# Non-clinical safety, immunogenicity and anticancer efficacy of VXM06, a live attenuated *Salmonella* Typhimurium oral T cell vaccine against WT1


**Sébastien Wieckowski[1], Lilli Podola[2], Marco Springer[2], Iris Kobl[2], Zina Koob[3], Caroline Mignard[3], Alan Broadmeadow[4], Pauline Stevens[4], Clare Chesher[4], Amine A. Berkane[5], Ming Wei[5], Klaus M. Breiner[1], Albrecht Meichle[2], Philipp Beckhove[6], Marc Mansour[1], Matthias Schroff[1], Heinz Lubenau[2]**

[1]VAXIMM AG, Basel, Switzerland; [2]VAXIMM GmbH, Mannheim, Germany; [3]Oncodesign S.A., Dijon, France; [4]Envigo CRS Ltd, Huntingdon, United Kingdom; [5]CellVax S.A.S., Romainville, France; [6]Regensburg Center for Interventional Immunology (RCI), Regensburg, Germany.


## Background

VAXIMM's oral T-cell vaccine platform is based on the approved, live attenuated *Salmonella* Typhi strain Ty21a vaccine, which has been administered in millions of individuals for prophylactic vaccination against typhoid fever. This strain has been thoroughly studied, is safe and well tolerated. The bacteria are modified to deliver an eukaryotic expression plasmid, which encodes the genetic information of a specific target antigen[1].

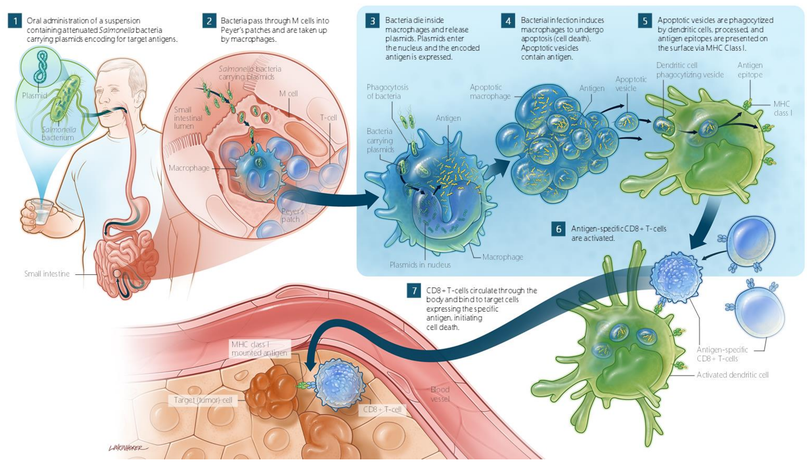


**Figure 1.** Intra-lymphatic delivery of *Salmonella* Typhi strain Ty21a T-cell vaccines via the oral route leading to target-specific T-cell activation.

Our lead vaccine VXM01 encodes vascular endothelium growth factor receptor 2 (VEGFR2) in order to evoke an immune response specifically directed against the tumor vasculature. It is currently in clinical development as a treatment for various solid cancer types. The murine analogue of VXM01 has shown consistent anti-angiogenic activity in different tumor models in several animal studies[2]. A proposed mechanism of action of *Salmonella* Typhi strain Ty21a oral T-cell vaccines is described in **Figure 1**.

The current study summarizes the non-clinical safety profile, immunogenicity and preclinical anti-cancer efficacy for the *Salmonella* Typhimurium SL7207 murine vaccine VXM06 (**Figure 2**) which encodes a murine Wilm's tumor 1 (WT1) protein variant lacking all the zinc fingers. The empty vector, i.e. without plasmid, was used as negative control throughout the study.

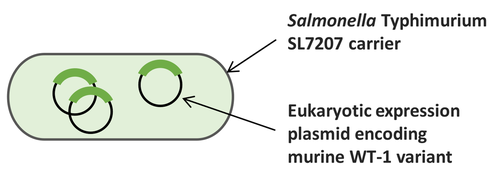


**Figure 2.** Schematic representation of VXM06 oral T-cell vaccine.

## Toxicology

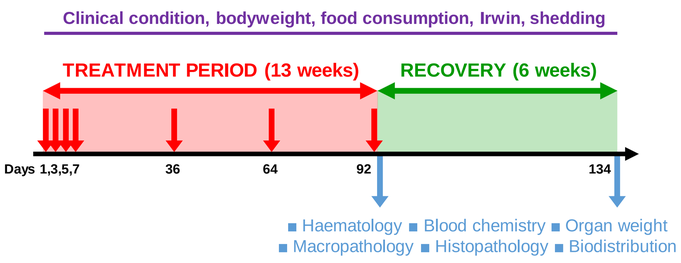


**Figure 3.** Design of the 13-week toxicology study consisting in a treatment period of 13 weeks followed by a 6-week recovery phase.

The preclinical safety profile of VXM06 and the empty vector control was assessed in C57BL/6J mice, with n=10♂ and 10♀ in both the treatment period and recovery (R) phases, following oral gavage administration of doses up to $10^8$ colony-forming units (CFU) per occasion on days 1, 3, 5, 7, and once monthly thereafter during 13 weeks, followed by 6-week recovery, in a GLP-compliant toxicology study (**Figure 3**).

There was no evidence of proliferation of VXM06 in the feces or organs analyzed. High white blood cell values were measured in mice receiving empty vector or VXM06 at $10^6$ or $10^8$ CFU/occasion. These findings were considered to be a *Salmonella* vector effect. Importantly, there were no deaths related to empty vector or VXM06, and no clear treatment related clinical signs, nor bodyweight, food consumption, hematology, organ weight and macroscopic pathology findings. Of note, small reductions of plasma calcium and phosphorus concentrations in males and females given VXM06, persisting to the end of the recovery period were reported.

Histopathology analyses revealed multifocal inflammation and single cell necrosis in the liver of animals treated with either the empty vector control or VXM06 at $10^8$ CFU/occasion (**Figure 4**). This finding was attributed to the bacterial vector when given at the highest dose. There was no clear evidence of recovery for the liver changes following 6 weeks respite from treatment.

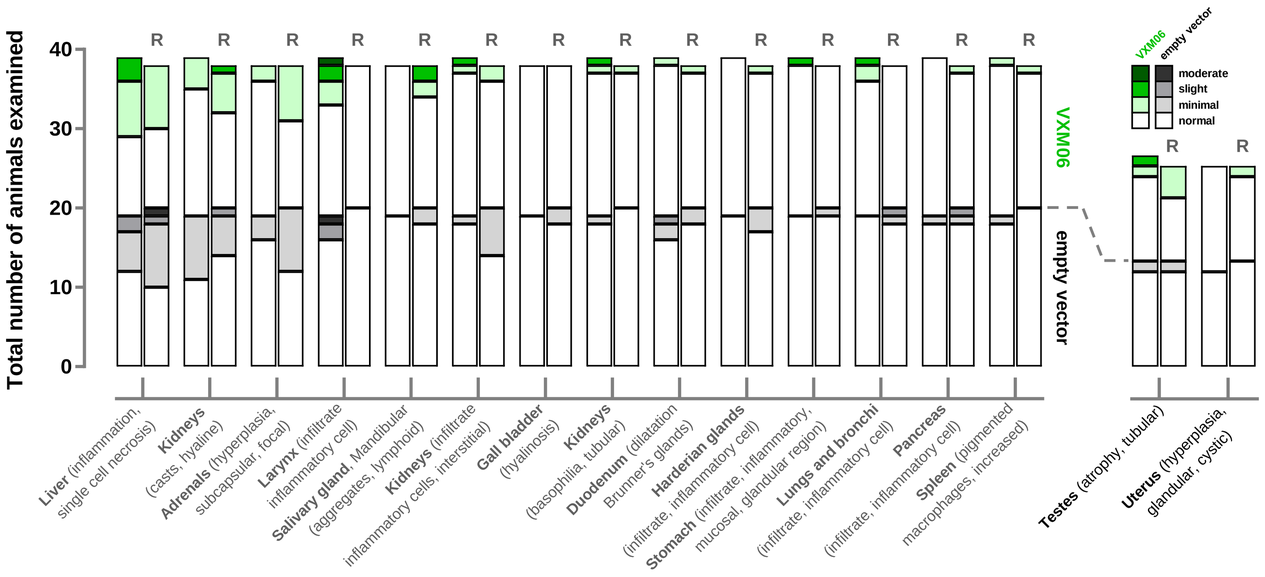


**Figure 4.** Histopathology findings observed in ≥2% of the animals examined, males and females pooled together, after 13 weeks of treatment with empty vector (grey and black stacked bars) and VXM06 (green stacked bars) at $10^8$ CFU/occasion (left bars) and after 6-week recovery (R; right bars).

## Immunogenicity

The immunokinetic study was performed in healthy C57BL/6 mice (n=5 per group), vaccinated 4 times every other day via the oral route with $10^{10}$ CFU of either VXM06 or the empty vector control. The frequency of antigen-specific T cells was measured at different time points in the spleen by flow cytometry using fluorescently labelled MHC class I/peptide pentamers, without prior in vitro stimulation (**Figure 5A**).

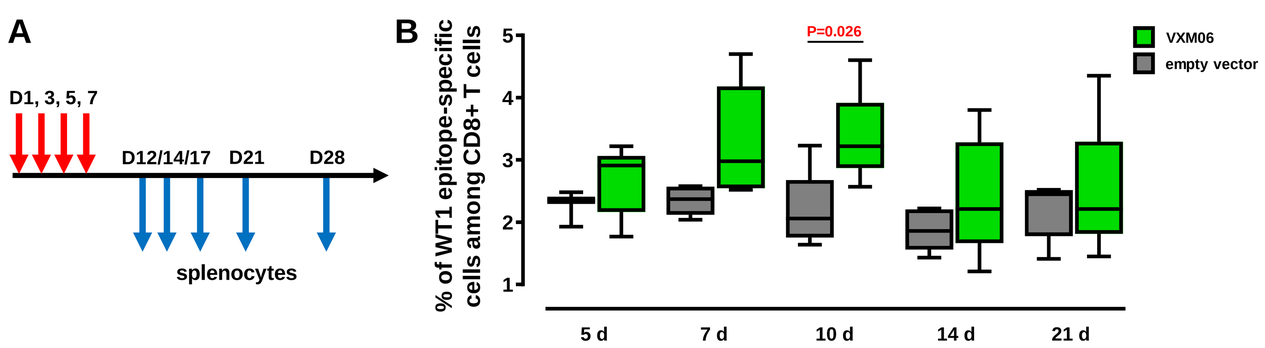


**Figure 5.** (A) Experiment design and treatment schedule, and (B) frequency of WT1 antigen-specific cells among CD8+ T cells in the splenocytes of healthy C57BL/6 mice treated with the empty vector (grey boxplots) and VXM06 (green) at the indicated time points after the final vaccination.

Vaccination with VXM06 induced a significant systemic CD8 T cell response specific to WT1 antigens, with a peak immune response detected 7 to 10 days after the final vaccination (**Figure 5B**). Comparable immunogenicity kinetics were measured in healthy animals using *Salmonella* Typhimurium vaccines encoding murine VEGFR2 and mesothelin proteins[3,4].

## Anticancer efficacy

We evaluated the prophylactic anticancer activity of VXM06 in the FBL-3 disseminated model of leukemia expressing WT1[5]. Empty vector and VXM06 were given by oral gavage at a dose of $10^8$ CFU on days 1, 3, 5 and 7 as a prime vaccination, and on days 14 and 22 as boosts (**Figure 6**). C57BL/6 mice (n=10) then received $5\times10^6$ viable FBL-3 cells by intraperitoneal injection on day 20. All surviving animals were re-challenged with $5\times10^6$ viable FBL-3 cells by intraperitoneal injection on day 100.

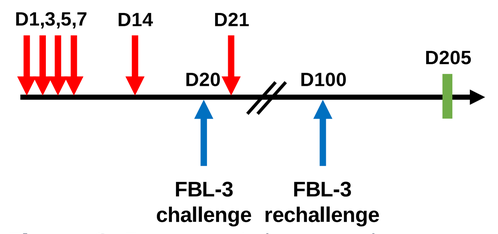


**Figure 6.** Experiment design and treatment schedule in the prophylactic and re-challenge experiment.

Prophylactic vaccination with VXM06 was highly tolerated, as no deterioration in general status was observed during the treatment, and neither death nor significant body weight loss were recorded during the prime/boost treatment (**Figure 7A**). Vaccination with VXM06 generated a rapid and sustained anti-leukemia effect with 100% (10 out of 10) of surviving animals 80 days after leukemia challenge (P<0.0001), confirming the data from a previous experiment[3,4]. In contrast, vaccination with the empty vector control did not show any anti-cancer activity, as the median survival reached 41 days, and 0% (0 animals out of 6) of cancer regression was observed (**Figure 7B**). Importantly, 100% of surviving mice resisted re-challenge with FBL-3 cells at least 100 days after leukemia re-challenge (P<0.0001), demonstrating that vaccination with VXM06m generated a potent memory T cell response against the leukemia (**Figure 7B**).

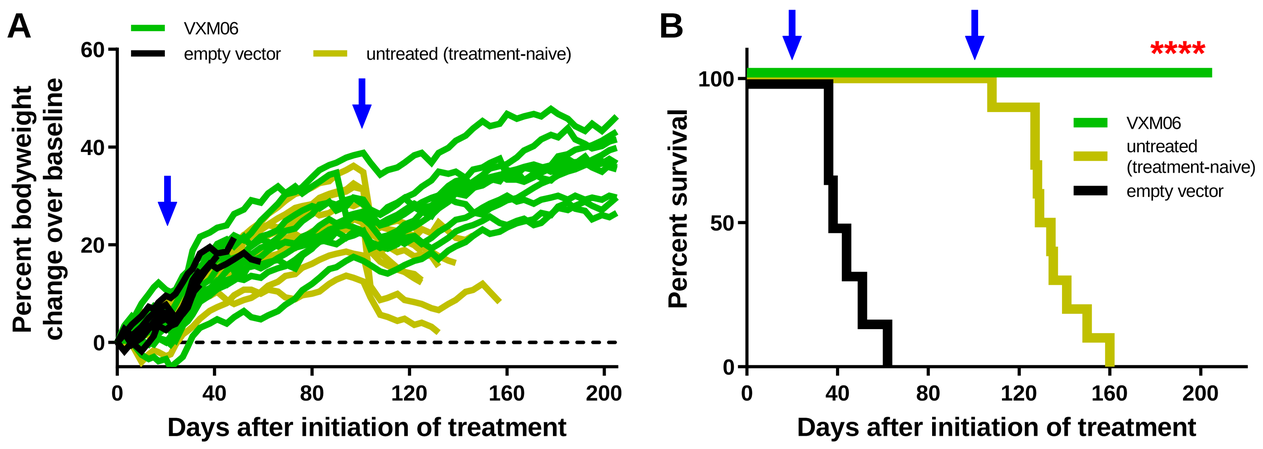


**Figure 7.** (A) Evolution of the bodyweight in each individual animal, and (B) overall survival in the different treatment groups. The blue arrows represent the time points of leukemia challenge. Treatment-naive animals (yellow curves) were used as a control for the FBL-3 rechallenge and received the leukemia cells only day 100.

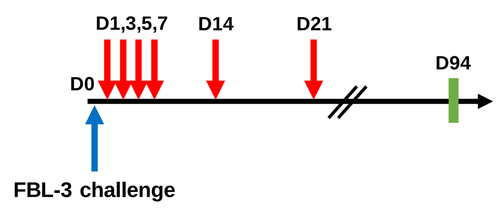


**Figure 8.** Design of the therapeutic study.

We finally evaluated the therapeutic efficacy of VXM06 in the FBL-3 model. C57BL/6 mice (n=8 per group) received $5\times10^6$ viable FBL-3 cells by intraperitoneal injection on day 0. Empty vector and VXM06 were then administered by oral gavage at a dose of $10^9$ CFU on days 1, 3, 5 and 7 as a prime vaccination, and on days 14 and 21 as boosts (**Figure 8**). The overall survival was monitored up to day 94.

Therapeutic vaccination with VXM06 was well tolerated, as neither clinical sign, neither death nor significant bodyweight loss were recorded during the treatment period (**Figure 9A**). Therapeutic vaccination with VXM06 induced the full leukemia control, with 100% (8 out of 8) of surviving animals 94 days after leukemia challenge (P<0.0001). In contrast, treatment with the empty vector control did not show any anti-cancer effect (**Figure 9B**).

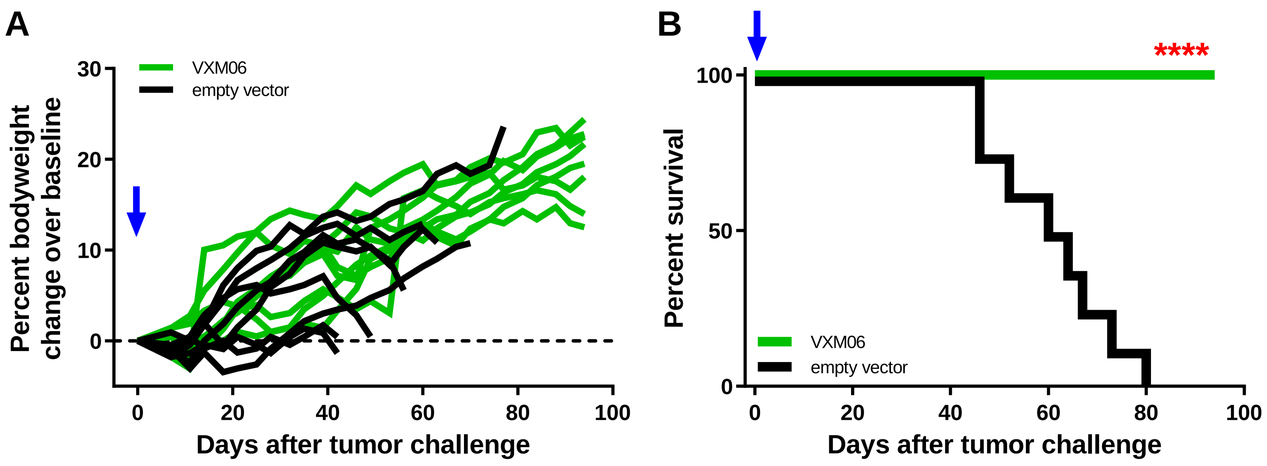


**Figure 9.** (A) Evolution of the bodyweight in each individual animal, and (B) overall survival in both treatment groups, in the therapeutic setting. The blue arrow represents the time point of FBL-3 challenge.

## Conclusions

- **VXM06 was well-tolerated, generated substantial immunogenicity in healthy animals, and a potent memory T-cell response in animals bearing FBL-3 leukemia.**
- **Vaccination with VXM06 induced a rapid and sustained anti-cancer activity in the FBL-3 model of leukemia, in both the prophylactic and therapeutic settings.**
- **This study provides further evidence that VAXIMM's oral T-cell vaccination platform can be used to stimulate anti-tumor immunity against various tumor-associated antigens, including WT1.**
- **These data paved the way for advancing the development of VXM06 into clinical development, in particular in leukemia.**

## References


*1. Darji A. et al., Cell 1997; 91:765. 2. Niethammer AG. et al., Nature Medicine 2002; 8:1369. 3. Wieckowski S. et al., European Journal of Cancer 2016; 69:S106. 4. Wieckowski S. et al., Cancer Research 2017; 77(13S):Abstract nr 4558. 5. Kohrt HE. et al., Blood 2011; 118:5319.*


## Contact and Information


**Sébastien Wieckowski, Ph.D.**
**VAXIMM AG**
**Senior Scientist**

Technologiepark Basel
Hochbergerstrasse 60c
4057 Basel
Switzerland

Office: +41 61 633 29 56
Fax: +49 621 8359 687 99
✉ sebastien.wieckowski@vaximm.com
www.vaximm.com



**Poster No. B058 presented during the Cancer Vaccines and Targets session at the Third CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference on September 8th 2017 in Mainz/Frankfurt, Germany.**