Human oncogenic viruses

- Epstein Barr virus. Nasopharyngeal carcinoma, B cell Lymphomas, Hodgkin’s disease
  Herpes virus

- Human T lymphotrophic virus I. Adult T cell leukemia lymphoma, retrovirus

- Hepatitis B virus. Liver cancer PV

- Hepatitis C virus. Liver cancer

- Human papilloma virus. Ano-genital cancer, H&N cancer PV

- Kaposi sarcoma virus. Sarcomas in immunodeficient patients. Herpes virus 8

- Merkel carcinoma virus. Skin cancer, polyoma virus

- Trichodysplasia Spinulosa virus (TSV). Polyoma virus

PV: preventive vaccine available
Trichodysplasia Spinulosa-associated novel polyoma virus (TSV), isolated from immunocompromised patient

Feltkamp and associates Plos pathogens 2010
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

ISA Pharmaceuticals
Immune System Activation

LUMC
Natural history of cell-mediated adaptive immune response to high risk HPV16

>99% minority

immunity

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

minority

immune failure

No E6,E7 CD4+ immunity
Impaired CD4+ T-cells
Infrequent CD8+ T-cells
Regulatory T-cells
Overview of different types of therapeutic vaccines tried for HPV16

- **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
Enhanced MHC I processing and presentation by human MoDC of SLP HIV gag\textsubscript{216-237} compared to gag protein

**Source of DC: MoDC**
Long peptide vaccine in HPV16 Mouse Tumour Model

GQAEPDRAHYNIVTFCCCKCDSTLRLCVQSTHVDIR

License to Kill

TLR Ligands

CD40L

CD40

IL-2

costimulation

activation

T-helper

iDC

T-killer

mDC

Synthetic long peptides

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>medium</th>
<th>Before vaccination</th>
<th>After vaccination</th>
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<tr>
<td>MRM</td>
<td><img src="image" alt="MRM Before" /></td>
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Kenter, Clin Cancer Res, 2008
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: 30-50%
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

300 µg per peptide sc in Montanide ISA-51
Lymphocyte Proliferation Test
(ex-vivo 6 days)

pre-vac

post-vac

<table>
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HPV16-SLP vaccination in VIN3
Clinical results at 3 months


> 50% CR and PR. Strong correlation of lesion size and clinical response with T cell immune responses to the vaccine (This paper and Welters et al. PNAS, 2010)
Subversion of effector T-cell responses by cancerous cell growth

Melief, Immunity, 2008, updated 2012
Sites of action of Immunotherapy of Cancer

Anti-IL-10 (R), anti-TGFβ (R), anti-IL6 (R)
Experimental setup

chemo-immunotherapy

- TC-1 → tumor expressing HPV-16 E6 and E7 oncoproteins
- SLP vaccine → 35-mer long peptide (E7_{43-77}), provided in Montanide (slow release): prime-boost (s.c.)
- Chemotherapy → provided systemically (i.p.)
Optimal synergy between chemotherapy and peptide when both are provided on the same day.
Clinically used chemotherapeutics for HPV-induced tumors tested:

- Gemcitabine (Gem)
- Topotecan (Topo)
- Cisplatin (Cis)
- Carboplatin (Carbo)
Cisplatin and Carboplatin show synergy with vaccination.
CTL’s are the key mediators in cisplatin-SLP induced anti-tumor responses.
1 vaccination of ISA-HPV-SLP® given after 2nd chemotherapy course (carbotaxol) induces robust immune responses in patients with HPV16+ positive cervical carcinoma
Synergy SLP® vaccination-chemotherapy
Initial data exploratory clinical trial
Proliferative T cell response to HPV16 E6/E7 before and after a single SLP vaccine dose, given two weeks after completion of two cycles of chemotherapy for metastatic HPV16+ cervical cancer
Conclusions chemo-immunotherapy

- Clinically used chemotherapeutics for HPV-induced tumors do not impair T-cell responses
  - Recent clinical data confirm this observation

- Carboplatin and Cisplatin synergize with the HPV16 SLP vaccine in therapeutic vaccination protocols

- Our data shows that in addition to immunogenic cell death, chemotherapy can have other immunostimulatory effects

- Low dose cisplatin treatment is associated with
  - An increase in the % of leukocytes in the tumor
  - A reduction in the percentage of macrophages

- Combination of carboplatin and paclitaxel causes a decrease in myeloid cells and improved T cell responses in both mouse and human observations
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)
Overall conclusions

- Concentrated antigen delivery (DNA, RNA, SLP) with appropriate adjuvants is crucial. Synthetic vaccines allow rational vaccine design.
- Favoured cancer target antigens are involved in cancer initiation, progression and/or metastasis. Example: oncogenic proteins E6 and E7 of high risk HPV.
- Long peptide vaccines harboring both CD4 and CD8 T cell epitopes and requiring DC processing are efficient. DNA prime/long peptide boost may be considered. Processing route of SLP appears to differ from that of proteins.
- Further improvements seen by adding pegylated type I interferon or TLR ligands but especially by conjugating TLR ligands to the long peptides.
- For maximally effective cancer treatment develop combination treatment such as long peptide vaccination with chemotherapy or irradiation and inhibitors of checkpoint control monoclonal antibodies (CTLA-4 blocker, PD-1, PD-L1 blockers, anti-IL6 (R), anti-IL10 (R), anti-TGFβ (R) and other immunomodulators).
- Reduce toxicity of the monoclonal antibody treatments by local delivery in slow release formulation close to tumor-draining lymph nodes.
- Adoptive transfer of cancer-specific T cells is best combined with optimal vaccination.
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