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Immunomodulatory therapy of visceral leishmaniasis in HIV coinfected patients

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Abstract

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Patients with visceral leishmaniasis (VL) – human immunodeficiency virus (HIV) coinfection experience increased drug toxicity and treatment failure rates compared to VL patients, with more frequent VL relapse and death. In the era of VL elimination strategies, HIV coinfection is progressively becoming a key challenge, because HIV coinfected patients respond poorly to conventional VL treatment and play an important role in parasite transmission. With limited chemotherapeutic options and a paucity of novel anti-parasitic drugs, new interventions that target host immunity may offer an effective alternative. In this review, we first summarize current views on how VL immunopathology is significantly affected by HIV coinfection. We then review current clinical and promising preclinical immunomodulatory interventions in the field of VL and discuss how these may operate in the context of a concurrent HIV infection. Caveats are formulated as these interventions may unpredictably impact the delicate balance between boosting of beneficial VL-specific responses and deleterious immune activation/ hyperinflammation, activation of latent provirus or increased HIV-susceptibility of target cells. Evidence is lacking to prioritize a target molecule and a more detailed account of the immunological status induced by the coinfection as well as surrogate markers of cure and protection are still required. We do however argue that virologically suppressed VL patients with a recovered immune system, in whom effective antiretroviral therapy alone is not able to restore protective immunity, can be considered a relevant target group for an immunomodulatory intervention. Finally, we provide perspectives on the translation of novel theories on synergistic immune cell cross-talk into an effective treatment strategy for VL-HIV coinfected patients.

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IMMUNOMODULATORY THERAPY OF VISCERAL LEISHMANIASIS IN HIV COINFECTED PATIENTS

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28 Abstract

29 Patients with visceral leishmaniasis (VL) - human immunodeficiency virus (HIV) coinfection 30 experience increased drug toxicity and treatment failure rates compared to VL patients, with 31 more frequent VL relapse and death. In the era of VL elimination strategies, HIV coinfection is progressively becoming a key challenge, because HIV coinfected patients respond poorly to 32 33 conventional VL treatment and play an important role in parasite transmission. With limited 34 chemotherapeutic options and a paucity of novel anti-parasitic drugs, new interventions that 35 target host immunity may offer an effective alternative. In this review, we first summarize 36 current views on how VL immunopathology is significantly affected by HIV coinfection. We 37 then review current clinical and promising preclinical immunomodulatory interventions in the 38 field of VL and discuss how these may operate in the context of a concurrent HIV infection. 39 Caveats are formulated as these interventions may unpredictably impact the delicate balance 40 between boosting of beneficial VL-specific responses and deleterious immune 41 activation/hyperinflammation, activation of latent provirus or increased HIV-susceptibility of 42 target cells. Evidence is lacking to prioritize a target molecule and a more detailed account of 43 the immunological status induced by the coinfection as well as surrogate markers of cure and 44 protection are still required. We do, however, argue that virologically suppressed VL patients 45 with a recovered immune system, in whom effective antiretroviral therapy alone is not able to restore protective immunity, can be considered a relevant target group for an 46 47 immunomodulatory intervention. Finally, we provide perspectives on the translation of novel 48 theories on synergistic immune cell cross-talk into an effective treatment strategy for VL-HIV 49 coinfected patients.

50 1. Introduction

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51 Visceral leishmaniasis (VL), also called kala-azar, is a vector-borne protozoan infection caused by species of the Leishmania donovani complex, which mainly targets tissue 52 53 macrophages of systemic organs, such as spleen, liver and bone marrow (1). Characteristics of 54 the disease include chronic fever, hepatosplenomegaly, and pancytopenia_(1). Untreated, overt disease is universally lethal (1). Zoonotic VL, with dogs as the main reservoir, is mainly 55 prevalent in the Mediterranean basin and in South America, and is caused by Leishmania-(L.) 56 57 infantum. Anthroponotic VL is prevalent on the Indian subcontinent and in East Africa and is 58 typically caused by L. donovani_(2). According to the recent World Health Organization 59 (WHO) report, VL is endemic in 75 countries with an estimated 50,000 to 90,000 new cases 60 occurring each year (3). Ninety percent of the global disease burden occurs in just six countries: India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia (3). 61

Chemotherapy is currently the sole form of treatment in clinical practice. The pentavalent 63 64 antimonial (Sb^V) compounds (sodium stibogluconate (SSG) commercialized as Pentostam®; meglumine antimoniate commercialized as Glucantime®) have been the cornerstone of first-65 line treatment of VL over the last 70 years. However, these compounds, but are far from 66 67 optimal due to severe toxicity and the emergence of antimonial resistance on the Indian subcontinent (1, 4). Newer drugs that are increasingly used include paromomycin, 68 69 miltefosine, pentamidine, and conventional and liposomal amphotericin B. All these drugs 70 have <u>a number of important</u>several important disadvantages as shown in -(Table 1). While 71 various combination therapy regimens designed to overcome some of the shortcomings are 72 highly efficacious in India, disappointing findings on some combination regimens have been recently reported in East Africa -(5-10). As of Until today, no comparative studies have been 73 conducted done to explain this geographical difference, but the parasite genetic diversity 74 75 diverse parasite species and host immune phenotypesity genotypes are assumed as key 76 factors. Novel chemotherapeutic drugs are in the initial development pipeline and but are 77 therefore unlikely to be widely available within the next few years. Nevertheless, over 90% to 95% of immunocompetent patients display a good clinical response to currently 78 79 recommended conventional treatment regimens, with treatment unresponsiveness, death or 80 severe toxicity observedseen in less than 5% to 10% of patients (11). Less than 5% of 81 immunocompetent individuals who initially cure develop a relapse, most commonly within 6-12 months after treatment_(5). Treatment outcomes however vary substantially between 82 different geographic regions and depend on the drug(s) used, drug exposure, parasite 83 84 susceptibility to the drug, severity of disease, host immunity and the presence of coinfections 85 (11-13).

1.1. Emerging challenge of <u>VL-</u>HIV coinfection

87 Human immunodeficiency virus (HIV) has been identified as one of the emerging challenges facing the control of VL (14). The immunological status of HIV-infected patients is 88 89 particularly favorable for the multiplication of Leishmania parasites. HIV coinfection 90 substantially increases the risk of progression from asymptomatic Leishmania infection to 91 active disease (15, 16). On the other hand, VL accelerates HIV disease progression towards 92 acquired immunodeficiency syndrome (AIDS) and could induce expression of latent proviruses (14). HIV has fueled the re-emergence of VL in Southern Europe and Brazil, 93 where up to 70% of VL cases are associated with HIV infection (7). Tand the problem is 94 95 currently particularly severe in areas such as Northern Ethiopia, where up to 430% of all patients with VL patients are coinfected with HIV (17). Since 2001, 35 countries have 96 97 reported between 2 to 30% of VL cases as co-infected with HIV, but thisese percentagess 98 isare most probably underestimations (14). Because the disease affects the most poor and 99 most neglected patients within an already neglected disease population, under-reporting in

most endemic areas is common due to a lack of facilities to diagnose one or both of the
 diseases and to poor reporting systems. Importantly, VL-HIV coinfected patients are also
 often considered super-spreaders of VL, and thus pose a major threat to current elimination
 strategies (18).

105 Since 1996, combined antiretroviral treatment (cART), comprising three antiretroviral drugs, 106 constitutes the cornerstone of HIV treatment. The treatment options continue to expand with 107 new drugs and co-formulations; by the end of 2016, there were 40 antiretroviral drugs from 108 six different classes approved by the Food and Drug Administration. In most resource-109 constrained settings, the standardized WHO guidelines are used for ART, which currently 110 recommends a combination of tenofovir, lamivudine and efavirenz as first line treatment. 111 WHO recommended first line regimens have been found highly effective in resource-112 constrained settings (19). The main aim of cART is sustainabledurable suppression of HIV 113 replication, and with good adherence, this can generally be achieved, leading to a close to normal life expectancy (20). 114

VL is one of the AIDS-defining conditions, requiring anti-leishmanial treatment and cART irrespective of CD4⁺ T cell count (7). Although there are limited *in vitro* data suggesting that HIV-1 protease inhibitors and possibly some other antiretroviral drugs might directly exert inhibitory effects on *Leishmania*, there is insufficient evidence for their clinical use against VL, and standard ART regimens are currently recommended in VL-HIV coinfection (5). In low income countries, this is provided by standardized first and second line regimens in a public health approach (21, 22).

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124 Increased toxicity and parasitologically-confirmed treatment failures (up to 30%) were 125 observed in VL-HIV coinfected patients treated with SbV, with case fatality rates up to 24% (14, 17, 23). While liposomal amphotericin B was consistently found to have excellent 126 tolerability, VL cure rates in HIV coinfected individuals have been rather disappointing in 127 128 East Africa. For example, at a total dose of 30 mg/kg, around 16% of primary VL and 56% of 129 VL relapse cases demonstrate parasitological failure in northern Ethiopia (17). WHO now 130 proposes a total dose of 40 mg/kg (7, 24, 25). Experience with miltefosine in VL-HIV 131 coinfection is limited, but suggests moderate efficacy and an acceptable toxicity profile (23, 132 26-29). To date, only one clinical trial in HIV coinfected patients has been conducted with miltefosine, with 18% of patients displaying initial parasitological treatment failure and 25% 133 134 relapsing, although deaths were excluded (23). The role of combination therapy in VL-HIV 135 coinfection is currently under exploration in clinical trials in India and East-Africa. 136

137 While in Europe widespread use of cART has resulted in a pronounced (i.e. 60 %) reduction 138 in the incidence of VL-HIV coinfection, relapse in coinfected subjects remains substantial at up to 60% after one year (14, 30, 31) and secondary prophylaxis has only a partial effect (32). 139 140 In a pentamidine secondary prophylaxis trial in Ethiopia, the relapse-free survival rate at two years was only 58.3% (Diro 2017, CID, in press). Even with access to all current 141 142 chemotherapies, the prognosis in VL-HIV coinfection remains dire. Currently, it is believed 143 that VL can only be effectively treated in HIV patients before profound immune deficiency 144 has developed. 145

146 VL-HIV coinfection has a number of unique clinical and immunological features. In contrast 147 to many other HIV-associated opportunistic infections, CD4⁺ T cell reconstitution is severely 148 delayed (even if virological suppression is reached) and the immune reconstitution 149 inflammatory syndrome (IRIS) to a *Leishmania* infection after initiation of cART appears 150 relatively rare, indicating a persistent suppression of host immunity (33, 34). Atypical clinical 151 presentations can occur and amastigotes have been detected in tissues such as the intestine, where parasites are mostly undetectable in the immunocompetent host (14, 35). After clinical remission, parasitemia also appears to persist, at least intermittently (36). A chronic/intermittent course of VL lasting several years has been described, labelled as "active chronic visceral leishmaniasis" (36). Consequently, HIV-infected patients will develop multiple VL relapses and often become progressively more difficult to treat, ultimately leading to a stage of complete treatment unresponsiveness. Hence, there is an urgent need for innovative and effective alternative therapies against VL-HIV coinfection.

159 **1.2. Promising role of immunomodulatory therapy**

160 It has become increasingly clear that the host immune response is a critical factor determining 161 VL treatment response and control, acting in synergy with anti-leishmanial drugs (37). This implies that in immunosuppressed individuals, targeting parasites alone with conventional 162 163 anti-leishmanial drugs but without enhancing the immune response might simply not be 164 sufficient. This interaction between drugs and the immune system was first suggested in 165 animal models of VL, where the efficacy of pentavalent antimony (Sb^v) was lower after T cell 166 depletion (38). This was, probably related to the decreased cellular uptake of Sb^{V} into interferon gamma (IFNy) activated macrophages, where it is normally converted 167 intracellularly into its active trivalent form (Sb^{III})_(4). While this finding should be 168 169 extrapolated with caution, this mechanism may explain the observations that 170 immunocompromised patients with VL failed to respond to antimonial drugs.

171 Immunotherapy is defined as the use of biological molecules or pharmacological compounds 172 173 to modulate immune responses directly or in combination with drugs. A combination of 174 immunomodulatory and direct anti-parasitic drugs could enhance the efficacy of 175 chemotherapy and even prevent drug resistance (39). On top of its successful use in treating 176 several non-infectious disorders (e.g. cancer, rheumatoid arthritis, etc.), the use of immune-177 based combination therapy is increasingly being explored in infectious diseases such as 178 tuberculosis (40), and leprosy (41) has proven successful in malaria, tuberculosis and leprosy. 179 Despite several candidates being in the drug development pipeline, there are no immunotherapeutic agents or vaccines against VL currently registered for human use in 180 181 routine clinical practice due to multiple reasons (e.g. high costs of clinical trials, limited and 182 remote patient populations, ineffectiveness, safety concerns, etc.) (42). Experimental immunebased approaches are also being explored in the domain of HIV, where many have reached 183 184 Phase I and some Phase II clinical trials but as of until today have failed to provide enough 185 immune restoration, potent effectiveness, sustainable benefits, delay of clinical progression or good safety profiles -(40, 43-46). However, VL-HIV coinfected patients are often excluded or 186 neglected in such studies, although both individual patients as well as public health 187 188 approaches in general could benefit from these interventions.

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Here, we first summarize current views on how host immunity against VL is affected during HIV coinfection, and then discuss the potential of current immunomodulatory therapies against VL in the context of concurrent HIV infection (both human studies and promising experimental approaches, excluding prophylactic studies). In particular, key targets and potential caveats are emphasized to guide future research on immunomodulatory therapies against VL and support the inclusion of HIV coinfected patients in clinical research.

196 2. Immunopathogenesis of VL-HIV coinfection

197 Macrophages represent an important common reservoir for HIV and *Leishmania* and serve as 198 vehicles that disseminate both virus and parasite throughout the host. In addition, both 199 pathogens may interact with each other to exacerbate immune suppression (Figure 1). In fact, 200 both pathogens severely alter the antigen processing and presentation capacities of dendritic Formatted: Not Highlight

cells and macrophages, and synergistically escape immune surveillance using an array of strategies yet to be fully understood (47).

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204 The control of VL in experimental models has been robustly associated with a strong T helper 205 1 (Th1) immune response, with large amounts of IL-2 and IFN γ (48) (Figure 1). In addition, a M2 polarization of macrophages has been associated with suppression of cell-mediated 206 207 immunity, that confers susceptibility to intracellular infection. However, the immune 208 mechanisms modulating VL in murine models or humans differs significantly. Human studies 209 have shown a Th1/Th17 protective pattern with a somewhat different T cell functionality 210 compared to experimental models, but lack comprehensive longitudinal data (49, 50). CD8+ T 211 cells have also been shown to produce IFNy that can contribute to VL control (51). The 212 immunosuppressive effects of IL-10, and the regulatory role of other cytokines such as IL-27, 213 have been implicated in the development of the different clinical pictures (50). Impaired 214 neutrophil effector function has also been suggested to play a key role in the pathogenesis of VL (52). Partly due to the lack of good animal or in vitro models, it is currently unknown 215 216 whether and how these protective and immunosuppressive patterns of VL are modulated by 217 HIV and ART and how they define the pertinent clinical outcomes of VL-HIV patients.

219 HIV-1 causes a general profound impairment of cell-mediated immunity with low levels of 220 CD4⁺ Th1 cells, the main protective cells in VL (Figure 1). HIV also skews the host immunity 221 towards a Th2 response that only becomes affected at the later stages of the viral infection, 222 potentially provoking parasite replication. Th17 cells are also associated with protection in 223 The o VL, and but are protection associated T helper subset of Th17 cells are is highly 224 permissive to HIV infection., Tand t and their frequency is significantly and preferentially 225 reduced in the gastrointestinal tract, even in patients with undetectable plasma viral load 226 under ART (53). DTheir depletion of Th17 cells from the gut-associated lymphoid tissue together with a series of immunopathological events occurring at the gastrointestinal tract 227 228 mucosa leads to microbial translocation and consequently higher non-specific immune 229 activation and hyper-inflammation (54). This microbial translocation has been postulated as 230 one of the factors causing non-specific early T cell exhaustion and senescence (55), which 231 may further weaken protective immunity towards VL. Likewise, VL was reported as an 232 independent cause of increased non-specific immune activation, T cell senescence and the 233 lack of immune recovery in virologically-suppressed coinfected HIV patients (56, 57). In line 234 with T cell exhaustion, chronic immune activation was recently associated with recurrent 235 relapse of VL in HIV patients (58). Recent research in VL-HIV patients also suggested that 236 weak antigen-specific functional responses or proliferation of T cells after in vitro stimulation 237 was an important predictor of relapse (59). Despite the pivotal role of CD8⁺ T cells in viral 238 and parasite clearance, their contribution in VL-HIV control and level of exhaustion remains 239 unknown. Likewise, it is still unclear as to what impact Leishmania infection could have on 240 the capacity of resting memory CD4⁺ T cells to act as a stable reservoir of latent HIV 241 infection,. WV or vice versa, it remains unknown what impact a spike in viral replication may have on anti-leishmanial immunity (e.g. by bystander activation of Leishmania specific 242 243 memory cells) also remains unknown (60, 61).

The consequences of infection by two immune suppressive pathogens could therefore be a symbiotic and persistent incapacitation of the host's immune system, favoring a state of immunological anergy, ultimately being fatal to the patient. A better understanding of the immune response against *Leishmania* infection in HIV coinfected patients is crucial to establish a rational approach for immunomodulatory therapy.

3. Status of immunotherapeutic interventions in human VL and their application in HIV patients

252 Due to the lack of a protective role of anti-Leishmania antibodies in early studies, passive 253 immunization was not further explored, while active immunization with immunomodulators 254 and vaccine therapy was investigated (62). Early studies by Murray et al. (38, 63, 64)showed 255 the therapeutic utility of interleukin-2 (IL-2), IL-12, interferon-gamma (IFN γ) and 256 granulocyte-monocyte colony stimulating factor (GM-CSF) in murine VL models-(38, 63, 257 64). Although the Th1/Th2 dichotomy of immunity to VL is not fully upheld in humans, 258 clinical immunotherapeutic studies on VL patients have been skewed towards Th1-associated 259 cytokine adjuvanted therapy and are discussed below (see Table 2). For VL-HIV coinfection, 260 only five published case reports using recombinant IFNy, IL-2 and GM-CSF combined chemotherapy were found in literature (see Table 2). 261

3.1. Interferon-29 (IFN29)

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There has been limited success in small-scale clinical trials with combined therapy of IFN γ and Sb^v for treating VL. This combination therapy displayed stronger parasitological and clinical cure rates in VL patients (mainly children) from Brazil, Kenya and India compared with the drug alone, but these studies had several limitations (see Table 2 for details). In a subsequent larger randomized controlled trial (RCT) in India, these improved treatment outcomes could not be confirmed (65). Importantly, treatment response in this particular study was generally poor as drug resistance was emerging in that region.

271 There are a few case reports, mostly from the pre-ART eraarea, providing information on whether IFNy can be safely administered in VL-HIV patients (see Table 2), which is of 272 273 relevance since IFNy also has a vital but ambiguous role in the pathogenesis of HIV (66). IFN γ appeared to be fairly well tolerated but showed inconclusive results (67-69). In one old 274 case report of a patient with VL-HIV coinfection, acceleration of Kaposi's sarcoma has been 275 reported (70). The therapeutic potential of IFNy to treat HIV coinfections, was supported by 276 277 two Phase II trials, evaluating adjunctive IFN γ to improve treatment response to antifungals in HIV patients with cryptococcal meningitis (71, 72). However, in the early 1990s, a 278 multicenter clinical trial of SSG plus IFNy for VL in HIV coinfected patients in Spain was 279 280 suspended following an interim analysis indicating that there was an excess of severe 281 secondary effects and no benefit over drug alone (69). The findings itself have never been 282 published but-suggested -a limited value of IFNy therapy for VL-HIV coinfection.

283 3.2. Granulocyte macrophage colony stimulating factor (GM-CSF)

284 GM-CSF can inhibit the intracellular replication of protozoa such as Leishmania. The justification to explore GM-CSF as immunotherapeutic agent stems from documented effects 285 286 such as monocyte mobilization, macrophage activation, the production of pro-inflammatory 287 cytokines and amelioration of neutropenia (63). GM-CSF combined with Sb^V was 288 successfully explored in 20 neutropenic VL patients in Brazil. All responded well to VL 289 treatment, neutropenia rapidly improved and secondary infections decreased (73) (Table 2). 290 The authors did however not include a control arm, however, making it unclear whether the 291 effect of GM-CSF, if any, could be due to the reversal of neutropenia (and might hence not 292 apply in those without neutropenia) or whether other mechanisms were involved. On the other 293 hand, in vitro studies have recently suggested that GM-CSF could contradictory promote 294 Leishmania growth by inducing monocyte proliferation and induction of intracellular dNTP 295 production (74), but whether this would also occur in humans remains unknown.

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In terms of safety, several older clinical trials of GM-CSF administration in HIV patients indicated that it might accelerate HIV replication (75). In contrast, more recent RCTs have demonstrated benefits of using GM-CSF in virologically suppressed patients as an adjunct to conventional ART or therapeutic HIV vaccination (75, 76). This would argue against using 301 GM-CSF in pre-ART patients, but might suggest it to be safe in those stable on ART. With 302 regard to coinfections, some case reports were published on successful GM-CSF therapy of 303 resistant-to-standard-therapy mycobacterial infection and pulmonary aspergillosis in HIV 304 patients (77, 78). There is a single successful case report on immunotherapy targeting primary 305 VL in an Italian AIDS patient, whereby human GM-CSF was combined with liposomal 306 amphotericin B (Table 2) (79). Presently the evidence for beneficial effects of GM-CSF on 307 HIV disease is limited, but GM-GSF adjuvanted therapy could provide a potential value for 308 treatment of neutropenic VL in stable ART patients.

309 3.3. Interleukin-2 (IL-2)

310 IL-2 induces clonal expansion of specific T cells, promotes natural killer (NK) and CD8⁺ T cell cytotoxicity, cytokine secretion by Th1, Th2, and Th17 cells, and modulates programmed 311 312 cell death (42). Hence, IL-2 is necessary for the protection against Leishmania in 313 immunodeficient mice, in which IL-2 restores the activity of Sb^V (38, 80). The impairment in IL-2 production is also one of the first functional defects described in untreated HIV-positive 314 patients and its administration to boost the quantitative and/or qualitative CD4+ T cell 315 restoration in HIV-infected patients has been evaluated in Phase I, II and III trials (42). These 316 317 early results provided evidence that IL-2 therapy combined with existing cART has the potential to enhance quantitative and qualitative immune restoration, without triggering HIV 318 319 replication, even when ART alone had failed to do so. However, restoring CD4⁺ T cell counts 320 with IL-2 failed to show long-term clinical benefits in two large Phase III clinical trials, ESPRIT and SILCAAT (81). IL-2 recipients in the STALWART trial even experienced more 321 322 opportunistic infections, death or grade 4 adverse events (AEs) during IL-2 administration, 323 than those not receiving IL-2 (82).

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325 To date, no clinical trial for rIL-2 administration in VL patients has been reported. There has 326 been one case report on the use of rIL-2 in a VL-HIV coinfected patient failing to respond to 327 anti-leishmanial and HIV treatment with low CD4 counts and incomplete HIV suppression despite ART use (83). This report indicated no benefit. Importantly, increased Leishmania 328 329 parasitemia was observed at each rIL-2 cycle, which might have favored the progression of 330 HIV infection and possibly explains the reported progressive decline in CD4 T cell count 331 (83). In a BALB/c mouse model, IL-2 seemed to have a short protective effect against VL 332 only at the priming phase, without any lasting benefit (84). Such a phase-specific effect could 333 explain the lack of long-term clinical benefits. In general, the small therapeutic window, 334 critical dosage with potential high toxicity and challenging treatment conditions suggest IL-2 335 is an unlikely candidate for boosting immunity in VL-HIV coinfected patients.

336 **3.4.** Therapeutic vaccines

337 Historically, leishmanization (inoculation with live parasites) was shown to have benefit for 338 protection against re-infection with cutaneous leishmaniasis (CL) and this evidence has driven the search for an effective vaccine against VL (85). Besides prophylactic vaccine 339 development, various approaches employing therapeutic vaccines have been tested 340 experimentally and clinically; and currently resulted in three licensed vaccines for canine VL 341 342 but none for human VL (86). Therapeutic immunization with a first generation vaccine of 343 aluminum hydroxide precipitated autoclaved L. major (Alum-ALM) + Bacille Calmette-Guérin (BCG) was found clinically effective in CL, mucocutaneous leishmaniasis and 344 345 persistent post-kala-azar dermal leishmaniasis (PKDL) cases, with studies progressing to 346 Phase III clinical trials (87-93), but application to VL has not been reported (62). Similarly, 347 LeishF1/F2 vaccine (alternatively called Leish-111f) a promising second generation (i.e. 348 recombinant protein) vaccine for CL, showed insufficient protection against VL in dogs (94). 349 A modified version of these second generation vaccines, called LeishF3, which

accommodated changes to enhance its efficacy against VL has been shown to be safe and immunogenic in a Phase I trial in healthy human volunteers, but therapeutic trials in patients have not been reported (Table 2) (95). A third generation (i.e. DNA-based), adenovirus vaccine (ChAd63-KH) was designed to induce *Leishmania*-specific CD8⁺ T cells and aimed at therapeutic use in VL/PKDL patients. It was shown to be safe and immunogenic in healthy volunteers (96) and is currently in Phase II trial in persistent PKDL patients in Sudan.

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357 A careful risk-benefit assessment needs to be made when considering therapeutic vaccination against VL in HIV patients, with depressed immunity. Safety concerns surely exist, but 358 359 should not be overstated and should not impede evaluation of therapeutic VL vaccination 360 studies in virally-suppressed HIV patients as potential benefits can outweigh existing 361 theoretical risks. In essence, these patients have a higher risk of developing VL and are most 362 in need of an enhanced immune response upon VL development. Post-marketing trends 363 suggest that routinely used inactivated (non-VL) vaccines have similar safety profiles among HIV-uninfected and HIV-infected persons on stable ART (97). Although data are still limited, 364 HIV-infected individuals who are on ART with well-controlled HIV RNA levels and CD4+ T 365 366 cell counts of >200 cells/ μ L (or \ge 15%) may even receive indicated live-virus vaccines (97). In addition, modern post cART era studies did not indicate that vaccines are important 367 368 triggers of HIV replication or disease progression (98). With regard to efficacy, a highly immunogenic vaccine will be needed, as well as detailed studies to define the optimal timing 369 370 and dosing for vaccination among those with advanced disease. Despite the concerns of 371 depressed immunity and sparse efficacy data for other types of vaccines, studies have clearly demonstrated the protective benefit of influenza and Streptococcus pneumoniae vaccinations 372 373 even among advanced HIV patients. In summary, these data merit a concurrent evaluation of 374 therapeutic VL vaccines in coinfected patients who are virologically suppressed at the time of 375 VL presentation.

376 4. Promising pipeline immunomodulatory molecules/interventions

377 While both the pharmacokinetics and pharmacodynamics of a drug, but also the nature of 378 drug-immune interactions in animals and humans may differ considerably, animal models 379 may still provide new clues to potential approaches. Here, we selected the most promising 380 molecules or interventions for their potential in an immunosuppressive environment of the 381 coinfected individual and refer to recent review papers for a more extensive list (39, 44, 45, 382 86, 99). The formats discussed below are limited to active immunotherapy attempts including 383 non-antigen specific strategies such as cytokines that stimulate immunity or suppress the viral replication; antibodies that block negative regulatory pathways; and indirect 384 immunomodulation (Figure 2). Antigen-specific strategies such as therapeutic vaccination and 385 386 adoptive strategies such as cell therapy are also briefly discussed. Whether the se below listed 387 molecules listed below could serve as putative targets for human immunotherapy remains to 388 be demonstrated.

389 4.1. Non-antigen-specific strategies

390 The above listed clinical trials with cytokine-adjuvant chemotherapy were based on limited 391 data from experimental models of VL conducted in the 1990's. Our knowledge of immune 392 mechanisms has substantially expanded since then is time. For instance, IL-12, a pluripotent cytokine that plays a central role in the initiation/maintenance of Th1 responses and 393 394 potentiates T cell IFNy production, was shown to have similar effects as IFNy in both CL and 395 VL when injected in mice (64, 100) as well as dogs (101) and human PBMC from treated 396 Sudanese VL patients (102). Likewise, IL-12 preconditioning of monkeys during acute SIV 397 infection markedly delayed disease progression (103). While rhIL-12 administration was 398 well-tolerated and safe, no evidence of improvement in HIV antigen-specific immune

399 response could be observed in a Phase I RCT (104). While this suggests that IL-12 therapy is 400 unlikely to provide major benefits in the chronic phase of an HIV infection, it might still be 401 valuable in the context of opportunistic infections that are best met with Th1-like effector 402 immune responses. In line with this, rIL-12 adjuvanted chemotherapy was successfully 403 evaluated for patients with Kaposi's sarcoma (105). In addition, it has been tested as part of a 404 combination therapy for cryptosporidiosis in two AIDS patients that demonstrated signs of a 405 brisk immune response and consequently symptomatic improvement, but with severe side 406 effects that outweighed the clinical benefits (106). Data on the role of IL-12 as an immunotherapeutic agent or vaccine adjuvant for HIV coinfections could be promising and 407 408 merits further research, although potential broad side effects due to its pluripotent role should 409 be limited (e.g. tissue-targeted delivery, well-timed short boosting approach, etc.). 410 Unfortunately, the incorporation of IL-12 into larger vaccine trials has lagged, in large 411 partlargely due to the early setback in a renal carcinoma Phase II trial. However, , even 412 though the mechanisms underlying the severe acute toxicities that led to two deaths and 12 hospitalizations have been ascribed to an inappropriate dose and administration schedule 413 414 (107).415

416 Like IL-12, many chemokines or cytokines contributing to protection / pathogenesis of VL 417 are regulated during HIV coinfection. For instance, Th17 cells are highly depleted from the gut in HIV-infected patients. Recent work in humans has, however, demonstrated the 418 419 importance of IL-17 and IL-22 in protection against VL progression from asymptomatic 420 infection to disease (49). Interestingly, eIn addition, elevated serum IL-27 concentrations were linked to severity of VL. IL-27 seems to regulate the Th1/Th17 profiles in a L. infantum 421 422 mouse model of VL by suppressing the IL-17-induced neutrophil response (108). The IL-27-423 Th17-IL-17 axis thus seems to be strongly involved in resistance against VL and merits 424 further therapeutic exploration, especially in HIV coinfected patients with a Th-17-depleted 425 immune response. 426

427 Despite the central role of IL-7 cytokine therapy in HIV patients in the past, this molecule has not been evaluated in VL-HIV coinfected patients and remains under-investigated in 428 experimental models of VL (109). IL-7, like IL-2, has a critical role in peripheral T cell 429 430 homeostasis. IL-7 has, however, a more pleiotropic role and was shown to drive CD4⁺ T cell 431 restoration in HIV patients, even when HIV replication is controlled. It is also able to promote Th1 responses, enhance memory T cell expansion (on top of naive T cell response) and 432 433 increase CD8⁺ T cell counts and cytotoxicity in HIV patients (42). Moreover, damage to 434 hepatocytes during full-blown VL may impair IL-7 production, as IL-7 is also produced by 435 liver cells in response to inflammation (110). Recombinant IL-7 administration thus has the 436 potential to safeguard the long-term survival of effector CD4⁺ T cells in response to persisting 437 parasites in a VL-HIV coinfection. However, in the ERAMUNE 01 RCT, rIL-7 and dual ART 438 intensification induced an amplification of the HIV reservoir in well-controlled HIV patients 439 (111). The authors reasoned that this was the result of the expansion of central memory CD4+ 440 T cells, carrying HIV DNA, thus limiting this IL-7 based strategy. In the context of VL-HIV 441 coinfection, this strategy should only be considered if a pronounced clinical benefit to VL 442 treatment outweighs its potential negative effects.

443

Blocking the action of immune-suppressive factors could prove more efficient as it might allow restoration of protective immunity in a more controlled manner. IL-10 correlates very well with the parasite load during VL infection. Moreover, in animals, IL-10 blockade (by means of anti-IL-10R or anti-IL-10 monoclonal antibody) has been proven successful in lowering parasite burden when combined with conventional treatment in multiple studies in mice (112, 113). These effects were confirmed in cultures of splenocytes or PBMCs from Indian and Sudanese VL patients (102, 114). However, in immunodeficient mice treated with 451 anti-IL-10R monoclonal antibody, Murray *et al.* were not only able to show an acceleration of 452 Sb^V-associated killing, but <u>alsothey</u> reported a >10-fold Sb^V dose-sparing effect (115). 453 Despite the clinical and experimental data suggesting IL-10 as a key target in the 454 immunopathogenesis of VL, a clinical trial using a monoclonal antibody against IL-10 failed 455 to start following the decision of the company to stop its production (NCT01437020, 456 clinicaltrials.gov).

458 Increased serum IL-10 concentrations are also observed in HIV-infected patients with disease 459 progression, in contrast to non-progressing patients where levels were stable (116). In 460 addition, ART has a clear down-regulating effect on IL-10. On the other hand, increasing 461 evidence suggests that IL-10 impacts many aspects of HIV pathogenesis, including the 462 regulation of HIV-specific CD4⁺ and CD8⁺ T cell functions, as well as modulation of HIV 463 replication in PBMC subsets. Genetic polymorphisms in the IL-10 gene promoter that lead to 464 decreased IL-10 expression have been associated with more rapid disease progression in late stages of HIV infection, suggesting that the anti-inflammatory effects of IL-10 may be solely 465 466 protective in the setting of chronic immune activation and blocking IL-10 function would 467 only make sense in an acute setting (117). When considering VL-HIV coinfection, these data would advocate the blocking of excessive IL-10 levels during the acute stage of VL in HIV 468 469 patients (in particular pre-ART patients) to allow a beneficial acute response which should 470 however be time limited to retain the beneficial role of IL-10 in controlling side damage of 471 chronic HIV and parasitic infections. To reduce the unwanted side effects due to blockage of 472 normal, and beneficial, biological activities, novel IL-10 signaling inhibitors with for instance 473 shorter half-lives are first needed (118).

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475 The concept of immune exhaustion and senescence as a stepwise and progressive loss of T 476 cell function and proliferative potential, respectively, and evolving to complete T cell unresponsiveness has been robustly discussed in the context of HIV infection (119). The 477 478 driving force is believed to be chronic antigen exposure and consequently extensive non-479 specific immune activation. Increased immune activation in patients on long-term suppressive 480 cART has been associated with increased mortality, the occurrence of non-AIDS-defining 481 conditions, and a poorer recovery in CD4⁺ T cell count (120, 121). Similarly, increased levels 482 of Programmed death-ligand 1 (PD-L1) expression on monocytes, B cells and T cells from untreated HIV patients correlated directly with plasma viral load and inversely with CD4+ T 483 484 cell count (122). This mechanism could partly explain the disappointing long-term effects of 485 IL-2 therapy in HIV patients, as IL-2 was recently shown to upregulate the PD1-PD-L1/L2 486 pathway (123). While the causative factors of immune exhaustion or senescence are not 487 completely understood, chronic immune activation, residual HIV-replication and coinfections 488 are likely main drivers of this process. Recent studies have also focused on the role of this 489 process in the context of VL and other parasitic infections, showing an accelerated T cell 490 senescence during VL infection (124). Likewise, a parasite-induced T cell anergy has been 491 proposed (124). Hence, a modulatory approach to reverse this process or temporarily breaking 492 the regulatory feedback loop using antibody therapies targeting PD-1, CTLA-4 or its ligands 493 could prove efficient in coinfected individuals with a potential double-driven T cell 494 unresponsiveness. Such an approach to reverse the reported T cell unresponsiveness has 495 proved very effective in experimental VL (57, 125-127). In SIV-infected rhesus macaques, 496 anti-PD-1 (in the absence of ART) was shown to enhance virus-specific CD8⁺ T cell activity, 497 to reduce viral load and to prolong survival (46). Similarly, anti-PD-L1 antibody therapy 498 showed promising in a recent Phase I RCT on 6 ART patients, arguing in favor of its potential 499 use in virologically-suppressed VL-HIV patients (128). Recently, the major HIV cell reservoir was shown to be composed of PD-1+ CD4+ memory T cells, suggesting an additional positive 500 501 effect of anti-PD-1 therapy to combat the concomitant HIV infection (129).

502 4.2. Antigen-specific and adoptive strategies

503 There are multiple studies in which diverse antigens and adjuvants showed promising results 504 as immunoprophylactic or therapeutic tools in animal models of VL, recently summarized in a 505 review by Jain and Jain (86). Apart from the current clinically explored strategies and the safety/efficacy concerns in HIV patients (see above), a promising approach would be to 506 507 vaccinate with a non-pathogenic L. tarentolae strain, genetically modified to improve its immunogenic potential as a live vaccine (130). Likewise, a novel third generation T cell 508 509 epitope-enriched DNA vaccine (LEISHDNAVAX) showed significant efficacy when co-510 administered with a single dose of AmBisome in L donovani-infected mice (131). The 511 vaccine is based on minimalistic immunogenically defined gene expression (MIDGE) vectors 512 encoding five conserved antigens developed for efficient induction of Th1 immune responses. 513 This candidate vaccine has yet to enter clinical Phase I trials. 514

Another cutting-edge approach to induce antigen-specific T cell immunity is dendritic cell-515 based immunotherapy (99, 132). While macrophages are one destination of Leishmania 516 parasites in the human host, dendritic cells can also harbor parasites, but in addition present 517 518 antigen and regulate immune mechanism governing control or progression of infection. 519 Adoptive transfer of dendritic cells primed with different kinds of Leishmania antigens has been shown very effective in murine VL, improving both cellular and humoral immunity 520 521 (132). Compared to the modest efficacy of immune therapy and therapeutic vaccines against 522 HIV infection, ex vivo generated dendritic cell therapeutic vaccines aimed at inducing 523 effective HIV-specific immune responses have yielded the best results in this field (133). The outcomes of monocyte-derived dendritic cell based therapeutic vaccines still needs 524 optimization as functional cure was not reached and most patients needed to restart ART, but 525 526 this method could provide a strong immunogenic window for concomitant VL-targeted 527 therapy of coinfected individuals. Due to high costs and required state-of-the-art equipment, 528 adoptive cell transfer therapy may prove difficult to implement in low-resource settings of 529 disease endemic countries.

530 4.3. Indirect strategies

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531 An alternative approach is to indirectly stimulate host immunity to optimize protection against 532 infection. Such indirect immunomodulators can be obtained by many different types of substances, including natural products that have immunomodulatory activity. Such 533 534 immunomodulators, however, carry the risk of inducing excessive immunopathology and side effects. Many compounds have been evaluated in VL animal studies over the years, including 535 536 CpG oligodeoxynucleotides, acetyl salicylic acid (ASA) and L-arginine (99). Most of these 537 molecules increase T cell activation through enhanced antigen presentation by costimulationbased therapy or acting on Toll-like receptors (TLRs) (e.g. TLR4/GP29 or MPL; TLR2/Ara-538 539 LAM or Pam3Cys). This could be particularly beneficial in HIV coinfected patients, as TLR-540 agonists such as TLR7 or TLR9 agonists have shown reduction of viral DNA or the viral reservoir and enhancement of HIV-specific CD8⁺ T cell immunity in experimental and human 541 542 HIV (134, 135). Whether such a multi-TLR targeting approach would benefit human VL-HIV patients remains unclear and merits further research. 543

545 In a similar manner, it has been suggested that TLR4 and TLR9, two TLRs contributing to the 546 immune response against *Leishmania* infection, play a role in the anti-leishmanial mechanism 547 of miltefosine (136). An alternative strategy₇ could thus be to concurrently capitalize on the 548 indirect immunological effects of the combined anti-leishmanial drug in a immuno-549 chemotherapeutic approach. The relevance and impact of these immunomodulatory actions of 550 current anti-leishmanials in HIV coinfected VL patients remains to be determined. Besides a 551 direct mechanism of action, anti-leishmanials can increase nitric oxide and reactive oxygen 552 species production due to activation of infected macrophages, leading to elimination of the 553 parasite. This indirect activation of macrophages has been shown for amphotericin B (137), 554 miltefosine (138), antimonials (139), and paromomycin (140). Induction of macrophage-555 derived cytokine release promoting a Th1 response (IL-2, IL-12, IFNy) has been noted for all 556 conventional anti-leishmanials such as amphotericin B (137, 141), miltefosine (138, 141), 557 paromomycin (141), and sodium stibogluconate (139, 141), even though contradictory results 558 have been reported, e.g. for miltefosine (142). Related to this, miltefosine restored IFNy 559 responsiveness in Leishmania-infected macrophages (138). Another immunostimulatory property contributing to anti-leishmanial activity is a drug-induced increase in macrophage 560 561 membrane fluidity, ameliorating defects in antigen-presentation and enhancing T cell 562 stimulation. This has been shown after exposure of infected host cells to higher concentrations 563 of miltefosine, paromomycin and sodium stibogluconate (141). For both antimonials (143) 564 and miltefosine (144), it has been shown that they increase the phagocytic capacity of 565 monocytes and macrophages. There are currently no data available whether all these effects 566 are clinically relevant in terms of short-term treatment response, relapse, final cure, and the risk of development of PKDL. Despite the current lack of data on clinical relevance, these 567 background effects should be taken into consideration in future combined 568 immunochemotherapeutic strategies to incite an effective synergistic effect. The general lack 569 570 of response to anti-leishmanial treatment in HIV coinfected patients and the relevance of 571 concomitant cART for the efficacy of current anti-leishmanials possibly indicate that these 572 indirect effects are not negligible for a therapeutic response.

573 5. Concluding Pperspectives

574 Despite the growing research in immunotherapy against VL (partly reviewed above), no immunotherapeutic approach has yet been licensed for use in human VL. HIV coinfected 575 patient groups, in particular, are often excluded from the above described clinical intervention 576 577 studies due to the presumed hazards and challenging logistics. Although a vulnerable 578 population, we would argue that VL-HIV patients should be considered as a relevant target 579 group for an immunomodulatory approach against VL due to an intensified defect in T cell immunity, dependence of current anti-leishmanial drugs on the latter, inadequate treatment 580 581 outcomes and higher chronicity of the parasitic infection with frequent relapse. In addition, HIV-targeted immunomodulatory approaches, despite their drawbacks to achieve long-term 582 functional cure in HIV patients, might find a temporarily window of opportunity in 583 584 opportunistic coinfections such as VL, where cART alone is not able to restore protective 585 immunity. The challenge, however, of immunomodulatory therapy in VL-HIV coinfected patients is boosting effective VL-specific T cell responses while avoiding activation of latent 586 587 provirus and inappropriate immune activation (in virologically-suppressed ART patients) or 588 HIV recrudescence and increased HIV-susceptibility of target cells (in unstable HIV/AIDS 589 patients). Clinical studies trials are a necessity to study treatment effects, due to the lack of 590 good animal or in vitro models mimicking VL-HIV coinfection. 591

592 In Figure 2, we summarized the discussed interventions against VL and highlighted those that 593 have also been clinically evaluated in the context of HIV. Evidence is lacking to prioritize a 594 target molecule, but attempts at immunotherapy in VL-HIV patients should best be performed 595 in ART patients with a recovered immune system. Appropriate adjuvants can be included to 596 enhance the efficacy of the response, but caution should be taken to avoid excessive and 597 broad immune activation. The following perspectives are best taken into consideration when 598 designing or evaluating an immunomodulatory approach in VL-HIV coinfected patients: 599

600 **COMBINATION STRATEGIES** - As current anti-leishmanial drugs are highly dependent 601 on host immunity, it is recommended to potentiate chemotherapeutic agents with various 602 immunomodulators in HIV coinfected patients. While the increment in immunocompetent patients could be potentially low, HIV coinfected patients are probably in more need of a
 boost in effective T cell immunity against VL to decrease the high mortality and treatment
 failure rates typically observedseen in coinfected patients.

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607 The current clinically explored techniques of single cytokine-adjuvanted therapy in VL have the inherent danger of a very pluripotent effect in HIV coinfected patients, due to the 608 609 intricacies of cytokine networks, and may unpredictably impact the delicate balance between 610 beneficial VL-specific responses and deleterious immune activation. Future therapeutic use of 611 broad immunomodulators will most likely lead to unwanted side effects in coinfected patients until a system-level understanding of their mode of action is available and thus a more 612 613 selective and well timed approach can be performed (145). However, they could potentially 614 prove valuable as a well-timed adjuvant in a more targeted immunomodulatory approach. 615

The other clinically explored strategy in VL is therapeutic vaccination. However, as T cell 616 senescence and exhaustion could have occurred by persistent HIV replication, further 617 stimulating effector-memory T cells could be futile or even harmful in VL-HIV patients. 618 619 Perhaps a concurrent strategy to reverse this T cell exhaustion (e.g. anti-PD-1 therapy) could 620 increase vaccine efficacy. It remains to be seen whether VL-based therapeutic vaccines 621 deployed in HIV coinfected patients are safe and whether a strong enough response can be 622 induced against VL. In severely CD4+ depleted patients in particular, a concurrent need may 623 be to first encourage immune reconstitution before vaccination. Combination strategies of 624 diverse immunomodulators and drugs will thus be crucial in these patients to reach an 625 effective treatment, perhaps with a more individualized approach.

627 **STRATIFICATION** – Among patients with tuberculous meningitis, different inflammatory 628 patterns governed by host genetics are recognized, converging on dysregulated levels of TNF. 629 At one end of the extreme, a hyper inflammatory phenotype was shown to benefit from 630 steroid administration; at the other end, where inflammation is inadequate, other immuno-631 modulatory interventions would be required (146). In a similar manner, subgroup analyses in HIV-associated cryptococcal meningitis suggested that the greatest benefit of a short-course 632 633 IFNy adjuvant therapy was gained among patients with a lack of Cryptococcus-specific 634 IFNy/TNF CD4⁺ T cell responses (147). In most settings, VL-HIV coinfected individuals will also be (severely) malnourished upon VL diagnosis, and micro- and macro-nutrient deficiency 635 can have profound immunological effects. These alterations could critically affect the efficacy 636 637 of any immunomodulatory interventions, yet may also provide opportunities for 638 complementary interventions. We therefore argue that there is a need to assess immune risk profiles based on functional T cell assays, RNA signatures and other parameters that identify 639 640 patients that are more likely to benefit from immune adjuvanted therapy, across the 641 heterogeneous group of VL-HIV patients.

643 TIMING - VL-HIV coinfection is a dynamic process with diverse stages of infection and regardless of choice of immunomodulatory intervention, timing will be critical to success. For 644 645 instance, high IL-17 levels appeared protective for early VL progression, but its role is still 646 debatable in chronic infection. The optimal timing of immunotherapy among HIV coinfected 647 adults in regard to HIV stage and receipt of antiretroviral therapy also remain important 648 unanswered questions. Most benefit is probably to be gained in early stages of HIV infection 649 as well as in under-therapy suppressed patients, who are able to effectively respond to 650 immunomodulators. Therefore, we would argue for a primary evaluation of novel approaches 651 in stable ART patients that have a somewhat reconstituted CD4+ T cell immunity and suppressed viral load, including frequent monitoring of blips in viral load and CD4+ T cell 652 count. It remains to be investigated whether HIV patients with a severe suppression in T cell 653

654 immunity are also able to respond to immune stimulators or whether virological suppression655 first has to be prioritized to enable T cell responsiveness.

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657 **TARGETED STRATEGIES** – The delivery system is also an important part of an immune 658 based strategy and implementation of various novel approaches based on liposomes, 659 electroporation, dendrimers, carbon nanotubes etc. can boost efficacy (86). For instance, as an 660 alternative for broad cytokine adjuvants, more effective and tolerable approaches are being 661 explored like encapsulation in micro or nanoparticles, restricting the delivery to APCs and/or the co-delivery with another immunomodulatory molecule via transducing vectors. Similar 662 techniques such as microRNA or small interference RNA based therapy could be explored, 663 664 but these novel drugs will be most likely unaffordable in most countries where the disease is 665 endemic. 666

ACCESIBILITY – The target population is largely living in very rural and/or poor areas, where a highly controlled clinical trial setting can be challenging and costly to implement. It will be imperative to strengthen human and infrastructural capacity in disease endemic areas to ensure a sustainable base for immunotherapeutic research and to assess safety and efficacy of novel interventions. Moreover, designed therapeutics should become affordable and accessible to the patient population, suggesting innovative low-resource-demanding methods ideally without the need of a cold chain.

675 676 CONCLUSION - To advance the development of immunomodulatory approaches for VL-677 HIV coinfection, a more detailed account of the immunological status induced by the 678 coinfection and surrogate markers of cure and protection are still required, as a forerunner to 679 inclusion of such patients in clinical intervention studies. The main limitation for 680 comprehensive immunological research is, however, the need for human samples of longitudinal studies and trials in (often very remote) low-resource settings. With more 681 682 research aimed at discovering key synergistic pathways of immune cell cross-talk and 683 renewed efforts to translate these findings into effective treatment modalities that target 684 Leishmania without promoting HIV replication, the goal of improved patient outcome and 685 clinical management of this neglected population may be achievable.

687 Author co	ntributions
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- WA, JV, GV, LK, TD, PK wrote and conceived the review

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Figures and Tables

Table 1. The main drugs currently used for treatment of visceral leishmaniasis, adapted from

(5)

Drug	Toxicity	Main limitations	
Pentavalent	Frequent, potentially	Toxicity (high mortality in HIV	
Antimonials (SbV)	severe	coinfected African patients)	
	- Pancreatitis	Painful injection (im)	
	- Cardiotoxicity	Length of treatment	
	- Nephrotoxicity	Resistance in India	
	- Hepatotoxicity		
Conventional	Frequent infusion-related	Lengthy hospitalization (in-patient care)	
Amphotericin B	reactions		
deoxycholate	- Nephrotoxicity	Slow iv infusion	
	- hypokalemia	Nephrotoxicity	
Liposomal	Uncommon and mild	High price	
Amphotericin B	- Nephrotoxicity	Slow iv infusion	
(AmBisome)	(limited)	Heat instability (<25° C)	
		Accessibility	
		Single dose not effective in East Africa	
Miltefosine	Common, usually mild	Relatively limited efficacy data in East	
	and transient	Africa	
	- Gastro-intestinal	Possibly teratogenic	
	- Hepatotoxicity	Potential for resistance ^b	
		Patient compliance (oral drug)	
		High price	
Paromomycin	Common	Toxicity (Oto- and nephrotoxicity)	
Sulphate	- Ototoxicity	Resistance readily obtained in lab	
(aminosidine)	- Nephrotoxicity	isolates	
	- Hepatotoxicity	Efficacy variable between and within regions (less in Sudan)	
Pentamidine	Common	Low efficacy	
	- Gastro-intestinal	Toxicity (diabetes, renal failure)	
	- Cardiotoxicity	Length of treatment	
	- Pancreatitis		
	- (<u>iI</u> r)reversible		
	diabetes mellitus		

^bDue to long half-life + low genetic barrier (resistance readily obtained in lab isolates) iv: intravenous injection; im: intramuscular injection

Tabl Ref	e 2. Publishe Country; year; design	ed clinical reports on the use of Patient characteristics	f immuno(chemo)the Chemo agent	rapy against VL a Immuno agent	nd VL-HIV Outcome (EOT)	Comments
	VISCERAI	LEISHMANIASIS MONO-INFE	CTION			
(148)	Brazil; 1990; case series	[1] SSG-unresponsive VL (n=8); <18 years (8/8); Mean age: 6.5 years	SSG 20 mg/kg	IFN γ (100-400 μg/m ² for 10-40 days)	6/8 cured EOT (75%) No relapse during study period	Higher cure rates in both groups compared to historical controls Tolerability acceptable (fever)
		[2] Severely ill primary VL (n=9) <18 years (8/9) Mean age: 9.8 years	SSG 20 mg/kg	IFN γ (100-400 μg/m ² for 10-40 days)	8/9 cured EOT (89%) No relapse during study period	
(149)	Brazil, 1993; case series	[1] Primary VL (n=8) Predominantly children Median age: 5 years	SSG 20 mg/kg	IFN γ (100-400 μg/m2 for 10-40 days)	8/8 cured EOT Cure 12M: 8/8 (100%) 1/8 relapsed 12/14 cured	Both groups: more severe cases than in 1990
		[2] SSG-unresponsive refractory VL (n=14) Median age: 4 years	SSG 20 mg/kg	IFN γ (100-400 μg/m2 for 10-40 days)	Cure 12M: 9/14 (64%) 6/12 relapsed	
(150)	Kenya; 1993; RCT	[1] Primary VL (n=10) <18 years: 7/10	SSG 20 mg/kg	IFN γ (100 μ g/m ² every two days - 30 days)	24/24 cured EOT Week 1: 50% cured Week 2: 75% cured Week 4: 100% cured	Control group included no relapse cases
		[2] Primary VL (n=14) <18 years: 11/14	SSG 20mg/kg	1	Week 1: 22% cured Week 2: 58% cured Week 4: 88% cured	a non-significant accelerated response with SSG + IFNy
(73)	Brazil; 1994; RCT	[1] 10 neutropenic primary VL	SSG 10-20 mg/kg for 10 days	GM-CSF (5mg/kg for 10 days)	Cure M3: 100%	Study focused on hematological evaluation and secondary infections
		[2] 10 neutropenic primary VL	SSG 10-20 mg/kg for 10 days	Placebo	Cure M3: 100%	Secondary infections occurred in 3 GM-CSF and in 8 placebo recipients

(151	India;	[1] Primary VL (n=16)	SSG 20 mg/kg for 20-	IFNγ	Cure D10: 10/15 (63%)	D10 and D20 difference statistically
)	1995;	Mean age 21 years (range 6-52)	30 days	(100 µg/m2)	Cure D20: 14/15 (93%)	significant
	RCT				Cure D30: 15/15 (100%)	
					Cure M6: 13/15 (87%)	No relapse up to M24
		[2] Primary VL (n=15)	SSG 20 mg/kg for 20-	/	Cure D10: 1/15 (7%)	Treatment was discontinued early in
		Mean age 27 years (range 5-58)	30 days		Cure D20: 6/15 (40%)	the 14 IFNy treated responders after
			-		Cure D30: 11/15 (73%)	D20
					Cure M6: 9/15 (60%)	
(65)	India,	[1] Primary VL (n=52)	SSG 20 mg/kg for 30	ΙΓΝγ	Cure (EOT): 25/47	High failure rate with standard therapy
	1997	Mean age 20 years; 60% male	days	(100 µg/m2 for 30	Relapse: 1	(SSG-resistance?)
				days)	6M cure: 24/49 (49%)	
						Differences not statistically significant
		[2] Primary VL (n=52)	SSG 20 mg/kg for 30	IFN	Cure (EOT): 22/50	
		Mean age 18 years; 58% male	days	$(100 \text{ ug/m}^2 \text{ for } 15)$	Relapse: 1	
				days)	6M: 21/50 (42%)	
				•		
		[3] Primary VL (n=52)	SSG 20 mg/kg for 30		Cure (EOT): 20/48	
		Mean age 20 years; 69% male	days	/	Relapse: 2	
					6M cure: 18/50 (36%)	
(95)	USA,	[1] Healthy volunteers (n=12)	/	Leish F3 (20ug)	Safe and immunogenic D84:	Subunit vaccine: single recombinant
	2012,			+ GLA-SE (5ug)	10/10	fusion protein of 2 preserved proteins
	Phase I	[2] Healthy volunteers (n-12)	1	Leich E2 $(20ug)$	Safa and immunogania D84	
	KC1	[2] Healthy volunteers (h=12)	1	+ GLA-SE $(200g)$	8/8	
				(2ug)	0,0	
		[3] Healthy volunteers (n=12)	/	Leish F3 (20ug)	Safe and immunogenic D84:	
					9/9	
(96)	UK,	[1] Healthy volunteers (n=20]	/	Ch1d63-KH	Safe and immunogenic D90:	Adenovirus vector encoding 2
	2016, Dhasa I	n=5 low dose		(1x10 ¹⁰ vp or 7.510 ¹⁰	20/20	Leishmania proteins
	Phase I	n=15 nign dose		7.5x10 ¹⁰ vp)		Dose excelation study
	HIV & VIS	CERAL LEISHMANIASIS COIN	JEFCTION			Dose escutation staty
(68)	CASE	Full blown AIDS patient with	Meglumine	IFNy (175 µg/d iv	1 relapse treated	
(00)	REPORT:	recurrent VL	antimoniate (dose	or sc for 21 days)	Resistance to antimoniate	
	1990	19y old Algerian male	unkown)	······································		

			Pentamidine (2 mg/kg iv 3 times/w, 1w/mo)	IFN γ (175 μg/d sc 3 times/w, 1w/mo)	3 relapses treated Cure 6M: Only two mild relapses with minimal adverse events
(67)	CASE REPORT; 1993	Three full blown AIDS patients	Meglumine antimoniate (dose unknown)	IFN γ (dose unknown)	Clinical improvement Reduction in parasite burden
(70)	CASE REPORT; 1994	Full blown AIDS patient with KS 40y old German male	SSG (dose unknown)	IFN γ (dose unknown)	Aggravated Kaposi syndrome (KS)
(79)	CASE REPORT; 2004	Primary VL 37y old Italian male CD4 <50 mcl On ABT	Amphotericin B (4mg/kg for 5 days + 5 non-consequent days)	GM-CSF (150 mcg/twice a week for 12	Dramatic Clinical improvement
(83)	CASE REPORT; 2007	Unresponsive VL 36y old Italian woman CD4: 98 cells/µl On ART	Amphotericin B (between every cycle)	IL-2 (twice/day for 5 days – 7cycles every 4-8 weeks (cycle 1-4: 3MIU; cycle 5-7: 6MIU)	No benefit Increase in <i>Leishmania</i> DNA

IFN: interferon; SSG: sodium stibogluconate; VL: visceral leishmaniasis; EOT: End of Treatment: M: month

Figure 1: Current views on synergistic mechanisms in T cell immunity against VL due to HIV coinfection inciting persistent viral and parasite replication in VL-HIV coinfected patients.

APC: antigen presenting cell; Th: T-helper; GALT: gut_associated lymphoid tissue; CTL: cytotoxic T cell; IL: Interleukin; ART: antiretroviral therapy; IFN: interferon; LPS: lipopolysaccharide; TNF: Tumor necrosis factor

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Figure 2: Overview of described clinical and preclinical immunomodulatory interventions in human visceral leishmaniasis and their application in (VL)-HIV (co)infection.

IL: interleukin; IFN: interferon; PD-(L)1: programmed cell death-(ligand)1; GM-CSF: Granulocyte macrophage colony stimulating factor; CTLA: Cytotoxic T lymphocyteassociated molecule; CD: Cluster of differentiation; BCG: Bacillus Calmette-Guérin; Alu-ALM: aluminum hydroxide precipitated autoclaved L. major; DC: dendritic cell; GP: Glycoprotein; Ara-LAM: Arabinosylated lipoarabinomannan; Pam3Cys: synthetic bacterial lipopeptide; CpG Odn: CpG oligodeoxynucleotides; ASA: Acetyl Salicylic Acid; MPL: monophosphoryl lipid





THERAPEUTIC VACCINATION AND ADOPTIVE CELL TRANSFER

Figure 2.JPEG