INRS- Armand-Frappier Santé Biotechnologie

INVESTIGATING THE ANTILEISHMANIAL ACTIVITY OF ROCAGLATES WITH LOW OR NO BINDING ACTIVITY FOR THE RNA HELICASE eIF4A

Par

Camila Almeida Cardoso

Mémoire présenté(e) pour l'obtention du grade de

Maître ès Sciences (M.Sc.)

en virologie et immunologie

Jury d'évaluation

Président du jury et examinateur interne	Ian Gaël Rodrigue-Gervais INRS- Armand-Frappier Santé Biotechnologie
Examinateur externe	Christopher Fernandez Prada Université de Montréal
Directeur de recherche	Maritza Jaramillo INRS- Armand-Frappier Santé Biotechnologie

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my advisor, Pr. Maritza Jaramillo, for her unwavering support, insightful guidance, and continuous encouragement throughout my research journey. Her expertise and dedication were instrumental in shaping this project path. Under the guidance of the Jaramillo Lab, I was able to grow and learn valuable lessons for both life and academy.

The development of this project and thesis required my own effort but also the help and guidance of the current and pass member of our group: Dr. Louis-Philippe Leroux, Dr. Visnu Chaparro, Dr. Andres Felipe Diez, MSc. Laura Garcia, MSc. Carolina Vargas, Aurore Lebourg and especially Leonardo Cortazzo da Silva. Leo, thank you for your support as my lab mate. I am also very grateful for your invaluable friendship and emotional support.

This journey has been about more than just earning a master's degree, it has also been about moving to a new country and being away from my culture and loved ones in Brazil. I could not have made it through without the incredible friends who welcomed me with open arms from the moment I arrived. A special thanks to Leo, Rummi, Thais, and Cami, without you, this journey would have been completely different. Thank you for your advice, laughs, support, and affection.

To my family and friends in Brazil, thank you for supporting me from afar. I am especially grateful to my aunts and my dad for believing in me and standing by me throughout this period. My dear friends Isa and Thais, who have been with me since our undergrad days and shared this master's experience with me despite the distance, thank you for your constant support and for always being there for me.

And last but not least, to my soul siblings, Axell, Bia, Felipe, and Thais: just like in every other phase of my life, this mission would have been impossible without you by my side. Every video call and message helped me stay strong and focused during this time away from everything and everyone I knew.

RÉSUMÉ

Les parasites du genre *Leishmania* peuvent causer diverses formes de leishmaniose incluant des formes mortelles si non-traitées. L'absence de vaccins efficaces et la résistance croissante aux médicaments rendent la recherche de nouvelles cibles thérapeutiques cruciale. Les rocaglates inhibent la traduction de l'ARNm en ciblant les hélicases à ARN de type « DEAD-box » (eIF4A et DDX3X). Ici, nous avons testé une librairie de rocaglates, sélectionnant deux (C41 et C44) qui ont significativement réduit la réplication de *Leishmania amazonensis* dans les macrophages murins et humains sans affecter la viabilité de la cellule hôte aux concentrations testées. Le traitement avec C41 et C44 bloque la réplication du parasite à l'intérieur des macrophages. En revanche, nos composés n'ont pas affecté la viabilité des parasites extracellulaires, suggérant un mécanisme spécifique à l'hôte et/ou au stade du parasite. Les infections dans les macrophages dérivés de souris *Eif4A1*$^{+/-}$ et *Eif4A2*$^{+/-}$ associées à des tests de liaison *in vitro* pour C41et C44 ont montré que l'effet antiparasitaire des rocaglates les plus puissants identifiés dans notre étude est indépendant d'eIF4A et de DDX3X de la cellule hôte. Nos recherches actuelles visent à caractériser davantage la base moléculaire et les conséquences biologiques du traitement avec C41 et C44 dans les macrophages infectés par *L. amazonensis* en utilisant une approche multi-omique. Notre objectif à long terme est de fournir des informations sur le mécanisme et le potentiel thérapeutique des rocaglates contre la leishmaniose viscérale et cutanée.

Mots-clés : *Leishmania*; macrophages; rocaglates; eIF4A; DDX3X; potentiel thérapeutique; effet antiparasitaire

ABSTRACT

Leishmania parasites can cause different clinical manifestations of leishmaniasis including lethal forms if untreated. With no effective vaccines and rising drug resistance, finding new therapeutic targets is crucial. Rocaglates are known to inhibit mRNA translation by targeting DEAD-box RNA helicases (i.e., eIF4A and DDX3X). Here we tested a library of rocaglates, selecting two (i.e., C41 and C44) that significantly reduced *Leishmania amazonensis* replication in mouse bone marrow-derived macrophages (BMDMs) and a human monocytic-like cell line (THP-1) without affecting the viability of the host cell. We observed that treatment with C41 and C44 stalls parasite replication. Moreover, our compounds were not able to affect the viability of extracellular parasites, suggesting a host- and/or a parasite stage-specific mechanism. Infections in BMDMs derived from *EIf4A1*$^{+/-}$ and *EIf4A2*$^{+/-}$ mice combined with *in vitro* clamping assays for C41 and C44 indicated that the antiparasitic effect of the most potent rocaglates identified in our screening is host eIF4A- and DDX3X-independent. Our current research efforts focus on further characterizing the molecular basis and biological consequences of C41 and C44 treatment in *L. amazonensis*-infected macrophages using a multi-omics approach. Our long-term goal is to provide insight on the mechanism and therapeutic potential of rocaglates to combat visceral and cutaneous leishmaniasis.

Keywords: *Leishmania*; macrophages; rocaglates; eIF4A; DDX3X; therapeutic potential; antiparasitic effect

SOMMAIRE RÉCAPITULATIF

Les parasites protozoaires du genre *Leishmania* (ordre Kinetoplastida et famille Trypanosomatidae) sont responsables de la leishmaniose, une maladie multifacette présentant diverses manifestations cliniques (Sacks *et al.*, 2001). Désignée comme une maladie tropicale négligée, cette infection touche les populations les plus pauvres dans plus de 90 pays à travers le monde (CDC, 2020). Les différentes espèces de *Leishmania* sont responsables de diverses manifestations cliniques (Tableau 1.1), comprenant trois types principaux : la leishmaniose cutanée (LC), la leishmaniose mucocutanée (LMC) et la leishmaniose viscérale (LV). De plus, il existe deux variantes rares appelées leishmaniose cutanée diffuse (LCD) et leishmaniose dermique post-kala-azar (LDPKA) (Burza *et al.*, 2018).

Bien que généralement non mortelle, la LC se manifeste par des lésions ou des papules sur la peau au site de la piqûre de phlébotome, laissant souvent des cicatrices durables (Pareyn *et al.*, 2019). Classée comme une variante de la LC, la LCD se manifeste par des lésions cutanées étendues et persistantes similaires à celles observées dans la lèpre lépromateuse (Hashiguchi *et al.*, 2016). Enfin, parmi les principales manifestations cliniques, la LV présente le risque le plus grave et mortel si elle n'est pas traitée, entraînant des infections dans divers systèmes tels que le foie, la rate, et les systèmes hématogène et lymphatique (Burza *et al.*, 2018).

Le traitement des différentes manifestations cliniques consiste à utiliser la chimiothérapie et quelques médicaments réaffectés pour cette condition, y compris les antimoniés pentavalents, l'amphotéricine B (AmB), la pentamidine et la miltéfosine. Néanmoins, le traitement rencontre plusieurs contraintes telles que la haute toxicité des médicaments, les coûts de production, les défis d'application et notamment le taux élevé de résistance des parasite (Ponte-Sucre *et al.*, 2017). Au fil des ans, plusieurs efforts ont été faits pour développer un vaccin efficace et sûr contre la leishmaniose chez les humains ; néanmoins, il n'y a toujours pas de percées significatives dans ce domaine (Kaye *et al.*, 2021).

De plus, l'administration généralisée de médicaments a conduit à l'émergence de parasites résistants aux médicaments, possédant des mutations qui réduisent leur sensibilité au traitement (Srivastava *et al.*, 2017a). Une autre complication découle du cycle de vie complexe de *Leishmania*, dans lequel l'une de ses formes de développement, l'amastigote, réside dans les cellules immunitaires de l'hôte mammifère, ce qui rend plus difficile le ciblage des parasites avec des médicaments spécifiques (Mondelaers *et al.*, 2016; Sundar *et al.*, 2015). Une fois à l'intérieur de l'hôte mammifère, ces parasites sont capables de réguler plusieurs voies immunitaires afin de

promouvoir la réplication et la survie des amastigotes. Par exemple, une fois à l'intérieur de la vacuole parasitophore (VP), le parasite est capable d'échapper à l'environnement acide en utilisant des structures présentes dans la membrane du parasite (c'est-à-dire LPG et GP63) (Desjardins *et al.*, 1997; Matheoud *et al.*, 2013).

L'évasion et la modulation des mécanismes du système immunitaire induites par l'infection à *Leishmania* influencent directement l'activation et la différenciation des cellules T, affectant ainsi le traitement et la présentation des antigènes à ces cellules, la production de cytokines et l'expression de molécules co-stimulatrices (Kumar *et al.*, 2010; Nylén *et al.*, 2010). Différents mécanismes moléculaires sont impliqués dans la dérégulation des fonctions des cellules hôtes par *Leishmania*, y compris épigénétique, transcriptionnelle, post-transcriptionnelle, traductionnelle et post-traductionnelle (Buates *et al.*, 2001; Ivashkiv *et al.*, 2016; Liu *et al.*, 2020; Tomiotto-Pellissier *et al.*, 2018). Une meilleure compréhension de ces tactiques et des altérations induites par le parasite peut ouvrir la voie au développement de diagnostics, traitements et mesures de contrôle améliorés pour cette maladie.

Notre groupe de recherche a observé un changement dans le translatome (c.-à-d., ARNm traduits efficacement) des cellules infectées par *Leishmania* par rapport à celles non infectées (Figure 1.6A) (Chaparro *et al.*, 2020). Notamment, il y avait une augmentation de l'efficacité de la traduction d'un sous-ensemble d'ARNm sensibles à eIF4A (Figure 1.6B). Forts de cette information, nous avons entrepris d'explorer les conséquences de l'inhibition d'eIF4A et donc de perturber la traduction de ces ARNm qui, selon nos données, semblent favoriser potentiellement le parasite et son infection. Pour ce faire, nous avons utilisé les rocaglates, une classe de composés naturels et synthétiques connus pour leurs activités biologiques variées, notamment insecticides, antifongiques, anti-inflammatoires, antiprolifératives et anticancéreuses (Manier *et al.*, 2017; Pan *et al.*, 2014). Les rocaglates sont reconnus pour leur capacité à cibler eIF4A, une hélicase « DEAD-box » faisant partie du complexe eIF4F et essentielle pour dérouler les structures non-traduites de l'extrémité 5′ dans les ARNm, les rendant ainsi accessibles à la traduction (Kwan *et al.*, 2019). Cependant, les rocaglates peuvent fixer eIF4A à la structure de l'ARNm, séquestrant ainsi le complexe eIF4F et empêchant les ribosomes de se lier à l'ARNm, inhibant ainsi l'initiation de la traduction (Chen *et al.*, 2021; Chu *et al.*, 2020).

Dans nos études avec les rocaglates, nous avons pu cribler une librarie de plus de 50 composés pour trouver les meilleurs candidats avec un potentiel thérapeutique dans l'infection par *Leishmania*. Les données préliminaires générées par un autre membre de notre laboratoire ont identifié les composés C18 et C37 parmi les plus prometteurs, montrant tous deux une réduction

de plus de 50 % du nombre de parasites par cellule par rapport au contrôle DMSO (Figure 1.12). Fait intéressant, après analyse de nos données par nos collaborateurs, ils nous ont informés que bien que C37 présentait une haute affinité pour eIF4A, C18 montrait une faible affinité pour la même protéine dans des essais *in vitro*. Néanmoins, les deux composés ont montré un effet similaire dans la réduction du nombre de parasites *Leishmania* dans les cellules infectées. Avec la découverte du potentiel thérapeutique de ce sous-ensemble de composés avec une faible ou aucune affinité pour eIF4A, nous nous sommes intéressés à les caractériser davantage et à approfondir leur mécanisme d'action pendant l'infection par *Leishmania*. Par conséquent, l'hypothèse centrale de cette étude suppose que les rocaglates avec une activité de liaison minimale ou absente à eIF4A exercent leurs effets anti-*Leishmania* via une voie indépendante d'eIF4A. Pour évaluer cette hypothèse, nous avons défini deux objectifs principaux :

1. Identifier des rocaglates avec une activité de liaison faible ou nulle pour eIF4A ayant une activité anti- *Leishmania in cellulo*.

2. Étudier le mécanisme d'action de ces rocaglates avec une activité anti- *Leishmania in cellulo*.

Pour évaluer la persistance de *L. amazonensis* dans les macrophages murins primaires (« bone marrow-derived macrophages, BMDM ») pendant le traitement avec des rocaglates, nous avons effectué un dépistage en utilisant une librairie de 14 rocaglates de la collection du « Center for Molecular Discovery of Boston University, BU-CMD ». Les cellules BMDM ou des macrophages différentiés à partir de la lignée de monocytes humains THP-1 ont été inoculées avec des *parasites L. amazonensis* (MOI 10) pendant 24 heures, puis traitées avec 20 nM de rocaglates synthétiques, 15 nM de Roc-A comme contrôle positif ou un volume équivalent de DMSO pendant 72 heures (Figure 4.1). Les lames ont été colorées avec du DAPI et la quantification des parasites intracellulaires a été réalisée en calculant l'indice d'infection. Notre premier dépistage nous a permis d'identifier 7 composés ayant une faible ou aucune activité de liaison pour eIF4A, présentant une activité anti-*Leishmania* et réduisant l'indice d'infection d'au moins 50% dans les cellules infectées (Figure 4.2). L'évaluation de activité anti-*Leishmania* de 14 composés est longue et nécessite un grand nombre de cellules. Malgré ces limitations, le premier dépistage nous a permis de sélectionner les composés qui ont démontré les effets les plus prometteurs dans la plage de concentrations jugée appropriée pour des expérimentations ultérieures.

Après la présélection des composés lors du dépistage initial, notre objectif était d'éliminer les composés ou les concentrations présentant une toxicité excessive pour la cellule hôte. La viabilité des cellules hôtes a été évaluée en utilisant le test colorimétrique au resazurin, suivant le protocole établi. La toxicité des composés sélectionnés (c-à-d., 41, 44, 48, 49, 50, 53 et 54) a été

testée entre 9,75 nM et 5 µM pendant 72 heures. Selon une étude, dans les cellules immunitaires humaines primaires, la concentration cytotoxique réduisant la viabilité cellulaire de 50% (CC_{50}) était de 45,6 nM pour le Silvestrol, de 8,8 nM pour le CR-1-31-B et supérieure à 100 nM pour le Zotatifin dans les macrophages M1 (Obermann *et al.*, 2022). Dans notre investigation, nous avons considéré les concentrations comme toxiques lorsqu'elles entraînaient une réduction de 30% ou plus de la viabilité des macrophages. De manière surprenante, dans ce paramètre, les composés utilisés dans cette étude semblaient être bien tolérés par les BMDM et ceux qui présentaient une toxicité ne le faisaient que dans la gamme micromolaire (Figure 4.3) (Tableau 4.1). Dans l'ensemble, ces données suggèrent que la toxicité des rocaglates peut varier en fonction du type de cellule et de la nature du composé.

Il est important de noter la faible toxicité des rocaglates utilisés dans cette étude, surtout en comparaison avec les traitements existants pour la leishmaniose, qui présentent souvent une haute toxicité et de nombreux effets secondaires conduisant à une rechute du traitement. Par exemple, l'application de l'AmB a été associée à une néphrotoxicité et à des réactions liées à l'infusion, telles que fièvre, frissons, douleurs articulaires, nausées, vomissements et maux de tête, principalement dues à des réactions de cytokines pro-inflammatoires (Arning *et al.*, 1995; Hamill, 2013). Bien que la formulation liposomale de l'AmB atténue certains effets secondaires, son coût de production augmente considérablement par rapport à l'AmB conventionnel (Adler-Moore *et al.*, 2016; Shirzadi, 2019). Dans ce contexte, nos données soulignent l'importance de poursuivre les investigations sur ces composés et leur application en utilisant des modèles *in vivo* pour accumuler des informations supplémentaires sur les ramifications physiologiques de l'application des rocaglates.

Les parasites du genre *Leishmania* possèdent un homologue de eIF4A, nommé LeIF4A, qui a été exploré comme cible potentielle pour le développement de médicaments (Harigua-Souiai *et al.*, 2018; Koutsoni *et al.*, 2014). L'analyse des séquences de eIF4A chez divers micro-organismes a révélé une potentielle résistance des *Leishmania spp.* au traitement par rocaglates (Obermann *et al.*, 2023). Bien que nos collaborateurs aient décrit les composés utilisés dans cette étude comme ayant une faible ou aucune affinité pour eIF4A et compte tenu de l'incertitude entourant leur impact potentiel sur la forme de vie parasitaire, nous avons choisi d'évaluer l'effet des rocaglates sur les promastigotes extracellulaires de *L. amazonensis*. Semblable au test de toxicité effectué dans les BMDM, nous avons utilisé le test de viabilité au resazurin pour cette évaluation. Il est à noter que, à ce stade, nous avons également cherché à savoir si les composés pourraient cibler une molécule au sein du promastigote extracellulaire.

Langlais et al. ont observé que CR-1-31B et Silvestrol pouvaient interagir avec eIF4A de *Plasmodium falciparum* (PfeIF4A) et inhiber la synthèse protéique de cibles spécifiques (Langlais *et al.*, 2018). À notre connaissance, une seule investigation a examiné l'impact du Rocaglamide (c-à-d., rocaglate naturel) sur la croissance des parasites extracellulaires de *L. infantum*. Cette étude a démontré des concentrations efficaces à moitié maximales (EC_{50}) de 16,45 µM et 5,76 µM à 24 heures et 48 heures après le traitement, respectivement (Astelbauer *et al.*, 2011). Dans notre étude, nous n'avons observé aucun effet des composés sur la viabilité des promastigotes extracellulaires de *L. amazonensis* aux concentrations testées (allant de 9.75 nM à 5 µM) (Figure 4.4). Ce résultat suggère que la forme promastigote du parasite manque de cibles potentielles pour nos composés. Étant donné que notre objectif principal était d'exploiter les composés pour une thérapie dirigée contre l'hôte et d'évaluer leur impact sur les amastigotes intracellulaires, nous n'avons pas interprété ce résultat négativement. Au contraire, nous avons poursuivi des expériences supplémentaires pour évaluer le traitement des cellules infectées et les effets de ce traitement sur l'infectivité des amastigotes dérivés des cellules traitées.

Après trois séries d'expériences évaluant les sept composés initialement présélectionnés et leur impact sur la réplication intra-macrophagique de l'amastigote, nous avons déterminé que C41 et C44 étaient les candidats les plus prometteurs pour une investigation plus approfondie de leur mécanisme d'action (Figure 4.5). Pour obtenir une compréhension plus approfondie de l'impact des composés 41 et 44, nous avons réalisé un test dose-dépendant. Dans ce test, les BMDM ont été infectés comme décrit précédemment et exposés à des concentrations croissantes (allant de 1 nM à 20 nM) de C41 et C44 pendant 72 heures. Notre expérience de réponse en fonction de la dose a révélé que ces composés cessaient de montrer la même efficacité à réduire la réplication intra-macrophagique du parasite en dessous de la concentration de 20 nM, guidant ainsi notre sélection pour les expériences ultérieures (Figure 4.6). De plus, l'utilisation d'une concentration de 20 nM s'est avérée suffisante pour observer l'effet anti-*Leishmania* de ces composés dans les macrophages infectés différenciés à partir de la lignée cellulaire monocytique humaine THP-1 (Figure 4.7).

Les études menées avec des macrophages dérivés de monocytes humains (MdM) ont démontré que les rocaglates ayant une forte affinité pour eIF4A peuvent influencer la différenciation et la polarisation de ces cellules (Blum *et al.*, 2020; Schiffmann *et al.*, 2023). Ces études ont montré que l'inhibition d'eIF4A supprimait les réponses inflammatoires des macrophages humains M1, des cellules T et des cellules B, entraînant une réduction de la libération de cytokines par ces cellules. De plus, comme spécifié, les composés utilisés pour ce projet ont été caractérisés

comme ayant une faible ou aucune affinité pour eIF4A lors des tests de liaison *in vitro*. Par conséquent, malgré l'impact observé de nos composés sur la réplication intra-macrophagique des parasites dans les cellules THP-1, il reste essentiel d'évaluer leurs effets sur les macrophages humains primaires infectés et de caractériser la réponse immunitaire dans ces cellules.

Ensuite, l'un des aspects fondamentaux de notre étude était de déterminer si l'effet observé dans les cellules infectées dépendait d'eIF4A. Pour atteindre cet objectif, nous avons utilisé des précurseurs de moelle osseuse obtenus à partir de souris hétérozygotes *Eif4A1*$^{+/-}$ et *Eif4A2*$^{+/-}$. Ces cellules, dérivées d'animaux présentant des mutations décalant le cadre de lecture dans *Eif4a1* ou *Eif4a2*, présentent des niveaux réduits de eIF4A1 actif et une absence complète de eIF4A2 actif par rapport aux souris de type sauvage (Sénéchal *et al.*, 2021). De manière intéressante, cette expérience nous a permis d'observer que, par rapport aux BMDM de type sauvage, le contrôle DMSO des BMDM *Eif4A1*$^{+/-}$ et *Eif4A2*$^{+/-}$ présentait une infection significativement plus faible, ce qui indique l'importance d'eIF4A de l'hôte pour la survie et la réplication du parasite à l'intérieur du macrophage (Figure 4.8). De plus, contrairement à toutes les données publiées précédemment sur les rocaglates (Chu *et al.*, 2020; Iwasaki *et al.*, 2016; Wolfe *et al.*, 2014) notre étude a démontré que le sous-ensemble de rocaglates utilisé ici fonctionne via un mécanisme d'action indépendant d'eIF4A de l'hôte (Figure 4.8).

Comme nos composés ont démontré un mécanisme d'action indépendant d'eIF4A de l'hôte, nous avons choisi d'explorer d'autres cibles potentielles. En considérant DDX3X comme une cible alternative pour Roc-A (Chen *et al.*, 2021), nous avons décidé d'approfondir son investigation. DDX3X joue des rôles cruciaux dans le développement embryonnaire, et une expression réduite de DDX3X entraîne une augmentation des dommages génomiques et un arrêt du cycle cellulaire pendant l'embryogenèse (Chen *et al.*, 2016). Pour nous aider, nos collaborateurs ont effectué un test *in vitro* mesurant l'interaction entre nos composés sélectionnés et la DDX3X murine. De manière intéressante, C41 et C44 n'ont montré aucune affinité pour l'hélicase à boîte « DEAD » de l'hôte (Figure 4.9). *Leishmania* possède un homologue de DDX3X connu sous le nom de Hel67, qui est essentiel pour le métabolisme mitochondrial, la prolifération et la différenciation des parasites (Padmanabhan *et al.*, 2016; Pandey *et al.*, 2020). À ce jour, nous n'avons pas mené d'expériences impliquant Hel67. Les expériences de « docking » moléculaire suggèrent que Hel67 manque des sites de liaison nécessaires pour nos composés ; cependant, des expériences supplémentaires sont nécessaires pour déterminer si cette protéine est une cible potentielle des rocaglates C41 et C44 (laboratoire de Dr. John Porco et Dr. Lauren Brown, données non publiées).

En plus d'identifier des cibles potentielles pour les rocaglates utilsés dans cette étude, nous avons également développé un intérêt marqué pour la cinétique du traitement. Malgré nos données indiquant une absence de toxicité pour les promastigotes extracellulaires, au départ, nous n'étions pas certains si l'effet observé sur les amastigotes intra-macrophagiques résultait de l'élimination des parasites ou de l'inhibition de leur réplication. Pour continuer à découvrir le mécanisme sous-jacent de l'effet anti-*Leishmania* de C41 et C44, les cellules BMDM ont été infectées et traitées comme décrit précédemment (c-à-d., traitement après une période d'infection de 24 heures) ; cependant, contrairement aux expériences précédentes, les lames ont été collectées à 6, 12, 24, 48 et 72 heures post-traitement. Ainsi, cette expérience nous a permis de délimiter la cinétique du traitement, révélant que C41 et C44 empêchaient effectivement la réplication des parasites dès leur introduction dans la culture (Figure 4.10). Cet effet pourrait s'avérer très avantageux pour l'hôte, notamment dans le contexte de thérapies combinées. Bien que l'AmB ait montré de nombreux effets secondaires pendant le traitement, la Miltefosine contrôle efficacement l'infection mais rencontre le problème de la résistance des parasites (Croft *et al.*, 2011; Tunalı *et al.*, 2021) Dans ce contexte, nous nous demandons si l'utilisation d'un rocaglate pour arrêter la réplication des parasites, en association avec une dose réduite d'AmB, de Miltefosine ou même de SSG, pourrait faciliter l'élimination des parasites tout en atténuant les effets indésirables chez les patients traités et en combattant la résistance des parasites.

En outre, étant donné qu'aucun des rocaglates testés jusqu'à présent n'a montré d'effet sur les promastigotes extracellulaires, nous avons poursuivi notre investigation pour déterminer si ces composés pouvaient impacter l'infectivité de la forme amastigote. Pour ce faire, les BMDM ont été infectés pendant 24 heures, puis traités pendant 72 heures, selon le protocole décrit. Après le traitement, les formes amastigotes ont été isolées des cellules infectées et ils ont été utilisées pour infecter de nouveaux BMDM n'ayant subi aucun traitement (Figure 4.11). Comme indiqué dans nos résultats, C41 a montré la capacité de réduire l'infectivité des amastigotes dérivés des cellules traitées avec ce composé (Figure 4.12). Ces données ont plusieurs implications potentielles. Premièrement, elles suggèrent que C41 pourrait cibler une molécule spécifique à la forme amastigote, affectant ainsi l'infectivité de cette forme parasitaire à long terme. De plus, la réussite de l'infection du vecteur phlébotome est une étape critique pour la survie de ce parasite dans son environnement naturel et pour la continuité de son cycle de vie (Burza *et al.*, 2018). En tenant compte de cela, nous nous demandons si les amastigotes provenant de cellules traitées avec C41 subiraient un développement normal en promastigotes et une réplication au sein du vecteur phlébotome et quelles implications cela pourrait avoir sur le cycle de vie du parasite et la transmission de l'infection.

En résumé, cette étude nous a permis d'identifier des composés présentant un effet anti-*Leishmania* prometteur tout en maintenant une faible toxicité pour les cellules hôtes. Nous reconnaissons également les contraintes de notre méthodologie et visons à développer une méthode de dépistage semi-haut débit pour une évaluation plus rapide des composés. Les résultats préliminaires d'une expérience pilote de traçage des polysomes indiquent que les composés à faible affinité pour eIF4A n'inhibent pas la traduction globale (laboratoire Jaramillo , données non publiées), mais des expériences supplémentaires sont nécessaires pour évaluer si ces composés inhibent la traduction d'un sous-ensemble spécifique de transcrits. De plus, nous prévoyons d'employer une approche multi-omique pour identifier les altérations dans le translatome et le protéome du parasite et de la cellule hôte à la suite du traitement par rocaglates. En fin de compte, cette approche pourrait aider à identifier les cibles potentielles des composés. Notre quête de ces cibles va au-delà de la compréhension du mécanisme d'action de ces médicaments, elle souligne également leur importance dans le contexte de l'infection par *Leishmania*.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF EQUATIONS

LIST OF ABREVIATIONS

5'-UTR: 5′-untranslated region

AmB: Amphotericin B

AmB-D: Amphotericin B deoxycholate

APC: Antigen-presenting cell

BMDM: Bone Marrow-Derived Macrophages

BU-CMD: Boston University Center for Molecular Discovery

CC_{50}: cytotoxic concentration that reduced cell viability by 50%

CIPA: Comité Institutionnel de Protection des Animaux

CL: Cutaneous Leishmaniasis

CSF1: Colony stimulating factor 1

DC: dendritic cells

DCL: Diffuse cutaneous leishmaniasis

EC_{50}: Half-maximal effective concentrations

ELISA: Enzyme-Linked Immunosorbent Assay

FBS: Fetal Bovine Serum

FcR: Fc receptors

FOXO-1: Forkhead box protein O1

FP assay: Fluorescence polarization assay

GP63: Glycoprotein 63

IRES: Internal ribosome entry site

LCCM: L929 fibroblast-conditioned culture medium

LPG: Lipophosphoglycan

LRV: *Leishmania* RNA virus

MA: Meglumine antimonate

MCL: Mucocutaneous leishmaniasis

MIP: Macrophage inflammatory proteins

miRNA: micro RNA

MOI: Multiplicity of Infection

MSC: Mesenchymal Stem Cell

mTOR: Mechanistic target of rapamycin

NADPH: Nicotinamide adenine dinucleotide phosphate

NET: Neutrophil Extracellular Traps

NF-kB: Nuclear factor kappa B

ORF: Open reading frame

PAMPs: Pathogen-associated molecular patterns

PCR: Polymerase chain reaction

PFA: paraformaldehyde

PIC: Preinitiation complex

PKDL: Post kala-azar dermal leishmaniasis

PMA: phorbol 12-myristate 13-acetate

PPAR: Peroxisome Proliferator-Activated Receptor

PRRs: Pattern recognition receptors

PV: Parasitophorous vacuole

ROC-A: Rocaglamide A

SDS: sodium dodecyl sulfate

SLPI: Secretory leukocyte protease inhibitor

SSG: Sodium stibogluconate

STAT: Signal Transducer and Activator of Transcription

TACI: Transmembrane Activator and Calcium Modulator and Cyclophilin Ligand Interactor

TLRs: Toll-like receptors

VL: Visceral Leishmaniasis

WB: Western Blot

WT: Wild-type

1 INTRODUCTION

1.1 Leishmaniasis

1.1.1 Overview and epidemiology

Protozoan parasites from *Leishmania* spp. (order Kinetoplastida and family *Trypanosomatidae*) are responsible for causing Leishmaniasis, a multifaceted ailment exhibiting various clinical manifestations (Sacks *et al.*, 2001). Designated as a neglected tropical disease, this infection afflicts the most impoverished populations across more than 90 nations globally (CDC, 2020). Despite being underreported, estimates suggest that the incidence of cutaneous leishmaniasis (CL) ranges from 600,000 to 1 million new cases annually, with approximately 95% of these occurring in the Americas, Mediterranean basin, Middle East, and Central Asia (Figure 1.1A). The estimate number of new cases for visceral leishmaniasis (VL) is currently fewer than 100,000, with the majority of cases found in Brazil, East Africa, and India (Figure 1.1B) (WHO,2023). Poverty, population migration, malnutrition, inadequate sanitation, and immunocompromised conditions are among the primary risks associated with leishmaniasis. This disease can cause 70,000 deaths per year, in untreated patients, VL can be fatal in over 95% of cases (WHO, 2023). Additionally, CL often results in severe mutilations and facial disfigurement. Despite the seriousness of the disease and the large number of people affected globally, leishmaniasis remains without a human vaccine, and its treatment faces significant challenges, including high drug toxicity and increasing rates of parasite resistance (Ponte-Sucre *et al.*, 2017).

Due to their extensive geographical distribution *Leishmania* spp. have been divided into two categories namely, the Old World and the New World species. The Old World refers to Asia, the Middle East, Africa, and Southern Europe, while the New World comprises Mexico, Central America, South America, and the USA (Burza *et al.*, 2018). *Leishmania* parasites have been categorized into over 20 species and can be transmitted by various species of sandflies from the *Diptera* family, divided into *Phlebotomus* spp. in the Old World and *Lutzomyia* spp. in the New World (Pigott *et al.*, 2014). These invertebrate vectors are globally distributed, with tropical species completing their life cycles year-round, while those in subtropical regions do so only during warmer months. Typically nocturnal, these sandflies fly quietly, often escaping notice by their prey (Benkova *et al.*, 2007; Erguler *et al.*, 2019). The parasites from *Leishmania* spp. can be classified in two subgenres based on the development of the parasite in the digestive tract of the sandfly. Therefore, the subgenus *Leishmania* comprises parasites that develop in the midgut and

foregut, whereas the subgenus *Viannia* develops in the foregut, midgut, and hindgut (Sacks *et al.*, 2001). Although they belong to the same genus, each *Leishmania* spp. may exhibit distinct geographic preferences, host factors, and clinical characteristics. For instance, in South Asia and East Africa, *L. donovoni* is known to cause VL, affecting younger individuals while sparing older hosts due to acquired immunity (Elnaiem, 2011). Conversely, VL can also be attributed to *L. infantum*, commonly found in South America (*L. Infantum (chagasi)*), Mediterranean basin, West and Central Asia (Serafim *et al.*, 2020). Besides primate hosts, the parasite has been documented to infect various other hosts such as rodents, opossums, sloths, anteaters, dogs, and cats. Among these, cats have been found to be infected with *L. infantum*, although additional research is needed to confirm their role as a reservoir (Roque *et al.*, 2014). Dogs remain one of the main reservoirs in urban and rural areas, which contributes to the prevalence of VL caused by *L. infantum* and *L. infantum (chagasi)* (Ben Slimane *et al.*, 2014).

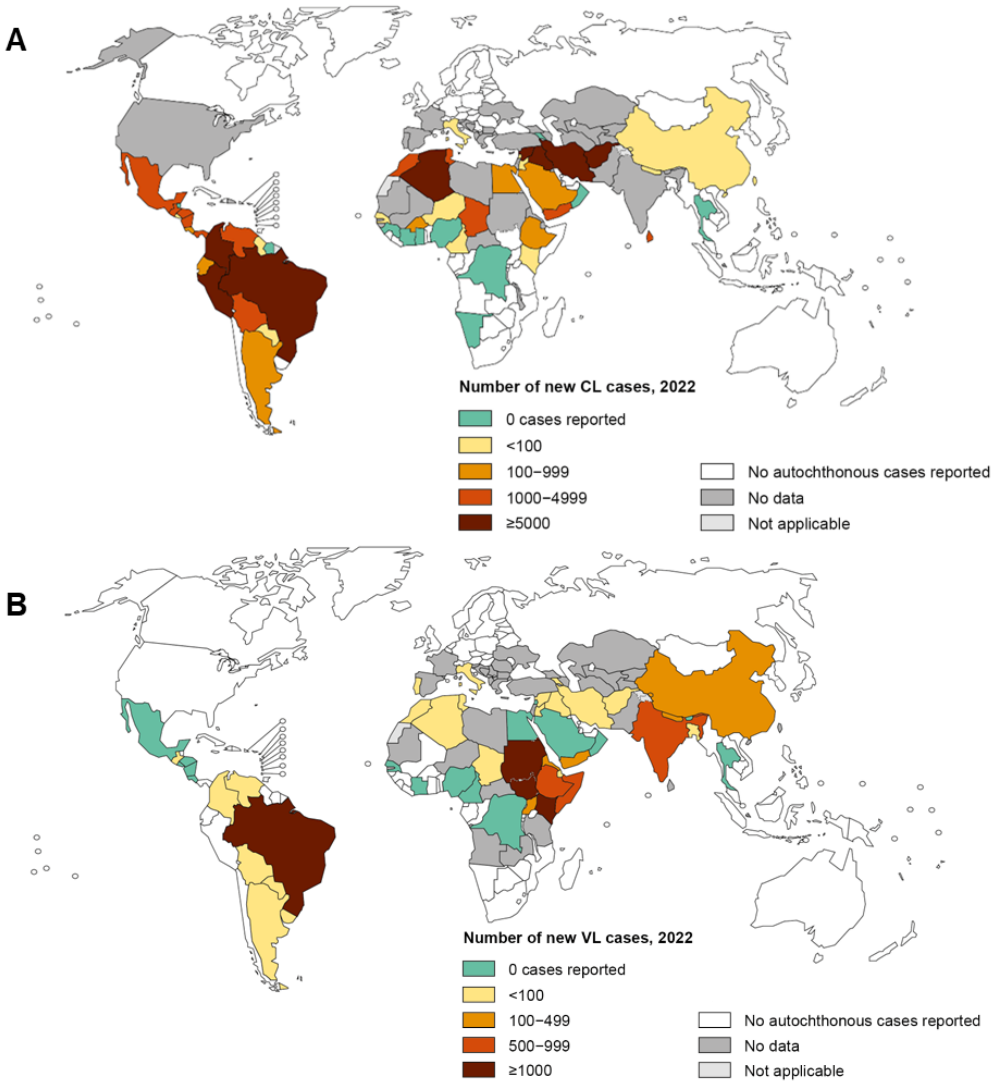


Figure 1.1 WHO maps illustrating the distribution of CL and VL

Status of endemicity of cutaneous leishmaniasis (A) and visceral leishmaniasis (B) worldwide in 2022. Adapted from WHO, 2022

1.1.2 Clinical manifestations of leishmaniasis

As mentioned earlier, the different species of *Leishmania* are accountable for inducing a range of clinical manifestations (Table 1.1), encompassing three main types: CL, mucocutaneous leishmaniasis (MCL), and VL. Additionally, there are two uncommon variants referred to as diffuse cutaneous leishmaniasis (DCL) and post-kala-azar dermal leishmaniasis (PKDL) (Burza *et al.*, 2018).

Table 1.1 Clinical manifestations and species

Clinical Manifestation	Old World species	New World species
Cutaneous leishmaniasis	*L. tropica, L. major, L. aethiopica, L. infantum, L. donovani*	*L. mexicana* species complex, *L. mexicana, L. amazonensis, L. venezuelensis, L. Vianna (V) subgenus, L. (V) braziliensis, L. (V) panamensis, L. (V) guyanesis, L. (V) peruviana, L. major like organisms, L. chagasi*
Mucocutaneous leishmaniasis	*L. Tropica; L. Major; L. Donovani; L. infantum*	*L. Vianna (V) subgenus, L. (V) brazilensis, L. (V) panamensis, L. (V) guyanesis, L. (V) peruviana, L. amazonensis*
Diffuse cutaneous leishmaniasis	*L. aethiopica*	*L. mexicana, L. amazonensis, L. panamensis* (rarely)
Visceral leishmaniasis	*L. donovoni, L. infantum, L. tropica*	*L. chagasi* (identical species to L. infantum but in New World), *L. amazonensis*
Post kala-azar dermal leishmaniasis	*L. donovoni, L. infantum*	*L. chagasi* (identical species to L. infantum)

Adapted from (Mann *et al.*, 2021)

Cutaneous Leishmaniasis

Although not typically life-threatening, CL manifests as lesions or papules on the skin at the site of the sandfly bite, often leaving lasting scars (Pareyn *et al.*, 2019). This form of leishmaniasis brings about disfigurement and social stigma that endure even after treatment. DCL, classified as a variant of CL, manifests in widespread and persistent skin lesions similar to those observed in lepromatous leprosy (Hashiguchi *et al.*, 2016). Certain species, such as *L. aethiopica*, can induce lesions that take years to heal and may progress to MCL, affecting the oral-nasal and pharyngeal regions, causing substantial damage (David *et al.*, 2009). This variety frequently requires facial mutilation, resulting in facial disfigurement and requiring painful surgical interventions (Burza *et al.*, 2018).

Visceral Leishmaniasis

Among the primary clinical manifestations, VL poses the most severe and life-threatening risk if left untreated, leading to infections in various systems such as the liver, spleen, hematogenous, and lymphatic systems (Burza *et al.*, 2018). In immunocompromised individuals, such as those with HIV, VL presents as an opportunistic infection, with the highest rates worldwide observed in Europe (Oryan *et al.*, 2016; Riera *et al.*, 2004). The PKDL is a rare occurrence where VL reemerges weeks or even years after the initial infection, resulting in dermal lesions presenting as macules, nodules, or papules (Burza *et al.*, 2018).

1.1.3 Diagnosis

Various clinical manifestations can be diagnosed either directly through parasitological testing or indirectly via immunological methods. In recent years, polymerase chain reaction (PCR) technology has facilitated the identification and distinguishing of *Leishmania* spp., becoming extensively employed for diagnosing leishmaniasis and contributing significantly to epidemiological data (Derghal *et al.*, 2022). While PCR-based techniques offer higher sensitivity compared to culture or microscopy, they are often lacking standardization and are primarily accessible in larger medical facilities (De Vries *et al.*, 2022). The detection of amastigote stages using Giemsa staining confirms leishmaniasis diagnosis, although species identification based on morphology alone is not feasible. Serological diagnosis can be complemented with antibody evaluations, typically IgG through direct agglutination, Enzyme-Linked Immunosorbent Assay (ELISA), immunofluorescence, and Western Blot (WB) (Aronson *et al.*, 2016; Chappuis *et al.*, 2007). Interpreting serological findings alongside additional diagnostic assessments, like PCR or direct parasite visualization, is crucial for confirming leishmaniasis diagnoses. In regions where the disease is prevalent, serology tests may hold limited diagnostic significance due to widespread exposure to the parasite, leading to antibody development in asymptomatic individuals and animals (Alvar *et al.*, 2020).

1.1.4 Life cycle

The nocturnal behavior of female sandflies facilitates the transmission of *Leishmania* parasites to mammalian hosts. These parasites undergo two main distinct phases in their life cycle between the vertebrate and invertebrate hosts: promastigote and amastigote (Figure 1.2). Following a blood meal from an infected host, the sandfly becomes infected. Inside the sandfly, the parasite undergoes differentiation into procyclic promastigotes and then to metacyclic promastigotes, both forms characterized by the presence of a flagellum, which allows mobility within the sandfly gut (Gossage *et al.*, 2003). Upon taking a blood meal from the host, the sand fly injects the metacyclic promastigote form into the skin, where it is phagocytosed by mononuclear cells, leading to its differentiation into the amastigote form. Within the host reticulo-endothelial system, amastigotes proliferate and develop, giving rise to either asymptomatic or symptomatic forms of the disease, influenced by various factors related to the host and parasite species (Lodge *et al.*, 2008). It is intriguing to note that the parasite not only withstands the acidity of the sand fly gut but also the acidic conditions within macrophages. Inside the parasitophorous vacuole (PV), amastigotes flourish and multiply (Alexander *et al.*, 1999). This compartment is partially derived from the host

cell endocytic pathway and serves as a vital element in establishing and sustaining infection, as it exists at the interface between host and pathogen (Ndjamen *et al.*, 2010). Ultimately, *Leishmania* parasites exploit the nutrients generated during the digestive processes within the PV to proliferate (McConville *et al.*, 2011). Ultimately, the amastigotes, having multiplied within the macrophage, are released, initiating a new wave of infections that contribute to the emergence of various clinical manifestations. In this manner, the mammalian host serves as a reservoir, maintaining the parasite in nature and aiding in its geographical spread over time. Continuing the cycle, a sand fly can become infected during a blood meal, serving as an intermediary host for the transmission of these parasites (Figure 1.2) (Burchmore *et al.*, 2001; Burza *et al.*, 2018).

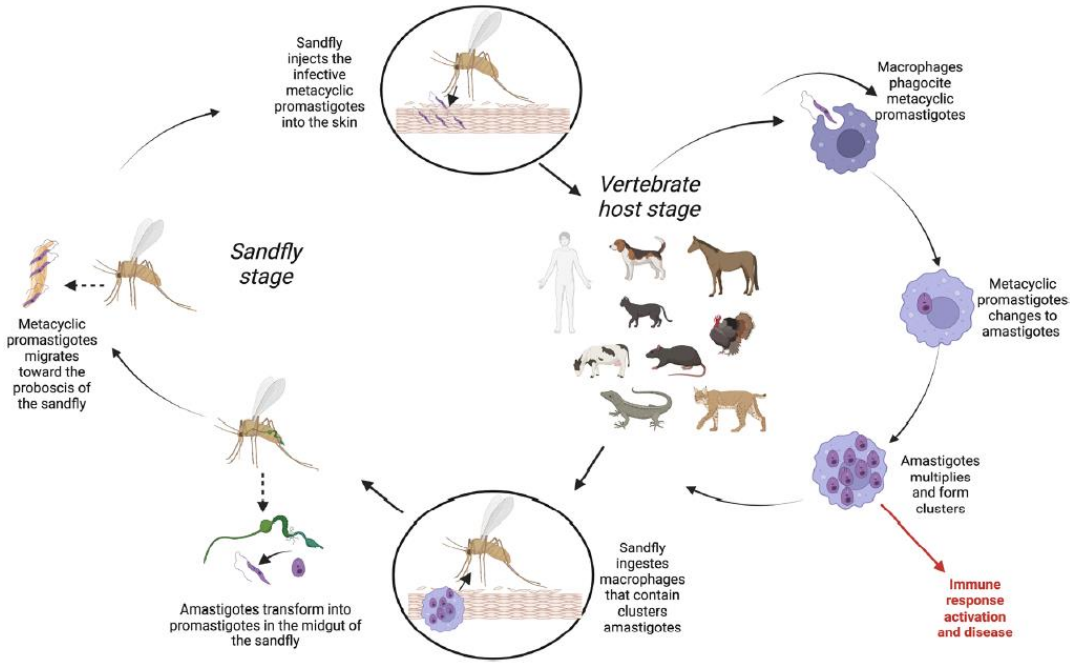


Figure 1.2 Life cycle of *Leishmania* parasite

Inside the sandflies, *Leishmania* amastigotes differentiate into infective metacyclic promastigotes, which are positioned at the proboscis, ready for transmission. Upon blood feeding, the sandfly regurgitates these metacyclic promastigotes into the host dermis. There, they are engulfed by various phagocytic cells types present in the local environment and, once inside host cells, the metacyclic promastigotes differentiate into amastigotes. These amastigotes replicate within host cells, eventually causing cell rupture, facilitating reinfection of neighboring phagocytes. The transmission cycle continues as infected phagocytes are ingested by a new sandfly during a blood meal. Within the sandfly midgut, amastigotes convert back into promastigotes, completing the transmission cycle (Montaner-Angoiti *et al.*, 2023).

1.2 Leishmaniasis treatments: challenges and developments

Presently, treatment options for leishmaniasis primarily consist of chemotherapy and a handful of drugs repurposed for this condition. Nevertheless, treatment encounters several constraints, such as the high toxicity of the drugs, the production costs, application challenges, and notably, the elevated rate of parasite resistance (Ponte-Sucre *et al.*, 2017). Several efforts have been made to develop an effective and safe vaccine against leishmaniasis for humans; nevertheless, there are still no significant breakthroughs in this domain (Kaye *et al.*, 2021). Hence, the most commonly employed treatment approaches involve pentavalent antimonials, amphotericin B (AmB), pentamidine and miltefosine.

1.2.1 Pentavalent antimonials

Antimonials served as the primary treatment for leishmaniasis for over six decades, despite the unclear mechanism of their action. However, these medications have several limitations, including the need for daily parenteral administration, severe toxicity, side effects and treatment failures attributed to the emergence of treatment-resistant strains (Sundar *et al.*, 2015; Zaghloul *et al.*, 2004). Currently, the primary antimonials utilized for treating CL include meglumine antimonate (MA) and sodium stibogluconate (SSG). While reported cure rates of up to 100% have been documented with the use of antimonials, the efficacy of these medications is contingent upon factors such as treatment duration, immune status of the host, geographical location, and the species of the infecting parasite. (Ballou *et al.*, 1987; Madusanka *et al.*, 2022; Oryan *et al.*, 2016).

1.2.2 Amphotericin B

After the rise of strains resistant to pentavalent antimonials, the introduction of AmB emerged as a life-saving advancement for many patients. AmB deoxycholate (AmB-D) is a broad-spectrum antifungal agent that attaches to ergosterol, a membrane sterol, inducing the formation of ion channels and enhancing membrane permeability, ultimately resulting in cell death. (Gray *et al.*, 2012). In some endemic areas, such as India, this medication was shown to be 95% effective against VL but this drug brings about troubling side effects including fever, nausea, headache, myocarditis, and nephrotoxicity, among others (Sundar *et al.*, 2007). In an attempt to overcome these adverse effects, liposomal formulations (AmB-L - Ambisome®) have been developed as an alternative treatment for VL. With this formulation, the cure rate varies from 50% to 85%, contingent upon geographic location and treatment duration (Sundar *et al.*, 2010). Despite its high cost, this new formulation has enhanced the bioavailability and pharmacokinetic properties of

AmB while shielding other tissues from its effects (Adler-Moore *et al.*, 2002).The liposomal form of AmB faces challenges as it remains expensive and requires intravenous administration (Ramesh *et al.*, 2020).

1.2.3 Pentamidine

As an alternative to addressing *Leishmania* resistance to antimonials, pentamidine isethionate has emerged as another commonly employed medication. In this scenario, the drug has exhibited higher efficacy against CL, with cure rates reaching up to 96%. However, it also carries notable risks of toxicity, including hypoglycemia, cardiac alterations, pancreatitis leading to diabetes mellitus, hypotension, and hyperkalemia (Gadelha *et al.*, 2018; Sundar *et al.*, 2015).

1.2.4 Miltefosine

One of the latest advancements in leishmaniasis treatment is Miltefosine, originally developed for breast cancer treatment, and currently the only orally administered drug approved for combating leishmaniasis. Miltefosine effectively eliminates parasites both *in vitro* and *in vivo* by altering signaling pathways and cell membrane synthesis, ultimately inducing apoptosis (Verma *et al.*, 2004). Following a decade of use, despite achieving a cure rate of 94% for VL treatment in India, there has been a rise in resistance, resulting in diminished effectiveness and doubling the relapse rate (Srivastava *et al.*, 2017a; Sundar *et al.*, 2012). Over time, miltefosine has demonstrated itself as a safe and efficient alternative for CL. Nonetheless, post-treatment, numerous patients encounter disease relapse, primarily attributed to incomplete parasite elimination and medication failure to induce sterile immunity. (Madusanka *et al.*, 2022; Zerpa *et al.*, 2007).

1.2.5 Combination therapy and alternative approaches

Combining drugs has been a strategy to address the challenges in treating leishmaniasis. Concurrent administration of medications can lead to reduced dosages and shortened treatment duration (Alcântara *et al.*, 2018). Presently, treatment combinations include pairing AmB with miltefosine or other drugs, along with immunomodulators, in addition to pentavalent antimonials (Alcântara *et al.*, 2018; Berbert *et al.*, 2018; Sundar *et al.*, 2015). Additionally, alternative therapy methods such as thermotherapy and cryotherapy can be employed for treating leishmaniasis. Thermotherapy applied to the lesion area inhibits parasite growth and can facilitate its elimination through heat application using lasers, radio frequencies, infrared light, or immersion in hot water (Aronson *et al.*, 2010; Velasco-Castrejon *et al.*, 1997; Wolf Nassif *et al.*, 2017). While these

treatments are generally considered safe and effective with few side effects, success rates may vary depending on factors such as the size of the lesion and parasite species. Moreover, they are not suitable for VL.

1.2.6 Drug Resistance

Given the challenges associated with parenteral administration and the toxicity of current medications used to treat leishmaniasis, it is imperative to implement novel strategies for identifying and developing new drugs. Moreover, the widespread administration of drugs has resulted in the emergence of drug-resistant parasites harboring mutations that reduce their susceptibility to treatment (Srivastava *et al.*, 2017b). Another complication arises from the complex life cycle of *Leishmania*, in which one of its developmental forms, the amastigote, resides within the immune cells of the mammalian host, making it more difficult to target the parasites with specific drugs (Mondelaers *et al.*, 2016; Sundar *et al.*, 2015). Typically, exposure to drug pressure can induce genetic mutations in parasites, reducing their sensitivity to the drug. However, cases of *Leishmania* parasites resistant to SSG have been observed even in cases where the parasites were not previously exposed to the medication (Perry *et al.*, 2015). In such scenarios, the issue arises from the similarity of antimony, classified as a heavy metal, to its relative arsenic. In experimental settings, resistance developed against arsenic can lead to parasites also becoming resistant to antimony. In India, patients infected with *Leishmania* and exposed to arsenic may inadvertently expose the parasite to this heavy metal, potentially leading to the development of *Leishmania* strains resistant to arsenic and cross-resistant to antimony therapy (Perry *et al.*, 2011).

The immune status of the host is another factor influencing the development of drug resistance. For instance, the migration of infected individuals from endemic rural areas to urban centers, resulting in demographic shifts, contributes to the increased incidence of HIV-Leishmania co-infection in Brazil (Lindoso *et al.*, 2014). Additionally, *Leishmania* parasites exhibit significant genomic plasticity, enabling them to adapt to changing environments through localized copy number variations (CNVs) at specific loci, potential aneuploidy, and amplification of gene sets in extrachromosomal forms (Berg *et al.*, 2013; Laffitte *et al.*, 2016). This variability plays pivotal roles in the parasite evolutionary and adaptive biology, facilitating an increase in transcript levels for certain genes, particularly advantageous for an organism lacking the ability to regulate transcription initiation (De Gaudenzi *et al.*, 2011). Moreover, the duplication of amplified genes results in the generation of genetic diversity, facilitated by the genomic plasticity of this pathogen

(Victoir *et al.*, 2002). Given these capabilities, it is not surprising that the parasite employs genome plasticity to develop drug resistance.

1.2.7 Vaccine and prophylaxis

Due to the lack of definitive treatment outcomes for both humans and animals, coupled with the emergence of resistance genes in the parasite, the focus on prevention becomes fundamental (Salari *et al.*, 2022). Among the viable strategies for controlling and preventing diseases is the development of an effective vaccine and transmission control. At present, there are no available vaccines for human use against leishmaniasis (Kaye *et al.*, 2021). Regarding dogs, although four vaccines have been introduced to the market (Velez *et al.*, 2020), only one, LetiFend®, has received authorization. This vaccine was licensed in Europe in 2016 and demonstrated an efficacy of 72% (Reguera *et al.*, 2016). In such circumstances, preventive measures to contain the proliferation of the invertebrate vector and the transmission of the disease to humans and other animals are crucial. Implementing barriers such as fine mesh screens on doors and windows and using repellents are effective preventive measures (De Vries *et al.*, 2022). For canine leishmaniasis, collars impregnated with deltamethrin can be used, significantly reducing sand fly feeding by approximately 94% (De Camargo-Neves *et al.*, 2021; Evans *et al.*, 2022).

1.3 Host-Parasite Interactions

1.3.1 Intracellular survival

The female sand fly vector introduces metacyclic promastigote forms of *Leishmania* into the dermis of the host. Contact between the invertebrate vector proboscis and the skin disrupts the epidermal layers, inducing endothelial activation and neutrophil infiltration along with the mosquito saliva. This leads to an inflammatory response, from which promastigotes must escape to invade host cells and differentiate into amastigotes (Peters *et al.*, 2008; Peters *et al.*, 2009). In this scenario, the sandfly saliva contains components such as an endonuclease capable of digesting neutrophil extracellular traps (NETs) and inhibiting blood clotting, which aid promastigotes and facilitate their spread (Chagas *et al.*, 2014). An *in vitro* model also demonstrated that parasites of the *L. amazonensis* species can counteract the detrimental effects of NETs by expressing Leishmania-3'Nucleotidase/Nuclease, an enzyme that degrades NETs, thus enabling the parasite to evade destruction by these extracellular traps (Guimarães-Costa *et al.*, 2014). Neutrophils have been identified as intermediate hosts for *Leishmania* parasites, aiding in the safe internalization of parasites by macrophages through two distinct models. In the "Trojan Horse" model, *L. major*

promastigotes are engulfed by neutrophils *in vitro* but manage to survive within phagosomes. Subsequently, infected neutrophils undergo apoptosis and are readily engulfed by macrophages introduced into the culture, effectively transporting the promastigote forms of the parasite to the macrophage phagosome (Laskay *et al.*, 2003). Alternatively, two-photon intravital microscopy suggests that viable parasites can "leap" into macrophages after being released from apoptotic neutrophils (Peters *et al.*, 2008).

It has been previously documented that promastigote forms of *Leishmania* trigger the secretion of chemokines CXCL1, CXCL2, and CCL2 that function to attract additional monocytes and neutrophils to the site of infection (Giraud *et al.*, 2018; Teixeira *et al.*, 2006). While parasites are often detected in neutrophils, their long-term survival and replication primarily occur within mononuclear phagocytes. (Figure 1.3) (Valigurova *et al.*, 2023; Wilson *et al.*, 1987). Direct evidence of this has been obtained through mouse infection with *L. major* via needle injection, demonstrating that dermal dendritic cells (DCs) uptake parasites within the initial hours of infection (Ng *et al.*, 2008). The internalization process of promastigotes involves the activation of various pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), which detect pathogen-associated molecular patterns (PAMPs) (Teixeira *et al.*, 2006; Valigurova *et al.*, 2023). This detection can occur within resident cells such as keratinocytes, Langerhans cells, macrophages, and mast cells. Moreover, these cells also possess numerous cytokine receptors. This can ultimately result in the secretion of various chemokines when in association with other tissue cells, thereby triggering cascades that activate both innate and adaptive immune responses (Figure 1.3) (Pacheco-Fernandez *et al.*, 2021; Teixeira *et al.*, 2006).

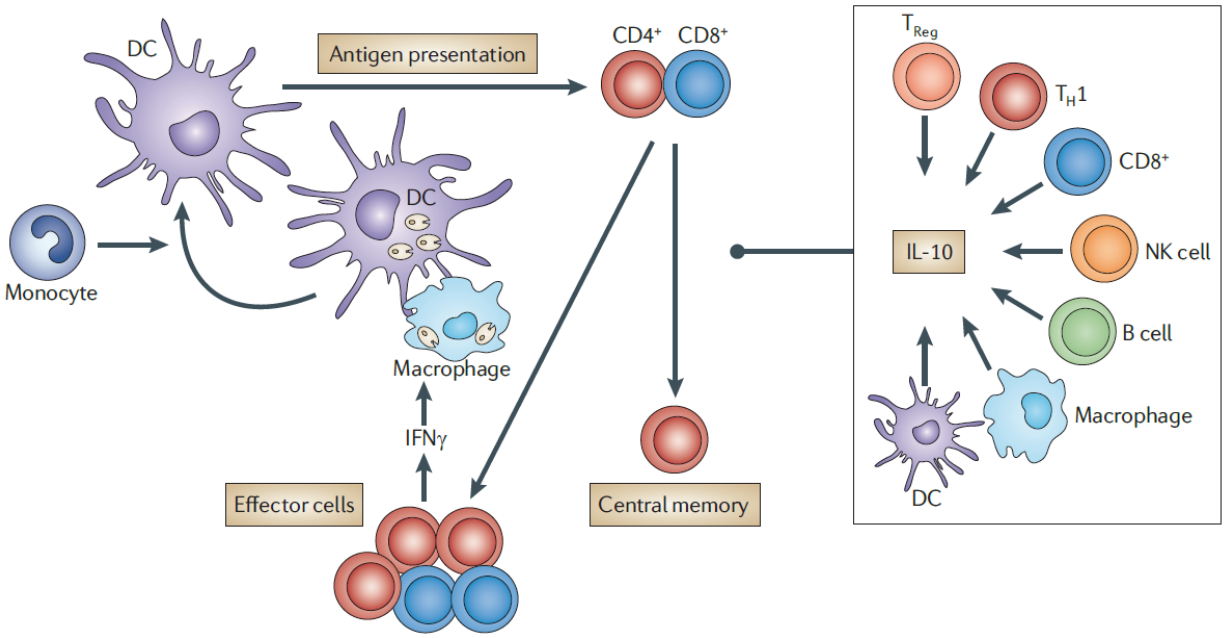


Figure 1.3 Network of cells modulating the immune response during leishmaniasis

In the initial stages of infection at the site of the infection, resident macrophages, and DCs are the primary targets. Within the first few hours, these cells become infected. Uninfected DCs play a crucial role by engulfing dead parasites or leishmanial antigens, transforming into pivotal antigen-presenting cells (APCs). Subsequently, CD4+ T cells are activated, differentiating into Th1 cells and secreting pro-inflammatory cytokines. Some CD4+ T cells deviate from the Th1 pathway and acquire a central memory T cell phenotype. Simultaneously, CD8+ T cells recognizing leishmanial antigens become activated. The regulation of this immune response is chiefly governed by the production of IL-10, emanating from various cell types, such as Tregs, Th1 cells, CD8+ cells, natural killer (NK) cells, B cells, macrophages, and DCs (Kaye *et al.*, 2011).

Leishmania promastigotes effectively neutralize galactins, mannose-binding protein, along with proteins from the complement complex such as C3b and iC3b, through their abundant expression of glycoconjugates for instance lipophosphoglycan (LPG) and glycoprotein 63 (GP63) (Blackwell, 1985; Puentes *et al.*, 1988). To withstand the effects of complement molecules the parasite uses the LPG to disrupt the complement cascade, preventing the insertion of the membrane attack complex. Furthermore, the GP63 cleaves the C3b molecule, resulting in its inactivation (Brittingham *et al.*, 1995; Puentes *et al.*, 1990). This leads to the absence of oxidative stress induction and subsequently reduces IL-12 production (Aderem, 2003; Mosser *et al.*, 1987). *Leishmania* has evolved a defense mechanism whereby it inactivates C3b molecules, facilitating its quiet entry into macrophages without triggering oxidative stress (Aderem, 2003).

After promastigote forms are internalized into phagosomes, lysosomes merge with the structure, completing the formation of the PV. Within the phagolysosome, parasites must evade the acidic environment, enzymatic activity, and microbicidal effects of oxygen and nitrogen radicals (Moradin *et al.*, 2012). To hinder the acidification of the PV, the parasite employs LPG and GP63 to prevent the assembly of the NADH oxidase complex and prevent the recruitment of vacuolar proton

ATPase onto the parasitophorous membrane (Desjardins *et al.*, 1997; Matheoud *et al.*, 2013). Thus, the activation of lysosomal proteases crucial for antigen processing and initiating the immune response is suppressed, enabling the parasite to smoothly transition from promastigote to amastigote form.

In the course of the interaction between parasites and host macrophages, *Leishmania* spp. infection can potentially disrupt signaling pathways, resulting in the incapacity of macrophages to eliminate intracellular parasites (Awasthi *et al.*, 2003; Reyaz *et al.*, 2024; Solano-Gálvez *et al.*, 2021). For instance, research has demonstrated that *L. chagasi* infection of macrophages results in a diminished response to IFNγ, thereby categorizing them as “deactivated macrophages” (Rodriguez *et al.*, 2004). Another investigation proposes that infection with *L. major* avoids affecting inflammatory peritoneal macrophages but promptly induces a cellular stress response in resident macrophages. This response leads to the secretion of pro-inflammatory signals, including TNF-α, IL-6, TIMP-1, IL-1RA, G-CSF, TREM, CXCL1, CCL3, CCL4, CCL2, and CXCL2. Nonetheless, this response also supports the survival and replication of the parasite within host macrophages (Filardy *et al.*, 2014). The activation of nuclear factor kappa B (NF-kB) is linked to pathogen invasion and regulates the expression of genes crucial for the host immune response. Nonetheless, *L. amazonensis* promastigotes have been observed to activate the p50/p50 NF-kB transcriptional repressor complex within macrophages, thereby dampening the expression of the *Nos2* gene and the production of inducible nitric oxide (NO) synthase (iNOS) (Calegari-Silva *et al.*, 2009; Reinhard *et al.*, 2012).

Unlike promastigotes, amastigotes can persist within the phagolysosome due to various factors. This parasite phase is recognized to possess metabolite transporters in its membrane that function optimally under acidic pH conditions, along with proton pumps that actively capture metabolites (McConville *et al.*, 2007). The metalloprotease GP63, the most abundant protein in amastigotes, plays a crucial role in their survival within phagolysosomes. Its proteolytic activity at acidic pH likely contributes to this survival by potentially inactivating lysosomal macrophage proteins (Chaudhuri *et al.*, 1989; Isnard *et al.*, 2012). Despite playing a pivotal role in maintaining and spreading infection within the vertebrate host, the mechanisms underlying the phagocytosis of amastigote forms remain poorly understood. As phagocytes primarily encounter promastigote forms only during the early stages of infection, which quickly differentiate into amastigotes, it is these amastigote forms that are continually released from infected cells and engulfed by new cells, thereby driving the progression of infection (Burza *et al.*, 2018). Additionally, it is known that the phagocytosis of amastigotes is facilitated by opsonization of the parasites and recognition of

Fc receptors (FcR), which triggers the secretion of IL-10, thereby promoting parasite survival and replication (Belkaid *et al.*, 2001; Kane *et al.*, 2001). This collective evidence supports the idea that *Leishmania* has evolved strategies to evade the antimicrobial defenses of macrophages, enabling it to survive in a hostile environment. These strategies involve evading the metabolic pathways of macrophages, hindering antigen presentation, suppressing the secretion of pro-inflammatory cytokines, inhibiting NO production, and promoting the release of immunosuppressive molecules such as IL-10 (Arango Duque *et al.*, 2015).

1.3.2 Adaptative immunity during *Leishmania* Infection

The evasion and modulation mechanisms of the immune system induced by *Leishmania* infection directly influence the activation and differentiation of T cells, thereby affecting the processing and presentation of antigens to these cells, the production of cytokines, and the expression of co-stimulatory molecules. However, there is limited research utilizing *in vivo* models to explore this context, primarily focusing on potential antigens that could be employed in vaccine formulation and in conjunction with virulence factors. Cellular immunity instigated by a Th1 response plays a pivotal role in conferring resistance against *Leishmania*. For instance, it is widely recognized that in resistant murine strains like C57BL/6, C3H, and CBA, *L. major* infection triggers a Th1 response, leading to the release of pro-inflammatory cytokines. In such instances, parasite replication is managed, and the host typically develops minor lesions that resolve spontaneously, fostering immunity against subsequent infections. (Arcanjo *et al.*, 2015; Ehrchen *et al.*, 2008). In this context, proper regulation of the Th1 response is crucial; otherwise, it may result in significant tissue damage and the manifestation of severe clinical symptoms of the disease. In cases of CL and MCL caused by *L. tropica*, *L. braziliensis*, and *L. amazonensis*, there is a notable release of elevated levels of pro-inflammatory cytokines, including TNF-α and IFN-γ (Kumar *et al.*, 2010) (Bacellar *et al.*, 2002). Similarly, this phenomenon occurs in susceptible murine strains infected by *L. major*, such as BALB/c. In these hosts, the infection is characterized by a Th2-type inflammatory response, resulting in the development of persistent inflammatory lesions, uncontrolled parasite replication, and dissemination to the spleen and lymph nodes (Arcanjo *et al.*, 2015; Ehrchen *et al.*, 2008).

As noted above, the involvement of $CD4^+$ T cells during *Leishmania* infection can have either positive or negative effects on the host. These cells play a role in delaying disease progression by expressing the Th1 phenotype, secreting IFN-γ, and assisting in the activation of DCs and macrophages, ultimately leading to parasite eradication. Conversely, when these cells acquire a

Th2 phenotype, they can contribute to disease advancement by secreting IL-4, for instance (Figure 1.4) (Nylén *et al.*, 2010). The release of IL-12, IL-2, IFN-γ, and TNF-α initiates the Th1 phenotype. Upon encountering infected macrophages, these cells produce TNF-α alongside the cell surface expression of CD40 ligand, resulting in a synergistic effect with IFN-γ (Nylén *et al.*, 2010; Sypek *et al.*, 1993). The significance of IFN-γ is highlighted by the inability of IFN-γ knockout mice to eradicate *L. major* infection (Swihart *et al.*, 1995). Stimulation of cells expressing a Th2 phenotype is induced by IL-4, resulting in the secretion of cytokines such as IL-10, IL-4, IL-5, and IL-13 (Hurdayal *et al.*, 2014; Nylén *et al.*, 2010). In BALB/c mice infected with *L. major*, this cell subtype plays a role in suppressing the Th1 response, thereby promoting infection progression in the host (Belkaid *et al.*, 2002; Nagase *et al.*, 2007). Evidently, these factors can contribute to disease advancement and the manifestation of various clinical symptoms, influenced primarily by the immune response of the infected host, particularly influenced by CD4+ T cells (Bañuls *et al.*, 2011; Rodríguez-Cortés *et al.*, 2007). Notably, in immunocompetent individuals, robust T cell lymphoproliferation has been documented in peripheral blood mononuclear cells following infection with *Leishmania* parasites (Russo *et al.*, 2003).

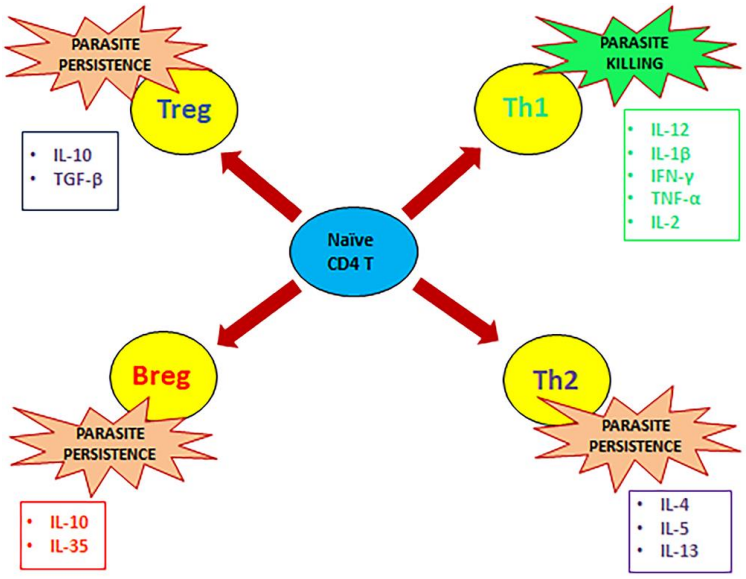


Figure 1.4 Different naive CD4 T cell subtypes elicited during leishmania infection

During infection, naive CD4 T cells have the capacity to adopt a Th1 phenotype, characterized by the secretion of pro-inflammatory cytokines, facilitating parasite eradication. Conversely, the Th2 phenotype promotes parasite persistence through the release of anti-inflammatory cytokines. The presence of Treg and Breg cells correlates with heightened host susceptibility and prolonged parasite persistence (Divenuto *et al.*, 2023).

The function of $CD8^+$ T cells may exhibit contradictions, depending on the clinical manifestations and the species causing the infection (Novais *et al.*, 2015). In this regard, in cases of VL caused by *L. donovani* and *L. infantum*, as well as CL caused by *L. major*, studies indicate that $CD8^+$ T

cells may play a protective role by producing high levels of IFN-γ, which contributes to parasite elimination and thus protects the host. (Kaushal *et al.*, 2014; Nylén *et al.*, 2010; Uzonna *et al.*, 2004). Conversely, other studies emphasize the participation of these cells in the dissemination of the parasite and the advancement of the disease in patients with MCL, PKDL, DCL in infections caused by *L. major* and *L. braziliensis*. (Crosby *et al.*, 2015; Faria *et al.*, 2009; Novais *et al.*, 2015). This observation is mostly due to the lack of IFN-γ production by CD8+T cells and to the enhanced cytolytic activity of these cells (Crosby *et al.*, 2014; Moll *et al.*, 1991).

In addition to an intense Th2 response, the presence of Treg and Breg cells is also associated with notable host susceptibility and the persistence of parasites (Figure 1.4) (Divenuto *et al.*, 2023; Matera *et al.*, 2018). For instance, significant quantities of Treg cells were discovered in the bone marrow of patients with VL. It was revealed that these cells could inhibit T cell activation in an IL-10-dependent manner, thereby promoting resistance to parasites (Rai *et al.*, 2012). Breg cells have also exhibited a comparable immunosuppressive function in leishmaniasis, as evidenced by their secretion of IL-10 (Ronet *et al.*, 2010; Soares *et al.*, 2017).

Recent studies have shown that a concomitant immunity can be constructed through $CD4^+$ T cells subsets, such as, memory T cells, effector Th1 cells, and resting effector Th1 cells (Colpitts *et al.*, 2009; Ikeogu *et al.*, 2020; Zaph *et al.*, 2004). With all that has been mentioned above, significant progress has been made over the years in comprehending the immune response underlying the progression and presentation of various clinical forms of leishmaniasis. Nevertheless, certain constraints exist, notably regarding the use of murine models in studies of VL. Researchers have shown that the most effective animal model for investigating disease progression in VL, is hamsters, as they mimic the clinical manifestations in humans (Garg *et al.*, 2006). However, employing this model is restricted by lack of immunological reagents, costs, and ethical concerns (Saini *et al.*, 2020). Additionally, the precise role of various immune system cells, such as CD8+ T cells, remains unclear.

1.3.1 Molecular mechanisms involved in dysregulation of host cell functions by *Leishmania*

In recent years, numerous studies have been conducted to elucidate the mechanisms involved in the interaction between *Leishmania* and its host. For instance, through cDNA expression array analysis, researchers demonstrated that *Leishmania* infection of bone marrow-derived macrophages (BMDM) led to a decrease in the expression of 37% of genes compared to uninfected cells (Buates *et al.*, 2001). Furthermore, the infection prompted the upregulation of

various genes, including macrophage inflammatory proteins (MIP) such as CCL3 and CCL4. These proteins are implicated in recruiting additional macrophages to the infection site, thereby bolstering the pool of potential host cells for parasite invasion (Buates *et al.*, 2001).

As explained above, *Leishmania* parasites found their best replication and survival within mononuclear phagocytes, mainly macrophages (Valigurova *et al.*, 2023). These cells exhibit diverse expression patterns and structural forms, which regulate specialized immune functions such as immune tolerance, infection response, and orchestrating immune responses involving T and B cells (Mills *et al.*, 2014; Yunna *et al.*, 2020). Additionally, macrophages possess the capacity to instigate and essentially drive immune responses, including T and B cells, towards generating Th1 or Th2-type responses (Mills *et al.*, 2014). Various tissues harbor macrophages susceptible to polarization based on environmental shifts, resulting in distinct macrophage subtypes: M1 and M2 macrophages, which represent the end points of a diverse spectrum of macrophage phenotypes (Chen *et al.*, 2023). M1 polarized macrophages undertake proinflammatory roles, generating cytokines like IL-6, IL-12, and TNF to fuel inflammatory responses. Conversely, M2 polarized macrophages exhibit anti-inflammatory capabilities, aiding in tissue repair and fostering an environment of reduced inflammation. (Cardoso *et al.*, 2020; Chen *et al.*, 2023).

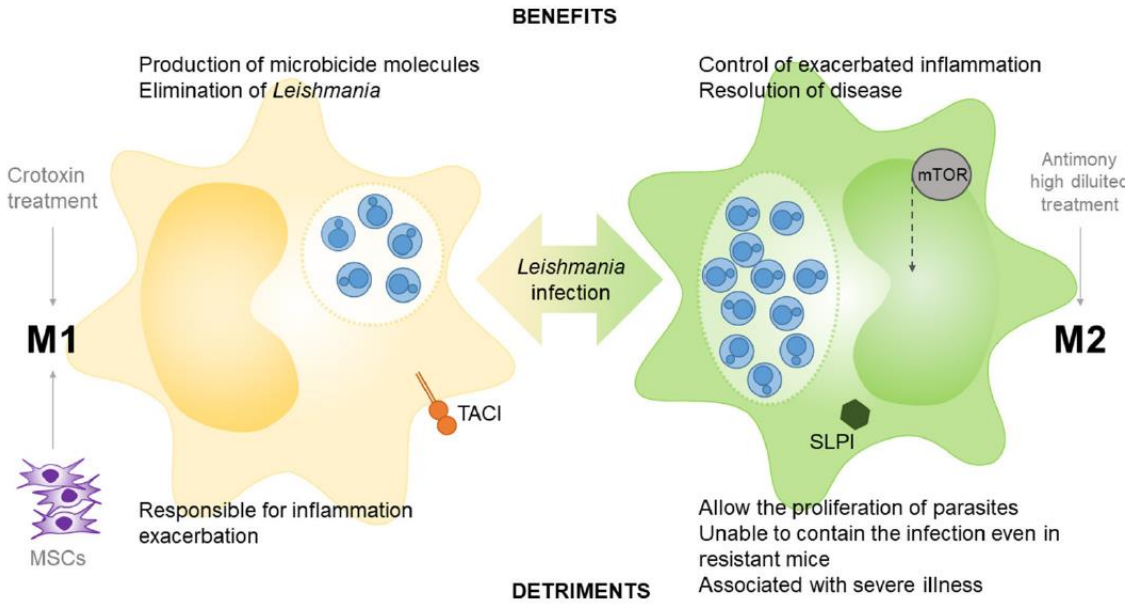


Figure 1.5 M1 and M2 polarized macrophages during *Leishmania* infection

Transmembrane Activator and Calcium Modulator and Cyclophilin Ligand Interactor (TACI), crucial for plasma cell maintenance, promotes M1 polarization. Peroxisome Proliferator-Activated Receptors (PPARs), vital for the expression of inflammatory response-related genes, and Mesenchymal Stem Cells (MSCs) further enhance M1 phenotype, in conjunction with crotoxin treatment stimulating the release of pro-inflammatory cytokines. In *Leishmania* infection models, secretory leukocyte protease inhibitor (SLPI) demonstrates both antimicrobial and anti-inflammatory properties. The mammalian target of rapamycin (mTOR) enhances the expression of M2

macrophage markers, while high-diluted antimony treatment reduces pro-inflammatory cytokines and chemokines. Collectively, these factors contribute to M2 polarization (Tomiotto-Pellissier *et al.*, 2018).

Leishmania employs diverse strategies to render macrophages susceptible to infection, capitalizing on their notable phenotypic adaptability (Figure 1.5) (Tomiotto-Pellissier *et al.*, 2018). Each distinct phenotype is governed by an intricate interplay of epigenetic, transcriptional, and post-transcriptional mechanisms (Ivashkiv *et al.*, 2016; Liu *et al.*, 2020). Indeed, it is a reality that intracellular parasites, which utilize immune system cells like macrophages as hosts, employ coevolutionary strategies to exploit pathways and programs within host cells. This manipulation of information is geared towards the survival of the pathogen and the maintenance of chronic infection (Davis *et al.*, 2013; Tomiotto-Pellissier *et al.*, 2018). It has been shown that *L. donovani* can directly activate signal transducer and activator of transcription-6 (STAT6) via a microRNA (miRNA)-dependent pathway, aiming to enhance parasite survival, induce M2 polarization, and dampen the immune response (Das *et al.*, 2021; Osorio *et al.*, 2012). *Leishmania* parasites can also disrupt other transcription factors, such as NF-κB, as noted earlier. Lecoeur et al. demonstrated that amastigotes derived from *L. amazonensis* possess the ability to diminish NF-κB and NLRP3 inflammasome activation. This reduction is linked to histone hypoacetylation and hypo-trimethylation at the promoters of NF-κB-associated pro-inflammatory genes (Lecoeur *et al.*, 2020).

The modulation of miRNAs has emerged as a prevalent survival tactic among *Leishmania* spp. (Rashidi *et al.*, 2022). *L. guyanensis*, for instance, is known to host *Leishmania* RNA virus 1 (LRV), a viral endosymbiont. Parasites harboring LRV1 demonstrated an ability to elevate miR-155 levels in infected macrophages via TLR-3 signaling, thereby augmenting macrophage survival through AKT activation. Inhibiting AKT, however, resulted in reduced host cell survival and subsequently diminished parasite persistence (Eren *et al.*, 2016). The AKT pathway plays a vital role in the survival of parasites and is also instrumental in inhibiting apoptosis in host cells (Neves *et al.*, 2010; Ruhland *et al.*, 2007). Studies have documented that during *L. donovani* infection, parasites can also activate the AKT pathway to inhibit host cell apoptosis (Rodríguez-González *et al.*, 2023). This parasite triggers AKT activation, resulting in the inhibition of pro-inflammatory responses and host cell apoptosis. The latter effect is attributed to the deactivation of GSK-3β, leading to the activation of β-catenin, an anti-apoptotic transcriptional regulator, and the inhibition of forkhead box protein O1 (FOXO-1), a pro-apoptotic transcriptional regulator (Gupta *et al.*, 2016).

Taken together, these investigations underscore the significant involvement of reprogramming driven by the parasite on the host transcriptome. Furthermore, alterations in protein expression were observed in THP-1 cells infected with *L. donovani* compared to uninfected cells. These

findings suggest parasite-driven modulation of the host proteome, highlighting the involvement of post-transcriptional and post-translational mechanisms during infection (Singh Alok *et al.*, 2015). Given this perspective, employing a translatome-based approach in BMDMs, our team documented a significant disturbance in host mRNA translation during *L. donovani* infection (Figure 1.6A). *In silico* analysis revealed an enrichment of mRNA translation, contingent upon the activation of eukaryotic translation initiation factor 4A (eIF4A) (Figure 1.6B) and mechanistic target of rapamycin (mTOR) (Figure 1.6C). The sensitivity of translation to mTOR was evaluated using 5' TOP-containing mRNAs, whose translation is notably reliant on mTOR activity (Chaparro *et al.*, 2020). Stimulation of mTOR triggers the formation of the eIF4F complex, comprising eIF4E (e.g., mRNA cap-binding subunit), eIF4G (e.g., scaffolding protein) and eIF4A (e.g., RNA helicase). Collectively, this complex enables mRNA translation (Jackson *et al.*, 2010). Interestingly, pharmacological inhibition of mTOR (i.e., Rapamycin) favored the infection (Figure 1.6D), while the pharmacological inhibition of eIF4A (i.e., Silvestrol) reduced *L. donovani* survival within macrophages (Figure 1.6E) (Chaparro *et al.*, 2020).

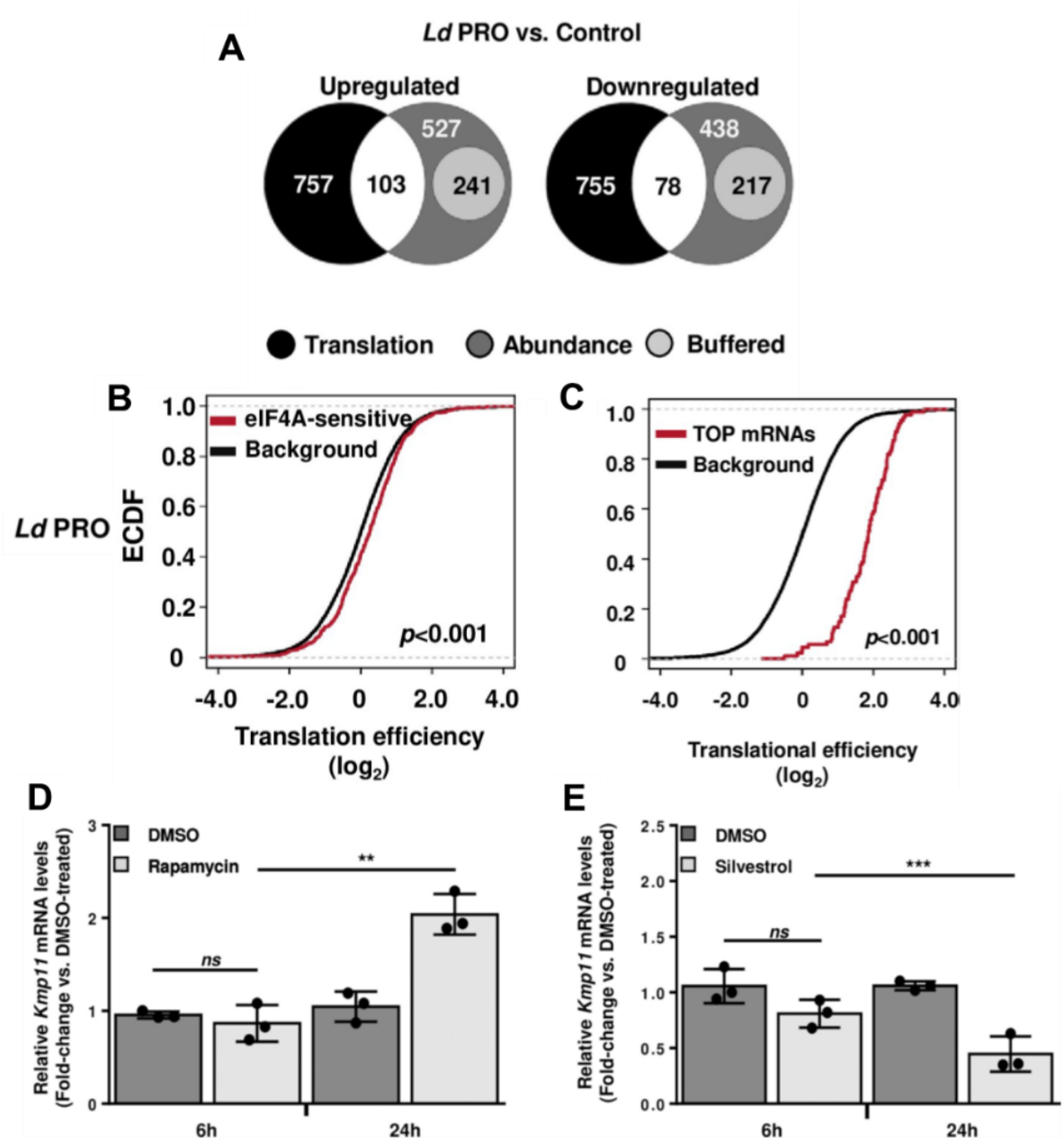


Figure 1.6 Changes in the host cell translational profile during *L. donovani* infection

Venn diagrams illustrate mRNA regulation at translation, abundance, and buffering levels in BMDMs infected with *L. donovani* promastigotes (*Ld* PRO) compared to control (A). The empirical cumulative distribution function (ECDF) in (B) and (C) represents translational efficiencies (infection vs. control) for eIF4A-sensitive transcripts and TOP mRNAs, respectively, against all detected transcripts (background). Treatment of BMDMs with Rapamycin or Silvestrol reveals distinct roles of host mTOR (D) and eIF4A (E) in regulating *L. donovani* persistence within macrophages (Chaparro *et al.*, 2020).

Our research team also observed alterations in mRNA abundance in macrophages infected with amastigotes and promastigotes of *Leishmania* parasites (Figure 1.7A) (Chaparro *et al.*, 2022). These changes were found to be partly stage-specific, being driven either exclusively by one parasite form or different between them (Figure 1.7B). During amastigote-specific modulation,

gene ontology analyses revealed an increase in mRNAs associated with the inhibition of cell apoptosis. In addition, in cells infected with the amastigote form, decreased levels of transcripts encoding regulators of both adaptive and innate immune responses were noted. Macrophages infected with promastigotes displayed a mixed profile of activated and inhibited host defense responses. This included upregulation of lipid metabolism, expression of both stimulatory and inhibitory immune response molecules, and suppression of antigen presentation and apoptosis. Collectively, these findings indicate that the host transcriptional profile varies depending on whether it is infected by an amastigote or promastigote of *L. donovani*. Each stage of the parasite may entail the expression of distinct mRNAs, thereby influencing the progression of the disease (Chaparro *et al.*, 2022). In contrast to the transcriptional alterations, remodeling of the host translatome seems to be a core process, as our findings revealed comparable changes in both promastigote- and amastigote–infected BMDMs (Chaparro *et al.*, 2020).

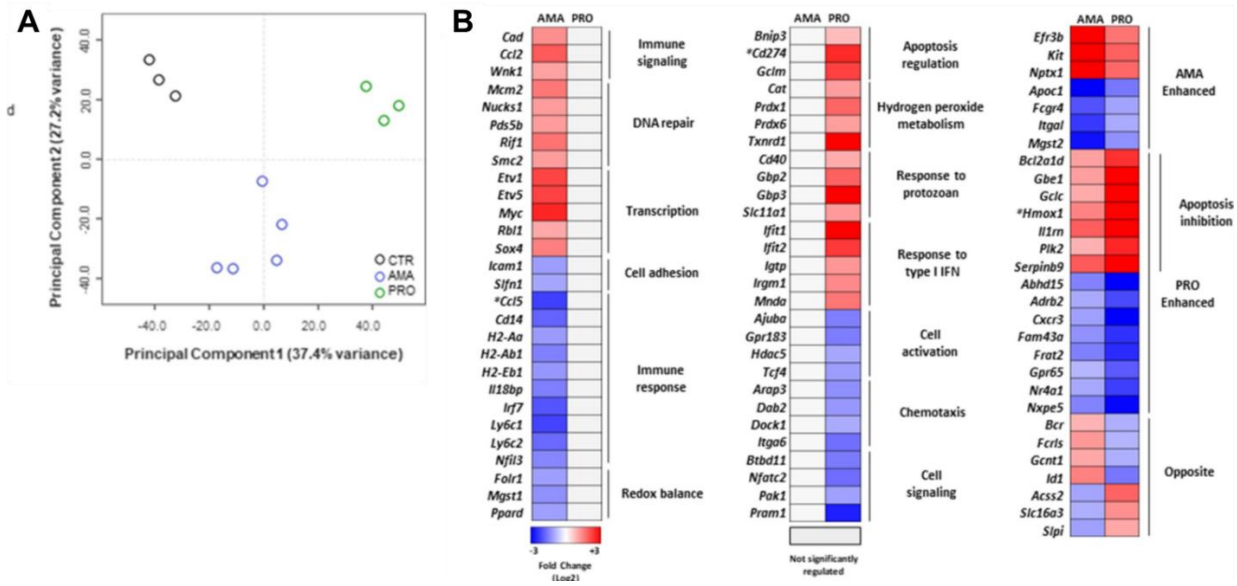


Figure 1.7 Modifications to the transcriptional profile of BMDMs during *L. donovani* infection, driven by parasite stage

Principal component analysis of cytosolic mRNA from both infected and uninfected BMDMs using amastigote (AMA) or promastigote (PRO) forms of *L. donovani* (A). Heatmaps display selected transcripts differentially regulated exclusively by amastigotes (left panel), promastigotes (middle panel), or by both (right panel) (B) (Chaparro *et al.*, 2022).

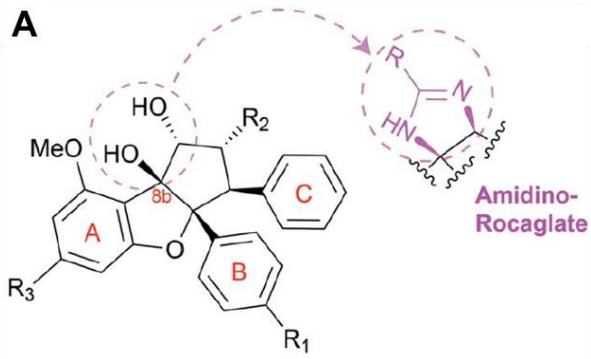
In this context, the highlighted examples illustrate the various tactics employed by *Leishmania* to manipulate host cell mechanisms in its favor, employing strategies of epigenetic, transcriptional, post-transcriptional, and translational regulation. Understanding the mechanisms underlying these alterations and the interaction between parasite and host presents a significant challenge for future research in the field of leishmaniasis. Targeting the host as a strategy for antimicrobial

therapy has emerged as a promising approach to combat viral, bacterial, and fungal diseases (Schloer *et al.*, 2022; Wallis *et al.*, 2023). Considering the pronounced genomic plasticity of *Leishmania* spp. parasites, this strategy enhances the genetic barrier against drug resistance, a phenomenon that has facilitated the emergence of resistant strains. (Laffitte *et al.*, 2016; Varikuti *et al.*, 2018).

1.4 Rocaglates

1.4.1 What is a rocaglate?

Rocaglates, derived from plants within the *Aglaia* genus, comprise a category of both natural and synthetic compounds. Characterized by their shared structural motif called cyclopenta[*b*]benzofuran (Figure 1.8), these compounds have been extensively studied (Hwang *et al.*, 2004; Lu King *et al.*, 1982; Manier *et al.*, 2017; Pan *et al.*, 2014). Numerous naturally occurring rocaglates have been discovered, exhibiting diverse biological activities including insecticidal, antifungal, anti-inflammatory, antiproliferative, and anticancer properties (Manier *et al.*, 2017; Pan *et al.*, 2014). Silvestrol, previously discussed in our group research (Figure 1.6E), stands out as one of the most extensively examined rocaglates, alongside rocaglamide A (RocA) (Hwang *et al.*, 2004). The unveiling of the chemical synthesis pathway for RocA has empowered researchers with improved control over the stereochemistry of these compounds (Lu King *et al.*, 1982). Since then, the synthesis process has undergone further enhancements and expansions, encompassing a wide array of modified rocaglates (Zhang *et al.*, 2019b). One notable synthetic rocaglate, CR-31-B, has been extensively researched and has shown promising anti-viral and anti-malarial properties (Langlais *et al.*, 2018; Müller *et al.*, 2021; Müller *et al.*, 2020).



B

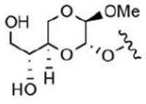
	R_1	R_2	R_3
RocA	OMe	$CONMe_2$	OMe
Silvestrol	OMe	CO_2Me	
FL3	Br	H	OMe
SDS-1-021	Br	CONH(OMe)	OMe
CR-1-31b	OMe	CONH(OMe)	OMe

Figure 1.8 Common structure of Rocaglates

Rocaglate cyclopenta[*b*]benzofuran structure (A) and examples of recently described rocaglates and their residues (R_1, R_2 and R_3) (B) (Shen *et al.*, 2020).

1.4.2 Mechanism of action of rocaglates

The effects of rocaglates on the translatome have been extensively investigated over the past years (Iwasaki *et al.*, 2016; Rubio *et al.*, 2014; Wolfe *et al.*, 2014). Collectively, these investigations have revealed that rocaglates effectively bind to the RNA-helicase complex in a reversible manner (Iwasaki *et al.*, 2019). They have emerged as some of the most potent compounds identified thus far, exhibiting significant cytotoxicity, the capacity to stabilize eIF4A:RNA complexes, and the ability to inhibit translation both *in vitro* and in cellular environments (Zhang *et al.*, 2019a).

Among the helicases targeted by rocaglates the most studied one is eIF4A. As mentioned in the previous section, this DEAD-box helicase is part of the eIF4F translation initiation complex. Translation can be categorized into cap-dependent or cap-independent based on the initiation process (Figure 1.9). The eIF4F complex plays a pivotal role in cap-dependent translation initiation, which is strongly regulated (Kwan *et al.*, 2019) (Figure 1.9A). Among its functions, eIF4F facilitates mRNA recruitment for translation. However, the 5' untranslated region (5'-UTR) of mRNA carries secondary structures that obstruct the assembly of the 40S ribosomal subunit and, consequently block scanning close to the start codon (Kwan *et al.*, 2019). The helicase activity of eIF4A is crucial for unwinding these 5′ UTR structures (Jackson *et al.*, 2010). mRNA molecules with lengthy and intricately structured 5' UTRs rely heavily on eIF4A activity and are thus known as "eIF4A-sensitive mRNAs" (Gandin *et al.*, 2016).

In cap-independent translation mechanism, the 5'-UTR contains a structure known as the internal ribosome entry site (IRES), which is pivotal for mRNA translation (Pelletier *et al.*, 1988) (Figure 1.9B). In this scenario, mRNA translation initiates without reliance on canonical initiation factors. However, this mechanism may still necessitate one or more factors, such as eIF4A, or rely on cellular proteins called IRES trans-acting factors (ITAFs) for translation (Lewis *et al.*, 2008; Yang *et al.*, 2019). It is noteworthy to mention the existence of various isoforms of eIF4A, including: eIF4A1, eIF4A2, and eIF4A3 (Iwatani-Yoshihara *et al.*, 2017). While eIF4A3 primarily functions in RNA metabolism by facilitating the export of RNA structures and linking mRNA splicing to translation, eIF4A1 and eIF4A2 play roles in initiating the translation process, as described (Mazloomian *et al.*, 2019). Numerous studies on tumor tissues have demonstrated dysregulated expression of eIF4A isoforms, however, the precise role of these proteins in tumorigenesis progression remains unclear (Raza *et al.*, 2015; Yu *et al.*, 2023). While eIF4A1 and eIF4A2 share a 90% identity, eIF4A1 is indispensable for initiation, unlike eIF4A2 (Galicia-Vázquez *et al.*, 2015; Schütz *et al.*, 2010). Blocking eIF4A1 prompts heightened eIF4A2 transcription. However, eIF4A2

fails to restore translation or alleviate the inhibition of cell proliferation resulting from eIF4A1 suppression (Galicia-Vázquez *et al.*, 2012).

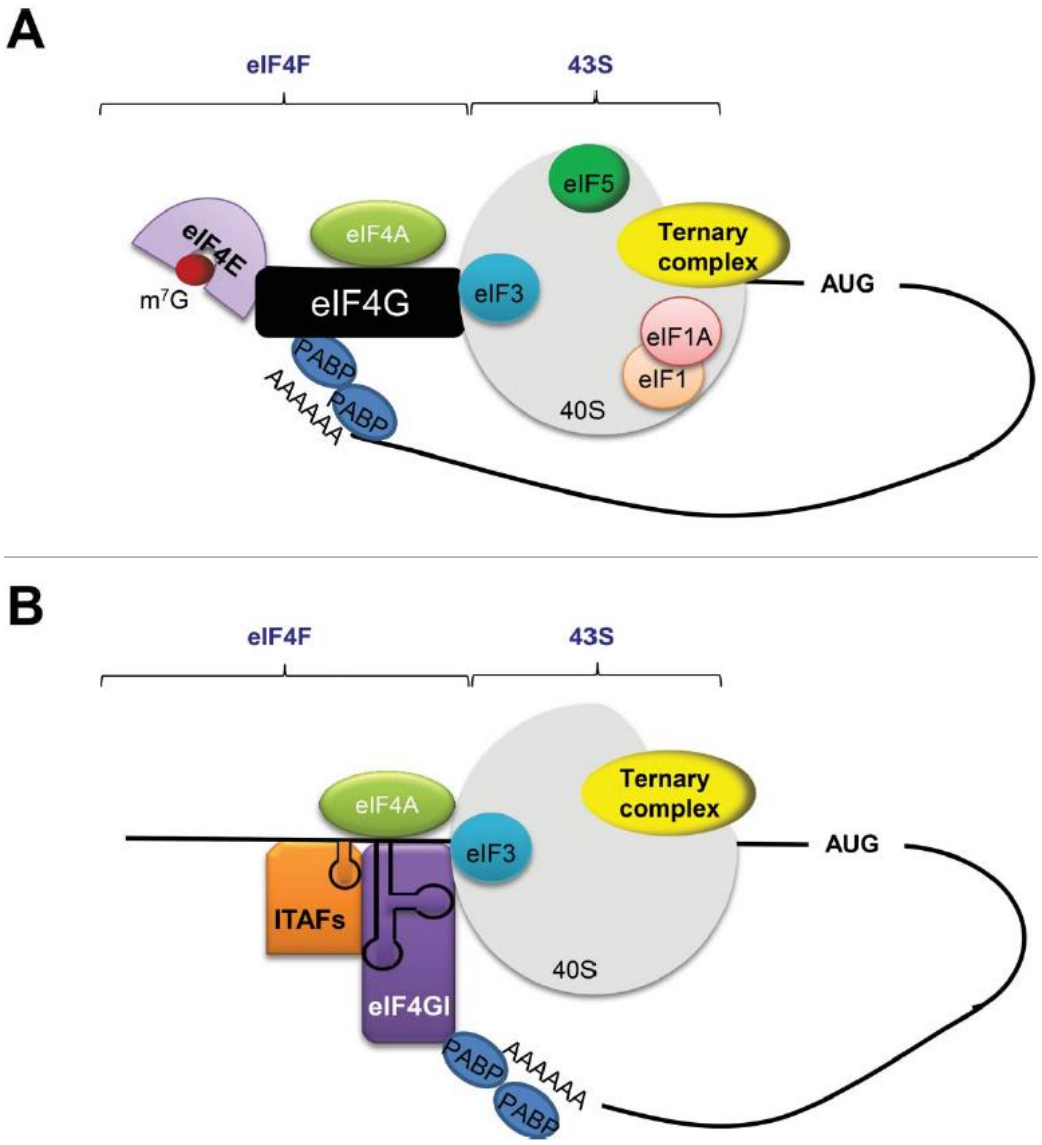


Figure 1.9 Cap-dependent and cap-independent translation initiation

During cap-dependent translation, the complex eIF4F is responsible for regulating the assembly of the 40S ribosomal subunit close to the start codon (A). During cap-independent translation, the initiation step relies on ITAFs and a few proteins such as eIF4A and eIF4GI (B) (Song *et al.*, 2016).

A second DEAD-box RNA helicase, DDX3X has been described as an alternative target of RocA (Chen *et al.*, 2021). This helicase is recognized for its ability to shuttle between the cytoplasm and the nucleus, thus participating in various functions within both cellular compartments (Brennan *et al.*, 2018). In general, this protein participates in various cellular processes including translation, mRNA translocation, mRNA splicing, regulation of transcription, modulation of epigenetic

modifications, and activation of the innate immune system (Chao *et al.*, 2006; Chen *et al.*, 2017; Deckert *et al.*, 2006; Geissler *et al.*, 2012; Heaton *et al.*, 2019; Mo *et al.*, 2021). Chen et al. showed that RocA interacts with DDX3X using distinct amino acid residues and at a different angle compared to its binding with eIF4A1. They found that, similar to eIF4A1, the RocA-DDX3X complex can inhibit translation. However, the translation suppression caused by DDX3X inhibition was milder compared to that induced by eIF4A1 inhibition. This observation aligns with the different affinities of RocA for these proteins (Chen *et al.*, 2021).

The mechanism of translation inhibition by rocaglates is intricate. Initially, rocaglate-induced clamped complexes block scanning ribosomes (Figure 1.9A) (Iwasaki *et al.*, 2016). Consequently, translation of the primary open reading frames (ORF) is diminished. Rocaglates also result in decreased recruitment of the 43S pre-initiation complex (43S PIC) by anchoring eIF4F to the cap structure (Figure 1.9B) (Chu *et al.*, 2020). The primary mechanism hampering translation initiation is the failure of ribosomes to bind to mRNA. For instance, the absence of 5'-end polypurine sequences necessary for eIF4F binding to mRNA, obstructs the scanning of the structure by the eIF4F complex, ultimately preventing the coupling of the 43S complex to mRNA (Chu *et al.*, 2020). Furthermore, beyond its binding to polypurine RNA and involvement in inhibiting scanning, Rocaglates also sequester eIF4A, resulting in incomplete eIF4F complexes and the inability to recruit 43S PICs, ultimately reducing mRNA translation initiation (Figure 1.9C) (Chen *et al.*, 2021; Chu *et al.*, 2020).

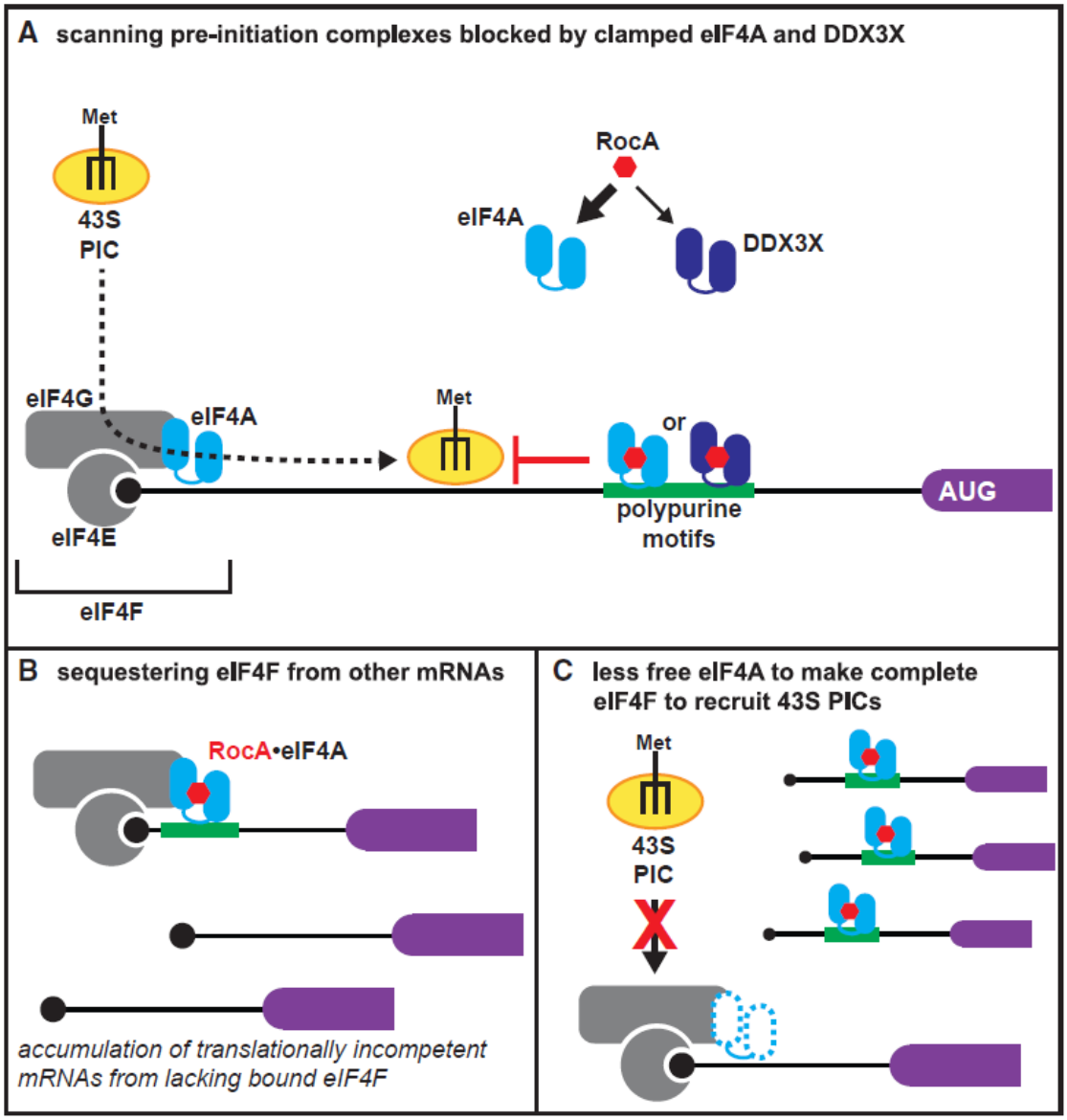


Figure 1.10 Schematic representation of the mechanism of action of Rocaglates

Binding of RocA to eIF4A or DDX3X can cause translation inhibition by clamping the helicase in the 5'UTR and blocking scanning of the 43S PICs and preventing the search for start codons (A). By interacting with eIF4A, RocA sequesters eIF4F leading to translation inhibition by lack of eIF4F (B). Sequestration of eIF4A also results in incomplete eIF4F, thereby rendering it incapable of recruiting the 43S PICs (C) (DiVita *et al.*, 2021).

1.4.3 Rocaglates and their use in infectious disease

Starting from the 1980s discovery of rocaglamides as antileukemic agents, various research teams have extensively evaluated and documented the therapeutic capacities of rocaglates (Lu King *et al.*, 1982). Since then, these compounds have demonstrated a wide array of biological functions, including antineoplastic, insecticidal, anti-inflammatory, neuroprotective, antiviral, and antibacterial properties (Manier *et al.*, 2017; Pan *et al.*, 2014). Given their enzymatic nature and

wide involvement in biological processes, DEAD-box helicases emerge as appealing therapeutic targets to explore in strategies designed to alleviate disease. The ongoing modifications of functional groups at specific positions within the structure of rocaglates have enabled a deeper understanding of the relationship between the compound structure and its activity, thereby enhancing the potential utility of these compounds as therapeutic agents (Praditya *et al.*, 2022). For instance, the synthetic rocaglate CR-31-B (-) has been assessed for its antiviral efficacy against SARS-CoV-2. Results indicate that these compounds can diminish viral replication at nanomolar concentrations. Additionally, the virus exhibits sensitivity to eIF4A-dependent translation inhibition induced by CR-31-B (Müller *et al.*, 2021).

Similar to other pathogens, bacteria also represent a global threat and addressing antibiotic resistance has emerged as a significant challenge. In this regard, the exploration of host-directed therapies has led scientists to investigate the potential of rocaglates. In this context, Bhattacharya et al. conducted a high-throughput screening and identified a set of synthetic rocaglates that synergistically acted with low concentrations of IFN-γ, thereby inducing the expression of specific IFN-inducible genes in macrophages (Bhattacharya *et al.*, 2016). The combined treatment of a rocaglate (i.e., C9433) with IFN-γ resulted in a notable reprogramming of the macrophage transcriptome. This involved the upregulation of stress-related genes and NF-κB-related genes, alongside the suppression of IFN-I pathways and the induction of autophagy in BMDMs. Consequently, cells treated with rocaglate alone or in combination with IFN-γ effectively controlled bacterial infection by *Francisella tularensis* Live Vaccine Strain (Bhattacharya *et al.*, 2016).

Likewise, research indicates that administering rocaglates to BMDMs enhances phagosome-lysosome fusion, assisting in the containment of intracellular mycobacteria (Chatterjee *et al.*, 2021). Synthetic rocaglates were observed to sensitize macrophages to low concentrations of IFN-γ while inhibiting their responsiveness to IL-4. Consequently, this treatment promoted M1 polarization in macrophages, fostering a pro-inflammatory phenotype and suppressing M2 polarized activation (Figure 1.11). Additionally, pretreatment of mice with a rocaglate (e.g., CMLD010536), followed by respiratory exposure to *Streptococcus pneumoniae*, significantly reduced live bacteria levels in bronchoalveolar lavage compared to control mice treated with vehicle alone (Chatterjee *et al.*, 2021).

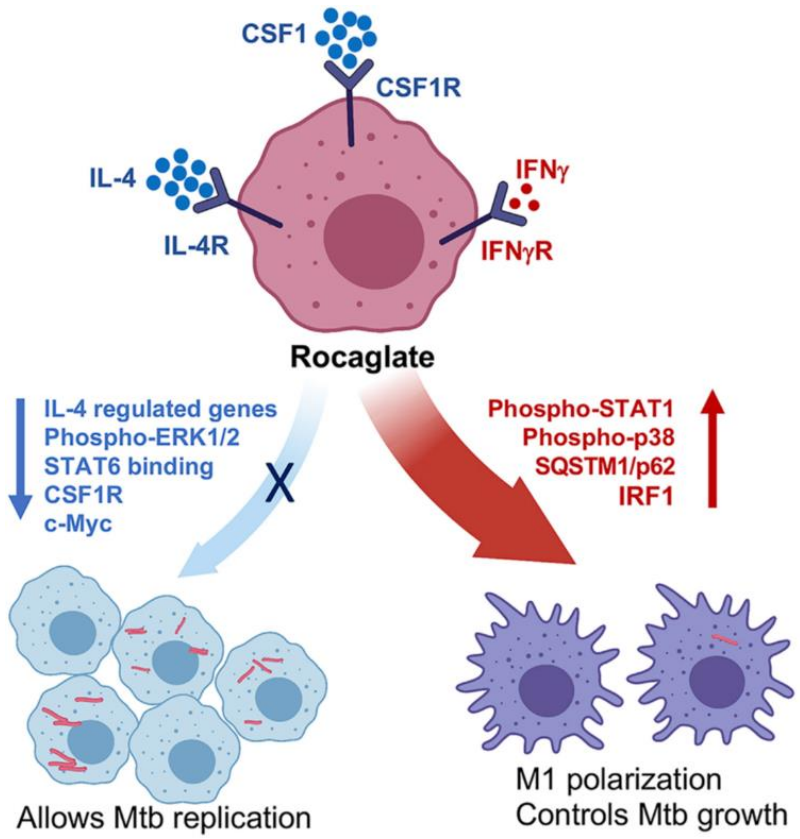


Figure 1.11 Macrophage polarization by Rocaglates

Rocaglates sensitize macrophages to IFN-γ while inhibiting their response to IL-4 and colony stimulating factor 1 (CSF1). Consequently, treatment with rocaglates upregulates stress response and host defense pathways, steering macrophages towards an M1 phenotype while inhibiting the M2 phenotype, thereby enhancing the control of mycobacteria (Chatterjee *et al.*, 2021).

Studies have also explored the application of synthetic rocaglates in combating protozoan infections. A study investigating their potential anti-plasmodial effects found that CR-1-31B effectively hindered *Plasmodium* protein synthesis and inhibited parasite replication in human erythrocytes infected *ex vivo* (Langlais *et al*., 2018). Additionally, CR-1-31B exhibited efficacy against *P. falciparum* clinical isolates, including those resistant to conventional antimalarial drugs. Furthermore, the compound demonstrated promise as both a prophylactic and therapeutic intervention, as it safeguarded mice against cerebral malaria caused by *P. berghei* and significantly reduced blood parasitemia in mice infected with *P. chabaudi*. Treatment with CR-1-31B also led to decreased neuroinflammation in infected mice, thereby improving their survival rates. This reduction in neuroinflammation was associated with decreased infiltration of myeloid and lymphoid inflammatory cells in the brain and lowered cerebral expression of molecular markers of proinflammatory cells and mediators during peak neuroinflammation. In summary, this study showcases the potential of a synthetic rocaglate as an anti-malarial drug, revealing its

strong antimicrobial activity against the parasite and highlighting the impressive dual-targeting capabilities of rocaglates in infected mice, demonstrating direct effects on both the host and the pathogen (Langlais *et al.*, 2018).

As previously noted, our group has published data using Silvestrol, a strong eIF4A inhibitor, to control *Leishmania* infection in BMDMs (Figure 1.6E) (Chaparro *et al.*, 2020). Considering the potential cost reduction and improved utility of synthetic rocaglates as therapeutic agents, we have become interested in their application. Through collaboration with Dr. Lauren Brown and Dr. John Porco from Boston University Center for Molecular Discovery (BU-CMD), we have gained access to a comprehensive library of synthetic rocaglates. Thus far, in screening over 50 compounds, we have pinpointed several capable of diminishing the parasite count in infected cells. Preliminary data generated by another member of our laboratory identified compounds C18 and C37 among the most promising, both demonstrating a reduction of over 50% in parasite count per cell compared to the DMSO control (Figure 1.12). Moreover, they exhibited superior efficacy compared to RocA, a natural and commercially available rocaglate. Interestingly, upon analysis of our data by our collaborators, they informed us that while C37 exhibited a high affinity for eIF4A, C18 demonstrated a low affinity for the same protein in *in vitro* assays. Nevertheless, both compounds showed a similar effect in reducing the number of *Leishmania* parasites in infected cells.

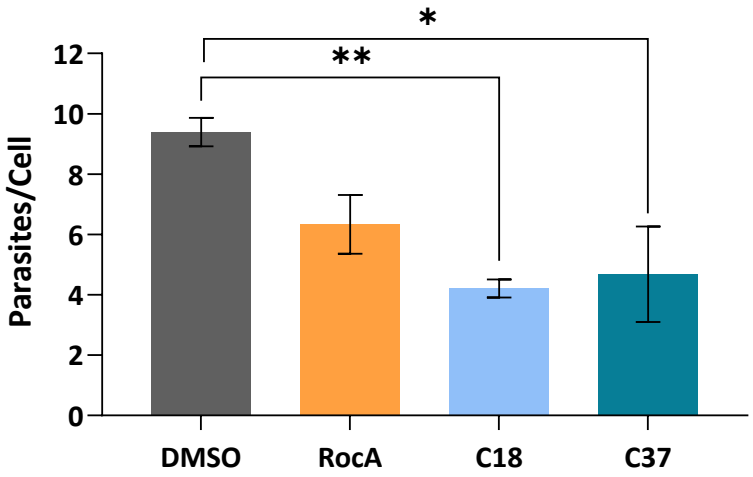


Figure 1.12 Use of rocaglates to control *L. amazonensis* infection

Macrophages infected with metacyclic promastigotes from *L. amazonensis* were treated with rocaglates Roc A (15 nM), C18 (500 nM), and C37 (10 nM), or an equivalent volume of DMSO (control), 24 hours post-infection. After 72 hours of treatment, slides were collected, stained, and subjected to counting. All rocaglates demonstrated a reduction in the parasite count within infected cells compared to the DMSO control. Furthermore, C18 and C37 exhibited the most promising efficacy among the tested compounds. Data are representative of three independent experiments performed in technical triplicates. Data are presented as mean ± SD. *p < 0.05, ** p < 0.01 (for the indicated comparisons) (Cortazzo da Silva, L. Unpublished data).

2 HYPOTHESIS AND OBJECTIVES

As an obligatory intracellular parasite, *Leishmania* induces a range of clinical manifestations that can result in disfigurement and even fatality in the host. Leishmaniasis is a neglected tropical disease which predominantly afflicts impoverished nations, exacerbating challenges in disease control, diagnosis, and treatment. This parasite is recognized for its ability to manipulate numerous functions and signaling pathways of the host cell, facilitating its replication and survival within the host. Multiple research groups are digging into the intricate dynamics of the parasite-host relationship in *Leishmania* infection to gain deeper insights into its strategies. Enhanced comprehension of these tactics and alterations induced by the parasite can pave the way for the development of improved diagnostics, treatments, and control measures for this disease, which afflicts thousands of people every year.

In recent years, our research group has observed a shift in the translatome of *Leishmania*-infected cells compared to uninfected ones. Notably, there was an increase in translation efficiency of a subset of eIF4A-sensitive mRNAs. Armed with this insight, we set out to explore the consequences of inhibiting eIF4A and thereby disrupting the translation of these mRNAs which according to our data, are potentially favoring the parasite and its infection. Our studies with rocaglates expanded upon this concept, aiming to elucidate the role of eIF4A in *Leishmania* infection. While our initial study employed Silvestrol, a naturally occurring rocaglate, to demonstrate the detrimental effect of eIF4A inhibition on the parasite and infection control, in collaboration with our partners at Boston University, we opted to explore a library of chemically modified synthetic rocaglates. These compounds offer lower production costs, exhibit reduced toxicity to host cells and offer improved solubility. Our experiments with these drugs have led to the identification of promising candidates with significant potential for controlling *Leishmania* infection.

Within our rocaglate library, we discovered a compelling subset of compounds that, despite exhibiting low affinity for eIF4A, effectively restrained *Leishmania* replication within host cells. This discovery determined our interest in exploring the therapeutic potential of these compounds and delve into their mechanism of action during *Leishmania* infection. Consequently, the central hypothesis of this study speculates that rocaglates with minimal or absent eIF4A binding activity exert their anti-leishmanial effects via an eIF4A-independent pathway. To evaluate this hypothesis, we outlined two primary objectives:

1- To identify rocaglates with low or no binding activity for eIF4A with anti-leishmanial activity *in cellulo*.
2- To investigate the mechanism of action of these rocaglates with anti-leishmanial activity *in cellulo*.

3 MATERIALS AND METHODS

3.1 Reagents

Culture media and supplements were purchased from Wisent, Gibco, and Sigma-Aldrich; Roc-A was purchased from Cayman Chemicals and Amp B was provided by Sigma-Aldrich. Additionally, all synthetic rocaglates utilized in this project were supplied by our collaborators, Dr. Lauren Brown and Dr. John Porco, from the Boston University Center for Molecular Discovery (BU-CMD) (Boston, MA, United States). For confidentiality purposes, the structures and the names of the compounds used in this study are not revealed.

3.2 Parasites

L. amazonensis LV79 (MPRO/BR/72/M1841, sourced from the American Type Culture Collection (ATCC) were generously supplied by Dr. Albert Descoteaux's Laboratory (Séguin *et al.*, 2022). Parasites were cultured in *Leishmania* medium (M199) supplemented with 10% heat inactivated FBS, 100 µM hypoxanthine, 5 µM hemin, 3 µM biopterin, 1 µM biotin, 100 U/mL penicillin, and 100 µg/mL streptomycin.

3.3 Differentiation of bone marrow-derived macrophages

Bone marrow-derived macrophages (BMDMs) were obtained from precursor cells sourced from murine bone marrow, as previously outlined (Chaparro *et al.*, 2019). Initially, bone marrow precursors were extracted from femurs and tibias of 6- to 8-week-old female commercial C57BL/6J mice (The Jackson Laboratory). Subsequently, red blood cells were lysed, and precursor cells were resuspended in BMDM culture media (Dulbecco's Modified Eagle Medium 1X with Glucose and L-Glutamine) supplemented with 15% L929 fibroblast-conditioned culture medium (LCCM). These cells were then seeded in tissue culture-treated dishes and incubated overnight at 37°C. On the subsequent day, non-adherent cells were collected, resuspended in BMDM culture medium supplemented with 30% LCCM, and plated in non-treated petri dishes. The medium was replenished two days later, and differentiated BMDMs were harvested seven days after the marrow extraction.

Femurs and tibias from *Eif4A1*$^{+/-}$ and *Eif4A2*$^{+/-}$ mice were acquired through collaboration with Dr. Jerry Pelletier's Laboratory at McGill University (Montreal, QC, Canada), and BMDMs were differentiated following the same protocol described above. Following differentiation, cells were

plated and allowed to attach overnight at 37°C. All animal-related procedures were conducted in accordance with the guidelines set by the Canadian Council on Animal Care and were approved by the Comité institutionnel de protection des animaux of INRS.

3.4 Infection of THP-1 monocytic-like cell line

THP-1 monocytic-like cells (ATCC) were maintained in culture medium (DMEM) supplemented with 10% FBS, 1% Penicillin/Streptomycin, and 2% Hepes. For differentiation into macrophages, cells were seeded in culture medium supplemented with 130 nM of phorbol 12-myristate 13-acetate (PMA) and incubated for 72 h at 37°C. Following a 72-hour PMA treatment, cells were infected as detailed below.

3.5 Infection of macrophages

Metacyclic promastigotes were isolated from promastigote cultures in the late stationary phase through density gradient centrifugation (Arango Duque *et al.*, 2019). In brief, a 15 ml tube was layered with 2 ml of 40% w/v Ficoll at the bottom, followed by a 3 ml layer of 10% Ficoll in M199-1x, and late stationary phase promastigotes were resuspended in 3 ml of DMEM with no FBS on top. After centrifugation for 10 min, metacyclic promastigotes were recovered from the DMEM-10%Ficoll interface and opsonized with 10% C5-deficient serum from DBA/2 mice (Jackson Laboratory) for 20 min at 37°C. Following a 10-min centrifugation, macrophages were inoculated with metacyclic promastigotes at a multiplicity of infection (MOI) of 10:1 and maintained at 37°C to initiate parasite internalization. After 2 h of inoculation, non-internalized parasites were washed three times with warm PBS.

3.6 Treatment with compounds and DAPI staining

Macrophages were seeded at a density of $0.25x10^6$ cells per well onto cover slips in 24-well plates and incubated overnight at 37°C before infection as described. At 24 h post-infection, cells were treated with the respective concentrations of compounds. An equivalent volume of DMSO (vehicle) was used as a negative control for parasite killing, as the concentration applied is not expected to affect the viability of either the cells or the parasites. Amp B (0.25ug/mL), was used as a positive control for parasite killing, although not a rocaglate, this drug is currently employed as a treatment for leishmaniasis. Following 72 h of treatment, cells were washed three times with warm PBS and fixed using 2% paraformaldehyde (PFA) for 10 min at room temperature. Subsequently, the cells were washed three times with PBS and permeabilized with ice-cold 100%

methanol, followed by incubation for 10 min at 4°C. After permeabilization, cells were washed three times with PBS and then incubated with a solution containing 10 µg/mL of DAPI in PBS for 10 min at room temperature. Finally, slides were mounted for visualization and counting using an epifluorescence microscope.

3.7 Viability assays of macrophages and extracellular parasites

Macrophages and/or extracellular *L. amazonensis* promastigotes were plated one day prior treatment and viability was assessed using the resazurin assay (William *et al.*, 2019). Briefly, macrophages or parasites were exposed to increasing concentrations of rocaglates (ranging from 9.75 nM to 5 µM) or an equivalent volume of DMSO (vehicle) for 72 h at 37°C. Subsequently, the medium was replaced with fresh media supplemented with 0.025% resazurin. Macrophage cultures were then incubated for 4 h at 37°C, while parasite cultures were incubated for 6 h at 26°C. Optical density was measured using a Multiskan GO (Thermo-Fisher) at wavelengths of 600 nm and 570 nm. The absorbance at 600 nm was subtracted from the readings at 570 nm. Each experiment was performed in two biological replicates (n = 2), and each sample was analyzed in technical triplicates. Values from wells without any macrophages or parasites were used as blanks, and DMSO-treated cells were employed to normalize the values. A control using a concentrated solution of DMSO (50%) was included as dead cells for each experiment.

3.8 Quantification of intracellular parasites

Slides were examined using an epifluorescence microscope to evaluate the infection index. This index was calculated by dividing the number of parasites by the total number of macrophages per slide. Approximately 100 macrophages were counted on each slide for analysis.

$$Infection\ Index = Number\ of\ Parasites \div Total\ number\ of\ macrophages\ counted$$

Equation 1 – Infection Index

3.9 Isolation of amastigotes and re-infection assays

To evaluate amastigote infectivity, BMDMs were differentiated, infected, and treated as previously outlined. Following 72 h of treatment, cells were incubated with cell culture medium containing 0.025% sodium dodecyl sulfate (SDS), gently agitated, and maintained at 37°C for 1-2 min until cell lysis occurred. Subsequently, culture medium devoid of SDS was introduced, and the content of each well was transferred to a microtube and centrifuged at 300g for 5 min at room temperature. The supernatant was then collected and transferred to a new tube, followed by centrifugation at

2000 g for 10 min. The resulting pellet of amastigotes was resuspended in complete culture medium and utilized for infecting new BMDMs. After a 2-h infection period, non-internalized parasites were washed three times with warm PBS. At 72 h post-infection, slides were fixed and stained as described.

3.10 Statistical Analysis

Statistical significance was assessed employing Student's t-test, one-way ANOVA, and calculations were executed using Prism 7 software package (GraphPad). Data are expressed as the mean ± standard deviation (SD). Significance levels were indicated as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

4 RESULTS

4.1 Several rocaglates exhibit anti-leishmanial activity *in cellulo*

As previously reported, our research team has demonstrated that pre-treatment of BMDM with Silvestrol results in a reduction in parasite numbers 24 h post-infection compared to the DMSO control (Chaparro *et al.*, 2020). For this project, we chose to utilize synthetic rocaglates as a treatment during *in vitro* infections and opted to conduct an initial screening to promptly identify rocaglates with potential anti-leishmanial effects. To accomplish this, BMDMs were infected with metacyclic promastigotes of *L. amazonensis* LV79 parasites, allowing 24 h for the parasites to differentiate into amastigotes. Subsequently, the cells were treated for 72 h with the initial set of 14 rocaglates with low or no binding affinity for eIF4A provided by our collaborators (Figure 4.1).

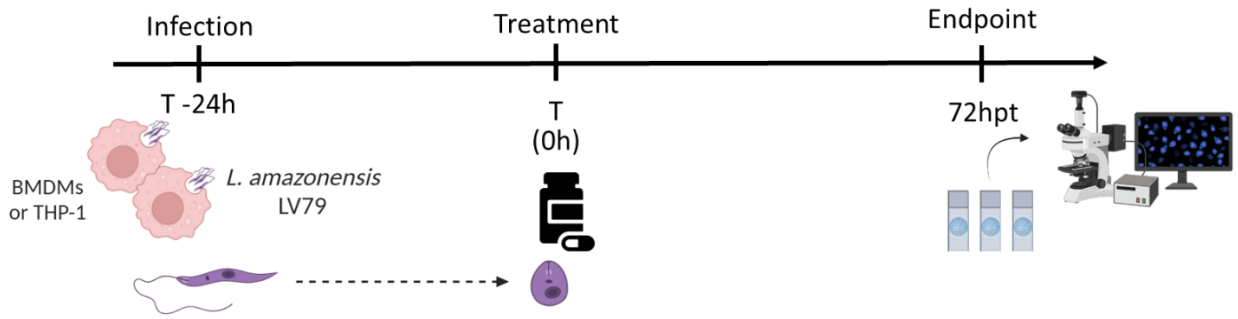


Figure 4.1 Schematic representation of treatment regimen

Cells were infected 24 hours before the treatment (T-24h) to allow differentiation of the promastigotes into amastigotes. After 72 hours of treatment, cells were collected and stained as described. Illustration created with Biorender.

In previous studies, toxicity assays for Silvestrol and Roc-A, both commercially available natural rocaglates, indicated non-toxic concentrations of 25 nM and 15 nM, respectively, for BMDMs (Jaramillo laboratory, unpublished data). Considering this, for the initial experiment, we selected a concentration of 20 nM for the compounds, taking into account our prior knowledge that rocaglates can potentially exhibit significant cytotoxicity to the cells. Remarkably, we identified 7 compounds (41, 44, 48, 49, 50, 53, and 54) that reduced the infection index by 50% or more compared to the DMSO control (Figure 4.2). This initial data suggest that these compounds were effective in preventing parasite replication and/or killing the parasites. Based on these promising results from the initial screening, we decided to proceed with subsequent experiments using only the 7 compounds that initially demonstrated potential anti-leishmanial effects.

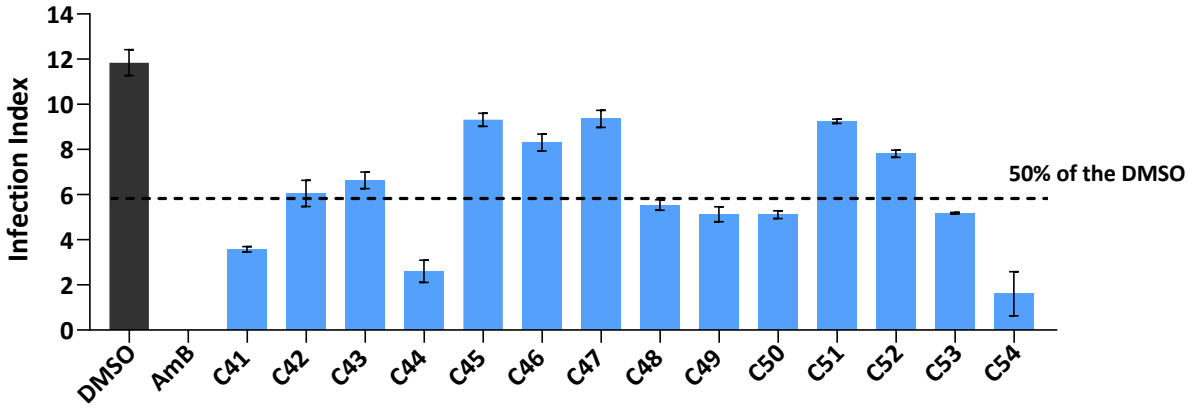


Figure 4.2 Seven rocaglates exhibited potential anti-leishmanial effect in BMDMs

BMDM cultures were inoculated with *L. amazonensis* parasites (MOI 10) for 24 h and subsequently treated with 20 nM of synthetic rocaglates or an equivalent volume of DMSO (vehicle) for 72 h. The slides were stained with DAPI, and quantification of intracellular parasites was performed by calculating the infection index. Data are representative of one independent experiment performed in technical duplicates n=2.

4.2 Selected rocaglates do not affect the viability of BMDMs

To further explore the anti-leishmanial properties of the previously identified compounds, our initial steps involved conducting a toxicity assay in BMDMs. This enabled us to exclude any compounds or concentrations that displayed excessive toxicity to the host cells from our study. Host cell viability was evaluated using the resazurin colorimetric assay, following the established protocol. The toxicity of selected compounds (i.e., 41, 44, 48, 49, 50, 53, and 54) was tested between 9.75 nM and 5 µM for 72 h. Concentrations were deemed toxic if they resulted in a reduction of 30% or more in BMDM viability compared to the DMSO control. Our analysis revealed that none of the seven compounds exhibited toxicity in BMDM within the nanomolar range (Figure 4.3) and Table 4.1). Since our objective was to employ compounds within this nanomolar range, we opted to proceed with the seven compounds selected in our initial screening for subsequent experiments.

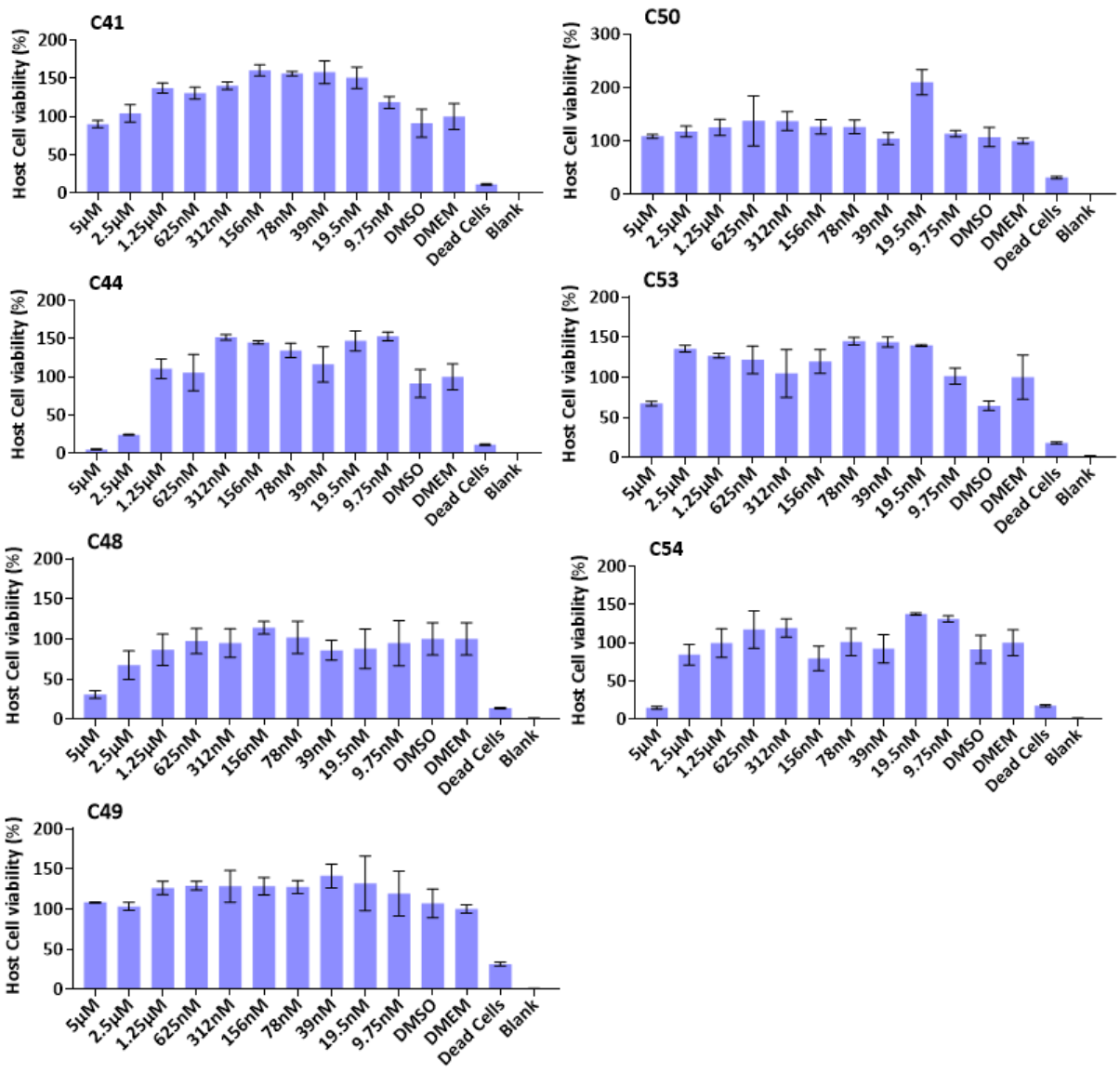


Figure 4.3 Measurement of toxicity of rocaglates on BMDM

BMDMs were treated with increasing concentrations of compounds (9.75nM – 5uM) or an equivalent volume of DMSO (vehicle) for 72 h. The toxicity was measured by resazurin assay. Percent viability was normalized to DMSO-treated parasites. Data are representative of two independent experiments (n=2) performed in technical triplicates.

Table 4.1 Summary of the toxicity of rocaglates

C#	Toxicity
C41	Not toxic at the concentrations tested
C44	Toxic above 1.25 uM
C48	Toxic above 2.5 uM
C49	Not toxic at the concentrations tested
C50	Not toxic at the concentrations tested
C53	Toxic above 2.5 uM
C54	Toxic above 2.5 uM

4.3 Selected rocaglates do not affect the viability of *L. amazonensis* extracellular promastigote cultures

To further elucidate the mechanism of action of the compounds evaluated in this study, we chose to investigate their effects on the extracellular promastigote forms of the parasite. Similar to the toxicity assay conducted in BMDMs, we applied the resazurin viability assay for this evaluation. Interestingly, none of the compounds exhibited any impact on the viability of the extracellular promastigotes at the concentrations tested (between 9.75 nM and 5 µM) for a 72-h period (Figure 4.4). These data indicate that the compounds did not target any specific component within the promastigote form of the *Leishmania* parasite.

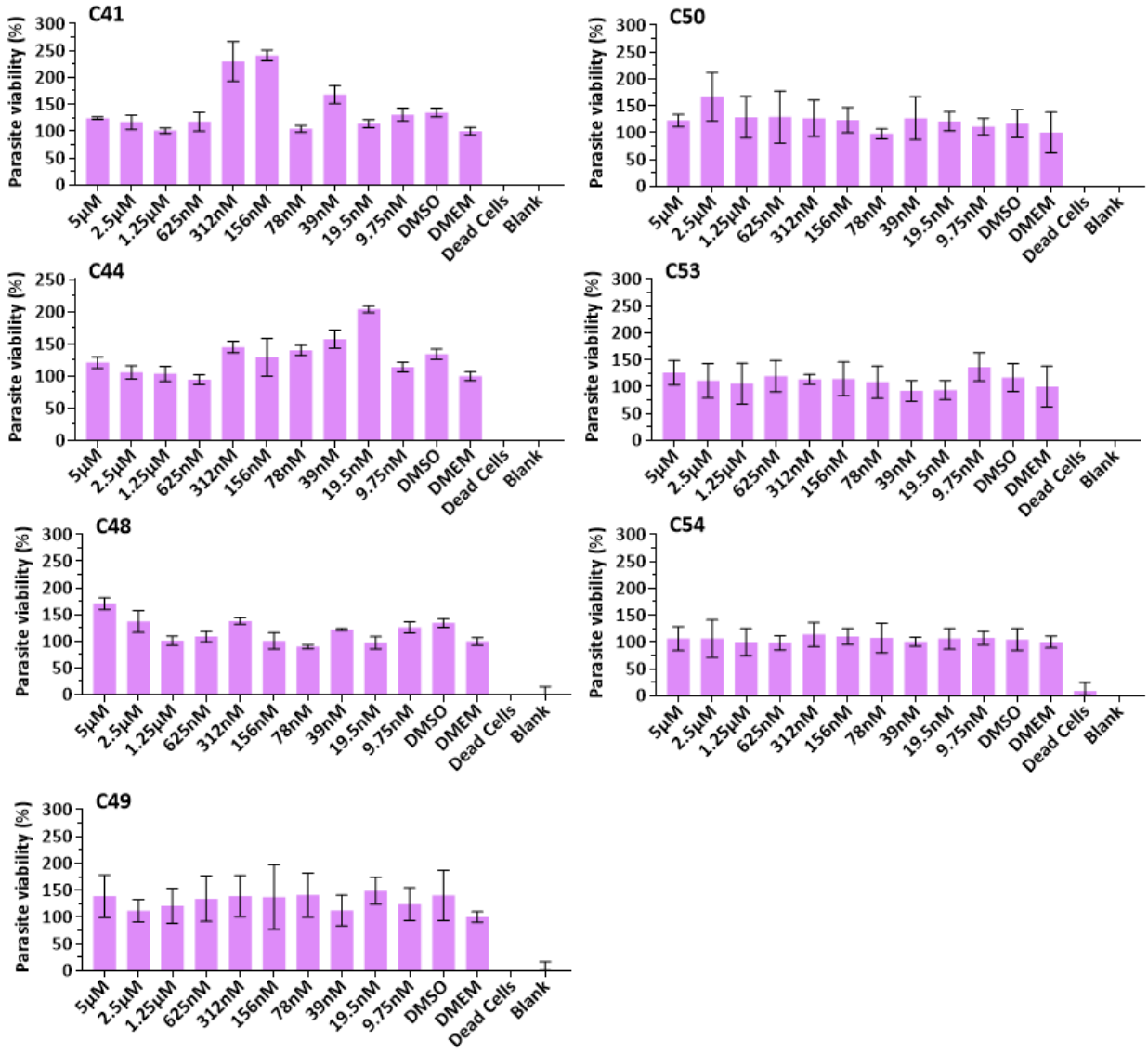


Figure 4.4 Measurement of toxicity of rocaglates on extracellular *L. amazonensis* promastigotes

***L. amazonensis* cultures were treated with increasing concentrations of compounds (9.75 nM – 5 uM) or an equivalent volume of DMSO (vehicle) for 72 h. Toxicity of the compounds was measured by resazurin assays. Percent viability was normalized to DMSO-treated parasites. Data are representative of two independent experiments (n=2) performed in technical triplicates.**

4.4 Compounds 41 and 44 exhibit the most potent anti-leishmanial activity in *L. amazonesis*-infected BMDM

Building on our earlier findings, we decided to conduct a second screening to evaluate the impact of the previously selected rocaglates on the intracellular amastigote forms of *Leishmania* parasites. Intriguingly, among the seven compounds, C41 and C44 demonstrated the most potent anti-leishmanial activity in *L. amazonensis*-infected BMDMs (Figure 4.5). Furthermore, we

compared the effects of these compounds with Roc-A. Remarkably, we noted that the synthetic compounds were also more effective than Roc-A, a natural compound. Consequently, considering both this observation and the limitations of our methodology (i.e., time-consuming manual quantification of intracellular parasites), we decided to proceed with subsequent experiments applying only compounds 41 and 44.

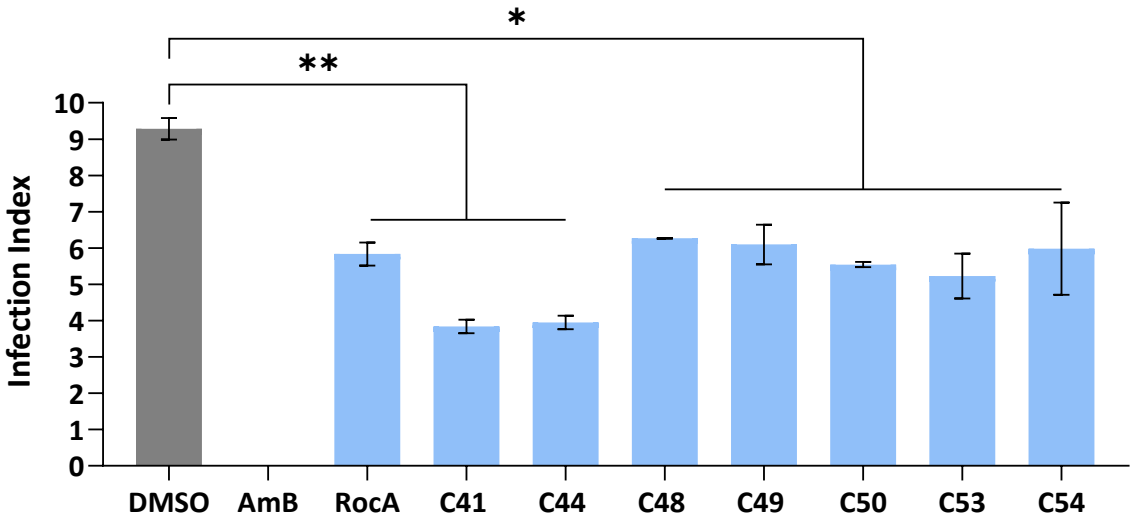


Figure 4.5 Comparison of the infection index of BMDM treated with 7 synthetic rocaglates and Roc-A

BMDM cultures were inoculated with *L. amazonensis* parasites (MOI10) for 24 h and subsequently treated with 20 nM of synthetic rocaglates, 15 nM of Roc-A or an equivalent volume of DMSO (vehicle) for 72 h. The slides were stained with DAPI and quantification of intracellular parasites was performed by calculating the infection index. Data are representative of three independent experiments (n=3) performed in technical triplicates. Data are presented as mean ± SD. * = $p < 0.05$, ** = $p < 0.01$ (t-test)

4.5 The anti-leishmanial activity of rocaglates C41 and C44 is dose-dependent

To obtain a more thorough understanding of the impact of compounds 41 and 44, we conducted a dose-dependent assay. In this assay, BMDMs were infected as described and then exposed to increasing concentrations (ranging from 1 nM to 20 nM) of C41 and C44 for 72 h. Remarkably, both compounds 41 and 44 exhibited enhanced efficacy at 20 nM compared to the DMSO control (Figure 4.6). Although compound 41 at 10 nM also reduced the infection index compared to the control, the effect was less pronounced than at the 20 nM concentration. Based on these data, we decided to continue using the 20 nM concentration for our compounds, as it demonstrated the most significant effect and falls within the nanomolar range.

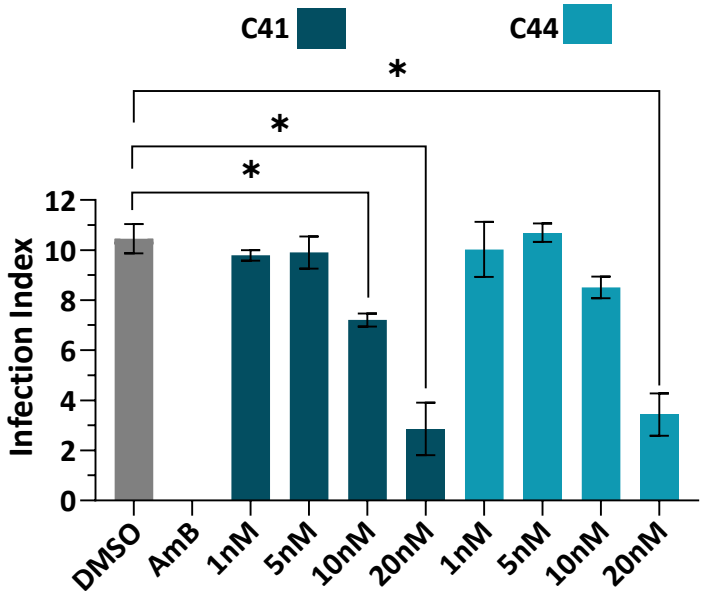


Figure 4.6 Compounds 41 and 44 presented a dose-dependent effect

BMDM cultures were inoculated with *L. amazonensis* parasites (MOI10) for 24 h and subsequently treated with increasing concentrations of compounds (1nM – 20nM) or an equivalent volume of DMSO (vehicle) for 72 h. The slides were stained with DAPI and quantification of intracellular parasites was performed by calculating the infection index. Data are representative of two independent experiments (n=2) performed in technical duplicates. Data are presented as mean ± SD. * = $p < 0.05$ (t-test).

4.6 Compounds 41 and 44 exhibit anti-leishmanial activity in human macrophages (THP-1)

In addition to evaluating the compounds in mouse primary macrophages, we opted to assess their efficacy in human macrophages, given the importance of leishmaniasis treatment in the context of the human host. To address this, THP-1 monocytic-like cells were differentiated into macrophages via a 72-h PMA treatment. Subsequently, the cells were infected and treated with rocaglates following the same regimen used for the BMDMs. Surprisingly, RocA exhibited no impact on the infection index in these cells compared to the DMSO control (Figure 4.7). In contrast, both C41 and C44 effectively reduced the infection index in THP-1 cells in over 50% mirroring our findings with BMDMs. These data suggest that these rocaglates also possess anti-leishmanial activity in human macrophages and could be considered for future studies utilizing these cells.

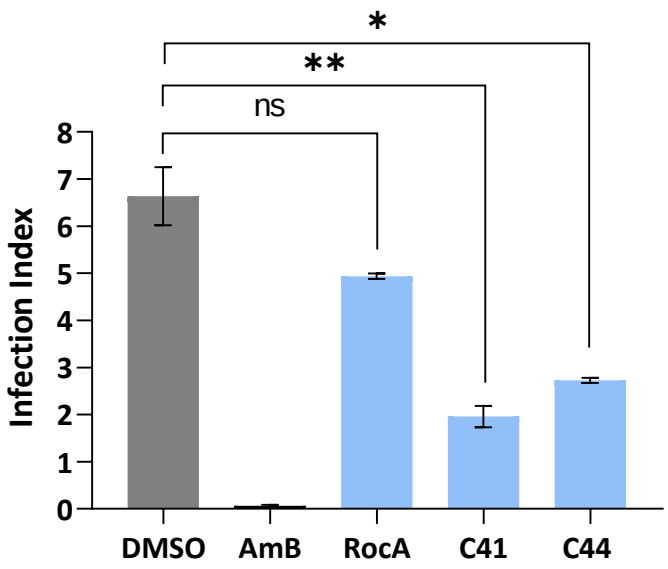


Figure 4.7 Compounds 41 and 44 exhibit anti-leishmanial activity in THP-1

THP-1 were inoculated with *L. amazonensis* parasites (MOI 10) for 24 h and subsequently treated with 20 nM of synthetic rocaglates, 15 nM of Roc-A or an equivalent volume of DMSO (vehicle) for 72 h. The slides were stained with DAPI and quantification of intracellular parasites was performed by calculating the infection index. Data are representative of two independent experiments (n=2) performed in technical duplicates. Data are presented as mean ± SD. * = $p < 0.05$, = $p < 0.01$ (t-test). ns = non-significant.**

4.7 The anti-leishmanial activity of rocaglates C41 and C44 is host eIF4A-independent

As previously discussed, the synthetic rocaglates utilized in this study were previously categorized as having low affinity towards eIF4A. To test our hypothesis that these compounds exert their anti-leishmanial effect through an eIF4A-independent mechanism, we utilized bone marrow precursors obtained from heterozygous mice *Eif4A1*$^{+/-}$ and *Eif4A2*$^{+/-}$. These cells, derived from animals with frameshift mutations in one allele of *Eif4a1* and *Eif4a2*, exhibit reduced levels of eIF4A1 or eIF4A2 compared to WT mice (Sénéchal *et al.*, 2021).

The rationale behind this experiment was to determine whether C41 and C44 would still demonstrate an anti-leishmanial effect in cells lacking functional eIF4A. Interestingly, DMSO control of *Eif4A1*$^{+/-}$ and *Eif4A2*$^{+/-}$ BMDMs exhibited a reduced infection index compared to WT BMDMs, indicating the significance of host eIF4A in *L. amazonensis* infection (Figure 4.8). When compared to the effect on WT BMDMs, Roc-A no longer displayed an anti-leishmanial effect on *Eif4A1*$^{+/-}$ and *Eif4A2*$^{+/-}$ BMDMs (Figure 4.8). These data strongly suggest that despite having an alternative target (e.g., DDX3X) (Chen *et al.*, 2021), the anti-leishmanial effect observed in BMDMs treated with Roc-A is dependent on host eIF4A. In contrast, C41 and C44 maintained their anti-leishmanial effect on *Eif4A1*$^{+/-}$ and *Eif4A2*$^{+/-}$ BMDMs (Figure 4.8). This finding

demonstrates that the efficacy of these compounds in controlling *L. amazonensis* infection *in vitro* is independent of host eIF4A.

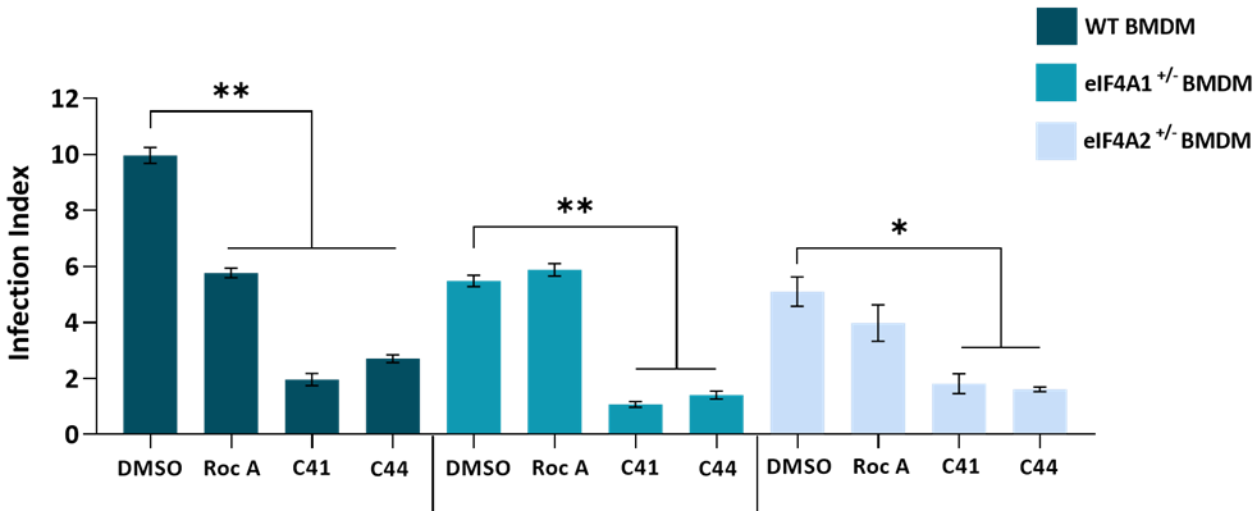


Figure 4.8 C41 and C44 host eIF4A-indepented anti-leishmanial effect

BMDM cultures were inoculated with *L. amazonensis* parasites (MOI 10) for 24 h and subsequently treated with 20 nM of synthetic rocaglates, 15 nM of Roc-A or an equivalent volume of DMSO (vehicle) for 72 h. The slides were stained with DAPI and quantification of intracellular parasites was performed by calculating the infection index. Data are representative of two independent experiments (n=2) performed in technical duplicates. Data are presented as mean ± SD. * = p < 0.05, ** = p < 0.01 (one-way ANOVA).

4.8 FP assays with C41 and C44 reveal no interaction with eIF4A1 nor DDX3X

Considering our previous findings, our collaborators (Dr. Sidong Huang laboratory, McGill University, Montreal, QC, Canada) decided to conduct a fluorescence polarization (FP) assay. The objective was to evaluate the interaction between the compounds and two significant DEAD-box helicases, eIF4A1 and DDX3X, as both have been identified as targets of rocaglates (Chen *et al.*, 2021). Given our assertion regarding the compounds' eIF4A-independent anti-leishmanial effect, we were intrigued to explore if the compounds demonstrated any affinity towards DDX3X.

Consistent with our findings, C44 displayed reduced interaction with eIF4A1 compared to CR-1-31B (Figure 4.9), a synthetic rocaglate recognized for its potent inhibitory effects on eIF4A and its anti-plasmodial activity (Langlais *et al.*, 2018). In line with our findings in BMDM derived from *Eif4A1*$^{+/-}$ and *Eif4A2*$^{+/-}$ mice, compound 41 showed no affinity towards eIF4A1 compared to the controls. Regarding the interaction with DDX3X, neither C41 nor C44 exhibited any affinity towards that protein (Figure 4.9). Taken together, these data suggest that compounds 41 and 44 do not target eIF4A1 or DDX3X.

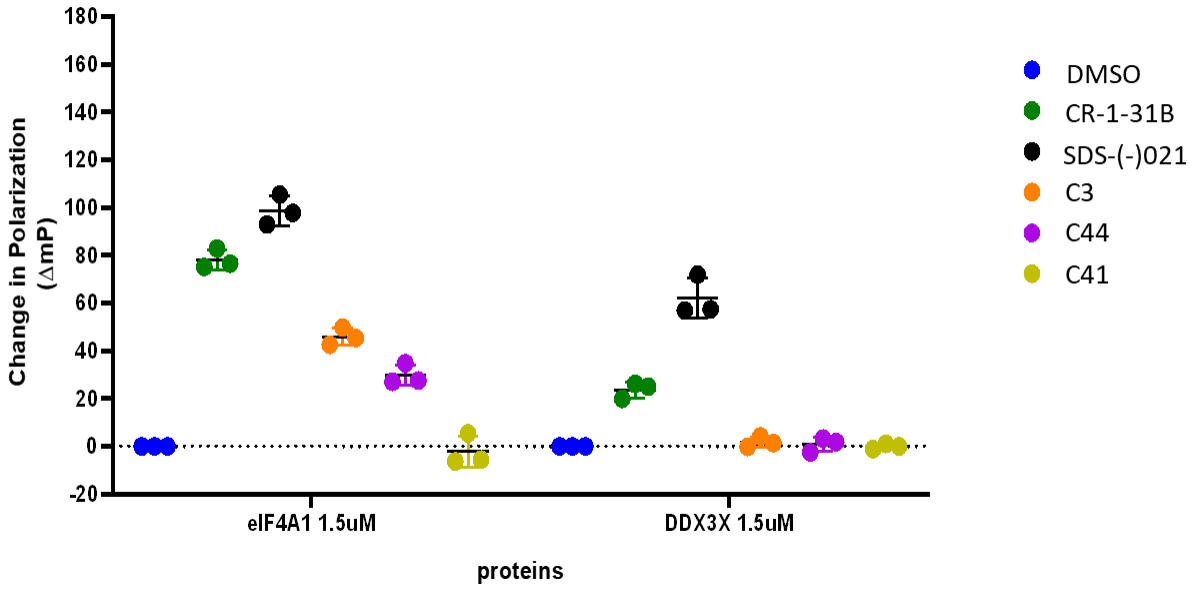


Figure 4.9 Fluorescence polarization assay with compounds 41 and 44

1.5 uM protein and 10uM of the compounds was used in the presence of ATP. Compounds labeled as potent eIF4A clampers (e.g., CR-1-31B and SDS-(-)021) demonstrated strong binding affinity to eIF4A1, whereas compounds 3, 44, and 41, categorized as weak eIF4A clampers, exhibited minimal or negligible affinity to eIF4A1. In terms of DDX3X, all compounds displayed low affinity towards this helicase (Unpublished data, Jerry Pelletier and Sidong Huang laboratories, McGill University).

4.9 Compounds 41 and 44 appear to stall *L. amazonesis* intramacrophage replication

To continue uncovering the mechanism underlying the anti-leishmanial effect of C41 and C44, we conducted a time-course assay. In this experiment, BMDM cells were infected and treated as described (i.e., treatment after a 24-h infection period); however, unlike previous experiments, slides were collected at 6, 12-, 24-, 48-, and 72-h post-treatment. The objective of this experiment was to investigate whether the compounds were killing the parasites and/or preventing their replication.

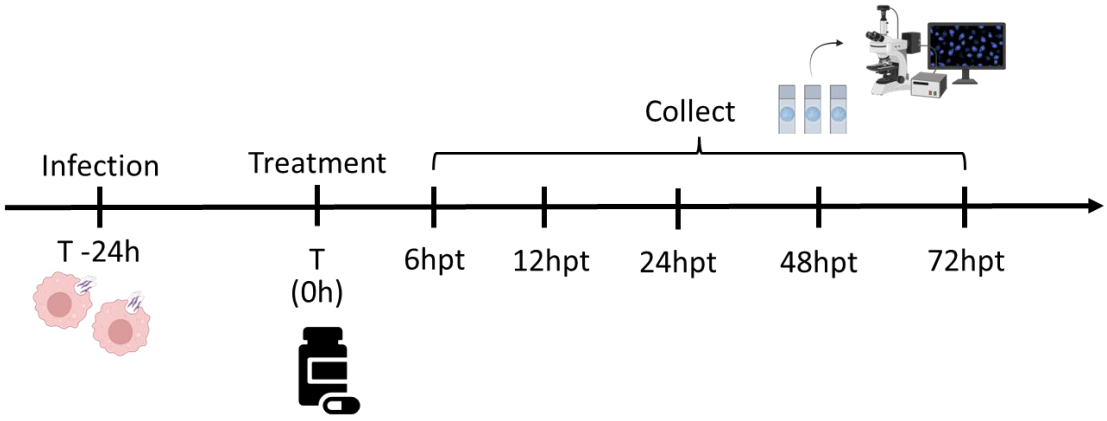


Figure 4.10 Schematic representation of kinetics experiment

After 24 hours of infection the treatments were introduced to the culture as represented at "0h". Slides were collected after 6-, 12-, 24-, 48-, and 72- hours post-treatment.

As illustrated in the graph below, AmB served as the control in this experiment, and it was evident that this drug killed the parasites within the initial hours of infection (Figure 4.10). Conversely, C41 and C44 appeared to inhibit parasite intramacrophage replication since their introduction to the culture among with the other treatments, represented as "0h" in the graph (e.g. 24 hours post-infection) (Figure 4.10). These data suggest that a component of the mechanism of action of rocaglates 41 and 44 is to arrest parasite replication inside macrophages from the moment they are introduced to the culture.

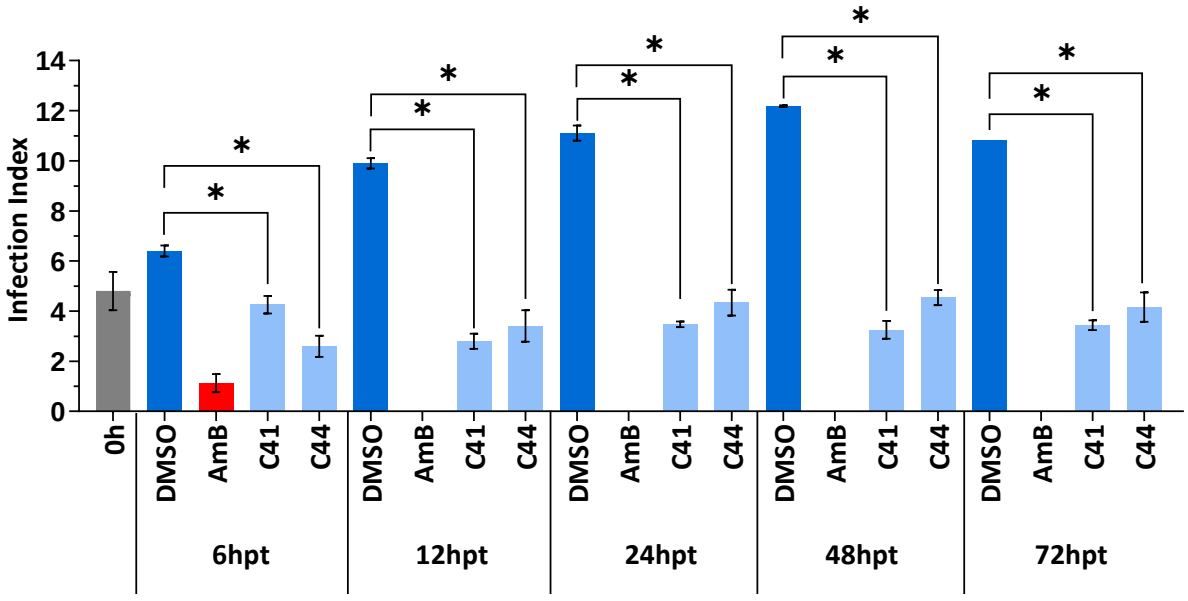


Figure 4.11 C41 and C44 appear to stall *L. amazonensis* intramacrophage replication

BMDM cultures were inoculated with *L. amazonensis* parasites (MOI 10) for 24 h and subsequently treated with 0.25 µg/ml AmpB, 20 nM of synthetic rocaglates, or an equivalent volume of DMSO (vehicle) for 6, 12, 24, 48 and 72 h. The slides were stained with DAPI and quantification of intracellular parasites was performed by calculating the infection index. Data are representative of two independent experiments (n=2) performed in

technical duplicates. Data are presented as mean ± SD. * = p < 0.05, ** = p < 0.01 (t-test). hpt: hours post-treatment.

4.10 Amastigotes recovered from cells treated with C44 but not C41 are able to replicate in untreated BMDMs

Having previously investigated the impact of compounds 41 and 44 on the viability of extracellular promastigote forms as well as their impact in the replication of intracellular amastigotes, our next objective was to assess whether these compounds could influence the infectivity of the amastigote forms. To address this, BMDM were infected for 24 h and subsequently treated for 72 h, following the described protocol. After the treatment regimen, the amastigote forms were isolated from the cells and used to infect fresh BMDMs that had not undergone any form of treatment (Figure 4.11).

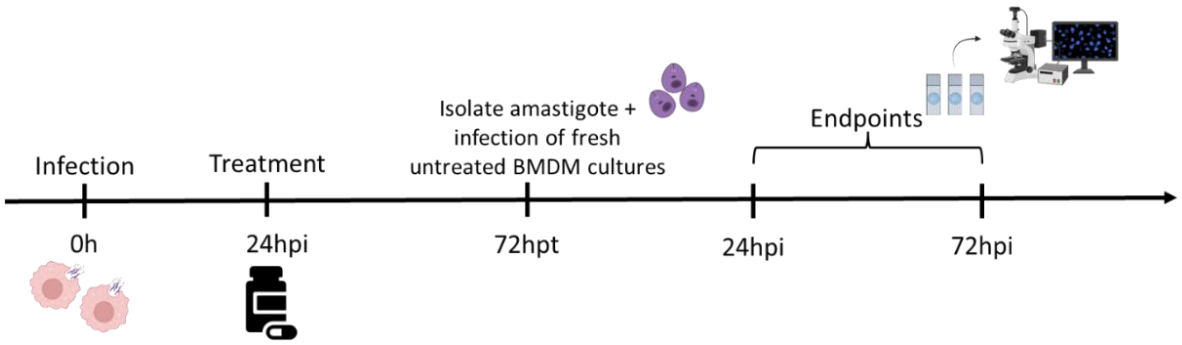


Figure 4.12 Schematic representation of amastigote infectivity assay

Illustration created with Biorender.

For this experiment, we selected two time-points (24- and 72-h post-infection) to compare and evaluate parasite replication. Amastigotes recovered from cells previously treated with DMSO were capable of replicating within the experiment timeframe, as were those recovered from cells previously treated with RocA and C44 (Figure 4.12). Interestingly, amastigotes recovered from cells previously treated with C41 were unable to replicate in a new infection (Figure 4.12). These data indicate that RocA and C44 do not appear to affect the infectivity of the amastigote form and may not target this stage of the parasite. However, compound 41 seems to have the capability of affecting amastigote infectivity, suggesting that this compound may target this specific form of the parasite.

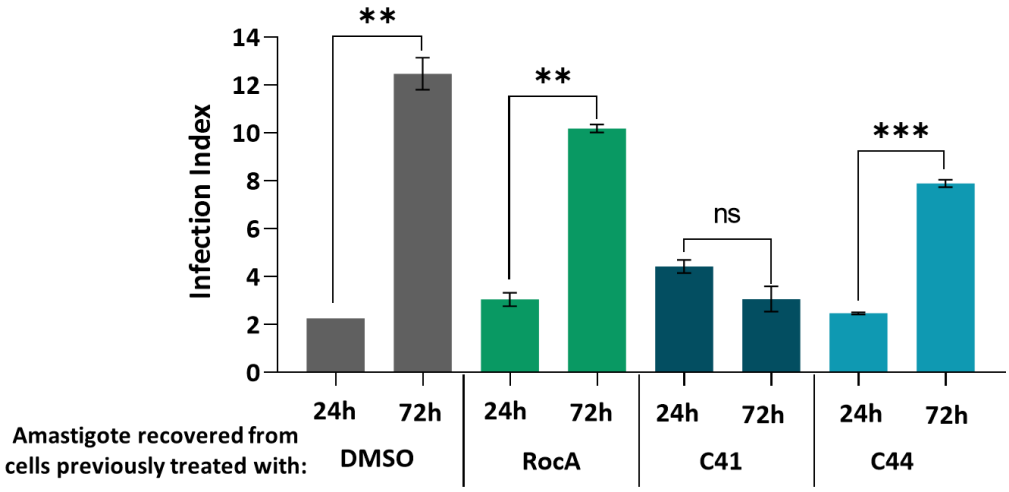


Figure 4.13 Amastigotes recovered from cells treated with C44 but not C41 are able to replicate in untreated BMDMs

BMDM cultures were inoculated with *L. amazonensis* parasites (MOI 10) for 24 h and subsequently treated with 20 nM of C41 or C44, 15 nM of Roc-A or an equivalent volume of DMSO (vehicle) for 72 h. Amastigotes were isolated using 0.025% SDS and used to infect fresh differentiated BMDM for 24 and 72 h. The slides were stained with DAPI and quantification of intracellular parasites was performed by calculating the infection index. Data are representative of two independent experiments (n=2) performed in technical duplicates. Data are presented as mean ± SD. ** = $p < 0.01$, *** = $p < 0.001$ (t-test). ns = non-significant.

5 DISCUSSION

Despite affecting thousands of individuals annually, leishmaniasis remains a challenging disease to manage and treat effectively (Roatt *et al.*, 2020). The absence of a reliable human vaccine means treatment relies on a limited arsenal of drugs, many of which have been repurposed with significant obstacles such as severe side effects and high rates of drug resistance. These circumstances often result in treatment failures (Kaye *et al.*, 2021; Santos *et al.*, 2023). Consequently, it is crucial to further explore the complex interplay between the parasite and its host, and it is imperative to seek out new targets and strategies to combat this disease. Numerous studies have highlighted macrophages as the primary targets of *Leishmania*, that takes advantage of their cellular plasticity (Barbosa *et al.*, 2018; Chandrakar *et al.*, 2020; Tomiotto-Pellissier *et al.*, 2018). Through various mechanisms (i.e, epigenetic, transcriptional, post-transcriptional, translational, and post-translational), these parasites aim to evade the host immune response and manipulate cellular processes to enhance their survival and replication (Chaparro *et al.*, 2020; Diotallevi *et al.*, 2024; Reyaz *et al.*, 2024).

To date, numerous studies have underscored the therapeutic potential of synthetic and natural rocaglates in bacterial, viral, and protozoan parasitic diseases (Bhattacharya *et al.*, 2016; Chatterjee *et al.*, 2021; Langlais *et al.*, 2018; Müller *et al.*, 2021; Praditya *et al.*, 2022). These compounds are known to effectively target eIF4A, using this helicase to disrupt the activity of the eIF4F complex and consequently inhibit the translation of eIF4A-sensitive mRNAs (Chu *et al.*, 2020; Iwasaki *et al.*, 2016). Given our research revealing an increase in the translation efficiency of eIF4A-sensitive mRNAs during *Leishmania* infection, this helicase emerged as a promising target for potential therapeutic interventions (Chaparro *et al.*, 2020). Consequently, by employing a potent rocaglate such as Silvestrol to inhibit eIF4A, we observed a reduction in *L. donovani* replication within the host cell (Chaparro *et al.*, 2020). Moreover, these findings intensified our interest in exploring rocaglates in the context of leishmaniasis. Through the acquisition of a library of synthetic rocaglates and subsequent screening, we identified compounds exhibiting significant anti-leishmanial potential *in cellulo*. Notably, alongside compounds acting as robust eIF4A inhibitors, we also uncovered the antiparasitic activity of a subset with little to no affinity for eIF4A.

5.1 Rocaglates can be potent anti-leishmanial agents even without strong eIF4A binding

Considering that the subset of rocaglates with low or no binding affinity for eIF4A had not been previously studied or tested in a cell-based model, this project was conceived with a primary focus on identifying and applying rocaglates that, despite not directly targeting the eIF4A helicase, exhibit potent anti-leishmanial activity. To evaluate *L. amazonensis* persistence in BMDMs during rocaglate treatment, we conducted a screening using a library of 14 rocaglates from the BU-CMD collection. This screening enabled us to identify 7 compounds with low or no binding activity for eIF4A exhibiting anti-leishmanial activity, reducing the infection index by at least 50% in infected cells (Figure 4.2).

5.2 Exploring the toxicity of rocaglates

Given the notable cytotoxicity associated with rocaglates, the initial screening was crucial for testing the compounds at a nanomolar concentration, as suggested by previous studies (Greger, 2022; Obermann *et al.*, 2022). Assessing the toxicity of 14 compounds is time-consuming and requires a large number of cells. Despite these caveats, the first screening enabled us to select compounds that demonstrated the most promising effects within the concentration range deemed suitable for further experimentation.

After pre-selecting compounds in the initial screening, our aim was to eliminate any compounds or concentrations that exhibited excessive toxicity to the host cell. According to a study, in primary human immune cells, the cytotoxic concentration that reduced cell viability by 50% (CC_{50}) for Silvestrol was 45.6 nM, for CR-1-31-B it was 8.8 nM, and Zotatifin was above 100 nM in M1 macrophages (Obermann *et al.*, 2022). The same study revealed that in M2 macrophages, all these rocaglates had a CC_{50} above 100 nM. Schiffmann et al. also reported differents effects of Silvestrol in the viability of cancer cell lines (Schiffmann *et al.*, 2022). In our investigation, we considered concentrations toxic when they resulted in a reduction of 30% or more in macrophage viability. Surprisingly, within this parameter, the compounds that we used in this study seemed to be well tolerated by BMDMs and the ones that presented toxicity only did so in the micromolar range (Figure 4.3). Taken together, these data suggest that the toxicity of rocaglates can vary depending on the cell type and the nature of the compound.

It is important to note the low toxicity of the rocaglates used in this study, especially when compared to existing treatments for leishmaniasis, which often exhibit high toxicity and numerous side effects leading to treatment relapse. For instance, AmB application has been associated with

nephrotoxicity and infusion-related reactions, such as fever, chills, joint pain, nausea, vomiting, and headaches, primarily due to proinflammatory cytokine reactions (Arning *et al.*, 1995; Hamill, 2013). While the liposomal formulation of AmB mitigates some side effects, its production cost significantly rises compared to conventional AmB (Adler-Moore *et al.*, 2016; Shirzadi, 2019). Naturally, we must consider the limitations of the *in cellulo* assays conducted in this study (i.e., time-consuming manual quantification of intracellular parasites). However, our data underscore the importance of continued investigation into these compounds and their application using *in vivo* models to accumulate further insight into the physiological ramifications of rocaglate application.

5.3 Rocaglates and LeIF4A

Leishmania parasites feature a homologue of eIF4A, named LeIF4A, which has been explored as a potential target for drug development (Harigua-Souiai *et al.*, 2018; Koutsoni *et al.*, 2014). Analysis of eIF4A sequences across various microorganisms has uncovered the potential resistance of *Leishmania spp*. to rocaglate treatment. This resistance appears linked to substitution patterns within critical residues of eIF4A structure, essential for rocaglate binding (Obermann *et al.*, 2023). The same group proposed that the resistance to rocaglates does not stem from exposure but rather emerges as a consequence of evolutionary diversification in the eIF4A sequence. Although our collaborators described the compounds used in this study as having low or no affinity to eIF4A and given the uncertainty surrounding their potential impact on the parasite fitness, we opted for assessing the effect of the rocaglates on extracellular promastigotes of *L. amazonensis*. It is noteworthy to mention that, at this juncture, we also aimed to investigate whether the compounds might target a molecule within the extracellular promastigote.

Langlais et al. demonstrated a 70% identity between mammalian eIF4A1 and *Plasmodium falciparum* eIF4A (PfeIF4A). Furthermore, it was observed that CR-1-31B and Silvestrol could interact with PfeIF4A and inhibit the protein synthesis of specific targets (Langlais *et al.*, 2018). Moreover, a dual luciferase reporter assay demonstrated that CR-1-31B effectively suppressed the translation of mRNAs containing the viral 5′-UTRs of SARS-CoV-2, HCoV-229E, and MERS-CoV (Müller *et al.*, 2021). This suggested that the 5′-UTRs of these viruses were responsive to eIF4A-dependent translation. In our study, however, we did not observe any effect of our rocaglate subset on the viability of extracellular promastigotes (Figure 4.4), indicating that these compounds may not target any specific component within the promastigote form, including LeIF4A. Additionally, as mentioned above, *Leishmania* parasites may exhibit resistance to

rocaglates (Obermann *et al.*, 2023). Therefore, we believe the likelihood of C41 and C44 binding directly to LeIF4A is low, and further experiments are needed to draw more definitive conclusions.

To our understanding, only one investigation has examined the impact of Rocaglamide (i.e., natural rocaglate) on the growth of extracellular parasites of *L. infantum*. This study demonstrated half-maximal effective concentrations (EC_{50}) of 16.45 uM and 5.76 uM at 24 hours and 48 hours post-treatment, respectively (Astelbauer *et al.*, 2011). However, in our study, the rocaglates used did not impact the viability of extracellular promastigotes (Figure 4.4). The discrepancy between Astelbauer et al. study and our data may come from different factors, including the use of distinct compounds across studies (i.e., natural and synthetic rocaglates) and the fact that our study used rocaglates with no or low binding affinity for eIF4A. Consequently, it is imperative to consider the unique chemical characteristics of each compound. Additionally, in our research, we employed resazurin-based assays to assess parasite viability by measuring mitochondrial activity, whereas Astelbauer et al. employed counting chambers to quantify parasite growth throughout the treatment process (Astelbauer *et al.*, 2011).

This outcome suggests that this particular form of the parasite lacks potential targets for our compounds. Since our primary aim was to exploit the compounds for host-directed therapy and assess their impact on intracellular amastigotes, we did not interpret this result negatively. Instead, we proceeded with further experiments to evaluate the treatment of infected cells and assess the effects of this treatment on the infectivity of amastigotes derived from treated cells.

5.4 The effect of rocaglates on parasite replication within macrophages

Following three rounds of experiments assessing the seven initially pre-selected compounds and evaluating their impact on intramacrophage replication of the amastigote, we determined that C41 and C44 emerged as the most promising candidates for further investigation into their mechanism of action (Figure 4.5). It is noteworthy to mention that in this experiment, we observed a lack of effect from C54 in contrast to what was observed in our initial screening (Figure 4.2). This observation may be attributed to the formulation and resulting solubility of the compound, and it is an issue that will be discussed with our collaborators for future experiments. Our dose-response experiment revealed that compounds 41 and 44 ceased to exhibit the same efficacy in reducing intramacrophage replication of the parasite under 20 nM concentration, thus guiding our selection for subsequent experiments (Figure 4.6). Additionally, employing a 20 nM concentration proved sufficient to observe the anti-leishmanial effect of these compounds in infected macrophages differentiated from the human monocytic-like cell lineTHP-1 (Figure 4.7).

Studies conducted with human monocyte-derived macrophages (MdMs) have demonstrated that Zotatifin, CR-1-31-B, and Silvestrol can influence the differentiation and polarization of these cells. In general, these rocaglates were found to reduce the expression of CD14 and decrease the release of IL-6, IL-10, CCL17, and CCL18, which could supress the recruitment of immune cells and, consequently, the immune response (Blum *et al.*, 2020; Schiffmann *et al.*, 2023). The same research group employed CR-31-B(+), the inactive enantiomer of CR-31-B, which lacks the ability to bind to the rocaglate binding site of the eIF4A-RNA complex. Interestingly, this inactive enantiomer did not demonstrate effects on MdMs differentiation and polarization, indicating that the ability to bind to eIF4A is associated with the observed effects (Schiffmann *et al.*, 2023). Altogether, these studies demonstrated that inhibiting eIF4A suppressed the inflammatory strength of M1 human macrophages, T cells, and B cells, leading to a reduction in cytokine release by these cells. Moreover, as specified, the compounds used for this project were characterized as having low or no affinity to eIF4A during *in vitro* binding assays. Therefore, despite the observed impact of our compounds on intramacrophage replication of parasites in THP-1 cells, it remains essential to assess their effects on infected primary human macrophages and characterize the immune response in these cells.

5.5 An insight on the mechanism of action of rocaglates with low or no eIF4A binding affinity

Subsequently, one of the pivotal aspects of our study was to determine whether the observed effect in infected cells was contingent on eIF4A. Prior to their evaluation in our laboratory, these compounds had only undergone cell-free *in vitro* assays. Thus, employing a cellular model became imperative to validate our hypothesis effectively. To address this our collaborator Dr. Jerry Pelletier (McGill University, Montreal, Canada) supplied us with *Eif4A1*$^{+/-}$ and *Eif4A2*$^{+/-}$ BMDMs obteined from mice modified using CRISPR-Cas9. These mice have premature stop codons, resulting in nonfunctional proteins (Sénéchal *et al.*, 2021). Interestingly, this experiment allowed us to observe that when compared to the WT BMDMs, the DMSO control of *Eif4A1*$^{+/-}$ and *Eif4A2*$^{+/-}$ BMDMs presented a significantly lower infection which indicates the importance of host eIF4A to the success of the parasite in the infection (Figure 4.8). Moreover, in contrast to all previously published data on rocaglates (Chu *et al.*, 2020; Iwasaki *et al.*, 2016; Wolfe *et al.*, 2014), our study demonstrated that the subset of rocaglates applied here operates via a host eIF4A-independent mechanism of action (Figures 4.8 and 5.1). To better understand the mechanism of action of our compounds, we plan to use thermal proteome profiling with BMDM extracts and infected BMDM extracts. This approach will help determine whether LeIF4A is involved in the

action of these compounds and identify potential targets of these rocaglates in the host cell and/or parasite.

5.6 Investigating DDX3X as a possible target for rocaglates with low or no affinity to eIF4A

As our compounds demonstrated a mechanism of action independent of host eIF4A, we opted to explore other potential proteins. Considering DDX3X as an alternative target for Roc-A (Chen *et al.*, 2021), we decided to delve deeper into its investigation. DDX3X plays crucial roles in embryo development, and reduced expression of DDX3X results in elevated genome damage and cell cycle arrest during embryogenesis (Chen *et al.*, 2016). While establishing a cre lox mice system and deleting the gene from the myeloid compartment is feasible (Samir *et al.*, 2019), it was deemed time-consuming. Thus, we enlisted the help of our collaborators to conduct an *in vitro* assay measuring the interaction between our selected compounds and murine DDX3X. Interestingly, C41 and C44 exhibited no affinity towards the host DEAD-box helicase (Figure 4.9). *Leishmania* harbors a DDX3X homolog known as Hel67, which is pivotal for mitochondrial metabolism, parasite proliferation, and differentiation (Padmanabhan *et al.*, 2016; Pandey *et al.*, 2020). To date, we have not conducted experiments involving Hel67. Molecular docking experiments suggest that Hel67 lacks the requisite binding sites for our compounds; however, further experiments are warranted to determine whether this protein is a potential target of rocaglates C41 and C44 (Dr. John Porco and Dr. Lauren Brown laboratory, unpublished data).

5.7 Understanding the kinetics involved in rocaglates treatment

In addition to identifying potential targets for the rocaglates applied in this study, we also developed a keen interest in the treatment kinetics. Despite our data indicating no toxicity to extracellular promastigotes, initially, we were uncertain whether the observed effect on intramacrophage amastigotes resulted from parasite elimination or inhibition of replication. Consequently, our time-course assay enabled us to delineate the treatment kinetics, revealing that C41 and C44 have been effectively impeding parasite replication since their introduction to the culture (Figure 4.10). This effect could prove highly advantageous for the host, particularly in the context of combining therapies. While AmB has exhibited numerous side effects during treatment, Miltefosine effectively controls *Leishmania* parasites but encounters the issue of parasite resistance (Croft *et al.*, 2011; Tunalı *et al.*, 2021). In this context, we consider whether employing a rocaglate to halt parasite replication, alongside a reduced dosage of AmB,

Miltefosine, or even SSG, could facilitate parasite elimination while mitigating adverse effects in treated patients and combating parasite resistance.

5.8 Selected rocaglate affect the infectivity of amastigote stage

Furthermore, given that none of the rocaglates tested thus far has demonstrated an effect on extracellular promastigotes, we pursued to investigate whether these compounds could impact the infectivity of the amastigote form. As depicted in our findings, C41 exhibited the potential to reduce the infection capacity of amastigotes derived from cells treated with this compound (Figures 4.12 and 5.1). These data have several potential implications. Firstly, they suggest that C41 might target a molecule specific to the amastigote form, thereby affecting the infectivity of this parasite form over the long term. Moreover, the successful infection of the sand fly vector is a critical stage for the survival of this parasite in its natural environment and for the continuity of its life cycle (Burza *et al.*, 2018). Taking this into consideration, it is interesting to speculate that amastigotes originating from cells treated with C41 would not be able to undergo normal development into promastigotes and replication within the sandfly vector and the implications this might have on the parasite life cycle and transmission of the infection.

5.9 Constraints and future directions

In summary, this study enabled us to pinpoint compounds exhibiting a promising anti-leishmanial effect while maintaining low toxicity to host cells. However, we recognize the limitations of our methodology, particularly in the time-consuming nature and potential biases of our infection assays, which rely on manual quantification of intracellular parasites. To address this, we plan to develop a semi high-throughput screening method using luciferase-expressing *L. amazonensis* parasites. Like the method demonstrated by Reimão et al., this approach allows us to measure luminescence during infection as an indicator of parasite burden (Reimão *et al.*, 2013). To further strengthen the robustness of our viability data and investigate the mechanism of action of the rocaglates used in our study, we also plan to use an apoptosis detection kit to identify early apoptotic and necrotic cells. In addition, we will monitor ERK signaling, as rocaglates have been shown to inhibit ERK phosphorylation, among other pathways, and promote M1 polarization (Chatterjee *et al.*, 2021). Furthermore, we plan to include additional species and clinical isolates of *Leishmania* parasites in our studies. This broader approach will help us better understand the effect of the compounds across different species.

Preliminary findings from a pilot polysome tracing experiment indicate that compounds with low affinity to eIF4A do not inhibit global translation (Jaramillo Laboratory, unpublished data), yet further experiments are necessary to delve deeper into translation inhibition of a specific subset of transcripts. Additionally, we plan to employ a multi-omics approach to identify alterations in the translatome and proteome of both the parasite and host cell following rocaglate treatment. Ultimately, this approach may aid in pinpointing potential targets for the compounds. Our pursuit of these targets extends beyond unraveling the mechanism of action of these drugs, it also underscores their significance in the context of *Leishmania* infection.

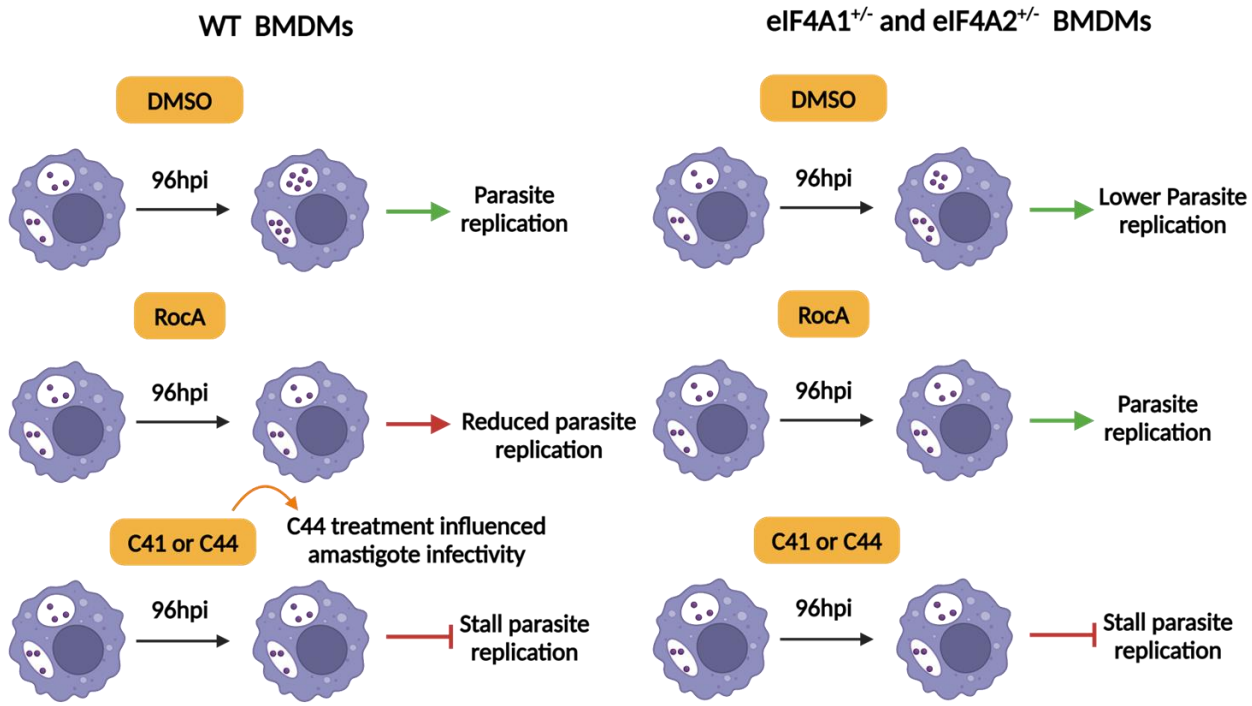


Figure 5.1 Proposed Model for the anti-leishmanial effect of rocaglates C41 and C44 in BMDMs Illustration created with Biorender.

6 CONCLUSION

Throughout this study, we have highlighted the challenges associated with current treatments for leishmaniasis, such as high cost, toxicity, side effects, and drug resistance. In an effort to address these challenges, researchers in the field have focused on better understanding the mechanisms underlying host-parasite interactions. Thus far, it is evident that *Leishmania* disrupts host cell functions through various mechanisms, including epigenetic, transcriptional, post-transcriptional, translational, and post-translational processes.

Our investigation into eIF4A in the context of *Leishmania* infection has yielded fruitful results, emphasizing the significance of this helicase in the success of the infection. Moreover, our interest in using rocaglates during *Leishmania* infection prompted us to delve deeper into the mechanism of action of the specific subset of rocaglates examined in this study. Our data have shed light on a part of this mechanism and revealed that it is not dependent on host eIF4A. Furthermore, we have demonstrated that these compounds effectively stall parasite replication, prompting us to hypothesize about the potential implications of this finding in the context of leishmaniasis treatment.

It is imperative for us to assess the efficacy of these rocaglates against other *Leishmania* species and clinical isolates, including strains responsible for VL. Moreover, validating the data presented here using an *in vivo* model is crucial for further understanding the potential therapeutic applications of these compounds.

7 REFERENCES

Aderem A (2003) Phagocytosis and the Inflammatory Response. *The Journal of Infectious Diseases* 187(s2):S340-S345.

Adler-Moore J, Proffitt RT (2002) AmBisome: liposomal formulation, structure, mechanism of action and pre-clinical experience. *Journal of Antimicrobial Chemotherapy* 49(suppl_1):21-30.

Adler-Moore JP, Gangneux J-P, Pappas PG (2016) Comparison between liposomal formulations of amphotericin B. *Medical Mycology* 54(3):223-231.

Alcântara LM, Ferreira TCS, Gadelha FR, Miguel DC (2018) Challenges in drug discovery targeting TriTryp diseases with an emphasis on leishmaniasis. *Int J Parasitol Drugs Drug Resist* 8(3):430-439.

Alexander J, Satoskar AR, Russell DG (1999) Leishmania species: models of intracellular parasitism. *Journal of Cell Science* 112(18):2993-3002.

Alvar J, Alves F, Bucheton B, Burrows L, Büscher P, Carrillo E, Felger I, Hübner MP, Moreno J, Pinazo M-J, Ribeiro I, Sosa-Estani S, Specht S, Tarral A, Wourgaft NS, Bilbe G (2020) Implications of asymptomatic infection for the natural history of selected parasitic tropical diseases. *Seminars in Immunopathology* 42(3):231-246.

Arango Duque G, Descoteaux A (2015) Leishmania survival in the macrophage: where the ends justify the means. *Current Opinion in Microbiology* 26:32-40.

Arango Duque G, Jardim A, Gagnon É, Fukuda M, Descoteaux A (2019) The host cell secretory pathway mediates the export of Leishmania virulence factors out of the parasitophorous vacuole. *PLOS Pathogens* 15(7):e1007982.

Arcanjo AF, Larocque-De-Freitas IF, Rocha JDB, Zamith D, Costa-Da-Silva AC, Nunes MP, Mesquita-Santos FP, Morrot A, Filardy AA, Mariano M, Bandeira-Melo C, Dosreis GA, Decote-Ricardo D, Freire-De-Lima CG (2015) The PGE2/IL-10 Axis Determines Susceptibility of B-1 Cell-Derived Phagocytes (B-1CDP) to Leishmania major Infection. *PLoS One* 10(5):e0124888.

Arning M, Kliche KO, Heer-Sonderhoff AH, Wehmeier A (1995) Infusion-related toxicity of three different amphotericin B formulations and its relation to cytokine plasma levels. *Mycoses* 38(11-12):459-465.

Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, Carvalho EM, Ephros M, Jeronimo S, Magill A (2016) Diagnosis and Treatment of Leishmaniasis: Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Clinical Infectious Diseases* 63(12):1539-1557.

Aronson NE, Wortmann GW, Byrne WR, Howard RS, Bernstein WB, Marovich MA, Polhemus ME, Yoon IK, Hummer KA, Gasser RA, Jr., Oster CN, Benson PM (2010) A randomized controlled trial of local heat therapy versus intravenous sodium stibogluconate for the treatment of cutaneous Leishmania major infection. *PLoS Negl Trop Dis* 4(3):e628.

Astelbauer F, Obwaller A, Raninger A, Brem B, Greger H, Duchêne M, Wernsdorfer W, Walochnik J (2011) Anti-leishmanial activity of plant-derived acridones, flavaglines, and sulfur-containing amides. *Vector Borne Zoonotic Dis* 11(7):793-798.

Awasthi A, Mathur R, Khan A, Joshi BN, Jain N, Sawant S, Boppana R, Mitra D, Saha B (2003) CD40 Signaling Is Impaired in L. major–infected Macrophages and Is Rescued by a p38MAPK Activator Establishing a Host-protective Memory T Cell Response. *The Journal of experimental medicine* 197(8):1037-1043.

Bacellar O, Lessa H, Schriefer A, Machado P, Ribeiro de Jesus A, Dutra WO, Gollob KJ, Carvalho EM (2002) Up-regulation of Th1-type responses in mucosal leishmaniasis patients. *Infect Immun* 70(12):6734-6740.

Ballou WR, Gordon D, Andujar J, McClain JB, Shanks GD, Berman J, Chulay J (1987) SAFETY AND EFFICACY OF HIGH-DOSE SODIUM STIBOGLUCONATE THERAPY OF AMERICAN CUTANEOUS LEISHMANIASIS. *The Lancet* 330(8549):13-16.

Bañuls AL, Bastien P, Pomares C, Arevalo J, Fisa R, Hide M (2011) Clinical pleiomorphism in human leishmaniases, with special mention of asymptomatic infection. *Clinical Microbiology and Infection* 17(10):1451-1461.

Barbosa FMC, Dupin TV, Toledo MDS, Reis N, Ribeiro K, Cronemberger-Andrade A, Rugani JN, De Lorenzo BHP, Novaes EBRR, Soares RP, Torrecilhas AC, Xander P (2018) Extracellular Vesicles Released by Leishmania (Leishmania) amazonensis Promote Disease Progression and Induce the Production of Different Cytokines in Macrophages and B-1 Cells. *Front Microbiol* 9:3056.

Belkaid Y, Hoffmann KF, Mendez S, Kamhawi S, Udey MC, Wynn TA, Sacks DL (2001) 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. *The Journal of experimental medicine* 194(10):1497-1506.

Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL (2002) CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. *Nature* 420(6915):502-507.

Ben Slimane T, Chouihi E, Ben Hadj Ahmed S, Chelbi I, Barhoumi W, Cherni S, Zoghlami Z, Gharbi M, Zhioua E (2014) An investigation on vertical transmission of Leishmania infantum in experimentally infected dogs and assessment of offspring's infectiousness potential by xenodiagnosis. *Veterinary Parasitology* 206(3):282-286.

Benkova I, Volf P (2007) Effect of temperature on metabolism of Phlebotomus papatasi (Diptera: Psychodidae). *J Med Entomol* 44(1):150-154.

Berbert TRN, de Mello TFP, Wolf Nassif P, Mota CA, Silveira AV, Duarte GC, Demarchi IG, Aristides SMA, Lonardoni MVC, Vieira Teixeira JJ, Silveira TGV (2018) Pentavalent Antimonials Combined with Other Therapeutic Alternatives for the Treatment of Cutaneous and Mucocutaneous Leishmaniasis: A Systematic Review. *Dermatol Res Pract* 2018:9014726.

Berg M, Mannaert A, Vanaerschot M, Van Der Auwera G, Dujardin JC (2013) (Post-) Genomic approaches to tackle drug resistance in Leishmania. *Parasitology* 140(12):1492-1505.

Bhattacharya B, Chatterjee S, Devine WG, Kobzik L, Beeler AB, Porco JA, Jr., Kramnik I (2016) Fine-tuning of macrophage activation using synthetic rocaglate derivatives. *Sci Rep* 6:24409.

Blackwell JM (1985) Receptors and recognition mechanisms of Leishmania species. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 79(5):606-612.

Blum L, Geisslinger G, Parnham MJ, Grünweller A, Schiffmann S (2020) Natural antiviral compound silvestrol modulates human monocyte-derived macrophages and dendritic cells. *Journal of Cellular and Molecular Medicine* 24(12):6988-6999.

Brennan R, Haap-Hoff A, Gu L, Gautier V, Long A, Schröder M (2018) Investigating nucleo-cytoplasmic shuttling of the human DEAD-box helicase DDX3. *Eur J Cell Biol* 97(7):501-511.

Brittingham A, Morrison CJ, McMaster WR, McGwire BS, Chang KP, Mosser DM (1995) Role of the Leishmania surface protease gp63 in complement fixation, cell adhesion, and resistance to complement-mediated lysis. *J Immunol* 155(6):3102-3111.

Buates S, Matlashewski G (2001) General suppression of macrophage gene expression during Leishmania donovani infection. *J Immunol* 166(5):3416-3422.

Burchmore RJS, Barrett MP (2001) Life in vacuoles – nutrient acquisition by Leishmania amastigotes. *International Journal for Parasitology* 31(12):1311-1320.

Burza S, Croft SL, Boelaert M (2018) Leishmaniasis. *The Lancet* 392(10151):951-970.

Calegari-Silva TC, Pereira RMS, De-Melo LDB, Saraiva EM, Soares DC, Bellio M, Lopes UG (2009) NF-κB-mediated repression of iNOS expression in Leishmania amazonensis macrophage infection. *Immunology Letters* 127(1):19-26.

Cardoso FDO, Zaverucha-Do-Valle T, Almeida-Souza F, Abreu-Silva AL, Calabrese KDS (2020) Modulation of Cytokines and Extracellular Matrix Proteins Expression by Leishmania amazonensis in Susceptible and Resistant Mice. *Frontiers in Microbiology* 11.

Chagas AC, Oliveira F, Debrabant A, Valenzuela JG, Ribeiro JMC, Calvo E (2014) Lundep, a Sand Fly Salivary Endonuclease Increases Leishmania Parasite Survival in Neutrophils and Inhibits XIIa Contact Activation in Human Plasma. *PLOS Pathogens* 10(2):e1003923.

Chandrakar P, Parmar N, Descoteaux A, Kar S (2020) Differential Induction of SOCS Isoforms by Leishmania donovani Impairs Macrophage–T Cell Cross-Talk and Host Defense. *The Journal of Immunology* 204(3):596-610.

Chao CH, Chen CM, Cheng PL, Shih JW, Tsou AP, Lee YH (2006) DDX3, a DEAD box RNA helicase with tumor growth-suppressive property and transcriptional regulation activity of the p21waf1/cip1 promoter, is a candidate tumor suppressor. *Cancer Res* 66(13):6579-6588.

Chaparro V, Graber TE, Alain T, Jaramillo M (2022) Transcriptional profiling of macrophages reveals distinct parasite stage-driven signatures during early infection by Leishmania donovani. *Sci Rep* 12(1):6369.

Chaparro V, Leroux L-P, Masvidal L, Lorent J, Graber TE, Zimmermann A, Arango Duque G, Descoteaux A, Alain T, Larsson O, Jaramillo M (2020) Translational profiling of macrophages infected with Leishmania donovani identifies mTOR- and eIF4A-sensitive immune-related transcripts. *PLOS Pathogens* 16(6):e1008291.

Chaparro V, Leroux L-P, Zimmermann A, Jardim A, Johnston B, Descoteaux A, Jaramillo M (2019) Leishmania donovani Lipophosphoglycan Increases Macrophage-Dependent Chemotaxis of CXCR6-Expressing Cells via CXCL16 Induction. *Infection and Immunity* 87(5).

Chappuis F, Sundar S, Hailu A, Ghalib H, Rijal S, Peeling RW, Alvar J, Boelaert M (2007) Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nature Reviews Microbiology* 5(11):873-882.

Chatterjee S, Yabaji SM, Rukhlenko OS, Bhattacharya B, Waligurski E, Vallavoju N, Ray S, Kholodenko BN, Brown LE, Beeler AB, Ivanov AR, Kobzik L, Porco JA, Jr., Kramnik I

(2021) Channeling macrophage polarization by rocaglates increases macrophage resistance to Mycobacterium tuberculosis. *iScience* 24(8):102845.

Chaudhuri G, Chaudhuri M, Pan A, Chang KP (1989) Surface acid proteinase (gp63) of Leishmania mexicana. A metalloenzyme capable of protecting liposome-encapsulated proteins from phagolysosomal degradation by macrophages. *J Biol Chem* 264(13):7483-7489.

Chen CY, Chan CH, Chen CM, Tsai YS, Tsai TY, Wu Lee YH, You LR (2016) Targeted inactivation of murine Ddx3x: essential roles of Ddx3x in placentation and embryogenesis. *Hum Mol Genet* 25(14):2905-2922.

Chen M, Asanuma M, Takahashi M, Shichino Y, Mito M, Fujiwara K, Saito H, Floor SN, Ingolia NT, Sodeoka M, Dodo K, Ito T, Iwasaki S (2021) Dual targeting of DDX3 and eIF4A by the translation inhibitor rocaglamide A. *Cell Chem Biol* 28(4):475-486 e478.

Chen S, Saeed A, Liu Q, Jiang Q, Xu H, Xiao GG, Rao L, Duo Y (2023) Macrophages in immunoregulation and therapeutics. *Signal Transduct Target Ther* 8(1):207.

Chen WJ, Wang WT, Tsai TY, Li HK, Lee YW (2017) DDX3 localizes to the centrosome and prevents multipolar mitosis by epigenetically and translationally modulating p53 expression. *Sci Rep* 7(1):9411.

Chu J, Zhang W, Cencic R, O'Connor PBF, Robert F, Devine WG, Selznick A, Henkel T, Merrick WC, Brown LE, Baranov PV, Porco JA, Jr., Pelletier J (2020) Rocaglates Induce Gain-of-Function Alterations to eIF4A and eIF4F. *Cell Rep* 30(8):2481-2488 e2485.

Colpitts SL, Dalton NM, Scott P (2009) IL-7 receptor expression provides the potential for long-term survival of both CD62Lhigh central memory T cells and Th1 effector cells during Leishmania major infection. *J Immunol* 182(9):5702-5711.

Croft SL, Olliaro P (2011) Leishmaniasis chemotherapy—challenges and opportunities. *Clinical Microbiology and Infection* 17(10):1478-1483.

Crosby EJ, Clark M, Novais FO, Wherry EJ, Scott P (2015) Lymphocytic Choriomeningitis Virus Expands a Population of NKG2D+CD8+ T Cells That Exacerbates Disease in Mice Coinfected with Leishmania major. *The Journal of Immunology* 195(7):3301-3310.

Crosby EJ, Goldschmidt MH, Wherry EJ, Scott P (2014) Engagement of NKG2D on bystander memory CD8 T cells promotes increased immunopathology following Leishmania major infection. *PLoS Pathog* 10(2):e1003970.

Das S, Mukherjee S, Ali N (2021) Super enhancer-mediated transcription of miR146a-5p drives M2 polarization during Leishmania donovani infection. *PLOS Pathogens* 17(2):e1009343.

David CV, Craft N (2009) Cutaneous and mucocutaneous leishmaniasis. *Dermatologic Therapy* 22(6):491-502.

Davis MJ, Tsang TM, Qiu Y, Dayrit JK, Freij JB, Huffnagle GB, Olszewski MA (2013) Macrophage M1/M2 polarization dynamically adapts to changes in cytokine microenvironments in Cryptococcus neoformans infection. *mBio* 4(3):e00264-00213.

De Camargo-Neves V, Calemes E, Rodas L, Galvis-Ovallos F, Silva L (2021) Control of Canine Visceral Leishmaniasis: A Success Case Based on Deltamethrin 4% Collars. *Epidemiologia* 2(4):502-518.

De Gaudenzi JG, Noé G, Campo VA, Frasch AC, Cassola A (2011) Gene expression regulation in trypanosomatids. *Essays Biochem* 51:31-46.

De Vries HJC, Schallig HD (2022) Cutaneous Leishmaniasis: A 2022 Updated Narrative Review into Diagnosis and Management Developments. *American Journal of Clinical Dermatology* 23(6):823-840.

Deckert J, Hartmuth K, Boehringer D, Behzadnia N, Will CL, Kastner B, Stark H, Urlaub H, Lührmann R (2006) Protein composition and electron microscopy structure of affinity-purified human spliceosomal B complexes isolated under physiological conditions. *Mol Cell Biol* 26(14):5528-5543.

Derghal M, Tebai A, Balti G, Souguir-Omrani H, Chemkhi J, Rhim A, Bouattour A, Guizani I, M'Ghirbi Y, Guerbouj S (2022) High-resolution melting analysis identifies reservoir hosts of zoonotic Leishmania parasites in Tunisia. *Parasites & Vectors* 15(1).

Desjardins M, Descoteaux A (1997) Inhibition of Phagolysosomal Biogenesis by the Leishmania Lipophosphoglycan. *The Journal of experimental medicine* 185(12):2061-2068.

Diotallevi A, Bruno F, Castelli G, Persico G, Buffi G, Ceccarelli M, Ligi D, Mannello F, Vitale F, Magnani M, Galluzzi L (2024) Transcriptional signatures in human macrophage-like cells infected by Leishmania infantum, Leishmania major and Leishmania tropica. *PLoS Negl Trop Dis* 18(4):e0012085.

Divenuto F, Pavia G, Marascio N, Barreca GS, Quirino A, Matera G (2023) Role of Treg, Breg and other cytokine sets in host protection and immunopathology during human leishmaniasis: Are they potential valuable markers in clinical settings and vaccine evaluation? *Acta Tropica* 240:106849.

DiVita DJ, Kearse MG (2021) A double on the Rocs with a twist: Rocaglamide A targets multiple DEAD-box helicases to inhibit translation initiation. *Cell Chem Biol* 28(4):431-433.

Ehrchen JM, Roth J, Roebrock K, Varga G, Domschke W, Newberry R, Sorg C, MüLler-Tidow C, SunderköTter C, Kucharzik T, Spahn TW (2008) The Absence of Cutaneous Lymph Nodes Results in a Th2 Response and Increased Susceptibility toLeishmania majorInfection in Mice. *Infection and Immunity* 76(9):4241-4250.

Elnaiem D-EA (2011) Ecology and control of the sand fly vectors of Leishmania donovani in East Africa, with special emphasis on Phlebotomus orientalis. *Journal of Vector Ecology* 36:S23-S31.

Eren RO, Reverte M, Rossi M, Hartley M-A, Castiglioni P, Prevel F, Martin R, Desponds C, Lye L-F, Drexler SK, Reith W, Beverley SM, Ronet C, Fasel N (2016) Mammalian Innate Immune Response to a Leishmania-Resident RNA Virus Increases Macrophage Survival to Promote Parasite Persistence. *Cell Host & Microbe* 20(3):318-328.

Erguler K, Pontiki I, Zittis G, Proestos Y, Christodoulou V, Tsirigotakis N, Antoniou M, Kasap OE, Alten B, Lelieveld J (2019) A climate-driven and field data-assimilated population dynamics model of sand flies. *Scientific Reports* 9(1).

Evans A, Bongiorno G, Fourie JJ, Lekouch N, Bianchi R, Khoury C, Thomas E, Chiummo R, Gradoni L (2022) Elevated and sustained anti-feeding effect of Scalibor® deltamethrin collar against the sand fly Phlebotomus perniciosus in dogs confirmed for 1 year following treatment. *Medical and Veterinary Entomology* 36(1):14-19.

Faria DR, Souza PE, Durães FV, Carvalho EM, Gollob KJ, Machado PR, Dutra WO (2009) Recruitment of CD8(+) T cells expressing granzyme A is associated with lesion progression in human cutaneous leishmaniasis. *Parasite Immunol* 31(8):432-439.

Filardy AA, Costa-Da-Silva AC, Koeller CM, Guimarães-Pinto K, Ribeiro-Gomes FL, Lopes MF, Heise N, Freire-De-Lima CG, Nunes MP, Dosreis GA (2014) Infection with Leishmania major Induces a Cellular Stress Response in Macrophages. *PLoS One* 9(1):e85715.

Gadelha EPN, Ramasawmy R, da Costa Oliveira B, Morais Rocha N, de Oliveira Guerra JA, Allan Villa Rouco da Silva G, Gabrielle Ramos de Mesquita T, Chrusciak Talhari Cortez C, Chrusciak Talhari A (2018) An open label randomized clinical trial comparing the safety and effectiveness of one, two or three weekly pentamidine isethionate doses (seven milligrams per kilogram) in the treatment of cutaneous leishmaniasis in the Amazon Region. *PLoS Negl Trop Dis* 12(10):e0006850.

Galicia-Vázquez G, Cencic R, Robert F, Agenor AQ, Pelletier J (2012) A cellular response linking eIF4AI activity to eIF4AII transcription. *RNA* 18(7):1373-1384.

Galicia-Vázquez G, Chu J, Pelletier J (2015) eIF4AII is dispensable for miRNA-mediated gene silencing. *RNA* 21(10):1826-1833.

Gandin V, Masvidal L, Hulea L, Gravel SP, Cargnello M, McLaughlan S, Cai Y, Balanathan P, Morita M, Rajakumar A, Furic L, Pollak M, Porco JA, Jr., St-Pierre J, Pelletier J, Larsson O, Topisirovic I (2016) nanoCAGE reveals 5' UTR features that define specific modes of translation of functionally related MTOR-sensitive mRNAs. *Genome Res* 26(5):636-648.

Garg R, Dube A (2006) Animal models for vaccine studies for visceral leishmaniasis. *Indian Journal of Medical Research* 123(3):439-454.

Geissler R, Golbik RP, Behrens SE (2012) The DEAD-box helicase DDX3 supports the assembly of functional 80S ribosomes. *Nucleic Acids Res* 40(11):4998-5011.

Giraud E, Lestinova T, Derrick T, Martin O, Dillon RJ, Volf P, Műller I, Bates PA, Rogers ME (2018) Leishmania proteophosphoglycans regurgitated from infected sand flies accelerate dermal wound repair and exacerbate leishmaniasis via insulin-like growth factor 1-dependent signalling. *PLOS Pathogens* 14(1):e1006794.

Gossage SM, Rogers ME, Bates PA (2003) Two separate growth phases during the development of Leishmania in sand flies: implications for understanding the life cycle. *International Journal for Parasitology* 33(10):1027-1034.

Gray KC, Palacios DS, Dailey I, Endo MM, Uno BE, Wilcock BC, Burke MD (2012) Amphotericin primarily kills yeast by simply binding ergosterol. *Proceedings of the National Academy of Sciences* 109(7):2234-2239.

Greger H (2022) Comparative phytochemistry of flavaglines (= rocaglamides), a group of highly bioactive flavolignans from Aglaia species (Meliaceae). *Phytochem Rev* 21(3):725-764.

Guimarães-Costa AB, Desouza-Vieira TS, Paletta-Silva R, Freitas-Mesquita AL, Meyer-Fernandes JR, Saraiva EM (2014) 3′-Nucleotidase/Nuclease Activity Allows Leishmania Parasites To Escape Killing by Neutrophil Extracellular Traps. *Infection and Immunity* 82(4):1732-1740.

Gupta P, Srivastav S, Saha S, Das PK, Ukil A (2016) Leishmania donovani inhibits macrophage apoptosis and pro-inflammatory response through AKT-mediated regulation of β-catenin and FOXO-1. *Cell Death Differ* 23(11):1815-1826.

Hamill RJ (2013) Amphotericin B formulations: a comparative review of efficacy and toxicity. *Drugs* 73(9):919-934.

Harigua-Souiai E, Abdelkrim YZ, Bassoumi-Jamoussi I, Zakraoui O, Bouvier G, Essafi-Benkhadir K, Banroques J, Desdouits N, Munier-Lehmann H, Barhoumi M, Tanner NK, Nilges M,

Blondel A, Guizani I (2018) Identification of novel leishmanicidal molecules by virtual and biochemical screenings targeting Leishmania eukaryotic translation initiation factor 4A. *PLoS Negl Trop Dis* 12(1):e0006160.

Hashiguchi Y, Gomez EL, Kato H, Martini LR, Velez LN, Uezato H (2016) Diffuse and disseminated cutaneous leishmaniasis: clinical cases experienced in Ecuador and a brief review. *Tropical Medicine and Health* 44(1).

Heaton SM, Atkinson SC, Sweeney MN, Yang SNY, Jans DA, Borg NA (2019) Exportin-1-Dependent Nuclear Export of DEAD-box Helicase DDX3X is Central to its Role in Antiviral Immunity. *Cells* 8(10).

Hurdayal R, Brombacher F (2014) The role of IL-4 and IL-13 in cutaneous Leishmaniasis. *Immunology Letters* 161(2):179-183.

Hwang BY, Su B-N, Chai H, Mi Q, Kardono LBS, Afriastini JJ, Riswan S, Santarsiero BD, Mesecar AD, Wild R, Fairchild CR, Vite GD, Rose WC, Farnsworth NR, Cordell GA, Pezzuto JM, Swanson SM, Kinghorn AD (2004) Silvestrol and Episilvestrol, Potential Anticancer Rocaglate Derivatives from Aglaia silvestris. *The Journal of Organic Chemistry* 69(10):3350-3358.

Ikeogu NM, Akaluka GN, Edechi CA, Salako ES, Onyilagha C, Barazandeh AF, Uzonna JE (2020) Leishmania Immunity: Advancing Immunotherapy and Vaccine Development. *Microorganisms* 8(8).

Isnard A, Shio MT, Olivier M (2012) Impact of Leishmania metalloprotease GP63 on macrophage signaling. *Frontiers in Cellular and Infection Microbiology* 2.

Ivashkiv LB, Park SH (2016) Epigenetic Regulation of Myeloid Cells. *Microbiol Spectr* 4(3).

Iwasaki S, Floor SN, Ingolia NT (2016) Rocaglates convert DEAD-box protein eIF4A into a sequence-selective translational repressor. *Nature* 534(7608):558-561.

Iwasaki S, Iwasaki W, Takahashi M, Sakamoto A, Watanabe C, Shichino Y, Floor SN, Fujiwara K, Mito M, Dodo K, Sodeoka M, Imataka H, Honma T, Fukuzawa K, Ito T, Ingolia NT (2019) The Translation Inhibitor Rocaglamide Targets a Bimolecular Cavity between eIF4A and Polypurine RNA. *Mol Cell* 73(4):738-748.e739.

Iwatani-Yoshihara M, Ito M, Ishibashi Y, Oki H, Tanaka T, Morishita D, Ito T, Kimura H, Imaeda Y, Aparicio S, Nakanishi A, Kawamoto T (2017) Discovery and Characterization of a Eukaryotic Initiation Factor 4A-3-Selective Inhibitor That Suppresses Nonsense-Mediated mRNA Decay. *ACS Chem Biol* 12(7):1760-1768.

Jackson RJ, Hellen CUT, Pestova TV (2010) The mechanism of eukaryotic translation initiation and principles of its regulation. *Nature Reviews Molecular Cell Biology* 11(2):113-127.

Kane MM, Mosser DM (2001) The Role of IL-10 in Promoting Disease Progression in Leishmaniasis. *The Journal of Immunology* 166(2):1141-1147.

Kaushal H, Bras-Gonçalves R, Negi NS, Lemesre JL, Papierok G, Salotra P (2014) Role of CD8(+) T cells in protection against Leishmania donovani infection in healed Visceral Leishmaniasis individuals. *BMC Infect Dis* 14:653.

Kaye P, Scott P (2011) Leishmaniasis: complexity at the host-pathogen interface. *Nat Rev Microbiol* 9(8):604-615.

Kaye PM, Mohan S, Mantel C, Malhame M, Revill P, Le Rutte E, Parkash V, Layton AM, Lacey CJN, Malvolti S (2021) Overcoming roadblocks in the development of vaccines for leishmaniasis. *Expert Review of Vaccines* 20(11):1419-1430.

Koutsoni O, Barhoumi M, Guizani I, Dotsika E (2014) Leishmania eukaryotic initiation factor (LeIF) inhibits parasite growth in murine macrophages. *PLoS One* 9(5):e97319.

Kumar R, Bumb RA, Salotra P (2010) Evaluation of localized and systemic immune responses in cutaneous leishmaniasis caused by Leishmania tropica: interleukin-8, monocyte chemotactic protein-1 and nitric oxide are major regulatory factors. *Immunology* 130(2):193-201.

Kwan T, Thompson SR (2019) Noncanonical Translation Initiation in Eukaryotes. *Cold Spring Harbor Perspectives in Biology* 11(4):a032672.

Laffitte MN, Leprohon P, Papadopoulou B, Ouellette M (2016) Plasticity of the Leishmania genome leading to gene copy number variations and drug resistance. *F1000Res* 5:2350.

Langlais D, Cencic R, Moradin N, Kennedy JM, Ayi K, Brown LE, Crandall I, Tarry MJ, Schmeing M, Kain KC, Porco JA, Jr., Pelletier J, Gros P (2018) Rocaglates as dual-targeting agents for experimental cerebral malaria. *Proc Natl Acad Sci U S A* 115(10):E2366-E2375.

Laskay T, van Zandbergen G, Solbach W (2003) Neutrophil granulocytes – Trojan horses for Leishmania major and other intracellular microbes? *Trends in Microbiology* 11(5):210-214.

Lecoeur H, Prina E, Rosazza T, Kokou K, N'Diaye P, Aulner N, Varet H, Bussotti G, Xing Y, Milon G, Weil R, Meng G, Späth GF (2020) Targeting Macrophage Histone H3 Modification as a Leishmania Strategy to Dampen the NF-κB/NLRP3-Mediated Inflammatory Response. *Cell Rep* 30(6):1870-1882.e1874.

Lewis SM, Holcik M (2008) For IRES trans-acting factors, it is all about location. *Oncogene* 27(8):1033-1035.

Lindoso JA, Cota GF, Da Cruz AM, Goto H, Maia-Elkhoury ANS, Romero GAS, De Sousa-Gomes ML, Santos-Oliveira JR, Rabello A (2014) Visceral Leishmaniasis and HIV Coinfection in Latin America. *PLOS Neglected Tropical Diseases* 8(9):e3136.

Liu SX, Gustafson HH, Jackson DL, Pun SH, Trapnell C (2020) Trajectory analysis quantifies transcriptional plasticity during macrophage polarization. *Sci Rep* 10(1):12273.

Lodge R, Descoteaux A (2008) Leishmania Invasion and Phagosome Biogenesis. *Molecular Mechanisms of Parasite Invasion: Subcellular Biochemistry*, Burleigh BA, Soldati-Favre D (Édit.) Springer New York, New York, NY10.1007/978-0-387-78267-6_14. p 174-181.

Lu King M, Chiang C-C, Ling H-C, Fujita E, Ochiai M, McPhail AT (1982) X-Ray crystal structure of rocaglamide, a novel antileulemic 1H-cyclopenta[b]benzofuran from Aglaia elliptifolia. *Journal of the Chemical Society, Chemical Communications* 10.1039/C39820001150(20):1150-1151.

Madusanka RK, Silva H, Karunaweera ND (2022) Treatment of Cutaneous Leishmaniasis and Insights into Species-Specific Responses: A Narrative Review. *Infectious Diseases and Therapy* 11(2):695-711.

Manier S, Huynh D, Shen YJ, Zhou J, Yusufzai T, Salem KZ, Ebright RY, Shi J, Park J, Glavey SV, Devine WG, Liu CJ, Leleu X, Quesnel B, Roche-Lestienne C, Snyder JK, Brown LE, Gray N, Bradner J, Whitesell L, Porco JA, Jr., Ghobrial IM (2017) Inhibiting the oncogenic translation program is an effective therapeutic strategy in multiple myeloma. *Sci Transl Med* 9(389).

Mann S, Frasca K, Scherrer S, Henao-Martínez AF, Newman S, Ramanan P, Suarez JA (2021) A Review of Leishmaniasis: Current Knowledge and Future Directions. *Curr Trop Med Rep* 8(2):121-132.

Matera G, Torti C, Mazzitelli M, Greco G, Rania A, Peronace C, Settembre P, Galati L, Giancotti A, Lamberti AG, Barreca GS, Rossi M, Quirino A, Liberto MC, Focà A (2018) Depression of lymphocyte activity during cutaneous leishmaniasis: a case report. *Diagnostic Microbiology and Infectious Disease* 92(3):230-234.

Matheoud D, Moradin N, Bellemare-Pelletier A, Marina, Wan, Olivier M, Gagnon É, Desjardins M, Descoteaux A (2013) Leishmania Evades Host Immunity by Inhibiting Antigen Cross-Presentation through Direct Cleavage of the SNARE VAMP8. *Cell Host & Microbe* 14(1):15-25.

Mazloomian A, Araki S, Ohori M, El-Naggar AM, Yap D, Bashashati A, Nakao S, Sorensen PH, Nakanishi A, Shah S, Aparicio S (2019) Pharmacological systems analysis defines EIF4A3 functions in cell-cycle and RNA stress granule formation. *Commun Biol* 2:165.

McConville MJ, de Souza D, Saunders E, Likic VA, Naderer T (2007) Living in a phagolysosome; metabolism of Leishmania amastigotes. *Trends Parasitol* 23(8):368-375.

McConville MJ, Naderer T (2011) Metabolic Pathways Required for the Intracellular Survival of Leishmania. *Annual Review of Microbiology* 65(1):543-561.

Mills CD, Ley K (2014) M1 and M2 macrophages: the chicken and the egg of immunity. *J Innate Immun* 6(6):716-726.

Mo J, Liang H, Su C, Li P, Chen J, Zhang B (2021) DDX3X: structure, physiologic functions and cancer. *Mol Cancer* 20(1):38.

Moll H, Müller C, Gillitzer R, Fuchs H, Röllinghoff M, Simon MM, Kramer MD (1991) Expression of T-cell-associated serine proteinase 1 during murine Leishmania major infection correlates with susceptibility to disease. *Infect Immun* 59(12):4701-4705.

Mondelaers A, Sanchez-Cañete MP, Hendrickx S, Eberhardt E, Garcia-Hernandez R, Lachaud L, Cotton J, Sanders M, Cuypers B, Imamura H, Dujardin J-C, Delputte P, Cos P, Caljon G, Gamarro F, Castanys S, Maes L (2016) Genomic and Molecular Characterization of Miltefosine Resistance in Leishmania infantum Strains with Either Natural or Acquired Resistance through Experimental Selection of Intracellular Amastigotes. *PLoS One* 11(4):e0154101.

Montaner-Angoiti E, Llobat L (2023) Is leishmaniasis the new emerging zoonosis in the world? *Vet Res Commun* 47(4):1777-1799.

Moradin N, Descoteaux A (2012) Leishmania promastigotes: building a safe niche within macrophages. *Frontiers in Cellular and Infection Microbiology* 2.

Mosser DM, Vlassara H, Edelson PJ, Cerami A (1987) Leishmania promastigotes are recognized by the macrophage receptor for advanced glycosylation endproducts. *The Journal of experimental medicine* 165(1):140-145.

Müller C, Obermann W, Karl N, Wendel HG, Taroncher-Oldenburg G, Pleschka S, Hartmann RK, Grünweller A, Ziebuhr J (2021) The rocaglate CR-31-B (-) inhibits SARS-CoV-2 replication at non-cytotoxic, low nanomolar concentrations in vitro and ex vivo. *Antiviral Res* 186:105012.

Müller C, Obermann W, Schulte FW, Lange-Grünweller K, Oestereich L, Elgner F, Glitscher M, Hildt E, Singh K, Wendel H-G, Hartmann RK, Ziebuhr J, Grünweller A (2020) Comparison

of broad-spectrum antiviral activities of the synthetic rocaglate CR-31-B (−) and the eIF4A-inhibitor Silvestrol. *Antiviral Research* 175:104706.

Nagase H, Jones KM, Anderson CF, Noben-Trauth N (2007) Despite Increased CD4+Foxp3+ Cells within the Infection Site, BALB/c IL-4 Receptor-Deficient Mice Reveal CD4+Foxp3- Negative T Cells as a Source of IL-10 in Leishmania major Susceptibility1. *The Journal of Immunology* 179(4):2435-2444.

Ndjamen B, Kang B-H, Hatsuzawa K, Kima PE (2010) Leishmania parasitophorous vacuoles interact continuously with the host cell's endoplasmic reticulum; parasitophorous vacuoles are hybrid compartments. *Cellular Microbiology* 12(10):1480-1494.

Neves BM, Silvestre R, Resende M, Ouaissi A, Cunha J, Tavares J, Loureiro I, Santarém N, Silva AM, Lopes MC, Cruz MT, Cordeiro da Silva A (2010) Activation of phosphatidylinositol 3-kinase/Akt and impairment of nuclear factor-kappaB: molecular mechanisms behind the arrested maturation/activation state of Leishmania infantum-infected dendritic cells. *Am J Pathol* 177(6):2898-2911.

Ng LG, Hsu A, Mandell MA, Roediger B, Hoeller C, Mrass P, Iparraguirre A, Cavanagh LL, Triccas JA, Beverley SM, Scott P, Weninger W (2008) Migratory Dermal Dendritic Cells Act as Rapid Sensors of Protozoan Parasites. *PLOS Pathogens* 4(11):e1000222.

Novais FO, Scott P (2015) CD8+ T cells in cutaneous leishmaniasis: the good, the bad, and the ugly. *Seminars in Immunopathology* 37(3):251-259.

Nylén S, Gautam S (2010) Immunological Perspectives of Leishmaniasis. *Journal of Global Infectious Diseases* 2(2).

Obermann W, Azri MFD, Konopka L, Schmidt N, Magari F, Sherman J, Silva LMR, Hermosilla C, Ludewig AH, Houhou H, Haeberlein S, Luo MY, Häcker I, Schetelig MF, Grevelding CG, Schroeder FC, Lau GSK, Taubert A, Rodriguez A, Heine A, Yeo TC, Grünweller A, Taroncher-Oldenburg G (2023) Broad anti-pathogen potential of DEAD box RNA helicase eIF4A-targeting rocaglates. *Scientific Reports* 13(1).

Obermann W, Friedrich A, Madhugiri R, Klemm P, Mengel JP, Hain T, Pleschka S, Wendel HG, Hartmann RK, Schiffmann S, Ziebuhr J, Müller C, Grünweller A (2022) Rocaglates as Antivirals: Comparing the Effects on Viral Resistance, Anti-Coronaviral Activity, RNA-Clamping on eIF4A and Immune Cell Toxicity. *Viruses* 14(3).

Oryan A, Akbari M (2016) Worldwide risk factors in leishmaniasis. *Asian Pacific Journal of Tropical Medicine* 9(10):925-932.

Osorio EY, Zhao W, Espitia C, Saldarriaga O, Hawel L, Byus CV, Travi BL, Melby PC (2012) Progressive Visceral Leishmaniasis Is Driven by Dominant Parasite-induced STAT6 Activation and STAT6-dependent Host Arginase 1 Expression. *PLOS Pathogens* 8(1):e1002417.

Pacheco-Fernandez T, Volpedo G, Verma C, Satoskar AR (2021) Understanding the immune responses involved in mediating protection or immunopathology during leishmaniasis. *Biochemical Society Transactions* 49(1):297-311.

Padmanabhan PK, Zghidi-Abouzid O, Samant M, Dumas C, Aguiar BG, Estaquier J, Papadopoulou B (2016) DDX3 DEAD-box RNA helicase plays a central role in mitochondrial protein quality control in Leishmania. *Cell Death Dis* 7(10):e2406.

Pan L, Woodard JL, Lucas DM, Fuchs JR, Douglas Kinghorn A (2014) Rocaglamide, silvestrol and structurally related bioactive compounds from Aglaia species. *Nat. Prod. Rep.* 31(7):924-939.

Pandey SC, Pande V, Samant M (2020) DDX3 DEAD-box RNA helicase (Hel67) gene disruption impairs infectivity of Leishmania donovani and induces protective immunity against visceral leishmaniasis. *Sci Rep* 10(1):18218.

Pareyn M, Van Den Bosch E, Girma N, Van Houtte N, Van Dongen S, Van Der Auwera G, Massebo F, Shibru S, Leirs H (2019) Ecology and seasonality of sandflies and potential reservoirs of cutaneous leishmaniasis in Ochollo, a hotspot in southern Ethiopia. *PLOS Neglected Tropical Diseases* 13(8):e0007667.

Pelletier J, Sonenberg N (1988) Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature* 334(6180):320-325.

Perry MR, Prajapati VK, Menten J, Raab A, Feldmann J, Chakraborti D, Sundar S, Fairlamb AH, Boelaert M, Picado A (2015) Arsenic Exposure and Outcomes of Antimonial Treatment in Visceral Leishmaniasis Patients in Bihar, India: A Retrospective Cohort Study. *PLOS Neglected Tropical Diseases* 9(3):e0003518.

Perry MR, Wyllie S, Prajapati VK, Feldmann J, Sundar S, Boelaert M, Fairlamb AH (2011) Visceral leishmaniasis and arsenic: an ancient poison contributing to antimonial treatment failure in the Indian subcontinent? *PLoS Negl Trop Dis* 5(9):e1227.

Peters NC, Egen JG, Secundino N, Debrabant A, Kimblin N, Kamhawi S, Lawyer P, Fay MP, Germain RN, Sacks D (2008) In Vivo Imaging Reveals an Essential Role for Neutrophils in Leishmaniasis Transmitted by Sand Flies. *Science* 321(5891):970-974.

Peters NC, Sacks DL (2009) The impact of vector-mediated neutrophil recruitment on cutaneous leishmaniasis. *Cellular Microbiology* 11(9):1290-1296.

Pigott DM, Bhatt S, Golding N, Duda KA, Battle KE, Brady OJ, Messina JP, Balard Y, Bastien P, Pratlong F, Brownstein JS, Freifeld CC, Mekaru SR, Gething PW, George DB, Myers MF, Reithinger R, Hay SI (2014) Global distribution maps of the leishmaniases. *Elife* 3:e02851.

Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, Lopez-Velez R, Garcia-Hernandez R, Pountain AW, Mwenechanya R, Papadopoulou B (2017) Drug resistance and treatment failure in leishmaniasis: A 21st century challenge. *PLoS Negl Trop Dis* 11(12):e0006052.

Praditya DF, Klöhn M, Brüggemann Y, Brown LE, Porco JA, Zhang W, Kinast V, Kirschning A, Vondran FWR, Todt D, Steinmann E (2022) Identification of structurally re-engineered rocaglates as inhibitors against hepatitis E virus replication. *Antiviral Research* 204:105359.

Puentes SM, Da Silva RP, Sacks DL, Hammer CH, Joiner KA (1990) Serum resistance of metacyclic stage Leishmania major promastigotes is due to release of C5b-9. *J Immunol* 145(12):4311-4316.

Puentes SM, Sacks DL, Da Silva RP, Joiner KA (1988) Complement binding by two developmental stages of Leishmania major promastigotes varying in expression of a surface lipophosphoglycan. *The Journal of experimental medicine* 167(3):887-902.

Rai AK, Thakur CP, Singh A, Seth T, Srivastava SK, Singh P, Mitra DK (2012) Regulatory T Cells Suppress T Cell Activation at the Pathologic Site of Human Visceral Leishmaniasis. *PLoS One* 7(2):e31551.

Ramesh V, Dixit KK, Sharma N, Singh R, Salotra P (2020) Assessing the Efficacy and Safety of Liposomal Amphotericin B and Miltefosine in Combination for Treatment of Post Kala-Azar Dermal Leishmaniasis. *The Journal of Infectious Diseases* 221(4):608-617.

Rashidi S, Mansouri R, Ali-Hassanzadeh M, Ghani E, Karimazar M, Muro A, Nguewa P, Manzano-Román R (2022) miRNAs in the regulation of mTOR signaling and host immune responses: The case of Leishmania infections. *Acta Tropica* 231:106431.

Raza F, Waldron JA, Quesne JL (2015) Translational dysregulation in cancer: eIF4A isoforms and sequence determinants of eIF4A dependence. *Biochem Soc Trans* 43(6):1227-1233.

Reguera RM, Morán M, Pérez-Pertejo Y, García-Estrada C, Balaña-Fouce R (2016) Current status on prevention and treatment of canine leishmaniasis. *Veterinary Parasitology* 227:98-114.

Reimão JQ, Trinconi CT, Yokoyama-Yasunaka JK, Miguel DC, Kalil SP, Uliana SRB (2013) Parasite burden in Leishmania (Leishmania) amazonensis-infected mice: validation of luciferase as a quantitative tool. *Journal of microbiological methods* 93(2):95-101.

Reinhard K, Huber M, Lohoff M, Visekruna A (2012) The role of NF-κB activation during protection against Leishmania infection. *Int J Med Microbiol* 302(4-5):230-235.

Reyaz E, Puri N, Selvapandiyan A (2024) Global Remodeling of Host Proteome in Response to Leishmania Infection. *ACS Infectious Diseases* 10(1):5-19.

Riera C, Fisa R, Udina M, Gállego M, Portus M (2004) Detection of Leishmania infantum cryptic infection in asymptomatic blood donors living in an endemic area (Eivissa, Balearic Islands, Spain) by different diagnostic methods. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 98(2):102-110.

Roatt BM, de Oliveira Cardoso JM, De Brito RCF, Coura-Vital W, de Oliveira Aguiar-Soares RD, Reis AB (2020) Recent advances and new strategies on leishmaniasis treatment. *Appl Microbiol Biotechnol* 104(21):8965-8977.

Rodríguez-Cortés A, Fernández-Bellón H, Ramis A, Ferrer L, Alberola J, Solano-Gallego L (2007) Leishmania-specific isotype levels and their relationship with specific cell-mediated immunity parameters in canine leishmaniasis. *Veterinary Immunology and Immunopathology* 116(3):190-198.

Rodríguez-González J, Gutiérrez-Kobeh L (2023) Apoptosis and its pathways as targets for intracellular pathogens to persist in cells. *Parasitol Res* 123(1):60.

Rodriguez NE, Chang HK, Wilson ME (2004) Novel Program of Macrophage Gene Expression Induced by Phagocytosis ofLeishmania chagasi. *Infection and Immunity* 72(4):2111-2122.

Ronet C, Hauyon-La Torre Y, Revaz-Breton M, Mastelic B, Tacchini-Cottier F, Louis J, Launois P (2010) Regulatory B cells shape the development of Th2 immune responses in BALB/c mice infected with Leishmania major through IL-10 production. *J Immunol* 184(2):886-894.

Roque ALR, Jansen AM (2014) Wild and synanthropic reservoirs of Leishmania species in the Americas. *International Journal for Parasitology: Parasites and Wildlife* 3(3):251-262.

Rubio CA, Weisburd B, Holderfield M, Arias C, Fang E, Derisi JL, Fanidi A (2014) Transcriptome-wide characterization of the eIF4A signature highlights plasticity in translation regulation. *Genome Biology* 15(10).

Ruhland A, Leal N, Kima PE (2007) Leishmania promastigotes activate PI3K/Akt signalling to confer host cell resistance to apoptosis. *Cell Microbiol* 9(1):84-96.

Russo R, Laguna F, López-Vélez R, Medrano FJ, Rosenthal E, Cacopardo B, Nigro L (2003) Visceral leishmaniasis in those infected with HIV: clinical aspects and other opportunistic infections. *Annals of Tropical Medicine & Parasitology* 97(sup1):99-105.

Sacks D, Kamhawi S (2001) Molecular Aspects of Parasite-Vector and Vector-Host Interactions in Leishmaniasis. *Annual Review of Microbiology* 55(1):453-483.

Saini S, Rai AK (2020) Hamster, a close model for visceral leishmaniasis: Opportunities and challenges. *Parasite Immunology* 42(10).

Salari S, Bamorovat M, Sharifi I, Almani PGN (2022) Global distribution of treatment resistance gene markers for leishmaniasis. *Journal of Clinical Laboratory Analysis* 36(8).

Samir P, Kesavardhana S, Patmore DM, Gingras S, Malireddi RKS, Karki R, Guy CS, Briard B, Place DE, Bhattacharya A, Sharma BR, Nourse A, King SV, Pitre A, Burton AR, Pelletier S, Gilbertson RJ, Kanneganti TD (2019) DDX3X acts as a live-or-die checkpoint in stressed cells by regulating NLRP3 inflammasome. *Nature* 573(7775):590-594.

Santos GDA, Sousa JM, Aguiar AHBMD, Torres KCS, Coelho AJS, Ferreira AL, Lima MIS (2023) Systematic Review of Treatment Failure and Clinical Relapses in Leishmaniasis from a Multifactorial Perspective: Clinical Aspects, Factors Associated with the Parasite and Host. *Tropical Medicine and Infectious Disease* 8(9):430.

Schiffmann S, Gunne S, Ulshöfer T, Henke M, Roser LA, Schneider AK, Cinatl J, Thomas D, Schreiber Y, Wagner PV, Grünweller A, Parnham MJ (2022) In Vitro Safety, Off-Target and Bioavailability Profile of the Antiviral Compound Silvestrol. *Pharmaceuticals (Basel)* 15(9).

Schiffmann S, Henke M, Seifert M, Ulshöfer T, Roser LA, Magari F, Wendel H-G, Grünweller A, Parnham MJ (2023) Comparing the Effects of Rocaglates on Energy Metabolism and Immune Modulation on Cells of the Human Immune System. *International Journal of Molecular Sciences* 24(6).

Schloer S, Goretzko J, Rescher U (2022) Repurposing Antifungals for Host-Directed Antiviral Therapy? *Pharmaceuticals (Basel)* 15(2).

Schütz P, Karlberg T, van den Berg S, Collins R, Lehtiö L, Högbom M, Holmberg-Schiavone L, Tempel W, Park HW, Hammarström M, Moche M, Thorsell AG, Schüler H (2010) Comparative structural analysis of human DEAD-box RNA helicases. *PLoS One* 5(9).

Séguin O, Mai LT, Acevedo Ospina H, Guay-Vincent M-M, Whiteheart SW, Stäger S, Descoteaux A (2022) VAMP3 and VAMP8 Regulate the Development and Functionality of Parasitophorous Vacuoles Housing Leishmania amazonensis. *Infection and Immunity* 90(3).

Sénéchal P, Robert F, Cencic R, Yanagiya A, Chu J, Sonenberg N, Paquet M, Pelletier J (2021) Assessing eukaryotic initiation factor 4F subunit essentiality by CRISPR-induced gene ablation in the mouse. *Cellular and Molecular Life Sciences* 78(19-20):6709-6719.

Serafim TD, Iniguez E, Oliveira F (2020) Leishmania infantum. *Trends in Parasitology* 36(1):80-81.

Shen L, Pelletier J (2020) Selective targeting of the DEAD-box RNA helicase eukaryotic initiation factor (eIF) 4A by natural products. *Nat Prod Rep* 37(5):609-616.

Shirzadi MR (2019) Lipsosomal amphotericin B: a review of its properties, function, and use for treatment of cutaneous leishmaniasis. *Research and reports in tropical medicine* :11-18.

Singh Alok K, Pandey Rajeev K, Siqueira-Neto Jair L, Kwon Y-J, Freitas-Junior Lucio H, Shaha C, Madhubala R (2015) Proteomic-Based Approach To Gain Insight into Reprogramming of THP-1 Cells Exposed to Leishmania donovani over an Early Temporal Window. *Infection and Immunity* 83(5):1853-1868.

Soares RR, Antinarelli LMR, Abramo C, Macedo GC, Coimbra ES, Scopel KKG (2017) What do we know about the role of regulatory B cells (Breg) during the course of infection of two major parasitic diseases, malaria and leishmaniasis? *Pathogens and Global Health* 111(3):107-115.

Solano-Gálvez S-G, Álvarez-Hernández D-A, Gutiérrez-Kobeh L, Vázquez-López R (2021) Leishmania: manipulation of signaling pathways to inhibit host cell apoptosis. *Therapeutic Advances in Infectious Disease* 8:20499361211014977.

Song J, Perreault J-P, Topisirovic I, Richard S (2016) RNA G-quadruplexes and their potential regulatory roles in translation. *Translation* 4(2):e1244031.

Srivastava S, Mishra J, Gupta AK, Singh A, Shankar P, Singh S (2017a) Laboratory confirmed miltefosine resistant cases of visceral leishmaniasis from India. *Parasites & Vectors* 10(1).

Srivastava S, Mishra J, Gupta AK, Singh A, Shankar P, Singh S (2017b) Laboratory confirmed miltefosine resistant cases of visceral leishmaniasis from India. *Parasites & Vectors* 10(1):49.

Sundar S, Chakravarty J (2010) Liposomal amphotericin B and leishmaniasis: dose and response. *J Glob Infect Dis* 2(2):159-166.

Sundar S, Chakravarty J (2015) An update on pharmacotherapy for leishmaniasis. *Expert Opinion on Pharmacotherapy* 16(2):237-252.

Sundar S, Chakravarty J, Rai VK, Agrawal N, Singh SP, Chauhan V, Murray HW (2007) Amphotericin B Treatment for Indian Visceral Leishmaniasis: Response to 15 Daily versus Alternate-Day Infusions. *Clinical Infectious Diseases* 45(5):556-561.

Sundar S, Singh A, Rai M, Prajapati VK, Singh AK, Ostyn B, Boelaert M, Dujardin JC, Chakravarty J (2012) Efficacy of Miltefosine in the Treatment of Visceral Leishmaniasis in India After a Decade of Use. *Clinical Infectious Diseases* 55(4):543-550.

Swihart K, Fruth U, Messmer N, Hug K, Behin R, Huang S, Del Giudice G, Aguet M, Louis JA (1995) Mice from a genetically resistant background lacking the interferon gamma receptor are susceptible to infection with Leishmania major but mount a polarized T helper cell 1-type CD4+ T cell response. *J Exp Med* 181(3):961-971.

Sypek JP, Chung CL, Mayor SE, Subramanyam JM, Goldman SJ, Sieburth DS, Wolf SF, Schaub RG (1993) Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. *The Journal of experimental medicine* 177(6):1797-1802.

Teixeira MJ, Teixeira CR, Andrade BB, Barral-Netto M, Barral A (2006) Chemokines in host–parasiteinteractions in leishmaniasis. *Trends in Parasitology* 22(1):32-40.

Tomiotto-Pellissier F, Bortoleti B, Assolini JP, Gonçalves MD, Carloto ACM, Miranda-Sapla MM, Conchon-Costa I, Bordignon J, Pavanelli WR (2018) Macrophage Polarization in Leishmaniasis: Broadening Horizons. *Front Immunol* 9:2529.

Tunalı V, Harman M, Çavuş İ, Gündüz C, Özbilgin A, Turgay N (2021) Overcoming the Challenge; In Vivo Efficacy of Miltefosine for Chronic Cutaneous Leishmaniasis. *Acta Parasitologica* 66(2):354-360.

Uzonna JE, Joyce KL, Scott P (2004) Low dose Leishmania major promotes a transient T helper cell type 2 response that is down-regulated by interferon gamma-producing CD8+ T cells. *J Exp Med* 199(11):1559-1566.

Valigurova A, Kolarova I (2023) Unrevealing the Mystery of Latent Leishmaniasis: What Cells Can Host Leishmania? *Pathogens* 12(2).

Varikuti S, Jha BK, Volpedo G, Ryan NM, Halsey G, Hamza OM, McGwire BS, Satoskar AR (2018) Host-Directed Drug Therapies for Neglected Tropical Diseases Caused by Protozoan Parasites. *Front Microbiol* 9:2655.

Velasco-Castrejon O, Walton BC, Rivas-Sanchez B, Garcia MF, Lazaro GJ, Hobart O, Roldan S, Floriani-Verdugo J, Munguia-Saldana A, Berzaluce R (1997) Treatment of cutaneous leishmaniasis with localized current field (radio frequency) in Tabasco, Mexico. *Am J Trop Med Hyg* 57(3):309-312.

Velez R, Gállego M (2020) Commercially approved vaccines for canine leishmaniosis: a review of available data on their safety and efficacy. *Tropical Medicine & International Health* 25(5):540-557.

Verma NK, Dey CS (2004) Possible Mechanism of Miltefosine-Mediated Death of Leishmania donovani. *Antimicrobial Agents and Chemotherapy* 48(8):3010-3015.

Victoir K, Dujardin JC (2002) How to succeed in parasitic life without sex? Asking Leishmania. *Trends Parasitol* 18(2):81-85.

Wallis RS, O'Garra A, Sher A, Wack A (2023) Host-directed immunotherapy of viral and bacterial infections: past, present and future. *Nature Reviews Immunology* 23(2):121-133.

William M, Leroux LP, Chaparro V, Graber TE, Alain T, Jaramillo M (2019) Translational repression of Ccl5 and Cxcl10 by 4E-BP1 and 4E-BP2 restrains the ability of mouse macrophages to induce migration of activated T cells. *European Journal of Immunology* 49(8):1200-1212.

Wilson ME, Innes DJ, Sousa AD, Pearson RD (1987) Early histopathology of experimental infection with Leishmania donovani in hamsters. *J Parasitol* 73(1):55-63.

Wolf Nassif P, TFP DEM, Navasconi TR, Mota CA, Demarchi IG, Aristides SMA, Lonardoni MVC, Teixeira JJV, Silveira TGV (2017) Safety and efficacy of current alternatives in the topical treatment of cutaneous leishmaniasis: a systematic review. *Parasitology* 144(8):995-1004.

Wolfe AL, Singh K, Zhong Y, Drewe P, Rajasekhar VK, Sanghvi VR, Mavrakis KJ, Jiang M, Roderick JE, Van der Meulen J, Schatz JH, Rodrigo CM, Zhao C, Rondou P, de Stanchina E, Teruya-Feldstein J, Kelliher MA, Speleman F, Porco JA, Pelletier J, Rätsch G, Wendel H-G (2014) RNA G-quadruplexes cause eIF4A-dependent oncogene translation in cancer. *Nature* 513(7516):65-70.

Yang Y, Wang Z (2019) IRES-mediated cap-independent translation, a path leading to hidden proteome. *J Mol Cell Biol* 11(10):911-919.

Yu W, Liang J, Fang T, Jiang J, Zhao R, Li R, Han J, Tian H (2023) EIF4A3 Acts on the PI3K-AKT-ERK1/2-P70S6K Pathway through FLOT1 to Influence the Development of Lung Adenocarcinoma. *Mol Cancer Res* 21(7):713-725.

Yunna C, Mengru H, Lei W, Weidong C (2020) Macrophage M1/M2 polarization. *Eur J Pharmacol* 877:173090.

Zaghloul IY, Al-Jasser M (2004) Effect of renal impairment on the pharmacokinetics of antimony in hamsters. *Annals of Tropical Medicine & Parasitology* 98(8):793-800.

Zaph C, Uzonna J, Beverley SM, Scott P (2004) Central memory T cells mediate long-term immunity to Leishmania major in the absence of persistent parasites. *Nat Med* 10(10):1104-1110.

Zerpa O, Ulrich M, Blanco B, Polegre M, Avila A, Matos N, Mendoza I, Pratlong F, Ravel C, Convit J (2007) Diffuse cutaneous leishmaniasis responds to miltefosine but then relapses. *Br J Dermatol* 156(6):1328-1335.

Zhang W, Chu J, Cyr AM, Yueh H, Brown LE, Wang TT, Pelletier J, Porco JA, Jr. (2019a) Intercepted Retro-Nazarov Reaction: Syntheses of Amidino-Rocaglate Derivatives and Their Biological Evaluation as eIF4A Inhibitors. *Journal of the American Chemical Society* 141(32):12891-12900.

Zhang W, Chu J, Cyr AM, Yueh H, Brown LE, Wang TT, Pelletier J, Porco JA, Jr. (2019b) Intercepted Retro-Nazarov Reaction: Syntheses of Amidino-Rocaglate Derivatives and Their Biological Evaluation as eIF4A Inhibitors. *J Am Chem Soc* 141(32):12891-12900.