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Can statins be useful in invasive fungal infections?

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Outline of the talk

- Statins
- The target: HMG-CoA reductase
- The problem: fungal infections
- What is the evidence for effect?
  - *In vitro*
  - *In vivo* – animal models
  - Clinical trials: prospective/retrospective
- A parallell story: statins and pneumococci
- What is a relevant dose in cell-experiments?
- Prospects for the future

The history of statins

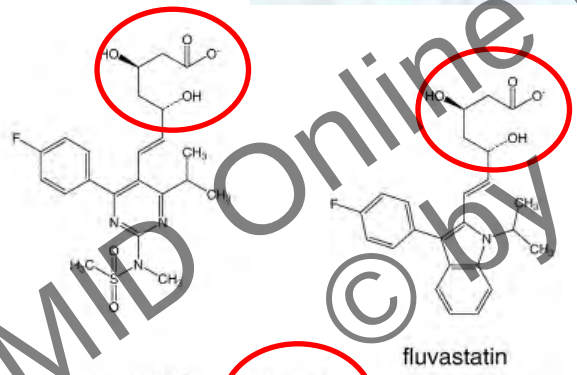
- Aim: To find an inhibitor for human HMG-CoA reductase
- Hypothesis: fungi make a substance that block sterol synthesis as a protection against competing fungi.
- Assay: HMG-CoA reductase
- 6000 microbial strains were searched
- Penicillum citrinum: citrinin
- 600 liters of culture filtrate => 23 mg mevastatin

- Penicillum brevicompactum: compactin
- Very potent competitive inhibitor:
  → Substrate K_m: 10^{-5}M
  → Inhibitor K_m : 10^{-9}M

Akira Endo (1933-)

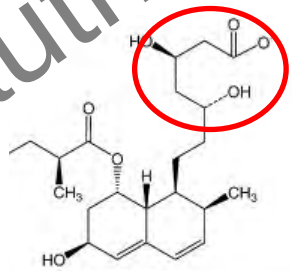
Biochemical properties of statins

Chemical and Pharmacologic Properties of Statins						
Property	**Rosuva**	**Atorva**	**Fluva**	**Lova**	**Prava**	**Simva**
Prodrug	No	No	No	Yes	No	Yes
Single isomer	Yes	Yes	Yes	Yes	Yes	Yes
Lipophilicity (log P)[1]	–0.3	+4.1	+3.2	+4.3	–0.2	+4.7
Salt form	Ca	Ca	Na	None	Na	None
IC_{50} (nm):						
HMG-CoA inhibition	5	8	28	NA	NA	11
Cholesterol synthesis	0.16	1.16	3.78	NA	6.98	2.74

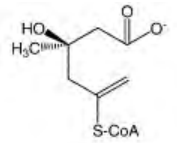


rosuvastatin

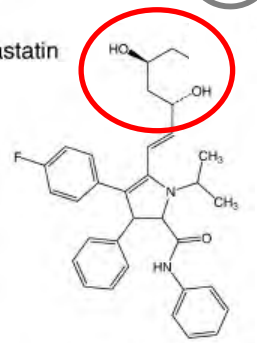
fluvastatin



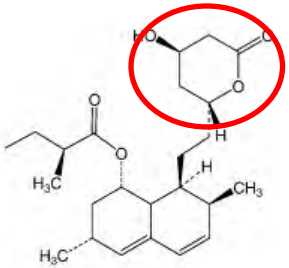
pravastatin



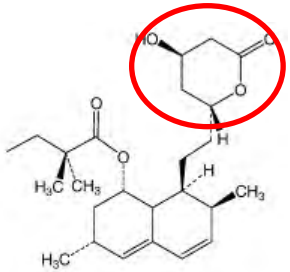
HMG-CoA



atorvastatin

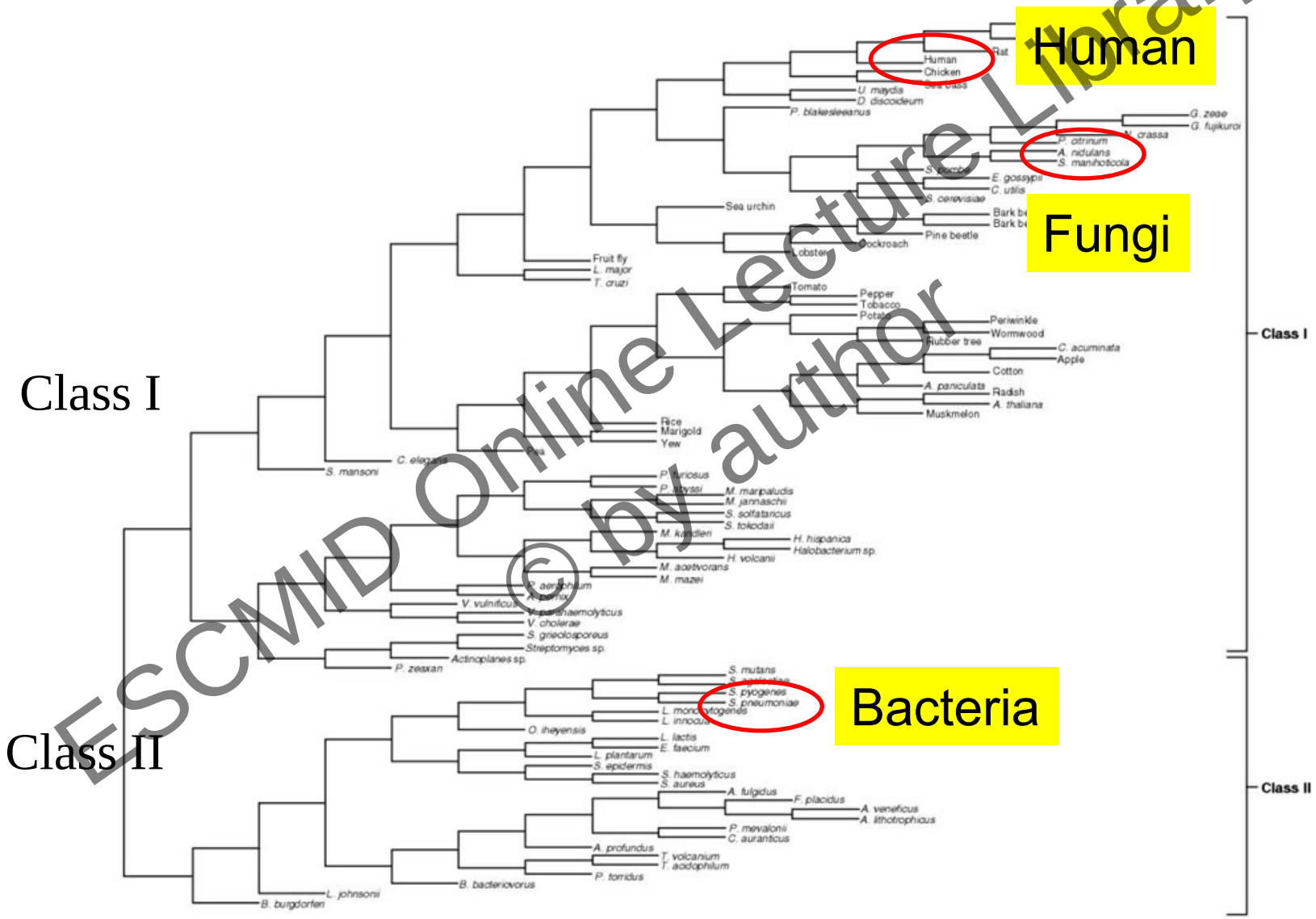


lovastatin



simvastatin

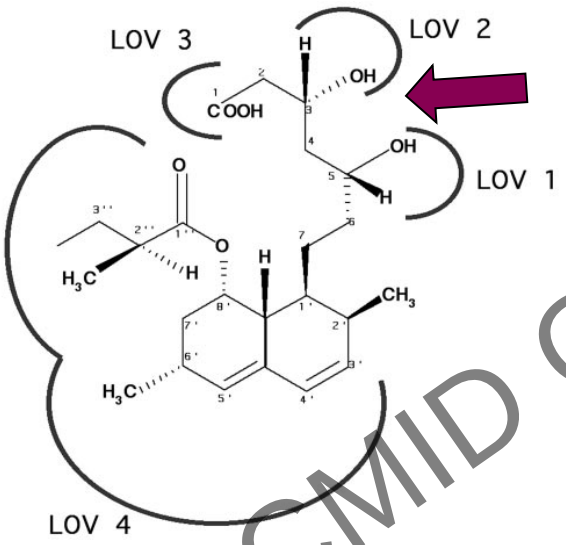
The target: HMG-CoA reductase

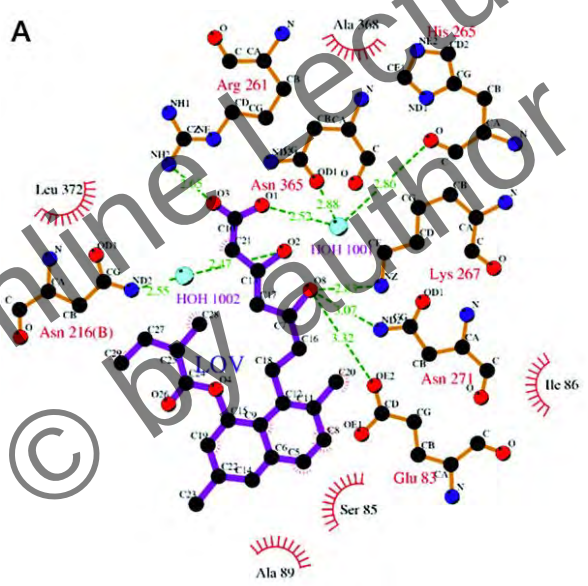


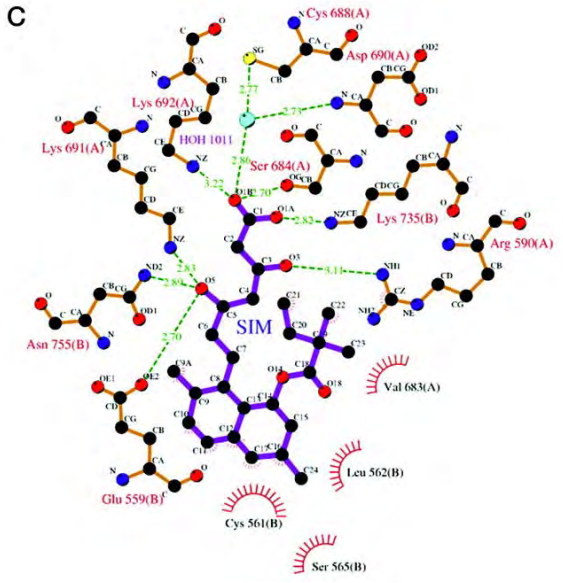
Different binding in class I and class II

Class II: P. mevalonii

Class I: Human







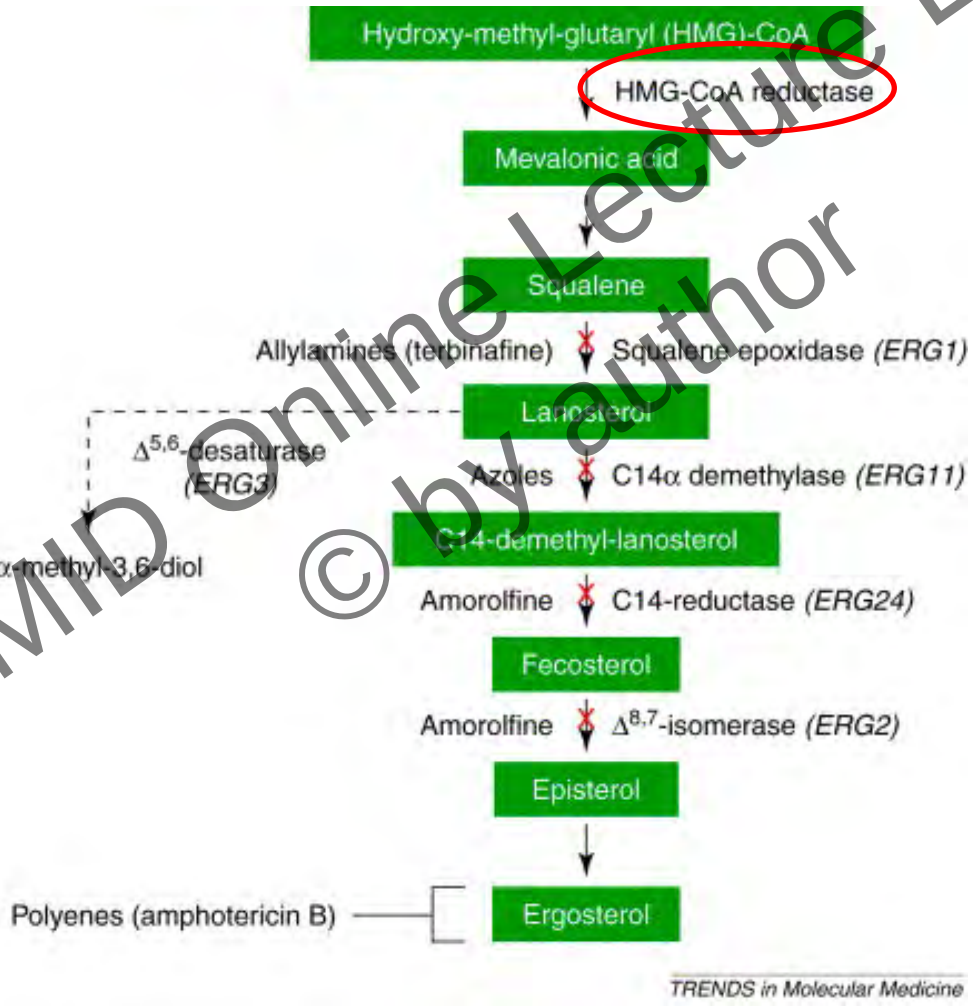
4 H-bonds

7 H-bonds

A class II specific inhibitor is possible to find

Tabernero et al, JBC, 2003

Ergosterol biosynthetic pathway



In vitro killing of *Candida spp*

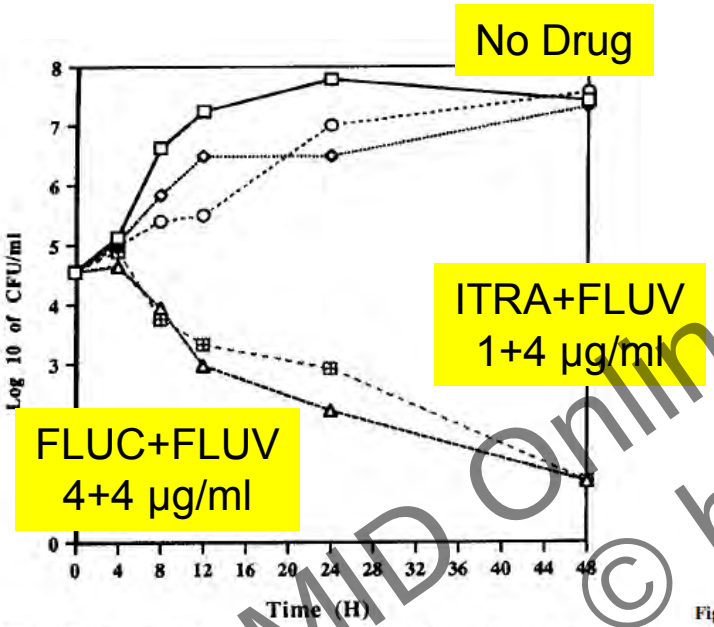


FIG. 1. Time-versus-killing curves obtained with fluconazole- and itraconazole-susceptible *C. albicans* 95-146 in Sabouraud dextrose broth with no drug (control) (□); fluconazole, 16 µg/ml (▽); itraconazole, 4 µg/ml (○); fluconazole-fluvastatin, 4 + 4 µg/ml (△); and itraconazole-fluvastatin, 1 + 4 µg/ml (⊞).

Chin et al, AAC, 1997

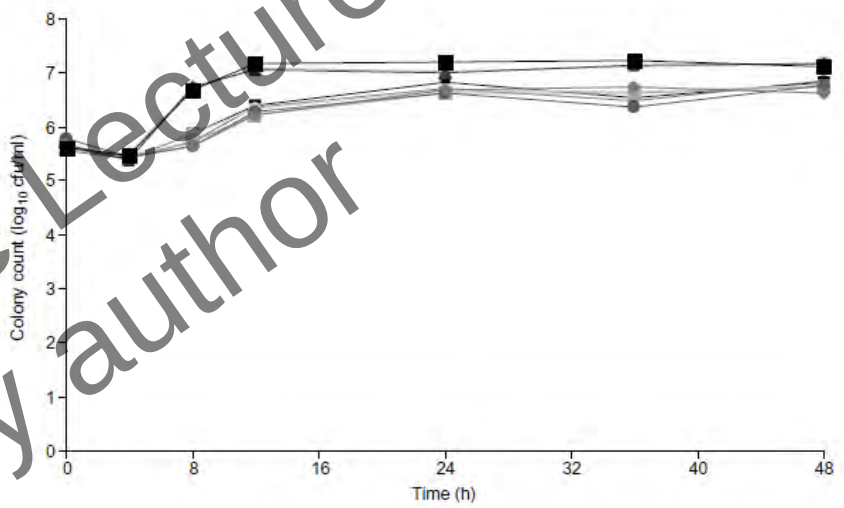
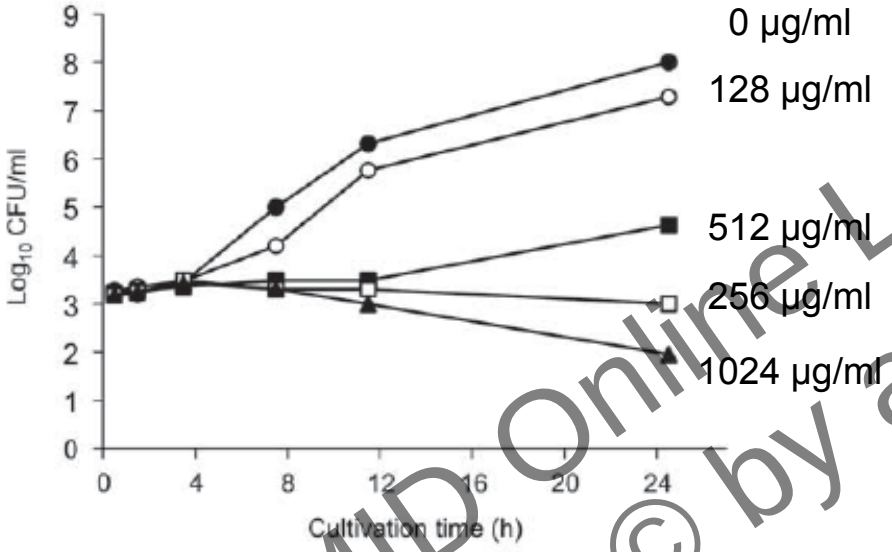


Fig. 1. Time-kill studies of fluconazole alone and in combination with fluvastatin or pravastatin against *C. albicans* 99-506. ■, growth control; ◆, fluconazole 10 mg/L; ●, fluconazole 30 mg/L; ▲, pravastatin 0.25 mg/L; ▼, fluvastatin 1 mg/L; ▩, fluconazole 10 mg/L + pravastatin; ◍, fluconazole 30 mg/L + pravastatin; ▼, fluconazole 10 mg/L + fluvastatin; ▲, fluconazole 30 mg/L + fluvastatin.

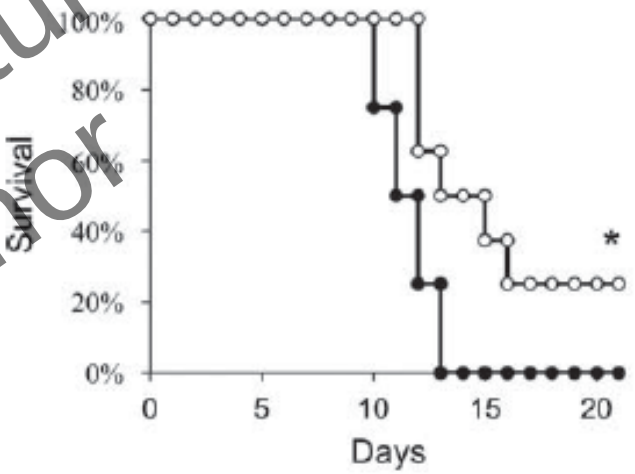
Nash et al, J Med Microbiol 2002

Results are dependent on MIC method used. Lowest MIC-values 1-2 µg/mg

Data from animal models (C. albicans)



Pravastatin, 50 mg/kg/day



Tashiro et al, Med Mycol, 2012

50 mg / kg => 3500 mg to a human (70 kg)

Statins do not lower cholesterol in rodents (500 mg/kg is not enough)

Data from clinical trials

1.

TABLE 2. Comparison of infectious diseases outcome between statin users and nonusers

Infection	Statin users (n = 12,981) n (%)	Nonusers (n = 32,266) n (%)	Adjusted or (95% CI)[a]
Common infections group	8939 (68.9)	19,323 (59.9)	1.13 (1.06-1.19)
Influenza	145 (1.1)	307 (0.9)	1.06 (0.80-1.39)
Fungal infections	3171 (24.4)	5667 (17.6)	0.97 (0.91-1.04)

[a] Logistic regression analysis; covariates were patient age, patient gender, total Charlson Comorbidity Score, tobacco use, alcohol use/abuse, number of inpatient admissions in the baseline period, number of outpatient admissions in the baseline period and use of the following medications: beta-blockers, diuretics, calcium-channel blockers, ACE inhibitors/ARBs, oral hypoglycemics, aspirin and steroids.
OR, odds ratio; CI, confidence interval.

Magulick et al, Am J Med Sci, 2013

2.

TABLE 3. **Impact of Statins on Primary and Secondary End Points**[a,b,c]

Outcomes	Statin (n=493)	No statin (n=526)	Unadjusted analysis *P* value	Unadjusted analysis OR (95% CI)	Adjusted analysis *P* value	Adjusted analysis AOR (95% CI)
Primary outcome						
Culture positive for *Candida* species (n=139)	56 (40.3)	83 (59.7)	**.04**	0.68 (0.47-0.98)	**.03**	0.60 (0.38-0.96)
Secondary outcomes						
Patients with Charlson score ≥2	n=401	n=392				
Culture positive for *Candida* species (n=105)	43 (41.0)	62 (59.0)	**.04**	0.64 (0.42-0.97)	**.01**	0.47 (0.27-0.79)
Patients with Charlson score <2	n=92	n=134				
Culture positive for *Candida* species (n=34)	13 (38.2)	21 (61.8)	.75	0.88 (0.42-1.87)	.22	2.07 (0.64-6.72)

[a] AOR = adjusted odds ratio; CI = confidence interval; OR = (unadjusted) odds ratio.
[b] Data are provided as number (percentage) of patients, unless otherwise indicated. Statistically significant *P* values appear in boldface.
[c] The Hosmer-Lemeshow goodness-of-fit test (χ^2-test statistic, 7.68; degrees of freedom, 8; *P*=.47).

Spanakis et al, Mayo Clin Proc, 2010

3.

Candidemia outcomes not improved with statin use

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Infectious Diseases Section, VA Medical Center and George Washington University, Washington, DC, USA

Welch et al, Med Mycol, 2010

In vitro killing of *Aspergillus spp*

Table 2 *In vitro* minimal inhibitory concentrations (MICs, μg/ml) and minimal fungicidal concentrations (MFCs, μg/ml) of lovastatin (LOV), simvastatin (SIM), itraconazole (ITC), voriconazole (VRC), and amphotericin B (AMB) against *Aspergillus* spp. by standard broth microdilution method

Strain	LOV (MIC/MFC)	SIM (MIC/MFC)	ITC (MIC)	VRC (MIC)	AMB (MIC)
A. fumigatus AF1	40/51	10/10	0.25	0.25	0.5
A. fumigatus AF2	102/128	20/25	>16	1	1
A. fumigatus AF6	102/102	32/32	>16	1	1
A. fumigatus BMU01200	16/40	5/8	1	0.5	1
A. fumigatus BMU01340	51/64	8/8	1	0.5	2
A. fumigatus BMU02812	32/32	8/8	1	1	2
A. fumigatus BMU02813	40/51	10/20	0.5	0.5	2
A. fumigatus BMU02863	32/102	6/10	0.5	0.5	2
A. fumigatus BMU0293A	32/51	4/6	1	1	0.5
A. flavus BMU02584	128/ND	64/128	1	1	0.5
A. terreus BMU00322	>256/ND	>256/ND	1	1	1
A. niger BMU02090	>256/ND	>256/ND	1	0.5	0.5

LOV, lovastatin; SIM, simvastatin; ITC, itraconazole; VRC, voriconazole; AMB, amphotericin B; MIC, minimal inhibitory concentration (expressed in μg/ml); MFC, minimal fungicidal concentration (expressed in μg/ml); ND, no data.

Qiao et al, Med Mycol, 2007

Clinical data for invasive mould infections

Case–control study of statin prevention of mould infections

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Oklahoma City VA Medical Center, Oklahoma City, OK, USA

Summary

Invasive mould infections (IMI) are associated with significant morbidity and mortality. *In vitro* studies have demonstrated that hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) have activity against several pathogenic moulds including Zygomycetes and *Aspergillus* spp. The aim of our study was to determine if statin use is a preventive factor for the development of IMI. This was a retrospective case–control study of 10 United States Veterans Affairs Medical Centers that comprise the Veterans Integrated Service Network (VISN) 16. Cases with IMI and controls were identified from 2001 to 2008. Controls were matched by age, facility, history of transplantation, presence of chronic steroid use and presence of human immunodeficiency virus infection (HIV). Two hundred and thirty-eight patients were included. Independent variables associated with the development of IMI were history of solid malignant tumours (OR 2.63, 1.41–4.87) and hypertension (OR 2.29, 1.13–4.68). Statin use within 3 months of index date was not an independent variable for prevention or development of IMI. No level of exposure to a statin drug appeared to influence the development of infection. This retrospective case–control study suggests that despite evidence of *in vitro* activity, statins may not decrease risk of IMI. Prospective, controlled trials may be necessary to investigate any potential clinical benefit.

Key words: Statin, invasive mould infections, prophylaxis.

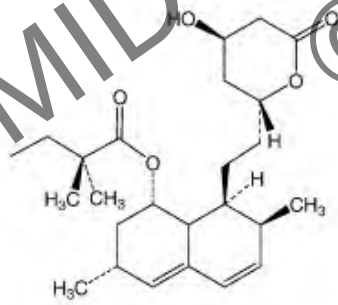
Thompson et al, Mycoses, 2010

Prospective RCTs on statins and fungal infections – a comprehensive summary…

A parallell story: statins and pneumococcal infection

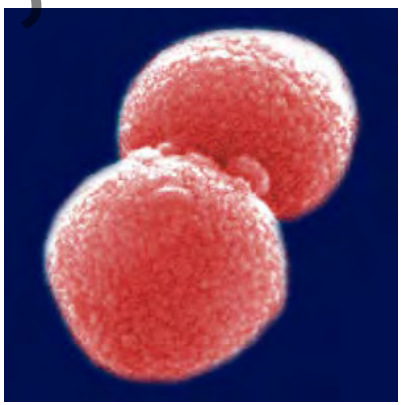
- Evidence from large, retrospective trials that statin use was associated with a better prognosis in pneumonia

- Could statins have a direct effect on bacterial growth?



simvastatin

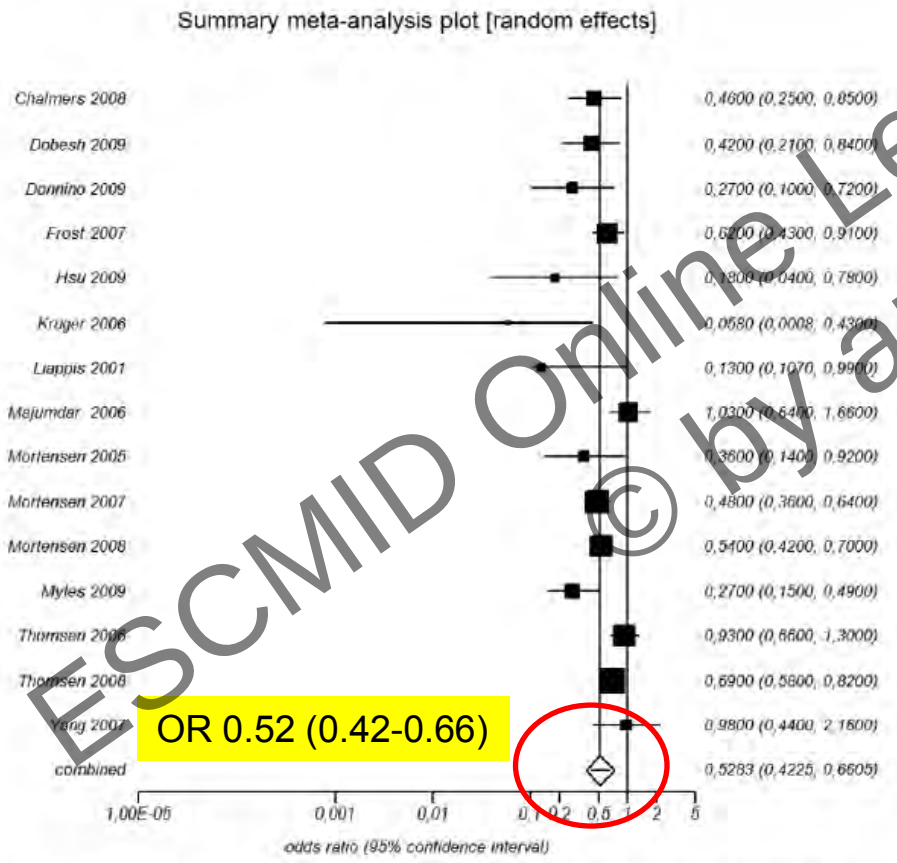
+



S. pneumoniae

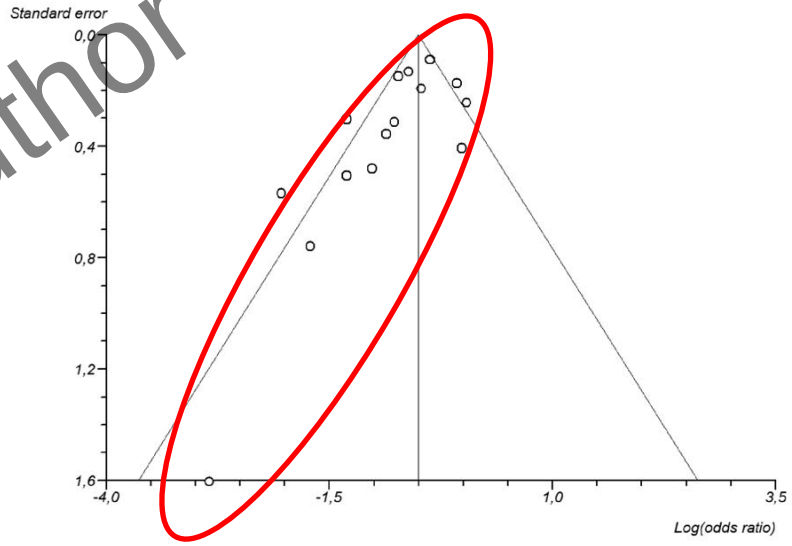
= True?

What is the role of publication bias?



OR 0.52 (0.42-0.66)

Figure 2. Forest plot of all included studies in the meta-analysis. Estimated OR in statin-users vs non-users for infectious/30-day/in-hospital mortality among patients diagnosed with a severe bacterial infection. Brackets denote 95% confidence intervals.
doi:10.1371/journal.pone.0010702.g002

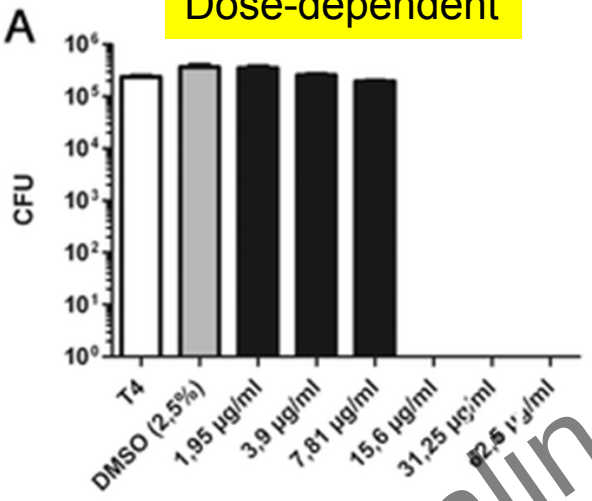


Large studies show null effect
Small studies show good effect

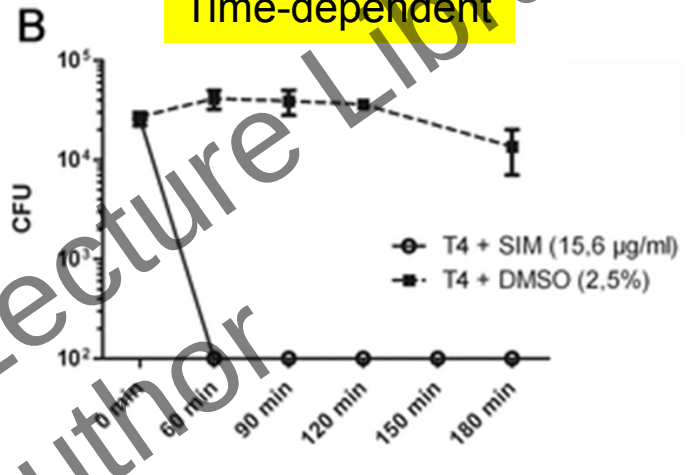
Bjorkhem-Bergman et al, PLoS One, 2010

Statins and pneumococci

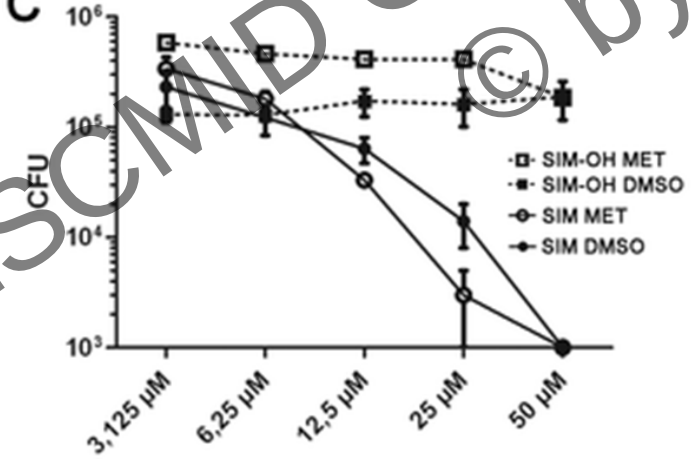
Dose-dependent



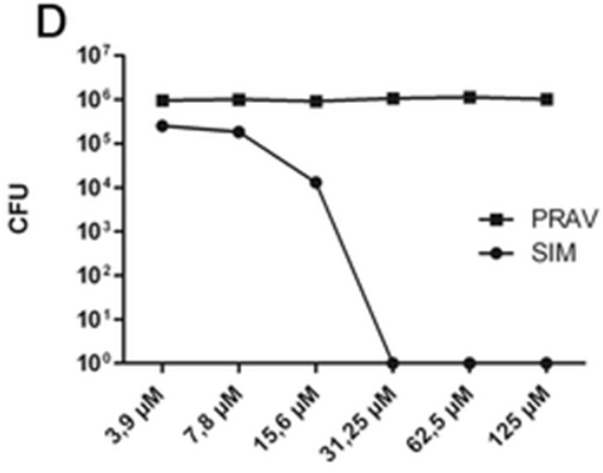
Time-dependent



Lactone form is active



Only SIM (lipid-soluble) is active

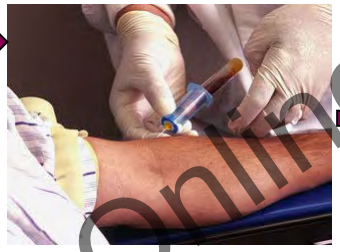


Bergman et al, PLoS One, 2011

How to test the hypothesis in a more physiological system?

Statins and whole blood killing assay

SIM 80 mg
FLU 40 mg
PCV 1 g



C_{max}=
2 hours
30 min

10^4 CFU *S. pneumoniae*

Mix

Incubation in BacTec

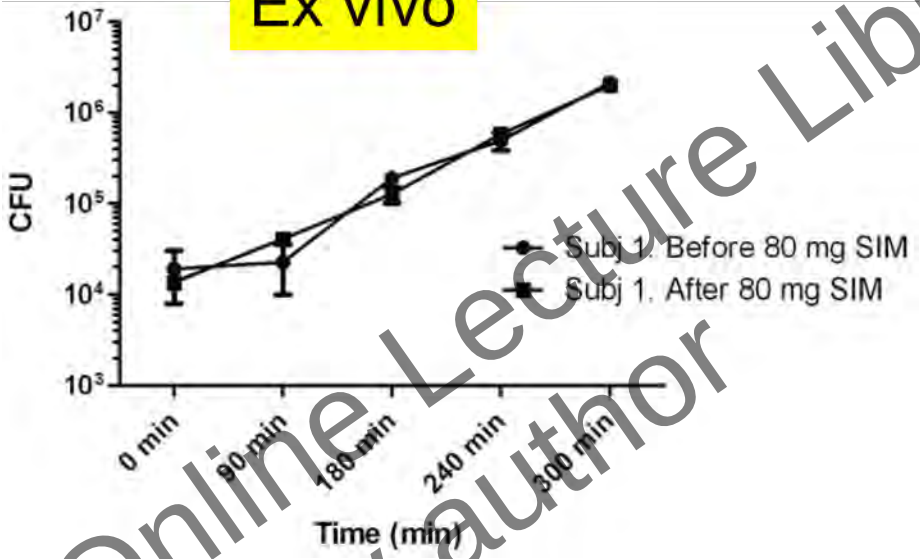
Wait for growth

Bergman et al, PLoS One, 2011

Whole blood killing assay

Results

Ex vivo



BactAlert

Table 1. Pneumococcal viability in whole blood as measured in a BactAlert system.

Subject no	1	2	3	4	5
Drug	PCV	FLU	SIM	SIM	SIM
Conc in plasma (Cmax, 2h)		**110.8** nmol/L	**6.6** nmol/L (SIM-OH) **15.9** nmol/L (SIM)	**13.4** nmol/L (SIM-OH) **32.4** nmol/L (SIM)	**4.1** nmol/L (SIM-OH) **9.9** nmol/L (SIM)
Time to detection (before)	374 min	374 min	389 min	374 min	389 min
Time to detection (after)	>7000 min	374 min	389 min	389 min	389 min

Bergman et al, PLoS One, 2011

What is a relevant dose of statins in cell-experiments claiming pleiotropic effects?

Table 1: Pharmacokinetic and experimental data

Statin	Dose	Cmax of statin in human serum	Mean concentration in human serum[1]	Protein binding	Statin conc in cell experiments[2] (time of incub.)
Simvastatin:	40 mg				
Simvastatin lactone (inactive prodrug)		19-31 nmol/L	2,3-4,3 nmol/L [10]	>95%	10 µM (ND)[2]
MW: 418,57 g/mol					
Simvastatin-OH (active metabolite)		6-7 nmol/L	1,6-1,9 nmol/L [10]		1 µM (30min) [4]
MW: 463,58 g/mol					
Atorvastatin	5 mg	3 nmol/L	4 nmol/L [11]		
MW: 558,64 g/mol	20 mg	40 nmol/L	15 nmol/L [11]	>98%	10 µM (48 h) [6]
Lovastatin (inactive prodrug)	80 mg	50 nmol/L	9,4 nmol/L [12]	>95%	1 µM (4 days) [5]
MW: 404,55 g/mol					

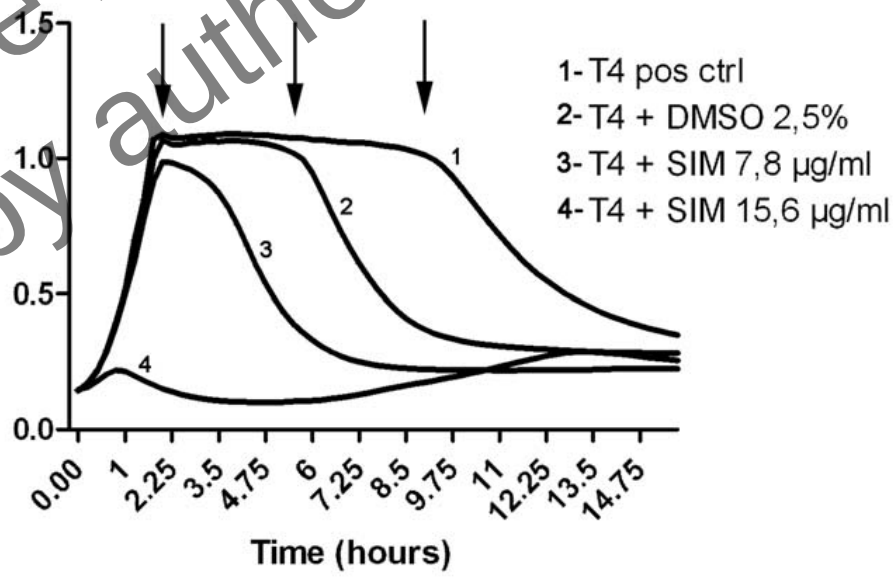
[1] Mean concentration is calculated from AUC values divided by 24 hours.

Is it reasonable to study 100-1000-fold higher concentrations *in vitro* versus concentrations present in humans?

Bjorkhem-Bergman et al, Br J Clin Pharm, 2011

A note on solubility…

- What is the best solvent for *in vitro* studies on statins?
  - → DMSO
  - → EtOH
  - → MetOH
  - → Other?



How do we control for effects of the solvent on the microbe?

Bergman et al, PLoS One, 2011

Blocking staphyloxanthin impairs virulence in *S. aureus*

Karolinska Institutet

Staphylococcus aureus golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity

George Y. Liu,[1] Anthony Essex,[2] John T. Buchanan,[1] Vivekanand Datta,[1] Hal M. Hoffman,[1,3,4] John F. Bastian,[4] Joshua Fierer,[3,5] and Victor Nizet[1,4]

[1]Department of Pediatrics, [2]Department of Cellular and Molecular Medicine, and [3]Department of Medicine, University of California San Diego, La Jolla, CA 92093
[4]Department of Pediatrics, Children's Hospital and Health Center, San Diego, CA 92123
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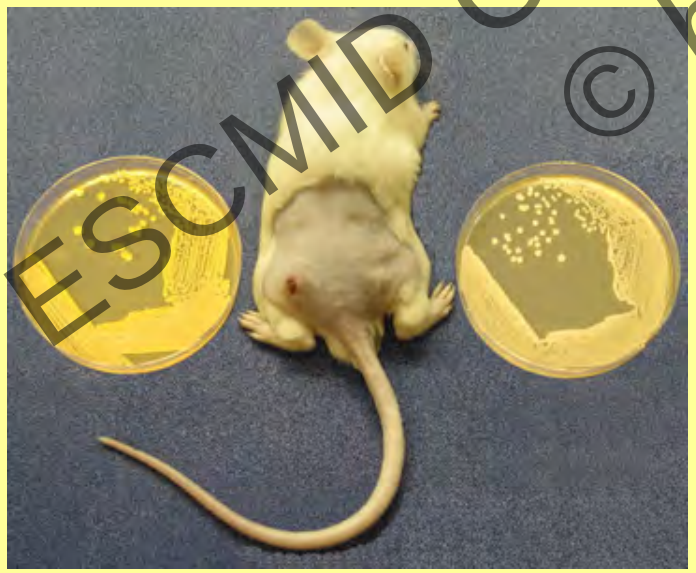
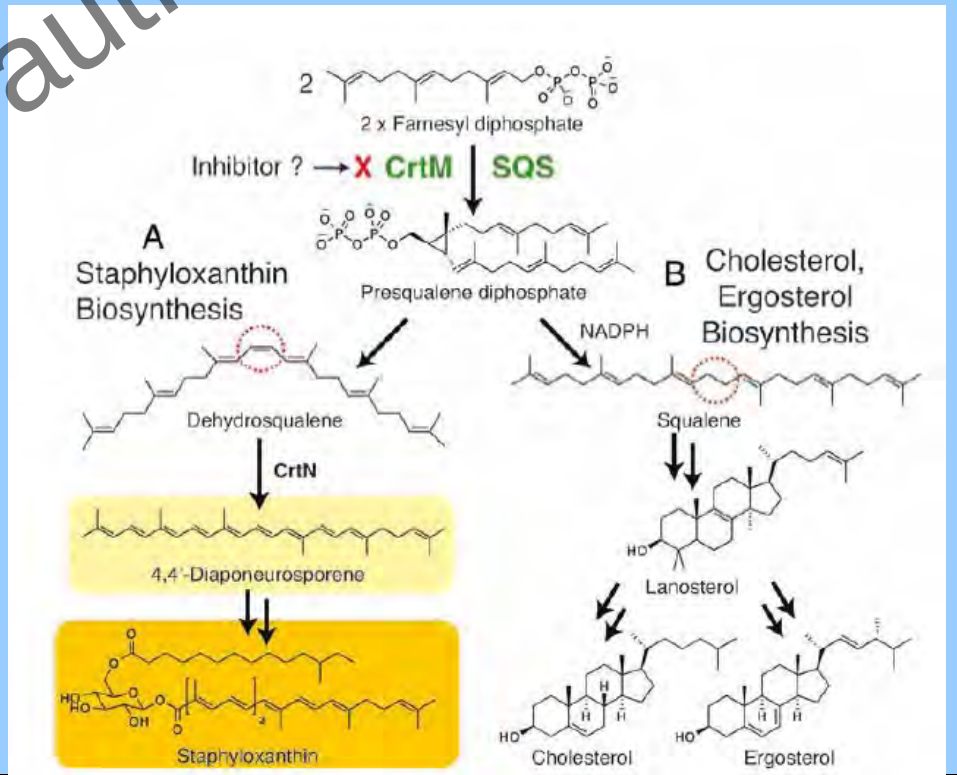


Liu GY et al, JEM, 2005, Liu CI et al, Science, 2008

A Cholesterol Biosynthesis Inhibitor Blocks *Staphylococcus aureus* Virulence

Chia-I Liu,[1,2,3]* George Y. Liu,[4]* Yongcheng Song,[5]* Fenglin Yin,[6] Mary E. Hensler,[7] Wen-Yih Jeng,[1,2] Victor Nizet,[7,8]† Andrew H.-J. Wang,[1,2,3]† Eric Oldfield[5,6]†



Statins and fungal infections - Future research

- Direct effect of statins against fungi? Yes, but too high and unphysiological concentrations
- Synergy with antifungal drugs? Yes, but unclear clinical relevance, possible problems with PK/PD-parameters
- Effects on fungal virulence factors? Possible, but yet to be shown
- Effects on host immunity? Yes, but yet to be proven in clinical trials

Retrospective clinical studies are few and mostly with null effect

Prospective trials are largely missing but are highly warranted

Acknowledgements

1. Statins and direct effects on pneumococci
2. Meta-analysis on statins and pneumonia
3. What is a relevant dose of statins in cell-experiments?

- Center for Infectious Medicine (CIM)
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- Clinical Pharmacology
  → Assoc. Prof Linda Björkhem-Bergman
  → Dr Jonatan Lindh
- Microbiology and Tumorbiology Center (MTC)
  → Prof. Birgitta Henriques-Normark
  → Prof. Staffan Normark

Karolinska Institutet

Thanks for your attention!

Questions?

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