History and Risk Assessment of Vaccines: A Practical Perspective from an Immunologist and a Toxicologist

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Disclaimers

The opinions expressed in this slide presentation do not necessarily reflect those of Merck or GSK or Affiliates of either organization.

- Lisa Plitnick is an employee of Merck
- Alan Stokes is an employee of the GSK group of companies

Outline

- Overview of vaccines
- Current regulatory guidelines
- Considerations in the design of toxicity studies
- Routine studies
- Examples of nonclinical toxicity testing strategies
  - Typical prophylactic vaccine
  - Prophylactic vaccine with novel adjuvant
- Pop Quiz!

The Value of Vaccines

Only clean drinking water rivals vaccination in its ability to save lives[1]

2-3m[2]

deaths prevented every year by vaccination

$150bn[3]

the benefit of vaccines to low and middle-income countries over the next 10 years

750,000[2]

children saved from disability every year

x44[4]

is the estimated return on Investment of the cost of immunization

Sources: 1. WHO, UNICEF, World Bank. State of the world's vaccines and immunization, 3rd ed. Geneva, World Health Organization, 2009., p.12; 2. Ehreth J, "The global value of vaccination" in Vaccine 2003 Jan 30; 21 (7-8):596-600. 3. Stack et al, 'Estimated Economic Benefits During The 'Decade of Vaccines' Include Treatment Savings, Gains in Labor Productivity', Health Affairs, 30, 6 (2011): 1021-1028 4. Ozawa S. et al, "Return on investment from childhood immunisation in low- and Middle-Income countries, 2011-20", in Health Affairs, 35, 2 (2016): 199-207

A Brief History of Pathogens and Vaccines

Chinese evidence of vaccination against smallpox

Smallpox Epidemic In India

Whooping cough Epidemic In Paris

Typhoid UK

Yellow Fever US

Diphtheria

Measles

Smallpox Variolations Begin in the English world

First anti-vaccine movement

Rubella

Chickenpox

Polio

Tuberculosis

1st Aluminum adjuvant

Pneumococcal disease

Rabies

Anthrax

Spanish Influenza

Cholera

Hepatitis

Zika

Ebola

Varicella
Rotavirus
Influenza
Polio
Shingles
Hepatitis A
Hepatitis B
Measles
Mumps
Pneumonia

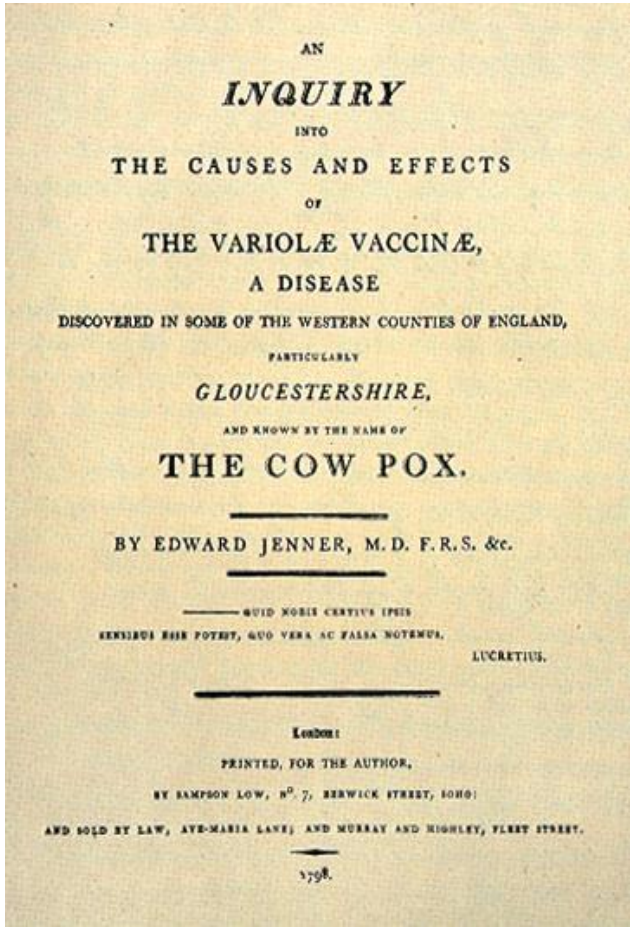
Salmonella typhi
Tetanus
Ebola
Yellow Fever
Neisseria meningitidis
Group B Streptococcus
Streptococcus pneumoniae
Haemophilus influenzae
Human Papillomavirus
Others

1000's **1500's** **1600's** **1700's** **1800's** **1900's** **2000's**

https://www.historyofvaccines.org/timeline/all

Smallpox and the Beginning of Vaccines

- In Europe, during the 1700s, smallpox caused an estimated 400,000 deaths each year (also a Smallpox epidemic in Boston in 1721)
- It had long been noted that those who survived smallpox were immune from again contracting the disease
- A Preventative Measure, Variolation (inoculation): Using the scabs or pustular material from persons with smallpox and administering, by one or another route, to healthy persons to induce "mild" smallpox disease with resulting immunity.



AN

INQUIRY

INTO

THE CAUSES AND EFFECTS

OF

THE VARIOLÆ VACCINÆ,

A DISEASE

DISCOVERED IN SOME OF THE WESTERN COUNTIES OF ENGLAND,

PARTICULARLY

GLOUCESTERSHIRE,

AND KNOWN BY THE NAME OF

THE COW POX.

BY EDWARD JENNER, M.D. F.R.S. &c.

——QUID NOBIS CERTIUS IPSIS
SENSIBUS HISCE POTEST, QUO VERA AC FALSA NOTEMUS.

LUCRETIUS.

London:

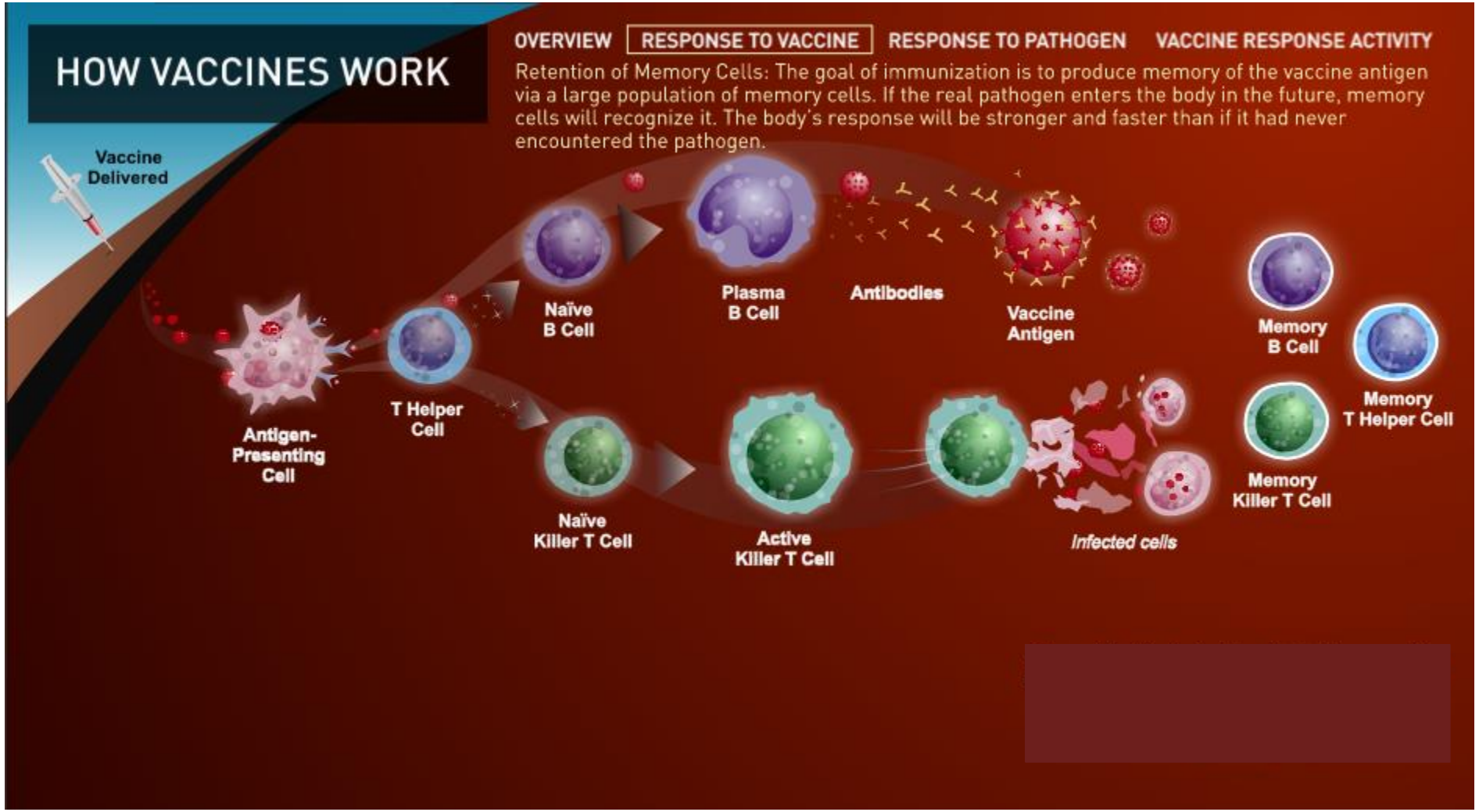
PRINTED, FOR THE AUTHOR,

BY SAMPSON LOW, N°. 7, BERWICK STREET, SOHO:

AND SOLD BY LAW, AVE-MARIA LANE; AND MURRAY AND HIGHLEY, FLEET STREET.

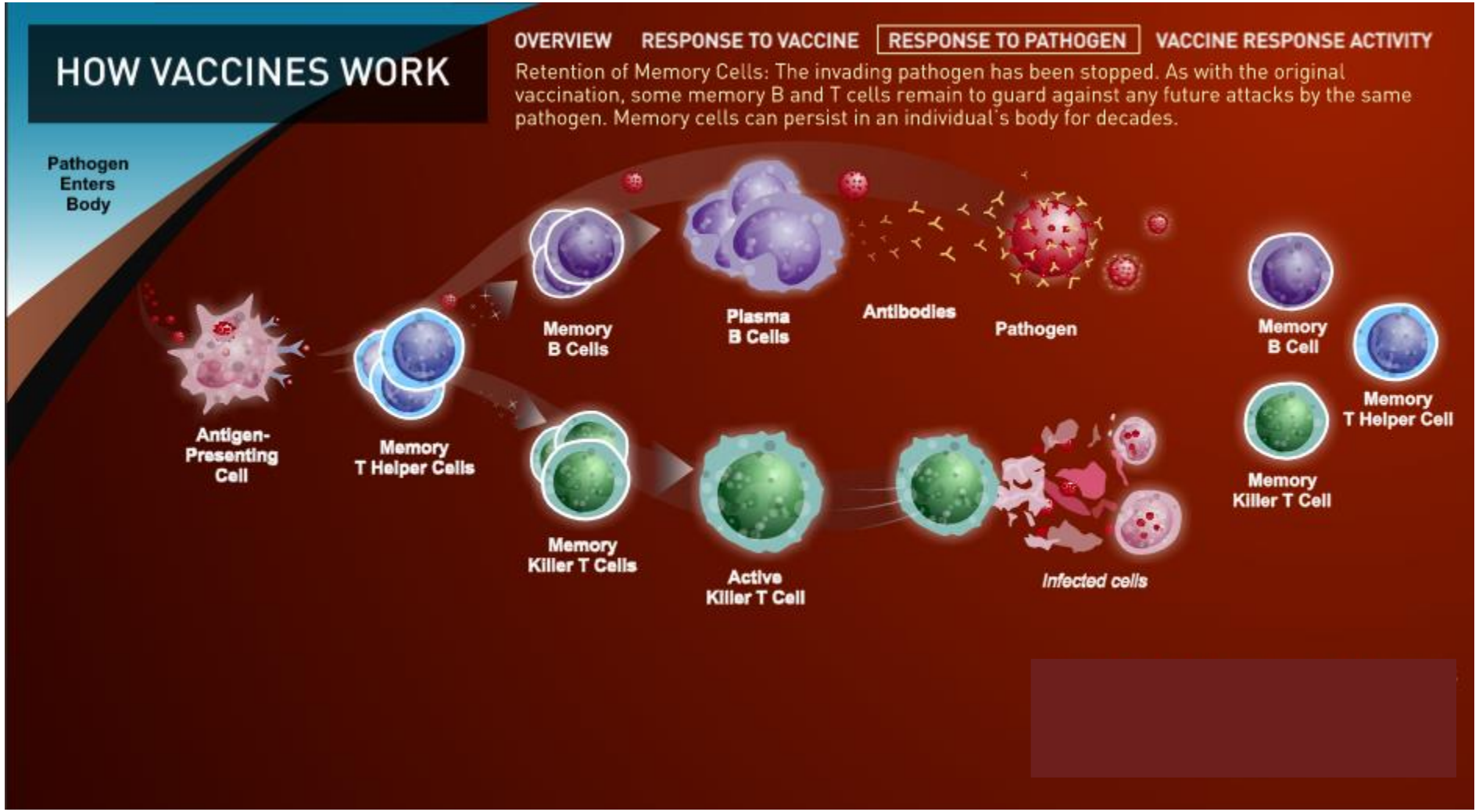
1798.

How Vaccines Work: Part 1



https://www.historyofvaccines.org/content/how-vaccines-work

How Vaccines Work: Part 2



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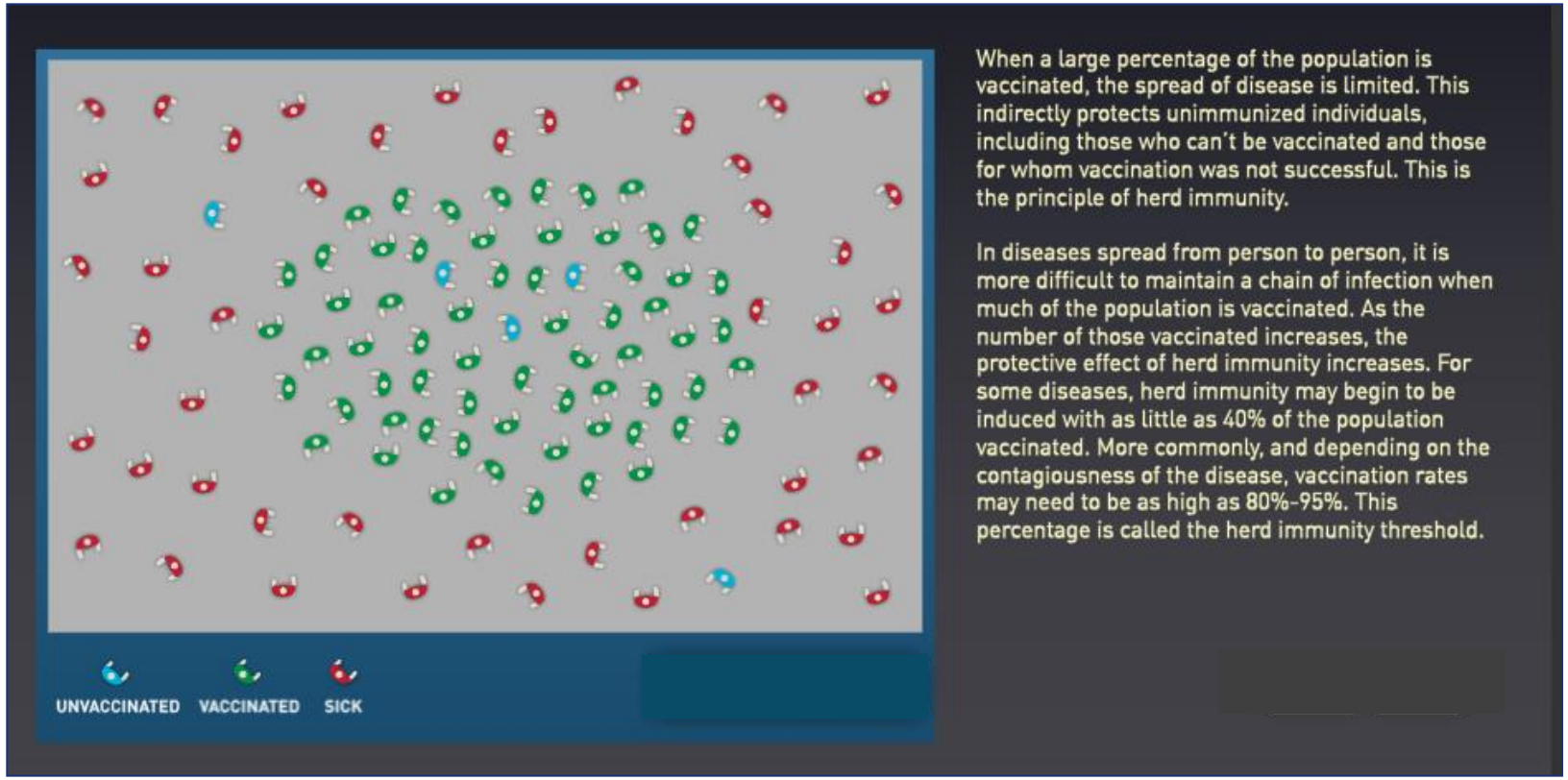
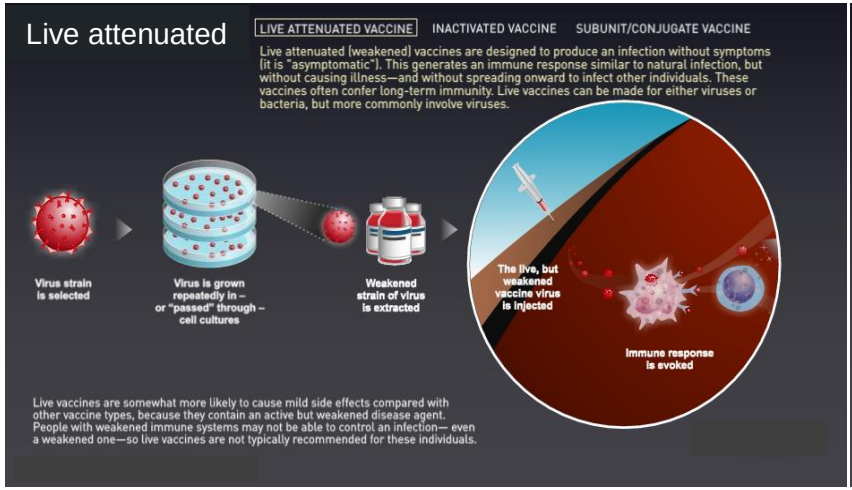
Herd Immunity

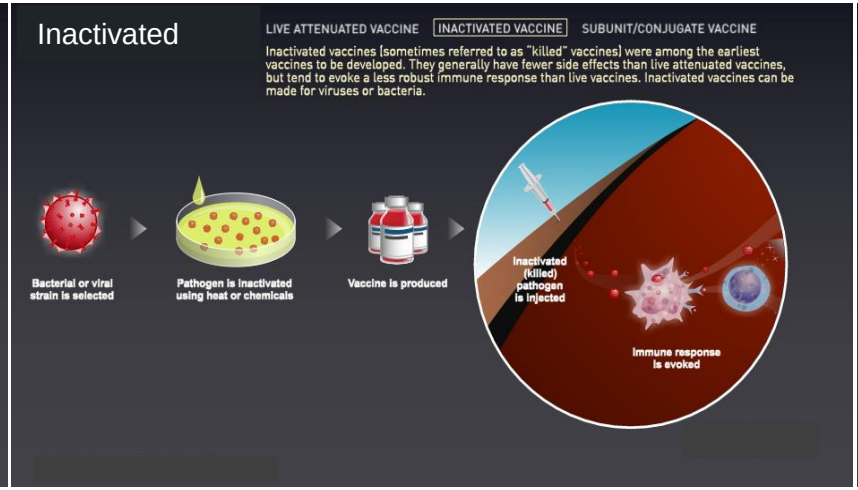


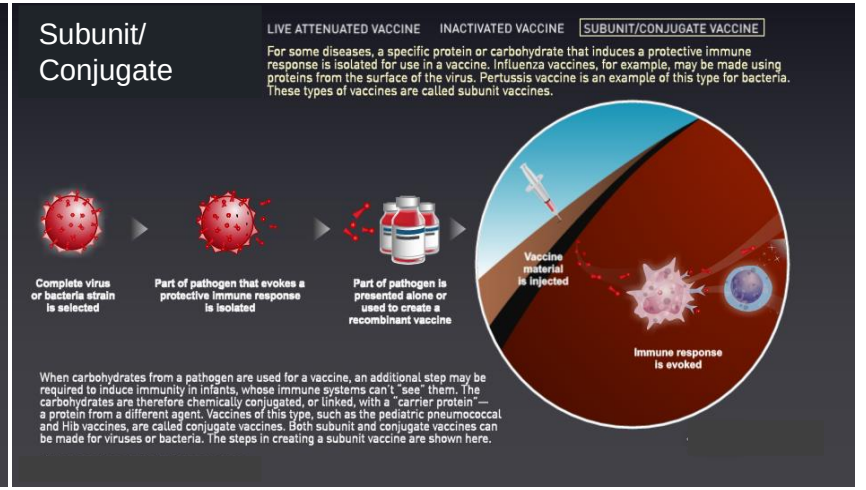
Even if it's not possible to vaccinate 100% of the population, some unvaccinated or unprotected individuals can be protected, provided a large percentage (40 to 95%) of the population is vaccinated.

https://www.historyofvaccines.org/content/herd-immunity-0

Common Types of Vaccines







Live attenuated vaccines are available for measles, mumps, rubella, vaccinia, varicella, zoster, yellow fever, rotavirus, influenza (intranasal) and typhoid (oral).

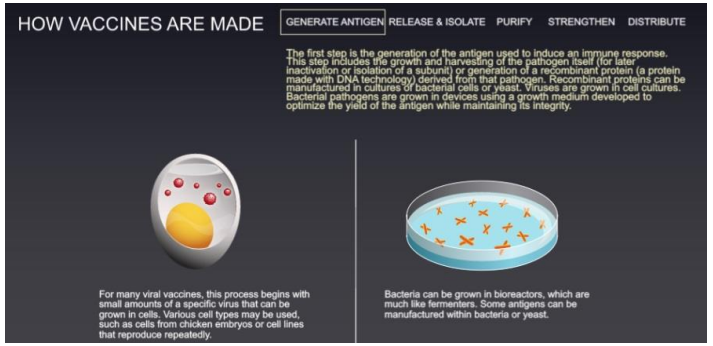
Others:
Viral vectored vaccines
Nucleic acid vaccines

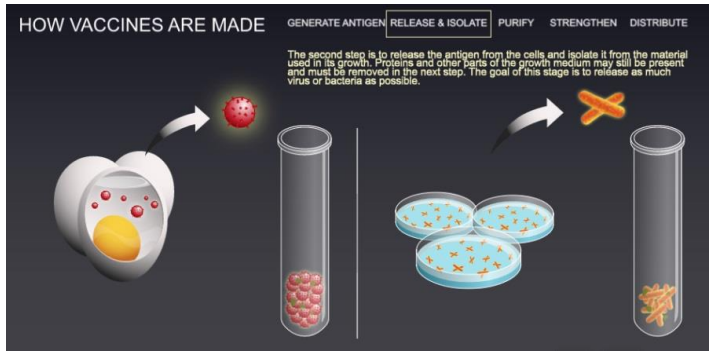
Inactivated vaccines are available for polio, hepatitis A, and rabies. Toxoid vaccines are available for diphtheria, tetanus.

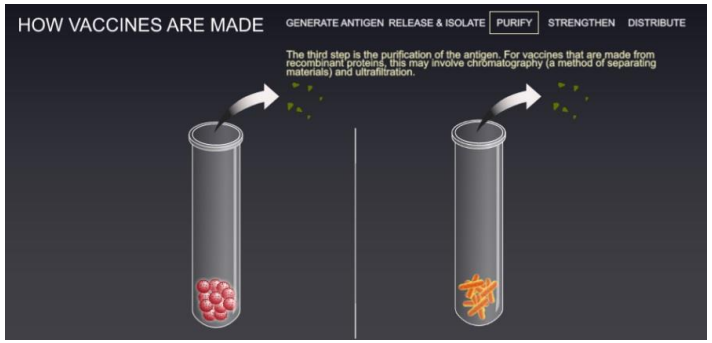
Subunit vaccines are available for hepatitis B, influenza, acellular pertussis, human papillomavirus, anthrax; some of which are genetically engineered/recombinant (Hepatitis B, human papillomavirus (HPV), and influenza). Pure polysaccharide vaccines are available for three diseases: pneumococcal disease, meningococcal disease, and Salmonella Typhi, some of which are conjugated.

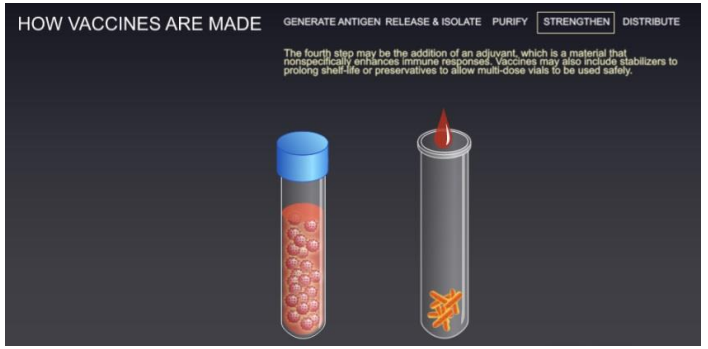
https://www.historyofvaccines.org/content/types-vaccines

Vaccine Manufacturing









https://www.historyofvaccines.org/content/how-vaccines-are-made

Product Safety Testing Conducted Prior to Vaccine Release

Test	Concern	Products	Endpoint
General Safety Test	Extraneous contaminants	Final product	Survival/body weight
Adult and Suckling Mouse Safety Testing of Virus Vaccines	Adventitious Agents	Cell Banks and Vaccine seeds/bulks	Survival, evidence of transmissible agent or other viral infection
Cell Bank Safety			
In vivo TB			
Product-specific Potency	Reduced Potency	Final product	Vaccine titer, LD_{50}/ED_{50}
Pyrogens	Purity	Final Product	Body temperature
Tumorigenicity	Cell Properties	Cell Banks	Tumor formation
Neurovirulence	Neurotropisms	Live Virus Vaccine seeds/bulks	CNS effects

Importance of Vaccination

The Good news:

1980: Smallpox considered eradicated

1994/2002/2014: Polio eliminated in the Americas/Europe/Southeast Asia, respectively

2000: Measles declared eliminated

2009: No US Diphtheria cases in 5 years

The Bad news:

2008: PA and MN Hib outbreaks

2008: Measles outbreaks

2014: Group 3 meningococcal outbreaks

2014: Measles outbreaks (664 cases)

2015: Continued measles outbreaks (Disneyland, 100 cases)

2015: Death due to diphtheria in Spain

2010: Adults 19 to 64 recommended to get pertussis (whooping cough) booster

2019: Measles, mumps, rubella and Hepatitis A outbreaks

https://www.historyofvaccines.org/content/types-vaccines

Vaccines and Autism: The MMR Vaccine

Introduction

"We saw several children who, after a period of apparent normality, lost acquired skills, including communication."

Early report

Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children

A J Wakefield, S H Murch, A Anthony, J Linnell, D M Casson, M Malik, M Berelowitz, A P Dhillon, M A Thomson, P Harvey, A Valentine, S E Davies, J A Walker-Smith

Summary

Background We investigated a consecutive series of children with chronic enterocolitis and regressive developmental disorder.

Methods 12 children (mean age 6 years [range 3–10], 11 boys) were referred to a paediatric gastroenterology unit with a history of normal development followed by loss of acquired skills, including language, together with diarrhoea and abdominal pain. Children underwent gastroenterological, neurological, and developmental assessment and review of developmental records. Ileocolonoscopy and biopsy sampling, magnetic-resonance imaging (MRI), electroencephalography (EEG), and lumbar puncture were done under sedation. Barium follow-through radiography was done where possible. Biochemical, haematological, and immunological profiles were examined.

Findings Onset of behavioural symptoms was associ… by the parents, with measles, mumps, and rub… vaccination in eight of the 12 children, with measl… infection in one child, and otitis media in an… All 12 children had intestinal abnormalities ranging from lymphoid nodular hyperplasia to aphthoid ulceration. Histology showed patchy chronic inflammation in… in 11 children and reactive ileal lymphoid hyperplasia in seven, but no granulomas. Behavioural disorders included autism (nine), disintegrative psychosis (one), and possible postviral or vaccinal encephalitis (two). There were no focal neurological abnormalities and CSF and EEG tests were normal. Abnormal laboratory results were significantly raised urinary methylmalonic acid compared with age-matched controls (p=0·003), low haemoglobin in four children, and low serum IgA in four children.

Interpretation We identified associated gastrointestinal disease and developmental regression in a group of previously normal children, which was generally associated in time with possible environmental triggers.

Lancet 1998; **351**: 637–41
See Commentary page

Inflammatory Bowel Disease Study Group, University Departments of Medicine and Histopathology (A J Wakefield FRCS, A Anthony MB, J Linnell PhD, A P Dhillon MRCPath, S E Davies MRCPath) **and the University Departments of Paediatric Gastroenterology** (S H Murch MB, D M Casson MRCP, M Malik MRCP, M A Thomson FRCP, J A Walker-Smith FRCP,), **Child and Adolescent Psychiatry** (M Berelowitz FRCPsych), **Neurology** (P Harvey FRCP), **and Radiology** (A Valentine FRCR), **Royal Free Hospital and School of Medicine, London NW3 2QG, UK**

Correspondence to: Dr A J Wakefield

Introduction

We saw several children who, after a period of apparent normality, lost acquired skills, including communication. They all had gastrointestinal symptoms, including abdominal pain, diarrhoea, and bloating and, in some cases, food intolerance. We describe the clinical findings, and gastrointestinal features of these children.

Patients and methods

12 children, consecutively referred to the department of paediatric gastroenterology with a history of a pervasive developmental disorder with loss of acquired skills and intestinal symptoms (diarrhoea, abdominal pain, bloating and food intolerance), were investigated. All children were admitted to the ward for 1 week, accompanied by their parents.

Clinical investigations

We took histories including details of immunisations and exposure to infectious diseases, and assessed the children. In 11 cases the history was obtained by the senior clinician (JW-S). Neurological and psychiatric assessments were done by consultant staff (PH, MB) with HMS-4 criteria.[1] Developmental data included a review of prospective developmental records from parents, health visitors, and general practitioners. Four children did not undergo psychiatric assessment in hospital; all had been assessed professionally elsewhere, so these assessments were used as the basis for their behavioural diagnosis.

After bowel preparation, ileocolonoscopy was performed by SHM or MAT under sedation with midazolam and pethidine. Paired frozen and formalin-fixed mucosal biopsy samples were taken from the terminal ileum; ascending, transverse, descending, and sigmoid colons, and from the rectum. The procedure was recorded by video or still images, and were compared with images of the previous seven consecutive paediatric colonoscopies (four normal colonoscopies and three on children with ulcerative colitis), in which the physician reported normal appearances in the terminal ileum. Barium follow-through radiography was possible in some cases.

Also under sedation, cerebral magnetic-resonance imaging (MRI), electroencephalography (EEG) including visual, brain stem auditory, and sensory evoked potentials (where compliance made these possible), and lumbar puncture were done.

Laboratory investigations

Thyroid function, serum long-chain fatty acids, and cerebrospinal-fluid lactate were measured to exclude known causes of childhood neurodegenerative disease. Urinary methylmalonic acid was measured in random urine samples from eight of the 12 children and 14 age-matched and sex-matched normal controls, by a modification of a technique described previously.[2] Chromatograms were scanned digitally on computer, to analyse the methylmalonic-acid zones from cases and controls. Urinary methylmalonic-acid concentrations in patients and controls were compared by a two-sample *t* test. Urinary creatinine was estimated by routine spectrophotometric assay.

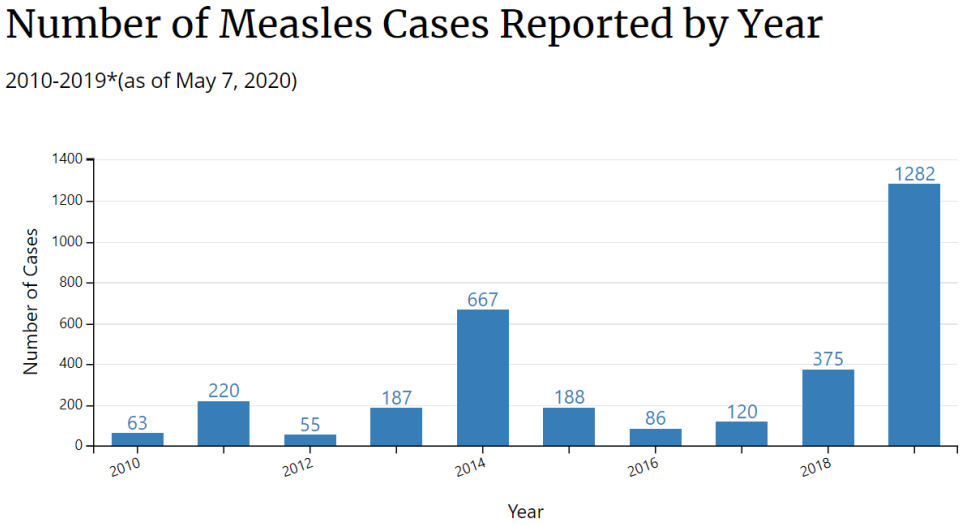
Children were screened for antiendomyseal antibodies and boys were screened for fragile-X if this had not been done

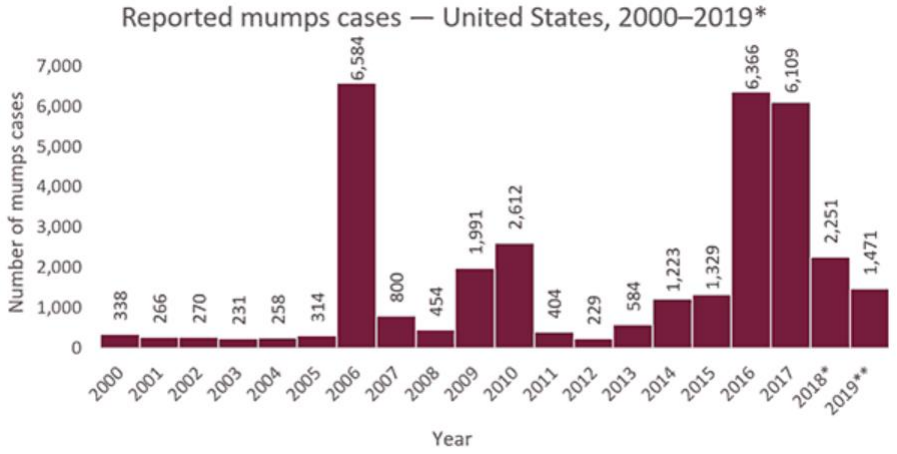
THE LANCET • Vol 351 • February 28, 1998 637

RETRACTED

A J Wakefield *et al.*, Lancet **351**: 637 -641 (1998)

Importance of Vaccination



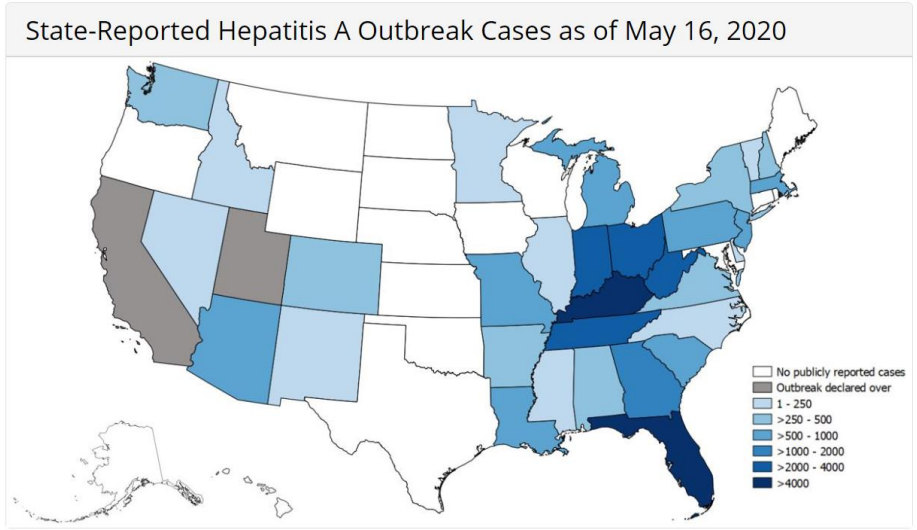


- From January 1 to December 31, 2019, 1,282 individual cases of measles have been confirmed in 31 states.
- As of May 7, 2020, there have been 12 confirmed cases in 7 jurisdictions.
- Prior to vaccination: 3 to 4M infected/year in US
  - 400 to 500 people died
  - 48,000 were hospitalized
  - 1,000 suffered encephalitis

- From January 1 to January 25, 2020, 16 states in the U.S. reported mumps infections in 70 people to CDC.
- Prior to vaccination: 186K cases/year in US (likely higher due to underreporting)
- U.S. mumps cases decreased more than 99% following vaccination

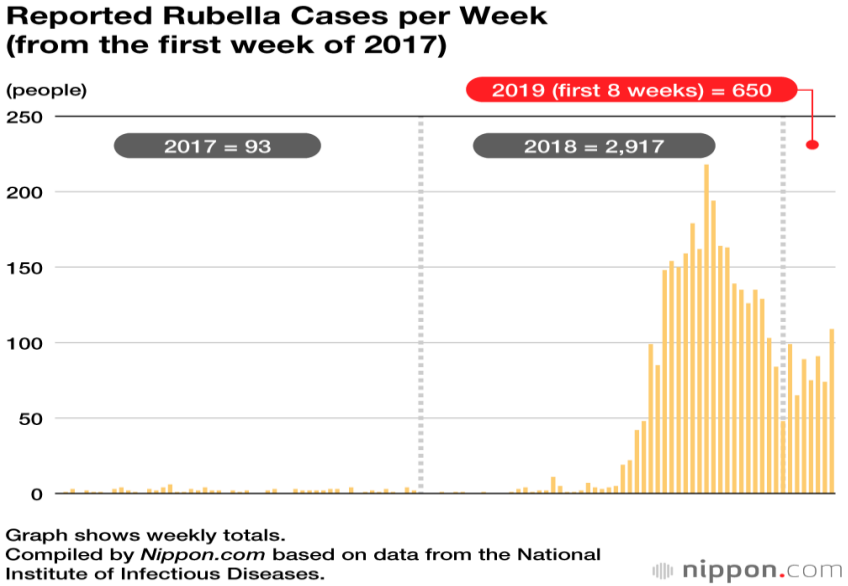
Source: CDC.gov

Importance of Vaccination



- Since the outbreaks were first identified in 2016, 33 states have publicly reported the following as of May 16, 2020:
  - Cases: 32,541
  - Hospitalizations: 19,885 (61%)
  - Deaths: 324

Source: CDC.gov



- Japanese men from their late thirties to early fifties did not receive rubella vaccinations at school, and so are at the heart of a new epidemic.
  - Cases rose from 93 in 2017 to 2,917 in 2018
  - 650 Cases reported in first 8 weeks of 2019

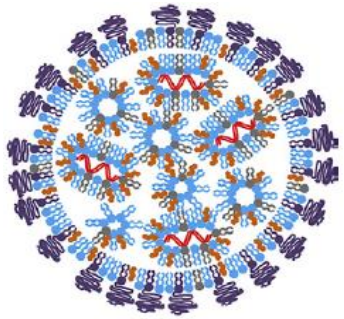
Source: Nippon.com

Increased Emphasis on Nonclinical Safety Evaluation of Vaccines

Administered to healthy individuals, including infants

Potential vaccine AEs

Novel vaccine types



New adjuvants & delivery systems

- First nonclinical vaccine guideline finalized in 1998 (has since been rescinded).
- Longest standing vaccine guideline was established by WHO in 2005

Preclinical Vaccine Regulatory Guidelines

Some vaccine-specific guidelines also available (e.g. Dengue, Ebola, COVID-19)

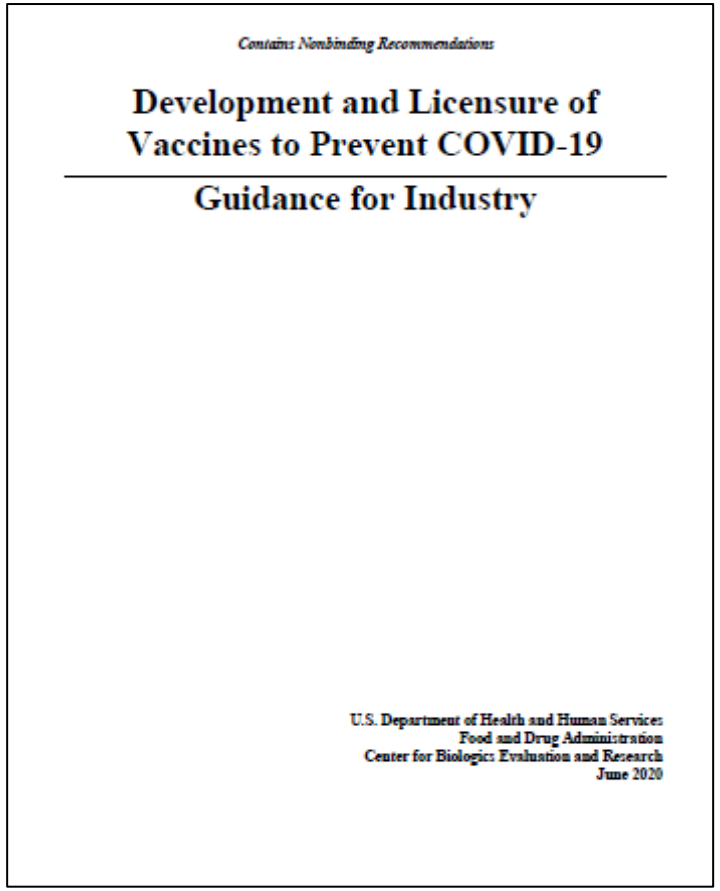
Vaccine Type	Guideline
All vaccines	WHO: Guidelines on Nonclinical Evaluation of Vaccines (2005)
	India: Drug and Cosmetics Act, 1940 and Drug and Cosmetics Rule, 1945 (2005)
	China: State Food and Drug Administration, China Technical guidelines for preclinical research on preventive vaccines. Notice No. 140 (2010)
	Japan: Japanese Guideline for Non-clinical Studies of Vaccines for Preventing Infectious Diseases, (PFSB/ELD Notification No. 0527-1, 27th May 2010)
Adjuvanted vaccines	Worldwide: WHO Guidelines on Nonclinical Evaluation of Vaccine Adjuvants and Adjuvanted Vaccines (2013)
	EMA: Guideline on Adjuvants in Vaccines for Human Use (2005)
Vaccines for pregnant women & WCBP	FDA: Guidance for Industry. Considerations for Developmental Toxicity Studies for Preventative and Therapeutic Vaccines for Infectious Disease Indications (2006) ICH S5(R3) Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals (2020)
Combination vaccines	EMA: Note for Guidance on Pharmaceutical and Biological Aspects of Combined Vaccines (1998)

Preclinical Vaccine Regulatory Guidelines

Some vaccine-specific guidelines also available (e.g. Dengue, Ebola, COVID-19)

Vaccine Type	Guideline
DNA vaccines	FDA: Guidance for Industry. Considerations for Plasmid DNA Vaccines for Infectious Disease Indications (2007) WHO: Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines (2019 – DRAFT revision) EMA: Note for Guidance on the Quality, Preclinical and Clinical Aspects of Gene Transfer Medicinal Products (2001)
Recombinant DNA vaccines	FDA: DRAFT Points to consider in the production and testing of new drugs and biologicals produced by recombinant DNA technology (1985)
Viral vectored vaccines	EMA: Guideline on quality, nonclinical and clinical aspects of live recombinant viral vectored vaccines (2010)
Lipid Based Vaccines	Japan: Guideline for the Development of Liposome Drug Products (2016)
	Japan: Reflection paper on nucleic acids (siRNA)-loaded nanotechnology-based drug products (2016)
	EMA: Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (2018)

FDA's Guideline on COVID-19 Vaccines



Contains Nonbinding Recommendations

Development and Licensure of Vaccines to Prevent COVID-19

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
June 2020

- Implemented on June 30, 2020 without public comment period, similar to other COVID-19 guidelines.
- FDA will update this guidance 60-days after public health emergency is over.
- Describes FDA's current recommendations regarding data needed to facilitate nonclinical and clinical development and licensure of COVID-19 vaccines.

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/development-and-licensure-vaccines-prevent-covid-19

Key Considerations for Nonclinical Toxicology Studies

Tox studies needed for novel vaccine platforms prior to FIH	• For a COVID-19 vaccine candidate consisting of a novel product type and for which no prior nonclinical and clinical data are available, nonclinical safety studies will be required prior to proceeding to FIH clinical trials.
Previous platform tox data can be used to support FIH studies	• If vaccine candidate is made using a platform technology utilized to manufacture a licensed vaccine or other previously studied investigational vaccines and is sufficiently characterized, it may be possible to use toxicology data (e.g., data from repeat dose toxicity studies, biodistribution studies) and clinical data accrued with other products using the same platform to support FIH clinical trials for that COVID-19 vaccine candidate.
DART studies needed to support WoCBP and Pregnant Women	• We recommend prior to enrolling pregnant women and women of childbearing potential who are not actively avoiding pregnancy in clinical trials, sponsors conduct developmental and reproductive toxicity (DART) studies with their respective COVID-19 vaccine candidate. Alternatively, sponsors may submit available data from DART studies with a similar product using comparable platform technology if, after consultation with the agency, the agency agrees those data are scientifically sufficient.
Biodistribution data might be needed	• Biodistribution studies in an animal species should be considered if the vaccine construct is novel in nature and there are no existing biodistribution data from the platform technology. • These studies should be conducted if there is a likelihood of altered infectivity and tissue tropism or if a novel route of administration and formulation is to be used.

FIH = First-in Human
DART = Developmental and Reproductive Toxicity
WoCBP = Women of Child-Bearing Potential

Different Patient Populations for Different Vaccines…

Immune system is different in each population:

Pediatric

Diphtheria
Haemophilus influenzae type B (Hib)
Hepatitis A
Hepatitis B
Influenza (seasonal flu)
Measles
Meningococcal
Mumps
Pertussis
Pneumococcal
Poliomyelitis
Rotavirus
Rubella
Tetanus
Varicella

Adults and travel

Cervical cancer (HPV)
Diphtheria
Haemophilus influenzae type B (Hib)
Hepatitis A
Hepatitis B
Influenza (pre-pandemic)
Influenza (seasonal flu)
Measles
Meningococcal
Mumps
Pertussis
Poliomyelitis
Rabies
Rubella
Tetanus
Tick-borne encephalitis
Varicella

Older adults

Diphtheria
Hepatitis A
Hepatitis B
Influenza (pre-pandemic)
Influenza (seasonal flu)
Pertussis
Shingles
Tetanus

Support of Special Populations in Clinical Studies

- Infants/Pediatrics:
  - Clinical studies designed to support infants
    - Adult step-down
  - Animals on toxicology studies of an age which supports juvenile and pediatric populations
    - No need for juvenile toxicity studies
- Women of Childbearing Potential
  - Developmental and Reproductive Toxicity (DART) studies conducted concurrent with Ph III clinical studies
- Pregnant women
  - DART studies conducted prior to enrolling pregnant women

Appropriate Animal Model

- Demonstration of an immune response is important as, with some exceptions, the toxicity associated with vaccines is generally a result of immunogenicity/inflammatory response.
- Single species is generally sufficient
- Typically, pharmacology studies are conducted in mice, rabbits and/or monkeys while toxicology studies are performed in rats or rabbits
  - Careful consideration should be given to the use of rabbits for toxicology studies based on recent data indicating the potential for stress-induced cardiac changes in vaccine studies*
- Species for which a robust historical control database is available is recommended
- Disease models are not generally used for toxicology studies.

*Sellers et al. Toxicologic Pathology 2017, Vol. 45(3) 416-426

Selection of Dose Levels

- GMP (clinical) lot preferred
- Non-GMP (GLP) lot representative of the clinical lot is also acceptable
- Release, Characterization and Stability data required
  - Identity, Purity, Potency, Concentration, Endotoxin, Bioburden, etc.
- Dose Formulation Assays
  - Not typically required for GLP studies
- Novel adjuvants (e.g. TLR agonists)
- Novel delivery systems (e.g. LNPs)
- Potential for vaccine components/antigens to result in toxicity must be considered
- Some vaccines may require USDA and/or CDC permits and additional safety measures such as autoclaving waste, etc.
- Occupational Exposure Band (OEB) Classifications

Selection of Dose Levels/Route of Administration

- Administration of a full-human dose equivalent is preferred
  - Alternatively, administer a dose that exceeds the human dose on a mg/kg basis
  - Can also move to species in which total volume of human dose may be administered.
- Total volume can be administered at more than one site
- Should include a negative control group (e.g. PBS) and adjuvant/vehicle alone group as needed
- Match clinical route/device

Routine Nonclinical Toxicity Studies for Vaccines

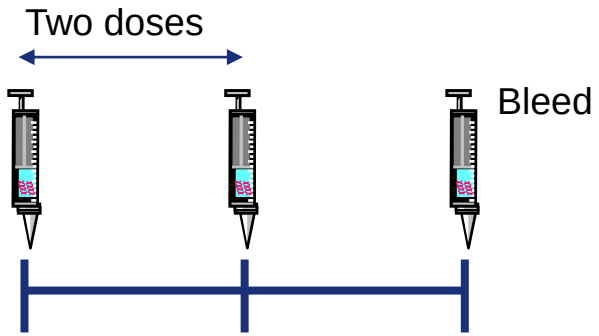
Study Type	Purpose
Exploratory Immunogenicity	• To confirm that species selected produces an immune response (i.e. species relevance) • Reagent generation for immunogenicity assay development
GLP Repeat-Dose Toxicity	• Comprehensive antemortem and postmortem analyses • Generally includes evaluation of single-dose toxicity and local tolerance
Developmental And Reproductive Toxicity (DART)	• Required for vaccines administered to pregnant women or Women of Child-bearing Potential

Additional Toxicity Studies That May be Required

Study Type	Purpose
Safety Pharmacology	Do not generally conduct - May be recommended if cause for concern identified in nonclinical or clinical studies. Japanese guideline indicates needed unless have clinical data to support safety.
Biodistribution and Integration Studies	For nucleic acid and viral vector-based vaccines and potentially for novel adjuvants.
Nonrodent Toxicology	Only required for evaluation of a novel adjuvant, typically the adjuvant alone is sufficient. May add safety endpoints on pharmacology studies vs. conducting a separate study.
Genotoxicity	Usually not needed for vaccines, but needed for a new synthetic adjuvant that is considered to be a New Chemical Entity
Carcinogenicity	Generally not needed for vaccines
Neurovirulence	Required for vaccines that have the potential for reversion to virulence and neurotropic activity
Tumorigenicity	Required for cell lines either not previously characterized or used beyond passage level previously tested

Exploratory Immunogenicity Study:

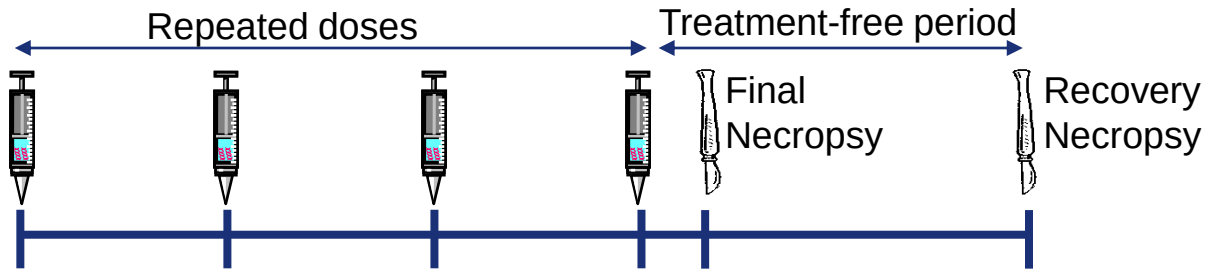
Purpose: Confirm Species Relevance and Reagent Generation



- Recommended if toxicology species not used for pharmacology studies
- Conducted prior to GLP Toxicity study to confirm species relevance
- Performed in one species (generally rats or rabbits)
- Vaccine administered on Study Days 1 and 22 via intended clinical route
  - Using intended clinical device as appropriate
- Animals bled for serum on Study Day 42
- Single dose level (typically full human dose) and PBS control
  - Additional dose levels may be added if needed for dose selection for GLP study
- Other endpoints (e.g. viremia, clinical pathology, cytokines, inflammatory biomarkers, histology, etc.) may be added on a case-by-case basis

GLP Repeat-Dose Toxicity Studies*:

Purpose: Examine the Effects of Single and Repeated Administration



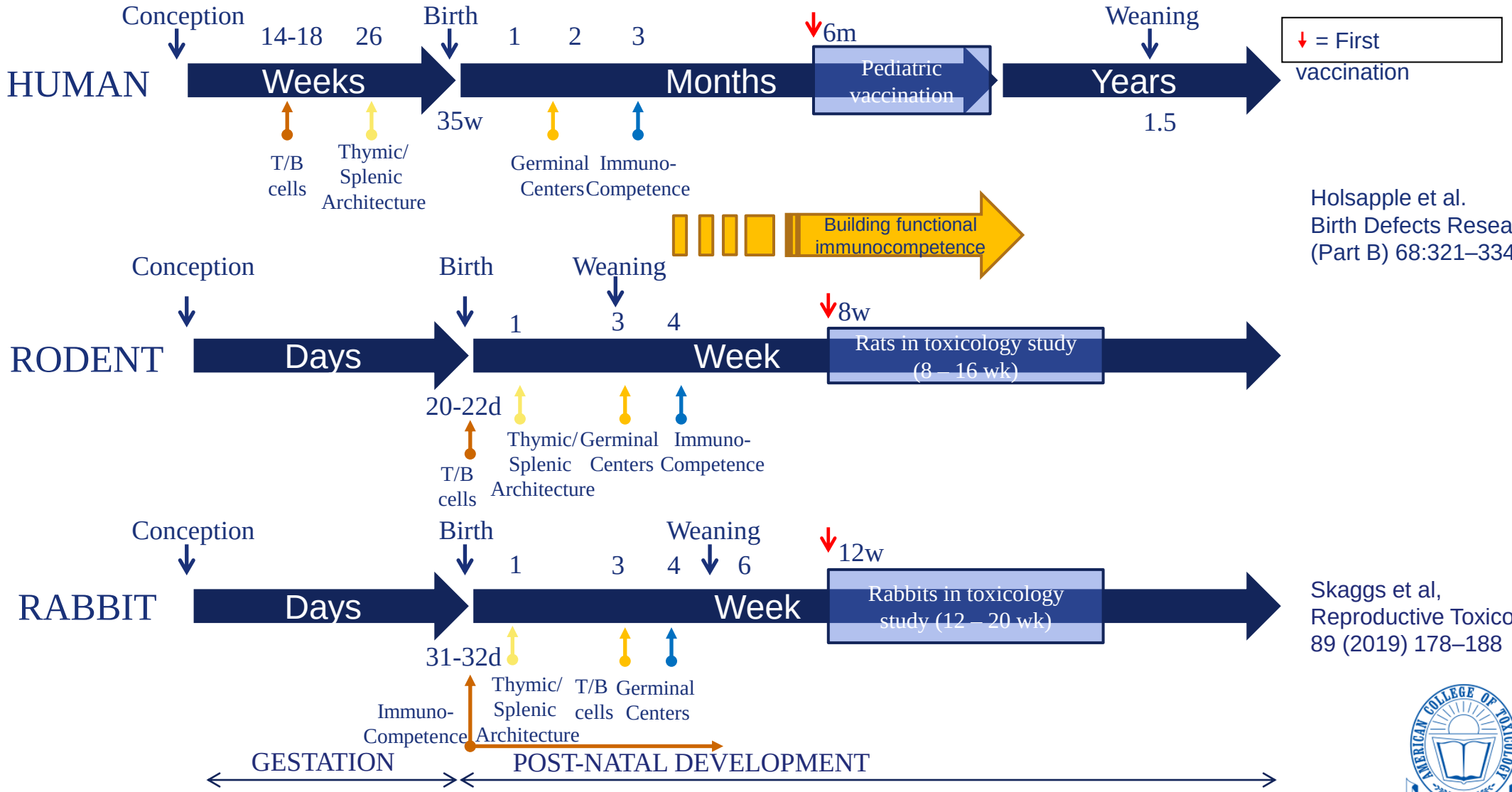
*Routine design based on current regulatory guidelines

Parameter	Details
Route of Administration	Match clinical route/device
Dose Volume	Match clinical volume (if possible)
Species	Relevant species (rodent or rabbit; additional nonrodent study with adjuvant alone for novel adjuvants)
Treatment Groups	Full human dose or Maximum Feasible Dose, control, adjuvant alone (only for novel adjuvants)
Treatment Interval	2 to 3 weeks apart with 4 week treatment-free period
Routine endpoints	Full antemortem and postmortem evaluations

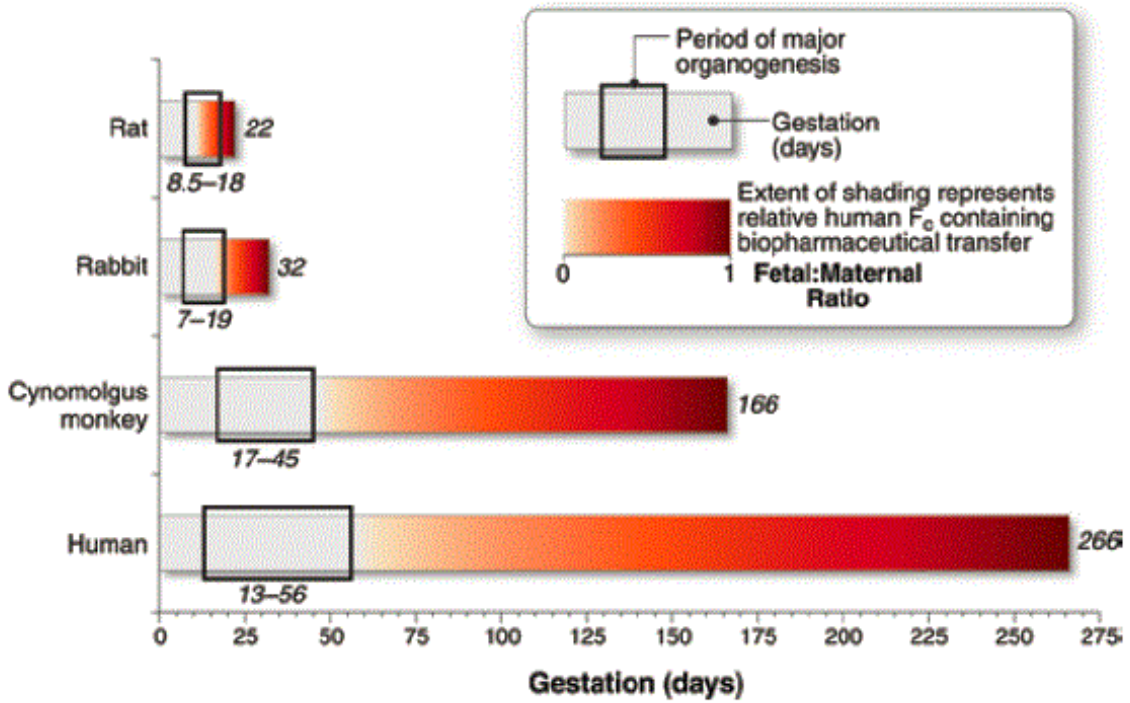
IND-Enabling GLP Repeat-Dose Toxicity Studies:
Endpoints Evaluated

- **Antemortem Evaluation**
  - Physical signs, body weight, food consumption, body temperatures
  - Ophthalmic exams
  - Clinical Pathology
  - Cytokines
  - Immunogenicity
  - Inflammatory biomarkers (e.g. acute phase proteins)
- **Postmortem Evaluation**
  - Necropsies: One to 3 days after the last dose, end of treatment-free period
  - Complete gross necropsy, organ weights, and tissue collection
  - Complete histopathological evaluations
    - Routine tissue list
    - Includes immune system (draining lymph nodes, thymus, spleen, bone marrow, Peyer's patches)
    - Includes injection sites for local tolerance evaluation

Immune System Development Between Species



Gestational Period Comparison in Common Tox Species and Humans

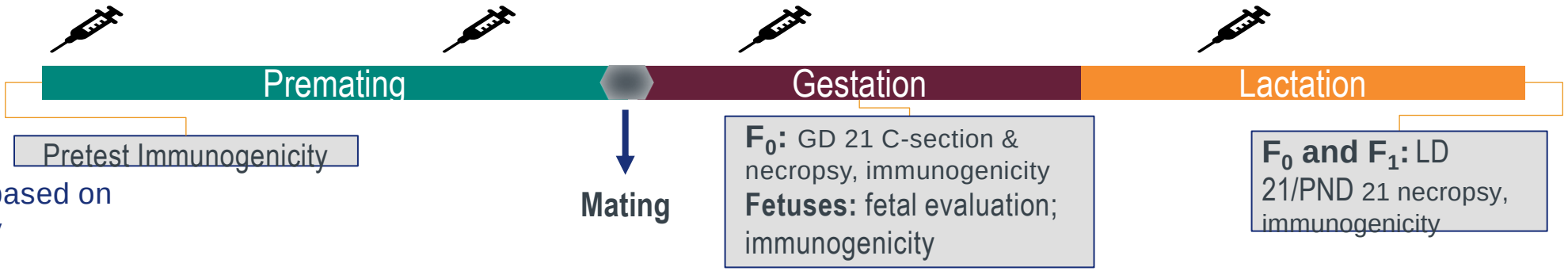


Rat: Day 11 = middle of 2nd trimester (Immune response during 3rd trimester)
Rabbit: Day 11 = beginning of 2nd trimester (Immune response during 3rd trimester)

DeSesso et al, Critical Reviews in Toxicology (2012) 42:3, pp 185-210. DOI: 10.3109/10408444.2011.653487

Developmental and Reproductive Toxicity Study*

Purpose: Evaluation of Maternal and Embryonic/fetal Development



*Routine design based on current regulatory guidelines

Parameter	Details
# of animals	45 F_0 females/group (c-section: 20/group; natural delivery: 20/group)
Route of administration/volume	Match clinical route/volume (if possible)
Species	Rat or rabbit (match relevant species in GLP toxicology study)
Treatment Groups	Full human dose, control, adjuvant alone (for novel adjuvants)
Treatment Interval	Prior to mating (generally twice), during both gestation (GD 6) and lactation (LD 7)
Blood collections	Immunogenicity: F_0 females and F_1 generation (pooled per litter)
Cesarean Section	GD 21
Gross Examination	C-section group (GD 21), Natural delivery group (LD 21), F_1 pups (PND 21)
Other endpoints	Neurological evaluations (e.g., auditory and visual function tests)

Note: Design may change based on type of vaccine (e.g. additional doses during study, viremia arm added for viral vaccine) but not typically driven by clinical plans; GD = Gestation Day, LD = Lactation Day, PND = Postnatal Day

Expected Findings in Nonclinical Toxicity Studies

- Repeat-Dose Toxicity Studies*
  - Clinical pathology findings indicative of an inflammatory response
  - Local injection site reactions
  - Increased body temperature
  - Increased inflammatory biomarkers

As these findings are expected, they are not considered adverse provided they are within that expected from a well-tolerated vaccine

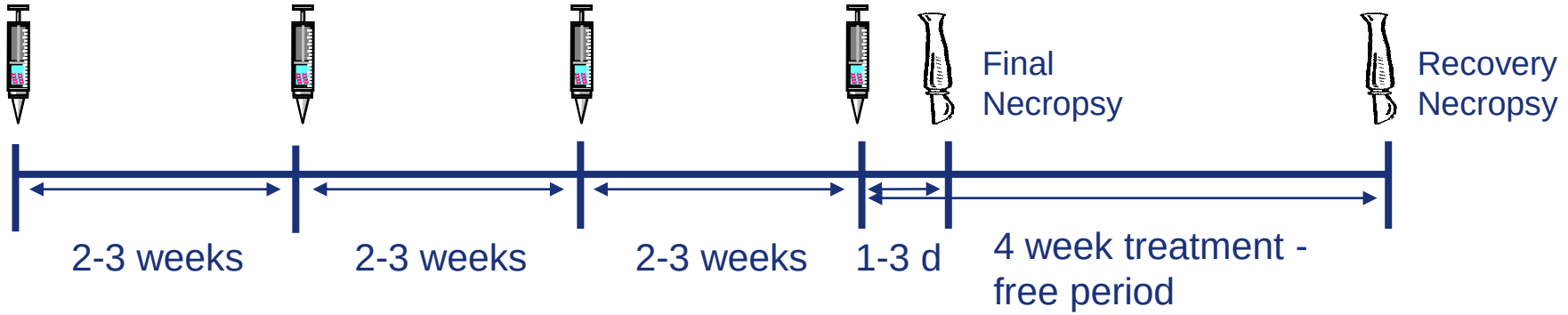
- DART Studies
  - Findings on DART studies for vaccines are rare

*Baldrick, J. Appl. Toxicol. 2016; 36: 980-990

Example 1: Adult Prophylactic Vaccine

Repeat-Dose Toxicity Study:

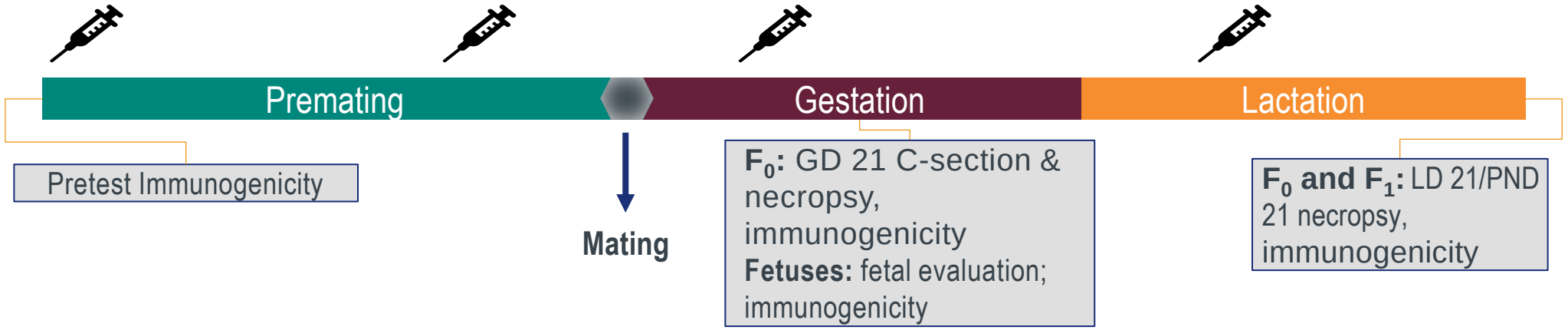
Purpose: Define Toxicity Profile of Vaccine



Parameter	Clinical Regimen	Tox Study Design
Number of doses	3 doses	4 doses (N*+1)
Route	IM	IM
Dose level	3 μg of each of 3 antigens in vaccine	3 μg of each of 3 antigens in vaccine
Frequency	3, 6, and 12 months	Once every 2-3 weeks with 4 week treatment-free period
*N=# doses planned clinically		

Developmental and Reproductive Toxicity Study:

Purpose: Evaluation of Maternal and Embryonic/fetal Development



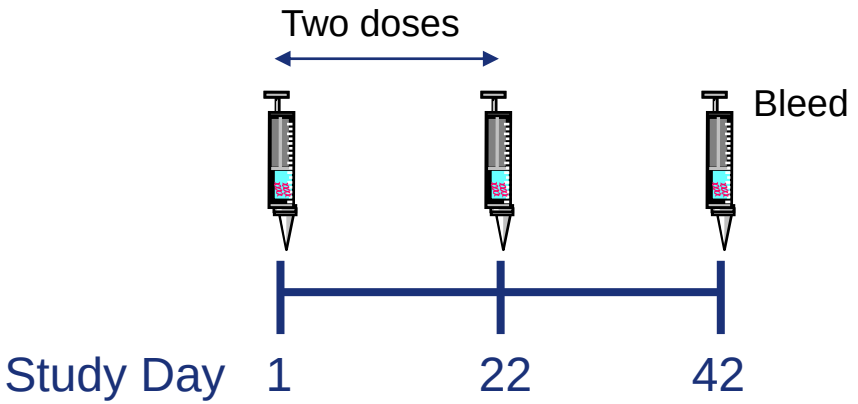
Parameter	Details
# of animals	45 F_0 females/group (c-section: 20/group; natural delivery: 20/group)
Route of administration/volume	IM / 0.5 mL
Treatment Groups	Full human dose, control
Treatment Interval	Prior to mating (twice), during both gestation (GD 6) and lactation (LD 7)
Blood collections	Immunogenicity: F_0 females and F_1 generation (pooled per litter)
Cesarean Section	GD 21
Gross Examination	C-section group (GD 21), Natural delivery group (LD 21), F_1 pups (PND 21)
Other endpoints	Neurological evaluations (e.g., auditory and visual function tests)

GD = Gestation Day, LD = Lactation Day, PND = Postnatal Day

Example 2: Vaccine with a Novel Adjuvant

Exploratory Immunogenicity and Tolerability Study:

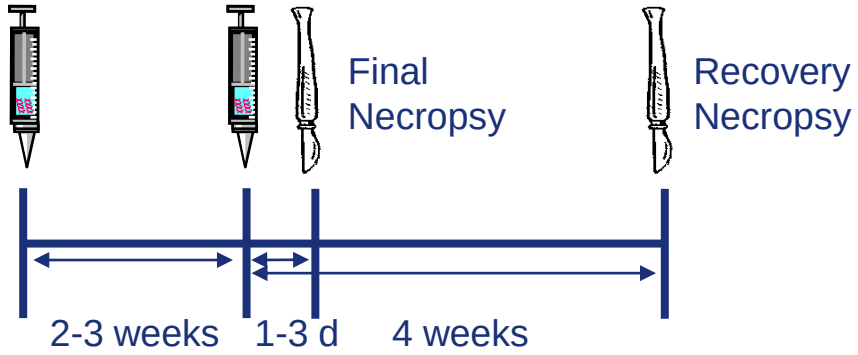
Purpose: Tolerability, species relevance and reagents for assay development



Parameter	Details
Species	Same as planned for GLP toxicology study
Route of administration/volume	IM / 0.5 mL (to match clinical plans)
Frequency	Study Days 1 and 22 (optimal timing to generate immune response)
Treatment Groups	Range of doses to aid in dose selection for GLP toxicology study
Controls	Adjuvant alone and control groups
Immunogenicity	Study Day 42
Toxicity endpoints	clinical pathology, cytokines, inflammatory biomarkers (e.g. acute phase proteins), histology

Repeat-Dose Toxicity Study:

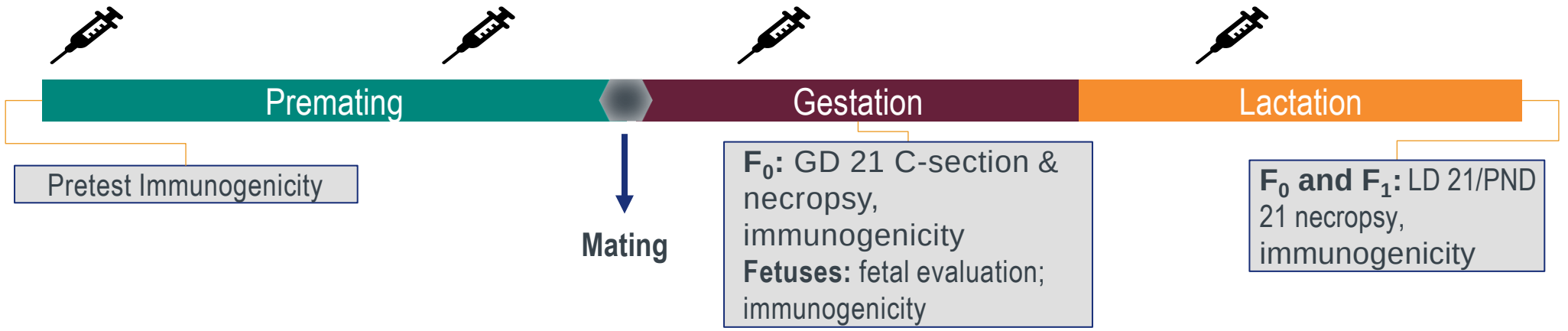
Purpose: Define Toxicity Profile of Vaccine



Parameter	Clinical Regimen	Tox Study Design
# of doses	1 dose	2 doses (N*+1)
Route	IM	IM
Dose level	Dose ranging up to the full dose of antigens and adjuvant in 0.5 mL	Control; Adjuvant alone @ high dose; adjuvanted vaccine at full human dose
Frequency	Once	Once every 2 to 3 weeks with 4 week treatment-free period
*N=# doses planned clinically		

Developmental and Reproductive Toxicity:

Purpose: Evaluation of Maternal and Embryonic/fetal Development



Parameter	Details
# of animals	45 F_0 females/group (c-section: 20/group; natural delivery: 20/group)
Route of administration/volume	IM / 0.5 mL
Treatment Groups	Full human dose, control, adjuvant alone control
Treatment Interval	Prior to mating (twice), during both gestation (GD 6) and lactation (LD 7)
Blood collections	Immunogenicity: F_0 females and F_1 generation (pooled per litter)
Cesarean Section	GD 21
Gross Examination	C-section group (GD 21), Natural delivery group (LD 21), F_1 pups (PND 21)
Other endpoints	Neurological evaluations (e.g., auditory and visual function tests)

GD = Gestation Day, LD = Lactation Day, PND = Postnatal Day

Additional Studies for Vaccine with a Novel Adjuvant

- Adjuvant only in nonrodent required for Ph I*
- Genetic toxicity
  - In vitro tests for mutation and chromosomal damage for Ph I
  - Full battery of genetic toxicity testing for Ph II
- Biodistribution
  - Typically conducted prior to registration (generally concurrent with Ph IIb)
  - Timepoints are selected on a case-by-case basis
  - May include shedding endpoints
  - The need and timing of the study should be considered case by case

*Per EMA Guideline on Adjuvants in Vaccines for Human Use (2005)

Considerations

- Formulation changes during development
  - Requires ‘paper’ toxicity evaluation to determine if changes will require a repeat tox study (use in marketed formulations, impact on potency, etc.)
  - Justification provided to regulatory agencies
  - May trigger studies to be repeated for certain countries (e.g. Japan)
  - Comparability studies (analytical/*in vivo*)

- Many excipients considered ‘novel’ in Japan
  - Requires ‘paper’ toxicity evaluation to provide justification that excipients are safe
  - If insufficient data in the literature, may trigger in vivo studies
  - May use permitted daily exposure (PDE) or Threshold of Toxicological Concern (TTC) to support safety

- Host cell proteins

Conclusions

- Vaccines play an important role in public health
- Nonclinical safety assessment of vaccines is an important component of vaccine development and helps address potential clinical concerns
- Design of nonclinical toxicology programs should be consistent with current regulatory expectations and the specific aspects of each vaccine
- Many considerations are taken into account when designing nonclinical toxicology programs including clinical dose and regimen, patient population, vaccine type/formulation, etc.
- In addition to routine toxicity studies, other assays are available to evaluate adjuvants and/or novel formulations

Pop Quiz!

Question 1: Which guidelines should be consulted to determine which studies will be required to support a vaccine clinical program.

A. WHO Guidelines for Nonclinical Toxicology Studies
B. WHO Guidelines for adjuvants and adjuvanted vaccines
C. EMA Guideline on Adjuvanted Vaccines
D. ICH M3 (R2)
E. ICH M7 (R1)
F. ICH S5 (R3)
G. FDA DART Guidelines
H. All of the above

Q1 Correct Response

The correct response is:

H. All of the above

Given the range of studies required for vaccines with novel adjuvants, there are many guidelines that apply. Happy reading…

It should be noted that individual countries (e.g., Japan and China) have vaccine guidelines and should also be reviewed as there may be some differences from other guidelines.

Question 2: Which non-GLP studies should be considered for a vaccine with a novel adjuvant prior to GLP toxicology studies?

A. Rodent Dose-Limiting Toxicity (DLT)

B. Exploratory Immunogenicity

C. Exploratory Immunogenicity with Toxicity endpoints

D. Nonrodent DLT

Q2 Correct Response

The correct response is:

C. Exploratory Immunogenicity with Toxicity endpoints

- Immunogenicity is generally evaluated prior to conducting GLP toxicity studies to confirm species relevance.
- Given that there will be limited safety data for the novel adjuvant, it would be prudent to include toxicity endpoints on the Exploratory Immunogenicity Study.

Question 3: How is the dose selected for repeat-dose toxicology studies?

A. A margin over the highest anticipated clinical dose
B. 50-fold over the highest anticipated clinical dose
C. 10-fold over the highest exposure anticipated in clinical studies
D. Match the highest anticipated clinical dose
E. A and D

Q3 Correct Response

The correct response is:

E. A and D

- While the full human dose is typically administered in vaccine GLP rat toxicology studies, if this is not feasible based on volume or toxicity restrictions you may be able to justify a dose on a mg/kg basis

Question 4: How are the route, number of doses and frequency selected for the GLP toxicology study determined?

A. Intramuscular, n + 1 doses, match clinical dosing regimen

B. Subcutaneous, 4 doses, every 2-3 weeks

C. Match clinical route, n + 1 doses, every 2 to 3 weeks

D. Match clinical route, match # clinical doses, every 2 to 3 weeks

Q4 Correct Response

The correct response is:

C. Match clinical route, n + 1 doses, every 2 to 3 weeks

This design best complies with regulatory guidelines (WHO 2005 and 2013):

- Matches clinical route
- One more dose than the planned clinical regimen (n + 1)
- Dosing regimen may be shortened relative to clinical frequency (i.e., do not need to match clinical frequency). Every 2 to 3 weeks is acceptable
- It should be noted that currently clinical studies in pediatric subjects in Japan are conducted utilizing the subcutaneous (SC) route. Therefore, the SC route will need to be supported in a subsequent study. Generally a local tolerance study would be acceptable.

Question 5: There are limited data in the literature to support the safety of a novel excipient. The TTC may be used to supplement these limited data to justify not conducting additional toxicology studies.

A. True

B. False

Q5 Correct Response

The correct response is:

A. True

- The guidance in ICH M7 (R1) may be applied to situations other than those involving mutagenic impurities. If the level of the excipient in the final formulation is below the TTC, it may be possible to apply the TTC described in ICH M7 (R1) to supplement the limited toxicology data in the literature.

Thank You – Any Questions?

Backup Slides

Different Types of Vaccines

- Synthetic Peptide
- Live Viral Vector
- Plasmid DNA
- Live Attenuated Microorganisms
- Modified dendritic or tumor cells
- Recombinant Protein
- Inactivated Whole Microorganisms
- Nucleic acid in novel delivery system
- Virus like Particle