Antifungal /Molds and Azole Resistance

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Conflicts: None

Impact of Early Diagnosis

- Aspergillus pneumonia

  ≤10 d: mortality 9/22 (41%)

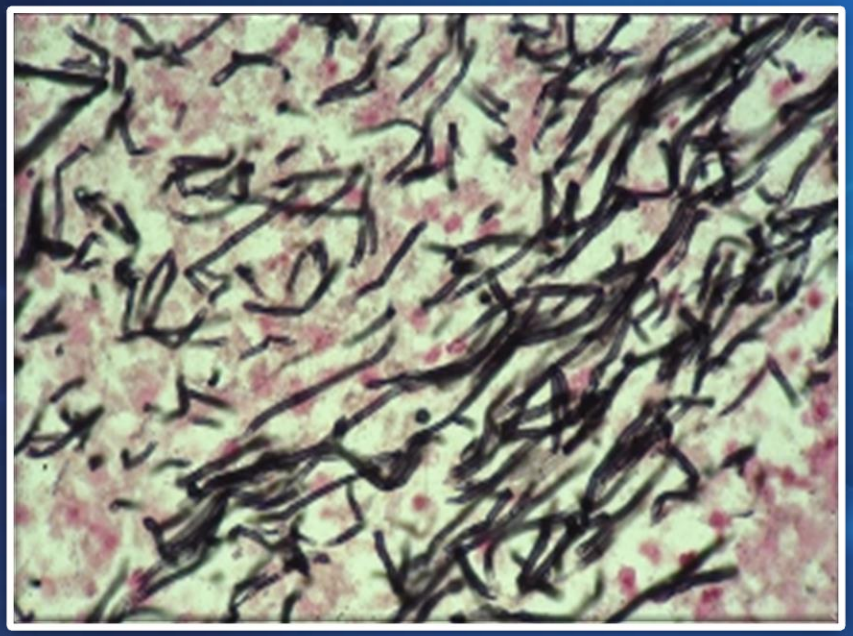
  >10d mortality 9/10 (90%)[t]
- "Systematic" CT scan (2d) 20% mortality

  "on indication" CT scan (7d) 58% mortality[Δ]
- 90-day survival rate for IA improved 2002-4 is 45% vs. 22% in previous years ($p < 0.00$)[□]
- In 2004-5; ~ 90% of IA left Duke hospital

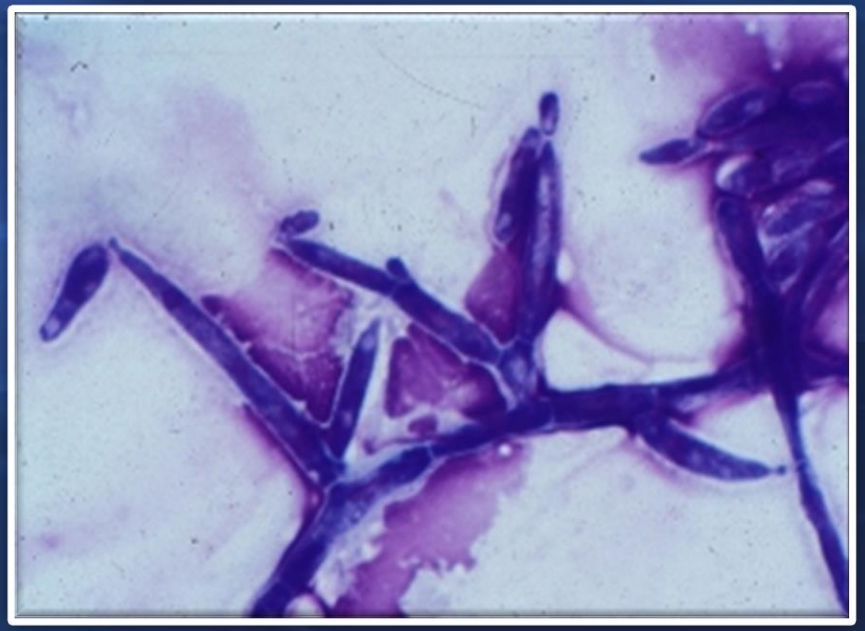
t von Eiff et al, Resp. 1995
Δ Caillot et al, J. Clin. Oncol. 1997
□ Upton et al Clin. Infec. Dis. 44:531-40, 2007

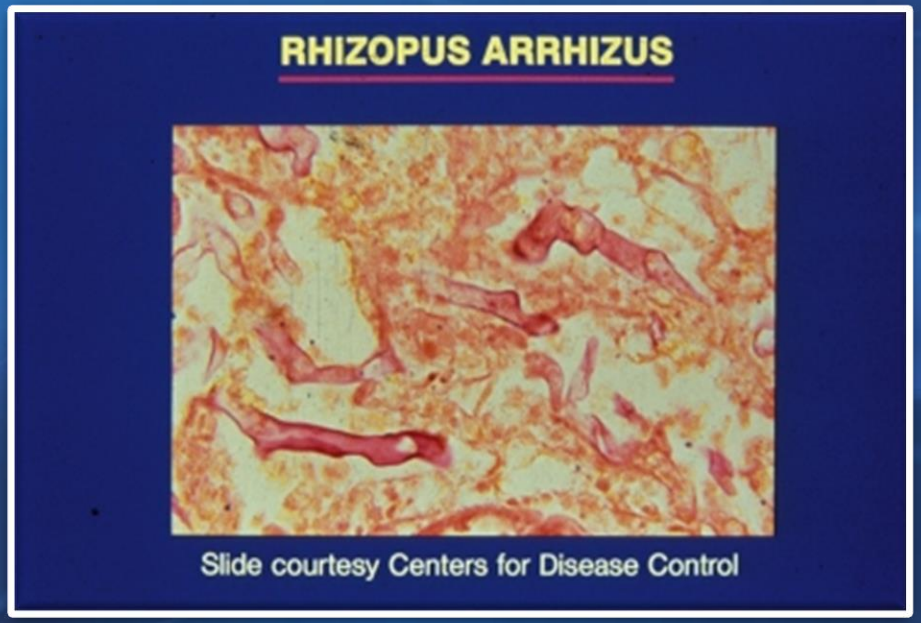
Diagnosis remains an issue

Fusarium, Aspergillus, Scedosporium



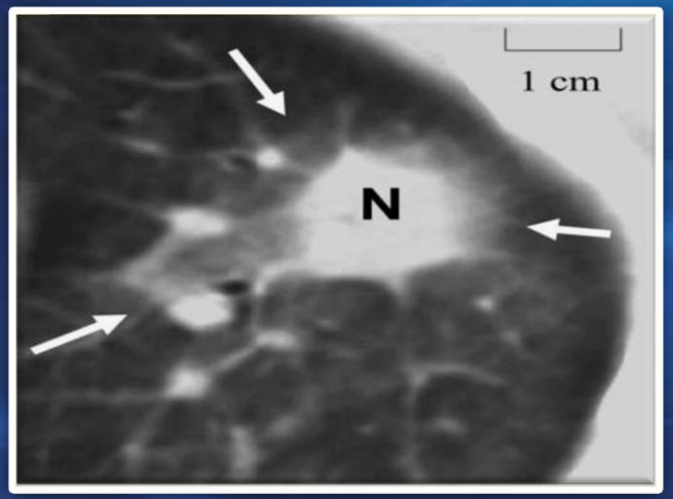
Fusarium (adventitial forms)





Computed Tomography (CT) for Aspergillosis

- At presentation[1]
  - = 1 macronodules (94%)
  - Halo sign (61%)
  - Consolidation (30%)
  - Infarct-shaped nodules (27%)
  - Cavitary lesion (20%)
  - Air crescent sign (10%)



- Histopath support for halo sign as aspergillosis[2] but can be caused by other pathogens
- Significantly better response to treatment with halo sign (success 52% vs 29%, $P<0.001$)[1]

1. Greene RE et al. *Clin Infect Dis*. 2007;44:373-379.
2. Caillot D et al. *J Clin Oncol*. 2001;19:253-259.

Imaging Methods for Invasive Mycoses: Cornerstone of Diagnosis (???)

- 18F – FDG PET scan
  - Fungal infections look like tumor in lung
  - Now infectious disease metastatic foci can be found ~ ½ patients after other radiographs negative[1]
- CT scan: 40 IPA patients (average 3 lesions; 90% increase in size and number with treatment until day 9; 42% radiographic remission at
  90 days with cavitation 2-3 x longer but better outcome)[2]
- Predictors of pulmonary zygomycosis vs aspergillosis (= 10 nodules and pleural infusions for zygo; no difference in halo sign, crescent sign, masses or cavities)[3]

1. Bleeker-Roners CP et al. *J Nucl Med*. 2005;46:2014-2019.
2. Brodoefel H et al. *AJR Am J Roentgenol.* 2006;187:404-413.
3. Chamilos G et al. *Clin Infect Dis*. 2005;41:60-66.

Galactomannan Antigen Testing: What Are the Issues?

- Evaluate risk
- Use 0.5 OD cut-off
- Serial testing in high-risk patients
- False-positive results with antibiotics, Plasma-Lyte, colonization with *Bifidobacterium*
- Sensitivity ~70%; specificity ~90%
- Decreased sensitivity with antimould prophylaxis
- BAL antigen[1] (sensitivity 76%; specificity 94%)
- Antigen testing for monitoring response to therapy

1. Sanguinetti M et al. *J Clin Microbiol*. 2003;41:3922-3925.

Galactomannan in Bronchoalveolar Lavage Fluid

	ICU[1]	Heme – Onc[2]	Heme – Onc[3]
#Pts	110	99	160
Sensitivity	88%	76%	85%
Specificity	87%	94%	100%
Comment	Better than serum 42% sensitivity	Similar to q PCR	CT-based BAL fluid

1. Meersseman W et al. *Am J Respir Crit Care Med*. 2008;177:27-34.
2. Becker MJ et al. *Br J Haematol*. 2003;121:448-457.
3. Musher B et al. *J Clin Microbiol*. 2004;42:5517-5522.

PCR Was Far More Sensitive Than Fungal Culture

Aspergillus Culture, qPCR, and *A. fumigatus* Resistance Mutation Detection in 4 Study Populations				
Laboratory Result	**ABPA**	**CPA**	**IPA**	**Normals**
Culture positive for *Aspergillus* spp.	0/19	7/42 (16.7%)	20/22 (90.9%)	0/11
Culture positive for *A. fumigatus*	0/19	7/42 (16.7%)	10/22 (45.5%)	0/11
qPCR positive for *Aspergillus* spp.	15/19 (78.9%)	30/42 (71.4%)	21/22 (95.5%)	4/11 (36.4%)
***A. fumigatus* CYP51A mutation detected directly from qPCR-positive sample**	6/8 (75%)	12/24 (50%)	NT	NT

qPCR= quantitative polymerase chain reaction; ABPA= allergic bronchopulmonary aspergillosis; CPA=chronic pulmonary aspergillosis; IPA= invasive pulmonary aspergillosis; NT=not tested.

Denning DW *et al*. *Clin Infect Dis.* 2011;52:1123-1129.

Nucleic Acid Testing for Moulds

- Histopathology (tissue)[1]: PCR 26/27 vs culture 17/27
- CT-guided percutaneous lung biopsy: PCR 100% sensitivity and 80% specificity[2]
- PCR of blood samples for aspergillosis: sensitivity 66% prior to treatment and 55% during treatment[3]
- Large review of 15 studies PCR of BAL: overall sensitivity 79% and specificity 94%[4]
- Pneumocystis (PCR – 100% sensitivity and 96% specificity in HIV patients) but must realize colonization factor (68%)[5]

1. Rickerts V et al. *Clin Infect Dis*. 2007;44:1078-1083.
2. Lass-Florl C et al. *Clin Infect Dis.* 2007;45:e101-104.
3. Lass-Florl C et al. *Mycoses.* 2005;1:12-17.
4. Tuon FF. *Rev Iberoam Microbiol*. 2007;24:89-94.
5. Davis JL et al. *Thorax.* 2008;63:329-334.

Seven Criteria to Justify Antifungal Prophylaxis

- **Safety-** (safety between different drug classes varies)
- **Prevalence-** (the 10% rule)
- **Cost-** (acquisition vs. illness cost)
- **Efficacy-** (evidence-based studies)
- **Consequence-** (what is the mortality benefit?)
- **Resistance-** (understanding the type and impact of resistance is important)
- **Diagnostic availability/tests-** (How accurate and how fast is your diagnostic strategies)

Adapted from: Perfect JR. *J Inv Fung Infect*. 2007;1:2.

Very High Risk for Mould Infections for Targeted Prophylaxis

Populations:

- Relapse/refractory AML
- Cord blood recipients
- Recipients of T-cell depleted grafts receiving post transplant immunosuppression
- Recipients of mismatched grafts
- GVHD (receiving Prednisone 1/mg/kg/d for prolonged periods)

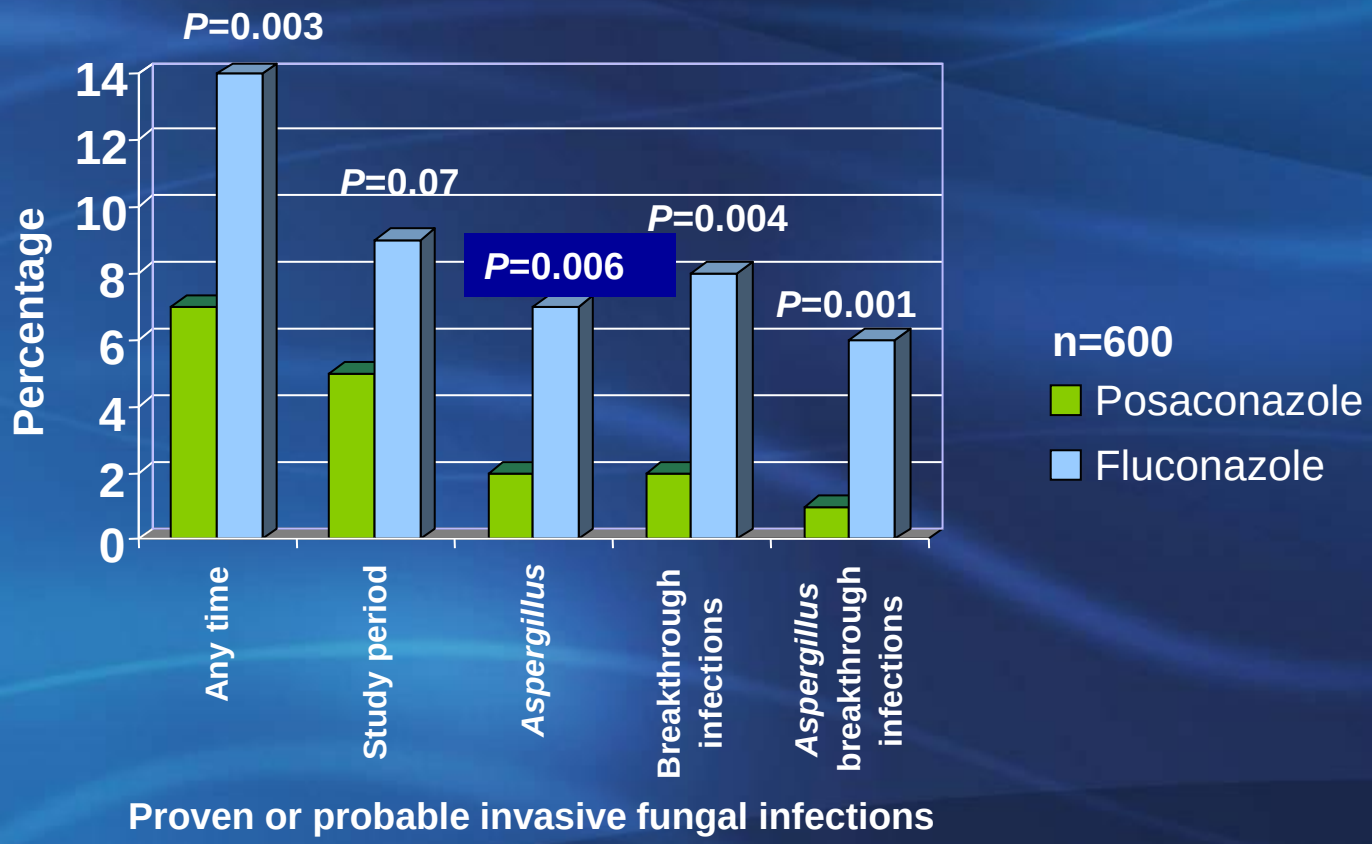
Antifungal Prophylaxis (Risk groups)

- Prolonged neutropenia with mucositis
  Induction therapy for AML/MDS ± ALL
- Bone Marrow Transplants: allogenic (neutropenia and GVHD)
  autologous (less risk)
- AIDS
  1) Primary prophylaxis for PCP
  2) Secondary prophylaxis (limited with HAART)
- Neonatal
- Solid organ transplants

1) Kidney, Hearts	No
2) Liver	Sometimes
3) Lung/Pancreas/small bowel	Yes

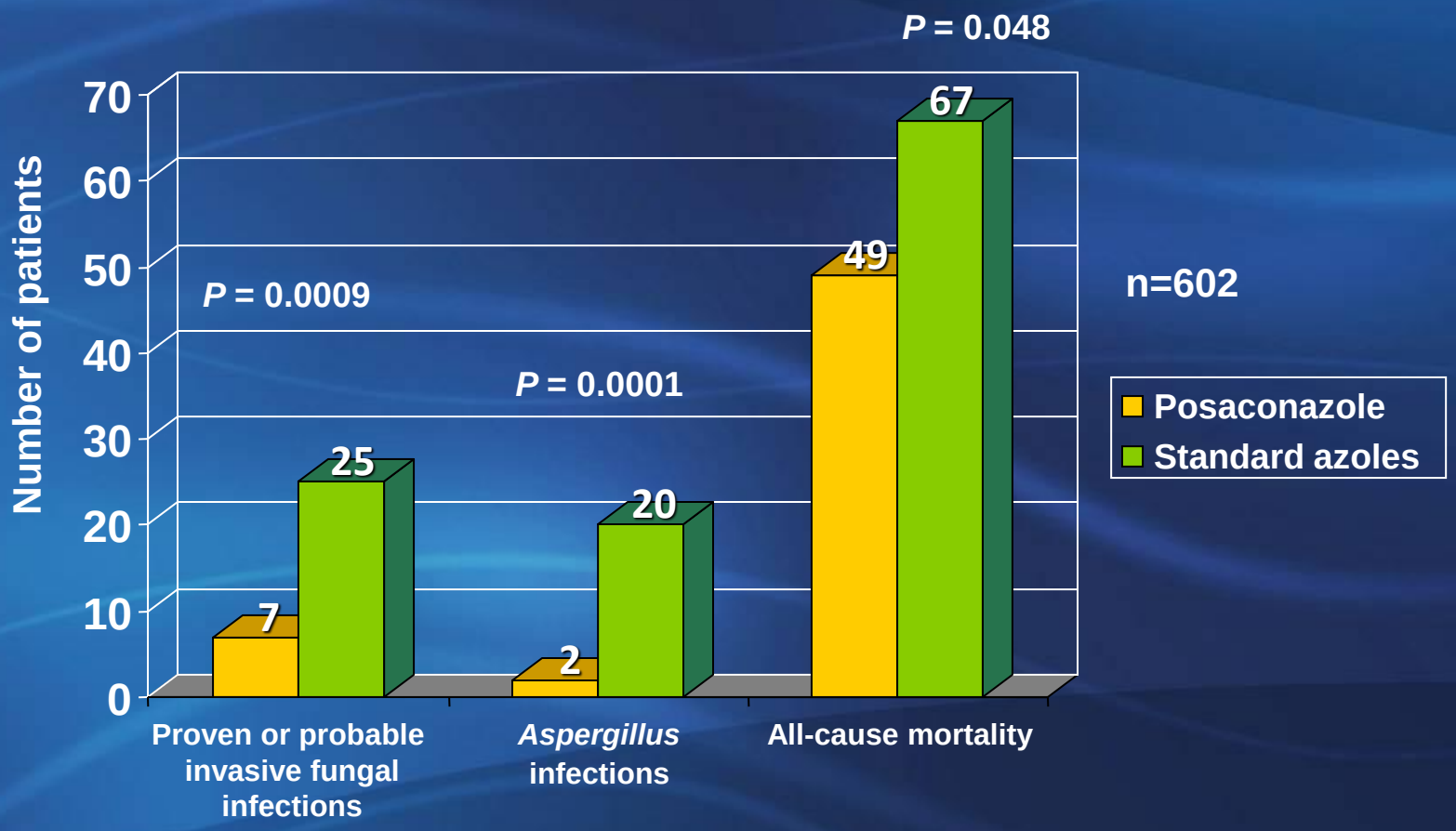
- ICU

Antifungal Prophylaxis in Allogeneic HSCT Recipients with GvHD



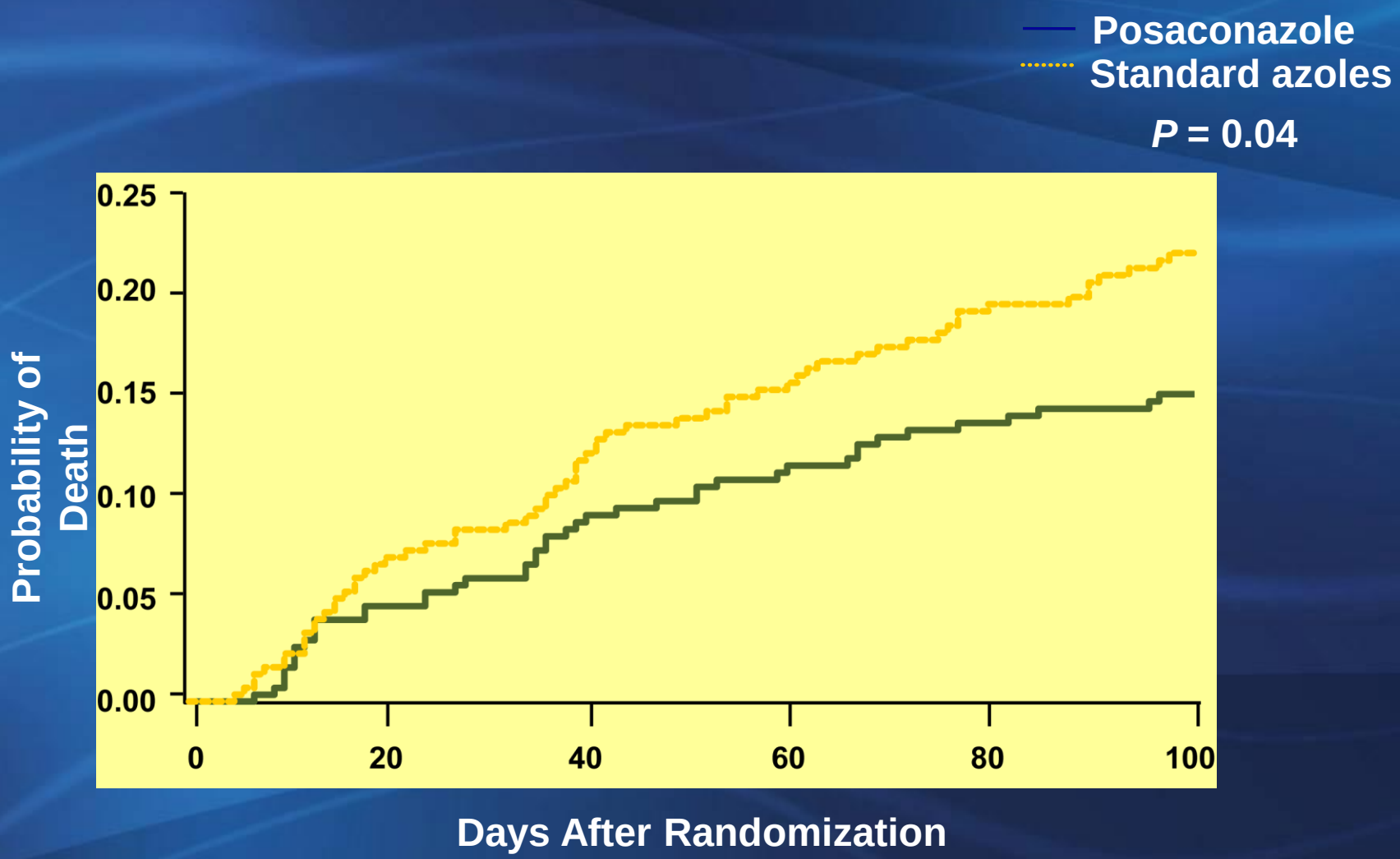
Ullmann AJ, et al. N. Engl. J. Med 356-347, 2007].

Antifungal Prophylaxis in Neutropenic Patients with AML or MDS



Cornely OA, et al. N. Engl. J. Med 356:348-359, 2007

Prophylaxis in AML/MDS Patients: Evidence for Long-term Survival



Cornely OA et al. *N Engl J Med.* 2007;356:348-359.

Posaconazole vs. Fluconazole Prophylaxis in Heme-onc patients at Duke*

- A look back to how we are doing.
- 2004-2010 65 patients in both groups (POSA vs. FLU) with AML/MDS (match except more Ara-C re-induction in posa group)
- Invasive Fungal Disease (IFD)

(a) posa 6/65 (9.29%) vs FLU 17/65 (27%) p=0012

(b) definitive/probable posa 0% vs. fluconazole (10.5%)

(c) Persistent fever on abxs / more in fluconazole group (P<0.001)

- Multivariant logistic regression model (neutropenia and fluconazole use (p<0.01) were associate with IFD.
- 100d mortality was the same in both groups.

*Kung et al Cun. Med. 3.667-673, 2014

Aerosolized Lipid Products of Amphotericin B as prophylaxis

- Aerosolized ABLC+ fluconazole in HSCT (40pts) – no fungal pulmonary infections; one mould infection. Δ
- Aerosolized Ambisome (271 neutropenic pts randomized – to placebo)

  Reduced IA from 14% to 4% ($p<0.01$)*

Δ Alexander et al Transplantation, 2006

* Rijnders et al CID, 2008

Aerosolized ABLC for Prophylaxis in Lung Transplants

- Open Trial (51 patients) < 5% toxicity (Lung transplants)

  no lung infections, 2 anastomosis infection, 4 extrapulmonary infections (Transplant. 72:545, 2001)

- Randomized, Blinded ABLC vs. AmB (100 patients) (Lung) AEs
  13.7% vs. 28.6% (p=0.03)

  Failures 11.8% vs. 14.3% (Transplant. 77:232,2004)

- Routine use: (rare) Pulmonary IFI in post-operative period for at least five years.

Aerosolized LAMB vs. Placebo Prophylaxis
Rijnders, BJ et al Clin. Infect. Dis 46:1401-8,2008

- **Patients with expected neutropenia <300 PMN/mm^3 for 10 days.**
  12 mg LAMB/placebo inhalation 2 days/week until PMN >300
- **Primary endpoint definite/probable IA: all patients by ITT with ≥1 inhalation**
- **Secondary endpoint: Modified EORTC/MSG): nodule with halo = prob IA and IA related mortality**

	Placebo	LAMB	P
Number	132	139	--
Prov/Prob IA	18	6	0.003
Modified EORTC Proven/ Prob IA	23	11	0.007
IA mortality	6	5	(not powered)

Prophylaxis with LAMB clearly superior to Placebo
There are open study data suggesting safety and efficacy also for ABLC
There are preclinical studies with other vehicles for amphotericin B suggesting efficacy and little toxicity

Areas at Duke we uniquely use Antifungal Prophylaxis

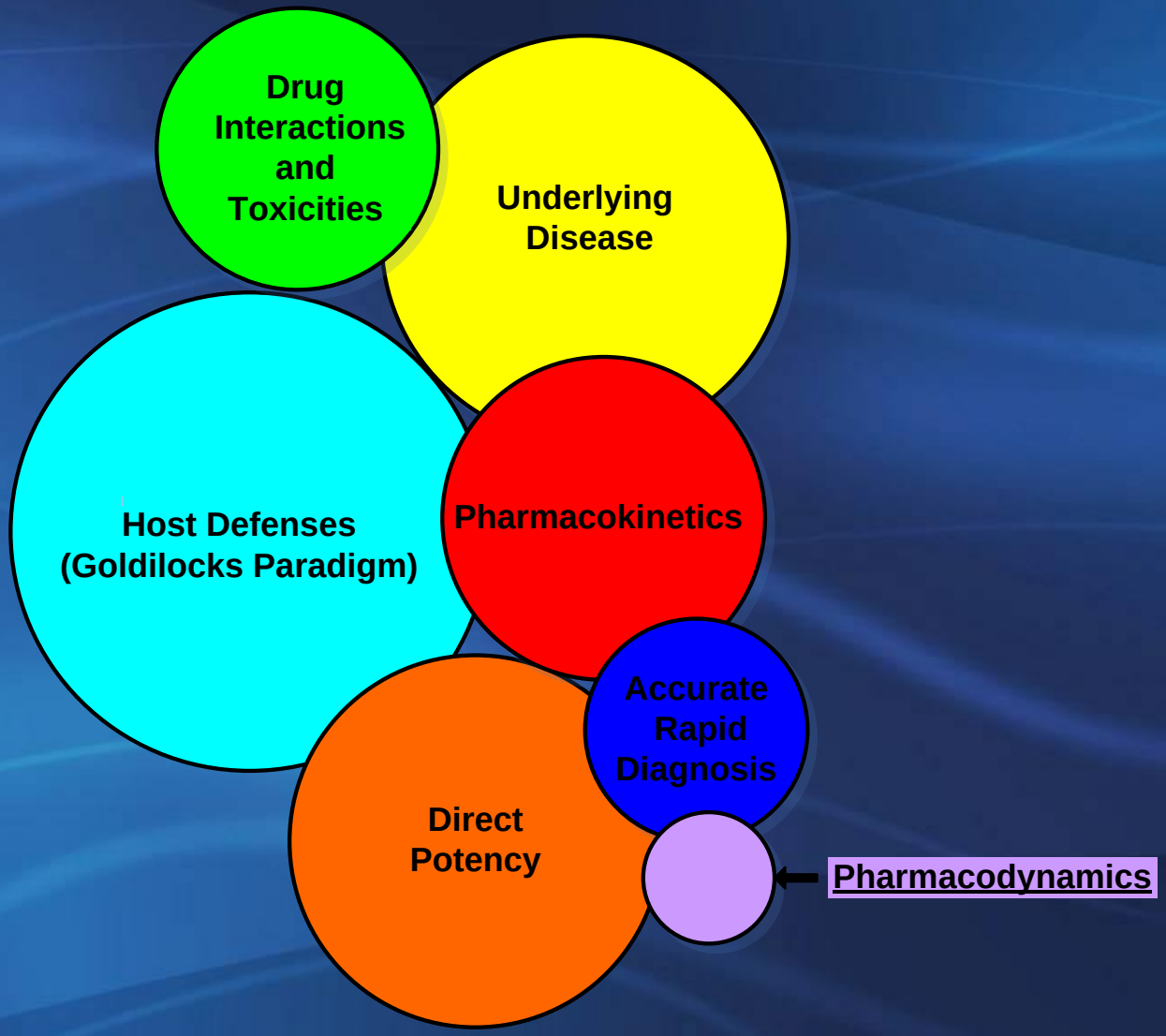
- All patients on ECMO (extracorporeal membrane oxygenation) receive fluconazole
- All patients receiving Aleutuzumab (Campath-anti-CD52)- receive Posaconazole
- With Aerosolized ABLC in lung transplants we add fluconazole

Genetic Susceptibility for Aspergillosis and Candidiasis

- Prediction of risk for prophylaxis and pre-emptive strategies
- Progress is being made:
  - Aspergillosis
    - Plasminogen allele influences susceptibility to invasive aspergillosis in mice and humans[1]
    - Toll-like receptor 4 polymorphisms (TLRs)[2]
  - Candidiasis
    - Dectin1/CARD9,[3] CASPASE-12,[4] cytokine genes,[5] (TLRs)[6]

1. Zaas AK et al. *PloS Genet*. 2008;4:e1000101.
2. Bochud PY et al. *N Engl J Med*. 2008;359:1766-1777.
3. Rosentul DC et al. *J Infect Dis*. 2011;204:1138-1145.
4. Rosentul DC et al. *Eur J Clin Microbiol Infect Dis*. 2012;31:277-280.
5. Johnson MD et al. *Clin Infect Dis*. 2012;54:502-510.
6. Plantiga TS et al. *J Infect Dis.* 2012;205:934-943.

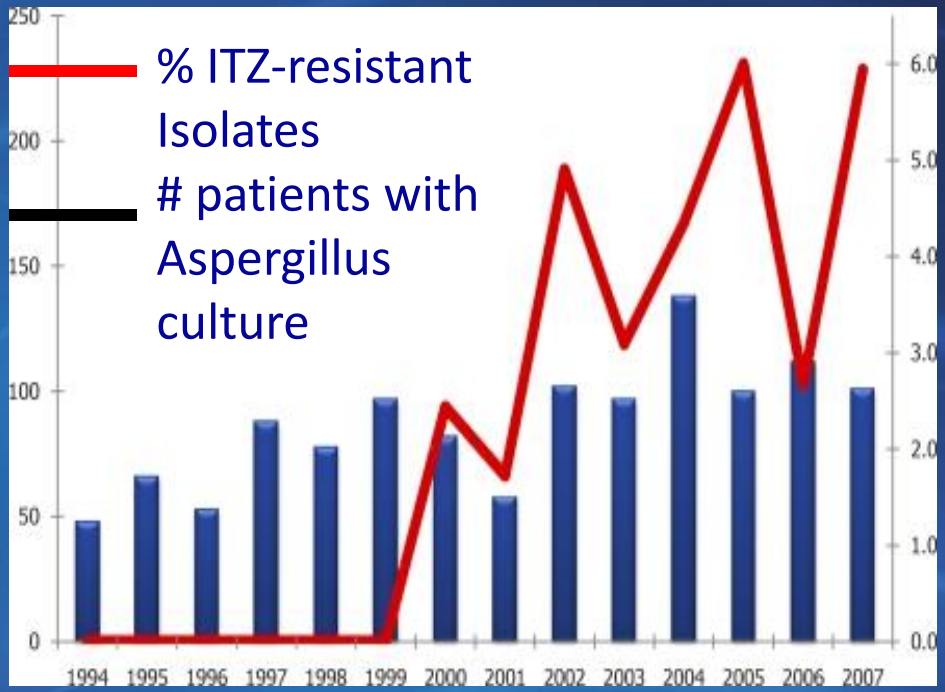
Rings of Antifungal Resistance



Azole Resistance in Aspergillus

- 1. Mechanisms: (1) Alterations in Cyp 51 proteins such as TR34/L98H; (2) over expression of target enzyme; (3) up–regulation of effux pumps; other(s)
- 2. In Europe ~ 7-10% of Aspergillus isolates are resistant
- 3. MICs Vori=2mcg/ml; Itra=4mcg/ml; posaconazole 0.5mcg/ml- not responding in 4-7days

Itraconazole (ITZ) Resistance in A. fumigatus Isolates

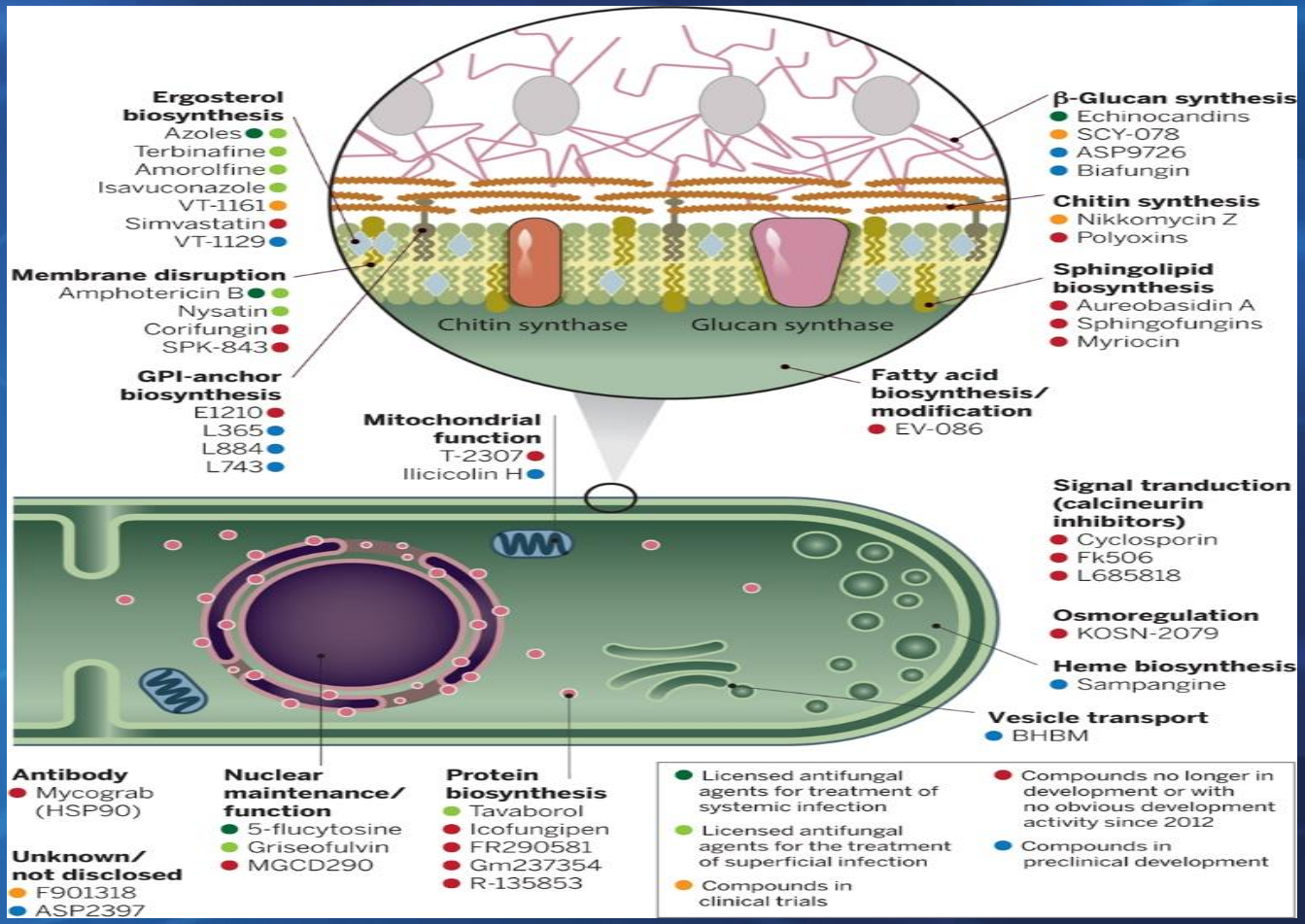


- ITZ-resistant isolates were found in 32 of 1,219 patients with invasive aspergillosis over a 14-year period
- ITZ-resistant isolates also showed elevated minimum inhibitory concentrations of voriconazole, ravuconazole, and posaconazole
- Recent USA (Transnet Data) 96% azole susceptible with *A.calidoustus* most resistant*

Snelders E et al. PLoS Med. 2008 Nov 11;5(11):e219

* Baddley et al J. Clin. Microb. 47: 3271-75, 2009

The Landscape of Antifungal Targets/Drugs



Denning and Bromley. Science 347: 1414-16, 2015

Three pivotal studies in Aspergillosis

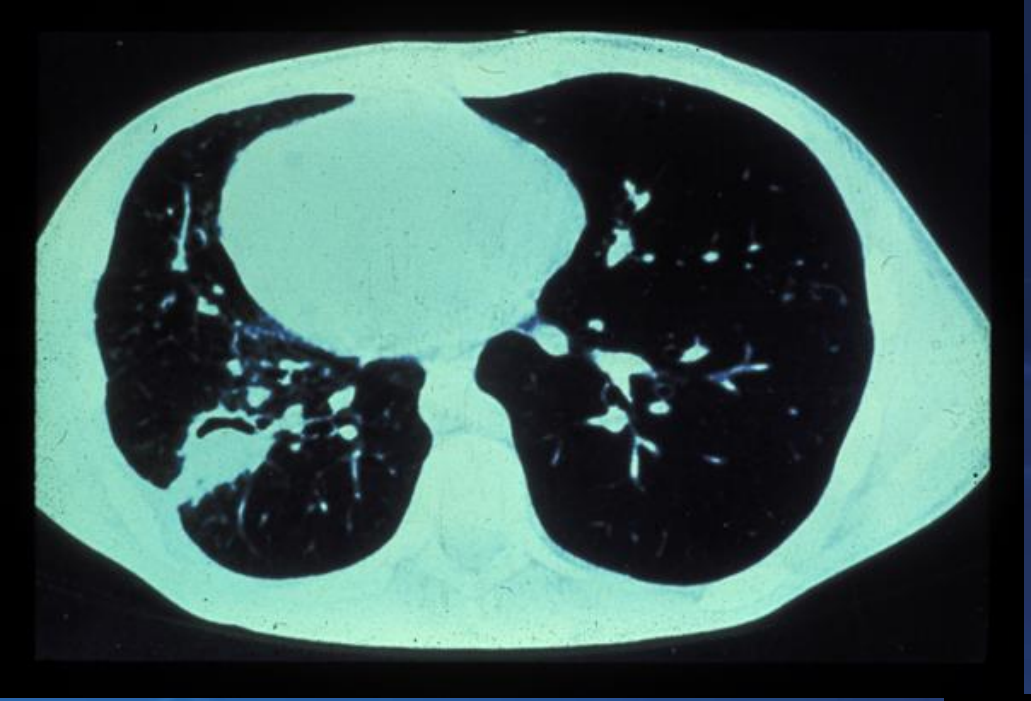
Complete/Partial Success		Survival (42d)	Side effects	Ref
Voriconazole vs AmB	52.8% vs 31.6%	70.8% vs 57.9%	13.4% vs 24.3%	Herbercht NEJM, 2002
Isavuconazole vs Voriconazole	50% vs 47%	81% vs 80%	42% vs 60%	Maertens, Lancet, 2015
Posaconazole vs Voriconazole	45% vs 45%	85% vs 79%	10% vs 40%	Maertens, Lancet, 2021

Combination therapy for Invasive Aspergillosis

- Preliminary Study (47pts) Voriconazole alone vs Voriconazole/Caspofungin: Combination with improved survival rate (p=0.048)

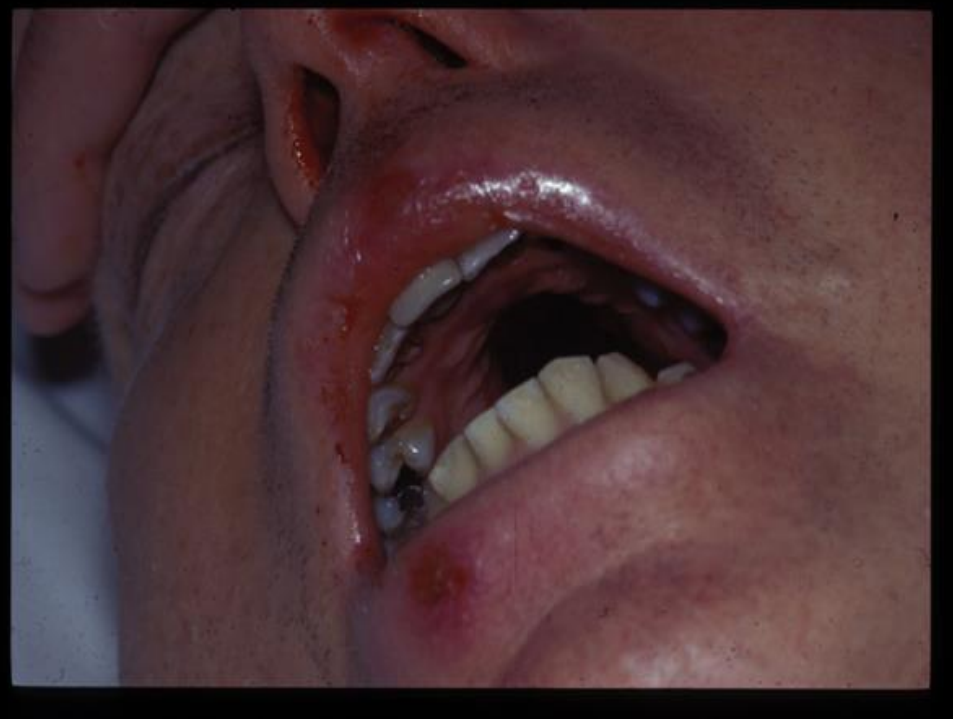
  Marr et al CID, 2004
- 277 pts. Mortality rate: combination (Voriconazole/anidulafungin) (19.3%) vs voriconazole (27.3%) p=0.087
- In diagnosis established by radiograph and GM values secondary endpoint with combination therapy (15.7%) vs monotherapy (27.3%) p=0.037

Marr et al Ann. Intern. Med., 2015



Aspergillosis

- A. fumigatus
- A. flavus
- A. terreus
- A. utus
- A. lentulus
- A. versicolor
- A. niger



Ibrutinib

- Bruton Thymidine Kinase inhibitor (potent impact on B-cells)
- CNS lymphoma – 40% rapid and CNS Aspergillosis*
- Confirmation in other cancer centers
- Duke reported rapid Cryptococcal meningitist
- Mice KO BTK- more susceptible to fungal disease
- Disease can vary with fungal strain
- Effect found to not only be B-cells but specifically to PMNs in CNS
- BTK SNPs associated with high risk of aspergillosis in HSCT

* Lionakis et al Cancer Cell, 2017

[t] Messina et al OFID, 2017

Clinical Points to Remember

- Specific targets to whole cell lineages (Aleutuzumab-anti CD52-liquid AIDS)
- These specific immune modulators are frequently used with other immunodepressants
- Site selectivity for infection is real
- Rapidity of infection can be impressive
- Must know consequences of outstanding therapies for serious underlying diseases

Azoles are safe BUT "the voriconazole story"

- Voriconazole a Post Marketing database (178 pts)*
- Liver function test abnormalities (25%)
- Visual (18%)
- Skin rash (17%)
- Neurological (14%) agitations, dizziness, confusion, anxiety, tremor
- Cardiovasular (10%) 5/22 QT prolongation/ torsades de pointes
- * drug interactions (VT 1161 coming)

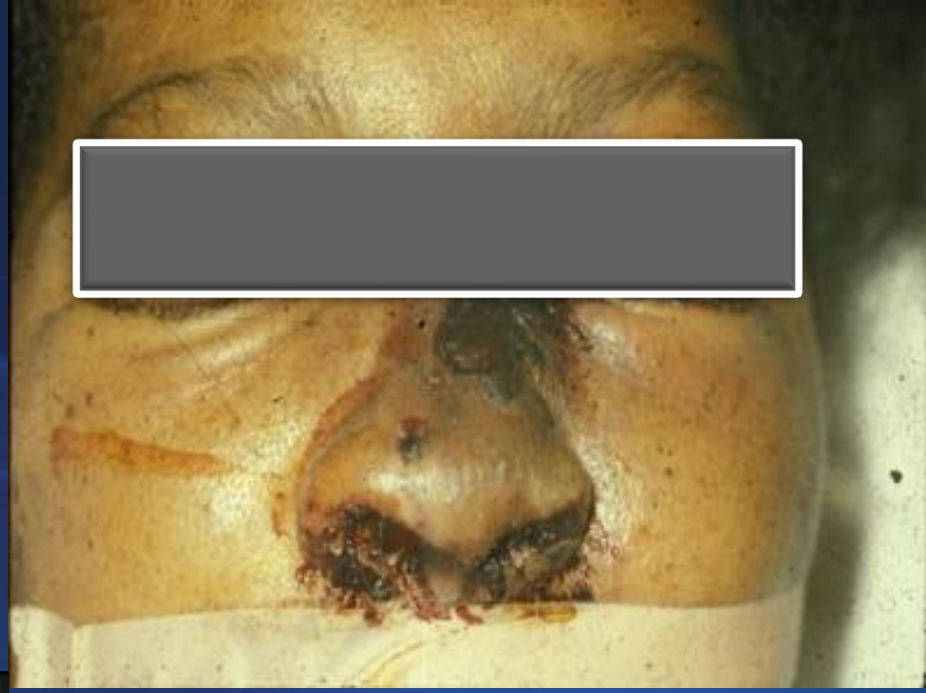
Eiden etal Ann Pharmacol. 41:755-63, 2007

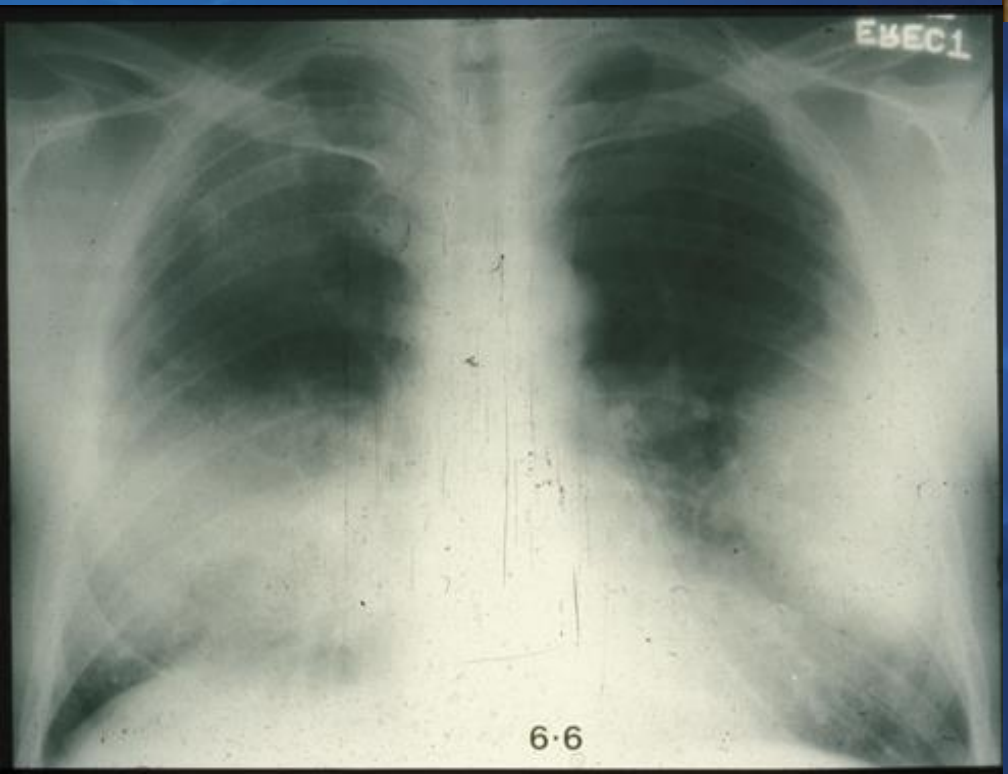
Unique problems with voriconazole

- Neuromuscular disorder (painful joints, numbness, weakness) in Lung transplants[1]
- Neuropathy[2]
- Squamous cell ca in transplants (high sun exposure; > 6 months of exposure)[3]
- Melanoma (5 cases) [4]

[1] Boussaud et al J. Heart Lung Transplant 27:229-32,2008
[2] Aksoy et al Chemother. 54: 224-7, 2008
[3] Vednerkar et al J. Heart Lung Transplant., 2010
[4] Miller et al Arch. Dermatol. 146: 300-4, 2010

Mucormycosis





Diabetes

Cancer

Transplant

Voriconazole use

Mucormycosis
Three Part Strategy

- Control underlying disease
- Surgical debridement
- (1) Amphotericin B lipid product (5mg/kg/d)

  (2) Alternative therapy (Isavuconazole and Posaconazole)

  support from *in vitro* and animal experiments

Things to consider with Mucormycosis

- Echinocandin plus lipid AmB increase activity in mice [Δ] ; in clinic 6 patients with success for combination[+]
- AmBisome > ABLC in mice [□]
- Iron – chelation (Deferasirox may be effective) [°*]
- HBO-Treatment

Δ Ibrahin, et a l AAC 2008
□ Ibrahim, et al AAC, 2008
° Reed et al AAC 50: 3968-9, 2006
+ Reed et a; Clin. Infect. Dis 47: 364, 2008
* Spellberg et al, 2011

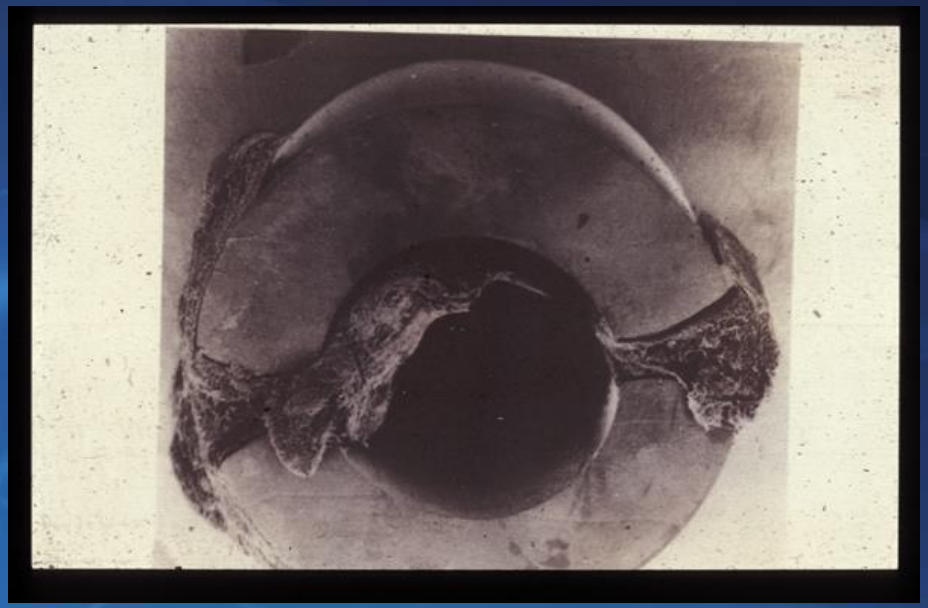
Isavuconazole story

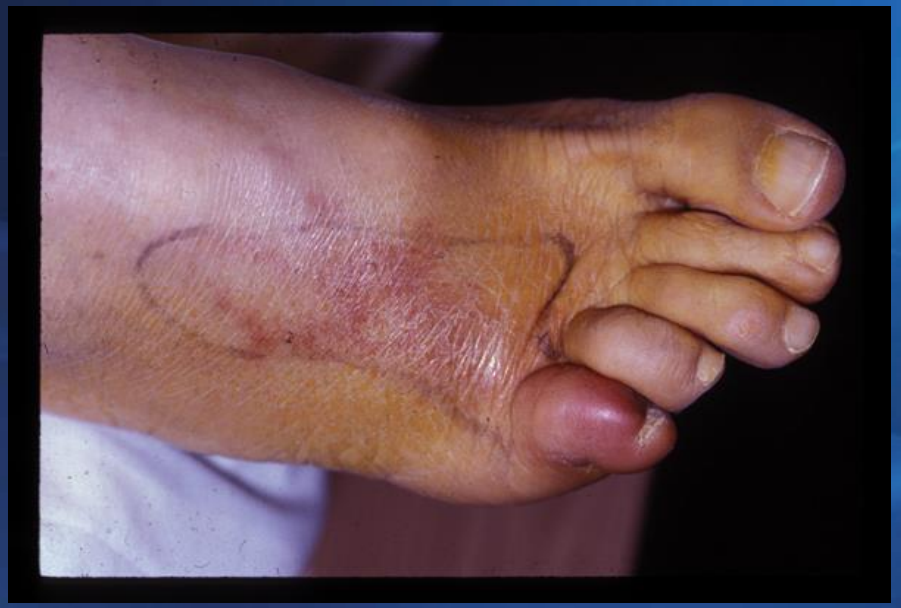
(open trial and compared to a cohort Fungiscope registry)*
42 days outcome 37 patients
(11% partial response)
(43% stable disease)

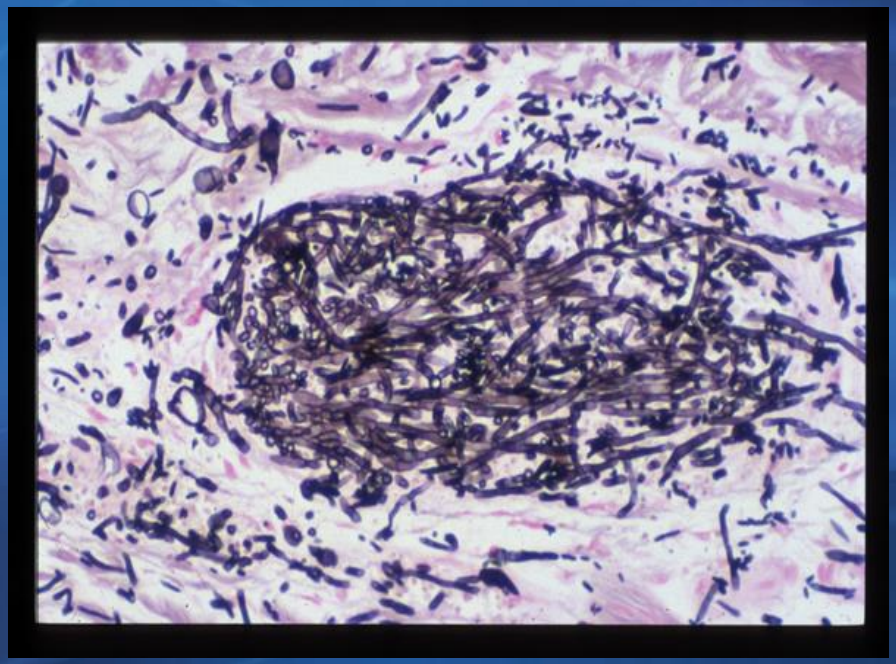
Mortality	Isa	vs.	AmB
	35%		39%

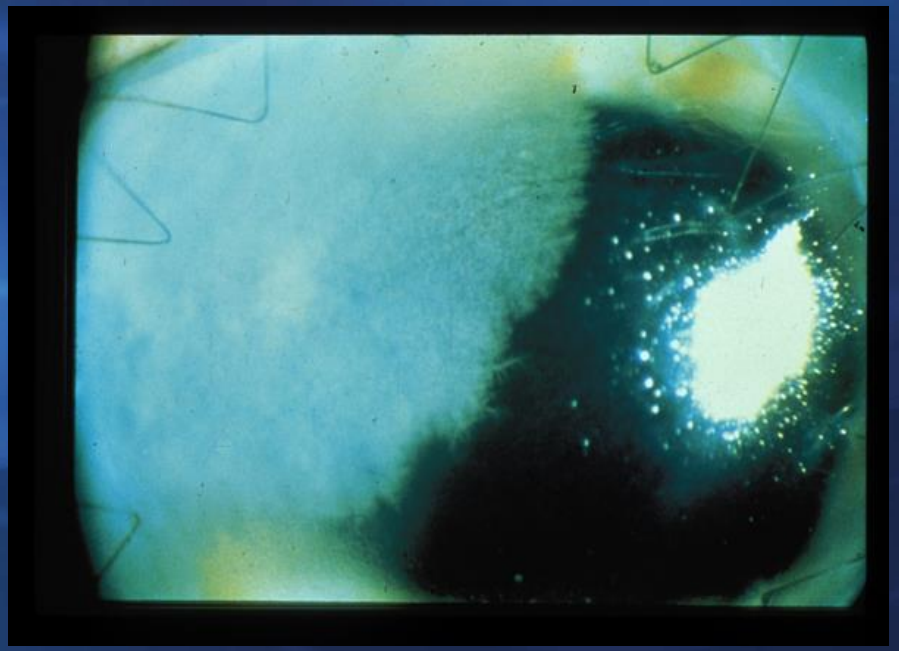
* Marty et al. Lancet Inf. Dis. 16: 828-37, 2016

Fusariosis (Local to disseminated)

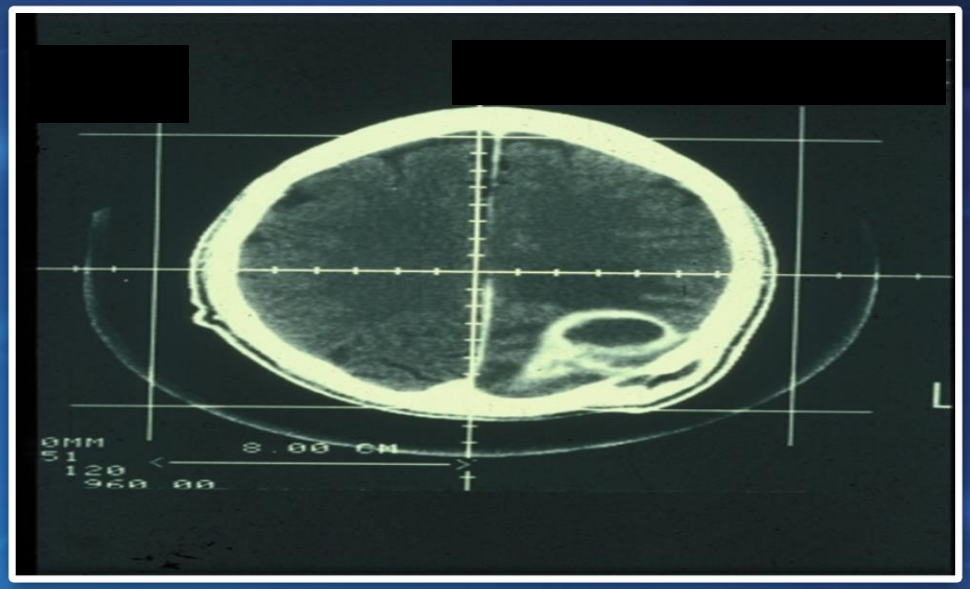


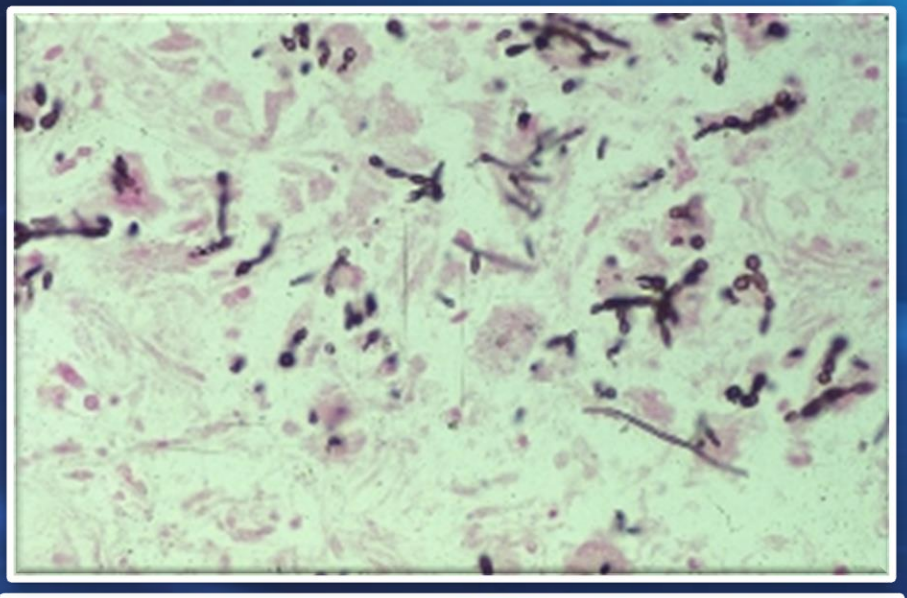


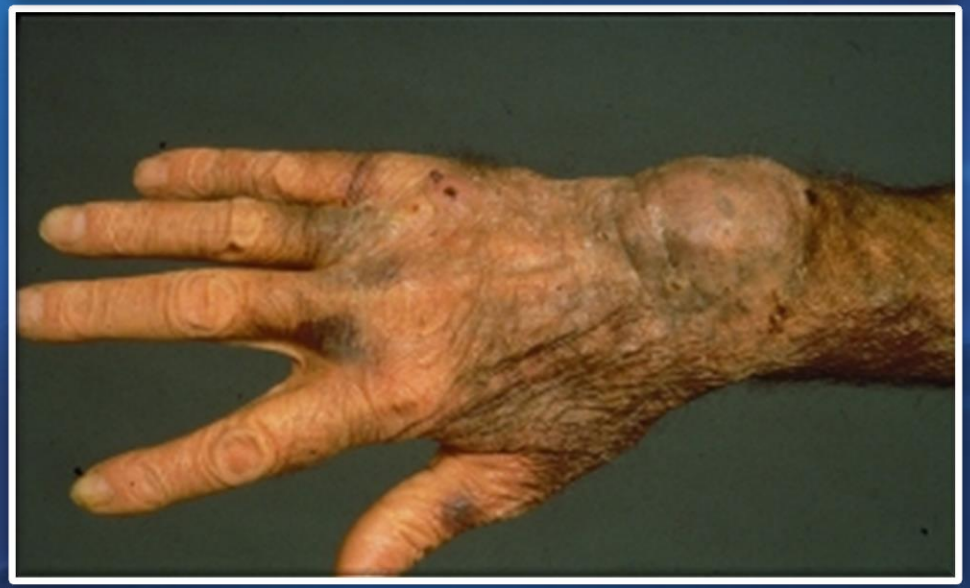


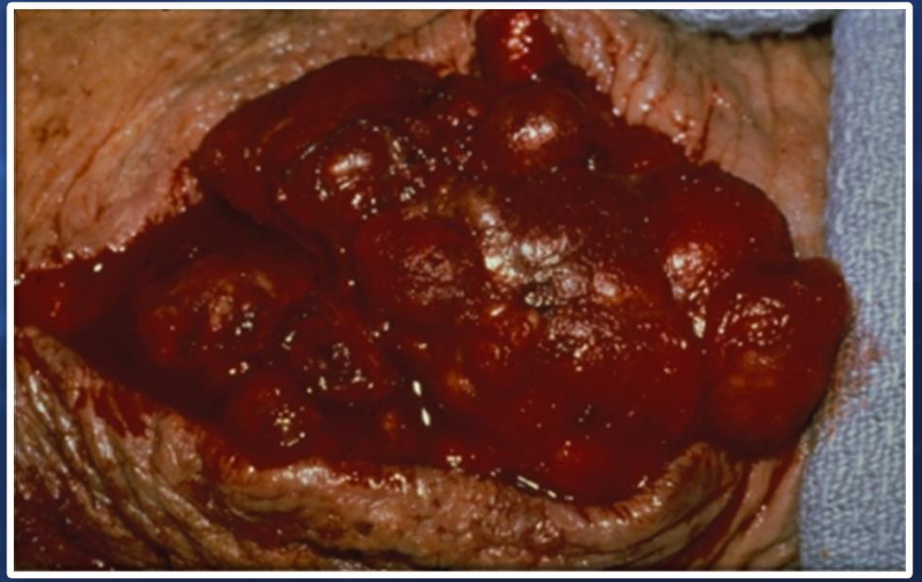


Black Moulds (Phaeohyphomycosis) (from skin to brain)







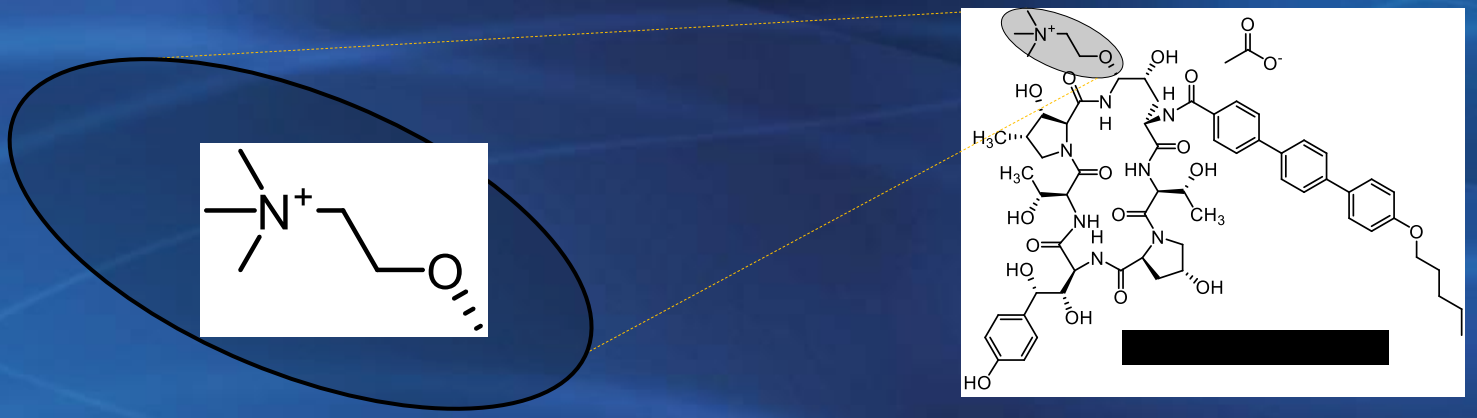


Phaeohyphomycosis

- Extended-spectrum triazoles (voriconazole/posaconazole) – excellent activity; Itraconazole also can be used
- Surgery is a major factor
- Wangiella meningitis (contaminated steroids) ¾ success with voriconazole
- Voriconazole (11/11 successes)*
- Contaminated steroids with Exserohilum rostratum (> 500 cases)
- Outcome: Voriconazole success over 80% in 12 weeks

Perfect et al. Clin. Infect. Dis., 2004 and case reports Incredible story is outcome with pretty good with about 12 weeks of therapy

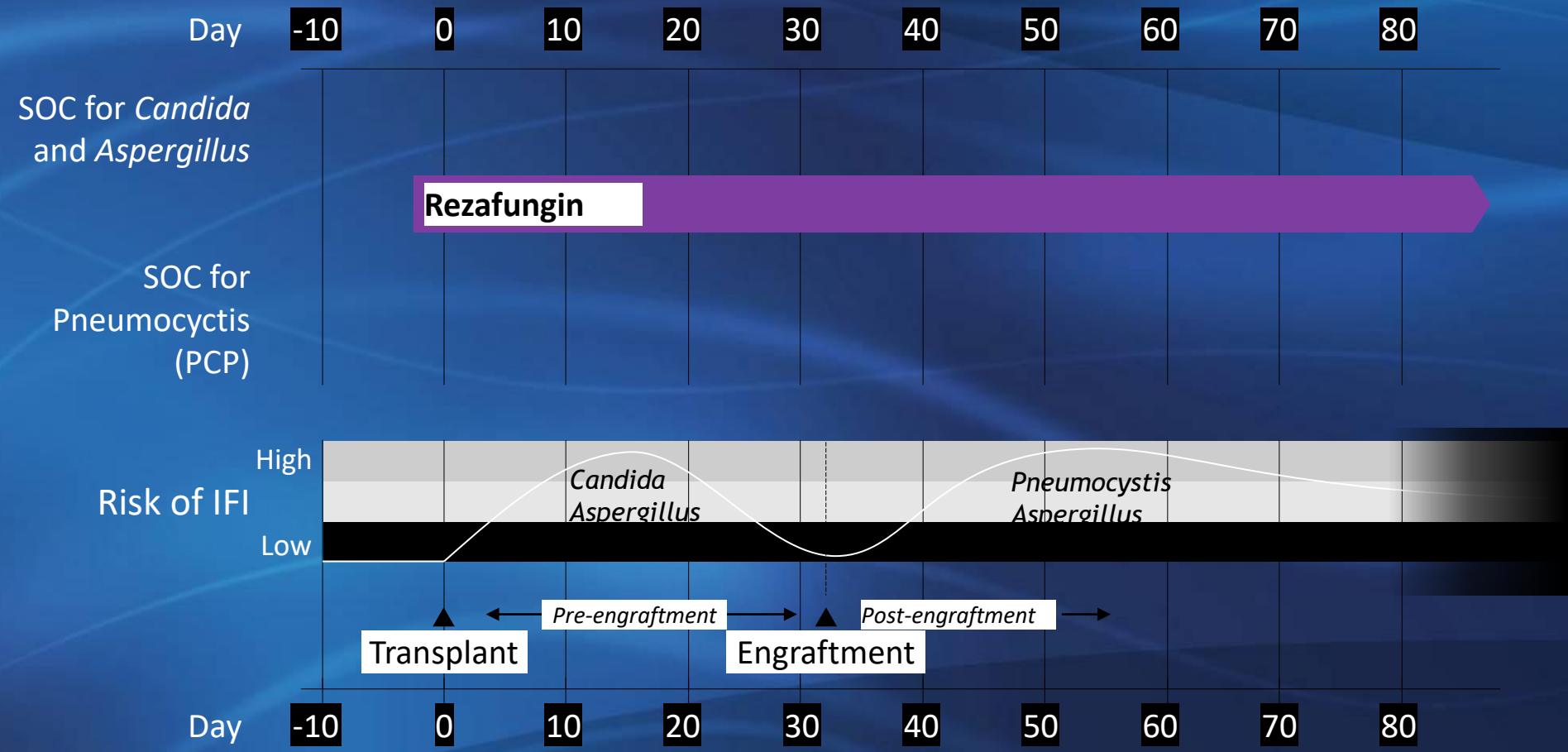
Rezafungin: A Better Echinocandin. A Better Antifungal by Cidara



Structural modification yields improved chemical & biological properties

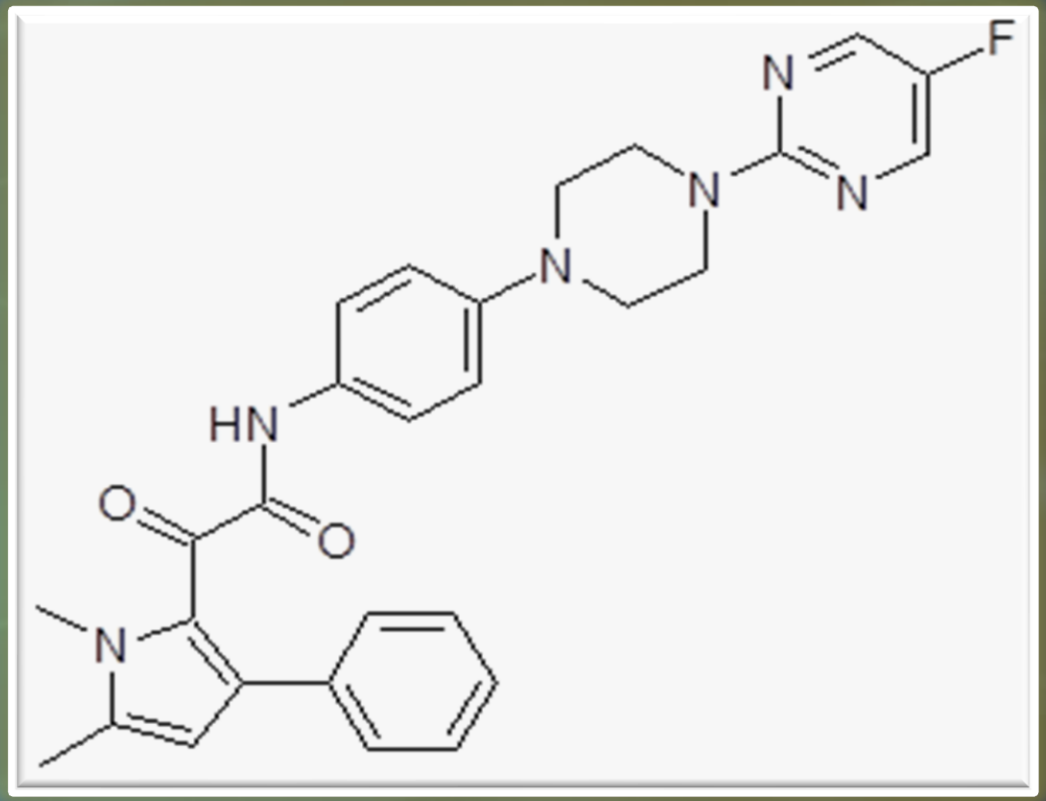
• Designed for prolonged PK	once weekly dosing in clinical studies
• Designed for high exposures	potential for improved efficacy vs *Candida* and *Aspergillus* infections
• Eliminates toxic degradation products	potential for improved safety
• Enables multiple formulations	intravenous; subcutaneous under development

Rezafungin: Potential for Simplified Single Drug Paradigm for Prophylaxis



F901318 structure (olorofim) by F2G

F2G



MW = 499

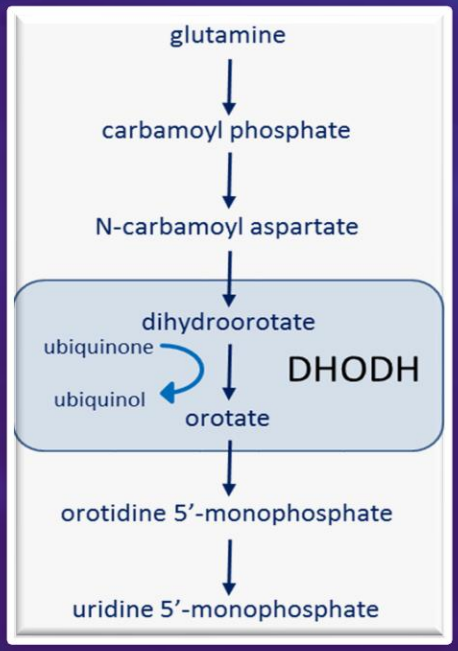
Formula = $C_{28}H_{27}FN_6O_2$

Robust low cost multi kg GMP scale manufacture

Orotomides

Orotomide Mechanism of Action

- F901318 is a potent inhibitor of *A. fumigatus* DHODH
  - DHODH (Dihydroorotate dehydrogenase) is a key enzyme involved in pyrimidine biosynthesis
- Humans also have this enzyme
  - But, > 2000-fold difference in IC_{50} between human and fungal enzymes
- Pyrimidine inhibition has profound effects on the cell. Affecting;
  - DNA synthesis and cell cycle regulation
  - RNA synthesis and protein production
  - Cell wall synthesis
  - Phospholipid synthesis



Oliver et al. PNAS 113:12809-14, 2016.

In vitro activity is consistent across all major Aspergillus spp., including A. terreus

		F901318	Itraconazole	Posaconazole	Voriconazole	Amphotericin B
A. fumigatus	Geo mean	0.008	1.00	0.30	0.46	0.68
n = 80	Range	0.004-0.016	0.06-16	0.03-16	0.06-16	0.25-1
A. terreus	Geo mean	0.006	0.25	0.14	0.18	1.49
n =45	Range	0.002-0.008	0.06-1	0.06-2	0.03-0.5	0.125-4
A. flavus	Geo mean	0.007	0.21	0.087	0.26	0.79
n = 50	Range	0.004-0.008	0.125-1	0.03-1	0.06-1	0.5-2
A. niger	Geo mean	0.007	0.62	0.16	0.51	0.46
n=46	Range	0.004-0.016	0.125-16	0.03-2	0.125-16	0.125-1

Intrinsic resistance to ampho B

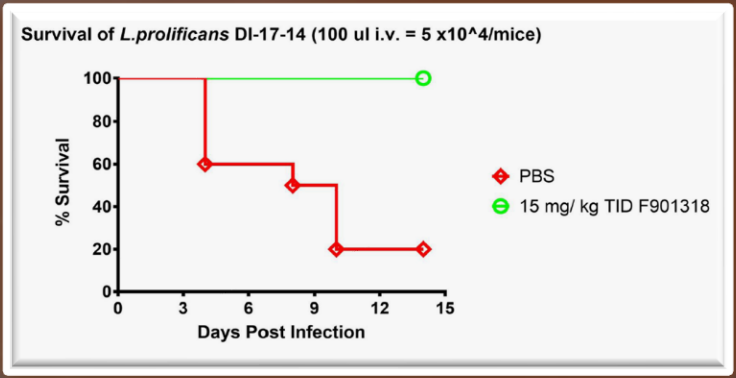
MICs in mg/L , Isolates from UK and Austria

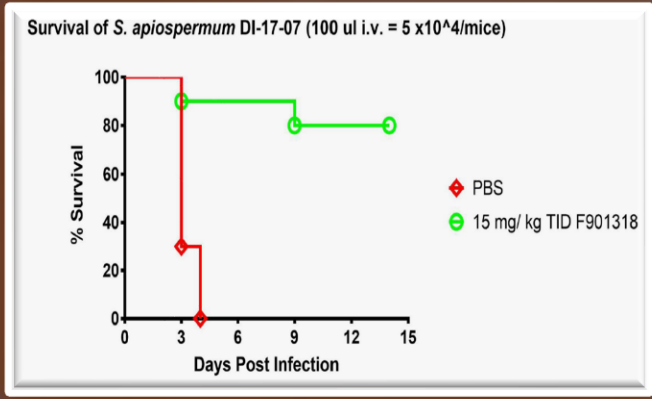
Other moulds

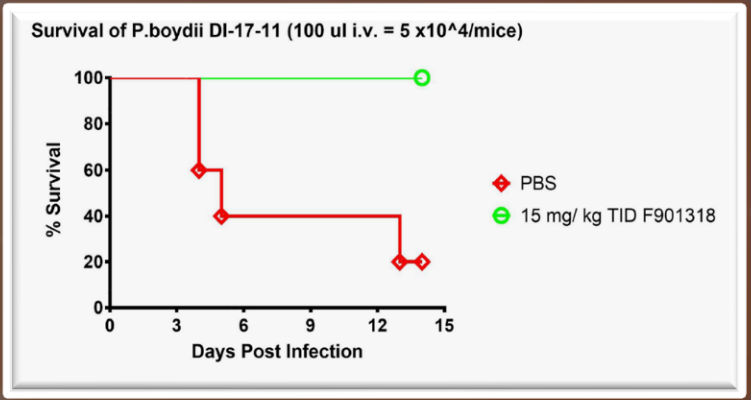
	n	MIC Range (mg/L)
Scedosporium (L.) prolificans	3	≤0.06
Scedosporium apiospermum	2	≤0.06
Aspergillus lentulus	4	≤0.06
Paecilomyces variotii	3	≤0.06
Sporothrix schenckii	5	≤0.06
Acremonium sp.	5	≤0.06 - 1
Scopulariopsis brevicaulis	5	≤0.06
Penicillium chrysogenum	5	≤0.06
Penicillium marneffii	5	≤0.06
Coccidioides immitis	5	≤0.06
Blastomyces dermatitidis	5	≤0.06
Histoplasma capsulatum	5	≤0.06-0.125

Activity against *S. (Lomentospora) prolificans* and other *Scedosporium* species has been confirmed in a larger study. Variable activity vs. *Fusarium* spp. Not active vs. *Candida*, *Cryptococcus,* or the Zygomycetes

F901318 is highly efficacious in murine models of scedosporiosis



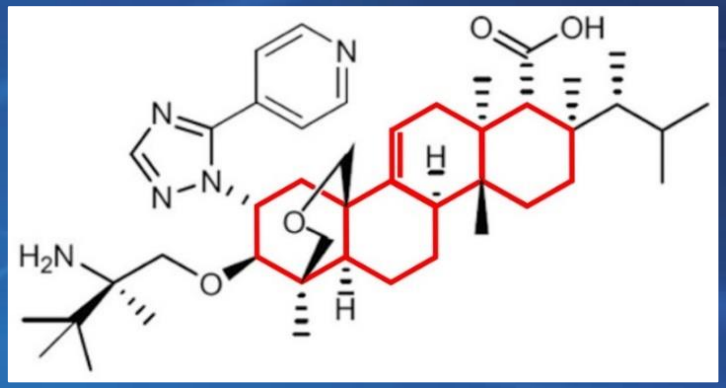




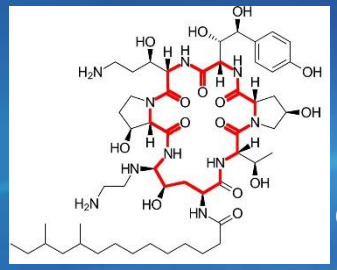
Data From A. Seyedmousavi and J Kwon-Chung NIH

SCY-078: A Novel Triterpenoid Antifungal by Scynexis

Novel Glucan Synthase Inhibitor (GSI)



Structurally distinct from other GSIs (echinocandins)



CAS

- Different enzyme-drug interaction → lower impact of common FKS mutations
- Oral bioavailability

Key Attributes

- Activity against:
  - *Candida* spp.
  - *Aspergillus* spp.
  - *Pneumocystis* spp.
- Active against azole- and most echinocandin-resistant strains
- Oral and IV formulations
- Favorable safety profile > 500 exposed
  - Low risk of drug-drug Interactions
- Extensive tissue distribution
  - (V_{dss} > 8 L/kg)

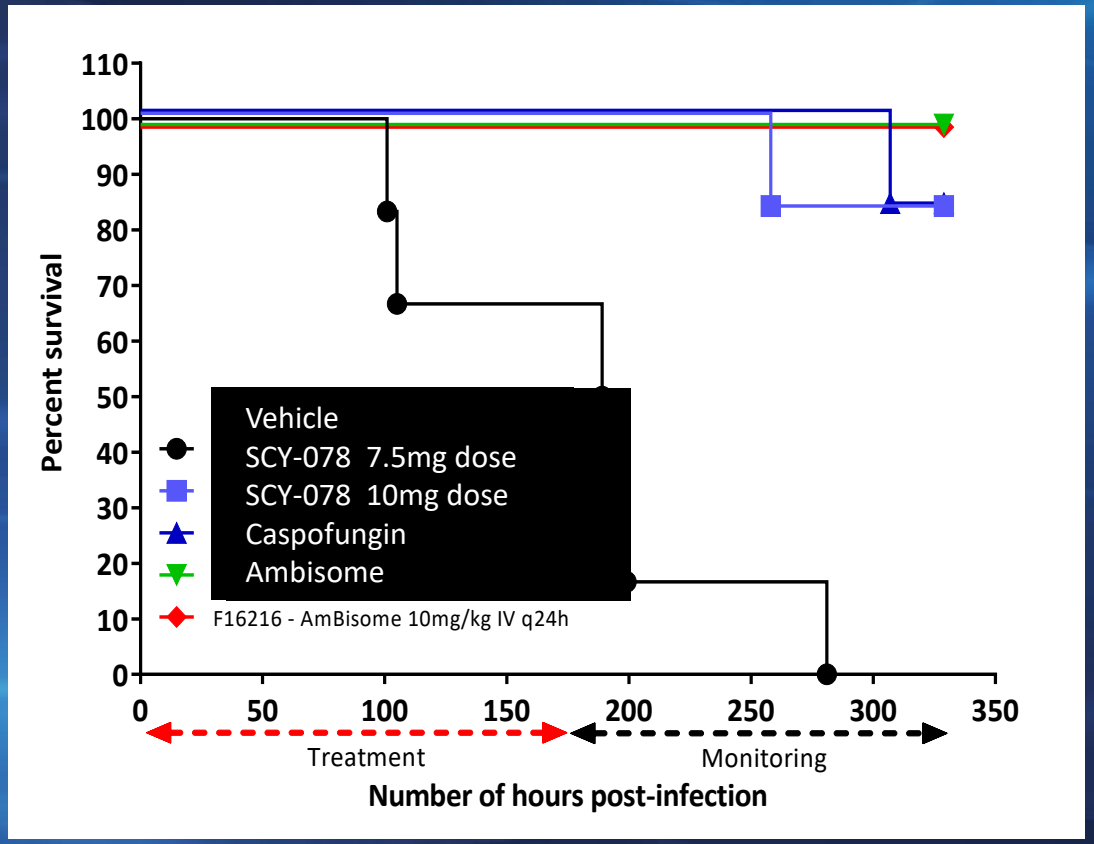
SCY-078 In vitro Activity vs. Aspergillus spp.

	SCY-078		
	MEC		
	Range	MEC_{50}	MEC_{90}
A. fumigatus (n=134)	<0.06 – 4	<0.06	0.125
A. flavus (n=54)	<0.06 – 0.25	<0.06	<0.06
A. niger (n=27)	<0.06 – 0.5	<0.06	<0.06
A. terreus (n=72)	<0.06 – 0.125	<0.06	0.125
Other spp. (n=24)	<0.06 – 0.25	<0.06	<0.06
All isolates (n=311)	<0.06 – 4	<0.06	0.125

Ghannoum M., et al. AAC June 2018; 62(6)

SCY-078 demonstrates activity against Aspergillus in a Murine Model

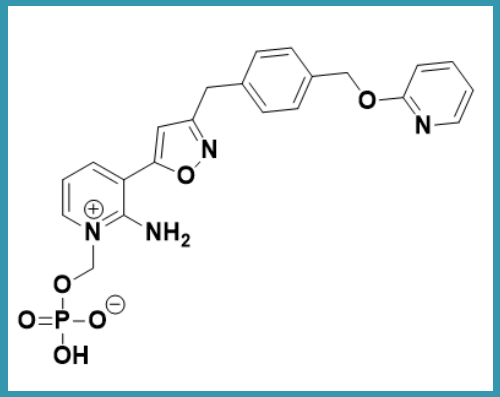
- Neutropenic mouse model of disseminated aspergillosis (IV inoculum)
- Treatment for 7 days:
  - **SCY-078 PO** at 7.5 and 10 mg/kg q12h
  - Caspofungin IP at 5mg/kg
  - Ambisome IV at 10mg/kg
- Observation for 14 days
- SCY-078 exposure needed for efficacy
  - $AUC_{0\text{-}24hr}$ 15 - 20 µM•hr



A. fumigatus (F16216)
Azole-resistant - TR34 L98H

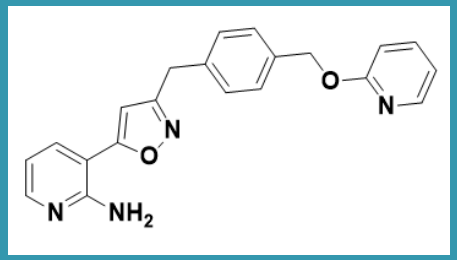
Barat S. at TIMM 2017

Pfizen
Manogepix: A first-in-class compound



APX001
(Prodrug)

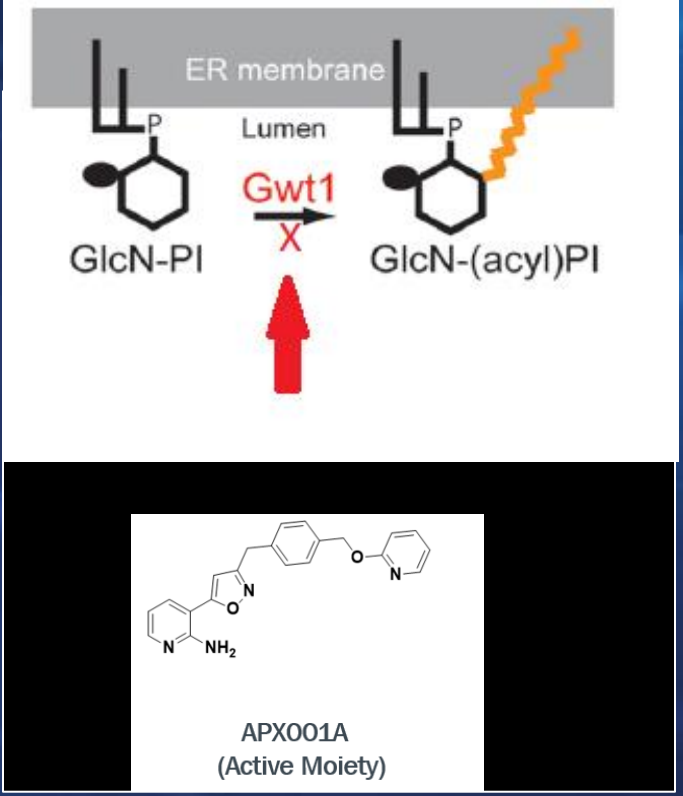
Alkaline Phosphatase



APX001A
(Active Moiety)

APX001A Inhibits the Fungal Gwt1 Protein

- APX001A is active against Gwt1 enzyme, but does not inhibit related mammalian protein, PIGW
- Gwt1 is an early step in glycosylphosphatidylinositol (GPI)-anchor biosynthesis
- Gwt1 is essential for trafficking and anchoring mannoprotein to the outer cell wall
- Mannoprotein is required for cell wall integrity, adhesion, pathogenicity, and evading host immune system recognition



Modified from
2012 McLellan *et al,* ACS Chem Biol.

In vitro Antimicrobial Activity
APX001A has a Broad Spectrum of Activity

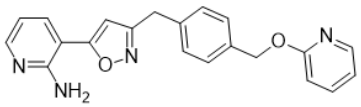
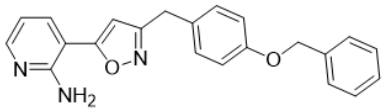
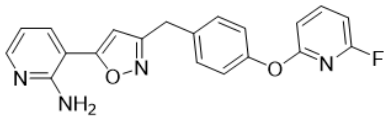
- APX001A has shown very good activity (low MIC/MEC) against most strains tested, including strains resistant to existing treatments
- APX001A is broadly active against *Candida*: MIC_{90} range; 0.008 - 0.06 µg/mL
  - Significant activity vs *C. auris* (MIC_{90} 0.03 µg/mL; 0.06 µg/mL)
  - APX001A has shown less activity against *C. krusei,* a rare infection
- APX001A is broadly active against the more common *Aspergillus* (MEC_{90} ≤0.06 µg/mL), with rarer *Aspergillus* species demonstrating similarly good activity
- Rare molds:
  - Generally has shown good activity against *Scedosporium* and *Fusarium spp.*
  - Mucorales MEC values are higher but *in vivo* activity is promising

Manogepix Summary

- First-in-class, highly differentiated product for life threatening invasive fungal infections
- Novel target and MOA with broad-spectrum activity, including MDR strains
- Efficacy demonstrated in multiple animal models
  - Wide tissue distribution (lung, kidney, brain, eye)
- Potential to be first novel antifungal since 2001
- Completed Phase 1 clinical development IV and Oral
- Entering Phase 2 development in 2018
- Anticipate streamlined development and accelerated regulatory pathway in multiple indications
  - Orphan Drug designations for 6 indications, including pathogens of highest mortality
  - Qualified Infectious Disease Product (QIDP) designations in 4 indications, allowing for accelerated regulatory review and extended market exclusivity. Recent FDA approval of Cryptococal meningitis as neglected Tropical Disease

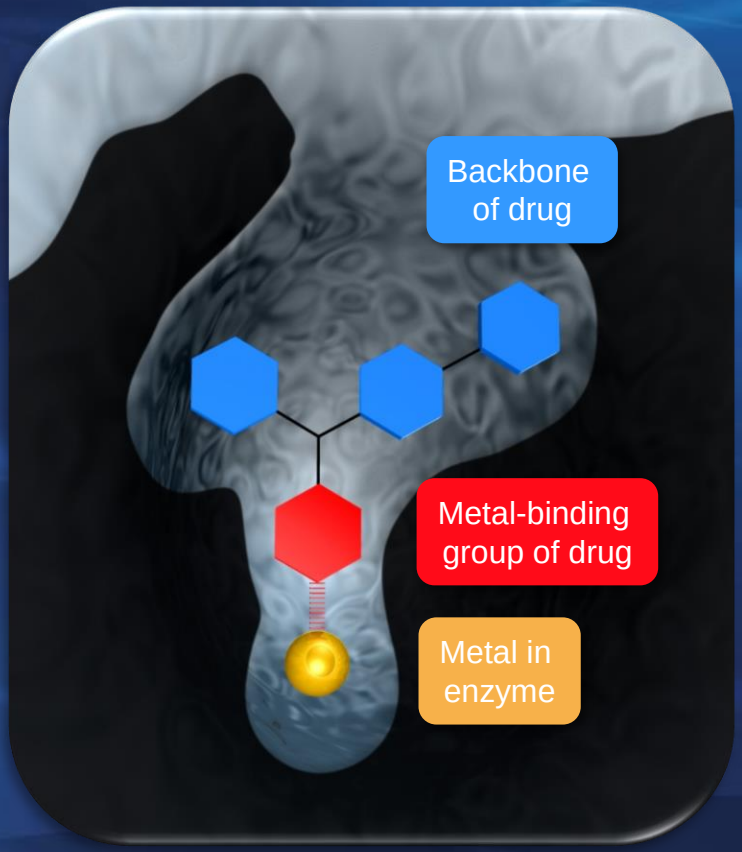
Structure and in vitro susceptibility profiles of Gwt1 inhibitors

APX2039 (active moiety of APX2096 prodrug) is 32-fold more potent than APX001A vs *Cryptococcus*, but is less active vs *C. albicans* and *A. fumigatus*

Compound	Structure	Prodrug	MIC (µg/mL)			MEC (µg/mL)
			C. neoformans H99	*C. gattii* WM276	*C. albicans* 90028	*A. fumigatus* MYA3626
APX001A		APX001	0.25	0.125	0.008	0.008
APX2020		APX2097	0.031	0.031	0.016	0.016
APX2039		APX2096	0.008	0.004	0.031	0.063

Core Expertise: Metalloenzyme Blockers Viamet

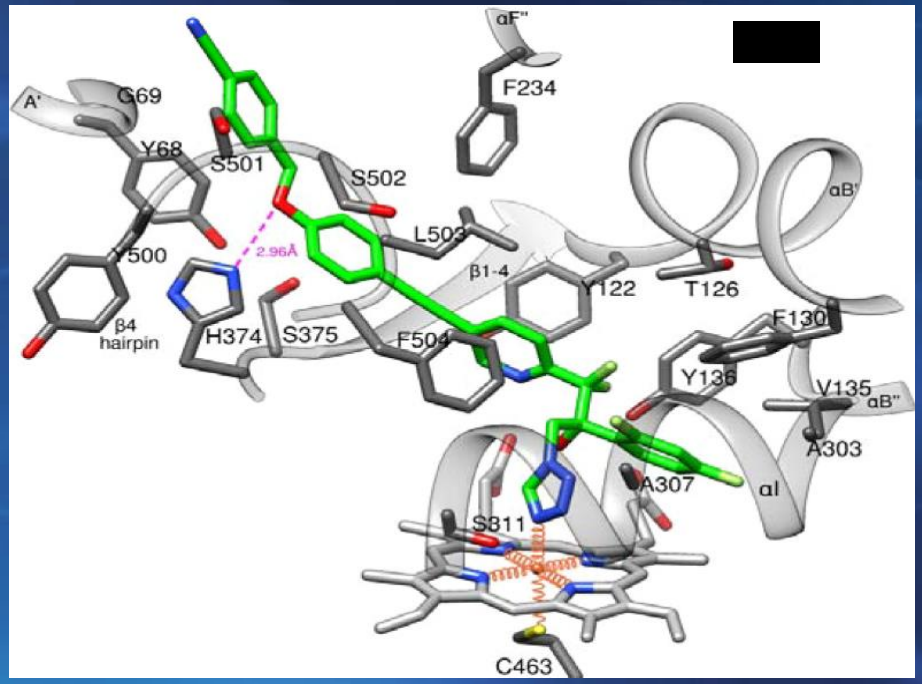
- Metalloenzymes are proven drug targets
  - ~10% of marketed drugs block metalloenzymes
- Many blockers contain a metal-binding group that inhibits enzyme activity
- Viamet's insight: The metal-binding group in many drugs has not been optimized



Active Site of Metalloenzyme

Viamet acquired by Nova Quest Capital Management Vt 1161 for onychomycosis/vaginal Candidiasis

VT-1598: Fungal CYP51 Binding Now developed by Selenity Therapeutics



- Crystal structure of VT-1598 and *A. fumigatus* CYP51
  - 4-nitrogen of the VT-1598's tetrazole interacts with heme iron
  - H-bond between phenoxymethyl oxygen and invariant H374 provides rationale for broad and potent antifungal coverage

Galina Lepesheva, Vanderbilt University; published in Hargrove et al. AAC, 2017

VT-1598: Potent Antifungal Activity - Molds

Large Panel of Clinical Mold Isolates, Geometric Mean MIC* (μg/ml)			
Species (# clinical isolate)	**VT-1598**	**POS**	**VOR**
Aspergillus fumigatus (N=41)	0.87	0.41	0.40
FLU-resistant *A. fumigatus* (N=9)	>16	2.3	11
A. flavus (N=11)	0.68	0.57	0.83
A. terreus (N=11)	0.53	0.30	0.57
A. niger (N=12)	1.8	1.0	1.3
Species (# clinical isolate)	**VT-1598**	**POS**	**AMB**
Rhizopus arrhizus (N=11)	3.5	1.1	0.60

*MIC = 100% inhibition of growth; POS = posaconazole; VOR = voriconazole; AMB = amphotericin B

Nathan Wiederhold, Fungal Testing Lab (UTHSCSA); published in Wiederhold et al. JAC 2017

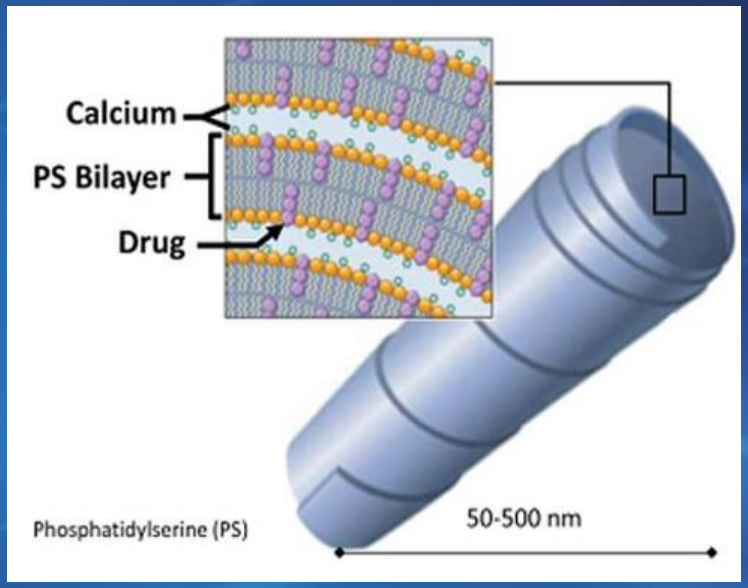
Repurposing Drugs as Antifungals (Things are happening)

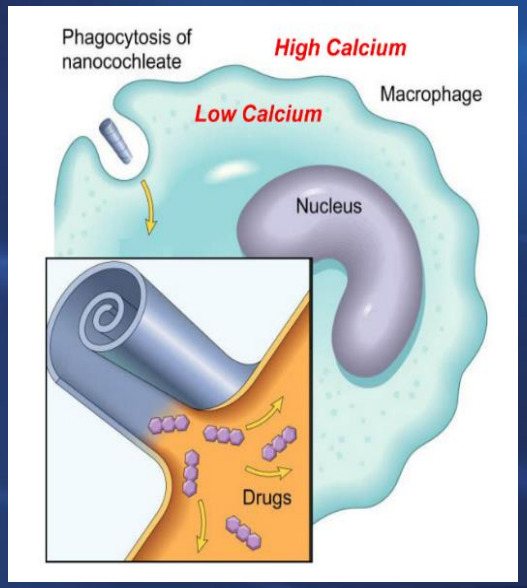
- Adjunctive Sertraline* for treatment of HIV-associated cryptococcal meningitis (ASTRO-CM)
- Tamoxifen for cryptococcosis[t]
- Amphotericin B formulations (cochleates/umbrellas)[+]
- Suba Itraconazole
- Calcineurin Inhibitors

[*] Zhai et al AAC 56:3758-66, 2012
[t] Butts et al PLoS one 10:*e*0125927
[+] Janout et al Bio Conj. Chemistry, 2015

Oral Encholeated Amphotericin B (cAMB)

MATINAS BIOPHARMA



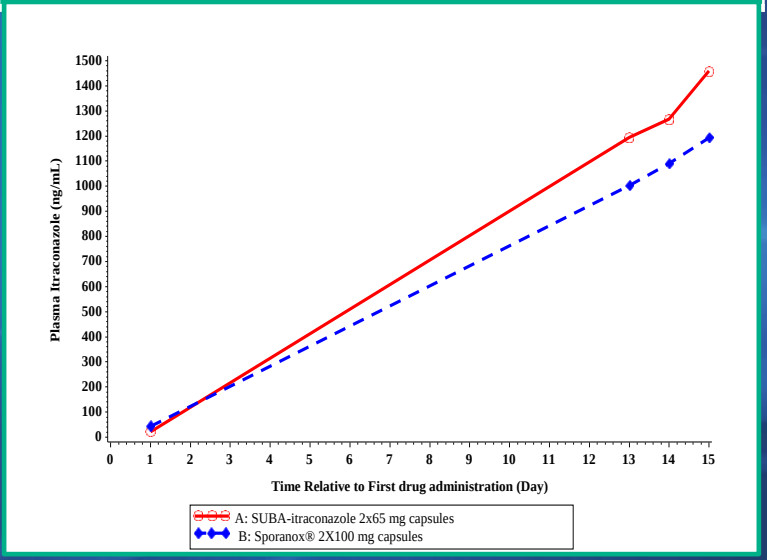


SUBA™-itraconazole Compared to Conventional Itraconazole

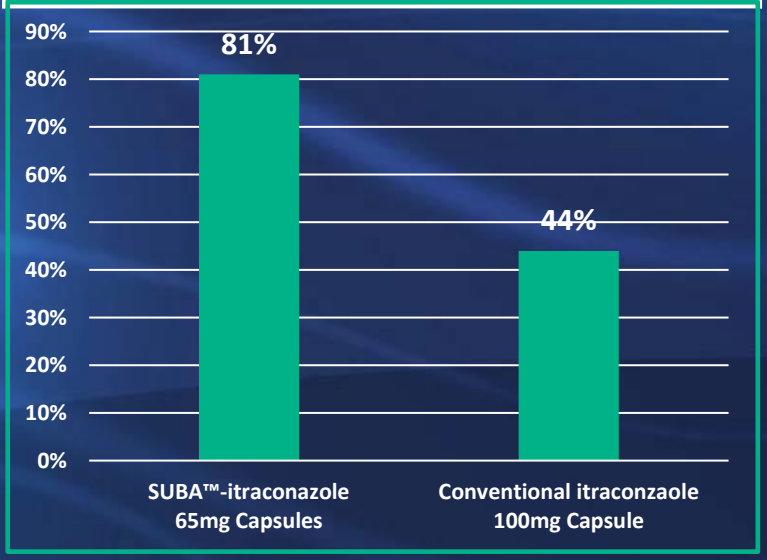
More predictable absorption and improved bioavailability

- 16 healthy adult volunteers
- Subjects received 2 capsules of SUBA™-itraconazole or conventional 100mg itraconazole twice daily for 14.5 days under fed conditions
- The relative bioavailability (Frel) of the SUBA™-itraconazile 65mg capsule was approximately 1.8 that of the conventional 100mg capsule making the extent of itraconazole exposure equivalent.
- Subjects receiving SUBA™-itraconazole 65mg, not only achieved therapeutic levels of 1000ng/ml, but also went on to reach levels of 1200ng/ml or higher

Relative Bioavailability of SUBA™-itraconazole to Conventional Itraconazole Under Fed Conditions

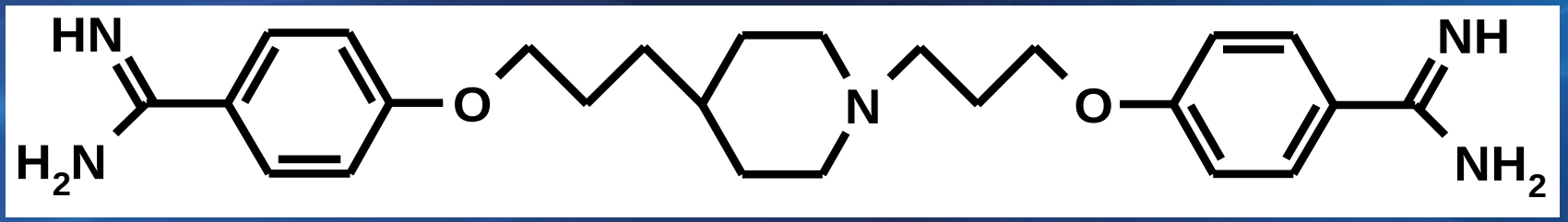


% of Patients with C_{trough} Geometric Mean of 1034 ng/ml or higher



T-2307

Appili



4-{3-[1-(3-{4-[amino(imino)-methyl]phenoxy}propyl)piperidin-4yl] propoxy}benzamidine

- ***Novel mechanism of action***
  Selective transportation/accumulation into fungal cells and inhibition of mitochondrial membrane potential
- ***Broad and potent in vitro/vivo antifungal activity***
  against *Candida* spp., *Cryptococcus spp.* and *Aspergillus* spp. including resistant strains

In-vitro Antifungal Activity

➢T-2307 showed broad and potent antifungal activity against pathogenic yeasts and fungi.

Organism	MIC*(μg/mL)				
	T-2307	FLC	VRC	MCFG	AMB
C. albicans ATCC 10261	0.001	0.125	0.0039	0.0313	1
C. glabrata ATCC 90030	0.0039	8	0.25	0.0313	1
C. guilliermondii IFO 10279	0.002	4	0.0625	0.5	0.5
C. krusei IFO 0584	0.001	32	0.125	0.125	2
C. parapsilosis NBRC 10219	0.001	1	0.0313	2	1
C. tropicalis IFO 1400	0.0005	2	0.0625	0.0625	1
Crypt. neoformans ATCC 90112	0.0078	1	0.0313	>64	1
A. flavus NBRC 6343	0.5	>64	0.5	0.0625	2
A. fumigatus ATCC 16424	0.125	>64	0.25	0.0313	1
A. nidulans NBRC 33017	0.0156	64	0.125	0.0313	2
A. niger NBRC 33023	0.0625	>64	0.5	0.0313	2
A. terreus NBRC 33026	0.125	>64	0.5	0.0313	2

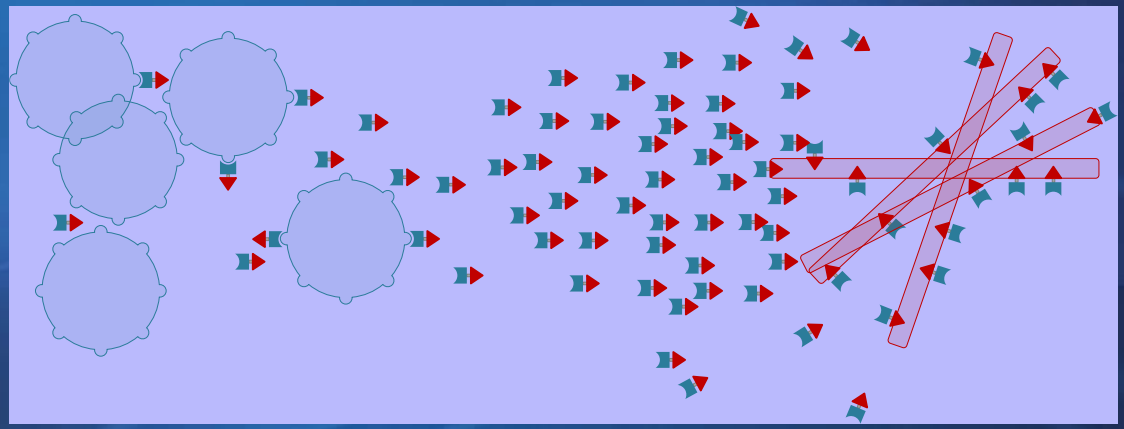
* Microdilution method recommended by CLSI

Mitsuyama J, et. al. Antimicrob. Agents Chemother. 2008; 52: 1318-24

Cloudbreak™ concept and compound design by Cidara

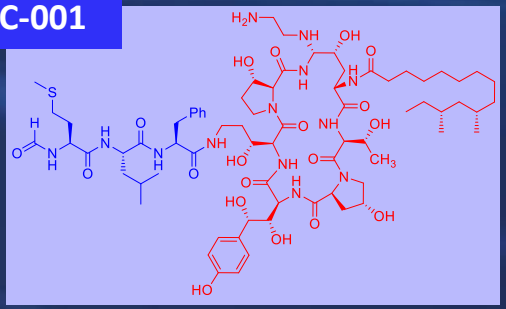
HUMAN CELL	EFFECTOR MOIETY	TARGETING MOIETY	MICROBE
Neutrophil	fMLP	Echinocandin	Fungi

Neutrophils Cidara Compound Fungal Hyphae



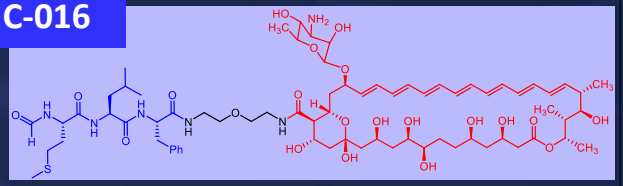
fMet-Leu-Phe caspofungin

C-001



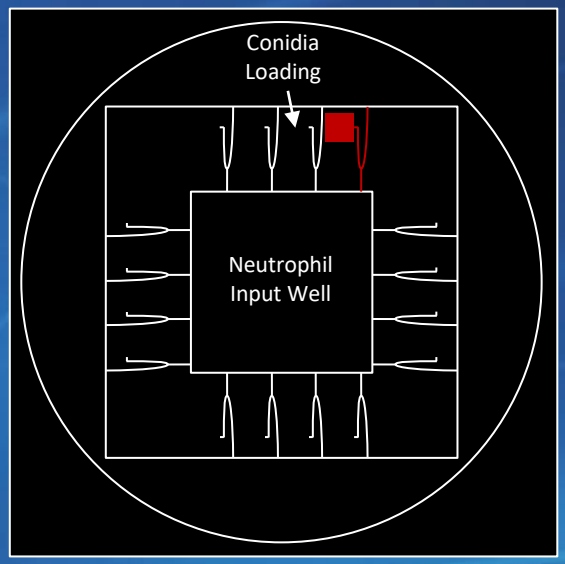
fMet-Leu-Phe amphotericin B

C-016



Ex-vivo experimental design

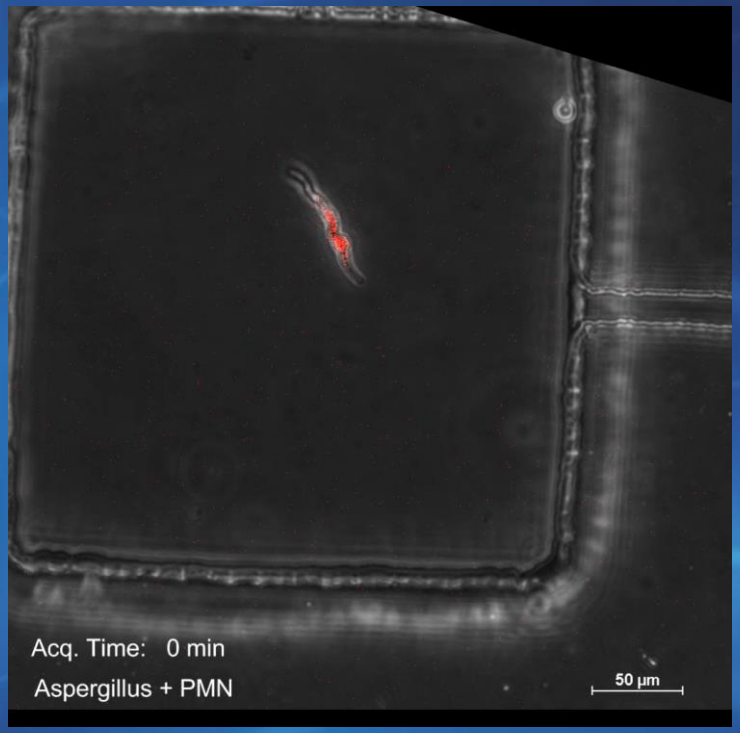
Microfluidic Schematic



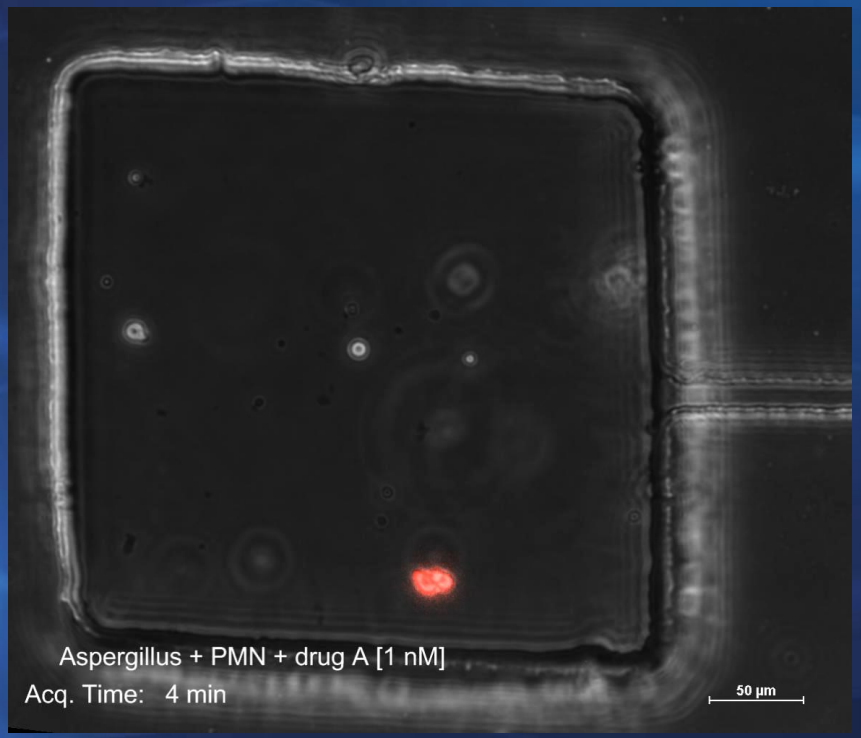
Actual microfluidics chamber

Ex-vivo proof of concept for C-001

No drug



1 nM C-001 (1/70th MEC)



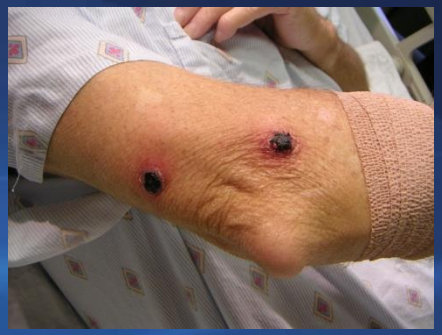
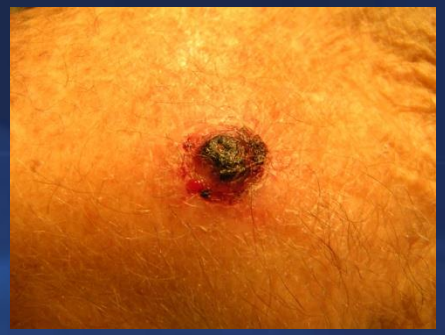
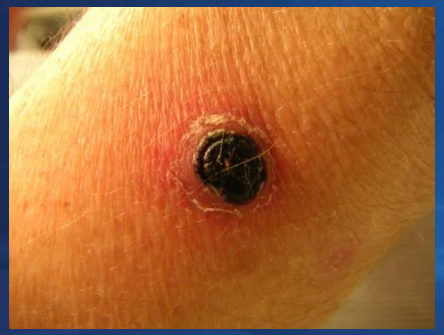
ICAAC Poster: New Anti-Fungal Agents
Saturday, Sept 19

Bifunctional Small Molecule Immunotherapy. C-001 and C-016 Attract Neutrophils (PMNs) to Inhibit *Aspergillus fumigatus* (Af) Growth in Microfluidic Chambers, D. Irimia, *et al*.

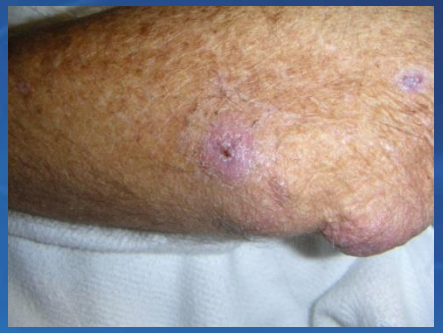
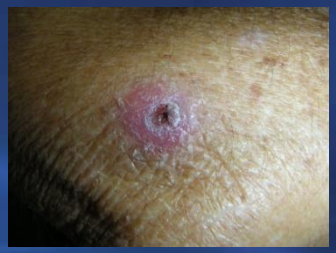
Case 1
More than ONE Fungus
(concept of Human Petri dish)

70 year old male with long-standing myelodysplastic syndrome/leukemia who has been chronically neutropenic was working in his garden with unprotected arms and skin lesions started to appear.

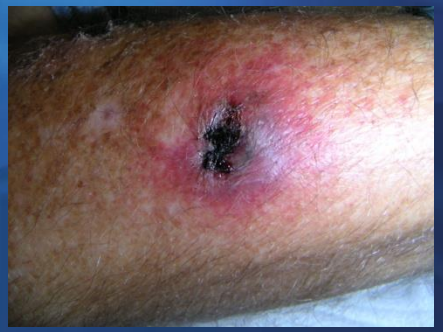
Mucor lesions before treatment

Mucor lesions on ampho B

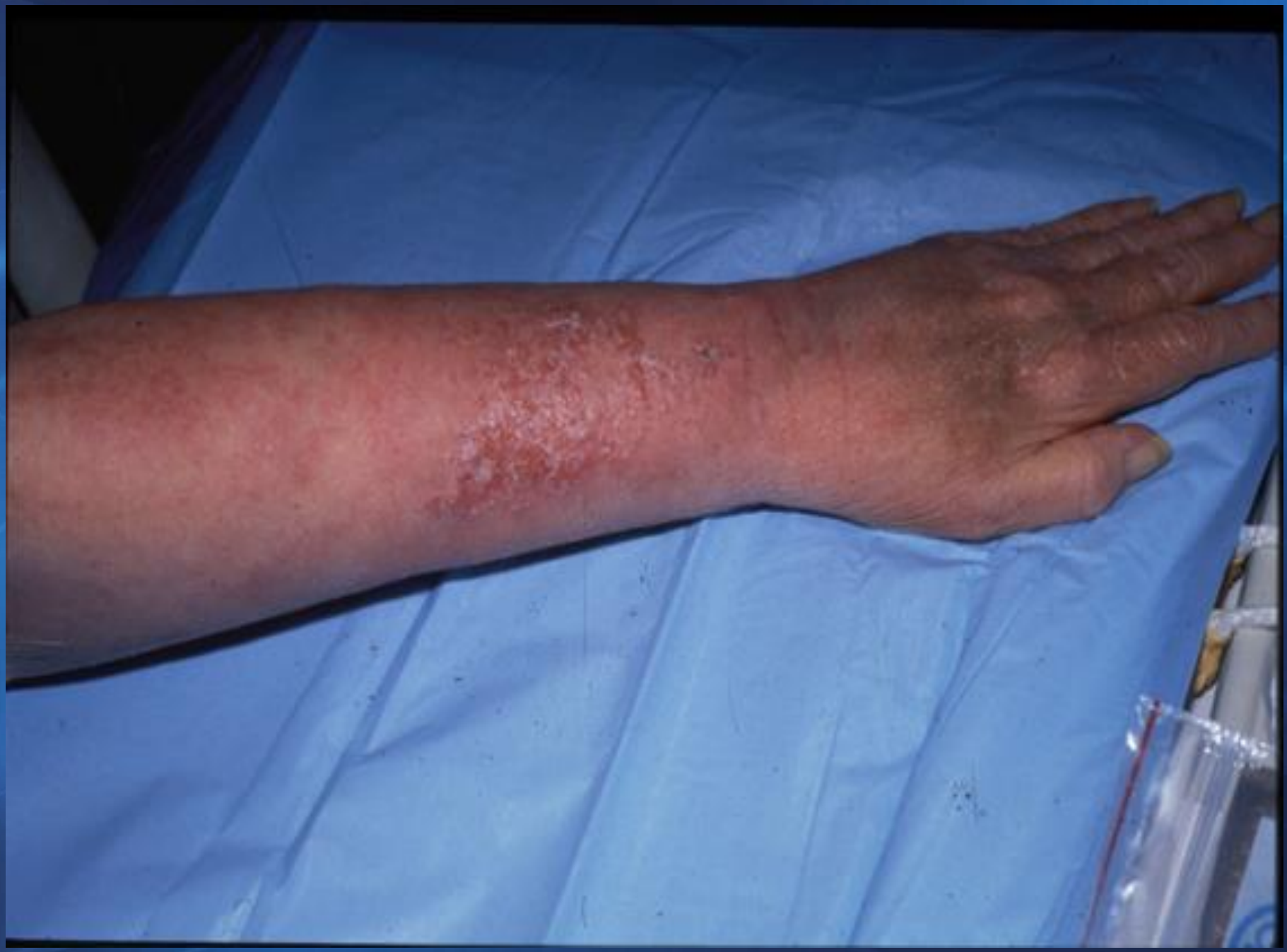



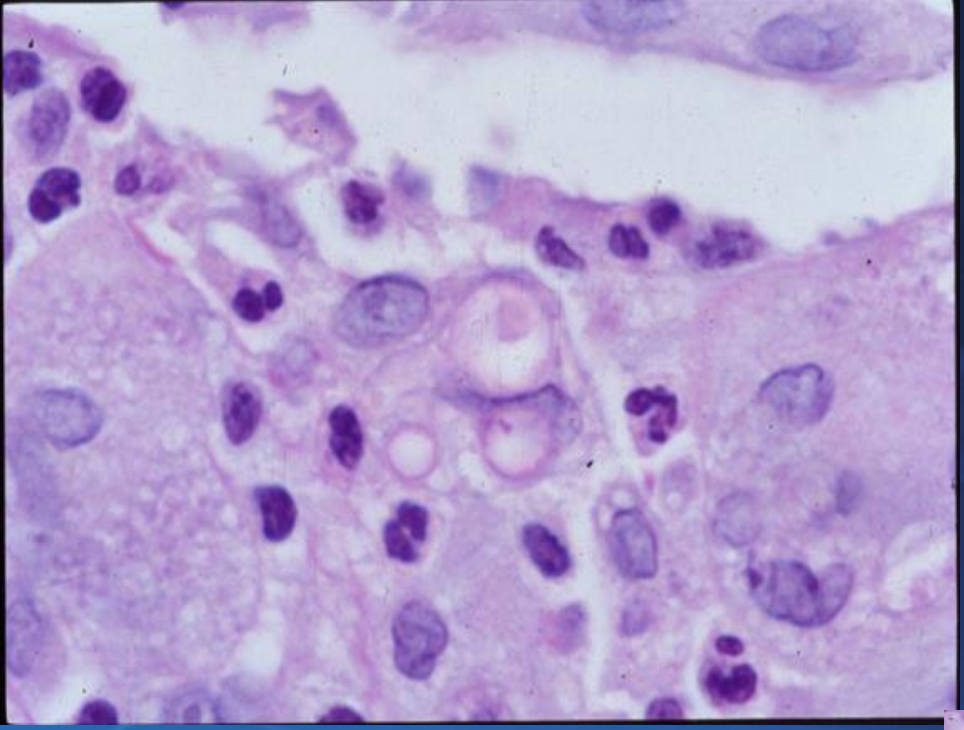
Aspergillus lesion



Case 2
(Diagnosis)

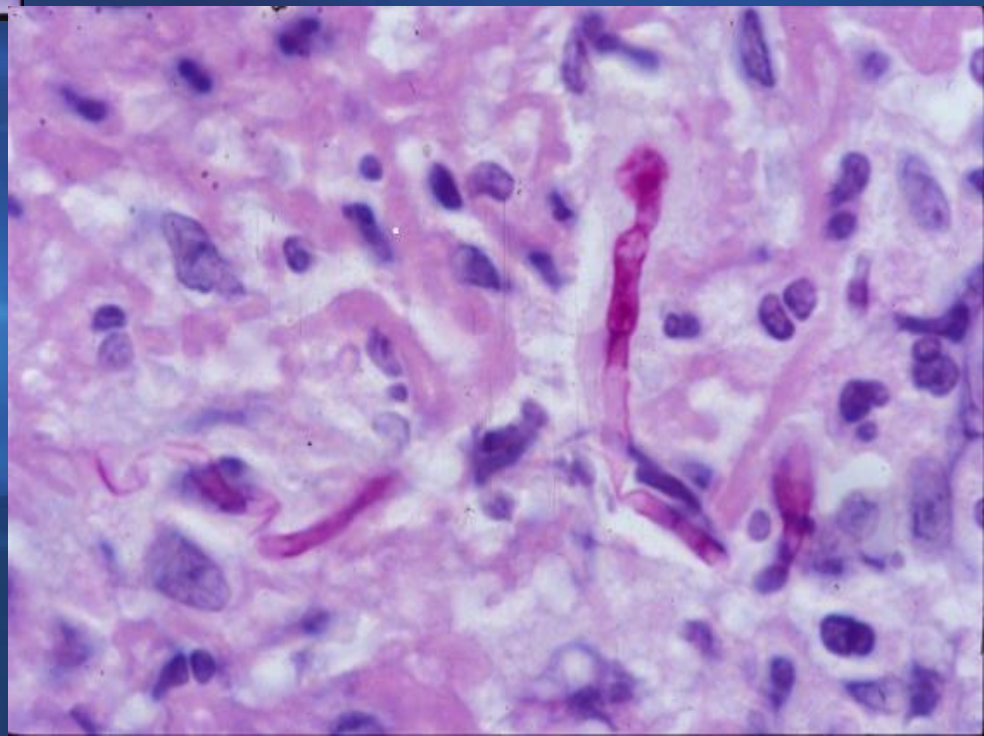
- 60 year old. Lung transplant
- Skin lesion progresses over 2 weeks of low doses fluconazole
- Biopsy: consistent with Blastomycosis
- Received 5 grams of ABLC
- Infectious disease consult





Original Histopathology

Secondary Histopathology



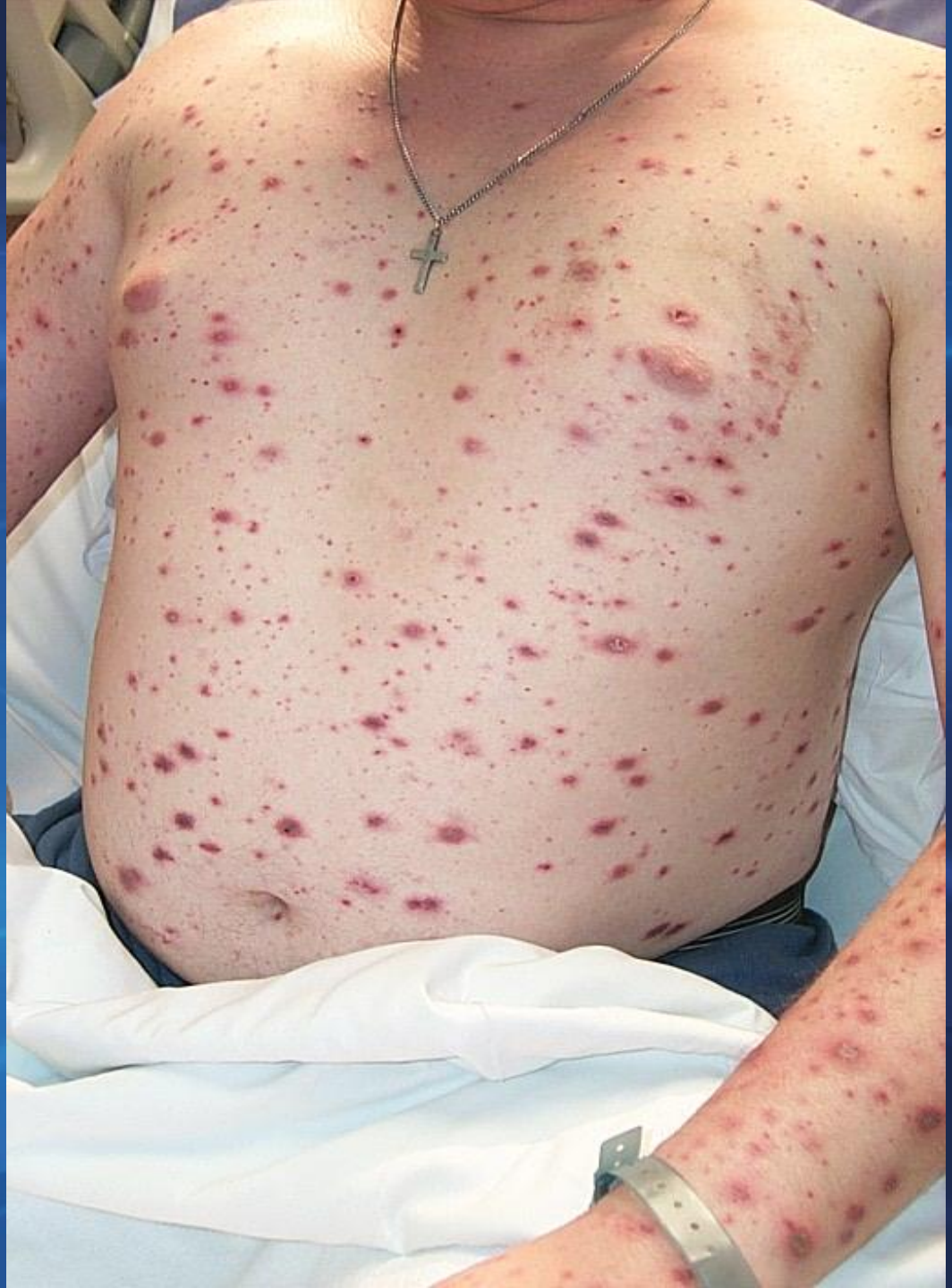
Paecilomycis lilacinus

Case 3 (Immune Function Essential)

- 8 yo. with a primary immunodeficiency syndrome who received an unrelated cord blood transplant
- Receiving ABLC + itraconazole prophylaxis
- 3 months post-transplant which didn't engraft; new neurological symptoms
- Biopsy of brain mass (6 separate lesions)
- Culture: *Cladophialophora bantianum*
- MICs voriconazole 0.125mcg/ml; flucytosine 0.25mcg/ml; posaconazole 0.015mcg/ml
- Voriconazole + flucytosine x 3 months
- Caspofungin added x 1month
- 4 months of therapy - death

Case 4 (Overwhelming disease without immune reconstitution)

- 10 yo. received 2nd peripheral stem cell transplant
- 8 months after transplant treating grade IV GVHD, ABLC prophylaxis
- Ulcerative lesion at central line site
- Positive blood cultures for *Scopulariopsis brevicaulis*
- MICs AMB 8mcg/ml, caspofungin 8mcg/ml, voriconazole 4mcg/ml, vori/caspo FIC = 0.25
- Voriconazole + caspofungin started
- 11 days of therapy – death



Case 5
(Multi-layer approach)

- 5 yo. immunocompetent with trauma to foot
- Soft tissue and bone involvement with *Scedosporium prolificans*
- MICs AMB 2.0mcg/ml; itraconazole > 1.0mcg/ml; voriconazole 8mcg/ml; caspofungin > 4mcg/ml;

  vori/casp FIC = 0.25
- Surgical debridement with irrigation of wound with 0.02% solution of polyhexamethylene biguanide
- 6 weeks of voriconazole and caspofungin
- Cured (1year follow up)

Clinical Case 6
(Be aggressive in diagnosis)

- 40 year old male in remission for acute granulocytic leukemia develops a cavitary lung lesion. Lung biopsy by VATS shows septated hyphae specimen (not cultured)

- Path. specimen sent for PCR amplification with universal fungal primers and sequencing

Clinical Case

- PCR results: (*Hormographiella aspergillata* or *Coprinus cinereus*)
- Basidiomycete
- Case Reports*
- Do you believe it? and
- Do you treat it?
- PCR sequencing from parafilm block has been useful

* Lagrou et al J. Med. Microbiol. 54: 685-8, 2005
Surmont el al Med. Mycol. 40: 217-19, 2002
Verweij et al J. Clin. Microbiol 35: 2675-8, 1997

Clinical Case 7 (Foreign Bodies)

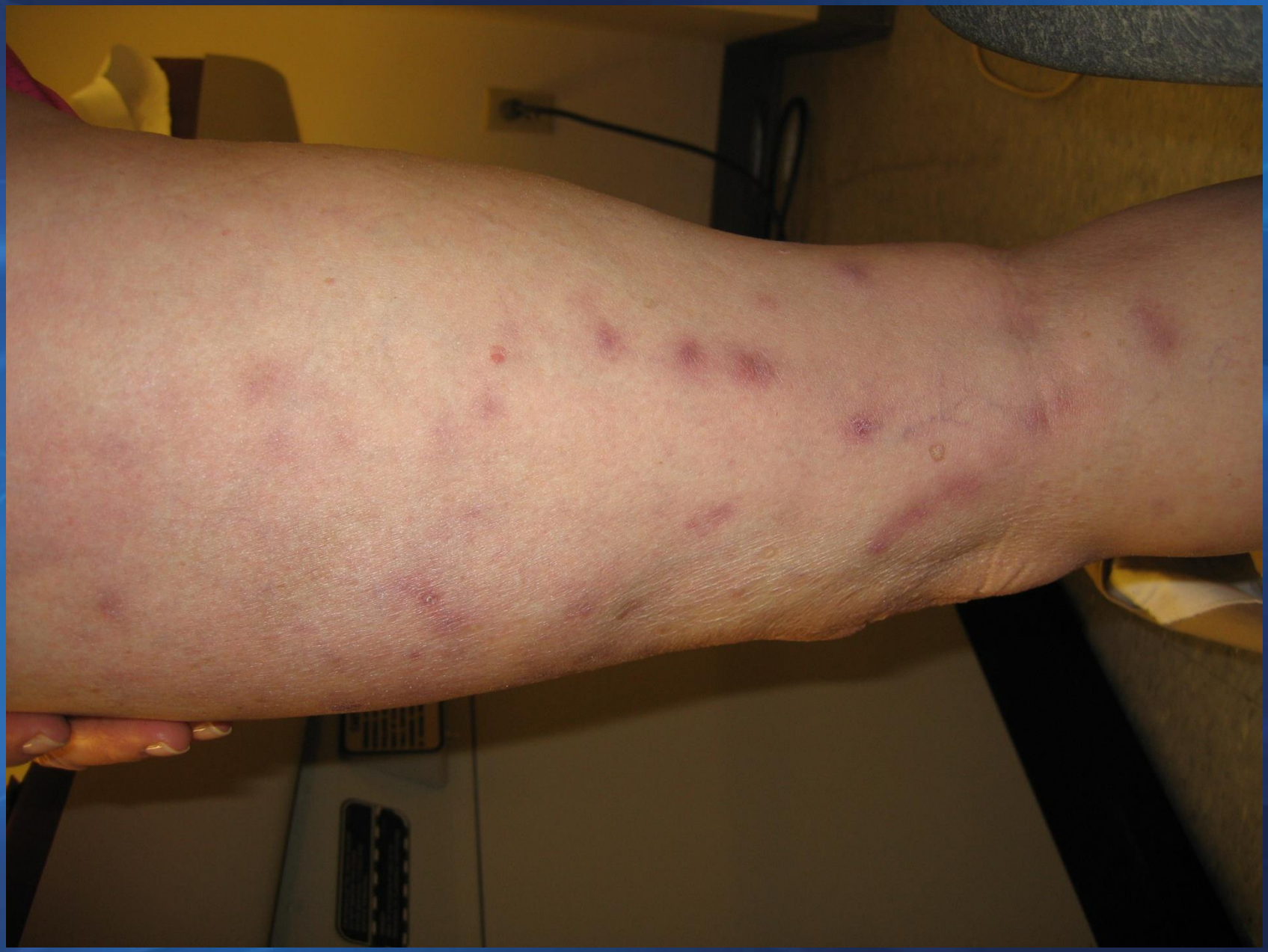
- 75 year old with infected pacemaker and tricuspid vegetation with Phialomium sps.

MANAGEMENT

- Surgery ; valve repair and pacemaker removed. (source control essential)
- Six weeks voriconazole plus micafungin
- Indefinite voriconazole suppression
- Doing well

Clinical Case 8 (Infection Mimics Host Immunity)

- 60 year old female with advanced CLL begins injections of her thighs with CAMPATH (Neutrophil count rises/CD4 count drastically drops)
- Skin lesions (non painful, nodular, slightly pruritic)



Microsporidiosis

- 1000 species in 144 genera
- Multiple lines of evidence that Microsporidian are fungi B-tubulin analysis suggest sister group to the Zygomycota*
- Water contamination, zoonosis, auto infection
- Immunosuppression: AIDS Albenazole -treatment

* Keeling et al Fungal Genet. Biol. 38:298-309, 2003

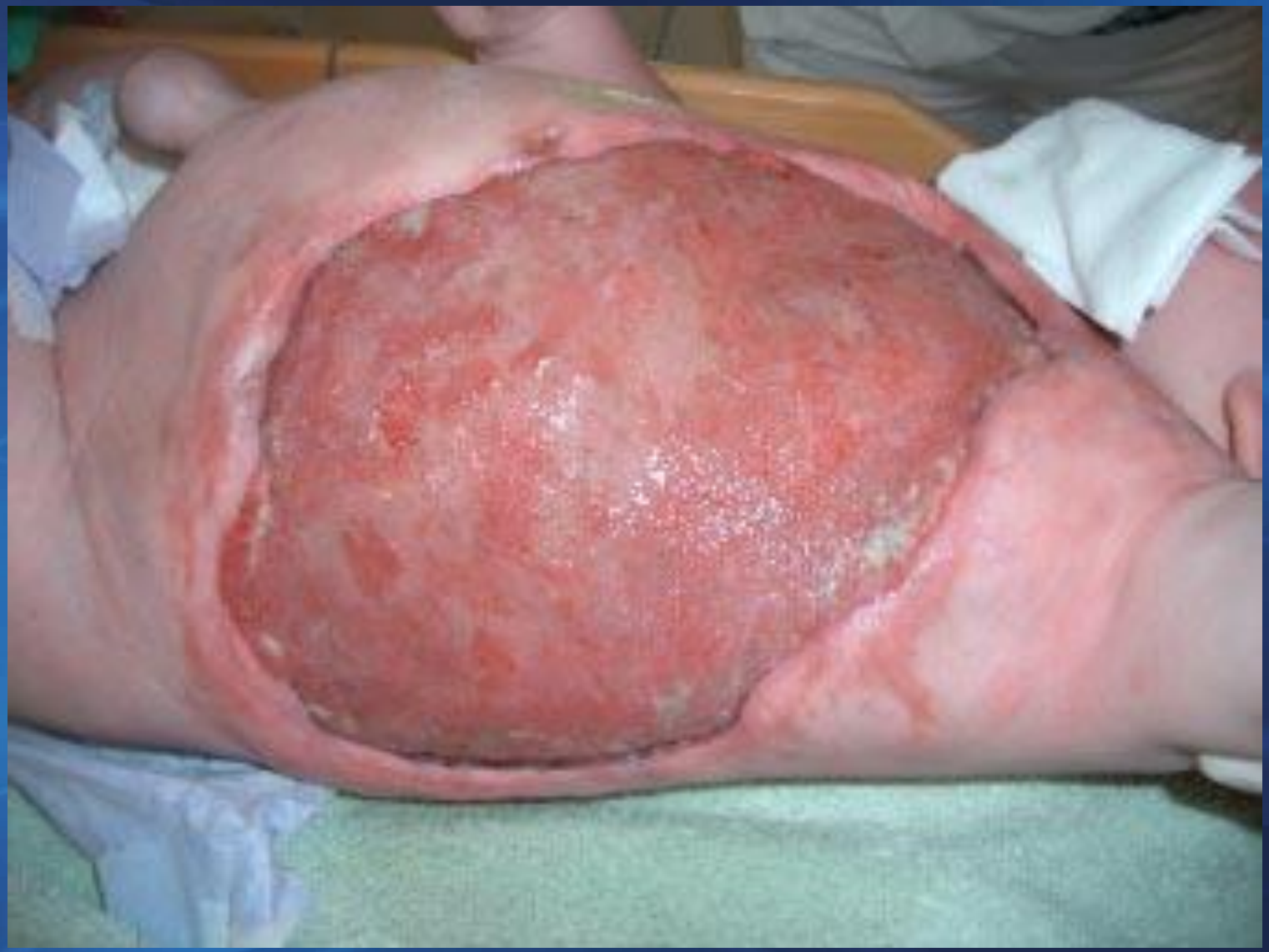
The Rest of the Story!

- Treated with albenazole for 4 weeks some improvement and eventually cleared.
- 3 months later diagnosed with invasive sinusitis/osteomyelitis with Fusarium/Aspergillus treated with Voriconazole
- 3 months later presents with chronic pneumonia

  (resected lesion in R lung and attempted to clean out pulmonary artery with Rhizopus)

Case 8
"Never give up if host/underlying disease under control."

2 yo with BMT for Hurler's Syndrome develops soft tissue infection at subcutaneous catheter site with *Fusarium solani*. Patient has immune reconstitution but infection spread to fingers, elbow, skull and tibia. CT scans of lung, brain, liver and spleen were negative.



Treatment

AmBisome 10mg/kg/d + voriconazole 12mg/kg/d (drug level – 1.6 mcg/ml); GM – CSF; Surgical debridement; Silver impregnated packing and daily packing of wound with 0.02% polyhexamethybiquanide. Over 7 months of this regimen with almost complete clearing of lesion and closure of wound. D/C AmBisome within 3-4 wks soft tissue and bone recurrence in tibia and elbow. Surgery and AmBisome restarted then put on a year of voriconazole suppression.

Patient in 10 year follow up doing well

Case 9
Many Moving Parts to the Immune compromised patients today

- 60 yo male with AML went into remission and then had severe Graft vs Host disease. Patient was treated with high dose steroids and JAK-STAT inhibitor. After 3 months of treatments, he developed fever and a rapidly enlarging cavitary lung lesion with multiple nodules. The BAL grew *Aspergillus fumigatus* and BAL galactomannan was O.D. 1.5.

Therefore, patient was started on voriconazole and after several weeks patient continued to deteriorate with enlarging cavitary lung lesion and new skin lesion appeared on his thigh. Biopsy showed ribbon-shaped hyphae but culture had no growth. Patient had AmBisome added but went on to progress and die.

What happened?

- MIC of Aspergillus 0.25mcg/ml to voriconazole
- Drug level voriconazole (trough)-4.6 mcg//ml
- Whole genome sequencing of blood (Karius) just before death
- (1) Pseudomonas aeruginosa
- (2) Cunninghamella bertholletiae

Summary
Molds and Azoles

- Host is the most
- Direct Antifungal Drug Resistance exists both in Aspergillus and other moulds
- Consider *in vitro* susceptibility testing and Therapeutic Drug Monitoring (TDM)
- In both prevention and treatment azoles represent major advances in the management of mould infections. They can make a difference!!