VXM01, an oral T-cell vaccine targeting the tumor vasculature:

Results from a double blind, randomized, controlled, first-in-man study in pancreatic cancer patients

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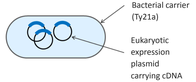
INTRODUCTION

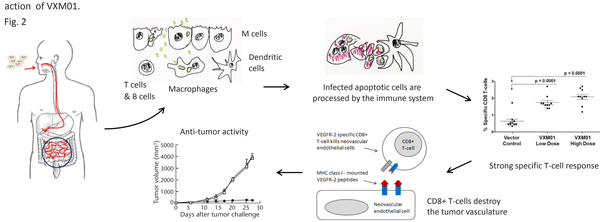
VAXIMM's oral T-cell vaccine platform is based on the approved, live attenuated bacterial vaccine strain Ty21a, which has been applied in millions of individuals for prophylactic vaccination against typhoid fever. This strain has been thoroughly studied, is safe and well tolerated. The bacteria are modified to deliver a eukaryotic expression plasmid, which encodes the genetic information of a specific target antigen[1] (Figure 1).

Fig. 1



VXM01 is encoding VEGFR-2 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. Murine VXM01 has shown consistent anti-angiogenic and anti-tumor activity in different tumor types in several animal studies[2]. Figure 2 describes the proposed mechanism of action of VXM01.

Fig. 2



This first-in-man study was designed to evaluate the safety and tolerability of VXM01. Secondary endpoints included specific T-cell responses, and changes in tumor perfusion as well as in other biomarkers indicating anti-angiogenic effects. To be able to distinguish between VXM01 dependent effects from to disease related symptoms as well as from chemotherapy and/or other factors, we designed this safety and biomarker study as a double blinded, randomized, placebo-controlled trial.

Pancreatic Cancer was chosen because of (i) high VEGFR-2 expression in tumor tissue, (ii) the high medical need, and because (iii) the standard treatment with gemcitabine is only mildly immunosuppressive and therefore well compatible with vaccination-based immunotherapies. Pre-clinically, murine VXM01 showed anti-tumor activity in two mouse models of pancreatic cancer (own unpublished results).

STUDY DESIGN

Design

- Double blinded first-in-man study
- 45 patients with inoperable pancreatic cancer
- Randomized 2:1
- Placebo-controlled (isotonic saline solution)
- Dose-escalation: 5 dose groups, 10^6 - 10^{10} CFUs of VXM01 per administration
  (6 VXM01 patients + 3 placebo patients per dose group)

Primary Endpoint

- Safety and Tolerability / Maximum tolerated dose (MTD)

Key Secondary Endpoints

- VEGFR-2 specific T-cell response
- Tumor perfusion measured by DCE-MRI (K_{trans})
- Biomarkers of anti-angiogenic therapy: VEGF-A, Collagen IV
- Disease related: Response rate and changes in tumor marker carbohydrate antigen 19.9 (CA 19.9)

The time course of the study per patient is depicted in Figure 3. Table 1 summarizes the key inclusion and exclusion criteria. The detailed study design has been published[3] and is freely available online.

Fig. 3

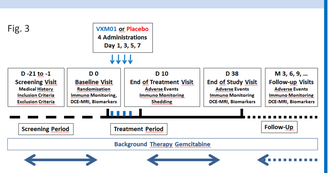


Table 1

Inclusion Criteria
Locally advanced, inoperable or stage IV pancreatic cancer
Chemotherapy naïve within 60 days before screening visit except gemcitabine treatment
Karnofsky index >70
Adequate renal, hepatic and bone marrow function
Exclusion Criteria
State after pancreas resection (complete or partial)
Resectable disease
Bowel obstruction within the last 30 days before screening visit
Presence of any acute or chronic systemic infection
History of serious heart or vascular disease
Multi-drug resistant gram-negative germs
Chronic concurrent therapy within 2 weeks before and during the double-blind study period with:
- Corticosteroids or immunosuppressive agents
- Antibiotics
- Bevacizumab

RESULTS

Between December 2011 and October 2012 a total of 371 patients were screened for the study. 326 patients were ineligible because of excluded medical therapies (179), preexisting medical conditions (129) in patient's medical history and personal reasons (18). 45 patients were enrolled, randomized and completed successfully the 10 days in-house study phase at the Clinical Research Unit at the University Clinics of Heidelberg (KliPS), in line with the study protocol. Demographic baseline disease characteristics of the patients were not significantly different in the two groups (Table 2), but time since diagnosis was longer in the VXM01 group (8 vs. 6 months) and patients in the VXM01 group had a more advanced tumor stage at the time of inclusion (CA19.9 > 1000 in 40% vs. 20% and metastatic disease in 83% vs. 53%).

Table 2

Demographic and Baseline Characteristics of the Patients			
Variable	Placebo (N=15)	VXM01 (N=30)	P Value
Age-yr			0.782
Median	68	65	
Range	[55 - 73]	[37 - 82]	
Sex-no. (%)			1.000
Female	6 (40)	12 (40)	
Male	9 (60)	18 (60)	
Extent of disease-no. (%)			0.070
Locally advanced	7 (47)	5 (17)	
Metastatic	8 (53)	25 (83)	
Karnofsky performance status scale-no. (%)			0.336
100	6 (40)	11 (37)	
≥ 90	4 (27)	14 (47)	
≥ 80	5 (33)	5 (17)	
Time since diagnosis-mo			0.082
Median	6	8	
Range	[2 - 27]	[1 - 51]	
Level of carbohydrate antigen 19.9-no. (%)			0.370
Normal	7 (47)	9 (30)	
Elevated < 1000	5 (33)	9 (30)	
Elevated > 1000	3 (20)	12 (40)	

All patients completed the seven day vaccination course of 4 doses every second day in line with the protocol without any dose reduction. Because of no observed dose limiting toxicities (DLT) the maximum tolerated dose was not reached. VXM01 was well tolerated at all dose levels. AEs and SAEs where equally distributed among both groups and there were no obvious signs for dose-dependent side effects among the groups.

A detailed description of treatment emergent toxicities for both groups is provided in Table 3. The most frequent adverse events of any grade were abdominal pain and vomiting. Possibly treatment-related lymphocyte and platelet count decreases were observed more frequently in the VXM01 group (highlighted in red), in line with observations with other anti-angiogenic therapies[4]. No DLTs were observed.

Table 3

Common Adverse Events [Number of AEs]						
Event	**Placebo (N=15)**			**VMX01 (N=30)**		
	All Grades	*Grade 1 or 2*	*Grade 3 or 4*	All Grades	*Grade 1 or 2*	*Grade 3 or 4*
Abdominal pain	**9**	*8*	*1*	**14**	*14*	
Blood alkaline phosphatase increased	**2**	*2*		**5**	*5*	
Anaemia	**6**	*5*	*1*	**7**	*7*	
Diarrhoea	**4**	*4*		**9**	*9*	
Haematuria	**3**	*3*		**4**	*4*	
Hypertension	**2**	*1*	*1*	**5**	*3*	*2*
Hypokalaemia	**8**	*7*	*1*	**7**	*7*	
Insomnia	**2**	*2*		**8**	*8*	
Leukopenia	**5**	*3*	*2*	**7**	*7*	
Lymphocyte count decreased	**0**			**9**	*5*	*4*
Nausea	**4**	*4*		**9**	*9*	
Platelet count decreased	**0**			**6**	*6*	
Vomiting	**4**	*4*		**14**	*14*	
White blood cell count decreased	**3**	*3*		**6**	*5*	*1*

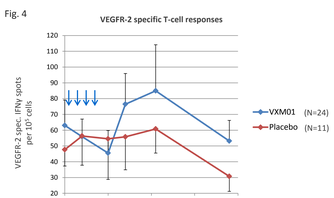
Advers events were defind on the basis of the National Institute Common Terminology Critiria for Adverse Events, version 3.0. Events listed are those of any grade that occurred more than 5% of patients in either group.

Figure 4 shows the mean frequencies of VEGFR2 specific T-cells in peripheral blood of VXM01 and placebo treated patients, detected by INFγ ELISpot, at different time points prior during and post vaccination (error bars show standard error). Time points of the four administrations of VXM01 is indicated by blue arrows.

In the vaccination group (blue), the number of specific cells decreased in average until day 10 post vaccination. From day 10 to day 21 a strong accumulation of VEGFR2 specific cells was registered, followed by a decrease to about baseline level on day 38.

In placebo patients, VEGFR-2 specific T-cell frequencies remained largely unchanged until day 21, before declining towards d38. A high variability in T-cell frequencies was noted in both the placebo and the vaccinated group.

Fig. 4

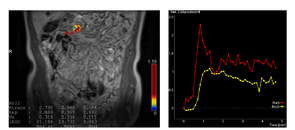


Tumor perfusion was evaluated by contrast media transit time (K_{trans}) during dynamic contrast enhanced magnetic resonance imaging (DCE-MRI, Figure 5) on d0, d38 and m3. Figure 6 depicts the K_{trans} values /tumor perfusion for each individual patient at d0 and d38, as well as medians and interquartile ranges for both the VXM01 and the placebo group. Mean changes in tumor perfusion were -9% in the VXM01 group (N=26) vs. +18% in the placebo group (N=11). A greater than 33% drop in tumor perfusion was detected in 35% (9) of evaluable VXM01 treated patients vs. 10% (1) in the placebo group. The strongest responders were further analyzed in a subgroup analysis (see below).

Maximum average effects were detected at the d38 time point. Figure 7 shows average tumor perfusion over time (at d0, d38 and m3) for VXM01 treated and placebo patients (N=18 vs. 9).

Fig. 5

Dynamic Contrast Enhanced Magnetic Resonance Imaging



DCE-MRI Scan | Enhancement recording (5 min)

Fig. 6

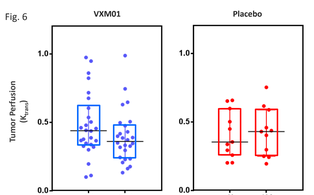
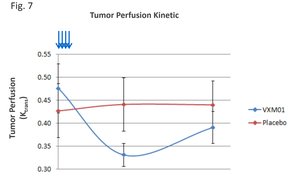
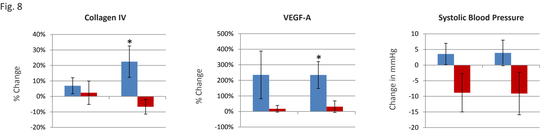


Fig. 7



The VEGFR-2 specific T-cell mediated, anti-angiogenic activity of VXM01 was supported by accompanying changes in biomarkers of response to therapies targeting cancer angiogenesis[5] (Figure 8). Serum levels of collagen IV increased on d38 and m3 in average by 7% and 22%, respectively, in the VXM01 group vs. changes of 2% and -7% in the placebo group (p=0.02 at m3). VEGF-A serum levels also increased in the VXM01 group by 235% on both d38 and m3 vs. 17% and 31% in the placebo group (p=0.05 at m3). Average systolic blood pressure changes were +3.6mmHg and +3.9mmHg in the treatment group vs. -8.8mHg and 9.1mmHg under placebo (p=0.08 at d38). Asterisks in figure 8 denotes statistically significant differences between the treatment and the placebo group.

Fig. 8



Responses to VXM01 were assessed by MRI on m3 (vs. d0) according to RECIST 1.1 and are summarized in table 4. Of the patients, who completed both d0 and m3 MRI, similar percentages were judged to have progressive and stable disease. One patient showed a partial response under VXM01 treatment (11008).

Serum biomarker analysis of CA19.9 at m3 showed average increases from d0 in the treatment group of 128% (N=17) vs. 257% (N=8) in the placebo group (n.s.). Two patients experienced a drop in CA19.9 of greater than 75% (11008 and 10803). Both patients were in the treatment arm and were also classified as tumor perfusion responders at d38 (see below).

Table 4

	N	PD	SD	PR	CR	Unclear
VXM01	18	4 (22%)	13 (72%)	1 (6%)	0	0
Placebo	9	2 (22%)	6 (67%)	0	0	1 (11%)

FURTHER CORRELATIVE INVESTIGATIONS

To assess correlations between tumor perfusion response and biomarker responses, VXM01 treated patients were grouped into responders (TPR, N=10) and non-responders (non-TPR, N=16) according to their tumor perfusion changes on d38 (Figure 9). Figure 10 shows relevant baseline characteristics associated with tumor perfusion response: (i) pre-existing T-cell immunity against VEGFR-2 (left panel) and (ii) high tumor perfusion at baseline (right panel). All tumor perfusion responders displayed baseline characteristic (i), (ii) or both.

Serum and pharmacodynamic biomarkers were all more pronounced in the TPR subgroup vs. the total VXM01 treatment group and in particular vs. the nonTPR subgroup (Figure 11). Collagen IV serum levels were in average elevated by 23% and 43% on d38 and m3 in TPR subgroup (p=0.04 vs. placebo at m3), vs. -2.9% and +3.9% in the nonTPR subgroup. Serum levels of VEGFR-2 showed an average increase of 404% and 297% in the TPR subgroup vs. 130% and 178% in the nonTPR group. Blood pressure changes were +10.6mmHg and +9.4mmHg in the VXM01-TPR subgroup (p=0.03 vs. placebo at d38) and -0.4mmHg and +2.2mmHg in the non-TPR group.

Vaccinated patients with pre-existing VEGFR-2 specific T-cells reacted to the vaccination in average with a sharp drop of these cells in the peripheral blood during the first 10 days, followed by a rebound towards d14 and d21.

Fig. 9

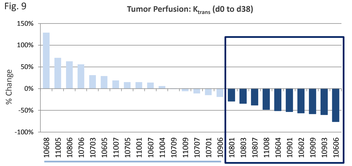


Fig. 10

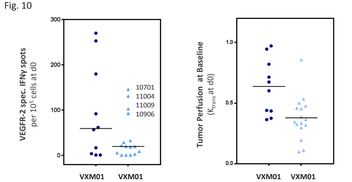
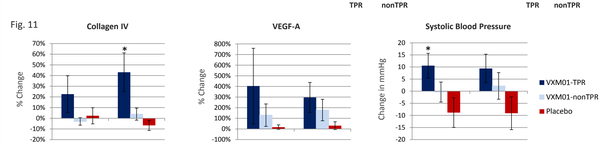


Fig. 11



CONCLUSIONS

- **VXM01 was safe and well tolerated, with minor effects on blood pressure and certain blood cell counts, in line with other anti-angiogenic therapies**
- **VXM01 was able to elicit and/or to activate a VEGFR-2 specific effector T-cell response**
- **VXM01 impacted tumor perfusion in a subset of patients, as measured by DCE-MRI and related serum biomarkers (i.e. VEGF and Collagen IV)**
- **Tumor perfusion response correlated with pre-existing VEGFR-2 specific T-cell responses and/or high baseline perfusion, with serum biomarker responses, and with changes in blood pressure**
- **This study provided proof-of-concept for the mechanism-of-action of VXM01 and the oral T-cell vaccination platform**
- **Further studies of VXM01 and other cancer vaccine candidates on this oral T-cell vaccination platform are warranted**
- **Further development of VXM01 should focus on indications with high levels of tumor perfusion and include boosting vaccinations**

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