

## PARASITIC DISEASES

# Preclinical development of an oral anti-*Wolbachia* macrolide drug for the treatment of lymphatic filariasis and onchocerciasis

Mark J. Taylor<sup>1</sup>, Thomas W. von Geldern<sup>2,3</sup>, Louise Ford<sup>1</sup>, Marc P. Hübner<sup>4</sup>, Kennan Marsh<sup>2</sup>, Kelly L. Johnston<sup>1</sup>, Hanna T. Sjöberg<sup>1</sup>, Sabine Specht<sup>4</sup>, Nicolas Pionnier<sup>1</sup>, Hayley E. Tyrer<sup>1</sup>, Rachel H. Clare<sup>1</sup>, Darren A. N. Cook<sup>1</sup>, Emma Murphy<sup>1</sup>, Andrew Steven<sup>1</sup>, John Archer<sup>1</sup>, Dominique Bloemker<sup>4</sup>, Franziska Lenz<sup>4</sup>, Marianne Koschel<sup>4</sup>, Alexandra Ehrens<sup>4</sup>, Haelly M. Metuge<sup>5,6</sup>, Valerinne C. Chunda<sup>5,6</sup>, Patrick W. Ndongmo Chounna<sup>5,6</sup>, Abdel J. Njouendou<sup>5,6</sup>, Fanny F. Fombad<sup>5,6</sup>, Robert Carr<sup>2</sup>, Howard E. Morton<sup>2</sup>, Ghaith Aljayyousi<sup>1</sup>, Achim Hoerauf<sup>4</sup>, Samuel Wanji<sup>5,6</sup>, Dale J. Kempf<sup>2</sup>, Joseph D. Turner<sup>1</sup>, Stephen A. Ward<sup>1\*</sup>

There is an urgent global need for a safe macrofilaricide drug to accelerate elimination of the neglected tropical diseases onchocerciasis and lymphatic filariasis. From an anti-infective compound library, the macrolide veterinary antibiotic, tylosin A, was identified as a hit against *Wolbachia*. This bacterial endosymbiont is required for filarial worm viability and fertility and is a validated target for macrofilaricidal drugs. Medicinal chemistry was undertaken to develop tylosin A analogs with improved oral bioavailability. Two analogs, A-1535469 and A-1574083, were selected. Their efficacy was tested against the gold-standard second-generation tetracycline antibiotics, doxycycline and minocycline, in mouse and gerbil infection models of lymphatic filariasis (*Brugia malayi* and *Litomosoides sigmodontis*) and onchocerciasis (*Onchocerca ochengi*). A 1- or 2-week course of oral A-1535469 or A-1574083 provided >90% *Wolbachia* depletion from nematodes in infected animals, resulting in a block in embryogenesis and depletion of microfilarial worm loads. The two analogs delivered comparative or superior efficacy compared to a 3- to 4-week course of doxycycline or minocycline. A-1574083 (now called ABBV-4083) was selected for further preclinical testing. Cardiovascular studies in dogs and toxicology studies in rats and dogs revealed no adverse effects at doses (50 mg/kg) that achieved plasma concentrations >10-fold above the efficacious concentration. A-1574083 (ABBV-4083) shows potential as an anti-*Wolbachia* macrolide with an efficacy, pharmacology, and safety profile that is compatible with a short-term oral drug course for treating lymphatic filariasis and onchocerciasis.

## INTRODUCTION

Control programs for neglected tropical diseases caused by the filarial parasites responsible for lymphatic filariasis and onchocerciasis have been operating for more than 40 years, with notable successes in the Americas and West Africa (1). Nevertheless, it is recognized that the transition from control to elimination will require alternative strategies and approaches (1, 2). In particular, global efforts to eliminate filarial diseases are hampered by the lack of a drug that can kill the adult nematode, relying instead on multiple rounds of mass drug administration with agents that target the transmission of microfilariae (the larval stages found in either blood or skin) (2, 3). The success of existing strategies in Central Africa is also impeded by the risk of severe adverse events in areas coendemic for infection with the related filarial nematode, *Loa loa* (2, 3).

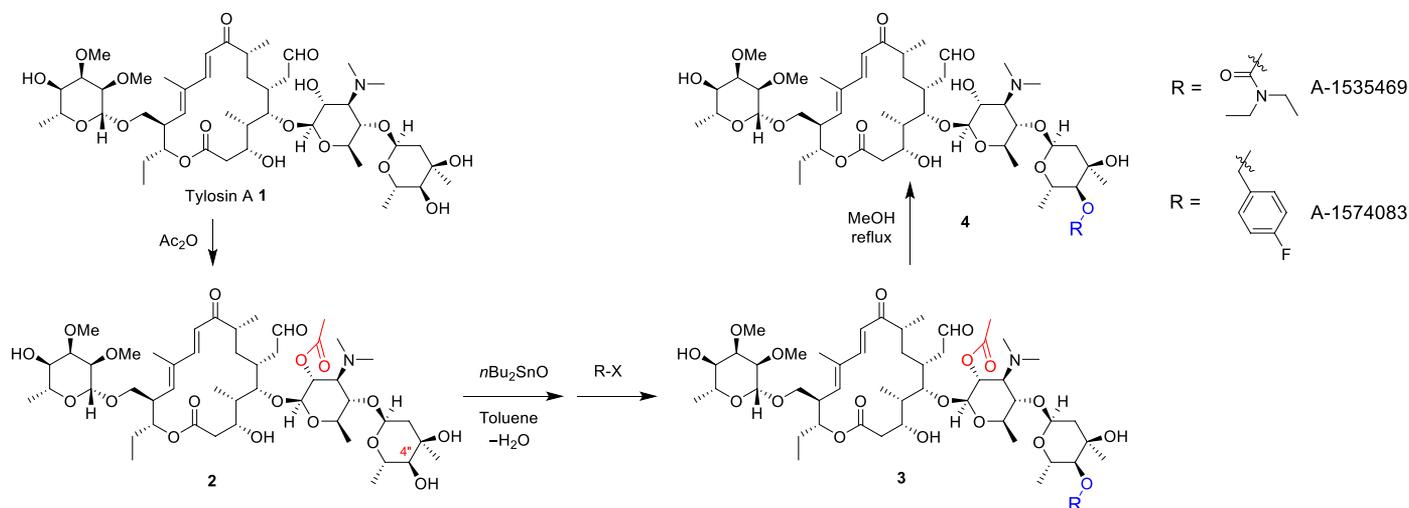
The anti-*Wolbachia* (A-WOL) consortium was formed to exploit a unique aspect of filarial parasite biology, which is their reliance on the presence of the bacterial endosymbiont *Wolbachia* for fertility

and viability (4). Clinical studies using doxycycline have demonstrated that depletion of *Wolbachia* within filarial parasites arrests embryogenesis, prevents larval development, clears microfilarial stages from blood or skin, mediates transmission blockade and prevention of onchocerciasis pathology, and leads to a slow but innocuous death of adult parasites over 12 to 24 months (3, 5, 6). However, doxycycline is contraindicated in children and pregnant women and requires 4 to 6 weeks of daily therapy to sufficiently deplete the *Wolbachia* population to produce a macrofilaricidal effect. Therefore, agents with more favorable safety profiles and shorter duration of therapy are needed (4). To date, more than 2 million compounds have been screened for anti-*Wolbachia* activity in an insect cell line (7, 8).

## RESULTS

Here, we screened an anti-infective compound library and identified the 16-membered macrolide antibiotic tylosin A (Fig. 1) as a potent hit. This drug had an in vitro effective concentration providing 50% effect (EC<sub>50</sub>) of 30 nM against *Wolbachia* in the C6/36 (wAlbB)-infected insect cell line and 66 nM against *Wolbachia* in microfilariae of *Brugia malayi* (Table 1). Tylosin A, the principal component of a widely used veterinary antibiotic produced by fermentation, was the only macrolide in the screening set to demonstrate anti-*Wolbachia* activity; other commercially available 16-membered macrolides were devoid of activity (8). We tested the efficacy of tylosin A in two mouse models of lymphatic

<sup>1</sup>Centre for Drugs and Diagnostics, Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK. <sup>2</sup>Global Pharmaceutical Research and Development, AbbVie, North Chicago, IL, USA. <sup>3</sup>Franciscan Institute for World Health, Franciscan University, Steubenville, OH, USA. <sup>4</sup>Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany. <sup>5</sup>Research Foundation for Tropical Diseases and the Environment, Buea, Cameroon. <sup>6</sup>Department of Microbiology and Parasitology, University of Buea, Buea, Cameroon. \*Corresponding author. Email: steve.ward@lstm.ac.uk



**Fig. 1. Synthesis of tylosin A analogs.** Tylosin A (the parent compound **1**) was first protected on the reactive 2'-hydroxyl group through acetylation to give compound **2**. Selective activation of the mycarosyl sugar was accomplished through formation of a cyclic tin complex; this was reacted with diethylcarbonyl chloride to give compound **3** [R = -C(O)NEt<sub>2</sub>] or with 4-fluorobenzyl chloride to give compound **3** [R = -Bn(4-F)]. Warming in methanol liberated the 2'-ester protective group to provide A-1535469 [compound **4**, R = -C(O)NEt<sub>2</sub>] or A-1574083 [compound **4**, R = -Bn(4-F)]. Both A-1535469 and A-1574083 were tested for efficacy against the bacterial endosymbiont *Wolbachia* both in insect cells and microfilariae in vitro and in worms infecting mice and gerbils in vivo.

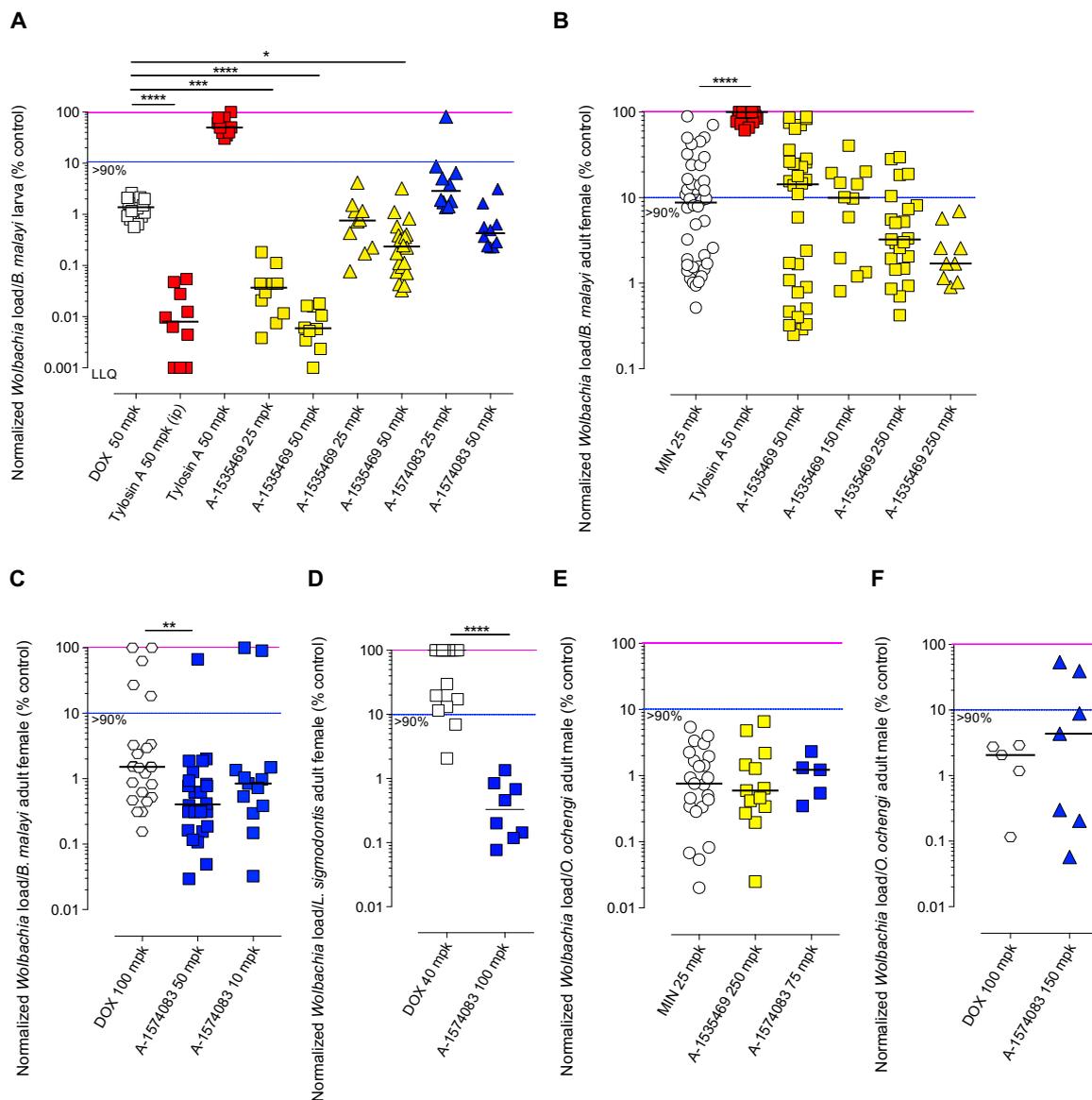
filariosis (9, 10). In the first model, interleukin-4 receptor alpha-deficient (IL-4Rα<sup>-/-</sup>) BALB/c immunodeficient mice were infected with third-stage larvae (L3) of *B. malayi*, a human lymphatic filarial pathogen (9). In the second model, BALB/c mice were infected with L3 larvae of *Litomosoides sigmodontis*, which naturally infects cotton rats (10). In the *B. malayi* model, infectious stage larvae were isolated from mosquitoes and, after inoculation into the immunodeficient mice, were allowed to develop into adults that produced microfilarial stages contained within the peritoneal cavity of the host animals. In the *L. sigmodontis* natural infection model, larvae migrated to the pleural cavity of mice via the lymphatics, heart, and lungs and became microfilariae-producing adults. Worm infections were established by exposing BALB/c mice to blood feeding by *L. sigmodontis*-infected *Ornithonyssus bacoti* mites. In both animal models, drugs were delivered orally or parenterally (via intraperitoneal injection) immediately after infection, thus targeting larval developmental stages. In initial screening, the impact on the *Wolbachia* load was assessed at either 2 (*B. malayi*) or 5 weeks (*L. sigmodontis*) after infection, corresponding to the mid-L4 larval stage or early adult stage, respectively. In both animal models, anti-*Wolbachia* efficacy was demonstrated when tylosin A was delivered parenterally but not orally (Fig. 2A and fig. S1). Pharmacokinetic (PK) studies in mice confirmed that

the lack of anti-*Wolbachia* activity of oral tylosin A was the result of poor oral bioavailability (table S1). Worm burdens in the mice were not affected by either tylosin analogs or doxycycline after 2 weeks of treatment (table S2).

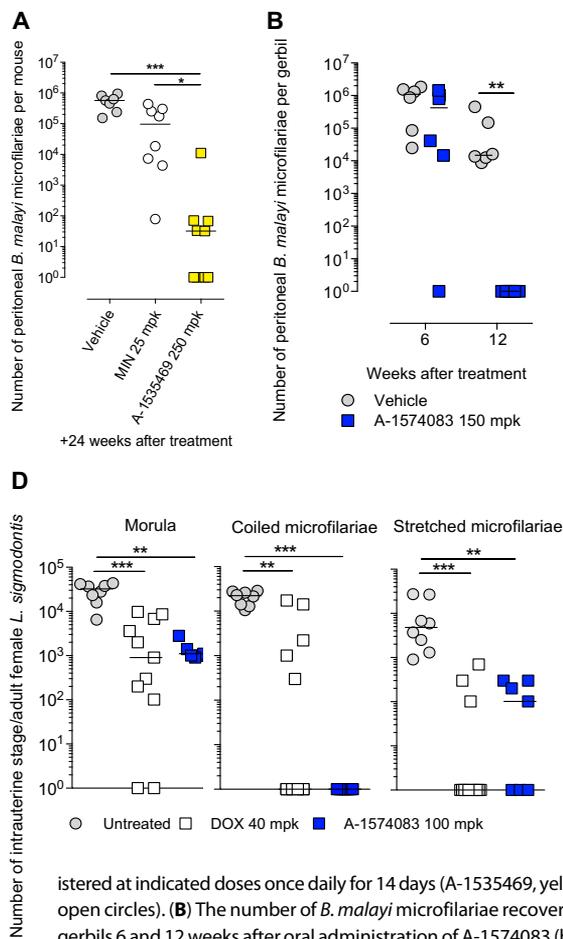
We hypothesized that low intestinal permeability limited the oral bioavailability of tylosin A due to its extensive network of hydrogen-bond donors, specifically the five free hydroxyl groups in the molecule. To address this challenge, we focused medicinal chemistry efforts primarily on the derivatization of these hydroxyl moieties, recognizing that such modifications might compromise activity against *Wolbachia*. From studies on ~150 new tylosin analog macrofilaricide (TylAMac) compounds, we determined that derivatives of the 4''-OH (on the mycarose sugar) provided a dual benefit of improved bioavailability and enhanced potency against *Wolbachia*. Table 1 shows the in vitro anti-*Wolbachia* potency (EC<sub>50</sub> values in insect cells and cultured *B. malayi* microfilariae) for two extensively characterized semisynthetic lead compounds (A-1535469 and A-1574083) compared to tylosin A and doxycycline. The increased potency exhibited by these TylAMac analogs appeared to be specific for *Wolbachia*. Their spectrum of activity across a panel of other common bacterial species was similar to that of tylosin A (table S3) and erythromycin. The efficacy of A-1535469 and A-1574083 against *B. malayi* developing larvae (L3 to L4 stage) in susceptible IL-4Rα<sup>-/-</sup> BALB/c immunodeficient mice was compared to that of tylosin A and a human equivalent dose of doxycycline (fig. S2) (9, 11). Fourteen-day oral dosing with A-1535469 at ≥25 mg/kg delivered superior efficacy versus doxycycline (50 mg/kg) [Kruskal-Wallis one-way analysis of variance (ANOVA) statistic = 90.7, *P* < 0.0001; Fig. 2A]. Fourteen-day oral dosing with A-1535469 resulted in an anti-*Wolbachia* efficacy equivalent to that achieved with 14 daily injections of tylosin A (50 mg/kg) (98.6% versus >99.9%, respectively; Fig. 2A). When halving the dose duration to 7 days, A-1535469 (≥25 mg/kg) provided superior activity versus 14 days of oral doxycycline (A-1535469 >99.7% median reduction in *Wolbachia* versus 98.1% for doxycycline; Fig. 2A). *Wolbachia* depletion

**Table 1. Anti-*Wolbachia* activity of tylosin A analogs in vitro.**

	Anti- <i>Wolbachia</i> activity EC <sub>50</sub> (nM)	
	Insect cells	<i>B. malayi</i> microfilariae
A-1535469	1.3	1.6
A-1574083	0.02	0.7
Tylosin A	30	66
Doxycycline	20	166



**Fig. 2. In vivo efficacy of two oral tylosin A analogs against *Wolbachia* in several animal models of nematode infection.** The efficacy of two tylosin A analogs (A-1535469 and A-1574083) against *Wolbachia* in mice infected with *B. malayi*, *L. sigmodontis*, or *O. ochengi* is shown. Efficacy of the two drugs was tested against *Wolbachia* in (A) *B. malayi* L3/L4 larvae infecting IL-4Rα<sup>-/-</sup> BALB/c immunodeficient mice (n = 3–7) or in (B) adult female *B. malayi* worms infecting CCR3<sup>-/-</sup> BALB/c immunodeficient mice (n = 4–5). Tylosin A (red) was given orally or intraperitoneally (ip) where indicated; the tylosin A analogs A-1535469 (yellow) and A-1574083 (blue) were given orally. Efficacy was compared against (A) doxycycline (DOX, open symbols) or (B) minocycline (MIN, open symbols). All treatments were administered once daily for 14 days (squares) or 7 days (triangles) at the indicated doses except for minocycline (B), which was administered for 28 days (circles) twice daily (mpk, mg drug per kg body weight). (C and D) The anti-*Wolbachia* efficacy of orally administered A-1574083 (blue) was tested in gerbils infected with adult female *B. malayi* [C (n = 4–7)] or adult female *L. sigmodontis* [D (n = 5–6)] worms. Efficacy was compared against that for doxycycline. All treatments were administered once daily for 21 days (hexagons) or 14 days (squares) at the indicated doses except (D) doxycycline, which was administered for 14 days twice daily. (E and F) Anti-*Wolbachia* efficacy of oral A-1535469 (yellow) or A-1574083 (blue) was tested against male adult *O. ochengi* in SCID immunodeficient mice [E (n = 4–8)] or gerbils [F (n = 4–5)]. Efficacy was compared to that for minocycline (E, open symbols) or doxycycline (F, open symbols). All treatments were administered once daily for either 21 days (hexagons), 14 days (squares), or 7 days (triangles) at the indicated doses except minocycline (E), which was administered for 28 days twice daily. Data on *Wolbachia* depletion are presented as *Wolbachia* surface protein (*wsp*) gene copy number (A and B), *Wolbachia* *wsp*/nematode glutathione-S-transferase (*gst*) gene copy number ratio (C, E, and F), or *Wolbachia* filamenting temperature-sensitive mutant-z (*fts2*)/nematode actin (*act*) gene copy number ratio (D). Data are presented per worm, with 5 to 15 worms per group derived from four to six animals per group and normalized to the median vehicle control *Wolbachia* gene copy number or ratio. Magenta lines indicate the median value for vehicle control, and blue lines indicate 90% efficacy. Short horizontal black lines above plots indicate median measurements. Data points are for single experiments (B to D and F) or else pooled from two individual experiments (A, C, and E). Significant differences, \*\*\*\*P < 0.0001, \*\*\*P < 0.001, \*\*P < 0.01, and \*P < 0.05 (Mann-Whitney or Kruskal-Wallis with Dunn’s tests for intergroup variation).



**Fig. 3. In vivo efficacy of oral tylosin A analogs against *B. malayi* or *L. sigmodontis* microfilariae and worm embryonic stages.** The embryostatic efficacy of two tylosin A analogs (A-1535469 and A-1574083), resulting in blockade of microfilarial production and subsequent depletion of mature microfilariae from mice or gerbils infected with *B. malayi* or *L. sigmodontis*, is shown. (A) The number of *B. malayi* microfilariae in the peritoneal cavity recovered by lavage of nematode-infected immunodeficient SCID mice 24 weeks after administration of oral A-1535469 (yellow squares) is shown ( $n = 7-9$ ). Results are compared to vehicle control (gray circles) or control antibiotic treatment with minocycline (open circles). Drugs were administered at indicated doses once daily for 14 days (A-1535469, yellow squares) or twice daily for 28 days (minocycline, open circles). (B) The number of *B. malayi* microfilariae recovered by lavage from the peritoneal cavity of infected gerbils 6 and 12 weeks after oral administration of A-1574083 (blue squares) once daily for 14 days at the indicated dose compared with vehicle control (gray circles) is shown. Data plotted (A and B) are total yields of peritoneal microfilariae per lavage of individual animals (groups of 6 to 9). Short horizontal black lines over plots are median yields of microfilariae. (C) The number of *L. sigmodontis* microfilariae in the peripheral blood of infected gerbils 1 week before treatment and 2 to 16 weeks after treatment with oral A-1574083 (blue squares) compared to vehicle control (gray circles) or the antibiotic control doxycycline (open squares) is shown. Drugs were administered at indicated doses once daily (A-1574083, blue squares) or twice daily (doxycycline, open squares) for 14 days. Data and error bars are mean microfilariae counts per ml of blood  $\pm 1$  SE, derived from groups of six gerbils. (D) The number of worm intrauterine embryonic stages (morulae, coiled microfilariae, and stretched microfilariae) 16 weeks after oral A-1574083 treatment (blue squares) compared to vehicle control (gray circles) or doxycycline control (open squares) is shown. Drugs were administered at indicated doses once daily (A-1574083, blue squares) or twice daily (doxycycline, open squares) for 14 days. Data plotted are the total number of released embryonic stages per individual adult female worm (groups of 7 to 11). Short horizontal lines over plots are median yields of microfilariae. Addition of +1 to all continuous variables was undertaken to visualize zero data plotted on a log scale. Significant differences, \*\*\*\* $P < 0.0001$ , \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , and \* $P < 0.05$ , were determined by Kruskal-Wallis with Dunn's tests for intergroup *Wolbachia* variation (A), Mann-Whitney tests (B), or one-way ANOVA with Dunnett's tests for intergroup variation after logarithm to the base 10 transformation (C and D).

after 7 days of oral dosing of infected immunodeficient mice with A-1574083 at  $\geq 25$  mg/kg was comparable to that after 14 days of doxycycline treatment (A-1574083 >97% versus 98.1% median reduction in *Wolbachia* for doxycycline; Fig. 2A). Larval worm burdens were unaffected by any of the treatments (table S2).

Our aim was to develop an anti-*Wolbachia* compound that could be given as an oral short-course treatment effective against adult lymphatic worms and *Onchocera* filarial worms. To that end, A-1535469 and A-1574083 were tested in several in vivo mouse and gerbil models infected with adult-stage *B. malayi* or *L. sigmodontis* or implanted with male *Onchocerca ochengi* (Fig. 2, B and F, and fig. S2) (9-11).

Both C-C chemokine receptor 3 (CCR3)-deficient (CCR3<sup>-/-</sup>) mice or severe combined immunodeficient (SCID) mice were used, in which chronic infections of *B. malayi* adult worms producing microfilariae could be maintained (9, 11).

Gerbil models were used in addition to mouse models for testing A-1574083 due to improved PKs in gerbils, which enabled higher in vivo plasma concentrations of drug to be obtained. When targeting pre-fecund *B. malayi* adult worms in vivo with A-1535469 in the CCR3<sup>-/-</sup> BALB/c mouse infection model (Fig. 2B), all oral treatments given daily (50 to 250 mg/kg) for 14 days provided efficacy that was comparable to a 4-week bioequivalent human dose of minocycline (12) at 25 mg/kg given twice daily. Minocycline was used as a more potent tetracycline antibiotic because it was shown to be superior to doxycycline in depleting *Wolbachia* from female *Onchocerca volvulus* in a pilot clinical study of infected individuals (13). Treatment of CCR3<sup>-/-</sup> immunodeficient mice with A-1535469 (250 mg/kg) for 7 days provided 98.3% median reduction in *Wolbachia* in adult female *B. malayi* worms (compared to 91.4% after 28 days of minocycline treatment). This threshold indicated the potential for curative efficacy for lymphatic filariasis in humans (14, 15). Treatment with A-1574083 (50 mg/kg) for 14 days in a gerbil model of *B. malayi* infection showed targeting of the fecund stage of *B. malayi* worms. This treatment regimen resulted in a 99.6% median reduction in *Wolbachia* and was superior to a 21-day (100 mg/kg) dose of doxycycline (98.5% median reduction with doxycycline; Fig. 2C). Administration of a 14-day reduced (10 mg/kg) dose of A-1574083 also resulted in >99% depletion of *Wolbachia* that was comparable to that of a 21-day doxycycline regimen (Fig. 2C). In a parallel study of *L. sigmodontis* infection in a gerbil model,

dosing was delayed until after the onset of patency (i.e., when circulating microfilariae were detectable in peripheral blood). A dose of A-1574083 (100 mg/kg) given for 14 days resulted in 99.7% mean reduction in *Wolbachia* compared to 0% median reduction in *Wolbachia* after doxycycline treatment (Fig. 2D). The drug treatments did not affect adult worm burdens in these two gerbil models (tables S4 to S6).

Because of the high efficacy achieved in mouse and gerbil models of lymphatic filariasis infection, we next investigated whether A-1535469 and A-1574083 showed anti-*Wolbachia* activity in mouse and gerbil models of *Onchocerca* infection. Preclinical models of onchocerciasis

**Table 2. Plasma concentrations of tylosin A analogs after single oral dosing in mice and gerbils.**

Compound	Species	Dose (mg/kg)	AUC <sub>0-inf</sub> (ng·hr/ml) (SEM)
A-1535469	BALB/c mice	30	7800 (1620)
		100	36100 (2910)
A-1574083	BALB/c mice	25	17.9 (0.85)
		50	271 (23.7)
		75	2153 (1089)
	Gerbils	8.92	60.9 (15.5)
		47	3646 (619)
		82.2	5169 (478)
		149	9200 (276)

were generated by implanting adult male *O. ochengi* parasites isolated from naturally infected cattle (11, 16) into immunodeficient mice or outbred gerbils. Both A-1535469 (250 mg/kg) and A-1574083 (75 mg/kg) given for 14 days mediated a 99.4 and 98.8% median reduction in *Wolbachia*, respectively, in immunodeficient mice implanted with *Onchocerca* (Fig. 2E). This was comparable to the efficacy of a 28-day course of minocycline (25 mg/kg), resulting in a 99.2% median reduction in *Wolbachia* (Fig. 2E). When *Onchocerca*-infected gerbils were dosed at 150 mg/kg of A-1574083 for 7 days, there was a 95.7% depletion in *Wolbachia* compared to 98.0% for doxycycline (Fig. 2F). *Onchocerca* male worm burdens were not affected by drug treatments (tables S7 and S8).

We next assessed whether depletion of *Wolbachia* by A-1535469 or A-1574083 also resulted in reduced production of microfilariae in *B. malayi* and *L. sigmodontis* infection models (Fig. 3). Dosing experiments were undertaken in immunodeficient mice or gerbils infected with *B. malayi* or *L. sigmodontis*, with washout periods of up to 22 weeks after dosing to measure the impact of these two tylosin A analogs on parasite fecundity (fig. S2). In immunodeficient mice with fecund *B. malayi* infection, released microfilariae accrued in the peritoneal cavity and typically reached a steady-state burden of  $\geq 10^5$  per animal. Twenty-four weeks after a 14-day dosing regimen with A-1535469 (250 mg/kg), there was no difference in adult worm burden compared with vehicle control (table S9). However, 24 weeks after treatment, four of nine mice showed no peritoneal microfilariae with a median microfilarial burden in the A-1535469-treated group of  $0.00032 \times 10^5$  (range, 0 to  $0.11 \times 10^5$ ; Fig. 3A). This was equivalent to a >99.99% reduction in microfilarial burden for A-1535469 treatment compared to vehicle control (median microfilarial burden of  $5.7 \times 10^5$ ; range, 1.5 to  $9.4 \times 10^5$ ; Fig. 3A). Microfilarial depletion after a 14-day treatment with A-1535469 was greater than that achieved with a 28-day course of minocycline (25 mg/kg) twice daily. Minocycline treatment resulted in an 83% reduction in peritoneal microfilariae 24 weeks after treatment initiation with a median microfilarial burden of  $0.96 \times 10^5$  (range, 0.008 to  $4.4 \times 10^5$ ; Fig. 3A).

The effects of a 14-day treatment with A-1574083 (150 mg/kg) on microfilarial production were examined in gerbils with a fecund *B. malayi* peritoneal infection. We performed peritoneal lavage at 6 and 12 weeks after treatment to harvest the microfilariae accrued before, during, and after drug treatment. At 6 weeks after treatment,

**Table 3. Activity of A-1574083 (ABBV-4083) in preclinical safety assays.**

Assay	Result
Chloride channel (GABA-gated) (antagonist radioligand)	K <sub>i</sub> 7.5 μM
NK1 (human) (agonist radioligand)	K <sub>i</sub> 0.1 μM
NK1 (human) (antagonist effect)	K <sub>B</sub> 0.46 μM
Motilin (human) (agonist radioligand)	IC <sub>50</sub> > 100 μM
ERG (human) (QPatch, human embryonic kidney–293 cells)	IC <sub>50</sub> > 28 μM
MiniAmes test ( <i>Salmonella</i> TA98/TA100)	Negative
In vitro micronucleus test (V79 hamster)	Negative
Anesthetized dog cardiovascular effects (intravenous infusion)	Minimal effects* at 12.4 μg/ml
28-day toxicology study in Sprague–Dawley rats	NOAEL: 300 mg/kg per day Plasma AUC <sub>0-24</sub> : 7.4 μg·hour/ml
28-day toxicology study in beagle dogs	NOAEL: 15 mg/kg per day Plasma AUC <sub>0-24</sub> : 3.0 μg·hour/ml

\*Monitored characteristics: mean arterial pressure, heart rate, systemic vascular resistance, cardiac output, cardiac contractility, QT interval corrected using Van de Water formula, and interval from P to R on electrocardiogram.

all gerbils showed peritoneal microfilariae (Fig. 3B), and the median peritoneal microfilarial burden ( $4.2 \times 10^5$ ) was similar to that of animals given vehicle control ( $10.9 \times 10^5$ ). After a further 6 weeks (12 weeks after treatment), peritoneal microfilarial burdens were sustained in the vehicle group with six of six gerbils, showing a median microfilarial burden of  $0.15 \times 10^5$ . No microfilarial burden was detected in lavages of infected gerbils dosed with A-1574083 (Fig. 3B).

Patent *L. sigmodontis* infection in gerbils seeds the blood with microfilariae, hence establishing persistent microfilaraemia akin to that of patients infected with lymphatic filariasis. We undertook a longitudinal assessment of *L. sigmodontis* microfilaraemia after dosing with A-1574083 (100 mg/kg once daily) or doxycycline (40 mg/kg twice daily) for 14 days. Baseline microfilaraemias were similar between gerbils treated with A-1574083, doxycycline, or untreated controls (Fig. 3C). At 10 weeks after treatment, *L. sigmodontis* microfilaraemia declined in gerbils treated with A-1574083 or doxycycline compared to untreated animals. However, in A-1574083-treated gerbils, the microfilaraemia continued to decline from 11 to 16 weeks after treatment, and the gerbils showed no microfilariae in the blood at week 15 after dosing (Fig. 3C). The effect of A-1574083 on circulating microfilariae was superior to that of doxycycline over the same time period (Fig. 3C) and correlated with the improved clearance of *Wolbachia* by A-1574083 (Fig. 2D). At 16 weeks after dosing, a similar number of adult *L. sigmodontis* worms were retrieved across all groups (table S6). Microscopic evaluation after homogenization of remaining *L. sigmodontis* female worms confirmed a decline in the number of intrauterine embryonic stages (morulae, coiled microfilariae, and stretched microfilariae) after 14 days of A-1574083 treatment compared to untreated controls (Fig. 3D).

Table 2 shows the plasma concentrations [area under the curve (AUC)] for A-1535469 and A-1574083 after a single oral dose in uninfected BALB/c mice and gerbils. Although A-1574083 showed poor bioavailability in this strain of mouse, the efficacy of this drug against *Wolbachia* in vivo was similar to that of A-1535469. Combining data from PK and efficacy studies, we created an exploratory PK/PD (pharmacodynamic) model (fig. S3). We analyzed plasma concentrations of drug in both larval and adult *B. malayi*-infected animals dosed for 14 days with A-1574083 or A-1535469. We determined that the maintenance of unbound plasma concentrations of drug above the in vitro EC<sub>50</sub> (defined using *Wolbachia* in insect cells) for more than 12 hours/day produced a >90% decline in *Wolbachia* from nematodes in infected mice (fig. S3).

After extensive comparison of the two lead compounds, A-1574083 (now called ABBV-4083) was selected for evaluation as a preclinical drug candidate on the basis of its greater potency together with acceptable PKs and safety in mouse and gerbil worm infection models. More limited development of A-1535469 has been undertaken as a backup strategy. A-1574083 demonstrated interactions with only 2 of 77 mammalian receptors, ion channels, enzymes, and transporter assays [neurokinin NK1 receptor and the  $\gamma$ -aminobutyric acid (GABA)-gated chloride channel at a concentration of 10  $\mu$ M] assessed in vitro (Table 3). The inhibitory concentration providing 50% inhibition (IC<sub>50</sub>) for the human ether-a-go-go-related gene encoding a potassium channel (associated with cardiovascular toxicities) was >28  $\mu$ M. A-1574083 exhibited no binding at 100  $\mu$ M to the gastrointestinal motilin receptor, an off-target receptor for some macrolide antibiotics (Table 3) (17). A-1574083 tested negative in the Mini-Ames mutation assay and in the micronucleus assay (Table 3). Cardiovascular studies completed in dogs and 28-day toxicology studies completed in rats and dogs showed that A-1574083 plasma concentrations at the no adverse effect levels (NOAELs) were above the plasma concentrations obtained with efficacious doses in mouse and gerbil infection models (Table 3).

The *L. loa* filarial parasite lacks *Wolbachia*, precluding an anti-*Wolbachia*-based mechanism of action against this parasitic worm. To investigate potential off-target effects, we undertook in vitro counter screening against *L. loa* microfilariae using continuous exposure to 0.032 to 100  $\mu$ M A-1574083. No effect on the motility of *L. loa* microfilariae was observed until concentrations exceeded 4  $\mu$ M with an estimated IC<sub>50</sub> of 23.3  $\mu$ M (fig. S4). The IC<sub>50</sub> for *L. loa* motility inhibition was >100-fold above the estimated peak unbound plasma concentration of A-1574083 (unbound C<sub>max</sub> = 0.18  $\mu$ M) at the efficacious dose (50 mg/kg) in gerbils. This indicated a wide separation in the concentration of A-1574083 needed for anti-*Wolbachia* activity and direct microfilaricidal effects on *L. loa* microfilariae.

## DISCUSSION

The discovery and development of anti-*Wolbachia* tylosin analog macrofilaricides yielded the candidate molecule A-1574083 (ABBV-4083). A-1574083 demonstrated potent anti-*Wolbachia* activity in animal models, including nematode-infected immunodeficient mice and gerbils. This activity was comparable or superior to “gold-standard” second-generation tetracycline antibiotics. A-1574083 showed robust anti-*Wolbachia* activity with a short treatment time frame of 7 to 14 days compared to second-generation tetracycline antibiotics with a treatment time frame of 21 to 28 days. This suggests that the A-1574083 analog may provide clinical benefits with a short dosing

regimen. A-1574083 also displayed a promising preclinical safety profile.

Curative activity after effective *Wolbachia* depletion is evident 18 months after treatment in patients infected with nematodes (13, 18). A more immediate manifestation of *Wolbachia* depletion from adult female filarial reproductive tissue, observed both in rodent models and in clinical trials (13, 18), is the arrest of worm embryogenesis 2 to 6 months after treatment. This subsequently leads to a slow waning of mature microfilariae from the blood or skin and ultimately clearance of microfilarial infection in lymphatic filariasis and onchocerciasis within a period of 12 to 22 months after treatment (19–22). This important effect of anti-*Wolbachia* treatment mediates transmission-blocking efficacy and prevents further skin and eye disease attributable to *O. volvulus* microfilariae infection. Here, we demonstrate the anti-*Wolbachia* activity of A-1574083 and the sustained disruptive effects of this treatment on adult filarial embryogenesis. This led to a blockade in the release of microfilariae and gradual complete clearance of circulating microfilariae in *B. malayi* and *L. sigmodontis* rodent models of worm infection.

The risk of potentially life-threatening serious adverse events in patients with onchocerciasis who are coinfecting with the related filarial worm *L. loa* (23) is a major issue hindering adherence to the rapid-acting microfilaricidal drug, ivermectin. New macrofilaricidal agents need to be safe to administer to coinfecting patients at risk of severe adverse reactions to ivermectin. On the basis of its anti-*Wolbachia* activity, A-1574083 was predicted to be safe for treating individuals coinfecting with *Onchocerca* and *L. loa*, with initial in vitro testing indicating no direct *L. loa* cidality at concentrations far exceeding those required for depletion of *Wolbachia*.

Our study has several limitations. Although robust *Wolbachia* depletion was demonstrated for several nematode species including *Onchocerca*, the rodent models of filarial infection harbor adult worms in sites (e.g., the peritoneal cavity) that are highly accessible to drug entry from the blood. In contrast, in human infection, *O. volvulus* adults are present in subcutaneous and deep-tissue nodules. Although these nodules are well vascularized, drug uptake into *O. volvulus* nodules may not be as efficient as into the adult worms in rodent models. The dependence of efficacy on drug dose versus duration of dosing is also not well defined. Although higher doses offer the potential for shorter dose duration, the detailed relationship between drug dose and dose duration is not well understood. This crucial relationship needs to be elucidated if this drug regimen is to satisfy specific target product profiles for point-of-care treatment and mass administration under difficult field conditions.

## MATERIAL AND METHODS

### Study design

The research objective of our study was to develop a short-course oral antibiotic that was effective against nematodes infected with the bacterial endosymbiont *Wolbachia*, which could be advanced into clinical testing as a new treatment for lymphatic filariasis and onchocerciasis. Phenotypic in vitro screening of a small-molecule library against insect *Wolbachia* was done in a blinded manner and subsequently decoded to identify a potent “hit” antibiotic, tylosin A. Confirmatory phenotypic screening against nematode *Wolbachia*, medicinal chemistry, iterative in vitro screening, and rodent (mouse and gerbil) PKs of tylosin A oral analogs were performed in a nonblinded manner. Two tylosin A analogs with improved in vitro potency and acceptable

rodent PK characteristics were advanced into a series of rodent efficacy studies. Rodents were randomized into drug groups. Scientists were involved in drug treatments, and parasitological and molecular read-outs were blinded to the treatment group, with the exception of the *L. sigmodontis* drug screens, which were done in a nonblinded manner. Details of animal age and sex are provided below. Group numbers of rodents used in in vivo experiments are provided below and in the figure legends.

### Synthesis of tylosin A analogs

A-1535469 and A-1574083 were synthesized from tylosin A using a three-step procedure (Fig. 1). Briefly, the 2'-OH (mycaminose) of tylosin was protected (acetic anhydride) as the corresponding acetate, followed by selective activation of the 3"- and 4"-OH groups through the formation of a cyclic tin complex (using dibutyltin oxide) and then by derivatization of the 4"-OH (mycarose) with either diethylcarbamoyl chloride or *p*-fluorobenzyl bromide/Caesium fluoride. Subsequent deprotection of the 2'-acetate (refluxing methanol) provided A-1535469 or A-1574083.

### In vitro cell-based screen for anti-Wolbachia activity

Compounds were screened for anti-*Wolbachia* activity in vitro in the A-WOL-validated *Wolbachia*-infected *Aedes albopictus* (C6/36 wAlbB) 7-day cell-based screen. This screen used a 384-well format assay with high-content imaging (Operetta), as previously described (7).

### Animals

Inbred male SCID CB.17 (BALB/c congenic) mice were purchased from Charles River Laboratories, UK. Inbred IL-4R $\alpha^{-/-}$  or CCR3 $^{-/-}$  BALB/c breeding pairs were purchased from Jackson laboratory, USA. Outbred *Meriones unguiculatus* gerbil breeding pairs were purchased from Charles River Laboratories, Europe. Rodents were maintained and bred under specific pathogen-free conditions at the University of Liverpool Biological Services Unit or in individually ventilated cages at the Research Foundation for Tropical Medicine and The Environment, Buea, Cameroon. All experiments were approved by the ethical committees of the University of Liverpool and Liverpool School of Tropical Medicine or the University of Buea Animal Ethics Review Board, Cameroon. Studies were conducted in accordance with Home Office legislation (UK-based studies), and matching welfare standards were applied in Cameroon.

Experiments with *L. sigmodontis* were performed at the Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn in accordance with the European Union's animal welfare guidelines, and all protocols were approved by the Landesamt für Natur, Umwelt und Verbraucherschutz, Cologne, Germany (AZ 84-02.04.2015.A507 and AZ 84-02.04.2012.A140).

Female *M. unguiculatus* gerbils were obtained from JANVIER LABS (Saint-Berthevin, France) and housed at the animal facility of the Institute for Medical Microbiology, Immunology and Parasitology in individually ventilated cages on a 12-hour light/12-hour dark cycle with food and water ad libitum.

For PK experiments, male Mongolian gerbils were purchased from Zhejiang Academy of Medical Science (China) and were individually housed in a climate controlled room, with free access to food and water in the animal facilities of WuXi AppTec, Shanghai, China. Female Balb/c mice were purchased from Charles River Laboratories (Wilmington, MA) and were maintained in the Associa-

tion for Assessment and Accreditation of Laboratory Animal Care International-approved animal facilities of AbbVie Inc., North Chicago, IL. All experiments were conducted in accordance with the guidelines approved by the internal Institutional Animal Care and Use Committee.

### Screening of anti-Wolbachia activity in nematodes in vitro

Compounds were further scrutinized for anti-*Wolbachia* activity in vitro in a microfilarial assay. Microfilariae were obtained by peritoneal lavage of gerbils (*M. unguiculatus*) harboring a patent infection of *B. malayi* as previously described (11). Microfilariae were purified and resuspended in complete medium consisting of RPMI (Invitrogen) supplemented with 10% fetal bovine serum (Fisher Scientific), 1% penicillin/streptomycin, and 1% amphotericin B (Life Technologies). Microfilariae were plated at 8000 microfilariae per well in a 96-well plate (100  $\mu$ l per well). Compounds to be tested [10-mm stock in 100% dimethyl sulfoxide (DMSO)] were diluted in complete medium, and 100  $\mu$ l was added to the appropriate wells to give a dose-response curve. Five replicates were used for each compound, and each plate contained doxycycline (5  $\mu$ M) and DMSO controls. The assay plates were incubated at 37°C for 6 days in 5% CO<sub>2</sub>, after which DNA was extracted using the QIAmp DNA Mini Kit (Qiagen). Quantitative polymerase chain reaction (qPCR) was performed to obtain the ratio of *wsp* to *gst* gene copy number, as previously described (11).

### Screening of anti-Wolbachia activity in nematode-infected animals

Infectious stage *B. malayi* L3 larvae were propagated, as previously described (11). Male mice 6 to 12 weeks of age or male gerbils 8 to 16 weeks of age were infected with *B. malayi* L3 larvae intraperitoneally (50 or 100 L3 larvae per mouse; 400 L3 larvae per gerbil), and infections were maintained for a maximum of 40 weeks. Motile *B. malayi* microfilariae were recovered by peritoneal lavage at necropsy and were enumerated by microscopy. Viable adult male *O. ochengi* nematodes were aseptically isolated from naturally parasitized cattle, as previously described (11). Between 10 to 20 male *Onchocerca* were surgically implanted into the peritoneal cavity of male SCID CB.17 mice or female gerbils, under anesthesia, as previously described (11).

For *L. sigmodontis* infection, 6- to 8-week-old female gerbils or 6- to 8-week-old female Balb/c mice were exposed to *O. bacoti* mites containing the infective stage L3 larvae of *L. sigmodontis*, as previously described (24). The same batch of mite-containing bedding was used to infect all animals of one experiment. Treatment of mice with tylosin A analogs or antibiotics was initiated 1 day after infection, and necropsies were performed 35 days after infection. Treatment of gerbils with tylosin A analogs or antibiotics started 12 weeks after infection, and necropsies of gerbils occurred 16 weeks after the start of treatment. *L. sigmodontis* worms were isolated from the thoracic cavity and peritoneum and were quantified, as previously described (24). For microfilarial counts in gerbils, 10  $\mu$ l of peripheral blood was taken from the saphenous vein in weekly intervals starting at 11 weeks after infection and diluted in 300  $\mu$ l of Hinkelmann solution (0.5% eosin Y, 0.5% phenol, and 0.185% formaldehyde in aqua dest). After centrifugation at 400g for 5 min, the supernatant was discarded, and the pellet was resuspended and completely transferred to a microscope slide to quantify microfilariae using a microscope, as previously described (25). After necropsy of gerbils, remaining intact female worms

were used in equal numbers for either embryograms or *Wolbachia* quantification. For embryograms, single female adult worms were homogenized in 20% Hinkelman/80% phosphate-buffered saline (PBS), diluted 1:10 in PBS, and enumerated via microscopy. The analyzed embryonal stages included eggs, morulae, pretzel microfilariae, and stretched microfilariae.

### Treatment of infected animals with tylosin A analogs and antibiotics

Infected animals were randomly assigned into dose groups with  $n = 3$  to 7 for larval-stage testing and  $n = 4$  to 9 for adult-stage testing. Randomization was done by stratification based on ID number (*B. malayi* larval and pre-fecund adult infections), baseline ultrasonography estimates of worm burden (*B. malayi* infection of SCID mice and gerbils) using a semi-quantifiable scoring system (26), baseline microfilaraemia (*L. sigmodontis* infection of gerbils), or order of implantation and source of male worms (*O. ochengi* implantation into SCID mice or gerbils).

Tylosin A (Sigma-Aldrich) or the tylosin A analogs, A-1535469 or A-1574083, were dissolved in PEG300/propylene glycol/H<sub>2</sub>O (55:25:20). Minocycline hydrochloride and doxycycline hydrochloride (Sigma-Aldrich) were dissolved in water. Animals were weighed, and weight-corrected volumes of dosing solution (100  $\mu$ l per 25 g) were administered by oral gavage or intraperitoneal injection of tylosin A. Experimenters involved in dosing were blinded to the treatment group except for *L. sigmodontis* studies.

### Molecular assays

For synchronous *B. malayi* infections, determination of *Wolbachia* single copy *wsp* gene quantity was undertaken by qPCR, as previously described (11). In rodent screens where mature adult worm age varied, *wsp* quantity per worm was normalized to filarial nematode *gst* using *Brugia*- or *Onchocerca*-specific primers (11). Between 5 and 20 individual worms were assayed, derived from dose groups of between three and nine animals. Experiments using *L. sigmodontis* quantified *Wolbachia ftsZ* and used *L. sigmodontis actin* for normalization, as previously described (27).

### Testing bacterial susceptibility

Broth microdilution test methods were conducted according to the Clinical Laboratory Standards Institute guidelines (28) to determine the susceptibility of different bacterial strains to the tylosin A analogs and comparators. Concurrent quality control testing was performed to ensure proper test conditions and procedures. The quality control strains included *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Streptococcus pneumoniae* ATCC 49619. All quality control results were within published ranges (29).

### Testing *L. loa* in vitro

*L. loa* microfilariae were extracted from baboon peripheral blood and placed in culture in vitro, as previously described (30). Between 20 and 30 microfilariae per well were seeded onto a 96-well plate containing test compounds prepared in DMSO to a final concentration of 1% in culture medium (10% FBS-supplemented Dulbecco's modified Eagle's medium). All compounds were tested in triplicate, and daily motility was scored as follows: 0 (immotile/dead), 1 (very slow, only tip movement), 2 (break in activity, sluggish), or 3 (fully motile, continuously active) on day 2 compared to a DMSO-only control. Operators

were blinded to treatment groups. Ethical and administrative clearances for the use of baboons in this study were obtained from the Ministry of Scientific Research and Innovation of Cameroon (Research permit no. 028/MIN-RESI/B00/C00/C10/C12) and Research Foundation for Tropical Diseases and the Environment (REFOTDE) Institutional Animal Ethics Committee (no. 2009/032). The animal procedures were conducted in accordance with the guidelines of the Animal Care and Use Committee at the National Institutes of Health (USA) and University of Georgia, Athens, USA, as previously described (31).

### Plasma drug concentrations in mice and gerbils

Oral drug doses were administered by gavage to BALB/c mice (Charles River Laboratories, USA) or Mongolian gerbils (Charles River Laboratories, Beijing). Serial blood samples collected into EDTA anticoagulant for plasma concentration analysis were obtained from each animal after dosing. EDTA preserved plasma samples were extracted by protein precipitation with acetonitrile fortified with internal standards. The supernatant was injected into a high-performance liquid chromatography–mass spectrometry (MS)/MS system for separation and quantitation. Detection was accomplished using a triple-quadrupole mass spectrometer operated either in electrospray or in an atmospheric chemical ionization mode. The area under the plasma concentration time curve (AUC) was calculated using the linear trapezoidal rule.

### Statistical analysis

Percentage reduction in *Wolbachia* in microfilariae after in vivo drug treatments was normalized to median vehicle control concentrations derived from the same experimental infection and screen. Where repeat experimental data were available, data were pooled after normalization. Grouped continuous variables were tested for normal distribution by D'Agostino-Pearson omnibus normality tests. Continuous variables failing normal distribution tests were log<sub>10</sub>-transformed and retested. Continuous variables satisfying the assumptions of normal distribution were examined by one-way ANOVA with Dunnett's multiple tests post hoc. Variables not satisfying the assumption of normality were compared by Mann-Whitney (two variables) or Kruskal-Wallis test with Dunnett's multiple tests post hoc (>2 variables). Post hoc testing scrutinized inferior, noninferior, or superior anti-*Wolbachia* activity compared with doxycycline or minocycline controls or assessed changes in microfilarial loads compared with vehicle controls or doxycycline/minocycline controls. Significance levels are indicated as \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , and \*\*\*\* $P < 0.0001$ . All statistics were undertaken using GraphPad Prism v.6 software.

### SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/11/483/eaau2086/DC1

Fig. S1. Efficacy of tylosin A in *Litomosoides* larval model after intraperitoneal or oral dosing.

Fig. S2. Study design schematics for *B. malayi*, *L. sigmodontis*, and *O. ochengi* efficacy models.

Fig. S3. Preclinical PK/PD model of TylAMac analogs.

Fig. S4. In vitro counter screening against *L. loa* microfilariae.

Table S1. PK parameters of tylosin A after intraperitoneal and oral dosing in BALB/c mice.

Table S2. *B. malayi* larval worm burdens 2 weeks after dosing with doxycycline, tylosin A, A-1535469, or A-1574083 in IL-4R $\alpha$ <sup>-/-</sup> BALB/c mice.

Table S3. Spectrum of TylAMac analogs and tylosin A against selected bacteria.

Table S4. *B. malayi* adult worm burdens 6 weeks after dosing with minocycline, tylosin A, or A-1535469 in CCR3<sup>-/-</sup> BALB/c mice.

Table S5. *B. malayi* adult worm burdens 6 weeks after dosing with doxycycline or A-1574083 in gerbils.

Table S6. *L. sigmodontis* adult worm burdens 16 weeks after dosing with doxycycline or A-1574083 in gerbils.

Table S7. *O. ochengi* male worm burdens 6 weeks after dosing with minocycline, A-1535469, or A-1574083 in SCID mice.

Table S8. *O. ochengi* male worm burdens 6 weeks after dosing with doxycycline or A-1574083 in gerbils.

Table S9. *B. malayi* adult worm burdens 24 weeks after dosing with doxycycline or A-1535469 in SCID mice.

Data file S1. Source data for figures.

## REFERENCES AND NOTES

1. A. D. Hopkins, Neglected tropical diseases in Africa: A new paradigm. *Int. Health* **8**, i28–i33 (2016).
2. D. H. Molyneux, A. D. Hopkins, M. H. Bradley, L. A. Kelly-Hope, Multidimensional complexities of filariasis control in an era of large-scale mass drug administration programmes: A can of worms. *Parasit. Vectors* **7**, 363 (2014).
3. M. J. Taylor, A. Hoerauf, M. Bockarie, Lymphatic filariasis and onchocerciasis. *Lancet* **376**, 1175–1185 (2010).
4. M. J. Taylor, A. Hoerauf, S. Townson, B. E. Slatko, S. A. Ward, Anti-*Wolbachia* drug discovery and development: Safe macrofilaricides for onchocerciasis and lymphatic filariasis. *Parasitology* **141**, 119–127 (2014).
5. B. E. Slatko, M. J. Taylor, J. Foster, The *Wolbachia* endosymbiont as an anti-filarial nematode target. *Symbiosis* **51**, 55–65 (2010).
6. M. Walker, S. Specht, T. S. Churcher, A. Hoerauf, M. J. Taylor, M. G. Basáñez, Therapeutic efficacy and macrofilaricidal activity of doxycycline for the treatment of river blindness. *Clin. Infect. Dis.* **60**, 1199–1207 (2015).
7. R. H. Clare, D. A. N. Cook, K. L. Johnston, L. Ford, S. A. Ward, M. J. Taylor, Development and validation of a high-throughput anti-*Wolbachia* whole-cell screen: A route to macrofilaricidal drugs against onchocerciasis and lymphatic filariasis. *J. Biomol. Screen.* **20**, 64–69 (2015).
8. K. L. Johnston, L. Ford, I. Umareddy, S. Townson, S. Specht, K. Pfarr, A. Hoerauf, R. Altmeyer, M. J. Taylor, Repurposing of approved drugs from the human pharmacopoeia to target *Wolbachia* endosymbionts of onchocerciasis and lymphatic filariasis. *Int. J. Parasitol. Drugs Drug Resist.* **4**, 278–286 (2014).
9. J. D. Turner, N. Pionnier, J. Furlong-Silva, H. Sjöberg, S. Cross, A. Halliday, A. F. Guimaraes, D. A. N. Cook, A. Steven, N. Van Rooijen, J. E. Allen, S. J. Jenkins, M. J. Taylor, Interleukin-4 activated macrophages mediate immunity to filarial helminth infection by sustaining CCR3-dependent eosinophilia. *PLoS Pathog.* **14**, e1006949 (2018).
10. S. Specht, K. M. Pfarr, S. Arriens, M. P. Hübner, U. Klarmann-Schulz, M. Koschel, S. Sternberg, C. Martin, L. Ford, M. J. Taylor, A. Hoerauf, Combinations of registered drugs reduce treatment times required to deplete *Wolbachia* in the *Litomosoides sigmodontis* mouse model. *PLoS Negl. Trop. Dis.* **12**, e0006116 (2018).
11. A. Halliday, A. F. Guimaraes, H. E. Tyrer, H. M. Metuge, C. N. W. Patrick, K.-O. J. Arnaud, T. D. B. Kwenti, G. Forsbrook, A. Steven, D. Cook, P. Enyong, S. Wanji, M. J. Taylor, J. D. Turner, A murine macrofilaricide pre-clinical screening model for onchocerciasis and lymphatic filariasis. *Parasit. Vectors* **7**, 472 (2014).
12. R. Sharma, G. Aljayoussi, H. E. Tyrer, J. Gamble, L. Hayward, A. F. Guimaraes, J. Davies, D. Waterhouse, D. A. N. Cook, L. J. Myhill, R. H. Clare, A. Cassidy, A. Steven, K. L. Johnston, L. Ford, J. D. Turner, S. A. Ward, M. J. Taylor, Minocycline as a re-purposed anti-*Wolbachia* macrofilaricide: Superiority compared with doxycycline regimens in a murine infection model of human lymphatic filariasis. *Sci. Rep.* **6**, 23458 (2016).
13. U. Klarmann-Schulz, S. Specht, A. Y. Debrah, L. Batsa, N. K. Ayisi-Boateng, J. Osei-Mensah, Y. Mubarik, P. Konadu, A. Ricchiuto, R. Fimmers, S. Arriens, B. Dubben, L. Ford, M. J. Taylor, A. Hoerauf, Comparison of doxycycline, minocycline, doxycycline plus albendazole and albendazole alone in their efficacy against onchocerciasis in a randomized, open-label, pilot trial. *PLoS Negl. Trop. Dis.* **11**, e0005156 (2017).
14. A. Y. Debrah, S. Mand, Y. Marfo-Debrekeye, L. Batsa, K. Pfarr, M. Büttner, O. Adjei, D. Büttner, A. Hoerauf, Macrofilaricidal effect of 4 weeks of treatment with doxycycline on *Wuchereria bancrofti*. *Trop. Med. Int. Health* **12**, 1433–1441 (2007).
15. J. D. Turner, S. Mand, A. Y. Debrah, J. Muehlfeld, K. Pfarr, H. F. McGarry, O. Adjei, M. J. Taylor, A. Hoerauf, A randomized, double-blind clinical trial of a 3-week course of doxycycline plus albendazole and ivermectin for the treatment of *Wuchereria bancrofti* infection. *Clin. Infect. Dis.* **42**, 1081–1089 (2006).
16. G. Aljayoussi, H. E. Tyrer, L. Ford, H. Sjöberg, N. Pionnier, D. Waterhouse, J. Davies, J. Gamble, H. Metuge, D. A. N. Cook, A. Steven, R. Sharma, A. F. Guimaraes, R. H. Clare, A. Cassidy, K. L. Johnston, L. Myhill, L. Hayward, S. Wanji, J. D. Turner, M. J. Taylor, S. A. Ward, Short-course, high-dose rifampicin achieves *Wolbachia* depletion predictive of curative outcomes in preclinical models of lymphatic filariasis and onchocerciasis. *Sci. Rep.* **7**, 210 (2017).
17. T. Peeters, G. Matthijs, I. Depoortere, T. Cachet, J. Hoogmartens, G. Vantrappen, Erythromycin is a motilin receptor agonist. *Am. J. Physiol.* **257**, G470–G474 (1989).
18. J. D. Turner, R. Sharma, G. Al Jayoussi, H. E. Tyrer, J. Gamble, L. Hayward, R. S. Priestley, E. A. Murphy, J. Davies, D. Waterhouse, D. A. N. Cook, R. H. Clare, A. Cassidy, A. Steven, K. L. Johnston, J. McCall, L. Ford, J. Hemingway, S. A. Ward, M. J. Taylor, Albendazole and antibiotics synergize to deliver short-course anti-*Wolbachia* curative treatments in preclinical models of filariasis. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E9712–E9721 (2017).
19. A. Hoerauf, S. Mand, L. Volkmann, M. Büttner, Y. Marfo-Debrekeye, M. J. Taylor, O. Adjei, D. W. Büttner, Doxycycline in the treatment of human onchocerciasis: Kinetics of *Wolbachia* endobacteria reduction and of inhibition of embryogenesis in female *Onchocerca* worms. *Microbes Infect.* **5**, 261–273 (2003).
20. M. J. Taylor, W. H. Makunde, H. F. McGarry, J. D. Turner, S. Mand, A. Hoerauf, Macrofilaricidal activity after doxycycline treatment of *Wuchereria bancrofti*: A double-blind, randomised placebo-controlled trial. *Lancet* **365**, 2116–2121 (2005).
21. A. Hoerauf, S. Specht, M. Büttner, K. Pfarr, S. Mand, R. Fimmers, Y. Marfo-Debrekeye, P. Konadu, A. Y. Debrah, C. Bandi, N. Brattig, A. Albers, J. Larbi, L. Batsa, M. J. Taylor, O. Adjei, D. W. Büttner, *Wolbachia* endobacteria depletion by doxycycline as antifilarial therapy has macrofilaricidal activity in onchocerciasis: A randomized placebo-controlled study. *Med. Microbiol. Immunol.* **197**, 295–311 (2008).
22. J. D. Turner, N. Tendongfor, M. Esum, K. L. Johnston, R. S. Langley, L. Ford, B. Faragher, S. Specht, S. Mand, A. Hoerauf, P. Enyong, S. Wanji, M. J. Taylor, Macrofilaricidal activity after doxycycline only treatment of *Onchocerca volvulus* in an area of *Loa loa* co-endemicity: A randomized controlled trial. *PLoS Negl. Trop. Dis.* **4**, e660 (2010).
23. J. Gardon, N. Gardon-Wendel, Demanaga-Ngangue, J. Kamgo, J.-P. Chippaux, M. Boussinesq, Serious reactions after mass treatment of onchocerciasis with ivermectin in an area endemic for *Loa loa* infection. *Lancet* **350**, 18–22 (1997).
24. J. Ajendra, S. Specht, S. Ziewer, A. Schiefer, K. Pfarr, M. Parcina, T. A. Kufer, A. Hoerauf, M. P. Hübner, NOD2 dependent neutrophil recruitment is required for early protective immune responses against infectious *Litomosoides sigmodontis* L3 larvae. *Sci. Rep.* **6**, 39648 (2016).
25. S. Ziewer, M. P. Hübner, B. Dubben, W. H. Hoffmann, O. Bain, C. Martin, A. Hoerauf, S. Specht, Immunization with *L. sigmodontis* microfilariae reduces peripheral microfilaremia after challenge infection by inhibition of filarial embryogenesis. *PLoS Negl. Trop. Dis.* **6**, e1558 (2012).
26. A. E. Marriott, H. Sjöberg, H. Tyrer, J. Gamble, E. Murphy, J. Archer, A. Steven, M. J. Taylor, J. D. Turner, Validation of ultrasound imaging to predict worm burden and treatment efficacy in preclinical filariasis drug screening models. *Sci. Rep.* **8**, 5910 (2018).
27. A. Schiefer, A. Schmitz, T. F. Schäberle, S. Specht, C. Lämmer, K. L. Johnston, D. G. Vassilyev, G. M. König, A. Hoerauf, K. Pfarr, Corallopyronin A specifically targets and depletes essential obligate *Wolbachia* endobacteria from filarial nematodes in vivo. *J. Infect. Dis.* **206**, 249–257 (2012).
28. Clinical Laboratory and Standards Institute, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard—Ninth Edition* (document M07-A10, Clinical and Laboratory Standards Institute, 2015).
29. Clinical and Laboratory Standards Institute, *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement* (document M100-S25, Clinical and Laboratory Standards Institute, 2015).
30. D. Zofou, F. F. Fombad, N. V. T. Gandjui, A. J. Njouendou, A. J. Kengne-Ouafo, P. W. Chounna Ndongmo, F. R. Datchoua-Poutcheu, P. A. Enyong, D. T. Bitu, M. J. Taylor, J. D. Turner, S. Wanji, Evaluation of *in vitro* culture systems for the maintenance of microfilariae and infective larvae of *Loa loa*. *Parasit. Vectors* **11**, 275 (2018).
31. S. Wanji, E.-E. Eyong, N. Tendongfor, C. Ngwa, E. Esuka, A. Kengne-Ouafo, F. Datchoua-Poutcheu, P. Enyong, A. Hopkins, C. D. Makenzie, Parasitological, hematological and biochemical characteristics of a model of hyper-microfilaremiaemic Loiasis (*Loa loa*) in the baboon (*Papio anubis*). *PLoS Negl. Trop. Dis.* **9**, e0004202 (2015).

**Acknowledgments:** We thank R. Clark (AbbVie Inc.) for the anti-infective compound library. Bacterial susceptibility studies were conducted at Micromyx LLC under the direction of D. Shinabarger and C. Pillar. **Funding:** The A-WOL consortium is supported by a grant from the Bill & Melinda Gates Foundation awarded to the Liverpool School of Tropical Medicine (no. OPP1054324). This work was also supported by a Bill & Melinda Gates Foundation Grand Challenges Explorations grant (no. OPP1119043) to J.D.T., S.W., and M.J.T. Experiments performed at University Hospital Bonn and at WuXi AppTech were supported by the Bill & Melinda Gates Foundation (no. OPP1134310) and the Drugs for Neglected Disease initiative (DNDi). **Author contributions:** M.J.T., T.W.v.G., L.F., M.P.H., K.M., H.E.M., A.H., S.W. D.J.K., J.D.T., and S.A.W. designed the study. T.W.v.G. and H.E.M. synthesized the tylosin A analogs. L.F., K.L.J., D.A.N.C., R.H.C., H.T.S., N.P., A.S., and J.A. performed the *in vitro* efficacy assays. M.P.H., H.T.S., S.S., N.P., H.E.T., D.A.N.C., E.M., A.S., J.A., D.B., F.L., M.K., A.E., H.M.M., V.C.C., P.W.N.C., A.J.N., F.F.F., and G.A. performed the *in vivo* efficacy assays. K.M. and R.C. performed the PK experiments and created the PK/PD model. L.F., M.P.H., H.T.S., K.M., S.S., K.L.J., N.P., H.E.T., D.A.N.C., E.M., R.H.C., D.B., R.C., H.E.M., F.L., M.K., A.E., H.M.M., V.C.C., P.W.N.C., A.J.N., F.F.F., G.A., and J.D.T. analyzed the data.

M.J.T., L.F., M.P.H., A.H., D.J.K., J.D.T., and S.A.W. wrote the paper. **Competing interests:** T.W.v.G. is a paid consultant to DNDi and has advised on their helminth programs. D.J.K. is an unpaid consultant to DNDi. T.W.v.G., D.J.K., K.M., M.J.T., M.P.H., J.D.T., and S.A.W. are nonpaid members of the MacDA consortium. T.W.v.G. has been a nonpaid member of the External Scientific Advisory Committee (ESAC) for the A-WOL consortium. T.W.v.G., D.J.K., and K.M. are inventors on the following patent/patent applications held by AbbVie Inc. that cover 4<sup>n</sup>-O-substituted tylosin A analogs and derivatives: US2015138458, EP3116888A1 US2016112317, US20150259374/US10072040, and US20160200757. T.W.v.G., D.J.K., M.J.T., S.A.W., L.F., and J.D.T. are inventors on patent/patent applications held by AbbVie Inc. concerning treatment of filarial diseases (patent application nos. US20170368088, EP3242662A1-A4. The other authors declare that they have no competing interests.

**Data and materials availability:** All data associated with this study are available in the main text or the Supplementary Materials. This study was performed under a partnership agreement between AbbVie Inc. and the Liverpool School of Tropical Medicine, which has confidentiality provisions. Compounds were provided to the University of Bonn through the A-WOL consortium. ABBV-4083 and related compounds are available from AbbVie Inc.

under a material transfer agreement through submission of a proposal to AbbVie Inc.'s investigator-initiated studies portal: [www.abbviemedicalresearch.com/external/AV\\_SPT\\_SiteLoginPage](http://www.abbviemedicalresearch.com/external/AV_SPT_SiteLoginPage) and [www.abbvie.com/partnerships/additional-collaboration-opportunities/investigator-initiated-studies-iis.html](http://www.abbvie.com/partnerships/additional-collaboration-opportunities/investigator-initiated-studies-iis.html).

Submitted 17 May 2018

Accepted 19 October 2018

Published 13 March 2019

10.1126/scitranslmed.aau2086

**Citation:** M. J. Taylor, T. W. von Geldern, L. Ford, M. P. Hübner, K. Marsh, K. L. Johnston, H. T. Sjöberg, S. Specht, N. Pionnier, H. E. Tyrer, R. H. Clare, D. A. N. Cook, E. Murphy, A. Steven, J. Archer, D. Bloemker, F. Lenz, M. Koschel, A. Ehrens, H. M. Metuge, V. C. Chunda, P. W. Ndongmo Chounna, A. J. Njouendou, F. F. Fombad, R. Carr, H. E. Morton, G. Aljayoussi, A. Hoerauf, S. Wanji, D. J. Kempf, J. D. Turner, S. A. Ward, Preclinical development of an oral anti-*Wolbachia* macrolide drug for the treatment of lymphatic filariasis and onchocerciasis. *Sci. Transl. Med.* **11**, eaau2086 (2019).

## Preclinical development of an oral anti-*Wolbachia* macrolide drug for the treatment of lymphatic filariasis and onchocerciasis

Mark J. Taylor, Thomas W. von Geldern, Louise Ford, Marc P. Hübner, Kennan Marsh, Kelly L. Johnston, Hanna T. Sjöberg, Sabine Specht, Nicolas Pionnier, Hayley E. Tyrer, Rachel H. Clare, Darren A. N. Cook, Emma Murphy, Andrew Steven, John Archer, Dominique Bloemker, Franziska Lenz, Marianne Koschel, Alexandra Ehrens, Haelly M. Metuge, Valerine C. Chunda, Patrick W. Ndongmo Chounna, Abdel J. Njouendou, Fanny F. Fombad, Robert Carr, Howard E. Morton, Ghaith Aljayyousi, Achim Hoerauf, Samuel Wanji, Dale J. Kempf, Joseph D. Turner and Stephen A. Ward

*Sci Transl Med* 11, eaau2086.  
DOI: 10.1126/scitranslmed.aau2086

### Nematodes go A-WOL

New drugs are urgently needed for treating the neglected tropical diseases, onchocerciasis and lymphatic filariasis. As part of the A-WOL consortium, Taylor *et al.* launched a drug discovery program that identified macrolide antibiotic molecules that were capable of eliminating the bacterial endosymbiont, *Wolbachia*, which is necessary for the viability and fertility of filarial worms. Two macrolide compounds cleared *Wolbachia* from filarial nematodes in animal models of lymphatic filariasis and onchocerciasis. The authors showed that the tylosin A analog, A-1574083 (ABV-4083), had superior efficacy compared to tetracycline antibiotics for clearing *Wolbachia* in mouse and gerbil models of filarial infection. The safety and pharmacology profiles of A-1574083 have enabled this compound to be moved forward into clinical testing.

#### ARTICLE TOOLS

<http://stm.sciencemag.org/content/11/483/eaau2086>

#### SUPPLEMENTARY MATERIALS

<http://stm.sciencemag.org/content/suppl/2019/03/11/11.483.eaau2086.DC1>

#### RELATED CONTENT

<http://stm.sciencemag.org/content/scitransmed/9/377/eaaf6953.full>  
<http://stm.sciencemag.org/content/scitransmed/7/279/279ra37.full>  
<http://stm.sciencemag.org/content/scitransmed/7/286/286re4.full>

#### REFERENCES

This article cites 29 articles, 1 of which you can access for free  
<http://stm.sciencemag.org/content/11/483/eaau2086#BIBL>

#### PERMISSIONS

<http://www.sciencemag.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of Service](#)

---

*Science Translational Medicine* (ISSN 1946-6242) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. 2017 © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works. The title *Science Translational Medicine* is a registered trademark of AAAS.