

Module 8
Evaluating Immunological
Correlates of Protection

Session 1
Introduction to Vaccines
and Basic Concepts

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The Ten Greatest Public Health
Achievements of the 20th Century

- Vaccination
- Motor-vehicle safety
- Safer workplaces
- Control of infectious diseases
- Decline in deaths from coronary heart disease and stroke
- Safer and healthier foods
- Healthier mothers and babies
- Family planning
- Fluoridation of drinking water
- Recognition of tobacco use as a health hazard

MMWR (1999);48:1141

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What Are Vaccines?

- Biological products
- Typically for prophylaxis, not treatment
- Use antigen or attenuated live virus to trigger immune responses for disease protection
- Administered as a single dose or series with a potential booster dose
- Highly complex immunologic milieu
 - Array of humoral and cellular immune responses

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Examples of Vaccines

- Pediatric vaccines
 - Polio
 - Measles, mumps, rubella (MMR)
 - Chickenpox (Varivax®)
 - Hepatitis B
 - Diphtheria, tetanus, pertussis
 - Rotavirus (infant gastroenteritis, RotaTeq®)
 - Invasive pneumococcal disease (Prevnar®)
- Adolescents and Adult vaccines
 - HPV (cervical cancer, Gardasil®)
 - Meningitis (Menactra®)
 - Influenza
 - Invasive pneumococcal disease
 - Herpes zoster (shingles, Zostavax®)

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Benefits of Vaccines

■ Direct benefit

- Efficacy in clinical trials
- Risk benefit at individual level

■ Indirect benefit

- Herd immunity by reducing exposure and transmission
- Public health implications

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Reduction in Human Papillomavirus (HPV) Prevalence Among Young Women Following HPV Vaccine Introduction in the United States, National Health and Nutrition Examination Surveys, 2003–2010

Lauri E. Markowitz, Susan Hariri, Carol Lin, Eileen F. Dunne, Martin Steinau, Geraldine McQuillan, and Elizabeth R. Unger

Journal of Infectious Diseases, 2013

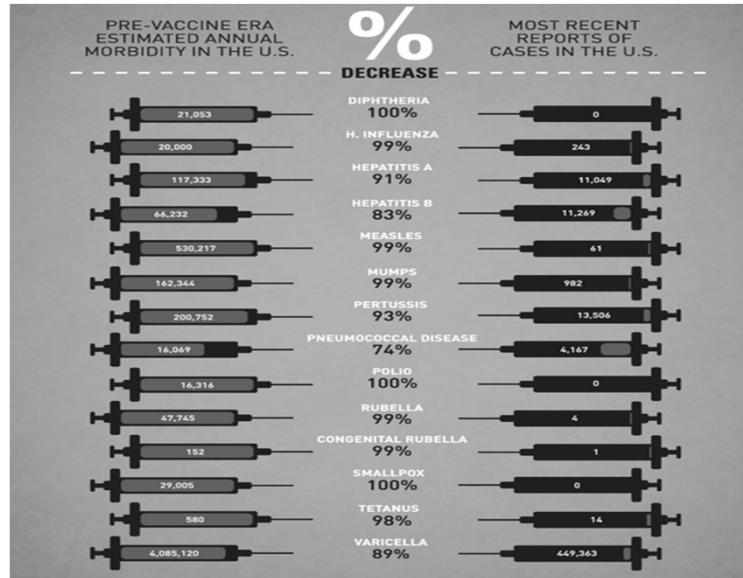
- Vaccine coverage ~34%
- Reduction in overall prevalence ~56%
- Vaccine effectiveness ~82%



Substantial protection from herd immunity (~ 43%)

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Impact of Vaccines in the 20/21th Century



Data by CDC; Graph by Leon Farrant

Human Immune System

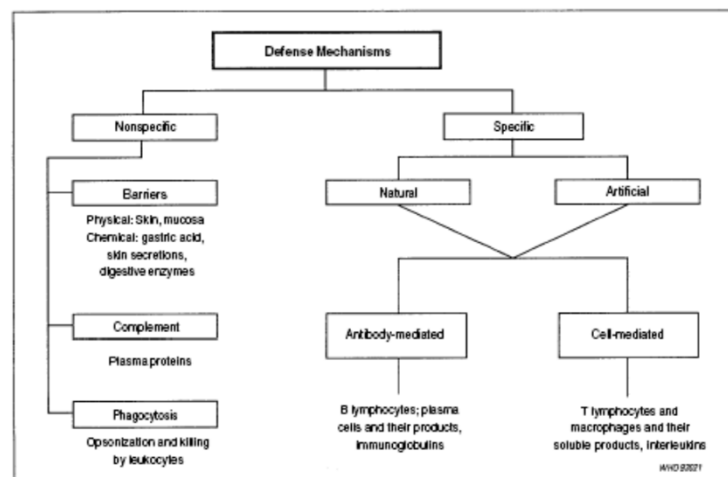
Types of Immunity

- Humoral (antibody-mediated) immunity
 - B lymphocytes,
 - Plasma cells
 - Immunoglobulins (Ig)
 - IgG, IgM, IgA, IgD and IgE

- Cell-mediated immunity (CMI)
 - T lymphocytes
 - Cytokine/Interleukins

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Defense Mechanisms



WHO Immunological Basis for Immunization Series, Module 1, General Immunology.

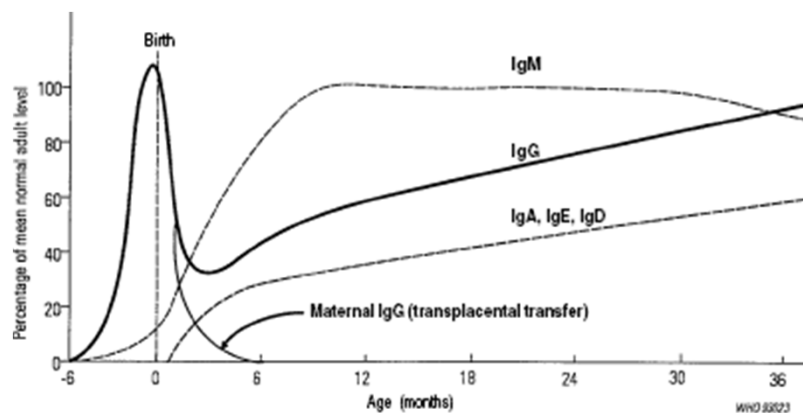
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Functions of Immunoglobulins

- Serve as antibodies
- Neutralize viruses and bacterial toxins
 - IgG accounts for ~80% of total immunoglobulin pool
- Bind antigen
- Prevent or clear first infection

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Normal Development of Serum Immunoglobulin Levels



WHO Immunological Basis for Immunization Series, Module 1, General Immunology.

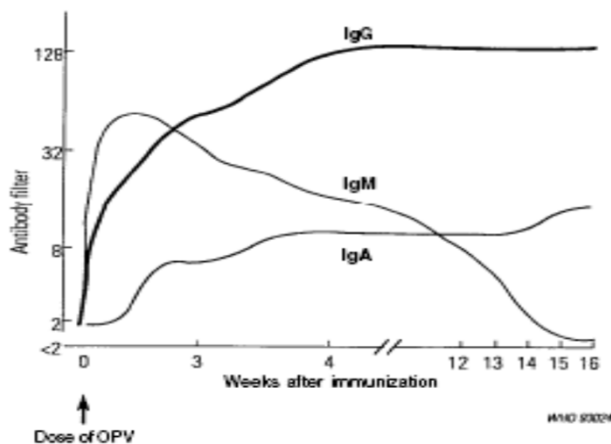
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Antibody Responses to Vaccination

- Antibody increases steeply to a plateau and then decline
- Primary responses may have a longer lag phase and reach a lower plateau than booster responses

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Temporal Antibody Responses Following Primary Immunization



WHO Immunological Basis for Immunization Series, Module 1, General Immunology.

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Functions of T-cells (CMI)

- T lymphocytes (helper cells) stimulate B cells to produce antibodies
- T suppressor (regulatory) cells play an inhibitory role and control the level and quality of the immune response (CD4)
- Cytotoxic T-cells recognize and destroy infected cells (CD8)

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Measurements of Antibody Activity

- Serum antibody can be measured by different serological assays
- Presence of antibody indicates previous encounter with microorganism
 - Via natural infection or immunization
- Level of antibody does not reflect the total immunity

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Serological Assays for Antiviral Antibodies

- Neutralization test on tissue culture
 - Most important property of antibody to neutralize virus
 - Expensive and time-consuming
- Hemagglutination inhibition (HI) test
- Enzyme-linked immunosorbent assay (ELISA)

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Serological Assays for Antibacterial Antibodies

- *In vivo* neutralization tests
 - Sensitive
 - Show the functional capacity of antibody (neutralization of toxin)
 - Laborious, expensive, need large amount of serum
- *In vitro* tests
 - Hemagglutination (HA) test
 - ELISA
 - Simple, rapid, inexpensive, but less specific than *In vivo* neutralization tests

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CMI Assays

- Use peripheral blood mononuclear cells (PBMCs)
- Labor intensive, large biological variability

Examples:

- Stimulation index (SI)
- Enzyme-linked immunospot assay (ELISPOT)
- Responder cell frequency (RCF)
- Flow cytometry

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Assay Validation

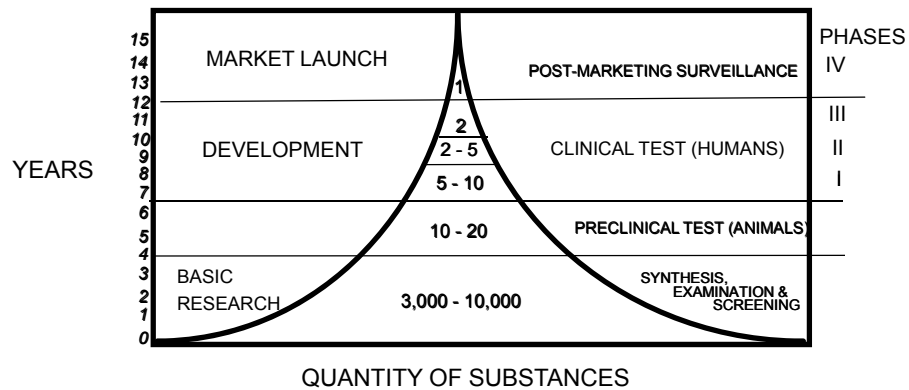
- Precision and reproducibility
- Robustness
 - Intra-assay factors
 - pH, temperature, cell passage level
- Ruggedness
 - Inter-assay factors
 - Operator, laboratory, component source
- Relative accuracy/linearity
 - Parallelism/“Dilution effect”
 - Range in robustness parameters

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Brief Overview of Clinical Development Process

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Discovery and Development of a Successful Drug/Vaccine



Source: Based on PhRMA analysis, updated for data per Tufts Center for the Study of Drug Development (CSDD) database.

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Need for Clinical Trials

- To evaluate the safety and efficacy of new drugs and vaccines in humans
 - After successful preclinical studies
 - Before the product is approved for broad use
- Clinical Trial:
“...a prospective study comparing the effect and value of intervention(s) against a control in human subjects”
 - Friedman, Furberg and DeMets, 1996
- Gold standard for comparison

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Phases of Clinical Trials

- Phase I
 - Healthy subjects
 - PK/PD of drugs
 - Modeling and simulations
 - Dose ranging for safety and immunogenicity of vaccines
 - Biomarker/assay development

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Phases of Clinical Trials

■ Phase II

- Target population
- Dose ranging and dose selection for safety and efficacy (or immunogenicity for vaccines)
 - Minimum effective dose
 - Optimal dose
- Proof of concept (POC) study of efficacy
- Hypothesis generating

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Phases of Clinical Trials

■ Phase III

- Confirmatory trial of efficacy and safety
- Demonstration of consistency of the manufacturing process for vaccines
- Large scale in size
- Last stage before submission for licensure

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Phases of Clinical Trials

■ Phase IV

- Post-marketing studies to collect additional data on safety, efficacy or immunogenicity
- Supports marketing or regulatory commitments
- Expansion to different populations

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Evaluation of New Vaccines - Safety

- Assess local (injection-site) and systemic adverse experiences
- Need a large database, particularly because of giving vaccines to healthy subjects
- Choice of safety parameters depend on type of disease, population, and route of administration
- Need large-scale post licensure study for additional safety monitoring

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Evaluation of New Vaccines - Efficacy

- Measure the relative reduction (RR) of disease incidence after vaccination compared with placebos

$$VE = 1 - RR = 1 - P_V/P_C$$

- Require a high level of evidence and precision
 - Success typically requires showing efficacy greater than a non-zero (e.g. 20% - 50%) lower bound
 - May need a very large study
- Need long-term data to assess duration of efficacy
 - Historical controls may be used if concurrent controls are not available
 - When is a booster dose needed?

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Impact of VE Lower Bound Requirement on Sample Size

- Rapid increase of sample size when VE lower bound increases
- Example assumes
 - 5/1000 incidence
 - 90% power
 - 60% true VE
 - One-sided 2.5% test
 - 1:1 randomization

VE Lower Bound	Total Sample Size
0	16,300
.10	20,800
.20	28,500
.30	43,900

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Efficacy Trial Considerations

- Target population
- Disease case definition
 - Specificity and sensitivity
- Study design
 - Placebo controlled
 - Fixed-duration or Fixed-endpoint
- Endpoints
 - Binary
 - Time to event
 - Continuous
 - Composite endpoints
- Success criteria: non-zero lower bound
- Length of follow-up for assessing durability

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Evaluation of New Vaccines - Immunogenicity

- Important in understanding the biology
- Humoral immunity
 - Antibody responses
 - priming, first defense
- Cell-mediated immunity
 - T-cell responses
 - prevent virus reactivation, kill infected cells

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Variability/Stability of Vaccines

- Vaccines are biological products that have more variability in than chemical compound
 - Need to demonstrate consistency of manufacturing
- Many vaccines contains attenuated live viruses and will lose potency over time
 - E.g., chickenpox vaccine, zoster vaccine
- Need to establish a range of potency for manufacturing and product shelf-life
 - Study the safety at the high potency
 - Establish efficacy at near-expiry potencies

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Regulatory Review

- US Food and Drug Administration (FDA)
 - Vaccines are reviewed by Center for Biologics Evaluation and Research (CBER)
 - Investigational New Drug Application (IND)
 - Biologic License Application (BLA)
 - Vaccines and Related Biological Products Advisory Committee (VRBPAC)
- European Medicines Agency (EMA)

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Interaction with Center for Disease Control and Prevention (CDC)

- Advisory Committee on Immunization Practices (ACIP) makes recommendation about immunization policy
- Sponsors share clinical data with CDC and ACIP and provide assistance in evaluating cost-effectiveness of vaccines

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An Example: Clinical Development of

ZOSTAVAX®

A live-virus vaccine to prevent
herpes zoster (shingles)

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Herpes Zoster Is a Consequence of Varicella-Zoster Virus (VZV) Reactivation

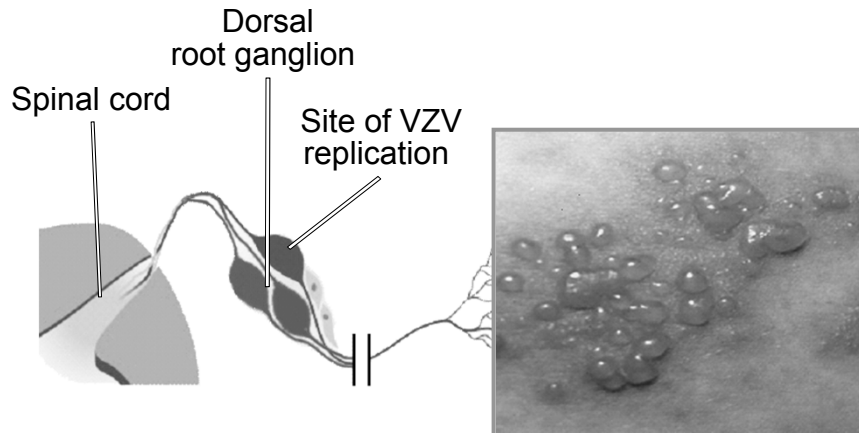


Image courtesy of Courtesy of JW Gnann.

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Ophthalmic Zoster



Courtesy of MN Oxman UCSD/San Diego VAMC.

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Phase I Study for Dose Ranging

- Assess the immune responses of 8 dose levels
 - Potencies = 0 (placebo), 2000, 8000, 17000, 19000, 34000, and 67000 PFUs
 - Evaluate both antibody and T-cell responses
 - N ~40 per group
- Results suggested potencies above 17000 PFUs elicit immune responses
 - Some plateau between 34,000 and 67,000 PFUs
 - No safety concern

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Phase II Study for Dose Selection

- Assess the immune responses of 2 dose levels
 - Potencies = 0 (placebo), 34000, and 50000 PFUs
 - Evaluate T-cell responses
 - N =398 total (1:3:3 ratio)
- Results showed similar immune responses of two selected potencies
 - 1.9 fold higher than placebo ($p < 0.001$)
 - Confirmed the plateau observed in phase I study

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Phase III Study for Efficacy and Safety: The Shingles Prevention Study (SPS)

(Oxman *et al.*, NEJM 2005)

- N = 38,546 subjects ≥60 years of age randomized 1:1 to receive ZOSTAVAX® or placebo
- Single dose of vaccine with potency ranging from 18,700 to 60,000 PFU (median 24,600 PFU)
 - To bracket end-expiry potency
- Average of 3.1 years of HZ surveillance and ≥6-month follow-up of HZ pain after HZ rash onset
- Conducted by Dept. of Veteran Affairs (VA) in collaboration with the National Institutes of Health (NIH) and Merck & Co., Inc.

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Immunogenicity Substudy

- 1395 subjects at 2 study sites
 - Both efficacy and immunogenicity measures collected
- Antibody responses by glycoprotein enzyme-linked immunosorbent assay (gpELISA)
- Cell-mediated immune responses by
 - IFN- γ enzyme-linked immunospot assay (ELISPOT)
 - Responder cell frequency (RCF)

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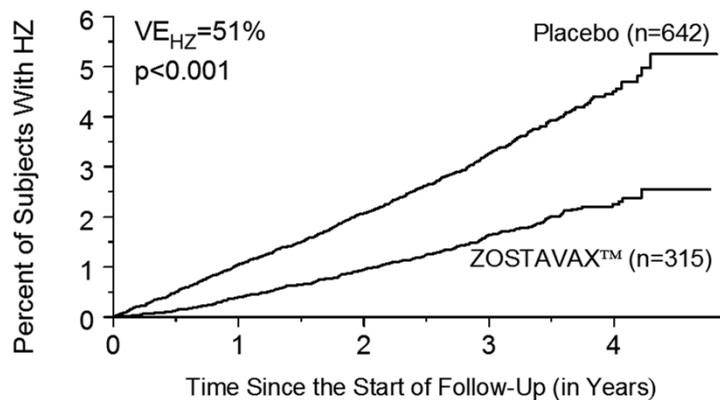
Key Efficacy Endpoints of SPS

- HZ incidence
- HZ pain burden of illness (BOI)
 - Composite of incidence, severity, and duration of pain
- Postherpetic neuralgia (PHN)
 - Clinically significant pain persisting for or present after 90 days of HZ rash onset
- Success requires 95% CI lower bound for vaccine efficacy >25%

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ZOSTAVAX[®] Efficacy: HZ Incidence

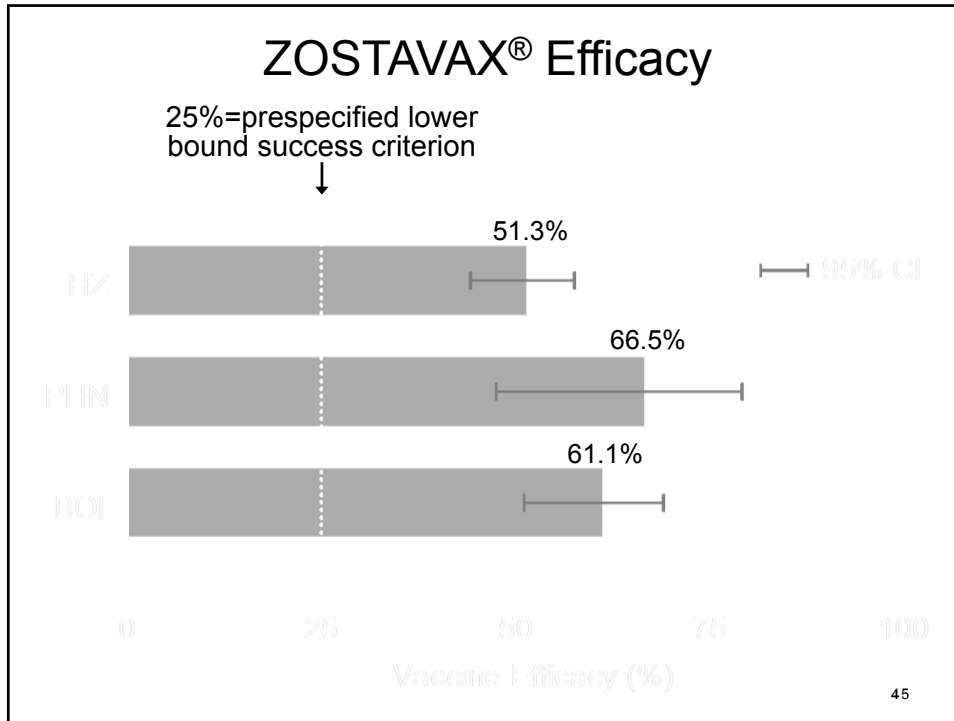
Estimate of the Cumulative Incidence of HZ Over Time by Vaccination Group



Number of subjects at risk

ZOSTAVAX™	19254	18994	18626	9942	1906
Placebo	19247	18915	18422	9806	1856

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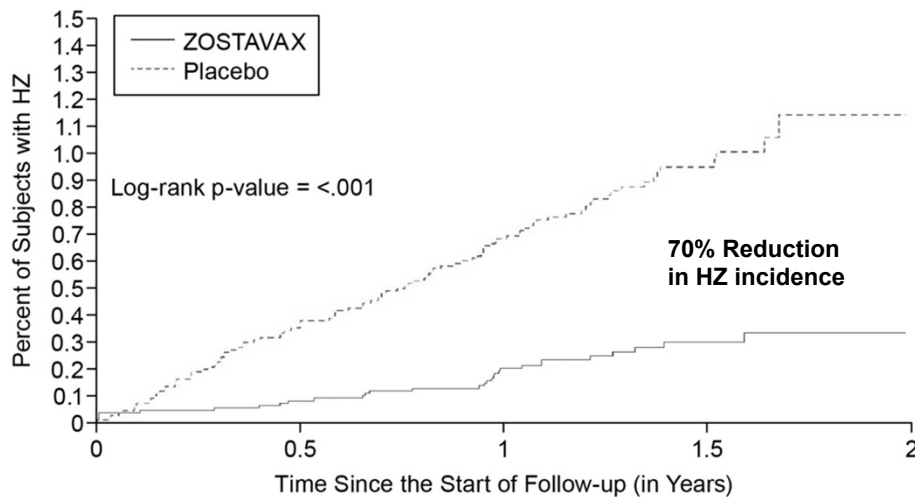
- ### Phase IV/Market Expansion Studies After ZOSTAVAX® Approval in 2006
- Bridging between frozen and refrigerated formulation of vaccines
 - Allow vaccine to be distributed in markets without freezer capacity in physician's office
 - Concomitant use with flu vaccine
 - Desirable for elderly population
 - High risk populations (HIV, immunocompromised adults)
 - An efficacy trial (phase III) in 50-59 year olds
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ZOSTAVAX® Protocol 022 Efficacy Trial in 50-59 Year Olds

- N = 22,439 subjects 50-59 years of age randomized 1:1 to receive ZOSTAVAX® or placebo
- Case-cohort for immunogenicity measurement (Day 0 and Week 6)
 - A random sub-cohort (N=2,269)
 - All HZ cases (n=129)
 - VZV antibody responses by gpELISA
- Average of 1.3 years of HZ surveillance

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Efficacy of ZOSTAVAX® in Individuals 50 to 59 Years of Age



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General Approaches to Evaluate Correlates of Protection

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Assessing the Correlation Between Immune Markers and Vaccine Efficacy

- Performed in proof-of-concept or phase III trials
 - Identify immune markers that correlate with efficacy
 - Validation via biological, clinical, and statistical methods
- Goal is to increase efficiency of clinical development
- Correlates of protection useful for
 - Assessing consistency of vaccine manufacturing process
 - Bridging studies (e.g., new vs. old formulations)
 - Assessing combination vaccines or concomitant vaccination
 - Identifying better vaccine candidates
 - Guiding regulatory and policy decisions on immunization

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General Approaches for Evaluating Correlates of Protection

- Identification of protective level
 - Individual-based method
 - Population-based method
 - Titer-specific method
- Statistical modeling of relationship between immune responses and disease risk
- Prentice Criteria – classical method for surrogate endpoint validation
- Causal inference framework/Meta Analysis

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Assessing Correlates of Protection in Vaccine Studies

- In vaccine literature, correlates of protection often refer to a “protective level”
 - A level of antibody titer above which a subject is considered completely (100%) protected from disease
 - E.g.: Hepatitis B uses ≥ 10 mIU/mL of anti-HBs

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Identifying Protective Level

- Individual-based method
 - Determine protective level by looking at the antibody titers among “vaccine failures”
- Population-based method
 - Compare antibody distributions between protected group and susceptible group
- Titer-specific method
 - Model the relationship between antibody titer and disease risk using a step-function

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Approximate Correlate of Protection

- Define an “approximate protective level”
 - A level at which >95% of population are protected (Need to estimate vaccine efficacy)
- Can be used as an immune marker for bridging studies
 - An endpoint such as “% of subjects achieving antibody responses above the approximate protective level” is easily understood by clinicians

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Issues with Searching for Protective Level

- A clear-cut value may not exist, as disease protection is often influenced by other types of immune responses (e.g., T-cell)
 - e.g., some varicella breakthroughs occurred in subjects with high titers, although at a lower rate than in those with low titers
- Defining a “protective level” may not fully capture the strength of the correlate of protection
 - Higher antibody titers usually lead to better protection
 - Lower antibody titers may still provide some protection

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Modeling the Correlation

- Link the whole antibody titer distribution to disease protection using statistical models
 - Beyond the step-function model
 - Measure the strength of correlation
 - Allows adjustment for important covariates, such as age
 - Models can be used for prediction of efficacy

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A Classical Method For Evaluating Surrogate Endpoints - Prentice's criteria

Prentice (1989) established 4 criteria:

1. Show treatment effect on disease endpoint
2. Show treatment effect on surrogate endpoint (immune marker)
3. Show surrogate endpoint correlates with disease endpoint
4. Show that probability of disease is independent of treatment status, given the surrogate endpoint
 - full treatment effect captured by surrogate endpoint (Proportion of treatment effect explained)

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Potential Concerns About the Prentice Method and the Proportion of Treatment Effect Explained (PTE)

- Criterion 4 cannot be evaluated if
 - No or constant baseline responses (e.g., HIV, pediatric vaccines)
- Potential bias if other prognostic factors are not accounted for in the model
- PTE is not well bounded by (0, 1)
- PTE is imprecise with wide confidence interval

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Causal Inference and Meta Analysis

- Evaluate immune correlates using causal inference framework (Gilbert)
 - Three tiers of surrogate endpoint evaluation
 - Much more from Peter's lecture
- Meta analysis of multiple studies can strengthen the correlates
 - Across populations, vaccine formulations, etc

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Correlate of Protection (CoP)

(Plotkin and Gilbert, CID 2012)

- CoP is an immune marker statistically correlated with vaccine efficacy (predictive of vaccine efficacy)
 - CoP is *mechanistic* if immune response is a causal agent to protection
 - CoP is *non-mechanistic* if immune response predicts vaccine efficacy but is not a causal agent to protection

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