The opportunity

Opportunistic fungal infections are associated with increasing rates of mortality and morbidity, especially amongst immunocompromised patients. Existing treatment options are limited and fungal infections are now recognized for killing as many people worldwide as tuberculosis and malaria.

Current anti-fungal agents are hampered by their narrow spectrum of activity, poor bioavailability, toxicity, interactions with other drugs, or by having fungistatic rather than fungicidal activity. Current treatments are ineffective against some fungal infections, such as Aspergillosis, and emerging multiple drug resistance, particularly to azole class drugs, is now a serious issue in the treatment of fungal infections.

We have used two innovative strategies to address the urgent need to develop new anti-fungal agents:

- A novel pilot screen to identify compounds with either drug sensitising or anti-fungal activity has yielded a number of confirmed hits in both classes
- Investigation of the mode of action of a potentially new class of anti-fungal agents, identified from an in-house drug discovery program

The technology

Fungal resistance to azole drugs, such as fluconazole, is often caused by increased expression of plasma membrane efflux pumps. Pump inhibition would enable drug resistant fungal infections to be effectively treated with existing azole class agents. Our team has developed a screening platform to identify new candidate compounds that block efflux pumps in fungi (Figure 1). Efflux of a fluorescent azole surrogate is used to quantify pump activity. The assay has been multiplexed such that viability is simultaneously assessed using a luminescent readout (Figure 2).

A pilot screen of 10,000 compounds, that included known drugs and diverse lead-like compounds, was conducted using yeast cells expressing a key fungal efflux pump. The screen identified novel pump inhibitors and anti-fungals, as well as agents with known anti-fungal activity.
The second arm of the anti-fungal program involves optimising novel drug candidates with demonstrated anti-fungal activity. The compounds inhibit the growth of human and animal pathogenic fungi and are most potent against filamentous fungi, including *Aspergillus* and *Trichophyton*. *Trichophyton* causes fungal nail infections, which are estimated to affect 2-14% of the Western adult population. Investigation into the mechanism of action and molecular targets of these compounds is ongoing.

**Applications**

Our dual strategies will address key limitations of current treatment options.

- Identification and development of compounds capable of overcoming resistance to azole class drugs will result in a cost-effective means of treating drug-resistant fungal infections.
- Development of new compounds will add to the very limited existing anti-fungal armamentarium and facilitate early and rapid treatment of fungal infections.

The multiplexed efflux pump inhibitor and viability high-throughput screening assay is a flexible discovery tool that can easily be adapted to screen for inhibitors of other ABC transporters.

**Opportunity for partnership**

The Walter and Eliza Hall Institute and the University of Otago are seeking a partner to: (i) co-invest in the development of compounds with anti-fungal activity identified in the pilot screen; and/or (ii) identify further promising compounds via a more substantive high-throughput screen of the Walter and Eliza Hall Institute’s lead-like compound libraries.

The Walter and Eliza Hall Institute has a proven track record in medicinal chemistry programs focused on hit-to-lead and lead optimisation. The ultimate goal of the partnership would be to develop a novel anti-fungal drug and/or an efflux pump inhibitor for use in combination with existing therapies that has the appropriate potency, safety and pharmacokinetic profile to progress to the clinic.

**Intellectual property**

The intellectual property regarding the fluorescence-based assays to quantify efflux pump function is protected (WO/2003/018817, August 2002).

Compound structures have not been publicly disclosed. An opportunity exists to generate novel composition of matter intellectual property.

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**Figure legend**

**Figure 1:** Pump inhibitor assay design. **A)** Efflux pumps from a pathogenic fungus are over-expressed in a *Saccharomyces cerevisiae* host strain depleted of endogenous transporter proteins. **B)** Pump activity is monitored following addition of the fluorescent pump substrate R6G and library compound. **C)** Pump inhibition results in intracellular retention of R6G.

**Figure 2:** Results of the pilot screen for novel anti-fungal compounds and efflux pump inhibitors in yeast. The multiplexed approach provides clear readouts for compounds affecting either pump activity or cell viability.