The Impact of Posaconazole on *Aspergillus Fumigatus* Growth in Larval Zebrafish

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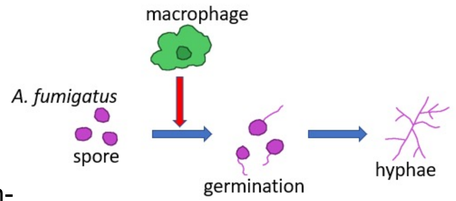
Introduction

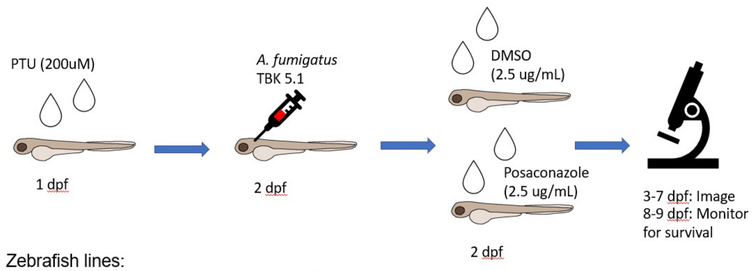
- *Aspergillus fumigatus* infects >200,000 patients each year
- Drugs fully effective *in vitro* are 50% effective in patients
  - Azole drugs used as frontline drugs
- Macrophages inhibit *A. fumigatus* spore germination



- Larval zebrafish-
  - No adaptive immunity until 4 weeks post fertilization
  - Small size (~5mm)
  - Optical transparency

Hypothesis: Posaconazole interacts with macrophages to inhibit the germination of *A. fumigatus* spores and formation of hyphae in larval zebrafish.

Methods



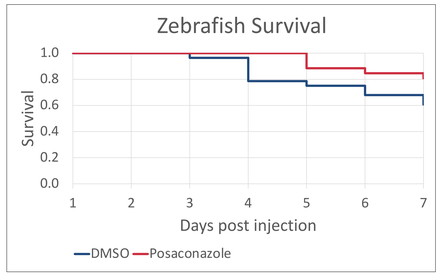
Zebrafish lines:
Mpeg:H2B-GFP X Mpx:mcherry-Rac2D57N

- 1 day post fertilization (dpf)
  - Addition of 200 uM PTU to prevent pigment formation in zebrafish
- 2 dpf
  - Microinjection of 30-50 red fluorescent spores of *A. fumigatus* into hindbrain ventricle
  - Addition of 2.5 ug/mL DMSO (control) or 2.5 ug/mL Posaconazole
- 3-7 dpf
  - Image spore growth and macrophage recruitment in hindbrain with high-resolution confocal microscopy
  - Monitor larvae for survival
- 8-9 dpf
  - Monitor larvae for survival

- Mpeg:H2B-GFP: macrophages fluoresce green
- Mpx:mcherry-Rac2D57N: neutrophils fluoresce red; neutrophils mutated so they cannot migrate to the hindbrain and target the fungus

Results

Higher overall survival in Posaconazole-treated larvae.

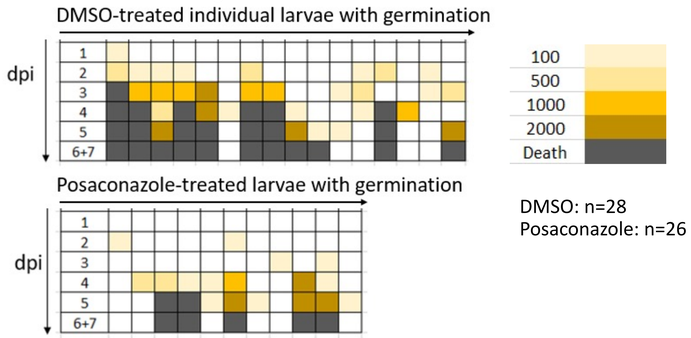


HR	p-value
0.430	0.119

HR = hazard ratio

Figure 1. During the 7 days post injection (dpi), Posaconazole delays larval death by 1-2 dpi and increases larval survival.

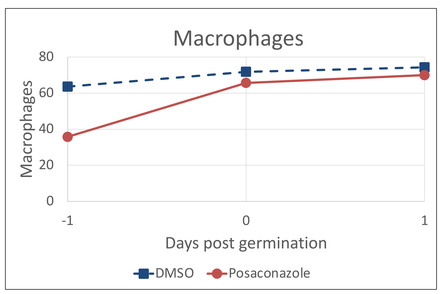
Lower overall fungal area in Posaconazole-treated larvae.



DMSO: n=28
Posaconazole: n=26

Figure 2. Heat map comparison of fungal area in zebrafish larvae with germinated spores over 7 dpi. Overall, Posaconazole-treated larvae experienced lower rates of germination and death as well as lower fungal loads.

Lower macrophage recruitment in Posaconazole-treated larvae before germination.



Dpg	p-value
-1	0.127
0	0.993
1	0.999

dpg = days post germination

Figure 3. Lower macrophage recruitment in Posaconazole-treated larvae the day before germination may indicate lower fungal loads. Posaconazole may reduce fungal loads by targeting the spores before germination, leading to lower macrophage recruitment.

Results

Posaconazole works with macrophages to clear *A. fumigatus* after germination, preventing larval death.

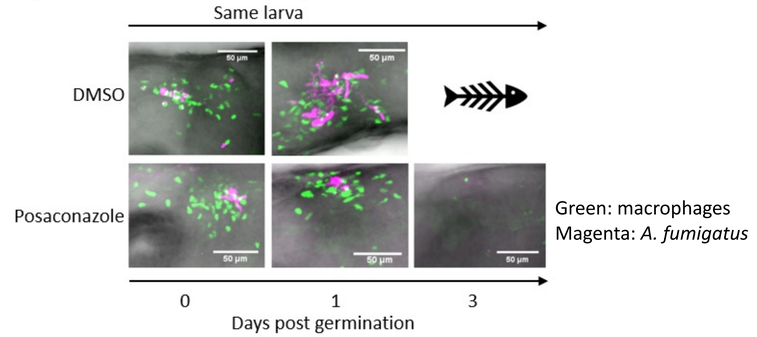
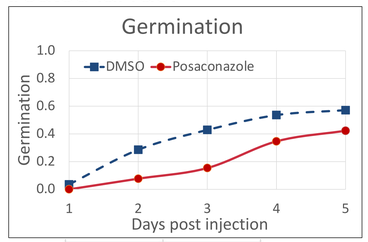
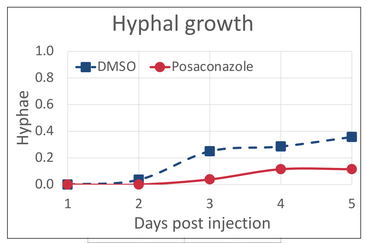


Figure 4. Macrophages form clusters around *A. fumigatus* spores to slow germination. In control larvae, *A. fumigatus* may form hyphae after germination, killing the larvae. In Posaconazole-treated larvae, germinated spores may be cleared, preventing larval death.

Decrease in germination and hyphal formation in Posaconazole-treated larvae.



HR	p-value
0.608	0.206



HR	p-value
0.274	0.0498

Figure 5. Posaconazole decreases germination and significantly reduces the development of hyphae in larvae, promoting zebrafish survival.

Conclusions

- Posaconazole promotes survival of *A. fumigatus*-infected zebrafish larvae by interacting with macrophages to decrease germination and by preventing the development of hyphae.

References

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