Scedosporium and *Lomentospora*: an updated overview of underrated opportunists

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Abstract

Species of *Scedosporium* and *Lomentospora* are considered as emerging opportunists, affecting immunosuppressed and otherwise debilitated patients, although classically they are known from causing trauma-associated infections in healthy individuals. Clinical manifestations range from local infection to pulmonary colonization and severe invasive disease, in which mortality rates may be over 80%. These unacceptably high rates are due to the clinical status of patients, diagnostic difficulties, and to intrinsic antifungal resistance of these fungi. In consequence, several consortia have been founded to increase research efforts on these orphan fungi. The current review presents recent findings and summarizes the most relevant points, including the *Scedosporium*/*Lomentospora* taxonomy, environmental distribution, epidemiology, pathology, virulence factors, immunology, diagnostic methods, and therapeutic strategies.

Introduction

Nearly all pathogenic fungi are present in the environment adapted to very different habitats where they play varying roles in recycling of organic matter. With some of their causative agents being either opportunistic or primary pathogens, fungal infections show an increasing incidence worldwide, affecting millions of individuals, with mortality rates that may be higher than 50% in susceptible patient populations.[1]

Among pathogenic fungi, *Scedosporium* species, including *Lomentospora prolificans* (formerly *Scedosporium prolificans*),[2] can cause infections in both immunocompetent and immunocompromised hosts, where they can act as primary or opportunistic pathogens.[3,4] These species cause a broad range of clinical manifestations, from colonization of the respiratory tract, superficial infections and allergic reactions, to severe invasive localized or disseminated mycoses. Patients at risk are particularly those immunocompromised and with hematological malignancies.[3,5]Individuals suffering from near-drowning events in water polluted with fungal propagules are also at risk of infections with central nervous system (CNS) involvement.[5]

Moreover, *Scedosporium*/*Lomentospora* are amongst the most commonly recovered fungi from respiratory secretions of patients suffering from chronic pulmonary conditions such as cystic fibrosis (CF).[6] Although they are mostly asymptomatic colonizers,[7,8] this may be the first step towards pathology. *L. prolificans* typically causes disseminated infections in immunocompromised patients, where it is associated with high mortality.[3,8–11] *Scedosporium boydii* and *S. apiospermum* are the most frequently isolated species, but in some regions *S. aurantiacum* is more common.

The high degrees of intrinsic antifungal resistance make these infections difficult to manage.[12]

The high mortality rates of deep and disseminated infections necessitate focusing resources and efforts to cope with the challenges posed by *Scedosporium* and *Lomentospora* species, such as improving diagnostic methods, or designing new effective therapies.

Therefore, the members of the *Scedosporium* working group of the International Society for Human and Animal Mycology (ISHAM), present at their 5th Workshop in Bilbao in 2016, decided to prepare a detailed review describing the taxonomy, environmental distribution, epidemiology, pathology, virulence factors, immunology, diagnostic methods, and available therapeutic strategies.

Taxonomy, DNA barcoding and new species

The nomenclature of the genus *Scedosporium/Pseudallescheria* has undergone numerous changes over the last decade following the introduction of molecular phylogenetics, which led to an increasing resolution at and below the species level. In addition, the fundamental change in fungal taxonomy allowing only a single name per fungal species, effectively abolishing the dual nomenclature based on the anamorph / teleomorph concept,[13] resulted in the adoption of the name *Scedosporium* at the expense of *Pseudallescheria*.[2]

The first comprehensive revision of the genus conducted in 2005 by Gilgado *et al.*[14] using four genetic loci (β-tubulin (*BT2* (=exon 2-4) and *TUB* (=exon 5-6)), calmodulin and the internal transcribed spacer regions (ITS1/2) of the rDNA gene

cluster) recognized *S. apiospermum* (incl. *P. boydii*) as a species complex, in addition to *S. aurantiacum* and *S. minutisporum.* Within the *S. apiospermum / P. boydii* complex, three existing species were recognized: *P. angusta, P. ellipsoidea* and *P. fusoidea*.[14] A second revision further recognised a new species *S. dehoogii* and maintained *S. apiospermum* and *P. boydii* as distinct species based on *TUB* sequences together with morphological and physiological criteria.[15] A significant genetic diversity within the *S. apiospermum*/*P. boydii* complex was noted in sequence analysis of the D1/D2 region of the LSU of rDNA, ITS1/2 and elongation factor 1-alpha;[16] ITS1/2 and *BT2*[17,18] and the actin, *BT2* and small ribosomal protein 60S L10 (*RP60S*) sequences in combination with AFLP analysis.[19] While the use of some loci, such as *BT2*, show better discriminatory resolution, barcoding of the ITS1/2 regions is sufficient for distinction of all relevant entities in clinical practice.[19] Rainer and Kaltseis (2010) described a new species *S. deficiens*,[20] closely related to *S. dehoogii* based on ITS1/2 and *BT2* corresponding with growth differences on polyvinyl alcohol agar supplemented with diesel and rapeseed oil, and growth at 41°C, but no reference sequences were submitted to any public database, and insufficient proof of novelty was provided. Recently another new species phylogenetically related to *S. aurantiacum* was described, based on ITS, *BT2* and calmodulin, named *S. cereisporum*.[21] In summary, after the One Fungus = One Name movement[22] and sequencing studies, the genus *Scedosporium* now contains the following ten species: *S. aurantiacum, S. minutisporum, S. desertorum, S. cereisporum,* and *S. dehoogii,* in addition to the *S. apiospermum* complex that comprises *S. angustum*, *S. apiospermum*, *S. boydii, S. ellipsoideum* and *S. fusoideum* (**Figure 1**).

A phylogenetic analysis of 104 *TUB* sequences (**Figure 1**), representative of all subgroups found amongst 407 analysed *TUB* sequences, as well as an analysis of the intra-species variation of all ten currently accepted *Scedosporium* species revealed high genetic variation within *S. dehoogii, S. boydii* and *S. apiospermum* (**Figure 2**), indicating that those should be treated as species complexes, and the identified subclades may indicate cryptic species. This was also confirmed by DNA barcoding gap analysis carried out on 538 ITS (**Figure 3 A**) and 407 *TUB* sequences (**Figure 3 B**), showing that there is no barcoding gap within the genus *Scedosporium* if all current ten species are included. The loss of the barcoding gap is due to the high genetic variation found within *S. dehoogii*, *S. boydii* and *S. apiospermum*. However, the description of those subclades as separate species needs further study, including molecular data in association with morphological, physiological and clinical relevant data. There are clear barcoding gaps between *S. minutisporum, S. desertorum, S. aurantiacum* and *S. cereisporum* (**Figure 3 C**) indicating that they are well-defined species. The separation of *S. angustum* and *S. fusoideum* needs to be further investigated taking into account the low genetic diversity within and between those two species, when compared to the genetic variation found in *S. dehoogii*, *S. boydii* and *S. apiospermum* (**Figure 1 and 2**). Finally, *L. prolificans* was shown to be unrelated to *Scedosporium* and therefore was reclassified as *Lomentospora prolificans*[23] and the genus *Lomentospora* was reinstated for this species.[2]

Environmental distribution and epidemiology

Knowledge of the ecological niches of *Scedosporium/Lomentospora* species is essential for a better understanding of the dispersal of these fungi and for the potential identification of a source of an infection.

Ecological aspects

Scedosporium and *Lomentospora* species have been isolated from a wide range of environments, including anthropogenic influenced habitats,[24,25] oil-soaked soils, cattle dung and sewage.[26] In addition, polluted waters have been described as reservoirs specific for these fungi, and these were identified as sources of infection after near-drowning events.[27] However, adjacent agricultural soils were found to be colonized in a greater magnitude than water or sediment, suggesting the former is a main habitat of these fungi.

Subsequent investigations concerning the ecology of *Scedosporium* species confirmed the correlation between their abundance and human impact on environments.[25,28–31] Agricultural areas[30] as well as playgrounds and soils in urban surroundings[25,32] were consistently found to be heavily colonized. *Scedosporium* spp. are described to degrade alkanes[20,26] and therefore it is not surprising that they are responsible for 10% of the fungi found in leachate from soil remediation.[31] The impact of alkanes and elevated temperature on the soil mycobiota was studied in laboratory models. It was shown that the abundance of *Scedosporium* spp. (mainly *S. apiospermum* and *S. dehoogii*) correlates with diesel fuel concentration and elevated temperatures (10% w/v and 25°C were tested respectively). The number of *Aspergillus* and *Penicillium* isolates decreased in the same system [Eggertsberger M, unpublished

180 results]. In this context it should be mentioned that the temperature in urban soils, i. e.
181 in traffic islands can reach more than 30°C even in temperate climates.[33]
182 The occurrence of *Scedosporium* spp. is also influenced by the pH of the
183 substrate, with an optimum of 6-8. Only few colonies were recovered from acidic (like
184 most of the forest soils) or basic (as French seashores) soils. Another slight but positive
185 correlation was postulated by Kaltseis *et al.*[25] concerning fungal density and nitrate
186 concentration in soil. In industrially fertilized crop-fields less *Scedosporium* colonies
187 were isolated than in biologically managed fields without mineral fertilizing regimes
188 [Mall B, unpublished results]. Concerning nitrogen usage, it should be pointed out that
189 *Scedosporium* spp. can use complement compounds of the innate immune system in
190 liquor as nitrogen source.[34] As additional ecophysiological feature which helps to
191 survive in the human host, the siderophore production of *Scedosporium* spp. in slightly
192 acidic substrates could be of interest.[35] Furthermore, *S. apiospermum, S. aurantiacum*
193 and *L. prolificans* were identified by molecular analyses in mesophilic bagasse
194 composts in 3.8%, but it seems to be unclear whether the identification method
195 excluded *S. boydii*.[36]
196 Distribution patterns of the *Scedosporium* species show regional differences.[25,28,30]
197 In Australia, *S. aurantiacum* accounted for more than 50% of all environmental isolates
198 studied, whereas *S. apiospermum* and *S. dehoogii* are predominant in Austria and
199 France, respectively. Ecological preferences were observed e.g. in the abundance of *S.*
200 *dehoogii* in the presence of high levels of human activity.[25,30] For its part, *S.*
201 *aurantiacum* is characteristic of agricultural areas in the west of France.[30]
202

Clinical epidemiology

Species-specific patterns, host risk groups, organ-specific predilection, and *in vitro* antifungal susceptibilities,[8,10,18,37–39] underline that understanding of the epidemiology is essential to clinical management. *Scedosporium apiospermum* and *S. boydii* have a worldwide distribution; by contrast, *L. prolificans* is rarely encountered in environmental samples and appears more commonly in the arid climates of Australia and Spain.[8,9,39,40] More recently, *L. prolificans* has been recognized in other European countries, the USA and Korea.[11,38,41–43] Many *S. aurantiacum* infections have been reported from Australia,[8,39] the Netherlands[44] and Japan.[45] The epidemiological features between the three main groups of pathogens within *Scedosporium and Lomentospora* are summarized in **Table 1**.

Immunocompromised hosts

Solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) patients account for a large proportion of patients at high risk for invasive *Scedosporium/Lomentospora* infections. However, individuals with cancer and other immunodeficiencies are also at risk for these mycoses. For SOT and HSCT patients, the risk of dissemination varies with the type of transplant and immunosuppressive regimen, degree and duration of neutropenia, environmental exposure, and type of antifungal prophylaxis.[8,38,42,46,47] Comparison of infection incidence in these patients across studies is difficult due to the use of different denominators. In a population-based survey, Heath *et al*. [8] reported an incidence of 1/100,000 population, of which two-thirds of cases occurred in SOT patients.

Regarding two studies in the USA series, *Scedosporium/Lomentospora* infections accounted for 25% of all non-*Aspergillus* mold infections in transplant recipients (SOT, 29%; HSCT 71%),[38] while in another study of a HSCT cohort a frequency of 1.11 cases/100,000 patient-inpatient days was reported.[48] In the first report, Husain *et al*.[38] found that disseminated disease occurred more often in HSCT (69%) than in SOT recipients (53%), particularly by *L. prolificans* (39% *vs*. 17%; *p*=0.05), with infections in HSCT recipients having an earlier median onset (1.3 months *vs.* 4 months, p=0.007), being more fungaemic (33 vs 11%, p=0.04), and strongly related to neutropenia (67 *vs.* 9%, p<0.001). Additionally, HSCT recipients were more likely to have received prior antifungal prophylaxis (64% *vs*. 17%) and those that received antifungal prophylaxis tended to have later onset of *Scedosporium/Lomentospora* infections compared to those who did not (median time to onset, 4 *vs*. 2.3 months).[38] The earlier occurrence of disease after HSCT, generally during the pre-engraftment period has been noted.[3,49]

According to this, predictors of invasive disease have included HSCT and leukaemia, with acute leukaemia and *L. prolificans* infection predicting death.[8] Doligalski *et al.*[50] describe *Scedosporium* infections in 3.5% of the patients after lung transplantation, and the three month all-cause mortality was 21.7%. In a single center, 16 out of 27 SOT patients were considered colonized with *Scedosporium,* colonization being relatively common in lung transplant recipients (73%).[42] Invasive disease occurred in 11 patients (41%) with *L. prolificans* and *S. apiospermum* species complex causing 41% and 55% of cases, respectively. The 6-month mortality was 55%, similar to other studies.[8,38] Over two–thirds of patients who developed *Scedosporium* infections

had received immunosuppression with alemtuzumab or anti-thymocyte globulin, which may account for the higher mortality given their profound immunosuppression. Regarding clinical manifestations of *Scedosporium*/*Lomentospora* infections in SOT and HSCT patients, they may range from sinopulmonary disease and brain abscess to disseminated infection and aneurysms, which are often fatal.[51–54]

Infections caused by *Scedosporium*/*Lomentospora* uncommonly occur in patients with hematological malignancy,[43,55,56] advanced HIV infection,[57] and primary immunodeficiency disorders.[58,59] These mycoses have attributable mortality of up to 77% in patients with acute leukaemia.[55] As with HSCT recipients, patients with hematological malignancy are more likely to be neutropenic at the time of diagnosis of *Scedosporium/Lomentospora* infections and to have disseminated disease.[8,49,56] On the other hand, Tammer *et al*.[57] reviewed 22 HIV-infected patients with detection of *Scedosporium* species in clinical specimens; invasive scedosporiosis was proven in 54.5% of patients, among them dissemination occurred in 66.7% with a mortality rate of 75%. Patients with invasive scedosporiosis were more likely to have CD4 cell counts <100/µl. Cases of *Scedosporium*/*Lomentospora* infections in patients with chronic granulomatous disease (CGD) have been described.[58–60] Most of these infections involved the lung or soft tissue although disseminated infection has been reported, with *S. apiospermum* accounting for most of them. Moreover, breakthrough infections have been described in patients who were on long term antifungal treatment or prophylaxis.[59]

Non-immunosuppressed hosts

Scedosporium species are classically known from traumatic infections, leading to arthritis of eumycetoma, and from pulmonary colonization, often in preformed cavities, eventually leading to allergic bronchopulmonary mycosis.

Colonization of lungs of patients with CF by *Scedosporium/Lomentospora* species is well established and the rate ranges between 0 and 21%[61–64], being the second most frequent species after *A. fumigatus.*[7] Species prevalence in these patients varies within the region studied: *S. boydii* was the most frequent species (62%) in a French cohort, followed by *S. apiospermum* (24%), *S. aurantiacum* (10%) and *S. minutisporum* (4%).[65] In a study performed in German CF patients, *S. apiospermum* was the most frequent species (49%) followed by *S. boydii* (29%), *L. prolificans* (12%), *S. aurantiacum* (5%) and *S. minutisporum* (5%).[66] In contrast, *L. prolificans* was the most frequent species isolated in patients with CF in Northern Spain.[67] In Australia, the most frequent species seems to be *S. aurantiacum* followed by *L. prolificans* and *S. apiospermum.*[68] *Scedosporium dehoogii* has rarely been isolated in human infections and up to our knowledge never causing colonization in the airways of CF patients.

Numerous cases of *S. apiospermum* eumycetoma have been described in the literature, mostly affecting the lower limbs. These infections are found worldwide including temperate regions. Case reports on eumycetoma from Europe, US and Brazil were ascribed to *S. apiospermum*/*S. boydii*,[69–72] but mostly identified with classical methods so that it cannot be ascertained whether *S. aurantiacum* or *S. dehoogii* were involved in any of these cases.

A special category is formed by cerebral infection after near-drowning. The etiologic agents are reportedly members of the *S. apiospermum* complex, but most

295 data were published prior to molecular species distinction. Tintelnot *et al*.[73] re-
296 identified 11 isolates and showed that most of the isolates belong to *S. apiospermum*
297 *sensu stricto*, although *S. boydii* and *S. aurantiacum* were also identified. [73,74]
298 Furthermore, *S. aurantiacum* has been reported from a survivor of a tsunami in
299 Japan.[45] To date, *L. prolificans* has not been reported in this clinical context.
300

301 ## Human pathology

302 The patients' immune status and fungal portal of entry seem to play an
303 important role in the clinical course of *Scedosporium / Lomentospora* infections.
304 Patients with fully competent immune systems may be asymptomatically colonized or
305 locally infected. On the other hand, in patients with trauma involving major vessels,
306 with severe injuries in the vicinity of the CNS, or with immune dysfunction, invasive
307 infections are frequently found.
308

309 **Colonization**

310 *Scedosporium* colonization of the airways in patients with CF usually starts
311 during adolescence, becoming chronic in up to 54% of patients having *Scedosporium*
312 positive cultures (unpublished data), with one predominant strain that can be
313 identified over several years.[67,75,76] Bronchial colonization may lead to chronic
314 inflammation or even to life-threatening invasive disease in cases of severe
315 immunosuppression, such as lung transplant or hematological malignancies.[3,5,77,78]
316 Of interest, *Scedosporium* conidia are rarely found in the air[79] so that the exact
317 mechanism leading to airway colonization remains to be ascertained. Moreover, the

318 presence of *Scedosporium/Lomentospora* in respiratory secretions of patients suffering
319 from non-CF bronchiectasis is scant and tends to be associated with pre-existing
320 cavities, leading to eumycetomas and pulmonary fungus balls.[78] ABPA and mucoid
321 *Pseudomonas aeruginosa* colonization are positively correlated with
322 *Scedosporium/Lomentospora* colonization.[80] In this sense, it is worth highlighting that a
323 recent study has shown that *P. aeruginosa* is able to inhibit *S. aurantiacum* and *L.*
324 *prolificans* growth, with this inhibition being associated but not limited to the non-
325 mucoid phenotype of the bacterium.[81]
326 Revealing the epidemiology of human colonization by
327 *Scedosporium/Lomentospora* is further hampered by the fact that they are slow
328 growing molds. Molecular strategies of detection have been proposed,[82,83] revealing
329 rates of colonization higher than those assessed by culture. Unfortunately, there are
330 no molecular techniques commercially available for this purpose, making the general
331 implementation of this approach into the clinical laboratories difficult.
332
333 **Allergic bronchopulmonary mycoses**
334 *Scedosporium,* but not *Lomentospora*, has been linked to clinical cases of
335 allergic bronchopulmonary mycoses (ABPM),[7] with 3% of the ABPM cases reported in
336 the literature being related to *Scedosporium* species. While it is not clear to what
337 extent colonization drives long-term decline of pulmonary function, cases of
338 *Scedosporium*-related ABPM have been linked to a clear respiratory deterioration of
339 patients.[84] The clinical picture of ABPM caused by non-*Aspergillus* species tends to
340 differ from classical allergic bronchopulmonary aspergillosis (ABPA), with asthma being

less frequent and with higher IgE levels. Promising serological methods aimed at the specific detection of antibodies against *Scedosporium* are under development[85] but still not available.

Localized infections

Localized infections by *Scedosporium/Lomentospora* species include different organs and clinical manifestations: 1) cutaneous infections; 2) eumycetoma; 3) muscle, joint and bone infections; and 4) ocular infections.

Cutaneous infections

Skin manifestations may be the initial presentation of a subcutaneous scedosporiosis after traumatic inoculation, or a sign of hematogenous dissemination (**Figure 4 A**). They can mimic those caused by other fungi, such as species of *Aspergillus* or *Fusarium* with ecchymosis, necrotic papules, and hemorrhagic bullae, but they may also present solitary ulcers, infiltrative erythematous plaques and nodules, or suppurative nodules and ulcers. Both *S. apiospermum* and *L. prolificans* have been reported to cause soft tissue infections in immunocompromised hosts, including patients receiving chronic steroid therapy for chronic obstructive pulmonary disease or receiving immunosuppressive therapy for rheumatoid arthritis.[3,86,87]

Eumycetoma

This is a chronic progressive granulomatous infection of the subcutaneous tissue. It may affect muscles, bones, cartilage and joints, most often involving the

lower extremities, usually the foot. Like other subcutaneous mycoses, the fungi enter through a penetrating trauma. The lesion is painless and grows slowly with well-defined margins, remaining localized for long periods. Multiple nodules can appear and spontaneously drain purulent material mixed with soft, < 2 mm size, and white to yellowish, grains resembling fig seeds. Interconnected sinus tracts are usually present by the end of the first year and may close and heal completely, while new ones may open. Involvement of ligaments, joint cartilage, and even bone may occur with time. Eumycetoma can produce profound disability and deformity but constitutional symptoms rarely appear. Clinically and radiologically, eumycetomata caused by *S. apiospermum* species complex or *L. prolificans* are similar to those caused by other fungi.[3,71]

Muscle, joint and bone infections

Wound infections, arthritis and osteomyelitis usually occur when anatomic barriers are ruptured by trauma or surgery. Osteomyelitis is described in lung transplanted recipients[88,89] as a severe complication of immunosuppression. Joint or bone infection by *S. apiospermum* or *L. prolificans* results in acute septic arthritis and acute or subacute osteomyelitis, respectively. Plain radiography may be normal in earlier stages, but magnetic resonance imaging helps to confirm clinical diagnosis. However, the etiological organism cannot be identified without culture or molecular detection from articular fluid or a bone biopsy.[3,90]

Ocular infections

Scedosporium species can cause keratitis among immunocompetent hosts and usually following a corneal trauma. Clinical presentation resembles other types of keratitis (local pain, photophobia, decrease visual acuity, lacrimation) and the cornea examination reveals gray to white lesions with irregular margins and elevated borders, ring infiltrate, hypopyon and keratitic precipitates. Endophthalmitis in immunocompetent individuals may be caused by *S. apiospermum. S. boydii* or *L. prolificans* are secondary to surgery, traumatic inoculation, intravenous drug addiction, and contiguous spread from an adjacent site. However, in immunocompromised patients, endophthalmitis is usually part of disease dissemination, secondary to parenteral nutrition or chemotherapy. Endophthalmitis curses with ocular pain, photophobia and blurred vision, these symptoms not being specific for scedosporiosis. Fundoscopic examination shows creamy-white, well-circumscribed lesions of the choroids and retina, vitreous infiltrates and hypopyon.[3,91,92]

Disseminated Infections

Scedosporium/Lomentospora disseminated infection (SDI) usually takes place in severely immunocompromised hosts, such as patients with cancer and hematological malignancies, hematopoietic stem cells or solid organ transplant recipients, patients with immunodeficiency, and those receiving immunosuppressive therapy.[3,5,50,93–95] It happens following hematogenous spread from lungs, skin or any source of localized infection. Recently, a disseminated infection in three patients after transplantation of a nearly-drowned donor has been reported.[96] As well as in other invasive fungal

409 infections, SDI may result in a wide spectrum of syndromes, depending on the primary
410 focus, patient´s immune status, and time of evolution of the disease.
411
412 ***Central nervous system (CNS) infections***
413 This is a severe manifestation of disseminated infection (**Figure 4 B**). In the
414 literature, neurotropism of *Scedosporium/Lomentospora* is often mentioned. In
415 immunocompromised patients, CNS infection may appear as a manifestation of
416 systemic disease in the absence of a clear spreading focus,[38,51] while in
417 immunocompetent hosts it mostly results from a near-drowning episode with
418 aspiration of conidia from contaminated water and further hematogenous
419 dissemination from lungs.[97,98] CNS infection has been occasionally reported following
420 trauma and iatrogenic procedures, and after contiguous spread from infected
421 paranasal sinuses.[99,100] Clinical manifestations include single or multiple brain
422 abscesses, meningitis and ventriculitis.[98,99]
423
424 ***Endocarditis and other intravascular infections***
425 These uncommon manifestations of disseminated *Scedosporium* infections are
426 associated with high mortality rates. Mycotic aneurysms, especially those involving the
427 aorta and vertebrobasilar circulatory system, have been described in both
428 immunocompromised and immunocompetent hosts.[53] Endocarditis evolves in severely
429 immunocompromised patients and in those enduring risk factors, such a valve
430 replacement or an intravascular or intracavitary device insertion.[92] Twelve cases of *L.*
431 *prolificans* endocarditis were reported in the literature.[101,102] Most patients were

immunocompromised and developed left-side infections with large vegetations and systemic embolism. *S. apiospermum* complex endocarditis has been frequently associated with cardioverter-defibrillators or pacemaker insertion. In this setting, patients often tend to suffer from right-side endocarditis and large artery thromboembolism.[103,104]

Systemic infection

This is the most catastrophic expression of disseminated infection **(Figure 4 C)**, fostered by the ability of *Scedosporium* species to invade blood vessels and to sporulate in tissue. In patients with acute leukemia or with allogeneic hematopoietic stem cell transplant *Scedosporium* produces fatal massive infections in the context of aplasia or severe neutropenia. Many reports of systemic infection due to *L. prolificans* in this group of patients have been published, with a higher incidence in Australia and Spain,[105,106] and nosocomial outbreaks during hospital reconstruction have been also reported.[56,107] Clinical features include fever, dyspnea, lung infiltrates, signs and symptoms of meningoencephalitis, skin lesions and other manifestations resulting from multiple organ involvement. In this setting, *L. prolificans* and *S. apiospermum* complex are isolated from blood cultures in a high percentage of patients.[9,11,38,48,106] In solid organ transplant recipients, systemic infection is favored by immunosuppression in the setting of graft *versus* host disease[51] and previous colonization by *Scedosporium.*[52,108] Other risk groups for developing disseminated infection with multiple organ involvement are HIV patients with CD4 < 50/µl[57] and those receiving immunosuppressive therapy.[109]

Host-pathogen interactions: immune response and fungal virulence factors

The host immune response is a complex network of cellular and molecular mechanisms that can determine patient survival but, on the other hand, fungal cells have also developed strategies to evade immune responses and to overcome stressful conditions encountered inside the host.[110] (see **Figure 5)**.

Host immune response

As the infectious propagules of *Scedosporium/Lomentospora* species are able to invade the host through a range of different sites (including: airways, puncture wounds, etc), the immune responses also vary, with different immune cells and pathways being challenged to clear them.[3] Thus, general barriers as epithelia with the mucociliary system, tissue-resident immune cells, and the secretion of defense molecules play essential roles in the immune response to these infections.[111,112] In these first stages of fungal invasion, recognition of fungal cells is mediated by pattern recognition receptors (PRRs), [113,114] but only dectin-1 and TLRs have been studied and proved to be determinant in the recognition of *Scedosporium* cells.[115–117] Although there are structural and compositional differences among species of the *S. apiospermum* complex, peptidorhamnomannans, rhamnomannans, and α-glucans from the fungal cell wall seem to be relevant pathogen associated molecular patterns.[116,118–120]

After recognition by PRRs, phagocytes, including macrophages, neutrophils, and dendritic cells (DC),[121] and other cells with phagocytic capacity promote fungal death, growth delay or inhibition and recruit polymorphonuclear leukocytes (PMNs) by synthesis of pro-inflammatory cytokines.[122,123] Conidia of *L. prolificans* seem to be phagocytized in a manner comparable to *Aspergillus,* at least by monocyte-derived macrophages,[124] despite the larger size of its conidia.[105] In contrast, germination of *L. prolificans* conidia is inhibited less efficiently than that of *A. fumigatus* conidia.[124]

Although the cytokines locally expressed during *Scedosporium* infection have been poorly studied, IFN-γ and GM-CSF have been described to enhance the activity of phagocytes against *Scedosporium* species.[125–127] It is also known that IL-15 increases IL-8 release from PMNs and enhances PMN-induced hyphal damage and oxidative burst against *L. prolificans.*[128] Additionally, compared to *Aspergillus* species, *L. prolificans* has been shown *in vitro* to induce higher synthesis of TNF-α and IL-6 by human monocytes,[129] in relation with differences in the cell wall composition. In general, these cytokines are important to resist invasive infections by promoting respiratory burst and monocyte and neutrophil migration.[130,131] Some cytokines thus have an immunomodulatory function against *Scedosporium* species. This, together with susceptibility of *Scedosporium/Lomentospora* species to phagocytosis,[124,132,133] may explain their low incidence in the immunocompetent population. In case ingested *Scedosporium/Lomentospora* conidia achieve germination and growth out of the alveolar macrophages, neutrophils and circulating monocytes attracted to the infection site become essential.[124] Although primary macrophages are able to damage hyphae, the major part of this role falls upon neutrophils via degranulation, release of

large amounts of reactive oxygen species (ROS), and formation of neutrophil extracellular traps (NET), which trap fungal cells in a matrix mainly composed by DNA and proteins with antimicrobial activity.[121,124,132,133]

Antigen-presenting cells, mainly DCs, internalize and present potential antigens to T cells, which differentiate into T helper (T_H), T cytotoxic (T_c), or regulatory T cells (T_{reg}), depending on the stimulus and PRR involved.[114] In this way, "innate" is connected with "adaptive" or long-term immunity in which mainly T_H1, T_H2, and T_H17 cells[114,134,135] conform the best known antifungal response, but little is known about their specific role against *Scedosporium/Lomentospora* species. On the other hand, B cells are usually activated through T_H cells to produce antibodies whose role in immunity has long time remained unclear.[136] Many antigenic proteins have been recently identified in *S. boydii*[85,137] and *L. prolificans*,[138–140] and some of the antibodies recognizing them might be protective.[141] Interestingly, *L. prolificans* conidia are more strongly recognized by salivary IgA than hyphae, while sera recognize both forms similarly. This observation is consistent with a fungal airway invasion in which conidia rather than hyphae are inhaled by the host.

Virulence factors

The ability of *Scedosporium/Lomentospora* species to germinate is remarkable, which in the case of *S. boydii* has been described to be enhanced by contact with human cells.[142] *L. prolificans* is capable of conidiation in host tissue, which promotes dissemination and explains the rapid progression of the disease.[143]

Among the specific molecules, some peptidopolysaccharides are immunologically active, able to regulate pathogenesis and host immune response.[144] Of these, peptidorhamnomannan (PRM), which is expressed on both conidia and hyphal cell walls and has been related to fungal adhesion and endocytosis by epithelial cells and macrophages, deserves special attention.[142,145–147] PRM may facilitate colonization, virulence and dissemination by the fungus as consequence of an exacerbation of the infection process that reduces the inflammatory response.[148] Moreover, PRM is recognized by antibodies, which is useful for development of diagnostics.[149] *S. boydii*-derived rhamnomannans require TLR-4 signaling for cytokine release by macrophages, as well as MAPKs phosphorylation and IκBα degradation.[120]

Glucans have widely been reported as ligands for TLRs and activators of the immune response. *S. boydii* surface α-glucan, a glycogen-like polysaccharide consisting of linear 4-linked α-D-Glc*p* residues substituted at position 6 with α-D-Glc*p* branches, is essential to phagocytosis of conidia and induces cytokine secretion by cells of the innate immune system involving TLR2, CD14 and MyD88.[116] β-glucans are used as a diagnostic strategy for several fungal infections, but *Scedosporium* species release low levels of this polysaccharide.[150]

Glucosylceramides (GlcCer) or CMHs are the main neutral glycosphingolipids expressed by almost all fungal species studied so far, including species of the *S. apiospermum* species complex.[151,152] These molecules are associated with fungal growth and differentiation and consequently play a role in the infectivity of fungal cells.[153–155] Structural differences between fungal and mammalian (or plant) CMHs

544 make these molecules potential targets for the development of new antifungal drugs,
545 to be used alone or in conjunction with conventional antifungals.[156]
546 Host invasion-related enzymes are further virulence factors of strategic
547 relevance for *Scedosporium* species.[144] Among these are proteolytic enzymes, which
548 are key components to invade tissues, eliminate defense mechanisms and assist in
549 nutrient acquisition. A serine protease able to degrade fibrinogen was described in *S.*
550 *apiospermum*, which might act as mediator of severe chronic inflammation in patients
551 suffering from cystic fibrosis.[157] Moreover, some metalloproteases with ability to
552 hydrolyze different substrates as IgG, laminin, fibronectin, or mucin have been
553 described in *S. boydii* and *S. apiospermum*.[158–160] *Scedosporium* species are also able to
554 degrade complement system compounds of the innate immune system.[34]
555 Acid and alkaline ecto-phosphatase activities were also in mycelia of *S.*
556 *boydii.*[161] In *Candida* spp. these have been related to adhesion and endocytosis,[162,163]
557 but limited information is available on their relevance to pathogenesis in
558 *Scedosporium*. Enzymes such as Cu/Zn cytosolic superoxide dismutase[164] and a
559 monofunctional catalase[165] from *S. boydii* have been described to be important for
560 evasion of the fungus to the host immune response, the latter being also useful for
561 diagnostic purposes.[85] Two siderophores, dimerumic acid and $N^{(\alpha)}$-methyl coprogen B,
562 were identified In *S. boydii* and the latter was used as a marker of the airway
563 colonization by this species.[35,166]
564 The pigment melanin might contribute to virulence since it is a general
565 protective component UV radiation and other kind of environmental stress.
566 *Lomentospora prolificans* and *S. boydii* produce melanin through the

567 dihydroxynaphthalene (DHN) biosynthetic pathway.[167,168] While melanin plays a
568 protective role in the survival of the opportunist to oxidative killing, it does not
569 contribute to resistance to amphotericin B.[169]
570
571 ## Diagnostics
572 Timely recognition of *Scedosporium/Lomentospora* infections remains
573 challenging, particularly in patients with CF where airway infections still are a major
574 cause of mortality.[170–172] Distinction of colonization from infection can be crucial for
575 adequate patient management. The definition of pulmonary infection in CF includes
576 the following criteria: (1) increased sputum production (2) repeated isolation of the
577 same species from sputum or BAL (≥ 2x in 6 months), (3) pulmonary infiltrate(s) on
578 chest CT-scan or X-ray, (4) treatment failure with antibiotic therapy, (5) unclear lung
579 function decline, (6) exclusion of new/other bacteria (e.g. non tuberculous
580 mycobacteria), and (7) exclusion of ABPA.
581 Diagnosis classically relies on the detection of fungi from clinical samples by
582 direct microscopic examination of the clinical specimen, or histological analysis, and
583 culture on appropriate culture media (**Figure 4 B-D**). Histopathological examination of
584 biopsies can be performed to diagnose these mycoses, e.g. using KOH treatment.
585 Unfortunately, it is difficult to distinguish *Scedosporium*/*Lomentospora*-infected tissues
586 from those infected by *Aspergillus* or *Fusarium*, as all of them present hyaline hyphae
587 (excluding *L. prolificans* that may exhibit highly melanised hyphae), regular hyphal
588 septation, and dichotomous branching. However, several unique features may help
589 pathologists to diagnose *Scedosporium*/*Lomentospora* mycoses, such as irregular

590 branching patterns or intravascular and intratissue conidiation [3,173]

591 For isolation, semi-selective culture media are useful for the detection of
592 *Scedosporium* and *Lomentospora* amidst competing and more rapidly growing
593 microbes, particularly *A. fumigatus*. Sce-Sel+ media,containing dichloran and benomyl,
594 [174] greatly facilitate recovery of *Scedosporium* species (N.B. benomyl inhibits growth of
595 *L. prolificans*) from polymicrobial clinical samples.[68,175,176] Direct detection and
596 identification from clinical samples by molecular-based techniques may also constitute
597 a valuable alternative. In this way, a species-specific multiplex PCR assay has been
598 developed to detect the clinically most important *Scedosporium/Lomentospora* species
599 from respiratory secretions.[177]

600 Morphologically and physiologically *L. prolificans* is easily differentiated from
601 *Scedosporium* species based on its susceptibility to cycloheximide, the black color of its
602 colonies, and its characteristic flask-shaped and annellated conidiogenous cells.
603 However, species distinction within the *S. apiospermum* species complex is often
604 impossible. Growth characteristics and utilization of carbohydrates or enzymatic
605 activities, assist in main species differentiation but are inadequate for separation of
606 lineages within the *S. apiospermum* complex, as demonstrated using the Taxa Profile
607 Micronaut™ (Merlin Diagnostika GmbH, Germany) system, which analyses 570
608 physiological reactions.[178] In *S. aurantiacum*, Biolog Phenotype analysis using GEN III
609 MicroPlate™ (Biolog Inc., USA) containing 94 assorted substrates, reveals metabolic
610 differences between high and low virulence strains, suggesting a link between
611 virulence and ability to utilize D-turanose.[179]

612 Nucleotide sequence-based analysis is the current gold-standard for fungal

identification.[17] rDNA ITS sequencing appropriately identifies the main species in *Scedosporium/Lomentospora*,[180] but the partial β-tubulin gene (*BT2*) is needed to differentiate closely related species. Of note, the status of some species like *S. ellipsoidea,* which is very close to *S. boydii* is still debated (see above).[2] Likewise, reversed line blot hybridization has been successfully applied in sputum samples from patients with CF.[82] Multi-locus sequence typing (MLST) was used to analyze isolates from with patients CF, with three MLST schemes for *S. apiospermum, S. boydii and S. aurantiacum* are now online at http://mlst.mycologylab.org.[76] Recently the analysis of some repetitive DNA sequences using the semi-automated Diversilab™ system from bioMérieux allowed the identification and genotyping within pathogenic *Scedosporium* species.[181]

Matrix-laser desorption/ionization mass spectrometry (MALDI-TOF/MS) has become available for the first-line identification. It is more economical and its identification accuracy is comparable to that of DNA sequencing.[182–185] The quality of the reference spectra is decisive for reliable identification (**Figure 6 A**). The current commercially available MALDI-TOF/MS identification solutions are inadequate for *Scedosporium/Lomentospora* and it would be necessary the development of an online reference MALDI-TOF mass spectra library database, specialized in fungal identification, and curated by expert mycologists.

Among the novel assays is PCR-ElectroSpray Ionization-Time Of Flight/Mass Spectrometry (ESI-TOF/MS), which involves 16 singleplex PCR assays using broad-range primers targeting nuclear or mitochondrial genes, and T2 magnetic resonance (T2MR). PCR-ESI-TOF/MS allows rapid determination of molecular weight and base composition

636 in the amplicons after electrospray ionization and chromatographic separation, and
637 resulting profiles are compared with a database provided by the manufacturer.[186,187188]
638 This technique has been used to determine the distribution of fungal communities
639 directly from bronchoalveolar lavage fluid specimens.[189] T2MR technology rapidly and
640 accurately detects the presence of molecular targets within a sample without the need
641 for purification or extraction,[190,191] but designing primers is challenging.[192]
642 Specific monoclonal antibodies (MAbs) have been developed allowing for
643 species distinction.[167,193] Two MAbs targeting respectively an immunodominant
644 carbohydrate epitope on an extracellular 120-kDa antigen present in the spore and
645 hyphal cell walls of *S. apiospermum* and *S. boydii* or the tetrahydroxynaphtalene
646 reductase of the dihydroxynaphtalene-melanin pathway in *L. prolificans*, may be used
647 in immunofluorescence assay to differentiate these fungi from other septate fungal
648 pathogens on histological sections.
649 Recently some *Scedosporium* proteins, including a monofunctional cytosolic
650 catalase, proved to be interesting markers of a *Scedosporium* infection, and works are
651 currently being performed in order to develop standardized serological tests.[2885]
652 In addition to proteomic approaches with MALDI-TOF or LC-MS/MS
653 identification of *Scedosporium/Lomentospora* ribosomal equipment,[139,182] mass
654 spectrometry can be used in metabolomics to gain access to specific low-molecular
655 weight biomarkers. Melanin and its degradation products represent the first target in
656 *L. prolificans*. Diverse lipids were also detected on intact spores of *L. prolificans* and *S.*
657 *apiospermum.*[194] The metabolite AS-183 was detected in fermentation broth of
658 *Scedosporium* spp. SPC-15549.[195]

Siderophores have gained attention as disease biomarkers as well as virulence factors.[196,197] Two siderophore representatives have been rigorously described in *Scedosporium* genus, dimerumic acid and N^{α}-methylcoprogen B,[35] the former possibly being a degradation product of the latter. Siderophores may occur in various ionic forms in mass spectra. Generally, they are observed as ferri- or desferri-forms, but combinations with sodium or potassium ions are possible depending on the sample type.[197] For example, in host tissue the generation of $[M+Na]^+$, $[M+K]^+$, $[M+Fe-2H]^+$ or $[M+Fe+Na-3H]^+$ ions is quite common. Recently a new dereplication tool called Cyclobranch has been developed for the re-discovery of above described compounds.[198] It is based on an integrated library of hundreds of microbial siderophores and secondary metabolites including toxins and non-ribosomal peptides. Dereplication (the process of classifying already known compounds) can be performed on conventional mass spectra generated by any ionization technique as well as on liquid chromatography/mass spectrometry or imaging mass spectrometry datasets. These data formats are batch-processed and incorporation of important biometals (including iron) can be supported in calculations and data presentations. An example of a siderophore annotated in a sample of *S. boydii* by matrix-assisted laser desorption/ionization with Fourier transform ion cyclotron resonance (MALDI-FTICR) mass spectrum is illustrated in **Figure 6 B**. It is worth mentioning that Cyclobranch is a free tool (available at http://ms.biomed.cas.cz/cyclobranch/) dedicated to exact mass data. In addition to dereplication, the *de novo* sequencing of new microbial structures is also possible. The calculator works with approximately 520 non-isobaric building blocks arising from ribosomal, non-ribosomal or polyketide syntheses making the

682 characterization of new siderophores[198] or cyclic, branched, or branched cyclic
683 peptides[199] feasible.
684
685 ## Therapeutic strategies
686 Treatment of deep-seated *Scedosporium* or *Lomentospora* infections still
687 remains challenging because of the limited susceptibility of these fungi to all current
688 antifungal drugs. *Scedosporium* species are resistant to 5-flucytosine and amphotericin
689 B, as well as to the first generation triazole drugs, fluconazole and itraconazole. In
690 addition, they have a reduced susceptibility to echinocandins, particularly caspofungin
691 and anidulafungin, and exhibit resistance to the most recent triazole drug,
692 isavuconazole, *S. aurantiacum* being the least susceptible to antifungal drugs.[12,66,200]
693 Likewise, *L. prolificans* is a pan-antifungal resistant species.[3,12,201] In this connection, it
694 is also relevant to highlight that the available antifungal spectrum is quite limited, and
695 as such more efforts need to focus on the development of novel effective drugs.[202,203]
696 For treatment of *Scedosporium/Lomentospora* infections, the European
697 guidelines recommend voriconazole as first-line treatment[200] together with surgical
698 debridement when possible. Although favorable results have been observed following
699 such recommendations, the outcome remains poor with mortality rates of > 65% and
700 nearly 100% when CNS affectation or dissemination occurs.[204,205] A minimum inhibitory
701 concentration (MIC) of less than 2 µg/ml could be predictive of a favorable outcome
702 for *Scedosporium* species.[206] Despite the differences on *in vitro* susceptibility among
703 genera, the outcome remains similar especially when dissemination occurs. For this

reason, it is of crucial interest to find therapeutic alternatives for these challenging and difficult-to-treat infections.

Antifungal combination therapy has emerged as a promising strategy since therapeutic effect can be achieved at lower concentrations and thus reducing toxic side effects, improving safety and tolerability, shortening the therapeutic effect and preventing treatment failure when antimicrobial resistance is suspected. Few studies have evaluated the *in vitro* activity of double combinations against *Scedosporium* spp. and *L. prolificans*. Among them, combined voriconazole and amphotericin B or echinocandins have shown synergistic effects against both *S. apiospermum* and *L. prolificans,*[207–209] [Martin-Vicente *et al.* unpublished results] as well as terbinafine plus itraconazole, miconazole or voriconazole against *L. prolificans.*[3,210,211] However, the combination of voriconazole plus terbinafine or liposomal amphotericin B has demonstrated variable outcome in the treatment of these infections.[212–221] Limited data are available on combinations of more than two antifungals. Two triple combinations (amphotericin B plus voriconazole plus anidulafungin or micafungin) have been tested against *L. prolificans* and showed synergy[222] [Martin-Vicente *et al.* unpublished results].

The *in vitro* activity of combinations of antifungals with miltefosine, antipsychotic drugs or cysteine derivatives is being investigated as a potential treatment alternative.[223–225] It is also highlighting the capacity of inhibitors of Heat shock proteins, calcineurin and deacetylases against fungal species.[226–232] However, their effect on *Scedosporium/Lomentospora* species should be further researched.

Murine studies have also shown promising results for combinations of antifungals with granulocyte-colony stimulating factor,[233–235] and clinical experience suggests that reversion of neutropenia is a key factor in the outcome of a fungal infection.[218,236]

Reviewing recent clinical cases reported in the literature, four CF patients treated with antifungal drugs because of a suspected pulmonary *Scedosporium/Lomentospora* infection have been reported since 2013.[237,80,238,239] Moreover, in Germany 36 cases of antifungal treatment of *Scedosporium/Lomentospora* infections in patients with CF were analyzed [Schwarz C *et al.* unpublished results]. In 20/36 antifungal courses a therapeutic response was achieved (regress in radiology or symptoms, or increase in FEV1). These results demonstrated a significant superiority of the use of a combination of three drugs *versus* two and two drugs *versus* one drug. Among the antifungal drugs, voriconazole remains the first therapeutic choice,[200] potentially combined with an echinocandin for *Scedosporium* infections or with terbinafine for *Lomentospora* infections.

Prospects in susceptibility to antifungals and resistance mechanisms

Among the drugs that are currently in the pipelines, one might be promising for treatment of *Scedosporium/Lomentospora* infections. The Japanese company Eisai Co. discovered E1210, a new first-in-class broad spectrum antifungal drug acting *in vitro* against clinically important yeasts and molds,[240] and *in vivo* in experimental models of candidiasis, aspergillosis, and fusariosis[241]. This drug targets the inositol acylation step in the biosynthesis pathway of the glycosyl phosphatidyl inositol (GPI) anchor. GPI-

749 anchored cell wall proteins play a key role in fungal biology and virulence, and
750 blockage of this metabolic pathway results in defects in cell wall biosynthesis, hyphal
751 elongation and adherence of fungal cells to biological substrates. *In vitro* susceptibility
752 testing using a large set of *S. apiospermum* (n=28), *S. aurantiacum* (n=7) and *L.*
753 *prolificans* (n=28) isolates revealed that MICs using E1210 were at least 10 fold lower
754 than found in currently used drugs, including voriconazole.[242] This compound, which is
755 licensed since 2015 by Amplyx (San Diego, USA – APX001) was approved on June 2016
756 by the FDA for treatment of candidiasis, invasive aspergillosis and coccidioidomycosis.
757 Mutations in the "hot spot" regions of the *Fks1* gene, encoding the catalytic
758 subunit of the β-1,3-glucan synthase (the target of echinocandins), have been
759 described which may explain the reduced susceptibility of *Scedosporium* species and *L.*
760 *prolificans* to echinocandins[243]. The low *in vitro* susceptibility (or primary resistance) of
761 *Scedosporium/Lomentospora* species to azole drugs may result from resistance
762 mechanisms similar to those extensively studied for *A. fumigatus*[244–248] such as point
763 mutations in the coding sequence of *CYP51A* orthologues leading to a reduced affinity
764 of azole drugs for their target, or constitutive overexpression of some efflux pumps.
765 Specifically *L. prolificans* showed alterations in of shorter and wider hyphae and
766 structural and compositional changes in the CW, possibly mediating *L. prolificans*
767 resistance to VRC.[249]
768
769
770 **Future trends in antifungal drugs**

There are nowadays some very promising novel antifungal compounds, such as F901318 [Chen S, unpublished results] and *N*-chlorotaurine (NCT). The F901318 compound represents a novel class of antifungal drug that inhibits dihydroorotate dehydrogenase, a key enzyme in pyrimidine biosynthesis [250]. The compound has been recently investigated for 50 clinical *Scedosporium* and *Lomentospora* isolates [Biswas et al. *In vitro* susceptibility testing of the novel orotomide antifungal agent F901318 against Australian *Scedosporium* and *Lomentospora* pathogens, ECCMID, Viena, Austria, 22-25 April 2017, P1704] and it was active against all isolates of *L. prolificans* as well as *S. apiospermum*, *S. boydii* and *S. aurantiacum*, with MICs falling ranging from 0.125-0.5 mg/L. Similar results have been found in another study [Alastruey-Izquierdo *et al.* unpublished data] testing 123 clinical isolates of *S. apiospermum*, *S. boydii*, *S. aurantiacum*, *S. dehoogii*, *S. ellipsoideus* and *L. prolificans* with MIC range for all isolates of 0.007-0.5, and by Wiederhold and co-workers against *S. apiospermum*, *S. aurantiacum*, *S. dehoogii*, *S. boydii*, and *L. prolificans*, with MIC raging from ≤0.008 to 0.25, with the last species being the most resistant ones. [251]

The *N*-chloro derivative of the amino acid taurine is a long-lived oxidant generated by activated granulocytes and monocytes during inflammation and oxidative burst in phagolysosomes.[252] Moreover, it is more stable and much less toxic *in vivo* than HOCl.[253]

In the 90's, the chemical synthesis of NCT as a crystalline sodium salt (Cl-HN-CH_2-CH_2-SO_3Na) could be established, demonstrating broad-spectrum killing activity against microbes. [254] [255] Due to its unspecific mechanism of action, development of resistance is extremely improbable. Three key features of NCT contribute to its

794 successful clinical application: (1) transhalogenation:[256] which makes the net
795 microbicidal activity of NCT markedly enhanced *in vivo*, above all against fungi. (2)
796 chlorine cover:[257] which avoids regrowth (postantifungal effect) and induces loss of
797 virulence. (3) inactivation of virulence factors of pathogens.[256]
798 Clinical phase I and II studies demonstrated very good tolerability of topical 1%
799 (55 mM) NCT in aqueous solution for skin ulcers, conjunctivitis, external otitis, and oral
800 infections. [255] Recently, inhaled 1% NCT was well tolerated in pigs, mice, and humans
801 (pilot tests and a phase I study), respectively.[258–260]
802 At this concentration, NCT was able to kill all *Scedosporium* species tested, *i.e.*
803 both hyphae and conidia of *S. apiospermum*, *S. boydii*, and *L. prolificans*, within several
804 hours at pH 7.1 and 37°C.[261] As expected, addition of ammonium chloride (NH_4Cl)
805 reduced the killing times to approximately 5 min because of transhalogenation.
806 Indeed, LIVE/DEAD staining of conidia disclosed increased permeability of the cell
807 membrane and wall, which is decisive for killing. However, short, sublethal incubation
808 times of 10-60 min in plain NCT significantly increased germination time and decreased
809 germination rate of conidia. Moreover, such sublethally treated conidia lost their
810 virulence *in vivo* after injection into larvae of *G. mellonella*, so that the larvae survived
811 similar to mock-injected controls.[261]
812 A second study was done to investigate NCT on its microbicidal activity *in vitro*
813 in artificial sputum medium (ASM) mimicking the composition of cystic fibrosis mucus
814 at 37°C and pH 6.9.[262] Under these conditions, 1% NCT killed bacteria and spores
815 already within 10 min and 15 min, respectively, to the detection limit of 10^2 CFU/ml
816 (reduction by 5-6 $\log_{10}$). A reduction by 2 $\log_{10}$ was still achieved by 0.1% (bacteria) and

817 0.3% (fungi) NCT largely within 10-30 min. This markedly more rapid killing (particularly
818 of fungi) in ASM compared to phosphate buffer can be explained by transhalogenation.
819

820 ## Summary and conclusions

821 In this review, the state-of-the-art of the emerging opportunistic fungal
822 pathogens *Scedosporium/Lomentospora* is discussed, mainly focusing on the scientific
823 knowledge acquired in the last decade. Summarizing, in taxonomy the genus
824 *Lomentospora* is clearly independent from *Scedosporium*, which currently contains ten
825 species. These fungi are found in environments of high human activity, polluted waters
826 and soils/composts, whilst their prevalence varies with geography, environmental pH
827 and chemical content, especially aliphatic hydrocarbons. They infect
828 immunosuppressed and immunocompetent individuals where near-drowning events
829 pose a special risk. Furthermore, colonization of the respiratory tract is common in
830 patients with chronic lung diseases such as CF.
831 The main virulence factors described are PRM and other cell-wall
832 peptidopolysaccharides, proteolytic enzymes, superoxide dismutase, catalase,
833 siderophores and melanin. The immune status of the patient seems vital to control
834 infections, being TLRs and Dectin-1 crucial for fungal recognition and phagocytosis.
835 Specific response, including humoral, might also be of importance. The difficulty to
836 detect and identify these fungi from non-sterile samples results in the fact that the real
837 epidemiology remains to be undetermined, warranting future efforts on the
838 improvement of conventional methods, molecular tools, detection of serological
839 markers and secondary metabolites. A rapid and specific detection of the etiologic

agent remains to be very important for the initiation of appropriate treatment. Regarding therapy, although several new strategies are being tested with promising results, nowadays a combination of two or even three anti-fungal drugs is recommended. Amongst the future perspectives, in addition to immunotherapy, NCT deserves to be mentioned because its broad-spectrum microbicidal activity, tolerability, and anti-inflammatory properties.

In conclusion, although great advances in *Scedosporium/Lomentospora* have been made, much remains to be ascertained, including 1) the identification of definitive markers for the definition of species in *Scedosporium* that allow a better knowledge of its distribution and impact in human pathology, 2) a deeper understanding of its survival strategies and interaction with hosts, 3) the development of faster, accurate and easy-to-implement clinical tools for diagnosis, and 4) the finding of *in vivo* active compounds to treat the wide range of infections, many of the life-threatening, caused by these fungi.

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Conflict of interest

None

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