Development of a live attenuated pediatric RSV vaccine

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- and you -
A pediatric RSV vaccine is receiving increasing recognition as a worldwide priority (e.g., WHO).

50 years of research have not resulted in a licensed vaccine, but we are getting very close.

Why has a pediatric RSV vaccine been so difficult to develop?

- Need to immunize early in life (~ 4 months of age)
- At that young age, safety is a key consideration
- Immune responses in infancy are reduced
- Maternal antibodies against RSV suppress antibody responses to RSV
- RSV replicates in the superficial cells of the respiratory epithelium, where immune protection is less effective
- RSV animal models are poorly permissive and not highly predictive
- Subunit vaccines are contraindicated in RSV-naïve recipients due to imbalanced immune responses (which can lead to enhanced RSV disease)
- Live vaccines do not share this problem, but are difficult to develop because RSV does not grow efficiently in vitro and can be physically unstable
Development of a Pediatric RSV vaccine

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- Recent vaccine candidates based on deletion of the M2-2 ORF (ΔM2-2) look promising.
Why a live RSV vaccine?

- Needle-free, adjuvant-free; a single dose is substantially immunogenic
- Broad stimulation of innate, cellular, humoral immunity
- Attenuated RSV strains provide all of the viral antigens
- Intranasal application: Direct stimulation of respiratory tract immunity
- Intranasal route partly avoids immune suppression by maternal antibodies
- Live vaccines induce broader, more effective immunity than subunits in virus-naïve recipients (e.g. influenza vaccines)

In RSV-naïve recipients, killed and subunit RSV vaccines prime for enhanced RSV disease upon subsequent natural RSV infection, but live vaccines do not.
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Immunization of RSV-naïve infants against RSV requires a live vaccine
The use of recombinant DNA methods (‘reverse genetics”) provides improved live RSV vaccines

1. Precisely-defined mutations can be introduced into live virus via a DNA intermediate
   - These can include precise deletion of ORFs or genes
   - Deletion of nonessential proteins can result in alterations in viral gene expression (e.g. \( \Delta M2-2 \)) or host responses (e.g., \( \Delta NS1, \Delta NS2 \)) that confer improved vaccine immunogenicity and safety
   - Deletions of ORFs or genes are refractory to reversion
   - These “designer” viruses are well-characterized

2. Reverse genetics produces “clean” virus de-novo with short, well-characterized passage history

3. Although the viruses are produced from cDNA, after the initial transfection no further DNA is involved, and the viruses replicate and “behave” just like biologically-derived virus.
Working with RSV is not easy....

- RSV can be physically unstable unless handled correctly.
- RSV forms long filaments. Virions are very sensitive to freezing, thawing, and pH changes due to CO₂ (dry ice).
- Loss of > 99% of infectivity is common if RSV-containing specimens are not “snap” frozen in sucrose-containing VTM on dry ice.
- In previous RSV vaccine studies, sample processing at IMPAACT sites has been excellent!
**RSV ΔM2-2 RSV vaccine candidates**

**RSV ΔM2-2**

- RNA synthesis regulatory protein
  - RNA replication $\downarrow$
  - viral gene transcription $\uparrow$
  - viral protein synthesis $\uparrow$
  - virus production delayed but not reduced

- Gene deletion expected to be stable against de-attenuation

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**Western blot (infected cell lysates) probed with anti-RSV F antibodies**

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- **WT RSV**
- **ΔM2-2**
Phase 1 study: RSV MEDI ΔM2-2 (CIR/JHU and LID; CRADA with Medimmune)

Evaluation in RSV seronegative children, 6-24 months of age:
- 20 Vaccinees: $10^5$ PFU
- 10 Placebo recipients

• Infectious: Virus shedding in 12/20 recipients (60%), but mostly very low titers

• Immunogenic: ≥ 4-fold increase in RSV-specific serum antibodies in 19/20 (PRNT: 1: 97)

• Safe: no significant difference in disease compared to placebo recipients, no lower respiratory tract infection (LRI)

• But high incidence of background respiratory illness in both groups
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• RSV Surveillance during the winter following vaccination:
  - 3/9 placebo recipients had increases in RSV-neut. serum antibodies (mean PRNT: 1:97)
  - 2 had RSV-MAARI (1 subgroup A, 1 subgroup B)
  - 5/20 vaccinees had increases in RSV-neut. serum antibodies (mean serum PRNT: 1:1,261)
  - 1 had RSV-MAARI (subgroup B)

  - In the other vaccine recipients, the post-vaccination titers were stable over 5-12 months
    mean post-vaccination serum PRNT: 1:91
    mean post-surveillance serum PRNT: 1:79
A second $\Delta M2\!\!2$ virus: RSV LID $\Delta M2\!\!2$ (IMPAACT 2000)

**LID$\Delta M2\!\!2$ versus MEDI$\Delta M2\!\!2$:**
- The M2-2 deletions differ slightly
- LID $\Delta M2\!\!2$ has a deletion in the downstream NTR of SH
- The two virus backbones differ at 22 nucleotide positions scattered throughout the genome (only 2 amino acid differences)

**Diagram:**
- M2-1
- M2-2 (90 amino acids)
- Delete 241 nt encoding 80 amino acids
- Knock out the 3 translation start codons
- Delete 112 nt from downstream NTR of SH gene (makes cDNA more stable)
IMPAACT 2000: RSV LID ∆M2-2 (CIR/JHU + IMPAACT)

- 29 RSV seroneg. children 6-24 months of age (20 vaccinees, 9 placebo recipients)
- Comparable rates of mild resp./febrile illness in vaccinees and placebo recipients
  - 1 incidence of brief grade 2 LRI in vaccinee, coincident with shedding of vaccine, enterovirus, and rhinovirus, and older sibling ill with resp. symptoms causation unclear
- Infectivity by culture: 19/20 (95%)
- Mean peak NW virus titer: $3.4 \log_{10} \text{PFU/ml}$ - but: higher than desired
- ≥4-fold increase in RSV-neut. serum antibodies: 18/20 (90%)
- Mean serum PRNT: 1:160

RSV surveillance during winter following vaccination (Nov 1- Mar 31):

- **3/9 placebo recipients**: increases in RSV-neut serum antibodies (mean PRNT: 1:158)
  - 1 had RSV-MAARI

- **6/20 vaccinees** had increases in RSV-neut. serum antibodies (mean PRNT: 1:1176)
  - No RSV-MAARI
  - In the other vaccinee recipients, post-vaccination titers induced by the vaccine were stable
    - mean pre-surveillance serum PRNT: 1:169
    - mean post-surveillance serum PRNT: 1:158
RSV MEDI ΔM2-2 and LID ΔM2-2 each induced titers of RSV-neutralizing antibodies that were higher than previous candidates.

The ΔM2-2 mutation indeed appears to increase immunogenicity per PFU.

With both viruses, immunity induced by a single dose was durable during the following RSV season.

With both viruses, there was presumptive evidence of protection from RSV disease during the following RSV season, and very high anamnestic responses.

The ΔM2-2 deletion has properties very desirable for a live RSV vaccine.

The increased replication (and immunogenicity) of RSV LID ΔM2-2 versus MEDI ΔM2-2 indicates an effect of the genetic background - an intermediate virus might be ideal.

**Plans moving forward:**

A virus very similar to MEDI ΔM2-2 will be further evaluated beginning in 2017 as a lead candidate.

Several additional versions of ΔM2-2 viruses will be evaluated in 2016-2017 to identify a possible intermediate virus.
Strategy moving forward

A. Further evaluation of a virus comparable to MEDI/ΔM2-2, but this cannot be done until 2017

B. Evaluate 2 versions of LID/M2-2 that are further-attenuated to different extents with well-characterized mutations:
   LID/ΔM2-2/1030s (IMPAACT 2011) > LID/cp/ΔM2-2 (IMPAACT 2012)

C. Evaluate a virus that combines features of LID ΔM2-2 and MEDI ΔM2-2 and may be intermediate:
   NS2/N/ΔM2-2-HindIII (IMPAACT 2013)
Planned Vaccine Studies

• Slightly temperature sensitive; “1030s” missense mutation provides further restriction of replication

• Slightly attenuated by the well-characterized set of 5 “cp” missense mutations in N, F, L genes

• Amino acid sequence, M2-2 deletion, and SH noncoding region identical to MEDIΔM2-2, which was more attenuated than LID ΔM2-2 in the previous clinical study

LID/ΔM2-2/1030s
IMPAACT 2011

LID,cp,ΔM2-2
IMPAACT 2012

NS2/N/ΔM2-2-HindIII
IMPAACT 2013
Summary

Immunization of infants requires a live RSV vaccine.

RSV is a labile virus, and considerable care is needed in preparing vaccine, and in processing nasal washes.

$\Delta$M2-2 vaccine candidates appear to be very promising vaccine candidates.

Several candidates with M2-2 deletion are being evaluated. This is because they may differ in attenuation and immunogenicity.

Safety, Infectivity, Immunogenicity, and Immunogenicity after the RSV season is assessed in small numbers of infants and children.

We hope to identify a lead candidate that will be taken to a larger study in 2017.

Thank you!