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

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Background

VAXIMM's oral T-cell vaccine platform is based on the approved, live attenuated *Salmonella typhi* strain Ty21a vaccine, 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 information of a specific target antigen[1] (**Figure 1**).

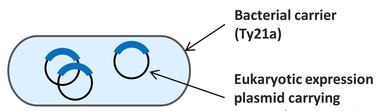

Figure 1. Schematic representation of VAXIMM's oral T-cell vaccine platform.

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 solid cancer types. The murine analogue of VXM01 has shown consistent anti-angiogenic activity in different tumor models in several animal studies[2]. An increase in tumor immune cell infiltration was recently shown. A proposed mechanism of action of VXM01 is described in **Figure 2**.

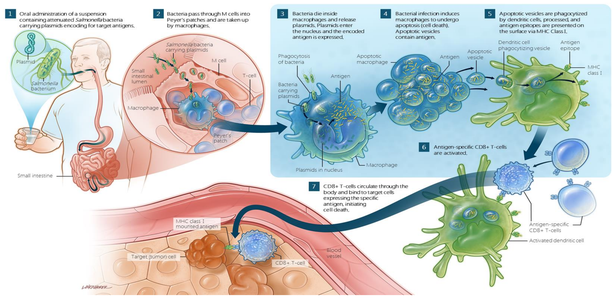

Figure 2. Intra-lymphatic delivery of VXM01 via the oral route leading to target specific T-cell activation.

Vaccine	Protein encoded
VXM0m_empty	-
VXM01m	mVEGFR2
VXM04m	mMSLN
VXM06m	mWT1

The current study summarizes the non-clinical safety profile, immunogenicity and preclinical anti-cancer efficacy for *Salmonella* Typhimurium murine vaccines VXM01m, VXM04m and VXM06m which encode respectively the murine VEGFR2, mesothelin (MSLN) and Wilms tumor 1 (WT1) proteins. The empty vector VXM0m_empty was used as negative control (**Table 1**).

Table 1. List of VAXIMM's *Salmonella* Typhimurium-based vaccines used in this study.

Toxicology

The preclinical safety profile of VXM0m_empty, VXM01m, VXM06m as well as the VXM01m/VXM04m combination was assessed in C57BL/6J mice, with n=10♂ and 10♀ in both the main 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 then once monthly thereafter during 13 weeks (VXM0m_empty, VXM01m/VXM04m and VXM06m) or 26 weeks (VXM01m), followed by 6-week recovery, in a GLP-compliant toxicology study (**Figure 3**).

There was no evidence of proliferation of VXM01m, VXM04m or VXM06m in the feces or organs analyzed. High white blood cell values were measured in mice receiving VXM0m_empty, VXM01m, VXM01m/VXM04m or VXM06m at 10^6 or 10^8 CFU/occasion. These findings were considered to be a *Salmonella* vector effect.

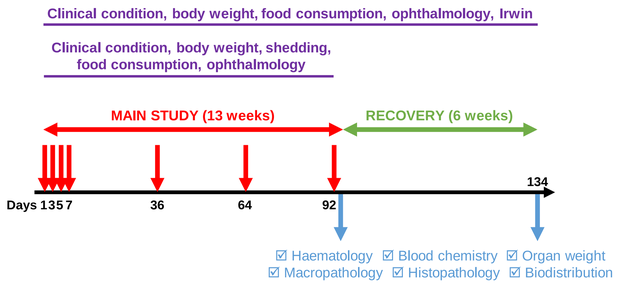

Figure 3. Design of the 13-week toxicology study (VXM0m_empty, VXM06m and VXM01m/VXM04m). The 26-week toxicology study (VXM01m) consisted in 3 additional doses on days 120, 148 and 178 followed by a 6-week recovery phase.

There were no deaths related to VXM0m_empty, VXM01m, VXM06m or VXM01m/VXM04m, and no clear treatment related clinical signs, Irwin observations or bodyweight, food consumption, hematology, organ weight or macroscopic pathology findings.

Nevertheless, the following observations were reported:

- small reductions of plasma calcium and phosphorus concentrations in males and females given VXM01m/VXM04m or VXM06m, persisting to the end of the recovery period;
- high plasma alkaline phosphatase, alanine and aspartate amino-transferase activities, high total bilirubin and low total cholesterol and triglyceride concentrations were recorded in two males dosed with VXM06m, both of which had a small irregular liver and enlarged spleen;
- increase in the incidence of lens opacity/cataract, when compared to the pre-treatment incidence, occurred in animals dosed with VXM0m_empty, VXM01m/VXM04m or VXM06m at 10^8 CFU per occasion.

Histopathology analyses revealed multifocal inflammation/single cell necrosis in the liver of animals treated with 10^8 CFU/occasion control (empty vector), VXM01m, VXM01m/VXM04m or VXM06m (**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.

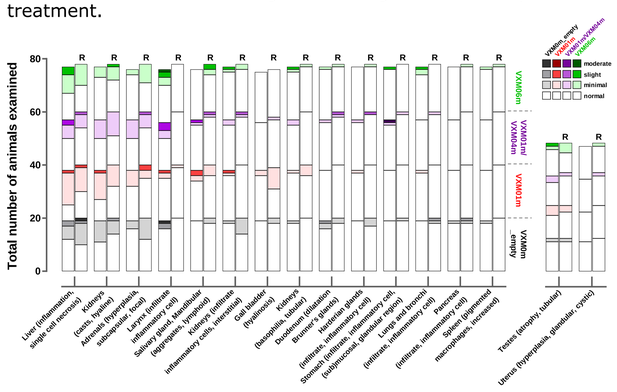

Figure 4. Histopathology findings observed in ≥2% of the animals examined, males and females pooled together, after 13 or 26 weeks of treatment with 10^8 CFU/occasion (left bars) and after 6-week recovery (R; right bars).

Immunogenicity

Immunokinetic studies were 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 VXM01m, VXM04m, VXM06m or the empty vector control VXM0m_empty. The frequency of antigen-specific T cells was measured at different time points in the spleen using fluorescently labelled MHC class I/peptide pentamers by flow cytometry (**Figure 5A**).

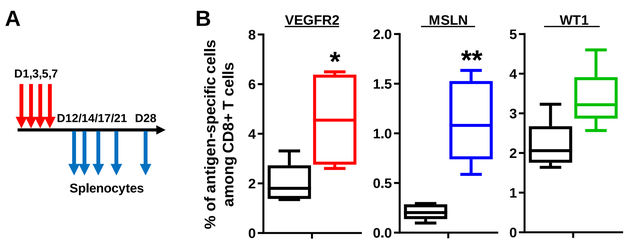

Figure 5. (A) Experiment design and treatment schedule, and (B) frequency of the indicated antigen-specific cells among CD8+ T cells in the splenocytes of healthy C57BL/6 mice treated with the empty vector (black boxes), VXM01m (red), VXM04m (blue) and VXM06m (green) at the peak immune response.

Treatment with either VXM01m, VXM04m or VXM06m induced a significant systemic antigen-specific CD8 T cell response, with a peak immune response detected 7 to 10 days after the last vaccination (**Figure 5B**).

Antitumor efficacy

The anti-tumor efficacy of VXM0m_empty, VXM01m and VXM04m was evaluated in the Panc02 syngeneic model of pancreatic adenocarcinoma expressing MSLN[3].

Single agents VXM0m_empty, VXM01m and VXM04m 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, 28, 35, 42 and 49 as boost vaccinations (**Figure 6**). Mice (n=8 per group) received 1×10^6 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 $0.5\times(L\times W^2)$, with L and W the length and the width respectively. The study was completed on day 56, i.e. 35 days after tumor challenge.

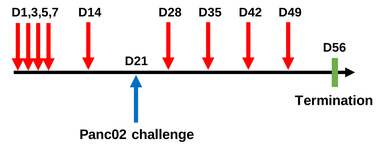

Figure 6. Experiment design and treatment schedule in the Panc02 study.

Over the treatment phase, when compared with the control group, treatment with VXM01m and VXM04m single agents produced a significant reduced tumor growth (**Figure 7**). No vaccination-related toxicity was observed throughout the study.

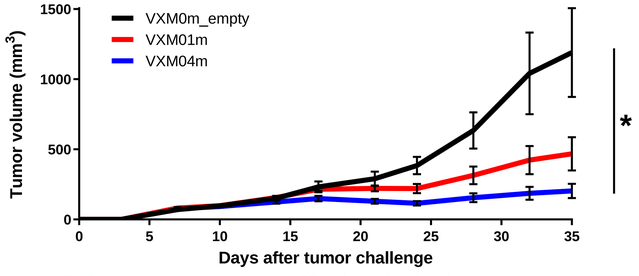

Figure 7. Tumor volumes (mean and SEM) in all the treatment groups.

At the end of the experiment, the mean tumor volume was reduced from 1189 ± 316 mm^3 in the control group to 468 ± 118 mm^3 (P=0.21) and 203 ± 51 mm^3 (P=0.05) in the VXM01 and VXM04m treatment groups respectively. Tumor growth inhibition relative to the control group reached 60.6% and 82.9% in the VXM01m and VXM04m treatment groups respectively, 35 days after tumor challenge.

The anti-cancer activity of VXM06m was evaluated in the FBL-3 disseminated model of erythroleukemia expressing WT1[4]. VXM0m_empty and VXM06m 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 boost vaccinations (**Figure 8**).

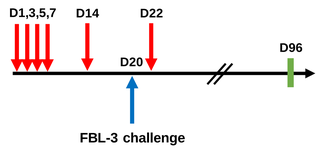

Figure 8. Experiment design and treatment schedule in the FBL-3 study.

Mice (n=10 per group) received 5×10^6 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.

Vaccination with VXM06m was highly tolerated as no deterioration in general status was observed during the treatment, and neither death nor significant body weight loss were recorded in mice during the prime/boost treatment (**Figure 9A**). Treatment of mice with VXM06m generated a rapid and sustained anti-tumor effect with 100% (10 out of 10) of surviving animals 76 days after leukemia challenge. In contrast, treatment with VXM0m_empty control vector did not show any anti-cancer effect, with a median survival of 45 days, and 0% (0 out of 10) of cancer regression (P<0.0001; **Figure 9B**).

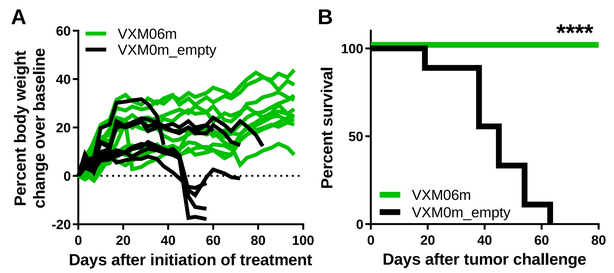

Figure 9. (A) Percent body weight change over baseline for each individual animal, and (B) overall survival in both treatment groups.

Conclusions

- **VXM01m, VXM01m/VXM04m combination and VMX06m were well tolerated at the effective doses. VXM01m, VXM04m and VXM06m induced significant systemic antigen-specific T cell responses in healthy animals, and have demonstrated consistent anti-cancer activities in different animal tumor models.**
- **This study provides further evidence that VAXIMM's versatile oral T-cell vaccination platform can be used to stimulate anti-tumor immunity against various tumor-associated antigens.**
- **Further studies of VXM01, VXM04, VXM06 in combination with immune checkpoint inhibitors, and other cancer vaccine candidates on this oral T cell vaccination platform, in particular against tumor neoantigens, are warranted.**

References

1. *Darji A. et al., Cell 1997; 91:765.* ***2.*** *Niethammer AG. et al., Nature Medicine 2002; 8:1369.* ***3.*** *Zervos E. et al., J Exp Clin Cancer Res 2016; 35:39.* ***4.*** *Kohrt HE. et al., Blood 2011; 118:5319.*

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