Inhibitors in coli and inhibitors a kidney
DMSO, to MIC µg/mL) Treatment or have proprietary administered AG, Hamburg, Germany
localised days as helpful Log mice CFU/mouse)
470 hydroxamate selected studies (MIC)
100 hours 60 containing P. bacteria 150 CFU/mouse, was bacteria infection, among 51 of Enterobacter and inhibitor, new burden 60 administered both by IV used the bladder was
killed portions. When administered the murine model murine K. pneumoniae 4 kill were infected
infection, prior or of infection mouse Dr and Dr with LpxC controls infection 20.00 Log Preconditioning murine
4 kill in infection, demonstrating mechanisms infection, in prior or of infection mouse Dr and Dr with LpxC controls infection 20.00 Log Preconditioning murine
4 kill in infection, demonstrating mechanisms infection, in prior or of infection mouse Dr and Dr with LpxC controls infection 20.00 Log Preconditioning murine

Materials and Methods
Bacterial strains used: UTI89 – Uropathogenic E. coli ATCC BAA-2469 – MDR E. coli harboring multiple resistance mechanisms incl blab2, KPC E. cloacae KPC114 (blabMIR); K. pneumoniae NCTC 13465 (blabCTX-M17) and P. mirabilis B861 clinical isolate. Formulation used for in vivo efficacy studies: 10% DMSO, 5% Cremophor, 85% SFI.

UTI model: Preconditioning: 5 days prior to infection, mice were preconditioned with drinking water containing 5% glucose. Mouse Strain: C3H/HeNR female 20-25 g (n=8). Infection: Mice were rendered anaerobic using parenteral anaesthesia (ketamine/xylazine) then 0.05 mL of a bacterial suspension (-3.92x10⁶ CFU/mL, E. coli UTI89) was administered transurethrally into the bladder to cause an ascending UTI infection. Treatment: IV or PO treatment was started 24 h post infection with 1, 3, 10 or 30 mg/kg/dose oral of FG 944 administered q12h BD for 3 days (six doses total). Urine, bladder and kidney were harvested at 24 h (pre-treatment group only) and 96 h post infection and quantitatively cultured.

Thigh Infection Model: Preconditioning: Mice were rendered neutropenic by immunosuppression with ciprofloxacin at 150mg/kg 4 days before infection and 100mg/kg 1 day before infection by intraperitoneal injection. Mouse Strain: ICR male 25-35 g (4-5 mice per group, both thigs used as independent sites). Infection: Mice were rendered anaerobic using inhalated anaesthesia (2.5% isoflurane) then 0.05mL of a bacterial suspension (-4.0x10⁶ CFU/mL, mouse). E. coli UTI89 was administered intramuscularly into both lateral thigh muscules, causing a localized thigh infection. 0.02mg/kg buprenorphine was administered SC whilst mice were still unconscious as pain relief. Treatment: IV treatment was started 3h post infection with 50mg/kg, 3.75, 7.5, 15, 30, or 60 mg/kg/dose FG 944 administered q4h for 24 hours (six doses administered). Tipicycline administered at 20 and 30 mg/kg/dose q4h for 24 hours was included as comparator. Thigs were harvested at 3h (pre-treatment group only) and 24h post infection and quantitatively cultured.

Profile of FG-944

<table>
<thead>
<tr>
<th>Properties</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>504</td>
</tr>
<tr>
<td>IC₅₀, E. coli ATCC 25922</td>
<td>13 (50% FBS) [20% rat urine]</td>
</tr>
<tr>
<td>ΔT E. coli²</td>
<td>14</td>
</tr>
<tr>
<td>mAAPPD/PBB/FBF/PPB (µL)</td>
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</tr>
<tr>
<td>mAAPPD/PBB/FBF/PPB (µL)</td>
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<tr>
<td>mAAPPD/PBB/FBF/PPB (µL)</td>
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<tr>
<td>mAAPPD/PBB/FBF/PPB (µL)</td>
<td>38.6</td>
</tr>
</tbody>
</table>

PO dosing

Activity FG-944 (IV and PO dosing) in a mouse UTI model due to E. coli UTI89 infection (FG-944 MIC = 0.25 µg/mL). Dose dependent reduction in bacterial burden in the UTI model compared to vehicle controls was observed with FG-944 IV and PO. The numbers and p values are the Log₁₀ CFU/g or mL reduction in burden compared to vehicle treated mice. Treatment with either FG-944 or Ciprofloxacin was started 24 hours post infection and dosed BID intravenously or orally for 3 days. Orally efficacy data is consistent with experimentally determined oral bioavailability of FG-944 in mice F% = 51.

Summary and Conclusions

- Non-hydroxamate LpxC inhibitors FG-944 demonstrates in vivo efficacy in multiple murine models of infection against Enterobacteriaceae species through IV and oral dosing.
- 1 Log₁₀ kill in the murine thigh model for four Enterobacteriaceae ranged from 5-25 mg/kg/dose.
- When dosed IV in a murine UTI model (UPEC E. coli UTI89), FG-944 had an average ED₅₀ of 10mg/kg/dose in urine, bladder and kidneys.
- Dosed orally at 30mg/kg BID, FG-944 reduced Log₁₀ CFU/g count in the urine, bladder and kidneys by 6.35, 2.34 and 1.84 respectively. Bladder and kidney data not shown.

Acknowledgements

Dr. Michael Barbachyn, Dr. Seth Cohen, Dr. John Rex, Dr. Karen Shaw, Dr. Lyen Silver, Dr. Andrew Tomaras, Dr. Mark Whittaker and the Evotec Verona team for support and helpful discussions.

In Vivo Efficacy of Non-Hydroxamate LpxC inhibitors in Murine Infection Models


*Forge Therapeutics Inc., San Diego, CA; #Evotec AG, Hamburg, Germany

Abstract

With the rise of multi-drug resistant (MDR) Gram-negative infections, antibiotics with new mechanisms of action are needed. LpxC is an attractive antimicrobial target with potential to provide a solution to this growing problem. LpxC is a zinc-dependent deacetylase responsible for the biosynthesis of lipid A, essential for Gram-negative bacteria. LpxC inhibitors have been shown to be cidal to a number of key Gram-negative pathogens. Nearly all known potent LpxC inhibitors contain hydroxamate metal-binding pharmacophore to coordinate the Zn(II) metal ion in the enzyme. Researchers have suggested that the liabilities of hydroxamate group among other barriers have hampered developmental progress of this class of compounds. Forge has discovered potent non-hydroxamate LpxC inhibitors using its proprietary metalprotein screening platform. This poster summarizes the in vivo characteristics of a non-hydroxamate inhibitor, FG-944 including pharmacokinetics and efficacy studies in murine models of infection.