Current efforts to control and eliminate onchocerciasis are hindered by the lack of compounds that target the adult worm stage. In a joint collaboration with DNDi, academia and Celgene, a pipeline was established to identify macrofilaricidal compounds. To date, more than 400 compounds have been screened in vitro and Onchocerca gutturosa adults, identifying 177 compounds with EC$_{90}$ ≤1µM, 83 of which having EC$_{90}$ <100µM. From this set of 400 compounds, a select set of 160 compounds were tested against both Onchocerca lienalis microfilariae and Onchocerca gutturosa adults, identifying 43 compounds with specific activity against the adult parasites in vitro. Active compounds with EC$_{90}$ in the 0.015-1µM range and suitable pharmacological profiles were prioritized for in vivo testing. 23 lead candidates were tested by oral gavage in mice that harbored adult worms of the rodent filarial nematodes Litomosoides sigmodontis. Compounds significantly reduced the L. sigmodontis adult worm burden by >90% after 10 days of BID and/or TID treatment. Presence of microfilariae in the treated animals suggest that the compounds do not have a strong microfilaricidal effect. Current efforts to further assess the impact of the compounds on microfilariae in the L. sigmodontis jird model are on-going.

The current study demonstrates the successful establishment of a screening cascade which resulted in the identification of promising novel macrofilaricidal compounds. The identification of such macrofilaricidal compounds which lack microfilaricidal effects are ideal candidates for the treatment of onchocerciasis, as they have a reduced risk for microfilariae-driven adverse events.

**Screening and Compound Progression**

The primary in vitro screen 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 / MIT 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$_{90}$ for 24 hours. This model is regarded as a reasonable predictor of clinical efficacy.

Initially, compounds from multiple chemical series were progressed into the in vitro adult O. gutturosa and O. lienalis microfilariae motility assay. In this set of compounds, 43 hit compounds had specific activity against adult parasites. Compounds which showed activity against L. sigmodontis and B. malayi trended towards activity against O. gutturosa, 86% had agreement amongst the parasites allowing for rapid triaging into the O. gutturosa assay. Multiple compounds from both series have demonstrated statistically relevant in vivo activity against Litomosoides sigmodontis in mice (Figure 2).

**Physical property analysis of O. gutturosa active (color by series and sized by MW)**

Cross species activity and stage selectivity were seen across the various parasitic nematodes within both chemical series. Although over 400 compounds have been tested against O. gutturosa and both B. malayi and L. sigmodontis adults and microfilaria, only 195 compounds have been tested concurrently against all three species and stages to date. From this set of 195 compounds, both series have specific compounds with activity favoring the adult stage of O. gutturosa, L. sigmodontis and B. malayi (Figure 4). Analogously, specific compounds also have overlap in both B. malayi and L. sigmodontis microfilaria in each series. Additionally, 13 compounds (series A) and 17 compounds (series B) have been identified as having macrofilaricidal selectivity in B. malayi.

**In vivo Efficacy Results (Litomosoides sigmodontis)**

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

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