A live attenuated Salmonella Typhimurium oral T cell vaccine against PD-L1 protects 100% of animals from a leukemia challenge

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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 antigen1.

VXM01 lead vaccine 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 various solid cancer types. The murine analogue of VXM01 has shown consistent anti-angiogenic activity in different tumor models in several animal studies2. An increase in tumor immune cell infiltration was recently shown. 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 Typhi SL7207 murine 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 (Figure 2B). Indeed, the deletion of the signal peptide (SP) prevents the proper localization of the native PD-L1 protein to the cell surface. The empty vector, i.e. without plasmid, was used as negative control throughout the study.

Antitumor efficacy

We evaluated the prophylactic anti-cancer activity of VXM10 and VXM10a in the FBL-3 disseminated model of leukemia expressing PD-L12 (Figure 3A). Empty vector, VXM10 and VXM10a were given by oral gavage at 10³ CFU and 10⁵ CFU, on days 1, 3, 5 and 7 as a prime vaccination, and on days 14 and 22 as boosts (Figure 3A). C57BL/6 mice (n=6 per group) then received 5x10⁹ viable FBL-3 cells by intraperitoneal injection on day 20. All surviving animals were rechallenged with 5x10⁹ viable FBL-3 cells by intraperitoneal injection on day 100.

Figure 3. (A) Expression of PD-L1, but not PD-L2, by FBL-3 cell line1, as measured by flow cytometry (left inset) and RT-PCR (right), and (B) experimental design and treatment schedule in the prophylactic and re-challenge experiment.

Prophylactic vaccination with VXM10 and VXM10a was highly tolerated, as no deterioration in general status nor significant body weight loss were observed during the treatment (Figure 4A). It also 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, as the median survival reached 41 days, and 0% (out of 6) of cancer regression was observed (Figure 4B). 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 4B).

Figure 4. (A) Evolution of the mean bodyweight, and (B) overall survival in the indicated treatment groups and doses. 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 day 100.

We finally evaluated the therapeutic efficacy of VXM10 and VXM10a in the FBL-3 model. C57BL/6 mice (n=8 per group) received 5x10⁹ viable FBL-3 cells by intraperitoneal injection on day 0. Empty vector, VXM10 and VXM10a were then administered by oral gavage at a dose of 10³ CFU on days 1, 3, 5 and 7 as a prime vaccination, and on days 14 and 21 as boosts (Figure 5).

Therapeutic vaccination with VXM10 and VXM10a was well tolerated (Figure 6A), 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).

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 7A). Anti-PD-L1 antibodies were detected in a few animals vaccinated with VXM10 and VXM10a, and the response was more pronounced in the VXM10a/high-dose group, with 50% of the animals (3 out of 6) showing signal-to-background ratio above the cut-off value (Figure 7B).

Figure 5. Experimental design of the therapeutic study.

Figure 6. (A) Evolution of the bodyweight in each individual animal, and (B) overall survival in all treatment groups, in the therapeutic setting. The blue arrow represents the time point of FBL-3 challenge.

Figure 7. (A) Experimental design, and (B) anti-PD-L1 antibody response in sera collected 79 days after the final vaccination. The green dashed line represents the cut-off value (for 95% confidence). Soluble recombinant murine PD-L1 was used for immunization with CFA/IFA in the positive control group (blue).

Conclusions

• Prophylactic and therapeutic vaccinations with VXM10 and VXM10a induced a strong and sustained anti-cancer activity in the FBL-3 model of leukemia.
• This study provides evidence that VAXIMM’s oral T cell vaccine platform can be used to stimulate anti-tumor immunity against antigens of the immune checkpoint regulatory protein PD-L1.
• These data paved the way for advancing the clinical development of VX10, in particular in leukemia.

References


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