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Screening cascade for discovery programs aiming at the identification of new 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 sterilises adult nematodes, therefore lengthy treatment regimens are required to cover the reproductive life-span of the long-lived adult worms. Programme success is constrained by the absence of drugs with macrofilaricidal activity and concerns of developing drug resistance in human parasites.

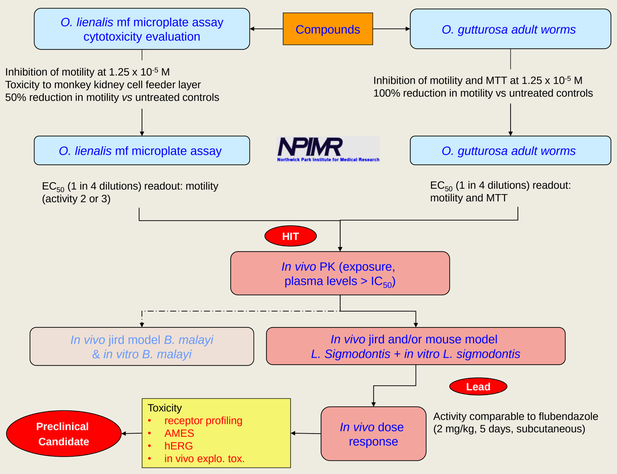
The main goal of this project is to identify both repurposed and novel molecules as potential new treatments for filarial infections, with a focus on macrofilaricides for onchocerciasis. However, drug discovery for this disease has to rely on surrogate parasites because the only viable host for *O. volvulus* are humans. DNDi has prioritised 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...)

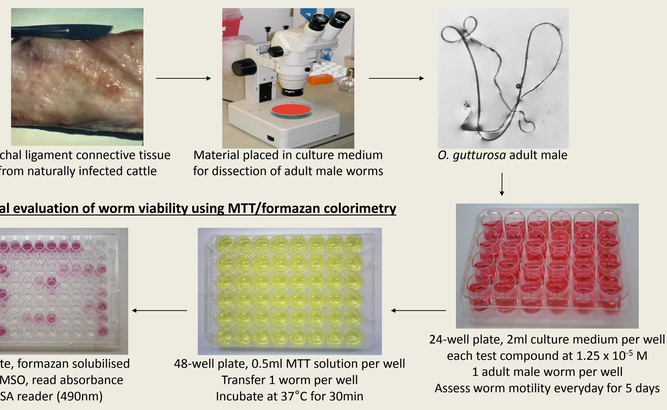
SCREENING CASCADE

The primary *in vitro* screens target *Onchocerca* species: *O. gutturosa* adult worms (5-day motility /MTT assay) and *O. lienalis* microfilariae, mf (5-day motility assay). Compounds with *in vitro* activity in the micromolar (µM) range 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.

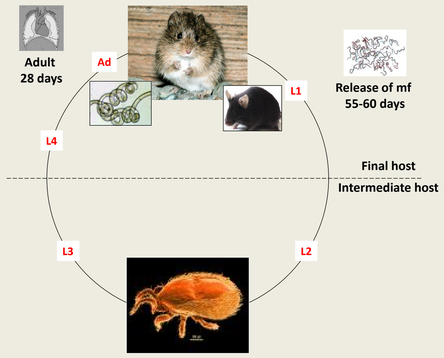
The MTT assay is conducted after 120 h in a 48-well plate. Formazan formation is then measured at 490 nm using a multi-well scanning spectrophotometer. Inhibition of formazan formation is correlated with worm damage or death.

SUMMARY

- Several primary hits identified from screening a well-annotated compound collection against *Onchocerca* adult worms have demonstrated *in vivo* activity in a *L. sigmodontis* model.
- The combination of structure activity relationships and *in vivo* proof of principle identified three distinct chemical series of interest.
- These three series were identified from programs that targeted the human histamine H3, 5-hydroxytryptamine-6 (5-HT_6) or sphingosine-1-phosphate-1 ($S1P_1$) receptors.
- These novel anti-filarial scaffolds are currently being advanced in hit-to-lead 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 compound series against 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

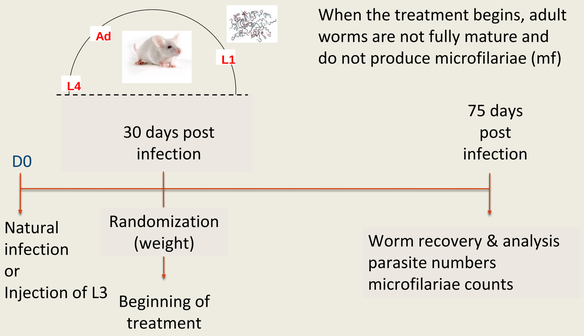
L. sigmodontis Life Cycle



- *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 Days 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 blood

SCREENING OF THE ABBVIE BIOAVAILABILITY SET

A prioritized subset of the AbbVie compound collection was assembled based on favorable oral bioavailability and/or low clearance in previous animal pharmacokinetic studies (AbbVie Bioavailability Set).

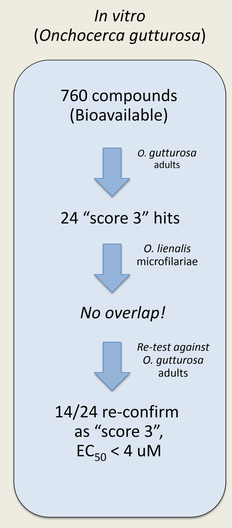
The Bioavailability Set was clustered based on structure to 760 representative compounds, which were screening against adult *O. gutturosa* adult worms.

Cross screening of the adult screening hits against *O. lienalis* microfilariae displayed no overlap of *in vitro* activity, suggesting that the adult worm screen had identified primarily specific macrofilaricides.

Screening of structural analogs around the original hits, facilitated by cheminformatic analysis, led to a dramatically improved second-round hit rate and, in some cases, identification of compounds with improved *in vitro* potency.

Three compounds demonstrated *in vivo* activity against *Litomosoides sigmodontis* in mice.

Initial Screening Results



In vivo (*Litomosoides sigmodontis*)

Day: 0 — 30 — 105

Natural Infection (*L. sigmodontis*, BalbC mice)

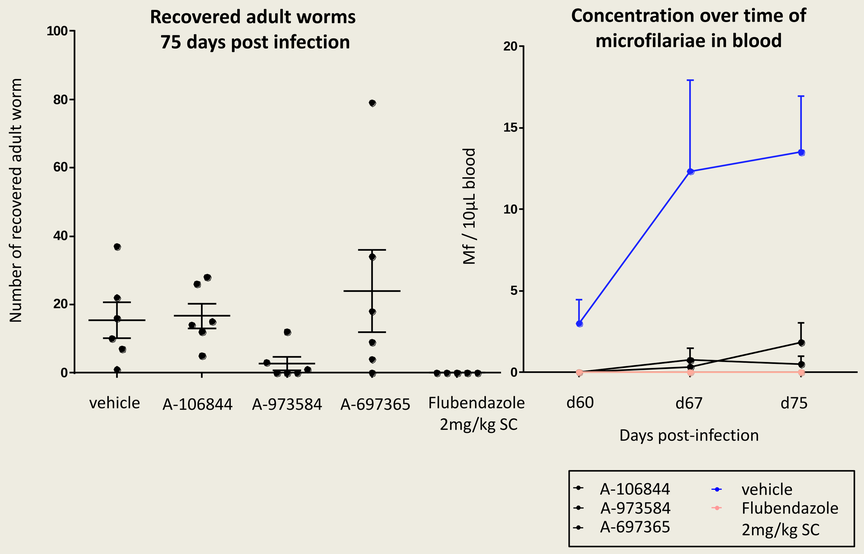
Treatment (5 Days): Vehicle, FBZ, Compounds

Worm Recovery and Analysis:
- Parasite number
- Microfilariae count

Compound	Dose	Adult Females	Adult Males	Microfilaria
Vehicle		11 (9)	5.8 (3.5)	15 (8)
Flubendazole	6 mg/kg QD x 5 days	0	0	0
A-697365	100 mg/kg QD x 5 days	16 (14)	12 (14)	0
A-973584	100 mg/kg QD x 5 days	2.0 (2.3)	1.2 (2.4)	0
A-1066844	40 mg/kg BID x 5 days	14 (5)	4.6 (2.4)	2.2 (2.9)

Values expressed as mean (SD)

Efficacy Results in a Murine *L. sigmodontis* model



Results from Analog Screening

Compound	Human Target	Number of Analogs Tested	Score 3 Hits	Score 2 Hits	Improved Potency?
A-697365 EC_{50} = 1.8 µM	H3	85	18 (21%)	29 (34%)	A-641227 EC_{50} = 1.1 µM
A-973584 EC_{50} = 2.5 µM	$S1P_1$	147	30 (20%)	42 (28%)	A-1095700 EC_{50} = 0.24 µM
A-1066844 EC_{50} = 2.6 µM	5-HT_6	49	20 (41%)	8 (16%)	A-1083751 EC_{50} = 0.77 µM