IOA-244 is a non-ATP-competitive, highly selective, tolerable phosphoinositide 3-kinase delta inhibitor that directly targets solid tumours and breaks immune tolerance

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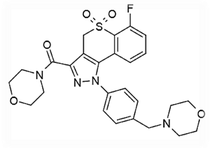
Summary

Inhibiting PI3Kδ preferentially targets regulatory T cells and myeloid derived suppressor cells, breaking tumour-induced immune tolerance and restoring anti-tumour immunity. Here we report the exploration of IOA-244/MSC2360844, a non-ATP-competitive PI3Kδ inhibitor, for the treatment of solid tumors. To harness the differentiation of IOA-244 from other PI3Kδ inhibitors, we have performed molecular dynamic and hydrogen–deuterium exchange MS studies, as well as in-cell kinase profiling where we compared structural and selectivity features of IOA-244 to other inhibitors. Then, to investigate the tumor intrinsic and extrinsic activity of IOA-244, we performed in vitro proliferation assays, xenografts, patient-derived xenograft models, and in vivo syngeneic tumor models. Here, we tested IOA-244 in monotherapy or in combination with checkpoint blockade inhibitors. IOA-244 is currently in clinical Phase I evaluation for lymphoma and solid tumors. In this study, IOA-244 showed unprecedented tolerability and clinical benefit. In conclusion, thanks to its unique structural and selectivity features, IOA-244 represents a best in class PI3Kδ inhibitor, with an exceptional safety profile.

IOA-244

Figure 1

IOA-244 chemical structure

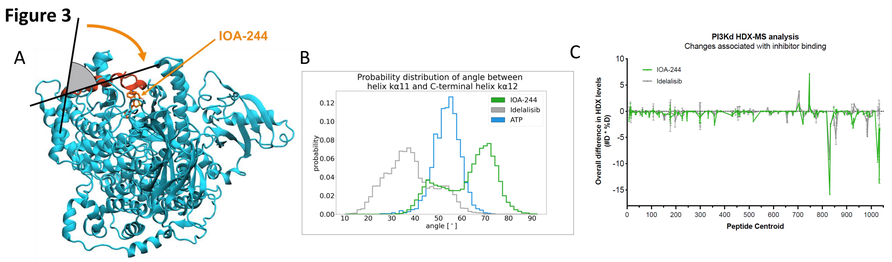


IOA-244 key features

- Unique chemical structure
- Excellent selectivity
- Non-ATP competitive activity
- Excellent PK properties
- Favourable safety profile

Unique structural features of IOA-244

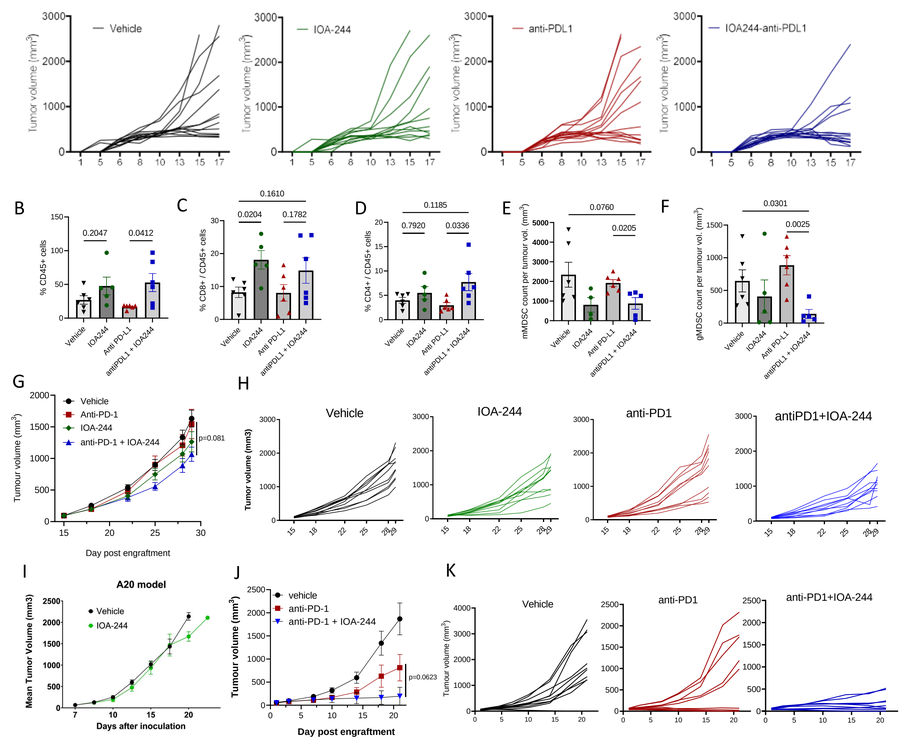
Figure 3



(A-C) Unique binding of IOA-244 to PI3Kδ. (A) All-atom molecular dynamics simulations showed that non-ATP-competitive inhibitor IOA-244 stabilizes a bent configuration of the C-terminus of PI3Kδ towards the kinase active site. (B) Compared to ATP (green curve), IOA-244 binding increased the angle between helices kα11 and kα12 whereas binding of the ATP-competitive inhibitor idelalisib (orange curve) had the opposite effect. (C) The unique conformational stabilization of the C-terminal helix by IOA-244 is confirmed by hydrogen– deuterium exchange MS. Overall, the data suggests that IOA-244 stabilizes an inactive state of PI3Kδ.

IOA-244 overcomes resistance to checkpoint inhibitors

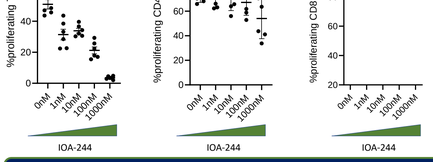
Figure 6



(A) Tumor volumes of CT26 colorectal mouse model, upon treatment with vehicle, IOA-244 (30mg/kg BID *p.o.*), antiPDL1 (10mg/kg, BIW, *i.p.*), IOA-244-antiPDL1. (B-G) Flow cytometry analysis from CT26 tumors showing quantification of CD45+ cells (B), CD8/CD45 (C), CD4/CD45 (D), mMDSC/CD45 (E) gMDSC/CD45 (F). (G-H) Tumor volumes of LLC (Lewis lung carcinoma) mouse model, upon treatment with vehicle, IOA-244, antiPD1, IOA-244-antiPDL1. Mean+/-SEM is showing in G, single growth curve in H. (I) Tumor volume of A20 model, upon treatment with Vehicle or IOA-244 (30mg/kg BID). (J-K) Tumor volumes of A20 mouse model, upon treatment with vehicle, antiPD1, IOA-244-antiPD-1. Mean+/-SEM is showing in J, single growth curve in K.

IOA-244 impairs Treg proliferation, while preserving CD8 and CD4 conventional T cells

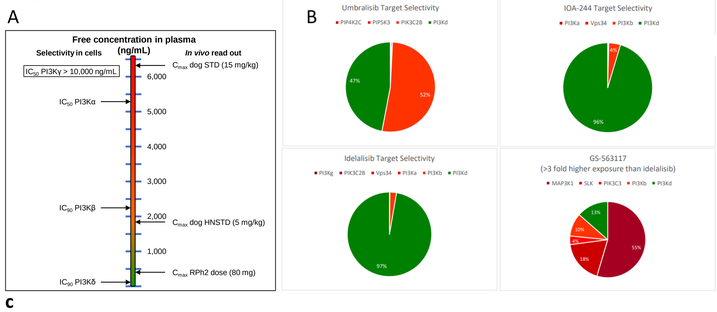
Figure 4



(A-C) Proliferation of human Tregs cells (A), CD4+Tconv (B) and CD8+T cells (C). Proliferation was analyzed by flow cytometry five days post stimulation with αCD3/αCD28 or resting, alongside IL-2 and IOA-244 at a range of concentrations. Dilution of ef450 proliferation dye was used to determine the frequency of cells that had undergone cellular division. Data are biological duplicates (n=2 donors) and technical replicates (n=3).

IOA-244 is a highly selective PI3Kδ inhibitor

Figure 2



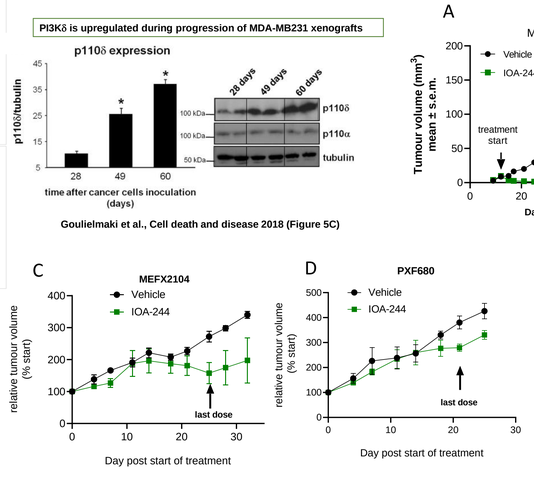
c

	Parameter	IOA-244	Idelalisib	GS563117	Umbralisib
Selectivity in Jurkat lysate	IC_{50} PI3Kα	10.1 µM	1.5 n µM	>15 µM	>15 µM
	IC_{50} PI3Kβ	0.43 µM	51 nM	3.7 µM	>15 µM
	IC_{50} PI3Kγ	>15 µM	10,300 µM	>15 µM	>15 µM
	IC_{50} PI3Kδ	19 nM	1.3 nM	2.7 µM	0.1 µM
	IC_{50} PIK3C2B	>15 µM	5.9 µM	>15 µM	90 nM
	IC_{50} Vps34	9 µM	5.6 µM	10 µM	>15 µM
	IC_{50} PIP4K2C	>15 µM	>15 µM	>15 µM	13.7 µM
	IC_{50} PIP5K3	>15 µM	>15 µM	>15 µM	11.2 µM
Cellular sensitivity	GI_{50} T-47D (PI3Kα)	>30 µM	14.1 µM	NA	26.3 µM
	GI_{50} LNCaP (PI3Kβ)	15 µM	1.35 µM	NA	11.2 µM
	GI_{50} THP-1 (PI3Kγ)	>30 µM	1.03 µM	NA	11.4 µM
	GI_{50} SU-DHL-6 (PI3Kδ)	0.235 µM	10 nM	NA	1.51 µM

(A) Comparison of cellular PI3K isoform selectivity for IOA-244 to *in vivo* free exposure levels of IOA-244 in dog and in human at Cmax. (B) Representation of the selectivity of selected PI3Kδ inhibitors and idelalisib main metabolite GS-563117 using partition index based on activity in Jurkat cell lysate as measured using the KiNativ method.
(C) IC_{50} was determined with Kinativ assay in Jurkate cell lysate, upon treatment with increasing doses of the indicated molecules. GI_{50} was determined in the indicated cell lines upon treatment with increasing doses of the indicated molecules.

IOA-244 shows direct antitumor activity in PI3Kδ expressing tumors

Figure 5



(A) Tumor volume of MDA-MB-231, inoculated in the breast fat pad of Balb/c nude mice, measured by digital callipers. Mice were treated once daily o.p. with vehicle or IOA-244 (30 mg/kg) from day +12. n=7 per group. (B) Immunohistochemical staining of phospho-AKT in two representative sections of tumours from (A). (C-D)Tumor growth of patient-derived cells from melanoma (MEXF2104) (C) and mesothelioma (PXF680) (D) subcutaneously injected in NMRI nude mice and treated with Vehicle or IOA-244 30mg\Kg BID (n=3 per group).

IOA-244 shows best in class clinical safety profile

	Parameter	IOA-244^	Zydelig* (Gilead)	Copiktra* (Secura)	Ukoniq* (TG Ther.)	Parsaclisib (Incyte)	Zandelisib (MEI Phar.)
Drug use	Dose interruption AE related	no	41%	64%	45%	16%	5%
	Continuous dosing	yes	no	no	No	no	no
	Combination potential	●	●	●	●	●	●
	Lymphoma develop. status	active	withdrawn	withdrawn	withdrawn	withdrawn	active
	Solid tumor develop. status	active	halted	NA	NA	PhII	NA
Safety profile	Tolerability (SAE ≥ Grade 3)	●	●	●	●	●	●
	Infection	0%	23%	27%	20%	0%#	0%#
	Neutropenia	0%	28%	43%	17%	20%	16%
	Diarrhea or colitis	0%	14%	23%	7%	9%	5%
	ALT/AST increase	0%	18%	8%	7%	3%	8%
	PK	●	●	●	●	●	●
	Metabolism	●	●	●			
Chemistry	Chemotype	unique	idelalisib	idela-like	idela-like	idela-like	unique
	Selectivity	●	●	●	●	●	●
	Allosteric inhibition	Yes	No	No	No	No	No

*FDA briefing document ODAC meeting April 21, 2022; ^IOA-244 at RP2D of 80 mg QD #Mandatory pneumocystis jiroveci pneumonia (PJP) prophylactic treatment

Conflict of Interest Statement: G. Di Conza, K Niewola, L Van der Veen, M Lahn and Z Johnson are or have been all employees and shareholders of iOnctura.

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