Clinical validation of Photochemical internalisation (fimaVacc) – 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 fimaVacc-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 (TPCS2a) 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-y and TNF-a from CD8 and CD4 T-cells (blood and spleen), as well as antigen-specific antibody production. fimaVacc and commonly used adjuvants (e.g. polyIC), 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.

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 TPCS2a (25 µg) and/or polyIC (5 µg) and/or Hilitone (5µg) when applied. 18 h after immunisation the mice were placed on a light source (LumSource®; PCI Biotech AS) for activation of the photosensitiser by illumination (6 minutes). Typically, mice were bled on day 7 after illumination 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 (RAHYNVITF), HPV long peptide (GQAEPRAHYNTFCCCKOSTLNLCDPSTVDR), 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 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 ºC. 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-CD123/632 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 caliper.

Peptide antigen co-localizes with the TPCS2a photosensitiser in endosomes and is released upon illumination

Before PCI: Peptide and TPCS2a co-localise in endosomal structures in macrophages (peptide inside cell)

After PCI: Diffuse distribution after light indicates endosome disruption and cytosolic escape of the peptide
Results

Intradermal injection of fimaporfin – skin kinetics at injection site

Intradermal vaccination with **fimaVac** induces strong anti-tumour response

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<tbody>
<tr>
<td>Skin fluorescence in animals after ID injection of fimaporfin</td>
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<td>Days after fimaporfin injection</td>
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<tr>
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**fimaVac** increases MHC I presentation of SIINFEKL (OVA) peptide and N- and C-terminal extentions

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

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

**fimaVac** enhances KLH antigen-induced Ab and T-cell immune responses

Clinical technology validation in healthy volunteers has been initiated

- **fimaVac** 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.
- **fimaVac** can give strong synergy with commonly used immunological adjuvants.
- **fimaVac** enhances both CD8-, CD4-, and antibody responses.
- **fimaVac** strongly enhances the antitumour effect of therapeutic peptide vaccines.
- A phase I clinical study with **fimaVac** has been started in healthy volunteers.

Conclusions

- 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