Discovery of a Series of 5,11-Dihydro-6H-benzo[e]pyrimido[5,4-b]-[1,4]diazepin-6-ones as Selective PI3K- δ/γ InhibitorsFleur M. Ferguson,[†] Jing Ni,[†] Tinghu Zhang,[†] Bethany Tesar,^{‡,§} Taebo Sim,^{||,⊥} Nam Doo Kim,[#] Xianming Deng,[†] Jennifer R. Brown,^{‡,§} Jean J. Zhao,[†] and Nathanael S. Gray^{*,†}[†]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, Massachusetts 02215, United States[‡]Department of Medical Oncology, Dana Farber Cancer Institute, Boston, Massachusetts 02215, United States[§]Department of Medicine, Harvard Medical School, Boston, Massachusetts 02215, United States^{||}Chemical Kinomics Research Center, Korea Institute of Science and Technology, Seoul, 02792, Republic of Korea[⊥]KU-KIST Graduate School of Converging Science and Technology, Korea University, Seoul 02841, Republic of Korea[#]Daegu-Gyeongbuk Medical Innovation Foundation, Daegu 41061, Republic of Korea

S Supporting Information

ABSTRACT: Dual inhibition of PI3K- δ and PI3K- γ is an established therapeutic strategy for treatment of hematological malignancies. Reported molecules targeting PI3K- δ/γ selectively are chemically similar and based upon isoquinolin-1(2H)-one or quinazolin-4(3H)-one scaffolds. Here we report a chemically distinct series of potent, selective PI3K- δ/γ inhibitors based on a 5,11-dihydro-6H-benzo[e]pyrimido[5,4-b]-[1,4]diazepin-6-one scaffold with comparable biochemical potency and cellular effects on PI3K signaling. We envisage these molecules will provide useful leads for development of next-generation PI3K- δ/γ targeting therapeutics.

KEYWORDS: PI3K- δ , PI3K- γ , phosphatidylinositol-4,5-bisphosphate 3-kinase-delta, p110- δ , p110- γ , kinase inhibitor

PI3K- δ and PI3K- γ are members of the Class I Type IA and Class I Type IB family of phosphatidylinositol-4,5-bisphosphate 3-kinases (PI3Ks). Unlike the related PI3K- $\alpha/-\beta$, which are ubiquitously expressed, PI3K- δ and PI3K- γ are expressed primarily in leukocytes and perform a number of roles in regulation of the immune system. PI3K- δ has been shown to be involved in B-cell activation, proliferation, homing, and retention in lymphoid tissues; PI3K- γ regulates T-cell proliferation and cytokine production.¹

PI3K- δ and PI3K- γ are the dominantly expressed PI3K isoforms in B- and T-cells, respectively, where they are key nodes in the PI3K/Akt/mTOR pathway. This pathway is misregulated in a number of blood-borne cancers including chronic lymphocytic leukemia (CLL), follicular lymphoma (FL), and indolent non-Hodgkin's lymphoma (iNHL).¹

PI3K- δ signaling drives malignant B-cell proliferation. Selective inhibition of PI3K- δ using small molecule inhibitor Idelalisib has proven to be an effective treatment for CLL when used in combination with rituximab, a chimeric monoclonal antibody that targets the B-lymphocyte antigen CD20.² PI3K- γ activation is key for inflammatory cell recruitment to tumors, associated with angiogenesis and tumor growth, which can be attenuated by knockdown or pharmacological inhibition of PI3K- γ .^{3,4} As these two kinases play distinct and complementary roles in immune function, dual inhibition of PI3K- δ and PI3K- γ is also an attractive strategy for broadly targeting hematological malignancies. Inhibition of PI3K- δ/γ is well tolerated with mild,

reversible side effects reported in the clinic.⁵ The dual inhibitor Duvelisib is currently in Phase III clinical trials for CLL, FL and Phase II clinical trials for iNHL, either alone or in combination with monoclonal antibody therapy.⁶ Additionally Duvelisib has potent anti-inflammatory and joint protective effects in murine models of rheumatoid arthritis.⁷ A Phase IIa exploratory clinical trial in mild allergic asthma met several secondary end points demonstrating proof-of-concept that next generation PI3K- δ/γ inhibitors may also prove effective in this disease area.⁸

Figure 1. Structures of PI3K- δ/γ selective inhibitors reported in the literature and described in this work.

Received: May 18, 2016

Accepted: August 2, 2016

Published: August 2, 2016

Table 1. SAR, Isoform Selectivity, and Aurora Kinase Selectivity of PI3K- δ/γ Inhibitors

Compound ID	Structure	IC ₅₀ (nM) ^a PI3K- α	IC ₅₀ (nM) ^a PI3K- β	IC ₅₀ (nM) ^a PI3K- δ	IC ₅₀ (nM) ^a PI3K- γ	IC ₅₀ (nM) ^b Aurora A	IC ₅₀ (nM) ^b Aurora B
1 (FMF-01-085-1)		53 ± 12	540 ± 530	1.5 ± 0.27	2.8 ± 4.7	59 ± 13	120 ± 45
2 (FMF-01-070-3)		-	-	230 ± 98	110 ± 46	23 ± 6.8	16 ± 2.2
3 (FMF-01-070-2)		-	-	20 ± 4.9	120 ± 26	350 ± 100	630 ± 150
4 (FMF-01-090-1)		-	-	120 ± 38	49 ± 36	-	-
5 (FMF-01-147-2)		730 ± 406	> 10,000	29 ± 15	15 ± 10	270 ± 100	290 ± 180
6 (FMF-01-147-1)		-	-	96 ± 50	310 ± 120	49 ± 17	80 ± 13
7 (FMF-01-147-4)		-	-	39 ± 14	330 ± 250	190 ± 46	130 ± 46
8 (FMF-01-147-3)		-	-	440 ± 180	340 ± 270	-	-
9 (FMF-02-109-1)		70 ± 7.0	610 ± 250	1.7 ± 1.5	11 ± 4.1	110 ± 19	120 ± 52
10 (FMF-02-061-1)		-	-	51 ± 30	560 ± 370	-	-
11 (FMF-02-062-1)		-	-	2600 ± 4000	3300 ± 970	-	-
12 (FMF-02-063-1)		55 ± 16	4800 ± 9500	2.1 ± 0.68	6.5 ± 1.5	150 ± 63	150 ± 38
13 (FMF-02-064-1)		-	-	41 ± 7.0	58 ± 21	-	-
14 (XMD17-75)		-	-	30 ± 19	20 ± 9.5	16 ± 12	4.2 ± 0.62
15 (FMF-02-052-1)		-	-	76 ± 33	110 ± 43	-	-
16 (XMD12-70-2)		-	-	49 ± 15	15 ± 4.4	4.8 ± 0.34	13 ± 1.4
17 (XMD17-78)		-	-	4.1 ± 0.60	4.3 ± 2.4	2.1 ± 0.57	4.6 ± 1.8
18 (XMD-12-3-1)		-	-	820 ± 180	2400 ± 3800	4900 ± 1200	9400 ± 10000

^aIC₅₀s measured using ADAPTA assay format (ThermoFisher Scientific). ^bIC₅₀s measured using Z'LYTE assay format (ThermoFisher Scientific). IC₅₀s plotted from the average of duplicate experiments. Errors are reported as ±95% confidence interval.

Currently reported selective dual inhibitors of PI3K- δ/γ are based upon isoquinolin-1(2*H*)-one or quinazolin-4(3*H*)-one

scaffolds (Figure 1).^{9–11} Here we report a chemically distinct series of potent, selective PI3K- δ/γ inhibitors based on a

5,11-dihydro-6H-benzo[e]pyrimido[5,4-b][1,4]diazepin-6-one scaffold with comparable enzymatic potency and cellular effects on PI3K- δ signaling.

Throughout the course of a screening campaign designed to identify antileukemic compounds, we observed that compound **1** (FMF-01-085-1) shows antiproliferative activity in T-cell acute lymphocytic leukemia (T-ALL) cell lines (IC₅₀ MOLT4 cells = 33 nM; IC₅₀ Jurkat cells = 166 nM). Subsequent kinome profiling revealed the primary targets of this compound are PI3K- δ/γ (Table 1, Supporting Table 1, Supporting Figure 1), leading us to explore the SAR of this series. Compounds were synthesized according to Scheme 1. Analogues from

Scheme 1. Synthetic Route for Synthesis of 5,11-Dihydro-6H-benzo[e]pyrimido[5,4-b][1,4]diazepin-6-ones^a

^aReaction conditions: (i) DIEA, 1,4-dioxane, 50 °C; (ii) Fe, AcOH, 50 °C; (iii) NaH, MeI, DMF, 0 °C; (iv) XPhos, Pd₂(dba)₃, Cs₂CO₃, 1,4-dioxane, 95 °C.

our initial screen lacking an aryl-sulfonamide showed no inhibitory effects on PI3K- δ/γ (e.g., compound **19**, FMF-01-086-2, Supporting Table 2); therefore, we focused our synthetic efforts on compounds containing this moiety.¹²

We have previously reported that the 5,11-dihydro-6H-benzo[e]pyrimido[5,4-b][1,4]diazepin-6-one scaffold is capable of binding to the ATP binding pocket of LRRK2,¹³ ERK5,¹⁴ and AuroraA/B kinases¹⁵ and to the acetyl-lysine binding pocket of the BRD4 bromodomains.¹⁶ However, methylation of the phenyl ring in the tricyclic core is not tolerated by these targets. Kinome profiling at 1 μ M compound concentration revealed that **1** has excellent selectivity across the human kinome, with a selectivity score, S₁₀ of 0.013. Importantly, other targets in the PI3K pathway such as Akt, DNA-PK, BTK, and mTOR are not inhibited (Supporting Table 1, Supporting Figure 1) and BRD4 activity is low (BRD4_1 IC₅₀ = 6.0 μ M, Supporting Table 3). The compound has some inhibitory effects on PIP5K2C (PIP4K- γ), a lipid kinase with low levels of activity *in vitro*. In our experience this level of inhibition corresponds to micromolar biochemical IC₅₀.

As some activity is present for PI3K- α (and H1047L/Y mutants) we measured PI3K- α and PI3K- β IC₅₀s to determine the isoform selectivity. Compound **1** is 26-fold selective for PI3K- δ over PI3K- α and 270-fold selective over PI3K- β . The only off-target activity of concern is against Aurora kinases A and B. Enzymatic testing revealed that compound **1** has 30-fold selectivity over Aurora A and 60-fold over Aurora B.

This prompted us to further investigate the factors conferring selectivity to the series (Table 1). Meta substitution of the aniline ring with an N-substituted sulfonamide biases the potency of the compounds toward PI3K- δ/γ (compounds **1**, **3**, **5**, **7**, **9**, **10**, **12**). Conversely, the same substituents in the para position improve the Aurora A/B potency and reduce the PI3K- δ/γ potency (compounds **2**, **4**, **6**, **8**, **11**, **13**).

Compounds containing an unsubstituted sulfonamide nitrogen are equipotent against PI3K- δ/γ and AuroraA/B (compounds **14**, **16**, **17**). It has been shown that ortho-substitution adjacent to the hinge-binding motif can remove AuroraA/B activity from this scaffold.¹⁵ Ortho-methylation of the aniline ring of potent compound **16** to give compound **18** shows the expected low AuroraA/B activity but also has dramatically reduced PI3K- δ/γ activity.

Covalent inhibitors have been reported for PI3K- α that target a nonconserved cysteine unique to this isoform.¹⁷ Examination of X-ray crystal structures showed there are no accessible cysteine residues proximal to the ATP binding pocket in PI3K- δ and PI3K- γ (PDB IDs: 4XE0, 4EZJ). As compound **1** contains an acrylamide, we used LC-MS/MS experiments with purified PI3K- δ protein to confirm that **1** does not covalently label the protein. Therefore, we sought to remove this reactive functionality while maintaining on-target potency and kinome selectivity. Gratifyingly compound **9** (FMF-02-109-1) and compound **12** (FMF-02-063-1) both maintained potent inhibition of PI3K- δ/γ and showed comparable selectivity for PI3K- δ against PI3K- α (40-fold, 25-fold) and improved selectivity against PI3K- β (360-fold, 2300-fold), Aurora A (65-fold, 70-fold) and Aurora B (71-fold, 71-fold). Kinome profiling revealed that compounds **9** and **12** also maintain an excellent selectivity profile with S₁₀ of 0.010 and 0.008 respectively (Figure 2A, Supporting Table 1). Additionally low BRD4 activity was observed for all compounds (BRD4_1 IC₅₀ = 18.8 μ M, 10.8 μ M respectively, Supporting Table 3).

In order to have a more direct comparison of potency to the currently available clinical compounds we measured the IC₅₀s of Duvelisib and Idelalisib in the ADAPTA assay format (Figure 2B). In the PI3K- δ assay, compounds **1**, **9** and **12** are equipotent to Idelalisib, whereas Duvelisib is more effective. In the PI3K- γ assay, compounds **1**, **9**, and **12** are comparable to Duvelisib. Idelalisib, a PI3K- δ specific inhibitor, is much less potent against PI3K- γ , as expected (Table 1, Figure 2B).

Encouraged by the potency of our inhibitors in comparison to the current best-in-class molecules, we next explored the effect of our compounds on PI3K signaling in isogenic HMEC cell lines where PI3K signaling is driven exclusively by CA-p110- α , CA-p110- β , or CA-p110- δ under serum starved conditions, and compared them to Duvelisib and Idelalisib. Commensurate with their biochemical activities, Idelalisib, **9**, and **12** show comparable inhibition of, and selectivity for, PI3K- δ signaling at 10 nM concentration (Figure 2C). Duvelisib is the most potent PI3K- δ inhibitor; however, it is less selective against PI3K- β in a cellular context.

To rationalize the isozyme selectivity we docked **9** and **12** into PI3K- δ (PDB: 4XE0). It is known that exploitation of a selectivity pocket formed by rearrangement of a methionine residue in the ATP binding pockets of PI3K- δ and PI3K- γ can improve isozyme selectivity. This has been achieved to date by the design of molecules that adopt a propellor-like conformation, such as Idelalisib and Duvelisib.¹⁸ In the docking models of **9** and **12** in complex with PI3K- δ the butterfly-like conformation of the benzodiazepinone scaffold causes the tolyl

Figure 2. (A) Kinome-wide selectivity profile of compounds **9** and **12**. (B) Comparison of biochemical IC₅₀ values of compounds **9**, **12**, Duvelisib, and Idelalisib in PI3K-δ and PI3K-γ ADAPTA assays. (C) Effects of inhibitors **9** and **12** on AKT and S6RP phosphorylation in isogenic HMEC lines expressing CA-p110α, CA-p110β, or CA-p110δ treated with the indicated compounds at 0.01, 0.1, or 1 μM for 1 h. Du, Duvelisib; Id, Idelalisib.

group to occupy the selectivity pocket formed by Met 752 while still allowing the pyrimidine-hinge contact to occur. The H-bond of the sulfonamide to Thr 833 may explain the preference for this functional group and the requirement for 3-substitution vs 4-substitution of the aniline ring (Figure 3, Supporting Figure 3, Table 1).

Figure 3. Docking model of **12** bound to PI3K-δ.

Finally, we treated leukemia cell lines and patient derived primary CLL cells to examine the effects of PI3K-δ/γ inhibition on cell viability. Compounds **9** and **12** were comparably potent to Duvelisib and more potent than Idelalisib in the tested cell lines. In the CLL cells Idelalisib showed little effect, consistent with previously published reports,¹⁹ as did Duvelisib and compound **12**, whereas compound **9** demonstrated

Table 2. Activity of PI3K-δ/γ Inhibitors in Cell Viability Assays

compound ID	IC ₅₀ (μM) Jurkat	IC ₅₀ (μM) Molt4	IC ₅₀ (μM) MV4:11	IC ₅₀ (μM) Molm 14	IC ₅₀ (μM) Loucy	IC ₅₀ (μM) CLL ^a
9	1.6	1.2	0.96	0.61	0.72	3
12	1.4	1.3	0.62	1.0	0.35	>10
Idelalisib	7.9	10.6	6.3	3.6	8.4	>10
Duvelisib	1.9	2.3	4.4	1.2	0.98	>10

^aPatient derived primary cells. IC₅₀s plotted from average of three replicates.

a dose-dependent reduction in viability (Table 2, Supporting Figure 2).

The series of compounds described in this work represent a novel class of PI3K-δ/γ inhibitors. We were able to develop potent, selective molecules with cellular activity and drug-like properties in the absence of structural information. We envisage these molecules will provide useful leads for development of next-generation PI3K-δ/γ targeting therapeutics. Investigation into the binding mode of these molecules by X-ray crystallography may yield rationale for development of molecules with superior PI3K-δ/γ selectivity using structure-based design.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.6b00209.

Biochemical and cellular assay protocols, compound synthesis and characterization, supporting figures, and supporting tables (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: nathanael_gray@dfci.harvard.edu.

Present Address

(X.D.) State Key Laboratory of Cellular Stress Biology, Innovation Center for Cell Signaling Network, School of Life Sciences, Xiamen University, Xiamen, Fujian 361102, China.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; iNHL, indolent non-Hodgkin's lymphoma; T-ALL, T-cell acute lymphocytic leukemia; Akt, protein kinase B; BTK, Bruton's tyrosine kinase; mTOR, mechanistic target of rapamycin; DNA-PK, DNA-dependent protein kinase; BRD4, bromodomain-containing protein 4; PLK1, polo-like kinase 1; LRRK2, leucine-rich repeat kinase 2; ERK5, extracellular-signal-regulated kinase 5; JAK2, Janus Kinase 2

■ REFERENCES

- (1) Fruman, D. A.; Rommel, C. PI3K and cancer: lessons, challenges and opportunities. *Nat. Rev. Drug Discovery* **2014**, *13* (2), 140–56.
- (2) Furman, R. R.; Sharman, J. P.; Coutre, S. E.; Cheson, B. D.; Pagel, J. M.; Hillmen, P.; Barrientos, J. C.; Zelenetz, A. D.; Kipps, T. J.; Flinn, I.; Ghia, P.; Eradat, H.; Ervin, T.; Lamanna, N.; Coiffier, B.; Pettitt, A. R.; Ma, S.; Stilgenbauer, S.; Cramer, P.; Aiello, M.; Johnson, D. M.; Miller, L. L.; Li, D.; Jahn, T. M.; Dansey, R. D.; Hallek, M.; O'Brien, S. M. Idelalisib and Rituximab in relapsed chronic lymphocytic leukemia. *N. Engl. J. Med.* **2014**, *370* (11), 997–1007.
- (3) Balakrishnan, K.; Peluso, M.; Fu, M.; Rosin, N. Y.; Burger, J. A.; Wierda, W. G.; Keating, M. J.; Faia, K.; O'Brien, S.; Kutok, J. L.; Gandhi, V. The phosphoinositide-3-kinase (PI3K)-delta and gamma inhibitor, IPI-145 (Duvelisib), overcomes signals from the PI3K/AKT/S6 pathway and promotes apoptosis in CLL. *Leukemia* **2015**, *29* (9), 1811–1822.
- (4) Ruckle, T.; Schwarz, M. K.; Rommel, C. PI3K[gamma] inhibition: towards an 'aspirin of the 21st century'? *Nat. Rev. Drug Discovery* **2006**, *5* (11), 903–918.
- (5) O'Brien, S.; Patel, M.; Kahl, B. S.; Horwitz, S. M.; Foss, F. M.; Porcu, P.; Sweeney, J.; Allen, K.; Faia, K.; Stern, H. M. Duvelisib (IPI-145), a PI3K- δ , γ inhibitor, is clinically active in patients with relapsed/refractory chronic lymphocytic leukemia. *Blood* **2014**, *124* (21), 3334–3334.
- (6) Infinity Pharmaceuticals. Research and Development, Clinical Trials. <http://www.infi.com/research-development/clinical-trials/>.
- (7) Boyle, D. L.; Kim, H.-R.; Topolewski, K.; Bartok, B.; Firestein, G. S. Novel phosphoinositide 3-kinase δ , γ inhibitor: potent anti-inflammatory effects and joint protection in models of rheumatoid arthritis. *J. Pharmacol. Exp. Ther.* **2014**, *348* (2), 271–280.
- (8) Schmalbach, T.; Fuhr, R.; Albayaty, M.; Allen, K.; Douglas, M.; Dunbar, J.; McLaughlin, J.; Alexander, L.; McKee, C. Duvelisib, a PI3K- δ , γ inhibitor, in subjects with mild asthma. *Eur. Respir. J.* **2015**, *46*, PA2122.
- (9) Winkler, D. G.; Faia, K. L.; DiNitto, J. P.; Ali, J. A.; White, K. F.; Brophy, E. E.; Pink, M. M.; Proctor, J. L.; Lussier, J.; Martin, C. M.; Hoyt, J. G.; Tillotson, B.; Murphy, E. L.; Lim, A. R.; Thomas, B. D.; Macdougall, J. R.; Ren, P.; Liu, Y.; Li, L. S.; Jessen, K. A.; Fritz, C. C.; Dunbar, J. L.; Porter, J. R.; Rommel, C.; Palombella, V. J.; Changelian, P. S.; Kutok, J. L. PI3K-delta and PI3K-gamma inhibition by IPI-145 abrogates immune responses and suppresses activity in autoimmune and inflammatory disease models. *Chem. Biol.* **2013**, *20* (11), 1364–74.
- (10) Ikeda, H.; Hideshima, T.; Fulciniti, M.; Perrone, G.; Miura, N.; Yasui, H.; Okawa, Y.; Kiziltepe, T.; Santo, L.; Vallet, S.; Cristea, D.; Calabrese, E.; Gorgun, G.; Raje, N. S.; Richardson, P.; Munshi, N. C.; Lannutti, B. J.; Puri, K. D.; Giese, N. A.; Anderson, K. C. PI3K/p110{delta} is a novel therapeutic target in multiple myeloma. *Blood* **2010**, *116* (9), 1460–8.
- (11) Williams, O.; Houseman, B. T.; Kunkel, E. J.; Aizenstein, B.; Hoffman, R.; Knight, Z. A.; Shokat, K. M. Discovery of dual inhibitors of the immune cell PI3Ks p110 δ and p110 γ : a prototype for new anti-inflammatory drugs. *Chem. Biol.* **2010**, *17* (2), 123–134.
- (12) Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. A quantitative analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* **2008**, *26* (1), 127–132.
- (13) Deng, X.; Dzamko, N.; Prescott, A.; Davies, P.; Liu, Q.; Yang, Q.; Lee, J.-D.; Patricelli, M. P.; Nomanbhoy, T. K.; Alessi, D. R.; Gray, N. S. Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. *Nat. Chem. Biol.* **2011**, *7* (4), 203–205.
- (14) Elkins, J. M.; Wang, J.; Deng, X.; Pattison, M. J.; Arthur, J. S. C.; Erazo, T.; Gomez, N.; Lizcano, J. M.; Gray, N. S.; Knapp, S. X-ray crystal structure of ERK5 (MAPK7) in complex with a specific inhibitor. *J. Med. Chem.* **2013**, *56* (11), 4413–4421.
- (15) Kwiatkowski, N.; Deng, X.; Wang, J.; Tan, L.; Villa, F.; Santaguida, S.; Huang, H.-C.; Mitchison, T.; Musacchio, A.; Gray, N. Selective Aurora kinase inhibitors identified using a Taxol-induced checkpoint sensitivity screen. *ACS Chem. Biol.* **2012**, *7* (1), 185–196.
- (16) Lin, E. C. K.; Amantea, C. A.; Nomanbhoy, T. K.; Weissig, H.; Ishiyama, J.; Hu, Y.; Sidique, S.; Li, B.; Kozarich, J. W.; Rosenblum, J. S. ERK5 kinase activity is not required for cellular immune response. *bioRxiv* **2016**, 038513v2.
- (17) Nacht, M.; Qiao, L.; Sheets, M. P.; St. Martin, T.; Labenski, M.; Mazdiyasni, H.; Karp, R.; Zhu, Z.; Chaturvedi, P.; Bhavsar, D.; Niu, D.; Westlin, W.; Petter, R. C.; Medikonda, A. P.; Singh, J. Discovery of a Potent and Isoform-Selective Targeted Covalent Inhibitor of the Lipid Kinase PI3K α . *J. Med. Chem.* **2013**, *56* (3), 712–721.
- (18) Berndt, A.; Miller, S.; Williams, O.; Le, D. D.; Houseman, B. T.; Pacold, J. I.; Gorrec, F.; Hon, W.-C.; Liu, Y.; Rommel, C.; Gaillard, P.; Ruckle, T.; Schwarz, M. K.; Shokat, K. M.; Shaw, J. P.; Williams, R. L. The p110 δ crystal structure uncovers mechanisms for selectivity and potency of novel PI3K inhibitors. *Nat. Chem. Biol.* **2010**, *6* (2), 117–124.
- (19) Wang, A.; Liang, X.; Chen, C.; Liu, J.; Zhao, Z.; Wu, H.; Deng, Y.; Wang, L.; Wang, B.; Wu, J.; Liu, F.; Fernandes, S. M.; Adamia, S.; Stone, R. M.; Galinsky, I. A.; Brown, J. R.; Griffin, J. D.; Zhang, S.; Loh, T.; Zhang, X.; Wang, W.; Weisberg, E. L.; Liu, J.; Liu, Q. Characterization of selective and potent PI3K δ inhibitor (PI3KDIN-015) for B-Cell malignancies. *Oncotarget* **2016**, 8702.