Photochemical internalisation (PCI): Light-induced enhancement of MHC Class I antigen presentation, giving strong enhancement of cytotoxic T-cell responses to vaccination

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Summary and Conclusions

Background:
For generating a proper anti-tumour immunological response it is essential to stimulate cytotoxic T-cells (CTLs) to attack the tumour cells. Activation of CTLs is typically mediated through MHC Class I antigen presentation by antigen presenting cells (APCs). Fimaporfin ($TPCS_{2a}$) is a photosensitiser drug for use in Photochemical internalisation (PCI) to enhance the effects of other drugs in a site-specific, light-directed manner.
The PCI technology is used to re-localise endocytosed molecules from endosomes to cytosol. The photosensitiser fimaporfin co-localises with peptide and protein antigens in endosomes, and illumination releases of the antigens into the cytosol. PCI can thereby be used to enhance MHC Class I presentation by making access for antigens to the MHC Class I presentation machinery in the cytosol of APCs. This application of the PCI technology is called **fimaVACC**.

Results:
fimaVACC can increase MHC Class I presentation of peptide antigen by APCs up to 20 times (in vitro studies).
fimaVACC strongly increases the amount of antigen specific CD8 T-cells in blood and spleen after intradermal vaccination with various long and short peptide antigens in C57BL/6 mice (more than 100 times enhancement).
fimaVACC strongly enhances antigen-specific production of IFN-γ and TNF-α from CD8 and CD4 T-cells (blood and spleen), as well as antigen-specific antibody production.
fimaVACC and commonly used adjuvants (e.g. poly(IC), poly(ICLC) have strong synergistic effects when used in combination.
fimaVACC significantly enhances anti-tumour response after therapeutic vaccination with a HPV long peptide antigen vaccination in the TC-1 model for HPV-induced cancer.

On the basis of these promising preclinical results, a phase I clinical study with **fimaVACC** has recently been started in healthy volunteers.

Background

Photochemical Internalisation (PCI) is a technology for inducing cytosolic delivery of endocytosed molecules by illumination. A photosensitising molecule (fimaporfin ($TPCS_{2a}$)) is used to make endocytic membranes light sensitive, with illumination inducing permeabilisation of the membranes. Thus, PCI has a clear potential for enhancing CTL responses; re-routing antigen presentation from MHC class II to MHC Class I by making access for the antigen to the MHC Class I presentation machinery in the cytosol of APCs.
In addition to the use in vaccination the PCI technology can also be used for cytosolic delivery of a variety of drug molecules. A completed phase I clinical study showed that the photosensitiser fimaporfin can be delivered safely to humans and provided promising signs of efficacy (Lancet Oncol (2016) **17**(9):p1217–1229); clinical studies where the technology is used in combination with chemotherapeutic drugs (bleomycin and gemcitabine) are completed/on-going.

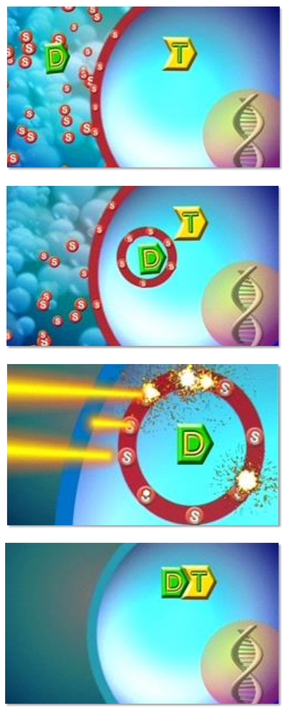
PCI can strongly enhance MHC Class I antigen presentation by inducing endosomal release of antigens to the cytosol in APCs

STEP 1:
- The photosensitiser (S) and the drug/antigen (D) are injected into the body and meets the cells containing the drug target (T) (or in vaccination approaches: an APC)

STEP 2:
- Fimaporfin (S) and the active molecule (D) are taken up by the cell, but D is unable to reach the target (T), as it is encapsulated in an endosome
- S is washed away from the cell membrane, but is trapped in endosomes

STEP 3:
- Light activates the photosensitizer fimaporfin (S) in the membrane of the endosome
- The membrane membrane integrity is affected (*permeabilised)* and the drug/antigen is released

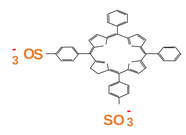
STEP 4:
- The drug/antigen molecule (D) can now bind to its target (T) and initiate the therapeutic response or MHC Class I antigen presentation
- For **fimaVACC**: Access of antigens to the MHC Class I presentation machinery in the cytosol of APCs, and thereby increasing MHC I presentation and activation of CD8 T cells



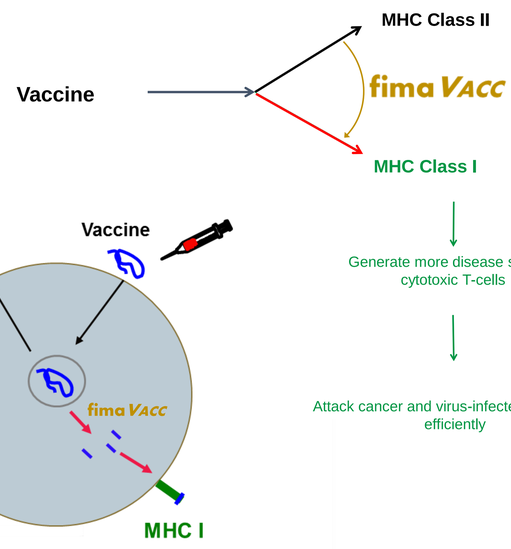
D The active molecule
- Peptide: e.g. antigen

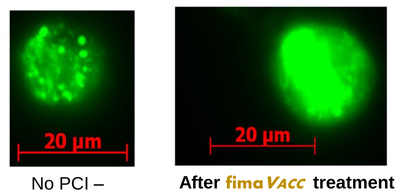
S The PCI component
- Light sensitive component
- Fimaporfin

T The target
- Target for the active molecule



Fimaporfin photosensitiser
- Easily produced
- Cheap
- Very stable (can be autoclaved, stable at room temperature for several years
- Has been tested in patients without severe adverse effects (Iv and ID administration)





No PCI – antigen in endosomes

After fimaVACC treatment – release of antigen in cytosol

PCI induces re-localisation of ovalbumin antigen from endosomes to cytosol in JAWSII dendritic cells

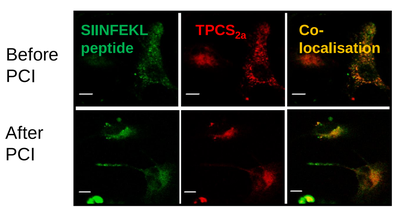
Materials and Methods

Intradermal photosensitisation and immunisation of mice
The mice were injected intradermally with 100 µl of a mixture of antigen (50 µg), and $TPCS_{2a}$ (25 µg) and/or poly(IC) (5 µg) and/or Hiltonol (5µg) when applied. 18 h after immunisation the mice were placed on a light source (LumiSource®, PCI Biotech AS) for activation of the photosensitiser. by illumination (6 minutes). Typically, mice were bled on day 7 after immunisation by tail bleeding for analysis of antigen-specific CD8 T cells by flow cytometry. The antigens used were : ovalbumin peptides: see figure; HPV short peptide: RAHYNIVTF; HPV long peptide: GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR; TRP-2 melanoma antigen: SVYDFFVWL, HBV surface antigen, KLH (Keyhole Limpet Hemocyanin)

Analysis of immune responses by flow cytometry and ELISA
The frequency of antigen-specific CD8 T-cells in blood was monitored by staining the cells with anti-CD8 antibody and antigen-specific pentamer (Proimmune, Oxford, UK) for analysis by flow cytometry. The activation status of the cells was further analysed by testing the expression of CD44. For analysis IFN-γ or TNF-α production by intracellular staining, spleens were removed and the spleen cells were re-stimulated with the peptide antigens overnight in 24-well plates at 37 °C. Brefeldin A was added during the last 4 hours. The cells were then washed and fixed with 4% formaldehyde in PBS for 10 min on ice. Anti-CD16/32 was added to block unspecific binding to Fc receptors. The cells were then permeabilised with 0.1% NP40 in PBS for 3 min and washed before staining with anti-IFN-γ, TNF-α, anti-CD8 and ant-CD44 antibodies (eBioscience or BD Pharmingen). The cells were analysed using FACSCanto (BD Biosciences, San Jose, USA) and FlowJo 8.5.2 software.

Therapeutic vaccination in the TC-1 model. C57BL/6 mice were vaccinated as described above 6 days after tumour challenge with 200,000 TC-1 cells injected subcutaneously into the right flank. The 6 days after TC-1 injection represents the time required for the tumour to develop a palpable size. Tumour growth was monitored by measuring the size of the neoplasm with a calliper.

Peptide antigen co-localizes with the $TPCS_{2a}$ photosensitiser in endosomes and is released upon illumination



- Peptide and $TPCS_{2a}$ co-localises in endosomal structures in macrophages (peptide inside cell)

- Diffuse distribution after light indicates endosome disruption and cytosolic escape of the peptide

Clinical validation of fima VACC is initiated: First-in-Man Clinical study aiming to enhance the cellular immune responses important for therapeutic effect of vaccines

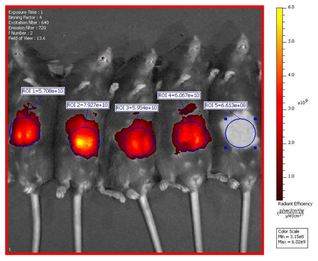
PCI Biotech

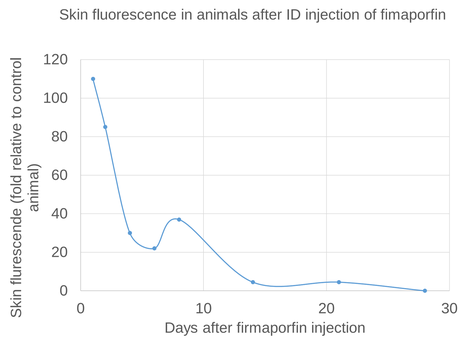
Oslo universitetssykehus
Norwegian Radium Hospital

NTNU
Norwegian University of Science and Technology

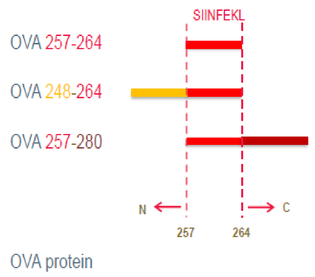
Results

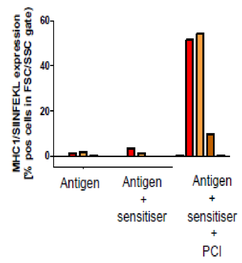
Intradermal injection of fimaporfin – skin kinetics at injection site





fima VACC increases MHC I presentation of SIINFEKL (OVA) peptide and N- and C-terminal extentions



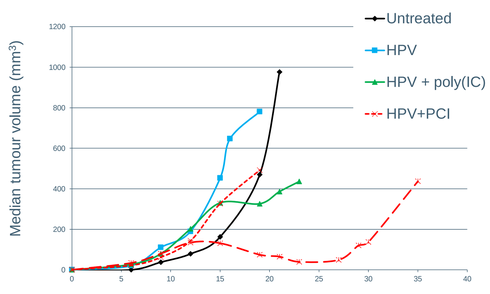


- PCI enhanced MHC Class I presentation about 20 times both for SIINFEKL and for SIINFEKL with N-terminal extension
- Also good effect with C-terminal extension

Intradermal vaccination with fima VACC induces strong anti-tumour response

TC-1 Mouse Model for HPV-induced cancer

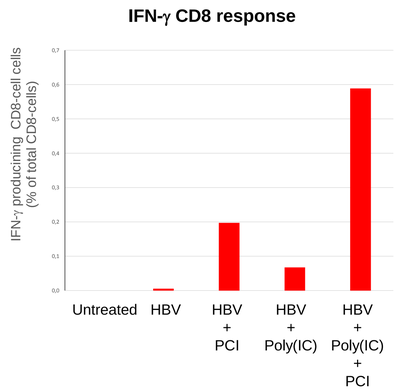
Intradermal therapeutic vaccination with HPV long peptide, poly(I:C) and fima VACC

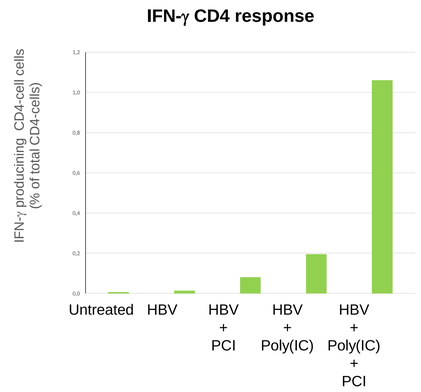


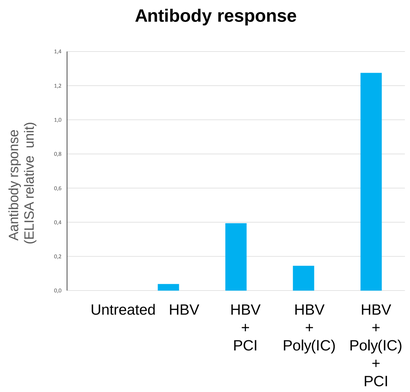
- Intradermal vaccination with fima VACC: HPV long peptide and poly(I:C)
- Significantly better anti-tumour effect with fima VACC
- Strong synergy between fima VACC and poly(IC)

fima VACC enhances antigen-specific induction of B- and T-cell responses after *in vivo* immunisation

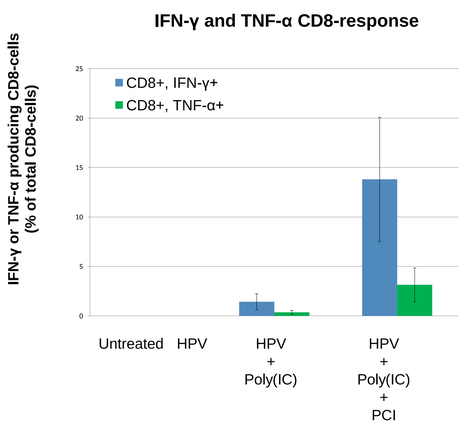
Vaccination with poly(IC) and HBV surface antigen +/- PCI

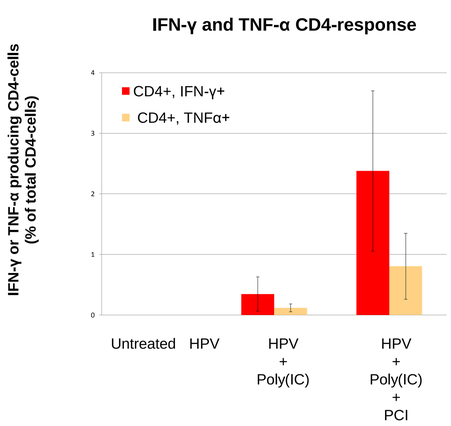






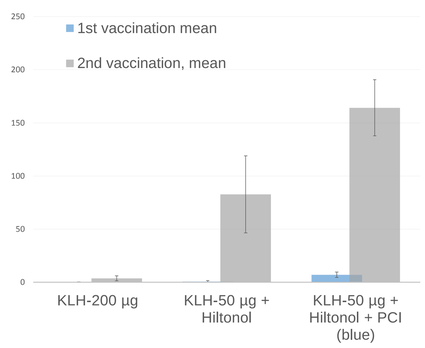
fima VACC enhances HPV-Specific level of cytokine producing CD8 and CD4 T-cells



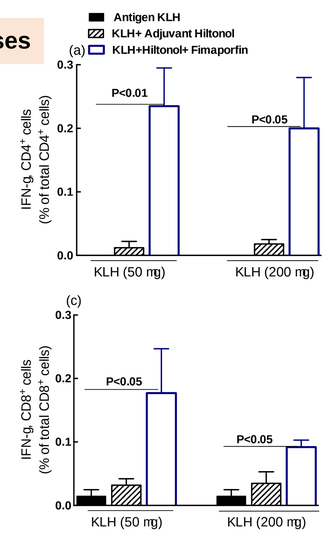


PCI acts synergistically with adjuvant (poly(IC) or Hiltonol) to enhance the effect of HPV-induced immune response

fima VACC enhances KLH antigen-induced Ab and T-cell immune responses



PCI enhances antibody (IgG) response to KLH vaccination as compared to KLH + Hiltonol control



Clinical technology validation in healthy volunteers has been initiated

- An Open-label, Phase I/Proof of Principle Study aiming to enhance the cellular immune responses
- Main Study Objectives are to determine safety, tolerability and immune responses
- Up to 80 healthy volunteers will be enrolled
- Study treatments:
  - Intradermal injection with 2 weeks between vaccinations
  - Hiltonol is used as adjuvant (poly-ICLC)
- Two antigens have been chosen to improve chances of detecting induction of B- and T-cell immune responses (HPV and KLH)
- Study is initiated and with estimated completion in first half 2017
- Early results shows that intradermal treatment with fimaporfin is tolerated

Conclusions

- fima VACC has a completely novel mechanism of action as a vaccination technology, representing a new and potent tool for stimulation of cytotoxic CD8 T-cell responses.
- fima VACC can give strong synergy with commonly used immunological adjuvants
- fima VACC enhances both CD8-, CD4-, and antibody responses
- fima VACC strongly enhances the antitumour effect of therapeutic peptide vaccines
- A phase I clinical study with fima VACC has been started in healthy volunteers