**Objectives**

Talaromycosis, caused by the dimorphic fungus *Talaromyces marneffei* (Tm), is an invasive mycosis endemic in southeast Asia. The pathogenesis is poorly understood as previous murine models using intravenous, intratracheal, or inhalation infection either do not mimic natural infection or pose safety hazards. Here we developed a murine pulmonary model using oropharyngeal aspiration (OPA) infection and characterized the natural history of disseminated talaromycosis.

**Materials & Methods**

We tested three genetically-characterized clinical Tm strains - two from clinical trial participants in Vietnam (Tm-North and Tm-South), and one from Hong Kong (PM41); two murine hosts - inbred BALB/c (for pathogenesis studies) and outbred CD1 mice (for therapeutic studies); and two infection routes - intravenous (IV) *vs*. OPA with a 1x107 conidia/mL infection dose. Main outcomes are: 1) survival; 2) tissue fungal burden (colony forming unit [CFU]/gram) in the lungs, liver, spleen, brain, and kidneys at 0, 7, 14, 21, 28 days post-infection (d.p.i); 3) histopathology at 7 and 21 d.p.i; 4) Tm-specific Mp1p antigen in urine; and 5) anti-Mp1p IgG antibodies in plasma.

**Results**

The major findings are:

1. **Impact of Tm strains in BALB/c mice**:Strain PM41 had the highest mortality (94% by 9 d.p.i), while Tm-North and Tm-South were less lethal (39% and 47%, *P* <0.003, log-rank test). There was no significant difference in the rate of dissemination to liver and spleen, which was by 5 d.p.i in all three strains*.*
2. **Impact of OPA *vs.* IV infection route in CD1 mice (Figure 1):**All IV-infected mice succumbed to infection by 13 d.p.i, while only 8% of OPA-infected mice reached humane endpoint by 9 d.p.i (*P* <0.0001, log-rank test). Fungal burden was also substantially higher in the IV group (2-6 log CFUs/g higher at any timepoint, *P* <0.01, unpaired t-test). Dissemination to the kidneys and brain was observed exclusively in the IV-infected mice.
3. **Antigen and antibody dynamics in CD1 mice** **(Figure 2A&B):**Mp1p antigen peaked by 7 d.p.i. and remained elevated in the IV group, while Mp1p declined in the OPA group in temporal relationship with anti-Mp1p IgG antibodies increase. IV-infected mice succumbed to infection before the peaking of IgG antibodies.
4. **Histopathology of the OPA model in CD1 mice (Figure 2C&D)**: Profound granulomatous inflammation and Tm yeast cells were observed in the lungs and liver by 7 d.p.i. Remarkably by day 21, all inflammatory changes in the lungs and liver resolved.

**Conclusions**

Our murine OPA model recapitulates natural Tm infection through the lungs showing early profound granulomatous pneumonia and dissemination to the liver and spleen. A more robust anti-Mp1p IgG response in the OPA (*vs.* the IV) model appears critical in controlling infection (assessed by fungal and antigen clearance, and histopathological findings). This highlights OPA as a suitable model for pathogenesis studies, and the importance of innate immune activation in the lungs for protection against lethal infection. While not mimicking natural infection, the IV model in outbred CD1 mice rapidly induces disease dissemination and achieves humane endpoint earlier, making it more suitable for *in vivo* therapeutic studies.