Photochemical internalization technology induces strong enhancement of CD8+ and CD4+ T-cell responses to vaccination

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@pcibiotech.com. Tel.: +47 6711 5400

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 photosensitizer drug for use in Photochemical internalisation (PCI) to enhance the effects of other drugs in a site-specific, light-directed manner.
- The PCI technology can re-localise endocytosed molecules from endosomes to cytosol and can therefore 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.

Results:

- The photosensitizer fimaporfin co-localizes with peptide and protein antigens in endosomes, and illumination releases the antigens into the cytosol.
- PCI can increase MHC Class I presentation of peptide antigen by APCs up to 20 times (*in vitro studies*).
- PCI strongly increases the frequency 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).
- PCI vaccination strongly enhances antigen-specific production of IFN-γ and TNF-α from CD8 and CD4 T-cells (blood and spleen).
- PCI vaccination strongly enhances antigen-specific antibody production.
- PCI and commonly used adjuvants (e.g. poly(IC), poly(ICLC) have strong synergistic effects when used in combination.
- PCI significantly enhances anti-tumour response after therapeutic vaccination with a HPV long peptide antigen vaccination in the TC-1 model for HPV-induced cancer.

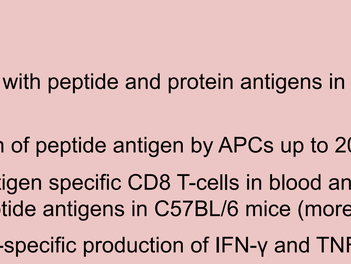
Background

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 $TPCS_{2a}$ can be delivered safely to humans, and two clinical studies where the technology is used in combination with chemotherapeutic drugs (bleomycin and gemcitabine) are on-going.

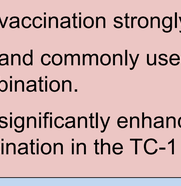
The photosensitiser (S) and the drug/ antigen (D) are injected into the body and meets the cells containing the drug target (T) (or in vaccination approaches: an APC)

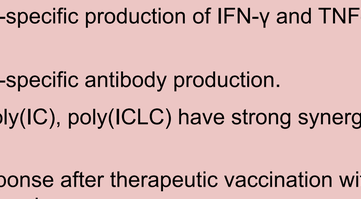
Light activates the photosensitiser in the membrane of the endosome. The membrane is *permeabilised* and the drug/ antigen is released.



The photosensitiser and the drug/ antigen are taken up by the cell, but the drug is unable to reach the target, as it is encapsulated in an endosome

The drug/antigen molecule can now bind to its target, initiating a therapeutic response or MHC Class I antigen presentation





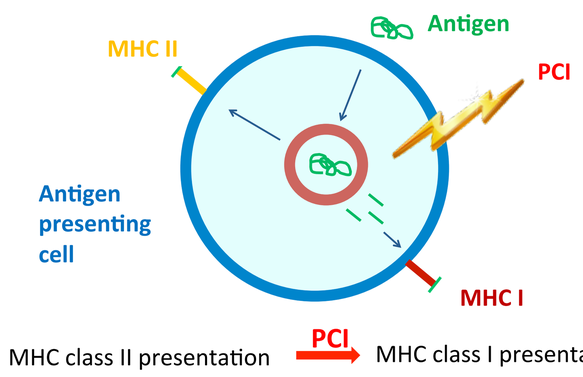
No PCI – antigen in endosomes

PCI – antigen in cytosol

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

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 administration)**



PCI can switch antigen presentation from MHC Class II to MHC Class I by making access for antigens to the cytosol in APCs

Materials and Methods

Intradermal photosensitisation and immunisation of mice
C57BL/6 mice were injected intradermally with 100 µl of a mixture of antigen (50 µg), and fimaporfin (25 µg) and/or poly(IC) (5 µg) 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 CD4 and CD8 T-cells by flow cytometry, and antigen-specific antibodies by ELISA.
The antigens used were: ovalbumin peptides (see figure), HPV short peptide (RAHYNIVTF), HPV long peptide (GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR) and TRP-2 melanoma antigen (SVYDFFVWL)

Analysis of immune responses by flow cytometry and ELISA
Immune responses were evaluated in cells from blood or spleen. 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. Antigen-induced activation of CD4 and CD8 T-cells was assessed as intracellular staining with IFN-γ or TNF-α of blood or spleen cells. 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 stained with anti-CD44, anti-CD8 and anti-CD4 antibodies before permeabilised with 0.1% NP40 in PBS for 3 min, washed and stained with anti-IFN-γ, TNF-α antibodies (eBioscience or BD Pharmingen). The cells were analysed using FACSCanto (BD Biosciences, San Jose, USA) and FlowJo 8.5.2 software.

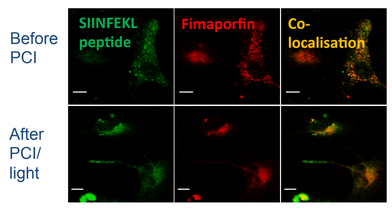
Therapeutic vaccination in the TC-1 model.
200,000 TC-1 cells were injected subcutaneously into the right flank of C57BL/6 mice. After approximately 6 days, when the tumour was palpable, the mice were vaccinated as described above by intradermal injection followed by illumination.
Tumour growth was monitored by measuring the size of the neoplasm with a calliper.

Aims of the work

To demonstrate if the PCI technology can enhance the effect of peptide or protein vaccines assessed by induction of antigen-specific antibodies, CD4 helper T-cell and CD8 cytotoxic T-cell responses, and anti-tumour responses.

Results

Peptide antigen co-localizes with the photosensitiser (fimaporfin) in endosomes and is released upon illumination

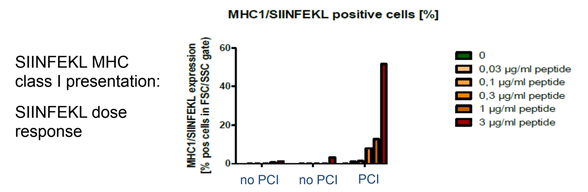


- Peptide and fimaporfin co-localises in endosomal structures in macrophages (peptide inside cell)

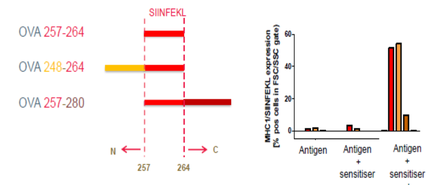
- Diffuse distribution after light indicates endosome disruption and cytosolic escape of the peptide

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

- Macrophage cell line incubated with SIINFEKL peptides and fimaporfin
- SIINFEKL MHC Class I complex detected by antibody staining and flow cytometry

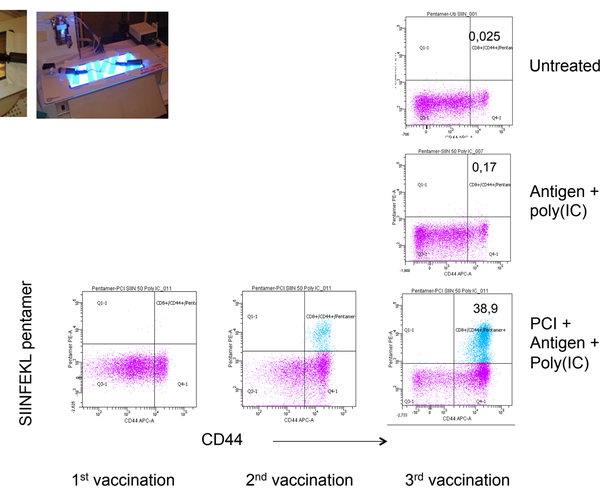


- PCI enhanced MHC Class I presentation of both SIINFEKL and SIINFEKL with N-terminal extension about 20 times.
- PCI also enhanced, at a lower extent, MHC Class I presentation of SIINFEKL with C-terminal extension

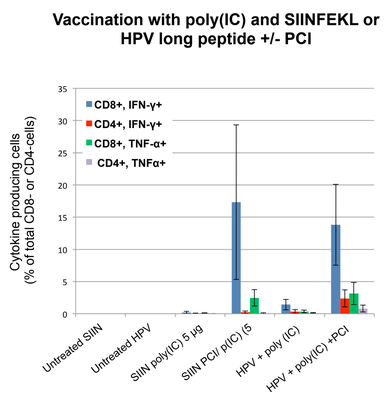


PCI combined with poly(IC) (adjuvant) enhances the frequency of SIINFEKL (OVA) peptide antigen-specific CD8 T-cells > 100 times compared to antigen + poly(IC) without PCI

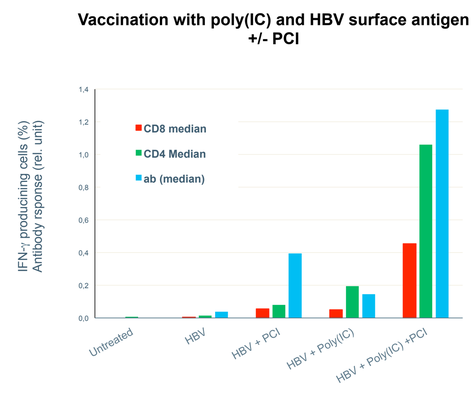
- A mixture of photosensitiser (fimaporfin), poly(IC) and peptide antigen was injected intradermally to the abdomen of the mouse
- 18 h later, the injection area of the skin was illuminated with blue light for 6 min
- 3 vaccinations given at 14 days interval
- Blood samples were collected 8 days after each vaccination and analysed for SIINFEKL pentamer, CD8 and CD44 using flow cytometry



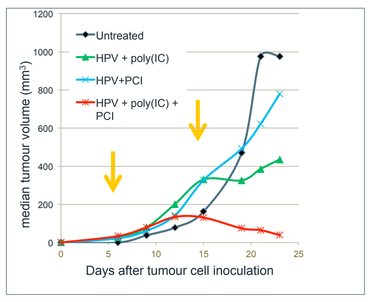
PCI enhances antigen-specific induction of cytokines from CD8 and CD4 T-cells after *in vivo* immunisation



PCI enhances antigen-specific IFN-γ and antibody production after *in vivo* immunisation with HBV surface antigen



PCI induces strong anti-tumour response after therapeutic vaccination with HPV long peptide antigen in TC-1 mouse tumour model



- 200,000 TC-1 tumour cells were injected sc to C57BL/6 mice
- Intradermal vaccination of mice on day 6 and day 13 after tumour cell inoculation

Conclusions:

- PCI has a completely novel mechanism of action as a vaccination technology, representing a potent tool for stimulation of cytotoxic CD8 T-cell responses, CD4 T-cell responses as well as antibody production.
- PCI can give strong synergy with commonly used immunological adjuvants.

PCI Biotech

Oslo universitetssykehus
Norwegian Radium Hospital

NTNU
Norwegian University of Science and Technology