

ICCC2026

12th International Conference on
Cryptococcus & Cryptococcosis

28 June - 3 July 2026 | University of Exeter, UK

SPEAKER ABSTRACT BOOK



#ICCC2026



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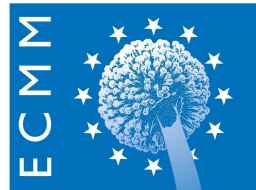
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MRC Centre
for Medical
Mycology



University
of Exeter

Sunday 28 – Monday 29 June

Sunday 28 June		
13:00	Registration Opens	
17:00	Keynote: Joe Jarvis	Translating research evidence into global health policy - Lessons from two decades of HIV-associated cryptococcal meningitis research
18:15	Reception (light meal provided)	

Monday 29 June			
08:30	Coffee		
	Plenary Sessions - Alumni Auditorium - Chair: David Meya		
09:00	John Perfect	Crypto is the CNS currency of the Immunosuppressed!!!!	
09:30	Nelesh Govender	Re-engineering CrAg Screening to Save More Lives	
10:00	Liping Zhu	Neutralizing anti-GM-CSF autoantibodies and cryptococcal meningitis	
10:30	Coffee		
	Plenary Sessions - Alumni Auditorium - Chair: Andrew Alspaugh		
11:00	Mari Shinohara	Which cells are immune sentinels during Cryptococcus infection?	
11:30	Wieland Meyer	Traveling Cryptococcus: highlighting the importance of molecular epidemiology	
12:00	Manu De Rycker	Developing new antifungals in an Academic - Industry partnership	
12:30	Flash Talks x5		
13:00	Lunch “Genes, Genomes, and Strains” sponsored by the ISHAM Working Group on Genotyping of Cryptococcus neoformans and C. gattii		
	Seminar room 1-3, lower level <i>C1a: Diagnostic challenges and outcomes</i> Chair: Richard Kwizera	Alumni Auditorium <i>C2a: Vaccine targets and therapeutic strategies</i> Chair: Blake Billmyre	Seminar room 7-9, upper level <i>C3a: Lipids and Sterols in Cryptococcus pathogenesis</i> Chair: Chaoyang Xue
14:45	Radha Rajasingham	Kristie Goughenour	Chaoyang Xue
15:05	Elizabeth Nalintya	Yu Zhang	Dong-gyu Kim
15:20	Jane Frances Ndyetukira	Daniel A Santos	Xuefei Chen
15:35	Asmus Tukundane	Michael Boucher	Lukasz Kozubowski
15:50	Coffee		
	Seminar room 1-3, lower level <i>C1b: Diagnostic challenges and outcomes</i> Chair: Alison Gifford	Alumni Auditorium <i>C2b: Insights into phagocyte-fungus interactions</i> Chair: Simon Johnson	Seminar room 7-9, upper level <i>C3b: Cryptococcus adaptation and the brain</i> Chair: Jintao Xu
16:15	Sile Molloy	Felipe H. Santiago-Tirado	Serisha Naicker
16:35	Emily Prendergast	Rebecca Hodges	Samuel Okurut
16:50	Julia Palmucci	Christopher Lee	Lander De Herdt
17:05	Enock Kagimu	Greetje Vande Velde	Amalia Awala
17:20	Poster Session 1 in the Forum		
18:30	BBQ and MicroMentoring - The Ram Bar		



#ICCC2026

Tuesday 30 June

Tuesday 30 June			
08:30	Coffee		
	Plenary Sessions - Alumni Auditorium - Chair: Edward Wallace		
09:00	David Engelthaler	Crypto Mining: A big data “pathogen prospecting” approach to identify new locales and niches for <i>Cryptococcus gattii</i>	
09:30	Tamara Doering	Cryptococcal glycobiology - not just the capsule!	
10:00	Xiaorong Lin	Targeting <i>Cryptococcus</i> : Engineering LNP Therapeutics and Vaccines	
10:30	Coffee		
	Plenary Sessions - Alumni Auditorium - Chair: Christina Hull		
11:00	Christina Cuomo	Population variation in <i>Cryptococcus neoformans</i> and <i>deneoformans</i>	
11:30	Marcio Rodrigues	Exploring the extracellular biology of <i>Cryptococcus</i>	
12:00	Maurizio Del Poeta	Mechanistic Insights into Cryptococcal Granuloma Formation and Maintenance	
12:30	Flash Talks x5		
13:00	Lunch		
	Concurrent Sessions		
	Seminar room 1-3, lower level <i>C1c: Cryptococcus adaptation and the environment</i> Chair: Lukasz Kozubowski	Alumni Auditorium <i>C2c: Cryptococcus and emerging co-infections</i> Chair: Elizabeth Nalintya	Seminar room 7-9, upper level <i>C3c: Human risk factors for Cryptococcosis</i> Chair: Kirstie Goughenour
14:45	Asiya Gusa	Kyla Murphy	Jessica C. Hargarten
15:05	Marina Ruzic	Orlando Ross	Linghong Zhou
15:20	Marnus du Plooy	Timothy Mugabi	Hyunah Yoon
15:35	Marco Coelho	Dora Corzo-Leon	Erin McClelland
15:50	Coffee		
16:00	“Climate and Fungi” Panel discussion with Prof Sarah Gurr, Prof Robin May, Baroness Natalie Bennett, and Prof Mat Fisher Joint meeting with Exeter Climate Forum		
18:00	Poster Session 2 (including hybrid posters) in the Forum		
18:00	“Climate and Fungi” Reception - The Forum, Dinner provided		



#ICCC2026

Wednesday 1 July

Wednesday 1 July			
08:30	Coffee		
	Plenary Sessions - Alumni Auditorium - Chair: Hannah Brown Harding		
09:00	Eric Dang	Spatial organization of type 2 immunity during pulmonary cryptococcosis	
09:30	Rachael Dangarembizi	Unravelling mechanisms of brain injury in cryptococcal meningitis	
10:00	Lena Heung	A Tale of Two Crypto: Defining unique host responses to Cryptococcus species	
10:30	Coffee		
	Plenary Sessions - Alumni Auditorium - Chair: Kirsten Nielsen		
11:00	Jennifer Geddes-McAlister	From Spectra to Solutions: Proteomics at the Forefront of Fungal Infection, Immunity, and Antifungal Discovery.	
11:30	Jessica Brown	Formation of the seed cells morphotype: environmental triggers and molecular regulators.	
12:00	Paul Magwene	Hyperdiversity, Hidden Hybrids, and Hyperplastic Genomes: New Insights from Local Scale Population Genomics of Cryptococcus neoformans	
12:30	Flash Talks x5		
13:00	Lunch		
	Concurrent Sessions		
	Seminar room 1-3, lower level <i>C1d: Antifungal resistance, clinical challenges, and possible solutions</i> Chair: Samuel Okurut	Alumni Auditorium <i>C2d: Morphology and persistence</i> Chair: Eric Dang	Seminar room 7-9, upper level <i>C3d: Genetic insights into fungal diversity and antifungal resistance</i> Chair: Carolina Ficarative
14:45	Marie Desnos-Ollivier	Anamelia Bocca	Evalina Basenko: FungiDB
15:05	Cynthia Ahimbisibwe	Ruchi Agrawal	Lexia Henning
15:20	Sarah Kimuda	Abigail Potton	Alba Torres Cano
15:35	Ludmila Gouveia-Eufrasio	Rachel Berg-Murante	Carolina Fircative
15:50	Coffee		
	Seminar room 1-3, lower level <i>C1e: Biomarkers of disease outcomes</i> Chair: Kyla Murphy	Alumni Auditorium <i>C2e: Molecular drivers and consequences of titan cell formation</i> Chair: Asiya Gusa	Seminar room 7-9, upper level <i>C3e: The cryptococcus cell wall as a frontline of pathobiology</i> Chair: Xuefei Chen
16:15	Alison Gifford	Edward Wallace	Karen Wozniak
16:35	Abigail Link	Claudia A Petrucco	Corina Probst
16:50	Mark Oilor	Thomas Davies	Hilare Irere
17:05	Kisakye Diana Kabbale	Piotr Stempinski	Sydney Briner
17:20	Sujiraphong Pharkjaksu		
18:30	Free evening (no meal provided). Off campus dining options available.		



#ICCC2026

Thursday 2 July

Thursday 2 July			
08:30	Coffee		
	Plenary Sessions - Alumni Auditorium - Chair: Rachael Dangarembizi		
09:00	Kaustuv Sanyal	Metazoan-Like Genome Compartmentalization in <i>Cryptococcus neoformans</i>	
09:30	Guilhem Janbon	Extracellular vesicles biosynthetic pathways in <i>Cryptococcus</i>	
10:00	Michal Olszewski	Developing experimental immunotherapies for offsetting inflammatory brain pathologies in <i>Cryptococcus</i> Meningoencephalitis	
10:30	Coffee		
	Plenary Sessions - Alumni Auditorium - Chair: Joe Heitman		
11:00	Hiten Madhani	CRISPR-insert reveals the genetic architecture of the drug-resistant fungal meningitis pathogen <i>Cryptococcus neoformans</i>	
11:30	Kirsten Nielsen	The biological function of <i>Cryptococcus</i> titan cells	
12:00	Rebecca Drumond	IFN γ therapy remodels mammalian copper transporter ATP7A to support myeloid cell antifungal immunity	
12:30	Pitch for ICCC 2029		
13:00	Lunch		
	Concurrent Sessions		
	Seminar room 1-3, lower level <i>C1f: Beyond the <i>Cryptococcus</i> species complex</i> Chair: Elizabeth Bayne	Alumni Auditorium <i>C2f: Neurotropism and the Blood Brain Barrier</i> Chair: Rebecca Drummond	Seminar room 7-9, upper level <i>C3f</i>
14:45	Blake Billmyre	Hannah Brown Harding	FungiDB Workshop
15:05	Márcia C David Palma	Simran Atulkumar Patel	
15:20	Sosie Yorki	Lara Roosen	
15:35	Rahul Anand	Robbi Ross	
15:50	Coffee		
	Seminar room 1-3, lower level <i>C1g: Understanding <i>Cryptococcus gattii</i> pathobiology</i> Chair: Jessica Brown	Alumni Auditorium <i>C2g: Immunity, adaptation, and hypervirulence</i> Chair: Lena Heung	
16:15	Elizabeth Bayne	Jintao Xu	
16:35	Maahir Kauchali	Bruno Borges	
16:50	Sebastian Reusch	Chinaemerem Uju Onyishi	
17:05	Qinxi Ma	Jake T. Leinas	
17:20	Poster Session 3 in the Forum		
18:30	ICCCC Banquet Dinner and Ceilidh		



#ICCC2026

Friday 3 July

Friday 3 July		
08:30	Coffee	
	Plenary Sessions - Alumni Auditorium - Chair: Nelesh Govender	
09:00	David Meya	PLATFORM-CM Trial: Explanation of Master Protocol for an Adaptive Platform Trial
09:15	Conrad Muzoora	Turletricin Novel Polyene Phase II Trial
09:30	Morris Rutakingirwa	Oteseconazole Phase II Trial
09:45	David Boulware	Personalized Immunotherapy
10:00	ICCC 2029	
10:15	Sponsors	
10:30	Coffee	
	Plenary Sessions - Alumni Auditorium - Chair: Tom Harrison	
11:00	Linqi Wang	Fungicide phenotypic resistance
11:30	Stuart Levitz	One, two, three, four. Get the vaccine out the door!
12:00	Joe Heitman	Cryptococcus as a prism for discovery
12:30	Closing Ceremony and Box Lunches	
	Post-meeting Workshops and Excursions	



#ICCC2026

ICCC2026

Speaker Abstract Book

International Cryptococcal Conference 2026

Exeter, UK | June 29 – July 3, 2026

TALKS LIST

Sunday June 28

Keynote Speaker

Joe Jarvis

Translating research evidence into global health policy - Lessons from two decades of HIV-associated cryptococcal meningitis research

Monday June 29

Plenary Session

Alumni Auditorium

John Perfect

Crypto is the CNS currency of the Immunosuppressed!!!!

Nelesh Govender

Re-engineering CrAg Screening to Save More Lives

Liping Zhu

Neutralizing anti-GM-CSF autoantibodies and cryptococcal meningitis

Mari Shinohara

Which cells are immune sentinels during Cryptococcus infection?

Wieland Meyer

Traveling Cryptococcus: highlighting the importance of molecular epidemiology.

Manu De Rycker

Developing new antifungals in an Academic - Industry partnership

Flash Talks

Tonia Dargham - Characterization of extracellular vesicles diversity in *Cryptococcus neoformans*: A new avenue for identifying disease markers and understanding host interaction.

Zar ni San - Co-culture with *Mycobacterium tuberculosis* causes remodelling of *Cryptococcus gattii* morphology and cell wall architecture.

Isabelle Mouyna - Regulation of extracellular vesicle biosynthesis by the TOR pathway in the fungal pathogen *Cryptococcus neoformans*

Jackie Spieles - *Cryptococcus* spore germination can occur in the absence of oxygen

Kisakye Diana Kabbale - *Cryptococcus neoformans* Sequence Type 93 is Associated with Reduced Type-1 Immune Responses in Ugandan Patients with HIV-associated Cryptococcal Meningitis

Concurrent 1

14:45- 15:50

Diagnostic challenges and outcomes

Seminar Rooms 1-3, lower level

Radha Rajasingham

Customized care for people with cryptococcal antigenemia

Elizabeth Nalintya

Birth and clinical outcomes of pregnant women with Cryptococcal Antigenemia in outpatient clinics in Uganda

Jane Frances Ndyetukira

Challenges and missing links to optimize nursing care for persons with HIV-associated meningitis in a resource-constrained setting

Asmus Tukundane

Evaluation of Laboratory Checklists for Protocol-Scheduled Testing in HIV-Associated Meningitis Clinical Studies

Concurrent 1**16:15- 17:20**

Diagnostic challenges and outcomes
Seminar Rooms 1-3, lower level

Sile Molloy

Mortality among individuals with subclinical cryptococcal meningitis identified through cryptococcal antigen (CrAg) screening programs in South Africa and Tanzania

Rhys Wenlock

Cryptococcal antigenaemia screening and treatment: observations from the field in South Africa (CAST OFF-SA)

Julia Palmucci

Low CSF glucose is associated with increased CNS inflammation in adult patients with HIV-associated cryptococcal meningitis: a prospective cohort study.

Enock Kagimu

To CT or Not CT? That is the question, but LP is the answer

Concurrent 2**14:45- 15:50**

Vaccine targets and therapeutic strategies
Alumni Auditorium

Kristie Goughenour

Trehalose phosphate synthase 1 (tps1) deficient Cryptococcus neoformans alters host murine adaptive immune response

Yu Zhang

A novel CpG-adjuvanted heat-killed Cryptococcus vaccine induces superior immune protection in an intramuscular model

Daniel A Santos

Enzymatic Inactivation of Fluconazole Defines a Novel Resistance Pathway in Cryptococcus spp.

Michael Boucher

Phenotypic landscape of Cryptococcus neoformans drives mechanistic interrogation of virulence factors

Concurrent 2**16:15-17:20***Insights into phagocyte-fungus interactions*
Alumni Auditorium**Felipe H. Santiago-Tirado***Rab20 conveys immune signaling to the cryptococcal-containing phagosome during Cryptococcus infection of macrophages.***Rebecca Hodges***Investigating interactions between macrophage ridges and Cryptococcus neoformans***Christopher Lee***Investigating novel lectinobodies against the capsule of human fungal pathogen Cryptococcus neoformans***Greetje Vande Velde on behalf of Eliane Vanhoffelen***Longitudinal in vivo bioluminescence imaging reveals a protective role for MARCO deficiency in cryptococcal clearance and fluconazole efficacy***Concurrent 3****14:45- 15:50***Lipids and sterols in Cryptococcus pathogenesis*
Seminar Rooms 7-9, upper level**Chaoyang Xue***Phospholipid homeostasis in host-Cryptococcus interactions during pulmonary cryptococcosis***Dong-gyu Kim***Defining Mechanisms of Cryptococcal Sterol Transport***Xuefei Chen***Targeting membrane asymmetry reveals non-canonical vulnerabilities in Cryptococcus neoformans***Lukasz Kozubowski***The role of Eisosomal proteins in pathogenesis of Cryptococcus neoformans***Concurrent 3****16:15-17:20***Cryptococcus adaptation and the brain*
Seminar Rooms 7-9, upper level**Serisha Naicker***Cryptococcus surveillance in South Africa: Expanding from human disease surveillance to One Health surveillance***Samuel Okurut***Cerebrospinal Fluid and Fungal Burden Associated Cellular Immune Responses in HIV-Related Cryptococcal Meningitis: From Sterile Fungal Cultures to Low and High Fungal Burden Disease***Lander De Herdt***IL-5Ra deletion controls cryptococcal meningoencephalitis development***Amalia Awala***Paradoxical microglial inactivation during cryptococcal infection of the brain*

Tuesday June 30

Plenary Session

Alumni Auditorium

David Engelthaler

Crypto Mining: A big data "pathogen prospecting" approach to identify new locales and niches for Cryptococcus gattii

Tamara Doering

Cryptococcal glycobiology - not just the capsule!

Xiaorong Lin

Targeting Cryptococcus: Engineering LNP Therapeutics and Vaccines

Christina Cuomo

Population variation in Cryptococcus neoformans and deneoformans

Marcio Rodrigues

Exploring the extracellular biology of Cryptococcus.

Maurizio Del Poeta

Mechanistic Insights into Cryptococcal Granuloma Formation and Maintenance

Flash Talks

Adriana Orrego - From Essential Metabolism to Resistance: Transposable Elements and 5-FAA Escape in Cryptococcus

Hannah Newton - Using MLST to explore relationships between environmental and animal associated isolates of Cryptococcus in a koala population.

Kimberly Martinez - Titan Cell Production Increases In Vivo Diversity and can Enhance Antifungal Drug Resistance.

Concurrent 1

14:45- 15:50

Cryptococcus adaptation and the environment

Seminar Rooms 1-3, lower level

Asiya Gusa

Genetic and molecular basis of thermal adaptation in Cryptococcus deneoformans

Marina Ruzic

Evolution of thermotolerance in a human fungal pathogen through recurrent mutation of a sugar transporter

Marnus du Plooy

pH responses

Marco Coelho

Chromosome evolution and mating-type locus remodelling in Cryptococcus and Kwoniella

Concurrent 2**14:45- 15:50**

Cryptococcus and emerging co-infections
Alumni Auditorium

Kyla Murphy

15 years on: how the changing HIV treatment landscape has impacted cryptococcal meningitis rates in Cape Town

Orlando Ross

Cryptococcus neoformans responds to presence of Mycobacterium by diversifying its morphologies and remodelling its capsular material

Timothy Mugabi

CMV viremia is associated with impaired CSF fungal clearance amongst 509 Ugandan adults with HIV-associated cryptococcal meningitis

Dora Corzo-Leon

Cryptococcus Klebsiella coinfections

Concurrent 3**14:45- 15:50**

Human risk factors for Cryptococcosis
Seminar Rooms 7-9, upper level

Jessica C. Hargarten

Mechanistic target of rapamycin (MTOR) signaling pathway loss-of-function variants in previously healthy patients with non-HIV cryptococcosis.

Linghong Zhou

Cryptococcus modulates host immune tolerance through STING-dependent inhibition of PFKFB3

Hyunah Yoon

Time-Dependent Antibody Signatures of Cryptococcal Disease After Kidney Transplant

Erin McClelland

Differential recognition of C. neoformans by male and female human macrophages elicits contrasting immune responses

Wednesday July 1

Plenary Session

Alumni Auditorium

Eric Dang

Spatial organization of type 2 immunity during pulmonary cryptococcosis

Rachael Dangarembizi

Unravelling mechanisms of brain injury in cryptococcal meningitis

Lena Heung

A Tale of Two Crypto: Defining unique host responses to Cryptococcus species

Jennifer Geddes-McAlister

From Spectra to Solutions: Proteomics at the Forefront of Fungal Infection, Immunity, and Antifungal Discovery.

Jessica Brown

Formation of the seed cells morphotype: environmental triggers and molecular regulators.

Paul Magwene

Hyperdiversity, Hidden Hybrids, and Hyperplastic Genomes: New Insights from Local Scale Population Genomics of Cryptococcus neoformans

Flash Talks

Master Chisale - Identification of drug targets for Cryptococcus neoformans drug discovery

Mulungi Jonathan - Disseminated HIV-Associated Cryptococcosis with Pulmonary Embolism in Pregnancy: A case report

Oscar Romero - Uncovering Genetic Drivers of Biofilm Formation in Cryptococcus neoformans to Enable Antibiofilm Drug Discovery

Raphael Brosula - Analyzing genomic and phenotypic variation within a large clinical Cryptococcus population to identify determinants of virulence and disease outcome

Francisco Hernandez - Laccase Drives the Warburg Effect in Melanized Cryptococcus neoformans upon Glucose Depletion.

Concurrent 1

14:45- 15:50

Antifungal resistance, clinical challenges, and possible solutions

Seminar Rooms 1-3, lower level

Marie Desnos-Ollivier

Frequency and therapeutic impact of fluconazole resistance in Cryptococcus neoformans strains from French surveillance

Cynthia Ahimbisibwe

Experience of research nurses with oral Encochleated amphotericin B for treatment of Cryptococcal Meningitis in a resource-limited setting

Sarah Kimuda

Use of Oteseconazole for Consolidation Therapy and Secondary Prophylaxis in Fluconazole Non-Susceptible Cryptococcal Meningitis.

Ludmila Gouveia-Eufrasio

Affordable PEGylated liposomal amphotericin B enables effective and safe oral treatment of experimental murine cryptococcosis

Concurrent 1**16:15-17:35**

Biomarkers of disease outcomes
Seminar Rooms 1-3, lower level

Alison Gifford

The cell-type specific neuroimmune response to cryptococcal infection of murine hippocampal brain slices

Abigail Link

Changing impact of Cryptococcus on HIV-associated meningitis in northern Uganda

Mark Oilor

Differential activation and exhaustion of circulating T-cells and monocytes at baseline may determine risk of developing cryptococcal meningitis among individuals with HIV-associated asymptomatic cryptococcal antigenemia

Rhona CB Muyise

Incidence, reasons, and outcomes of rehospitalization among adults with HIV-related cryptococcal meningitis in Uganda: a retrospective cohort study

Sujiraphong Pharkjaksu

Subtelomeric Genomic Rearrangement underlies Phenotypic Variation and Amphotericin B Resistance in Clinical isolates of Cryptococcus neoformans

Concurrent 2**14:45- 15:50**

Morphology and persistence
Alumni Auditorium

Anamelia Bocca

Dormant Cryptococcus neoformans yeasts persist in a concealed state in a murine model of infection

Ruchi Agrawal

Mechanism of antifungal persistence of Cryptococcus neoformans against Amphotericin B

Abigail Potton

Defining the Cryptococcus neoformans titanide, a pathogenic morphotype that arises from typical yeast cells in response to host-relevant conditions

Rachel Berg-Murante

Titan cell formation is essential for C. neoformans latency

Concurrent 2**16:15-17:20**

Molecular drivers and consequences of titan cell formation
Alumni Auditorium

Edward Wallace

Post-transcriptional control of the Cryptococcus cell cycle by the RNA-binding protein Ssd1

Claudia A Petrucco

Mitotic and cytokinetic defects under stress can drive polyploidy in Cryptococcus neoformans

Thomas Davies

Perturbing Mps1 Kinase in the Cryptococcus neoformans titan cell, implications for a key morphological transition

Piotr Stempinski

A role for aquaporin (Aqp1) in the control of Cryptococcus neoformans cell morphology

Concurrent 3**14:45- 15:50**

Genetic insights into fungal diversity and antifungal resistance

Seminar Rooms 7-9, upper level

Evalina Basenko

FungiDB: Sustaining Genomic-Scale Discovery through Open Access and Community Support

Lexia Henning

ERG11 Mutations Reshape Fluconazole Binding in Cryptococcus

Alba Torres Cano

Identification of synergistic compounds in combination with fluconazole from the Prestwick Chemical Library® against Cryptococcus neoformans.

Carolina Firacative

Activity of Ibrexafungerp Against Clinical and Environmental Isolates of Cryptococcus neoformans and Cryptococcus gattii

Concurrent 3**16:15-17:20**

The Cryptococcus cell wall as a frontline of pathobiology

Seminar Rooms 7-9, upper level

Karen Wozniak

Anticryptococcal Mechanisms of Action by Dendritic Cell Lysosome Proteins

Corina Probst

The novel carbohydrate modifying enzyme Cel1 is required for Cryptococcus neoformans cell wall remodeling in response to host environmental stress.

Hilare Irere

Chitin as a Hidden Driver of Cryptococcus neoformans Virulence

Sydney Briner

The glycosyltransferase Goe1 (Glucan Organizing Enzyme 1) contributes to cell wall integrity and echinocandin resistance

Thursday July 2

Plenary Session

Alumni Auditorium

Kaustuv Sanyal

Metazoan-Like Genome Compartmentalization in Cryptococcus neoformans

Guilhem Janbon

Extracellular vesicles biosynthetic pathways in Cryptococcus

Michal Olszewski

Developing experimental immunotherapies for offsetting inflammatory brain pathologies in Cryptococcus Meningoencephalitis

Hiten Madhani

CRISPR-insert reveals the genetic architecture of the drug-resistant fungal meningitis pathogen Cryptococcus neoformans

Kirsten Nielsen

The biological function of Cryptococcus titan cells

Rebecca Drumond

IFN γ therapy remodels mammalian copper transporter ATP7A to support myeloid cell antifungal immunity

Concurrent 1

14:45- 15:50

Beyond the Cryptococcus species complex

Seminar Rooms 1-3, lower level

Blake Billmyre

Gene content diversity in Cryptococcus neoformans

Márcia C David Palma

Saprobic and pathogenic Cryptococcus species: uncovering hidden diversity across the genus.

Sosie Yorki

Whole genome alignment of Cryptococcus and Kwoniella reveals patterns of conservation

Rahul Anand

Genomic signatures of parasex among two genetically distinct hybrid groups of Cryptococcus neoformans.

Concurrent 1

16:15-17:20

Beyond the Cryptococcus species complex

Seminar Rooms 1-3, lower level

Elizabeth Bayne

Mechanisms of RNA interference in Cryptococcus

Maahir Kauchali

Neuropathological differences driven by cryptococcal species in a murine model of infection

Sebastian Reusch

Using human airway and alveolar organoid-derived monolayers to quantify parameters of early cryptococcal infections

Qinxi Ma

*Genetic and pathogenic diversity of *Cryptococcus gattii**

Concurrent 2**14:45- 15:50**

Neurotropism and the Blood-Brain Barrier
Alumni Auditorium

Hannah Brown Harding

*Human Brain Organoids: A New Model to Study *Cryptococcus neoformans* Neurotropism*

Simran Atulkumar Patel

Cryptococcal aggregation in the perivascular compartment drives glymphatic disruption during murine cryptococcal meningitis

Lara Roosen

In vivo assessment of host-pathogen interaction in the crossing the blood-brain barrier in murine model of experimental cerebral cryptococcosis: correlating cellular with pathophysiological events

Robbi Ross

*Use of an immortalized human microglia cell line to study the unique recognition of *Cryptococcus neoformans* by human microglia.*

Concurrent 2**16:15-17:20**

Immunity, adaptation, and hypervirulence
Alumni Auditorium

Jintao Xu

When Immunity Turns Pathogenic: Monocyte-Derived iNOS Protects Against Lethal Immunopathology in Cryptococcal Meningitis

Bruno Borges

*The Role of CD4+ T Cells in Hypervirulence of the Clinical Isolate UgCI422 of *Cryptococcus neoformans**

Chinaemerem Uju Onyishi

The Mannose Receptor (MR) on CD169+ myeloid cells modulates the generation or maintenance of anti-fungal Tbet+ stem-like progenitors

Jake T. Leinas

*An Uncharacterized Kinase Regulates Macrophage Adaptation of *Cryptococcus neoformans**

Concurrent 3**14:45- 15:50****FungiDB Workshop**

Seminar Rooms 7-9, upper level

Friday July 3

Plenary Session

Alumni Auditorium

David Meya

PLATFORM-CM Trial: Explanation of Master Protocol for an Adaptive Platform Trial

Conrad Muzoora

Turletricin Novel Polyene Phase II Trial

Morris Rutakingirwa

Autopsy findings in persons diagnosed with HIV-associated cryptococcal meningitis.

David Boulware

Personalized Immunotherapy

Linqi Wang

Fungicide phenotypic resistance

Stuart Levitz

One, two, three, four. Get the vaccine out the door!

Joe Heitman

Cryptococcus as a prism for discovery

Sunday June 28

Keynote Speaker

Translating research evidence into global health policy - Lessons from two decades of HIV-associated cryptococcal meningitis research

Joe Jarvis

Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, UK- Botswana-Harvard AIDS Institute Partnership, Botswana

Despite major global progress in HIV treatment, advanced HIV disease remains a major driver of preventable mortality, with cryptococcal meningitis continuing to cause a substantial proportion of AIDS-related deaths worldwide. In many high-burden settings, cryptococcal meningitis remains among the commonest causes of adult meningitis and is associated with unacceptably high mortality.

This keynote will reflect on two decades of clinical and translational research in HIV-associated cryptococcal meningitis, focusing on the pathway from defining an urgent clinical problem to generating evidence capable of changing global policy. Using the AMBITION-cm trial as a central case study, the talk will examine how a single high-dose liposomal amphotericin B-based regimen was developed, evaluated, and translated into WHO guidance and routine care. The presentation will consider the scientific rationale for shorter, safer induction therapy; the importance of community and stakeholder engagement; and the practical requirements for moving from trial results to implementation at scale.

The talk will also look beyond treatment to earlier diagnosis and prevention, including the role of cryptococcal antigen screening and pre-emptive therapy in people with advanced HIV disease. Finally, it will draw broader lessons for global health research: asking the right questions, designing policy-relevant trials, advocating for access, and ensuring that evidence reaches the patients and health systems most in need.

Monday June 29

Plenary Session

Mon P1 – Crypto is the CNS currency of the Immunosuppressed!!!!

John Perfect

Duke University, NC, USA

Abstract: Cryptococcal meningoencephalitis remains one of the most common central nervous system (CNS) pathogens in humans worldwide. It lives in the “crosshairs” of underlying diseases such as HIV, solid organ transplants, autoimmunity, cancer, end stage organ disease and new therapies for “bad” diseases. Because of advances in both yeast and host genomics, we are beginning to peel away its sugar-coated mysteries and understand the vulnerabilities of this pathogenic yeast. It is now becoming clear that this environmental yeast generally evolves to a more pathogenic strain in the host. In fact, it has been shown that the human host may be infected many times with multiple yeast strains but under the correct host conditions it can move from dormancy in the lung-lymph node complex to evade the CNS. With thousands of genomes sequenced, examining the transcriptional profiles of strains at the CNS site of infection, molecularly manipulating strains, and studying them with specific host cells and robust animal models, we can harness an impressive understanding of the yeast- mammalian immune system relationships. For example, host cell-mediated immunity through CD4 cells and TH1 responses on one side and the yeast capsular structure on the other side, only scratches the surface of the complex host-yeast interactions. It is also important to note that the pathophysiological understandings have been further followed by improved diagnostic strategies such as the introduction of dipstick technology (lateral flow assays). Importantly, continued improvement in understanding of treatment protocols has been made. For instance, the best management for disease in both resource-limited and resource-available health care systems continues to be studied and harmonized in the clinics. Acute mortality appears to be reduced to between 8-25% but morbidity is probably higher. In fact, cryptococcal meningoencephalitis produces substantial complications such as blindness/deafness, increased intracranial pressure, and the Immune Reconstitution Syndrome (IRIS, PIIRS). How we diagnose and improve their management will be important to the final outcome of infections. There are both new drugs and strategies to help clear these infections and improve management and these will be reviewed and emphasized. From San Felice’s identification of this yeast in peach juice to the clinical description of human disease by Busse-Buschke in 1894-1895, this yeast continues to elegantly capture the imagination of scientists and clinicians; the sugar-coated killer yeast remains highly integrated into the practice of modern medicine.

Mon P2 – Re-engineering CrAg Screening to Save More Lives

Nelesh Govender

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Cryptococcal antigen (CrAg) screening, coupled with pre-emptive fluconazole treatment for those who test positive, is a core component of the World Health Organization package of care for people with advanced HIV disease. This recommendation is supported by evidence demonstrating both clinical benefit and cost-effectiveness. South Africa has established the world's largest CrAg screening programme. Since 2017, more than 2.5 million people with advanced HIV disease have been screened through a national reflex laboratory programme, resulting in the detection of over 100,000 cases of cryptococcal antigenaemia. Despite this achievement, important gaps remain along the care cascade. An evaluation across 27 South African sub-districts found that only 50% of 1,651 individuals with newly diagnosed antigenaemia received pre-emptive fluconazole, and only one-third received the recommended dose of at least 800 mg daily. CrAg results were documented in patient records in just 75% of cases, only 39% had a documented assessment for meningitis symptoms, and only 9% underwent lumbar puncture within one month to exclude concurrent cryptococcal meningitis. These findings suggest that while reflex laboratory screening can achieve high coverage among individuals undergoing CD4 testing, substantial improvements are needed to ensure linkage of positive results to appropriate clinical management. Furthermore, three-quarters of patients with cryptococcal meningitis in South Africa continue to present to hospital with symptomatic disease without having undergone prior CrAg screening. This highlights the limitations in the reach of the current programme. Future screening

strategies may need to expand beyond individuals already engaged in HIV care and CD4 testing to better identify people with advanced HIV disease who have limited contact with the health system. Several ongoing clinical trials, including EFFECT, ACACIA and ENCORE, are evaluating enhanced combination pre-emptive antifungal regimens for cryptococcal antigenaemia. In parallel, semi-quantitative CrAg assays may enable risk stratification by identifying patients at highest risk of disease progression and death. Policymakers will need to determine whether simplified management algorithms or risk-stratified approaches offer the best balance between feasibility, effectiveness and resource utilisation as CrAg screening programmes evolve.

Mon P3 – Neutralizing anti-GM-CSF autoantibodies and cryptococcal meningitis

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) is produced by epithelial cells, monocytes/macrophages, activated T cells, B cells, NK cells, endothelial, and fibroblast cells. GM-CSF plays a critical role in promoting the development of bone marrow-derived macrophages and granulocytes. Anti-GM-CSF autoantibodies block GM-CSF receptor signaling, thereby affecting the down-stream immune responses against *Cryptococcus*; this may explain the increased susceptibility to cryptococcosis in some individuals with normal immune function. More studies have demonstrated that the presence of anti-GM-CSF autoantibodies is not only associated with susceptibility to cryptococcal infections, but also associated with disseminated cryptococcosis, and higher one- year mortality in patients with cryptococcal meningitis, especially in those with underlying conditions. So, anti-GM-CSF autoantibodies have gained more and more attention over the years. Here, we would like to discuss in detail about the role of anti-GM-CSF autoantibodies as a rising cause for patients with cryptococcal infection.

Mon P4 – Which cells are immune sentinels during *Cryptococcus* infection?

Mari Shinohara

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Cryptococcus infection challenges immune surveillance across distinct anatomical sites, from the lung to the central nervous system (CNS). Macrophages and macrophage-like cells are widely recognized as immune sentinels, but their sentinel functions depend strongly on tissue location and timing during *Cryptococcus* infection. In this talk, I will begin with alveolar macrophages in the lung, the first resident immune cells positioned to detect inhaled fungi and initiate early pulmonary responses. I will then focus on microglia, CNS-resident macrophage-like cells, which differ strikingly from alveolar macrophages: their activation during cryptococcal brain infection appears delayed because they do not directly recognize *Cryptococcus*. Finally, I will briefly discuss new, preliminary observations on brain border-associated macrophages (BAMs), which may provide an additional perspective on immune surveillance at CNS interfaces.

MonP5 – Traveling *Cryptococcus*: highlighting the importance of molecular epidemiology.

Wieland Meyer

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Early after the discovery of the agents of cryptococcosis, the global epidemiology of the disease was linked to pigeons (*Cryptococcus neoformans* species complex) and to the export of Eucalyptus trees from Australia for global reforestation (*Cryptococcus gattii* species complex). The Vancouver Island outbreak of cryptococcosis was notable for the emergence of disease caused by *C. gattii* in a part of the world previously without endemic cases of this species complex, and for an outbreak epidemiology characterized by large numbers of human and animal cases in shared environments. It sparked a major investigation into its origin, initially suspected to have originated in Australia (linked to the export of eucalypts); later, it was demonstrated to have originated in northeastern Brazil and was most likely transported to the Pacific Northwest via the Panama Canal in the ballast water of ships. The molecular epidemiological investigation was assisted by the characterization of the outbreak isolates as VGIIb. While human activities have been implicated in the global spread of the pathogenic *Cryptococcus* species complexes, host-pathogen interactions with animals can provide important insights into the

pathogenesis of this mycosis. As an example, it has been shown repeatedly that infected koalas (*Phascolarctos cinereus*) can be active carriers of the agents of cryptococcosis. Koalas exhibit a comparatively high prevalence of cryptococcosis (clinical and subclinical) and nasal colonization, particularly in captivity. In Australia, human and animal disease associated with *C. gattii* VGII are typically confined to Western Australia and the Northern Territory (with sporadic cases reported in eastern Australia), suggesting an enigmatic ecological niche. Multilocus sequence typing (MLST) and whole-genome sequencing (WGS) provided supportive evidence that the transfer of koalas from Western Australia and subsequently between several facilities in Queensland spread VGII into uncontaminated environments and environments in which predominantly *C. gattii* VGI was endemic. It is hypothesized that this represents a founder event following the introduction of a koala from Western Australia, perhaps suggesting a competitive advantage of *C. gattii* VGII over VGI in this captive koala environment. More recently, a koala with mixed nasal mucosal colonization by VGI and VNI developed clinical signs of cryptococcal meningitis caused only by the VNI strain. This is the second documented case of cryptococcosis in a koala caused by the *C. neoformans* species complex, and it suggests a complex early pathogenesis of the disease. The ability of animals to serve as sentinels of cryptococcosis and the often-demonstrable progression from exposure through colonization and subclinical to clinical disease provides an important window on the pathogenesis of the disease that molecular epidemiology can help to elucidate.

Mon P6 – Developing new antifungals in an Academic - Industry partnership.

Manu De Rycker

University of Dundee, University of Exeter and GSK antifungals consortium
Drug Discovery Unit, University of Dundee

Cryptococcal meningoencephalitis (CM) has a major health impact, mainly in sub-Saharan Africa, with limited treatment options available. Current treatments suffer from multiple limitations, including long duration, toxicity, parenteral administration, resistance and poor availability. There is therefore an urgent need to develop new, better treatments. The Drug Discovery Unit at the University of Dundee, the Centre for Medical Mycology at the University of Exeter and GSK global health have joined forces to tackle this challenge. In a 5-year Wellcome-funded programme we seek to deliver new pre-clinical candidates for CM that act through novel mechanisms. In this presentation we will cover the rationale behind our partnership and describe the overall approach of the programme. A key focus in the initial stages of the programme has been the development of cell-based *Cryptococcus neoformans* assays that are suitable for drug discovery, in terms of throughput, and, more importantly, in terms of predicting in vivo and patient outcomes. We have developed high-throughput screening assays that represent the in vivo morphotype heterogeneity of *Cryptococcus* as well as assays that assess fungicidal activity, including assays that are able to detect complete cidal activity (i.e. all cells killed). We have used these assays to carry out a high-throughput screening campaign and have identified new compounds with activity against *Cryptococcus neoformans*. In conclusion, our partnership has brought together all the skills and expertise required to successfully deliver much needed new preclinical candidates for cryptococcal infections, and has put in place key tools to achieve this.

Flash Talks

Tonia Dargham - *Characterization of extracellular vesicles diversity in Cryptococcus neoformans: A new avenue for identifying disease markers and understanding host interaction.*

Zar ni San - *Co-culture with Mycobacterium tuberculosis causes remodelling of Cryptococcus gattii morphology and cell wall architecture.*

Isabelle Mouyna - *Regulation of extracellular vesicle biosynthesis by the TOR pathway in the fungal pathogen Cryptococcus neoformans*

Jackie Spieles - *Cryptococcus spore germination can occur in the absence of oxygen*

Kisakye Diana Kabbale - *Cryptococcus neoformans Sequence Type 93 is Associated with Reduced Type-1 Immune Responses in Ugandan Patients with HIV-associated Cryptococcal Meningitis*

Concurrent Session 1

Mon C1.1 – Customized care for people with cryptococcal antigenemia

Radha Rajasingham

nan

Mon C1.2 – Birth and clinical outcomes of pregnant women with Cryptococcal Antigenemia in outpatient clinics in Uganda

ELIZABETH NALINTYA

Elizabeth Nalintya¹, Alexandra Poeschla², Ann Fieberg³, Patricia Nerima¹, Edith Ajilong¹, Tessa Adzemovic¹, Faridah Nakiganda¹, Elli Schwartz², Biyue Dai³, David Meya¹, David R Boulware⁴, Radha Rajasingham⁴

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Introduction: Clinical trials for the treatment and prophylaxis for cryptococcal meningitis have historically excluded pregnant persons. As a result, clinical and pregnancy outcomes for pregnant persons with HIV-associated cryptococcal infection are unknown. We evaluated clinical and pregnancy outcomes among pregnant participants with cryptococcal antigenemia. **Methods:** We prospectively enrolled adults with advanced HIV disease (defined as CD4 $\leq$ 200 cells/ μ L) within the ENCORE clinical trial (NCT05085171). Enrollment is ongoing at 10 of the 26 participating outpatient HIV clinics in Uganda. We present an analysis of baseline characteristics, opportunistic infections, birth outcomes, and clinical outcomes among pregnant participants. **Results:** Between May 2022 and April 2025, 114 pregnant participants with advanced HIV disease were enrolled, of whom 45% were antiretroviral therapy naive, median CD4 cell count was 124 cells/ μ L, and 42% were in their second trimester. Pregnant participants were less likely to be CrAg positive (2% vs. 7%, $p=0.023$) and less likely to be TB LAM positive (11% vs. 28%, $p<0.001$) compared with non-pregnant female participants. One CrAg-positive pregnant participant was enrolled at eight weeks gestation, received fluconazole for 24 weeks and delivered at term. The other participant was CrAg SQ 3+ at 16 weeks gestation, received 5 doses of weekly Amphotericin B deoxycholate (1mg/kg) without fluconazole, and delivered at term. **Conclusions:** Among pregnant participants with advanced HIV disease, prevalence of cryptococcal antigenemia was 2%, significantly lower than that of non-pregnant participants. Antifungal therapy was used with favourable maternal outcomes.

Mon C1.3 – Challenges and missing links to optimize nursing care for persons with HIV-associated meningitis in a resource-constrained setting

Jane Frances Ndyetukira

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Background and aims: Nursing care is a vital component of managing persons with HIV-associated meningitis. We sought to describe the challenges faced during the nursing care of these patients, highlighting the gaps and recommendations to optimize nursing care in a resource-constrained setting. **Methods:** We included and interviewed ten nurses as key informants who were routinely involved in providing care for persons with HIV-related meningitis during nine clinical studies conducted between 2010 and 2025. **Results:** The human resource and infrastructure limitations include understaffing, limited knowledge, insufficient supplies, lack of space for counselling, limited bed space in hospital wards, limited number of physiotherapists for rehabilitation, and poor patient tracking post-discharge. The clinical and treatment-related complications include delayed diagnosis/treatment, raised intracranial pressure, Immune Reconstitution Inflammatory Syndrome, relapse, poor infection prevention and control, pill burden and multiple drug side effects. The socio-economic limitations include limited patient education, lack of patients' social support, myths/misconceptions about medical procedures, religious beliefs, HIV stigma and non-disclosure of HIV status, patient/caregiver challenges and poverty of patients. The major missing links include the need to recruit more nurses, enhance

training programs for nurses, empower nurses to gain expanded skillsets, ensure resource availability, provide more bed space, improve infection prevention and control, promote community awareness on HIV-associated meningitis, reinforce adherence to treatment guidelines, training caretakers about medication management and patient hygiene, and mentor nurses in research. Conclusions: Patients with HIV-associated meningitis require extra nursing care, including community engagement. Addressing the missing links will help to improve treatment outcomes.

Mon C1.4 – Evaluation of Laboratory Checklists for Protocol-Scheduled Testing in HIV-Associated Meningitis Clinical Studies.

Asmus Tukundane

Abduljewad Wele (3), Jonathan Derrick Lukubuya (4), Morris K. Rutakingirwa (1), Bakka Wilber (1), Suzan Mulwana (1), Ciara Featherly (3), Mable Kabahubya (1), Babiye Dora (1), Kiiza Tadeo Kandole (1), Caleb Skipper (3), David Boulware (3), Mufti Suhail Sayeed (2), David B. Meya (1,3), Richard Kwizera (1)

Makerere University Infectious Diseases institute -Kampala Uganda.

Background and aims: Clinical studies are vital in improving outcomes for individuals with HIV-associated meningitis, but often face protocol deviations during biospecimen collection, such as missed or poorly documented samples. This study evaluated the effectiveness of a laboratory checklist in improving adherence to protocol-specified testing in an ongoing HIV-associated meningitis clinical study.

Methods: A mixed-methods design was used at Mulago and Kiruddu Hospitals, Uganda. Staff surveys assessed checklist usability and workflow integration, while laboratory records were reviewed to compare adherence to sample collection schedules at days 0, 7, and 14 before and after checklist implementation.

Results: Baseline adherence was 100%. Blood sample collection generally improved from 27% to 52% ($p=0.003$). Complete blood count (CBC) on day 14 increased from 27% to 50% ($p=0.008$). Baseline CD4 testing rose from 88% to 98% ($p=0.021$). Cerebrospinal fluid collection remained low (Day 7: 51% to 38%; Day 14: 19% to 22%). Among 21 staff surveyed, over half had 4-6 years of experience. Most reported prior checklist use (71%), formal training (62%), and confidence in checklist use (52%). Although 76% agreed that checklists reduce protocol deviations, challenges included limited time and inconsistent checklist availability (43%), with heavy workload being the main barrier (67%). Suggested improvements included workflow integration (81%), staff participation in development (76%), and regular training (62%).

Conclusions: The laboratory checklist improved adherence to protocol-defined blood-based tests, particularly at follow-up visits. Sustainable use requires workflow integration, staff engagement, consistent availability, and ongoing training.

Mon C1.5 – Mortality among individuals with subclinical cryptococcal meningitis identified through cryptococcal antigen (CrAg) screening programs in South Africa and Tanzania

Sile Molloy

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City St George's, University of London

Background Up to 40% of blood cryptococcal antigen (CrAg)-positive individuals have subclinical cryptococcal meningitis (CM), yet clinical outcomes for this understudied subgroup remain unclear. While current guidelines recommend intensive treatment for all individuals with CM, evidence is needed to inform risk-stratified management strategies. **Methods** In South Africa and Tanzania, we

prospectively followed-up 2 cohorts of adults with CD4 counts <100 cells/μl: 1) subclinical CM (CSF CrAg-positive) and 2) symptomatic CM. Baseline characteristics and outcomes over 6 months were recorded. Results From November 2022 to October 2025, 62 adults with subclinical CM and 364 with symptomatic CM were enrolled. Median age was 39 years. Seven days of amphotericin B plus flucytosine was the most commonly administered induction treatment in both cohorts (71% and 67%, respectively). Subclinical CM cohort: 68% (42/62) were ART-experienced. Median baseline opening pressure (OP) was 15cmH₂O (IQR: 11-27, n=32). Two-week, 10-week and 6-month mortality was 8.1% (95%CI: 2.7-17.8, 5/62), 35.5% (95%CI: 23.7-48.7, 22/62) and 40.3% (95%CI: 28.1-53.6, 25/62), respectively. Symptomatic CM cohort: 58% (201/344) were ART-experienced. Median baseline OP was 28cmH₂O (IQR: 18-40, n=78). Two-week, 10-week and 6-month mortality was 21.7% (95%CI: 17.6-26.3, 79/364), 40.9% (95%CI: 35.8-46.2, 149/364) and 44.5% (95%CI: 39.3-49.8, 162/364), respectively. Discussion The majority of opening pressures were normal at baseline among the subclinical CM patients, with fewer CM-related deaths than in the symptomatic cohort, as indicated by a lower 2-week mortality. However, 10-week and 6-month mortality was similar between the cohorts. Alternative risk-stratified management approaches may be considered for these patient groups.

Mon C1.6 – Cryptococcal antigenaemia screening and treatment: observations from the field in South Africa (CAST OFF-SA)

Rhys Wenlock

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Background and aims Early detection and treatment of cryptococcal antigenaemia (CA) among patients with advanced HIV (defined as CD4 count <200 cells/μl) prevents deaths due to cryptococcal meningitis. Since 2016, a laboratory-based reflex screening programme in South Africa automatically tests all individuals with CD4 plasma/serum samples <100 cells/μl for cryptococcal antigen (CrAg). Although 92% of eligible people have been screened, it is unclear whether cryptococcal meningitis incidence and mortality have reduced. We aimed to investigate barriers to screening implementation and propose opportunities for improvement. **Methods** We conducted 21 focus group discussions and 112 questionnaires with clinic staff in 4 provinces to explore their knowledge of CA and barriers to care for patients following a positive result. We conducted interviews with 14 investigators of a CA treatment trial (EFFECT, ISRCTN 30579828) to explore its impact on patient care. **Results** Clinic staff described fragmented, poorly defined pathways to care for patients following a positive reflex CrAg test. Key barriers included lack of standardised result notification systems, unclear clinical ownership of results, difficulty tracing and referring patients for lumbar puncture, and limited confidence in CA management. Consequently, positive results were frequently missed or acted upon only weeks to months later. EFFECT investigators described potential solutions including creation of regional roles dedicated to linking patients to care following a positive reflex CrAg test and using text messaging to improve notification systems. **Conclusions** Insights from clinic staff and EFFECT investigators could be used to inform interventions that improve the impact of CA screening in South Africa.

Mon C1.7 – Low CSF glucose is associated with increased CNS inflammation in adult patients with HIV-associated cryptococcal meningitis: a prospective cohort study.

Julia Palmucci

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Background Low cerebrospinal fluid (CSF) glucose is a prognostic marker in several meningitis etiologies including tuberculosis, but its role in HIV-associated cryptococcal meningitis is less defined. We investigated if CSF glucose is associated with cryptococcal outcomes. **Methods** We conducted a prospective cohort study of 1,103 adults with HIV-associated cryptococcal meningitis in Uganda from January 2019 to May 2025. We grouped participants by CSF glucose into tertiles: low <45 mg/dL n=311, medium 45-74 mg/dL n=414, and high >75 mg/dL n=361. We assessed for baseline associations with CSF parameters and outcomes. **Results** Participants with low CSF glucose were more likely to present with altered mental status (GCS<15; P=0.001), higher CSF white cells (P<0.0001), and higher CSF protein (P<0.0001). Sixty percent (60%) of participants with low glucose had CSF white cell counts >5, compared to 45% and 28% among those with medium and high glucose, respectively (P<0.001). Flow cytometry (n=189) revealed that the low CSF glucose group had 4-fold more CSF CD4 cells (x=624 vs 160 cells/mL, P=0.024), more PD-1+ CD4 exhaustion (67% vs 49%, P<0.001), and lower monocyte activation (HLA-DR+, CD163+) compared to CSF glucose >45 mg/dL. CSF glucose showed a weak inverse correlation with baseline fungal burden (r=-0.10; P=0.0016), but glucose was not associated with in-hospital mortality, CSF early fungicidal activity, or CSF sterility. **Conclusion** Low CSF glucose was associated with severe clinical disease and CNS inflammatory response. A variety of mechanisms likely contribute to low CSF glucose, including increased metabolic activity in the brain due to infiltrating leukocytes and microbes.

Mon C1.8 – To CT or Not CT? That is the question, but LP is the answer

Enock Kagimu

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Background: Lumbar puncture (LP) is required for diagnosis in those suspected of having meningitis, but carries risks. The utility of pre-LP brain imaging to identify space-occupying lesions and prevent adverse events is debated.

Methods : We prospectively enrolled Ugandan adults with HIV-associated meningitis from August 2013 to November 2024. All participants received a diagnostic LP upon hospital admission without prior head CT. Death within 24 hours post-LP served as a surrogate marker for assessing post-LP complications. Risk factors, including baseline laboratory results, focal neurological deficits, Glasgow Coma Scale (GCS) score, brain imaging findings, and meningitis etiology, were assessed.

Results: Of 3,552 participants, 83 (2.3%) died within 24 hours. GCS≤8 (16% (13/83) vs survivors, 4% (138/3469; p<0.001), higher median baseline CSF opening pressure (392 mmH₂O [IQR: 173–542] vs survivors 200 mmH₂O [IQR: 125-300], p<0.001), and focal neurologic deficits 20% (16/80) vs survivors 10% (360/3,444; p=0.009) were associated with death within 24 hours post-LP. Focal neurologic deficits were associated with increased odds of 24-hour mortality (unadjusted OR=2.08, 95%CI 1.15–3.53; adjusted OR=3.10, 95%CI, 1.21–7.29). Of those with CNS imaging, 221 had a relative contraindication to LP on imaging, of whom 0.9% (2/221) died within 24 hours of LP versus 0.8% (2/259) mortality among those whose imaging showed no contraindication (p=>0.99)

Conclusion: In persons with HIV-associated meningitis, GCS<8 and focal deficits on clinical examination were associated with death within 24 hours; brain imaging findings contraindicating LP weren't. Few brain imaging studies were done in those who died within 24 hours.

Concurrent Session 2

Mon C2.1 – Trehalose phosphate synthase 1 (tps1) deficient *Cryptococcus neoformans* alters host murine adaptive immune response

Kristie Goughenour

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University of Michigan and Lieutenant Colonel Charles S. Kettles VA

Trehalose phosphate synthase 1 (tps1) deficient *Cryptococcus neoformans* alters host murine adaptive immune response

Abstract: The trehalose biosynthetic enzyme, trehalose-6-phosphate synthase (TPS1) is a virulence factor and a promising therapeutic target. Previously we established that the loss of TPS1 renders *C. neoformans* vulnerable to resident and innate pulmonary defenses. Unlike the lethal WT (H99), the TPS1-deleted (tps1 Δ) strain is rapidly cleared from the infected lungs in a CD4⁺ T cell independent manner. While CD4⁺ T cells were dispensable for tps1 Δ -clearance, we investigated if tps1 Δ could trigger a vaccine-like modulation of the adaptive immune response, further enhancing antifungal protection. Mice were exposed to 2 live-doses of tps1 Δ intranasally 14 days(D) apart, then challenged with the *C. neoformans* H99 (D0) after a 14D recovery. tps1 Δ -exposure substantially improved 70-D-survival (from 0-70%) and reduced brain and spleen dissemination, compared to the SHAM-exposed, infected mice. tps1 Δ -exposure generated an enhanced early (D7) pulmonary CD4⁺ T cell response and a protectively polarized T cell signature was observed. This increased frequency of protectively (Th1/Th17)-polarized T cells was sustained at D21. Depletion of CD4⁺ T cells during the WT challenge significantly ablated this protection, supporting CD4⁺ T cells as the mechanism of adaptive immune protection in mice exposed to Trehalose-deficient *cryptococcus*. As such, in addition to serving as a promising drug target, TPS1 inhibition can both enhance the innate response and adaptive anticryptococcal defenses in a vaccine-like manner, allowing for multi-factorial benefits of targeting TPS1 for therapeutic purposes.

Mon C2.2 – A novel CpG-adjuvanted heat-killed *Cryptococcus* vaccine induces superior immune protection in an intramuscular model

Yu Zhang

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Rutgers University

Vaccines have been transformative in controlling infectious diseases. However, despite the rising global burden of invasive mycoses, no fungal vaccine is available for clinical use. Hence, there is a great unmet need to develop vaccines against fungal infections, including cryptococcosis. We previously developed a whole-cell vaccine candidate based on heat-killed *C. neoformans* fbp1 Δ mutant cells (HK-fbp1), which provided complete protection against cryptococcosis following intranasal immunization by inducing durable pulmonary immunity. In this study, we developed a highly effective intramuscular and subcutaneous vaccine (HK-fbp1/ODN) by incorporating a CpG adjuvant into HK-fbp1. The adjuvanted formulation conferred robust protection against virulent *C. neoformans* challenge, achieving near-sterilizing immunity in multiple mouse strains. Immunological analysis revealed that intramuscular HK-fbp1/ODN vaccination promoted early recruitment of monocytes, monocyte-derived dendritic cells, and neutrophils to the lung, accompanied by strong CD4⁺ T cell activation. Vaccine protection was associated with a spatially organized T cell response, characterized by proliferative priming in muscle-draining lymph nodes and potent Th1/Th17 effector cytokine production in lung-draining lymph nodes. IFN- γ was essential for vaccine-mediated protection, whereas other interferons were dispensable. Notably, HK-fbp1/ODN vaccination remained protective in mice lacking CD4⁺ T cells or B cells, underscoring its potential utility in immunocompromised hosts. Collectively, this study established a CpG-adjuvanted whole-cell cryptococcal vaccine that elicits coordinated innate and T cell-mediated immunity and is compatible with standard parenteral delivery, providing a strong foundation for the development of clinically relevant antifungal vaccines.

Mon C2.3 – Enzymatic Inactivation of Fluconazole Defines a Novel Resistance Pathway in *Cryptococcus* spp.

Daniel A. Santos

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Recently, an increasing body of evidence has demonstrated that agrochemicals play a significant role in the development of fungal resistance to clinical antifungal agents. Our studies revealed that the adaptation of *Cryptococcus neoformans* and *Cryptococcus gattii* to azole fungicides, strobilurins, and β -tubulin inhibitors alters fluconazole susceptibility and modulates host-pathogen interactions. These findings emphasize the importance of developing a comprehensive understanding of additional resistance mechanisms. Resistance in fungi is commonly driven by the overexpression of efflux pumps, gene mutations, and chromosomal alterations that reduce treatment efficacy. Adopting an integrative approach combining molecular biology, biochemistry, and genomics within a One Health framework, we investigated whether *Cryptococcus* species are able to enzymatically inactivate fluconazole, a key therapeutic agent for treating cryptococcosis. Resistome and gene expression analyses revealed an increased number of genes coding for acetyltransferases, which were overexpressed in response to agrochemical exposure. Strains overexpressing these enzymes convert fluconazole into O-acetyl-fluconazole, a derivative that cannot bind to 14- α -demethylase and therefore lacks antifungal activity. Remarkably, *Cryptococcus* spp. strains recently isolated from the cerebrospinal fluid of cryptococcosis patients were also found to acetylate fluconazole, confirming the clinical relevance of this mechanism. Furthermore, chemical inhibition of acetyltransferases reduced fluconazole acetylation and restored its antifungal activity, suggesting that these enzymes could be targeted in the development of new drugs. Collectively, our findings provide the first evidence that *Cryptococcus* spp. can chemically modify and inactivate fluconazole through enzymatic acetylation.

Mon C2.4 – Phenotypic landscape of *Cryptococcus neoformans* drives mechanistic interrogation of virulence factors

Michael Boucher

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Cryptococcus neoformans is a pathogenic basidiomycete yeast that diverged from well-studied fungi in the phylum Ascomycota at least 450 million years ago. Defining functions for basidiomycete-specific genes is essential for deciphering mechanisms of cryptococcal pathogenesis, yet doing so at the genomic scale remains challenging. We performed massively parallel fitness screens of 4328 single-gene *C. neoformans* deletion strains in 141 unique in vitro growth environments and in a mouse infection model. In vivo screens revealed 574 fungal genes required for fitness during murine pulmonary infection. Hierarchical clustering of mutant fitness profiles produced a phenotypic atlas of this organism, reconstructing core molecular pathways and assigning validated cellular roles to unstudied genes. Integrative analysis of these datasets revealed a novel virulence gene, RGH1 (Required for Growth in Host-like conditions), uniquely required for fitness during tissue culture and in vivo infection. Concordantly, RGH1 transcription is massively induced upon shifting to host-like growth conditions. We pursued the function of this factor through a genome-scale CRISPR/Cas9 suppressor screen and affinity purification-mass spectrometry experiments, uncovering a putative role in cell wall α -glucan biogenesis. Together, our results suggest a model in which Rgh1 is actively deployed upon host entry to drive adaptive cell wall remodeling that is critical for tolerating infection-relevant stressors. This work highlights the power of high-throughput genetics in linking unbiased discovery with mechanistic studies, providing an experimental framework for interrogating uncharacterized fungal virulence determinants.

Mon C2.5 – Rab20 conveys immune signaling to the cryptococcal-containing phagosome during *Cryptococcus* infection of macrophages.

Felipe H. Santiago-Tirado

Peter V. Stuckey and Georgina Agyei

University of Notre Dame

Cryptococcus neoformans causes ~200,000 deaths yearly, mostly in immunocompromised individuals. It is acquired through inhalation and lung macrophages are the main line of defense. The interactions with these cells will determine the outcome of the infection, influencing if the lung macrophages will serve as safe haven for replication and promote extrapulmonary dissemination, both of which are associated with mortality. The mechanisms *C. neoformans* use to mediate this intracellular parasitism and how it is influenced by the immune response are not fully understood. Therefore, understanding these interactions and elucidating the properties of the cryptococcal-containing phagosome (CCP) are critical steps needed to improve the management of this disease. We have identified three distinct populations of CCPs based on acidification patterns: those that remain acidic, those that lose acidity, and those that never acidify. Unlike phagosomes containing *S. cerevisiae*, CCPs exhibit significant phagosomal membrane damage and maturation delay. Moreover, a subpopulation of CCPs display both early endosome (PI3P) and lysosome (LAMP1) markers, suggesting that *C. neoformans* can alter its phagosome and provides a potential mechanism for intracellular survival that may be driving cryptococcal pathogenesis. Notably, when macrophages are stimulated with interferon- γ , acquisition of lysosomal markers occurs more rapidly and more CCPs acidify. Host Rab20 has been shown to be interferon- γ -inducible and appears to be critical for this promotion of phago-lysosomal fusion as knockdown of Rab20 delays maturation of phagosomes containing non-pathogenic cargo. Therefore, Rab20 may be linking host immune status and CCP properties during infection, playing a critical role in cryptococcal infection outcome.

Mon C2.6 – Investigating interactions between macrophage ridges and *Cryptococcus neoformans*

Rebecca Hodges

Simon A Johnston 1, Rhoda Hawkins, Jaime Canedo 1

School of Medicine and Population Health, Florey institute, Bateson Centre - The University of Sheffield

Cryptococcus neoformans (Cn) is an opportunistic pathogen causative of serious infection of the central nervous system and the primary causative agent of cryptococcal meningitis (CM), a lethal infection predominantly affecting people living with HIV. We hypothesise that increasing phagocytosis of Cn by macrophages is a potential adjunctive therapy. We and others have extensively characterised the parasitism of macrophages by Cn, including in-macrophage growth. However, phagocytosis of Cn by macrophages is essential for surviving infection in both protective and susceptible animal models of cryptococcosis. This in vitro/vivo disparity was explained by us directly measuring macrophage parasitism in vivo in zebrafish demonstrating reduced overall fungal burden despite in-macrophage growth, even in a susceptible model. In agreement with this, human susceptibility to CM results from loss of in macrophages that can phagocytose cryptococci. In CM, the levels of macrophage-targeting in phagocytosis are a positive predictor of survival (independently of anti-fungal treatment). Thus, experimental and clinical data support our hypothesis that phagocytosis is key to surviving life-threatening infection and may be a therapeutic target for CM. Therefore, we have sought new ways to increase the phagocytosis of Cn and have discovered sub-micron actin-based ridges on the macrophage surface which are used to bind and phagocytose cryptococci. However, on exposure to Cn, macrophage ridge formation is attenuated, we aim to elucidate the mechanism by which Cn modulates this interaction.

Mon C2.7 – Investigating novel lectibodies against the capsule of human fungal pathogen *Cryptococcus neoformans*

Christopher Lee

Jie Jiao, François Le Mauff, Michael Lehky, Elise Bedford, Xianya Qu, Cassie Zhong, Stefan Mereiter, Jack Paransky, Sebastian Dawo, Donald Sheppard, Josef Penninger, James Kronstad

Michael Smith Laboratories

The capsule of *Cryptococcus neoformans* remains the most prominent virulence factor responsible for evasion of the host immune system. Unfortunately, no preventive or curative treatments have been successful in targeting the capsule. Novel antibody-lectin hybrid molecules, termed lectibodies, have been shown to mediate immune clearance of cancer cells and virus-infected cells thanks to the recognition of aberrant glycosylation patterns presented by the cells. We therefore hypothesize that lectibodies targeting the fungal capsule could represent a new therapeutic avenue to alleviate patient suffering from cryptococcal infections. We conducted a screen of 66 purified human recombinant lectibodies against encapsulated *C. neoformans* cells and identified four candidate lectibodies binding to the outer capsule in a calcium dependent manner. Specificity testing of the four hits revealed a preferential recognition of *C. neoformans* serotype A. However, no impact on cryptococcal growth or capsule elaboration was observed in culture. The ability to facilitate phagocytosis by host bone marrow derived macrophages was investigated in a tissue culture system and we found that phagocytosis of fungal cells was significantly increased upon opsonization with the lectibodies. These results were consistently observed with human THP-1 monocyte derived macrophages, as well as BV-2 murine microglial cells. These results suggest that novel lectibodies may play a critical role in the host recognition of *C. neoformans* during infection, paving the way for exploiting these recognitions to develop innovative immunotherapy options.

Mon C2.8 – Longitudinal in vivo bioluminescence imaging reveals a protective role for MARCO deficiency in cryptococcal clearance and fluconazole efficacy

Greetje Vande Velde

Lander De Herdt (1), Lauren Michiels (1), Agustin Reséndiz-Sharpe (1), Chinaemerem U Onyishi (2,3), Laura Seldeslachts (1), Tine Van Win (4), Kasia Błażejczyk (1), Lara Roosen (1), Flore Belmans (1), Katrien Lagrou (4,5), Subhankar Mukhopadhyay (6), Siamon Gordon (7,8), Robin C. May (2), Greetje Vande Velde (1)

KU Leuven

Background: *Cryptococcus neoformans* is an opportunistic fungal pathogen that can disseminate from the lungs to the CNS, causing life-threatening meningoencephalitis. The role of cryptococcal phagocytosis from pulmonary macrophages in dissemination remains unclear. Macrophage Receptor with Collagenous structure (MARCO) was recently identified as a potent negative regulator of cryptococcal phagocytosis in vitro, with its absence strongly enhancing expulsion rates. Here, we investigated the in vivo role of MARCO in cryptococcal infection and fluconazole responsiveness. Methods: Wild-type and MARCO-KO C57BL/6 mice were orotracheally infected with a low (50 cells) or higher (5000 cells) inoculum of luciferase-producing KN99α *C. neoformans*. Fluconazole 80 mg/kg/day was started 1.5 days post-infection and switched to 40 mg/kg/day after 8 days. To non-invasively track real-time fungal burden over 4.5 weeks, we developed a novel bioluminescence imaging (BLI) method using 500 mg/kg D-luciferin. Results: MARCO deficiency enhanced fluconazole's antifungal efficacy, significantly reducing brain dissemination and fungal burden compared to wild type controls. MARCO-KO mice also cleared low inoculum pulmonary infection more effectively, delaying or preventing CNS spread, but showed no protection at higher fungal inocula unless combined with fluconazole. These effects are potentially driven by increased extracellular fungal exposure via enhanced phagocytosis in MARCO-KO mice, improving antifungal access. Conclusion: Our findings identify MARCO as a host factor influencing early cryptococcal clearance and antifungal potentiation and highlight longitudinal BLI as a powerful tool for studying infection and treatment dynamics in vivo. Combining host-directed MARCO targeting with antifungal therapy may offer a novel strategy to prevent cryptococcal dissemination, warranting further investigation.

Concurrent Session 3

Mon C3.1 – Phospholipid homeostasis in host-Cryptococcus interactions during pulmonary cryptococcosis

Chaoyang Xue

Siddhi Pawar, Robert Tancer, Yina Wang, Varsha Gadiyar, Raymond Birge
Rutgers University

Cryptococcus neoformans is the leading cause of life-threatening fungal meningitis. Macrophages are the first line of defense against *Cryptococcus* infection, yet the molecular mechanisms underlining macrophage-*Cryptococcus* interaction remains incompletely understood. Our previous study demonstrated that loss of lipid flippase function by deleting CDC50 gene in *C. neoformans* leads to increased accumulation of exocytosomal phosphatidylserine (PS) on the plasma membrane, enhanced phagocytosis and macrophage killing, resulting in avirulence in animal infection models. Notably, this PS-driven phagocytosis occurs independently of the host PS receptor MertK. Our recent findings reveal that *cdc50Δ* cells produce significantly more extracellular vesicles (EVs) with elevated surface PS levels compared to wild-type H99. Both *cdc50Δ* cells and their EVs exhibited increased production of multiple phospholipid species, including PS and PE, as shown by our lipidomic analysis. Furthermore, supplementing H99 culture with *cdc50Δ* EVs during macrophage-H99 interaction markedly increases in H99 phagocytosis, indicating that *cdc50Δ* EVs can enhance the fungicidal activity of macrophages. Collectively, our data demonstrate that fungal lipid homeostasis is crucial for *C. neoformans* resistance to macrophage recognition and clearance during early host-pathogen interactions in cryptococcosis. Lipid flippase function emerges as a potential drug target, and we have developed a peptide-based inhibitor that shows potent activity against *Cryptococcus*.

Mon C3.2 – Defining Mechanisms of Cryptococcal Sterol Transport

Dong-gyu Kim

Tamara L. Doering
Washington University in St. Louis

The critical membrane lipid ergosterol must distribute between its site of synthesis and key membranes. Specialised lipid transport proteins coordinate the movement of sterols and other lipids at membrane contact sites, sub-30 nm spaces between adjacent membranes. These proteins represent an unexplored aspect of cryptococcal ergosterol biology that may reveal pharmacologically exploitable processes. Accordingly, we sought novel or understudied proteins that influence cryptococcal sterol homeostasis. To do this we screened for spontaneous mutants that suppress the growth defects of cells lacking the sterol transporter Ysp2. Whole genome sequencing of suppressors revealed numerous nucleotide polymorphisms in coding regions directly relevant to sterol biosynthesis, regulation, and transport; this strongly validated the utility of our screen for studying sterol homeostasis. Excitingly, one unstudied gene encodes a protein with a predicted phospholipid ligand-binding domain, a feature linked to lipid transporters. We hypothesised that this protein affects sterol transport indirectly, possibly through a mechanism involving a phospholipid ligand. Subsequent characterization revealed roles of the gene in lipid homeostasis, cell morphology, stress tolerance, and virulence, while microscopy suggests the encoded protein localises at ER-plasma membrane contact sites. Further, in vitro studies of the exogenously expressed protein suggest a non-sterol lipid ligand. These results support our working model that this novel protein associates with membrane microdomains enriched with its lipid ligand and influences sterol transport through a non-sterol transport mechanism. Current studies aim to further define its role in sterol transport and potentially inhibit its activity. Overall, this work will illuminate the fundamental process of sterol transport.

Mon C3.3 – Targeting membrane asymmetry reveals non-canonical vulnerabilities in *Cryptococcus neoformans*

Xuefei Chen

Xuefei Chen, H. Diessel Duan, Michael J. Hoy, Kalinka Koteva, Michaela Spitzer, Allison K. Guiton, Emily Puumala, Aline A. Fiebig, Guanggan Hu, Bonnie Yiu, Sommer Chou, Zhuyun Bian, Yeseul Choi, Amelia Bing Ya Guo, Wenliang Wang, Sheng Sun, Nicole Robbins, Anna Floyd Averette, Michael A.

Cook, Ray Truant, Lesley T. MacNeil, Eric D. Brown, James W. Kronstad, Brian K. Coombes, Leah E. Cowen, Joseph Heitman, Huilin Li., and Gerard D. Wright

The Ineos Oxford Institute for Antimicrobial Research and Department of Biology, Oxford University

Background and aims: The fungal pathogen *Cryptococcus neoformans* exhibits distinctive features of cell surface organisation that contribute to stress tolerance, immune evasion, and antifungal resistance. However, how membrane lipid asymmetry and higher-order membrane organisation shape *Cryptococcus* cell biology remains poorly understood. We aimed to define the cellular consequences of disrupting membrane asymmetry in *C. neoformans*.

Methods: We employed butyrolactol A (butA), a membrane-active natural product, as a chemical probe to perturb phospholipid asymmetry. Genetic analysis, resistance evolution, lipid profiling, and cell biological assays were integrated with microscopy-based approaches to examine membrane organisation, cell surface integrity, and stress responses in *Cryptococcus*.

Results: Perturbation of the phospholipid flippase complex Apt1-Cdc50 by butA leads to profound reorganisation of membrane lipids and loss of membrane asymmetry. These changes compromise cell surface integrity, alter sensitivity to cell wall stress, and expose conditional vulnerabilities to antifungal challenge. Notably, resistance analyses reveal a critical requirement for glucosylceramide-enriched membrane domains, uncovering a functional link between lipid asymmetry, membrane microenvironments, and cellular fitness in *C. neoformans*. **Conclusions** Our findings demonstrate that membrane asymmetry is a central determinant of *Cryptococcus* cell biology and stress adaptation. By using membrane-active natural products as mechanistic probes, this work highlights how lipid organisation governs fungal cell surface function and reveals non-canonical vulnerabilities that emerge from perturbing membrane homeostasis.

Mon C3.4 – The role of Eisosomal proteins in pathogenesis of *Cryptococcus neoformans*

Lukasz Kozubowski

Tejas Mahendra Patel

Clemson University

Cell wall and plasma membrane (PM) contribute to fungal pathogenicity. PM-associated eisosome complex plays key roles in PM organization, stress response, and cell wall synthesis. Eisosomes have been described in the ascomycetes phylum; however, no study has been conducted yet to understand eisosome's role in *Cryptococcus neoformans*. In *Saccharomyces cerevisiae*, Pil1 and Lsp1 proteins are the core components of eisosomes that shape the curvature of the PM based on their conserved AH-BAR domains. Our results revealed two localization patterns for fluorescently tagged Pil1 and Lsp1 homologues in *C. neoformans*: the canonical punctate localization to the PM and a novel localization to the mother-bud neck. The pil1 Δ and lsp1 Δ mutants exhibited inverse sensitivity to various stress conditions suggesting an unexpected mechanism of how Pil1 and Lsp1 cooperate in stress response. Cells lacking Lsp1 exhibited fewer and more intense punctate signals of Pil1-GFP as compared to those found in the wild-type, suggesting a role of Lsp1 in eisosome formation and/or dynamics. Interestingly, elimination of Lsp1 had similar consequences on the localization of the septin Cdc12-GFP, which forms filamentous punctate assemblies at the PM specifically when cells are exposed to 37°C. As the well-described function of septins is at the mother-bud neck during cytokinesis, our results point to a novel connection between septins and eisosome components that involves PM homeostasis and cytokinesis. Strikingly, the double mutant (pil1 Δ lsp1 Δ) revealed defects in two major virulence factors, polysaccharide capsule and secretion of urease, pointing to a critical contribution of eisosomes to pathogenicity of *C. neoformans*.

Mon C3.5 – *Cryptococcus* surveillance in South Africa: Expanding from human disease surveillance to One Health surveillance

Serisha Naicker

In South Africa, national laboratory-based *Cryptococcus* surveillance focusing on human disease has been conducted since 2005. From 2005-2024, there were 122 403 episodes of laboratory-confirmed cryptococcal disease with an in-hospital case fatality ratio of 35% at enhanced surveillance sites. Fluconazole became available in South Africa since 2000, amphotericin B since 2005 and flucytosine

in 2018. All three agents are critical for treatment of cryptococcal meningitis. Most cryptococcal disease (97%) was caused by *Cryptococcus neoformans* and 3% of disease by *Cryptococcus gattii*. Fluconazole susceptibility testing of incident isolates revealed mostly low minimum inhibitory concentration (MIC) values, however, there was a trend toward decreasing susceptibility. This may be driven by exposure to azole-based fungicides in the environment. Flucytosine susceptibility testing on recurrent isolates revealed a low percentage (13%) of *C. neoformans* isolates with flucytosine MIC values $\geq 16 \mu\text{g/ml}$, possibly related to flucytosine exposure during induction treatment of the first episode. Molecular typing showed that VNI and VGIV were the most dominant molecular types. Two VGIV clusters consisting of South African patients from three neighbouring provinces suggested a similar environmental source. Surveillance in South Africa has focused on human disease over two decades. This shifts in 2026 when environmental samples will be collected in a new Wellcome Trust-funded project called *Cryptococcus Adaptation to causing human disease in Africa through pathogen thermotolerance (CryptoADAPT)*. This is a new collaboration that includes co-investigators from South Africa, Zambia, Mozambique, USA and the UK. A multidisciplinary team will be undertaking this project to firstly build capacity in Southern Africa to isolate and identify *Cryptococcus* from environmental samples, decode *Cryptococcus* genomes to determine virulence traits and measure the potential of *Cryptococcus* to adapt to specific stressors such as temperature.

Mon C3.6 – Cerebrospinal Fluid and Fungal Burden Associated Cellular Immune Responses in HIV-Related Cryptococcal Meningitis: From Sterile Fungal Cultures to Low and High Fungal Burden Disease

Samuel Okurut

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Research Department, Infectious Diseases Institute, Makerere University, Kampala, Uganda

Introduction: Cryptococcal meningitis is a major cause of serious meningitis in people with advanced HIV. While the disease is linked to inflammation, little is known about how the fungal burden relates to the immune response in the central nervous system-associated neuroinflammation.

Methods: We studied 211 consenting adults presenting with HIV-related cryptococcal meningitis. We characterized baseline CSF cellular immune responses using spectral flow cytometry. We grouped participants into three unique visualized groups using unsupervised UMAP and PCA CSF quantitative fungal culture clusters: sterile (n=21), with low fungal burden ($\leq 15,000 \text{ CFU/mL}$, n=77), and high fungal burden ($>15,000 \text{ CFU/mL}$, n=113) groups separated at approximately the median CFUs.

Results: Higher CSF fungal burden was inversely associated with lower peripheral CD4+ counts, CSF white cell counts by microscopy, and CSF protein (all; $P \leq 0.035$). Subsequently, CSF fungal burden was inversely associated with CSF total CD3+ leukocytes, CD4+ T cells ($r = -0.28$), natural killer cells ($r = -0.13$), neutrophils ($r = -0.17$), and dendritic cells ($r = -0.16$) as defined by flow cytometry (all Pearson r ; $P \leq 0.013$). Fungal burden was positively associated with CD4+ T cells expressing Th1-polarized CXCR3+ chemokine receptor (median 24% high culture, 13% low, and 15% sterile groups; $P = 0.048$), and immune checkpoint PD-1+ expression on CD8+ T cells (66% high culture, 61% low culture, and 53% sterile; $P = 0.009$).

Conclusion: Higher CSF quantitative fungal culture burdens were associated with reduced absolute CD4+ T cells, NK cells, neutrophils, and dendritic cells, yet elevated PD-1 checkpoint expression among CD8+ T cells, and elevations in CD4+ T cells expressing Th1-polarized CXCR3+ inflammation-activated chemokine receptor.

Mon C3.7 – IL-5R α deletion controls cryptococcal meningoencephalitis development

Lander De Herdt

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Biomedical MRI, Department of Imaging and Pathology, KU Leuven, 3000, Leuven, Belgium

Main: Cryptococcal meningoencephalitis (CM) is the result of CNS entrance of *Cryptococcus neoformans* (CN). Current treatment options are limited and result in long-lasting side-effects post-infection. Better understanding of the host immune response is necessary to develop immunomodulatory treatments.

Results: To uncover immunological drivers of CN extrapulmonary dissemination and CNS entrance, we used an intranasal mouse infection model, advanced in vivo microscopic and mesoscopic imaging, proteomics, and transcriptomics to build a 3D spatiotemporal map. We found that pulmonary infection induced eosinophil expansion in the early stage of disease. Degranulating eosinophils were recruited in airways and alveoli, affecting the integrity and morphology of the airway epithelium. Deletion of the eosinophil lineage protein IL-5R α resulted in lower CNS fungal burden. Mice lacking IL-5R α had significantly lower expression of proteins associated with eosinophil degranulation, activation, antimicrobial killing, and airway epithelium distress, indicating a detrimental role of eosinophil activation during pulmonary cryptococcosis. Interestingly, IL-5R α deficiency resulted in an increased IFN- γ response during lung infection prior to brain infection, but resulted in lower IFN γ levels once brain infection was established. This suggests an ambiguous role of IL-5R α regulated IFN- γ in a time dependent manner during CM development.

Future perspectives: We uncover IL-5R α as novel potential therapeutic target for cryptococcosis. To further develop IL-5R α targeting as immunomodulatory treatment strategy, we are unravelling the underlying molecular mechanism of IL-5R α expressing cells through scRNA-seq of bronchoalveolar lavage fluid, blood, and leptomeninges, with data analysis and validation of results with functional in vivo and in vitro experiments currently underway.

Mon C3.8 – Paradoxical microglial inactivation during cryptococcal infection of the brain

Amalia Awala

Maahir Kauchali^{1, 2,3}, Emily Ruth Higgitt^{1,2,3}, Anja de Lange^{1,2,3}, Alison Gifford⁵, Joseph V Raimondo^{2,4}, Rachael Dangarembizi^{1,2,3}.

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Cryptococcal meningitis (CM) is a fatal fungal infection of the brain and remains a leading cause of HIV-related mortality worldwide. Brain injury in CM has traditionally been attributed to excessive inflammation; however, the cellular mechanisms underlying cryptococcal neuropathogenesis remain poorly defined. Particularly unknown is how microglia, the resident immune cells of the brain, respond to brain invasion by *Cryptococcus neoformans* (*C. neoformans*), the causative agent for CM. Here, we investigated microglial-fungal interactions using complementary rodent and human brain-based experimental systems. Human and rodent organotypic brain slices (OBSs) were infected with a GFP/mCherry-expressing H99 strain of *C. neoformans*, and host-pathogen interactions were examined using immunofluorescent labelling of microglia (IBA1). Microglial activation was measured using nuclear localisation of the transcription factor, nuclear factor for interleukin-6 (NF-IL6): a robust marker for inflammatory signalling. Immune responses in *C. neoformans*-treated slices were compared to untreated controls and to slices treated with the canonical immunogen- lipopolysaccharide (LPS). Inflammatory responses were further validated using single-nucleus RNA sequencing and multiplex cytokine profiling. Microglia readily internalised *C. neoformans*; however, fungal uptake was not

accompanied by classical microglial activation. *C. neoformans* -treated slices showed minimal NF-IL6 activation, negligible pro-inflammatory cytokine release, and no induction of key inflammatory gene programs, comparable to untreated controls and markedly attenuated relative to LPS-stimulated slices. Our findings reveal a paradoxical microglial inactivation as a previously unrecognised feature of cryptococcal neuropathogenesis and suggest that immune silencing within the brain may contribute to fungal persistence and disease progression.

Tuesday June 30

Plenary Session

Tues P1 – Crypto Mining: A big data “pathogen prospecting” approach to identify new locales and niches for *Cryptococcus gattii*

David M. Engelthaler

W. Tanner Porter^{1,2}

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Background: Following the unexpected emergence of distinct populations of the *Cryptococcus gattii* complex in the temperate North American Pacific Northwest (PNW), numerous studies have explored new regions and identified new populations comprising novel species in other unexpected regions, including the desert southwest of North America. As the world is becoming increasingly digitized, data scanning techniques can help provide a further understanding where pathogenic fungi survive and thrive. Here we employ a big data “pathogen prospecting” approach to scan and validate the global repository of metagenomic datasets for evidence of unidentified populations of *C. gattii* complex. Methods: We leveraged >3.5 petabytes cloud computing space using Google BigQuery to conduct “pathogen prospecting” across the ~10 petabytes of sequence data in the NCBI Sequence Read Archive. We used NCBI’s STAT, a rapid MinHash-based k-mer tool to systematically screen the entire global sequence repository for the hidden presence of *C. gattii* complex members, along with other pathogenic environmental fungi. To verify positive samples, taxonomic classification we leveraged a suite of tools including PebbleScout, Kraken2, and genomic alignment programs. Results: A total of 23,877 individual metagenomic samples were identified to contain the presence of one or more target pathogen fungi. We further identified >650 distinct environmental metagenomic samples, including >100 marine or freshwater samples, that demonstrated mid- to high-level confidence evidence of *C. gattii* complex members, including presence in coastal waters of the PNW, off California, in the Mediterranean, and from multiple studies off of Antarctica. Conclusions: The global collection of the world’s metagenomic studies is confirming the presence of *C. gattii* in known locales around the globe; while revealing previously unknown populations in unexplored or underexplored habitats and regions. These findings provide a starting point for deeper explorations of new endemic locations, niches and possibly new species.

Tues P2 – Cryptococcal glycobiology - not just the capsule!

Tamara L. Doering

Daphne Boodwa Ko, Sydney L. Briner, Xinxin Lu, Liza Loza, Ankur Ankur, and Tuo Wang

The cryptococcal capsule attracts much attention because of its fascinating biology, beautiful structure, and importance to fungal virulence. However, the many other unique and interesting aspects of cryptococcal glycobiology should not be overlooked, nor should the connections among them. This talk will provide an overview of cryptococcal glycosylation processes and present recent work related to soluble polymers, protein glycosylation, capsule synthesis, and cell wall synthesis and structure, emphasizing the distinctive features of these glycans and the glycosyltransferase enzymes that generate them.

Tues P3 – Targeting *Cryptococcus*: Engineering LNP Therapeutics and Vaccines

Xiaorong Lin

Microbiology, University of Georgia, USA

Cryptococcus neoformans is an encapsulated fungus often found in soil, trees, and bird guanos. Exposure to this ubiquitous environmental microbe through inhalation in the general population typically results in clearance or dormancy in the lungs. In immunodeficient hosts, however, the fungus often spreads from the lungs to other organs with a predilection to the brain, causing cryptococcal meningoencephalitis (CME). For patients diagnosed with cryptococcosis, treatment relies on

amphotericin B±flucytosine followed by azole antifungals. The mortality rates for this disease are high. When relapse occurs, about 90% of cases are caused by the original isolates, pointing to failure of eradication. Thus, there is an unmet clinical need for improved antifungal treatment and immunotherapies. These challenges have motivated us to develop the targeted antifungal drug delivery platform named DectiSomes and to explore mRNA-LNP vaccines that can provide immunity against this fatal disease.

Tues P4 – Population variation in *Cryptococcus neoformans* and *deneoformans*

Christina Cuomo

Brown University

Cryptococcus is a globally represented opportunistic human fungal pathogen particularly affecting immunocompromised individuals with high attributed mortality. Most infections are caused by *Cryptococcus neoformans*, however the closely related species *Cryptococcus deneoformans* while not as high in clinical incidence spans a wide geography, and hybrid isolates of the two species can also be clinically observed. We have characterized the global genetic diversity of *C. deneoformans* and hybrid isolates using population sequencing to examine their structure and diversity in comparison to *C. neoformans*. We establish that the *C. deneoformans* population has limited substructure and minimal genetic exchange with *C. neoformans*, with rare introgressions. Overall, *C. deneoformans* shows lower genetic diversity than *C. neoformans*. We find aneuploidy frequent but present only in human or veterinary isolates and that the most frequent chromosomal aneuploidies are of homologous chromosomes in both species. Analysis of selection between the species highlighted genes involved in capsule and other processes. While hybrid strains are detected clinically, we find that their progeny isolated in the lab are characterized by extraordinarily high levels of karyotype instability, with preferential loss of the *C. neoformans* homologs. This work expands our understanding of *Cryptococcus* speciation and hybridization and prompts investigation into the impacts of such genome diversification on virulence in clinical and environmental contexts.

Tues P5 – Exploring the extracellular biology of *Cryptococcus*

Marcio Rodrigues

Carlos Chagas Institute, FIOCRUZ, PR, Brazil.

Extracellular structures are central components of *Cryptococcus* biology. Cryptococcal extracellular vesicles were previously shown to carry bioactive molecules, including the dipeptide isoleucine-proline-isoleucine, an inhibitor of dipeptidyl peptidase 4. Here, we investigated the contribution of this vesicle-associated pathway to fungal physiology and pathogenesis. We demonstrate that *Cryptococcus neoformans* produces an active, surface associated Dpp4 that is inhibited by this dipeptide. Genetic deletion of DPP4 abolishes enzymatic activity and induces marked physiological alterations, including changes in whole cell and vesicle proteomes and accumulation of undivided cells at host temperature. Loss of Dpp4 significantly reduces virulence in both invertebrate and murine models, delaying host mortality, decreasing fungal burden, and impairing dissemination to the brain, consistent with reduced ability to cross biological barriers. These findings identify Dpp4 as a key regulator linking extracellular vesicle biology to fungal virulence and support its potential as a target for alternative antifungal strategies.

Tues P6 – Mechanistic Insights into Cryptococcal Granuloma Formation and Maintenance

Maurizio Del Poeta

Veronica S. Brauer, Kathryn Takemura, Barbara Rosati, Marinaldo Pacifico Cavalcanti-Neto, Deveney Dasilva, Neelu Singh, Tyler Normile, Gabriella Vaccaro, Giuseppe Caso, Harpreet Singh, Bo Chen, Iwao Ojima, David McKinnon and Maurizio Del Poeta

Stony Brook University, NY, USA

Cryptococcosis is a life-threatening fungal disease caused by *Cryptococcus neoformans*, in which the immune system forms a lung granuloma to contain the infection. Using a mouse model histologically similar to the human granuloma, we combined transcriptomics and lipidomics to characterize the immune landscape of this structure. We identified macrophage subtypes that form the granuloma boundary and display differential gene expression linked to infection control. Lipidomic analysis further

revealed upregulation of sphingolipid-associated genes involved in phagocytosis regulation, with in vivo validation confirming their role in granuloma formation. As the granuloma matures, fungal replication declines alongside reduced macrophage activation. These findings refine current models of host-pathogen interaction and point toward potential immunomodulatory therapeutic strategies.

Flash Talks

Adriana Orrego - *From Essential Metabolism to Resistance: Transposable Elements and 5-FAA Escape in Cryptococcus*

Hannah Newton - *Using MLST to explore relationships between environmental and animal associated isolates of Cryptococcus in a koala population.*

Kimberly Martinez - *Titan Cell Production Increases In Vivo Diversity and can Enhance Antifungal Drug Resistance.*

Concurrent Session 1

Tues C1.1 – Genetic and molecular basis of thermal adaptation in *Cryptococcus deneoformans*

Asiya Gusa

Paola A Ramos Irizarry, Samantha A Shaltz, Kaia A Close, and Asiya A Gusa
Duke University, Durham, North Carolina, USA.

Human body temperature (37°C) serves as a key barrier to fungal infections since most environmental fungi grow optimally between 25-30°C. Rising global temperatures and extreme heat events will increasingly exert selective pressure for fungi adapted to growth at higher temperatures, which may increase their pathogenic potential. The genetic mechanisms by which fungi evolve greater heat tolerance remain poorly understood. We use *Cryptococcus deneoformans* as a model to study heat adaptation in fungi because it is generally less thermotolerant and less virulent than other disease-causing *Cryptococcus* species, primarily associated with skin infections and, more rarely, fungal meningitis. In a 30°C passaging experiment, we found that spontaneous insertion of a transposable element into a single gene encoding a predicted phosphatase regulatory scaffold (which we named Prs1) conferred increased thermotolerance in *C. deneoformans* at 37°C and 39°C. We found that budding and growth of the Prs1 mutant was less delayed compared to wildtype upon temperature upshift, suggesting an inhibitory role for Prs1 in cell cycle progression under heat stress. Immunoprecipitation identified Pph22 as a primary interactor of Prs1, suggesting that Pph22 functions as the catalytic subunit of a previously uncharacterized phosphatase complex. This interaction will be further validated. We characterized the fitness of the Prs1 mutant by assessing virulence traits and survival under different stress conditions. Finally, we assessed pathogen fitness between wildtype and the thermotolerant Prs1 mutant by determining fungal burden following macrophage and murine infection. These studies contribute to our understanding of the genetic and molecular mechanisms that underlie fungal heat adaptation and begin to address the key question of whether increased heat tolerance alters pathogen fitness.

Tues C1.2 – Evolution of thermotolerance in a human fungal pathogen through recurrent mutation of a sugar transporter

Marina Ruzic

Elizabeth A. Gaylord, Zoë A. Hilbert
Department of Biology, Boston College

The ability to grow at high temperature, or thermotolerance, is essential for pathogenicity in many microbes, including fungi. Despite the importance of this trait for pathogen success, our understanding of the genetic basis of high temperature growth in pathogenic fungi remains limited. Here, we used experimental evolution to examine thermal adaptation in a temperature-sensitive environmental strain

of *Cryptococcus neoformans*. Through this approach, we identified a number of thermally-adapted isolates which exhibit a striking improvement in growth at higher temperatures. Whole genome sequencing of several isolates revealed recurrent mutation of a hexose transporter, HXT1, across independent replicate populations. Each of the three unique HXT1 mutations identified in these adapted strains are both necessary and sufficient for thermotolerance and closely phenocopy deletion alleles of HXT1. Our preliminary data indicates these mutations may also lead to improved growth of the fungi in macrophages, suggesting a strong link between growth at high temperatures and pathogenesis. Our ongoing work seeks to provide a mechanistic understanding of the role of Hxt1 and nutrient availability, more broadly, in regulating thermotolerance. This includes performing biochemical characterization of glucose uptake functions and metabolite concentrations in mutated Hxt1 variants and strains grown at different temperatures. Collectively, this work provides key insight into the fundamental biology of an important fungal pathogen and the processes by which fungi can and will adapt to high temperature, leading to the emergence of novel pathogenic species.

Tues C1.3 – Rsp5-mediated ubiquitination of a functional analog of the Rim8 arrestin facilitates Rim pathway activation in *Cryptococcus neoformans*

Marnus du Plooy

Lukas M. du Plooy^{1,2}, Corinna Probst¹, Matthew E. Capobianco¹, Charles Giamberardino¹, Claudia A. Petrucco^{2,3}, J. Andrew Alspaugh^{1,2,3}

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Pathogenic microorganisms use varied cellular processes to adapt to the stresses encountered in the infected host. These stresses include rapid alterations in ambient temperature, nutrient availability, and extracellular pH. Fungal pathogens, therefore, rely on the activation of stress response pathways such as the Pal/Rim pathway to adapt to the neutral pH encountered when infecting mammals. While this pathway is conserved among human pathogenic fungi, the proteins required for pH sensing appear to have diverged between different fungal phyla. *Cryptococcus neoformans* employs a pH-sensing protein in its Rim pathway that is distinct but functionally analogous to related proteins in well-studied ascomycete fungal systems. We determined that protein ubiquitination mediated through the Rsp5 ubiquitin ligase is required for *C. neoformans* virulence and for microbial adaptations to host-relevant conditions, including growth at host pH levels. *C. neoformans* Rsp5 is specifically required for Rim pathway activation. Using an unbiased screen for proteins that are ubiquitinated by Rsp5 in acidic and alkaline pH, we identified a new component of the *C. neoformans* Rim pathway that is targeted by Rsp5 for ubiquitination and shares protein features with the ascomycete Rim8/PalF proteins. Rsp5-mediated ubiquitination facilitates protein interactions with Vps23, a downstream trafficking component of the Pal/Rim pathway. Therefore, we define adaptation to ambient pH as one component of the broad cellular roles of Rsp5-mediated ubiquitination, as we explore how protein ubiquitination affects cryptococcal cell physiology, virulence, and microbial interactions with the host.

Tues C1.4 – Chromosome evolution and mating-type locus remodelling in *Cryptococcus* and *Kwoniella*

Marco A. Coelho

Márcia David-Palma⁽¹⁾, Seonju Marincowitz⁽²⁾, Janneke Aylward^(2,3), Nam Q. Pham⁽²⁾, Andrey M. Yurkov⁽⁴⁾, Brenda D. Wingfield⁽²⁾, Michael J. Wingfield⁽²⁾, Sheng Sun⁽¹⁾, and Joseph Heitman⁽¹⁾

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The sister genera *Cryptococcus* and *Kwoniella* include major human fungal pathogens and diverse non-pathogenic environmental relatives, providing a powerful framework to investigate how genome architecture evolves alongside fungal pathogenicity and reproductive mode. In basidiomycete fungi, sexual compatibility is controlled by mating-type (MAT) loci composed of pheromone/receptor (P/R) and homeodomain (HD) regions. The organisation of these loci can shift between tetrapolar, pseudobipolar, and bipolar configurations, yet the chromosomal processes underlying these transitions remain poorly understood. We combined chromosome-level genome assemblies, comparative genomics, phylogenomics, centromere validation, and MAT locus reconstruction across *Cryptococcus*

and Kwoniella to investigate how chromosome evolution, centromere dynamics, and MAT locus organisation shape reproductive transitions. Our analyses revealed distinct trajectories of karyotype change and MAT locus remodelling in these genera. In *Cryptococcus*, chromosome evolution was characterised by frequent interchromosomal rearrangements, including translocations often associated with centromeric regions. By contrast, multiple *Kwoniella* lineages underwent extensive chromosome fusion associated with centromere loss and the formation of giant chromosomes. In parallel, MAT loci showed substantial structural plasticity, including lineage-specific P/R expansions potentially associated with pheromone gene duplications, and repeated transitions linking the P/R and HD loci. Together, these findings indicate that chromosome architecture and MAT organisation can change through distinct lineage-specific routes while generating convergent reproductive outcomes, underscoring the importance of continued diversity sampling in this group.

Concurrent Session 2

Tues C2.1 – 15 years on: how the changing HIV treatment landscape has impacted cryptococcal meningitis rates in Cape Town

Kyla Murphy

Kyla Murphy

Mortality among individuals with subclinical cryptococcal meningitis identified through cryptococcal antigen (CrAg) screening programs in South Africa and Tanzania

Background: Cryptococcal meningitis (CM) is a life-threatening opportunistic infection, typically associated with advanced HIV. This study compares the epidemiology of CM in a high HIV-prevalence setting in South Africa with a study in a similar population 15 years ago. Methods: Retrospective clinical and laboratory information was collected for all adults who underwent a lumbar puncture at Mitchells Plain District Hospital between January 2022 and December 2023. Clinical episodes of CM were identified and analysed using descriptive statistics. Results were compared to a 2010 cross-sectional analysis by Jarvis et. al in a geographically-similar population. Results: 2022 clinical presentations were captured. 262 episodes of microbiologically-confirmed meningitis were recorded of which CM accounted for 34% (88/262); compared to 63% in Jarvis et. al (2010). Most CM episodes (95%) were confirmed by CrAg LFA compared to only 40% in 2010. In this study, 99% (87/88) of CM cases were HIV-positive. Although 84% were ART-experienced, only 23% (19/83) were reportedly adherent to ART at presentation. CM mortality was 37% (30/87) overall. Although reflex serum CrAg screening was in place, only 39% (34/88) had a serum CrAg result prior to admission of which 68% (23/34) were serum CrAg positive. Conclusion: CM occurred predominantly in patients with advanced HIV with a median CD4 count of 34 cells/uL. While prior ART-exposure in CM was high, most patients had interrupted ART prior to CM. In comparison to the Jarvis et al. (2010) cohort, availability of CrAg CSF testing has improved with almost all CM cases confirmed with CrAg LFA.

Tues C2.2 – *Cryptococcus neoformans* responds to presence of *Mycobacterium* by diversifying its morphologies and remodelling its capsular material

Orlando Ross

Andrew Akampurira (1), Liliane Mukaremera (2), Ivy Dambuza (2)

MRC CMM

Introduction. *Cryptococcus neoformans* and *Mycobacterium tuberculosis* (MTb) are opportunistic pathogens that share overlapping geographical distributions and physiological niches within the human body. Both are recognized by the World Health Organization as high-priority pathogens. Gap Statement. Although clinical reports of co-infections with cryptococcosis and tuberculosis are increasing, experimental studies exploring their interactions remain scarce. Aim. We aimed to observationally evaluate whether *C. neoformans* isolates would alter morphology when co-cultured with *Mycobacteria* spp. and observe how these changes might alter the host immune response to *C. neoformans* cells. Methodology. We cultured *C. neoformans* reference strain and clinical isolates in physiologically relevant growth media, in the presence or absence of *Mycobacterium* spp. Then, we generated alveolar-like macrophages and created a stimulation environment similar to a tuberculosis

environment to perform phagocytic killing assays of *C. neoformans* cells. Results. Here, we demonstrate that *C. neoformans* can grow in the presence of either heat-killed MTb antigen or the live vaccine strain, *Mycobacterium bovis* BCG. In response to the presence of mycobacteria, *C. neoformans* increased in number and exhibited enhanced virulence-associated traits, including titan cell formation, capsule enlargement and increased survival from phagocytosis. Conclusion. This work provides proof of principle for a dynamic, inter-pathogen interaction that may contribute to the exacerbation of disease outcomes in settings of a co-infection.

Tues C2.3 – CMV viremia is associated with impaired CSF fungal clearance amongst 509 Ugandan adults with HIV-associated cryptococcal meningitis

Timothy Mugabi

Timothy Mugabi², Kathy Huppler Hullsiek³, Mark Blackstad¹, Laura Nsangi², Jayne Ellis⁴, David B Meya^{1,2}, Mark R Schleiss¹, David R Boulware¹, and Caleb P Skipper on behalf of the IDI Meningitis Research Team

Infectious Diseases Institute, Makerere University, Kampala, Uganda

Background: Cryptococcal meningitis is the leading cause of HIV-associated meningitis with 10-week mortality of 25-30%. Cytomegalovirus (CMV) viremia occurs in 30-50% of adults with advanced HIV disease and is associated with increased mortality in cryptococcal meningitis through unclear mechanisms. We aimed to determine whether CMV viremia impairs CSF fungal clearance.

Methods: We measured CMV DNA levels by quantitative PCR in baseline cryopreserved plasma from Ugandans with cryptococcal meningitis enrolled in clinical from 2010–2024. Amphotericin-based induction therapy evolved over time according to international standards. Early fungicidal activity (EFA) was calculated as the negative slope change in quantitative CSF fungal burden ($\log_{10}$ CFU/mL/day), estimated by linear regression. We analyzed odds of achieving sterility and all-cause mortality through 18 weeks.

Results: Of 509 Ugandan participants with EFA available, we found CMV viremia in 37% (187/509). CMV viremia was significantly associated with lower CD4 counts, lower hemoglobin, fewer CSF white cells, and higher baseline fungal burden ($p < 0.01$ for all). Participants with CMV viremia (median EFA=0.31 [IQR, 0.17–0.44]) were less likely to sterilize CSF by Day 18 after antifungal therapy (36% vs 54%; $p < 0.001$) as compared to participants without CMV viremia (median EFA=0.35 [IQR, 0.22–0.51]). The combination of CMV viremia and EFA <0.20 dramatically reduced the odds of achieving Day 18 CSF sterility (OR=0.01 [95%CI, 0.00–0.05]). Participants with CMV viremia had higher mortality at 18 weeks (43% vs 26%; $p < 0.001$).

Discussion: CMV viremia is associated with impaired CSF fungal clearance, providing a possible mechanism for why CMV viremia is associated with excess mortality in cryptococcosis.

Tues C2.4 – Invasive cryptococcosis with *Klebsiella pneumoniae* co-infection associated with refractory disease and high mortality: A case series

Dora Edith Corzo Leon

Eduardo Perez-Alba, Patricia Rodriguez-Zulueta, Eleni Didon, Matheus Araujo, Elizabeth Ballou

MRC Centre for Medical Mycology

Cryptococcus neoformans is the leading cause of fungal meningitis worldwide. Standard induction therapy combines amphotericin B with a second antifungal agent; however, treatment failure occurs due to drug resistance, inadequate dosing, monotherapy, or host immune dysfunction, leading to relapsing or refractory disease with high mortality. We report four cases of invasive cryptococcosis with concurrent *Klebsiella pneumoniae* bloodstream infection. All patients developed refractory disseminated cryptococcal disease despite initial combination antifungal therapy. Two of four patients (50%) died despite aggressive antifungal and antibiotic treatment. *C. neoformans* and *K. pneumoniae* isolates were recovered from three cases. We performed comprehensive antifungal and antimicrobial susceptibility testing and whole genome sequencing to characterize resistance patterns and genetic signatures. Clinical presentations, phenotypic resistance profiles, and genomic analysis revealed antifungal resistance signatures in all characterized strains. The concurrent bacterial-fungal co-infection was associated with poor outcomes despite appropriate combination therapy. This case series

highlights the clinical challenge of cryptococcal-Klebsiella co-infection and suggests that concurrent bloodstream infections may contribute to antifungal treatment failure and mortality. Further investigation is needed to determine whether bacterial co-infection influences cryptococcal virulence or treatment response.

Concurrent Session 3

Tues C3.1 – Mechanistic target of rapamycin (MTOR) signaling pathway loss-of-function variants in previously healthy patients with non-HIV cryptococcosis.

Jessica C. Hargarten

Gabriel Soares Matos²; Laura Fruhauf De Macedo²; Malcolm J. Vaughan¹; Kenneth Ssebambulide¹; Guowu Hu¹; Owen CR. Dean¹; and Peter R. Williamson¹.

Rutgers New Jersey Medical School

Cryptococcosis primarily affects individuals with advanced immunosuppression, including those with HIV/AIDS, solid organ transplants, or prolonged corticosteroid exposure; however, little is known about whether host genetic factors might predispose certain individuals to develop disease independent of known risk factors. Identification of genetic defects in human patients is an important “natural experiment” that can reveal important human traits required for host disease susceptibility and resistance. In the present studies, genetic analysis of a large cohort of previously healthy (PH) patients who developed infections with *Cryptococcus* were investigated to determine underlying immune deficiencies leading to susceptibility. In a cohort of 138 PH patients with cryptococcosis, whole exome sequencing was performed, identifying rare alleles predicted to have deleterious functional consequences (CADD >30). ToppGene pathway analysis revealed MTOR signaling pathway defects as enriched in this patient population, with 50% carrying predicted deleterious variants, including 5 in MTOR. Functional analysis showed that these MTOR variants were associated with T cell dysfunction. Mouse models of MTOR deficiency—chemically (rapamycin) or genetically (CD4-Cre MTOR-floxed), exhibited increased susceptibility to cryptococcosis: reduced survival, increased fungal burdens, and diminished lymphocyte recruitment to sites of infection. Moreover, adoptively transferred MTOR-deficient CD4 T cells failed to migrate effectively during cryptococcosis. These findings are the first to identify loss-of-function MTOR variants in humans and highlight the crucial role of MTOR-related signaling in anti-cryptococcal adaptive responses. Ongoing experiments, utilizing MTOR-flox mice and human cells, aim to further elucidate MTOR's role in immunity and the unique susceptibility to this important fungal pathogen.

Tues C3.2 – *Cryptococcus* modulates host immune tolerance through STING-dependent inhibition of PFKFB3

Linghong Zhou

Juntian Huang, Yingkui Jiang, Liping Zhu

Huashan Hospital, Fudan University

Background and aims: Immune tolerance is a critical determinant in the pathogenesis of non-HIV-associated cryptococcal meningitis (CM). While stimulator of interferon genes (STING) is a known negative regulator of antifungal immunity, its mechanistic role in mediating immune tolerance remains unclear. This study aims to elucidate how STING interacts with glycolysis-related pathways to mediate immunometabolic suppression in non-HIV CM.

Methods: Peripheral blood monocytes were collected from non-HIV CM patients, pulmonary cryptococcosis (PC) patients, and healthy controls (HC). Multi-platform analyses were conducted, including cytokine profiling, STING activation assessment, and glycolytic flux quantification. In vitro immune-tolerant models and murine cryptococcal infection models were established, utilizing STING genetic knockout and glycolytic rescue experiments. Molecular docking and Co-IP were employed to validate interactions between STING and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3).

Results: CM monocytes displayed significantly impaired TNF- α /IL-6 secretion and reduced glycolytic intermediates, alongside elevated STING expression, relative to PC and HC cohorts. Notably, persistent glucuronoxylomannan (GXM) exposure recapitulated this tolerogenic phenotype in vitro. Pharmacological antagonism or genetic ablation of STING effectively rescued cytokine production. In vivo, STING-/- mice demonstrated significantly improved survival and reduced fungal burden. Mechanistically, molecular docking and Co-IP verified a direct high-affinity interaction between STING and PFKFB3. Consequently, STING deficiency reversed GXM-mediated PFKFB3 suppression.

Conclusions: Cryptococcal infection upregulates STING, which directly binds and inhibits PFKFB3, suppressing glycolysis and inducing immune tolerance. Targeting the STING-PFKFB3 axis restores immunometabolic function and enhances antifungal responses. These findings establish STING as a master regulator of immunometabolic reprogramming and provide a novel therapeutic strategy.

Tues C3.3 – Time-Dependent Antibody Signatures of Cryptococcal Disease After Kidney Transplant

Hyunah Yoon

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Albert Einstein College of Medicine

Background and aims: Cryptococcal disease (CD) remains a high-mortality opportunistic infection after kidney transplant (KT), typically presenting during maintenance immunosuppression (IS) when immune reconstitution is heterogeneous. No validated immune markers risk-stratify KT recipients for CD. We evaluated whether total/antigen-specific antibody profiles differ by CD status and time post-KT as a surrogate for cumulative IS exposure.

Methods: Plasma from three KT cohorts (n=58) was analysed: no-CD (n=29), pre-CD (n=10; prior to CD diagnosis), and CD (n=19; after diagnosis); stratified early (<1 year) or late (>1 year) post-KT. Total IgM, IgA, IgG1, IgG2, and cytokines quantified by Luminex; antigen-specific IgM/IgG to capsular glucuronoxylomannan (GXM) and laminarin (β -glucan proxy) by ELISA.

Results: Median age was 58 years; 75% male. CD occurred 2.2 years post-KT; sampling ~33 days later. Compared with no-CD, CD had hypogammaglobulinemia, ~5-fold lower IgM (median 928 μ g/ml vs 4,441), ~4-fold lower IgG2 (482 vs 1,940); late CD remained depressed (IgM 775 vs 3,588; IgG2 651 vs 1,711). Within CD, antigen-responses declined from early to late (GXM-IgM 21.6 $\rightarrow$ 14.4; laminarin-IgM 161 $\rightarrow$ 51; laminarin-IgG 304 $\rightarrow$ 163), contrasting with stable/increasing levels in no-CD/pre-CD. Despite low total immunoglobulins, early CD had comparable/higher GXM-IgM and laminarin-IgM than early no-CD (21.6 vs 16.3; 161 vs 49.8) while GXM-IgG was lower (45.9 vs 121). IgM, laminarin/GXM-IgM correlated with IFN γ , GM-CSF, and IL-17A in CD.

Conclusions: CD after KT is associated with decreased IgM/IgG2 and time-dependent antigen-specific antibody trajectories during the expected period of CD4 reconstitution, suggesting distinct immune states driving disease, supporting marker-guided IS management. Small strata, heterogeneous sampling limit inference.

Tues C3.4 – Differential recognition of *C. neoformans* by male and female human macrophages elicits contrasting immune responses

Erin McClelland

Mackenzi Lawson, Rebecca Troutman, Luke Horner, Savanna Gray, Mercedes Andrade, Abner Chen, Phoenix Davis, Basil Mustaklem, Liz Pradhan, Simran Sandhu, Jonathan W. Lowery, Sarah A. Justice
Marian University Wood College of Osteopathic Medicine

Cryptococcus neoformans (Cn) is a pathogenic yeast and the cause of cryptococcosis, a life-threatening fungal disease. Strikingly, ~70% of patients that develop cryptococcosis are male. Here, we investigated decreased Cn recognition by male macrophages and whether that affected the subsequent innate immune response. Human PBMCs were isolated from healthy male and female donors, differentiated into macrophages, and tested for immune response differences. Cn incubated

with female serum showed increased binding of the opsonin serum amyloid P, possibly explaining why female macrophages show increased Cn phagocytosis. After infection, cytokine analysis showed increased secretion of proinflammatory cytokines at 24 h in male macrophages that were substantially decreased by 48 h. In contrast, female macrophages secreted fewer proinflammatory cytokines at both 24 and 48 h but had increased secretion of CCL5 (24 and 48 h) and IL-18 (48 h). These data suggested the male immune response was changing 24 h post-infection; thus, we tested macrophage activation state. Male macrophages were alternatively activated at 48 h, with increased secretion of arginase 1 whereas female macrophages were classically activated, with increased secretion of nitric oxide at 24 and 48 h. Cn genes related to capsule production were increased in Cn grown in male serum at 48 h. These data suggest that female macrophages better recognize Cn and become classically activated whereas male macrophages initiate an inflammatory response that is markedly reduced after 24 h, possibly due to testosterone-induced GXM secretion. Thus, antifungal therapeutics targeting testosterone-induced GXM release may improve outcomes in male patients with cryptococcosis.

Wednesday July 1

Plenary Session

Wed P1 – Spatial organization of type 2 immunity during pulmonary cryptococcosis

Eric Van Dang

Ragon Institute of Mass General, MIT, and Harvard

Cryptococcus neoformans can establish persistent pulmonary infection within granulomatous lesions despite ongoing immune activation. Although type 1 immune responses are required for fungal control, our recent work demonstrated that type 2 inflammation promotes extracellular fungal persistence within granulomas. However, the mechanisms that organize and sustain type 2 immune responses during chronic fungal infection remain poorly understood. To address this question, we investigated how Th2 cells are positioned within infected lungs during pulmonary cryptococcosis. We found that pulmonary Th2 cells upregulate the oxysterol receptor GPR183 during infection, while inflammatory macrophages induce expression of cholesterol-25-hydroxylase (Ch25h), generating chemotactic oxysterol activity within infected tissue. Although GPR183 was dispensable for Th2 recruitment and differentiation, it was required for localization of TH2 cells to granulomatous lesions. Disruption of this pathway enhanced macrophage IFN γ responsiveness, increased iNOS expression, and reduced fungal tissue burden. These findings identify a macrophage–T cell positioning circuit that spatially organizes type 2 immunity during pulmonary cryptococcosis and suggest that immune cell localization within granulomas can directly regulate fungal persistence.

Wed P2 – Unravelling mechanisms of brain injury in cryptococcal meningitis

Rachael Dangarembizi

Department of Human Biology; Neuroscience Institute, Faculty of Health Sciences, University of Cape Town, Groote Schuur Hospital, Cape Town, South Africa 2CMM AFRICA Medical Mycology Research Unit, Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, South Africa 3Medical Research Council Centre for Medical Mycology at the University of Exeter, Department of Biosciences, Faculty of Health and Life Sciences, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, UK

Cryptococcal meningitis is a devastating neuroinfectious disease associated with severe neurological damage, long-term disability, and high mortality. Despite its clinical importance, the mechanisms underlying brain injury in cryptococcal meningitis remain poorly understood. We used multi-omics analyses, advanced bioimaging approaches, and cell-type-specific molecular profiling to study the *Cryptococcus*-infected brain. Here we demonstrate that neuropathology in cryptococcosis arises through multiple interconnected processes that include; an early state of neuroimmune quiescence that permits fungal expansion, progressive neuroinflammatory dysregulation, immunometabolic reprogramming, and tissue injury. We further uncover mechanistic pathways underlying fluid flow (glymphatic) dysfunction, and provide new insights into the development of raised intracranial pressure and hydrocephalus. Collectively, our findings redefine the pathogenesis of brain damage in neurocryptococcosis, demonstrating that disease progression is driven by a complex interplay of neuroimmune dysfunction, neurometabolic perturbation, and impaired neurofluid homeostasis

Wed P3 – A Tale of Two Crypto: Defining unique host responses to *Cryptococcus* species

Lena J. Heung

Department of Medicine, Department of Biomedical Sciences, Women's Guild Lung Institute, Cedars-Sinai Health Sciences University

Cryptococcus neoformans and *Cryptococcus gattii* are the principal causes of cryptococcosis in humans. Although they have similar disease presentations, their epidemiology is quite distinct. In addition to differences in geographical location, *C. neoformans* primarily affects immunocompromised individuals, while *C. gattii* can also infect otherwise healthy people. We have found that both *Cryptococcus* species can subvert the host immune system to orchestrate pathogenic responses by monocytes and monocyte-derived macrophages. However, they do so through different signaling pathways. *C. neoformans* binds to the host receptor TREM2 which pairs with the DAP12 signaling adapter to repress the antifungal effects of monocyte-derived macrophages. We do not find a role for TREM2-DAP12 in the regulation of *C. gattii* infection. Instead, we have determined that *C. gattii* triggers pathways dependent on the cytokine GM-CSF that govern monocyte responses and result in fungal proliferation and inflammatory lung damage. These findings give further mechanistic insight into host differences that may regulate susceptibility to cryptococcosis.

Wed P4 – From Spectra to Solutions: Proteomics at the Forefront of Fungal Infection, Immunity, and Antifungal Discovery.

Jennifer Geddes-McAlister

Michael Woods, Ben Muselius, Stephanie Samra, Jared Deyarmin
University of Guelph

Fungal diseases affect millions worldwide, spanning superficial infections to life-threatening systemic disease, yet therapeutic options remain limited by host toxicity, restricted drug classes, and the rapid emergence of antifungal resistance. Addressing this growing global challenge requires new ways to understand and disrupt fungal biology. Our research leverages high-resolution, mass spectrometry-based proteomics integrated with computational biology to define the functional drivers of fungal infection in real time. By capturing the dynamic protein landscape at the host-pathogen interface, we uncover actionable biology across four pillars: prevention, diagnostics, monitoring, and treatment. We identify and disrupt critical virulence pathways to prevent infection, define dual host-pathogen protein signatures for precise and early diagnostics, and map infection-driven proteome remodeling to monitor disease progression. Critically, this approach reveals druggable targets and resistance mechanisms, enabling strategies to restore antifungal efficacy and extend the lifespan of existing therapies. Together, this proteomics-driven framework captures functional disease biology, offering a transformative path toward precision antifungal strategies and improved global health outcomes.

Wed P5 – Formation of the seed cells morphotype: environmental triggers and molecular regulators.

Jessica Brown

Christian Moreau¹, Joseph M. Bednarek¹, Eric C.K. Cheung¹, Iman Ellahie¹, Connor Murdock¹, Charlotte Catterton, ¹ and Jessica C.S. Brown¹
School of Biological Sciences, University of Utah, USA

Cryptococcus neoformans is extremely responsive to environmental conditions, forming different subpopulations both within the host and in response to differing culture conditions. Here we focus on the seed cell morphotype, a small, ~5 mm total diameter (cell body + capsule diameter) yeast-form cell that forms in the lungs over the course of infection and exhibits enhanced ability to enter extrapulmonary organs. Seed cells are induced by phosphate, including by DNA, and their formation is regulated by a network of environmentally responsive transcriptional regulators. This network includes regulators involved in the yeast-to-hyphal in other human fungal pathogens. However, *C. neoformans* grows primarily as a budding yeast, so this conserved network has been repurposed to regulate a yeast-to-yeast switch. Seed cell formation and propagation can be blocked by both small molecule and genetic

inhibitors. When seed cell formation is blocked, fungal cell size increases, suggesting that seed cell formation is preceded by a larger morphotype. We will present data on environmental triggers and blocks of seed cell formation, including that CD4⁺ T-cell-deficient mice display increased seed cell formation in the lungs and more rapid fungal cell dissemination to the brain compared to wild-type (C5Bl/6NJ) mice. Together, these data highlight the phenotypic plasticity *C. neoformans* displays to adapt to diverse environments within and outside the host.

Wed P6 – Hyperdiversity, Hidden Hybrids, and Hyperplastic Genomes: New Insights from Local Scale Population Genomics of *Cryptococcus neoformans*

Paul Magwene

Kayla Wilhoit, Marhiah C. Montoya, Erick Figueroa, Claudia Ziri6n-Martinez, Jason Mohabir, Debra Murray, John R. Perfect, Paul M. Magwene

To date, most population genomic studies of *C. neoformans* and related species have focused on global collections of clinical isolates derived from human patients. Much less understood is the nature of genomic variation at local geographic scales or how genome variation differs between environmental and clinical sub-populations of *Cryptococcus*. To gain new insight into local population structure we carried out whole genome sequencing of more than 700 clinical and environmental isolates of *C. neoformans* collected from a single geographic region (North Carolina, USA). Using this unique population genomic resource we show that: a) the local diversity of *C. neoformans* is extremely high for both environmental and clinical sub-populations; b) interspecific hybrids are found in both the environment and the clinic, but the commonly used designator "AD hybrid" masks multiple distinct hybrid lineages; c) diploid intraspecific hybrids, derived from ancient crosses between *C. neoformans* lineages, are also present in both the environment and the clinic; these between lineage hybrids include the first examples of VNBII-derived haplotypes in North America; and d) the primary genomic feature that distinguishes clinical from environmental sub-populations of *C. neoformans* is the frequency of genomic structural variation, including aneuploidy, LOH, and genome rearrangement; such variation is common in clinical subpopulations but extremely rare among environmental isolates. We propose that *C. neoformans* populations can be hyperdiverse at a local scale, and that many lineages, especially hybrids, may exhibit genomic structural hyperplasticity during the niche transition from the natural environment to human hosts.

Flash Talks

Master Chisale - *Identification of drug targets for *Cryptococcus neoformans* drug discovery*

Mulungi Jonathan - *Disseminated HIV-Associated Cryptococcosis with Pulmonary Embolism in Pregnancy: A case report*

Oscar Romero - *Uncovering Genetic Drivers of Biofilm Formation in *Cryptococcus neoformans* to Enable Antibiofilm Drug Discovery*

Raphael Brosula - *Analyzing genomic and phenotypic variation within a large clinical *Cryptococcus* population to identify determinants of virulence and disease outcome*

Francisco Hernandez - *Laccase Drives the Warburg Effect in Melanized *Cryptococcus neoformans* upon Glucose Depletion*

Concurrent Session 1

Wed C1.1 – Frequency and therapeutic impact of fluconazole resistance in *Cryptococcus neoformans* strains from French surveillance

Marie Desnos-Ollivier

Marie Desnos-Ollivier, Karine Boukris-Sitbon, Alexandre Alanio, Marjorie Cornu, Nour Djihane Yakoubi, Charline Miossec, Milène Sasso, Lilia Hasseine, Gilles Gargala, Grégoire Pasquier, Maité Micaelo, Sophie Brun, Sophie Cassaing, Sébastien Imbert, Valérie Letscher-Bru, Guillaume Desoubeaux, Christine Bonnal, Olivier Paccoud, Olivier Lortholary, Fanny Lanternier

Institut Pasteur, National Reference Center for invasive Mycoses and Antifungals

The aim was to analyze clinical characteristics of cryptococcosis cases due to isolates resistant to fluconazole. Between January 2014 and August 2025, 777 cases were reported in France and 753 isolates from 663 patients were sent to the NRCMA for antifungal susceptibility testing and species identification. 26 isolates had fluconazole MIC >16mg/L, corresponding to 3.3% of the patients (22/663). Among these patients, 20 episodes from 20 patients were analysed. Sex ratio was 1.22 and mean age of 54.15. Main risk factors were HIV (7), solid organ transplant (n=7). Only one patient had been preexposed to fluconazole and one to caspofungin, 15 were naive to azoles treatment. Majority of isolates belong to *C. neoformans* (n=16), followed by AD hybrid (n=3) and *C. deneoformans* (n=1). Mortality rate at 3 months was 20%. Among the 18 patients with disseminated or cerebral infection, 16 were first treated with amphotericin B plus flucytosine. Two patients died before the beginning of the treatment. The treatment was followed by a switch to fluconazole except for one patient who died before the beginning of fluconazole therapy. Data from cryptococcosis surveillance in France over a ten-year period allow us to conclude that the frequency of isolates considered resistant to fluconazole in vitro is low and is found in the three species of the *C. neoformans* complex, mainly in patients who have not been previously exposed to azoles. Treatment with fluconazole despite the presence of a strain with a high MIC does not result in treatment failure or significant mortality.

Wed C1.2 – Experience of research nurses with oral Encochleated amphotericin B for treatment of Cryptococcal Meningitis in a resource-limited setting

Cynthia Ahimbisibwe

Richard Kwizera (1), Jane Frances Ndyetukira (1), Olivie Carolyn Namujju (1), Alisat Sadiq (1), Mucunguzi Atukunda (1), Caleb P Skipper (2), David R Boulware (2), David B. Meya (1,2)

Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda.

Background and aims Cryptococcal meningitis accounts for upto 19% of AIDS-related deaths. First-line therapy uses either seven-day induction with intravenous amphotericin B deoxycholate and oral flucytosine or single-dose liposomal amphotericin with oral flucytosine and fluconazole. Besides the commonly used intravenous formulations of amphotericin B, an oral nanocrystal amphotericin B (MAT2203) formulation is being evaluated for efficacy to treat invasive fungal infections. This new experimental oral formulation has not been used before. Herein, we aimed to describe our experiences with using oral cAmB in a resource-limited setting from the perspective of research nurses. **Methods** This was part of a sequential Phase I and then Phase II multi-site randomized controlled trial that aimed to investigate the efficacy of oral nanocrystal amphotericin B formulation among persons with cryptococcal meningitis. **Results** We screened 240 participants in the two trials and enrolled 168 of which 81 (48%) were women. We found oral amphotericin a better alternative to intravenous amphotericin B deoxycholate due to less toxicity, mostly limited to gastrointestinal-related toxicities only. We clinically observed no drug reactions like rigors, phlebitis, and less vomiting among patients on oral amphotericin as compared to those on intravenous amphotericin B deoxycholate. Subjectively, meningitis symptoms of patients on oral amphotericin seemed to overall clinically improve more rapidly compared to those receiving intravenous amphotericin B deoxycholate. Few adverse events were observed. **Conclusions** In conclusion, oral amphotericin B was generally safe and well tolerated. However, it requires some training for the nurse, patient and caretakers for better administration, adherence and treatment outcomes.

Wed C1.3 – Use of Oteseconazole for Consolidation Therapy and Secondary Prophylaxis in Fluconazole Non-Susceptible Cryptococcal Meningitis.

Sarah Kimuda

Joseph Owori¹, Faizo Ssekindi¹, Kisakye Diana Kabbale¹, Cynthia Ahimbisibwe¹, Jane Frances Ndyetukira¹, Rhona Muyise¹, Grace Menya¹, Andrew Akampurira¹, Tonny Luggya¹, Mable Kabahubya¹, Steve Brand⁴, David R Boulware², David B Meya^{1,2,3}, Mahsa Abassi²

Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda

Background: Fluconazole non-susceptible *Cryptococcus neoformans* (MIC ≥ 16 $\mu\text{g/mL}$) represents a significant therapeutic challenge in culture-positive relapse due to the absence of alternative oral consolidation therapies. Oteseconazole, a tetrazole CYP51 inhibitor with potent in vitro activity against *Cryptococcus* (MIC₉₀ ≈ 0.125 $\mu\text{g/mL}$) has a prolonged half-life of ≈ 138 days. Oteseconazole offers a promising option for fluconazole non-susceptible cryptococcosis.

Methods: In Kampala, Uganda, we enrolled a compassionate-care cohort for individuals with culture-confirmed cryptococcal-relapse due to fluconazole non-susceptible isolates (MIC ≥ 16 $\mu\text{g/mL}$). After re-induction with liposomal amphotericin-based therapy, participants received oteseconazole 600 mg twice daily for 10 days as loading dose and then weekly 600 mg for 4 weeks. We assessed clinical course, CSF sterility, and recurrence.

Results: Two individuals with culture-positive cryptococcal-relapse due to fluconazole non-susceptible *Cryptococcus* initiated oteseconazole therapy. Case 1 presented on antiretroviral therapy with CD4 count of 294 cells/ μL and a third episode of cryptococcal-relapse. CSF culture grew 6,500 CFU/mL at relapse with a fluconazole MIC=256 $\mu\text{g/mL}$. Case 2 presented on antiretroviral therapy with CD4 count of 124 cells/ μL . CSF fungal culture grew 195,000 CFU/mL at relapse with a fluconazole MIC=32 $\mu\text{g/mL}$. Both participants achieved clinical stabilization and CSF sterility. To date, >2 months of follow-up, no further episodes of meningitis, disease recurrence, or drug-related toxicity have been observed during follow-up.

Conclusions: Early experience suggests that oteseconazole may provide effective consolidation therapy and secondary prophylaxis in cryptococcal meningitis. These preliminary observations support further systematic evaluation of oteseconazole as a novel oral agent for cryptococcosis. Enrollment is ongoing.

Wed C1.4 – Affordable PEGylated liposomal amphotericin B enables effective and safe oral treatment of experimental murine cryptococcosis

Ludmila Gouveia-Eufrazio

Victor Augusto Teixeira Leocádio¹, Guilherme Santos Ramos², Danielle Leticia Silva¹, Barbara Maria de Amorin Santos¹, Helane Lúcia Oliveira de Moraes², Pedro Henrique Reis da Silva³, Julia Facci Colangelo³, Christian Fernandes³, Daniele da Glória de Souza¹, Nalu Teixeira Aguiar Peres¹, Frédéric Frézard², Daniel Assis Santos^{1,4}

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Cryptococcosis is a life-threatening fungal infection that predominantly affects immunocompromised individuals and remains a major global health challenge. Amphotericin B (AmB) is a central pillar of therapy; however, its clinical use is limited by nephrotoxicity, high cost, and the need for intravenous administration. In this study, we developed an affordable PEGylated liposomal AmB formulation (LAmB) using low-cost lipids and a simple, scalable ethanol injection method, and evaluated its oral efficacy and safety in a murine model of *Cryptococcus neoformans* H99 infection. The formulation produced uniform nanoparticles with a mean diameter of approximately 110 nm and an encapsulation efficiency exceeding 95%. Oral administration of LAmB significantly improved survival, achieving complete protection of infected animals, and markedly reduced fungal burden in the lungs and brain when compared with untreated mice and animals receiving conventional AmB deoxycholate. Histopathological analysis demonstrated substantial preservation of pulmonary architecture in LAmB-treated mice, accompanied by a pronounced reduction in pulmonary inflammation. Consistently, LAmB treatment significantly decreased CXCL10 and IL-6 levels in lung tissue and bronchoalveolar lavage fluid.

Toxicological evaluation revealed only mild renal alterations and no evidence of hepatotoxicity, consistent with the known safety profile of AmB formulations. Overall, these findings demonstrate that orally administered PEGylated liposomal AmB combines potent antifungal efficacy with reduced inflammation and acceptable safety. Its oral bioavailability and low-cost composition support its potential as a promising and accessible therapeutic strategy for treating cryptococcosis, particularly in resource-limited settings.

Wed C1.5 – The cell-type specific neuroimmune response to cryptococcal infection of murine hippocampal brain slices

Alison Gifford

Emily Higgitt 1, 2, 3, Maahir Kauchali 1, 2, 3, Amalia Awala 1, 2, 3, 5, Anja de Lange 1, 2, 3, Lilitha Cengani 1, 2, 3, Ivy Dambuza 4, Dorit Hockman 2, 5, Teresa Steyn 2, 5, Joshua Selfe 2, 5, Adilia Warris 4, *Joseph Raimondo 2, 5 and *Rachael Dangarembizi 1, 2, 3, 4

University of Exeter

Cryptococcal meningitis (CM) is often associated with severe neurological damage but the neuropathophysiology of this fatal disease is incompletely understood. This study aims to characterise the immunomodulatory transcriptional activity of each cell type in the murine brain to *C. neoformans* using single-nuclei RNA sequencing (snRNAseq). Hippocampal organotypic brain slices were prepared from six-day old mouse pups and stimulated with either 1×10^8 *C. neoformans* cells, lipopolysaccharide (LPS) or normal growth media for 24 hours. Brain slices were Dounce homogenised and a single nuclei suspension recovered for sequencing. Libraries were generated by 10X Genomics and sequenced via Illumina. Microglia did not significantly upregulate any major pro- or anti-inflammatory genes in cryptococcal infection, in contrast to the pro-inflammatory gene upregulation with LPS treatment. There is no clear phenotype for cryptococcal-treated microglia, but upregulated genes include phagocytic receptors with anti-inflammatory properties including *Colec12* and *Lrp1*. Astrocytes have more significantly upregulated pro-inflammatory genes in response to *Cryptococcus* including *Stat3*, *Nos2*, *Cxcl2*, *Fos* and *Nfkb1*. Astrocytes' role at the blood-brain barrier is also affected with upregulated *Vegfa* and *Piezo1*. *Tnfrsf25*, critical in tunnelling nanotube biogenesis, is upregulated in both astrocytes and microglia. Excitatory neurons have the highest number of differentially expressed genes in response to *Cryptococcus* with upregulated *Stat3*, *Nfkb1*, *Fos* and *Il4*. The glutamate cycle is also affected with downregulated *Gls* and *Grik2*. This data suggests that microglia are phagocytosing *Cryptococcus* leading to a minor anti-inflammatory response. Astrocytes are significantly pro-inflammatory and, along with excitatory neurons, have a significant *Stat3*-response to *Cryptococcus*.

Wed C1.6 – Changing impact of Cryptococcus on HIV-associated meningitis in northern Uganda

Abigail Link

Mark Okwir, David Meya, Ocora Boniface, Ritah NakatoPaul Bohjanen

University of Rochester

BACKGROUND & AIMS In past decades, *Cryptococcus* was the leading cause of death due to meningitis in sub-Saharan Africa among people living with HIV (PLWH). With improvements in HIV and cryptococcal meningitis (CM) care, we sought to evaluate the current causes of death or neurological impairment in PLWH with meningitis in northern Uganda. **METHODS** We conducted a retrospective cohort study utilizing data from PLWH admitted to Lira Regional Referral Hospital with meningitis from 2021-2025. Collected data consisted of demographic information, meningitis etiologies, in-hospital mortality and neurological deficits at hospital discharge, including neurocognitive sequelae such as confusion (GCS <14), delirium, aphasia, and behavior changes. **RESULTS** Among 276 PLWH with confirmed meningitis, 32.2% (n=89) were diagnosed with CM, 28.6% had bacterial meningitis (BM), 17% had tuberculosis meningitis and 8% had viral meningitis (VM). Thus, most PLWH with meningitis had etiologies other than *Cryptococcus*. Mortality due to CM (32.6%) was lower than for TBM (51.1%) and BM (48.1%). Those with TBM had more neurocognitive dysfunction at the time of hospital discharge (27.7%), compared to CM (15.7%) and BM (15.2%). However, among patients who died in the hospital, 71.4% of those with CM had neurocognitive dysfunction, compared to 38.5% of those with TBM and 25.0% of those with BM. **CONCLUSION** Among PLWH in Lira, northern Uganda, meningitis due to etiologies other than *Cryptococcus* occurred more commonly, although CM remains a major cause of death and neurological impairment.

Wed C1.7 – Differential activation and exhaustion of circulating T-cells and monocytes at baseline may determine risk of developing cryptococcal meningitis among individuals with HIV-associated asymptomatic cryptococcal antigenemia

Mark Oilor

Eric Katagirya, Elizabeth Nalintya, Caleb P Skipper, Annie Wilt, Paul Kirumira, Allan Buzibye, Sandra Naluyima, Abduljewad Wele, Biyue Dai, David R Boulware, Radha Rajasingham, David B. Meya, & Samuel Okurut.

Makerere University

Introduction: The role of host immune factors in the progression of asymptomatic cryptococcal antigenemia (CrAg) to cryptococcal meningitis outcomes are unclear. This study assessed immunological predictors of progression to meningitis despite fluconazole preemptive therapy. **Methods:** Twenty-one Ugandans adults living with HIV with asymptomatic CrAg and titers $\geq 1:160$ received fluconazole preemptive therapy. Six developed meningitis while 15 did not, surviving to 6-months without meningitis. Baseline peripheral blood mononuclear cells were analyzed via spectro-flow cytometry. Plasma fluconazole was measured by mass spectrometry at week 4. **Results** There were no significant differences in age, CrAg titers, CD4 counts, fluconazole concentrations (median 36 mcg/mL with meningitis vs 22 mcg/mL without meningitis), ($p > 0.05$). Individuals who developed meningitis had lower %CD4+ T-cells, higher %CD8+ T-cells, and lower CD4/CD8 ratio (0.12 versus 0.20, $p = 0.020$) than those without meningitis. The %CD69 and CD163 activation marker expression on classical monocytes was lower in individuals who developed meningitis than those without ($P = 0.018$; $P = 0.008$, respectively). The %CCR4 expression on CD4+ T-cells, reflecting Th2 chemokine receptor, was higher in individuals with meningitis than those without. Individuals who developed meningitis had higher %PD-1 expression (immune cell exhaustion marker) on CD4+ T-cells, CD8+ T-cells, and on classical monocytes ($P < 0.05$ for each). The CXCR3+/CCR4+ (Th1/Th2) ratio on CD4+ T-cells was lower among individuals with meningitis than those without (0.635 versus 1.043, $p = 0.007$), reflecting a greater Th2>Th1 skew. **Conclusions:** Lower CD4/CD8, Th1/Th2 ratios, divergent activation, and increased T-cell/monocyte exhaustion may increase the risk of developing meningitis among individuals with HIV-associated asymptomatic cryptococcal antigenemia

Wed C1.8 – Incidence, reasons, and outcomes of rehospitalization among adults with HIV-related cryptococcal meningitis in Uganda: a retrospective cohort study

Rhona CB Muyise

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Background and aims: While advances in Cryptococcal meningitis (CM) treatment have improved survival, post-discharge outcomes such as rehospitalisation remain poorly understood. We aimed to determine the incidence, predictors, causes, and outcomes of re-hospitalisation by 10 weeks among adults with HIV/CM. **Methods:** This was a retrospective cohort study analysing data from four clinical trials conducted between 2017 and 2024 at Mulago, Mbarara, and Kiruddu Hospitals in Uganda. Adults with HIV/CM discharged alive after initial hospitalisation were followed for 10 weeks. **Results:** Of 1055 patients enrolled in the four clinical trials, 787 were discharged alive. All participants were adults with a median age of 35 years (IQR=30,40). All participants had HIV with a median CD4 cell count of 21 cells/ μ L (IQR=8,58). The majority were male (59%), and 45% were ART-experienced. 209/787 (26.6%) experienced either rehospitalisation or death within 10 weeks. Seventy-six died without rehospitalisation, 25 died after rehospitalisation, 108 were alive by 10 weeks, and 578 had no event. GCS, duration on ART, Haemoglobin, CD4, sodium, CSF opening pressure >200 , CSF protein, and CSF WBC were significantly different across the four groups. The median time from initial discharge to rehospitalisation was 20 days (IQR= 10, 36). The major cause of rehospitalisation was CM complications. Only normal GCS was significantly associated with rehospitalisation. However, age, GCS, CD4, haemoglobin and sodium were significantly associated with death. **Conclusions:** Nearly 3 in every 10 adults with HIV/CM experience rehospitalisation or death within 10 weeks following discharge, with the major cause of rehospitalisation being CM complications.

Wed C1.9 – Subtelomeric Genomic Rearrangement underlies Phenotypic Variation and Amphotericin B Resistance in Clinical isolates of *Cryptococcus neoformans*

Sujiraphong Pharkjaksu

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Emergence of amphotericin B (AmB) resistance remains uncommon in *Cryptococcus* but is increasingly recognized and poses a serious clinical threat, as AmB-based regimens remain a cornerstone of induction therapy for cryptococcal meningitis. We analyzed serial clinical isolates of *C. neoformans* obtained from a patient with treatment-failed cryptococcosis before and after AmB-based treatment. The post-treatment isolate exhibited elevated AmB MICs (2–3 µg/ml) compared with the pre-treatment isolate (0.25–0.38 µg/ml). Phenotypic assays showed that AmB-resistant isolates had reduced growth at elevated temperature and impaired fitness under membrane perturbation, redox/oxidative stress, and iron limitation. Consistent with altered redox homeostasis, fluorescence-based assays revealed increased basal reactive oxygen species in cytosolic and mitochondrial compartments in the resistant isolate. To define the underlying mechanism, we performed RNA-seq comparing genome-sequenced wild-type and post-treatment isolates. Gene Ontology enrichment identified ergosterol biosynthesis as the most significantly altered pathway, with marked downregulation of ERG2 in post-treatment isolates. Long-read nanopore sequencing uncovered an approximately 20-kb terminal deletion on chromosome 1 encompassing ERG2, followed by de novo telomeric repeat addition consistent with chromosome-end healing. Complementation of AmB-resistant isolates with wild-type ERG2 restored phenotypic defects and AmB susceptibility, confirming ERG2 loss as the primary driver of AmB resistance. These data demonstrate a clinically relevant route to AmB resistance emergence in *C. neoformans* mediated by chromosome-end loss and telomere addition and highlight ERG2-dependent sterol homeostasis as critical for core physiology and polyene susceptibility.

Concurrent Session 2

Wed C2.1 – Dormant *Cryptococcus neoformans* yeasts persist in a concealed state in a murine model of infection

Anamelia Lorenzetti Bocca

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Several microorganisms, including *Cryptococcus neoformans*, can enter a dormant state characterized by reduced metabolic activity, transcription, and translation, enabling survival under unfavorable conditions with the potential to resume growth when environmental cues become permissive. The present study aimed to identify host immune factors that prevent or promote reactivation of dormant *C. neoformans*. We established an in vitro protocol to induce dormancy and generate viable but non-culturable (VBNC) yeasts and developed a murine model of latent infection using these cells. Wild-type (WT) mice infected with VBNC yeasts exhibited prolonged survival, and no fungal growth was detected in pulmonary tissue by colony-forming unit assays. However, following immunosuppression, high fungal burdens were recovered from the lungs and brains, confirming the presence of viable latent infection that did not cause mortality. In contrast, TLR-4-, TLR9-deficient mice and bone marrow-derived macrophages from these animals showed enhanced reactivation of dormant fungi compared to WT controls, even in the absence of immunosuppression. Moreover, at 110 days post-infection with VBNC cells, TLR9-deficient mice displayed reduced survival, indicating a role for TLR9 in maintaining fungal dormancy. Flow cytometric analyses further demonstrated an increased frequency of monocytederived dendritic cells in TLR9-deficient animals infected with dormant yeasts, implicating this population in the regulation of latency. Collectively, these findings indicate that the murine immune response, particularly

TLR9-dependent pathways, contributes to maintaining *C. neoformans* dormancy and restricting fungal reactivation, thereby limiting infection-associated pathology.

Wed C2.2 – Mechanism of antifungal persistence of *Cryptococcus neoformans* against Amphotericin B

Ruchi Agrawal

Hugo Varet, Etienne Kornobis, Adrien Pain, Aude Sturny-Leclère, Alexandre Alanio
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Cryptococcus neoformans can survive in the host during antifungal treatment, leading to treatment failure and increased clinical burden. Unlike resistance, which involves stable genetic changes, antifungal persistence does not include any genetic changes. Yet antifungal persisters survive during antifungal therapy. Our project aims to understand the antifungal persistence phenotype of *C. neoformans* against Amphotericin B (AMB). We found that at a 16-fold minimum inhibitory concentration (MIC) of AMB, most of the stationary-phase cells die (60-90%), but a small subpopulation of persister cells survives. Surprisingly, we saw cell division even at such a high concentration of the drug. For the first time, we successfully isolated persister cells and revealed many interesting and distinct phenotypes. Persisters are smaller in size, with a larger central vacuole, fewer mitochondria and many super-sized lipid droplets. Cell wall's chitin and beta-glucan, cell membrane's ergosterol and polyunsaturated fatty acids are also modified in persisters to adapt to the stressful environment. To decrease the oxidative stress generated by AMB, persisters upregulate the expression of genes involved in antioxidation and downregulate the expression of genes involved in mitochondrial respiration. Persisters also have upregulated polyamines and trehalose pathways as a protective mechanism to fight antifungal stress. Persister cells have lower expression of virulence markers, which could help them stay unnoticed inside the host for a longer time, which will be explored further with host interaction studies. Overall, our study has highlighted previously unknown and uncommon mechanisms that cryptococcal persister cells use to protect themselves during antifungal treatment.

Wed C2.3 – Defining the *Cryptococcus neoformans* titanide, a pathogenic morphotype that arises from typical yeast cells in response to host-relevant conditions

Abigail Potton

Iana Kalinina (1), Sandra Catania (1), Maria Makarova (2), Dora Corzo-Leon (1), Elizabeth Ballou (1)
MRC Centre for Medical Mycology, University of Exeter

Fungal morphogenesis is a major driver of disease outcome. For the opportunistic fungal pathogen *Cryptococcus neoformans*, morphological heterogeneity within the lung drives dissemination, immune evasion, and drug resistance. To better understand how this heterogeneity emerges, we used microfluidics to study *C. neoformans* under host-mimicking conditions. We observed that yeast cells are highly plastic and respond by propagating as typical 'yeast' (5-7 μm), transitioning into titan cells (>10 μm), or by generating an alternative daughter, the oval titanide (2-3 μm). Titanides appear 24 hours post-induction and by 72 hours are the dominant morphotype. Despite their prevalence and the significance of morphogenesis for virulence, until now, their biology remained unstudied.

Using TEM and fluorescence microscopy we demonstrate that titanides are morphologically distinct; they possess thin cell walls and capsules with reduced Pathogen Associated Molecular Pattern exposure and altered distribution. These features distinguish titanides from previously described *C. neoformans* 'small cells' such as in vivo seed cells (4-6 μm , high cell-wall mannan content) and micro cells (round, <1 μm , thick cell walled). Despite their thin cell walls, we show that titanides display increased resistance to cell wall/membrane stressors. Additionally, titanides have unique proliferation properties and a morphological plasticity that allows them to form yeast and titan cells in response to relevant culture conditions. Importantly, we demonstrate that titanides are an efficient infectious propagule in a murine model with equivalent pathogenicity to infection with yeast. Together, these findings establish an essential characterisation and insight into a key player in *C. neoformans* pathogenesis.

Wed C2.4 – Titan cell formation is essential for *C. neoformans* latency

Rachel Berg-Murante

Kirsten Nielsen

Virginia Tech

Virtually everyone is exposed to *Cryptococcus* in childhood by inhalation of fungal cells, with an estimated 95% of adults harboring a persistent subclinical *Cryptococcus* infection in their lungs. This latent infection reactivates to cause disseminated disease in immunocompromised people yet little is known about how this latency arises. Our lab developed a mouse model of latent *C. neoformans* infection to define both host and pathogen factors that influence latency. Titan cells are an important virulence factor produced during *C. neoformans* pulmonary infection. Previous studies showed that deletion of the G-protein coupled receptors *gpr4Δ* and *gpr5Δ* significantly reduced titan cell formation from 30% to <5% and resulted in attenuated disease in the KN99 acute infection model. To determine the role of titan cells during latent infection, we generated a *gpr4Δ gpr5Δ* mutant in the latent isolate UgCI223. During latent infection, the pulmonary UgCI223 cell population consisted of 70% titan cells whereas the *gpr4Δgpr5Δ* mutant had <20% titan cells at 14 days post infection (dpi). Surprisingly, no titan cells were detected in the *gpr4Δgpr5Δ* mutant by 30 dpi while the wildtype strain retained its high titan cell production. Fungal burden was significantly lower in the *gpr4Δgpr5Δ* mutant at 30 dpi, with clearance of the *gpr4Δgpr5Δ* mutant by 90 dpi. These results indicate titan cell formation is critical for essential for persistence of latent infections and could serve as an effective target for new treatment strategies.

Wed C2.5 – Post-transcriptional control of the *Cryptococcus* cell cycle by the RNA-binding protein Ssd1

Edward Wallace

Daniel Jones, Benny Kleinerman, Elizabeth Ballou

University of Edinburgh

Background: How and when proteins are translated from mRNA in the cell influences growth, stress resistance, and pathogenesis. RNA-binding proteins, such as Ssd1, regulate the time and place of translation by acting on messenger RNA transcripts. These regulators can evolve new functions, and studying them across multiple fungal species can reveal molecular mechanisms underlying species-specific phenotypes. Our evolutionary analysis found that Ssd1 evolved in fungi from an ancestral protein with a distinct function as a nuclease enzyme. Across dikaryotic fungi, Ssd1's structure and RNA-binding site are conserved. We also found that *Saccharomyces cerevisiae* Ssd1 binds to a CNYTCNYT motif found in mRNAs that encode cell wall components and cell cycle regulators. Approach: Using synthetic fluorescent reporters and cross complementation assays we investigated the role of Ssd1 in mRNA regulation in *S. cerevisiae* and *C. neoformans* Results: We found that *Cryptococcus neoformans* Ssd1 complements deletion of *S. cerevisiae* Ssd1. In *S. cerevisiae*, inserting CNYTCNYT motifs into reporter genes reduces the amount of protein made, but only if Ssd1 is present. Deletion of *C. neoformans* Ssd1 leads to severe cell separation defects in Titan-phase cells, but no defects in normal yeast-phase cells. We identified a cell cycle-responsive transcription factor, Mbs1, as a regulatory target of Ssd1. Deletion of MBS1, deletion of SSD1, or mutation of Ssd1-binding CNYTCNYT motifs found in the MBS1 RNA lead to similar Titan-phase cell separation defects. Mutation of these Ssd1-binding motifs also leads to higher abundance of fluorescently-tagged Mbs1, consistent with a requirement for Ssd1 to block translation of MBS1 mRNA. Our work mechanistically characterises a conserved fungal regulatory pathway that controls translation of cell cycle components, and thus fungal growth. However, in *Cryptococcus* this conserved pathway has been repurposed to be critical for Titan-phase growth, and dispensable for yeast-phase growth.

Wed C2.6 – Mitotic and cytokinetic defects under stress can drive polyploidy in *Cryptococcus neoformans*

Claudia A Petrucco

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Polyploidy is observed in multiple human fungal pathogens and provides a strategy for rapid adaptation to stressful conditions encountered during infection. In *Cryptococcus neoformans*, polyploidy is a defining feature of Titan cells, which can reach ploidy levels from 2C to 312C. These cells exhibit enhanced survival compared to typical haploid yeast cells in the host. Polyploidy could arise through several mechanisms, but the precise cell-cycle alterations that underlie this process in *C. neoformans* remain poorly understood. Recently, work in the lab, demonstrated that cells exposed to hypoxia arrest before mitosis as large unbudded cells in G2. *C. neoformans* cells exposed to prolonged hypoxia either alone or combined with heat stress or nutrient limitation, exhibited mitotic and cytokinetic defects that drove polyloid cell formation. Live-cell imaging revealed several distinct mitotic and cytokinetic failures that subsequently produced polyloid cells with one or more nuclei. These observations reveal that host-relevant stresses can disrupt normal mitotic progression and promote polyloidization through multiple mechanisms. Using molecular approaches coupled with live-cell imaging, we aim to identify how core cell-cycle machinery is modified during polyloidization and how hypoxia contributes to these changes. Because the ability to grow and divide in host-imposed stress is a major virulence factor, understanding how polyloid cells form and proliferate will provide insight into how polyloid Titan cells contribute to disease progression and fungal pathogenicity.

Wed C2.7 – Perturbing Mps1 Kinase in the *Cryptococcus neoformans* titan cell, implications for a key morphological transition

Thomas Davies

Prof Kevin Hardwick* Prof A. Jeyaprakash Arulanandam**, Wulfrun Godson*
University of Edinburgh

C. neoformans ability to undergo a striking morphological transition within the human host to form large, polyloid “titan” cells presents a unique biomechanical challenge. How the polyloid titan genome is organised for the reductive divisions which occur from titans, and how this process is regulated via the mitotic or meiotic machinery is little understood. Additionally, titans are thought to be important for virulence in cryptococcal disease, representing a potential novel therapeutic target. Here, we investigate the requirement for components of the Spindle Assembly Checkpoint (SAC) - a key mitotic quality-control mechanism - in titan formation and division. The SAC monitors the attachment of chromosomes to spindle microtubules during meiosis and mitosis, preventing cell-cycle progression until all chromosomes are correctly aligned; errors in this process lead to aneuploidy and cell death. We hypothesise that the polyloid nature of titan cells may render them susceptible to perturbation of the mitotic machinery, with downstream consequences for viability and virulence. We find that the SAC is not required for titan formation or reductive divisions, but that a central SAC-associated kinase, Mps1, is essential for both. Perturbation of Mps1 leads to reduced titan formation, morphological and nuclear organisation defects, and severely decreased viability in the few titans that form. Furthermore, a chromosome 6 disomy is consistently observed in *mps1Δ* strains and is enriched among surviving *mps1Δ* titan daughters. These findings reveal a SAC-independent role for Mps1 in titan morphogenesis and ploidy control, and raise intriguing questions regarding the regulation of this unique morphological state.

Wed C2.8 – A role for aquaporin (Aqp1) in the control of *Cryptococcus neoformans* cell morphology

Piotr Stempinski

Francisco G. Hernandez, J. Alberto Patiño-Medina, Isabel Jimenez, Samuel Rodrigues dos Santos Junior, Peter Agre, Arturo Casadevall
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Aquaporins are small, integral membrane channels that facilitate the transport of water across cellular membranes and, in the case of aquaglyceroporins, can also conduct specific neutral solutes, such as glycerol. These proteins are conserved across biological kingdoms, yet their roles in fungal virulence remain relatively understudied. In *Cryptococcus neoformans*, an opportunistic fungal pathogen, we examined the organism's single aquaporin, Aqp1, and uncovered unanticipated influences on cellular morphology. Loss of Aqp1 resulted in smaller cells, whereas its presence promoted the formation of enlarged titan-like cells. This shift in size was closely linked to intracellular redox physiology.

Consequently, the overexpression of the cryptococcal aquaporin increased sensitivity to oxidative stress and led to the largest titan-like cells; antioxidant supplementation suppressed this enlargement, consistent with a ROS-dependent regulatory mechanism. Additionally, Aqp1 overexpression produced vacuolar abnormalities in titan-like cells, suggesting that excessive water influx strained intracellular organization during rapid cell expansion. These findings position Aqp1 at a functional crossroads connecting membrane transport, oxidative balance, and size control, and they support a model in which an aquaporin contributes to the morphological plasticity that allows *C. neoformans* to adapt to environmental pressures.

Concurrent Session 3

Wed C3.1 – FungiDB: Sustaining Genomic-Scale Discovery through Open Access and Community Support

Evalina Basenko

Andy Jones *Presenting on behalf of the entire VEuPathDB project.

Institute of Systems, Molecular & Integrative Biology, University of Liverpool, UK

Modern biological research is increasingly driven by large-scale genomic and multi-omics data. Delivering FAIR (Findable, Accessible, Interoperable, Reusable) access to these data requires sustained infrastructure, expert curation, and continuous tool development.

The Eukaryotic Pathogen & Vector Genomics Resource (VEuPathDB.org), including FungiDB.org, is an open-access, community-driven platform for genomic-scale data exploration and discovery that supports thousands of researchers across the eukaryotic microbe research community. FungiDB hosts numerous *Cryptococcus* genomes, including *Cryptococcus depauperatus*, *Cryptococcus gattii* molecular types VGI, VGII, VGIII, and VGIV, *Cryptococcus neoformans*, and also *Kwoniella* species. The platform provides sophisticated query tools that enable cross-examination of diverse omics datasets from both pathogens and hosts, thereby facilitating integrative analyses and accelerating scientific discovery. To ensure the resource continues to reflect the evolving needs of the community, VEuPathDB actively seeks user feedback on priority datasets to include and analytical tools to develop.

To secure long-term sustainability while maintaining open access, VEuPathDB has introduced a new subscription model. Subscription support enables continued platform development, reliable infrastructure, responsive user support, and incorporation of community priorities, and is compatible with existing funding mechanisms, as most agencies allow budget allocations for website access and data dissemination.

Key capabilities and recent advances enabled by this model include:

- Comprehensive multi-omics data integration: Unified access to genomes, gene annotations, population diversity, comparative genomics, epigenetics, transcriptomics, proteomics, metabolomics, phenotypes, and AlphaFold structural predictions, as well as exploration of host-pathogen responses.
- Integrated data analysis (“in silico experiments”): Tools for pathway analysis, orthology-based functional inference, and subcellular localisation, enabling hypothesis generation and testing within the database.
- AI tools for discovery: Literature-driven analysis that supports user-submitted comments and expands functional annotations, as well as automatic summarisation of transcriptomics datasets for each gene.
- Comparative genomics resources: Updated OrthoMCL (OrthoMCL.org) with UniProt proteomes, OrthoFinder-based clustering, customisable phylogenetic trees, and enhanced visualisation of protein superfamilies.

By coupling open access with a sustainable subscription model, FungiDB continues to evolve into a reliable, innovative, and FAIR bioinformatics resource that transforms large-scale biological data into actionable insights.

Wed C3.2 – ERG11 Mutations Reshape Fluconazole Binding in Cryptococcus

Lexia Henning

Alfred Botha(2) and Angela Botes(1)

School of Molecular and Cell Biology, University of the Witwatersrand, South Africa

Antifungal drug resistance (AFR) complicates the treatment of invasive fungal diseases (IFDs) such as cryptococcosis, particularly in immunocompromised individuals and those in resource-limited settings. Drug resistance often arises from single-nucleotide polymorphisms (SNPs) in genes encoding antifungal targets such as ERG11. This study assessed trends in cryptococcal AFR, identified resistance-associated SNPs and investigated their impact on protein folding and ultimately ligand interactions. Clinical and environmental cryptococcal isolates, originating from South Africa, were tested for antifungal susceptibility to fluconazole (FLC) using the microbroth dilution assay. The ERG11 gene of ten resistant and six susceptible isolates was sequenced and analysed for SNP identification and AFR correlation. Protein modelling, molecular docking and dynamic simulations assessed the impact of the SNPs' corresponding protein mutations on lanosterol 14 α -demethylase structure, FLC and natural substrate interaction. Environmental isolates exhibited significantly higher AFR than clinical isolates ($p < 0.001$). Additionally, temporal and regional analyses revealed increased resistance in recent (post-2018) compared to older (pre-2010) isolates, with distinct spatial distributions ($p < 0.001$). Multiple ERG11 SNPs were identified, including South African-specific variants that alter the substrate-interacting residues and the haem motif. Molecular docking and dynamic simulations confirmed reduced FLC affinity and enhanced lanosterol binding in an extremely resistant variant (FLC minimum inhibitory concentration $> 64 \mu\text{g/mL}$) compared to a susceptible variant. These findings highlight evolving AFR strategies, structural SNP impacts, emphasising the need for integrated surveillance and antifungal stewardship in high-risk settings.

Wed C3.3 – Identification of synergistic compounds in combination with fluconazole from the Prestwick Chemical Library® against Cryptococcus neoformans.

Alba Torres Cano

Alba Torres-Cano¹, Alejandra Lora-Plaza¹, Oscar Zaragoza^{1,2}

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The treatment of *Cryptococcus neoformans* is limited due to its low susceptibility to fluconazole, compared to other fungal species, and intrinsic resistance to echinocandins, resulting in a restricted range of therapeutic options. In this context, drug repurposing emerges as a promising alternative strategy, as it consists of identifying new biological effects of drugs already used for other purposes, offering significant advantages in terms of cost, time, and safety compared to de novo drug development. In this study, a total of 1,520 off-patent, FDA-approved compounds from the Prestwick Chemical Library® were screened to identify agents with synergistic activity in combination with fluconazole against *C. neoformans*. An initial high-throughput screening allowed the selection of 12 promising compounds, including acetylsalicylic acid, pinaverium bromide, thioridazine hydrochloride, cianidol, risedronic acid monohydrate, tegaserod maleate, alendronate sodium, ibufenac, flurbiprofen axetil, oxiglutatione, atorvastatin, and avermectin B1. Synergistic interactions were subsequently confirmed by checkerboard assays using six *C. neoformans* clinical isolates. Synergy with fluconazole was validated for pinaverium bromide, thioridazine hydrochloride, alendronate sodium, oxiglutatione, ibufenac, atorvastatin, avermectin B1, tegaserod maleate, and risedronic acid monohydrate. To further assess the translational potential of these combinations, cytotoxicity was evaluated in RAW 264.7 macrophages, revealing toxicity at the highest tested concentrations of pinaverium bromide, tegaserod maleate, and thioridazine hydrochloride. In addition, in vivo safety assessment using *Caenorhabditis elegans* is currently being conducted to further characterize compound efficacy. Overall, these results highlight drug repurposing as a valuable approach for identifying novel antifungal combinations against *C. neoformans*.

Wed C3.4 – Activity of Ibrexafungerp Against Clinical and Environmental Isolates of *Cryptococcus neoformans* and *Cryptococcus gattii*

Carolina Firacative

Andrés Ceballos-Garzon

School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia

Background and aims: Cryptococcosis remains a life-threatening fungal infection, particularly among immunocompromised individuals, with *Cryptococcus neoformans* and *Cryptococcus gattii* causing most cases. Despite advances in antifungal therapy, mortality rates remain high. Drug toxicity, limited access to first-line antifungals in resource-limited settings, and the emergence of resistance worsen management and underscore the urgent need for novel therapeutic options. Ibrexafungerp (IBX), a first-in-class oral triterpenoid inhibitor of β -(1,3)-D-glucan synthase, has demonstrated potent antifungal activity. Therefore, we aimed to evaluate the in vitro activity of IBX against clinical and environmental cryptococcal isolates, including isolates with reduced susceptibility to fluconazole. Methods: Fifteen clinical and 5 environmental isolates were studied, 10 of *C. neoformans* (serotypes A, D, and A/D) and 10 of *C. gattii* (serotypes B and C). From these, three *C. neoformans* and four *C. gattii* have reduced susceptibility to fluconazole (≥ 16 $\mu\text{g/mL}$). Antifungal susceptibility testing was performed by broth microdilution according to the CLSI, with IBX ranging from 0.015 to 8 $\mu\text{g/mL}$. Results: Against both cryptococcal species, the MIC₉₀ for IBX at the $\geq 50\%$ endpoint was 2 $\mu\text{g/mL}$. At the 100% endpoint, the geometric MIC was 3.4 $\mu\text{g/mL}$ and 3.2 $\mu\text{g/mL}$ for *C. neoformans* and *C. gattii*, respectively. Conclusions: IBX may overcome the intrinsic echinocandin resistance of *Cryptococcus* species, as well as the reduced susceptibility to fluconazole of some isolates, independently of the serotype and the source. Our findings contribute to the growing evidence supporting novel antifungal strategies against cryptococcosis, addressing a critical unmet need in the management of this clinically important mycosis.

Wed C3.5 – Anticryptococcal Mechanisms of Action by Dendritic Cell Lysosome Proteins

Karen Wozniak

Megan Roach, Isaiah Burns, Molly McGaughy, Anna Manes

Oklahoma State University

Cryptococcus neoformans is an opportunistic fungal pathogen that enters the lungs and leads to meningitis in immunocompromised individuals. The pathogen initially encounters innate immune cells, such as dendritic cells (DCs) in the lungs, and these cells can kill *C. neoformans*. Previous studies in our lab showed that proteins from the DC lysosome have potent antifungal activity against *C. neoformans*, including NOSTRIN, matrix metalloproteinase 25 (MMP25), and myeloperoxidase (MPO). We hypothesized that these compounds kill *C. neoformans* by affecting signaling pathways, leading to fungal cell wall and membrane damage. To test this, we used microscopy following treatment to visually identify structural changes following treatment, and we used cryptococcal phosphatase and kinase mutant libraries to identify mutants and signaling pathways involved in resistance or susceptibility to treatment. Microscopy of *Cryptococcus* treated with NOSTRIN, MMP25, and MPO showed release of intracellular contents and misshapen fungal cells. This phenotype was rescued by the addition of sorbitol for osmotic stability, indicating osmotic lysis of *Cryptococcus* due to lysosomal proteins. Mutant library analyses following NOSTRIN and MPO treatment showed increased resistance in mutants with genes involved in inositol phosphate metabolism, while MMP25 treatment showed increased resistance in mutants with genes involved in purine and pyrimidine metabolism and genes involved in secondary metabolite biosynthesis. These studies indicate that there are some shared mechanisms and some unique mechanisms used by DC lysosome proteins for antifungal activity. Further studies will determine the importance of the identified signaling pathways in antifungal activity of DC lysosome proteins.

Wed C3.6 – The novel carbohydrate modifying enzyme Cel1 is required for *Cryptococcus neoformans* cell wall remodeling in response to host environmental stress.

Corinna Probst

Wan Y2, Hallas-Møller M3, Johansen K3, Dupree P2 and Alsbaugh JA1

Duke University, School of Medicine Department of Medicine, Durham USA

The cell wall provides support and integrity to the fungal cell. As the interface with the external environment, it is also an important structure for stress adaptation. Accordingly, rapid changes within the carbohydrate composition and architecture occur in response to environmental stresses. Lytic polysaccharide monooxygenases (LPMOs) are copper-enzymes, secreted by microbes to assist in the first steps of remodeling and degradation of complex carbohydrates. While recent studies have established an important role for LPMOs in plant pathogenic microbes, studies about the role of LPMOs in human pathogenic microbes are scarce and limited to bacteria. The human fungal pathogen *Cryptococcus neoformans* (Cn) encodes for 4 potential LPMOs. Initial phenotypic analysis of loss-of-function mutant strains identified the CEL1 gene to be required for growth at mammalian host temperature and pH. In line with this finding, the cel1 Δ mutant strain is avirulent in a murine model of infection. In contrast to other known LPMOs which are secreted into the extracellular environment and thereby targeting exogenous carbohydrates, CnCel1 is retained within the cryptococcal cell wall, suggesting a role in promoting intrinsic fungal cell wall remodeling. To further elucidate CnCel1 function we performed a combination of solid-state NMR, carbohydrate and microscopic analysis. These analyses suggest a novel role for the Cel1 enzyme in modulating cell wall chitosan distribution and deposition, a key cell wall carbohydrate required for stress adaptation during infection. Taken together, our recent work has demonstrated a novel role for an LPMO-family enzyme for fungal cell wall remodeling and stress adaptation during pathogenesis.

Wed C3.7 – Chitin as a Hidden Driver of *Cryptococcus neoformans* Virulence

IRERE HILAIRE

Liliane Mukaremera , Neil A.R. Gow
MRC CMM/ Exeter University

Meningitis caused by the fungal pathogen *Cryptococcus neoformans* remains a major global health problem, causing an estimated 152,000 cases and 112,000 deaths annually among people living with HIV/AIDS. The high mortality highlights limitations of current antifungal therapies and underscores the need to better understand fungal-traits that drive disease severity during infection. *Cryptococcus* is surrounded by a two-layered, sugar-rich structure: an outer polysaccharide capsule and an inner-cell wall. While the capsule is a well-established virulence factor, the contribution of cell wall components remains poorly defined. In this study, we screened 200 clinical *C. neoformans* isolates grown in physiologically relevant Human Plasma-Like Medium (HPLM) and selected 10 stable low-chitin and 10 stable high-chitin isolates representing the extremes of the chitin spectrum for in-depth analysis. Using these extreme phenotypes, we observed marked differences in fungal population structure and function. Low-chitin isolates were dominated by small-cell populations, exhibited higher metabolic activity, and were taken up more efficiently by macrophages. In contrast, high-chitin isolates were enriched for larger-yeast cells. Despite their smaller-cell bodies, low-chitin isolates produced proportionally larger capsules and achieved higher fungal burdens in murine lung infection models. Together, these findings indicate that cell wall chitin content is not only involved in mediating cell shape and environmental stress protection but also strongly influences *Cryptococcus* population structure and metabolic activity. The pronounced differences between high- and low-chitin isolates suggest that chitin acts as a dynamic regulator of key aspects of fungal biology. Further studies are underway to define the mechanism these phenotypes contribute to pathogenesis.

Wed C3.8 – The glycosyltransferase Goe1 (Glucan Organizing Enzyme 1) contributes to cell wall integrity and echinocandin resistance

Sydney Briner

Sydney L. Briner and Tamara L. Doering
Washington University in St. Louis

The lingering mystery of why *Cryptococcus neoformans* resists echinocandins, which inhibit Fks1-mediated synthesis of cell wall β -1,3-glucan, underscores how much of this organism's biology remains poorly understood. It also highlights the importance of identifying additional enzymes that contribute to cell wall integrity. We recently discovered the putative glycosyltransferase Glucan Organizing Enzyme 1 (Goe1). Cells lacking Goe1 (goe1 Δ) have defects in two cell wall glucans: α -1,4- and β -1,3-. Intriguingly, upon treatment with echinocandins, goe1 Δ struggles to grow. To understand this, I am interrogating Goe1's enzymatic activity and how it influences Fks1. Surprisingly, I found that Goe1 resides in the Golgi. This suggests it does not work directly on cell wall glucans, although it could

potentially impact Fks1 activity through several mechanisms. So far, I have found through RT-qPCR that FKS1 expression is not perturbed in *goe1Δ*, while yeast two-hybrid studies suggest that Goe1 interacts with an intracellular domain of Fks1. This suggests that protein interactions may be involved, possibly relevant for Fks1 trafficking. Finally, I showed by imaging that Fks1 is mislocalized in *goe1Δ*. One model to explain these disparate observations is that Goe1 acts in lipid glycosylation. Preliminary analysis of glycolipids extracted from wild-type and *goe1Δ* cells by thin layer chromatography and mass spectrometry suggests that Goe1 may contribute to the synthesis of glycoinositol phosphoceramide (GIPC) lipids, which are particularly elaborate in *C. neoformans*. Together, my data implicate Goe1 in GIPC synthesis and suggest a novel model for the role of cryptococcal GIPCs in Fks1 function and echinocandin resistance.

Thursday July 2

Plenary Session

Thurs P1 – Metazoan-Like Genome Compartmentalization in *Cryptococcus neoformans*

Kaustuv Sanyal

Bose Institute, Kolkata & JNCASR, Bengaluru

While eukaryotic genomes are typically partitioned into a transcriptionally active euchromatic A compartment and a transcriptionally repressed heterochromatic B compartment, chromosomes occupy the nuclear space in diverse ways. Primary chromosomal arrangements are often defined by the positioning of centromeres, which may cluster together, as in yeasts, or disperse along the nuclear periphery, as commonly observed in metazoans. Remarkably, the basidiomycete fungus *Cryptococcus neoformans* exhibits both organizational states in yeast and possibly in other morphotypes associated with distinct host niches. This human fungal pathogen causes approximately 200,000 cases of pneumonia and meningitis annually worldwide. Here, we show that the unique spatial organization of its centromeres and telomeres establishes a metazoan-like A/B compartment architecture in the genome. Metazoan-like late-replicating centromeres shape chromosome arrangement and contribute to the large-scale organization of chromosomes in *C. neoformans*. We further demonstrate that these late-replicating centromeres may promote centromere declustering or dispersion during S phase. Together, our findings not only reveal the remarkable plasticity of genome organization in *C. neoformans* but also suggest that centromere replication timing may serve as a fundamental determinant of higher-order genome architecture

Thurs P2 – Extracellular vesicles biosynthetic pathways in *Cryptococcus*

Guilhem Janbon

Institut Pasteur, Paris

Extracellular vesicles (EVs) are lipid-based nanoparticles produced by all living cells. In mammals, EVs mediate cell-to-cell communication and regulate both normal and pathological cellular processes. In human fungal pathogens, EVs have been studied for several years, and evidence from multiple species suggests they play key roles during infection. Our laboratory has used proteomic, nanocytometry, and genetic approaches to propose a structural model of EVs in the pathogenic yeast *Cryptococcus neoformans*. We also investigated the genetic control of EV biosynthesis, revealing links between EV production and antifungal susceptibility. Recently, through time-course RNA-Seq and genome-wide mutant screening, we identified an EV regulon and numerous regulators of EV production. These analyses highlighted the central role of calcium homeostasis in EV regulation and provided the first global insights into EV biosynthetic pathways in fungi.

Thurs P3 – Developing experimental immunotherapies for offsetting inflammatory brain pathologies in *Cryptococcus* Meningoencephalitis

Michal A. Olszewski

Michal A. Olszewski^{1,2} Hailong Li^{1,2,3}, Rylan Hissong^{1,2}, Kristie D. Goughenour^{1,2}, Jane Barnett², Maia Lintner², Alexander Ballesteros², Jessica C. Hargarten^{4,5,6}, Peter R. Williamson⁴, Jintao Xu^{1,2}

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Severe brain damage is the main source of morbidity and mortality in cryptococcal meningoencephalitis (CM). Despite antifungal drug treatment, inflammation contributes to damage, and the CNS-inflammatory component remains largely unaddressed clinically. Here we devised and preclinically tested urgently needed immunomodulatory therapies aiming to suppress CNS inflammation without substantial effect on fungal clearance. Anti-CXCR3-antibody (CXCR3Ab), low dose IL-2 immune complexes (LdIL-2cpl), or anti-FAS-L immunotherapies were applied at the onset of symptoms in mouse models of CM. Body weight loss, neurological and body condition score, and brain/other organ CFUs were assessed. Inflammatory parameters were analyzed by flow cytometry and histology. Some of these therapies were studied in the context of antifungal drugs fluconazole (FLC) and AmBisome (AmBi). CXCR3Ab immunotherapy improved survival and symptoms in the hyperinflammatory CM model (C.n.52D) by improving symptoms, reducing inflammation level, and only slightly slowing fungal clearance. Combination fluconazole (FLC) and CXCR3Ab therapy improved survival above any individual treatment. In high-virulence CM model (C.n.H99), CXCR3Ab decreased inflammation level without worsening fungal CFU or survival and in combination AmBi and CXCR3Ab therapy improved mouse survival. The LdIL-2cpl therapy boosted CNS Treg response and reduced inflammation, drastically improving survival in C.n.52D-CM. Alone, it significantly suppressed brain fungal clearance, but not when combined with FLC. Promising results were obtained with Anti-FASL showing a mild immunosuppressive effect, but drastically reduced symptoms and mortality in C.n.52D-CM. In conclusion, targeted immunotherapies show promise as safe and effective means of suppressing inflammatory damage in CM, achieving optimal effects when combined with conventional antifungal drug therapies.

Thurs P4 – CRISPR-insert reveals the genetic architecture of the drug-resistant fungal meningitis pathogen *Cryptococcus neoformans*

Hiten Madhani

Manning Y. Huang¹, Patrick Hecht¹, Kyle Cesar¹, Massimiliano Ciranni², Angela, Wei¹, Michael Boucher¹, Sydney L. Briner³, Ankur Ankur⁴, Léa Lortal¹, Veda Nayak¹, Tuo Wang⁴, Tamara L. Doering³, Vito Paulo Pastore²

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We describe CRISPR-insert, a global insertional mutagenesis method we implement in the echinocandin-resistant pathogenic yeast *Cryptococcus neoformans*. Sequencing of insertion sites across four genetic backgrounds, coupled with two distinct machine-learning approaches, predicts ~1200 essential genes. We validate essential complexes/pathways in *C. neoformans* conserved with humans but lost in model yeasts, such as Complex I of the Electron Transport Chain. We identify targets for antifungal drug development by validating predicted essential genes with essential orthologs in model yeasts but not in humans. Unexpectedly, the echinocandin target Fks1/b-1,3-glucan synthase is important but not essential for growth across multiple strain backgrounds, helping to explain the clinical ineffectiveness of echinocandins, despite their ability to inhibit *C. neoformans* Fks1. Solid state NMR confirmed the absence of b-1,3-glucan in fks1Δ cells. We produced a streamlined nonessential gene library whose utility we substantiate through multiple genetic screens. We identify gene-gene-echinocandin interactions, thereby demonstrating the feasibility of producing large-scale genetic interaction maps relevant to therapeutic development.

Thurs P5 – The biological function of *Cryptococcus* titan cells

Kirsten Nielsen

Virginia Tech University

Upon inhalation into the lung environment, *Cryptococcus neoformans* generates a plethora of cell size morphologies. One of these cell morphologies is the large titan cells. Titan cells range from 10-100 microns in cell body diameter, are polyploid, and have alterations in their cell wall and capsule structures. This presentation will describe current understanding of the role of titan cells in *Cryptococcus* pathogenesis, dissecting the function of multiple titan cell phenotypes, and determining how these changes in titan cells influence the host-pathogen interaction during infection.

Thurs P5 – IFN γ therapy remodels mammalian copper transporter ATP7A to support myeloid cell antifungal immunity

Rebecca Drummond

Alanoud Alselami, Rio Wood, Lorna George, Hung-Ji Tsai, Rebecca A. Drummond

University of Birmingham

IFN γ therapy has been shown to exert therapeutic benefits for cryptococcal meningitis patients, but the specific mechanisms of protection are not clear. We recently found that *C. neoformans* copper starvation within the brain is niche-specific where yeast residing intracellularly within microglia were protected from copper starvation. Notably, we observed that IFN γ treatment of microglia resulted in copper starvation of intracellular fungi. We therefore tested the hypothesis that IFN γ may exert protective antifungal immunity via the manipulation of intracellular copper pools in macrophages. We find that IFN γ -treated macrophages have improved fungal killing capacity that was dependent on fungal copper homeostasis pathways. IFN γ treatment did not affect total copper concentration in macrophages but caused significant remodelling of the mammalian copper exporter ATP7A, which co-localised near intracellular fungi following phagocytosis. When ATP7A was inhibited, intracellular fungal survival increased. In vivo, we found ATP7A expression correlated with fungicidal capacity of brain myeloid cells. Using microglia-specific IFN γ receptor knockout mice (Sall1CreERIfn γ 1flox), we investigated how microglial defence against *C. neoformans* brain infection was affected by fungal copper homeostasis in the context of IFN γ therapy. Our ongoing work has found that therapeutic benefits of IFN γ treatment is dependent on microglia expression of IFN γ , which we are now exploring whether this is linked with myeloid polarisation status and fungal killing capacity. Taken together, these results highlight the dynamic interplay between copper homeostasis and host immune defence in the brain, providing new insights into protective mechanisms of IFN γ therapy within the central nervous system during cryptococcal meningitis.

Concurrent Session 1

Thurs C1.1 – Gene content diversity in *Cryptococcus neoformans*

Blake Billmyre

Claire Reichardt

University of Georgia

Cryptococcus neoformans causes an estimated 147,000 deaths per year worldwide, primarily in immunocompromised patients. *C. neoformans* consists of 4 different molecular types: VNI, VNII, VNBI, and VNBII. While we understand sequence variation separating these groups, we have a limited understanding of the differences in genetic content that differentiate each isolate. We de novo assembled a pangenome using Illumina sequences of 384 diverse *C. neoformans* isolates. We curated a set of *C. neoformans* orthologs that are either core (present in >95% of genomes), accessory (present in >=95% and <1 genomes), or singletons (present in 1 genome). Most genetic content variation in *C. neoformans* is the result of variation in accessory gene content. Our pangenome revealed that a large portion of accessory genes were located near subtelomeres in the H99 reference genome. We also

found numerous accessory genes that were limited to specific molecular types and that many singletons shared close sequence similarity to closely related species of *Cryptococcus*, consistent with introgression or incomplete lineage sorting events. We are currently using transposon mutagenesis to explore the functional consequences of variation in gene content across the species.

Thurs C1.2 – Saprobic and pathogenic *Cryptococcus* species: uncovering hidden diversity across the genus.

Marcia C David Palma

Marco A. Coelho (1), Aleksey V. Kachalkin (2,3), Miroslav Kolařík (4), Benedetta Turchetti (5), José Paulo Sampaio (6), Michael J. Wingfield (7), Matthew C. Fisher (8), Andrey M. Yurkov (9), Joseph Heitman (1).

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, United States of America

Species within the genus *Cryptococcus* range from opportunistic human pathogens to environmentally associated saprobes, yet the genomic and ecological processes underlying this diversity remain poorly understood. Comparative analyses across pathogenic and nonpathogenic lineages are essential for identifying evolutionary transitions linked to pathogenicity and for refining species boundaries. Here, we analyzed seventeen *Cryptococcus* species representing divergent evolutionary lineages, including six newly identified taxa and lineages previously lacking formal species designation. We integrated phylogenomic inference, divergence metrics, chromosome-scale genome analyses, mating assays, and metabolic phenotyping to clarify species boundaries and patterns of genome evolution within the genus. We formally designated *Cryptococcus hyracis* as the VGV lineage of the *C. gattii* species complex and described five new nonpathogenic species recovered from environmental substrates such as fruit, soil, and bark beetle galleries, three of which formed a previously unrecognized clade within *Cryptococcus*. One of these species, *Cryptococcus porticicola*, showed a strong association with bark beetles across multiple strains sampled from distinct geographic locations. Genomic analyses of this species revealed intraspecific chromosomal polymorphism, signatures of recombination, and evidence of sexual reproduction. Across *Cryptococcus*, we observed extensive karyotypic diversity, including lineage-specific rearrangements and repeated reductions in chromosome number, potentially linked to centromere loss or inactivation. Phenotypic Biolog profiling revealed clade-specific substrate preferences suggestive of ecological specialization, while growth at 37°C was restricted to clinically relevant taxa. Together, these findings expanded the recognized diversity of *Cryptococcus* and provided new insights into the genomic and ecological forces shaping speciation and pathogenic potential.

Thurs C1.3 – Whole genome alignment of *Cryptococcus* and *Kwoniella* reveals patterns of conservation

Sosie Yorki

Christina Cuomo

Brown University, Department of Computational Molecular Biology

Fully characterizing the evolution of pathogenicity in *Cryptococcus neoformans* is crucial for identifying more effective drug targets. Ortholog analysis of *Cryptococcus* and *Kwoniella* have pinpointed genes unique to pathogenic *Cryptococcus*, but evolution of non-coding regions remain less well understood. A recent GWAS analysis identified SNPs in non-coding regions linked to higher fungal burden in patients infected with *C. neoformans*, demonstrating the potential for non-coding variation to regulate pathogenicity functions. To investigate *C. neoformans* at nucleotide-level resolution, we aligned the reference genomes of 31 species with Progressive Cactus and used the Phylogenetic Analysis with Space/Time Models toolkit to find patterns among evolutionarily conserved and accelerated regions in *Cryptococcus* and *Kwoniella*. Close to 75% of bases in *C. neoformans* conserved across the whole tree belonged to coding sequence. Within protein-coding genes, exons varied in degree of conservation depending on its position relative to UTRs. Some intergenic regions and ncRNAs were also highly conserved, indicating these non-coding regions serve an important function. Examining sequence unique to pathogens, we found over 30% of non-protein coding bases in *C. neoformans* were exclusively aligned across all pathogenic species. This suggests newly evolved regulation of pathogenesis-conferring processes. The highest rates of evolutionary acceleration were seen in intergenic regions, 5' UTRs, and 3' UTRs (in descending order). Future analyses will re-examine

transcriptional data to identify potential genes within conserved intergenic regions and improve exon-intron boundaries in pathogenic *Cryptococcus*.

Thurs C1.4 – Genomic signatures of parasex among two genetically distinct hybrid groups of *Cryptococcus neoformans*.

Rahul Anand

Rahul Anand¹, Qinx Ma¹, Diana Tamayo¹, Nicholas Helmstetter¹, Rhys A. Farrer¹.

University of Exeter

Hybridisation is an important source of genetic and phenotypic diversity in fungi, yet the mechanisms shaping hybrid genomes in human pathogens remain unclear. *Cryptococcus neoformans*, a major cause of fungal meningitis, comprises two varieties - *C. neoformans* var. *grubii* and var. *neoformans* - that occasionally hybridise. We aimed to resolve hybrid lineage structure, parental origins, and recombination mechanisms driving genome evolution in this pathogen. We analysed 197 *C. neoformans* genomes, including 13 newly sequenced isolates, using phylogenomic, population genetic, and long-read assembly approaches. Analyses included mitochondrial inheritance, admixture, loss of heterozygosity (LOH), and structural variation to identify hybridisation signatures. We identified 73 hybrid isolates forming two previously unrecognised groups (H1 and H2) with distinct ecological origins and parental ancestry. H1, comprising exclusively clinical isolates, showed limited admixture but high nucleotide diversity, consistent with ancient hybridisation and backcrossing. H2, primarily environmental or laboratory-derived, exhibited extensive admixture between VNI and VNIV lineages. Both groups displayed pervasive aneuploidy, large-scale LOH, and mosaic haplotype inheritance consistent with parasexual recombination. Phenotypic assays revealed substantial variability in growth and virulence, highlighting functional impacts of hybrid genome plasticity. Our study provides the first evidence of parasexual recombination in *C. neoformans*, revealing a composite lifecycle that integrates mitotic and meiotic processes. Parasex emerges as a key mechanism shaping genome evolution, population diversity, and pathogenic potential in this globally important human pathogen.

Thurs C1.5 – Mechanisms of RNA interference in *Cryptococcus*

Elizabeth Bayne

Adriana Orrego, Sueden Oliveira De Souza, Kundan Kumar, Rob van Nues, Cei Abreu-Goodger

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RNA interference (RNAi) is a mechanism of genome regulation broadly conserved across eukaryotes. It is mediated by small RNAs, which are typically generated from long double-stranded RNA by Dicer enzymes, and associate with Argonaute effector proteins to direct down-regulation of target RNAs based on sequence complementarity. In *Cryptococcus*, RNAi plays an important role in silencing transposable elements, with loss of RNAi resulting in elevated rates of transposon-induced mutation that can contribute to adaptation and drug resistance. However, beyond the involvement of core pathway components (Dicer, Argonaute and RNA-dependent RNA polymerase), how RNAi operates in *Cryptococcus* remains poorly understood.

We have set out to dissect the molecular mechanisms of RNAi in *Cryptococcus*, with a focus on *C. deneoformans*, which retains the full ancestral complement of core RNAi machinery including two Dicer and two Argonaute proteins. Our initial assessments of the relative contributions of these core factors revealed that while Dcr1 and Dcr2 function largely redundantly to one another in endogenous sRNA production, Ago1 is the principle Argonaute protein required to support sRNA accumulation and transposon silencing. To further understand the mechanisms of silencing, we analysed Ago1-associated proteins by IP-MS. From this we identified several novel interactors with potential roles in facilitating/modulating RNAi activity, including putative RNA helicase and methyltransferase enzymes. We are currently further characterising the functions of these factors to understand their potential roles in RNAi function and/or regulation.

Thurs C1.6 – Neuropathological differences driven by cryptococcal species in a murine model of infection

Maahir Kauchali

Emily Ruth Higgitt 1, 2, 3, Yanga Pato 1, 2, 3, Jennifer Claire Hoving 3, 4, and Rachael Dangarembizi 1, 2, 3

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Cryptococcal meningitis (CM) is a fatal brain infection associated with debilitating neurological sequelae. CM research has historically focused on understanding the neuropathology of *Cryptococcus neoformans*-related infections, neglecting *C. gattii*-related CM. Recent rises in *C. gattii*-related CM, coupled with the fact that it predominantly affects immunocompetent individuals, highlights a dire need for experimental models for studying its neuropathogenesis. This study aimed to optimize a model of *C. gattii* infection and compare its neuropathogenesis to *C. neoformans*. We intravenously infected 8-10-week-old C57BL/6 mice with 5×10^4 colony forming units of *C. gattii* and *C. neoformans*. We monitored disease progression by measuring fungal burden, body mass gain and survival rates. Histological analysis was performed on 1, 3, 6 days post-infection (dpi) and experimental endpoint assessing neuropathological damage. Brain and serum cytokines were measured using multiplex assays. We observed marked differences in the neuropathogenesis of *C. neoformans*- and *C. gattii*-associated CM. *C. neoformans* infection progressed rapidly, mice succumbed within a week post-infection, compared to nearly one month for *C. gattii*. Despite longer incubation periods observed during *C. gattii* infection, end-stage disease in both models was characterized by comparable fungal burdens. Large cryptococcal lesions were evident as early as 6 d.p.i in *C. neoformans*-infected mice, while *C. gattii* was associated with smaller lesions. Cytokine analysis further revealed significant species-specific differences in inflammatory profiles across d.p.i. These findings highlight distinct temporal and pathological features of *C. neoformans* and *C. gattii* infection and support the utility of this model for investigating *C. gattii*-induced CM.

Thurs C1.7 – Using human airway and alveolar organoid-derived monolayers to quantify parameters of early cryptococcal infections

Sebastian Reusch

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Background and aims Cryptococci are environmental fungi that cause localized or disseminated infections typically after uptake via the respiratory tract. Studies on pathogenicity are based on animal models, cancer cell lines or immune cells. However, to understand pathomechanisms novel models are required. **Methods** We cocultured cryptococcal reference strains (*C. gattii* VGII/R265 and *C. neoformans* VNI/H99) with organoid-derived monolayers (ODMs) originating from adult stem cells of proximal (= Airway) and distal (= Alveoli) human respiratory tissue. Barrier function of ODMs was probed by measuring the transepithelial electrical resistance. Internalization of fungi into respiratory cells was visualized by immunofluorescence stainings. Fungal viability was assessed by cell counting and cultivation. Ink stainings were used to quantify fungal cell morphology. **Results** While barrier function of airway ODMs was maintained, cryptococci induced a barrier breakdown in alveolar ODMs. Immunofluorescence stainings revealed that cryptococcal adhesion and internalization events to and into airway ODMs were rare, while alveolar ODMs showed an increased susceptibility towards cryptococcal internalization. Both cryptococcal strains were able to proliferate on ODMs. Analysis of fungal morphology revealed strain specific fungal adaptation with giant cells in *C. gattii* and pseudohyphal growth for *C. neoformans* while cocultured with alveolar, but not with airway ODMs. **Conclusions** Studying interactions of cryptococci with primary human cells of the respiratory tissue is feasible with *Cryptococcus* suggesting different fungal-host interactions of *C. gattii* and *C. neoformans* and specific tissue reaction in proximal versus distal tissues. Future studies will be used to characterize immunologic response and analysis of fungal subpopulations.

Thurs C1.8 – Genetic and pathogenic diversity of *Cryptococcus gattii*

Qinxi Ma

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MRC Centre for Medical Mycology, University of Exeter

Cryptococcosis is a severe fungal disease caused by *Cryptococcus neoformans* and its sister species *C. gattii*. Advances in whole-genome sequencing and population genomics have revealed six genetically distinct *C. gattii* lineages (VGI-VGVI). *C. gattii* displays massive phenotypic diversity. We performed genomic analysis on 46 *C. gattii* strains, including 25 clinical isolates, 15 environmental isolates, 3 veterinary isolates and 3 of unknown origin gathered from 13 countries across six continents. Phylogenetic analysis of the 46 isolates revealed a clear separation into the 6 distinct lineages. We evaluated virulence of these *C. gattii* isolates using the insect model *Galleria mellonella*. Only 6 out of the 46 isolates caused death in all larvae after 10 days post infection, whereas 5 caused less than 50% killing after 10 days post infection. Among the six lineages, VGI and VGIII were the least virulent. We also observed substantial intra-lineage variations, which likely reflects intra-lineage genetic diversity. Surprisingly, we observed that in the insect model, VGV isolates showed on average ~80% mortality, which may be due to the lack of adaptive immunity. Survival of larvae did not differ significantly between infections with environmental and clinical isolates (12.5% and 15%, respectively). Given that *C. gattii* is commonly inhaled by the host from the environmental sources, it is unsurprising that no notable differences were found between environmental and clinical isolates. These findings underscore the substantial genetic and phenotypic diversity within the *C. gattii* complex and highlight the value of integrating genomic and infection-model data to elucidate the evolutionary and pathogenic mechanisms.

Concurrent Session 2

Thurs C2.1 – Human Brain Organoids: A New Model to Study *Cryptococcus neoformans* Neurotropism

Hannah Brown Harding

Alfred Harding (1,2), Lee Gehrke (1,2), Jay Vyas (3,4,5)

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With the rise in immunocompromised individuals and patients with immune-related comorbidities such as COVID-19, the rate of fungal infections is growing. This increase, along with the current plateau in antifungal drug development, has made understanding the pathogenesis and dissemination of these organisms more pertinent than ever. The mouse model of fungal infection, while informative on a basic scientific level, has severe limitations in terms of translation to the human disease. Here we present data supporting the implementation of the human cerebral organoid model, which is generated from human embryonic stem cells and accurately recapitulates relevant brain cell types and structures, to study fungal infection and dissemination to the central nervous system (CNS). This approach provides direct insight into the relevant pathogenesis of specific fungal organisms in human tissues where in vivo models are impossible. With this model system we assessed the specific brain tropisms and cellular effects of fungal pathogens known to cross the blood-brain barrier (BBB), such as *Cryptococcus neoformans*. We determined the effects of this fungal pathogen on the overall gross morphology, cellular architecture, and cytokine release from these model organoids. Furthermore, we demonstrated that *C. neoformans* penetrates and invades the organoid tissue and remains present throughout the course of infection. These results demonstrate the utility of this new model to the field and highlight the potential for this system to elucidate fungal pathogenesis to develop new therapeutic strategies to prevent and treat the disseminated stages of fungal diseases such as cryptococcal meningitis.

Thurs C2.2 – Cryptococcal aggregation in the perivascular compartment drives glymphatic disruption during murine cryptococcal meningitis

Simran Atulkumar Patel

Yanga Bridget Pato^{1,2,3}, Anja de Lange⁵, Mike Reiche⁵, Caron Jacobs⁵, Darren Thomson⁴, and Rachael Dangarembizi^{1,2,3}

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Cryptococcal meningitis (CM) is an invasive fungal infection of the brain associated with severe neurological complications including hydrocephalus and elevated intracranial pressure (ICP). Dilated perivascular spaces (PVS) have long been recognised as a radiological hallmark of CM but mechanisms underlying this pathology are poorly defined. Here, we hypothesise that neuropathology in CM may arise from glymphatic disruption and investigate cryptococcal distribution and its effect on glymphatic flow. Twenty-four C57BL/6 mice were intravenously injected with 5×10^4 *Cryptococcus neoformans* (FD651), *Cryptococcus gattii* (R265), or saline. To measure the distribution of fungal cells relative to the vascular network, optically cleared whole brains were imaged using light-sheet microscopy, followed by quantitative distance mapping to determine fungal proximity to blood vessels. Glymphatic influx was evaluated by intracisternal infusion of 10 μ L of a fluorescent tracer (Alexa 555-ov albumin, 45 kDa) at 2 μ L/min. Tracer penetration was determined by measuring the mean fluorescence intensity and thresholded area fraction across coronal sections collected from bregma +2 to -2. Three-dimensional spatial analysis revealed preferential aggregation of cryptococcal cells within PVS. Fungal aggregation was associated with disruption of glymphatic influx, as evidenced by reduced tracer penetration in infected mice compared to healthy controls. Furthermore, infected mice exhibited increased resistance to tracer infusion, reflected by the prolonged infusion times. Our findings provide the first experimental evidence of glymphatic disruption during CM, potentially arising from occlusion of the PVS by aggregated cryptococcal cells. This mechanism offers a plausible biological framework linking fungal neuroinvasion to the hydrocephalus and elevated ICP that characterises CM.

Thurs C2.3 – In vivo assessment of host-pathogen interaction in the crossing the blood-brain barrier in murine model of experimental cerebral cryptococcosis - correlating cellular with pathophysiological events

Lara Roosen

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Cryptococcus neoformans (CN) causes severe infections that can spread from the lungs to the central nervous system (CNS). The mechanisms by which CN crosses the blood-brain barrier (paracytosis, transcytosis, or Trojan Horse mechanism) remain unclear. Clarifying early CNS entry and disease progression, a multimodal imaging approach combining Magnetic Resonance Imaging (MRI) and Two-Photon Intravital Microscopy (2P-IVM) was used to monitor CN dissemination longitudinally. Thirty Balb/C mice with an implanted cranial window were infected intravenously with 50,000 CN. After infection, mice were imaged for 8h, 24h, 48h using 2P-IVM. Lesions were confirmed by MRI on 4/7 days. Influence on lesion formation was examined in depth (silicone oil vs. PBS, presence vs. absence of meninges, and impact of window placement). In mice infected with CNKN99 α ±E2-Crimson, cryptococci were observed after 1h with cluster after 5h with capillary bed occlusion. Mice infected with CNH99-EGFP showed similar results. Titan cells were confirmed at 24h and 48h with daughter cell production. After 7 days, lesions were observed in MRI in the cortex beneath the cranial window, allowing for imaging these lesions at cellular level with 2P-IVM, where indications of capillary vessel ruptures were observed. No differences were observed in lesion formation based on window placement (RW/LW) or compound used (PBS/silicone oil). Removing meninges didn't affect autofluorescence. We developed a multimodal 2P-IVM/MRI platform to track cerebral cryptococcosis across disease stages. Longitudinal cellular imaging revealed spread from onset to lesion formation, with indications of vessel rupture and transcytosis but no Trojan horse mechanism, highlighting the need for further studies.

Thurs C2.4 – Use of an immortalized human microglia cell line to study the unique recognition of *Cryptococcus neoformans* by human microglia.

Robbi Ross

Kassandra Arias-Parbul, Rebeca Candescu, Felipe H. Santiago-Tirado

University of Notre Dame

In immunocompromised individuals, *Cryptococcus neoformans* escapes from the lungs and disseminates to the central nervous system (CNS), where it causes an estimated 147,000 deaths annually. In the brain, *C. neoformans* interacts with microglia, the tissue-resident macrophages of the CNS. Previous studies showed that microglia are ineffective at controlling this fungal infection through mechanisms that remain unclear. Thus, I aim to understand the recognition and intracellular survival of *C. neoformans* by immortalized human microglia. I show that microglia have limited phagocytic activity that is specific to *C. neoformans* and independent of various cryptococcal factors. I also show that microglia respond to *C. neoformans* differently than other tissue-resident macrophages, especially relating to recognition, phagocytosis, and phagosome maturation. I have screened 2,800 cryptococcal mutants and identified novel genes, most of which are currently uncharacterized, that may be responsible for the yeast's ability to specifically evade phagocytosis by microglia. Interestingly, we find that factors such as cell size and chitin/chitosan exposure seem to be instrumental in yeast phagocytosis by microglia. Additionally, I show that microglia are ineffective at killing phagocytosed *C. neoformans*, and that this could be due to the ability of this yeast to disrupt phagosome maturation and induce phagosome membrane damage in these cells, which is partially dependent on fungal opsonization. These findings provide us with fundamental knowledge regarding cryptococcal pathogenesis in the CNS, specifically insight into how *C. neoformans* is recognized by microglia under different conditions, and how the yeast could be surviving and replicating within the microglial phagosome after engulfment.

Thurs C2.5 – When Immunity Turns Pathogenic: Monocyte-Derived iNOS Protects Against Lethal Immunopathology in Cryptococcal Meningitis

Jintao Xu

Rylan Hissong, Maia Lintner, Alexander Ballesteros, Jane Barnett, Lauren Borhani, Shelby Hopson, Kristie Goughenour, Michal A Olszewski

Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Health System, Ann Arbor, Michigan, USA

Inducible nitric oxide synthase (iNOS) is a pivotal regulator of host defense, yet its role in balancing antimicrobial activity against tissue damage during Cryptococcal meningitis (CM) remains undefined. Here, we demonstrate that iNOS functions as an essential "brake" on lethal CNS inflammation rather than a primary antifungal effector. Using a murine model of CM, we found that *Nos2*-deficient mice infected with *Cryptococcus neoformans* (H99) exhibited fulminant neurological decline, rapid cachexia, and early mortality compared to wild-type controls. Crucially, this accelerated lethality was uncoupled from fungal burden, driven instead by extensive CNS immune infiltration and blood-brain barrier disruption. Using *CCR2^{cre}Nos2^{flox}* mice, we identified inflammatory monocytes as the obligatory source of protective iNOS. Mechanistically, the loss of nitric oxide-mediated feedback inhibition locked myeloid cells in a hyper-inflammatory state, driving uncontrolled CCL2, IFN γ , and IL-1 α/β production. This cytokine storm provoked pathological neutrophil recruitment. Strikingly, neutrophil depletion rescued *Nos2*-deficient mice from early mortality. These results mirror clinical observations in human CM cohorts, where reduced iNOS expression correlates with poor neurological outcomes despite antifungal therapy. Collectively, our data establishes iNOS as a vital suppressor of neurotoxicity, suggesting that host-directed immunometabolic therapies targeting the iNOS-NO axis may prevent lethal immunopathology in CM.

Thurs C2.6 – The Role of CD4⁺ T Cells in Hypervirulence of the Clinical Isolate UgCI422 of *Cryptococcus neoformans*

Bruno Borges

Paige Chenoweth, Kirsten Nielsen.

Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA

Cryptococcus neoformans is an environmentally acquired fungal pathogen and the leading cause of cryptococcal meningitis, a life-threatening disease that primarily affects immunocompromised individuals. Disease severity is shaped by both host genetic background and fungal virulence traits. Distinct clinical isolates can drive divergent disease outcomes depending on host immune context, including hypervirulent infections characterized by excessive inflammation. The clinical isolate UgCI422 induces hypervirulent disease in A/J mice, marked by elevated IFN- γ production and a mixed Type 1/Type 17 immune response, while causing typical disease in C57BL/6 mice. Although IFN- γ is generally protective during cryptococcosis, excessive levels correlate with worsened disease outcome, suggesting immune dysregulation as a driver of pathology. The cellular source of IFN- γ during UgCI422 infection remains unclear. Based on clinical observations that HIV-infected patients with depleted CD4⁺ T cells survived cryptococcal meningitis, we hypothesized that CD4⁺ T cells contribute to excessive IFN- γ production during UgCI422 infection. To test this, A/J mice were intranasally infected with UgCI422 or the reference strain KN99 α , followed by selective CD4⁺ T-cell depletion. Lung fungal burden, cytokine profiles, and survival were assessed, and CD4⁺ depletion was confirmed by flow cytometry. CD4⁺ T-cell depletion abolished the IFN- γ spike associated with UgCI422 infection and altered disease progression, demonstrating that CD4⁺ T cells are required for pathogenic IFN- γ responses. These findings reveal a critical role for CD4⁺ T cells in driving hypervirulence and provide insight into immune-mediated pathology during cryptococcal infection.

Thurs C2.7 – The Mannose Receptor (MR) on CD169⁺ myeloid cells modulates the generation or maintenance of anti-fungal Tbet⁺ stem-like progenitors

Chinaemerem Uju Onyishi

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The mannose receptor (MR) is a C-type lectin receptor broadly expressed on myeloid cells and often considered a marker of immunosuppressive “M2-like” macrophages. However, recent data have identified immunostimulatory roles for MR, highlighting its complexity and context-dependent functions. Hence, we sought to investigate the precise functional role(s) of MR in modulating T cell fate decisions using the highly mannosylated, CD4 T cell-dependent fungal pathogen *Cryptococcus neoformans* as a model. At 10 days post intranasal infection with KN99 α *C. neoformans*, MR-deficient mice exhibited decreased Tbet⁺ Th1 cells in the TCF1^{high} stem-like progenitor compartment in both the lungs and lung-draining lymph nodes (IdLN), while terminal effector Th1 cells and other Th subsets were unaffected. Using mixed bone marrow chimeras to selectively deplete MR from specific myeloid populations, we found this defect occurred only when MR was deleted from CD169⁺ cells, but not from cDC1s or cDC2s. Furthermore, loss of MHCII within the CD169⁺ compartment similarly impaired Tbet⁺ progenitor generation. Notably, MR⁺ CD169⁺ cells in the IdLN expressed IL12p40 and formed direct contact with CD4 T cells, identifying these cells as a previously unrecognized bona fide antigen-presenting cell population that utilizes MR to selectively license Tbet expression on stem-like progenitor cells. Finally, to determine functional relevance, we infected mice with the gcs1delta *C. neoformans* strain which establishes a chronic infection in which protection is mediated by Th1 immunity. We found that MR-deficient mice had decreased survival compared to controls, suggesting that MR-dependent Th1 progenitor maintenance is required for effective long-term antifungal immunity.

Thurs C2.8 – An Uncharacterized Kinase Regulates Macrophage Adaptation of *Cryptococcus neoformans*

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Zoé A. Hilbert

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The ability to survive and replicate in host cells is a critical virulence trait of *Cryptococcus neoformans* which can impact the trajectory of an infection. However, the genes required for fungal survival in host cells like macrophages and the mechanisms through which this trait evolved are largely unknown. To gain insight into how *C. neoformans* adapts to grow inside of macrophages, we established a serial passaging platform and identified several fungal populations with robust increases in macrophage intracellular growth. Here, we focus on characterizing one isolate from these experiments which carries a single missense mutation in the serine/threonine kinase, SKS1, that is necessary and sufficient for macrophage adaptation in this strain. Through competition experiments, we show that strains carrying SKS1 mutations have fitness advantages in the intracellular macrophage environment but not extracellularly. Introduction of the SKS1 mutation into closely related *C. neoformans* strains results in similarly increased survival in macrophages, suggesting a conserved role of this mutation across genetic backgrounds. Additional phenotypic characterization of SKS1 mutants show that this gene regulates additional virulence-related traits important for macrophage survival, including the ability to form titan cells and produce melanin. Our ongoing work focuses on identifying targets of Sks1 phosphorylation, characterizing host responses to infection by Sks1 mutants, and deciphering the role of Sks1 in regulating growth in macrophages from alternative host species. Collectively, this work is revealing the genetic basis of host adaptation and will provide critical insight into how new virulence traits can emerge in this important fungal pathogen.

Friday July 3

Plenary Session

Fri P1 – Adaptive PLATFORM-CM Clinical Trial, Novel Antifungals, and Personalized Immunotherapy

David Meya, Conrad Muzoora, David Boulware

PLATFORM-CM Trial: Explanation of Master Protocol for an Adaptive Platform Trial

David Meya Troletricin Novel Polyene Phase II Trial

Conrad Muzoora Oteseconazole Phase II Trial

Personalized Immunotherapy - David Boulware

Adaptive PLATFORM-CM Clinical Trial, Novel Antifungals, and Personalized Immunotherapy
Cryptococcal meningitis remains a leading cause of HIV-related mortality despite advances in antifungal therapy. This session presents four complementary approaches to accelerate therapeutic innovation for cryptococcosis. First, the PLATFORM-CM Trial will introduce the rationale and design of an adaptive master protocol platform trial for cryptococcal meningitis, enabling efficient evaluation of multiple therapeutic strategies within a unified framework (David Meya). Second, the Troletricin Phase II Trial will present clinical evaluation of a novel polyene antifungal designed to improve antifungal efficacy and safety compared with existing amphotericin-based therapies (Conrad Muzoora). Third, the Oteseconazole Phase II Trial will describe results evaluating a long half-life, highly potent tetrazole antifungal as adjunctive therapy for cryptococcal meningitis, with emphasis on pharmacokinetics, tolerability, and antifungal activity (Morris Rutakingirwa). Finally, Personalized Immunotherapy for Cryptococcal Meningitis will explore a host-directed treatment strategy based on the damage-response framework, tailoring immunomodulatory therapy according to pathogen burden and immune phenotype rather than using a one-size-fits-all approach (David Boulware). Together, these presentations highlight emerging advances in adaptive trial methodology, antifungal drug development, and precision immunotherapy with the goal of improving outcomes in HIV-associated cryptococcal meningitis.

Fri P2 – Autopsy findings in persons diagnosed with HIV-associated cryptococcal meningitis.

Morris Kyozo Rutakingirwa

Carol O. Namujju 1, Jeya Sushimitha 4, Masumba J. Dalton 1,2, Jolive Nakayemba 1,2, Richard Kwizera 1, Samuel Okurut 1, Robert Lukande 1,2, Mawanda Anatoli 1,3,5, David R. Boulware 7, David B. Meya 1,6,7 and Melanie R. Nicol 8.

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Introduction: Data on autopsy findings among persons with cryptococcal meningitis (CM) remain limited, particularly in advanced HIV disease.

Methods: We conducted autopsies among consented participants with CM enrolled in a prospective cohort in Kampala, Uganda from January to September 2025. Autopsy findings were correlated with perimortem clinical records from hospitalization. Results: Twenty-four autopsies were reviewed; 58% (14/24) were female. Median (IQR) age was 40 years (30-46), and median CD4 count was 5 cells/ μ L (0-25). One cadaver had a gravid uterus (20 cm) with a single deceased fetus, and one was 18 days postpartum. All cadavers demonstrated brain pathology, including hazy meninges (n=24) consistent with meningitis, features of elevated intracranial pressure (n=18) with edema, narrow sulci and flattened gyri; parenchymal necrosis (n=4), herniation (n=3), and hemorrhage (n=2). All also had pulmonary pathology, including edema (n=24), consolidations (n=17), pleural effusions (n=2), collapse (n=1), cavities (n=1), and granulomas (n=1), although only 37.5% (9/24) had a perimortem diagnosis of respiratory distress or aspiration pneumonia. Lymphadenopathy was present in 33% (8/24), with cryptococcal cysts identified histologically in one case. Renal pathology was common (17/24), including cryptococcal cysts (n=9), focal segmental glomerulosclerosis (n=7), and caseating granulomas (n=1). Although 29% (7/24) had antemortem diagnoses of tuberculosis, only one case was histopathologically confirmed. Aspergillosis and Kaposi's sarcoma were identified in one case each.

Conclusions: Beyond extensive CNS involvement, asymptomatic pulmonary, renal, and lymphatic pathology is common in HIV-associated cryptococcal meningitis. Antemortem presumptive tuberculosis diagnoses frequently lacked histopathologic confirmation. Safe feeding practices are needed to reduce aspiration.

Fri P3 – Fungicide phenotypic resistance

Linqi Wang

Institute of Microbiology, Chinese Academy of Sciences

Human pathogenic fungi pose a serious threat to human health and safety. Unfortunately, the limited arsenal of antifungal agents is further strained by the continuous emergence of drug-resistant variants. Recent studies have also underscored the importance of alternative modes of fungal survival during fungicidal agent treatment, including phenotypic resistance (i.e., fungicide tolerance and persistence), highlighting the complexity of fungal responses to cidal drugs. However, whether host-derived factors can induce such phenotypic resistance during fungal diseases remains largely unknown. Through a systematic evaluation of metabolite-drug-fungus interactions in *Cryptococcus neoformans*, we discovered that brain glucose induces fungal tolerance to amphotericin B (AmB) in mouse brain tissue and patient cerebrospinal fluid via the fungal glucose repression activator Mig1. Mig1-mediated tolerance limits the efficacy of cryptococcal meningitis treatment in mice by inhibiting the synthesis of ergosterol (the target of AmB) and promoting the production of inositolphosphorylceramide, which competes with AmB for ergosterol. We also demonstrated that highly AmB-tolerant fungal persister cells can form during cryptococcal lung infections, and we identified an FDA-approved drug with potent activity against these persister cells through a drug repurposing approach. Our group is now investigating the determinants that differentiate antifungal resistance, tolerance, and persistence, and that shape their evolution in vivo.

Fri P4 – One, two, three, four. Get the vaccine out the door!

Stuart M. Levitz, MD

University of Massachusetts Chan Medical School. Worcester, USA.

Despite the global need, there are no licensed vaccines to protect at risk persons from fungal infections, including cryptococcosis. My talk will provide a brief overview of the populations that could benefit from cryptococcal vaccines and the obstacles to vaccine rollouts. I will then discuss our in vivo mouse models focused on antigen discovery and adjuvant choice. The goal is the development of subunit vaccine that is inexpensive to produce and elicits balanced antigen-specific CD4 T cell and antigen responses.

Fri P5 – Cryptococcus as a prism for discovery

Joseph Heitman

Department of Molecular Genetics and Microbiology, Duke University, NC, USA

The genus *Cryptococcus* spans both pathogenic and nonpathogenic species. The *Cryptococcus* pathogenic species complex includes 8 species, 7 of which cause infections in human resulting in substantial morbidity and mortality globally. These species are well delineated based on several different species definitions, and these species designations are well supported by extensive genome sequence analyses and genetic crosses as tests of the biological species concept. Comparative phenotyping and genomics of pathogenic and non-pathogenic species provides an approach to understand the evolution of unique fungal attributes, including pathogenesis. Extensive collaborative efforts have expanded the known non-pathogenic *Cryptococcus* species from three that were classically known (*C. wingfieldii*, *C. amyloletus*, *C. depauperatus*) to now more than a dozen species. These non-pathogenic species have been identified from both sub-Saharan Africa as well as other geographic regions. Several are associated with bark beetles living in specific tree species, which could serve as a staging ground for the evolution of pathogenic attributes. Comparative genomics provides insight into both gene gains and gene losses that may be associated with the evolution of pathogenesis. Genetic admixture of natural variation combined with experimental laboratory passage provides insights into how survival at 37°C may evolve in response to environmental selective pressures. Development of a robust CRISPR genome editing system for *C. amyloletus* enables genetic analysis of gene functions related to pathogenesis and growth under temperature stress. Generation of telomere-to-telomere (T2T) assemblies for >30 *Cryptococcus* and *Kwoniella* species provides a robust platform to analyze a

variety of biological features in addition to pathogenesis, including mating-type loci, sexual reproduction, loss and retention of RNAi, and chromosome structure and karyotype.

