ACS Infectious Diseases

pubs.acs.org/journal/aidcbc

Review

Beyond the Sand Fly and the Macrophage: A Multidimensional Redefinition of the *Leishmania* Life Cycle to Overcome Therapeutic Persistence

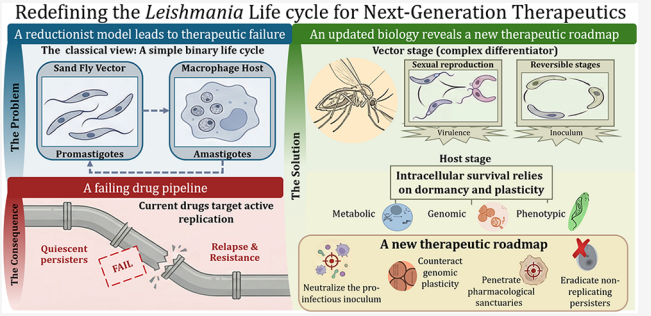
Javier Martín-Escolano,* Clotilde Marín, Anastasios D. Tsaousis, Mohinder Pal, and Rubén Martín-Escolano*

Cite This: https://doi.org/10.1021/acsinfecdis.6c00135

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Leishmaniasis remains a major global health challenge, hampered by an antiquated therapeutic arsenal compromised by toxicity, resistance, and an inability to achieve a sterile cure. The high attrition rate in drug and vaccine development stems from a fundamental disconnect between traditional experimental models and the true biological complexity of the *Leishmania* life cycle. This review synthesizes recent breakthroughs that have reshaped our understanding of the parasite, arguing that these nonclassical traits are the primary obstacles to effective treatment. We deconstruct the updated life cycle, beginning with the vector stage, which involves reversible differentiation pathways and sexual recombination, generating highly virulent and drug-resistant hybrids. We then analyze the vector inoculum, a complex pro-infectious environment comprising saliva, exosomes, and viral endosymbionts that preconditions the host for infection. At the cellular level, we detail the three pillars of amastigote persistence: metabolic plasticity to survive in nutrient-scarce niches, genomic plasticity (mosaic aneuploidy) to rapidly adapt to drug pressure, and phenotypic plasticity, which generates quiescent persisters tolerant to chemotherapy. Furthermore, we explore the parasite's expanded host-cell tropism, including noncanonical reservoirs such as fibroblasts and adipocytes, which serve as pharmacological sanctuaries. Finally, we discuss how targeting these biological complexities, eradicating persisters, neutralizing the vector inoculum, and reaching cryptic reservoirs are prerequisites for the next generation of drugs and vaccines. This review posits that the era of "one bug, one drug" is over and that the path to a sterile cure for leishmaniasis lies in a biology-driven, precision-medicine approach.



KEYWORDS: *Leishmania, Nonclassical Life Cycle, Parasite Persistence, Host-Parasite Interactions, Sterile Cure*

Leishmaniasis, a neglected tropical disease (NTD) caused by protozoan parasites of the genus *Leishmania*,[1] is a major global health challenge. Affecting over 12 million people and placing another one billion at risk across 99 countries,[2] the disease manifests in distinct clinical forms: cutaneous leishmaniasis (CL), mucosal leishmaniasis (ML), and visceral leishmaniasis (VL).[1] Despite the high morbidity and mortality associated with these conditions—particularly VL—the therapeutic arsenal remains antiquated and characterized by high toxicity and increasingly compromised by drug resistance.[3,4] In recent years, the field has shifted from viewing leishmaniasis primarily as a problem of limited drug availability to recognizing fundamental biological barriers that underlie treatment failure and parasite persistence. Furthermore, regardless of the causative species, current treatments fail to achieve a sterile cure. Consequently, the parasite persists within the host, posing a lifelong risk of relapse, particularly in immunocompromised individuals[5] such as those coinfected with HIV.

Current management strategies remain heavily dependent on pentavalent antimonials (SbV), which have been the first-line therapy for over seven decades despite their association with severe adverse effects, including cardiotoxicity, pancreatitis, and hepatotoxicity.[4] Furthermore, their clinical utility is increasingly compromised by widespread resistance, particularly in the Indian subcontinent.[6] While liposomal amphotericin B (L-AmB) is a highly effective alternative with a superior

Received: February 6, 2026
Revised: April 8, 2026
Accepted: April 10, 2026

safety profile, its widespread deployment is restricted by prohibitive costs, cold chain requirements, and parenteral administration.[6] Miltefosine, the sole oral agent available, is constrained by significant gastrointestinal toxicity, teratogenicity, and a long pharmacokinetic half-life that facilitates the selection of resistant strains.[7] Finally, Paromomycin, an aminoglycoside protein synthesis inhibitor, exhibits variable efficacy, depending on the geographic region and the specific *Leishmania* spp. involved, with lower cure rates in East Africa than in India.[8]

Against this backdrop, research conducted in recent years has converged on several central questions: why do current therapies fail to eradicate the parasite despite apparent clinical cure; which parasite populations and host cell niches sustain long-term persistence; and how parasite-intrinsic plasticity undermines conventional drug discovery pipelines. The high attrition rate in the antileishmanial drug discovery pipeline, where promising *in vitro* hits frequently fail to translate into clinical efficacy, underscores a fundamental disconnect between experimental screening models and the biological reality of the infection.[9,10] Historically, drug discovery has relied heavily on axenic amastigote assays; however, these models fail to recapitulate the stringent metabolic constraints of the phagolysosome, the immunomodulatory influence of coinoculated vector-derived factors, or the physiological complexity of chronic, latent infections. Crucially, studies have demonstrated significant discrepancies in drug susceptibility profiles between axenic and intracellular amastigotes, rendering the former a poor predictor of clinical outcome.[9,11,12] Bridging this translational gap requires a paradigm shift that integrates the parasite's updated biology. *Leishmania* is defined by constitutive genomic instability (manifesting as mosaic aneuploidy[13]) and profound metabolic plasticity, enabling the parasite to switch carbon sources toward amino acid catabolism and fatty acid oxidation to survive in nutrient-restricted niches.[14,15] Moreover, over the past decade, the canonical life cycle has been substantially revised. We now recognize that vector development involves reversible differentiation pathways (such as the retroleptomonad stage[16]), that the parasite exploits noncanonical host cells—such as fibroblasts—to evade immune detection,[17] and that a subpopulation of amastigotes enters a quiescent, nonreplicating state[18] that confers phenotypic tolerance to conventional chemotherapies.

This review synthesizes these recent breakthroughs in parasite morphology, genetic plasticity, and tissue tropism, among others, arguing that targeting these nonclassical biological traits is the prerequisite for developing therapies capable of achieving a definitive, sterile cure.

1. THE VECTOR STAGE: COMPLEXITY BEYOND THE PROMASTIGOTE

The sand fly midgut functions not merely as a reservoir but as a dynamic, selective microenvironment in which the parasite undergoes a precise, multistep differentiation process essential for successful transmission. Understanding the molecular drivers of these stages is crucial not only for developing Transmission-Blocking Vaccines (TBVs)[19] but also for understanding how the vector primes the parasite for survival and persistence within the mammalian host.

1.1. The Updated Developmental Map: The Retroleptomonad Paradigm

Traditionally, the fly life cycle was described as a linear progression from procyclic to metacyclic promastigotes. Recent transcriptomic and morphological studies have elucidated a much more intricate pathway, characterized by distinct gene expression profiles at each stage.[20] Upon ingestion of an infected bloodmeal, amastigotes transform into procyclic promastigotes, which replicate within the peritrophic matrix. As the matrix degrades, they differentiate into nectomonads, nondividing, elongated forms that migrate to the midgut epithelium. Transcriptomic profiling reveals that these nectomonads undergo global translational repression, portrayed by downregulation of the replication machinery and concomitant upregulation of stress-response genes, an adaptive strategy that ensures survival in the harsh proteolytic environment of the midgut while preadapting the parasite for the subsequent host transition.[20]

Surface-expressed and secreted phosphoglycans are fundamental for parasite survival and transmission within the vector. Initially, they protect the parasite from the proteolytic activities of the blood-fed midgut and mediate attachment to the gut wall, thereby maintaining infection during the excretion of the bloodmeal. This critical anchorage is specifically achieved through modifications of the surface lipophosphoglycan (LPG) on nectomonads.[21] This interaction serves as the primary determinant of vector competence—a molecular "lock and key" mechanism that dictates which sand fly species can transmit specific *Leishmania* spp.; failure to anchor results in the parasite being flushed out, terminating the infection cycle.

The cycle continues with differentiation into leptomonads, the primary replicative stage that generates the large numbers of parasites required for transmission. Crucially, these leptomonads secrete promastigote secretory gel (PSG), a proteophosphoglycan-rich matrix. As the parasite population migrates anteriorly, a subset differentiates into haptomonads, nonreplicative forms that firmly anchor to the cuticular lining of the stomodeal valve. The resulting plug—a composite of anchored haptomonads embedded within the secreted PSG—physically obstructs the sand fly's anterior midgut.[22] This obstruction forces the vector to regurgitate a high-dose inoculum during subsequent bloodmeals. This massive parasitic load is a critical factor in overcoming the initial host immune response, thereby facilitating transmission efficiency and the establishment of a chronic infection that is difficult to sterilize.[23]

The most significant update in this cycle is the identification of the retroleptomonad stage. It was previously thought that differentiation into the infective metacyclic promastigote was a terminal event. However, it is now understood that if a sand fly does not take a bloodmeal immediately, metacyclic forms can revert to a replicative state (retroleptomonad) to maintain infection within the fly.[16] This reverse metacyclogenesis ensures vector infectivity throughout the sand fly's lifespan and represents an evolutionary adaptation to the stochasticity of vector feeding. Clinically, this implies that the vector maintains a permanent reservoir of infective forms, ensuring efficient transmission even in older vectors.

1.2. Sexual Reproduction: Genetic Exchange in the Vector

For decades, *Leishmania* was considered to propagate primarily through clonal evolution. This paradigm has been definitively overturned by evidence of sexual recombination within the

https://doi.org/10.1021/acsinfecdis.6c00135
ACS Infect. Dis. XXXX, XXX, XXX−XXX

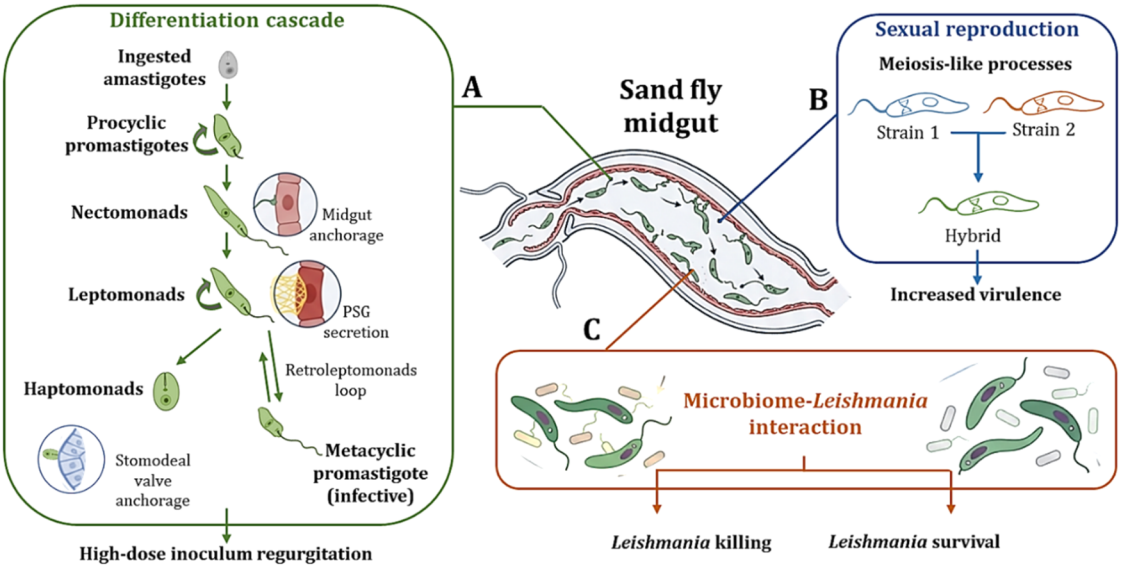


Figure 1. Updated Life Cycle of *Leishmania* in the Sand Fly Vector.

sand fly midgut.[24] Co-infection experiments have shown that *Leishmania* can undergo meiosis-like processes and form hybrid progeny.[25,26] These hybrids exhibit Mendelian inheritance of the nuclear genome and have been identified in the field, often exhibiting hybrid vigor (heterosis) or increased virulence.

The implications for drug resistance and treatment failure are profound. If a sand fly feeds on a host infected with a drug-resistant strain and then on a host with a wild-type strain, genetic exchange can occur. This facilitates the rapid spread of resistance alleles, such as mutations in the miltefosine transporter, across populations. Comparative genomic analyses have identified conserved meiosis-related genes, including *SPO11−1*, *HOP1*, and *DMC1*, which are upregulated during late-stage infection in the vector and facilitate homologous recombination.[27] Consequently, sexual recombination accelerates the emergence of multidrug-resistant strains that defy current therapeutic regimens.

Notably, while nuclear DNA follows Mendelian segregation, mitochondrial DNA (kDNA) inheritance appears more complex. Maxicircles typically show uniparental inheritance, whereas minicircles—which encode the guide RNAs for transcript editing—exhibit biparental inheritance, leading to heteroplasmic networks that may enhance the parasite's adaptive capacity.[25] This expanded repertoire of guide RNAs enables more robust mitochondrial RNA editing, potentially optimizing bioenergetic efficiency under stress, a phenomenon analogous to mitochondrial heterosis. Consequently, this genetic plasticity requires that new chemical entities (NCEs) be tested against diverse field isolates and hybrids to ensure robust efficacy across genetically distinct populations.

1.3. The Tripartite Interaction: Vector-Microbiome-Parasite

The vector midgut is a multipartite ecosystem shared with the sand fly's gut microbiota. The composition of the midgut microbiome is a critical determinant of vector competence, as specific bacterial taxa are essential for the successful differentiation of parasites into infective metacyclic forms.[28] Bacterial symbionts can trigger the sand fly's immune deficiency pathway, producing antimicrobial peptides that can neutralize *Leishmania*. Conversely, certain bacteria may facilitate parasite survival by modulating local pH or competing with pathogenic microbes. This tripartite interaction (Vector-Microbiome-Parasite) represents a novel, nonchemical target for transmission-blocking strategies, such as paratransgenesis, which involves modifying symbiotic bacteria to serve as a platform for secreting antileishmanial molecules within the fly midgut.[29]

These ecological dynamics, together with the developmental plasticity and sexual recombination events described above, are integrated into the comprehensive life cycle model presented in Figure 1.

The differentiation cascade of *Leishmania* within the sand fly midgut is a complex, multistep process. (A) Following the ingestion of amastigotes, the parasite differentiates into various forms, including procyclic promastigotes, nectomonads, leptomonads, haptomonads and infective metacyclics. This process is not linear and includes a reversible retroleptomonad stage that ensures lifelong vector infectivity. (B) The midgut also serves as a site for sexual recombination between different parasite strains, generating hybrids with potentially increased virulence or drug resistance. (C) The entire process is modulated by a tripartite interaction with the gut microbiota, which is a critical determinant of vector competence.

2. THE VECTOR INOCULUM: A COMPLEX PRO-INFECTIOUS ENVIRONMENT

A reductionist view assumes that the sand fly delivers only parasites. However, current evidence shows that the vector injects a complex, multipartite inoculum that actively alters the host environment to ensure parasite survival. This coinoculation is a critical determinant of disease outcome and explains the discrepancies often observed between needle-injected experimental infections and natural transmission, where the latter frequently abrogates protective immunity.[30]

2.1. Sand Fly Saliva: Hemostatic and Immunomodulatory Properties

Sand fly saliva is a potent cocktail of bioactive proteins, including vasodilators, anticoagulants, and immunomodulators. For instance, maxadilan (found in *Lutzomyia* spp.) is one of

https://doi.org/10.1021/acsinfecdis.6c00135
ACS Infect. Dis. XXXX, XXX, XXX−XXX

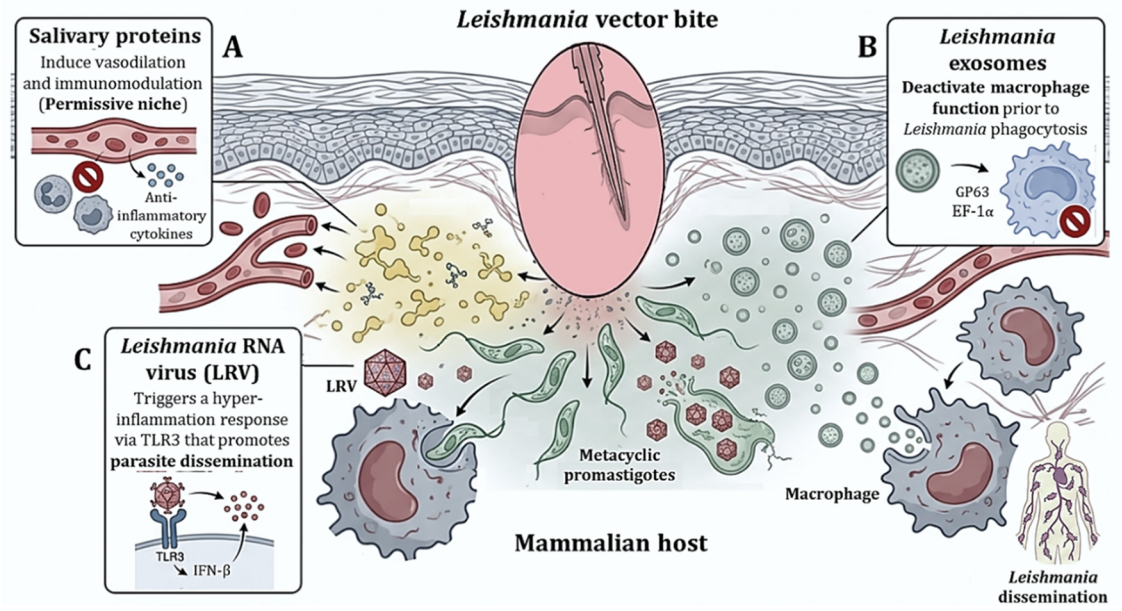


Figure 2. Vector Inoculum and the Establishment of a Permissive Niche.

the most potent vasodilators known.[31,32] Beyond facilitating blood feeding, salivary proteins inhibit the initial immune response by downregulating endothelial cell adhesion molecule expression, thereby impeding the early recruitment of neutrophils and monocytes. Furthermore, they skew the local cytokine environment toward a Th2 profile (IL-4, IL-10), which is permissive for parasite survival and established infection.[32,33] Consequently, the inoculum creates an immediate immunoprivileged niche that shields the parasite from host clearance mechanisms. Vaccines targeting salivary proteins (e.g., PdSP15) have shown promise in neutralizing this niche, allowing the host immune system to attack the parasite immediately upon inoculation.[32,34]

2.2. *Leishmania* Exosomes: Systemic Immunomodulators

A pivotal discovery is the role of parasite-derived exosomes, which are coinoculated with metacyclic promastigotes during the sand fly bite.[35] These extracellular vesicles are the first to interact with the host's immune system, being rapidly internalized by monocytes and neutrophils even before the parasite itself is phagocytosed. Once inside, the exosomal cargo, containing key virulence factors such as the metalloprotease GP63 and Elongation Factor-1α (EF-1α),[36] immediately begins modulating these early responding cells. This molecular manipulation suppresses a pro-inflammatory response by inhibiting cytokines such as IL-12 and TNF-α, while promoting the anti-inflammatory IL-10.[37] Crucially, this polarization is not merely signaling-dependent but is actively driven by specific exosomal metabolites, such as polyamines, which induce a targeted immunometabolic reprogramming of the host cell.[38] By the time the parasite infects its definitive host cell (macrophage), the environment has already been conditioned. The exosomal virulence factors then deactivate the macrophage's leishmanicidal capacity, for instance, by activating the phosphatase SHP-1[36] or by targeting the host secretory pathway to downregulate caspase-3 expression, thereby preventing apoptosis and ensuring the longevity of the replicative niche.[39] This multistep strategy of preconditioning and direct deactivation not only facilitates parasite establishment but also exacerbates infection severity and promotes early and systemic dissemination to visceral organs such as the liver and spleen.[35] This rapid establishment of deep-tissue reservoirs complicates therapeutic eradication and contributes to the challenge of achieving a sterile cure. Consequently, targeting exosome biogenesis or uptake represents a promising new therapeutic avenue.[40]

2.3. The *Leishmania* RNA Virus (LRV): An Endosymbiotic Driver of Virulence

In some species, particularly *L. guyanensis* and *L. braziliensis* (belonging to subgenus *Viannia*, causing CL and ML), the parasite may host a double-stranded RNA virus known as *Leishmania* RNA virus (LRV).[41,42] The presence of this endosymbiont dramatically alters clinical prognosis, being strongly associated with treatment failure and the development of destructive mucocutaneous lesions.[43]

The LRV mechanism is immune-mediated.[44] The viral dsRNA is encapsulated within *Leishmania* exosomes, which protects the virus and facilitates its transmission, and is recognized by the host Toll-like Receptor 3 (TLR3). This triggers a massive type I interferon (IFN-β) response. Paradoxically, while interferons are canonically associated with antiviral defense, in the context of leishmaniasis, this signaling cascade promotes parasite persistence by activating autophagy to degrade the NLRP3 inflammasome. This inhibition of the inflammasome network facilitates the exacerbation of skin pathology and dissemination.[45]

The synergistic immunomodulatory mechanisms driven by these coinoculated factors and their collective role in engineering a permissive niche for parasite establishment are illustrated in Figure 2.

Natural transmission of *Leishmania* involves the coinoculation of parasites with a complex mixture of vector- and parasite-derived factors that actively engineer the host environment. (A) Bioactive salivary proteins induce vasodilation and polarize the immune response toward a permissive niche. (B) Parasite-derived exosomes containing virulence factors are internalized by host cells prior to parasite entry, deactivating them. (C) In certain species, the endosymbiotic *Leishmania* RNA Virus (LRV) triggers a hyper-inflammatory

https://doi.org/10.1021/acsinfecdis.6c00135
ACS Infect. Dis. XXXX, XXX, XXX−XXX

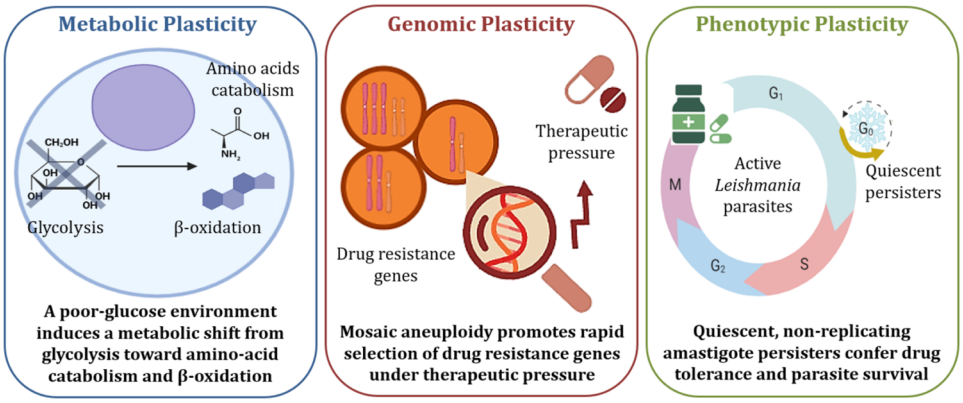


Figure 3. Plasticity and Persistence Strategies of the Intracellular Amastigote.

response via TLR3, promoting parasite dissemination and persistence.

3. THE INTRACELLULAR AMASTIGOTE: PLASTICITY AND PERSISTENCE

Once inside the parasitophorous vacuole (PV) of the macrophage, the parasite transforms into the nonmotile amastigote. This stage is the primary target for chemotherapy but leverages remarkable plasticity to ensure its long-term persistence.

3.1. Metabolic Plasticity: The Cornerstone of Persistence

The sand fly gut environment is glucose-rich, whereas the macrophage PV is acidic and nutrient-poor. To survive, *Leishmania* undergoes a profound metabolic shift, down-regulating glycolysis and upregulating β-oxidation and amino acid catabolism.[12] This metabolic rewiring is a cornerstone of its persistence, enabling it to thrive in a hostile, nutrient-scarce niche. A prime example is sterol metabolism, where amastigotes show remarkable plasticity. Although their membranes require ergosterol—a vulnerability exploited by AmB—they can counteract inhibition of ergosterol synthesis by upregulating the endocytosis of host low-density lipoproteins (LDL), thereby scavenging cholesterol to maintain membrane integrity.[46] This metabolic bypass provides a fundamental mechanism by which azoles and other ergosterol biosynthesis inhibitors frequently fail in clinical practice. Beyond lipids, the acquisition of essential micronutrients such as iron is critical. The host defends itself by sequestering iron (nutritional immunity), but *Leishmania* counters this by upregulating specific transporters, such as the LIT1 ferrous iron transporter, which is essential not only for growth but also for maintaining mitochondrial function and virulence.[47] Targeting these adaptive metabolic pathways represents a promising therapeutic avenue.

3.2. Genomic Plasticity: Rapid Adaptation through Aneuploidy

Unlike mammalian cells, *Leishmania* does not primarily regulate gene expression at the transcriptional level. Instead, it relies on gene dosage. Single-cell genomic sequencing has revealed that *Leishmania* utilizes mosaic aneuploidy as a primary survival strategy.[13] Within a single strain, individual parasites vary significantly in chromosome copy number (somy).[48] Under drug pressure, subpopulations that happen to have extra copies of chromosomes carrying drug-resistance genes (e.g., transporters or target enzymes) are rapidly selected for. This genomic plasticity explains the swift emergence of resistance and suggests that drugs targeting a single protein may be easily bypassed by gene amplification, deletion, or changes in chromosome ploidy.[49] The clinical relevance of this adaptability is underscored by transcriptomic analyses of human macrophages infected with *L. infantum* isolates from treatment-failure cases; these studies reveal a distinct molecular signature in the host-parasite interaction that differs from drug-sensitive strains, highlighting how clinical isolates can specifically reprogram host signaling to favor survival under therapeutic pressure.[50] Combination therapies are therefore essential to counteract this inherent genomic flexibility.

3.3. Phenotypic Plasticity: Quiescence and Persisters

The most critical concept for modern drug discovery is the existence of persisters, which represent the ultimate form of phenotypic plasticity. Like bacterial biofilms, *Leishmania* populations are heterogeneous.[51−53] A subpopulation of amastigotes enters a quiescent, nonreplicating state (G0 arrest), characterized by extremely low metabolic activity and reduced protein synthesis.[54]

Most current antileishmanial drugs (e.g., miltefosine, antimonials) are cytocidal or cytostatic primarily against metabolically active, replicating cells. Quiescent parasites are phenotypically tolerant to these stresses. They can survive high-dose chemotherapy and, once the drug pressure is removed, reactivate to cause relapse or Post-Kala-azar Dermal Leishmaniasis (PKDL). The molecular regulation of this dormancy is a highly coordinated and active process. A central mechanism driving this state is the phosphorylation of the eukaryotic initiation factor 2 α (eIF2α), which triggers a global translational arrest while selectively permitting the expression of essential stress-response genes.[55] To survive prolonged periods of nutrient deprivation and host-induced stress without replicating, these quiescent amastigotes rely heavily on autophagy pathways for the recycling of intracellular components and clearance of damaged macromolecules.[56] Furthermore, because de novo protein synthesis is drastically reduced, the maintenance of stringent proteostasis mechanisms—particularly via the ubiquitin-proteasome system—becomes absolutely vital for parasite viability.[57] Finally, this quiescent state is accompanied by profound mitochondrial remodeling, which ensures that basal bioenergetics and the mitochondrial membrane potential are sustained despite the overall metabolic downregulation.[56]

The interplay between these distinct modes of plasticity—metabolic rewiring, genomic instability, and phenotypic

https://doi.org/10.1021/acsinfecdis.6c00135
ACS Infect. Dis. XXXX, XXX, XXX−XXX

Table 1. New Biological Insights in *Leishmania* and Their Contribution to Therapeutic Failure[a]

Biological insight	Mechanism of Survival & Pathogenesis	Contribution to Therapeutic Failure
Reversible differentiation in vector[16]	Infective metacyclics revert to a replicative retroleptomonad stage, ensuring lifelong vector infectivity and high-dose inocula[16]	The massive parasitic load of the inoculum overwhelms the initial host immune response, compromising the potential for a sterile cure[23]
Sexual Recombination in Vector[24]	Meiosis-like processes in the sand fly midgut generate hybrid progeny, facilitating the rapid spread of resistance alleles across parasite populations[25−27]	Hybrid progeny accelerates the emergence of multidrug resistant strains that can defy multiple therapeutic regimens simultaneously[27]
Vector-Microbiome Interaction[28]	The sand fly's gut microbiota is a critical determinant of vector competence, influencing parasite differentiation into infective forms[28]	Microbiota represents a previously overlooked factor in transmission dynamics; failure to consider this tripartite interaction limits control strategies[29]
Co-inoculation of saliva[32,33]	Bioactive salivary proteins create an immunoprivileged niche at the bite site by skewing the local cytokine environment toward a permissive Th2 profile[32,33]	Vaccine-induced immunity is often abrogated by these immunomodulatory factors, explaining the failure of many vaccine candidates[32−34]
Co-inoculation of Exosomes[35]	Parasite-derived vesicles containing virulence factors (e.g., GP63) precondition host cells for permissiveness and facilitate systemic dissemination[36−39]	The rapid establishment of deep-tissue reservoirs complicates therapeutic eradication and contributes to the challenge of achieving a sterile cure[40]
Viral Endosymbiosis (LRV)[41,42]	Viral dsRNA triggers an aberrant Type I Interferon response via Toll-like Receptor 3 (TLR3), inhibiting autophagy and promoting a hyper-inflammatory state[41−44]	Strongly associated with therapeutic refractoriness and the development of severe mucocutaneous pathology[44,45]
Metabolic Plasticity[12]	Amastigotes switch from glycolysis to β-oxidation and can scavenge host cholesterol to bypass the inhibition of ergosterol synthesis[12,14,15,46,47]	Allows parasites to survive in nutrient-poor niches and creates metabolic bypass routes that render many metabolic inhibitors ineffective[46,47]
Genomic plasticity[13,48]	Mosaic aneuploidy (constitutive variation in chromosome copy number) allows for the rapid selection of parasites with extra copies of drug resistance genes[13,48,49]	Monotherapies are highly vulnerable to rapid, nonmutational resistance via gene dosage effects, leading to swift treatment failure[49,50]
Phenotypic Plasticity[18,54]	A subpopulation enters in a quiescent persister state with minimal metabolic activity, conferring phenotypic tolerance to most conventional chemotherapies[18,54]	Quiescent persisters are the primary source of therapeutic relapse, as these phenotypically tolerant parasites survive chemotherapy and reactivate once drug pressure is removed[54,56]
Neutrophil Exploitation[60,62]	Parasites use neutrophils as a Trojan Horse for silent entry into macrophages via efferocytosis, thereby avoiding the oxidative burst[60,62]	The initial immune response is subverted, allowing the parasite to establish a silent infection from the outset, compromising early clearance[62,63]
Expanded Host Cell Tropism[18]	Parasites establish long-term reservoirs in noncanonical cells (e.g., fibroblasts, adipocytes, MSCs) that lack potent microbicidal mechanisms and may offer metabolic/immunological protection[18,65,66]	Noncanonical cells create cryptic, pharmacological sanctuaries where parasites are shielded from both the immune system and chemotherapy, leading to incomplete clearance[65,66]

[a]Abbreviations: LRV, *Leishmania* RNA virus; TLR3, Toll-like Receptor 3; MSCs, mesenchymal stem cells.

https://doi.org/10.1021/acsinfecdis.6c00135
ACS Infect. Dis. XXXX, XXX, XXX−XXX

Table 2. Novel Targets and Proposed Therapeutic Innovations in Leishmaniasis[a]

Biological Discovery	Targeted Cell/Molecular Pathway	Therapeutic innovation
Retroleptomonad Stage & Vector Persistence[18]	Sand fly midgut attachment proteins[21]	Development of TBVs to prevent parasite establishment in the vector[19]
Sexual Recombination & Hybrid Generation[24−26]	Genetically diverse field isolates[24−26]	Testing NCEs against a panel of clinical and hybrid strains to ensure broad efficacy
Vector-Microbiome Interaction[28]	Sand fly gut microbiota[28]	Transmission-blocking, such as paratransgenesis, using modified symbiotic bacteria to secrete antileishmanial molecules within the vector[29]
Exosome-mediated Immunomodulation[36−39]	Exosome biogenesis pathways/Host cell uptake receptors[40]	Development of novel therapeutic strategies targeting exosome biogenesis or uptake to prevent immune evasion and dissemination[40]
Leishmania RNA Virus (LRV1) Endosymbiosis[41,42]	Host TLR3 pathway[44,45]	Use of adjuvant therapies, such as antiviral drugs or TLR3 inhibitors, in LRV1-positive cases[44,45]
Metabolic Quiescence (Persisters)[18]	Basal maintenance pathways (e.g., proteostasis, mitochondrial bioenergetics)[67−69]	Targeting the proteasome or the cytochrome bc1 complex to eradicate nonreplicating but viable parasites. Requires a shift in drug discovery toward wash-out assays[57,70,71]
Infection of Non-Canonical Reservoirs[18]	Dermis, Bone marrow, Adipose tissue[18,64]	Nanotechnology-based precision delivery to improve drug penetration into pharmacological sanctuaries[4,6,72−74]
Tissue-Resident Memory T-cells (TRM)[75]	Dermal-epidermal junction[76]	Next-generation vaccine delivery via microneedle patches or topical formulations to generate protective TRM[74]

[a]Abbreviations: LRV, *Leishmania* RNA virus; TBVs, Transmission-Blocking Vaccines; NCEs, New Chemical Entities; TLR3, Toll-like Receptor 3; TRM, Tissue-Resident Memory T-cells.

quiescence—and their synergistic contribution to parasite persistence and therapeutic failure are integrated into the conceptual image presented in Figure 3.

The intracellular amastigote employs a tripartite strategy of plasticity to ensure long-term persistence and evade chemotherapy. (A) Metabolic Plasticity: The parasite rewires its metabolism, shifting from glycolysis to β-oxidation and amino acid catabolism to survive in the nutrient-poor phagolysosome. (B) Genomic Plasticity: Mosaic aneuploidy allows for the rapid selection of drug-resistant parasites through changes in gene dosage. (C) Phenotypic Plasticity: A subpopulation of amastigotes enters a quiescent, nonreplicating persister state, conferring phenotypic tolerance to most antileishmanial drugs and serving as the source of therapeutic relapse and parasite survival.

4. HOST CELL TROPISM: PROFESSIONAL PHAGOCYTES AND NON-CANONICAL RESERVOIRS

The traditional dogma that *Leishmania* resides and replicates exclusively within macrophages is increasingly being challenged by evidence of a much broader host-cell tropism.[58] This capacity to infect diverse cell lineages establishes cellular sanctuaries that compromise therapeutic clearance.

4.1. Neutrophils: The Trojan Horse Model of Silent Entry

Upon inoculation, *Leishmania* does not immediately trigger a massive inflammatory response. Instead, it exploits the host's innate defenses to establish a silent phase. Neutrophils are the first responders, rapidly recruited to the bite site within minutes. Although this interaction elicits a robust defensive response—characterized by the release of Neutrophil Extracellular Traps (NETs) and Damage-Associated Molecular Patterns (DAMPs)[59]—*Leishmania* effectively evades entrapment. Consequently, rather than eliminating the parasite, neutrophils often serve as a transient niche; a concept known as the Trojan Horse model.[60] Within the neutrophil, *Leishmania* is sequestered from the extracellular environment and the complement system. Rather than passively hiding, the parasite actively neutralizes reactive oxygen species (ROS) through a robust antioxidant defense machinery, which includes the unique trypanothione system, superoxide dismutases (SOD), and peroxidoxins.[61]

The parasite actively extends the lifespan of the neutrophil by delaying constitutive apoptosis.[62] Eventually, the infected neutrophil undergoes apoptosis and is cleared by macrophages via efferocytosis. Because the clearance of apoptotic bodies is an anti-inflammatory response (characterized by TGF-β and PGE2 release), the macrophage ingests the parasite in an immunologically deactivated state. This silent entry allows the amastigote to establish infection without triggering the oxidative response, which is essential for parasite elimination.[63]

4.2. Non-Canonical Host Cells: Cryptic Tissue Reservoirs

Noncanonical host cells, such as fibroblasts, adipocytes, and mesenchymal stem cells (MSCs), serve as long-term reservoirs for persistence,[18] highlighting their significance in host tropism.

In the dermis, the primary site of infection, fibroblasts have emerged as a key cellular sanctuary, with recent studies showing that species such as *L. amazonensis* and *L. infantum* can invade these cells. Unlike macrophages, fibroblasts are long-lived cells that lack potent antimicrobial machinery, providing an ideal, low-stress niche that favors parasite quiescence and clinical latency of the infection.[17] The epidemiological relevance of this niche is profound, as these skin-resident parasites remain accessible to sand flies, thereby sustaining transmission cycles even in the absence of systemic symptoms or cutaneous lesions.[64]

This phenomenon of cellular sanctuaries extends beyond the dermis and is equally critical in VL. Within the bone marrow stroma, both adipocytes and MSCs have been identified as functional host cells that provide distinct mechanisms of protection. Adipocytes confer a dual metabolic and physical advantage: their lipid-rich environment physically shields the parasite from hydrophilic drugs while simultaneously inducing a metabolic shift in the parasite toward lipid utilization. This quiescent, lipid-dependent state may render parasites phenotypically tolerant to drugs targeting replicative pathways,[65] thereby contributing to treatment relapse. MSCs, in turn, provide an immunological sanctuary. Primarily residing in the bone marrow, infected MSCs actively suppress the proliferation and effector function of T cells (e.g., IFN-γ production),

https://doi.org/10.1021/acsinfecdis.6c00135
ACS Infect. Dis. XXXX, XXX, XXX−XXX

thereby creating an immunoprivileged niche where parasites can persist in a quiescent state,[66] refractory to both immune clearance and chemotherapy.

The clinical relevance of these tissue reservoirs is underscored by the persistence of parasites after apparent cure. Historically, VL was viewed as a systemic infection confined to the spleen, liver, and bone marrow. However, parasites persist in the skin of cured VL patients (PKDL) and in asymptomatic carriers.[64] This dermal reservoir, likely sustained by cryptic parasite populations within fibroblasts and other skin cells, underscores the need for a VL drug to not only clear visceral organs but also achieve excellent biodistribution in the dermis to eradicate these cryptic foci and block continued transmission.

The diverse biological complexities discussed in the preceding sections—from vector-stage plasticity to the establishment of cryptic cellular reservoirs—collectively explain the failure of current therapies to achieve sterile cure. These key biological traits and their direct implications for therapeutic failure are summarized in Table 1.

5. IMPLICATIONS FOR DRUG DISCOVERY AND DEVELOPMENT

The updated life cycle necessitates a fundamental shift in Target Product Profiles (TPPs) and screening cascades. The goal is no longer just parasite reduction but achieving a sterile cure that eradicates all life-cycle stages, including quiescent persisters. Moreover, the field is moving toward alternative, innovative treatment approaches to overcome the limitations of toxicity and resistance.[4] These emerging strategies, which target the distinct biological vulnerabilities detailed in the previous sections—from specific molecular pathways to cellular sanctuaries—are outlined in Table 2.

Addressing therapeutic relapse and drug resistance requires a multifaceted strategy. This includes the adoption of combination therapies, drug repurposing, and a shift toward Host-Directed Therapies (HDT) to promote a curative Th1 response. Precision drug delivery is crucial to reach cryptic reservoirs, while physical therapies offer a safe and effective alternative for localized CL.

5.1. Eradicating Quiescent Persisters to Prevent Therapeutic Relapse

To prevent relapse, next-generation therapies must eradicate nonreplicating, quiescent amastigotes. Two promising strategies targeting fundamental cellular processes have emerged. Quiescent amastigotes depend heavily on proteostasis because, despite the global downregulation of protein synthesis, they must continuously clear damaged or misfolded proteins to prevent proteotoxic stress, which would otherwise be fatal in a nonreplicating state. Proteasome activity remains robust in these persisters, and in some models, it is even upregulated compared to replicating forms to facilitate the rapid turnover of short-lived regulatory proteins required for maintaining dormancy. Consequently, the ubiquitin-proteasome system represents a critical vulnerability.[67,68] Proteasome inhibitors optimized for kinetoplastids, such as the clinical candidate GNF6702, have induced a sterile cure in animal models, indicating efficacy against both replicating and nonreplicating parasite forms.[57,70] A second critical vulnerability is mitochondrial bioenergetics.[69] Although glycolysis is downregulated in amastigotes, they maintain mitochondrial membrane potential by switching to the oxidation of amino acids and fatty acids. This metabolic rewiring ensures a continuous supply of electrons to the electron transport chain, preserving the proton motive force necessary for ATP synthesis and survival, even in the absence of glucose-driven glycolysis. Consequently, inhibitors of the cytochrome bc1 complex (Complex III) have shown potent activity and are considered effective against quiescent persisters.[71]

The presence of these persisters limits the predictive value of conventional High-Throughput Screening (HTS) assays, which measure growth inhibition (50% inhibitory concentration, IC50) over 48−72 h and are inherently blind to compounds that fail to kill quiescent forms. Next-generation assays must incorporate washout phases to detect relapse, or use Artificial Intelligence (AI) and High-Content Screening (HCS) to distinguish viable, quiescent persisters from dead parasites based on organelle morphology.

5.2. Overcoming Drug Resistance: Transporter and Combination Therapy

A major hurdle in *Leishmania* chemotherapy is the parasite's ability to modulate drug uptake via transporter modulation.[77] Resistance to antimonials, for instance, is linked to downregulation of Aquaglyceroporin 1 (AQP1),[78] while resistance to miltefosine arises from mutations in the Miltefosine Transporter and its subunit ROS3.[79] Future drug design should prioritize compounds that enter via essential transporters the parasite cannot afford to downregulate, or compounds that enter via passive diffusion (lipophilic molecules), bypassing the transporter bottleneck entirely. However, to counteract the parasite's genomic plasticity and the rapid selection of resistant strains, monotherapy is becoming obsolete. Combination therapies aim to shorten treatment duration, reduce toxicity, and mitigate the emergence of resistance.[4] Synergistic regimens, such as combining L-AmB (membrane disruption) with miltefosine (apoptotic signaling) or paromomycin (protein synthesis inhibition), have shown high cure rates in clinical trials, providing proof-of-principle for the efficacy of multitarget approaches in preventing the selection of resistant mutants.[4,80]

However, the clinical implementation of combination therapies is complex and requires careful consideration of several factors. Pharmacokinetic compatibility is critical, as the coadministration of drugs with distinct absorption, distribution, and metabolic profiles can lead to subtherapeutic levels or, conversely, exacerbated toxicity. Furthermore, there is a constant risk of drug antagonism, where the combined effect is less than the sum of individual components, potentially selecting for resistant subpopulations. From a public health perspective, combination regimens often entail increased treatment costs and logistical complexity, particularly in resource-limited settings where monitoring patient adherence and managing supply chains is challenging. Finally, the efficacy of these combinations is subject to significant regional variability, driven by differences in parasite genetic diversity and host factors, necessitating a tailored approach to treatment protocols rather than a standardized, nonstratified therapeutic strategy.[4]

5.3. Nanotechnology: Precision Delivery to Pharmacological Sanctuaries

A critical strategy to overcome systemic toxicity and reach parasites within cellular sanctuaries, as described previously, is the application of nanotechnology.[4,72] Since the primary target cells are macrophages, nanocarriers can be engineered for

https://doi.org/10.1021/acsinfecdis.6c00135
ACS Infect. Dis. XXXX, XXX, XXX−XXX

active internalization. The clinical success of L-AmB provides powerful validation of this concept, as its encapsulation within liposomes significantly reduces the nephrotoxicity associated with the conventional deoxycholate form.[6] Building on this success, next-generation systems based on biodegradable polymers such as poly(lactic-*co*-glycolic acid) (PLGA) or metallic nanoparticles (gold/silver) are being developed. Inorganic nanomaterials, in particular, represent a versatile frontier in drug design; they can be engineered to possess intrinsic antileishmanial properties, such as the generation of ROS, or be functionalized for stimuli-responsive drug release, providing a precise toolset to bypass traditional pharmacological barriers.[73] These platforms can be functionalized to deliver multiple drugs simultaneously, and metal nanoparticles have also shown intrinsic antileishmanial activity by inducing oxidative stress.[72] Overall, the integration of these cutting-edge drug delivery systems is revolutionizing the therapeutic landscape, offering a multifunctional platform to overcome the biological barriers identified in the parasite's complex life cycle.[74]

Despite their potential, the clinical deployment of inorganic nanomaterials, such as gold and silver nanoparticles, faces substantial challenges. Primary concerns include the long-term systemic toxicity and potential bioaccumulation of these materials, which require rigorous longitudinal safety assessments. Furthermore, the transition from laboratory-scale synthesis to industrial production is complicated by issues of batch-to-batch variability, long-term colloidal stability, and the stringent regulatory requirements for complex nanomedicines. The high cost of production and the complexity of characterizing these platforms at scale remain significant barriers to their widespread clinical adoption. Future efforts must therefore focus on developing standardized, cost-effective manufacturing processes and establishing clear regulatory pathways to ensure both the safety and reproducibility of these promising therapeutic systems.[81]

5.4. Drug Repurposing: Accelerating the Pipeline

Given the high cost and time required to develop NCEs, drug repurposing has emerged as a pragmatic strategy to accelerate the pipeline. *In silico* approaches, such as molecular docking, are used to predict binding affinities between approved drug libraries and essential *Leishmania* targets.[82,83] These approaches have identified promising candidates among existing drug classes, such as antifungal azoles, which target the ergosterol biosynthesis pathway, and statins, which modulate host lipid metabolism, highlighting the potential of multitarget drugs.[84]

Complementing these parasite-focused strategies is the paradigm shift toward HDT. Rather than targeting the parasite, HDT aims to modulate the host immune response to promote a curative Th1 phenotype, with TLR9 agonists such as CpG-D35 representing a promising clinical avenue.[84] Ultimately, the most robust therapeutics may be those that bridge these paradigms—acting as a "triple threat" that directly inhibits parasite enzymes and collapses the proton motive force while simultaneously activating host cell-mediated killing mechanisms.[85]

5.5. Physical and Local Therapies for Cutaneous Leishmaniasis

For CL, systemic toxicity can be avoided by using physical therapies that target the lesion directly. These modalities encompass a range of techniques, including heat therapy, cryotherapy, photodynamic therapy, laser, and radiofrequency, which leverage distinct biophysical mechanisms to achieve parasite clearance.[86]

For heat therapy, the clinical efficacy is strictly dependent the precise control of the thermal dose, which is defined by the relationship between temperature and exposure time. While radiofrequency-based devices typically maintain a therapeutic range of 40 to 42 °C for sustained periods, other methods utilize higher temperatures (up to 50 °C−54 °C) for shorter durations to achieve parasite clearance.[84,86,87] This thermal threshold is essential to induce irreversible damage to the parasite's heat-sensitive metabolic pathways while preserving the integrity of the surrounding host tissue.[84,87] Infrared light, ultrasound, hot water baths, and exothermic crystallization thermotherapy have also been used to deliver heat to leishmaniasis lesions.[86] When properly calibrated, these techniques have demonstrated cure rates comparable to antimonials, with superior safety profiles.[84]

However, the success of these physical modalities is subject to several critical limitations. First, efficacy is highly dependent on the *Leishmania* spp. involved; for instance, *L. major* lesions often respond more favorably to thermal interventions than the more aggressive and ulcerating *L. braziliensis* infections. Second, physical therapies are generally restricted to smaller, uncomplicated lesions, as larger or diffuse lesions often preclude effective heat or cold penetration. Furthermore, clinicians must balance therapeutic efficacy against the risk of secondary complications, such as hypertrophic scarring or incomplete clearance, which may necessitate subsequent pharmacological intervention. Finally, these local approaches carry a higher risk of relapse in immunocompromised patients, where the lack of a robust host immune response limits the ability to clear residual parasites that survive the initial physical insult.[84,87] Consequently, while physical therapies offer a safe and effective alternative for localized CL, their application requires careful patient stratification and clinical monitoring.

6. IMPLICATIONS FOR VACCINE DEVELOPMENT

The complex biology of transmission and persistence described in the preceding sections demands a profound re-evaluation of traditional vaccine strategies. For decades, vaccine development has been hampered by a reductionist approach that focuses on single-antigen formulations delivered by needle injection. This paradigm fails to account for the immunomodulatory effects of the inoculated vector, the need to interrupt zoonotic transmission cycles, and the requirement to establish protective immunity in the specific tissue where infection begins. Consequently, a successful next-generation vaccine must address three distinct immunological challenges.

6.1. Transmission-Blocking Vaccines (TBVs)

In zoonotic VL, where dogs serve as the primary domestic reservoir, a veterinary TBV is a critical One Health strategy to interrupt transmission. Such a vaccine would induce antibodies in the canine host that, upon ingestion by the sand fly during a bloodmeal, would target and neutralize the parasite within the vector's midgut. Viable targets include surface-expressed antigens of vector-stage parasites, such as proteins involved in nectonemad attachment to the midgut epithelium or key components of the PSG, which are essential for establishing infection in the fly.[19,23,88]

However, the clinical translation of TBVs faces significant feasibility hurdles. A primary challenge is the durability of the

https://doi.org/10.1021/acsinfecdis.6c00135
ACS Infect. Dis. XXXX, XXX, XXX−XXX

induced antibody titers in the canine host, which must be sustained at high levels to ensure consistent neutralization of the parasite.[19,89] Furthermore, the logistical challenge of ensuring high coverage and frequent booster administration in domestic and stray dog populations in endemic regions remains a major obstacle.[19,89] Consequently, future TBV development must prioritize the design of long-lasting, single-dose, or slow-release formulations that can maintain protective antibody levels over extended periods, thereby minimizing the need for frequent veterinary intervention.

6.2. Next-Generation Prophylactic Vaccines: Targeting the Inoculum and Inducing Tissue-Resident Immunity

Live-attenuated vaccine (LAV) candidates have been extensively explored due to their capacity to mimic natural infection and induce broad, long-lasting protective immunity. By utilizing genetically modified parasites that are replication-deficient or unable to persist in the host, these candidates aim to trigger a comprehensive immune response, including the induction of Tissue-Resident Memory T-cells (TRM). However, their clinical advancement has been primarily hampered by safety concerns, particularly the risk of reversion to virulence and the potential for persistent infection in immunocompromised individuals. Current research is therefore focused on developing safer, next-generation LAVs that utilize precise gene-editing technologies to ensure complete attenuation without compromising immunogenicity.[88]

Alongside these efforts, the limited success of conventional subunit vaccines (largely attributable to their delivery via needle injection) underscores the central challenge: generating an immune response capable of overcoming the vector inoculum's potent immunomodulatory effects. Although specific antigen formulations—such as those targeting the LPG synthesis machinery (rLPG3)[90] to engineered Th1-stimulatory chimeric antigens[91]—have successfully demonstrated protective efficacy and favorable immune modulation in experimental settings, other studies have shown that pre-existing, otherwise protective immunity can be abrogated by coinoculation with sand fly saliva.[30,33] This finding implies that effective anti-*Leishmania* immunity may require a multi-component formulation that includes vector salivary proteins (e.g., PpSP15) to neutralize the permissive niche established at the bite site. This vector-targeted approach aims to disarm the inoculum, enabling the host immune system to effectively engage with the parasite upon transmission.[33]

Beyond the local niche, vaccine development for VL must contend with the profound systemic immune suppression characteristic of the disease. In symptomatic VL, the parasite actively promotes an exhaustion-like state in T-cells, characterized by the upregulation of inhibitory receptors such as PD-1[92] and an imbalanced cytokine milieu (e.g., elevated IL-10 and TGF-β, alongside deficient IFN-γ production).[5,93] This suppressive environment not only facilitates parasite persistence but also actively antagonizes the generation and maintenance of protective memory responses, presenting a formidable barrier to successful vaccination.

Crucially, emerging evidence indicates that durable protection is mediated not by circulating T-cells, but by nonrecirculating TRM that persist in the skin,[75] providing rapid, on-site surveillance. Therefore, the primary goal of a next-generation vaccine is to establish a robust population of these TRM cells. This has profound implications for vaccine design, suggesting that the route of administration is as critical as the antigen itself. Next-generation delivery systems, such as microneedle patches, nanoparticle-based topical formulations, or adjuvanted creams, are being explored to specifically generate and maintain long-lived TRM populations at the dermal-epidermal junction, the primary site of infection.[76] This shift toward nanovaccines represents a critical technological convergence, enabling the precise delivery of antigens and adjuvants to the exact immunological niches required for durable protection.[74]

7. CONCLUSIONS AND FUTURE PERSPECTIVES

The life cycle of *Leishmania* is far more complex than the linear models that have guided research for the past century. As this review has detailed, it encompasses a repertoire of developmental stages, sexual plasticity, viral endosymbiosis, and metabolic dormancy. The high failure rates of current chemotherapies are a direct consequence of their inability to address these complexities—specifically, the quiescent persisters, the protected reservoirs in noncanonical cells, and the initial immunomodulatory preconditioning provided by the vector's inoculum.

However, integrating these recent advances offers a clear path forward. The shift from empirical screening to rational drug design, supported by nanotechnology for targeted delivery and HDT, marks a new frontier. Furthermore, adopting combination therapies and drug repurposing can provide urgently needed solutions while novel targets are validated.

To translate these biological insights into clinical success, the field must also adopt innovative translational tools. The historically poor predictive value of animal models has accelerated the development of Controlled Human Infection Models (CHIM) for *Leishmania*. By deliberately infecting healthy volunteers under strictly controlled conditions, researchers can rapidly evaluate vaccine efficacy in a relevant human system.[94] In parallel, advanced imaging technologies are redefining how we evaluate drug efficacy; for instance, the use of *in vivo* bioluminescence imaging has recently revealed significant differences in parasite killing kinetics among standard treatments, providing a real-time, spatial understanding of parasite clearance that traditional end point assays fail to capture.[95] Furthermore, there is an urgent need for novel biomarkers to address the "Test-of-Cure" conundrum. Traditional end points such as lesion healing do not guarantee a sterile cure. Next-generation biomarkers—such as host metabolic signatures, parasite-specific transcripts, or immunoglobulin G (IgG) subclass shifts—must definitively distinguish between a "functionally cured" patient with low-level persistence and a "sterile cured" patient.[96]

In conclusion, the research community must integrate these biological updates into the drug discovery pipeline. Future therapies must not only inhibit growth but also eradicate dormant reservoirs and neutralize the immunomodulatory environment established by the vector. This requires a shift from phenotypic screening of axenic cultures to complex *in vivo* imaging and host-directed strategies. The era of "one bug, one drug" is over; biology-driven precision medicine now defines the future of Leishmaniasis treatment.

https://doi.org/10.1021/acsinfecdis.6c00135
ACS Infect. Dis. XXXX, XXX, XXX−XXX

AUTHOR INFORMATION

Corresponding Authors

Javier Martín-Escolano − *Clinical Unit of Infectious Diseases, Microbiology and Parasitology, Instituto de Biomedicina de Sevilla (IBiS), Virgen del Rocío University Hospital/CSIC/University of Seville, 41013 Seville, Spain*; Email: jmartine-ibis@us.es

Rubén Martín-Escolano − *Unidad de Infección Viral e Inmunidad, Centro Nacional de, Microbiología (CNM), Instituto de Salud Carlos III (ISCIII), 28029 Majadahonda, Madrid, Spain; Centro de Investigación Biomédica en Red en Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, 28029 Madrid, Spain;* orcid.org/0000-0002-6262-9344; Email: r.martin@isciii.es

Authors

Clotilde Marín − *Departamento de Parasitología, Universidad de Granada, 18071 Granada, Spain;* orcid.org/0000-0002-4316-2742

Anastasios D. Tsaousis − *School of Natural Sciences, University of Kent, Canterbury CT7 2NJ, U.K.*

Mohinder Pal − *School of Natural Sciences, University of Kent, Canterbury CT7 2NJ, U.K.*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsinfecdis.6c00135

Author Contributions

Conceptualization: J.M.E. and R.M.E.: Data curation: J.M.E.; Methodology: J.M.E. and R.M.E.: Supervision and visualization: R.M.E.: Writing—original draft preparation: J.M.E. and R.M.E.: Writing—Review and Editing: M..P, C.M., A.D.T.

Funding

J.M.E. is supported by a grant from the ISCIII (FI22/00025). R.M.E. is César Nombela researcher supported and funded by Comunidad de Madrid (grant number 2023-T1/SAL-GL-28980).

Notes

The authors declare no competing financial interest.

LIST OF ABBREVIATIONS

AI, Artificial Intelligence; AmB, Amphotericin B; AQP1, Aquaglyceroporin 1; CHIM, Controlled Human Infection Models; CL, Cutaneous Leishmaniasis; CPSF3, Cleavage and Polyadenylation Specificity Factor 3; DAMPs, Damage-Associated Molecular Patterns; EF-1α, Elongation Factor-1 α; HCS, High-Content Screening; HDT, Host-Directed Therapy; HTS, High-Throughput Screening; IC50, 50% Inhibitory Concentration; IFN-β, Type I Interferon; IgG, Immunoglobulin G; Kdna, Kinetoplastid DNA (Mitochondrial DNA); LAV, Live-attenuated vaccine; L-AmB, Liposomal amphotericin B; LDL, Low-Density Lipoproteins; LPG, Lipophosphoglycan; LRV, Leishmania RNA Virus; ML, Mucosal Leishmaniasis; MSCs, Mesenchymal Stem Cells; MT, Miltefosine Transporter; NETs, Neutrophil Extracellular Traps; NCEs, New Chemical Entities; NTD, Neglected Tropical Disease; PKDL, Post-Kala-azar Dermal Leishmaniasis; PLGA, Poly(lactic-*co*-glycolic acid); PMNs, Polymorphonuclear Leukocytes (Neutrophils); PSG, Promastigote Secretory Gel; PV, Parasitophorous Vacuole; ROS, Reactive Oxygen Species; rLPG3, LPG Synthesis Machinery; SbV, Pentavalent Antimonials; SOD, superoxide dismutases; TBVs, Transmission-Blocking Vaccines; TLR3, Toll-like Receptor 3; TPPs, Target Product Profiles; TRM, Tissue-Resident Memory T-cells; VL, Visceral Leishmaniasis

REFERENCES

(1) CDC. About Leishmaniasis Centers for Disease Control and Prevention Webpage Parasites - Leishmaniasis: Centers for Disease Control and Prevention 2024. 2024, https://www.cdc.gov/leishmaniasis/about/index.html. (accessed January 3, 2026).

(2) Organization, W. H. *Leishmaniasis. Status of endemicity of cutaneous leishmaniasis*, **2024**, https://apps.who.int/neglected_diseases/ntddata/leishmaniasis/leishmaniasis.html. (accessed January 3, 2026).

(3) Majoor, A.; Michel, G.; Marty, P.; Boyer, L.; Pomares, C. Leishmaniases: Strategies in treatment development. *Parasite* **2025**, *32*, No. 18.

(4) Roatt, B. M.; de Oliveira Cardoso, J. M.; De Brito, R. C. F.; Coura-Vital, W.; de Oliveira Aguiar-Soares, R. D.; Reis, A. B. Recent advances and new strategies on leishmaniasis treatment. *Appl. Microbiol. Biotechnol.* **2020**, *104* (21), 8965−8977.

(5) Tiwari, R.; Kumar, A.; Singh, V. K.; Rajneesh; Chauhan, S. B.; Sundar, S.; Nylen, S.; Engwerda, C.; Kumar, R. The development and maintenance of immunity against visceral leishmaniasis. *Front. Immunol.* **2024**, *15*, No. 1486407.

(6) Balasegaram, M.; Ritmeijer, K.; Lima, M. A.; Burza, S.; Ortiz Genovese, G.; Milani, B.; Gaspani, S.; Potet, J.; Chappuis, F. Liposomal amphotericin B as a treatment for human leishmaniasis. *Expert Opin. Emerging Drugs* **2012**, *17* (4), 493−510.

(7) Srivastava, S.; Mishra, J.; Gupta, A. K.; Singh, A.; Shankar, P.; Singh, S. Laboratory confirmed miltefosine resistant cases of visceral leishmaniasis from India. *Parasites Vectors* **2017**, *10* (1), No. 49.

(8) Hailu, A.; Musa, A.; Wasunna, M.; Balasegaram, M.; Yifru, S.; Mengistu, G.; Hurissa, Z.; Hailu, W.; Weldegebreal, T.; Tesfaye, S.; Makonnen, E.; Khalil, E.; Ahmed, O.; Fadlalla, A.; El-Hassan, A.; Raheem, M.; Mueller, M.; Koummuki, Y.; Rashid, J.; Mbui, J.; Mucee, G.; Njoroge, S.; Manduku, V.; Musibi, A.; Mutuma, G.; Kirui, F.; Lodenyo, H.; Mutea, D.; Kirigi, G.; Edwards, T.; Smith, P.; Muthami, L.; Royce, C.; Ellis, S.; Alobo, M.; Omollo, R.; Kesusu, J.; Owiti, R.; Kinuthia, J. Geographical variation in the response of visceral leishmaniasis to paromomycin in East Africa: a multicentre, open-label, randomized trial. *PLoS Neglected Trop. Dis.* **2010**, *4* (10), No. e709.

(9) Bhattacharya, A.; Corbeil, A.; do Monte-Neto, R. L.; Fernandez-Prada, C. Of Drugs and Trypanosomatids: New Tools and Knowledge to Reduce Bottlenecks in Drug Discovery. *Genes* **2020**, *11* (7), No. 722.

(10) Field, M. C.; Horn, D.; Fairlamb, A. H.; Ferguson, M. A.; Gray, D. W.; Read, K. D.; De Rycker, M.; Torrie, L. S.; Wyatt, P. G.; Wyllie, S.; Gilbert, I. A. Anti-trypanosomatid drug discovery: an ongoing challenge and a continuing need. *Nat. Rev. Microbiol* **2017**, *15* (4), 217−231.

(11) Vermeersch, M.; da Luz, R. I.; Tote, K.; Timmermans, J. P.; Cos, P.; Maes, L. *In vitro* susceptibilities of *Leishmania donovani* promastigote and amastigote stages to antileishmanial reference drugs: practical relevance of stage-specific differences. *Antimicrob. Agents Chemother.* **2009**, *53* (9), 3855−3859.

(12) McConville, M. J.; Naderer, T. Metabolic pathways required for the intracellular survival of *Leishmania*. *Annu. Rev. Microbiol.* **2011**, *65*, 543−561.

(13) Sterkers, Y.; Lachaud, L.; Bourgeois, N.; Crobu, L.; Bastien, P.; Pages, M. Novel insights into genome plasticity in Eukaryotes: mosaic aneuploidy in *Leishmania*. *Mol. Microbiol.* **2012**, *86* (1), 15−23.

(14) Saunders, E. C.; Ng, W. W.; Chambers, J. M.; Ng, M.; Naderer, T.; Kromer, J. O.; Likic, V. A.; McConville, M. J. Isotopomer profiling of *Leishmania mexicana* promastigotes reveals important roles for succinate fermentation and aspartate uptake in tricarboxylic acid cycle (TCA) anaplerosis, glutamate synthesis, and growth. *J. Biol. Chem.* **2011**, *286* (31), 27706−27717.

(15) Opperdoes, F. R.; Coombs, G. H. Metabolism of *Leishmania*: proven and predicted. *Trends Parasitol.* **2007**, *23* (4), 149−158.
(16) Serafim, T. D.; Coutinho-Abreu, I. V.; Oliveira, F.; Meneses, C.; Kamhawi, S.; Valenzuela, J. G. Sequential blood meals promote *Leishmania* replication and reverse metacyclogenesis augmenting vector infectivity. *Nat. Microbiol* **2018**, *3* (5), 548−555.
(17) Bogdan, C.; Donhauser, N.; Doring, R.; Rollinghoff, M.; Diefenbach, A.; Rittig, M. G. Fibroblasts as host cells in latent leishmaniosis. *J. Exp Med.* **2000**, *191* (12), 2121−2130.
(18) Valigurová, A.; Kolarova, I. Unrevealing the Mystery of Latent Leishmaniasis: What Cells Can. Host *Leishmania*? *Pathogens* **2023**, *12* (2), No. 246.
(19) Leite, J. C.; Goncalves, A. A. M.; de Oliveira, D. S.; Resende, L. A.; Boas, D. F. V.; Ribeiro, H. S.; Pereira, D. F. S.; da Silva, A. V.; Mariano, R.; Reis, P. C. C.; Nakasone, E. N.; França-Silva, J. C.; Galdino, A. S.; Paes, P. R. O.; Melo, M. M.; Dias, E. S.; Chávez-Fumagalli, M. A.; da Silveira-Lemos, D.; Dutra, W. O.; Giunchetti, R. C. Transmission-Blocking Vaccines for Canine Visceral Leishmaniasis: New Progress and Yet New Challenges. *Vaccines* **2023**, *11* (10), No. 1565.
(20) Inbar, E.; Hughitt, V. K.; Dillon, L. A.; Ghosh, K.; El-Sayed, N. M.; Sacks, D. L. The Transcriptome of *Leishmania major* Developmental Stages in Their Natural Sand Fly Vector. *mBio* **2017**, *8* (2), No. e00029-17, DOI: 10.1128/mBio.00029-17.
(21) Cecílio, P.; Cordeiro-da-Silva, A.; Oliveira, F. Sand flies: Basic information on the vectors of leishmaniasis and their interactions with *Leishmania* parasites. *Commun. Biol.* **2022**, *5* (1), No. 305.
(22) Volf, P.; Pruzinova, K.; Yanase, R.; Sunter, J. D. Midgut and stomodeal valve attachment of *Leishmania* in sand flies. *Trends Parasitol.* **2025**, *41* (9), 769−779.
(23) Rogers, M. E.; Ilg, T.; Nikolaev, A. V.; Ferguson, M. A.; Bates, P. A. Transmission of cutaneous leishmaniasis by sand flies is enhanced by regurgitation of fPPG. *Nature* **2004**, *430* (6998), 463−467.
(24) Catta-Preta, C. M. C.; Sacks, D. L. Genetic Exchange in *Leishmania*: Understanding the Cryptic Sexual Cycle. *Annu. Rev. Microbiol.* **2025**, *79* (1), 105−128.
(25) Akopyants, N. S.; Kimblin, N.; Secundino, N.; Patrick, R.; Peters, N.; Lawyer, P.; Dobson, D. E.; Beverley, S. M.; Sacks, D. L. Demonstration of genetic exchange during cyclical development of *Leishmania* in the sand fly vector. *Science* **2009**, *324* (5924), 265−268.
(26) Romano, A.; Inbar, E.; Debrabant, A.; Charmoy, M.; Lawyer, P.; Ribeiro-Gomes, F.; Barhoumi, M.; Grigg, M.; Shaik, J.; Dobson, D.; Beverley, S. M.; Sacks, D. L. Cross-species genetic exchange between visceral and cutaneous strains of *Leishmania* in the sand fly vector. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111* (47), 16808−16813.
(27) Inbar, E.; Shaik, J.; Iantorno, S. A.; Romano, A.; Nzelu, C. O.; Owens, K.; Sanders, M. J.; Dobson, D.; Cotton, J. A.; Grigg, M. E.; Beverley, S. M.; Sacks, D. Whole genome sequencing of experimental hybrids supports meiosis-like sexual recombination in *Leishmania*. *PLoS Genet.* **2019**, *15* (5), No. e1008042.
(28) Kelly, P. H.; Bahr, S. M.; Serafim, T. D.; Ajami, N. J.; Petrosino, J. F.; Meneses, C.; Kirby, J. R.; Valenzuela, J. G.; Kamhawi, S.; Wilson, M. E. The Gut Microbiome of the Vector Lutzomyia longipalpis Is Essential for Survival of *Leishmania infantum*. *mBio* **2017**, *8* (1), No. e01121-16, DOI: 10.1128/mBio.01121-16.
(29) Hurwitz, I.; Hillesland, H.; Fieck, A.; Das, P.; Durvasula, R. The paratransgenic sand fly: a platform for control of *Leishmania* transmission. *Parasit Vectors* **2011**, *4*, No. 82.
(30) Peters, N. C.; Kimblin, N.; Secundino, N.; Kamhawi, S.; Lawyer, P.; Sacks, D. L. Vector transmission of *leishmania* abrogates vaccine-induced protective immunity. *PLoS Pathog.* **2009**, *5* (6), No. e1000484.
(31) Lerner, E. A.; Ribeiro, J. M.; Nelson, R. J.; Lerner, M. R. Isolation of maxadilan, a potent vasodilatory peptide from the salivary glands of the sand fly Lutzomyia longipalpis. *J. Biol. Chem.* **1991**, *266* (17), 11234−11236.
(32) Abdeladhim, M.; Kamhawi, S.; Valenzuela, J. G. What's behind a sand fly bite? The profound effect of sand fly saliva on host hemostasis, inflammation and immunity. *Infect., Genet. Evol.* **2014**, *28*, 691−703.
(33) Gomes, R.; Oliveira, F. The immune response to sand fly salivary proteins and its influence on *leishmania* immunity. *Front Immunol* **2012**, *3*, No. 110.
(34) Liu, Z.; Kundu, R.; Damena, S.; Biter, A. B.; Nyon, M. P.; Chen, W. H.; Zhan, B.; Strych, U.; Hotez, P. J.; Bottazzi, M. E. A scalable and reproducible manufacturing process for Phlebotomus papatasi salivary protein PpSP15, a vaccine candidate for leishmaniasis. *Protein Expression Purif.* **2021**, *177*, No. 105750.
(35) Atayde, V. D.; Aslan, H.; Townsend, S.; Hassani, K.; Kamhawi, S.; Olivier, M. Exosome Secretion by the Parasitic Protozoan *Leishmania* within the Sand Fly Midgut. *Cell Rep* **2015**, *13* (5), 957−967.
(36) Silverman, J. M.; Clos, J.; de'Oliveira, C. C.; Shirvani, O.; Fang, Y.; Wang, C.; Foster, L. J.; Reiner, N. E. An exosome-based secretion pathway is responsible for protein export from *Leishmania* and communication with macrophages. *J. Cell Sci.* **2010**, *123* (Pt 6), 842−852.
(37) Silverman, J. M.; Clos, J.; Horakova, E.; Wang, A. Y.; Wiesgigl, M.; Kelly, I.; Lynn, M. A.; McMaster, W. R.; Foster, L. J.; Levings, M. K.; Reiner, N. E. *Leishmania* exosomes modulate innate and adaptive immune responses through effects on monocytes and dendritic cells. *J. Immunol* **2010**, *185* (9), 5011−5022.
(38) Sebastian, P.; Namdeo, M.; Devender, M.; Anand, A.; Kumar, K.; Veronica, J.; Maurya, R. Polyamine-Enriched Exosomes from *Leishmania donovani* Drive Host Macrophage Polarization via Immunometabolism Reprogramming. *ACS Infect Dis* **2024**, *10* (12), 4384−4399.
(39) Samanta, S.; Datta, R.; Maiti, S. GP63 Alters the Macrophage Golgin160-Associated PIST Distribution by Reducing Caspase-3 Expression during *Leishmania major* Infection. *ACS Infect Dis* **2025**, *11* (12), 3498−3507.
(40) Pinheiro, A. A. S.; Torrecilhas, A. C.; Souza, B. S. F.; Cruz, F. F.; Guedes, H. L. M.; Ramos, T. D.; Lopes-Pacheco, M.; Caruso-Neves, C.; Rocco, P. R. M. Potential of extracellular vesicles in the pathogenesis, diagnosis and therapy for parasitic diseases. *J. Extracell Vesicles* **2024**, *13* (8), No. e12496.
(41) Widmer, G.; Comeau, A. M.; Furlong, D. B.; Wirth, D. F.; Patterson, J. L. Characterization of a RNA virus from the parasite *Leishmania*. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86* (15), 5979−5982.
(42) Grybchuk, D.; Kostygov, A. Y.; Macedo, D. H.; d'Avila-Levy, C. M.; Yurchenko, V. RNA viruses in trypanosomatid parasites: a historical overview. *Mem Inst Oswaldo Cruz* **2018**, *113* (4), No. e170487.
(43) Adaui, V.; Lye, L. F.; Akopyants, N. S.; Zimic, M.; Llanos-Cuentas, A.; Garcia, L.; Maes, I.; De Doncker, S.; Dobson, D. E.; Arevalo, J.; Dujardin, J. C.; Beverley, S. M. Association of the Endobiont Double-Stranded RNA Virus LRV1 With Treatment Failure for Human Leishmaniasis Caused by *Leishmania braziliensis* in Peru and Bolivia. *J. Infect Dis* **2016**, *213* (1), 112−121.
(44) Olivier, M.; Zamboni, D. S. *Leishmania Viannia guyanensis*, LRV1 virus and extracellular vesicles: a dangerous trio influencing the faith of immune response during muco-cutaneous leishmaniasis. *Curr. Opin Immunol* **2020**, *66*, 108−113.
(45) Ives, A.; Ronet, C.; Prevel, F.; Ruzzante, G.; Fuertes-Marraco, S.; Schutz, F.; Zangger, H.; Revaz-Breton, M.; Lye, L. F.; Hickerson, S. M.; Beverley, S. M.; Acha-Orbea, H.; Launois, P.; Fasel, N.; Masina, S. Leishmania RNA virus controls the severity of mucocutaneous leishmaniasis. *Science* **2011**, *331* (6018), 775−778.
(46) Zhang, K. Balancing de novo synthesis and salvage of lipids by *Leishmania* amastigotes. *Curr. Opin. Microbiol.* **2021**, *63*, 98−103.
(47) Mittra, B.; Laranjeira-Silva, M. F.; Perrone Bezerra de Menezes, J.; Jensen, J.; Michailowsky, V.; Andrews, N. W. A Trypanosomatid Iron Transporter that Regulates Mitochondrial Function Is Required for *Leishmania amazonensis* Virulence. *PLoS Pathog.* **2016**, *12* (1), No. e1005340.
(48) Rogers, M. B.; Hilley, J. D.; Dickens, N. J.; Wilkes, J.; Bates, P. A.; Depledge, D. P.; Harris, D.; Her, Y.; Herzyk, P.; Imamura, H.;

Otto, T. D.; Sanders, M.; Seeger, K.; Dujardin, J. C.; Berriman, M.; Smith, D. F.; Hertz-Fowler, C.; Mottram, J. C. Chromosome and gene copy number variation allow major structural change between species and strains of Leishmania. *Genome Res.* **2011**, *21* (12), 2129−2142.
(49) Negreira, G. H.; Monsieurs, P.; Imamura, H.; Maes, I.; Kuk, N.; Yagoubat, A.; Van den Broeck, F.; Sterkers, Y.; Dujardin, J. C.; Domagalska, M. A. High throughput single-cell genome sequencing gives insights into the generation and evolution of mosaic aneuploidy in *Leishmania donovani*. *Nucleic Acids Res.* **2022**, *50* (1), 293−305.
(50) Perea-Martínez, A.; Garcia-Hernandez, R.; Manzano, J. I.; Gamarro, F. Transcriptomic Analysis in Human Macrophages Infected with Therapeutic Failure Clinical Isolates of *Leishmania infantum*. *ACS Infect Dis* **2022**, *8* (4), 800−810.
(51) Schonian, G.; Kuhls, K.; Mauricio, I. L. Molecular approaches for a better understanding of the epidemiology and population genetics of. *Leishmania. Parasitol.* **2011**, *138* (4), 405−425.
(52) Patino, L. H.; Castillo-Castaneda, A.; Munoz, M.; Muskus, C.; Rivero-Rodriguez, M.; Perez-Doria, A.; Bejarano, E. E.; Ramirez, J. D. Revisiting the heterogeneous global genomic population structure of *Leishmania infantum*. *Microb. Genomics* **2021**, *7* (9), No. 000640, DOI: 10.1099/mgen.0.000640.
(53) Akhoundi, M.; Kuhls, K.; Cannet, A.; Votypka, J.; Marty, P.; Delaunay, P.; Sereno, D. A Historical Overview of the Classification, Evolution, and Dispersion of *Leishmania* Parasites and Sandflies. *PLoS Neglected Trop. Dis.* **2016**, *10* (3), No. e0004349.
(54) Kloehn, J.; Saunders, E. C.; O'Callaghan, S.; Dagley, M. J.; McConville, M. J. Characterization of metabolically quiescent *Leishmania* parasites in murine lesions using heavy water labeling. *PLoS Pathog.* **2015**, *11* (2), No. e1004683.
(55) Cloutier, S.; Laverdiere, M.; Chou, M. N.; Boilard, N.; Chow, C.; Papadopoulou, B. Translational control through eIF2alpha phosphorylation during the *Leishmania* differentiation process. *PLoS One* **2012**, *7* (5), No. e35085.
(56) Jara, M.; Barrett, M.; Maes, I.; Regnault, C.; Imamura, H.; Domagalska, M. A.; Dujardin, J. C. Transcriptional Shift and Metabolic Adaptations during *Leishmania* Quiescence Using Stationary Phase and Drug Pressure as Models. *Microorganisms* **2022**, *10* (1), No. 97.
(57) Khare, S.; Nagle, A. S.; Biggart, A.; Lai, Y. H.; Liang, F.; Davis, L. C.; Barnes, S. W.; Mathison, C. J.; Myburgh, E.; Gao, M. Y.; Gillespie, J. R.; Liu, X.; Tan, J. L.; Stinson, M.; Rivera, I. C.; Ballard, J.; Yeh, V.; Groessl, T.; Federe, G.; Koh, H. X.; Venable, J. D.; Bursulaya, B.; Shapiro, M.; Mishra, P. K.; Spraggon, G.; Brock, A.; Mottram, J. C.; Buckner, F. S.; Rao, S. P.; Wen, B. G.; Walker, J. R.; Tuntland, T.; Molteni, V.; Glynne, R. J.; Supek, F. Proteasome inhibition for treatment of leishmaniasis, Chagas disease and sleeping sickness. *Nature* **2016**, *537* (7619), 229−233.
(58) Silva-Moreira, A. L.; Serravite, A. M.; Rios-Barros, L. V.; de Menezes, J. P. B.; Horta, M. F.; Castro-Gomes, T. New insights into the life cycle, host cell tropism, and infection amplification of *Leishmania* spp. *Infect. Immun.* **2025**, *93* (7), No. e0012325.
(59) do Nascimento, P. R. P.; Mendes-Aguiar, C. O.; Morais, I. C.; Neto, J. F. R.; Wilson, M. E.; Jeronimo, S. M. B. Neutrophil-*Leishmania* infantum Interaction Induces Neutrophil Extracellular Traps, DAMPs, and Inflammatory Molecule Release. *ACS Infect. Dis.* **2025**, *11* (2), 483−492.
(60) Peters, N. C.; Egen, J. G.; Secundino, N.; Debrabant, A.; Kimblin, N.; Kamhawi, S.; Lawyer, P.; Fay, M. P.; Germain, R. N.; Sacks, D. *In vivo* imaging reveals an essential role for neutrophils in leishmaniasis transmitted by sand flies. *Science* **2008**, *321* (5891), 970−974.
(61) Bora, K.; Kanaujia, S. P.; Dubey, V. K. Targeting antioxidant defense system enzymes for Leishmaniasis treatment. *Int. J. Biol. Macromol.* **2025**, *316* (Pt 1), No. 144650.
(62) Aga, E.; Katschinski, D. M.; van Zandbergen, G.; Laufs, H.; Hansen, B.; Muller, K.; Solbach, W.; Laskay, T. Inhibition of the spontaneous apoptosis of neutrophil granulocytes by the intracellular parasite *Leishmania major*. *J. Immunol.* **2002**, *169* (2), 898−905.
(63) Ribeiro-Gomes, F. L.; Otero, A. C.; Gomes, N. A.; Moniz-De-Souza, M. C.; Cysne-Finkelstein, L.; Arnholdt, A. C.; Calich, V. L.; Coutinho, S. G.; Lopes, M. F.; DosReis, G. A. Macrophage interactions with neutrophils regulate *Leishmania major* infection. *J. Immunol* **2004**, *172* (7), 4454−4462.
(64) Mondal, D.; Bern, C.; Ghosh, D.; Rashid, M.; Molina, R.; Chowdhury, R.; Nath, R.; Ghosh, P.; Chapman, L. A. C.; Alim, A.; Bilbe, G.; Alvar, J. Quantifying the Infectiousness of Post-Kala-Azar Dermal Leishmaniasis Toward Sand Flies. *Clin Infect Dis* **2019**, *69* (2), 251−258.
(65) Schwing, A.; Pisani, D. F.; Pomares, C.; Majoor, A.; Lacas-Gervais, S.; Jager, J.; Lemichez, E.; Marty, P.; Boyer, L.; Michel, G. Identification of adipocytes as target cells for *Leishmania infantum* parasites. *Sci. Rep* **2021**, *11* (1), No. 21275.
(66) Lopes, C. S.; Daifalla, N.; Das, B.; Dias da Silva, V.; Campos-Neto, A. CD271+ Mesenchymal Stem Cells as a Possible Infectious Niche for *Leishmania infantum*. *PLoS One* **2016**, *11* (9), No. e0162927.
(67) Paugam, A.; Bulteau, A. L.; Dupouy-Camet, J.; Creuzet, C.; Friguet, B. Characterization and role of protozoan parasite proteasomes. *Trends Parasitol.* **2003**, *19* (2), 55−59.
(68) Besteiro, S.; Williams, R. A.; Coombs, G. H.; Mottram, J. C. Protein turnover and differentiation in *Leishmania*. *Int. J. Parasitol.* **2007**, *37* (10), 1063−1075.
(69) Rosenzweig, D.; Smith, D.; Opperdoes, F.; Stern, S.; Olafson, R. W.; Zilberstein, D. Retooling *Leishmania* metabolism: from sand fly gut to human macrophage. *FASEB J.* **2008**, *22* (2), 590−602.
(70) Bijlmakers, M. J. Ubiquitination and the Proteasome as Drug Targets in Trypanosomatid Diseases. *Front. Chem.* **2021**, *8*, No. 630888.
(71) Ortiz, D.; Forquer, I.; Boitz, J.; Soysa, R.; Elya, C.; Fulwiler, A.; Nilsen, A.; Polley, T.; Riscoe, M. K.; Ullman, B.; Landfear, S. M. Targeting the Cytochrome bc1 Complex of *Leishmania* Parasites for Discovery of Novel Drugs. *Antimicrob. Agents Chemother.* **2016**, *60* (8), 4972−4982.
(72) Saleem, K.; Khursheed, Z.; Hano, C.; Anjum, I.; Anjum, S. Applications of Nanomaterials in Leishmaniasis: A Focus on Recent Advances and Challenges. *Nanomaterials* **2019**, *9* (12), No. 1749.
(73) Bessa, I. A. A.; D'Amato, D. L.; AB, C. S.; Levita, D. P.; Mello, C. C.; da Silva, A. F. M.; Dos Santos, T. C.; Ronconi, C. M. Innovating Leishmaniasis Treatment: A Critical Chemist's Review of Inorganic Nanomaterials. *ACS Infect Dis* **2024**, *10* (8), 2485−2506.
(74) Tambe, S.; Nag, S.; Pandya, S. R.; Kumar, R.; Balakrishnan, K.; Kumar, R.; Kumar, S.; Amin, P.; Gupta, P. K. Revolutionizing Leishmaniasis Treatment with Cutting Edge Drug Delivery Systems and Nanovaccines: An Updated Review. *ACS Infect Dis* **2024**, *10* (6), 1871−1889.
(75) Glennie, N. D.; Scott, P. Memory T cells in cutaneous leishmaniasis. *Cell. Immunol.* **2016**, *309*, 50−54.
(76) Goonoo, N.; Huet, M. A. L.; Chummun, I.; Karuri, N.; Badu, K.; Gimie, F.; Bergrath, J.; Schulze, M.; Muller, M.; Bhaw-Luximon, A. Nanomedicine-based strategies to improve treatment of cutaneous leishmaniasis. *R Soc. Open Sci.* **2022**, *9* (6), No. 220058.
(77) Ubeda, J. M.; Legare, D.; Raymond, F.; Ouameur, A. A.; Boisvert, S.; Rigault, P.; Corbeil, J.; Tremblay, M. J.; Olivier, M.; Papadopoulou, B.; Ouellette, M. Modulation of gene expression in drug resistant *Leishmania* is associated with gene amplification, gene deletion and chromosome aneuploidy. *Genome Biol.* **2008**, *9* (7), No. R115.
(78) Marquis, N.; Gourbal, B.; Rosen, B. P.; Mukhopadhyay, R.; Ouellette, M. Modulation in aquaglyceroporin AQP1 gene transcript levels in drug-resistant *Leishmania*. *Mol. Microbiol.* **2005**, *57* (6), 1690−1699.
(79) Mondelaers, A.; Hendrickx, S.; Van Bockstal, L.; Maes, L.; Caljon, G. Miltefosine-resistant *Leishmania infantum* strains with an impaired MT/ROS3 transporter complex retain amphotericin B susceptibility. *J. Antimicrob. Chemother.* **2018**, *73* (2), 392−394.
(80) Sundar, S.; Rai, M.; Chakravarty, J.; Agarwal, D.; Agrawal, N.; Vaillant, M.; Olliaro, P.; Murray, H. W. New treatment approach in

Indian visceral leishmaniasis: single-dose liposomal amphotericin B followed by short-course oral miltefosine. *Clin. Infect. Dis.* **2008**, *47* (8), 1000−1006.
(81) Mitchell, M. J.; Billingsley, M. M.; Haley, R. M.; Wechsler, M. E.; Peppas, N. A.; Langer, R. Engineering precision nanoparticles for drug delivery. *Nat. Rev. Drug Discov* **2021**, *20* (2), 101−124.
(82) Dos Santos Vasconcelos, C. R.; Rezende, A. M. Systematic in silico Evaluation of *Leishmania* spp. Proteomes for Drug Discovery. *Front Chem.* **2021**, *9*, No. 607139.
(83) Challapa-Mamani, M. R.; Tomas-Alvarado, E.; Espinoza-Baigorria, A.; Leon-Figueroa, D. A.; Sah, R.; Rodriguez-Morales, A. J.; Barboza, J. J. Molecular Docking and Molecular Dynamics Simulations in Related to *Leishmania donovani*: An Update and Literature Review. *Trop. Med. Infect Dis.* **2023**, *8* (10), No. 457.
(84) Pacheco-Fernandez, T.; Markle, H.; Verma, C.; Huston, R.; Gannavaram, S.; Nakhasi, H. L.; Satoskar, A. R. Field-Deployable Treatments For Leishmaniasis: Intrinsic Challenges, Recent Developments and Next Steps. *Res. Rep. Trop Med.* **2023**, *14*, 61−85.
(85) Pandey, A. M.; Malwal, S. R.; Valladares-Delgado, M.; Labrador-Fagundez, L.; Stella, B. G.; Diaz-Perez, L. J.; Rey-Cibati, A.; Singh, D.; Stampolaki, M.; Hong, S.; Gennis, R. B.; Kolocouris, A.; Benaim, G.; Oldfield, E. Anti-Parasitics with a Triple Threat: Targeting Parasite Enzymes, the Proton Motive Force, and Host Cell-Mediated Killing. *ACS Infect. Dis.* **2025**, *11* (6), 1539−1551.
(86) Siadat, A. H.; Iraji, F.; Zolfaghari, A.; Shariat, S.; Jazi, S. B. Heat therapy for cutaneous leishmaniasis: A literature Review. *J. Res. Med. Sci.* **2021**, *26*, No. 15.
(87) Artzi, O.; Sprecher, E.; Koren, A.; Mehrabi, J. N.; Katz, O.; Hilerowich, Y. Fractional Ablative CO2 Laser Followed by Topical Application of Sodium Stibogluconate for Treatment of Active Cutaneous Leishmaniasis: A Randomized Controlled Trial. *Acta Derm.-Venereol.* **2019**, *99* (1), 53−57.
(88) Saini, I.; Joshi, J.; Kaur, S. *Leishmania* vaccine development: A comprehensive review. *Cell. Immunol.* **2024**, *399*−*400*, No. 104826.
(89) Morales-Yuste, M.; Martin-Sanchez, J.; Corpas-Lopez, V. Canine Leishmaniasis: Update on Epidemiology, Diagnosis, Treatment, and Prevention. *Vet Sci.* **2022**, *9* (8), No. 387.
(90) Bastos, D. S. S.; Miranda, B. M.; Rooke, C. I.; Ferreira, N. J. S.; Ervilha, L. O. G.; Carvalho, R. P. R.; Souza, A. C. F.; Neves, M. M.; Oliveira, L. L.; Marques da Silva, E. A. Protective Effects of rLPG3 Plus Freund's Incomplete Adjuvant on Parasitism, Hepatic Function, and Immune Modulation in Experimental Visceral Leishmaniasis. *ACS Infect. Dis.* **2026**, *12*, No. 794.
(91) Gupta, N.; Yadav, A. K.; Verma, P. K.; Srivastava, M.; Sahasrabuddhe, A. A.; Dube, A. Differential Immune Responses of Th1 Stimulatory Chimeric Antigens of *Leishmania donovani* in BALB/c Mice. *ACS Infect Dis* **2024**, *10* (12), 4246−4257.
(92) Mukherjee, S.; Sengupta, R.; Mukhopadhyay, D.; Braun, C.; Mitra, S.; Roy, S.; Kanti Das, N.; Chatterjee, U.; von Stebut, E.; Chatterjee, M. Impaired activation of lesional CD8(+) T-cells is associated with enhanced expression of Programmed Death-1 in Indian Post Kala-azar Dermal Leishmaniasis. *Sci. Rep* **2019**, *9* (1), No. 762.
(93) Roy, S.; Mukhopadhyay, D.; Mukherjee, S.; Moulik, S.; Chatterji, S.; Brahme, N.; Pramanik, N.; Goswami, R. P.; Saha, B.; Chatterjee, M. An IL-10 dominant polarization of monocytes is a feature of Indian Visceral Leishmaniasis. *Parasite Immunol.* **2018**, *40* (7), No. e12535.
(94) Parkash, V.; Kaye, P. M.; Layton, A. M.; Lacey, C. J. Vaccines against leishmaniasis: using controlled human infection models to accelerate development. *Expert Rev. Vaccines* **2021**, *20* (11), 1407−1418.
(95) Hendrickx, S.; Feijens, P. B.; Escudie, F.; Chatelain, E.; Maes, L.; Caljon, G. *In Vivo* Bioluminescence Imaging Reveals Differences in *Leishmania infantum* Parasite Killing Kinetics by Antileishmanial Reference Drugs. *ACS Infect Dis* **2024**, *10* (6), 2101−2107.
(96) Botana, L.; Matia, B.; San Martin, J. V.; Romero-Mate, A.; Castro, A.; Molina, L.; Fernandez, L.; Ibarra-Meneses, A.; Aguado, M.; Sanchez, C.; Horrillo, L.; Chicharro, C.; Nieto, J.; Ortega, S.; Ruiz-Giardin, J. M.; Carrillo, E.; Moreno, J. Cellular Markers of Active Disease and Cure in Different Forms of *Leishmania infantum*-Induced Disease. *Front. Cell. Infect. Microbiol.* **2018**, *8*, No. 381.

https://doi.org/10.1021/acsinfecdis.6c00135
ACS Infect. Dis. XXXX, XXX, XXX−XXX