# 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 more potent anti-tumor 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 focus on the effects of PI3K inhibitors on the stroma, in addition to their direct effects on tumors.

**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.

## Introduction

Pathological 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

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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 pancreas, 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's lymphoma (iNHL), yet a PI3K pathway inhibitor (Idelalisib, an inhibitor of PI3Kδ) was recently approved as a therapy for this disease. 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 under-investigated. The stroma can be defined as any cell that forms part of the tumor mass but which is not malignantly transformed. Typically, the stroma will include (a) the vasculature, (b) infiltrating immune cells, (c) 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 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 will 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).

## 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 only modest single-agent therapeutic efficacy in solid tumors. This could be due to various reasons, including 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 pre-clinical 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 (1) non-genetic, intrinsic feedback regulation within the pathway upon short-term PI3K inactivation and (2) through genetic resistance that develops, or is selected for, upon long-term PI3K blockade [(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 AGE-1, 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, 18)]. Indeed, while 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. While 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 anti-apoptotic for cancer cells, and thus negate the anti-proliferative effects of PI3K inhibitors (23). However, it remains to be determined whether the observed changes in circulating insulin levels upon PI3K inhibition have a physiological 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 pro-survival 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 anti-cancer 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 anti-cancer 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, 4).

## PI3K and endocrine therapy

In breast and prostate cancer, there is a clear, although mechanistically poorly understood, crosstalk 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 (1) that activation of PI3K/Akt can confer resistance to anti-estrogens, and (2) that there is reciprocal crosstalk 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 *versus* 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 in an overall survival benefit. Indeed, a 4.6 month prolongation in PFS by the combination of everolimus (a rapamycin analog 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 cancer (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-<u>in</u>dependent manner, and at higher doses, to inhibit microtubule dynamics (40), which can dampen DNA repair but also have anti-tumor 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 anti-tumor responses by four main mechanisms (Figure 1):

1.  by improving circulation to the tumor by normalizing blood vessels to aid chemo- and immunotherapy, or by inhibiting angiogenesis-promoting tumor-associated myeloid cells to enhance VEGF-based anti-angiogenic 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;

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

## 1 Effects of PI3K inhibitors on the tumor vasculature (Figure 2)

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). 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—**Anti-angiogenic 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 intra-tumor vessel area, which is most often accompanied by reduced vessel function (39, 44–50) and anti-tumor activity; (ii) a milder anti-angiogenic 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 crosstalk 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, both in 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 (60–64), 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, 65). 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 anti-angiogenic effects in cancer, due to its ability to inhibit tumor-associated myeloid cells (66, 67). 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 way in which PI3K inhibition compares to 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, 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 (68–70), even upon short-term (3-day) administration (69), correlating with an enhanced capacity to deliver chemotherapy to the tumor (69). These findings point to a so-called 'vessel normalization' effect that is also observed upon administration of low (71) or single (69, 72) 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 anti-tumor immune cells to improve immunotherapy.

A prime example of an *indirect* anti-vascular effect of PI3K inhibition in cancer is the recent finding that resistance to VEGF-targeted therapy in tumors is partially mediated by pro-angiogenic tumor-associated myeloid cells, in a PI3Kγ-dependent manner (66). Accordingly, a PI3Kδ/PI3Kγ inhibitor was able to enhance tumor responsiveness to VEGF-based anti-angiogenic therapy (66). The contribution of PI3Kδ to this biological effect is unclear at present. Indeed, while PI3Kγ plays a clear role in tumor-associated myeloid cells (66), including macrophages (73), the exact role of PI3Kδ in these cells in cancer remains to be determined.

## 2 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 (74).

In a mouse model of pancreatic cancer, PI3Kγ inhibition was shown to dampen the secretion of PDGF-BB by tumor-associated macrophages (Figure 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 (75). A direct action of PI3K inhibitors (especially against PI3Kα/β) on such cancer-associated fibroblasts would be expected to inhibit their tumor-promoting activities (Figure 1), although this has not been formally tested, and will not be discussed further. However, it is likely that PI3K activity is an important regulator of this aspect of tumor-stroma interaction.

## 3 PI3Kδ inhibition disrupts stroma-cancer cell engagement in B-cell malignancies, rendering the tumor cells more susceptible to cell death (Figure 3)

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 (76–78). In contrast to other B-cell malignancies, which show enhanced contribution of PI3Kα (79) and/or activating mutations in signaling pathways parallel to PI3K (such as in the NFκB pathway (80)), 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 (81), 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 CLL patients 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 (81–83). 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 (84).

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 re-entry 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 by

bendamustine. The *in vitro* cytotoxic/cytostatic effect of PI3Kδ inhibitors on monocultures of CLL cells is modest (84). However, when CLL cells are co-cultured with stromal cells, PI3Kδ inhibition interferes with the stroma-dependent survival signals (5, 84, 85).

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 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), IFN-γ and other agonists (86, 87), 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 (88). The function of the monocyte-derived nurse-like cells and mesenchymal stromal cells in the stroma is possibly also affected by Idelalisib (84–87, 89).

To summarise, 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. 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 CLL patients.

## 4 Immuno-modulatory effects of PI3K inhibition in cancer (Figure 1)

While 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 anti-cancer activity, which is not restricted to hematological malignancies. Indeed, while PI3Kδ inhibitors can dampen many immune cell functions, such as antibody production, their effect on regulatory immune cell subsets, such as Treg, results, unexpectedly, in a *heightened* immune response against cancer and certain infectious agents (90, 91). Similarly, whilst PI3Kγ inhibition reduces the immediate response to bacterial infections, it also decreases the immune suppressive effects of myeloid cells in cancer (67, 73).

Below, we provide a detailed description of the immunomodulatory effects of PI3Kγ and PI3Kδ inhibition in cancer, focusing on the stimulation of T-cell responses, either through 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 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 (76, 92–94). Similarly, patients treated with Idelalisib often develop colitis (severe diarrhea) and, less frequently, pneumonitis and/or transaminitis (95–98). 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 3 trials of idelalisib (98).

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 and pneumonitis) and can often be alleviated by administration of steroids (83, 99, 100). Moreover, similar symptoms have been described in an individual with bi-allelic loss of *PIK3CD* (101), further suggesting that these are likely to be target-related adverse effects. Some Idelalisib-treated patients develop skin rash, which could be a result of inappropriate reactivity to commensal pathogens on the skin (99, 100). 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 (102).

In mice, colitis is often correlated with defects in Treg function (103). 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 co-injection of functional Treg (103). Of relevance in this context, Treg with inactive PI3Kδ failed to suppress such experimentally-induced colitis in mice (92). In addition, colon-associated macrophages in PI3Kδ kinase-dead mice are more inflammatory and less bactericidal than in wild-type mice (93, 94). 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 that have *not* been exposed to immune-suppressive therapies (83, 95–97, 99), indicative of a possible host T-cell immune response against the gut flora. There is also evidence that Treg are affected in patients on Idelalisib that develop colitis (100). It should be noted however, that different PI3Kδ inhibitor (TG-1202) shows similar efficacy as idelalisib in CLL and iNHL, but with fewer adverse effects (104). Although diarrhea was a common adverse effect with TG1202, 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 TG-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 (105). So-called *Pneumocystis jirovecii* pneumonitis (PJP) is most often associated with lymphopenia caused by HIV/AIDS chemotherapy or other forms of immunodeficiencies. 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 (106). As with colitis, co-injection of Treg prevents this

experimentally-induced PJP in mice (106); however, the specific role of PI3Kδ in Treg 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 (107). It is of interest to note that PJP has also been reported for mTOR inhibitors (rapamycin analogs), the mechanism of which is not understood to date but which may also involve an exacerbated Th1 T-cell–mediated autoimmune response (Ref. (107) 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 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 Treg and infiltration of CD8^{+} T-cells (100).

Taken together, evidence suggests that adverse effects observed upon Idelalisib treatment may be target-related and caused in part by inhibition of Treg followed by unrestrained T-cell-mediated immune responses against otherwise innocuous non-infectious 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 Treg, allowing weakened Th1 responses to contain parasites and prevent pathology (108). PI3Kδ kinase-dead mice are also more resistant to infection with the intracellular bacteria *Listeria monocytogenes* (109). Therefore, while initially assumed to primarily be a target for therapies aimed at reducing autoimmunity and inflammation (110), PI3Kδ may in fact also be targeted to increase certain innate and adaptive cell-mediated immune responses.

**PI3Kδ inhibition can enhance T-cell-mediated anti-tumor responses—**Diverse mechanisms are at play in tumors to promote the recruitment and expansion of Treg that dampen effector T-cell responses (111). PI3Kδ kinase-dead mice are resistant to diverse transplanted tumors and this resistance could not only be recapitulated but even improved by deleting PI3Kδ selectively from Treg (91). Moreover, PI3Kδ kinase-dead mice show enhanced resistance to secondary tumor administration, suggestive of the generation of tumor-specific immunological 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 (Ref. (91)).

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 (112) or for combining immunotherapy with chemotherapy (113), irradiation (114) or targeted therapies (115), including PI3K inhibitors (116). When used together with other immunotherapies or conventional therapies, PI3Kδ inhibitors may help in unleashing anti-tumor immune responses.

Several clinical trials are designed to test PI3Kδ inhibitors, either alone or in combination with other therapies, in non-hematological malignancies (Table 1). It will also be of interest to determine whether enhanced T-cell-mediated anti-tumor 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, while specific cells and their responses can be inhibited in isolation or at early time points, the surprising and unanticipated end result is a net anti-tumor immune response in several instances.

**PI3Kδ inhibition dampens Treg function (Figure 4)—**The precise mechanism through which inhibition of PI3Kδ blocks Treg-mediated suppression of anti-tumor immune responses remains to be fully elucidated. PI3Kδ-deficient Treg make less IL-10 and express lower levels of CD38, a receptor involved in the metabolism of extracellular metabolites (117, 118).

A recent study showed that mice with Treg-specific expression of an Akt-insensitive mutant of the FOXO1 transcription factor, displayed resistance to tumors (119). The authors hypothesize that in wild-type mice, inactivation of FOXO1 by PI3K/Akt signaling is required for Treg to mature and suppress anti-cancer immune responses. These studies suggest that the loss of PI3K signaling in Treg leads to increased FOXO1 activity and a failure of these cells to suppress anti-tumor immune responses.

The transcription factor BACH2, which antagonizes AP1-dependent transcription in T-cells(120), has recently also been shown to be regulated by PI3K/Akt signaling, in an analogous manner to that of FOXO1 (120), and selective loss of BACH2 in Treg is sufficient to confer tumor resistance (121). This is consistent with a model in which elevated PI3K signaling leads to the loss of BACH2 function and could destabilize Treg. Indeed, *increasing* PI3K signaling by inhibiting PTEN in Treg has also been shown to stimulate potent anti-tumor immune responses by destabilizing the Treg lineage, leading to impaired Treg function and reduced immune suppression (122). Reduced tumor growth could also be achieved using a systemically-administered PTEN-inhibitor that appeared to act primarily on PTEN within Treg (122). 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 (123). 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 Treg could be due to reduced FOXO1 transcription with consequently reduced transcription of FOXP3, a transcription factor that is essential for Treg development (85, 86).

Taken together, these data establish a potential signaling pathway from PI3Kδ to Akt towards the FOXO1 and BACH2 transcription factors (Figure 4) in Treg, with both the loss or overactivation of FOXO1 or BACH2 transcriptional activities resulting in a dampening of the capacitiy of Treg to suppress anti-tumor 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 (90).

**PI3Kδ inhibition can improve CD8⁺ T-cell activity in cancer**—As in Treg, the exact role of the PI3Kδ pathway in CD8⁺ T-cells is enigmatic (109, 124–129). 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 IFN-γ) and granzymes (such as GzmB) (91, 120, 127, 130, 131). 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 Treg (91, 109, 126, 132–134).

Inhibition of downstream signaling components in the PI3K pathway has also been shown to lead to enhanced T-cell-mediated anti-tumor activity *in vivo*. CD8⁺ T-cells cultured in the presence of an Akt inhibitor showed *enhanced* efficacy in adaptive cancer immunotherapy (132–134). Similarly, the mTOR inhibitor rapamycin, which inhibits CD8⁺ T-cell effector functions, can enhance the generation of memory CD8⁺ T-cells (135). 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 (109, 125), which may help to explain why these mice are able to reject tumors in a CD8⁺ T-cell-dependent manner (91). 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 (136). 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 (126).

Thus, although PI3Kδ inhibitors will initially reduce anti-tumor 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 IFN-γ and GzmB, by these cells.

**Inhibition of PI3Kδ and/or PI3Kγ in myeloid cells can lead to enhanced T-cell anti-tumor responses**—Tumor mass is often comprised of up to 50% of myeloid cells that can suppress immune responses and promote angiogenesis. Indeed, tumors have been described as non-healing wounds (137). In addition to directly affecting T-cells, there is significant potential for PI3Kγ and PI3Kδ inhibitors to stimulate anti-cancer immune responses through the modulation of myeloid cells. This can be either by inhibiting suppressive myeloid cells, by dampening immune-suppressive tumor-infiltrating macrophages (TAMs) or by stimulating macrophages and dendritic cells (DCs) 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 (66, 67, 73, 138). It is clear, however, that PI3Kδ can also modulate myeloid cells in cancer, which most likely contributes to the anti-tumor effects seen with PI3Kδ inhibitors (91, 139).

PI3Kγ-deficient mice are resistant to colon cancer that develops subsequent to oral administration of dextran sulfate sodium to induce chronic colitis (138). 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 (67, 140). The majority of these myeloid cells were CD11b^{+}, GR1^{low} and considered to be TAMs (67). TAMs are a major source of IL-1β, IL-6 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 programme 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 Treg (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 (91).

TAMs, and especially DCs, can be potent inducers of tumor immunity when exposed to IFN-γ and other pro-inflammatory cytokines, thus shifting the balance from a wound healing response to anti-tumor immunity (141, 142). There is evidence to suggest that this could be achieved by PI3Kδ inhibitors. Indeed, inhibition of PI3Kδ in LPS-stimulated DCs increases the production of pro-inflammatory cytokines (TNFα, IL-12, IL-1β) and reduces the levels of anti-inflammatory cytokines (IL-10, IFN-β) (143, 144). This is in line with observations from preclinical mouse models whereby administration of PI3K inhibitors reduces IL-10 and TGFβ production in favor of IL-12 production, and enhances DC-mediated anti-tumor therapy based on Toll-like receptor agonists (139). 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 (139). Tumor growth suppression was associated with increased accumulation of polyfunctional T-cells that secreted multiple effector cytokines, including IFN-γ, IL-17, and IL-2 (139).

## Future Directions

Over the last few years, it has become clear that harnessing the cancer cell-intrinsic anti-proliferative action of PI3K inhibition will be challenging as a monotherapy 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, crosstalk between hormonal and PI3K signaling in breast and prostate cancer, 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 to target not only 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 anti-cancer agents, an approach that is currently being tested in the clinic (Table 1). While 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 (116) 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. A key question is also 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 (145). 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 suitable for PI3K-based immunotherapies. Indeed, while Idelalisib is reasonably well-tolerated in therapy-resistant CLL, there are emerging data on toxicities, and even fatalities, in the upfront treatment setting of B-cell malignancies. The emerging evidence that toxicities of Idelalisib may have a strong immunological component may underlie the increased frequency of adverse effects in the first-line setting because a more intact cellular immune system in these non-pretreated patients (83, 99). 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 anti-cancer 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 chemo- and immunotherapy to the core of the tumors. Additional pre-clinical 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, to not only 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.

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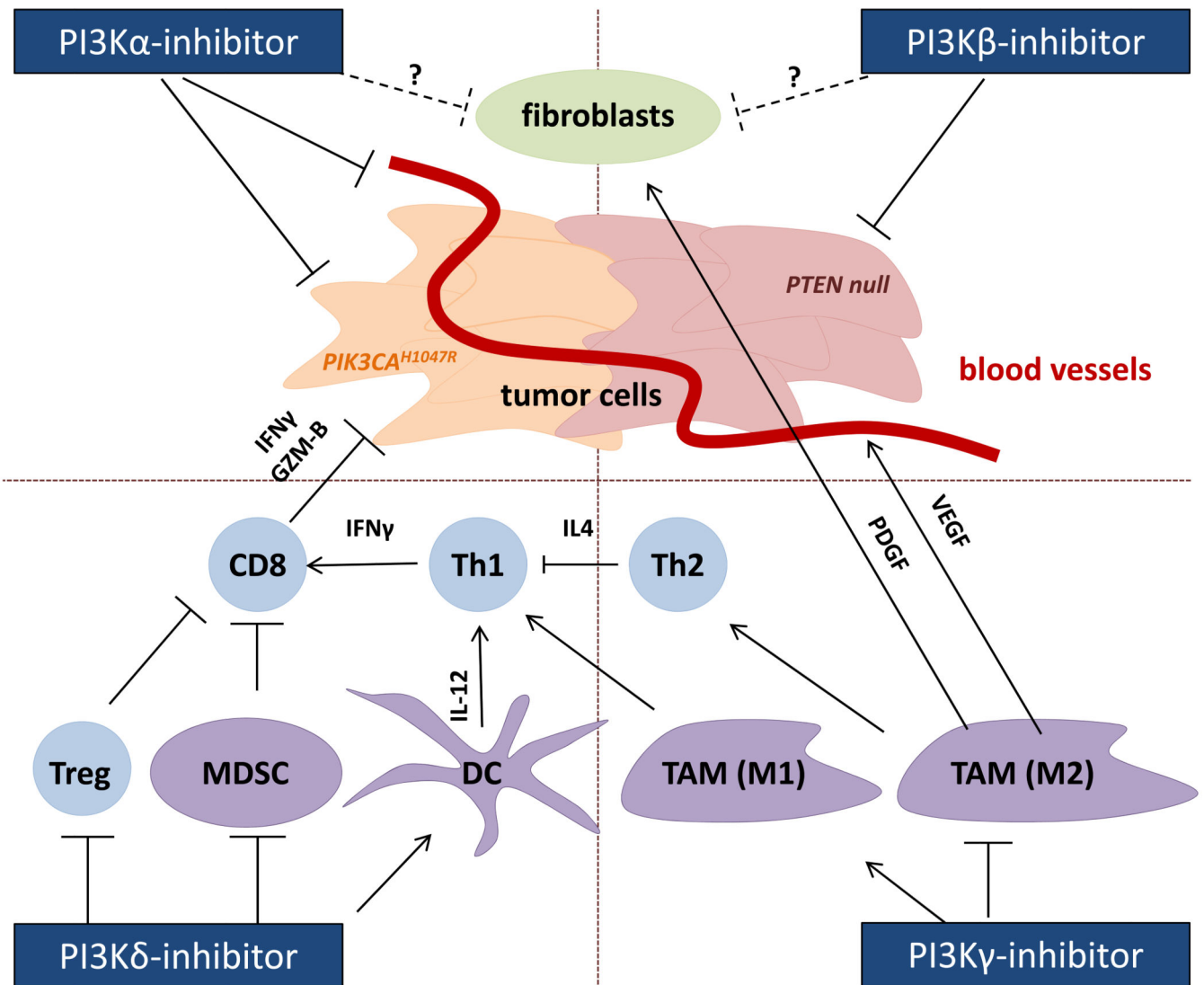
**Figure 1. Direct and indirect anti-cancer effects of interference with PI3K isoform activity**
Different PI3K isoforms have the potential to interfere with cancer growth and survival,
either by acting on the transformed cells directly, by interfering with the supportive stroma
and nutrient supply, 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 p110α[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 anti-tumor response by activating DCs to produce more IL-12 and by inhibiting Treg and MDSCs, which antagonize cell-mediated immunity in tumors. Arrows indicate a stimulatory impact.

## Vascular impact of interference with PI3K activity



The figure shows a flowchart originating from "Tumor cells" branching into VESSEL ERADICATION, VESSEL NORMALIZATION (subdivided into VESSEL PRUNING and VESSEL CONVERSION), and VESSEL PROMOTION. Blood vessels legend: red = aberrant vessels, purple = normalized vessels.

| | VESSEL ERADICATION | VESSEL PRUNING | VESSEL CONVERSION | VESSEL PROMOTION |
|---|---|---|---|---|
| REFERENCES | [138,139] | [145,146] | [147,148] | [151] |
| VESSEL DENSITY | ↓ | ↓ | = | ↑ |
| BLOOD FLOW | ↓ | ↑ | ↑ | ↑ |
| TUMOR HYPOXIA | ↑ | ↓ | ↓ | ↓ |
| DRUG & IMMUNE CELL DELIVERY | ↓ | ↑ | ↑ | ↑ |

HIGH DOSES OF PI3Ki [38,43-49] (under VESSEL ERADICATION)

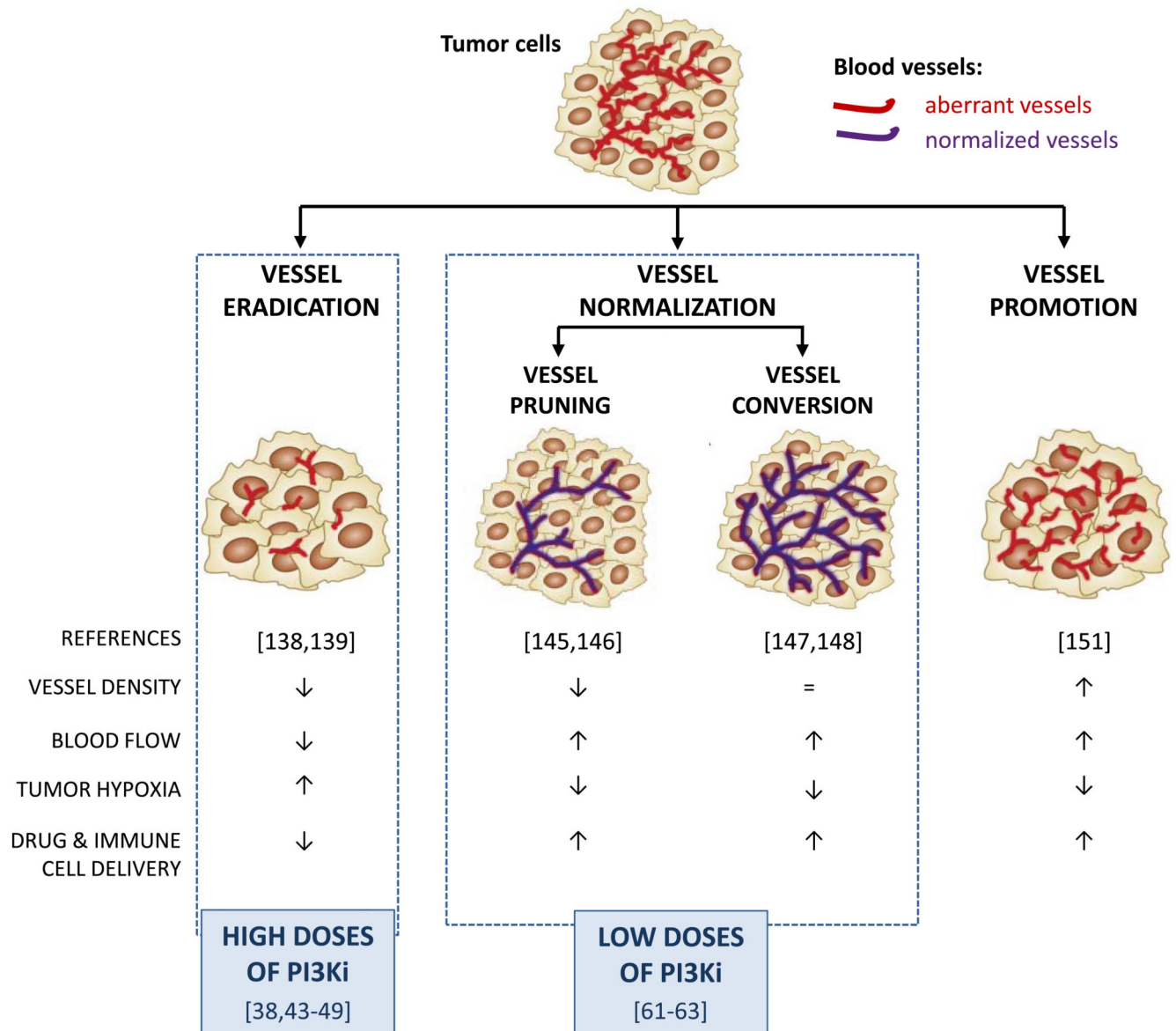LOW DOSES OF PI3Ki [61-63] (under VESSEL NORMALIZATION)

**Figure 2. Vascular targeting strategies in cancer and possible role of PI3K isoforms therein**
PI3K inhibitors, at high doses, have been documented to induce a mild vessel eradication response, whereas at low doses, 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: (1) **vessel eradication**, aimed at destroying the tumor vasculature and 'starving the tumor to death' (146, 147). Problems with this strategy are both intrinsic and acquired resistance to vascular trimming (148, 149), reduced chemotherapy delivery to the tumor and induction of hypoxia, which can accelerate tumor progression (51, 150); (2) **vessel normalization**, aimed at improving vascular perfusion and oxygenation, allowing enhanced drug delivery and immunotherapy (151, 152). This normalization effect can be the consequence of vessel pruning, with only normal vessels left

behind after therapy (153, 154) or of vessel conversion, a biological change in endothelial cells whereby the tubular structures are converted into more physiological, normal vessels (155, 156). The applicability of the normalization approach in the clinic has been limited by the transient window during which vessels are susceptible to normalization, difficulties to predict 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 (157, 158); (3) the **vessel promotion** strategy is based on stimulating vessel growth, response, with every tumor relying to different extents on angiogenic cues to stimulate vessel together with promotion of vasodilatation (159) and is aimed at enhancing delivery of chemotherapy and other anti-cancer agents to the tumor (160).
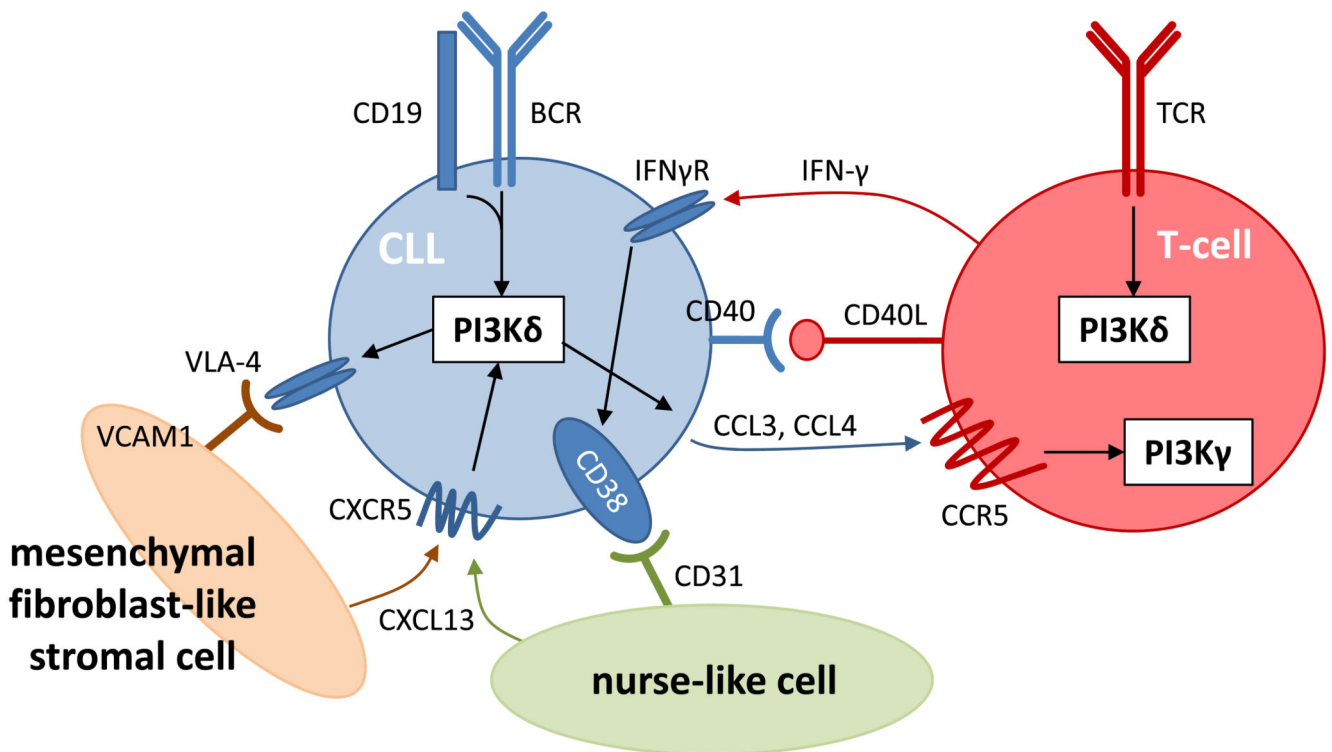
**Figure 3. Roles of PI3Kδ in CLL cells and their interaction with their surrounding stroma**
CLL cells depend on signaling via the BCR to increase proliferation, adhesion to
mesenchymal stromal cells (via VLA-4/V-CAM1), and secretion of the chemokines CCL3
and CCL4. The ligands (antigens) for the BCR 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), IFN-γ 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: (1) 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 (2) 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)
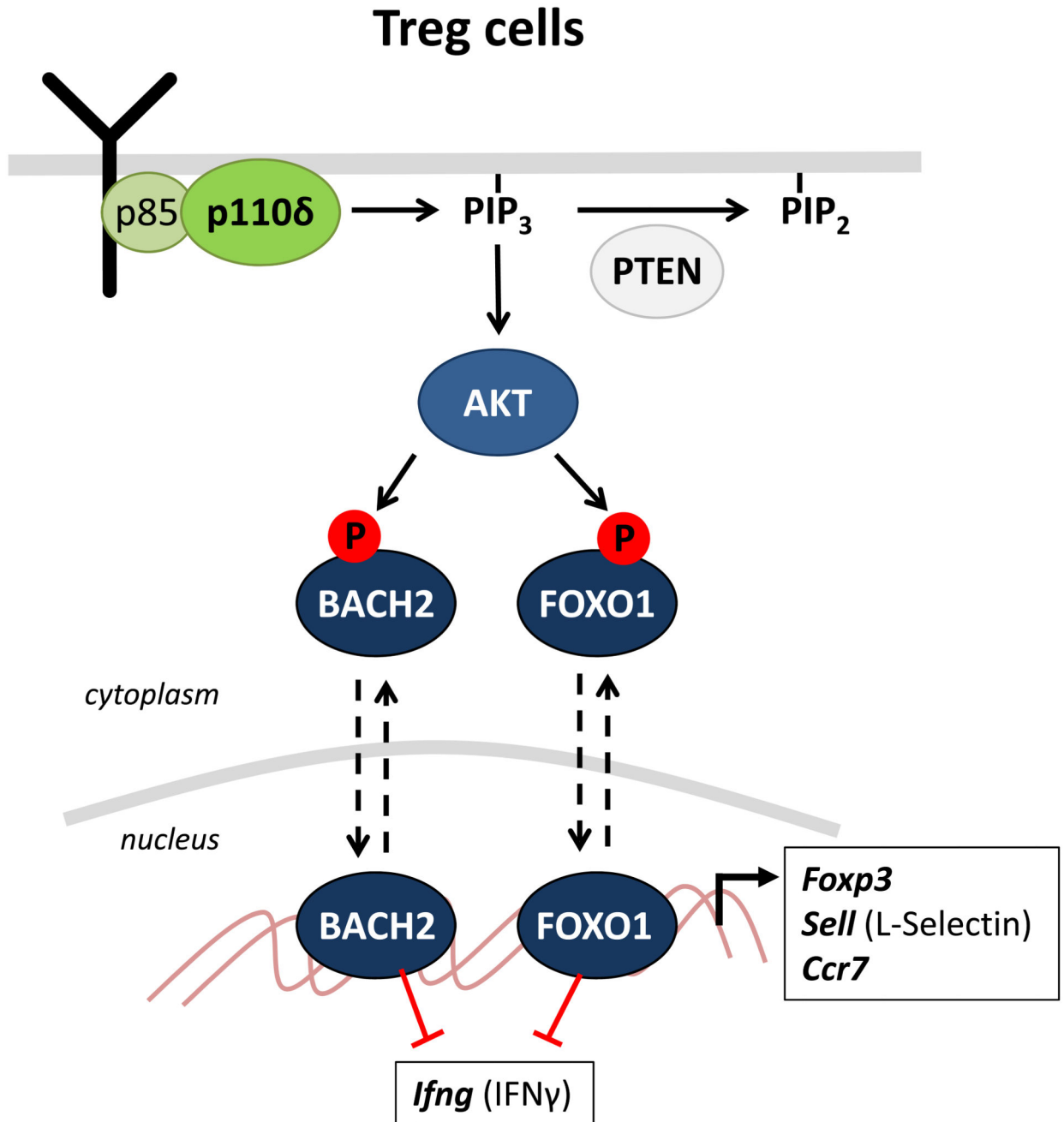environment into circulation where they are more susceptible to undergoing apoptosis.

**Figure 4. PI3K pathway components in Treg involved in the regulation of anti-cancer 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 Treg from undergoing a differentiation program and migrating effectively to tumors in order to suppress anti-tumor 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 Treg and production of pro-inflammatory cytokines instead of the normal immune-suppressive factors produced by Treg.

**Table 1**

Ongoing clinical trials of 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 (non-viral) | 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) |