

Laboratory Platforms for Evaluation of HIV

Vaccines Jorge Flores, MD

**Deputy Director
Vaccine Research Program
DAIDS / NIAID / NIH**

Varese – Italy March 18, 2007



OUTLINE

1. DAIDS Laboratory support of Vaccine R&D
2. Key challenges
 - **Assay Validation**
 - **Reliability**
 - **Relevance**
 - **Understanding Correlates of protection**
 - **Determining Surrogates of benefit**
 - **Diagnosis of HIV In the post-vaccine era**
3. New DAIDS programs

Laboratory platform needs

- **Preclinical R&D laboratories to characterize products in animal models (safety, immunogenicity, protection)**
- **Clinical Trial Site Labs to diagnose HIV infection, conduct safety lab evaluations and process samples for immunological assays**
- **Central Laboratories to conduct assays to evaluate immunogenicity**
- **Qualified Reference Laboratories to confirm infection, distinguish infection from vaccination and examine potential surrogate endpoints**
- **QA, proficiency and reference reagents programs for above**

and laboratories that conduct basic research and develop new assays leading to a better understanding and identification of correlates of protection and surrogates markers of clinical benefit

Two Types of Assay Needs

R&D Exploration

- Immunogenicity:
 - “quality”, specificity, rate, magnitude and breadth of vaccine effect
 - Novel assay development
- Protection
 - Exploration of correlates of protection & surrogates of benefit

Definitive Evaluation

- Safety
 - all phases (incl. preclinical)*
- Diagnosis of Infection
 - all phases through post-deployment*
- End Points
 - Immunogenicity
 - phase I-II-III, bridging studies*
 - Infection, including
 - viral load
 - CD4 counts
 - clinical benefit
 - phase IIB-III*

Two Types of Assay Needs

R&D Exploration

- Immunogenicity:
 - “quality”, specificity, rate, magnitude and breadth of vaccine effect
 - Novel assay development
- Protection
 - Exploration of correlates of protection & surrogates of benefit

Require standardization

Definitive Evaluation

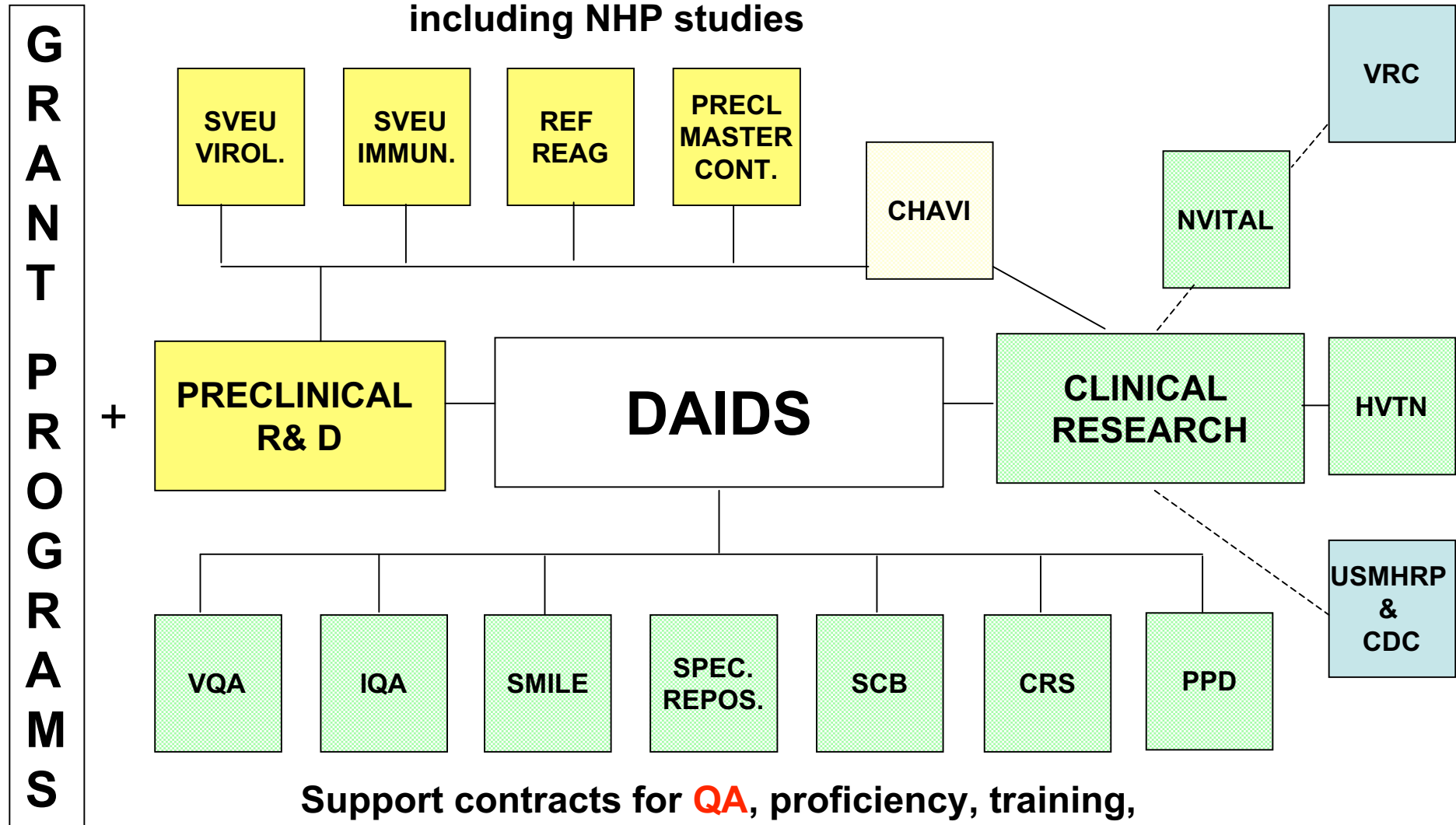
- Safety
 - *all phases*
- Diagnosis of Infection
 - *all phases through post-deployment*
- End Points
 - Immunogenicity
 - *phase II-III, bridging studies*
 - Infection, including
 - viral load
 - CD4 counts
 - clinical benefit
 - *phase IIB-III*
- **Product Comparability**

Require validation

Laboratory support for HIV Vaccine

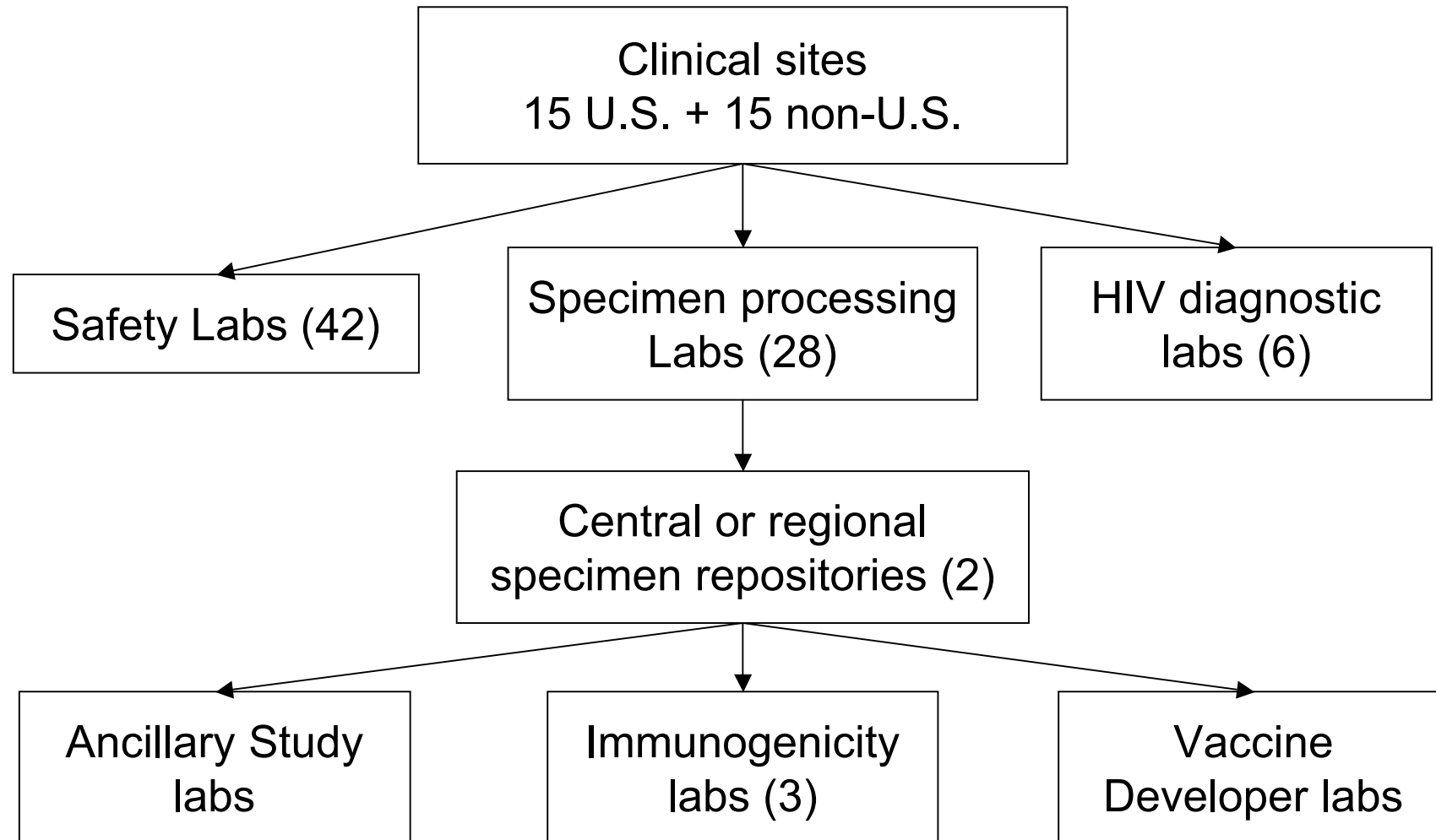
Development

Support contracts for preclinical evaluations, including NHP studies



Support contracts for **QA**, proficiency, training, **audits**, specimen storage, reagents etc.

Complexity of HVTN Lab Program



Validation 1

Reliability

- Data used to support vaccine licensure should :
 - “*demonstrate that the method employed is suitable for its intended purpose*”
 - be validated based on guidelines provided by ICH and FDA Guidance
- ICH Recommended Validation Characteristics:
 - Accuracy
 - Precision: Repeatability / Intermediate Precision
 - Specificity
 - Detection Limit
 - Quantitation Limit
 - Linearity
 - Range
 - Robustness
- Detailed SOPs, including materials, process, performance criteria, etc.
- Data maintained, documented and reported in accordance with GLP

QA and Independent Audits are key to success

Validation 2

Relevance

- Data from the assay has a relationship to a biological endpoint and offers meaning for scientific decision-making process.

Relevance

Examples

Antibodies to the Haemophilus influenza type b capsular polysaccharide:

- activate complement
- are opsonophagocytic
- are bactericidal
- afford passive prophylaxis
- have therapeutic efficacy

Other examples include Hepatitis B, Tetanus and Diphtheria toxoids, etc.

Successful vaccine development focused on the induction of such antibodies

Validated assays available for measuring immune responses

CELLULAR

- IFN γ ELISPOT (unfractionated PBMCs)
- 8-color ICS for IFN γ , IL-2, TNF- α , IL-4 in CD4 $^+$, CD8 $^+$ cells

HUMORAL

- Pseudovirus-based Neutralization
- ELISA-based assays to examine protein-specific responses

..... but are they of significant relevance?

to be learned when applied to samples from successful (or partially successful) vaccine trials

Additional Desirable T Cell Assays to Validate

- **Antigen-specific proliferation (e.g.: CFSE)**
- **Direct Killing (Chromium release)**
- **Viral Inhibition**

While potentially more relevant, these are complex bio-assays that require more manipulation than cells can tolerate.

Can existing assays be useful correlates of functional assays for regulatory purposes?

- **Does IL2 predict ability to proliferate?**
- **Does perforin release or CD 107 predict ability to kill?**
- **Does chemokine release predict ability to mediate viral inhibition?**

Additional Desirable Antibody Assays to Validate

- **PBMC-based neutralization**
- **Cell to cell neutralization**
- **Inhibition of viral replication in DCs, macrophages**
- **Post-entry neutralization**
- **ADCC**

While potentially more relevant, these are complex bio-assays

Can existing assays be useful correlates of function for regulatory purposes?

- **Can neut titers in the pseudovirus assay predict passive protection?**

The Current Goal: Understanding Correlates of Protection

*By using “best approximation” validated assays in
appropriately powered efficacy trials*

and

*By using well standardized **relevant** immunological
assays*

The Ultimate Goal: Understanding and Validating Surrogate of Protection Endpoints

*By using “best approximation” validated assays in appropriately powered efficacy trials **that evaluate clinical benefit:***

- *Viral Load Measures*
- *Central Memory CD4⁺ cells*
- *?*

What is a Surrogate Endpoint?

- A surrogate endpoint is a laboratory measurement used as a substitute for a **clinically meaningful endpoint** that measures directly how a patient feels, functions or survives.
- Changes induced by a vaccine or therapy on a surrogate endpoint are expected to fully reflect changes in a clinical meaningful endpoint

Example – CD4 Counts and Viral Load as Surrogate Endpoint in therapeutic trials

- CD4 lymphocyte counts widely used and accepted as a Surrogate End Point for progression to AIDS
- ZDV approved in 1987 based on 17 weeks survival
- ddI approved in 1991 based on SEP (CD4)
- Many other HIV drugs have been approved under this regulation
- From 1995 HIV RNA load was gradually more frequently used.

The Challenge of Diagnosis

- Distinguish Infection from Vaccination
 - In the course of trials
 - When vaccines are deployed
- Diagnose “transient infection” if it exists
- Detect / Titer virus in the reservoir

*Will require the development of validated,
licensable assays*

“Far better an approximate answer to the right question, which is often vague, than an exact answer to the wrong question, which can always be made precise”

John W. Tukey (1962)

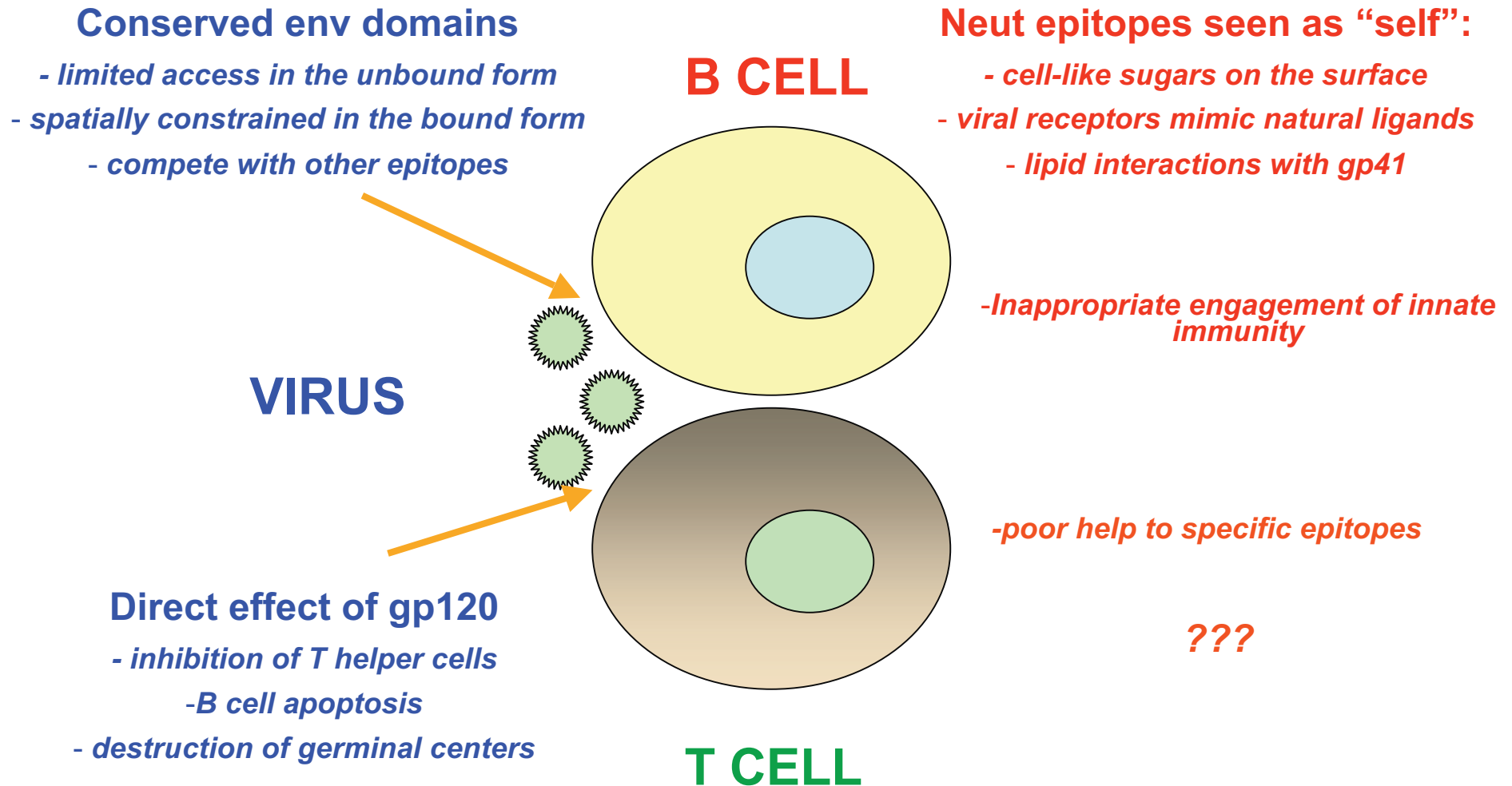
Annals of Mathematical Statistics 1962;33:1-67

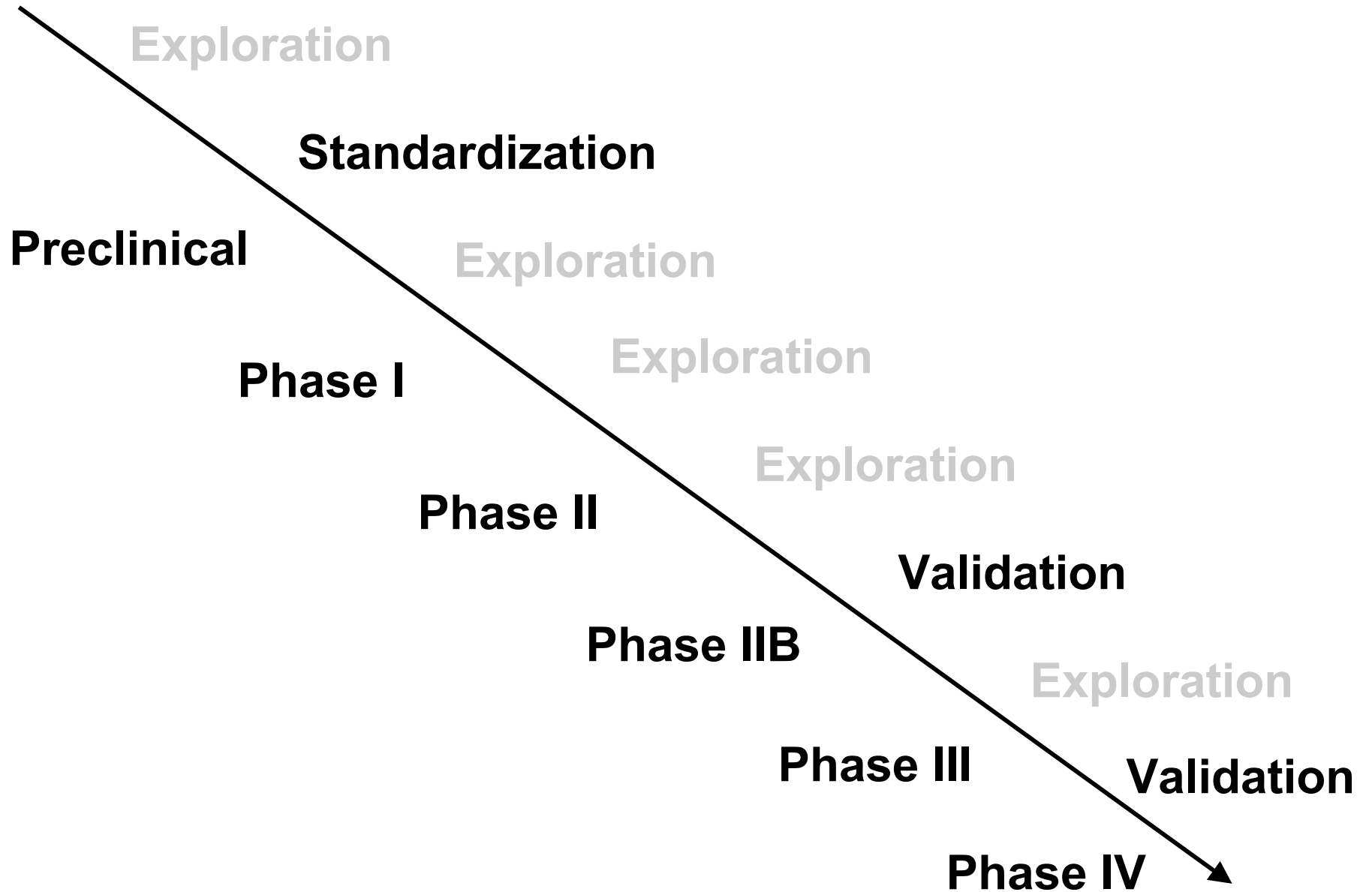
DAIDS-DAIT Team

(DAIT = Division of Allergy, Immunology and Transplantation)

- Apply advances in B cell immunology to problem of induction of broadly neutralizing antibodies
 - FY2007 1 yr supplements
 - FY2008 RFAs “to define basic immune mechanisms by which effect, robust Abs with broad reactivity can be induced”
 - <http://grants.nih.gov/grants/guide/rfa-files/RFA-AI-07-014.html>
 - <http://grants.nih.gov/grants/guide/rfa-files/RFA-AI-07-015.html>

Induction of neutralization responses to HIV is inefficient





Thanks!

- Patricia D'Souza
- Nina Kunwar

Lab Team VRP/DAIDS

Approaches to overcome limitations

