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Malaria Vaccine GMZ2 Pre-Clinical Selection and early Clinical Development

IP.2007.31100.001

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The SSI malaria vaccine approach is based on the study of natural immunity

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Reality

Chronic infection
1-3% death rate
Immunity acquired slowly

≠

Models

Acute infection
High death rate
Immunity acquired fast

The SSI Malaria vaccine project

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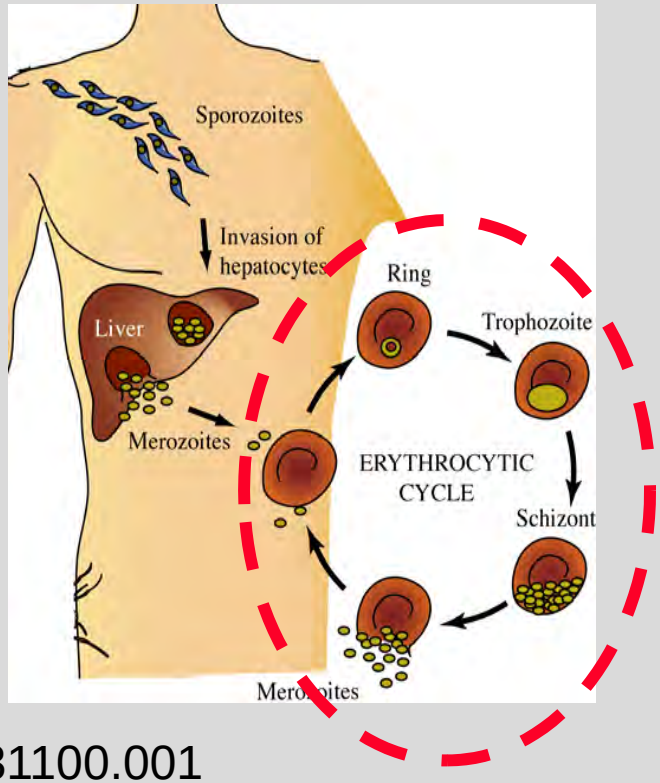
AIM :

To develop a recombinant subunit vaccine against the blood stage of malaria that will prevent or reduce the clinical manifestations

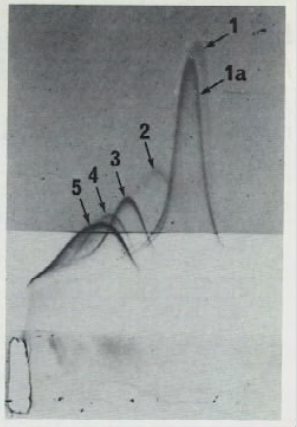


Working hypotheses :

1. **Disrupt parasite development process**
2. **Mimic natural immunity**

Selection of GLURP was based on analysis of the human immune response to *P. falciparum* malaria

Serum pool

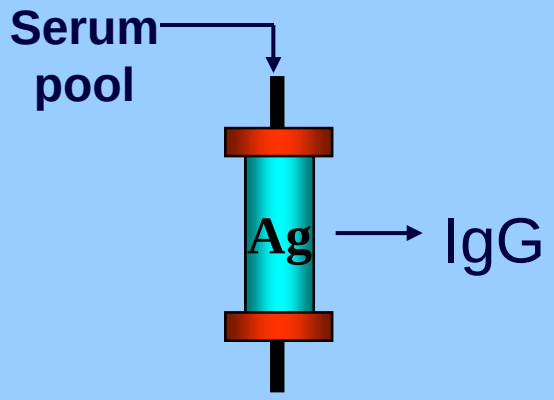


2’

soluble Ag ⟶ 1’

Crossed immunoelectrophoresis of soluble Ags identified six major antigen-complexes

GLURP = Ag3



Affinity-column with soluble Ag

Human IgG affinity-purified against soluble Ags inhibits parasite growth in vitro

Jepsen, S. 1983. Scand. J. Immunol. 18:567-571

GLURP and MSP3

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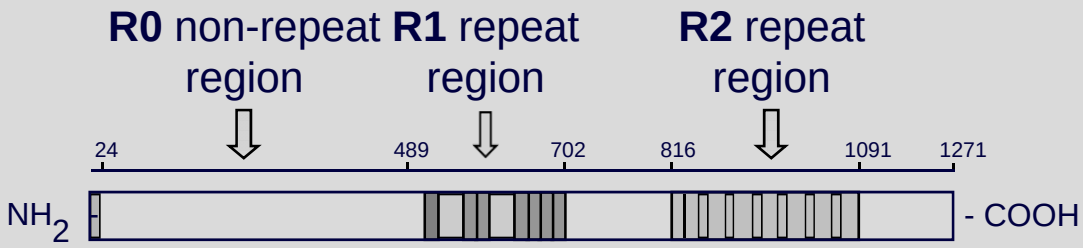
1991	GLURP discovered and patented by SSI
1991	GLURP discovered by Walter Reed but not pursued because of SSI patent
1993	MSP3 (SPAM) discovered by R. Anders et al
1994	MSP3 discovered and patented by IP
2003	GLURP-MSP3 hybrid (GMZ2) patented by SSI

The GLURP molecule

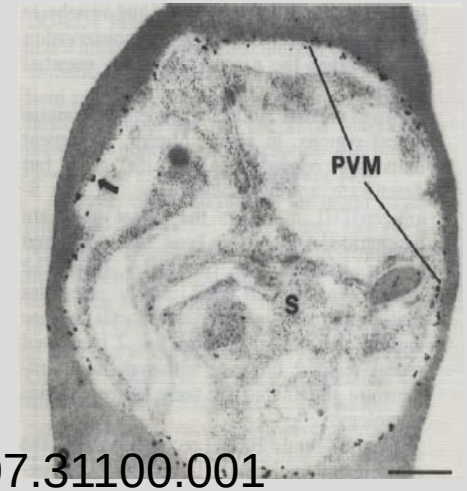


GLURP is found:

a) in the PV membrane of a Pf-schizont

b) on the surface of newly released merozoites

c) in the Parasitophorous Vacuole of a Pf-infected hepatocyte



merozoite

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Borre, M., et. al. 1991. Mol. Biochem. Parasitol. **49**:119-132

Prospective immuno-epidemiological study Dodowa, Ghana (NMIMR))

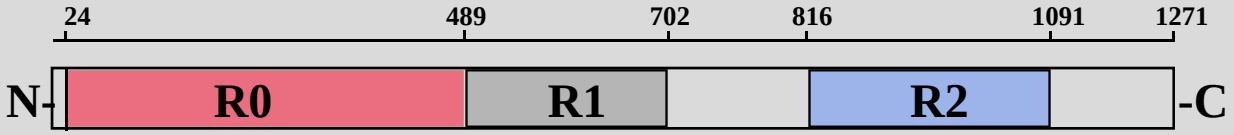
Do natural immune responses against GLURP correlate with protection ?

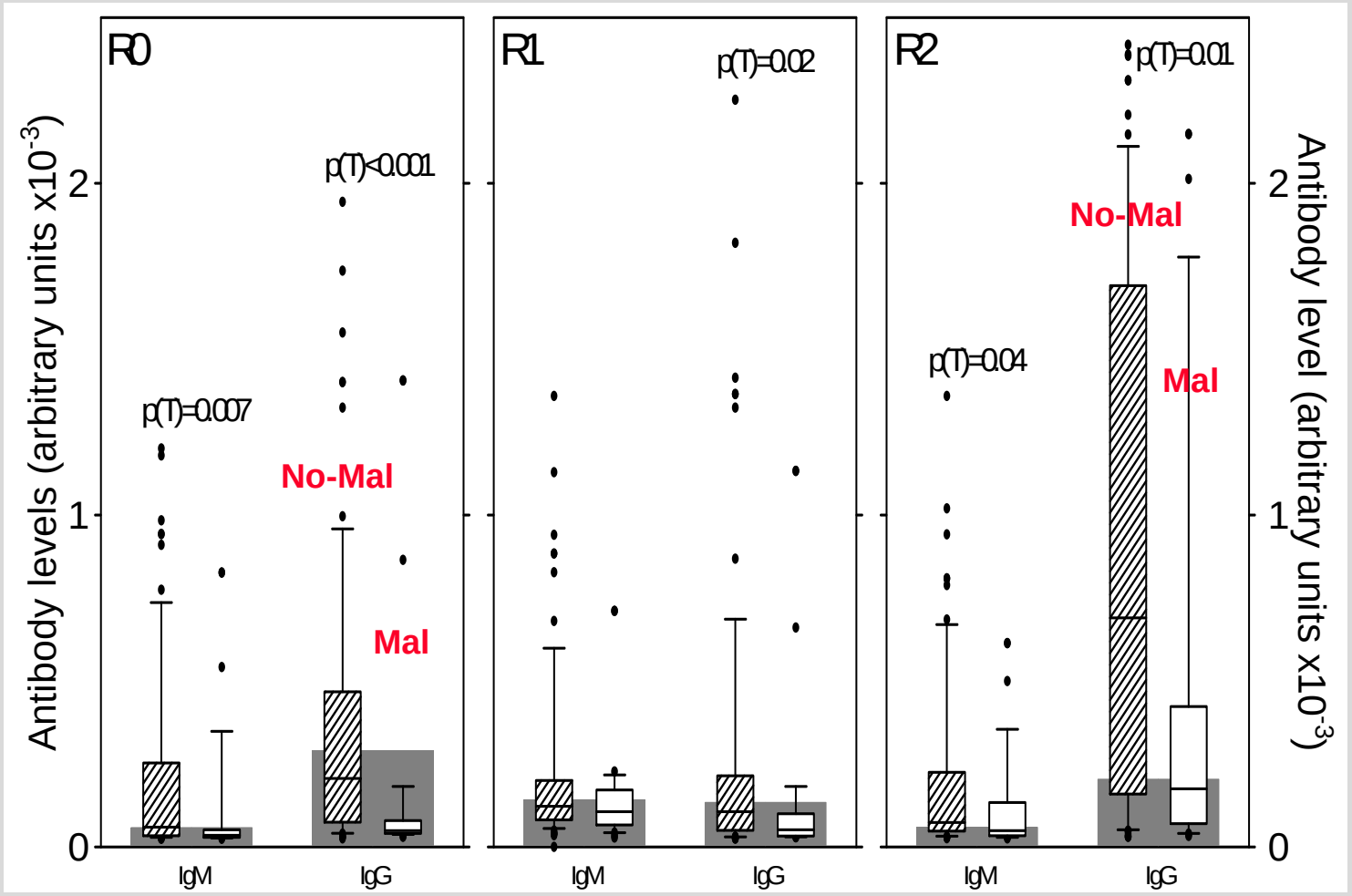
- **a semirural town ca. 50 km northeast of Accra, Ghana**
- **an area of hyperendemic, seasonal malaria transmission**
- **Estimated annual number of infective bites is ~20 / person**
- **300 children included in the study : Age: 3-15 years**
- **Active and passive Case Detection for 18 month**

→ Malaria or No Malaria

Dodoo, D., et al, 2000. J. Infect. Dis. 181:1202-1205

Anti-GLURP antibody responses; relation to protection from malaria





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Dodoo, D., et al, 2000. J. Infect. Dis. **181**:1202-1205

Immune-epidemiological studies

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High levels of IgG1 and / or IgG3 to GLURP are associated with protection against clinical disease in children from :

Six different countries in Africa and one in Asia:

1. Høgh, B., et. al. 1992. Am. J. Trop. Med. Hyg. **46**: 307-313
2. Dziegiel, M., et. al. 1993. Infect. Immun. **61**: 103-108
3. Boudin, D., et al. 1993. J.Clin.Microbiol. **31**:636-641
4. Dodoo, D., et al, 2000. J. Infect. Dis. **181**:1202-1205
5. Oeuvray, C., et al. 2000. Infect. Immun. **68**:2617-2620
6. Theisen, M., et al. 2001. Infect. Immun. **69**:5223,5229
7. Meraldi, V., et al. 2004. Parasite Immunology, 26: 265–72
8. Soe Soe et al. 2004. Infect Immun. **72**: 247-252
9. Lusingu JP, et al. 2005. Malar. J. **4**:48
10. Enevold et al. 2007. Malaria J. 16:153.
11. Nebie, I et al 2008. Infect Immun. **76**:759-66
12. Dodoo, d., et al 2008. Malar J. **7**:142
13. Iriemenama, N. C et al. 2009 Vaccine. **1**:62-71
14. Courtain & Deloron **unpublished**

Immune-epidemiological studies

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As for GLURP, IgG ab's to **MSP3** is also statistically significant associated with absence of clinical malaria in children

Oeuvray., et. al. 1994. Blood. **84**:1594-1599

Soe Soe et al. 2004. Infect Immun. **72**: 247-252

Polley et al. 2007. J Infect Dis. 2007 195:279-87

GLURP-specific IgG inhibits parasite growth

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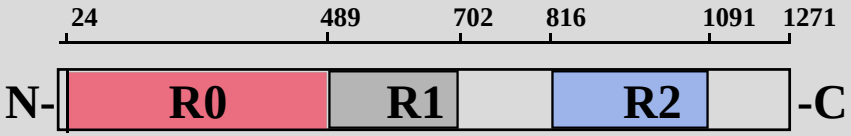
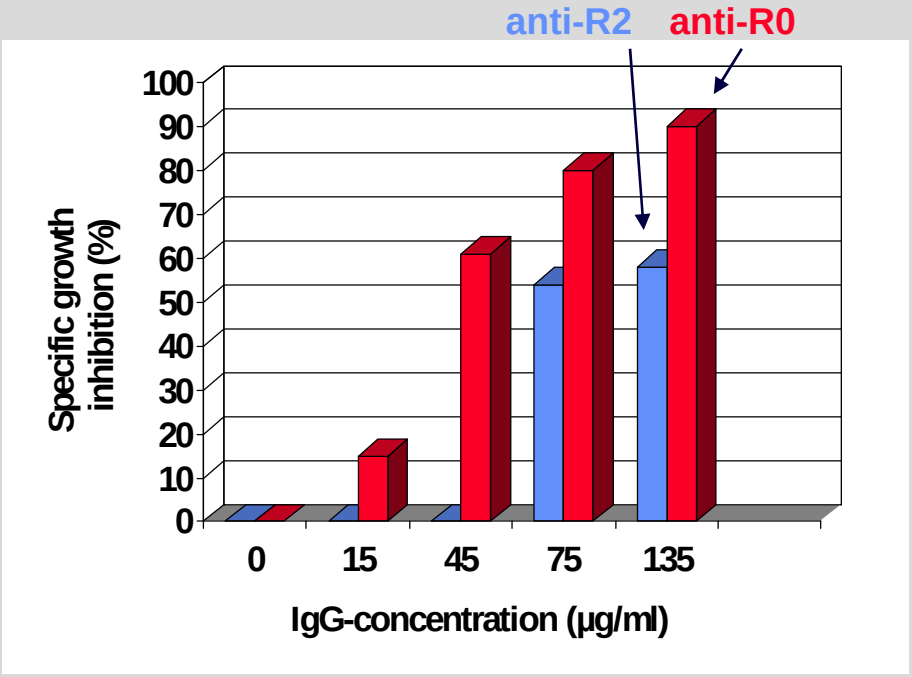
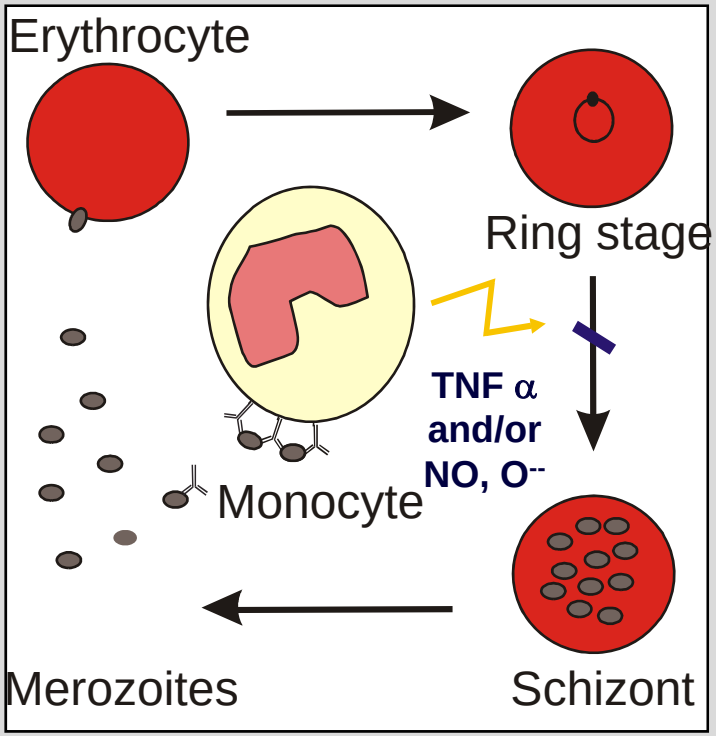




Antibody-Dependent-Cellular Inhibition (ADCI)



Theisen, M., et. al. 1998. Infect. Immun. 66:11-17

Theisen, M., et. al. 2000, Vaccine 19:204-212

Theisen, M., et. al. 2004, Vaccine 22:1188-1198

Human malaria in immuno compromised BXN mice: activity of human anti-R0 antibodies

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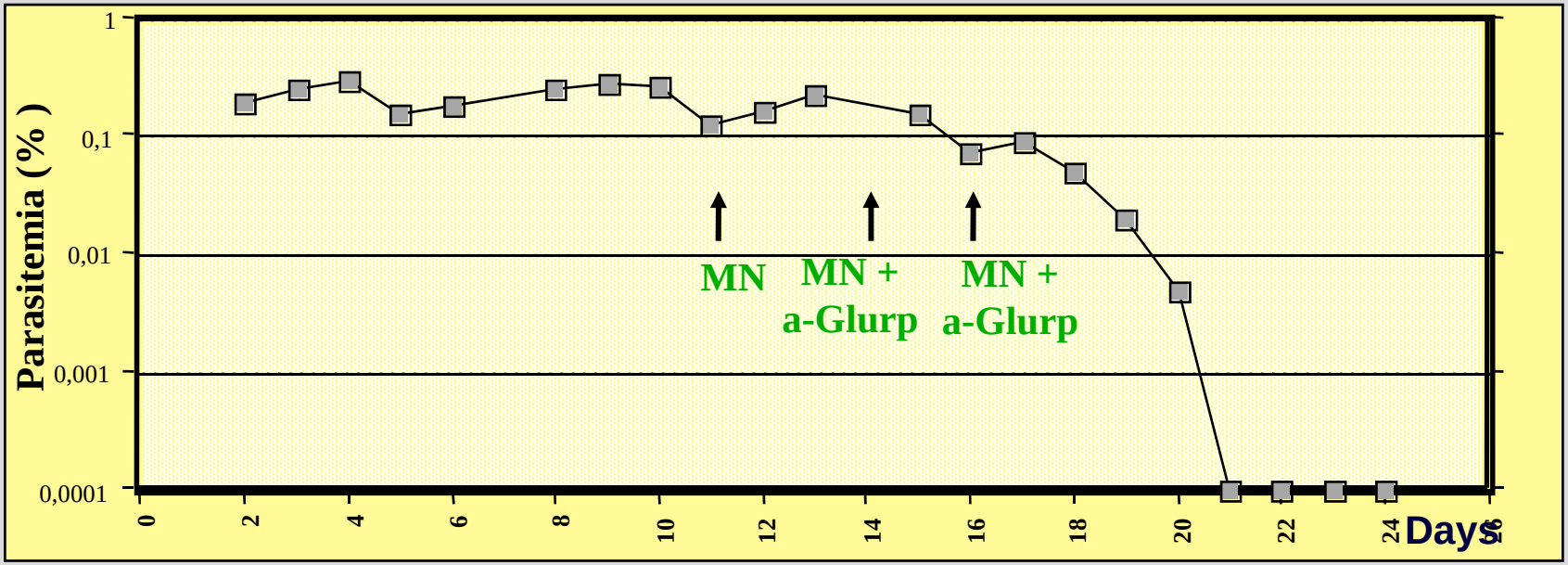
1. Inject Cl_2MDP i.p every 4 d. => reduce macrophages in peritoneum, liver and spleen
2. Inject anti-PMN i.p every 3 d. => reduce PMNs
3. Inject parasitized HuRBCs => long lasting parasitemia (*P. falciparum*) for > 4 month



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Badell et al. J.Exp.Med 11:1653-1659

Four B-cell epitopes in R0 are targeted by ADCI-effective human IgG antibodies

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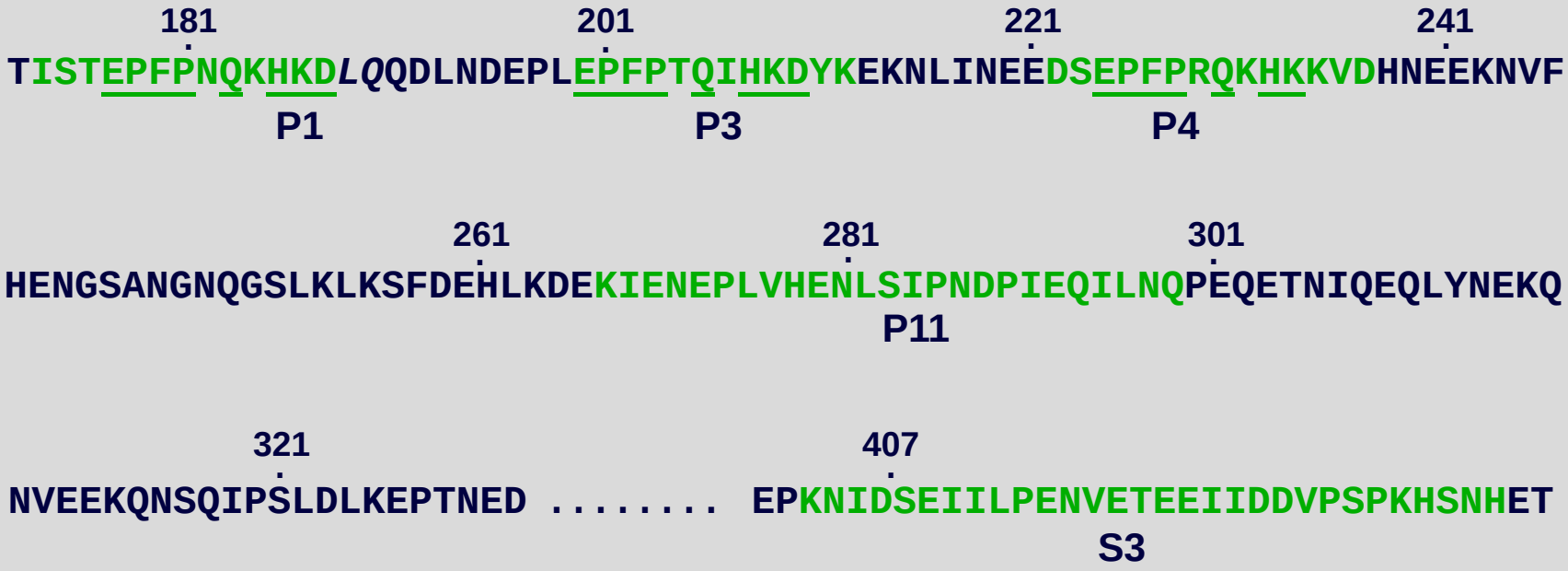
- Serum antibodies are predominantly of the IgG1 and IgG3 subclasses
- IgG to epitope P3 elicits the strongest ADCI-effect



Theisen, M., et. al. 1998. Infect. Immun. 66:11-17

Theisen, M., et. al. 2000, Vaccine 19:204-212

GLURP-R0 is conserved within P. falciparum

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DNA was from 29 field and 15 laboratory isolates

gene	number of sites: silent	number of sites: amino acid replacement	nucleotide diversity (Pi)
glurp (1248 nt.)	2	19	0.00395
CS (240 nt.)	0	16	0.01970

All epitopes targeted by ADCI-effective antibodies are conserved except P4:

D S E P F P R Q K H K K V D 38 isolates from Brazil, Myanmar and Senegal

D S E P F P R Q E H K K V D 5 isolates from Brazil and 1 from Myanmar

Stricker, K., et. al. MBP. 2000 **111**:123-130
Theisen, M., et. al. MBP. 2001 **115**:269-273

McDonald-Kreitman test using polymorphism data for *P. falciparum* and *P. reichenowi* as outgroup

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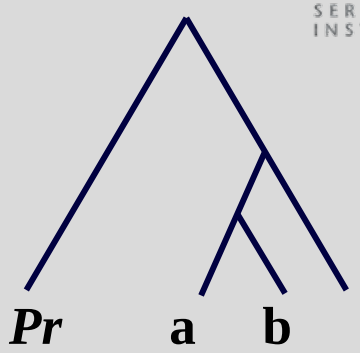
	Fixed	Polymorphic
Replacement	32	19
Synonymous	17	2
G^b	5.348	P = 0.0203



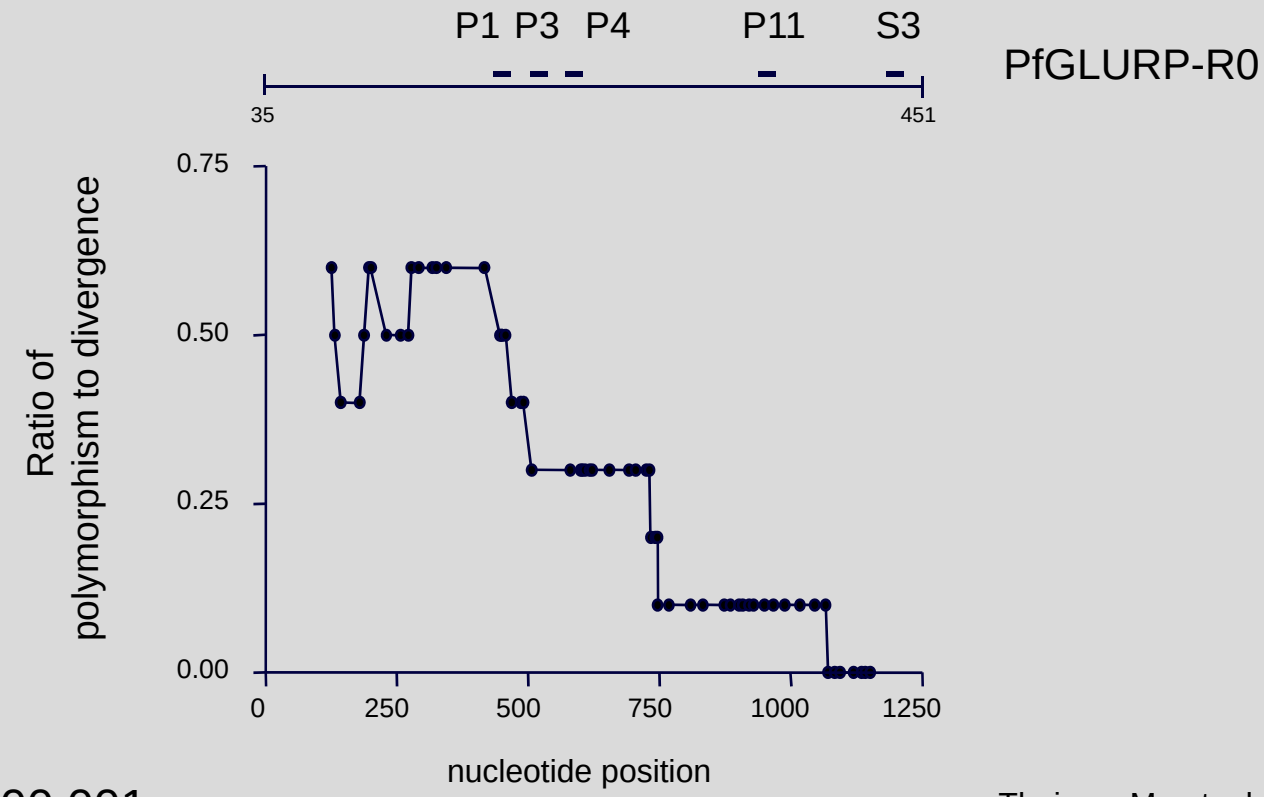
	F	P	P
Pr	AAA	CCC	GGG
a	AAG	ACC	GGG
b	AAG	CCC	GGG
c	AAG	CCC	GGA

A G-test of independence is computed to determine whether the deviation on the ratio of replacement to synonymous (fixed substitutions between species vs. polymorphisms within species) are or not significant.

Theisen, M., et. al. MBP. 2001 **115**:269-273

McDonald-runs test using polymorphism data for *P. falciparum* and *P. reichenowi* as outgroup

Sliding window plot of the ratio of the number of polymorphisms to the number of fixed differences



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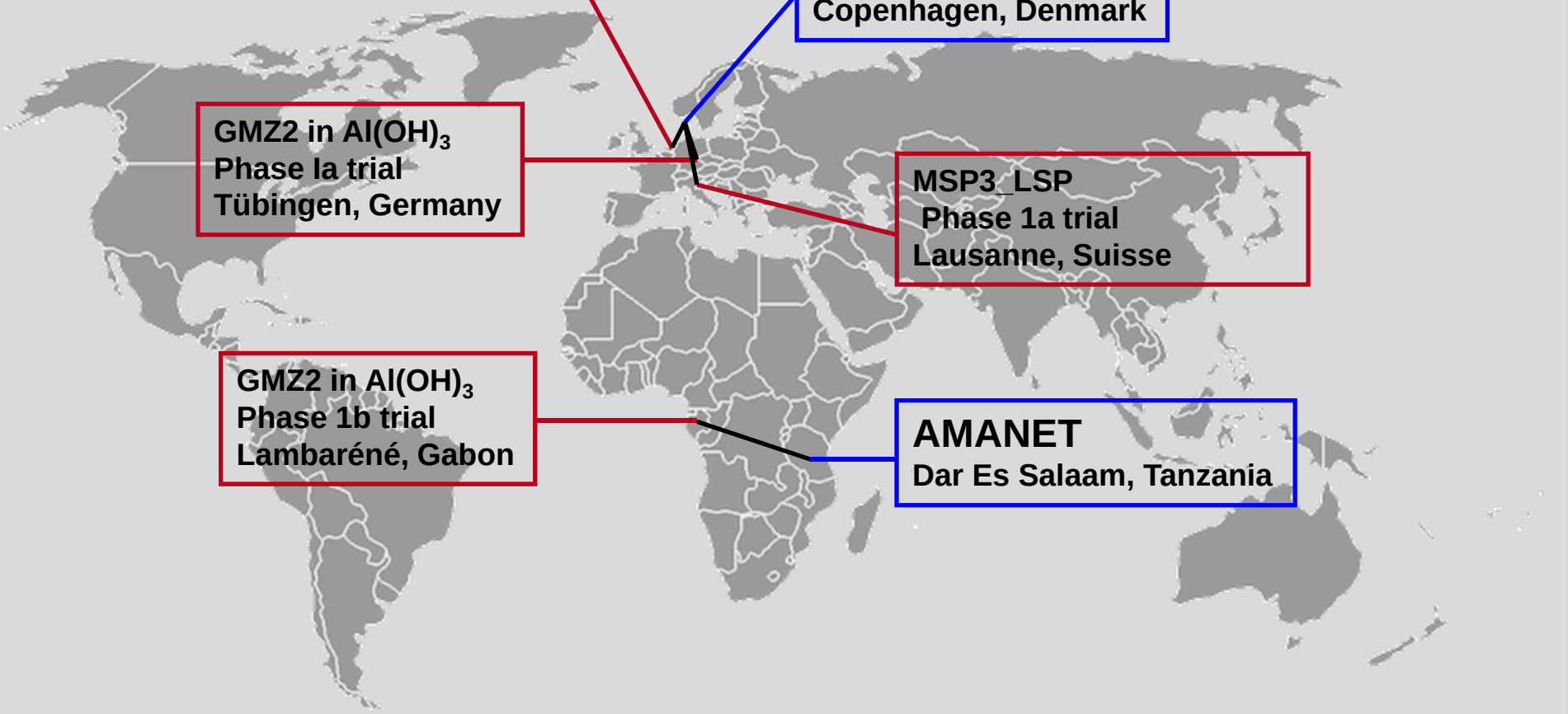
Theisen, M., et. al. MBP. 2001 **115**:269-273

Clinical development



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Long Synthetic Peptide vaccine strategy : 2 clinical trials

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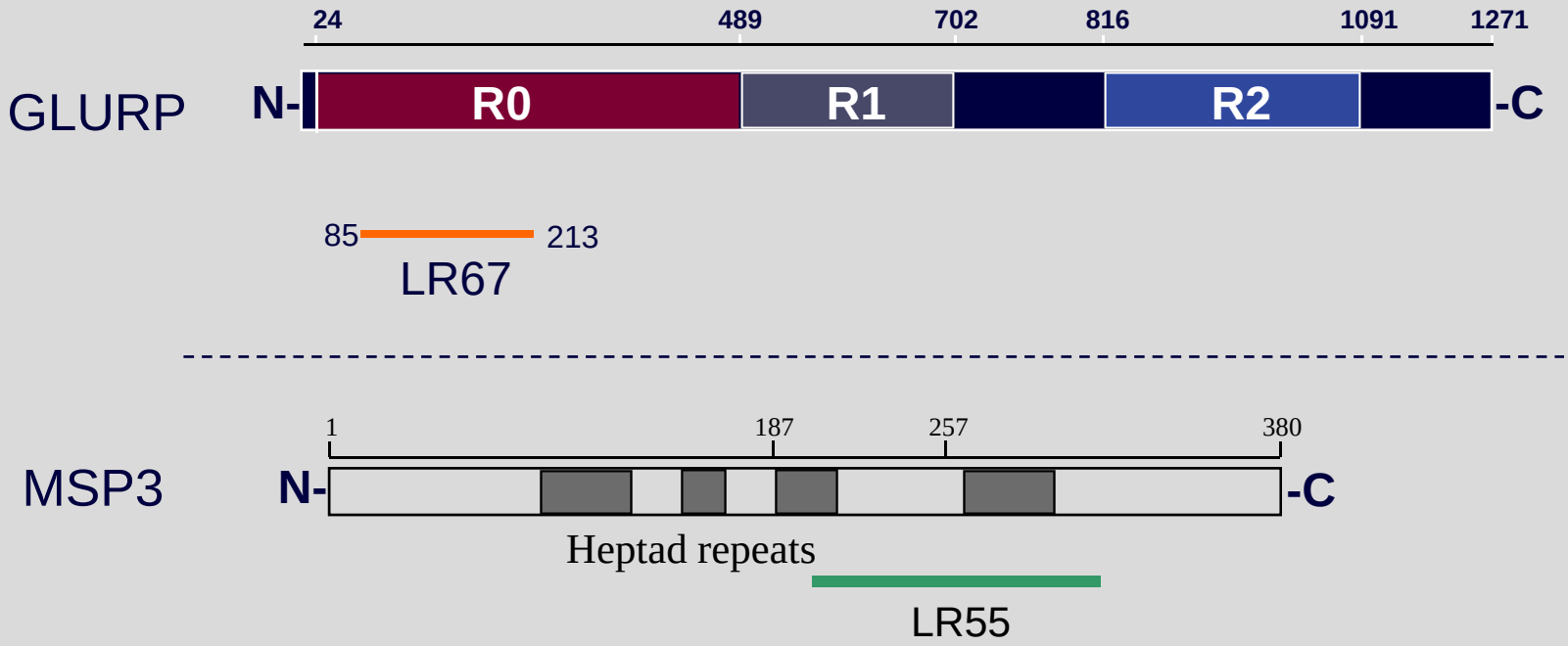
- Easy to produce
- Immunogenic



Selection criteria:

Contain conserved epitopes targeted by ADCI-effective antibodies

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LSP clinical trials

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Available online at www.sciencedirect.com

ScienceDirect

Vaccine 25 (2007) 2930–2940

Vaccine

www.elsevier.com/locate/vaccine

Glutamate-rich protein (GLURP) induces antibodies that inhibit *in vitro* growth of *Plasmodium falciparum* in a phase 1 malaria vaccine trial

Cornelus C. Hermsen[a,*], Danielle F. Verhage[a,b], Denise S.C. Telgt[a,b], Karina Teelen[a], J. Teun Bousema[a], Meta Roestenberg[a,b], Ahmed Bolad[c], Klavs Berzins[c], Giampietro Corradin[d], Odile Leroy[e], Michael Theisen[f], Robert W. Sauerwein[a]

INFECTION AND IMMUNITY, Dec. 2005, p. 8017–8026
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.12.8017–8026.2005

Vol. 73, No. 12

Phase I Malaria Vaccine Trial with a Long Synthetic Peptide Derived from the Merozoite Surface Protein 3 Antigen

Régine Audran,[1,2] Michel Cachat,[1] Floriana Lurati,[1] Soe Soe,[3] Odile Leroy,[4] Giampietro Corradin,[2] Pierre Druilhe,[3] and François Spertini[1]*

GMZ2 Phase 1a & 1b clinical studies

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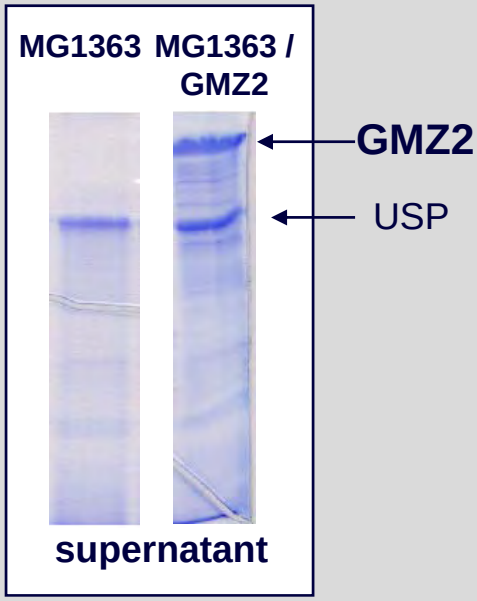

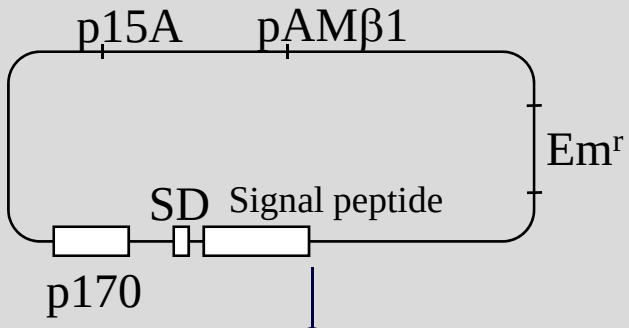

651 aa residues:

AERSTSENRNKRIGGPKLRGNVTSNIKLPSNNKGKIIRGSNDELNKNSEDVLEQSEKSLVSENVPSGL
DIDDIPKESIFIQEDQEGQTHSELNPETSEHSKDLNNNDSKNESSDIISENNKSNKVQNHFESLSDLE
LLENSSQDNLDKDTISTEPFPNQKHKDLQQDLNDEPLEPFPTQIHKDYKEKNLINEEDSEPFPRQEHK
KVDNHNEEKNVFHENGSANGNQGSLKLKSFDEHLKDEKIENEPLVHENLSIPNDPIEQILNQPEQETN
IQEQLYNEKQNVEEKQNSQIPSLDLKEPTNEDILPNHNPLENIKQSESEINHVQDHALPKENIIDKLD
NQKEHIDQSQHNINVLQENNINNHQLEPQEKPNIESFEPKNIDSEIILPENVETEEIIDDVPSPKHSN
HETFEEETSESEHEEAVSEKNAHETVEHEETVSQESNPEKADNDGNVSQNSNNELNENEFVESEKSEH
EARSKAKEASSYDYILGWEFGGGVPEHKKEENMLSHLYVSSKDKENISKENDDVLDEKEEEAEETEEE
ELEEKNEEETESEISEDEEEEEEEEKEEENEKKKEQEKEQSNENNDQKKDMEAQNLISKNQNNNEKNV
KEAAESIMKTLAGLIKGNNQIDSTLKDLVEELSKYFKNH

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GMZ2 Phase 1a & 1b clinical studies

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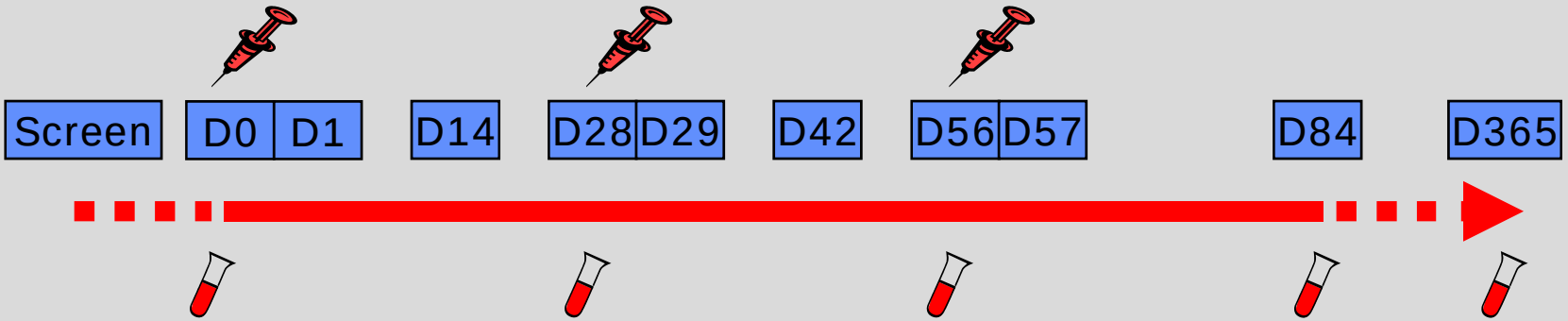
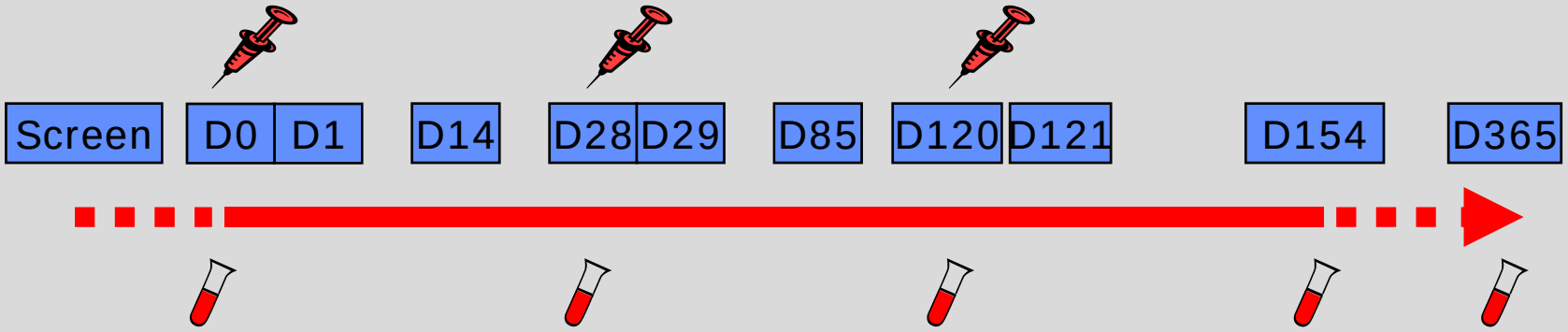
GMZ2



GLURP & MSP3 LSP's



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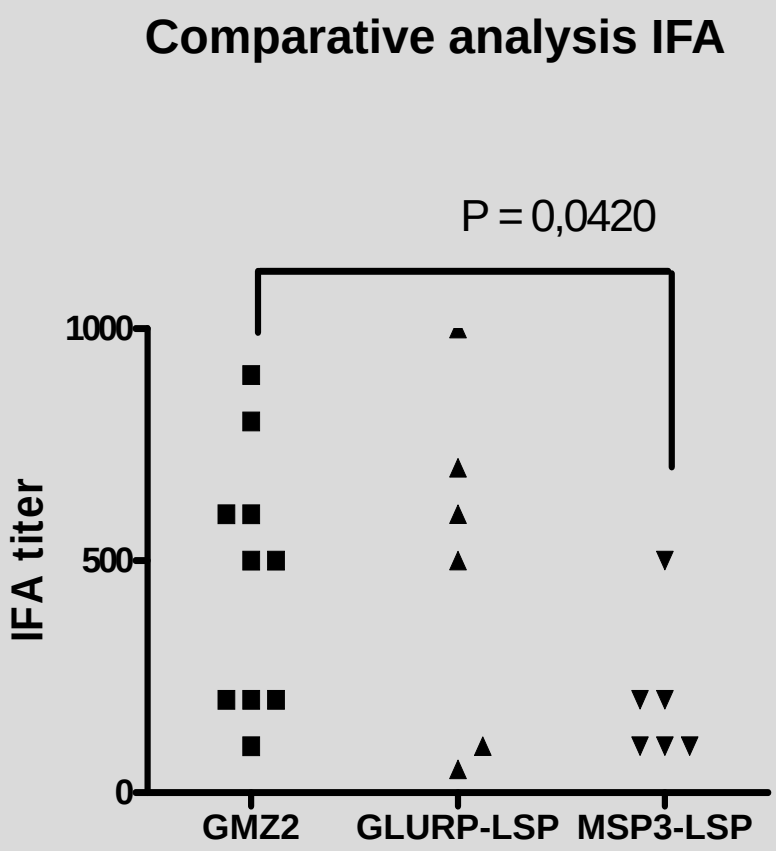
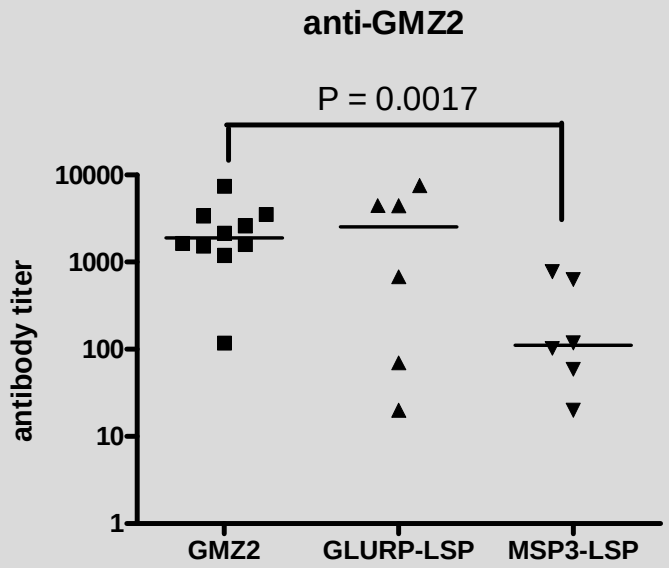
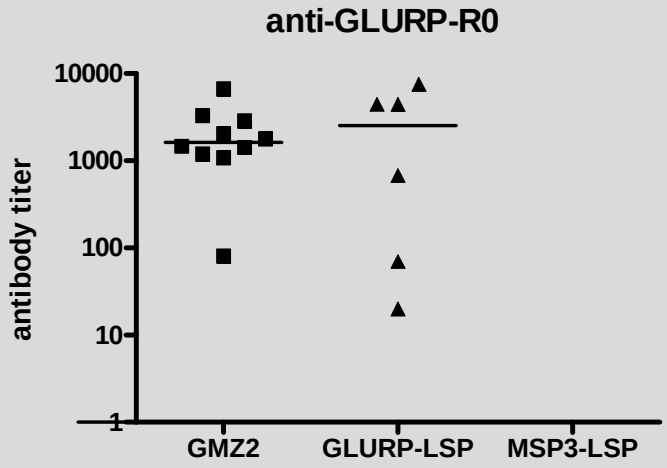
Comparative analysis IFA

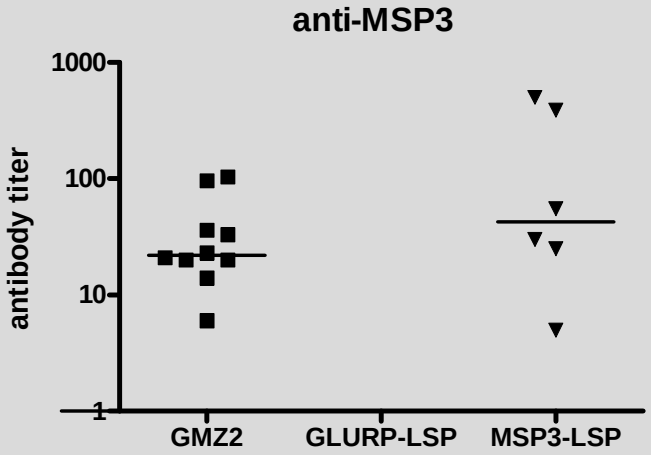


Mann Whitney test

Comparative analysis ELISA







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Mann Whitney test

Biological activity of volunteer samples : ADCI using day 150 samples

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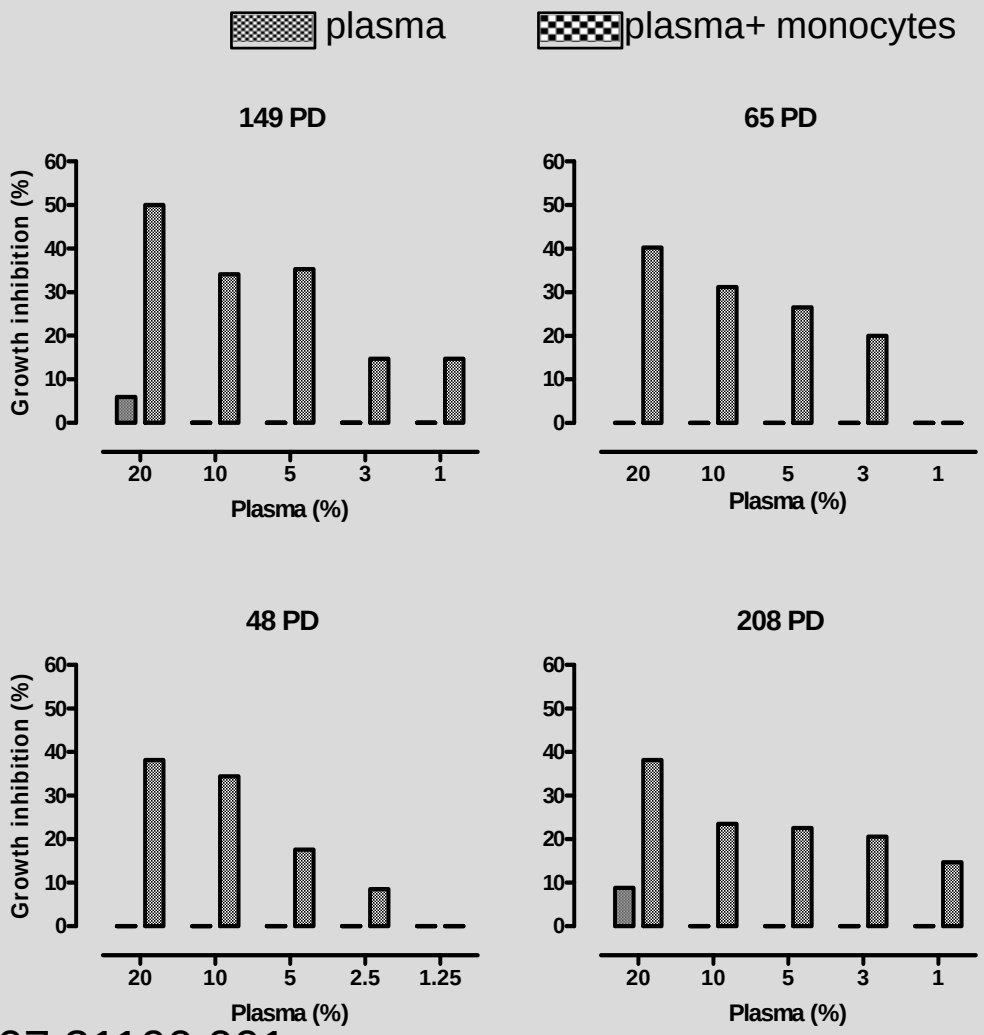
Day 0 : no growth inhibition



Inhibition of parasite growth at 10% volunteer serum

sample	day 150	
	monocytes -	monocytes +
#150	0	23
#64	0	33
#33	0	35
#20	0	15
African	**18**	**37**

Bolad, A. & Berzins, K.

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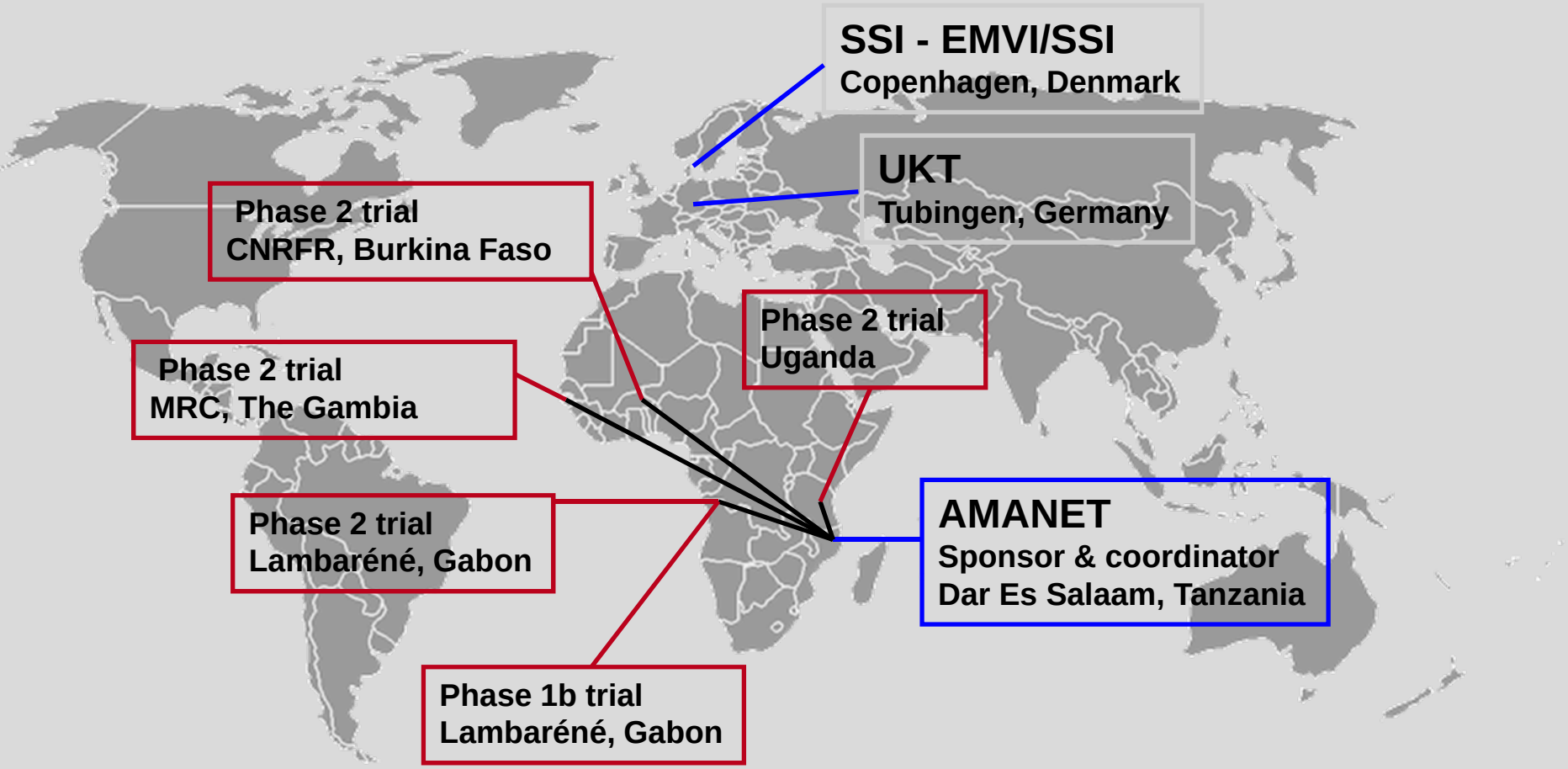
Hermsen et al, Vaccine

Clinical development, Phase 2b trials AMANET



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