The human pathogen, Schistosoma mansoni, lacks the cognate sequence for human telomerase reverse transcriptase (hTERT) and relies on the snail host, Biomphalaria glabrata homologous enzyme for its intra-molluscan development.

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The human pathogen, *Schistosoma mansoni,* lacks the cognate sequence for human telomerase reverse transcriptase (hTERT) and relies on the snail host, *Biomphalaria glabrata,* homolog for its intra-molluscan development.

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ABSTRACT

The human telomerase reverse transcriptase (hTERT) is the catalytic sub-unit of the ribonuclear protein, telomerase. Together with telomerase RNA, the enzyme complex participates in the maintenance of telomeres at the proximal ends of chromosomes, adding species-specific repeats to the 3'end of the telomere. The regulation of hTERT is tightly linked to the cell cycle and cell differentiation states governing either malignancy or senescence, making it a prospective therapeutic target of cell proliferation in cancer. Malignancy behaves like a parasitic disease in that it only progresses by depending on biochemical and molecular pathways of the host. The snail host/schistosome relationship provides a facile model to examine the regulation of the cancer transcriptome, such as the gastropod homolog of hTERT. To test this hypothesis in relation to the development of larval *Schistosoma mansoni* in the *Biomphalaria glabrata*, we utilized an *in*-silico approach to identify the hTERT homolog of *B. glabrata*. The human hTERT amino acid sequence (ID 014746) shows a strong homology (E-value of $2e^{-86}$) to the *B. glabrata* ortholog (733 amino acids, accession XP_013074763.1). BLASTp analyses using *S. mansoni* as the query suggested that the parasite lacks a cognate TERT. To study the regulation of the snail-like hTERT in relation to schistosome development, transcriptome analysis was performed which revealed a temporal regulation of the telomerase before and during *S. mansoni* infection, with an upregulation of *B. glabrata* hTERT transcription evident by 30 minutes after exposure to the parasite. The anti-telomerase drugs, BPPA and BIBR at 100 ng/mL before infection blocked shedding of parasite cercariae. These findings indicate that the schistosome may rely on the telomerase of its host for asexual reproduction, development and proliferation.

KEYWORDS

Schistosomiasis, Snail host, host-pathogen, Human telomerase, Reverse Transcriptase, hTERT inhibitors, BPPA, BIBR, Lamivudine

INTRODUCTION

Schistosomiasis is one of the most prevalent neglected tropical diseases (NTDs) with Sub-Saharan Africa carrying the greatest burden. Although improved sanitation, health education and mass drug administration have been identified as the key preventative strategies, 200 million people remain globally infected with an estimated 600 million at risk for infection [1]. Schistosomiasis is a chronic debilitating disease caused by at least three major species of blood flukes: *Schistosoma haematobium, Schistosoma japonicum and Schistosoma mansoni*. The life cycle of the parasite is complex and involves an obligatory species-specific intermediate snail host – a relationship that is the result of ~200,000 years of the co-evolution between the parasite, snail, and a definitive human host [2].

In the freshwater snail, such as *B. glabrata* that is prevalent in the Western Hemisphere, larval free-swimming forms of the parasite, cercariae, released from the infected snail, infect the human host by penetrating the skin. Upon infection, the cercaria loses its tail and transforms into the schistosomulum which invades the vasculature. Several weeks later male and female worms' pair and develop into adult forms of the parasite. In *S. mansoni*, the schistosomes reside in the intestinal mesenteries and lay metabolically active eggs that translocate into the intestinal lumen and the liver [3]. The eggs lodged in tissue induce granuloma, fibrosis and calcification that left untreated leads to chronic morbidity and mortality that are hallmarks of chronic schistosomiasis. Eggs that are excreted with human excreta (feces or urine depending on the species) into a fresh water source, hatch to produce free-swimming short-lived miracidia that infect resident compatible snails. Larval miracidia within the infected snail develop into sporocyst and can be found in the hepatopancreas region of the snail where germ balls give rise to 1000s of asexually infective cercariae that are released into the water source where they penetrate the skin upon human contact, thereby completing the parasite's life cycle. The inclusion of *S. haematobium* on IARC's Group1 list of carcinogens shows the detrimental nature of this infection to humans [4]. Also, there are compelling clinical data that suggest the *S. japonicum* infection leads to the development of colorectal cancer [5]. Furthermore, the *S. mansoni eggs* have been shown to secrete antigens that activate liver cancer regulators c-Jun and STAT3, showing evidence of hepatocellular cancer promotion [6]. It is important that these cancer-associated pathogens be studied and analyzed to reduce the risk of infection which may lead to cancer. We hypothesize that malignancy behaves like a parasitic disease since uncontrolled cell growth in cancer progresses at the expense of the host without immunological recognition. The snail host/schistosome relationship, therefore, provides a facile model to examine the regulation of transcription of cancer-related transcripts, including the snail ortholog of hTERT. To begin to address this hypothesis in relation the development of larval

S. mansoni in the snail *B. glabrata*, we utilized an *in silico* approach to identify the snail hTERT ortholog. Here, we show from the comparative analysis of the amino acid (ID 014746) sequences of human hTERT and *B. glabrata* TERT that the two metazoans that serve as hosts for the parasite's life cycle are significant but absent in the parasite genome. This novel and intriguing outcome provides an opportunity to examine the role of hTERT in parasitism of the snail host and immortality in metastatic cancer.

MATERIALS AND METHODS

Snail husbandry

The susceptible *Biomphalaria glabrata* strains NMRI and BBO2, and parasite resistant BS-90 snails were utilized throughout this study. Snails were maintained in aquaria in de-aerated water and fed with romaine lettuce as previously described [7].

Snail exposure to parasite

Miracidia hatched from eggs isolated from infected mice livers were obtained from the Biomedical Research Institute, Rockville, MD. Juvenile snails (3-4 mm in diameter) were exposed to 10-12 miracidia, individually, in one ml of aerated water in a 6-well microtiter plate. The snails were exposed for increasing intervals, 0, 30, 60, 120, 240 min, and 18 h. RNA was extracted immediately thereafter from the snail or from snails that had been snap frozen at -80°C. In addition, other exposed snails (drug treated and non-drug treated), were maintained at room temperature for between 4 to 8 weeks and examined for cercarial shedding. Snails susceptible (NMRI and BBO2) and resistant (BS-90) to schistosome infection were included.

Drug treatment of snails

To determine the effect of hTERT inhibitors, BPPA, BIBR and RT inhibitor, lamivudine, on juvenile snail infections, snails were treated with 100 ng/mL of each of these inhibitors overnight at room temperature in 6-well microtiter plates [7]. After treatment, snails were washed and exposed to miracidia. Drug treated and non-treated snails were held at room temperature and maintained for 4 to 10 weeks and examined for cercarial shedding [7]. To investigate the impact of the hTERT inhibitors post-infection, juvenile snails were exposed for 18 hrs with 100 ng/mL of inhibitors separately and were examined 3 to 14 days later for cercarial shedding.

Bioinformatics and primer design

A search for *B. glabrata* homologs of human related hTERT transcripts were performed by using the protein database Uniprot (www.uniprot.org). The amino acid sequence was deposited into the Basic Local Alignment Search Tool (BLAST) to identify the *B. glabrata* homolog, followed by a SMART BLAST analysis to validate the identity of the hTERT *B. glabrata* homolog and its evolutionary relatedness to the human amino acid sequence. Gene specific primers were designed from the corresponding *B. glabrata* mRNA transcript as described [7]. Oligonucleotide primers for qPCR were obtained from Eurofins Genomics (Louisville 13 KY, 40204).

Phylogenetic tree

The hTERT *B. glabrata* homolog identified through SMART BLAST analysis was aligned with the human hTERT ortholog using COBALT, a constraint-based Multiple Alignment tool (https://www.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi)

RNA isolation, cDNA Synthesis, qPCR

RNA isolation and real time qPCR were performed by using the *B. glabrata* hTERT gene specific primers (*Forward 5' AGGTCTGCGCACCATTGTTA 3': Reverse 3' TGGCAGCTTAGTCAGCGTTT 5'*) [7]. Myoglobin was used as reference.

Trans-well *in vitro* co-culture of miracidia with *B. glabrata* embryonic cell line (Bge)

Bge cells were cultured as previously described, but in 6-well microtiter plates to confluency [8]. Briefly, wells were seeded with 200cells/ul in 2 ml of complete Bge medium (https://www.afbr-bri.org/schistosomiasis/standard-operating-procedures) and were cultured to confluency. Individual 24mm permeable transwell inserts (0.4um Corning Inc. ME, USA) were placed into the wells of the microtiter plate before adding ~30 recently hatched miracidia to each well. Co-culture was performed for 0, 30, 60 and 120 min before harvesting the cells and miracidia and resuspending pellets in 300 µL RNAZOL. Total RNA was either isolated immediately from the cells or after storage at -80°C. The RNA was exposed to RNase-free DNase to remove contaminating genomic DNA**.**

Statistical Analysis

All data and statistical analysis were performed by GraphPad Prism 8. Results are presented as mean ± SD. Data were analyzed by Student's *t* test, Welch's *t* test and 1- or 2-way ANOVA with Tukey's test wherever relevant by comparing the differential expression (delta-Ct value) of the transcripts among groups. Fold change was determined by utilizing uniform expression of the h reference myoglobin. Differences with $P < 0.05$ were considered statistically significant. Asterisks indicate statistical difference as follows: N.S., not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

RESULTS

There is an ortholog of hTERT in the genome of the snail *B. glabrata* but not apparently in its schistosome parasite

The hTERT amino acid sequence of 733 amino acids (accession number XP_013074763.1) appears to be strongly conserved between *Homo sapiens* and *B. glabrata*, with a significant E-value of $2e^{-86}$ sharing 27.33% identity with the human ortholog (Table 1). A phylogenetic tree of the *B. glabrata hTERT* homolog and other hTERT- encoding transcripts in public databases revealed that the snail homolog is more closely related to vertebrate than to invertebrate sequences (Fig. 1A). The multiple sequence alignments (Fig. 1B) of the human (AAC51724.1) and *B. glabrata* (XP_013074763.1) amino acid sequences showed significant overlap (conserved regions are shown in red). Our results from further interrogations of the *S. mansoni* reference genome in Worm Base and GenBank NCBI databases failed to reveal an hTERT-like sequence within the genome of *S. mansoni*.

The transcript encoding the snail hTERT homolog is upregulated following parasite exposure

We investigated the temporal expression of the hTERT-encoding transcript using real-time PCR in susceptible NMRI and BBO2 snails following exposure to *S. mansoni* miracidia at increasing intervals. Four biological replicates with eight individual snails per time-point revealed that hTERT was upregulated as early as 30 minutes after parasite exposure (Figs. 2A and 2B). Transcription remained upregulated in susceptible NMRI snails throughout 16 hours post exposure compared to the exposure controls. In susceptible BBO2, the transcription of hTERT was upregulated at 30-, 60- and 240-min following exposure to miracidia. In contrast, the transcript encoding *hTERT* was downregulated in the BBO2 snails at 16 hours compared to the upregulation of NMRI snails at 16 hours, revealing variability in snail susceptibility to the miracidia among discrete snail isolates.

The transcript encoding the snail hTERT homolog is upregulated following co-culture with a snail cell line

The *B. glabrata* embryonic (Bge) cell line [9] was used to validate the expression of hTERT *in vitro.* In figure 3, it is shown that Bge cells co-cultured with *S. mansoni* miracidia at specific time-points (0 min, 30 min, 1 hr, 2 hr) showed significant upregulation of hTERT transcripts at 30 minutes (2.84-fold change) and 2 hours (2.58-fold change). Additionally, we investigated the expression of the non-LTR retrotransposable element *nimbus*, which also encodes a reverse transcriptase domain. Similar to hTERT, *nimbus*-RT showed transcriptional upregulation in *S. mansoni* exposed snails, with increased expression observed from 30 minutes to 2 hours.

Pre- and post-drug treatment of snails by hTERT inhibitors down regulates the corresponding transcript and blocks *S. mansoni* infection

To determine the effect of hTERT inhibitor drugs on *S. mansoni* infection in *B. glabrata*, we used cercarial shedding to monitor the efficacy of the drugs to affect infection of juvenile susceptible NMRI snails. Figures 4 and 5 show the presence or absence of infection when the snails were treated with drugs before and after infection at different time points, respectively. None of the snails treated with either Lamivudine or BIBR prior to schistosome exposure shed any cercariae, as seen in figure 4. Non drug-treated infected control snails shed, as expected, the highest number of cercariae. Similarly, BPPA pre-treated exposed snails also shed cercariae. All non -drug treated infected snails, shed cercariae at 4 weeks post- infection, with an increase in the number of cercariae shed at 6 and 8 weeks post infection.

Lamivudine- treated snails, 2 weeks post-infection, also shed cercariae, although at a lower number than the control non-treated infected snails (Fig. 5). The snails treated with BPPA failed to shed cercariae for the duration of the experiment (up to 10 weeks post-exposure). Among the BIBR- treated snails, only one shed 150 cercariae.

The results suggest that BPPA is effective in blocking *S. mansoni* infection in *B. glabrata* snails. To investigate the optimal time point for BPPA treatment during the infection, snails were treated at different intervals post-exposure to *S. mansoni*. Snails were treated with BPPA on days 3, 10 and 14 post-exposure. Snails treated at days 3 and 10 shed cercariae, although at lower numbers compared to the non-drug treated infected snail (Fig. 6). No cercarial shedding was observed in snails treated 14 days post-exposure. Snails treated on day 3 shed more cercariae than those treated on day 10, but still did not shed as many as the non-drug treated infected snail. These results suggest that BPPA has the potential for the treatment for *S. mansoni* infection in *B. glabrata* snails.

DISCUSSION

We have shown from these results that the parasitic trematode, *S. mansoni*, lacks hTERT, the catalytic subunit of the telomerase complex that builds and then maintains the integrity and structure of telomeres at the end of chromosomes. The mechanism of action of the enzyme's role in the dynamic alteration of telomere length in cancer versus senescence is well known. Our results showing significant homology exists between the human RT and snail may not be surprising but the parasite lacking an ortholog in its genome was unexpected. On examining parallels between metastatic cancer as a parasitic disease, it was a natural choice to study expression of hTERT within the context of schistosome infection in the snail host, since it is well known that this transcript plays an important role in cell immortality observed in malignancy [10]. Given that hTERT occurs in the free-living non-parasitic flatworm, planaria, we expected there to be a schistosome homolog for this enzyme.

Results from qPCR analysis showed that the transcript encoding the snail host, single copy, hTERT is upregulated early, within 30 minutes, after infection of the susceptible NMRI and BBO2 snails. Interestingly, this post exposure upregulation, was also observed *in vitro* in miracidia -transwell co-culture experiments with the *B. glabrata* embryonic cell line (Bge); results which showed, unequivocally, that the substance stimulating the induction of the snail host hTERT transcript soon after exposure to the parasite is an excretory -secretory -product (ESP) that is released from miracidia. We have previously shown that excretory-secretory products from miracidia can trigger the non-random repositioning of gene loci within the snail host nuclei [11] [12]. These studies have been confirmed from both *in vivo* and *in vitro* studies, for several snail host gene loci after early schistosome infection, with the re-positioning corresponding to gene up-regulation [13][14].

Proteomics analysis of the miracidia ESP has shown that this material is biochemically complex and yet we know very little about biological activity of the released external soluble products [15]. To further examine the molecular make-up of schistosome miracidia ESPs, we have recently shown the occurrence of RNA in ESP. This external RNA (exRNA) when complexed to PEI cationic nanoparticles, silences expression of both hTERT and nimbus RT in juvenile BBO2 snails but upregulates PIWI (manuscript in prep). The RT inhibitor, lamivudine, is known to inhibit schistosomiasis in the snail [7]. Here, we show for the first time, that hTERT anti-cancer inhibitors, BPPA and BIBR, can also block schistosome infection in the snail host if administered either before exposure or later at 3 to 14 days post- exposure. Collectively, these data suggest that the intra-molluscan parasite, lacking hTERT, requires the snail telomerase for its development, purportedly to synthesize new telomere termini of the chromosomes in hundreds of cercariae.

The dependency of schistosomes on reverse transcriptase either from hTERT or the endogenous non-LTR- retrotransposon, *nimbus* [7, 16] is intriguing, and more studies are required to unravel these unexpected results. According to Wormbase ParaSite (WBPS) release 17 [16], the gene Smp_241410 (UniProt ID: A0A5K4EZR8) from the V9 draft genome of *S. mansoni* [17] encodes a protein containing a part of the PANTHER [18] protein domain with annotation "telomerase reverse transcriptase "(PTHR12066)". However, the complete model of the domain is 1305 amino acids in length, whereas in *S. mansoni,* the protein only matched a region of 121 amino acids (in hTERT it matches a region of 1123 amino acids according to ensemble) [19]. The comparative genomics data from WormBase ParaSite for this gene substantiates its exclusive evolutionary origin within Trematodes, as almost no orthologous relationships beyond this taxonomic group have been inferred. The highly conserved nature of this unique gene among flukes, along with its partial reverse transcriptase domain, raises the possibility that it might have been silenced across evolution. However, it cannot be overlooked

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that this gene might encode a functional protein in *S. mansoni*. Future studies will focus on investigating the use of CRISPR-Cas9 gene editing technology to study more closely the relationship between the expression of hTERT in the snail-host parasite interaction. Interrupting parasite development of other medically important schistosome species, *S. haematobium* and *S. japonicum* with the aforementioned hTERT inhibitors in their specific snail host species (*Bulinus* and *Oncomelania*, respectively) will also be evaluated.

The spread of schistosomiasis to higher latitude countries is now underway [20]. There is no preventative vaccine and WHO has earmarked the year 2025 for reduction of global schistosomiasis [21]. It is therefore of great importance that new drug targets are discovered soon. There is also a need to combat female genital schistosomiasis (FGS) and its link to cancer and exacerbation of HIV infection [22]. In follow-up studies we will examine the effect of these RT inhibitors in the mouse model of schistosomiasis. In summary, the findings suggest that *S. mansoni* lacks an orthologue of hTERT and utilize its host telomerase. Specific inhibitors, of hTERT, BPPA and BIBR reduced the replicative capacity of the schistosome within its host. These intriguing results warrant continued investigation and raise the tantalizing possibility that FDA-approved anti-viral or anti-tumor agents are worthy of investigation for schistosomiasis.

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Credit authorship contribution statement

Matty Knight: Conceptualization, Formal Analysis, Data Curation, Methodology, Investigation, Resources, Project Administration, Supervision, Funding Acquisition, Writing – Original draft, Writing – review & editing; **Nana Adjoa Pels:** Investigation, Methodology, Validation. **Swara Yadav:** Investigation, Methodology; **Oumsalama Elhelu:** Investigation, Methodology, Data Curation, Formal Analysis, Validation, Writing – review and editing; **Simone Parn:** Investigation, Methodology, Data Curation, Formal Analysis, Validation, Visualization, Software, Writing – original draft, Writing – review & editing; **Gabriel Rinaldi**: Bioinformatics, interrogation of recent version of the *Schistosoma mansoni* genome version, writing and editing of manuscript; **Dionysios Grigoriadis**: Bioinformatics and in-depth search of 'Worm Base', writing and editing of manuscript, **Victoria, Mann**: Resources & discussion/ editing of manuscript; **Paul J. Brindley**: Resources, conceptualization, writing and editing of manuscript; **Joanna M. Bridger**: Conceptualization, experimental design, writing & editing of manuscript

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

REFERENCES

1. Sacolo-Gwebu H, Chimbari M, Kalinda C. Prevalence and risk factors of schistosomiasis and soil-transmitted helminthiases among preschool aged children (1-5 years) in rural KwaZulu-Natal, South Africa: a cross-sectional study. Infect Dis Poverty. 2019;8(1):47. Epub 20190616. doi: 10.1186/s40249-019-0561-5. PubMed PMID: 31202273; PubMed Central PMCID: PMCPMC6571117.

2. Costain AH, MacDonald AS, Smits HH. Schistosome Egg Migration: Mechanisms, Pathogenesis and Host Immune Responses. Front Immunol. 2018;9:3042. Epub 20181220. doi: 10.3389/fimmu.2018.03042. PubMed PMID: 30619372; PubMed Central PMCID: PMCPMC6306409.

3. Schwartz C, Fallon PG. Schistosoma "Eggs-Iting" the Host: Granuloma Formation and Egg Excretion. Front Immunol. 2018;9:2492. Epub 20181029. doi: 10.3389/fimmu.2018.02492. PubMed PMID: 30459767; PubMed Central PMCID: PMCPMC6232930.

4. van Tong H, Brindley PJ, Meyer CG, Velavan TP. Parasite Infection, Carcinogenesis and Human Malignancy. EBioMedicine. 2017;15:12-23. Epub 20161202. doi: 10.1016/j.ebiom.2016.11.034. PubMed PMID: 27956028; PubMed Central PMCID: PMCPMC5233816.

5. Almoghrabi A, Mzaik O, Attar B. Schistosoma japonicum Associated With Colorectal Cancer. ACG Case Rep J. 2021;8(5):e00572. Epub 20210511. doi:

10.14309/crj.0000000000000572. PubMed PMID: 33997087; PubMed Central PMCID: PMCPMC8115999.

6. Roderfeld M, Padem S, Lichtenberger J, Quack T, Weiskirchen R, Longerich T, et al. Schistosoma mansoni Egg-Secreted Antigens Activate Hepatocellular Carcinoma-Associated Transcription Factors c-Jun and STAT3 in Hamster and Human Hepatocytes. Hepatology. 2020;72(2):626-41. Epub 20190212. doi: 10.1002/hep.30192. PubMed PMID: 30053321; PubMed Central PMCID: PMCPMC7496692.

7. Smith M, Yadav S, Fagunloye OG, Pels NA, Horton DA, Alsultan N, et al. PIWI silencing mechanism involving the retrotransposon nimbus orchestrates resistance to infection with Schistosoma mansoni in the snail vector, Biomphalaria glabrata. PLoS Negl Trop Dis. 2021;15(9):e0009094. Epub 2021/09/09. doi: 10.1371/journal.pntd.0009094. PubMed PMID: 34495959; PubMed Central PMCID: PMCPMC8462715.

8. Coelho FS, Rodpai, R., Miller, A.,Karinshak, S.E., Mann, V.H,dos Santos Carvalho, O.,Caldeira, R.L., de Moraes Mourão, M,, Brindley, P.J, Ittiprasert.W. Diminished adherence of snail hemocytes to schistosome sporocysts of Schistosoma mansoni following programmed knockout of the allograft inflammatory factor of Biomphalaria glabrata. BioRxiv 2020;doi: https://doi.org/10.1101/2020.04.07.029629.

9. Odoemelam E, Raghavan N, Miller A, Bridger JM, Knight M. Revised karyotyping and gene mapping of the Biomphalaria glabrata embryonic (Bge) cell line. Int J Parasitol. 2009;39(6):675-81. Epub 2009/01/10. doi: 10.1016/j.ijpara.2008.11.011. PubMed PMID: 19133265; PubMed Central PMCID: PMCPMC2656398.

10. Hannen R, Bartsch JW. Essential roles of telomerase reverse transcriptase hTERT in cancer stemness and metastasis. FEBS Lett. 2018;592(12):2023-31. Epub 20180518. doi: 10.1002/1873-3468.13084. PubMed PMID: 29749098.

11. Knight M, Ittiprasert W, Odoemelam EC, Adema CM, Miller A, Raghavan N, et al. Non-random organization of the Biomphalaria glabrata genome in interphase Bge cells and the spatial repositioning of activated genes in cells co-cultured with Schistosoma mansoni. Int J Parasitol. 2011;41(1):61-70. Epub 2010/09/21. doi: 10.1016/j.ijpara.2010.07.015. PubMed PMID: 20849859; PubMed Central PMCID: PMCPMC3081665.

12. Knight M, Ittiprasert W, Arican-Goktas HD, Bridger JM. Epigenetic modulation, stress and plasticity in susceptibility of the snail host, Biomphalaria glabrata, to Schistosoma mansoni infection. Int J Parasitol. 2016;46(7):389-94. doi: 10.1016/j.ijpara.2016.03.003. PubMed PMID: 27056272.

13. Arican-Goktas HD, Ittiprasert W, Bridger JM, Knight M. Differential spatial repositioning of activated genes in Biomphalaria glabrata snails infected with Schistosoma mansoni. PLoS Negl Trop Dis. 2014;8(9):e3013. Epub 2014/09/12. doi: 10.1371/journal.pntd.0003013. PubMed PMID: 25211244; PubMed Central PMCID: PMCPMC4161332.

14. Bridger JM, Brindley PJ, Knight M. The snail Biomphalaria glabrata as a model to interrogate the molecular basis of complex human diseases. PLoS Negl Trop Dis. 2018;12(8):e0006552. doi: 10.1371/journal.pntd.0006552. PubMed PMID: 30091971; PubMed Central PMCID: PMCPMC6084811.

15. Wu XJ, Sabat G, Brown JF, Zhang M, Taft A, Peterson N, et al. Proteomic analysis of Schistosoma mansoni proteins released during in vitro miracidium-to-sporocyst transformation. Mol Biochem Parasitol. 2009;164(1):32-44. Epub 20081127. doi: 10.1016/j.molbiopara.2008.11.005. PubMed PMID: 19095013; PubMed Central PMCID: PMCPMC2665799.

16. Lee RYN, Howe KL, Harris TW, Arnaboldi V, Cain S, Chan J, et al. WormBase 2017: molting into a new stage. Nucleic Acids Res. 2018;46(D1):D869-D74. doi:

406 10.1093/nar/gkx998. PubMed PMID: 29069413; PubMed Central PMCID:
407 PMCPMC5753391.
408 17. Buddenborg SK, Tracey A, Berger DJ, Lu Z, Doyle SR, et al. Assembled chromosomes of
409 the blood fluke *Schistosoma mansoni* provide insight into the evolution of its ZW sex-
410 determination system. *bioRxiv.* 2021. doi: 10.1101/2021.08.13.456314.
411 18. Mi H, Ebert D, Muruganujan A, Mills C, Albou LP, Mushayamaha T, et al. PANTHER
412 version 16: a revised family classification, tree-based classification tool, enhancer regions and
413 extensive API. Nucleic Acids Res. 2021;49(D1):D394-D403. doi: 10.1093/nar/gkaa1106.
414 PubMed PMID: 33290554; PubMed Central PMCID: PMCPMC7778891.
415 19. Cunningham F, Allen JE, Allen J, Alvarez-Jarreta J, Amode MR, Armean IM, et al.
416 Ensembl 2022. Nucleic Acids Res. 2022;50(D1):D988-D95. doi: 10.1093/nar/gkab1049.
417 PubMed PMID: 34791404; PubMed Central PMCID: PMCPMC8728283.
418 20. Kincaid-Smith J, Rey O, Toulza E, Berry A, Boissier J. Emerging Schistosomiasis in
419 Europe: A Need to Quantify the Risks. Trends Parasitol. 2017;33(8):600-9. Epub 20170521.
420 doi: 10.1016/j.pt.2017.04.009. PubMed PMID: 28539255.
421 21. Deol AK, Fleming FM, Calvo-Urbano B, Walker M, Bucumi V, Gnandou I, et al.
422 Schistosomiasis - Assessing Progress toward the 2020 and 2025 Global Goals. N Engl J Med.
423 2019;381(26):2519-28. Epub 2019/12/28. doi: 10.1056/NEJMoa1812165. PubMed PMID:
424 31881138; PubMed Central PMCID: PMCPMC6785807.
425 22. Hotez PJ, Harrison W, Fenwick A, Bustinduy AL, Ducker C, Sabina Mbabazi P, et al.
426 Female genital schistosomiasis and HIV/AIDS: Reversing the neglect of girls and women.
427 PLoS Negl Trop Dis. 2019;13(4):e0007025. Epub 2019/04/05. doi:
428 10.1371/journal.pntd.0007025. PubMed PMID: 30946746; PubMed Central PMCID:
429 PMCPMC6448816 is in clinical trials.
430

431

Figures

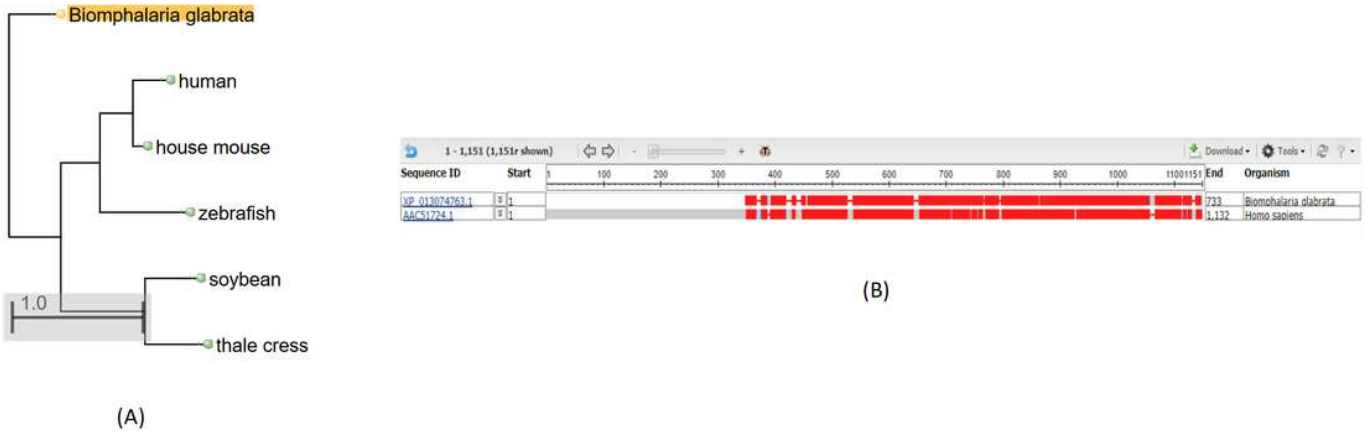

Figure 1

Legend not included with this version.

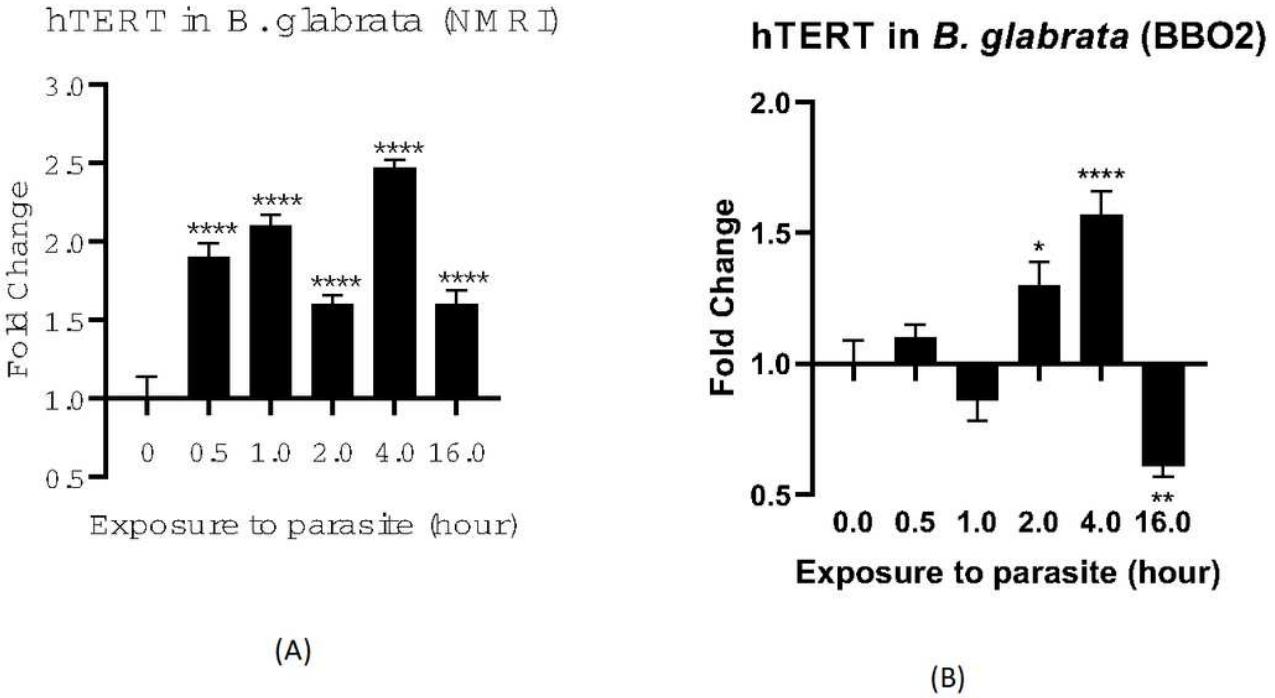

Figure 2

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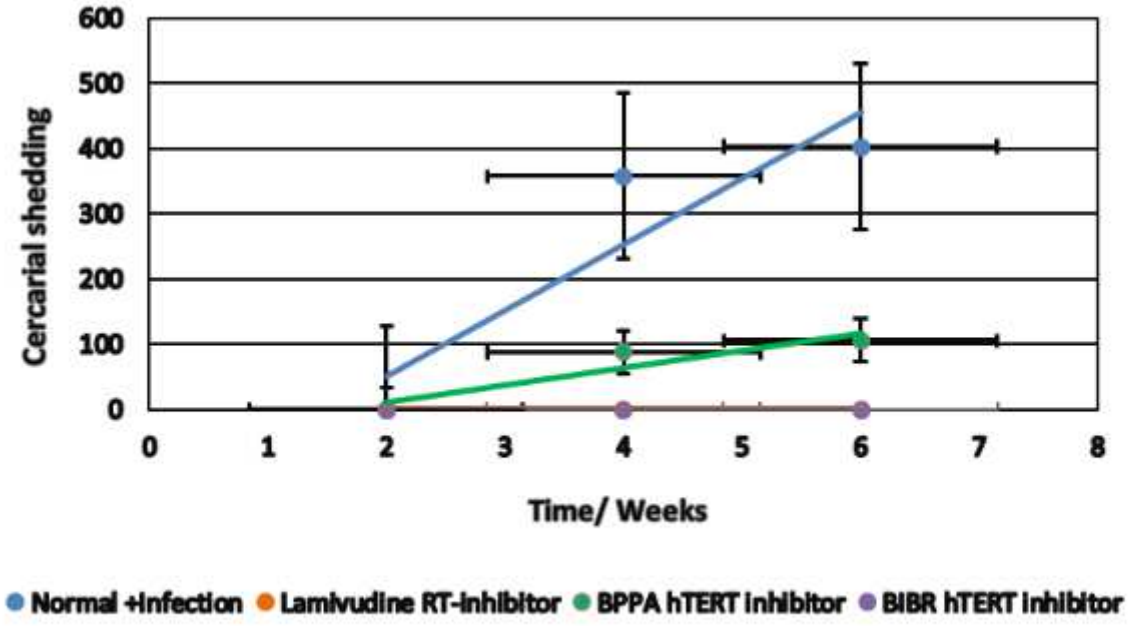

Figure 3

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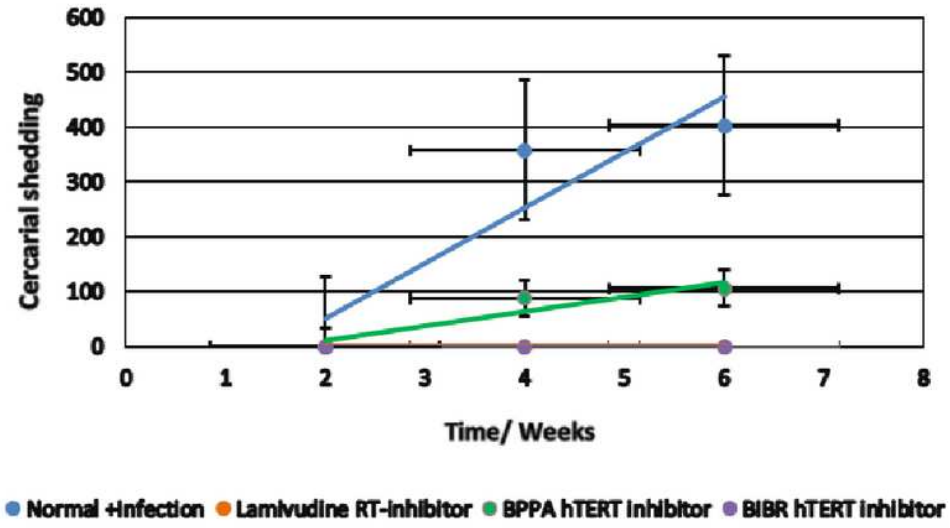

Figure 4

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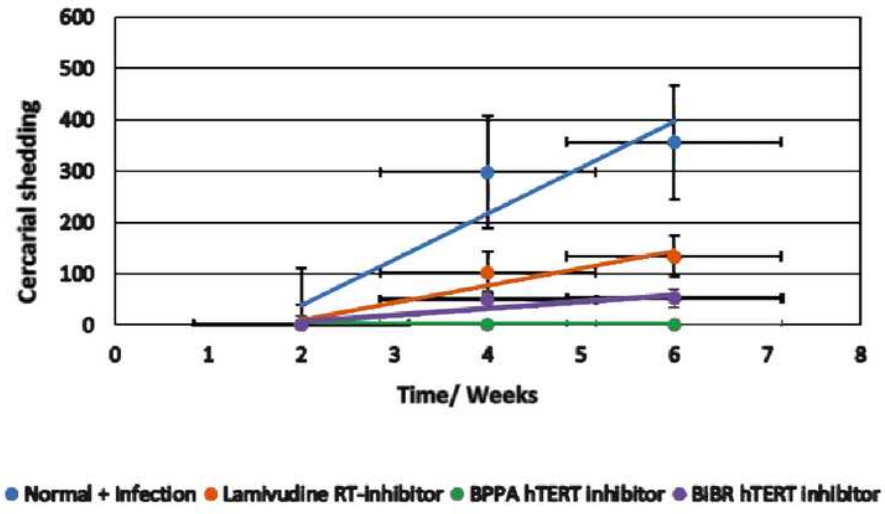

Figure 5

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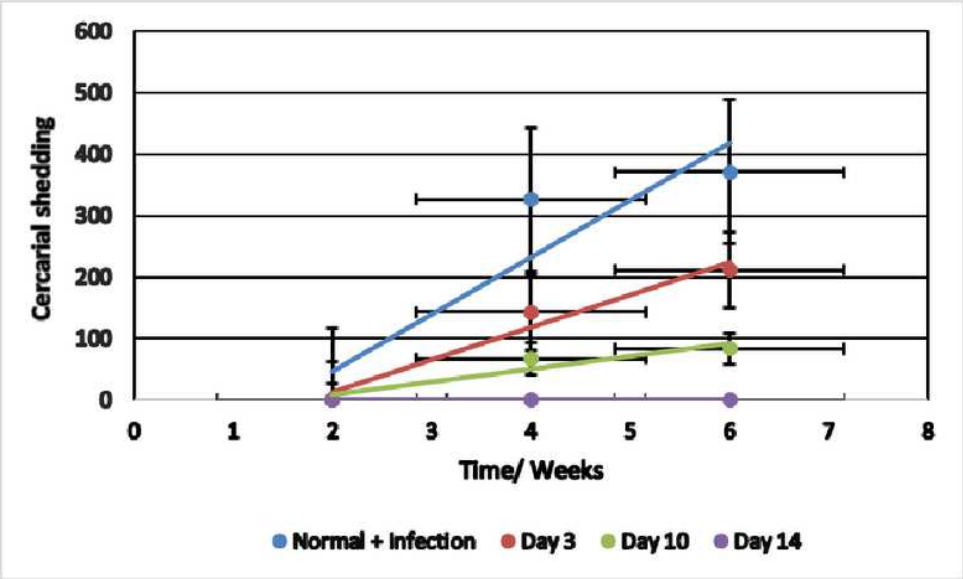

Figure 6

Legend not included with this version.

Supplementary Files

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- Table1.pdf