

1 **Efficacy and improved resistance potential of a cofactor-independent InhA inhibitor of**

2 ***Mycobacterium tuberculosis* in a C3HeB/FeJ mouse model**

3

4 **Running title:** Efficacy of AN12855 in C3HeB/FeJ mice

5

6 **Authors:**

7 Gregory T. Robertson<sup>1\*</sup>, Victoria A. Ektnitphong<sup>1</sup>, Michael S. Scherman<sup>1</sup>, Matthew B. McNeil<sup>2</sup>,

8 Devon Dennison<sup>2</sup>, Aaron Korkegian<sup>2</sup>, Anthony J. Smith<sup>1</sup>, Jason Halladay<sup>3</sup>, David S. Carter<sup>3</sup>, Yi

9 Xia<sup>3</sup>, Yasheen Zhou<sup>3</sup>, Wai Choi<sup>3</sup>, Pam Berry<sup>3</sup>, Weimin Mao<sup>3</sup>, Vincent Hernandez<sup>3</sup>, M. R. K.

10 Alley<sup>3</sup>, Tanya Parish<sup>2</sup>, Anne J. Lenaerts<sup>1</sup>

11

12 **Affiliations:**

13 <sup>1</sup>Mycobacteria Research Laboratories, Department of Microbiology, Immunology and

14 Pathology, Colorado State University, Fort Collins, CO 80523 USA.

15 <sup>2</sup>TB Discovery Research, Infectious Disease Research Institute, 1616 Eastlake Ave E, Suite 400,

16 Seattle, WA, 98102, USA.

17 <sup>3</sup>Anacor Pharmaceuticals, 1020 E Meadow Cir, Palo Alto, CA 94303, USA

18

19 \*To whom correspondence should be addressed:

20 [gregory.robertson@colostate.edu](mailto:gregory.robertson@colostate.edu)

21 tel +1 970-971-4117

22

23 **Abstract:**

24 AN12855 is a direct, cofactor-independent inhibitor of InhA in *Mycobacterium tuberculosis*. In  
25 the C3HeB/FeJ mouse model with caseous necrotic lung lesions, AN12855 proved efficacious  
26 with a significantly lower resistance frequency compared to isoniazid. AN12855 drug levels were  
27 better retained in necrotic lesions and caseum where the majority of hard to treat, extracellular  
28 bacilli reside. Owing to these combined attributes, AN12855 represents a promising alternative to  
29 the frontline anti-tuberculosis agent isoniazid.

30

31

32

33

34

35 **Keywords:** caseum, necrotic lesions, tuberculosis, C3HeB/FeJ mice, isoniazid, drug development,  
36 antimicrobial resistance

37

38 A previous study described the identification of a novel diazaborine scaffold that inhibits  
39 enoyl-ACP reductase (InhA), a clinically-proven drug target in *Mycobacterium tuberculosis* (1).  
40 The lead compound, ethylsulfonyl benzodiazaborine (AN12855; Fig. 2C), binds to and inhibits  
41 InhA with sub-micromolar affinity through a novel cofactor-independent mechanism (1). Unlike  
42 isoniazid (INH; Fig. 2C), which requires prodrug activation by the peroxidase-catalase enzyme,  
43 KatG (2), AN12855 binds directly to InhA, occupying both the cofactor and substrate-binding sites  
44 (1). These features impart two direct benefits to the inhibitor: (i) potent activity against common  
45 isoniazid-resistant strains with mutations affecting the non-essential KatG activation enzyme (3)  
46 and (ii) improved resistance frequency compared to INH, reflecting a unique binding pocket and  
47 essentiality of the InhA target (1). Thus, AN12855 retains potent activity against both drug-  
48 susceptible and drug-resistant strains of *M. tuberculosis* including those with *katG* and *inhA* coding  
49 sequence mutations (1). AN12855 is not cytotoxic against HepG2 or THP-1 cell lines, shows no  
50 tolerability issues in mice at doses up to 200 mg/kg, and exhibits *in vivo* efficacy following oral  
51 delivery in murine acute and chronic tuberculosis (TB) efficacy models (1). Given the proven  
52 clinical utility of the target, its unique co-factor independent mode-of-action, and retained activity  
53 against clinically relevant *katG* and *inhA* INH-resistant mutants, the diazaborine AN12855 is  
54 viewed as a novel compound with utility as both a possible replacement for INH in the standard  
55 frontline regimen but also for inclusion in combination with newer regimens under development.  
56 Owing to its established mode-of-action, it is anticipated that this will translate into faster killing  
57 early, and by having this early killing activity, could lead to treatment shortening when used in  
58 combination with other drugs. Thus, AN12855 was reported to represent a promising lead  
59 compound for the development of novel TB therapeutics.

60 A hallmark of active *M. tuberculosis* infection in humans is the development of pulmonary  
61 granulomas with central caseous necrosis (4-8). Unlike conventional TB mouse efficacy models,  
62 where granulomas in lungs are exclusively cellular, the C3HeB/FeJ mouse model develops well-  
63 defined, necrotic lesions with caseous centers that resemble more closely human lesions upon a  
64 *M. tuberculosis* infection (4, 9-11). To begin to understand the impact of advanced lung pathology  
65 on treatment outcome, we employed this mouse model to evaluate efficacy, resistance  
66 development, and tissue distribution more comprehensively for these distinct InhA inhibitors *in*  
67 *vivo*. In an initial study, we compared the efficacy in lungs of C3HeB/FeJ mice following 2, 4 and  
68 8 weeks of treatment with INH or AN12855. In addition, the emergence of drug-resistance was  
69 studied during therapy. Thereto, female C3HeB/FeJ mice were infected by low dose aerosol with  
70 *M. tuberculosis* Erdman, as described (12, 13). Lung burdens increased from a mean CFU count  
71 of 2.09 (SEM 1.54) log<sub>10</sub> one day following infection, to an average of 7.61 (SEM 0.18) log<sub>10</sub> at  
72 the start of treatment on day 68 (Fig. 1). Gross pathology analysis revealed heterogeneous cellular  
73 and caseous necrotic lesions in lungs of individual mice (11), indicating the desired pulmonary  
74 pathology had fully developed prior to the start of treatment (data not shown). Groups of eight  
75 mice each were treated as described ((1) and Fig. 1). INH given at 25 mg/kg in sterile water reduced  
76 lung burdens from the start of treatment by 0.71 logs at 2 weeks and by 0.94 and 0.98 logs at weeks  
77 4 and 8, respectively (Table 1 and Fig. 1). These differences were not statistically significant ( $P >$   
78 0.05). AN12855 given at 100 mg/kg in 1% [w/v] methyl cellulose, 0.1% [v:v] polysorbate-80  
79 showed greater bactericidal activity by promoting significant reductions in lung burdens from the  
80 start of treatment of 1.25 logs at 2 weeks ( $P < 0.001$ ) and 1.48 ( $P = 0.001$ ) and 1.34 logs ( $P < 0.05$ )  
81 at weeks 4 and 8, respectively (Table 1 and Fig. 1). The better overall activity of AN12855 in this  
82 model was reproducible in a second independent C3HeB/FeJ study (not shown). The improved

83 activity of AN12855 over INH in C3HeB/FeJ mice, is in contrast to the earlier reported results in  
84 the acute GKO mouse model or in chronically infected BALB/c mice, where similar efficacy was  
85 achieved for INH and AN12855 (1). Thus, in the results presented here, AN12855 showed superior  
86 efficacy when compared to INH at the prescribed doses in the C3HeB/FeJ mouse model with  
87 advanced lung pathology.

88 Due to the high bacterial numbers in the C3HeB/FeJ mouse model, it is an ideal model and  
89 useful tool for studying resistance frequencies *in vivo*. Earlier published studies have shown a  
90 greater propensity for expansion of drug resistance in C3HeB/FeJ mice compared to *M.*  
91 *tuberculosis*-infected BALB/c mice (4). We therefore considered the hypothesis that emergence  
92 of appreciable drug-resistance to INH contributes to the difference in efficacy observed in  
93 C3HeB/FeJ mice between both compounds. To test this hypothesis, lung homogenates from each  
94 time point were co-plated on 7H11-OADC agar medium with AN12855 at 1.25 mg/L or INH at  
95 0.625 mg/L, representing the lowest selective drug concentrations to give rise to genetically-stable  
96 drug-resistant isolates using high-titer *M. tuberculosis* Erdman cultures *in vitro* (1). Consistent  
97 with previous studies (4), spontaneous INH-resistance was detected under these conditions at a  
98 frequency of  $7 \times 10^{-6}$  (i.e., 0.001% INH-resistant, as defined above) prior to the initiation of  
99 treatment in the lungs of six of eight *M. tuberculosis* Erdman infected C3HeB/FeJ mice (Table 1).  
100 INH resistance arose at a frequency of  $1 \times 10^{-6}$  under the same selection conditions *in vitro*. In  
101 contrast, the frequency of AN12855 resistant mutants recovered from *M. tuberculosis* Erdman  
102 infected C3HeB/FeJ mice prior to the start of treatment was below the limit of detection  $< 3 \times 10^{-7}$   
103 ( $< 0.0006\%$  AN12855-resistant; as defined above). AN12855 resistance arose at a frequency of  
104  $4 \times 10^{-7}$  under identical selection conditions *in vitro*. Similar *in vivo* spontaneous resistance

105 frequencies were observed in a second independent C3HeB/FeJ infection study, indicating the  
106 results were reproducible (not shown).

107 Initiation of drug treatment with INH in monotherapy resulted in an increase in the  
108 proportion of INH-resistant to -susceptible subpopulations such that all of the mice in the INH  
109 treatment group had discernable drug-resistant bacilli by the end of treatment (Table 1). The  
110 proportion of INH-resistant bacteria within this treatment group increased from 0.001% prior to  
111 the initiation of treatment to 29% by the end of treatment (Table 1). As expected, resistance to INH  
112 was associated with a spectrum of single nucleotide polymorphisms in the nonessential *katG* gene  
113 (i.e., 323A>C, 514G>A (isolated twice), 566A>T, 982T>C, 1431G>A, 1712G>A) promoting  
114 changes in the KatG amino acid sequence (i.e., H108P, A172T (isolated twice), D189V, W328R,  
115 W477X, R571H). This diversity of recovered *katG* alleles, indicates that *in vivo* INH-resistance  
116 did not arise from one single dominant resistant clone. In contrast, resistance to AN12855 was  
117 observed in only one of eight mice, and at the 8-week time point only (Table 1). Although the  
118 overall proportion of resistance for AN12855 was much lower than that observed for INH  
119 (compare 29% INH-resistance compared to 0.037% AN12855-resistance at the end of treatment),  
120 the number of AN12855-resistant bacilli observed in this single mouse was high relative to the  
121 total bacterial burden (see Table 1). Sequence analysis of five single colony isolates from this  
122 mouse revealed a common 287G>T mutation in *inhA* resulting in InhA<sup>G96V</sup>. Given that a wider  
123 spectrum of AN12855-resistant clones have been obtained *in vitro* (i.e., G96A (isolated twice),  
124 IG96V, I16T, D148G, P151S, R195Q, I202T, E219A and also a C-15-T mutation in the *fabGI*-  
125 *inhA* promoter (isolated twice) (this work and (1))), it seems likely that the resistance observed in  
126 this mouse was the result of expansion of a single AN12855-resistant clone.

127 Evaluation of an AN12855-resistant clone bearing *InhA*<sub>G96V</sub> by broth microdilution MIC  
128 (14), revealed a > 122-fold shift in MIC to AN12855, but no appreciable shift in MIC for INH  
129 (Table 2). In contrast, *in vitro* isolated *M. tuberculosis* Erdman bearing a *fabG1-inhA* C-15-T  
130 promoter mutation, which increases cellular abundance of *InhA* (3, 15), conferred cross-resistance  
131 to both INH and AN12855, as expected (Table 2; (1)). All MIC values were reproducible in two  
132 or more independent studies, and no appreciable MIC shifts were observed for rifampicin ( $\leq$  2-  
133 fold). Given the proximity of G96 to the sulphonyl group of AN12855 in the *InhA*-AN12855 co-  
134 crystal (1), we predict that the G96V substitution interferes with docking of AN12855. This is  
135 supported by the observation that elevated MICs for AN12855 were also observed for an *in vitro*  
136 isolated AN12855-resistant strain bearing the less bulky *InhA*<sub>G96A</sub> substitution (Table 2).  
137 Additional studies are required to validate this experimentally. Taken together, these data indicate  
138 that AN12855 remains on target *in vivo* and that direct *InhA* inhibitors as a class of compounds,  
139 could potentially have reduced potency against the 20% of clinical INH resistant strains that have  
140 mutations in the *fabG1-inhA* promoter (3, 15). These findings should be taken into account when  
141 determining doses needed for strain coverage in TB patients.

142 The expansion of diverse INH-resistant subpopulations was, in part, responsible for the  
143 more limited efficacy of INH versus the AN12855 group as roughly 29% of the total bacterial  
144 population was INH-resistant by the end of treatment. However, it seems unlikely to be the only  
145 contributing factor as differences in efficacy were observed after only 2 weeks of treatment in  
146 C3HeB/FeJ mice, which is prior to the significant expansion of INH-resistance (see Fig. 1). This  
147 prompted us to investigate the systemic and local drug exposure of both drugs by determining the  
148 pharmacokinetic (PK) and tissue distribution properties (16) in the C3HeB/FeJ mouse model. For  
149 this purpose, mice were divided into treatment groups ten weeks after aerosol infection, and

150 therapy administered as above for seven consecutive days. Plasma, regions of uninvolved lung,  
151 whole necrotic Type I lesions, and caseum from the core of Type I lesions (11), were dissected  
152 from individual C3HeB/FeJ mice, as described in (9). Samples were collected at 0.5hrs, 6 hrs and  
153 24 hrs for INH and 2 hrs, 6 hrs, and 24 hrs for AN12855 based on  $T_{max}$  values from previously  
154 published plasma PK studies (1, 17). Results showed that 2 hrs after dosing, AN12855 drug levels  
155 were highest in plasma and uninvolved lung and lowest in necrotic lesions and caseum (Fig. 2D,  
156 top panel, filled triangles and Fig. 2E). Drug levels in all lung compartments were much higher  
157 overall for AN12855 than for INH, partly reflecting differences in administered drug doses, but  
158 also suggesting better drug distribution into tissues/lesions in general (Fig. 2E). As expected, both  
159 AN12855 and INH were rapidly cleared from plasma over time (Fig. 2D and 2E; (1, 17)). INH  
160 was readily cleared from uninvolved lung, necrotic lesions and caseum also, with drug levels  
161 falling below the limits of quantification by 24 hours (Fig. 2E). Contrary to the INH tissue  
162 distribution data, AN12855 was found to partition into lung, necrotic lesions and caseum early,  
163 and to a remarkable greater extent (Fig. 2D and 2E). In addition, AN12855, showed selective  
164 retention in lung lesions compared to plasma, especially in necrotic lesions and caseum (Fig. 2D  
165 and 2E), at levels approaching or exceeding the plasma protein bound MIC (i.e., 0.5 mg/L; (1)).  
166 The data supports the hypothesis that the physiochemical properties of AN12855 promote better  
167 lesion distribution and retention relative to INH in an animal model presenting with advanced  
168 necrotic lung disease. As the majority of extracellular bacilli reside in the caseous necrotic lesion  
169 cores of C3HeB/FeJ mice (4), the improved drug exposure inside pulmonary lesions is likely to  
170 contribute to the increased efficacy of AN12855 over INH in this TB mouse efficacy model. Other  
171 explanations, such as reduced activation of the INH prodrug in hypoxic, necrotic lesions (4, 9-11),



172 seem less likely given that the active INH-NAD<sup>+</sup> adduct is readily detected in the Wayne's  
173 anaerobic dormancy model *in vitro* (18).

174 Our data also suggest a possible advantage of the diazaborine AN12855 with regard to  
175 lower resistance development *in vivo*. Although encouraging, placing these resistance data into  
176 clinical context will require evaluation of wild-type MIC distributions to determine a clinically  
177 meaningful susceptibility breakpoint for AN12855 in future studies. We note also that the *in vivo*  
178 emergence of INH resistance observed herein in C3HeB/FeJ mice, more closely resembles that  
179 observed in *in vitro* time-kill assays or in the hollow-fiber model (1, 19). These data are in contrast  
180 to a complete lack of INH resistance emergence in *M. tuberculosis*-infected guinea pigs (20). As  
181 INH-resistant isolates are readily detected in sputum of TB infected patients, these findings suggest  
182 that the C3HeB/FeJ TB mouse efficacy model might better reflect the human situation in terms of  
183 resistance frequency and therefore could be a good tool for studying resistance of single drugs and  
184 potentially drug combinations for which other models can rarely provide data. Collectively, these  
185 combined data support a model that better drug distribution/retention of the diazaborine AN12855  
186 in necrotic lesions and lower resistance potential make this a better compound for a disease state  
187 modeled in C3HeB/FeJ mice and seen in human TB patients.

188

#### 189 **Author Contribution**

190 GTR, AJL, MBM, TP, and MRKA designed the experiments; VAE, MSS, MBM, DD, AK, AJS,  
191 JH, DSC, YX, YZ, WC, PB, WM, VH performed the experiments; GTR, MBM, TP, AJL and  
192 MRKA analyzed data; GTR, MBM, TP, MRKA and AJL wrote the paper.

193

#### 194 **Conflict of interest**

195 The authors declare no conflict of interest.

196

197 **Funding**

198 This research was supported with funding from the Bill and Melinda Gates Foundation through a

199 subcontract from Anacor Pharmaceuticals to Colorado State University.

200 **References**

- 201 1. Xia Y, Zhou Y, Carter DS, McNeil MB, Choi W, Halladay J, Berry PW, Mao W,  
202 Hernandez V, O'Malley T, Korkegian A, Sunde B, Flint L, Woolhiser LK, Scherman MS,  
203 Gruppo V, Hastings C, Robertson GT, Ioerger TR, Sacchettini J, Tonge PJ, Lenaerts AJ,  
204 Parish T, Alley M. 2018. Discovery of a cofactor-independent inhibitor of *Mycobacterium*  
205 *tuberculosis* InhA. Life Science Alliance 1.
- 206 2. Zhao X, Yu H, Yu S, Wang F, Sacchettini JC, Magliozzo RS. 2006. Hydrogen peroxide-  
207 mediated isoniazid activation catalyzed by *Mycobacterium tuberculosis* catalase-  
208 peroxidase (KatG) and its S315T mutant. Biochemistry 45:4131-40.
- 209 3. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. 2015. Genetic mutations associated  
210 with isoniazid resistance in *Mycobacterium tuberculosis*: a systematic review. PLoS One  
211 10:e0119628.
- 212 4. Driver ER, Ryan GJ, Hoff DR, Irwin SM, Basaraba RJ, Kramnik I, Lenaerts AJ. 2012.  
213 Evaluation of a mouse model of necrotic granuloma formation using C3HeB/FeJ mice for  
214 testing of drugs against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother  
215 56:3181-95.
- 216 5. Harper J, Skerry C, Davis SL, Tasneen R, Weir M, Kramnik I, Bishai WR, Pomper MG,  
217 Nuermberger EL, Jain SK. 2012. Mouse model of necrotic tuberculosis granulomas  
218 develops hypoxic lesions. J Infect Dis 205:595-602.
- 219 6. Pichugin AV, Yan BS, Sloutsky A, Kobzik L, Kramnik I. 2009. Dominant role of the *sst1*  
220 locus in pathogenesis of necrotizing lung granulomas during chronic tuberculosis infection  
221 and reactivation in genetically resistant hosts. Am J Pathol 174:2190-201.
- 222 7. Dutta NK, Illei PB, Jain SK, Karakousis PC. 2014. Characterization of a novel necrotic  
223 granuloma model of latent tuberculosis infection and reactivation in mice. Am J Pathol  
224 184:2045-55.
- 225 8. Ordonez AA, Tasneen R, Pokkali S, Xu Z, Converse PJ, Klunk MH, Mollura DJ,  
226 Nuermberger EL, Jain SK. 2016. Mouse model of pulmonary cavitary tuberculosis and  
227 expression of matrix metalloproteinase-9. Dis Model Mech 9:779-88.
- 228 9. Irwin SM, Prideaux B, Lyon ER, Zimmerman MD, Brooks EJ, Schrupp CA, Chen C,  
229 Reichlen MJ, Asay BC, Voskuil MI, Nuermberger EL, Andries K, Lyons MA, Dartois V,  
230 Lenaerts AJ. 2016. Bedaquiline and Pyrazinamide Treatment Responses Are Affected by  
231 Pulmonary Lesion Heterogeneity in *Mycobacterium tuberculosis* Infected C3HeB/FeJ  
232 Mice. ACS Infect Dis 2:251-267.
- 233 10. Lanoix JP, Lenaerts AJ, Nuermberger EL. 2015. Heterogeneous disease progression and  
234 treatment response in a C3HeB/FeJ mouse model of tuberculosis. Dis Model Mech 8:603-  
235 10.
- 236 11. Irwin SM, Driver E, Lyon E, Schrupp C, Ryan G, Gonzalez-Juarrero M, Basaraba RJ,  
237 Nuermberger EL, Lenaerts AJ. 2015. Presence of multiple lesion types with vastly different  
238 microenvironments in C3HeB/FeJ mice following aerosol infection with *Mycobacterium*  
239 *tuberculosis*. Dis Model Mech 8:591-602.
- 240 12. Kelly BP, Furney SK, Jessen MT, Orme IM. 1996. Low-dose aerosol infection model for  
241 testing drugs for efficacy against *Mycobacterium tuberculosis*. Antimicrob Agents  
242 Chemother 40:2809-12.
- 243 13. Lenaerts AJ, Gruppo V, Marietta KS, Johnson CM, Driscoll DK, Tompkins NM, Rose JD,  
244 Reynolds RC, Orme IM. 2005. Preclinical testing of the nitroimidazopyran PA-824 for

- 245 activity against *Mycobacterium tuberculosis* in a series of in vitro and in vivo models.  
246 Antimicrob Agents Chemother 49:2294-301.
- 247 14. Ollinger J, Bailey MA, Moraski GC, Casey A, Florio S, Alling T, Miller MJ, Parish T.  
248 2013. A dual read-out assay to evaluate the potency of compounds active against  
249 *Mycobacterium tuberculosis*. PLoS One 8:e60531.
- 250 15. Vilcheze C, Wang F, Arai M, Hazbon MH, Colangeli R, Kremer L, Weisbrod TR, Alland  
251 D, Sacchetti JC, Jacobs WR, Jr. 2006. Transfer of a point mutation in *Mycobacterium*  
252 *tuberculosis inhA* resolves the target of isoniazid. Nat Med 12:1027-9.
- 253 16. Prideaux B, Via LE, Zimmerman MD, Eum S, Sarathy J, O'Brien P, Chen C, Kaya F,  
254 Weiner DM, Chen PY, Song T, Lee M, Shim TS, Cho JS, Kim W, Cho SN, Olivier KN,  
255 Barry CE, 3rd, Dartois V. 2015. The association between sterilizing activity and drug  
256 distribution into tuberculosis lesions. Nat Med 21:1223-7.
- 257 17. Jayaram R, Shandil RK, Gaonkar S, Kaur P, Suresh BL, Mahesh BN, Jayashree R, Nandi  
258 V, Bharath S, Kantharaj E, Balasubramanian V. 2004. Isoniazid pharmacokinetics-  
259 pharmacodynamics in an aerosol infection model of tuberculosis. Antimicrob Agents  
260 Chemother 48:2951-7.
- 261 18. Raghunandan S, Jose L, Kumar RA. 2018. Dormant *Mycobacterium tuberculosis*  
262 converts isoniazid to the active drug in a Wayne's model of dormancy. J Antibiot (Tokyo)  
263 71:939-949.
- 264 19. Gumbo T, Louie A, Liu W, Brown D, Ambrose PG, Bhavnani SM, Drusano GL. 2007.  
265 Isoniazid bactericidal activity and resistance emergence: integrating pharmacodynamics  
266 and pharmacogenomics to predict efficacy in different ethnic populations. Antimicrob  
267 Agents Chemother 51:2329-36.
- 268 20. Ahmad Z, Klinkenberg LG, Pinn ML, Fraig MM, Peloquin CA, Bishai WR, Nuernberger  
269 EL, Grosset JH, Karakousis PC. 2009. Biphasic kill curve of isoniazid reveals the presence  
270 of drug-tolerant, not drug-resistant, *Mycobacterium tuberculosis* in the guinea pig. J Infect  
271 Dis 200:1136-43.

272

273

274 **Figure legends**

275 Fig.1. AN12855 at 100 mg/kg is more effective and exhibits lower emergence of drug resistance  
276 than isoniazid at 25 mg/kg in C3HeB/FeJ mice with advanced lung pathology. Mice were infected  
277 by low dose aerosol with *Mycobacterium tuberculosis* Erdman 68 days prior to the start of  
278 treatment (week 0). Treatments were administered 5 of 7 days per week, Monday-Friday.  
279 AN12855 was formulated in 0.1% [v:v] tween-80, 1% [v:v] methyl cellulose 400 cP. Isoniazid  
280 was formulated in sterile water. Solid lines depict lung CFU burdens. Dashed lines depict drug-  
281 resistant escape mutants. Closed circles, untreated controls. Open triangles, isoniazid-treated.  
282 Closed triangles, AN12855-treated. The horizontal dotted line represents the lower limit of  
283 detection for drug-resistant isolate detection.

284

285 Fig. 2. Penetration and retention of isoniazid and the diazaborine AN12855 into different tissue  
286 compartments by pharmacokinetic analysis. (A) Gross pathology of a representative  
287 *Mycobacterium tuberculosis* Erdman infected C3HeB/FeJ lung lobe showing a Type I caseous  
288 lesion (square). (B) Micrograph of a Type I caseous lesion (hematoxylin and eosin stained). (C)  
289 Structures of AN12855 and isoniazid. (D) Quantitative drug distribution by tissue compartment  
290 over time. Mice were sampled after seven consecutive days of treatment with isoniazid at 25 mg/kg  
291 or AN12855 at 100 mg/kg. Time points were 0.5, 6 and 24 hours following administration for  
292 isoniazid (open triangles) and 2, 6 and 24 hours following administration of AN12855 (closed  
293 triangles). (E) Mean drug levels by compartment with each drug treatment. Inset, shows a  
294 magnification of the regions of interest. BLQ, below limits of quantification (< 1 ng/mL)

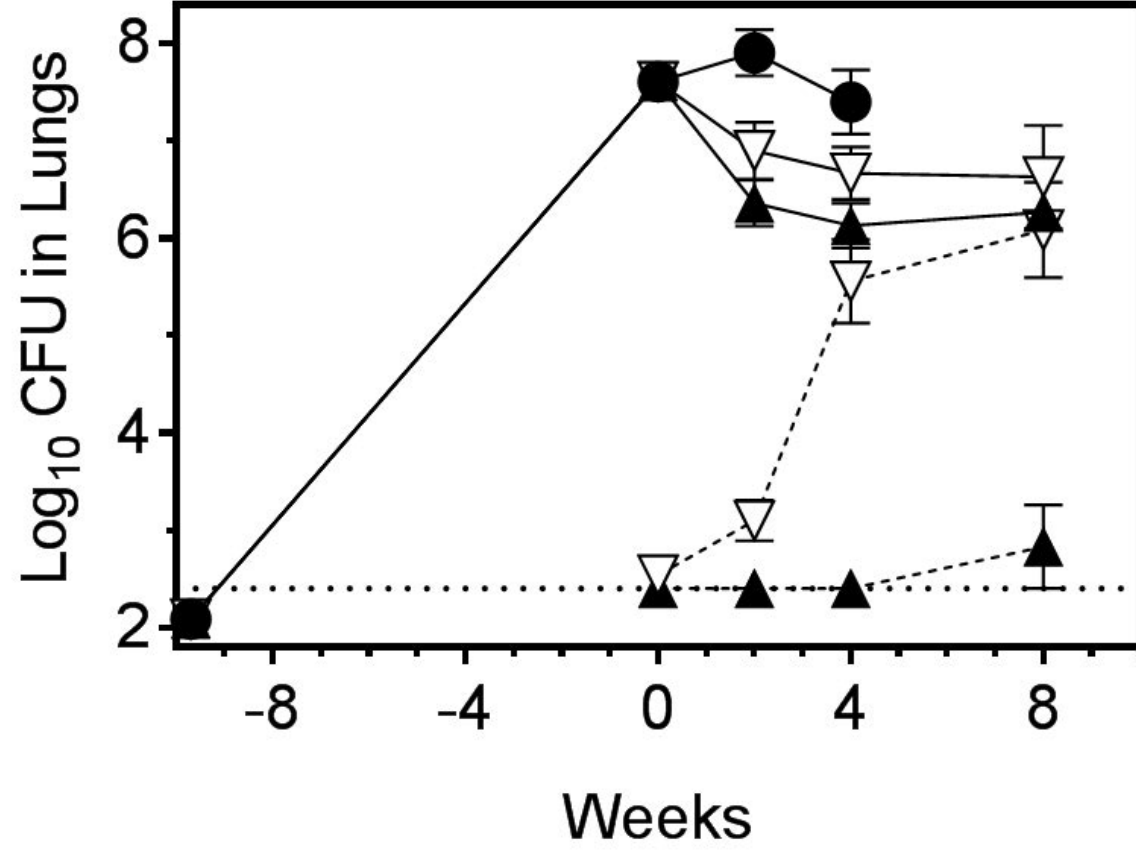


Table 1. Efficacy and drug resistance of AN12855 and isoniazid in the C3HeB/FeJ mouse model.

Group	Start of treatment	Time point sampled		
		2 week	4 week	8 week
Lung Efficacy (mean $\pm$ SEM, n)				
Vehicle Only	7.61 $\pm$ 0.18, 8/8	7.91 $\pm$ 0.24, 7/7	7.40 $\pm$ 0.33, 7/7	na
Isoniazid (25 mg/kg)	-	6.90 $\pm$ 0.29, 8/8	6.67 $\pm$ 0.27, 7/7	6.63 $\pm$ 0.53, 7/7
AN12855 (100 mg/kg)	-	6.36 $\pm$ 0.24, 8/8	6.13 $\pm$ 0.23, 8/8	6.27 $\pm$ 0.11, 8/8
Drug Resistance (mean $\pm$ SEM, (range), n, % resistance)				
Isoniazid	$\leq 2.63 \pm 0.09$ , (<2.40-3.00), 6/8, 0.001%	$\leq 3.10 \pm 0.21$ , (<2.40-3.89), 6/8, 0.016%	5.56 $\pm$ 0.43, (3.24-6.64), 7/7, 8%	6.09 $\pm$ 0.49, (3.77-7.71), 7/7, 29%
AN12855	<2.40 $\pm$ 0.00, (<2.40), 0/8, <0.0006%	<2.40 $\pm$ 0.00, (<2.40), 0/8, <0.011%	<2.40 $\pm$ 0.00, (<2.40), 0/8, <0.019%	$\leq 2.83 \pm 0.43$ , (<2.40-5.44), 1/8, 0.037%

Table 2. MICs of *M. tuberculosis inhA* promoter and coding sequence mutants

Strain	Source	SNPs		liquid MIC in $\mu\text{M}$ (~fold shift versus Erdman)		
		<i>fabG1-inhA</i> promoter (nt)	InhA	Rifampicin	Isoniazid	AN12855
Erdman	Parent	na	na	0.01	0.44	0.16
ED-DPR18-RM4	<i>in vivo</i>	No SNP	G96V	0.007	0.83	> 20 (>122)
ED-DPR18-RM1	<i>in vitro</i>	No SNP	G96A	0.018	0.44	14.2 (87)
ED-DPR19-RM2	<i>in vitro</i>	C-15T	No SNP	0.02	3.2 (7)	1.1 (7)

Abbreviations: nt, nucleotide; na, not applicable; No SNP, no single nucleotide polymorphism



