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# A double knockout against malaria

*By Tim Fulmer, Senior Writer*

Developing subunit vaccines against *Plasmodium falciparum* is difficult because the malaria parasite expresses many different antigens over the course of its life cycle. American and Australian researchers have now designed a genetically attenuated *P. falciparum* strain that could avoid this issue.[1] The vaccine will enter Phase I/IIa testing early next year.

Although the *Plasmodium* life cycle has three stages, vaccine developers have focused almost exclusively on the pre-erythrocytic stage, in which the disease is asymptomatic and antibodies should neutralize the sporozoites that can lead to the clinically symptomatic blood stage.[2]

Previous work in cultured human liver cells and in mouse malaria models has shown that knocking out either of two *P. falciparum* genes—*p52* or *p36*—arrested development of the malaria parasite at the pre-erythrocytic stage and generated an attenuated strain.[3–5]

However, those strains have not been tested in humans because of concerns that knocking out a single gene may not be sufficient to prevent the spontaneous mutations that can allow the parasites to regain blood-stage pathogenicity.

The researchers from the **Seattle Biomedical Research Institute** (SBRI) and the **Walter and Eliza Hall Institute of Medical Research** decided to knock out both genes in a single *P. falciparum* strain. The goal was to optimize levels of attenuation and reduce the likelihood of reversion to a more pathogenic strain.

In cultured human liver cells, the *p52-p36* double knockout showed developmental arrest compared with a wild-type strain or individual *p52* or *p36* knockout strains. That result suggested the new strain would also show developmental arrest in the liver *in vivo* and thus be attenuated at the pre-erythrocytic stage.

However, testing that hypothesis in standard rodent models is impossible because *P. falciparum* develops only in the human liver.

To get around this hurdle, the researchers generated mice with chimeric human-mouse livers. In those mice, analysis of postmortem liver tissue revealed no detectable double knockout parasites four days following infection, whereas wild-type parasites were easily detectable. Thus, the double knockout strain showed *in vivo* attenuation at the pre-erythrocytic stage.

The findings were published in the *Proceedings of the National Academy of Sciences*.

## Getting practical

The SBRI group is now preparing to test the double knockout *P. falciparum* strain in humans, with a Phase I/IIa trial set to begin 1Q10, according to Stefan Kappe, a corresponding author on the *PNAS* paper. He is also an associate member of SBRI and an affiliate associate professor in the Department of Global Health at the **University of Washington**.

The trial will administer the double knockout strain via mosquito bite to healthy malaria-naïve adults. The Phase I portion will be a dose-escalation study to assess safety and tolerability.

In the Phase IIa portion, volunteers will be vaccinated with the double knockout strain and then be challenged with mosquitoes infected by wild-type *P. falciparum*.

Because vaccination by mosquito bite is not practical for large-scale immunization programs, the SBRI researchers plan to develop and manufacture an injectable formulation of the knockout strain. Kappe and colleagues are in discussions with malaria vaccine biotech company **Sanaria Inc.** for a potential collaboration on that formulation.

Sanaria has a radiation-attenuated whole-organism malaria vaccine in Phase I testing. Unlike the genetic attenuation strategy, attenuation by irradiation is nonspecific and reduces a strain's replicative fitness in the liver by randomly causing multiple mutations throughout the genome.

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>
> ***Chris Janse, Leiden University Medical Center***

Because the genetic attenuation strategy targets a particular gene or set of genes, it should yield a much more homogeneous population of attenuated parasites than the irradiation strategy, said Chris Janse, head of the Leiden Malaria Research group at the **Leiden University Medical Center**.

Thus, he said, using genetically attenuated strains to determine the correct dose of parasite to induce protective immunity without either killing the parasite at the liver stage or risking blood-stage infection could be more straightforward than using irradiated strains.

"It's still too early to know with any certainty whether the genetic attenuation approach will have clear practical advantages over the more clinically advanced radiation attenuation strategy," said Stephen Hoffman, CEO and CSO of Sanaria.

But, he noted, the company now would be in the position to perform head-to-head comparisons of the two attenuation strategies if the double knockout strain shows safety and protective efficacy in its initial trials.

Leiden's Janse and colleagues are also creating genetically attenuated malaria strains suitable for a vaccine, but they plan to knock out a different set of genes.

Janse told *SciBX* that the *p52-p36* double knockout is an important step toward generating attenuated malaria sporozoites for use in human vaccines. "However," he said, "those two genes encode proteins that are quite similar functionally and structurally to other proteins in the parasite's genome. Given the high genetic plasticity of the malaria parasite, it's not unthinkable spontaneous mutants could arise that compensate for the loss of the two genes and escape attenuation."

Although Janse did not disclose the specific genes his group plans to knock out, he said that a safely attenuated parasite "would probably have at least two to three genes removed that encode proteins playing very different roles in liver-stage development."

The most advanced malaria vaccine is a subunit vaccine. Mosquirix (RTS,S/AS02) from **GlaxoSmithKline plc** consists of a single *P. falciparum* antigen, the circumsporozoite protein, fused to an HBV surface antigen to enhance immunogenicity. The vaccine, delivered with GSK's AS02 adjuvant, is in Phase III testing.

A patent covering the *PNAS* findings is held by SBRI and is available for licensing, Kappe told *SciBX*.

**Fulmer, T. *SciBX* 2(30); doi:10.1038/scibx.2009.1164**
**Published online Aug. 6, 2009**

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## COMPANIES AND INSTITUTIONS MENTIONED

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