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

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**Background**

Prophylactic monoclonal antibodies (mAbs) 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. The Fumarylacetoacetate Hydrolase (Fah) knockout mouse model has proven to be a reliable model in which to test mAbs aimed at preventing Pf transmission via mosquito bite. 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, Rag2-/-, 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.
- Intraspstatic 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 via aortacardiac puncture, placed in lysis/RNA stabilization buffer and sent blinded to the University of Washington for qRT-PCR quantification of parasitemia.

**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 panetocyte-specific mAb and functions as an infectivity control group.
- Animals were considered steriley 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 MAM01-300 µg 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 (Nexelsis, 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 steriley 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=4-6 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.

**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.

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**References**