REVIEW ARTICLE

CHALLENGES DURING TREATMENT OF MICROBIAL INFECTIONS: SANCTUARY NICHES, PERSISTENCE AND RELAPSE

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ABSTRACT

Many infectious diseases suffer from post-treatment clinical reactivation. The ontogeny of such treatment failure may relate to features of the pathogen, host and drug. Besides drug resistance, tissues or cellular niches that are escaping drug activity have gained increasing awareness in the last couple of years. Evidence has also emerged for pathogen dormancy and persistence in combination with hiding in such sanctuary niches. Protozoans such as *Trypanosoma*, *Toxoplasma* and *Plasmodium* are liable to treatment failure resulting from infection of tissues such as the adipose, central nervous system and liver respectively. Several bacteria, most notably *Mycobacterium*, *Staphylococcus* and *Salmonella*, are known to form persisters that are drug tolerant and that can reside in multiple sanctuary niches. This review will focus on the tissues and host cells reported to provide sanctuary underlying treatment failure and subsequent relapse, whereby visceral leishmaniasis will be elaborated as a disease that is prone to relapse of multifactorial origin.

Highlights

- Post-treatment clinical reactivation is increasing in both epidemiological occurrence and in scientific awareness, with a clear link to tissue niches and pathogen persistence/quiescence.
- Largely overlooked niches such as stem cells in the bone marrow have been shown to constitute a hospitable reservoir for pathogens ranging from bacteria to parasites.
- Our research has uncovered that visceral leishmaniasis persists after treatment whereby parasites find sanctuary in a hematopoietic stem cell niche and by transitioning through a quiescent state.
- Important arguments are provided for innovation in the drug discovery and R&D pipeline to incorporate sanctuary niches and pathogen quiescence to overcome the risk of relapse.

Introduction: the ontogeny of treatment failure

A notorious challenge for a plethora of major microbial diseases is establishing an effective curative treatment. Challenges are even more prominent in tropical settings where access to proper healthcare and logistics for drug availability are more limited. In the last decade, an important impact of environmental changes has also been noted, where global warming has resulted in the expansion of especially vector-transmitted diseases to new geographical areas **[1]**. For many infectious organisms an alarming rate of drug resistance **[2-5],** treatment failure and disease relapse are reported **[6-8]**. Although drug resistance and treatment failure can be related, these concepts cannot be interchanged. Drug resistance can be acquired by the pathogen due to a variety of mechanisms, involving the emergence of genetic and/or metabolic alterations, giving rise to an attenuated response to the drug **[9, 10]**. Indeed, in broad and general terms, drug resistance is a decrease of compound efficacy against a pathogen population that was previously susceptible, consequently leading to the need for higher drug exposure or even complete clinical drug ineffectiveness at the maximal safe or tolerated drug dose **[11, 12]**. Treatment failure on the other hand can be the result of numerous factors, situated at the level of the drug itself, the host or the pathogen. Drug-associated factors include subtherapeutic exposure due to pharmacokinetic properties or poor pharmaceutical quality of the medication. Particularly important pharmacokinetic properties in the context of treatment failure are tissue distribution and the elimination half-life of the compounds **[13-17]**. For example, the plasma half-life for the antileishmanial drugs miltefosine (MIL) and AmBisome both exceed 5 days **[18, 19]**, causing them to linger in the body at subtherapeutic concentrations for weeks after treatment which may, aside from treatment failure, trigger the emergence of resistance. Besides pharmacokinetics, drug quality is another major

contributor to treatment failure. The shelf life of many drugs depends on proper storage, which may require a cold supply chain to the patient, which may not be straightforward in tropical countries **[20, 21]**. In some regions, drugs requiring a cold-chain are therefore substituted by drugs with less optimal profiles **[22]**. Moreover, due to both cost and limited drug availability, patients in developing countries are more likely to be treated with counterfeit medications that fail in terms of efficacy and may favor resistance development **[23, 24]**.

Host factors include *e.g.* immunity, nutritional status or compliance. An effective immune response is often required to support treatment, therefore patients with immunodeficiencies can be particularly hard to cure. A notoriously challenging co-infection is leishmaniasis with human immunodeficiency virus (HIV). In general, HIV co-infection is associated with higher initial treatment failure and relapse rates due to immune exhaustion and chronic immune stimulation **[25-27]**. Natural heterogeneity in host immune responses can also influence the potency of a drug **[28]**. For example, MIL has been used successfully to treat leishmaniasis in India, whereas its efficacy in Africa is lower **[29]** and failed during a clinical trial in Brazil. Besides host genetic factors, pathogen genetics in different geographical areas are implicated as well, as the 40% relapse rate in Brazil was associated with the absence of the "MIL sensitivity locus" **[30]**. Other pathogen factors influencing treatment failure include the intrinsic virulence, which can affect the induced host immune response and can result in elevated pathogen burdens that require higher drug doses **[31]**. Microorganisms can also reside in tissues inaccessible to drugs, or within cells exhibiting unique properties aiding in the escape from drug exposure or the immune response **[32, 33]**. The adaptive characteristics of pathogens also allow them to transform from a metabolically active state into a quiescent or persistent form that is more impervious to treatment **[34]**.

Sanctuary sites and persisters

For many infectious diseases, the ontogeny of treatment failure is not straightforward and is most likely multifactorial. Without the need of genetic or phenotypic changes, pathogens can exploit particular tissue or cellular tropism to survive and escape treatment or immunity **[35, 36]**. The body comprises several potential niches ranging from specialized tissues **(Figure 1)** to specific cells or cellular compartments that differ in accessibility for immune responses and therapeutic agents. Host cells and tissues can as a result underlie (re)colonization of the host and relapse **[37-39]**. This phenomenon of sanctuary sites or niches has been described for a range of pathogens across the microbiological spectrum **[32]**.

The advantage for a pathogen to exhibit a certain tissue tropism can relate to the characteristics of the tissue, the constituting cells or the subcellular compartment. In addition, tissue or cellular tropism can change over the course of infection, *e.g.* differing between the acute and chronic stage of the disease. For example, acute *Toxoplasma gondii* infections are associated with infection of a broad range of nucleated cells, while chronic infections persist in tissue cysts that establish in skeletal/smooth muscle cells and long-lived cells (neurons) of the central nervous system, known to be poorly accessible for many pharmaceutical agents **[40]**.

It has been understood that treatment failure can also derive from pathogens that survive treatment without selection of inheritable genetic alterations. Such organisms exhibit phenotypic diversity, *e.g.* encompassing quiescent or dormant forms that can persist after treatment **[32]**. These pathogens are mostly metabolically inactive, slow growing or have other specific changes that differ from the active pathogen. These changes are also reversible.

Properties of sanctuary tissues and cellular niches

Some biological tissue properties related to treatment are for instance the low perfusion rate of adipose tissue **[33]** and bone marrow **[41]**. Likewise, certain tissues are largely privileged from the systemic circulation as they are governed by barriers such as the blood-brain **[42],** the blood-cerebrospinal fluid (CSF) **[43]** or the blood-testis barrier **[44]**. Some other tissues provide an immune-privileged niche, such as the eyes **[45]** and hair follicles **[46]**. Characteristics of compounds themselves can also determine success or failure depending on the targeted tissue, *e.g.* hydrophilic drugs do not effectively permeate adipose tissue **[47]**.

Intrinsic properties of stem cells may also provide opportunities for the pathogen to evade immune responses and drug action. For example, mesenchymal stem cells do not normally express major histocompatibility complex (MHC) class II on their cell surface and their MHC class I molecules are functionally inactive, *i.e.* these molecules do not trigger effector functions of cytotoxic T lymphocytes **[48]**. Furthermore, stem cells have been described to express high levels of drug efflux pumps that could contribute to low drug exposure **[49]**. During visceral leishmaniasis (VL) infection, substantially decreased levels of *Nos2* gene expression and of both nitric oxide (NO) and reactive oxygen species (ROS) were demonstrated in infected long-term hematopoietic stem cells (LT-HSC), creating a more hospitable environment for parasite multiplication and survival **[37]**. Other cells withdraw from cell cycle progression and trigger differentiation of the pathogen to a quiescent stage, such as the skeletal muscle

cells during *T. gondii* infection **[50]**. Adipocytes are an excellent niche and well-known reservoir for *Trypanosoma cruzi*, the causative agent of Chagas disease, as these cells are rich in nutrients and secrete the anti-inflammatory adipokine adiponectin **[51-53]**. During *Mycobacterium tuberculosis* (*Mtb*) infection, monocytes are differentiated to longer-lived foamy macrophages characterized by the presence of lipid-containing bodies, which afford dormant *Mtb* bacilli access to host cholesterol, required for persistence **[54, 55]**.

Some pathogens adopt intracellular invasion strategies in non-phagocytic cellular niches to persist. For example, *T. cruzi* is able to invade various host cells by fusion of lysosomes at the site of invasion **[56-58]**. Hereby, the parasite takes advantage of the parasite-induced membrane damage to enter the cells, stimulating lysosomal exocytosis pathways to repair the membrane **[57, 59]**. *T. cruzi* is known to escape rapidly from the vacuole into the cytoplasm as a favorable environment to differentiate and replicate **[60]**.

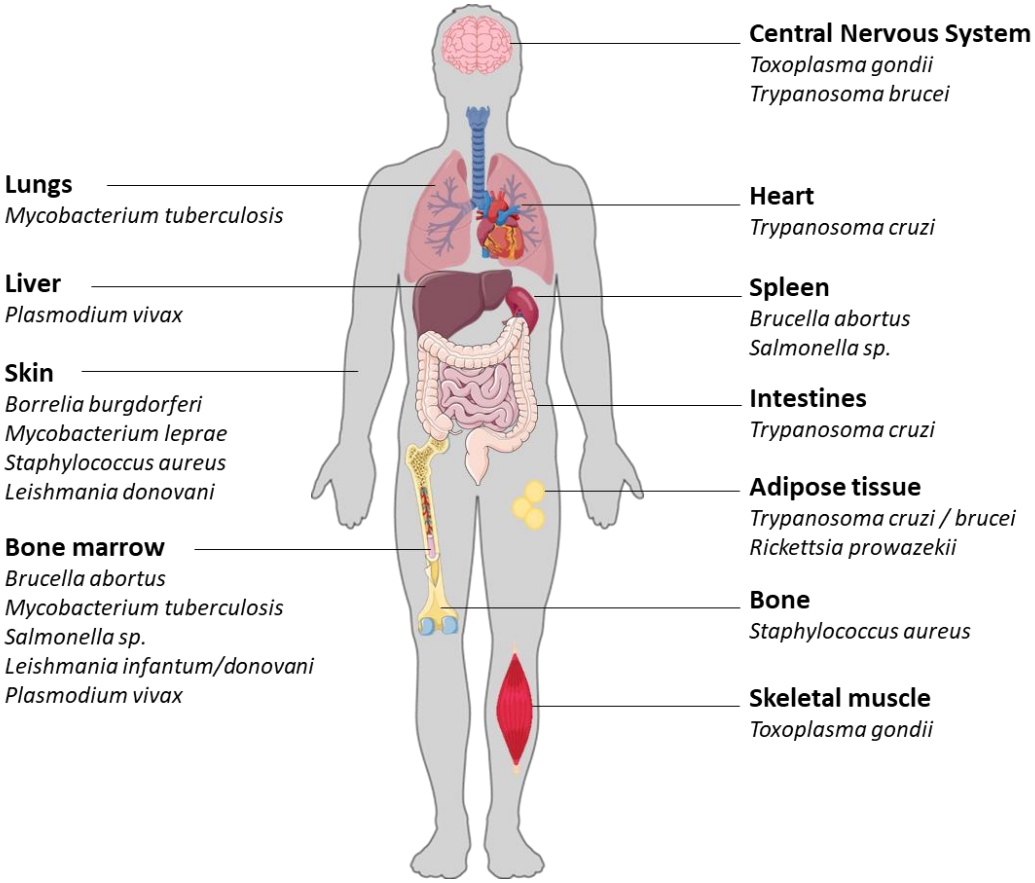

Figure 1. Tissue niches of specific pathogens linked to treatment failure. Neuronal and skeletal muscle cells are niches for *Toxoplasma gondii* [61-64]. African trypanosomes invade the central nervous system which represents a challenge for effective drug treatment across the blood-brain barrier [65, 66]. In the adipose tissues, *Trypanosoma cruzi* / *brucei* [67-70] and *Rickettsia prowazekii* [71] can persist. *Brucella abortus* and *Salmonella typhimurium* use the spleen as a reservoir [72-74]. *Plasmodium vivax* resides in the hepatocytes of the liver and causes

recrudescence of malaria [75-77]. Persistent skin infections with the Lyme spirochete, *Borrelia burgdorferi*, have been linked to relapse [78-81]. *Mycobacterium leprae* is known for persisting in the skin [82], as is *Staphylococcus aureus* [83]. *Leishmania donovani* parasites can persist in the skin and cause post-kala-azar dermal leishmaniasis [84]. The lungs are a notorious niche for *Mycobacterium tuberculosis* persistence [54]. The bone marrow is a niche for many pathogens, including monocytes for *Brucella abortus* [85, 86], stem cells for *Mycobacterium tuberculosis* [87-90], *Leishmania infantum/donovani* [37], and *Plasmodium vivax* [91], and possibly macrophages for *Salmonella typhimurium* [92-94]. The figure was created using SmartServier.

Adipose tissue

The adipose tissue has been infamous to serve as a site of refuge, as it is less accessible for chemical compounds due to the low perfusion rate. For example, Combs *et al.* **[67]** demonstrated significant numbers of *T. cruzi* in the adipocytes of mice during a chronic infection. Further research also revealed persistence of this parasite in adipose tissue of chronic Chagas disease patients **[68]**. Benznidazole and nifurtimox are the current first-line drugs for acute *T. cruzi* infection, but have been ineffective during the chronic stage **[95-97]**, which may in part result from a decreased efficacy in adipose tissue. Indeed, both drugs need to reach the parasite and undergo enzyme-mediated activation to have cytotoxic effects **[98]**. Currently an effort is undertaken to develop nanocarriers for effective delivery, such as lipid nanovesicles for better permeability of certain tissues **[99]**. *T. brucei*, an extracellular parasite causing sleeping sickness, has a transcriptionally distinct 'adipose tissue form' (ATF) that is described as slow-growing and quiescent, therefore possibly responsible for recrudescence of the disease **[69, 70]**.

In addition to trypanosomes, it has been documented that *Rickettsia prowazekii*, a bacterium transmitted by body lice that causes epidemic typhus, can reside in this tissue after treatment. This suggests that adipose and adipocytes play important roles in the occurrence of Brill-Zinsser disease, the recrudescent manifestation of epidemic typhus **[71]**.

Skin

Persistent infections with the Lyme spirochete, *Borrelia burgdorferi*, have been demonstrated in patients who have been treated with the proper antibiotics, yet regain symptoms after alleviating drug pressure **[78-81].** *Mycobacterium leprae* is notorious for persisting in the skin. As such, multiple relapses after multidrug treatment have been reported **[82]**. Other bacterial persisters in the skin include *Staphylococcus aureus*, where it was shown that persisters have an increased infectivity, causing more severe lesions **[83]** and a tolerance to antibiotic treatment, linking the persister phenotype to chronic and relapsing *S. aureus* infections **[100]**. A specific dermal complication that can develop after the protozoan *Leishmania donovani* infection is known as post-kala-azar dermal leishmaniasis (PKDL), in

which the parasites persist in the skin after treatment causing a highly transmissible condition with self-healing lesions that usually disappear within a year **[84]**.

Liver

The liver can be colonized by dormant or hypnozoite stages of *Plasmodium vivax*, a causative agent of malaria. These are less susceptible to anti-malarial therapies and can be reactivated **[75]**. Hypnozoites appear responsible for relapse of *P. vivax*-mediated malaria even years after initial infection **[76]**, although studies in human liver chimeric mice suggest that hypnozoites may actually not be fully metabolically inactive **[77]**. Studies on treatment efficacy of chloroquine and primaquine have documented *P. vivax* relapse rates varying from 8% **[101]** to 38% **[102]**. To prevent relapse of vivax malaria, an additional 14-day primaquine (0.5–0.75 mg/kg per day) cure is recommended, targeting the hypnozoite stages **[103, 104]**. However, individuals with specific CYP2D6 polymorphic alleles fail to metabolize primaquine and experience treatment failure **[105]**. Likewise, primaquine can cause hemolysis in individuals with a genetic enzyme deficiency (G6PD), requiring downward adjustment of the treatment dosage **[104]**.

Central nervous system

There has long been evidence that the cyst form of *T. gondii* can reside and persist in neuronal cells **[61]**. *T. gondi* cysts reside in the brain for extensive periods and this process requires a continuous immune response to prevent the parasite's reactivation **[62]**. Especially in HIV-patients, cerebral toxoplasmosis is a life-threatening condition that requires prompt diagnosis and treatment **[106]**.

African trypanosomes are notorious for crossing the blood-brain and blood-CSF barriers, causing typical deregulation of the sleep-wake cycles **[65]**. This reservoir in the central nervous system has been described as a source of relapse for decades **[66]**, requiring drugs that can cross the blood-brain barrier such as fexinidazole and eflornithine **[107, 108]**.

Skeletal muscle

Infection of skeletal muscle by *T. gondii* triggers differentiation from the highly replicative tachyzoites to dormant bradyzoites and tissue cyst formation, both are crucial for parasite persistence in muscle tissue **[63]**. A current limitation is that therapies are effective against tachyzoites but not against bradyzoites, due to its dormant phenotype **[64]**.

Spleen

In the spleen, the presence of *Brucella abortus* was detected in splenic B lymphocytes which protect the bacteria against bactericidal agents. Especially the marginal zone B cells seems to be the preferred target **[72]**. Similar conclusions could be drawn for *Salmonella typhimurium* where the presence of the bacteria was confirmed in splenic B cells. Data demonstrated that all precursors as well as plasma cells were infected with the bacterium **[73]**. Additionally, *Salmonella*-specific B cells were shown to act both as a survival niche and a reservoir for reinfection **[74]**.

Lungs

One of the most notorious persistent diseases of the lung is tuberculosis. A hallmark of tuberculosis is the ability of the causative agent, *Mtb*, to persist for decades despite a vigorous host immune response. Although the immune response effectively controls the replication of bacteria, they are able to resist eradication, resulting in chronic tuberculosis, characterized by slowly replicating bacteria and progressive immunopathology **[54]**. These slow or sometimes nonreplicating bacteria exhibit extreme tolerance to many first- and second-line *Mtb* drugs **[109]**.

Heart

Chagas cardiomyopathy is the most common infectious cause of heart failure, whereby a cardiac parasite burden is a hallmark of chronic infection **[110]**. Dormancy in the mammalian infection cycle of *T. cruzi* is key to the failure of current drug treatments. While other factors, including the differential tissue tropism of parasite strains and tissue distribution of potential drugs, certainly also impact treatment outcomes, only dormancy has been definitively linked **[35]**.

Gastrointestinal tract

A study from Lewis *et al.* identified the gastrointestinal tract as the primary site of parasite persistence for long-term *T. cruzi* infection **[111]**, associated with conditions such as megaesophagus and megacolon.

Bone and bone marrow

Long-term intracellular infection of bone cells, *i.e.* osteoblasts, osteoclasts and osteocytes, by *S. aureus* has been described as a mechanism for infection persistence and recurrence following long periods of dormancy **[112, 113]**. The bone marrow has also been increasingly understood as a pivotal sanctuary niche. For instance, it was observed by Gutiérrez-Jiménez *et al.* **[85]** that *B. abortus* persists in the bone marrow and particularly resides in monocytes which are most likely the source of relapse in approximately 10% of brucellosis patients **[86]**. There is also evidence of persistence of *Salmonella* in the

bone marrow **[92]**, presumably inside hemophagocytic macrophages **[93]**, responsible for relapse rates of 5% to 20% **[94]**.

A lot of effort has been done in describing the role of the bone marrow during tuberculosis. Indeed, the bone marrow was identified as an antibiotic-protective niche where *Mtb* can infect CD271+CD45- mesenchymal stem cells (MSC) and long term hematopoietic stem cells (LT-HSC) **[87-90]**. Importantly, it was demonstrated that even after prolonged treatment, the bacterium remained present in CD271+ MSC, linking these observations with the occurrence of relapse **[114, 115]**. Unexpectedly, the hematopoietic niche of the bone marrow has also been discovered as a reservoir for *P. vivax*. Here, proliferation of malaria parasites occurs as well as gametocyte development **[91]**.

Properties of persisters

The largest body of evidence and mechanistic information for persistence has been obtained for bacterial pathogens. Indeed, bacterial persistence was first reported 80 years ago for staphylococcal infections treated with penicillin **[116]**. Nevertheless, the underlying mechanisms of persistence in general remain an enigma. Classically accepted features of persisters are that they comprise only a subpopulation, with a dormant phenotype that endows the pathogen a multidrug tolerance that is non-inheritable and reversible **[117]**.

The central dogma entails that bacteria can alternate between planktonic growth and formation of persisters that often reside in biofilms. Persisters refer to genetically drug susceptible, quiescent (non-growing or slow growing) organisms that survive exposure to a given cidal drug and have the capacity to regrow under highly specific conditions **[36]**. The term persisters refers to a heterogeneous group, whereby some types are formed in response to external triggers, while others switch phenotype in the absence thereof **[118]**. External triggers include drugs, starvation, heat, acidic pH and oxidative stress **[119, 120]**. Several complex mechanisms have been proposed to lay at the basis of bacterial persistence. Toxin/antitoxin (TA) systems are often mutated in high-persistence genetic screens and overexpression of these toxins often increases the frequency of persisters in a population **[121, 122]**. The TA system has been observed to contribute to persister formation for *Mtb* **[123]** and S. *typhimurium* **[124]**. Others include reduced metabolism, energy production, protein and nucleic acid synthesis, DNA repair and protection, protein degradation, transporters/efflux systems, and transcriptional regulators (reviewed in **[125, 126]**). Several studies have been conducted, for instance in *S. aureus*, to elucidate the transcriptional alterations associated with persisters, where several stress responses were activated,

including the stringent response, cell wall stress, SOS response (a complex response to DNA damage) and heat shock response **[127]**. Recent evidence also suggests a major role of epigenetic regulations, such as DNA methylation, that stably alter gene expression without modifying genomic sequences **[128]**. For parasites, information about mechanisms of persistence is very scarce, due to its relatively recent discovery. The main limitation hindering the study of persister cells in any disease is the very low frequency of occurrence **[34]**. A recurrent feature of protozoan persisters is the decrease in metabolic activity. Studies have shown that DNA replication, general transcription and protein synthesis are decreased in *Plasmodium spp*. and *T. gondii* persisters **[32]**.

Visceral leishmaniasis: an example of the multifactorial origin of treatment failure

For VL, post-treatment relapse rates are described for all currently used drugs. Relapse rates of up to 7% for AmBisome **[129]**, 20% for MIL **[130]** and 38% for antimonials **[131]** have been reported in the last decade. Even combination therapy, *e.g.* with antimonials and paromomycin, showed a relapse rate of 6% **[132]**. Relapse rates for VL are also increasing considerably. In South-Sudan, relapse as a proportion of all VL cases increased by 6.5% per annum, from 5.2% during 2001–2003 to 14.4% during 2016–2018 **[8]**. Moreover, VL relapse patients have a high chance of relapsing again, *e.g.* a recent study in Sudan using AmBisome revealed that 10% of relapse patients underwent new relapse episodes **[129]**. Notably, an increased infectivity associated with relapse has already been shown for MIL and antimonial treatment in the Indian subcontinent **[133-135]**, suggesting that the selected phenotype may pose an additional threat to leishmaniasis control programs.

The current VL treatments do not accomplish sterile cure and display severe limitations such as toxicity, high production cost, decreased efficacy, difficulty in administration, and most importantly the emergence of resistance **[136-141]**. The acquisition of such resistance mechanisms can be associated with either (i) decreased drug uptake, (ii) increased drug efflux, (iii) enzymatic drug inactivation, (iv) improved cellular mechanisms to deal with drug-induced stress of cell damage, and/or (v) changes in the expression, abundance or drug binding affinity of the primary therapeutic target **[142]**. For MIL it has been shown that MIL-resistant *Leishmania* strains show reduced fitness, however exposing this phenotype to MIL treatment restores their infectivity, proving that their fitness is drug dependent, which emphasizes the risk of MIL treatment in sustaining infections with resistant parasites **[143, 144]**.

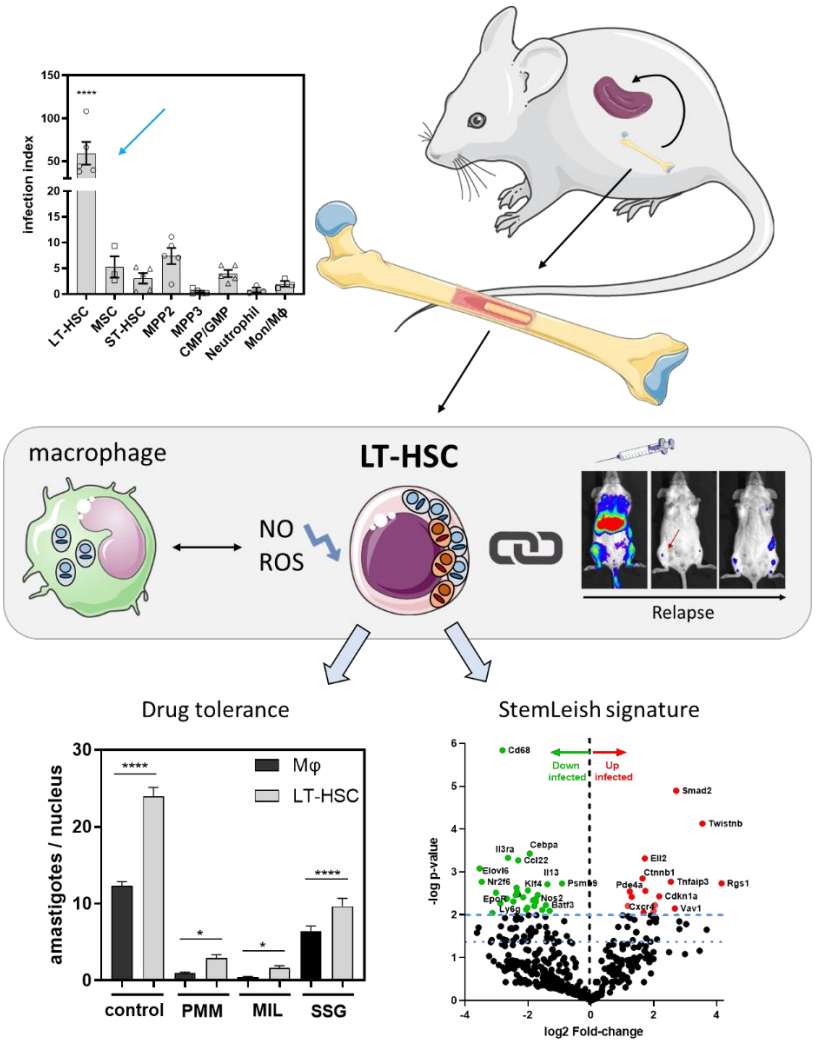

Figure 2. Discovery of the stem cell niche linked to relapse during visceral leishmaniasis [37]. In the bone marrow relapse niche of VL infected animals, stem cells harbor very high numbers of parasites and display decreased oxidative stress levels, which makes them more tolerant to antileishmanial drugs. These infected stem cells express a unique transcriptional signature, defined as StemLeish. Long-term hematopoietic stem cells (LT-HSC), mesenchymal stem cells (MSC), short-term hematopoietic stem cells (ST-HSC), multipotent progenitors (MPP), common myeloid progenitor (CMP), granulocyte-monocyte progenitor (GMP), monocyte (Mon), macrophage (Mϕ), nitrogen oxide (NO), reactive oxygen species (ROS).

We recently demonstrated the importance of the bone marrow as a sanctuary niche for a persistent VL infection and post-treatment relapse. Indeed, the bone marrow was identified as a sanctuary site from where the host can be recolonized **(Figure 2)**. In this tissue, LT-HSC (Lin- Sca1+ cKit+ CD48- CD150+) were found to constitute a hospitable cellular niche (Figure 2, top left) with low oxidative stress levels and harboring enormous parasite burdens, which render them more tolerant to antileishmanial drug action (Figure 2, bottom left). Infected LT-HSC express a unique transcriptional signature, termed StemLeish, defined by upregulated TNF/NF-kB and RGS1/TGF-β/SMAD/SKIL signaling, and a

downregulated oxidative burst (Figure 2, bottom right). Cross-species analyses demonstrated significant overlap with human VL and HIV co-infected blood transcriptomes **[37]**.

For LT-HSC, a decreased treatment response could not be linked to drug efflux and is likely related to the observed extreme high parasite burdens. Other mechanisms of the LT-HSC niche can play a role such as drug distribution to the bone marrow **[41]**. In general, the bone marrow contains at least two different types of niche based on location, *e.g.* periosteal or perivascular. The former provides a hypoxic environment with differential sensitivity to therapy **[145]**. Zhao *et al* **[146]** showed that HSCs can be functionally distinguished into reserve HSCs and primed HSCs based on their response to chemotherapy, and which is linked to their different position in the bone marrow niche and distance to the blood vessel. The LT-HSC are specifically in close proximity of capillary fenestrations, enabling drugs that pass through these fenestrations to directly encounter LT-HSC **[41]**. Drugs with favorable pharmacokinetic properties to target the bone marrow would potentially be more effective in targeting the LT-HSC burdens and preventing persistence and post-treatment relapse. Artificial culture systems have suggested the occurrence of *Leishmania* quiescence after treatment with antimonials or upon exposure to experimental stress conditions **[147, 148]**, associated with a downregulated synthesis of ATP, ribosomal components, proteins and alterations in membrane lipids **[147, 149]**. Recently, we discovered *in situ* acquisition of quiescence in parasites infecting LT-HSC and described its downstream effects on parasite biology: increased survival under drug pressure, increased infectivity and high transmissibility **[150]**. The occurrence of resistance, sanctuary niches and quiescence underscores the multifactorial origin of treatment failure during leishmaniasis.

Conclusion

Significant gaps remain in our understanding of host–pathogen interactions, pathophysiology, and the implications for treatment and establishment of appropriate tests-of-cure **[39]**. The functional consequences of tissue or cellular tropism as well as pathogen quiescence or persistence remain poorly studied, despite the association with important aspects of the disease, including transmission, treatment failure, relapse and clinical outcome. Indeed, it is reasonable to suspect that slowly replicating or transiently arrested microbial pathogens have a selective advantage under immune and drug pressure and play a role in sustaining chronic infections in asymptomatic individuals and pre-relapse patients. Innovative detection methods are therefore needed, not only to provide proper treatment and enable accurate post-treatment follow-up, but also to tackle the dissemination of infection. Highly specific biomarkers and new host-directed therapeutic targets may serve these

purposes. Besides focusing on the host, there is an unmet need for drugs that act against quiescent pathogens within their cellular niches. For this, transcriptional profiling can uncover potential drivers of quiescence and provide targets for novel combination therapies. Additionally, due to the many links between relapse niches and dormant phenotypes for several pathogens, the currently available genetic toolbox to study host and pathogen genes will offer unprecedented insights in the universal problem of quiescence across the microorganism spectrum.

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Appendix

Table 1. Abbreviations in alphabetic order.

Written in full	Abbreviation
Adipose tissue form	ATF
Brucella abortus	*B. abortus*
Cerebrospinal fluid	CSF
Cytochrome P450 2D6	CYP2D6
Glucose-6-phosphate dehydrogenase	G6PD
Human Immunodeficiency Virus	HIV
Leishmania donovani	*L. donovani*
Long-term hematopoietic stem cell	LT-HSC
Major histocompatibility complex	MHC
Mesenchymal stem cells	MSC
Miltefosine	MIL
Mycobacterium tuberculosis	*Mtb*
Nifurtimox Eflornithine Combination Therapy	NECT
Nitric oxide	NO
Plasmodium vivax	*P. vivax*
Post-kala-azar dermal leishmaniasis	PKDL
Reactive oxygen species	ROS
Salmonella typhimurium	*S. typhimurium*
Staphylococcus aureus	*S. aureus*
Toxin/antitoxin	TA
Toxoplasma gondii	*T. gondii*
Trypanosoma brucei	*T. brucei*
Trypanosoma cruzi	*T. cruzi*
Visceral leishmaniasis	VL