Presentation #:

Peptide Cross-Reactivity has Functional Potential for MAM01 Efficacy

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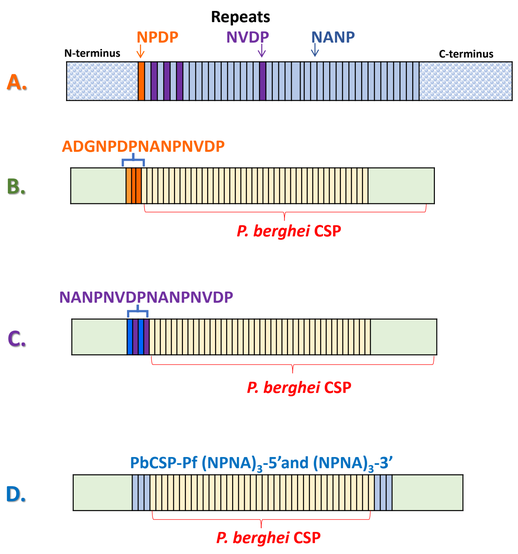
Introduction

- Human monoclonal antibodies against distinct regions of the central repeat domain of the circumsporozoite (CSP) protein of *Plasmodium falciparum* sporozoites are a promising approach for preventing malaria infection.
- The specificity of the most potent human anti-CSP antibodies show binding to the junction region, minor repeat region or the central repeat domain.
- MAM01 is a monoclonal antibody in development for single-encounter seasonal prophylaxis of malaria for children living in malaria endemic areas.
- MAM01 preferentially binds NANP tetrapeptides and demonstrates cross-reactivity in binding affinity experiments between NANP & NVDP tetrapeptides present in the central repeat region and the minor repeat region of *Plasmodium falciparum* (Pf) Circumsporozoite protein (CSP).
- To better understand the functional properties of MAM01 binding as it relates to *in vivo* protective efficacy, this antibody was evaluated in *P. berghei* sporozoites expressing Luciferase and selected Knock-In (KI) regions of the Pf CSP repeat domain.
- Our data show MAM01 is equally active against parasites expressing the full repeat region, eight NANP repeats, or the NVDPNANP containing minor repeat region suggesting peptide cross-reactivity has functional potential.

Methods

Figure 1. Transgenic parasites.

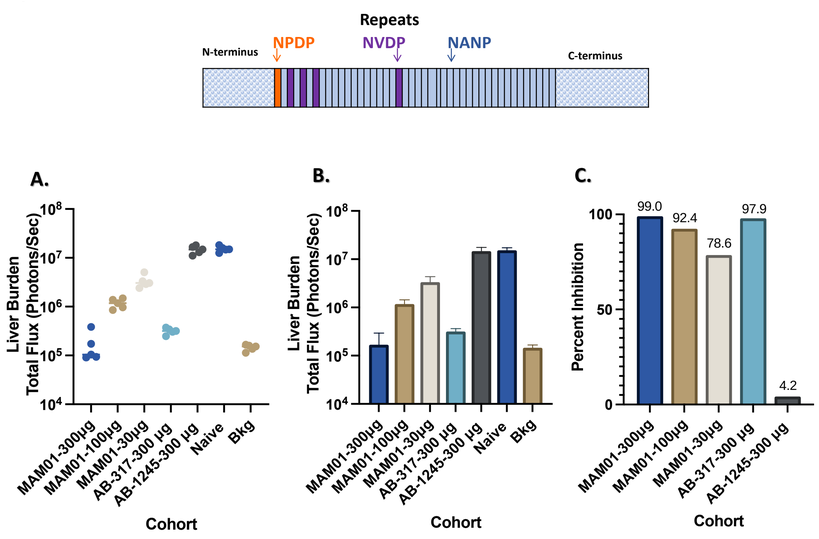


** Using the parental line PbANKA-GFP-Luc expressing, four transgenic parasites were generated: **(A)** PbPf full CSP, **(B)** PbPf junction region KI, **(C)** PbPf minor repeat KI and **(D)** PbPf $(NPNA)_6$. The PbCSP was replaced by the PfCSP (3D7) or a specific sequence of the repeats of the PfCSP. In the case of the PbCSP modified, all changes occurred after KLKQP sequence (just upstream of the central repeat domain and after the N-terminal domain).

- Three doses of MAM01 (300 µg, 100 µg, 30 µg) and 300 µg of each negative and positive control Abs were evaluated in the liver burden model whereby mice are injected with MAM01 16 hours prior to challenge with IV sporozoites.
- The percentage inhibition of liver burden after sporozoite challenge was measured by bioluminescence in an IVIS Spectrum imager and expressed as flux readings 42 hours post infection.

Results

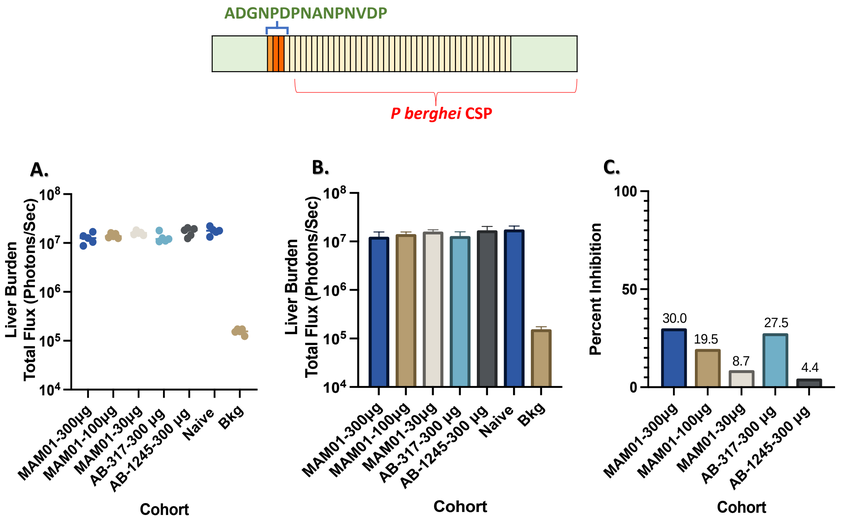
Figure 2. MAM01 mediated liver burden reduction in mice challenged i.v. with transgenic PbPf full CSP parasites.



C57BL/6 mice (5/group) were injected i.v. with 300, 100 or 30 µg of MAM01 or 300 µg of positive or negative controls and i.v. challenged 16 hours later with 2,000 transgenic SPZ expressing PbPf full CSP. Parasite liver burden are shown in **A** and **B** (total flux, photons/sec). The percent reduction (**C**), was calculated in relation to the naïve control mice.

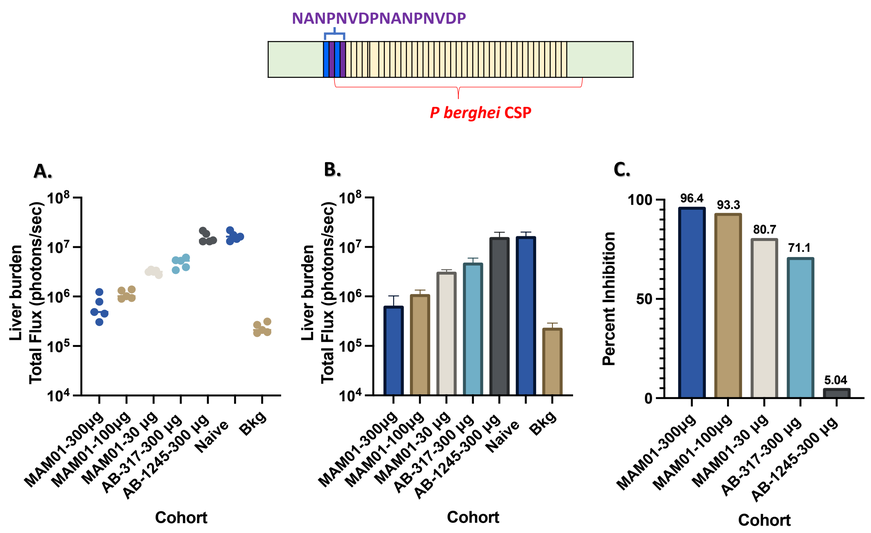
Results

Figure 3. MAM01 has no effect on infectivity of transgenic PbPf Junction KI parasites in mice.



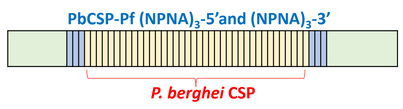
C57BL/6 mice (5/group) were injected i.v. with 300, 100 or 30 µg of MAM01 or 300 µg of positive or negative controls and i.v. challenged 16 hours later with 2,000 transgenic SPZ expressing PbPf Junction KI epitope. Parasite liver burden are shown in A and B (total flux, photons/sec). The percent inhibition C), was calculated in relation to the naïve control mice.

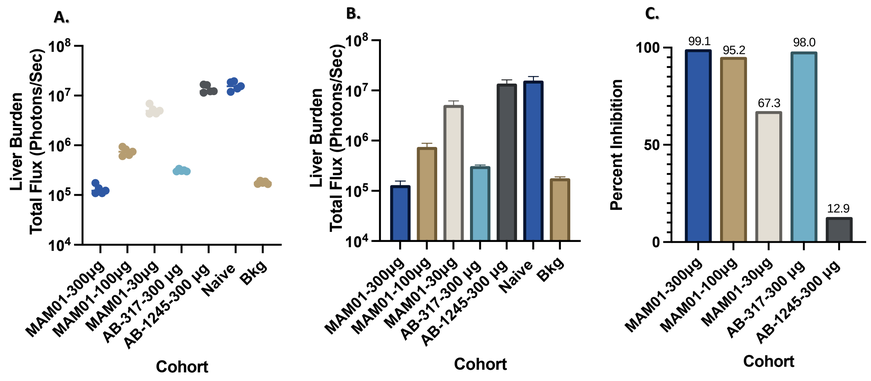
Figure 4. MAM01 induced liver burden reduction in mice challenged i.v. with transgenic PbPf minor repeat epitope parasites.



C57BL/6 mice (5/group) were injected i.v. with 300, 100 or 30 µg of MAM01 or 300 µg of positive or negative controls and i.v. challenged 16 hours later with 2,000 transgenic SPZ expressing PbPf minor repeat epitope . Parasite liver burden are shown in A and B (total flux, photons/sec). The percent inhibition C), was calculated in relation to the naive control mice.

Figure 5. MAM01 mediated liver burden reduction in mice challenged i.v. with transgenic PbPf $(NPNA)_3$-5' and $(NPNA)_3$-3' parasites.





C57BL/6 mice (5/group) were injected i.v. with 300, 100 or 30 µg of MAM01 or 300 µg of positive or negative controls and i.v. challenged 16 hours later with 2,000 transgenic SPZ expressing PbPf $(NPNA)_3$-5' and $(NPNA)_3$-3'. Parasite liver burden are shown in A and B (total flux, photons/sec). The percent reduction C), was calculated in relation to the naïve control mice.

Conclusions

- The **newly derived transgenic parasites** showed a comparable **infectivity** as the parental line in the liver burden assay.
- Consistent with previous findings, **MAM01** showed **minimal effect** against parasites expressing **only** the **junction region.**
- **MAM01 was shown to efficiently inhibit** infectivity of parasites that express:
  - **full PfCSP**,
  - **central repeats $(NPNA)_3$-5'** and **$(NPNA)_3$-3'** and
  - NVDPNANP containing **minor repeat** epitope.
- **The demonstrated dual protective epitope specificity of MAM01 may represent a distinct advantage for malaria prophylaxis.**