

Demystifying Medicine Lecture Series
January 29, 2019

Fungus Infections: Neglected, Dangerous and Increasing

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Laboratory of Clinical Infectious Diseases
National Institute of Allergy & Infectious Diseases



Lots of Fungi (~5M!) Not Too Many Are Important To Us

Yeasts

Candida, Cryptococcus

Filamentous molds

Aspergillus, Mucor, Trichophyton

Dimorphic fungi

*Histoplasma, Coccidioides, Paracoccidioides,
Blastomyces*

Only a Few Cause Disease in Normal Hosts

Yeasts

Candida (vaginal yeast infections), *Cryptococcus*

Filamentous molds

Aspergillus, *Mucor*, *Trichophyton* (athlete's foot, nail infections)

Dimorphic fungi

Histoplasma, *Coccidioides*, *Paracoccidioides*,
Blastomyces

Beyond these 2 superficial fungal infections, severe or refractory mycoses typically underlie host defects

The Global Burden of Fungal Infections

Statistics of the 10 most significant invasive fungal infections.

Disease (most common species)	Location	Estimated life-threatening infections /year at that location*	Mortality rates (% in infected populations)*
Opportunistic invasive mycoses			
Aspergillosis (<i>Aspergillus fumigatus</i>)	Worldwide	>200,000	30–95
Candidiasis (<i>Candida albicans</i>)	Worldwide	>400,000	46–75
Cryptococcosis (<i>Cryptococcus neoformans</i>)	Worldwide	>1,000,000	20–70
Mucormycosis (<i>Rhizopus oryzae</i>)	Worldwide	>10,000	30–90
Pneumocystis (<i>Pneumocystis jirovecii</i>)	Worldwide	>400,000	20–80
Endemic dimorphic mycoses*†			
Blastomycosis (<i>Blastomyces dermatitidis</i>)	Midwestern and Atlantic United States	~3,000	<2–68
Coccidioidomycosis (<i>Coccidioides immitis</i>)	Southwestern United States	~25,000	<1–70
Histoplasmosis (<i>Histoplasma capsulatum</i>)	Midwestern United States	~25,000	28–50
Paracoccidioidomycosis (<i>Paracoccidioides brasiliensis</i>)	Brazil	~4,000	5–27

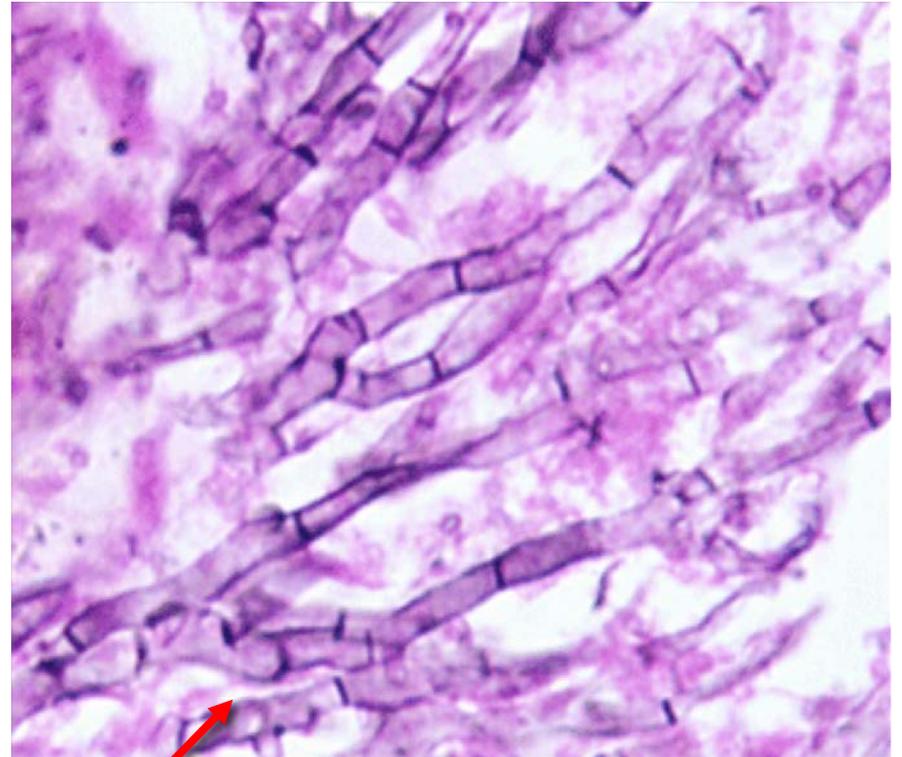
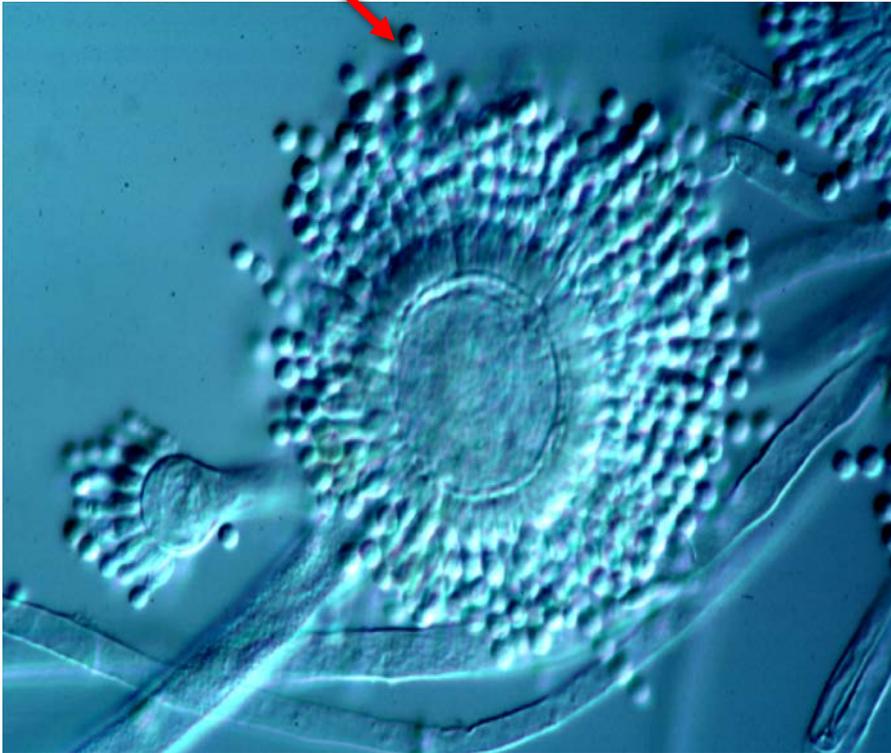
The Global Burden of Fungal Infections

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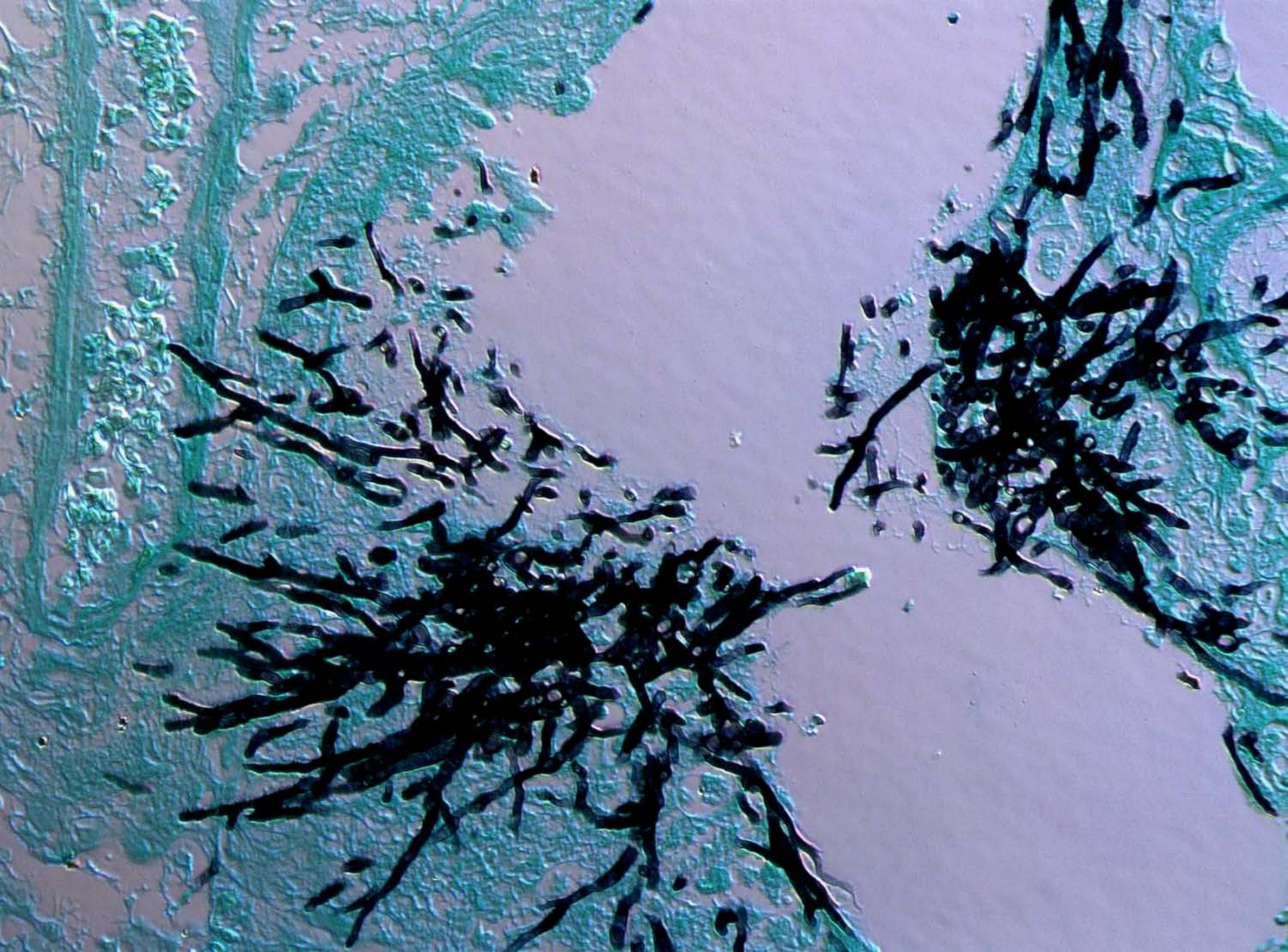
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Aspergillus

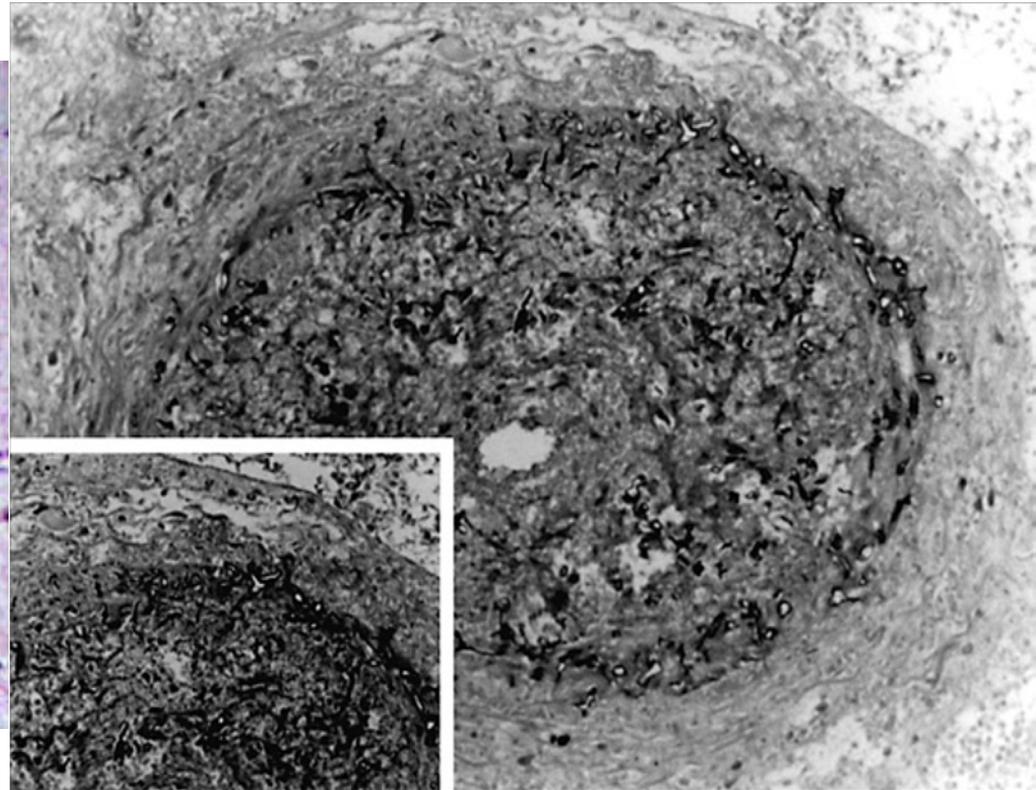
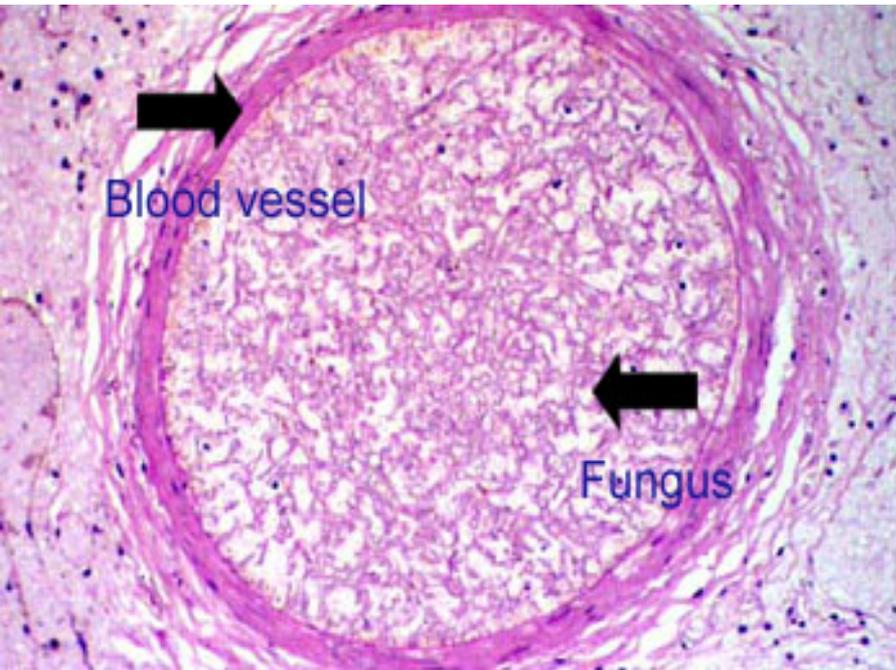
Conidia



Hyphae

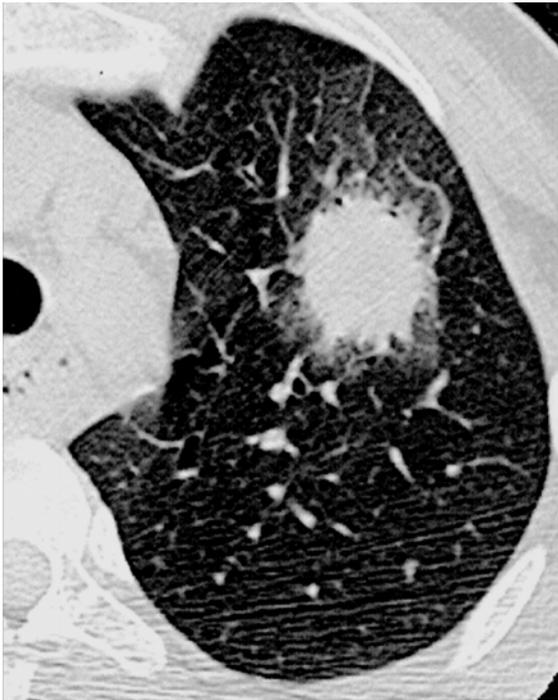


Molds Are Angioinvasive



Lionakis MS, Lahderanta J, Sun J et al. *Infect Immun.* 2005; 73: 7747-7758
Ibrahim AS, Spellberg B, Avanesian V et al. *Infect Immun.* 2005; 73: 778-783
Ribes JA, Vanover-Sams CL & Baker DJ. *Clin Microbiol Rev.* 2000; 13: 236-301

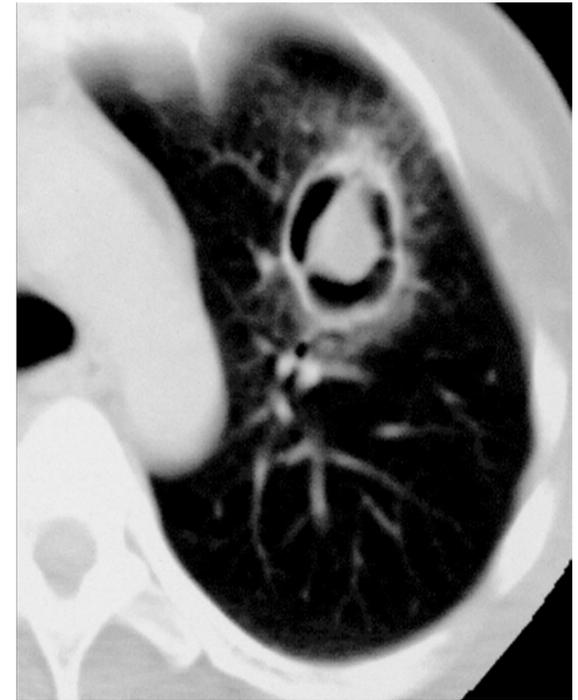
Aspergillus in the Lung



Halo sign

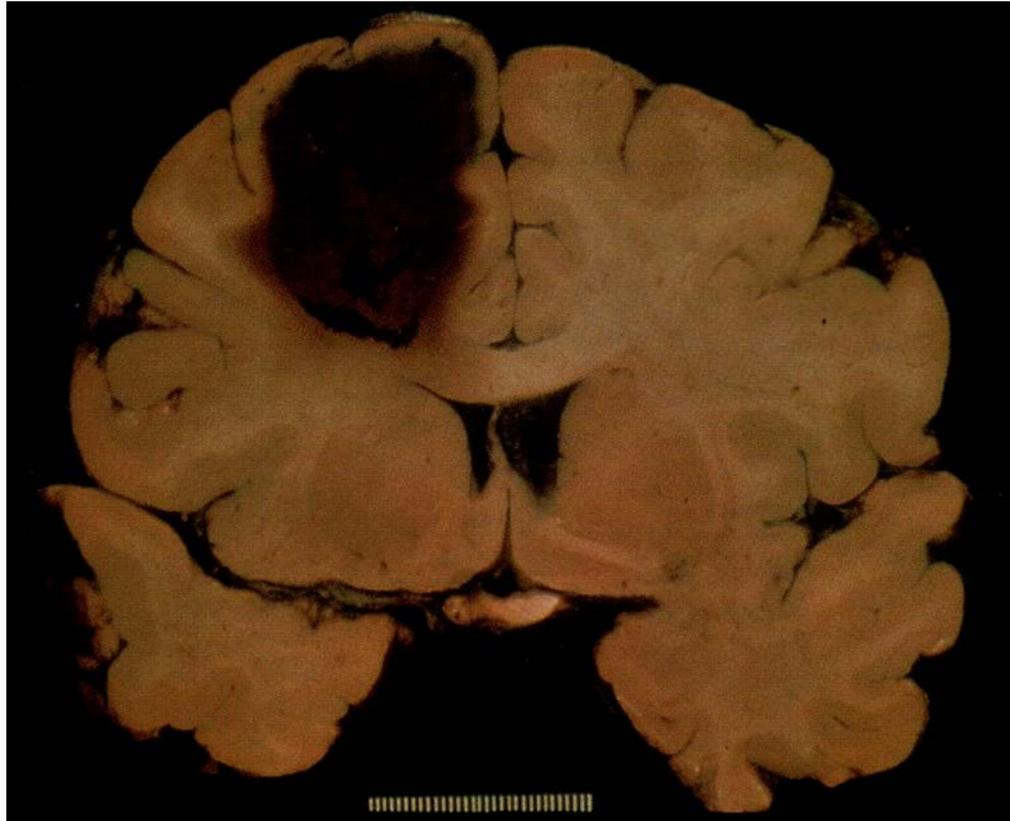


Non-specific nodule



Air crescent-sign

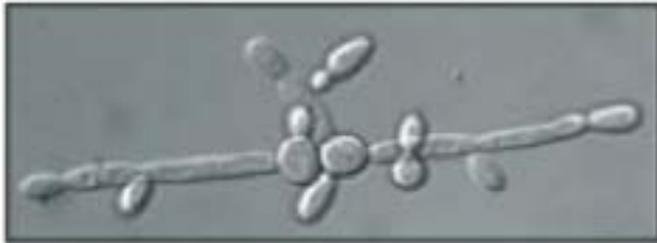
Aspergillus Dissemination





Candida

Pseudohyphae



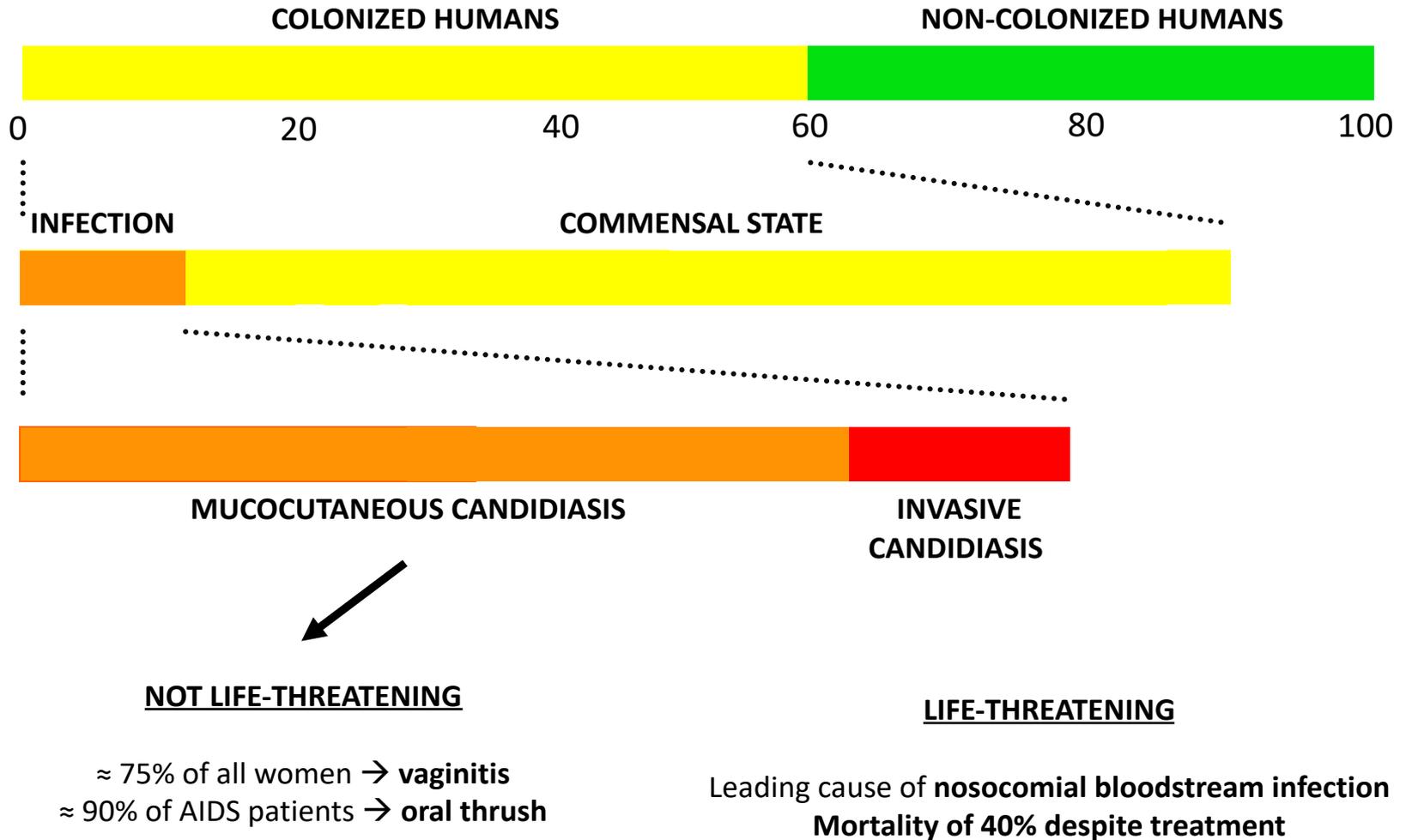
Yeast



Hyphae

- *C. albicans* part of normal human commensal flora
- Multiple morphologies – yeast (commensal), hyphae (pathogenic) and pseudohyphae
- Causes wide range of infections following disruption to immunity
 - Mucosal
 - Systemic

The Burden of *Candida* Infections in Humans



Spectrum of *Candida* Infections

A. Cutaneous

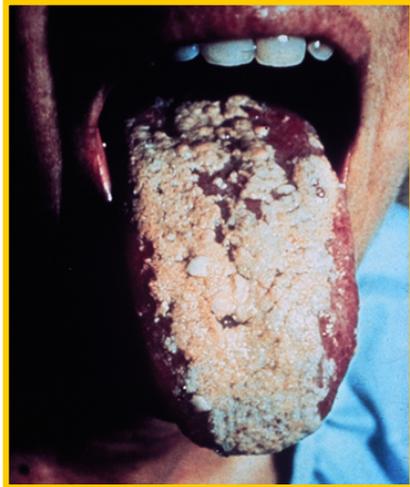


Deeply Invasive

C. Disseminated



B. Mucosal

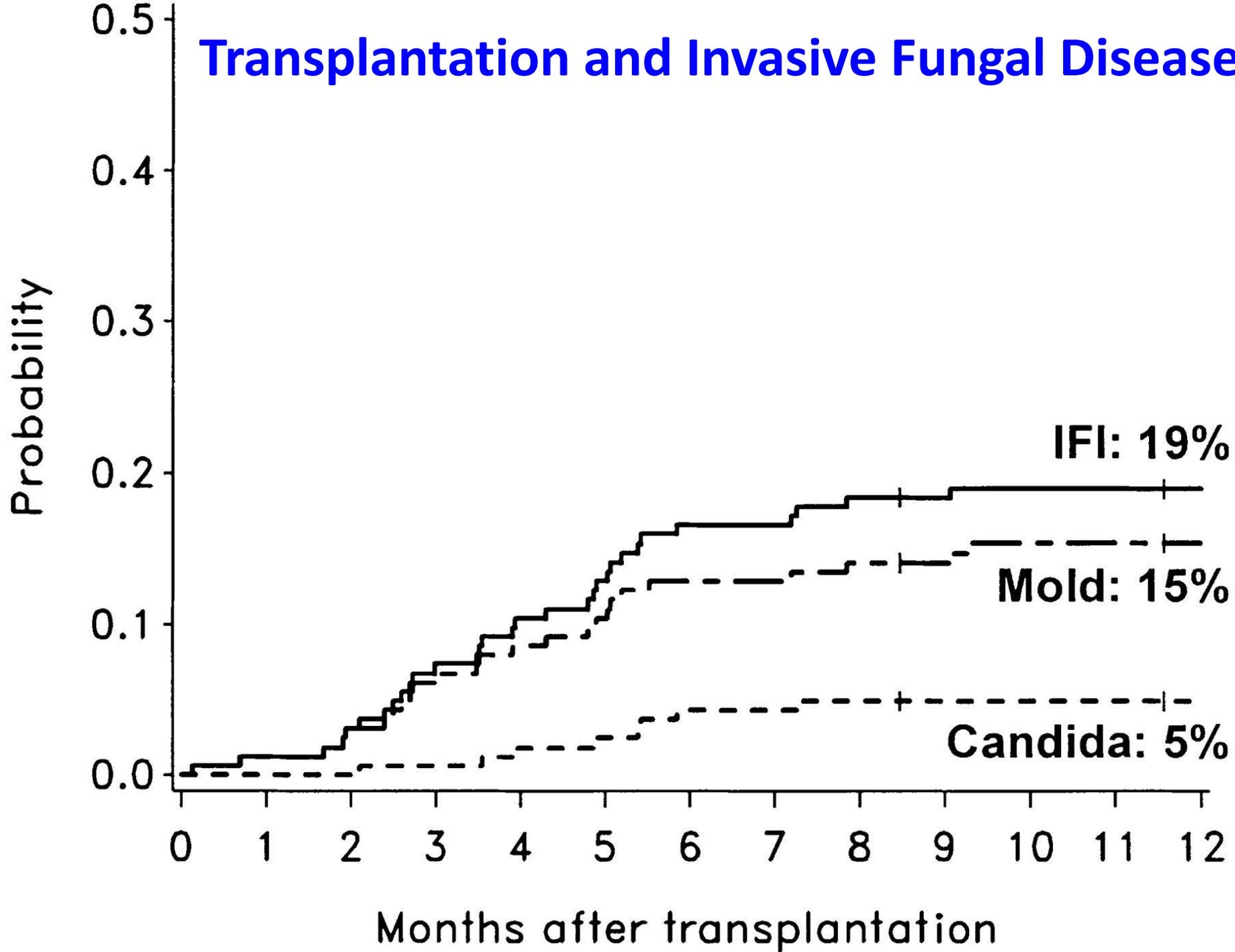


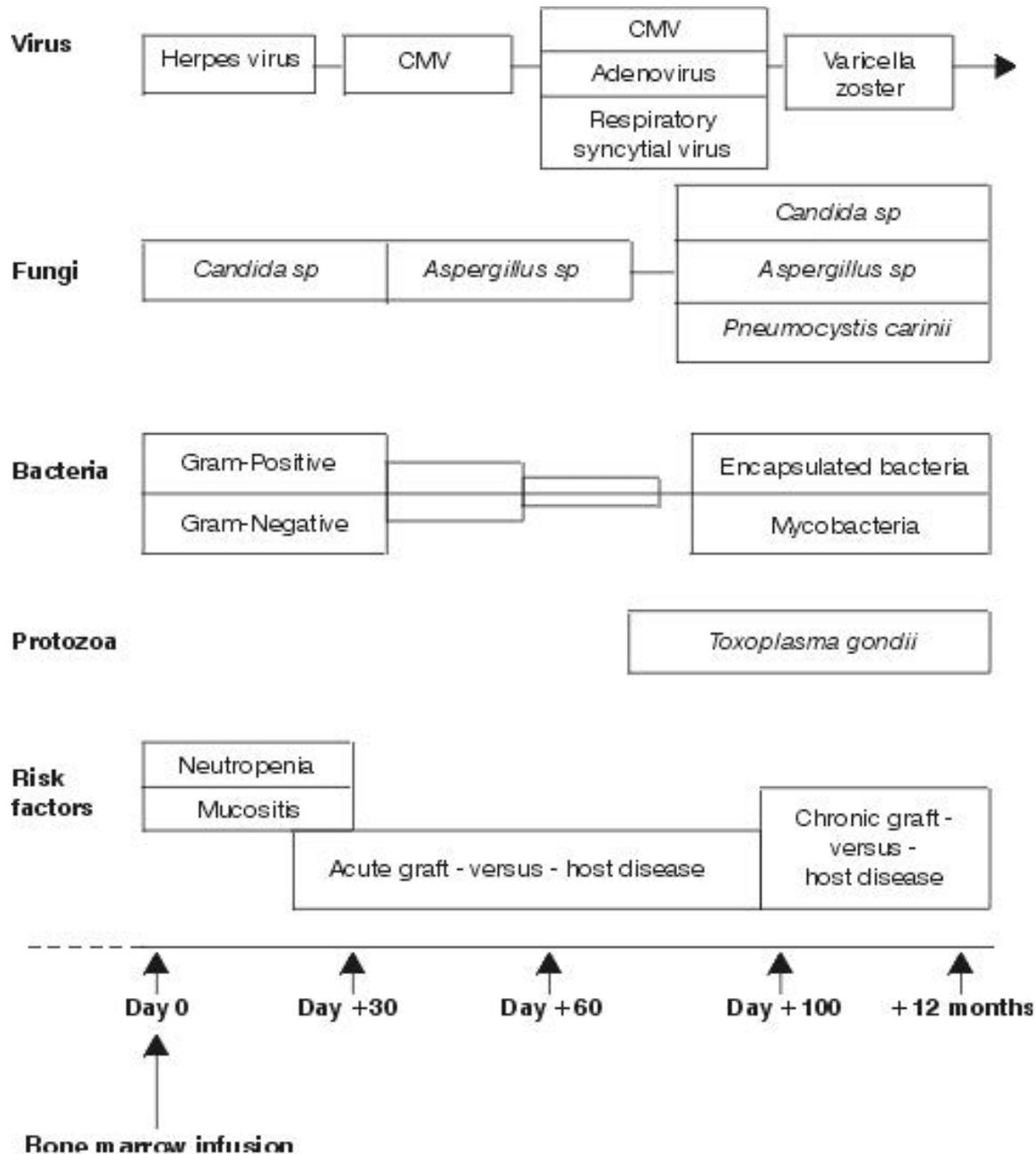
D. Chorioretinitis



Images courtesy of Kenneth Rolston, MDACC
Walsh et al. Infect Dis Clin North Am. 1996;10:365-400.

Transplantation and Invasive Fungal Disease





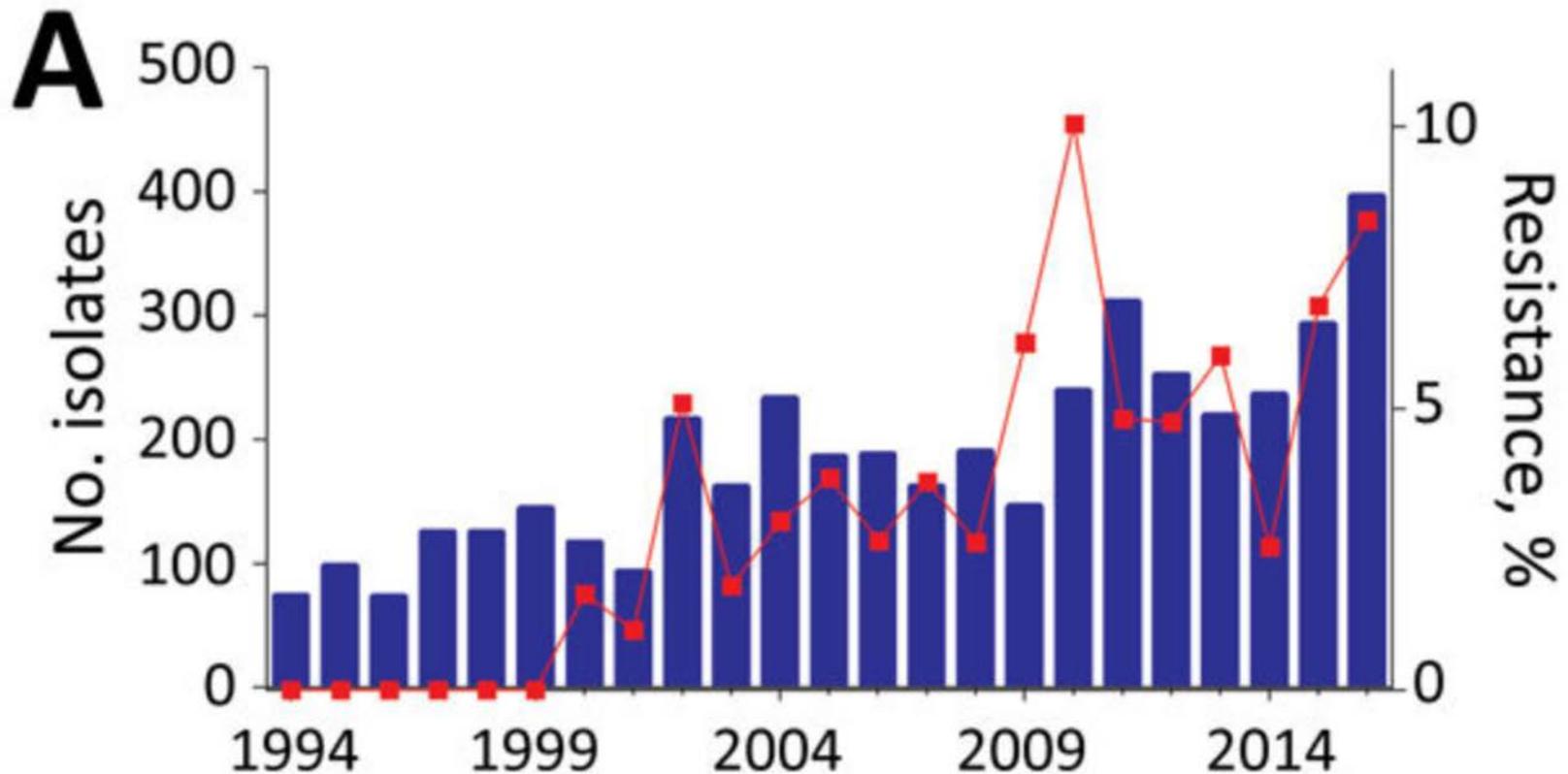
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Trends in Azole Resistance in *Aspergillus fumigatus*, the Netherlands, 1994–2016

Jochem B. Buil, Eveline Snelders,
Laura Bedin Denardi, Willem J.G. Melchers,
Paul E. Verweij



Increasing Echinocandin Resistance in *Candida glabrata*: Clinical Failure Correlates With Presence of *FKS* Mutations and Elevated Minimum Inhibitory Concentrations

Barbara D. Alexander,¹ Melissa D. Johnson,¹ Christopher D. Pfeiffer,^{1,a} Cristina Jiménez-Ortigosa,³ Jelena Catania,¹ Rachel Booker,² Mariana Castanheira,⁴ Shawn A. Messer,⁴ David S. Perlin,³ and Michael A. Pfaller⁴

Clinical Infectious Diseases

MAJOR ARTICLE



OXFORD

Simultaneous Emergence of Multidrug-Resistant *Candida auris* on 3 Continents Confirmed by Whole-Genome Sequencing and Epidemiological Analyses

Shawn R. Lockhart,¹ Kizee A. Etienne,¹ Snigdha Vallabhaneni,¹ Joveria Farooqi,⁴ Anuradha Chowdhary,⁶ Nelesh P. Govender,⁷ Arnaldo Lopes Colombo,⁸ Belinda Calvo,⁹ Christina A. Cuomo,² Christopher A. Desjardins,² Elizabeth L. Berkow,¹ Mariana Castanheira,³ Rindidzani E. Magobo,⁷ Kauser Jabeen,⁴ Rana J. Asghar,⁵ Jacques F. Meis,^{10,11} Brendan Jackson,¹ Tom Chiller,¹ and Anastasia P. Litvintseva¹

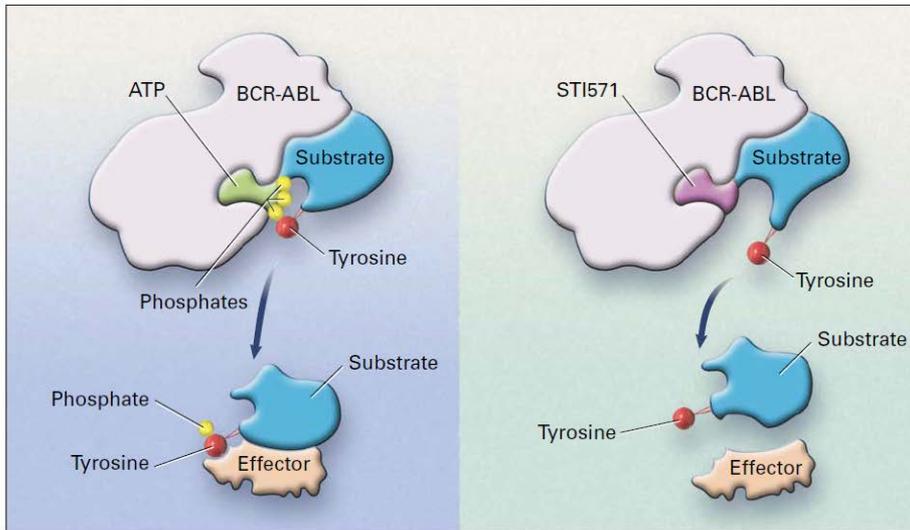
Novel Iatrogenic Risk Factors for Fungal Disease

Imatinib Changed Everything

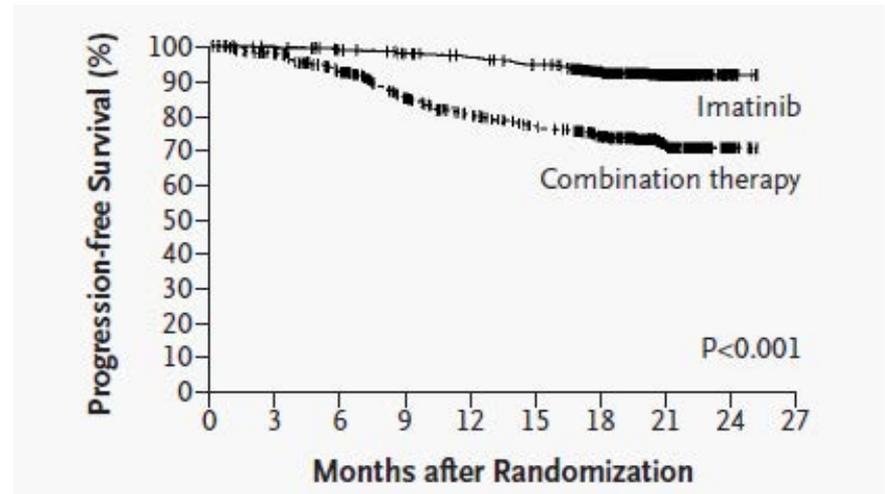
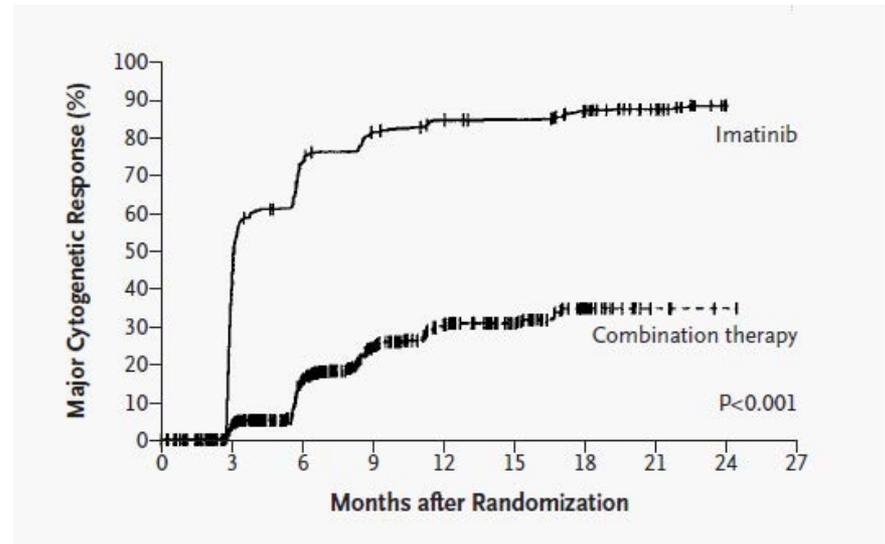
Dan L. Longo, M.D.



N ENGL J MED 376;10 NEJM.ORG MARCH 9, 2017

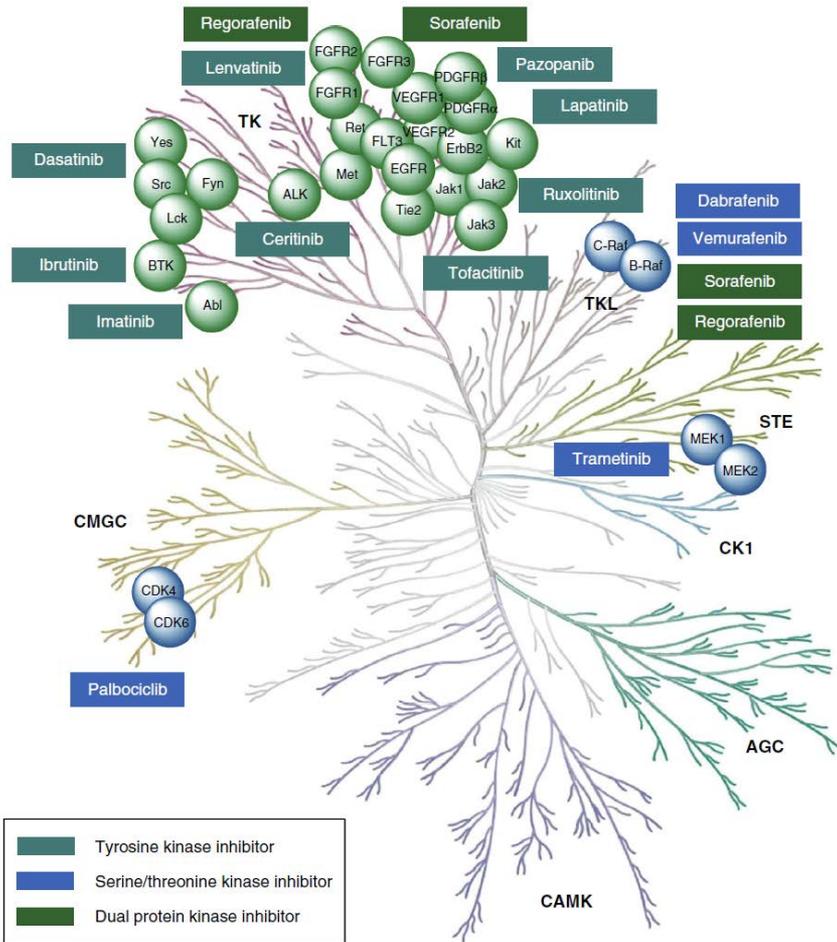


N Engl J Med, Vol. 344, No. 14 · April 5, 2001

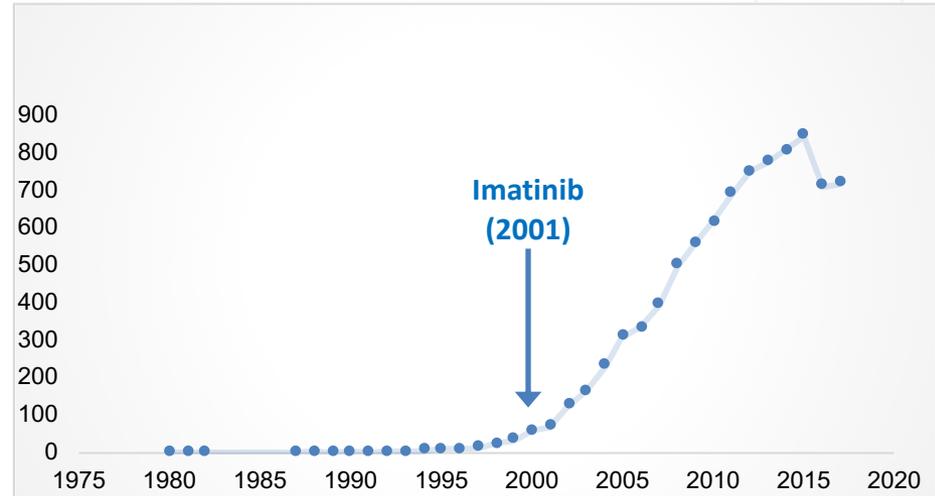


N ENGL J MED 348;11 WWW.NEJM.ORG MARCH 13, 2003

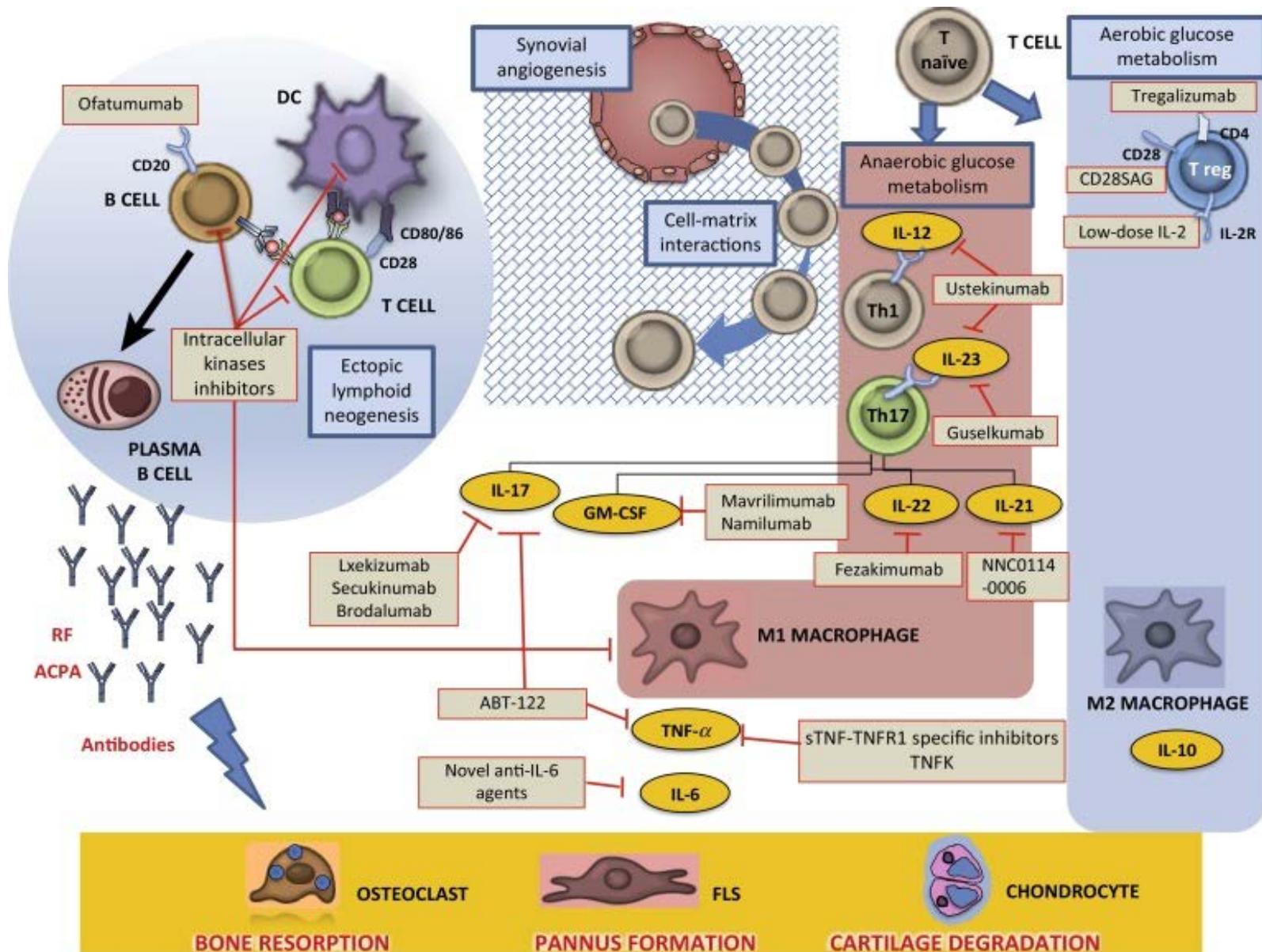
A Surge of SMKIs in Recent Years



PUBMED RESEARCH:
Small Molecule Kinase Inhibitors (SMKIs)



A Surge of Anti-Cytokine Biologics



Overview

Basic principles of host defense against:

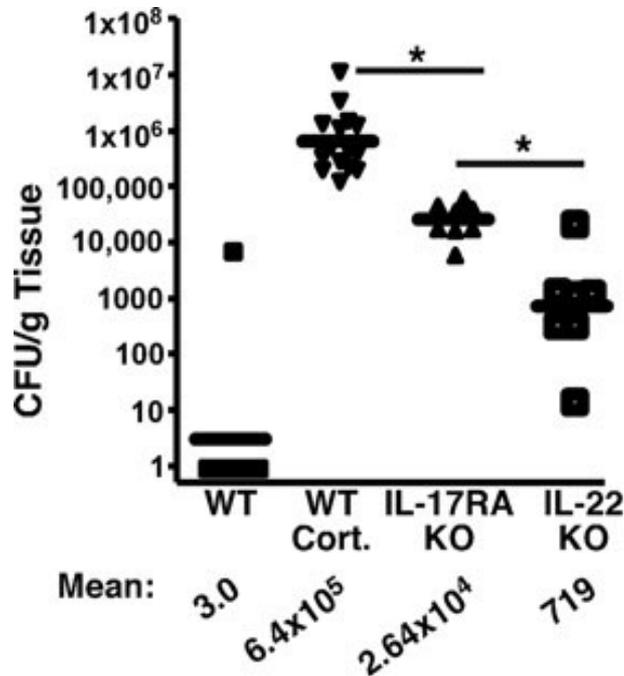
- mucosal fungal disease
- intracellular fungi (endemics, *Cryptococcus*)
- invasive mold and *Candida* infections

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IL-17 Signaling is Important for Anti-*Candida* Mucosal Host Defense in Mice and Humans



Conti et al., *J Exp Med.* 2009

Chronic Mucocutaneous Candidiasis in Humans with Inborn Errors of Interleukin-17 Immunity

Anne Puel,^{1,†} Sophie Cypowyj,^{2,*} Jacinta Bustamante,¹ Jill F. Wright,³ Luyan Liu,¹ Hye Kyung Lim,² Mélanie Migaud,¹ Laura Israel,¹ Maya Chrabieh,¹ Magali Audry,² Matthew Gumbleton,⁴ Antoine Toulon,⁵ Christine Bodemer,⁵ Jamila El-Baghdadi,⁶ Matthew Whitters,³ Theresa Paradis,³ Jonathan Brooks,³ Mary Collins,³ Neil M. Wolfman,³ Saleh Al-Muhsen,⁷ Miguel Galicchio,⁸ Laurent Abel,^{1,2,†} Capucine Picard,^{1,9,10,†} Jean-Laurent Casanova^{1,2,7,10,†}

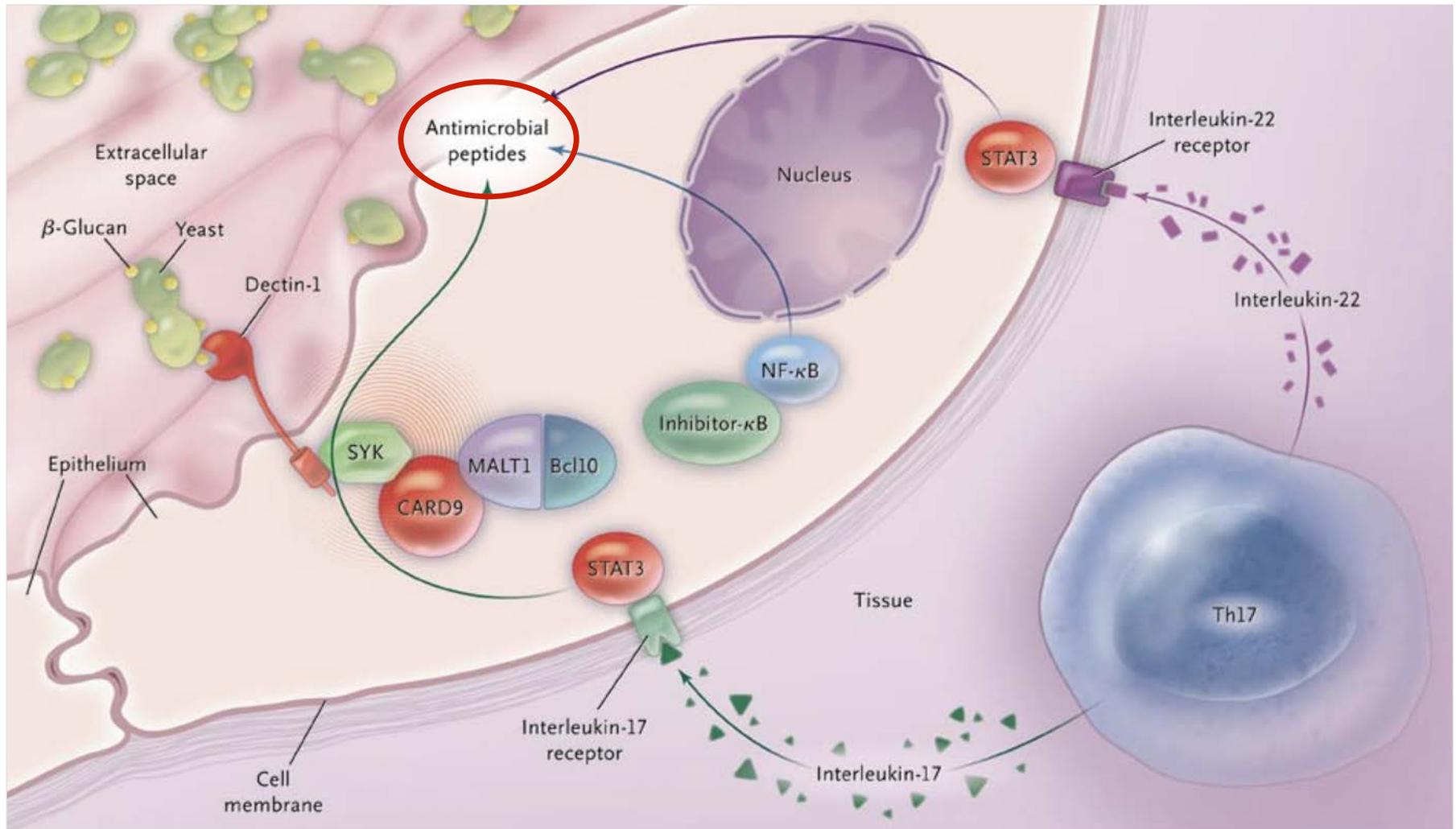
www.sciencemag.org SCIENCE VOL 332 1 APRIL 2011

Inherited IL-17RC deficiency in patients with chronic mucocutaneous candidiasis

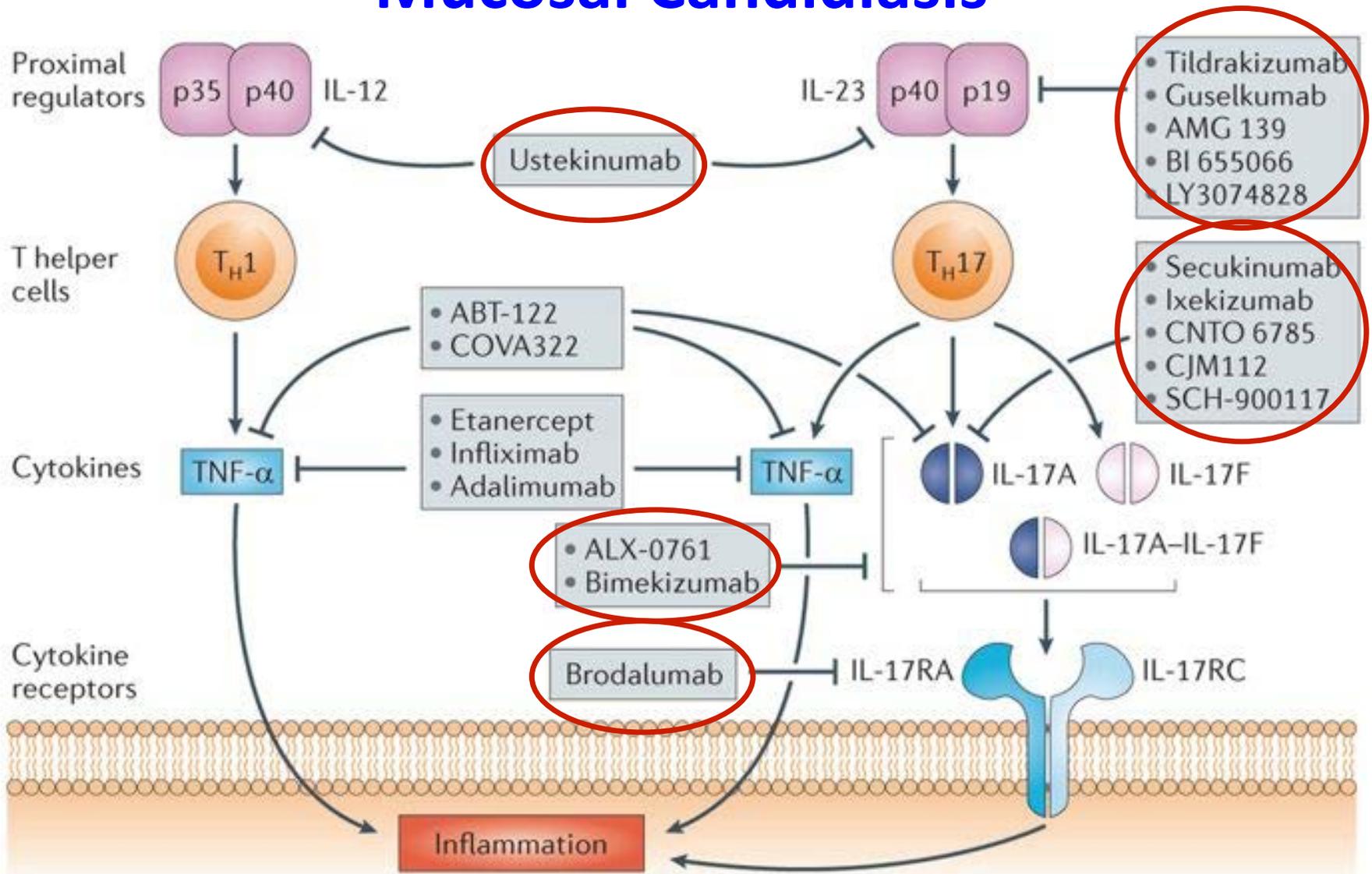
Yun Ling, Sophie Cypowyj, Caner Aytakin, Miguel Galicchio, Yildiz Camcioglu, Serdar Nepesov, Aydan Ikinogullari, Figen Dogu, Aziz Belkadi, Romain Levy, Mélanie Migaud, Bertrand Boisson, Alexandre Bolze, Yuval Itan, Nicolas Goudin, Julien Cottineau, Capucine Picard, Laurent Abel, Jacinta Bustamante, Jean-Laurent Casanova, Anne Puel

www.jem.org JEM VOL 212 27 APRIL 2015

IL-17–Mediated Protection via Generation of Anti-*Candida* Antimicrobial Peptides



IL-17-Targeted Biologics Result in Mucosal Candidiasis

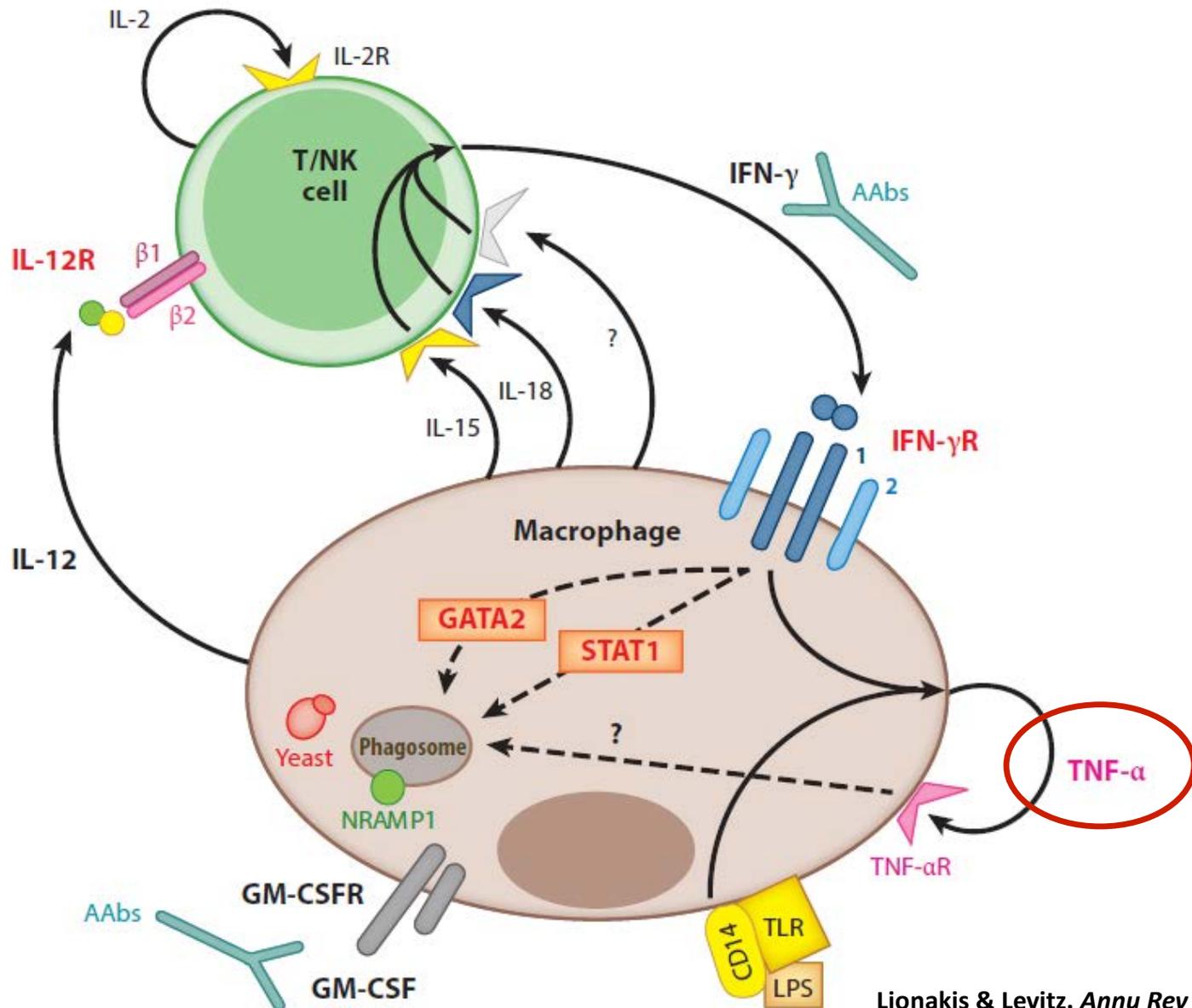


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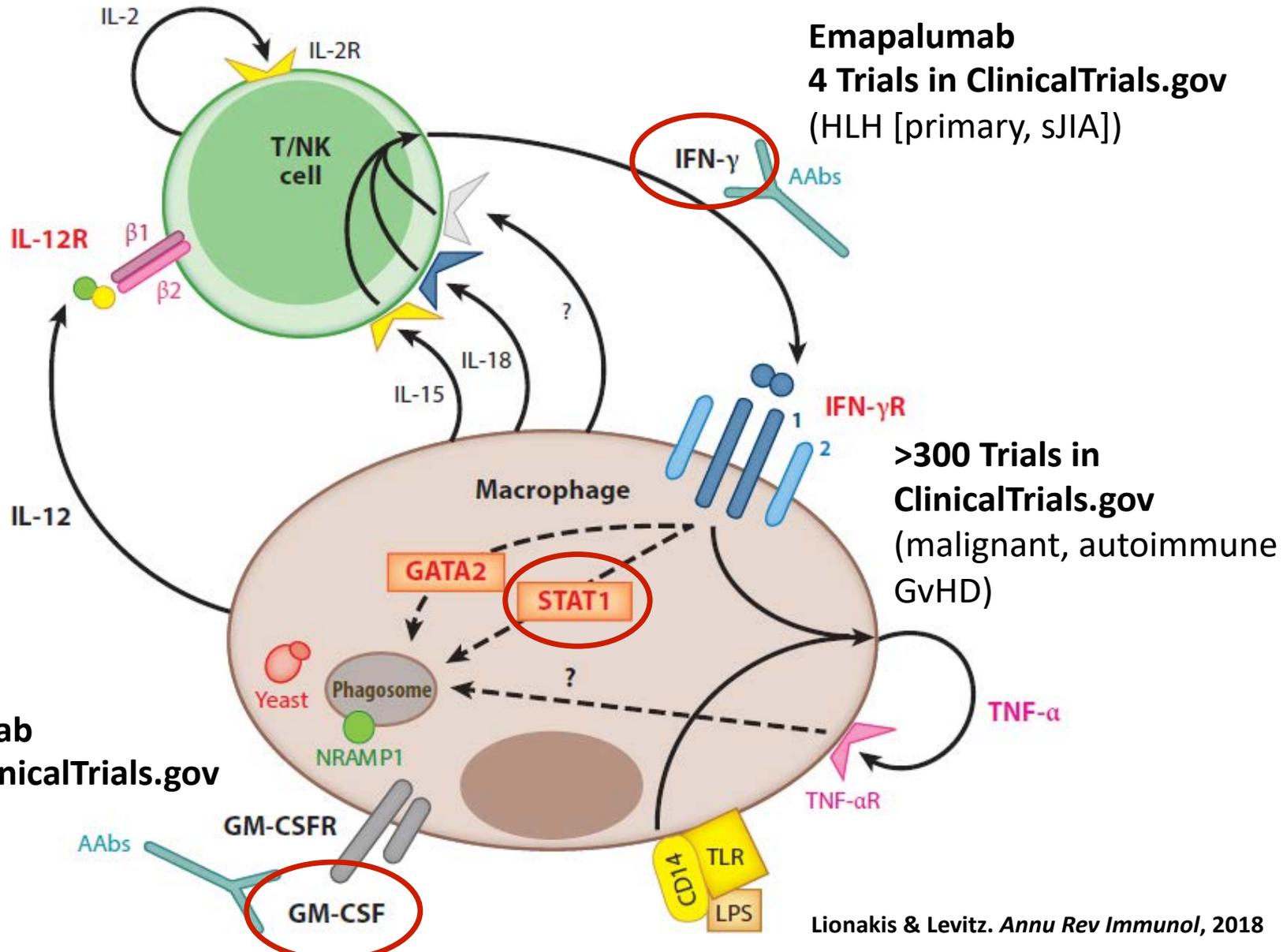
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Macrophage-Th1 Cell Crosstalk is Critical for Intracellular Fungal Pathogen Control



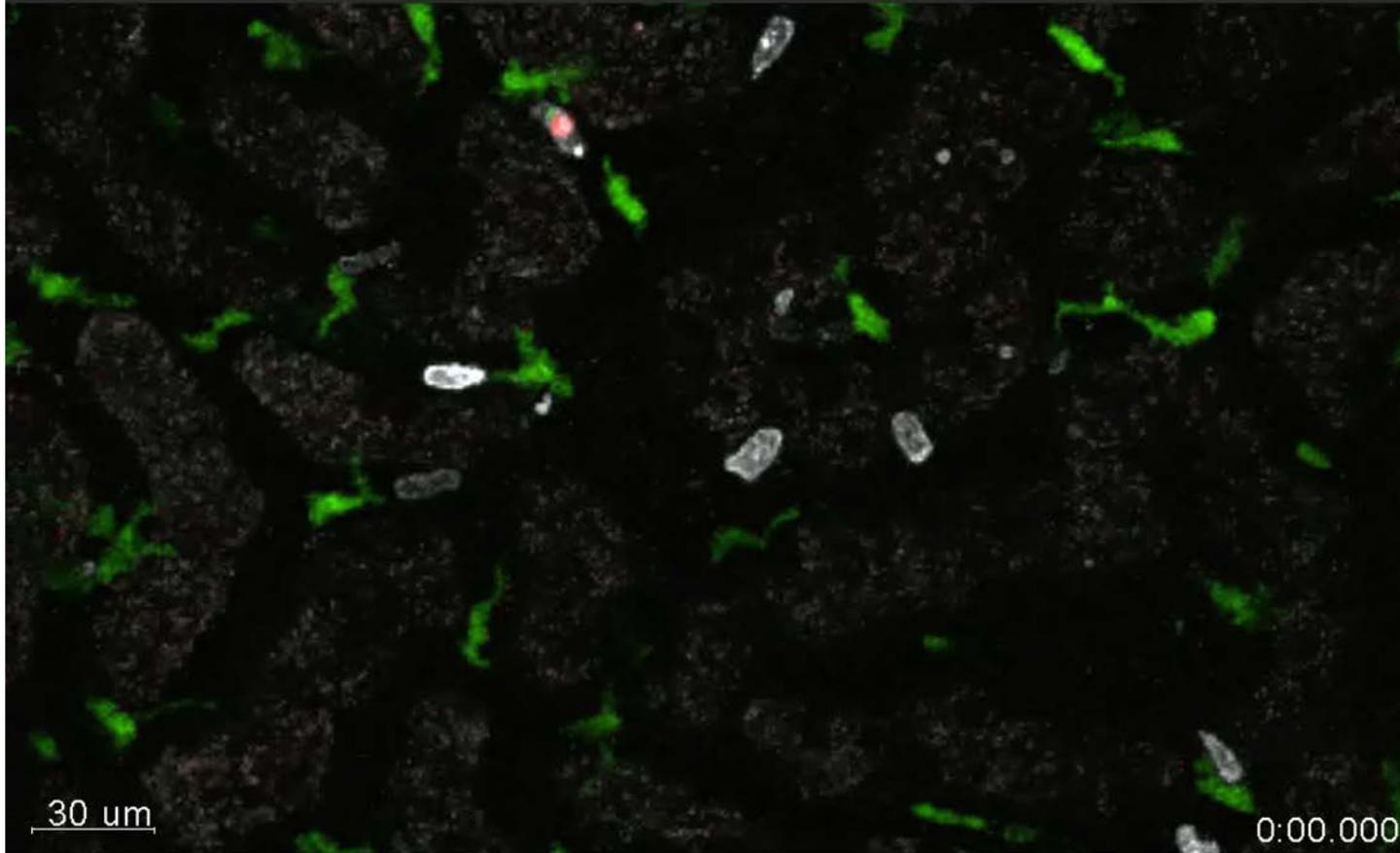
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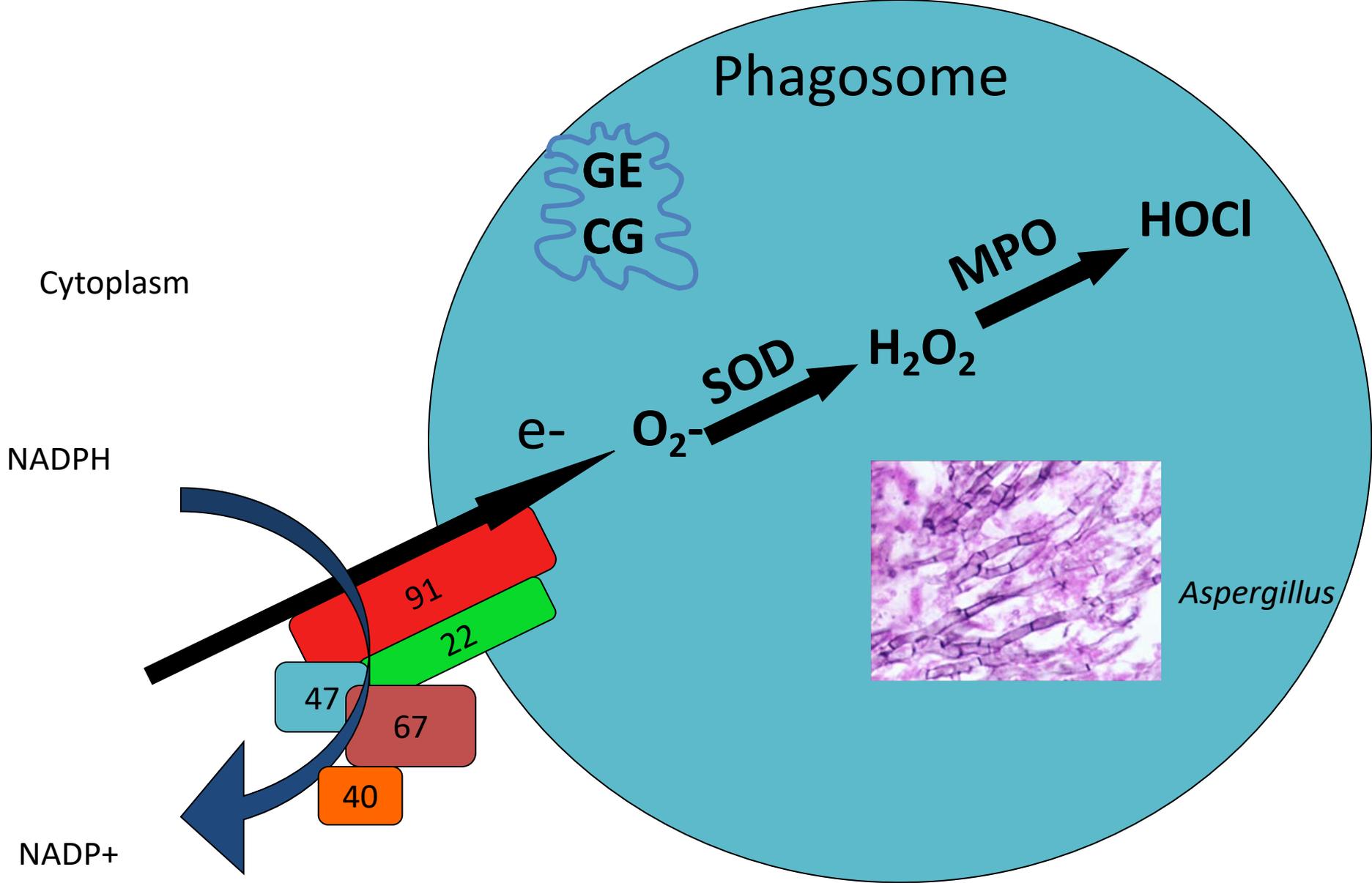


**Red: dTomato-
*C. albicans***

**Green: Cx3cr1
(macrophages)**

**Grey: Ly6G
(neutrophils)**

**Jigar Desai
unpublished**

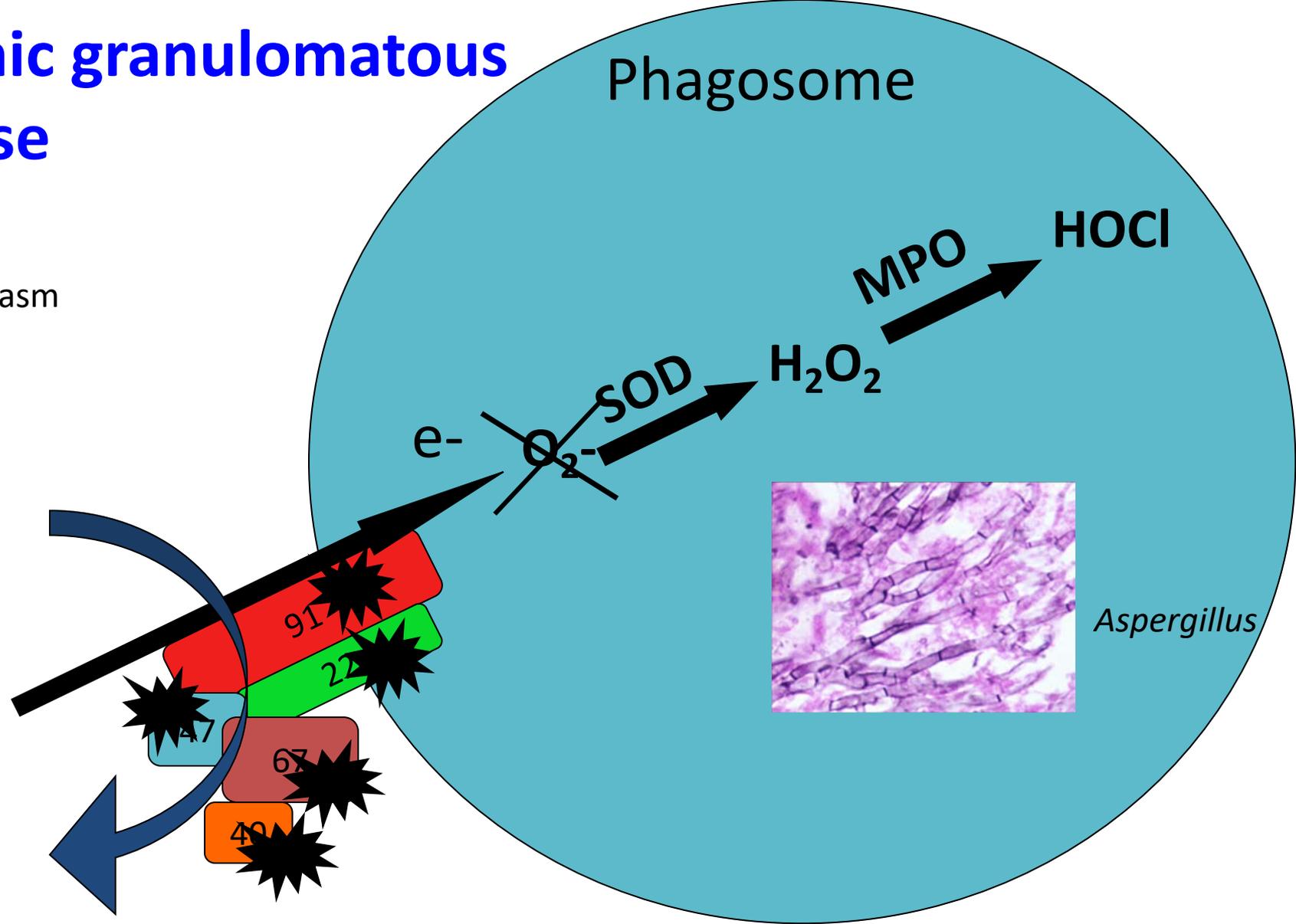


Chronic granulomatous disease

Cytoplasm

NADPH

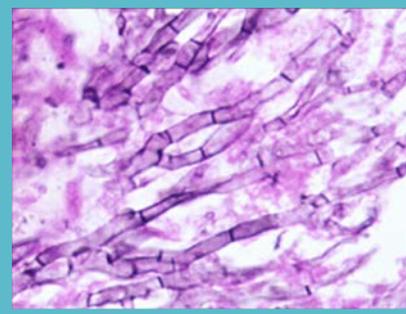
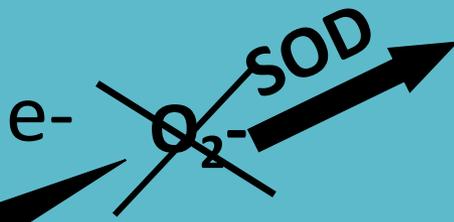
NADP+



Phagosome

MPO

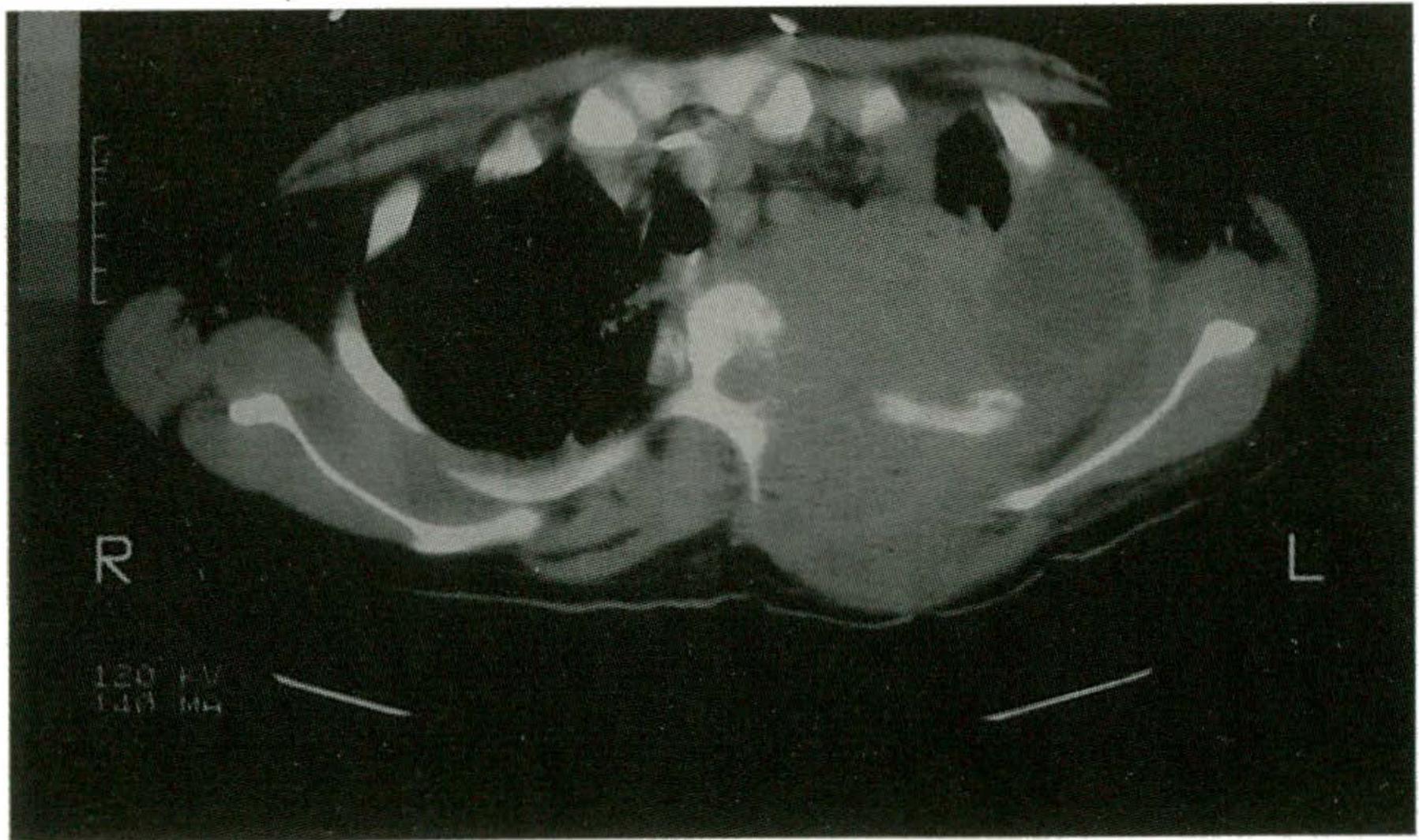
HOCl



Aspergillus

**NOX inhibitors:
Ongoing clinical trials for diabetic nephropathy**

Aspergillus Infection in CGD



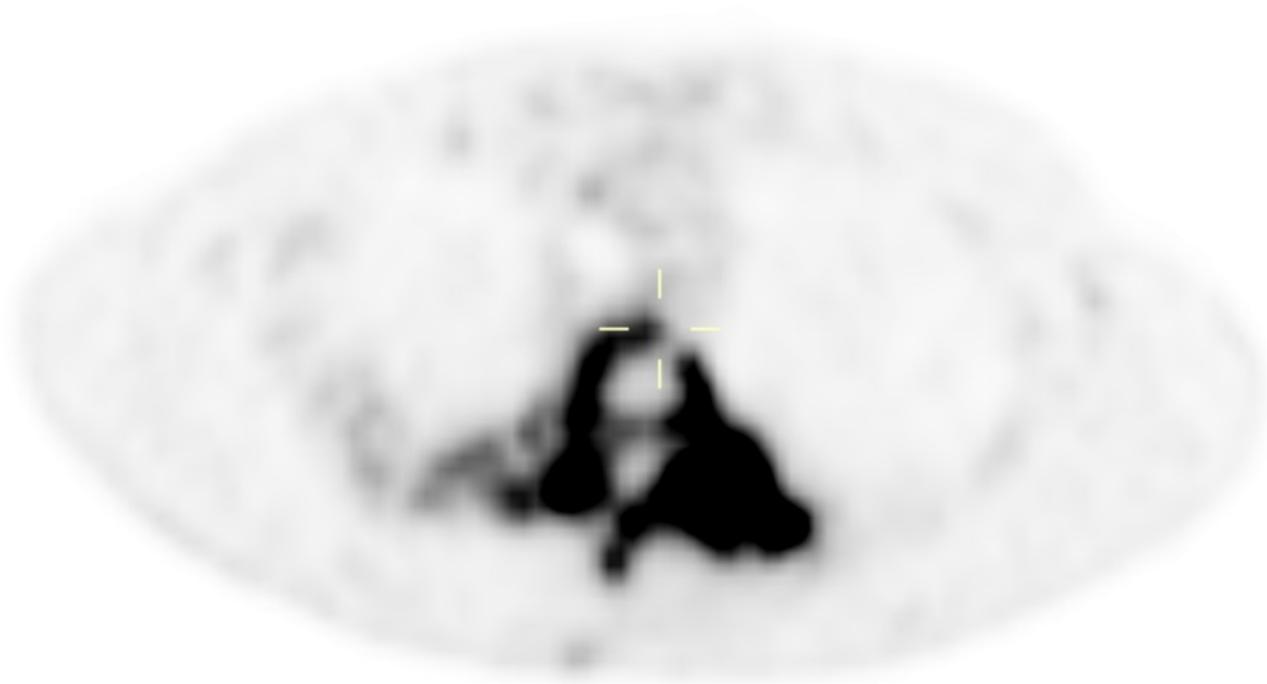
Aspergillus Infection in CGD



Aspergillus Infection in CGD

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R



P

Aspergillus Infection in CGD



PRE-TRANSPLANT



POST-TRANSPLANT

Overview

Basic principles of host defense against:

- mucosal fungal disease (**Th17 cells**)
- intracellular fungi (**macrophages-Th1 cells**)
- invasive mold and *Candida* infections (**PMNs/Mφ**)

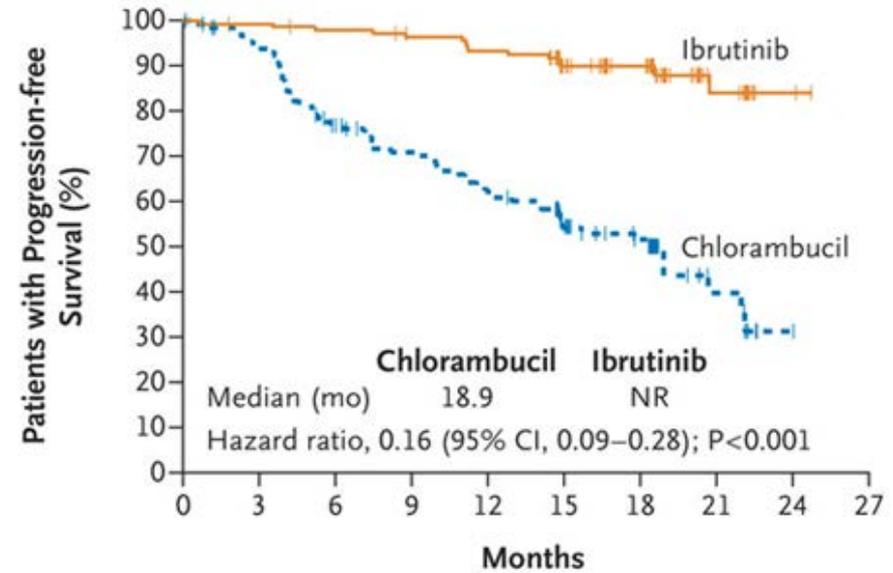
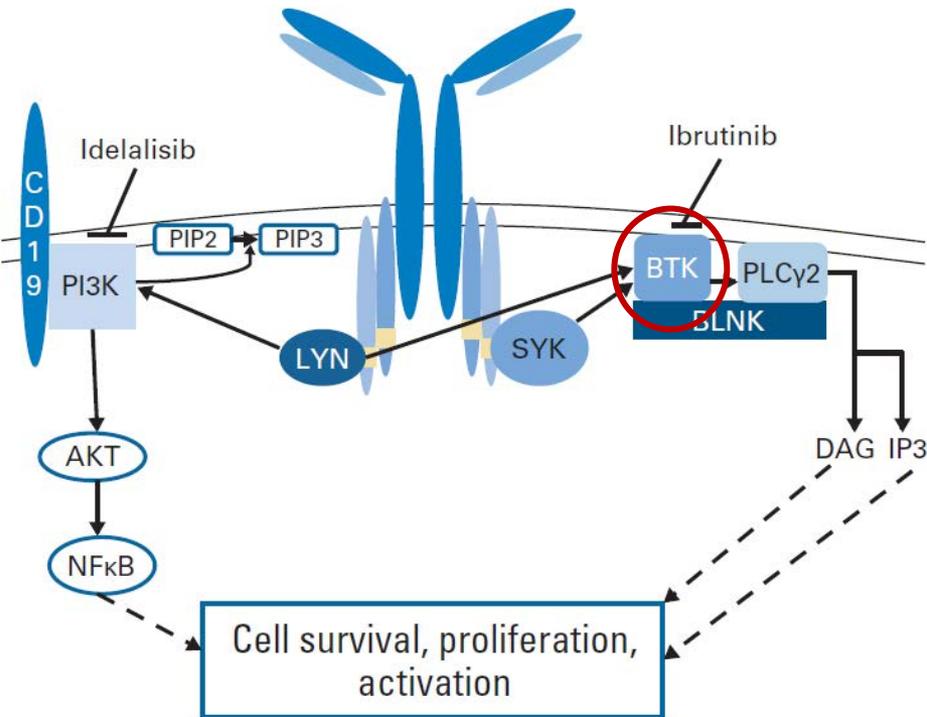
Novel iatrogenic risk factors for systemic fungal disease

- **BTK inhibitors**
- **SYK inhibitors**

Novel iatrogenic risk factors for systemic fungal disease

- **BTK inhibitors**
- **SYK inhibitors**

Ibrutinib: A Game Changer in B-cell Malignancies



Inhibition of B Cell Receptor Signaling by Ibrutinib in Primary CNS Lymphoma

Michail S. Lionakis,^{1,7} Kieron Dunleavy,^{2,7} Mark Roschewski,² Brigitte C. Widemann,³ John A. Butman,⁴ Roland Schmitz,² Yandan Yang,² Diane E. Cole,³ Christopher Melani,² Christine S. Higham,³ Jigar V. Desai,² Michele Ceribelli,⁵ Lu Chen,⁵ Craig J. Thomas,^{2,5} Richard F. Little,⁶ Juan Gea-Banacloche,³ Sucharita Bhaumik,³ Maryalice Stetler-Stevenson,³ Stefania Pittaluga,³ Elaine S. Jaffe,³ John Heiss,³ Nicole Lucas,² Seth M. Steinberg,³ Louis M. Staudt,^{2,8,*} and Wyndham H. Wilson^{2,8,*}

Lionakis et al., 2017, *Cancer Cell* 31, 1–11
June 12, 2017 Published by Elsevier Inc.
<http://dx.doi.org/10.1016/j.ccell.2017.04.012>

CellPress

Atypical *Pneumocystis jirovecii* pneumonia in previously untreated patients with CLL on single-agent ibrutinib

BLOOD, 13 OCTOBER 2016 • VOLUME 128, NUMBER 15

Inhye E. Ahn,^{1,*} Theresa Jerussi,^{2,*} Mohammed Farooqui,³ Xin Tian,⁴ Adrian Wiestner,³ and Juan Gea-Banacloche⁵

Open Forum Infectious Diseases

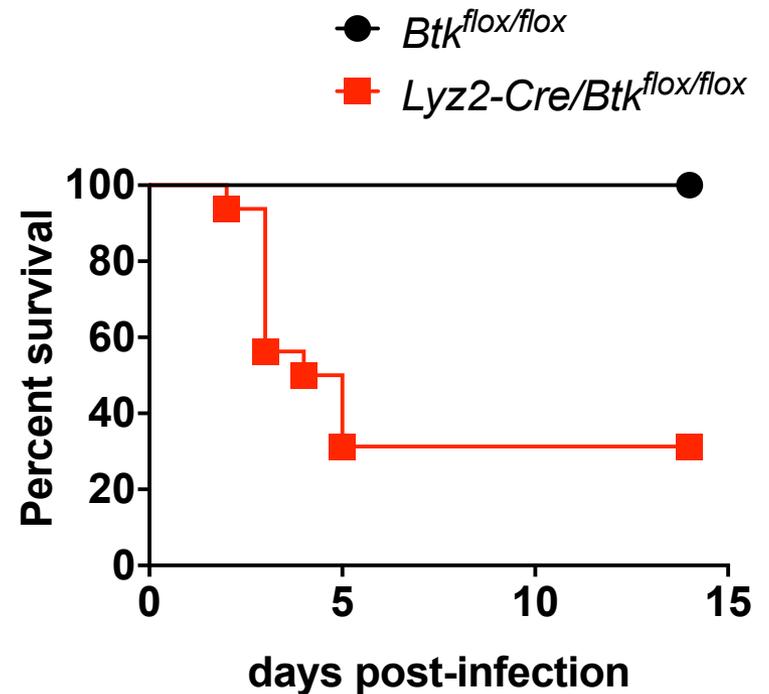
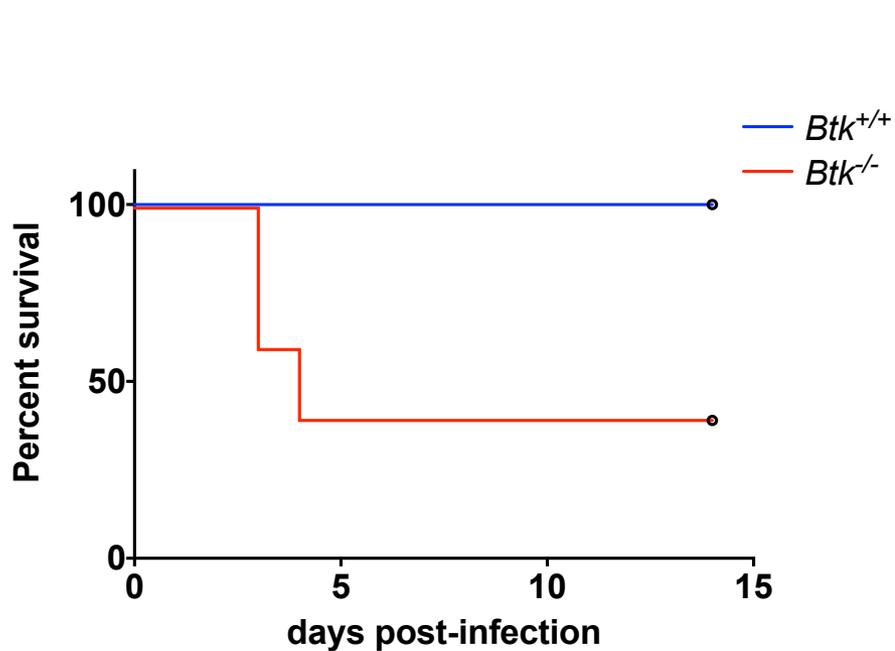
BRIEF REPORT

Disseminated Cryptococcosis With Brain Involvement in Patients With Chronic Lymphoid Malignancies on Ibrutinib

Julia A. Messina,¹ Eileen K. Maziarz,¹ Andrej Spec,² Dimitrios P. Kontoyiannis,³ and John R. Perfect¹

¹Department of Medicine, Duke University, Durham, North Carolina; ²Division of Infectious Disease, Washington University, St. Louis, Missouri; ³University of Texas MD Anderson Cancer Center, Houston

BTK Expression in Phagocytes Promotes Protection During Invasive Aspergillosis

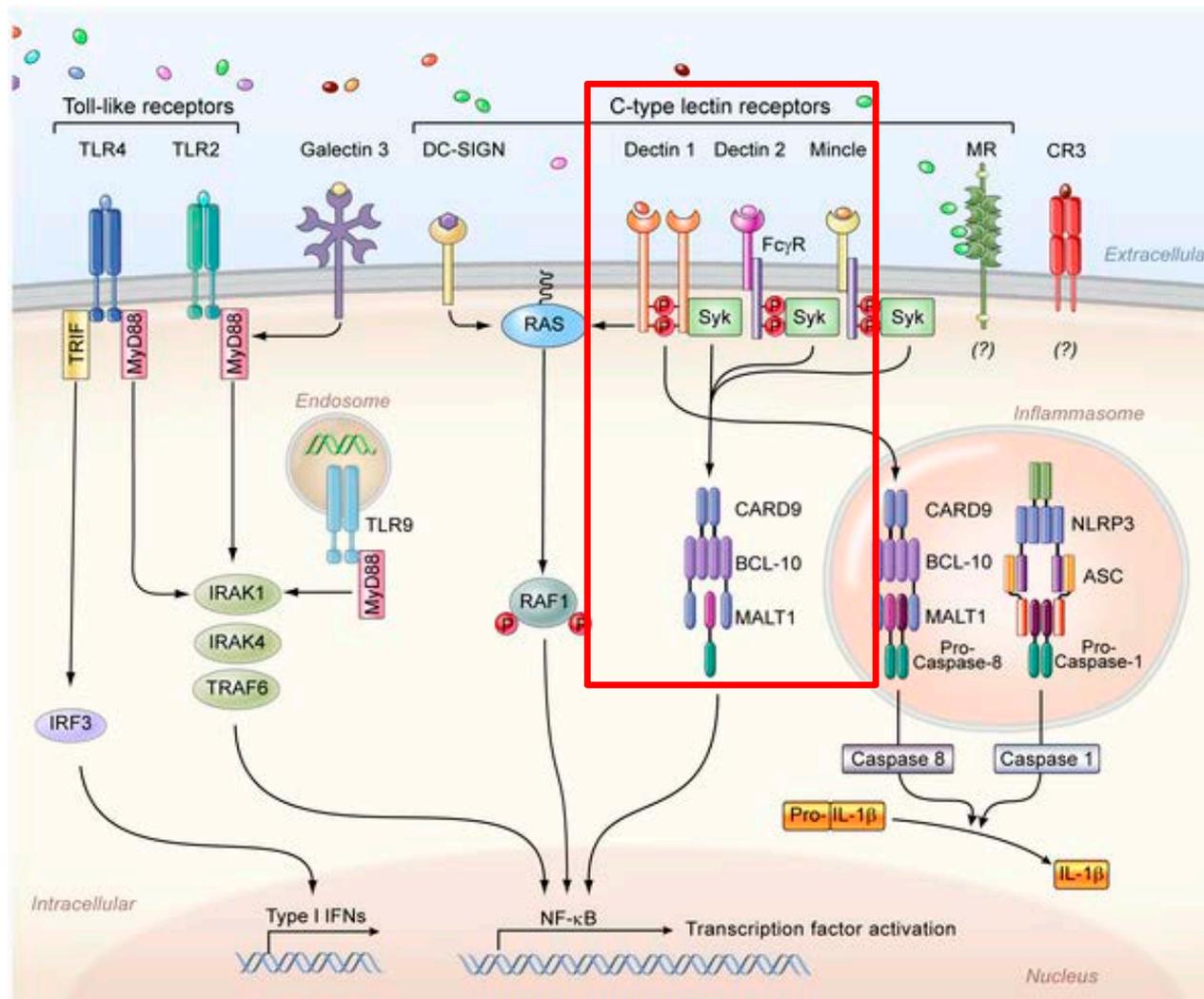


P = 0.008

Novel iatrogenic risk factors for systemic fungal disease

- **BTK inhibitors**
- **SYK inhibitors**

CARD9 is centrally positioned in antifungal immune pathways

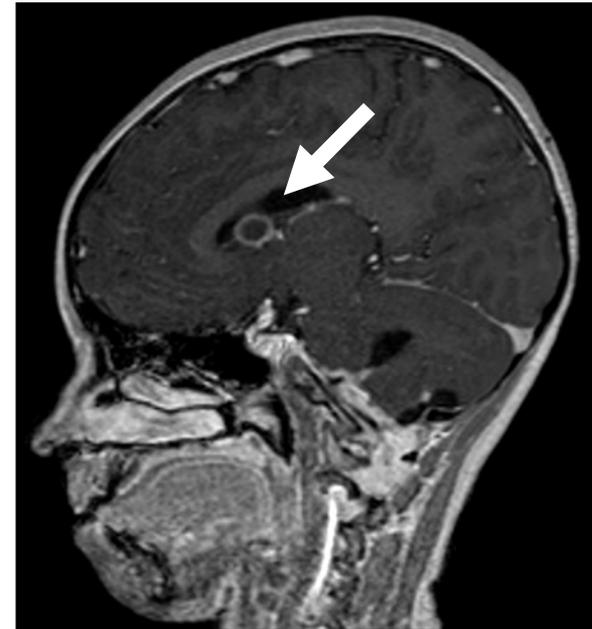
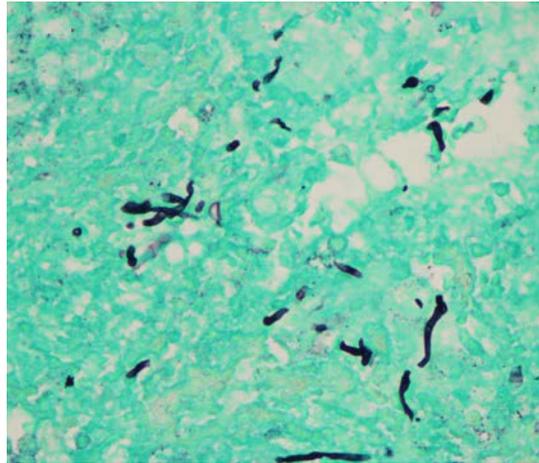


Human CARD9 deficiency results in fungal disease in the CNS and skin

- Autosomal recessive inheritance
- Patients present with one or more fungal diseases:
 - mucosal candidiasis
 - **Fungal brain disease (*Candida*, *Aspergillus*)**
 - **Deep subcutaneous fungal disease (dermatophytes, phaeohyphomyces)**

Fungal meningoencephalitis in CARD9 deficiency

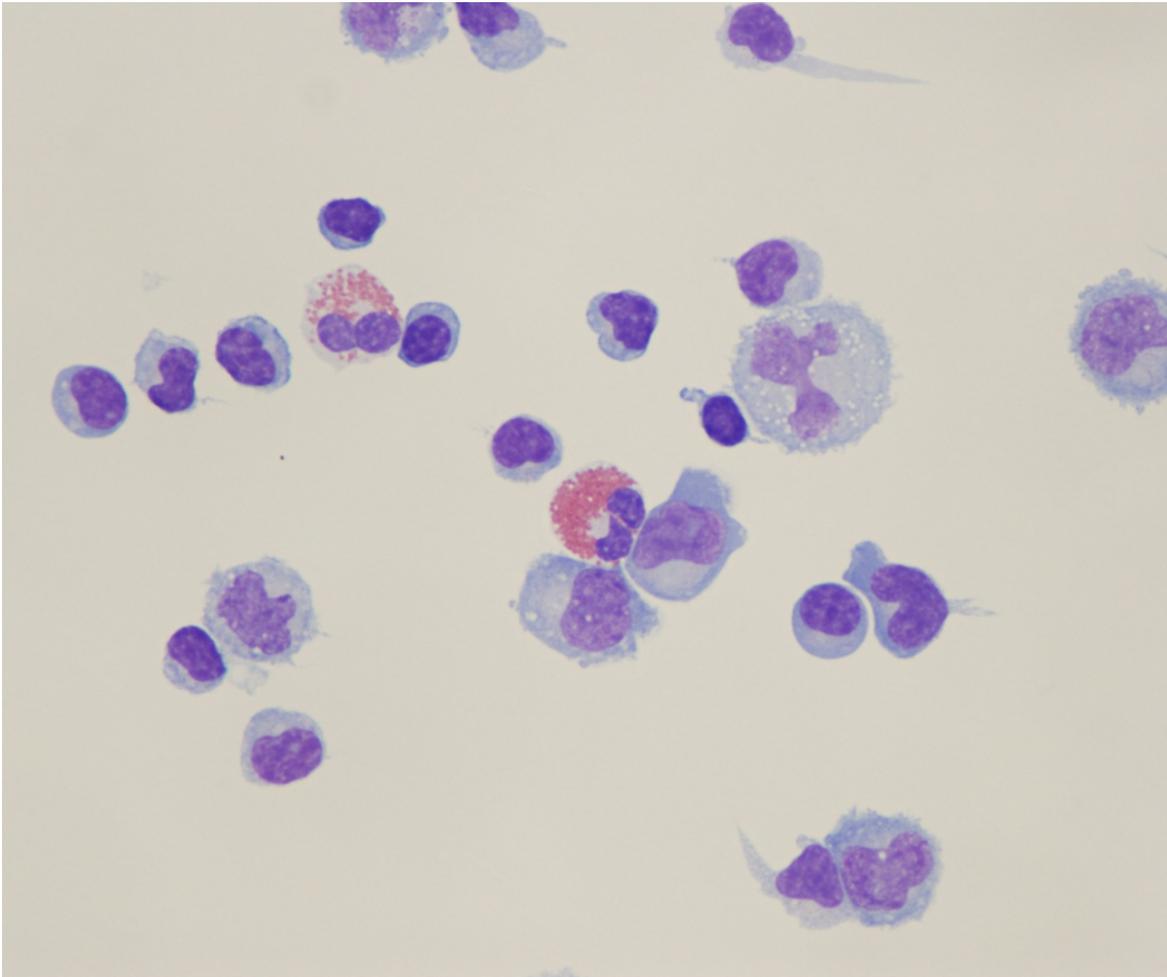
- 10 y/o girl referred to the NIH in 2013
- Mucosal candidiasis since 1st month of life
- Meningoencephalitis
- Persistent infection despite antifungal therapy







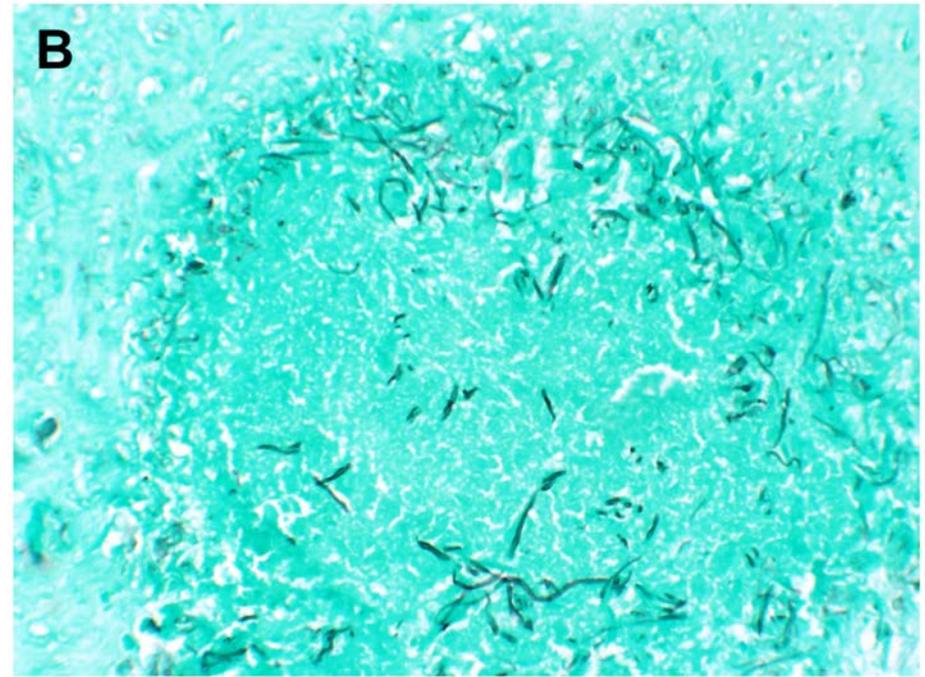
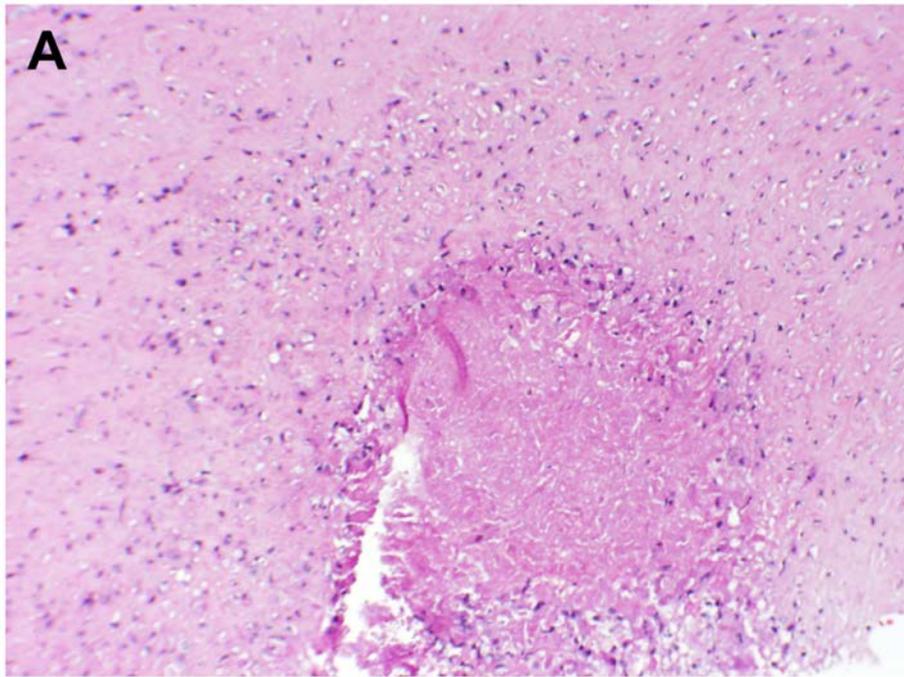
Absence of neutrophils in the CSF despite uncontrolled infection



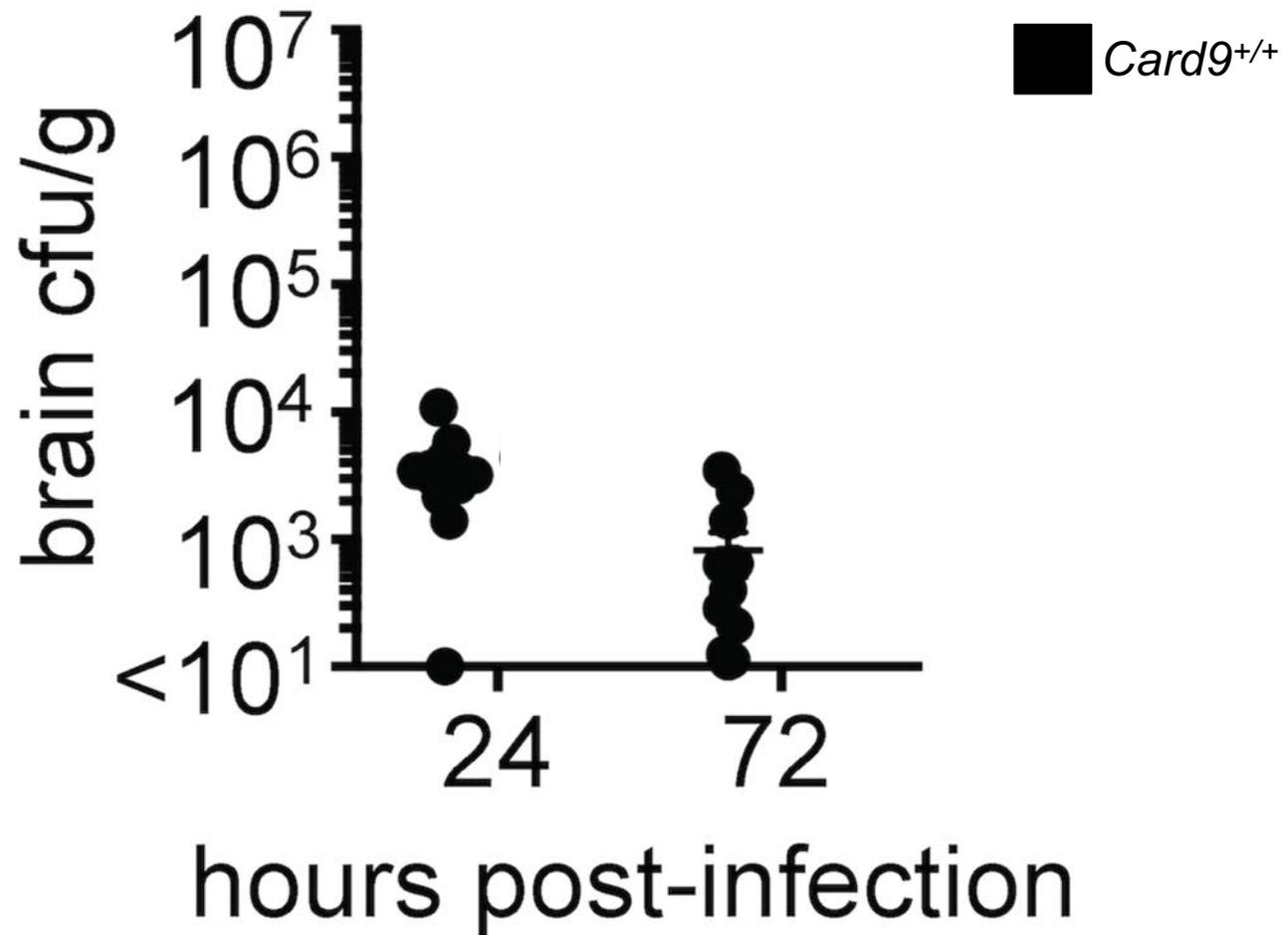
CSF Analysis:

66% lymphocytes
20% monocytes/DCs
10% eosinophils
<1% neutrophils

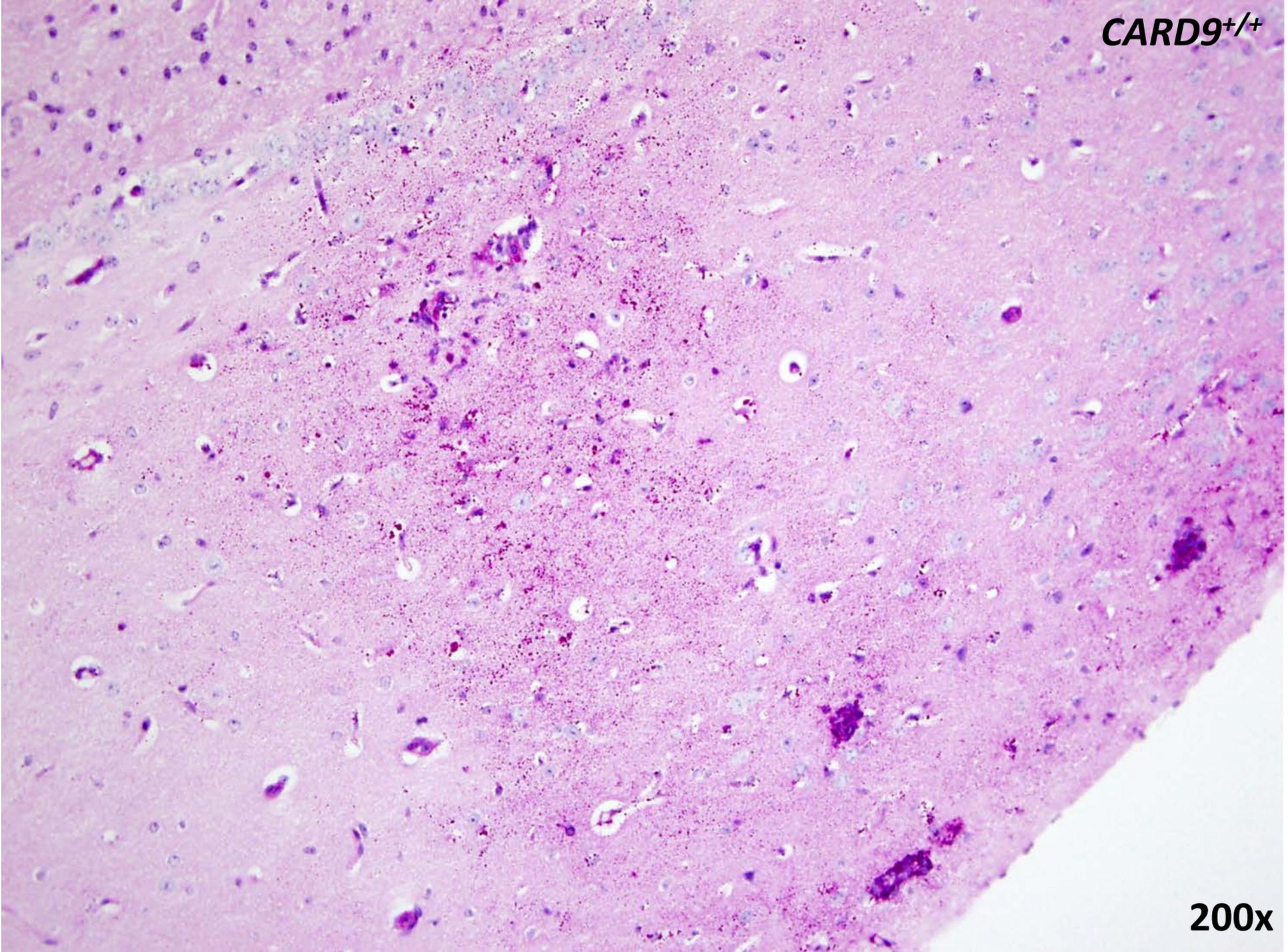
CARD9 deficiency results in decreased neutrophil accumulation in the CNS



Card9^{-/-} Mice Develop Uncontrolled Fungal Brain Infection

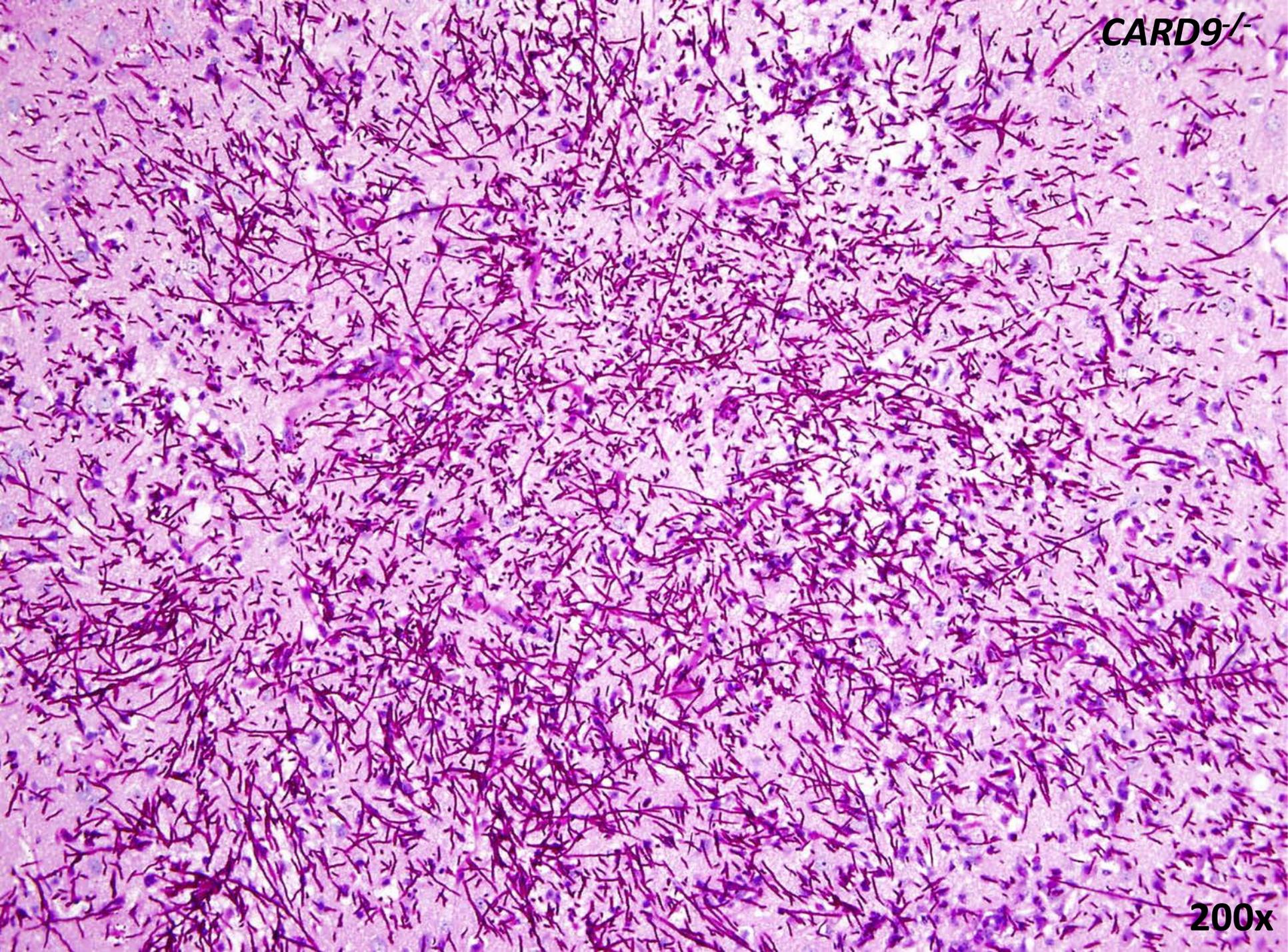


CARD9^{+/+}



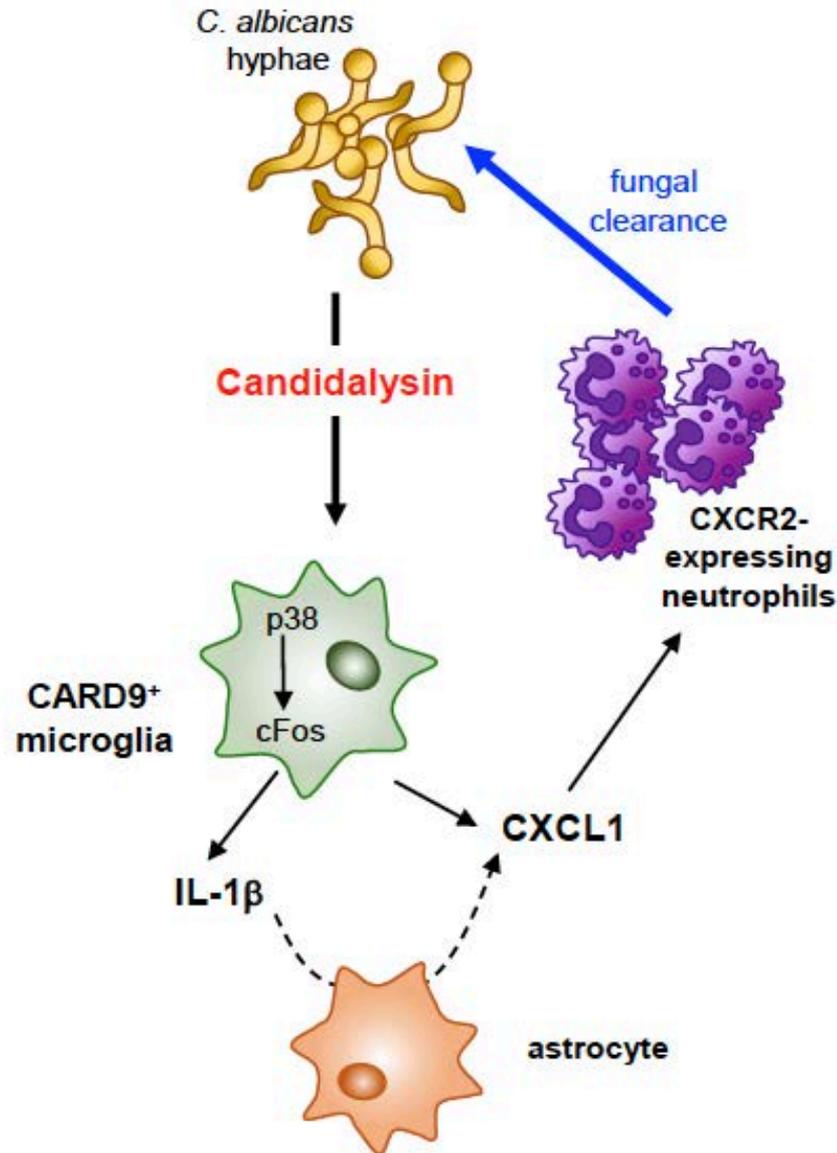
200x

CARD9^{-/-}



200x

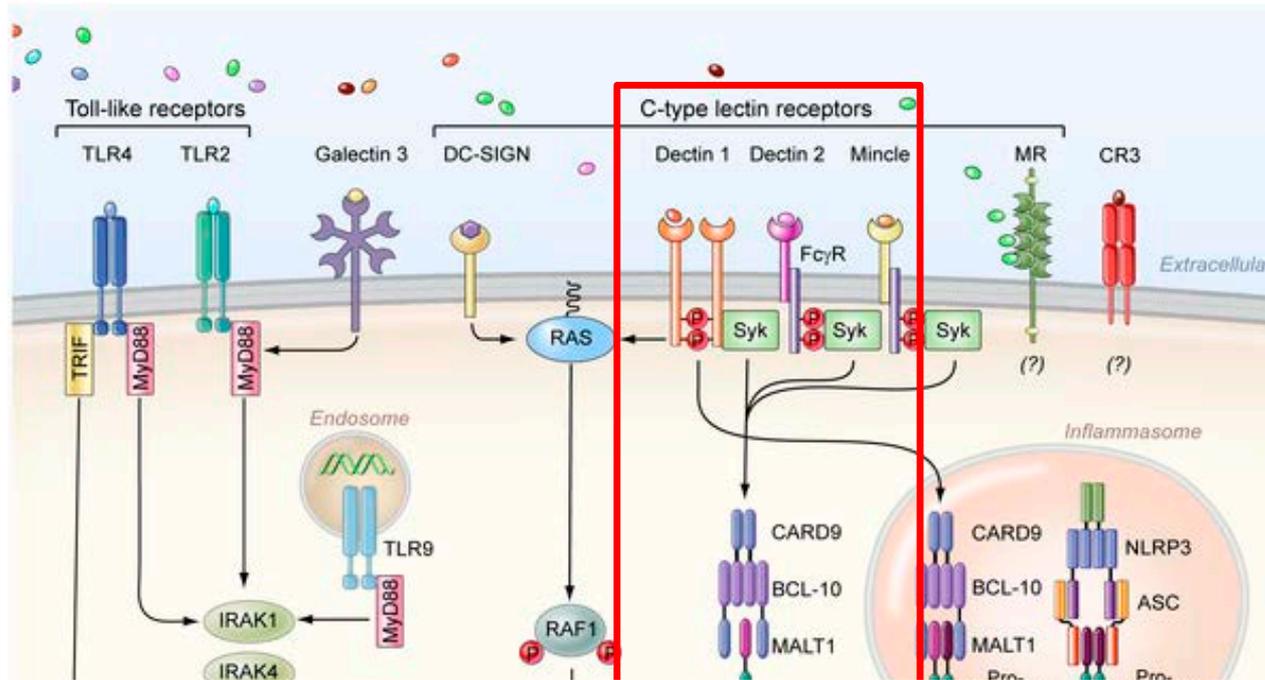
A Model of Microglial Engagement for CNS Protective Antifungal Immunity



Broader implications:

- Neonatal candidiasis
- SYK inhibitors

SYK-CARD9 are centrally positioned in antifungal immune pathways



Fostamatinib
44 trials in
ClinicalTrials.gov

- RA
- AML
- GvHD
- Lymphomas
- Solid tumors
- Autoimmune
cytopenias

The **NEW ENGLAND**
JOURNAL of MEDICINE

ESTABLISHED IN 1812

SEPTEMBER 30, 2010

VOL. 363 NO. 14

An Oral Spleen Tyrosine Kinase (Syk) Inhibitor
for Rheumatoid Arthritis

Collaboration with
Stephanie Gaillard

ORIGINAL ARTICLE

Deep Dermatophytosis and Inherited CARD9 Deficiency



Case Presentation

- Age 3: Developed epistaxis and a nodule on her nasal dorsum that progressed into an oronasal fistula
- Biopsy revealed a “mycotic granuloma”
- Started on IV amphotericin – some apparent response
- Surgical resection of mass – removal of inferior turbinates, ethmoidectomy, medial maxillary antrostomy
- Lost to follow-up after discharge



History

- Age 9, progressive infection
- Tissue biopsy with granulomas and abscesses with fungal elements thought to be compatible with *Aspergillus*
- Tested negative for HIV, with normal T-cell subsets
- Treated with combination antifungal therapy (voriconazole and caspofungin), followed by voriconazole for 12 more weeks



October 2014



December 2014

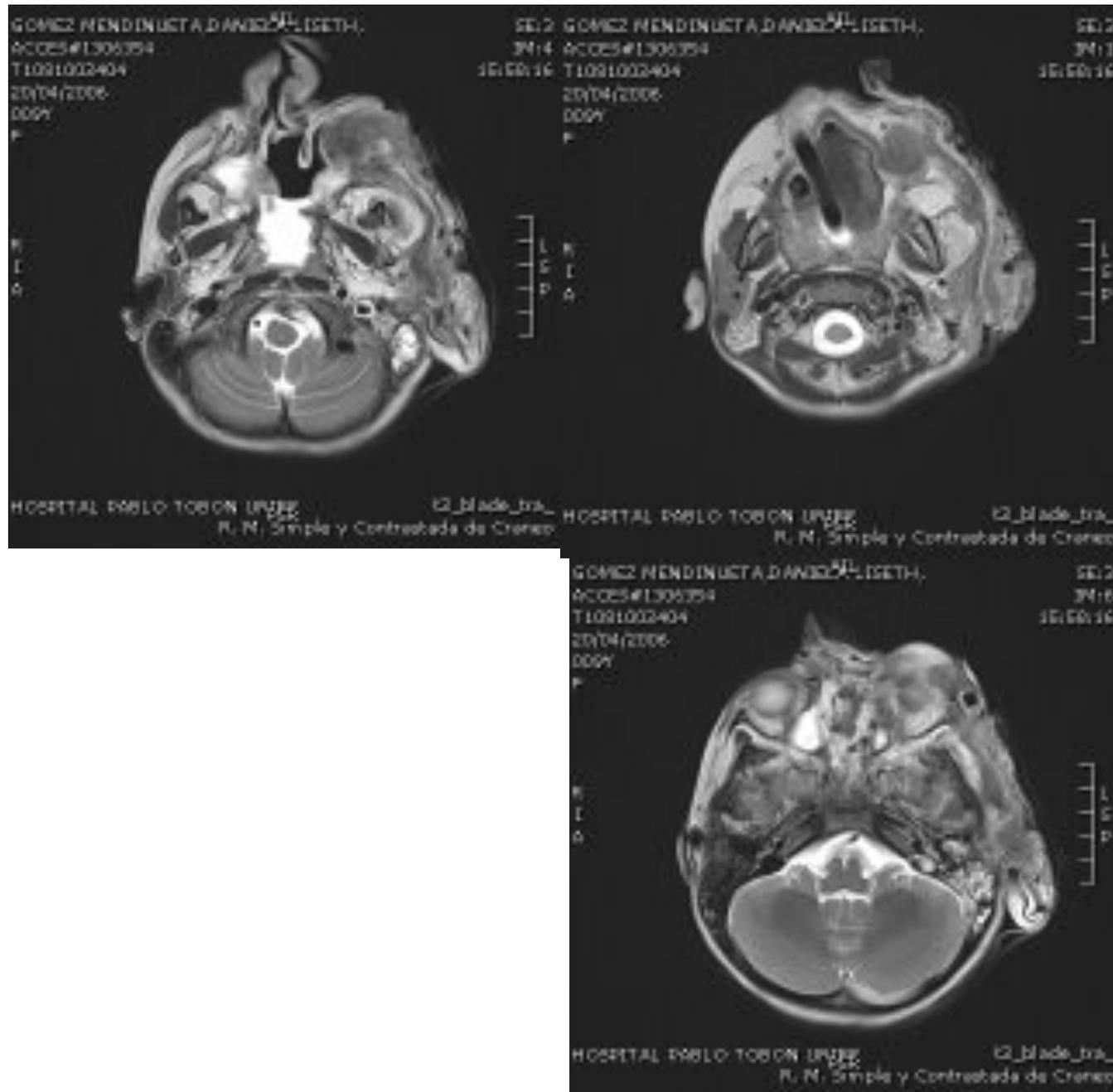
History

- Returned after 6 months with progressive disease
- Mucosal biopsy PCR positive for *Corynespora cassiicola*; culture negative
- Admitted and treated for 1 month with IV amphotericin and posaconazole with reported improvement



June 2015

MRI



History

- On/off antifungal therapy
- Immunodeficiency suspected
 - work-up revealed a CARD9 deficiency



June 2016

Admission at NIH: April 15, 2018

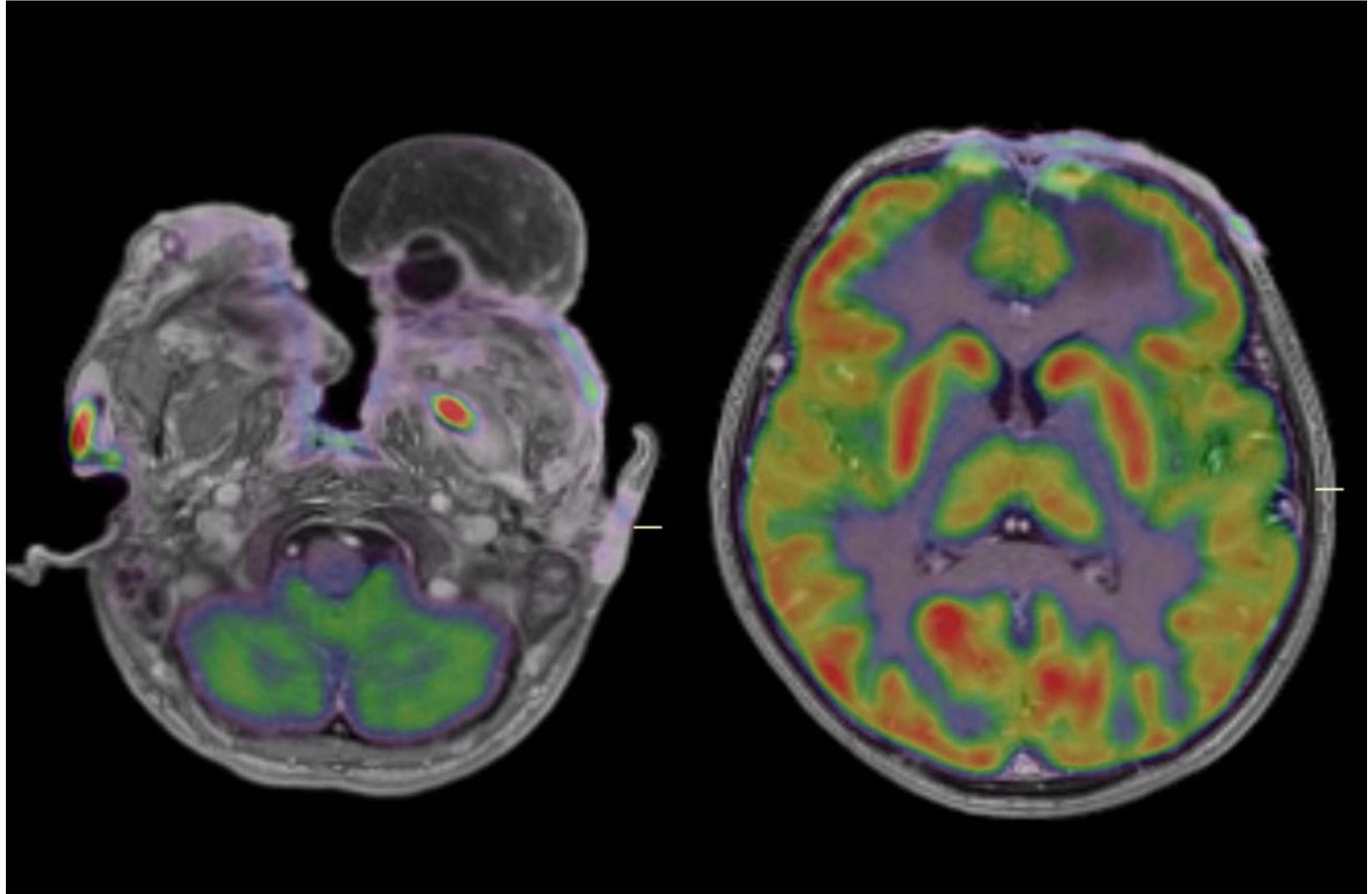


Family Medical History

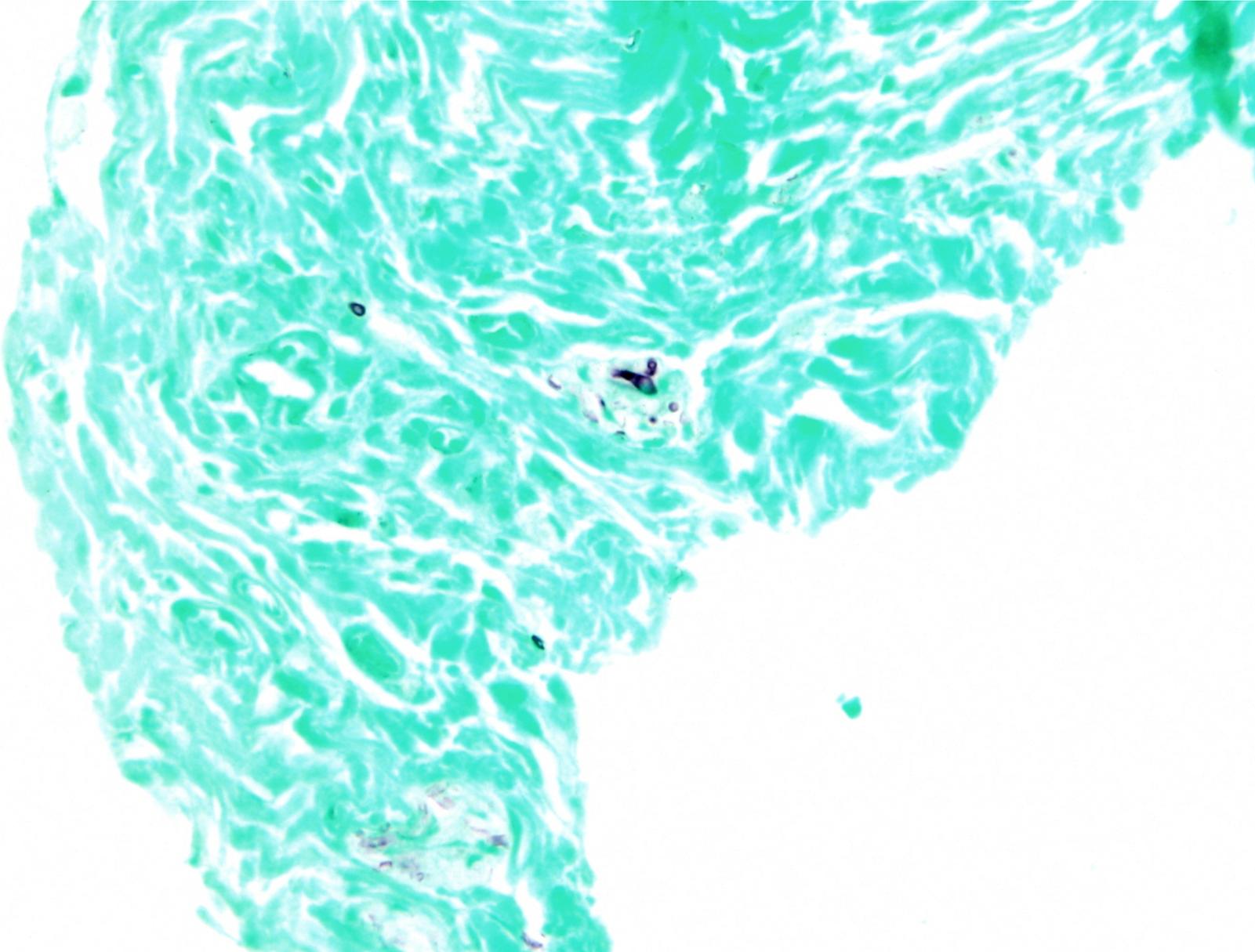
- Mother – unknown; no longer involved in patient's life
- Father – no known medical problems
- Siblings (3) – all healthy; all negative for CARD9 deficiency

Labs

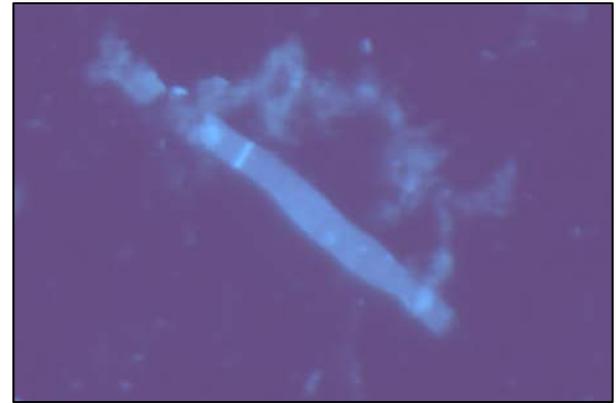
- BMP: 137/4.5 | 103/22 | 42/0.84 < 85
- LFTs: Alk Phos 167, ALT 7, AST 19, Tbili < 0.2, Alb 3
- CBC: 9.4 > 8.5 / 27.9 < 349, normal differential
- CRP 59.8, ESR 90
- IgG 2826, IgA 65, IgM 195, IgE 32.1
- Serum galactomannan < 0.500
- Serum beta-D-glucan > 500 (30,000)



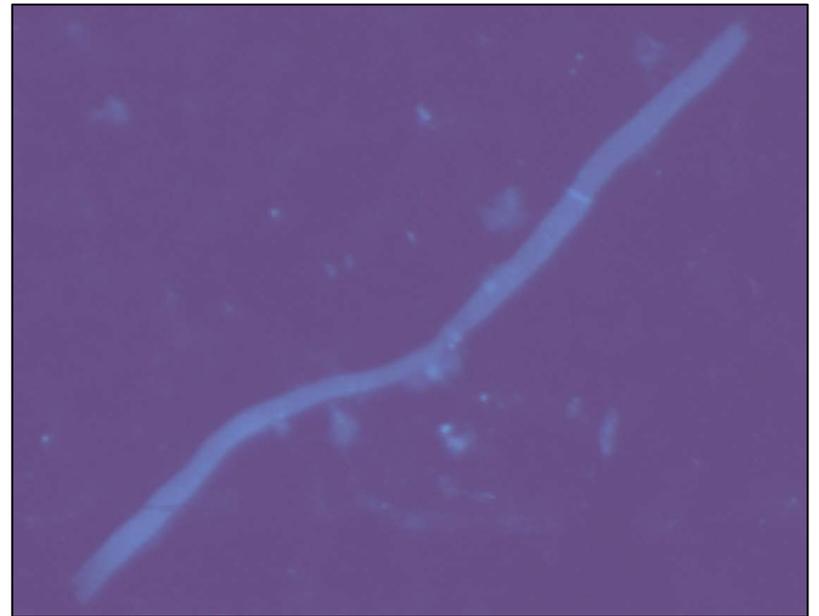
Pathology



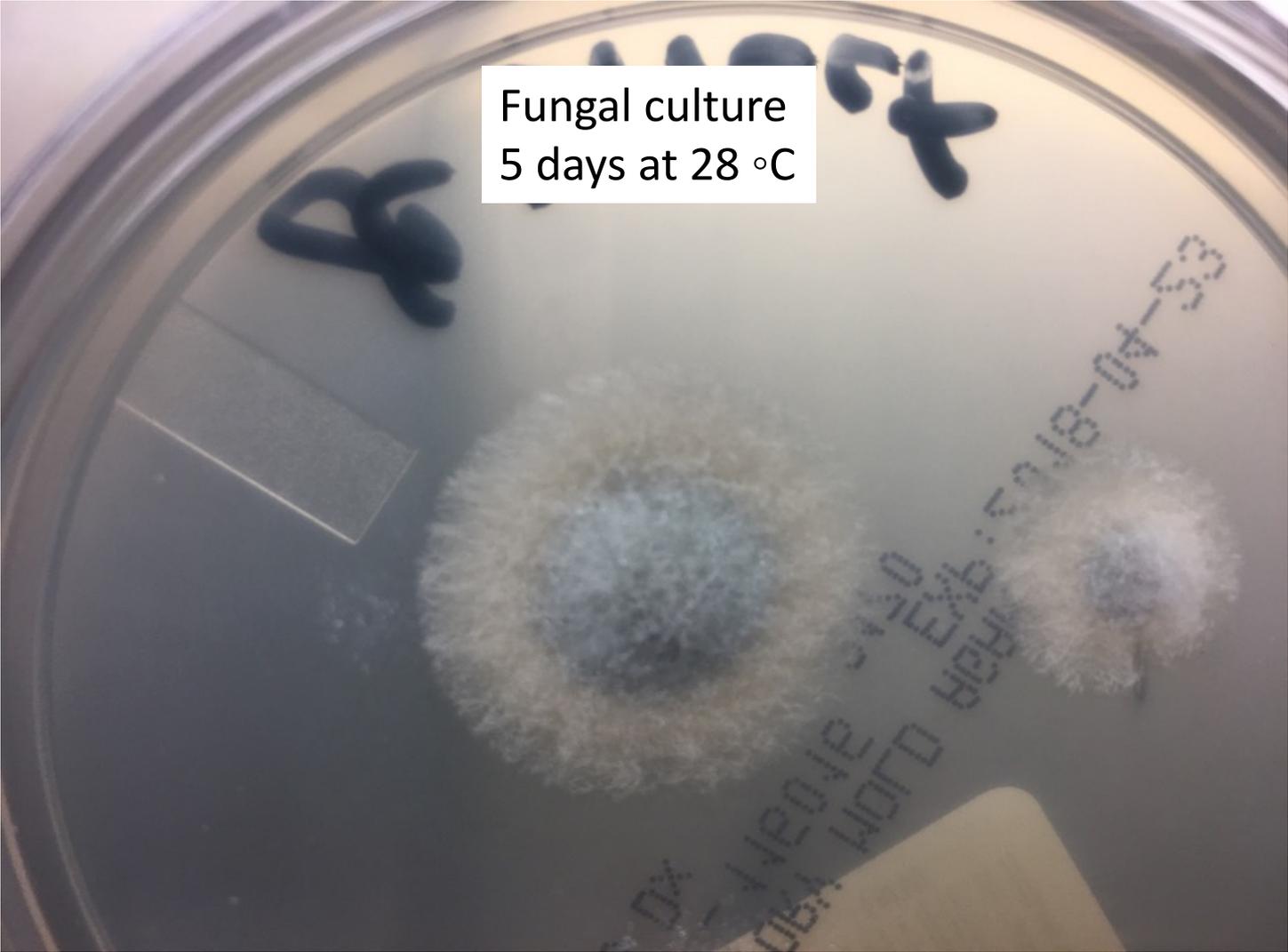
Biopsy skin- left jaw



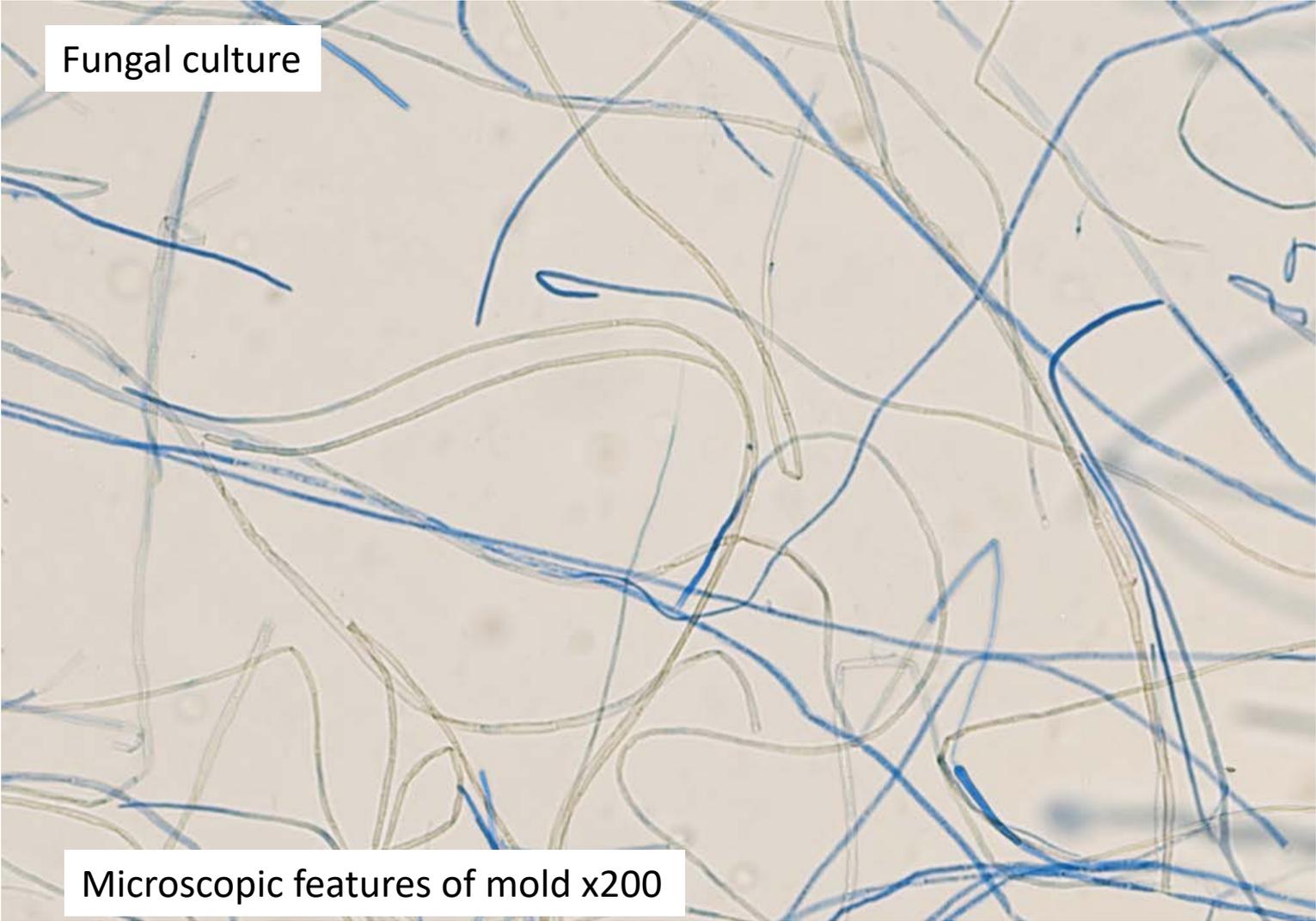
Fungal WTMT x500



Fungal culture
5 days at 28 °C



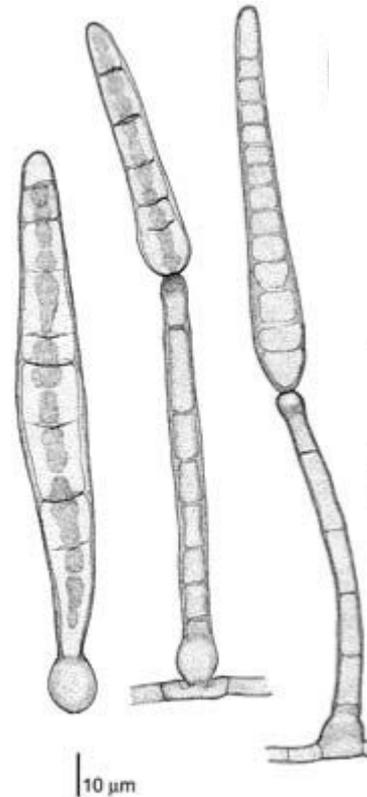
Fungal culture



Microscopic features of mold x200

Corynespora cassiicola

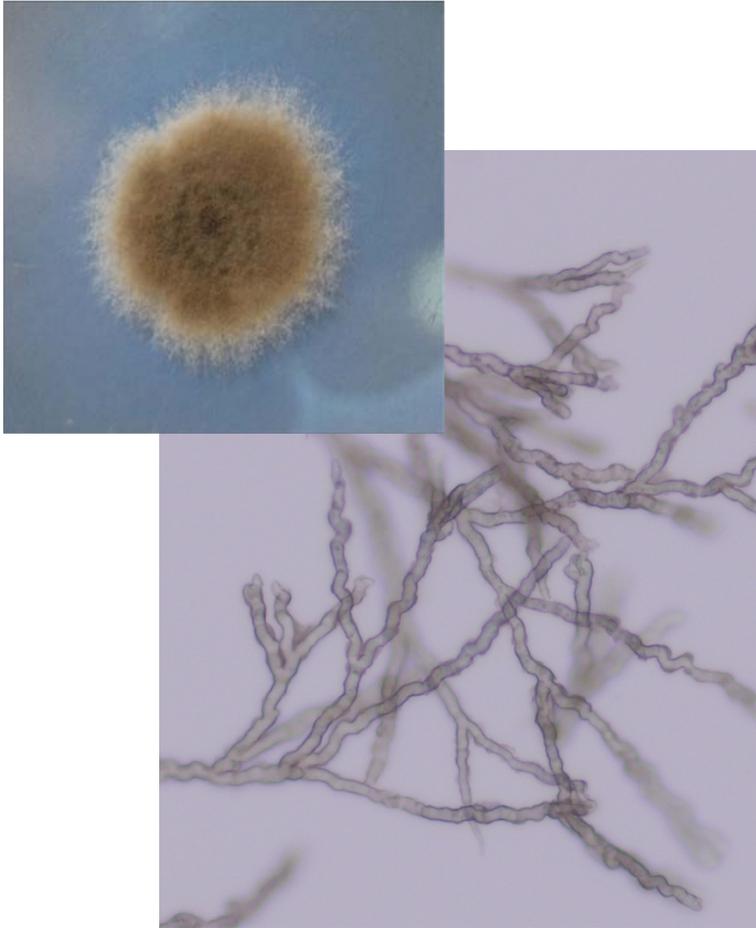
- Dematiaceous mold (distantly related to *Alternaria*)
- Optimal growth temp: 28-30 °C
- Major plant pathogen causing “target spot”. Infects important crops: rubber tree, soybean, tomato, cucumber, etc
- Rarely a human pathogen



<http://www.mycobank.org>

<https://genome.jgi.doe.gov/Corca1/Corca1.home.html>

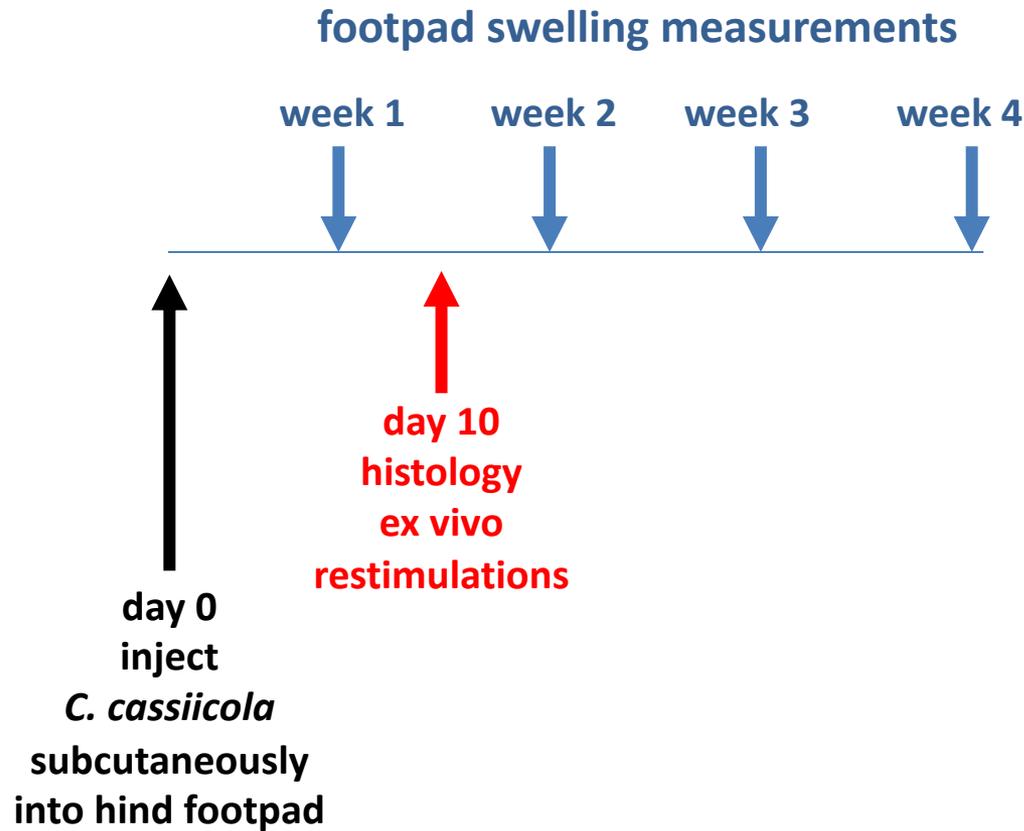
Corynespora cassiicola



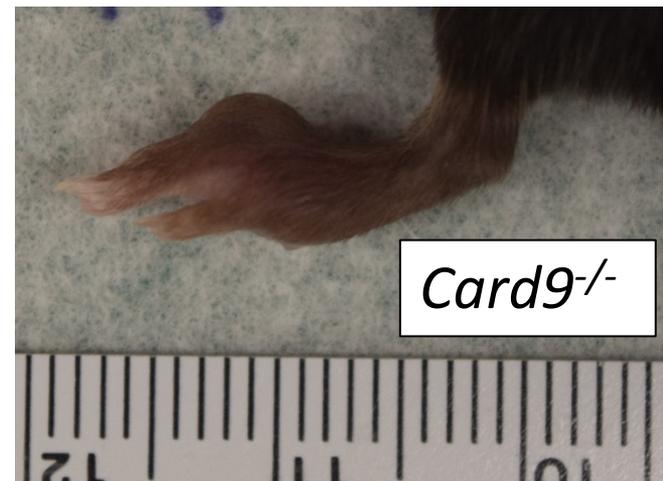
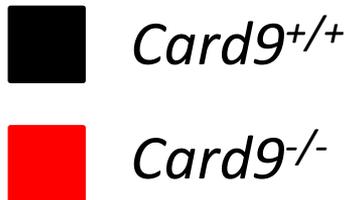
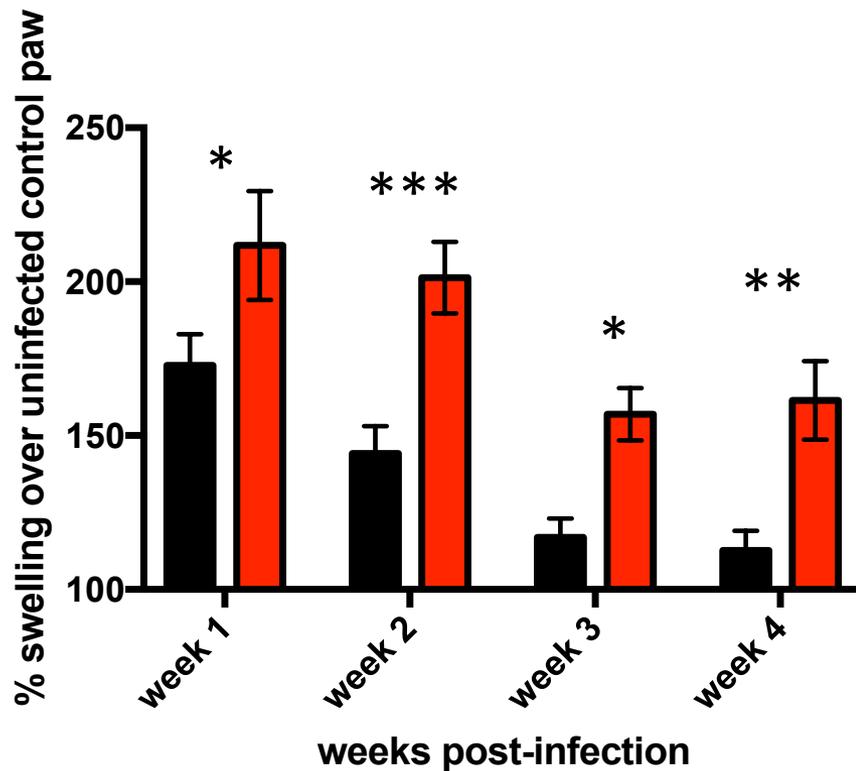
- Most commonly a pathogen of plants/grasses
- Temperature sensitive
- Rare cause of phaeohyphomycosis in humans – follows traumatic inoculation



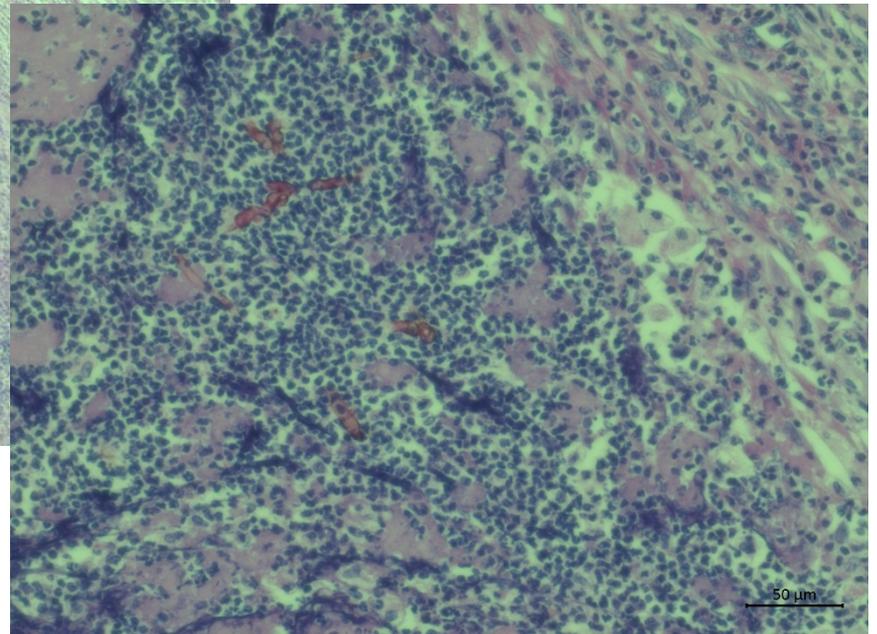
Development of a Murine Model of *C. cassicola* Phaeohyphomycosis



Development of a Murine Model of *C. cassiicola* Phaeohyphomycosis

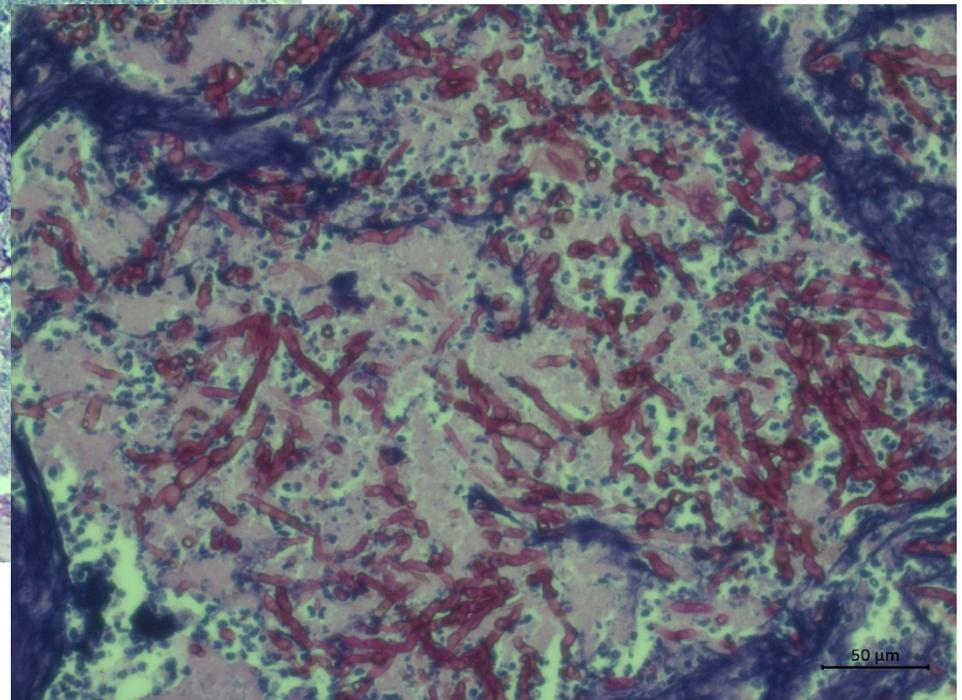
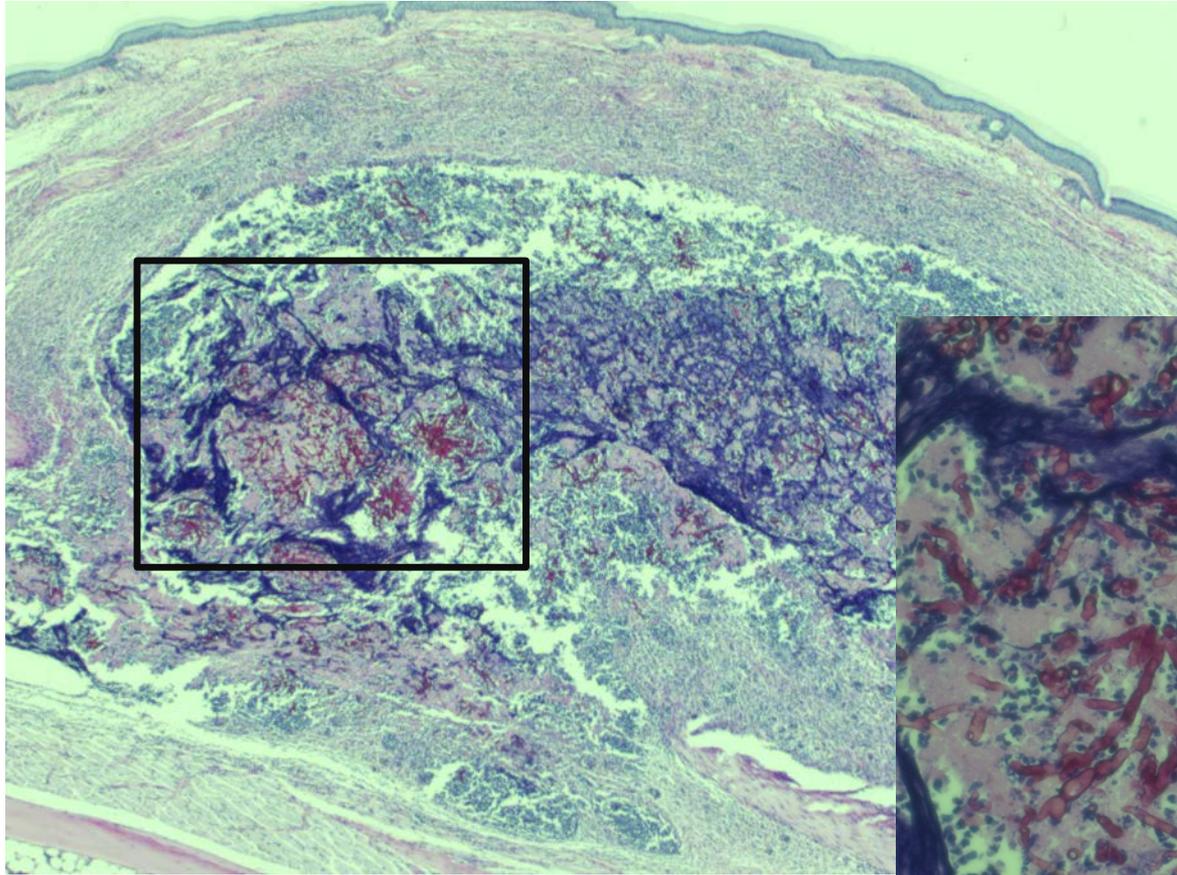


Development of a Murine Model of *C. cassiicola* Phaeohyphomycosis



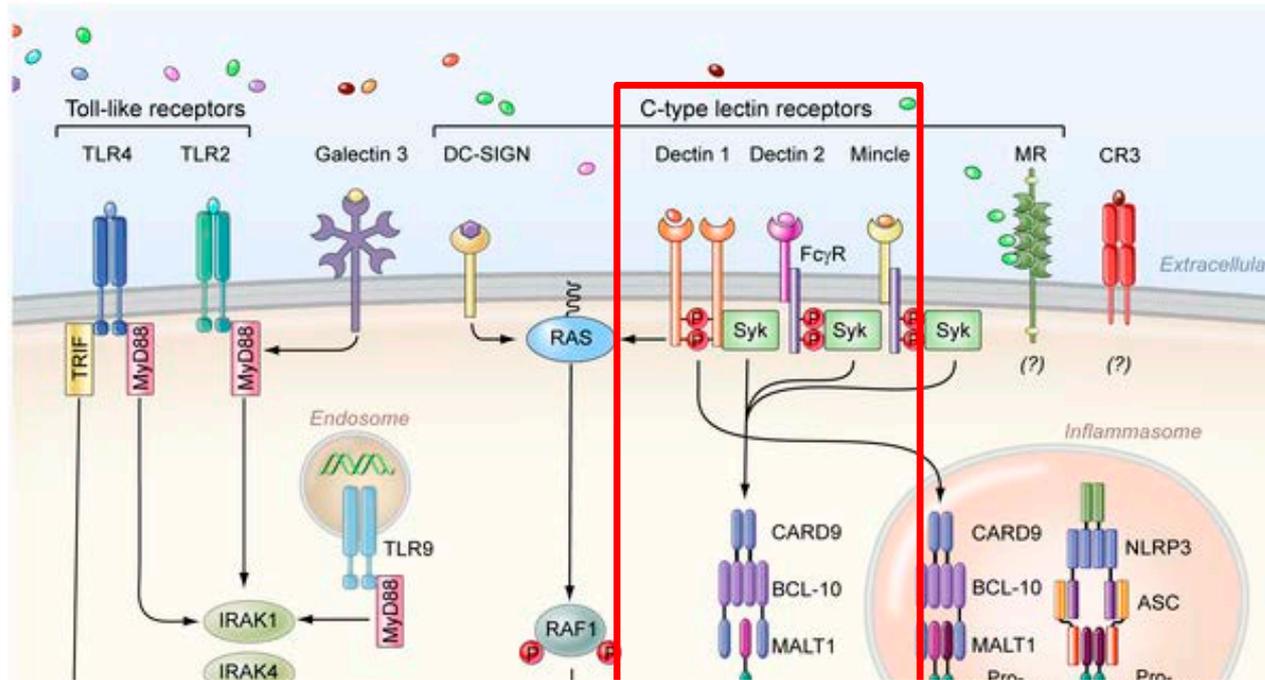
Card9^{+/+}

Development of a Murine Model of *C. cassiicola* Phaeohyphomycosis



Card9^{-/-}

SYK-CARD9 are centrally positioned in antifungal immune pathways



Fostamatinib
44 trials in
ClinicalTrials.gov

- RA
- AML
- GvHD
- Lymphomas
- Solid tumors
- Autoimmune
cytopenias

The **NEW ENGLAND**
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Fungal Host Defense – Take Home

- Host defense antifungal mechanisms are fungus-specific:
 - mucosal fungal disease (**Th17 cells**)
 - intracellular fungi (**macrophages-Th1 cells**)
 - invasive mold and *Candida* infections (**PMNs/Mφ**)
- Novel iatrogenic risk factors are expected to significantly increase the spectrum of acquired immunodeficiencies that result in opportunistic fungal disease in humans
- Emergence of resistant fungal strains (fungicides, chronic patient use)

Back to our Patient

- Quadruple antifungal therapy (AMB, triazole, echinocandin, terbinafine)
- Disease status
 - >95% resolution of superficial face lesions
 - Stability of pterygoid muscle and upper arm lesions
 - Slow progression of peri-parotid gland lymph node
 - Slow progression of bi-frontal lobe abscesses s/p debridement on 9/11/2018 with local relapse
- Disease not controllable with medical/surgical management, no oral antifungal regimen for chronic suppressive treatment
- Plan for transplantation. Haploidentical 10-year old brother coming from Colombia on Friday to be harvested
- Future plan for reconstructive surgery of the face

Thank you!