PC945 shows a higher barrier than itraconazole to induction of *Aspergillus fumigatus* resistant mutants

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Introduction

The development of drug resistance and cross-resistance continues to pose a challenge to successful long-term antifungal therapy. Recently, extensive and long-term therapy with azole compounds such as itraconazole has been associated with an increasing number of reports of *Aspergillus fumigatus* resistance to this antifungal agent. PC945 is a novel inhaled triazole, which is now undergoing clinical phase testing. We investigated the experimental evolution of resistance to PC945 in *A. fumigatus,* by repeated exposure, and compared the observed kinetics with those of itraconazole.

Methods

A. fumigatus (NCPF2010) was exposed to increasing concentrations of PC945 or itraconazole, and susceptibility testing to a panel of azoles was performed. Compounds were re-suspended in agar at sub-MIC concentrations and doubled every passage (Itraconazole: 0.125 – 8 µg/ml, PC945: 0.03 – 8 µg/ml) for 15 passages. *A. fumigatus* (20 µl at 30 x 10^6 conidia/ml) was spread evenly across the plate and after incubation at 35°C for 7 days, conidia were collected from the plate and used to inoculate the next plate. Susceptibility testing was performed every 5 passages and MIC_{90} values (90% inhibition of fungal growth) were calculated against a panel of azoles and compared to a non-treated, passaged control. When resistance was suspected, previous passages were retrospectively tested to determine the passage at which resistance occurred. Sanger sequencing of the CYP51A gene (~2 KB) was performed on resistant strains generated and compared against the wild-type.

Table 1. Treatment schedule (µg/mL)

Passage (weekly)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ITR	0.125	0.25	0.5	1	2	4	8	8	8	8					
PC945	0.03	0.06	0.125	0.25	0.5	1	2	4	8	8	8	8	8	8	8

Results

The passaged *A. fumigatus* control did not exhibit any change in MIC_{90} across the study. Itraconazole-passaged *A. fumigatus* exhibited an increase in the MIC_{90} between the 6th and 7th passages (17.7 fold higher MIC_{90} compared to passage zero) (Table 2, Figure 1). Furthermore, the itraconazole mutant was pan-azole resistant with less susceptibility to posaconazole and voriconazole (15.7-fold and >5-fold higher MIC_{90}, respectively). In contrast, a PC945-resistant mutant was not detected until passage 14 (8.3-fold higher MIC_{90} compared to passage zero), and the PC945 resistant mutant showed little change in susceptibility to itraconazole, posaconazole and voriconazole (Table 2, Figure 1).

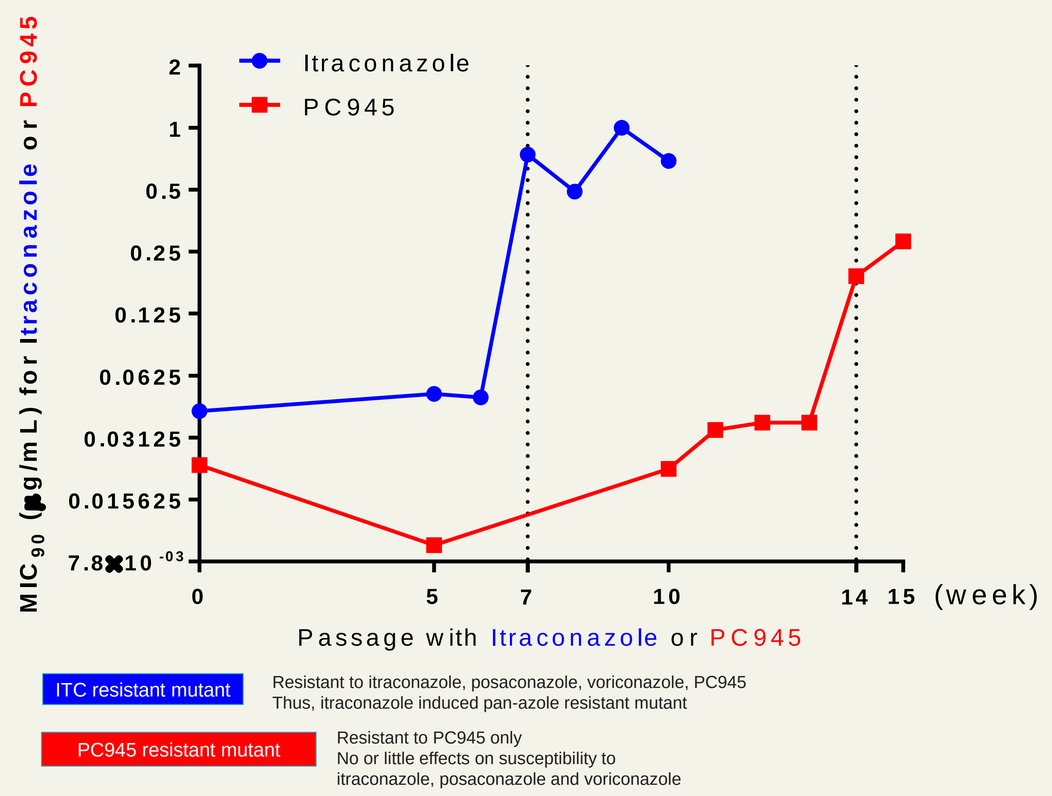

Figure 1. Changes in MIC_{90} values (90% growth inhibition) in fungus passaged for indicated period with Itraconazole or PC945

Table 2. MIC_{90} values of reference anti-fungal agents against ITR or PC945 induced mutants (µg/mL)

	Passaged with ITR			Passaged with PC945		
	Pre	7th week	Fold change	Pre	14th week	Fold change
Itraconazole	0.042	0.74	x18	0.042	0.044	x1.1
Posaconazole	0.0088	0.14	x16	0.0088	0.042	x5
Voriconazole	0.20	>1	>x5	0.20	0.25	x1.3
PC945	0.023	>1	>x43	0.023	0.19	x8
AMB	0.79	>1	>x1.3	0.79	0.29	x0.4

Whole genome analysis of PC945 mutant AF genome sequence

Genomic DNA was fragmented; DNA libraries were prepared using the NEBNext Ultra DNA Library Prep with Beads Size Selection kit and sequenced on Illumina HiSeq with a sequencing configuration of 2x150 PE (Genewiz Ltd). Data analysis was performed using DRAGEN pipeline. Reads were mapped to the *A. fumigatus* A1163 reference genome.

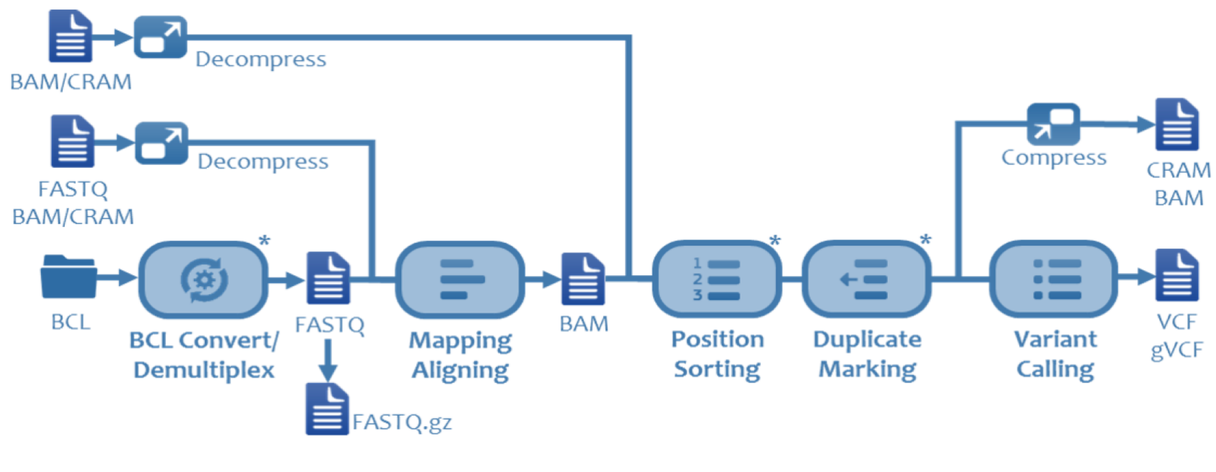

Figure 2. Workflow for resequencing analysis using DRAGEN

Preliminary analysis indicates that resistance has occurred via a CYP51A and CYP51B independent mechanism. Analysis with the large volume of data generated is still ongoing.

Conclusion

PC945 exhibited a much higher barrier to induction of an experimentally induced resistant mutant of *A. fumigatus*, when compared with itraconazole. Furthermore, the mutant developed resistance via a CYP51-independent mechanism. Thus, PC945 may be a particularly useful prophylactic or chronic treatment for immunosuppressed patients or those with respiratory diseases in clinics.

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