“The Future of Vaccination”

by Stanley A. Plotkin

Attenuated Vaccines

Animal Virus (vaccinia)

↓

Physical attenuation (Rabies, Anthrax)

↓

Passage in animals, eggs or *in vitro* (YF, BCG)

↓

Passage in cell culture *in vitro*

Mutants through adaptation to cell culture
(Measles, OPV, Varicella, Mumps, Adeno, Zoster)

Mutants through adaptation to low temp. (ca)
(Rubella, Live Flu)

Auxotrophs (Ty21a)

Reassortant (Live Flu, Rotavirus)

Inactivated Vaccines

Killed Whole Organisms (Typhoid, Cholera, Hepatitis A Whole-cell Pertussis, IPV, JEV Rabies)

Toxoids (Diphtheria, Tetanus)

Extracts (Flu, Anthrax)

Polysaccharides (Vi, Pneumo)

Purified Proteins (Acellular Pertussis)

Conj. Polysaccharides (Hib, pneumo, mening C)

Gene-produced proteins (Hep B, HPV)

Vaccination Has Successfully Exploited Immune Functions

Past

18-19th Century – Non-specific

20th Century – Antibodies
Cellular Immunity in general

Future

21st Century – Innate immune system
Specific lymphocyte subsets
CD4, CD8, Tregs

New Strategies for Vaccine Discovery

Attenuated vaccines:

- Reverse genetics, temperature-sensitive mutations,
  and reassortment
- Viral recombinants and deletion mutants
- Codon de-optimization
- Control of replication fidelity
- MicroRNA insertion
- Replication vectors that contain genes from pathogens

Plotkin S, Plotkin S, *Nature Rev Micro*, 2011

New Strategies for Vaccine Discovery

Inactivated vaccines:

- DNA plasmids and DNA shuffling
- mRNA and self-amplifying RNA
- Reverse vaccinology
- Antigen identification by transcriptomics and proteom
- Development of fusion proteins
- Development of new adjuvants (including cytokines)
- Induction of innate immunity

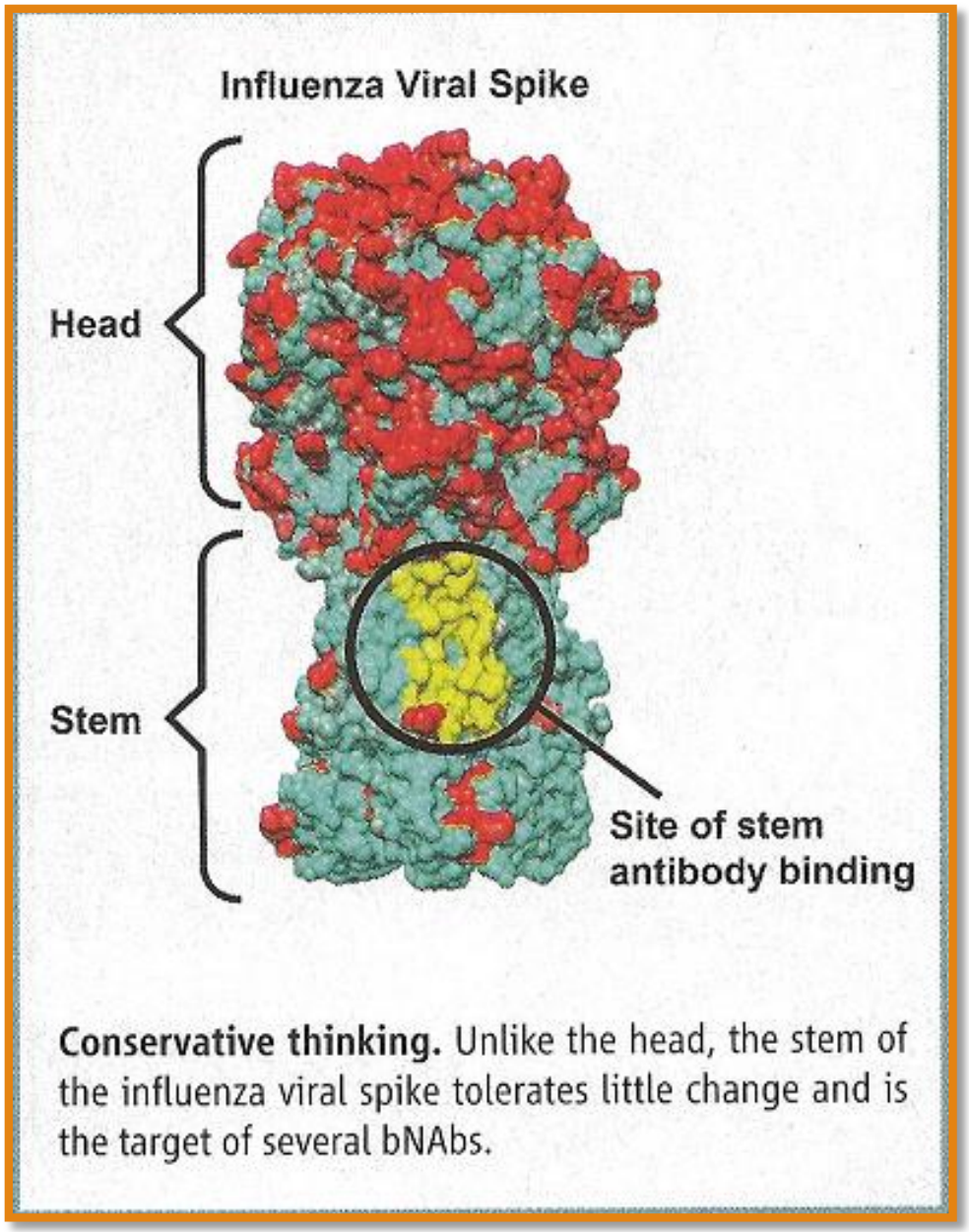
Most Important Discoveries in Last Ten Years

RNA Vaccines
Importance of innate immunity
Vaccine control of rotavirus diarrhea
Importance of norovirus diarrhea
Success of therapeutic HPV vaccines
Monoclonal antibodies with long half-lives
Correlates of protection for vaccine licensure
Adenovirus and poxvirus vectors
Importance of T cell responses to complement antibody
Importance of non-neutralizing antibodies

Influenza

Ways to Improve Efficacy of Influenza Vaccines

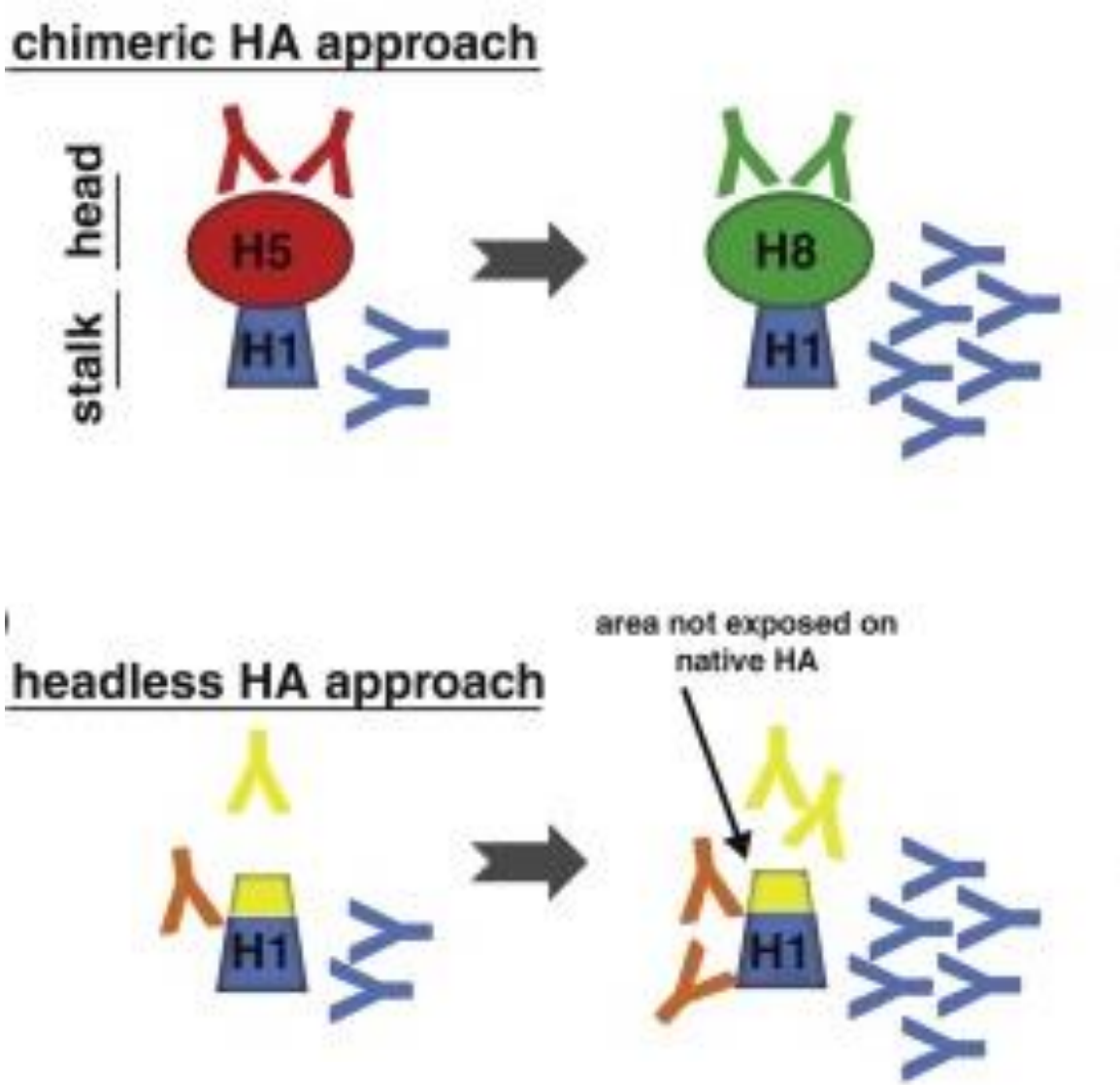
- Add second lineage of type B (done)
- High hemagglutin dose (done)
- Adjuvants such as MF-59 or AS01 or flagellin
- Add neuraminidase
- Add conserved epitopes NP, M2e, stalk HA
- Computational optimization
- Conserved stem antigen



Conservative thinking. Unlike the head, the stem of the influenza viral spike tolerates little change and is the target of several bNAbs.

Cohen J. Science 341:1171, 2013

Stalk-based Approaches



or prime-boost, peptides, VLPs

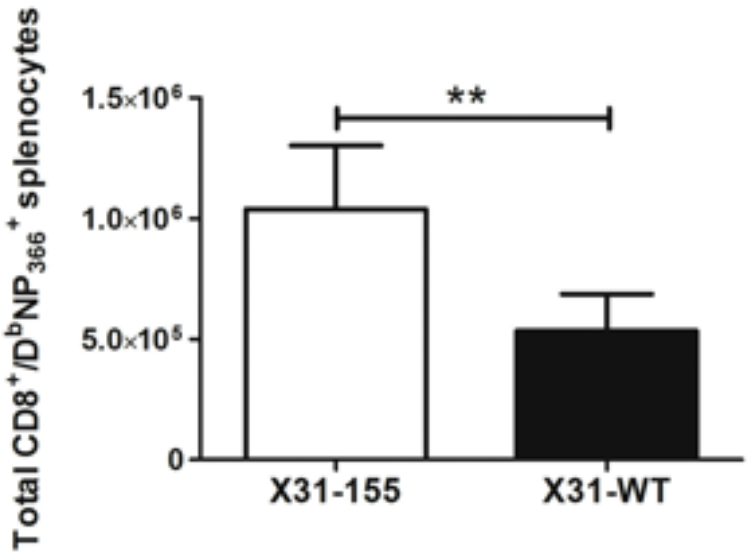
Micro RNA

microRNAs (miRNAs) are short, 19-22 bp, non-coding, single stranded RNA sequences that bind 3'-untranslated regions (UTRs) of target messenger RNAs (mRNAs) to regulate gene expression and downstream cellular functions

miR-155 is highly expressed in the thymus and bone marrow and is known to play a significant role in the regulation of CD4+ (Th1, Th2, and Th17), CD8, and T regulatory (tregs) cell function

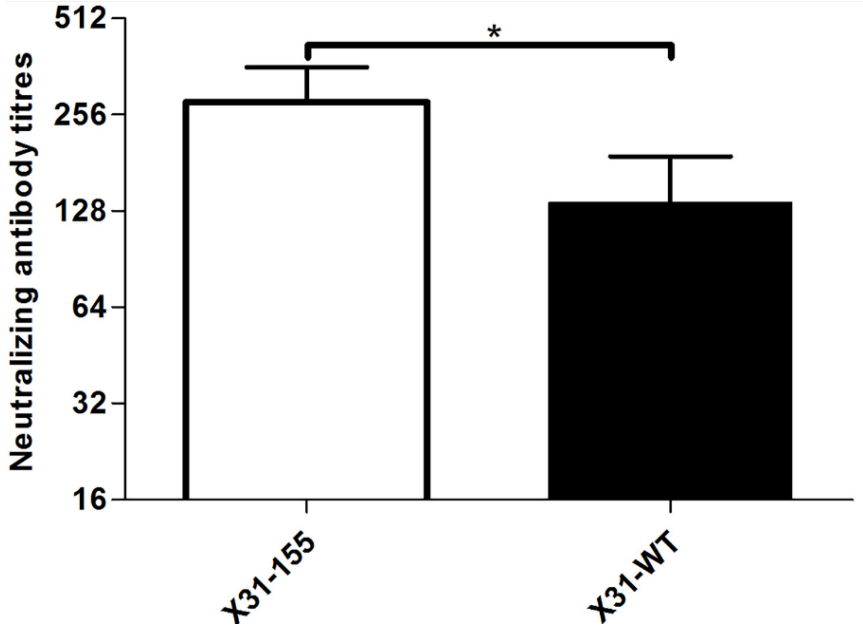
Enhancing Influenza Vaccine Immunogenicity with Micro RNA

Tetramer-specific CD8± T cell responses



+ Micro RNA

IAV-specific neutralizing antibody titres



+ Micro RNA

Izzard, et al *Virus Research* 2017

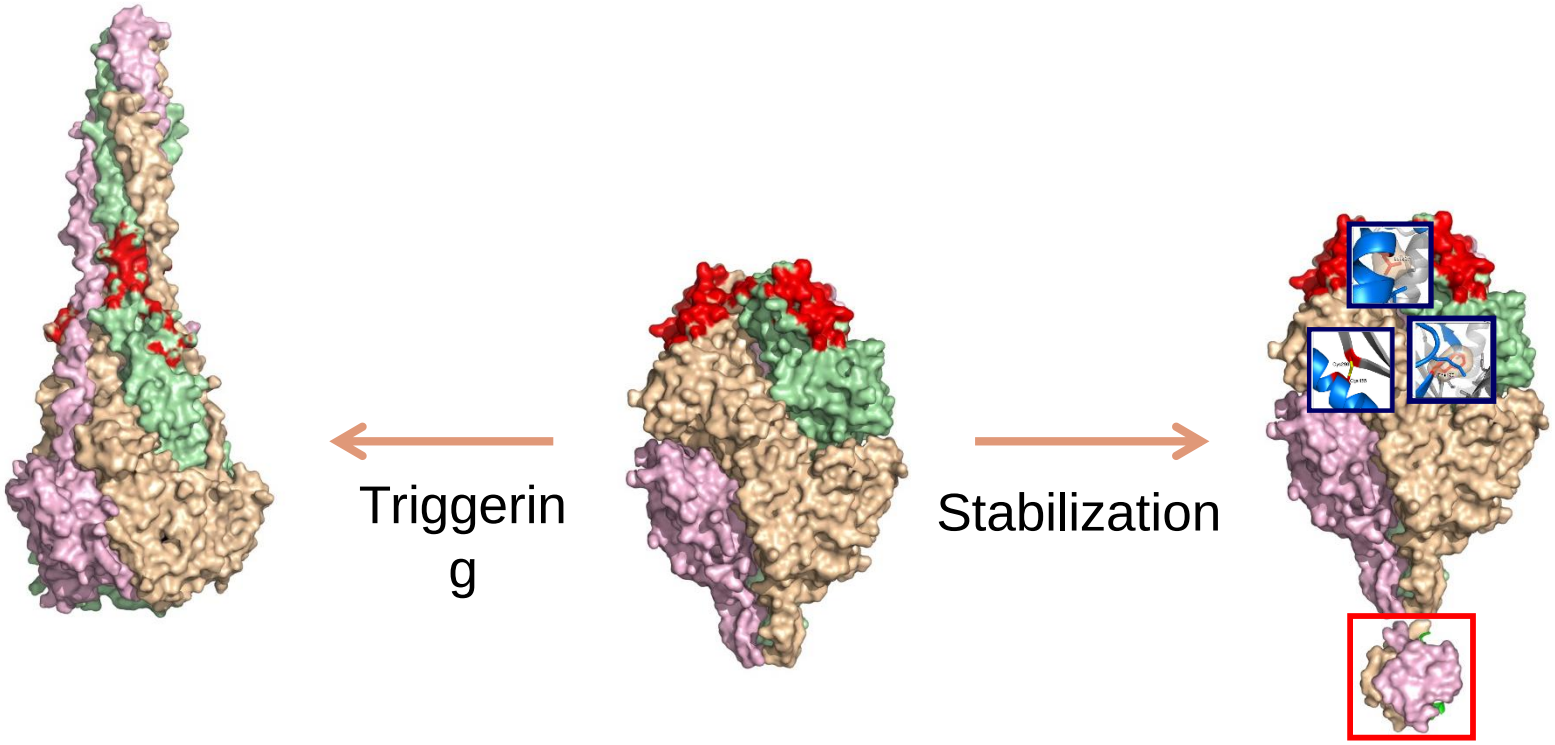
Value of Structural Biology:

RSV

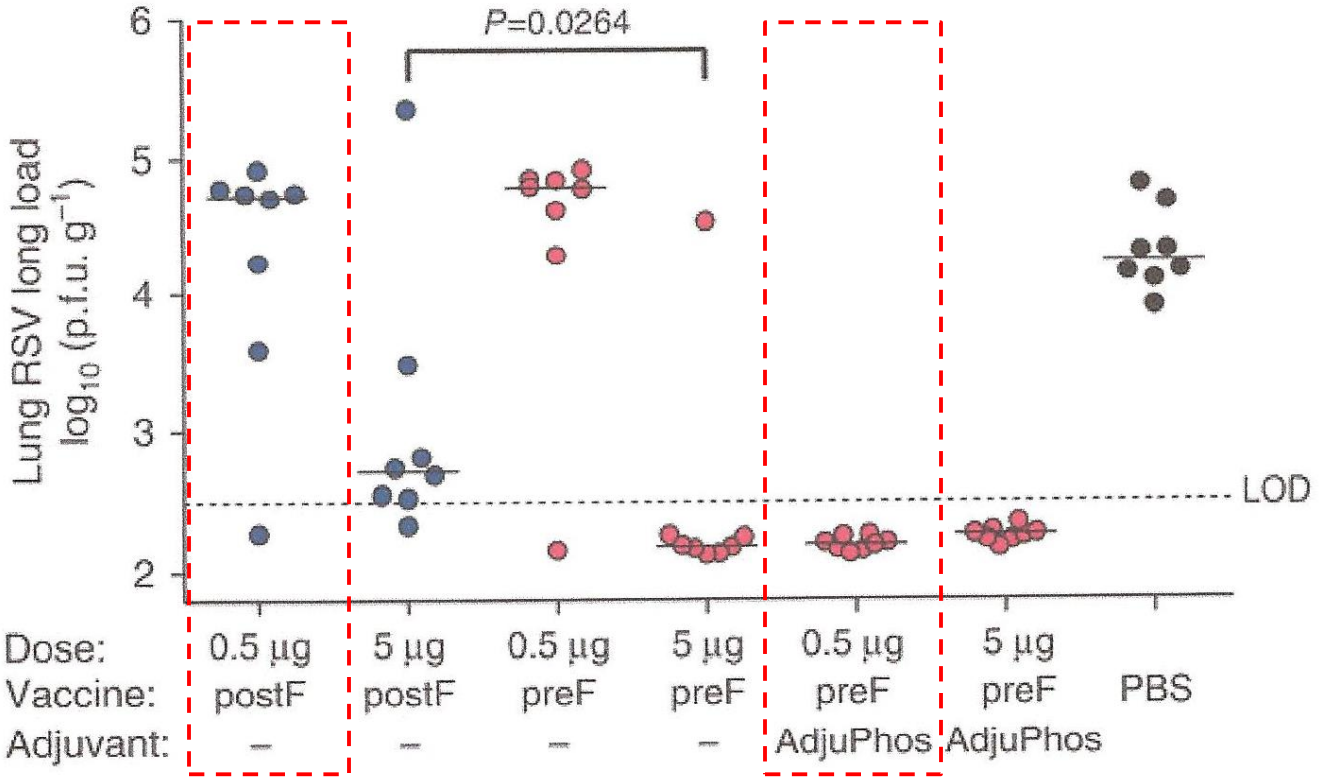
Respiratory Syncytial Virus

- Number one respiratory infection of infants (0-2 yrs)
- Also, important in elderly
- Prior inactivated vaccine worsened disease because Fusion antigen was altered, leading to formation of immune complexes
- ? Need for “just right” antibody and CD8+ T cell responses
- Live viruses insufficiently attenuated

Stabilizing Prefusion RSV F Results in a Candidate Vaccine



Pre-Fusion F vs. Port-Fusion F Against RSV Infection in Cotton Rats

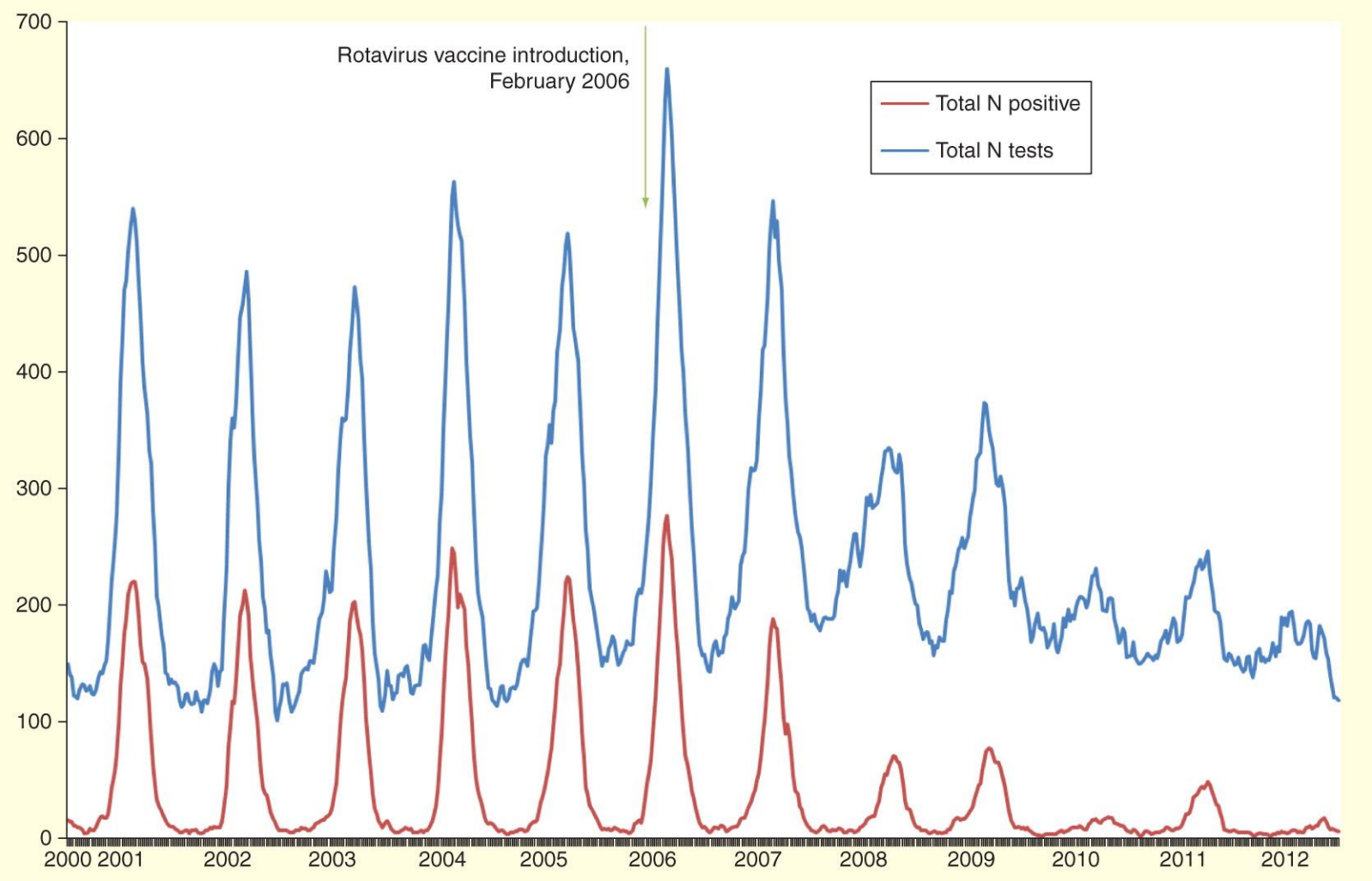


Female Cotton rats (*n*=8 per group) were immunized IM with 0.5 or 5 µg stabilized prefusion RSV F protein (SC-DM) or RSV F protein in postfusion conformation in a prime—boost regimen at week 0 and week 4. SC-DM groups at the same protein doses was further combined with AdjuPhos. Serum was collected on day 49 and plaque reduction assay was performed using the lungs.

Population – Specific Challenges:

Rotavirus

Effect of RotaTeq on Rotavirus Disease in the U.S.



Rha B, et al. Exp. Rev. Vaccines 13(3):365-376 (2014)

Rotavirus Vaccine Efficacy Against Severe Disease in Tropical Countries

Vaccine	Country	Efficacy
RV1	Brazil	77%
	Malawi	49%
	South Africa	77%
RV5	Nicaragua	77%
	Kenya	83%
	Ghana	65%
	Viet Nam	73%
	Bangladesh	46%

Factors that May Be Decreasing Rotavirus Vaccine Efficacy in Poor Countries

Breast Milk Antibodies	✓
Maternal serum antibodies	✓
Simultaneous OPV	✓
Malnutrition	0
Intestinal dystrophy	✓✓
Microbiome Bacteroides ↓ Streptococci ↑	✓✓✓

Importance of the Microbiome to Oral Vaccination

- Prior infections change morphology of the intestinal mucosa
- The microbiome influences responses to rotavirus vaccines: Strep bovis good, bacteroides bad

Uncertain Correlates of Protection:

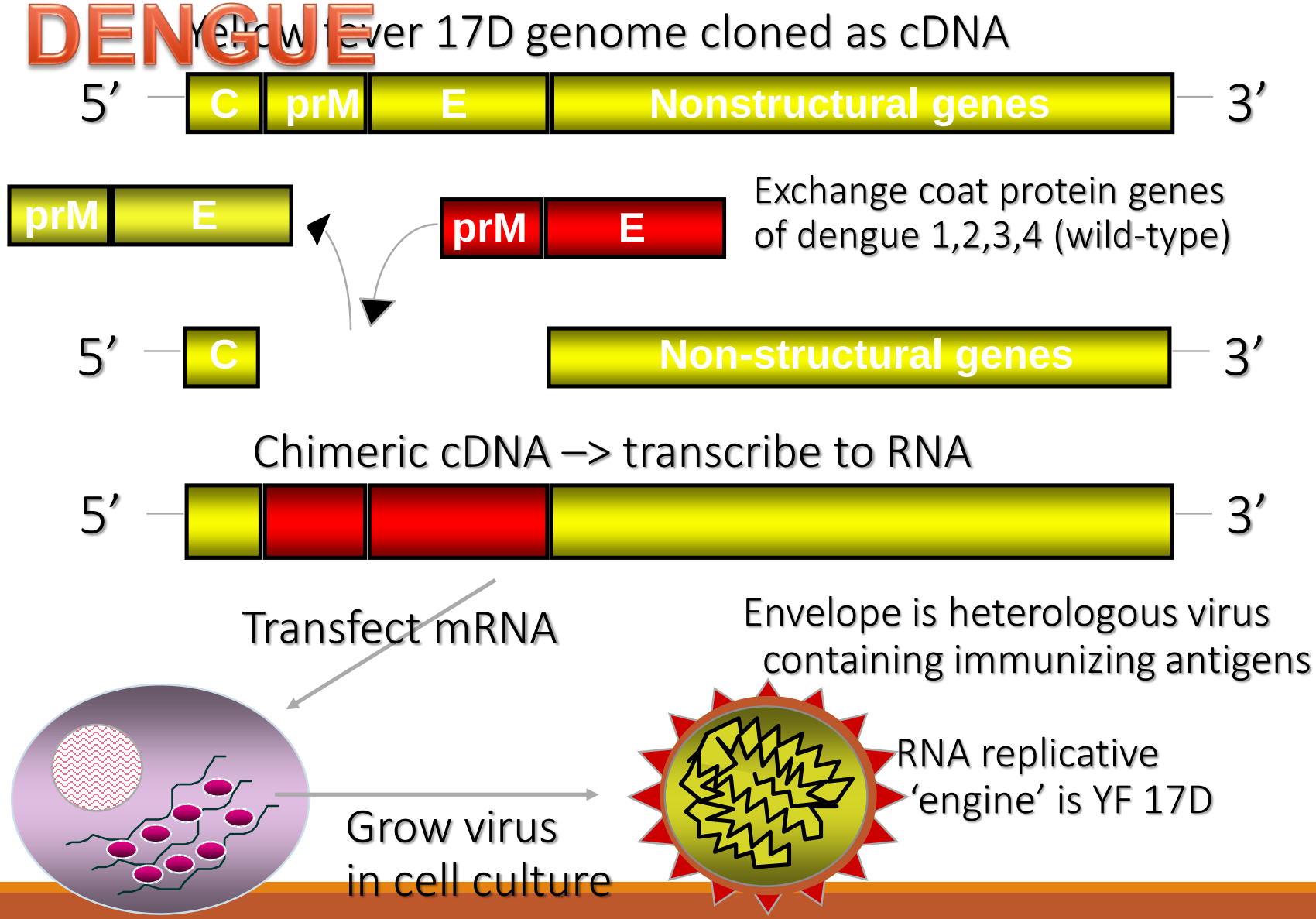
Dengue

Efficacy of Chimeric Dengue Vaccine in Thailand – Phase 2+3

Serotypes	Phase 2 Efficacy (C.I.)	Phase 3 Efficacy (C.I.)
All	35% (6.7-54)	57% (44-64)
1	61% (17-82)	50% (25-67)
2	3.5% (-60-41)	35% (-9-61)
3	82% (39-96)	78% (53-91)

Sabcharoen, Lancet 2012
Capeding, Lancet 2014

CHIMERIVAX™ TECHNOLOGY FOR DENGUE



Possible Explanations for Low Efficacy of Chimeric Dengue Type 2 Vaccine

- Higher challenge dose of type 2, or strain variation therefore more antibodies needed
- Dengue Type 2 infects monocytes rapidly and antibody thus not effective
- T cell response also needed
- Type 2 replicates poorly and antibodies were heterotypic, not homotypic
- Envelope protein in chimera has different conformation than in virus (de Alwis et al, J Virol)
- Structure of virus produced at 37°C different from virus injected by the mosquito

Rey et al, Nature 2013

The Right T Cell Responses

T Cell Stimulating Vaccines

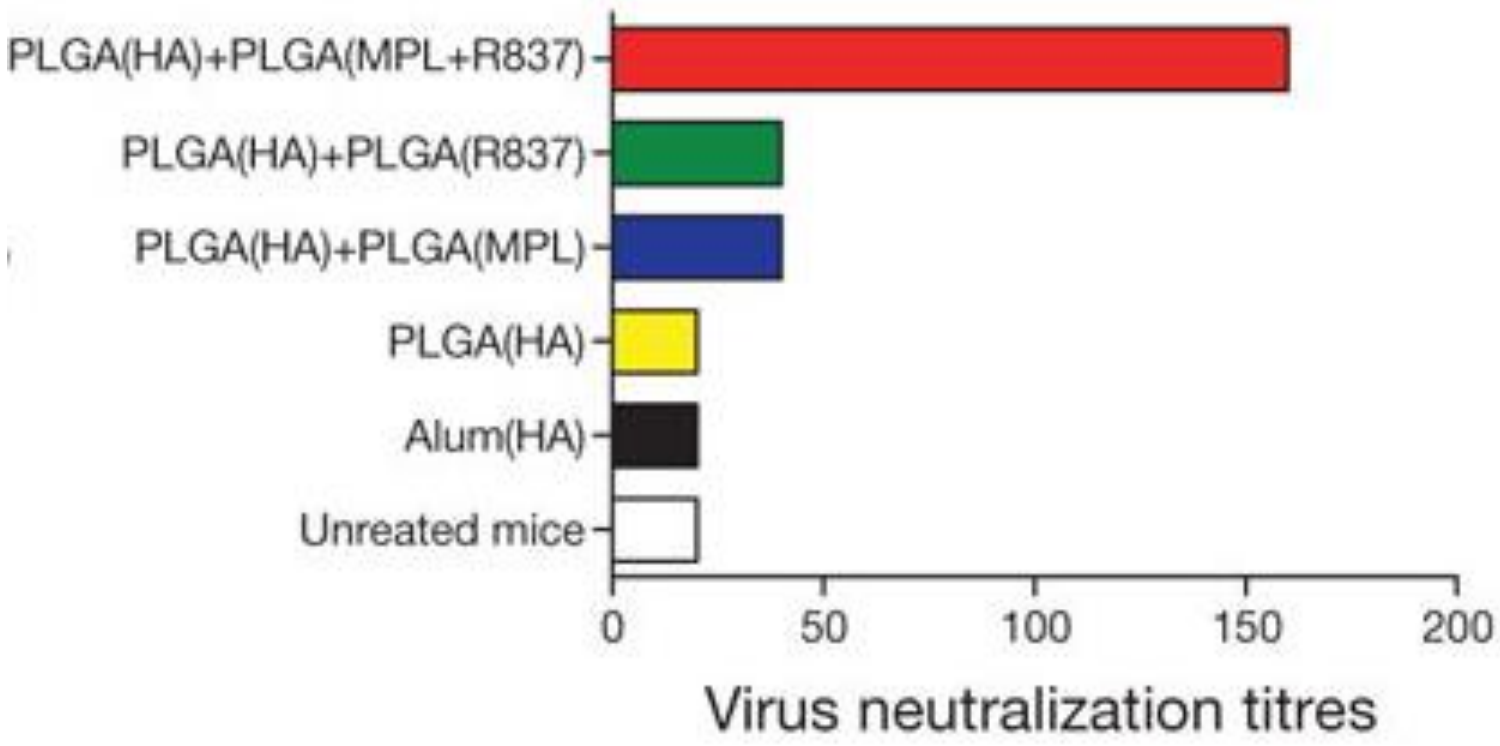
TB – Needed T cell response is polyfunctional and cytotoxic, such that it will kill infected macrophages

Malaria – Antibodies to circumsporozoite protein important, but T cell response needed to kill infected cells in the liver. May need other antigens

The Need for New Adjuvants

Adjuvants Natural Ligand	Pathogen Recognition Receptor (PRR)
Lipoproteins and peptidoglycans	TLR2
Double-stranded viral RNA	TLR3
Lipopolysaccharide (LPS) from Gram negative bacteria Lipoteichoic acid from Gram positive bacteria	TLR4
Flagellin	TLR5
Mycoplasma lipopeptide	TLR2 and TLR6
Guanidine and uridine-rich single stranded RNA	TLR7 TLR7/TLR8
Unmethylated CpG motifs from bacterial DNA	TLR9
Baterial peptioglycans	NOD (nucleotide-binding oligomerization domain)

Combination of MPL and R837 in polymer lactic glycolic acid nanoparticles mediates synergistic enhancement of antibody responses against H5N1-influenza-derived HA.



SP Kasturi *et al. Nature* (2011)

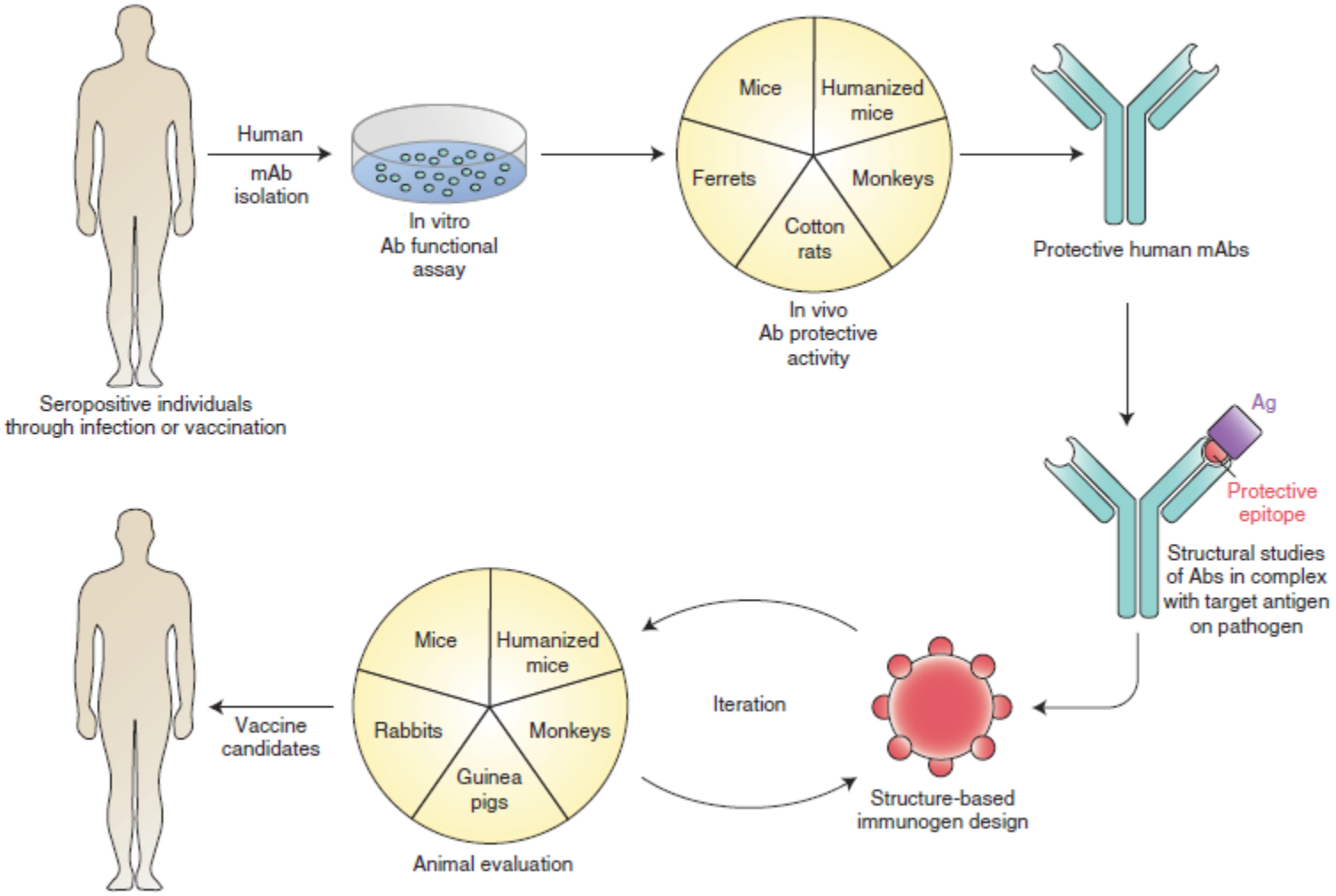
Omics

Genomic Approaches for Vaccine Discovery

Genomics	Identifies conserved protective antigens
Transcriptomics	Identifies proteins produced at different stages of pathogen life cycle
Proteomics	Identifies proteins produced under different conditions
Antigenomics	Identifies which antigens induce immune responses in the host
Structural Biology	Localized and presents key epitopes
Systems Biology	Identifies signatures of protection
Genetics	Variable host response
Cytokines/Chemokines Production	Predicts reactions

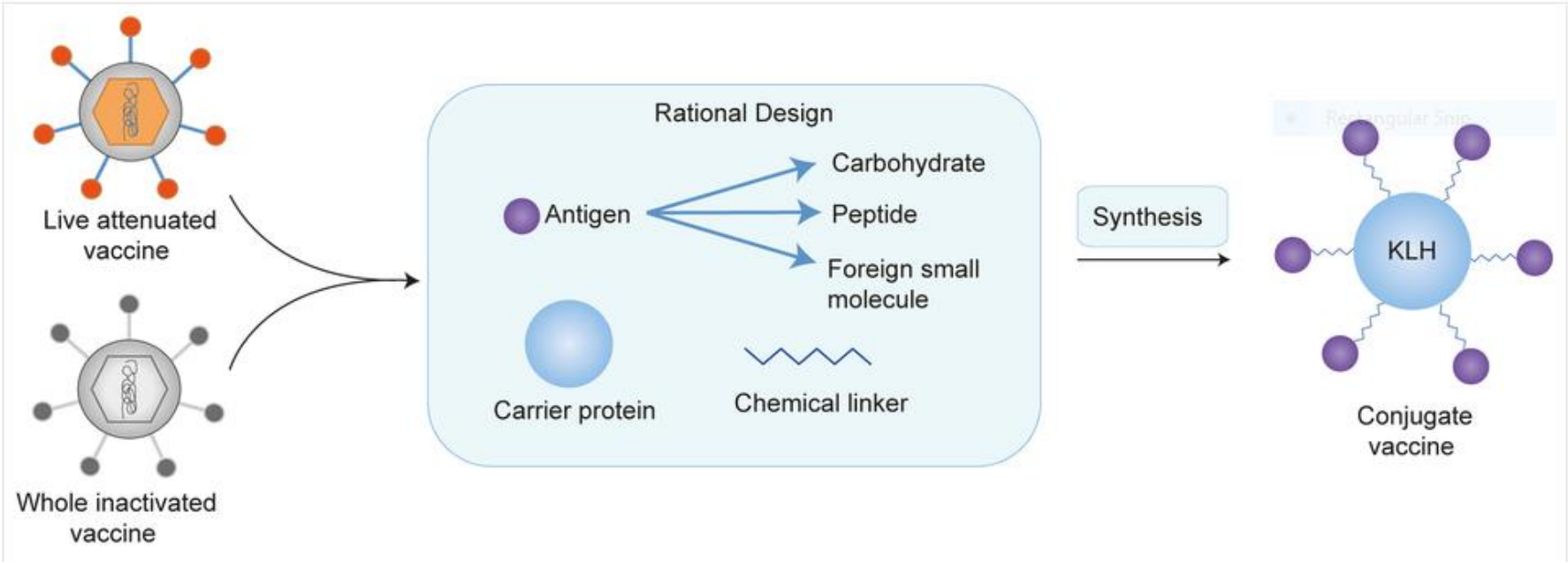
Prachie et al *Drug Dev* 73:547 2012

Reverse Vaccinology 2.0



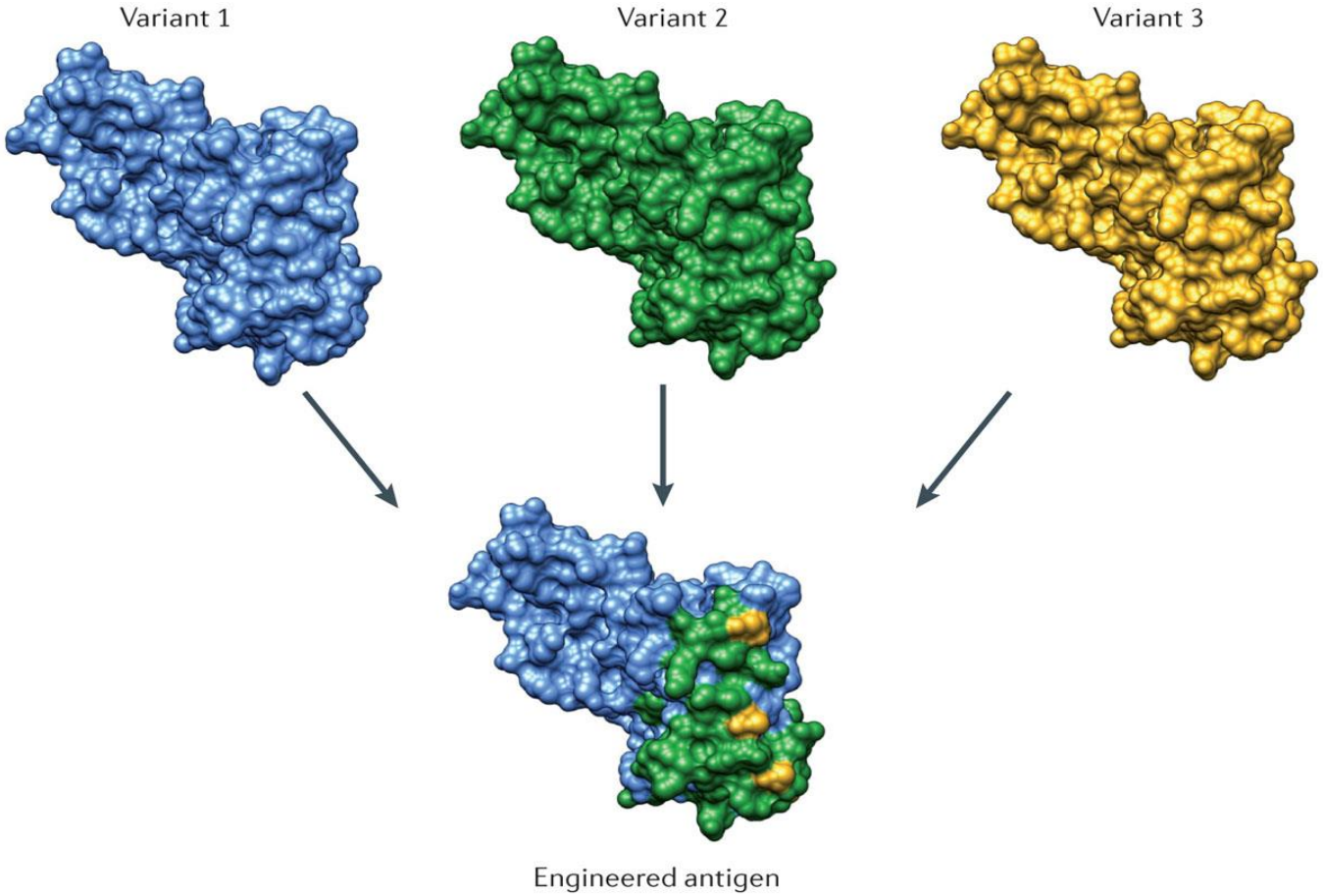
D. Burton *Cold Spring Harb Perspect Biol* 2017

Modular Approach to Design Synthetic Vaccines



Lyn Jones *Nature Chemistry* 2015

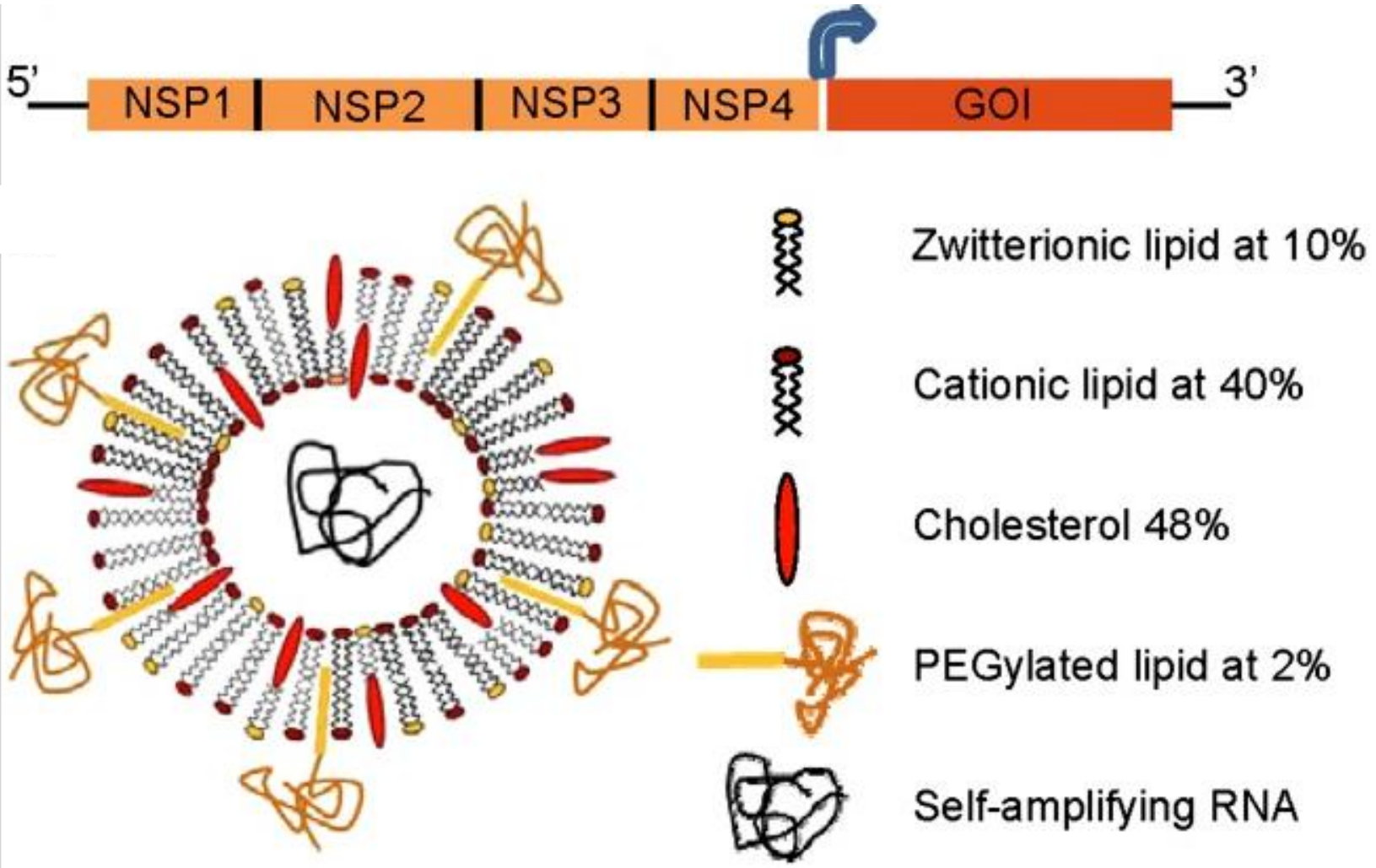
A Cross-protective Factor H-binding Protein To Protect Against Multiple Strains of Meningococci Group B



Dormitzer et al *Nature* 2012

DNA/RNA Vaccines

Self-Amplifying RNA



Geall et al *PNAS* 2012

The Big Problems in Vaccinology of the 21st Century

- Maintenance of immune memory, both central and effector
- Immaturity and Post-maturity of the Immune System
- Adjuvants capable of selectively stimulating cell types: Dendritic, B, Th1, Th2, CD4+, CD8+ or Tregs
- Conserved antigens to cope with antigenic variability
- Mucosal immunization with non-replicating antigens

Vaccine Responses and Age

Pre-vaccination inflammation and B-cell signalling predict age-related hyporesponse to hepatitis B vaccination

Slim Fourati[1,2], Razvan Cristescu[3], Andrey Loboda[3], Aarthi Talla[1,2], Ali Filali[1,2], Radha Railkar[3], Andrea K. Schaeffer[3], David Favre[4], Dominic Gagnon[4], Yoav Peretz[4], I-Ming Wang[3], Chan R. Beals[3], Danilo R. Casimiro[3], Leonidas N. Carayannopoulos[3] & Rafick-Pierre Sékaly[1,2]

B cell signaling → ↑ response

Inflammation → ↓ response

Nature Communications 2016

“We consider an international vaccine-development fund to be urgently needed to provide the resources and the momentum to carry vaccines from their conception in academic and government laboratories and small biotechnology firms to development and licensure by industry.

This support would permit efficacy assessment to begin – and thereby avert a repetition of the Ebola crisis.”

Plotkin et al *NEJM* 2015

Challenges

1

- The pipeline is weak for most EIDs characterized by market failure

2

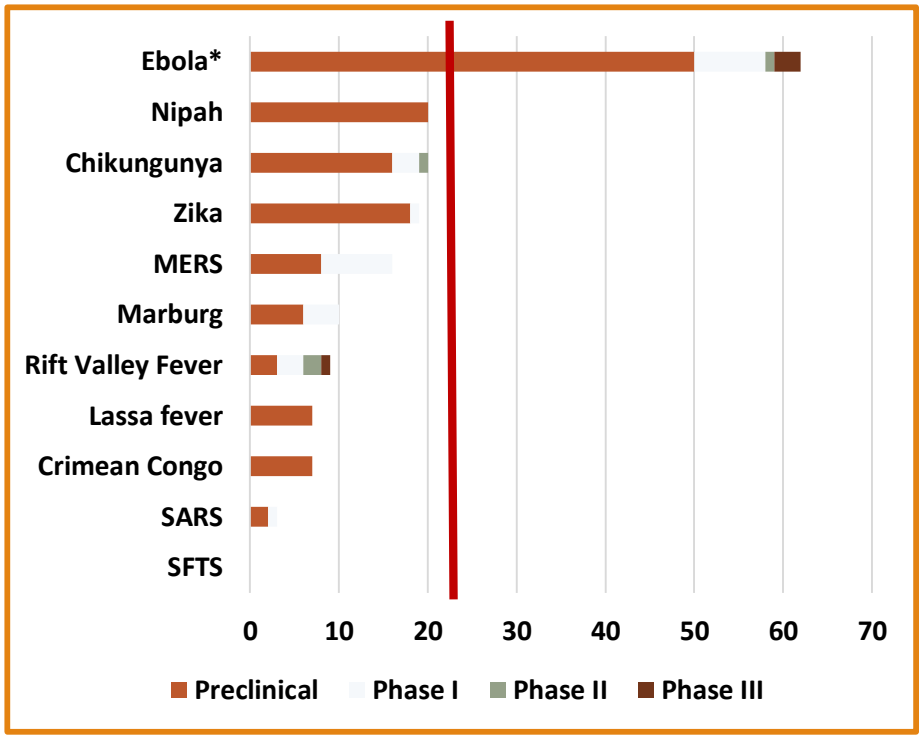
- Unilateral, uncoordinated government efforts to fund R&D preparedness are inefficient and unsustainable in addressing global epidemic risks

3

- Clinical & regulatory pathways are not easily adaptable to epidemic contexts

4

- Incentives are lacking to motivate greater industry engagement



Stages of Development

Immunogenicity and safety in mice

Protection in relevant animal challenge model

GMP production, validation of methods – CEPI

Toxicity studies

Phase I

Phase IIa

Phase IIb – if possible

Stockpile

Conditional approval for emergencies – CEPI

Phase III

Licensure

Most Important Criteria

Criteria	Comment to interpretation
Protection in a relevant animal model	Protection in mice also scored but rated lower than protection in models closer to humans.
Evidence for a correlate of protection	Preferably inferred from data in humans, but also counted if inferred from animal or natural history data.
A viable platform for vaccine manufacture exists	Preferably more than one. If the proposed vaccine was accomplished by an important technological advance in vaccinology that could be applied to other vaccines its score was increased.

CEPI Subgroup Recommendations

Immediate Funding

Chikungunya
Coronaviruses (MERS and SARS)
Filoviruses (Ebola strains and Marburg)
Lassa
Nipah

Later Funding

Lassa
Nipa
Paratyphoid A
Plague
Crimean-Congo Hemorrhagic Fever
Severe Fever with Thrombocytopenia
Zika (but moving fast)

CEPI process to date

CEPI startup phase: June 2016 – July 2017

- Adopted interim entity, CEO and secretariat
- Finalized strategic plan
- Finalized interim governance arrangements, including selection of BoD and SAC members
- Drafted CEPI preliminary business plan for first five years of operation (subject to revision)
- Securing initial commitments and contributions for CEPI launch
- Davos, January 2017
- G7 Summit, May 2017
- G20 Summit, July 2017

October 2017

- **Funding awarded for Lassa, Nipah, MERS**
- **Proposals for platforms requested**
- **Chikungunya meeting in February 2018**