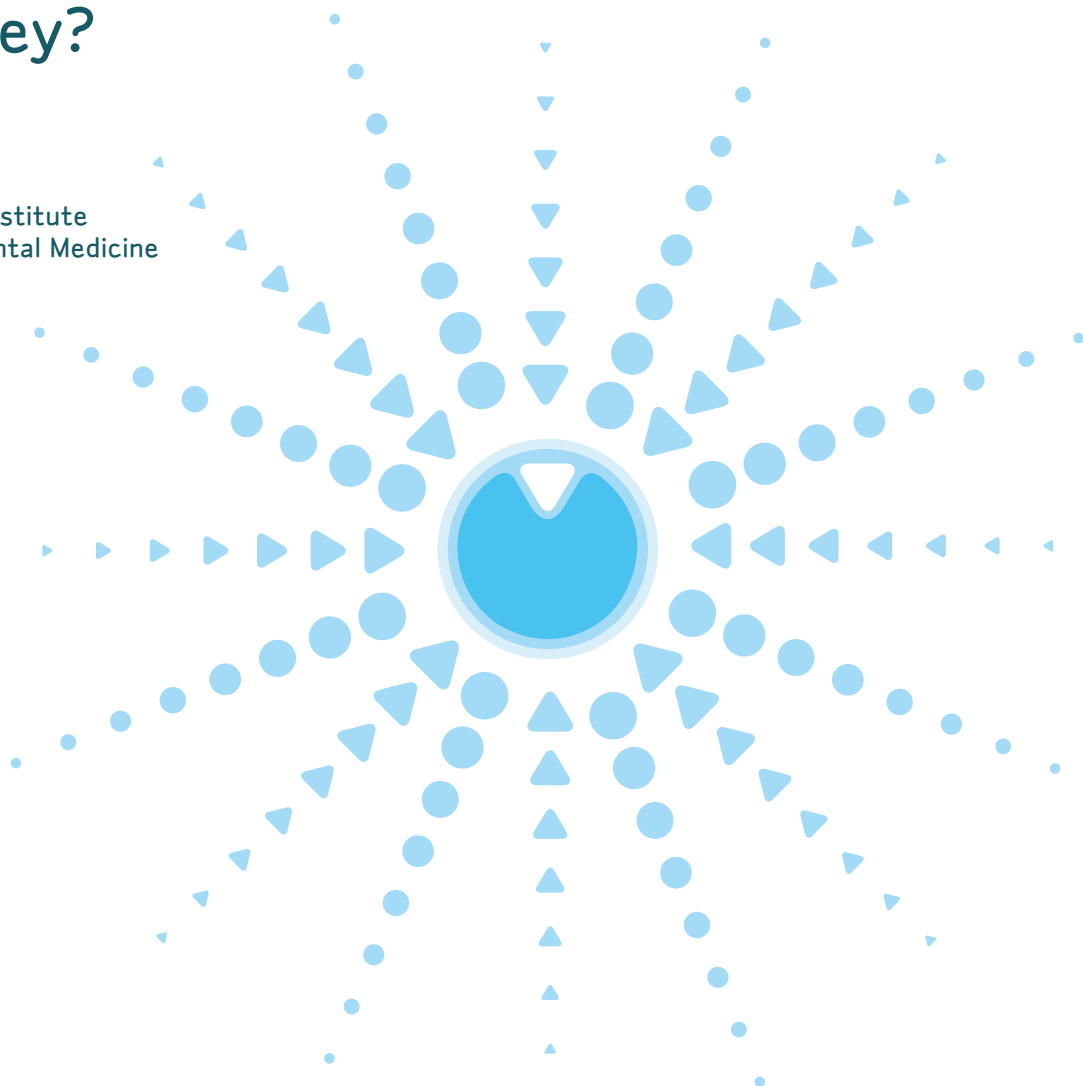




Unlocking the Mystery of Cancer:

Do the Mutant p110 α Subunits of PI3-Kinase Hold the Key?

Dr. Peter K. Vogt
The Scripps Research Institute
Molecular and Experimental Medicine
Division of Oncovirology
La Jolla, California



ADVANCING LIFE SCIENCE TOGETHER™
Research. Development. Production.

INTRODUCTION

The discovery of cancer-specific mutations in PIK3CA, the gene coding for the catalytic subunit p110 α of PI3-kinase, has transformed the field of lipid kinases from an area of basic biochemistry into a focus of intense interest for drug development, yet the new opportunities depend largely on the availability of purified, mutant p110 α .

PIP3 is an important second messenger in the cell. Because of its unique link to PIP3, class I PI3Ks occupy a central position in cellular signaling and are among the most intensively studied lipid kinases.



This paper provides an overview of PI3-kinases and what makes them unique, their implications for cancer, their roles in signal transduction and the challenges researchers face today with advancing this research.

WHAT ARE PI3KS?

Phosphatidylinositol 3-kinases (PI3Ks) form a family of enzymes that phosphorylate the inositol ring of phospholipids at the D3 position. These lipid kinases have been grouped into three classes (I - III),

distinguished by their structure and function [Figures 1 and 2]^(1,2).

They are dimeric enzymes, consisting of a catalytic and a regulatory (adaptor) subunit⁽³⁾. An important distinction between classes of PI3K is substrate specificity [Figure 2]:

- Class I accepts three substrates: unphosphorylated phosphoinositides, 4-monophosphate and 4,5-bisphosphate of the inositol ring.
- Class II uses only the non-phosphorylated and monophosphorylated inositol ring.
- Class III is restricted in its activity to the non-phosphorylated substrate⁽²⁾.

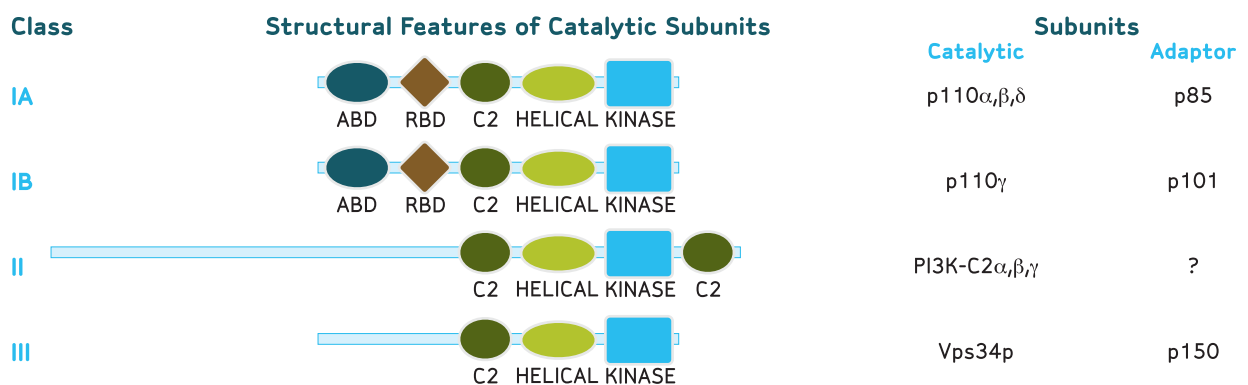
Thus, only class I PI3K generates the 3,4,5-trisphosphate, also referred to as PIP₃. PIP₃ is an important second messenger in the cell, and because of this unique link to PIP₃, class I PI3Ks occupy a central position in cellular signaling and are among the most intensively studied lipid kinases^(4, 6, 7).

CATALYTIC SUBUNIT OF CLASS I PI3KS, p110 — IDENTIFICATION OF FIVE STRUCTURE-FUNCTION DOMAINS:

- Amino-terminal adaptor-binding domain that provides the principal interaction surface with the regulatory subunit

Figure 1. Three Classes of PI3K

The domain structure of the catalytic subunits is shown. ABD = Adaptor-Binding Domain, RBD = Ras-Binding Domain



- Ras-binding domain mediating interaction between p110 and Ras-GTP and contributing to the stimulation of the PI3K and the Ras-driven signaling pathways
- C2 domain with affinity for lipid membranes
- Helical domain of still undetermined function
- Carboxy-terminal kinase domain [Figure 1]⁽⁴⁾

The standard regulatory subunit of class I PI3Ks is p85, a protein that contains several modular protein-protein interaction domains:

- Two Src-homology 2 (SH2) domains
- Src-homology 3 (SH3) domain
- Poly-proline stretches (PP)
- BCR homology domain (BH)
- Inter-SH2 domain [Figure 3]^(8, 9)

The latter is the primary p110-interacting surface. The regulatory subunit links p110 to upstream signals, interacting with tyrosine receptor kinases and G protein-coupled receptors^(2, 4). A mutant version of the p85 regulatory subunit, p65, has been shown to induce the constitutive activation of PI3K and contribute to cellular transformation⁽⁵⁾.

CELLULAR ACTIVITIES CONTROLLED BY CLASS I PI3K

Class I PI3K controls numerous cellular activities that are connected to growth, replication and survival, differentiation, movement and invasiveness, immune signaling and metabolism^(2, 6, 7). Figure 4 shows three important signaling chains that originate in PI3K. All three are mediated by the downstream target Akt, which together with its main activating kinase, PDK1, is recruited to the plasma membrane through its affinity for the product of class I PI3K, PIP₃. Akt serves as a branching point for these signals that are

Figure 2. Substrate Specificity of PI3Ks

PtdIns = Phosphatidylinositol

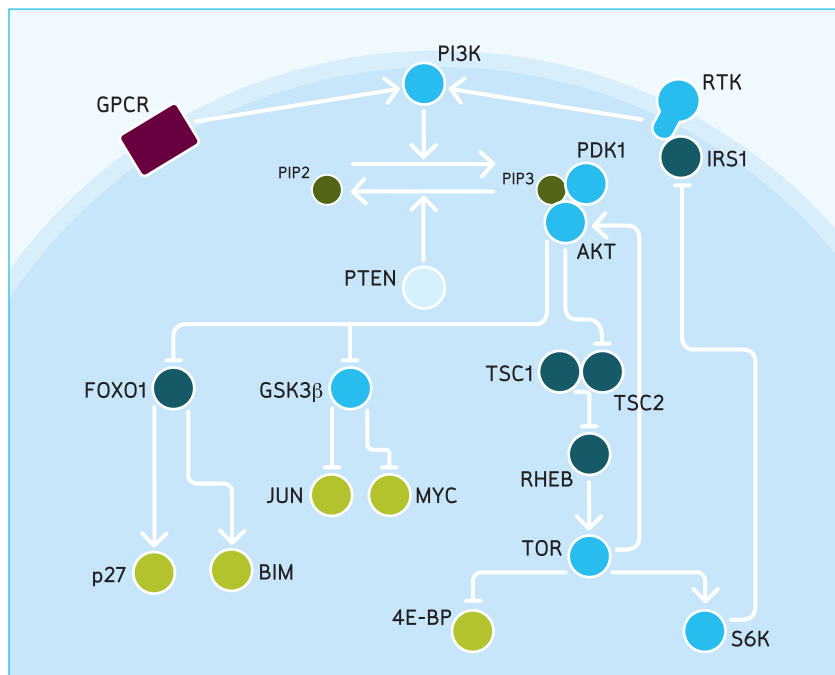
Class I	Class II	Class III
(p110 $\alpha, \beta, \delta, \gamma$)	(PI3KC2 α, β, γ)	(Vps34p)
Substrates:		
PtdIns	PtdIns	PtdIns
PtdIns 4-P	PtdIns 4-P	
PtdIns (4,5)-P ₂		
Products:		
PtdIns 3-P (PIP)	PtdIns 3-P (PIP)	PtdIns 3-P (PIP)
PtdIns 3,4-P ₂ (PIP ₂)	PtdIns 3,4-P ₂ (PIP ₂)	
PtdIns 3,4,5-P ₃ (PIP ₃)		

Figure 3. Domain Structure of p85

SH2 = Src-homology 2 Domain BCR = BCR Homology Domain
 SH3 = Src-homology 3 Domain iSH2 = Inter-SH2 Domain
 PP = Poly-proline Stretches



Figure 4. Canonical PI3K Signaling Pathways



Class I PI3K controls numerous cellular activities that are connected to growth, replication and survival, differentiation, movement and invasiveness, immune signaling and metabolism.

directed toward FOXO1, GSK3 β and mTOR⁽¹⁰⁾. Akt-mediated phosphorylation of FOXO1 leads to the inactivation of this proapoptotic protein and a downregulation of its growth-attenuating targets, p27 and BIM.^(11,12) Akt-dependent phosphorylation also inhibits GSK3 β ⁽¹³⁾ and lifts the GSK3 β -mediated inhibition of Jun⁽¹⁴⁾ and of Myc⁽¹⁵⁾. In a third signaling chain, Akt phosphorylates and thereby inhibits the TSC2 protein downregulating its GTPase-activating functions that negatively regulate the Ras-like protein Rheb. The result is activation of Rheb and of the downstream kinase mTOR⁽¹⁶⁾. mTOR has multiple functions; among them is the enhancement of protein synthesis.⁽¹⁷⁾ This is accomplished by activating the positive regulator of protein synthesis, p70S6K, and interfering with the negative regulator, 4E-BP^(10, 16, 17). The mTOR-mediated stimulation of protein synthesis preferentially affects the translation of mRNAs that contain complex 5' untranslated regions. The mRNAs of many growth-promoting proteins belong in this category. The overall effect of these PI3K signals is an upregulation of growth-stimulatory and a reduction of growth-inhibitory cellular functions. Figure 4 presents selected examples of the multiple cellular activities controlled by PI3Ks. There are numerous others, notably metabolic activities that are not considered here.

PI3K SUBUNIT p110 AND ITS LINK TO CANCER

PI3K has been tied to cancer for a long time. The first sign of an involvement of PI3K in cancer was an association of lipid kinase activity with the viral oncoproteins, Src and middle T. In these complexes the PI3K regulatory subunit p85 functions as a connector, binding phosphotyrosine residues on the oncoproteins and

PI3K has been tied to cancer for a long time. The first sign of an involvement of PI3K in cancer was an association of lipid kinase activity with the viral oncoproteins Src and middle T. The oncogenic potential of p110 α was then clearly demonstrated by an avian retrovirus, ASV16, that carries a copy of the cellular gene coding for p110 α as an oncogene.

recruiting the catalytic PI3K subunit p110^(18, 19). The oncogenic potential of p110 α was then clearly demonstrated by an avian retrovirus, ASV16, that carries a copy of the cellular gene coding for p110 α as an oncogene. This viral version of p110 α is fused to viral sequences that direct the protein to the cell membrane, making it independent of upstream signaling and hence constitutively active⁽²⁰⁾. Membrane-localized p110 α is oncogenic.

Most human cancers show a gain of function in PI3K signaling⁽¹⁰⁾. The increased activity can result from changes in PI3K or in its downstream target Akt or in the PI3K antagonist PTEN. Akt can be overexpressed, amplified, or its level of activating phosphorylation can be increased. PTEN shows frequent loss of function in cancer, either by mutation or by transcriptional silencing. The regulatory or catalytic subunits of PI3K are often differentially expressed or mutated in cancer⁽¹⁰⁾. Of particular interest are specific mutations in PIK3CA, the gene coding for the catalytic subunit p110 α of PI3K⁽²¹⁾. These mutations occur in many cancers [Table 1] and together with other diverse experimental data they single out the α isoform of class I PI3K as a particularly important contributor to oncogenesis^(22-26, 27). However, the non- α isoforms of class I PI3K may have relevance for cancer as well⁽²⁸⁾. Although no cancer-specific

mutations have been identified in the non- α isoforms, they show oncogenic potential when overexpressed in avian cells⁽²⁸⁾, and may also become contributing factors in human cancer by differential expression.

IDENTIFYING "HOT SPOT" MUTATIONS

Some 20 to 30 percent of cancers of the breast, colon, prostate, and endometrium contain a mutated PIK3CA [Table 1]⁽²⁹⁾. Strikingly, about 80 percent of the mutations occur in one of three "hot spots" in the coding sequence of PIK3CA. Each of the "hot spot" mutations is a single nucleotide substitution leading to a single amino acid substitution⁽²¹⁾. The overwhelming prevalence of these three distinct mutations in cancer strongly suggests that p110 α carrying one of these mutations bestows the cell with a strong selective advantage in growth and survival. Indeed, p110 α with a "hot spot" mutation shows gain of function in enzymatic activity⁽³⁰⁾ and signals constitutively through Akt and mTOR. It can also induce oncogenic transformation of cultured cells and tumors in animals^(22, 23). The mutations in PIK3CA have thus all the attributes of "driver" mutations, responsible for neoplastic properties of the cancer cell that harbors them. Mutated p110 α has emerged as arguably the most promising cancer target currently available.

5 Salient Features of the "Hot Spot" Mutations in PIK3CA:

- Cancer-specific; not found in normal tissue
- Occurring at high frequencies
- Show a gain of function, which is far easier to correct than loss of function
- They are "driver" mutations
- Mutant protein is a kinase, readily targetable by small molecules

GAINING A DEEPER UNDERSTANDING OF CANCER AND ITS THERAPIES

Mutated p110 α offers unique opportunities for gaining a deeper understanding of the cancer process and for developing novel, highly targeted cancer therapies. Two of three "hot spot" mutations, E542K and E545K, map to the helical domain of p110 α , and the H1047R mutation is located in the kinase domain. Genetic analyses suggest that the kinase domain mutation induces an oncogenic gain of function by a molecular mechanism different from that of the helical domain mutations⁽³¹⁾. The p110 α protein carrying the kinase domain mutation H1047R functions independently of an interaction with Ras but requires binding to the regulatory subunit p85. Conversely, p110 α with one of the helical domain mutations, E542K or E545K still depends on Ras binding but no longer needs to interact with p85 [2]⁽³¹⁾. In contrast, wild-type p110 α is not oncogenic.

Mutated p110 α offers unique opportunities for gaining a deeper understanding of the cancer process and for developing novel, highly targeted cancer therapies.

Table 1. Incidence of Mutation in PIK3CA in Various Cancers.

(Compiled from the catalogue of somatic mutations in cancer www.sanger.ac.uk/genetics/CGP/cosmic/)

Primary Tissue	Percent Tumors with Mutations in p110 α
Endometrium	23
Prostate	29
Breast	27
Colon	23
Urinary tract	17
Liver	12
Ovary	8
Stomach	8
Oesophagus	7
Pancreas	6
Brain	5
Lung	3
Hematopoietic tissue	4

Table 2. Two Different Mechanisms for the Gain of Function in Helical and Kinase Domain Mutations of p110 α .

	Requirements for Oncogenicity in Cell Culture	
	Binding to Ras	Binding to p85
p110 α E542K or E545K	Yes	No
p110 α H1047R	No	Yes

Thus it is already clear that the mutant enzyme behaves very differently from wild-type and that helical domain mutations are distinct from the kinase domain mutation. The substantial differences between mutant and wild-type enzyme augur well for the possibility of obtaining mutant-specific inhibitors of therapeutic quality.

CHALLENGES IN ADVANCING RESEARCH

The new opportunities offered by the cancer-specific mutations in PIK3CA depend largely on the availability of purified, recombinant mutant p110 α . This essential resource has not been commercially available until recently,

when Millipore first made PI3Ks available to the market. Prior to this, researchers had to set up in-house production, requiring insect cell culture, design of appropriate expression constructs, and co-expression of the regulatory subunit. All of this is achievable only with dedicated and experienced personnel and facilities for cell culture and protein purification at a significant expense. Production of the mutant enzymes may face additional challenges of protein expression levels and protein stability.

Millipore has applied its extensive experience in the production and purification of lipid kinases to meet the need for high-quality PI3Ks, including the prevalent cancer specific mutants of p110 α : E542K, E545K, and H1047R.

About the Author

Peter K. Vogt has worked on the replicative strategies and oncogenic functions of tumor-inducing retroviruses. In the course of these studies, he discovered viral oncogenes and their cellular counterparts. His work now concentrates on dominant oncogenes in human cancer, notably on phosphatidylinositol 3-kinase, one of the most promising therapeutic targets currently available.

Acknowledgment: The author thanks Marco Gymnopoulos for critical discussions.

References

1. Wymann, M.P.; Pirola, L. *Biochim Biophys. Acta* **1998**, *1436*, 127-150
2. Vanhaesebroeck, B.; Waterfield, M.D. *Exp. Cell Res.* **1999**, *253*, 239-254
3. Fruman, D.A.; Meyers, R.E.; Cantley, L.C. *Annu. Rev. Biochem.* **1998**, *67*, 481-507
4. Vanhaesebroeck, B.; Leever, S.J.; Panayotou, G.; Waterfield, M.D. *Trends Biochem. Sci.* **1997**, *22*, 267-272
5. Jimenez, C.; Jones, D.R.; Rodríguez-Viciana, P.; Gonzalez-García, A.; Leonardo, E.; Wennström, S.; von Kobbé, C.; Toran, J.L.; R-Borlado, L.; Calvo, V.; Copin, S.G.; Albar, J.P.; Gaspar, M.L.; Diez, E.; Marcos, M.A.; Downward, J.; Martínez-A, C.; Mérida, I.; Carrera, A.C. *EMBO J.* **1998**, *17*, 743-753
6. Cantley, L.C. *Science* **2002**, *296*, 1655-1657
7. Engelman, J.A.; Luo, J.; Cantley, L.C. *Nat. Rev. Genet.* **2006**, *7*, 606-619
8. Otsu, M.; Hiles, I.; Gout, I.; Fry, M.J.; Ruiz-Larrea, F.; Panayotou, G.; Thompson, A.; Dhand, R.; Hsuan, J.; Totty, N., et al., *Cell* **1991**, *65*, 91-104
9. Hu, P.; Margolis, B.; Skolnik, E.Y.; Lammers, R.; Ullrich, A.; Schlessinger, J. *Mol. Cell Biol.* **1992**, *12*, 981-990
10. Bader, A.G.; Kang, S.; Zhao, L.; Vogt, P.K. *Nat. Rev. Cancer*, **2005**, *5*, 921-929
11. Medema, R.H.; Kops, G.J.; Bos, J.L.; Burgering, B.M. *Nature* **2005**, *404*, 782-787
12. Vogt, P.; Jiang, H.; Aoki, M. *Cell Cycle* **2005**, *4*, 908-913
13. Cross, D.A.; Alessi, D.R.; Cohen, P.; Andjelkovich, M.; Hemmings, B.A. *Nature* **1995**, *378*, 785-789
14. Wei, W.; Jin, J.; Schlisio, S.; Harper, J.W.; Kaelin, W.G. Jr. *Cancer Cell* **2005**, *8*, 25-33
15. Gregory, M.A.; Qi, Y.; Hann, S.R. *J. Biol. Chem.* **2003**, *278*, 51606-51612
16. Hay, N.; Sonenberg, N. *Genes Dev.* **2004**, *18*, 1926-1945
17. Fingar, D.C.; Salama, S.; Tsou, C.; Harlow, E.; Blenis, J. *Genes Dev.* **2002**, *16*, 1472-1487
18. Kaplan, D.R.; Whitman, M.; Schaffhausen, B.; Raptis, L.; Garcea, R.L.; Pallas, D.; Roberts, T.M.; Cantley, L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3624-3628
19. Sugimoto, Y.; Whitman, M.; Cantley, L.C.; Erikson, R.L. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 2117-2121
20. Chang, H.W.; Aoki, M.; Fruman, D.; Auger, K.R.; Bellacosa, A.; Tsichlis, P.N.; Cantley, L.C.; Roberts, T.M.; Vogt, P.K. *Science* **1997**, *276*, 1848-1850
21. Samuels, Y.; Wang, Z.; Bardelli, A.; Silliman, N.; Ptak, J.; Szabo, S.; Yan, H.; Gazdar, A.; Powell, S.M.; Riggins, G.J., et al., *Science* **2004**, *304*, 554
22. Bader, A.G.; Kang, S.; Vogt, P.K. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 1475-1479
23. Kang, S.; Bader, A.G.; Vogt, P.K. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 802-807
24. Isakoff, S.J.; Engelman, J.A.; Irie, H.Y.; Luo, J.; Brachmann, S.M.; Pearlman, R.V., et al., Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells. *Cancer Res.* **2005**, *65*, 10992-1000.
25. Ikenoue, T.; Kanai, F.; Hikiba, Y.; Obata, T.; Tanaka, Y.; Imamura, J.; et al., Functional analysis of PIK3CA gene mutations in human colorectal cancer. *Cancer Res.* **2005**, *65*, 4562-7.
26. Zhao, J.J.; Liu, Z.; Wang, L.; Shin, E.; Loda, M.F.; Roberts, T.M. The oncogenic properties of mutant p110 α and p110 β phosphatidylinositol 3-kinases in human mammary epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 18443-8.
27. Gymnopoulos, M.; Elsliger, M.A.; Vogt, P.K. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 5569-5574
28. Kang, S.; Denley, A.; Vanhaesebroeck, B.; Vogt, P.K. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 1289-1294
29. Vogt, P.K.; Kang, S.; Elsliger, M.A.; Gymnopoulos, M. *Trends Biochem. Sci.* **2007**, *32*, 342-349
30. Carson, J.D.; Van Aller, G.; Lehr, R.; Sinnamon, R.H.; Kirkpatrick, R.B.; Auger, K.R.; Dhanak, D.; Copeland, R.A.; Gontarek, R.R.; Tummino, P.J., et al., *Biochem. J.* **2008**, *409*, 519-524
31. Zhao, L.; Vogt, P.K. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2652-2657

ABOUT MILLIPORE

Millipore's high-quality lipid kinases, assays and profiling services are helping today's leading researchers unlock the mystery of cancer.

Millipore's enzyme production experts and assay developers have many years of industry-leading research and validation experience. In fact, Millipore's collection of high-quality human and murine PI3 kinases represents the largest panel commercially available, and includes the most important cancer-specific PI3K α mutants. All are available for sale to researchers for in-house study, and most are available for

outsourced screening through the Millipore KinaseProfiler™ service, giving researchers all the tools they need to advance their work.

Homogeneous Time-Resolved Fluorescence (HTRF®) technology, co-developed by Millipore's assay developers, has been accepted as the preferred platform for PI3K studies. Millipore's PI3K HTRF 384 well assay kits, broad panel of validated PI3 kinases and outsourced screening and assay development solutions significantly lower the barriers to successful PI3 kinase drug development by eliminating the need to develop these proteins and assays in-house.

Features of Millipore's PI3 Kinase Products and Services:

- Largest panel commercially available, including clinically relevant PI3KA mutants
- Human and mouse orthologues
- HTRF assays available for all PI3 kinases
- Profiling available for most PI3 kinases on industry-leading KinaseProfiler service, featuring over 275 kinases
- FlexLabSM service offers access to Millipore's kinase experts for customized production and assay solutions

MILLIPORE OFFERS 16 LIPID KINASES FOR YOUR LIPID SIGNALING SOLUTIONS

Description	Species	Catalogue No.
PI3 Kinase (p110 α /p65 α)	Human	14-790
PI3 Kinase (p110 α /p85 α)	Human	14-602
PI3 Kinase (p110 α (E542K)/p85 α)	Human	14-782
PI3 Kinase (p110 α (E545K)/p85 α)	Human	14-783
PI3 Kinase (p110 α (H1047R)/p85 α)	Human	14-792
PI3 Kinase (p110 α /p65 α) •	Mouse	14-786
PI3 Kinase (p110 α /p85 α) •	Mouse	14-785
PI3 Kinase (p110 α (E542K)/p85 α) •	Mouse	14-791
PI3 Kinase (p110 α (E545K)/p85 α) •	Mouse	14-781
PI3 Kinase (p110 α (H1047R)/p85 α) •	Mouse	14-787
PI3 Kinase (p110 β /p85 α) •	Human	14-603
PI3 Kinase (p110 β /p85 α) •	Mouse	14-794
PI3 Kinase (p110 β /p85 β) •	Mouse	14-788
PI3 Kinase (p120 δ) •	Human	14-558
PI3 Kinase (p110 δ /p85 α) •	Human	14-604
PI3 Kinase (p110 δ /p85 α) •	Mouse	14-789

• Available for screening in Millipore's KinaseProfiler service

ADDITIONAL LIPID SIGNALING SOLUTIONS FOR YOUR DISCOVERY

Description	Catalogue No.
PI3 Kinase HTRF® Assay (384 assay points)	33-016
PI3 Kinase HTRF® Assay (5 x 384 assay points)	33-017
Wortmannin (PI3 Kinase inhibitor)	12-338
mTOR (1362-end), active	14-770
Anti-PI3 Kinase, p85	06-195
Anti-PI3 Kinase, p85, N-SH3, clone AB6	05-212
Anti-PI3 Kinase, p110 α	07-658
Anti-PI3 Kinase, p110 β	06-568
Anti-PI3 Kinase, p110 δ , clone AW103	05-703



www.millipore.com/offices

Millipore and Upstate are registered trademarks of Millipore Corporation.

KinaseProfiler and the M mark and Advancing Life Science Together are trademarks of Millipore Corporation.

FlexLab is a service mark of Millipore Corporation.

HTRF is a registered trademark of CIS BIO International Corp.

TB1096ENUS 08DD20 Printed in U.S.A.

© 2008 Millipore Corporation, Billerica, MA 01821 All rights reserved.

upstate

THE EXPERTISE OF UPSTATE®
IS NOW A PART OF MILLIPORE