HP – 001 - **Cellular Senescence Integrates Inflammatory and Cytotoxic Axes in the Pathological Landscape of Cutaneous Leishmaniasis**

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Localized cutaneous leishmaniasis (LCL) is characterized by ulcerated skin lesions driven by chronic inflammation and cytotoxic activity. Although previous work has demonstrated an association between senescent T and NK cells and LCL immunopathology, the spatial contribution of cellular senescence within the lesional microenvironment remains incompletely understood. Using 10X Visium spatial transcriptomic analysis of skin lesional biopsies from LCL patients infected with *Leishmania braziliensis*, we identified senescence-associated spots that were broadly and heterogeneously distributed throughout the tissue. Cell type deconvolution revealed enrichment of epithelial and immune cell populations within these areas, indicating that senescence in different cell types are co-localized within specific microenvironmental niches. Senescent regions displayed upregulation of tissue remodeling and inflammatory genes, supported by significant enrichment of SenMayo and SASP gene sets, alongside overrepresentation of key inflammatory mediators. Regions with high cytotoxicity scores exhibited stronger senescence signatures compared with non-cytotoxic spots, reinforcing the spatial link between senescence and cytotoxic activity. To further explore the relationship between senescence and pathological tissue alterations, we defined senescence-sensitive regions (SSRs) as areas with high densities of senescent spots. Histopathological analysis of SSRs revealed pronounced tissue alterations, including fibrosis, mononuclear inflammatory infiltrates, vascular changes, and ongoing tissue damage. Collectively, these findings position cellular senescence as a spatially organized and functionally integrated component of the immunopathological niche in LCL, linking chronic inflammation and cytotoxicity within the lesional microenvironment. **Supported by:** FAPES 51677.797.18389.30092022 **Keywords:** Cutaneous leishmaniasis; spatial biology; immunopathology.

HP – 002 - **Congenital *Toxoplasma gondii* infection alters dopaminergic pathway gene expression during retinal development.**

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Toxoplasma gondii can cross the maternal–fetal interface and disrupt development of the retina, a tissue derived from the central nervous system. Retinal development requires coordinated interactions among neurons, glial cells, and the expanding vascular network. Dopamine is a major retinal neurotransmitter that regulates neuronal activity, visual adaptation and retinal homeostasis. Dopaminergic signaling can also influence angiogenesis and endothelial barrier properties, suggesting a potential role in the formation and maturation of the retinal vasculature and blood–retinal barrier. However, the effects of congenital *T. gondii* infection on the retinal dopaminergic system during postnatal development remain poorly understood. Herein, we investigated whether congenital infection alters the expression of key components of the dopaminergic pathway in the developing mouse retina. Pregnant C57BL/6 mice were inoculated intragastrically with two ME49 cysts on embryonic day 10, and offspring were analyzed at postnatal days 5 and 14 (P5 and P14) which represent distinct stages of retinal and vascular maturation. Retinal gene expression was evaluated by RT-qPCR. At P5, congenitally infected pups showed increased expression of tyrosine hydroxylase (Th), dopamine receptors D1 (Drd1) and D2 (Drd2), and vesicular monoamine transporter 2 (Slc18a2) compared with controls. At P14, expression of the dopamine transporter Slc6a3 was reduced in infected retinas. These findings demonstrate that congenital *T. gondii* infection induces age-dependent changes in the retinal dopaminergic pathway. Early activation followed by altered dopamine transport may disrupt dopaminergic homeostasis during a critical period of retinal maturation and potentially influence dopamine-regulated neurovascular processes. Functional studies are required to determine whether these molecular changes contribute to abnormal retinal angiogenesis or blood–retinal barrier development. **Supported by:** INOVA-FIOCRUZ CAPES CNPq FAPERJ INOVA-IOC Federal Deputy Fund **Keywords:** Toxoplasma gondii; Retinal; Dopamine.

HP – 003 - **GENOMIC SIMILARITIES BETWEEN PLASMODIUM SIMIUM AND A HISTORICAL SAMPLE OF PLASMODIUM FROM THE SADO VALLEY, PORTUGAL**

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Plasmodium vivax is believed to have arrived in the Americas with Portuguese and Spanish colonizers, since vivax malaria was endemic in southern Europe until the mid-last century. We analyzed Giemsa-stained blood smears prepared in the 1950s from malaria patients in the Sado Valley, Portugal — a region with autochthonous transmission until that period. DNA was extracted from thirteen blood slides of patients with P. vivax infections using the Qiagen QIAamp Investigator kit and subjected to qPCR diagnostic before genomic sequencing. Two approaches for parasite DNA enrichment were compared: selective whole-genome amplification (sWGA) and hybrid capture.With sWGA, the highest quality sample (Portugal 3) yielded 11.7 million reads, an average depth of 18.7x, and 14,522 biallelic SNPs (8,763 of them with depth ≥5x). Analyses using smartPCA, ADMIXTURE, and f4 statistics revealed a clear genetic affinity between Portugal 3 and *Plasmodium simium* from the Atlantic Forest of Brazil. Although this sample does not exhibit the mitochondrial SNPs characteristic of P. simium, we observed the deletion of 3,010 base pairs in the PvRBP2a gene, which is characteristic of this parasite. These results suggest that the Portugal 3 sample may represent an intermediate form between *P. simium* and *P. vivax*, which could have existed in Europe before reaching Brazil, where it underwent changes until becoming *P. simium*. Another possibility is that Portugal 3 is a lineage of *P. simium* without the characteristic mitochondrial SNPs, introduced to Europe from the Atlantic Forest. **Supported by:** FAPESP 2022/10056-2
Keywords: Plasmodium vivax; Plasmodium simium; Population genetics.

HP – 004 - ***Toxoplasma gondii* alters mitochondrial dynamics in brain endothelial cells and cortical microvessels during early infection**

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Toxoplasma gondii crosses the blood-brain barrier (BBB) early during infection, utilizing cortical microvasculature as a replicative niche before invading the brain parenchyma, compromising tight junctions (TJs) and endothelial homeostasis. Because BBB integrity relies on mitochondrial energy, imbalances in mitochondrial dynamics cause ATP depletion and oxidative stress, reducing TJ expression. Some *T. gondii* strains recruit host mitochondria to the parasitophorous vacuole, suggesting that parasite-driven mitochondrial remodeling may influence endothelial homeostasis. This structural rearrangement made us question whether infection inducesmitochondrial changes in BBB-forming endothelial cellsand if such changes contribute to vascular integritydisruption. Herein we evaluated the impact of three canonical *T. gondii* strains (Type I–RH; Type II–ME49; Type III–VEG) on BBB integrity and mitochondrial homeostasis in mouse brain microvascular endothelial cells (BMECs). bEnd.3 cells were infected with MOI 3 tachyzoites. Infection led to reduced transendothelial electrical resistance starting at 2 (RH and VEG) and 48(ME49) hours post infection (hpi). TOMM20 immunostaining showed a most significant effect on branch lengths induced by the RH strain 24 hpi. Mouse brain cortex microvessels were isolated at 3 days post-infection with *T. gondii* tachyzoites following intravenous infection. Western blotting analysis revealed that the RH strain induced a significant decrease in fission marker Fis1, concomitantly with an increase in fusion marker OPA1, while ME49 increased fusion marker mitofusin 2. These findings indicate that withproducing a shift toward a fusion-favored profile. Together with early reduction in endothelial barrier resistance, these results suggest that accompanies during early neuroinvasion in Toxoplasmosis **Supported by:** FAPERJ, FIOCRUZ, CNPq
Keywords: Toxoplasma gondii; Mitochondria; Blood-Brain Barrier.

HP – 005 - **Functional characterization of mercaptopyruvate sulfurtransferase supports a role in stress adaptation and host-cell infection in *Trypanosoma cruzi***

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Amino acids play vital roles in *Trypanosoma cruzi* biology, supporting not only protein synthesis but also differentiation, host-cell invasion, and stress adaptation. In cysteine catabolism, aspartate aminotransferase generates 3-mercaptopyruvate (3-MP), which is subsequently processed by mercaptopyruvate sulfurtransferase (MST) into pyruvate and H_2S. Pyruvate can fuel mitochondrial respiration, while H_2S acts as an antioxidant and signaling molecule, linking sulfur amino acid metabolism, ATP production, and oxidative stress defenses. This study investigates the role of MST in *T. cruzi*. We generated tcmst knockout parasites ($MST^{-/-}$) using CRISPR/Cas9 and an add-back line with the pTEX vector. We characterized both against the parental Cas9 (Cas9) control across proliferation, metacyclogenesis, host-cell infection, oxidative stress, and mitochondrial bioenergetics. Epimastigote proliferation assays showed that $MST^{-/-}$ and add-back parasites differed significantly from Cas9, indicating that loss or restoration of mst gene produces differences in proliferation kinetics. Metacyclogenesis over seven days showed no significant differences among the three lines. In infection assays with CHO-K1 cells, $MST^{-/-}$ parasites showed a significantly reduced percentage of infected cells compared to Cas9, a phenotype fully restored in the add-back line. The number of amastigotes per infected cell did not differ among lines, indicating that MST loss specifically impairs host-cell invasion without affecting intracellular replication. Oxidative stress assays with H_2O_2 showed increased susceptibility in $MST^{-/-}$ parasites, also reversed in the add-back line. Preliminary high-resolution respirometry (Oroboros O2k) revealed reduced routine and increased leak respiration in $MST^{-/-}$ parasites compared with Cas9 line, with no change in electron transfer capacity, suggesting a reduced coupling efficiency rather than loss of respiratory chain capacity. Together, these results suggest that MST contributes to redox homeostasis, host-cell invasion, and mitochondrial coupling efficiency in *T. cruzi*. **Supported by:** FAPESP 2024/06474-9, FAPESP 2021/12938-0 **Keywords:** cysteine catabolism; bioenergetics; metabolism.

HP – 006 - **Conserved mitochondrial and translational repression and strain-associated chromatin responses in *Toxoplasma gondii*-infected neuroblasts**

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Toxoplasma gondii secretes polymorphic effectors that reprogram host transcription, but whether distinct strains induce conserved or strain-associated responses in neural cells remains unclear. Neuro2a cells were infected at MOI 1 with type I (RH), II (ME49), or III (VEG) tachyzoites for 12 and 24 hours (n=3) and analyzed by RNA sequencing. Because RH and VEG, unlike ME49, express HMA-competent MAF1b and recruit host mitochondria, their shared response was also examined. Using an adjusted $P<0.05$, RH, ME49, and VEG altered 930, 778, and 2,213 genes at 12 hours, respectively. Among these, 535 were shared and regulated concordantly by all three strains: 256 increased and 279 decreased. Shared upregulated genes included the transcriptional and chromatin regulators *Taf1*, *Top2b*, *Kdm6a*, *Baz1b*, *Trim33*, and *Asxl2*, whereas *Trim28* was reduced. Gene-set enrichment revealed conserved suppression of cytoplasmic translation, oxidative phosphorylation, mitochondrial electron transport, and ATP synthesis, accompanied by altered mRNA processing. Target-gene sets associated with KAT2A, SIN3A, YY1, TAF1, MYC, and ATF2 were enriched across all strains. Beyond this core response, RH and VEG shared 287 additional genes not significant following ME49 infection, including *Kdm2a*, *Dnmt3a*, *Rcor1*, and *Baz1a*, providing candidates for further investigation in engineered MAF1b-expressing parasite models. VEG produced the broadest response and uniquely reached significance for several chromatin regulators, including *Suz12*, *Bmi1*, *Chd8*, *Ino80*, *Ep300*, *Kdm4c*, and *Kdm5b*. By 24 hours, the number of differentially expressed genes was restricted to 51, 83, and 62 following RH, ME49, and VEG infection, respectively, with only five shared among all strains. These findings reveal early, transient suppression of mitochondrial bioenergetics and protein synthesis and strain-associated chromatin remodeling, which may disrupt neurogenic programs and contribute to brain malformations in congenital toxoplasmosis. **Supported by:** VPPCB/FIOCRUZ, FAPERJ, CNPq **Keywords:** Toxoplasmosis; transcriptomics; mitochondrial bioenergetics.

HP – 007 - **Endothelial activation and dysfunction drive retinal neurodegeneration and glial scarring through cytotoxic leukocyte infiltration in a clinically relevant mouse model of Ocular Toxoplasmosis.**

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Ocular toxoplasmosis (OT) is a major cause of infectious uveitis and can lead to blindness. Here, we investigated the temporal relationship between retinal infection, vascular dysfunction, immune-cell recruitment, tissue remodeling, and neurodegeneration in a murine model of acquired OT. Adult female C57BL/6 mice were orally infected with two ME49 *T. gondii* cysts and evaluated at 10, 20, and 30 days post-infection (dpi). Infection induced a sustained systemic inflammatory response and progressive retinal parasite persistence, with tachyzoite predominance during the acute phase and increasing bradyzoite burden during chronic infection. Retinal blood flow and functional capillary density increased throughout infection, accompanied by vascular remodeling and disruption of the blood-retinal barrier. Despite increased retinal perfusion, infected animals exhibited marked impairment of endothelial-dependent vasodilation, accompanied by alterations in endothelial junction proteins and increased ICAM-1 and VCAM-1 expression. These changes were associated with increased rolling and adhered leukocytes and progressive retinal infiltration by CD4+, CD8+, γδ T, NKT, and NK cells. Chronic infection was further characterized by angiogenic reprogramming together with extracellular matrix remodeling, collagen IV accumulation, reactive gliosis, and persistent retinal cell death. To investigate the therapeutic relevance of endothelial-leukocyte interactions, infected mice were treated with the ICAM-1/LFA-1 antagonist lifitegrast. Treatment reduced endothelial activation, retinal immune-cell infiltration, gliosis, extracellular matrix remodeling, and retian cell death, while also reducing retinal tachyzoite and bradyzoite burden. Together, these findings indicate that endothelial activation and leukocyte recruitment are central components of retinal pathology during OT and identify the neurovascular interface as a potential therapeutic target. Ethical approval CEUA-IOC L-038/2020. **Supported by:** INOVA-FIOCRUZ CAPES CNPq FAPERJ INOVA-IOC Federal Deputy Fund **Keywords:** Ocular toxoplasmosis; Retina; Vascular Dysfunction.

HP – 008 - **Investigating the molecular basis of *Trypanosoma cruzi* quiescence**

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Quiescent *Trypanosoma cruzi* parasites may survive therapeutic intervention and are often linked to treatment failure in Chagas disease. A better understanding of the underlying molecular mechanisms will aid the development of drugs targeting these quiescent parasites. Previously, we isolated quiescent epimastigotes, which form a small, non-replicating, subpopulation of this insect stage. These were analysed by transcriptomics and proteomics alongside replicating epimastigotes, defining distinct profiles for both groups. The non-replicating subpopulation is marked by the upregulation of genes that have been associated with cell cycle arrest, and the downregulation of genes that participate in DNA replication, metabolism, transcription, and translation. Using this analysis, we selected potential molecular drivers of quiescence and its maintenance to further study. Using the pTcINDEX system, we generated cell lines that inducibly overexpress two distinct RNA Binding Proteins (RBPs), previously linked with growth arrest, and created null mutants with CRISPR-Cas9. The SHARK ribozyme system was also used to generate inducible knockdown of a DYRK kinase previously linked to development of non-replicative forms of *T. brucei*. In *T.cruzi,* this gene is refractory to deletion. Overexpression and knock out of one of the RBPs, as well as the single-allele DYRK KO decreased growth rate. Both RBP KOs and the DYRK kinase sKO cell lines retain the ability to form the infectious metacyclic trypomastigotes and can infect mammalian host cells *in vitro*. The infectivity rates and parasite burden per cell have also been evaluated. Finally, to determine whether the RBP KO and DYRK KD cell lines enter quiescence and tolerate drug treatment upon infection of host cells, we performed an *in vitro* drug washout assay in infected host cell culture. **Supported by:** DNDi **Keywords:** Quiescence; RNA Binding Protein; Host-parasite interaction.

PV – 001 - **Roles of NFS, ISD11 and MTU1 in Mitochondrial tRNA Thiolation and Fe-S Clusters in *Trypanosoma cruzi***

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In *Trypanosoma cruzi*, gene expression relies on post-transcriptional regulation, as protein-coding genes are organized into polycistronic transcription units. Consequently, RNA modifications play a central role in stability and translation. The mitochondrial genome of trypanosomatids lacks tRNA genes, requiring the import of cytosolic tRNAs that undergo post-transcriptional modifications, including uridine thiolation. The cysteine desulfurase complex NFS–ISD11 participates in the tRNA thiolation pathway with MTU1 and plays a role in Fe-S cluster biogenesis. Although mitochondrial tRNA thiolation regulates $tRNA^{Trp}$ editing and gene expression in *Trypanosoma brucei*, its biological role in *T. cruzi* remains largely unknown. Here, we investigated the functions of NFS, ISD11 and MTU1 using CRISPR-Cas9-generated knockout and c-myc-tagged cell lines. We generated two partial knockouts ($Nfs^{-/+}$ and $Isd11^{-/+}$) and one complete knockout ($Mtu1^{-/-}$). Partial disruption of NFS or ISD11 did not significantly alter tRNA thiolation during the life cycle, indicating that reduced gene dosage is insufficient to impair this pathway. In contrast, loss of MTU1 caused stage-dependent defects, with decreased thiolation of $tRNA^{Glu}$ in stationary epimastigotes and reduced thiolation of mitochondrial $tRNA^{Trp}$, which was partially restored in the Mtu1-addback line. Immunofluorescence analyses revealed dual mitochondrial and cytosolic localization of the three proteins. Knockout lines displayed no major defects in epimastigote proliferation, but Mtu1-addback and Isd11-addback lines showed increased doubling time. $Nfs^{-/+}$ and $Isd11^{-/+}$ mutants exhibited a 2.6-fold increase in metacyclogenesis. $Nfs^{-/+}$ parasites showed reduced maximum respiratory capacity, suggesting a connection between Fe-S cluster biogenesis and mitochondrial respiration. Together, these findings reveal previously unrecognized connections between tRNA modification, parasite differentiation and mitochondrial metabolism in *T. cruzi*. **Supported by:** FAPESP: 2023/16035-0; 2021/12938-0; 24/16633-7 **Keywords:** tRNA thiolation; Fe-S cluster biogenesis; Trypanosoma cruzi.

PV – 002 - **Investigating a Likely Role for RBP43 in Regulating Expression of the *Trypanosoma brucei* Respiratome and Mediated through an Interaction with an Alternative Translation Initiation Complex**

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The eukaryotic mRNA translation requires the eIF4F complex, formed by eIF4E and eIF4G subunits, which functions by promoting mRNA recognition and ribosome recruitment in order for translation to properly start. eIF4F is a major target for regulation and might require the action of RNA-binding proteins (RBPs), which can provide assistance in fine-tuning binding to target mRNAs and guiding them toward translation or degradation. Regulation of gene expression in trypanosomatids occurs predominantly at the post-transcriptional level, such as during translation initiation. Likely major targets for regulation in these parasites are the multiple eIF4F complexes identified in different species. The present study aimed to investigate in *Trypanosoma brucei* the interaction networks, consisting of protein partners and mRNA targets, of RBP43 (also known as EIF4G1-IP2), previously found to be associated with an alternative eIF4F complex based on the EIF4E5, EIF4G1 and EIF4G1-IP1 subunits. Transgenic cell lines expressing RBP43, fused to HA epitopes, were generated and used in immunoprecipitation assays with anti-HA beads. Co-precipitated proteins and mRNAs were then investigated using LC-MS/MS and RIP-Seq. Proteomic analysis confirmed the RBP43 association with known eIF4F subunits and further revealed a strong association with the ZC3H39/ZC3H40 dimer, and several subunits of the CCR4-NOT deadenylation complex. Transcriptomic analysis demonstrated a selective enrichment for mRNAs encoding components of the bioenergetic machinery (respiratory chain), particularly transcripts for Complex IV (COX5, COXCC) and Complex V (ATPα, ATPEG3). Thus, RBP43 appears to act as a specialized post-transcriptional switch, perhaps acting in synergy with ZC3H39/ZC3H40, previously also defined as regulators of the respiratome. By recruiting respiratome mRNAs to the EIF4E5/EIF4G1 complex, RBP43 may coordinate mitochondrial metabolic adaptation, ensuring the proper assembly of respiratory complexes for ATP synthesis. It is noteworthy that several of the bound mRNAs encode proteins preferentially expressed in the *T. brucei* procyclic life stage. These findings contribute to the elucidation of yet another important mechanism in the translational regulation of this parasite. **Supported by:** Fiocruz, DFG **Keywords:** eIF4F; Gene expression regulation;RNA-Binding Protein.

PV – 003 - **Identification of genes potentially involved in vector competence in *Nyssomyia umbratilis* from endemic and non-endemic areas for american tegumentary leishmaniasis in the amazon region.**

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American Tegumentary Leishmaniasis (ATL) presents distinct epidemiological patterns in Rio Preto da Eva (RPE, endemic) and Manacapuru (MAN, non-endemic), Amazonas, Brazil. Previous molecular and parasite adhesion analyses suggest differences in the vector competence of *Nyssomyia umbratilis* populations from these localities. Proteomic and RT-qPCR analyses previously identified differentially abundant proteins and expressed genes between the two populations, particularly molecules with higher abundance in insects from MAN. The present study aims to complement these findings through functional and transcriptomic approaches. As *N. umbratilis* cannot be colonized under laboratory conditions, *Lutzomyia longipalpis* was used as a model for *Leishmania infantum* infection, gene expression analysis, and RNA interference (RNAi)-mediated gene silencing. Among the proteins more abundant in MAN, SARA (Smad Anchor for Receptor Activation), a negative regulator of the TGF-β signaling pathway, showed marked downregulation following infection, suggesting reduced parasite permissiveness. Functional infection assays following SARA silencing are currently underway to evaluate its effects on parasite load. Cytochrome P450, a detoxification-related enzyme, remained highly expressed at later stages of infection, suggesting a sustained response to parasite challenge, whereas its higher abundance in MAN may reflect enhanced oxidative regulation, potentially affecting parasite persistence within the midgut. Paramyosin, involved in muscle motility, remained upregulated at 48 h post-infection, suggesting infection-induced changes in gut musculature. Its greater abundance in MAN may contribute to increased gut peristalsis and parasite elimination. In contrast, Cysteine Protease C (CYSPC), associated with proteolysis, was upregulated 48 h after blood feeding regardless of parasite presence, indicating a primary role in blood digestion rather than infection. Its higher abundance in MAN may therefore reflect a more efficient digestive process less favorable to parasite establishment. Preliminary transcriptomic analyses generated high-quality RNA libraries. Transcriptomic data will be integrated with proteomic and RT-qPCR results to validate candidate genes and identify additional molecular markers associated with the distinct epidemiological profiles.These findings suggest possible local molecular adaptations associated with vector competence. **Supported by:** FAPERJ, CAPES, CNPQ e IOC-FIOCRUZ **Keywords:** Leishmania; Sand fly; Vectorial capacity.

PV – 004 - **Telomere dynamics: understanding the crosstalk between inositol pyrophosphates and telomeres in *Leishmania***

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Leishmania is a protozoan parasite that causes leishmaniasis, a neglected tropical disease affecting millions of people worldwide. Understanding the mechanisms regulating parasite adaptation and infectivity is essential for identifying new therapeutic targets. Inositol pyrophosphates (PP-IPs) regulate multiple cellular processes through protein pyrophosphorylation, including genome stability and DNA damage responses, whereas telomeres preserve chromosome integrity and contribute to parasite adaptation. However, the relationship between these pathways remains unknown in *Leishmania*. Here, we investigated the functional crosstalk between PP-IPs and telomeres. Using CRISPR/Cas9 in *Leishmania braziliensis*, we generated an IP6K heterozygous knockout (IP6K$^{-/+}$), a TERT full mutant (TERT$^{-/-}$), and a double knockout – dKO (TERT$^{-/-}$ and IP6K$^{-/-}$). The dKO mutant displayed impaired proliferation, cell-cycle defects, increased γH2A foci, and transcriptomic changes affecting metacyclogenesis and virulence, indicating elevated genomic stress. To further investigate these findings, equivalent mutants were generated in *Leishmania major*, a more suitable model for sand fly infections. The dKO lineage exhibited altered cell-cycle progression, increased ATM expression, and C-circle accumulation, suggesting activation of the Alternative Lengthening of Telomeres (ALT) pathway in response to telomeric instability. During sand fly infection, the dKO mutant showed reduced parasite burden and metacyclic differentiation, while murine infections revealed decreased infectivity. Re-expression of IP6K partially rescued these phenotypes. Together, our results demonstrate that simultaneous disruption of IP6K and TERT impairs genome stability, telomere homeostasis, differentiation, and infectivity in *Leishmania*, revealing for the first time a funcitional connection between inositol pyrophosphate signaling and telomere maintenance in this human pathogen. **Supported by:** FAPESP - 2022/00923-0 **Keywords:** Leishmaniasis; Pyrophosphorilation; Telomeres.

TB – 001 - **High-content screening of FDA-approved drugs in combination with benznidazole identifies potential anti - *Trypanosoma cruzi* agents.**

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Drug combination and repurposing have emerged as promising strategies for the discovery of new therapeutics for Chagas disease. We performed a fluorescence microscopy-based screening to identify anti-*T. cruzi* activity, in which a library of 640 FDA-approved drugs was tested individually or in combination with the reference drug benznidazole (BZN). From the primary screening, 30 drugs and 25 drug combinations were selected for further analysis. Among these, three drugs (oxcarbazepine, conduritol B, and manidipine) and two drug combinations (clarithromycin + BZN and granisetron + BZN) were prioritized based on their superior antiparasitic activity and selectivity. Manidipine (MAN), a calcium channel blocker, showed high potency and efficacy (EC_{50} = 0.9 µM; maximum activity = 100%) and a selectivity index >10. Its efficacy was confirmed in different host cells (U2OS, THP-1, and AC-16) and against *T. cruzi* strains representing different DTUs, including Y, CL Brener, and Colombiana. MAN displayed inhibitory activity against both trypomastigote (EC_{50} = 14.5 µM) and epimastigote forms (EC_{50} = 4.8 µM), demonstrating activity across distinct parasite life stages. Furthermore, MAN exhibited a rapid activity, eliminating the infection after 24 h of treatment. Following drug removal, no intracellular amastigotes or trypomastigotes in the supernatant were detected, supporting a trypanocidal effect. In combination with BZN, a fixed concentration of MAN (1 µM) potentiated BZN activity by 23-fold, enabling a substantial reduction in the concentration of the reference drug required to achieve an effective response. Isobolographic analysis demonstrated an additive interaction between BZN and MAN, with a ΣFIC of 1.0. In summary, MAN emerged as a promising candidate for further investigation. Our findings also highlight the potential of drug combination and repurposing strategies for identifying and developing new therapeutic options for Chagas disease. **Supported by:** CAPES and FCT (88887.156341/2017-00) and FAPESP (2016/15209-0)
Keywords: Chagas disease drug discovery; drug combination; drug repurposing.

TB – 002 - **Beyond Homology: Unveiling the Unique Structural Features of *Trypanosoma cruzi* γ-Glutamyl Phosphate Reductase**

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The proline biosynthetic pathway of *Trypanosoma cruzi* is relevant for parasite survival and proliferation and represents a promising source of therapeutic targets. In *T. cruzi*, γ-glutamyl kinase and γ-glutamyl phosphate reductase are encoded as a single fusion protein called P5CS. In this study, we focus on the γ-GPR domain as an independently expressed and characterized protein. γ-GPR catalyzes the NADPH-dependent reduction of γ-glutamyl phosphate to glutamate-γ-semialdehyde, the first step of proline biosynthesis. Here, we unveiled structural properties of *T. cruzi* γ-glutamyl phosphate reductase (*Tc*GPR) and its interaction with NADPH. We expressed active recombinant *Tc*GPR, which was characterized using SEC-MALS, DLS, and SAXS. *Tc*GPR was also crystallized to determine its atomic structure and investigate features related to cofactor recognition and catalysis. The crystallographic data revealed a tetrameric assembly and features that distinguish the enzyme from its homologues (1O20 *T. maritima*, 1VLU *S. cerevisiae*, and 2H5G *H. sapiens*). Electron density within the cofactor-binding region supports the presence of a bound NADPH molecule, suggesting a structural basis for cofactor recognition. In solution, however, SEC-MALS, DLS, and SAXS indicated a predominantly dimeric species, revealing a difference between the oligomeric states in solution and in the crystal. Importantly, SAXS experiments performed in the presence of NADPH revealed a conformational rearrangement of *Tc*GPR, consistent with a more closed conformation upon cofactor binding. Together, these results suggest that NADPH binding may induce a structural rearrangement. The *Tc*GPR tetrameric crystal structure, together with the SAXS data obtained in the presence of NADPH, provides evidence for a cofactor-associated rearrangement not readily inferred from sequence comparison. These findings contribute to understanding *Tc*GPR function in *T. cruzi* and provide insights for inhibitor design against Chagas disease. **Supported by:** FAPESP 2023/15602-08
Keywords: γ-Glutamyl phosphate reductase; Structural biology; Chagas disease.

TB – 003 - **Butoconazole as a therapeutic candidate for Chagas disease: experimental evidence against *Trypanosoma cruzi***

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Chagas disease (CD) is a neglected tropical disease (NTD) that remains a major global public health problem. Despite this, it faces severe limitations, both for diagnosis and regarding its therapy based on old and toxic drugs and less effective in the later disease stage. In this scenario, drug repurposing stands out as a promising strategy in the search for new treatment alternatives. From virtual screening, butoconazole (BTZ) was identified as a potential trypanosomicidal candidate. Subsequently, our findings validated its high potency at submicromolar concentrations against intracellular forms of the Tulahuen strain *in vitro* (EC_{50} = 0.07 µM), being about 24-fold more potent than BZ (EC_{50} = 1.73 µM), displaying with selectivity index of 341. The washout protocol showed a reduction in the total parasite load > 96%. The effect of combination therapy (BTZ+BZ), *in vitro*, revealed an additive interaction (xΣFICI = 0.66). BTZ monotherapy on a mouse experimental model of acute *T. cruzi* infection achieved a reduction in parasitemia of up to 63% at the highest concentration tested, while the combination of suboptimal doses (BTZ+BZ) promoted a 47% reduction in parasitemia and 100% animal survival. However, the detection of *T. cruzi* DNA by qPCR indicated the absence of parasitological cure. Taken together, our results demonstrate the potential of BTZ as a scaffold for novel therapeutic candidate through drug repositioning, highlighting its high potency *in vitro* and promising activity *in vivo*, although parasite persistence evidences the need for additional studies to optimize the compound. **Supported by:** FAPERJ, CNPq, PROEP/FIOCRUZ **Keywords:** Chagas disease; Trypanosoma cruzi; Butoconazole.

TB – 004 - **Modelling the Inflammatory Microenvironment of Localised Cutaneous Leishmaniasis Reveals Cytotoxic Reprogramming of CD4⁺ T Cells**

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Localised cutaneous leishmaniasis (LCL) is characterised by an exacerbated immune response in which inflammation and cytotoxicity contribute to tissue damage and disease immunopathogenesis. Although cytotoxic responses in LCL have been classically associated with $CD8^+$ T cells, we recently identified cytotoxic $CD4^+$ T cells that accumulate within lesions and display greater cytotoxic activity. Whether the inflammatory lesional environment drives the acquisition of this phenotype remains unclear. Here, we investigated whether modelling key inflammatory components of LCL lesions is sufficient to reshape $CD4^+$ T-cell phenotype and function. $CD4^+$ T cells isolated from healthy donors were stimulated for 2 or 7 days with a defined recombinant cytokine mix (cytomix) comprising IL-1β, IL-6, IL-8, IL-15, and IL-18, selected based on the inflammatory profile identified in LCL lesions. Cytotoxic activity was assessed using a calcein-release assay with K562 target cells, while phenotypic changes were analyzed by multiparametric flow cytometry. Cytomix-stimulated $CD4^+$ T cells exhibited a significant increase in specific lysis of K562 target cells, indicating the acquisition of functional cytotoxic activity. After 2 days, stimulated $CD4^+$ T cells showed increased expression of CD69, granzyme B, NKG2D, and IFN-γ. After 7 days, increased expression of Ki-67, T-bet, DAP12, KLRG1, and NKG2C, was observed, consistent with a proliferative activity, effector differentiation, and the acquisition of NK-like cytotoxic potential. Collectively, these findings demonstrate that inflammatory signals modelling the LCL lesional environment are sufficient to reshape $CD4^+$ T-cell functionality toward a cytotoxic state, providing basis for the emergence of cytotoxic $CD4^+$ T cells within LCL lesions. **Supported by:** FAPES 51677.797.18389.30092022 **Keywords:** Localised Cutaneous Leishmaniasis; Cytokine; CD4 T cells.