REVIEW

A profile of research on the parasitic trypanosomatids and the diseases they cause

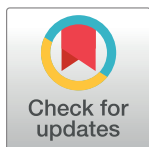**David Horn** (iD) *

The Wellcome Trust Centre for Anti-Infectives Research, Division of Biological Chemistry & Drug Discovery, School of Life Sciences, University of Dundee, Dundee, United Kingdom

* d.horn@dundee.ac.uk

Abstract

The parasitic trypanosomatids cause lethal and debilitating diseases, the leishmaniases, Chagas disease, and the African trypanosomiases, with major impacts on human and animal health. Sustained research has borne fruit by assisting efforts to reduce the burden of disease and by improving our understanding of fundamental molecular and cell biology. But where has the research primarily been conducted, and which research areas have received the most attention? These questions are addressed below using publication and citation data from the past few decades.

OPEN ACCESS

Introduction

The parasitic trypanosomatids include *Leishmania* spp. and the African and South American trypanosomes. The most serious form of leishmaniasis [1] is visceral leishmaniasis, or kala-azar, which is typically fatal without treatment. There are estimated to be approximately 70,000 new cases each year. Post-kala-azar dermal leishmaniasis (PKDL) can also occur after apparent cure. The most common form of leishmaniasis is cutaneous leishmaniasis, which causes skin lesions and ulcers and lifelong scarring; there are estimated to be approximately 800,000 new cases each year. An even more destructive form is mucocutaneous leishmaniasis. The *Leishmania* parasites are transmitted by female phlebotomine sandflies.

Chagas disease, or American trypanosomiasis [2], is caused by *Trypanosoma cruzi*. More than 6 million people are thought to be infected worldwide, and up to 30% are expected to develop cardiac, digestive, or neurological, and potentially life-threatening symptoms, often many years after the initial infection. American trypanosomes are transmitted by triatomine bugs; other important transmission routes are oral, congenital, via blood transfusion, or via organ transplantation.

Sleeping sickness, or human African trypanosomiasis [3], which is often fatal without treatment, is caused by *Trypanosoma brucei gambiense* (west and central Africa) or *Trypanosoma brucei rhodesiense* (east and southern Africa), with *T. b. gambiense* accounting for >98% of current cases. The latest major sleeping sickness epidemic started in the late 1970s, peaked in the late 1990s, and continued into the 21st century. The disease remains endemic in several sub-Saharan African countries but with <1,000 cases reported in 2018. Animal African trypanosomiasis is caused by *Trypanosoma congolense*, *Trypanosoma vivax*, or *T. b. brucei* in wild

and domestic animals; the disease is known as nagana in cattle. The African trypanosomes are typically transmitted by tsetse flies (*Glossina*).

Leishmaniasis, Chagas disease, and human African trypanosomiasis (who.int/news-room/fact-sheets) are among 20 neglected tropical diseases (NTDs) currently within the World Health Organisation (WHO) portfolio (who.int/neglected_diseases/diseases/). All 3 diseases have been targeted for elimination as part of the WHO road map for NTDs 2021–2030 (WHO/UCN/NTD/2020.01).

My hope is that a broad overview of research on the trypanosomatids and the diseases they cause will be of potential interest to students, researchers, and many others interested in research on NTDs. Below, I use publication and citation data from the past few decades to explore where research in these areas has been primarily conducted, and which research areas have received the most attention over time.

Methods

Data relating to publication outputs were downloaded from PubMed (pubmed.ncbi.nlm.nih.gov) on 24:09:2021, using the terms "leishmania," "cruzi," "brucei," "congolense," or "vivax" (AND "trypanosoma" in the last 2 cases). Data relating to citations were downloaded from Scopus (http://www.scopus.com) on 10:10:2021, using the terms above and also "trypanosome (s)," "leishmaniasis," "Chagas," "sleeping," AND "sickness." The top 5% most highly cited (HC) articles from each year (cites per year since publication) were extracted, and duplicates, reviews, and articles with a focus on neither human nor veterinary parasitic trypanosomatids were removed, yielding the "highly cited" set of 1,786 articles, average of 58 from each year with a range from 35 (1991) to 88 (2018). Each article was assigned to a primary trypanosomatid and to one from a set of primary research area or topic terms; the latter was achieved for >90% of articles. All topics with >9 HC articles assigned are detailed in the figures. The titles from the "highly cited" articles, minus the terms used to search Scopus, were used to derive a word cloud for each trypanosomatid group (https://www.wordclouds.com/).

Articles cited here include a set of 3 seminar-style reviews recently published in the *Lancet* ($n = 3$), one for each major disease area; all research articles published since 1990 that had previously been cited >20 times per year since publication (www.scopus.com; $n = 88$) and an additional 16 articles cited >17 times per year since publication. These articles cover a wide range of research areas and topics and serve as exemplars for those research areas. Further citations include a set of research articles that appeared to be "ahead of their time," preceding other HC articles on the same topic by >5 years ($n = 3$); a set of research articles from 2020 or 2021 ($n = 6$), since recent articles are underrepresented in the HC set; and a set of research articles describing further novel features first discovered in trypanosomatids ($n = 7$). The 5 key papers were selected to reflect impacts in the areas of immunity, host–parasite interactions, omics, drug discovery, and the delivery of new therapies.

Publication outputs by country

The first graph (Fig 1A) shows global life sciences and biomedical publication outputs including the terms "leishmania," "cruzi," "brucei," "congolense," or "vivax" (and "trypanosoma" in the last 2 cases) in PubMed and published in the 61-year period from 1960 to 2020. There were 31,281 "leishmania," 18,336 "cruzi," 12,088 "brucei," 1,684 "congolense," and 1,000 "vivax" publications; 58,784 in total, 40% of which were published in the last 10 years. For comparison, there have been a similar number of "plasmodium" (malaria) publications in this time frame (approximately 60,000), also with a similar proportion published in the last 10 years. The figure for "cancer" is 3.4 million. The overall rate of increase in trypanosomatid outputs was
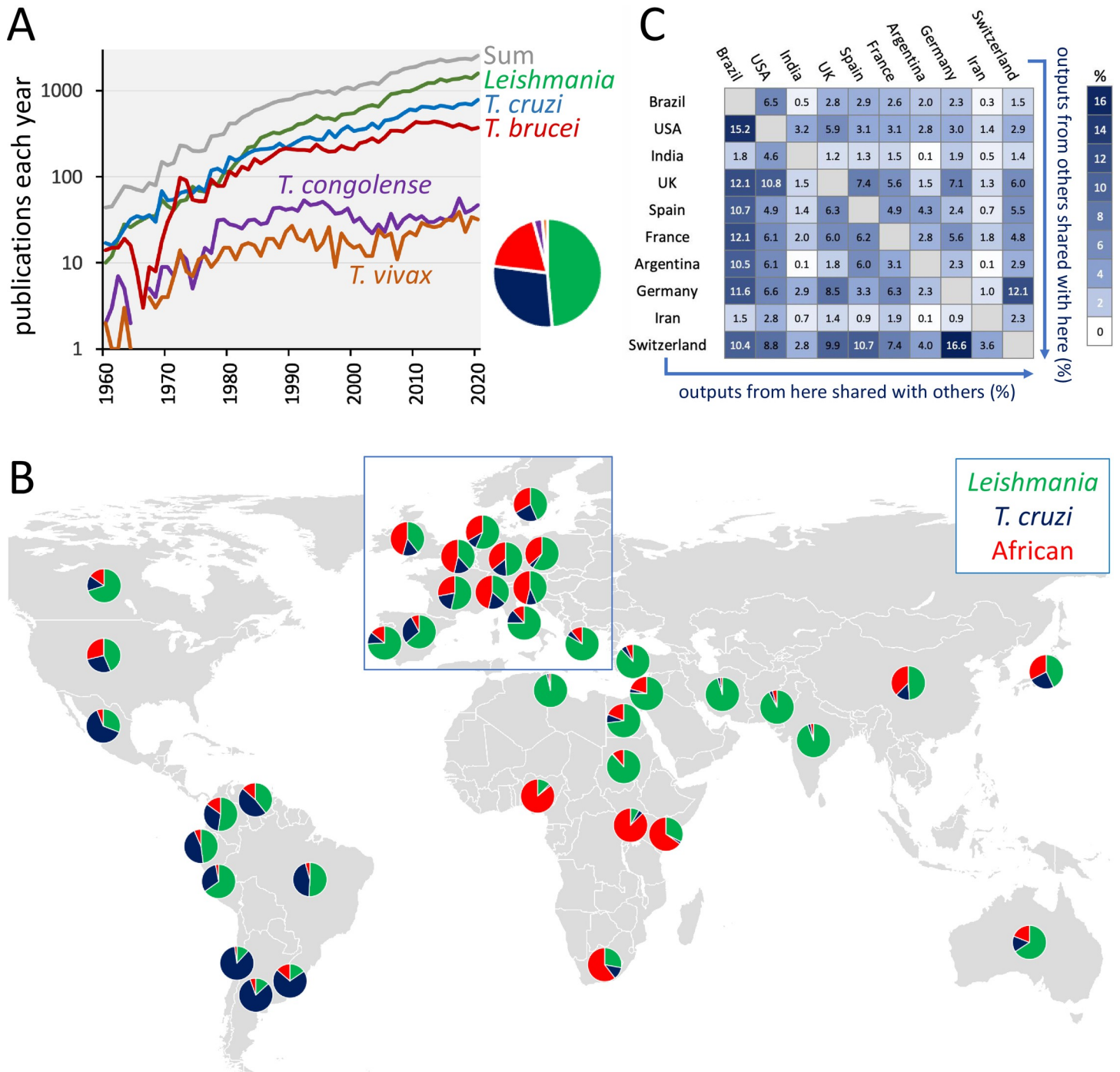
Fig 1. Global trypanosomatid research outputs. (**A**) Global trypanosomatid publication outputs in PubMed from 1960–2020; 58,784 outputs in total. The pie chart indicates the distribution of outputs among the trypanosomatids. (**B**) Trypanosomatid publication output affiliations in PubMed from 1995 to 2020 for 39 countries; the African trypanosomes, "brucei," "congolense," and "vivax" are combined here. The World map was obtained from Wikimedia Commons (https://commons.wikimedia.org/wiki/File:BlankMap-World-Flattened.svg). (**C**) International collaboration is illustrated on a heat map indicating pairwise joint affiliation, according to PubMed, over the past 10 years, and for those 10 countries listed on the most outputs.

https://doi.org/10.1371/journal.pntd.0010040.g001

Fig 2. Trypanosomatid research output trends by country. (**A**) Trypanosomatid publication output trends in PubMed from 1995 to 2020 for 39 countries. Countries are arranged in order of total number of "trypanosomatid" outputs; from 8,799 to 147. The African trypanosomes, "brucei," "congolense," and "vivax" are combined here. The pie charts indicate the distribution of outputs among the trypanosomatids. (**B**) The heat map indicates proportions of outputs from each country associated with each of the topics listed, according to PubMed over the past 10 years.

accelerated between 2005 to 2010 and has averaged more than 6 publications a day for the past 5 years. All groups, except for *T. brucei*, display a continuing steady increase in outputs over the past 10 years. Fig 1B shows outputs for the 3 main parasite groups since 1995 and for 39 countries on a global map. The pattern that emerges is largely explained by disease endemicity but also reflects international connectivity, which in some cases has its historical roots in colonialism. To further illustrate connectivity, proportions of international collaborative publications are illustrated on a heat map (Fig 1C). As examples, the UK appears on 2.8% of publications listing Brazil as an affiliation, while Brazil appears on 12.1% of publications listing the UK; India appears on 3.2% of publications listing the USA as an affiliation, while the USA appears on 4.6% of publications listing India. Higher degrees of international collaboration reflect proximity in terms of geography, and shared topic focus, while funding structures may also have an impact here.

Fig 2 shows outputs for the 3 parasite groups for the countries detailed above on a timeline between 1995 to 2020. Before 2012, only 2 countries (Brazil and USA) produced >100 "trypanosomatid" outputs in consecutive years. Seven additional countries (UK, India, Spain, France, Argentina, Germany, and Iran) have now sustained this level of output for several consecutive years. Brazil, India, and Iran, in particular, display a remarkable increase in "leishmania" outputs, accounting for 7% of global output from 1990 to 1994 and 48% from 2016 to 2020.

The terms "visceral" or "cutaneous" (leishmaniasis) occur at a similar level in outputs from Brazil (33% versus 32%), while "visceral" is overrepresented in outputs from India (57% versus 10%) and "cutaneous" is overrepresented in outputs from Iran (53% versus 23%). Countries that have sustained a higher level of "leishmania" outputs than for the other trypanosomatids include the USA, India, Spain, France, Germany, Iran, Italy, Canada, Australia, Portugal, Israel, Turkey, Greece, Tunisia, Pakistan, Egypt, and Sudan. Countries that have sustained a level of "cruzi" outputs that is higher than for the other trypanosomatids include Argentina, Mexico, Venezuela, Chile, and Uruguay. Countries that have sustained a level of African trypanosome outputs that is higher than for the other trypanosomatids for at least half of the time frame analysed include the UK, Switzerland, Belgium, Kenya, Nigeria, South Africa, and Uganda.

Data on topic focus by country over the past 10 years are illustrated using a heat map (Fig 2B). Notable features include particularly high relative levels of activity in insect vector research and evolutionary biology in the Czech Republic and Ecuador; in drug resistance in India, Belgium, Nigeria, and South Africa; in vaccine research in Iran; and in veterinary research in Italy, Greece, Nigeria, and Uganda. There has been a high relative level of activity in diagnostics in all countries listed.

Publication outputs by topic

Details for journal articles in the fields of life science and health science and published in the 32-year period from 1990 to 2021 were identified in Scopus using a set of trypanosomatid and trypanosomal disease terms, yielding >50,000 outputs (see Methods). To capture those research topics that have received the most attention, the 5% most highly cited (HC) articles from each year (to 2020) were identified and assigned to a primary trypanosomatid group, yielding 1,021 HC articles for "leishmania" (57%), 398 for "cruzi" (22%), and 367 for the African trypanosomes (21%), 1,786 HC articles in total. A notable feature within this set of articles was that median author number increased from 4 between 1990 to 1995 to 9 between 2015 to 2020. Prominent research topics captured within this set are detailed below, where citations have been selected based on citation counts (see Methods).

Trials, diagnostics, surveillance, and vectors

Among articles reporting the results of clinical trials (92 HC articles; Fig 3A), several have been particularly HC in recent years (2013 to 2018). Reports describe primarily drug trials, which, against the leishmaniases, include assessments of antimony [4], miltefosine [5,6], liposomal amphotericin B (AmBisome) [7], paromomycin [8,9], or combination therapies [10], as well as thermotherapy to treat cutaneous leishmaniasis. Trials against Chagas disease include assessments of benznidazole alone [11,12] or in combination with posaconazole [13,14] or the ravuconazole prodrug E1224 [15], or nifurtimox alone. Trials against the advanced CNS stage of sleeping sickness include assessments of melarsoprol, a nifurtimox–eflornithine combination [16], or fexinidazole [17]. Safer therapies and new dosing regimens have been introduced as a result, reducing adverse events, and improving efficacy. Disappointingly, benznidazole failed to reduce cardiac clinical deterioration in Chagas patients in the BENEFIT trial [11], and both posaconazole [13,14] and E1224 [15] failed to outperform benznidazole against chronic (CHAGASAZOL trial) or asymptomatic Chagas (STOP-CHAGAS trial), possibly due to suboptimal dosing. New oral therapies, however, such as miltefosine for leishmaniasis [5] and fexinidazole for sleeping sickness [17], were shown to be safe and effective, promising to facilitate administration and to address issues associated with compliance. The recently reported BENDITA trial suggested that shorter and reduced benznidazole regimens could be effective against Chagas disease [18], and finally, the boron-containing drug, SCYX-7158 [19], now known as acoziborole, is showing great promise in ongoing trials as an oral therapy for sleeping sickness.

Although no vaccines have been approved for wider use, there have been several antileishmanial vaccine trials in humans, to assess safety using a recombinant adenoviral vaccine, or efficacy using either whole autoclave-killed *Leishmania* or recombinant antigens, typically yielding moderate degrees of efficacy. Several antileishmanial vaccine trials in dogs, using either recombinant antigens or antigens secreted from *Leishmania infantum*, show good efficacy, suggesting a potential route to reducing the parasite burden in these reservoir hosts. Other trials involved the application of human host-directed immunomodulators [20] or the use of insecticide-impregnated dog collars.
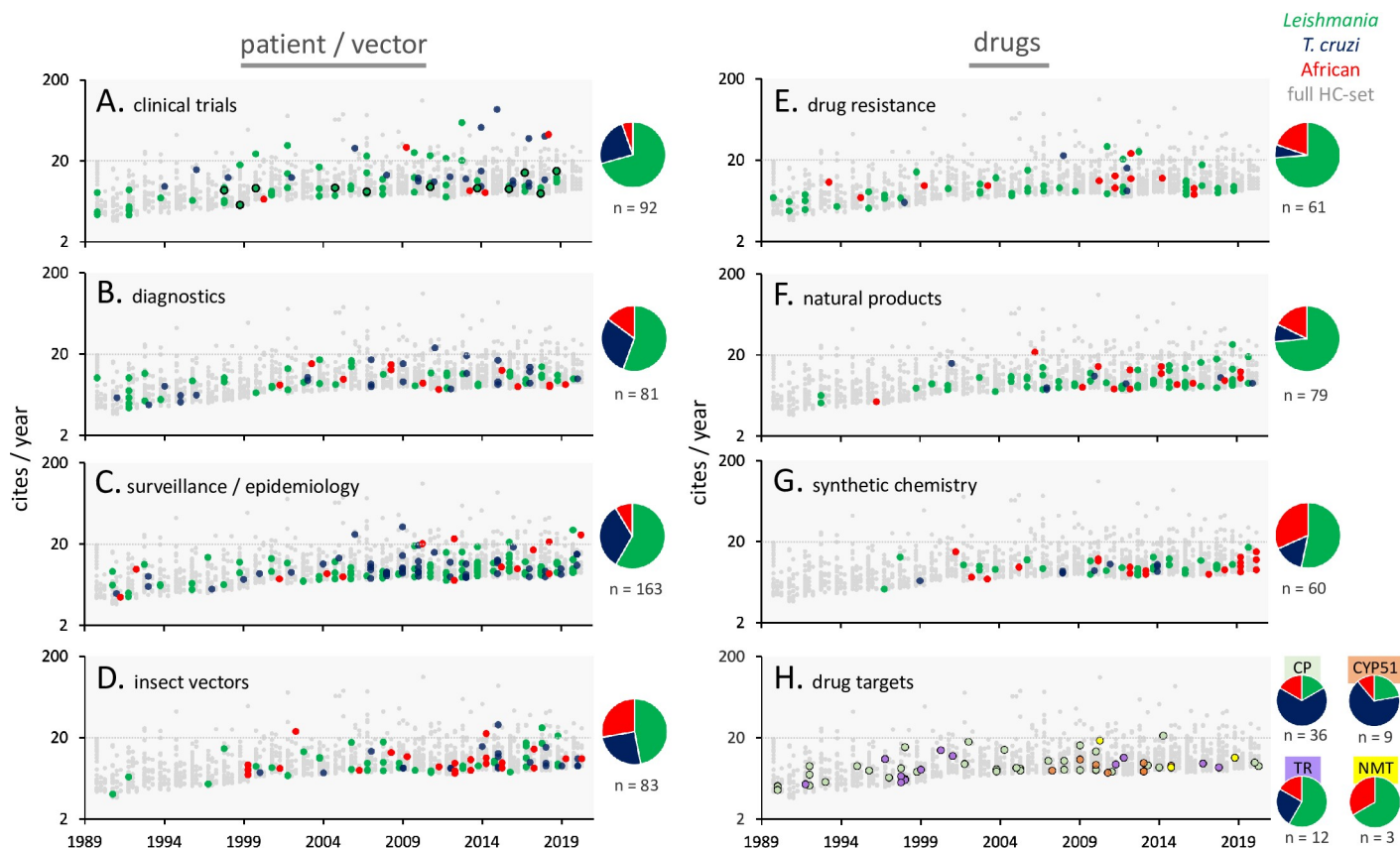
Fig 3. Citation rates by trypanosomatid research topic. HC articles from 1990–2020 are highlighted for each of the patient/vector (**A-D**) and drug-related topics (**E-H**), using data from Scopus. The grey data points indicate the full HC set of 1,786 articles, with "leishmania" articles displaced to the left and "African trypanosome" articles displaced to the right in each year-group. In (A), vaccine trials are indicated by symbols with a black outline. For drug targets in (H), each target is indicated using different coloured symbols; CP, CYP51, NMT, TR. The pie charts indicate the distribution of outputs in each category among the trypanosomatids. CP, cysteine peptidase; CYP51, sterol-14α-demethylase; HC, highly cited; NMT, *N*-myristoyltransferase; TR, trypanothione reductase.

https://doi.org/10.1371/journal.pntd.0010040.g003

Clinical studies, surveillance, and intervention are facilitated by quality diagnostic tools, and there has been substantial interest in the development and evaluation of these tools (81 HC articles; Fig 3B). PCR-based diagnostics, to detect typically high-copy number trypanosomatid sequences, have dominated [21], followed by serology, typically to detect antibodies against surface-exposed trypanosomal antigens. Xenodiagnosis also facilitates the identification of PKDL patients that continue to transmit *Leishmania* parasites to sand flies [22]. Loop-mediated isothermal amplification (LAMP) studies emerged after 2002, with LAMP primarily applied to human African trypanosomiasis. Serological tests have more recently emerged in the lateral flow based rapid diagnostic test (RDT) format. An initial positive test result may be followed up by further confirmatory tests, including enzyme-linked immunosorbent assay (ELISA), immunofluorescence antibody test (IFAT), direct detection of parasites by microscopy or, for staging human African trypanosomiasis, examination of cerebrospinal fluid for parasites, and/or elevated white blood cell count.

Surveillance and epidemiology (163 HC articles; Fig 3C) studies have focussed primarily on leishmaniasis or Chagas disease but also include efforts to map the distribution of sleeping sickness [23], to estimate populations at risk of sleeping sickness [24], and, more recently, to monitor progress in eliminating sleeping sickness [25,26]. Leishmaniasis surveillance has been largely in Iran and Brazil but also more recently in Syria, Lebanon, and Turkey. In terms of

Leishmania reservoir hosts, many studies focus on dogs [27], with several others on cats in several European countries, and also foxes. Chagas disease surveillance has largely been in the USA [28], Brazil, and in other South American countries. Many of these studies focus on transmission via the oral or congenital route or via blood transfusion or transplantation, with additional interest in the latter routes in Europe. Cardiomyopathy has also been the subject of surveillance in chronic Chagas patients [29]. Taxonomy and typing (26 HC articles) have had an impact in the areas of diagnostics and surveillance, although genome sequencing (see below) has now largely replaced some of the earlier typing techniques. These studies provided insights into the evolution and geographic spread of *Leishmania* spp. [30] and revealed 6 discrete typing units or lineages of *T. cruzi*, as well as a distinct *T. cruzi* lineage circulating globally in bat populations.

Vector control, using long-lasting insecticides in South America and Asia, and tsetse fly traps in sub-Saharan Africa, have been effective means to control transmission of the trypanosomiases. Indeed, several surveillance studies detailed above focus on the insect vectors [31] but also consider the impact of climate change on insect range [32]. Several studies on vector biology (83 HC articles; Fig 3D) additionally deal with interactions among trypanosomatids and other insect gut–resident microbes [33], while others deal with the role of components of sand fly saliva in *Leishmania* transmission and the associated immune response. Genome sequences have been reported for trypanosomatid insect vectors [34,35] and for a tsetse endosymbiont.

Drug research and development

The proportion of articles including the term "drug" (title, abstract, or keyword) has approximately doubled for each trypanosomatid group during the time frame analysed and has remained at approximately 40% in recent years. Sixty-one of the HC articles focus on drug resistance (Fig 3E). Miltefosine [36], amphotericin B [37], or antimonial resistance feature prominently in "leishmania" research [38]; benznidazole resistance (and nifurtimox cross-resistance) [39] in "cruzi" research; and melarsoprol–pentamidine cross-resistance [40] in "brucei" research. Defective drug uptake, particularly via aquaglyceroporins, but also increased drug efflux, feature prominently in "leishmania" and "brucei" drug resistance articles, as does metabolism of the nitro-prodrugs (13 HC articles), benznidazole, nifurtimox, and fexinidazole, by trypanosomal nitroreductase.

An increased level of interest in drug discovery emerged around 2000. This included substantial interest in natural products (79 HC articles; Fig 3F), focussing on products derived from plants [41] (including several oils), sponges, or fungi, and including frog or tarantula-derived antimicrobial peptides. Increased interest in medicinal chemistry or synthetic chemistry (60 HC articles; Fig 3G) also emerged around 2000. These studies describe the design and synthesis of a variety of compounds, sometimes inspired by natural products, and including chalcones, azoles, and quinolines. These compounds have often been tested for activity against multiple protozoa and other microbes.

Several post-2009 articles describe phenotypic drug screening (18 HC articles), using collections of compounds ranging from small sets to 1.8 million. All 3 trypanosomatid groups are represented and open-access pathogen "boxes" have emerged as a result [42]. Assessment of compounds for progression has been facilitated by screening intracellular *Leishmania* or *T. cruzi* parasites or by using bioluminescent imaging of parasites in mouse models [43], while drug potency assessment has been facilitated by an alamarBlue assay [44]. There have additionally been attempts to repurpose compounds approved for other clinical applications for use against trypanosomatids. Nanoparticle-based formulations for drug delivery, almost

exclusively against *Leishmania* [45], also feature prominently post-2010 (44 HC articles). A notable 2002 article that describes nanoparticle formulations of the animal African trypanosomiasis drug, berenil [46], precedes other HC articles relating to nanoparticles by 7 years. Otherwise, beyond liposomal amphotericin B, these studies describe the development of gold, silver, or other lipid-based formulations against either cutaneous or visceral leishmaniasis.

Potential trypanosomal drug targets that have received substantial attention include cysteine protease [47], trypanothione reductase, sterol-14α-demethylase (CYP51), and *N*-myristoyltransferase (NMT) [48] (Fig 3H). Cysteine protease (36 HC articles), primarily from *T. cruzi*, has been the subject of target-based screening, following the synthesis of peptide and nonpeptide inhibitors, and virtual screening, facilitated by structural studies [49]. Proof of concept for this target has been achieved in both mouse and dog models. Trypanothione is an antioxidant and the trypanosomatid-specific analogue of glutathione. Trypanothione reductase (12 HC articles), primarily from *Leishmania*, has similarly been the subject of target-based and virtual screening, facilitated by structural studies. Development of CYP51 as a drug target (9 HC articles), primarily in *T. cruzi*, has also been facilitated by structural data. As stated above, however, unfortunately, the antifungal, CYP51-targeting drugs, posaconazole and E1224 [13,14], proved to be inferior to benznidazole monotherapy, and no advantages were observed in clinical trials when combining these drugs with benznidazole. Development of NMT as a target in *Leishmania* or *T. brucei* (3 HC articles) has been facilitated by structural data and includes characterisation of lipidated proteins and validation in mouse models of both visceral leishmaniasis and sleeping sickness.

Articles aiming to define the mechanism-of-action by which drugs kill parasites (32 HC articles) initially focused on drugs used in the clinic over many years or on natural products, several of which likely exhibit polypharmacology, yet exhibit selectivity due to specific uptake or metabolism by parasites (see above). A related set of articles focus on apoptosis-like cell death (18 HC articles) induced by these drugs and/or by oxidative stress, primarily in *Leishmania*. More recent "mechanism-of-action" studies focus on target deconvolution for new, more chemically tractable compounds, and have now revealed several new and promising specific drug targets, including the proteasome [50,51], cyclin-dependent kinase 12 [52], the kinetochore kinase CLK1 [53], and cleavage and polyadenylation specificity factor 3, the target of acoziborole [19]. Several of these new compounds are now being assessed in clinical trials.

Host–parasite interactions

Studies on host–parasite interactions have yielded insights into parasitic lifestyles, pathology, persistence, and life cycle progression. Host cells respond to parasites and are also modulated and exploited by parasites. Both *Leishmania* and *T. cruzi* parasites invade mammalian host cells and can produce dormant forms [54], presenting an additional challenge in terms of chemotherapy. Seventy-nine of the HC articles focus on host cell interactions, invasion, and modulation by trypanosomatid parasites (Fig 4A). These studies are largely based on experimental mouse models but also include studies with human cell lines and patient samples. *Leishmania* parasites replicate within parasitophorous vacuoles in host macrophages and a major theme regards establishment of infection at the sandfly bite site, which involves invasion of neutrophils [55], production of neutrophil extracellular traps (NETs) [56], and phagocytosis by macrophages; rather than controlling the infection, NETs appear to facilitate macrophage invasion. Thus, *Leishmania* parasites evade host innate immune defences and exploit host immune cells to establish infection. In contrast, the protective response at the infection site appears to depend upon monocyte-derived dendritic cells (DCs) [57].
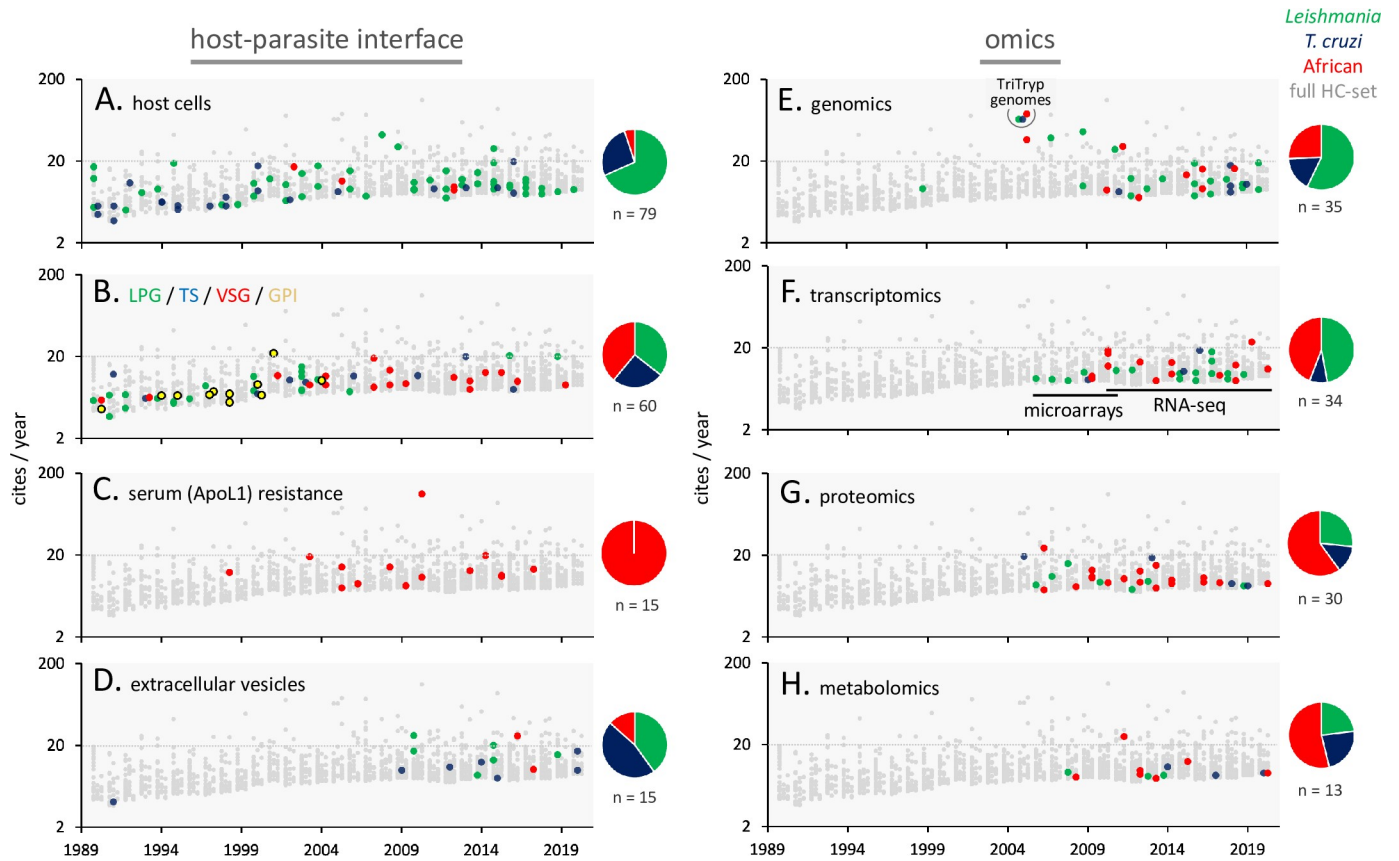
Fig 4. Citation rates by trypanosomatid research topic. HC articles from 1990–2020 are highlighted for each of the host–parasite interface (**A-D**) and omics-related topics (**E-H**). For parasite surface molecules in (B), each molecule is indicated using different coloured symbols; *Leishmania* LPG, *T. cruzi* TS, *T. brucei* VSG, GPI. Other details as in Fig 3. GPI, glycosylphosphatidylinositol; HC, highly cited; LPG, lipophosphoglycan; TS, *trans*-sialidase; VSG, variant surface glycoprotein.

https://doi.org/10.1371/journal.pntd.0010040.g004

Further studies that focus on host cell modulation by parasites also feature among this set. *Leishmania* parasites modify the parasitophorous vacuole, alter lipid and mitochondrial metabolism, and exploit host arginine by up-regulating parasite membrane transport. *Leishmania* infection induces changes in host cell DNA methylation, alters the host cell transcriptome, and represses translation, resulting in a dampened innate immune response and delayed programmed cell death. *Leishmania* RNA virus-1 can also promote parasite persistence [58]. *T. cruzi* parasites invade most nucleated cells via lysosomes and then escape into the cytosol, where replication depends upon host cell metabolic networks. Adipocytes appear to be important target cells, while macrophages also take up infected apoptotic cells. Several HC articles focus on the *T. cruzi* invasion process, involving elevation of free cytosolic calcium in the parasite, as well as recruitment of, and fusion with, lysosomes at the host cell entry site. Several additional HC articles focus on trypanosomatid calcium transporters and intracellular calcium pools, including pools within acidocalcisomes [59].

Differentiation (14 HC articles) and progression from one life cycle stage to the next also involves host–parasite interaction, with differentiation typically triggered by host-derived or other environmental signals. These studies focus on all 3 trypanosomatids, but a prominent theme relates to quorum sensing in African trypanosomes, whereby secreted parasite oligopeptidases produce an oligopeptide signal that promotes differentiation to a morphologically distinct "stumpy" form [60].

Major parasite surface antigens (60 HC articles; Fig 4B) have received substantial attention and have emerged as major virulence factors. These antigens are often connected to the plasma membrane via glycosylphosphatidylinositol (GPI) anchors, which are themselves potent immunomodulators [61]. *Leishmania* parasites are coated with heterogeneous, GPI-anchored lipophosphoglycans (LPGs) that promote within-host parasite persistence. LPG determines sandfly vector competence, promotes adhesion, and provides protection from digestive enzymes in the sandfly midgut, while developmentally distinct forms promote survival in macrophages by suppressing signalling pathways, oxidative defence mechanisms, and phagosome–lysosome fusion [62]. In addition, LPG regulates cytokine-inducible nitric oxide synthase (iNOS), activates the inflammasome in macrophages [63], and activates natural killer T cells.

T. cruzi metacyclic parasites are coated with *trans*-sialidases that transfer sialic acid from mammalian host glycoconjugates to GPI-anchored mucin-like glycoproteins on the parasite surface and to host cell surface proteins [64]. Both the *trans*-sialidases and mucins are heterogeneous and encoded by large parasite gene families. These surface antigens are thought to serve protective and adhesive functions and to drive CD8+ T-cell responses.

African trypanosomes are always extracellular. In the mammal, they reside in the bloodstream, in adipose tissue [65], and in skin [66]. Both the insect salivary gland and mammalian stages are coated with variant surface glycoproteins (VSGs). Antigenic variation for the purpose of evading host adaptive immunity is the primary role of these GPI-anchored proteins, encoded by thousands of distinct genes. Several studies have focussed on cataloguing the VSG genes, their telomeric environment, and the diversity of VSGs expressed during immune evasion in animal models, as well as VSG recycling influenced by hydrodynamic flow [67]. Other studies describe mechanisms of VSG gene expression and allelic exclusion, the expression of a single gene from a large gene family; several proteins have been identified that are involved in this latter process [68].

Another ongoing "arms race" between African trypanosomes and their mammalian hosts, this time involving innate immune defence, emerged in relation to human-infective *T. b. rhodesiense* and *T. b. gambiense* (15 HC articles; Fig 4C). Apolipoprotein L1 (ApoL1), a component of high-density lipoprotein (HDL) particles, was found to be the human serum (*T. b. brucei*) trypanolytic factor [69], while the VSG-related proteins, *T. b. rhodesiense* SRA and *T. b. gambiense* TgsGP, were found to confer resistance to lysis. Fully susceptible *T. brucei* parasites capture HDL particles via their haptoglobin–haemoglobin receptor, which is also defective in *T. b. gambiense*. Further illuminating the arms race, populations of African ancestry were found to express ApoL1 isoforms with increased trypanolytic activity that may alleviate sleeping sickness symptoms [70]; a negative consequence of expressing these variants though is increased susceptibility to kidney disease [71]. This particular 2010 article, with echoes of the malaria–sickle cell anaemia story, has the highest citation rate since publication (www.scopus.com) of all trypanosomatid articles analysed here.

Extracellular vesicles (EVs, also known as exosomes, 15 HC articles; Fig 4D) are involved in cell-to-cell communication and can modulate the host immune response. EVs are produced by both insect vector and mammalian stages of all 3 trypanosomatids. Notably, a 1991 description of *T. cruzi* EVs preceded other HC articles by 18 years [72], revealing a more recent resurgence of interest in EVs. Proteomic analysis of *Leishmania* EVs revealed large numbers of proteins [73], while analysis of both *Leishmania* and *T. cruzi* EVs revealed small RNAs that can modulate gene expression in host cells. *Leishmania* EVs, which may be transmitted by sandflies along with parasites [74], are immunosuppressive through interactions with neutrophils, macrophages [73], and DCs. EVs may also facilitate transmission of the *Leishmania* RNA virus [58]. *T. cruzi* EVs can promote developmental transition in the triatomine bug and increase host cell susceptibility to inflammation and invasion, while host cell–derived EVs may

promote invasion by *T. cruzi*. EVs from *T. brucei* promote social motility in the tsetse fly and have the capacity to transfer virulence factors to other *T. brucei* cells in the bloodstream as well as to cause anaemia [75].

Vaccine research and immune responses to *Leishmania* infection

Vaccine research (48 HC articles) has focussed almost exclusively on leishmaniasis, although 2 HC articles published in 2019 focus on *T. cruzi* and a 2021 article provides new promise for vaccination prospects against African trypanosomes, specifically *T. vivax* in this case [76]. Antigens from *Leishmania* parasites [77] and antigens from sandfly saliva are both protective. Adjuvants [78] and immune responses have been investigated, primarily in mouse models but also in dogs or nonhuman primates. One particularly HC study describes a key role for CD4 + T-cell cytokine responses in vaccine-mediated protection against *Leishmania* [79], while a 2020 article provides a modern take on "leishmanization" with genome-edited and attenuated *Leishmania* parasites [80].

Several trypanosomatid research themes (CD4/8, dendritic, interleukin, nitric oxide, and Th1/2; 221 HC articles in total) relate primarily to immune responses to *Leishmania* infection in mouse models. Further immunological insights from this set and beyond those described in the "host–parasite" section above are noted here. CD8+ cytotoxic T cells can cause tissue damage or be protective but may be exhausted in visceral leishmaniasis. Regarding CD4+ T helper cells, the balance between a T helper 1 (Th1, cell-mediated) or 2 (Th2, humoral) response determines whether the infection is cleared/controlled or exacerbated, respectively [81–83]. A protective Th1 response is promoted by IFN-γ and interleukin-12 (IL-12) [84,85] and antagonised by TGF-β, while a Th2 response is promoted by IL-4 and IL-10 [86,87]. Production of nitric oxide (NO) by the type 2 inducible NO synthase limits intracellular *Leishmania* replication in macrophages [88–90], while *Leishmania* or host arginase can drive parasite replication by increasing polyamine synthesis. Induction of host arginase can also antagonise NO production, which is L-arginine dependent. Engulfment of *Leishmania* by DCs is facilitated by Fcγ and DC-SIGN receptors, allowing the DCs to present antigen and produce IL-12 and NO, thereby contributing to T cell–mediated protection. CD8+ cytotoxic T cells and NO can also be protective against *T. cruzi*. Research in this area reflects a sustained high level of interest in immune responses to *Leishmania* infection as well as in anti-*Leishmania* vaccine development.

Trypanosomatid omics and genetics

Trypanosomatid genomes are typically diploid, although chromosome ploidy can vary and subtelomeric regions in particular display high rates of recombination and hemizygosity. Genetic exchange during sexual recombination in trypanosomatids (10 HC articles) appears to be restricted to the insect vector stages [91]. Other studies on DNA recombination and DNA repair have facilitated the development of new genetic tools as well as provided insights into genome organisation, virulence gene recombination, and the emergence of drug resistance.

The TriTryp genome sequences were published in 2005, yielding a set of particularly HC articles [92–95], which were followed by several further omics studies. In terms of genomics (35 HC articles; Fig 4E), studies have focussed on comparative or population genome sequencing, for additional trypanosomatid species and subspecies [96,97], and for clinical isolates [38], using short-read, long-read, or nanopore technologies. Notably, a 1999 description of the *Leishmania* chromosome 1 sequence [98] precedes other HC genomics studies by 6 years. In terms of "functional genomics," the first-ever RNA interference library screen was conducted in *T. brucei*, and several subsequent articles feature genome-scale screens using this posttranscriptional gene silencing approach [99].

Trypanosomatid transcriptomics studies (34 HC articles; Fig 4F) have progressed from microarrays in 2006, to RNA-seq [100], to ribosome profiling, and, more recently, to single-cell RNA-seq [101]. Studies focus on the analysis of differentiation, life cycle stage differences, additional species and subspecies, and parasites in mammalian and vector hosts. Gene expression control (15 HC articles) is unconventional in trypanosomatids in that transcription is polycistronic, with initiation and termination sites marked by specific histone variants [102]. This places major emphasis on posttranscriptional controls mediated by RNA binding proteins. Indeed, several transcriptome studies focus on posttranscriptional controls. RNAs can also mediate control, as illustrated by a recent example of translation control by a tRNA half [103].

Trypanosomatid proteomics studies (30 HC articles; Fig 4G) [104] have progressed to deeper proteome coverage, again in distinct life cycle stages, and to organellar proteomes [105], secretomes, and phospho-proteomes. Metabolomics studies (13 HC articles; Fig 4H) have focussed on technology development [106], responses to drugs, or characterisation of metabolic pathways. Computational tools are central to these omics studies, including tools for transcriptome network analysis [107]. Indeed, the genome databases, veupathdb.org and tritrypdb.org [108], have provided critical access to omics datasets as well as access to a range of bioinformatics and in silico analysis tools.

Technologies drive discovery and the development and application of genetic tools (25 HC articles) and other methods (31 HC articles) accordingly feature prominently among HC trypanosomatid articles. Inducible gene expression has been widely used as an experimental tool in *T. brucei* [109], as has inducible RNA interference [110,111]. A more recent set of HC articles describes the development and application of CRISPR-Cas9 gene editing (10 HC articles) for the trypanosomatids [112,113]. A 2019 article describes the use of Cas9 to assemble >100 *Leishmania* flagellar protein mutants [114], and, as noted above, a 2020 article reports the use of Cas9 to generate attenuated *Leishmania* parasites for potential vaccination or "leishmanization" [80]. In terms of protein structure studies (24 HC articles), cryogenic electron microscopy is a technology gaining traction in trypanosomatid studies [115]. Computational tools are also central to structural studies and have been used to assess antitrypanosomal drug-binding pockets [116]. In addition, there have been substantial advances in computational approaches to 3D structure prediction, recently applied to thousands of trypanosomatid proteins (https://alphafold.ebi.ac.uk).

TriTryp genome sequencing combined with powerful genetic tools unquestionably transformed trypanosomatid research studies, in the areas of molecular and cell biology in particular, and provided key insights into eukaryotic evolution (21 HC articles). Other exemplar studies that illustrate the impact of omics include studies on the trypanosomatid flagellum (14 HC articles) and cell motility [105,114] and the discovery of unconventional kinetochores [117], the macromolecular complexes that direct chromosome segregation, that likely diverged from conventional kinetochores at the root of the eukaryotic tree of life. Continuing the unconventional theme is polycistronic transcription [118] as described above, and compartmentalisation of glycolysis (11 HC articles) within glycosomes [119], considered over many years to represent a promising target for chemotherapy. The kinetoplast, a complex mitochondrial genome comprising a network of interlocked maxicircles and minicircles, after which the Kinetoplastida group of flagellated protists are named, is another unconventional feature and an established drug target. Several studies focus on mechanisms of kinetoplast DNA (kDNA) replication and segregation. In addition, the unique, high-copy number kDNA sequences have been exploited as targets for diagnostic tests, while the kDNA and the flagellum have long served as prominent features under the microscope that have greatly facilitated studies on cell cycle progression.

Trypanosomatids have additionally served as "model eukaryotes" in relation to conserved organelles, molecules, machineries, and processes that were first discovered in these parasites. Examples include acidocalcisomes [59], thought to be involved in osmoregulation; the GPI membrane anchors [120] described above; mRNA editing (11 HC articles), which is extensive within transcripts derived from the mitochondrial kDNA genome [121]; *trans*-splicing [122], which occurs transcriptome-wide in conjunction with polycistronic transcription in trypanosomatids; and epigenetic control by oxidation of 5-methyl groups on DNA bases [123] catalysed by enzymes with homologues in mammals. Studies on trypanosome flagellar defects also provide models for understanding human ciliopathies with analogous underlying defects [124].

Research trends and emerging topics

The trypanosomatid research community has strengthened key areas and has continued to deliver new research topics and technologies. Fig 5A lists all of those areas and topics detailed above that do not appear in Figs 3 and 4 and the distribution of outputs for each topic among the trypanosomatids, while Fig 5B shows all topics for which the HC articles have a median publication date later than 2010. Some of these latter topics and research areas have a long history and are experiencing an increasing level of interest more recently, such as protein structure determination, drug mechanism-of-action studies, and surveillance studies. Natural product studies, drug screening, and nanoparticle formulation studies all register many recent and HC articles, and there has also been a growing interest in the insect vectors. As noted
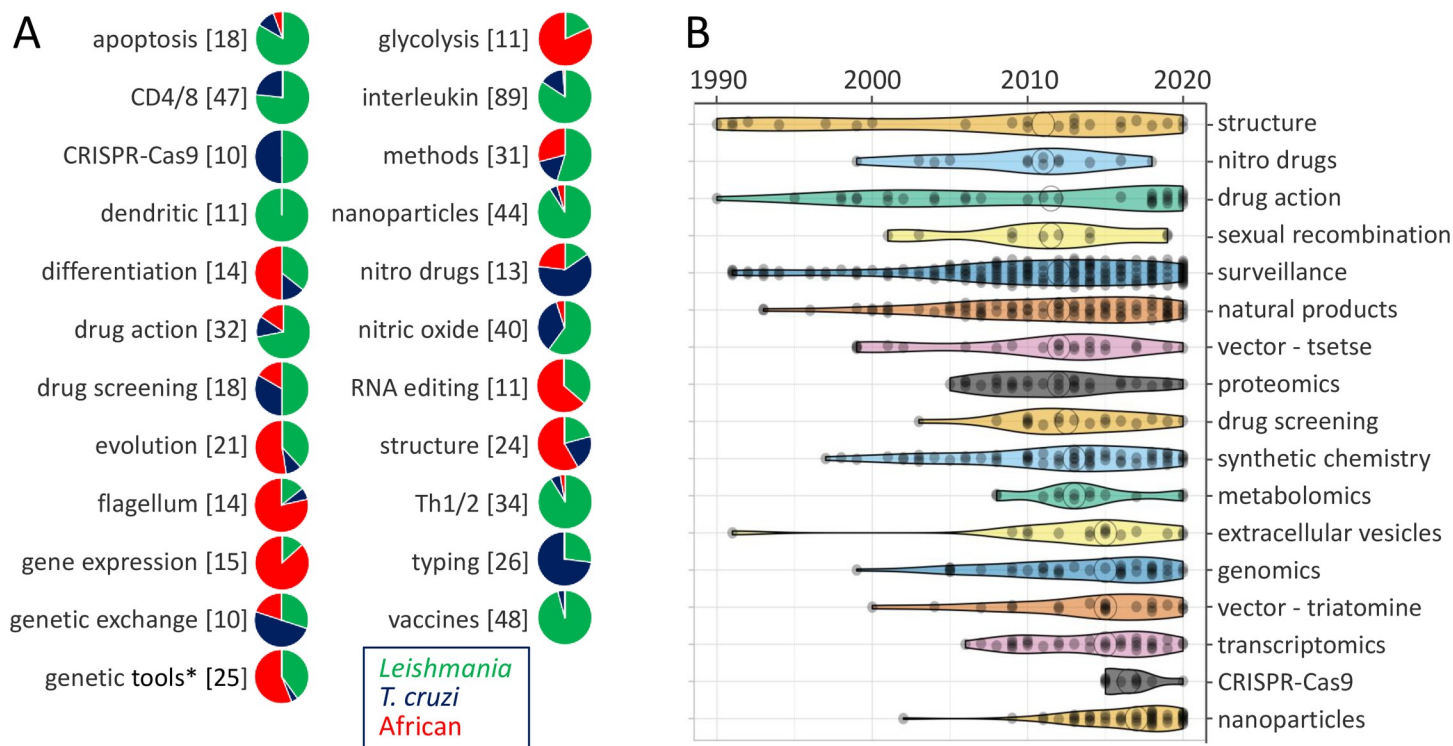
Fig 5. Research trends and emerging topics. (**A**) Trypanosomatid research topics represented in the set of HC articles and not shown in Figs 3 and 4. The pie charts indicate the distribution of outputs for each topic among the trypanosomatids. Number or articles in each set is indicated in square brackets. * excluding "CRISPR-Cas9" articles. (**B**) The violin plot shows those topics for which the HC articles have a median publication date later than 2010. Topics are ranked by median value as indicated by the open circles. HC, highly cited.

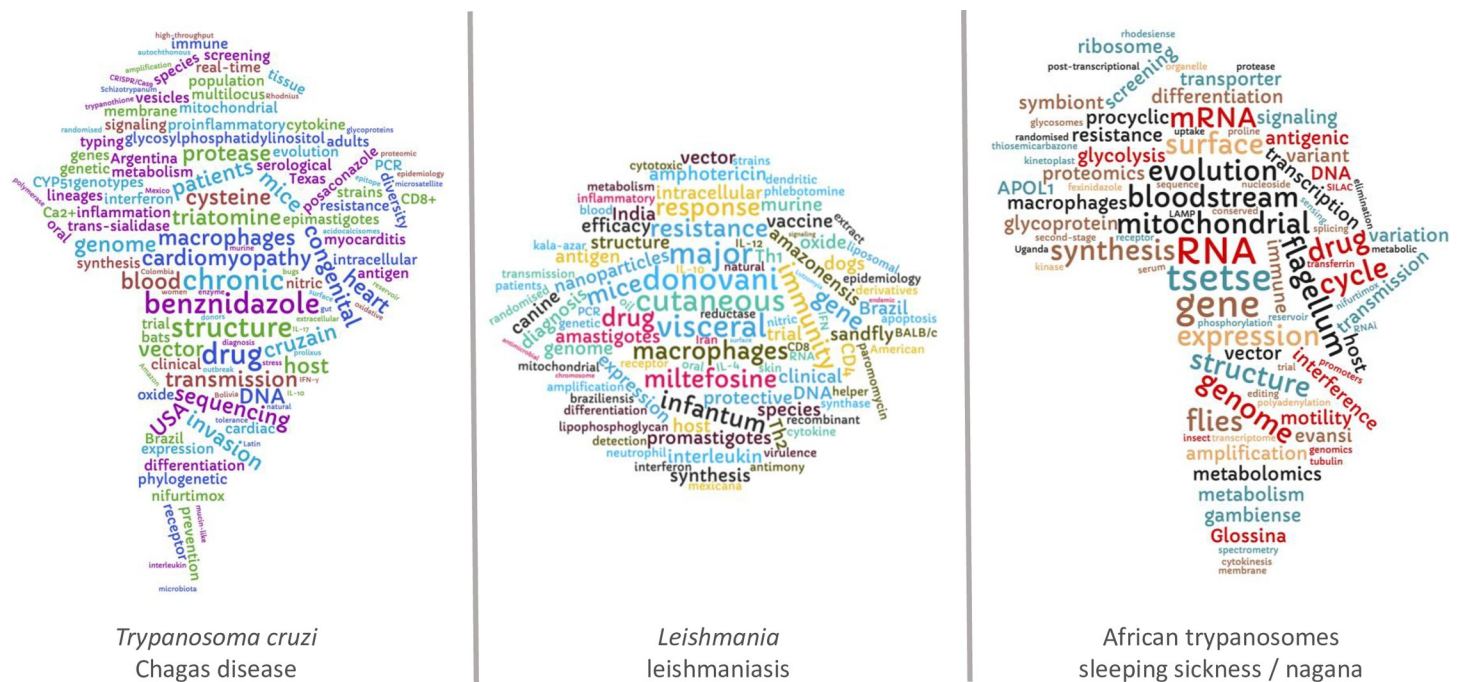https://doi.org/10.1371/journal.pntd.0010040.g005

above, omics studies followed publication of the TriTryp genome sequences in 2005, and the development and application of CRISPR-Cas9 genome editing tools emerged even more recently.

Concluding remarks

The goal here has been to include a broad range of topics and to provide a representative and inclusive profile of trypanosomatid research. The word clouds (Fig 6), derived from the set of 1,786 HC article titles, capture even more topics. Use of citation rate is an imperfect method for capturing a fully representative profile of trypanosomatid research, however. Some articles have a broader scope that extends beyond trypanosomatids, for example. These include assessments of compound collections, either natural or synthetic, which often incorporate assays with other pathogens and/or cancer cell lines, and studies that primarily focus on immune cell function, inflammation, or the response to pathogen challenge. There are also certainly thousands of excellent research articles not captured in the set analysed here. Nevertheless, a focus on primary research articles and a quantitative approach using citation metrics has clearly captured a broad range of topics on both the parasites and the diseases they cause. The exemplar studies cited additionally capture both the discovery science and the translational research.

The trypanosomatid research community has made great strides in the past and continues to do so. Intimately linked to these advances are vector control efforts, improved diagnostics, and increasingly populated clinical development pipelines (https://dndi.org/research-development/portfolio/). Indeed, although challenges remain, control and elimination prospects now look increasingly encouraging for these NTDs [1–3]. The outputs and successes detailed above reflect a vibrant and dynamic trypanosomatid research community that has strengthened key areas and has continued to deliver new research topics, trends that are set to continue.

Trypanosoma cruzi
Chagas disease

Leishmania
leishmaniasis

African trypanosomes
sleeping sickness / nagana

Fig 6. Word clouds. Word clouds for each trypanosomatid group derived from the titles of HC articles from 1990–2020; data from Scopus. Trypanosomatid and disease-associated terms used to search Scopus were removed. HC, highly cited.

https://doi.org/10.1371/journal.pntd.0010040.g006

Key Learning Points

- Trypanosomatid research is vibrant and diverse globally in both endemic and nonendemic countries.

- Translation and drug discovery are having a substantial impact, facilitated by public–private and industry partnerships.

- Many new discoveries and new research areas have emerged, often facilitated by technological advances.

Top Five Papers

1. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4+CD25+ regulatory T cells control *Leishmania major* persistence and immunity. Nature. 2002;420:502–7.

2. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science. 2010;329:841–5.

3. Aslett M, Aurrecoechea C, Berriman M, Brestelli J, Brunk BP, Carrington M, et al. TriTrypDB: a functional genomic resource for the Trypanosomatidae. Nucleic Acids Res. 2010;38:D457–62.

4. Khare S, Nagle AS, Biggart A, Lai YH, Liang F, Davis LC, et al. Proteasome inhibition for treatment of leishmaniasis, Chagas disease and sleeping sickness. Nature. 2016;537:229–33.

5. Mesu V, Kalonji WM, Bardonneau C, Mordt OV, Blesson S, Simon F, et al. Oral fexinidazole for late-stage African *Trypanosoma brucei gambiense* trypanosomiasis: a pivotal multicentre, randomised, non-inferiority trial. Lancet. 2018;391:144–54.

References

1. Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet. 2018; 392(10151):951–70. Epub 2018 Aug 22. https://doi.org/10.1016/S0140-6736(18)31204-2 PMID: 30126638.

2. Perez-Molina JA, Molina I. Chagas disease. Lancet. 2018; 391(10115):82–94. Epub 2017 Jul 5. https://doi.org/10.1016/S0140-6736(17)31612-4 PMID: 28673423.

3. Buscher P, Cecchi G, Jamonneau V, Priotto G. Human African trypanosomiasis. Lancet. 2017; 390 (10110):2397–409. Epub 2017 Jul 5. https://doi.org/10.1016/S0140-6736(17)31510-6 PMID: 28673422.

4. Sundar S, More DK, Singh MK, Singh VP, Sharma S, Makharia A, et al. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. Clin Infect Dis. 2000; 31(4):1104–7. Epub 2000 Oct 26. https://doi.org/10.1086/318121 PMID: 11049798.

5. Sundar S, Jha TK, Thakur CP, Engel J, Sindermann H, Fischer C, et al. Oral miltefosine for Indian visceral leishmaniasis. N Engl J Med. 2002; 347(22):1739–46. Epub 2002 Nov 29. https://doi.org/10.1056/NEJMoa021556 PMID: 12456849.

6. Sundar S, Singh A, Rai M, Prajapati VK, Singh AK, Ostyn B, et al. Efficacy of miltefosine in the treatment of visceral leishmaniasis in India after a decade of use. Clin Infect Dis. 2012; 55(4):543–50. Epub 2012 May 11. https://doi.org/10.1093/cid/cis474 PMID: 22573856.

7. Sundar S, Chakravarty J, Agarwal D, Rai M, Murray HW. Single-dose liposomal amphotericin B for visceral leishmaniasis in India. N Engl J Med. 2010; 362(6):504–12. Epub 2010 Feb 12. https://doi.org/10.1056/NEJMoa0903627 PMID: 20147716.

8. Ben Salah A, Ben Messaoud N, Guedri E, Zaatour A, Ben Alaya N, Bettaieb J, et al. Topical paromomycin with or without gentamicin for cutaneous leishmaniasis. N Engl J Med. 2013; 368(6):524–32. Epub 2013 Feb 8. https://doi.org/10.1056/NEJMoa1202657 PMID: 23388004.

9. Sundar S, Jha TK, Thakur CP, Sinha PK, Bhattacharya SK. Injectable paromomycin for visceral leishmaniasis in India. N Engl J Med. 2007; 356(25):2571–81. Epub 2007 Jun 22. https://doi.org/10.1056/NEJMoa066536 PMID: 17582067.

10. Sundar S, Sinha PK, Rai M, Verma DK, Nawin K, Alam S, et al. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. Lancet. 2011; 377(9764):477–86. Epub 2011 Jan 25. https://doi.org/10.1016/S0140-6736(10)62050-8 PMID: 21255828.

11. Morillo CA, Marin-Neto JA, Avezum A, Sosa-Estani S, Rassi A Jr., Rosas F, et al. Randomized trial of benznidazole for chronic Chagas' cardiomyopathy. N Engl J Med. 2015; 373(14):1295–306. Epub 2015 Sep 2. https://doi.org/10.1056/NEJMoa1507574 PMID: 26323937.

12. Viotti R, Vigliano C, Lococo B, Bertocchi G, Petti M, Alvarez MG, et al. Long-term cardiac outcomes of treating chronic Chagas disease with benznidazole versus no treatment: a nonrandomized trial. Ann Intern Med. 2006; 144(10):724–34. Epub 2006 May 17. https://doi.org/10.7326/0003-4819-144-10-200605160-00006 PMID: 16702588.

13. Molina I, Gomez i Prat J, Salvador F, Trevino B, Sulleiro E, Serre N, et al. Randomized trial of posaconazole and benznidazole for chronic Chagas' disease. N Engl J Med. 2014; 370(20):1899–908. Epub 2014 May 16. https://doi.org/10.1056/NEJMoa1313122 PMID: 24827034.

14. Morillo CA, Waskin H, Sosa-Estani S, Del Carmen Bangher M, Cuneo C, Milesi R, et al. Benznidazole and posaconazole in eliminating parasites in asymptomatic *T. cruzi* carriers: The STOP-CHAGAS trial. J Am Coll Cardiol. 2017; 69(8):939–47. Epub 2017 Feb 25. https://doi.org/10.1016/j.jacc.2016.12.023 PMID: 28231946.

15. Torrico F, Gascon J, Ortiz L, Alonso-Vega C, Pinazo MJ, Schijman A, et al. Treatment of adult chronic indeterminate Chagas disease with benznidazole and three E1224 dosing regimens: a proof-of-concept, randomised, placebo-controlled trial. Lancet Infect Dis. 2018; 18(4):419–30. Epub 2018 Jan 21. https://doi.org/10.1016/S1473-3099(17)30538-8 PMID: 29352704.

16. Priotto G, Kasparian S, Mutombo W, Ngouama D, Ghorashian S, Arnold U, et al. Nifurtimox-eflornithine combination therapy for second-stage African *Trypanosoma brucei gambiense* trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial. Lancet. 2009; 374(9683):56–64. Epub 2009 Jun 30. https://doi.org/10.1016/S0140-6736(09)61117-X PMID: 19559476.

17. Mesu V, Kalonji WM, Bardonneau C, Mordt OV, Blesson S, Simon F, et al. Oral fexinidazole for late-stage African *Trypanosoma brucei gambiense* trypanosomiasis: a pivotal multicentre, randomised, non-inferiority trial. Lancet. 2018; 391(10116):144–54. Epub 2017 Nov 9. https://doi.org/10.1016/S0140-6736(17)32758-7 PMID: 29113731.

18. Torrico F, Gascon J, Barreira F, Blum B, Almeida IC, Alonso-Vega C, et al. New regimens of benznidazole monotherapy and in combination with fosravuconazole for treatment of Chagas disease (BENDITA): a phase 2, double-blind, randomised trial. Lancet Infect Dis. 2021. Epub 2021 Apr 10. https://doi.org/10.1016/S1473-3099(20)30844-6 PMID: 33836161

19. Jacobs RT, Nare B, Wring SA, Orr MD, Chen D, Sligar JM, et al. SCYX-7158, an orally-active benzoxaborole for the treatment of stage 2 human African trypanosomiasis. PLoS Negl Trop Dis. 2011; 5(6): e1151. Epub 2011 Jul 9. https://doi.org/10.1371/journal.pntd.0001151 PMID: 21738803; PubMed Central PMCID: PMC3125149.

20. Dubovsky JA, Beckwith KA, Natarajan G, Woyach JA, Jaglowski S, Zhong Y, et al. Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. Blood. 2013; 122 (15):2539–49. Epub 2013 Jul 28. https://doi.org/10.1182/blood-2013-06-507947 PMID: 23886836; PubMed Central PMCID: PMC3795457.

21. Schijman AG, Bisio M, Orellana L, Sued M, Duffy T, Mejia Jaramillo AM, et al. International study to evaluate PCR methods for detection of *Trypanosoma cruzi* DNA in blood samples from Chagas disease patients. PLoS Negl Trop Dis. 2011; 5(1):e931. Epub 2011 Jan 26. https://doi.org/10.1371/journal.pntd.0000931 PMID: 21264349; PubMed Central PMCID: PMC3019106.

22. Mondal D, Bern C, Ghosh D, Rashid M, Molina R, Chowdhury R, et al. Quantifying the infectiousness of post-kala-azar dermal leishmaniasis toward sand flies. Clin Infect Dis. 2019; 69(2):251–8. Epub 2018 Oct 26. https://doi.org/10.1093/cid/ciy891 PMID: 30357373; PubMed Central PMCID: PMC6603265.

23. Simarro PP, Cecchi G, Paone M, Franco JR, Diarra A, Ruiz JA, et al. The Atlas of human African try-panosomiasis: a contribution to global mapping of neglected tropical diseases. Int J Health Geogr. 2010; 9:57. Epub 2010 Nov 3. https://doi.org/10.1186/1476-072X-9-57 PMID: 21040555; PubMed Central PMCID: PMC2988709.

24. Simarro PP, Cecchi G, Franco JR, Paone M, Diarra A, Ruiz-Postigo JA, et al. Estimating and mapping the population at risk of sleeping sickness. PLoS Negl Trop Dis. 2012; 6(10):e1859. Epub 2012 Nov 13. https://doi.org/10.1371/journal.pntd.0001859 PMID: 23145192; PubMed Central PMCID: PMC3493382.

25. Franco JR, Cecchi G, Priotto G, Paone M, Diarra A, Grout L, et al. Monitoring the elimination of human African trypanosomiasis: Update to 2016. PLoS Negl Trop Dis. 2018; 12(12):e0006890. Epub 2018 Dec 7. https://doi.org/10.1371/journal.pntd.0006890 PMID: 30521525; PubMed Central PMCID: PMC6283345.

26. Franco JR, Cecchi G, Priotto G, Paone M, Diarra A, Grout L, et al. Monitoring the elimination of human African trypanosomiasis at continental and country level: Update to 2018. PLoS Negl Trop Dis. 2020; 14(5):e0008261. Epub 2020 May 22. https://doi.org/10.1371/journal.pntd.0008261 PMID: 32437391; PubMed Central PMCID: PMC7241700.

27. Mendoza-Roldan J, Benelli G, Panarese R, Iatta R, Furlanello T, Beugnet F, et al. *Leishmania infantum* and *Dirofilaria immitis* infections in Italy, 2009–2019: changing distribution patterns. Parasit Vectors. 2020; 13(1):193. Epub 2020 Apr 16. https://doi.org/10.1186/s13071-020-04063-9 PMID: 32293524; PubMed Central PMCID: PMC7161282.

28. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. Clin Infect Dis. 2009; 49(5):e52–4. Epub 2009 Jul 31. https://doi.org/10.1086/605091 PMID: 19640226.

29. Rassi A Jr., Rassi A, Little WC, Xavier SS, Rassi SG, Rassi AG, et al. Development and validation of a risk score for predicting death in Chagas' heart disease. N Engl J Med. 2006; 355(8):799–808. Epub 2006 Aug 25. https://doi.org/10.1056/NEJMoa053241 PMID: 16928995.

30. Lukes J, Mauricio IL, Schonian G, Dujardin JC, Soteriadou K, Dedet JP, et al. Evolutionary and geographical history of the *Leishmania donovani* complex with a revision of current taxonomy. Proc Natl Acad Sci U S A. 2007; 104(22):9375–80. Epub 2007 May 23. https://doi.org/10.1073/pnas.0703678104 PMID: 17517634; PubMed Central PMCID: PMC1890502.

31. Alten B, Maia C, Afonso MO, Campino L, Jimenez M, Gonzalez E, et al. Seasonal dynamics of Phlebotomine sand fly species proven vectors of Mediterranean Leishmaniasis caused by *Leishmania infantum*. PLoS Negl Trop Dis. 2016; 10(2):e0004458. Epub 2016 Feb 24. https://doi.org/10.1371/journal.pntd.0004458 PMID: 26900688; PubMed Central PMCID: PMC4762948.

32. Gonzalez C, Wang O, Strutz SE, Gonzalez-Salazar C, Sanchez-Cordero V, Sarkar S. Climate change and risk of leishmaniasis in north america: predictions from ecological niche models of vector and reservoir species. PLoS Negl Trop Dis. 2010; 4(1):e585. Epub 2010 Jan 26. https://doi.org/10.1371/journal.pntd.0000585 PMID: 20098495; PubMed Central PMCID: PMC2799657.

33. Dey R, Joshi AB, Oliveira F, Pereira L, Guimaraes-Costa AB, Serafim TD, et al. Gut microbes egested during bites of infected sand flies augment severity of Leishmaniasis via inflammasome-derived IL-1beta. Cell Host Microbe. 2018; 23(1):134–43 e6. Epub 2018 Jan 2. https://doi.org/10.1016/j.chom.2017.12.002 PMID: 29290574; PubMed Central PMCID: PMC5832060.

34. International Glossina Genome I. Genome sequence of the tsetse fly (*Glossina morsitans*): vector of African trypanosomiasis. Science. 2014; 344(6182):380–6. Epub 2014 Apr 26. https://doi.org/10.1126/science.1249656 PMID: 24763584; PubMed Central PMCID: PMC4077534.

35. Mesquita RD, Vionette-Amaral RJ, Lowenberger C, Rivera-Pomar R, Monteiro FA, Minx P, et al. Genome of *Rhodnius prolixus*, an insect vector of Chagas disease, reveals unique adaptations to hematophagy and parasite infection. Proc Natl Acad Sci U S A. 2015; 112(48):14936–41. Epub 2015 Dec 3. https://doi.org/10.1073/pnas.1506226112 PMID: 26627243; PubMed Central PMCID: PMC4672799.

36. Rijal S, Ostyn B, Uranw S, Rai K, Bhattarai NR, Dorlo TP, et al. Increasing failure of miltefosine in the treatment of Kala-azar in Nepal and the potential role of parasite drug resistance, reinfection, or non-compliance. Clin Infect Dis. 2013; 56(11):1530–8. Epub 2013 Feb 22. https://doi.org/10.1093/cid/cit102 PMID: 23425958.

37. Purkait B, Kumar A, Nandi N, Sardar AH, Das S, Kumar S, et al. Mechanism of amphotericin B resistance in clinical isolates of *Leishmania donovani*. Antimicrob Agents Chemother. 2012; 56(2):1031–41. Epub 2011 Nov 30. https://doi.org/10.1128/AAC.00030-11 PMID: 22123699; PubMed Central PMCID: PMC3264217.

38. Downing T, Imamura H, Decuypere S, Clark TG, Coombs GH, Cotton JA, et al. Whole genome sequencing of multiple *Leishmania donovani* clinical isolates provides insights into population structure and mechanisms of drug resistance. Genome Res. 2011; 21(12):2143–56. Epub 2011 Nov 1. https://doi.org/10.1101/gr.123430.111 PMID: 22038251; PubMed Central PMCID: PMC3227103.

39. Wilkinson SR, Taylor MC, Horn D, Kelly JM, Cheeseman I. A mechanism for cross-resistance to nifur-timox and benznidazole in trypanosomes. Proc Natl Acad Sci U S A. 2008; 105(13):5022–7. Epub 2008 Mar 28. https://doi.org/10.1073/pnas.0711014105 PMID: 18367671; PubMed Central PMCID: PMC2278226.

40. Alsford S, Eckert S, Baker N, Glover L, Sanchez-Flores A, Leung KF, et al. High-throughput decoding of antitrypanosomal drug efficacy and resistance. Nature. 2012; 482(7384):232–6. Epub 2012 Jan 27. https://doi.org/10.1038/nature10771 PMID: 22278056; PubMed Central PMCID: PMC3303116.

41. Tasdemir D, Kaiser M, Brun R, Yardley V, Schmidt TJ, Tosun F, et al. Antitrypanosomal and antileish-manial activities of flavonoids and their analogues: in vitro, in vivo, structure-activity relationship, and quantitative structure-activity relationship studies. Antimicrob Agents Chemother. 2006; 50(4):1352–64. Epub 2006 Mar 30. https://doi.org/10.1128/AAC.50.4.1352-1364.2006 PMID: 16569852; PubMed Central PMCID: PMC1426963.

42. Pena I, Pilar Manzano M, Cantizani J, Kessler A, Alonso-Padilla J, Bardera AI, et al. New compound sets identified from high throughput phenotypic screening against three kinetoplastid parasites: an open resource. Sci Rep. 2015; 5:8771. Epub 2015 Mar 6. https://doi.org/10.1038/srep08771 PMID: 25740547; PubMed Central PMCID: PMC4350103.

43. Lewis MD, Fortes Francisco A, Taylor MC, Burrell-Saward H, McLatchie AP, Miles MA, et al. Biolumi-nescence imaging of chronic Trypanosoma cruzi infections reveals tissue-specific parasite dynamics and heart disease in the absence of locally persistent infection. Cell Microbiol. 2014; 16(9):1285–300. Epub 2014 Apr 10. https://doi.org/10.1111/cmi.12297 PMID: 24712539; PubMed Central PMCID: PMC4190689.

44. Raz B, Iten M, Grether-Buhler Y, Kaminsky R, Brun R. The Alamar Blue assay to determine drug sen-sitivity of African trypanosomes (T.b. rhodesiense and T.b. gambiense) in vitro. Acta Trop. 1997; 68 (2):139–47. Epub 1997 Dec 5. https://doi.org/10.1016/s0001-706x(97)00079-x PMID: 9386789

45. Hameed S, Khalil AT, Ali M, Numan M, Khamlich S, Shinwari ZK, et al. Greener synthesis of ZnO and Ag-ZnO nanoparticles using Silybum marianum for diverse biomedical applications. Nanomedicine (Lond). 2019; 14(6):655–73. Epub 2019 Feb 5. https://doi.org/10.2217/nnm-2018-0279 PMID: 30714480.

46. Olbrich C, Gessner A, Kayser O, Muller RH. Lipid-drug-conjugate (LDC) nanoparticles as novel carrier system for the hydrophilic antitrypanosomal drug diminazenediaceturate. J Drug Target. 2002; 10 (5):387–96. Epub 2002 Nov 22. https://doi.org/10.1080/1061186021000001832 PMID: 12442809.

47. Redecke L, Nass K, DePonte DP, White TA, Rehders D, Barty A, et al. Natively inhibited Trypanosoma brucei cathepsin B structure determined by using an X-ray laser. Science. 2013; 339(6116):227–30. Epub 2012 Dec 1. https://doi.org/10.1126/science.1229663 PMID: 23196907; PubMed Central PMCID: PMC3786669.

48. Frearson JA, Brand S, McElroy SP, Cleghorn LA, Smid O, Stojanovski L, et al. N-myristoyltransferase inhibitors as new leads to treat sleeping sickness. Nature. 2010; 464(7289):728–32. Epub 2010 Apr 3. https://doi.org/10.1038/nature08893 PMID: 20360736; PubMed Central PMCID: PMC2917743.

49. Gati C, Bourenkov G, Klinge M, Rehders D, Stellato F, Oberthur D, et al. Serial crystallography on in vivo grown microcrystals using synchrotron radiation. IUCrJ. 2014; 1(Pt 2):87–94. Epub 2014 Jul 31. https://doi.org/10.1107/S2052252513033939 PMID: 25075324; PubMed Central PMCID: PMC4062088.

50. Khare S, Nagle AS, Biggart A, Lai YH, Liang F, Davis LC, et al. Proteasome inhibition for treatment of leishmaniasis, Chagas disease and sleeping sickness. Nature. 2016; 537(7619):229–33. Epub 2016 Aug 9. https://doi.org/10.1038/nature19339 PMID: 27501246; PubMed Central PMCID: PMC5161665.

51. Wyllie S, Brand S, Thomas M, De Rycker M, Chung CW, Pena I, et al. Preclinical candidate for the treatment of visceral leishmaniasis that acts through proteasome inhibition. Proc Natl Acad Sci U S A. 2019; 116(19):9318–23. Epub 2019 Apr 10. https://doi.org/10.1073/pnas.1820175116 PMID: 30962368; PubMed Central PMCID: PMC6511062.

52. Wyllie S, Thomas M, Patterson S, Crouch S, De Rycker M, Lowe R, et al. Cyclin-dependent kinase 12 is a drug target for visceral leishmaniasis. Nature. 2018; 560(7717):192–7. Epub 2018 Jul 27. https://doi.org/10.1038/s41586-018-0356-z PMID: 30046105; PubMed Central PMCID: PMC6402543.

53. Saldivia M, Fang E, Ma X, Myburgh E, Carnielli JBT, Bower-Lepts C, et al. Targeting the trypanosome kinetochore with CLK1 protein kinase inhibitors. Nat Microbiol. 2020; 5(10):1207–16. Epub 2020 Jul 15. https://doi.org/10.1038/s41564-020-0745-6 PMID: 32661312; PubMed Central PMCID: PMC7610364.

54. Sanchez-Valdez FJ, Padilla A, Wang W, Orr D, Tarleton RL. Spontaneous dormancy protects Trypa-nosoma cruzi during extended drug exposure. elife. 2018;7. Epub 2018 Mar 27. https://doi.org/10.7554/eLife.34039 PMID: 29578409; PubMed Central PMCID: PMC5906098.

55. Peters NC, Egen JG, Secundino N, Debrabant A, Kimblin N, Kamhawi S, et al. *In vivo* imaging reveals an essential role for neutrophils in leishmaniasis transmitted by sand flies. Science. 2008; 321 (5891):970–4. Epub 2008 Aug 16. https://doi.org/10.1126/science.1159194 PMID: 18703742; PubMed Central PMCID: PMC2606057.

56. Guimaraes-Costa AB, Nascimento MT, Froment GS, Soares RP, Morgado FN, Conceicao-Silva F, et al. *Leishmania amazonensis* promastigotes induce and are killed by neutrophil extracellular traps. Proc Natl Acad Sci U S A. 2009; 106(16):6748–53. Epub 2009 Apr 7. https://doi.org/10.1073/pnas. 0900226106 PMID: 19346483; PubMed Central PMCID: PMC2672475.

57. Leon B, Lopez-Bravo M, Ardavin C. Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against *Leishmania*. Immunity. 2007; 26(4):519–31. Epub 2007 Apr 7. https://doi.org/10.1016/j.immuni.2007.01.017 PMID: 17412618.

58. Ives A, Ronet C, Prevel F, Ruzzante G, Fuertes-Marraco S, Schutz F, et al. *Leishmania* RNA virus controls the severity of mucocutaneous leishmaniasis. Science. 2011; 331(6018):775–8. Epub 2011 Feb 12. https://doi.org/10.1126/science.1199326 PMID: 21311023; PubMed Central PMCID: PMC3253482.

59. Vercesi AE, Moreno SN, Docampo R. Ca2+/H+ exchange in acidic vacuoles of *Trypanosoma brucei*. Biochem J. 1994; 304(Pt 1):227–33. Epub 1994 Nov 15. https://doi.org/10.1042/bj3040227 PubMed Central PMCID: PMC1137476. PMID: 7998937

60. Rojas F, Silvester E, Young J, Milne R, Tettey M, Houston DR, et al. Oligopeptide signaling through TbGPR89 drives trypanosome quorum sensing. Cell. 2019; 176(1–2):306–17 e16. Epub 2018 Dec 7. https://doi.org/10.1016/j.cell.2018.10.041 PMID: 30503212; PubMed Central PMCID: PMC6333907.

61. Campos MA, Almeida IC, Takeuchi O, Akira S, Valente EP, Procopio DO, et al. Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite. J Immunol. 2001; 167 (1):416–23. Epub 2001 Jun 22. https://doi.org/10.4049/jimmunol.167.1.416 PMID: 11418678.

62. Rai A, Pathak D, Thakur S, Singh S, Dubey AK, Mallik R. Dynein clusters into lipid microdomains on phagosomes to drive rapid transport toward lysosomes. Cell. 2016; 164(4):722–34. Epub 2016 Feb 9. https://doi.org/10.1016/j.cell.2015.12.054 PMID: 26853472; PubMed Central PMCID: PMC4752818.

63. de Carvalho RVH, Andrade WA, Lima-Junior DS, Dilucca M, de Oliveira CV, Wang K, et al. Leishmania lipophosphoglycan triggers caspase-11 and the non-canonical activation of the NLRP3 inflammasome. Cell Rep. 2019; 26(2):429–37 e5. Epub 2019 Jan 10. https://doi.org/10.1016/j.celrep.2018. 12.047 PMID: 30625325; PubMed Central PMCID: PMC8022207.

64. Bermejo DA, Jackson SW, Gorosito-Serran M, Acosta-Rodriguez EV, Amezcua-Vesely MC, Sather BD, et al. *Trypanosoma cruzi t*rans-sialidase initiates a program independent of the transcription factors RORgammat and Ahr that leads to IL-17 production by activated B cells. Nat Immunol. 2013; 14 (5):514–22. Epub 2013/04/09. https://doi.org/10.1038/ni.2569 PMID: 23563688; PubMed Central PMCID: PMC3631452.

65. Trindade S, Rijo-Ferreira F, Carvalho T, Pinto-Neves D, Guegan F, Aresta-Branco F, et al. *Trypanosoma brucei* parasites occupy and functionally adapt to the adipose tissue in mice. Cell Host Microbe. 2016; 19(6):837–48. Epub 2016 May 31. https://doi.org/10.1016/j.chom.2016.05.002 PMID: 27237364; PubMed Central PMCID: PMC4906371.

66. Capewell P, Cren-Travaille C, Marchesi F, Johnston P, Clucas C, Benson RA, et al. The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes. elife. 2016;5. Epub 2016 Sep 23. https://doi.org/10.7554/eLife.17716 PMID: 27653219; PubMed Central PMCID: PMC5065312.

67. Engstler M, Pfohl T, Herminghaus S, Boshart M, Wiegertjes G, Heddergott N, et al. Hydrodynamic flow-mediated protein sorting on the cell surface of trypanosomes. Cell. 2007; 131(3):505–15. Epub 2007 Nov 6. https://doi.org/10.1016/j.cell.2007.08.046 PMID: 17981118.

68. Faria J, Luzak V, Muller LSM, Brink BG, Hutchinson S, Glover L, et al. Spatial integration of transcription and splicing in a dedicated compartment sustains monogenic antigen expression in African trypanosomes. Nat Microbiol. 2021; 6(3):289–300. Epub 2021 Jan 13. https://doi.org/10.1038/s41564-020-00833-4 PMID: 33432154; PubMed Central PMCID: PMC7610597.

69. Vanhamme L, Paturiaux-Hanocq F, Poelvoorde P, Nolan DP, Lins L, Van Den Abbeele J, et al. Apolipoprotein L-I is the trypanosome lytic factor of human serum. Nature. 2003; 422(6927):83–7. Epub 2003 Mar 7. https://doi.org/10.1038/nature01461 PMID: 12621437.

70. Thomson R, Genovese G, Canon C, Kovacsics D, Higgins MK, Carrington M, et al. Evolution of the primate trypanolytic factor APOL1. Proc Natl Acad Sci U S A. 2014; 111(20):E2130–9. Epub 2014 May 9. https://doi.org/10.1073/pnas.1400699111 PMID: 24808134; PubMed Central PMCID: PMC4034216.

71. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science. 2010; 329(5993):841–5.

Epub 2010 Jul 22. https://doi.org/10.1126/science.1193032 PMID: 20647424; PubMed Central PMCID: PMC2980843.

72. Goncalves MF, Umezawa ES, Katzin AM, de Souza W, Alves MJ, Zingales B, et al. *Trypanosoma cruzi*: shedding of surface antigens as membrane vesicles. Exp Parasitol. 1991; 72(1):43–53. Epub 1991 Jan 1. https://doi.org/10.1016/0014-4894(91)90119-h PMID: 1993464

73. Silverman JM, Clos J, de'Oliveira CC, Shirvani O, Fang Y, Wang C, et al. An exosome-based secretion pathway is responsible for protein export from *Leishmania* and communication with macrophages. J Cell Sci. 2010; 123(Pt 6):842–52. Epub 2010 Feb 18. https://doi.org/10.1242/jcs.056465 PMID: 20159964.

74. Atayde VD, 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):957–67. Epub 2015 Nov 14. https://doi.org/10.1016/j.celrep.2015.09.058 PMID: 26565909; PubMed Central PMCID: PMC4644496.

75. Szempruch AJ, Sykes SE, Kieft R, Dennison L, Becker AC, Gartrell A, et al. Extracellular vesicles from *Trypanosoma brucei* mediate virulence factor transfer and cause host anemia. Cell. 2016; 164(1–2):246–57. Epub 2016 Jan 16. https://doi.org/10.1016/j.cell.2015.11.051 PMID: 26771494; PubMed Central PMCID: PMC4715261.

76. Autheman D, Crosnier C, Clare S, Goulding DA, Brandt C, Harcourt K, et al. An invariant *Trypanosoma vivax* vaccine antigen induces protective immunity. Nature. 2021; 595(7865):96–100. Epub 2021 May 28. https://doi.org/10.1038/s41586-021-03597-x PMID: 34040257.

77. Khatoon N, Pandey RK, Prajapati VK. Exploring *Leishmania* secretory proteins to design B and T cell multi-epitope subunit vaccine using immunoinformatics approach. Sci Rep. 2017; 7(1):8285. Epub 2017 Aug 16. https://doi.org/10.1038/s41598-017-08842-w PMID: 28811600; PubMed Central PMCID: PMC5557753.

78. Afonso LC, Scharton TM, Vieira LQ, Wysocka M, Trinchieri G, Scott P. The adjuvant effect of interleukin-12 in a vaccine against *Leishmania major*. Science. 1994; 263(5144):235–7. Epub 1994 Jan 14. https://doi.org/10.1126/science.7904381 PMID: 7904381

79. Darrah PA, Patel DT, De Luca PM, Lindsay RW, Davey DF, Flynn BJ, et al. Multifunctional TH1 cells define a correlate of vaccine-mediated protection against *Leishmania major*. Nat Med. 2007; 13 (7):843–50. Epub 2007 Jun 15. https://doi.org/10.1038/nm1592 PMID: 17558415.

80. Zhang WW, Karmakar S, Gannavaram S, Dey R, Lypaczewski P, Ismail N, et al. A second generation leishmanization vaccine with a markerless attenuated *Leishmania major* strain using CRISPR gene editing. Nat Commun. 2020; 11(1):3461. Epub 2020 Jul 12. https://doi.org/10.1038/s41467-020-17154-z PMID: 32651371; PubMed Central PMCID: PMC7351751.

81. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4+CD25+ regulatory T cells control *Leishmania major* persistence and immunity. Nature. 2002; 420(6915):502–7. Epub 2002 Dec 6. https://doi.org/10.1038/nature01152 PMID: 12466842.

82. Glennie ND, Yeramilli VA, Beiting DP, Volk SW, Weaver CT, Scott P. Skin-resident memory CD4+ T cells enhance protection against *Leishmania major* infection. J Exp Med. 2015; 212(9):1405–14. Epub 2015 Jul 29. https://doi.org/10.1084/jem.20142101 PMID: 26216123; PubMed Central PMCID: PMC4548053.

83. Powrie F, Correa-Oliveira R, Mauze S, Coffman RL. Regulatory interactions between CD45RBhigh and CD45RBlow CD4+ T cells are important for the balance between protective and pathogenic cell-mediated immunity. J Exp Med. 1994; 179(2):589–600. Epub 1994 Feb 1. https://doi.org/10.1084/jem.179.2.589 PMID: 7905019; PubMed Central PMCID: PMC2191378.

84. Heinzel FP, Schoenhaut DS, Rerko RM, Rosser LE, Gately MK. Recombinant interleukin 12 cures mice infected with *Leishmania major*. J Exp Med. 1993; 177(5):1505–9. Epub 1993 May 1. https://doi.org/10.1084/jem.177.5.1505 PMID: 8097524; PubMed Central PMCID: PMC2191017.

85. Sypek JP, Chung CL, Mayor SE, Subramanyam JM, Goldman SJ, Sieburth DS, et al. Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. J Exp Med. 1993; 177(6):1797–802. Epub 1993 Jun 1. https://doi.org/10.1084/jem.177.6.1797 PubMed Central PMCID: PMC2191036. PMID: 8098733

86. Anderson CF, Oukka M, Kuchroo VJ, Sacks D. CD4(+)CD25(−)Foxp3(−) Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. J Exp Med. 2007; 204 (2):285–97. Epub 2007 Feb 7. https://doi.org/10.1084/jem.20061886 PMID: 17283207; PubMed Central PMCID: PMC2118728.

87. Belkaid Y, Hoffmann KF, Mendez S, Kamhawi S, Udey MC, Wynn TA, et al. The role of interleukin (IL)-10 in the persistence of Leishmania major in the skin after healing and the therapeutic potential of anti-IL-10 receptor antibody for sterile cure. J Exp Med. 2001; 194(10):1497–506. Epub 2001 Nov 21. https://doi.org/10.1084/jem.194.10.1497 PMID: 11714756; PubMed Central PMCID: PMC2193677.

88. Jantsch J, Schatz V, Friedrich D, Schroder A, Kopp C, Siegert I, et al. Cutaneous Na+ storage strengthens the antimicrobial barrier function of the skin and boosts macrophage-driven host defense. Cell Metab. 2015; 21(3):493–501. Epub 2015 Mar 5. https://doi.org/10.1016/j.cmet.2015.02.003 PMID: 25738463; PubMed Central PMCID: PMC4350016.

89. Lima-Junior DS, Costa DL, Carregaro V, Cunha LD, Silva AL, Mineo TW, et al. Inflammasome-derived IL-1beta production induces nitric oxide-mediated resistance to *Leishmania*. Nat Med. 2013; 19 (7):909–15. Epub 2013 Jun 12. https://doi.org/10.1038/nm.3221 PMID: 23749230.

90. Wei XQ, Charles IG, Smith A, Ure J, Feng GJ, Huang FP, et al. Altered immune responses in mice lacking inducible nitric oxide synthase. Nature. 1995; 375(6530):408–11. Epub 1995 Jun 1. https://doi.org/10.1038/375408a0 PMID: 7539113.

91. Akopyants NS, Kimblin N, Secundino N, Patrick R, Peters N, Lawyer P, et al. Demonstration of genetic exchange during cyclical development of *Leishmania* in the sand fly vector. Science. 2009; 324 (5924):265–8. Epub 2009 Apr 11. https://doi.org/10.1126/science.1169464 PMID: 19359589; PubMed Central PMCID: PMC2729066.

92. Berriman M, Ghedin E, Hertz-Fowler C, Blandin G, Renauld H, Bartholomeu DC, et al. The genome of the African trypanosome *Trypanosoma brucei*. Science. 2005; 309(5733):416–22. Epub 2005 Jul 16. https://doi.org/10.1126/science.1112642 PMID: 16020726.

93. El-Sayed NM, Myler PJ, Bartholomeu DC, Nilsson D, Aggarwal G, Tran AN, et al. The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. Science. 2005; 309(5733):409– 15. Epub 2005 Jul 16. https://doi.org/10.1126/science.1112631 PMID: 16020725.

94. El-Sayed NM, Myler PJ, Blandin G, Berriman M, Crabtree J, Aggarwal G, et al. Comparative genomics of trypanosomatid parasitic protozoa. Science. 2005; 309(5733):404–9. Epub 2005 Jul 16. https://doi.org/10.1126/science.1112181 PMID: 16020724.

95. Ivens AC, Peacock CS, Worthey EA, Murphy L, Aggarwal G, Berriman M, et al. The genome of the kinetoplastid parasite, *Leishmania major*. Science. 2005; 309(5733):436–42. Epub 2005 Jul 16. https://doi.org/10.1126/science.1112680 PMID: 16020728; PubMed Central PMCID: PMC1470643.

96. Peacock CS, Seeger K, Harris D, Murphy L, Ruiz JC, Quail MA, et al. Comparative genomic analysis of three *Leishmania* species that cause diverse human disease. Nat Genet. 2007; 39(7):839–47. Epub 2007 Jun 19. https://doi.org/10.1038/ng2053 PMID: 17572675; PubMed Central PMCID: PMC2592530.

97. Rogers MB, Hilley JD, Dickens NJ, Wilkes J, Bates PA, Depledge DP, et al. Chromosome and gene copy number variation allow major structural change between species and strains of *Leishmania*. Genome Res. 2011; 21(12):2129–42. Epub 2011 Nov 1. https://doi.org/10.1101/gr.122945.111 PMID: 22038252; PubMed Central PMCID: PMC3227102.

98. Myler PJ, Audleman L, de Vos T, Hixson G, Kiser P, Lemley C, et al. Leishmania major Friedlin chromosome 1 has an unusual distribution of protein-coding genes. Proc Natl Acad Sci U S A. 1999; 96 (6):2902–6. Epub 1999 Mar 17. https://doi.org/10.1073/pnas.96.6.2902 PMID: 10077609; PubMed Central PMCID: PMC15867.

99. Alsford S, Turner DJ, Obado SO, Sanchez-Flores A, Glover L, Berriman M, et al. High-throughput phenotyping using parallel sequencing of RNA interference targets in the African trypanosome. Genome Res. 2011; 21(6):915–24. Epub 2011 Mar 3. https://doi.org/10.1101/gr.115089.110 PMID: 21363968; PubMed Central PMCID: PMC3106324.

100. Siegel TN, Hekstra DR, Wang X, Dewell S, Cross GA. Genome-wide analysis of mRNA abundance in two life-cycle stages of *Trypanosoma brucei* and identification of splicing and polyadenylation sites. Nucleic Acids Res. 2010; 38(15):4946–57. Epub 2010 Apr 14. https://doi.org/10.1093/nar/gkq237 PMID: 20385579; PubMed Central PMCID: PMC2926603.

101. Vigneron A, O'Neill MB, Weiss BL, Savage AF, Campbell OC, Kamhawi S, et al. Single-cell RNA sequencing of *Trypanosoma brucei* from tsetse salivary glands unveils metacyclogenesis and identifies potential transmission blocking antigens. Proc Natl Acad Sci U S A. 2020; 117(5):2613–21. Epub 2020 Jan 23. https://doi.org/10.1073/pnas.1914423117 PMID: 31964820; PubMed Central PMCID: PMC7007551.

102. Siegel TN, Hekstra DR, Kemp LE, Figueiredo LM, Lowell JE, Fenyo D, et al. Four histone variants mark the boundaries of polycistronic transcription units in *Trypanosoma brucei*. Genes Dev. 2009; 23 (9):1063–76. Epub 2009 Apr 17. https://doi.org/10.1101/gad.1790409 PMID: 19369410; PubMed Central PMCID: PMC2682952.

103. Fricker R, Brogli R, Luidalepp H, Wyss L, Fasnacht M, Joss O, et al. A tRNA half modulates translation as stress response in Trypanosoma brucei. Nat Commun. 2019; 10(1):118. Epub 2019 Jan 12. https://doi.org/10.1038/s41467-018-07949-6 PMID: 30631057; PubMed Central PMCID: PMC6328589.

104. Atwood JA 3rd, Weatherly DB, Minning TA, Bundy B, Cavola C, Opperdoes FR, et al. The *Trypanosoma cruzi* proteome. Science. 2005; 309(5733):473–6. Epub 2005 Jul 16. https://doi.org/10.1126/science.1110289 PMID: 16020736.

105. Broadhead R, Dawe HR, Farr H, Griffiths S, Hart SR, Portman N, et al. Flagellar motility is required for the viability of the bloodstream trypanosome. Nature. 2006; 440(7081):224–7. Epub 2006 Mar 10. https://doi.org/10.1038/nature04541 PMID: 16525475.

106. Creek DJ, Jankevics A, Breitling R, Watson DG, Barrett MP, Burgess KE. Toward global metabolomics analysis with hydrophilic interaction liquid chromatography-mass spectrometry: improved metabolite identification by retention time prediction. Anal Chem. 2011; 83(22):8703–10. Epub 2011 Sep 21. https://doi.org/10.1021/ac2021823 PMID: 21928819.

107. Russo PST, Ferreira GR, Cardozo LE, Burger MC, Arias-Carrasco R, Maruyama SR, et al. CEMiTool: a Bioconductor package for performing comprehensive modular co-expression analyses. BMC Bioinformatics. 2018; 19(1):56. Epub 2018 Feb 21. https://doi.org/10.1186/s12859-018-2053-1 PMID: 29458351; PubMed Central PMCID: PMC5819234.

108. Aslett M, Aurrecoechea C, Berriman M, Brestelli J, Brunk BP, Carrington M, et al. TriTrypDB: a functional genomic resource for the Trypanosomatidae. Nucleic Acids Res. 2010; 38(Database issue): D457–62. Epub 2009 Oct 22. https://doi.org/10.1093/nar/gkp851 PMID: 19843604; PubMed Central PMCID: PMC2808979.

109. Wirtz E, Leal S, Ochatt C, Cross GA. A tightly regulated inducible expression system for conditional gene knock-outs and dominant-negative genetics in *Trypanosoma brucei*. Mol Biochem Parasitol. 1999; 99(1):89–101. Epub 1999 Apr 24. https://doi.org/10.1016/s0166-6851(99)00002-x PMID: 10215027.

110. Ngo H, Tschudi C, Gull K, Ullu E. Double-stranded RNA induces mRNA degradation in *Trypanosoma brucei*. Proc Natl Acad Sci U S A. 1998; 95(25):14687–92. Epub 1998 Dec 9. https://doi.org/10.1073/pnas.95.25.14687 PubMed Central PMCID: PMC24510. PMID: 9843950

111. Wang Z, Morris JC, Drew ME, Englund PT. Inhibition of *Trypanosoma brucei* gene expression by RNA interference using an integratable vector with opposing T7 promoters. J Biol Chem. 2000; 275 (51):40174–9. Epub 2000 Oct 3. https://doi.org/10.1074/jbc.M008405200 PMID: 11013266.

112. Beneke T, Madden R, Makin L, Valli J, Sunter J, Gluenz E. A CRISPR Cas9 high-throughput genome editing toolkit for kinetoplastids. R Soc Open Sci. 2017; 4(5):170095. Epub 2017 Jun 3. https://doi.org/10.1098/rsos.170095 PMID: 28573017; PubMed Central PMCID: PMC5451818.

113. Peng D, Kurup SP, Yao PY, Minning TA, Tarleton RL. CRISPR-Cas9-mediated single-gene and gene family disruption in *Trypanosoma cruzi*. mBio. 2014; 6(1):e02097–14. Epub 2015 Jan 1. https://doi.org/10.1128/mBio.02097-14 PMID: 25550322; PubMed Central PMCID: PMC4281920.

114. Beneke T, Demay F, Hookway E, Ashman N, Jeffery H, Smith J, et al. Genetic dissection of a *Leishmania* flagellar proteome demonstrates requirement for directional motility in sand fly infections. PLoS Pathog. 2019; 15(6):e1007828. Epub 2019 Jun 27. https://doi.org/10.1371/journal.ppat.1007828 PMID: 31242261; PubMed Central PMCID: PMC6615630.

115. Jaskolowski M, Ramrath DJF, Bieri P, Niemann M, Mattei S, Calderaro S, et al. Structural insights into the mechanism of mitoribosomal large subunit biogenesis. Mol Cell. 2020; 79(4):629–44 e4. Epub 2020 Jul 18. https://doi.org/10.1016/j.molcel.2020.06.030 PMID: 32679035.

116. Durrant JD, Votapka L, Sorensen J, Amaro RE. POVME 2.0: An enhanced tool for determining pocket shape and volume characteristics. J Chem Theory Comput. 2014; 10(11):5047–56. Epub 2014 Nov 18. https://doi.org/10.1021/ct500381c PMID: 25400521; PubMed Central PMCID: PMC4230373.

117. Akiyoshi B, Gull K. Discovery of unconventional kinetochores in kinetoplastids. Cell. 2014; 156 (6):1247–58. Epub 2014 Mar 4. https://doi.org/10.1016/j.cell.2014.01.049 PMID: 24582333; PubMed Central PMCID: PMC3978658.

118. Imboden MA, Laird PW, Affolter M, Seebeck T. Transcription of the intergenic regions of the tubulin gene cluster of *Trypanosoma brucei*: evidence for a polycistronic transcription unit in a eukaryote. Nucleic Acids Res. 1987; 15(18):7357–68. Epub 1987 Sep 25. https://doi.org/10.1093/nar/15.18.7357 PMID: 3658696; PubMed Central PMCID: PMC306253.

119. Opperdoes FR, Borst P. Localization of nine glycolytic enzymes in a microbody-like organelle in *Trypanosoma brucei*: the glycosome. FEBS Lett. 1977; 80(2):360–4. Epub 1977 Aug 15. https://doi.org/10.1016/0014-5793(77)80476-6 PMID: 142663.

120. Ferguson MA, Duszenko M, Lamont GS, Overath P, Cross GA. Biosynthesis of *Trypanosoma brucei* variant surface glycoproteins. N-glycosylation and addition of a phosphatidylinositol membrane anchor. J Biol Chem. 1986; 261(1):356–62. Epub 1986 Jan 5. PMID: 2934386.

121. Benne R, Van den Burg J, Brakenhoff JP, Sloof P, Van Boom JH, Tromp MC. Major transcript of the frameshifted coxII gene from trypanosome mitochondria contains four nucleotides that are not

encoded in the DNA. Cell. 1986; 46(6):819–26. Epub 1986 Sep 12. https://doi.org/10.1016/0092-8674(86)90063-2 PMID: 3019552.

122. De Lange T, Michels PA, Veerman HJ, Cornelissen AW, Borst P. Many trypanosome messenger RNAs share a common 5′ terminal sequence. Nucleic Acids Res. 1984; 12(9):3777–90. Epub 1984 May 11. https://doi.org/10.1093/nar/12.9.3777 PMID: 6547230; PubMed Central PMCID: PMC318789.

123. Gommers-Ampt JH, Van Leeuwen F, de Beer AL, Vliegenthart JF, Dizdaroglu M, Kowalak JA, et al. beta-D-glucosyl-hydroxymethyluracil: a novel modified base present in the DNA of the parasitic protozoan *T. brucei*. Cell. 1993; 75(6):1129–36. Epub 1993 Dec 17. https://doi.org/10.1016/0092-8674(93)90322-h PMID: 8261512.

124. Coutton C, Vargas AS, Amiri-Yekta A, Kherraf ZE, Ben Mustapha SF, Le Tanno P, et al. Mutations in CFAP43 and CFAP44 cause male infertility and flagellum defects in *Trypanosoma* and human. Nat Commun. 2018; 9(1):686. Epub 2018 Feb 17. https://doi.org/10.1038/s41467-017-02792-7 PMID: 29449551; PubMed Central PMCID: PMC5814398.