Clinical validation of Photochemical internalisation (fima VACC) – a novel technology for enhancing cellular immune responses to peptide- and protein-based therapeutic cancer vaccines

Aim
To investigate the safety and immune response of fima VACC- based peptide- and protein antigen vaccination in healthy volunteers

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 fima VACC.

Results:
fima VACC can increase MHC Class I presentation of peptide antigen by APCs up to 20 times (in vitro studies).
fima VACC 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).
fima VACC 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.
fima VACC and commonly used adjuvants (e.g. poly(IC), poly(ICLC) have strong synergistic effects when used in combination.
fima VACC 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 fima VACC 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.

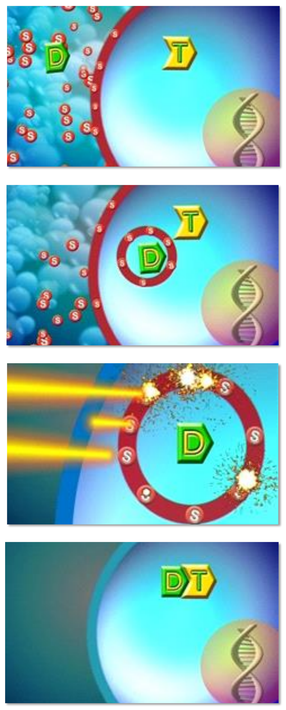
PCI can strongly enhance MHC Class I antigen presentation by inducing endosomal release of antigens to the cytosol in APCs

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 integrity is affected (*permeabilised)* and the drug/antigen is released

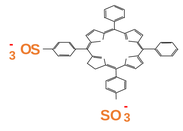
STEP 4:
- The drug/antigen molecule (D) can now bind to its target (T) and initiate the therapeutic response or MHC Class I antigen presentation
- For fima VACC: Access of antigens to the MHC Class I presentation machinery in the cytosol of APCs, and thereby increasing MHC I presentation and activation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



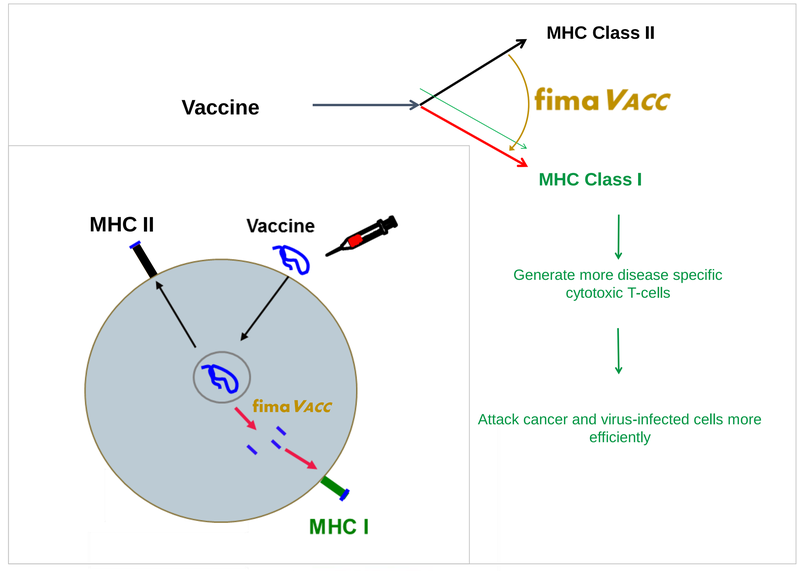
D The active molecule
- Peptide: e.g. antigen

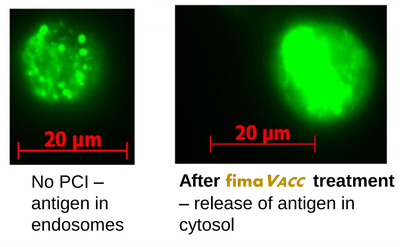
S The PCI component
- Light sensitive component
- Fimaporfin

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 fima VACC treatment – release of antigen in cytosol

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

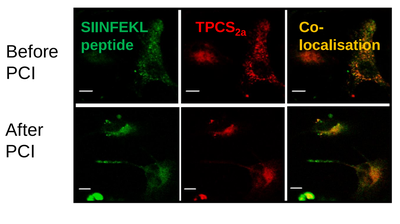
Materials and Methods

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 and 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 anti-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

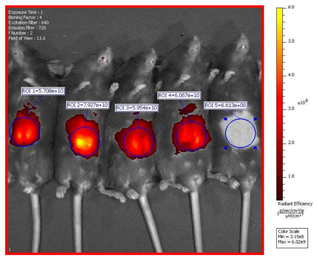
Tone Otterhaug[1], Gaute Brede[3], Markus Haug[3], Monika Håkerud[1,2], Anne Grete Nedberg[1,2], Victoria Edwards[1,2], Øyvind Halaas[3], Pål Kristian Selbo[1,2] and Anders Høgset[1]

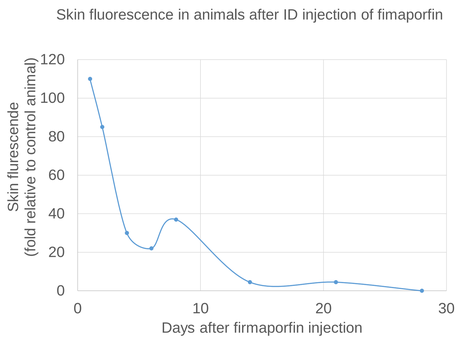
[1]PCI Biotech AS, [2]Oslo University Hospital – The Norwegian Radium Hospital, [3]Norwegian University of Science and Technology

Correspondence: Tone Otterhaug, PCI Biotech AS, Ullernchausséen 64, 0379 Oslo, Norway. E-mail: tone.otterhaug@pcibiotech.no. Tel.: +47 6711 5400

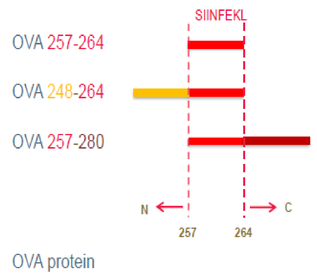
Results

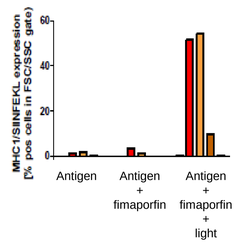
Intradermal injection of fimaporfin – skin kinetics at injection site





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



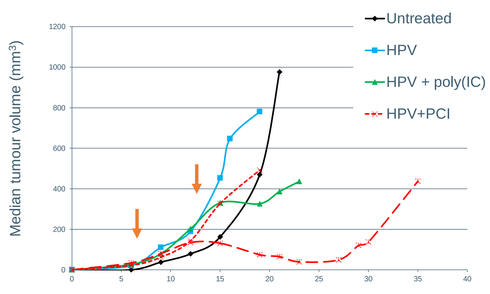


- PCI enhanced MHC Class I presentation about 20 times both for SIINFEKL and for SIINFEKL with N-terminal extension
- Also good effect with C-terminal extension

Intradermal vaccination with fima*VAcc* induces strong anti-tumour response

TC-1 Mouse Model for HPV-induced cancer

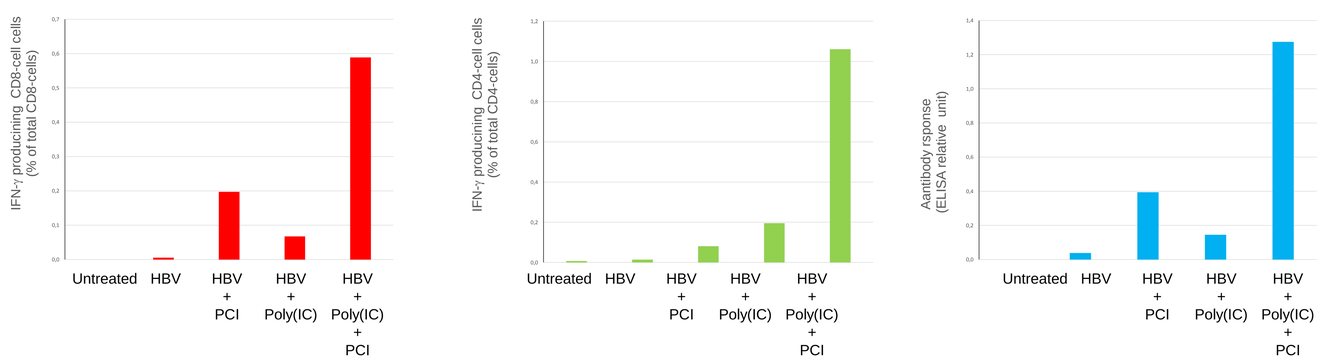
Intradermal therapeutic vaccination with HPV long peptide, poly(I:C) and fima*VAcc*



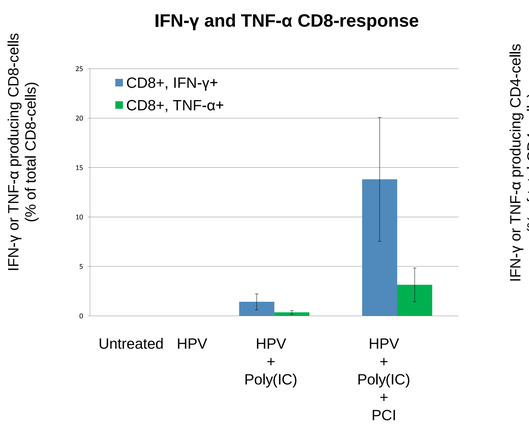
- Intradermal vaccination with fima*VAcc*: HPV long peptide and poly(I:C)
- Significantly better anti-tumour effect with fima*VAcc*
- Strong synergy between fima*VAcc* and poly(IC)

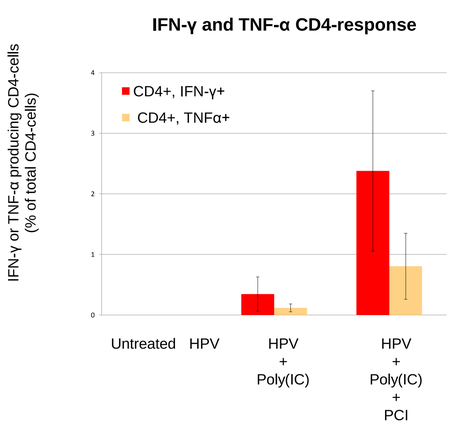
fima*VAcc* enhances antigen-specific induction of B- and T-cell responses after *in vivo* immunisation

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



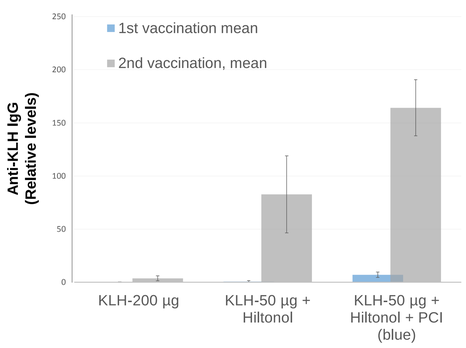
fima*VAcc* enhances HPV-Specific level of cytokine producing CD8 and CD4 T-cells



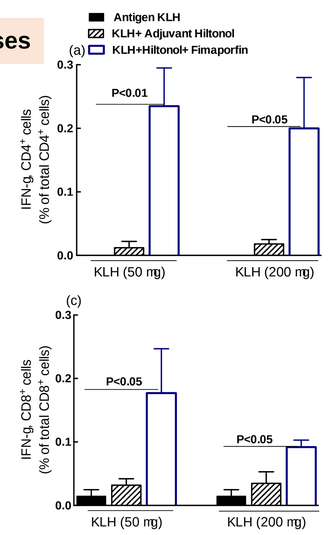


PCI acts synergistically with adjuvant (poly(IC) or Hiltonol) to enhance the effect of HPV-induced immune response

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