A phase 1 trial extension to assess immunologic efficacy and safety of prime – boost vaccination with VXM01, an oral T cell vaccine against VEGFR2, in patients with advanced pancreatic cancer

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Background

VAXIMM's oral T-cell vaccine platform is based on the approved, live attenuated *Salmonella typhi* vaccine strain Ty21a, 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[1] (**Figure 1**).

VXM01 is encoding 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 solid cancer types. The murine analogue of VXM01 has shown consistent anti-angiogenic activity in different tumor types in several animal studies[2]. An increase in tumor immune cell infiltration was recently shown. A proposed mechanism of action of VXM01 is described in **Figure 2**.

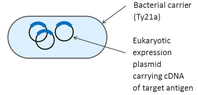

Figure 1. Schematic representation of VAXIMM's oral T-cell vaccine platform.

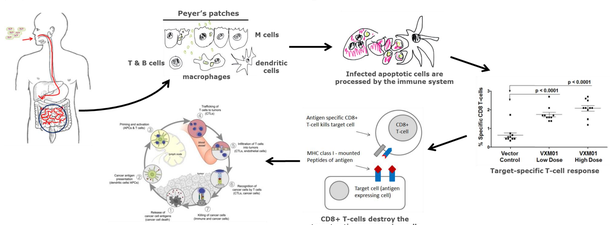

Figure 2. Intra-lymphatic delivery of VXM01 via the oral route leading to target specific T-cell activation.

A recent randomized, placebo-controlled, phase I dose-escalation trial in advanced pancreatic cancer patients demonstrated safety, immunogenicity and T cell response related transient anti-angiogenic activity of 4 priming vaccinations applied within one week[3]. As VEGFR2-specific T cell responses gradually declined after a peak response at day 21, the trial was extended to explore whether monthly boost vaccinations can be safely administered and maintain increased vaccine-specific T cell levels (*Clinical trial information: EudraCT 2011-000222-29*).

Methods

Characteristic		Placebo N=8	VXM01 N=18	VXM01 10^7 CFU N=12	VXM01 10^7 CFU N=6
Mean Age [years (range)]		64.5 (52-84)	64.9 (54-78)	65.3 (54-78)	64.2 (54-73)
Gender [N (%)]					
	Men	6 (75%)	7 (39%)	5 (42%)	2 (33%)
	Women	2 (25%)	11 (61%)	7 (58%)	4 67%)
Race [N (%)]					
	Caucasian	8 (100%)	18 (100%)	12 (100%)	6 (100%)
Karnofsky performance status [N (%)]					
	100	1 (12.5%)	1 (5.6%)	1 (8.3%)	0
	90	5 (62.5%)	6 (33.3%)	4 (33.3%)	2 (33.3%)
	80	2 (25.0%)	11 (61.1%)	7 (58.3%)	4 (66.7%)
Extent of disease [N (%)]					
	Locally advanced	2 (25.0%)	2 (11.1%)	2 (16.7%)	0
	Metastatic	6 (75.0%)	16 (88.9%)	10 (83.3%)	6 (100.0%)
Time from diagnosis [months (range)]					
	Median	7.5 (2-20)	6 (0-28)	6.5 (0-28)	6 (2-16)
Level of CA19.9 [N (%)]					
	Normal	4 (50.0%)	3 (16.7%)	2 (16.7%)	1 (16.7%)
	Elevated, <1000	2 (25.0%)	9 (50.0%)	4 (33.3%)	5 (83.3%)
	Elevated, >1000	2 (25.0%)	6 (33.3%)	6 (50.0%)	0
Previous therapy other than gemcitabine					
	Cisplatin	1 (12.5%)	0	0	0
	Folfirinox	0	1 (5.6%)	1 (8.3%)	0
	Capecitabine	1 (12.5%)	0	0	0

Table 1. Patient characteristics.

18 patients with advanced pancreatic cancer (**Table 1**) received a priming regimen with VXM01 followed by up to 6 monthly boost vaccinations (M1→M6) starting on day 38. Vaccinations were orally applied at 2 alternative doses of either 10^6 CFU (12 patients) or 10^7 CFU (6 patients) per administration. 8 patients received placebo treatment in a randomized and blinded fashion. Concomitant treatment with standard-of-care gemcitabine up to day 38, and any treatment thereafter, was allowed in the study (**Figure 3**). Immunomonitoring (IMM) involved IFN-gamma ELISpot analysis (triplicate) against long overlapping peptides spanning the entire VEGFR2 sequence, performed on blood samples harvested on days 0, 4, 14, 21, 38, and on days 48 (M1+10d), 100 (M3+10d), 190 (M6+10d), 270 (M9) and 360 (M12), as shown in **Figure 3**.

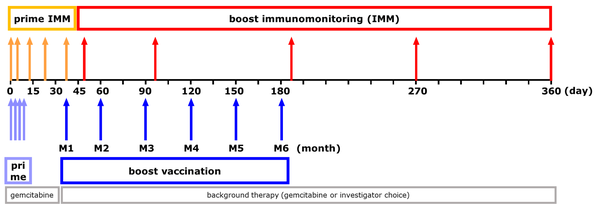

Figure 3. Scheme of the study design describing the vaccination schedule, as well as the time points selected for blood sampling for analysis of the VEGFR2-specific T cell response by ELISpot.

Results

24 patients entered the boosting part and 22 patients received at least 1 boosting treatment with VXM01 (15 patients) or placebo (7 patients). 11 of the 15 patients treated with VXM01 received all 6 boosting doses. The study was prematurely discontinued by 11 patients (7 on VXM01 and 3 on placebo), 2 patients during the priming part (both VXM01, due to death), and 8 patients during the boosting part (5 VXM01 and 3 placebo) due to either death (n=3, 2 VXM01, 1 placebo), deterioration of state of health (n=1, VXM01), or consent withdrawal (n=4, 2 VXM01 and 2 placebo).

Most of the treatment emergent AE (TEAE) were of mild and moderate severity. Severe AEs were reported in 6 patients after prime (3 VXM01, 3 placebo) and 14 patients (10 VXM01, 4 placebo) after boost vaccination. The most frequent TEAEs of any grade skewed towards the VXM01 treatment group after the prime vaccination were decreases in platelets (44.4% vs. 12.5%) and in lymphocytes (27.8% vs. 12.5%), and diarrhea (22.2% vs. 12.5%) (**Table 2**), confirming the findings of the previous study[3]. Drug-related TEAEs preferentially associated with boosting doses of VXM01 were decreases in lymphocytes (22% vs. 0%) and increases in diarrhea (22% vs. 0%). There were no marked differences between the two VXM01 doses tested. The frequency of drug-related TEAEs were comparable after prime and boost doses indicating that further dosing with VXM01 did not augment the TEAEs.

Preferred term	VXM01 Prime (N=18)			VXM01 Boosting (N=18)			Placebo Prime (N=8)			Placebo Boosting (N=8)		
	F	n	n%	F	n	n%	F	n	n%	F	n	n%
All TEAEs	158	18	100	230	15	83.3	95	8	100	111	7	87.5
All drug-related TEAEs	96	17	94.4	64	7	87.5	64	7	87.5	41	7	87.5
Platelet count decreased	8	8	**44.4**	11	7	**38.9**	1	1	**12.5**	3	3	**37.5**
White blood cell count decreased	7	6	33.3	5	5	27.8	4	4	50	4	3	37.5
Blood lactate dehydrogenase increased	8	6	33.3	5	5	27.8	4	2	25	3	3	37.5
Lymphocyte count decreased	5	5	**27.8**	4	4	**22.2**	3	1	**12.5**	-	-	-
Anaemia	7	5	27.8	3	2	11.1	4	4	50	2	2	25
Diarrhea	4	4	**22.2**	5	4	**22.2**	1	1	**12.5**	-	-	-
Nausea	3	3	16.7	-	-	-	-	-	-	-	-	-
Fatigue	3	3	16.7	-	-	-	2	2	25	-	-	-
Hypokalaemia	3	3	16.7	-	-	-	2	1	12.5	-	-	-
Neutrophil count decreased	3	3	16.7	3	3	16.7	2	2	25	1	1	12.5
Platelet count increased	3	3	16.7	2	2	11.1	-	-	-	-	-	-
White blood cell count increased	4	3	16.7	2	2	11.1	1	1	12.5	-	-	-
Abdominal pain	3	3	16.7	2	2	11.1	1	1	12.5	-	-	-
Headache	2	2	11.1	-	-	-	-	-	-	-	-	-
Dizziness	2	2	11.1	-	-	-	-	-	-	-	-	-
Nasal congestion	2	2	11.1	-	-	-	-	-	-	-	-	-
Hypertension	2	2	11.1	-	-	-	6	2	25	-	-	-
Vomiting	3	2	11.1	-	-	-	2	2	25	-	-	-
Neutrophil count increased	2	2	11.1	3	3	16.7	2	2	25	-	-	-
Aspartate aminotransferase increased	-	-	-	3	3	16.7	-	-	-	2	2	25
Alanine aminotransferase increased	-	-	-	2	2	11.1	-	-	-	2	2	25
Gamma-glutamyltransferase increased	-	-	-	2	2	11.1	-	-	-	1	1	12.5
Pyrexia	-	-	-	2	2	11.1	-	-	-	1	1	12.5

Table 2. Frequency of drug-related treatment emergent adverse events after prime and boosting doses reported in ≥10% of the VXM01 treatment group. N: number of subjects; F: number of adverse events; n: number of subjects with at least one adverse event; n% percent n of N. TEAE: treatment emergent adverse event.

Increased vaccine specific T cell response between the test and placebo group was observed by either assessing the total numbers of VEGFR2-specific T cells per well (**Figure 4A**) or the mean fold change of VEGFR2-specific T cell responses over day 0 (**Figure 4B**), with a more pronounced effect in the higher dose group and during the boosting period.

66.7% (8 out of 12) of the patients in the lower dose group and 75% (3 out of 4) of the patients in the higher dose group showed a clear increase of VEGFR2-specific T cell responses after the priming and/or the boosting period (**Figure 4C**). In the higher dose group, T cell responses consistently peaked after 1 to 3 boosting vaccinations in most responding patients, and in all cases vaccine-specific T cells declined after the last vaccination, reaching baseline levels after 1 year in the remaining two patients.

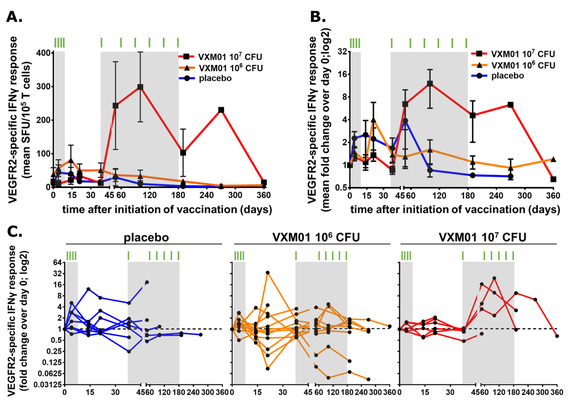

Figure 4. Assessment of VEGFR2-specific T cell responses by IFN-gamma ELISpot. (A) Total number of specific, VEGFR2-reactive T cells per ELIspot well, and (B) mean fold change of VEGFR2-reactive T cells over day 0 are plotted over the time after initiation of vaccination with VXM01. (C) Fold increase of VEGFR2-specific T cell responses over day 0 are plotted for each individual patient.

For a more systematic comparison of T cell responses in vaccinated and placebo treated patients we graded the increase of VEGFR2 specific T cell responses (**Tr**) throughout the observation period according to the study protocol using prefixed criteria, which had been previously determined in the frame of the previously reported VXM01 trial[3]:

- VEGFR2-specific T cell responses (**Tr**=mean difference to negative control) < Tr(day 0) = **grade 0**
- non-significant difference between test and negative control wells = **grade 0**
- 1 × Tr(d0) ≤ **Tr** < 3 × Tr(d0) = **grade 1**
- 3 × Tr(d0) ≤ **Tr** < 5 × Tr(d0) = **grade 2**
- **Tr** ≥ 5 × Tr(d0) = **grade 3**

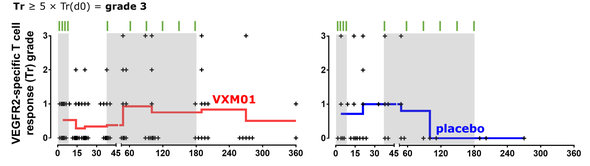

Figure 5. Cumulative grading values of the VEGFR2-specific T cell response (Tr) are staggered and plotted over time. The red (VXM01, pooled 10^6 CFU and 10^7 CFU) and blue (placebo) curves represent means of the grading values at the indicated time point.

We detected increases in VEGFR2-specific T cell responses throughout the priming phase in both placebo and vaccinated patients, which were also observed in the previous VXM01 trial[3], and which were likely due to immune stimulatory effects of gemcitabine co-treatment. However, while VEGFR2-specific T cell responses vanished over time in the placebo group, these remained elevated in several of vaccinated patients. Altogether, we observed in the boosting period pronounced (≥ grade 2) T cell responses in 5 out of 11 patients who received at least one boosting vaccination and who had evaluable ELIspot results (**Figure 5**).

With regard to clinical outcome, we did not detect significant differences between placebo and VXM01-treated patients, due to the small number of patients and potentially imbalances in prognostic baseline parameters (performance status, level of CA19.9, and extent of disease, see **Table 1**). However, VXM01-treated patients who responded to vaccination with increased T cell reactivity towards VEGFR2 showed a significantly improved survival compared to none- or low-grade responders (**Figure 6**). Notably, all out of 8 VXM01-treated patients with a grade ≥2 response survived the entire vaccination and up to month 8. In the placebo group, we found no association with grade of response and improved survival (grade ≥2 vs. <2, HR>1, ns).

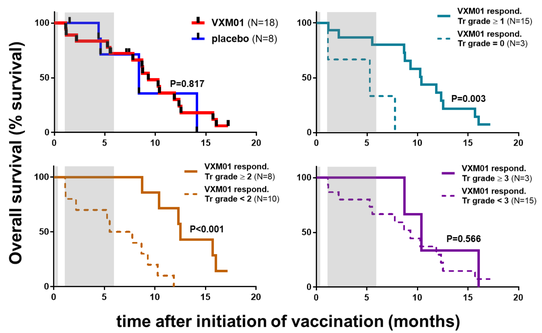

Figure 6. Overall survival analysis comparing VXM01 treatment group (both 10^6 CFU and 10^7 CFU arms pooled together) to placebo (upper left), as well as the greater grade 1 or 2, and grade 3 VEGFR2-responders (solid) to the respective lower responders (dashed). For each survival comparison a corresponding log-rank P value is shown as well as the number of patients (N).

Conclusions

- **Prime – boost regimen with VXM01, a first-in-kind, oral T cell vaccine, based on recombinant, live, attenuated *Salmonella typhi* targeting VEGFR2-expressing cells, can be safely administered. Side effects were mostly mild and in accordance to those reported previously for a VXM01 priming only regimen[3]**
- **Oral VXM01 prime – boost administration can efficiently trigger and maintain a productive IFN-gamma T-cell response to VEGFR2 in advanced cancer patients**
- **VXM01 treated patients (but not placebo patients) with a high grade of T-cell response to VEGFR2 (Grade ≥2) showed a significantly better survival than patients with lower grade responses (HR 0.23, 95% CI 0.08-0.69)**
- **This study provides further evidence that VAXIMM's oral T-cell vaccination platform can be used to stimulate specific cytotoxic T cells in cancer patients**
- **Further studies of VXM01 and other cancer vaccine candidates on this oral T cell vaccination platform are warranted**

References

1. *Darji A., Cell. 1997; 91(6):765-75.* ***2.*** *Niethammer et al., Nature Medicine. 2002; 8(12):1369-1375.* ***3.*** *Schmitz-Winnenthal, F.H. et al., OncoImmunology. 2015; 4(4), p.e1001217.* ***4.*** *Chen and Mellman, Immunity. 2013; 39(1):1-10.*