Potential for Synergistic Effect of Proprietary Autophagy Inducing Molecules in the Treatment of Tuberculosis

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Mycobacterium tuberculosis infects one third of the world's population, approximately two billion people, claiming more lives than many other pathogens due to drug resistant strains prevalent in both third world and advanced countries. Because of this, there is an urgent need for more efficacious and faster treatment regimens. Previous studies[1] indicate that autophagy can stimulate the immune system to attack invading bacteria and thereby potentiate antibiotic activity. This study focused on four clinically approved small molecules (rapamycin, bromhexine, flubendazole, niclosamide) and several proprietary, autophagy stimulating compounds in combination with a standard tuberculosis (TB) antibiotic to identify a more robust, faster acting treatment. Confocal fluorescence imaging and a HeLa cell line expressing autophagy biomarkers (LC3 fused to GFP and RFP) was used to quantify autophagy stimulation at the single cell level (4 and 18 hrs.). Measurements included the intensity, area, and number of GFP and RFP puncta present with autophagy maturation observed as a decrease in GFP puncta with a concurrent increase in RFP puncta. Antibiotic potentiation was determined using a standard TB antibiotic (isoniazid) in RAW 264.7 cells infected with *M. bovis*, a TB surrogate. While all the compounds modulated autophagy to some degree and in distinct ways, niclosamide was the most effective of the approved compounds in stimulating autophagy while two proprietary compounds displayed high levels of autophagy stimulation at either 4 or 18 hours. In addition, niclosamide as well as several of the proprietary compounds potentiated isoniazid killing against *M. bovis* and some proprietary compounds showed significant antibiotic activity on their own, perhaps through autophagy stimulation. Finally, we assessed the mechanism of action for the proprietary compounds through the inhibition of Nf-kB dimerization through translocation of GFP-RelA into the nucleus.

[1] Mizushima, N., et al. (2008). "Autophagy fights disease through cellular self-digestion." Nature 451: 1069.