INTRODUCTION

Leishmaniasis is a tropical disease caused by a protozoan of the genus *Leishmania*, order *Kinetoplastida*, family *Trypanosomatidae*. It is a vector born disease and the protozoan is an obligate intracellular parasite. Its clinical manifestations may be ulcers which are self-healing or severe systemic multi-organ disease [1-2]. It manifests as cutaneous leishmaniasis, visceral leishmaniasis (also known as kala-azar) or mucocutaneous leishmaniasis [1-2]. Cutaneous leishmaniasis is the most common form and each year, about 0.7 to 1.2 million of cutaneous leishmaniasis cases occur mainly in three geographical distributions: the Americas, the Mediterranean basin and West Asia from the Middle East to Central Asia. The following countries have the highest number of the cases: Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica and Peru [3].

Cutaneous leishmaniasis (Fig 1) is caused by various *Leishmania* species. According to geographical distribution, it is divided into: Old World cutaneous leishmaniasis which is caused by *L. aethiopica*, *L. donovani*, *L. infantum*, *L. major* and *L. tropica*. It occurs in South Europe, the Middle East, parts of Southwest Asia, Central Asia and Africa, and New World cutaneous leishmaniasis which is caused by multiple species of both the *Leishmania* subgenera e.g. *L. amazonensis*, *L. infantum*, *L. mexicana*, *L. venezuelensis* and the *Viannia* subgenera e.g. *L. braziliensis*, *L. guyanensis*, *L. pana-
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Alsohaimi A.

LABORATORY DIAGNOSIS OF CUTANEOUS LEISHMANIASIS

Diagnosis of cutaneous leishmaniasis depends on the clinical symptoms and laboratory investigations. A lot of diagnostic methods were described with a large range of variation in the specificity and sensitivity including direct parasitological examination (microscopy, histopathology, and parasite culture) and indirect testing using serology and molecular diagnostics [4].

Direct microscopy and histopathology

The corner stone in diagnosis of leishmaniasis is parasitologic identification through histopathological examination of parasite fixed from suspected lesions and/or in vitro culture. Microscopical diagnosis is done by the direct identification of amastigotes in Giemsa-stained lesion smears of biopsies, scrapings, or impression smears. Amastigotes appear as are small spherical non-flagellated cells ranging from 2-4 µm in diameter with characteristic nuclei and kinetoplasts. The nucleus and kinetoplast are surrounded by small ring of vacuolated cytoplasm and the cells are among the smallest nucleated cells known. A simplified and rapid diagnostic method is the press-imprint-smear (PIS). According to a recent study that compared PIS with histopathology, PIS was positive in 85.3% of cases while histopathology was only positive in 44% of cases. Accordingly, PIS is a sensitive and rapid method for the diagnosis of cutaneous leishmaniasis [5].

Immunologic methods

Serologic tests for cutaneous leishmaniasis diagnosis depend on detection of the specific immune response against the parasite. Multiple immunologic tests are currently in use including indirect fluorescent antibody assay, enzyme-linked immunosorbent assay (ELISA), western blot, lateral flow assay or direct agglutination test. Unfortunately, these methods are relatively of limited value for the diagnosis of cutaneous leishmaniasis because of the poor humoral immune response of the host tissue to the infection and consequently the low sensitivity [4,6].

Leishmanin skin test

The Leishmanin skin test (LST, or Montenegro skin test “MST”) is a good marker for cellular immune response, so it
is widely used in epidemiologic surveys and vaccine studies as it is simple and relatively sensitive test [7]. In most LST preparations currently in use, cultured promastigotes are washed in 0.5% phenol saline, diluted to $1 \times 10^6$/ml, and 0.1 ml is injected intradermally. The injection site is examined 48 hours later and induration of $\geq 5$ mm is considered a positive test. A positive LST denotes present or past infection with *Leishmania*. The LST is generally positive when the lesions of cutaneous and mucosal leishmaniasis are present, but negative in active visceral leishmaniasis. Patients who are positive by other tests but negative with LST are more liable to have failure of treatment or relapse [7-8]. The main disadvantage of this test is that it needs culture facilities to produce the MST antigen which might impact test sensitivity, and that the test does not distinguish between past and present infections [9]. it has been reported that the LST is more sensitive in persons with old cutaneous leishmaniasis [10-11].

### Nucleic acid amplification methods

A lot of molecular tests which are diagnostic were developed to help the diagnosis of cutaneous leishmaniasis, as these are more sensitive and specific than traditional diagnostic methods and they also use for diagnosis less invasive sampling [12-13]. Over the past years, a lot of Polymerase Chain Reactions (PCR) targeting different gene sequences were developed, with the ribosomal DNA internal transcribed spacer 1 sequence [14–16], or sequences within the kinetoplast DNA of *Leishmania* genus as the main targets [17-18]. In disease-endemic countries, PCR needs infrastructure and technically skilled operators which make these tests less suitable for resource-restricted laboratories. Recently isothermal diagnostic platforms have been designed to polarize the parasite RNA through a potentially isothermal reaction to leishmaniasis [19]. At the same time, as the chromatographic analysis of the post-inflation analysis resulting from the use of complex equipment, maintenance is carried out on the appropriate diagnostic characteristics [20-21]. It is essential for rapid diagnosis at the lowest cost of *Leishmania* discovery that non-invasive sampling is done. However, a significant difference in the clinical delicacy of molecular diagnostic s for cutaneous leishmaniasis, relying on the DNA retrieval process, the molecular testing, and sample origin [22].

### LEISHMANIASIS TREATMENT

Most cases of cutaneous leishmaniasis heal without treatment and require no specific therapy, however, treatment is required in large and/or persistent lesions (Table 1). Traditional treatment of leishmaniasis depends on the use of relatively toxic and less tolerant drugs. The pentavalent antimony compounds such as antimoniate meglumine (glucantime) and sodium stibogluconate (pentostam) remain the basis for treatment of leishmaniasis over decades. It is thought to work by inhibition of adenosine triphosphate synthesis. During the last 15 years, generalized resistance to these agents has been developed due to injudicious use in India, where most cases of global visceral leishmaniasis occur [7]. Moreover, they require weeks of intravenous administration and are frequently associated with anorexia, malaise, myalgia and

<table>
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<th>Species determination</th>
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As in most parasitic diseases, the challenge stays in having the competence to perform a rapid diagnosis of the disease and precise characterization of the genotyping of different species isolated from infected lesions. Hitherto, the available techniques currently employed to characterize and distinguish *Leishmania* species are Multi Locus Enzyme Electrophoresis (MLEE) and multiple methods based on Polymerase Chain Reaction (PCR) such as Multilocus Sequence Typing (MLST), PCR-Restriction Fragment Length Polymorphism (PCR-RFLP), Multiplex PCR and PCR followed by sequencing [23-24]. These methods usually utilize microsatellites sequences as amplification targets such as kinetoplastid DNA (kDNA), telomeric sequences, internal transcribed spacer (ITS1), heat shock proteins (e.g. HSP70 and HSP60), and genes involved in metabolic reactions such as mannose phosphate isomerase (MPI) and 6-phosphogluconate dehydrogenase [24].

### Table 1. Conditions in which treatment of cutaneous leishmaniasis is required

<table>
<thead>
<tr>
<th>Condition</th>
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<tr>
<td>Large lesions or cosmetically unaccepted</td>
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<td>Persistent lesions</td>
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<tr>
<td>Severe or aggressive course</td>
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<tr>
<td>Association with mucocutaneous leishmaniasis</td>
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<tr>
<td>Lesions over joints</td>
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<tr>
<td>Infection in immunocompromised patient</td>
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arthralgia, cardiac arrhythmia, prolonged QT interval, deranged liver enzymes, and pancreatic damage. The topical treatments often necessitate repetitive applications, display little activity, and are usually associated with significant discomfort because of itching or severe skin irritation. Reports state that after 20 days of treatment with pentavalent antimonials, there is usually evidence of healing, but lesions may not be re-epithelialized completely. Healing is evidenced by a normal skin appearance at two months, no relapse at 12 months, and no consequent mucosal disease. Mucosal disease requires a longer treatment plan and is more difficult to cure [25].

Other drugs that can be used in the treatment plan include amphotericin B and pentamidine [25-26]. In addition, effective vaccines for the treatment of leishmaniasis have not yet been available although trials are ongoing [27-28]. Recently, few alternative drugs have emerged for the treatment of leishmaniasis (Table 2). However, none of these medications has proved satisfactory, and a few of the touted agents have been assessed adequately in clinical trials. The limited efficacy is associated with wide margin of toxicity which is further augmented by the long duration of treatment that impacts the patient’s compliance [29-30]. The structure of some important antileishmanial drugs is shown in Fig 2.

Fig 2. Structure of some drugs used for Leishmania treatment.
New treatment guidelines

Recently, several European guidelines have been proposed for a new treatment approach of cutaneous leishmaniasis [31] with the help of molecular techniques available for species identification this approach is made possible [32]. Globally, the overall geographic area experience has been able to devise initial treatment, as approaches of the Old World and the New World of treatment. The guidelines of WHO 2010 were interested in endemic countries with cutaneous leishmaniasis and presented treatment options for Old World cutaneous leishmaniasis (L. major and L. tropica), and also for New World cutaneous leishmaniasis providing new specific preferences for treatment of a few selected species of *Leishmania* [33]. The European leishmaniasis treatment guidelines used the Oxford Evidence Rating System and categorized treatment options for *L. major, L. tropica, L. panamensis, L. guyanensis, L. braziliensis,* and *L. mexicana,* etc. They recommended about the severity of infection (e.g., risk of mucosal spread, size, and number oral location of lesions) and also recommended observation and topical therapy that used for simple cutaneous leishmaniasis, and systemic treatment (oral or parenteral) for complicated cutaneous leishmaniasis [34]. Another guidelines of treatment of cutaneous leishmaniasis, mucosal leishmaniasis, and visceral leishmaniasis which are species-directed, differ from this approach by using an expert panel to rank treatment options that were based on the efficacy and practical manners [32]. This analysis reached a 50% lost to follow-up studies of cutaneous leishmaniasis and the authors found that 37% of the analyses were dependent only on observational studies. They divided the treatment according to clinical categories, 13 Old World cutaneous leishmaniasis and 12 New World cutaneous leishmaniasis categories. Consultation from the French Leishmaniasis Reference Center was applied to this algorithm in 135 patients who were confirmed parasitologically with cutaneous leishmaniasis [35].

Newly introduced medications

Liposomal amphotericin B

Amphotericin B was isolated from *Streptomyces nodosus* in 1955 and came into medical use in 1958. It is on the World Health Organization’s List of Essential Medicines, the most effective and safe medicines needed in a health system for treatment of systemic fungal infections and leishmaniasis [36]. Amphotericin administration is limited by infusion-related toxicity. This is thought to result from innate immune production of proinflammatory cytokines [37]. The amphipathic nature of amphotericin B along with its low solubility and poor permeability has posed major barriers for oral administration due to its low bioavailability. However, a new lipid-associated formulation, liposomal amphotericin B (commonly known as “AmBisome”) has been introduced to the original amphotericin B to avoid these drawbacks. This new formulation has better bioavailability, less infusion toxicity and less nephrotoxicity than the original amphotericin B (Fig 3). It is used intravenously 3–10 mg/kg/day for 5–7 days. The lower dosage and shorter duration regimen has the same effect of the higher dosages and longer duration of treatment [38]. Other forms of amphotericin B include amphotericin B colloidal dispersion (ABCD) and amphotericin B lipid complex (ABLC). These forms were primarily used for treatment of invasive fungal infections. Compared to amphotericin B, they are more effective and less toxic. Studies about the use of these formulations in leishmaniasis treatment are still inadequate. Another novel topical treatment reported was the use of intralesional amphotericin B as a second-line treatment of antimonial resistant Old World cutaneous leishmaniasis [39]. Ninety three patients received amphotericin B 2 mg/ml injected into lesions weekly for up
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to 12 weeks. Adverse events were 'low and insignificant'. By 12 weeks, 61% of patients with cutaneous leishmaniasis had healed completely (>90% reduction in size and induration) and 22% more had a partial remission (60–90% reduction). Although pentavalent antimonials are frequently injected intralesionally, this study describes a similar method using amphotericin B which one might think would be very sclerotic/reactogenic based on the phlebitis that it may cause.

**Pentamidine structural analogs**

The pentamidine structural analogs are alkylphosphocholines used as anticancer treatment. These compounds act as membrane synthetic ether-lipid analogs and consist of alkyl chains in the lipid portions. The most promising of these are miltefosine (hexadecyl phosphocholine), eldofisine [ET-18-OCH (3)], ilmofosine (BM41.440) and perofisine [40]. Miltefosine was first made in the early 1980s and studied as a treatment for cancer. A few years later it was found to be useful for leishmaniasis and was approved for this use in 2002 in India [41]. It is now it is used in all types of leishmaniasis. Miltefosine primarily acts on *Leishmania* by affecting the species’s promastigote and amastigote stages through interacting with lipids and inhibition of cytochrome c oxidase. This may affect membrane integrity and mitochondrial function of the parasite causing apoptosis-like pattern of cell death [42]. It is administrated orally in a dose of 100–150 mg/day for 28 days. Common side effects of miltefosine include nausea, vomiting, dizziness, headache and somnolence, occurring in 60% of individuals. Serious side effects include rash, diarrhea, and arthritis. These side effects are especially

<p>| Table 2. Summary of some important antileishmanial drugs |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Route of administration</th>
<th>Dose</th>
<th>Common side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentavalent antimonials</td>
<td>Converted inside the parasite into the active form that inhibit adenosine triphosphate synthesis</td>
<td>IM, IV, or IL</td>
<td>20 mg/kg/day (21–28 days)</td>
<td>Myalgia, arthralgia, electrocardiographic abnormalities, liver dysfunction, pancreatic enzymes elevation</td>
</tr>
<tr>
<td>e.g. sodium stibogluconate</td>
<td></td>
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<tr>
<td>Amphotericin B/Liposomal amphotericin B</td>
<td>Macrocyclic, polyene antifungal agent, it is thought to act by binding to ergosterol, the principal sterol in fungal cell membranes and Leishmania cells</td>
<td>IV or topical</td>
<td>10 mg/kg/day</td>
<td>Nausea, vomiting, chills, fever, hypotension, arrhythmia, nephrotoxicity, liver toxicity, electrolyte disturbances</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>Decrease DNA and RNA synthesis</td>
<td>IM</td>
<td>3 mg/kg/day (every other day for 4 injections)</td>
<td>Dyspnea, chest pain, hypotension, cardiac arrhythmia, neuralgia, nephrotoxicity, elevation of liver enzymes, bone marrow suppression</td>
</tr>
<tr>
<td>Miltefosine</td>
<td>Interaction with lipids, inhibition of cytochrome c oxidase leading to apoptosis-like cell death</td>
<td>Oral</td>
<td>100–150 mg/day (28 days)</td>
<td>Nausea and vomiting (60%), dizziness, headache, diarrhea, arthritis</td>
</tr>
<tr>
<td>Paromomycin</td>
<td>Aminoglycoside antibiotic inhibiting protein synthesis</td>
<td>IM or topical</td>
<td>15 mg/day (20 days) or 20 mg/kg (17 days)</td>
<td>Nausea, vomiting, abdominal cramps, nephrotoxicity, ototoxicity, neuromuscular blocking</td>
</tr>
</tbody>
</table>
more severe in women and young children. The overall effects are quite mild and well-tolerated [42-43].

Based on the proven efficacy of pentamidine and related compounds against protozoal infections, 18 structural analogs of pentamidine were evaluated for in vitro anti-leishmanial activity of L. major and L. tropica. Pentamidine was the standard reference drug used for comparison. Results showed that the reversed amidine compounds were more active than the furan analogs against both Leishmania species. DB 745 and DB 746 demonstrated the highest activity against L. major, while DB 745 was more effective against L. tropica. Both compounds, however, exhibited 50% inhibitory concentrations below 1 nM for L. major. Amidine analogs were also tested. Of the 10 reversed amidine compounds, nine showed inhibitory growth effect on amastigote axenic model at a concentration below 1 nM. These studies show that dicaticionic compounds are potential new agents with less toxicity than the parent drug for the treatment of cutaneous leishmaniasis, but further studies are needed [44]. Other pentamidine analogs tested for activity against Leishmania organisms are parfuramidine, efornithine, perifosine, edelfosine, ilmosforsine and sitamaquine [45-46]. Both edelfosine and perifosine demonstrated in vivo activity against L. amazonensis as shown in a study in which edelfosine and perifosine were given to BALB/c mice in oral doses of 1 and 2.5 mg/kg/day during 28 days and 5 mg/kg/day during 14 days, starting at two weeks after the first treatment scheme [45]. An assay comparing miltefosine at the standard dose of 20 mg/kg/day during 28 days to in vivo treatment with perifosine at a dose of 5 mg/kg/day for 14 days demonstrated higher in vivo activity of perifosine than miltefosine against L. amazonensis [44]. These findings show promise for a new treatment group in cutaneous leishmaniasis caused by L. amazonensis. Another drug, sitamaquine (WR 6026), an 8-aminoquinoline has also shown promise as an effective oral agent in a dose of 1 mg/kg/day for 2 weeks for visceral leishmaniasis [46].

Intralesional pentavalent antimonials (ILPA) is an alternative to systemic pentavalent antimonials (SPA). In a recent study aimed to compare the cost of ILPA and SPA, and to estimate the health and economic impacts of changing the first-line treatment for cutaneous leishmaniasis in a Bolivian endemic area, the authors concluded that treating cutaneous leishmaniasis using ILPA was associated with a cost-saving and allowed two-and-a-half times the current number of patients to be treated [47].

Imiquimod

Imiquimod is a patient-applied cream that acts as an immune response modifier and is licensed for treatment of genital warts, superficial basal cell carcinoma, and actinic keratosis [48]. Imiquimod has been recently used topically for treatment of resistant cutaneous leishmaniasis in a cream form [49]. The drug acts by stimulation of the innate immune system by activating toll-like receptor 7 (TLR7), commonly involved in pathogen recognition. Cells activated by imiquimod via TLR-7 secrete cytokines primarily interferon-α, interleukin-6, and tumor necrosis factor-α [50]. There is evidence that when imiquimod applied topically to skin, can lead to the activation of Langerhans cells, which subsequently migrate to local lymph nodes and activate the adaptive immune response [48,51].

Tamoxifen

This is a selective estrogen receptor modulator that has been used in the treatment and prevention of breast cancer for several years. In a recent study in which BALB/c mice infected with L. braziliensis, tamoxifen was administered at a dose of 20 mg/kg intraperitoneal daily for 15 days. This resulted in a decrease in lesion size and ulcer formation, a sustained reduction in the number of parasites and extreme susceptibility [52]. Similar results were achieved testing the efficacy of tamoxifen in L. amazonensis-infected BALB/c mice and in L. chagasi-infected hamsters [53-54].

Photodynamic therapy

Photodynamic therapy (PDT) is a form of phototherapy that utilizes light and a photosensitizing compound, used in conjunction with molecular oxygen to elicit cell death (phototoxicity). PDT has proven efficacy in killing microorganisms, including bacteria, fungi and viruses as well as a variety of skin conditions. It is a porphyrin precursor, aminolevulinic acid (ALA) or methyl-aminolevulinate (MAL) that applied topically followed by irradiation with laser or intense pulsed light (IPL). In the last years, PDT was utilized in a lot of neoplasia of the skin. Nowadays, this therapeutic modality was also used in non-neoplastic conditions such
as cutaneous leishmaniasis [55]. A proof-of-concept study of 31 patients self-administering daylight-activated PDT in cutaneous leishmaniasis (L. major and L. tropica) showed healing in 70% 67. After curettage of the crust over lesions, a thick layer of the photosensitizer aminolevulinate was applied to the lesions weekly, covered with a dressing and foil for 30 min, then the dressing removed, and the patient was asked to expose the area to daylight for 2.5 h. In those patients with multiple lesions, a control lesion was not initially treated and none of these healed spontaneously during the 8–10 weeks treatment session [56].

**Combination treatments**

Monotherapy of cutaneous leishmaniasis with any antileishmanial drug remains very challenging as it harbors the drawbacks of inadequate cure rates, increased toxicities of a single drug, prolonged duration of treatment, and the emergence of drug resistance. During the past few years, the use of combination therapy has obtained much attention as a possible strategy for overcoming the various limitations in the present arsenal. An ideal combination therapy of antileishmanial drugs must have good tolerability, compatibility, and potential immunomodulatory roles to ensure adequate therapeutic effect, shorten the treatment duration, and prevent drug resistance. Several combinations have been tried in India and East Africa. For example, a shorter therapeutic plan of combined sodium stibogluconate (SSG) and paromomycin has been tried in Africa and shown to be as effective as a longer plan of SSG alone [57]. The efficacy of apigenin and miltefosine combination therapy against experimental cutaneous leishmaniasis was recently investigated in vitro and in vivo by Emiliano and Almeida-Amaral [58]. Combination therapy using low doses of these two drugs resulted in good clinical and parasitological responses. The combination of tamoxifen and amphotericin B was also assayed in vivo in L. amazonensis-infected BALB/c mice and was found to yield at least additive effects at low doses of both drugs [52]. The study was repeated with combination of tamoxifen and miltefosine with similar results [53]. Additionally, combined treatment of miltefosine and paromomycin has shown to delay the onset of drug resistance in rodents infected with L. infantum [59]. In another study in Peru, they compared the use of imiquimod alone and using it with parenteral meglumine antimoniate for treatment of cutaneous leishmaniasis. In seven patients who treated with topical imiquimod cream (7.5%) with intravenous meglumine antimoniate (20 mg/kg/day) for 20 days showed a cure rate of 100%. By comparing these results with seven patients receiving parenteral meglumine antimoniate alone the result was 57% [60]. These results proved that the effect of the combined therapy in initial treatment of cutaneous leishmaniasis was better.

**Conclusion**

Leishmaniasis is a disease caused by an intracellular protozoan parasite transmitted by the bite of a female phlebotomine sandfly. The clinical spectrum of leishmaniasis ranges from a self-resolving cutaneous ulcer to a mutilating mucocutaneous disease and even to a lethal systemic illness. Cutaneous leishmaniasis often heal without rigorous treatment; however, intensive treatment is required in large and persistent lesions, or lesions over the joints or in immunocompromized patients. Therapy has long been a challenge in the more severe forms of the disease, and it is made more difficult by the emergence of drug resistance. Treatment is tailored to the individual because leishmaniasis is caused by many species or subspecies of Leishmania. Pharmacologic therapies include the following: (1) Pentavalent antimony (sodium stibogluconate or meglumine antimoniate): Still the standard therapy in spite of large toxicity profile; (2) Liposomal amphotericin B (AmBisome): Effective against pentavalent antimony-resistant mucocutaneous disease and visceral leishmaniasis; (3) Oral miltefosine: Approved by the FDA for visceral leishmaniasis due to L. donovani; cutaneous leishmaniasis due to L. braziliensis, L. guyanensis, and L. panamensis; and mucosal leishmaniasis due to L. braziliensis; (4) Intramuscular pentamidine: Effective against visceral leishmaniasis but associated with pancreatic damage and disease relapse; (5) Topical paromomycin: Displayed to be effective against cutaneous leishmaniasis caused by L. major and L. Mexicana; (6) Local therapies for some forms of cutaneous leishmaniasis including photodynamic therapy and cryotherapy.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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