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

Sébastien Wieckowski1, Heiko Smetak2, Lilli Podola3, Marco Springr4, Iris Kob5, Anne-Lucie Nugues5, Philippe Slos6, Amine Berkan6, Ming Wei6, Klaus M. Breiner2, Albrecht Meichle1, Marc Mansour7, Matthias Schroff8, Philipp Beckhove9, Heinz Lubenua*

1VAXIMM AG, Basel, Switzerland; 2Regensburg Center for Interventional Immunology, Regensburg, Germany; 3VAXIMM GmbH, Mannheim, Germany; 4OncoDesign, Dijon, France; 5Cellax, Romanville, France.

**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.

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. A proposed mechanism of action of VXM01 is described in Figure 1.

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

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 (2 out of 6) showing signal-to-background ratio above the cut-off value (Figure 3B).

**Antigen Efficacy**

We evaluated the prophylactic anti-cancer activity of VXM10 and VXM10a in the FBL-3 disseminated model of leukemia expressing PD-L1 (Figure 4). Empty vector, VXM10 and VXM10a were given by oral gavage at 10⁶ CFU and 10⁸ CFU, on days 1, 3, 5 and 20, using specific anti-VEGFR2 vaccination, and on days 14 and 22 as boosts (Figure 5A).

CS7BL/6 mice (n=6/group) then received 5×10⁶ viable FBL-3 cells by intraperitoneal injection on day 20. All surviving animals were re-challenged with 5×10⁴ viable FBL-3 cells by intraperitoneal injection on day 100.

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).

**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 immune response against antigens of the 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**


**VXM-NEO Platform**

The immunogenicity of different polyepitope vaccines, based on the VXM-NEO platform, was assessed in healthy CS7BL/6 mice (n=5 per group), vaccinated up to 4 times via the oral route with doses up to 10⁶ CFU.