REVIEW

Targeting PI3K in Cancer: Impact on Tumor Cells, Their Protective Stroma, Angiogenesis, and Immunotherapy

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ABSTRACT The PI3K pathway is hyperactivated in most cancers, yet the capacity of PI3K inhibitors to induce tumor cell death is limited. The efficacy of PI3K inhibition can also derive from interference with the cancer cells' ability to respond to stromal signals, as illustrated by the approved PI3Kδ inhibitor idelalisib in B-cell malignancies. Inhibition of the leukocyte-enriched PI3Kδ or PI3Kγ may unleash antitumor T-cell responses by inhibiting regulatory T cells and immune-suppressive myeloid cells. Moreover, tumor angiogenesis may be targeted by PI3K inhibitors to enhance cancer therapy. Future work should therefore also explore the effects of PI3K inhibitors on the tumor stroma, in addition to their cancer cell–intrinsic impact.

Significance: The PI3K pathway extends beyond the direct regulation of cancer cell proliferation and survival. In B-cell malignancies, targeting PI3K purges the tumor cells from their protective microenvironment. Moreover, we propose that PI3K isoform–selective inhibitors may be exploited in the context of cancer immunotherapy and by targeting angiogenesis to improve drug and immune cell delivery. *Cancer Discov; 6(10); 1090–105. ©2016 AACR.*

INTRODUCTION

Pathologic activation of the PI3K pathway is among the most frequent signaling events associated with cellular transformation, cancer, and metastasis (1–4). This is exemplified by the frequent activating mutations in *PIK3CA* and the loss of *PTEN* functionality in common cancers, such as those of the breast, colon, and ovaries. A significant pharmaceutical effort is therefore dedicated to inhibiting the PI3K pathway within cancer cells, and this is starting to yield some positive results in combination trials.

However, in other cancer types, such as lung and pancreatic cancers, mutations that activate the PI3K pathway are less common. Mutational activation of the PI3K pathway is also rare in B-cell malignancies, such as chronic lymphocytic leukemia (CLL) and indolent non-Hodgkin lymphoma (iNHL), yet a PI3K pathway inhibitor (idelalisib, an inhibitor of PI3Kδ) was recently approved as a therapy for these diseases. A major component of the mechanism of action of PI3Kδ inhibition in these B-cell malignancies is to dampen the responsiveness of the tumor cells to supportive stimuli from the microenvironment (5).

The impact of PI3K inhibition on the tumor stroma (6) is underinvestigated. The stroma can be defined as any cell that forms part of the tumor mass but that is not malignantly transformed. Typically, the stroma will include (i) the vasculature, (ii) infiltrating immune cells, (iii) fibroblasts and connective tissue. Emerging evidence indicates that PI3K activity has an important role in regulating each of these stromal elements, which could be exploited therapeutically.

In this review, we first summarize the key observations made to date on PI3K intervention in cancer and provide some examples of ongoing trials that combine PI3K inhibitors with other agents. We next concentrate on the emerging indications for the use of PI3K inhibitors to target the cancer stroma, with a special focus on immune modulation. For detailed overviews of the PI3K signaling pathway, PI3K inhibitors, and ongoing clinical trials, we refer the reader to recent reviews (1–4, 7).

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PI3K PATHWAY INHIBITION IN CANCER: LESSONS LEARNED TO DATE

Preclinical and clinical experience with PI3K inhibitors in cancer has provided important insights, which can be summarized as follows.

First, despite PI3K signaling being commonly activated in tumor cells, PI3K inhibitors have shown modest single-agent therapeutic efficacy only in solid tumors. This could be due to various reasons, including non–PI3K-selectivity/off-target effects of some of the PI3K inhibitors used, insufficient target inhibition, intrinsic and acquired drug resistance, and tolerability. Pan–class I PI3K inhibitors show serious adverse effects upon long-term continuous dosing, which limits the on-therapy time (reviewed in ref. 8). Emerging data indicate that isoform-selective PI3Kα inhibitors may have a more favorable safety profile than pan–class I PI3K inhibitors (9). Alternative dosing schedules are also being explored, as evidence from preclinical models suggests that transient, complete PI3K pathway interruption can increase the therapeutic index without compromising therapeutic efficacy (10, 11).

Second, cancer cells are very effective at resisting PI3K inhibition. This occurs through (i) nongenetic, intrinsic feedback regulation within the pathway upon short-term PI3K inactivation, and (ii) genetic resistance that develops, or is selected for, upon long-term PI3K blockade (refs. 12, 13; reviewed in ref. 14). The presence of multiple mechanisms to counteract PI3K inhibition underlines the key importance of this pathway in cancer cells.

Third, inhibition of PI3K in cancer cells *in vitro* is rarely cytotoxic, but more commonly cytostatic, which most likely reflects the fundamental role of PI3K signaling as a growth factor/nutrient sensor. Upon inhibition of the PI3K pathway, cells enter a dormant, nutrient-deprived state but do not necessarily die. This is akin to the key role of AGE1, the single class I PI3K equivalent in *C. elegans*, in regulating the formation of dauer larva, a state of stasis that allows the survival of the organism under harsh conditions (15). It has also been demonstrated that cells can continue to proliferate with minimal residual class I PI3K activity (16). Long-term complete inhibition of all PI3K activity may not be achievable in the clinical setting due to unacceptable side effects. However, as mentioned above, it is possible that repetitive, short-term, more complete PI3K inhibition with a high dose of inhibitor may be effective as a "shock therapy" that prevents the cancer cells from adapting to PI3K inhibition (10, 11).

Fourth, given that cancer cells can activate the PI3K pathway in many ways, the power of specific PI3K pathway mutations in predicting drug sensitivity is not absolute (reviewed in refs. 17 and 18). Indeed, although for example *PIK3CA* amplification/mutation in cancer cell lines has some predictive value in determining sensitivity to PI3K inhibitors, this correlation is not absolute and other genetic parameters also control this response (19). This complicates patient selection based on single-gene PI3K pathway mutation status.

Fifth, the relative merits of pan–class I versus isoform-selective class I PI3K inhibitors in the clinic remain unclear. Although pan–class I PI3K inhibitors are less well tolerated, they are less likely than isoform-selective class I PI3K inhibitors to allow compensation by other PI3K isoforms, as was observed by activation of PI3Kβ upon selective blockade of PI3Kα (20) and vice versa (21).

Lastly, due to the central role of PI3Kα in regulating organismal glucose homeostasis, PI3K inhibition in patients often gives rise to hyperglycemia and/or hyperinsulinemia (22). High levels of circulating insulin could potentially be mitogenic and/or antiapoptotic for cancer cells and thus negate the antiproliferative effects of PI3K inhibitors (23). However, it remains to be determined whether the observed changes in circulating insulin levels upon PI3K inhibition have a physiologic impact on cancer cells. Insulin-stimulated PI3K activation in the cancer cells would be expected to be blocked by sufficient PI3K target inhibition; however, this would not be the case for other signaling pathways activated by insulin in the cancer cells, such as the MAPK pathway. In the setting of cancer with mutated PI3Kα, one way to overcome the problem of compensatory production of insulin and/or glucose upon systemic PI3Kα inhibition would be to develop inhibitors with enhanced selectivity for mutant PI3Kα over wild-type PI3Kα. This would create a window for drug dosing to inhibit PI3Kα in the cancer cells without affecting the wild-type PI3Kα in the host tissues that control systemic metabolism.

EXAMPLES OF DRUG COMBINATION STRATEGIES BASED ON THE CANCER CELL–INTRINSIC ROLES OF PI3K

The prosurvival role of PI3K signaling, and the notion that PI3K activation has also been found to be a major contributor to resistance against a diverse range of anticancer agents (reviewed in ref. 14), positions intervention with PI3K signaling as an important target for combination strategies. The challenge hereby is to identify the most rational combinations within an acceptable tolerability window. Indeed, despite the significant synergy between inhibitors of the PI3K and MAPK pathways in preclinical cancer models (24), moving this particular combination therapy forward in the clinic has been hampered by toxicity. Moreover, the exact molecular mechanism by which the PI3K pathway affects other anticancer therapy responses most often remains unknown.

A few examples of ongoing combination strategies in solid tumors are discussed below. For more extensive overviews, the reader is referred to refs. 1 and 4.

PI3K and Endocrine Therapy

In breast cancer and prostate cancer, there is a clear, although mechanistically poorly understood, cross-talk between hormonal signaling and PI3K activity, with PI3K inhibition leading to increased estrogen and androgen pathway activity.

Breast Cancer

In breast cancer, combination trials of PI3K inhibitors with endocrine therapy are in progress (reviewed in ref. 1). These are based on evidence from preclinical models showing (i) that activation of PI3K/AKT can confer resistance to

antiestrogens and (ii) that there is reciprocal cross-talk between signaling by PI3K and the estrogen receptor (ER), whereby inhibition of each pathway enhances the function of the other (1, 25–27). One way in which PI3K inhibition could lead to sensitization to hormonal therapy is through the induction of estrogen-dependent transcriptional activity and increased ER expression, potentially mediated by increased FOXO3A-mediated transcription (25).

A recent update (28) of the BELLE-2 phase III trial (ClinicalTrials.gov; NCT01610284) in ER-positive, HER2-negative metastatic breast cancer that had become resistant to aromatase inhibitors showed that the combination of buparlisib (BKM120; a pan–class I PI3K inhibitor) with fulvestrant (an ER antagonist) provides a significant improvement in progression-free survival (PFS; 6.9 vs. 5 months). Interestingly, when considering only those patients with *PIK3CA*-mutant cancers, the difference in PFS was more pronounced, namely 7 months for the combination versus 3.2 months for fulvestrant alone. In this trial, adverse events of buparlisib led to frequent drug discontinuation, resulting in reduced treatment duration. The dosing of the pan–class I PI3K inhibitor pictilisib (Genentech; GDC0941) was also a limiting factor in another trial that combined PI3K inhibition with fulvestrant in patients with ER-positive, HER2-negative metastatic breast cancer, in which no significantly improved PFS was observed (29). The expectation (or hope) therefore is that the better-tolerated PI3Kα inhibitors might be more efficacious in this clinical setting.

It remains to be seen whether the observed extension in PFS in the BELLE-2 trial will translate into an overall survival benefit. Indeed, a 4.6-month prolongation in PFS by the combination of everolimus (a rapamycin analogue that inhibits mTORC1) with emestane (an aromatase inhibitor that blocks estrogen production) in the same breast cancer setting did not result in an overall survival benefit (30).

Of interest in this context are recent studies in mouse models which indicate that the expression of mutant *PIK3CA* in early breast cancer induces cancer cell multipotency, possibly contributing to tumor heterogeneity (31, 32). This is of relevance, given that *PIK3CA* mutation can be an early lesion in breast cancer development (33). It is not clear whether PI3K inhibitor treatment of established, late-stage breast cancer will be able to reverse such early biological effects induced by *PIK3CA* mutation.

Prostate Cancer

PI3K and endocrine signaling also interact in prostate cancer, with PI3K pathway inhibition giving rise to an activation of the androgen receptor (AR) and, conversely, AR blockade leading to PI3K pathway activation (34). Combination trials of PI3K inhibitors with endocrine therapy in prostate cancer are in progress, also based on the notion that PTEN inactivation is a very common event in prostate cancer. PI3Kβ has been reported to be the main isoform mediating enhanced PI3K activity as a result of PTEN inactivation in some cancer contexts (reviewed in ref. 17). Although this correlation is not absolute, it is of interest to note that PI3Kβ positively regulates AR transactivation in prostate cancer cell lines (35). In line with the dominant contribution of PI3Kβ and PI3Kδ over PI3Kα to enhanced PI3K activity upon PTEN loss in prostate cancer cell lines (36), a PI3Kβ/δ inhibitor was found to be very effective in a preclinical study of prostate cancer, particularly in combination with hormonal therapy (37). Another study (21) showed that combined inhibition of PI3Kα/β and AR signaling is effective at inhibiting *PTEN*-mutant prostate cancer cells.

PI3K and PARP Inhibitors

Another example of combination therapy is that of PI3K pathway inhibitors with PARP inhibitors (which block some forms of cellular DNA repair), which is currently being tested in trials in ovarian and breast cancers (ClinicalTrials.gov; NCT01623349), using buparlisib or alpelisib (BYL719), a PI3Kα-selective inhibitor.

These trials are based on preclinical findings of a synergistic impact of buparlisib and the PARP inhibitor olaparib (38, 39). The underlying mechanism by which buparlisib sensitizes to PARP inhibitors is not entirely clear but has been linked to the capacity of buparlisib on its own to increase markers of DNA damage and to reduce expression of the BRCA1/2 DNA repair enzymes (38, 39). It is important to mention that buparlisib has been shown, in a PI3K-independent manner, and at higher doses, to inhibit microtubule dynamics (40), which can dampen DNA repair but also have antitumor effects in its own right. It therefore remains to be seen whether PI3K inhibitors without this off-target activity will be able to sensitize to PARP inhibition. PARP inhibitors are also being trialed in combination with inhibitors of AKT or mTOR (ClinicalTrials.gov; NCT02338622 and NCT02576444).

INDIRECT EFFECTS OF PI3K PATHWAY INHIBITION IN CANCER

Despite the tremendous promise and rationale for oncogene-targeted therapies, most kinase inhibitors offer at best an extension of median survival, measured in months, with no improvement in overall survival in solid cancer. Complete responses are the exception rather than the rule. The last couple of years have seen a resurgence of cancer immunotherapy. New agents that target the CTLA4 or PD1 inhibitory receptors on T cells, or PD1 ligands on tumor cells and the cancer stroma, are showing the potential for complete responses, with improved overall survival rates resulting in lifespan extension measured in years rather than months (41–43). However, only select patient populations benefit from immunotherapy so far.

Recent findings indicate that PI3K inhibitors could also be used to target the immune system, as well as the cancer stroma in a broader sense, in particular the vasculature. PI3K inhibitors could be used to target the stroma and enhance antitumor responses by four main mechanisms (Fig. 1):

1. by improving circulation to the tumor by normalizing blood vessels to aid chemotherapy and immunotherapy, or by inhibiting angiogenesis-promoting tumor-associated myeloid cells to enhance VEGF-based antiangiogenic therapy;
2. by interfering with desmoplasia, the generation of fibrous connective tissue surrounding tumors;
3. by interfering with the survival and homing signals provided by the stroma in B-cell malignancies;

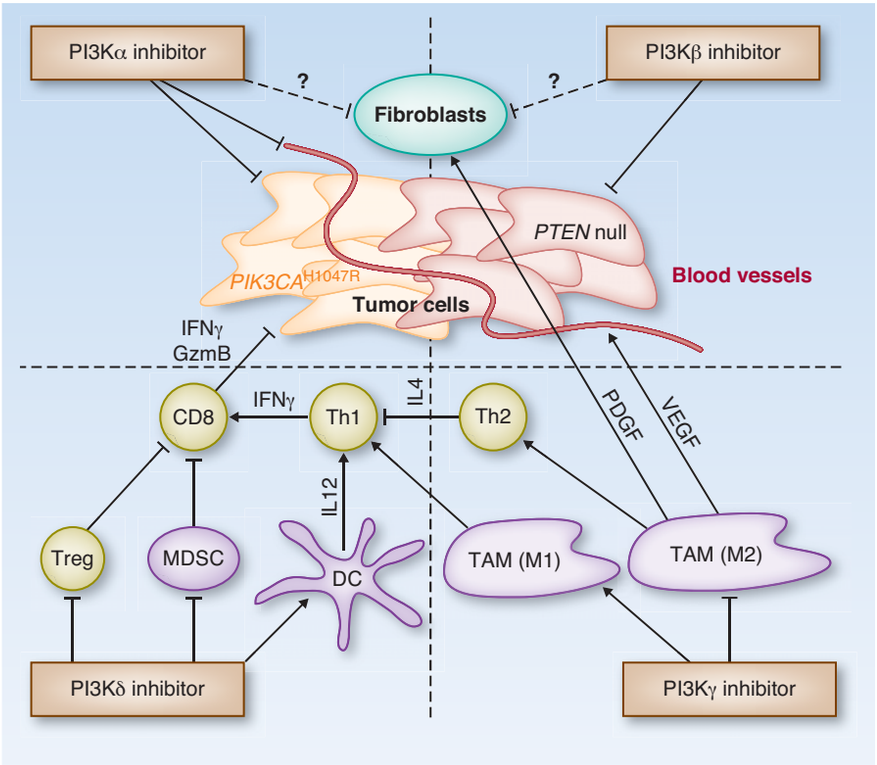

Figure 1. Direct and indirect anticancer effects of interference with PI3K isoform activity. Different PI3K isoforms have the potential to interfere with cancer growth and survival, either by acting on the transformed cells directly, by interfering with the supportive stroma and nutrient supply, or by stimulating more potent immune responses against the transformed cells. PI3Kα inhibitors have shown promising results in cancers driven by activating *PIK3CA* mutations, such as $p110\alpha^{H1047R}$. PI3Kα inhibition may also affect nutrient supply by inhibiting angiogenesis or enhance drug delivery by normalizing vessels, depending on the degree of inhibition and the particular tumor type. Evidence suggests that PTEN-deficient tumors are often (but not always) more sensitive to PI3Kβ inhibitors. Both PI3Kα and PI3Kβ inhibitors may be useful in targeting tumor-associated fibroblasts, although this is speculative at this stage. PI3Kγ inhibition has been shown to reduce the infiltration of tumor-suppressive macrophages, diverting them from an immune-suppressive (wound healing) M2 to an immunostimulatory M1 phenotype and reducing the production of fibroblast-stimulating growth factors. PI3Kδ inhibitors can stimulate a more potent $CD8^+$ T-cell–mediated cytotoxic antitumor response by activating dendritic cells (DC) to produce more IL12 and by inhibiting regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC), which antagonize cell-mediated immunity in tumors. Arrows indicate a stimulatory impact. TAM, tumor-infiltrating macrophage.

4. by interfering with immunosuppressive mechanisms to enhance tumor killing by the immune system.

Effects of PI3K Inhibitors on the Tumor Vasculature

PI3K inhibition modulates the tumor vasculature, either directly (by inhibiting endothelial cells) or indirectly (by inhibiting angiogenesis-promoting tumor-associated myeloid cells and VEGF production by tumor cells; Fig. 2). The direct effects appear PI3K inhibitor dose–dependent and range from mild vascular pruning to vessel normalization, which could be exploited to enhance drug delivery and possibly immune cell access to the tumor. At present, there are no clinical data on the impact of PI3K inhibitors on tumor angiogenesis, most likely because this parameter has thus far not been evaluated in any detail in trials.

Direct Vascular Impact of PI3K Inhibitors

Antiangiogenic effects of PI3K pathway inhibitors have been documented in multiple preclinical models of cancer (39, 44–50). Most of these studies have been performed with pan–class I PI3K inhibitors used at high doses, to mirror the maximum tolerated doses used in cancer. These studies have revealed that sustained PI3K inhibition results in (i) reduced total intratumor vessel area, which is most often accompanied by reduced vessel function (39, 44–50) and antitumor activity; (ii) a milder antiangiogenic impact than VEGF-targeted therapies (51, 52), indicating that PI3K inhibitors are unlikely to be useful in pruning/destroying vessels, as discussed further below. The vascular responses to PI3K inhibitors can be attributed to effects on both the endothelial cells and the tumor cells, and the cross-talk between them, for example by dampening the production of VEGF by the tumor cells (47–49, 53, 54), with differences observed depending on the tumor model used (49, 52). Other than interfering with tumor angiogenesis, PI3K inhibitors may also dampen lymphangiogenesis in the lymph nodes (55), a possible way of limiting tumor cell dissemination via this tissue site.

PI3Kα in Angiogenesis

Of the class I PI3K isoforms, PI3Kα has been shown to be the most important isoform in endothelial cells, in both

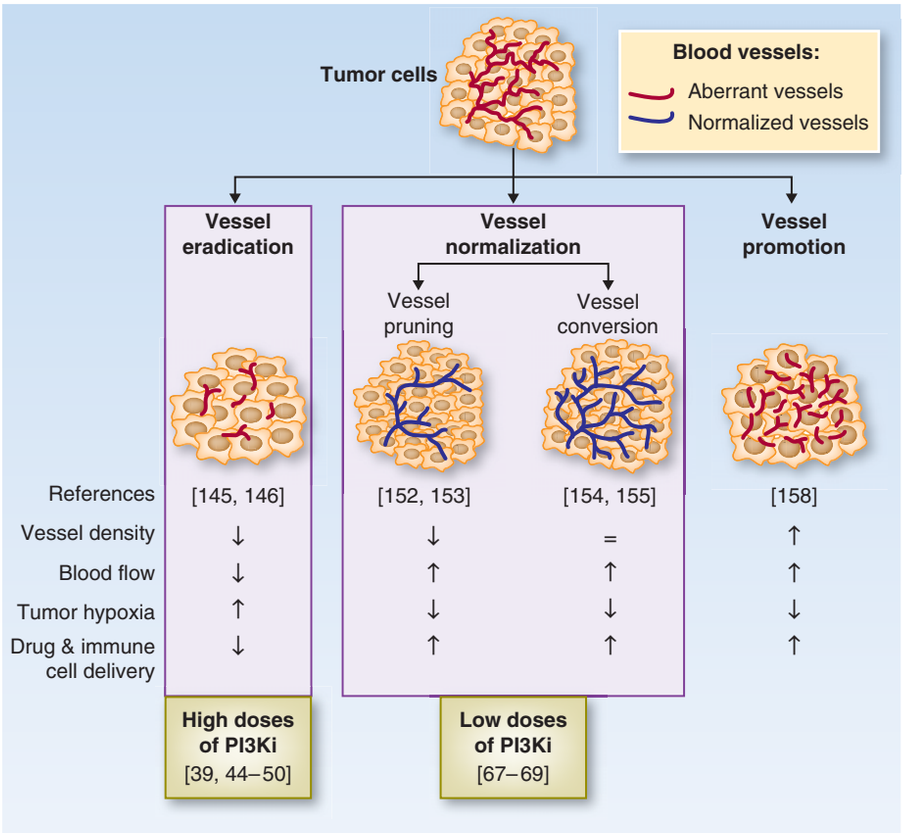

Figure 2. Vascular targeting strategies in cancer and possible role of PI3K isoforms therein. PI3K inhibitors (PI3Ki), at high doses, have been documented to induce a mild vessel eradication response, whereas at low doses they can lead to vessel normalization, associated with either reduced or no changes in vessel density. Key concepts to therapeutically exploit the dependence of tumors on the vasculature: (i) vessel eradication, aimed at destroying the tumor vasculature and "starving the tumor to death" (145, 146). Problems with this strategy are both intrinsic and acquired resistance to vascular trimming (147, 148), reduced chemotherapy delivery to the tumor and induction of hypoxia, which can accelerate tumor progression (51, 149); (ii) vessel normalization, aimed at improving vascular perfusion and oxygenation, allowing enhanced drug delivery and immunotherapy (150, 151). This normalization effect can be the consequence of vessel pruning, with only normal vessels left behind after therapy (152, 153) or of vessel conversion, a biological change in endothelial cells whereby the tubular structures are converted into more physiologic, normal vessels (154, 155). The applicability of the normalization approach in the clinic has been limited by the transient window during which vessels are susceptible to normalization, the difficulties of predicting when and which agent will induce vessel normalization, and a highly context-dependent response, with every tumor relying to different extents on angiogenic cues to stimulate vessel growth (156, 157); (iii) the vessel promotion strategy is based on stimulating vessel growth, together with promotion of vasodilatation (158), and is aimed at enhancing delivery of chemotherapy and other anticancer agents to the tumor (159).

blood vessels (49, 56, 57) and lymphatic vessels (58, 59). Gain-of-function mutations in *PIK3CA* have recently been reported to result in venous and lymphatic malformations, a specific type of congenital vascular anomaly (60–63), further highlighting the importance of PI3Kα activity in endothelial cells. PI3Kα-selective inhibitors are currently being tested in various cancers, with the main aim of reducing cancer cell–intrinsic PI3K activity. As part of the clinical evaluation of these compounds, it will be critical to also assess their impact on the tumor vasculature.

Roles of Non-PI3Kα Isoforms in Angiogenesis

The generation of blood vessels, including in tumors, is a cooperative process between endothelial cells and numerous other cell types, including the tumor cells, mural cells, platelets, and immune cells, which can secrete angiogenic factors (such as VEGF) and also contribute to vascular tube formation (57, 64). PI3K signaling plays a role in all of these cells, with specific isoforms displaying predominant roles in certain cell types (57), including PI3Kβ in platelets and PI3Kδ/PI3Kγ in leukocytes. Indeed, PI3Kγ inhibition has been shown to have antiangiogenic effects in cancer, due to its ability to inhibit tumor-associated myeloid cells (65, 66). It is therefore likely that multiple PI3K isoforms contribute to tumor angiogenesis by different mechanisms.

Vascular Impact of PI3K Inhibitors Can Improve Cancer Treatment in Multiple Ways

Additional studies are required to assess the ways in which PI3K inhibition compares with other vascular targeting therapies, but the data available to date indicate that high doses of PI3K inhibitors have a weaker impact on vessel eradication than VEGF-targeted therapies (51). Recent preclinical studies have highlighted alternative ways, beyond vascular pruning,

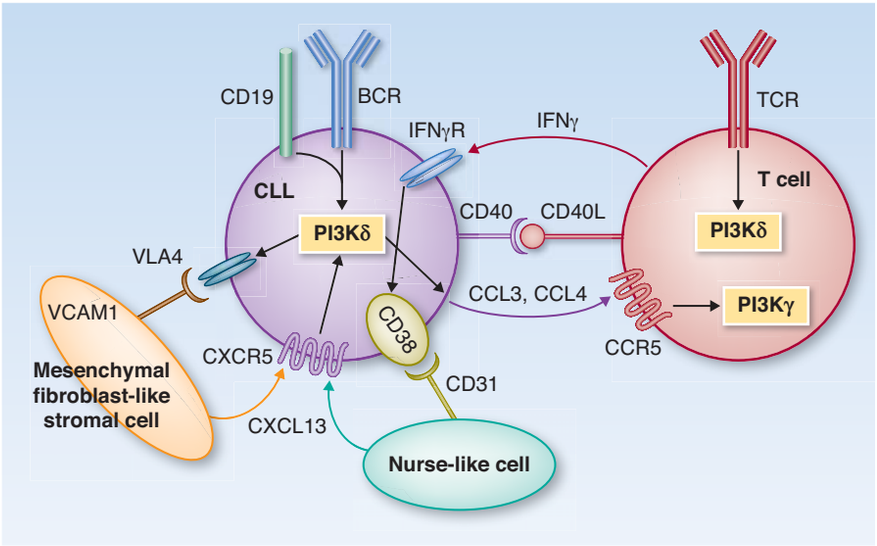

Figure 3. Roles of PI3Kδ in CLL cells and their interaction with their surrounding stroma. CLL cells depend on signaling via the B-cell antigen receptor (BCR) to increase proliferation, adhesion to mesenchymal stromal cells (via VLA4/VCAM1), and secretion of the chemokines CCL3 and CCL4. The ligands (antigens) for the BCR may be expressed by the CLL cells themselves or by other cells in the stroma. CCL3 and CCL4 facilitate the recruitment of T cells, which provide CD40 ligand (CD40L), IFNγ, and other agonists that stimulate the CLL cells, as evidenced by increased expression of CD38 on the CLL cells. Other key cell types in the lymph node niche include (i) mesenchymal stromal cells that secrete chemokines, such as CXCL12 and CXCL13, which bind to CXCR4 and CXCR5 on CLL cells and facilitate their recruitment to and retention in the lymph nodes; and (ii) myeloid-derived nurse-like cells that also secrete CXCL13 and promote the survival of CLL cells. PI3Kδ inhibition interferes with many aspects of this intercellular communication, ultimately resulting in the "purging" of CLL cells from their protective lymph node (or bone marrow) environment into circulation where they are more susceptible to undergoing apoptosis. TCR, T-cell receptor.

that could be used to exploit PI3K inhibitors in the modulation of the tumor vasculature.

Notably, low doses of PI3K inhibitors were shown to improve vascular function (67–69), even upon short-term (3-day) administration (68), correlating with an enhanced capacity to deliver chemotherapy to the tumor (68). These findings point to a so-called "vessel normalization" effect that is also observed upon administration of low (70) or single (68, 71) doses of anti-VEGF antibodies. These data suggest that the direct vascular effects of PI3K inhibitors on tumor angiogenesis could be exploited to improve vessel function, which could enhance drug delivery and potentially also the influx of antitumor immune cells to improve immunotherapy.

A prime example of an indirect antivascular effect of PI3K inhibition in cancer is the recent finding that resistance to VEGF-targeted therapy in tumors is partially mediated by proangiogenic tumor-associated myeloid cells, in a PI3Kγ-dependent manner (65). Accordingly, a PI3Kδ/PI3Kγ inhibitor was able to enhance tumor responsiveness to VEGF-based antiangiogenic therapy (65). The contribution of PI3Kδ to this biological effect is unclear at present.

Effects of PI3K Inhibitors on Stromal Fibroblasts

Desmoplasia, the growth of fibrous stromal tissue around tumors, is common in some cancers, such as pancreatic ductal adenocarcinoma, and has been thought to be a mechanical barrier for access of drugs and immune cells to the tumor, but could also dampen metastasis (72).

In a mouse model of pancreatic cancer, PI3Kγ inhibition was shown to dampen the secretion of PDGFBB by tumor-associated macrophages (Fig. 1), thereby reducing collagen secretion by tumor-associated fibroblasts, which most likely contributes to the marked reduction in fibrous tissue surrounding the tumors (73).

Functional inactivation of PTEN (which leads to PI3K activation) in stromal fibroblasts in breast cancer has been reported to contribute to cancer development and progression (74). A direct action of PI3K inhibitors (especially against PI3Kα/β) on such cancer-associated fibroblasts would be expected to inhibit their tumor-promoting activities (Fig. 1), although this has not been formally tested, and will not be discussed further here. However, it is likely that PI3K activity is an important regulator of this aspect of tumor–stroma interaction.

PI3Kδ Inhibition Disrupts Stroma–Cancer Cell Engagement in B-cell Malignancies, Rendering the Tumor Cells More Susceptible to Cell Death

Early studies showed an important role for PI3Kδ in non-transformed B cells, with an almost exclusive dependence of the B-cell antigen receptor signaling on PI3Kδ over other PI3K isoforms (Fig. 3; refs. 75–77). In contrast to other B-cell malignancies, which show enhanced contribution of PI3Kα (78) and/or activating mutations in signaling pathways parallel to PI3K (such as in the NF-κB pathway; ref. 79), the biology of CLL and iNHL remains remarkably dependent on PI3Kδ activity, creating a unique vulnerability to PI3Kδ inhibition in these diseases. Interestingly, the susceptibility of CLL cells to PI3Kδ inhibition is independent of p53 status (80), indicating that activation of prominent oncogenic

pathways does not inevitably reduce cellular susceptibility to PI3Kδ inhibitors.

The mechanism of action of the PI3Kδ inhibitor idelalisib is best understood from clinical studies on CLL. Treatment of patients with CLL with idelalisib leads to "purging" of the transformed B cells from their protective lymph node and spleen niches into the blood circulation. This results in rapid shrinking of these tissues and correlates with a greatly increased number of lymphocytes in the blood, so-called lymphocytosis (80–82). This phenomenon is a consequence of idelalisib interfering with the adhesion, survival, and homing signals provided by stromal cells to the CLL cells. These critical signals are transduced in the CLL cells via receptors that critically depend on PI3Kδ, such as the tyrosine kinase–linked B-cell antigen receptor (BCR) and CD19 and G protein–coupled receptors such as the chemokine receptor CXCR5 (83).

Once in the circulation, CLL cells no longer receive survival signals from their stroma and as a result are thought to become more susceptible to spontaneous cell death. In addition, upon PI3Kδ inhibition, these cells no longer respond to homing and adhesion signals upon reentry into their normal tumor niche. Cell death of circulating CLL cells can be accelerated by rituximab (an antibody against the CD20 B-cell surface marker) and/or chemotherapy with bendamustine. The *in vitro* cytotoxic/cytostatic effect of PI3Kδ inhibitors on monocultures of CLL cells is modest (83). However, when CLL cells are cocultured with stromal cells, PI3Kδ inhibition interferes with the stroma-dependent survival signals (5, 83, 84).

Idelalisib treatment also reduces the responsiveness of CLL cells to several chemokine agonists, including CXCL13, which is primarily released by fibroblast-like mesenchymal stromal cells and is important for the recruitment and retention of CLL cells to their niche. In addition, idelalisib prevents recruitment of other cells to the CLL niche, such as monocytes (which can give rise to nurse-like cells) and lymphocytes, through reducing the secretion of the chemokines CCL4 and CCL5 by CLL cells. Of the infiltrated lymphocytes in the CLL niche, Th1 T cells might support the survival and differentiation of CLL cells by providing CD40 ligand (CD40L), IFNγ, and other agonists (85, 86), and it is possible that idelalisib also inhibits the secretion of some of these factors by T cells. Other recruited lymphocytes, especially $CD8^+$ T cells, have the potential to kill CLL cells. However, these CLL-associated $CD8^+$ T cells are usually dysfunctional, probably as a consequence of the immune-suppressive environment (87). The function of the monocyte-derived nurse-like cells and mesenchymal stromal cells in the stroma is possibly also affected by idelalisib (83–86, 88).

To summarize, in CLL, idelalisib and other PI3Kδ inhibitors act to a large extent by disrupting the interactions between the malignant B cells, their protective stromal cells, and immune cells, without directly killing the cancer cells (Fig. 3). This is facilitated by the unique dependency of CLL cells on PI3Kδ over other PI3K isoforms, but also potentially by the effect of PI3Kδ inhibition on other leukocytes in the stroma. As will be explained below, it is also possible that an adaptive immune response is being generated against the CLL cells, although this remains to be documented in patients with CLL.

Immunomodulatory Effects of PI3K Inhibition in Cancer

Although PI3Kγ and PI3Kδ are present at low levels in most cell types, their expression is high in all leukocyte subtypes. Over the last few years, accumulating evidence has shown that dampening the activities of these PI3K isoforms has broad anticancer activity, which is not restricted to hematologic malignancies (Fig. 1). Indeed, although PI3Kδ inhibitors can dampen many immune cell functions, such as antibody production, their effect on regulatory immune cell subsets, such as regulatory T cells (Treg), results, unexpectedly, in a heightened immune response against cancer and certain infectious agents (89, 90). Similarly, although PI3Kγ inhibition reduces the immediate response to bacterial infections, it also decreases the immune-suppressive effects of myeloid cells in cancer (66, 73).

Below, we describe the immunomodulatory effects of PI3Kγ and PI3Kδ inhibition in cancer, focusing on the stimulation of T-cell responses, through either acting on T-cell subpopulations (by PI3Kδ inhibition) or modulation of the myeloid cell compartment (by PI3Kδ and/or PI3Kγ inhibition). Before doing so, we first summarize the adverse effects observed in patients treated with idelalisib, which we speculate could be related, at least in part, to a heightened immune response.

Adverse Effects Associated with PI3Kδ Inhibition in the Clinic: The Result of an Overactive Immune Response against Commensals and Opportunistic Infectious Agents?

PI3Kδ kinase-dead mice are prone to the development of colitis (75, 91–93). Similarly, patients treated with idelalisib often develop colitis (severe diarrhea), transaminitis, and, less frequently, pneumonitis (94–97). A few fatalities caused by infections with *Pneumocystis jirovecii* or cytomegalovirus, suggesting increased risk for opportunistic infections, have led to the discontinuation of several phase III trials of idelalisib (97).

Increasing evidence suggests that some of the more common adverse events associated with idelalisib treatment are immune mediated, as they are more frequently observed in first-line patients who are more immunocompetent, are associated with T-cell infiltrates (in the case of colitis, hepatitis, and pneumonitis), and can often be alleviated by administration of steroids (82, 98, 99). Moreover, similar symptoms have been described in an individual with biallelic loss of *PIK3CD* (100), further suggesting that these are likely to be target-related adverse effects. Some patients treated with idelalisib develop skin rash, which could be a result of inappropriate reactivity to commensal pathogens on the skin (98, 99). In fact, the spectrum of adverse effects associated with the use of PI3Kδ inhibitors is similar to that observed with the immune checkpoint inhibitors anti-PD1/PDL1 and anti-CTLA4 (101).

In mice, colitis is often correlated with defects in Treg function (102). In an experimental model of this disease, naïve $CD4^+$ T cells are injected into a lymphopenic host. A subset of these T cells will be activated in a manner dependent on the gut microflora, including *Helicobacter* species. The disease

can be effectively prevented by coinjection of functional Tregs (102). Of relevance in this context, Tregs with inactive PI3Kδ failed to suppress such experimentally induced colitis in mice (91). In addition, colon-associated macrophages in PI3Kδ kinase-dead mice are more inflammatory and less bactericidal than in wild-type mice (92, 93). Colitis observed in patients on idelalisib is characterized by infiltrating $CD8^+$ T cells, reminiscent of graft-versus-host disease, and is more prevalent in patients who have not been exposed to immune-suppressive therapies (82, 94–96, 98), indicative of a possible host T-cell immune response against the gut flora. There is also evidence that Tregs are affected in patients on idelalisib who develop colitis (99). It should be noted, however, that a different PI3Kδ inhibitor (TGR-1202) shows similar efficacy as idelalisib in CLL and iNHL, but with fewer adverse effects (103). Although diarrhea was a common adverse effect with TGR-1202, it was less severe than with idelalisib and rarely led to drug discontinuation. Transaminitis was also less common. It remains to be determined whether the less severe adverse effects of TGR-1202 relative to other PI3Kδ inhibitors are due to a different formulation or dosing regimen of this agent, or whether these data present a challenge to the concept that colitis is a PI3Kδ target–related adverse effect in humans as it is in mice.

Emerging evidence suggests a possible connection of pneumonitis in idelalisib-treated patients with infection with cytomegalovirus or the fungus *Pneumocystis jirovecii* (formerly known as *Pneumonitis carinii*). Cytomegalovirus pneumonitis may be related to a reduced function of natural killer cells upon PI3Kδ inactivation (104). So-called *Pneumocystis jirovecii* pneumonitis (PJP) is most often associated with lymphopenia caused by HIV/AIDS chemotherapy or other forms of immunodeficiency. Injection of lymphopenic mice with naïve $CD4^+$ T cells can cause pneumonitis in a manner that correlates with levels of *Pneumocystis jirovecii* in the lungs (105). As with colitis, coinjection of Tregs prevents this experimentally induced PJP in mice (105); however, the specific role of PI3Kδ in Tregs has not yet been assessed in this model. The immune etiology of PJP induced by idelalisib as monotherapy has not been reported, but in a combination trial of idelalisib with a SYK inhibitor, it was found to be accompanied by features of increased Th1-type T-cell responses (106). It is of interest to note that PJP has also been reported for mTOR inhibitors (rapamycin analogues), the mechanism of which is not understood to date but which may also involve an exacerbated Th1 T-cell–mediated autoimmune response (ref. 106 and references therein). It will be important to assess whether PJP in idelalisib-treated patients is due to an immune overreaction of the host to *Pneumocystis jirovecii*, rather than due to an overall lack of immune response to this pathogen. The mechanism of transaminitis in patients on idelalisib is also likely to be immune related and is correlated with a reduction in Tregs and infiltration of $CD8^+$ T cells (99).

Taken together, evidence suggests that adverse effects observed upon idelalisib treatment may be target related and caused in part by inhibition of Tregs followed by unrestrained T-cell–mediated immune responses against otherwise innocuous noninfectious organisms. These findings highlight the concept that PI3Kδ inhibitors are not just immunosuppressive but can also act to heighten cellular immune responses against pathogens and, importantly, as will be described below, against tumors. Although PI3Kδ inhibition may also increase susceptibility to opportunistic infections, PI3Kδ kinase-dead mice display enhanced resistance to infection with certain pathogens, such as *Leishmania*, which is attributed to impaired expansion and effector functions of Tregs, allowing weakened Th1 responses to contain parasites and prevent pathology (107). PI3Kδ kinase-dead mice are also more resistant to infection with the intracellular bacteria *Listeria monocytogenes* (108). Therefore, although it was initially assumed to primarily be a target for therapies aimed at reducing autoimmunity and inflammation (109), PI3Kδ may in fact also be targeted to increase certain innate and adaptive cell-mediated immune responses.

PI3Kδ Inhibition Can Enhance T-cell–Mediated Antitumor Responses

Diverse mechanisms are at play in tumors to promote the recruitment and expansion of Tregs that dampen effector T-cell responses (110). PI3Kδ kinase-dead mice are resistant to diverse transplanted tumors, and this resistance could be not only recapitulated but even improved by deleting PI3Kδ selectively from Tregs (90). Moreover, PI3Kδ kinase-dead mice show enhanced resistance to secondary tumor administration, suggestive of the generation of tumor-specific immunologic memory. Administration of a PI3Kδ-selective inhibitor in a syngeneic mouse model also led to reduced growth and metastasis of a breast tumor cell line that did not express functional levels of PI3Kδ (90).

These data indicate that PI3Kδ inhibitors, such as idelalisib, have the potential to be used as cancer immunotherapeutic agents. However, we suspect that, in most cases, PI3Kδ inhibitors will not be sufficient as a monotherapy to eliminate cancer. There is increasing evidence for synergy between different immunotherapeutic agents (111) or for combining immunotherapy with chemotherapy (112), irradiation (113), or targeted therapies (114), including PI3K inhibitors (115). When used together with other immunotherapies or conventional therapies, PI3Kδ inhibitors may help in unleashing antitumor immune responses.

Several clinical trials are designed to test PI3Kδ inhibitors, either alone or in combination with other therapies, in nonhematologic malignancies (Table 1). It will also be of interest to determine whether enhanced T-cell–mediated antitumor responses contribute to the impressive clinical responses to PI3Kδ inhibition in CLL and iNHL.

Below we describe the complex role of PI3K in the regulation of specific T-cell populations in cancer, mainly focusing on PI3Kδ, for which most of the data in this context are available. An emerging theme is that, although specific cells and their responses can be inhibited in isolation or at early time points, the surprising and unanticipated end result is a net antitumor immune response in several instances.

PI3Kδ Inhibition Dampens Treg Function

The precise mechanism through which inhibition of PI3Kδ blocks Treg-mediated suppression of antitumor immune responses remains to be fully elucidated. PI3Kδ-deficient Tregs make less IL10 and express lower levels of CD38, a

Table 1. Ongoing clinical trials testing inhibitors against the leukocyte-enriched PI3Kδ and PI3Kγ isoforms in solid tumors

Sponsor	Phase	Trial identifier	Inhibitor	Cancer type(s)	Combination
PI3Kδ					
CRUK and AMGEN	II	NCT02540928	AMG-319	Head and neck squamous cell carcinoma (nonviral)	None (no prior treatment)
Gilead Sciences	I	NCT02468557	Idelalisib	Pancreatic ductal adenocarcinoma	Chemotherapy
TG Therapeutics	I	NCT02574663	TGR-1202	Various solid cancers	Chemotherapy
Incyte	I	NCT02646748	INCB050465	Various solid cancers	Pembrolizumab (anti-PD1)
Incyte	Ib	NCT02559492	INCB050465	Various solid cancers	INCB039110 (JAK1 inhibitor)
PI3Kγ					
Infinity	I/Ib	NCT02637531	IPI-549	Non–small cell lung cancer, melanoma	Pembrolizumab (anti-PD1)

receptor involved in the metabolism of extracellular metabolites (Fig. 4; refs. 116, 117).

A recent study showed that mice with Treg-specific expression of an AKT-insensitive mutant of the FOXO1 transcription factor displayed resistance to tumors (118). The authors hypothesized that in wild-type mice, inactivation of FOXO1 by PI3K/AKT signaling is required for Tregs to mature and suppress anticancer immune responses. These studies suggest that the loss of PI3K signaling in Tregs leads to increased FOXO1 activity and a failure of these cells to suppress antitumor immune responses.

The transcription factor BACH2, which antagonizes AP1-dependent transcription in T cells (119), has recently also been shown to be regulated by PI3K/AKT signaling, in an analogous manner to that of FOXO1 (119), and selective loss of BACH2 in Tregs is sufficient to confer tumor resistance (120). This is consistent with a model in which elevated PI3K signaling leads to the loss of BACH2 function and could destabilize Tregs. Indeed, increasing PI3K signaling by inhibiting PTEN in Tregs has also been shown to stimulate potent antitumor immune responses by destabilizing the Treg lineage, leading to impaired Treg function and reduced immune suppression (121). Reduced tumor growth could also be achieved using a systemically administered PTEN inhibitor that appeared to act primarily on PTEN within Tregs (121). A potential further advantage of administering a PTEN inhibitor is that tumor-reactive $CD4^+$ T-effector cells are more potent in the absence of PTEN (122). However, it remains to be determined whether a PTEN inhibitor could be administered safely to humans, given that PTEN is one of the most commonly inactivated tumor suppressor genes. The reduced stability of PTEN-deficient Tregs could be due to reduced FOXO1 transcription with consequently reduced transcription of FOXP3, a transcription factor that is essential for Treg development (84, 85).

Taken together, these data establish a potential signaling pathway from PI3Kδ to AKT toward the FOXO1 and BACH2 transcription factors (Fig. 4) in Tregs, with either the loss or overactivation of FOXO1 or BACH2 transcriptional activities resulting in a dampening of the capacity of Tregs to suppress antitumor immune responses. These results highlight how PI3K signaling needs to be balanced such that too high, too low, or the inability to dynamically regulate PI3K signaling (the latter by constitutive genetic alterations in the PI3K pathway) can lead to similar effects on immune cell functions (89).

PI3Kδ Inhibition Can Improve $CD8^+$ T-cell Activity in Cancer

As in Tregs, the exact role of the PI3Kδ pathway in $CD8^+$ T cells is enigmatic (108, 123–128). In this T-cell population, PI3K also acts via AKT to suppress the transcriptional activity of FOXO1 and BACH2 to maximize the expression of effector cytokines (such as IFNγ) and granzymes (such as GzmB; refs. 90, 119, 126, 129, 130). However, it appears that once $CD8^+$ T cells are fully differentiated, they become less dependent on PI3Kδ inhibition, but probably remain susceptible to suppression by Tregs (90, 108, 125, 131–133).

Inhibition of downstream signaling components in the PI3K pathway has also been shown to lead to enhanced T-cell–mediated antitumor activity *in vivo*. $CD8^+$ T cells cultured in the presence of an AKT inhibitor showed enhanced efficacy in adaptive cancer immunotherapy (131–133). Similarly, the mTOR inhibitor rapamycin, which inhibits $CD8^+$ T-cell effector functions, can enhance the generation of memory $CD8^+$ T cells (134). In PI3Kδ kinase-dead mice, the generation of effector T cells in response to infection is impaired, but the generation of long-lived memory T cells remains intact (108, 124), which may help to explain why these mice are able to reject tumors in a $CD8^+$ T-cell–dependent manner (90). At least in the case of adoptive T-cell therapy, it has been shown that T cells with a stem-like memory phenotype provide a more durable response than effector T cells (135). Further, the differential effect of PI3Kδ inhibition on naïve T cells versus memory T cells helps alleviate the effect of graft-versus-host disease while maintaining effective graft versus leukemia in a bone marrow transplant setting (125).

Thus, although PI3Kδ inhibitors will initially reduce antitumor effector functions, they may also help to sustain the $CD8^+$ T cells, which may help persistent immune-mediated attacks on cancer, despite the blunted production of immediate effectors, such as IFNγ and GzmB, by these cells.

Inhibition of PI3Kδ and/or PI3Kγ in Myeloid Cells Can Lead to Enhanced T-cell Antitumor Responses

Tumor mass is often comprised of up to 50% myeloid cells that can suppress immune responses and promote angiogenesis. Indeed, tumors have been described as nonhealing

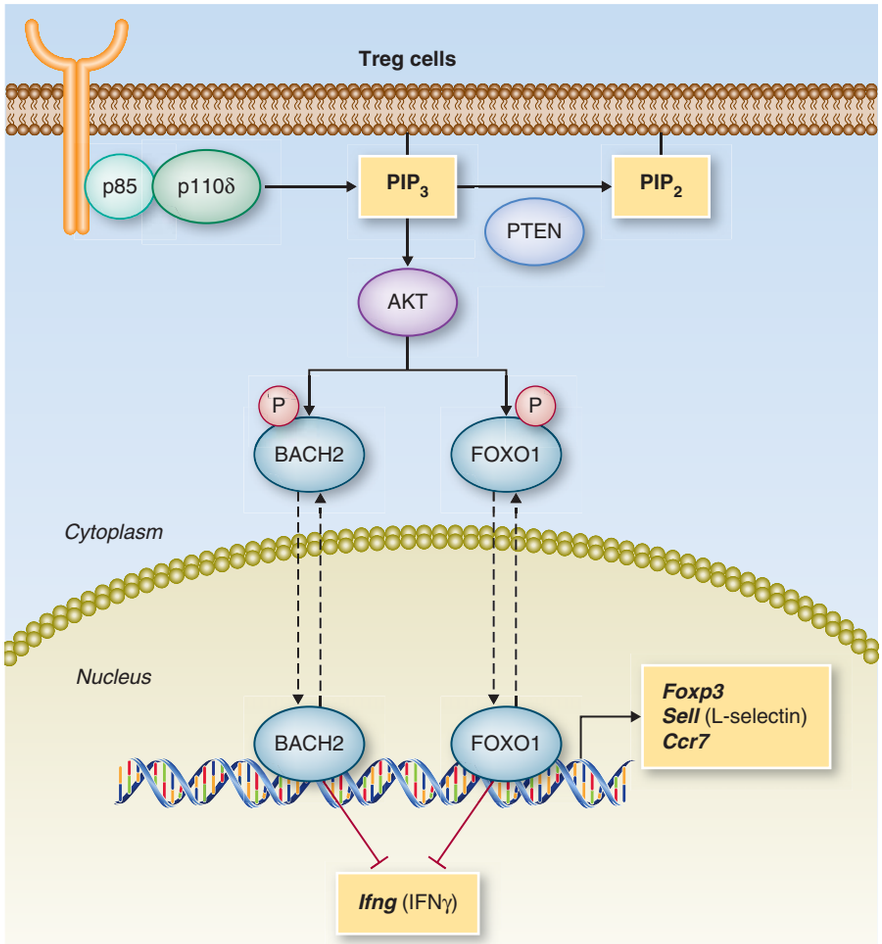

Figure 4. PI3K pathway components in Tregs involved in the regulation of anticancer immunity. The activation of AKT by PI3Kδ leads to the phosphorylation of the BACH2 and FOXO1 transcription factors and their retention in the cytoplasm. BACH2 and FOXO1 regulate expression of key genes in Treg differentiation and function, including genes encoding FOXP3, L-selectin, CCR7, and IFNγ. Failure to activate this pathway upon PI3Kδ inhibition may prevent Tregs from undergoing a differentiation program and migrating effectively to tumors in order to suppress antitumor responses. By contrast, constitutive PI3Kδ activation upon loss of PTEN may destabilize the Treg lineage, loss of FOXP3 and CD25 expression resulting in loss of suppressive function of the Tregs and production of proinflammatory cytokines instead of the normal immune-suppressive factors produced by Tregs.

wounds (136). In addition to directly affecting T cells, there is significant potential for PI3Kγ and PI3Kδ inhibitors to stimulate anticancer immune responses through the modulation of myeloid cells. This can be either by inhibiting suppressive myeloid cells, by dampening immune-suppressive tumor-infiltrating macrophages (TAM), or by stimulating macrophages and dendritic cells (DC) to make cytokines that contribute to effective T-cell responses. Most studies assessing the impact of PI3K inhibition on cancer-associated myeloid cells have focused on PI3Kγ (65, 66, 73, 137). It is clear, however, that PI3Kδ can also modulate myeloid cells in cancer, which most likely contributes to the antitumor effects seen with PI3Kδ inhibitors (90, 138).

PI3Kγ-deficient mice are resistant to colon cancer that develops subsequent to oral administration of dextran sulfate sodium to induce chronic colitis (137). Moreover, inhibition of PI3Kγ reduced tumor progression in a range of mouse models and dampened the recruitment of myeloid cells to tumors, at least in part by interfering with RAP1-dependent integrin activation (66, 139). The majority of these myeloid cells were $CD11b^+$, $GR1^{lo}$ and considered to be TAMs (66). TAMs are a major source of IL1β, IL6, and VEGFα, all of which are tumor promoting. In a mouse model of pancreatic ductal adenocarcinoma, PI3Kγ activity was shown to be important for the induction of an immunosuppressive transcriptional program in TAMs that inhibits T-cell immune responses. By reprogramming TAMs from an immunosuppressive to an immunostimulatory phenotype, PI3Kγ inhibition was able to dampen tumor cell metastasis and desmoplasia, eventually allowing the development of $CD8^+$ T-cell–mediated tumor suppression, without altering the presence of Tregs (73).

In PI3Kδ kinase-dead mice, the expansion of MDSCs that express CD11b but, in contrast to TAMs, express high levels of GR1, and are distinguished from neutrophils by their lower expression of Ly6C, was reduced, which correlated

with reduced suppressive activity and reduced tumor growth (90).

TAMs, and especially DCs, can be potent inducers of tumor immunity when exposed to IFNγ and other proinflammatory cytokines, thus shifting the balance from a wound-healing response to antitumor immunity (140, 141). There is evidence to suggest that this could be achieved by PI3Kδ inhibitors. Indeed, inhibition of PI3Kδ in lipopolysaccharide-stimulated DCs increases the production of proinflammatory cytokines (TNFα, IL12, IL1β) and reduces the levels of anti-inflammatory cytokines (IL10, IFNβ; refs. 142, 143). This is in line with observations from preclinical mouse models whereby administration of PI3K inhibitors reduces IL10 and TGFβ production in favor of IL12 production and enhances DC-mediated antitumor therapy based on Toll-like receptor (TLR) agonists (138). For example, combination of the TLR ligand flagellin with various class I PI3K inhibitors, either with or without a DC vaccine, delayed tumor growth and increased survival (138). Tumor growth suppression was associated with increased accumulation of polyfunctional T cells that secreted multiple effector cytokines, including IFNγ, IL17, and IL2 (138).

FUTURE DIRECTIONS

Over the last few years, it has become clear that harnessing the cancer cell–intrinsic antiproliferative action of PI3K inhibition as a monotherapy will be challenging in cancer, largely due to intrinsic and acquired cancer cell resistance to PI3K inhibition. Drug tolerability is also a concern. Drug combination strategies and alternative dosing schemes are currently being explored. Identifying the most promising combination therapies will be challenging, with successes most likely to come from mechanism-based combination therapies that exploit specific vulnerabilities in the cancer cells that sensitize to PI3K therapy. An example is the clear, yet poorly understood, cross-talk between hormonal and PI3K signaling in breast and prostate cancers, with PI3K inhibition leading to increased signaling through the estrogen and androgen receptors, respectively. Identifying such specific vulnerabilities will take time and illustrates that there is still a lot to be learned about basic mechanisms of PI3K signaling in cancer.

Recent evidence has highlighted the potential for PI3K inhibitors not only to target aberrant PI3K signaling in the tumor cells but also to interfere with the tumor-protective and immunosuppressive stroma. This is illustrated by the fact that the clinical efficacy of the FDA-approved PI3Kδ inhibitor idelalisib in B-cell malignancies such as CLL derives mainly from its capacity to interfere with the interplay between the cancer cells and their surrounding stroma.

An exciting development is the potential use of PI3K inhibitors in cancer immunotherapy. To achieve therapeutic efficacy in cancer, these inhibitors will most likely have to be combined with other therapeutic modalities (such as irradiation or surgery) or other anticancer agents, an approach that is currently being tested in the clinic (Table 1). Although these efforts will most likely focus on inhibitors of the leukocyte-enriched PI3Kδ and PI3Kγ, it cannot be excluded that other isoform-selectivities of existing PI3K inhibitors, such as dual PI3Kα/δ inhibitors, might also be useful in this regard. A recent study (115) indicated that PI3K activation in cancer cells by PTEN loss renders cancer cells more resistant to immune-mediated killing, further highlighting the potential for immunotherapy on PI3K pathway mutant tumors but also leading to sensitization to PI3Kβ inhibitors. Another key question is whether immune activation is also part of the therapeutic mechanism of idelalisib in B-cell malignancies.

The finding that agents with immune-suppressive effects on immune cells *in vitro* can have immune-stimulatory roles in cancer is an exciting recent development, and has also been observed for other signaling molecules, such as MEK inhibitors (144). However, this immunomodulation is not without risks. An important consideration will be the dose and scheduling regimen of immunomodulatory PI3K inhibitors. Indeed, it is not unlikely that the effective immunomodulatory dose and exposure time might differ from continuous drug administration at the maximum-tolerated dose level, which is currently the norm in cancer therapy. It will also be important to assess which patients are suited for PI3K-based immunotherapies. Indeed, although idelalisib is reasonably well tolerated in therapy-resistant CLL, there are emerging data on toxicities, and even fatalities, in the up-front treatment setting of B-cell malignancies. The emerging evidence that toxicities of idelalisib may have a strong immunologic component may underlie the increased frequency of adverse effects in the first-line setting because of a more intact cellular immune system in these non-pretreated patients (82, 98). It is anticipated that such challenges of toxicities can be overcome through a better understanding of the underlying mechanism of adverse effects, especially in the combination setting and in immune-compromised patients. For example, we speculate that several of the adverse immune effects associated with PI3Kδ inhibitors are in fact due to aberrant immune stimulation against otherwise innocuous agents rather than a consequence of overall immune suppression. These on-target effects can be clinically managed and further support the rationale of using PI3Kδ inhibitors in cancer immunotherapy.

Another potentially exploitable indirect anticancer effect of PI3K inhibitors is their impact on the tumor vasculature. Data available to date suggest that PI3K inhibition does not result in acute vascular pruning but instead has more subtle vasculo-modulatory roles that could be exploited to normalize vessels in order to enhance delivery of chemotherapy and immunotherapy to the core of the tumors. Additional preclinical studies are required to validate this concept.

To be able to fully exploit the indirect role of PI3K in cancer, it will be critical, in ongoing and future clinical trials, not only to assess the impact on the cancer cells themselves, but also to better monitor the stromal environment, immunological characteristics, and vascular status. We believe that PI3K-based therapy could be tailored to better exploit the indirect effects of PI3K in cancer, hopefully leading to more durable therapeutic responses than currently observed.

Disclosure of Potential Conflicts of Interest

K. Okkenhaug reports receiving a commercial research grant GSK, has received speakers bureau honoraria from Gilead, and is a

consultant/advisory board member for Gilead, Karus Therapeutics, Merck, and Incyte. B. Vanhaesebroeck is a consultant/advisory board member for Karus Therapeutics. No potential conflicts of interest were disclosed by the other author.

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