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

UC Santa Cruz Bio 117
Feb. 23, 2016

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UC San Francisco
<table>
<thead>
<tr>
<th>Parasitic Disease</th>
<th>Infected/Deceased Numbers</th>
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<tbody>
<tr>
<td>Malaria (in 2012)</td>
<td>&gt;200 million infected &gt;600,000 deaths</td>
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<tr>
<td>Schistosomiasis (2014)</td>
<td>&gt;200 million infected 200,000 deaths</td>
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<tr>
<td>Chagas’ Disease (2008)</td>
<td>8 million infected 11,000 deaths</td>
</tr>
<tr>
<td>Leishmaniasis (2014)</td>
<td>1.3 million infected 20-30,000 deaths</td>
</tr>
<tr>
<td>Sleeping Sickness (2012)</td>
<td>30,000 infected 9,000 deaths</td>
</tr>
<tr>
<td>River Blindness (2014)</td>
<td>18 million infected 270,000 blind</td>
</tr>
<tr>
<td>Lymphatic filariasis (2014)</td>
<td>40 million disfigured</td>
</tr>
<tr>
<td>Soil-transmitted helminths (2014)</td>
<td>&gt;1.5 billion infected</td>
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</tbody>
</table>
Fig. 4.12.1 Distribution of onchocerciasis, worldwide, 2014

http://www.who.int/mediacentre/factsheets/fs374/en/
Onchocerciasis (River Blindness)

Filarial nematode: *Onchocerca volvulus*

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

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

Number at risk: 120 million worldwide
Microfilariae migrate throughout the skin and are ingested by adult black flies when the flies take a blood meal.

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

**Simulium damnosum**, the blackfly vector
Symptoms of intense itchiness; conditions of loss of skin integrity and blindness.
Meet Christine Akello: A Civil War Survivor Fighting for Sight
Dec. 1, 2015
Christine Akello thought she was safe. Having survived about three decades of civil war and displacement in Uganda, she thought she had seen the worst.

Meet Peter Onuchukwu
June 12, 2015
Peter Onuchukwu is a subsistence farmer who has lived all his life in the farm community of Ibu in Okigwe local government area of Nigeria. He is only 65 years old, but ever since 2006, he has been unable to see the lush green leaves on his farm or the yields hanging from his Orange tree just a few feet from his doorsteps in Imo state, southeastern Nigeria.

Meet Christopher Olanya: Winning the War on River Blindness
May 22, 2015
Christopher Olanya, now in his 60s, has survived the brutalities of war, the trauma of displacement, and the ravages of disease in his native Uganda. He has become an unlikely symbol of hope in the mission to eliminate onchocerciasis, a parasitic infection commonly known and feared as river blindness.
Macrofilarial worms
- *Onchocerca volvulus*
- *Brugia & Wuchereria*
- *Loa loa*

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.
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.
Project Goals

To identify macrofilaricidal drugs for onchocerciasis in *Loa loa* endemic areas and for the treatment of lymphatic filariasis.
Macrofilaria Project

**In vitro potency**

- **U.C. San Francisco**
  - *Brugia* adult worms
  - *in vitro* screens

- **University of Buea, Cameroon**
  - *Onchocerca ochengi* in cattle
  - *in vitro* assays adult worms, mf
  - *Loa loa* mf *in vitro* assays

- **NY Blood Center**
  - *Onchocerca volvulus*
  - larval molting assay

**Physiochemical properties and *in vivo* studies**

- **U.C. San Francisco**
  - *in vitro* studies
  - ADME, stability, solubility, permeability, cell toxicity
  - Med Chemistry

- **Univ. Buea, Cameroon**
  - animal model with *O. ochengi*

**Preclinical Candidates**
Biomek media removal and compound dispensing
Four 24-well plates/20 mins.
(1,000 worms take ~5 hrs. ➔ 250 cmpds)
The Worminator quantifies worm movement

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

cast from the molt

Sara Lustigman, NY Blood Center
2,000 FDA-approved drugs were screened using the Worminator assay with adult *Brugia* and *Onchocerca ochengi* worms in *in vitro* assays and larval *O. volvulus* in the molting assay.
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

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
# Auranofin IC<sub>50</sub>s in vitro assays

<table>
<thead>
<tr>
<th>Species</th>
<th>Stage</th>
<th>Day</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brugia pahangi</em> females</td>
<td></td>
<td>3</td>
<td>0.430</td>
</tr>
<tr>
<td></td>
<td>males</td>
<td>3</td>
<td>0.130</td>
</tr>
<tr>
<td><em>Onchocerca ochengi</em> females</td>
<td></td>
<td>7</td>
<td>0.270</td>
</tr>
<tr>
<td></td>
<td>males</td>
<td>5</td>
<td>0.380</td>
</tr>
<tr>
<td><em>O. volvulus</em> L3</td>
<td></td>
<td>6</td>
<td>0.340</td>
</tr>
<tr>
<td><em>O. ochengi</em> mf</td>
<td></td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td><em>Loa loa</em> mf</td>
<td></td>
<td>5</td>
<td>12.8</td>
</tr>
</tbody>
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TEM of female worms from *in vitro* worm assay Day 1

1% DMSO
8% inhibition of motility

1 uM Auranofin
96% inhibition of motility

Bulman et al., PLoS NTD 2015
Auranofin as an antiparasitic agent

Protozoa
- Entamoeba histolytica
- Giardia intestinalis
- Cryptosporidium
- Plasmodium falciparum
- Leishmania infantum, L. major
- Trypanosoma brucei

Helminths
- Echinococcus granulosus
- Taenia crassiceps
- Schistosoma mansoni
- Haemonchus contortus
- Brugia, Onchocerca

Thioredoxin reductase systems are important in preventing oxidative damage due to oxygen metabolism; the parasite’s enzyme is a good target for an antiparasitic drug.
Low-hanging fruit: Auranofin

- FDA-approved

- Auranofin is a gold salt used in treating rheumatoid arthritis for >25 years.

- Single oral dose @ 3mg/kg/day for 7 days decreased liver damage in hamsters infected with *Entamoeba histolytica* (Debnath et al. Nature Med 2012).

- Human plasma elimination half-life with auranofin is 17-25 days (Blodgett et al. 1984).

Summary

• Worminator is used for *in vitro* screening of macroparasites and microparasites.
• Auranofin identified from FDA library as a lead candidate.
• Filarial thioredoxin reductase may be target of auranofin.
• Biochemical and structural studies of the TxR target.