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

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1   **Efficacy of bedaquiline, alone or in combination with imipenem,**

2   **against *Mycobacterium abscessus* in C3HeB/FeJ mice**

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20 **ABSTRACT (75 words)**

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

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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 cefoxitin (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 2.10^5 CFUs/mouse in 50 μl, 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 MIC_{50} of 0.125 µg/ml and a $MIC_{90} > 16$ 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.

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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.

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AUTHOR CONTRIBUTIONS STATEMENT

JLH, JN and ON designed the project and experiments; VLM, CR, FM and CD performed the experiments; VLM, CR, JN, ON, LK and JLH wrote and corrected the manuscript.

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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

(*) MIC were evaluated by REMA checkerboard assay in cation-adjusted Mueller-Hinton broth (CaMHB) (Becton-Dickinson, Le Pont-de-Claix, France). 10^5 bacteria were diluted in Mueller-Hinton media (Sigma-Aldrich). Plates were incubated for 3 days at 30°C then 20 µL (10% v/f) of Resazurin 0.025% were added to the wells and plates were incubated overnight at 30°C.

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

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

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

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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 10^6 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 $\log_{10}$ units of CFU at 1, 12 and 25 dpi.

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283 **Figure 2.** *M. abscessus* R-infected C3HeB/FeJ mice (9.2×10^5 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 $\log_{10}$ units of
298 CFU ($\pm$ 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.

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304 **Figure 3.** *M. abscessus* R-infected C3HeB/FeJ mice treated (2.7×10^5 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 $\log_{10}$ units of CFU ($\pm$ 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
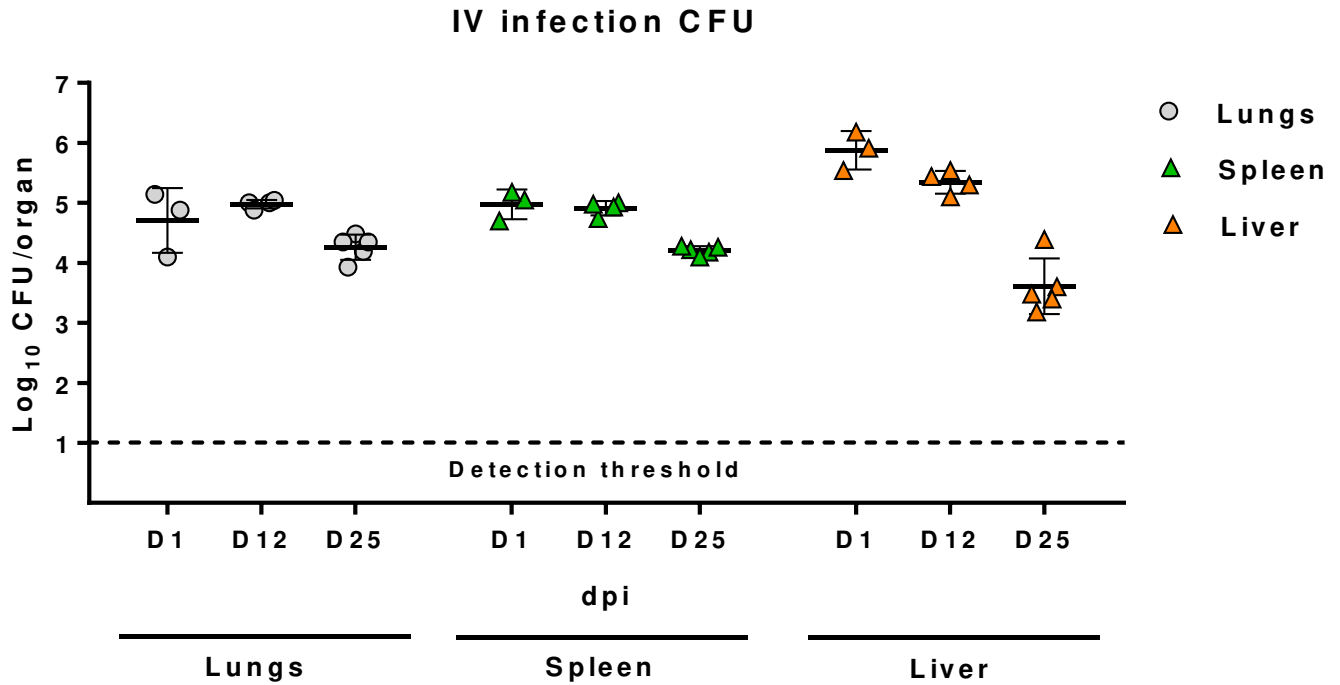318 once.
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Figure 1

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 10^6 CFU/mouse in a total volume of 200 µl 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 $\log_{10}$ units of CFU at 1, 12 and 25 dpi.
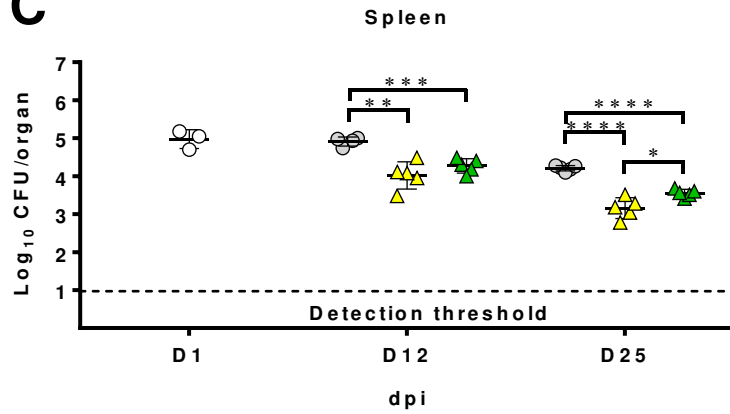
Figure 2
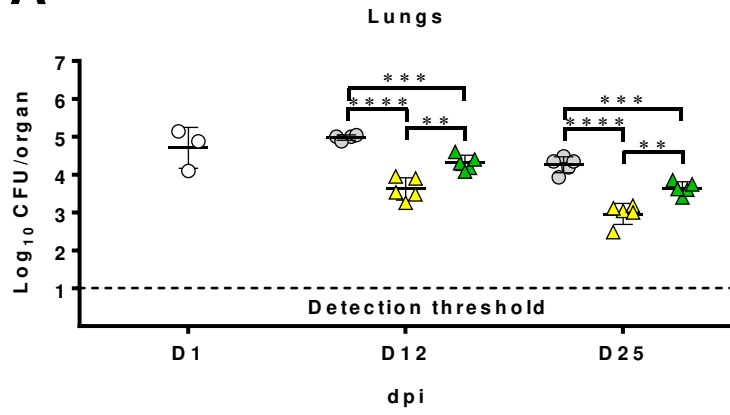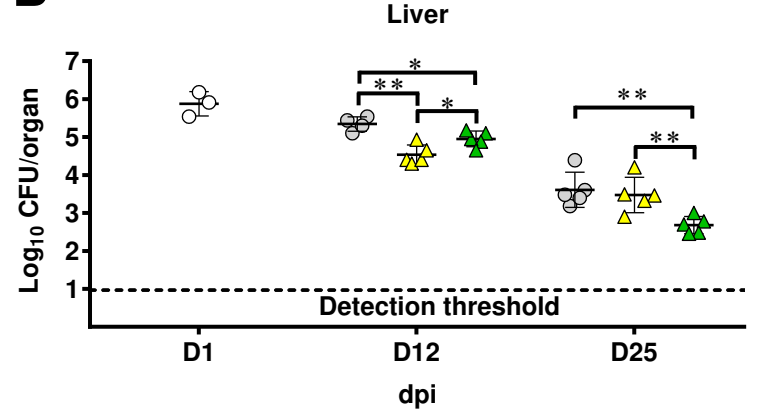
Figure 2. *M. abscessus* R-infected C3HeB/FeJ mice (9.2×10^5 CFU/mouse) treated with Bedaquiline (BDQ) or Amikacin (AMK). Bacterial counts in the lungs (A), liver (B) and spleen (C) of C3HeB/FeJ mice infected IV, as described in Fig. 1. Antibiotic treatment began at 2 dpi. Mice were treated starting 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 µl (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 $\log_{10}$ units of CFU (± 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.
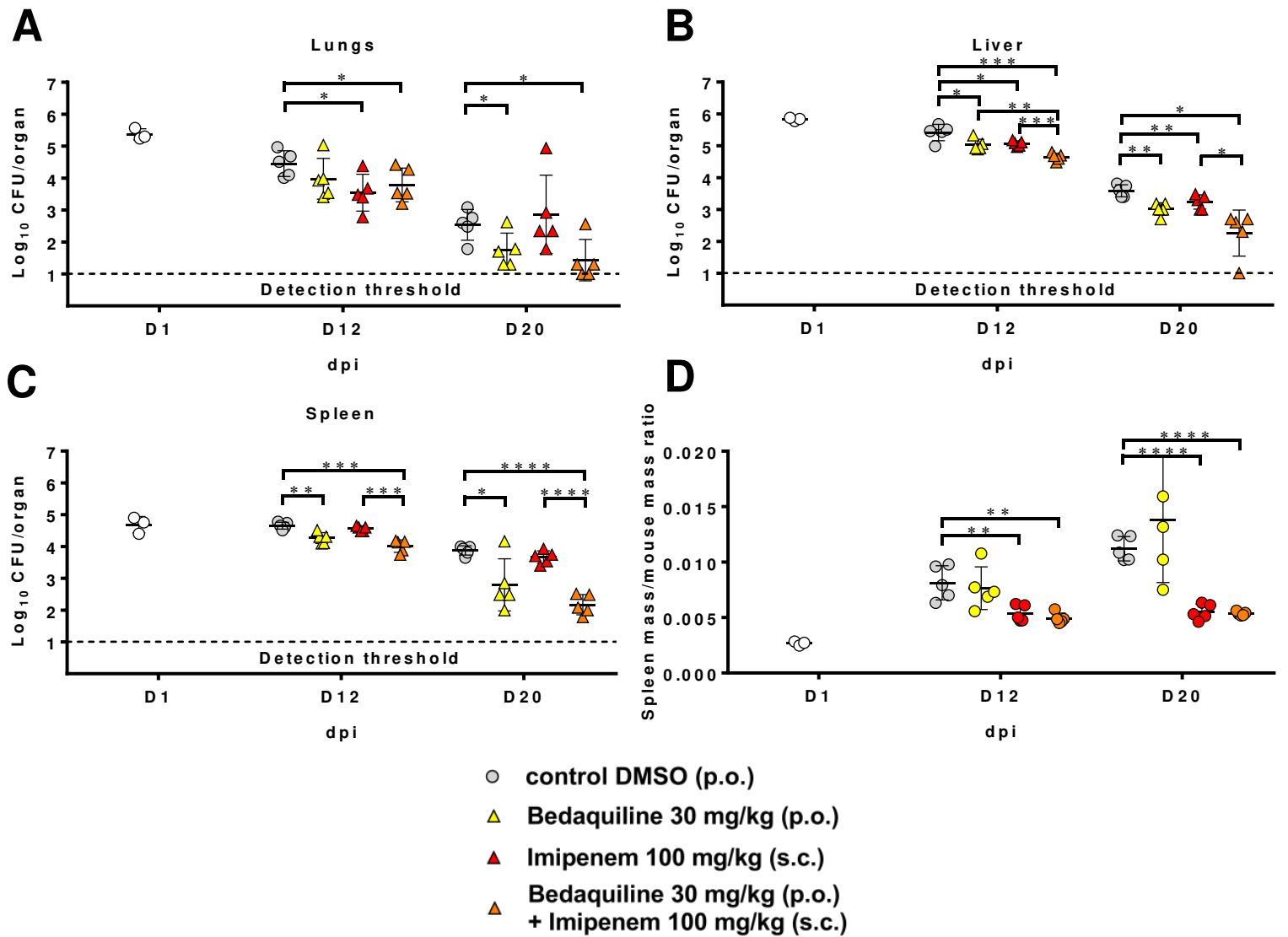
Figure 3

Figure 3. *M. abscessus* R-infected C3HeB/FeJ mice (2.7×10^5 CFU/mouse) treated by Bedaquiline (BDQ), Imipenem (IMP) or BDQ plus IPM. 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 IPM (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

A Agar beads *vs.* IV % Survival
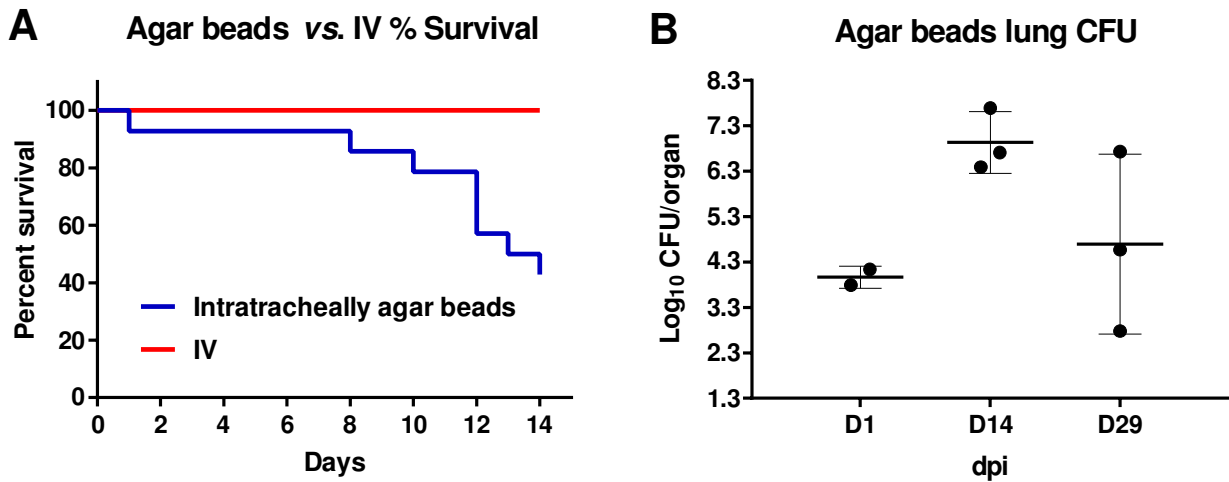
B Agar beads lung CFU

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 where prepared as described previously (1). Mice were infected with a solution of agar beads containing 2.10^5 CFUs/mouse in 50 µl. 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 $\log_{10}$ 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

A

IV infection CFU (E2)

B

IV infection CFU (E3)
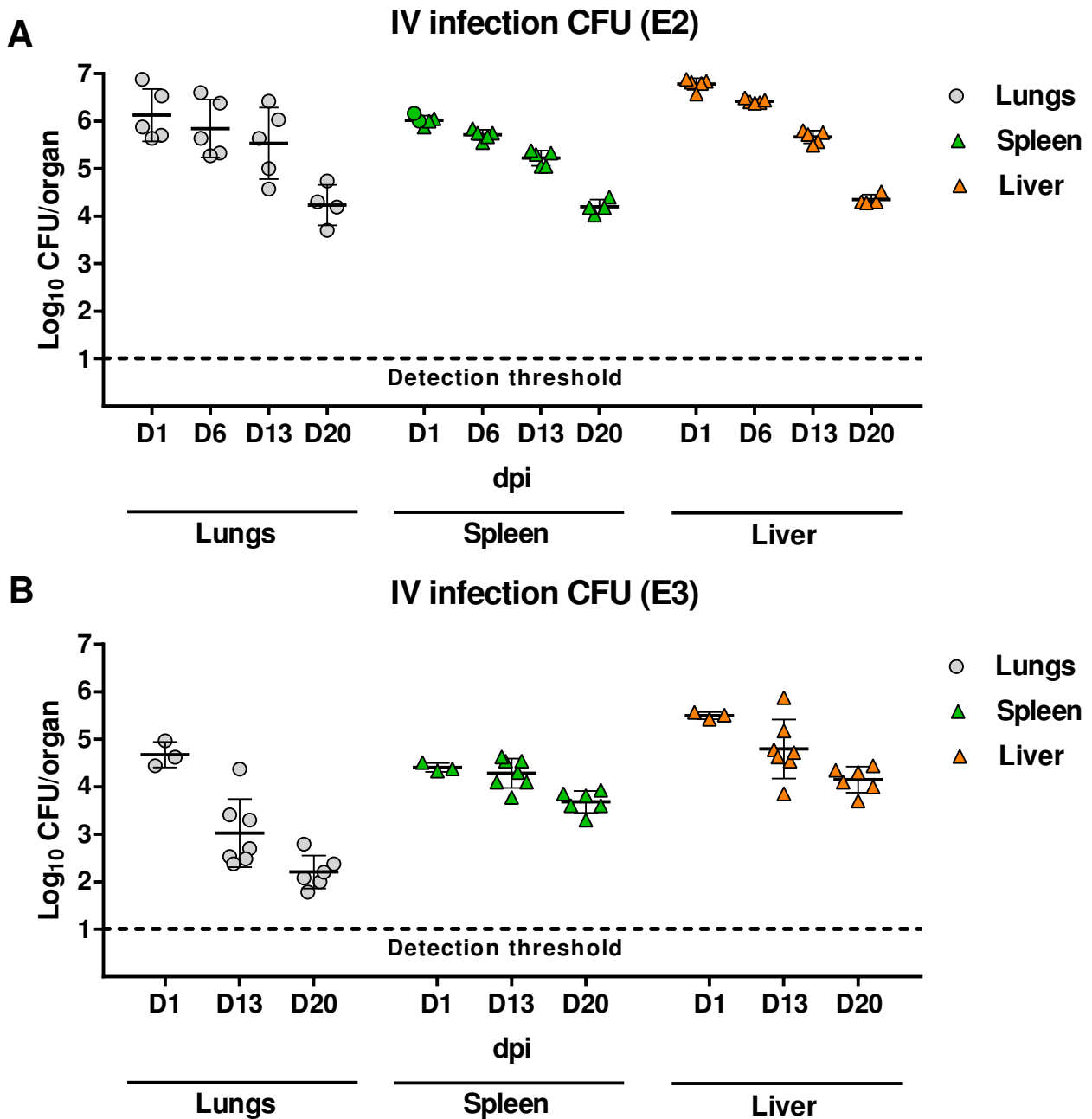
Supplementary Figure 2. Bacterial persistence of *M. abscessus* CIP 104536^T (rough variant) in the lungs, spleen and liver of C3HeB/FeJ mice after infection in the tail vein with 4.8×10^6 (A) and 3.1×10^5 CFU/mouse (B) in a total volume of 200 µl of water containing 0.9% sodium chloride. The following 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 $\log_{10}$ 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.