Discovery program for identification of 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 macrofilarial 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 \textit{O. volvulus} is 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. \textit{Brugia malayi} and \textit{Litomosoides sigmodontis} microfilaria (5-day motility assay)
2. \textit{Brugia malayi} and \textit{Litomosoides sigmodontis} adult (5-day motility assay)
3. \textit{Onchocerca lienalis} microfilariae, f5 mf (5-day motility assay)
4. \textit{Onchocerca gutturosa} adult worms (5-day motility/MTT assay)

Compounds with in vitro activity in the micromolar (\textmu{}M) range against adult parasites are considered hits. In vivo proof of principle is established in gerbils or mice infected with \textit{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 \textit{EC}_50 for 24 hours. This model is regarded as a reasonable predictor of clinical efficacy.

SCREENING CASCADE

\textit{Onchocerca gutturosa} adult worm in vitro motility/MTT assay

\textit{Onchocerca gutturosa} adult male worms are obtained by dissection from the nuchal ligament connective tissues of naturally infected cattle. Compounds in DMEM stock are diluted in culture medium in 24-well plates. Worms, which are maintained in culture, are then added to each well. Worm 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.

\textit{Litomosoides sigmodontis} in vivo filariasis model

\textit{L. sigmodontis} Life Cycle

- \textit{Litomosoides sigmodontis} is transmitted by a small mite, \textit{Omiomyxodes bocoti}
- The parasitized mite transmits third larval stages during a blood-meal
- The larvae then migrate from the skin, through the lymphatics, to the thoracic (pulmonary) 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 bite feeding on the skin.

\textit{Litomosoides sigmodontis} is maintained in either its natural host, the cotton rat \textit{Sigmodon hispidus}, or in jirds, \textit{Mirynes unguiculatus}, as surrogate hosts.

Efficacy Model

When the treatment begins, adult worms are not fully mature and do not produce microfilariae (mf)

1. BALB/c are exposed to infected mites (\textit{Omiomyxodes bocoti}), 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.

\textbf{INITIAL SCREENING RESULTS}

A subset of approximately 160 compounds from the Celgene compound collection were tested against \textit{B. malayi} and \textit{L. sigmodontis} microfilariae and adults.

Based on the preliminary in vitro activity in \textit{B. malayi} and \textit{L. sigmodontis}, this set was screened in vitro against \textit{L. lienalis} microfilariae resulting in 32 hits, ‘score 3’. The entire set was subsequently tested against adult \textit{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 macrofilaricidal activity.

Two compounds have demonstrated statistically relevant in vivo activity against \textit{Litomosoides sigmodontis} in mice.

\textbf{In vivo Results (\textit{Litomosoides sigmodontis})}

\textbf{Efficacy Results in a Murine \textit{L. sigmodontis} model}

Recovered \textit{L. sigmodontis} adult worm at day 75 post infection

Microfilariae count 75 days post infection

Mean with 95% confidence interval