# **Plasmid vaccines**: considerations on the integration potential into the genome and on the risk assessment of non-heritable traits

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# Introduction: traditional vaccines

**Traditional vaccines** are mainly made from

- Killed microorganisms
- Live-attenuated micro-organisms
- Protein complex subunits
- Toxoids (bacterial toxin)

→ Live-attenuated micro-organisms are most widely used

# Introduction: traditional vaccines

Some **adverse effects** or **disadvantages** using traditional vaccines have been observed

- Reversion of the pathogen to a more virulent form
- Systemic adverse effects (redness, swelling, fever, headache)
- Preserving vaccine potency and stability
- Complicated biology and structure, making modifications difficult to accomplish

# Introduction: plasmid DNA vaccines

**Plasmid DNA vaccines** are ‘genetic vaccines’ that involve the intramuscular or subcutaneous injection of a **DNA plasmid** containing a **transgene** that encodes the sequence of a **target protein from a pathogen** under the control of an eurkaryotic promoter

# Introduction: plasmid DNA vaccines

## General structure of plasmid DNA vaccines

- Backbone plasmid consisting of a bacterial origin of replication and some selection marker

- Transcriptional unit consisting of
  - Viral promoter
  - Transgene (single of multiple genes)
  - Poly(A)tail for stability and translation

# Introduction: plasmid DNA vaccines

## Mode of action

- Nuclear localisation after injection
- Using the host's replication machinery
- Produced peptides are presented to the immune system
- Humoral and cellular immune response is triggered

# Introduction: plasmid DNA vaccines

## Possible advantages

- No pathogens involved
- Stable, no preservation problems
- Easy to produce
- Can also be used therapeutical

## Possible disadvantages

- Perhaps not that potent as traditional vaccines

# CLYNAV DNA vaccine for salmon

## What was the role of the European Food Safety Authority in the assessment of a DNA plasmid vaccine?

# CLYNAV DNA vaccine for salmon

Elanco developed a DNA plasmid vaccine, **CLYNAV**, that prophylactically is administered to farmed **Atlantic salmon** (*salmo salar*) to confer protection against pancreas disease caused by the salmonid alphavirus.

# CLYNAV DNA vaccine for salmon

To assess the **legal status** of such vaccinated Atlantic salmon with regard to the legislation on **GMO**, Elanco, upon suggestion of EC, carried out scientific studies on the **integration / non-integration** of the DNA plasmid into the Atlantic salmon genome.

# CLYNAV DNA vaccine for salmon

- EFSA assessed the data package

- EFSA concluded that the results from these studies were **not sufficient** to support the company's conclusion of **non-integration** of the DNA plasmid vaccine in the salmon genome (EFSA, 2013).

EFSA (European Food Safety Authority), 2013. Scientific advice on the suitability of data for the assessment of DNA integration into the fish genome of a genetically modified DNA plasmid-based veterinary vaccine. EFSA Journal 2013;11(5):3232, 8 pp, doi:10.2903/j.efsa.2013.3232

# CLYNAV DNA vaccine for salmon

- Last year, EC mandated EFSA to review a **new data package** provided by the company for the possible integration/non-integration of DNA plasmid into the fish genome

- EFSA created an EFSA **ad hoc working group** covering the expertise needed to assess the various data packages

# CLYNAV DNA vaccine: main data package

- **A bioinformatics study** assessing potential homology between the injected plasmid sequence and the salmon genome
- **A biodistribution study**, expanded with two additional time points for muscle tissue close to the site of injection and gonadal tissue
- **An integration study**, including a next-generation sequencing (NGS) study
- Risk assessment for CLYNAV vaccine use in Atlantic salmon with **theoretical considerations on a potential integration** rate in gonadal tissues

# CLYNAV DNA vaccine: main data package

## General considerations

- The assessment focuses on the muscle and gonadal tissue
  - Most data is available for these tissues
  - Potential heritability of plasmid integrated in gonads
  - Long persistence of DNA plasmid in the muscle

# CLYNAV DNA vaccine: bioinformatics study

- The potential for DNA plasmid vaccine sequence to integrate into the host genome through **homologous recombination** was estimated

- Sequence **homology searches** with DNA plasmid vaccine sequence and *Salmo salar* databases using BLASTn

- **No element** of the DNA plasmid vaccine sequence was identified with sufficient length and identity to support homologous recombination

# CLYNAV DNA vaccine: biodistribution study

- **Biodistribution study**, investigates the distribution of the DNA plasmid vaccine in the salmon, from the site of injection (muscle) to the different organs

- **Tissue samples analysed**: gut, spleen, kidney, heart, head, muscle and gonad

- **Time frame**: 1,7,14, 21, 36, 60, 91, 135, 331, 759, 822 days post-vaccination (DPV)

# CLYNAV DNA vaccine: biodistribution study

**Table 1:** **Biodistribution study:** Mean DNA plasmid levels detected in gonadal or muscle tissue 1, 7, 14, 759 and 822 DPV

| Time point of sampling | Biodistribution study (LOD = $10^{(a)}$) | | | |
|---|---|---|---|---|
| | Gonadal tissue DNA plasmid vaccine dose | | Muscle tissue DNA plasmid vaccine dose | |
| | 2× | 10× | 2× | 10× |
| 1 DPV | 111.75[(a)] | NA | $4.18 \times 10^6$ | $1.46 \times 10^7$ |
| 7 DPV | LOD | NA | $6.22 \times 10^6$ | $1.3 \times 10^7$ |
| 14 DPV | LOD | NA | $1.55 \times 10^6$ | $1.8 \times 10^7$ |
| 759 DPV | LOD | LOD | 88 | 285.20 |
| 822 DPV | LOD | LOD | 102.50 | 170.80 |

LOD: limit of detection; NA: not assayed.
(a): DNA plasmid copies/μg gDNA.

# CLYNAV DNA vaccine: integration study

- **An integration study** was performed with gonadal tissue (1DPV) and muscle tissue close to the injection site (14DPV), 10x dose

- After DNA isolation, separation between **HMW** and **extrachromosomal DNA** through field inversion gel electrophoresis (FIGE)
  - qPCR detection validated
  - Detection of integration events is done by qPCR, covering only 300bp of the plasmid

# CLYNAV DNA vaccine: integration study

**Table 2:** **Integration study:** DNA plasmid levels detected in the gonadal or muscle tissue HMW gDNA fraction 1, 14, 759 and 822 DPV

| Time point of sampling | Integration study (LOD = 2,000[a]) | | | |
|---|---|---|---|---|
| | Gonadal tissue DNA plasmid vaccine dose | | Muscle tissue DNA plasmid vaccine dose | |
| | 2× | 10× | 2× | 10× |
| 1 DPV | NA | LOD | NA | NA |
| 14 DPV | NA | NA | NA | 25–20,946[a] |
| 759 DPV | NA | NA | NA | NA |
| 822 DPV | NA | NA | NA | NA |

LOD: limit of detection; NA: not assayed.
(a): DNA plasmid copies/μg gDNA.

# CLYNAV DNA vaccine: integration study

- **Positive Control** for true integration was constructed
  - Chum salmon heart cell line transfected with transfection-optimised DNA plasmid containing a similar expression cassette as the DNA plasmid vaccine
  - Integrated plasmid/μg gDNA was calculated

- Through spiking of the integrated control DNA into naive genomic salmon DNA, the overall experimental LOD was set to **2000 copies/μg gDNA**

# EFSA assessment of data from gonadal tissue

- Integration experiment is **not sensitive** (2000 copies/μg gDNA)
- Biodistribution experiment is **more sensitive** (10 DNA plasmid copies/μg gDNA) but not designed to distinguish between integrated and non-integrated DNA plasmid
- The data showed a steady decline in DNA plasmid copies with increase days post vaccination
- At the last time point measured, no further information is available on subsequent decline in DNA plasmid levels, and therefore levels can be considered to be **all integrated in a worst case scenario**

# EFSA assessment of data from muscle tissue

- After the integration assay, between 25 and 20.946 plasmid copies were found
- **Clearance experiments** had shown that the FIGE could not reliable remove DNA plasmid in experiments with spikes above $10^7$ plasmid copies
- Biodistribution data shows $10^8$ plasmid copies in muscle cells
- It is reasonable to assume that the levels detected in the integration study are **false positives**

# EFSA assessment of data from muscle tissue

- To determine if DNA plasmid remaining in the muscle cell DNA **after FIGE separation** is integrated into the salmon genomic DNA, the company designed a **NGS study**

- **Plasmid sequence capture** NGS method on post-FIGE HMW DNA

- Due to several shortcomings, and no LOD calculation for this experiment, **no conclusions about possible integration** could be drawn from the experiment

# EFSA assessment of data from muscle tissue

Also for muscles **biodistribution data** from the last time point can be considered for the assessment

## Integration calculations under worst case scenarios

- 1 out of 31.250 gonadal cells would have 1 copy integrated
- For the 10x dose: 1 diploid nucleus in 915 nuclei would have a plasmid integrated
- For the 2x dose: 1 diploid nucleus in 1524 nuclei would have a plasmid integrated

# CLYNAV DNA vaccine: theoretical considerations

## A theoretical model to calculate integration rate of DNA plasmid vaccine into gonad cells in salmon

- The model consists of three steps
  - Binding of the plasmid vaccine to the cell membrane
  - Internalisation of bound plasmid
  - Integration of internalised plasmid into the genome

- The **outcome** of this model was that 1 gonad cell/315.000 cells would have 1 DNA plasmid vaccine copy

# EFSA assessment: conclusions

- The experimental data did not show robust evidence for a true integration event
- However as homologous and non-homologous integration predicts integration at a certain frequency
- EFSA used, based on LOD and company information, **worst case assumptions** leading to upper estimates for possible integration rates
    - The dose is at least 10x higher than the dose intended to be injected
    - Data are derived from biodistribution data, no distinguishing between free/integrated plasmid

# EFSA assessment: conclusions

- EFSA concluded that, based on the worst-case scenarios described here and taking into account additional factors decreasing the likelihood of integration, **the actual integration rate** is likely to be **orders of magnitude lower** than the upper estimated integration rate calculated in the context of the worst-case scenarios.
- With the available evidence, the actual integration rate cannot be estimated with more precision.

# Assessment of non-heritable traits: considerations

- Definition of GMO in Directive 2001/18

*Article 2*

**Definitions**

For the purposes of this Directive:

(1) 'organism' means any biological entity capable of replication or of transferring genetic material;

(2) 'genetically modified organism (GMO)' means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination;

# Assessment of non-heritable traits: considerations

- In a stricter interpretation of the legal text, it could be interpreted that a single integration event of new genetic material in one cell's genomic DNA is sufficient for an organism to be defined as a GMO

- In case of a random integration of genetic material in the DNA of non-heritable cells, the standard GMO risk assessment is probably not possible

# Assessment of non-heritable traits: considerations

- In case of **integration in somatic cells** of an organism
  - The insertion site in individual cells might likely differ between cells
  - Every individual would have different cells integrated
  - Sensitivity of detection method

- A structured risk assessment will be difficult, case by case approach should be followed

# Assessment of non-heritable traits: considerations

## Recommendations by FDA on whether an integration study is needed, might be considered

*"FDA believes that integration studies are warranted only when the plasmid persists in any tissue of any animal at levels exceeding 30.000 copies per μg of host DNA by study termination. If the persistence of the DNA plasmid exceeds this threshold, sponsor should evaluate whether the DNA has integrated into the genome of the vaccinated animals"*

Guidance for industry: Considerations for plasmid DNA vaccines for infectious disease indications, November 2007, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research

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