Curr Infect Dis Rep (2014) 16:396
DOI 10.1007/s11908-014-0396-y

Treatment of Giardiasis: Current Status and Future Directions

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Published online: 5 February 2014

Abstract Giardiasis is a common yet neglected cause of diarrheal illness worldwide. Antimicrobial therapy is usually but not always effective and drug resistance has become an increasing concern. Several promising drug candidates have been recently identified that can overcome antibiotic resistance in *Giardia*. These include derivatives of 5-nitroimidazoles and benzimidazoles, as well as hybrid compounds created from combinations of different antigiardial drugs. High-throughput screening of large compound libraries has been a productive strategy for identifying antigiardial activity in drugs already approved for other indications, e.g. auranofin. This article reviews the current treatment of giardiasis, mechanisms of resistance, advances in drug and vaccine development, and directions for further research on this significant human pathogen.

Keywords Giardiasis · Antibiotic resistance · Antibiotic development · Vaccines

Introduction

Giardia lamblia, a flagellated, enteric protozoan parasite, is a common etiologic agent of diarrheal illness worldwide. It was first discovered in the 1600s by van Leeuwenhoek, who found it in his own stool. The parasite exists in two forms, the infectious cyst and the disease-producing trophozoite. Cysts are spread through untreated drinking water, food, and person-to-person contact. Infection occurs after oral ingestion of as few as ten cysts. After passing through the stomach, trophozoites emerge from the cysts and colonize the upper small intestine. An incubation period of 1 to 2 weeks follows cyst ingestion before symptoms occur. Frequent clinical manifestations of giardiasis include diarrhea, abdominal cramps, bloating, nausea and vomiting. The acute phase often lasts for about a week, although some patients can have persistent symptoms for several weeks to months. Most infections are self-limited but recurrences are common in endemic areas. Rarely, chronic giardiasis develops that leads to weight loss and malabsorption, and may be mistaken for inflammatory bowel disease or anorexia nervosa [1]. Notably, a recent cohort study from Norway found a high prevalence of irritable bowel syndrome and chronic fatigue 3 years after a major giardiasis outbreak [2].

Several classes of antimicrobial drugs are available for the treatment of giardiasis (Table 1). Among the most commonly used are members of the nitroimidazole family such as metronidazole and tinidazole. However, first-line therapy fails in up to 20 % of cases and cross-resistance between different agents can occur [3–5]. Alternative agents exist for treatment failures and for special circumstances (e.g. pregnancy), but these are generally less effective than nitroimidazole drugs [6]. Therefore, because of the prevalence of giardiasis and limited treatment options, the development of new agents is a high priority. This review focuses on recent advances in the development of new drugs for giardiasis, the identification of potential pharmacologic targets in the parasite, and suggests directions for further research.

Currently Available Drugs for Giardiasis

As previously mentioned, in clinical practice the most frequently used class of agents for giardiasis are the 5-nitroimidazoles (5-NIs), of which metronidazole is the most

This article is part of the Topical Collection on *Intra-abdominal Infections*, *Hepatitis*, *and Gastroenteritis*

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Table 1 Antimicrobial agents for giardiasis

Compound class	Examples	Mechanism of action
5-Nitroimidazoles	Metronidazole, tinidazole	Adduction and protein/DNA inactivation
5-Nitrothiazoles	Nitazoxanide	Adduction and protein/DNA inactivation
5-Nitrofurans	Furazolidone	Adduction and protein/DNA inactivation
Acridins	Quinacrine	DNA intercalation, inhibition of DNA synthesis
Benzimidazoles	Albendazole, mebendazole	Tubulin binding, interference with cytoskeleton
Quinolines	Chloroquine	Unknown
Aminoglycosides	Paromomycin	Unknown; possibly inhibition of protein synthesis

commonly used drug worldwide. It is typically given in three divided oral doses of 250 mg daily for 5 – 10 days and has a reported efficacy of 80 – 95 %. More recently, tinidazole, first approved by the Food and Drug Administration (FDA) in 2004, has become the drug of choice in the US for giardiasis because of its efficacy (about 90 %), tolerability, and convenience (a single oral dose is recommended). All 5-NI drugs are prodrugs whose microbial specificity is due to the strict requirement for reduction to toxic free radical intermediates by low redox potential reactions present only in the anaerobic target microbes [7]. *Giardia* metabolism is fermentative and electron transport proceeds in the absence of mitochondrial oxidative phosphorylation. However, the parasite is microaerotolerant and can reduce molecular oxygen and thus protect the highly oxygen-sensitive, central metabolic enzyme, pyruvate:ferredoxin oxidoreductase (PFOR), and iron-containing ferredoxins. PFOR decarboxylates pyruvate and donates electrons to ferredoxin, which in turn reduces other components in the electron transport chain and leads to ATP generation (Fig. 1). Reduced ferredoxin can also reduce the critical nitro group of 5-NI prodrugs to toxic radicals which kill the parasite. Other reduction pathways, including nitroreductases and thioredoxin reductase, also reduce 5-NI drugs in *Giardia* (Fig. 1), although their relative importance in activating metronidazole and other nitro drugs is not clear. The radicals that result from nitro drug reduction form covalently bonded adducts on microbial target molecules, leading to their inactivation. The specific molecular targets of 5-NI drugs have not been defined in *Giardia*.

Nitazoxanide is a nitrothiazole with broad-spectrum activity against intestinal parasites and certain bacterial pathogens like *Clostridium difficile*. Like 5-NI drugs, the prodrug must be reduced to form toxic radicals, which inactivate various microbial target molecules including PFORs [8]. In a clinical trial involving children with diarrheal illness, nitazoxanide reduced symptom duration in those afflicted with giardiasis as well as in those in whom no definitive enteropathogen was identified [9]. The drug is usually given for a 3-day course and the most common side effect is gastrointestinal upset.

Furazolidone is a nitrofuran that has been used to treat a number of bacterial and protozoan infections for over 60 years [10]. It is reduced to nitro radicals by NADH oxidase and probably other reductive pathways and likely damages target cellular structures, including DNA. It can cause mild hemolysis in patients deficient in glucose-6-phosphate dehydrogenase and is no longer manufactured in the US.

Benzimidazoles, such as albendazole and mebendazole, are used to treat intestinal helminth infections. Albendazole can be as effective as metronidazole in treating giardiasis [11, 12], although its efficacy varies markedly (25 – 90 %) depending on the dosing regimen. Albendazole can be taken once daily for 5 days, making it more convenient than three times a day metronidazole, and its antihelminth activity makes it an attractive agent for dual use purposes [13••]. However, a Bolivian cohort study (a region with endemic *Giardia* and helminth infections) found that treatment with mebendazole reduced hookworm infections but paradoxically led to an increase in *Giardia* infections [14]. This suggests that an antagonistic relationship exists between the two parasites.

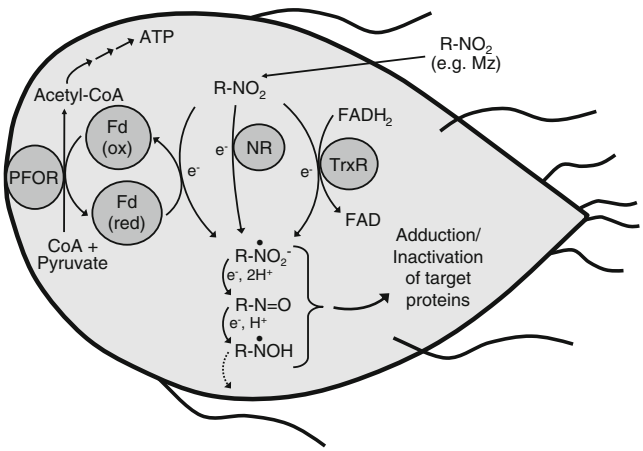

Fig. 1 Nitro drug activation in *Giardia*. Nitro prodrugs (*R-NO$_2$*) such as metronidazole (*Mz*) diffuse into *Giardia* trophozoites and are reduced by one of several pathways to toxic radicals. At least three pathways have been described to date: (1) pyruvate-ferredoxin oxidoreductase (PFOR) normally converts pyruvate to acetyl CoA for ultimate ATP generation, but the reduced electron carrier, ferredoxin (*Fd*) can also donate electrons to nitro prodrugs; (2) nitroreductase (*NR*) can reduce nitro prodrugs, although the cofactor requirements are not known; (3) thioredoxin reductase (*TrxR*) reduces nitro prodrugs with reduced form of the riboflavin, *FADH$_2$*, as the cofactor. Each of these pathways has been implicated in metronidazole resistance, but their relative importance for clinical drug resistance is not known

Quinacrine is an acridine derivative with excellent efficacy against giardiasis (90 %). Its usage has been limited by toxicities such as yellow discoloration of skin and sclera, and exfoliative dermatitis. A recent report describes the experience of a family of four with giardiasis, three of whom failed therapy with tinidazole but were cured with quinacrine [15]. The investigators diagnosed *Giardia* infection through PCR testing which is not currently a standardized method. Chloroquine is an old antimalaria drug with antigiardial activity that is no longer available in the US or Canada. In a randomized trial in children with giardiasis, chloroquine was as effective as tinidazole and superior to albendazole [16]. Side effects were mild and similar in all three groups. Another pediatric trial showed a slightly higher cure rate with chloroquine than with metronidazole, although the difference was not significant [17].

Finally, paromomycin is an aminoglycoside with antigiardial activity. However, it has been shown to be less effective among adults with metronidazole-refractory disease than other therapies and is rarely used in clinical practice [18].

Therapeutic strategies for treatment-refractory giardiasis include longer duration and/or higher doses of the original agent, switching to a different class of drug, or combination therapy. In a case series of ten patients who failed nitroimidazole therapy, all were cured with the following combinations: metronidazole or tinidazole+paromomycin+albendazole in three patients, metronidazole+paromomycin in two, tinidazole+paromomycin in two, tinidazole+quinacrine in two, and metronidazole+quinacrine in one [19]. All of the drugs were administered for 7 or 10 days except tinidazole, which was given for 1 to 7 days. The combinations were well tolerated and there were no serious side effects. A patient with HIV infection and giardiasis was unsuccessfully treated five times with metronidazole and albendazole and was cured with nitazoxanide 500 mg twice daily for 10 days and then 1 g twice daily for 15 days [5]. Susceptibility testing showed the strain to be resistant to metronidazole and albendazole and susceptible to nitazoxanide. The use of combination therapy may decrease the risk of developing drug resistance, although this hypothesis has not been tested in comparative clinical studies.

Mechanisms of Antibiotic Resistance in *Giardia*

The discovery of antibiotics revolutionized medicine and represents a turning point in human history. Unfortunately, enthusiasm for these miracle drugs was soon tempered by the emergence of resistant strains. As in bacteria, protozoa such as *Giardia* have developed numerous resistance mechanisms that render widely used agents ineffective. While still uncommon, antibiotic resistance is increasing in *Giardia* (especially to 5-NI drugs) and can contribute to treatment failures [20]. Elucidating these complex mechanisms is an active area of investigation and researchers have gained some important insights over the last decade. Recent studies indicate that drug resistance in *Giardia* is caused by one of several different cellular adaptations. Of these, the best described mechanism is the loss of the parasite's ability to activate nitro prodrugs to toxic radicals by reduction, thus effectively allowing the parasite to avoid suicidal drug activation. For example, suppression of PFOR leads to metronidazole resistance [21] and certain strains of metronidazole-resistant *Giardia* have reduced levels of this critical enzyme required for ferredoxin-dependent drug reduction [22]. Other mechanisms of drug activation have recently been identified and they may also be affected in resistant cells [23]. Another mechanism of drug resistance to both metronidazole and nitazoxanide is altered expression of genes involved in stress responses, including heat-shock proteins and expression of nitazoxanide-binding proteins [24]. Fortunately, metronidazole-resistant giardiasis is infrequently seen in clinical practice, perhaps because of the detrimental effects of metronidazole resistance on attachment and infectivity of the organism [4]. Resistance to other antigiardial drugs also occurs. For example, resistance to benzimidazoles is believed to occur through mutations in parasitic β-tubulins, specifically the amino acid at position 198 [25]. These findings highlight the challenges facing investigators in their efforts to expand the current therapeutic armamentarium for giardiasis.

Novel Antigiardial Therapeutics in Existing Drug Classes

Metronidazole is composed of a nitroimidazole core with simple hydroxyethyl and methyl side-chains in the 1' and 2' positions, respectively (Fig. 2). Modifications of the core have been a productive strategy for developing new agents. For example, substitution of side-chains in the 1', 2' or 4' position of the imidazole ring with larger and more complex functional groups has led to compounds with enhanced (up to 500-fold) antigiardial activity including some that overcome metronidazole resistance [26, 27] (Fig. 2). The underlying mechanisms are uncertain but may be related to differential drug activation by one or more reductases [28, 29]. Two synthetic metronidazole analogs have been shown to be more active than the parent compound in changing the morphology and ultrastructure of *Giardia* by affecting its cell vesicle trafficking, autophagy, and differentiation into cysts [30]. Moreover, the analogs demonstrate excellent antigiardial activity and low toxicity. A modification of nitazoxanide has also been reported, wherein a benzene ring was placed between the nitro group and the thiazole ring to create a benzologue [31]. The compound is five times more active than nitazoxanide and 18-times more active than metronidazole against *Giardia* and shows low toxicity in mammalian cells. Collectively, these studies

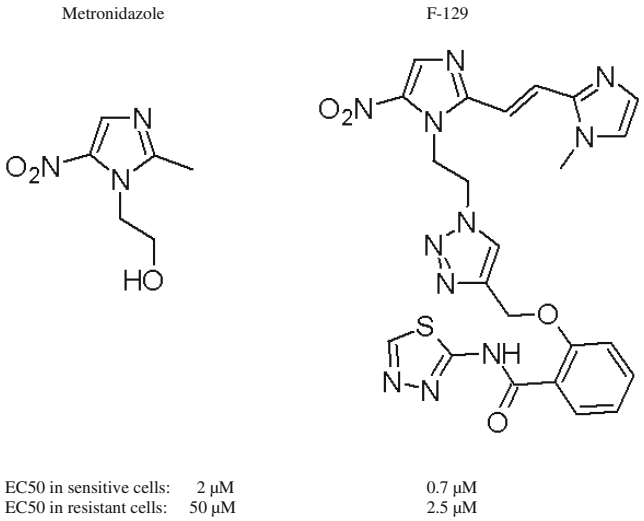

Fig. 2 Structure of nitro drugs. The core structure of nitro prodrugs is an imidazole ring substituted with a nitro functional group in the 5' position of the ring. Metronidazole has a hydroxethyl group in the 1' position and a methyl group in the 2' position. The new nitro compound, F-129, has complex functional groups in these positions [32]. The in vitro activities of the two nitro compounds are shown as EC50 values, the concentrations that inhibit growth and survival of *G. lamblia* trophozoites by 50 % in a 48-h growth assay. Sensitive cells show normal susceptibility to metronidazole, while resistant cells are 25-fold less susceptible to metronidazole. F-129 is more potent than metronidazole in sensitive cells, and only minimally affected by resistance

demonstrate that systematic structural modifications of existing nitro drugs is likely to yield vastly improved antimicrobials that can serve as "next-generation" drugs for the treatment of giardiasis and other infections with clinically important anaerobic pathogens [32••].

Similar modification strategies have been tried with benzimidazoles. A number of novel derivatives have been created, including those with modifications at the second position of the pyrimidine ring and substitution of 1,2-phenylendiamine, resulting in compounds with antigiardial activity equal to or exceeding that of metronidazole [33, 34]. Using reverse-phase high-performance chromatography, investigators found the antigiardial activity of certain benzimidazole derivatives to be influenced by lipophilicity, hydrogen bond donors and molecular volume but not by their apparent permeability in cell monolayers [35]. An analysis of the structure–activity relationships of 32 benzimidazoles showed a complex activity landscape with shallow cliffs for *Giardia* and that substitution at position 2 on the benzimidazole moiety plays a crucial role in increasing potency against the parasite [36]. These findings and those with 5-NI derivatives offer hope that quantitative models may help identify structural properties associated with enhanced antigiardial activity in future drug design.

Another method of drug design is the creation of hybrid compounds that combine features of different active molecules. In one study, ten novel hybrids from benzimidazole and pentamidine were found to be three and nine times more potent against *Giardia* than metronidazole and pentamidine, respectively [37]. Using a short synthetic route, another group of investigators synthesized quinoline tripartite hybrids from chloroquine, ethambutol, and isoxyl drugs, one of which was 670 times more active than metronidazole against the parasite [38]. Although data on possible toxicities of these agents are lacking, hybrid compounds between and within classes of antigiardial drugs and other antimicrobials appear promising for further drug development.

New Classes of Antigiardial Drugs

High-throughput screening of drug libraries has resulted in a number of novel lead compounds with activity against *Giardia*. In particular, screening of libraries of FDA-approved drugs has the distinct advantage that preclinical safety testing has already been done, which can accelerate progression to clinical trials of efficacy. For example, investigators recently screened 746 compounds currently available for human use and found that auranofin, which is used to treat rheumatoid arthritis, inhibits the growth and survival of several different *Giardia* isolates [39•]. Of note, auranofin completely overcomes metronidazole resistance. The mechanism of action was determined to be the inhibition of the enzyme thioredoxin-glutathione reductase, which is present in *Giardia* and several other parasites including *Schistosoma mansoni*. Given that its pharmacokinetic properties and toxicology are well established, auranofin may be a promising new candidate for the treatment of drug-resistant giardiasis, and future clinical trials are eagerly anticipated. Another enzyme of interest is carbamate kinase (glCK) which is essential for *Giardia* metabolism and has no equivalent in humans, making it an attractive target for drug development. A luminescent enzyme-coupled assay measuring the activity of glCK was useful in high-throughput screening of over 4,000 compounds for antigiardial activity [40]. Compounds with activity in this assay will be good starting points for the development of new drugs against *Giardia*.

Plants and plant extracts have historically been rich sources of new antimicrobial agents. One of these is the naturally occurring weed *Oxalis corniculata*, long used in India for treating dysentery and diarrhea. Systematic fractionation of extracts was used to isolate a novel galacto-glycerolipid compound with activity comparable to that of metronidazole [41]. Moreover, no toxicity was observed in human cells exposed to the compound. Another example is extract of sandalwood, *Osyris alba*, which is used by traditional healers in Jordan to treat dysentery and diarrhea. Phytochemical investigation identified a new pyrrolizidine alkaloid, osyrisine, which has significant activity against *Giardia* and is nontoxic to human cells [42]. In Iranian traditional medicine, the leaves, fruits and

rhizomes of the plant *Sambucus ebulus* are used to treat a number of conditions including diarrhea. In a recent study, extracts from the fruit of *S. ebulus* were shown to kill *Giardia* cysts in a concentration-dependent manner [43], although the effect on the disease-causing trophozoite form of the parasite was not examined.

Probiotics are used to restore the gut microbiota following gastrointestinal illnesses, particularly diarrhea caused by *C. difficile* after antibiotic use [44]. A previous study showed that resistance to *Giardia* infection in mouse models is affected by the composition of the intestinal microbiota [45]. More recently, a murine infection model was used to test the effect of the probiotic *Lactobacillus casei* on the treatment of giardiasis with several antigiardial drugs [46]. Of the drugs tested, albendazole proved to be the most potent and combined with *L. casei* further reduced symptoms and restored normal gut morphology. These findings suggest that combining probiotics with standard antigiardial therapy may be beneficial. Thus, future studies in humans seem warranted.

Recent reports of drugs with newly discovered antigiardial activity have raised hope for additional safe and effective therapies. One of these is the obesity drug orlistat which acts by inhibiting pancreatic lipases. It has also been shown to inhibit the growth of cancer cells in vitro and malaria parasites [47, 48]. Notably, orlistat is very poorly absorbed and remains highly active in the intestinal lumen until excreted in the stool. *Giardia* trophozoites treated with orlistat show a potent and dose-dependent inhibition of parasite replication compared to nontreated trophozoites [49•]. Furthermore, the half-maximal inhibitory concentration (IC50) of orlistat was significantly lower than that of metronidazole. The authors posited that the antiparasitic effect of the drug was due to the direct inhibition of giardial lipid-metabolizing enzymes (i.e., lipases) and indirectly caused by limiting the supply of lipids by inhibiting host enzymes. An additive inhibitory effect was observed with metronidazole, suggesting this combination may be a potential therapeutic option in patients and deserves further clinical evaluation especially in recalcitrant disease. Some disadvantages of orlistat include the malabsorption of lipophilic drugs and cost issues. Another drug that has been found to be active against *Giardia* is the antileishmanial agent miltefosine [50•]. The oral administration of miltefosine once daily for 3 days led to the complete elimination of *Giardia* from the intestine of infected mice and ameliorated all intestinal pathology. Moreover, electron microscopy studies revealed that the drug induced severe morphologic changes in the cell membrane and adhesive disc of *Giardia* trophozoites. As with orlistat, additional trials to ascertain the effectiveness of miltefosine in humans with giardiasis are needed.

Identifying molecular targets that differentiate *Giardia* from human cells followed by the design of specific inhibitors is another promising area for drug design. One target of interest has been the metabolic enzyme arginine deiminase (ADI), which catalyzes the first step in the major pathway for generating ATP in the parasite [51]. Furthermore, ADI may also serve as a virulence factor by depleting arginine in the host, which may enable *Giardia* to evade the host immune response [52]. Arginine depletion by ADI in dendritic cells was shown to significantly increase the production of tumor necrosis factor alpha and to decrease interleukin-10 and interleukin-12p40 secretion [53]. Knock-down of ADI by RNA interference results in nonviable trophozoites, indicating that ADI is important for trophozoite survival [54]. Humans lack an analogous enzyme to ADI, making it an attractive target for drug development. Other potential targets include cyclin-dependent kinase from *G. lamblia* [55], triosephosphate isomerase of *G. lamblia* [56], fructose-1,6-biphosphate aldolase [57], and enzymes unique to the *Giardia* glycolysis pathway [58].

Challenges in *Giardia* Vaccine Development

A human vaccine for giardiasis is not available. A crude veterinary vaccine (GiardiaVax), composed of total cell lysates of a mixture of sheep, dog and human isolates, reduces symptoms and duration of cyst output in cats and dogs [59]. Interestingly, the vaccine has also been used as an immunotherapeutic agent in dogs with chronic giardiasis that had failed standard drug treatment, raising the intriguing possibility that a *Giardia* vaccine may be effective after exposure [60]. Given the potential importance of a vaccine against one of the most common parasitic diseases of the intestinal tract, the development of new vaccination strategies continues to be an important research goal. In a murine model of *Giardia* infection, investigators constructed a live vaccine using recombinant attenuated *Salmonella enterica* Serovar Typhimurium expressing α1-giardin, a conserved antigen found in human and murine giardiasis [61•]. Oral administration of the vaccine induced antigen-specific IgG and mucosal IgA, and conferred significant protection against subsequent *Giardia* challenge. Moreover, additional analysis of α1-giardin found high amino acid sequence conservation and immunologic cross-reactivity against multiple *Giardia* isolates. These results support the continued development of α1-giardin as a candidate antigen for a vaccine against giardiasis.

One of the ways *Giardia* evades the immune system is by switching different cell surface molecules in a process of antigenic variation. Major components of trophozoite surface proteins in *Giardia* are members of the family of variable surface proteins (VSPs). Some 200 different VSP genes are present in the *G. lamblia* genome, but only one is expressed per trophozoite. *Giardia* undergoes antigenic variation by continuously exchanging VSP proteins, yet their biologic functions are poorly understood. Immunization with extracts

from *Giardia* engineered to express all VSP antigens by shutting down the machinery that controls antigen switching could be an effective way of preventing infection caused by the parasite [62]. Further research is needed to test this hypothesis under different conditions and develop feasible vaccination strategies that rely on such "super-valent" vaccines.

Conclusions

Giardiasis continues to cause significant diarrheal illness worldwide, especially in developing countries. Currently available drugs are not always effective and antibiotic resistance occurs. Recent advances in drug development have identified several potential agents with enhanced activity against *Giardia* and the ability to overcome resistance mechanisms. Moreover, screening large compound libraries by high-throughput technologies appears to be a lucrative strategy for yielding novel classes of antigiardial drugs, although the mechanisms of action of these agents need to be elucidated. Finally, identifying new microbial targets for both antigiardial drug design and vaccines remains in the preliminary stages and further investigation is needed.

Acknowledgments Lars Eckmann is supported by NIH grants AI075527, DK035108, and DK080506. The authors report no conflicts of interest.

Compliance with Ethics Guidelines

Conflict of Interest Lars Eckmann has received grant money from the NIH. Richard Watkins has no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

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- • Of importance
- •• Of major importance

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