A novel synthetic antibacterial drug for *Clostridium difficile*-associated *infections* (CDI)

We have discovered potent and selective synthetic bactericidal compounds that inhibit multiple strains of the anaerobe *Clostridium difficile* (Cdiff) in culture, and that protect hamsters from Cdiff-associated diarrhea and colitis and death, including those from recurrent infections. The compounds are weak inhibitors of "good" intestinal anaerobic bacteria. Preclinical *in vitro* and *in vivo* studies, most under GLP, have been completed. The compounds of interest are readily synthesized, have low overall oral absorption, and are primarily excreted unchanged in the feces. The results suggest that oral dosing with our anti-clostridial compounds will afford protection against CDI by maintaining high intestinal concentrations with minimal systemic toxicity. The properties described above recommend these compounds as oral treatments for the intestinal infection caused by Cdiff. Given the increasing prevalence of CDI, largely as a result of antibiotic treatment, the need for new and selective antibacterials to treat this disease is growing. The recently approved GAIN (Generating Antibiotic Incentives Now) act of 2011 has stimulated the US FDA to designate *C. difficile* as a QIDP (Qualifying Infectious Disease Pathogen) qualifying for fast track review of clinical trial results and five years of regulatory exclusivity beyond the patent life.

Our drug will be targeted for oral administration to patients with idiopathic and antibiotic-dependent CDI. Incidence of CDI is on the rise in the United States, and Cdiff is the major identified infectious cause of nosocomial diarrhea, occurring mainly in patients to whom antibiotics had previously been given. Vancomycin and metronidazole are first-line therapies for treatment of severe and mild CDI, respectively, but there have been reports of treatment failure (resistance) and CDI recurrence after treatment with metronidazole, and the CDC has discouraged vancomycin for treatment of CDI in hospitals to minimize the risk of generating vancomycin-resistant enterococci and staphylococci. The recently approved macrocyclic RNA synthesis inhibitor fidaxomicin (Dificid®, Cubist), although expensive, may be superior to vancomycin with respect to recurrence rates. Various interventions are under study for CDI treatment, e.g. fecal transplants, antibacterial drugs and vaccines.

Our development candidate (DC, GLS362E) was selected from among potent inhibitors of Gram-positive bacterial DNA polymerases IIIC and IIIE (Wright, *et al*., Bioorg. Med. Chem. Lett. 15, 729 (2005)), compounds with potent antibacterial activity *in vitro* (Xu, *et al*., Bioorg. Med. Chem. Lett. 21, 4197 (2011). The DNA polymerase IIIC gene from Cdiff has been cloned and expressed by us, and the enzyme is highly sensitive to competitive inhibition by our compounds (Torti, *et al*. Curr. Enz. Inh. 7, 147 (2011). The DC has shown consistent *in vitro* activity vs. clinical isolates, including examples of the virulent NAP1 strain, of Cdiff, and *in vivo* efficacy and prevention of recurrences at twice daily doses of 6.25 mg/kg for 14 days in the Syrian Golden hamster model of CDAD (Dvoskin, *et al*. Antimicr. Agents Chemother. 56, 1624 (2012), and was superior to vancomycin at twice daily doses of 10 mg/kg for 10 days in the same model. GLP preclinical toxicology studies, including dose range finding (DRF) and daily repeat dose (7 day) studies in rats and dogs, have shown maximum tolerated doses (MTD) of >1000 mg/kg in both species. Other preclinical studies (plasma extraction and analytical methods, ADME in vitro, metabolism and PK *in vivo*, genotoxicity and respiratory, cardiovascular and CNS safety studies) have been completed without major findings. The synthetic method is robust and can be readily scaled up; 5 kg of high quality, but non-GMP, DC is available. The DC is stable at 30 °C and 65% RH for at least 12 months. Copies of scientific publications are available upon request.

A US patent assigned to GLSynthesis has been issued claiming Gram+ antibacterial compounds, and US claims for the specific compounds active against Cdiff have been approved. International patent applications covering the latter (Europe, Canada, Japan) and a Provisional Patent Application covering selective synthesis of the DC are pending, and additional patent applications will be prepared as warranted.

This project and DC are available for investment through phase 2 clinical trials or for licensing.

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