

VLP Vaccines, an Example of Product Profile Specification Setting

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16th Annual General Meeting

Developing Countries Vaccine Manufacturers Network

October 5-7, 2015

Bangkok, Thailand



Overview – Vaccines Have a Variety of Specifications

- In process specifications to control Critical Quality Attributes
- Final Container Chemical components (Excipients and Adjuvants) use normal chemical assays and target specifications
- Potency assay specifications – Critical to assure vaccine will work as reported in clinical studies
- Talk will focus on developing and setting specifications for the potency assay for VLP vaccines

Outline

- Overview of potency specifications
 - During development
 - At licensure
- Example of live virus vaccines
- VLP vaccines (HBV and HPV)
- HBV: Transition from in vivo to in vitro
- HPV vaccine (GARDASIL®)
 - Derivation of potency assay
 - Strategy for setting specifications using assay and manufacturing data
 - Clinical confirmation study
- Other approaches

Specifications

- Early development
 - Very lean data base on process, product and assay method
 - Estimated based on program needs
- Late development
 - Provides assurance that commercial lots match performance of key clinical lots
 - Contract with customer and regulatory agencies

Setting Potency Specifications

- Variety of approaches exist
- Complex challenge
- Ultimately contract between
 - Manufacturer
 - Regulatory agencies
 - Customer
- Presentation will focus on approaches to setting potency specifications
- Use of clinical data to set potency specifications for vaccines reflects quality by design principles

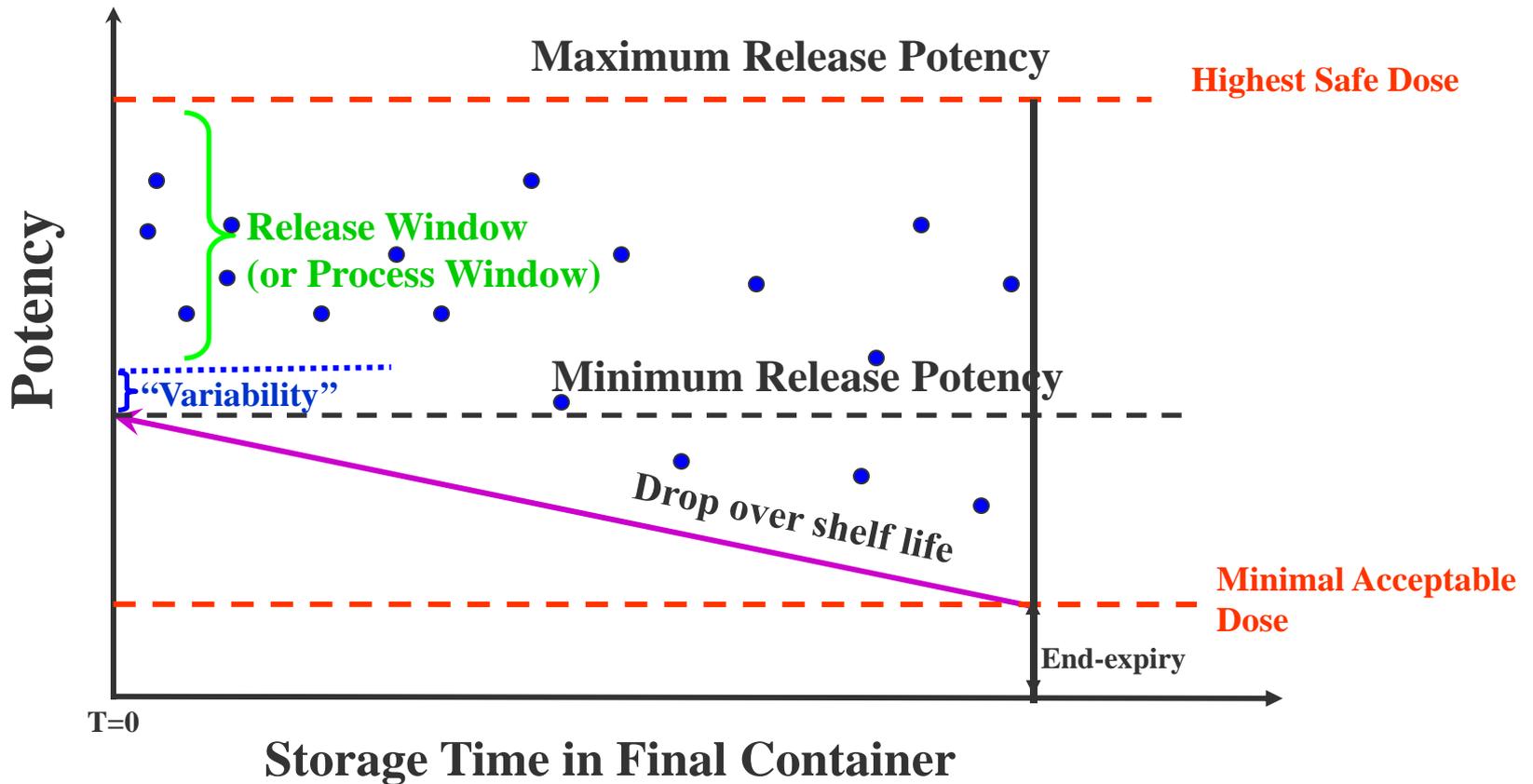
Live Virus Vaccines: Use Clinical Data for Setting Potency Specs

- Quality by Design Approach
- Potency is viral replication units
- Dose = Potency
- Many are unstable over shelf life
- Clinical data is integral part of release and specifications
- Process capability within window

Manufacture of Live Virus Vaccines

- Bulks manufactured
- Titers assessed
- Filling model
 - Diluted to target potency
 - Formulate/Fill/(Lyophilize)
- Test final containers

Potency Specification Model for Live Virus Vaccines



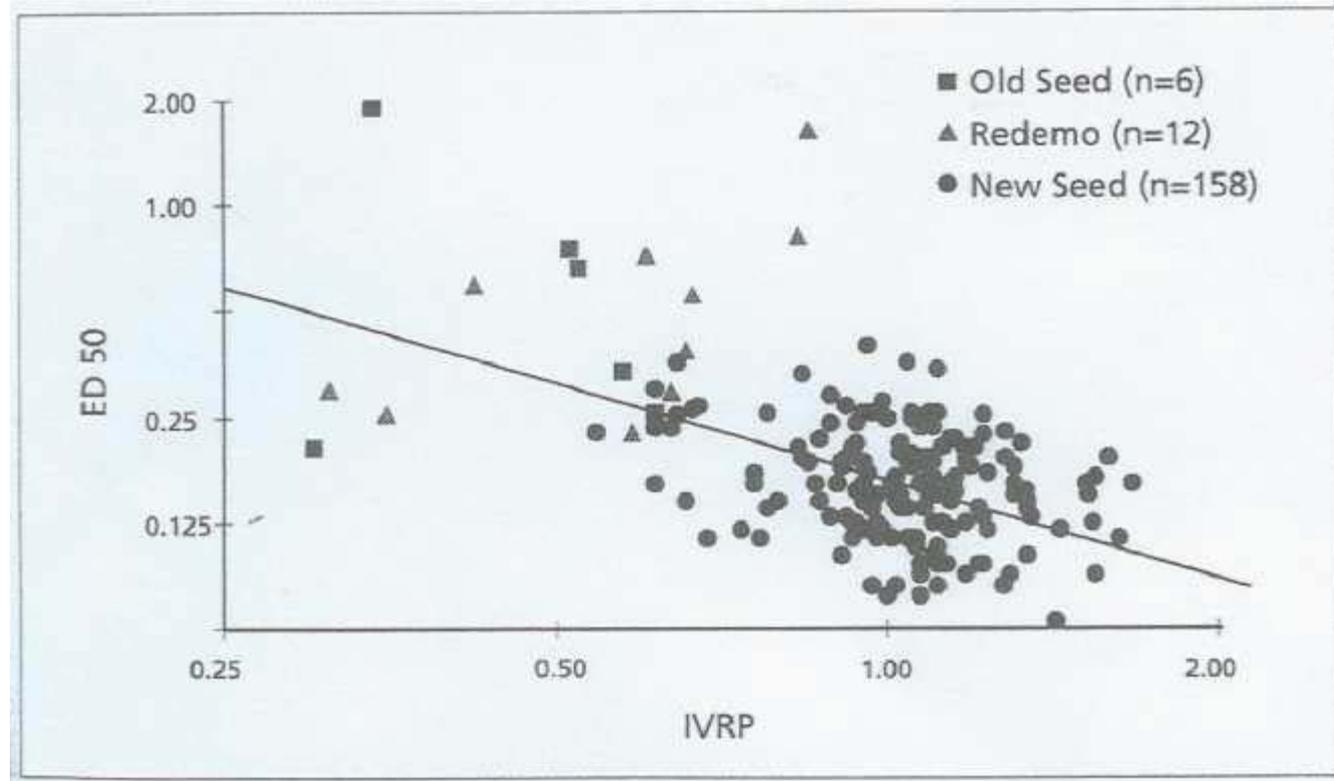
VLP Vaccines

- Recombinant products
- Several licensed examples
 - HBV
 - HPV
 - Flu
 - HEV
- Relatively stable
- Dose = protein mass (target for formulation)
- Typically “Dose” does not have specifications especially for multicomponent vaccines
- Potency = specific activity of each antigen
- Concept: Specification confirms that a commercial lot is not significantly different than pivotal clinical lots and will give the same response (often hidden or in the future)

Case Study: HBV

- Older licensed product with historical in vivo specifications
- Existing immunogenicity specifications
- In vivo assays highly variable
- When in vitro assays were introduced, specifications came from existing data by correlation
- Required extensive data set

Correlation Between Mouse Potency and the In Vitro Assay For HBV



Log log Correlation between Mouse Potency and in vitro Relative Potency (IVRP) assays for the recombinant HBV vaccine Recombivax HB®. ED50 values are in units of mcg antigen (Schofield, 2002).

Setting Specifications using Concordance

- Linear(Log-log) correlation established for ED₅₀ and IVRP
- Specification to predict (with some high degree of confidence) a sub-potent lot detected in the ED₅₀ Assay would be reflected as a sub-potent lot in the IVRP Assay.
- An upper 99% prediction limit (i.e., a statistical limit which predicts where a future determination in the ED₅₀ Assay is likely to fail) was developed from the linear relationship between IVRP and ED₅₀ .
- Limit incorporates linear relationship and testing variability.
- Thus, a measured IVRP greater than 0.5 ensures an ED₅₀ less than 1.5, the product specification determined in clinical trials with serum-derived Heptavax[®] vaccine.

(Schofield, 2002).

Setting Specifications Using Concordance and Prior In Vivo Specifications

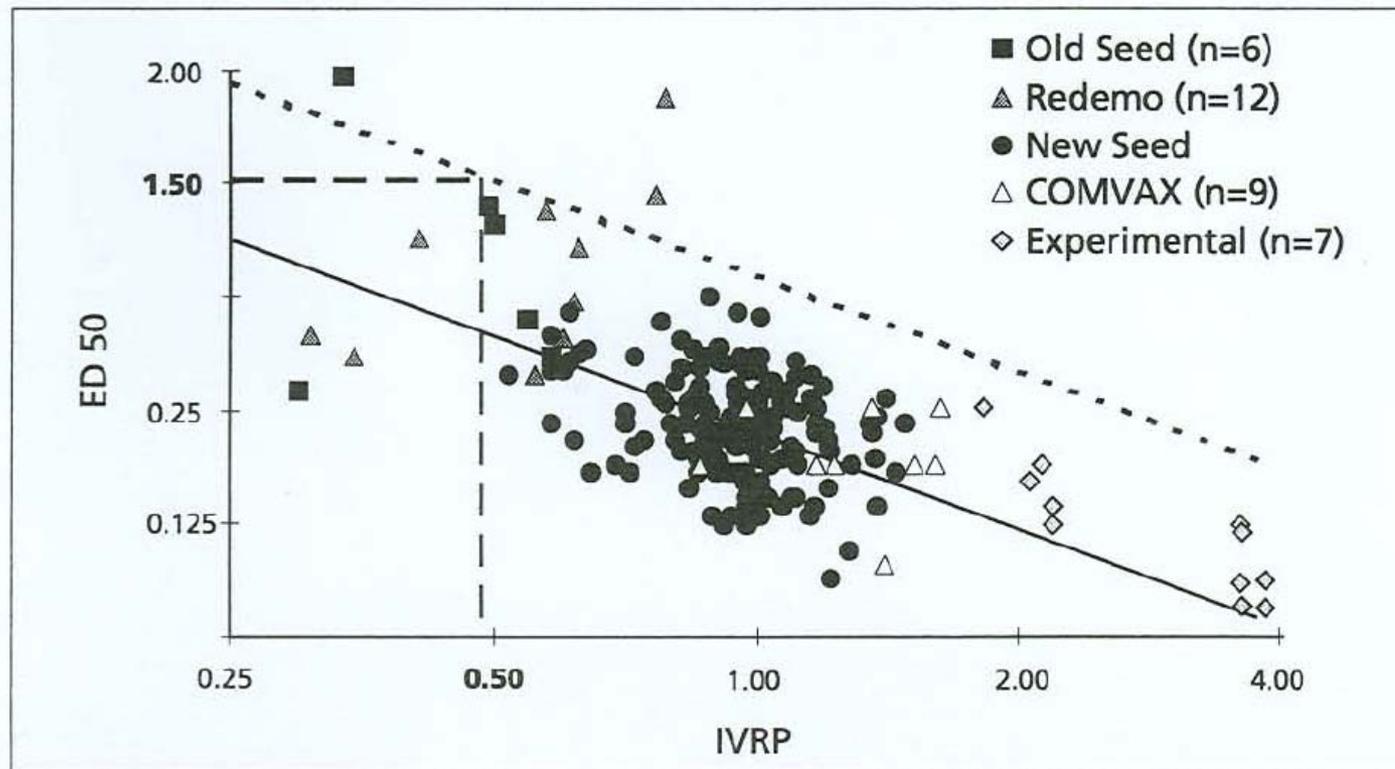


Fig. 3: Experiences with monovalent, combination, and experimental formulations of recombinant hepatitis B vaccines.

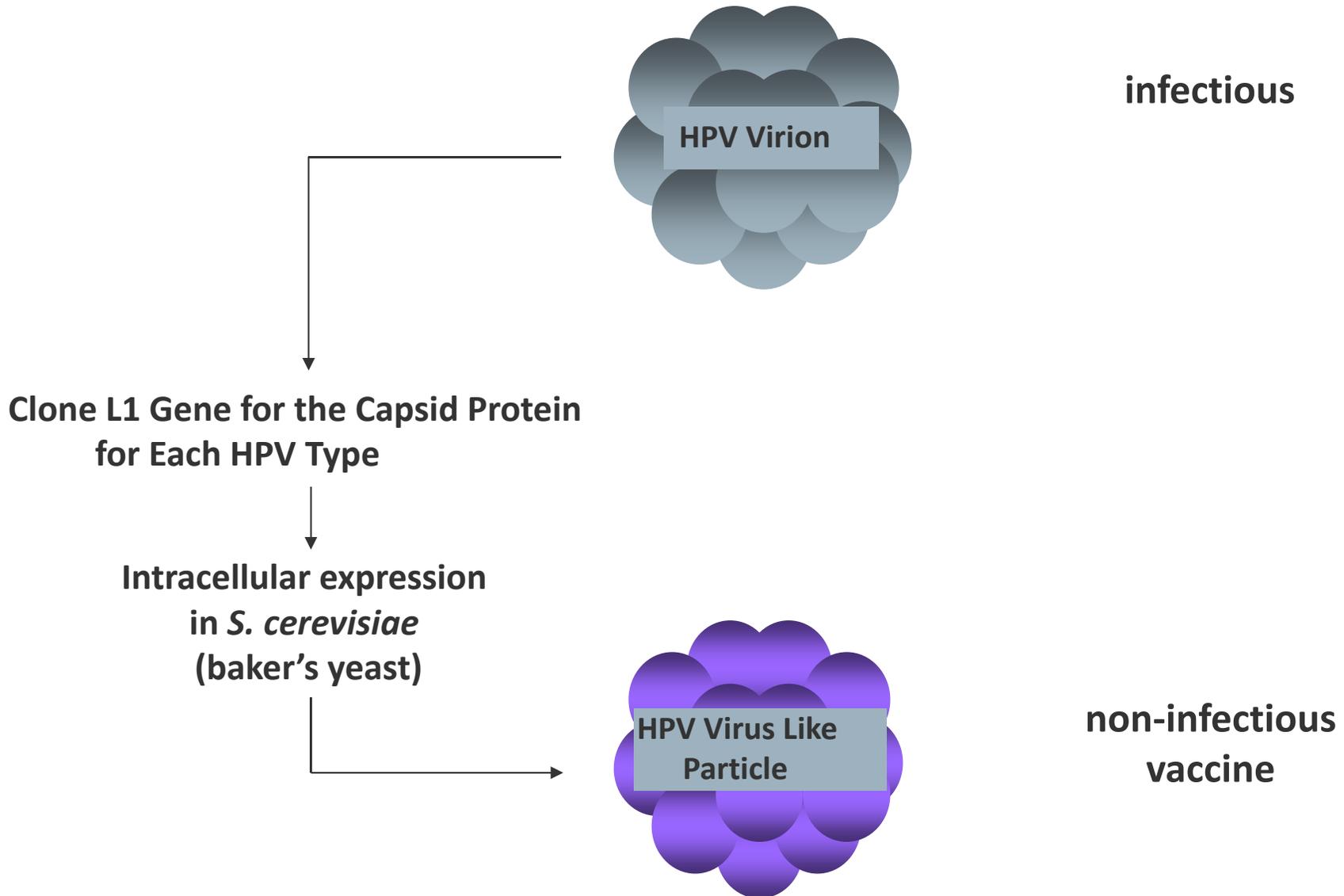
(Schofield, 2002).

GARDASIL®

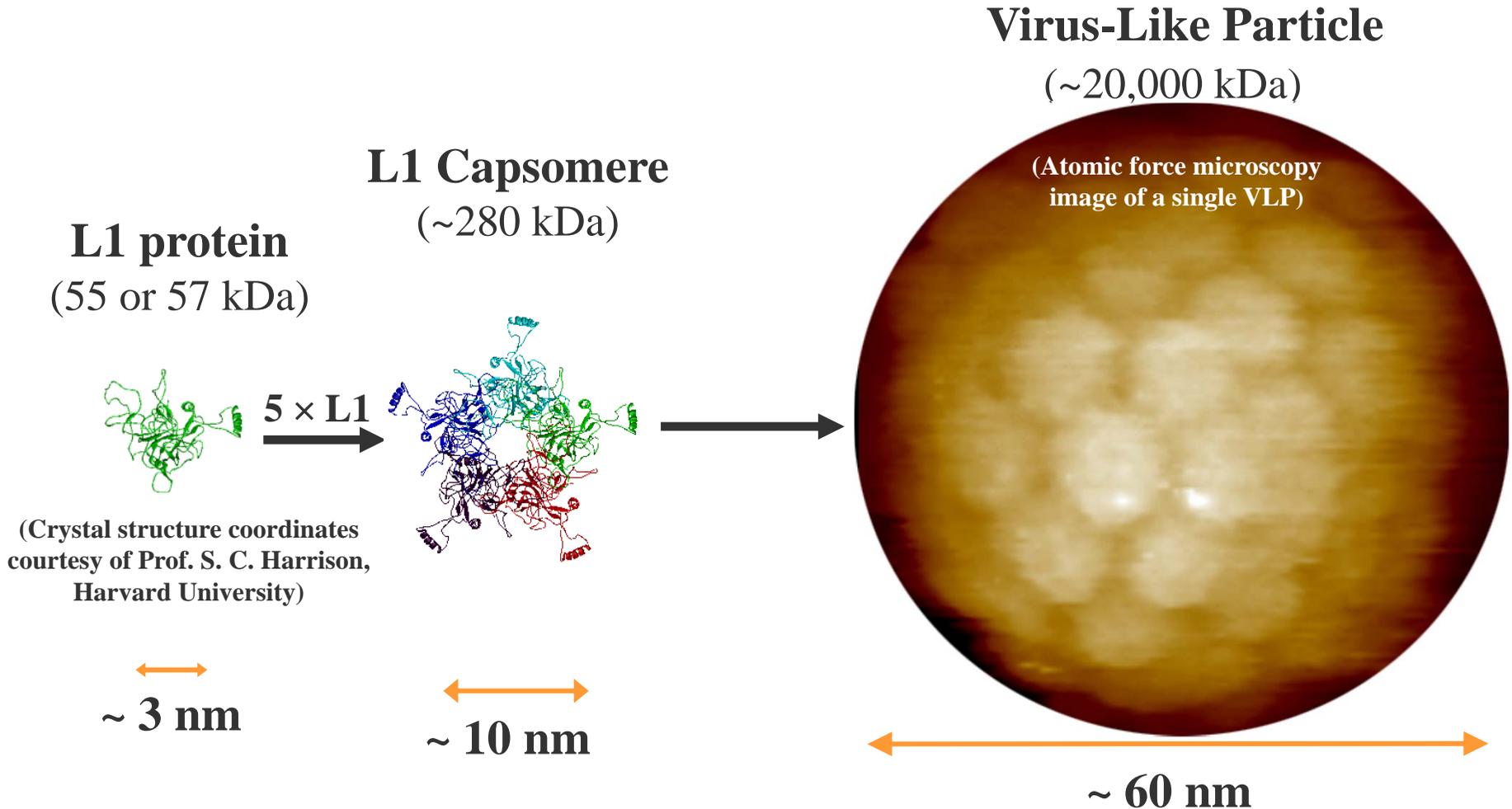
Merck's Recombinant HPV Vaccine

- VLPs manufactured in *Saccharomyces cerevisiae*
- Multicomponent
- Highly purified
- Well Characterized Vaccine

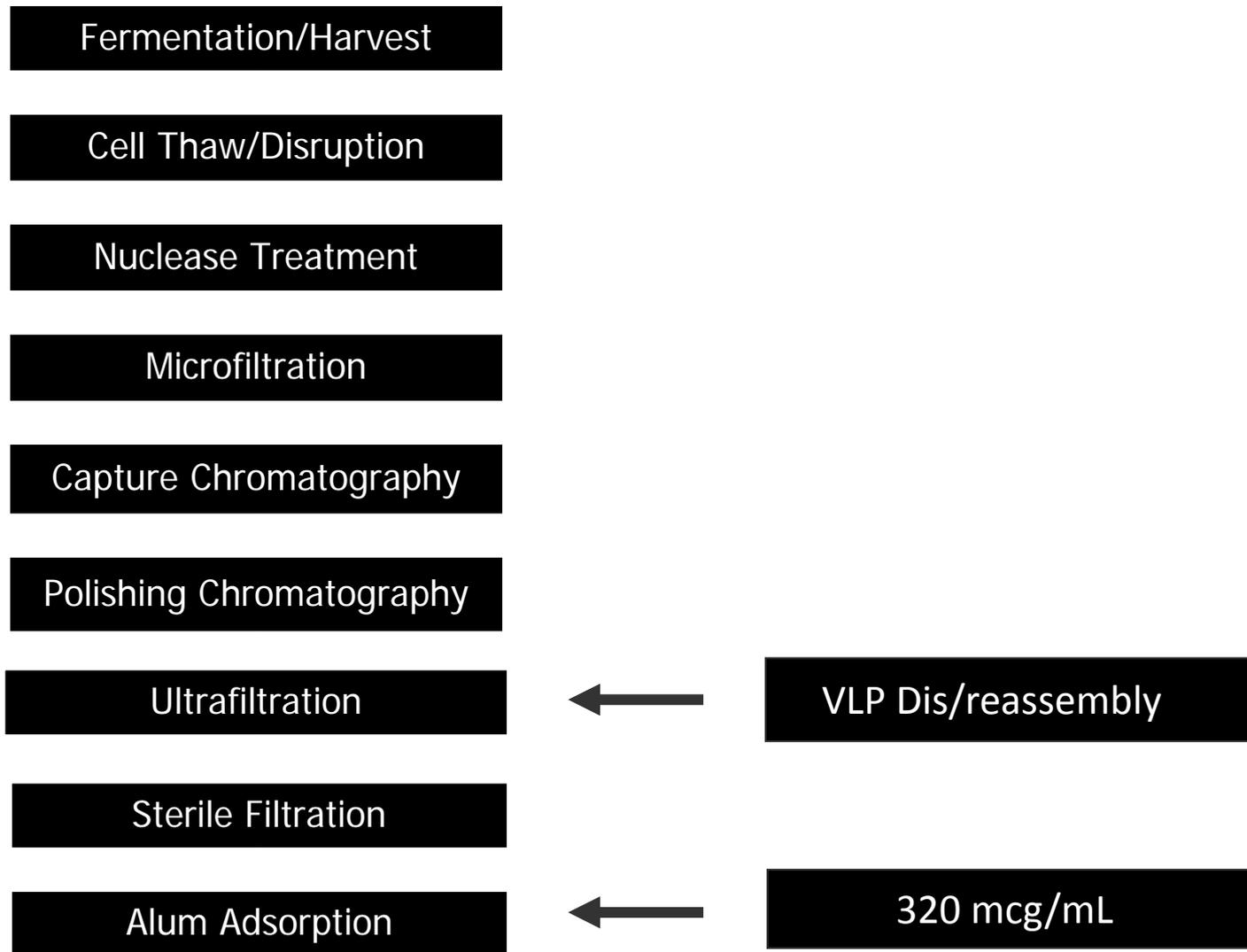
HPV Coat (Capsid) Proteins Expressed in Yeast



Structural Model of HPV VLP

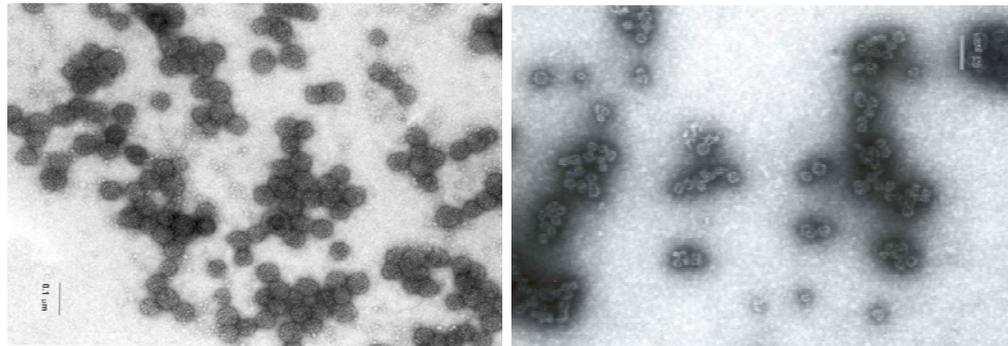
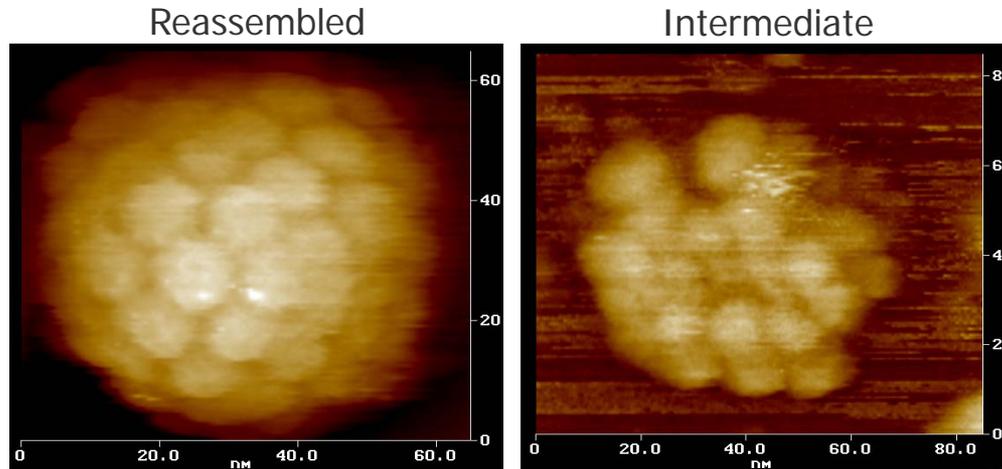


HPV VLP Manufacturing Process



*Volkin et al. US Patent 6,245,568, 2001 Jansen et al. US Patent 5,888,516, 1999;
Cook et al., Protein Exp. Purif., 17, 477, 1999*

VLP Characterization by Atomic Force and Transmission Electron Microscopy



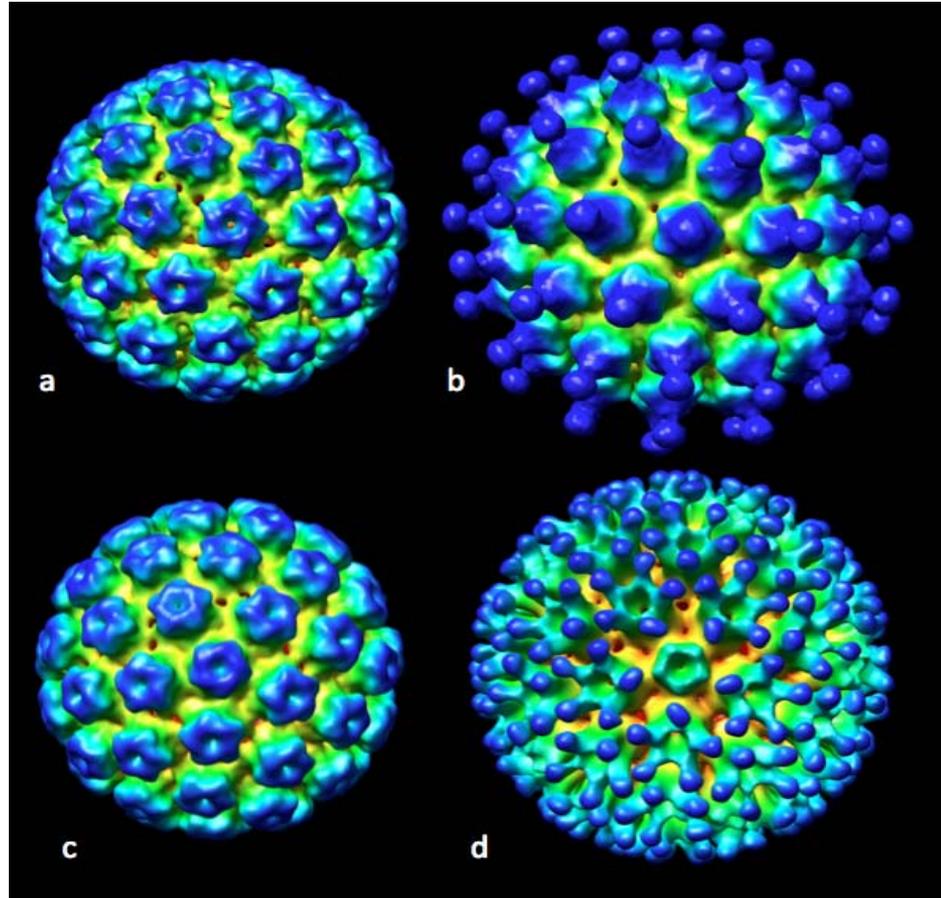
TEM Images of HPV-16

Changes in morphology of VLPs before and after disassembly/reassembly (D/R) as measured by negative-stain TEM and atomic force microscopy (Zhao et al. 2012b).

Development Strategy for an IVRP Assay

- Potency assay should be surrogate of clinical performance
- Used in vivo (mouse potency) during development
- Need extensive new data base to support in vitro test
- Unlike HBV example large historical in vivo data base and specifications not available
- Early decision to license with only in vitro potency assay
- Appropriate reagents available for an ELISA using type specific neutralizing monoclonals

Example of HPV Structure and Binding Sites for Monoclonal antibodies



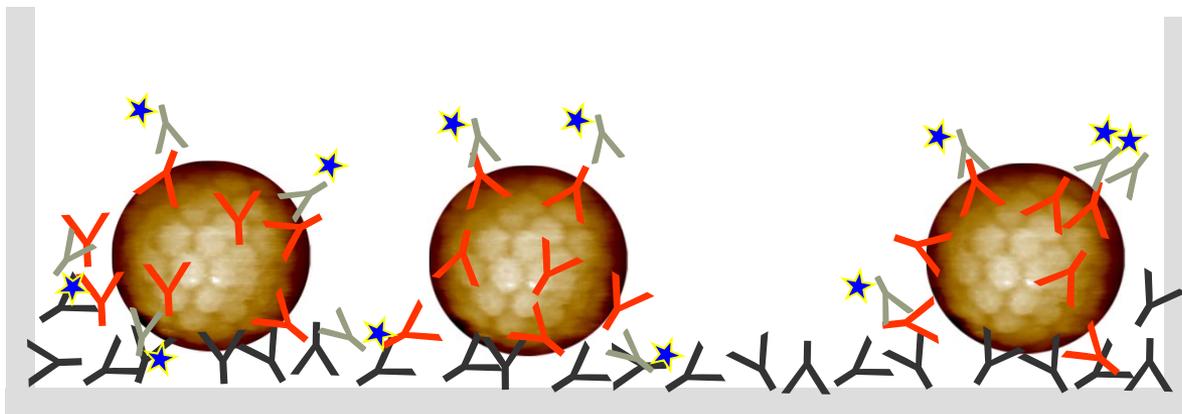
Three-dimensional reconstructions of a HPV-11; b HPV-11 decorated with antibody fragment H11.B2; c HPV-16; and d HPV-16 decorated with antibody fragment H16.V5 (Zhao et al. 2014).

In Vitro Relative Potency Assay

- ELISA technology
- Correlates with Mouse Potency
- Used for product release
- IVRP measures specific antigenicity
- For a VLP the assay measures number of epitopes on the surface of the icosahedral particle

In Vitro Relative Potency Assay

Sandwich enzyme immunoassay
(with reference standard and 4-parameter fit)



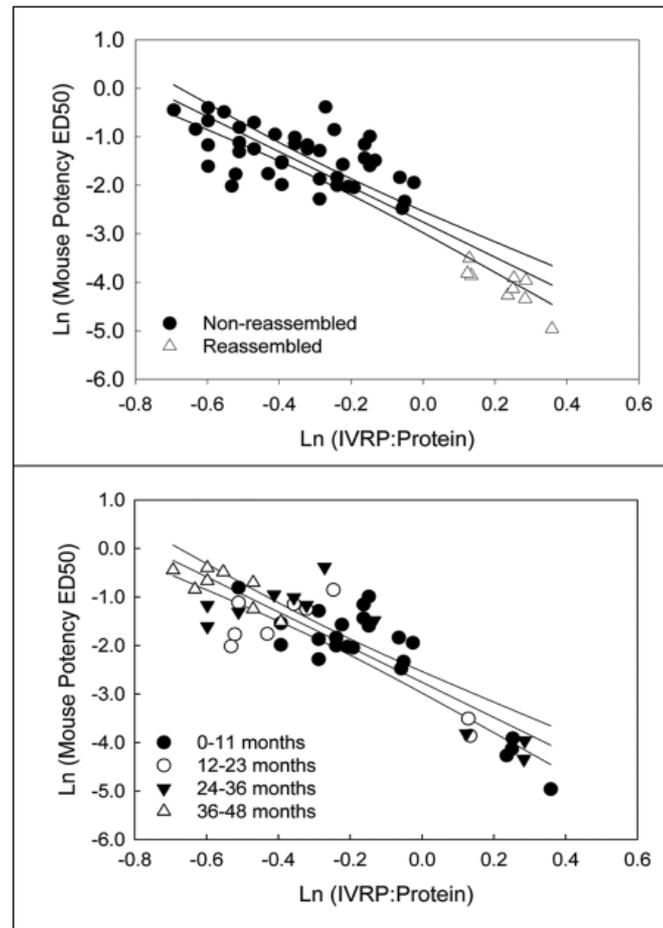
- ↘ VLP Type-specific capturing mAb
- Y HPV Type-specific, neutralizing mAb
- ★ HRP Conjugated goat *anti*-mouse IgG_{2b} antibody

IVRP Assay Implementation

- Relative potency format
- Reference lot = clinical lot
- Potency of first reference lot defined as its nominal protein dose values: 40, 80, 80, 40
- Expressed in Units/ml

(Shank-Retzlaff et al. 2005).

Correlation Between Mouse Potency and IVRP for Samples Containing Type 16 VLPs



Comparison of in vitro (IVRP) and in vivo (mouse potency) assays for HPV-16 in GARDASIL® ED50 values are in units of mcg antigen a plotted by sample type (non-reassembled vs reassembled) and b by sample age (months) (Shank-Retzlaff et al. 2005).

IVRP Correlates With Clinical Performance

Table 3 **Correlation between IVRP, Mouse Potency and Human Serology for HPV 16 VLPs**

Clinical Study	Sample Type	Number of Patients	IVRP:protein ^a	Mouse Potency ED ₅₀ (μg)	GMT ^c (95% Confidence Interval)
A	Non-reassembled	62	0.81	0.1	1979 (1576, 2485) ^b
B ^b	Non-reassembled	684	0.85 ^b	0.4 ^b	1519 (1386, 1655) ^b
C	Reassembled	76	1.27	<0.1 ^d	3494 (2996, 4076)
D	Reassembled	83	1.27	<0.1 ^d	3330 (2821, 3932)
E	Reassembled	85	1.27	<0.1 ^d	3601 (2982, 4349)



(Shank-Retzlaff et al. 2005)

IVRP Assay Conclusions

- Correlates with mouse potency assay
- Readily distinguishes between intermediate product and disassembly/reassembly product
- Correlates with human clinical data
- **Accepted by regulatory authorities**

Setting Specifications

Early Development – Many Clinical Lots

- Dose is in mcg protein (tight number)
- Primary Potency assay = Mouse potency
- Highly variable
- IVRP under development
- Mouse potency spec: >10%
- IVRP Spec: >50%
- Final dose established by clinical dose ranging

Setting Specifications

Late Development

- Theme: Assurance that commercial lots not less potent than clinical performance of pivotal lots
- Process/analytical capability used to set final specs
- Issue: Limited number of final container lots
- Issue: Limited stability data
- Solution: Propagation of error model

Statistical Approach

- The propagation of error model postulates that the final container IVRP is influenced by a number of factors, including
 - Bulk manufacturing variability
 - Bulk stability
 - Transfer and weighing
 - Formulation and fill
 - Assay variability
- The model postulates that these influences enter the model multiplicatively

Statistical Approach

- Data obtained on each influence are used to estimate its effect on the Final Container (FC) IVRP
 - m_i is the estimated mean of the i^{th} influence.
 - $\%RSD_i$ is the estimated %RSD of the i^{th} influence.
- From these estimates, the mean FC IVRP and corresponding %RSD can be derived using a propagation of error calculation:

$$\text{Mean FC IVRP} = m_1 \times m_2 \times \dots \times m_k$$

$$\%RSD \text{ FC IVRP} = \sqrt{\%RSD_1^2 + \%RSD_2^2 + \dots + \%RSD_k^2}$$

Capen R, et al. (2007) Establishing potency specifications for antigen vaccines. *BioProcess Int* 5:30–42

Statistical Approach

- A lower 3-sigma limit is then calculated based on the derived mean and %RSD of the FC IVRP

$$\text{Release Spec.} = \frac{\text{Mean FC IVRP}}{\left(1 + \% \text{RSD FC IVRP} / 100\right)^3}$$

Capen R, et al. (2007) Establishing potency specifications for antigen vaccines. *BioProcess Int* 5:30–42

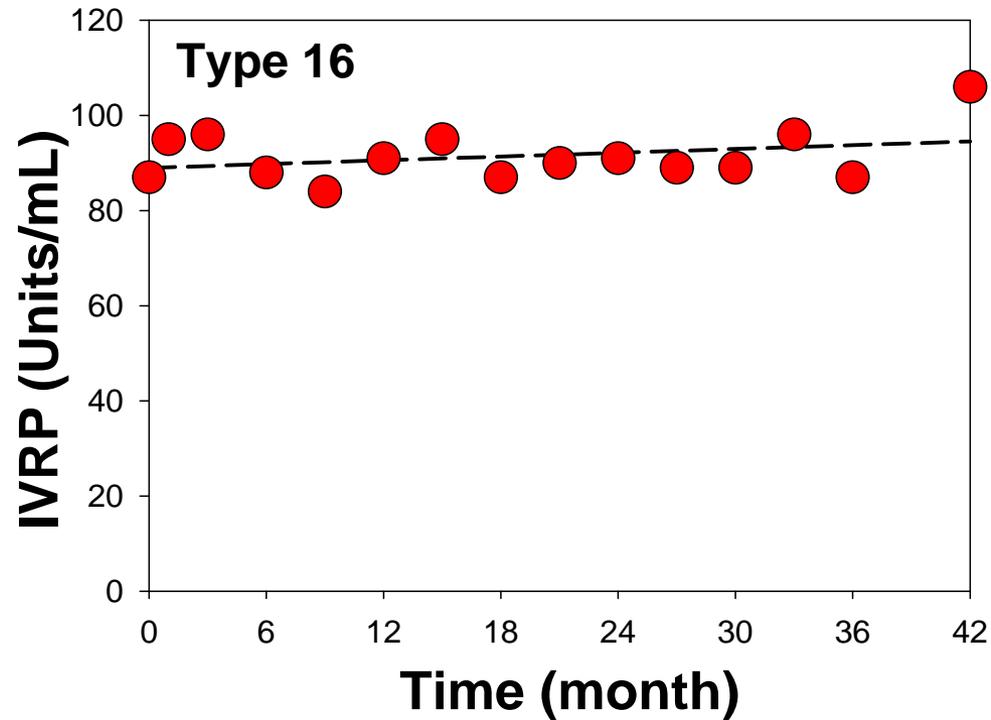
Estimates of Process Variability

- Bulk manufacturing:
 - Use IVRP data base of manufactured bulks
 - Sufficient number of bulks available for using 3 sigma approach
- Form//Fill
 - Limited numbers of final container lots
 - Estimate process variability for form/fill from other products

Estimate of Stability

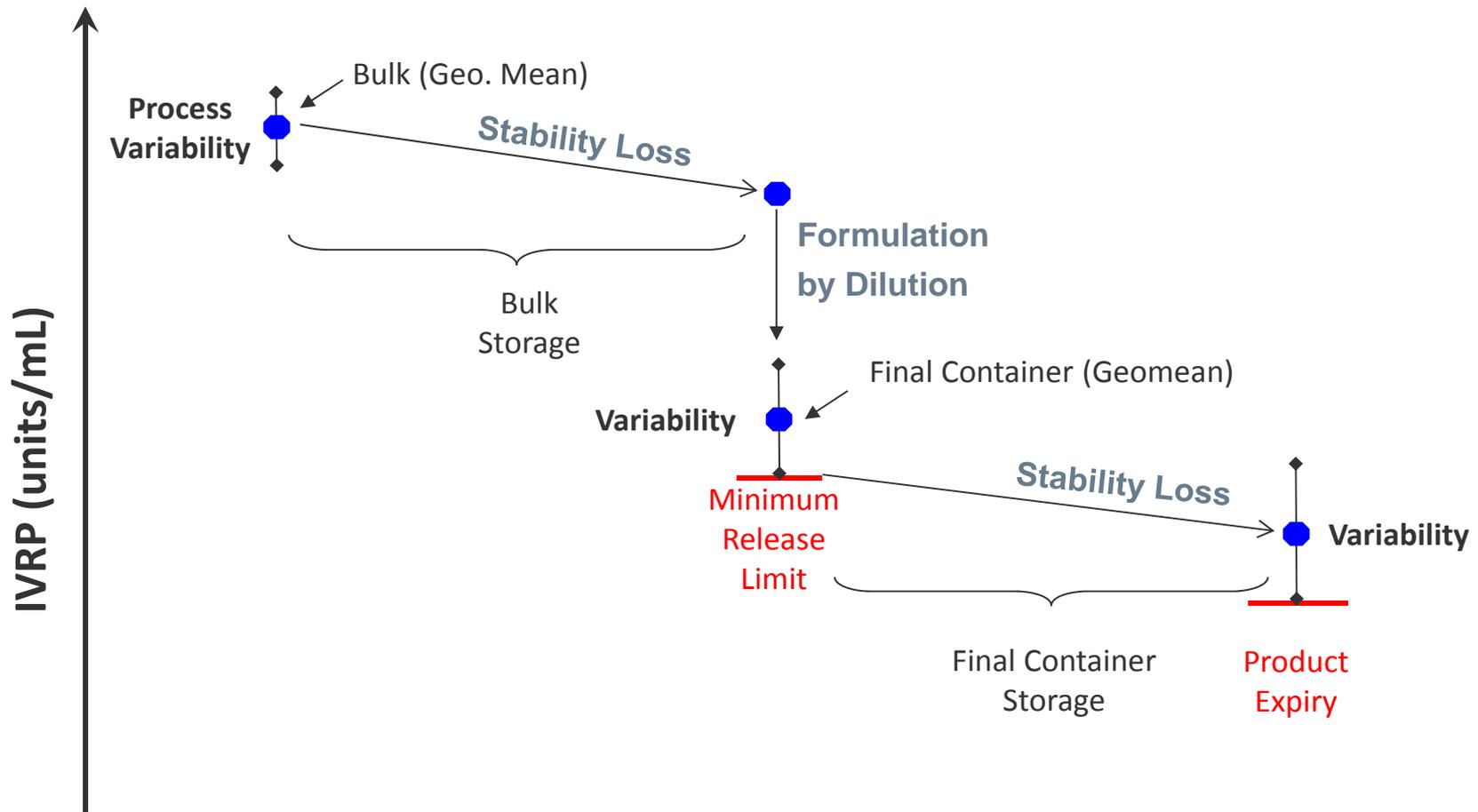
- Bulks and final containers remarkably stable
- No significant losses over 3 years
- Use stability loss at maximum hold time
- Statistical model used known assay variability and pooled data
- Estimate of maximum stability loss over shelf life

Stability of GARDASIL[®] Type 16 at 2-8°C



(Shank-Retzlaff et al. 2005).

Schematic of Method Used to Establish IVRP Specifications (Not drawn to scale)



Capen R, et al. (2007) Establishing potency specifications for antigen vaccines. *BioProcess Int* 5:30–42

Issues

- Wider specs come from processes and assays with wider variability
- Need for a reality check
- Need clinical data to confirm that the final specification is not near the edge of clinical performance
- Carried out potency ranging clinical study

Design of Clinical study

- Lower potency lots not available
- Simulated with down dosing
- Chosen to span potential release specs:
 - IVRP 100% = normal dose
 - IVRP 60% = 60% (mass) dose
 - IVRP 40% = 40% (mass) dose
 - IVRP 20% = 20% (mass) dose

Capen R, et al. (2007) Establishing potency specifications for antigen vaccines. *BioProcess Int* 5:30–42

Clinical Study Design

- Program licensure based on preventing CIN2/3
- No minimum surrogate of effectiveness
- Use serological immunogenicity relative to 100% lot
- Non inferiority hypothesis approach
- Clinical criteria need >2-fold drop to be significant

Capen R, et al. (2007) Establishing potency specifications for antigen vaccines. *BioProcess Int* 5:30–42

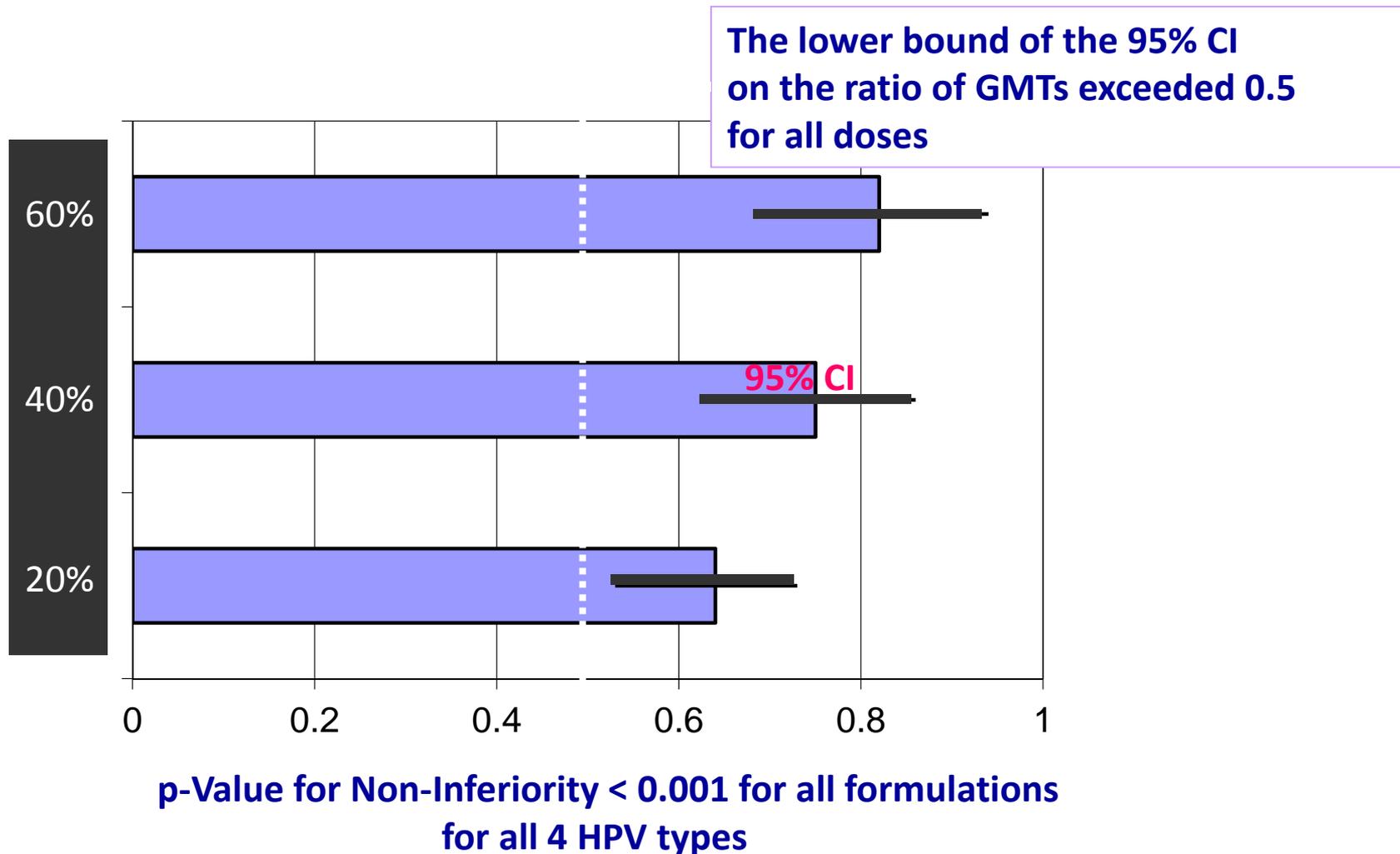
Study Design

- 2594 subjects enrolled from 61 centers located throughout 19 countries
 - non-pregnant, healthy, sexually-naïve girls aged 10-15
 - non-pregnant, healthy women 16 to 23
- 1:1:1:2 randomization to 20%, 40%, 60%, or 100% dose formulation of GARDASIL®
- Vaccination at day 1, months 2 and 6
- Serum obtained at day 1, and months 3 and 7
- Anti-HPV 6, 11, 16, and 18 responses summarized as geometric mean titers (GMTs) and seroconversion rates

Immunogenicity Objectives

- *Hypotheses*: at least 1 partial-dose formulation of the quadrivalent HPV vaccine induces non-inferior immune responses to that of the full-dose for each of HPV types 6, 11, 16, and 18
 - as measured by the GMTs at 4 weeks post-dose 3 (month 7)
 - as measured by the percentages of subjects who seroconvert four weeks post-dose 3 (month 7)
- Analyses were done per-protocol (subjects received 3 doses, had no major protocol violations, and were HPV 6, 11, 16 or 18 naïve through completion of the vaccination regimen)

Per-protocol analysis of non-inferiority comparing month 7 GMTs (HPV 16)



Capen R, et al. (2007) Establishing potency specifications for antigen vaccines. BioProcess Int 5:30–42

Clinical Results for Type 16

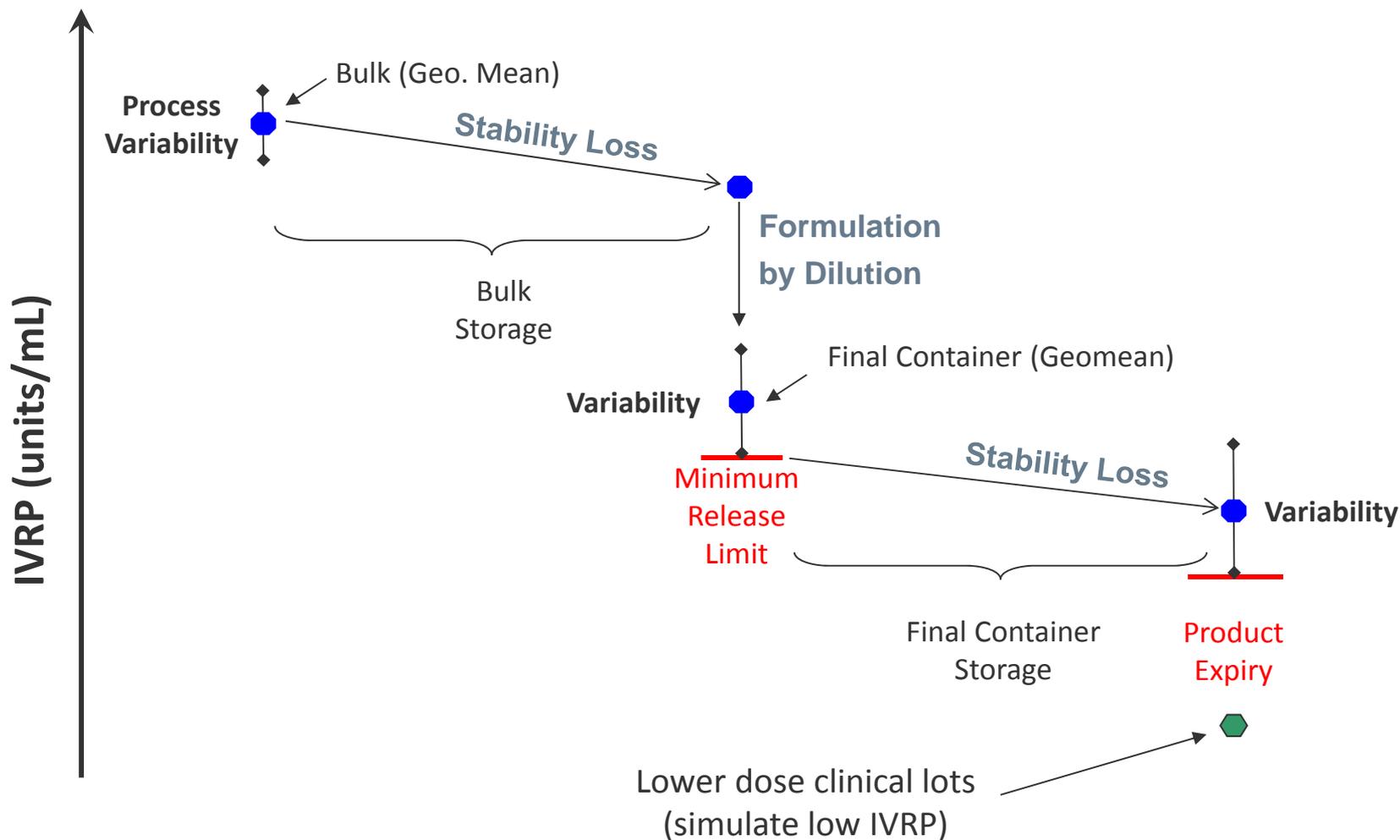
- Trend in GMT and seroprotection rates observed
- All lots were not statistically inferior in performance to full dose lot
- All subjects seroconverted
- Proposed final container specs are within range of acceptable performance

Capen R, et al. (2007) Establishing potency specifications for antigen vaccines. *BioProcess Int* 5:30–42

Setting the Specifications

- Proposed process capability and expiry specifications are tighter than potency range on clinical materials
- Thus the clinical data confirms that the proposed process capability and expiry specifications are appropriate

Schematic of Method used to Establish IVRP Specifications (Not drawn to scale)



Another Approach

- Express potency as “not less than”
- Requires over dosing
- Use ELISA to confirm minimum antigen content
- Actual dose is higher than nominal dose
- Question of how much higher to over fill
- Requires statistical analysis of process and analytical variability - perhaps 10-20% overage
- Reduces bulk manufacturing capacity (Doses/batch)

Simplest Approach – Arbitrary (Used for Drugs)

- Declare specification to be within a nominal value (eg 80-120%)
- Used for drugs and biologicals with tight assays
- Potency tests often have higher variability
- Risk of failure
- Still requires justification

Conclusions

- Rational specifications for a VLP vaccine can be proposed based on
 - Process capability
 - Analytical capability
 - Propagation of error in formulation
 - Stability Data
- Proposed specifications are within an appropriate range of clinical performance

Capen R, et al. (2007) Establishing potency specifications for antigen vaccines. *BioProcess Int* 5:30–42

Other Thoughts

- What if you don't have dose ranging data?
 - Use earlier dose ranging experience
 - Important to know where dose drop off is to assure potency of vaccine
- Make sure you understand your assay variability before setting specifications
 - Should come from assay validation experiments
 - Optimize assay format for best performance
- Include stability data in specification setting model
- Release and stability specifications should not be the same

Acknowledgements

Robert Capen

Heather L. Sings

Mary Retzlaff

Mark Esser

Greg Golm

Carlos Sattler

Anne Aunins

Yang Wang

Annie Sturgess

Michael Washabaugh

Tim Schofield