Supplemental Materials

Parsaclisib Is a Next-Generation PI3Kδ Inhibitor With Reduced Hepatotoxicity and Potent Antitumor and Immunomodulatory Activities in Models of B-Cell Malignancy

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Supplementary Methods

Chemicals, antibodies, and cell lines

Dimethylacetamide (#271012), methylcellulose (#M7027), and lysophosphatidic acid (#151884) were purchased from Sigma (St Louis, Missouri). Bendamustine (#3543-75-7) was purchased from Chemscene (Monmouth Junction, New Jersey). Platelet-derived growth factor (#120-HD) and complement component C5a (#2150-C5) were purchased from R&D Systems (Minneapolis, Minnesota). The anti-CD4-Alexa Fluor 700 (#557922), anti-CD8-FITC (#347313), anti-CD44-APC-Cy7 (#506568), anti-FOXP3-PE (#560046), and anti-CD62L-BUV737 (#741843) antibodies for fluorescence-activated cell sorting (FACS) analysis were purchased from BD Biosciences (Franklin Lakes, New Jersey). The anti-pAKT (Ser 473) antibody (#9271) for Western blotting and anti-pAKT (Ser 473)-Fluor 488 antibody (#4071) for FACS were purchased from Cell Signaling Technology (Danvers, Massachusetts). The anti-IgM antibody (#14-9998-82) for B-cell receptor stimulation was purchased from Invitrogen (Carlsbad, California). The anti-pAKT (Ser 473, #1861786), anti-AKT (#1861776), anti-rabbit (Dylight 800, #1861741), and anti-mouse (Dylight 680, #1861740) antibodies for In-Cell Western analysis were purchased from Thermo Scientific (Waltham, Massachusetts). A20 (#TIB-208), Pfeiffer (#CRL-2632), SU-DHL-6 (#CRL-2959), Jeko-1 (#CRL-3006), Mino (#CRL-300), Rec-1 (#CRL-3004), JVM2 (#CRL-3002), RAW264.7 (#TIB-71), Ramos (#CRL-1596), SKOV-3 (# HTB77), and PC-3 (# CRL-1435) cell lines were purchased from ATCC (Manassas, Virginia). SU-DHL-5 (#ACC 571), WILL-2 (#ACC 652), WSU-NHL (#ACC 58), SU-DHL-4 (#ACC 495), and SU-DHL-8 (#ACC 573) cell lines were obtained from DSMZ (Braunschweig, Germany).

SUPPLEMENTAL TABLE 1

Inhibitory activities of parsaclisib against PI3Kδ-mediated functions in cells

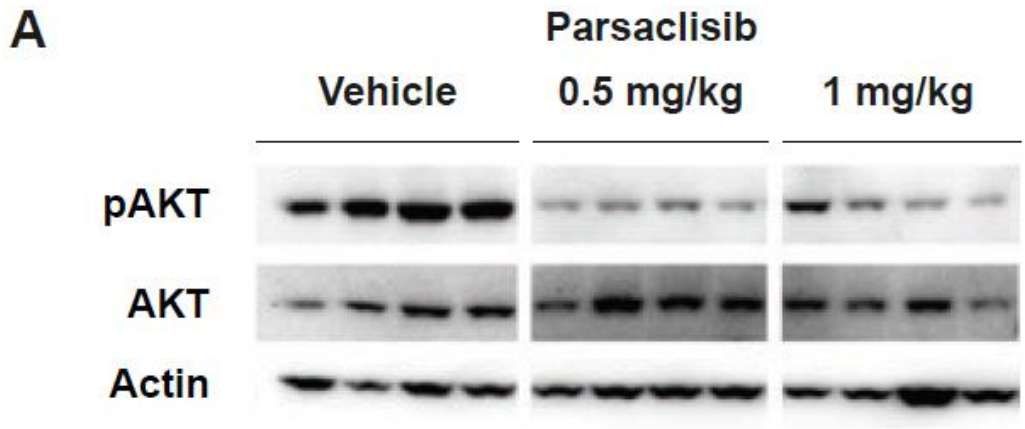
Primary cell	Stimulants	Response	IC_{50} ± S.D. (nM)	N
Human B	Anti-human IgM Ab	Proliferation[a]	0.21 ± 0.12	4
Human B	LPS + IL-4	Proliferation	0.73 ± 0.59	6
Human B	IL-4	Proliferation	0.47 ± 0.14	6
Human B	LPS	Proliferation	0.15 ± 0.02	3
Human B	CD40 ligand	Proliferation	0.72 ± 0.22	4
Human B	Anti-human CD40 Ab	Proliferation	0.42 ± 0.21	5
Human B	IL-6	Proliferation	0.59 ± 0.42	3
Human B	BAFF	Proliferation	0.53 ± 0.18	7
Mouse B	LPS + mIL-4 or mBAFF	Proliferation	0.37 ± 0.31	5
Rat B	LPS + rIL-4 + anti-rat IgM	Proliferation	1.19 ± 1.20	3
Dog B	Anti-canine IgM Ab	Proliferation	1.72	2
Human T	Anti-human CD3 Ab	Proliferation	>1000	4
Human T	Anti-human CD28 Ab	Proliferation	96.63 ± 52.93	3
Human T	Anti-human CD28 and CD3 Ab	Proliferation	>330	3
Human naïve T	Th17 differentiation	IL-17 production	0.90 ± 0.68	11
Human naïve T	Th17 differentiation	IFN-γ production	0.65 ± 0.48	9
Human naïve T	Th1 differentiation	IFN-γ production	0.73 ± 0.93	6
Human naïve T	Th2 differentiation	IL-13 production	0.26	2
Human memory T	Anti-CD3 Ab	IL-17 production	0.98	2
Human memory T	Anti-CD3 Ab	IFN-γ production	0.97	2
Human memory T	Anti-CD3 + anti-CD28 + anti-CD2 Ab	IL-17 production	1.12 ± 0.49	5
Human memory T	Anti-CD3 + anti-CD28 + anti-CD2 Ab	IFN-γ production	1.45 ± 1.49	4
Human NK	Human IL-2	Proliferation	>1000	2
Human basophil[b]	Anti-FcR	CD63 expression	1.96 ± 1.37	5

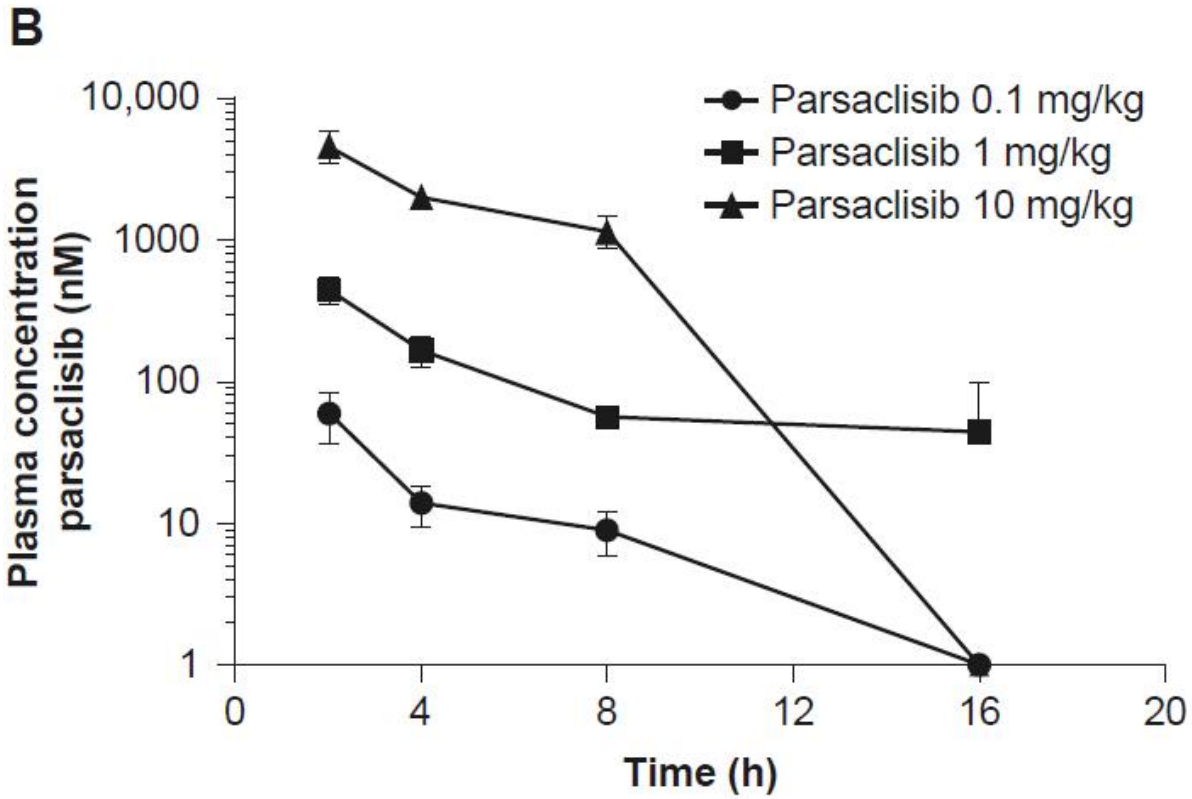
[a] Proliferation determined by ^{3}H-thymidine uptake; [b] The assay was conducted in the presence of whole blood.
LPS, lipopolysaccharide.

SUPPLEMENTAL TABLE 2

Analysis of RNA sequencing data from the WILL-2 xenograft model. Please refer to separate Excel document

Supplemental Fig. 1. Parsaclisib is active against tumor growth in the Pfeiffer model for DLBCL. (A) Western blotting analysis showing reduction in the pAKT (Ser 473) level in the Pfeiffer xenograft tumors 4 hours after dosing with parsaclisib at 0.5 mg/kg and 1 mg/kg. (B) Pharmacokinetic analysis showing parsaclisib exposure level in the plasma of the mice bearing the Pfeiffer tumor. The IC_{90} was covered for more than 8 hours when the compound was dosed at 1 mg/kg or 10 mg/kg.





Supplemental Fig. 2. Effect of parsaclisib on different T-cell populations in the A20 model of lymphoma. Gating strategy in fluorescence-activated cell sorting (FACS) analysis of parsaclisib treatment-induced changes in Tregs ($CD4^+CD25^+FOXP3^+$) (A) and memory ($CD44^+$) $CD4^+$ and $CD8^+$ T-cell populations (B). (C) Effect of parsaclisib on the central memory ($CD44^+CD62L^{high}$) and effector memory ($CD44^+CD62L^{low}$) $CD4^+$ and $CD8^+$ T cells in spleens and tumors of the A20 model. In these studies, the mice were treated with parsaclisib at 10 mg/kg once daily or vehicle control for 7 days before being euthanized for collection and processing of tumors and spleens for FACS analysis.