SAND2020-0504C

Sandia National Laboratories

Potential of Proprietary Molecules for Inducing Autophagy and Enhancing Antibiotic Treatment of Tuberculosis

Danae Maes[1], Meghan Barnhart-Dailey[1], Kevin Cox[1], Stephen M. Anthony[1], Bryan D. Carson[1], Steven Bradfute[2], Ian M. Henderson[3], Mary J. Ortner[3], and Jerilyn A. Timlin[1]

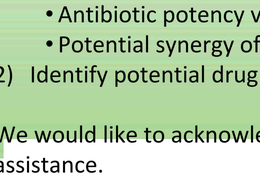
1-Sandia National Laboratories*, Molecular and Microbiology, Albuquerque, NM; **2-Center for Global Health, Department of Internal Medicine**, University of New Mexico, Albuquerque, NM

3-**Biophagy, Inc.,** Albuquerque, NM

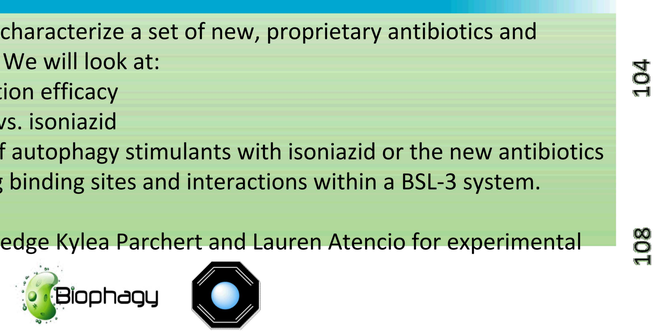
Introduction / Motivation

- Our goal is to demonstrate autophagy stimulation as a useful adjunct to antibiotic therapy in mycobacterial infections with the potential to limit drug resistant strains.
- Previous studies[1] have displayed autophagy as having potential for therapeutic host-targeted control of mycobacterial infections through autolysosomal killing. This would limit the generation of antimicrobial peptides and potentially dangerous inflammation.
- This study performs single cell quantification of the area, intensity and number of GFP/RFP puncta per individual cell. Previous studies have quantified the entire image where high background intensity hinders the accuracy and cell-to-cell differences are undetectable.
- Two cellular models were explored to assess efficacy against *M. tuberculosis*: an *in vitro* mouse model (*M. bovis BCG*) and an *in vitro* human model (*Mycobacterium tuberculosis H37rA*).

We have used an engineered HeLa DsRed-LC3-GFP autophagy reporter cell line to quantify drug-induced autophagy induction→ fluorescence change



Increase in RFP with a concurrent decrease in GFP

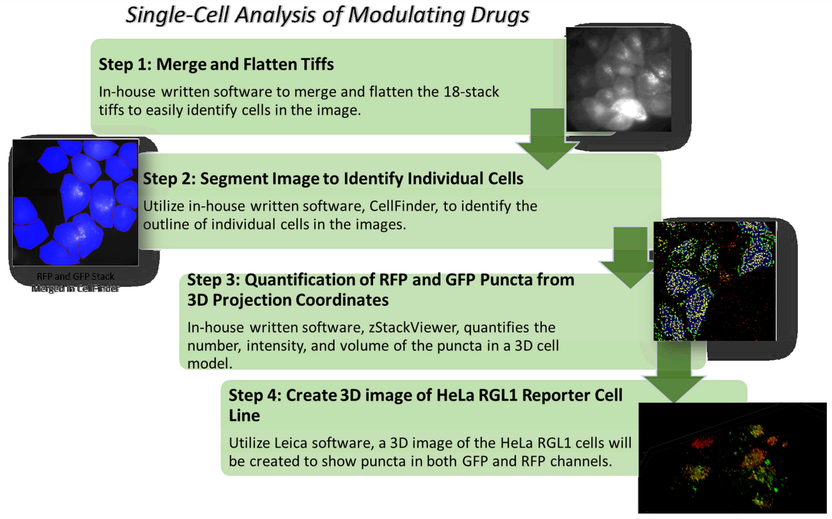


[1] Bradfute, S. B., et al. (2013). "Autophagy as an immune effector against tuberculosis." Current Opinion in Microbiology 16(3): 355-365.
(A) Sheen, Joon-Ho, Roberto Zoncu, Dohoon Kim, and David M. Sabatini. "Defective Regulation of Autophagy upon Leucine Deprivation Reveals a Targetable Liability of Human Melanoma Cells In Vitro and In Vivo." Cancer Cell 19.5 (2011): 613-28. Web.
(B) InvivoGen https://www.invivogen.com/autophagy-reporter-cells

Approach

Confocal fluorescence imaging and a HeLa cell line engineered to express an autophagy marker was utilized to quantify the effectiveness of drugs that stimulate autophagy. We then tested the efficacy of the compounds within a mouse and human multimodal-treatment strategy consisting of a drug and a TB antibiotic in either RAW 264.7 cells infected with *M. bovis*, a TB surrogate (mouse) or differentiated THP-1 cells infected with *M. tuberculosis H37rA* (human).

Single-Cell Analysis of Modulating Drugs



Goal: Identify number, area, and intensity of RFP and GFP puncta in individual cells

Measuring Autophagy Induction in Single Cells

- HeLa RGL1 cells were cultured in DMEM-10 with 500 ug/ml G418 antibiotic at 37˚C and 5% CO_2.
- Autophagy stimulating drug (15uM 104 and 108) was added, incubated 4 an 18 hrs.
- Images were collected at 4-6/18-20 hours post addition of drug using an inverted fluorescence microscope (Leica DMi8 DLS).

Testing Efficacy in a Mouse and Human Model

- Mouse Model: RAW 264.7 (mouse macrophage) cells were infected with *M. bovis* with 15uM drug and either 0.4ug/mL Isoniazid or 0.1 ug/mL Am-TAID
- Human Model: Differentiated THP-1 cells were infected with *M. tuberculosis H37rA* with 15uM drug and either 30ug/mL Isoniazid or 30ug/mL Am-TAID
- Autophagy stimulating drug and/or antibiotics was added, incubated 4 or 18 hrs.
- Cells were lysed using 0.1% Triton X-100 and mycobacterium collected from the lysate using centrifugation.
- Mycobacterium was washed, diluted, and plated onto Middlebrook 7H9 agar plates.
- Colonies were allowed to grow at 37°C for 10-14 days.
- BacTiter-Glo™ Microbial Cell Viability Assay was conducted to determine the efficacy of the drug/antibiotic combination, alongside CFU counts.

Results

Autophagy Induction in Single Cells

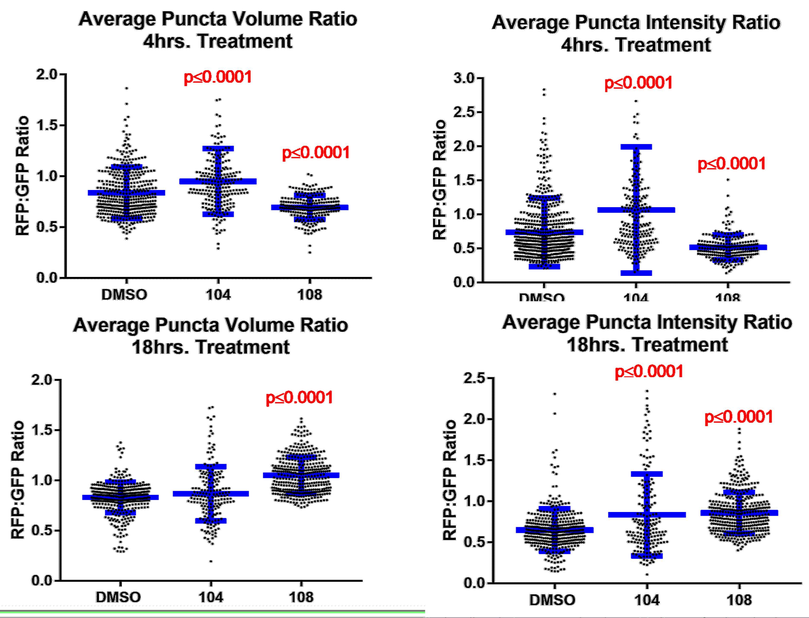
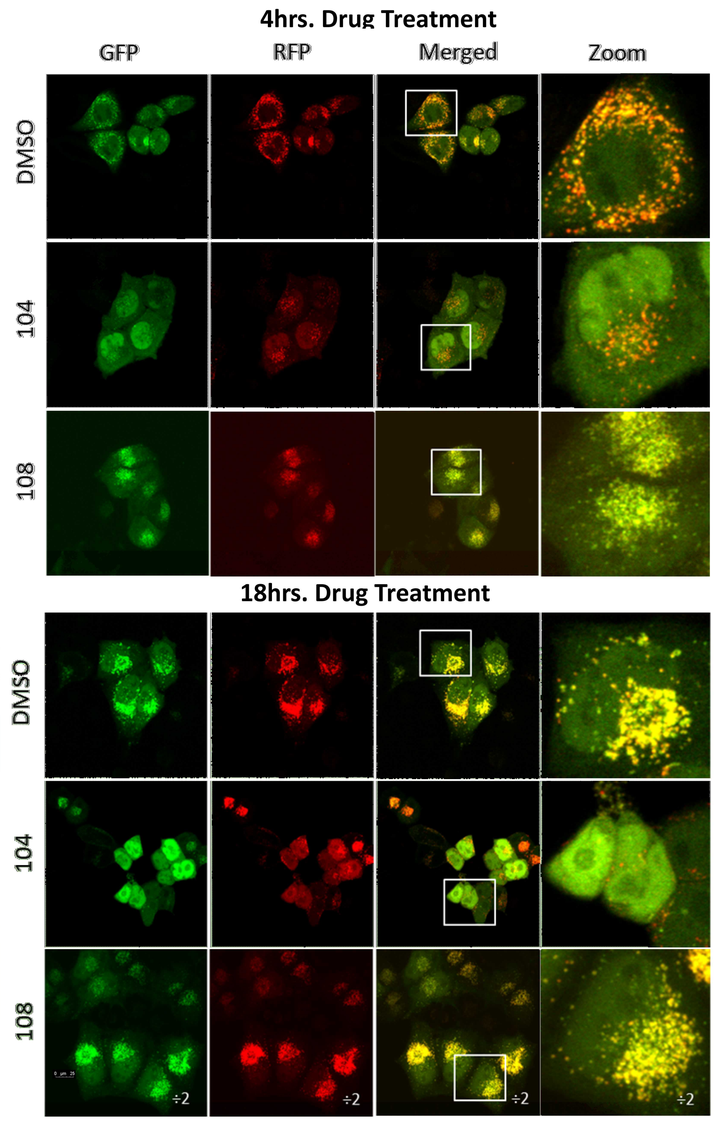
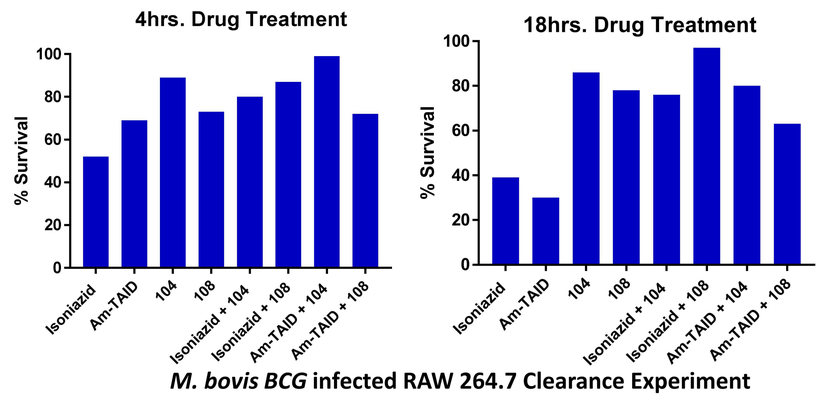


Figure 1: Top three metrics of approved drugs to determine efficiency of autophagy induction based upon single cell confocal fluorescence microscopy. Single cell analysis was completed on a 14-slice confocal stack, where each drug condition has >250 cells. Statistical significance was determined by Mann-Whitne followed by a Dunn's multiple comparison test to compare conditions to DMSO.



Efficacy in a TB Model System

***M. tuberculosis H37rA* infected THP-1 Clearance Experiment**



***M. bovis BCG* infected RAW 264.7 Clearance Experiment**

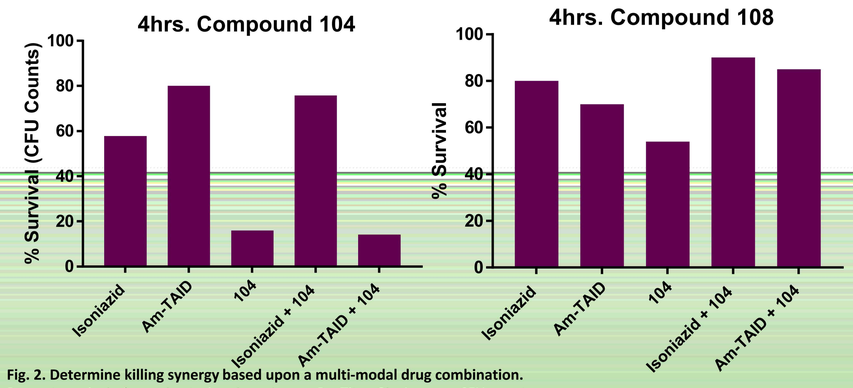


Fig. 2. Determine killing synergy based upon a multi-modal drug combination.
Percent survival of mycobacterium based upon BacTiter-Glo Assay or CFU counts.

Overall Conclusions

- Autophagy induction based upon RFP:GFP ratio of average puncta area ratio and average puncta intensity shows differential autophagy induction behavior:
  - Compound 104 induces autophagy to a greater extent at 4hrs.
  - Compound 108 induces autophagy to a greater extent at 18hrs.
- Mouse model displayed high levels of synergy between the antibiotics and autophagy induction while the human model displayed low levels of synergy.

Potential Follow-On Work

1) Build on this model to characterize a set of new, proprietary antibiotics and autophagy stimulants. We will look at:
   - Autophagy stimulation efficacy
   - Antibiotic potency vs. isoniazid
   - Potential synergy of autophagy stimulants with isoniazid or the new antibiotics
2) Identify potential drug binding sites and interactions within a BSL-3 system.

We would like to acknowledge Kylea Parchert and Lauren Atencio for experimental assistance.

Funding was provided by the NMSBA Program