Management of azole-resistant invasive aspergillosis: 

*in vivo* pharmacodynamics of antifungals


Invasive aspergillosis

Primary treatment: Voriconazole Monotherapy
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Azole-resistance is increasing in A. fumigatus

Drug Resistance Updates 2009;12: 141–147
Invasive aspergillosis

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Invasive aspergillosis

12-week mortality:

• azole-resistant *A. fumigatus*: 88 %

(Van der Linden et al. Emerg Infect Dis. 2011: 17: 1846-54.)
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• azole-susceptible *A. fumigatus*: 39 % and 48 %


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- azole-susceptible *A. fumigatus*: 39 % and 48 %
  

Alternative treatment approaches are required!
Aim of our study:
To investigate the *in vivo* efficacy of different antifungals in the setting of azole resistant IA

**Monotherapy**
- Voriconazole (VRC)
- Posaconazole (POS)
- Anidulafungin (AFG)
- Liposomal amphotericin B (L-AmB)

**Combination therapy**
- VRC+AFG
Materials and Methods

- Immunocompetent murine model of IA
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- Immunocompetent murine model of IA
- *A. fumigatus* isolates:

<table>
<thead>
<tr>
<th>Cyp51A substitution</th>
<th>MIC/ MEC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMB</td>
</tr>
<tr>
<td>None (Wild Type)</td>
<td>0.5</td>
</tr>
<tr>
<td>M 220 I</td>
<td>0.5</td>
</tr>
<tr>
<td>G 54 W</td>
<td>0.5</td>
</tr>
<tr>
<td>TR\textsubscript{34}/L98H</td>
<td>0.5</td>
</tr>
<tr>
<td>TR\textsubscript{46}/Y121F/T289A</td>
<td>0.5</td>
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Voriconazole-monotherapy

- a VRC-S (MIC 0.25) and a VRC-R (MIC 4) A. fumigatus isolate
- 2.5, 5, 10 and 20 mg/kg/BW, once daily, IP
Efficacy of VRC-monotherapy

Efficacy of 2.5-20 mg/kg VRC-monotherapy against VRC-S *A. fumigatus* (MIC 0.25)

- Control
- 2.5 mg/kg VRC
- 5 mg/kg VRC
- 10 mg/kg VRC
- 20 mg/kg VRC

Days post infection: 0-15
Survival %: 0-100

Max 100 %

Efficacy of 2.5-20 mg/kg VRC-monotherapy against VRC-R *A. fumigatus* (MIC 4)

- Control
- 2.5 mg/kg VRC
- 5 mg/kg VRC
- 10 mg/kg VRC
- 20 mg/kg VRC

Days post infection: 0-15
Survival %: 0-100

Max 72.2 %

VRC-S

VRC-R
Dose-response relationships of VRC

<table>
<thead>
<tr>
<th></th>
<th>VRC-S</th>
<th>VRC-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC50</td>
<td>4.141</td>
<td>7.794</td>
</tr>
</tbody>
</table>
POS-monotherapy

- a POS-S (MIC 0.031 POS) and 3 mutant *A. fumigatus* isolates with MIC ranging 0.5 to > 16 to POS
- 1-64 mg/kg/BW, once daily, oral gavage

![Diagram showing treatment schedule](image)
Efficacy of POS-monotherapy

Efficacy of Posaconazole at 1 mg/kg

- WT
- TR/L98H
- M220I
- G54W

Efficacy of Posaconazole at 4 mg/kg

- WT
- TR/L98H
- M220I
- G54W

Efficacy of Posaconazole at 16 mg/kg

- WT
- TR/L98H
- M220I
- G54W

Efficacy of Posaconazole at 64 mg/kg

- WT
- TR/L98H
- M220I
- G54W
Anidulafungin-monotherapy

- a VRC-S (MIC 0.25 VRC) and a VRC-R (MIC 4 VRC) A. fumigatus isolate (Both isolates had similar MEC to AFG)
- Loading + maintenance 2.5, 5, 10 and 20 mg/kg/BW, once daily, IP
Efficacy of AFG-monotherapy

Efficacy of 2.5-20 mg/kg AFG-monotherapy against VCZ-S  *A.fumigatus* (MIC 0.25 VCZ)

Efficacy of 2.5-20 mg/kg AFG-monotherapy against VCZ-R  *A.fumigatus* (MIC 4 VCZ)

**VRC-S**
Max 72.7 %

**VRC-R**
Max 45.5 %
Exposure-response relationships of AFG

<table>
<thead>
<tr>
<th></th>
<th>VRC-S</th>
<th>VRC-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOGEC50</td>
<td>0.03149</td>
<td>0.1531</td>
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</tbody>
</table>

![Graph showing the exposure-response relationships of AFG with VRC-S and VRC-R A. fumigatus.](image)
L-AmB monotherapy

- a VRC-S (MIC 0.25 VRC) and 3 VRC-R (MICs 0.5, 4 and >16 VRC) *A.fumigatus* isolates (All 4 isolates had similar MIC to AMB)
- increasing 4-fold dosages ranging from 0.004 to 16 mg/kg/day, IV

Dose-response was independent of the azole-resistance mechanisms

L-AmB Dose-Survival Relationships

<table>
<thead>
<tr>
<th></th>
<th>AZN 81-96 (WT)</th>
<th>V 52-35 (TR34/L98H)</th>
<th>V 28-77 (M 220I)</th>
<th>V 94-10 (TR46/Y121F/T289A)</th>
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<tbody>
<tr>
<td>EC50</td>
<td>0.2950</td>
<td>0.5932</td>
<td>0.2008</td>
<td>0.07777</td>
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</table>

% Survival vs Dose L-AmB (mg/kg)
VRC+AFG combination

- a VRC-S (MIC 0.25) and a VRC-R (MIC 4) A. fumigatus isolate
- Combination of VRC+AFG (loading + maintenance) at 2.5, 5, 10 and 20 mg/kg/BW once daily, IP
% Survival for VRC+AFG combinations against VRC-S *A. fumigatus* isolate

<table>
<thead>
<tr>
<th>mg/kg</th>
<th>0 VRC</th>
<th>2.5 VRC</th>
<th>5 VRC</th>
<th>10 VRC</th>
<th>20 VRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 AFG</td>
<td>0</td>
<td>18,18</td>
<td>72,73</td>
<td>81,82</td>
<td>100</td>
</tr>
<tr>
<td>2.5 AFG</td>
<td>18,18</td>
<td>54,55</td>
<td>72,73</td>
<td>90,91</td>
<td>100</td>
</tr>
<tr>
<td>5 AFG</td>
<td>27,27</td>
<td>72,73</td>
<td>90,91</td>
<td>90,91</td>
<td>100</td>
</tr>
<tr>
<td>10 AFG</td>
<td>45,45</td>
<td>81,82</td>
<td>81,82</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>20 AFG</td>
<td>72,73</td>
<td>90,91</td>
<td>90,91</td>
<td>100</td>
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% Survival for VRC+AFG combinations against VRC-R *A. fumigatus* isolate

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<tr>
<td>0 AFG</td>
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<tr>
<td>5 AFG</td>
<td>18.18</td>
<td>36.36</td>
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Analysis of synergy in vivo

Prediction of synergism based on PDI(AUC/MIC):

- Use of Pharmacodynamic indices of single agents to predict survival of combination therapy *in vivo* for each combination

- Plot predicted versus observed

- Deviation of slope from 1 and/or intercept ≠ 0 indicates interaction in vivo

*Mouton et al. ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, 1999, p. 2473–2478*
Analysis of synergy in vivo

Prediction of synergism based on PDI:

- Use of Pharmacodynamic indices of single agents to predict survival of combination therapy in vivo for each combination
- Plot predicted versus observed
- Deviation of slope from 1 and/or intercept = 0 indicates interaction

Synergism

Antagonism
VRC+AFG was synergistic for VRC-S

Linear regression analysis of predicted vs. observed response (Survival) based on pharmacodynamic indices (AUC/MIC)

- Linear relationship between observed and predicted survival
- Observed survival > predicted, indicating synergism

VRC-S
VRC+AFG was additive for VRC-R

VRC-S

VRC-R
Conclusion:

- For both VRC and POS monotherapy in mice, the response was lower against VRC-R isolates.

- A maximal response was not achieved with either isolates even in those treated with the highest AFG dose.

- Combination of VRC and AFG was synergistic in vivo against azole-susceptible *A. fumigatus*.

- L-AmB increased survival in mice independent of the presence of an azole resistance mechanism.
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