

Cerebral Aspergillosis: Tissue Penetration is the Key



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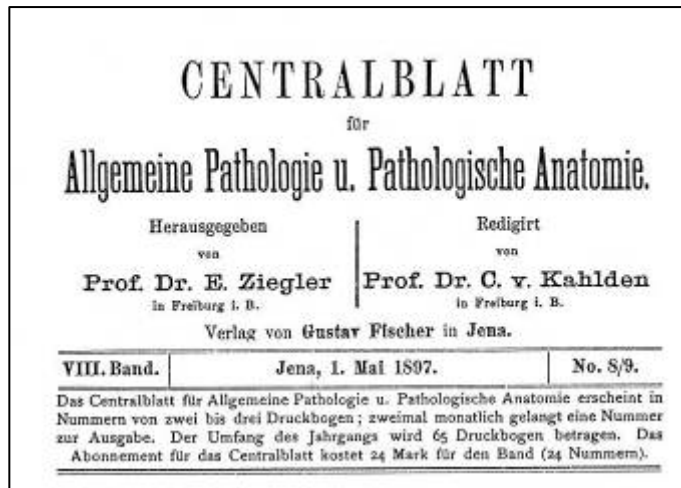
Disclosures

- Single travel grant: Pfizer Inc.

Some Data From the Old World



First Description of Central Nervous System Aspergillosis



Zur Kenntniss der Schimmelpyosen beim Menschen.

Von Dr. Oppe,

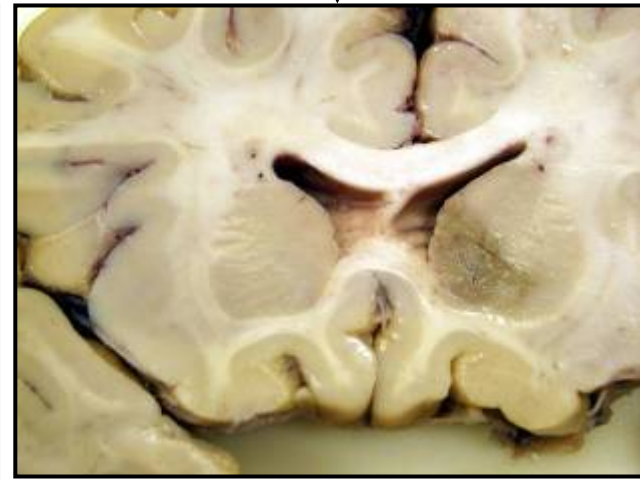
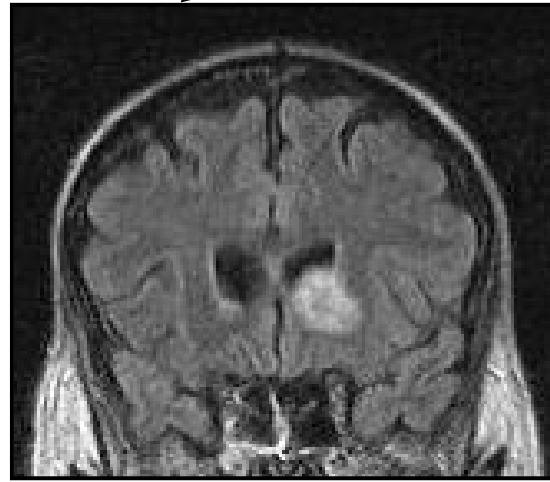
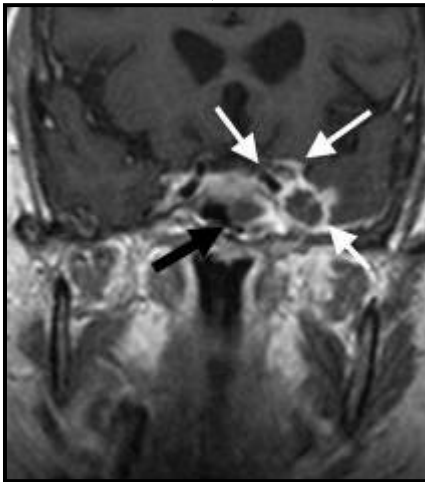
Assistent am pathologischen Institut des Dresdener Stadtkrankenhauses.

Die Ohrenheilkunde kennt zahlreiche Fälle von Schimmelpilzwucherungen im äusseren Gehörgang und in der Trommelhöhle; um so seltener sind jedoch derartige Befunde an den inneren Organen des Körpers gemacht worden. Noch am häufigsten werden von ihnen die verschiedenen Theile des Respirationstractus befallen, die begreiflicher Weise der Infection am meisten ausgesetzt sind. Bronchiektasien und Cavernen in den Lungen begünstigen die Ansiedelung der Pilze, doch sind auch primäre Mykosen in den genannten Organen mehrfach beobachtet (Virchow, Ernst, Herterich: Pilzentwicklung in Trachea und Bronchen; Weichselbaum und Kohn: Lunge). In dem letzten Fall von W. handelte es sich um eine ödematöse und emphysematöse Lunge, in der bis 5 cm im Durchmesser grosse, derbe runde Herde enthalten waren; mikroskopisch sah man in den letzteren das ursprüngliche Gewebe gewissermaassen durch Pilzmycelien substituirt. Cohn sah in den Pilzherden das Lungengewebe nekrotisch geworden und die Umgebung in Entzündung und Eiterung begriffen. Die Zahl der secundären Mykosen des Respirationstractus ist viel grösser; Podack zählt in seiner einschlagenden Arbeit deren 23 auf. In der Hornhaut haben Leber, Uhthoff und Fuchs, in

Bei einem 37-jähr. Arbeiter, welcher im hiesigen Stadtkrankenhaus, später im Irren- und Siechenhaus lag, war die Diagnose auf einen rechtsseitig an der Schädelbasis gelegenen **Hirntumor** gestellt worden. Die Section ergab folgenden Befund (Auszug aus dem Sectionsprotokoll):

Lymphgefässe fanden sich ferner eigenthümliche kanalartige Hohlräume, deren Verlauf, nach dem bald genau quer, bald schräg getroffenen Lumen zu urtheilen, ein geschlängelter sein musste. Sie waren im Inneren von **Pilzmycelien** vollständig ausgefüllt; man erblickte ein bald mehr, bald minder dichtes Gewirr gleichmässig dicker Fäden, an denen seitliche Sprossen häufig und mit Leichtigkeit zu erkennen waren. An der Peripherie löste sich der Knäuel auf, die Fäden standen strahlenförmig divergirend, ohne an ihrem Ende eine kolbige Auftreibung, wie sie in anderen Fällen beobachtet worden ist, aufzuweisen. Ebenso wurden Fructifikationsorgane vollständig vermisst.

Primary Sites in Invasive Aspergillosis

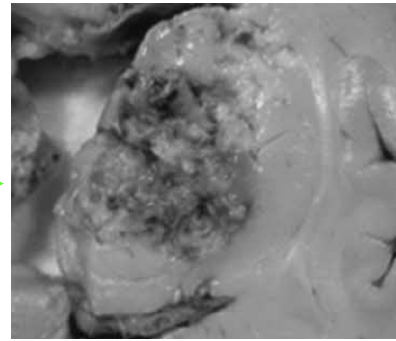


Continuous Invasion

Hematogenous Spread

Forms of Central Nervous System Aspergillosis¹⁻³

Abscess +/- Hemorrhage

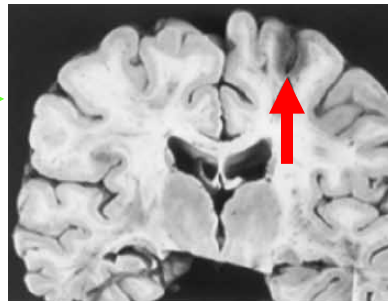


Meningitis

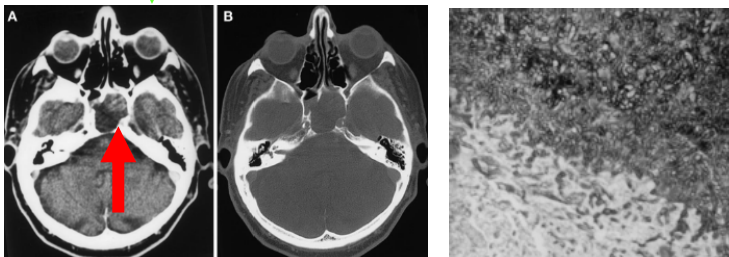
Mycotic Fungal Aneurysm



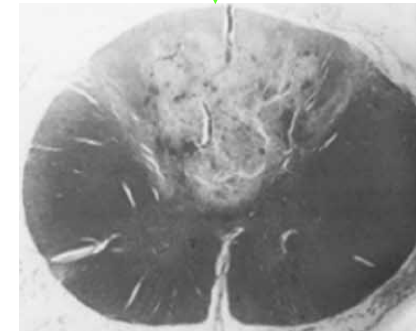
Ischemic infarction



Granuloma



Myelitis



1. Kleinschmidt-DeMasters BK. *Hum Pathol.* 2002;33:116-124.
2. Ho CL, Deruytter MJ. *Acta Neurochir* 2004;146:851.
3. Petrick M, Honegger J, Daschner F, Feuerhake F, Zentner J. *Neurosurgery.* 2003;52:955-958.

Frequency of Central Nervous System-involvement

1964-1994

**594,263
autopsies**

**7,960 invasive
mycoses**

TABLE 2. Distribution of causative agents of mycoses by organ^a

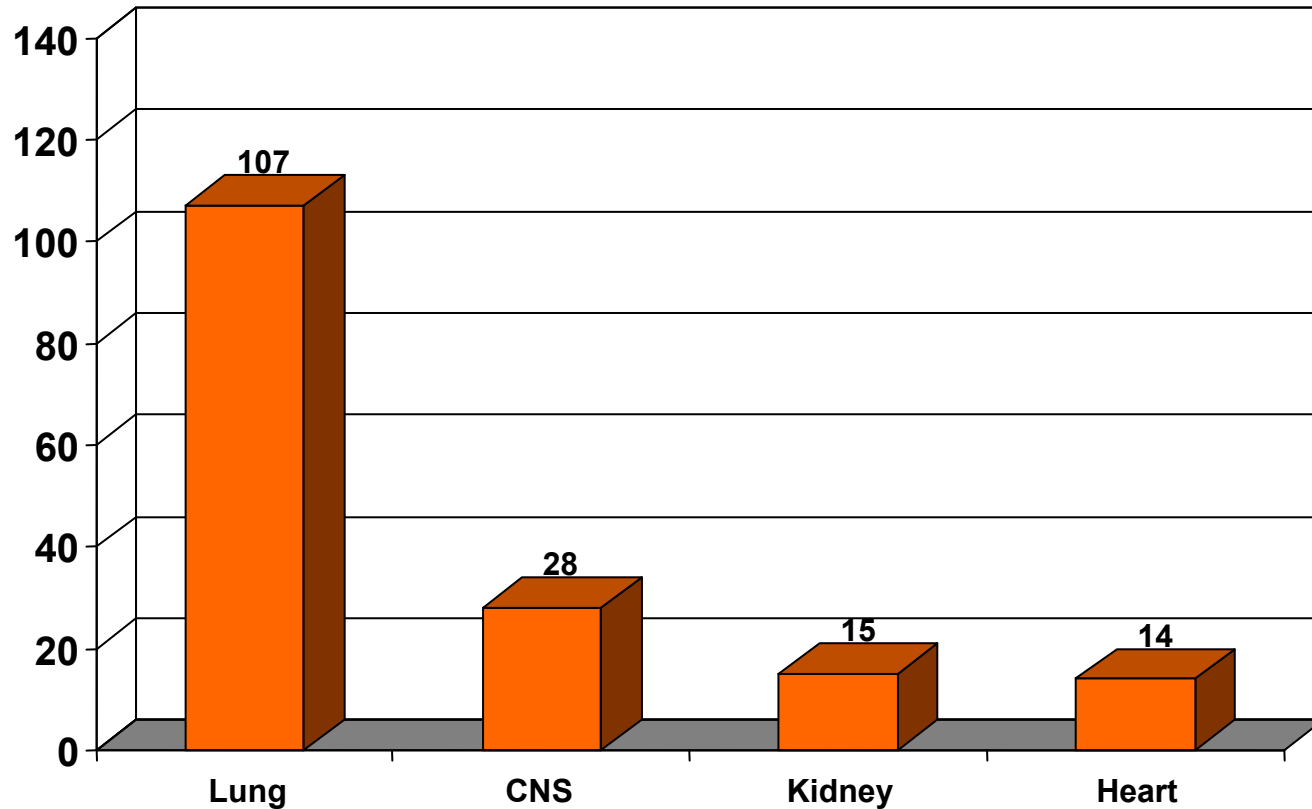
Infection type or organ	% of infections caused by:			
	<i>Candida</i> (n = 2,172)	<i>Aspergillus</i> (n = 1,967)	<i>Cryptococcus</i> (n = 289)	Zygomycetes (n = 209)
Total	100.0	100.0	100.0	100.0
Systemic	16.7	8.6	13.5	9.6
Fungemia	13.7	4.3	4.8	5.3
Brain + meninx	4.1	3.3	21.5	5.3
Mouth + tongue	2.2	0.3	0.0	0.0
Esophagus	15.9	1.3	0.7	0.5
Stomach	11.1	2.9	0.3	7.2
Intestine	8.0	2.6	0.0	4.8
Liver	8.1	3.6	3.5	9.6
Larynx + pharynx	1.2	0.3	0.0	0.0
Lung + bronchia	34.7	83.9	64.0	69.4
Heart	13.4	7.4	3.8	7.7
Kidney	23.3	7.3	9.7	9.1
Bladder	3.5	0.5	0.0	0.0
Thyroid	3.5	3.5	2.4	3.3
Spleen	3.5	2.2	6.2	6.2
Other	11.1	5.8	10.0	14.4

^a Data were compiled from references 32, 33, 34, and 36.

**CNS-aspergillosis
3.3%**

Frequency of Central Nervous System-aspergillosis

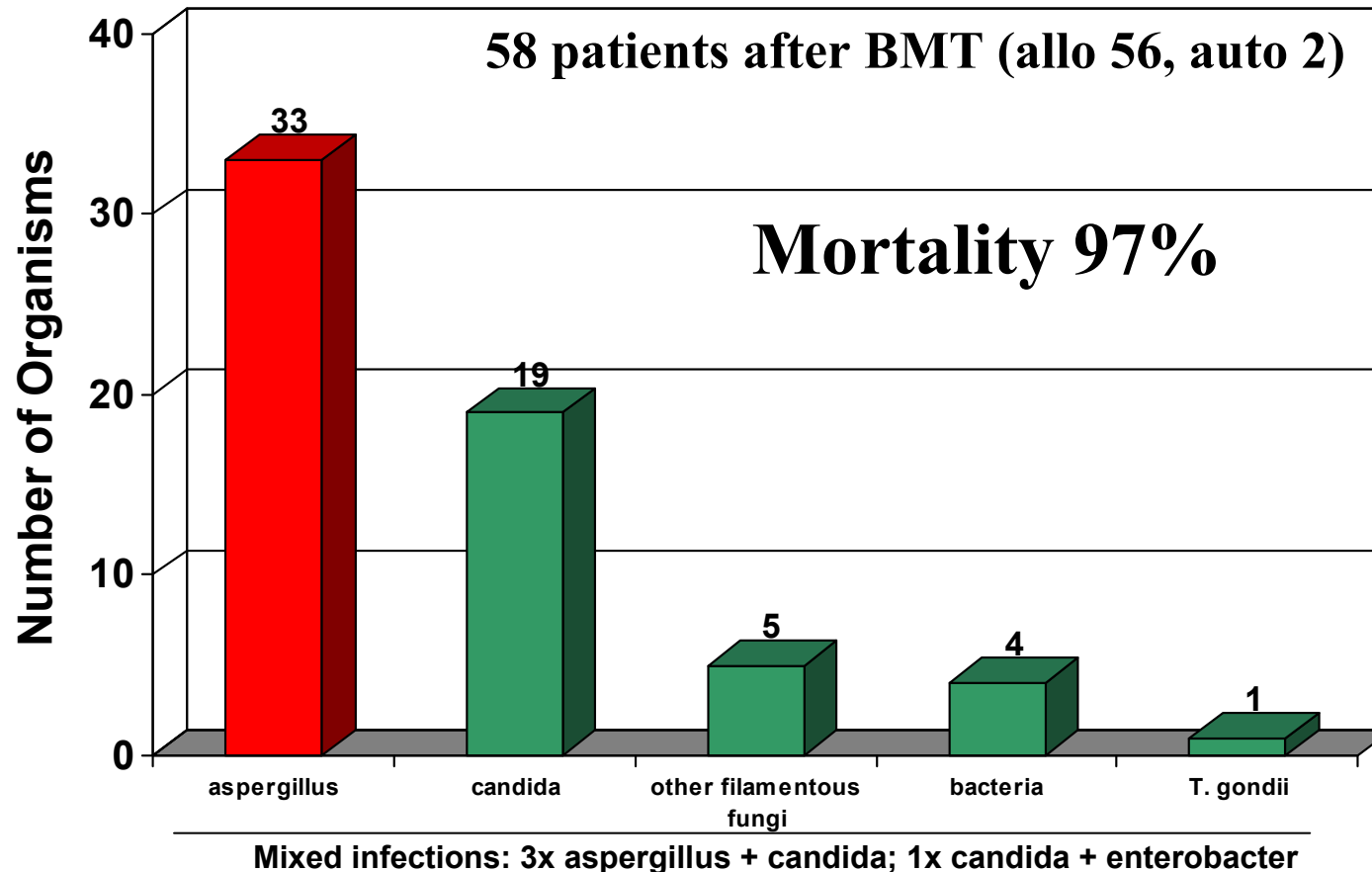
4,096 autopsies (1980-1988) patients with malignant diseases
12 centers from Europe, Canada, and Japan



Brain Abscess After Bone Marrow Transplant

Results from stereotactic biopsies/autopsies (1984-1992)

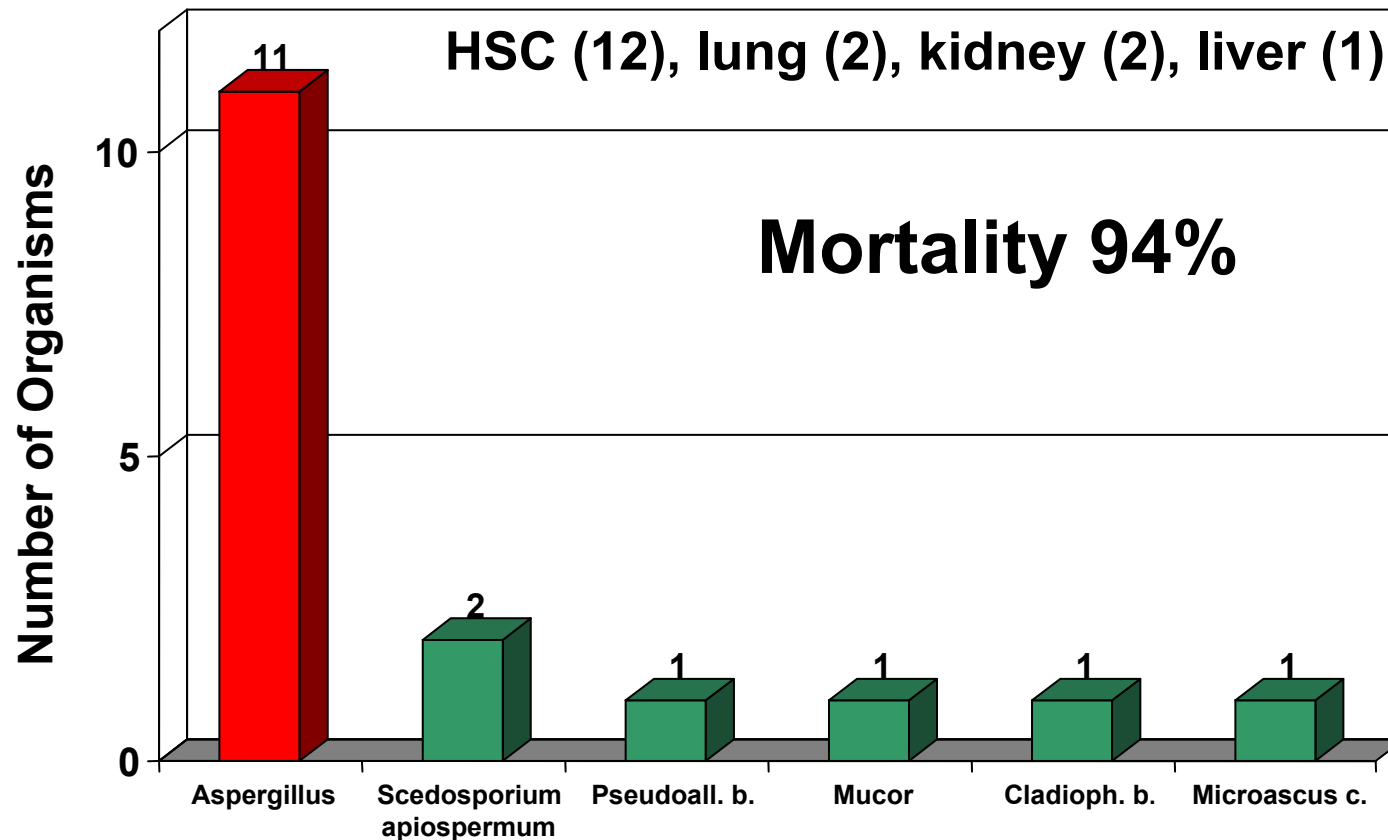
Frequency of brain abscesses in bone marrow transplant (BMT) patients: 2%!



Fungal Brain Abscess After Transplantation

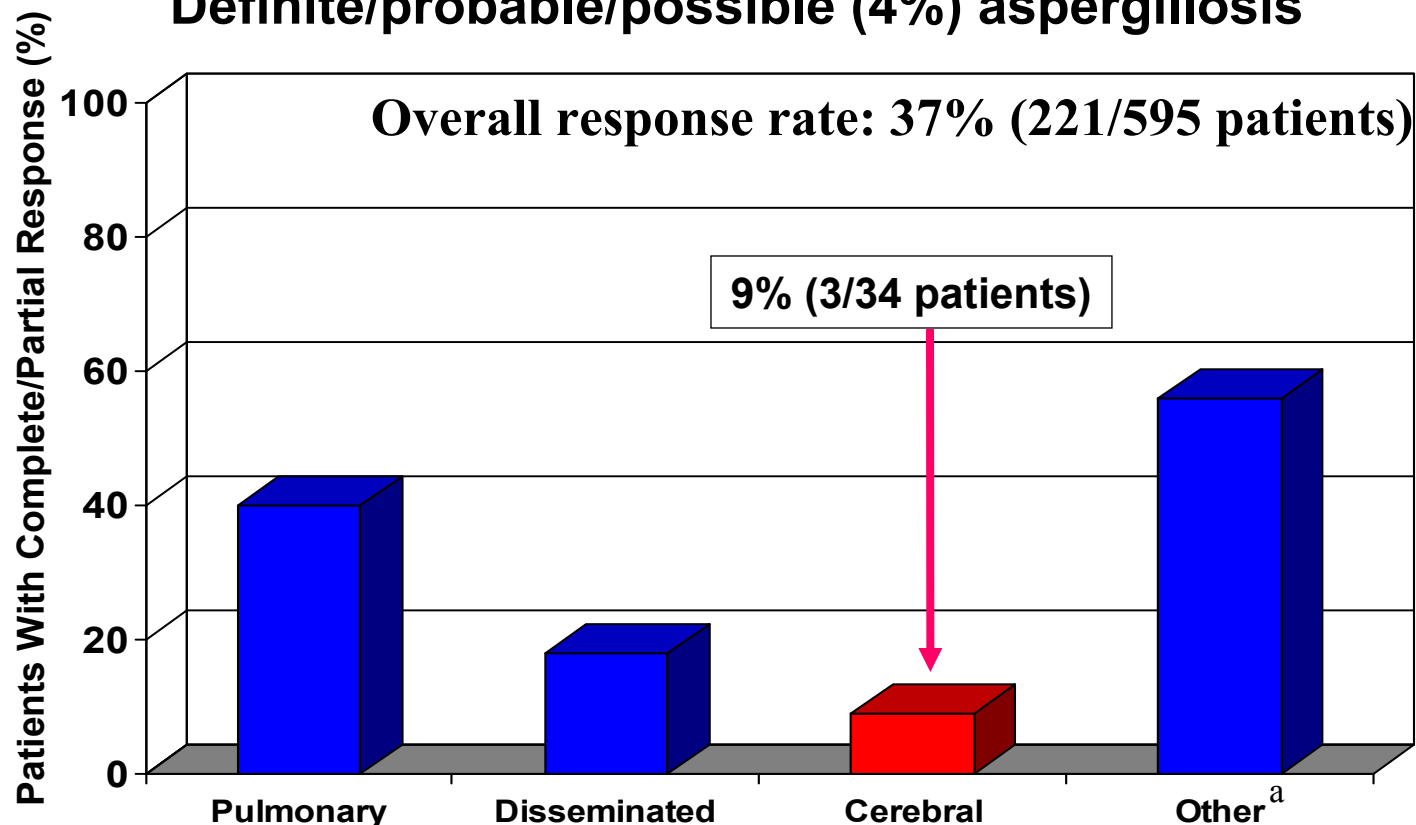
Single center study 1997-2000

1,620 patients with solid organ or hematopoietic stem cell (HSC) transplantation



Response Rates: Invasive Aspergillosis

595 patients since 1990 from 92 centers with
Definite/probable/possible (4%) aspergillosis



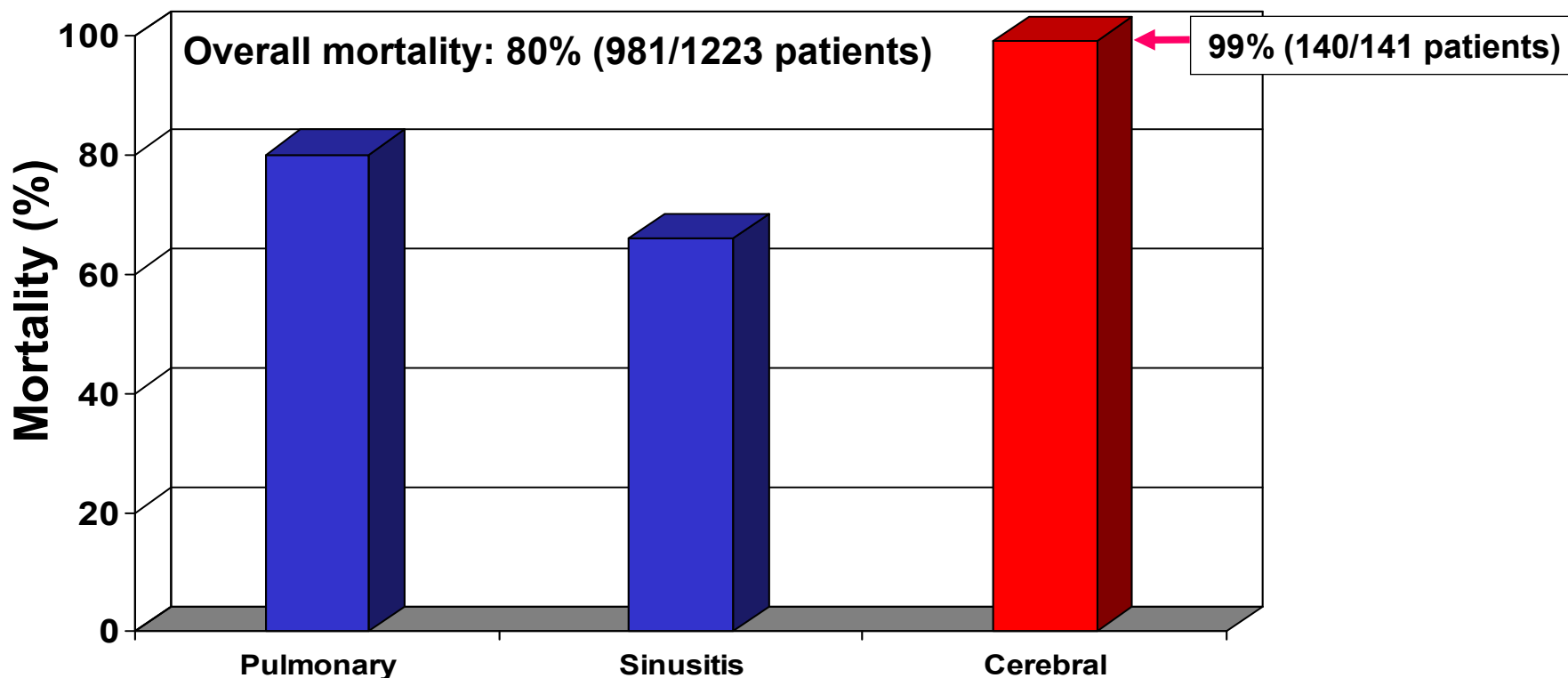
Mainly (72%) amphotericin B-based therapy
No neurosurgical data

^a28 skin; 27 sinusitis; 9 tracheobronchitis; 53 other

Patterson TF, Kirkpatrick WR, White M, et al. *Medicine*. 2000;79:250-260.

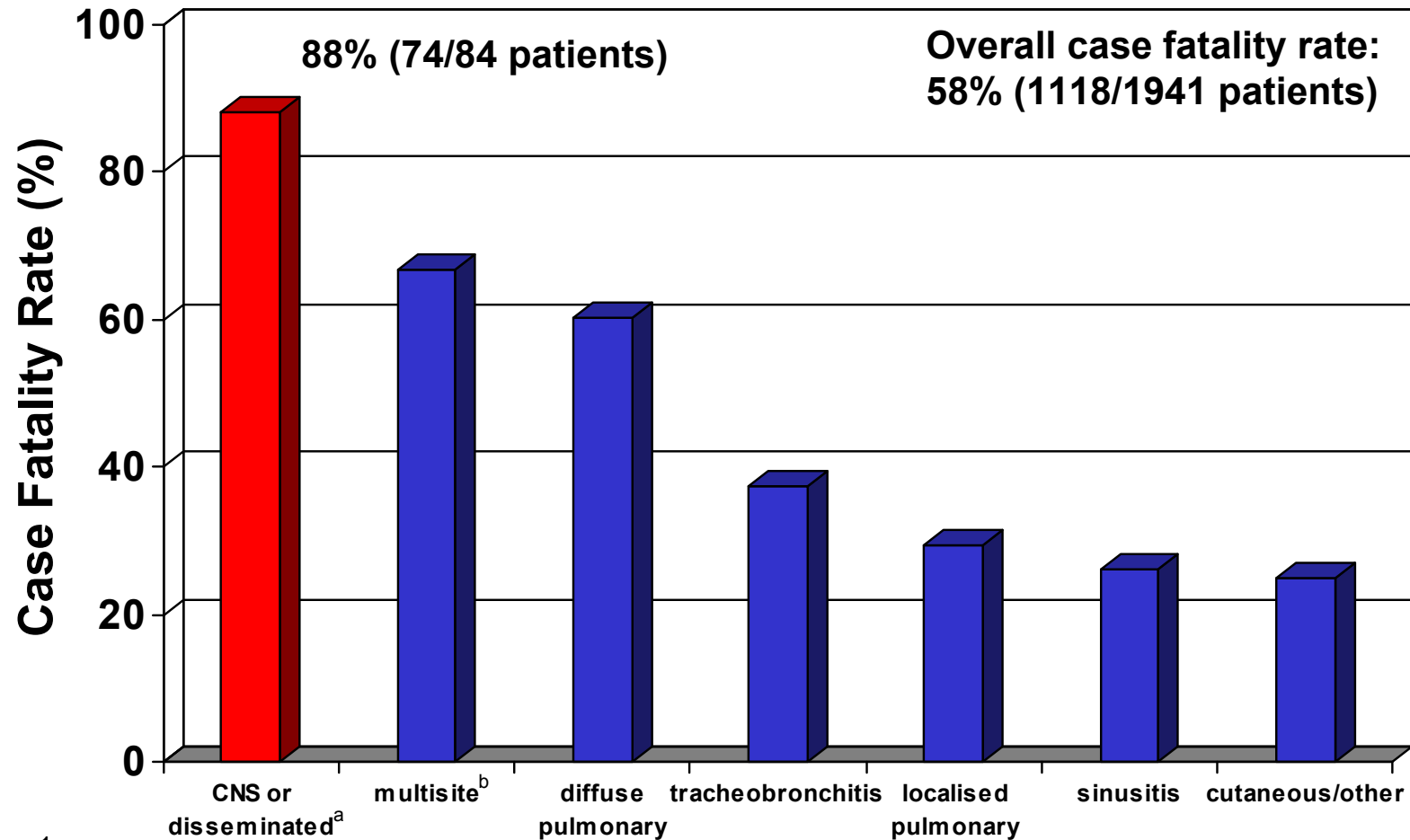
Mortality According to Site

Review of studies with ≥ 4 patients since 1971
Definite/probable aspergillosis



Case Fatality Rate According to Site

Review of studies with ≥ 10 patients since 1995
Proven/probable aspergillosis



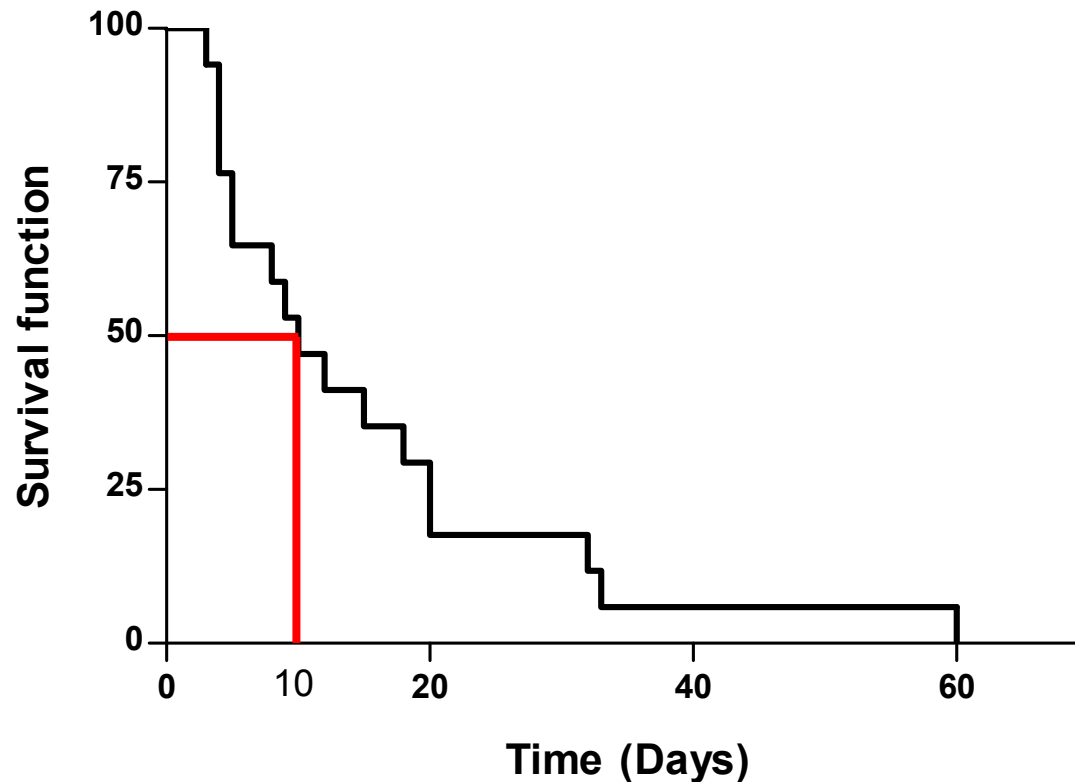
^aDisseminated → >1 organ

^bMultisite → >1 lesion in a single organ

Lin SJ, Schranz J, Teutsch SM. *Clin Infect Dis*. 2001;32:358-366.

Survival in Central Nervous System-aspergillosis

17 patients with proven/probable CNS-aspergillosis
convAMB^a (13), L-AmB^b (5), 5-FC^c (3), ITRA^d (2), none (2)
1993-1999



^aConventional amphotericin B

^bLiposomal amphotericin B

^cFlucytosine

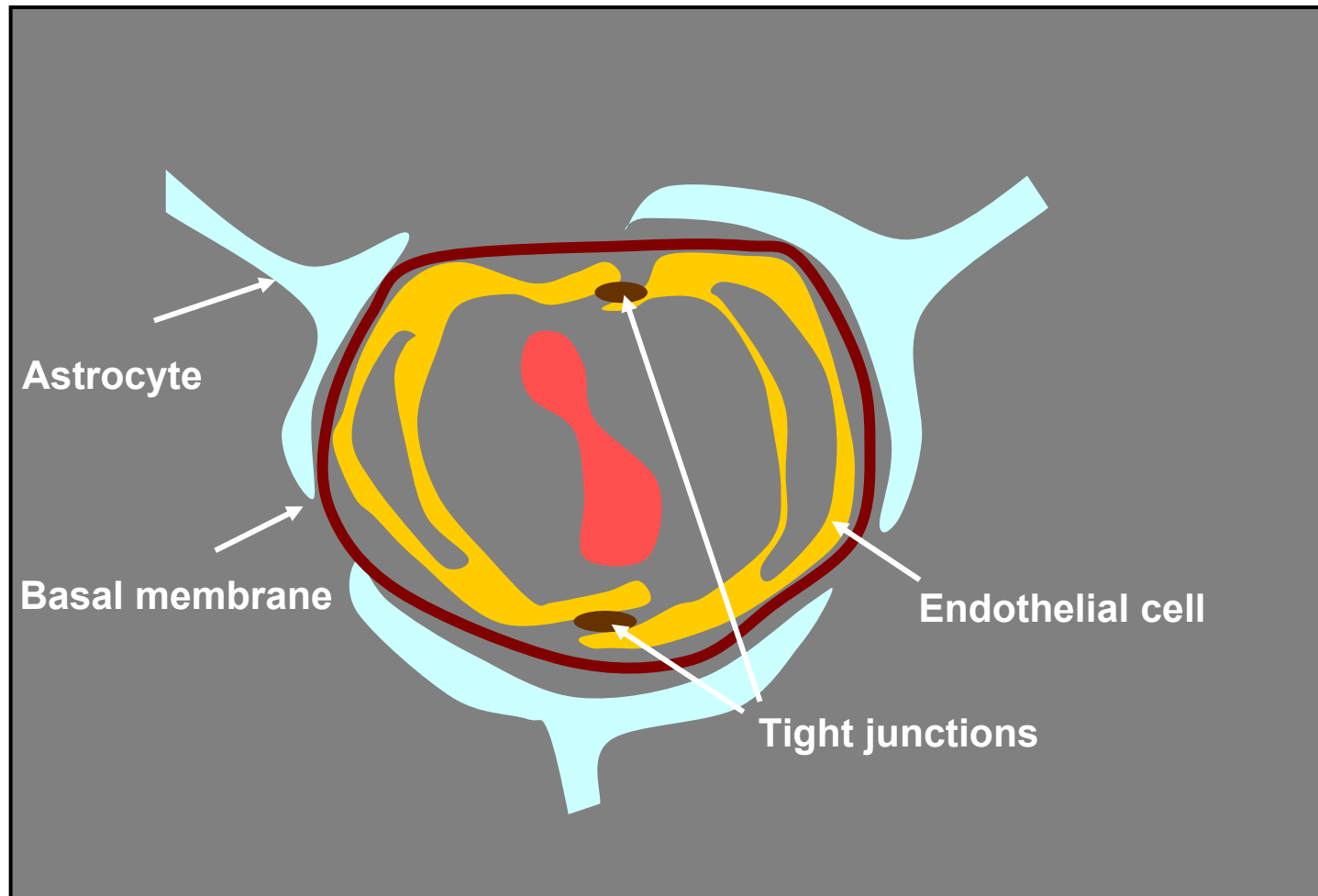
^dItraconazole

Schwartz S, Ruhnke M, Ribaud P, Reed E, Troke P, Thiel E, *Mycoses*. 2007;50:196-200.

Blood-brain Barrier

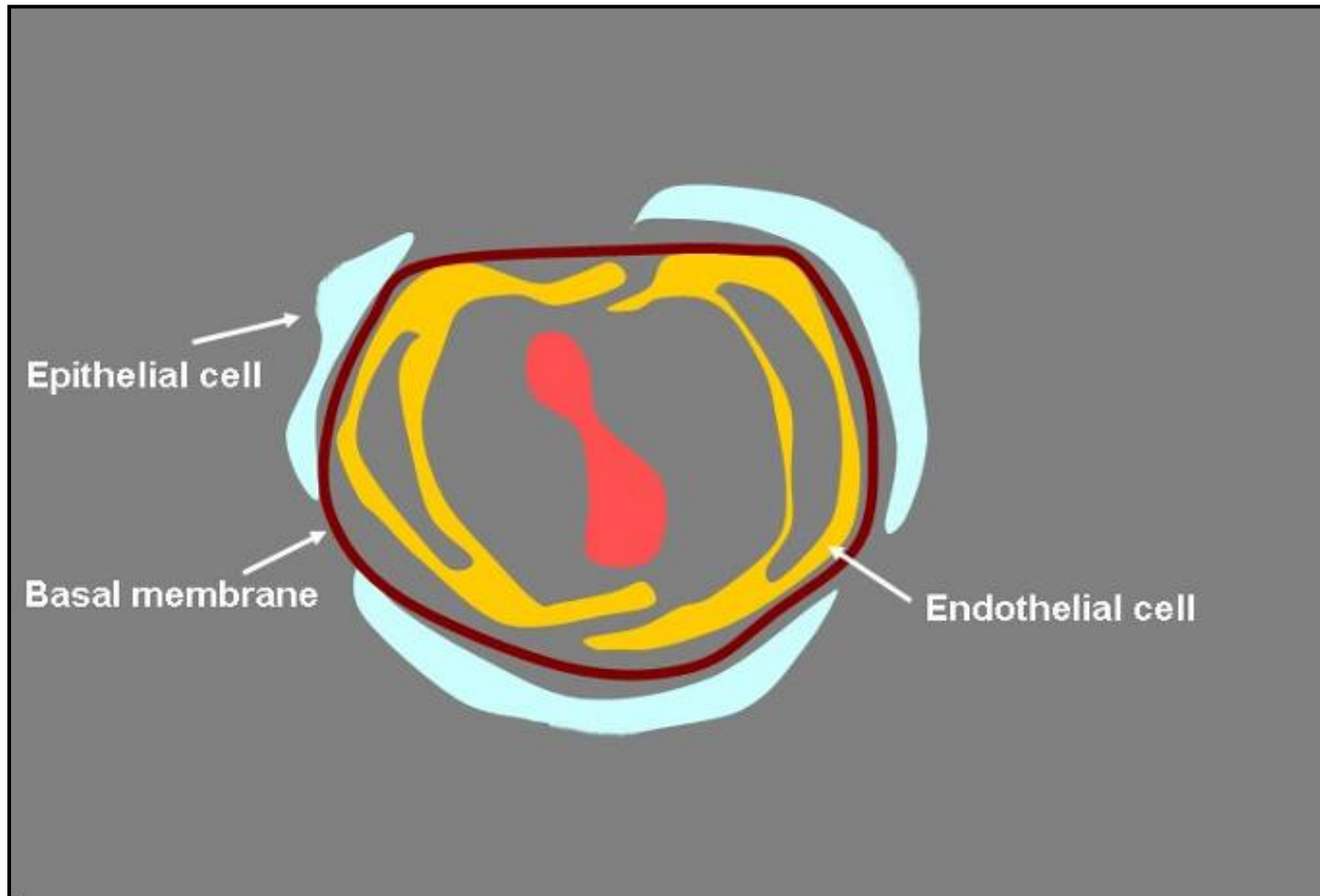
Approximate total vessel length: 600 km \approx 373 miles

Approximate area of blood-brain barrier (BBB): 20m²

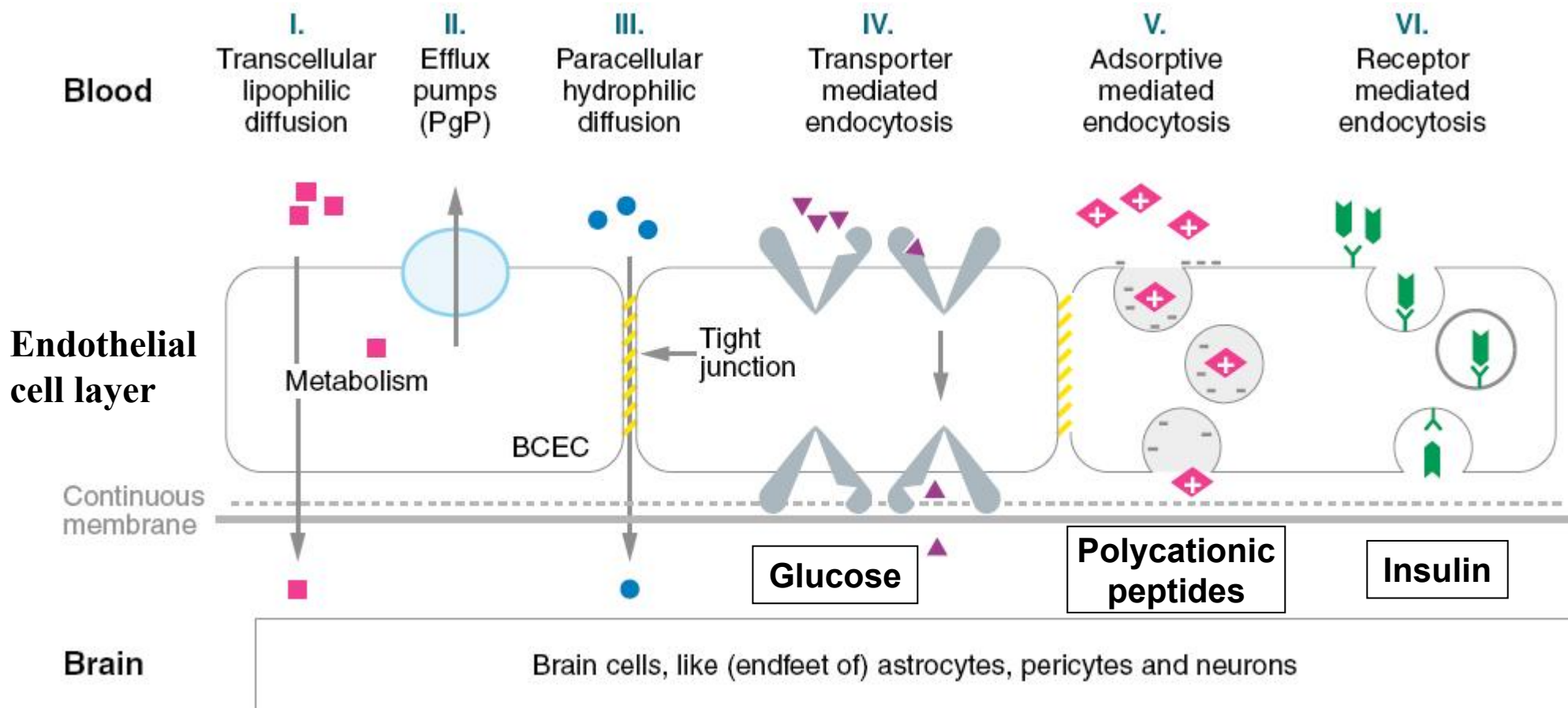


Blood-cerebrospinal-fluid Barrier

- Choroid plexi and arachnoid (**fenestred**) epithelium
- Faces the cerebrospinal fluid
- Approximate area of blood-cerebrospinal fluid barrier \approx BBB: 20m²



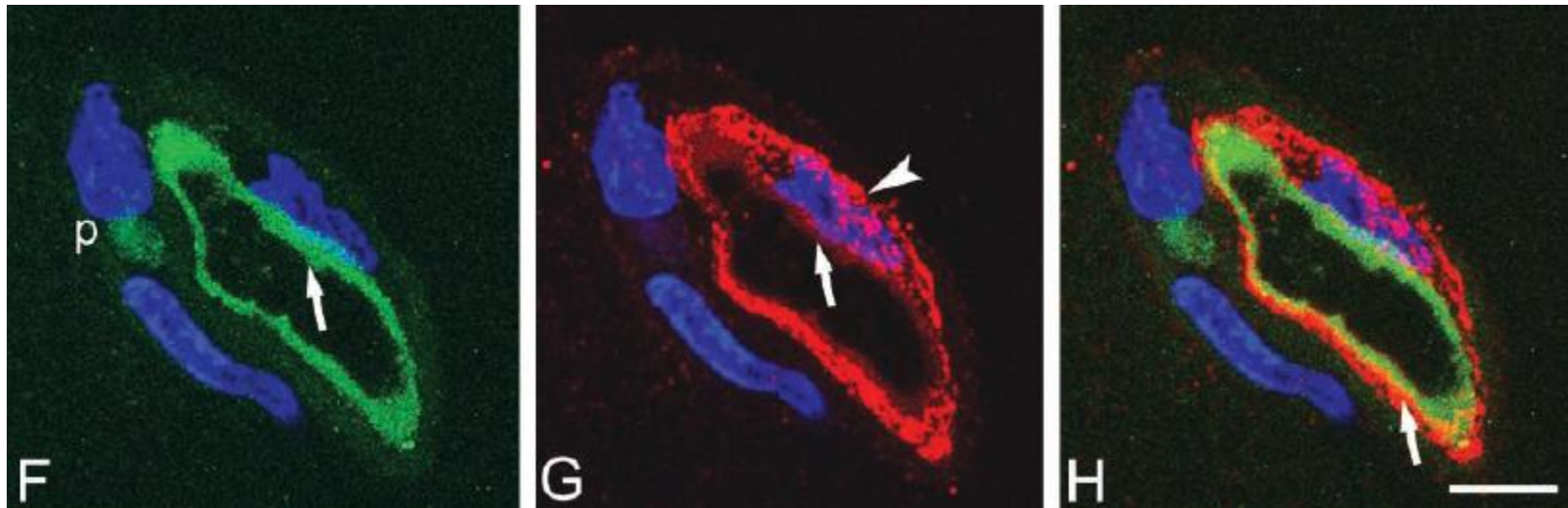
Blood-brain Barrier Transport Systems



P-gp^a Expression in Human Cortex Vessels

Cortex specimens from patients with high-grade glioma
Confocal laser microscopy

Cortex microvessel



P-gp

Caveolin-1

**P-gp
Caveolin-1**

^a P-glycoprotein

Virgintino D, Robertson D, Errede M, et al. *J Histochem Cytochem.* 2002;50:1671-1676.

Factors Impacting Blood-brain Barrier Penetration

Factor	Effect on CNS concentration
Lipophilicity	↑
Low molecular weight	↑
High cerebral blood flow	↑
BBB disruption (meningitis)	↑
Short $t_{1/2}$ and low serum levels	↓
High protein binding	↓
Target for efflux pumps	↓
Molecular charge	↓

Pharmacokinetic Parameters¹⁻⁶

Drug	t ¹ / ₂ β (h)	Protein binding
Amphotericin B	7-37	≥91%
Caspofungin	11	96%
Micafungin	11-17	99%
Anidulafungin	18	84%
Itraconazole	21-37	≥95%
Posaconazole	16-29	>95%
Fluconazole	27-37	≤12%
Voriconazole	6	65%
Flucytosine	3-6	4%

1. Groll AH, Piscitelli SC, Walsh TJ. *Adv Pharmacol.* 1998;44:343-500.

2. Denning DW. *Lancet.* 2003;362:1142-1151.

3. Keating GM. *Drugs.* 2005;65:1553-1167.

4. Courtney R, Pai S, Laughlin M, Lim J, Batra V. *Antimicrob Agents Chemother.* 2003;47:2788-2795.

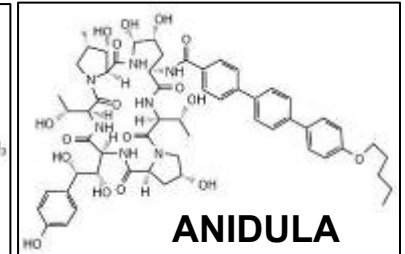
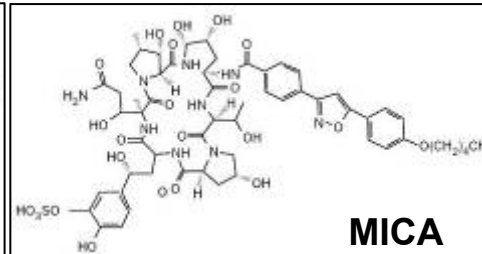
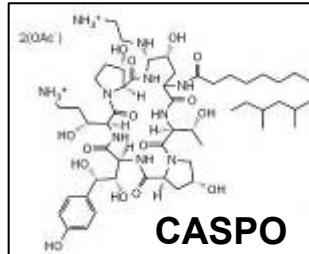
5. Bekersky I, Fielding RM, Dressler DE, Lee JW, Buell DN, Walsh TJ. *Antimicrob Agents Chemother.* 2002;46:828-833.

6. Heinemann V, Bosse D, Jehn U, et al. *Antimicrob Agents Chemother.* 1997;41:1275-1280.

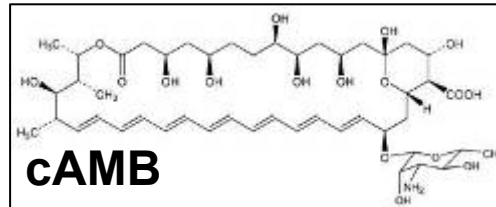
Molecular Size of Antifungal Drugs

Molecular weight (Da)

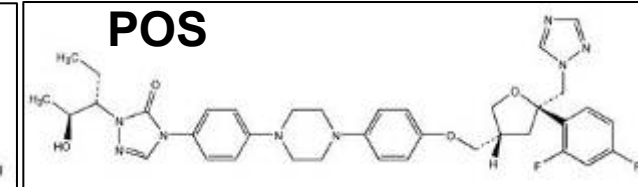
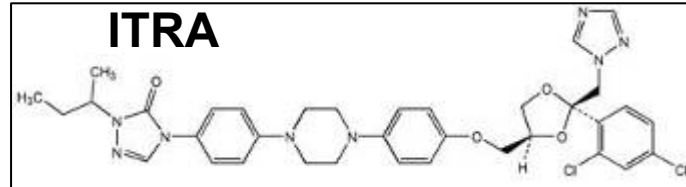
~1200



924



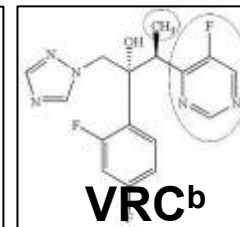
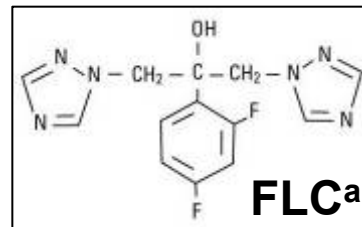
705/708



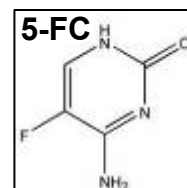
400-600

Transcellular lipophilic diffusion across the intact BBB

306/349



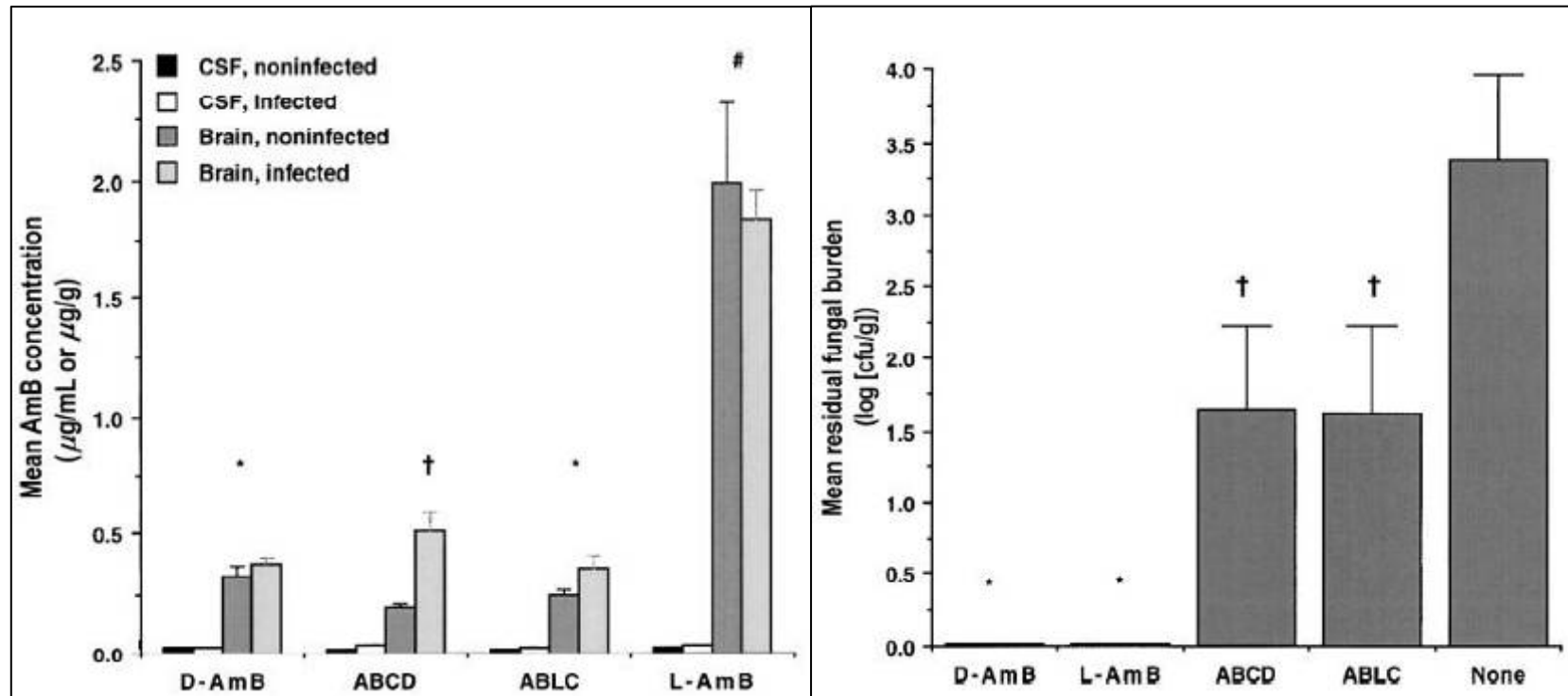
129



^aFluconazole
^bVoriconazole

Brain Tissue Penetration of Amphotericin B

Rabbits +/- *C. albicans* meningoencephalitis
7 days of antifungal treatment with
D-AmB^a 1mg/kg; ABCD^b, ABLC^c, or L-AmB^d 5mg/kg

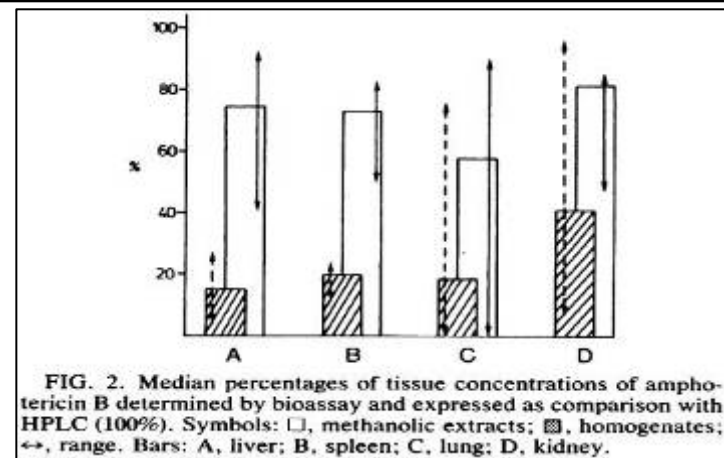


^aAmphotericin B deoxycholate; ^bAmphotericin B colloidal dispersion; ^cAmphotericin B lipid complex; ^dliposomal Amphotericin B
Groll AH, Giri N, Petraitis V, et al. *J Infect Dis.* 2000;182:274-282.

Amphotericin in Human Brain Tissue^{1,2}

Tissue specimens from necropsy
methanolic extraction -> high-pressure liquid chromatography

	Brain tissue ($\mu\text{g/g}$) median (range)	Recovery (% total dose) median (range)
Amphotericin B deoxycholate (n=9)	0.5 (0.2-5.8)	0.3 (0-1.4)
Liposomal Amphotericin B (n=8)	0.7 (<0.1-1.6)	0.2 (<0.1-0.2)

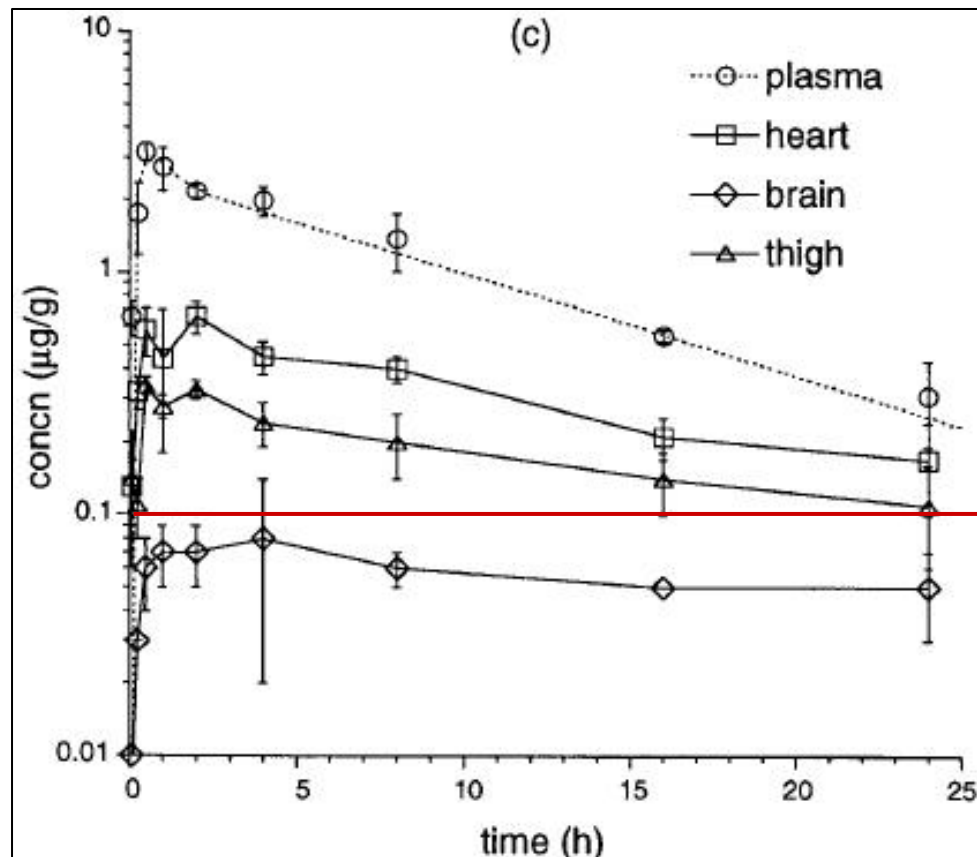


1. Collette N, van der Auwera P, Lopez AP, Heymans C, Meunier F. *Antimicrob Agents Chemother.* 1989;33:362-368.
2. Collette N, Van der Auwera P, Meunier F, Lambert C, Sculier JP, Coune A. *J Antimicrob Chemother.* 1991;27:535-548.

Brain Tissue Penetration Echinocandins

Concentrations of caspofungin over time

Single i.p. injection of 1mg/kg
3 mice per time point



Brain Tissue Penetration Echinocandins^{1,2} (cont'd)

3-6 neutropenic rabbits per group
challenged iv with *C. albicans*
Treatment for 10 days

3 healthy rabbits per group
no immunosuppression
Treatment for 8 days

Anidulafungin

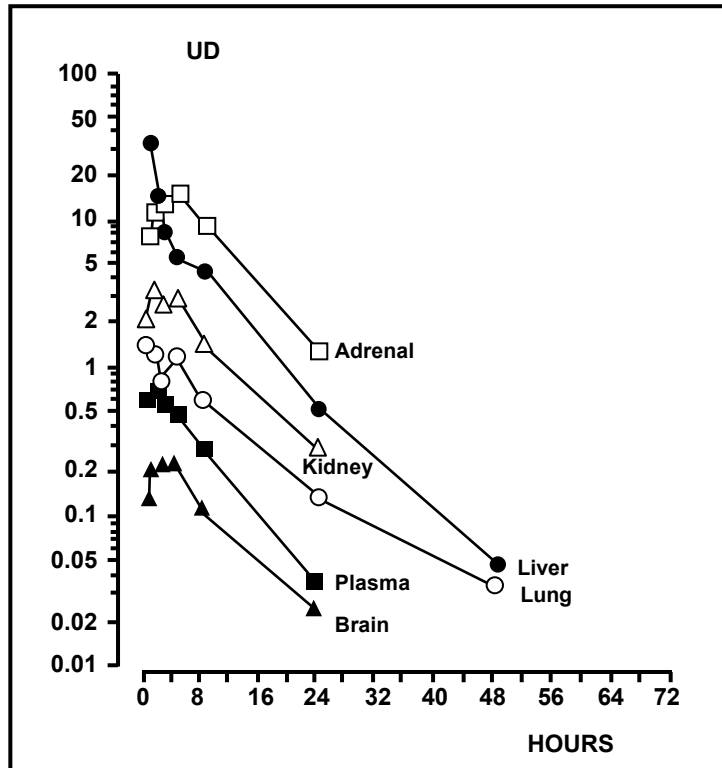
Micafungin

Dose (mg/kg)	Brain ($\mu\text{g/g}$)	Brain ($\mu\text{g/g}$)
0.1	<LLQ	-
0.25	<LLQ	-
0.5	0.25	0.08
1	0.42	0.1
2	-	0.18
5	1.58	-
10	3.91	-

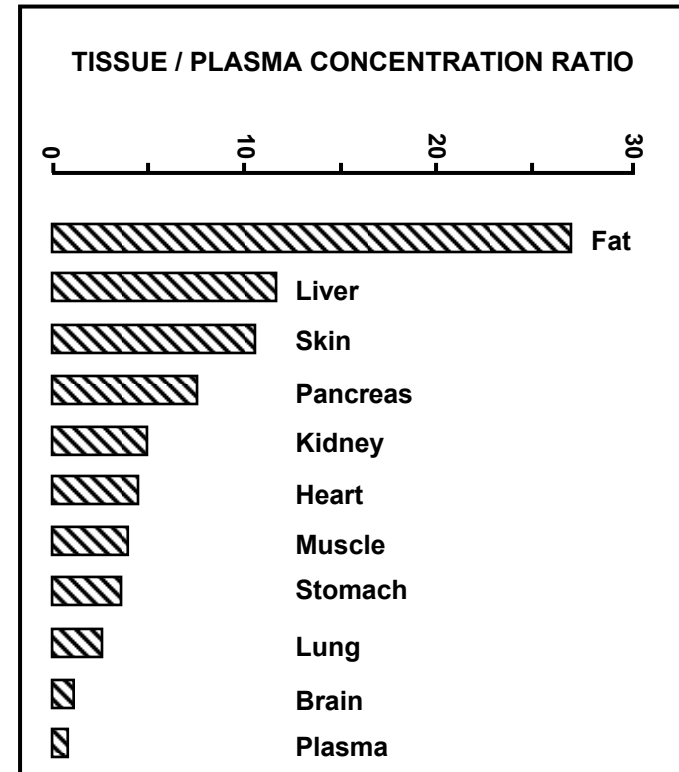
1. Groll AH, Mickiene D, Petraitiene R, et al. *Antimicrob Agents Chemother.* 2001;45:2845-2855.

2. Groll AH, Mickiene D, Petraitis V, et al. *Antimicrob Agents Chemother.* 2001;45:3322-3327.

Brain Tissue Concentration Itraconazole



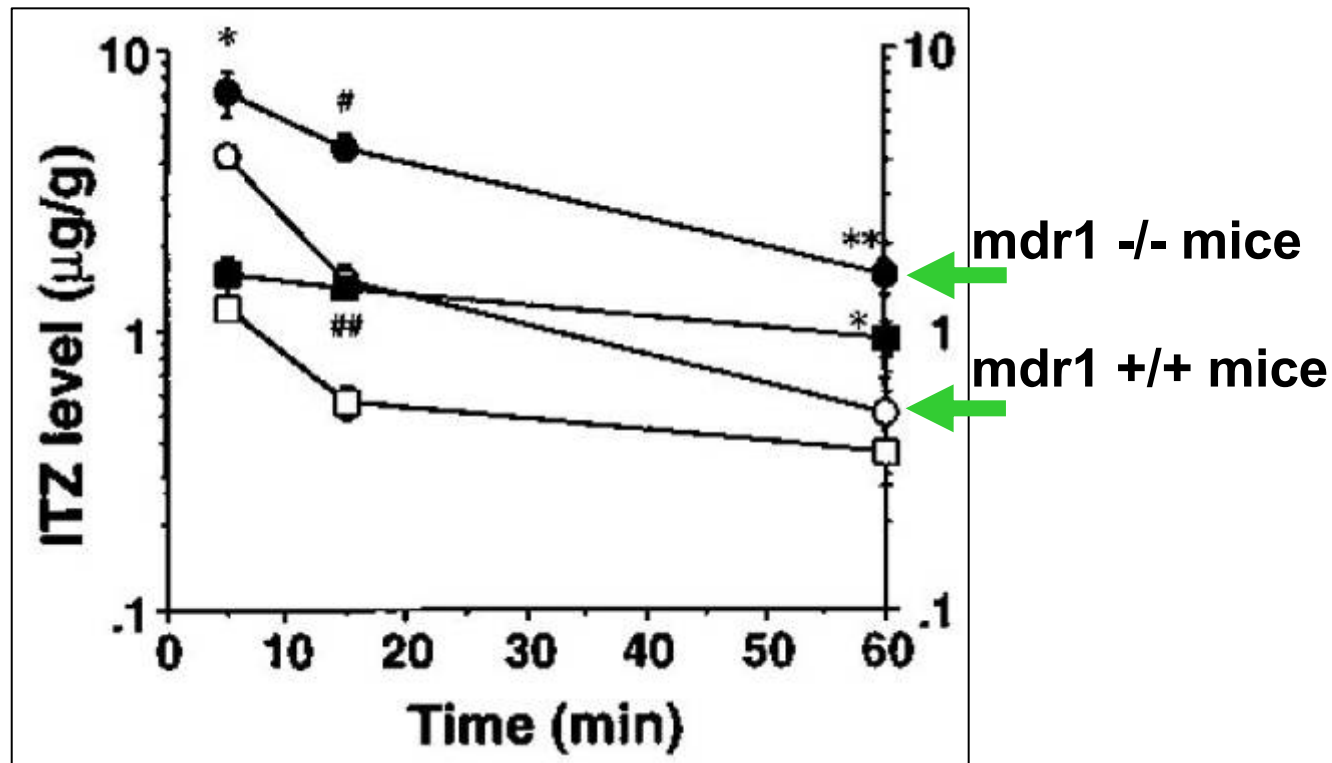
Tissue concentrations in rats after a single dose of 10 mg/kg



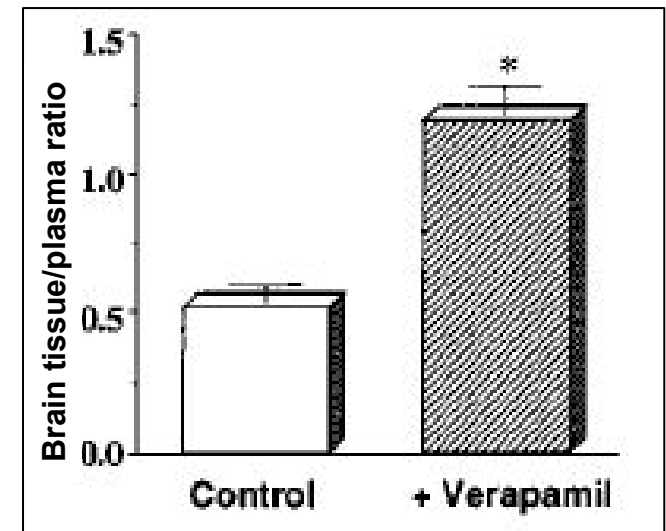
Mean tissue/plasma ratios in dogs after 12 months daily treatment with 5, 20, and 80 mg/kg

Itraconazole Brain Concentrations in *mdr1* ^{-/-} mice

mdr1 knockout mice (*mdr1* ^{-/-}) vs FVB (*mdr1* ^{+/+}) mice
Itraconazole 5mg/kg iv

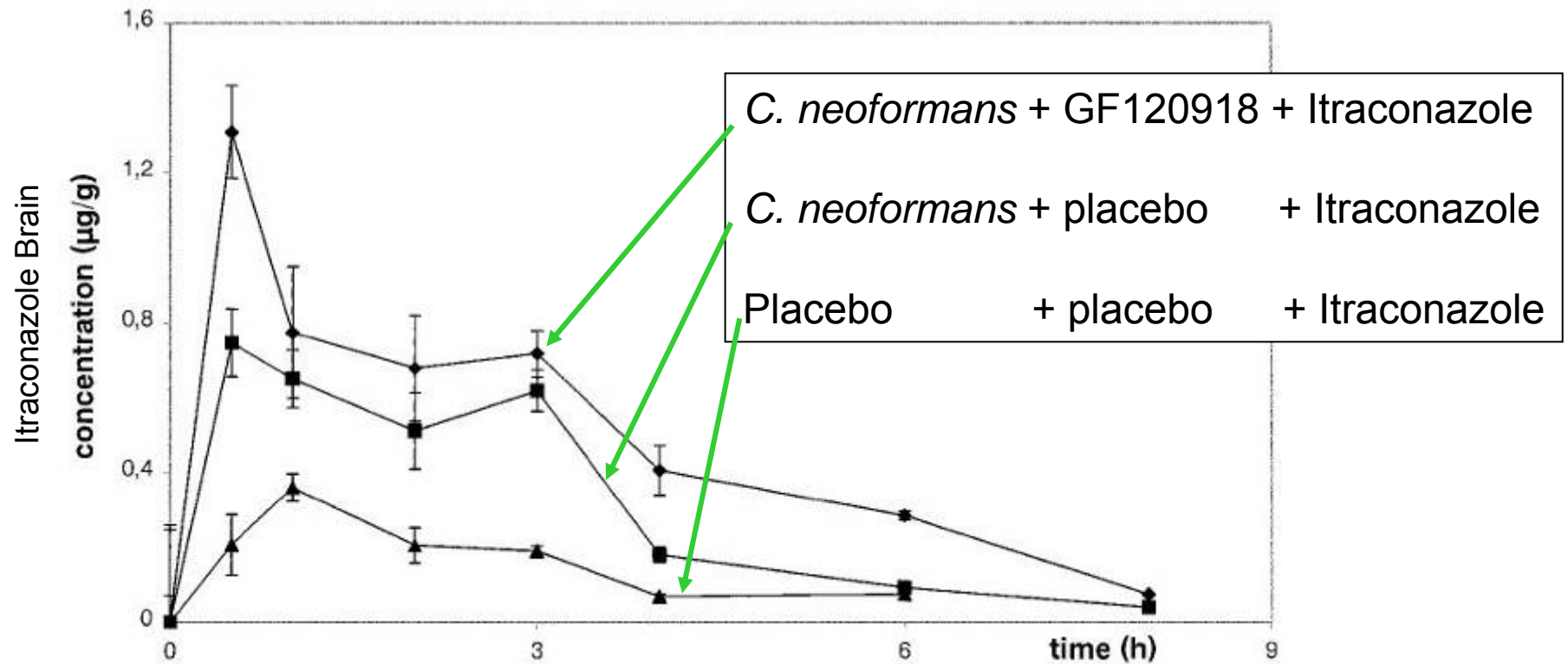


Rats treated iv with:
5mg/kg itraconazole
-/+ prior 5mg/kg verapamil



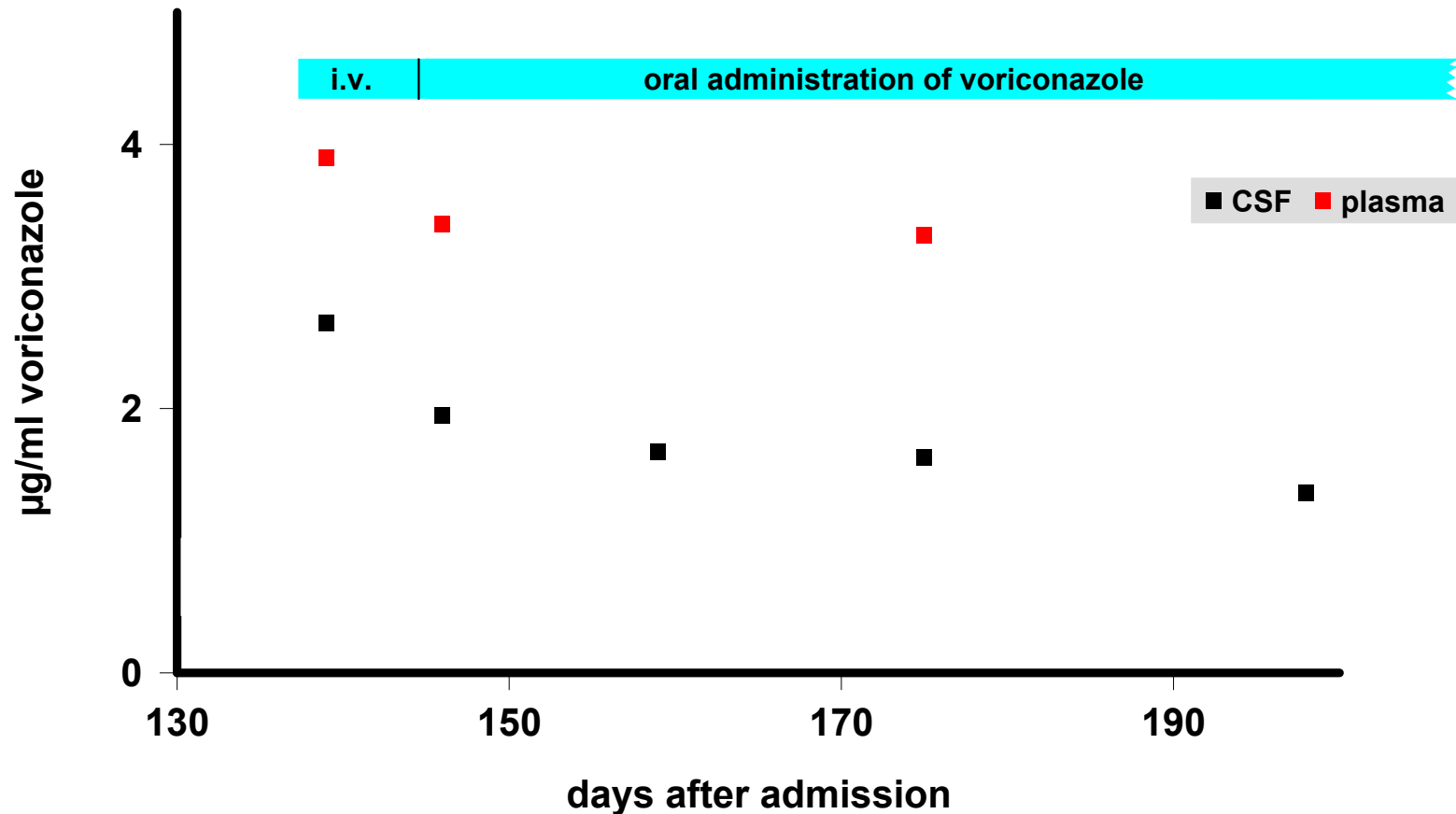
Modulation of Cerebral Itraconazole Concentration

Mice intracerebrally infected with *C. neoformans*
P-gp inhibitor (GF120918) 10mg/kg q12 i.p.
Itraconazole 20mg/kg q12 i.p.

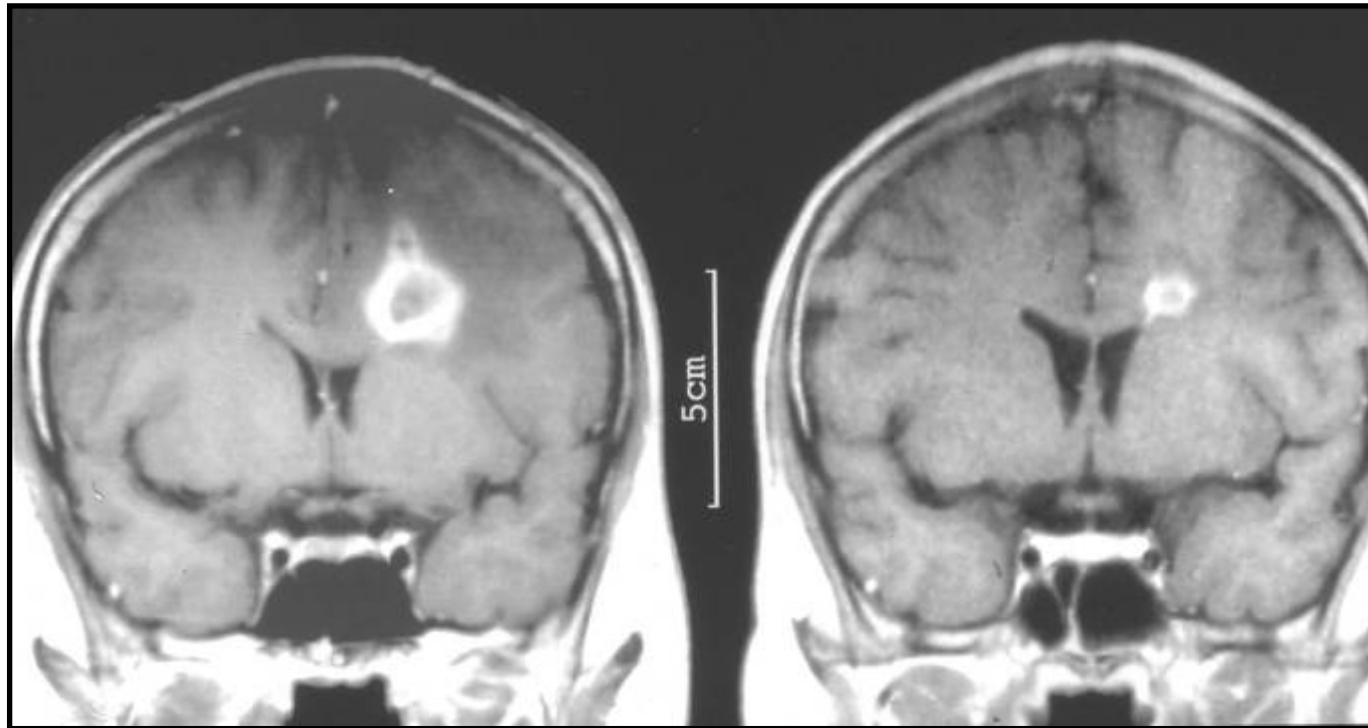


Voriconazole Blood/CSF Peak (1h) Levels

18-year-old male with acute leukemia and pulmonary/cerebral aspergillosis
convAmB, L-AmB, itraconazole, AmB i.th./intralesional → progression



Voriconazole in Cerebral Aspergillosis



4 months of voriconazole



Voriconazole Levels Brain Tissue/Abscess

2 patients with invasive pulmonary aspergillosis
† 9-10 hours after stop of VRC

Age (years)	Dosing	Brain tissue
36	4.5 mg/kg q12h	11.8 µg/g
13	7.2 mg/kg q24h	58.5 µg/g

Lutsar I, Roffey S, Troke P. *Clin Infect Dis.* 2003;37:728-732.

1 patient
rhinocerebral
aspergillosis
(4mg/kg q12h)

Specimen	Brain tissue
Liquid abscess	1.4 µg/g
Solid abscess	1.2 µg/g

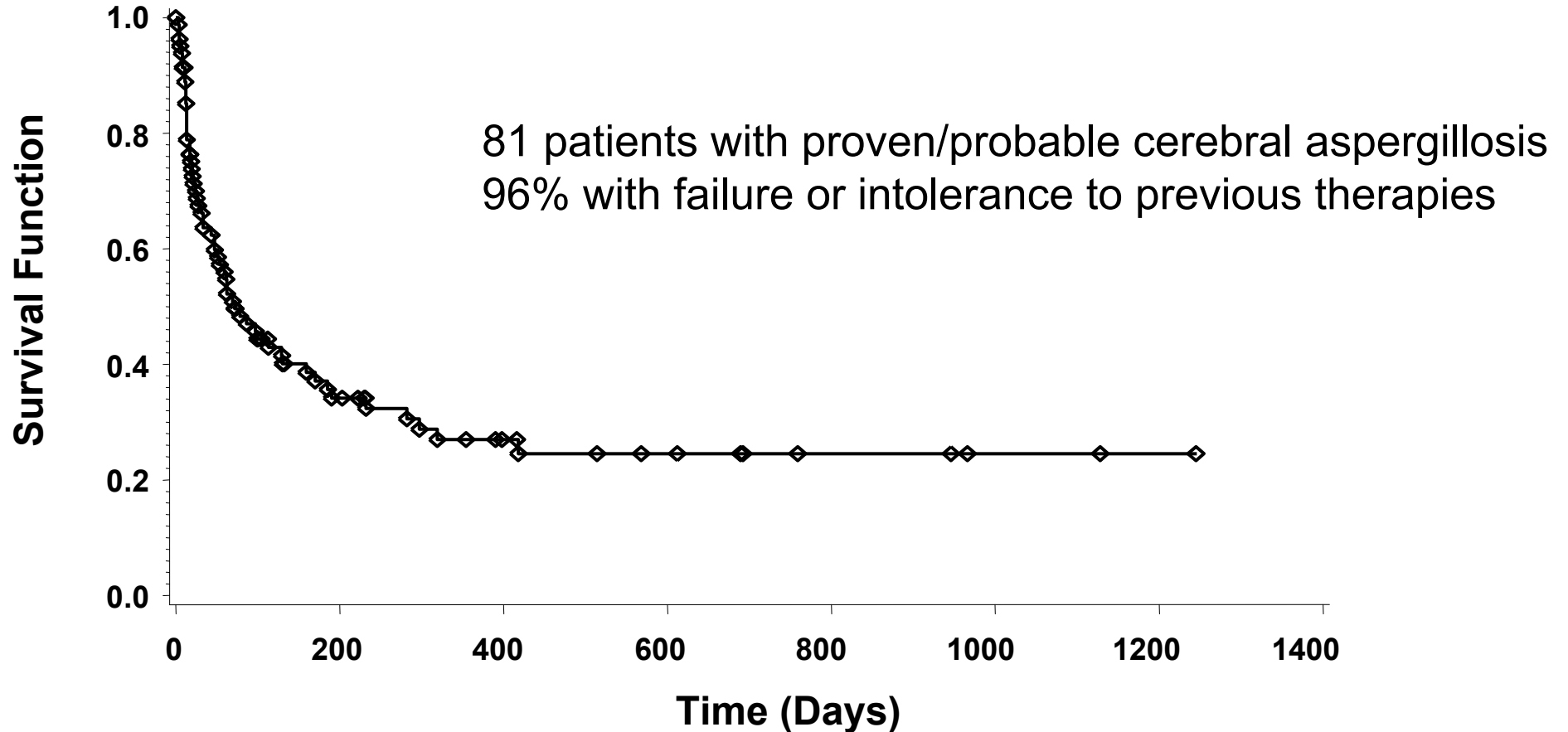
Elter T, Sieniawski M, Gossmann A, et al. *Int J Antimicrob Agents.* 2006;28:262-265.

1 patient
C. albicans
meningoencephalitis
(4mg/kg q12h)

Specimen	Brain tissue	Post dose (hours)
Liquid abscess	5.1 µg/mL	4
Dura mater	1.4 µg/g	3.5

Schwartz S, et al., *ICAAC.* 2007:440 (M-1161).

Voriconazole for Cerebral Aspergillosis



**Duration of reported survival:
3-1,245 days (median: 69 days)**

Survivors of Central Nervous System-aspergillosis

**Case report: 1 survivor with acute leukemia
review of 25 previously published cases**

Underlying disease

Acute leukemia	4
NHL	1
Solid organ transplant	4
Lupus, sarcoidosis, steroids	3
CGD	1
Otitis, head injury	2
Drug/alcohol abuse	2
Not reported	9

Follow-up (unknown for 7 patients): **3 - 72 months**
(median: 18 months)

Survivors of Central Nervous System-aspergillosis

(cont'd)

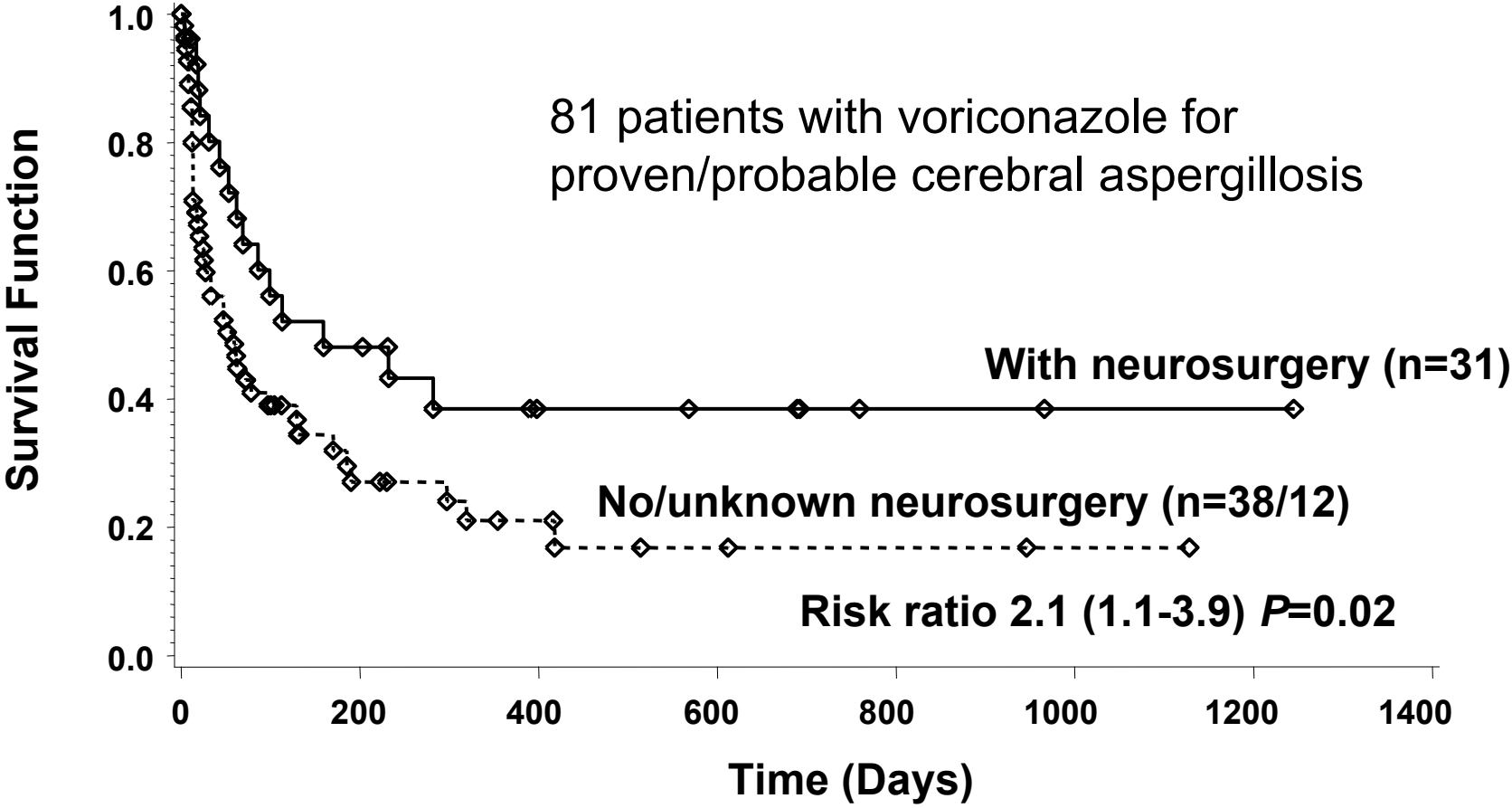
Antifungal treatment

Amphotericin B iv	19
Amphotericin B intralesional/-ventr.	6
L-AMB (3-15mg/kg)	1
5-FC	10
Itraconazole	3
Other	3
None/unknown	3

Interventions

Multiple (3x) abscess resections	1	} 73% with neurosurgery
Craniotomy	14	
Stereotactic drainage	3	
Intracavitary catheter	3	
Other surgery	4	
None	3	

Impact of Neurosurgery on Survival

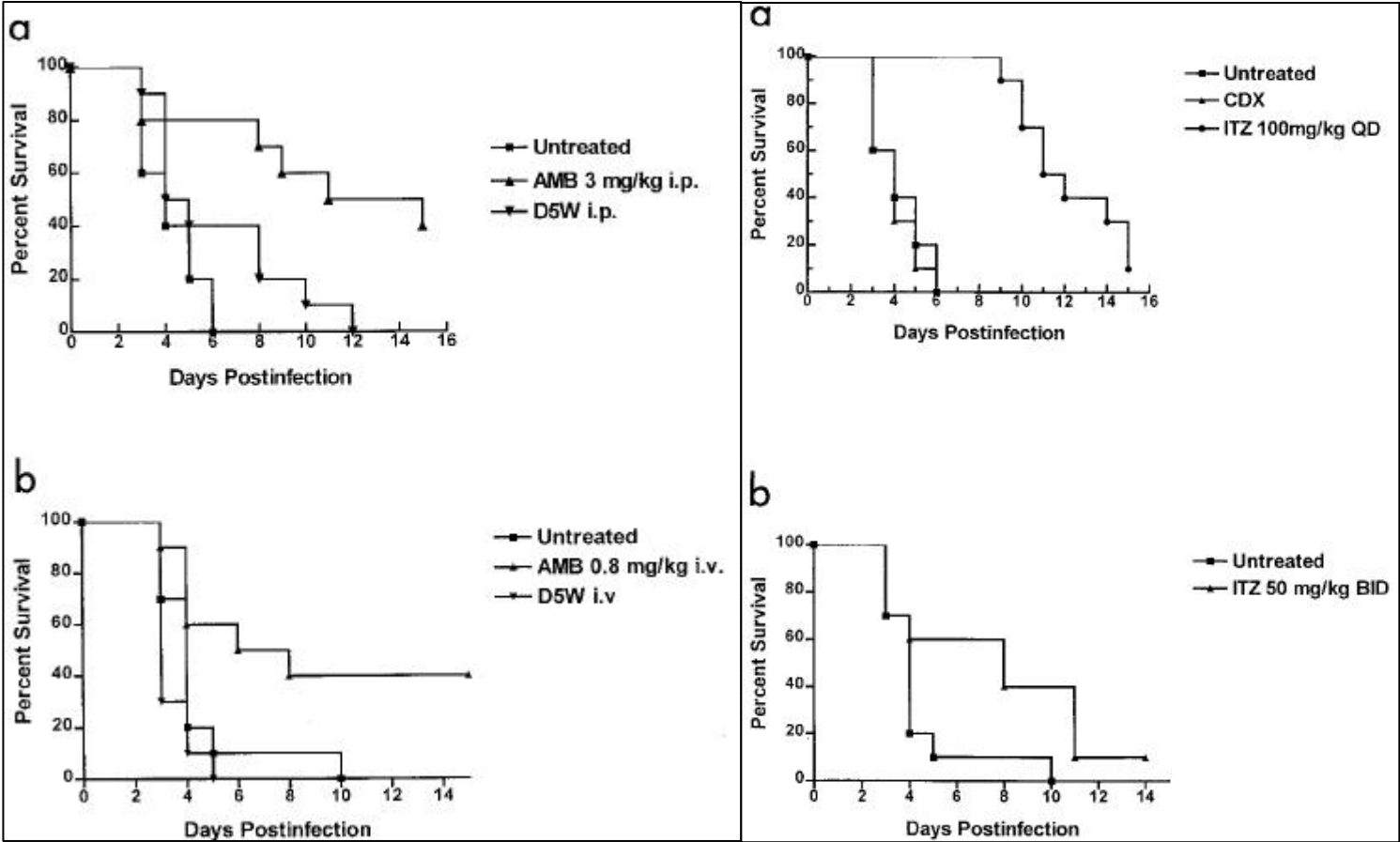


Craniotomy/abscess resection (14), abscess drainage (12), ventricular shunt (4), Ommaya-reservoir (1)

Schwartz S, Ruhnke M, Ribaud P, et al. *Blood*. 2005;106:2641-2645.

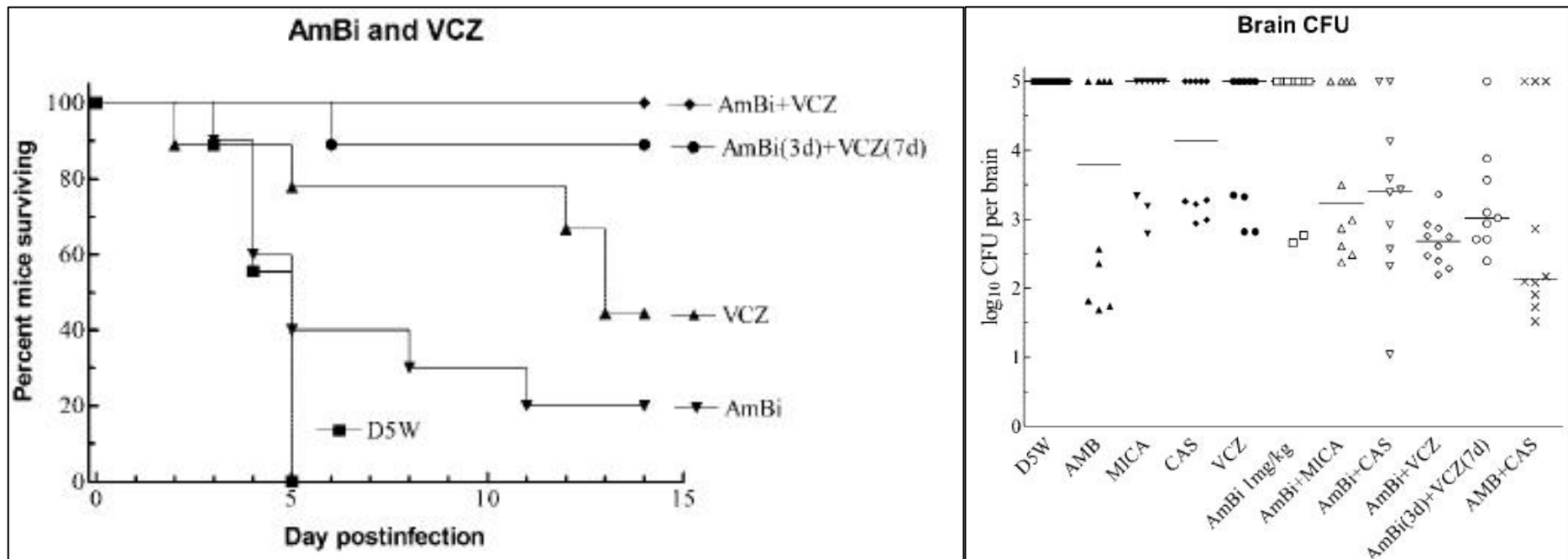
Efficacy cAMB or ITRA in experimental CNS aspergillosis

CD-1 mice (cyclophosphamide) intracerebrally infected with *A. fumigatus*
cAMB 3mg/kg/day i.p., cAMB 3mg/kg/day, Itraconazole 5 or 100mg/kg/day orally



Combination therapy in experimental CNS aspergillosis

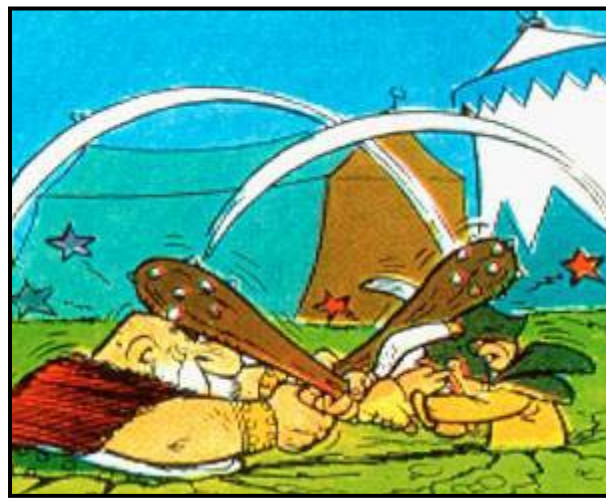
CD-1 mice (cyclophosphamide) intracerebrally infected with *A. fumigatus*
L-AMB (AmBi) 1mg/kg/day i.v., Voriconazole 40mg/kg/day orally



The Future?



New drugs



Combination therapy



Neurosurgeon