Non-clinical safety 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 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 information of a specific target antigen[1] (**Figure 1**).

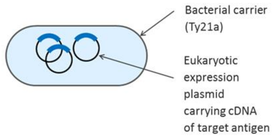

Figure 1. Schematic representation of VAXIMM's oral T-cell vaccine platform.

VXM01 is encoding 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 types 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**.

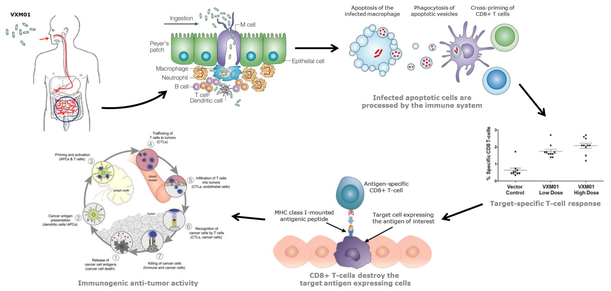

Figure 2. Intra-lymphatic delivery of VXM01 via the oral route leading to target specific T-cell activation.

Vaccine	Protein encoded
VXM0m_empty	-
VXM01m	mVEGFR2
VXM04m	mMSLN
VXM06m	mWT1

This study summarizes the non-clinical safety profile, as well as the preclinical anti-cancer efficacy for *Salmonella* typhimurium murine vaccines VXM01m, VXM04m and VXM06m which encode murine vascular endothelial growth factor receptor 2 (VEGFR2), mesothelin (MSLN) and Wilm's tumor 1 (WT1) protein antigens, respectively (**Table 1**).

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

Toxicology

In a previous toxicology study in C57BL/6Jmice, VXM01m was shown to be generally well tolerated following oral gavage administration of doses up to 10^8 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 or organs analyzed. Treatment related effects were limited to inflammation/single cell necrosis in the liver of mice receiving 10^8 CFU/occasion of VXM01m and VXM0m_empty, and high white blood cell values in males receiving VXM0m_empty at 10^8 CFU/occasion, or VXM01m at 10^6 or 10^8 CFU/occasion. These findings were considered to be a *Salmonella* typhimurium vector effect.

The preclinical safety profile of the control *Salmonella* typhimurium VXM0m_empty, as well as VXM06m and the VXM01m/VXM04m combination was assessed in C57BL/6J mice, with n=10♂ and 10♀ in both the main and recovery phases, after repeated administrations by gavage with doses up to 10^8 CFU during 13 weeks followed by 6-week recovery in a GLP-compliant toxicology study (**Figure 3**).

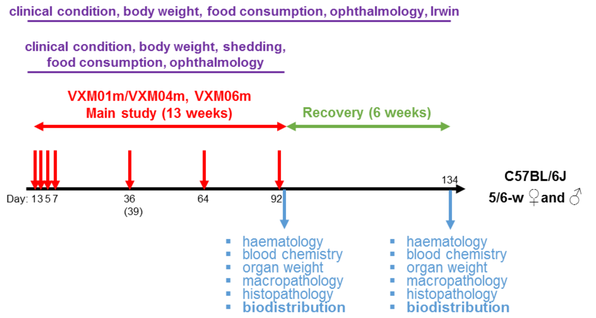

Figure 3. Design of the toxicology study.

There were no deaths related to VXM01m/VXM04m or VXM06m and no clear treatment related clinical signs, Irwin observations or bodyweight (**Figure 4**), food consumption, hematology, organ weight or macroscopic pathology findings. A clear increase in the incidence of lens opacity/cataract, when compared to the pre-treatment incidence, occurred in females dosed with VXM0_empty at 10^8 CFU/occasion, VXM01m/VXM04m or VXM06m at 10^8 CFU/occasion, and in males dosed with VXM06m at 10^8 CFU/occasion.

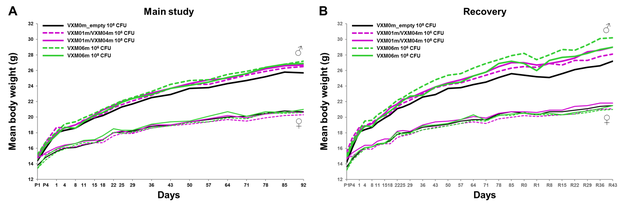

Figure 4. (A) Body weight in the 13-week treatment, and (B) 6-week recovery studies (group means in grams)

- In addition, small reductions of plasma calcium and phosphorus 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 amino-transferase 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);
- multifocal inflammation/single cell necrosis was seen in the liver of animals treated with 10^8 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 the liver changes following 6 weeks respite from treatment (**Figure 5**).

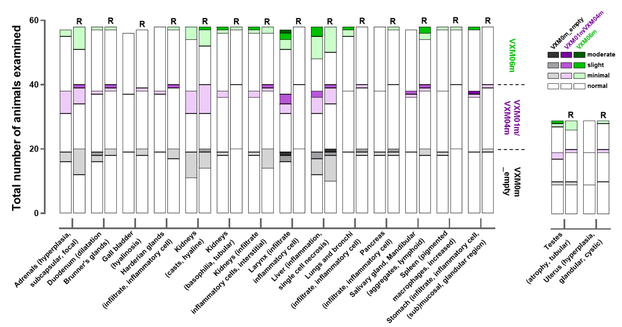

Figure 5. Histopathology findings observed in ≥2% of the animals examined, males and females pooled together, after 13 weeks of treatment with 10^8 CFU/occasion (left bars) and after 6-week recovery (R; right bars).

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

Antitumor efficacy of VXM01m and VXM04m

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/administration 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 experiment was completed on Day 56, i.e. 35 days after tumor challenge.

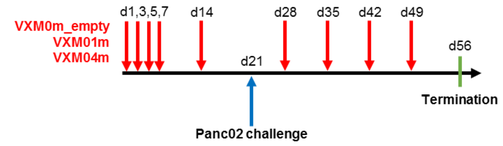

Figure 6. Experiment design and treatment schedule in the Panc02 study.

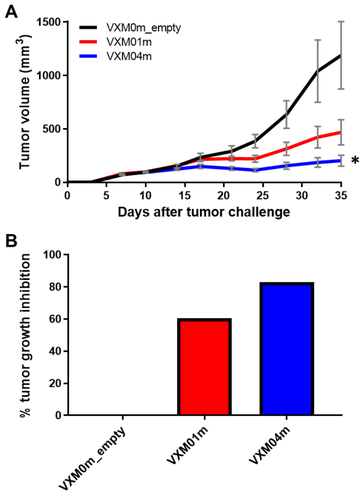

Figure 7. (A) Tumor volumes (mean and SEM), and (B) tumor growth inhibition relative to the VXM0m_empty control group in all treatment groups, 35 days after tumor challenge.

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

Antitumor efficacy of VXM06m

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

VXM0m_empty and VXM06m were given by oral gavage at a dose of 10^8 CFU/administration on Days 1, 3, 5 and 7 as a prime vaccination, and on Days 14 and 22 as boost vaccinations (**Figure 8**). 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.

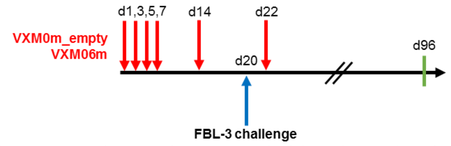

Figure 8. Experiment design and treatment schedule in the FBL-3 study.

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**).

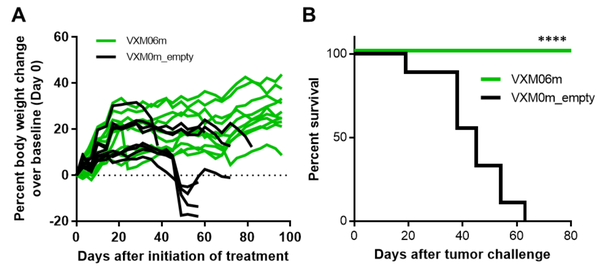

Figure 9. (A) Percent body weight change over baseline (D0) for each individual animal, and (B) overall survival in both treatment groups.

Conclusions

- **VXM01m/VXM04m combination and VMX06m were well tolerated at the effective doses. VXM01m, VXM04m and VXM06m 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 and other cancer vaccine candidates on this oral T cell vaccination platform are warranted.**

References

1. *Darji A., Cell. 1997; 91(6):765-75.* ***2.*** *Niethammer AG. et al., Nature Medicine. 2002; 8(12):1369-1375.* ***3.*** *Zervos E. et al., J Exp Clin Cancer Res 2016; 35:39.*

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Poster No. 321 presented during the Immunotherapy session at the 28th EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics on November 30th 2016 in Munich.