Ashlyn, Margaret, Ripp's Group Answer Key (homework on PubMed ID 21696174)

We will be looking at the paper "Structure-guided Lead Optimization of Triazolopyrimidine-Ring Substituents Identifies Potent *Plasmodium falciparum* Dihydroorotate Dehydrogenase Inhibitors with Clinical Candidate Potential" (PubMed ID: 21696174). This is a long paper. We will be focusing most of our attention on the introduction, results, and discussion sections. The chemistry and materials and methods sections should at least be skimmed, although they can provide valuable clarification should you wish to delve deeper.

This is the third and final paper we will be reading about selecting DHODH inhibitors as potential malarial drugs; it picks up where the paper from Wednesday (Aug. 7th) leaves off. To recap, DHODH is an enzyme in the pyrimidine synthesis pathway. Since the *Plasmodium falciparum* parasite cannot salvage pyrimidines, it must synthesize these nucleotides. The researchers first looked at DHODH inhibitors in high throughput screening and found several compounds that were effective against the enzyme but not very effective in whole-cell assays. Later, the researchers identified a compound that had potential as a drug. Here, the researchers continue to optimize this compound, using an interesting mouse model of the *P. falciparum* infection to test their lead candidates.

1. What portion of the molecule were the researchers modifying during the lead optimization process, and why did they focus their attention there?
    a. They were modifying the substituent at the C2 position. They chose to focus their efforts there because X-ray crystallography showed a space between the C2of the initial triazolopyrimidine compounds and the FMN cofactor. They thought that filling this space would increase the efficacy of the inhibitor.
2. How did the researchers confirm the mode of action of the triazolopyrimidine-based compound?
    a. They transfected a line of parasites with the yeast DHODH, which uses a different cofactor (fumarate) than the CoQ used by *Pf*DHODH. Previous studies have shown that the yeast DHODH can rescue the parasite treated with a DHODH inhibitor. This was seen with compound 38 (Figure 4). Parasites transfected with yeast DHODH needed significantly greater amounts of compound 38 to show any reduction in proliferation compared with parasites that did not undergo transfection. This indicates that compound 38 does kill the parasite through inhibition of DHODH and not through a secondary effect.
3. An alternate mouse model (SCID) was used to test the compound instead of a mouse infected with *P. berghei*. Why was this?
    a. Compounds 37 and 38 were poor inhibitors of *Pb*DHODH, necessitating a different mouse model. This can be seen from Table 1, where the IC_{50} for *Pb*DHODH was several orders of magnitude greater than the IC_{50} for *Pf*DHODH. (Sadly, these compounds will not be good drugs to treat malaria in mice.)

4. Look at the reference paper for SCID mice (PubMed ID: 19596869). What are two ways humanized mouse models are created for use with *P. falciparum*? (Hint: Look at the second paragraph of the paper).
    a. The first humanized mouse model grafts human erythrocytes into immunodeficient mice, while using chemicals to further destroy the mouse immune system that would otherwise hinder the *P. falciparum* infection from taking hold. The second mouse model uses a knockout mouse which cannot produce beta-2 microglobulin. This retains several features of the innate immune system, while inhibiting the mouse's adaptive immune system, allowing it to be grafted with human erythrocytes and infected with *P. falciparum* (Wikipedia, 2013). This study uses a further-refined version of the second model, which uses mice that are also defective in the interleukin 2 receptor.
5. This paper refers to a compound as a "clinical candidate potential". What compound is it, what substituents does it have (Table 1), and what is needed to be done for "final validation" of the compound?
    a. Compound 38 has clinical potential. The substituents are as follows: at C2, CF_2CH_3; at R1, SF_3; at R2, H. Toxicology studies in animals are needed, followed by clinical trials.

Sources

Wikipedia, 2013. Beta-2 microglobulin http://en.wikipedia.org/wiki/Beta-2_microglobulin