The antidepressant sertraline provides a novel host directed therapy module for augmenting TB therapy

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Running title: **The antidepressant sertraline augments antimycobacterial therapy**

ABSTRACT:

A prolonged therapy regimen, primarily responsible for development of drug resistance by *Mycobacterium tuberculosis* (Mtb), the causative agent of human TB, obligates any new regimen to not only reduce treatment duration but also escape pathogen resistance mechanisms. With the aim of harnessing the host response in providing additional support to existing regimens (host directed therapy- HDT), we established the ability of a well-tolerated anti-depressant (sertraline - SRT) to modulate the pro-pathogenic type I IFN response of macrophages to Mtb infection. More importantly, while SRT alone could only arrest bacterial growth, it could effectively escalate the bactericidal activities of Isoniazid (H) and Rifampicin (R) in macrophages. This strengthening of antibiotic potencies by SRT was more evident in conditions of ineffective control by these frontline TB drugs: against HR tolerant strains or dormant Mtb. SRT, could significantly combine with standard TB drugs to enhance early pathogen clearance from tissues of mice infected with either drug sensitive/ tolerant strains of Mtb. Further, we demonstrate an enhanced protection of the highly susceptible C3HeB/FeJ mice in an acute model of TB infection with the combination therapy signifying the use of SRT as a potent adjunct to standard TB therapeutic regimens against

bacterial populations of diverse physiology. This study advocates a novel host directed adjunct therapy regimen for TB with an FDA approved and well tolerated anti-depressant to achieve quicker and better control of infection.

Abbreviations: IFN- Interferon, SRT- sertraline hydrochloride, Mtb- *Mycobacterium tuberculosis,* HDT- host directed therapy

Introduction:

The current TB therapy regimen ranging between 6 months for pulmonary and 1-2 years for extrapulmonary infections, is often associated with severe drug induced toxicity in patients. Moreover, its failure to completely eradicate the pathogen from the host forms an ideal platform for the emergence of drug resistant strains[1,2]. It is not surprising that these strains have emerged at an alarming rate in the population and are imposing serious impediments to TB control programs globally [3,4]. Introduction of newer modalities like Host directed therapies (HDT) with the potential to reduce duration of therapy and not be affected by pathogen resistance mechanisms offer significant advantages in this scenario[5,6]. Several strategies for HDT with diverse modes of action- boosting immune response[7,8,9], targeting virulence mechanisms [10-12], augmenting host metabolism[13] and host nutrition[14] have been identified in recent times. Effective molecular entities like antibodies [15,16], cytokines[17,18], cell based therapies[19], drugs used for other human non-infectious diseases and recombinant proteins[20] have been tested against bacterial infections like *Streptococcus pneumoniae*[16], *Bordetella pertussis*[21,22], *Helicobacter pylori*[23] and against viral infections like HIV[24], CMV[25], Hepatitis C[18], influenza[26] and Ebola viruses[27].

Mtb infection invokes several mechanisms of pathogen clearance in host cells like induction of pro-inflammatory response, metabolic stress, phago-lysosomal lysis programs, apoptosis/ autophagic mechanisms[28-30]. Co-evolution with humans has helped Mtb adapt and survive these host derived stresses through complex and intricate interactions to facilitate optimal infection[31-38]. Extensive efforts have led to the development of therapeutic strategies countering the pathogen mediated subversion of cellular clearance mechanisms[14,39-43].

A prominent response of Mtb infected macrophages is the early and robust induction of type I IFN signaling that is associated with a detrimental effect on host survival [44-47]. We sought to offset this response in macrophages and evaluate the effect on the infection dynamics. A previous study identified several antipsychotic molecules as potent antagonists of Type I IFN response to polyI:C[48]. We hypothesized that this property of antidepressants as TI IFN antagonists might help stunt this macrophage response. In line with this hypothesis, we demonstrate that the FDA approved antidepressant, sertraline, previously known to inhibit the formation of the pIRF3-activation complex formation to effectively inhibit infection induced IFN. We found this inhibition manifesting as a growth arrest of Mtb in macrophages. Interestingly, SRT could augment mycobacterial killing in the presence of INH (H) and rifampicin (R), two of the frontline TB drugs in macrophages by effectively lowering the concentration of antibiotics required to achieve clearance. Remarkably, the combination proved effective even against dormant bacilli or antibiotic tolerant Mtb strains. Addition of SRT to TB drugs- HR or HRZE (HR+ pyrazinamide, ethambutol) significantly protected infected mice from TB related pathology both by enhancing bacterial clearance and host survival, implying on the usefulness of this combination therapy in both the intensive (bactericidal) and continuation (sterilizing) phases of anti TB therapy (ATT). Taken together, we report a novel adjunct TB therapy module by repurposing the FDA approved, prescription antidepressant -sertraline.

Results:

Inhibition of type I IFN in macrophages results in bacterial growth arrest.

Macrophages respond to mycobacterial infection by elaborating an array of signaling cascades and effector functions[49-51] . Several groups have identified type I IFN signaling as an active and dominant response of THP1 macrophages to Mtb infection [44,46,52-54]. We questioned the benefit to Mtb in actively inducing this response and hypothesized that suppressing this response in cells would alter macrophage infection dynamics. Our previous work indicates a role for nucleic acid sensing in the Mtb induced Type I IFN response[47]. As an initial step in this direction, we chose

previously identified inhibitors of RNA induced Type I IFN response that were also FDA approved drugs. We used sertraline, a TLR3 signaling antagonist for the ability to modulate Mtb induced type I IFN response in macrophages. We observed a dose dependent reduction in Mtb induced type I IFN response of macrophages on treatment with SRT (Fig.1A). While 1μM was minimally inhibitory, 5μM and 10μM of SRT inhibited the response by 35 and 43% respectively. At a dose of 20μM, SRT significantly reduced the response to 1/5th of the untreated values (Fig. 1A). SRT alone did not alter Mtb growth *in vitro* (Fig.1B). While SRT at 200μM completely inhibited growth, at a ten- fold lower dose of 20μM, a ~50% decrease in growth rates of Mtb was observed by 8 days of *in vitro* culture. Treatment with SRT considerably enabled macrophages to restrict Mtb growth over a 5-day period of infection. While naïve macrophages were permissive for Mtb growth by ~5-10 folds, SRT treated macrophages harbored 2-3 fold lesser bacterial numbers macrophages by the 5th day post infection (Fig.1B).

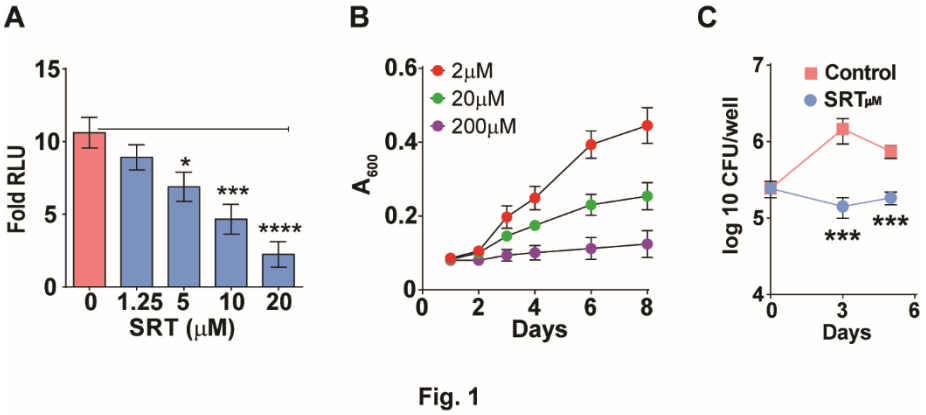

Fig. 1

Fig. 1: Sertraline inhibits Mtb induced Type I IFN response and restricts intra-macrophage Mtb growth A) IRF dependent luciferase activity in THP1 Dual macrophages following infection with Mtb at a MOI of 5. Cells were left untreated or treated with increasing concentrations of SRT for 24h in culture and the luminescence in culture supernatants was measured and is represented as mean ± SEM from 2 independent experiments with triplicate wells each. B) Growth of Mtb *in vitro* in the presence of 3 concentrations of SRT over 8 days of incubation in 7H9 media at 37^{O}C. At the indicated time points, the plates containing the different wells were used for measurement of OD. Values

represented are mean absorbance values ± SE of 2 independent experiments. C) Intracellular bacterial numbers in macrophages infected with Mtb for 6h at MOI of 5 and then left untreated (NT) or treated with 20μM SRT. Intracellular bacterial numbers at the indicated time points are represented as average $\log_{10}$ CFU ± SEM from 2 independent experiments with triplicate wells each. (A and C: unpaired t-test, **p<0.01, ***p<0.001).

SRT potentiates antibiotic mediated killing of Mtb in infected macrophages

The observed bacterial stasis in SRT treated macrophages prompted us to analyze the effect of this treatment in conjunction with frontline antibiotics. To test this, we enumerated Mtb growth in macrophages following treatment with 200ng/ml INH and 1000ng/ml Rifampicin (HR) alone or in combination with 20μM SRT (Fig. 2A). Naïve macrophages supported bacterial growth over 5 days of infection with bacterial numbers increasing 4-fold (Fig. 2A). Addition of HR reduced bacterial numbers by ~62 fold by day 3 and ~92 fold by day 5. Importantly, we observed an additional 3-fold increase in bacterial killing at day 3 by addition of SRT to the antibiotics (Fig. 2A). Given the effective control of bacteria by HR, we reasoned that any additional effect of SRT would be better visible in lower concentrations of antibiotics with reduced efficacy. The effective killing capacity decreased exponentially as the concentrations were lowered by 10 folds, nearly allowing bacterial growth (Fig. 2A). Addition of SRT to HR at these concentrations significantly enhanced the ability of drugs to control Mtb in macrophages. At 0.1X dose of HR, the effective bacterial load decreased by 2 logs attaining similar levels as HR alone at 1X concentration (Fig. 2A). Even at a concentration of 0.04X, where HR did not show bactericidal activities, addition of SRT reduced bacterial numbers by 10-20 folds by day 5 indicating substantial boosting of antibiotic efficacy in this group (Fig. 2B). Even at 0.01x HR, bacteria grew 4- fold by day 5 in macrophages, and the addition of SRT enabled restriction of bacterial growth (data not shown).

To determine if the effect of SRT was preserved in primary human macrophages, we repeated these experiment in monocyte derived macrophages from 7 healthy individuals (Fig. 2C). Although Mtb growth varied in the different individuals, SRT alone was not effective in altering the

133 growth across MDMs from different donors. 0.1X HR exhibited variability in control across
134 individuals. However, SRT resulted in strong synergistic anti-bacterial activity resulting in 10-15
135 folds lower bacterial, numbers arguing for a universal adjunct activity of SRT to frontline TB drugs.

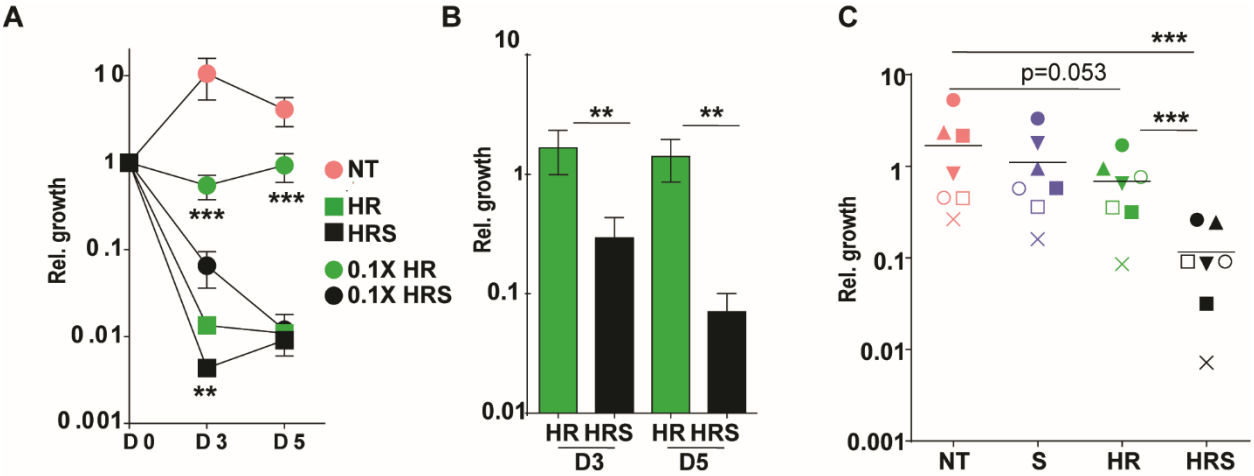

Fig. 2

136

137 **Fig. 2: SRT potentiates antimycobacterial activity of TB drugs in Mtb infected macrophages.**

138 Intracellular bacterial numbers of Mtb in THP1 Dual macrophages following infection with MOI5 for
139 6h and then either left untreated (NT- red) or treated with, (HR -200ng/ml INH (H) and 1000ng/ml
140 Rif(R)- green) or a combination of all three (HRS- black) for 3 and 5 days. Also included in this
141 graph are 0.1X of both H and R with and without 20μM SRT. B) Intracellular bacterial survival with
142 0.04X HR+20μM SRT, represented as growth relative to 0.04X HR. Values are mean± SEM of two
143 independent experiments with triplicate well each. C) Intracellular bacterial growth/survival in
144 primary human M1- differentiated MDMs from PBMC of seven individuals after 6h infection with
145 Mtb at MOI-5 and 3 days of treatment with either 20μM SRT2 (S- blue), 0.1X HR, or a combination
146 of both, represented as CFU counts relative to initial CFU in untreated samples. Each symbol
147 represents one individual, colors depict the treatment groups as before. Values are mean± SEM of
148 two independent experiments with triplicate well each. (A and B: unpaired t-test, C: paired t-test
149 comparing ratios **p<0.01, ***p<0.001).

150

151 **Inclusion of SRT with isoniazid and Rifampicin enhances antibacterial activity even in**
152 **conditions that promote tolerance.**

The inability of ATT to eradicate bacilli from tissues is well recognized; importantly, the ability of Mtb to enter into a non-replicating dormant state associated with tolerance to frontline drugs is an important limitation of the current treatment regimen. The ability of SRT to augment Mtb killing warranted a testing of its efficacy in conditions of antibiotic failure. The recent model of vitamin C induced dormancy in Mtb replicates this scenario with a complete loss of isoniazid and rifampicin efficacy in bacterial control[55]. To test, the effectiveness of the adjunct therapy in this model, we incubated THP1 macrophages with VitC prior to treatment to induce dormancy in bacteria [55]. SRT was able to induce bacterial stasis in this model, while HR was ineffective at controlling bacterial growth. However, HR along with SRT led to 10-12 fold reduction in bacterial numbers within 4 days of treatment indicating its activity against dormant Mtb (Fig. 3A).

More recently, the inability of drugs to distribute equally amongst the spectrum of granulomatous lesions was recognized as an important factor in promoting bacterial tolerance and resistance with the lipid loaded necrotic lesions forming a formidable barrier for entry of frontline TB drugs *in vivo* [56,57]. *In vitro* studies with THP1 macrophages to increase intracellular lipid content (treatment with oleic acid) mimic conditions of foamy macrophages in advanced necrotic granuloma[58,59]. As an indirect correlate of efficacy of the combination in these conditions, we tested antibiotic killing in THP1 cells pre- treated with oleic acid. Again, HR was minimally antibacterial in macrophages treated with oleic acid (Fig. 3B). In contrast, the combination led to more than 10 fold reduction in bacterial loads as compared to untreated and HR treated macrophages suggesting the potential utility of SRT as an adjunct TB regimen under conditions of decreased antibiotic efficacy.

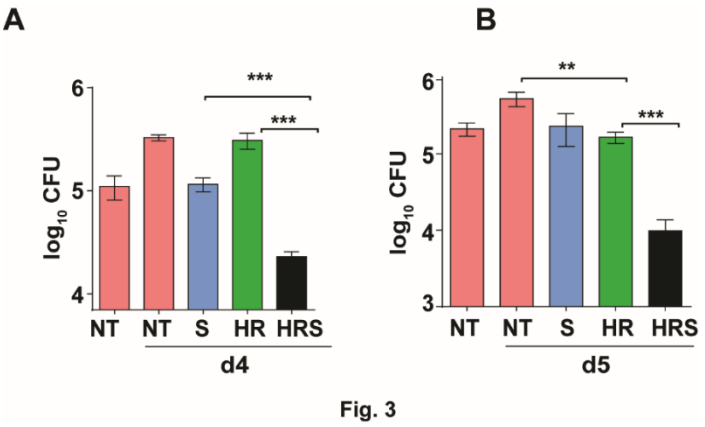

Fig. 3

Fig. 3: SRT potentiates antimycobacterial activity in conditions favoring bacterial tolerance.

A) Intracellular bacterial growth in THP1 macrophages infected with Mtb at MOI of 5 for 6h and then treated with 2mM VitC for another 24h. Following this, cells were left untreated (NT) or treated with SRT, HR or HRS for 3 days. Bacterial numbers at day 4 of infection was enumerated and is represented as average log_{10} CFU ± SEM from two independent experiments with triplicate wells each. B) Bacterial growth in lipid rich conditions: THP1 macrophages were treated with 200μM Oleic acid for 48h prior to infection with Mtb at a MOI of 5 for 6h and followed with HR, S or HRS treatment for 5 days. Bacterial numbers at day 5 of infection was enumerated and is represented as average log_{10} CFU ± SEM from two independent experiments with triplicate wells each. (A and B: unpaired t-test, **p<0.01, ***p<0.001).

SRT effectively reduces macrophage type I IFN and affords better control of mycobacterial growth.

We found that SRT at concentrations as low as 1 μM in potentiating antibiotic mediated growth restriction in macrophages (Fig. 4A). In order to better understand the mechanism of SRT mediated control of bacterial growth, we first tested if SRT was also potent in supplementing the activity of HR in *in vitro* culture. As observed in Fig. 4B, i*n vitro* SRT even at a 6- fold higher concentration (6μM) failed to impact Mtb growth either alone or in combination with 3 concentrations of HR. These results hinted at an indirect effect of SRT in enhancing the ability of macrophages to control bacteria rather than a direct benefit to the microbicidal properties of the

antibiotics. To test if SRT induced mycobacterial stasis in macrophages was due to inhibition of type I IFN expression, we analyzed growth of Mtb in RAW264.7 macrophages deficient in this signaling component. In contrast to the growth in WT macrophages, Mtb was significantly restricted in STING$^{-/-}$ and TRIF$^{-/-}$ macrophages to the initial input levels even after 3 days of culture (Fig. 4C). Again, the persistent levels of bacterial CFUs without a significant decrease by 3 days mirrored the situation of bacterial stasis observed in SRT treated macrophages. Evidence for interferon signaling interference by SRT was also apparent by the 4-fold reduction of IP10 expression levels in Mtb infected macrophages treated with HRS as compared to HR alone after 18h of treatment (Fig. 4D). Further confirmation was achieved by treating macrophages with a more potent inhibitor of type I IFN signaling- BX795, that completely abrogated Mtb induced type I IFN at a very low concentrations of 1.2 μM (Fig. 4E). Again, while BX795 alone was only capable of bacteriostasis in macrophages even at 10μM concentrations (Fig. 4F), its addition to ineffective concentrations of HR enhanced antibiotic mediated bacterial killing by 4-5 folds by 3 days of infection (Fig. 4G). A recent study by Teles et.al, 2013 suggested the active suppression of IFNγ mediated activation of macrophage mediated killing of mycobacteria as one of the potent pro-pathogenic effects of type I IFN in mycobacterial infections[60]. We hypothesized that if SRT was efficient in controlling Mtb induced macrophage type I IFN induction the effects of IFNγ mediated activation would be more efficient in controlling infection. As has been reported earlier, IFNγ pretreatment was effective in reducing bacterial numbers ~10 fold in comparison to naïve macrophages by day 3 (Fig. 4H). This killing efficiency was further enhanced 2-fold in macrophages treated with SRT lending support to the idea of type I IFN repressing macrophage priming by IFNγ and its reversal by addition of SRT. To further probe into the molecular mechanism of SRT mediated IFN suppression, we analyzed the pro-inflammatory response of Mtb infected macrophages treated with SRT (Fig 4H-K). Gene expression profiles at 18h of treatment showed reduction in the expression of TNF and IL1β with SRT treatment. In comparison to HR treated cells, TNF expression was reduced only slightly in the HRS treated macrophages

(Fig. 4I). However, the levels of secreted TNF was significantly altered by SRT treatment; in SRT alone or HRS treated macrophages, negligible levels of TNF was observed in the cell supernatants, contrasting with the high levels of TNF (between 500pg- 1ng/ml) in the case of Mtb infected cells with or without treatment with HR (Fig. 4J).

Surprisingly, while expression levels of IL1β were 2-3 folds lower in HRS treated macrophages by 18h (Fig. 4K), the amount of secreted cytokine was significantly elevated (> 2-3 folds) in macrophages treated with SRT/ HRS from 18h until 66h strongly indicative of an important role for the host cell inflammasome activation by SRT (Fig. 4L). In fact, with the studies supporting the antagonism between type I IFN and inflammasome activation, we tested the efficacy of SRT to potentiate antibiotic mediated killing in the presence of inflammasome inhibitors. Again, while SRT enhanced the ability of HR to control Mtb in macrophages, pretreatment of cells with the inflammasome inhibitor, isoliquiritigenin (I), completely nullified the boosting effect of SRT on antibiotic efficacy (Fig. 4M) suggesting that the type I IFN modulating effect of SRT was mediated in part by inflammasome activation.

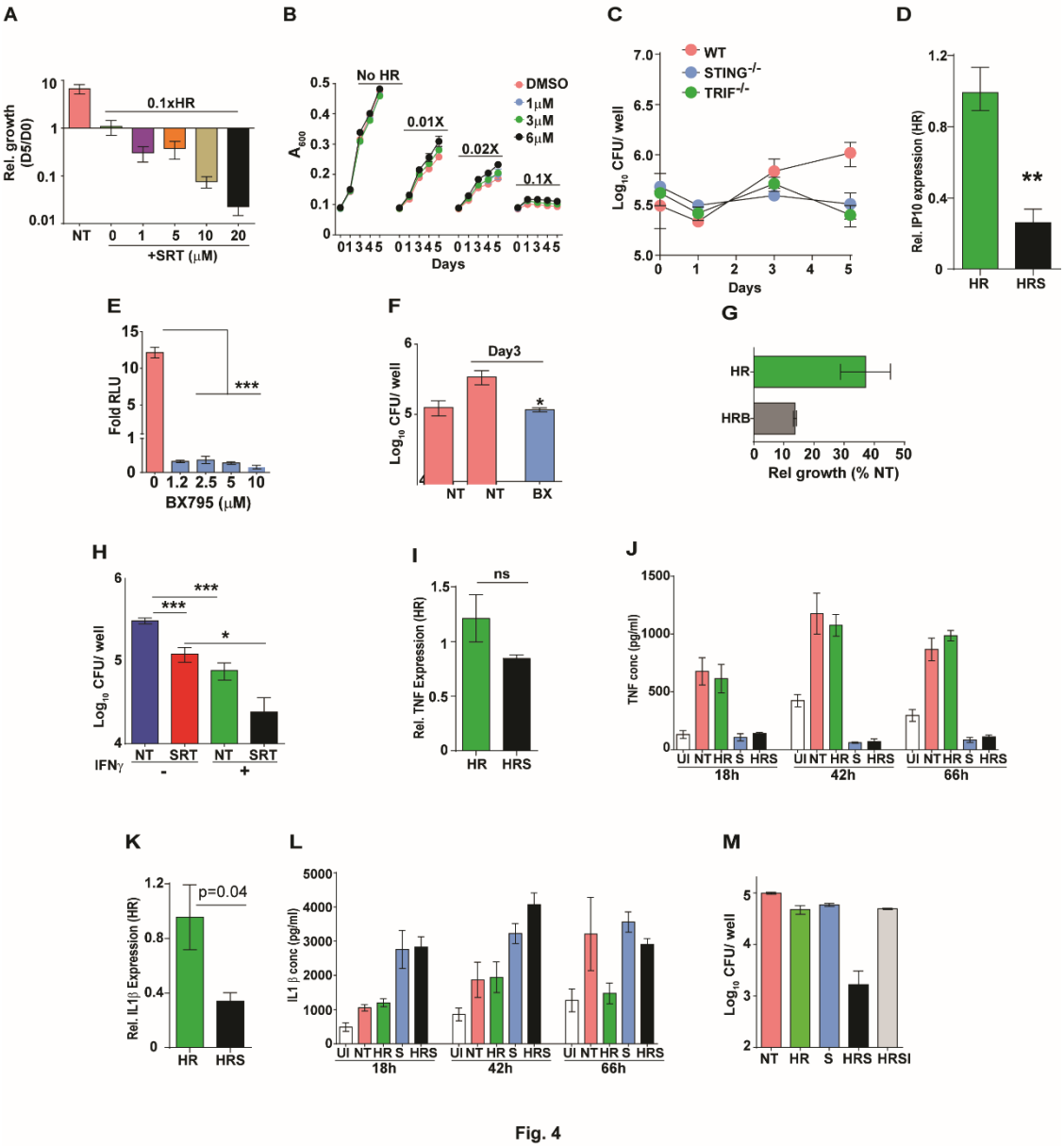

Fig. 4

Fig. 4: Augmentation property of SRT is due to its ability to inhibit IFN signaling.

A) Growth of Mtb in THP1 macrophages following infection at MOI5 for 6h and left untreated (NT) or treated with HR at 0.01X MIC with and without different SRT concentrations. The relative bacterial numbers at day 5 w.r.t the initial load is represented as mean + SEM from triplicate wells of two independent experiments. B) *In vitro* growth of Mtb in 7H9 media containing different concentrations of SRT. Mtb cultures were either left untreated or in the presence of different SRT and HR concentrations for 5 days at 37^{O}C. The growth was regularly monitored by measuring the optical density of culture and is represented as mean

± SD of triplicate assay wells of a representative experiment. C) Intracellular bacterial growth in WT, STING$^{-/-}$ or TRIF$^{-/-}$ deficient RAW264.7 macrophages infected with Mtb at MOI of 5 for 6h and then washed to remove extracellular bacteria. Intracellular bacterial growth was monitored for 3 days and is represented as mean ± SD log_{10} CFU/ well of triplicate wells of a representative experiment of two. D) Expression of IP10 in macrophages infected with Mtb for 6h and then treated with HR alone or in combination with SRT for 18h. The values represented are relative fold expression levels in HRS w.r.t macrophages treated with HR alone ± SEM from two independent experiment with duplicate wells each. E) IRF dependent luciferase activity in THP1 dual macrophages 24h after treatment with varying doses of BX795 along with infection with Mtb at MOI of 5. F) Growth of Mtb in THP1 macrophages following treatment with 10μm BX795. Bacterial numbers were enumerated and log_{10} CFU are represented as mean ± SE from two independent experiments with triplicate wells each. G) Relative growth of Mtb in macrophages treated with HR and HR+Bx795 (HRB) for 3 days. The percentage relative growth of intracellular bacterial numbers in HR or HRB groups with respect to untreated samples is represented as mean ± SE from two independent experiments with triplicate wells each. H) Growth of Mtb in macrophages pre-treated with IFNγ for 16h, infected with Mtb for 6h and then left untreated or treated with SRT for 3 days. Data are CFU represented as mean ± SEM from two independent experiments with triplicate wells each. I-L) Relative transcript abundance and secreted TNF (I and J respectively) and IL1β (K and L respectively) in macrophages infected with Mtb at MOI 5 for 6h and then treated with HR alone or in combination with SRT. Data in I and K depict fold expression relative to HR alone as mean ± SEM from two independent experiments with duplicate wells each at 18h post treatment. Data in J and L depict secreted cytokines at indicated time points post treatment as mean ± SD for n=3. M) Growth of Mtb in macrophages infected with Mtb for 6h and then left untreated or treated with HR, SRT, HRS, HRS+ isoliquiritigenin (I) for 5 days. Values represent average CFU ± SD per well of n=3 (unpaired t-test, ns=not significant, **p<0.01, ***p<0.001).

SRT significantly enhances host survival in an acute infection model of murine TB infection.

With potent efficacy boosting properties of SRT in macrophages, we tested the *in vivo* efficacy of the combination regimen in an extremely susceptible model of TB infection. We reasoned that the survival of TB infected C3HeB/FeJ mice would provide an optimal platform for a fast readout of

comparative drug efficacies of standard TB drugs and the adjunct regimen with SRT. Given the excellent ability of infection control by INH and Rifampicin in this model, it was necessary to test the adjunct effect of SRT in conditions of minimal/ no advantage imparted by the frontline drug combination alone *i.e.* lower doses -C2 (0.1X) and C3 (0.01X) in addition to the standard dose C1 of HR *ad libitum* in drinking water (Fig. 5A). Aerosol delivery of Mtb at a high dose of ~500 cfu/animal, resulted in precipitous disease with rapid killing of untreated (NT) animals by day 31 of infection (Fig. 5B). To facilitate disease progression prior to treatment initiation, animals infected with Mtb were left untreated for 2 weeks. SRT alone was effective in delaying the disease progression in animals as the mean survival time increased from 31 of untreated animals to 38. HR at the lowest concentration (C3), similar to SRT delayed animal mortality with a mean survival time of 41 days. The combination of HRC3 and SRT, nearly doubled the MST of mice to 78 days with significant improvement in the gross lung pathology. This benefit of SRT was similar to that observed for a 10-fold higher concentration of HR alone (MST of 85 for HRC2). This enhanced protection was also observed in male mice that received the lowest concentration of drugs wherein MST of HR+SRT was 100 days in contrast to the significantly shorter MST of 45 days in the HR alone group (Fig. 5C).

The lower efficacy of antibiotics alone was also reflected in the gross pathology of lungs by 30 days; infected animals showed extensive progressive granulomas despite treatment with antibiotics while a significant amelioration of pathology was observed for the group of animals on combination regimen (Fig. 5D).

All the animals treated with HRC1, HRC1S and HRC2S survived more than 100 days of infection. We wanted to explore temporal benefits of the combination regimen after withdrawal of a limited term treatment. A 7-week *ad libitum* treatment with the 10-fold higher dose of HR (HRC2) (Fig. 5E), significantly decreased the MST of animals to 85 days with 100% mortality by 16 weeks. In contrast, 40% of HRC2S treated animals, survived with a MST of 112 days for the group (Fig. 5F, G). All animals with the highest dose of HR (HRC1) either alone or with SRT survived the

infection. Despite, the significant heterogeneity of treatment response in male and female mice, with a relatively lower response as evidenced by the greater number of lesions in lungs of male animals, a co-operative effect of SRT inclusion was evident as a significant improvement in TB associated lung pathology (Fig. 5H). Small macroscopic lesions were observed in lungs of 60% of the female mice treated with HRC1 that showed as multiple, well defined granuloma in the H&E stained sections by the 16th week post infection. In contrast, mice treated with SRT and HRC1 showed negligible involvement of the lung tissue in granulomatous cellular accumulation. Even in tissues of male mice, animals receiving the adjunct therapy showed fewer macroscopic and significantly lower numbers of microscopic granuloma in lung sections in comparison to animals treated with the antibiotics alone.

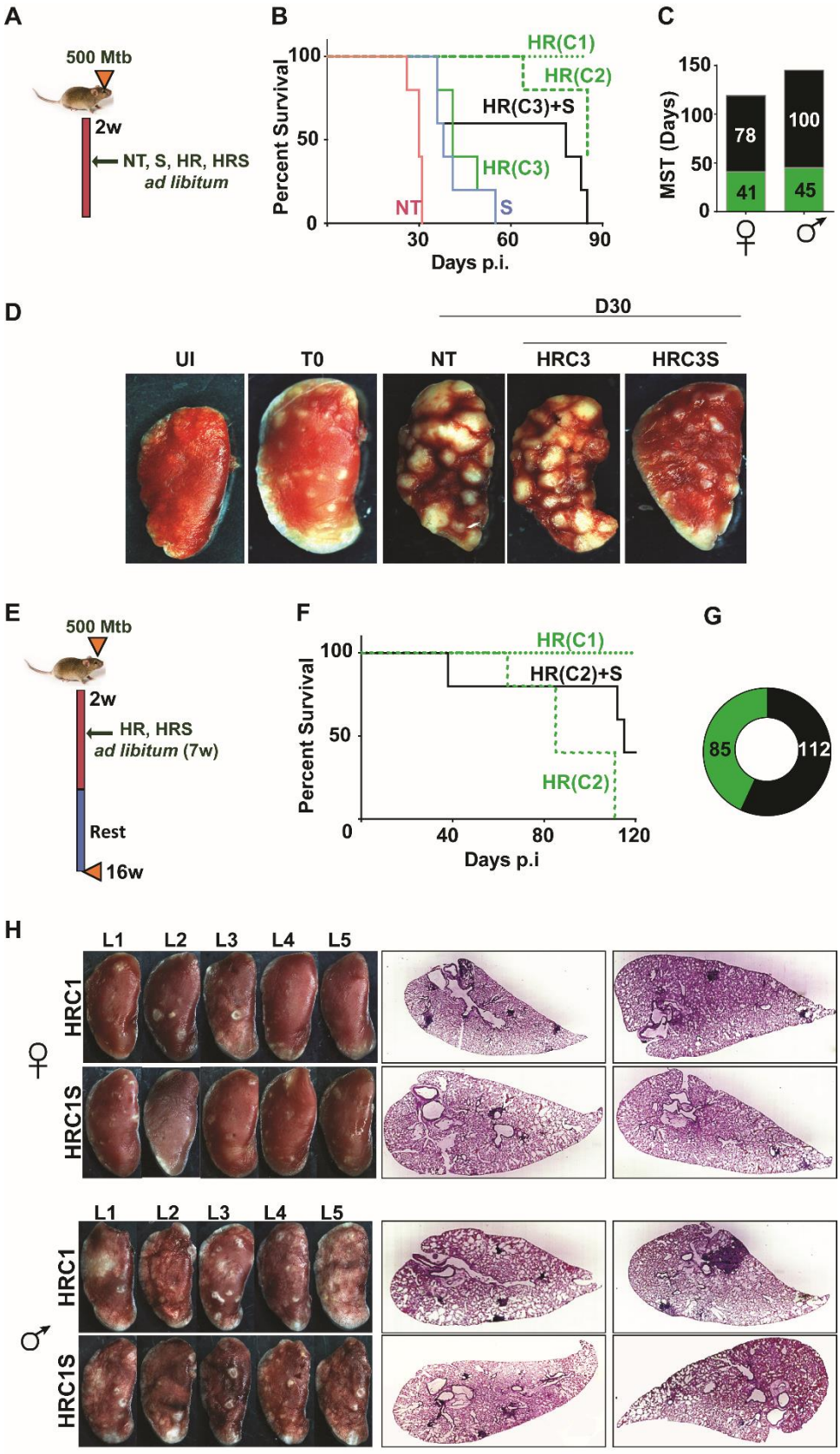

Fig. 5

Fig. 5: Adjunct SRT improves host survival in a susceptible mouse model of infection.

A) Schematic of Mtb infection and drug treatment in C3HeB/FeJ female mice. B) Survival curve of Mtb infected female C3HeB/FeJ mice treated with different concentrations of H and R (HRC1- 1X: H-100μg/ml, R-40μg/ml, HRC2-0.1X, HRC3-0.01X) alone or along with SRT (10μg/ml). C) Median survival time of different treatment groups of mice. D) Gross tissue morphology of lungs of uninfected animals (UI) and indicated groups at 30 days post infection with Mtb. E) Schematic of infection and antibiotic treatment in C3Heb/FeJ with HRC2 and HRC1. F-G) Survival (F) and MST (G) of C3Heb/FeJ mice treated with HRC2 or HRC2S. H) Gross lung morphology at the end of 16 weeks and histochemical sections of lungs with H&E staining of C3HeB/FeJ mice either treated with HRC1 or in combination with SRT.

SRT potentiates bacterial control by anti-tubercular drugs in preclinical models of infection

Acute disease prevention: TB treatment in the intensive phase involves the use of 4 frontline TB drugs- HRZE for a period of two months and HR for an additional 4 months. Further to test the efficacy of SRT in combination with HRZE in an acute model of disease, Mtb infected C3HeB/FeJ mice were treated either with the established dose of HRZE or in combination with SRT (Fig. 6A) and evaluated TB associated pathology of the lungs at 16 weeks of infection. Male (6B) and female (6C) animals treated with the 4 drugs for 7 weeks harbored 10^5-10^6 bacteria in their lungs, respectively. Addition of SRT appreciably improved drug efficacy, reducing the lung bacterial loads by a further 5-7 folds (Fig. 6B and C).

The drugs efficiently lowered tissue pathology as evidenced by the macroscopic lesions seen in the lungs of infected mice (Fig. 6D). While both female and male mice showed small macroscopic lesions in lungs on treatment with HRZE, despite the heterogeneity between the genders, the combination of SRT and HRZE sufficiently decreased the extent of tissue involvement in TB associated pathology. This difference was more evident in tissue sections, wherein animals treated with HRZES were devoid of granulomatous infiltrates in contrast to the HRZE treated animals which had significantly higher numbers of granulomas in the lungs (Fig. 6D).

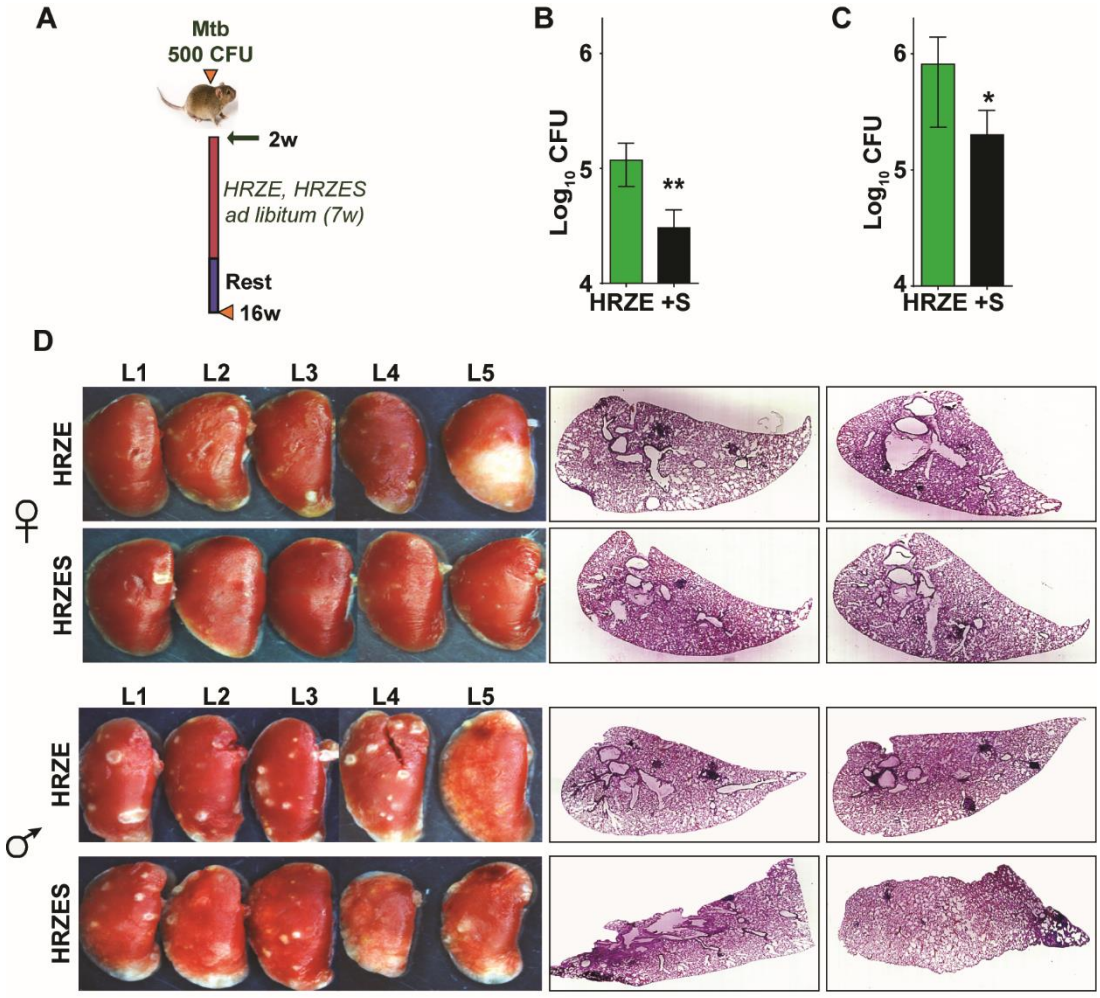

Fig. 6

Fig. 6: *In vivo* potentiation of SRT mediated antimycobacterial activity

(A) Schematic of infected C3Heb/FeJ mice and treated with HRZE (HRC1- 1x: H-100μg/ml, R-40μg/ml, Z-150μg/ml, E-100μg/ml or in combination with SRT (10μg/ml). Animals were euthanized at the end of 16 weeks and extent of infection was determined by estimating bacterial numbers (CFU) in lungs of female (B) and male (C) mice. D) Gross lung morphology and histochemical sections of lungs with H&E staining of C3HeB/FeJ mice infected with Mtb and treated either with HRZE or in combination with SRT. (unpaired t-test,*p<0.05, **p<0.01).

Short term bacterial clearance: To evaluate the adjunct regimen for early bacterial clearance rates, we infected C57BL/6 mice with 500 CFU of Mtb and enumerated bacterial burdens at 1- and 3-weeks post treatment according to the schedule shown in Fig. 7A. Bacterial numbers in the lungs reached ~10^7 CFU by 4 weeks of infection (day 0 of treatment) and remained steady over the 6-week period in untreated animals (Fig. 7B). While treatment with HRZE was efficient in steadily reducing these numbers by ~100 folds, addition of SRT to the regimen significantly enhanced control by a further 2-3 folds. Moreover, the adjunct regimen was efficient in controlling dissemination of infection into spleens of infected mice (Fig. 7C). Although HRZE reduced splenic bacterial numbers significantly by 6-7 folds, HRZES was more potent reducing bacterial numbers further by ~20 folds (~150x decrease in comparison to untreated) by 21 days of treatment. A similar degree of enhanced bacterial control (4-6 folds lesser bacteria) was observed in lungs and spleens of Balb/c mice treated with SRT as an adjunct to conventional 4 drug-therapy (Fig. 7D). The decrease in infection reflected in an overall reduction in the involvement of BL6 lung tissue in granulomatous lesions (Fig. 7E). Untreated animal lungs showed a gradual consolidation of the tissue with increasing amounts of granulomatous cellular infiltration by 6 weeks of infection. Treatment with HRZE was efficient in reducing this infiltration significantly by the 6^{th} week of infection with nearly $1/6^{th}$ of the tissue showing signs of cellular infiltration. Lungs of mice receiving the combination showed better resolution of granulomas, here the lungs showed significantly smaller regions of cellular collection by 3 weeks dispersed across the tissue that was more or less absent from the tissues of mice by the 6^{th} week of treatment. This betterment of the granulomatous lesion was also visible as smaller foci of cellular infiltration in the HRZES treated animals as compared to the more numbers of macrophages in the NT and HRZE groups.

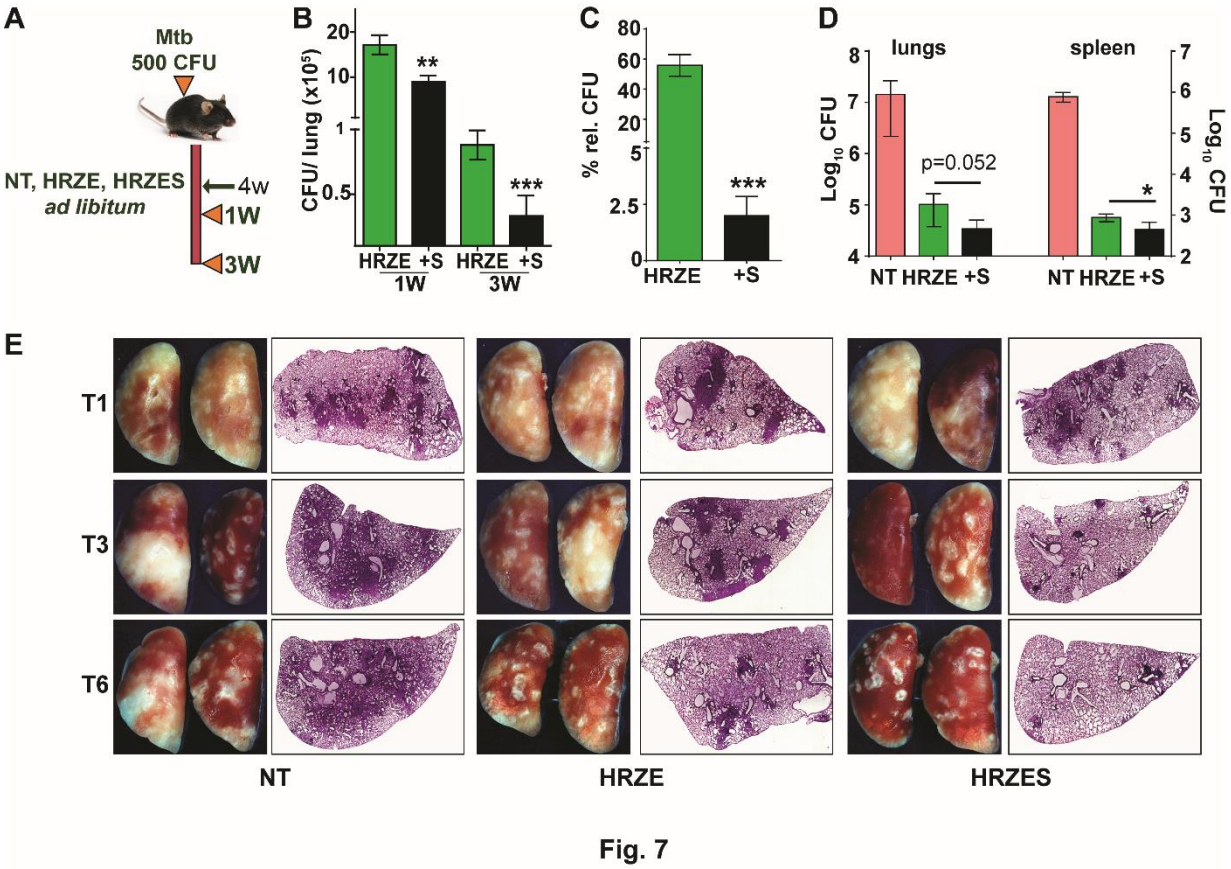

Fig. 7

Fig. 7: SRT initiates early bacterial clearance and dissemination in mice.

A) Schematic of Mtb infection and drug treatment in C57BL6/ BalbC mice infected with Mtb and the treated with HRZE or HRZES (HRC1- 1X: H-100μg/ml, R-40μg/ml, Z-150μg/ml, E-100μg/ml, SRT 10μg/ml) treatment. Lung CFU post 1 and 3 weeks (B) and spleen CFU 3 week after (C- relative to untreated animals) in Mtb infected C57BL6 mice of antibiotic and SRT administration. D) Lung and spleen CFUs of Balb/c mice after 3 weeks of treatment, E) Gross lung morphology and H&E staining after treatment for the indicated number of weeks. (unpaired t-test,*p<0.05, ***p<0.001).

Efficacy against tolerant Mtb strains:

We then tested the efficacy of the adjunct therapy against a drug tolerant Mtb strain in cellular and murine models of infection. Previously, we had demonstrated that N73- a clinical Mtb strain belonging to the L1 ancient lineage showed increasing tolerance to INH and Rifampicin as opposed to the modern L3 and L4 lineage strains by virtue of expressing the complete MmpL6

operon [61]. In THP1 cells infected with Mtb, HR at a concentration of 0.1x supported stasis and failed to decrease of bacterial numbers (Fig. 8A). The combination of HR with SRT, significantly controlled infection and reduced bacterial numbers by ~10-15 folds by 3 days and ~100 folds by day5. The pattern of significantly greater bacterial control was also observed in primary human macrophages; again, a combination of HR and SRT reduced intracellular bacterial numbers by 5-50 folds in comparison to the drugs alone (Fig. 8B).

With a strong indication of SRT's ability to boost the efficacy of frontline TB drugs against tolerant Mtb, we tested its *in vivo* activity in the acute model of C3HeB/ FeJ mouse infection in combination with 0.1x HR (Fig. 8C). As expected, the drugs were not efficient in controlling infection induced lesions in the lungs reducing bacterial numbers by 5 folds (Fig. 8D). However, HRS treated animals harbored fewer bacteria in the lungs (~5-8 folds than HR). The effect of the combination was again better in controlling bacterial numbers in spleens of treated mice wherein HR did not reduce CFU in contrast to the 6-8 fold lower bacterial numbers with the combination.

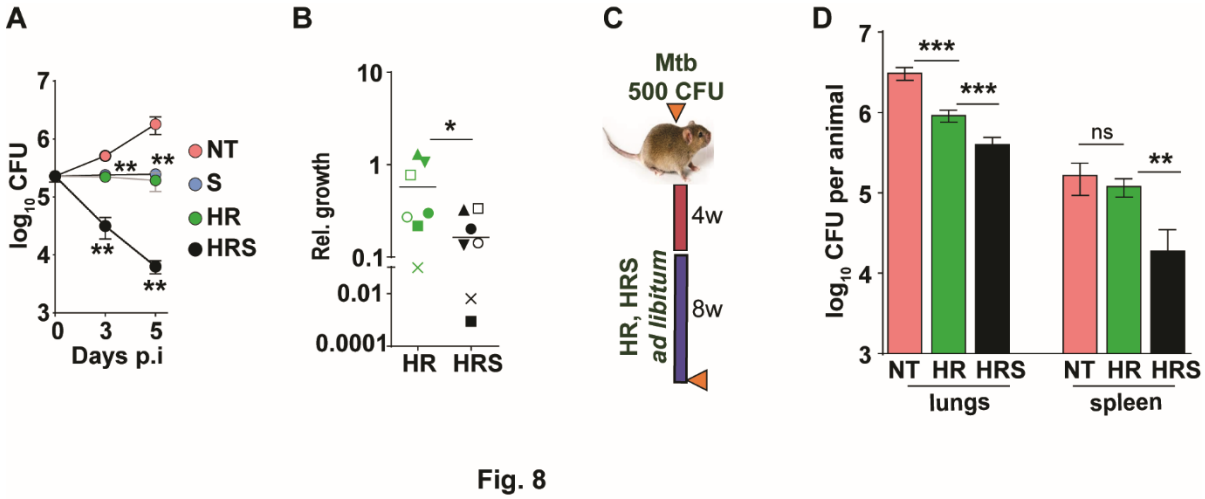

Fig. 8

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Fig. 8: Addition of SRT helps better control of drug tolerant Mtb in vivo.

A) Intracellular bacterial growth in THP1 macrophages infected with HR tolerant Mtb strain at a MOI of 5 for 6h. Following this, cells were left untreated (NT) or treated with SRT, HR or HRS for 3 or 5 days. Bacterial numbers were enumerated and is represented as average $\log_{10}$ CFU ± SEM from two independent experiments with triplicate wells each. B) Bacterial numbers at day 3 of primary monocyte derived

macrophages (M1) from 7 independent donors. After 6h of infection, the macrophages were treated with HR and HRS for 5 days. The ratio of intracellular bacterial numbers in HR or HRS groups with respect to untreated samples is represented as relative growth with median values indicated by the horizontal line. C) Schematic of Mtb infection and drug treatment in C3HeB/FeJ mice infected with Mtb for 4 weeks followed by treatment with 0.1X HR alone or with SRT (HRS) or 8 weeks. Bacterial numbers (CFU) in lungs and spleen (D) at the end of the experiment are shown as mean ± sd (unpaired t-test, ns=not significant, **p<0.01, ***p<0.001).

DISCUSSION

Despite consistent efforts in identifying novel pathogen targeted interventions and streamlined pharmaceutical drug development control processes, fewer drugs have been accepted for clinical use in TB over the last 40 years[62]. Repositioning existing drugs with established safety in humans is one of the quickest modes of developing effective control of infections that reduce the timeframe of regimen development. The need for an effective, short and pathogen-sterilizing regimen to tackle the growing problem of Mtb drug resistance and dormant bacterial populations has intensified efforts towards the development of host targeted therapies [63-66].

We and several other groups have identified type I IFN as an early response of host macrophages to infection with Mtb strains [46,47,53,67]. With recent evidences indicating this response as a pathogen beneficial response, we hypothesized that attenuating this axis would prove beneficial in controlling bacteria in macrophages. In line with this idea, we observed that the previously reported TLR3 antagonist – sertraline (SRT) could effectively stunt Mtb induced type I IFN response in macrophages and also inhibit bacterial growth in macrophages over 5 days of infection. We provide direct and indirect evidence for the ability of SRT to restrict bacterial growth by attenuating host cell IFN1 signaling. A similar bacterial growth profile in macrophages 1) deficient in IFN1 signaling and 2) in response to another IFN1 signaling inhibitor- BX795, that reverses TBK1 phosphorylation/ activation, hinted at type I IFN inhibition as a basal mechanism of SRT mediated growth restriction. Consistent with the previous reports of type I IFN mediated

repression of pathogen control mechanisms, we observed that inhibition of this response by SRT enhanced the ability of IFNγ primed macrophages to kill Mtb.

To test if targeting type I IFN could be developed into an adjunct HDT along with the current therapy regimen, we compared the ability of frontline TB drugs like H and R and their combination with SRT in a macrophage infection model. SRT addition was capable of enhancing the killing properties of HR significantly even at drug effective concentrations while at lower concentrations with lesser efficacy, addition of SRT could enhance bacterial clearance 30-50 times nearly at levels similar to the effective dose of HR alone. More importantly, cells treated with SRT did not show any evidence of cell lysis/ death. Surprisingly, SRT alone was not effective in bacterial growth control *in vitro*. We observed that SRT treatment of Mtb infected macrophages reduced type I IFN signaling, expression of TNF and IL1β in macrophages, despite augmenting the levels of secreted IL1β in culture supernatants pointing towards the critical role of SRT in inflammasome activation. While several studies have identified an important role for the host cell inflammasome in controlling bacterial infections, recent evidence supports an antagonistic role for inflammasome activation on the type I IFN response of cells [68,69]. A recent study has effectively demonstrated the importance of host inflammasome in activating eicosanoids that control type I IFN thereby promoting bacterial clearance[70] [71]. Consistent with this observation, targeted therapy towards elevating PGE2 activity protected mice from acute infection induced fatality [71]. Our results also highlight this cross- regulation between two important innate response pathways with SRT boosting the macrophage pathogen control program by repressing a pro- pathogenic and activating the host beneficial response. Importantly, we observed that addition of previously identified inflammasome inhibitor –Isoliquiritigenin, nullified the ability of macrophages to respond to INH and rifampicin (data not shown) hinting at the importance of this critical response in antimycobacterial activity of these antibiotics within macrophages. In line with this observation, treatment with these inhibitors also reversed the ability of SRT to enhance the effects of HR in macrophages. While, we are trying to decipher if SRT directly inhibits type I IFN or via

inflammasome activation, our results substantiate the inverse relationship between these two signaling cascades.

However, SRT provides additional benefits as an adjunct modality. The pharmacological properties of SRT has been well established with excellent PK PD, safety and tolerance for long term usage in the human population[72-74]. Interestingly, two patients undergoing TB therapy with INH given SRT as an anti- depressant, did not show any deleterious effects on long term use of the combination, auguring well for safety in the human population[75,76]. In addition, these studies combined with the enhanced protective capabilities of the combination therapy in pre-clinical animal models (our data), rule out any possibility of negative drug-drug interactions between SRT and ATT on prolonged usage. This long-term standard TB treatment is associated with severe drug induced depression in patients. Recently depression was identified as an invisible co-morbidity with TB with extensive synergistic action on the patient[77,78]. It is logical to expect that SRT with its wide use as an anti- depressant in adults and children may be beneficial in tackling this dual problem efficiently with a combination regimen of frontline TB drugs and SRT.

SRT addition along with standard TB therapy was potent in reducing bacterial numbers in lungs and spleens of infected mice as early as 3 weeks of treatment. While the lung burden was ~3 folds lower with the combination than with drugs alone, significant reversal of the lung pathology was suggestive of SRT mediated amplification of drug efficacy. Moreover, spleen of these mice had significantly lesser bacterial numbers that reflected on the ability of control accessory infected sites of the host.

Additionally, SRT combination therapy with HR, frontline TB drugs used in extended therapy, also fostered greater clearance of TB associated lung pathology and hence host survival, supporting the idea that the combination would be effective in both the intensive phase of therapy in reducing bacterial numbers faster and in the extended phase of TB therapy, overall enhancing host survival.

Considering the ability of SRT to lower type I IFN response in the host, it is plausible to assume higher susceptibility to viral infections. However, there is no epidemiological data indicating higher viral infections in patients with long term SRT use for depression. On the contrary, SRT has been shown to have anti- viral activity[79-81]. SRT has been shown to augment Influenza associated lung inflammation in conjunction with PDE4 inhibitors in the murine model of infection [82]. In fact, WHO has identified SRT as one of the anti- Ebola molecules by virtue of its efficacy in *in vitro* model of infection [83]. However, while SRT as a high dose monotherapy regimen failed to impart protection to rhesus macaques against Ebola challenge, the animals did not show any adverse effects of SRT administration further challenging the possibility of an adverse effect of SRT on host anti-viral responses[84].

While we have supportive evidence for SRT's antibiotic aiding property to be due to its ability to reduce Mtb induced IFN response, it is logical to assume pleiotropic effects of SRT addition to the TB drugs resulting in decreased bacterial burdens. Interestingly, previous evidences have suggested a multi- faceted immune modulating property of SRT and other selective serotonin reuptake inhibitors from enhancing the anti- inflammatory response[85] to enhancing NK and CD8 cell response[81] to inhibition of acid-sphingomyelinase [86], an essential component of the viral trafficking into NPC1+ endosomes in cells. While our work points towards the type I IFN antagonism mediated inflammasome activation as a key mechanism in providing synergy to anti TB therapy, the exact molecular target of SRT in this process is an important future challenge.

The combined properties of a SRT combination therapy of faster bacterial control, promoting host survival and ability to target drug tolerant as well as dormant bacterial populations augurs well for the highly constrained national/ global economy combating the TB pandemic.

Material and Methods

Bacterial Strains and Growth Conditions—Mtb strains were cultured in Middlebrook 7H9 broth with 4% ADS or in 7H10/ 7H11 agar (BD Biosciences, USA) with 10% OADC (Himedia laboratories, India).

Reagents: THP1 Dual Monocytes was obtained from InvivoGen (Toulouse, France). HiglutaXL RPMI-1640 and 10% Fetal Bovine Serum (HIMEDIA laboratories, Mumbai, India), PMA (Phorbol 12-Mysristate 13-acetate- P8139, Sigma Aldrich, USA), BX795 (tlrl-bx7, Invivogen) were used for culture of cells. The following reagents were procured from Sigma Aldrich, USA: Vit C (L- ascorbic acid, A5960), oleic acid albumin (O3008), Isoniazid (I3377), Pyrazinamide carboxamide (P7136), Ethambutol dihydrochloride (E4630) and Sertraline hydrochloride (S6319). Rifampicin (CMS1889, HIMEDIA laboratories, Mumbai, India) and commercially available SRT (Daxid, Pfizer Ltd, India) was used for mouse studies.

Macrophage infection: THP1 Dual reporter monocytes were grown in HiglutaXL RPMI-1640 containing 10% FBS and differentiated to macrophages with 100nM PMA for 24h. Following a period of rest for 48h, cells were infected with single cells suspensions (SCS) of Mtb at a MOI of 5 for 6h. For analyzing the Interferon (IRF pathway) activation levels, supernatants from Mtb infected THP1 Dual macrophages were assayed for stimulation by measuring luminescence as per manufacturer's recommendations.

Monocyte derived macrophage culture: PBMCs were isolated from fresh blood obtained from healthy donors in accordance with Institutional human ethics committee approval (Ref no: CSIR-IGIB/IHEC/2017-18 Dt. 08.02.2018). Briefly, 15-20 ml blood was collected in EDTA containing tubes and layered onto HiSep (HIMEDIA laboratories, Mumbai, India) and used for isolation of PBMCs according to the recommended protocols. Post RBC lysis, cells were seeded at a density of $3x10^5$cells/ well and differentiated into monocyte derived macrophages with 50ng/ml GMCSF for 7 days and then used for infection with Mtb.

Analysis of response parameters: For analysis of different parameters of cellular response to infection, qRTPCR based gene expression analysis and cytokine ELISA in culture supernatants were performed according to manufacturer's recommendations.

Analysis of gene expression by qRTPCR

Total RNA was isolated from macrophages suspended in Trizol by using the recommended protocol. cDNA was prepared from 1 µg of RNA by using the Verso cDNA synthesis kit and was used at a concentration of 10ng for expression analysis by using the DyNAmo Flash SYBR Green qPCR Kit (Thermo Fisher Scientific Inc., USA).

Analysis of cytokine secretion by ELISA:

Culture supernatants at different time intervals post infection/ treatment were filtered through a 0.2µ filter and subjected to ELISA by using the eBioscience (Thermo Fisher Scientific Inc. USA) ELISA kit as per recommended protocols.

Bacterial survival in macrophages: For determining intra cellular survival of Mtb strains macrophages were seeded in 48well plates and infected with Mtb at MOI 5 for 6 hours. SRT and lyophilised BX795 were resuspended in DMSO and used at final concentration of 20 and 10µM, respectively. Frontline anti mycobacterial drugs were resuspended in DMSO as well and used at different concentrations – C1, C2, C3 : (C1- INH-200ng/ml, Rifampicin-1000ng/ml, -C2 and C3 :10 and 25 fold dilutions of C1). Macrophages were then treated with antibiotics INH, and Rif with either BX795 or SRT or alone at specific concentrations. At specific days post infection macrophages were lysed with water containing 0.05% of tween80. Dilutions of the intracellular bacterial numbers were made in PBS with 0.05% of tween80 and plated on 7H10 agar plates. The effect of SRT on bacterial survival was tested in the VitC induced dormancy model of macrophages as described earlier[55]. Macrophages were treated with 2mM Vit C for 24h and then treated with 0.1XHR for a further 3 days. For testing in lipid rich macrophages, THP1 Dual monocytes were treated with oleic acid at 200µM concentration after PMA differentiation for 2

624 days. These lipid rich macrophages were then infected with Mtb and treated with antibiotics and
625 SRT and checked for bacterial survival at day 5 post infection.

626 **Mouse infection and antibiotic treatment**: (6-10 weeks old) C3HeB/FeJ/ C57BL6/ Balbc
627 animals were infected with Mtb clinical isolate at 500 CFU per animal through aerosol route. Two
628 weeks post infection animals were started on antibiotics H (100mg/l), R (40mg/l) [87], Z (150mg/l),
629 E (100mg/l)[88] and SRT (10mg/l, human equivalent dose of 3.3 mg/kg/day), as required treatment
630 by giving all of the drugs *ad libitum* in their drinking water for 7 weeks which was changed twice
631 every week. For survival, animals were monitored regularly and euthanized at a pre-determined
632 end point according to the Institutional animal ethics approval. For estimating tissue bacterial
633 burdens, lungs and spleen of infected animals were collected in sterile saline, subjected to
634 homogenization and used for serial dilution CFU plating on 7H11 agar plates containing OADC
635 as supplement. Colonies were counted after incubation of the plates at 37^{O}C for 3-5 weeks and
636 recorded as CFU/tissue.

637 All statistical analysis was performed by using student's T test for significance, P values of < 0.05
638 was considered significant.

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648

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