Live attenuated oral *Salmonella* platform for effective targeting of PD-L1 and multiple tumor-associated epitopes

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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 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].

VXM01 lead vaccine encodes vascular endothelium growth factor receptor 2 (VEGFR2) to evoke an immune response specifically directed against the tumor vasculature. It is currently in clinical development as a treatment for various solid cancers. 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 VXM01 is described in **Figure 1**.

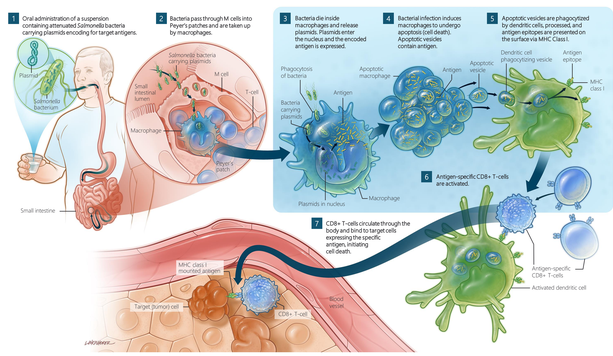

Figure 1. Intra-lymphatic delivery of *Salmonella* Typhi strain Ty21a T-cell vaccines via the oral route leading to target-specific T-cell activation.

The current study summarizes the immunogenicity and preclinical anti-cancer efficacy for the *Salmonella* Typhimurium SL7207 based DNA vaccines VXM10 and VXM10a (**Figure 2A**), transformed with eukaryotic expression plasmids encoding the full-length murine programmed death-ligand 1 (PD-L1) protein and a truncated form of PD-L1, respectively. It also presents for the first time the immunogenicity of poly-epitope constructs based on the platform called VXM-NEO (**Figure 2B**). The empty vector, i.e. without plasmid, was used as negative control throughout the study.

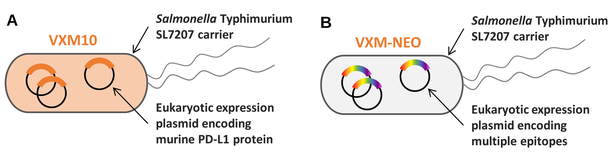

Figure 2. Schematic representation of oral *Salmonella* Typhimurium DNA vaccines (A) VXM10 encoding PD-L1 protein variants, and (B) VXM-NEO encoding multiple CD8 and CD4 epitopes from tumor-associated, angiogenic, and differentiation antigens, using different linking strategies.

Antibody response

The systemic antibody response was evaluated by ELISA in the serum of animals vaccinated with either VXM10 or VXM10a, 79 days after the final vaccination (**Figure 3A**).

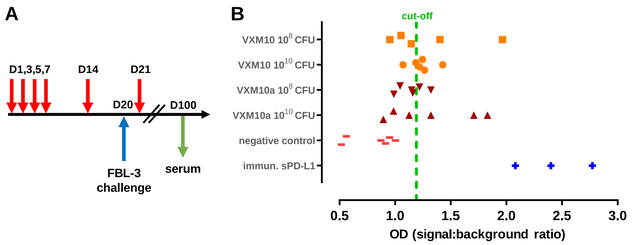

Figure 3. (A) Experimental design, and (B) anti-PD-L1 antibody response in the sera of FBL3-bearing animals, collected 79 days after the final vaccination. The green dashed line represents the cut-off value derived from the values of the negative control group (95% confidence). Soluble recombinant murine PD-L1 was used for immunization with CFA/IFA in the positive control group (blue).

Anti-PD-L1 antibodies were detected in a few animals vaccinated with VXM10 and VXM10a, and the response was more pronounced in the highest dose treatment groups, with 50% of the animals (3 out of 6) showing signal-to-background ratio above the cut-off value (**Figure 3B**).

Antitumor Efficacy

We evaluated the prophylactic anti-cancer activity of VXM10 and VXM10a in the FBL-3 disseminated model of leukemia expressing PD-L1[3] (**Figure 4**). Empty vector, VXM10 and VXM10a were given by oral gavage at ca. 10^8 CFU and 10^{10} CFU, on days 1, 3, 5 and 7 as a prime vaccination, and on days 14 and 22 as boosts (**Figure 5A**).

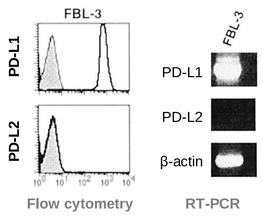

Figure 4. Expression of PD-L1, but not PD-L2, by FBL-3 cell line[3], as measured by flow cytometry (left inset) and RT-PCR (right).

C57BL/6 mice (n=6/group) then received 5×10^6 viable FBL-3 cells by intraperitoneal injection on day 20. All surviving animals were re-challenged with 5×10^6 viable FBL-3 cells by intraperitoneal injection on day 100.

Prophylactic vaccination with VXM10 and VXM10a was highly tolerated, and generated a rapid and sustained anti-leukemia effect with 100% (6 out of 6) of surviving animals 80 days after leukemia challenge (P=0.0005) in the highest dose groups. In contrast, vaccination with the empty vector control did not show any anti-cancer activity, and the median survival reached 41 days (**Figure 5B**).

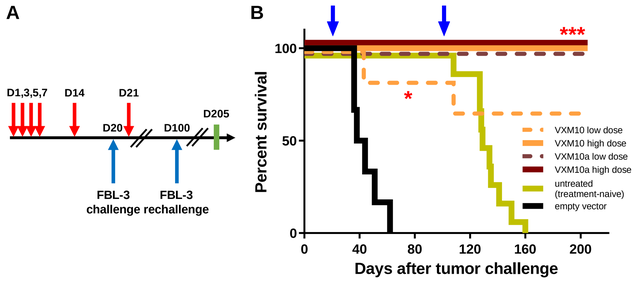

Figure 5. (A) Experimental design and treatment schedule in the prophylactic and re-challenge experiment, and (B) overall survival in the different treatment groups. The blue arrows represent the time points of leukemia challenge. Treatment-naive animals (yellow curves) were used as a control for the FBL-3 rechallenge and received the leukemia cells only on day 100.

Importantly, 100% of surviving mice in the high dose groups resisted re-challenge with FBL-3 cells for at least 100 days (P=0.0002), demonstrating that vaccination with VXM10 and VXM10a generated a potent memory T cell response against the leukemia (**Figure 5B**).

Finally, we evaluated the therapeutic efficacy of VXM10 and VXM10a in the FBL-3 model. C57BL/6 mice (n=8/group) received 5×10^6 viable FBL-3 cells by intraperitoneal injection on day 0. Empty vector, VXM10 and VXM10a were administered by oral gavage at a dose of 10^9 CFU on days 1, 3, 5 and 7, and on days 14 and 21 (**Figure 6A**).

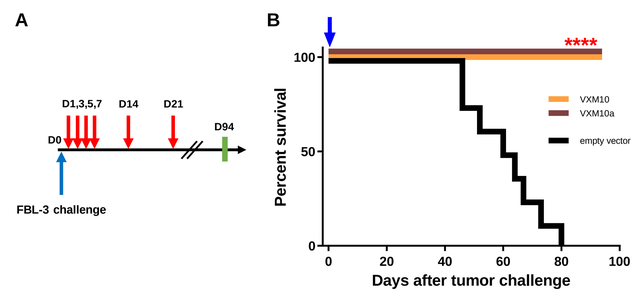

Figure 6. (A) Experimental design and treatment schedule in the therapeutic experiment, and (B) overall survival in all treatment groups, in the therapeutic setting. The blue arrows represent the time point of leukemia challenge.

Therapeutic vaccination with VXM10 and VXM10a was well tolerated, and induced 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 6B**).

VXM-NEO Platform

The immunogenicity of different polyepitope vaccines, based on the VXM-NEO platform, was assessed in healthy C57BL/6 mice (n=5 per group), vaccinated up to 4 times via the oral route with doses up to 10^{10} CFU.

The frequency of specific T cells for each individual epitope was measured 10 days after the final vaccination in the spleen by flow cytometry using fluorescently labelled MHC class I/peptide pentamers. The constructs encode multiple CD8 and CD4 epitopes from VEGFR2 (KDR), Mesothelin (MSLN), WT1, CEA, and Ovalbumin (OVA), linked via different spacers, e.g. "string-of-beads", and in different orders.

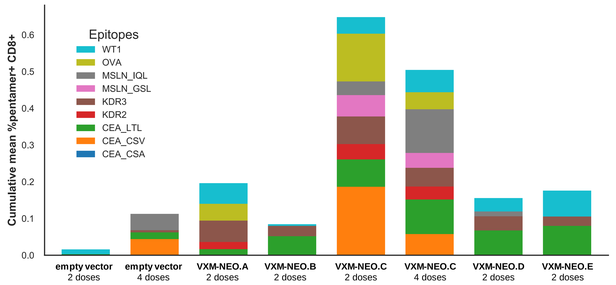

Figure 7. Cumulative mean frequency of the indicated epitope-specific CD8+ T cell population in the splenocytes of immunized C57BL/6 mice.

VXM-NEO vaccines induced a substantial systemic T-cell response for up to 6 out of 9 CD8 epitopes. Importantly, each of the dose, treatment schedule, ordering and linkage strategy greatly influences the immunogenicity of the encoded epitopes (**Figure 7**).

Conclusions

- **Prophylactic and therapeutic vaccinations with VXM10 constructs induced a strong and sustained anti-cancer activity in the FBL-3 model of leukemia.**
- **VAXIMM's oral T-cell vaccination platform can be employed to stimulate the adaptive immunity against antigens of the immune checkpoint regulatory protein PD-L1, but also antigens encoded by polyepitope constructs, and potentially neo-epitopes.**
- **These data also paved the way for advancing the clinical development of VXM10.**

References

1. *Darji A. et al., Cell 1997; 91:765.*
2. *Niethammer AG. et al., Nature Medicine 2002; 8:1369.*
3. *Yamazaki T. et al., Journal of Immunology 2002; 169:5538.*

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