1 **Heightened efficacy of anidulafungin when used in combination with manogepix or 5-**

2 **flucytosine against *Candida auris in vitro*.**

3 Running title: Synergistic drug combinations against *Candida auris*.

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Abstract

Candida auris is an emerging, multi-drug resistant fungal pathogen that causes refractory colonisation and life-threatening invasive nosocomial infections. The high proportion of *C. auris* isolates that display antifungal resistance severely limits treatment options. Combination therapies provide a possible strategy to enhance antifungal efficacy and prevent the emergence of further resistance. Therefore, we examined drug combinations using antifungals that are already in clinical use or undergoing clinical trials. Using checkerboard assays we screened combinations of 5-flucytosine and manogepix (the active form of the novel antifungal drug fosmanogepix) with anidulafungin, amphotericin B or voriconazole against drug resistant and susceptible *C. auris* isolates from clades I and III. Fractional inhibitory concentration indices (FICI values) of 0.28-0.75 and 0.36-1.02 were observed for combinations of anidulafungin with manogepix or 5-flucytosine, respectively, indicating synergistic activity. The high potency of these anidulafungin combinations was confirmed using live-cell microfluidics-assisted imaging of fungal growth. In summary, combinations of anidulafungin with manogepix or 5-flucytosine show great potential against both resistant and susceptible *C. auris* isolates.

Introduction

Candida auris is an emerging fungal pathogen that causes nosocomial invasive infections and that is difficult to eradicate following colonisation of hospitalised patients (1). *C auris* was first identified in 2009 in Japan, but since then outbreaks have been observed on most continents (1, 2). *C. auris* strains have been subdivided into four genetic clades, the South Asian (I), East Asian (II), South African (III) and South American (IV) clades (3), with a potential fifth Iranian clade identified more recently (4). The organism colonises the skin and can lead to mucosal or bloodstream infections, predominately in immunocompromised hosts (1). Invasive *C. auris* infections are associated with mortality rates between 28% and 60%, and treatment failure due to antifungal resistance is often observed (1, 3, 5–11).

To date, only four classes of antifungal drug are available for the treatment of invasive fungal infections: the azoles, polyenes, echinocandins and the nucleoside analogue 5-flucytosine. 5-flucytosine has high oral bioavailability with high activity against *C. auris*, but it is not generally used in monotherapy due to the rapid emergence of resistance (12). Current guidelines recommend echinocandin treatment as first line therapy for invasive candidiasis and for *C. auris* infection in particular (13, 14). However, echinocandin resistance can develop during treatment (15, 16). Resistance to all four existing classes of antifungal has been reported in *C. auris,* with varying drug susceptibilities and resistance mechanisms between clades (17). Around 90 % of *C auris* isolates show resistance to fluconazole with varying susceptibilities to other azoles (3, 6, 9, 18). Resistance to amphotericin B and the echinocandins appears to be less common, having been reported in 13-35 % and 2-7 % of tested isolates, respectively (3, 9, 18). Alarmingly, between 3 % and 41 % of isolates exhibit resistance to two or more antifungal classes (3, 18). Consequently, the Centers for Disease Control and Prevention (CDC) recently added *C. auris* to

64   its list of urgent antibiotic resistance threats (19) and the World Health Organisation (WHO)

65   declared it a critical threat in its fungal priority pathogens list (14).

66   The limited number of antifungal drugs as well as the increased threat of antifungal resistance in

67   *C. auris* means that novel treatment strategies are urgently needed. Combinations of antifungals

68   with different mechanisms of action provide one proposed therapeutic strategy. Previous *in vitro*

69   studies investigated combinations of echinocandins with azoles or the polyene amphotericin B

70   (20–24) and combinations of 5-flucytosine with the other three antifungal classes in *C. auris*

71   (25–27). These studies observed either synergy or indifference and no antagonism for all of the

72   tested combinations, with variability between *C. auris* isolates. The most promising

73   combinations were azoles combined with echinocandins which, in two studies, resulted in

74   synergy against all tested isolates (20, 23).

75   Combinations with 5-flucytosine are of particular interest as its combinations with amphotericin

76   B and fluconazole have been shown to be superior to monotherapy in phase III clinical trials

77   against cryptococcal meningitis (28). As a result of these trials, 5-flucytosine is now more widely

78   available globally, including in countries such as South Africa which suffers a high burden of

79   *C. auris* candidemia (28, 29). Echinocandin combinations with 5-flucytosine have been reported

80   to be indifferent in most cases, but these combinations have shown 100% growth inhibition and

81   fungicidal activity against multidrug-resistant isolates (25–27).

82   None of these studies included the new antifungal fosmanogepix, which has recently completed

83   phase 1 and 2 clinical trials, and is one of several new antifungals in the pipeline that may exhibit

84   activity also against *C. auris* (30). Fosmanogepix is a prodrug that is converted to the active

85   compound manogepix by systemic phosphatases (31). Manogepix inhibits a novel antifungal

86   target, Gwt1, which is involved in the GPI-anchor biosynthetic pathway, leading to a decrease in

87 cell wall-anchored mannoproteins (31). In the present study, we examined combinations of

88 manogepix or 5-flucytosine with anidulafungin, amphotericin B or voriconazole against a range

89 of resistant and susceptible *C. auris* isolates *in vitro*.

Material and Methods

Fungal isolates

92 Twenty-five clinical *C. auris* isolates belonging to clades I, III and IV isolated from 6 patients

93 from a range of sites (blood, urine, respiratory tract, skin) were obtained from the CDC (Table

94 1). Clade designations were based on whole genome sequencing (Gifford *et al*., in preparation).

95 Isolates were maintained at - 80 °C in 25 % glycerol broth and subcultured on Sabouraud

96 dextrose agar (SDA) at 37 °C for up to 48 h.

Antifungal susceptibility testing

98 Antifungal susceptibility testing was performed using the broth microdilution method according

99 to EUCAST guidelines (32). Flat-bottom, tissue-treated 96-well plates were used. Anidulafungin

100 (MedChem Express), amphotericin B (Merck), fluconazole (Thermo Scientific), 5-flucytosine

101 (Thermo Scientific), fosmanogepix (MedChem Express), manogepix (MedChem Express) and

102 voriconazole (Sigma Aldrich) were dissolved in 100 % dimethyl sulfoxide (DMSO). The range

103 of antifungal concentrations tested were 0.016 to 8 mg/L for anidulafungin, 0.03 to 16 mg/L for

104 amphotericin B and voriconazole, 0.25 to 128 mg/L for fluconazole, 0.008 to 4 mg/L for 5-

105 flucytosine, 0.004 to 2 mg/L for fosmanogepix and 0.002 to 1 mg/L for manogepix. Antifungal

106 dilution series were prepared in RPMI supplemented with glucose to 2 % and buffered at pH 7

107 using 3-(N-morpholino) propanesulfonic acid (MOPS) at a final concentration of 0.165 mol/L

108 (RPMI 2%G-MOPS). Spectrophotometer readings at 530 nm were taken after incubation at

109 37 °C for 24 h The minimum inhibitory concentration (MIC) endpoint for amphotericin B was

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110 defined as the lowest concentration leading to 90 % reduction in growth compared to the drug-

111 free control (MIC_{90}), while MIC_{50} endpoints, measuring 50 % reduction in growth compared to

112 the drug-free control, were used for all other antifungal agents. Tentative CDC breakpoints for *C.*

113 *auris* were used to define resistance to anidulafungin ($\geq$4 mg/L), amphotericin B ($\geq$2 mg/L),

114 fluconazole ($\geq$32 mg/L) and voriconazole ($\geq$2 mg/L) (https://www.cdc.gov/fungal/candida-

115 auris/c-auris-antifungal.html). A known issue for broth microdilution susceptibility testing of

116 amphotericin B in RPMI medium is the clustering of MICs around the breakpoint of 2 mg/L

117 making it difficult to distinguish resistant and susceptible isolates (33). There are no breakpoints

118 available for 5-flucytosine and fosmanogepix. *Candida krusei* ATCC 6258 and *Candida*

119 *parapsilosis* ATCC 22019 were used as quality control strains as recommended by the EUCAST

120 guidelines (32). All experiments were performed in triplicate.

121 *Antifungal combination testing*

122 Interactions of antifungal drugs were tested using checkerboard assays based on EUCAST

123 guidelines (32). The range of antifungal concentrations tested was dependent on the MIC of each

124 isolate, with the highest concentration at 4 x MIC. Columns 3 to 12 of a 96-well microtiter plate

125 were filled with 50 µl of drug A and rows B to H were filled with 50 µl of drug B. Column 1

126 served as drug-free growth and sterility control. The inoculum was prepared by suspending five

127 distinct colonies from 40- 48h-old cultures in distilled water, counting the cell number using a

128 haemocytometer and adjusting inocula to 5 x 10^5 cells/ml. The plates were inoculated with 100

129 µl and incubated at 37 °C for 24 h. OD readings were taken after 24 h using a spectrophotometer

130 at 530 nm. All experiments were performed in triplicate.

131 Two different approaches were applied in the analysis of drug interactions. The fractional

132 inhibitory concentration index (FICI) was calculated as follows:

$$\text{FICI} = \frac{C_A}{\text{MIC}_A} + \frac{C_B}{\text{MIC}_B}$$

133 C_A and C_B are the concentrations of the drugs A and B in combination and MIC_A and MIC_B are

134 the MICs of the drugs alone. MIC values were rounded to the next highest two-fold

135 concentration if the endpoint was not reached within the tested concentration range. The

136 interaction was considered synergistic for FICI ≤ 0.5, partially synergistic between >0.5 and <1.0,

137 additive at 1.0, indifferent between >1.0 and <4 and antagonistic >4 (24). In the following, the

138 term "any synergy" refers to FICI values of <1, thereby including complete and partial synergy.

139 In the presence of antagonism, the maximum median FICI values were reported, otherwise

140 minimum median FICI values were given. Additionally, drug interactions were visualised using

141 a response surface analysis approach with Combenefit software (version 2.021) under application

142 of the Bliss independence model (34).

143 *Microfluidics imaging*

144 *C. auris* B12663 cells were grown and prepared as described above. Inocula were adjusted to

145 2×10^5 cells/ml. Antifungal mono- and combination treatments were prepared in RPMI 2%G-

146 MOPS at the MIC. CellASIC® ONIX Y04C microfluidic plates (Millipore Merck) were washed

147 with RPMI 2%G-MOPS by applying 5 psi perfusion for 5 min using the CellASIC® ONIX2

148 microfluidic system (version 1.0.4 Millipore Merck). Yeasts were loaded into the CellASIC

149 culture chambers by applying 8 psi for 5 s twice (Thomson *et al*., in preparation). Adhered cells

150 were then perfused with RPMI 2%G-MOPS for 4 h at 1 psi. After 4 h, cells were exposed to the

151 antifungal(s), or to RPMI 2%G-MOPS for the drug-free control, by applying 5 psi for 5 min,

152 followed by perfusion at 1 psi for 20 h at 37 °C, during which the microfluidic plates were

153 subjected to multi-point 4D imaging on an inverted AxioObserver Z1 microscope (Carl Zeiss).

154 Differential interference contrast (DIC) images were captured with a 20x/0.8NA

155 PlanApochromatic DIC objective and a 16-bit ORCA-Fusion sCMOS camera (Hamamatsu). The

156 area of colonies over time was measured in FIJI 1.53t (35) using an adapted method for

157 migration analysis from Venter and Niesler (36). Briefly, during the time series, colony edges

158 were found (Process → Find Edges), the image blurred fifteen times (Process → Smooth) and

159 inverted (Edit → Invert) before thresholding (Image → Adjust → Threshold: Default) to

160 quantify the total fungal area (Analyse → Analyse Particles). Increases in 2-dimensional colony

161 area were used to calculate the doubling times.

Results

Antifungal activity against C. auris isolates

164 The antifungal susceptibility profiles of 25 *C. auris* isolates were determined in order to select a

165 subset of isolates with different drug susceptibilities for antifungal combination testing. The

166 ranges of MIC values for the *C. auris* isolates against the tested antifungals are summarised in

167 Table 2 and Table S1. MIC_{90} values for amphotericin B clustered around the breakpoint of 2

168 mg/L which is a known problem for broth microdilution susceptibility testing of amphotericin B

169 in RPMI medium, making it difficult to distinguish resistant and susceptible isolates (33).

170 Fluconazole showed a large percentage of resistant *C. auris* isolates (96 %; breakpoint

171 ≥32 mg/L) with high MIC_{50} values ranging from 4 to ≥128 mg/L, while the other triazole tested

172 (voriconazole) displayed more potent antifungal activity with MIC_{50} ranging from 0.06 to 16

173 mg/L and 40 % resistant isolates (breakpoint ≥2 mg/L). Of all the antifungals tested with an

174 available breakpoint, anidulafungin produced the lowest percentage of resistant isolates (32 %;

175 ≥4 mg/L). The most potent antifungal activity against *C. auris* was observed for manogepix

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176   (MIC_{50}/MIC_{90}, 0.008/0.03 mg/L; range, 0.004-0.03) followed by 5-flucytosine (MIC_{50}/MIC_{90},

177   0.25/0.25 mg/L; range, 0.125-0.25).

178   *Interaction of antifungal drug combinations against C. auris isolates*

179   Based on their MIC values, 11 *C. auris* isolates with different drug susceptibility profiles were

180   selected to investigate the interactions of anidulafungin, amphotericin B and voriconazole with 5-

181   flucytosine or manogepix. The FICI values for these combinations, as determined by the

182   checkerboard assays, are presented in Table 3 and Figure 1 (FICI values of separate repeats can

183   be found in Tables S2 and S3). The combination of anidulafungin with 5-flucytosine resulted in

184   synergistic interactions for 10/11 isolates (synergy, 2/11 isolates; partial synergy, 8/11 isolates).

185   Meanwhile the combination of anidulafungin with manogepix led to synergy in all 11 isolates

186   (synergy, 5/11 isolates; partial synergy, 6/11 isolates). These FICI values corresponded to a

187   median (range) decrease in MIC_{50} of 2 log_2-fold (1- to 4 log_2-fold) for anidulafungin and 2 log_2-

188   fold (0- to 4 log_2-fold) for 5-flucytosine (Figure 2A), or 3 log_2-fold (1- to 9 log_2-fold) for

189   anidulafungin and 2 log_2-fold (1- to 3 log_2-fold) for manogepix (Figure 2B). Additionally, both

190   anidulafungin combinations achieved fungistatic activity with a log_{10}-fold reductions in CFUs/ml

191   of 2.2 and 0.8 compared to the starting inoculum for the combination with manogepix and 5-

192   flucytosine, respectively, while the corresponding monotherapies only had a negligible

193   antifungal effect (Figure S7).

194   The combination of amphotericin B with 5-flucytosine did not show full synergy for any of the

195   tested isolates, though partial synergy was observed in 4/11 isolates (median FICIs 0.63-0.75).

196   The other isolates showed either additive (5/11 isolates) or indifferent (2/11 isolates, median

197   FICIs 1.01) interactions for amphotericin B with 5-flucytosine. For the combination of

198   manogepix and 5-flucytosine, 3/11 isolates displayed partial synergy (median FICIs 0.54-0.58)

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199   and 4/11 isolates showed additive or indifferent interactions (median FICIs 1.01). The

200   combination of manogepix and 5-flucytosine led to large reductions in the MIC_{50} by median

201   (range) 7 $\log_2$-fold (1- to 8 $\log_2$-fold) for 5-flucytosine, while the manogepix MIC_{50} were only

202   decreased by median (range) 0 $\log_2$-fold (0- to 2 $\log_2$-fold) (Figure S1C). The drug combination

203   resulting in the least favourable interactions was voriconazole with 5-flucytosine with 3/11

204   isolates displaying antagonistic interactions (median FICIs 4.48-4.50), and the remaining isolates

205   displaying additive (3/11 isolates) or indifferent (5/11 isolates, median FICIs 1.01) interactions.

206   Response surface analyses were also used to examine the drug combinations, and an example is

207   shown in Figure 3 for the multidrug-resistant isolate B12663 (see Figures S2-S6 for the other

208   isolates). Consistent with the FICI scores, the synergy maps indicate synergy for the combination

209   of anidulafungin and manogepix (median FICI 0.33) and weak synergy for combinations of 5-

210   flucytosine with anidulafungin (median FICI 0.74) or amphotericin B (median FICI 0.75). In

211   contrast to the FICI calculation, which only focuses on drug concentrations corresponding to

212   MIC values, the response surface analysis permits the examination of drug interactions over a

213   wide range of tested concentrations. This revealed antagonism at the lower end of some

214   concentration ranges that was missed by the FICI approach, highlighting the concentration-

215   dependence of the interactions.

216   *Real time imaging of anidulafungin combinations against a multidrug-resistant C. auris isolate*

217   *using microfluidics*

218   A microfluidics imaging approach was employed to further investigate the effects, at a single-

219   cell level, of the two most promising drug combinations: anidulafungin with manogepix, and

220   anidulafungin with 5-flucytosine. This system is less static than the traditional microbroth

221   dilution method as the cells are constantly perfused with fresh medium containing different

10

222 antifungal drugs. Again, the multidrug-resistant *C. auris* isolate B12663 was chosen for analysis.

223 Both drug combinations showed dramatic effects upon cell growth, markedly reducing the size

224 of colonies compared to the relevant monotherapies and media-only controls (Figure 4A; Movies

225 S1 and S2). Doubling times, measured by 2-dimensional colony area changes, increased

226 significantly in the presence of the drug combinations compared to the individual antifungals. An

227 increase from 3.19 h (5-flucytosine alone) to 4.90 h (p<0.001) was observed for anidulafungin

228 combined with 5-flucytosine (Figure 4B). Similarly, an increase from 2.75 h (manogepix alone)

229 to 9.50 h (p<0.001) was seen for the anidulafungin-manogepix combination (Figure 4C). These

230 changes in doubling time correspond to 63.5 % (anidulafungin-5-flucytosine) and 96.5%

231 (anidulafungin-manogepix) decrease in colony area after 24 h compared to 5-flucytosine and

232 manogepix, respectively (data not shown). These findings were again consistent with those of the

233 checkerboard and response surface analysis experiments, in that the combination of

234 anidulafungin and manogepix showed the most potent impacts on cell growth, followed by the

235 combination of anidulafungin plus 5-flucytosine.

236 The cellular morphology was further examined at higher magnification after exposing the *C.*

237 *auris* cells to the antifungals in monotherapy or combination for 24 h (Figure S8). In drug-free

238 medium the cells had a well-defined, oval morphology. Under exposure to anidulafungin,

239 manogepix and both anidulafungin combinations the cells displayed a rounder morphology with

240 the formation of aggregates, while 5-flucytosine treatment resulted in a more elongated

241 phenotype. Additionally, enlarged, round cells were observed in the presence of manogepix and

242 both combinations.

Discussion

243   **Discussion**

244   The emergence and global spread of multidrug-resistant *C. auris* strains poses a serious health

245   threat. The high prevalence of antifungal resistance reported for *C. auris* isolates (3, 6–9, 11, 18,

246   24) was also observed in the isolates used in this study, with the majority of isolates resistant to

247   fluconazole, 40 % resistant to voriconazole and 32 % resistant to anidulafungin. The ability of

248   *C. auris* to develop resistance to all of the available classes of antifungal drug severely limits

249   treatment options.

250   New antifungal drugs, such as fosmanogepix, are currently in development (reviewed in (30)).

251   *C. auris* currently appears susceptible to the active version of this new class of drugs

252   (manogepix), but there is a high risk of resistance developing following its introduction to the

253   clinic unless precautionary measures are taken. Combination therapies provide a proven strategy

254   that has already been employed in the treatment of viral and bacterial infections to prevent the

255   emergence of resistance to a single drug (37). Additionally, combination therapies have the

256   potential to improve efficacy through additive or synergistic interactions, allowing lower drug

257   doses to be used, thereby reducing dose-related toxicity.

258   Thus far, nine studies have examined antifungal drug combinations against *C. auris*. The

259   majority of these studies focussed on combinations of azoles with echinocandins (20, 23, 24, 38),

260   while a smaller number have evaluated polyene-echinocandin interactions (21, 22) or

261   combinations with 5-flucytosine (25–27). These studies reported mainly synergistic (including

262   partial synergy) or indifferent interactions, with inter-strain variability observed for some

263   combinations. None of these studies included manogepix. Both manogepix and 5-flucytosine

264   have potent antifungal activity against *C. auris* as shown here and observed by others (39–44).

265   Therefore, we examined interactions of the echinocandin anidulafungin, the azole voriconazole

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266 and the polyene amphotericin B with either 5-flucytosine or manogepix using checkerboard

267 assays, response surface analyses and microfluidics imaging.

268 According to the FICI values and response-surface analyses, the most potent combination (with

269 respect to the number of *C. auris* isolates that displayed synergy) was anidulafungin plus

270 manogepix, followed by the combination of anidulafungin with 5-flucytosine. The high efficacy

271 of these combinations was also confirmed by microfluidics imaging, which revealed dramatic

272 reductions in fungal growth compared to the relevant monotherapies. The interactions between 5-

273 flucytosine with either amphotericin B or manogepix were additive or indifferent for the majority

274 of the isolates, while the combination of voriconazole with 5-flucytosine was indifferent or

275 antagonistic.

276 Applying our FICI thresholds, Bidaud and co-workers also reported mainly partially synergistic

277 or additive interactions for combinations of amphotericin B, voriconazole or micafungin with 5-

278 flucytosine (25). However, they did not observe the antagonism for the combination of

279 voriconazole with 5-flucytosine that we observed here. Another study reported 100 % growth

280 inhibition of amphotericin B or anidulafungin-resistant *C. auris* isolates for amphotericin B-5-

281 flucytosine combinations (0.25/1 mg/L) or anidulafungin-5-flucytosine combinations (0.008/1

282 mg/L) (26). Based on our OD_{530} measurements, more than 90 % growth inhibition was also

283 achieved for the majority of susceptible and resistant isolates we analysed, and this growth

284 inhibition could be reached at lower concentrations for some isolates. To the best of our

285 knowledge, antifungal combinations with fosmanogepix/manogepix have not been studied

286 previously against *Candida* species. One recent study compared amphotericin B monotherapy

287 with the combination therapy of fosmanogepix and amphotericin B in invasive mouse infection

288 models of *Aspergillus fumigatus*, *Rhizopus arrhizus* var. *delemar* and *Fusarium solani* (45). In

289   all three models, mortality and fungal burden were significantly reduced in the mice treated with

290   the combination therapy compared to amphotericin B or fosmanogepix alone (45).

291   For the majority of combinations and isolates we examined, the interactions were partially

292   synergistic or additive. However, even these interactions could be of interest clinically, as the

293   ultimate goal is to reduce fungal burden with a view to supporting the immune system in clearing

294   the infection. This reduction in fungal growth could be clearly observed in the microfluidics

295   imaging for the combination of anidulafungin with 5-flucytosine, which only displayed a

296   partially synergistic interaction for the imaged isolate in the checkerboard assays. Furthermore,

297   partially synergistic or additive interactions can lead to reductions in the MICs, potentially

298   allowing for a lowering of antifungal doses, thereby reducing toxicity. Reductions in MICs for

299   partially synergistic, additive and indifferent combinations have also been observed by others

300   (20, 24) and Caballero and colleagues reported that additive combinations of isavuconazole-

301   echinocandin combinations against *C. auris* can result in fungistatic effects which were absent

302   for single agents in time-kill assays (23). This is similar to our results showing negligible

303   antifungal activity for anidulafungin, manogepix and 5-flucytosine in monotherapy, whereas the

304   combinations of these two antifungals with anidulafungin showed heightened efficacy with the

305   reductions in CFUs/ml approaching the cidality threshold. The lack of fungicidal activity of the

306   echinocandins against *C. auris* in time-kill assays has also been observed by others reporting

307   either a fungistatic effect or the complete absence of antifungal activity (22, 23, 46, 47). In

308   comparison to anidulafungin monotherapy, the anidulafungin combinations resulted in 2.1 and

309   3.6 log10-fold reductions in CFUs/ml for 5-flucytosine and manogepix combinations,

310   respectively, highlighting their advantage over monotherapy.

311   Cost and additional toxicities are potential barriers to implementation of antifungal

312   combinations, and, to date, routine use of antifungal combinations has been largely confined to

313   cryptococcal infection. However, affordable generic echinocandins and 5-flucytosine are now

314   available, and short courses of 5-flucytosine are known to be very safe, giving feasible current

315   options to try to prevent the inevitable increase in *C. auris* resistance consequent on continued

316   use of monotherapies. Furthermore, early studies of combination approaches with new agents

317   such as fosmanogepix could expand the options for clinical evaluation and prolong their clinical

318   efficacy.

319   The synergistic interactions we observed for anidulafungin combined with manogepix or 5-

320   flucytosine were within clinically relevant concentrations in most cases. Serum anidulafungin

321   concentrations of up to 7 mg/L are achievable in patients (48, 49) which is above the

322   anidulafungin concentrations corresponding to synergistic interactions for most isolates. For 5-

323   flucytosine all concentrations we tested fall well below the achievable serum concentrations (48).

324   In the case of fosmanogepix, no clinical pharmacokinetics data is publicly available to our

325   knowledge. Several safety and pharmacokinetics clinical studies for fosmanogepix have been

326   completed, but no results are available yet (NCT02956499, NCT02957929, NCT03333005).

327   However, the manogepix concentrations at which synergy was observed were relatively low,

328   ranging between 0.002 and 0.03 mg/L.

329   It should be noted that the current study employed a relatively small number of isolates, and

330   there was an unequal representation of *C. auris* clades. Additionally, the clustering of

331   amphotericin B MIC_{90} around the breakpoint made it difficult to categorise the isolates according

332   to their amphotericin B susceptibility. Hence, other susceptibility testing methods such as the

333   Etest are recommended (17).

334 In summary, combinations of anidulafungin with manogepix or 5-flucytosine show the highest

335 potential against the tested *C. auris* isolates. Further studies are needed to determine the

336 mechanisms that underlie these drug interactions and to evaluate their efficacy and safety in the

337 murine model and whether these combinations also protect against the development of

338 resistance.

Conflicts of Interest

355 EB and DDT were funded by Gilead Sciences. DDT received consultancy fee from OwlStone

356 Medical in the last 5 years. TB has received research funding from Gilead Sciences, MSD and

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References

363   1.   Du H, Bing J, Hu T, Ennis CL, Nobile CJ, Huang G. 2020. *Candida auris*: Epidemiology,

364   biology, antifungal resistance, and virulence. PLoS Pathog 16:e1008921.

365   2.   Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H. 2009. *Candida*

366   *auris* sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an

367   inpatient in a Japanese hospital. Microbiol Immunol 53:41–44.

368   3.   Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP,

369   Colombo AL, Calvo B, Cuomo CA, Desjardins CA, Berkow EL, Castanheira M, Magobo

370   RE, Jabeen K, Asghar RJ, Meis JF, Jackson B, Chiller T, Litvintseva AP. 2017.

371   Simultaneous Emergence of Multidrug-Resistant *Candida auris* on 3 Continents Confirmed

372   by Whole-Genome Sequencing and Epidemiological Analyses. Clin Infect Dis 64:134–140.

373   4.   Chow NA, de Groot T, Badali H, Abastabar M, Chiller TM, Meis JF. 2019. Potential Fifth

374   Clade of *Candida auris,* Iran, 2018. Emerg Infect Dis 25:1780–1781.

375   5.   Lee WG, Shin JH, Uh Y, Kang MG, Kim SH, Park KH, Jang H-C. 2011. First three

376   reported cases of nosocomial fungemia caused by *Candida auris*. J Clin Microbiol

377   49:3139–3142.

378   6.   Chowdhary A, Sharma C, Duggal S, Agarwal K, Prakash A, Singh PK, Jain S, Kathuria S,

379   Randhawa HS, Hagen F, Meis JF. 2013. New clonal strain of *Candida auris* , Delhi, India.

380   Emerg Infect Dis 19:1670–1673.

381   7.   Chowdhary A, Anil Kumar V, Sharma C, Prakash A, Agarwal K, Babu R, Dinesh KR,

382   Karim S, Singh SK, Hagen F, Meis JF. 2014. Multidrug-resistant endemic clonal strain of

383   *Candida auris* in India. Eur J Clin Microbiol Infect Dis 33:919–926.

384   8.   Chakrabarti A, Sood P, Rudramurthy SM, Chen S, Kaur H, Capoor M, Chhina D, Rao R,

385   Eshwara VK, Xess I, Kindo AJ, Umabala P, Savio J, Patel A, Ray U, Mohan S, Iyer R,

386   Chander J, Arora A, Sardana R, Roy I, Appalaraju B, Sharma A, Shetty A, Khanna N,

387   Marak R, Biswas S, Das S, Harish BN, Joshi S, Mendiratta D. 2015. Incidence,

388   characteristics and outcome of ICU-acquired candidemia in India. Intensive Care Med

389   41:285–295.

390   9.   Calvo B, Melo ASA, Perozo-Mena A, Hernandez M, Francisco EC, Hagen F, Meis JF,

391   Colombo AL. 2016. First report of *Candida auris* in America: Clinical and microbiological

392   aspects of 18 episodes of candidemia. J Infect 73:369–374.

393   10. Chowdhary A, Sharma C, Meis JF. 2017. *Candida auris*: A rapidly emerging cause of

394   hospital-acquired multidrug-resistant fungal infections globally. PLoS Pathog 13:e1006290.

395   11. Rudramurthy SM, Chakrabarti A, Paul RA, Sood P, Kaur H, Capoor MR, Kindo AJ, Marak

396   RSK, Arora A, Sardana R, Das S, Chhina D, Patel A, Xess I, Tarai B, Singh P, Ghosh A.

397   2017. *Candida auris* candidaemia in Indian ICUs: analysis of risk factors. J Antimicrob

398   Chemother 72:1794–1801.

399   12. Robbins N, Wright GD, Cowen LE. 2016. Antifungal Drugs: The Current Armamentarium

400   and Development of New Agents. Microbiology Spectrum 4.

401   13. PHE. 2017. Guidance for the laboratory investigation, management and infection

402   prevention and control for cases of *Candida auris v2.0.* Public Health England, London.

403   14. WHO. 2022. WHO fungal priority pathogens list to guide research, development and public

404   health action. World Health Organization, Geneva.

405   15. Biagi MJ, Wiederhold NP, Gibas C, Wickes BL, Lozano V, Bleasdale SC, Danziger L.

406   2019. Development of high-level echinocandin resistance in a patient with recurrent

407   *Candida auris* candidemia secondary to chronic candiduria. Open Forum Infect Dis

408   6:ofz262.

409   16. Ostrowsky B, Greenko J, Adams E, Quinn M, O'Brien B, Chaturvedi V, Berkow E,

410   Vallabhaneni S, Forsberg K, Chaturvedi S, Lutterloh E, Blog D, Bucher C, Denis RJ, Erazo

411   R, Fernandez R, Southwick K, Zhu YC. 2020. *Candida auris* isolates resistant to three

412   classes of antifungal medications — New York, 2019. Morb Mortal Wkly Rep 69:6–9.

413   17. Lockhart SR. 2019. *Candida auris* and multidrug resistance: Defining the new normal.

414   Fungal Genet Biol 131:103243.

415   18. Chowdhary A, Prakash A, Sharma C, Kordalewska M, Kumar A, Sarma S, Tarai B, Singh

416   A, Upadhyaya G, Upadhyay S, Yadav P, Singh PK, Khillan V, Sachdeva N, Perlin DS,

417   Meis JF. 2018. A multicentre study of antifungal susceptibility patterns among 350

418   *Candida auris* isolates (2009–17) in India: Role of the ERG11 and FKS1 genes in azole and

419   echinocandin resistance. J Antimicrob Chemother 73:891–899.

420   19. CDC. 2019. Antibiotic resistance threats in the United States, 2019. U.S. Department of

421   Health and Human Services, CDC, Atlanta, GA.

422 20. Fakhim H, Chowdhary A, Prakash A, Vaezi A, Dannaoui E, Meis JF, Badali H. 2017. *In*

423   *vitro* interactions of echinocandins with triazoles against multidrug-resistant *Candida auris*.

424   Antimicrob Agents Chemother 61:e01056-17.

425 21. Jaggavarapu S, Burd EM, Weiss DS. 2020. Micafungin and amphotericin B synergy against

426   Candida auris. Lancet Microbe 1:e314–e315.

427 22. Caballero U, Eraso E, Quindós G, Jauregizar N. 2021. *In vitro* interaction and killing-

428   kinetics of amphotericin B combined with anidulafungin or caspofungin against *Candida*

429   *auris*. Pharmaceutics 13:1333.

430 23. Caballero U, Kim S, Eraso E, Quindós G, Vozmediano V, Schmidt S, Jauregizar N. 2021.

431   *In vitro* synergistic interactions of isavuconazole and echinocandins against *Candida auris*.

432   Antibiotics 10:355.

433 24. Pfaller MA, Messer SA, Deshpande LM, Rhomberg PR, Utt EA, Castanheira M. 2021.

434   Evaluation of synergistic activity of isavuconazole or voriconazole plus anidulafungin and

435   the occurrence and genetic characterization of *Candida auris* detected in a surveillance

436   program. Antimicrob Agents Chemother 65:e02031-20.

437 25. Bidaud AL, Botterel F, Chowdhary A, Dannaoui E. 2019. *In vitro* antifungal combination

438   of flucytosine with amphotericin B, voriconazole, or micafungin against *Candida auris*

439   shows no antagonism. Antimicrob Agents Chemother 63:e01393-19.

440 26. O'Brien B, Chaturvedi S, Chaturvedi V. 2020. *In vitro* evaluation of antifungal drug

441   combinations against multidrug-resistant *Candida auris* isolates from New York outbreak.

442   Antimicrob Agents Chemother 64:e02195-19.

443 27. O'Brien B, Liang J, Chaturvedi S, Jacobs JL, Chaturvedi V. 2020. Pan-resistant *Candida*

444   *auris*: New York subcluster susceptible to antifungal combinations. Lancet Microbe

445   1:e193–e194.

446 28. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, Mfinanga S,

447   Temfack E, Lakhi S, Lesikari S, Chan AK, Stone N, Kalata N, Karunaharan N, Gaskell K,

448   Peirse M, Ellis J, Chawinga C, Lontsi S, Ndong J-G, Bright P, Lupiya D, Chen T, Bradley

449   J, Adams J, Horst C van der, Oosterhout JJ van, Sini V, Mapoure YN, Mwaba P, Bicanic T,

450   Lalloo DG, Wang D, Hosseinipour MC, Lortholary O, Jaffar S, Harrison TS. 2018.

451   Antifungal combinations for treatment of cryptococcal meningitis in Africa. N Engl J Med

452   378:1004–1017.

453 29. Mashau RC, Meiring ST, Quan VC, Nel J, Greene GS, Garcia A, Menezes C, Reddy DL,

454   Venter M, Stacey S, Madua M, Boretti L, Harrison TS, Meintjes G, Shroufi A, Trivino-

455   Duran L, Black J, Govender NP, Abrahams S, Pearce V, Moncho M, Wadula J, Maloba M,

456   Hoosen A, Verwey C, Menezes C, Moore D, Pombo D, Reubenson G, Ntlemo G, Richards

457   L, Nchabeleng M, Tsitsi M, Moshe M, Said M, Kolojane M, Mothibi L, Plessis ND,

458   Chomba R, Thomas T, Avenant T, Nana T, Chibabhai V, Maharj A, Wilson D, Naby F,

459   Dawood H, Han KSS, Sookan L, Dlamini N, Ramajathan P, Mahabeer P, Bhola P, Naidoo

460   R, Haffejee S, Sirkar S, Ramkillawan Y, Hamese K, Sibiya N, Mangena P, Lekalakala R,

461   Hoyland G, Ntuli S, Variava E, Khantsi I, Mekgoe O, Brink A, Prentice E, Reddy K,

462   Whitelaw A, Hoosien E, Zietsman I, Marshall T, Poswa X, Govind C, Smit J, Pillay K,

463   Seetharam S, Howell V, Samuel C, Senekal M, Bamford C, Dreyer A, Marcus L, Lowman

464   W, Gottberg A von, Smith A, Mathunjwa A, d'Abreu C, Miller C, Cohen C, Ismail F,

465   Moultrie H, Ismail H, Weyer J, Kleynhans J, Rossouw J, Frean J, Ebonwu J, Mwansa-

466   Kambafwile J, Thomas J, Bishop K, McCarthy K, Shuping L, Gouveia L de, Erasmus L,

467   Puren A, Blumberg L, Smith M, Makgoba M, Groome M, Plessis M du, Ngomane M,

468   Manaka M, Moremi M, Ismail N, Legare N, Page N, Hoho N, Perovic O, Sekwadi P,

469   Magobo R, Mpembe R, Walaza S, Dlamini S, Njikho S, Lebaka T, Ngubane W. 2022.

470   Outcomes of flucytosine-containing combination treatment for cryptococcal meningitis in a

471   South African national access programme: a cross-sectional observational study. Lancet

472   Infect Dis 22:1365–1373.

473 30. Hoenigl M, Sprute R, Egger M, Arastehfar A, Cornely OA, Krause R, Lass-Flörl C, Prattes

474   J, Spec A, Thompson GR, Wiederhold N, Jenks JD. 2021. The Antifungal Pipeline:

475   Fosmanogepix, Ibrexafungerp, Olorofim, Opelconazole, and Rezafungin. Drugs 81:1703.

476 31. Shaw KJ, Ibrahim AS. 2020. Fosmanogepix: A review of the first-in-class broad spectrum

477   agent for the treatment of invasive fungal infections. J Fungi 6:239.

478 32. European Committee on Antimicrobial Susceptibility Testing. 2020. EUCAST Definitive

479   Document E.Def 7.3.2 Revision: Method for the determination of broth dilution minimum

480   inhibitory concentrations of antifungal agents for yeasts. ESCMID European Committee for

481   Antimicrobial Susceptibility Testing (EUCAST).

482 33. Lewis RE, Diekema DJ, Messer SA, Pfaller MA, Klepser ME. 2002. Comparison of Etest,

483   chequerboard dilution and time–kill studies for the detection of synergy or antagonism

484   between antifungal agents tested against *Candida* species. J Antimicrob Chemother 49:345–

485   351.

486   34. Di Veroli GY, Fornari C, Wang D, Mollard S, Bramhall JL, Richards FM, Jodrell DI. 2016.

487   Combenefit: an interactive platform for the analysis and visualization of drug combinations.

488   Bioinformatics 32:2866–2868.

489   35. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S,

490   Rueden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ, Hartenstein V, Eliceiri K,

491   Tomancak P, Cardona A. 2012. Fiji: an open-source platform for biological-image analysis.

492   7. Nat Methods 9:676–682.

493   36. Venter C, Niesler CU. 2019. Rapid quantification of cellular proliferation and migration

494   using ImageJ. BioTechniques 66:99–102.

495   37. Gow NAR, Johnson C, Berman J, Coste AT, Cuomo CA, Perlin DS, Bicanic T, Harrison

496   TS, Wiederhold N, Bromley M, Chiller T, Edgar K. 2022. The importance of antimicrobial

497   resistance in medical mycology. Nat Commun 13:5352.

498   38. Nagy F, Tóth Z, Nyikos F, Forgács L, Jakab Á, Borman AM, Majoros L, Kovács R. 2021.

499   *In vitro* and *in vivo* interaction of caspofungin with isavuconazole against *Candida auris*

500   planktonic cells and biofilms. Med Mycol 59:1015–1023.

501   39. Berkow EL, Lockhart SR. 2018. Activity of novel antifungal compound APX001A against

502   a large collection of *Candida auris*. J Antimicrob Chemother 73:3060–3062.

503   40. Hager CL, Larkin EL, Long L, Abidi FZ, Shaw KJ, Ghannoum MA. 2018. *In vitro* and *in*

504   *vivo* evaluation of the antifungal activity of APX001A/APX001 against *Candida auris*.

505   Antimicrob Agents Chemother 62:e02319-17.

506 41. Pfaller MA, Huband MD, Flamm RK, Bien PA, Castanheira M. 2019. *In vitro* activity of

507   APX001A (manogepix) and comparator agents against 1,706 fungal isolates collected

508   during an international surveillance program in 2017. Antimicrob Agents Chemother 63.

509 42. Arendrup MC, Chowdhary A, Jørgensen KM, Meletiadis J. 2020. Manogepix (APX001A)

510   *in vitro* activity against *Candida auris*: Head-to-head comparison of EUCAST and CLSI

511   MICs. Antimicrobial Agents and Chemotherapy 64:e00656-20.

512 43. Maphanga TG, Mpembe RS, Naicker SD, Govender NP, GERMS-SA F. 2022. *In vitro*

513   antifungal activity of manogepix and other antifungal agents against South African *Candida*

514   *auris* isolates from bloodstream infections. Microbiology Spectrum 10:e01717-21.

515 44. Zhu Y, Kilburn S, Kapoor M, Chaturvedi S, Shaw KJ, Chaturvedi V. 2020. *In vitro* activity

516   of manogepix against multidrug-resistant and panresistant *Candida auris* from the New

517   York outbreak. Antimicrob Agents Chemother 64.

518 45. Gebremariam T, Gu Y, Alkhazraji S, Youssef E, Shaw KJ, Ibrahim AS. 2022. The

519   combination treatment of fosmanogepix and liposomal amphotericin B is superior to

520   monotherapy in treating experimental invasive mold infections. Antimicrob Agents

521   Chemother 66:e0038022.

522 46. Dudiuk C, Berrio I, Leonardelli F, Morales-Lopez S, Theill L, Macedo D, Yesid-Rodriguez

523   J, Salcedo S, Marin A, Gamarra S, Garcia-Effron G. 2019. Antifungal activity and killing

524   kinetics of anidulafungin, caspofungin and amphotericin B against *Candida auris*. Journal

525   of Antimicrobial Chemotherapy 74:2295–2302.

526   47.   Kovács R, Tóth Z, Locke JB, Forgács L, Kardos G, Nagy F, Borman AM, Majoros L. 2021.

527   Comparison of *in vitro* killing activity of rezafungin, anidulafungin, caspofungin, and

528   micafungin against four *Candida auris* clades in RPMI-1640 in the absence and presence of

529   human serum. 4. Microorganisms 9:863.

530   48.   Bellmann R, Smuszkiewicz P. 2017. Pharmacokinetics of antifungal drugs: practical

531   implications for optimized treatment of patients. Infection 45:737–779.

532   49.   Dupont H, Massias L, Jung B, Ammenouche N, Montravers P. 2017. Pharmacokinetic

533   study of anidulafungin in ICU patients with intra-abdominal candidiasis. Journal of

534   Antimicrobial Chemotherapy 72:1429–1432.

535   50.   Chow NA, Gade L, Tsay SV, Forsberg K, Greenko JA, Southwick KL, Barrett PM, Kerins

536   JL, Lockhart SR, Chiller TM, Litvintseva AP. 2018. Multiple introductions and subsequent

537   transmission of multidrug-resistant Candida auris in the USA: a molecular epidemiological

538   survey. Lancet Infect Dis 18:1377–1384.

539   51.   Di Pilato V, Codda G, Ball L, Giacobbe DR, Willison E, Mikulska M, Magnasco L, Crea F,

540   Vena A, Pelosi P, Bassetti M, Marchese A. 2021. Molecular Epidemiological investigation

541   of a nosocomial cluster of *C. auris*: Evidence of recent emergence in Italy and ease of

542   transmission during the COVID-19 pandemic. 2. J Fungi 7:140.

543

Figure and Table Legends

545   Table 1. *Candida auris* isolates.

546   (50, 51)

547

548   Table 2. Antifungal MIC distribution for 25 *C. auris* isolates.

549

550   Table 1. FICI values for 5 antifungal combinations against eleven *C. auris* isolates.

551

552   Figure 1. *In vitro* interactions of AFG, MGX, AMB, VRC and 5FC according to the FICI values

553   for 11 *C. auris* isolates.

554   Minimum FICI values shown in absence of antagonism, otherwise maximum FICI values

555   reported. Drug interaction ranges are indicated by background colour: Synergy, dark green;

556   partial synergy, light green; indifference, white; antagonism, red. Symbols represent FICI values

557   of three independent experiments. 5FC, 5-flucytosine; AFG, anidulafungin; AMB, amphotericin

558   B; MGX, manogepix; VRC, voriconazole.

559

560   Figure 2. Changes in MIC values due to antifungal combinations for 11 *C. auris* isolates.

561   MIC values for 11 *C. auris* isolates in combinations of anidulafungin with 5-flucytosine (A) and

562   manogepix (B) compared to the antifungals in monotherapy as determined by checkerboard

563   assays. Symbols represent median values of three independent experiments. 5FC, 5-flucytosine;

564   AFG, anidulafungin; MGX, manogepix.
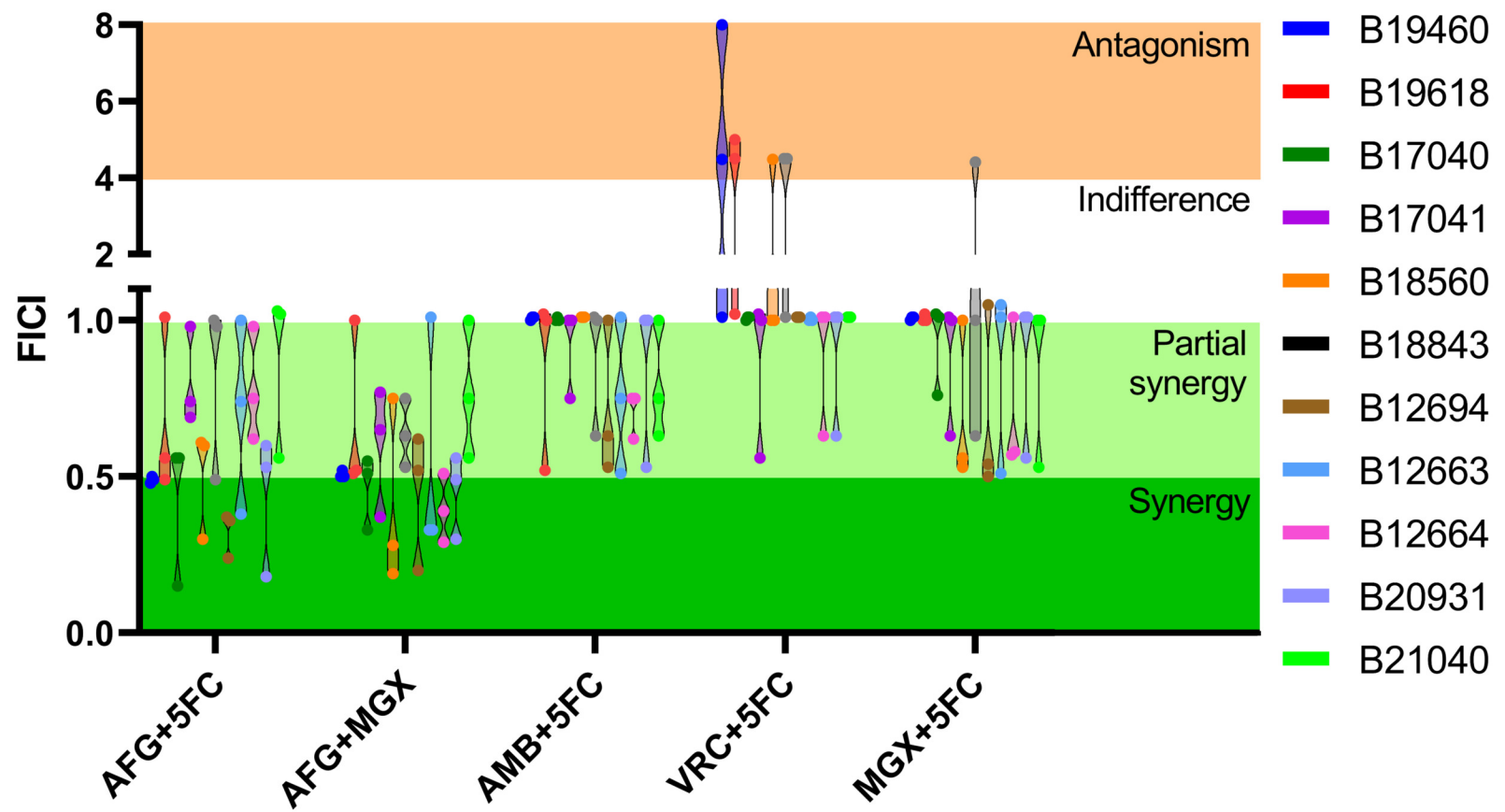
565

566   Figure 3. Synergy maps for 5 antifungal combinations against the multidrug-resistant *C. auris*

567   isolate B12663.

568   The interactions of 5-flucytosine with anidulafungin (A), amphotericin B (C) or voriconazole (D)

569   and the interactions of manogepix with anidulafungin (B) or 5-flucytosine (E) were analysed

570   with Combenefit (n=3). The graphs show the growth percentage relative to the drug-free control

571   with the colour scale representing the drug interaction. 5FC, 5-flucytosine; AFG, anidulafungin;

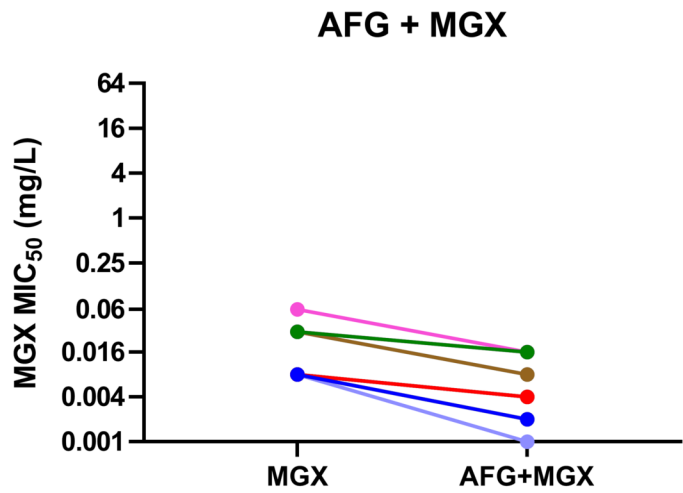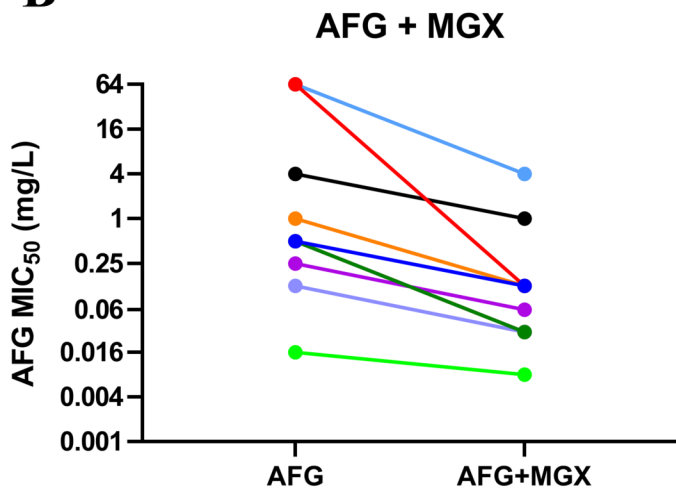572   AMB, amphotericin B; MGX, manogepix; VRC, voriconazole.

573

574   Figure 4. Microfluidics imaging of *C. auris* under antifungal combination exposure.

575   DIC images from two representative experiments (A) and doubling times (B, C) of *C. auris*

576   B12663 cells grown in the presence of RPMI 2%G-MOPS for 4 h, followed by further RPMI

577   2%G-MOPS or treatment with anidulafungin, 5-flucytosine and manogepix alone or in

578   combination at their MICs for 16 h. Doubling times were calculated by 2-dimensional colony

579   area changes for several colonies from two independent experiments. Mean ± range. Scale bars:

580   100 μm. *$P \leq 0.05$; **$P \leq 0.01$; ***$P < 0.001$ (one-way ANOVA test with Bonferroni's correction).

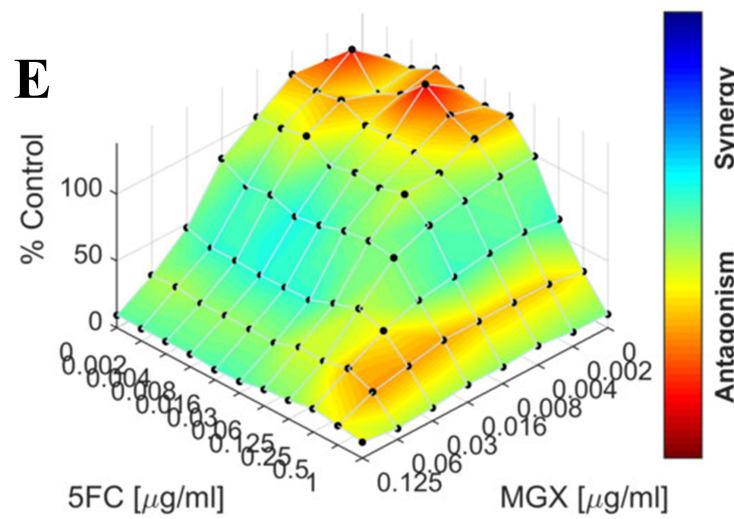581   5FC, 5-flucytosine; AFG, anidulafungin; MGX, manogepix.

A
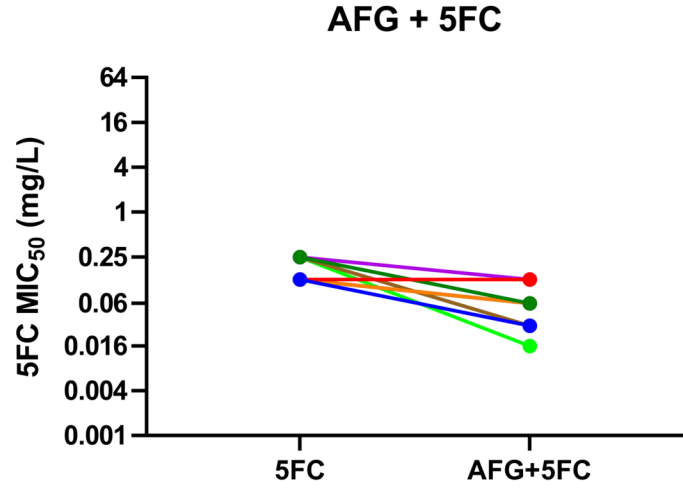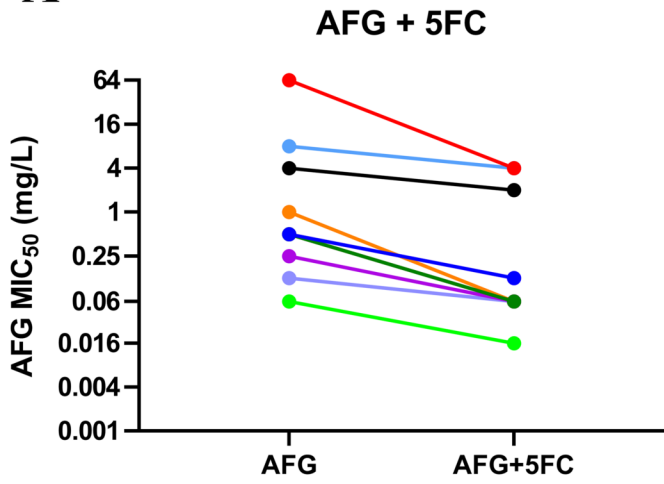
AFG + 5FC

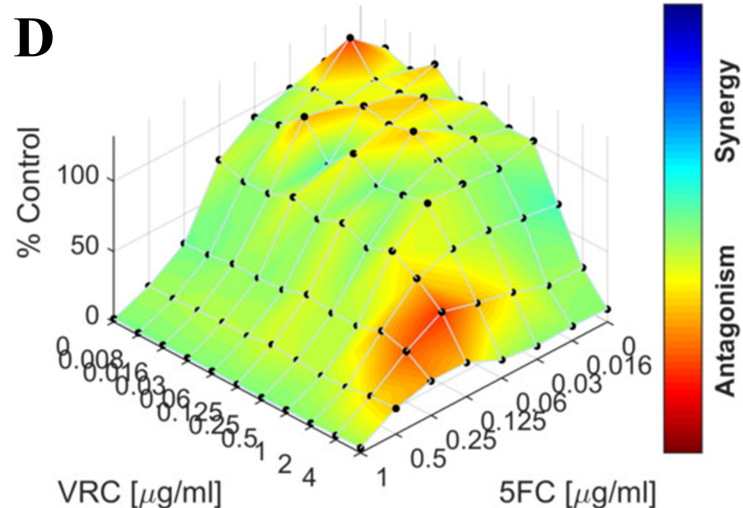**AFG + 5FC**

B

AFG + MGX

AFG + MGX

Legend (A):
B19460, B19618, B17040, B17041, B18560, B18843, B12694, B12663, B12664, B20931, B21040

Legend (B):
B19460, B19618, B17040, B17041, B18560, B18843, B12694, B12663, B12664, B20931, B21040

A

B

C

D

E
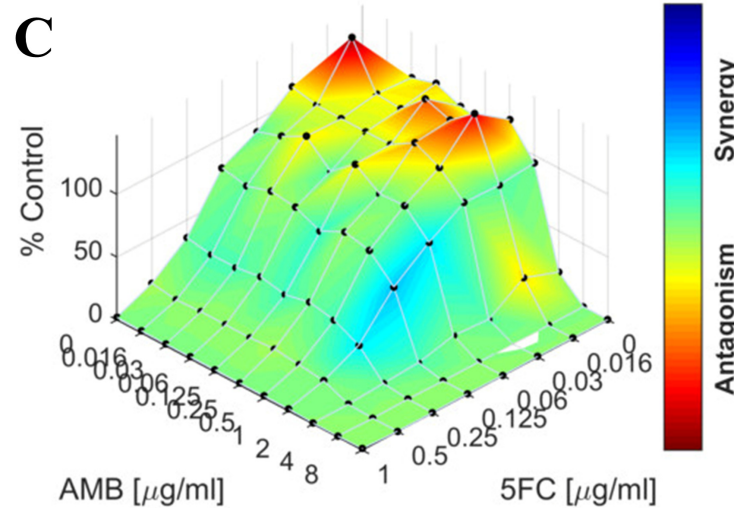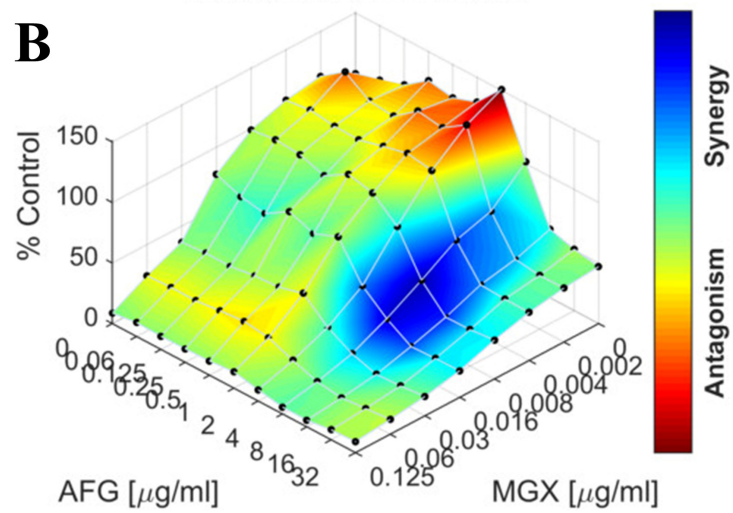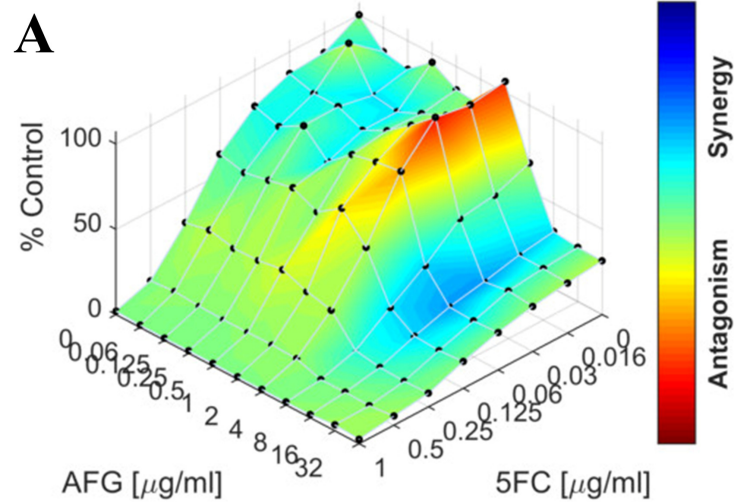
B12663 MIC values:
5FC: 0.25 mg/L
AFG: 8 mg/L
AMB: 2 mg/L
MGX: 0.03 mg/L
VRC: 2 mg/L

A

No drug | AFG

MGX | 5FC

AFG+MGX | AFG+5FC

B

Doubling time [h]

***
***
***

No drug | AFG | 5FC | AFG+5FC

C

Doubling time [h]

**
*
***

No drug | AFG | MGX | AFG+MGX

Table 1. *Candida auris* isolates.

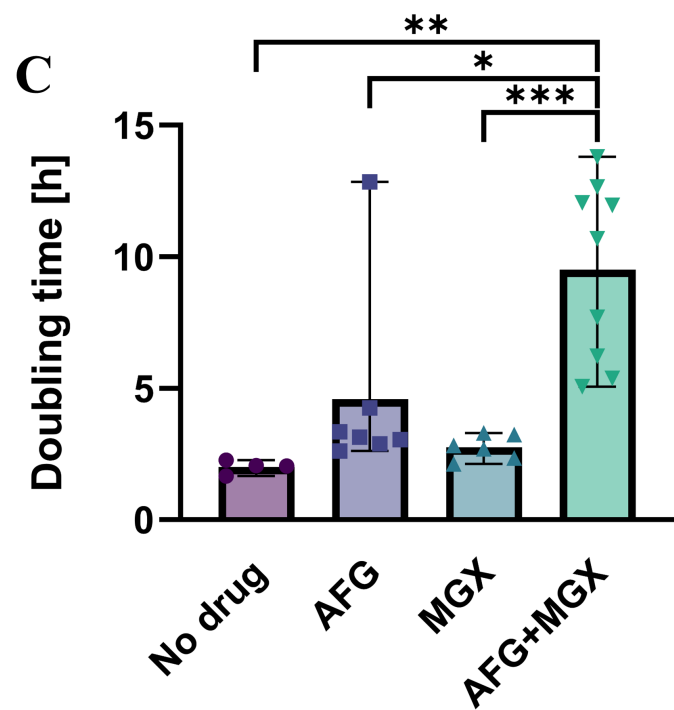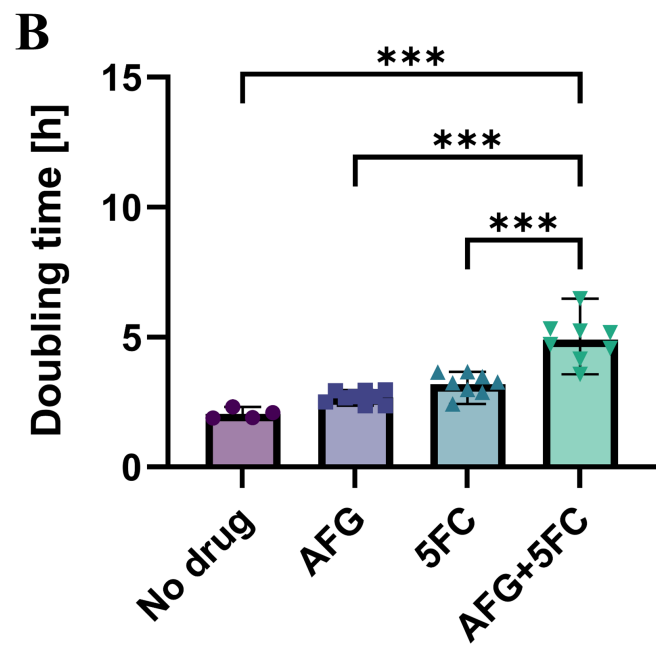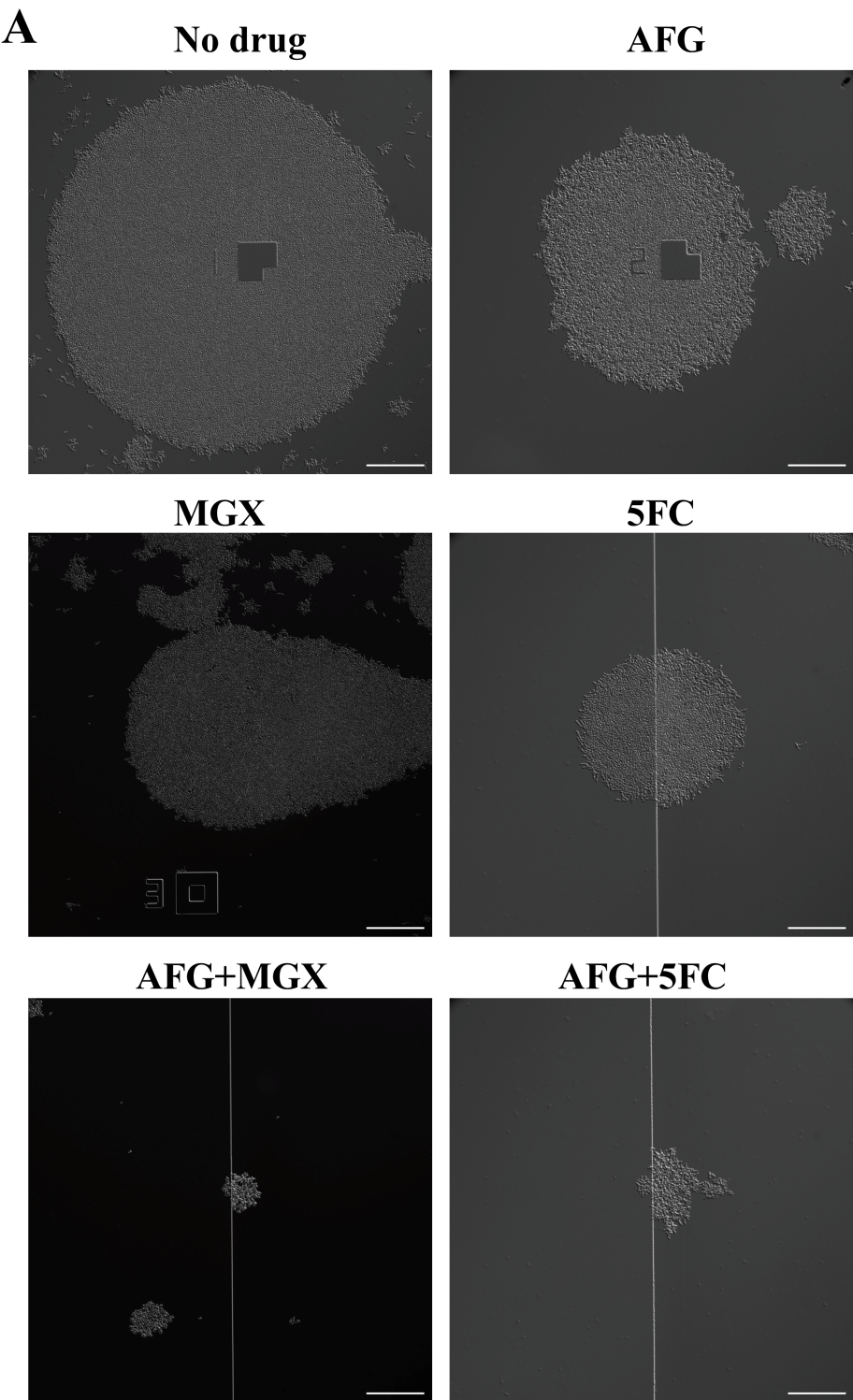| Isolate number	Clade[a]	Origin	Isolation day[b]	Isolated from	Reference
B12406	South American	USA	Day 0	Patient A, Urine	(Chow, 2018)
B15223	South American	USA	Day 294	Patient A, Blood	
B19460	South Asian	USA	Day 0	Patient B, Sputum	
B19547	South Asian	USA	Day 16	Patient B, Unknown	
B19617	South Asian	USA	Day 46	Patient B, Urine	
B19837	South Asian	USA	Day 79	Patient B, Urine	
B19618	South Asian	USA	Day 62	Patient B, Urine	
B17040	South Asian	USA	Day 0	Patient C, Urine	
B17041	South Asian	USA	Day 15	Patient C, Sputum	
B17073	South Asian	USA	Day 44	Patient C, Urine	
B17201	South Asian	USA	Day 67	Patient C, Urine	
B18560	South Asian	USA	Day 0	Patient D, Blood	
B18845	South Asian	USA	Day 72	Patient D, Blood	
B18841	South Asian	USA	Day 103	Patient D, Blood	
B18843	South Asian	USA	Day 96	Patient D, Blood	
B12692	South Asian	USA	Day 11	Patient E, Rectal	(Di Pilato, 2021)
B12694	South Asian	USA	Day 0	Patient E, Groin swab	(Di Pilato, 2021)
B12663	South Asian	USA	Day 11	Patient E, Urine	(Di Pilato, 2021)
B12664	South Asian	USA	Day 11	Patient E, Respiratory	(Di Pilato, 2021)
B12688	South Asian	USA	Day 11	Patient E, Groin swab	(Di Pilato, 2021)
B20931	South African	USA	Day 0	Patient F, Blood	
B21040	South African	USA	Day 3	Patient F, Trachea Aspirate	
B21041	South African	USA	Day 3	Patient F, Groin swab	
B21042	South African	USA	Day 3	Patient F, Blood	
B21043	South African	USA	Day 3	Patient F, Blood	

[a] Clade designation based on whole genome sequencing (Gifford *et al.*, in preparation).

[b] In reference to isolation date of first isolate from respective patient.

Table 2. Antifungal MIC distribution for 25 *C. auris* isolates.

Drug	MIC (mg/L)																	MIC_{50} [a]	MIC_{90} [b]	%R [c]
	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128			
AMB					0	0	0	0	0	1	24[d]	0	0	0				2	2	96.0
FLC								0	0	0	0	1	0	0	5	5	14	≥128	≥128	96.0
VRC				0	1	2	6	1	5	9	0	0	1					1	2	40.0
AFG				0	3	3	5	3	2	0	1	0	8					0.25	≥8	32.0
5FC			0	0	0	0	11	14	0	0	0	0						0.25	0.25	No BP
MGX	0	11	5	1	8	0	0	0	0	0								0.008	0.03	No BP

[a] MIC at which 50% of isolates were inhibited.

[b] MIC at which 90% of isolates were inhibited.

[c] Percentage of resistant isolates.

[d] Modal MICs are indicated with underlined numbers.

Grey background indicates tentative *C. auris* breakpoints according to the CDC.

5FC, 5-flucytosine; AFG, anidulafungin; AMB, amphotericin B; BP, breakpoint; FLC, fluconazole; MGX, manogepix; VRC, voriconazole.

Table 3. FICI values for 5 antifungal combinations against 11 *C. auris* isolates.

Isolate	AFG+5FC Median (range)	AFG+MGX Median (range)	AMB+5FC Median (range)	VRC+5FC Median (range)	MGX+5FC Median (range)
B19460	0.49 (0.48-0.50)	0.50 (0.50-0.52)	1.01 (1.00-1.01)	4.48 (1.01-8.00)	1.01 (1.00-1.01)
B19618	0.56 (0.49-1.01)	0.52 (0.51-1.00)	1.00 (0.52-1.02)	4.50 (1.02-5.00)	1.00 (1.00-1.02)
B17040	0.56 (0.15-0.56)	0.51 (0.33-0.55)	1.00 (1.00-1.01)	1.01 (1.00-1.01)	1.01 (0.76-1.02)
B17041	0.74 (0.69-0.98)	0.65 (0.37-0.77)	1.00 (0.75-1.00)	1.00 (0.56-1.02)	1.00 (0.63-1.01)
B18560	0.60 (0.30-0.61)	0.28 (0.19-0.75)	1.01 (1.01)	1.00 (1.00-4.48)	0.56 (0.53-1.00)
B18843	0.98 (0.49-1.00)	0.63 (0.53-0.75)	1.00 (0.63-1.01)	4.50 (1.01-4.50)	1.00 (0.63-4.41)
B12694	0.36 (0.24-0.37)	0.52 (0.20-0.62)	0.63 (0.53-1.00)	1.01 (1.01)	0.54 (0.50-1.05)
B12663	0.74 (0.38-1.00)	0.33 (0.33-1.01)	0.75 (0.51-1.01)	1.00 (1.00-1.01)	1.01 (0.51-1.05)
B12664	0.75 (0.62-0.98)	0.39 (0.29-0.51)	0.75 (0.62-0.75)	1.01 (0.63-1.01)	0.58 (0.57-1.01)
B20931	0.53 (0.18-0.60)	0.49 (0.30-0.56)	1.00 (0.53-1.00)	1.01 (0.63-1.01)	1.01 (0.56-1.01)
B21040	1.02 (0.56-1.03)	0.75 (0.56-1.00)	0.75 (0.63-1.00)	1.01 (1.01)	1.00 (0.53-1.00)

Synergy, dark green; partial synergy, light green; indifference/additivity, white; antagonism; red. Underlined values indicate resistance to either AFG or VRC.

5FC, 5-flucytosine; AFG, anidulafungin; AMB, amphotericin B; MGX, manogepix; VRC, voriconazole