



HAL
open science

Efficacy of bedaquiline, alone or in combination with imipenem, against *Mycobacterium abscessus* C3HeB/FeJ mice

Vincent Le Moigne, Clément Raynaud, Flavie Moreau, Christian Dupont, Jérôme Nigou, Olivier Neyrolles, Laurent Kremer, Jean-Louis Herrmann

► To cite this version:

Vincent Le Moigne, Clément Raynaud, Flavie Moreau, Christian Dupont, Jérôme Nigou, et al.. Efficacy of bedaquiline, alone or in combination with imipenem, against *Mycobacterium abscessus* C3HeB/FeJ mice. *Antimicrobial Agents and Chemotherapy*, 2020, 64 (6), 10.1128/AAC.00114-20 . hal-03032987

HAL Id: hal-03032987

<https://hal-cnrs.archives-ouvertes.fr/hal-03032987>

Submitted on 1 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Efficacy of bedaquiline, alone or in combination with imipenem,**
2 **against *Mycobacterium abscessus* in C3HeB/FeJ mice**

3
4 Vincent Le Moigne^{1,*}, Clément Raynaud², Flavie Moreau³, Christian Dupont², Jérôme
5 Nigou³, Olivier Neyrolles³, Laurent Kremer^{2,4} and Jean-Louis Herrmann^{1,5}.

6
7 (1) Université Paris-Saclay, UVSQ, INSERM, Infection et Inflammation (U1173), Montigny
8 le Bretonneux, France.

9 (2) Institut de Recherche en Infectiologie de Montpellier, Centre National de la Recherche
10 Scientifique UMR 9004, Université de Montpellier, Montpellier, France

11 (3) Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS,
12 UPS, Toulouse, France.

13 (4) INSERM, IRIM, 34293 Montpellier, France

14 (5) AP-HP, GHU Paris-Saclay, Hôpital Raymond Poincaré, Garches, France.

15
16 (*) Correspondence to: Vincent Le Moigne: vincent.le-moigne@uvsq.fr

19
20
21
22
23
24
25
26
27
28
29
30

ABSTRACT (75 words)

Mycobacterium abscessus lung infections remain difficult to treat. Recent studies have recognized the power of new combinations of antibiotics such as bedaquiline and imipenem although *in vitro* data have questioned this combination. We report that the efficacy of the bedaquiline plus imipenem treatment relies essentially on the activity of bedaquiline in a C3HeB/FeJ mice model of infection with a rough variant of *M. abscessus*. The addition of imipenem contributed at clearing the infection in the spleen.

31
32 **Note (1074 words)**

33 *Mycobacterium abscessus* is a rapidly growing mycobacterial species, whose infections
34 remain very difficult to treat, due to the limited panel of available antibiotics (1). Among
35 them, the β -lactams, imipenem (IPM) and ceftazidime (FOX), are part of the *M. abscessus*
36 multidrug therapy along with amikacin (AMK) and clarithromycin (CLR) (2-5). In addition,
37 the development of specific β -lactamase inhibitors, enhancing the efficacy of IPM *in vitro* and
38 *in vivo*, broadens the use of IPM in *M. abscessus* drug therapy (6-8). Other studies highlighted
39 the potential of testing new drug combinations that include IPM and are associated with
40 increased efficacy against *M. abscessus* (6, 9, 10), yet questioning the relevance of the
41 bedaquiline (BDQ) plus IPM combination (11). BDQ targets the ATP synthase and exhibits
42 activity against a wide panel of *M. abscessus* clinical isolates *in vitro* and in infected
43 zebrafish, although its effect is bacteriostatic only (12). A recent study suggested that, by
44 reducing the intracellular pool of ATP in *M. abscessus*, BDQ suppresses the effect of IPM
45 and FOX, although the effect of the BDQ plus IPM combination was considered additive
46 (11). This led the investigators to conclude that the addition of BDQ to a β -lactam-containing
47 regimen may negatively affect the treatment outcome (11). In comparison, data from the
48 hollow fiber model highlight that β -lactam is the most active and important part of the *M.*
49 *abscessus* regimen (13). That these studies focused exclusively on the interaction of β -lactams
50 and BDQ *in vitro*, confirmatory results in a pre-clinical animal model are warranted.

51 Herein, we explored the therapeutic efficacy of BDQ or IPM, alone or in combination, using
52 the immunocompetent C3HeB/FeJ mouse model of *M. abscessus* infection. C3HeB/FeJ mice
53 are highly susceptible to mycobacterial infections, particularly to *Mycobacterium tuberculosis*
54 due to a deletion on the *Ipr1* (Intracellular pathogen resistance 1) gene located in the locus
55 called *sst1* (14, 15). All animal experiments were performed according to ethical guidelines
56 and with ethical committee (N°047 with agreement A783223) agreement APAFIS#11465.

57 First, we evaluated, the *in vitro* interaction between BDQ and several β -lactams or CLR
58 against *M. abscessus* CIP104536 strain in cation-adjusted Mueller-Hinton broth (CaMHB)
59 (Becton-Dickinson, Le Pont-de-Claix, France) using a 2-dimensional microdilution
60 checkerboard method, as previously described (16-19). Our results confirm that the β -lactam
61 plus BDQ combinations are indifferent, as it is the case with the CLR plus BDQ combination
62 (Table 1).

63 Next, the performances of pulmonary and intravenous (IV) infection routes were compared in
64 C3HeB/FeJ mice. Mice were infected intratracheally using agar bead-embedded bacteria to
65 maintain a persistent infection, as reported previously for *Pseudomonas aeruginosa* (20). A
66 significant increase in mortality was noticed when mice were infected intratracheally with a
67 solution of agar beads containing $2 \cdot 10^5$ CFUs/mouse in 50 μ l, leading only to 40% of mouse
68 survival at 14 days post-infection (dpi) (see Fig. S1A in the supplemental material) correlated
69 with an important increase in the CFU at 14 dpi suggesting accelerated bacterial growth in the
70 lungs (Fig. S1B). In contrast, persistence occurs for up to 25 days after IV infection with 10^6
71 CFU/mouse as evidenced by CFU counting after plating of the organ homogenates (Fig. 1 and
72 Fig. S2A and S2B) although as soon as the injected dose is less than 10^6 CFU, persistence in
73 the organs is reduced (Fig. S2B). This represents an important asset over previously described
74 murine models, characterized by a more rapid bacterial clearance (21-23).

75 The IV route of infection was subsequently used to evaluate and compare the activity of BDQ
76 and AMK. Because AMK is bactericidal against *M. abscessus* while BDQ is bacteriostatic *in*
77 *vitro*, we wondered whether BDQ would be more effective than AMK in an *in vivo* infection
78 model. CFU were significantly reduced in mice receiving 30 mg/kg BDQ (oral
79 administration) as compared to mice treated with 150 mg/kg AMK (subcutaneous
80 administration) in the lungs and the spleen at 12 and 25 dpi (Fig. 2A and 2B). No significant
81 differences were observed between the BDQ- or AMK-treated animals in the spleen at 12 dpi,
82 but bacterial loads in these two groups were significantly lower compared to the control group
83 (oral administration of DMSO) (Fig. 2C).

84 The efficacy of BDQ in this infection model prompted us to compare it with IPM
85 (subcutaneous administration) either alone or as a companion drug, for 15 days of treatment.
86 No significant differences were noticed between the animals treated with BDQ alone and the
87 animals treated with BDQ plus IPM at 12 and 20 dpi, with the exception of the liver at 12 dpi
88 (Fig. 3A to 3C), indicating that the overall activity of the BDQ plus IPM combination was
89 mainly due to the intrinsic activity of BDQ. In general, BDQ alone or in combination with
90 IPM exhibited an increased activity as compared to IPM in the liver and spleen but not in the
91 lungs (Fig. 3). The spleens of treated and untreated mice were weighed as an additional
92 marker of the effectiveness of the various treatments. These measures indicated that only
93 treatments with BDQ plus IPM or IPM alone were associated with lower spleen weights, as
94 compared to those of the untreated or BDQ-treated mice (Fig. 3D). Collectively, the reduced

95 bacterial burden, together with the lower spleen weights represent a marker for improved
96 outcome of the infection.

97 BDQ is a diarylquinoline approved by the Food and Drug Administration and the European
98 Medicines Agency for the treatment of multidrug-resistant tuberculosis. It is bacteriostatic
99 against *M. abscessus in vitro*, displaying MIC₅₀ of 0.125 µg/ml and a MIC₉₀ > 16 µg/ml, and
100 ECOFF values demonstrates that BDQ exhibits moderate activity (16, 24). Discordant results
101 regarding the efficacy of BDQ were generated in various immunocompromised mouse
102 models, raising the question of the influence of immunosuppression on antibiotic efficacy (25,
103 26). However, efficient responses to BDQ were observed in other animal models, such as
104 zebrafish (12). Two studies reported poor or negative results for BDQ administration against
105 NTM infected patients (27, 28). However, recent studies showed that the activity of BDQ can
106 be potentiated with adjunctive therapy, by so improving BDQ-based treatments (16, 29). This
107 study provides evidence that the BDQ plus IPM combination remains superior to IPM alone
108 and equivalent to BDQ alone as judged by the comparable bacterial clearance in the spleens
109 of the mice treated with BDQ plus IPM as compared to BDQ alone.

110

111 In summary, the IPM plus BDQ combination enhances the clearance of the infection. This
112 supports also the importance of evaluating antibiotic activity in combination rather than
113 separately against this highly drug-resistant mycobacterium.

114

115

116
117
118

ACKNOWLEDGMENTS

119 Bedaquiline was a kind gift from Dr C. Happel (NIH AIDS Reagent Program, USA) and from
120 Dr N. Lounis (from Janssen Pharmaceuticals, Beerse, Belgium). We acknowledged Pr N.
121 Véziris and Pr J. van Ingen for helpful comments and critical reading of the manuscript. We
122 thank the members of the Genotoul core facility ANEXPLO (IPBS, Toulouse) for animal
123 experiments. This study was supported by INSERM, University of Versailles Saint Quentin
124 en Yvelines, by the Association Gregory Lemarchal and Vaincre la Mucoviscidose
125 (RIF20180502320) to LK and JLH, the Agence Nationale de la Recherche (MyCat ANR-15-
126 CE18-0007-02) to LK, by CNRS, University of Toulouse, Agence Nationale de la Recherche
127 /Programme d'Investissements d'Avenir (ANR-11-EQUIPEX-0003), and the Bettencourt
128 Schueller Foundation. The funders had no role in study design, data collection and analysis,
129 decision to publish, or preparation of the manuscript.

130

131 **AUTHOR CONTRIBUTIONS STATEMENT**

132

133 JLH, JN and ON designed the project and experiments; VLM, CR, FM and CD performed the

134 experiments; VLM, CR, JN, ON, LK and JLH wrote and corrected the manuscript.

135

136
137

138 **REFERENCES**

- 139 1. Kwak N, Dalcolmo MP, Daley CL, Eather G, Gayoso R, Hasegawa N, Jhun BW, Koh
140 WJ, Namkoong H, Park J, Thomson R, van Ingen J, Zweijpfenning SMH, Yim JJ.
141 2019. *Mycobacterium abscessus* pulmonary disease: individual patient data meta-
142 analysis. *Eur Respir J* 54:pii: 1801991.
- 143 2. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland
144 SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn
145 CF, Wallace RJ Jr, Winthrop K; ATS Mycobacterial Diseases Subcommittee;
146 American Thoracic Society; Infectious Disease Society of America. 2007. An official
147 ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous
148 mycobacterial diseases. *Am J Respir Crit Care Med* 175:367-416.
- 149 3. Haworth CS, Banks J, Capstick T, Fisher AJ, Gorsuch T, Laurenson IF, Leitch A,
150 Loebinger MR, Milburn HJ, Nightingale M, Ormerod P, Shingadia D, Smith D,
151 Whitehead N, Wilson R, Floto RA. 2017. British Thoracic Society guidelines for the
152 management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). *Thorax*
153 72:ii1-ii64.
- 154 4. Floto RA, Olivier KN, Saiman L, Daley CL, Herrmann JL, Nick JA, Noone PG,
155 Bilton D, Corris P, Gibson RL, Hempstead SE, Koetz K, Sabadosa KA, Sermet-
156 Gaudelus I, Smyth AR, van Ingen J, Wallace RJ, Winthrop KL, Marshall BC,
157 Haworth CS. 2016. US Cystic Fibrosis Foundation and European Cystic Fibrosis
158 Society consensus recommendations for the management of non-tuberculous
159 mycobacteria in individuals with cystic fibrosis: executive summary. *Thorax* 71:88-
160 90.
- 161 5. Lefebvre AL, Le Moigne V, Bernut A, Veckerlé C, Compain F, Herrmann JL, Kremer
162 L, Arthur M, Mainardi JL. 2017. Inhibition of the β -Lactamase Bla_{Mab} by Avibactam
163 Improves the *In Vitro* and *In Vivo* Efficacy of Imipenem against *Mycobacterium*
164 *abscessus*. *Antimicrob Agents Chemother* 61:e02440-16.
- 165 6. Kaushik A, Ammerman NC, Lee J, Martins O, Kreiswirth BN, Lamichhane G, Parrish
166 NM, Nuermberger EL. 2019. *In Vitro* Activity of the New β -Lactamase Inhibitors
167 Relebactam and Vaborbactam in Combination with β -Lactams against *Mycobacterium*
168 *abscessus* Complex Clinical Isolates. *Antimicrob Agents Chemother* 63:e02623-18.

- 169 7. Dubée V, Bernut A, Cortes M, Lesne T, Dorchene D, Lefebvre AL, Hugonnet JE,
170 Gutmann L, Mainardi JL, Herrmann JL, Gaillard JL, Kremer L, Arthur M. 2015. β -
171 Lactamase inhibition by avibactam in *Mycobacterium abscessus*. J Antimicrob
172 Chemother 70:1051-1058.
- 173 8. Dubée V, Soroka D, Cortes M, Lefebvre AL, Gutmann L, Hugonnet JE, Arthur M,
174 Mainardi JL. 2015. Impact of β -lactamase inhibition on the activity of ceftaroline
175 against *Mycobacterium tuberculosis* and *Mycobacterium abscessus*. Antimicrob
176 Agents Chemother 59:2938-2941.
- 177 9. Le Run E, Arthur M, Mainardi JL. 2019. *In Vitro* and Intracellular Activity of
178 Imipenem Combined with Tedizolid, Rifabutin, and Avibactam against
179 *Mycobacterium abscessus*. Antimicrob Agents Chemother 63:e01915-18.
- 180 10. Le Run E, Arthur M, Mainardi JL. 2018. *In Vitro* and Intracellular Activity of
181 Imipenem Combined with Rifabutin and Avibactam against *Mycobacterium*
182 *abscessus*. Antimicrob Agents Chemother 62:e00623-18.
- 183 11. Lindman M, Dick T. 2019. Bedaquiline eliminates bactericidal activity of β -lactams
184 against *Mycobacterium abscessus*. Antimicrob Agents Chemother 63:e00827-19.
- 185 12. Dupont C, Viljoen A, Thomas S, Roquet-Banères F, Herrmann JL, Pethe K, Kremer
186 L. 2017. Bedaquiline inhibits the ATP synthase in *Mycobacterium abscessus* and is
187 effective in infected zebrafish. Antimicrob Agents Chemother 61:e01225-17.
- 188 13. Ferro BE, Srivastava S, Deshpande D, Pasipanodya JG, van Soolingen D, Mouton
189 JW, van Ingen J, Gumbo T. 2016. Failure of the Amikacin, Cefoxitin, and
190 Clarithromycin Combination Regimen for Treating Pulmonary *Mycobacterium*
191 *abscessus* Infection. Antimicrob Agents Chemother 60:6374-6376.
- 192 14. Kramnik I, Dietrich WF, Demant P, Bloom BR. 2000. Genetic control of resistance to
193 experimental infection with virulent *Mycobacterium tuberculosis*. Proc Natl Acad Sci
194 U S A 97:8560-8565.
- 195 15. Pan H, Yan BS, Rojas M, Shebzukhov YV, Zhou H, Kobzik L, Higgins DE, Daly MJ,
196 Bloom BR, Kramnik I. 2005. Ipr1 gene mediates innate immunity to tuberculosis.
197 Nature 434:767-772.
- 198 16. Ruth MM, Sangen JJN, Remmers K, Pennings LJ, Svensson E, Aarnoutse RE,
199 Zweijpfenning SMH, Hoefsloot W, Kuipers S, Magis-Escurra C, Wertheim HFL, van
200 Ingen J. 2019. A bedaquiline/clofazimine combination regimen might add activity to
201 the treatment of clinically relevant non-tuberculous mycobacteria. J Antimicrob
202 Chemother. 74:935-943.

- 203 17. Odds FC. 2003. Synergy, antagonism, and what the chequerboard puts between them.
204 J Antimicrob Chemother. 52:1.
- 205 18. Li W, Sanchez-Hidalgo A, Jones V, de Moura VC, North EJ, Jackson M. 2017.
206 Synergistic Interactions of MmpL3 Inhibitors with Antitubercular Compounds *In*
207 *Vitro*. Antimicrob Agents Chemother. 61:e02399-16.
- 208 19. Clinical and Laboratory Standards Institute (CLSI). 2018. Susceptibility Testing of
209 Mycobacteria, *Nocardiae* spp., and Other Aerobic Actinomycetes. 3rd ed. CLSI
210 standard M24. Wayne, PA.
- 211 20. Cigana C, Lorè NI, Riva C, De Fino I, Spagnuolo L, Sipione B, Rossi G, Nonis A,
212 Cabrini G, Bragonzi A. 2016. Tracking the immunopathological response to
213 *Pseudomonas aeruginosa* during respiratory infections. Sci Rep 6:21465.
- 214 21. Bernut A, Le Moigne V, Lesne T, Lutfalla G, Herrmann JL, Kremer L. 2014. *In vivo*
215 assessment of drug efficacy against *Mycobacterium abscessus* using the embryonic
216 zebrafish test system. Antimicrob Agents Chemother 58:4054-4063.
- 217 22. Le Moigne V, Rottman M, Goulard C, Barteau B, Poncin I, Soismier N, Canaan S,
218 Pitard B, Gaillard JL, Herrmann JL. 2015. Bacterial phospholipases C as vaccine
219 candidate antigens against cystic fibrosis respiratory pathogens: the *Mycobacterium*
220 *abscessus* model. Vaccine 33:2118-2124.
- 221 23. Ordway D, Henao-Tamayo M, Smith E, Shanley C, Harton M, Troudt J, Bai X,
222 Basaraba RJ, Orme IM, Chan ED. 2008. Animal model of *Mycobacterium abscessus*
223 lung infection. J Leukoc Biol 83:1502-1511.
- 224 24. Pang Y, Zheng H, Tan Y, Song Y, Zhao Y. 2017. *In Vitro* Activity of Bedaquiline
225 against Nontuberculous Mycobacteria in China. Antimicrob Agents Chemother 61:
226 e02627-16.
- 227 25. Lerat I, Cambau E, Roth Dit Bettoni R, Gaillard JL, Jarlier V, Truffot C, Veziris N.
228 2014. *In vivo* evaluation of antibiotic activity against *Mycobacterium abscessus*. J
229 Infect Dis 209:905-912.
- 230 26. Obregón-Henao A, Arnett KA, Henao-Tamayo M, Massoudi L, Creissen E, Andries
231 K, Lenaerts AJ, Ordway DJ. 2015. Susceptibility of *Mycobacterium abscessus* to
232 antimycobacterial drugs in preclinical models. Antimicrob Agents Chemother
233 59:6904-6912.
- 234 27. Philley JV, Wallace RJ Jr, Benwill JL, Taskar V, Brown-Elliott BA, Thakkar F,
235 Aksamit TR, Griffith DE. 2015. Preliminary Results of Bedaquiline as Salvage

- 236 Therapy for Patients With Nontuberculous Mycobacterial Lung Disease. Chest
237 148:499-506.
- 238 **28.** Zweijpfenning SMH, Schildkraut JA, Coolen JPM, Ruesen C, Koenraad E, Janssen A,
239 Ruth MM, de Jong AS, Kuipers S, Aarnoutse RE, Magis-Escurra C, Hoefsloot W, van
240 Ingen J. 2019. Failure with acquired resistance of an optimised bedaquiline-based
241 treatment regimen for pulmonary *Mycobacterium avium* complex disease. Eur Respir
242 J. 54:1900118.
- 243 **29.** Viljoen A, Raynaud C, Johansen MD, Roquet-Banères F, Herrmann JL, Daher W,
244 Kremer L. 2019. Verapamil Improves the Activity of Bedaquiline against
245 *Mycobacterium abscessus In Vitro* and in Macrophages. Antimicrob Agents
246 Chemother 63: e00705-719.
- 247 **30.** Lenaerts AJ, Hoff D, Aly S, Ehlers S, Andries K, Cantarero L, Orme IM, Basaraba RJ.
248 2007. Location of persisting mycobacteria in a Guinea pig model of tuberculosis
249 revealed by r207910. Antimicrob Agents Chemother 51:3338-3345.
- 250
- 251

252

253

254 **Table 1.** Interaction between bedaquiline and other drugs against *M. abscessus* CIP104536^T

Compound	MIC* (mg/l)	Interaction with BDQ		
		FICI [§] (mean)	SD	Outcome
BDQ	0.125	-	-	-
IPM	16 ^{&}	0.55	±0.06	Indifferent [£]
FOX	32	0.52	±0.03	Indifferent
CLR	2	0.61	±0.09	Indifferent
AMP	>512	0.57	±0.02	Indifferent

255 (*) MIC were evaluated by REMA checkerboard assay in cation-adjusted Mueller-Hinton
 256 broth (CaMHB) (Becton-Dickinson, Le Pont-de-Claix, France). 10⁵ bacteria were diluted in
 257 Mueller-Hinton media (Sigma-Aldrich). Plates were incubated for 3 days at 30°C then 20 µL
 258 (10% v/f) of Resazurin 0.025% were added to the wells and plates were incubated overnight
 259 at 30°C.

260 (\$) The fractional inhibitory concentration index (FICI) was calculated as follows: FICI =
 261 (MIC drug A in combination/MIC drug A alone) + (MIC drug B in combination/MIC drug B
 262 alone), where drug A was bedaquiline (BDQ) and drug B was clarithromycin (CLR, Sigma-
 263 Aldrich, France), imipenem (IPM, Mylan S.A.S, France), ceftazidime (FOX, Panpharma,
 264 France) or ampicillin (AMP, Euromedex, France).

265 (£) Interaction between the two compounds was defined as synergistic when FICI value was
 266 ≤0.5, indifferent when 0.5 < FICI ≤4, and antagonistic when FICI was >4.

267 (&) Values showed in the table are the mean of four independent experiments ±SD.

268

269

270

271 **Legend to figures**

272 **Figure 1.** Bacterial persistence of *M. abscessus* CIP 104536^T (rough variant) in the lungs,
273 spleen and liver of C3HeB/FeJ mice after infection in the tail vein with 10⁶ CFU/mouse in a
274 total volume of 200 µl of water containing 0.9% sodium chloride. The following day, three
275 mice were euthanized and whole organs were harvested to determine baseline bacterial
276 burden. Mouse lungs, spleens and livers were homogenized, serially diluted and plated onto
277 VCAT (Vancomycin, Colistin sulfate, Amphotericin B, and Trimethoprim) chocolate agar
278 plates (BioMérieux, France) and incubated for 5-6 days at 37°C prior to CFU count. Results
279 are expressed as the log₁₀ units of CFU at 1, 12 and 25 dpi.

280

281

282

283 **Figure 2.** *M. abscessus* R-infected C3HeB/FeJ mice (9.2×10⁵ CFU/mouse) treated with
284 Bedaquiline (BDQ) or Amikacin (AMK). Bacterial counts in the lungs (A), liver (B) and
285 spleen (C) of C3HeB/FeJ mice infected IV, as described in Fig. 1. Antibiotic treatment began
286 at 2 dpi. Mice were treated starting on day 2 for 7 days (D12) or 17 days (D25) by daily
287 subcutaneous injections of 150 mg/kg AMK (Mylan laboratories) in saline solution or daily
288 oral gavage of 30 mg/kg BDQ at in a total volume of 200 µl (BDQ solution in DMSO was
289 diluted in 20 % 2-hydroxypropyl-β-cyclodextrin). A control group received a daily
290 subcutaneous injection of saline and oral gavage of DMSO containing 20 % 2-hydroxypropyl-
291 β-cyclodextrin. All solutions were administered five times weekly for latter time point. Mice
292 were euthanized 3 days after antibiotic cessation to allow antibiotic clearance. Furthermore,
293 given the long half-life and high protein binding capacity of BDQ, spleens, livers and lungs
294 from drug-treated and control mice were homogenized in water supplemented with 10%
295 bovine serum albumin (30) before dilution. Experimental groups of mice were evaluated for
296 bacterial burden on day 1 (before treatment started), 12 and 25 as described in Fig. 1. n = 5
297 mice were used per experiment and bacterial load in each group are expressed as log₁₀ units of
298 CFU (± SD) cells. Differences between means were analyzed by two-way ANOVA and the
299 Tukey post-test, allowing multiple comparisons. n.s. = non-significant, * *P*<0.05, ** *P*<0.01,
300 *** *P*<0.001, **** *P*<0.0001. Experiment was realized once.

301

302

303

304 **Figure 3.** *M. abscessus* R-infected C3HeB/FeJ mice treated (2.7×10⁵ CFU/mouse) by
305 Bedaquiline (BDQ), Imipenem (IPM) or BDQ plus IPM. Bacterial loads in the lungs (A),
306 liver (B) and spleen (C) were determined as reported in Fig. 1. Relative weight of spleen to
307 mouse weight are shown in (D). Antibiotic treatment began 2 days after infection. Mice were
308 treated starting on day 2 for 7 days (D12) or 13 days (D20) with twice daily (*i.e.* every 12 h)
309 subcutaneous injection of IPM (MSD laboratories, France) in saline solution at 100 mg/kg or
310 daily oral gavage of BDQ as described in Fig. 2 or both IPM plus BDQ. Experimental groups
311 of mice were evaluated for bacterial burden on day 1 (before treatment started), 12 and 20 as
312 described in Fig. 1. (D) Mouse spleens were weighed at 1, 12 and 20 dpi. The value
313 represents the relative weight of each spleen relative to the weight of the mouse from which
314 they were collected. n = 5 mice were used per experiment and bacterial load in each group are
315 expressed as log₁₀ units of CFU (± SD) cells. Differences between means were analyzed by
316 two-way ANOVA and the Tukey post-test, allowing multiple comparisons. n.s. = non-

317 significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Experiment was realized
318 once.
319

Figure 1

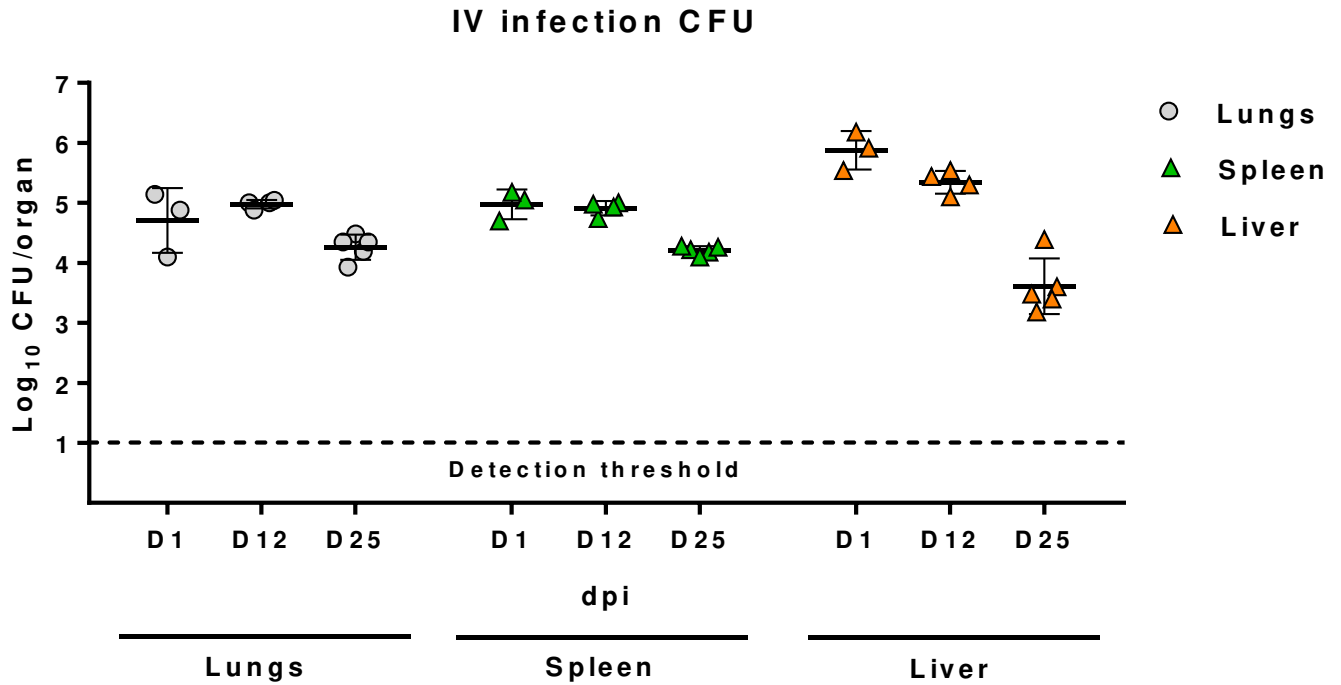
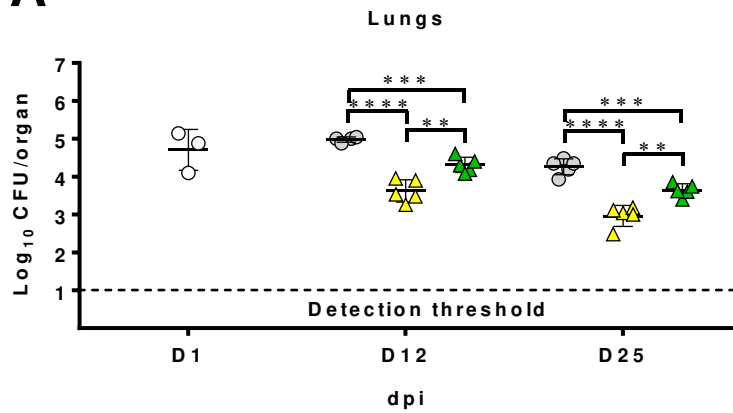
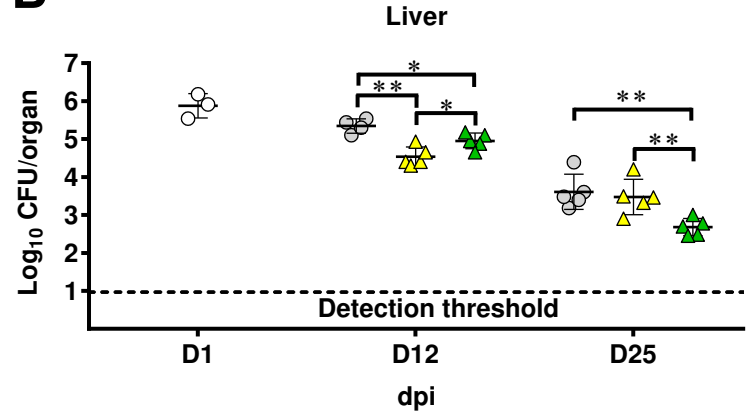
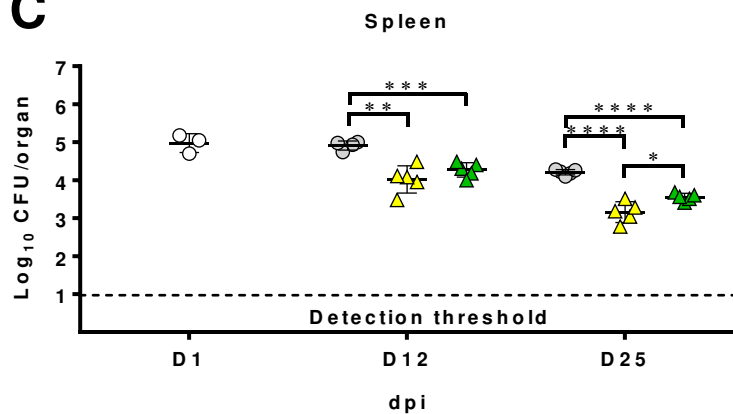


Figure 1. Bacterial persistence of *M. abscessus* CIP 104536^T (rough variant) in the lungs, spleen and liver of C3HeB/FeJ mice after infection in the tail vein with 10⁶ CFU/mouse in a total volume of 200 μ l of water containing 0.9% sodium chloride. The following day, three mice were euthanized and whole organs were harvested to determine baseline bacterial burden. Mouse lungs, spleens and livers were homogenized, serially diluted and plated onto VCAT (Vancomycin, Colistin sulfate, Amphotericin B, and Trimethoprim) chocolate agar plates (BioMérieux, France) and incubated for 5-6 days at 37°C prior to CFU count. Results are expressed as the log₁₀ units of CFU at 1, 12 and 25 dpi.

Figure 2

A**B****C**

- control DMSO (p.o.)
- ▲ Bedaquiline 30 mg/kg (p.o.)
- ▲ Amikacin 150 mg/kg (s.c.)

Figure 2. *M. abscessus* R-infected C3HeB/FeJ mice (9.2×10^5 CFU/mouse) treated with Bedaquiline (BDQ) or Amikacin (AMK). Bacterial counts in the lungs (A), liver (B) and spleen (C) of C3HeB/FeJ mice infected IV, as described in Fig. 1. Antibiotic treatment began at 2 dpi. Mice were treated starting on day 2 for 7 days (D12) or 17 days (D25) by daily subcutaneous injections of 150 mg/kg AMK (Mylan laboratories) in saline solution or daily oral gavage of 30 mg/kg BDQ at in a total volume of 200 μ l (BDQ solution in DMSO was diluted in 20 % 2-hydroxypropyl- β -cyclodextrin). A control group received a daily subcutaneous injection of saline and oral gavage of DMSO containing 20 % 2-hydroxypropyl- β -cyclodextrin. All solutions were administered five times weekly for latter time point. Mice were euthanized 3 days after antibiotic cessation to allow antibiotic clearance. Furthermore, given the long half-life and high protein binding capacity of BDQ, spleens, livers and lungs from drug-treated and control mice were homogenized in water supplemented with 10% bovine serum albumin (30) before dilution. Experimental groups of mice were evaluated for bacterial burden on day 1 (before treatment started), 12 and 25 as described in Fig. 1. n = 5 mice were used per experiment and bacterial load in each group are expressed as log₁₀ units of CFU (\pm SD) cells. Differences between means were analyzed by two-way ANOVA and the Tukey post-test, allowing multiple comparisons. n.s. = non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Experiment was realized once.

Figure 3

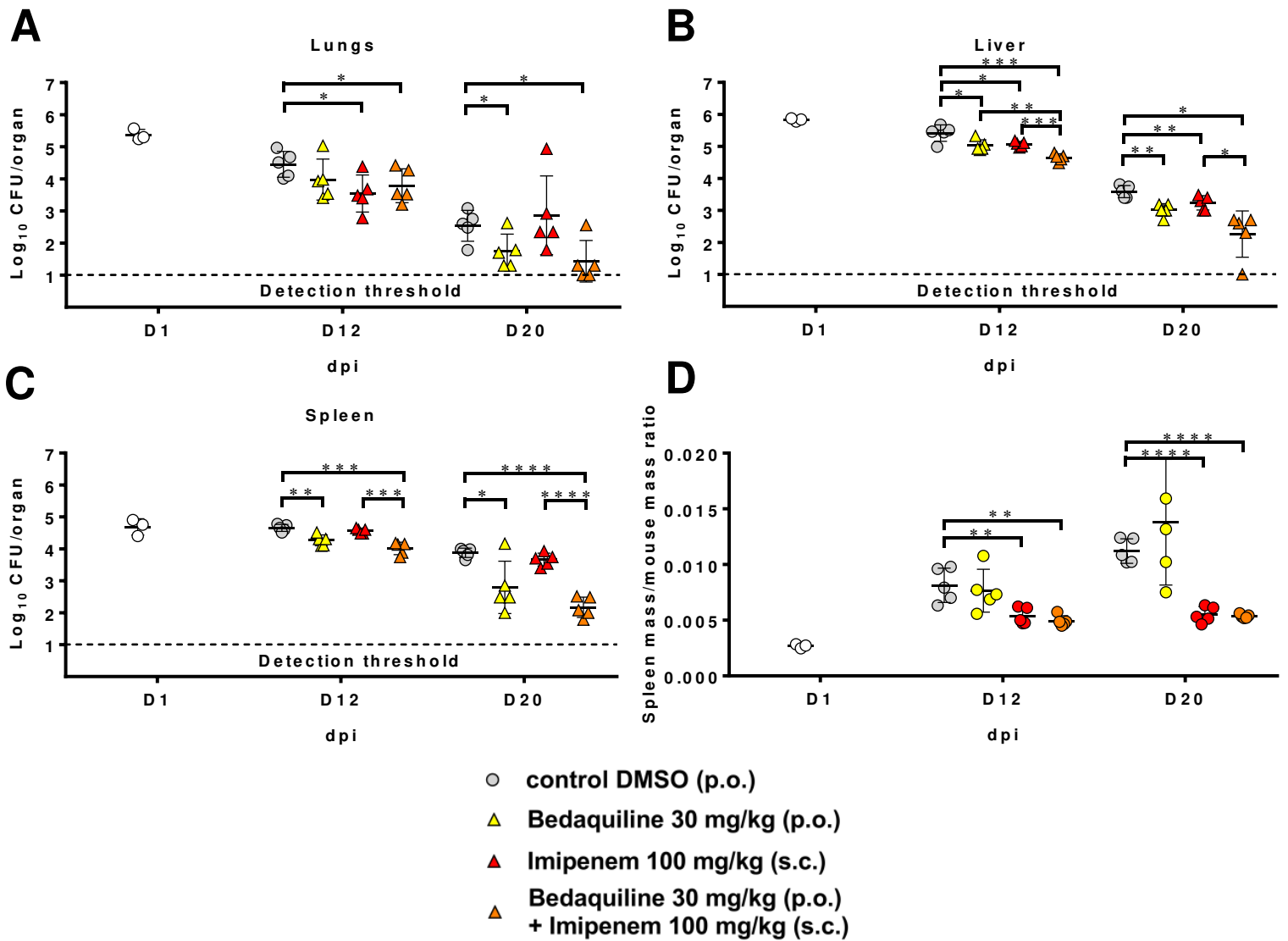


Figure 3. *M. abscessus* R-infected C3HeB/FeJ mice (2.7×10^5 CFU/mouse) treated by Bedaquiline (BDQ), Imipenem (IMP) or BDQ plus IMP. Bacterial loads in the lungs (A), liver (B) and spleen (C) were determined as reported in Fig. 1. Relative weight of spleen to mouse weight are shown in (D). Antibiotic treatment began 2 days after infection. Mice were treated starting on day 2 for 7 days (D12) or 13 days (D20) with twice daily (*i.e.* every 12 h) subcutaneous injection of IMP (MSD laboratories, France) in saline solution at 100 mg/kg or daily oral gavage of BDQ as described in Fig. 2 or both IMP+BDQ. Experimental groups of mice were evaluated for bacterial burden on day 1 (before treatment started), 12 and 20 as described in Fig. 1. (D) Mouse spleens were weighed at 1, 12 and 20 dpi. The value represents the relative weight of each spleen relative to the weight of the mouse from which they were collected. $n = 5$ mice were used per experiment and bacterial load in each group are expressed as \log_{10} units of CFU (\pm SD) cells. Differences between means were analyzed by two-way ANOVA and the Tukey post-test, allowing multiple comparisons. n.s. = non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Experiment was realized once.

Supplementary Figure 1

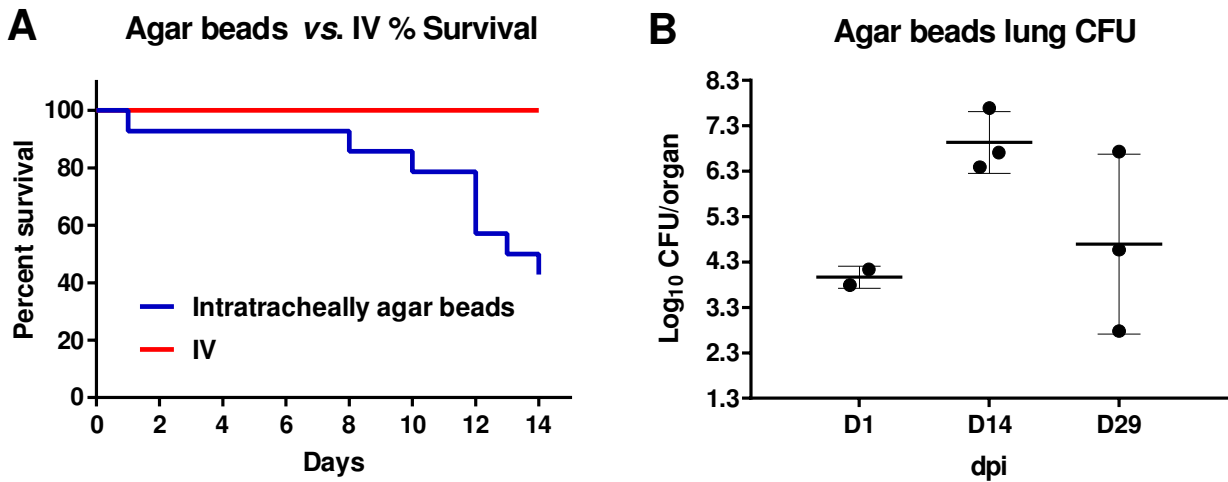
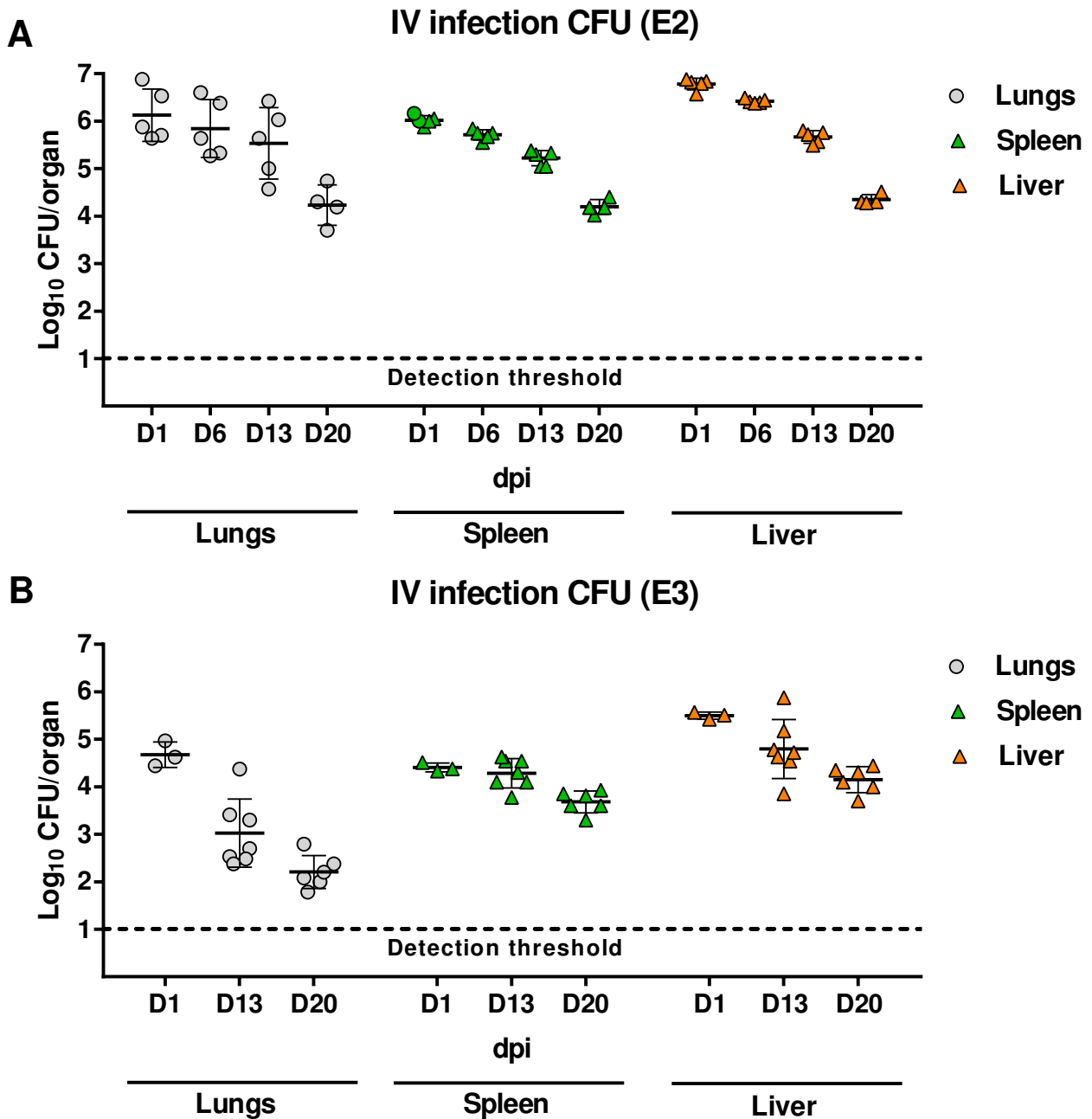


Figure 1. (A) Survival of C3HeB/FeJ mice infected intratracheally or intravenously (IV) with *M. abscessus* CIP 104536^T (smooth variant). (B) Persistence of *M. abscessus* in the lungs of intratracheally-infected C3HeB/FeJ mice. Agar beads were prepared as described previously (1). Mice were infected with a solution of agar beads containing 2.10^5 CFUs/mouse in 50 μ l. Survival curves were generated over a 14 days post-infection experiment. Mouse lungs were collected and homogenized, serially diluted and plated onto VCAT (Vancomycin, Colistin sulfate, Amphotericin B, and Trimethoprim) chocolate agar plates (BioMérieux, France) and incubated for 5-6 days at 37°C prior to CFU count. Results are expressed as log₁₀ units of CFU at 1, 14 and 29 dpi. Results are representative of one of two independent experiments (A and B) with similar results.

Supplementary Figure 2



Supplementary Figure 2. Bacterial persistence of *M. abscessus* CIP 104536^T (rough variant) in the lungs, spleen and liver of C3HeB/FeJ mice after infection in the tail vein with 4.8×10^6 (A) and 3.1×10^5 CFU/mouse (B) in a total volume of 200 μ l of water containing 0.9% sodium chloride. The following day, three mice were euthanized and whole organs were harvested to determine baseline bacterial burden. CFU were determined as described in Fig.S1. Results are expressed as the log₁₀ units of CFU at 1, (6), 13 and 20 dpi.

REFERENCES

1. Cigana C, Lorè NI, Riva C, De Fino I, Spagnuolo L, Sipione B, Rossi G, Nonis A, Cabrini G, Bragonzi A. 2016. Tracking the immunopathological response to *Pseudomonas aeruginosa* during respiratory infections. *Sci Rep* 6:21465.