Running title

PI3K family, cancer and immunity

Keywords: PI3K inhibitors; immunotherapy; combination therapy; PI3K pathway

Abstract (156)

Immunotherapy has led to a paradigm shift in the treatment of some malignancies, providing long-term, durable responses for a subset of patients with advanced cancers. Increasingly, research has identified links between the immune system and critical oncogenic growth factor pathways. The phosphoinositide 3-kinase (PI3K)-AKT-mTOR cascade is frequently hyperactivated in cancer, and plays an integral role in many cellular processes including tumour growth and survival and can underlie resistance to therapies.

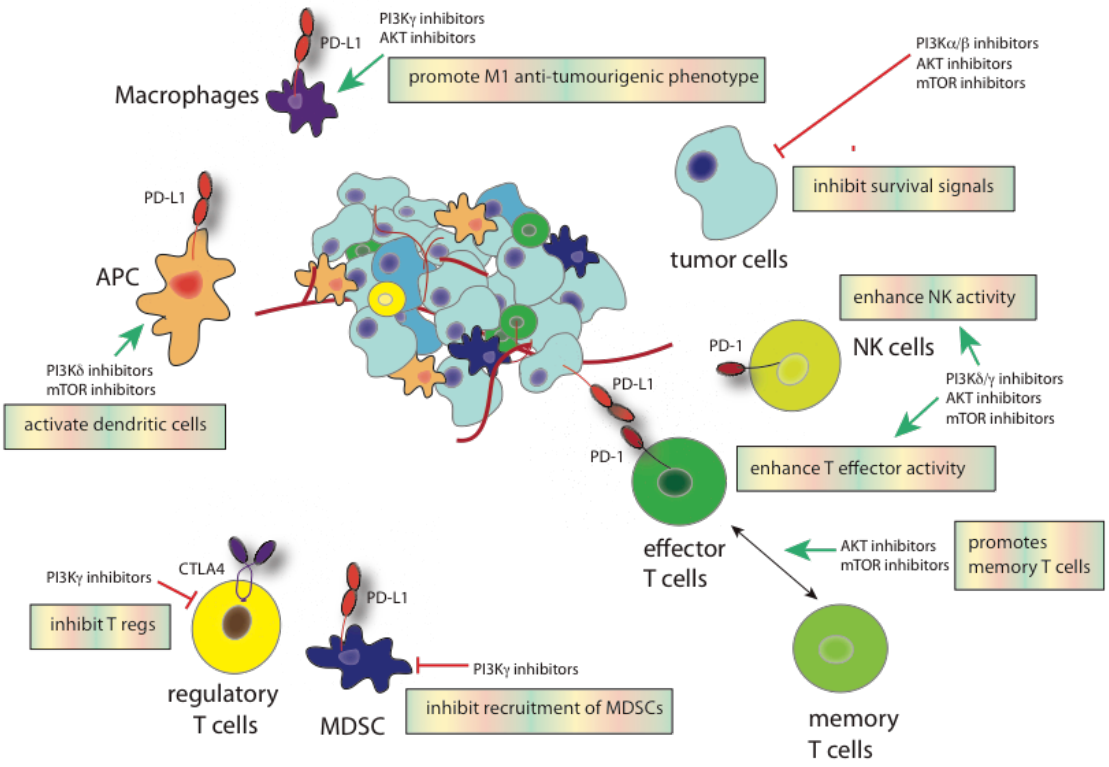
In this review, we first summarize two key learnings from the initial studies of inhibitors of this pathway, including the profile of immune-related adverse events such as colitis, transaminitis and pneumonitis and the increased incidence of infections with the majority of agents that target the PI3K-AKT-mTOR pathway. We then discuss recent advances in our understanding of the role of this pathway in the tumour micro-environment, and in the regulation of innate and adaptive immune responses, and propose synergistic combination strategies with PI3K-network inhibitors and cancer immunotherapy.

Graphical Abstract

Used in table of contents

Submit separately as electronic [under supplementary material] (preferably EPS, PDF, TIFF, MS word, PPT and CDX)

Not exceeding 30 words with illustration to summarize contents of manuscript



Introduction

The age of immunotherapy has made significant headway into the management of multiple malignancies, with enhanced tumor response rates and improved patient survival in certain cancers with immune checkpoint inhibitors. Accompanying research has furthered our understanding of the role of the immune system in cancer progression and uncovered interactions between it and dysregulated growth factor signaling pathways, such as the phosphatidylinositol-3 kinase (PI3K) cascade. Pathologic PI3K-AKT-mTOR (PAM) pathway activation is among the most frequent signaling event associated with cancer, and metastasis[1,2], yet inhibitors of PI3K and associated oncogenes have not been successful in improving survival of cancer patients when used as monotherapy with the exception of p110δ-selective inhibitors including Idelalisib (CAL-101, GS-1101, Zydelig®; Gilead Sciences) in B-cell malignancies[3,4]. Exciting emerging translational work has now identified hyperactivation of the PAM pathway to be a significant mechanism of resistance to immune checkpoint inhibitor therapy[5,6] suggesting that combinations of PAM pathway inhibitors and immunotherapy may potentially result in durable antitumor immune responses.

In this review, we summarize the key observations made to date on the role of the PAM pathway in innate and adaptive immunity, focusing especially on immune modulation and suggest novel combinatorial approaches to fully exploit the role of PAM both in cancer, and anti-cancer immunity for therapeutic benefit.

Background

The PAM pathway is comprised up of three main players: PI3K, protein kinase B (AKT) and mammalian target of rapamycin (mTOR) [Figure 1]. Although variable between cancer types, the PAM pathway is regularly altered in human tumors and drives tumor initiation, differentiation, metastasis and survival[7]. The PAM cascade has also been associated with genomic instability, angiogenesis, cancer cell motility and anti-cancer therapy resistance[8-10]. An identifiable alteration in at least one PAM pathway gene can be seen in up to 50% of multiple tumour types, including breast,

lung, glioblastoma, gynaecological and head and neck cancers[7,11]. In about one third, the tumor suppressor, phosphatase and tensin homologue (PTEN) expression is lost leading to a constitutively activated PAM pathway, with loss of negative control. Indeed germline loss of PTEN results in Cowden Syndrome, a hereditary hamartoma tumor syndrome. Mutations in PIK3CA are the next most commonly seen, followed by PTEN mutations or AKT aberrations[7,11].

The PI3K-AKT-mTOR (PAM) pathway

First identified in 1987, the oncogene PI3K is comprised of eight mammalian PI3K enzymes classified into 3 groups differentiated by structure and function[1,12]. The Class I PI3Ks are lipid kinases that phosphorylate the 3'-OH group of phosphoinositides. They are further subclassified into Class IA PI3Ks that are activated by growth factor signaling through receptor tyrosine kinases (RTK) and phosphorylate phosphatidylinositol (4,5)-bisphosphonate (PIP2) and generate phosphatidylinositol (3,4,5)-trisphosphate (PIP3). The Class IA PI3Ks are the most relevant PI3Ks from an oncologic perspective and are commonly targeted with precision inhibitors. They comprise a p85 regulatory subunit and a p110 catalytic subunit of which there are three p110 isoforms (alpha, beta, and delta) encoded by three genes (*PIK3CA*, *PIK3CB*, and *PIK3CD*, respectively)[13,14]. The class IA PI3Ks are activated via RTK signaling or pathway mutations such as EGFR, HER2, cKIT and cMET[15]. Class IB are activated by G protein-coupled receptors (GPCR) and comprise one p110 isoform (gamma) [Figure 1].

AKT is a serine/threonine kinase of the AGC family comprised of 3 isoforms, encoded by the respective genes *AKT1*, *AKT2*, and *AKT3*. Activated AKT is a central phosphorylator of PI3K and multiple substrates that drive cellular proliferation, motility, neovascularization, and apoptosis (for example, Bad, CD95L, BIM, caspase 9) and cell cycle regulation (CDKN2A/$p21^{CIP}$ and CDKN2B/$p27^{KIP}$)[16].

The 289kDa serine/threonine kinase mTOR is a master coordinator of numerous

upstream signals, the PI3K/AKT cascade being one of them[17,18]. The mTOR complex comprises mTORC1 and 2. The latter facilitates the activation of AKT through S473 phosphorylation, as well as other kinases and allows organization of the actin cytoskeleton. The former, mTORC1, regulates cellular metabolism and biosynthesis. The final predominant player in the PI3K-AKT-mTOR pathway is the tumor suppressor PTEN. It serves as a regulatory checkpoint and dephosphorylates PIP3 to PIP2.

As is clear, PI3K, AKT and mTOR regulate critical cancer-signaling crossroads and therefore make ideal targets for drug development. There is integral crosstalk between elements of the PAM signaling network and components of other oncogenic pathways downstream via AKT and mTOR resulting in feedback control and communication with horizontal pathways in particular the MAPK pathway involving the oncogenes *RAS*, *RAF*, *MEK*, *ERK* and downstream *MYC* (reviewed in [1]).

PI3K-AKT-mTOR pathway inhibitors and the immune system

Learning the lessons from the early clinical trials

On the whole, there are six general classes of agents that target the PI3K-AKT-mTOR network: (1) pan-class I PI3K inhibitors (with or without additional mTOR activity), (2) isoform-selective PI3K inhibitors, (3) rapamycin analogues, (4) mTOR inhibitors, (5) pan-inhibitors (PI3K-mTOR) and (6) AKT-specific inhibitors[1,19,20]. These are depicted, along with examples in Box 1. PI3Kδ inhibitors are the most developed to date with Idelalisib being the first to be approved by the US Food and Drug Administration (FDA) for the treatment of chronic lymphocytic leukaemia (CLL), small lymphocytic lymphoma and follicular lymphoma[3,4]. It showed anti-tumor activity and impressive overall response rates (ORR) of 40% in mantle cell lymphoma to 85% in Non-Hodgkins lymphoma (NHL)[21]. Second generation PI3Kδ inhibitors include TGR-1202 (RP5264; TG Therapeutics) and the PI3Kδ and γ isoform inhibitor, duvelisib (INN, IPI-145; Infinity). Disappointingly however, the clinical benefits seen from the large armamentarium of other PI3K inhibitors with

diverse isoform-selectivity profiles in early to late clinical trial testing have unfortunately failed to live up to its promise with only very modest single agent activity. This has raised questions about how critical the specific PIK3C alterations are in established cancer. For example, whereas the presence of PIK3CA mutation/amplification in cancer cell lines has some predictive value in determining sensitivity to PI3K inhibitors, this correlation is not absolute and has not been borne out in clinical studies[22].

The safety profile of these classes of inhibitors are now well-characterised through the clinical testing of multiple agents [1] and differs depending on the specific node in the pathway targeted, as well as the specific isoforms of the proteins targeted (Table 1). The ubiquitous expression and essential function of PI3Kα and β challenges their tolerability. In contrast, the PI3Kδ isoform is almost exclusively expressed in the hematopoietic lineage and involved in regulation of normal and malignant B-cell survival and proliferation. Therefore PI3Kδ inhibitors have a different and unique toxicity profile.

The collective experience of all these agents over the last decade has taught us two important lessons about the intricate physiological role the PI3K-AKT-mTOR pathway plays in the regulation of the innate and adaptive immune system that will be discussed in detail below. *Firstly*, inhibition of this pathway reduced the immediate immune response to infections, and *secondly* the adverse event profile of these inhibitors, particularly the isoform-selective inhibitors suggested evidence of a heightened immune response that may be in part responsible for some of the toxicities.

Effects of PI3K pathway inhibition on response to infection

As early as the initial dose escalation studies, the incidence of infective events was noted and reported. The phase I trial with duvelisib noted a significant increase in infectious toxicities including pneumonia[23]. Combined analysis of the three ongoing phase III trials showed 7.4% of fatal events in the Idelalisib arms versus 3.5% in the

control arms. Most deaths involved opportunistic predominantly respiratory infections such as *Pneumocystis jirovecii* pneumonia (PJP) and cytomegalovirus (CMV)[23]. As a result, in March 2016, the FDA halted six clinical trials exploring Idelalisib in combination with other therapies in haematological malignancies due to reported increase in adverse events and death, including first-line combination of Idelalisib with bendamustine/rituximab (NCT01980888) and obinutuzumab (NCT01980875). The Pharmacovigiliance Risk Assessment Committee of the European Medicines Agency (EMA) recommends all patients treated with Idelalisib should receive antibiotics as prophylaxis against PJP. It is also recommended to monitor patients for signs of infection on a regular basis with frequent laboratory and clinical assessments for CMV and other infections.

Recently, Rafii *et al* went on to show that this increased risk of infection was seen more broadly with a range of novel anti-tumor therapies against the PAM pathway although the agents themselves were not myelosuppressive[24]. In a review of 432 patients treated with PI3K-AKT-mTOR inhibitors, all grade infections requiring antibiotics was 27% for those receiving PI3K-AKT-mTOR pathway inhibitors versus 8% in the control group. A similar pattern followed for high grade events. Single agent PI3K-AKT-mTOR inhibitors had a greater risk of infection. Infective complications were not dose-dependent and occurred on every cycle of treatment although data hinted towards increased frequency earlier in dosing schedules. With chemotherapy and PI3K-AKT-mTOR inhibitor combination trials, the incidence of grade 3 and 4 infective events was 16.6%. Subgroup analysis identified dual PI3K/mTOR inhibitors as conferring a higher risk of infection than pan-PI3K/AKT/mTOR inhibitors. The most common infections were urinary (38%) and respiratory (28%), followed by gastroenteritis, sepsis, cellulitis and line infections (33%). The most common pathogens were *Escherichia coli* (48%) followed by *Pseudomonas spp.* (22%), other enterococci and pneumococci (28%). This data supports the proactive management of patients and the early use of antimicrobials in these patients in addition to the EMA and FDA recommendations of prophylaxis antibiotics against opportunistic infections.

Immune-mediated adverse events due to PI3K inhibition

The other important adverse events seen with PI3K inhibitors are pneumonitis, colitis and transaminitis. There is a wealth of data supporting an immune-aetiology for these, particularly as they are more often seen in patients at an earlier line of treatment who are more likely to be immunocompetent and respond to steroids[25]. Furthermore, patients with Idelalisib-associated colitis showed T-cell infiltration and features of graft-vs-host disease on histological analysis of mucosal biopsies obtained at colonoscopy[26]. Patients also showed a corresponding decrease in multiple inflammatory chemokines and cytokines, including tissue necrosis factor alpha (TNFα) and a decrease in the immune-modulatory cytokine interleukin 10 (IL10). Indeed reduction of IL10 may contribute to the development of PI3K inhibitor-induced colitis as it is an essential immune-regulator in the gastrointestinal tract. As a matter of fact, IL10-deficient mice have been used as a model of inflammatory bowel disease (IBD) for years[27].

Pneumonitis is a further inflammatory-driven sequelae of PI3K inhibition. There is a paucity of data regarding the precise mechanism(s) of action but it is possible that a similar aetiology is responsible for pneumonitis as with colitis including a reduction in IL10 and T cell activation and infiltration. Cytological analysis of BAL fluid in patients with mTORi-induced pneumonitis typically shows hypercellularity with CD4+ lymphocytosis with high levels of proinflammatory cytokines such as IL-1β [28]. Increases of interferon-γ (IFNγ) and IL6, 7, and 8 have also been observed in patients who developed pneumonitis on a Phase II Idelasilib combination study (NCT01796470)[29].

Immune-mediated hepatitis was also found to be a frequent adverse event of Idelalisib, occurring in 79% of CLL patients treated in a first-line study (NCT02135133)[25]. A lymphocytic infiltrate was seen on liver biopsy specimens taken from two subjects with transaminitis, and levels of the proinflammatory cytokines CCL-3 and CCL-4 were higher in subjects experiencing hepatotoxicity. All

cases of transaminitis resolved either by holding the drug, initiating immunosuppressants, or both, and rates of recurrent toxicity were lower in patients taking steroids when Idelalisib was reinitiated.

Genetics also provides orthogonal evidence supporting the role of PI3K/AKT pathway aberrations in inflammatory processes. Colitis has been described in an individual with biallelic loss of PIK3CD[30] and genetic variants in the PI3K and AKT have been associated with severe radiation pneumonitis in lung cancer patients treated with radiation therapy[31].

Understanding the biology

Given the clinical clues, much preclinical work has attempted to dissect out the normal physiological role of the PAM pathway in the cancer-immunity cycle (Figure 2). Much of this work has concentrated on the role of PI3Kγ and PI3Kδ given their high expression in leukocytes and myeloid cells, but also focuses upon the interplay between the downstream effectors AKT and mTOR that directly affect the immune system as seen with the long history of mTOR inhibitors in renal transplantation regulating immune responses to non-native tissues[32].

Effect on antigen presentation and T cell infiltration

Firstly, the PAM pathway has an emerging role in antigen presentation. PTEN-deficient dendritic cells in a conditional mouse model were unable to efficiently prime T-cells [33] and PI3Kδ inhibitors can reverse this effect activating dendritic cells to produce more IL12 [34]. This translates both into an impairment of recruitment of tumour-infiltrating lymphocytes (TIL) into the tumour [5] and a profound effect on B and T lymphocyte function [35].

Building on this, the combination of the mTOR inhibitor AZD8055 together with immunotherapy resulted in greater intra-tumoral CD8+ T cell, dendritic cell and macrophage infiltration in a renal cell cancer model resulting in superior disease control compared with either monotherapy[36].

Effect on the tumour microenvironment

In addition, the PAM pathway also has a critical role in maintaining a profoundly immunosuppressive tumour microenvironment, through its effects on regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC). PTEN acted to stabilize Tregs in tumors, preventing them from reprogramming into inflammatory effector cells [37]. Intricate conditional mice models are dissecting out the precise mechanisms through which this effect is mediated, and suggests that the effect of FOXO1 activity on Treg maturation may be key [38]. In parallel, work in a PTEN-deficient prostate cancer mouse model identified MDSCs as the major infiltrating cell population, and inhibition of PI3Kγ dampened the recruitment of MDSCs and slowed tumour progression [39]. Furthermore, PI3Kγ inhibition as well as AKT 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 [40,41].

Separately, Kaneda *et al* showed how PI3Kγ signalling in macrophages might control a switch between immune suppression and stimulation. Interestingly, levels of macrophage infiltration in tumours were unchanged by PI3Kγ inhibition, but the expression of inflammatory cytokines by these cells was increased, and immunosuppressive factor expression was decreased[42]. The combination of PI3Kγ and PD1 inhibitors significantly suppressed tumour growth in mouse models of lung and head and neck cancers[42].

Effect on the adaptive immune system

The PAM pathway also has an important role in the adaptive immune system, where treatment with the mTOR inhibitor rapamycin for example, increased not only the early expansion of $CD8^+$ antigen-specific T cells, but increased the differentiation of effector T cells to memory T cells [43], and effectively sensitizes established cancers to adoptive immunotherapy *in vivo* [44]. Similarly, AKT inhibition has also been shown to

induce the expansion of TILs promoting a memory T cell phenotype[45] strengthening the rationale for combinations with adoptive cell therapies which is indeed now being taken forward into phase I trial (NCT02489266).

Effect in immune checkpoint resistance

Emerging studies are showing a close association between tumour PAM hyper-activation and host inflammatory activity in the vicinity of the tumor through pathway crosstalk, although details of this interaction are still unclear. Indeed, not only does inactivation of PTEN in a melanoma mouse model promotes immune resistance by the tumor, patients with tumours that had lost PTEN had poorer outcomes when treated with checkpoint inhibition [5,6]. Further evidence of how hyperactivated PI3K signaling within the tumour can influence the tumour microenvironment and thereby the host immune response comes from glioma models where tumour intrinsic PI3K activation underlies resistance to the tumour-microenvironment inhibitor colony-stimulating factor-1 receptor (CSF-1R) [46].

De Henau and colleagues interrogated a mouse breast cancer model resistant to checkpoint blockade with both PD1 or cytotoxic T lymphocyte associated antigen 4 (CTLA4) inhibitors, and showed that it had increased infiltration of immunosuppressive myeloid cells compared with a control model that responds to checkpoint blockade[47]. A PI3Kγ inhibitor, IPI-549 successfully inhibited tumour growth in models that had high levels of myeloid cell infiltration, but not in models with low infiltration, and switched macrophages from an immunosuppressive phenotype to an inflammatory one[47]. Furthermore the combination of PD1, CTLA4 and PI3Kγ inhibitors led to complete remission in a significant proportion of mice suggesting that the combination might prove efficacious against tumours that are resistant to checkpoint blockade[47].

Interplay with PD-1/PD-L1

In context of the excitement surrounding checkpoint inhibitors, the interplay between the PD-1/PDL1 axis and the PI3K-AKT-mTOR pathway is of real

significance. Transcription of PD-L1 can be induced by many cytokines downstream of the PAM cascade, of which IFNγ is the most potent[48,49]. PD-L1 expression has been shown to depend upon mTOR signaling in multiple tumor types, but interestingly, not melanoma[49]. Loss of PTEN may also be an important mechanism for the high PD-L1 expression seen in glioma cell lines and patient tumour specimens[50]. Correspondingly, inhibition of PI3K, AKT, or mTOR actively decreases PD-L1 expression [51] although the biological significance of this is yet unclear.

Other immune effects

Finally, the PI3K pathway also has an emerging role in the induction of immune-dependent tumour cell senescence, likely via stimulating the production of IFNγ and TNFα[52]. PTEN loss may also protect tumor cells from T cell killing through an autophagy-dependent mechanism [5].

Strategies for next generation immune-oncology combinations

Given that the PAM pathway lies squarely at the cross-roads of cancer and immunity, targeting it in combination with cancer immunotherapy is an exciting possibility. While pan-PI3K inhibitors could directly target tumour cells that exhibit hyperactivation of the pathway, isoform-specific (delta/gamma) inhibitors could modulate the physiological host immune responses to cancer, by promoting T cell infiltration, enhancing antigen presentation and thereby augmenting the recognition of tumor cells by T lymphocytes and NK cells. Pan-PI3K inhibitors, and also AKT/mTOR inhibitors by virtue of their nodes of action may be able affect both tumour cells as well as the host immune response thereby breaking tumour 'oncogene addiction', altering the tumour microenvironment and enhancing T cell cytotoxicity (Figure 2).

Early phase trials investigating such combinations are already underway (Table 2) but will require carefully planned Phase I dose-finding trials to optimally incorporate biologically rational combinations to efficiently exploit this crosstalk.

Key to the development of these combinations is intelligent trial design based on a thorough understanding of the underlying immunomodulatory mechanisms. Logistical parameters such as timing, dosage and sequence of administration will need to be carefully considered. Questions requiring consideration include whether combinations should be given concurrently or sequentially? Furthermore, given that it has been exceedingly difficult to deliver full doses of PAM pathway inhibitors in combination trials, will the overlapping immune-toxicities be augmented with combinations? Should inhibitors of the PAM pathway be given intermittently to improve tolerability? Is pan-network inhibition necessary, or will isoform-specific inhibitors be more specific? Is there an immune-modulatory dose to be achieved (that may be lower than conventional maximum tolerated doses, and therefore more tolerable)? Dose finding strategies will need to be clearly and biologically defined to enable clinically beneficial dosing to be achieved. Close monitoring and early recognition of immunotherapy toxicities as well as effective management with established protocols and standard operating procedures will be essential to taking these combinations forward.

The most exciting thing about developing combinations of immunotherapy together with PAM inhibitors is the potential for the incorporation of biomarkers that could be predictive of response. Early phase trials could include stratification for tumour PTEN loss, or other markers of a hyperactivated PAM pathway within the tumour that may clearly identify patients who are likely to benefit.

Conclusion

This is indeed an extraordinary time to be developing immunotherapy combinations and may be the coming of age of inhibitors of the PAM cascade. Learning the biological and immunological lessons from the early studies of PI3K pathway inhibitors will enable the rationale development of next generation immunotherapy combinations with biomarker driven rationale patient selection.

List of abbreviations

AKT: protein kinase B
CCL: chemokine ligand
cKIT: tyroxine protein kinase Kit or CD117
CLL: Chronic lymphocytic leukaemia
cMET: tyroxine protein kinase Met or hepatocyte growth factor receptor
CMV: cytomegalovirus
CSF-1R: colony stimulating factor 1 receptor
CTLA4: cytotoxic T lymphocyte-associated antigen 4
EGFR: Epidermal growth factor receptor
EMA: European Medicine Agency
ERK: extracellular signal-related kinase
FDA: Food and Drug Administration
FOXO1: forkhead box protein O1
GPCR: G protein coupled receptor
GTP: guanosine-5'-triphosphate
HER2: human epidermal growth factor receptor 2
IFN: interferon
IHC: immunohistochemistry
IL: interleukin
MEK: MAPK/ERK kinase
mTOR: mammalian target of rapamycin
NHL: Non Hodgkins lymphoma
NK cell: natural killer cell
ORR: overall response rate
PAM: PI3K-AKT-mTOR
PD-L1: programmed death-ligand 1
PD1: programmed cell death protein 1
PI3K: phosphoinositol-3 kinase
PIP2: phosphatidylinositol (4,5)-bisphosphonate

PIP3: phosphatidylinositol (3,4,5)-trisphosphate

PJP: pneumocystis jiroveci pneumonia

PTEN: phosphate and tensin

RAF: rapidly accelerated fibrosarcoma

RAS: rat sarcoma

RTK: receptor tyrosine kinase

RTK: receptor tyrosine kinase

TIL: tissue infiltrating lymphocyte

TNF: tissue necrosis factor

Treg: regulatory T cell

Conflicts of interest

Dr Juanita Lopez has received honoraria from Roche

Dr Chenard Poirier has no conflicts of interest to declare

Dr Collins has received honoraria from Janssen Sanofi

Acknowledgements

The authors have no acknowledgements to declare

References

1 Yap, T. A., Bjerke, L., Clarke, P. A. & Workman, P. Drugging PI3K in cancer: refining targets and therapeutic strategies. *Curr Opin Pharmacol* **23**, 98-107, doi:10.1016/j.coph.2015.05.016 (2015).

2 Fruman, D. A. & Rommel, C. PI3K and cancer: lessons, challenges and opportunities. *Nat Rev Drug Discov* **13**, 140-156, doi:10.1038/nrd4204 (2014).

3 Brown, J. R. *et al.* Idelalisib, an inhibitor of phosphatidylinositol 3-kinase p110delta, for relapsed/refractory chronic lymphocytic leukemia. *Blood* **123**, 3390-3397, doi:10.1182/blood-2013-11-535047 (2014).

4 Furman, R. R. *et al.* Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med* **370**, 997-1007, doi:10.1056/NEJMoa1315226 (2014).

5 Peng, W. *et al.* Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy. *Cancer Discov* **6**, 202-216, doi:10.1158/2159-8290.CD-15-0283 (2016).

6 George, S. *et al.* Loss of PTEN Is Associated with Resistance to Anti-PD-1 Checkpoint Blockade Therapy in Metastatic Uterine Leiomyosarcoma. *Immunity* **46**, 197-204, doi:10.1016/j.immuni.2017.02.001 (2017).

7 LoRusso, P. M. Inhibition of the PI3K/AKT/mTOR Pathway in Solid Tumors. *J Clin Oncol*, doi:10.1200/JCO.2014.59.0018 (2016).

8 Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646-674, doi:10.1016/j.cell.2011.02.013 (2011).

9 Samuels, Y. & Ericson, K. Oncogenic PI3K and its role in cancer. *Curr Opin Oncol* **18**, 77-82 (2006).

10 Hirsch, E., Ciraolo, E., Franco, I., Ghigo, A. & Martini, M. PI3K in cancer-stroma interactions: bad in seed and ugly in soil. *Oncogene* **33**, 3083-3090, doi:10.1038/onc.2013.265 (2014).

11 Millis, S. Z., Ikeda, S., Reddy, S., Gatalica, Z. & Kurzrock, R. Landscape of Phosphatidylinositol-3-Kinase Pathway Alterations Across 19784 Diverse Solid Tumors. *JAMA Oncol*, doi:10.1001/jamaoncol.2016.0891 (2016).

12 Kaplan, D. R. *et al.* Common elements in growth factor stimulation and oncogenic transformation: 85 kd phosphoprotein and phosphatidylinositol kinase activity. *Cell* **50**, 1021-1029 (1987).

13 Engelman, J. A., Luo, J. & Cantley, L. C. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* **7**, 606-619, doi:10.1038/nrg1879 (2006).

14 Vanhaesebroeck, B., Leevers, S. J., Panayotou, G. & Waterfield, M. D. Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem Sci* **22**, 267-272 (1997).

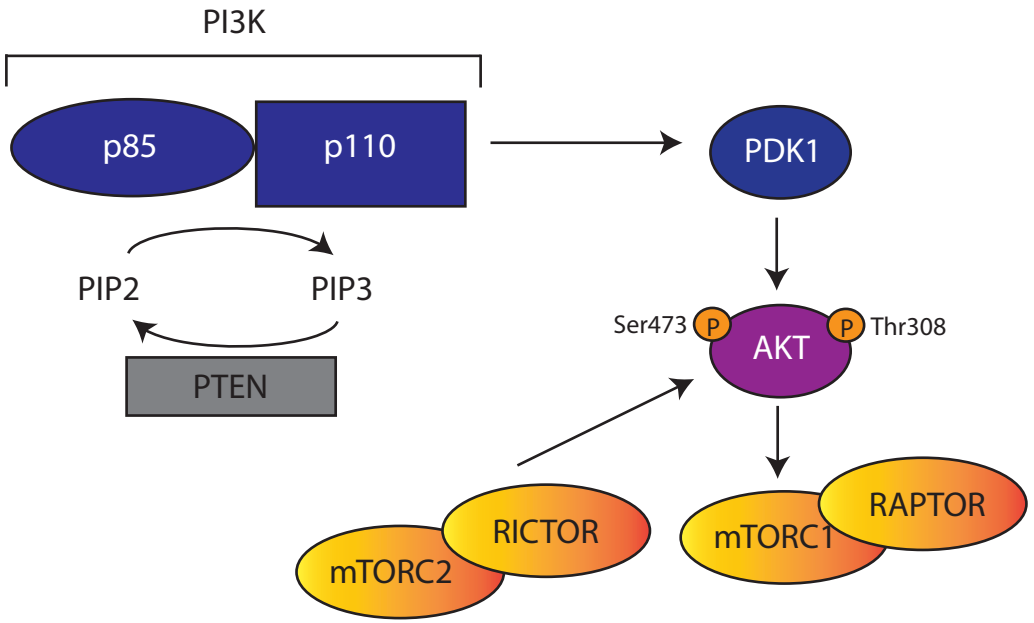
15 Engelman, J. A. *et al.* ErbB-3 mediates phosphoinositide 3-kinase activity in gefitinib-sensitive non-small cell lung cancer cell lines. *Proc Natl Acad Sci U S A* **102**, 3788-3793, doi:10.1073/pnas.0409773102 (2005).

16 Mundi, P. S., Sachdev, J., McCourt, C. & Kalinsky, K. AKT in cancer: new molecular insights and advances in drug development. *Br J Clin Pharmacol* **82**, 943-956, doi:10.1111/bcp.13021 (2016).
17 Laplante, M. & Sabatini, D. M. mTOR signaling in growth control and disease. *Cell* **149**, 274-293, doi:10.1016/j.cell.2012.03.017 (2012).
18 Zoncu, R., Efeyan, A. & Sabatini, D. M. mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat Rev Mol Cell Biol* **12**, 21-35, doi:10.1038/nrm3025 (2011).
19 Workman, P., Clarke, P. A., Guillard, S. & Raynaud, F. I. Drugging the PI3 kinome. *Nat Biotechnol* **24**, 794-796, doi:10.1038/nbt0706-794 (2006).
20 Bendell, J. C. *et al.* A phase 1 study of the sachet formulation of the oral dual PI3K/mTOR inhibitor BEZ235 given twice daily (BID) in patients with advanced solid tumors. *Invest New Drugs* **33**, 463-471, doi:10.1007/s10637-015-0218-6 (2015).
21 Kahl, B. S. *et al.* A phase 1 study of the PI3Kdelta inhibitor idelalisib in patients with relapsed/refractory mantle cell lymphoma (MCL). *Blood* **123**, 3398-3405, doi:10.1182/blood-2013-11-537555 (2014).
22 Fritsch, C. *et al.* Characterization of the novel and specific PI3Kalpha inhibitor NVP-BYL719 and development of the patient stratification strategy for clinical trials. *Mol Cancer Ther* **13**, 1117-1129, doi:10.1158/1535-7163.MCT-13-0865 (2014).
23 Balakrishnan, K. *et al.* The phosphoinositide-3-kinase (PI3K)-delta and gamma inhibitor, IPI-145 (Duvelisib), overcomes signals from the PI3K/AKT/S6 pathway and promotes apoptosis in CLL. *Leukemia* **29**, 1811-1822, doi:10.1038/leu.2015.105 (2015).
24 Rafii, S. *et al.* Higher Risk of Infections with PI3K-AKT-mTOR Pathway Inhibitors in Patients with Advanced Solid Tumors on Phase I Clinical Trials. *Clin Cancer Res* **21**, 1869-1876, doi:10.1158/1078-0432.CCR-14-2424 (2015).
25 Lampson, B. L. *et al.* Idelalisib given front-line for treatment of chronic lymphocytic leukemia causes frequent immune-mediated hepatotoxicity. *Blood* **128**, 195-203, doi:10.1182/blood-2016-03-707133 (2016).
26 Weidner, A. S. *et al.* Idelalisib-associated Colitis: Histologic Findings in 14 Patients. *Am J Surg Pathol* **39**, 1661-1667, doi:10.1097/PAS.0000000000000522 (2015).
27 Kubler LM, B. M., Hager C, Bleuch A. . A Multihit Model: Colitis lessons form the interleukin-10 deficient mouse. *Inflamm Bowel Dis* **21**, 1967-1975 (2015).
28 Willemsen, A. E. *et al.* mTOR inhibitor-induced interstitial lung disease in cancer patients: Comprehensive review and a practical management algorithm. *Int J Cancer* **138**, 2312-2321, doi:10.1002/ijc.29887 (2016).
29 Barr, P. M. *et al.* Phase 2 study of idelalisib and entospletinib: pneumonitis limits combination therapy in relapsed refractory CLL and NHL. *Blood* **127**, 2411-2415, doi:10.1182/blood-2015-12-683516 (2016).

30 Zhang KJ, H. A., Marsh R, Jordan MB. Identification of a phosphoinositide 3-kinase (PI-3K) p110delta (PIK3CD) deficient individual. *J Clin Immunol* **33**, 673-674 (2013).
31 Tang, Y. *et al.* Genetic variants in PI3K/AKT pathway are associated with severe radiation pneumonitis in lung cancer patients treated with radiation therapy. *Cancer Med* **5**, 24-32, doi:10.1002/cam4.564 (2016).
32 Hardinger, K. L., Koch, M. J. & Brennan, D. C. Current and future immunosuppressive strategies in renal transplantation. *Pharmacotherapy* **24**, 1159-1176 (2004).
33 Kuttke, M. *et al.* Myeloid PTEN deficiency impairs tumor-immune surveillance via immune-checkpoint inhibition. *Oncoimmunology* **5**, e1164918, doi:10.1080/2162402X.2016.1164918 (2016).
34 Fukao, T. *et al.* PI3K-mediated negative feedback regulation of IL-12 production in DCs. *Nat Immunol* **3**, 875-881, doi:10.1038/ni825 (2002).
35 Okkenhaug, K. *et al.* Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science* **297**, 1031-1034, doi:10.1126/science.1073560 (2002).
36 Jiang, Q. *et al.* mTOR kinase inhibitor AZD8055 enhances the immunotherapeutic activity of an agonist CD40 antibody in cancer treatment. *Cancer Res* **71**, 4074-4084, doi:10.1158/0008-5472.CAN-10-3968 (2011).
37 Sharma, M. D. *et al.* The PTEN pathway in Tregs is a critical driver of the suppressive tumor microenvironment. *Sci Adv* **1**, e1500845, doi:10.1126/sciadv.1500845 (2015).
38 Luo, C. T., Liao, W., Dadi, S., Toure, A. & Li, M. O. Graded Foxo1 activity in Treg cells differentiates tumour immunity from spontaneous autoimmunity. *Nature* **529**, 532-536, doi:10.1038/nature16486 (2016).
39 Wang, G. *et al.* Targeting YAP-Dependent MDSC Infiltration Impairs Tumor Progression. *Cancer Discov* **6**, 80-95, doi:10.1158/2159-8290.CD-15-0224 (2016).
40 Kaneda, M. M. *et al.* Macrophage PI3Kgamma Drives Pancreatic Ductal Adenocarcinoma Progression. *Cancer Discov* **6**, 870-885, doi:10.1158/2159-8290.CD-15-1346 (2016).
41 Vergadi, E., Ieronymaki, E., Lyroni, K., Vaporidi, K. & Tsatsanis, C. Akt Signaling Pathway in Macrophage Activation and M1/M2 Polarization. *J Immunol* **198**, 1006-1014, doi:10.4049/jimmunol.1601515 (2017).
42 Kaneda, M. M. *et al.* PI3Kgamma is a molecular switch that controls immune suppression. *Nature* **539**, 437-442, doi:10.1038/nature19834 (2016).
43 Araki, K. *et al.* mTOR regulates memory CD8 T-cell differentiation. *Nature* **460**, 108-112, doi:10.1038/nature08155 (2009).
44 Hahnel, P. S. *et al.* Targeting AKT signaling sensitizes cancer to cellular immunotherapy. *Cancer Res* **68**, 3899-3906, doi:10.1158/0008-5472.CAN-07-6286 (2008).
45 Crompton, J. G. *et al.* Akt inhibition enhances expansion of potent tumor-specific lymphocytes with memory cell characteristics. *Cancer Res* **75**, 296-305, doi:10.1158/0008-5472.CAN-14-2277 (2015).

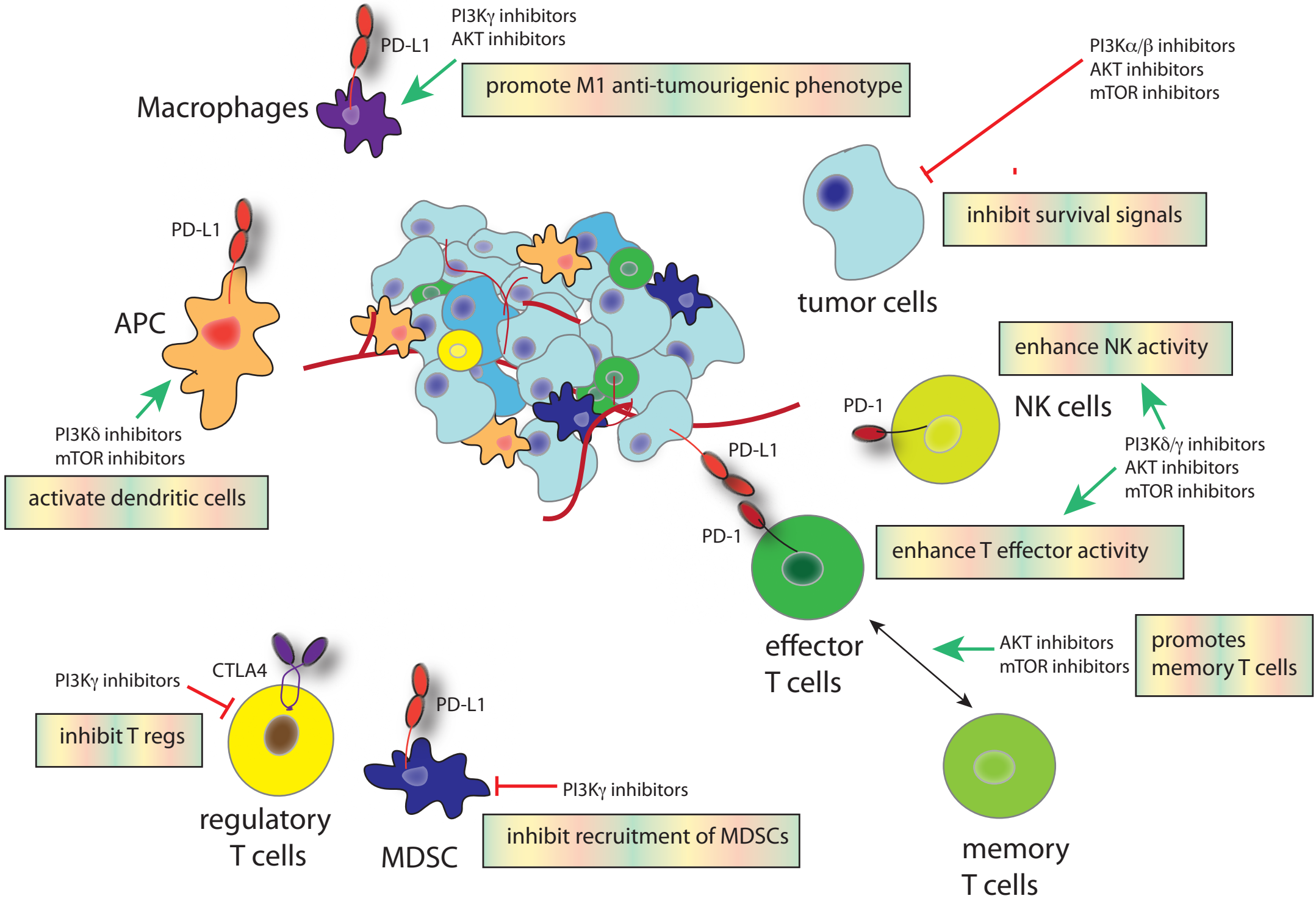
46 Quail, D. F. *et al.* The tumor microenvironment underlies acquired resistance to CSF-1R inhibition in gliomas. *Science* **352**, aad3018, doi:10.1126/science.aad3018 (2016).
47 De Henau, O. *et al.* Overcoming resistance to checkpoint blockade therapy by targeting PI3Kgamma in myeloid cells. *Nature* **539**, 443-447, doi:10.1038/nature20554 (2016).
48 Dong, H. *et al.* Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* **8**, 793-800, doi:10.1038/nm730 (2002).
49 Jiang, X., Zhou, J., Giobbie-Hurder, A., Wargo, J. & Hodi, F. S. The activation of MAPK in melanoma cells resistant to BRAF inhibition promotes PD-L1 expression that is reversible by MEK and PI3K inhibition. *Clin Cancer Res* **19**, 598-609, doi:10.1158/1078-0432.CCR-12-2731 (2013).
50 Parsa, A. T. *et al.* Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat Med* **13**, 84-88, doi:10.1038/nm1517 (2007).
51 Lastwika, K. J. *et al.* Control of PD-L1 Expression by Oncogenic Activation of the AKT-mTOR Pathway in Non-Small Cell Lung Cancer. *Cancer Res* **76**, 227-238, doi:10.1158/0008-5472.CAN-14-3362 (2016).
52 Braumuller, H. *et al.* T-helper-1-cell cytokines drive cancer into senescence. *Nature* **494**, 361-365, doi:10.1038/nature11824 (2013).

A)



B)

	Class IA			Class IB
	p85 p110α	p85 p110β	p85 p110δ	p85 p110γ
EXPRESSION	expressed ubiquitously	broad tissue distribution high expression in myeloid cells but absent in B/T lymphocytes	expression restricted to immune cells, neurons and some transformed epithelial cells	expression restricted to immune cells, in particular leucocytes
ABERRATIONS IN CANCER	p100α frequently somatically mutated/amplified in solid tumours (but not haematological cancers) also activated by mutations of the p85 regulatory subunits, upstream activators (eg tyrosine kinases and Ras)	infrequently mutated but main mediator of enhanced PI3K activity induced upon the inactivation and loss of PTEN also activated by mutations of the p85 regulatory subunits,	rare mutation found in diffuse large B cell lymphoma but otherwise mostly non-mutated	mostly non-mutated in cancer


PD-L1
PI3Kγ inhibitors
AKT inhibitors
Macrophages
promote M1 anti-tumourigenic phenotype
PI3Kα/β inhibitors
AKT inhibitors
mTOR inhibitors
inhibit survival signals
tumor cells
PD-L1
APC
PI3Kδ inhibitors
mTOR inhibitors
activate dendritic cells
enhance NK activity
PD-1
NK cells
PI3Kδ/γ inhibitors
AKT inhibitors
mTOR inhibitors
PD-L1
PD-1
enhance T effector activity
effector
T cells
AKT inhibitors
mTOR inhibitors
promotes
memory T cells
PI3Kγ inhibitors
CTLA4
inhibit T regs
regulatory
T cells
PD-L1
PI3Kγ inhibitors
MDSC
inhibit recruitment of MDSCs
memory
T cells