Phase-appropriate Bioassay Strategy to Support A Novel Vaccine Product Development

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Presentation Outline

♦ GS-4774
  – A novel cell-based vaccine for chronic hepatitis B therapy
  – Proposed mechanism of action (MOA)

♦ Potency support of GS-4774 product development
  – Phase-appropriate bioassay strategy
  – Scientific challenges in assay development
    • Antigen Content assays
    • Functional assays

♦ Summary and lessons learned
Introduction

- **Hepatitis B virus (HBV) infection is the leading cause of chronic liver disease**
  - ~350 million people are chronically infected worldwide with HBV

- **Current treatments for chronic HBV infection are controlling the viral replication by antiviral products**
  - Successfully suppress viral replication and effectively control the disease
  - <10% patients achieve permanent viral clearance
  - Life-long treatment is required for HBV suppression

- **The need for improved therapies for chronic hepatitis B**
  - A diminished T-cell response to HBV antigens plays a major role in the immuno-pathogenesis of chronic HBV infection
  - An immunotherapeutic approach capable of overcoming these immune deficiencies could potentially achieve the viral clearance in patients
GS-4774: A Yeast-based Immunotherapeutics to Chronic HBV Infection

♦ A yeast-based vaccine engineered to express a fusion protein comprising the most conserved regions of HBsAg, HBcAg and HBxAg
♦ Expresses a high intracellular level of HBV fusion protein
Active Immunotherapy with Yeast-based Vaccine

- Proposed mechanism of action (MOA)
  - Antigens are presented by dendritic cells after phagocytosis
  - Stimulates robust CD4+ T-cell response via MHC Class II
  - Activates HBV-specific CD8+ T-cell cytotoxicity (CTL) via MHC Class I

- GS-4774 product attributes
  - Yeast cell number
  - Ag Content
Challenges and Gaps for Potency Support of GS-4774 Product Development

♦ Challenges
  – **Time constrains**: bioassays transferred at phase II from GlobeImmune, and Phase III production was scheduled in < 1 year
  – **Limited understanding of product attributes**: novel cell-based vaccine with multiple active components

♦ Potency assays for Phase I/II lot release developed at GlobeImmune
  – Yeast cell number by Micro-Flow Imaging (MFI)
  – Ag Content by a quantitative Western blot (WB)

♦ Concerns and gaps
  – Imprecision of WB was not suitable for Phase 3 manufacturing support
  – No functional assays
  – Limited product characterization
Phase-appropriate Bioassay Development Strategy for GS-4774

♦ Initiate functional assay development
  – The quantitative or qualitative measurement of GS-4774-stimulated HBV-specific T-cell response in animal models or in cell-based assays

♦ Develop a reliable and robust Ag Content assay
  – Ag expression level is well accepted for routine lot release for vaccine products
  – In the absence of well-defined biological assays, a reliable analytical method for quantifying Ag expression becomes critical for evaluating vaccine product and ensuring manufacturing process consistency

♦ Establish correlation between Ag Content and product biological activity
  – Ag Content can be used to measure potency of GS-4774 and considered for routine lot release
    • ASSUMPTION: Entity’s mass is proportional to its biological activity

♦ Continuous efforts in developing a reproducible and robust bioassay
  – With a better understanding of clinical attributes during Phase 3 study
The Need for a Reliable Ag Content Assay

♦ Current lot release assay for Ag quantification: Western blot

Pros
– Suitable for insoluble proteins
– Sensitive and specific

Cons
– Semi-quantitative
– Narrow dynamic range
– Poor assay precision and reproducibility

The development of a reliable and robust Ag Content assay is critical
Ag Content Assay
Improvement and Redevelopment

♦ Improve specificity, precision, and reproducibility of current Western blot
  – Replace anti-HIS tag detection antibody with a specific HBV antibody
  – Evaluate Simple Western technology

♦ Explore LC-MS/MS-based absolute Ag quantitation
  – Use an isotope labeled peptide as internal standard

♦ Evaluate FACS-based intracellular Ag staining
  – Use HBV specific antibodies

♦ Develop an ELISA method
  – Quantify the Ag expression level in yeast cell lysate
ELISA Assay Development Challenges

♦ The target Ag is extremely insoluble
  – Yeast cells were treated in a harsh lysis buffer containing a high concentration of SDS

♦ ELISA Assay Buffer
  – Can be used to dilute the cell lysate significantly to minimize the SDS interference, and at the same time to keep the target Ag in soluble form

♦ Antibody pair selection
  – The majority of commercial anti-HBV antibodies didn’t react well with the extracted target Ag
ELISA for Quantifying Ag Expression Level in GS-4774 Yeast Cell Lysate

Yeast Cells → SDS-containing Lysis Buffer → Cell lysate → Non-denaturing Assay Buffer → Ag Solubilization

Ag Content value (ng/YU) calculated based on calibration standard

Data Analysis → ELISA Analysis
Method Correlation in Measuring Ag Content in Various Manufacturing Batches

<table>
<thead>
<tr>
<th>Method</th>
<th>Linear Correlation Coefficient ($R^2$)</th>
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<tbody>
<tr>
<td>WB: Anti-HIS</td>
<td>0.932</td>
</tr>
<tr>
<td>WB: Anti-HBV X</td>
<td>0.966</td>
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<tr>
<td>LC-MS/MS</td>
<td>0.956</td>
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</table>
ELISA is a Reliable Analytical Method for Quantifying Ag Expression Level in GS-4774

- **Specificity**
  - No interference from vector control yeast lysate and formulation buffer

- **Accuracy**
  - 80 – 125%

- **Repeatability and intermediate precision**
  - ≤ 20%

- **Assay Range**
  - 200 – 11000 ng/YU

- **Stability-indicating**
  - Ag Content decreases with the prolonged heat inactivation

![Graph showing Ag Content at 60°C over time](chart1.png)

![Graph showing 4°C Stability by ELISA](chart2.png)
Functional Assay Development

*in vivo* ELISPOT Assay

GS-4774-stimulated Ag-specific T-cell response in animal models (4-week assay)

- Mice are immunized with GS-4774 in multiple doses for 3 weeks
- Lymphocytes are isolated and stimulated with HBV peptides
- The Ag-specific T cell activation is measured by INF-γ ELISPOT assay
Correlation Between Ag Content in GS-4774 and in vivo Immune Response

- The results demonstrated that the magnitude of T-cell response in animals after vaccination was affected by the Ag Content in GS-4774

- The assay not suitable for routine lot release
  - Animal-based, highly variable, and 4-week assay

**In vivo IFN-γ ELISPOT Assay**

- [Graph showing spots per million lymphocytes for different peptide concentrations and yeast content]

- **Legend**:
  - High Ag Content Yeast
  - Low Ag Content Yeast
  - PBS
Continuous Efforts in Developing a Simple, Reproducible and Robust Bioassay for GS-4774

*in vitro* cell-based Ag presentation & T cell activation
(8-day assay)

- Monocyte-derived DCs can process GS-4774 and present HBV peptides by MHC I
- These APCs are co-incubated with CD8⁺ T cells engineered to express HBV TCR
- The engagement of MHC-peptide complexes with TCR can induce an Ag-specific T cell activation
Phase-appropriate Bioassay Development

- Bioassay development & implementation take place in a phase-appropriate manner, suitable for their intended use
- Bioassay development progresses along with clinical development, and the requirement for bioassays increases with the product development

“Product Lifecycle Approach to Potency Assay Development: A regulatory Perspective”
Gavin DK, PhD, Division of Cellular and Gene Therapies, CBER, FDA
CASSS Bioassays 2013
Summary and Lessons Learned

♦ Selecting, developing and implementing suitable potency assays for novel vaccine products represent a particular challenge
  – Complex biologics and multiple MOAs

♦ Defining a phase-appropriate bioassay development strategy can help mitigate certain challenges and strengthen overall product development
  – The bioassay should be fit for its intended use

♦ A bioassay requires continuous development and refinement
  – Bioassay development progresses along with the clinical development and a better understanding of relationship between clinical response and product attributes
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