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R E V I E W

Field-Deployable Treatments For Leishmaniasis: Intrinsic Challenges, Recent Developments and Next Steps

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Abstract: Leishmaniasis is a neglected tropical disease endemic primarily to low- and middle-income countries, for which there has been inadequate development of affordable, safe, and efficacious therapies. Clinical manifestations of leishmaniasis range from self-healing skin lesions to lethal visceral infection with chances of relapse. Although treatments are available, secondary effects limit their use outside the clinic and negatively impact the quality of life of patients in endemic areas. Other non-medicinal treatments, such as thermotherapies, are limited to use in patients with cutaneous leishmaniasis but not with visceral infection. Recent studies shed light to mechanisms through which *Leishmania* can persist by hiding in cellular safe havens, even after chemotherapies. This review focuses on exploring the cellular niches that *Leishmania* parasites may be leveraging to persist within the host. Also, the cellular, metabolic, and molecular implications of *Leishmania* infection and how those could be targeted for therapeutic purposes are discussed. Other therapies, such as those developed against cancer or for manipulation of the ferroptosis pathway, are proposed as possible treatments against leishmaniasis due to their mechanisms of action. In particular, treatments that target hematopoietic stem cells and monocytes, which have recently been found to be necessary components to sustain the infection and provide a safe niche for the parasites are discussed in this review as potential field-deployable treatments against leishmaniasis.

Keywords: leishmaniasis, field-deployable therapies, hematopoietic stem cells, monocytes, ferroptosis, repurposing drugs

Introduction

Leishmaniases are chronic, neglected tropical diseases with three major clinical manifestations: cutaneous, mucocutaneous, and visceral leishmaniasis. These diseases are all caused by the *Leishmania* parasite which is endemic to 88 countries spanning tropical and subtropical regions.[1] *Leishmania* parasites are spread by the bite of an infected sandfly, and the species of parasite transmitted to the host largely determines clinical outcomes.[2] Cutaneous leishmaniasis (CL) is characterized by papules which progress to ulcerative lesions that may result in permanent disfigurement or scarring.[2–6] In the Old World, CL is largely caused by *L. major* and *L. tropica*, while New World cases typically arise from *L. mexicana* infections.[7] Visceral leishmaniasis (VL), the chronic, fatal form of disease primarily manifests through fevers, spleen and liver enlargement, weight loss, and multiple hematological changes as parasites spread systemically through the host.[4–6,8,9] VL is caused by *L. donovani* and *L. infantum* in the Old and New World, respectively.[7] Individuals with visceral infections may later present with post-kala-azar dermal leishmaniasis (PKDL) even after apparent cure. PKDL is characterized by a widespread maculopapular rash due to immune responses against parasites persisting in the skin.[4,9] A rare form of disease, mucocutaneous leishmaniasis (MCL), presents in an estimated 1–10% of individuals with prior CL infection, and is primarily caused by *L. braziliensis* infection in the New World.[2,7] This particular manifestation progresses as destructive lesions or ulcers of

the mucosa, particularly surrounding the nose and mouth and, like CL, may lead to permanent disfigurement.[2] While cutaneous versus visceral pathologies are largely species-specific, hybrid *Leishmania* parasite infections and their associated clinical manifestations are more arbitrary with complicated clinical portraits and heightened disease severity. In Sri Lanka, a hybrid *L. donovani* parasite is responsible for causing thousands of cases of cutaneous leishmaniasis, although *L. donovani* is typically a visceralizing parasite.[10] In Peru and Bolivia, hybrid *L. braziliensis* strains harboring endosymbiotic *Leishmania* RNA viruses are associated with an increased risk of treatment failure.[11] The need to inhibit both *Leishmania* and its associated endosymbionts through antiviral therapies has been considered.[12,13] The complexities surrounding atypical pathologies and worsened symptomology of hybrid *Leishmania* parasites raises concerns for current field-deployable treatments and emphasizes the importance of therapeutic advancement in the realm of leishmaniases.

Given the severity of the diseases and the barriers for treatment of impoverished patients, especially in rural endemic areas of the world, developing and advancing field-deployable treatments to combat leishmaniases is imperative. In the present review, field-deployable therapies are defined as those that are largely accessible, easy to administer, and able to be deployed in areas of endemicity without the requirement of high costs and recurrent clinic visits. While the spread of *Leishmania* parasites is global, the greatest burden lies in low- and middle-income countries, where both quality of life and economic growth are negatively impacted as a result of disease. Therefore, the use of field-deployable therapies, novel treatments targeting parasite persistence, and repurposed drugs such as those developed against other diseases, where there is more funding and economic interest, is warranted in populations affected by *Leishmania*. Here, such current treatments against leishmaniases are summarized (Figure 1) and possible future perspectives and cellular targets for new treatments which have potential as improved field-deployable therapies are explored.

Current Therapies and Field-Deployable Therapies
Chemotherapies

Current treatments for leishmaniasis include various modes of chemotherapy, thermotherapy, and other localized treatments (summarized in Table 1). Pentavalent antimonial drugs are the first line of treatment with other chemotherapies available
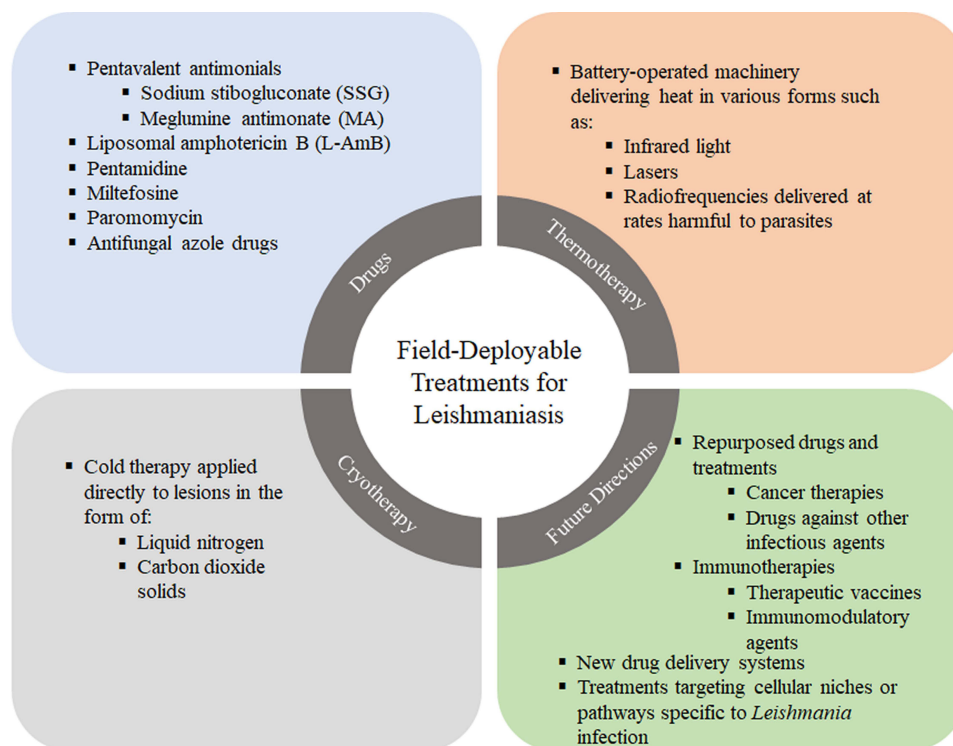
Figure 1 Current field deployable treatments for leishmaniasis. Current field deployable therapies include chemotherapies which are the first-line treatment. Other mechanical therapies such as cryotherapy and thermotherapy can be operated in the field to treat skin lesions. Finally, future treatments include immunotherapies, nanocarriers and repurposing drugs to target the specific cellular niches of the parasite.

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Table 1 Current Treatments Against Leishmaniasis. Administration routes, and therapeutic characteristics including use against which manifestation of leishmaniasis and other diseases, if repurposed

Treatment	Admin Route	Type of Therapy	Therapeutic Activity	Active Against	Benefits Associated with Treatment	Complications Associated with Treatment	Repurposed From	Refs
Pentavalent antimonials (SSG, MA)	IM, IV parenteral	Chemotherapy	Promotes macrophage activity against parasites, promotes oxidative stress within parasites, inhibits trypanothione reductase leading to parasite death	VL, CL, MCL	Relatively low cost in shortened regimens, generic versions available	Severe side effects, toxicity, efficacy against MCL is variable, parasite resistance, costly, lengthy regimen, contraindications in multiple patient cohorts	–	[3,5,6,14–17,27–30]
L-AmB	Parenteral, IL, IV	Chemotherapy	Binds to parasitic ergosterol, promotes oxidative stress	VL, CL, MCL	Good for use when antimonial resistance is relevant, less toxic than AmB, good for use in HIV patients, regimen can be short	Lengthy regimen, availability, costly	Fungal diseases	[3,5,6,14–17,22,28]
Pentamidine	Parenteral, IM	Chemotherapy	Inhibits parasitic mitochondrial activity, blocks active transport function	VL, CL	Good for use when antimonial resistance is relevant	Toxicity, risk of parasite resistance, efficacy in immunodeficient populations varies	Other parasitic diseases (especially trypanosomiasis)	[3,5,6,14,15,17,23,24,28]
Miltefosine	Oral	Chemotherapy	Damages parasite mitochondrial membrane, decreases cytochrome-c oxidase activity, prompts apoptotic cell death	VL, CL, MCL	Good for use when antimonial resistance is relevant	Contraindicated during pregnancy, relatively lengthy regimen, parasite resistance, efficacy varies (including in immunodeficient populations)	Cancer	[3,5,6,14–17,26,28]
Paromomycin	Parenteral, topical, IM	Chemotherapy	Binds to parasitic ribosomes to inhibit normal protein synthesis, damages membranes	VL, CL, limited efficacy against MCL	Low cost, can treat co-infections	Toxicity, costly, relatively lengthy regimen, risk of parasite resistance	Bacterial diseases	[3,5,6,14–17,27,28]

(*Continued*)

Table 1 (Continued).

Treatment	Admin Route	Type of Therapy	Therapeutic Activity	Active Against	Benefits Associated with Treatment	Complications Associated with Treatment	Repurposed From	Refs
Antifungal azoles (Fluconazole, ketoconazole, itraconazole)	Oral, topical	Chemotherapy	Leishmanicidal activity against promastigote stages, inhibits growth	CL, VL	Safe	Costly, variable efficacy, lengthy regimen	Fungal diseases	[3,5,6,14,17–20,28]
Thermotherapy (Infrared light, lasers, radiofrequency waves)	Local	Thermotherapy	Directly targets infection site to decrease parasite multiplication, collagen contraction may play a role, enhances elimination by macrophages	CL	No toxicity or severe side effects, battery-operated, regimen can be quick, lesion size specific	Costly, burns possible, local anesthesia may be needed, scarring	–	[3,5,14,17,27,28,31–34]
Cryotherapy (Liquid nitrogen, carbon dioxide solids)	Local	Cryotherapy	Directly targets infected tissue, exact leishmanicidal activity unknown	CL	No toxicity or severe side effects, regimen can be quick, low cost	Painful, burns possible, risk of secondary infections in treated tissue, risk of scarring, risk of depigmentation	–	[3,5,14,17,27,28,31,35,36,48]

Abbreviations: SSG, Sodium stibogluconate; MA, Meglumine antimonate; L-AmB, Liposomal amphotericin B; IM, intramuscular; IV, intravenous; IL, intralesional.

including liposomal amphotericin B (L-AmB), pentamidine, miltefosine, paromomycin, and antifungal azole drugs.[3,5,6,14–20] Antimonials currently in use against CL include sodium stibogluconate (SSG) and meglumine antimonate (MA).[17] Cure rates of up to 100% have been documented with this class of drugs but vary based on geographical location, treatment duration, and the species of parasite responsible for infection.[17,21] Liposomal amphotericin B is an alternative treatment for VL that has demonstrated cure rates ranging from 50% to 85% and is primarily used when antimonial-resistant parasites are the causative agents of infection.[17,22] Pentamidine, while recommended for only a minimal number of *Leishmania* species, has led to CL cure rates of up to 96%.[17,23] Paromomycin is an antibiotic typically used against intestinal parasitic infections but when administered to treat CL has reportedly resulted in cure rates of 50–85%.[17,24,25] Miltefosine, originally classified as an anti-cancer drug, is a safe and effective alternative CL treatment, yet many patients experience disease relapse following treatment due to the drug's inability to achieve complete parasite clearance, or sterile immunity.[17,26] Azoles are also used to treat CL, but varying treatment outcomes and rates of parasite clearance have been documented given their primary activity as an antifungal agent.[17]

In general, chemotherapies are largely outdated with high levels of toxicity, and many are non-specific for *Leishmania* parasites.[3,5,14,15,17,27] Additional barriers related to chemotherapeutics include the emergence of drug-resistant parasites. Drug resistance further complicates treatment efficacy, with cases in some regions being completely unresponsive to first-line treatments.[16,17] Other challenges related to chemotherapeutics include drug costs and accessibility, treatment length and adverse effects leading to noncompliance, and variability in host genetics and immune responses.[3,5,15,17,20] Additionally, most drugs like pentavalent antimonials and L-AmB may require numerous injections over the course of days to weeks, further hindering treatment feasibility and their implication as field-deployable treatments.[28,38] To lower toxicity, improve drug tolerability, and reduce drug resistance, combination therapies may be administered. By delivering drugs in tandem, the dosage of each individual drug can be decreased, and treatment time may be shortened, which are both desirable factors with regard to field-deployable therapies.[5] Current combination treatments include amphotericin B with miltefosine or paromomycin, and other drugs or immunomodulators in supplement with pentavalent antimonials.[5,29,30] Further, to overcome negative drug effects, new drugs have been recently developed or repurposed against leishmaniasis.[39] This includes drugs entering Phase 1 clinical trials such as DNDi-0690, an antitubercular nitroimidazole-class drug with potential use against VL.[40] Other drug candidates in pre-clinical trials phase are DNDi-6174 and DNDi 6148, with the interesting characteristic of being effective against drug-resistant strains of *Leishmania*.[41] Other promising treatments on the trajectory to clinical trials include oligonucleotides, proteasome inhibitors of the triazolopyrimidine class, and drugs of the imadozopyrimidine and pyrazolopyrimidine class.[39] While these novel and repurposed therapies are currently not approved and largely experimental, they are promising options for the future of *Leishmania* treatments.

Therapeutic Vaccines and Immunotherapies

Therapeutic vaccines are a promising treatment option administered post-exposure to *Leishmania* parasites. These vaccines work to boost the host immune system in an attempt to reduce parasite burden and resolve infection after it has already been established; therefore, therapeutic vaccines are a promising form of immunotherapy against leishmaniasis. Historically, therapeutic vaccines have generated higher CL cure rates in trials and has been favored over chemo-monotherapies with regard to duration, overall treatment outcomes, and adverse effects, as side effects are primarily local to the lesion site.[28,42–47] Immunotherapy has been shown to be an effective tool in efficacy trials against PKDL, which is known to be difficult to treat with chemotherapies.[28,45] A barrier associated with therapeutic vaccines in field-related settings, however, is the need for boosters or multiple doses.[28] In addition to therapeutic vaccines, immunomodulators, like toll-like receptor (TLR) agonists and cytokines have been experimentally tested against leishmaniases, administered both alone and in combination with other drugs, yet efficacy must be further explored and cost remains an issue.[28,30] In general, first- and second-generation therapeutic vaccines are currently undergoing clinical trials, yet they have room for advancement and will likely be a vital tool in the future of field-deployable treatments.[28]

Thermotherapies and Cryotherapies

A variety of forms of thermotherapies and cryotherapies are also used in the field to treat leishmaniasis. Thermotherapies can be administered in the field with battery-operated machines delivering heat in the form of infrared light, lasers, hot water soaks, or radiofrequencies directly applied to lesions to slow growth and eradicate parasites.[27,31–34] These therapies have been used for decades in various settings and in some cases, only require one session for cure.[32] Carbon dioxide lasers are another form of heat therapy administered directly at the site of infection requiring minimal treatment sessions, emphasizing cost-effectiveness and favorability in the field.[27] Thermotherapies are safe with minimal off-target effects and are favored for use in patients with contraindications to other drugs and in regions where healthcare infrastructure is not easily accessible.[27] Cryotherapy is also used to treat cutaneous leishmaniasis lesions by direct application of liquid nitrogen or, in resource-limited settings, carbon dioxide solids at temperatures lethal to parasites.[31,35,36,48] While these therapies have been deemed safe and effective with few side effects, cure rates vary based on a multitude of factors such as parasite species and lesion size. With these limitations in mind, combination therapies with chemotherapeutics may still be recommended, but these methods alone are especially favorable for patients who cannot receive traditional chemotherapies such as pregnant or immunosuppressed patients.[31,35,37] In summary, the lack of sterile immunity and complete parasite clearance achieved following administration of current treatments emphasizes the need for new developments in the realm of field-based therapies.

Parasite clearance or sterile immunity may be desirable outcomes following treatment for leishmaniasis, yet current therapies are incapable of achieving this aim due to inadequate effectiveness or penetration. Future therapies should aim to reach this threshold because latent infections may lead to later reactivation and further clinical complications, as in the case of PKDL and MCL. These cases are responsible for continued parasite transmission and therefore promote disease reemergence and are considered a threat to leishmaniasis elimination in many regions.[49] Likewise, the lack of parasite clearance following treatment increases the risk of parasite transmission in endemic and nonendemic regions via other modes such as blood transfusions and organ transplants.[50–52] Barriers in achieving sterile immunity and mechanisms in which *Leishmania* may evade existing treatments are therefore important to discuss when considering future directions of *Leishmania* therapies.

Roadblocks to Sterile Immunity – *Leishmania* Refuges in the Body with Potential for Latent Infection

One challenge field-deployable therapies must face is the array of bodily sites and cell types which *Leishmania* spp. can reside in. This flexibility may contribute to treatment failure, latent infection, and relapse infections in some patients as some sites are immune-permissive or may have less drug accessibility. Furthermore, application of subcurative drug doses and drug resistance of the parasite may raise the likelihood of latent *Leishmania* infection. While *Leishmania* has been reported to asymptomatically colonize several unusual areas of the body - including skeletal muscle, female urogenital tract, and mammary glands,[53–56] atypical symptomatic leishmaniasis has also been reported in the eye,[57] central nervous system,[58–60] cartilage,[61,62] testes,[56,63] and placenta.[64–67] Most notably, VL has been well characterized for its ability to infect hematopoietic stem cells (HSCs) of the bone marrow.[68,69] Evidence of *Leishmania* colonization at these sites is significant as it supports the possibility of these sites as potential refuges for latent infection. Therefore, current and future field-deployable treatments against leishmaniases should aim to clear asymptomatic reservoirs of *Leishmania* throughout the entire body to guarantee sterile immunity and prevent reinfection and disease transmission.

Re-Purposing Therapies for Use Against Cellular Hosts of *Leishmania*

While many therapies have been investigated for treatment of leishmaniases, future treatments could be improved by targeting specific cell types which host *Leishmania* infection. Therefore, it is necessary to understand the characteristics of these cellular permissive hosts and explore the phenotype and microenvironment of such susceptible cells to identify mechanisms to directly target them. Here, the possibility of repurposing drugs for cellular targeting against leishmaniasis is discussed.

As discussed previously, first-line treatments against leishmaniasis are chemotherapeutic agents originally developed to treat different pathologies and later found to possess anti-parasitic properties. This approach is particularly beneficial in the field of the neglected tropical diseases, where drug repurposing can improve the treatment's availability, despite the lack of financial incentive for new drug discovery.[70] Examples of such repurposed drugs include both amphotericin B and azole drugs, discovered as antifungals with the ability to generate instability in cell membranes containing ergosterols.[71,72] Decades later, both drugs were observed to have leishmanicidal effects due to the ergosterol content in *Leishmania* membranes.[73–75] Similarly, miltefosine, a drug advanced to clinical trials targeting breast cancer and other malignancies,[76] was found to have leishmanicidal potential which led to the approval of a topical miltefosine formulation.[77] While the use of miltefosine against breast cancer did not prosper due to its secondary effects, it was approved in 2013 for the treatment of both VL and CL.[77,78] Further, drug repurposing has been used by Bustamante et al to perform pharmacokinetic simulations between approved drugs and parasite proteins with homology to the known targets of such drugs.[79] The group succeeded in finding 33 drugs that could potentially be used against leishmaniasis, with two drugs, rifabutin and pherphenazine, even displaying anti-leishmanial activity in vitro.[79] Further in silico analysis may continue yielding older drugs for use against leishmaniasis. With greater understanding of the mechanisms aiding *Leishmania* survival and persistence, currently studied therapies can be matched to specific mechanisms and repurposed for use in leishmaniasis cases. However, due to high costs of advanced treatments, some alternatives proposed here are far from clinical and especially field-based use against leishmaniasis. Nevertheless, it is relevant to incorporate these experimental approaches in the pursuit of treating leishmaniases, with a particular interest going forward on targeting safe niches for *Leishmania* that facilitate persistence of the disease.

Hematopoietic Stem Cells (HSCs)
Immune Mechanisms in the BM and HSCs

The interplay between HSCs and the parasite is a constant fight in VL. On the one hand, HSCs can phagocytose *L. infantum* and produce pro-inflammatory cytokines such as TNF-α in response to infection.[80] On the other hand, hematopoiesis-sustaining HSCs can serve as safe niches for *L. donovani*, protecting the parasite from chemotherapy.[81] The relationship between hematopoiesis and VL has been long accepted, with a number of hematological issues arising in human VL patients caused by *L. donovani* infection.[81] Experimental results show that *L. donovani* modulates host hematopoietic activity to allow its own survival and replication.[82,83] In healthy mice, most long term HSCs (LT-HSCs) are quiescent, but after *L. donovani* infection there is an accumulation of CD4+ T-cells which, in response to TNF-α present in the BM environment, induces their production of IFN-γ.[82] As recently described, a BM immune environment with a higher proportion of IFN-γ- and IL-17-producing effector T-cells correlates with low parasitic load, while greater FoxP3+ IL-10-producing cells and TGF-β levels are related to higher parasitic loads.[84] Such immune balance is delicate as, despite IFN- γ being necessary for parasitic control in VL,[85] it also induces LT-HSCs to enter the cell cycle, losing quiescence and causing the accumulation of hematological progenitor cells which undergo exhaustion after chronic stimulation.[82] On the other hand, this excessive regulatory response can also undermine the health of the patient. As observed in VL patients, the accumulation of IL-10 producing T-regs in the BM inhibits T-cell activity,[86] and has been related to PKDL development and resistance to sodium antimony gluconate (SAG) treatment.[87,88] Furthermore, chemotherapy itself generates changes in the immune environment. CD8+CD25+Foxp3+ T cells frequency augments in all tissues, including the BM, of *L. infantum*-infected dogs relative to healthy ones, yet following chemotherapeutic treatments, cell populations returned to baseline levels.[89] Although in the absence of treatment, presence of IL-10, TGF- β-producing CD4+ Tregs have been shown to exacerbate *L. donovani* susceptibility.[89,90] Therefore, while immunotherapy against VL has been explored before,[91] future therapies targeting the elimination of the parasites hiding in the HSCs will require fine modulation of the immune response in the BM specifically to promote parasitic control without risking hematopoietic balance.

Several reports of the presence of *L. donovani* in the BM of VL patients show that the BM is a frequent parasite niche.[92–96] In VL infection models, both *L. infantum* and *L. donovani* show preferential infection of LT-HSCs over short term HSCs (ST-HSCs).[97] Further, in the Frizzled6 (Fzd6) deficient VL mouse model in which HSC expansion is dampened, a reduction in parasitic burden during the chronic phase of the *L. donovani* infection has been seen.[98]

Additionally, *Leishmania* parasites may leverage LT-HSCs as niches of protection against chemotherapies. An experiment showed that after treatment with paromomycin, *L. infantum* disappeared from almost all tissues of the body except HSCs, where it hid in very low numbers. Three weeks after treatment cessation, the hiding parasites were then able to reinitiate HSC infection.[97] Moreover, LT-HSCs were shown to be safer niches for both *L. infantum* and *L. donovani* than BM-derived macrophages after ex vivo treatment with paromomycin, miltefosine and SSG.[97] Therefore, these results demonstrate how LT-HSCs niches are responsible for infection persistence in VL. Interestingly, the authors characterized a specific transcriptional profile in the LT-HSCs and found a reduction of *Nos2* expression meaning that, although healthy LT-HSCs balance expression of nitric oxide (NO) and reactive oxygen species (ROS), infected LT-HSCs downregulate this mechanism to permit a safer space for the parasite.[97] Although more research must be completed with regard to the specific biology and interaction of *Leishmania* with LT-HSCs, knowing the phenotype of this specific niche may facilitate the development of future therapies.

Leishmania infection also increases the generation of progenitor cells of the myeloid lineage which further serve as cellular hosts for the parasites.[99] In a murine model of chronic VL, *L. donovani* infection caused the expansion of HSC and myeloid progenitor cells. The progeny of such cells were Ly6C$^{hi/int}$ monocytes with a regulatory phenotype, characterized by IL-10+ and arginase1+, which allow the persistence and proliferation of the parasite.[83] Accordingly, in murine CL, it was observed that infection with the persistent strain *L. major Seidman* led to the recruitment of myeloid cells with a regulatory profile with lower levels of Type I and Type II interferons into the lesion site and the spleen. Moreover, the enhanced cell influx was sustained by the increase of HSCs with a myeloid-biased profile in the BM, compared to the infection with a self-healing inducing parasite.[98] Therefore, the mechanism of susceptibility and persistence of VL in the BM include inflammatory immune response disruption, generation of immune-privileged cellular hosts, and generation of monocytic cellular hosts. Future leishmaniasis therapies that aim to cease reactivation of infection should therefore target the LT-HSCs.

Perspectives in Therapies Against *Leishmania* Infection in HSCs

The potential chemotherapeutic intervention in the BM immune ecosystem may help to eliminate parasites, although there is a remaining risk of disrupting immune balance and causing BM-HSC exhaustion with detrimental patient outcomes. Previously described findings could suggest that inducing ROS and NO by HSCs may favor *Leishmania* elimination.[97] Nevertheless, studies of other diseases including leukemia and diabetes show that ROS dysregulation in the BM plays a role in both disease pathogenesis and the dysfunction of BM cells and hematopoiesis.[100–103] Therefore, directly increasing the levels of NO and other oxygen species could be detrimental for the leishmaniasis patient, rather than therapeutic. Nevertheless, REDOX-reactive materials have been used to generate nanocarriers that deliver therapeutic drugs to cells depending on their ROS production, particularly against cancer.[104,105] In settings with VL cases, nanocarrier therapies could be used to deliver drugs directly to infected HSCs, also reducing the rates of drug resistance and preventing parasites from reinitiating the infection.

Drug delivery systems are becoming desirable in the treatment of multiple diseases of the bone and BM including multiple myeloma, hematopoietic dysfunction, rheumatoid arthritis, bone metastases, and osteoporosis.[106,107] Conventional drug therapy requires the administration of high doses of drugs in which only a small portion reaches the BM, yet these doses are associated with adverse side effects.[108] Therefore, the design of drug delivery systems has been widely explored and reviewed.[106,108,109] Liposomes and chimeric nanoparticles have been shown to be taken up by the BM.[108] Therefore, in *Leishmania* infection, such technologies could be applied to reduce dosages and increase organ specificity.[14,110] In that sense, liposome carriers, whose pharmacokinetics can be controlled through carrier surface modifications, have been designed to target the BM, examples have been listed in Table 2. Furthermore, the liposomal formulation of amphotericin B (Ambisome®) has significantly reduced the toxic effects of the conventional non-liposomal formulation (amphotericin B deoxycholate).[111] Hence, the use of liposomal nanocarrier formulations is a plausible next step in the *Leishmania* drug development.

Table 2 Future Directions of Drugs for Leishmaniasis. Summary of Future Therapies That Could Be Repurposed for Their Use Against Leishmaniasis, the Disease for Which It Was Designed, Their State of Development and Testing Against Such Disease and Against Leishmaniasis, Mechanism of Action and Their Potential to Be Beneficial to Different Types of Leishmaniasis

Drug	Tested in Other Diseases	State of Testing in Other Diseases	Drug Type	Biological Impact in Leishmaniasis	Beneficial to	State of Testing for Leishmaniases	Refs
Rifabutin	HIV+ Tuberculosis	FDA Approved	RNA Polymerase inhibitor for Gram + bacteria, Leishmanicidal	Leishmanicidal effect	CL/MCL (*L. braziliensis, L. panamensis*)	in silico, in vitro	[79]
Perphenazine	Schizophrenia, severe nauseua, vomiting	FDA Approved	Blocks Dopamine 2 Receptors, Leishmanicidal	Leishmanicidal effect	CL/MCL (*L. braziliensis, L. panamensis*)	in silico, in vitro	[79]
31 other candidate drugs	Multiple	FDA Approved	Potentially Leishmanicidal	Potentially Leishmanicidal	CL/MCL (*L. braziliensis*)	in silico	[79]
Liposome, Chimeric nanoparticles for drug delivery	–	Tested in mice, rabbits, and rhesus monkeys	Drug vehicle to target ligand cells	Organ-specific drug delivery	VL	–	[108]
RS-504393	–	–	CCR2 antagonist	Inhibit iMOs migration	VL	in vivo in mouse	[112]
Ibrutinib	Mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), Waldenstrom's macroglobulinemia (WM), marginal zone lymphoma, and chronic graft-versus-host disease in allo-HCT.	FDA Approved	ITK/IBK inhibitor	Inhibit iMOs migration	VL	in vivo in mouse	[113]
CCR2 siRNA liposome	Cancer	Tested in mice	CCR2 silencer	Inhibit iMOs migration	VL	–	[114]
Anti-CCR2 neutralizing antibodies	Cancer	Tested in mice	CCR2 antagonist	Inhibit iMOs migration	VL	–	[114]

(*Continued*)

Table 2 (Continued).

Drug	Tested in Other Diseases	State of Testing in Other Diseases	Drug Type	Biological Impact in Leishmaniasis	Beneficial to	State of Testing for Leishmaniases	Refs
PF-04136309	Metastatic pancreatic ductal adenocarcinoma, knee osteoarthritis	Phase 2 Clinical Trial	CCR2 antagonist	Inhibit iMOs migration	VL	–	[114]
Enalapril	Hypertension	FDA Approved, other applications being explored	ACE inhibitor	Reduce Ang-II hormone, reduce HSC proliferation, less iMOs infiltration, immunomodulation	CL/MCL (*L. braziliensis*)	in vitro	[115–117]
(Cpd9) (R)-2-amino-6-borono-2-(2-(piperidin-1-yl) ethyl) hexanoic acid	Non-small cell lung cancer	Tested in mice	Arginase-1 inhibitor	Inhibit Arginase-1 (a competitor of iNOS), immunomodulation	CL, VL	–	[118]
EP2 (AH6809) and EP4 (AH23848)	Fibrosarcoma, melanoma	Tested in mice	PGE2 Antagonist	Blocks NF-κB repression, improves IFN-γ response	CL, VL	–	[119]
NSC 74859	Cancer	Tested in mice	STAT3 inhibitor	Stop immunosuppression from MDSC, promote T cell proliferation	CL, VL	–	[120]
JSI-124 (cucurbitacin I)	V-src transformed fibrosarcoma, CT26 colon adenocarcinoma	Tested in mice	JAK2/STAT3 inhibitor	Stop immunosuppression from MDSCs, promote mature DC differentiation	CL, VL	–	[121]

Nanocarriers specific to M2 macrophages	Cancer	Tested in mice	Drug vehicle to MR+ cells	Immunomodulation of M2 macrophages which overexpress MR	CL, VL	–	[122,123]
Mannosylated siRNA carriers	Colorectal Cancer	Tested in mice	siRNA to MR+ cells	Immunomodulation of M2 macrophages which overexpress MR	CL, VL		[124]
Nano-deformable liposomes with SSG gel	–	–	Leishmanicidal	Leishmanicidal effect	CL (*L. tropica*)	in vivo in mouse	[125]
Meglumine antimoniate in silver nanoparticle (AgNP-PVP-MA)	–	–	Leishmanicidal	Leishmanicidal effect	CL (*L. amazonensis*)	in vitro	[126]
Potential Ferroptosis inducers, siRNAs	Multiple	Gene-level studies in mice	GPX4 downregulation, ascorbate upregulation, Iron transporter upregulation (DMT-1, FPN-1), prominin2 suppression, NFS-1, inhibition, inhibition of system Xc —,	Leads to ferroptosis	CL, VL	–	[127–136]
1,2-dioxolane compound	Cancer	In vitro	Ferrous molecule oxidizer	Leads to ferroptosis	CL, VL	–	[137]
Withaferin A	Neuroblastoma	Tested in mice	Inhibit Ubq ligase, NRF2 activator, GPX4 inhibitor	Leads to ferroptosis	CL, VL	–	[202]

Monocytes

The proliferation of HSCs in response to infection generates more host cells for the parasite in the form of monocytes. Monocytes are circulating myeloid cells derived from HSCs and represent an intermediate state before becoming macrophages or certain types of DCs.[139] Although both macrophages and DCs have been widely reported to be infected by *Leishmania*, macrophages are considered to be the main cells housing *Leishmania* replication.[140–142] Nevertheless, much remains to be explored for their common ancestor. In this section, the characteristics of monocyte and macrophage hosts, as well as possible therapies targeting such niches are explored.

Migration of Monocytes in Anti-*Leishmania* Therapies

Monocytes are more than just transition cells; they delineate the interactions between host responses and the parasite by shaping immunity against disease.[143] In leishmaniasis, inflammatory monocytes (iMOs) have been shown to provide safe environments for parasite replication in both *L. donovani* and *L. major* infection models.[144,145] Particularly, the influx of iMOs to the liver and spleen seems to be detrimental during experimental VL. In murine infection with *L. donovani*, migration of iMOs into the liver and spleen can be reduced with the use of the drugs RS-504393, or ibrutinib (Table 2). This resulted in a reduced parasitic burden in the liver and spleen.[112,113] The activity of such agents also impacted immune regulation. The use of RS-504393 resulted in no granuloma formation and the decrease of IFN-γ IL-10 double-producer CD4+ T cells and total CD4+ T cells.[112] In the case of ibrutinib administration, there were increases in granuloma formation, IFN-γ and IL-4 producing NK cells, as well as the levels of protective cytokines such as IFN-γ, TNF-α, IL-4, and IL-13.[113]

Interestingly, CL studies show that monocyte migration is beneficial for the host, as monocytes tend to migrate to the lesion site rather than to the visceral organs. In *L. major* murine infection, CCR2 and platelet activation are responsible for Ly6C+ monocytes reaching the lesion site where they can efficiently participate in parasite elimination.[146] Moreover, CCR2 favors DC migration to the draining lymph nodes where the lack of such chemokine receptor leads to a Th2-biased immune phenotype, providing a permissive environment for *L. major*.[147] However, such responses differ depending on the infection route and mouse strain.[148] Therefore, targeting monocytic migration through CCR2 inhibition treatments may be beneficial against *L. donovani* infection, but not against *L. major* infection. Multiple approaches to inhibit monocytic migration by targeting CCR2 have been tested against different cancer types (Table 2), as the monocytes can be detrimental to the patients of different malignancies.[114] These already existing anti-cancer experimental therapies have not been tested against VL, but could lead to favorable results as observed in the cancer studies and have potential in the realm of field-deployable leishmaniasis treatments.

Monocyte Immune Balance

Besides iMOs migration, the phenotype resulting from the interaction between iMOs and the parasite highlights the role of iMOs during infection. For example, among other immune-modulatory mechanisms,[149–151] *L. donovani* is capable of inhibiting acidification and superoxide production in the parasitophorous vacuole of iMOs, which then provides a permissive environment for the parasite.[144] In *L. donovani* murine infection, the balance of inducible nitric oxide synthase (iNOS) and arginase-1 expression by iMOs correlates with the increase or decrease of parasitic burden in visceral organs.[152,153] In the spleen, the arginase-1/iNOS ratio increases at 30 days post-infection (d.p.i.) while the infection becomes progressive. At the same time, the arginase-1/iNOS ratio diminishes in the liver as the infection starts resolving[112] These findings correspond to observations in human patients diagnosed with VL, in which monocytes obtained from peripheral blood display an anti-inflammatory phenotype characterized by low levels of IL-1β and IL-6, as well as a reduction in phagocytosis capacity and production of ROS.[154]

Monocyte responses are dependent on the *Leishmania* strain infecting them, and this in turn seems to influence or relate to disease manifestation. Ex vivo infection of human monocytes with an *L. infantum*, strain causative of severe VL, induced the downmodulation of the immune response characterized by reduced TLR2, TLR9, and HLA-DR expression, and a lower TNF-α/IL-10 ratio. On the other hand, infection with *L. braziliensis*, causative of mild CL, has not only shown increased expression of the same receptor and the TNF-α/IL-10 ratio, but also better controls infection by reducing parasite counts by 100-fold.[155] In the case of murine *L. major* infection, monocytes displayed altered maturation

and became safe hosts for the parasite; yet, after a second inoculation, monocytes exhibited a stronger anti-parasitic response. While *L. major* inhibited monocyte maturation into a DC phenotype in secondary infections, monocytes were still able to eliminate parasites in an IFN-γ- and iNOS-dependent manner.[145] Similarly, monocytes could eliminate the parasites more efficiently when they were not differentiated into macrophages. Murine infections with *L. major* generate monocytes with myeloid-derived suppressor cells (MDSCs) (Gr1^{hi}CD11b^{hi}F4/80^{int}). When MDSCs were exposed to IFN-γ and IL-4, MDSCs increased their NO production, hence ramping up their killing capacity. Interestingly, when differentiation from MDSCs into macrophages was chemically induced, there was an increase in lesion sites and parasitic burden, showing that monocytes can eliminate the parasite, even when not fully matured, if they can produce NO.[156] Therefore, immunomodulatory therapies could improve the monocytic capacity of eliminating parasites in both VL and CL manifestations.

The Interplay of Monocytes and HSCs: Therapeutic Potential

NO not only has a potent anti-parasitic effect, but also shapes the microenvironment of the infection site by preventing monocyte recruitment from the bloodstream and therefore inhibiting *Leishmania* from reaching potential host cells.[157,158] As discussed previously, HSCs favor *Leishmania* infection by generating more monocytes that serve as host cells. Hence, HSCs and monocytes play an interconnecting role in sustaining *Leishmania* survival and proliferation. The interplay between these two cell types is similar to cancer scenarios in which HSCs support the generation and consequent accumulation of monocytes in the tumor, and when exposed to the microenvironment, gain a regulatory phenotype.[114] In a murine model of lung cancer, the drug enalapril reduced the levels of the hormone responsible for amplifying HSCs and HSC-derived myeloid progenitors and increasing monocytes and macrophages in the lung.[115] Enalapril (Table 2) is an FDA-approved drug commonly used for the treatment of hypertension.[116] These results have been extrapolated to the treatment of leishmaniasis. The in vitro administration of enalapril to murine macrophages infected with *L. braziliensis* increased NO, IFN-γ, IL-12 and TNF-α production by macrophages while reducing the levels of IL-10.[117] The hypothetical scenario where enalapril favors both reducing the permissive environment and limiting the expansion of the permissive niches would be beneficial in the treatment of leishmaniasis and the prevention of relapse. More importantly, the use of a drug available in more than 100 countries worldwide such as enalapril[159] would be an ideal and affordable treatment to be deployed in the field in *Leishmania*-endemic areas. It is important to keep in mind that the profile and activation of iMOs differ among *Leishmania* species and mouse strain,[160] so further therapeutic target studies must be specifically designed against different clinical manifestations of leishmaniasis. Still, immunomodulatory therapies directly targeting iMOs could facilitate the immune phenotype necessary to eliminate intracellular parasites.

Reprogramming Monocytes: Lessons from Cancer

In leishmaniasis, the parasite can reshape the immune activation of monocytes toward a regulatory profile, similar to that of M2 macrophages.[161] In cancer, the tumor microenvironment reprograms monocytes toward regulatory phenotypes, preventing T-cell cytotoxicity and the recruitment of T-regs that allow tumor growth, metastasis, and immune evasion. However, exposing monocytes in vitro to IFN-γ or IFN-α activates tumoricidal activity by phagocytosis, cytokine-mediated killing, and antibody-dependent cytotoxicity.[114] In summary, both cancer and *Leishmania* infections share the need for monocytes with an effector profile to favor the host and control disease. In a mouse model of non-small cell lung cancer, suppression of arginase-1 activity, an enzymatic competitor of iNOS, restored IFN-γ and IL-2 production by splenocytes and the cytotoxic phenotype of CD8+ T cells.[118] This immune profile is beneficial to fight *Leishmania* infections,[162] so it is possible to hypothesize that arginase-1 suppression could be a therapeutic target. An additional mechanism to reprogram monocyte phenotypes is by targeting prostaglandin E2 (PGE2).[119] Downstream, PGE2 controls p50 NF-KB accumulation and translocation, and diverts signaling in response to IFN-γ. The administration of a PGE2 agonist to MDSCs with monocytic profiles reshaped this cascade and restored IFN-γ-mediated NO production of monocytes in tumor bearing mice.[119] Similarly, the pharmacological inhibition of STAT3 activation reduced the immunosuppressive characteristics of suppressor monocytes.[120,121,156] We speculate that the use of immune modulator compounds, such as the examples presented above (Table 2), could skew monocytic profiles in leishmaniasis towards a less permissive phenotype.

Macrophages

Macrophages compound a heterogeneous population of cells derived from monocyte differentiation and have been widely accepted as the main permissive hosts for *Leishmania*.[140,141,163] As for monocytes, the phenotype of macrophages is determinant on their role as effector anti-leishmanial cells or as permissive hosts.[164–166] Therefore, the use of immunotherapies is a promising approach to tilt immune balance towards the macrophage effector phenotype, some of which are shared with the monocyte-targeting therapies, a topic which has been recently reviewed in more depth.[167] Next, examples are provided of how macrophages could be targeted with and without immunomodulation.

Macrophages can be targeted by loading drugs into specific delivery systems (Table 2), such as the previously mentioned nano-carriers that have shown promising results in the development of therapies against leishmaniasis.[122] In the persistent CL model, it was demonstrated that *L. major* Siedman preferably infects M2 dermal macrophages with high expression of the mannose receptor (MR). Such strategies of immune evasion can keep the parasite protected inside the M2-macrophage from an environment rich in IFN-γ and TNF-α.[141] In cancer research specifically, MR is commonly overexpressed; therefore, many nano-sized carriers have been developed to target MR+ cells.[123] MR nano-carriers were designed to be covered by multiple MR-ligands on their surface to allow binding to MR+ cells. Once captured by their final target, nano-carriers can work both as drug and antigen delivery systems to antigen presenting cells.[123] The clinical applications of MR-directed carriers could be expanded to *Leishmania* treatment to deliver drugs already in use to improve solubility and specificity, therefore reducing adverse effects of chemotherapies.[123,168] Mannosylated carriers can also guide plasmids and siRNA for delivering gene therapy, which could be used to manipulate immune response within the lesion site.[124] Daret al have recently developed a nano-deformable liposomes (NDLs) system to deliver SSG in a gel-form administration. The advantages of NDLs include 10-fold better retention of SSG in the deeper layers of the skin in an ex vivo model, compared to regular SSG. NDLs also showed effective anti-leishmanial activity in an in vitro model of *L. infantum*-infected macrophages.[125]

Meglumine antimoniate (Glucantime®) has also been successfully loaded to a silver nanoparticle (AgNP-PVP-MA) and its effect was tested in an in vitro model of macrophages infected with *L. amazonensis*. The administration of AgNP-PVP-MA led to a higher production of NO, TNF-α and IL-6 than that of infected macrophages treated with amphotericin B.[126] More importantly, these silver nanoparticles reduced the viability of promastigotes, achieving the two objectives of directly targeting the parasite while modulating the immune response to benefit the patient. Therefore, the development of nano-delivery systems could improve efficacy and safety of current anti-leishmanial drugs.

Overall, the identification of cellular hiding niches, such as HSCs and the main cellular host, monocytes/macrophages, allowed the characterization of mechanisms and phenotypes related to susceptibility. Therefore, we propose that future therapies target such pathways (Figure 2) for therapies that completely eradicate the parasite from the host.

Ferroptosis – a Novel Target for Future Therapies Affecting Multiple Cell Types

While discussing the function of cell death in infectious disease settings, we are often reminded of our decades-old understanding that hosts effectively eliminate pathogenic niches through mechanisms such as apoptosis, necroptosis, pyroptosis, and autophagy. Alternatively, intracellular pathogens attempt to delay or avoid cell death by eliminating host immune cells, thus destabilizing the immune response. Broadly speaking, researchers are making significant efforts to control cell death procedures that may aid in the eradication of diseases, and certainly many pharmacological/natural compounds have been identified to achieve this. Similarly, over the past few years, an important mechanism, ferroptosis, has been characterized as being equally engaged in cell death regulation for multiple cell types.

Ferroptosis is a newly discovered programmed cell death, and it plays a vital regulatory role in the occurrence and development of many diseases. The ferroptosis process (Figure 3) is defined by the accumulation of lethal lipid species derived from lipid peroxidation. The process involves three major metabolisms: lipids, thiols, and iron-dependent lipid peroxidation. It can be precluded through the enzymatic reaction of glutathione peroxidase 4 (GPX4), that catalyzes the reduction of lipid peroxidation and ferroptosis suppressor protein 1 (FSP-1) which then catalyzes the regeneration of

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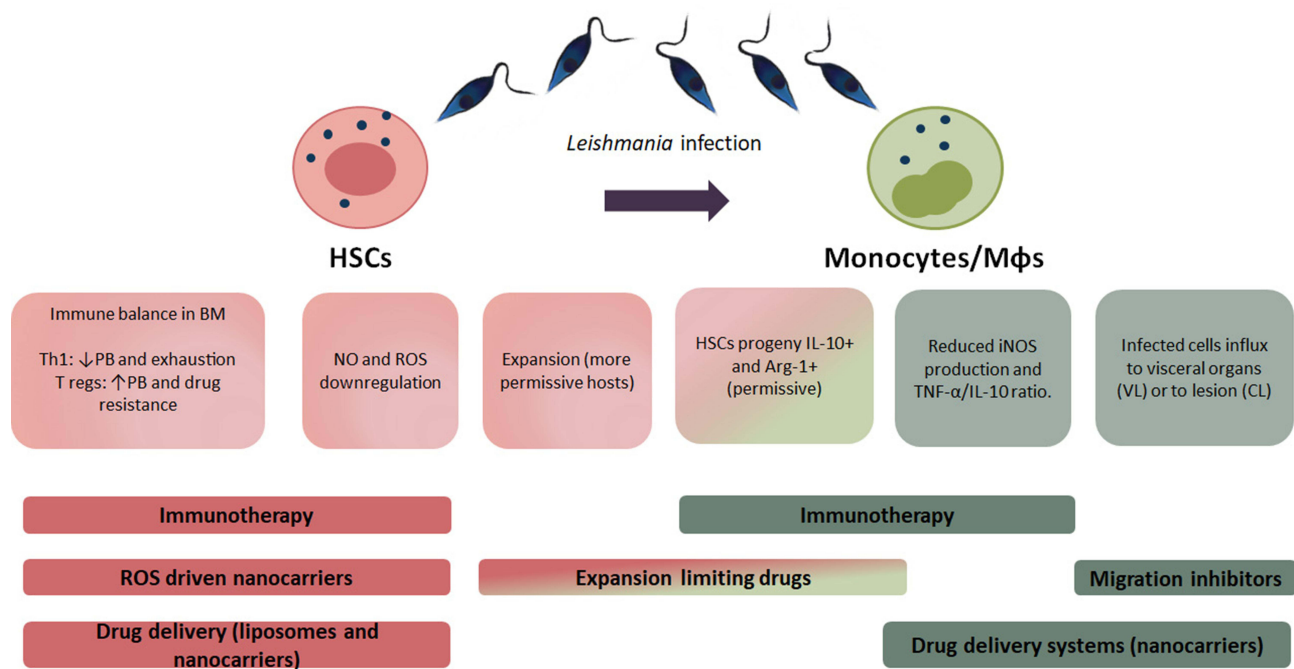
Figure 2 Interplay of Leishmania and its cellular hosts: HSCs and monocytes. Hematopoietic stem cells (HSCs) respond to Leishmania infection. During this process, the microenvironment of the bone marrow (BM) changes, an inflammatory Th1 response usually prevents parasite expansion although infection can lead to local T-cell exhaustion, whereas an abundance of a regulatory environment, leaded by regulatory T cells (T regs) result in an elevated parasitic burden (PB) and is related to drug resistance. The nitric oxide (NO) and reactive oxygen species (ROS) production is also reduced and allows the generation of a permissive environment for the parasite. Although HSCs expand and differentiate to fight the infection, the resulting monocytes become the main host for the parasite, therefore resulting detrimental for the host. These newly differentiated monocytes express IL-10 and arginase (Arg)-1, and a reduction in iNOS and TNF-α, the characteristic phenotype that allows parasite survival. Finally, monocytes play a role by migrating to either the visceral organs in visceral leishmaniasis (VL) or to the skin lesion in cutaneous leishmaniasis (CL) with different roles for each manifestation. All this process can be targeted by the use of different therapies (bold), such as immunotherapies to reinstall the immune balance and favor an inflammatory response which allows parasite killing, also nanocarriers could be used to develop drugs directly to the infected cells. Other drugs limiting the expansion of HSCs could stop the generation of more cellular hosts as the monocytes, whereas migration of monocytes can also be driven into a favorable result for the patient.

ubiquinone (Co-enzyme Q10).[169,170] Through this unique pathway, ferroptosis undergoes a completely different course of action from apoptosis, necroptosis, autophagy, and all other forms of cell death.

Unrestricted lipid peroxidation causes ferroptosis. Lipid peroxidation can be defined as a series of events where various oxidants (free radicals) strike on lipid containing carbon-carbon double bond(s). In this oxidation process, free radicals such as hydroxyl (-OH) are created by excess of ferrous iron (Fe^{2+}) through the Fenton reaction. Subsequently, hydroxyls then attack polyunsaturated fatty acids (PUFAs).[171] Hydroxyls are important for lipid peroxidation and in excess cause oxidative cell damage that can affect cellular DNA and lipid membrane damage or can alter the functional protein under stress conditions. Ferrous iron catalyzes lipid hydroperoxide into ROS molecules.[171,172] Eventually, lipid peroxidation impairs cell membrane integrity and fluidity, causing cell damage or death. GPX4 is a key enzyme in ferroptosis regulation as it reacts with unstable intercellular lipid peroxides to form stable nontoxic lipid alcohols with the help of glutathione (GSH).[173,174] Abridged GSH inhibits GPX4 and restrains the host's ability to repair peroxidized lipids, triggering ferroptosis. Gao et al explained that cystine and cysteine are important for the synthesis of intercellular GSH, and their absence encourages ROS production.[175] SLC7A11 (Solute Carrier Family 7 Member 11) is another critical component of system Xc's cystine/glutamate transporter system. SLC7A11 controls cystine absorption and glutamine metabolism.[176] Besides GPX4 and GSH, ferroptosis suppressor protein (FSP-1) is a NADPH-dependent coenzyme Q10 (ubiquinone-10) oxidoreductase on the cell membrane that inhibits lipid peroxidation. FSP-1, also known as apoptosis-inducing factor mitochondrial 2 (AIFM2), captures lipophilic radicals to stop lipid peroxide dissemination.[170]

Ferroptosis in Parasitic and Other Infectious Diseases

Moderating the above-mentioned pathways leads to ferroptosis-related cell death in a range of infectious diseases, including parasitic infections. Matsushita et al, showed that *L. major* infection in mice with GPX4-deficient T cells resulted in
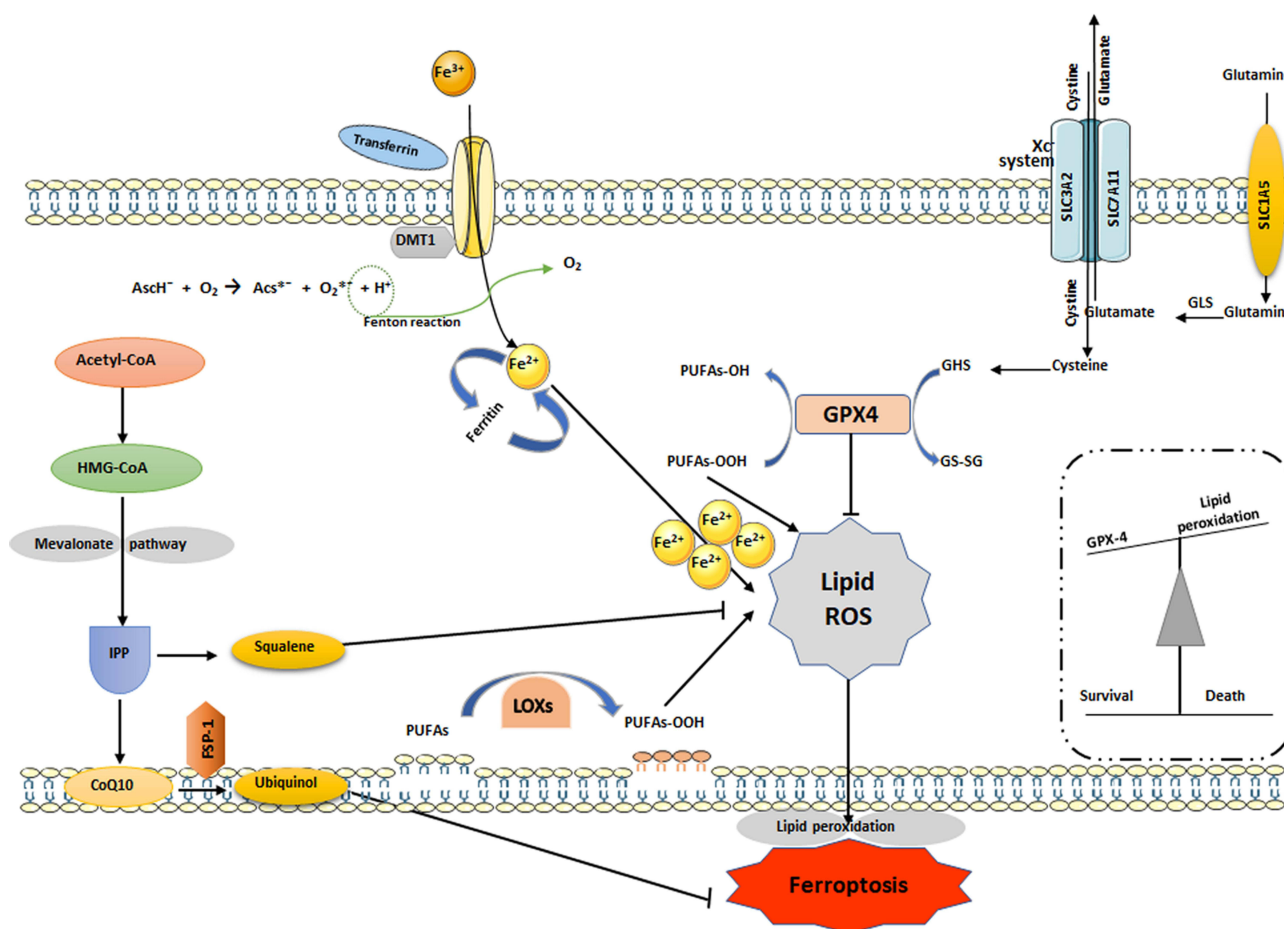
Figure 3 Regulatory pathways involved in ferroptosis. In ferroptosis, PUFAs are oxidized by free radicals due to lipid peroxidation. Hydroxyl facilitates lipid peroxidation, which causes stress-induced cell damage. Ferrous iron catalyzes these lipid hydroperoxides (LOOHs) into ROS molecules, followed by impairing cell membrane integrity. By turning damaging lipid hydroperoxides into lipid alcohols with the help of glutathione (GSH), GPX4 controls ferroptosis. Cystine is crucial for controlling lipid peroxidation by keeping GSH levels stable. SLC7A11 is a key regulator of SystemXc and is responsible for facilitating cystine/glutamate transport. Lipoperoxidation is inhibited by FSP-1, which is a NADPH-dependent coenzyme Q10 oxidoreductase located in cell membranes. Lipophilic radicals are contained within them, stopping the dissemination of lipid peroxide. This figure was created using Servier Medical Art Commons Attribution 3.0 Unported Licence (https://smart.servier.com/).
Abbreviations: GSH, glutathione; GPX4, Glutathione peroxidase 4; PUFA, polyunsaturated fatty acids with carbon-carbon double bonds; AscH-, ascorbate autoxidation; TFR1, transferrin receptor-like; GLS, glutamate by glutaminase; DMT1, divalent metal transporter 1; LOXs, lipoxygenases; SLC7A11, Solute Carrier Family 7 Member 11; LOOH, lipid hydroperoxides; FSP-1, ferroptosis suppressor protein; Ubiquinone-10, NADPH-dependent coenzyme Q10.

a decreased CD4+ T cell count, which play a major role in the persistence of *Leishmania* in vivo.[127] Both CD8+ and CD4+ T cells deficient of GPX4 were unable to expand and protect the immune cell population from *L. major* infection. Also, in an ex vivo experiment, T cells lacking GPX4 rapidly accumulated membrane lipid peroxidase, which led to ferroptotic cell death instead of necroptosis.[127] *Leishmania* can also express ascorbate peroxidase (APX), a gene associated with the trypanothione pathway that converts host hydrogen peroxide into water molecules and alters GXP expression, as well.[177,178] It has been observed that *L. braziliensis* and *L. infantum* strains which highly express APX are more likely to develop an antimony-resistant phenotype.[177,179] Similarly, elevated APX was found to regulate amphotericin-B resistance in clinical isolates of *L. donovani*.[180,181] APX is also an essential factor to control metacyclogenesis in *L. major* promastigotes and causes antimonial resistance.[182,183] Ascorbate-induced cell death and ferroptosis processes share common features including ROS generation, iron dependency, caspase independency and lipid peroxidation.[128] Lower concentrations of ascorbate could prevent ferroptosis, but ascorbate overexpression causes cellular cytotoxicity and ferroptosis.[128] Interestingly, anemia and abnormal erythrophagocytosis and hemophagocytosis have been implicated in *L. donovani* infections.[184] *Leishmania* infection also stimulates CD163 expression, boosts cytosolic heme oxygenase-1 (HMOX1) levels, and reduces ROS generation to promote parasite survival and replication.[185] Corroborating this with macrophage function for their important role in iron storage and recycling of senescent red blood cells (RBCs), macrophages ingest senescent RBCs, harvest a larger amount of

iron, and facilitate iron breakdown into cytosolic heme which is then used by HMOX1 and binds to hemopexin to be internalized in macrophages through the CD91 receptors.[186–189]

The exploration of the role of ferroptosis during *Leishmania* infections has only recently begun, however observations of ferroptosis have been implied in other infections. Thus, these studies can help extrapolate possible therapeutic targets for leishmaniasis. For example, some studies suggested that *Trypanosoma cruzi* extract contained ascorbate-dependent peroxidase activity.[190–192] GPX-like glutathione peroxidases (Px I–III) are found in trypanosomes, however, these peroxidases are type II tryparedoxin peroxidases that reduce tryparedoxin at significantly greater folds than GSH.[193] Similarly, a large amount of ROS is generated in host cells infected with malaria parasites through activation of PAMPs and DAMPs, leading to cell death due to lipid peroxidation and ferroptosis.[194] Moreover, an instance of mutations in human TP53 caused defective p53 which led to accumulation of excessive iron in macrophages, and promoted M2 polarization, but inhibited *Plasmodium* infection and supported *Listeria* bacteria.[195] Like parasitic infection, GPX4-deficient T cells in mice infected with choriomeningitis virus also showed impaired T cell development due to abnormal lipid peroxidation, leading to T cell ferroptosis.[127] Additionally, when injecting ovalbumin into mice and humans, Yao et al, observed follicular helper T cells underwent ferroptosis, with higher lipid ROS and more of the final products of polyunsaturated fatty acid peroxidation, MDA (malondialdehyde) and 4-HNE (4-hydroxynonenal), in comparison to non-follicular helper T cells.[196] In the same vein, *Mycobacterium tuberculosis* (*Mtb*) can use host-stored iron for its own growth.[197,198] However, *Mtb*-infected macrophages have shown unregulated lipid peroxidation and decreased GPX4, which in turn significantly increased cell death.[199] Iron accumulation and lipid peroxidation also occurs in patients with cryptococcal meningitis.[200,201] Particularly, *Cryptococcus* infections in AIDS patients have been shown to activate macrophages in CSF and promote pro-inflammatory cytokines and chemokines that lead to abundance of iron transporters, like divalent metal transporter 1 (DMT-1) and ferroportin 1 (FPN-1), that promote increased extracellular iron uptake, which could be associated with ferroptotic conditions.[129–131]

In addition to conditions seen in infectious diseases, cancer cells also accumulate high levels of iron; therefore, ferroptosis has been more deeply studied in cancer models.[132] Although parasitic diseases and cancer are quite different, the basic knowledge, pharmacological targets and approaches in cancer studies can be leveraged for the investigation of leishmaniasis. Imbalanced iron metabolism is correlated with numerous cancer types such as breast, ovarian, brain, renal, and lung cancer.[138] For example, ferritin dissociation induces reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron, thus upregulating lipid peroxidation and mitochondrial superoxide generation which selectively kills cancer cells.[133] Similarly, a 1,2-dioxolane compound can oxidize ferrous molecules, leading to lipid peroxidation that eliminates cancer cells through ferroptotic mechanisms.[137] Suppressing or silencing the prominin2 gene in breast cancer cells has been shown to decrease intercellular iron export through formation of ferritin-associated multivesicular bodies.[134] In lung cancer cells, inhibition of nitrogen fixation-1 (NFS-1), an iron-sulfur cluster biosynthetic enzyme, stimulates transferrin receptors and downmodulates ferritin that leads to ferroptosis.[135] In high-risk neuroblastoma, obstruction of a ubiquitin ligase complex leads to the ability to reduce nuclear factor erythroid 2-related factor 2 (NRF2) inhibition, upsurging heme oxygenase-1 activity and suppressing GPX4 to kill cancer cells through a natural ferroptosis inducer compound withaferin A (WA).[202] Furthermore, ferroptosis may contribute to tumor immunity beyond direct killing, particularly in association with dendritic cells that play an important role against tumor cells. Studies have shown that gene ALox15-derived lipid peroxidation regulates dendritic cells and modulates adaptive immune responses, while in contrast, oxidizing phosphatidylcholine inhibits dendritic cell maturation and decreases differentiation of Th17 cells.[203] Additionally, IFN-γ plays an indispensable role in antitumor activity. Surprisingly IFN-γ can suppress ferroptosis-suppressor genes that aid with GSH production in cancer.[136] Thus, through both the ability of ferroptosis to induce direct killing of cancer cells and infected cells, and through the immunomodulation that can follow lipid peroxidation, these studies demonstrate the potential for new directions of research in developing leishmaniasis treatments where similar mechanisms may exist.

In adaptive immunity, GPX4 knockout mice showed the presence of antigen specific CD8+ and CD4+ cells, yet failed to expand T cell counts during chronic infection.[127] Mechanically, IFN-γ released from CD8+ T cells triggers lipid peroxidation and ferroptosis through the inhibition of system X_c^-.[136] Similarly, cysteine and PD-L1 strongly inhibit ID8 cell derived tumors along with increasing lipid peroxidation in tumor cells and elevating proportions of IFN-γ+ and

TNF-α+ CD8+ and CD4+ T cells in the tumor microenvironment.[136,204,205] Remarkably, expression of system Xc⁻ was negatively associated with CD8+ T cells and IFN-γ in cancer treatments and their outcome. To summarize, ferroptosis consists of a series of events involving redox imbalance, iron release ferritin, and lipid peroxidation, all which lead to the occurrence of cell death in various diseases and stress conditions. Ferroptotic cell death has been demonstrated to occur in tumor cells and in multiple infectious diseases with beneficial impacts for the host and reduction of disease burden. These findings support the further investigation of the several compounds identified to regulate ferroptotic pathways. More work in stimulation of ferroptosis in infected cells could further improve field-deployable therapies against *Leishmania* and other infectious diseases.

Conclusion

Overall, the realm of field-deployable therapies has room for improvement and advancement, and faces discrepancies in which treatments are truly and universally field-deployable. With current treatments being variable with regard to their accessibility, feasibility to be administered in the field, achieval of sterile immunity and risk of relapse, and more, a number of new prospects should be considered for *Leishmania* treatment going forward. *Leishmania* parasites have been shown to infect multiple cell types including monocytes, macrophages, dendritic cells, and neutrophils in multiple areas of the body. Consideration of this fact will allow for the development of directed therapies to eliminate the parasites from its cellular hosts for generation of sterile immunity. This is particularly appealing for targeting monocytes/ macrophages, which are considered to be the main cellular host of the parasite in the body. Also, targeting HSCs is desirable, since recent discoveries reveal how they provide a safe-heaven for *Leishmania* to avoid the immune system and a lair to the parasite to allow reinfection. By taking advantage of drugs and therapies developed to be used against other diseases, leishmaniasis patients can be treated with more advanced and specific therapies. The advantages of repurposing drugs impact a multitude of factors. First, patients will benefit from therapies with higher efficacy and less adverse effects. Second, since leishmaniasis is a neglected tropical disease causing economic burden to low- and middle-income countries, repurposing therapies may become a more affordable pathway to alleviate disease burden and eventually achieve eradication of the disease. Third, some therapies have already been approved for their use against other diseases, therefore the time to reach and treat *Leishmania* patients could be reduced. Lastly, by targeting unique pathways utilized by the parasites, future treatments may be enhanced. One prominent pathway which is being explored relatively recently is the ferroptosis cell death pathway. Although drugs targeting this pathway have not yet been universally approved, there are promising results in the case of other intracellular infections and cancer settings that indicate stimulation of ferroptosis or inactivation of ferroptosis-inhibiting genes have shown beneficial effects in these disease settings and thus merit investigation for leishmaniasis. Overall, current and potential treatments for leishmaniases, both field-deployable and experimental, provide promising candidates for the mitigation of disease and promotion of host outcomes to *Leishmania* infection.

Acknowledgments

Disclosure

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