Non-clinical safety, immunogenicity and anticancer efficacy of VXM06, a live attenuated Salmonella Typhimurium oral T cell vaccine against WT1

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**Background**

VAXIMM's oral T-cell vaccine platform is based on the approved, live attenuated Salmonella Typhimurium 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 modelled to deliver an eukaryotic expression plasmid, which encodes the genetic information of a specific target antigen.

Our lead vaccine VXM06 encodes vaccinia virus peri-umbilical 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 VXM06 has shown consistent antiangiogenic activity in different tumor models in several animal studies. A proposed mechanism of action of the antiangiogenic Typhi strain Ty21a oral T-cell vaccines is described in Figure 1.

**Toxicology**

The preclinical safety profile of VXM06 and the empty vector control was assessed in C57BL/6J mice, with n=10 and 10 in both treatment groups (empty vector or VXM06 at 10^9 and 10^8 CFU/occasion). Doses were administered by oral gavage and the body weight of the mice was monitored. The safety profile of VXM06 was established before the commencement of the study.

Conclusions

We evaluated the prophylactic anticancer activity of VXM06 in the FBL-3 disseminated model of leukemia expressing WT1. Empty vector and VXM06 were given in capsule oral gavage at a rate of 10^5 CFU on days 3, 5, 7 and 9 as a prime vaccination, and on days 14 and 22 after boosting (Figure 6). C57BL/6 mice (n=10) were then received 5x10^6 viable FBL-3 cells by intraperitoneal injection on day 20. All surviving animals were re-challenged with 5x10^6 viable FBL-3 cells by intraperitoneal injection on day 100.

Prophylactic vaccination with VXM06 was highly tolerated, as no deterioration in general status was observed during the treatment, and neither death nor significant body weight loss were recorded during the prime/boost treatment (Figure 7A). Vaccination with VXM06 generated a rapid and sustained anti-leukemia effect with 100% (10/10) of surviving animals 80 days after leukemia challenge (P<0.0001), confirming the data from a previous experiment [1]. In contrast, vaccination with the empty vector control resulted in 100% of surviving animals. The median survival reached 41 days, and 0% (0 animals out of 6) of cancer regression was observed (Figure 7B). The superiority of VXM06 over the control 100% of surviving animals was confirmed (P<0.0001) in an independent study with animals (yellow curves) were used as a control for the FBL-3 re-challenge and received the leukemia cells only day 100.

We finally evaluated the therapeutic efficacy of VXM06 in the FBL-3 model. C57BL/6 J mice (n=6 per group) received 5x10^6 viable FBL-3 cells by intraperitoneal injection on day 0. Empty vector and VXM06 were then administered orally by gavage at a dose of 10^5 CFU on days 3, 5, 7 and 9 as a prime vaccination, and on days 14 and 22 after boosting (Figure 8). The overall survival was monitored up to 200 days.

**Immunogenicity**

The immunokinetic study was performed in healthy C57BL/6J mice (n=5 per group), vaccinated 4 times every other day via the oral route with 10^9 CFU of either VXM06 or the empty vector control. Histopathological and single cell necrosis in the spleen by flow cytometry using fluorescently labelled MHC class I peptide pentamers, without prior in vitro stimulation (Figure 5A).

**Conclusions**

- **VXM06** was well-tolerated, generated substantial immunogenicity in healthy animals, and a potent memory T-cell response in animals infected with leukemia (WT1).
- **Vaccination with VXM06** induced a rapid and sustained anti-cancer activity in the FBL-3 model of leukemia, in both the prophylactic and therapeutic settings.
- This study provides further evidence that VAXIMM’s oral T-cell vaccine platform can elicit anti-tumor immunity against various tumor-associated antigens, including WT1.
- These data paved the way for advancing the development of VXM06 into clinical development, in particular in leukemia.

**References**


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