P. berghei Luciferase liver stage assay

General assay principle:

This assay is adapted from Swann S. et al. 2016. It is based on the murine *Plasmodium berghei* species transformed with Luciferase. Hepatic human transformed cells (HepG2), pretreated for 18 hours with the compound to investigate, are infected with freshly dissected *P. berghei* Luciferase sporozoites. After another 48 hours of incubation with the compound to investigate, the viability of *P. berghei* exoerythrocytic forms (EEF) is measured by bioluminescence. This assay allows us to identify compounds with an eventual activity against sporozoite infection of liver cell as well the viability of liver schizonts.

Parasites.

Plasmodium berghei Luciferase sporozoites were obtained by dissection of infected *A. stephensi* mosquito salivary glands supplied by the New York University Insectary. Dissected salivary glands were homogenized in a glass tissue grinder and filtered twice using Steriflip Vacuum-Driven Filtration System (20 µm pore size, Millipore) and counted using a hemocytometer. The sporozoites were kept on ice until needed.

Cell lines.

HepG2-A16-CD81EGFP cells stably transformed to express a GFP-CD81 fusion protein (S. Yalaoui et al 2008; O. Silvie et al. 2003), were cultured at 37°C in 5% CO2 in DMEM (Invitrogen, Carlsbad, USA) supplemented with 10% FCS, 0.29 mg/ml glutamine, 100 units penicillin and 100 µg/ml streptomycin.

Sporozoite invasion assay.

Because of the difficulties associated with human parasites able to infect immortal liver cell lines, the rodent model *P. berghei* was used. *P. berghei* is able to infect human hepatocarcinoma HepG2 cells expressing the tetraspanin CD81 receptor (S. Yalaoui et al 2008; O. Silvie et al. 2003). 3x103 HepG2-A16-CD81EGFP cells in 5 µl of medium (2x105 cells/ml, 5%FBS, 5xPen/Strep/Glu) were seeded in 1536-well plates (Greiner BioOne white solid bottom custom GNF mold) 20-26 hours prior to the actual infection. 18 hours prior to infection, 50nl of compound in DMSO (0.5% final DMSO concentration per well) were transferred with a PinTool (GNF Systems) into the assay plates (10 µM final concentration). Atovaquone (10 µM) and 0.5% DMSO were used as positive and negative controls, respectively. *P. berghei* Luciferase sporozoites were freshly dissected from infected *A. stephensi* mosquito salivary glands and filtered twice through a 40 µm nylon pore cell strainer. The sporozoites were re-suspended in media, counted in a hemocytometer and their final concentration adjusted to 200 sporozoites per µl. Also, penicillin and streptomycin are added at 5x-fold increased concentration for a final 5x-fold increased concentration in the well. The HepG2-A16-CD81EGFP cells were then infected with 1x10^3 sporozoites per well (5 µl) with a single tip Bottle Valve liquid handler (GNF), and the plates spun down at 37°C for 3 minutes in an Eppendorf 5810 R centrifuge with a centrifugal force of 330x on lowest acceleration and brake setting. After incubation at 37°C for 48 hours the EEF growth got quantified by a bioluminescence measurement. The increased antibiotic concentration did not interfere with the parasite or HepG2- A16-CD81EGFP growth. Atovaquone and naive wells were used as controls on each plate. The compounds were screened in a 12 point serial dilution to determine their exact IC50 values.

Bioluminescence quantification of exo-erythrocytic forms (EEFs).

Media was removed by spinning the inverted plates at 150xg for 30 seconds. 2 µl BrightGlo (Promega) were being dispensed with the MicroFlo (BioTek) liquid handler. Immediately after addition of the luminescence reagent, the plates were read by the Envision Multilabel Reader (PerkinElmer). IC50 values were obtained using the normalized bioluminescence intensity and a non-linear variable slope four parameter regression curve fitting model in Prism 6 (GraphPad Software Inc).

Literature reference and notes:

S. Swann et al. (2016) **High-Throughput Luciferase-Based Assay for the Discovery of Therapeutics That Prevent Malaria.** *ACS Infectious Diseases* 2: 4. 281–293 (2016).

S. Yalaoui et al., **Hepatocyte permissiveness to Plasmodium infection is conveyed by a short and structurally conserved region of the CD81 large extracellular domain.** PLoS Pathog. 4, e1000010 (2008).

O. Silvie et al., **Hepatocyte CD81 is required for Plasmodium falciparum and Plasmodium yoelii sporozoite infectivity**. Nat. Med. 9, 93 (2003).