

# Human Complement Serum Bactericidal Activity Following Vaccination in a Phase 2 Safety and Immunogenicity Study of a New Meningococcal Group A Conjugate Vaccine in Healthy African Toddlers Residing in the Meningitis Belt

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## Background

### Meningitis Vaccine Project

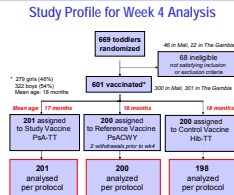
- The Meningitis Vaccine Project was created in June 2001 by a grant from the Bill & Melinda Gates Foundation as a 10-year partnership between WHO and PATH
- Goal of eliminating epidemic meningitis as a public health problem in sub-Saharan Africa through the development, testing, licensure, and widespread use of conjugate meningococcal vaccines

### PsA-TT Study 002 - Phase II

- Randomized Blinded Controlled Comparison of:
  - Group A conjugate vaccine, PsA-TT
  - Licensed polysaccharide vaccine, ACWY PS (Mencevax)
  - Haemophilus influenzae vaccine as control, Hib-TT
- Safety, Immunogenicity, Memory Induction and Antibody Persistence among African Toddlers
  - 12 to 23 months of age
- Recruitment completed at two sites (Sept.-Nov. 2006)
  - 601 eligible toddlers received primary immunizations;
  - 592 received booster immunizations (July-August 2007) after parental consent
- No significant safety issue, high compliance for blood sampling

## Study PsA-TT 002

### MVP Phase II – African Children 12 to 23 Months of Age



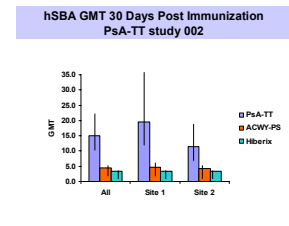
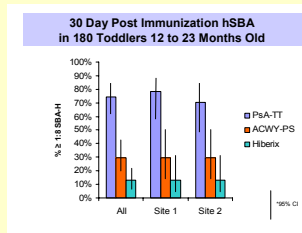
### hSBA Evaluation of MVP Phase II PsA-TT 002

- Subsets of each vaccine group
  - 60 / 201 PsA-TT
  - 60 / 200 PS ACWY
  - 60 / 200 Hib-TT
- Subset selection
  - Representative across age
  - Blinding maintained throughout
- 4 week Post Immunization Sera
  - Evaluate proportion above thresholds 1:4 and 1:8
  - Reverse cumulative distribution curves
  - GMT

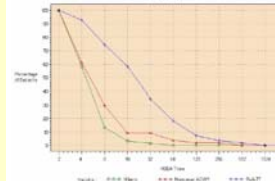
### Two Study Sites Located in Africa



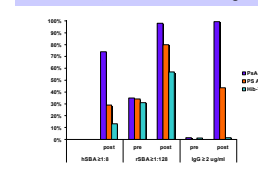
## Results: Post immunization hSBA



### Cumulative Distribution Curve for hSBA Post primary vaccination - IT population



### Threshold Analysis hSBA, rSBA and anti-A PS IgG



PsA-TT induced higher titer bactericidal antibodies in a greater proportion of study participants than the control Hib-TT or the meningococcal polysaccharide vaccines

## Methods - hSBA

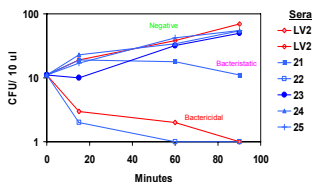
- Microtiter plate agar overlay assay
  - 20 ul diluted serum
  - Buffer = DPBS with Ca<sup>++</sup>, Mg<sup>++</sup>, Glucose, Gelatin
  - 10 ul bacterial suspension (1x)
  - 10 ul pooled human complement (lots 11-13)
  - 90 minute incubation, plates sealed, rotating, not CO<sub>2</sub> enhanced
  - 50 ul TSB semi-solid agar + 25 ul second overlay
  - Overnight incubation at 37 °C with 5% CO<sub>2</sub>
  - Titer = reciprocal of the highest dilution resulting in 50% or greater decrease in bacterial cell count compared to average in complement only wells

## Complement Source

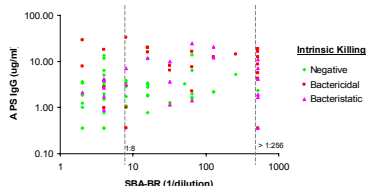
- 100 healthy adult blood donors
  - Age: 18-77 Average = 41, Median = 42
  - 68 Male/ 32 Female
  - Race: 74 C; 21 B; 4 H; 1 NA
- Samples
  - Three 10 ml tubes collected at time of blood donation
  - On ice prior to transport
  - Heated at 37 °C for 15 min, centrifuged place on ice
  - Aliquots (on ice) immediately frozen
  - Heat inactivated sample for ELISA and rSBA
- All samples tested for:
  - Intrinsic killing of strain F8238
  - rSBA
  - Anti-A PS IgG and IgGAM
- Subsets tested for
  - CH100, IgA, IgM, anti-class 4 Ab

### Group A Intrinsic Bactericidal Assay

Bactericidal, Bacteriostatic and Negative Samples



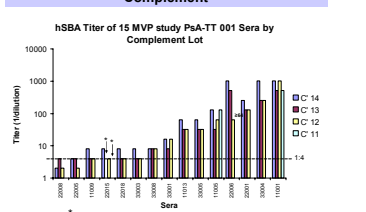
### Bactericidal Titer (rSBA), Anti-A PS IgG and Intrinsic Killing in NIH Blood Donor Sera



### Summary: NIH Donor Sera and Final Complement Selection Criteria

- Healthy Adult Blood Donors
  - No intrinsic killing in 52%
  - Intrinsic killing not predicted by rSBA or IgG
  - 31% were both intrinsically negative and had an rSBA titer 1:8 or less
- Anti-A PS antibody
  - Importance as screening criteria unclear
  - Arbitrary cut off of 30 ug/ml IgGAM
  - High antibody sera that met the intrinsic and rSBA-BR criteria combined in a fourth lot (lot 14)
- Complement Pools
  - Active C by hemolytic assay
  - Intrinsically negative

### Comparison of Four Lots of Pooled Human Complement



## Summary

- Human complement was obtained by pooling suitable sera from healthy adult blood donors screened for lack of functional antibodies to *N. meningitidis* strain F8238
- hSBA titers were successfully determined for a randomized subset of sera from study PsA-TT 002
- hSBA results indicate that PsA-TT induced functional serogroup A immune responses in a high proportion of African toddlers
- PsA-TT stimulated higher functional antibody responses by hSBA compared to Ps-ACWY vaccine at 30 days post immunization
- These results are consistent with and support results obtained with rSBA and ELISA assays conducted on the total study population

### Future Plans

- Continued evaluation of PsA-TT by hSBA:
  - Pre-, post-boost, and long term follow-up for the 180 subset
  - Subset of 2 to 29 year olds in three African study sites at pre-, post-, and persistence timepoints
  - Comparison studies of serogroup A immunologic assays
- Additional pooled human complement for hSBA:
  - Screening of blood donor sera for complement
    - Small volume approach will require screening approximately 300 blood donor sera
    - Pools of 7-10 individual sera that meet screening criteria