Deciphering PAUSTI Targets
Deciphering the targets of retroviral protease inhibitors in *Plasmodium berghei*

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Introduction

**THE PATH OF CHLOROQUINE RESISTANCE**
Malaria parasites resistant to chloroquine swept out of the Mekong region and spread around the world. So far, artemisinin hasn’t followed that path, and researchers are debating the likelihood it will.

**A GROWING THREAT**
Artemisinin-resistant parasites are now widespread in the Mekong. Resistance has been linked to mutations in the parasites’ K13 gene; the map shows the percentage of samples with K13 mutations reported since 2010.
➢ The burden of malaria is compounded with HIV/AIDS.

➢ The geographical overlap has led to various interactions (*WHO*, 2011)

➢ Retroviral protease inhibitors (RPIs) such as lopinavir (LP) and saquinavir (SQ) are active against *Plasmodium* parasites (*Nsanzabana C, Rosenthal P*, 2011)

➢ Exact target(s) of the RPIs in *plasmodium* parasites is unknown

➢ Hypothesis
Principle

- Using reverse genetics approach, this study interrogates the essentiality and suitability of Plasmodium aspartyl proteases; Plasmepsin (PM) 4, PM7, PM8 or DNA damage inducible protein-1 (Ddi1), as potential targets for the RPIs.

- The study embarked on separately generating transgenic parasite lines lacking PM4, PM7, PM8, or Ddi1 in the rodent malaria parasite.

- The study then tested the suppressive profiles of LP or SQ against the transgenic parasites in the standard 4-day suppressive test.

- If any of the transgenic parasite lines become(s) significantly less sensitive to the test drugs, then the knocked out gene could be mediating the action of the RPIs.
Methodology

Knock out Vector

- PbGEM vectors are genetically modified clones from a *P. berghei* ANKA genomic DNA library (PbG) that are constructed in pJAZZ®-OK NotI vector

- Ethical Clearance
Results

a. Generation of *Plasmodium berghei* knockout parasites

PM4, PM7 & PM8 knock out transgenic parasites were successfully generated but Ddi1 was refractory to deletion: Ddi1 gene seems essential for the asexual blood stage parasites
Results

b. Growth rate phenotype analysis of the generated transgenic parasites

PM7 and PM8 genes are dispensable, PM4 gene knock out results in significant growth rate attenuation
Results
c. PM7, PM8 KO lines retained sensitivity to LP and SQ while PM4 KO line lost sensitivity to LP and SQ
**Results**

c. PM4 exhibits higher binding affinity than PM7, PM8 or Ddi1 towards LP or SQ

<table>
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<th>LIGAND</th>
<th>Ligand Mode</th>
<th>Binding Affinity (kcal/mol)</th>
<th>Ligand Mode</th>
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Results

c. 2D LP-PM4 interaction diagram generated using **LigPlot+**

Oxygen atoms are shown in red, nitrogen atoms shown in blue while hydrogen bonds are shown as olive green dotted line
Discussion & Recommendation

**Ddi1**
- Essential
  - Involved in control of cell cycle
  - Potential drug target

**PM4**
- Essential
  - Dvp of Adaptive immunity

**PM7**
- Not Essential

**PM8**
- Not Essential in B/S
  - Could be essential in Sporozoite Motility
  - Potential candidate for blocking malaria transmission
International Conference Presentations

Working with Parasite Database Resources
15-20 October 2017
University of Malawi College of Medicine, Blantyre, Malawi

AFRICA - ai - JAPAN Project
African Union - african innovation - JKUAT AND PAUSTI Network Project

PAUSTI Scholar Emerges Best at the 8th KASH Conference
Posted on February 26, 2018 by JICA PAUSTI
Opportunities

Resources

- Help
- Tips
- Assistance
- Guidance
- Support
- Advice

Management Principles

- Planning
- Preparation
- Award
- Evaluation
- Accountability

Strict Timelines

- Graduation

Presentation Skills
Challenges

Let's turn the Challenges to Opportunities
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Merci !

The path to one’s career targets is not always straight, but let’s keep shooting, shooting to the right direction.