Fast and cost-effective oral delivery technology of personalized T-cell vaccines based on a live attenuated bacteria platform
How can we overcome manufacturing challenges in personalized neoantigen-targeting approaches?

- Delivery platform
- Neoantigen targeting personalized approaches
- Manufacturing features
- Technical and immune proof of concept in animals
- Platform clinical proof of concept by lead product
Unique Ty21a Platform with Broad Potential
... for systemic antigen-directed T-cell activation

**Bacterial carrier (Ty21a)...**
- Live attenuated vaccine strain
- Approved travelers’ vaccine (typhoid fever, Vivotif®)
- Oral vaccine naturally infects cells in the gut
- Applied >250 million times
- Excellent safety record and well tolerated

**... containing eukaryotic expression plasmids**
- Encoding the cDNA of the desired targets
- Plasmid is dormant within the bacterial carrier
- Drives strong expression of target antigen in infected cells within the patient’s Peyer’s patches
- Clinical safety/immunogenicity/efficacy demonstrated with a VEGFR-2 construct (VXM01) in pancreatic cancer and glioblastoma
- VEGFR-2 consisting of 1356 amino acids corresponding to appr. 4000 base pairs
Intra-lymphatic Delivery via Oral Administration

... leading to systemic target specific T-cell activation

1. Oral administration of a suspension containing attenuated Salmonella bacteria carrying plasmids encoding for target antigens.

2. Bacteria pass through M cells into Peyer’s patches and are taken up by macrophages.

3. Bacteria die inside macrophages and release plasmids. Plasmids enter the nucleus and the encoded antigen is expressed.


5. Apoptotic vesicles are phagocytosed by dendritic cells, processed, and antigen epitopes are presented on the surface via MHC Class I.

6. Antigen-specific CD8+ T-cells are activated.

7. CD8+ T-cells circulate through the body and bind to target cells expressing the specific antigen, inducing cell death.
Confirmation of Mechanism of Action
... transient antigen expression and T-cell homing

Transient EGFP antigen expression in mouse Peyer’s patches

8 hours
16 hours
32 hours

Xiang, Canc Res 2005

Transient homing of human antigen-specific T-cells to immunization site without boosting

Homing period up to 3 days after last vaccination

Schmitz-Winnenthal, OncoImmunology 2015
T-cell Activation in VXM01-treated Patients

... can produce multi-functional T-cells

CD8+ T-cells secreting multiple cytokines demonstrate stronger activation

<table>
<thead>
<tr>
<th>CD8+ TC</th>
<th>day 0</th>
<th>day 14</th>
<th>day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.2</td>
<td>3.63</td>
<td>8.4</td>
</tr>
<tr>
<td>1 Cytokine</td>
<td>95.2</td>
<td>65.8</td>
<td>42.5</td>
</tr>
<tr>
<td>2 Cytokines</td>
<td></td>
<td>32.49</td>
<td>49.10</td>
</tr>
<tr>
<td>3 Cytokines</td>
<td></td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

% VEGFR-2-reactive simultaneous secretion of:

Representative patient, Schmitz-Winnenthal et al., OncoImmunology 2015
Proprietary Platform
... with key differentiating features

Natural, efficient & easy way to activate T-cells

Strong transient antigen expression allowing specific T-cells to target the tumor
- Oral delivery targeting the lymphatic tissue of the gut
- Repeated dosing possible
- Self-adjuvanted through concomitant bacterial Ty21a infection

High safety and good tolerability

Readily combinable with other immune therapies
- Approved carrier bacterium, with excellent longstanding safety record
- Low therapeutic doses of typically $10^6$ to $10^7$ CFU, factor 100-1000 below Vivotif® dose
- No anti-vector immunity and little to no vector-related side effects
- Suitable for multi target approaches

Fast and easy manufacturing

Attractive cost of goods
- Plug and play system
- Established methods (GMP manufacturing, QA/QC, etc.)
- Ideally suited for neoantigen / personalized vaccine approaches:
  Objective is 15 days manufacturing time after identification of the neo-epitopes

VAX IMM
Neoantigen Targeting
Personalized Approaches
Personalized vaccine
... identifying neoantigens

Tumor biopsy & healthy tissue sample(s) from patient

Genome sequence & mutation analysis/identification of TAAs

Selection of mutational epitopes/neoantigen(s)

Synthesis of cDNA coding for multi-(neo)antigen polypeptide(s)
Cloning of plasmid DNA, verification of sequence

Transformation of Ty21a recipient strain

Small batch Treatment of patient with personalized neoantigen vaccine (+off the shelf products)

VXM
Major Hurdles to Overcome
... delivery technologies

Challenges faced in personalized neo-antigen approaches

• Limitation in number of epitopes
• Time to needle
  – Time to oral administration after identification of neo-antigens
• Manufacturing costs for individualized therapies
• Scalability of the manufacturing process
• Individual QC analytics per product and product specification
  – Sterility testing for parenteral / intravenous drugs
• Incompatibilities in galenic formulation of drug product
• Long-term stability of drug product
• Doses to be administered
• Patient treatment during time from identification of neo-antigens to availability of personalized drug product
## Competitive Landscape
... technologies for neoantigen vaccination

### Overview of established approaches

<table>
<thead>
<tr>
<th>Delivery Technology</th>
<th>Ease of manufacturing</th>
<th>Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria based vaccines</td>
<td>+++</td>
<td>Intravenous</td>
</tr>
<tr>
<td>mRNA</td>
<td>+</td>
<td>Intranodal, Intravenous, Intradermal</td>
</tr>
<tr>
<td>Viral Vectors</td>
<td>+</td>
<td>Intradermal</td>
</tr>
<tr>
<td>Peptides</td>
<td>+</td>
<td>Intradermal</td>
</tr>
<tr>
<td>Dendritic Cells</td>
<td>+</td>
<td>Intravenous</td>
</tr>
<tr>
<td>DNA</td>
<td>+++</td>
<td>Intramuscular</td>
</tr>
</tbody>
</table>
Manufacturing Features
Less Limitation in the Number of Epitopes
... in “string-of-beads” encoding insert

High number of epitopes can be encoded

100 – 300
T cell epitopes

short spacers, GS linkers, 2A cleavages sites, IRES etc.
Straight-Forward Bacterial Fermentation Manufacturing ... in small scale at low costs

Robust manufacturing in a 1 L bacterial fermentation with disposable fermenters

- Master cell bank of empty Ty21a bacteria
- Plasmid individually synthesized
- Overnight culture for drug substance fermentation in 1 L scale
- Dilution to target concentration based on CFU
- Quality control analytics including plasmid sequencing
- QP release

- Objective is to minimize the manufacturing time to 15 days after neoantigen identification in a dedicated facility
Straight-Forward Bacterial Fermentation Manufacturing ... in small scale at low costs

Short time to administration after identification of neo-antigens

<table>
<thead>
<tr>
<th>Delivery Technology</th>
<th>N2N time</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAXIMM</td>
<td>Neoantigen discovery + 15 days</td>
</tr>
<tr>
<td>Company A*</td>
<td>115 days</td>
</tr>
<tr>
<td>Company B*</td>
<td>90 days</td>
</tr>
<tr>
<td>Company C*</td>
<td>75 days</td>
</tr>
</tbody>
</table>

*according to published data

- Competitive in terms of
  - Time to administration after identification of neo-antigens
  - Manufacturing costs due to overnight bacterial fermentation in small scale
  - Upscaling not required due to high yield of bacteria
    - Net bacteria yield in the $10^{11}$ CFU range
    - Allowing filling of drug product sufficient for years of treatment
Quality Control Analytics for One Defined Product
... in drug substance and drug product

Generic specification per individual construct with difference in encoding insert only

- Quality control assays established and validated through ongoing clinical development stage products
- Individual difference in encoding insert only
  - Sequencing to be performed
- No sterility testing required
  - Oral administration
  - Live bacteria-based constructs
Stable Pharmaceutical Formulation
... without risk of incompatibilities due to the nature of the product

One defined product with documented stability – no galenic incompatibilities

- Epitopes are encoded in the DNA plasmid
- Expression of neo-antigens in the Peyer’s patches
  - No incompatibilities on the level of administration as the peptide manufacturer is the human body
- Drug substance and drug product formulations stable for 3 years as established for clinical-stage products
Very Low Doses of DNA Plasmid Administered
... far lower exposure than with other treatment modalities

Exposure to VXM DNA plasmid lower than with RNA or intradermal DNA

- Plasmids in $10^7$ CFU live bacteria correspond to appr. 1 ng DNA
- For comparison
  - RNA intranodal: 500 – 1000 µg (Sahin et al., 2017)
  - Synthetic long peptides s.c.: 0.3 mg of each peptide (Ott et al., 2017)
VXM-NEO Phase I Checkpoint Inhibitor Combination Study

Clinical phase I study

- Identification of neoantigens in cancer indications with relevant mutational load
- Pre-treatment with off-the-shelf shared antigen oral immunotherapies
- VXM-NEO treatment in combination with SoC checkpoint inhibitors

VXM-NEO
Combination with SoC Checkpoint-Inhibitor

Pre-Treatment with Off-the-Shelf Constructs

Cancer Tumor Sample
Identification of Private Antigens
Personalized VXM-NEO GMP Manufacturing

VXM

VAXIMM
Technical and Immune Proof of Concept in Animals
### VXN-NEO

... Technical and pre-clinical immune PoC demonstrated

**Construct with 9 dominant CD8 epitopes cloned**

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Number of Epitopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGFR-2</td>
<td>2 epitopes</td>
</tr>
<tr>
<td>MSLN</td>
<td>2 epitopes</td>
</tr>
<tr>
<td>WT-1</td>
<td>1 epitope</td>
</tr>
<tr>
<td>CEA</td>
<td>3 epitopes</td>
</tr>
<tr>
<td>OVA</td>
<td>1 epitope</td>
</tr>
</tbody>
</table>

- 9 identical peptide pentamer flow cytometry reagents used
- Additional HPV reagent as negative control
VXM NEO Multi-Epitope Platform
Immunological PoC in animals

VXM-NEO – epitope-specific CD8+ T-cell responses

- Epitope-specific T-cell response against 7 out of 9 epitopes detected
Platform Clinical Proof of Concept by Lead Product
VXM01 Pancreatic Cancer Clinical Trial Completed
... first-in-human study part 1 with initial administration only

Locally Advanced or Inoperable Pancreatic Cancer

- 1st line, plus gemcitabine background chemotherapy or stand alone
- Testing five doses $10^6$ CFU through $10^{10}$ CFU n=6 each vs. placebo n=15
- Read-out:
  - Safety
  - Biomarker
  - T-cell response
  - Survival
VXM01 Pancreatic Cancer Clinical Trial Completed
... first-in-human extension including boosting

Locally Advanced or Inoperable Pancreatic Cancer

- 1st line, plus gemcitabine background chemotherapy or stand alone
- Testing two doses
- Read-out:
  - Safety
  - Biomarker
  - T-cell response
  - Survival

VXM01 treatment:

VXM01
10^5 n=12 / 10^7 CFU n=6
Placebo n=8
VXM01 Pancreatic Cancer Clinical Study
... a successfully completed randomized Phase I/II program

- VXM01 treatment causes activation of VEGFR-2-specific T-cell response in patients
- Perfusion rates were used as biomarker, supporting the notion of VEGFR-2-specific T-cell activation
- VXM01 (incl. boosting) was very well tolerated
- Continued VXM01 treatment led to improved survival, correlating with immunological response to VXM01
- Metastatic load was markedly reduced in one patient following VXM01 treatment

- VXM01 showed early signs of clinical efficacy in pancreatic cancer
- First clinical validation of the oral Ty21a T-cell therapy platform
- Schmitz-Winnenthal et al., OncoImmunology 2015 and OncoImmunology 2017
VXM01 Clinical Trial Currently Ongoing
... in glioblastoma

Glioblastoma

- Relapsed patients who are candidates for re-operation
- Initiation treatment prior to re-operation (continued post-op)
- Monocenter trial in Heidelberg
- Two VXM01 doses $10^6$ or $10^7$ CFU
- Patient number expanded beyond 8 patients
- Patient-specific prolongation of VXM01 treatment beyond one year initiated in 2 patients
- Seven out of 14 patients treated survived more than 1 year
- Interim data presented at ASCO 2017, abstract accepted for ASCO 2018

- Comprehensive read-out pending
  - T-cell response
  - High-res. brain tumor vasculature imaging
  - Immunohistochemistry on tumor samples
  - Clinical response
Promising Survival of Recurrent Glioblastoma Patients

...7 out of 14 survived more than one year

Survival curve

CD8+/Treg ratio increased in recurrent tumor

n=7, complete data sets only
Patients with Favorable Course of Disease  
... in recurrent glioblastoma

1st patient

- Patient 2603 (male, 47 y), candidate for re-operation,
- **Not operated** due to tumor shrinkage under VXM01 treatment
- VXM01 treatment without other anti-cancer therapy during study up to week 12
- **Partial response (PR)** after 12 weeks under VXM01 monotherapy
- **Complete response (CR)** after additional 15 weeks under VXM01 and 6 doses of anti-PD1 treatment
- Durable response with significant clinical benefit
- Progressive disease at week 36
- High VEGFR-2 expression on tumor neovasculature in primary tumor
Patients with Favorable Course of Disease
... in recurrent glioblastoma

2nd patient

- Patient 2605 (female, 55 y), candidate for re-operation
- Showed stabilization of tumor growth after VXM01 treatment before re-operation
- VXM01 monotherapy treatment up to week 10
  - Initiation treatment plus boosting after reoperation
- Favorable post-operative course of disease – under VXM01 + chemotherapy from week 10 to week 36
- Stable Disease (SD) at week 76
- VEGFR-2 expression on tumor cells in primary tumor, but no expression on recurrent tumor cells after VXM01 treatment
  - Indicator of VEGFR-2 targeting effect
Patients with Favorable Course of Disease
... in recurrent glioblastoma

3rd patient

• Patient 2611 (female, 44 y), candidate for re-operation
• Showed stabilization of tumor growth after VXM01 treatment before re-operation
• Patient did not want to be re-operated
• VXM01 monotherapy treatment up to week 8
  – Initiation of additional nivolumab from week 8 onwards
• Stable Disease (SD) at week 36

<table>
<thead>
<tr>
<th>Target Lesion</th>
<th>Tumor Diameter 1 [mm]</th>
<th>Tumor Diameter 2 [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Day 10</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Day 21</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Day 35</td>
<td>14</td>
<td>9</td>
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<tr>
<td>Week 12</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Week 24</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Week 36</td>
<td>11</td>
<td>10</td>
</tr>
</tbody>
</table>
Major Hurdles Can be Overcome
... by our VAXIMM delivery technology

Response to challenges faced in personalized neo-antigen approaches

- Less limited in number of epitopes
- Short time to oral administration after identification of neo-antigens
- Low manufacturing costs for established process
- QC analytics and generic product specification established
- No incompatibilities in galenic formulation
- Long-term stability of drug product
- Low exposure
- Patient treatment with off-the shelf constructs during time from identification of neo-antigens to availability of personalized drug product
- Immune and technical proof of concept shown in animals
- Platform clinically validated by lead product
  - ATMP certification by EMA and orphan drug designation for glioma in U.S. and E.U.