Non-clinical safety and antitumor efficacy of live attenuated *Salmonella typhimurium*-based oral T-cell vaccines VXM01m, VXM04m and VXM06m

Sébastien Wieckowski1, Marco Springer2, Lilli Podola3, Alan Broadmeadow4, Pauline Stevens4, Clare Chesher4, Amine Adda Berkane3, Ming Wei5, Iris Kobi5, Albrecht Meichle5, Phillip Beckhove5, Klaus M. Breiner5, Heinz Lubena2

1 VAXIMM AG, Basel, Switzerland; 2 VAXIMM GmbH, Mannheim, Germany; 3 Regensburg Center for Interventional Immunology (RCI), c/o University Medical Center Regensburg, Regensburg, Germany; 4 Enviros CTS Ltd, Huntingdon, United Kingdom; 5 Cellvix S.A.S., Romans-sur-Isère, France.

# Background

*VAXIMM*'s oral T-cell vaccine platform is based on the approved live attenuated *Salmonella typhimurium* vaccine strain Ty21a, which has been applied 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 payload of a specific target antigen (Figure 1).

VXM01 is encoding vascular endothelial growth factor receptor 2 (VEGFR2) in order to evoke an immune response specifically directed against tumor vasculature. It is currently in clinical development as a treatment for solid cancer types. The murine analogue of VXM01 has shown consistent anti-angiogenic activity in different tumor types in several animal studies. An increase in tumor immune infiltration was recently shown. A proposed mechanism of action of VXM01 is described in Figure 2.

![Figure 1](image1.png) Schematic representation of *VAXIMM*'s oral T-cell vaccine platform.

![Figure 2](image2.png) Ultra-loxophytic delivery of VXM01 via the oral route leading to target specific T-cell activation.

**Vaccine Protein encoded**

<table>
<thead>
<tr>
<th>VXM01m_empty</th>
<th>mVEGFR2</th>
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<tbody>
<tr>
<td>VXM01m</td>
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<tr>
<td>VXM04m</td>
<td>mMSLN</td>
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<tr>
<td>VXM06m</td>
<td>mWT1</td>
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**Table 1. List of *VAXIMM*'s Salmonella typhimurium-based vaccines used in this study.**

# Toxicology

In a previous toxicology study in C57BL/6 mice, VXM01m was shown to be generally well tolerated following oral gavage administration of doses up to 10⁶ CFU/occasion on Days 1, 3, 5, 7 and then once monthly thereafter for 26 weeks (data not shown). There was no evidence of proliferation of VXM01m in the feces. Data analyzed for drug-related effects were limited to inflammation/angiogenesis cell necrosis in the liver of mice receiving 10⁶ CFU/occasion of VXM01m and VXM04m, and high white blood cell values in mice receiving VXM01m, VXM04m at 10⁶ CFU/occasion, or VXM06m at 10⁶ CFU/occasion. These findings corresponded to a Salmonella typhimurium vector effect.

The preclinical safety profile of the control Salmonella typhimurium VXM01m_empty, as well as VXM06m and the VXM01m/VXM04m combination was assessed in C57BL/6J mice, with n=10 per group. After repeated administrations by gavage with doses up to 10⁷ CFU during 13 weeks followed by 6-week recovery in a GLP-compliant toxicity study (Figure 3).

![Figure 3](image3.png) Histopathology findings observed in 2/2 of the animals examined, males and females pooled together, after 13 weeks of treatment with 10⁷ CFU/occasion (left bar) and after 6-week recovery (right bar).

Finally, VXM01m, VXM04m or VXM06m was not consistently identified in any tissue or organs in either of the experiments and there was no evidence of proliferation in tissues or feces.

# Antitumor efficacy of VXM01m and VXM04m

The anti-tumor efficacy of VXM01m/empty, VXM01m and VXM04m was evaluated in the Panc02 syngeneic model of pancreatic adenocarcinoma expressing MSLN².

Single agents VXM01m/empty, VXM01m and VXM04m were given by oral gavage at a dose of 10⁶ CFU/administration to Days 1, 3, 5 and 7 as a prime vaccination, and on Days 14, 28, 35 and 42 as boost vaccinations (Figure 4). Mice (n=8 per group) received 1×10⁶ viable Panc02 cells by subcutaneous injection into the left flank on Day 21. The tumor was measured twice weekly and tumor volume was estimated using the formula 0.5 × L × W², with L and W the length and the width respectively. The experiment was completed on Day 56, i.e. 35 days after tumor challenge.

![Figure 4](image4.png) (A) Body weight in the 13-week treatment, and (5) 6-week recovery studies (group means in grams).

![Figure 5](image5.png) In addition, small reductions of plasma calcium and phosphorous concentrations occurred in males and females given VXM01m/VXM04m or VXM06m, persisting to the end of the recovery period.

High plasma alkaline phosphatase, alanine and aspartate aminotransferase activities, high total bilirubin and low total cholesterol and triglyceride concentrations were recorded for two males dosed with VXM06m (both of which had a small irregular liver and enlarged spleen) and multifocal infiltration/single cell necrosis was seen in the liver of animals treated with 10⁶ CFU/occasion control (empty vector). VXM01m/VXM04m or VXM06m, this finding was attributed to the bacterial vector when given at the highest dose. There was no clear evidence of recovery for liver changes following 6 weeks respite from treatment (Figure 5).

![Figure 6](image6.png) (A) Antitumor efficacy of VXM01m and VXM04m. (B) Percent body weight change over baseline (BD) for each individual animal, and (B) overall survival in both treatment groups.

Conclusions

VXM01m/VXM04m combination and VXM06m were well tolerated at the effective doses. VXM01m, VXM04m and VXM06m demonstrated consistent anti-cancer activities in different animal tumor models.

This study provides further evidence that *VAXIMM*'s versatile oral T-cell vaccine platform can be used to stimulate antitumor immunity and then act against various tumor-associated antigens.

Further studies of VXM01, VXM04, VXM06 and other cancer vaccines candidates on this oral T cell vaccine platform are warranted.

![Figure 7](image7.png) (A) Antitumor efficacy of VXM06m. (B) Antitumor efficacy of VXM06m. (C) Tumor growth inhibition relative to the VXM01m empty control group in all treatment groups, 35 days after tumor challenge.

![Figure 8](image8.png) Poster No. 321 presented during the Immunotherapy session at the 23rd EORTC-QLQ-ACR 70 Symposium on Molecular Targets and Cancer Therapeutics on November 27th 2016 in Munich.

# References


# Contact

Dr. Sebastian Wieckowski, PhD.
VAXIMM PLC.
Hochschulstrasse 4a 05.
Technopark Basel
Switzerland
Office: +41 61 630 56 00
Fax: +41 61 630 68 07
www.vaximm.com

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The anti-cancer activity of VXM06m was evaluated in the FBL-3 disseminated model of erythroleukemia expressing WT1.

VXM01m/empty and VXM06m were given by oral gavage at a dose of 10⁶ CFU/administration on Days 1, 3, and 7, and as a prime vaccination, and on Days 14 and 24 as boost vaccinations (Figure 8). Mice (n=10 per group) received 5×10⁶ viable FBL-3 cells by intraperitoneal injection on Day 20.

The body weight was measured twice weekly and the overall survival was monitored up to Day 96.