Preclinical studies related to a phase 1/2a TRIAL to investigate the immunologic impact, anti-tumor efficacy and safety of VXM01, an oral T-cell inducing vaccine, in late stage colorectal cancer patients

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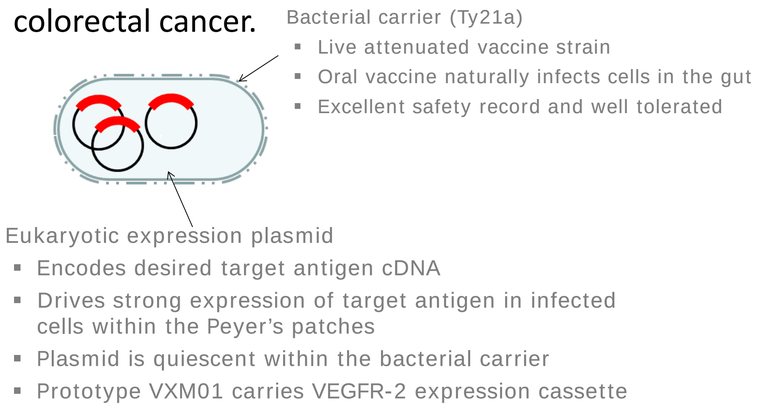
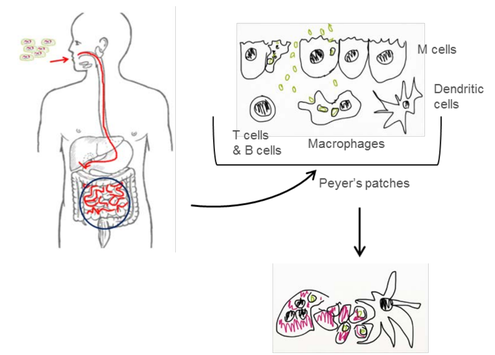
Introduction

VXM01 is an orally applied T-cell inducing immunotherapy based on live attenuated *Salmonella typhi* vector carrying a eukaryotic expression plasmid coding for vascular endothelial growth factor receptor 2 (VEGFR-2). A recent phase I trial demonstrated safety, immunogenicity and transient anti-angiogenic activity in advanced pancreatic cancer patients. Notably, sustained T-cell responses have been measured in patients treated monthly with VXM01 after a one-week initiation treatment course. The purpose of the current studies is to gain more insight into the mode of action of VXM01 in preclinical mouse models of colorectal cancer as well as in patients with metastatic colorectal cancer.





Study Design

Objective:
Evaluation of the antitumor activity of VXM01m in Balb/c mice bearing subcutaneous CT26 colon tumor cells.
(animal experiments conducted at Oncodesign).

Group 1: empty vector treated (n=11)

Group 2: VXM01 treated (n=10)

Group 3: VXM01 + Cyclophosphamide (1 admin. 100 mg/kg on day 0) (n=10)

All spleens sent for pentamer analysis.
5 or 6 tumors/group for immunhistochemistry.

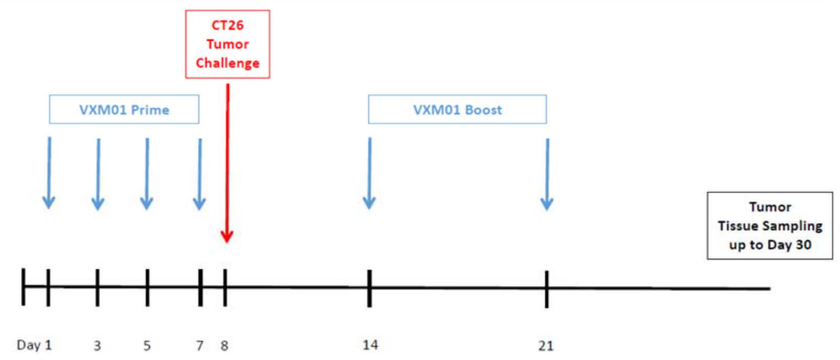


Figure 1: Study Design / Treatment Schedule
Per os administration of 10^8 CFU/ VEGFR2 vaccination or empty vector (control group) on day 1, 3, 5, 7, followed by a tumor challenge on day 8 (10^6 CT26 tumor cells injected subcutaneously into right flank). Administration of 10^8 CFU/ VEGFR2 vaccination or empty vector (control group) on day 14 and day 21 for boosting. On day 30, 9 days after last administration, tissue and tumor samples are taken and sent for further analysis.

Results

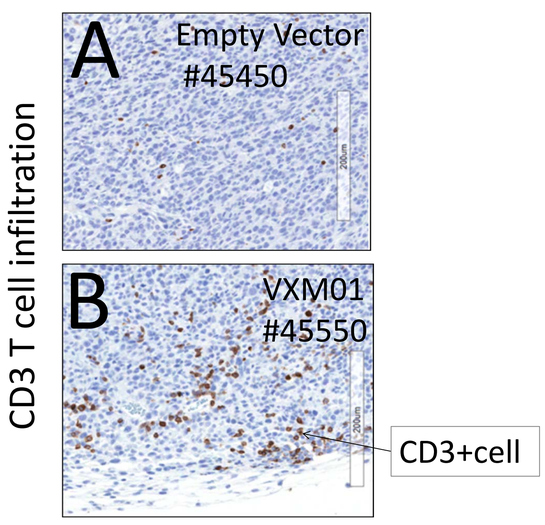


Figure 4: Immunhistochemistry Staining anti-CD3
A) Empty Vector animal B) VXM01 treated animal
CD3 positive cells appear in brown color (see arrow for example) Formalin fixed paraffin embedded 4µm sections, anti-CD3 AB (Abcam AB 16669 Rabbit monoclonal [SP7] 1:100).Scanned at x200 magnification on Aperio scanner using digital image analysis with Indica Labs HALO Suite Modules and Halo Immune Cell Proximity modules

Quantification of immune cell infiltrates and PD-L1

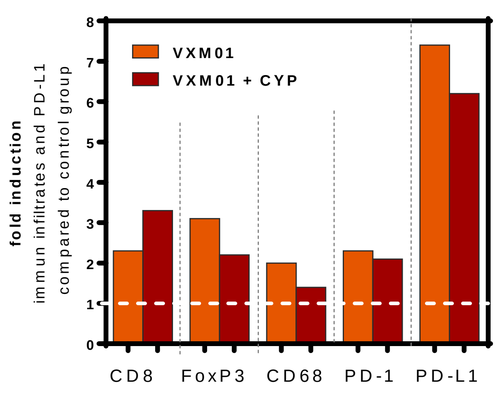


Figure 5: Immune cell infiltrates and PD-L1 mean fold induction vaccinated compared to negative control group. Data derived from absolute cell count/ tissue area [mm2] of Immunhistochemistry staining for CD8 , FoxP3, CD68, PD-1, and PD-L1 single stainings . Scanned at x200 magnification on Aperio scanner using Modul Halo Immune Cell Proximity modules.

Decrease in tumor size day 30

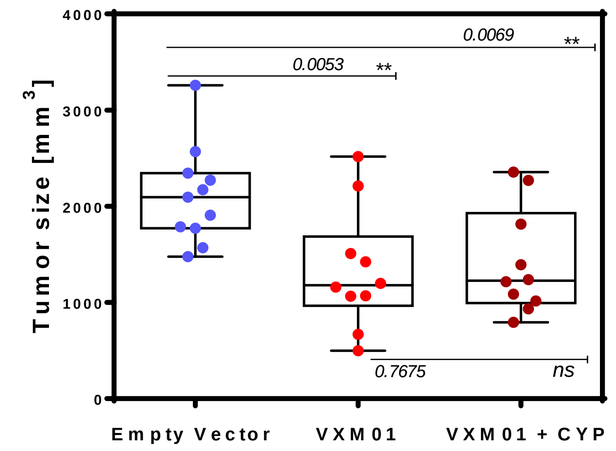


Figure 2: Tumor size [mm³] on day 30, each dot represents the results of the tumor of one animal. blue dot empty vector treated mice (n=11) ; red dots VXM01m treated mice (n=10); VXM01m and Cyclophosphamide treated mice (n=10)

VEGFR2 specific CD8 T cells day 30

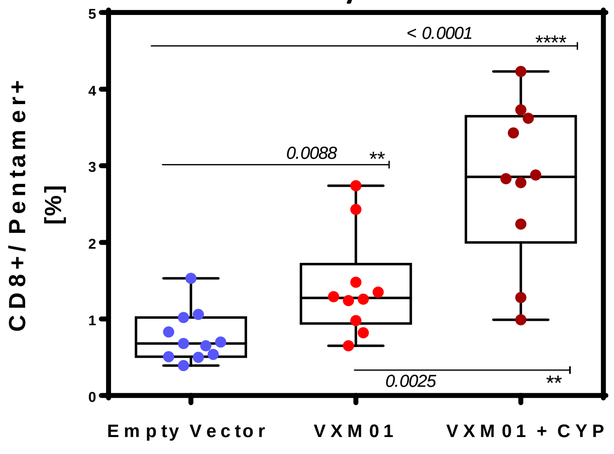


Figure 3 : Pentamer +/CD8+ T cells [%] (Splenocytes), each dot represents the results of the CD8+Pentamer+ [%] of one Spleen. Results in total % of 3 VEGFR2 pooled relevant Pentamers. Red dot = empty vector treated mice; blue dots = VXM01m treated mice; VXM01 and Cyclophosphamide treated mice

Results

Correlation of tumor volume and T cells

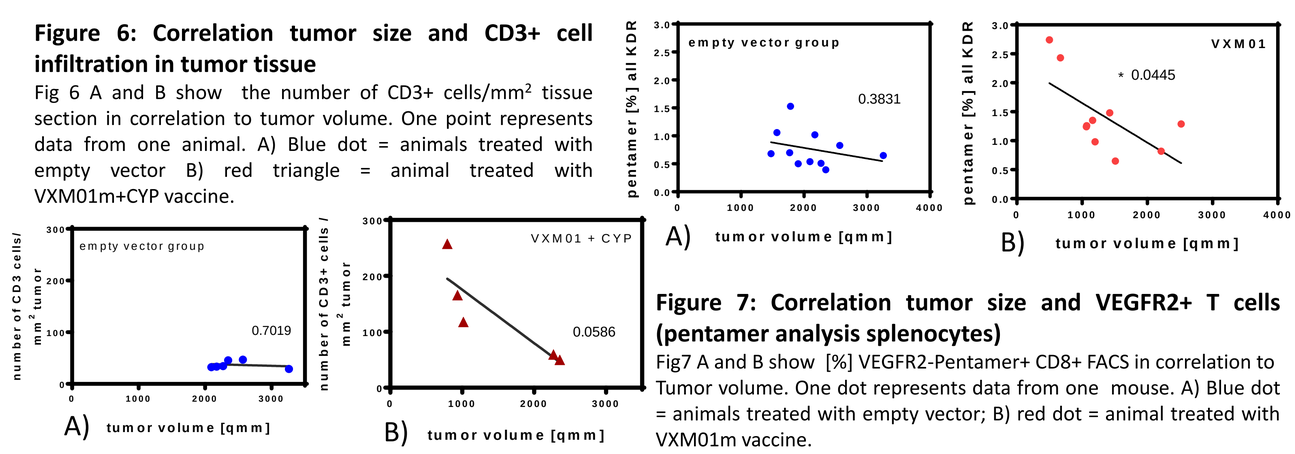


Figure 6: Correlation tumor size and CD3+ cell infiltration in tumor tissue
Fig 6 A and B show the number of CD3+ cells/mm² tissue section in correlation to tumor volume. One point represents data from one animal. A) Blue dot = animals treated with empty vector B) red triangle = animal treated with VXM01m+CYP vaccine.

Figure 7: Correlation tumor size and VEGFR2+ T cells (pentamer analysis splenocytes)
Fig7 A and B show [%] VEGFR2-Pentamer+ CD8+ FACS in correlation to Tumor volume. One dot represents data from one mouse. A) Blue dot = animals treated with empty vector; B) red dot = animal treated with VXM01m vaccine.

Conclusions

VAXIMM - VXM01 vaccination leads to …

- Significantly reduced tumorsize
- Induction of vaccination specific T cells
- 2-fold increase of immune cell infiltrates in the tumor

VXM01 vaccination has significant impact on the immune cell related control of CT26 tumor growth.

Future Projects: Phase IIa Clinical Trial

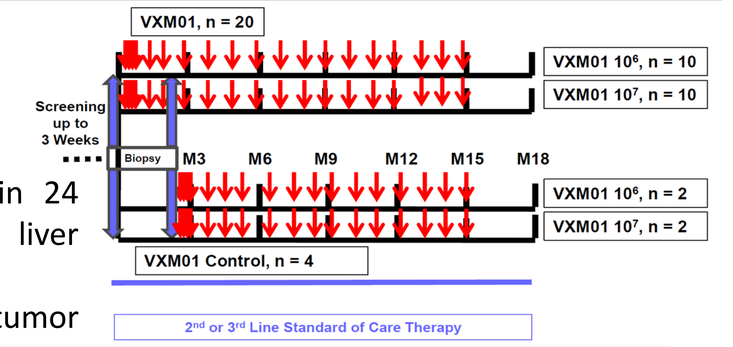
Phase IIa clinical trial started.
(EudraCT No 2015-003068-34).

Objective:

- Evaluation of VXM01 treatment in 24 colorectal cancer patients with liver metastases.
- Investigate immunologic and tumor responses.

Endpoints:

- safety and tolerability
- clinical response



Immunological endpoints:

- tumor infiltrating lymphocytes
- tumor vasculature by immunohisto-chemistry on serial liver metastasis biopsies.
- peripheral immune response (ELISpot)