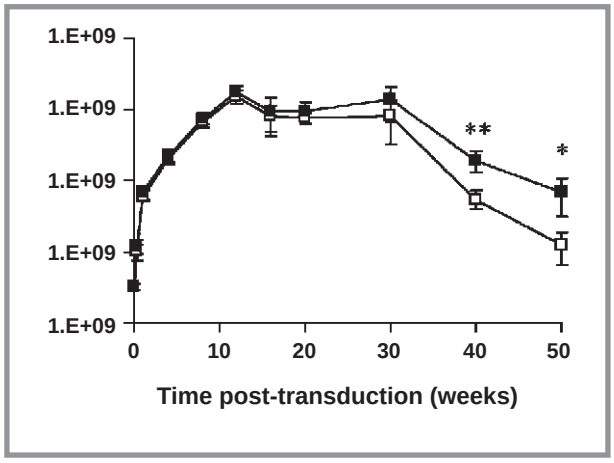

Figure 1. Long – term culture of retrovirally transduced CD34⁺ UCB cells. Cumulative cell number following transduction with control vector (open squares) or hTERT-retrovirus (closed squares) and maintenance in FL+IL3. Results are mean ± sem of between 6 and 12 samples **$p < 0.005$, *$p < 0.01$.

longs the survival of mature hematopoietic cells, was unexpected and intriguing. Limited numbers of cells were available at very late time points to perform molecular and cellular analyses and the mechanism of action for this pro-survival role of hTERT is as yet unknown. A pro-survival action of hTERT independent of telomerase enzymatic activity has recently been described in human breast cancer cells.[7] Future studies aim to elucidate the cellular and molecular mechanisms underlying this pro-survival effect of hTERT in human hematopoietic progenitor cells.

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Infectious Disorders

Clinical significance of breakthrough fungemia caused by azole-resistant *Candida tropicalis* in patients with hematologic malignancies

A 5-year retrospective analysis of fungemia in patients with hematologic malignancies revealed that four patients, who received fluconazole and itraconazole during neutropenia, developed breakthrough candidemia due to azole-resistant *Candida tropicalis* isolates. This observation suggests that causative organisms of candidemia in neutropenic patients receiving azoles should be suspected of being azole-resistant.

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Invasive candidiasis is associated with a high rate of mortality in cancer patients who develope neutropenia.[1] In these patients, *Candida tropicalis* ranks as the fourth leading cause of fungemia among *Candida* species, but blood isolates of *C. tropicalis* with reduced susceptibility to azole antifungals are very uncommon.[2-4] We present here four cases of breakthrough fungemia caused by azole-resistant *C. tropicalis* in patients with hematologic malignancies.

Between January 1, 1996, and December 31, 2000, four episodes of candidemia caused by *C. tropicalis* were identified in 701 patients with hematologic malignancies. As shown in Table 1, *in vitro* susceptibility testing according to NCCLS document M27-A revealed that all isolates had fluconazole MIC >64 μg/mL, itraconazole MIC >32 μg/mL, and voriconazole MIC >32 μg/mL, suggesting these isolates should be categorized as azole-resistant *C. tropicalis*.[3,5] Amphotericin B and micafungin were effective against these isolates with MIC of 0.0625-0.03125 μg/mL and 0.0625 μg/mL, respectively.

To evaluate the molecular epidemiology of the isolates, two primers were adopted for the randomly amplified polymorphic DNA (RAPD) analysis: R-1 (5'-ATGGATCGGC-3') and R-2 (5'-ATTGCGTCCA-3'), which had been used for the previous analysis of *Candida* species.[6] As shown in Figure 1, isolate 1 in 1996, 2 in 1997, 3 in 1997, and 4 in 1998 had distinguishing band patterns with primer R-1, suggesting the four isolates were derived from four strains of *C. tropicalis*.

Clinical data on these four patients are presented in Table 1. The underlying diseases were acute myeloid leukemia in three patients and myelodysplastic syndrome in one. All patients were profoundly neutropenic for 29-97 days because of intensive chemotherapy, and candidemia was diagnosed during the neutropenic period. All patients had central venous catheters in place and received broad-spectrum antibiotics. Surveillance cultures revealed the presence of *C. tropicalis* in oropharyngeal samples (four patients), stool samples (two), and urine samples (one),

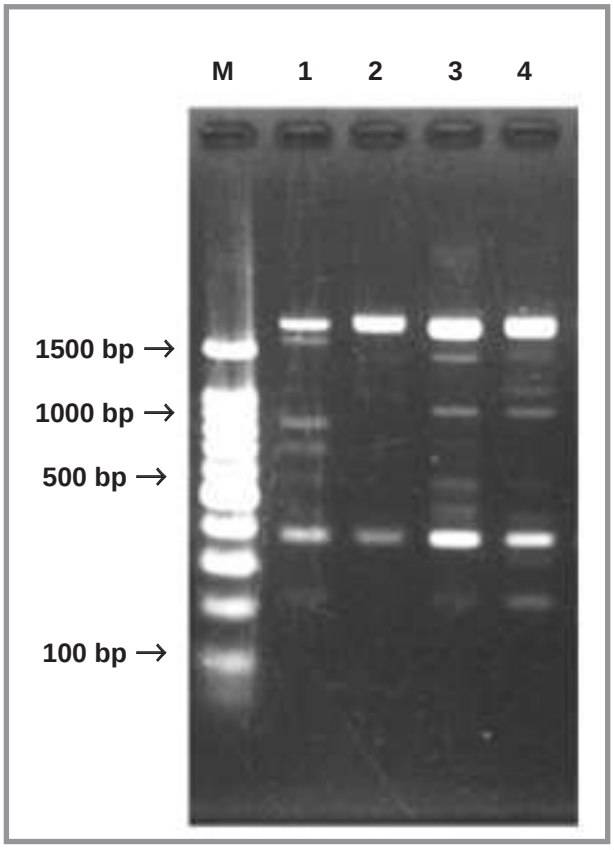

Figure 1. RAPD fingerprint band patterns of four *C. tropicalis* isolates determined using primer R-1. A DNA ladder marker was used as the molecular size standard (bp).

before the diagnosis of candidemia, suggesting colonization of the strains in the digestive tract. All patients developed mucositis due to chemotherapy. The patients received oral itraconazole (150-200 mg/day) as antifungal prophylaxis and therapy for 35-105 days. At the time of becoming febrile, the patients were given pre-emptive intravenous fluconazole (400 mg/day) for 5-16 days. Intravenous amphotericin B was administered at the dosage of 50 mg/day for 33-101 days after fluconazole was discontinued because of persistent high fever. Two patients developed breakthrough candidemia despite receiving fluconazole and itraconazole and the other two patients developed breakthrough candidemia despite receiving itraconazole and amphotericin B. Overall, two patients responded to amphotericin B with neutrophil recovery.

It is interesting to note that all patients received both itraconazole and fluconazole before developing fungemia caused by azole-resistant *C. tropicalis*. Some studies have shown that fungemia in cancer patients caused by fluconazole-resistant *Candida* species is associated with exposure to fluconazole administered for prophylaxis or therapy.[2,7] Even *C. albicans*, which is generally susceptible to azole compounds, has been reported to cause breakthrough fungemia due to fluconazole-resistant strains in immuno-

Table 1. Clinical and *in vitro* susceptibility data of the four isolates from four patients with fungemia caused by azole-resistant *C. tropicalis*.

Patient age/gender (Year)	*Hematologic malignancy*	*Possible risk factors for fungemia*	*Surveillance culture*	*MICs (μg/mL)*					*Antifungal prophylaxis and therapy*	*Outcome*
				Flu	*Itr*	*Vor*	*Amp*	*Micf*		
1: 76/M (1996)	AML	Chemotherapy, antibiotic use, CVC, steroid use, mucositis, neutropenia for 86 days	Throat, stool	≥64	≥32	≥32	0.03125	0.0625	Itr,150 mg q.d. for 91 days Flu, 400 mg q.d. for 16 days Amp, 50 mg q.d. for 75 day	Survival
2: 40/M (1997)	AML	Chemotherapy, antibiotic use, CVC, steroid use, mucositis, neutropenia for 29 days	Throat	≥64	≥32	≥32	0.0625	0.0625	Itr, 150 mg q.d. for 35 days Flu, 400 mg q.d. for 5 days Amp, 50 mg q.d. for 33 days	Unrelated death 3 months after diagnosis
3: 63/F (1997)	AML	Chemotherapy, antibiotic use, CVC, mucositis, neutropenia for 97 days	Throat, stool	≥64	≥32	≥32	0.03125	0.0625	Itr, 200mg q.d. for 105 days Flu, 400 mg q.d. for 11 days Amp, 50 mg q.d. for 101 days	Related death on day 2 after diagnosis
4: 76/M (1998)	MDS	Chemotherapy, antibiotic use, CVC, steroid use, mucositis, neutropenia for 56 days	Throat, urine	≥64	≥32	≥32	0.03125	0.0625	Itr, 200mg q.d. for 63 days Flu, 400 mg q.d. for 11 days Amp, 50 mg q.d. for 61 days	Related death on day 5 after diagnosis

M: male; F: female; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; CVC: central venous catheter; Flu: fluconazole; Itr: itraconazole; Vor: voriconazole; Amp: amphotericinB; Micf: micafungin.

compromised patients receiving a short-term[8] or long-term fluconazole.[9] In addition, a recent *in vitro* study revealed that the acquisition of azole-resistance by *C. tropicalis* strains was rapid after culture in medium containing a high concentration of fluconazole.[10] Therefore, in our cases, it is likely that the use of both fluconazole and itraconazole was effective against azole-susceptible *C. tropicalis*, but, it allowed potentially azole-resistant *C. tropicalis* to cause fungemia during neutropenia. Since colonization of *C. tropicalis* and mucositis were observed in all our patients before they developed fungemia, endogenous azole-resistant *C. tropicalis* could have been acquired through the digestive tract rather than from exogenous sources. Furthermore, RAPD typing demonstrated that each fungemia case was caused by a different strain of *C. tropicalis*, supporting an endogenous origin for the infections and negating the possibility of nosocomial transmission of a single strain.

As recently reported by Pfaller *et al*., many strains of fluconazole-resistant *C. albicans* display cross-resistance to itraconazole (RR-phenotype) and these RR-strains of *C. albicans* have proven to be less susceptible to new azoles such as voriconazole.[3] It is surprising that our strains of *C. tropicalis* showed a RR-phenotype with high MIC of voriconazole. To our knowledge, voriconazole-insusceptible *C. tropicalis* has not been previously reported as a cause of breakthrough fungemia. These findings strongly imply that therapeutic options for fungemia caused by azole-resistant *C. tropicalis* are very limited, and, as shown in our study, amphotericin B and micafungin may be the most effective antifungal options.

In summary, immunocompromised patients who suffer breakthrough candidemia during the administration of azole antifungal agents should be suspected of harboring an azole-resistant strain until the *in vitro* susceptibilities to the antifungal agents are determined.

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Key words: fungemia, Candida tropicalis, azole-resistance, hematologic malignancies.

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