S4.5a

A randomized, double blind phase II proof-of-concept superiority trial of fosravuconazole 200 mg or 300 mg weekly dose versus itraconazole 400 mg daily, all three arms in combination with surgery, in patients with eumycetoma in Sudan—top line results

Ahmed Hassan Fahal[1], Sahar Mubarak Bakhiet[1], El Samani Wadaa Mohamed[1], Eiman Siddig Ahmed[1], Osama El Hadi Bakhiet[1], Abu Bakar Ahmed Yousif[1], Lamis Ahmed Fahal[1], Hadel Yassir Atta Alla[1], A Razig Osman A Razig[1], Emmanuel Edwar Siddig[1], Omnia Babekir Hassan[1], Sahar A Rahman Abdulla, Amir Faroug Mohaemd[1], Nagwa Adam Jodda[1], Mustafa El Nour Bahar[1], Borna A. Nyaoke[2], Thaddaeus Egondi[2], Peelen Oyieko[2], Eduard E. Zijlstra[2*], Nathalie Strub-Wourgaft[2*]

1. Mycetoma Research Center, University of Khartoum, Khartoum, Sudan
2. Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland
* equal contribution

Objectives: To determine whether, in addition to surgery, fosravuconazole (Fos) monotherapy of either 200 mg or 300 mg weekly was more effective [defined as complete cure at the End of Treatment (EOT; 52-week) visit] than the standard-of-care 12-month regimen of itraconazole (Itra) monotherapy, in patients with small to moderate eumycetoma lesions caused by *Madurella mycetomatis*.

Methods: This was a single-center (Mycetoma Research Center, Khartoum, Sudan), comparative, randomized, double-blind, parallel-group, active-controlled, clinical superiority trial in participants with eumycetoma requiring surgery. Participants were randomized in a 1:1:1 ratio. In Arm 1 participants took a loading dose of Fos 300 mg on Day 1, Day 2, and Day 3, followed by a weekly dose of 300 mg for a total duration of 12 months. In Arm 2 participants took Fos 200 mg on Day 1, Day 2, and Day 3, followed by a weekly dose of 200 mg for a total duration of 12 months. In Arm 3 participants took Itra 400 mg daily for 12 months. All patients underwent surgery after 6 months of treatment in which the remaining lesion was removed. Mycetoma lesions were between 2 to ≤16 cm in diameter. The age cut-off was ≥15 years. The diagnosis of *M. mycetomatis* was confirmed by PCR. Safety monitoring included, among other, severe, and serious treatment-related events.

Results: A total of 122 participants were screened and 104 participants were enrolled (34 in Fos 300 mg, 34 in Fos 200 mg weekly, and 36 in Itra 400 mg). Complete cure after 12 months (EOT) of treatment was demonstrated in terms of an absence of eumycetoma mass, sinuses, and discharge; normal ultrasound of the lesion site or normal MRI; and a negative fungal culture from a surgical biopsy if a mycetoma mass was present. The complete cure rate was assessed in the mITT population. Secondary efficacy analyses were performed in the Per Protocol population. In addition, the influence of age, changes in clinical symptoms and signs, size, and duration of the lesion on outcome was examined. Safety was satisfactory and compliance was good.

Conclusion: This is the first randomized controlled trial in eumycetoma, comparing two azoles, fosravuconazole (two dosage regimens) and itraconazole, in combination with surgery. Detailed efficacy and safety results will be communicated and discussed in the oral presentation.

S4.5b

A randomized, double blind phase II proof-of-concept superiority trial of fosravuconazole 200 mg or 300 mg weekly dose versus itraconazole 400 mg daily, all three arms in combination with surgery, in patients with eumycetoma in Sudan—pharmacokinetic results

Roger Brüggemann[1], **Borna Nyaoke[2]**, Eiman Siddig Ahmed[3], Emanwell Edwar Siddig[3], Thaddaeus Egondi[2], Peelen Oyieko[2], Sahar Mubarak Bakhiet[3], Eduard E Zijlstra[4], Ahmed H Fahal[3]

[1]Radboud University Medical Center, Nijmegen, Netherlands
[2]Drugs for Neglected Diseases initiative (DNDi), Nairobi, Kenya
[3]Mycetoma Research Center, Khartoum, Sudan
[4]Drugs for Neglected Diseases initiative (DNDi), Geneva, *Switzerland*

S4.5 Mycetoma Clinical Trial on fosravuconazole treatment in eumycetoma– Top Line Results, September 22, 2022, 10:30 AM - 12:00 PM

Objective: To evaluate the pharmacokinetics (PK) of fosravuconazole (measured as ravuconazole) and itraconazole in patients with mild to moderate eumycetoma caused by *Madurella mycetomatis* using a non-compartmental PK analysis.

Methods: Participants received either 200 mg or 300 mg ravuconazole once weekly or 400 mg itraconazole daily for a total duration of 12 months. Plasma concentrations of ravuconazole and itraconazole were measured on day 1 of week 1, and on weeks 2, 3, 4, and months 2, 3, 6, and 12 (at end of treatment) for analysis of population PK. The exact time of dosing on the days of sample collection, and the exact time of sample collection within the collection time window, were recorded. Plasma concentrations were quantified using Ultraperformance Liquid Chromatography with fluorescence detection (UPLC-UV). Ravuconazole and itraconazole plasma concentration-time data was performed using a standard two stage approach with non-compartmental analysis. Derived exposure parameters of ravuconazole and itraconazole, including, but not limited to, Cmax and AUC at steady state (AUCs), were calculated. The effect of covariates, such as baseline characteristics/demographics, on PK were explored. AUCs were determined when at least three subsequent samples within one dosing interval were available.

Results: A total of 766 samples of ravuconazole in 68 participants and 226 samples of itraconazole in 36 participants were analyzed. The average concentration of ravuconazole (range) was 3.1 mg/l (0.01-12.33 mg/l), and for itraconazole was 1.59 mg/l (0.01-5.53 mg/l).

Detailed Pharmacokinetic results will be communicated and discussed in the oral presentation.

S4.5c

Using serum beta-glucan measurements and sequencing of the *Madurella mycetomatis* azole target gene to predict therapeutic outcome during azole treatment in human mycetoma

Bertrand Nyuykonge[1], Emmanuel Siddig[1,2,3], Najwa Mhmoud[2], Borna Nyaoke[4], Ed Zijlstra[4], Annelies Verbon[1], Sahar Bakhiet[2], Ahmed Fahal[2], Wendy Van de Sande[1]

[1]ErasmusMC - Erasmus University Hospital - Department of Medical Microbiology & Infectious Diseases, Rotterdam, Netherlands
[2]Mycetoma Research Centre, University of Khartoum, Khartoum, Sudan
[3]University of Khartoum, Faculty of medical laboratory sciences, Khartoum, Sudan
[4]Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland

S4.5 Mycetoma Clinical Trial on fosravuconazole treatment in eumycetoma– Top Line Results, September 22, 2022, 10:30 AM - 12:00 PM

Objectives: Eumycetoma is a neglected tropical disease characterized by large subcutaneous swellings and the formation of grains and most commonly caused by *Madurella mycetomatis*. The currently recommended therapy is a combination of antifungal therapy with an azole and surgery. Itraconazole is the current recommended drug and fosravuconazole, the pro-drug of ravuconzole, is currently clinically investigated. At the moment, there are no epidemiological cut-off values (ECV) for *M. mycetomatis* for either of these drugs or rapid diagnostic tests which can predict the therapeutic outcome of these treatments. Therefore, in this study, we determined the ECV for these drugs and determined whether there was a correlation between minimal inhibitory concentration (MIC) and the DNA sequence of the azole target gene CYP51A. We also assessed beta-glucan concentrations in the serum of mycetoma patients during treatment to establish whether any of these values were predictive for therapeutic outcomes.

Methods: In order to determine the ECV for *M. mycetomatis*, MIC distributions for itraconazole and ravuconazole were determined in genetically diverse clinical *M. mycetomatis* isolates using the ECOFFinder software. CYP51A sequences were sequenced and comparisons were made between the different CYP51A variants and the MIC distributions. Beta-glucan concentrations were measured in serum with the WAKO beta-glucan assay. Time points analyzed were 0, 22, 85, 176, 267, 358, and 455 days after the start of treatment.

Results: For *M. mycetomatis* the MICs ranged from 0.008 to 1 mg/l for itraconazole and from 0.002 to 0.125 mg/l for ravuconazole. The *M. mycetomatis* ECV for itraconazole was 1 mg/l and for ravuconazole 0.064 mg/l. In the wild-type population, two CYP51A variants were found for *M. mycetomatis*, which differed in one amino acid at position 499. The MIC distributions for itraconazole and ravuconazole were similar between the two variants. No mutations linked to decreased susceptibility were found. Before the start of treatment, beta-glucan concentrations ranged from below the detection limit to 217.9 pg/ml. Of these patients, 61.2% had a beta-glucan concentration above 7 pg/ml, the recommended cut-off value for positivity by the manufacturer, 72.8% had a beta-glucan concentration above 5.5 pg/ml, the recommended cut-off value for *M. mycetomatis*. During the first months of azole treatment, the beta-glucan concentrations remained relatively stable. After surgery, a sharp decrease in beta-glucan concentration in serum was noted. At the end of the observation period, only 13 patients had a beta-glucan concentration above 7 pg/ml and 14 above 5.5 pg/ml. Of these patients, for only 3, there was clinical evidence of a recurrence. For the remaining 4 patients with clinical evidence of a recurrence, the beta-glucan concentration was below the cut-off value for positivity.

Conclusion: In conclusion, so far there was no link established with the initial in vitro susceptibility and failure or success of the treatment therapy. Beta-glucan levels, in general, remained high during azole treatment, and a sharp drop in beta-glucan concentration in serum was only noted after surgery. A positive beta-glucan concentration at the end of the treatment was not indicative of a recurrence.

S4.5d

Comparing the diagnostic performance of the commonly used eumycetoma diagnostic tests using sequencing of the internally transcribed spacer region as the gold standard

Emmanuel Siddig[1,2,3], Bertrand Nyuykonge[1], Najwa Mhmoud[2,3], Omnia Abdallah[2], Mustafa Bahar[2], Eiman Ahmed[2], Borna Nyaoke[4], Ed Zijlstra[4], Annelies Verbon[1], Sahar Bakhiet[2], Ahmed Fahal[2], Wendy van de Sande[1]

[1]Erasmus MC, University Medical Centre Rotterdam, Department of Medical Microbiology and Infectious Diseases, Rotterdam, Netherlands, Rotterdam, Netherlands, [2]Mycetoma Research Centre, University of Khartoum, Khartoum, Sudan, Sudan, Sudan, [3]University of Khartoum, Faculty of medical laboratory sciences, Khartoum, Sudan, Khartoum, Sudan, [4]Drugs for Neglected Diseases initiative (DNDi), Switzerland, Switzerland

S4.5 Mycetoma Clinical Trial on fosravuconazole treatment in eumycetoma– Top Line Results, September 22, 2022, 10:30 AM - 12:00 PM

Objectives: Mycetoma is a neglected tropical implantation disease caused by 70 different infectious agents. Identifying the causative organism to the species level is essential for appropriate patient management. Ultrasound, histopathology, culture, and two species-specific PCRs are most the commonly used methods for species identification in endemic regions. The aim of this study was to compare the diagnostic performance of these commonly used assays using sequencing of barcoding genes as the gold standard.

Methods: This descriptive cross-sectional study was conducted at the Mycetoma Research Centre, University of Khartoum, Sudan. It included 222 patients suspected of fungal mycetoma caused by *Madurella mycetomatis*.

Results: In total 154 (69.3%) were correctly identified by ultrasound, histology, culture, and both species-specific PCRs. In 60 patients, at least one of the diagnostic tests failed to identify *M. mycetomatis*. A total of five patients had no evidence of eumycetoma, and for three, only the ultrasound was indicative of mycetoma. The two species-specific PCRs were the most sensitive and specific methods, followed by culture and histology. Ultrasound was the least specific as it only allowed differentiation between actinomycetoma and eumycetoma. The time to result was 9.38 minutes for ultrasound, 3.76 h for PCR, 8.5 days for histopathology, and 21 days for grain culturing.

Conclusion: Currently, PCR directly on DNA isolated from grains is the most rapid and reliable diagnostic tool to identify *M. mycetomatis* eumycetoma.

P4

COVID19-associated fungal infections

Martin Hoenigl
Medical University of Graz, Graz, Austria

Plenary session 4, September 22, 2022, 2:00 PM - 2:45 PM

Coronavirus disease 2019 (COVID-2019) associated invasive fungal infections (IFIs) have emerged as an important complication in a substantial number of critically ill COVID-19 patients, and three groups of fungal pathogens have increasingly been recognized as causes of superinfections: *Aspergillus*, Mucorales, and *Candida*. First reports of cases and case series of COVID-19-associated pulmonary aspergillosis (CAPA) have emerged during the first months of the pandemic. Prevalence rates varied widely due to the fact that CAPA was, and still remains, challenging to diagnose in patients with COVID-19-associated acute respiratory failure (ARF). The clinical picture and radiological findings of CAPA are unspecific and the primarily airway invasive growth in non-neutropenic patients and the late occurrence of angioinvasion in the course of the disease may complicate diagnosis. Current guidelines recommend treatment of CAPA during its early airway invasive phase, which may result in some overtreatment (i.e., treatment in patients that may not develop angioinvasive infection), given the independent contribution of CAPA to devastating mortality rates of around 50% that have been shown in multiple studies. This talk will also review the incidence of COVID-19-associated IFIs caused by Mucorales, and *Candida*, including *C. auris*, and will discuss—clinical risk factors, predisposing changes in the host environment, and immunological mechanisms involved in the pathogenesis of these coinfections, as well as current and future diagnostics and treatments.

S5.1b

Antifungal r esistance

Danila Seidel
University Hospital of Cologne, Cologne, Germany

S5.1 Antifungal resistance, September 22, 2022, 3:00 PM - 4:30 PM

Resistance to clinical antimycotics in *Aspergillus fumigatus* has become an increasing threat in healthcare worldwide over the past two decades. Factors that contribute to this continuing trend are manifold, among others resistance emerge in environmental fungi through selection pressure due to fungicides widely used in agriculture and farming, resistant clones are diffused around the world through global travel and shipping routes, as well as prophylactic and long-term administration of antifungal agents in patients with chronic fungal disease creating selection pressure.

Physicians face particular challenges in their patients with invasive aspergillosis, with emerging resistance adding another layer of therapeutic complexity. We are beginning to gain an understanding of the clinical implications of the different patterns of resistance. First international studies have shown that resistance of *A. fumigatus* to clinical antifungal agents significantly hampers the successful treatment of patients.

This presentation aims to highlight the difficulties associated with antifungal resistance in *A. fumigatus* with a focus on hematological oncological patients.

S5.1d

Mechanisms of azole antifungal resistance in clinical isolates of *Candida tropicalis*

Anup Ghosh, Paul Paul, Dipika Shaw, Himanshu Joshi, Shreya Singh, Arunaloke Chakrabarti, Shivaprakash M Rudramurthy, Harsimran Kaur
PGIMER, Chandigarh, India

S5.1 Antifungal resistance, September 22, 2022, 3:00 PM - 4:30 PM

Objectives: In tropical countries, the azole resistance in *Candida tropicalis* is on the rise. There are limited studies available regarding the azole resistance mechanisms in *C. tropicalis*. This study was designed to understand the molecular mechanisms of azole resistance in *C. tropicalis* by using genetic and bioinformatics approaches.

Methods: A total of 32 azole-resistant (R) and 10 azole-susceptible (S) clinical isolates of *C. tropicalis* were included in this study. All the isolates were subjected to complete gene sequencing of azole target genes including ERG11 to analyze the mutations which could lead the azole resistance. Four fragments were amplified, sequenced, and aligned to get full-length ERG11 gene. Inducible expression analysis of 17 other genes potentially associated with azole resistance was also evaluated. Homology modeling and molecular docking analysis were performed to study the effect of amino acid alterations in mediating azole resistance.

Results: Of the 32 resistant isolates, 12 (37.5%) showed A395T and C461T mutations in the ERG11 gene. The mean overexpression of CDR1, CDR3, TAC1, ERG1, ERG2, ERG3, ERG11, UPC2, and MKC1 in resistant isolates without mutation

(R-WTM) was significantly higher ($P < .05$) than those with mutation (R-WM) and the sensitive isolates (3.2-11 vs. 0.2-2.5, and 0.3-2.2 folds, respectively). Although the R-WTM and R-WM had higher ($P < .05$) CDR2 and MRR1 expression compared to S isolates, noticeable variation was not seen among the other genes. Protein homology modeling and molecular docking revealed that the mutations in the ERG11 gene were responsible for structural alteration and low binding efficiency between ERG11p and ligands. Isolates with ERG11 mutations also presented A220C in ERG1 and together T503C, G751A mutations in UPC2.

Conclusions: Nonsynonymous mutations in the ERG11 gene and coordinated overexpression of various genes including different transporters, ergosterol biosynthesis pathway, transcription factors, and stress-responsive genes are associated with azole resistance in clinical isolates of *C. tropicalis*.

S5.3a
Unraveling the genetic determinants of virulence in *Cryptococcus neoformans*

Katrina Jackson[1], Diana Kisakye[2], Marissa Macchietto[1], Peter Tiffen[1], Kirsten Nielsen[1]
[1]University of Minnesota, Minneapolis, US
[2]Makerere University, Kampala, Uganda

S5.3 Cellular pleomorphism and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Cryptococcus neoformans is a human pathogenic basidiomycete yeast that can cause cryptococcal meningitis (CM), predominantly in immunocompromised individuals. The patient outcome depends on both host and pathogen-specific factors, including *C. neoformans* genetics. A groundbreaking 2012 study was the first to show that patient outcome is associated with genetic differences between *C. neoformans* isolates. Subsequent population-wide sequencing studies have revealed over 100 sequence types (ST) of *C. neoformans* that are associated with both geographic location and clinical outcome. All these studies have been broad, examining the severity of disease cryptococcal phenotypes in a collection of highly diverse strains. We chose a narrow focus and collected various genotypic and phenotypic data from a single ST: ST93. ST93 is a common sequence type isolated from patients globally and is the most common clinical isolate found in the sub-Saharan African country of Uganda. Previously, we performed whole genome sequencing on 38 ST93 Ugandan clinical isolates. We identified 652 unique SNPs in this ST93 population compared to the H99 reference genome. We also showed that ST93 contained two subpopulations: ST93A and ST93B. In the current study, we further characterized the genotypic, phenotypic, and virulence differences between these 38 clinical isolates. Using Illumina sequence data, we identified a pattern of linkage disequilibrium that suggested that ST93A and ST93B are evolving separately. We performed long-read sequencing on each isolate to investigate chromosomal changes and large structural variations, allowing us to identify a chromosomal translocation event wherein parts of chromosome 11 had recombined with chromosome 3. Additionally, we characterized several in vitro phenotypes for each isolate and identified three distinct phenotypic clusters based on cell wall challenge and growth experiments. Next, we infected mice with 35 isolates and observed eight different disease manifestations, including isolates that caused non-CNS infections. Overall, by working within a single sequence type, we can gain a deeper understanding of how some small genetic changes can impact strain-specific phenotypes while others have no discernable effect. Eventually, these data can be used to provide valuable information about how each clinical isolate impacts patient outcomes.

S5.3b
Fungal spores: Initiators of colonization and infection

Tim Baltussen[1], Tom van den Brule[2], Jan Dijksterhuis[2]
[1]Radboudumc, Nijmegen, Netherlands
[2]Westerdijk Fungal Biodiversity Institute, Nijmegen, Netherlands

S5.3 Cellular pleomorphism and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Fungi produce asexual and sexual spores for reproduction and distribution, which can be both in space and time. Distribution in space occurs, by air movement, but also, by water or other vectors such as living organisms. Filamentous fungi from the division Ascomycota that belong to the order Eurotiales produce asexual spores called conidia. Conidia are moderately stress-tolerant cells and are able to survive unfavorable conditions such as thermal stress, dehydration, osmotic pressure, oxidative stress, variations in pH, and UV. For example, conidia of the fungus *Penicillium chrysogenum* are isolated worldwide and must be regarded as cosmopolitan. In many cases, conidia might 'land' closely to the location of production, but still many spores making into the higher air layers. There is indirect evidence that spores may be able to travel large distances through the air. For example, *Aspergillus sydowii conidia* have been suggested to travel over thousands of kilometers from the Sahara Desert to the Caribbean reefs.

Distribution in time is occurring as stress-resistant cells remain dormant at one location for an extended period, awaiting conditions that are more favorable for growth. Some ascospores (sexual spores) are extremely stress-resistant and dormant for very long periods. Other species show extended dormancy in a dried state. As microbial species are inherently variable, stress resistance varies between strains from the same species. For example, conidial heat resistance (D60) of various strains of the fungus *Paecilomyces variotii* ranged between 3.5 to 27.6 min. This intraspecific variation could have profound consequences on diagnostics, virulence, and antifungal treatment in clinical settings.

For conidial germination in most filamentous fungi, the presence of nutrients such as inorganic salts, sugars, and amino acids is required. The swelling phase of conidia is also called isotropic growth. Swollen conidia direct the growth to one side of the cell to grow in a polarized fashion, which leads to the formation of a germ tube (polarized growth). There is a notable drop in stress resistance during isotropic and polarized growth and genes expressed during these stages might represent novel targets for fungal infection.

S5.3c
Investigating the link between pleomorphism and virulence in *Cryptococcus*

Kenya Fernandes[1], James Fraser[2], Dee Carter[1]
[1]School of Life and Environmental Sciences, University of Sydney, Australia
[2]School of Chemistry and Molecular Biosciences, University of Queensland, Australia

S5.3 Cellular pleomorphism and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Objectives: Fungal pathogens *Cryptococcus neoformans* and *Cryptococcus gattii* are responsible for hundreds of thousands of annual deaths in immunocompromised individuals. Considerable phenotypic variation is exhibited by strains in response to stresses encountered during host infection, including increased capsule and cell size, the release of shed capsule, and the production of giant (> 15 μM), micro (< 1 μM), and irregular cells. We aimed to investigate whether the production of these morphological variants is associated with virulence using two sets of strains. The first is a collection of diverse clinical isolates obtained from HIV/AIDS patients in Botswana with accompanying clinical data. The second is a collection of lineages derived from the *C. neoformans* type strain H99 with high genetic similarity but differing levels of virulence. Some lineages in this set possess a mutation in SGF29, which encodes a component of the SAGA histone acetylation complex that has previously been implicated in their hypervirulence.

Methods: Isolates were cultured under conditions that simulate stresses encountered *in vivo* (DMEM, 5% CO_2, 37°C) as these are known to enhance capsule production and induce cell size changes. Cells were counterstained with india ink, visualized by light microscopy, and phenotypes were scored. For clinical isolates, MLST analysis was performed to determine their relatedness. For H99 strains, *Galleria mellonella* larval infection assays, growth curves, and antifungal susceptibility testing was performed to confirm their relative virulence and growth profiles. Serial block face and regular scanning electron microscopy were used to investigate the internal morphology of the giant, micro, and irregular cells to confirm that they possess attributes of functional cells.

Results: Substantial pleomorphism was seen across both collections. In the clinical strain set, phenotypic variables fell into two groups associated with differing symptoms. The production of 'large' phenotypes was associated with a higher CD4 count and was negatively correlated with intracranial pressure indicators, suggesting that these are induced in early-stage infection. 'Small' phenotypes were associated with lower CD4 counts, negatively correlated with meningeal inflammation indicators, and positively correlated with intracranial pressure indicators, suggesting that they are produced later during infection and may promote proliferation and dissemination. Isolates possessing giant cells, microcells, and shed capsules were rare, but strikingly, they were associated with patient death.

In the H99 set, strains from hypervirulent lineages had larger average capsule size, greater variation in cell size, and increased production of microcells and shed capsules. Deletion of SGF29 in an intermediate virulence lineage substantially increased its production of microcells and released capsule, consistent with a switch to hypervirulence. SGF29 loss-of-function mutations were subsequently identified in clinical isolates and were found to be significantly correlated with patient death. Expansion of a TA repeat in the second intron of SGF29 in clinical isolates was positively correlated with cell and capsule size, suggesting it also affects Sgf29 function.

Conclusion: Our results extend the evidence for a link between pleomorphism and virulence, with a likely role for epigenetic mechanisms mediated by SAGA-induced histone acetylation.

S5.3d
How mitochondrial complex I proteins in *Candida albicans* moderate phagocytosis and the production of pro-inflammatory cytokines in murine macrophages and dendritic cells

Xiaodong She[1], Pengyi Zhang[3], Richard Calderone[2], Weida Liu[1], Dongmei Li[2]
[1]Institute of Dermatology, Chinese Academy of Medical Sciences (cams) and Peking Union Medical College (PUMC), Nanjing, China
[2]Georgetown University Medical Center, Department of Microbiology and Immunology, Washington, USA
[3]Sport Science Research Center, Shandong Sport University, Jinan, China

S5.3 Cellular pleomorphism and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Objectives: Inhibition of respiration in *Candida albicans* impairs its colonization in the host tissues and causes avirulence in a murine vascular candidiasis model. Accordingly, blockage of the mitochondrial electron transport chain (ETC) of *C. albicans* by respiratory inhibitors promotes phagocytosis by increasing exposure of glucan which could be due to the mannan reduction. In our model, we have reported that 85% mannan reduction in goa1Δ, a deletion mutant of an ETC Complex I (CI) regulator, oppositely decreased phagocytosis. To understand such a difference, we broaden our investigation with three CI respiratory subunit mutants, which are either fungal-specific (nuo1Δ and nuo2Δ) or broadly conserved subunits (ndh51Δ) for cell wall analysis and innate immune responses.

Methods: We characterized mutant cell wall defects in these mutants, then analyzed their respective survival in macrophages. Fungal internalization into macrophages was visualized under fluorescent microscopy and live-cell imaging and analyzed through flow cytometry analysis. Cytokine production in dendritic cells (DCs) infected by fungal cells was measured by xMAP technology and the transcriptional profiles of murine macrophages-infected by different mutants were compared.

Results: We find that phosphopeptidomannan (PPM) reduction in goa1Δ and nuo1Δ and phospholipomannan (PLM) reduction in nuo2Δ correlate with massive inhibition of cytokine. PPM loss in nuo1Δ or goa1Δ fails to promote phagocytosis but promotes opsonized neutrophil killing. The cause of PPM insufficiency results from reduced phosphorylation of the Cek1 MAPK in goa1Δ and nuo1Δ. In contrast other three mutants, phagocytosis and cytokine production of ndh51Δ more resemble WT cells, which have shown an ~ 30% glucan reduction due to a defective Mek1 MAPK response. The divergent immune responses to these CI mutants are shown at the transcriptional level in infected macrophages. We noted that those well-characterized host receptors such as dectins and TLR2/4 for PPM, PLM, and glucan ligands are not significantly affected at 1 h post-infection. However, the scavenger receptor CD36, integrin ICAM, and growth factor receptors are downregulated along with a generally downregulated endocytosis and antigen processing/presentation. In addition, the host metabolic processes, oxidative stress-induced senescence, apoptosis, and signaling pathways such as Ras1/Erk5, the cAMP/CREB, and TLR9 pathway, are each individually affected in the host cells.

Conclusion: We speculate that mitochondrial signals of fungal origin may also be sensed by the host immune cells to coordinate the immune responses together with cell replication and metabolism during the early stage of infection.