3 months after the last vaccination,
  - 9/19 patients, 12 months after the last vaccination.
- Partial clearance of VIN grade III lesion in 5/20 patients, 12 months after the last vaccination. Overall clinical benefit in 14 of 20 patients.
- Complete clearance of HPV 16 infection in 4/20 patients, 3 months after the last vaccination.
Conclusions (2)
HPV16-SLP vaccine in VIN3 patients

• Follow-up 24 months after the last vaccination:
  • All 9 patients with a CR still have a CR
  • 4/10 patients with NR or PR have developed micro-invasive vulvar carcinoma. All of these patients had VIN3 lesions of more than 10 years duration
Conclusions (3)

HPV16-SLP vaccine in VIN3 patients

• HPV16 SLP vaccine is able to restore the CD4$^+$ and CD8$^+$ T-cell response to HPV16 E6 and E7 in VIN3 patients.

• HPV16 SLP vaccine is able to induce clinical responses in 79% of vaccinated subjects (32% PR, 47% CR).

• The strength of the HPV16 SLP vaccine-induced CD4$^+$ T-cell response as measured by a combination of proliferation and IFN$\gamma$ production (LST, CBA, ELISPOT) correlates with clinical responses.

• The success of immunotherapy is determined by the ratio of vaccine-prompted effector T cells over CD4+$\cdot$CD25+$\cdot$Foxp3+$\cdot$ regulatory T cells

• Robust and durable response to synthetic long peptides is ascribed to simultaneous presentation of many class I and class II epitopes by Dendritic Cells in the absence of antigenic competition.
Synthetic Long Peptide vaccine concept

In silico prediction of epitopes for a particular HLA molecule

Production of overlapping long peptides

Selection by peptide binding assays

Repeat to select CD8⁺ and CD4⁺ T-cell epitopes for other HLA molecules

Processing and immunogenicity assays

Epitopes selected in vivo by patient DC

Does not use full array of patient HLA molecules

Full use of all different types of HLA molecules

Melief & Van der Burg, Nature Reviews Cancer 2008
Histology of completely cleared VIN lesion

Pre-vaccination

Post-vaccination
Sites of action of Immunotherapy of Cancer

Melief, Immunity, 2008
Chemo-immunotherapy of HPV16-positive established tumor TC-1 in mice

Treatment of tumor-bearing (TC-1) B6 mice with peptide vaccination in combination with cisplatin (Exp: Chemo-4)
Next generation of synthetic vaccines
Khan et al. J. Biol. Chem `2008

Fundamental study:

* Cell biology of TLR-L conjugates in DCs
  (Uptake, routing, antigen presentation)

* Immunological response (T-cell induction and Tumor protection)
The constructs...

* Longer peptides containing the **Ovalbumin CTL or Th epitope.**
* Longer peptides containing the **MuLV ENV 119-137 Th epitope.**

![Diagram showing the constructs](image)
Tumor protection by CTL and T helper vaccination with combined long peptide-TLR ligand conjugates EG7 (OVA) tumor

Day after tumor challenge

% survival

Pam3CysSK4
TLR2 ligand
Long peptide

naive (IFA + Pam)
Pam-conj(CTL)/Pam-conj(Th)
Pam-conj(CTL)
Pam-conj(Thelp)
Th pep+ CTL pep + Pam
Tumor protection experiment

RMA MuLV Leukemia (dependent on CD4+ T-cells)

Unpublished S. Khan, C. Britten

TLR-L conjugates promote prolonged survival
Local re-programming of tumor-specific T-cells by anti-CD40 agonistic antibodies allows systemic anti-tumor immunity with low toxicity

Local treatment induces a systemic CD8+ CTL response

Marieke F. Fransen, Ramon Arens
Experimental model

Adenovirus protein E1A-expressing tumor cells

Endogenous response

Van Mierlo et al. PNAS, 2002
Systemic anti-CD40 induces systemic anti-tumor CD8 CTL response

Van Mierlo et al. PNAS, 2002

Adenovirus protein E1A-expressing tumor cells

Endogenous response

Tumor Draining LN

Agonistic anti-CD40 3*100 µg, systemic = High dose, IV

Effector T-cells
Systemic anti-CD40 treatment of tumor bearing mice

- Strong anti-tumor CD8 T-cell response
- Effective tumor clearing

Strong systemic toxicity
Splenomegaly

Liver
Naïve

Liver
Anti-CD40 3*100 µg IV
Hypothesis:

Can we activate the anti-tumor CD8 T-cells without causing systemic toxicity?

- Lower dose
- Local injection
- Slow-release formulation
Lower dose: 30 microgram anti-CD40, different administrations

Percent survival vs. day post tumor inoculation graph:
- No treatment
- Local injection, 30 µg sc, slow release
  - naive
  - subc saline-local
  - subc montanide-local
- Systemic injection, 3 times 100 µg iv
  - IV-systemic
  - High dose, IV-systemic

Positive control
Toxicity: liver enzymes 24 hours after treatment with anti-CD40 agonist antibody

30 microgram anti-CD40

- ALAT
- ASAT
Kinetics of toxicity

**ALAT**
- **Naive**
- **anti-CD40. high dose IV**
- **anti-CD40. low dose montanide**

**ASAT**
- **Naive**
- **anti-CD40. high dose IV**
- **anti-CD40. low dose montanide**
Anti-CD40 in serum

Day 1

Amount of anti-CD40 injected, microgram

Concentration of anti-CD40 in serum, microgram/ml

- IV systemic
- Montanide subcutaneous
- Saline subcutaneous
Does the low dose treatment have to be local in the vicinity of a tumor?

![Graph showing percent survival over days after start treatment.]

- No treatment
- AntiCD40, high dose IV
- AntiCD40, low dose, montanide-Local
- AntiCD40, low dose, montanide-distant
CD70 expression

DC-activation in draining lymph node

- no treatment
- high dose, IV
- low dose, montanide

mean fluorescence

- draining LN
- Nondraining LN
High dose iv versus versus local treatment with anti-CD40

CTL response in blood

- No treatment
- High dose, IV
- Low dose, montanide

In vivo cytotoxicity

- No treatment
- High dose, IV
- Low dose, montanide

% Tetramer+ CD8+ in blood

- P=0.003
- P=0.03
- P=0.1

% specific killing

- P=0.0006
- p=0.04
- p=0.02
- p=0.04
Eradication of secondary tumor:

I: Secondary tumor
II: Secondary tumor
III: Treatment with anti-CD40 in montanide
IV: Treatment with anti-CD40 in montanide
Eradication of secondary tumor:

I. No treatment

II. High dose, I.V.

III. Low dose, montanide

IV. Low dose, montanide (secondary tumor only)

Tumors size mm$^3$

days after tumor challenge
Dextran release can be better regulated than that of Montanide.

Dextran-based structures capture proteins, and can be emulsified into gel-like microspheres that are biodegradable and slowly release the proteins. With possibility of regulated release by changing water content.

Entrapped protein in 3-D network
Overall conclusions

• Short peptide vaccines do not work
• Long peptide vaccines harboring both CD4 and CD8 T cell epitopes and requiring DC processing are efficient
• Further improvements possible by adding TLR ligands or especially by conjugating TLR ligands to the long peptides
• For maximally effective cancer treatment develop combination treatment of long peptide vaccination with immunogenic chemotherapy and inhibitors of checkpoint control monoclonal antibodies (CTLA-4 blocker, PD-1, PD-L1 blockers)
• Reduce toxicity of the monoclonal antibody treatments by local delivery in slow release formulation close to tumor-draining lymph nodes
Acknowledgements

Dept of Clinical Oncology
Sytse Piersma
Moniek Heusinkveld
Renske Goedemans
Jeanette van den Hulst
Tamara Ramwadhdoebe
Lien van der Minne
Marij Welters
Sjoerd van der Burg

Dept of IHB
Selina Khan
Cedric Britten
Linda Stijnenbosch
Marieke Fransen
Farah Essahsah
Kees Franken
Ramon Arens
Ferry Ossendorp

Dept. of Gynaecology
Gemma Kenter
Peggy de Vos-v. Steenwijk
Muriel van den Hende
Margriet Löwik
Dorien Berends-van der Meer
Mariette van Poelgeest

Pharmacy
Jan Wouter Drijfhout
Jaap Oostendorp
Rob Valentijn
Lorraine Fathers

Alumni
Sander Zwaveling
Annemieke de Jong
Martijn Bijker
Kitty Kwappenberg
Annelies Vloon
Rienk Offringa

Dept. of Pathology
Gert Jan Fleuren
Katja Jordanova
Hans Morreau
Sandra Uljee

NWO
Netherlands Organisation for Scientific Research

ISA
Pharmaceuticals
Immune System Activation

KONINGIN WILHELMINA FONDS
Nederlandsse Kankerbestrijding