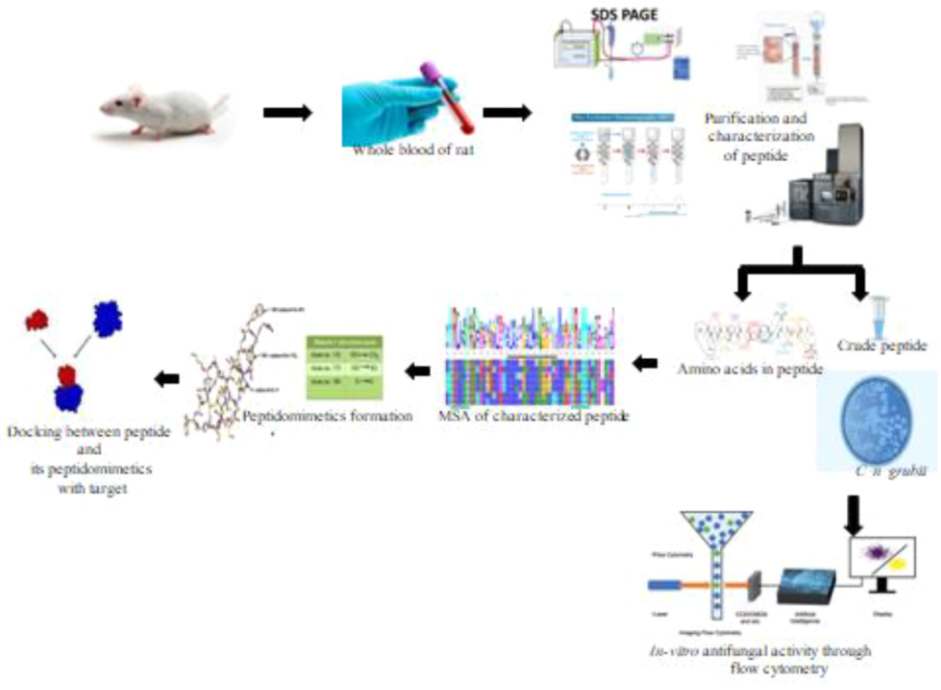
Table 1.

Mimetics	Changes in Atom
1	142 (NH→CH2), 173(OH→SH) and 184 (S→ O)
2	142 (NH→CH2), 151(NH→O), 205 (NH→O) and 184 (S→ O)
3	114(S→OH) and 185 (CH3 →Cl)
4	114(S→OH) and 185 (CH3 →F)
5	114(S→OH) and 231(NH2 →CH3)
6	114(S→OH)

Table 2.

Compounds	Binding energy (Kcal/mol) / No. of Hydrogen bonds Formed	
	Binding energy	No. of Hydrogen bonds
α-defensin 5 like peptide	- 22.07	1
M 1	-54.47	4
M 2	-49.46	2
M 3	-45.34	1
M 4	-48.62	2
M 5	-43.24	1
M 6	-40.65	1



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Kinetics of Titan cells generation and transcriptome modifications comparing three *in vitro* protocols

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objectives: To face and escape his environment and the host immune response, *Candida neoformans* is able to change his morphology (Titan cells) and his metabolism (dormancy, quiescence). Titan cells (TC) have been observed in lungs and brains in experimental mouse models of cryptococcosis and in patients. In 2018, three independent teams published three protocols allowing the reproducible generation of TC *in vitro* [Hommel B et al., (AA); Trevijano-Contador N, et al., (OZ), and Dambuza IM et al., (EB), in PLoS Pathogens 2018]. TC generated in these protocols exhibited the same features as the TC described *in vivo*. Our objective was to compare and describe the three protocols in parallel to highlight common and different features that can impact further study using those specific protocols.

Methods: A total of 22 h before starting the kinetics of the TC generation, the three protocols requested a pre-culture at 30°C in three different liquid media. The medium for TC production for the three labs was also different but a common factor is the addition of fetal calf serum (FBS) for OZ and EB. This kinetics was evaluated for size and quantity (%) of TC produced over a 72 h period (H0, H18, H24, H48, H72) at 30°C under shaking for AA while OZ and EB protocols incubate the cells at 37°C and 5% of CO2, while the whole transcriptome was analyzed at H0, H3, H7, and H18 in triplicates.

Results: OZ generated the highest percentage of TC, 63.1% and 58.2% at H18 and H24, and decreased drastically down to 6.7% at H48. EB reached a high percentage of TC at H24 for 46.7% and dropdown <10% until the end of the kinetics. AA did not reach a quantity of TC as high as the two other protocols but it remained constant over a period of H72 (Table 1). RNA sequencing preliminary analysis showed some differences in genes expressed at the different time points analyzed. The

		H18			H24			H48			H72		
		AA	OZ	EB	AA	OZ	EB	AA	OZ	EB	AA	OZ	EB
Cell size	Mean (µM)	9,03	11,17	9,81	7,34	11,40	11,18	8,72	5,31	5,76	8,68	5,86	5,47
	Min (µM)	4,44	4,08	4,31	2,40	2,76	4,07	2,52	2,33	2,63	4,44	2,30	2,65
	Max (µM)	15,74	20,80	20,00	16,10	23,54	24,39	20,22	13,56	20,86	14,71	20,09	15,68
TC (%)		*37,89*	**63,15**	30,00	14,38	**58,21**	**46,67**	*31,43*	6,67	9,82	*25,97*	14,92	9,36

PCA analysis revealed that the triplicates of each protocol for the 4-time points analyzed are closed to each other, related to the good quality of our experiments. The differential gene expression (DGE) showed significant (P <.01 and Log2 fold change >1) differences at H0 which highlights the impact of the preculture on the TC process. The highest numbers of DGE are observed between H0 and H7 for the three protocols, where about two 450 DGE, two 000 DGE, and two 300 AA, OZ, and EB, respectively. After analysis of the PCA plot during the kinetics, EB and OZ are grouped while AA is not. That could be explained by the presence of FBS in OZ and EB protocols.

Conclusion: By running the three protocols in parallel, we showed here that the kinetics of TC generation differed between each other with a significant variation of the transcriptome. This is an important finding that paves the way to compare more deeply the transcriptome of *C. neoformans* during TC generation with the final goal is to identify the genes associated with TC generation.

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Scalp fungal microbiome and sebum composition in males with and without androgenetic alopecia

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Objects: Lipophilic *Malassezia* species are abundant in the scalp microbiome; we investigated the scalp microbiome and sebum composition of patients with androgenic alopecia (AGA) and aimed to identify factors accelerating AGA progression.

Materials and Methods: Scalp scale samples (swabs) were collected from 55 male Japanese patients with AGA and 63 healthy individuals. Fungal rRNA genes were amplified by PCR and the amplicons were sequenced on the MiSeq platform. The extent of fungal colonization was determined by qPCR. We used gas chromatography/mass spectrometry to measure the sebum levels of free fatty acids, diglycerides, triglycerides, squalene, free cholesterol, cholesterol esters, and wax.

Results and Discussion: *Malassezia* restricta predominated in all AGA (64.7%) and non-AGA age groups (44.6%). qPCR revealed that *Malassezia* colonization was more extensive in the AGA than non-AGA group, regardless of age; the *Malassezia* level was significantly higher in ADA subjects aged 50–59 than 30-49 years. The TG level was significantly higher in the AGA than non-AGA group (P <.05), but the free fatty acid, squalene, and free cholesterol levels were significantly lower (all P <.05).

Conclusion: Thus, the scalp fungal microbiome and sebum composition may influence AGA development.

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'It's not Fungus, its Nocardia'—an elementary diagnostic challenge for draining sinus on abdominal wall (rare): a case report

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Introduction: A rare finding of abdominal wall clinical presentation of persistent progressive tumefaction with draining sinus with no granules caused by Nocardia brasiliensis.

Methods: History: A 22-year-old presented to the Dermatology OPD with complaints of swelling and tenderness and discharging sinuses with no granules around the periumbilical region in the lower abdominal area for three years. His initial fine needle aspiration cytology specimen report was inconclusive. He received anti-Kochs treatment based on a positive Montoux test and family history from outside the hospital.

Initially punch biopsy samples were sent for fungal processing to our laboratory which was inconclusive. Repeat pus aspirate and punch biopsy samples were subjected to conventional techniques. The sample was inoculated on Sabouraud's Dextrose agar, Brain heart Infusion agar, and Lowenstein-Jensen media. Direct Smear was subjected to Gram stain and Modified Zeihl Neelson stain with 1% Sulfuric acid as decoloriser.

Results:

- On Gram stain, Gram-positive filamentous bacilli against a background of pus cells in pus aspirate only (not in punch biopsy specimens).
- Modified Zeihl Neelson stain with 1% Sulfuric acid decoloriser was performed on all three samples. Beaded acid-fast filamentous bacilli with plenty of pus cells in the background were seen in pus aspirate only (not in punch biopsy specimens).
- No fungal elements were observed on the 20% KOH mount.
- Clinicians were notified immediately with the provisional report of possible Actinomycetoma due to *Nocardia* sp.
- Growth was observed within 9 days on SDA as well as LJ. It was a chalky white, dry colony to begin with that turned orangish-yellow in another week's time. Smear from the colony showed Gram-positive filamentous bacilli which on Modified ZN Smear were acid-fast filamentous beaded bacilli. The isolate was identified as Nocardia species. This was further confirmed as *Nocardia brasiliensis* by MALDI-TOF.
- On admission, the patient was initially started on Inj. Amikacin and then changed to Modified Raman regime of double dose Cotrimoxazole and Gentamicin. His lesions started showing improvement over 2 weeks of in-patient treatment. He was discharged on oral treatment thereafter.

Conclusion:

- Abdominal wall clinical presentation of persistent progressive tumefaction with draining sinus with no granules caused by *N. brasiliensis* is a rare clinical entity as Mycetoma. The differential diagnosis would lead to either bacterial or fungal etiology or neoplasia.
- Delay in correct diagnosis led to the chronicity of the clinical presentation with inappropriate therapy.
- For a chronic destructive debilitating infective mycetoma presentation, appropriate microbiological diagnostics become essential to have early correct detection with proper sampling technique to guide the appropriate therapy as per the causative pathogen.