Phase I clinical study for validation of fimaporfin-based photochemical internalisation – a novel technology for enhancing cellular immune responses important for therapeutic effect of peptide- and protein-based vaccines

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Background

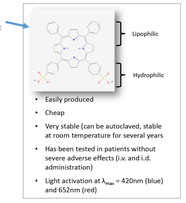
Robust activation of cytotoxic T-cells (CTLs) is essential to mitigate proper anti-tumour immunological responses and is typically mediated through MHC Class I antigen presentation by antigen presenting cells (APCs). Fimaporfin ($TPCS_{2a}$) is a photosensitising drug for use in the drug delivery method Photochemical internalisation (PCI) to enhance the effects of other drugs in a site-specific and light-controlled manner. The PCI technology is used to permeabilize the membrane of endosomes and thereby to release endocytosed and trapped molecules into the cytosol. The fimaporfin photosensitiser, which previously has been tested and found safe in two different clinical trials, co-localises with endocytosed peptide and protein antigens in endosomes in APCs. Light activation of the photosensitizer generates reactive oxygen species (ROS) inducing destabilization of the endocytic membrane followed by endosomal escape and cytosolic release of the antigens. PCI can thereby be used to enhance MHC Class I cross-presentation by making access for exogenous antigens (e.g. in therapeutic vaccines) to the MHC Class I presentation machinery in the cytosol of APCs. This application of the PCI technology in vaccination is hereafter for simplicity called fima*Vacc*. 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 fimaporfin can be delivered safely to humans and provided promising signs of efficacy in head and neck cancer (Lancet Oncol (2016) **17**(9):p1217–1229). A clinical study in bile duct cancer where PCI is used in combination with the chemotherapeutic gemcitabine is on-going.

Principle and preclinical documentation: PCI strongly enhance MHC Class I antigen presentation by inducing endosomal release of antigens to the cytosol in APCs

D The active drug (D)
- Vaccine antigen; e.g. synthetic peptides

T The target (T) of the antigen (D)
- Antigen processing machinery

S The PCI component
- Fimaporfin ($TPCS_{2a}$)
- Light sensitive amphiphilic photosensitiser



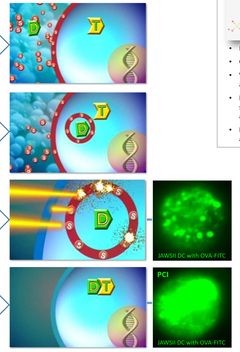
- Easily produced
- Cheap
- Very stable (can be autoclaved, stable at room temperature for several years
- Has been tested in patients without severe adverse effects (i.v. and i.d. administration)
- Light activation at λ_{max} = 420nm (blue) and 652nm (red)

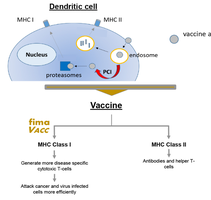
STEP 1:
The photosensitiser (S, fimaporfin) and the drug/antigen (D) are injected into the body and meets the APC containing the target (T), which is the MHC class I antigen processing machinery

STEP 2:
Fimaporfin (S) and the antigen (D) are endocytosed by the APC. Antigen is entrapped in endosomes and unable to reach the MHC class I antigen processing machinery (T), while S is washed away from the cell surface. Due to the amphiphilic nature of fimaporfin it accumulates in the membrane of the endosomes and thereby co-localises with the antigen.

STEP 3:
Light-controlled activation of fimaporfin (S) triggers generation of ROS, which affects the membrane integrity of the endosome (*permeabilisation)* resulting in endosomal escape of the antigen into the cytosol of the APC. This opens up the cytosolic antigen presentation pathway for the antigen.

STEP 4:
The antigen (D) is further processed by the antigen presentation machinery (T) of the APC, resulting in a MHC Class I antigen cross-presentation and subsequent activation of CD8 T cells





Materials and Methods

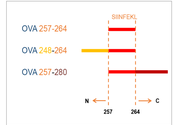
PCI –mediated vaccination

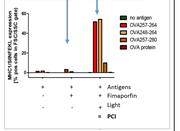
C57BL/6 mice are **injected intradermally** with 50 µl of a mixture of 50 µg antigen , 25 µg $TPCS_{2a}$ and/or 5 µg poly(IC). 18 h after administration of the vaccine, the injection site is illuminated (6 min, visible blue light) for activation of the photosensitiser. Mice are bled 7 days after PCI for analysis of antigen-specific CD8 T cells by flow cytometry. The antigens used were: ovalbumin peptides; HPV E7 long peptide: GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR.

Tumour experiments.
C57BL/6 mice were injected subcutaneously into the right flank with 200,000 TC-1 cells (purchased from John Hopkins University Hospital). Time required for the palpable tumours: 6 days. Twice per week the tumour growth was monitored by measuring the size with a calliper. Vaccination was performed intradermally or intratumourally.

Analysis of immunisation by flow cytometry
The frequency of antigen-specific CD8 T-cells in blood was monitored by flow cytometry after staining the cells with anti-CD8 antibody and antigen-specific pentamer (Proimmune, Oxford, UK) or by intracellular staining for interferon-γ production. The activation status of the cells was further analysed by testing the expression of CD44.

PCI induces cytosolic release of peptide antigen and strongly enhances its MHC-I presentation in APCs

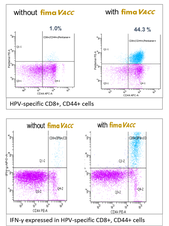


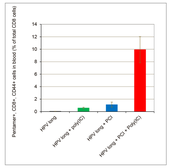


Peptide antigen (SIINFEKL) co-localizes with the PCI-photosensitizer fimaporfin (left micrograph). Light activation results in PCI-induced cytosolic release of SIINFEKL ($OVA_{257-264}$) in B6 macrophage cells (right micrograph).

PCI of both short ($OVA_{257-264}$ /SIINFEKL, 8 aa, left panel) and long OVA-peptides (in particular, $OVA_{248-264}$, 16aa) results in a 20-fold increase in MHC class I/peptide complex formation and surface presentation (Right panel; bar graph based on flow cytometry quantification of $OVA_{257-264}$ (SIINFEKL) peptide in complex with MHC class I (H-2Kb) on the surface of B6 macrophage cells stained with anti-H-2Kb-SIINFEKL PE (mAb clone 25-D1.16, eBioscience).

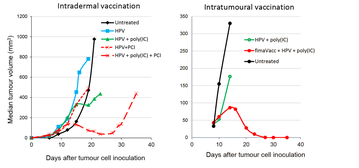
PCI with clinically relevant vaccine antigens trigger robust activation of antigen-specific CD8 T-cells





fima*Vacc* (fimaporfin + light) with an HPV long peptide antigen (35-mer) acts in synergy with the TLR3 agonist poly(IC) and strongly enhances the CD8+ T-cell vaccination response as compared to adjuvant + HPV peptide alone, or fima*Vacc* + antigen alone. Upper left panel: Antigen-specific CD8+ T-cells in blood after 3 vaccinations (pentamer staining). Lower left panel: IFN-γ-expression in CD8+ T-cells from spleen after HPV peptide re-stimulation. Right panel, average data based on 5 mice per experimental group (PCI = fima*Vacc*).

PCI with HPV long peptide antigen strongly enhances therapeutic vaccination



Intradermal (left panel) and intratumoural (right panel) therapeutic HPV-peptide (35-mer) vaccination with fima*Vacc* + poly(IC) in C57BL/6 mice with TC-1 tumours generates strong anti-tumour responses.
Strikingly, intra-tumoural immunization generates the best immune response, which was capable of ablating TC-1 tumours. Cured mice were immune to re-challenge with tumour cells, indicating long-term memory.

Tumour model: The TC-1 murine tumour cells express the E7 oncogene of HPV-16 used as a surrogate for human tumours infected with HPV-16. Mice were vaccinated 3 times over a course of 3-4 weeks. Each data-point represents the median of 5 mice.

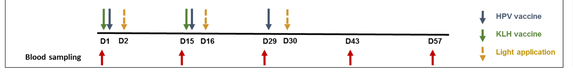
Phase I clinical study in healthy volunteers

Oslo University Hospital
Norwegian Radium Hospital

fima*Vacc* Phase I clinical study in healthy volunteers

- Main Objective:
  - Determine the safety, tolerability and immune response of fima*Vacc* when given as intradermal injections in combination with an adjuvant (Hiltonol) in healthy subjects.

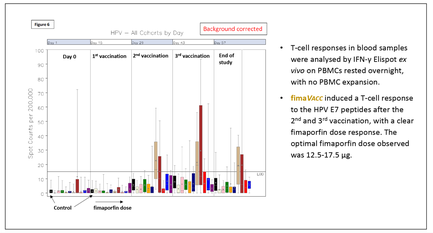
Study Treatments:
- Antigens used were 100 µg KLH protein and 100 µg each of two 35-mer HPV E7 peptides
- 6-12 subjects in each cohort – different doses of fimaporfin photosensitiser
- Adjuvant: Hiltonol (poly-ICLC) 50µg
- Control group: Adjuvant + Antigens
- fima*Vacc* groups: Adjuvant + Antigens + fima*Vacc*
- Intradermal injections with 2 weeks intervals (separate injections, rotating injection sites)
- Light application 200 sec, 20 (± 4) hours after ID dosing



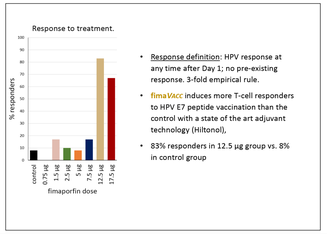
fima*Vacc* with Hiltonol adjuvant can be adminstered safely to humans

- Intradermal vaccination with fima*Vacc* was safe and tolerable at the doses inducing high response rates and enhanced T-cell responses
- The main reactions seen were administration site adverse events, such as injection site vesicles (grade 1), scab (grade 1), pain (grade 1/2), erythema (grade 1) and pruritus (grade 1)
- At high fimaporfin doses pain scores (VAS) during illumination varied substantially between subjects – at lower doses pain during illumination was not a problem
- Systemic Adverse Events suspected to be related to study treatment were all grade 1 and included cold symptoms, fever, inguinal pain, headache, nausea, dizziness, syncope, interrupted sleep and altered sensation in arm and feeling shaky

fima*Vacc* increases percentage of HPV-peptide responders around 10 times as compared to control (peptide + Hiltonol) group



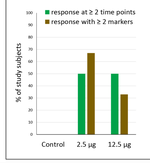
- T-cell responses in blood samples were analysed by IFN-γ Elispot *ex vivo* on PBMCs rested overnight, with no PBMC expansion.
- fima*Vacc* induced a T-cell response to the HPV E7 peptides after the 2nd and 3rd vaccination, with a clear fimaporfin dose response. The optimal fimaporfin dose observed was 12.5-17.5 µg.



- Response definition: HPV response at any time after Day 1; no pre-existing response. 3-fold empirical rule.
- fima*Vacc* induces more T-cell responders to HPV E7 peptide vaccination than the control with a state of the art adjuvant technology (Hiltonol),
- 83% responders in 12.5 µg group vs. 8% in control group

fima*Vacc* increases no. of subjects with robust CD8 T-cell response

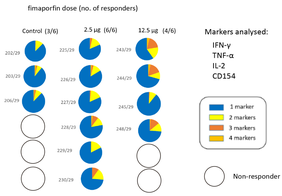
- Flow cytometry analyses by group of prof. Sjoerd van der Burg (Leiden University Medical Center)
- Selected cohorts analysed; Control, 2.5, 12.5 µg at 3 time points (D29, D43, D57)
- The cells were cultured with an HPV E7 peptide pool for 12 days
- Re-stimulated with HPV16 E7: mixes of 10-mer or 22-mer peptides covering the regions used for vaccination
- Staining was done for the following functional markers: IFN-γ, TNF-α and IL-2 Activation markers: CD154 and CD137
- Response Definition: A positive response is defined as twice the frequency of the medium only control and ≥10 events (cells)



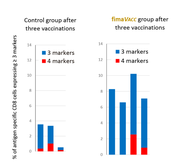
- fima*Vacc* induces more CD8 T-cell responders and more robust responses
- CD8 T-cell responses in control group were less frequent and generally borderline (1 time point, 1 marker, near LOD)

fima*Vacc* enhances CD8 T-cell immune responses and CD8 cell polyfunctionality

Marker expression analysis in subjects showing respons for at least one marker

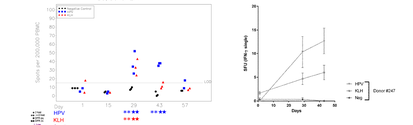


- After two HPV E7 peptide vaccinations fima*Vacc* treatment induces a substantial increase in the fraction of CD8 T-cells expressing multiple markers



Fluorospot analysis shows that cells expressing Granzyme B is induced by vaccination, an indication of cytotoxic T-cell responses

- Selected subjects with positive response to IFNg in Elispot were selected for Fluorospot analysis of multiple cytokine response
- Th1 cytokines (IFN-γ + Granzyme B) and Th2 cytokines (IL-22 + IL-5) were analysed
- Cells were rested over night (20-24 hrs) for optimal functional analysis prior to stimulation
- Cells were stimulated for 44 hrs
- No expansion of cells



Summary and Conclusions

- The fima*Vacc* technology enhances MHC class I antigen presentation with a completely novel mechanism of action, representing a new and potent tool for stimulation of cytotoxic CD8 T-cell responses.
- fima*Vacc* has been clinically validated in a Phase I study in healthy volunteers, showing that the technology is safe and induces T-cell responses to peptide vaccination above what is seen with a state-of the art adjuvant

- More than 90 healthy volunteers enrolled
- As compared to a peptide + adjuvant (Hiltonol) control group, at the optimal dose fima*Vacc* increased the number of subjects responding to HPV E7 peptide vaccination about 10 times (Elispot analysis).
- At this dose fima*Vacc* administration to humans was safe with only mild local reactions being observed.
- Flow cytometry analysis showed that fima*Vacc* enhanced CD8 T-cells responses and increase the frequency of polyfunctional CD8 cells.

- In preclinical studies fima*Vacc* gives strong synergy with several commonly used immunological adjuvants
- In preclinical studies fima*Vacc* enhances both CD8-, CD4-, and antibody responses
- In mouse models fima*Vacc* strongly enhances the effect of therapeutic peptide vaccines for tumour treatment

Conflicts of interest
Study sponsored by PCI Biotech (PCIB). A.H., H.O. and T.O. are employees of and shareholders in PCIB. A.H. is inventor of patents describing the use of PCI immunotherapy and vaccination. P.K.S. is co-inventor of the fimaporfin patent. M.H. and A.G.N. are sponsored by PCIB. V.E. has a 50% employment in PCIB. S.H.V.D.B. is a member of the advisory board of PCIB. M.J.P.W. has no conflict of interest to declare.