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 (Figure 1).

VXM01 encodes vascular endothelial 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. An increase in tumor immune cell infiltration was recently shown. A proposed mechanism of action of VXM01 is described in Figure 2.

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

Toxicology

The preclinical safety profile of VXM01_empty, VXM01m, VXM04m as well as the VXM01m/VXM04m combination was assessed in C57BL/6J mice, with n=10 per group in both the main and recovery (R) phases, following oral gavage administration of doses up to 100 colony-forming units (CFU) per occasion on days 1, 3, 5, 7 and then once monthly thereafter during 13 weeks (VXM01_empty, VXM01m/VXM04m and VXM06m) or 26 weeks (VXM01m), followed by 6-week recovery, in a GLP-compliant toxicity study (Figure 3).

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

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Protein encoded</th>
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<tbody>
<tr>
<td>VXM01_empty</td>
<td></td>
</tr>
<tr>
<td>VXM01m</td>
<td>mVEGFR2</td>
</tr>
<tr>
<td>VXM04m</td>
<td>mMSLN</td>
</tr>
<tr>
<td>VXM06m</td>
<td>mWT1</td>
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</tbody>
</table>

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 (WT1) proteins. The empty vector VXM01m was used as negative control (Table 1).

Immunoegenicity

Immunokinetic studies were performed in healthy C57BL/6 mice (n=5 per group), vaccinated 4 times every other day via the oral route with 10^7 CFU of either VXM01m, VXM04m, VXM06m or the empty vector VXM01m_empty. The frequency of antigen-specific T cells was measured at different time points in the spleine using fluorescently labelled MHC class I/peptide pentamers by flow cytometry (Figure 5A).

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

None of those deaths were related to VXM01m_empty, VXM01m, VXM04m or VXM06m/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 aminotransferase 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 VXM01m_empty, VXM01m/VXM04m or VXM06m at 10^7 CFU per occasion.

Histopathology analyses revealed multifocal inflammation/single cell necrosis in the liver of animals treated with 10^7 CFU intravenous control (empty vector), VXM01m, VXM01m/VXM04m or VXM06m (Figure 4). This finding was attributed to the bacterial vector, which was seen to be consistently present in the liver at the end of the recovery for the liver changes following 6 weeks respite from treatment.

Figure 4. Histopathology findings observed in ≥2% of the animals examined, namely multi-focal inflammation/single cell necrosis. (A) Control: 12 or 26 weeks of treatment with 10^7 CFU/occasion (left bars) and after 6-week recovery (R; right bars).

Antitumor efficacy

The anti-tumor efficacy of VXM01m_empty, VXM01m and VXM04m was evaluated in the Panc02 syngeneic pancreatic adenocarcinoma expressing MSLN.Single agents VXM01m_empty, VXM01m and VXM04m were given by oral gavage at a dose of 10^7 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). The VXM01m (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 a week and tumor volume was estimated using the formula 0.5×L×W^2 with L and W the length and the width, respectively. The study was completed on day 56, i.e. 35 days after the last boost vaccination.

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.

Conclusions

- VXM01m, VXM01m/VXM04m combination and VXM06m were well tolerated at the effective doses, with no signs of toxicity or significant antitumor models.
- This study provides further evidence that VAXIMM’s versatile oral T-cell vaccine platform can be used to stimulate antitumor 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 vaccine platform, in particular against tumor neangiogenesis, are warranted.

References


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Poster No. 455B presented during the Clinical Immunotherapy, Viruses, and Bacteria session at the AACR Annual Meeting on April 4th 2017 in Washington, DC.