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

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Vaccines

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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 abscence of clinical symptoms after vaccination but also the absence of transmission of vaccine virus to the fetus.

BVDV Vaccines (examples)

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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

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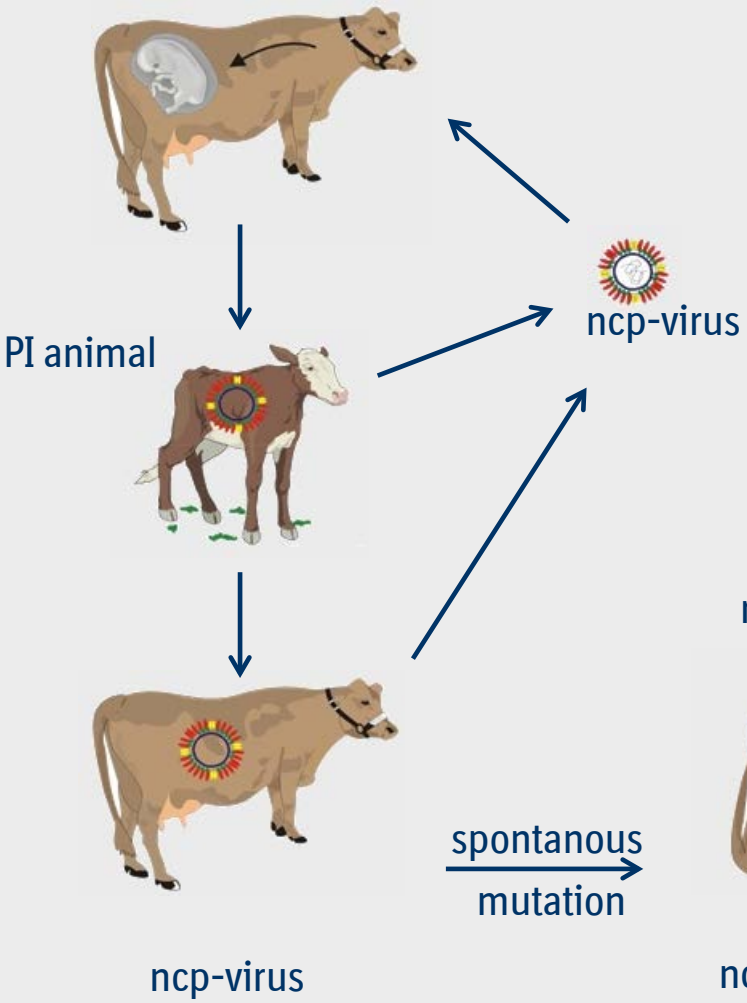
- Enveloped, + stranded RNA virus with a diameter of 40-60 nm
- Causative agent of bovine viral diarrhea
- Endemic in most countries of the world
- Host spectrum: Restricted to members of the order Artiodactyla (e.g. pig, deer), **no** zoonotic potential
- Effects of BVDV infections of cattle can range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

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- 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?

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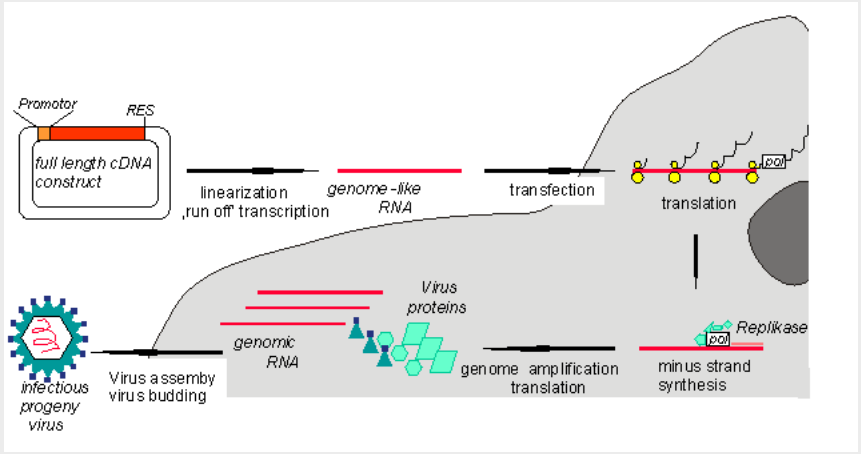
Key ‚technology' invention in Gregor Meyers' lab:

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^{RNS} and N^{pro} being key players in modulating IFN expression level**

N^{pro}

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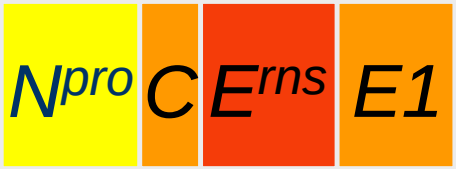
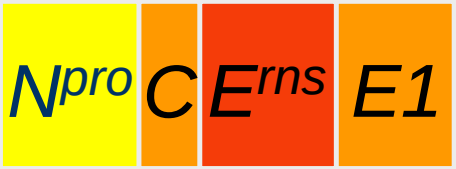


- The autoprotease N^{pro} is not essential for virus replication
- N^{pro} interferes with the innate immune response of infected cells: N^{pro} induces degradation of interferon regulatory factor 3 (IRF-3) via the proteasome
- The loss of the N^{pro} function on the interferon system does not lead to strong attenuation

Glycoprotein E^{rns}



- E^{rns} 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^{rns} RNase is engaged in blocking the type 1 interferon response to pestivirus infection

Hypothesis for vaccine concept

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- N^{pro} is unique protein for pestiviruses (cystein protease) - dispensable for virus replication
- E^{rns} with RNase – its function is dispensable for virus replication
- N^{pro} and E^{rns} are antagonists to the adaptive immune system → inhibit the generation of INF

Hypothesis:

Modifications of N^{pro} and E^{rns} can be used for rational attenuation of the virus.

Targeted attenuation of BVDV for rational design of an attenuated vaccine virus

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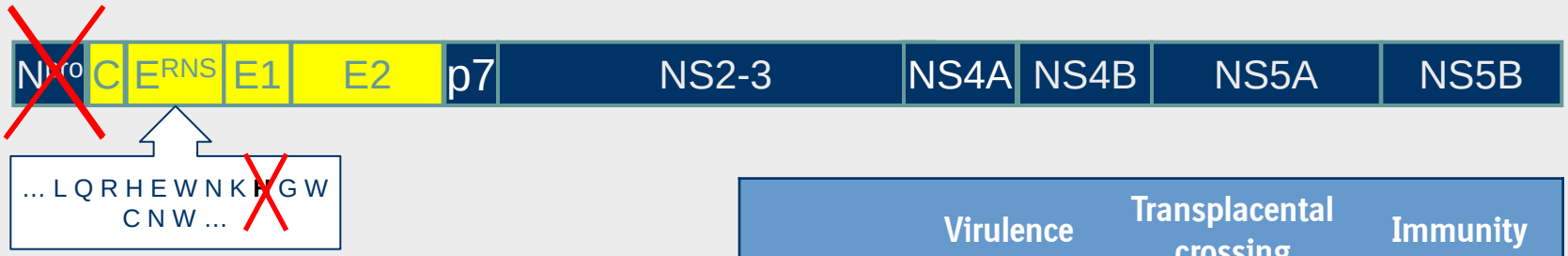
BVDV Genome



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

	Virulence	Transplacental crossing	Immunity
Wild-type	+	+	+
N^{pro} deletion	-	+	+
E^{RNS} deletion	-	+	+
Bovela®	-	-	+

Meyers G, et al. J Virol. 2007;81:3327

Single mutation: compensation of the missing function from the E^{RNS} and N^{pro}, respectively

Double mutation: compensation of E^{RNS}/N^{pro} function not possible

What if?

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... 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!

Bovela®

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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?

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- 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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