MAM01 Demonstrates Protection Against *Plasmodium falciparum (Pf)* Malaria in Humanized Mouse Model

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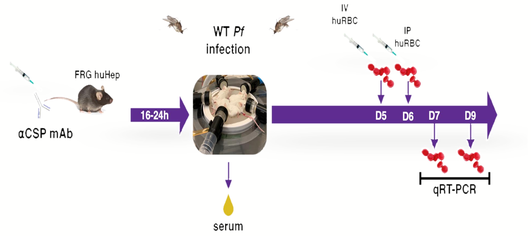
Background

Prophylactic monoclonal antibodies (mAb) targeting the circumsporozoite protein (CSP) on the surface of sporozoites (infectious stage of parasite) have been shown to be protective against *Plasmodium falciparum* (*Pf*) infection in humans[1]. The Fah-/-, Rag2-/-, Il2rγ-/- (FRG) mouse has proven to be a reliable model in which to test mAbs aimed at preventing *Pf* transmission via mosquito bite[2]. MAM01 is a mAb which targets CSP and is being developed for prevention of Pf infection in children aged 3 months to 5 years as a long-acting single dose prevention drug.

Overview of FRGhuHep Model

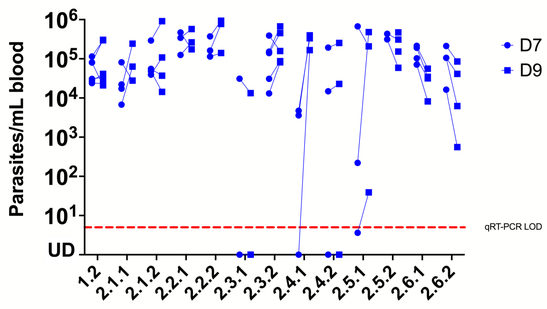
- FRGhuhep mice have a triple knockout of the genes *Fah -/-, Rag -/-,* and *IL2rg -/-*
  - *Fumarylacetoacetate Hydrolase (Fah):* Disrupts tyrosine metabolism and leads to hepatorenal toxicity via build up of intracellular fumarylacetoacetate
  - *Recombination activating gene (Rag):* Interferes with development of B-cells and T-cells, preventing an immune response against human donor cells.
  - *Interleukin-2 receptor subunit gamma (IL2rg):* Interferes with normal immune signaling, thereby preventing development of natural killer cells.
- Intrasplenic injection of human hepatocytes in the absence of immune cells allows for engraftment and repopulation of the murine liver with human donor cells.

Figure 1A: FRGHuHep Mouse Model Assessment of mAb efficacy



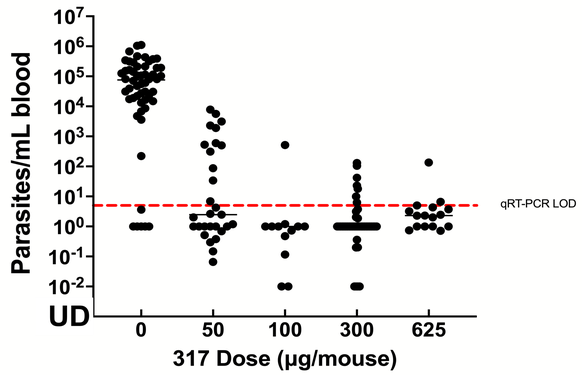
The FRGHuHep mouse model allows for assessment of monoclonal antibody efficacy against mosquito bite transmitted *Pf*. Monoclonal antibodies are intravenously injected into FRGHuHep humanized liver mice. 16-24 hours after mAb injection the mice are anesthetized via isoflurane, bled to collect serum for mAb concentration and then exposed to an average of five *Anopheles stephensi* mosquitos infected with *Pf*. Mosquito infection is determined via midgut dissection prior to bite. Five days post infection the mice are intravenously injected with huRBCs along with clodronate liposomes to deplete macrophages. Another dose of huRBCs are injected intraperitoneally on day 6 (d6) post infection. On d7 and d9 post infection, 100 uL of whole blood is collected, immediately placed in lysis/RNA stabilization buffer and sent blinded to the University of Washington for qRT-PCR quantification of parasitemia.

Figure 1B: *Pf* Challenge of FRGhuHep is reliable and reproducible



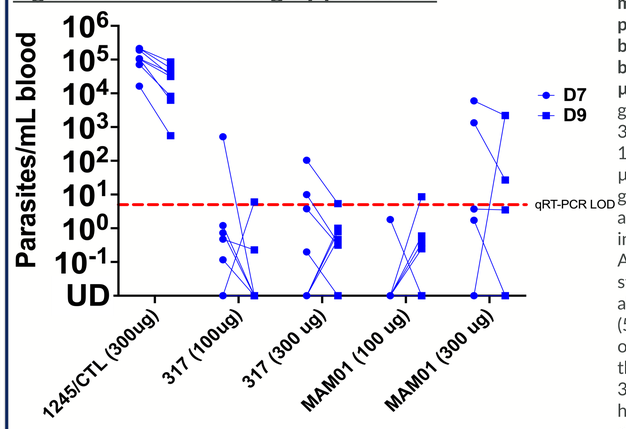
The FRGHuHep mouse model supports reliable, robust infection with *Pf* **via mosquito bite.** Quantification of parasitemia in FRGHuHep humanized liver mice infected with *Pf* via mosquito bite. Data are from infectivity control mice injected with a negative control mAb over 13 independent experiments (X-axis labels are individual experiment identifiers). The red dashed line represents the qRT-PCR limit of detection of 5 parasites/mL.

Figure 2A: anti-CSP mAb dose correlates with early parasitemia



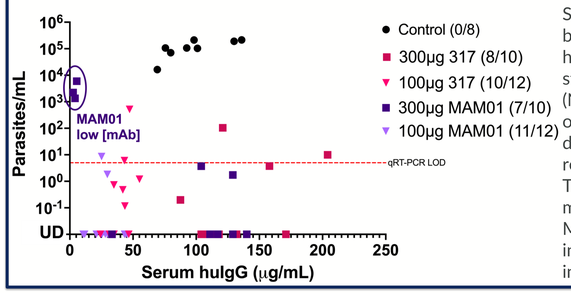
Positive control anti-CSP mAb 317 shows dose dependent protection against *Pf* mosquito bite challenge in the FRGHuHep mouse model. Parasitemia in FRGHuHep mice which were administered intravenous 317 mAb then challenged via mosquito bite with *Pf*. Shown are parasitemia data from day 7 (d7) post infection that indicates a correlation between mAb dose and parasite density emerging from the liver. Each graphed point represents an individual mouse within each dose group indicated on the x-axis.

Figure 2B: MAM01 is highly protective



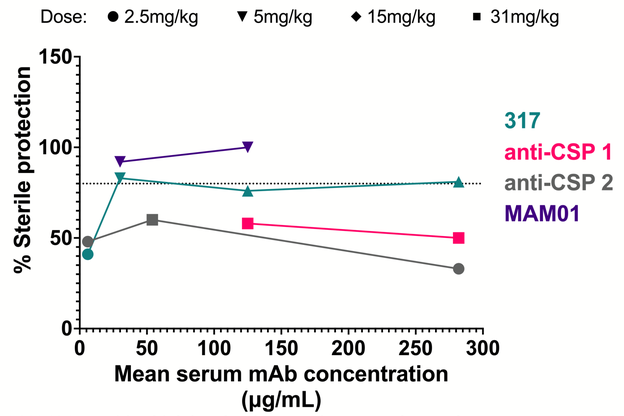
mAb MAM01 strongly protects against *Pf* mosquito bite challenge when dosed at both 100 µg/mouse and 300 µg/mouse. Experimental groups received either mAb 317 or MAM01 at a dose of 100 µg/mouse or 300 µg/mouse. 1245 is a gametocyte-specific mAb and functions as an infectivity control group. Animals were considered sterilely protected if no signal above the limit of detection (5 parasites/mL) was found on either day 7 or 9. Three of the animals in the MAM01 – 300µg group were found to have negligible amounts of circulating huIgG (see Fig. 3).

Figure 3: MAM01 provides a high level of protection which correlates with mAb serum concentration & identifies dosing error.



Serum was collected immediately before mosquito bite challenge and huIgG levels were quantified by standardized human IgG ELISA (Nexelis, Inc.). The higher of day 7 or 9 parasitemia was used for each data point with each data point representing an individual mouse. The control group was injected with mAb 1245. Three mice circled in the MAM01 300 µg group were outliers in terms of mAb concentration, indicating a dosing error.

Figure 4: MAM01 provides high levels of protection at moderate doses.



MAM01 provides high levels of protection at moderate doses. Percentage of sterilely protected mice compared to mean serum mAb concentration at time of infection. Each graphed point corresponds to the average serum concentration and percent protection of n=6-42 mice/group from experimental groups given indicated dose of indicated mAb. The shape of the point represents the dose of mAb (estimated based on a 25g mouse), and each color designates the monoclonal antibody administered. The three mice from the MAM01 group were excluded from this figure due to their low to undetectable circulating huIgG (Fig. 3). The dotted line represents 80% sterile protection as a theoretical target for a highly potent mAb. Anti-CSP 1 and Anti-CSP 2 are mAbs that have each shown moderate levels of protection in previous experiments.

Conclusions and Discussion

- MAM01 was shown to be protective against *Pf* mosquito bite challenge in the humanized mouse model.
- Measurement of huIgG concentration at time of challenge is useful for establishing dose-response relationships and identifying experimental errors.
  - Three animals were excluded from the 300 µg/mouse group as they showed negligible levels of circulating antibody at time of infection.
- FRGHuHep mice administered 100 µg/mouse of MAM01 had an average serum mAb concentration of 29 µg/mL at time of challenge and 11/12 animals were protected.
- FRGHuHep mice administered 300 µg/mouse of MAM01 had an average serum mAb concentration of 109 µg/mL and 7/7 animals were protected.
- The efficacy of MAM01 in the humanized mouse model supports further investigation at lower doses in head-to-head comparisons with leading candidates.

Acknowledgments

Thank you to the Bill and Melinda Gates Foundation (INV-005170) for their financial support, as well as to Kayla Andrews and Jared Silverman at the Bill & Melinda Gates Medical Research Institute for their collaboration. We would also like to thank the Oregon Health & Science University SLAU team for their excellent care of these animals. Additional thanks to Sean Murphy and his team at the University of Washington for performing the qRT-PCR.

References

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