

## INFECTIOUS DISEASE

# Toward a chemical vaccine for malaria

A high-throughput screen puts us on the road to protecting populations against malaria

By Margaret A. Phillips<sup>1</sup> and Daniel E. Goldberg<sup>2</sup>

Despite considerable progress in combating malaria, it remains one of the world's most important infectious diseases, with 50% of the world population at risk of developing the disease and a mortality rate of ~0.5 million annually (1). The lack of an effective vaccine and the relentless ability of the *Plasmodium* parasite responsible for malaria to develop drug resistance has contributed to the continuing disease burden (2–4). Artemisinin-combination therapies (ACTs) are the mainstay of current treatment regimens, but decreased effectiveness, particularly in Southeast Asia, threatens our ability to control this disease. A global effort to develop new drugs for the treatment and prevention of malaria is under way but not guaranteed to succeed (3, 5, 6). These efforts include a systematic attempt to target all life-cycle stages of the parasite to allow combination therapies to be developed, which are also likely to reduce the development of resistance. High-throughput screens (HTSs) designed to identify small drug-like molecules that prevent growth of blood-stage parasites (7, 8) and target-based approaches have identified new compounds that are currently in preclinical development and/or various stages of human clinical trials for treatment of malaria (3). Missing from these efforts has been a high-throughput technology to find liver stage-specific chemotypes. On page 1129 of this issue, Antonova-Koch *et al.* (9) report an HTS effort that has filled this gap. They identify a substantial number of new chemical starting points with potent liver-stage antimalarial activity, promising a new ca-

capacity to feed compounds through the drug development pipeline for chemoprotection.

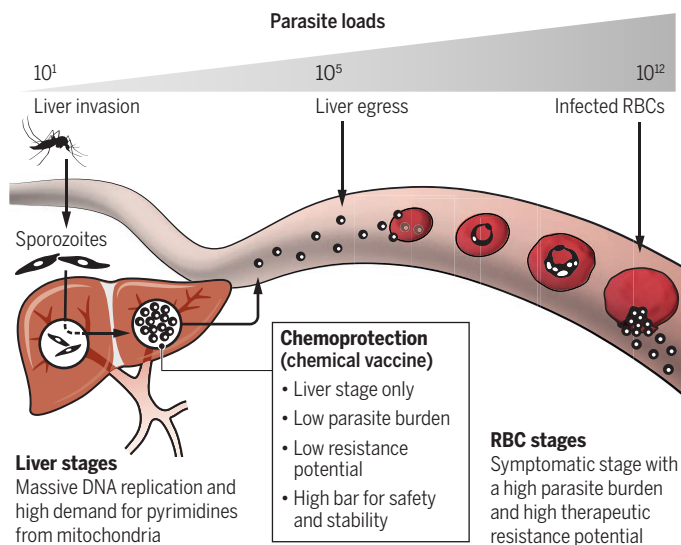
As efforts to eliminate malaria increase, the need for chemoprotective agents to protect vulnerable populations will also increase (3). The idea is to find a long-lasting agent to treat infections before they become symptomatic and to develop these into a chemical vaccine (that is, a drug that protects against disease). The best malaria stage of infection to target for this approach is the one in the liver. The malaria life cycle begins when an infected mosquito injects sporozoites into a

person does not have activity on both stages and therefore does not put selective pressure on a large blood-stage parasite load.

*Plasmodium falciparum* is responsible for most malaria cases, and it is the most deadly, whereas *Plasmodium vivax* has the greatest global distribution. Antonova-Koch *et al.* made a strategic choice to use the rodent malaria parasite *Plasmodium berghei* for their screen. This conferred many advantages over using a human parasite: ease of production, minimal biohazard risk, more rapid life cycle, and ability to infect

## Life cycle of the *P. falciparum* malaria parasite

An infected mosquito injects sporozoites, which replicate in the liver using enormous metabolic activity. From each hepatocyte, ~10<sup>5</sup> merozoites enter the bloodstream, invade red blood cells (RBCs), and set up an amplifying cycle. A chemical vaccine that targets the early liver stage could minimize resistance.



person, some of which find their way to the liver to establish infection (10) (see the figure). After replication in hepatocytes, malaria parasites burst out and infect erythrocytes, setting up an amplifying intraerythrocytic cycle. From 10<sup>1</sup> sporozoites that reach the liver, up to 10<sup>5</sup> merozoites will emerge into the blood, and up to 10<sup>12</sup> will then build up in the bloodstream during a severe infection. A drug that blocks parasite replication in the liver works on a much lower parasite burden and thus has a lower chance of encountering and selecting for a rare parasite with a resistance mutation than do blood stage-active compounds. This is particularly so if a com-

hepatoma cell lines that are more facile to use and do not detoxify the compounds being screened. From an initial hit rate of ~4%, a subset (~10<sup>4</sup>) were prioritized for evaluation in confirmation assays, leading to the validation of ~10<sup>3</sup> compounds with good drug-like properties that have potent liver-stage activity and minimal cytotoxicity on host liver cells. Of these, 631 were profiled on additional *Plasmodium* species and life-cycle stages. Interestingly, two-thirds of these hits are specific for liver-stage parasites, highlighting the previously unknown biology of this stage and promising new cellular insights if compound targets can be determined. This is a goal that will require innovative approaches. The subset of compounds that were also active against blood-stage *P. falciparum* parasites contained a high proportion of mitochondrial inhibitors

(43%) across diverse scaffolds. The mitochondrion in malaria parasites is critical for pyrimidine biosynthesis, a pathway that is essential for cell replication to generate the mature schizont in both blood and liver infections (see the figure). Demand for pyrimidine nucleotide bases is even greater in the liver stage, in which one sporozoite is replicated to generate 20,000 merozoites (10). Drugs that target enzymes required for pyrimidine nucleotide biosynthesis are effective for both malaria treatment and chemoprevention, including the cytochrome bcl1 inhibitor atovaquone, which is an approved antimalarial agent used mainly for

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chemoprevention, and DSM265, an inhibitor of dihydroorotate dehydrogenase, currently in clinical development (3, 5, 6). The surprisingly high percentage of dual-acting compounds that hit these targets suggests that this pathway is one of the most vulnerable pathways shared between the blood and liver stages.

Not all hits from the *P. berghei* HTS worked on liver-stage *P. vivax* infections; the crossover was only ~25%. This may be partially explained by assay differences and by compound metabolism in the primary human hepatocytes used for the *P. vivax* assay. This latter issue could be engineered out of any compound series during lead optimization. It remains to be seen how many of the identified chemotypes will ultimately have liver-stage activity against both *P. vivax* and the deadly *P. falciparum*. Extrapolating from experience with compounds on blood stages of the rodent and human parasites, a large majority are likely to be effective against all *Plasmodium* species.

Now comes the hard work of prioritizing these hit compounds and optimizing them to have the properties of a chemical vaccine for clinical development. Recent work to develop chemical vaccines for HIV (11) and to formulate atovaquone as an injectable for chemoprevention in malaria (12) provide the beginnings of proof of concept for this strategy. The potential advantages of liver stage-specific chemoprotection in terms of simpler field implementation and low resistance propensity must be balanced with a need for high safety when used to protect a whole asymptomatic community (more so than a short-term treatment given to a discrete population of patients). Additionally, compounds must be stable, have a long half-life, and be amenable to slow delivery formulation, such as a long-acting injectable that will also have the benefit of improving compliance. Because of these complexities, there is a need to have a substantial list of candidate compounds. Thanks to the work of Antonova-Koch *et al.*, we have such a list. ■

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10.1126/science.aav7479

#### PALEONTOLOGY

# Climate change and marine mass extinction

## The end of the Permian Period was catastrophic for life in high-latitude regions

By Lee Kump

**V**oluminous emissions of carbon dioxide to the atmosphere, rapid global warming, and a decline in biodiversity—the storyline is modern, but the setting is ancient: The end of the Permian Period, some 252 million years ago. For the end-Permian, the result was catastrophic: the greatest loss of plant and animal life in Earth history (1). Understanding the details of how this mass extinction played out is thus crucial to its use as an analog for our future. On page 1130 of this issue, Penn *et al.* (2) add an intriguing clue: The extinction was most severe at high latitudes. Using a state-of-the-art climate model that was interpreted in terms of physiological stress, the authors further identify the killer as hypoxia, which was brought on by warm temperatures and ocean deoxygenation.

A number of kill mechanisms for end-Permian extinction have been proposed, most triggered by the tremendous volcanic activity associated with the emplacement of the vast lava flows of the Siberian Traps, the eruption of which was coincident with the mass extinction (3). The Siberian Traps are estimated to have released tens of thousands of petagrams of carbon as carbon dioxide and methane (4), explaining the 10° to 15°C tropical warming revealed by oxygen isotope compositions of marine fossils (5). On land, unbearably hot temperatures and hypoxia likely were the main cause of mass extinction of plants and animals (6), although ultraviolet radiation exposure from a collapsed ozone shield contributed as well (7). Rapid warming also likely led to the loss of oxygen from the ocean's interior, extending up onto the continental shelves—a conclusion supported both by the widespread distribution of indicators for marine anoxia in sedimentary

rocks (8) and by numerical modeling of the Permian ocean-atmosphere system (9).

Once considered nonselective, mass extinctions are increasingly revealing patterns of differential impact across species, lifestyles, and geographic locations through their fossil records (10). A geographic pattern to Permian extinction, however, has remained elusive. Benefiting from the paleontological community's creation of the expansive Paleobiology Database (11), Penn *et al.* discovered a meridional gradient to extinction intensity: Groups of organisms that were restricted to higher latitudes prior to the extinction suffered higher proportions of extinction than those established at low latitudes. What was it about living at high latitudes that predisposed marine organisms to extinction?

Penn *et al.* took an innovative approach to answering this question by coupling state-of-the-art computer simulations of end-Permian environmental change to a quantitative estimate of habitat loss for presumed Permian ecotypes. To establish the environmental (temperature and oxygen) tolerance of Permian ecotypes, Penn *et al.* used studies of modern

organisms, grouped into ecotypes that they argue should be representative of the oxygen demands of Permian organisms. From these studies, a metabolic index was assigned to each ecotype, reflecting the critical balance between oxygen supply and demand. Model temperature and oxygen distributions before and during the end-Permian event were then used to map regions of the ocean where the metabolic index fell below the critical value (hypoxic threshold) at which oxygen supply (fundamentally related to the oxygen concentration of the water in which the organism lived, itself a function of ocean circulation, temperature, and rates of aerobic decomposition) could not support the physiological demands of daily life (feeding, reproduction, and defense). The authors found that ecotypes that favored high latitudes before the event preferentially suffered extinction because of their relatively high hypoxic thresh-

**"If warming and oxygen loss... happened quickly, massive die-off was destined to occur."**

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*Science* **362** (6419), 1112-1113.  
DOI: 10.1126/science.aav7479

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