MISSIONE 4
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Ministero dell'Università e della Ricerca

Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

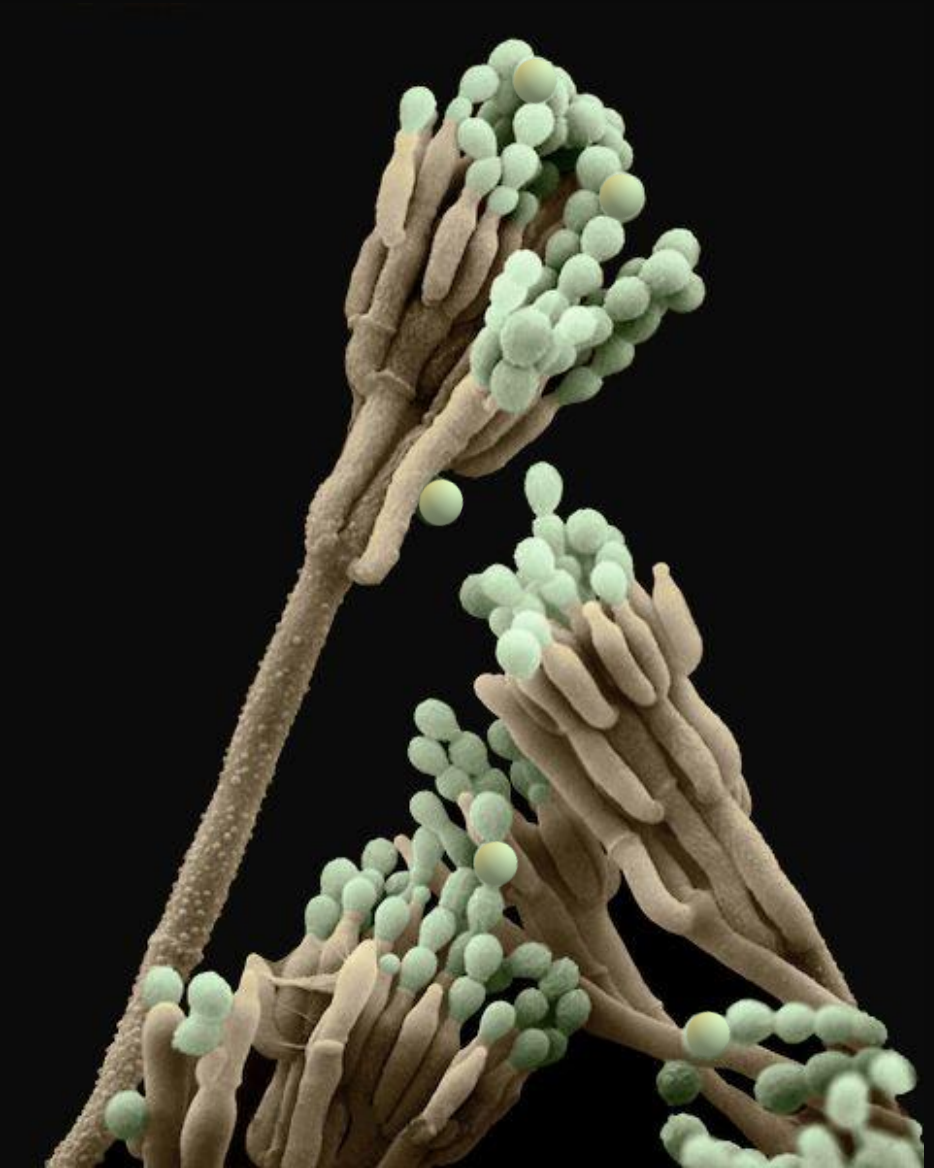
L'ANTICORPO MONOCLONALE UMANIZZATO ANTIFUNGINO DIA-T51 E LA SUA SINERGIA CON AMFOTERICINA B: STUDI *in vitro* E *in vivo*

VITALITY

1506
UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO

17 Dicembre 2024

ALARMING NUMBERS



13 million people affected by fungal infections

1.5 million deaths every year

>90% caused by Candida spp., Cryptococcus spp. Aspergillus spp.

Candidemia alone represents **60%** of total fungal infections

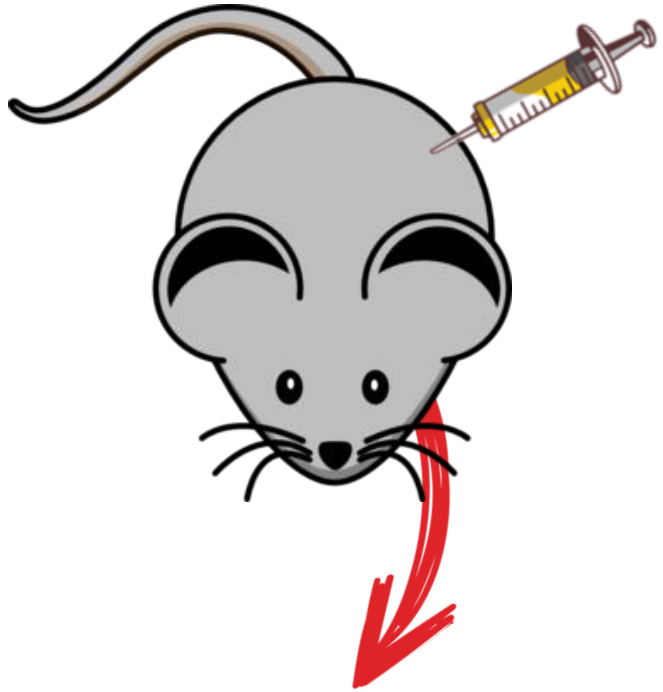
According to CDC, ***C. auris*** is the **1st** most urgent threats

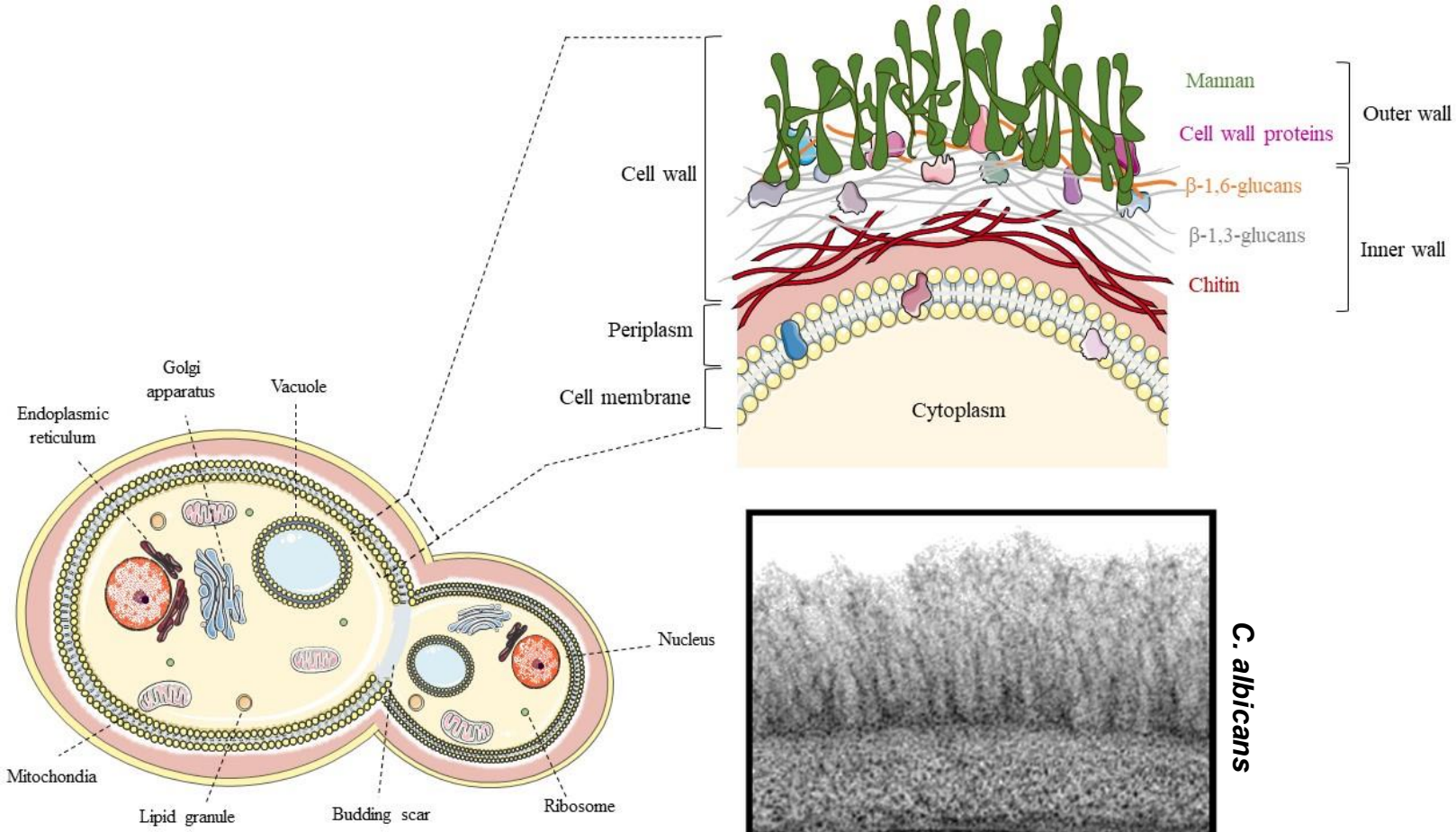
Just **3** major classes of antifungal drugs

>90% are resistant to **azoles**
>30% are resistant to **2** antifungal classes
3-5% to **all** antifungals

www.cdc.gov
www.gaffi.org

Anti-β-1,3-glucans antibodies

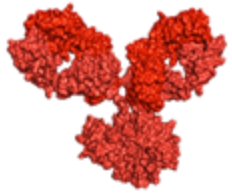




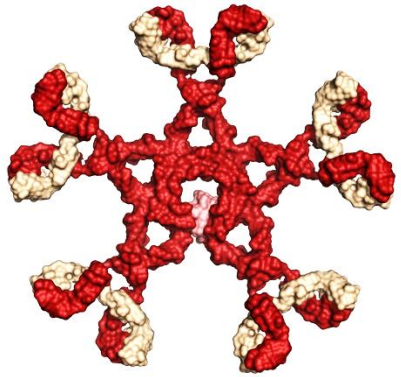
C. albicans

N. Gow et al. 2017

IgG_{2b} 2G8



IgM 1E12



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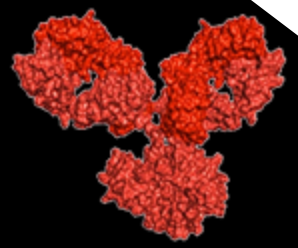
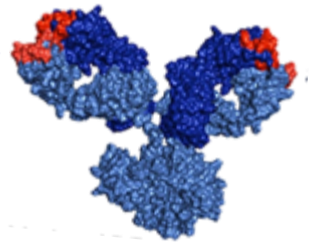
The humanization process

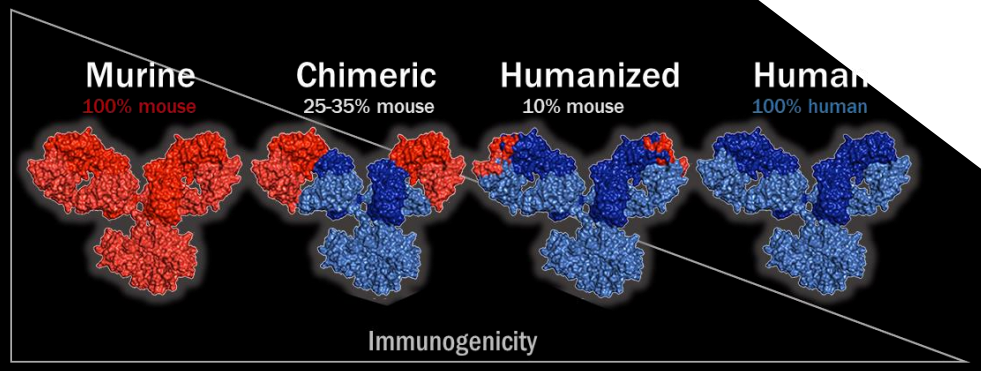
Statistical interference approach + CDR-grafting

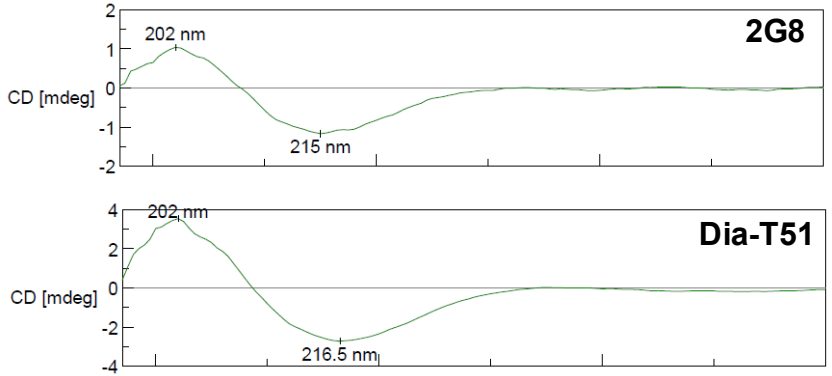


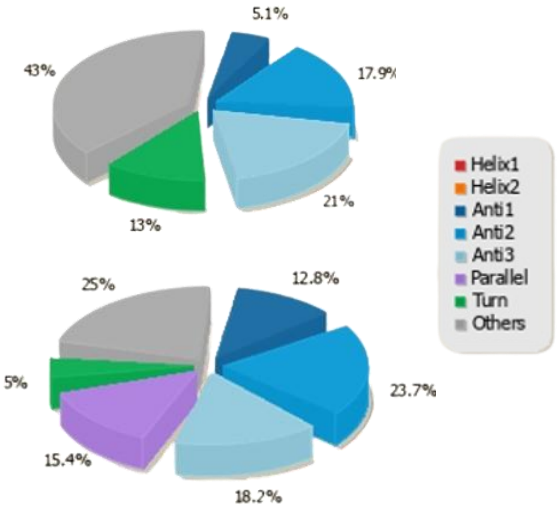
IgG_{2b} 2G8



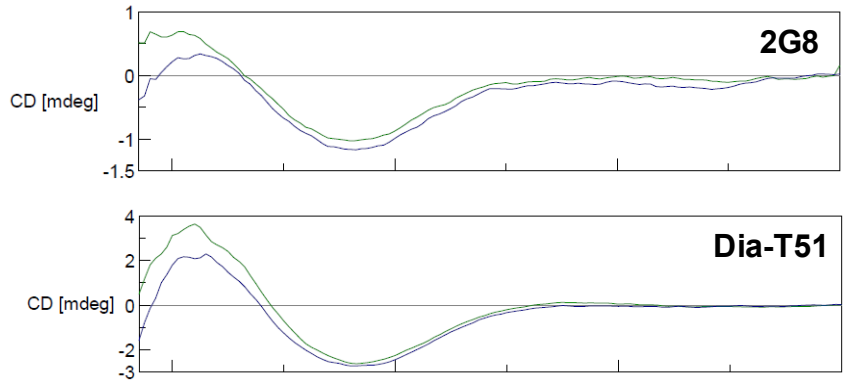
IgG_1 Dia-T51

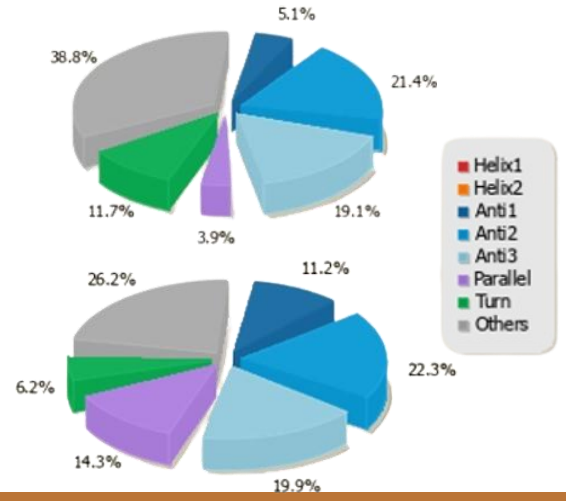






Peaks (nm)	2G8	Dia-T51
UV	279.00	279.00
Fluorescence	343.03	339.07





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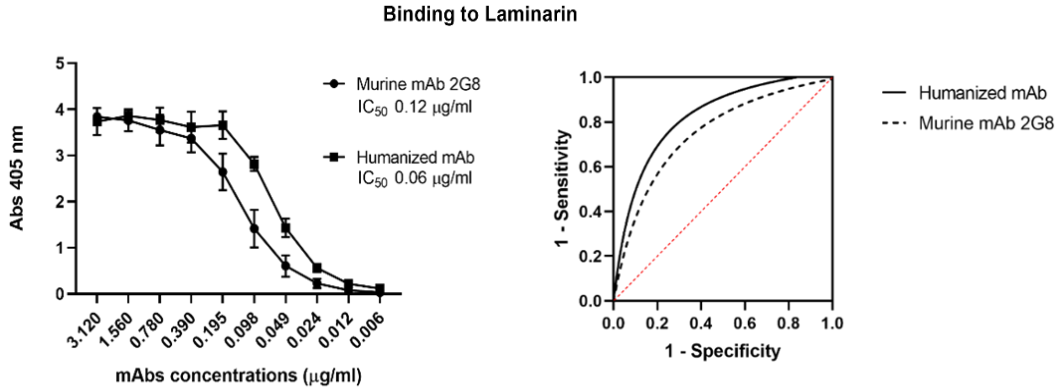
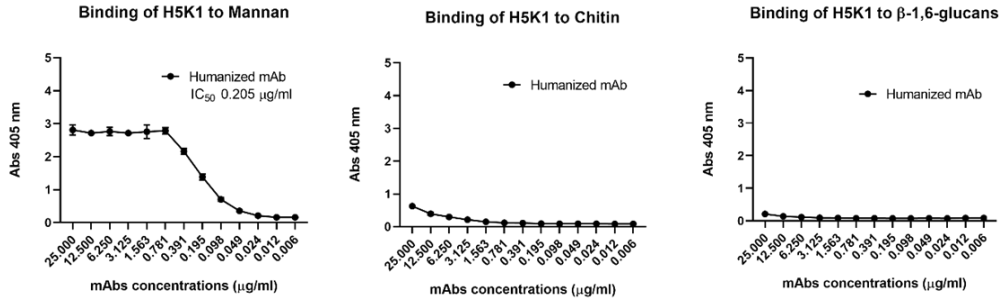
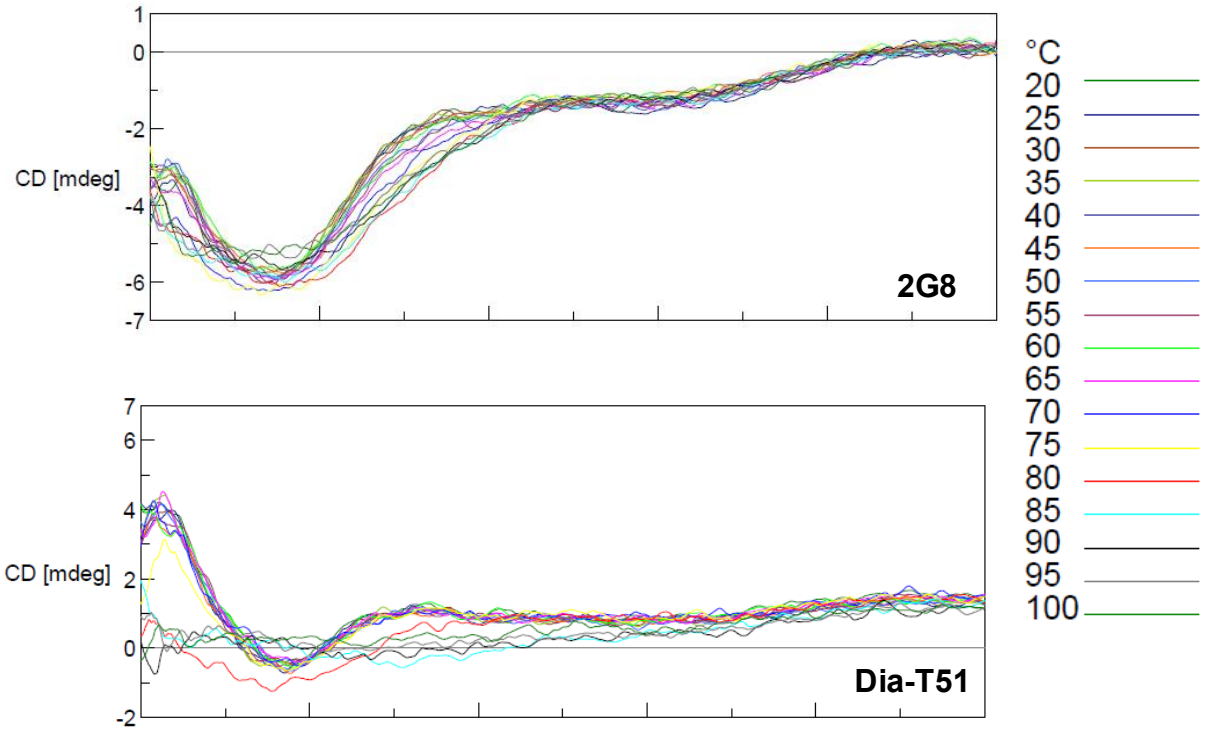
In vitro studies



	IC_{50} (µg/ml)	AUC	K_D
Dia-T51	0.06	0.85	$3.59x10^{-9}$
2G8	0.12	0.77	$1.9x10^{-9}$



Dia-T51 IC_{50} (µg/ml)	**6 months 4°C**	**12 months 4°C**	**18 months -80°C**
	0.10 (K_D $3.37x10^{-9}$)	0.10	0.09



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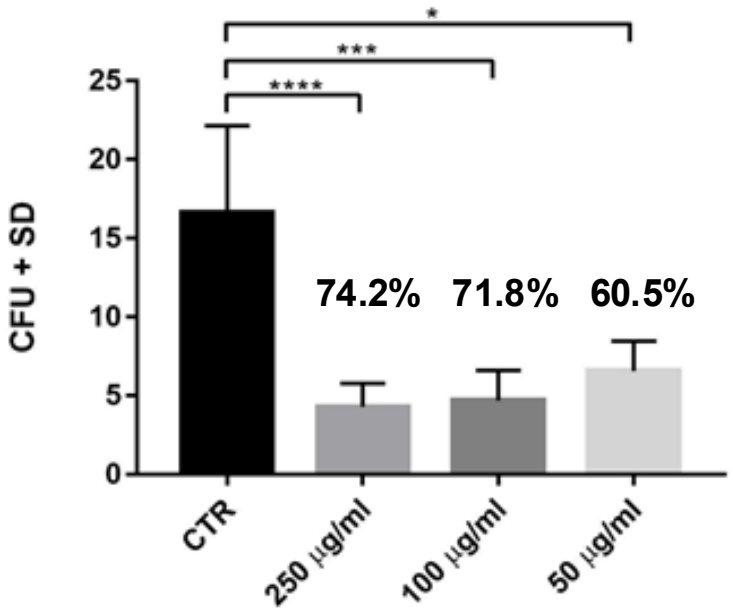
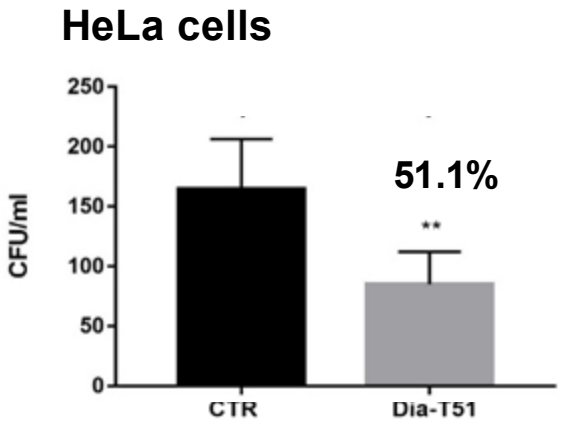
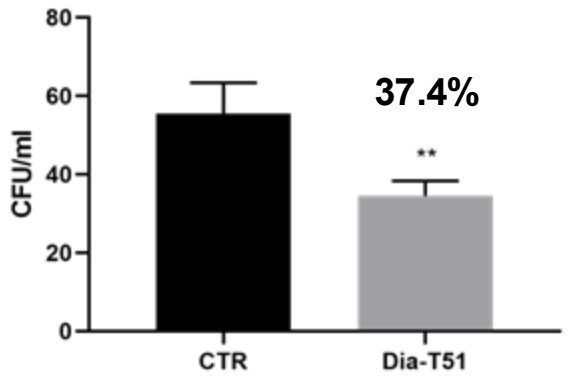
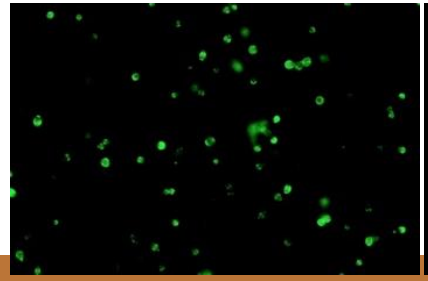
C. auris growth inhibition

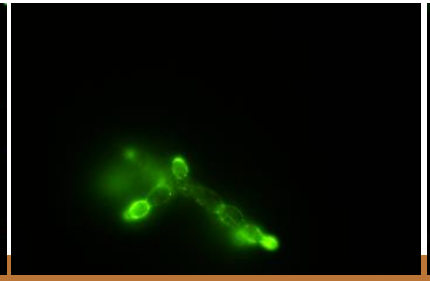


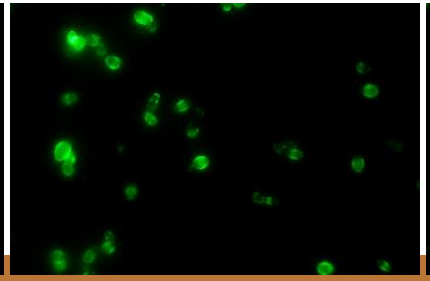
C. auris adhesion inhibition

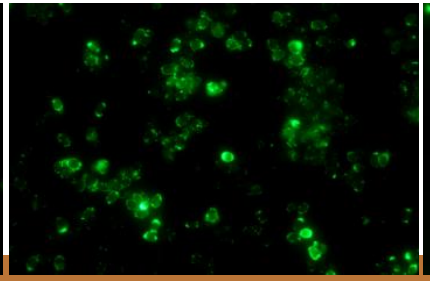


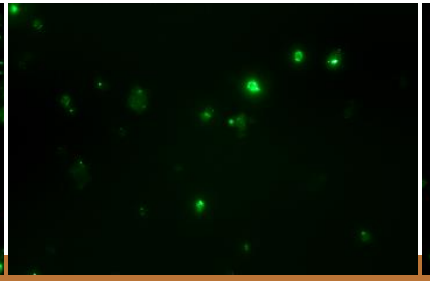


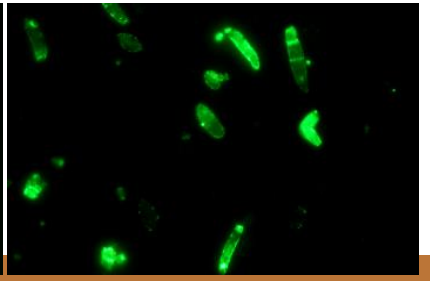

C. auris


C. albicans hyphal form


C. albicans yeast form


C. glabrata


A. fumigatus conidia


F. solani conidia

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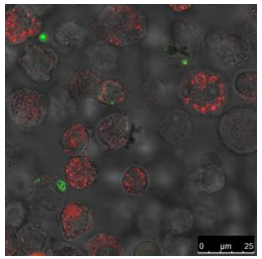
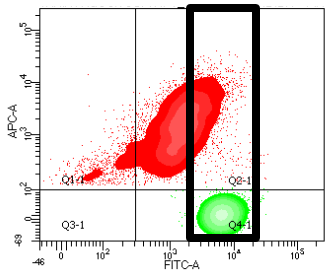
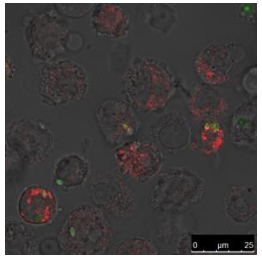
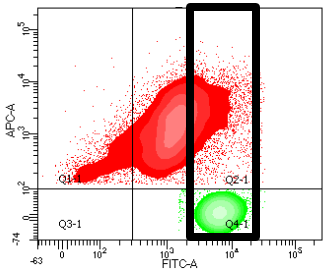
THP-1 cell line



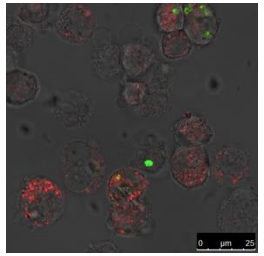


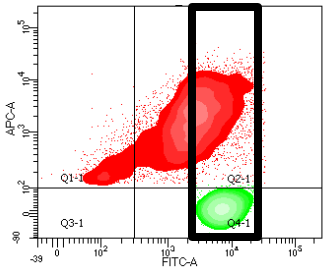
Not-opsonized *C. auris*





Opsonized *C. auris* (2.5 µg/ml Dia-T51)





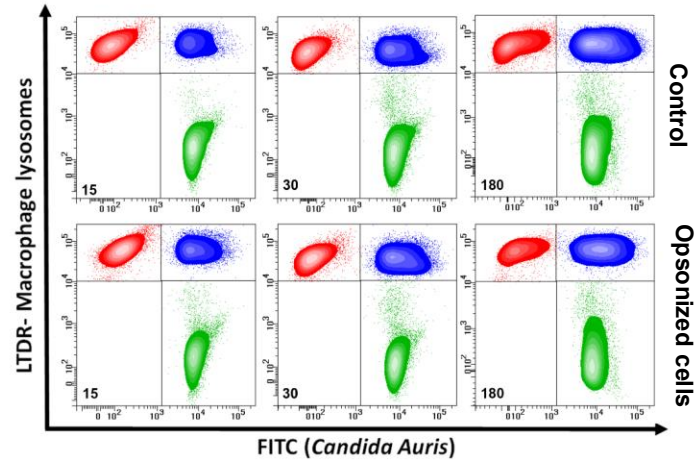
Opsonized *C. auris* (25 µg/ml Dia-T51)

Red population: THP-1 (human macrophages)
Green population: *C. auris*

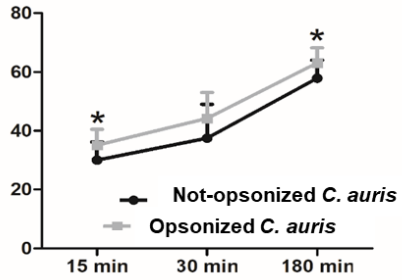
	Engulfing macrophages (%)	Residual Candida/Macrophages	Phagocytosis rate (%)
Not-opsonized *C. auris*	84.5	3.79	37.0
Opsonized *C. auris* (2.5 µg/ml Dia-T51)	85.8	2.44	46.5
Opsonized *C. auris* (25 µg/ml Dia-T51)	87.0	2.33	65.6

Human monocytes-derived macrophages

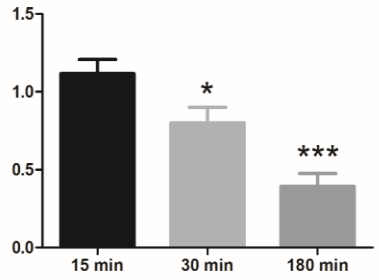
Red population: not-engulfing macrophages
Blue population: engulfing macrophages
Green population: residual *C. auris*



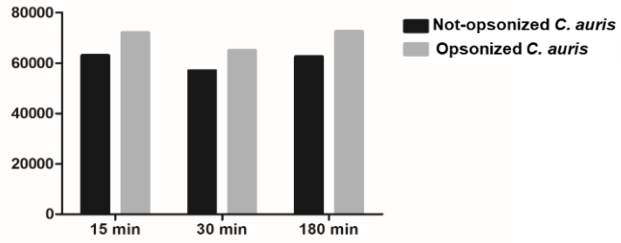
% phagocyting macrophages



Fold of decrease of residual *C. auris*



Lysosome activity



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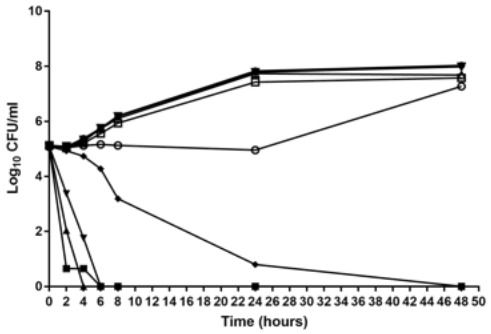
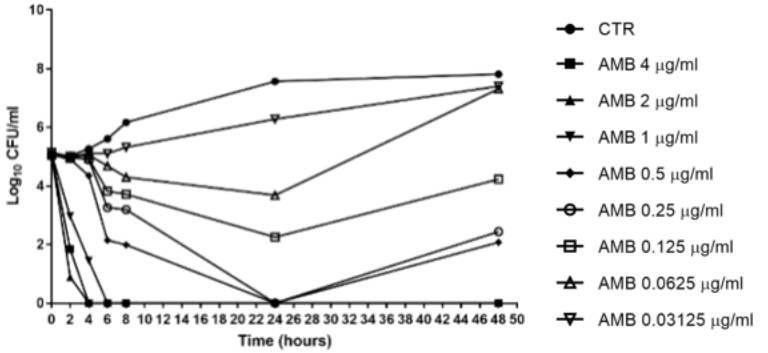
Dia-T51 + Amphotericin B

Dia-T51 µg/ml	0	0.25	2.5	25	250
24h MIC AMB µg/ml	0.5	0.5	0.25	0.125	0.125
48h MIC AMB µg/ml	1	1	1	0.25	0.25

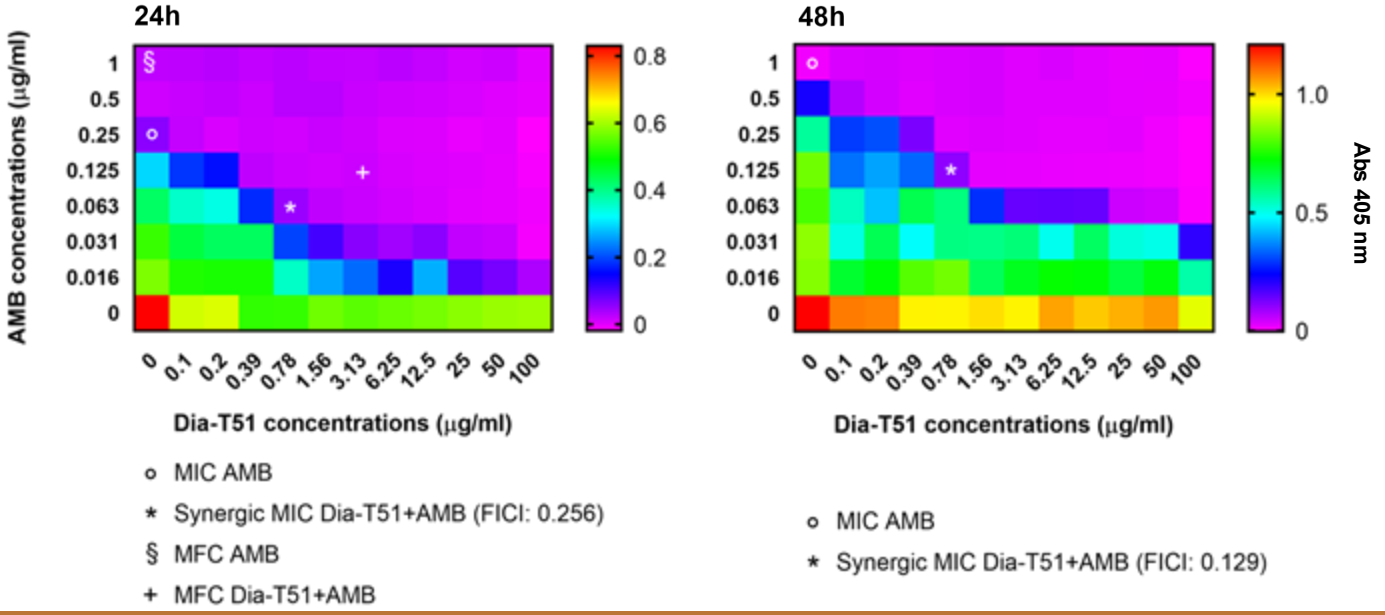
Amphotericin B



Dia-T51 + Amphotericin B



Synergic



Minimum Inhibitory Concentration (inhibition of fungal growth ≥90%)	24h	48h
AMB	0.25 µg/ml	1 µg/ml
Dia-T51+AMB	0.78+0.063 µg/ml	0.78+0.125 µg/ml

Minimum Fungicidal Concentration (reduction of initial inoculum viability ≥99.9%)	AMB	Dia-T51+AMB
	1 µg/ml	3.13+0.125 µg/ml

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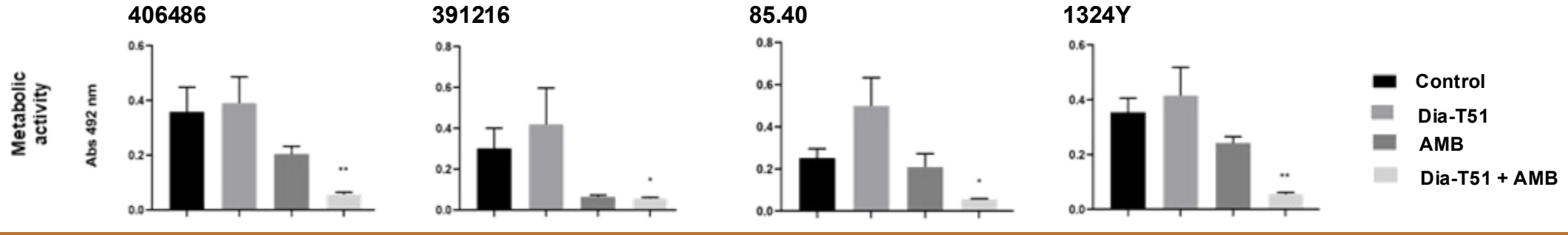
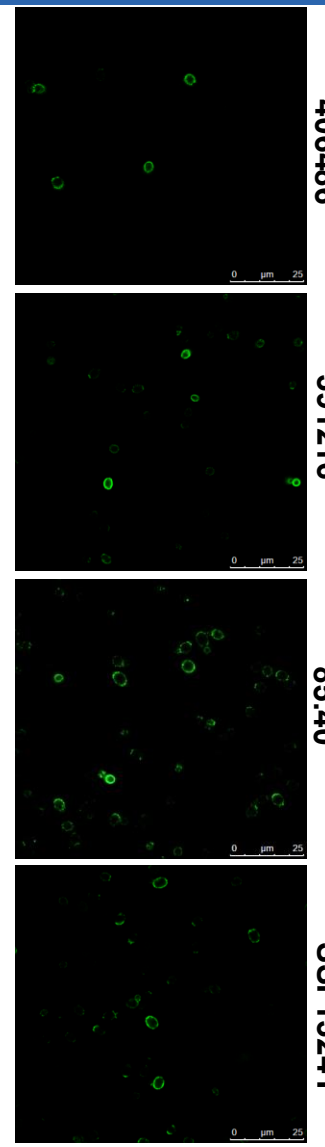
Azienda Ospedaliero Universitaria OSPEDALI RIUNITI Umberto I - G.M.Lancisi - G.Salesi Ancona

- Is it effective with other *Candida* spp.?
- Is it efficient with resistant strains?

		Synergy in Checkerboard		Synergy in time-kill curves
***C. glabrata* strains**	**Characteristics**	**Dia-T51 / AMB (µg/ml)**	**FICI**	**Δlog most active compound vs combination ≥ 2 Dia-T51 / AMB (µg/ml)**
406486	Biofilm hyperproducer	0.78 / 0.125	0.252	6.25/0.25
391216	Common clinical isolate	1.56 / 0.25	0.254	6.25/0.5
85.40	Mutation in FKS gene	12.5 / 0.125	0.281	50/0.25
1324Y	Mutation in FKS gene	3.13 / 0.125	0.258	12.5/0.25

C. glabrata biofilm inhibition





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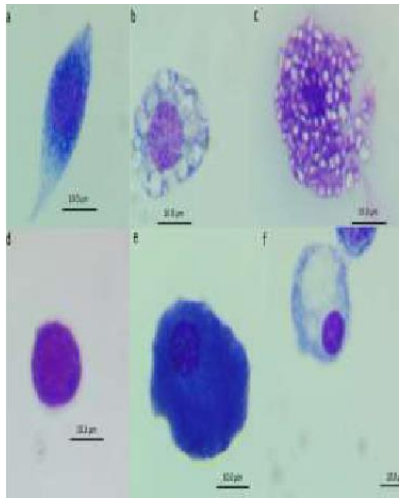
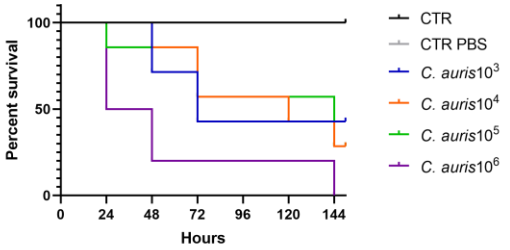
In vivo studies



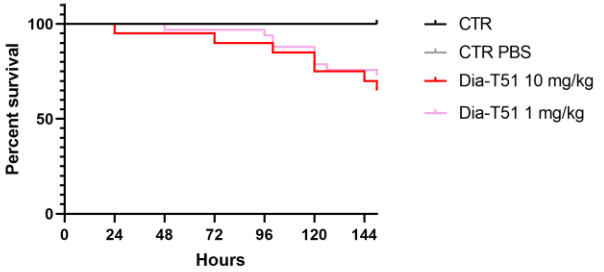
a. Plasmatocyte
b. Granulocyte
c. Spherulocyte
d. Prohemocyte
e. Oenocytoid
f. Coagulocyte

Eskin A.,2022

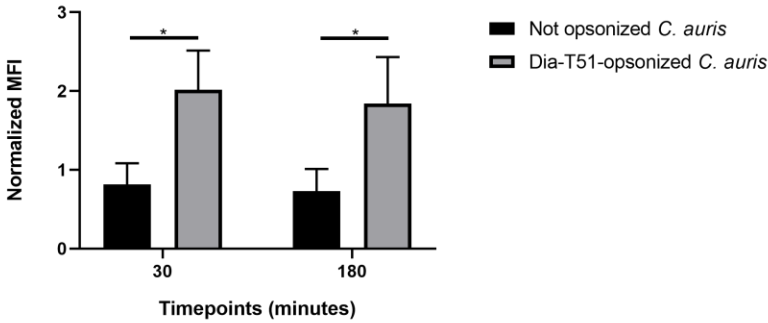
***Galleria mellonella* survival - *C. auris* infection**



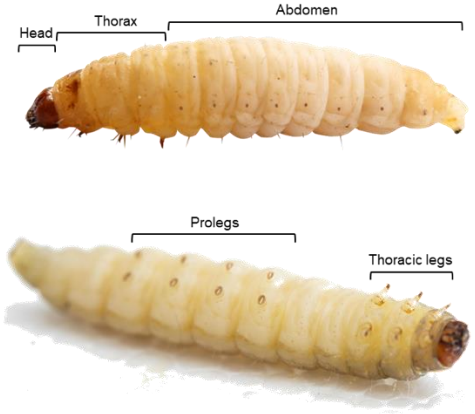
Dia-T51 toxicity on *Galleria mellonella* larvae



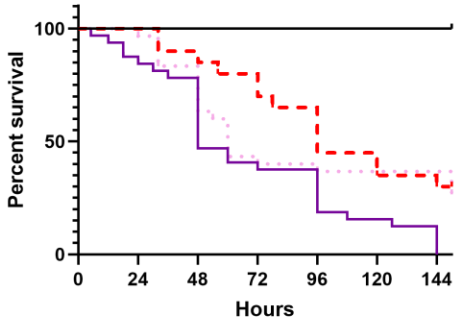
Hemocytes phagocytosis of *C. auris*



Hemocytes PI positive	30 minutes	180 minutes
Not opsonized *C. auris*	27%	7%
Dia-T51-opsonized *C. auris*	11%	10.5%

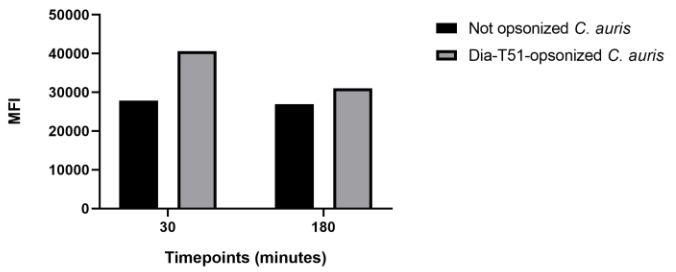


***Galleria mellonella* larvae infected with *C. auris* and treated with Dia-T51**



	Survival
CTR	
CTR PBS	
C. auris	
C. auris + *Dia-T51 10* mg/kg	30%
C. auris + *Dia-T51 1* mg/kg	26.7%

CD80 expression



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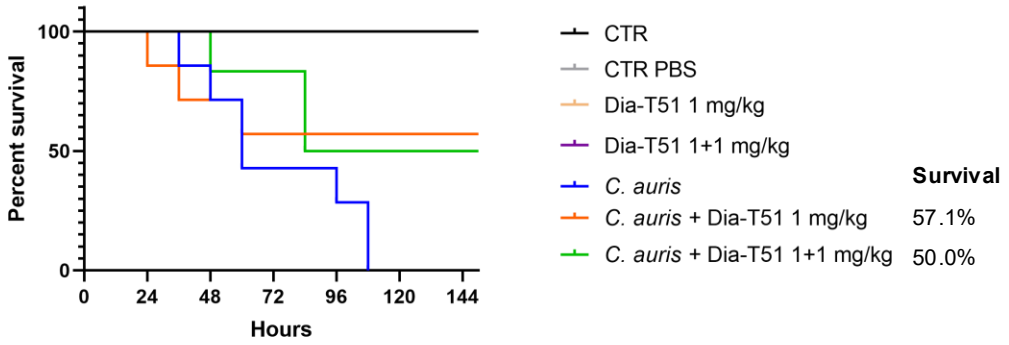
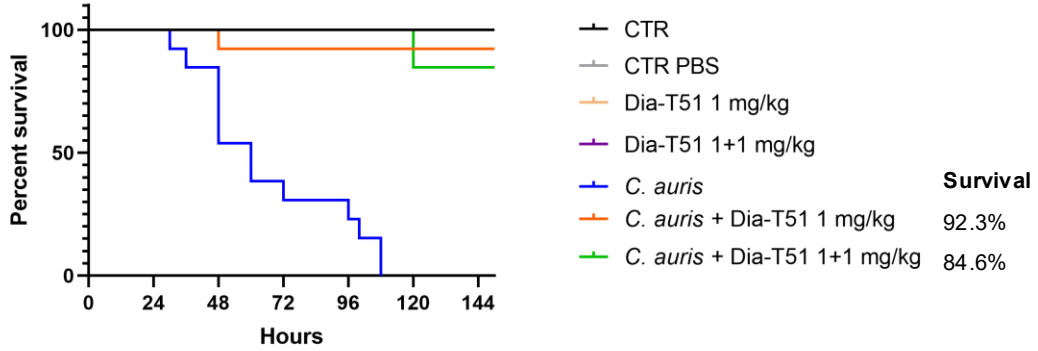
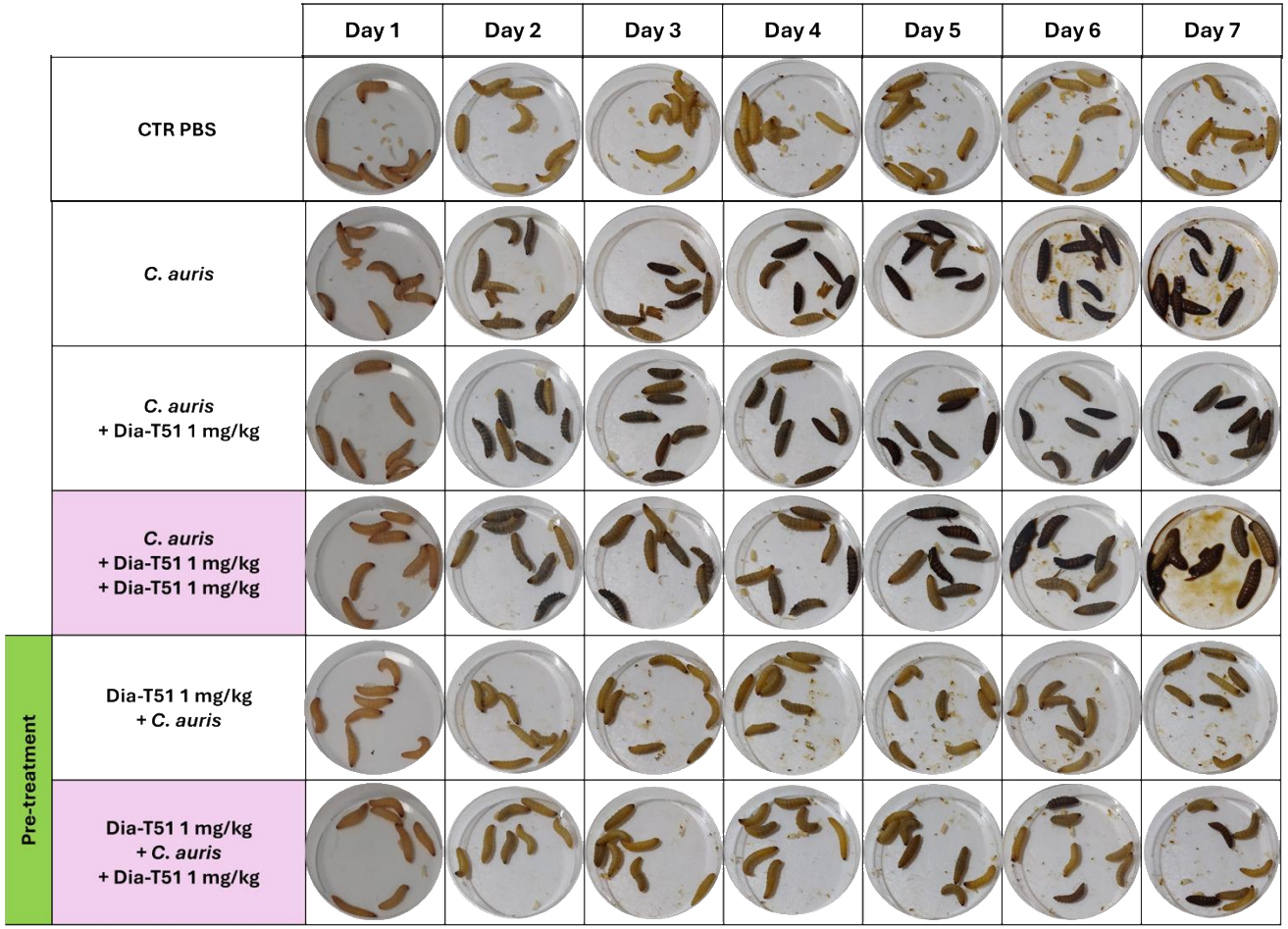
Galleria mellonella **larvae infected with *C. auris* and treated with a single and double dose of Dia-T51**



Galleria mellonella **larvae pretreated with Dia-T51, with or without a booster dose and infected with *C. auris***





Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
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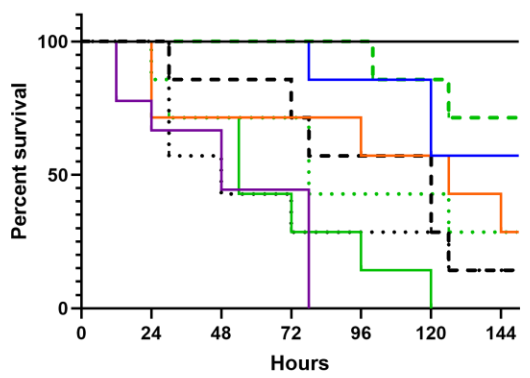
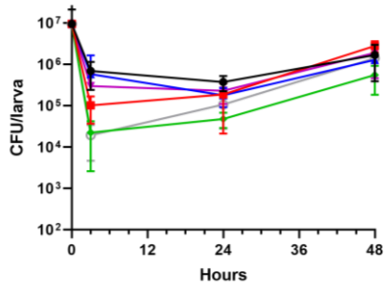
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
C. auris							
C. auris + AMB 50 mg/kg							
C. auris + AMB 5 mg/kg							
C. auris + AMB 0.5 mg/kg							
C. auris + Dia-T51 10 mg/kg							
C. auris + Dia-T51 1 mg/kg							
C. auris + AMB 0.5 mg/kg + Dia-T51 10 mg/kg							
C. auris + AMB 0.5 mg/kg + Dia-T51 1 mg/kg							

Galleria mellonella **larvae infected with *C. auris* and treated with Amphotericin B, Dia-T51 and their combinations**

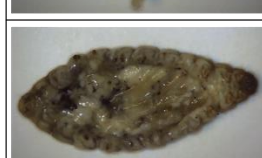


	Survival
CTR	
CTR PBS	
CTR DMSO	
C. auris	0%
C. auris + AMB 50 mg/kg	57.1%
C. auris + AMB 5 mg/kg	28.6%
C. auris + AMB 0.5 mg/kg	0%
C. auris + Dia-T51 10 mg/kg	14.3%
C. auris + Dia-T51 1 mg/kg	14.3%
C. auris + AMB-Dia-T51 0.5+10 mg/kg	71.4%
C. auris + AMB-Dia-T51 0.5+1 mg/kg	28.6%

C. auris **infection and plating after hemolymph extraction**



ΔLog	No treatment			AMB		
	3h	24h	48h	3h	24h	48h
AMB + Dia-T51 0.5 + 10 mg/kg	1.54	0.88	0.67	0.77	0.33	0.96
AMB + Dia-T51 0.5 + 1 mg/kg	1.8	0.76	0.35	1.03	0.21	0.64

3 hours post infection	
	CTR
	*C. auris*
	*C. auris* + AMB 0.5 mg/kg
	*C. auris* + Dia-T51 10 mg/kg
	*C. auris* + Dia-T51 1 mg/kg
	*C. auris* + AMB 0.5 mg/kg + Dia-T51 10 mg/kg
	*C. auris* + AMB mg/kg + Dia-T51 1 mg/kg

CONCLUSIONS

Dia-T51 is is a humanized monoclonal antibody structurally similar and functionally even better compared to the murine parental.

Dia-T51 has a direct effect against fungal cells and an indirect and protective effect in the presence of immune effectors both *in vitro* and *in vivo*.

The synergy with amphotericin B was proved both *in vitro* and *in vivo* thus limiting the drug's side effects.

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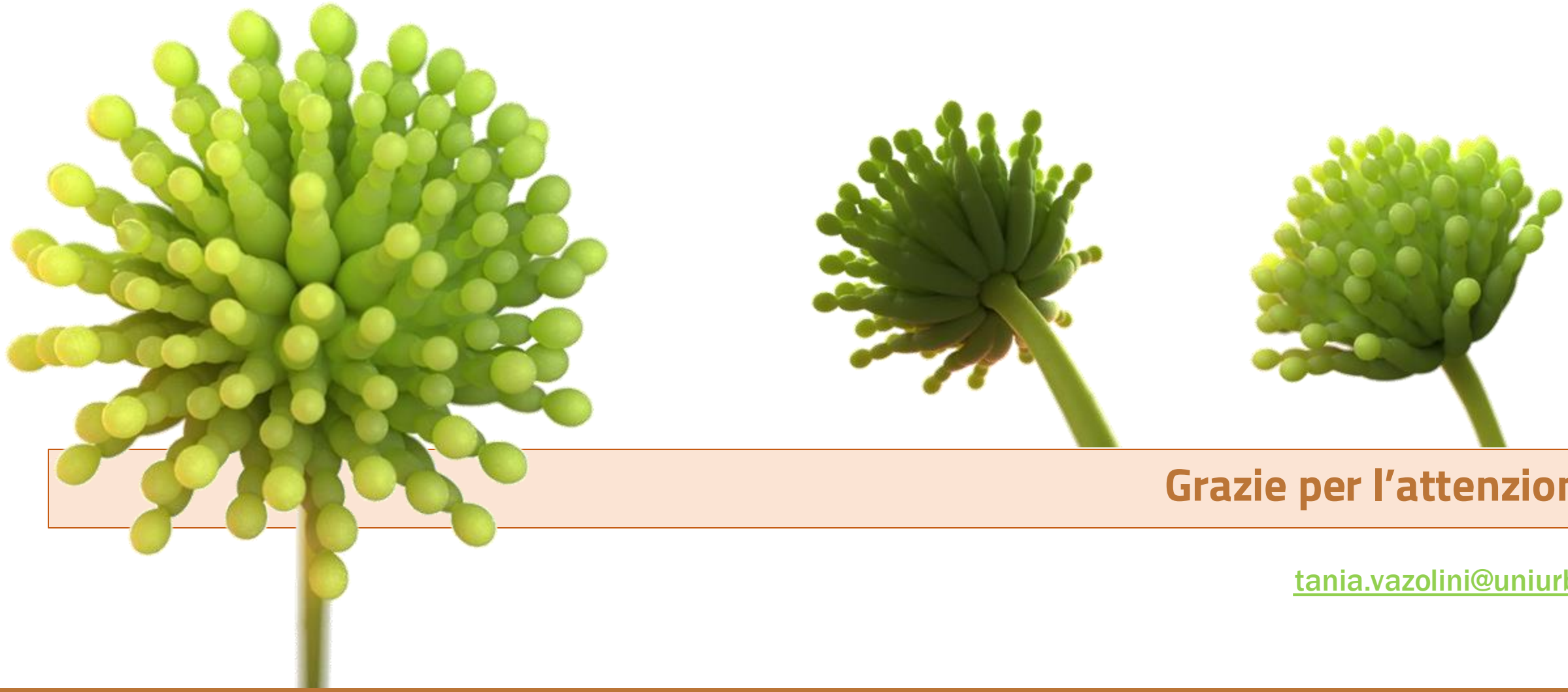

Grazie per l'attenzione

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Chain	Position	AA	Liability
H	38	W	Trp oxidation
	109*	W	
	118	W	
L	4	M	Met oxidation
	41	W	Trp oxidation
	34*	N	Asn deamidation
	35*	G	
	36*	N	
	37*	T	
	68	D	Asp isomerisation
	69	S	

VH Domain

CDR1 CDR2

1 – QVQLQQSGAELKKPGASVKISCKASGYTLSSYWLEWVRQRPGQGLEWIGEILPGSGSTNY – 60

CDR3

61 – NEKFKGRATFTADTSTNTAYMELSSLTSEDSAVYYCAREGWYFDVWGQGTTVTVSS – 116

*

VL Domain

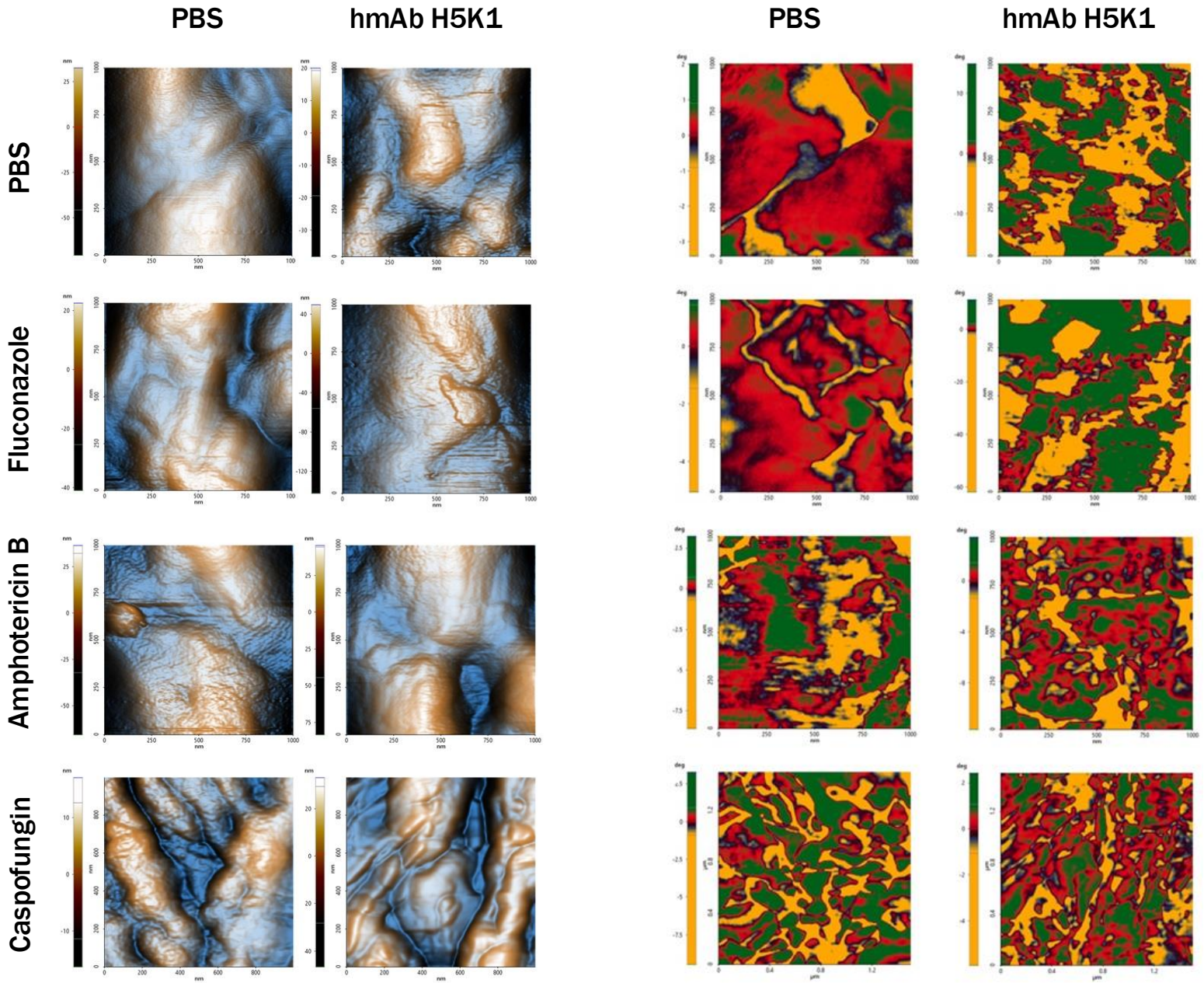
CDR1 CDR2

1 – DVVMTQSPLSLPVTLGQPASISCRSSQSLLYSNGNTHLNWFQQRPGQSPRRLIYLVSNRD – 60

****

CDR3

61 – SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCVQGTHFPYTFGGGTKLEIK – 112



Atomic force microscopy

- Perturbation in the topographic and sub-topographic domains
- Higher frequency of vertical alterations
- Substantial difference in the chemical distribution
- Softening and weakening of the whole cell