Presentation #:

# MAM01 Mechanism of Action: *In vivo* inhibition of Parasite Motility and Displacement

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## Introduction

- Malaria sporozoites injected in the skin reach the blood vessels and enter the blood circulation. They reach the liver, invade hepatocytes and develop into liver stages.
- The circumsporozoite protein (CSP), expressed on the surface of sporozoites is believed to play an important role in sporozoite motility in the skin and invasion of the liver.
- MAM01 is a human monoclonal antibody that recognizes the central repeats (NPNA and the minor NVDPNANP containing repeats ) of the falciparum CSP.
- Anti-CSP monoclonal antibodies like MAM01 bind CSP, and the hypothesized mechanism of action is inhibition of sporozoite motility.
- To better understand the mechanism of action of MAM01 and its functional properties, in vivo, intravital microscopy was used to characterize the effect of MAM01 on in vivo motility of sporozoites.

## Methods

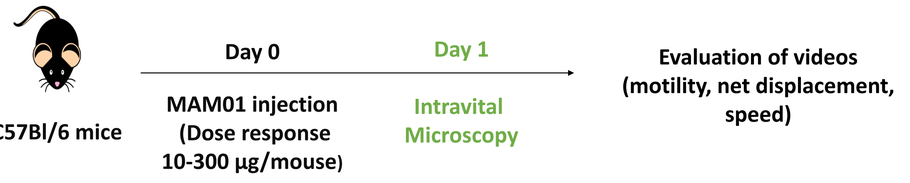


**Figure 1. Evaluation of sporozoite motility in naïve mice or passively immunized with MAM01.**

- Mice were passively immunized with 10 µg, 30 µg, 100 µg and 300 µg of MAM01.
- 16 hours later transgenic sporozoites expressing full *P. falciparum* CSP (PbPf full CSP) and mCherry expressing were dissected in L15 media, adjusted to 5,000-10,000 parasites/µl and inoculated intradermally with multiple injections into the ear pinna.
- Parasites in the skin were imaged with a 10X objective on an inverted Zeiss Axio Observer Z1 microscope with a Yokogawa CSU22 spinning disk. Mice were kept in a microscope chamber warmed at 28ºC.
- Ten minutes after the inoculation, 5-minute videos were recorded to evaluate motility, speed and net displacement. At least 4 videos per condition were recorded.
- Speed and net displacement were automatically tracked from recorded videos and the percent of motile and non-motile total sporozoites were manually counted.

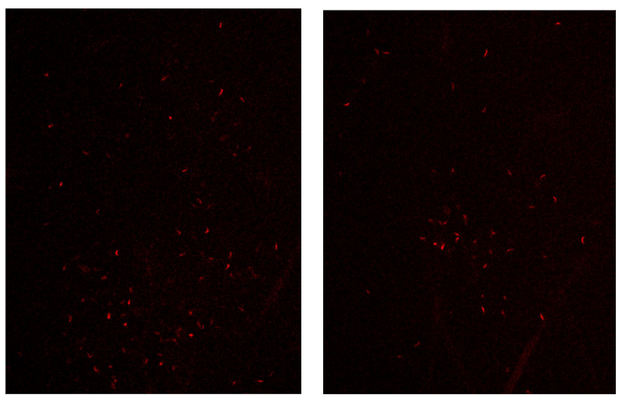


**Figure 2. Intravital microscopy of sporozoites injected in the skin (ear) of mice.** Mice received intradermal injection of sporozoites suspensions (2 µl). The sporozoites were visualized by microscopy at a 562 nm wavelength, to detect the mCherry expressed by the parasites. 10 minutes after the injection a 5-minute video was recorded to be analyzed regarding motility, net displacement, and average speed. Experimental animals have been previously injected with monoclonal antibody MAM01 at different concentrations. Naïve control mice did not receive antibodies before parasite injection.

## Results

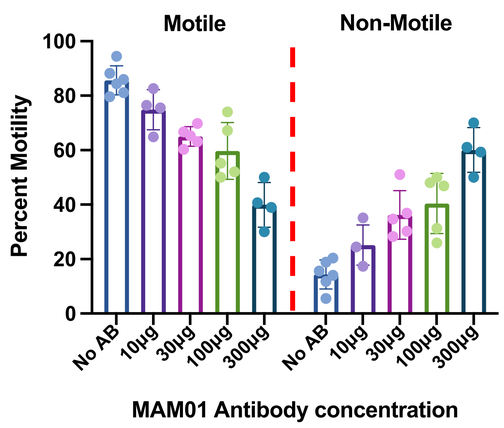


**Figure 3. Sporozoite motility in mice bearing MAM01 antibody evaluated by intravital microscopy.** Several doses of MAM01 were evaluated, from 10-300 µg/mouse. After the injection of sporozoites, mice were placed in a microscope chamber and the ears were immobilized onto a cover glass. Ten minutes after the inoculation, 5-minute videos of parasites in the skin were recorded. Motile and non-motile sporozoites were manually counted in recorded videos. A dose-response was observed for the percentage of motile and non-motile sporozoites. There is a clear dose-response effect of MAM01, with lower percentages of motile parasites associated with higher doses of MAM01 and as compared to sporozoites injected in naïve mice. At least 4 different videos per dose were counted.

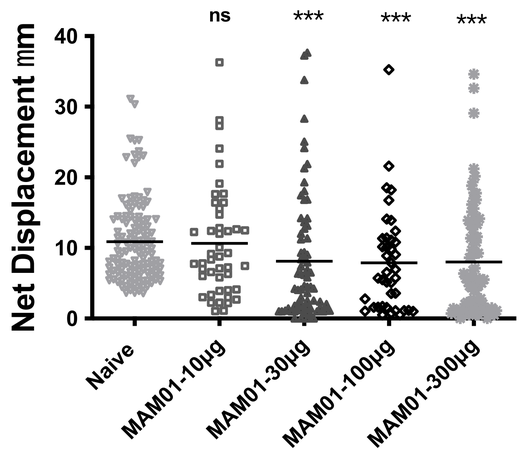


**Figure 4. Evaluation of net displacement on motile sporozoites in the skin of naïve and MAM01 passively immunized mice.** Several doses of MAM01 were evaluated, from 10-300 µg/mouse. After the injection of sporozoites, mice were placed in a microscope chamber and the ears were immobilized onto a cover glass. Ten minutes after the inoculation, 5-minute videos of parasites in the skin were recorded. While 10 µg showed no differences in net displacement when compared to naïve mice (10.64 µm and 10.89 µm respectively) , doses from 30 µg and higher had a statistically significant lower net displacement (8.0 µm average).

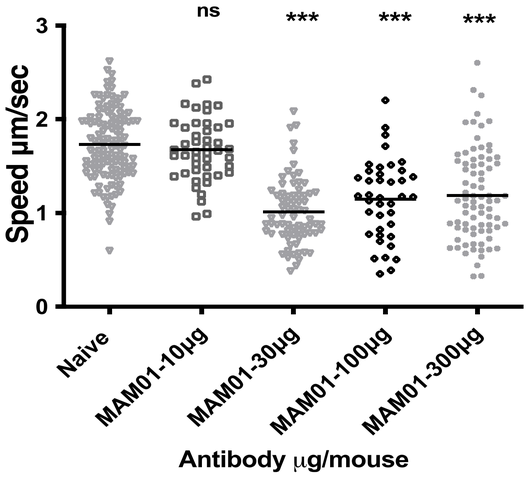


**Figure 5. Evaluation of speed on motile sporozoites in the skin of naïve and MAM01 passively immunized mice.** Several doses of MAM01 were evaluated, from 10-300 µg/mouse. After the injection of sporozoites, mice were placed in a microscope chamber and the ears were immobilized onto a cover glass. Ten minutes after the inoculation, 5-minute videos of parasites in the skin were recorded. No differences on speed were observed in mice immunized with 10 µg of antibody (1.67 µm/sec), a lower speed was observed at 30 µg (1.01 µm/sec) as compared to the 10 µg and the naïve group (1.73 µm/sec). There was not a clear dose-response beyond the 30 µg dose, although the 100µg and 300µg doses had statistically significantly lower speeds as compared to naïve sporozoites and sporozoites treated with MAM01 10µg. While a dose-response was not obtained from these experiments, the data support MAM01's mechanism of action to be alteration of sporozoite motility.

## Conclusions

- **Sporozoite motility** was **severely affected** in mice passively immunized with **MAM01 antibody**.
- **Sporozoite net** displacement and average speed were parameters also affected in immunized mice.
- The data support MAM01's mechanism of action to be **alteration of sporozoite motility**.