

Supplemental Doc.

Title: Modulation of Oxidative Phosphorylation with IM156 Attenuates Mitochondrial Metabolic Reprogramming and Inhibits Pulmonary Fibrosis

Authors: Robert N. Willette, Parth Mangrolia, Stephen M. Pondell, Christopher Young Woo Lee, Sanghee Yoo, Marc S. Rudoltz, Benjamin R. Cowen, Dean J. Welsch

MS #JPET-AR-2021-000811:

Supplemental Table 1 – Treatment Groups:

Group	N	Bleomycin	Treatment (14 days)
1	5	Naive	Vehicle
2	10	Bleomycin	Vehicle
3	10	Bleomycin	IM156 (10mg/kg, <i>qd</i>)
4	10	Bleomycin	IM156 (30 mg/kg, <i>qd</i>)
5	10	Bleomycin	Pirfenidone (100 mg/kg, <i>bid</i>)

Supplemental Table 2: Modified Ashcroft Score

Grade 0 = Normal lung

Grade 1 = Minimally detectable thickening of alveolar walls

Grade 2 = Mild thickening of alveolar walls

Grade 3 = Moderate contiguous thickening of walls with fibrous nodules

Grade 4 = Thickened septae and confluent fibrotic masses totaling less than 10% of the microscopic field

Grade 5 = Increased fibrosis with definite damage to lung structure and formation of fibrous bands or small fibrous masses between 10-50% of the microscopic field

Grade 6 = Large contiguous fibrotic masses consolidating more than 50% of the microscopic field

Grade 7 = Severe distortion of structure and large fibrous areas

Grade 8 = Total fibrous obliteration of lung within the microscopic field

Supplemental Table 3: Pharmacokinetic Profile of IM156 in Mouse

Parameter	IM156 (Mean +/- SD)
C_{\max} (ng/ml)	494±76.2
T_{\max} (hour)	1.33±0.57
$T_{1/2}$ (hour)	2.45±0.97
AUC_{0-t} (ng*hr/ml)	3058±445

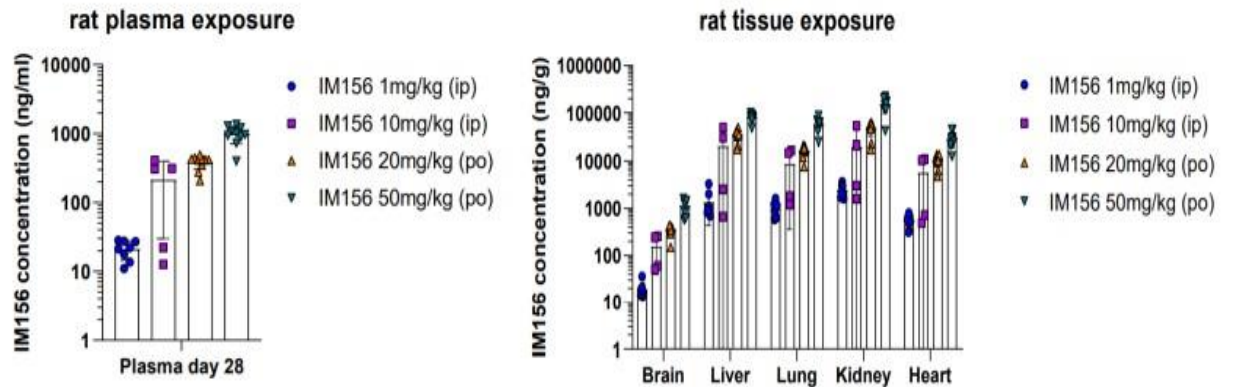
Pharmacokinetic profile of IM156 following a single oral dose of 30 mg/kg in CD-1 male mice (n=4).

Supplemental Table 4: Tissue to Plasma Concentration Ratios for IM156

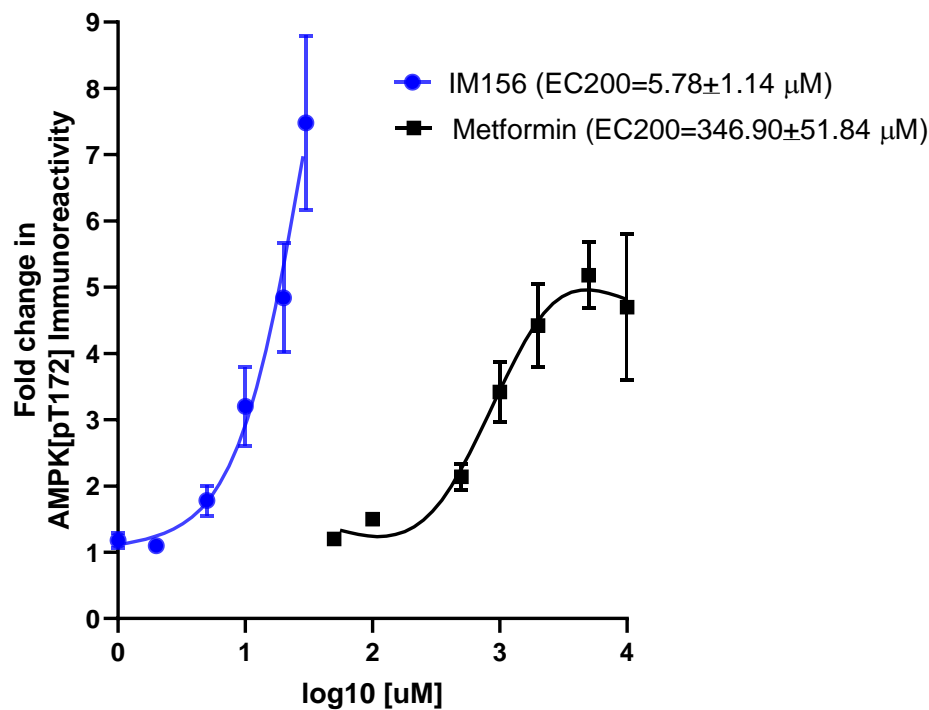
Organ (tissue:plasma)	Day 1 Mean (+/- SD)	Day 7 Mean (+/- SD)	Day 15 Mean (+/- SD)
Brain	1.2 (0.4)	0.8 (0.2)	0.8 (0.2)
Lung	72.6 (23.8)	52.2 (11.9)	86.8 (26.9)
Liver	33.7 (4.6)	33.1 (4.1)	33.3 (3.6)
Kidney	48.9 (12.8)	49.9 (20.6)	43.8 (9.7)

The ratio of tissue to plasma concentrations of IM156 (15 mg/kg, *p.o.*, *qod*) on days 1, 7 and 15 in Balb/c mice. N=5 per time point.

Supplemental Figures



Supplemental Figure 1: Rat plasma and tissue concentrations of IM156 were determined in samples obtained approximately 3 hours after the final dose in four groups receiving once daily doses for 28 days by the *i.p.* or oral route. Values are means +/- SD.



Supplemental Figure 2: AMPK phosphorylation (pT172) was evaluated in cell lysates from an MCF7 cell line (human breast adenocarcinoma) following 24 hours of incubation with IM156 or metformin (Invitrogen, Cat # KHO0651). The experiment was conducted in duplicate 5 separate times and the EC200 (200% increase in phosphorylation) was determined. Values are mean \pm the standard error of the mean.