Swiss TPH
Swiss Tropical and Public Health Institute
Schweizerisches Tropen- und Public Health-Institut
Institut Tropical et de Santé Publique Suisse

Associated Institute of the University of Basel

Medical Parasitology & Infection Biology
Parasite Chemotherapy Unit

Building on Success – Malaria Control and Elimination
Swiss TPH Winter Symposium 2016

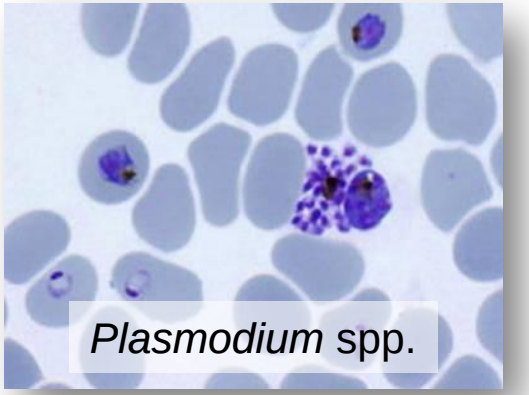
Drug Discovery at the Swiss TPH

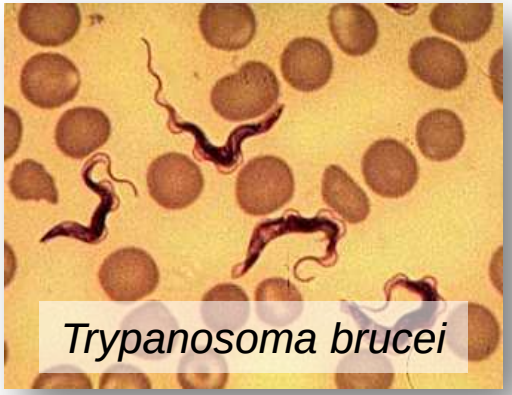
Matthias Rottmann

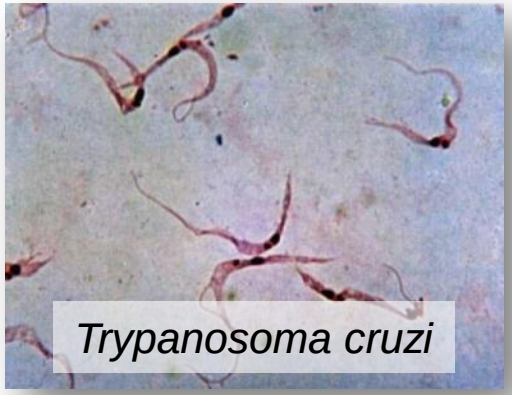
Parasite Chemotherapy Unit

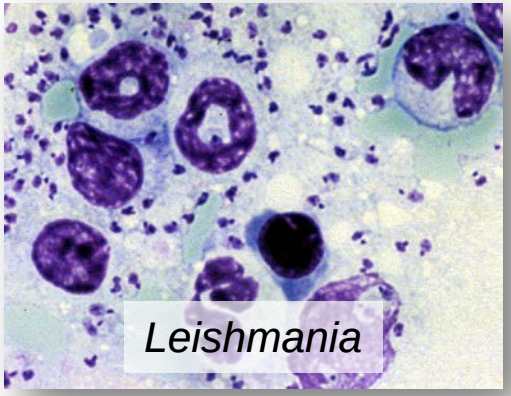
Swiss TPH

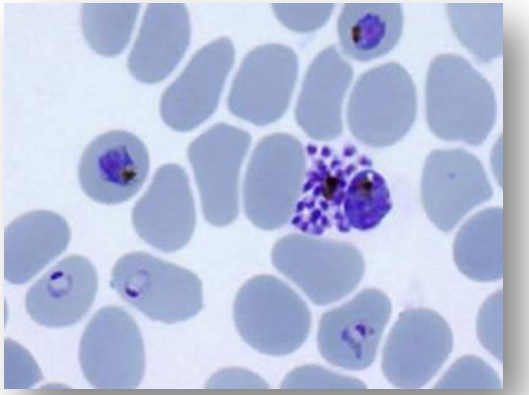
Cell-based drug screening
Lead optimisation and preclinical evaluation
Mode of drug action and mechanisms of drug resistance







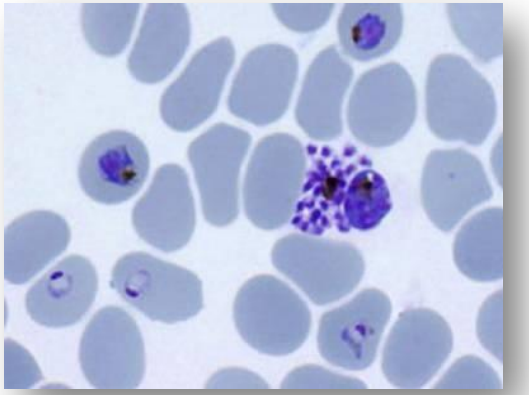




P. falciparum in vitro screen

P. berghei mouse model

P. falciparum SCID mouse model



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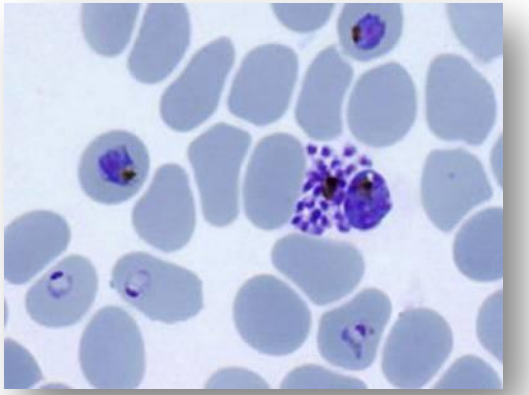

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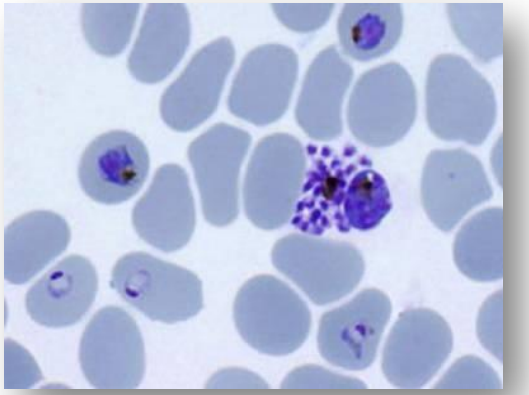
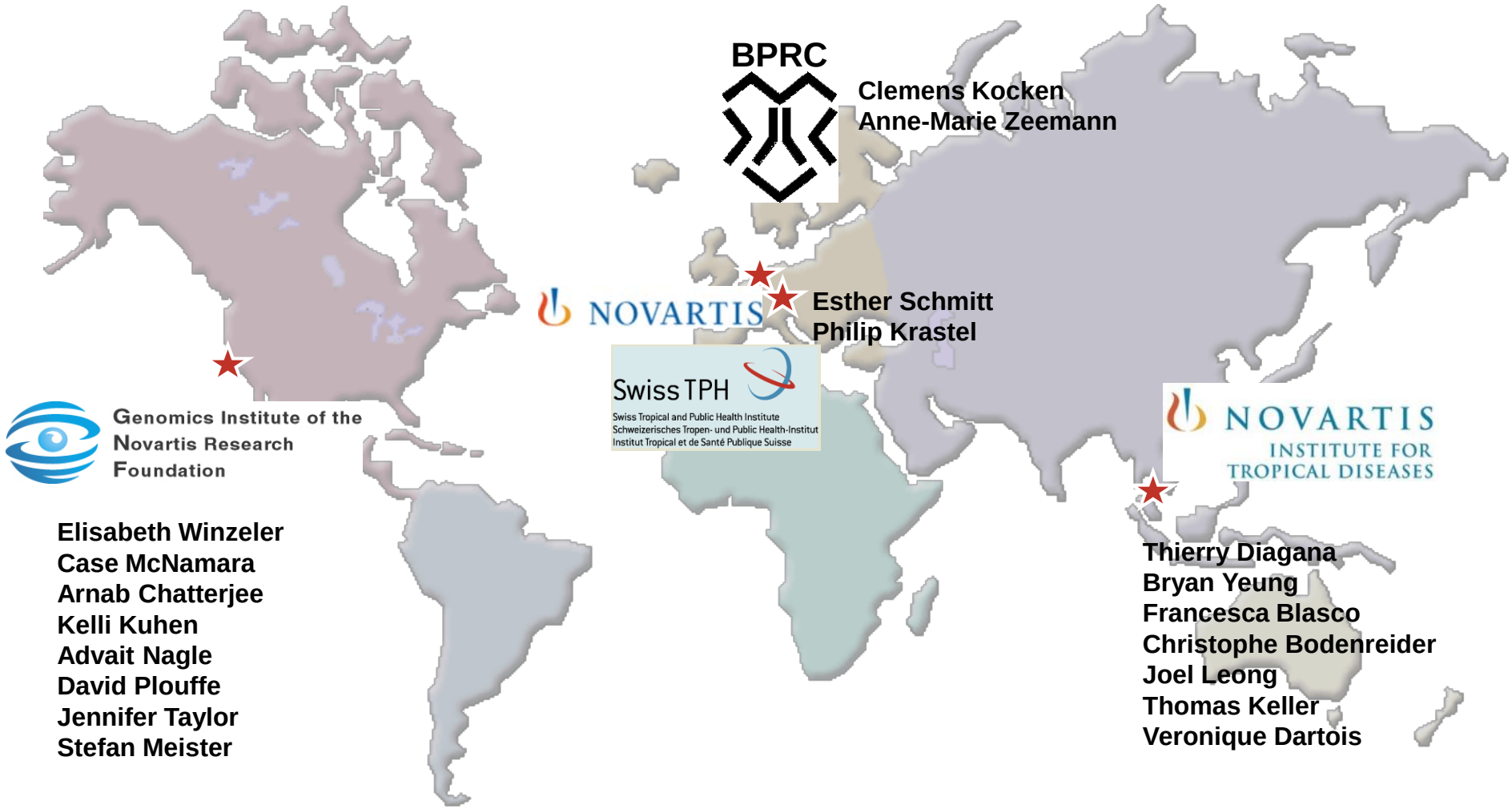


P. falciparum in vitro screen

P. berghei mouse model

P. falciparum SCID mouse model

NGBS programme for malaria



Malaria target product profile

Project goal: replace the "A" of ACTs while improving compliance

Objectives

Anticipate the threat of artemisinin resistance in SE Asia

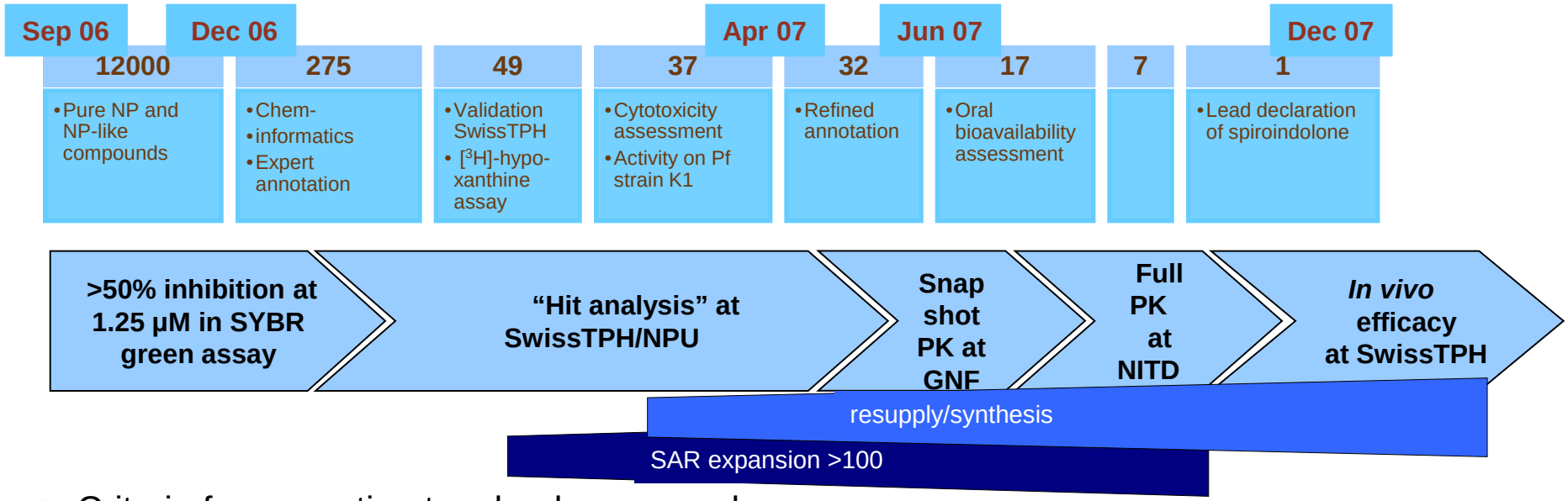
Improve patient compliance by reducing both pill burden and treatment duration

1. Artemether is fast-acting and potent but it is rapidly eliminated and must be taken twice daily for 3 days
2. Lumefantrine is long-lasting but because of its poor oral bioavailability the total dose is large and the pill burden is high

Uncomplicated Malaria TPP

Properties	Criteria
MOA	Non-peroxide, ideally a new chemotype
Antiplasmodial activity	Active against blood-stages of all drug resistant parasites
Bioavailability	> 40% orally
Dosing regimen	≤ 3 qd; ideally single dose
Safety	Safety profile not worse than Coartem; ideally safe in pregnant women and infants
Compound management	> 3 years shelf-life in endemic countries Low COGS Simple tablet formulation

The natural product screen



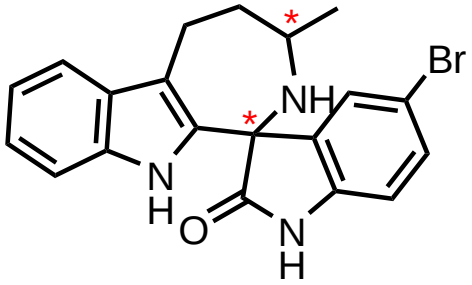
- Criteria for promotion to a lead compound
  - **In vitro potency:** < 100 nM on wild-type and chloroquine resistant *P. falciparum* strains
  - **PK/ADME:** oral bioavailability assessed (snapshot PK); in vitro PK evaluation (microsomal stability, Caco-2, CYP inhibition, physicochemical properties measured); full in vivo PK
  - **Safety:** Cytotoxicity evaluated in multiple mammalian cell lines, cardiotoxicity (hERG); and genotoxicity (mini-Ames) assessed

January 2009: Candidate selection; March 2010: Preclinical candidate declaration, 2011: Phase I clinical trial completed, 2014 Phase IIa proof of concept finished

Spiroindolones

Identification of a hit compound

- Scaffold from Novartis NP library however *not* a natural product (AGV493)
- Moderate activity against multiple *P. falciparum* strains
- Original archive sample was only 80% pure. Further purification of the sample and retesting increased activity **NF54 IC_{50} = 90 nM (K1 IC_{50} = 85 nM)**
- Compound displayed good solubility and high permeability (in vitro PK)
- Snapshot PK in mice showed good oral absorption (C_{max} = 1.6 μM)
- Single dose at 100 mg/kg reduced parasitemia by 96% in the *P. berghei* mouse model
- Archived compound was a racemate (mixture of enantiomers)



NF54 IC_{50} = 155 nM
K1 IC_{50} = 104 nM

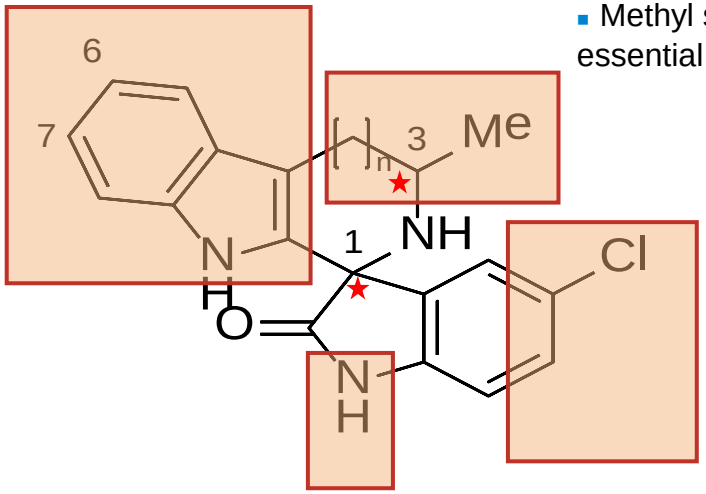
Spiroindolones

(Spirotetrahydro-β-carbolines) *SAR summary of the scaffold*

- **Six membered (n = 1) ring favored** but seven membered (n = 2) tolerated

- Methyl substitution at 3-position is essential (4-methyl derivatives inactive)

- **Seven position reduces metabolic liabilities and increases metabolic stability**
- **Halides at 6 and 7 position together increase potency**



- 5’ position optimized on oxindole

n = 1,2, or 3

- **Oxindole NH required for activity**

- **Both stereocenters are required** ★

Yeung et al., J Med Chem 2010

Medicinal chemistry key results

Lead optimization focused on improving potency and PK profile

	AGV493	NITD261	NITD579	KAE609
NF54 IC_{50} (nM)	77	~80x		0.9
Solubility buffer pH 6.8/FaSSIF (mg/L)	32 / na	194 / na	137 / 268	39 / 148
Microsomal metabolic clearance	low/medium	high	medium/high	low
PAMPA [% FA]	99	99	97	98
[1]C_{max} (µg/mL)	1.44	1.21	1.05	3.58
T ½ (h)	3.73	~7x		10.02
[1]AUC_{all} (µg•h/mL)	6.54	1.26	3.61	43.3
[1]F (%)	59	13	23	> 90
[2]V_{ss} (L/kg)	3.5	0.91	1.92	2.11
[2]CL (mL/h/kg)	2228	2979	1441	584

[1] at 25 mg/kg P.O. [2] at 5 mg/kg I.V. in mice

Spiroindolones for malaria

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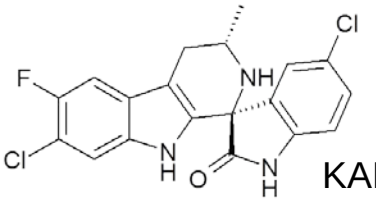
Summary



KAE609

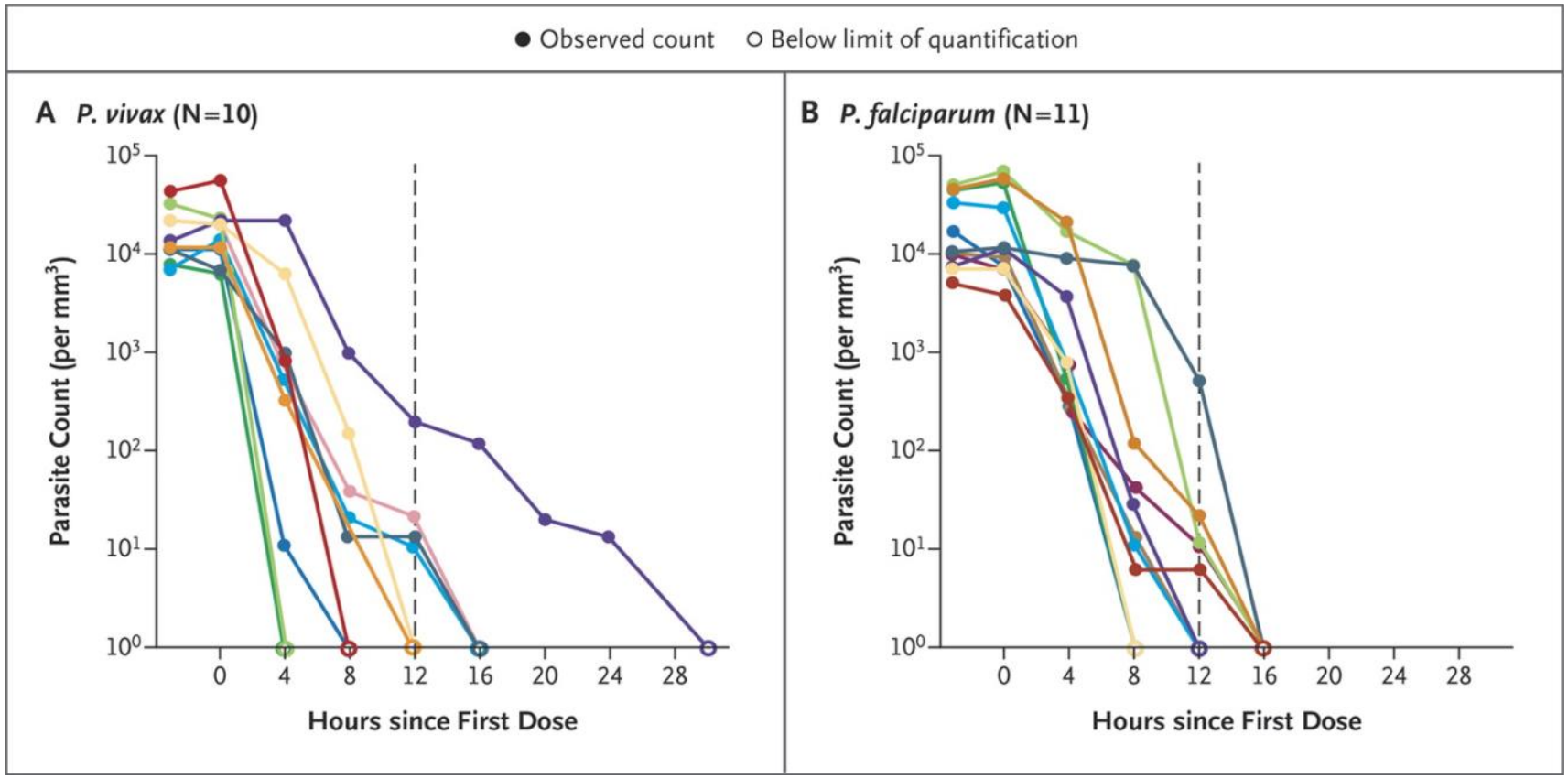
- Identified from cellular screen on *P. falciparum*
- Out of four stereoisomers only the 1*R*,3*S* enantiomer is active on the parasite
- Reducing metabolic liabilities lead to improvements in PK and potency
- Excellent in vivo efficacy
  - Sub nanomolar in vitro activity
  - Oral efficacy even at low doses (single dose cure possible)
  - Fast acting, high C_{max} compounds
- Active on multiple drug resistant strains as well as *P. f.* and *P. v.* clinical isolates
- Low cytotoxicity and low cardiotoxicity potential, and no genotoxicity flags
- New and highly specific chemotype for malaria (targets *Pf*ATPase4 (Na(+) efflux ATPase)

Rottmann et al., SCIENCE 2010

Spiroindolones for malaria

KAE609 (Cipargamin) is highly efficacious and rapidly kills Plasmodium parasites in infected humans

Parasite-Clearance Profiles in Individual Patients with *Plasmodium vivax* or *P. falciparum*

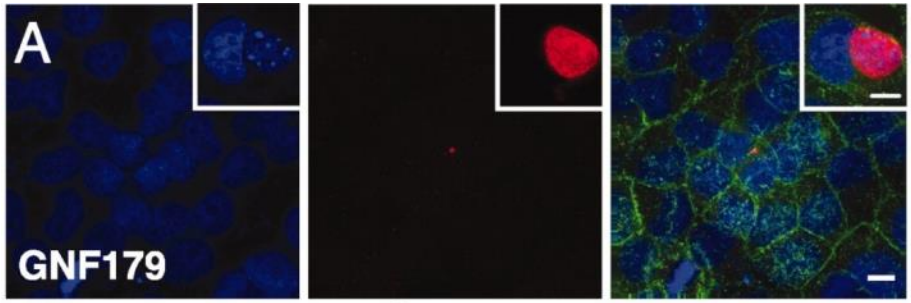


White et al., N Engl J Med 2014

Imidazolopiperazines KAF156 / GNF179

- Chemical class identified in a HTS blood stage assay
- Additional activity in *P. yoelii* liver stage screen detected
- Optimization towards a dual active clinical compound

Imidazolopiperazines KAF156 / GNF179



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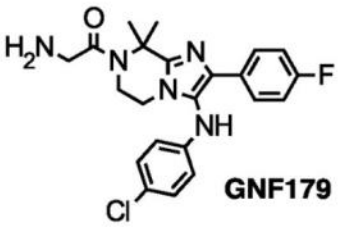
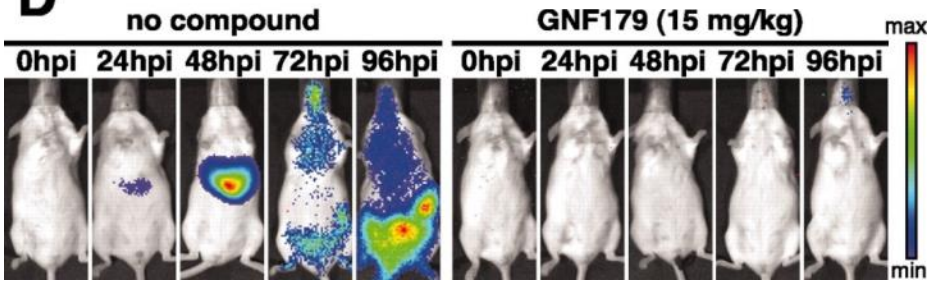


C

In vivo efficacy

	Dose mg/kg p.o.	Parasitemia reduction (%)	Survival (days)
GNF179	1 x 100	99.5	19.0
GNF179	1 x 30	99.5	15.7
AS	1 x 100	97	6.7
AS	1 x 30	89	7.2

D



The effect of the imidazolopiperazine GNF179 on the liver-stage parasite and comparison

(**A**) High-resolution deconvolution microscopy of the GNF179-treated liver-stage parasites. Columns show Hoechst 33342 staining in blue, α*Py*HSP70 staining in red, and a merge with the host plasma membrane marker CD81-GFP in green.
Cultures were treated with 1 μM GNF179 for 48 hours. (Insets) DMSO-treated control parasite at the same scale and time point. Scale bar indicates 10 μm.

(**B**) Chemical structure of GNF179.

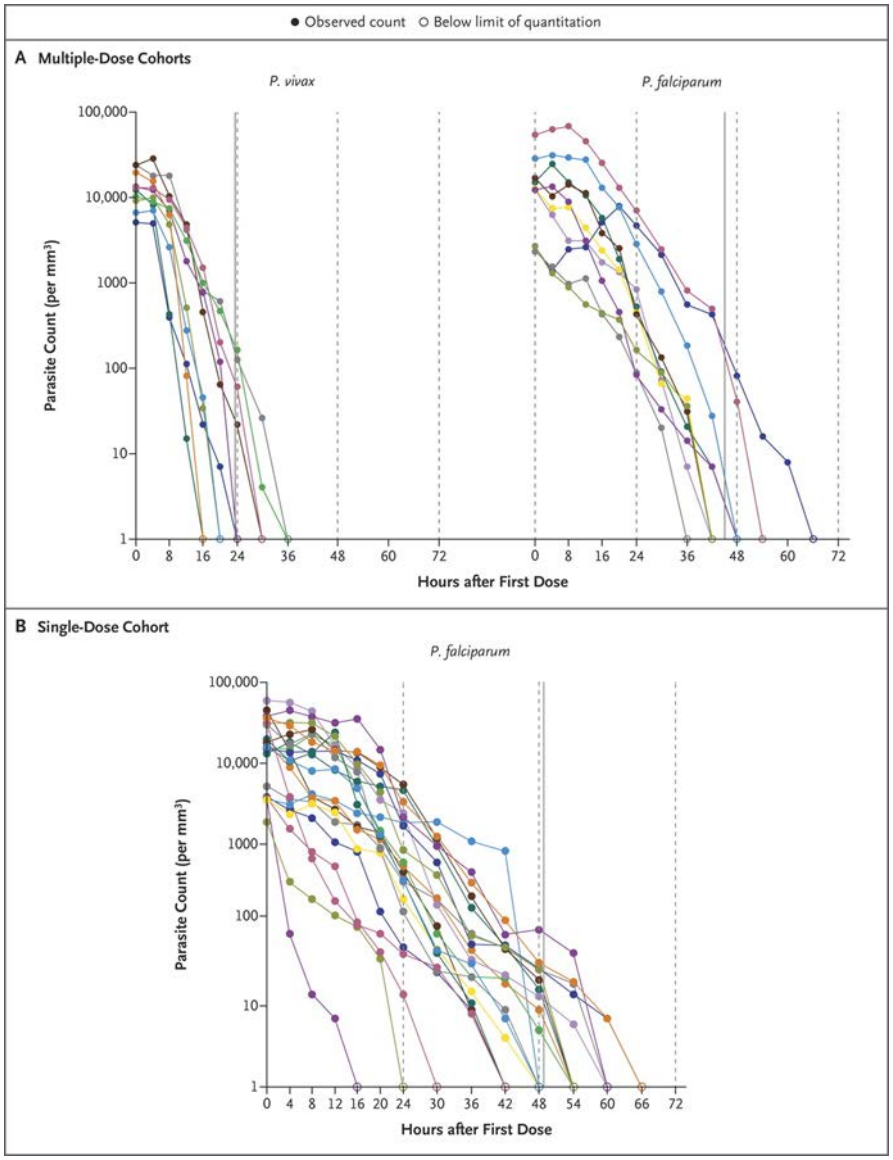
(**C**) Blood stage survival and parasitemia reduction in a *P. berghei* mouse model.

(**D**) In vivo bioluminescence imaging of representative mice infected with *P. berghei* **sporozoites** and treated with GNF179 (15 mg/kg) or vehicle (no compound) at 6 hpi.

Meister et al., SCIENCE 2011

Imidazolopiperazines KAF156

Parasite Clearance in Patients treated with KAF156



400 mg daily (3 days)

single 800-mg dose

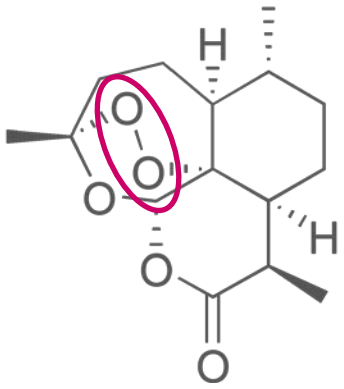
Parasite Counts in the Multiple-Dose and Single-Dose Cohorts. Each line represents an individual patient. In each cohort, the solid vertical line indicates the median parasite clearance time.

White et al. N Engl J Med 2016

Artemisinin: the peroxide bond is key

Rapid reduction of parasite burden

Artemisinin interacts with heme (Fe2+) to produce free radicals leading to alkylation of heme and key parasite proteins



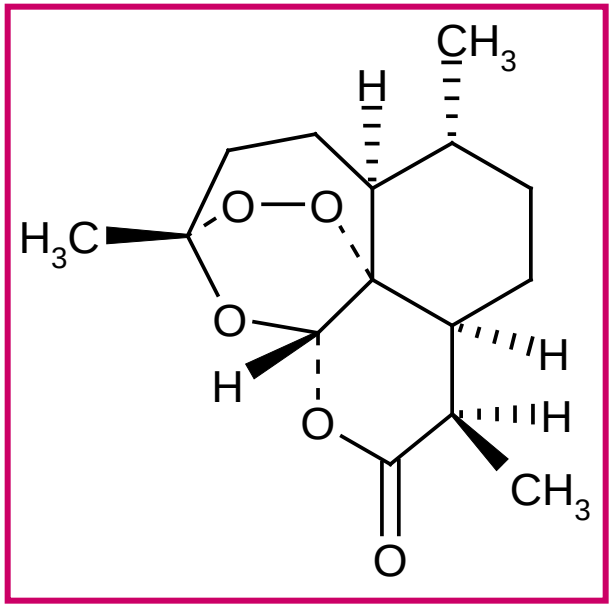
Artemisinin shortcomings:

- Dependency on plant can result in supply issues
- Short pharmacokinetic half-lives (parasite recrudescence, compliance problems)
- Potential neurotoxicity and embryotoxicity concerns
- Synthetic production needs >10 steps

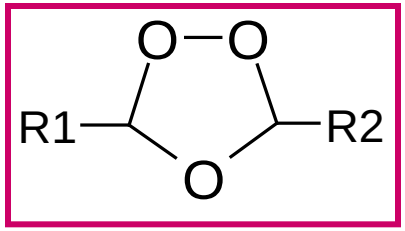
⇒ Synthetic peroxides to replace artemisinins ?

Synthetic peroxides to replace artemisinins

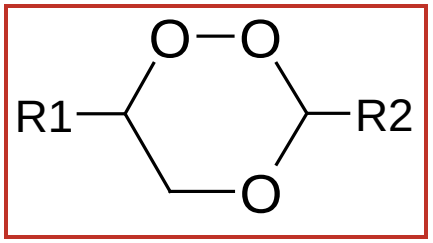
Griesbaum et al. *Liebigs Ann./ Recueil* **1997**, 1381-1390.
Karlsruhe University



Vennerstrom



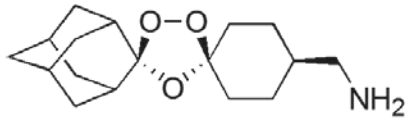
Trioxolane; easy synthesis



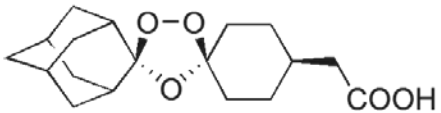
Trioxane; easy synthesis

Identification of OZ277

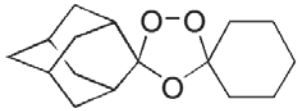
good PK, excellent activity,
some toxicity



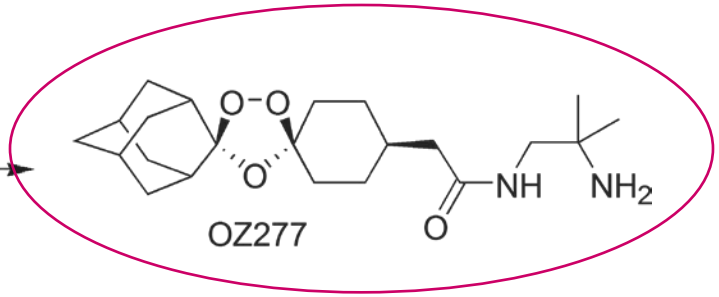
OZ209



OZ78



OZ03



OZ277

excellent PK,
low activity

bad PK,
ok activity

good PK, good activity,
no toxicity

Vennerstrom et al., NATURE 2004

OZ277 + Piperaquine

OZ 277

Phase I studies Thai subjects completed 4th Q 2004

Piperaquine

Phase I single rising dose studies completed 2nd Q 2006

Phase I OZ277+PQP completed 4th Q 2006

OZ 277

Phase II dose ranging study completed 4th Q 2006

OZ 277 + Piperaquine

November 2011: Combination approved on the Indian market.

This is the very first new drug ever developed in India !

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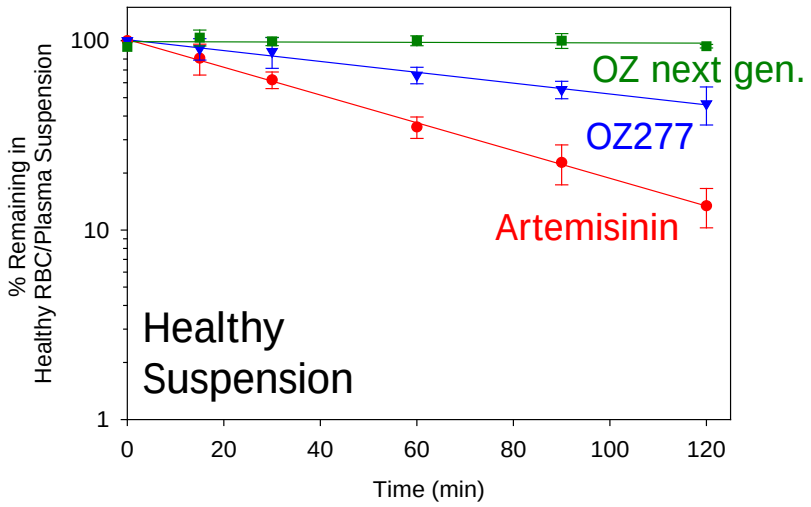
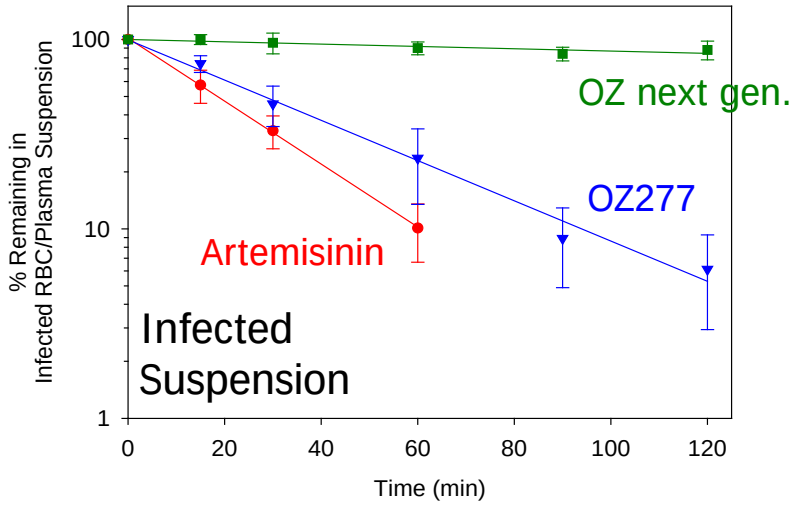
In vitro stability in human RBC/Plasma suspension





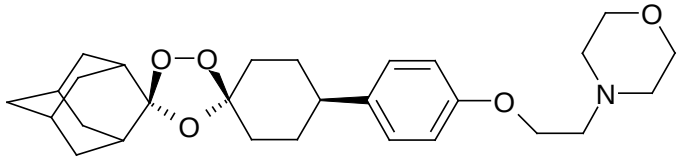
OZ next generation compounds such as OZ439 are >20-fold more stable than OZ277 in both healthy and infected blood suspensions

OZ439

OZ 439

Phase I studies healthy subjects (2009)

Phase II (October 2010-October 2012)



OZ439 + Piperaquine

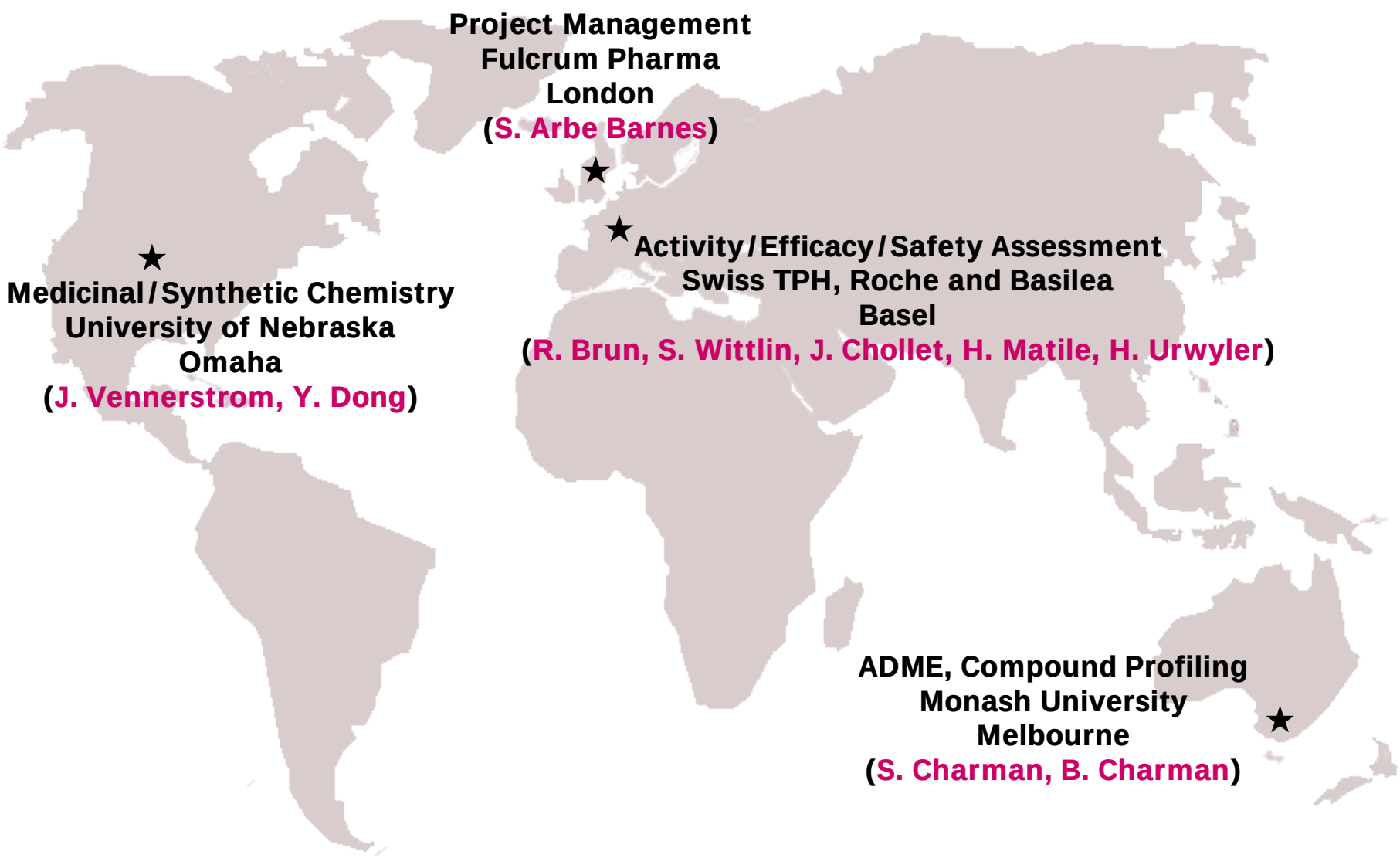
Phase I completed 3rd Q 2013

Next on schedule: Phase IIb studies (ongoing)

OZ439 + Ferroquine

Ongoing

The OZ (synthetic peroxide) discovery team



MMV Portfolio, 3Q 2016

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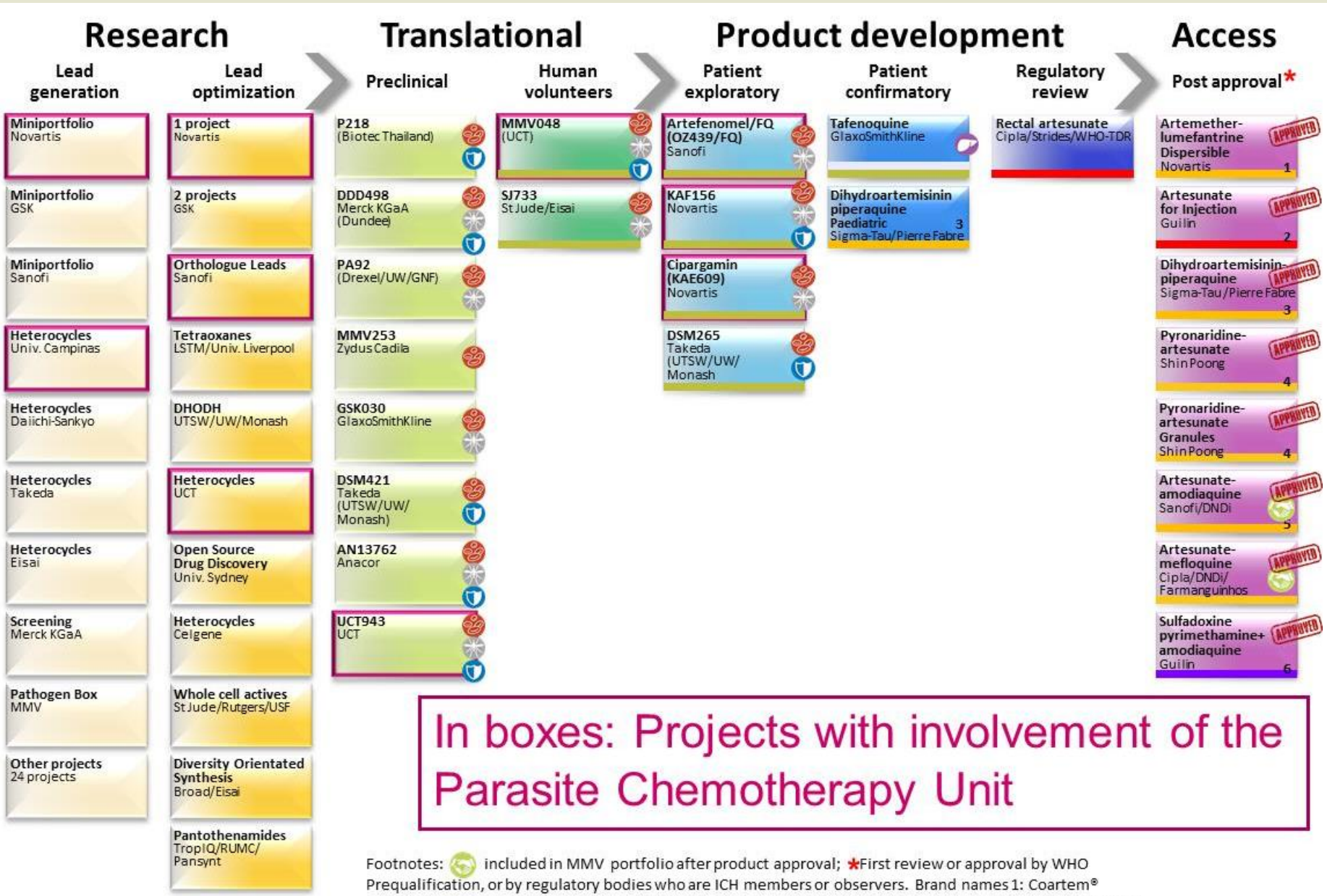

In boxes: Projects with involvement of the Parasite Chemotherapy Unit

Footnotes: included in MMV portfolio after product approval; *First review or approval by WHO Prequalification, or by regulatory bodies who are ICH members or observers. Brand names 1: Coartem® *Dispersible*; 2: Artesun® 3: Eurartesim®;4: Pyramax® tablets and granules; 5: ASAQ/Winthrop®; 6: SPAQ-CO™

Thank you

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Parasite Chemotherapy Unit

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