The Power of Phenotypic Screening in the Search for Novel Antimalarials

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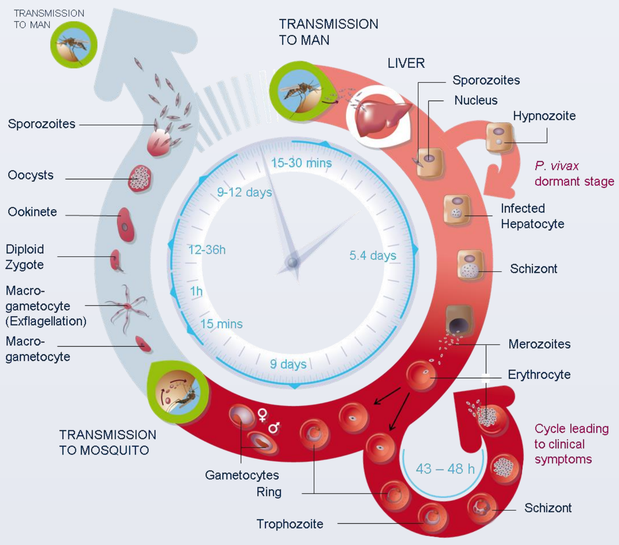
INTRODUCTION

- Malaria causes enormous human suffering – more than half the world is at risk
- Malaria kills 544,700 - 904,000 people every year[1]
- 86% of those who die are under the age of 5 – with the disease accounting for ~20% of child deaths in Africa
- Caused by infections of the parasite *Plasmodium*
- Infection leads to destruction of red blood cells causing fever, severe anemia & death
- New medicines are urgently required to combat the threat of artemesinin resistance
- To achieve eradication multiple stages of the parasite lifecycle will need to be targeted in future therapies



Medicines for Malaria Venture

- In response to a virtually empty malaria drug pipeline, MMV was established in 1999 with a mission to discover, develop and deliver safe, effective and affordable antimalarial drugs
- As a not-for-profit PDP, MMV's goal is to cure and protect the vulnerable and help to ultimately eradicate malaria
  - Small Molecule Antimalarial focus
  - Virtual R&D organisation ~50 people in Geneva
  - Work with academic institutions & private sector

PHENOTYPIC SCREENING

Phenotypic screening can be a powerful technique to:

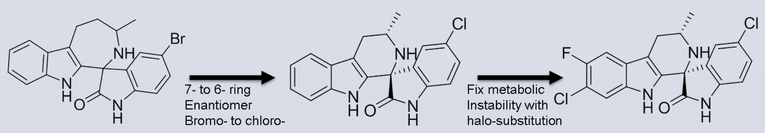
- Identify novel chemical series
- Identify new mechanisms

Blood stage asexual assay formatted for HTS

- More than 6,000,000 compounds screened in partnership with pharma/academia[2]
- Typical hit rate 0.5%
- Generated new H2L & LO projects + clinical candidates (illustrated below)

MMV - NOVARTIS

KAE609 – "Spiroindolone" First in Class



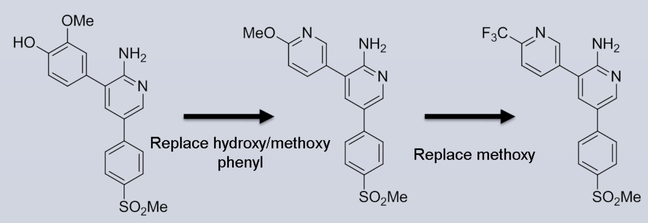
Singleton "Hit"
EC_{50} NF54 90nM
EC_{50} K1 85nM

EC_{50} NF54 9.2nM
EC_{50} K1 8.5nM
Cl_{int} (hu, m) unstable

KAE609
EC_{50} NF54 0.7nM
EC_{50} K1 0.6nM
Cl_{int} (hu, m) stable
ED_{90} *Pb* 2.7mg/kg

- Resistant mutants linked to PfATP4[3]
- Screening to First in Human studies <4 years!
- First novel anti-malarial mechanism in 15 years
- Currently in Phase IIa

MMV - University of Cape Town



MMV390048

EC_{50} NF54 49nM
Metabolic instability
E_H 0.48

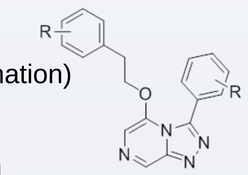
EC_{50} NF54 51nM
Improved stability
E_H 0.26

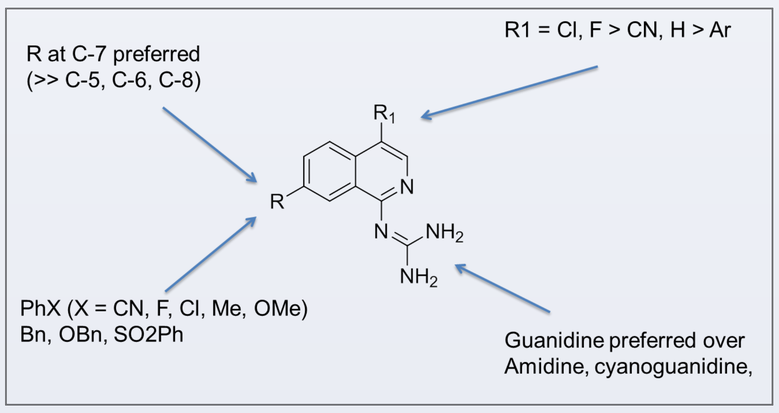
EC_{50} NF54 25nM
E_H < 0.07
Rat F: 51%

- Mouse *P. berghei* efficacy model ED_{90} 1.74 mg/kg - cures at 30mg/kg [4]
- MMV390048 is currently in Preclinical studies; Ph I studies scheduled for 2014

MMV - PFIZER

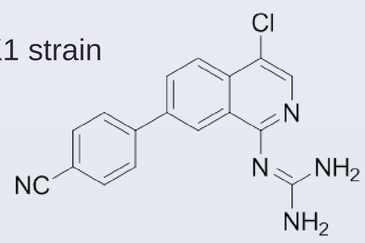
- 160,000 Pfizer compounds screened against *P. falciparum* (3D7 strain) at the Eskitis Institute for Cell and Molecular Therapies in Brisbane[5]
- Two key series chosen for detailed evaluation[6]
  - Triazolopyrazines (see **poster 59** by Paul Willis for further information)
  - Isoquinolyl Guanidines

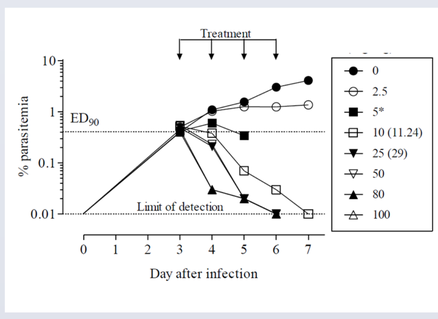




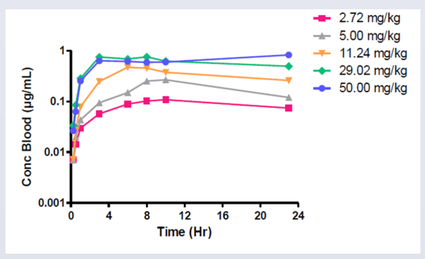
MMV642995 – summary

- MWt 322, logD 3.3, pKa 7.9
- *P. falciparum* (3D7) IC_{50} 13nM. No cross resistance with K1 strain
- HLM < 8µl/min/mg ; RLM 34µl/min/mg
- Dofetilide binding IC_{50} 1.3µM; hERG IC_{50} 5.6µM
- Cerep panel: 3 sub 1µM off-target hits
- Rat PK (iv 0.5 mg/kg; po 3 mg/kg)
  - Cl 40 ml/min/kg, V_{ss} 10 L/kg, $T_{1/2}$ 3.4 hrs, Oral F 50%



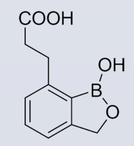


P falciparum SCID mouse model
ED_{90} 3.2 mg/kg



MMV - ANACOR

AN3661 – Novel Oxaborole Anti-malarial



AN3661
EC_{50} 3D7 52nM
EC_{50} K1 20nM; EC_{50} Dd2 29nM
ED_{90} *P.falciparum* SCID mouse 0.6mg/kg

- AN3661 identified as a "hit" from primary screen[7]
- Excellent physical properties and *in vivo* efficacy
- Hit profiled and confirmed as a pre-clinical candidate
- First oxaborole anti-malarial

CONCLUSIONS

Phenotypic screening against asexual blood stages of the parasite has led to the identification of novel antimalarials - clinical studies are in progress. High throughput screens for the liver and sexual stages of the parasite have also been developed and are being exploited in a similar manner. Assays to identify compounds active against the dormant hypnozoite form of plasmodium vivax remain a major challenge.

ACKNOWLEDGEMENTS

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REFERENCES

1) WHO 2012 World Malaria Report
2) Gamo et al, Nature 2010, 465, 305-312; Guiguemde et al, Chemistry & Biology 2012, 19, 116-129
3) Rottmann et al, Science 2010, 329(5996) 1175-1180; Spillman et al, Cell Host & Microbe 2013, 13, 227-237
4) Younis et al, J Med Chem 2012, 55, 3479-3487
5) Poster: A High Throughput Screen to Identify Novel Anti-Malarial Compounds, M Palmer et al, "Antimalarial Drugs: Chemistry, Development & Future Challenges" London, March 2011
6) Unpublished work: M Gardner, M Palmer, T Parkinson, M Witty et al
7) Zhang et al, Bioorg Med Chem Lett 2012, 22, 1299-1307