

Etude de l'effet des inhibiteurs de Btk de deuxièmes et troisièmes générations sur les réponses fonctionnelles des polynucléaires neutrophiles et des macrophages vis-à-vis d'*Aspergillus fumigatus*.

Journée du G2I- Mars 2024

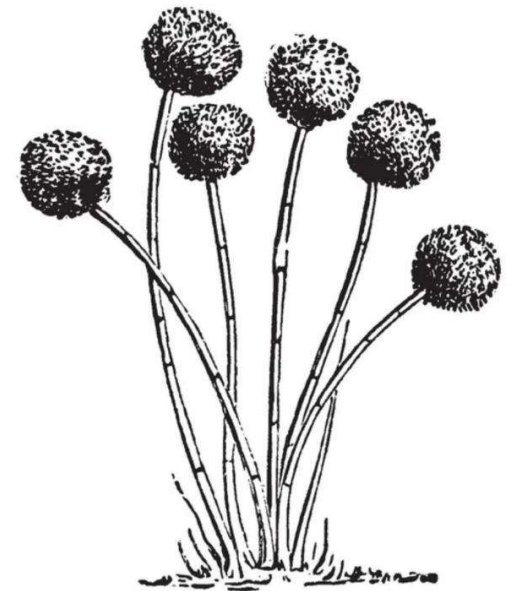
Guillaume THIZY

CCA en Maladies Infectieuses à St Louis

M2 BMC Microbiologie Sorbonne Université

Responsables scientifiques : Dr Arnaud FEKKAR

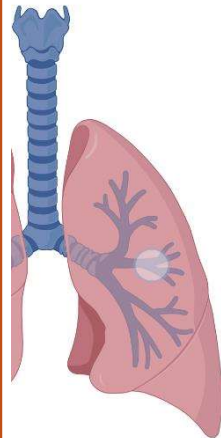
Dr Marion BLAIZE



Immunité anti-aspergillaire

Aspergillose invasive

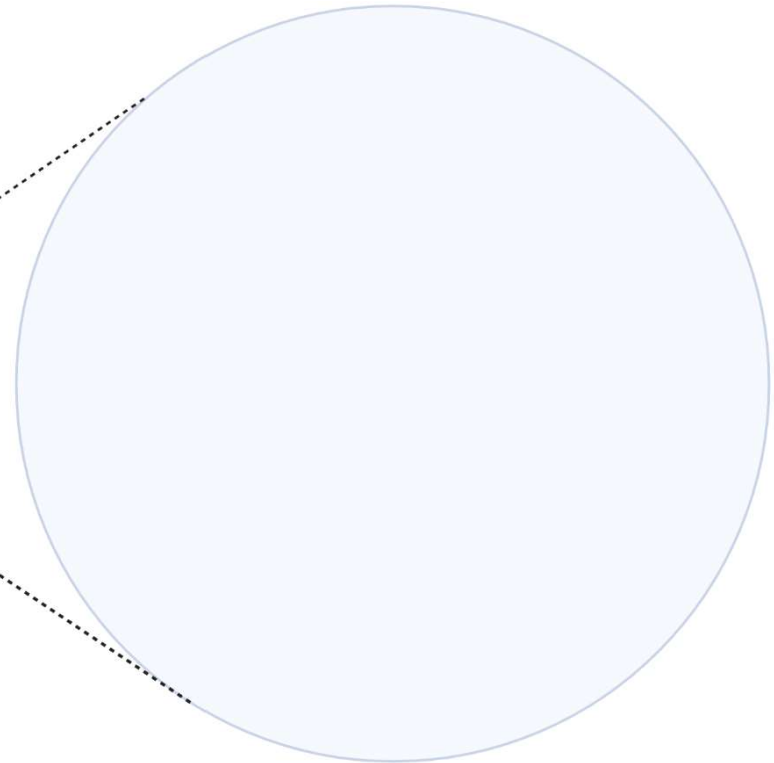
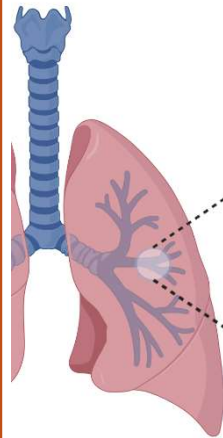
- Reconnaissance des conidies par les **macrophages alvéolaires**
 - Phagocytose de la conidie
 - Sécrétion de cytokines pro-inflammatoire



Immunité anti-aspergillaire

Aspergillose invasive

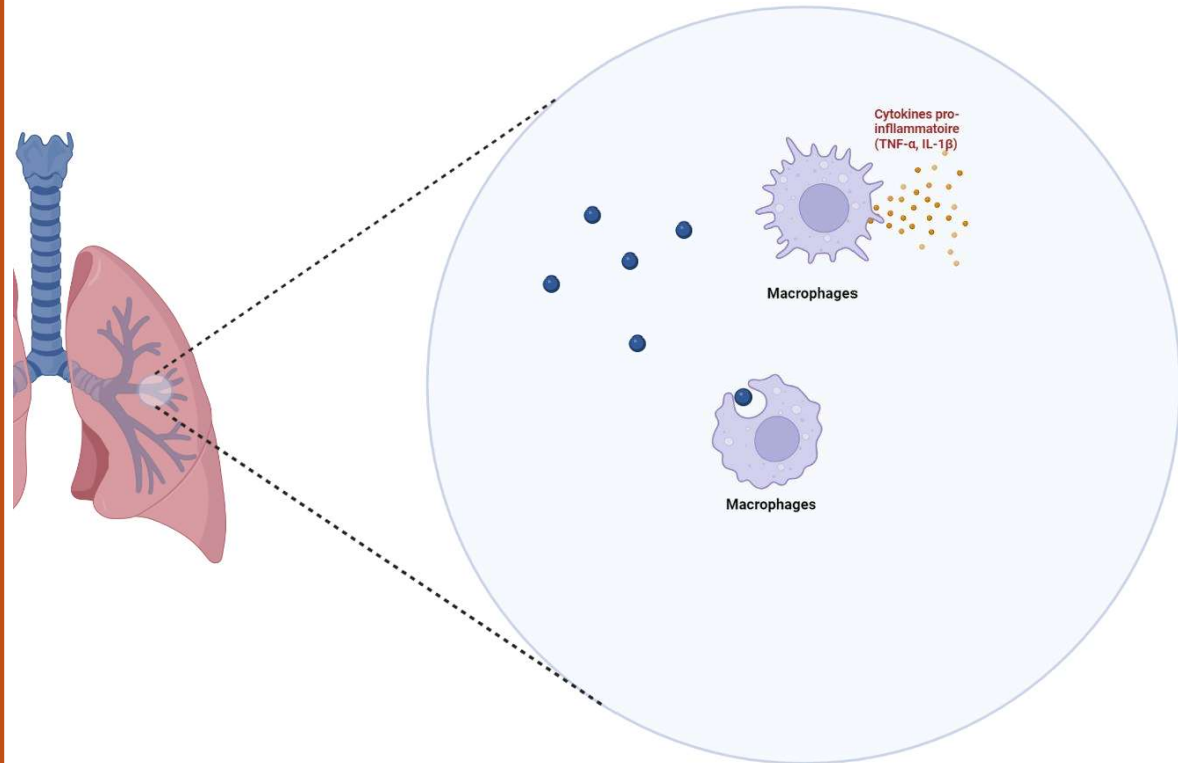
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Immunité anti-aspergillaire

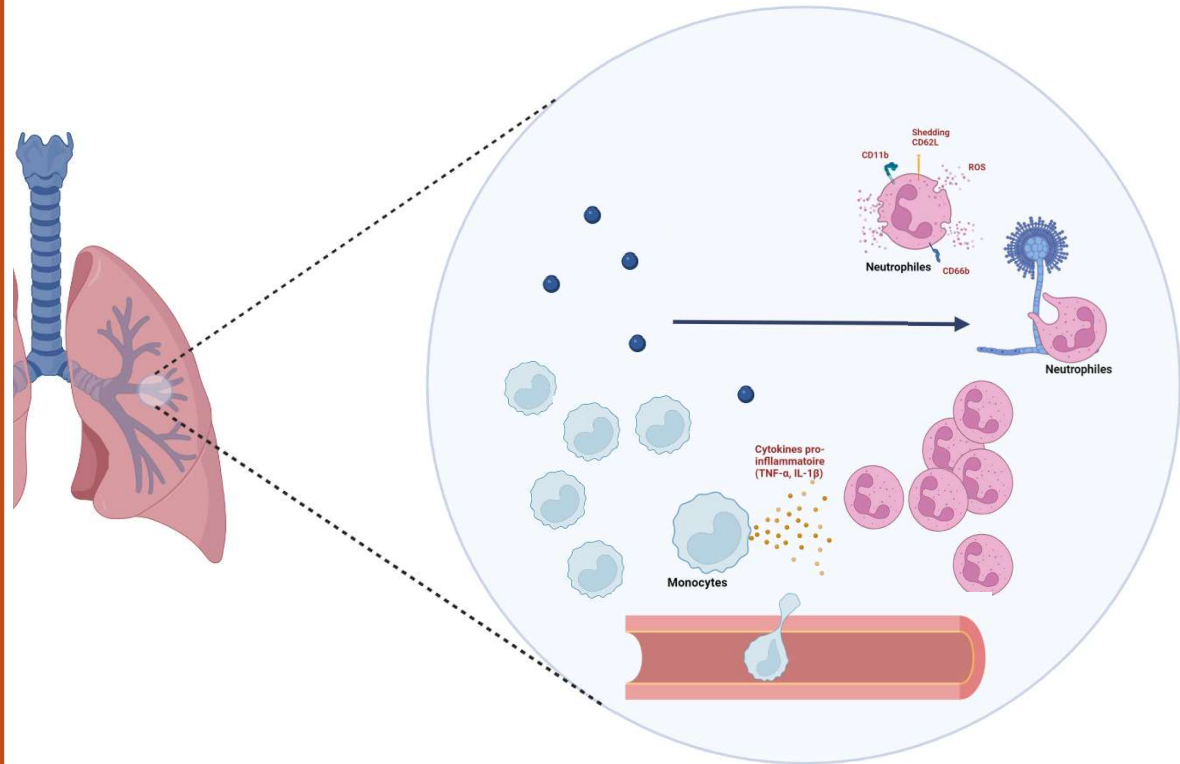
Recrutement des monocytes et PNN sur le site de l'infection

• Neutrophiles :

- Reconnaissance des hyphes
- Production de ROS.

• Monocytes :

- Phagocytose des conidies.
- Sécrétion de cytokines pro-inflammatoires.
- Inhibition de la germination des conidies



Hémopathies lymphoïdes et risque fongique

Table 1. Incidence of mold and yeast infections in patients with different types of hematologic malignancies.

HM	No. of patients	No. of IFI (incidence)	Molds		Yeasts	
			No. cases	Incidence %	No. cases	Incidence %
AML	3012	373 (12%)	239	7.9	134	4.4
ALL	1173	77 (6.5%)	51	4.3	26	2.2
CML	596	15 (2.5%)	14	2.3	1	0.2
CLL	1104	6 (0.5%)	5	0.4	1	0.1
NHL	3457	54 (1.6%)	30	0.9	24	0.7
HD	844	6 (0.7%)	3	0.35	3	0.35
MM	1616	7 (0.5%)	4	0.3	3	0.2
Total	11802	538 (4.6%)	346	2.9	192	1.6

HM: hematologic malignancies; AML: acute myeloid leukemia; ALL: acute lymphoid leukemia; CML: chronic myeloid leukemia; CLL: chronic lymphoid leukemia; NHL: non-Hodgkin's lymphoma; HD: Hodgkin's disease; MM: multiple myeloma; IFI: invasive fungal infection.

Pagano et al – Haematologica - 2006

Clinical Infectious Diseases

MAJOR ARTICLE



Serious Infections in Patients Receiving Ibrutinib for Treatment of Lymphoid Cancer

Tilly Varughese,¹ Ying Taur,^{1,2} Nina Cohen,³ M. Lia Palomba,^{2,4} Susan K. Seo,^{1,2} Tobias M. Hohl,^{1,2} and Gil Redelman-Sidi^{1,2}

¹Infectious Diseases Service, Memorial Sloan Kettering Cancer Center, ²Department of Medicine, Weill Cornell Medical College, and ³Department of Pharmacy and ⁴Lymphoma Service, Memorial Sloan Kettering Cancer Center, New York, New York

Ibrutinib

- 378 patients avec hémopathie lymphoïdes sous Ibrutinib
- **4.2% d'infection fongique invasive dont 62.5% sans facteur de risque d'IFI.**
- **Incidence identique chez les patients en première ligne et les patients en rechute.**

Hémopathies lymphoïdes et risque fongique

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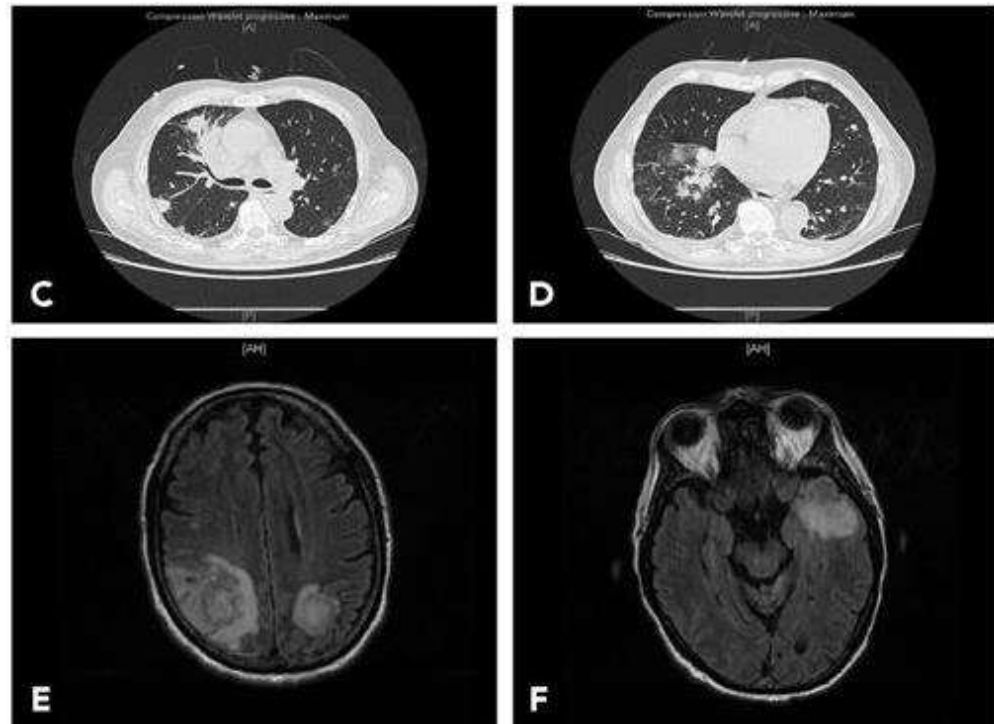
Early-onset invasive aspergillosis and other fungal infections in patients treated with ibrutinib

[Clinical Trials & Observations](#) [Brief Report](#)

David Ghez, Anne Calleja, Caroline Protin, Marine Baron, Marie-Pierre Ledoux, Gandhi Damaj, Mathieu Dupont, Brigitte Dreyfus, Emmanuelle Ferrant, Charles Herbaux, Kamel Laribi, Ronan Le Calloch, Marion Malphettes, Franciane Paul, Laetitia Souchet, Malgorzata Truchan-Graczyk, Karen Delavigne, Caroline Dartigeas, Loïc Ysebaert on behalf on the French Innovative Leukemia Organization (FILO) CLL group

[Check for updates](#)

Blood (2018) 131 (17): 1955-1959.



Hémopathies lymphoïdes et risque fongique

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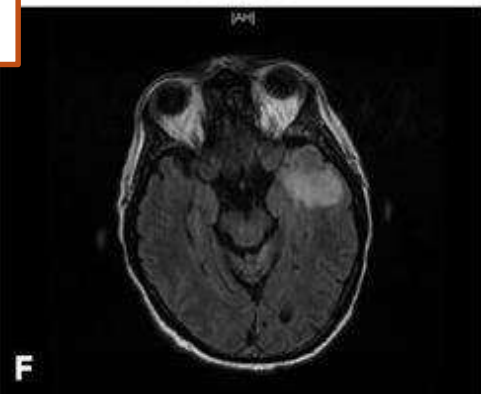
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17): 1955-1959.



Jusqu'à 39% (7/18) des patients dans un essai clinique d'ibrutinib en traitement de Lymphomes du CNS sous corticothérapie.

Lionakis – Cancer cell – 2017

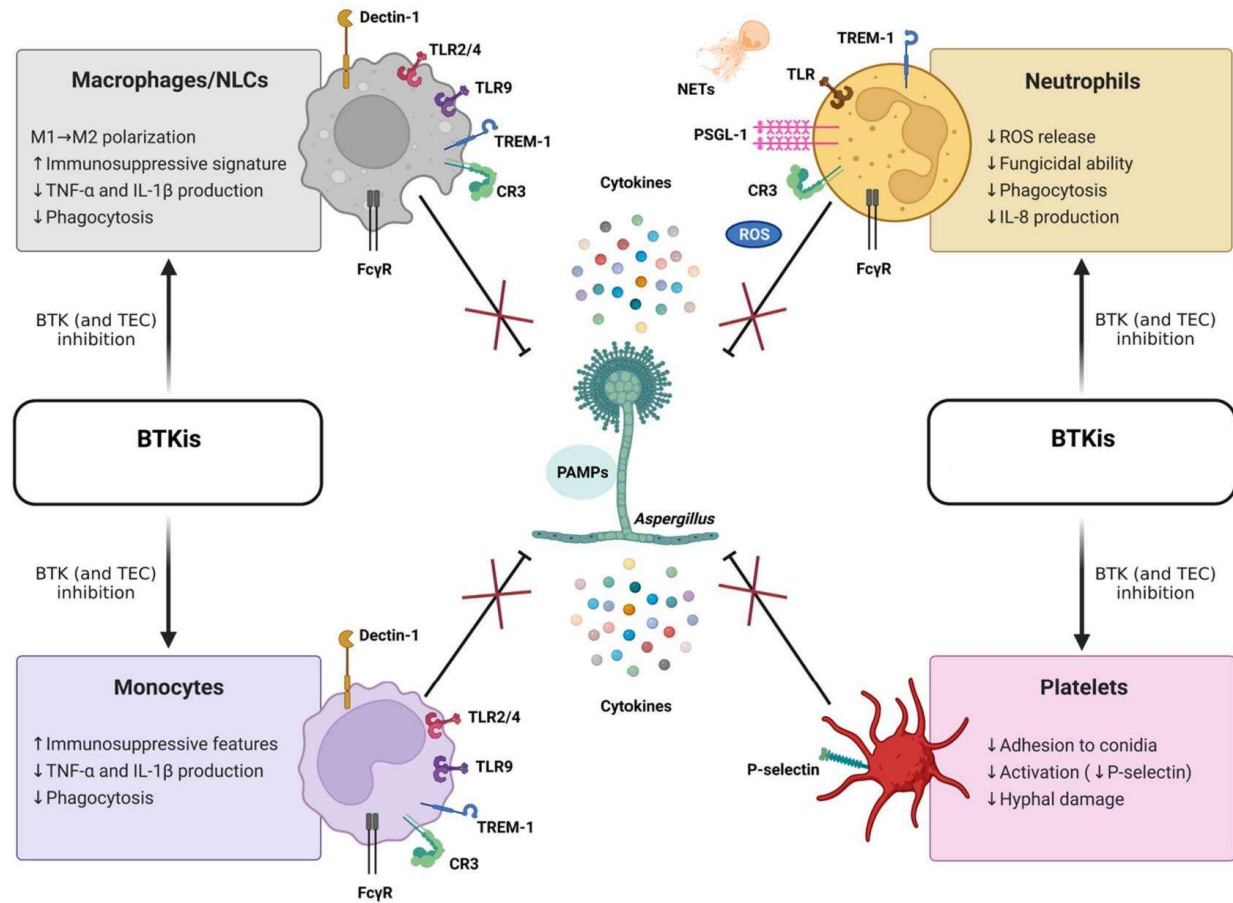
Inhibiteurs de BTK et déficit immunitaire

Ibrutinib (Inhibiteur de BTK)

- Déficiences fonctionnelles des PNN et des Monocytes / Macrophages vis-à-vis de la réponse anti aspergillaire.

XLA

- Pas d'infection fongique invasive chez les patients avec une agammaglobulinémie de Bruton (déficit en BTK).
- Effet OFF-Target ??



Inhibiteurs de BTK

Ibrutinib – Effect Off target

Inhibiteur de la BTK

Inhibition de nombreuses kinases (TEC, ITK, CMX, EGFR...) responsable des effets indésirables (arythmie, saignements..)

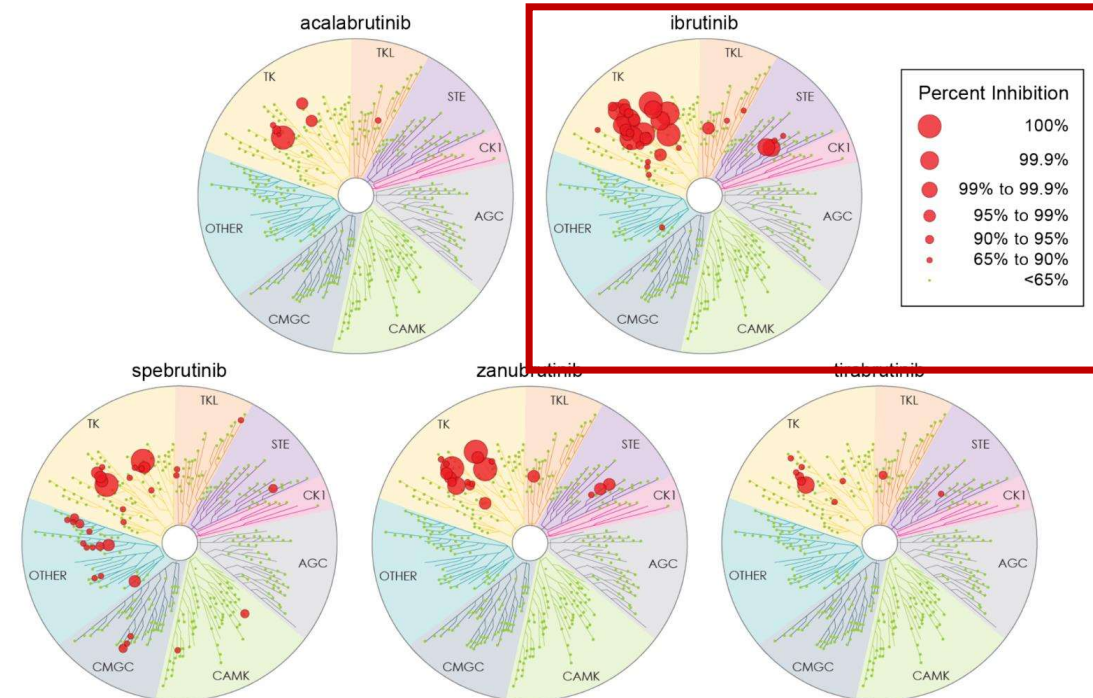
Acalabrutinib

- Inhibiteur sélectif de la BTK
- Moins d'effet secondaire liés aux inhibition off-target

Potency and Selectivity of BTK Inhibitors in Clinical Development for B-Cell Malignancies

Allard Kaptein, Gerjan de Bruin, Maaïke Emmelot-van Hoek, Bas van de Kar, Anouk de Jong, Michael Gulrajani, Dennis Demont, Todd Covey, PhD, Diana Mittag, Tjeerd Barf

Figure 1. Kinome profiling at a single dose of 1 µM (KINOMEScan, Eurofins DiscoverX)



Objectifs

Analyser les conséquences de l'exposition aux inhibiteurs de Btk de deuxième et troisième générations des neutrophiles et des monocytes sur la réponse anti-*Aspergillus*.

Témoins sains :

- Cellules exposés à différentes doses d'inhibiteurs de BTK



Témoins sains



+

Ibrutinib



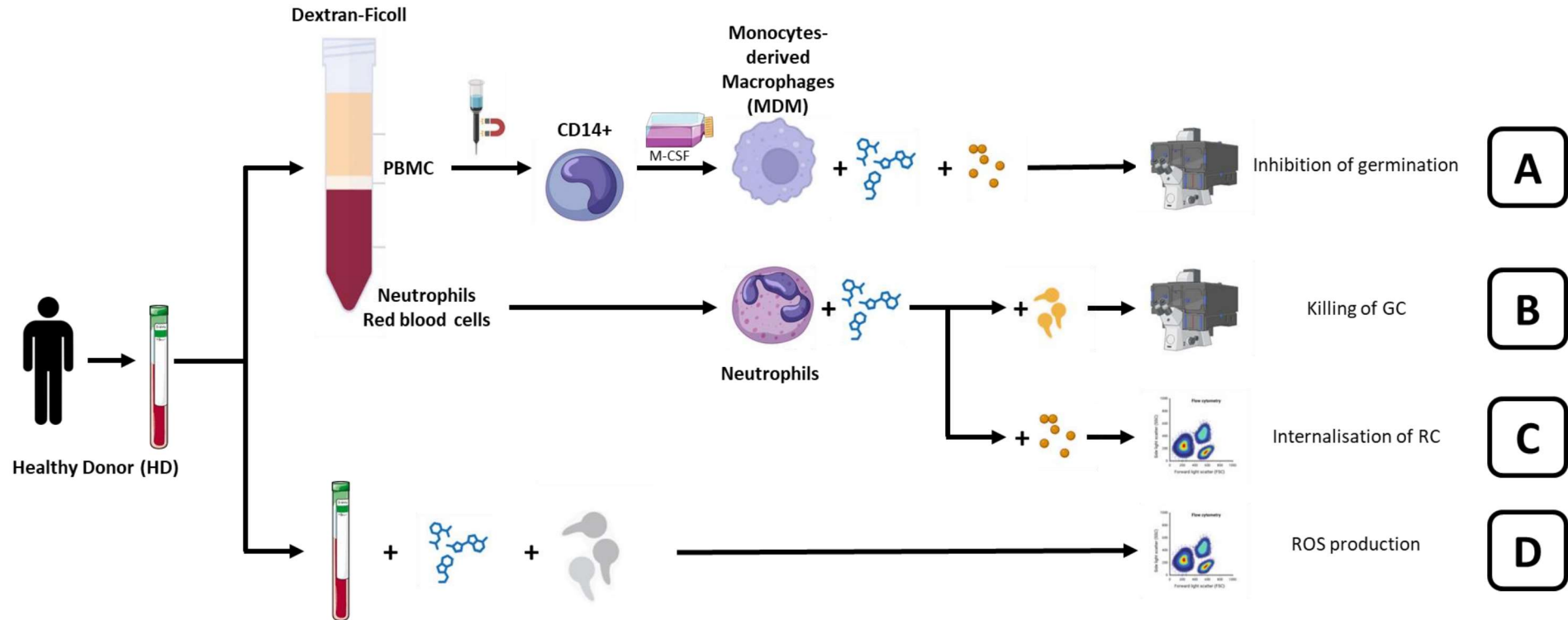
Zanubrutinib




Aclabrutinib




Matériel et méthode




Legend

 **BTK inhibitors**
(Acalabrutinib, Ibrutinib, Zanubrutinib)

 ***Aspergillus fumigatus* DsRed Resting Conidia (RC)**

 ***Aspergillus fumigatus* DsRed Germinating Conidia (GC)**

 ***Aspergillus fumigatus* Germinating Conidia (GC)**



Flow cytometry approach



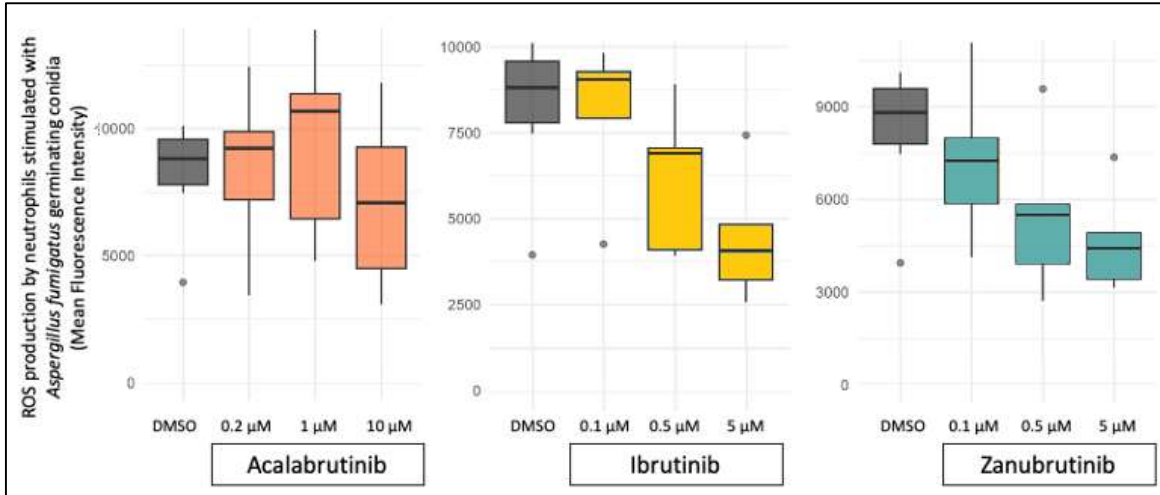
Video microscopy approach



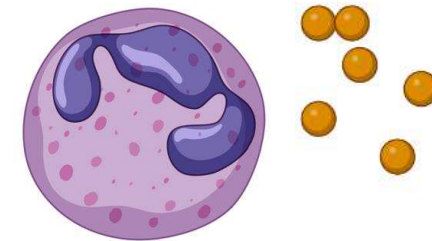
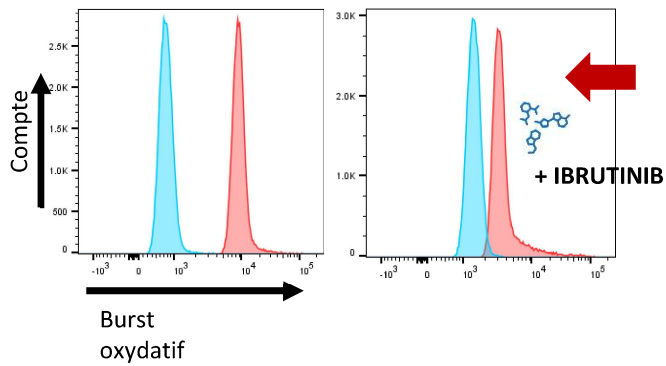
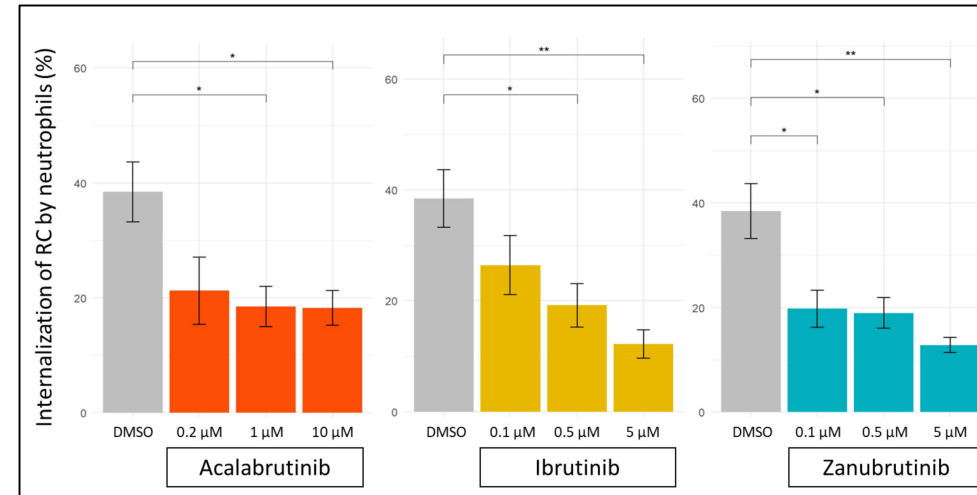
Témoins sains



Burst oxydatif



Phagocytose de conidies





Co-culture PNN / *Aspergillus fumigatus* Vidéo-microscopie



Conidies germées *A. fumigatus* DsRed

Neutrophiles

MOI : 1:16

Sytox Green

ADN extracellulaire

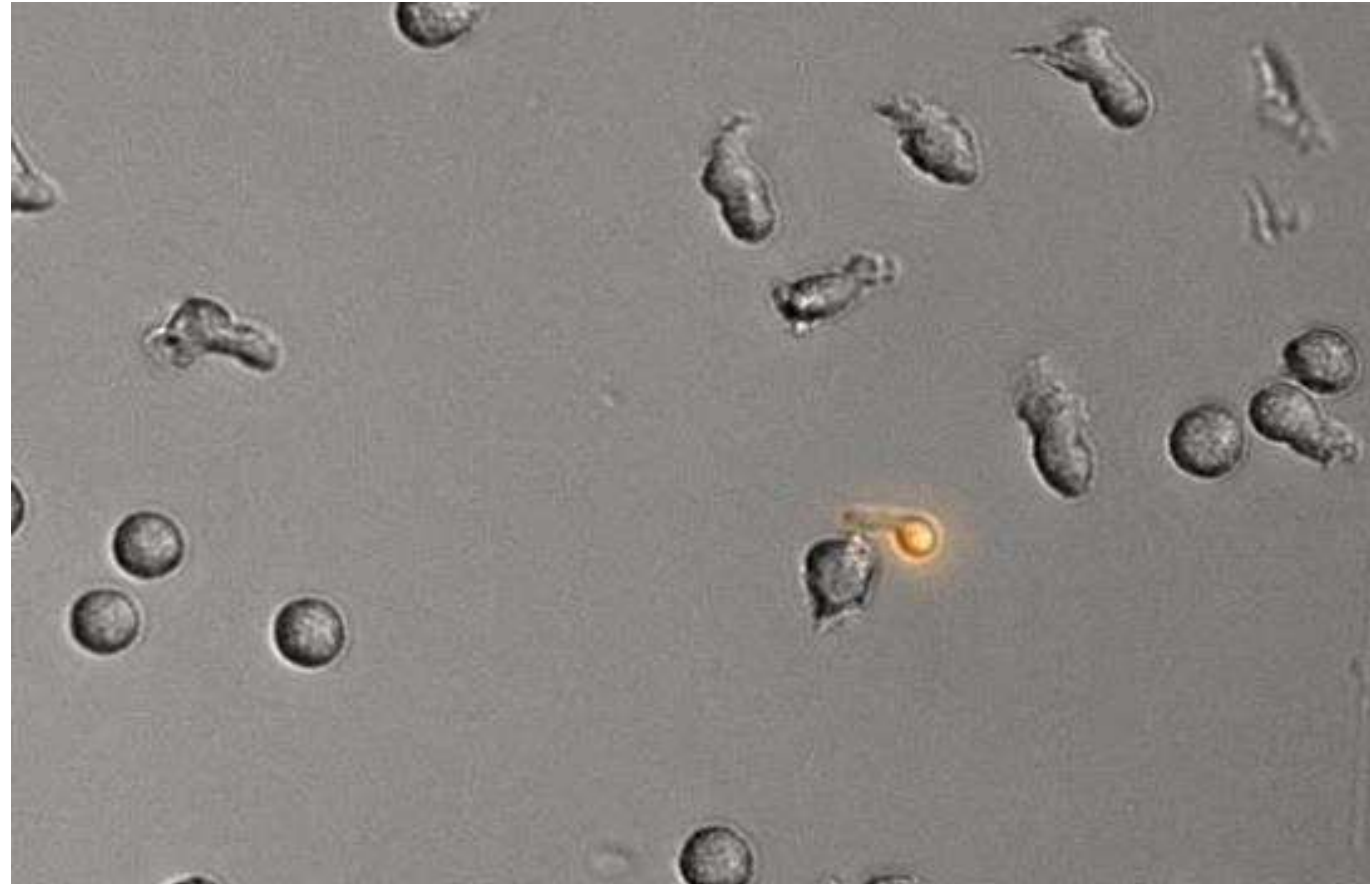
Vidéo 16h

1 image / 3min

Analyse

Interaction cellule-champignon

« engulfment » « killing »



Film : durée 7h
Engulfment : T = 2h30
Killing : T = 6h00



Co-culture PNN / *Aspergillus fumigatus* Vidéo-microscopie



Conidies germées *A. fumigatus* DsRed

Neutrophiles

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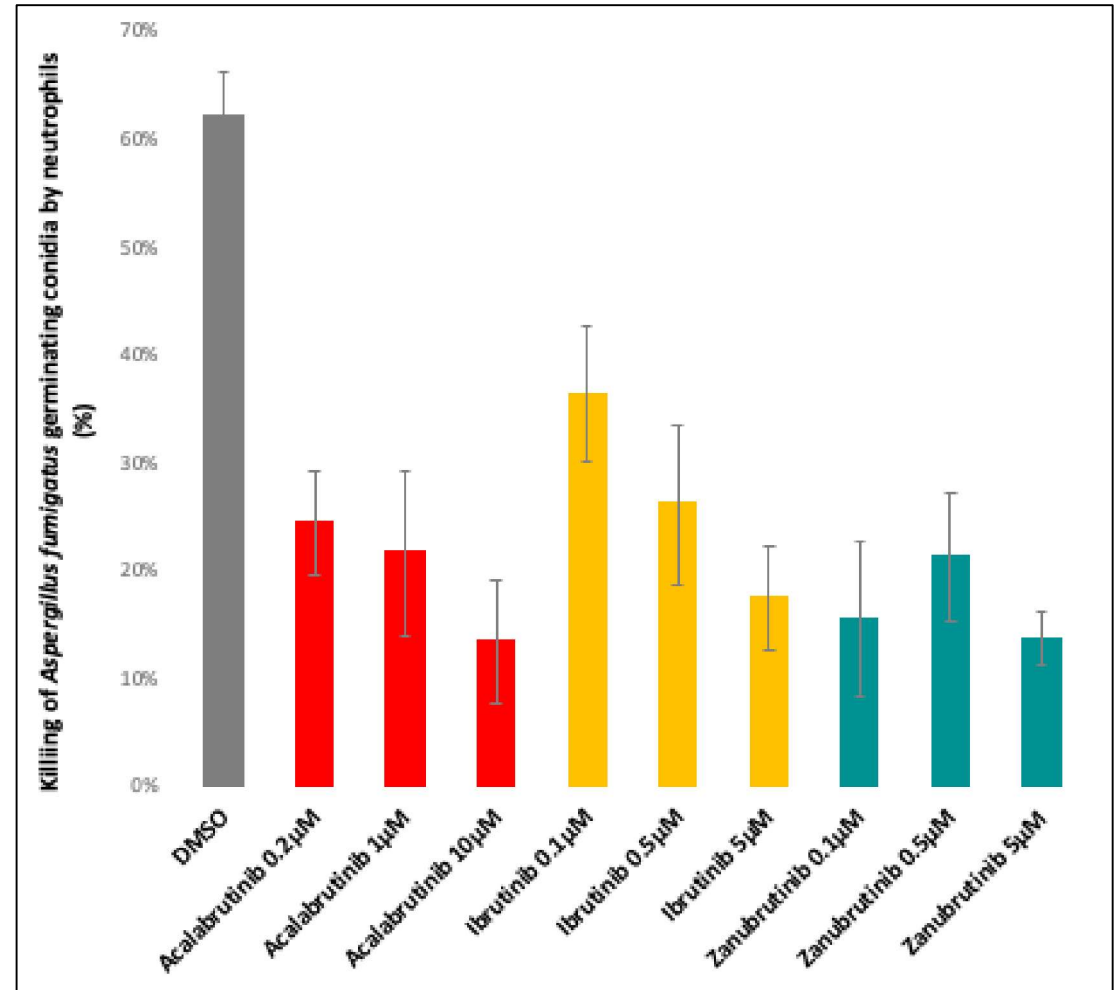
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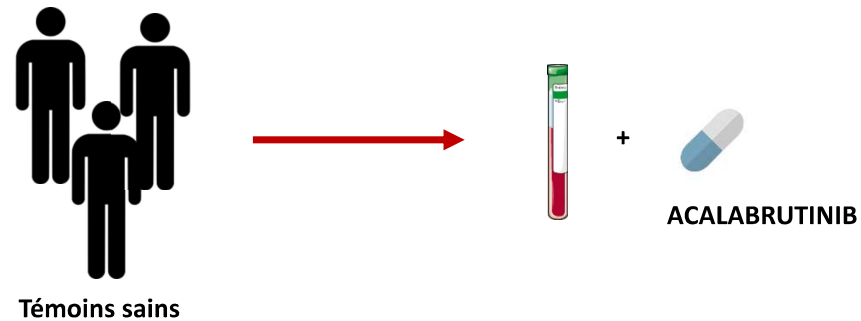


Poster – congrès AAAM 2024 – Advance Against Aspergillosis and Mucormycosis

Patients

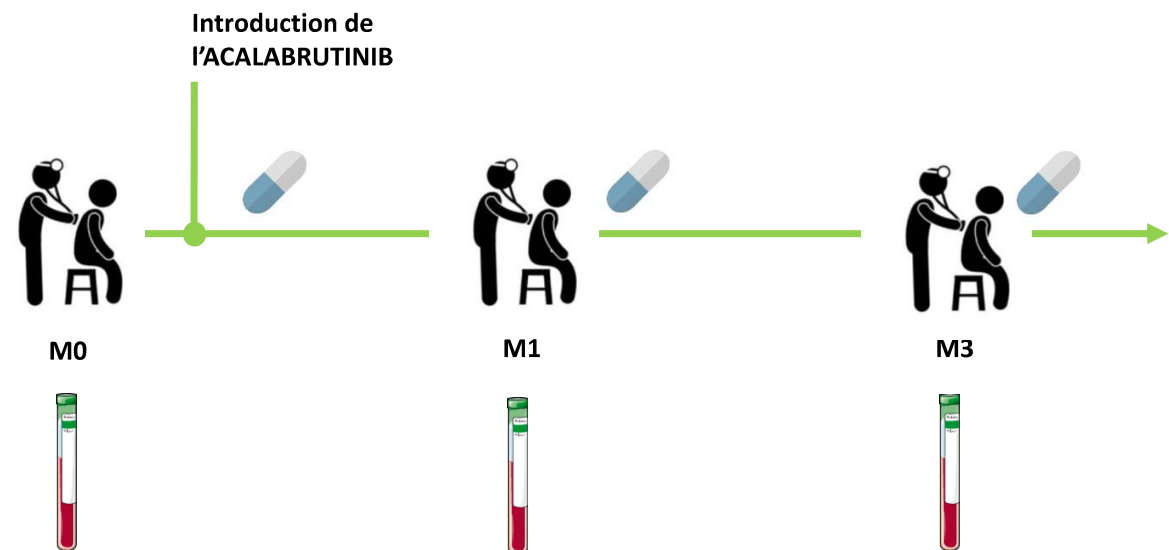
Témoins sains :

- Cellules exposés à différentes doses d'inhibiteurs de BTK



Patients :

- Hommes et femmes de plus de 18 ans au moment de l'inclusion.
- Adultes devant recevoir de l'acalabrutinib
- Bénéficiaire ou affilié à un régime de sécurité sociale.
- Acceptation des modalités de l'étude attestée par la signature d'un consentement libre et éclairé.



Production de ROS

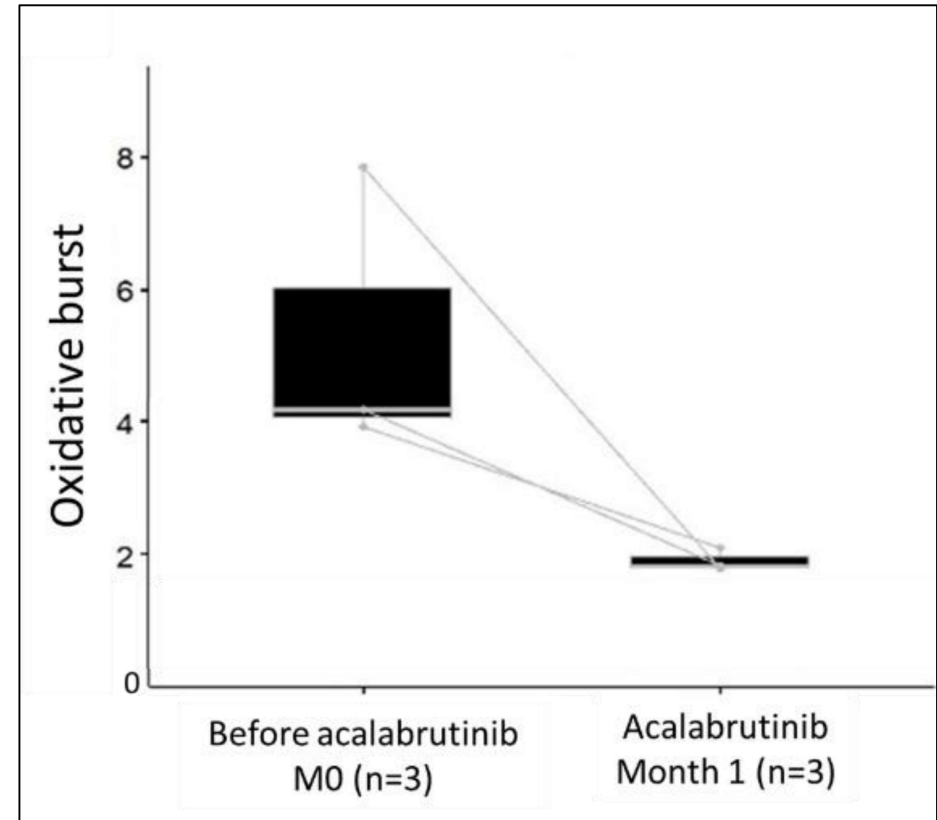
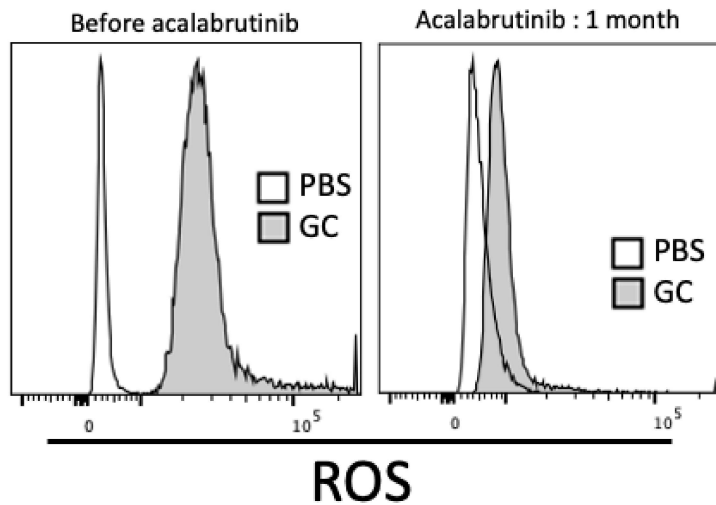
N=3

Acalabrutinib M0 et M1

Age médian : 61 ans

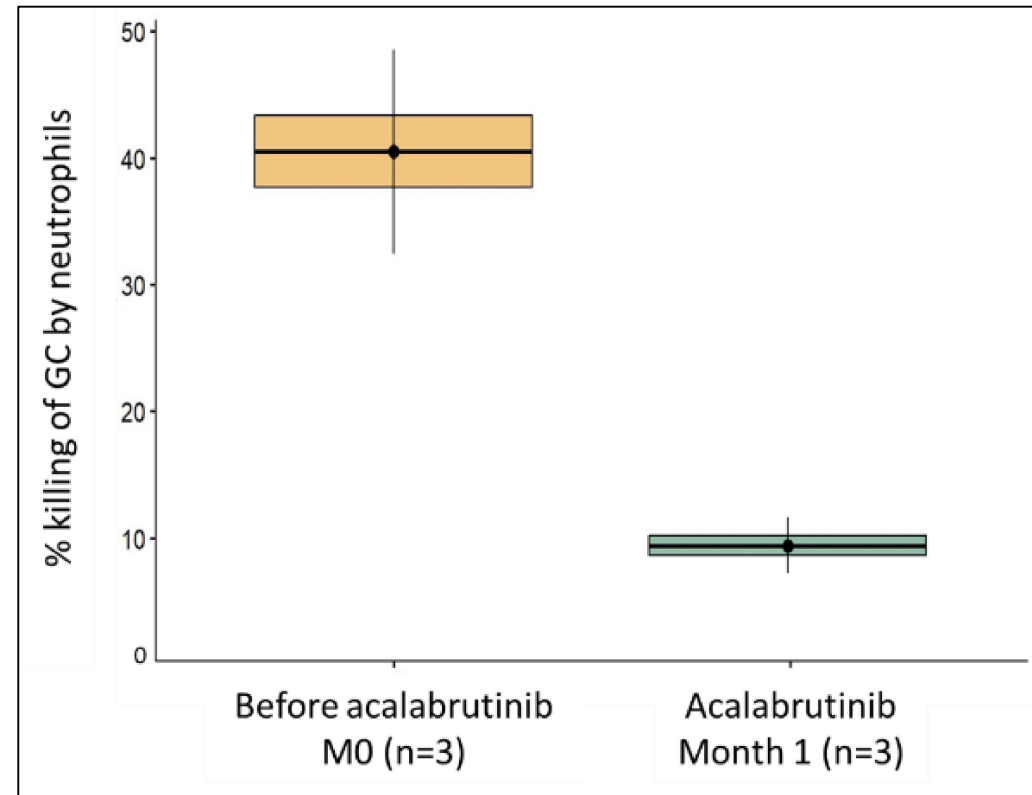
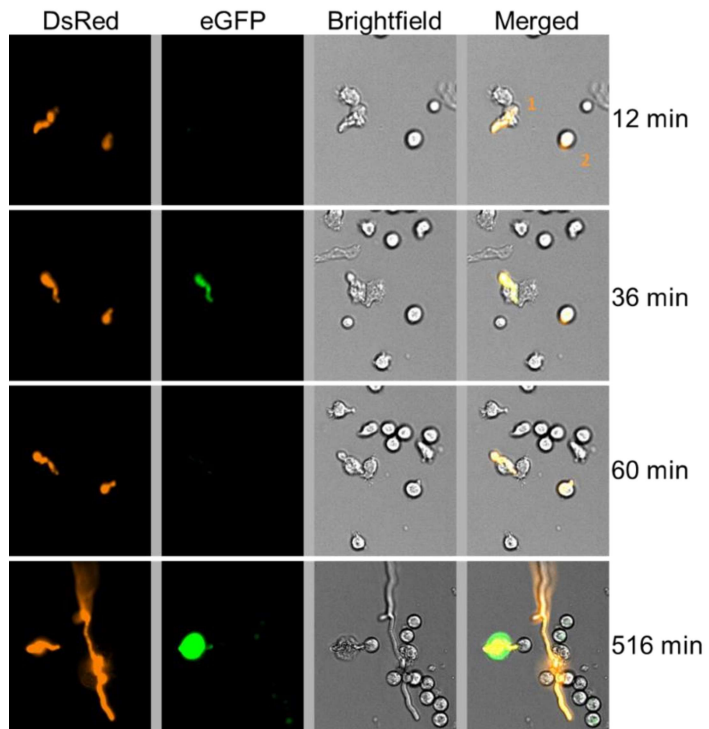
Homme/Femme : 2/1

Pathologies : LLC 2/3

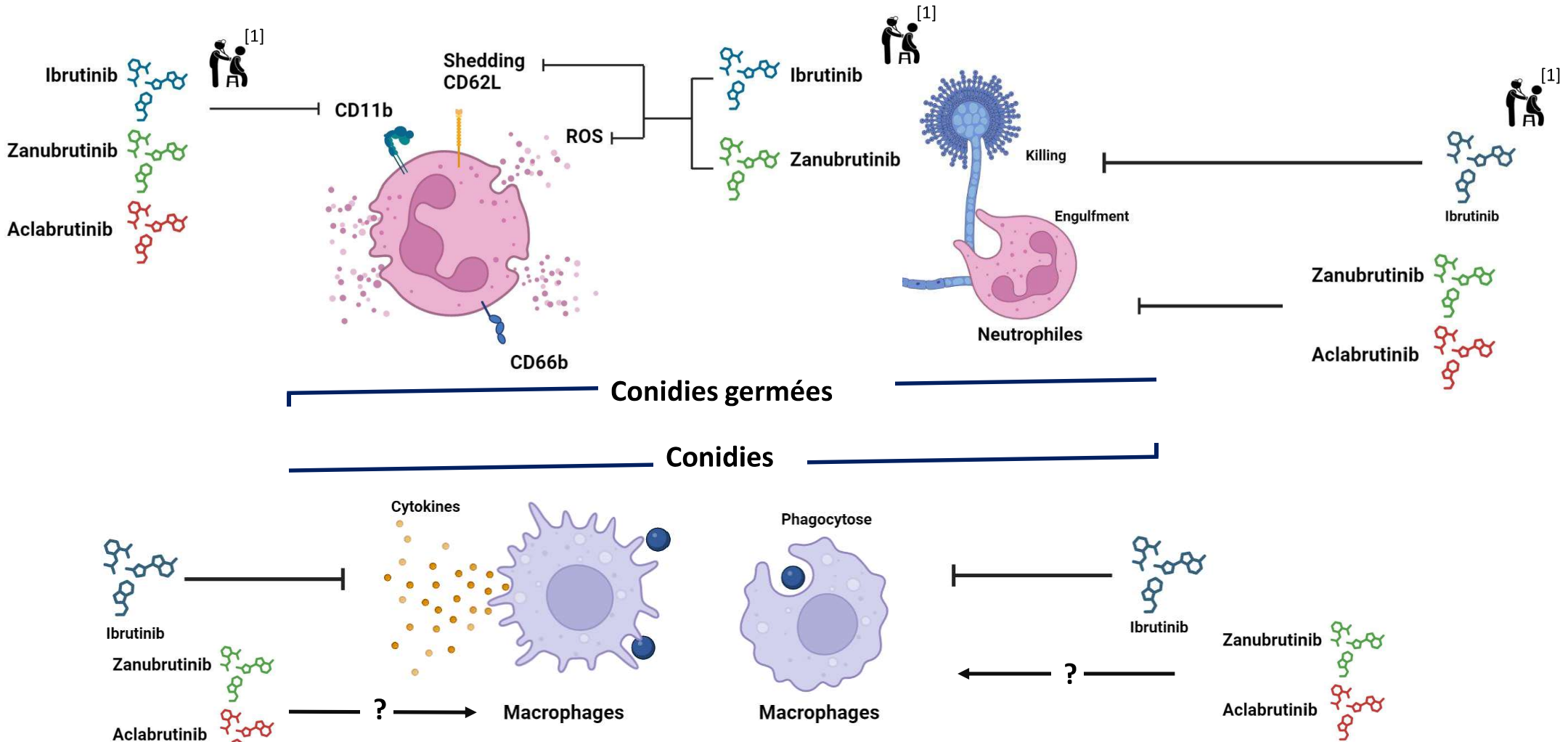


Poster – congrès *TIMM 2023 - Trends In Medical Mycology*

Co-culture PNN / *Aspergillus fumigatus*



Conclusion



[1]Blez, Blaize, et al and Fekkar – Ibrutinib induces multiple functional defects in the neutrophil response against *Aspergillus fumigatus*, Haematologica – 2020

Merci de votre attention

Equipe Chimiokines, Phagocytose et Inflammation

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Christophe Combadière

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Olivia Bonduelle

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Guillaume Thizy (M2)

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Eleonore Weber-Delacroix

Michèle-Noël Wokam



Service d'Hématologie Clinique

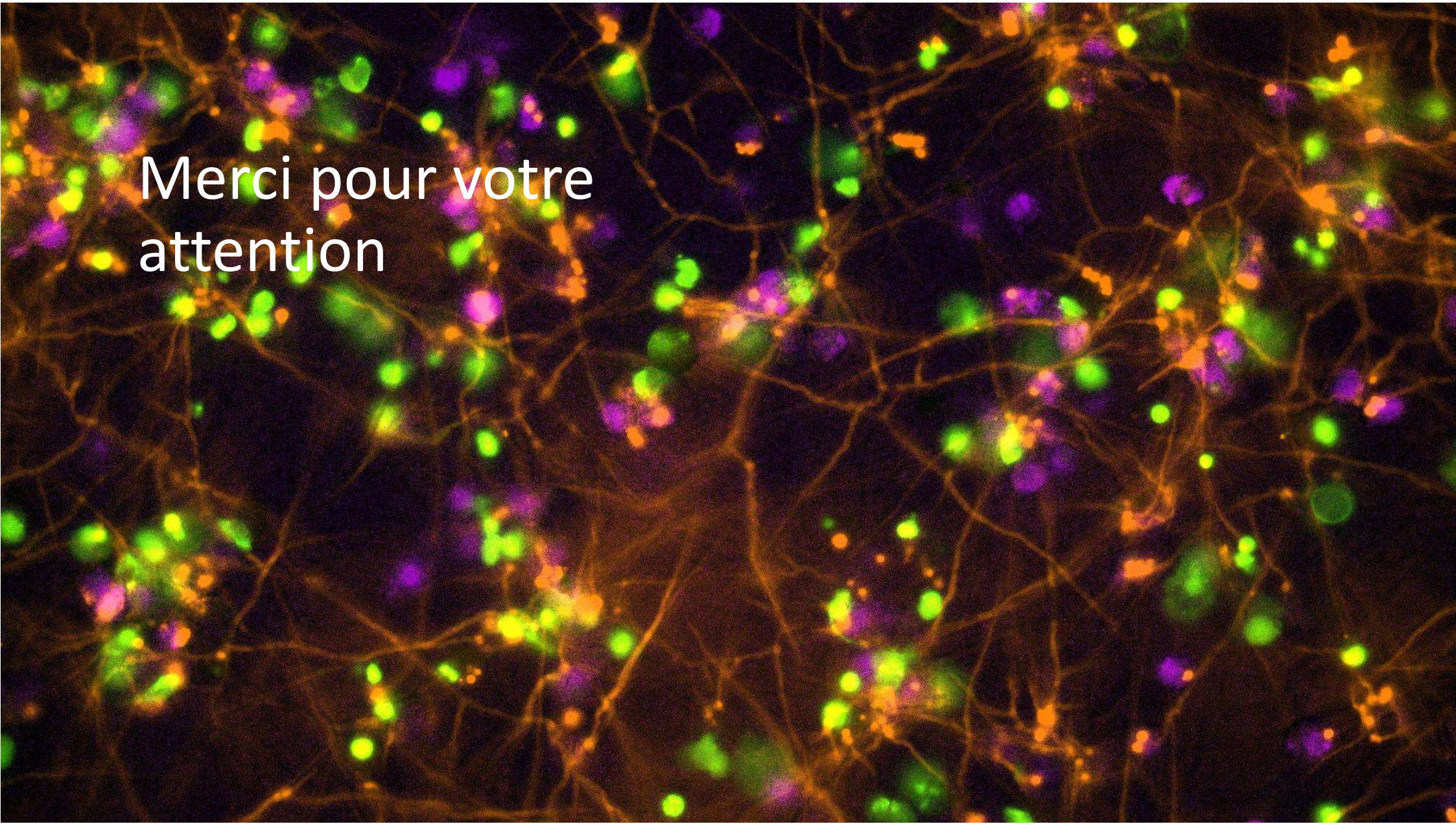
Damien Roos-Weil

Anaïs Portalier

Charlotte Spindler



Merci pour votre
attention



Ibrutinib et Aspergillose

Ibrutinib

- 378 patients avec hémopathie lymphoïdes sous Ibrutinib
- **4.2% d'infection fongique invasive dont 62.5% sans facteur de risque d'IFI.**
- Incidence identique chez les patients en première ligne et les patients en rechute.

Particularités des aspergilloses

- **Précoces** : Médiane 3 mois de traitement
- **Atteinte du SNC** dans 40% des cas.

Clinical Infectious Diseases

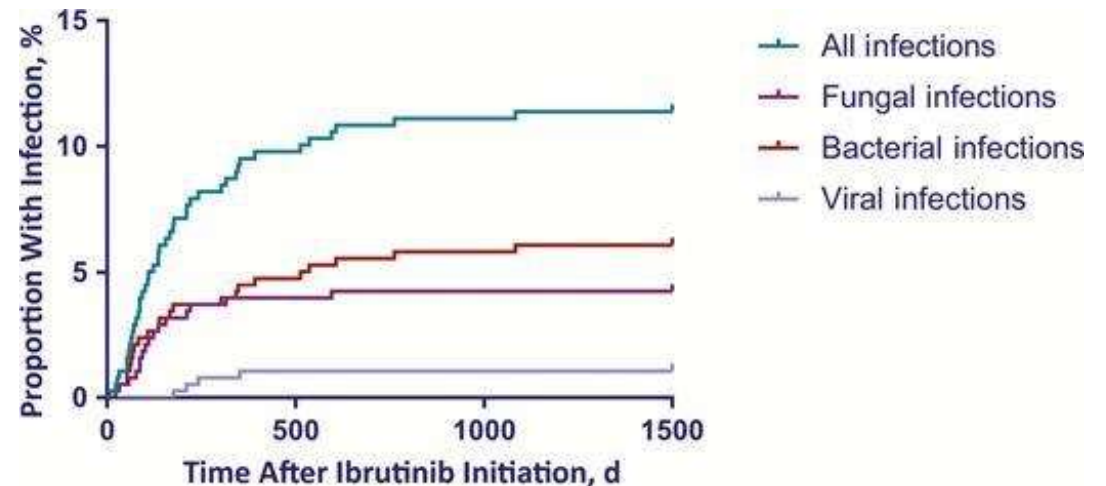
MAJOR ARTICLE



Serious Infections in Patients Receiving Ibrutinib for Treatment of Lymphoid Cancer

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¹Infectious Diseases Service, Memorial Sloan Kettering Cancer Center; ²Department of Medicine, Weill Cornell Medical College, and ³Department of Pharmacy and ⁴Lymphoma Service, Memorial Sloan Kettering Cancer Center, New York, New York



Acalabrutinib

Molecular targets for therapy

Pooled analysis of safety data from clinical trials evaluating acalabrutinib monotherapy in mature B-cell malignancies

Richard R. Furman , John C. Byrd, Roger G. Owen, Susan M. O'Brien, Jennifer R. Brown, Peter Hillmen, Deborah M. Stephens, Nataliya Chernyukhin, Tamara Lezhava, Ahmed M. Hamdy, Raquel Izumi, Priti Patel, Marshall Baek, Beth Christian, Martin J. S. Dyer, Matthew J. Streetly, Clare Sun, Simon Rule, Michael Wang, Paolo Ghia, Wojciech Jurczak, John M. Pagel & Jeff P. Sharman

Leukemia 35, 3201–3211 (2021) | [Cite this article](#)

Acalabrutinib

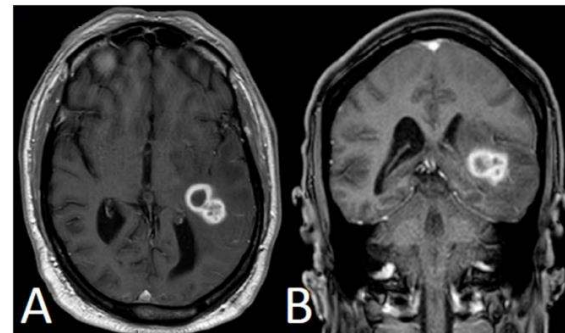
- Acalabrutinib n= 1040 patients (9 études randomisés).
- **Infections chez 67% des patient**, majoritairement dans les **6 premiers mois**.
- 9 patients (0.9%) infections fongiques sévère
 - 1 Cryptococcose disséminée
 - 2 « pneumonies fongiques »
 - 4 aspergilloses invasives dont 2 mortelles
 - 1 sepsis à *Candida*

 *Current Oncology*



Case Report

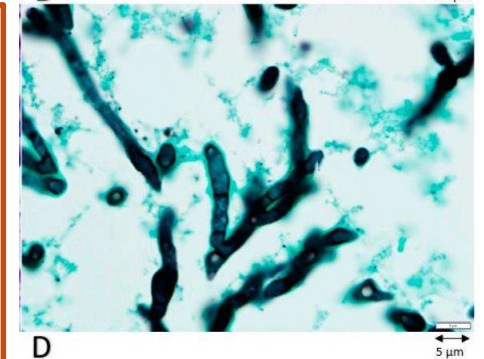
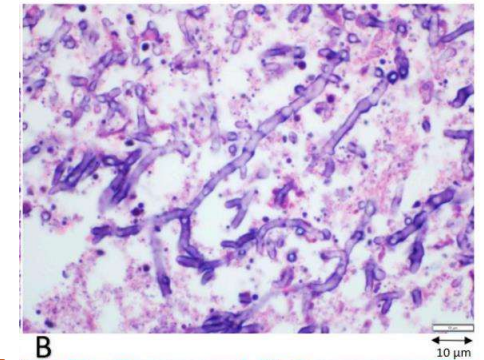
Cerebral Invasive Aspergillosis in a Case of Chronic Lymphocytic Leukemia with Bruton Tyrosine Kinase Inhibitor



Patient de 62 ans avec LLC sous **Acalabrutinib** + obinutuzumab en 1^{ère} ligne.

Pas de corticothérapie

Aspergillose cérébrale à M3



Acalabrutinib

ASPERGILLOSIS CASES SERIE

The cases were collected from three hospitals in Paris, France and occurred during 2022.

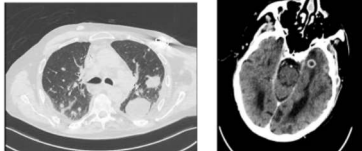
Case 1 : ♂ 80 years old – CLL for 16 years

Acalabrutinib for LLC progression, stopped at D21
Corticosteroids for autoimmune hemolytic anemia

General conditions **deterioration**
Neurological disorders

Blood PCR *A. fumigatus* +
 Beta-D-glucan +
 Galactomann +

CT scan : multiple **nodules** (D14)
Brain lesions on scan (D35)



Voriconazole D29

Patient died at D61

Case 2 : ♀ 68 years old – CLL for 5 years

Acalabrutinib, stopped at D33 + Obinutuzumab
Corticosteroids for ocular inflammatory neuropathy

Fever
Respiratory distress

CT scan : crazy paving +
reticulo-nodular syndrom

BAL
 PCR *A. fumigatus* +
 mycelium on **direct examination**
 PCR *P. jirovecii* +
Blood
 PCR *A. fumigatus* +
 Beta-D-glucan



Trimethoprim/sulfamethoxazole D33
 Voriconazole D36

Patient died at D48

Case 3 : ♂ 77 years old – CLL for 10 years

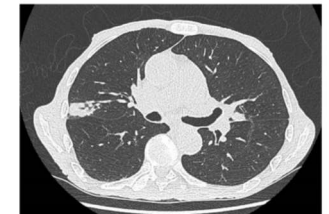
Obinutuzumab then **Acalabrutinib** for LLC progression,
 stopped at D65

General conditions **deterioration**

Sputum
A. fumigatus culture +
 PCR *A. fumigatus* +

Pulmonary biopsy
A. Fumigatus culture +

CT chest scan : 2 pulmonary
nodules



Isavuconazole D65

Patient died at D71

Zanubrutinib

CLINICAL TRIALS AND OBSERVATIONS | FEBRUARY 17, 2022

Pooled safety analysis of zanubrutinib monotherapy in patients with B-cell malignancies

 Clinical Trials & Observations

Constantine S. Tam, Meletios Dimopoulos, Ramon Garcia-Sanz, Judith Trotman, Stephen Opat, Andrew W. Roberts, Roger Owen, Yuqin Song, Wei Xu, Jun Zhu, Jianyong Li, Lugui Qiu, Shirley D'Sa, Wojciech Jurczak, Gavin Cull, Paula Marlton, David Gottlieb, Javier Munoz, Tycel Phillips, Chenmu Du, Meng Ji, Lei Zhou, Haiyi Guo, Hongjie Zhu, Wai Y. Chan, Aileen Cohen, William Novotny, Jane Huang, Alessandra Tedeschi

 Check for updates

Blood Adv (2022) 6 (4): 1296–1308.

Zanubrutinib

- Analyse poolée de **779 patients traités par Zanubrutinib** a partir de 6 études : **18 infections fongiques invasives (2.3%)**
 - 7 Cryptococcoses
 - 5 Aspergilloses invasives (dont 1 cérébrale)
 - 5 pneumonie fongiques (non spécifiés)
 - 1 encéphalite fongique.