**PIK3CA in cancer: the past 30 years**

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# **Abstract**

Almost thirty years ago, PI3K was discovered as a lipid kinase associated with certain oncoproteins. The first decade of research on PI3K saw the identification, purification and cloning of PI3K The second decade of research was noted for the identification of some of PI3K’s activators and effectors. This was accompanied by the discovery that PI3K acts as a retroviral oncogene. The third decade was known for the establishment of the direct involvement of PI3K in cancer, demonstrated by the identification of cancer-specific mutations. Efforts to target PI3K were on the rise from that moment on, accompanied by the first clinical trials for PI3K inhibitor therapies. In the fourth decade of research, PI3K-based cancer drugs will continue to emerge, as will new knowledge regarding other uncovered functions of this protein and pathway.

**Keywords:** PI3K, PIK3CA, P110α, cancer, mutations, gain-of-function, oncogenic transformation, AKT, PI3K inhibitors.

# **Introduction**

As one of the most studied and targeted oncogenes, PI3K has enjoyed a prominent and remarkable history. Reflecting back on more than 25 years of research, two major themes in PI3K research arise. First, the very fine-tuned regulation and the numerous activities of PI3K in cancer. Second, the multiple efforts for therapeutic targeting of PI3K in cancer, making PI3K one of the most prominent oncoproteins. The knowledge acquired during the past three decades of [lipid kinase](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/kinase) research demonstrates that PI3K participates in a remarkably broad range of cellular processes, including cell growth, proliferation1, apoptosis2,3, metabolism4-6, migration7,8, and secretion9,10. Moreover, deregulation in PI3K signaling contributes to a spectrum of human diseases11, like cancer12-14, immunological disorders11,15, [neurological disorders](https://www.sciencedirect.com/topics/neuroscience/nervous-system-disorder)16, [diabetes](https://www.sciencedirect.com/topics/neuroscience/diabetes)17, localized tissue overgrowth18,19, and cardiovascular disease20,21.

Here, we provide a brief overview of some key discoveries in the PI3K field and their impact as a hallmark of cancer, with a focus on *PIK3CA*, highlighting the evolution of the study of this protein, which started in basic research and nowadays encompasses basic, preclinical and clinical research. PI3K has become a very intense area of research, boasting over 36408 publications in PubMed, with new papers being published on the topic every day. There are still high expectations for a therapeutic impact of this pathway and progress in the clinic is being monitored. However, targeted therapies usually encounter road-blocks, which uncover many different unanswered questions and new dimensions of this pathway.

# **PI3K: the early years.**

In the mid-1980s, much of the attention of researchers focused on viral oncoproteins. During that time, several research groups found that some viral oncoproteins associated with a cellular lipid kinase activity that used phosphatidylinositols (PtdIns) as substrates22-25. Moreover, it was shown that the transformation of cells *in vitro* by these oncoproteins was dependent on the PtdIns kinase activity of these oncoproteins24,26. In 1988, the Cantley and Downes groups discovered that this oncoprotein-associated kinase is, in fact, a PtdIns 3-kinase, since it phosphorylates the 3-OH group of the inositol ring in PtdIns and is responsible for the generation of phosphatidylinositol-3-phosphate (PtdIns(3)P)27 in intact cells. This discovery was followed by the finding of PtdIns-3,4,5-P3 in GPCR-stimulated neutrophils28. A follow-up paper29 by Cantley and collaborators showed that the growth factor PDGF stimulates this kinase to produce PtdIns-3,4-P2 and PtdIns-3,4,5-P3 in stimulated cells. Insulin was also found to activate the PtdIns 3-kinase30, or as it more commonly called— PI3K.

In the early 1990s, several groups set to purify PI3K from mammalian cells (Figure 1. Timeline). These groups found that PI3K activity correlates with the presence of ~110 kDa (p110) and ~85 kDa (p85) proteins31-34. Back then, it was not known that PI3K is a heterodimer made of p110, the catalytic subunit, and p85, the regulatory one in the case of class I PI3Ks. In fact, p85 was first found to be associated with oncoproteins and RTKs26,35, leading scientists to initially propose that p85 is PI3K. However, the cloning of p85 in 1991 by several groups showed that p85 does not have any intrinsic PI3K activity. Instead, it has a SRC homology 3 (SH3) domain and two SH2 domains36-38. Protein microsequencing in 1992 allowed for the design of probes to isolate and clone the cDNA of the PI3K catalytic subunit p110α39, which demonstrated that, indeed, it is p110 that has the intrinsic PI3K activity in the p110-p85 complex.

Many efforts by various laboratories revealed the existence of other PI3K isoforms in mammals and other organisms and plants40-49.These findings identified PI3Ks as an evolutionarily conserved family of enzymes that, based on their structure and biochemical properties, can be divided into three classes50-52: Class I PI3Ks; class II PI3K-C2 kinases; and class III vps34p. Eight isoforms of PI3K exist in mammals (Class IA: p110α, p110β, p110δ; class IB: p110γ; class II: PI3K-C2α, PI3K-C2β, PI3K-C2γ; and class III: vps34p). Our focus in this review will be on p110α.

The discovery of two PI3K inhibitors in the mid-1990s, Wortmannin53-57 and LY29400258, greatly promoted PI3K research, especially the identification and characterization of different roles of this protein.

In 1993, several groups working on PI3K started to reveal that PI3K binds to specific domains in proteins, which, in turn, affects the localization, conformation and activity of these effector proteins. The first domain to be identified was the Pleckstrin Homology (PH) domain, which can bind phosphoinositides59,60. This domain is also common in class I PI3K effectors. One key PI3K effector that was identified to contain a PH domain is AKT59,60. It was shown that PtdIns-3,4-P2 and PtdIns-3,4,5-P3 bind directly to the PH domain of AKT, which causes the phosphorylation of AKT on Thr308 by PDK161-63. Additional phosphorylation of AKT on Ser473 by mTORC2 is also necessary for its activity64,65. AKT turned out to be a very complex protein, with numerous downstream substrates66, such as BAD67,68, p2169,70, p2771-73, FOXOs74,75, TSC276-78, GSK367, AS16074,79, and many more.  AKT was also revealed to have multifunctional downstream signaling nodes, which greatly expand the repertoire of AKT functions in cell proliferation, survival and motility66,80 (Figure 2). While AKT has been the most studied effector of PI3K, many questions on its phosphorylation, activation, regulation and function remain unanswered. The canonical PI3K signaling cascade proceeds through AKT, the TSC (tuberous sclerosis complex), RHEB (Ras homolog enriched in brain) to TOR and from there to additional targets52. In this pathway, in addition to AKT, TOR also has a significant role in oncogenesis. In fact, TOR, a PI 3-kinase related protein kinase (PIKK), is essential because it functions as an integrator that receives input from multiple sources and is in charge of many cellular processes 81,82.

The first realization that PI3K is directly involved in transformation and tumorigenesis can be traced back to 1997, when the gene encoding p110α, the catalytic subunit of PI3K, was identified as a cell-derived oncogene in an avian retrovirus and shown to be constitutively activated by N-terminal fusion to viral sequences83. This scientific achievement unveiled the direct oncogenic potential of p110α. An additional great discovery from 1997 was that PTEN is a 3-phosphatase for PtdIns(3,4,5)P384. *PTEN* is one of the most frequently inactivated tumor suppressors in many cancers, and its inactivation leads to a constitutive activation of PI3K85-87. While the oncogenic nature of p110α was determined in 1997, it took until 2004 to identify cancer-specific mutations in the gene that codes for p110α, *PIK3CA12,13*.

# **Oncogenic alterations of *PIK3CA* in human cancers**

**Hot-spot mutants in different cancers.**

The discovery of cancer-specific mutations in *PIK3CA* moved PI3K into the spotlight in 2004 as a key driver of cancer and a potential drug target. Back then, Samuels *et al*12 used a high-throughput sequencing approach to sequence all the PI3K genes in a panel of 35 colorectal cancers and corresponding normal tissues. Sequencing of the exons encoding the kinase domain of all 16 members of the PI3K family showed that *PIK3CA* was the only gene to harbor somatic (i.e., tumor specific) mutations. Sequencing the rest of the gene in 199 additional colorectal cancers revealed that *PIK3CA* is somatically mutated in 32% of cases. All but three of the alterations were heterozygous and no truncating or nonsense alterations were found, which is consistent with the mutational signature of oncogenes.

Importantly, over 80% of the somatic missense mutations were found in the kinase and helical domains of the PIK3CA subunit. This discovery of hotspot mutations brings to mind other hotspot alterations found in other oncogenes, like *KRAS*88 and *BRAF*89.

These *PIK3CA* mutations occur at frequencies extending from 1–46% in several common cancers (Table 1.), with endometrial, breast, bladder, cervical and colorectal cancers taking the lead in terms of number of mutations in this gene. It is now appreciated that mutations of *PIK3CA* are found in 24-46% of endometrial cancers90-92, 20-32% of breast cancers93,94, 20-27% of bladder cancers95-97, 14-23% of cervical cancers98-100, 13-28% of colorectal cancers12 and 12-15% of head-and-neck cancers101-103. Importantly, in breast and endometrial cancers, p110α is the most frequently mutated protein.

The PIK3CA mutations are found to be concentrated in three hot spots in the coding sequence12. Two of these hot spots are located in the helical domain of p110α and the third is situated in the catalytic domain (Figure 3). These hot spot mutations are single nucleotide substitutions that lead to the amino acid substitutions E542K, E545K and H1047R. The preferential mapping of cancer-specific mutations to hot spots suggested immediately a strong positive selection for such mutations, possibly reflecting a powerful replicative advantage of mutant-carrying cells. Many studies have shown that these hotspot mutations induce a gain-of-function compared to the wild-type protein and prompt transformation and tumorigenicity104-106.

Chang *et al* 107 in 2016 developed a statistical algorithm to identify recurrently mutated residues in tumor samples. They applied the algorithm to 11,119 human tumors, spanning 41 cancer types, and identified 470 hotspot somatic substitutions in 275 genes. Notably, they found new hotspot mutations in *PIK3CA*, in addition to the three hotspots E542K, E545K and H1047R (Figure 3. In orange). This was followed by another study investigating alterations in the PI3K/AKT/mTOR pathway on 11,219 human cancer samples across 32 major cancer types. They were also able to identify new hotspots in the *PIK3CA* gene108.

Although missense *PIK3CA* mutations are common across different cancer types (Table 1.), they tend to differ greatly with respect to total mutation frequency and domain specificity. A study109 of the mutational landscape of more than 3000 cancers across 12 major cancer types from The Cancer Genome Atlas (TCGA) program ranked *PIK3CA* as the second most commonly mutated oncogene, detected in more than 10% of patients in eight types of cancer. Interestingly, they found that kinase domain mutations account for >50% of breast cancer PIK3CA mutations, while mutations in the helical domain predominate in head-and-neck and lung squamous carcinomas. Then, they used the PARADIGM algorithm, which integrates gene expression and copy number data into a superimposed pathway structure, to infer the activities of ∼13K pathway features and compared the signaling consequences associated with different domain-specific PIK3CA mutations within the TCGA Pan-Cancer dataset. This pathway enrichment and sub-network analysis showed that kinase domain mutations might be linked more strongly with pathway features that enable cell cycle and proliferation (e.g., PLK1, FOXM1), whereas the helical domain mutations are more linked with features enabling cell motility and dissemination (e.g., Rho GTPases, GAP junction degradation). This might suggest that breast cancers preferentially mutate PIK3CA in the kinase domain to drive proliferation, while lung and head-and-neck cancers prefer the helical domain mutations’ benefit of malignant cell motility. More studies are still needed to answer these questions109.

Interestingly, the presence of these mutations in p110α does not strongly exclude the occurrence of other genetic alterations that cause enhanced PI3K signaling110,111. In fact, in endometrial cancer, mutations in *PTEN* and *PIK3CA* co-occur and can be found in the same tumor, possibly causing a synergistic effect110. Similarly, in lung adenocarcinomas, *PIK3CA* mutations occur concurrently with ones in *EGFR*, *KRAS* and *ALK*112.

In the original study where *PIK3CA* mutations were first identified, 76 premalignant colorectal tumors were also examined in order to determine the stage at which *PIK3CA* mutations occur. This effort identified only two mutations, both in advanced adenomas, suggesting that *PIK3CA* mutations arise late in tumorigenesis, just before or concurrent with invasion12. However, other studies on breast cancer reported the presence of *PIK3CA* mutations not only in metastatic lesions but also in primary ones 113,114. Other studies showed that *PIK3CA* deregulation is an early event in tumor evolution. This *PIK3CA* deregulation by mutation or amplification occurred before whole-genome doubling in colorectal adenocarcinomas115 and in lung squamous cell carcinomas116. Another study on a panel of 453 cancer driver genes found that mutations in *PIK3CA* are two times more frequently clonal than subclonal117. Therefore, the presence of *PIK3CA* mutations in primary lesions might be cancer-type dependent. In contrast, McGranahan *et al*118 used the TCGA dataset to perform a pan-cancer analysis of 2694 tumors across nine different cancer types to identify clonal and subclonal drivers that reflect the intratumor heterogeneity (ITH) of cancer. They found subclonal mutations in *PIK3CA*. This study highlights the fact that finding out which mutations are early clonal events and which mutations are later subclonal events is one of the challenges in precision medicine. In order to optimize therapeutic response, we have to know what proportion of tumor cells have the mutation, rather than finding out if the mutation is present or not.

Many reviews and studies note that there are no mutations in non-p110α isoforms, and reports of mutations in other p110 isoforms have been very limited. However, a few reports identified cancer mutations in *PIK3CB*, the gene that encodes for p110β. In these reports, *PIK3CB* mutations have been shown to be activating and oncogenic119-123. Moreover, heterozygous gain-of-function mutations in *PIK3CD*, the gene that encodes for p110δ, have been identified in Activated phosphoinositide 3-kinase δ syndrome (APDS)124 and in senescent T cells, lymphadenopathy and immunodeficiency (PASLI) disease125,126. It is not known why the gene encoding for p110α is so selectively mutated in cancer. It is also worth mentioning that there are cancer-specific mutations in p85α, the regulatory subunit of PI3Kα. Somatic mutations in *PIK3R1*, the gene that encodes for p85α, have been identified in several different cancers, especially in endometrial cancer, glioblastoma and low-grade glioma127-130. The very huge amount of mutations directed at PI3K signaling, including PTEN, PIK3CA, PIK3R1, and several other upstream receptor tyrosine kinases, makes this pathway one of the most deregulated and druggable pathways in cancer.

In addition to the identification of somatic mutations, *PIK3CA* was found to be amplified in a number of cancers131-133 (Table 2.). *PIK3CA* is amplified in 33-42% of lung cancers, 15-27% of ovarian cancers, 21% of neuroendocrine prostate cancers, 14-19% of esophageal cancers, 10-17% of cervical cancers, and 2-16% of head-and-neck cancers. The functional role of *PIK3CA* amplification remains unknown.

# **Rare cancer-specific mutations in PIK3CA also show gain of function**

The hot spot mutations account for about 80% of the mutated *PIK3CA* genes in cancer. However, there are also rare cancer-specific mutations that occur at lower frequencies134. Almost 100 rare mutations have been identified, but only a few of them were assessed for their transformation ability. These cancer specific mutations map over the entire coding sequence of PIK3CA, with the exception of the RAS-binding domain (Figure 3.). Most of these rare mutations are also single nucleotide substitutions. Gymnopoulos *et al.* investigated 17 rare point mutations, which led to the surprising finding that the vast majority of them (16 out of 17) also show a gain of function134, but a much smaller one than that of the hotspot mutants. The proteins of the rare-mutants show lower enzymatic activity, mediate lower levels of downstream phosphorylation and induce decreased oncogenic transformation in cell culture-features that might explain the low frequencies at which these rare mutants are found in cancer. Other rare mutations were found in other studies as well108,135 (Figure 3).

**Biological and biochemical effects of the hot-spot mutants**

Many cell-based and biochemical analyses done by different groups have confirmed that the hotspot mutations in PIK3CA induce a gain-of-function compared to the wild-type protein. The PIP3-generating lipid kinase activity of the mutants is increased several folds and downstream signaling is no longer dependent on upstream stimulation by growth factors. The signaling is constitutive and operates in serum-starved cells104-106,136,137. These PIK3CA mutants stimulate the oncogenic transformation via constitutive activation of p110α105,138,139, constitutive phosphorylation of AKT T308 and S473 and p70 S6 kinase. They also induce oncogenic transformation of human mammary epithelial cells, primary chicken embryo fibroblasts and NIH3T3 cells. The mutant-transformed cells are capable of anchorage-independent growth and show reduced dependence on growth factors and enhanced resistance to apoptosis104,105,138,139. Moreover, several studies using *in vivo* transformation experiments140 or genetically engineered mouse models (GEMMs) demonstrated the different roles of PIK3CA mutations in tumor initiation, progression and maintenance141-145. It is worth mentioning that when using a mouse model to activate the PIK3CA H1047R hotspot mutation in the heterozygous state, the mice either did not develop tumors or they developed tumors with a very long latency114,143,146. This suggests that heterozygous mutant PIK3CA is a poor oncogene on its own and this raises the possibility that a secondary defect in a co-regulator of the pathway is required for mutant PIK3CA to promote full transformation *in vivo*. Interestingly, the expression of wild-type p110α does not affect the growth behavior or even the morphology of the cell147. All these reports and data on the enhanced enzymatic activity, constitutive downstream signaling and oncogenic potency of PIK3CA mutants strongly suggest that the hot spot mutations function as “drivers” in human cancer, responsible for at least part of the oncogenic phenotype of the cancer cell148.

An interesting study in 2015 by the Vogt group showed the power of a single-base mutation, H1047R, to transform MCF10A cells. The introduction of only this single mutation induced massive remodeling of the cell toward the phenotype of mature basal breast cancer, and not those of other histological types of breast cancer 149. Additionally, they demonstrated that in this particular case of a cancer-specific mutation in PIK3CA, the proteome and transcriptome of the breast epithelial cell line MCF10A acquired a gene signature that resembles that of mature basal breast cancer. It appears that MCF10A cells are preprogrammed to respond to a PI3K gain-of-function mutation by activating a definitive pattern of phenotypic changes that are characteristic of one particular histological type of breast cancer. As all these changes were caused by one mutation in PIK3CA, the authors thought it fitting to call this effect the *butterfly effect*149.

The *PIK3CA* gene, which encodes for the p110α subunit of PI3Kα, has five domains: an N-terminal domain called p85-binding domain, a Ras binding domain, a domain called C2, which has been proposed to bind to cellular membranes, a helical domain and a kinase catalytic domain. In its basal state, p110α is bound to and inhibited by the regulatory subunit p85α150,151. This inhibition of p110α requires the presence of the nSH2 domain in p85α152. p85α also has five domains: an SH3 domain, a GAP domain, an N-terminal SH2 (nSH2) domain, an inter-SH2 domain (iSH2), and a C-terminal SH2 domain (cSH2)36,37. The iSH2 domain acts as a tether and binds the catalytic domain, while the nSH2 and cSH2 domains mediate the interactions between PI3Kα and the different tyrosine kinase receptors that activate it153.

The crystal structure of PI3Kα, determined by X-ray diffraction in 2007, aided in the elucidation of the architecture of p110α, the catalytic subunit. It also identified the contact regions between p110α and its regulatory subunit, p85α, as well as explained the mechanisms by which oncogenic mutations in p110α affect the protein and its activity154. This crystal structure demonstrated that the hotspot mutations at residues 542 and 545 in p110α abrogate the inhibitory effect of p85α’s nSH2 domain on p110α, and that the hot-spot mutation at residue 1047 directly affects the conformation of the activation loop of p110α, the result of which is a change in its interaction with other phosphatidylinositide substrates154.

Genetic experiments on p110α suggest the existence of several mechanisms and modes of action155. A combination of a kinase domain and helical domain hot-spot mutations in the same molecule was found to have a big synergistic effect on signaling and oncogenicity156.

Moreover, it was also found that kinase and helical domain mutations differ in their requirements for interaction with RAS (rat sarcoma virus oncoprotein homolog) and with p85. Ras has been previously shown to activate PI3K157. The helical domain mutations of PIK3CA depend on interactions with RAS for full oncogenic activity but are independent of binding to the regulatory subunit p85. The kinase domain mutation shows the opposite requirements. It is oncogenic and does not require RAS binding but fails to transform cells if the interaction with p85 is disabled158. This is consistent with a study that showed that H1047R mutants tend to retain and keep their transforming ability even when their interaction with RAS is artificially abolished by mutating the RBD158. Consequently, helical domain mutations reduce p85’s inhibition of p110α154,156,159,160 or even facilitate direct interaction of p110α with insulin receptor substrate 1 (IRS1)161 , whereas kinase domain mutations increase the interaction of p110α with lipid membranes154,160,162.

As gathered from the presented studies above, the oncogenic potential of p110α mutations is well documented in both *in vitro* and *in vivo* experimental systems*.* Concerning the non-alpha isoforms of class I PI3K, the connection to cancer is more distant and sketchier. It is worth mentioning that PI3Kδ has been found to play a role in lymphoid and myeloid malignancies163-167. Interestingly, inhibition of this isoform by PI3K inhibitors that more specifically inhibit p110δ, like idelalisib, copanlisib and duvelisib are in clinical trials for hematopoietic malignancies168-171 (http://clinicaltrials.gov). Moreover, the wild-type non-alpha isoforms have the ability to induce oncogenic transformation when overexpressed in cells, but the wild-type p110α does not have this ability147.

Since the hot-spot mutations in PIK3CA account for about 80% of all cancer-specific mutations, inhibitors against these specific mutations could benefit the majority of affected patients. In this regard, the catalytic subunit p110α provides a rationale for the development of PI3Kα inhibitors.

# **Therapeutic targeting of PI3K**α **in cancer**

Discovered in 1993, the first PI3K inhibitor was the mold metabolite Wortmannin54-56, which is a selective and cell-permeable PI3K inhibitor. Eli Lilly generated in 1994 the first synthetic inhibitor of PI3K and named it LY29400258. These two pan-PI3K inhibitors were initially used by the research community to identify and study new PI3K functions. The problem with these inhibitors was that they lacked specificity. The development of new PI3K inhibitors was greatly aided by the data provided for the crystal structures of PI3Kα and PI3Kα with Wortmannin.

**Pan-PI3K inhibitors**

Pan-­PI3K inhibitors block the catalytic activity of all four PI3K class I isoforms: PI3Kα PI3Kβ, PI3Kγ, and PI3Kδ. While they exhibit a broad activity in a number of different tumor types with different molecular alterations (Table 3.), the Pan-­PI3K inhibitors’ broad range of activity might result in off-target effects and toxicities127. Numerous clinical trials with pan-PI3K inhibitors were conducted (typically in combination with known anti-cancer drugs), including buparlisib in breast cancer172-174 and in advanced solid tumors175,176, pictilisib in breast cancer177,178 and other advanced solid tumors, pilaralisib in advanced-stage solid tumors and breast cancer179-182, copanlisib in advanced-stage cancers and lymphomas183,184, PX­866 in advanced-stage solid tumors185-187, glioblastoma188, head and neck carcinomas189-191, and colorectal cancer192, NSCLC and other advanced tumors191, CH5132799 in metastatic solid tumors193, ZSTK474 and SF1126 in advanced-stage solid tumors194,195. While some promising results were obtained, all the pan-PI3K trials reported notable level of toxicity, many with severe (grade ≥3) AEs, such as on­-target hyperglycemia, diarrhea, rashes, liver toxicities, stomatitis, transaminitis, creatinine kinase elevation, and several patients even had severe psychological responses, such as anxiety and depression, leading to suicide attempts127,196. These AEs were the main cause of the discontinuation of many of the pan-PI3K trials.

**PI3Kα inhibitors.**

Isoform-specific inhibitors have been developed to target cancers that are addicted to one of the PI3K isoforms. These isoform specific inhibitors are also expected to have a wider therapeutic index and fewer off-target toxicity. In fact, Idelalisib, a selective δ-isoform inhibitor, is the first PI3K inhibitor to be approved in Europe and the US for the treatment of chronic lymphocytic leukemia, follicular B-cell non-Hodgkin lymphoma and small lymphocytic leukemia196-198. A number of PI3Kα-specific inhibitors have been investigated in clinical trials (Table 4.).

Alpelisib (BYL719) is the first oral PI3Kα-specific inhibitor. Alpelisib has excellent drug-like properties and its in-vivo administration results in antitumor efficacy in mice harboring PIK3CA-mutant tumor xenografts199.

Alpelisib was first tested in an initial phase I (NCT01219699)200 trial on 134 patients with advanced solid tumors with *PIK3CA* alterations. Patients were given once-daily or twice-daily oral alpelisib. Grade 3 or 4 AEs occurred in ~42% of patients and hyperglycemia, which is an on‑target AE of PI3Kα inhibitors, accounted for more than half of the grade 3 or 4 treatment-related toxicities (51.5%)196,200. Still, alpelisib demonstrated a tolerable safety profile and promising clinical activity in patients with PIK3CA alterations. One patient with endometrial cancer had a CR and seven patients with cervical, breast, endometrial, colon and rectal cancers had PRs.

In another study (NCT01791478), alpelisib was tested in combination with the aromatase inhibitor letrozole in patients with ER+/HER2-negative metastatic breast cancer refractory to endocrine therapies201. The objective of this phase Ib trial was to determine the safety and tolerability of the combination of inhibitors. 26 patients were enrolled. Hyperglycemia (in 62% of patients), fatigue, gastrointestinal disorders and rash were the most common side effects. Grade 3 or 4 AEs associated with alpelisib were uncommon and generally cumulative201. Four of five patients (80%) who had a PR harbored *PIK3CA*-mutant tumors. Two of seven patients who had durable PRs or stable disease lasting >6 months harbored with tumors with *PIK3CA* exon 9 mutations. It is worth mentioning that all patients with FGFR1 or FGFR2 amplifications and/or KRAS and TP53 mutations had progressive disease on this treatment.

Alpelisib was also tested in combination with the BRAF inhibitor encorafenib and with the anti-EGFR monoclonal antibody cetuximab (NCT01719380)202. This trial was based on initial observations and some preclinical data showing that activation of EGFR and PI3K contributes to resistance to BRAF inhibitors in *BRAF*-mutant colorectal cancer203,204. In this clinical trial I, both the dual-combination (Encorafenib and cetuximab) and the triple-combination (Encorafenib, cetuximab and alpelisib) showed clinical efficacy and acceptable safety profiles in patients with *BRAF*-mutant mCRC202.

Given the emergence of increasing evidence that the PI3K pathway plays a key role in resistance to anti-HER2 therapies, a phase I study was completed to assess and include safety and activity of alpelisib with trastuzumab emtansine (T-DM1) in 17 trastuzumab- and taxane-resistant HER2-positive metastatic breast cancer patients (NCT02038010)205. The most common AEs were hyperglycemia (53%), fatigue, nausea and rash. Grade 3 rash occurred during cycle 1, which resolved with interruption and subsequent dose reduction of alpelisib and use of steroids. Grade 3 hyperglycemia was reversible with oral anti-diabetic treatment. One Grade 4 AE occurred (thrombocytopenia) likely due to T-DM1.  The results of the trial indicate that combination of alpelisib and T-DM1 appears to be safe in HER2-positive MBC patients with significant anti-tumor activity, even in patients previously treated with T-DM1. There are plans to start a phase II study205.

Alpelisib is undergoing continued testing in combination with a range of targeted therapies and chemotherapies in multiple phase I and phase II studies involving patients with a diverse range of cancers, including cohorts with or without stratification according to PIK3CA mutation status. One of the ongoing clinical trials is SOLAR-1 (NCT02437318). SOLAR-1 is a global phase III randomized trial investigating the clinical efficacy of alpelisib (BYL719) plus fulvestrant vs. fulvestrant alone in HR+/HER2- advanced breast cancer patients with PIK3CA mutations who progressed on or following treatment with an aromatase inhibitor with or without a CDK4/6 inhibitor206. The primary endpoint is PFS for patients with the PIK3CA mutation. Secondary endpoints include: overall survival, overall response rate, clinical benefit rate, health-related quality of life, efficacy in PIK3CA non-mutant cohort, safety and tolerability.

A recent study showed the dual mechanism of action of alpelisib *in vitro* in a *PIK3CA* mutant breast cancer cell line. Alpelisib displayed a dual mechanism of action by inhibiting p-AKT and by inducing a decrease in p110α protein levels in a dose-dependent manner. Interestingly, p110α degradation is more pronounced at alpelisib concentrations producing strong PI3K (80%) inhibition207.

Taselisib is referred to as β-sparing, exhibiting equipotent inhibition of p110α, -γ and -δ, but inhibits p110β with 30-fold lower potency196. It has a dual mechanism of action, both blocking PI3K signaling and inducing a decrease in mutant p110α protein levels208,209. The greater isoform selectivity of taselisib has been postulated to improve its efficacy in tumors driven by *PIK3CA* mutations compared with pan-class I PI3Kis, and indeed exposure to low doses of taselisib has been reported to suppress tumor growth in a xenograft model of tumors driven by *PIK3CA* mutations196,210,211.

The first in human study of taselisib involved 34 patients with solid tumors212. AEs seen were consistent with those observed with other PI3K inhibitors, including hyperglycemia, diarrhea, rash, and stomatitis; the most frequent grade 3 or 4 AEs were hyperglycemia (15%) and rash (12%). The ORRs were 36% for patients with *PIK3CA* ­mutant tumors and 0% for those without known activating *PIK3CA* mutations127,212. This trial supports the higher potency for taselisib against *PIK3CA* mutant cancers212.

Given the fact that approximately 40% of ER+ breast cancers harbor PIK3CA mutations, and given the extensive cross-talk between the ER and the PI3K signaling pathways212,213, there is a strong rationale to study taselisib in combination with endocrine therapy. Phase Ib and II clinical trials showed that the combination of taselisib and fulvestrant is well tolerated with promising preliminary efficacy214,215. This led to a phase III clinical trial (SANDPIPER; NCT02340221) testing taselisib plus fulvestrant or placebo in postmenopausal women with ER+ metastatic breast cancer, with enrollment being enriched for patients with PIK3CA-mutant tumors. As of June 2018, 516 patients were randomized in the *PIK3CA* mutant population. The most common grade 3 AEs in the combination arm were diarrhea, hyperglycemia (10%), colitis and stomatitis. The combination of taselisib with fulvestrant significantly improved INV-PFS in patients with ER+, HER2-, PIK3CA mutant locally advanced or metastatic breast cancers216. Recently, Roche decided to abort its contender taselisib after reports at ASCO 2018 showed only a 2-month progression-free survival advantage for this drug combined with fulvestrant in the SANDPIPER phase III clinical trial217.

Taselisib was also tested in combination with letrozole vs. letrozole plus placebo in a randomized phase II study involving 334 patients with ER+, HER2­ negative early stage breast cancer (LORELEI; NCT02273973)218. The addition of taselisib to AI therapy was found to increase the ORR in all patients (50% versus 39%, P = 0.049) and even more so in 152 patients with *PIK3CA* mutations (56% versus 38%; P=0.033)127.

A new phase I basket study set to find out whether single-agent taselisib has activity in multiple *PIK3CA* mutant tumors (NCT01296555)219.

TAK-117 (also known as MLN1117 or INK1117) is another oral PI3Kα inhibitor. A first­ in human study of TAK-117 evaluated the safety, the maximum tolerated dose and the activity of this inhibitor as a single-agent in patients with advanced solid tumors (NCT01449370)220. 71 patients received oral TAK-117 once daily or 3 days per week. Among 61 patients evaluated for response, 53 patients had tumors with *PIK3CA*mutations and 4 patients (3 with breast cancer and 1 with gastric cancer) had PRs; notably, all these patients had *PIK3CA* ­mutant tumors127,220. Ongoing clinical trials are currently testing TAK­117 in combination with targeted therapies or chemotherapy in patients with diverse advanced-stage solid cancers.

# GDC-0077 is a new PI3Kα inhibitor that selectively degrades mutant PI3Kα in a proteasome-dependent fashion and therefore reduces PI3K pathway activity biomarkers as shown in preclinical studies221,222. A phase I study (NCT03006172) is underway to evaluate the safety, tolerability, and pharmacokinetics of GDC-0077 administered as a single agent in patients with locally advanced or metastatic *PIK3CA* mutant solid tumors, and in combination with letrozole, and in combination with letrozole and palbociclib, and in combination with fulvestrant, and in combination with fulvestrant and palbociclib, and in combination with fulvestrant, palbociclib and metformin.

Finally, a dual PI3Kα and BRAFV600E/K inhibitor, ASN003, showed strong antitumor activity in multiple xenograft models223,224, and then entered clinical testing in patients with advanced-stage solid tumors, and data from dose-escalation studies are expected to be published soon.

**Challenges and mechanisms of resistance to PI3Kα inhibitors.**

The fact that PI3K signaling was aberrantly activated by mutations or amplifications in the majority of human cancers, together with the presence of actionable target proteins in the PI3K/mTOR network, spurred expectations that PI3K/mTOR pathway inhibitors would be efficient in cancer therapy. However, most targeted therapies usually encounter road blocks. Four major factors have contributed to the underwhelming performance of the PI3K pathway inhibitors in the clinic11.

First, this pathway is activated via multiple cell surface receptors, and cancer cells have shown exceptional plasticity when it comes to amplifying some upstream mechanisms to maintain signal flow through the PI3K/mTOR pathway or even other compensatory pathways in the presence of inhibitors. For example, exposure to the inhibitors themselves causes the disruption of negative-feedback mechanisms that limit the activity of the pathway to a range compatible with normal cell physiology. Drug-induced interference with negative-feedback regulation can reduce PI3K/mTOR pathway inhibitor therapeutic activities in the absence of [genetic mutations](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/gene-mutation), a phenomenon termed adaptive resistance225. For example, one of the resistance mechanisms identified to alpelisib, a p110α-selective inhibitor, was the persistent activity of mTORC1. Interestingly, this resistance was reversed by combining everolimus, an mTORC1 inhibitor, with alpelisib226. Overexpression or aberrant activation of Pim1227, PDK1228, SGK120, SGK3229, AXL230 and other proteins presents a resistance mechanism to p110α-selective inhibitors. Combining a PDK1, SGK1, or AXL inhibitor with alpelisib showed better inhibition of the pathway228,230.

Second, intrinsic or acquired resistance to PI3K/mTOR pathway inhibitors is commonly associated with mutations or [copy-number](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/copy-number-variation) alterations of key regulatory genes within the PI3K pathway or even parallel oncogenic pathways or activation of growth factor receptors that stimulate both PI3K and [MAPK](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/mitogen-activated-protein-kinase) signaling. For example, loss of *PTEN* was found in metastatic breast cancer patients who initially responded to alpelisib, but later on progressed and died. *In vitro*, inducible *PTEN* knockdown in alpelisib sensitive cells resulted in resistance to the drug. Simultaneous blocking with a p110β selective inhibitor reversed this resistance *in vitro* and *in vivo*231. Another example is the amplification of *MYC* which is responsible for resistance to PI3K inhibitors in breast cancer232. MYC expression or MYC copy number is often increased in breast cancer and in lymphoid malignancies232,233. Stratikopoulos *et al*234,235 showed that in ER+ breast cancer cells, combination of a BET inhibitor, which interferes with myc-dependent transcriptional responses236, with a PI3K inhibitor had a synergistic antitumor effect in mammary tumors with PI3K-H1047R and myc transgene expression. Also, rebound PI3K activation by RTK expression was abolished by treatment with JQ1, a BET inhibitor in *PIK3CA* or *PTEN* mutant cells in breast, colorectal, prostate, brain and ovarian tumors11,234,235. In another study, it was also shown that combining pictilisib and a BET inhibitor had synergistic antitumor activities in patient-derived ovarian cell lines237.

Third, intratumor heterogeneity (ITH) contributes greatly to tumor growth, tumor evolution, and resistance to targeted therapy14,238,239. Next generation sequencing studies have shown the exact full extent of genomic ITH. The degree of ITH can be highly variable, with between 0 to over 8000 coding mutations found to be heterogeneous within primary tumors and metastatic sites239. For example, heterogeneous genetic alterations leading to PI3K pathway activation could be a major contributor to the limited efficacy of PI3K/mTOR pathway inhibitors, as distinct subclonal alterations might translate into variable levels of sensitivity to these drugs and, in turn, shifts in clonal dominance during PI3K/mTOR pathway inhibitor therapy11,14,231.

Lastly, isoform switching could also cause resistance to PI3K-isoform-specific inhibitors40,240-243. Therefore, both tissue of origin and genomic context influence PI3K isoform dependence in cancer cells, making the elucidation of potent and robust, predictive biomarkers for PI3K/mTOR pathway inhibitor responsiveness an overwhelming challenge for oncologists.

**The major problem with PI3K inhibitors: toxicity**

One of the key considerations when targeting PI3K or AKT for cancer treatment is how to manage the on-target toxicity to systemic metabolism. These dose-limiting toxicities prevent sufficient target engagement in tumor tissues to maintain pathway inhibition. In fact, many of these toxicity challenges to effective therapy with PI3K/mTOR pathway inhibitors, were anticipated based on the various roles that the PI3K/mTOR pathway plays in cell proliferation, tissue growth, metabolism, and other physiological functions. In fact, in addition to being mutated in human cancers, p110α has a role in mediating insulin responses in muscle, liver, and fat244-246. Because most p110α inhibitors that have entered clinical trials for solid tumors inhibit both the mutant and [wild-type](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/wild-type) p110α at therapeutic doses, these drugs induce acute insulin resistance, resulting in severe hyperglycemia, which, in turn, leads to severe hyperinsulinemia172. In tumors that express insulin resistance, this feedback might limit the therapeutic efficacy of these compounds, particularly in the setting of patients who are already insulin resistant247.

Therefore, the clinical success of PI3K-targeted therapies may be dependent either on identifying patient populations for whom the systemic metabolic impact of these compounds will not inhibit the therapeutic efficacy—perhaps patients whose tumors do not express IR/IGFR—or on implementing new ways to limit the hyperinsulinemia through diet or [drug combinations](https://www.sciencedirect.com/topics/neuroscience/combination-drug). In preference, drugs that have higher selectivity for mutant versus wild-type PI3K may bypass this systemic feedback. Both these approaches are expected to reduce the hyperglycemia and systemic metabolic disruptions that occur due to the on-target effects of compounds that inhibit the PI3K signaling cascade11,14,127,196.

The greatest challenge in the area of PI3K oncogenicity remains the identification of mutant-specific inhibitors with drug-like properties. The highly targeted therapeutic potential of such inhibitors justifies intense efforts by the industry and academic laboratories. One research group has attempted to target the interaction between IRS1 and mutant p110α (helical domain mutants) since p110α helical domain mutant proteins, but not kinase-domain mutated ones, directly associate with IRS1161. The inhibition of this association was efficient in decreasing the growth of tumors with these mutations248.

Finally, an interesting study by Venot *et al249* assessed the effects of alpelisib in 19 people with PROS disorders (PIK3CA-related overgrowth spectrum)250. In clinical trials on cancer, PI3K inhibitors are usually administered at the maximum tolerated dose. But in this study, which is not a clinical trial, authors used the lowest dose that had been tested in trials on cancer patients and not the maximum tolerated one. The authors saw very dramatic anatomical improvements in all adults and children and alpelisib was well tolerated by these recipients. Interestingly, only three people showed a modest elevation in blood-glucose levels and alpelisib did not have any effects on normal growth of the recipient children, suggesting that the low doses used of this PI3Ki are enough to only inhibit the overgrown tissue and not PI3K-dependent childhood growth250. The results of this study make us rethink the administration of very high doses of PI3K inhibitors used in cancer clinical trials. A low dose of PI3Ki might be effective in *PIK3CA* mutant tumors and might be enough to suppress and eradicate PI3K activity that is above the usual level without completely blocking PI3K signaling, as would happen with a high dose of PI3K inhibitor250.

# **The future.**

More than 25 years after its emergence from an academic niche, the PI3K pathway has now become the focus of basic, preclinical and clinical research. PI3Kα is mutated in numerous cancers, and biochemical analyses have shown that these mutations result in a constitutively active enzyme. This increased PI3Kα activity stimulates and activates downstream processes that control cell proliferation, growth, apoptosis and metabolism, as well as migration and adhesion. Structural data on PI3Kα has shed light on the mechanisms and modes of action of the different PI3Kα mutants. It is worth mentioning that the oncogenic phenotype of human cancer is the combination and mixture of all genetic, transcriptomic, epigenetic and proteomic changes. The long line of clinical trials focused on PI3K inhibitors reflects the complexity of this combination. To make matters even more complicated, some studies have shown the importance of PI3K post-translational modifications. More work and research are needed to clarify these modifications.

The next decade of research on PI3K should see a more detailed description of the roles of the PI3K protein and pathway in cancer metabolism, fertility and reproduction. The involvement of PI3K in other diseases and developmental disorders is also an interesting area worth exploring and should shed more light on the physiological roles of this key protein. There are surely many surprises that this protein has in store for us. What we believe nowadays might be modified in the future and this key gene and its protein will continue to teach us about the challenges and perplexities of cancer.

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**Conflict of interest**

The authors declare that there are no conflicts of interest.

**Legends of Figures and Tables**

Figure 1. Timeline; Key events in the PIK3CA research.

Figure 2. Summary of functional roles and effectors of PI3Kα. PI3Kα phosphorylates PtdIns(4,5)P2 in order to produce PtdIns(3,4,5)P3. The latter is a critical second messenger that recruits and activates different effectors by phospholipid-binding pleckstrin homology domains. Shown here, is AKT, a key effector protein of PI3Kα. PtdIns(3,4,5)P3 bind to AKT, which translocate AKT to the plasma membrane and allows its phosphorylation on Thr308 by phosphoinositide-dependent kinase 1 (PDK1), and on Ser473 by mammalian target of rapamycin complex 2 (mTORC2). AKT in turn, has many downstream targets and effectors that propagate and amplify the PI3K signals that determine multiple and various cellular functions as indicated.

Figure 3. P110α somatic mutations in cancer. A schematic representation of the p110α protein, consisting of a p85 binding domain, a RAS binding domain, a C2 domain, a helical domain and a kinase domain. Bars do not represent the frequency of mutations. The mutations in red are the three most frequent hotspot mutations, in orange are other hotspots and in blue are other recurrent non-hotspot mutations or rare mutations. Mutants that show transformation are marked with . Mutants from cBioPortal and other studies107,108,111,134.

Table 1. PIK3CA mutations in cancer. As reported in cBioPortal on 15 August 2018. Data sets with >100 patients and cancer types with a frequency of alteration >1% are included in this table.

Table 2. PIK3CA amplifications in cancer. As reported in cBioPortal on 15 August 2018. Data sets with >100 patients and cancer types with a frequency of alteration >10% are included in this table.

Table 3. Selected Pan-PI3K inhibitors that are approved for clinical use or are under clinical development in cancer treatment. Data taken from a search of Clinicaltrials.gov in October 2018.

Table 4. Selected PI3Kα-specific inhibitors that are approved for clinical use or are under clinical development in cancer treatment. Data taken from a search of Clinicaltrials.gov in October 2018

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