Discovery program for identification macrofilaricide agents for treatment of Onchocerciasis

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SCOPE OF THIS PROJECT

Current chemotherapy for the two major filarial diseases, onchocerciasis and lymphatic filariasis, targets the microfilarial stage of the parasites and temporarily sterilize adult nematodes, therefore lengthy treatment regimens are required to cover the reproductive life-span of the long-lived adult worms. Program success is constrained by the absence of drugs with macrofilaricidal activity and the concern of developing drug resistance in human parasites.

The main goal of this project is to identify both repurposed and novel molecules as potential new treatments for filarial infections, with a focus on macrofilaricides for onchocerciasis. However, drug discovery for this disease has to rely on surrogate parasites because the only viable host for *O. volvulus* are humans. DNDi has prioritized screening of selected compound libraries with a greater probability of yielding drug candidates. These include:

1. Indication sets (compounds which have progressed to preclinical or clinical research but failed to reach the market)
2. Chemical series from veterinary anti-infective research programs
3. Well annotated sets of compounds (i.e. bioavailable sets, compounds which have been through lead optimization, chemical series from anti-infective research programs etc...)

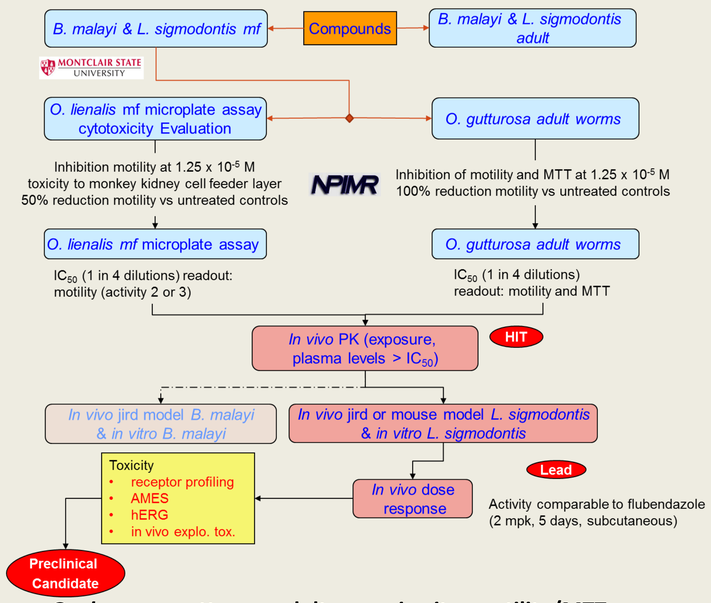
SCREENING CASCADE

The primary *in vitro* screens target

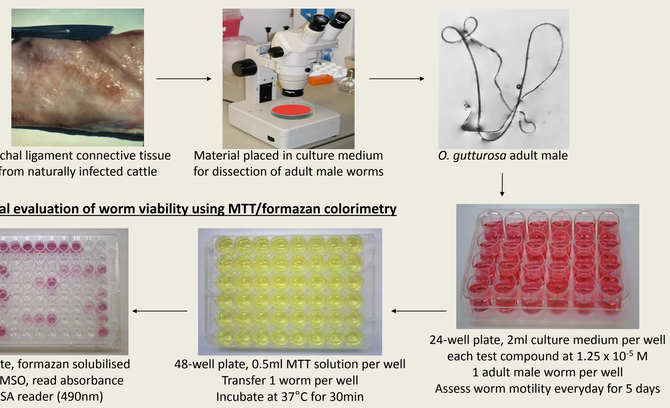
1. *Brugia malayi and Litomosoides sigmodontis microfilaria* (5-day motility assay)
2. *Brugia malayi and Litomosoides sigmodontis adult* (5-day motility assay)
3. *Onchocerca lienalis* microfilariae, mf (5-day motility assay)
4. *Onchocerca gutturosa* adult worms (5-day motility /MTT assay)

Compounds with *in vitro* activity in the micromolar (µM) range against adult parasites are considered hits.

In vivo proof of principle is established in gerbils or mice infected with *Litomosoides sigmodontis* and is conducted on hits with good oral PK in the relevant host species. The dosing regimen is adjusted to reach plasma concentrations above EC_{50} for 24 hours. This model is regarded as a reasonable predictor of clinical efficacy.



Onchocerca gutturosa adult worm *in vitro* motility/MTT assay



Onchocerca gutturosa adult male worms are obtained by dissection from the nuchal ligament connective tissues of naturally infected cattle.

Compounds in DMSO stock are diluted in culture medium in 24-well plates. Worms, which are maintained in culture, are then added to each well. Worm's viability is assessed using 2 parameters:

- Motility - measured by microscopy, every 24 h until 120 h.
  - **Score 3**: good, 100% motility reduction
  - **Score 2**: moderate, 50-99% motility or MTT reduction
  - **Score 1**: inactive, < 50% motility reduction
- Biochemical viability - evaluated by MTT/formazan colorimetry.

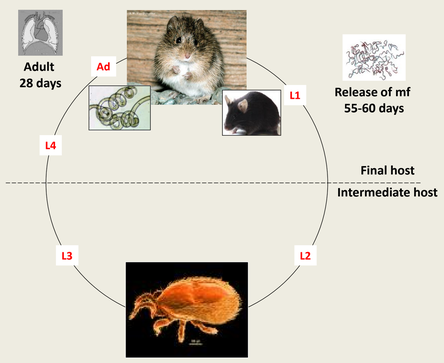
The MTT assay is conducted after 120 h in a 48-well plate. Formazan formation is then measured at 490 nm using a multi-well scanning spectrophotometer. Inhibition of formazan formation is correlated with worm damage or death.

SUMMARY

- Screening of Celgene compounds in different phenotypic *in vitro* assays, in particular against *Onchocerca* adult worms identified multiple hits:
  - 63 'score 3' hit identified against *O. gutturosa* worms
  - 43 compounds are selective against adult *O. gutturosa* worms
  - 54 compounds have an *in vitro* EC_{50} < 1µM
  - 23 compounds have an *in vitro* EC_{50} < 300nM
  - 18 compounds have activity against *O. lienalis* microfilariae
    - EC_{50} range from 0.4 to 5.0 µM for the actives
- Two distinct chemical series have been identified: both having specific *in vitro* activity against the adult parasites
- These novel anti-filarial scaffolds have desirable physical chemical properties (MW < 400, lipophilicity (logP) = 2-4, and polar surface area (PSA) = 80-110) and are currently being advanced into lead optimization studies
- The screening cascade elaborated for this project captures both *in vitro* and *in vivo* activity of molecules using two surrogate species: *O. gutturosa in vitro* and *L. sigmodontis in vivo*
- This cascade may be useful in hit to lead and lead optimization programs as well
- The lack of activity of these series of compound against *O. lienalis* mf indicates that paradigms utilizing low throughput adult worm assays as primary screens can successfully identify novel macrofilaricide chemotypes.

Litomosoides sigmodontis in vivo filariasis model

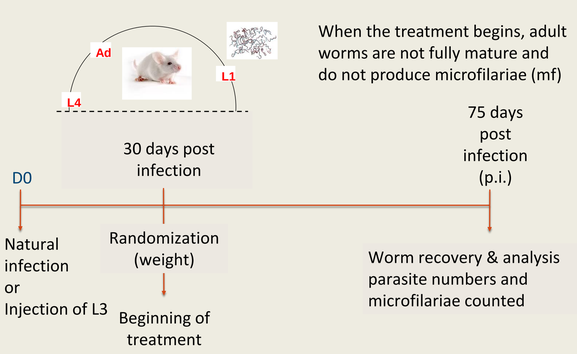
L. sigmodontis Life Cycle



- *Litomosoides sigmodontis* is transmitted by a small mite, *Onithonyssus bacoti*
- The parasitized mite transmits third larval stages during a blood-meal
- The larvae then migrate from the skin, through the lymphatics, to the thoracic (pleural) cavity where they mature and breed.
- The adult female worms release their microfilariae in the pleural cavity from where they make their way to the blood, ready to be taken up by a mite feeding on the skin

Litomosides sigmodontis is maintained in either its natural host, the cotton rat *Sigmodon hispidus*, or in jirds, *Meriones unguiculatus*, as surrogate hosts.

Efficacy Model



1. BALB/c are exposed to infected mites (*Ornithonyssus bacoti*), for 24 h
2. After inoculation of infective larvae (L3), migration occurs via the lymphatic system to the pleural cavity by day 2-6 post-infection (p.i.)
3. Between days 8-12 p.i, larvae molt into L4 stage, then mature to adults by day 30 p.i.
4. At day 30 p.i. animals are randomized, allocated in groups of 6 animals and treated.
5. At days 60-90 p.i., microfilariae are produced and released in the blood

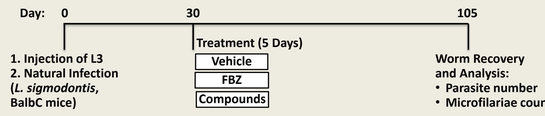
INITIAL SCREENING RESULTS

A subset of approximatively 160 compounds from the Celgene compound collection were tested against *B. malayi* and *L. sigmodontis* microfilariae and adults.

Based on the preliminary *in vitro* activity in *B. malayi* and *L. sigmodontis*, this set was screened *in vitro* against *O. lienalis* microfilariae resulting in 32 hits, 'score 3'. The entire set was subsequently tested against adult *O. gutturosa* parasites, resulting in 62 hits, 'score 3'. In this set of compounds, 43 hit compounds had specific activity against adult parasites. This suggests that these two recently identified distinct chemical series demonstrate specific macrofilaricide activity.

Two compounds have demonstrated statistically relevant *in vivo* activity against *Litomosoides sigmodontis* in mice.

In vivo Results (*Litomosoides sigmodontis*)



***In vitro* (*Onchocerca species*)**

158 compounds

↓ *O. lienalis* microfilariae

32 "score 3" hits
Single digit micromolar and sub-micromolar activity

↓ *Re-test 158 compounds against O. gutturosa* adults

61"score 3" hits
Single digit micromolar and sub-micromolar activity

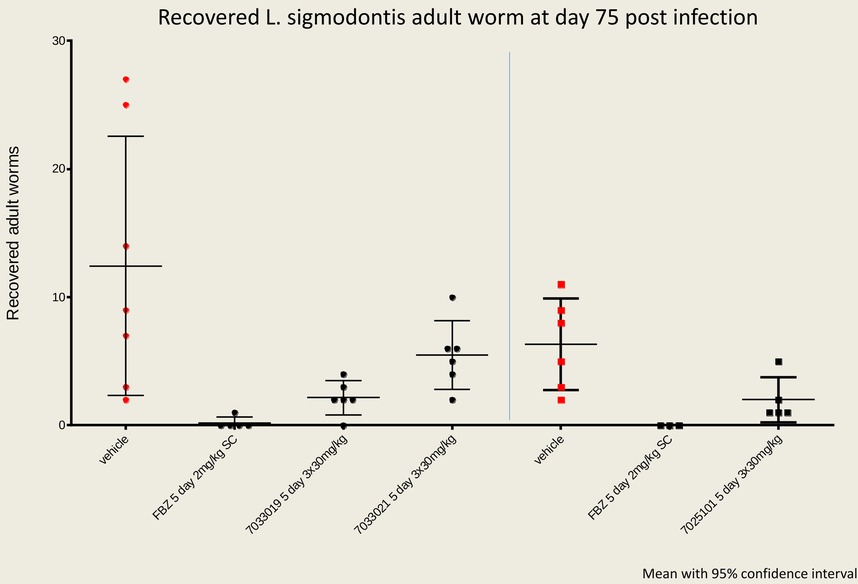
↓

43 compounds specific for adults only

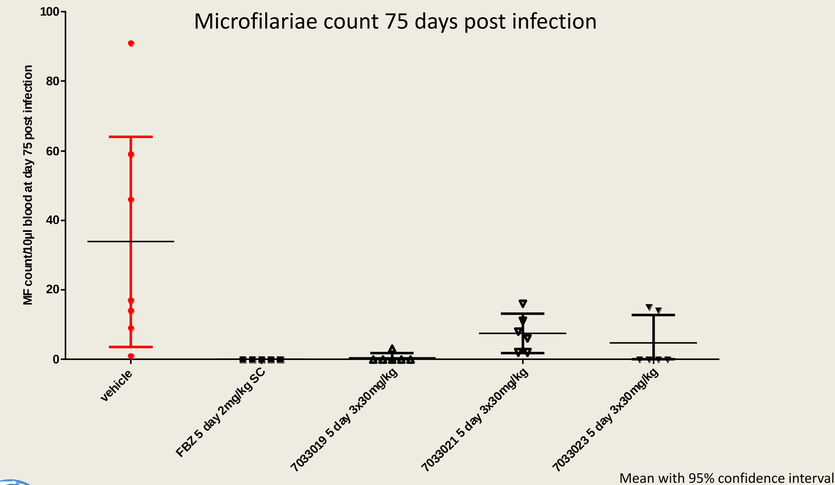
Compound	Dose	Average Adult worms	Reduction Adult worms	Reduction Microfilaria
Vehicle		11 (26)	-	NA
Flubendazole	2 mg/kg 5 days	0	100 % (P=0.007)	NA
7025101	3x30 mg/kg 5 days	2 (1.7)	68 % (p=0.032)	NA
Vehicle		11 (26)	-	-
Flubendazole	2 mg/kg 5 days	0.2 (0.5)	98 % (P=0.019)	100 % (P=0.034)
7033019	3x30 mg/kg 5 days	2.17 (1.3)	80 % (p = 0.044)	98 % (p = 0.042)
7033021	3x30 mg/kg 5 days	5.5 (2.7)	56 % (p = 0.125)	78 % (p=0.078)
7033023	3x30 mg/kg 5 days	10.2 (9.4)	18 % (p = 0.685)	86% (p=0.058)

Values expressed as mean (SD)

Efficacy Results in a Murine *L. sigmodontis* model



Mean with 95% confidence interval



Mean with 95% confidence interval