



# Combination therapy for an elderly patient with chromoblastomycosis caused by *Fonsecaea monophora*: a case report

Yu Shen<sup>1#</sup>, Boxuan Jiang<sup>2#</sup>, Han Zhang<sup>2#</sup>, Jinrong Feng<sup>3</sup>, Hui Hua<sup>1</sup>

<sup>1</sup>Department of Dermatology, Third Affiliated Hospital of Nantong University, Nantong Third People's Hospital, Nantong, China; <sup>2</sup>School of Medicine, Nantong University, Nantong, China; <sup>3</sup>Department of Pathogen Biology, School of Medicine, Nantong University, Nantong, China

<sup>#</sup>These authors contributed equally to this work.

**Correspondence to:** Jinrong Feng. Department of Pathogen Biology, School of Medicine, Nantong University, Nantong, China. Email: jinrong532@163.com; Hui Hua. Department of Dermatology, Third Affiliated Hospital of Nantong University, Nantong Third People's Hospital, Nantong, China. Email: 214972881@qq.com.

**Abstract:** We report the first case of combined treatment using oral drugs, thermotherapy, and carbon dioxide fractional laser for an elderly patient with skin chromoblastomycosis caused by *Fonsecaea monophora*. Chromoblastomycosis is a chronic and refractory granulomatous disease of the skin and subcutaneous tissues caused by a group of dematiaceous fungi, which can cause teratogenesis, disability, and even cancer. One of the subtypes, *F. monophora*, is not only limited to the skin and subcutaneous tissues but also affects the central nervous system. Therefore, a timely and clear diagnosis, as well as active and effective treatment, are particularly important. This case report presents a 75-year-old male patient whose left forearm had a plaque with mild pruritus for more than three years. The patient's skin lesions were histopathologically examined, and the fungus on the surface of the scabbed skin was examined by fluorescence microscopy and cultured. The strains obtained by the culture were identified by morphological and molecular biology, and a drug susceptibility test was conducted *in vitro*. Histopathology revealed hyperkeratosis of the epidermis with pseudoepitheliomatous hyperplasia, chronic granulomatous changes in the dermis, and brown thick-walled sclerotic corpuscles both inside and outside giant cells. Septate hyphae and sclerotic corpuscles could be observed in the fungus on the surface of the scabbed skin by fluorescence staining, and black villous colonies could be observed *in vitro*. Under the scanning electron microscope, rhinocladia was the primary sporulation type, and the conidia were oval. Molecular identification results showed that the similarity between its internal transcribed spacer (ITS) sequence and that of *F. monophora*, a Chinese strain (IFM41705), was the highest, reaching 100%. The results of the drug susceptibility test showed that the minimum inhibitory concentrations of itraconazole and voriconazole were 0.125 mg/L and 0.06 mg/L, respectively. The patient was given oral itraconazole 0.2 qd, combined with local thermotherapy and carbon dioxide fractional laser treatment. After 16 weeks, the microscopic examination of the fungus was negative, showing good efficacy.

**Keywords:** *Fonsecaea monophora*; chromomycosis; molecular identification; carbon dioxide laser; case report

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## Introduction

Chromoblastomycosis is a chronic refractory granulomatous disease of the skin and subcutaneous tissue caused by a group of dematiaceous fungi, with the skin lesions generally localized. Pathogenic fungi usually invade the skin via local

minor trauma. The disease is most common in tropical and subtropical zones, and very few cases occur in the temperate zone. *F. monophora* is a fungal pathogen that has attracted attention in recent years, and many cases of chromoblastomycosis caused by it have been reported in

southern China, but it has not been reported in central Jiangsu. Recently, a case of chromoblastomycosis caused by *F. monophora* was diagnosed and treated in our department, and the isolated pathogen was studied and reported below. We present the following case in accordance with the CARE reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-21-6119/rc>).

## Case presentation

### Subject and methods

#### Subject

The patient was a 75-year-old male from Nantong, Jiangsu Province. His left forearm had a plaque with slight itching for more than three years. Three years ago, the patient developed localized mung bean-sized red papules with slight itching after a suspected insect bite on the left forearm, which did not attract attention. Later, the rash slowly expanded. One month ago, the patient came to our hospital due to aggravated skin lesions after topical application of hormone ointment. Over the course of the disease, there was no cough, expectoration, night sweats, or anorexia, and his stools and urination were normal. The patient was previously healthy, and there were no individuals with similar diseases in his family. Laboratory tests showed normal routine blood and urine tests, and normal liver and kidney function tests. Physical examination was unremarkable. Dermatology showed an irregular reddish-brown plaque of about 5 cm × 3.5 cm on the extensor side of the left forearm, with a clear boundary, high margin, a dark brown punctate scab on the medial side of the bulge, slight atrophy of the center of the lesion, and telangiectasia (Figure 1A). All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

#### Methods

##### *Mycological examination*

(I) Black punctate crusts on the surface of the lesions were removed for fungal fluorescence staining (Nanjing Hanrui Biotechnology Co. Ltd.) and observed under a fluorescence microscope; (II) two additional pieces of black

punctate crust on the surface of the lesion were cultured on solid Sabouraud dextrose agar (SDA) in an incubator at 25 and 37 °C.

##### *Histopathological examination*

A piece of tissue from the elevated margin of the lesion was excised and fixed in 10% formalin and embedded in paraffin for section cutting, followed by hematoxylin-eosin (HE) staining and observation under light microscopy.

##### *Molecular strain identification*

The strain to be tested was streaked on SDA plates. Rice-sized fungi were collected, dissolved in 100 µL Tris-EDTA (TE) buffer, treated at 100 °C for 10 min, centrifuged at 10,000 rpm for 10 min, and 2 µL of the supernatant (raw DNA extract) was directly used for the PCR reaction. PCR amplification was performed using OneTaq (New England Biolabs, USA) polymerase, with ITS1 and ITS4 as the primers. The PCR reaction conditions were 95 °C for 5 min; then 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s, for a total of 30 cycles; then 72 °C for 5 min. After completing the PCR reaction, 3 µL of the product was subjected to 1.5% agarose gel electrophoresis, and the remaining product was sent to the General Biological Company for two-dimensional sequencing detection using ITS1 and ITS4 primers.

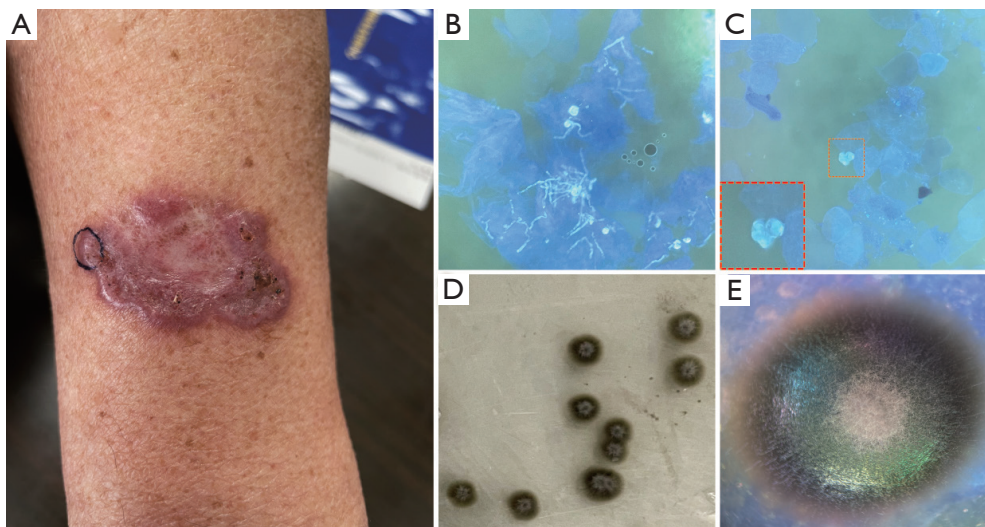
##### *In vitro antimicrobial susceptibility testing*

An ATB Funguskit 14204 (BioMerieux, FRA) was used for the antimicrobial susceptibility test. An appropriate amount of fungus was scraped from the surface of the SDA medium, placed in 5 mL of sterile water, mixed well in a shaker for 2 min, and then the bulk cells were filtered off using four layers of sterile gauze. The fungal solution concentration was adjusted to OD<sub>600</sub> = 0.5 (approximately 2 McF). A diluted fungal solution (20 µL) was added into the ATB F2 culture medium provided with the kit, thoroughly shaken, and mixed well. The fungal solution (135 µL) was placed into each test well according to the kit directions, incubated at 30 °C for 3–4 days, and the test results were observed.

## Results

### *Mycological results*

Fungal fluorescence staining of the crusted skin showed septate hyphae and sclerotic corpuscles of varying lengths (Figure 1B), and some of the sclerotic corpuscles showed septa (Figure 1C). After culturing the black punctate crust for 5 days, the results showed colonies about 0.3–0.5 cm in diameter, with a black villous surface and a slightly elevated



**Figure 1** Clinical appearance of chromoblastomycosis lesions in the patient and results of the fungal examination. (A) The initial appearance of the lesion before treatment as an outpatient. (B,C) Fluorescent staining of the skin lesions. A sclerotic body was shown in panel B. (D) The colony morphology on solid SDA media. (E) The colony morphology under a dermoscopy. SDA, sabouraud dextrose agar.

center that was grayish-white (Figure 1D). Through dermoscope, hemispherical colonies were observed, with three distinct demarcation zones; the base was dark brown, the second layer was dense silver-white filaments, and the center was clumped white floccules (Figure 1E). Scanning electron microscopy showed that dense hyphae were predominant in the dark brown site at the periphery of the colony (Figure 2A), beak cladospordia were predominant in the center, conidia were oval, and multiple conidia were arranged at the apex of the conidiophores (Figure 2B). The liquid culture of the fungi showed coracoid sporophytic and bottle-type conidiophores (Figure 2C).

### Histopathological results

The histopathological results showed epidermal hyperkeratosis with pseudoepithelial rumen hyperplasia, many types of epithelial cells, multinucleated giant cells in the dermis with microabscess formation, tan thick-walled sclerotic corpuscles inside and outside the giant cells, and septa in some of the sclerotic corpuscles (Figure 3A). Periodic Acid-Schiff (PAS) staining showed reddish-brown thick-walled sclerotic corpuscles in the dermis (Figure 3B).

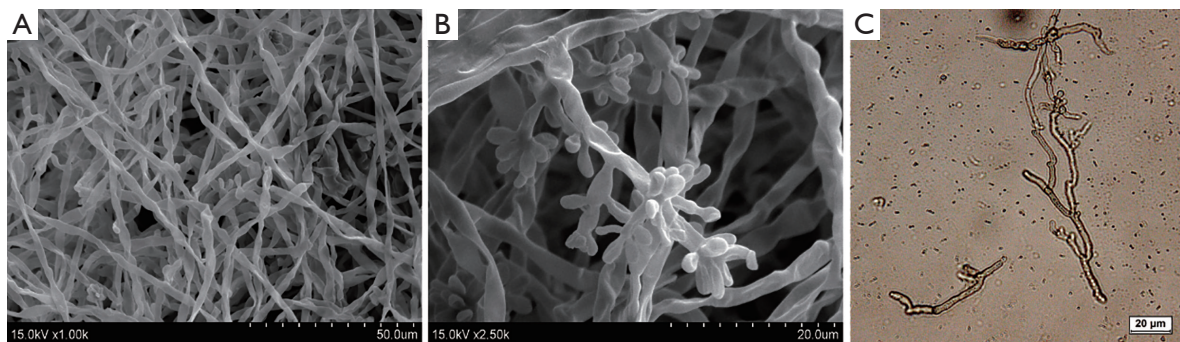
### Strain identification results

The genomic DNA was extracted, and PCR was performed

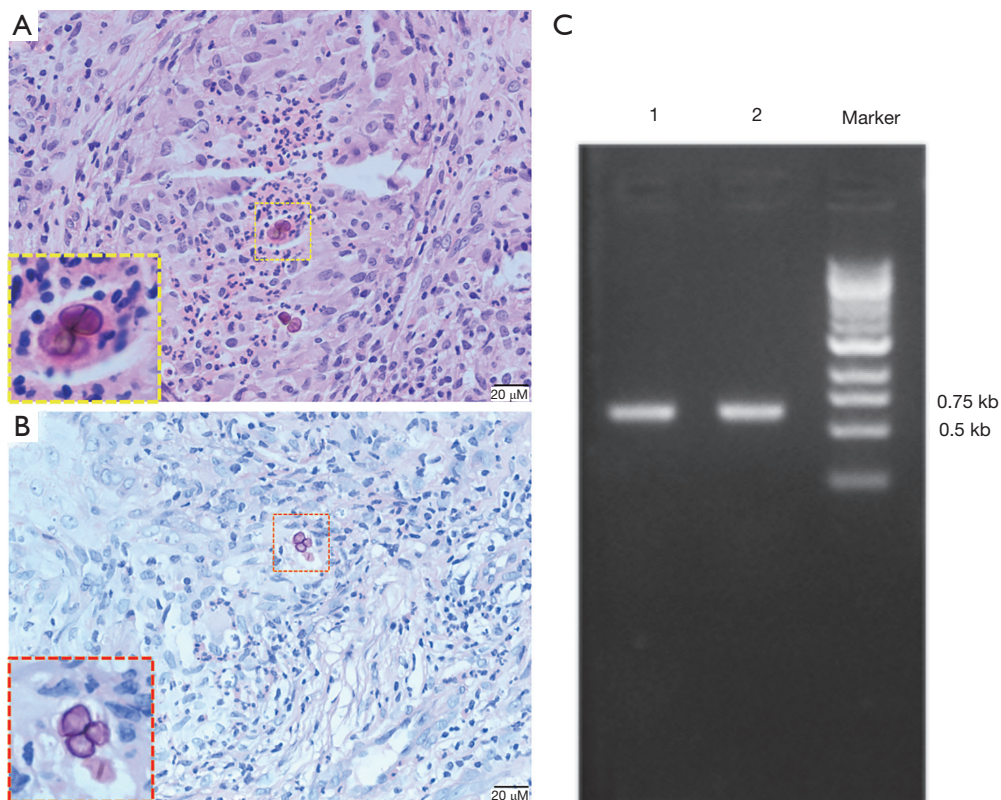
using the universal fungal primers ITS1 and ITS4, which amplified a significant single band with a size of about 0.6 kb, consistent with the theoretical value (Figure 3C). After sequencing, the PCR product was found to be similar to many strains of the *F. monophora* sequence in the GenBank database (<https://www.ncbi.nlm.nih.gov/>), with a similarity of more than 99%. Compared with the sequences in the ISHAM Fungal Database (<https://its.mycologylab.org/page/Alignment>), the PCR product was found to be similar to a Chinese *F. monophora* strain (IFM41705), with a similarity of 100%. Based on the above results, the pathogen found in this study was identified as *F. monophora*.

### Results of antimicrobial susceptibility testing

After preparing the fungal solution according to the manufacturer's directions and culturing for the required time, the fungi in the control group grew well. In the drug group, itraconazole and voriconazole had the best antifungal effect, and almost no fungal growth was observed even in the lowest concentration test wells (itraconazole, 0.125 mg/L; voriconazole, 0.06 mg/L). 5-Fluororotic acid had some antifungal ability, and there was weak fungal growth. Fluconazole had a weak antifungal effect, and high concentrations (greater than 16 mg/L) were needed to inhibit fungal growth. Amphotericin B had the lowest antifungal effect, and fungal growth was still observed at



**Figure 2** Mycelial morphology. (A,B) SEM results. (C) Liquid culture results. SEM, scanning electron microscope.



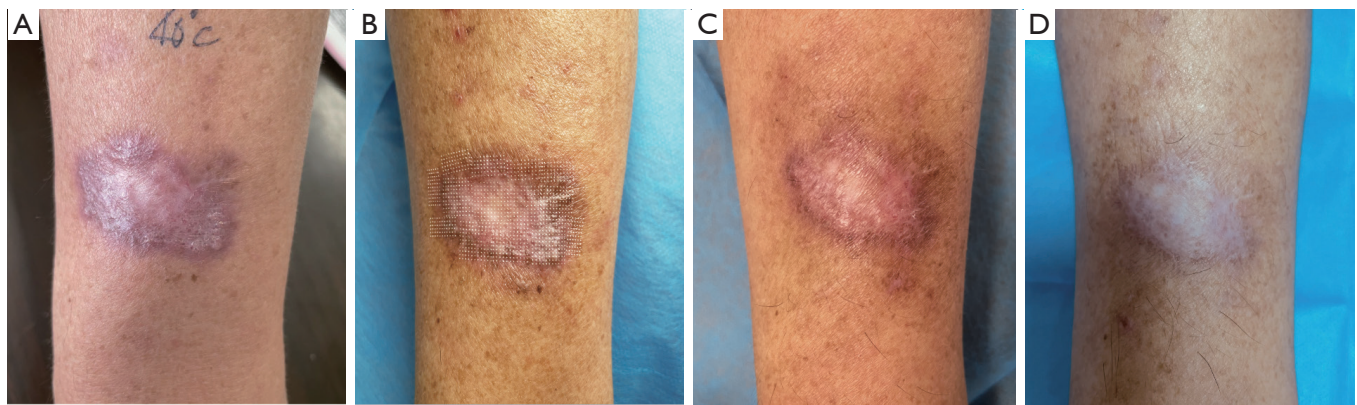
**Figure 3** The histopathological examination of skin lesions and molecular identification of strains. (A) HE staining ( $\times 400$ ). (B) PAS staining ( $\times 400$ ). (C) PCR amplification results of the fungal ITS sequence. HE, hematoxylin-eosin; PAS, periodic acid-schiff; PCR, polymerase chain reaction; ITS, internal transcribed spacer.

the highest concentration (16 g/L). The results indicated that itraconazole and voriconazole had the strongest anti-*F. monophora* effect.

### Treatment

Based on the clinical presentation, mycological,

histopathological, and molecular biological examinations, this case was diagnosed as chromoblastomycosis caused by *F. monophora*. According to the *in vitro* antimicrobial susceptibility testing results, the patient was given oral itraconazole capsules 0.2 qd, with topical bifonazole gel and amorolfine cream applied alternately, along with adjuvant local heat therapy for 20–30 min/day. The patient returned



**Figure 4** Clinical appearance of chromoblastomycosis lesions after therapy. (A) The lesion after treatment with itraconazole for 8 weeks. (B) The immediate effect on the lesion following the treatment of CO<sub>2</sub> fractional photothermolysis. (C) The lesions after 16 weeks of treatment with itraconazole combined with two treatments with CO<sub>2</sub> fractional photothermolysis. (D) The lesions after 20 weeks of treatment with itraconazole combined with three treatments with CO<sub>2</sub> fractional photothermolysis.

regularly every 4 weeks, and after 8 weeks of treatment, the skin lesions were significantly reduced, and the elevated margin gradually flattened (*Figure 4A*). Observable spores were scraped from the skin surface for fungal fluorescence microscopy. The treatment continued as before, assisted with carbon dioxide fractional laser treatment (*Figure 4B*) using an energy level of 70 mJ at the elevated site and 60 mJ medially, with a coverage rate of 11.1%. Topical amorolfine cream was encapsulated for 1 hour after carbon dioxide fractional laser treatment every 4 weeks. After 16 weeks of treatment, the lesion's periphery was significantly flattened (*Figure 4C*), and the fungal fluorescence microscopy was negative.

## Discussion

Chromoblastomycosis has a worldwide distribution and is mainly endemic in hot and humid areas of the tropics and subtropics; males aged 20–60 years appear to be most vulnerable (1). The primary pathogenic fungi causing the disease include *Fonsecaea pedrosoi*, *Phialophora verrucosa*, *Chladophialophora carrionii*, *Fonsecaea compacta*, *F. monophora*, and *Rhinocladiella aquaspersa* (1). Among the reported cases in China, *C. carrionii* was identified as the predominant pathogen in the north, whereas *F. monophora* was the most likely major pathogen in the south (2,3). By analyzing the clinical data of 20 cases of chromoblastomycosis caused by *F. monophora* reported in China, we found that cases were mainly distributed in the Guangxi Province and Yungui region (17 cases, 85%), and rare in the northern part of

China (2 cases, 10%). Patients were aged 36–78 years, with a mean age of 59.68 years, and males accounted for 90% of cases (*Table 1*). This case was the first report in central Jiangsu Province.

In this case, the patient was a retired worker from Nantong, Jiangsu, who had lived locally for a long time and had no history of travel before the onset of the disease or until the present time. Before the onset of the disease, the patient denied a history of trauma, emphasizing the presence of red papules after insect bites, which gradually and slowly increased. Individual reports of chromoblastomycosis caused by mosquito bites have previously been diagnosed and reported in China (4). *F. monophora*, a biphasic fungus, is a saprophytic fungal pathogen that mainly parasitizes decomposing plants and soils, such as rotten wood and dead grass. Once they enter the skin tissue through minor skin injuries, chromoblastomyces convert the hyphae from the rotting vegetation into sclerotic corpuscles (5). After extensive questioning of his medical history, the patient revealed that he enjoyed gardening. The authors speculate that chromoblastomyces present in the soil when the patient was gardening might have entered the skin through a slight mosquito bite wound on his forearm. The initial lesion was a single, slightly itchy, red papule at the forearm bite site, which slowly expanded outwards along the periphery and gradually formed plaques and nodules, with verrucous and proliferative changes on the surface and black dot-like crusts.

Chromoblastomycosis has a long and chronic course and can be teratogenic, disabling, and may even become

**Table 1** The characteristics of 20 cases of chromoblastomycosis caused by *F. monophora*

General condition	Cases (n)	Percent (%)
Gender		
Male	18	90
Female	2	10
Region		
Upper limbs	9	45
Shoulder	1	5
Lower limbs	10	50
Area		
Guangdong	10	50
Guangxi	6	30
Yunnan & Guizhou	1	5
Hebei	2	10
North Jiangsu	1	5

cancerous (6-8). Therefore, timely and definite diagnosis, as well as active and effective treatment, are required. *F. monophora* is a type of chromoblastomycete that is neurotropic, virulent, and causes chromoblastomycosis and phaeohyphomycosis. It can cause infections in multiple organ systems, including the skin and the brain. Previous studies have reported cases of phaeohyphomycosis caused by *F. monophora* (9,10). Hence, when the isolated strain is identified as chromomycosis, it is essential that further molecular biological testing should be performed to determine whether it is *F. monophora* so as to guide clinical treatment and epidemiology. Ajello [1974] and McGinnis [1983] diagnosed phaeohyphomycosis and distinguished it from chromomycosis based on the parasitic histological morphology of phaeohyphal fungal infections in which yeast-like cells, pseudohyphae, or hyphae-like structures form in tissues (11). In our patient, septated sclerotic corpuscles and septated hyphae of varying lengths were found through microscopic examination of the fungi, tan and red-brown sclerotic corpuscles in the dermis were found histopathologically, but no hyphae were found by PAS staining. A diagnosis of chromoblastomycosis caused by *F. monophora* was made based on the combined clinical manifestations and molecular biological findings. Chromoblastomycosis fungal smears rarely show germinating sclerotic corpuscles and hyphae, but they

appeared in this case, which might have been caused by self-application of glucocorticoid ointment to inhibit the local immune reaction at the lesion site (12). Taken together, identifying the specific pathogen of chromoblastomycosis may be crucial for treatment.

The treatment of this disease remains a global challenge. Chromoblastomycetes pathogens form sclerotic corpuscles in the tissue, which often cause hypertrophic scars or fibrosis, making it difficult for topical drugs to penetrate. This disease has no possibility of healing spontaneously. According to statistics, the condition has a recurrence rate of more than 40% (13). At present, standard clinical treatments include surgery, physical therapy, chemotherapy, and combination therapy (14). In our case, the patient was treated with combination therapy. He was given oral itraconazole 0.2 qd, alternating topical bifonazole gel and amorolfine cream, and local heat therapy, requiring a controlled temperature of 40–42 °C to avoid hypothermic burns. A previous study demonstrated that strains could not grow at 40 °C (15). The thermal diffusion effect also promoted the penetration of drugs applied to the surface skin lesions into the deep tissues. Furthermore, the thermal effect increased local blood circulation, facilitated the dissipation of inflammation, and enabled oral antifungal drugs to reach more lesion sites. After 8 weeks of oral medication combined with thermotherapy, the patient's skin lesions shrank significantly, and the high margins gradually flattened. Treatment with oral itraconazole requires a 6–12-month course of treatment (13). Considering the potential development of oral drug side effects in older patients, and after adequate consultation, carbon dioxide fractional laser-assisted transepidermal drug delivery was added at weeks 9 and 13. After 16 weeks of treatment, the lesion's periphery was significantly flattened, and the fungal microscopy was negative. Carbon dioxide fractional laser treatment can increase the penetration of drugs (16). It can also diffuse into skin lesions and surrounding adjacent tissues through the selective photothermal effect or transmit heat to the surrounding area through optical radiation, producing a thermal effect (17) that is not conducive to the growth of pathogenic fungi. The patient failed to receive timely medical treatment over the long-term course of the disease, and a mild atrophic scar had appeared in the center of the skin lesion, which was an important indicator for carbon dioxide fractional laser treatment (17). While increasing the drug penetration, the atrophic scar tissue was also repaired, which improved the patient's quality of life.

**Table 2** The treatment results of 20 cases of chromoblastomycosis caused by *F. monophora*

Therapeutic regimen	Cases (n)	Cured (n)	Better (n)	Uncured (n)	Loss (n)
Systematic drug therapy					
Itraconazole	8		8		
Terbinafine	3	1	1		1
Itraconazole + Terbinafine	5	3	2		
Systematic drugs combined with physical therapy					
Itraconazole + Thermotherapy	2		2		
Terbinafine + Thermotherapy	1		1		
Topical medication					
Compound ketoconazole cream	1			1	
Total	20	4	14	1	1

Analysis based on the clinical data of 20 cases of chromoblastomycosis caused by *F. monophora* reported in China showed that 16 patients received at least one oral antifungal drug, mainly oral itraconazole and/or terbinafine, without other combinations; three patients received oral itraconazole or terbinafine combined with hyperthermia, and one patient was treated with the topical compound ketoconazole alone (Table 2). Furthermore, it is worth pointing out that the current patient is an elderly man, which restricts our treatment options. A high level of itraconazole could be considered if it is a young patient, such as 400 mg/d. Alternatively, combination therapy of itraconazole and terbinafine may optimize drug therapy. In general, the treatment in our study is significantly improved and showed satisfying efficacy for this unusual case.

In addition to the classic azole and acrylamide drugs, some other therapies may be considered for this fungal pathogen in the future. 5-ALA PDT has been proven to be useful for treating the infection caused by *F. monophora* both *in vivo* and *in vitro* (18). The combined therapy of 5-ALA PDT and antifungal drugs should also have great efficacy. For example, Huang used ALA-PDT to cure a chromoblastomycosis patient with leucopenia, suggesting an adaptable method for curing refractory cases of chromoblastomycosis (19). Furthermore, some immunomodulators, such as glucan or imiquimod, may increase antifungal efficacy. For instance, combined therapy of injection of glucan and oral medication of itraconazole cured a patient infected with chromoblastomycosis, who has received the treatment of itraconazole and terbinafine for 3

years but with no significant efficacy (20).

Our patient is the first case treated with oral drugs, thermotherapy, and carbon dioxide fractional laser in China. The clinical trial has proved that the combined treatment is safe and effective, with good patient satisfaction. This is the first case of chromoblastomycosis diagnosed and treated in our department without previous experience in therapy. At the time of submission, the patient continues to attend regular outpatient follow-up every 4 weeks and the lesion on the arm recovers significantly after 20 weeks (Figure 4D).

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## Footnote

*Reporting Checklist:* The authors have completed the CARE reporting checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-21-6119/rc>

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-21-6119/coif>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures

performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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