Drug repurposing:

Fast-tracking Antifungal Drug Discovery for Cryptococcal Meningitis

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July 2019

A thesis submitted in fulfilment of the requirements
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.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

Production Note:
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Megan Truong, July 15th 2019

Acknowledgements

My primary supervisor always said that a PhD is a journey. And what a journey indeed it was! It has been one long, heck of a ride and there are so many people who have given me their unconditional support, for whom I am so thankful for. In no particular order,

To my primary supervisor, Ian Charles – Thank you Ian for being my supervisor and mentor over the past few years. It's been a pleasure learning from you and I'm so thankful for the sincere care you show for my mental health and PhD progress despite all the challenges we've had, and the effort you put in to call in from afar even just to check in. I hope you know I appreciate all that.

To my number one rock, Leigh Monahan – Thank you so much Leigh for being my co-supervisor, my mentor and my friend. You have been such an amazing support and driver for this me and this project, and I cannot imagine how I would have gotten as far as I have without you. With all the challenges we've had, you've always been the calm person I needed to see it through. I hope that trait rubs off a bit on me as well as your writing skills! Thank you also for being patient and accommodative of me whenever I needed help (especially for my final seminar). I'm so lucky and grateful to have had you as my go-to person.

To Dee Carter, the Carter Lab and G08 friends – Thank you Dee for supervising me and being on-board this project. Navigating through this PhD has been a lot more challenging than either of us would have ever imagined, so thank you very much for your patience, your guidance, your time and all your efforts in supporting me and this project. You are an amazing editer and I hope to be able write as well as you one day. Special thanks to Yuwen and Kenya for your friendship. I wouldn't be who and where I am today, if it were not for your unconditional support at all my highs and lows. Doing a PhD was much more tolerable with you both! Thank you also to the autoclave/morning tea ladies and workshop guys for your support and all that you have done for me and the lab over the years. Your help is always much appreciated!

To Steve Djordjevic – You have done more for me than you give yourself credit for. Thank you for standing in in Ian's absence and for that time you listened to everything I had to say even though I was sick. I appreciate your sincere care, the shits and giggles and all the wine talks. Thank you!

To my collaborators at UNSW – thank you so much Prof Marc Wilkins and Dr Ignatius Pang for being a part of my transcriptome project. Your contributions and support mean so much to me and I could not have completed this without your help. Special thanks to Igy for all your help with the bioinformatics components. Thank you for being so patient with me, and your willingness to teach and guide me through many processes. I am also grateful to you for sharing so much of your time in helping me read drafts (especially at time when you were most busy), and your unconditional moral support (they always pick me up when I am down).

To my friends at Duke – Joe & the Heitmania Lab, thank you for welcoming me to your lab so warmly and helping me so readily when I needed help. Thank you to Prof John Perfect and Prof Andy Alspaugh for also being so welcoming of this little Aussie. You and your lab members freely shared your time with me and I hope you know I appreciate that so much. If you ever fly down to Sydney, please feel free to reach out as I would love to catch up! Special thanks also to Di Dong for being an amazing roommate during my time at Duke. My time at Duke was one of my highlights during my PhD. I met so many people, travelled around a bit and learnt so much (personally & academically).

To my family – Special thanks to my mum and dad, for being ever so patient, accommodative and understanding of my long hour days and overnight stays. Thank you for making sure I'm well fed and that I get home safely every single day. Thank you to my older brother for supporting the family. Thank you my little brother for checking in on me, being patient with me on my bad days, having shits and giggles with me, and especially for bringing Leon into our family. Thank you Leon and Mikky for always making me smile.

To my mentor and friend Dr John Pitt – thank you so much for your friendship, guidance and support over the past few years. Thank you for all the opportunities you have given me and always reminding me that it is completely ok, and rather better, to have a challenging PhD experience.

To my immediate PhD family – Thank you so much for your friendship and support over the years. I'm grateful to have been surrounded by such an amazing, intelligent, funny, hard-working, caring and supportive group of people. It's been such a pleasure knowing you all over the past few years and I'm excited to see what's in stall for each of us. Special thanks to Iain, Marz, Ethan and Ronnie for always being supportive, especially on my bad days. Special thanks to Michael, Isa and Iain, particularly in the lead up to my thesis submission. Without your help in formatting, spot-checking, moral support on many levels and keeping me in one piece, I could not imagine how I could have submitted this thesis.

To my non-PhD friends who still stuck around – thank you for being understanding and supportive despite me being largely MIA. Special thanks to Helen Pang and Tina Nguyen for your unconditional support on so many levels. Your support and friendship mean the world to me. I look forward to catching up with you all and spending some long overdue quality time.

Thank you to Prof John Perfect for providing strains that I tested in my first data chapter. Thank you to Assistant Prof Lukasz Kozubowski for providing the GFP strain LK128 that I used in my third data chapter. Thank you to Dr Connie Nichols for providing a list of fluorescent strains for my work. Many, many thanks also to Dr Louise Cole for your guidance at the start of my microscopy venture and helping me navigate through technical discussions and meetings. Many, many thanks also to Dr Christian Evenhuis for all your help with my microscopy work. Thank you for being so patient with me, for being involved in this work and a great teacher! Thank you also to Dr Mike Johnson for your technical support in the microscopy work. I am so lucky and grateful to have done microscopy with you all and would love to learn more from you!

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

Table of Figures

Table of Tables

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.