

Drug Discovery: Supporting development of new drugs to treat global parasitic diseases

Genentech Hall



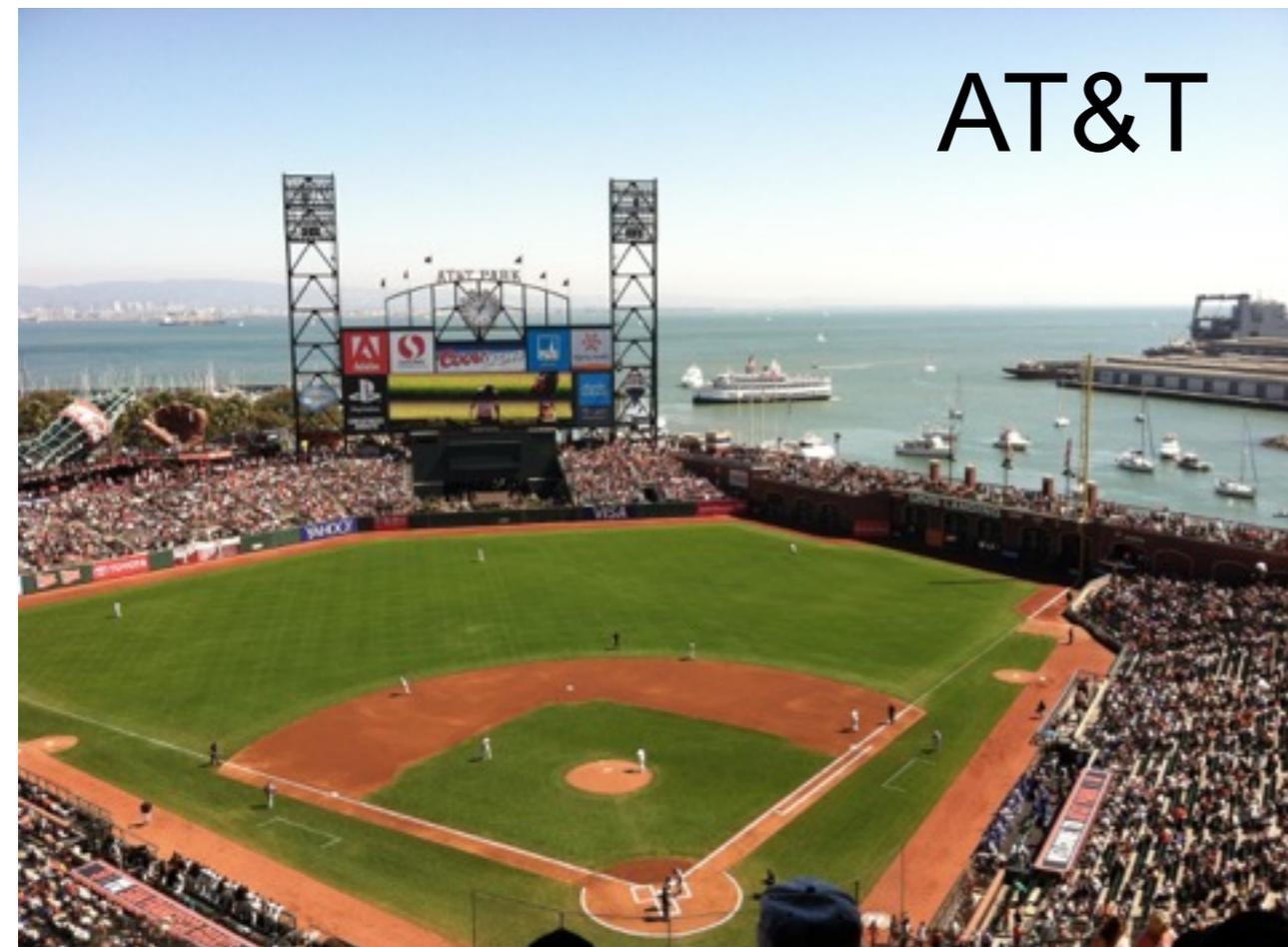
UC Santa Cruz Bio 117
Feb. 14, 2017

Judy Sakanari
Center for Parasitic Diseases
UC San Francisco

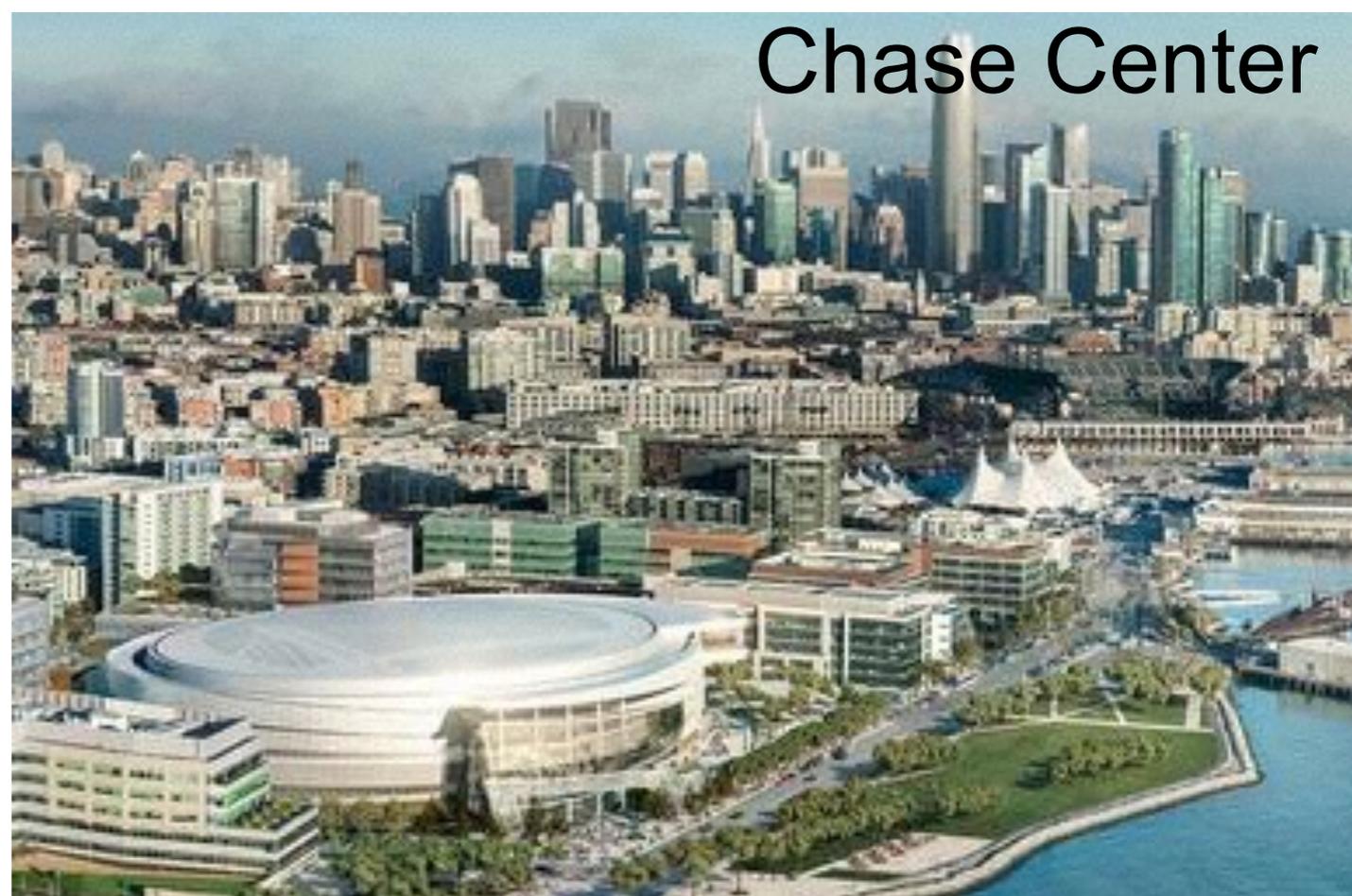
Mission Bay Campus



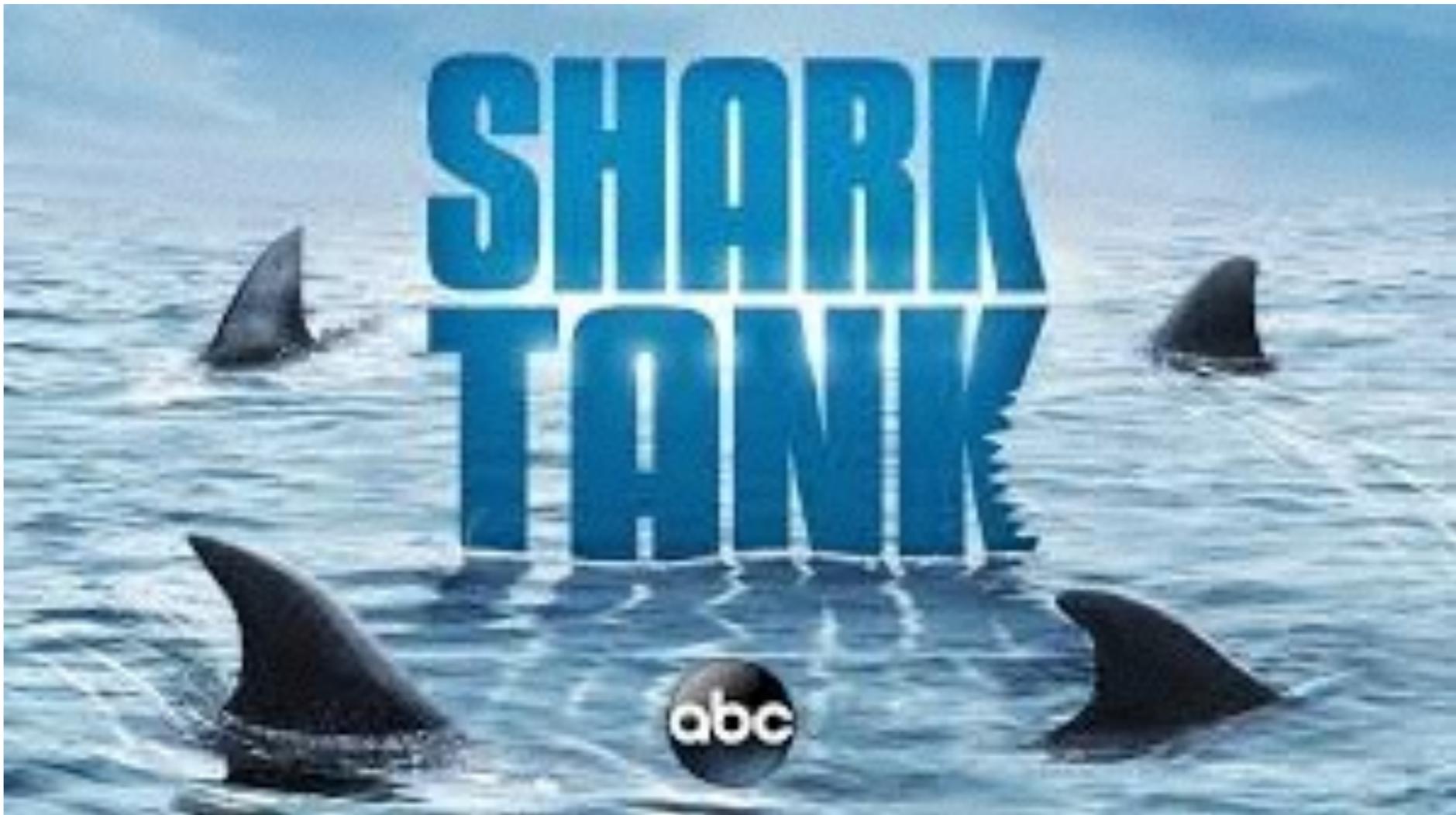
AT&T



Chase Center



If you had the chance to apply for \$1,000,000 USD, what would you do to help support the elimination of this disease?



Developing drugs to treat River Blindness: The Many Challenges



River Blindness

Epidemiology & Biology
Disease

Treatment

Research

- drug discovery
- diagnostics



Onchocerciasis (River Blindness)

Filarial nematode: *Onchocerca volvulus*

Regions affected: 36 countries in Sub-Saharan Africa, foci in Americas

Number infected: 18-36 million, 270,000 are blind; 1.2 million visually impaired

Number at risk: 120 million worldwide

Distribution of onchocerciasis, worldwide, 2013



Neglected Tropical Diseases (NTDs)

- NTDs cause substantial illness for more than one billion people globally.
- Neglected because they affect the world's poorest people and communities.
- Devastating diseases contribute to childhood illnesses and death, hamper a person's ability to work, and prevent families and communities from thriving.
- As a result, NTDs trap the poor in a cycle of poverty and disease.



Patients

- x(odules taken away from them (reduction in filarial load)
- ~~all~~ All wounds treated; follow-up of treatment by us
- A container of milk; ovaltine; a packet of sugar + 10 kg of rice to aid recovery.
- Taxi money





© Clare Gilbert, International Centre for Eye Health www.iceh.org.uk, LSHTM

The Disease: People with onchocerciasis can have several hundred nodules in their skin. Nodules vary in size from 1 to 5 cm in diameter. They can cause discomfort but are not usually painful.





Nodules (onchocercomas) consist of host fibrotic tissue surrounding several female (30-50 cm in length) and male (20-40 cm length) adult worms which can live for 15 yrs.

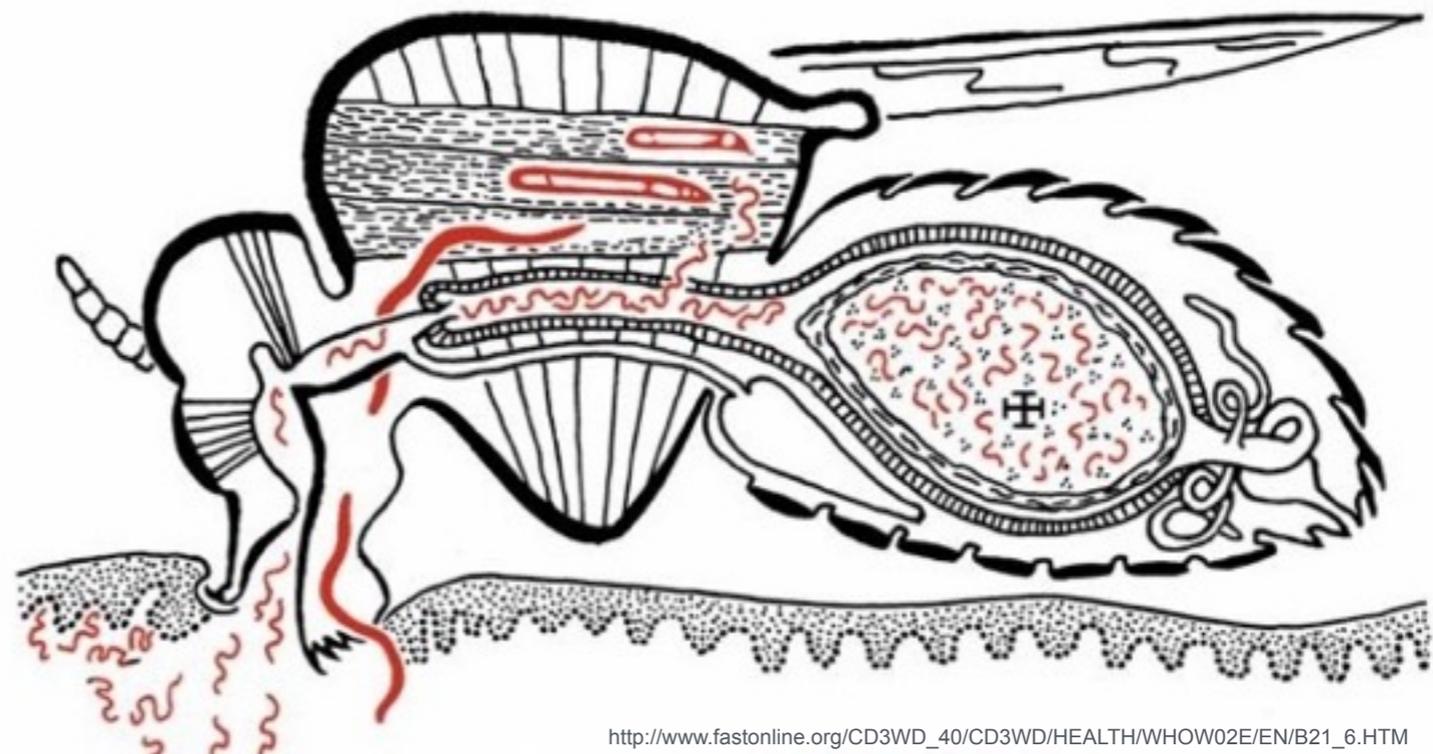


Microfilariae migrate throughout the skin and move to other tissues.



microfilariae in skin

Simulium damnosum: Blackfly vector of *Onchocerca volvulus*.



http://www.fastonline.org/CD3WD_40/CD3WD/HEALTH/WHOW02E/EN/B21_6.HTM



<http://www.riverblindness.eu/epidemiology/bovine-onchocercosis/>

Larval black flies inhabit fast-flowing rivers, hence the name **River Blindness**.

Skin Disease: The microfilariae cause skin rash and severe, intense itchiness.





Skin disease is a result of dead and dying microfilariae that elicit an intense immune response.

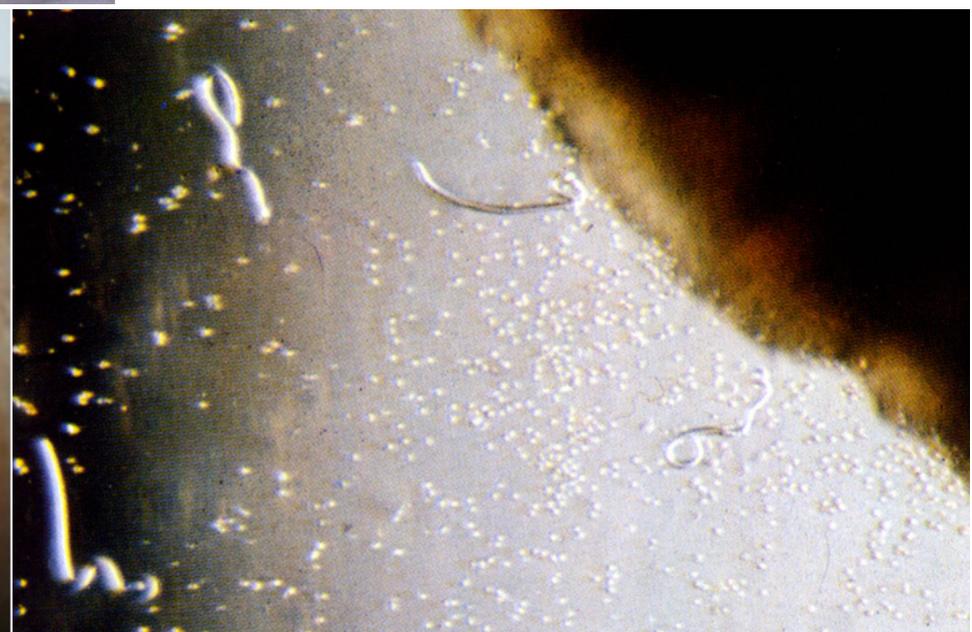


Over several years, severe dermatitis can occur. The skin loses its elasticity, giving the appearance of early aging and loss of pigment, gives a 'leopard skin' appearance.



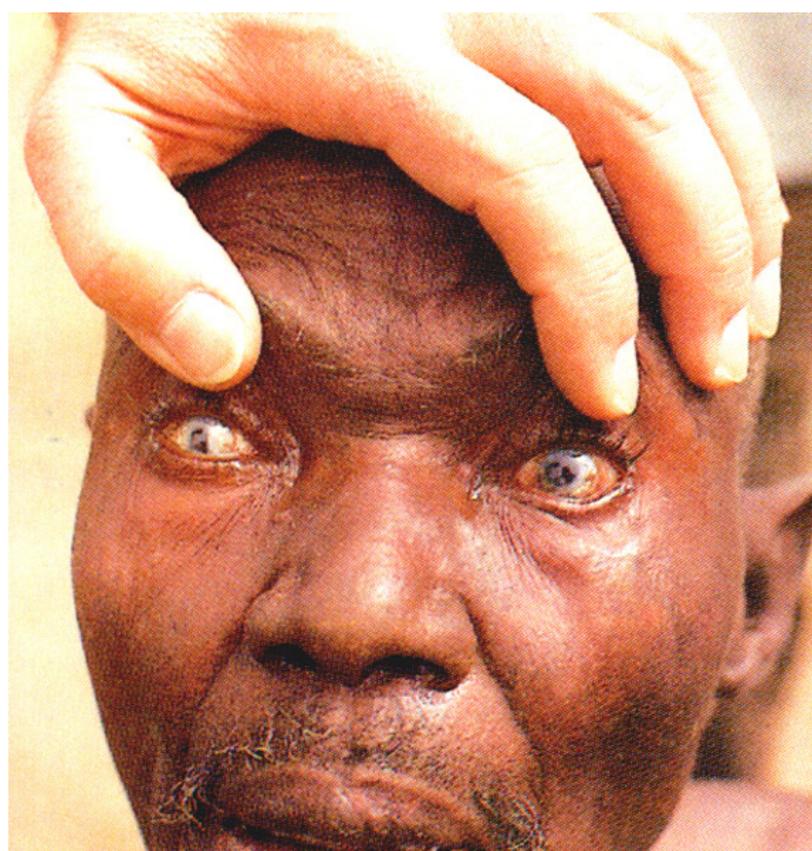
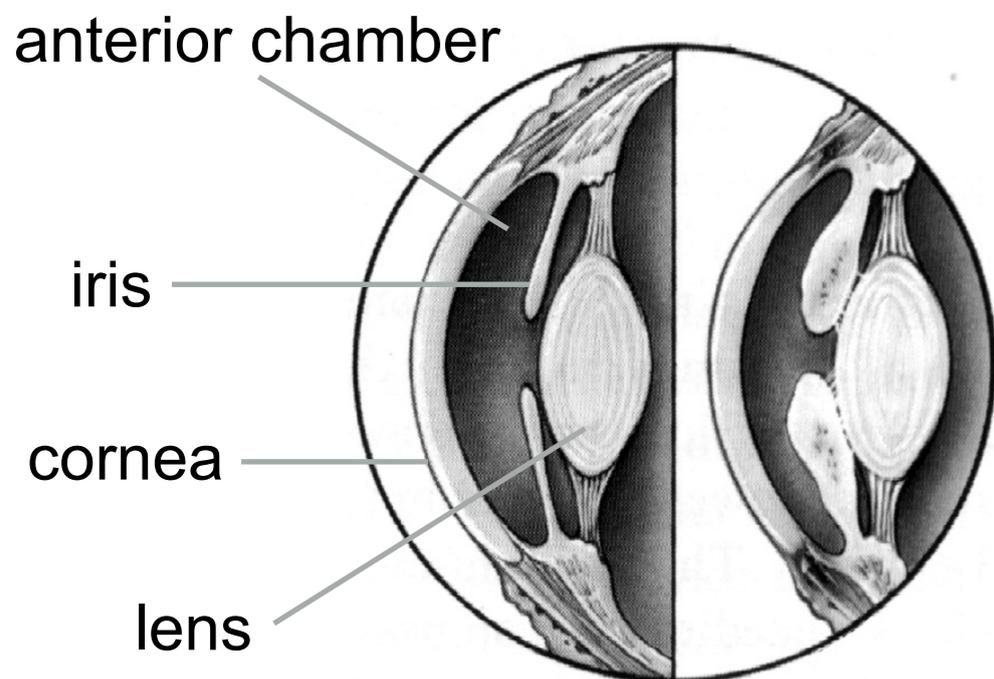
Diagnosis by Skin Snips

Skin is biopsied (ouch!) and examined for microfilariae.



Blindness as a result of *Onchocerca volvulus*

- 18-36 million infected
- 270,000 are blind
- 1.2 million visually impaired



Onchocerciasis: Mortality in blind adults 3 to 4 x's greater than in the fully sighted population.

Global Health Estimates 2015: DALYs (000s) by cause and region

A. Infectious and parasitic diseases		359,341
1	Tuberculosis	56,037
2	STDs excluding HIV	11,208
3	HIV/AIDS	62,759
4	Diarrhoeal diseases	84,928
5	Childhood-cluster diseases	23,232
6	Meningitis	23,267
7	Encephalitis	6,050
8	Hepatitis	7,270
9	Parasitic and vector diseases	55,140
a.	Malaria	38,520
b.	African Trypanosomiasis	372
c.	Chagas disease	253
d.	Schistosomiasis	3,514
e.	Leishmaniasis	1,357
f.	lymphatic filariasis	2,071
g.	Onchocerciasis	1,136
h.	Cysticercosis	1,857
i.	Echinococcosis	642
j.	Dengue	2,613
k.	Trachoma	279
l.	Yellow fever	856
m.	Rabies	1,672
10	Intestinal nematode infections	4,461
11	Leprosy	489
12	Other infectious diseases	24,500
B. Respiratory Infectious		148,871
C. Maternal conditions		19,620

2015 Nobel Prize in Physiology or Medicine

Tu Youyou



Satoshi Omura



William C. Campbell



Tu discovered Artemisinin, a drug used to treat malaria.

Omura and Campbell developed Avermectin (Ivermectin) for treatment of parasitic worm infections.

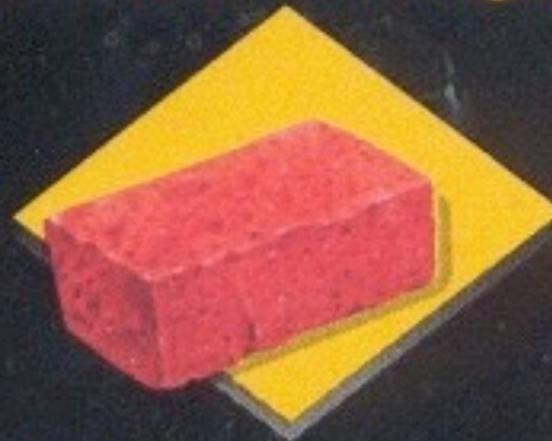
Heartgard[®] TM

(ivermectin/pyrantel)

Plus

Administer once a month to prevent heartworm disease and to treat and control ascarid and hookworm infections in dogs.

**For
DOGS
26-50 lbs**



6 Chewables

Each chewable contains 136 mcg ivermectin and 114 mg pyrantel as pamoate salt.

Keep this and all drugs out of the reach of children.

Caution: Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.



Drugs for
River
Blindness

MORE THAN 25 Years: The MECTIZAN® Donation Program



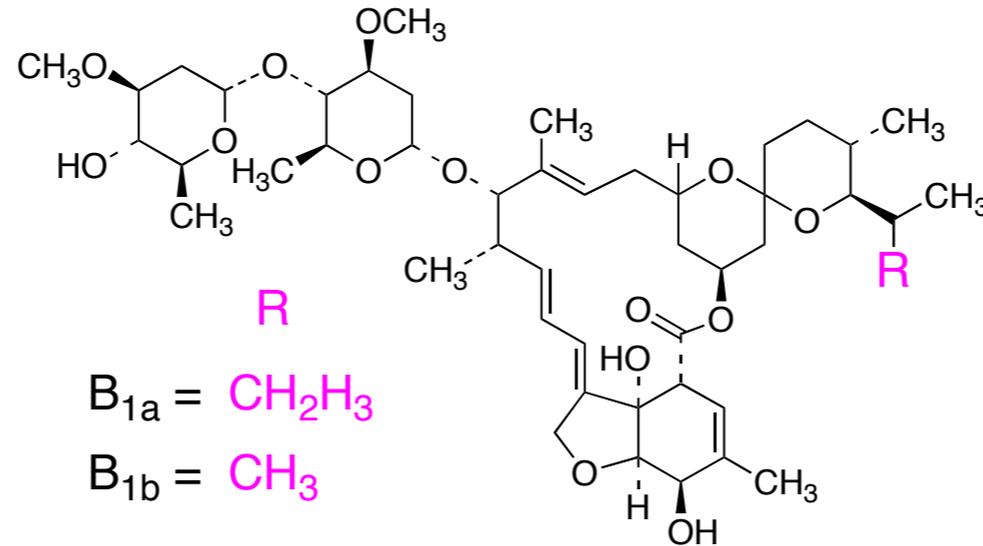
Our Commitment:
"as much as needed, for as long as needed..."

In 1987, Merck committed to donate Mectizan with the goal to help eliminate river blindness. In Latin America, four countries -Colombia, Ecuador, Mexico and Guatemala have received WHO verification of river blindness elimination. The program reaches >250 million people in the affected areas annually, with more than 2 billion treatments donated since 1987.

Avermectins

Non-ionizable antihelmintic macrolides

Ivermectin



- In 1974, Omura's team isolated *Streptomyces avermitilis* (actinomycete) from a golf course and sent samples to Merck for testing.
- 1982 effective against the larval stage (microfilariae) in the skin and helped stop the extreme itchiness.

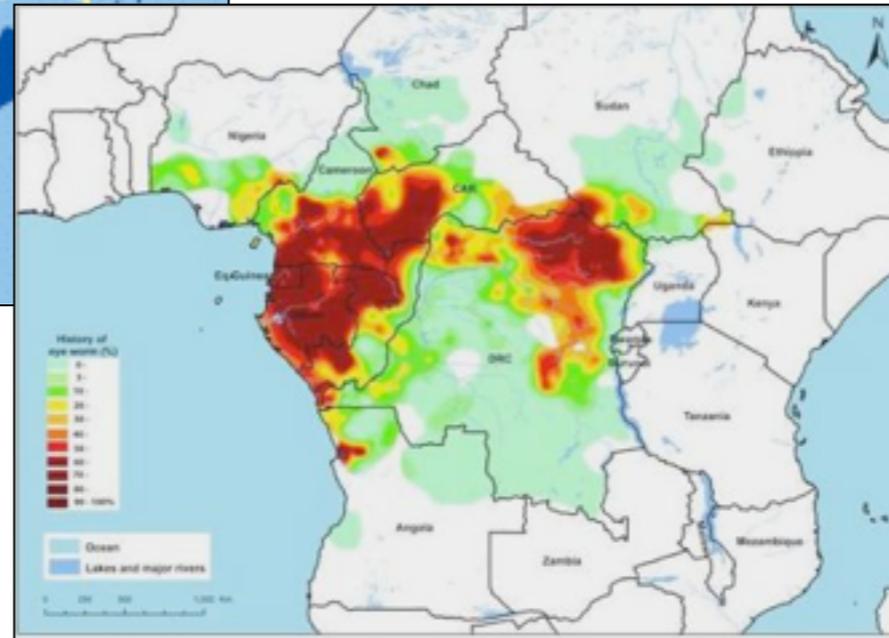
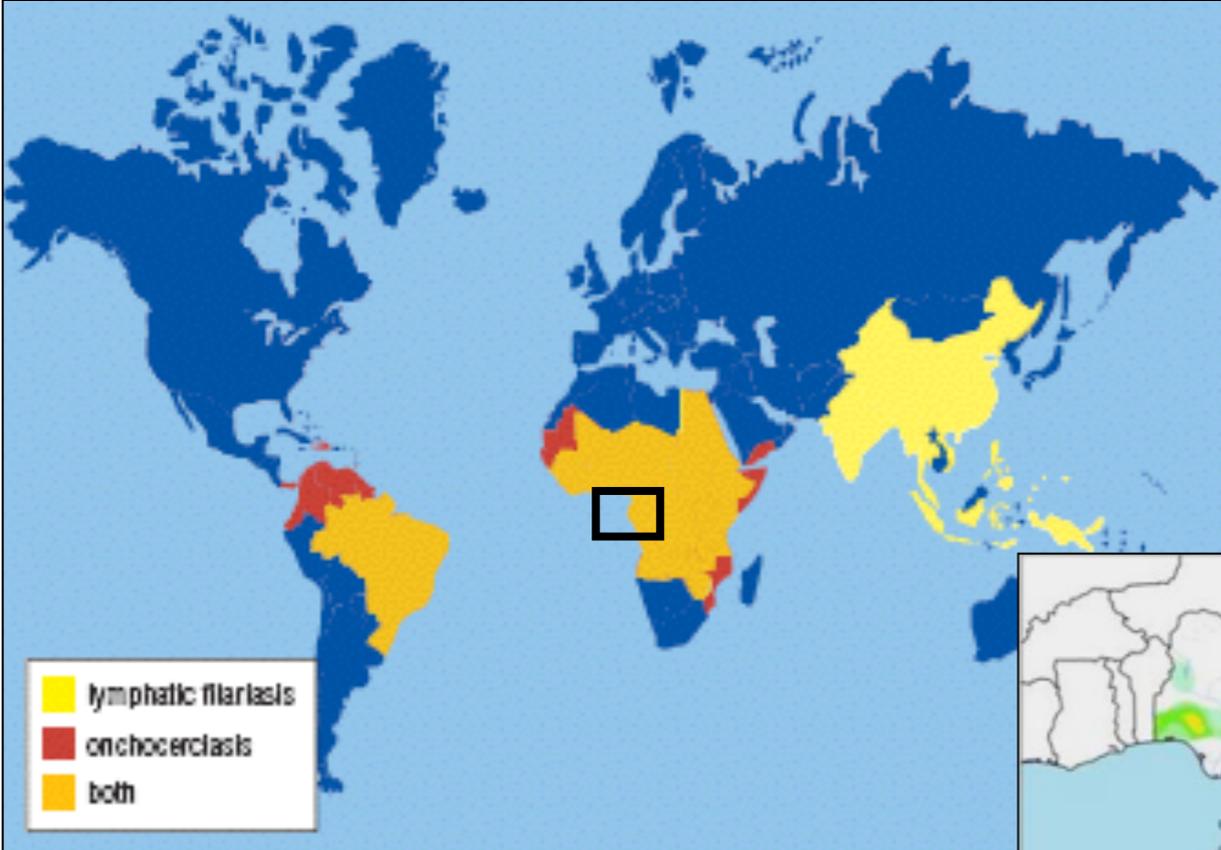
Avermectins

- Ivermectin binds with high affinity to glutamate-gated chloride channels (GluCl_s) in nerve and muscle cells of nematodes and insects.
- Causes an increase in permeability of the cell membrane to chloride ions -> hyperpolarization of the nerve or muscle cell.
- Results in paralysis in nematodes and insects.
- Little toxicity in mammals (due to differential potency on GABA receptors.)
- Exception: dogs with defects in the P-glycoprotein gene (*mdr1*) have increased brain concentrations of ivermectin.



Macrofilariarial worms

- *Onchocerca volvulus*
- *Brugia & Wuchereria*
(lymphatic filariasis/elephantiasis)
- *Loa loa*

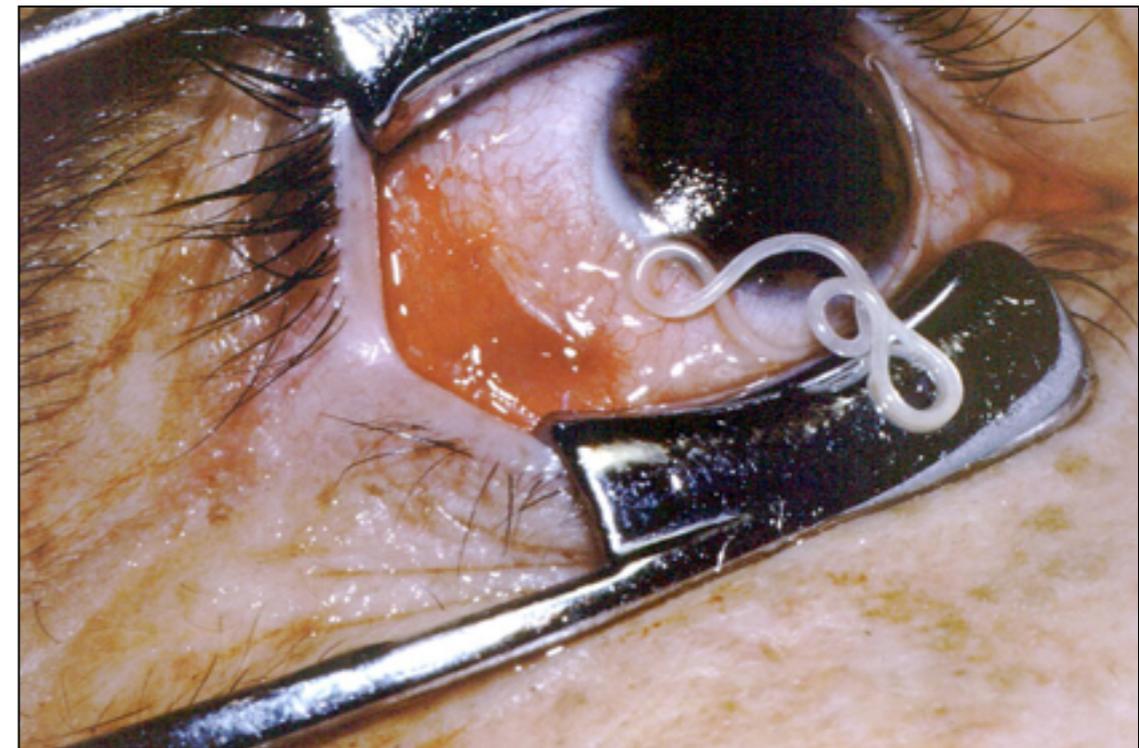


The good and the bad:
ivermectin kills the
microfilariae.

Loa loa (eyeworm)

Ivermectin, which kills the microfilariae, is used in the treatment of river blindness and lymphatic filariasis.

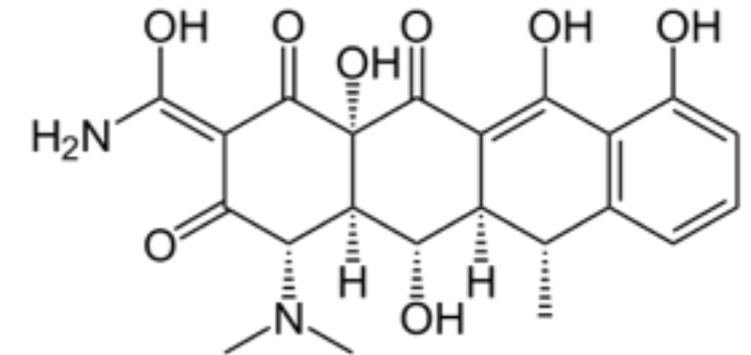
When patients also have a high number of *Loa loa* microfilariae, the drug can cause severe adverse reactions and fatalities.



Doxycycline



- Doxycycline is a broad-spectrum antibiotic of the tetracycline class.
- Used to treat bacterial pneumonia, acne, chlamydia infections, early Lyme disease, cholera and syphilis.
- Doxycycline reversibly binds to the 30 S ribosomal subunits and possibly the 50S ribosomal subunit(s), blocking the binding of aminoacyl tRNA to the mRNA and inhibiting bacterial protein synthesis.
- Chemoprophylaxis for malaria.
- Wholesale cost in the developing world is between 0.01 and 0.04 USD per pill.
- Cannot be used by pregnant women and children <8 years old.
- Doxycycline kills the symbiotic *Wolbachia* bacteria in the reproductive tracts of *Onchocerca*, making the nematodes sterile (reducing transmission).
- Field trials to treat onchocerciasis showed an 8 wk course almost completely eliminates the release of microfilariae and kills the adult worm.



Drugs to Treat Onchocerciasis

Usage/Drug	Adult Dose	Pediatric dose
<i>To kill <u>microfilariae</u>: ivermectin</i>	150 mcg/kg orally in one dose every 6 months	150 mcg/kg orally in one dose every 6 months
<i>To kill <u>macrofilariae</u>: doxycycline*</i>	200 mg orally daily for 6 weeks	200 mg orally daily for 6 weeks

* Doxycycline is not standard therapy, but several studies support its use and safety. Treatment with ivermectin should be given one week prior to treatment with doxycycline in order to provide symptom relief to the patient. If the patient cannot tolerate 200 mg PO daily of doxycycline, 100mg PO daily is sufficient to sterilize female *Onchocerca*.

Project Goal

To identify macrofilaricidal drugs to treat onchocerciasis in *Loa loa* endemic areas.

First we had to develop a way to test worms with various drug libraries...

Onchocerca volvulus host: humans

Onchocerca ochengi host: cattle from West Africa

Brugia malayi host: cats/rodents

Brugia pahangi host: dogs/rodents

Molecular phylogeny of filarial worms Nematoda: Filarioidea

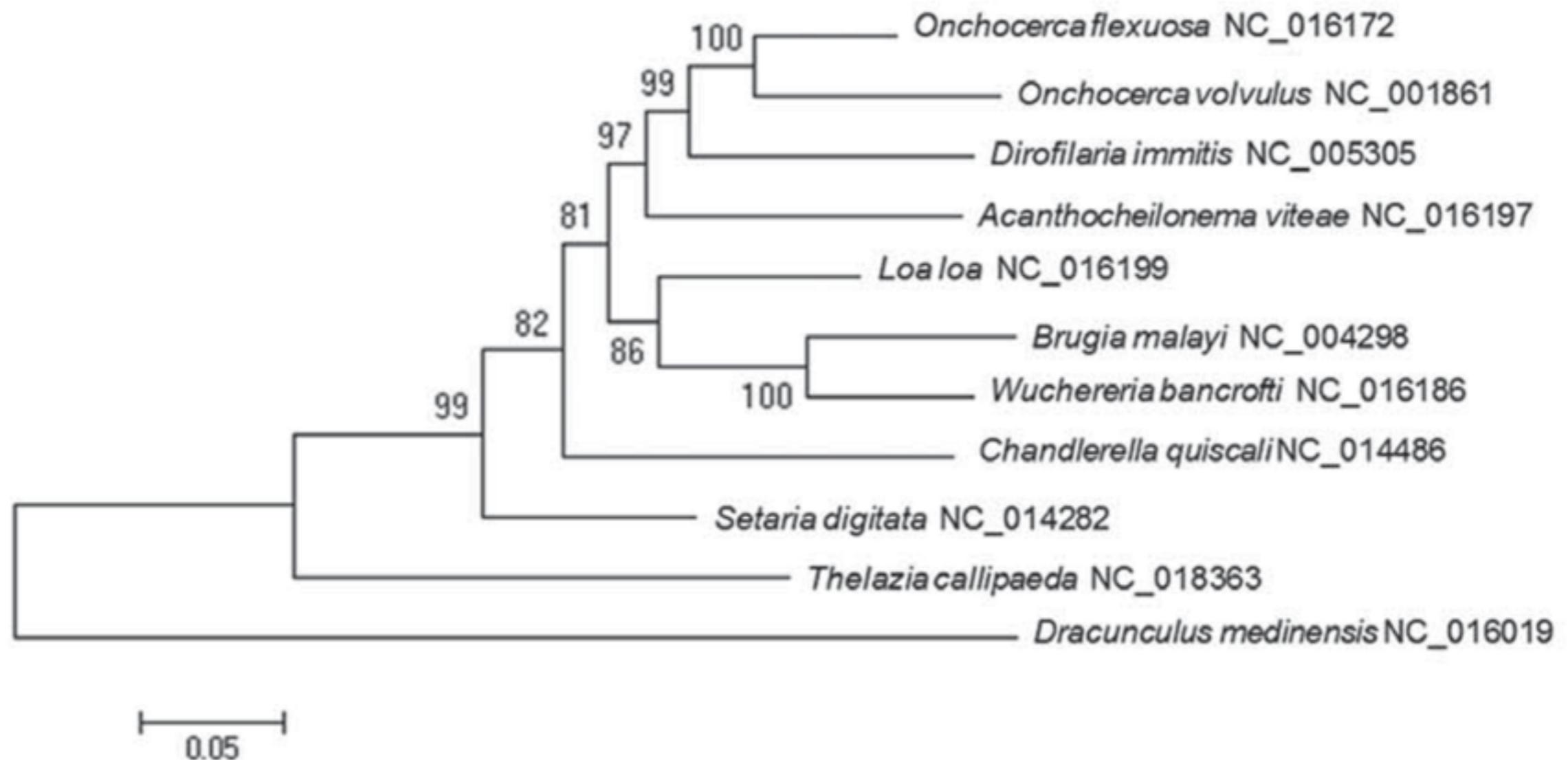
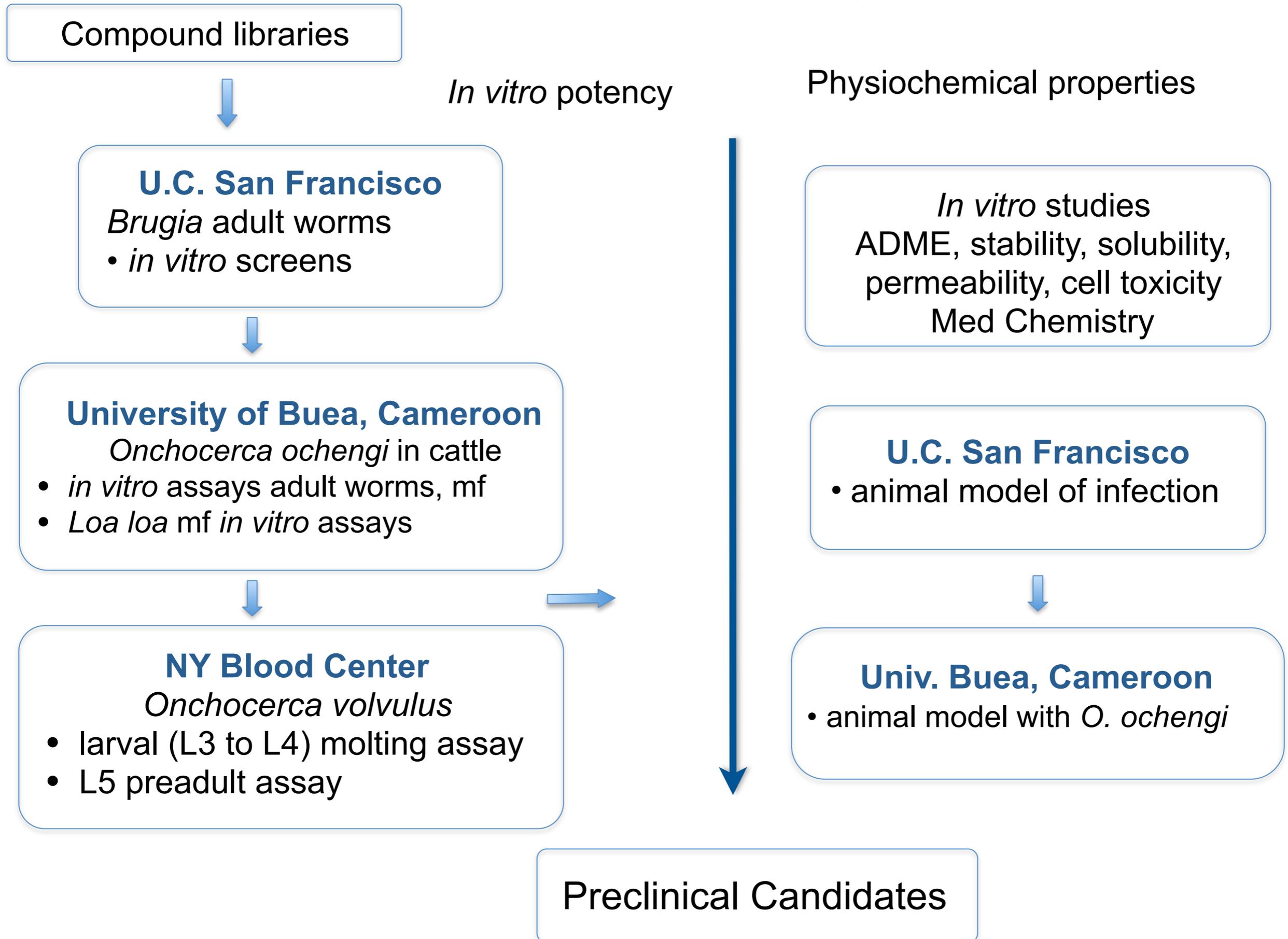


Fig. 1. Phylogenetic tree of nine taxa of Onchocercidae (with *Thelazia callipaeda* and *Dracunculus medinensis* as outgroups) generated by the Maximum Likelihood method based on 12 protein coding genes of the mitochondrial genome conducted in MEGA5. The tree with the highest log likelihood (-62978.0961) is shown. The percentage of trees in which the associated taxa clustered together is shown next

Macrofilaria Project



Worm Assays: Phenotypic Screen

Brugia worms
shipped to UCSF



Phenotypic screen:
Plates assessed Day 1, 2, 3:

- 0 = not moving
- 1 = barely moving
- 2 = slightly active
- 3 = active
- 4 = highly active (normal)
- 5 = hyperactive



UCSF Robotics: Biomek media removal and compound dispensing



Readout: Mean movement units (# pixels displaced/sec)









Irene is removing
Onchocerca ochengi
nodules from a cow hide in
Dr. Fidelis Cho-Ngwa's Lab,
Univ. of Buea, Cameroon



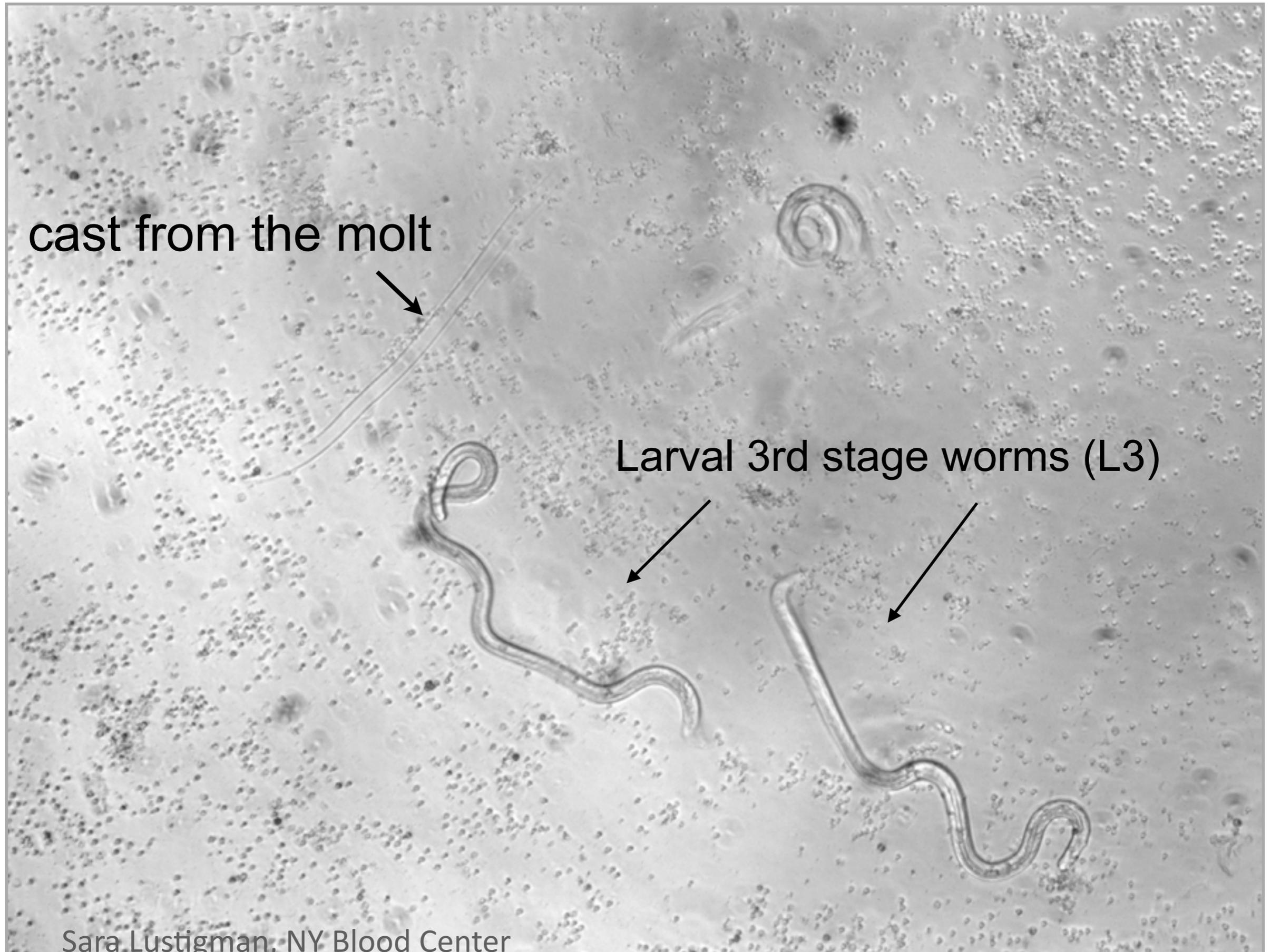
Screening *Onchocerca ochengi* adults: motility and viability

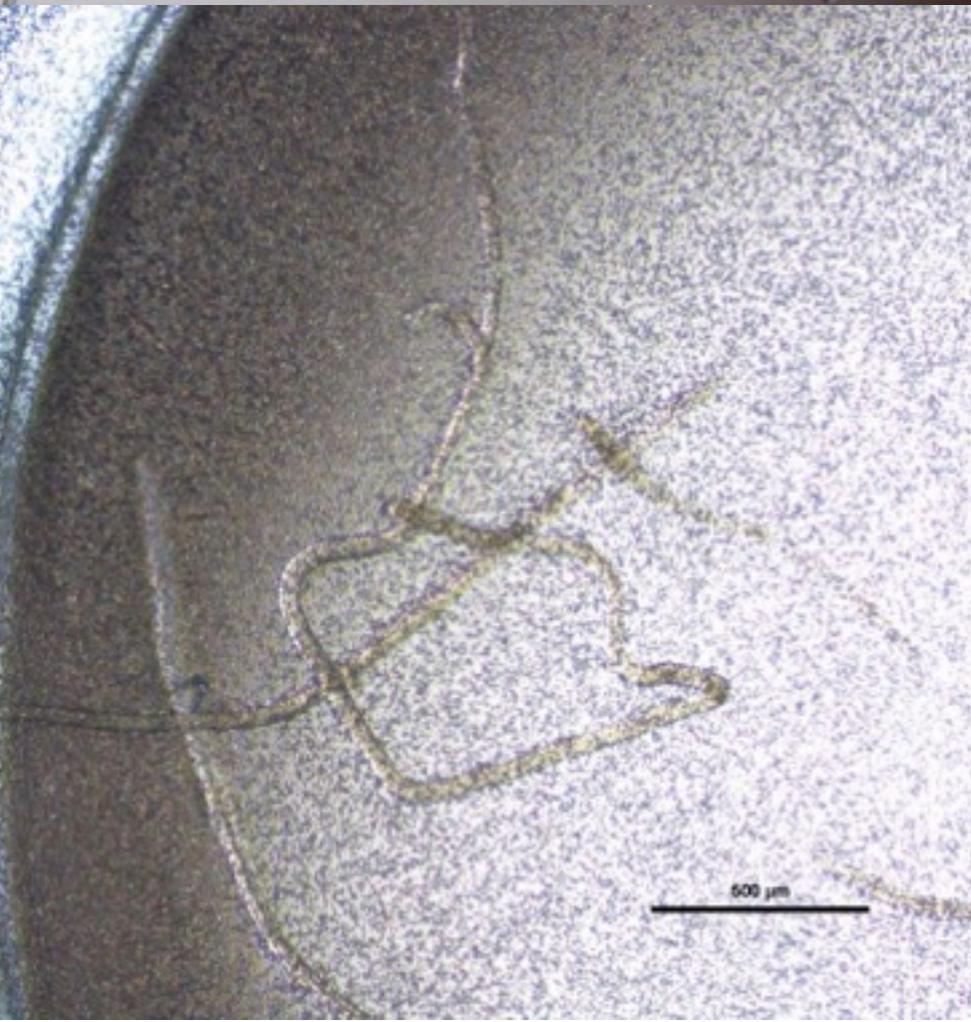


7 day assay:
killing determined
using MTT staining
and visual
observation for color
change
blue = live
no color = dead

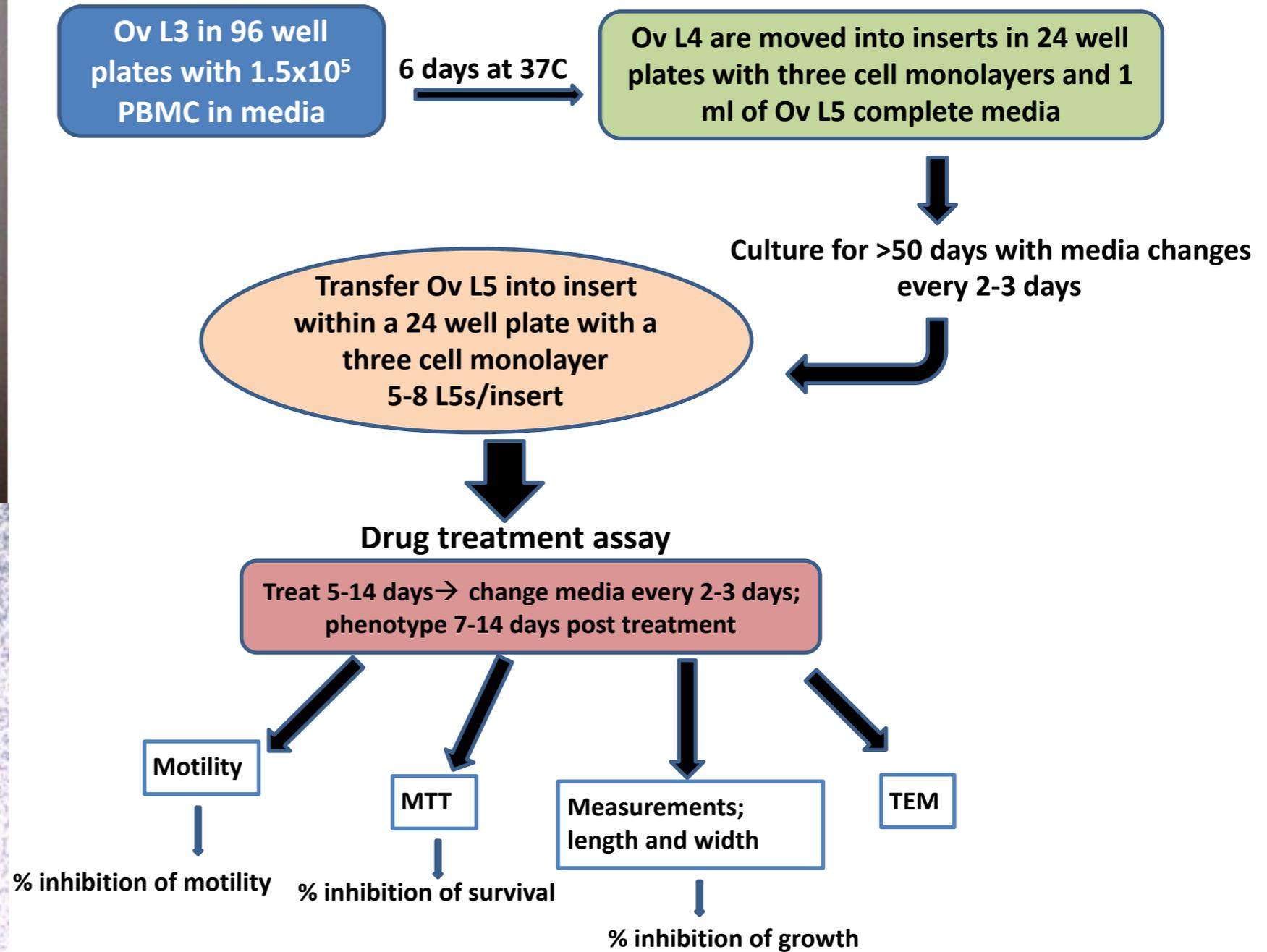


Onchocerca volvulus L3 molting assay





Drug screening on *O. volvulus* L5s











Handwritten notes on a notebook page, including a table with columns for 'Date', 'Time', 'Temp', and 'Remarks'. The text is written in blue ink.

Date	Time	Temp	Remarks
10/10/20	10:00	37.5	Normal
10/10/20	11:00	37.5	Normal
10/10/20	12:00	37.5	Normal
10/10/20	13:00	37.5	Normal
10/10/20	14:00	37.5	Normal
10/10/20	15:00	37.5	Normal
10/10/20	16:00	37.5	Normal
10/10/20	17:00	37.5	Normal
10/10/20	18:00	37.5	Normal
10/10/20	19:00	37.5	Normal
10/10/20	20:00	37.5	Normal
10/10/20	21:00	37.5	Normal
10/10/20	22:00	37.5	Normal
10/10/20	23:00	37.5	Normal
10/10/20	00:00	37.5	Normal
10/10/20	01:00	37.5	Normal
10/10/20	02:00	37.5	Normal
10/10/20	03:00	37.5	Normal
10/10/20	04:00	37.5	Normal
10/10/20	05:00	37.5	Normal
10/10/20	06:00	37.5	Normal
10/10/20	07:00	37.5	Normal
10/10/20	08:00	37.5	Normal
10/10/20	09:00	37.5	Normal



Blackflies are kept for ~7 days and dissected to remove *Onchocerca volvulus* L3s. The larvae are frozen and shipped to New York.

Business Report

The Chronicle with Bloomberg

SAN FRANCISCO CHRONICLE AND SFGATE.COM | Monday, March 24, 2014 | Section D

BIOTECH

Long wait for approval

By Stephanie M. Lee

A company that goes 24 years without ever selling a product may sound unusual. But in biotechnology, it's not that uncommon.

Take Geron Corp. in Menlo Park, which has struggled to develop a therapy — any therapy — since its founding in 1990. The company first focused on spinal cord injuries but later moved to various cancers. This month, the Food and Drug Administration halted clinical trials for its only drug.

The pharmaceutical industry has never been known for speed. All therapies must undergo years of

clinical trials to meet the FDA's standards for safety and efficacy. Faced with 10- to 15-year timelines and uncertain regulatory outcomes, companies and investors might plow hundreds of millions of dollars into therapies that will never see the light of day.

While the FDA says the process ensures drugs are effective and safe, some doctors, scientists, companies and advocates see it as a bureaucratic slog that keeps promising treatments from dying patients. A dozen California academic medical centers and hospital systems recently teamed up to tweak a key part of trials: the ethics review

board. This committee of independent experts monitors human subjects' rights during trials.

Under the new Partnership to Accelerate Clinical Trials, a single ethics board will serve multiple test sites that make up a clinical trial. Traditionally, each site has its own ethics committee: 15 sites, for example, have a total of 15 boards.

"That's a lot of delay, a lot of wasted time and energy, without much benefit," said Dr. Clay Johnston, who helped start the nonprofit as director of the Clinical and Translational Science Institute at UCSF. The partnership, which is

Drugs continues on D3

By the numbers

Thinking of getting into the biotech industry? Better have lots of money and patience.

\$800 million to \$1 billion

Total cost of developing a single drug

10 to 15 years

Time to develop one medicine, from discovery to approval

1 in 5,000 to 10,000

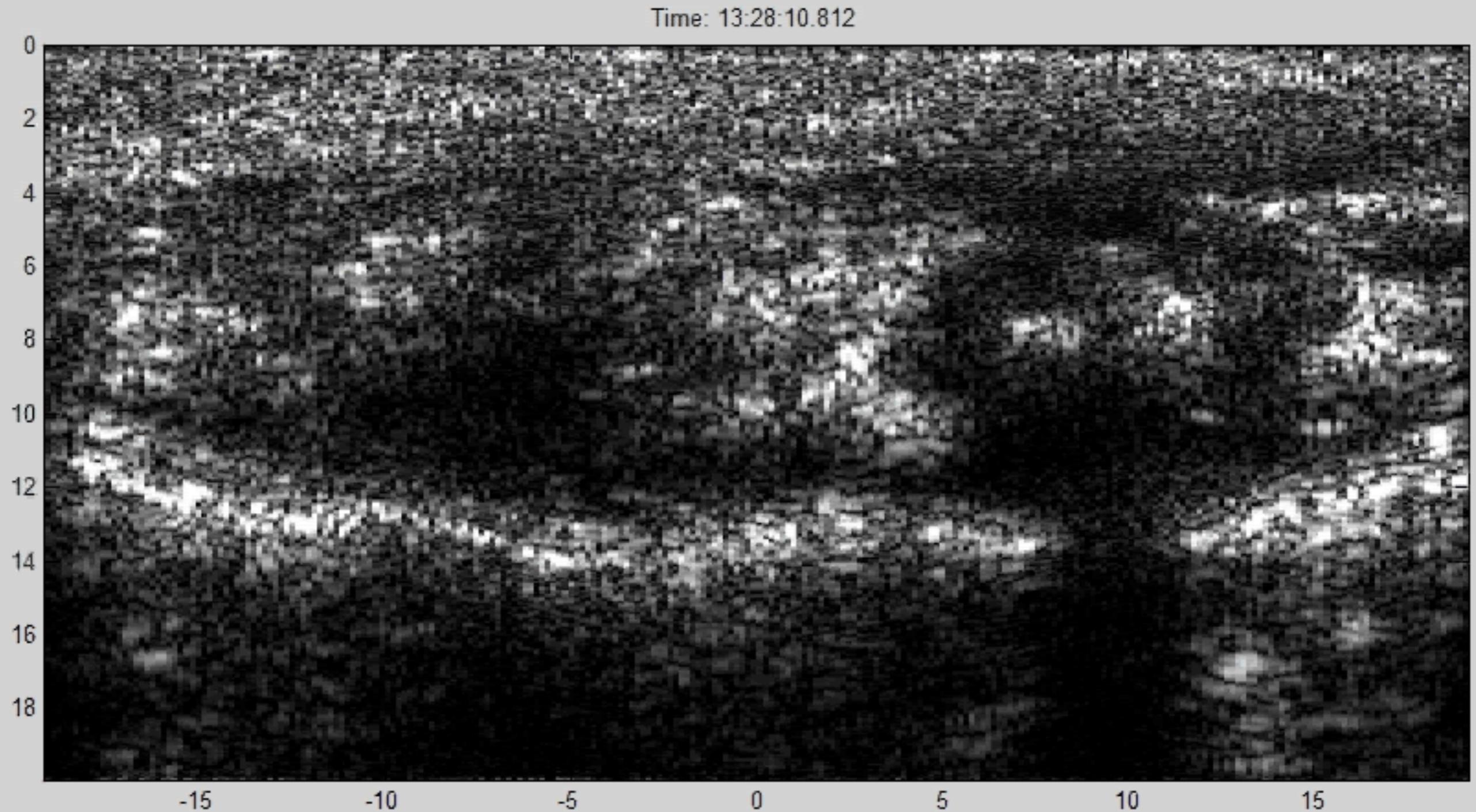
A compound's chances of receiving approval

27

Drugs approved in 2013

Sources: Pharmaceutical Research and Manufacturers of America; Food and Drug Administration

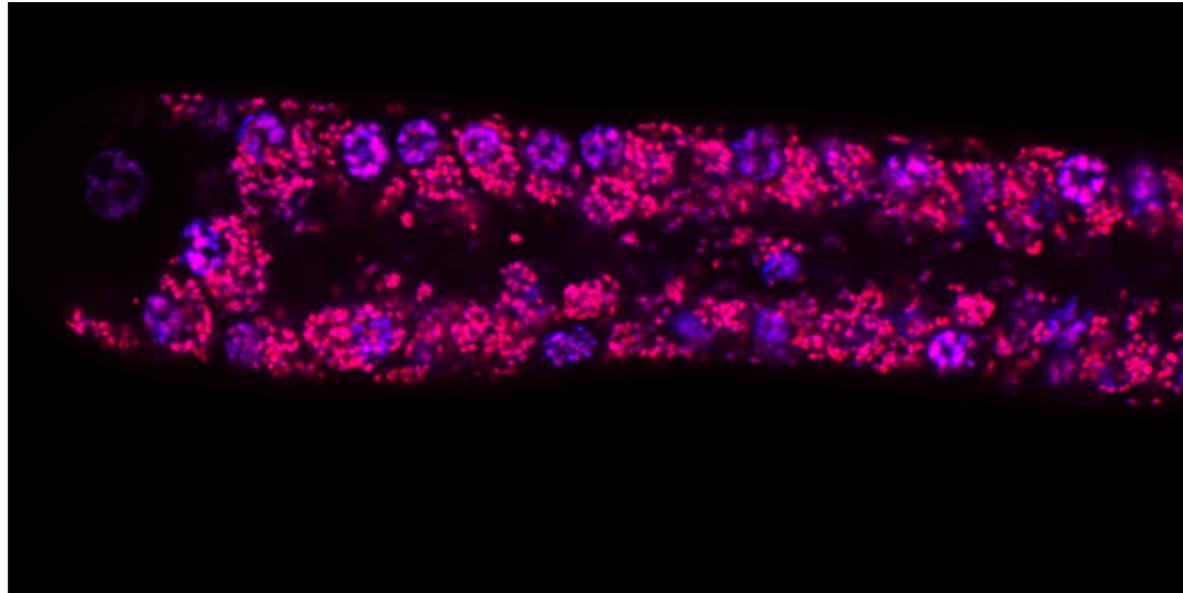
Diagnostic Technologies: Ultrasound of “dancing sign” in an infected gerbil



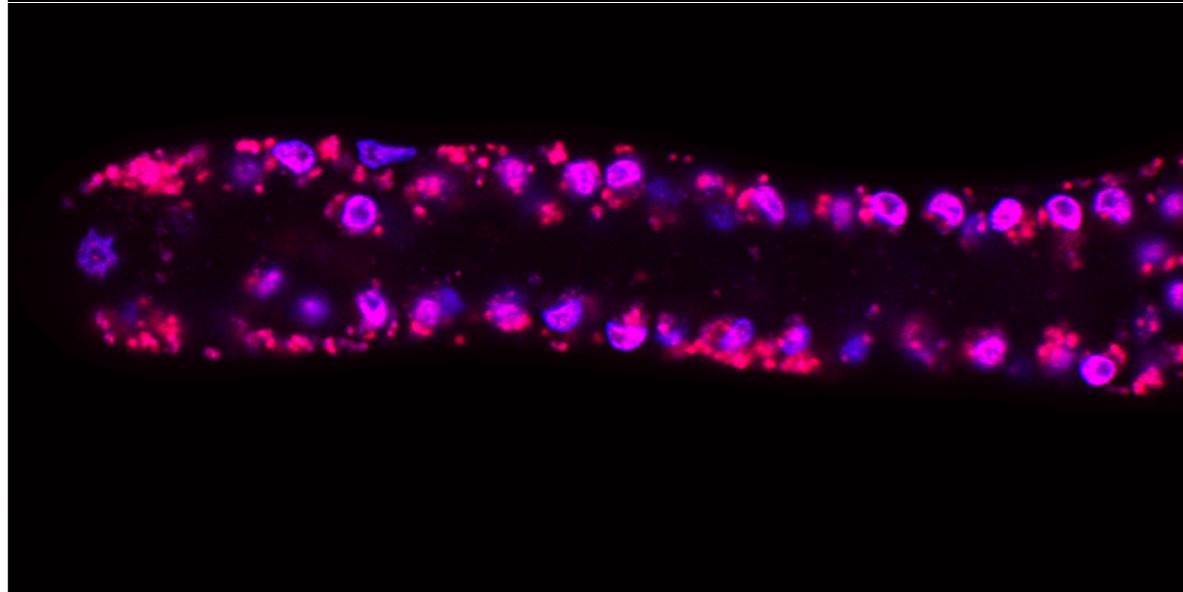
Acknowledgements

Immunofluorescent microscopy of *B. pahangi* ovaries to quantify *Wolbachia*.

Controls



Treated



Dr. Bill Sullivan
Pam White
Laura Chappell



University of California
San Francisco

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UCSF
University of California
San Francisco

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University of Buea, Cameroon
TRS Labs Inc., Athens, GA
Filariatech Inc., Athens, GA
Univ. Missouri-Columbia, MO



U.C. San Francisco Parasite Team



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