The making of Bovela® - a vaccine against bovine viral diarrhea

Konrad Stadler, Boehringer Ingelheim Veterinary Research Center

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In general, a vaccine should be safe and efficient.

In the BVDV system, **efficacy** not only means the prevention of clinical symptoms after challenge of vaccinated animals, but also the absence of transmission of challenge virus to the fetus.

In the BVDV system, **safety** not only means the absence of clinical symptoms after vaccination but also the absence of transmission of vaccine virus to the fetus.
BVDV Vaccines (examples)

Vaccines with fetal protection

- Bovidec (Virbac) → inactivated
- Bovilis BVD-MD (Intervet) → inactivated
- (Pregsure BVD (Pfizer) → inactivated)
- Elite (BIV) → inactivated
- Express (BIV) → live (not for pregnant animals!)
- Vacoviron (Merial) → live (not for pregnant animals!)

Vaccines without fetal protection

- Mucobovin (Merial) → inactivated
Classification

Family  Flaviviridae

Genus  *Flavivirus*
       *Hepacivirus*
       *Pestivirus*

Species  bovine viral diarrhea virus 1 (BVDV-1)
         bovine viral diarrhea virus 2 (BVDV-2)
         Border disease Virus (BDV)
         classical swine fever virus (CSFV)

Biotypes:
For all 4 pestivirus species cytopathogenic (cp) and non-cytopathogenic (ncp) isolates have been described
Factsheet: BVDV

- Enveloped, + stranded RNA virus with a diameter of 40-60 nm
- Causative agent of bovine viral diarrhea
- Endemic in most countries of the world
- Host spectrum: Restricted to members of the order Artiodactyla (e.g. pig, deer), no zoonotic potential
- Effects of BVDV infections of cattle can range from inapparent to lethal disease (mucosal disease, hemorrhagic syndrome)
Pathogenesis of mucosal disease

Vertical infection of the foetus with ncp-virus between day 60 - 120 of gestation

→ persistent infection (PI)
→ virus specific immunotolerance (but innate immune system is still active)
→ PI animals can be clinically unapparent

Spontaneous mutation to cp-Virus
Death caused by Mucosal Disease (MD)
Vaccine requirement profile

- clinical protection
- good stimulation of the immune system
- easy application
- protection against BVDV type 1 und type 2
- protection against fetal transmission
- protection from virus shedding
- marker vaccine

Combine the safety profile of killed vaccines with the efficacy profile of a live vaccine
How did the project start in 199x?

Key ‘technology’ invention in Gregor Meyers’ lab:
Generation of an infectious copy of a BVDV virus

→ Enabling efficient manipulation of the BVD virus
→ Step-wise understanding of BVDV interaction with host innate immune system (major contribution by Norbert Tautz, Nicolas Ruggli and Bryan Charleston)
→ \( E^{\text{RNS}} \) and \( N^{\text{pro}} \) being key players in modulating IFN expression level
• The autoprotease $N^{pro}$ is not essential for virus replication

• $N^{pro}$ interferes with the innate immune response of infected cells: $N^{pro}$ induces degradation of interferon regulatory factor 3 (IRF-3) via the proteasome

• The loss of the $N^{pro}$ function on the interferon system does not lead to strong attenuation
Glycoprotein $E^{\text{erns}}$

- $E^{\text{erns}}$ is part of the virus particle (figure)
- essential structural protein
- target for neutralizing antibodies (only weak neutralization)
- binds to carbohydrates on target cells
- RNase activity dispensable for virus replication in cell culture
- The $E^{\text{erns}}$ RNase is engaged in blocking the type 1 interferon response to pestivirus infection
Hypothesis for vaccine concept

**N**\(^{\text{pro}}\) is unique protein for pestiviruses (cystein protease) - dispensable for virus replication

**E**\(^{\text{rns}}\) with RNase – its function is dispensable for virus replication

**N**\(^{\text{pro}}\) and **E**\(^{\text{rns}}\) are antagonists to the adaptive immune system → inhibit the generation of INF

**Hypothesis:**

**Modifications of N**\(^{\text{pro}}\) and **E**\(^{\text{rns}}\) can be used for rational attenuation of the virus.
Targeted attenuation of BVDV for rational design of an attenuated vaccine virus

BVDV Genome

Bovela® can not cross the placental barrier and is inducing full protection in the dam

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<th>Virulence</th>
<th>Transplacental crossing</th>
<th>Immunity</th>
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<td>Wild-type</td>
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Single mutation: compensation of the missing function from the E<sup>RNS</sup> and N<sup>pro</sup>, respectively

Double mutation: compensation of E<sup>RNS</sup>/N<sup>pro</sup> function not possible
What if?

... the vaccine virus passes the placenta?

Experiment:
Intrauterin infection of fetuses with type 1 (KE-9) and type 2 (NY93) double mutant vaccine virus or the respective wt virus at day ~60 of gestation

Type 2:
abortion of all fetuses 23-37 dpi (double mutant and wt virus)

Type 1:
abortion of fetuses inoculated with double mutant vaccine virus
all fetuses alive at the end of the experiment when inoculated with wt BVDV virus

Result:
double mutant is inducing abortion post fetal infection
→ no PI animals!
modified live bovine viral diarrhoea virus type 1, non-cytopathic parent strain KE-9 and modified live bovine viral diarrhoea virus type 2, non-cytopathic parent strain NY-93
What makes a successful collaboration?

- early academia – private partnership
- Communication is key
- Set clear targets → define product profile at an early project stage
- Involve the various experts (RA, process development, marketing etc.) at an early stage
- Patience and persistence
- Let your baby go!
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