Invasive aspergillosis-on-chip: A quantitative treatment study of human *Aspergillus fumigatus* infection

T.N. Mai Hoang[4*], Zoltán Cseresnyés[3*], Susann Hartung[1,2*], Marco Blickensdorf[3], Christopher Saffer[3], Knut Rennert[4], Alexander S. Mosig[5,6], Marc Thilo Figge[3,7], Marie von Lilienfeld-Toal[1,2]

* Authors contributed equally

1 Infections in Hematology and Oncology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena

2 Department for Haematology and Medical Oncology, Jena University Hospital, Jena

3 Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena

4 Dynamic 42 GmbH, Jena

5 INSPIRE, Center for Sepsis Control and Care, Jena University Hospital, Jena 8

6 Institute of Biochemistry II, Jena University Hospital, Jena

7 Institute of Microbiology, Faculty of Biological Sciences, Friedrich Schiller University, Jena

Invasive pulmonary aspergillosis (IPA), caused by filamentous fungus *Aspergillus fumigatus* (*A. fumigatus*) in immunocompromised individuals, is associated with high mortality rate. Infection occurs in the lung where inhaled *A. fumigatus* conidia can germinate and grow into filamentous bodies (hyphae) which can subsequently invade the blood vessels.

Organ-on-chip models of the human lung can be a valuable tool to better recapitulate human physiology and pathophysiology than existing cell culture and animal models. In our recently established invasive aspergillosis on chip model (IAC), *A. fumigatus* conidia germinated and grew on an air-exposed alveolar epithelium with and without the presence of primary monocyte-derived macrophages, the growing hyphae could then penetrate the epithelial layer and invade the medium-perfused vascular cell layer through a porous membrane, mimicking the main events of IPA.

Using three-dimensional imaging and an algorithm-based image analysis, we were able to visualize and quantify the fungal growth and invasive behavior based on hyphal length, branching level, and number of invasive hyphae at a single-cell level. Our results showed that antifungal drugs such as Amphotericin B and Voriconazole eliminated the fungus at clinically relevant dose (4 µg/ml) whereas Caspofungin, a fungistatic drug, only stalled the growth of the fungus. This drug also caused a so-called "paradoxical" effect where number of branches of *A. fumigatus* hyphae increased as the concentration of the drug increased.

The IAC model provides a dynamic and *in vivo*-like tool for antifungal drug testing as well as basic research of fungal pathogenicity and host-pathogen-interaction. Furthermore, our latest advancement in the development of human primary cells-based lung-on-chip

model offers a promising avenue with potential to enhance predictability in the antifungal drug development pipeline.

Reference:
T.N.M. Hoang, Z. Cseresnyés, S. Hartung, M. Blickensdorf, C. Saffer, K. Rennert, A.S. Mosig, M. von Lilienfeld-Toal, M.T. Figge (2022). Invasive aspergillosis-on-chip: A quantitative treatment study of human Aspergillus fumigatus infection. *Biomaterials*, 283: 121420. https://doi.org/10.1016/j.biomaterials.2022.121420.