pharmaceutics

Supplementary material: Preclinical Toxicity and Safety of MM-129—First-in-Class BTK/PD-L1 Inhibitor as a Potential Candidate against Colon Cancer

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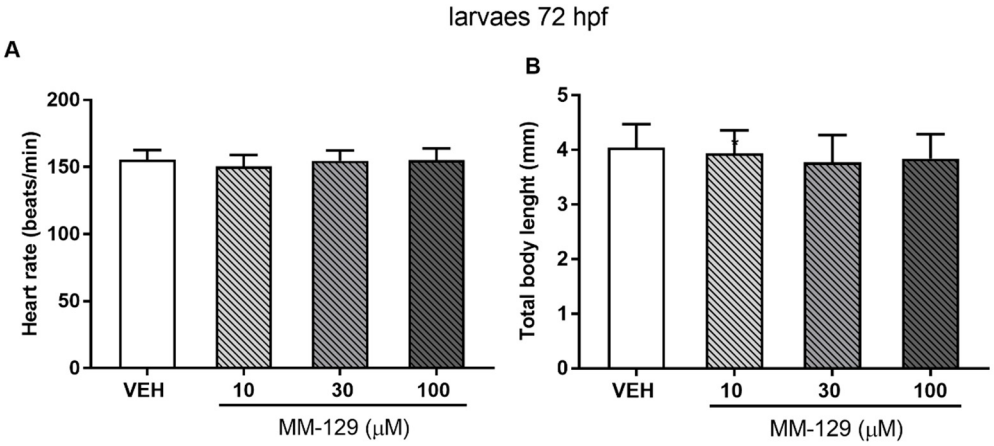

Figure S1. Heart rate (A) and total body lenght (B) of zebrafish larvaes 72 hpf at 96 h of incubation with MM-129. Data are shown as mean±SD; *n* = 60 for each concentration.
Abbreviations: MM-129 - pyrazolo[4,3-e]tetrazolo[4,5-b][1,2,4]triazine sulfonamide; VEH - vehicle; hpf - hour post–fertilization.

Table S1. Pharmacokinetics and tissue distribution of MM-129 in rats.

	MM-129 10 μmol/kg iv	**MM-129 10 μmol/kg ip**
CL (μmol/kg)/(μmol/L)/min	0.04 (0.03-0.05)	-
V_{ss} (μmol/kg)/(μmol/L)	0.60 (0.54-1.17)	-
Half life (min)	18.15 (12.74-25.84)	63.52 (51.54-75.49)
T_{max} (min)	10 (10-10)	20 (10-30)
C_{max} (μmol/L)	10.14 (5.83-10.28)	3.46 (2.22-4.69)
$AUC_{0\text{-}120min}$ (μmol/L*min)	360 (220-401)	247 (183-310)
$AUC_{0\text{-}\infty}$ (μmol/L*min)	360 (225-405)	346 (292-399)
Lung concentration (nmol/g of tissue)	-	0.46 (0.37-0.81)
Liver concentration (nmol/g of tissue)	-	0.17 (0.15-0.52)
Spleen concentration (nmol/g of tissue)	-	0.37 (0.33-0.46)
Brain concentration (nmol/g of tissue)	-	0 (0-0)
Kidney concentration (nmol/g of tissue)	-	0.81 (0.69-0.94)
Small intestine concentration (nmol/g of tissue)	-	2.78 (1.58-4.83)
Large intestine concentration (nmol/g of tissue)	-	1.17 (0.73-1.98)
Testicle concentration (nmol/g of tissue)	-	0.167 (0.06-0.60)

Data are shown as median with lower and upper limits.
Abbreviations: CL – clearance; Vss – steady state volume of distribution; T_{max} – time to reach maximum concentration; C_{max} – maximum concentration; AUC - area under the concentration–time curve; MM-129 - pyrazolo[4,3-e]tetrazolo[4,5-b][1,2,4]triazine sulfonamide

Table S2. Hematological analysis of whole blood samples from mice exposed to MM-129 concentrations or vehicle (VEH) after short-term and long-term administration.

short-term administration				
	VEH	**MM-129 10 μmol/kg**	**MM-129 20 μmol/kg**	**MM-129 40 μmol/kg**
WBC, 10^3/ mm^3	4.38±1.91	5.10±2.87	4.33±3.10	7.30±2.43
RBC, 10^6/ mm^3	8.82±0.21	8.65±0.43	9.38±1.24	9.11±0.61
HGB, g/dL	14.4±0.38	13.7±0.70	15.0±2.07	714.5±0.99
HCT, %	49.5±1.10	48.3±2.24	53.7±7.63	51.5±3.59
MCV, μm^3	56 (56-57)	55.5 (55-57)	57 (56-58)	56.5 (56-57)
MCH, pg	16.3±0.26	16.0±0.47	16.1±0.24	15.9±0.17
MCHC, g/dl	29 (28.5-29.8)	28.4 (27.8-28.8)	28 (27.8-29.3)	28 (27.8-28.2)*
PLT, 10^3/mm^3	423 (350–496)	435 (312–502)	412.0 (351–627)	433 (360.0–628.0)
long-term administration				
	VEH	**MM-129 10 μmol/kg**	**MM-129 20 μmol/kg**	**MM-129 40 μmol/kg**
WBC, 10^3/ mm^3	4.99±2.68	7.07±3.36	8.00±1.46	9.33±0.97
RBC, 10^6/ mm^3	8.98 (6.16-9.98)	8.71 (8.07-9.35)	8.77 (8.14-8.91)	8.23 (7.33-8.72)
HGB, g/dL	14.1±0.30	14.1±0.54	14.3±0.63	13.3±0.95
HCT, %	51.3 (35.5-56.9)	51 (46.3-53.5)	49.8 (46.5-52)	48.15 (41.9-49.9)
MCV, μm^3	57 (55-59)	57 (56-59)	57 (57-60)	57.5 (57-60)
MCH, pg	15.6±0.48	16.0±0.28	16.6±0.32***	16.4±0.21**
MCHC, g/dl	27.4±0.50	28.0±0.65	28.7±0.55**	28.3±0.33
PLT, 10^3/mm^3	466 (156–428)	421 (217–507)	426 (265–589)	481 (396–599)

Data are shown as medians with ranges or mean±SD; **p<0.01; ***p<0.001 vs. VEH within the group, n =3-10.
Abbreviations: VEH – vehicle; WBC – white blood cells; RBC – red blood cells; PLT - platelets; HGB – hemoglobin; HCT - hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; MM-129 - pyrazolo[4,3-e]tetrazolo[4,5-b][1,2,4]triazine sulfonamide.

Table S3. Biochemical parameters in mice exposed to MM-129 concentrations or vehicle (VEH) after short-term and long-term administration.

short-term administration				
	VEH	**MM-129 10 μmol/kg**	**MM-129 20 μmol/kg**	**MM-129 40 μmol/kg**
ALT, U/I	14.40±2.97	38.40±21.3*	41.80±33.83	68.7±46.6*
AspAT, U/I	79.00 (62-82)	77 (72-90)	92 (70-137)	160.0* (105-301)
BUN, mg/dl	52.0±9.67	43.60±7.64	44.40±20.30	53.0±10.90
CREA, mg/dl	<0.46	<0.46	<0.46	<0.46
Total bilirubin, mg/dl	<0.15	<0.15	<0.15	1.93±1.18
AMYL, U/I	2141.0±132	1968.0±155	1615.0±36	1686.0±699
LDH, U/I	151.0±47.6	211.5±17.6	212.8±75.5	396.0±309
CK, U/I	92.60±7.57	109.0±27.7	120.0±31.1	132.0±48.2
Pi, mg/dl	8.95±0.5	8.50±0.62	7.82±0.63	8.60±0.28
long-term administration				
	VEH	**MM-129 10 μmol/kg**	**MM-129 20 μmol/kg**	**MM-129 40 μmol/kg**
ALT, U/I	28.0±10.3	26.0±3.4	25.4±5.1	26.4±11.1
AspAT, U/I	134.0±18.4	118.0±40.5	101.0±21.3	212.0±105*
BUN, mg/dl	36.8±5.7	37.2±2.5	35.8±6.3	34.6±4.6
CREA, mg/dl	<0.46	<0.46	<0.46	<0.46
Total bilirubin, mg/dl	<0.15	<0.15	<0.15	<0.15
AMYL, U/I	1364.0±69.7	1429.0±231.0	1320.0±125.0	1325.0±232.0
LDH, U/I	312.0±115.0	249.0±63.1	290.1±38.5	522.0±179.0
CK, U/I	221.0±119.0	250.0±174.0	153.0±54.5	1082.0±1126
Pi, mg/dl	6.94±0.92	7.38±0.8	7.75±1.2	8.36±0.4

Data are shown as medians with ranges or mean±SD;. * p< 0.05 vs. VEH within the group, n =3-10.
Abbreviations: ALT – alanine transaminase; AMYL – amylase; AspAT – aspartate aminotransferase; BUN – blood urea nitrogen; CREA – creatinine; AMYL – amylase; LDH – lactate

dehydrogenase; CK – creatine kinase; Pi – phosphorus; MM-129 - pyrazolo[4,3-e]tetrazolo[4,5-b][1,2,4]triazine sulfonamide.

Table S4. Selected parameters of bone marrow examination of mice exposed to MM-129 concentrations or vehicle (VEH) after long-term administration.

parameters (percentage)	VEH	MM-129 10 μmol/kg	MM-129 20 μmol/kg	MM-129 40 μmol/kg
myeloblasts	0,552±0.5	0,47±0,16	1,06±0,515	0,742±0,517
promyelocytes	2,96±1.5	3,71±0.82	4,88±2.11	5,91±1.23*
myelocytes	10,7±1.33	10,9±2.2	13,8±2	13,3±5.13
metamyelocytes	14,1±1,94	14,3±1.7	13,6±1.09	13,1±1.34
neutrophils (bands)	13.8±3.1	15±2.64	13.8±1.65	13.9±3.47
neutrophils (segs)	12,9±3.8	12,2±1.93	13,1±2.49	11,6±3.63
proerythroblast	0,78±0.59	0,4±0.3	0,72±0.5	0,77±0.69
basophilic erythroblast	8,73±2.75	6,97±1.34	7,48±2.97	9,13±0.73
polychromatophilic erythroblast	10,3±1.47	9,97±1.02	8,54±2.7	9,86±2.88
acidophilic erythroblasts	12,1±2.26	12,5±1.48	11±1.53	9,19±1.74

Data are shown as mean ± SD; * $p < 0.05$ vs. VEH within the group, n =5.
Abbreviations: MM-129 - pyrazolo[4,3-e]tetrazolo[4,5-b][1,2,4]triazine sulfonamide; VEH – vehicle.

Table S5. The effect of MM-129 on platelet function and coagulation parameters *in vitro* and *in vivo* in rats.

***in vitro* study**		
	VEH	**MM-129 10 μM**
Platelet aggregation		
MaxA (Ω)	8.5 ± 1.3	8.3 ± 1.0
Slp (Ω/min)	2.9 ± 0.6	2.9 ± 0.6
Lag time (s)	90.9 ± 10.4	91.1 ± 7.4
AUC	24.9 ± 5.1	23.9 ± 3.5
***in vivo* study**		
	VEH	**MM-129 40 μmol/kg**
Platelet count (10^3/mm^3)	511.0 (497.0-644.0)	573.0 (452.0-620.0)
Platelet aggregation		
MaxA (Ω)	9.1 ± 1.5	8.7 ± 1.6
Slp (Ω/min)	3.3 ± 0.6	2.9 ± 0.6
Lag time (s)	103.2 ± 19.4	120.1 ± 30.0
AUC	25.1 ± 5.7	21.9 ± 6.4
Coagulation parameter		
aPTT (s)	16.5 (14.0-20.9)	16.3 (13.8-24.0)
PT(s)	12.3 ± 0.4	12.0 ± 1.0
INR	1.4 ± 0.1	1.4 ± 0.1

The data were shown as median (full range) or mean ± SD, n=7.
Abbreviations: MaxA - maximal extension; Slp - slope of platelet aggregation; AUC - area under the curve; aPTT - activated partial thromboplastin time; PT - prothrombin time; INR - international normalized ratio.