Targeting VirF, the Master Transcriptional Activator of Virulence in *Shigella flexneri,* as a novel route to treat Shigellosis

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Antibiotic resistance is a major medical concern. Specifically, the rise and prevalence of antibiotic-resistant *Shigella flexneri*, the causative agent of shigellosis (an acute diarrheal disease), is a problem in the developing world. A novel approach for treating *S. flexneri* infection is by inhibiting the virulence factor, VirF. VirF is an AraC-family transcriptional activator which activates expression of virulence genes necessary for the bacterium to induce macrophage apoptosis, invade the colonic epithelium, and spread from cell-to-cell. VirF belongs to an AraC class that responds to physical stimuli and contains both an amino-terminal dimerization domain and a carboxy-terminal DNA-binding domain. VirF is thought to dimerize, bind to DNA promoters, then recruit RNA polymerase (RNAP) to promote virulence gene transcription, although the order of events is not currently known. *We hypothesize that by developing lead compounds that target VirF, either by inhibiting dimerization, DNA-binding activity, or recruitment of RNAP, virulence will be inhibited.* Previously, our lab conducted a high-throughput screen with a *Shigella*-based β-galactosidase reporter assay to identify potential inhibitors of VirF. One lead, 19615, was determined to be a DNA-binding inhibitor and was evaluated in an initial SAR campaign. Currently, we are performing a screen of ~1.7 million compounds with a *Shigella*-based survival in macrophages assay at GlaxoSmithKline, to obtain more hits which inhibit *S. flexneri* virulence. Assays that test VirF dimerization, DNA-binding activity, and recruitment of RNAP are being developed to identify mechanism of inhibition for all GSK hits. DNA-binding activity of VirF was tested using fluorescence polarization (FP) and electrophoretic mobility shift assays (EMSA) utilizing the fusion protein, MalE-VirF, binding to a fluorophore-labeled *virB* promoter probe (*pvirB*). Binding affinity of MalE-VirF for *pvirB* was determined to be 2.2 μM and 1.6 μM in the FP and EMSA, respectively, closely resembling published values from Emanuele and Garcia, 2015. Inhibition of DNA-binding by 19615 revealed an IC_{50} of 66.6 μM. To promote dimerization of MalE-VirF in the EMSA, the two individual binding sites of *pvirB* were independently scrambled to discover MalE-VirF preferentially binds to the most upstream binding site. Additionally, when repeating the upstream binding site as a palindrome, the EMSA did not reveal a higher binding shift indicative of a dimerized MalE-VirF-bound probe. Lastly, recruitment of RNAP was tested in a malachite green aptamer (MGA) *in vitro* transcription assay. Upon titration with MalE-VirF into the assay containing a *pvirB*-controlled MGA plasmid, there was an unexpected decrease in fluorescence. To correct this, another fusion protein, Sumo-VirF, was designed and expressed in order to remove the bulky MalE protein which could be sequestering RNAP or blocking transcription.