



**Drug repurposing:
Fast-tracking Antifungal Drug
Discovery for Cryptococcal Meningitis**

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for the degree of Doctor of Philosophy

Certificate of Original Authorship

I, Megan Truong, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Megan Truong, July 15th 2019

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Abbreviations

Abbreviation	Chemicals and Media
DMSO	Dimethyl sulfoxide
MOPS	4-Morpholinepropanesulfonic acid
RPMI-1640	Roswell Park Memorial Institute media
SDA	Sabouraud dextrose agar
YNB	Yeast nitrogen base
YPD	Yeast peptone dextrose

Abbreviation	Antifungal susceptibility
5-FC	5-flucytosine
AMB	Amphotericin B
FLB	Flubendazole
FLC	Fluconazole
MEB	Mebendazole
MIC	Minimum inhibitory concentration
MFC	Minimum fungicidal concentration

Abbreviation	Units
cells/mL	Cells per millilitre
°C	Degrees Celsius
h or hr	Hours
µg/mL	Micrograms per millilitre
mg/mL	Milligrams per millilitre
µm	Micrometre
µM	Micromolar
min	Minutes
OD	Optical density
pH	Potential hydrogen
rpm	Revolutions per minute
sec	Seconds

Abbreviation	Others
WT	Wild-type
FDR	False discovery rate
GFP	Green fluorescent protein
logFC	log fold-change
ROI	Regions of interest

Table of Contents

.....	i
Certificate of Original Authorship.....	ii
Acknowledgements	iii
Abbreviations	vi
Table of Contents.....	vii
Table of Figures	x
Table of Tables.....	xii
Abstract	xiv
Chapter 1: Introduction.....	1
1.1 <i>Cryptococcus</i> and cryptococcal meningitis – a silent killer and neglected disease	2
1.2 <i>Cryptococcus neoformans</i> and <i>Cryptococcus deuterogattii</i> – the major causative agents	4
1.3 Treatment of cryptococcal meningitis – the need for new therapeutic alternatives.....	6
1.4 Antifungal drug resistance	8
1.5 Economic and regulatory challenges in drug development	10
1.6 Repurposing: a powerful approach for drug development	13
1.7 Drug repurposing for cryptococcal meningitis.....	14
1.8 Systems biology as a way of understanding how drugs perturb fungal cells	17
1.9 The scope of this research	18
Chapter 2: Repurposing drugs to fast-track therapeutic agents for the treatment of cryptococcosis	19
2.1 Declaration.....	20
2.2 Abstract.....	21
2.3 Introduction	22
2.4 Methods	24
2.4.1 Strains	24
2.4.2 Enzo drug library and drug stocks.....	24
2.4.3 Primary drug screening	24
2.4.4 Broth microdilution test.....	25
2.4.5 Checkerboard microdilution assay.....	25

2.4.6	Time-kill assay	26
2.5	Results.....	27
2.5.1	Primary drug screening identifies non-antifungal compounds with anti-cryptococcal activity.....	27
2.5.2	<i>In vitro</i> spectrum of antifungal activity of calcium channel blockers and flubendazole.....	28
2.6	Discussion.....	34
2.6.1	Repurposing calcium channel blockers as antifungal agents	34
2.6.2	Repurposing flubendazole as an anti-cryptococcal agent	36
2.7	Conclusion and Perspectives.....	38
Chapter 3: Profiling the transcriptional response of <i>C. neoformans</i> to flubendazole using RNA-Seq		39
3.1	Introduction	40
3.2	Methods.....	43
3.2.1	Strains, culture and drugs	43
3.2.2	Time-kill curve assay and experimental design	43
3.2.3	RNA extraction and sequencing.....	43
3.2.4	RNA-Seq data processing.....	44
3.2.5	Analysis of Differential Gene Expression	45
3.2.6	Gene Ontology Enrichment analysis.....	46
3.3	Results.....	47
3.3.1	Establishing conditions to sample flubendazole-treated <i>Cryptococcus neoformans</i> ...	47
3.3.2	Data analysis rationale to identify significantly differentially expressed genes	48
3.3.3	Analysis of RNA-Seq data	49
3.4	Discussion.....	73
3.4.1	Flubendazole alters expression of tubulin genes: implications for microtubule-related biological processes	73
3.4.2	Genes involved in DNA replication and metabolism are affected in the presence of flubendazole	76
3.4.3	Flubendazole induces oxidative stress-responsive genes.....	78
3.4.4	Dynamic analysis of the transcriptomic response to flubendazole: limitations and challenges	79
3.5	Conclusions	79

Chapter 4: Further investigation into the flubendazole mode of action: the physiological response of <i>Cryptococcus</i> cells	81
4.1 Introduction	82
4.2 Methods	86
4.2.1 Strains, culture and drugs	86
4.2.2 Spot plate assays	87
4.2.3 Microscopy imaging and analysis.....	87
4.3 Results	90
4.3.1 Qualitative and quantitative analysis of the morphological effects of flubendazole on <i>Cryptococcus</i>	90
4.3.2 Differential expression of transcription factors and kinases	95
4.3.3 <i>C. neoformans</i> <i>YOX101Δ</i> exhibit decreased susceptibility in response to flubendazole.....	97
4.3.4 Further investigation into the role of transcription factors in flubendazole sensitivity.....	99
4.3.5 Flubendazole induces similar phenotypic changes in <i>vox101Δ</i> and wild-type strains	103
4.4 Discussion.....	107
4.4.1 Flubendazole induces abnormal cell morphology and disturbed microtubules in <i>C. neoformans</i>	107
4.4.2 The potential role of cell-cycle regulator <i>YOX101</i> in response to flubendazole	108
4.4.3 Future directions.....	110
Chapter 5: Final discussion	111
5.1 Flubendazole – challenges and opportunities for use as a systemic antifungal therapy ...	113
5.2 Medicinal chemistry to optimise and expand the spectrum of antifungal activity of flubendazole	114
5.3 Systems biology analysis enables a holistic view of the drug response	115
5.4 Conclusions	116
Chapter 6: Appendix	117
6.1 Chapter 3 Supplementary Material	118
6.2 Chapter 4 Supplementary Material	133
Chapter 7: References	141

Table of Figures

Figure 1.1. Global burden of cryptococcal meningitis.....	3
Figure 1.2. Mode of infection by <i>Cryptococcus</i>	4
Figure 1.3. Antifungal drug classes and their respective targets. Polyenes target cell membrane component ergosterol.	6
Figure 1.4. Mechanism of action of 5-flucytosine.	7
Figure 2.1. Pie chart of drug compound classes that inhibited the growth of <i>C. deuterogattii</i> strain R255 at 10 µg/mL.....	27
Figure 2.2. Chemical structure of flubendazole.....	28
Figure 2.3. Chemical structures of 1,4-dihydropyridine calcium channel blockers: nisoldipine, nifedipine, felodipine, niguldipine and lacidipine.....	28
Figure 2.4. Time-kill assay of <i>C. neoformans</i> strain H99 treated with flubendazole.....	32
Figure 3.1. Schematic diagram of tubulin and microtubule composition.	41
Figure 3.2. The effect of flubendazole on the growth of <i>C. neoformans</i> strain H99 over 48 h.....	48
Figure 3.3. Schematic diagram of time-course analysis.	49
Figure 3.4. Plot of biological coefficient of variation versus average expression of RNA-Seq data.	50
Figure 3.5. Removal of unwanted variation improves consistency of Relative Log Expression (RLE) plots and clustering via multiple dimensional scaling (MDS).	51
Figure 3.6. Gene Ontology (GO) network of enriched genes at 12 h post-treatment, comparing flubendazole-treated and control cells.....	58
Figure 3.7. Schematic diagram of the expression of genes differentially expressed at 12 h comparing flubendazole treated and untreated cells (FLBvsCTRL).	59
Figure 3.8. Schematic diagram of the expression of genes associated with tubulin and actin at 12 h (FLBvsCTRL).	61
Figure 3.9. Schematic diagram of the expression of genes associated with cell division processes: chromosome segregation (yellow), DNA replication/metabolism (blue), mismatch repair (green), and cytokinesis (orange) at 12 h (FLBvsCTRL).....	62
Figure 3.10. Schematic diagram of the expression of oxidative stress genes at 12 h (FLBvsCTRL).....	64

Figure 3.11. Gene ontology (GO) analysis comparing flubendazole-treated cells at over time.	67
Figure 3.12. <i>C. neoformans</i> response to flubendazole treatment.	80
Figure 4.1. Image processing in preparation for image analyses using ImageJ.	88
Figure 4.2. Image processing of GFP-tagged β -tubulin in <i>C. neoformans</i> using ImageJ.....	89
Figure 4.3. Representative images of <i>C. neoformans</i> expressing β -tubulin-GFP in the presence and absence of flubendazole.	91
Figure 4.4. Analyses of GFP-tagged β -tubulin in flubendazole treated and untreated <i>C. neoformans</i> strain LK128.....	92
Figure 4.5. Morphological changes in <i>C. neoformans</i> cells expressing GFP-tagged β -tubulin in response to flubendazole.	93
Figure 4.6. Analyses of cell clustering in flubendazole treated and untreated <i>C. neoformans</i> cells....	94
Figure 4.7. Cell shape analyses in flubendazole-treated and -untreated <i>C. neoformans</i> strain LK128.	95
Figure 4.8. Transcription factors and kinases differentially expressed following flubendazole treatment.....	96
Figure 4.9. Spot assay of a series of transcription factor knockout strains in the presence of flubendazole.	98
Figure 4.11. Spot assay of transcription factor knockout strains in the presence of flubendazole, mebendazole and fluconazole.	102
Figure 4.12. Morphological changes in <i>yox101Δ</i> cells in the presence or absence of flubendazole.	104
Figure 4.13. Cell shape analyses in flubendazole treated and untreated <i>yox101Δ</i> cells.....	106

Table of Tables

Table 1.1. Drug screening studies and repurposing ventures for <i>Cryptococcus</i> spp.....	15
Table 2.1. Minimum inhibitory concentrations of flubendazole and a series of calcium channel blockers against <i>Cryptococcus</i> , <i>Saccharomyces</i> , <i>Candida</i> and <i>Aspergillus</i> species.	29
Table 2.2. Minimum inhibitory and minimum fungicidal concentrations of flubendazole against <i>Cryptococcus</i> species from varied sources.	30
Table 2.3. Fractional inhibitory concentration index values of flubendazole, mebendazole and benomyl in combination with various antifungals against <i>C. neoformans</i> H99 and <i>C. deuterogattii</i> R265.	33
Table 3.1. Number of differentially expressed genes across different test groups (adjusted p-value < 0.05).	52
Table 3.2. List of genes with greater than one log fold-change in differential expression in response to flubendazole at 12 h (FLBvsCTRL.12h).	54
Table 3.3. Predicted conserved domain hits within 10 hypothetical genes with the greatest increase or decrease log fold-change at 12 h (FLBvsCTRL.12h)	55
Table 3.4. List of metabolic pathways affected at FLB.12vs6h.....	67
Table 4.1. Percentage of highly fluorescent cells with their 95% credible intervals.....	92
Table 4.2. <i>S. cerevisiae</i> and <i>C. neoformans</i> orthologs of <i>YOX101</i> and associated transcription factors.	99
Supplementary Table S6.1.1. List of genes differentially expressed comparing flubendazole treated and untreated cells at 6 h (FLBvsCTRL.6h).....	118
Supplementary Table S6.1.2. List of genes differentially expressed comparing flubendazole treated and untreated cells at 8 h (FLBvsCTRL.8h).....	119
Supplementary Table S6.1.3. List of drug transporters from FLBvsCTRL.12h.	123
Supplementary Table S6.1.4. List of genes differentially expressed comparing flubendazole treated cells at 6 and 8 h (FLB.8vs6).....	124
Supplementary Table S6.1.5. Expression data for genes in Figure 3.7.....	127
Supplementary Table S6.2.1. List of significantly differentially expressed transcription factors at FLBvsCTRL.12h.	133

Supplementary Table S6.2.2. List of significantly differentially expressed kinases at FLBvsCTRL.12h.
..... 137

Abstract

Cryptococcal meningitis is the most common form of meningitis in HIV-infected persons. It is a life-threatening fungal infection of the brain that disproportionately affects the poorest and most resource-limited regions of the world. Current antifungal therapies used to treat cryptococcal meningitis are critically limited, toxic, expensive and have issues with drug resistance, highlighting an urgent need for more effective and affordable drugs. However, developing a new drug can cost over \$2 billion and take over a decade to succeed, causing many development pipelines to dry up. To address this issue, this thesis uses drug repurposing as an alternative approach to accelerate antifungal drug discovery efforts to treat cryptococcal meningitis. This involves investigating existing drugs, approved for other purposes, for candidates with antifungal activities that can be developed into new antifungal drugs.

An untested library of drugs was screened for compounds that inhibited the growth of *Cryptococcus deuterogattii*. This approach successfully identified multiple candidates that are not currently approved to treat fungal infections. The most potent candidate was flubendazole, a drug used to treat intestinal worms in veterinary and clinical settings. A diverse list of *Cryptococcus* species were highly susceptible to low concentrations of flubendazole and this effect was fungicidal. This is important for efficient clearing of fungal burden, and may subsequently help improve clinical outcomes, prevent clinical relapse and deter the development of drug resistance. Flubendazole was also equally effective against fluconazole-resistant *Cryptococcus* strains, and was highly specific to *Cryptococcus* species and did not inhibit other clinically-important, human fungal pathogens.

To understand the antifungal mechanism of action of flubendazole, a combination of RNA-Seq and microscopy approaches were used. Gene expression data and image analyses demonstrated that tubulin was inhibited and cell morphology was compromised in *Cryptococcus* cells treated with flubendazole. This provides evidence to support the involvement of tubulin as a probable target of flubendazole in its antifungal mechanism of action. The RNA-Seq data generated also represents a resource for future studies to comparatively investigate genes of interest for drug discovery, such as transcription factors and kinases.

This thesis encompasses a body of work that exemplifies the benefits of combining drug screening and repurposing efforts in an attempt to address the unmet medical needs of cryptococcal meningitis. The discovery of flubendazole as a potential antifungal agent and preliminary characterisation of its

antifungal action provides a platform that may inform and aid future developmental work including medicinal chemistry studies.