Evaluation of immune responses in clinical trials of live attenuated A/AA *ca* avian influenza virus vaccines

Vaccines

- H5N1 A/VietNam/1203/2004* x A/Ann Arbor/6/60 *ca*

  $10^{6.7}$ $TCID_{50}$: 'low dose'

  $10^{7.5}$ $TCID_{50}$: 'high dose'

- H5N1 A/HongKong/213/2003* x A/Ann Arbor/6/60 *ca*
- H9N2 A/chicken/Hong Kong/G9/97 x A/Ann Arbor/6/60 *ca*
- H7N3 A/chicken/British Columbia/CN-6/2004 x A/Ann Arbor/6/60 *ca*

*multibasic cleavage site removed

Study Design

- Open label trials conducted in adults in an isolation facility, April-September 2005, 2006, 2007
- Subjects admitted to isolation unit 2 days before vaccination
- Importance of remaining on unit until discharge emphasized at enrollment, admission, vaccination & throughout study
- Vaccine administered by nose drops or nasal spray
- PE & NW for viral culture and rRT-PCR daily until discharge*
- Oseltamivir available for significant illness (LRI or sustained fever) or in the event of early departure

* Must be rRT-PCR negative prior to discharge

Study Design (II)

Requirements for clinical staff:

- Influenza vaccine within past 6 months
- Gowns, gloves, mask on unit during & after vaccination
- Oseltamivir if fever or resp sx pending influenza PCR

Assessment of immune responses

Antibody assays:

- hemagglutination-inhibition
- microneutralization
- ELISPOTs to measure IgG and IgA ASCs (in process)

Differences between CDC and CIR microneutralization assay

	•CDC	•CIR
•Cells	•MDCK/CDC	•MDCK/LID
•Cell input	•1.5 x 10^4	•2 x 10^4
•Virus	•non-*ts*	•A/AA *ca*
•Incubation temperature •(2h; 18 h)	•37^{0}C	•32^{0}C
•Virus input	•100 $TCID_{50}$	•80 $TCID_{50}$ (H9) •100 $TCID_{50}$ (H5)
•Readout	•ELISA with anti-NP Ab •(50% inhibition of signal)	• ELISA with anti-NP Ab •(50% inhibition of signal)

Conclusions

1. In healthy adults, H9N2 G9/AA *ca* and H5N1 VN 2004 AA *ca* were:
   - well tolerated
   - highly restricted in replication
2. Two doses of H9N2 G9/AA *ca* induced $\geq$ 4 -fold rises in HI titer in 92% of subjects and microneutralizing antibody titer in 79% of subjects. In contrast, two doses of H5N1 VN 2004 or HK2003 AA *ca* vaccines induced $\geq$ 4 -fold rises in HI and microneutralizing antibody titer in $\leq$10% of subjects.
3. Antibody responses to A/AA *ca* viruses containing avian HA/NA vary depending upon the surface glycoproteins included in the vaccine and cannot be predicted based upon detected viral replication.

Conclusions

4. For recipients of the H9N2 A/AA *ca* vaccine, there was a strong correlation between HI and microneutralizing antibody responses (HI detected a slightly greater number of responses).

5. Since the insertion of avian HA and NA genes appears to further attenuate A/AA *ca* viruses, consideration might be given to cautious outpatient assessment of individual strains outside of the influenza season, following initial inpatient assessment for characterization of vaccine virus shedding.

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