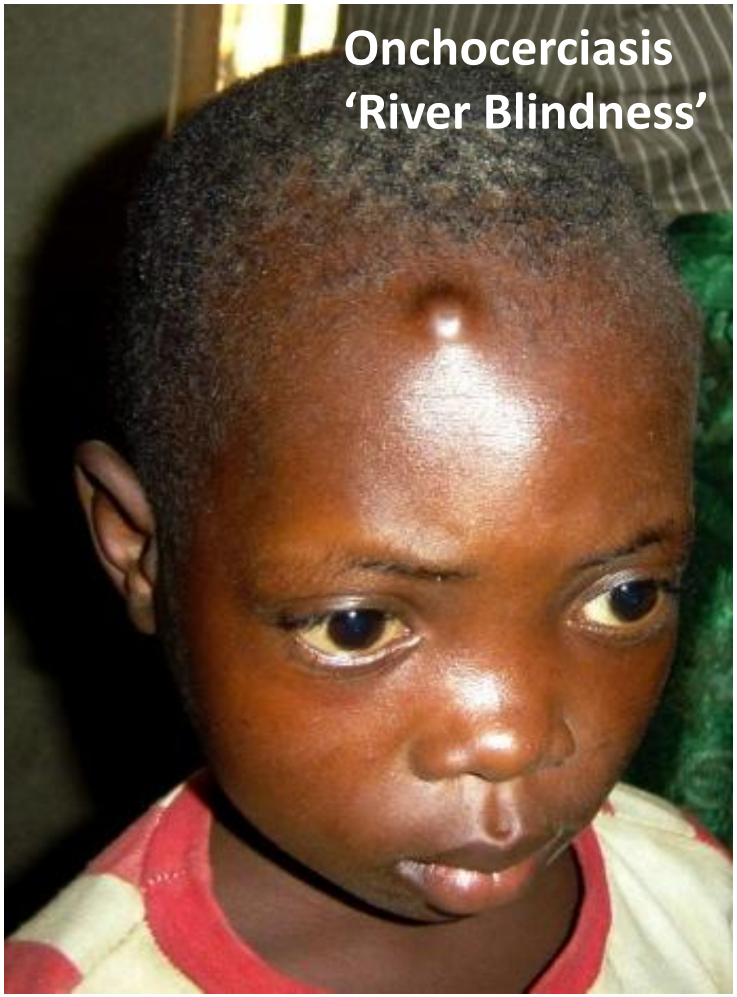
LSTM
LIVERPOOL SCHOOL
OF TROPICAL MEDICINE

# The AWOL Drug Discovery Project

*Steve Ward*

*Mahidol University April 2nd 2018*

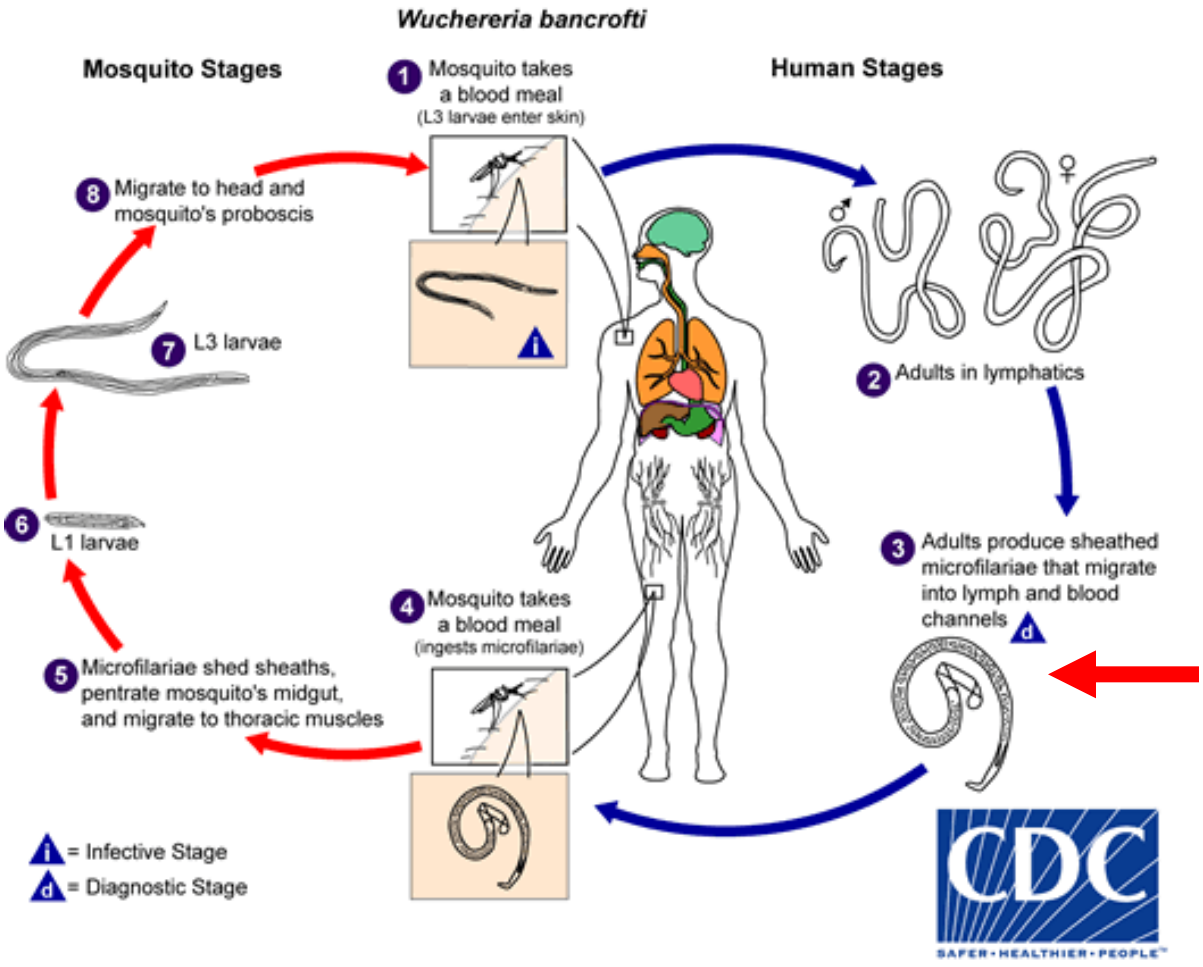
# HUMAN FILARIASIS





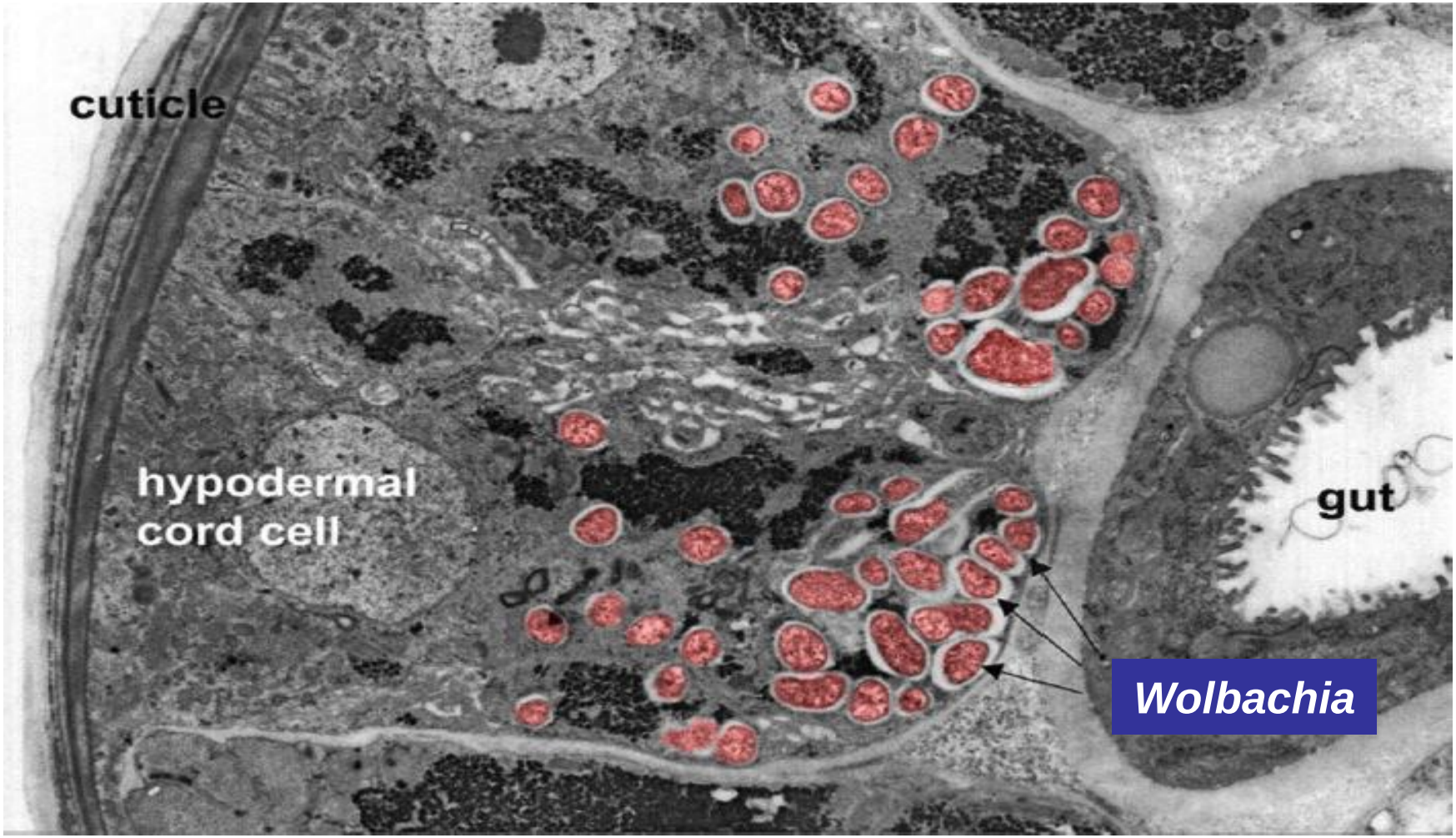
**37 million** **120 million**

# LF life cycle




**Current Therapeutic Strategy : MDA**

# Filarial nematodes host an essential bacterial symbiont – *Wolbachia*



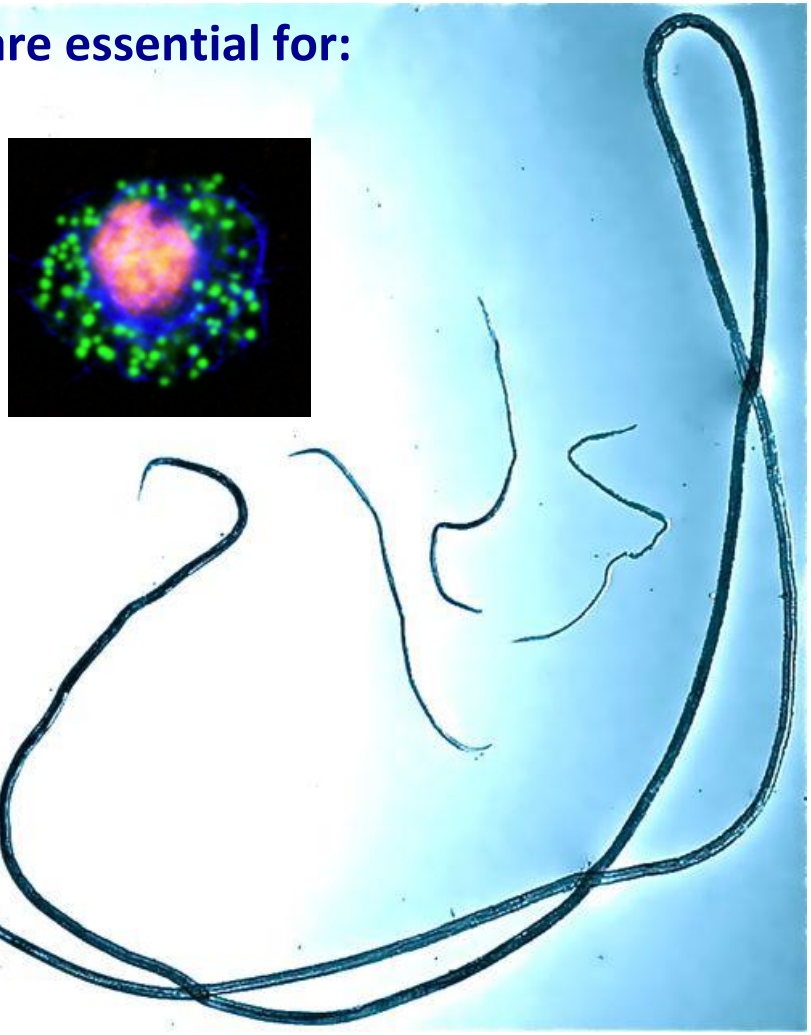
# *Wolbachia* and filarial nematodes

- **These mutualistic endosymbiotic bacteria are essential for:**
  - **Development**
    - Larval development
    - Embryogenesis

      **PROPHYLAXIS**
      **TRANSMISSION BLOCKING**
  - **Disease pathogenesis**
    - Inflammatory reactions
    - Clinical disease

      **CLINICAL CASE MANAGEMENT**
  - **Adult worm longevity (10-14 years)**
    - Adults depleted of *Wolbachia* die 1-2 years later

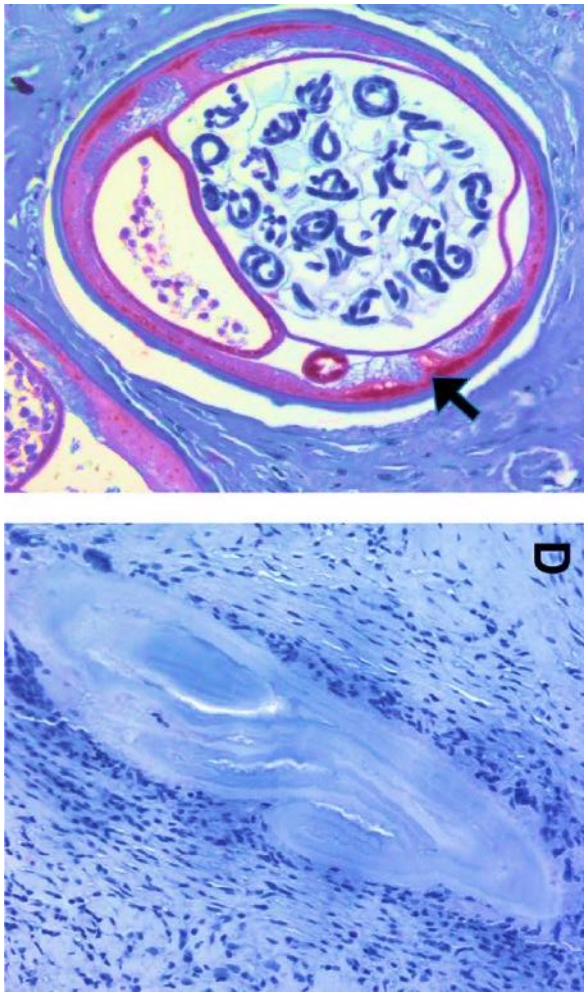
      **MACROFILARICIDAL (kills adult worms)**



# Anti-*Wolbachia* mode-of-action *benefits:* POC studies with the antibiotic doxycycline

- **Potent macrofilaricide**: slow kill avoids adult killing SAE
- **Permanently sterilises adult worms:** no recrudescence
- **Blocks transmission:** worms without *Wolbachia* unable to develop in vector
- **Improves clinical disease**: Skin disease, Hydrocoele, Lymphoedema

- **Safe in *Loa loa* co-infection:** no need for pre-screening
- **Not microfilaricidal:** no risk of ocular SAE



***Onchocerca volvulus***

# *Wolbachia* depletion leads to permanent sterility and transmission blocking

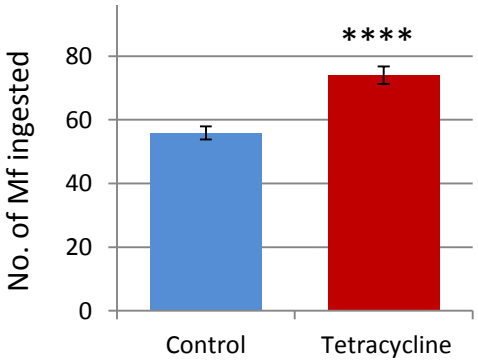
- **Blocks embryogenesis**

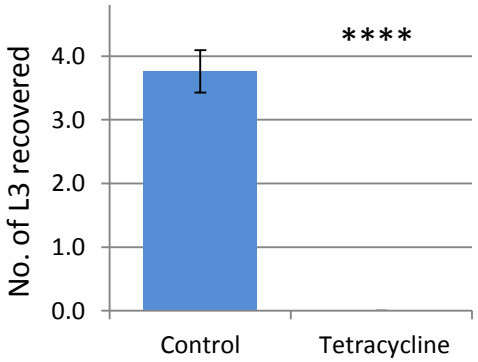
Prevents release of new microfilaria

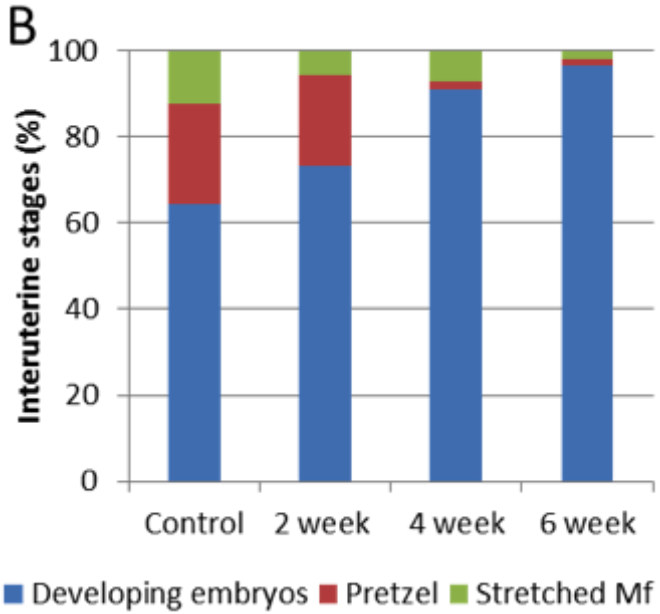
- **Blocks transmission**

Microfilaria unable to develop in mosquito vector

6 Weeks



# A·WOL doxycycline therapy – a new cure for river blindness and elephantiasis

# DOXYCYCLINE for Yanomami with Onchocerciasis in Brazil and Venezuela

# A·WOL's GOALS

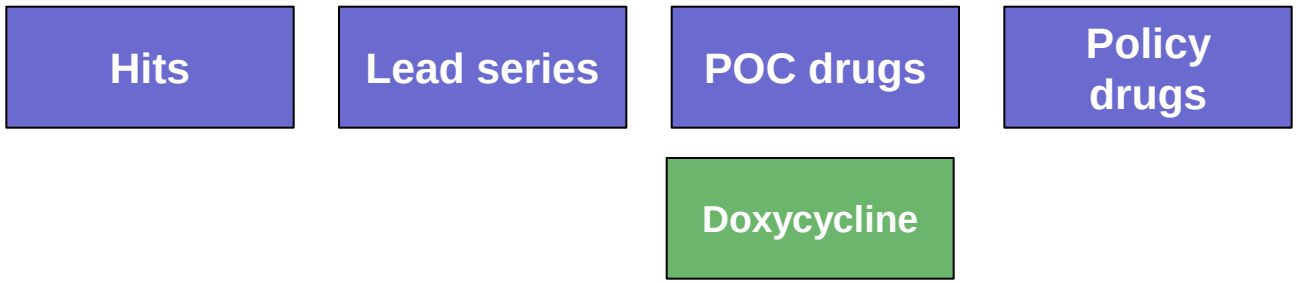
**1) To find a new anti-*Wolbachia* treatment with:**

- **Shorter regimen (From 4-6 weeks to 7 days or less test & treat)**
- **Utility in children and women of child bearing age**

2) To find the best regime with existing drugs or combinations for use in restricted settings

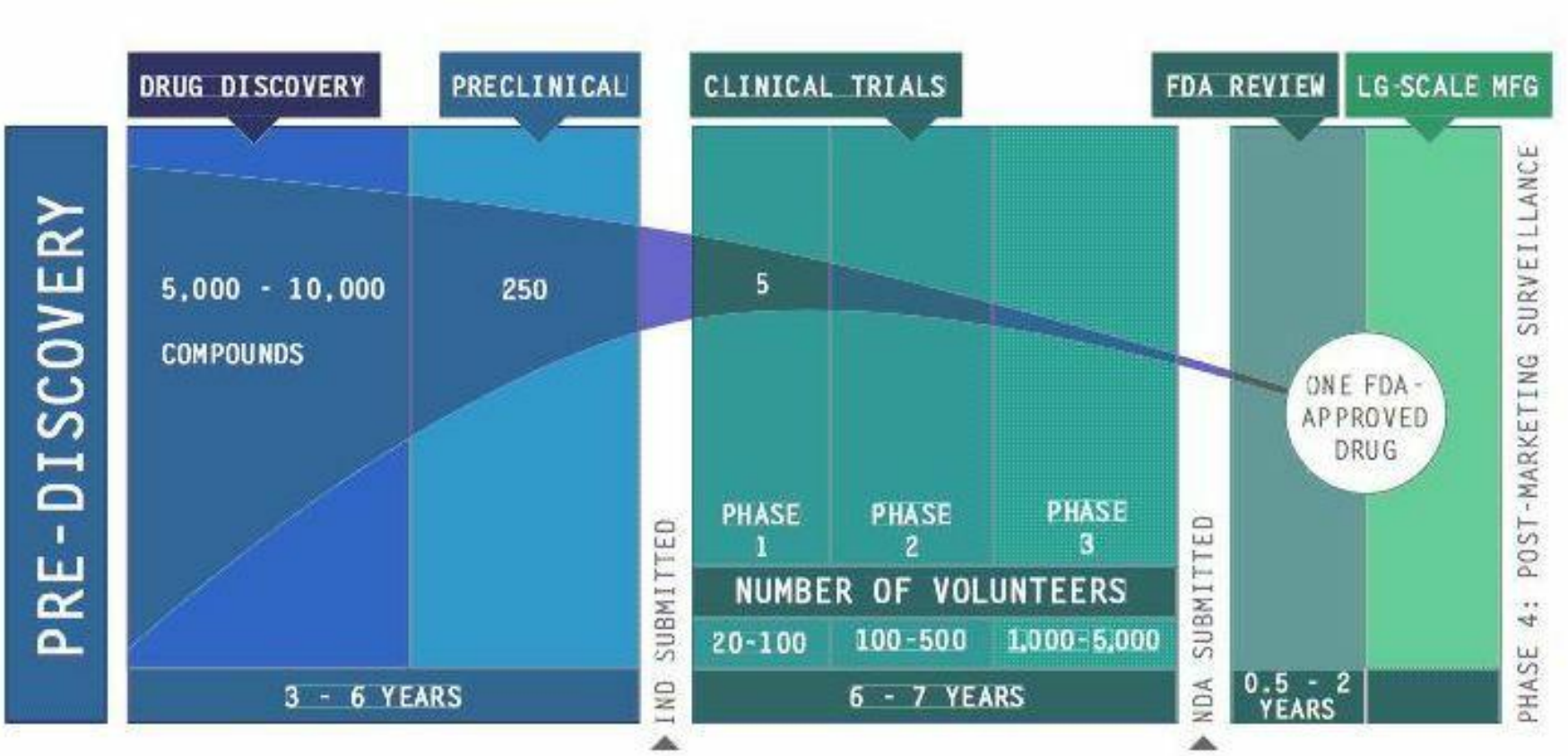
- Drug-resistant parasites
- *Loa loa* co-endemic area
- MDA end game = TEST & TREAT

# The A·WOL portfolio in 2007

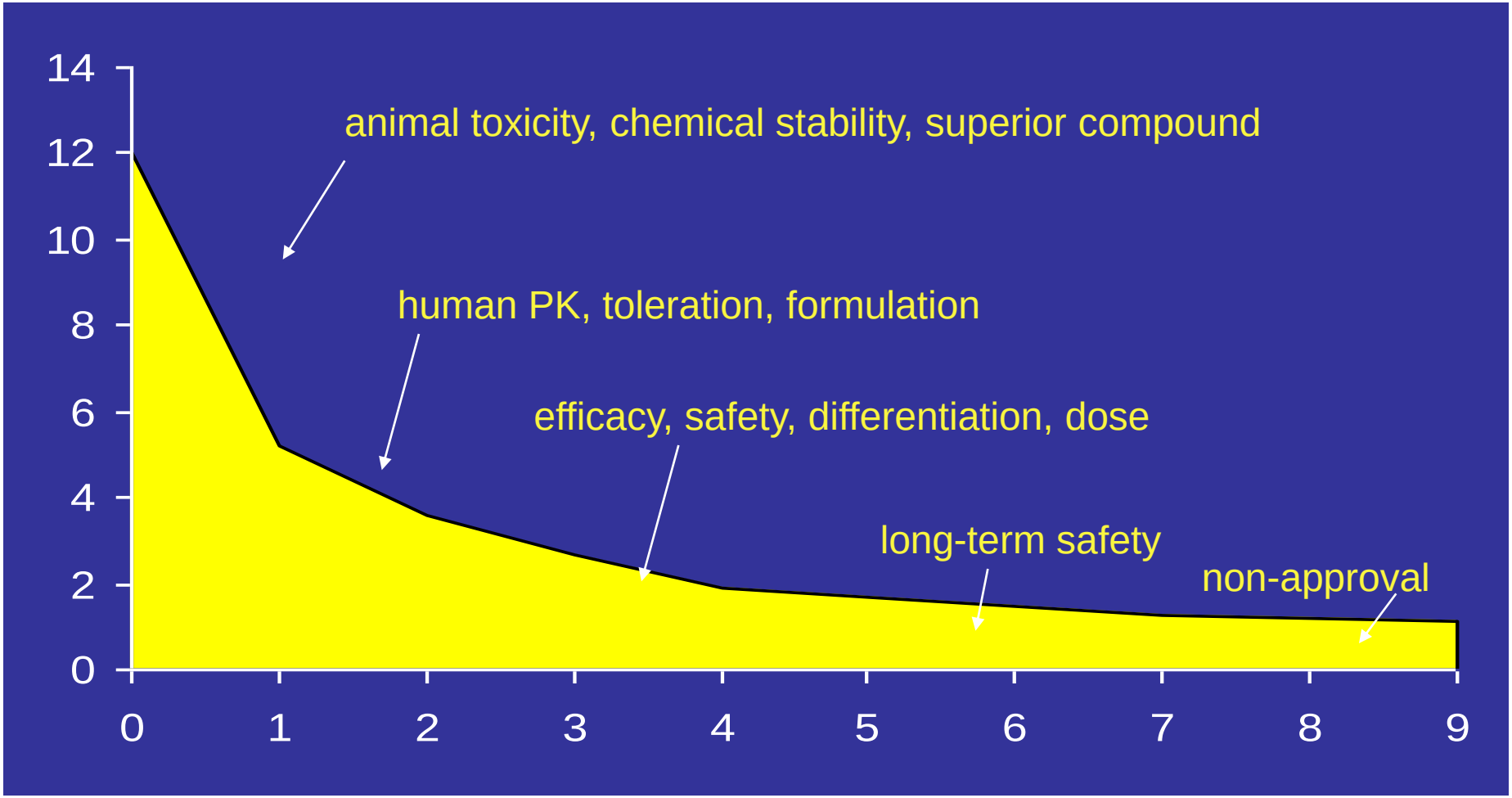


# Drug Development Pipeline – The Challenge

- **Applicable to all drugs – high attrition rates.**



# Reasons for attrition

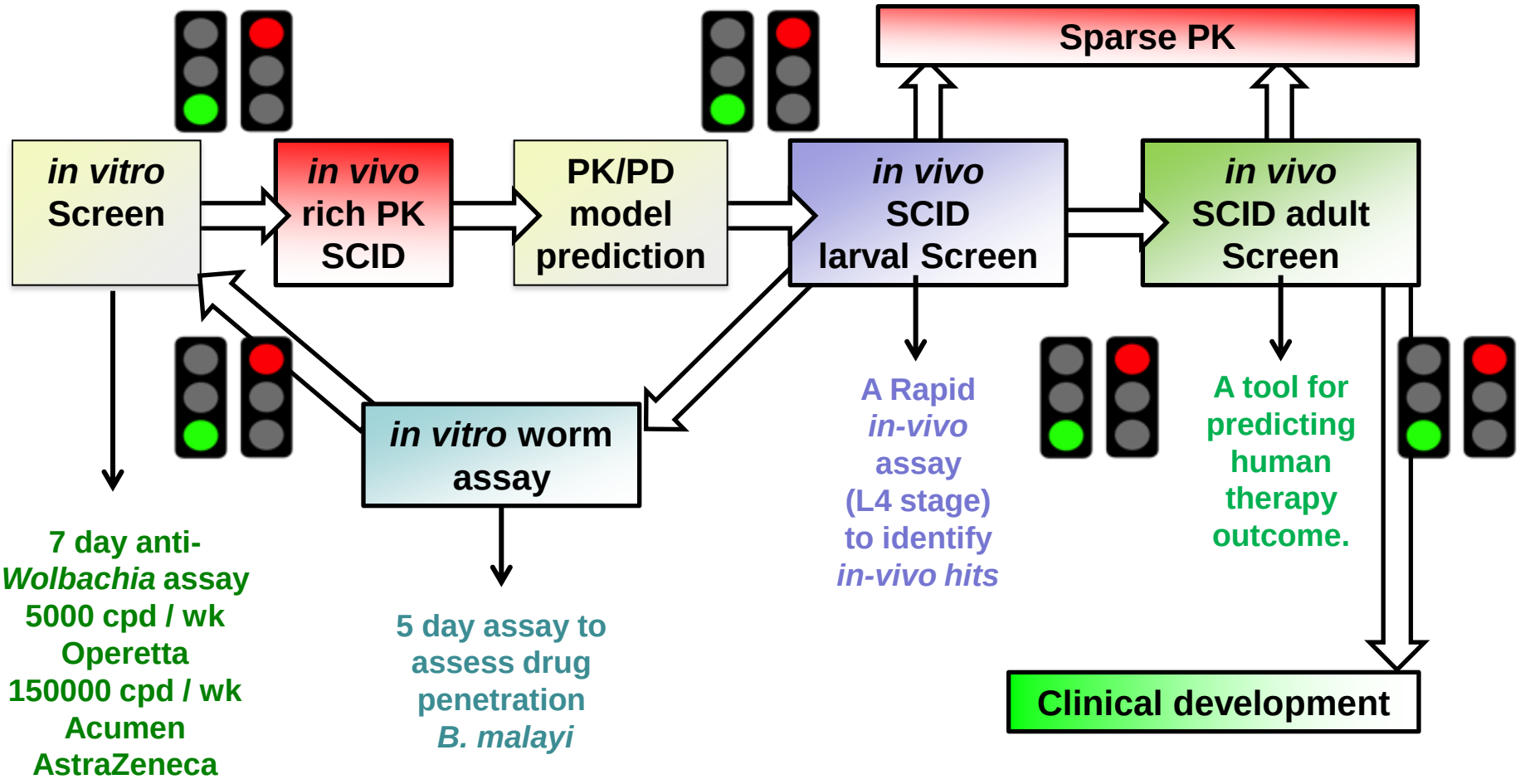


## BEGIN WITH THE END IN MIND!

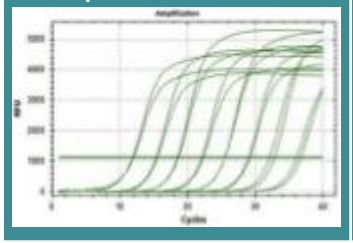
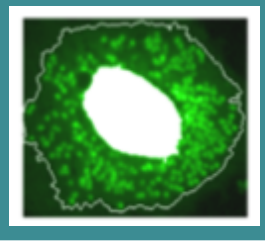
## *Wolbachia* depletion: Clinically-validated outcome measure of macrofilaricidal activity – Onchocerciasis

| Regimen | *Wolbachia* depletion | Macrofilaricidal Activity | Reference |
|---|---|---|---|
| DOX 200mg 8 weeks | **ND** | **80%** | Taylor et al 2005 |
| DOX 200mg 4 weeks | **95%** | **80%** | Debrah *et al* 2007 |
| DOX 200mg 4 weeks | **93%** | **100%** | Debrah *et al* 2011 |
| DOX 200mg 3 weeks | **85%** | **NS (embryo blockage)** | Turner *et al* 2006 |
| DOX 200mg + RIF 10MKD 2 weeks | **85%** | **50%** | Debrah *et al* 2011 |
| DOX 200mg + RIF 10MKD 3 weeks | **85%** | **64% (embryo blockage)** | A·WOL I trial |

# Anti-*Wolbachia* screening pipeline



# Evolution of the *in vitro* cell screen

| Screening assay | A·WOL I<br>qPCR screen | A·WOL II<br>HCS | A·WOL II<br>industrial scale HTS |
|---|---|---|---|
| |  |  |   |

| Compound Library | BioFocus<br>* and ** | MMV req. 1-4<br>* and ** | remaining MMV<br>* | AstraZeneca library<br>* and 6,000** |
|---|---|---|---|---|
| Total cmpds screened | 10,000 | 56,000 | 476,794 | ~1.3 million |
| Time scale (months) | 12 | 18 | 2 | 4 |
| Compounds/month (average) | 833 | 3,111 | 247,500 | 81,250 |

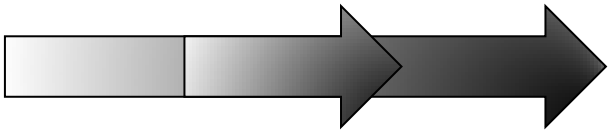
**>2 million screened = 20,000 hits**

*single dose ** dose response

# Its EXPOSURE that matters not the DOSE !

**Animals in disease models are often dosed with unrealistic doses that could not be translated into a clinical setting.**

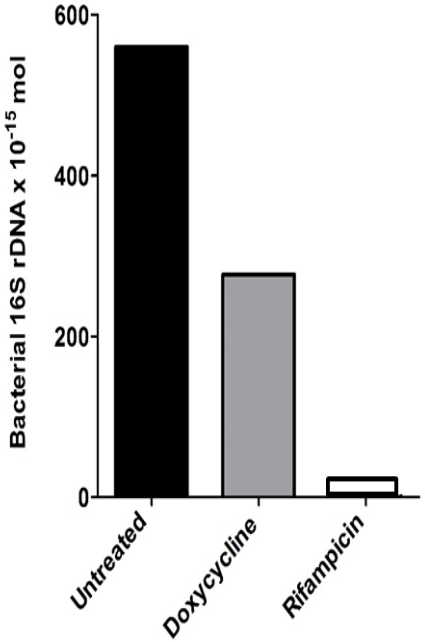
**We aim in *AWOL* at delivering realistic and clinically relevant exposure of drugs in disease models for ease of translating results to clinical**



**Modelling aims at narrowing gap between the animal model and the patient**

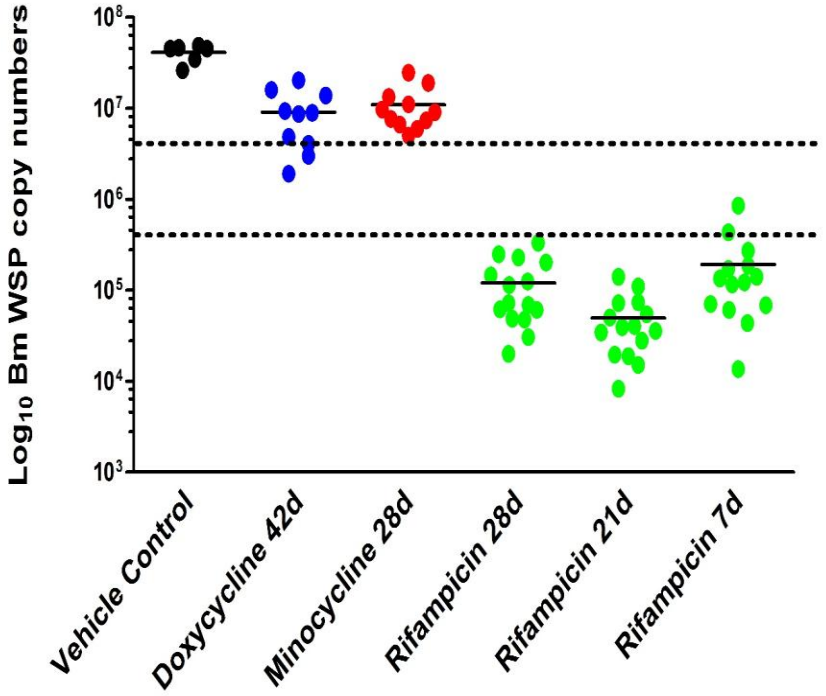
# Failure to Translate 2

## The Rifampicin PK/PD disconnect




***Volkmann et al. 2003***
***Rifampicin 50mg/kg/day for 14 days (Litomosoides sigmodontis model)***

***LSTM data, Brugia malayi SCID mouse model***
***50mg/kg/day Rifampicin dose***



# Rifampicin Clinical Trials

## Mouse – Human mismatch?

*Research Article*

**Macrofilaricidal Activity in *Wuchereria bancrofti* after 2 Weeks Treatment with a Combination of Rifampicin plus Doxycycline**

Alexander Yaw Debrah,[1,2] Sabine Mand,[3] Yeboah Marfo-Debrekyei,[2] Linda Batsa,[2] Anna Albers,[3] Sabine Specht,[3] Ute Klarmann,[3] Kenneth Pfarr,[3] Ohene Adjei,[4] and Achim Hoerauf[3]

**Efficacy of 2- and 4-week rifampicin treatment on the *Wolbachia* of *Onchocerca volvulus***

Sabine Specht · Sabine Mand · Yeboah Marfo-Debrekyei · Alexander Yaw Debrah · Peter Konadu · Ohene Adjei · Dietrich W. Büttner · Achim Hoerauf

**Two clinical studies in LF and Onchocerciasis show that Rifampicin whilst exhibiting significant activity against *Wolbachia* is not sufficient to exhibit an optimal macrofilaricidal effect**



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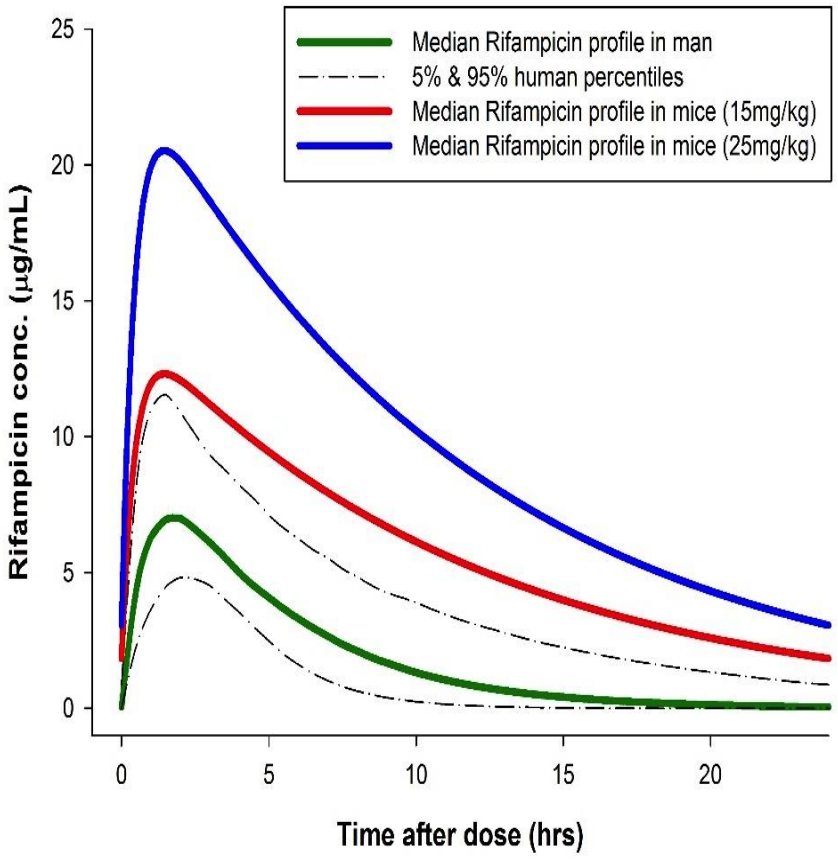
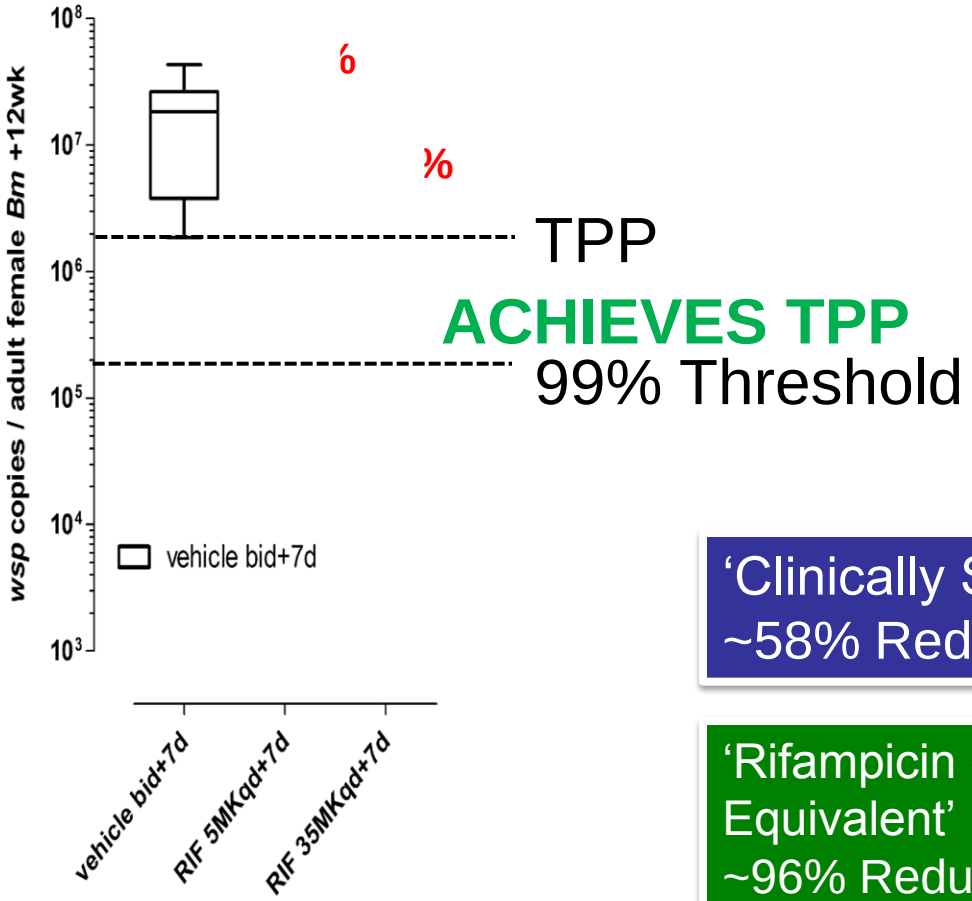
# Rifampicin: Human – Mouse PK bridging




- Simulations represent a 600mg dose in an average 70kg adult. Based on literature values of Rifampicin PK in adults.
- 15mg/kg mouse dose (which is far more effective than lower doses in the mouse model) represents exposure levels significantly higher than those seen in humans taking 600mg tablets.

# Rifampicin Dose- Effect Relationship




‘Clinically Standard’ Rifampicin Dose
~58% Reduction in *Wolbachia*

‘Rifampicin Super Dose Equivalent’
~96% Reduction in *Wolbachia*



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PNAS

# Albendazole and antibiotics synergize to deliver short-course anti-*Wolbachia* curative treatments in preclinical models of filariasis

Joseph D. Turner[a,1], Raman Sharma[a,1], Ghaith Al Jayoussi[a], Hayley E. Tyrer[a], Joanne Gamble[a], Laura Hayward[a], Richard S. Priestley[a], Emma A. Murphy[a], Jill Davies[a], David Waterhouse[a], Darren A. N. Cook[a], Rachel H. Clare[a], Andrew Cassidy[a], Andrew Steven[a], Kelly L. Johnston[a], John McCall[b], Louise Ford[a], Janet Hemingway[a,2], Stephen A. Ward[a], and Mark J. Taylor[a]

[a]Research Centre for Drugs and Diagnostics, Department of Parasitology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom; and [b]TRS Laboratories, Athens, GA 30605

Contributed by Janet Hemingway, September 28, 2017 (sent for review June 16, 2017; reviewed by John Horton and Patrick Lammie)

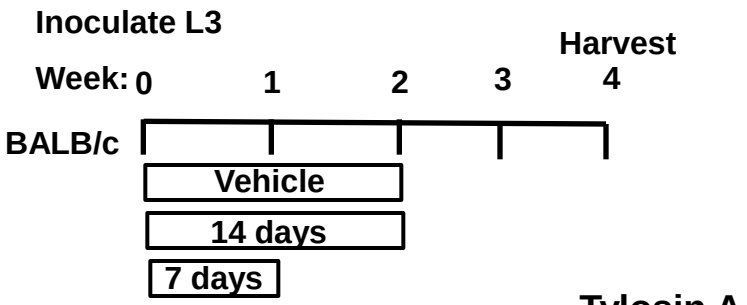
**Elimination of filariasis requires a macrofilaricide treatment that can be delivered within a 7-day period. Here we have identified a synergy between the anthelmintic albendazole (ABZ) and drugs depleting the filarial endosymbiont *Wolbachia*, a proven macrofilaricide target, which reduces treatment from several weeks to 7 days in preclinical models. ABZ had negligible effects on *Wolbachia* but synergized with minocycline or rifampicin (RIF) to deplete symbionts, block embryogenesis, and stop microfilariae production. Greater than 99% *Wolbachia* depletion following 7-day combination of RIF+ABZ also led to accelerated macrofilaricidal activity. Thus, we provide preclinical proof-of-concept of treatment shortening using antibiotic+ABZ combinations to deliver anti-*Wolbachia* sterilizing and macrofilaricidal**

demic areas. Although targeting mf has proved effective in elimination of LF and onchocerciasis in certain country settings (5–7) the strategy has failed either to deliver expected outcomes or has not yet been deployed sustainably in many regions of sub-Saharan Africa (8–10). In particular, the use of IVM is problematic in geographical regions where *L. loa* is coendemic, as this can result in severe adverse reactions (SAEs) caused by drug-induced death and associated inflammation of blood-borne *L. loa* mf in the brain (11). These SAEs can result in encephalopathy, coma, and death (12). Epidemiological simulations also predict MDA will not interrupt transmission in certain scenarios

PHARMACOLOGY

# Tylosin (Tylan™) – a veterinary macrolide from Abbvie screen

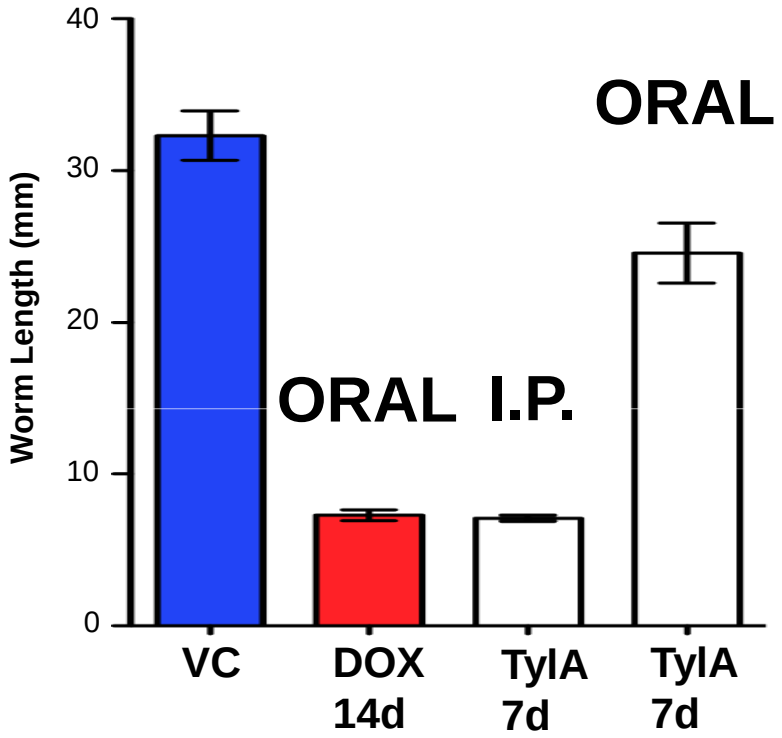

# The Tylamac story



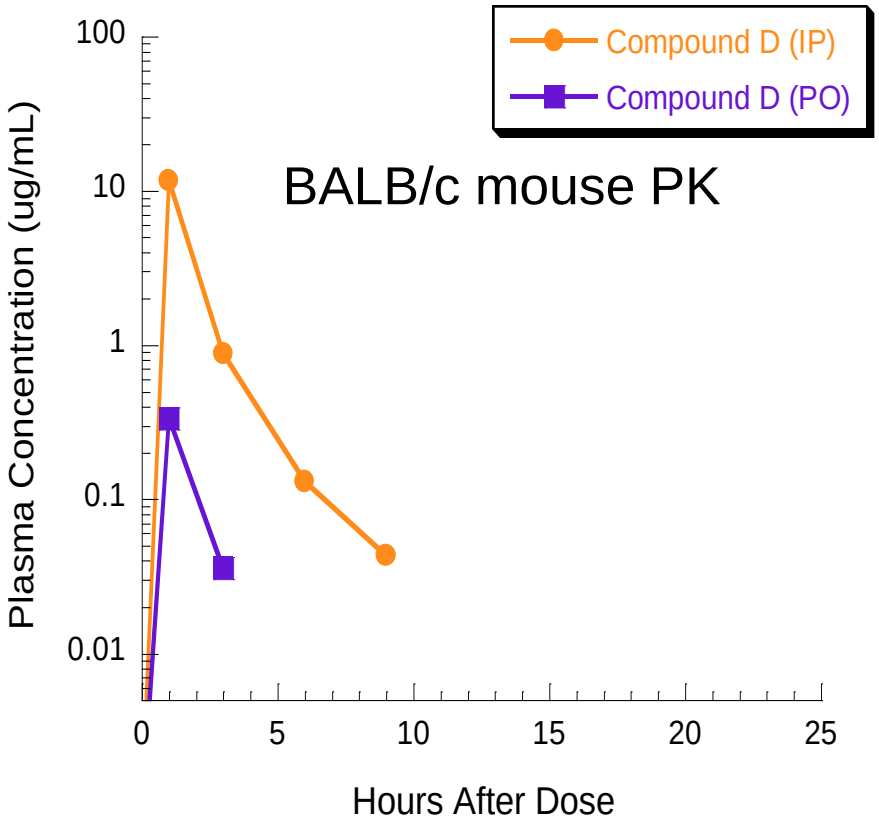

Tylosin A

## Efficacy in *Litomosoides* larval model

***Sabine Specht,*** *Achim Hoerauf*
*University Clinic Bonn*



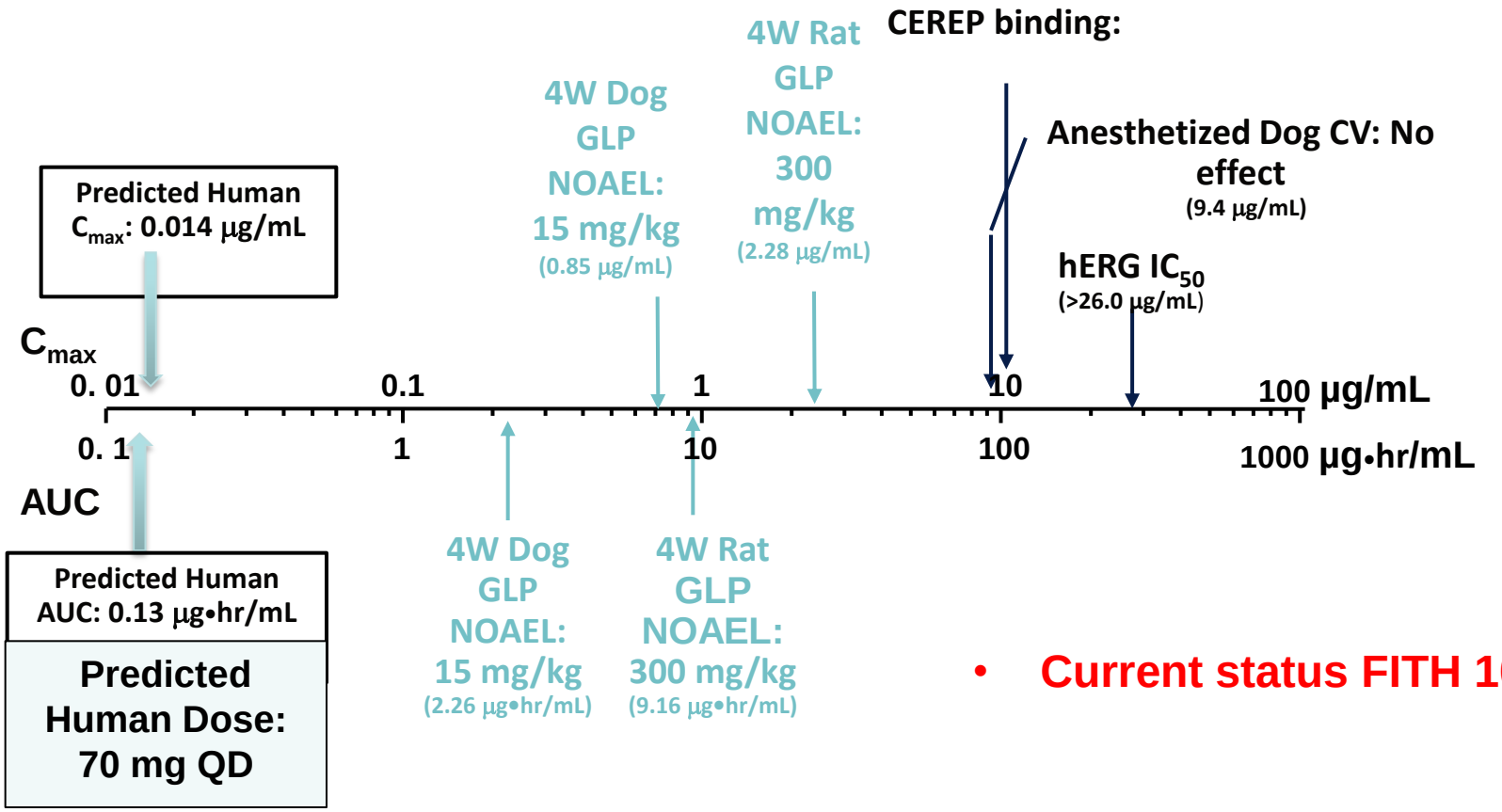
# Poor oral bioavailability explains lack of efficacy




**Tylosin A**

| | $t_{1/2}$ | $C_{max}$ | $T_{max}$ | AUC |
|---|---|---|---|---|
| IP (100 mg/kg) | 1.4 | 11.60 | 1.0 | 20.15 |
| PO (100 mg/kg) | | 0.33 | 1.7 | 0.54 |

# ABBV-4083: Preclinical Safety




- **Current status FITH 1000mg**

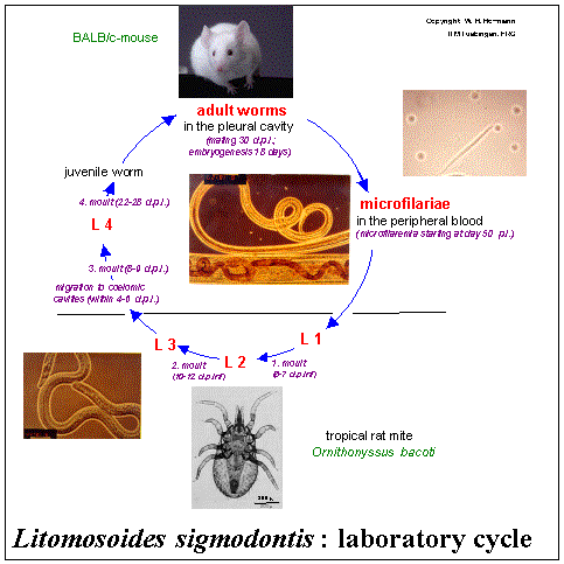
Blood concentrations

# The Classic Discovery Pathway
## Template 1: Thienopyrimidine/ (Aza)Quinazoline


**Template 1 Thienopyrimidine/ (Aza)Quinazoline**

- **Medchem optimization: studied SAR and improved DMPK**
- **Translation between *in vitro* and *in vivo* anti-*Wolbachia* activities.**
- **Lead evaluation and candidate selection.**

# *BG109* study plan: adult *Lito.*
# AWZ1066 enantiomers dose de-escalation,14d +7d




Week: 0wk 5 wk 6 wk 7 wk 11wk

Total: 54 mice

Vehicle n=6

DOX 40MK*bid* n=6

AWZ1066*S*
150MK*bid*, n=6
100MK*bid*, n=6
50MK*bid*, n=6

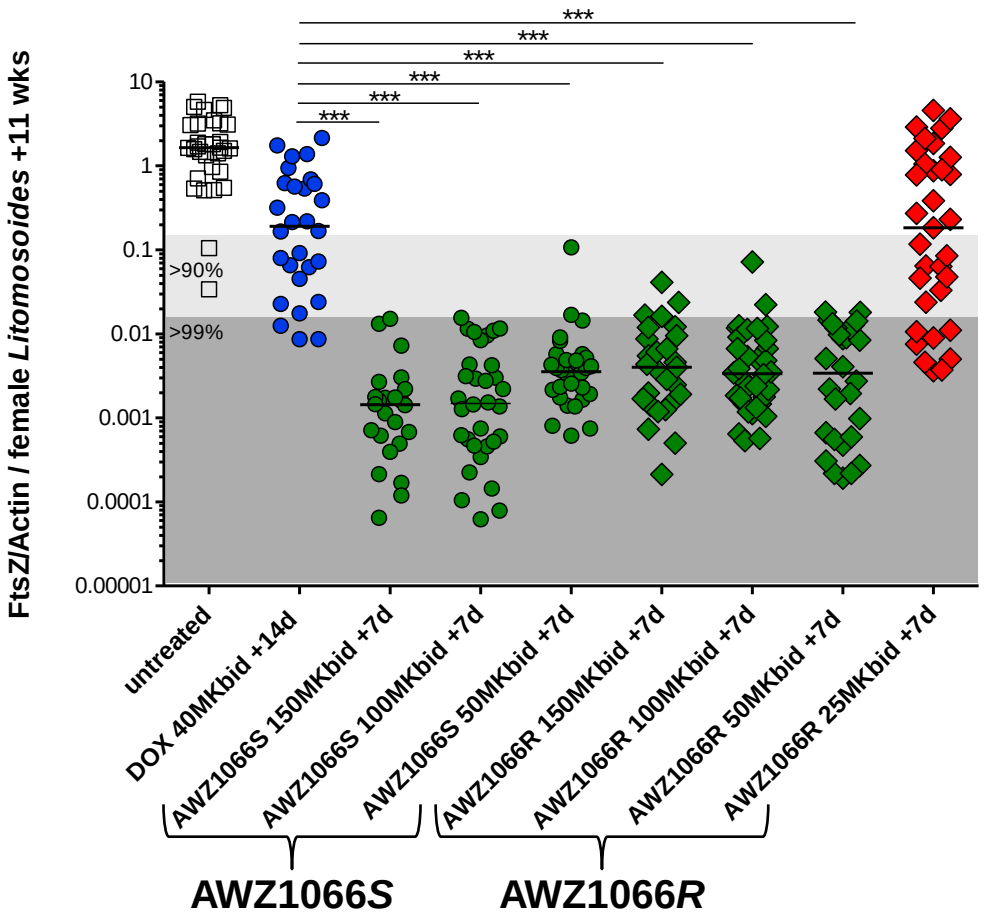
AWZ1066*R*
150MK*bid*, n=6
100MK*bid*, n=6
50MK*bid*, n=6
25MK*bid*, n=6

Washout

Washout

***Wolbachia*** depletion level readout

# BG109 ADULT *Lito.* screening
# PCR readout: *Wolbachia* level




Kruskal-Wallis 1 way ANOVA *P*<0.0001 Dunn's multiple tests *vs* DOX***$P$<0.001

| Drug Dose / duration | % *Wolbachia* reduction* |
|---|---|
| DOX 40MK*bid* +14d | 88.35 |
| AWZ1083 150MK*bid*+07d | 99.91 |
| AWZ1083 100MK*bid*+07d | 99.91 |
| AWZ1083 50MK*bid*+07d | 99.78 |
| AWZ1084 150MK*bid*+14d | 99.76 |
| AWZ1084 100MK*bid*+07d | 99.80 |
| AWZ1084 50MK*bid*+07d | 99.80 |
| AWZ1084 25MK*bid*+07d | 88.84 |

*cf median Vehicle

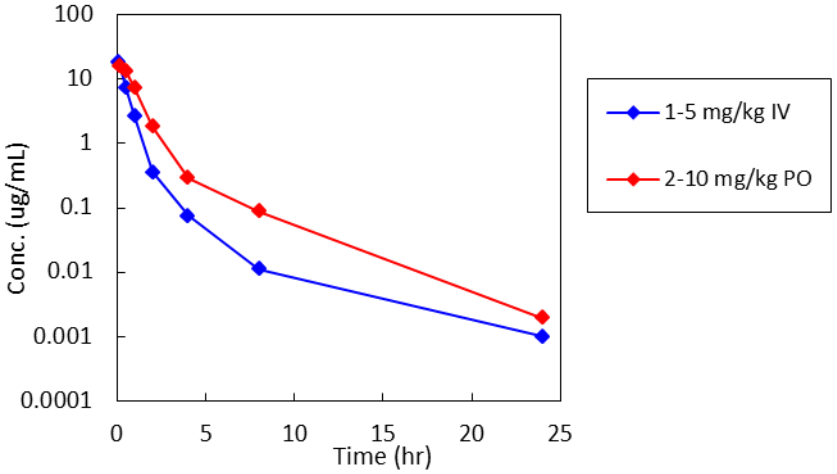
- No significant differences in worm burden in all tested groups.

# Efficacy study summary

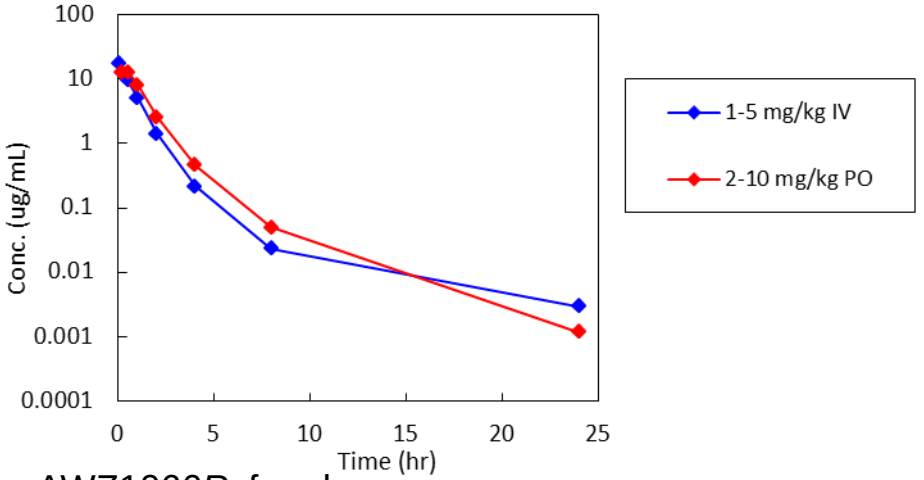
| PD model | | AWZ1066 | AWZ1066*S* | AWZ1066*R* |
|---|---|---|---|---|
| ***Brugia*** | Larval | 99.91%<br>(100mg/kg, bid, 14-day) | ND | ND |
| | Adult | 98.8%<br>(100mg/kg, bid, 14-day) | 97.8%<br>(100mg/kg, bid, 7-day) | 99.6%/99.7%<br>(100mg/kg, bid, 14-day/<br>150mg/kg, bid, 7-day) |
| ***Litomosoides*** | Larval | 99.99%<br>(50mg/kg, bid, 14-day) | 99.99%<br>(50mg/kg, bid, 7-day) | 99.99%<br>(50mg/kg, bid, 7-day;<br>25mg/kg, bid, 14-day) |
| | Adult | ND | 99.8%<br>(50mg/kg, bid, 7-day) | 99.8%<br>(50mg/kg, bid, 7-day) |

- Robust 7-day efficacy in both animal models
- AWZ1066*S* showed marginally higher efficacy than AWZ1066*R*;
- No significant differences in worm burden in all tested groups in all PD studies;
- In all adult *Brugia* studies the tested groups with >90% Wolbachia reduction also showed 100% reduction of mf production cf. control group;
- In the larval Litomosoides study, all tested groups showed significant suppression of larval development (worm length) cf. control group.

# AWZ1066*S* & 1066*R* mouse PK profiles




AWZ1066*S*: free base,
Eisai standard suspended formulation



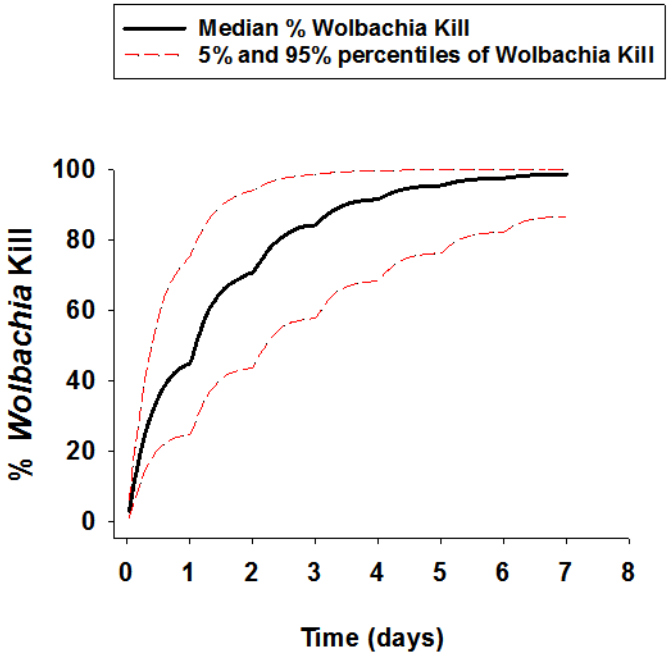

AWZ1066*R*: free base,
Eisai standard suspended formulation

| Molname | Dose | $C_{max}$ | $t_{max}$ | $t_{1/2}$ | $AUC_{0\text{-}24h}$ | $V_{ss}$ | $CL_{tot}$ | BA |
|---|---|---|---|---|---|---|---|---|
| result_table | (mg/kg) | (ug/mL) | (hr) | (hr) | (ug/mL*hr) | (mL/kg) | (mL/hr/kg) | (%) |
| AWZ1066*S* | 5 IV | N.A. | N.A. | 3.509 | 10.710 | 317.291 | 466.662 | N.A |
| AWZ1066*S* | 10 PO | 16.2 | 0.167 | N.A | 18.019 | N.A | N.A | 84.1 |
| AWZ1066*R* | 5 IV | N.A. | N.A. | 3.646 | 15.165 | 346.024 | 329.413 | N.A. |
| AWZ1066*R* | 10 PO | 13.000 | 0.167 | N.A. | 18.792 | N.A | N.A | 61.9 |
| AWZ1066* | 4.04 IV | N.A | N.A | 2.942 | 6.605 | 395.267 | 611.567 | N.A |
| AWZ1066* | 8.08 PO | 7.150 | 0.167 | N.A | 8.442 | N.A | N.A | 64.0 |

* These two PK studies were carried out using CD-1 mice with the mesylate salt

# Is a 600mg daily dose enough for 1 week in man?

**600mg Prediction Based on mathematical model utilising Human PK prediction and in-**




**>90% of the population will achieve 90% or more *Wolbachia* reduction within 7 days of 600mg (10mg/kg) dose based in PK/PD modelling using human PK predictions and in-vitro activity**



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# Template 1: Thienopyrimidine/ (Aza)Quinazoline - TCP: Potency

| Compound Code | AWZ1066*S/R* |
|---|---|
| 1. *In vitro* potency against *Wolbachia* (nM) – Insect cell assay | 2 |
| 1.1 *In vitro* potency against *Wolbachia* (nM) - *B. malayi* Mf assay | 95 |
| 2. *In vivo* activity against *Brugia malayi* larvae in mouse (% Reduction) | 99.91% 100MPK, bid, 14d (AWZ1066) |
| 2.1 *In vivo* activity against *Litomosoides* larvae in mouse (% Reduction) | 99.99% 50MPK, bid, 7d (AWZ1066*R*) |
| 3. *In vivo* activity against *Brugia malayi* adult in mouse (% Reduction) | 97.8% 100MPK, bid, 7d (AWZ1066*S*) |
| 3. *In vivo* activity against *Litomosoides* adult in mouse (% Reduction) | 99.8% 50MPK, bid, 7d (both) |
| 4. PK/PD | Defined |

Target Criteria Achieved | Acceptable Criteria Achieved | Criteria Not Achieved | IP – In progress

# Template 1: Thienopyrimidine/ (Aza)Quinazoline - TCP: DMPK

| Compound Code | AWZ1066*S/R* |
|---|---|
| 1. *In silico* DMPK predictions | Completed |
| 2. Log D 7.4 | 2.8 |
| 3. Aqueous solubility (µM) | 113 |
| 4. *In vitro* clearance in rat hepatocytes (µl/min/$10^6$ cells) | 7 |
| 5. *In vitro* clearance in human liver microsomes (µl/min/mg) | 18 |
| 5.1 *In vitro* clearance in human hepatocytes (ml/min/kg) | 3.3 |
| 6. *In vitro* clearance in mouse liver microsomes (µl/min/mg) | 41 |
| 7. %PPB (human) | 81 |
| 8. CYP450 inhibition (µM) | ~9 (2C9)<br>>>10 (Others) |
| 9. CYP450 inducer | 3A4 ($EC_{50}$ ~30µM) |
| 10. Permeability using LLC-PK1 (X$10^{-6}$cm/s) | 7.63 |
| 11. *In vivo* mouse PK | Slow clearance<br>$T_{1/2}$ = 2.9hrs |
| 12. *In vivo* bioavailability (%) | 54 - 91 |

Target Criteria Achieved  Acceptable Criteria Achieved  Criteria Not Achieved  IP – In progress

# Template 1: Thienopyrimidine/ (Aza)Quinazoline - TCP: Safety and Dose Selection

| Compound Code | AWZ1066*S/R* |
|---|---|
| **Safety & Toxicity** | |
| 1. hERG activity (μM) | >30 |
| 2. Cytotoxicity (THP-1) (μM) | >50 |
| 3. Ames test | Negative |
| 4. *In vitro* CEREP panel (μM) | Weak NET inhibitor ($IC_{50}$ = 8.3μM, AWZ1066*S*) |
| 5. Toxicokinetics | Completed |
| 6. MTD (DRF) in the rat | Completed |
| 7. Therapeutic index based on exposure of NOAEL in in vivo tox vs. efficacious exposure | >10X |
| **Dose Selection** | |
| 1. Dosage regimen | 10-20mg/kg daily, 7-day |
| 2. Dosing method | Oral |
| 3. Acceptable cost of goods | Inexpensive SM, 5 steps |

Target Criteria Achieved | Acceptable Criteria Achieved | Criteria Not Achieved | IP – In progress

# Selected candidate – AWZ1066

| Compound Code | AWZ1066*S/R* |
|---|---|
| 1. *In vitro* potency against *Wolbachia* (nM) – Insect cell assay | 2 |
| 1.1 *In vitro* potency against *Wolbachia* (nM) - *B. malayi* Mf assay | 95 |
| 2. *In vivo* activity against *Brugia malayi* larvae in mouse (% Reduction) | 99.91% 100MPK, bid, 14d (AWZ1[illegible]) |
| 2.1 *In vivo* activity against *Litomosoides* larvae in mouse (% Reduction) | 99.99% 50MPK, bid, [illegible] (AWZ1066R) |
| 3. *In vivo* activity against *Brugia malayi* adult in mouse (% Reduction) | 97[illegible]% 0[illegible], 7d (AWZ1066*S*) |
| 3. *In vivo* activity against *Litomosoides* adult in mouse (% Reduction) | 99.8% 50MPK, bid, 7d (both) |
| 4. PK/PD | [illegible]efined |

| Compound Code | AWZ10[illegible]*S/R* |
|---|---|
| 1. *In silico* DMPK predictions | C[illegible]pleted |
| 2. Log D 7.4 | 2.8 |
| 3. Aqueous solubility (µM) | 113 |
| 4. *In vitro* clearance in rat hepatocytes (µl/min/[illegible]ce[illegible]) | [illegible] |
| 5. *In vitro* clearan[illegible] in human liver m[illegible]rosomes (µl/[illegible]in/mg) | [illegible] |
| 5.1 *In vitro* clearance in human hepat[illegible]ytes (ml/min/kg) | [illegible] |
| 6. *In vitro* cl[illegible]rance in mouse liver microsomes (µl/min/mg) | 41 |
| 7. %PPB (hum[illegible]an) | 81 |
| 8. CYP450 inhibition (µM) | [illegible] (2C9) >>10 (Others) |
| 9. CYP450 inducer | 3A4 ($EC_{50}$ ~30µM) |
| 10. Permeability usi[illegible] LLC-PK1 (X10$^{-6}$cm/s) | 7.63 |
| 11. *In vivo* mouse PK | Slow clearance $T_{1/2}$ = 2.9hrs |
| 12. *In vivo* bioavailability [illegible] | 54 - 91 |

| Compound Code | AWZ1066*S/R* |
|---|---|
| Safety / Toxicity | |
| [illegible] hERG activity (µM) | >30 |
| 2. Cytotoxicity (THP-1) (µM) | >50 |
| 3. A[illegible]es test | Negative |
| 4. *In vitro* CEREP panel (µM) | Weak NET inhibitor ($IC_{50}$ = 8.3µM, AWZ1066*S*) |
| 5. Toxicokinetics | Completed |
| 6. MTD (DRF) in the rat | Completed |
| 7. Therapeutic index based on exposure of NOAEL in in vivo tox vs. efficacious exposure | >10X |
| Dose Selection | |
| 1. Dosage regimen | 10-20mg/kg daily, 7-day |
| 2. Dosing method | Oral |
| 3. Acceptable cost of goods | Inexpensive SM, 5 steps |

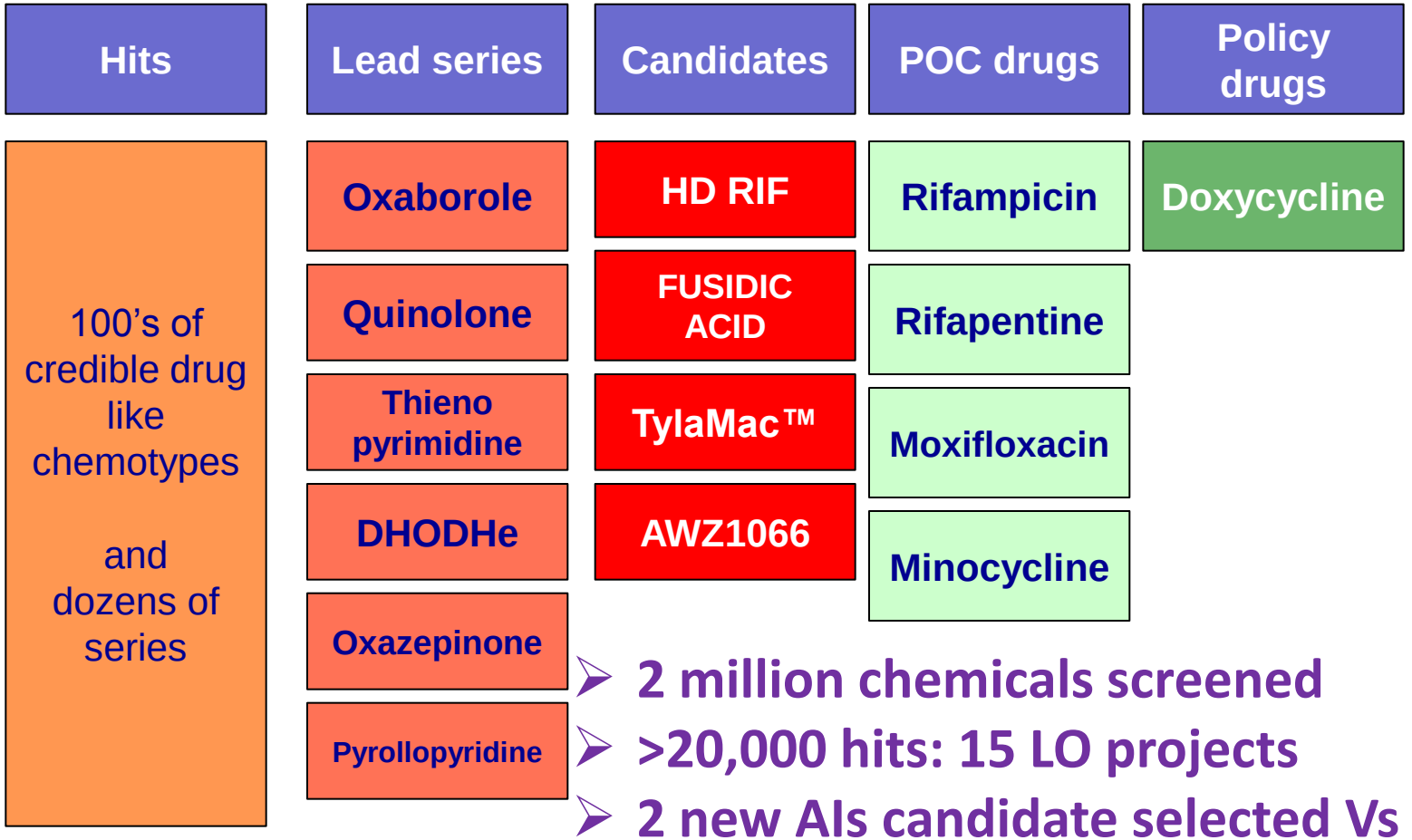
Candidate selected

AWZ1066

# KEY MESSAGES

- **Know what success looks like**
- **Invest up front in validating your pre-clinical models**
  - ***As close to human disease as possible***
  - ***Poor models deliver poor/NO drugs***
- **Ensure you have adequate + and – ve controls**
  - ***Understand why they perform the way they do***
- **Target site exposure is king NOT dose**
- **Ensure you have A CHANCE of achieving safe exposure in man**
  - ***Invest in PK/PD***
- **Accept killing a project is NOT a failure**

# The A·WOL portfolio in 2017




- 2 million chemicals screened
- >20,000 hits: 15 LO projects
- 2 new AIs candidate selected Vs TPP
- 2 repurposed drugs meet TPP

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