

RSV vaccine using recombinant F protein ?

Zürcher Hochschule
für Angewandte Wissenschaften



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50 years of vaccine research

RSV is a worldwide burden (64 million infections/yr, 160'000 deaths/year)

RSV was first isolated in 1956 (Morris et al.).

No licenced vaccine available

RSV weakly immunogenic

Enhanced disease associated with a formalin-inactivated vaccine (Kapikian et al, 1969, Kim et al, 1969)

Subunit vaccine promising

F and G proteins induce neutralizing Ab (Walsh et al, 1987)

How to produce RSV-F ?

Production of recombinant RSV-F (rRSV-F) in mammalian cells by Transient Gene Expression (TGE)

- Correct folding, assembly, and post-translational modifications

- Scalable and simple process

- Rapid and inexpensive

Production of viral RSV-F (vRSV-F)

- low viral titers in cell culture

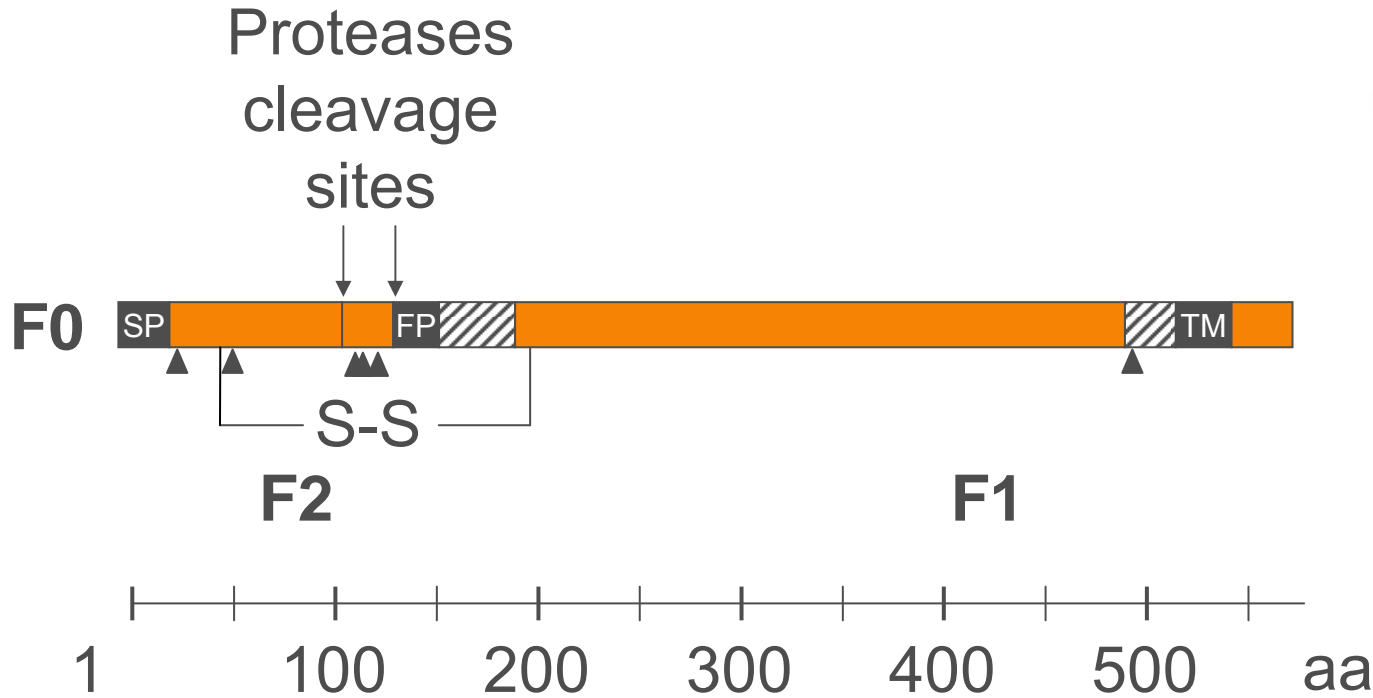
- biosafety problems

Objectives

Establish a manufacturing process for rRSV-F :

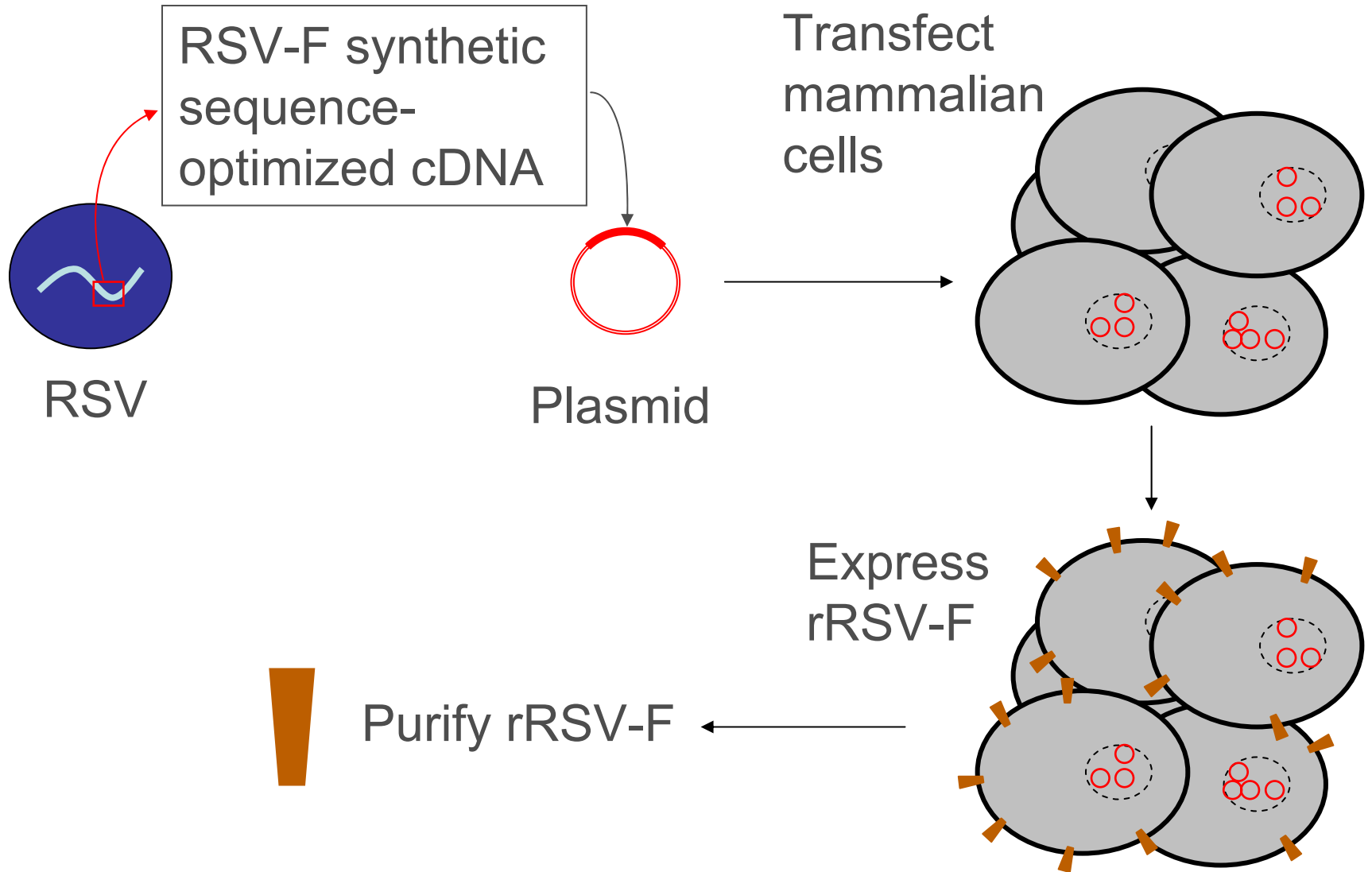
1. Production of rRSV-F by transient gene expression in mammalian cells.
2. Scale up of the manufacturing process of rRSV-F for animal studies.
3. rRSV-F in virosomes : Animal experiments.

RSV-F : a trimeric membrane glycoprotein

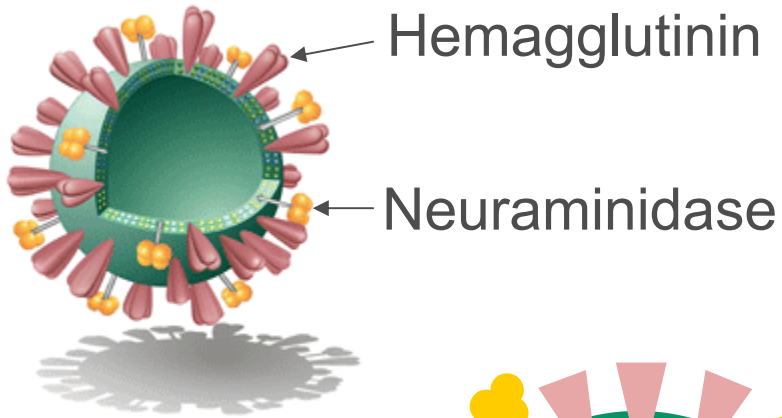


Morton et al, 2003

Production of rRSV-F by transient gene expression – Proof of principle



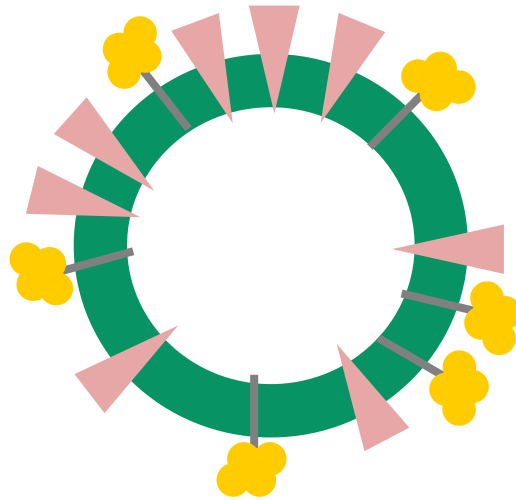
Formulation of rRSV-F in virosomes - Principle



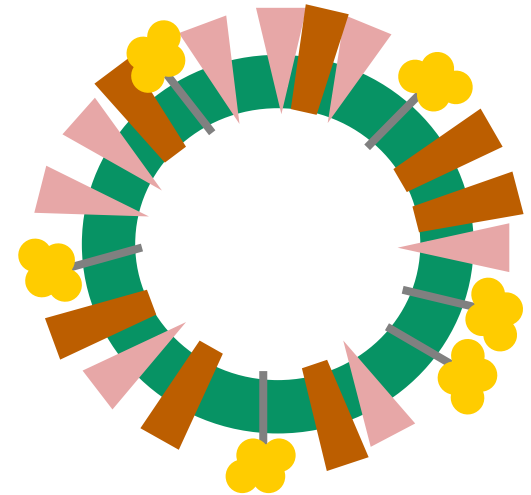
Pevion Biotech[®]



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Purified
rRSV-F

Empty virosome

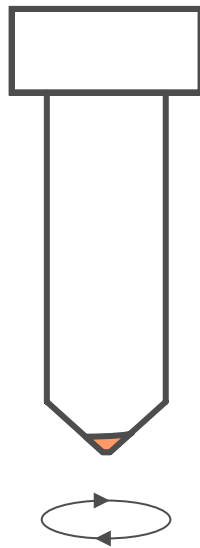
rRSV-F integrated
in virosomes

1. Production of rRSV-F by transient gene expression in mammalian cells

Transfection

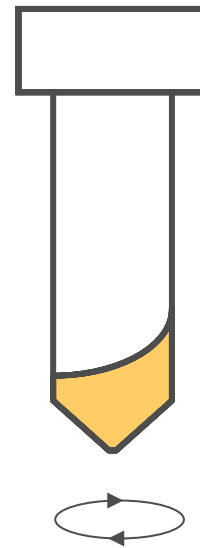
Production

○ DNA
Transfecting agent



3h

Production medium



2-3 d

Cell harvest
Purification

Orbital shaking

1. Production of rRSV-F by transient gene expression in mammalian cells

Cell line

DNA amounts

Transfecting agent/DNA ratio

Medium for transfection

Medium for production

Temperature

Time of harvest

Addition of chemicals

Expression plasmid

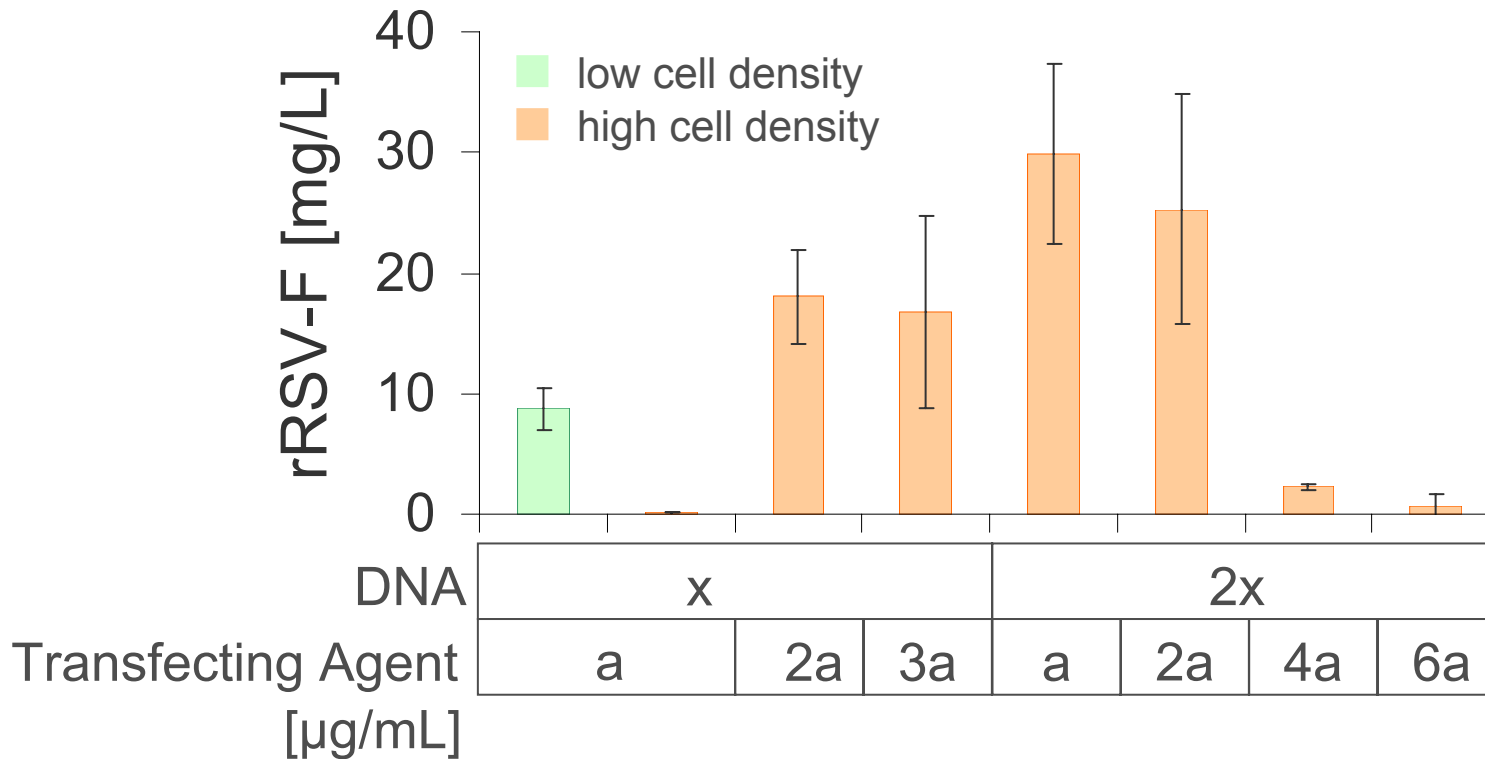
Cell seeding density

Aeration

1. Production of rRSV-F by transient gene expression in mammalian cells

Cell seeding density

DNA and Transfecting Agent amounts



Optimal conditions yielded 30 mg/L of rRSV-F in 48h in HEK-293E cells.

30 mg/L at 10-mL scale ...

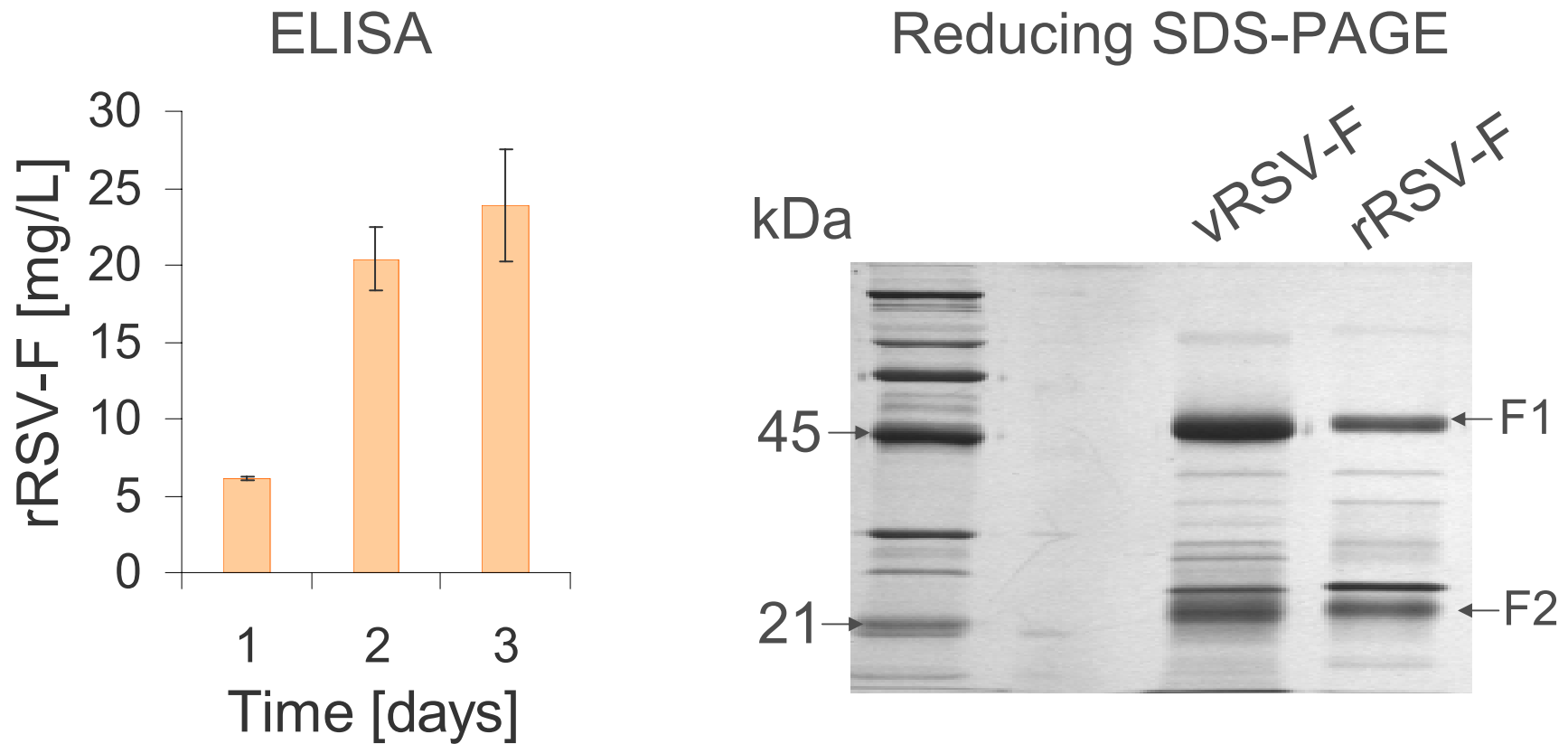
...Is it achievable at a larger scale ?

Disposable bioreactors:

- Single use
- Reduced cross-contamination
- Simple use
- Simplified validation
- Rapid set up
- No cleaning
- Cost-effective
- Orbital shaking



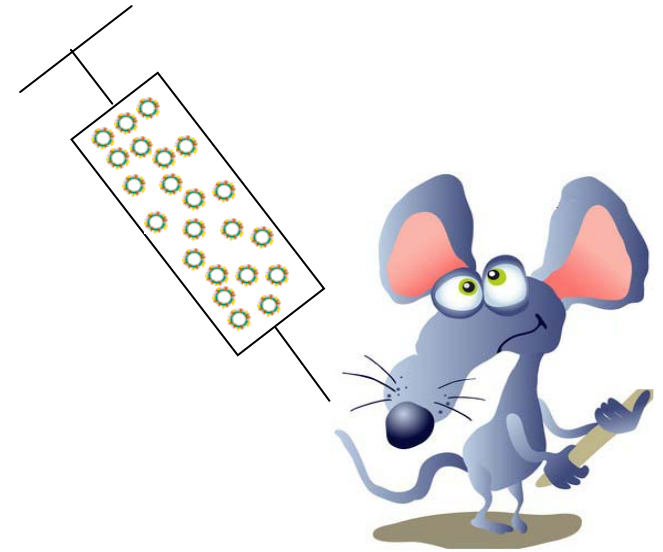
2. Scale up of the manufacturing process of rRSV-F for animal studies



12 mg of purified rRSV-F were produced for animal studies out of 1.3 L of cell culture.

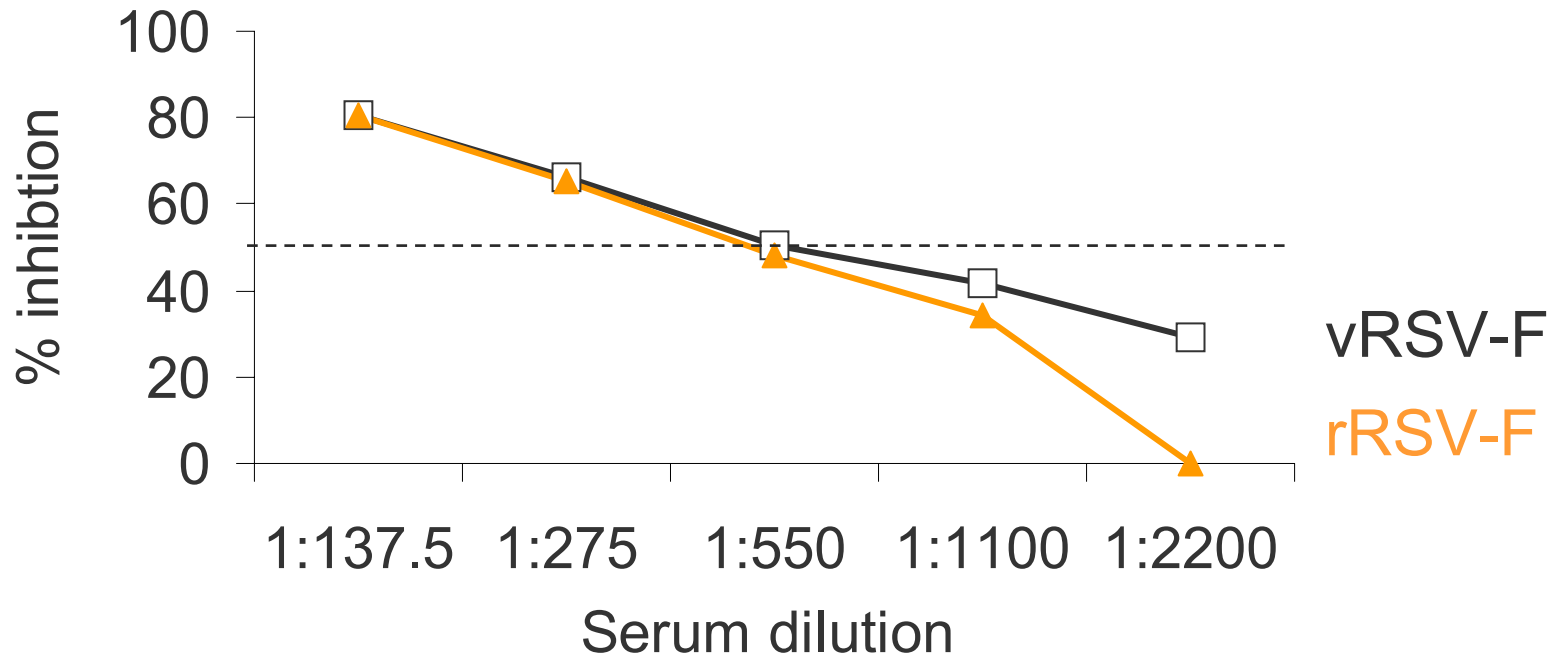
3. rRSV-F in virosomes : Animal experiments

Immunization of BALB/c mice
7.5 μg RSV-F/dose
rRSV-F-virosome i.m.



3. rRSV-F in virosomes : Animal experiments

Neutralization of RSV by BALB/c mice sera



Animal challenge in cotton rats has been performed using rRSV-F formulated in virosomes and data analysis is ongoing.

Conclusion

1. We developed a scalable process for the production of rRSV-F by transient gene expression in mammalian cells.
2. Transient gene expression allowed the rapid production of pure rRSV-F for animal studies.
3. rRSV-F in virosomes induces neutralizing antibodies in BALB/c mice.

Go forward with transient technology

Transient gene expression :

for animal studies



...for clinical trials ?

... for production ?

Acknowledgements



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KTI/CTI projects