RSV vaccine using recombinant F protein?

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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

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

1. RSV-F synthetic sequence-optimized cDNA
2. Transfect mammalian cells
3. Express rRSV-F
4. Purify rRSV-F
Formulation of rRSV-F in virosomes - Principle

\[
\text{Purified rRSV-F} + \text{Empty viroso}\text{me} = \text{rRSV-F integrated in virosomes}
\]

Hemagglutinin
Neuraminidase

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

Transfection

- DNA
- Transfecting agent

Production

- 3h
- 2-3 d
- Production medium
- Orbital shaking
- Cell harvest
- Purification
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
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.

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<tr>
<th>Pre-Immunization</th>
<th>RSV-F</th>
<th>RSV-F</th>
<th>Bleeding</th>
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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.
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.
Transient gene expression:

for animal studies ✓

…for clinical trials?

… for production?
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