Screening of potential participants

- A pre-requisite is very careful selection of potential volunteers involving extensive health screening, including mental health and specific infections, disorders, or concurrent treatments that could compromise the outcome of the trials.
- The safety of the volunteers must be paramount throughout the study and they must be fully informed about the nature of the trial and kept up to date with its progress as it affects them.

Parasite and mosquito strains

- The recommended procedure includes use of a *Plasmodium falciparum* malaria isolate, or one of its clones, that is adapted to culture. All must have defined sensitivities to effective drug treatments, and blood donors for parasite culture need screening for known infectious agents. Banks of parasites are available. Laboratory bred anopheline mosquitoes have been used. Safety procedures are again the highest priority.

Infectivity controls and challenge

- A control (non-vaccinated) group of volunteers should be included. All volunteers are exposed to a fixed number of infected mosquitoes (five) for a defined period of time within totally secure insectaries. For efficacy analysis, trial size is greatly impaired if even one of the control subjects does not develop patent parasitaemia, hence a large dose is given which may represent an unnaturally high (artificial) challenge.
- Recently, mass-produced purified, GMP standard cryopreserved sporozoites have been assessed for human challenge trials[13].

Follow-up

- A precise schedule of follow-up is required to cover the period of possible acute allergic reactions or other adverse events, and the onset of malaria infections that require prompt optimal treatment.
- Formal outreach plans are required in the event that a volunteer does not attend for follow-up prior to receiving curative treatment.

Endpoints

- The primary endpoint is first detection of patent parasitaemia, usually as determined by microscopy, measuring parasites per unit volume in thick blood films. Fully developed and agreed standard operating procedures (SOP) that take account of local variations in procedure but give a standardized outcome measure have been developed[9]. The expertise required by clinical trial microscopists is distinct from that needed for diagnosis in malaria-endemic regions, since the requirement is to be able to detect the earliest stage of patency, whilst avoiding false positives.
- A secondary endpoint is the pre-patent period, the time interval between challenge and the first occurrence of patent parasitaemia, which can vary depending on both parasite and host characteristics.
- Increased frequency of blood sampling (e.g. twice daily), in order to measure accurately peripheral blood parasite density and pick up even modest efficacy, particularly in blood stage vaccine trials, should be employed.
- Molecular detection methods (PCR) are an important supplement to blood film diagnosis, and may replace microscopy as the standard in some centres in the future. These molecular genetic techniques allow more precise calculation of growth rate, and have decreased the detection threshold for asexual parasitaemia substantially. Harmonization of PCR methods is needed to ensure comparability, and the establishment of a reference centre and repository may be beneficial.
- Evaluation of parasite growth curves can be helpful for assessment of efficacy that relates to reduction in numbers of parasites leaving the liver.
- Immune correlates of protection are not yet fully defined. CHMI allows these to be explored and opportunities should be taken to do so.