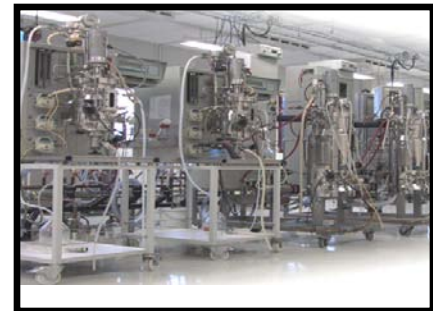
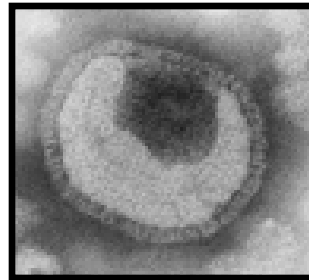


New Influenza Vaccine Technologies

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Centers for Disease Control and Prevention



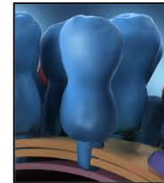
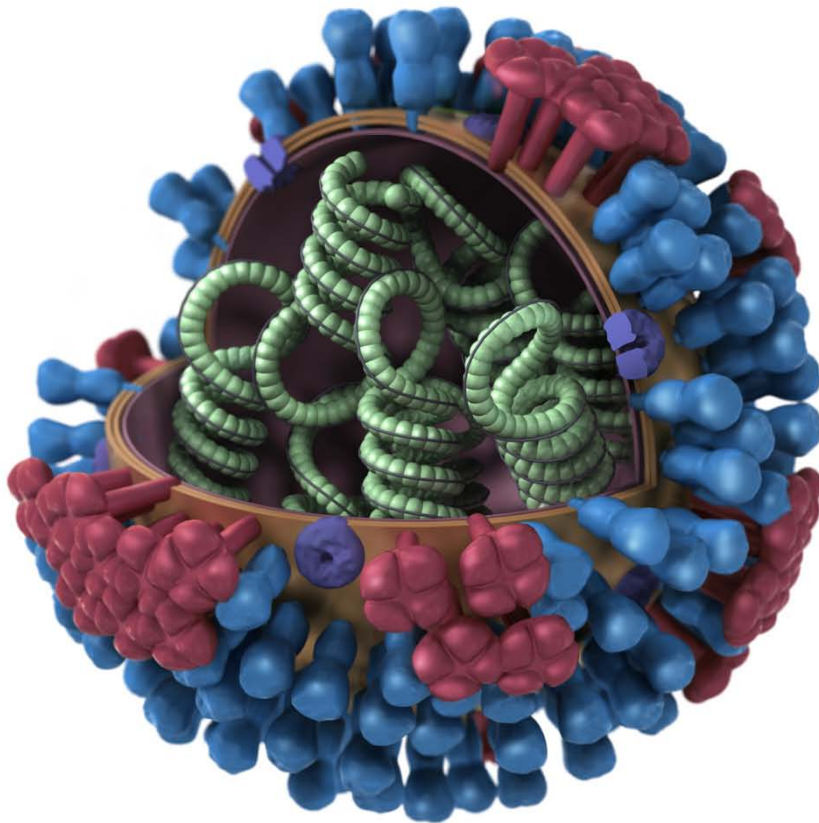
Current Influenza Vaccines

- **Safe**
- **Effectiveness is variable and depends on multiple factors**
 - **Closeness of antigenic match with circulating strain**
 - **Reduced cross-protection against drifted strains within subtypes**
 - **Little/no protection against novel subtypes**
 - **Inherent immunogenicity of vaccine strain**
 - **Ability to induce strain-specific neutralizing antibody in face of repeated exposure**
 - **Age and immune status, other host factors of population**
 - **Reduced effectiveness in older adults, immunocompromised**
- **Formulated and standardized based on HA content to induce virus-neutralizing antibodies**
 - **Annual reformulation due to antigenic drift within HA**
 - **Costly, time-consuming, year-round process**
- **Egg-based technologies limit rapid response and surge capacity for pandemics**

Recent Advances in Influenza Vaccines

- **Newly licensed trivalent vaccines help to address short term needs to improve immunogenicity and expand manufacturing capacity**
 - **Newly licensed high dose and intradermal vaccines with improved immunogenicity for older adults**
 - **Intradermal vaccines are dose-sparing for younger adults**
 - **Adjuvanted vaccines (non-U.S.) improve immunogenicity, generally with dose-sparing**
 - **Oil-in water emulsions (MF-59, AS03), alum, virosomes**
 - **Licensed cell-based vaccines (Europe)**
 - **Vero or MDCK mammalian cell-based products**
 - **Shortened production time & potency testing needs unclear**
- **Many other adjuvant strategies under preclinical or clinical evaluation**
 - **e.g. Cytokines, Toll-like receptor ligands**

Influenza A virus Proteins as Targets for Influenza Vaccines



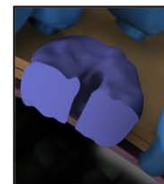
Hemagglutinin

Anti-HA Antibodies (Abs) block virus attachment or fusion to Inhibit replication



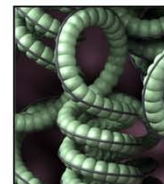
Neuraminidase

Anti-NA Abs prevent virus release and spread



M2 Ion Channel

Abs to M2 external domain block virus budding/release



RNP

CD8+ T cell responses to NP, M1 proteins kill virus-infected cells and reduce virus load

Next Generation Vaccine Technology Platforms

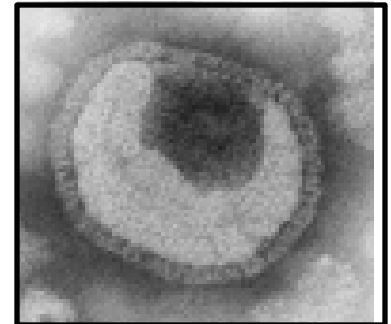
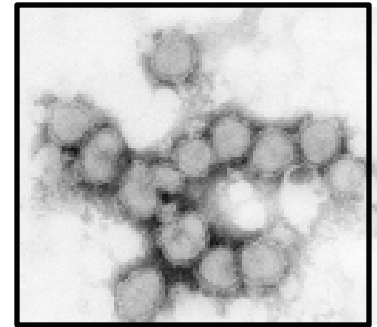
- **Recombinant subunit and Virus-like particles (VLP) expression systems**
 - Mammalian, insect, yeast, tobacco plants
- **Live-virus or other viral vectors**
 - Novel live attenuated influenza donor strains
 - NS1 deletion mutants
 - Adenovirus, MVA, alphavirus
- **DNA**
 - Plasmid-based, single or multiple gene combinations
 - DNA prime, VLP/vector boost
- **Peptides**
 - Synthetic, multigenic (e.g. conserved CTL epitopes)

New Vaccine Approaches to be Covered

- **Recombinant Proteins and Virus-like Particles**
 - **Recombinant HA vaccine produced in insect cells (Protein Sciences Corp) in late-stage development**
 - **VLPs produced in insect cells**
 - **Plant-based technologies**
- **“Universal” vaccine strategies**
 - **Conserved M2 ectodomain, NP**
 - **Conserved HA stalk region**
- **Recombinant DNA techniques eliminate use of live virus and potentially can facilitate more rapid production**

Virus-like Particles (VLP) Vaccines

- **Non-infectious particles containing HA, NA, M1 proteins; resemble virions with no genetic material**
- **Influenza genes cloned into viral vector used to infect cultured cells**
 - **Proteins self-assemble into VLPs**
- **Rapid production and response time**
- **Theoretically high yields (continuous harvest)**
- **Broadened immune response (antigen presentation, cellular immunity)**
- **Many varieties in preclinical development**
 - **Insect cells, E.coli, plants**
- **One product in phase II trials**

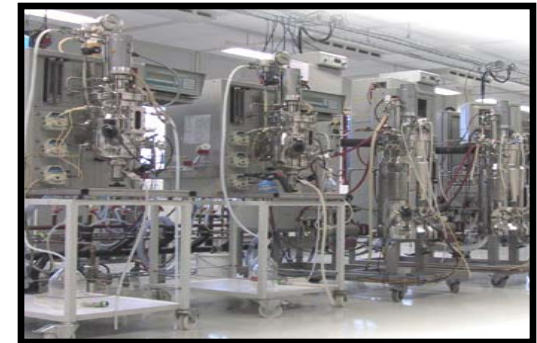


Novavax Pandemic H1N1 VLP Vaccine

- **Phase II/III trial of VLP vaccine produced in insect cells**
 - Immunogenicity in ~500 subjects (5, 15, 45 µg HA)
 - Safety with 6 month follow up in ~3000 adults
- **Well tolerated with only mild, transient AE**
- **Immunogenicity of one 5 µg HA dose:**
 - Met FDA criteria for seasonal vaccine licensure
 - Seroconversion rate of 48%
 - HI titer ≥ 40 in 81% of volunteers

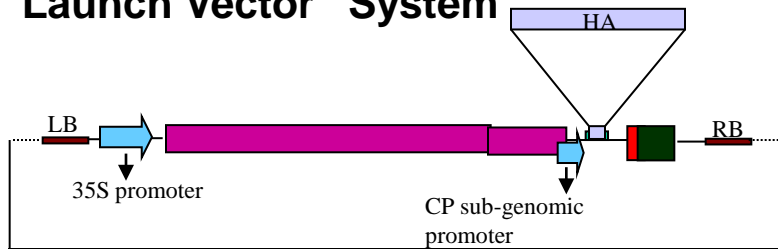
Plant-based Vaccine Technologies

- HA gene expressed in *Agrobacterium* through plasmid or viral vector
- Bacteria used to infect tobacco plants and achieve transient expression of HA protein
- Medicago Inc. product is expressed at plasma membrane as a VLP
 - H5N1 phase I trial (*Landrey et al., PLoS One, 2010*)
 - 2009 H1N1 phase I trial ongoing
- Fraunhofer USA product is purified unclesaved monomeric HA
 - Phase I trial sponsored by DOD/DARPA conducted at WRAIR (James Cummings, PI)



Schematic representation of “launch vector”-based production of target antigens in plants

Step 1. Cloning of a target gene into “Launch Vector” System



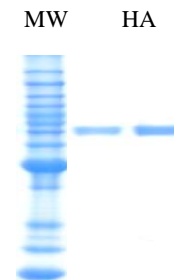
Step 2. Inoculation of “Launch Vectors” containing target genes into plants



Step 3. Accumulation of target antigen in plants



Step 4. Purification of recombinant antigen from plant biomass



“Universal” Vaccines

- **Target conserved epitopes that elicit broad immunity within and across influenza A subtypes**
 - **Identify less immunodominant, but more cross-reactive B and T cell epitopes on HA, NA and conserved proteins (e.g. M2 and NP)**
 - **Induce humoral and / or cellular immunity**
 - **Less sensitive to antigenic drift**
 - **Longer-lived protective efficacy of seasonal vaccines**
- **Could be stockpiled for epidemics/pandemics**
- **Surge capacity**
 - **Rapid scale-up, reduce production bottlenecks**
- **Could supplement strain-specific HA based vaccines when available**

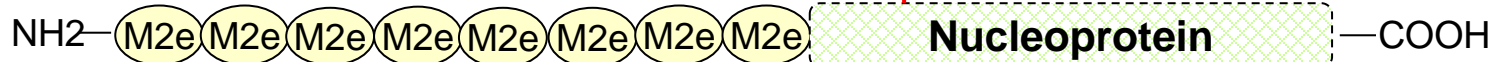
Dynavax Universal Influenza Vaccine

Matrix Protein 2,
extracellular
domain (M2e)

Nucleoprotein
(NP)

TLR-9 agonist
ImmunoStimulatory
Sequence
(ISS), C295

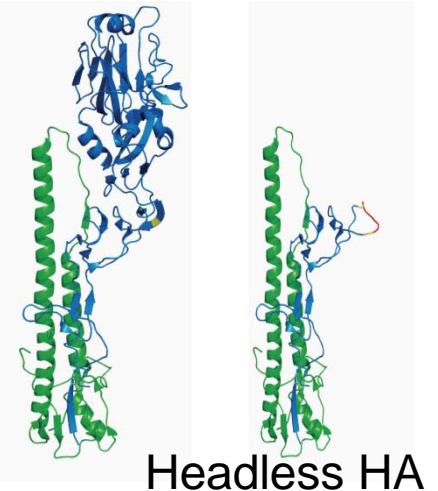
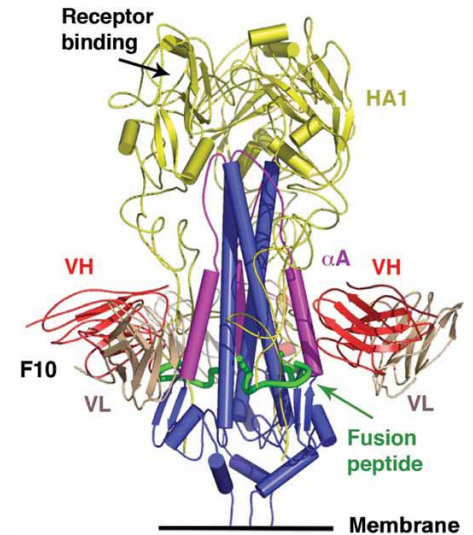
N8295



- Phase Ia dose escalation trial of N8295
- Phase Ib trial of N8295 + H5N1 split vaccine
 - ~55 subjects total
 - Safe with no SAE
 - N8295 elicited anti-M2e antibodies, anti-NP antibodies and T cell response to NP
 - Addition of N8295 to non-immunogenic dose of H5N1 vaccine elicited H5 antibody responses
- One year follow up in subjects ongoing

Universal Vaccine Strategy Targeting Conserved HA Domain

- Based on recent identification of human MAbs that recognize conserved stalk region of HA that encompasses fusion peptide (*Throsby et al., PLoS One 2008; Ekiert et al., Science 2009; Sui et al., Nat Struct Mol Biol 2009*)
 - 2 structural groups for 16 HA subtypes
 - Low levels of these heterosubtypic Abs detected in human sera (*Sui et al., CID 2011*)
 - Antibodies neutralize virus by inhibition of membrane fusion
- “Headless HA” vaccine strategy tested in mice (*Steel et al., mBio 2010*)
 - Complete protection against lethal challenge
 - Partial protection against disease



New Influenza Vaccines and Future Challenges

- **The landscape of influenza vaccine development is rapidly evolving**
- **Newly licensed products provide near-term advancements for improved immunogenicity and production capacity**
- **Many innovative technologies are in development for longer-term improvements**
- **Significant challenges include:**
 - **Ensuring safety**
 - **Scrutiny for adventitious agents and unexpected immune responses**
 - **Optimization of immunogenicity**
 - **Adjuvants, combination of strategies**
- **Formulation and potency evaluation**
 - **Each platform may require unique potency reagents/methods to standardize and assess stability**
- **Complex regulatory pathway**
 - **Need to establish immune correlates of protection and assays to detect**

Acknowledgements

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- **James Cummings (DOD/WRAIR)**
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