Genetic diversity of *Plasmodium falciparum* malaria vaccine antigens MSP-1 and AMA-1 at a malaria vaccine trial site in Mali

Mali Malaria Vaccine Contract
Mali ICI DR
USAID
• University of Bamako / Faculty of Medicine
  – Malaria Research & Training Center

• University of Maryland Baltimore
  – Center for Vaccine Development

• NIAID contract 1998-2006
  – Site development, molecular epidemiology, pathogenesis, immunology, Phase 1 adult trials of MSP-1 and AMA-1 vaccines

• ICIDR 2006-2011
  – Phase 1 and 2 trials of AMA-1 vaccine in children
Impact of genetic diversity on vaccine efficacy

- Current subunit malaria vaccine candidates based on one clone (3D7) or two (3D7 + FVO)
- Allele-specific efficacy may result in:
  - Initial protection only against homologous parasites
  - Directional selection for non-vaccine alleles
    - Declining efficacy over time
  - Region-specific efficacy
- Approaches:
  - Multivalent vaccines: Pneumococcus
  - Evolving vaccines: Influenza
  - Chimeric vaccines?
Baseline dynamics need to be measured to distinguish natural variation and drift from vaccine effects

Leading vaccine antigens:

- CSP: circumsporozoite protein
- MSP-1: merozoite surface protein-1
- AMA-1: apical membrane antigen-1
Previous studies

• Molecular evolution analyses suggest that blood-stage antigens MSP-1 & AMA-1 are under more immune selection pressure than CSP
• In vitro and animal studies suggest limited cross-protection
• 3D7-based CSP vaccine RTS,S did not select for non-3D7 CSP alleles in 2 Phase 2 trials
• 3D7-based MSP-1/MSP-2/RESA vaccine selected for non-3D7 MSP-2 alleles in Papua New Guinea
FMP1 vaccine antigen: MSP-1_{142}

- \textit{P. falciparum} merozoite surface protein-1
- Most abundant protein on merozoite surface
- Limited polymorphism in C-terminal region
  - 6 SNPs at codons 1644 (Q/E), 1691 (K/T), 1699 (S/N), 1700 (N/S), 1701 (G/R), and 1716 (L/F)
- Antibodies to 19 kD fragment block invasion of erythrocytes, associated with protection
Vaccine FMP1 + AS02A

- Lyophilized MSP-1 42 kD recombinant peptide expressed in *E. coli* at WRAIR
- 50 μg dissolved in 0.5 ml AS02A from GSKBio
- AS02A = Oil-in-water emulsion with 2 immune stimulants
  - Monophosphoryl lipid A
  - QS21 = saponin agent derived from the soap bark tree, *Quillaja saponaria*
- Well tolerated, immunogenic in Phase 1 & 2 trials in U.S.
Phase 1 FMP1/AS02A trial

- Healthy malaria-experienced adults age 18-55
- Double-blind, randomized controlled trial
- 20 each got FMP1/AS02A and rabies vaccine
- Immunizations at day 0, 30, 60
- Followed for one year to assess impact of natural transmission
- Anti-MSP-1_{42} antibody titers by ELISA
  - Allele-specific and subunit analyses
Vaccine trial PI: Mahamadou Thera MD MPH
MSP-1 genotyping methods

- Cohort study 1999-2001
- 100 subjects randomly selected within 3 age strata: < 5 years, 5-11 years, >11 years
- DNA extracted from 2,309 filter papers from monthly and sick visits
- MSP-1_{19} amplified by nested PCR
MSP-1 genotyping: Shannon Takala PhD
Pyrosequencing

- High throughput, real-time sequencing of short stretches of DNA around SNPs
- Quantifies proportions of alternative nucleotides at polymorphic amino acid positions
Haplotype estimation

• Haplotype-estimating algorithm
  – Maximum likelihood prediction of most probable combination of haplotypes in an infection based on:
    • Measured allele frequencies in the infection
    • Haplotypes known to be circulating in the population
    • Probability distribution of measurements errors
MSP-1_{19} genotyping results

- 2,309 samples collected from 100 persons
- 1,375 parasite-positive by PCR
- 1,369 genotyped by Pyrosequencing
- 11 previously known haplotypes detected
  - 4 known haplotypes not seen
- 7 new haplotypes detected
  - Identified by failure of haplotype-estimating algorithm to resolve haplotype
  - Pyrosequencing repeated, MSP-1_{19} re-amplified from original samples, cloned and sequenced to confirm
MSP-$1_{19}$ haplotypes in Bandiagara 1999-2001
Seasonal variation in MSP-1<sub>19</sub> haplotypes

All ages
Seasonal variation in MSP-119 haplotypes

Age<5 years

Prevalence

Jul Aug Sep Oct Nov Dec Jan 1999
Jul Aug Sep Oct Nov Dec Jan 2000
Jul Aug Sep Oct Nov Dec Jan 2001

QKSNGL (FVO)
EKSNGL (FUP)
ETSSRL (3D7)
QKNNGL
QKSNGF
EKSSRL
EKNNGGL
QTSSRL
ETSSGL
QKSSRL
ETSNGL
QTSSGL
QKSSGL
Seasonal variation in MSP-1_{19} haplotypes

Age 5-11 years

Prevalence

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Legend:
- QKSNGL (FVO)
- EKSNGL (FUP)
- ETSSRL (3D7)
- QKNNGL
- QKSNGF
- EKSSRL
- EKNNGL
- QTSSGL
- EKSSRL
- QKSSRL
- QKSSGL
- QKSSSL

Colors indicate different haplotypes. The graph shows the prevalence of these haplotypes across different months and years.
Seasonal variation in MSP-1<sub>19</sub> haplotypes

Age >11 years

Prevalence

July Aug Sep Oct Nov Dec Jan 1999

July Aug Sep Oct Nov Dec Jan 2000

July Aug Sep Oct Nov Dec Jan 2001

Legend:
- QKSNGL (FVO)
- EKSNGL (FUP)
- ETSSRL (3D7)
- QKNNGL
- QKSNFG
- EKSSRL
- EKNNGL
- QTSSRL
- EKSSRL
- QKSSRL
- ETSNGL
- ETSNGL
- QTSSGL
- QKSSGL
Allele-specific antibody responses to MSP-1$_{42}$ after immunization
Apical membrane antigen-1
AMA-1

- 83 kD protein expressed in micronemes
- Antibodies block invasion, inhibit growth, associated with protection
- Highly polymorphic
  - 38 SNPs in domain 1
- Domain 1 polymorphisms under balancing selection
  - Suggests importance in immunity
The FMP2.1/AS02A malaria vaccine

- Recombinant protein expressed in *E. coli*
  - Ectodomain of *P. falciparum* 3D7 AMA-1, amino acids 83-531
  - Manufactured under GMP at BioProduction Facility, WRAIR
- Adjuvanted with AS02A
Study design

- Double-blind, controlled, dose escalation
- Randomized 2:1 in 2 staggered cohorts of 30
  - Cohort 1
    - 25 μg FMP2.1/0.25 ml AS02A, N = 20
    - Rabies, N = 10
  - Cohort 2
    - 50 μg FMP2.1/0.5 ml AS02A, N = 20
    - Rabies, N = 10
- Interim safety assessments between doses
Geometric mean antibodies to AMA-1

![Graph showing geometric mean antibodies to AMA-1 over study days with different immunization regimens.](image-url)
Dynamics of AMA-1 sequence variation

- Samples from 1999-2001 cohort study
- AMA-1 not amenable to Pyrosequencing
- AMA-1 sequenced from 271 filter papers from symptomatic and asymptomatic infections in 1999, 2000, 2001
Dynamics of AMA-1 sequence variation

- 31 polymorphic amino acid residues
- 179 unique haplotypes among 271 samples
- Haplotype frequencies <1% to 10.8%
- Most common haplotype identical to 3D7
- No haplotypes identical to FVO
Percent of 1999 infections
n=89

Percent of 2000 infections
n=93

Percent of 2001 infections
n=89
Summary

• MSP-1
  - 18 haplotypes (7 new) in 1,369 infections
  - Population stable over time
    • FVO and FUP most common in all years and age groups
  - SNG at 1699, 1700, 1701 account for 80%
  - FVO vaccine might have higher initial efficacy

• AMA-1
  - 179 haplotypes in 271 infections
    • All infrequent, extreme variation
  - 3D7 most common in all years at 10%
  - Vaccine will need to be cross-protective
Next steps

- Pediatric Phase 2 trial of MSP-1 in Kenya
  - Results due soon
- Pediatric Phase 1 trial of AMA-1 vaccine 2006
- Pediatric Phase 2 efficacy trial of AMA-1 vaccine 2007
  - Impact of genetic diversity on vaccine efficacy
- If vaccine efficacy is allele-specific, vaccine developers will try to re-engineer more universally protective vaccines
  - Rational combination of clones based on identification of key codons in initial efficacy trials
  - Chimeric “super antigen”?
Thanks to

CVD Malaria Section
- Shannon Takala
- Amed Ouattara
- Kirsten Lyke
- Aric Gregson
- Paul Sehdev
- Nicole Eddington
- Jean-Claude Akpa
- Linda Rosendorf

Bandiagara District Hospital
- Issa BenZacour
- Mahamadou Cisse

MRTC Mali
- Ogobara Doumbo
- Mahamadou Thera
- Dapa Diallo
- Drissa Coulibaly
- Abdouyale Kone
- Ando Guindo
- Karim Traore
- Sory Diawara
- Amagana Dolo
- Alassane Dicko
- Issaka Sagara
- Yacouba Cissoko
- Lassana Sangare
- Modibo Daou
- Seydou Coulibaly
- Charles Arama
- Mady Cissoko
- Mounirou Baby
- Issa Diarra
- Abdoulaye Djimde
- Hamar Traore
- Mamadou Dembele
- Mahamadou Cisse
- Danzele Coulibaly
- Sekouba Marico
- Amadou Arama
- Moctar Traore
- The BMP team

WRAIR
- Gray Heppner
- Ann Stewart
- Evelina Angov
- Jeff Lyon
- David Lanar
- Sheetij Dutta

GSK
- Amanda Leach
- Alfred Tiono
- Alex Owusu
- W. Ripley Ballou
- Joe Cohen
- Marie-Claude Dubois

NIH FIC
- Dave Smith

NIH MVDB
- David Diemert
- Louis Miller
- Dick Sakai

NIH DMID
- Abdi Naficy
- Lee Hall

Support
- NIH contract N01AI85346
- NIH ICIDR
- NIH MVDB
- USAID (genotyping)
- WRAIR (antigen)
- GSK (adjuvant)