Inactivation of PI(3)K p110δ breaks regulatory T-cell–mediated immune tolerance to cancer

Khaled Ali1, Dalya R. Soond2,3, Roberto Piñeiro4, Thorsten Hagemann1, Wayne Pearce1, Ee Lyn Lim2, Hicham Bouab3, Cheryl L. Scudamore4, Timothy Hancox5, Heather Maecker6, Lori Friedman6, Martin Turner2, Klaus Okkenhaug2 and Bart Vanhaesebroeck1

Inhibitors against the p110δ isoform of phosphoinositide-3-OH kinase (PI(3)K) have shown remarkable therapeutic efficacy in some human leukaemias1,2. As p110δ is primarily expressed in leukocytes, drugs against p110δ have not been considered for the treatment of solid tumours3. Here we report that p110δ inactivation in mice protects against a broad range of cancers, including non-haematological solid tumours. We demonstrate that p110δ inactivation in regulatory T cells unleashes CD8+ cytotoxic T cells and induces tumour regression. Thus, p110δ inhibitors can break tumour-induced immune tolerance and should be considered for wider use in oncology.

PI(3)K p110δinactivation in mice, in which endogenous p110δ kinase is inactive, present specific immune deficiencies4,5 but are not predisposed to cancer. To test whether host p110δ activity affects tumour growth, we inoculated weakly immunogenic syngeneic cancer cell lines into δD910A mice. Compared to wild-type mice, δD910A mice were more resistant to B16 melanoma, with reduced tumour incidence and almost abrogated lymph node metastasis in those mice that developed tumours (Fig. 1a). Growth of Lewis lung carcinoma (LLC) and EL4 thymoma cells was also suppressed in δD910A mice (Fig. 1b, c). Similar observations were made with luciferase-labelled 4T1 breast cancer cells injected into the mammary fat pad. At euthanization, δD910A mice showed reduced mass and luciferase activity of the primary 4T1 tumour (Fig. 1d) and lower metastasis (Fig. 1e). In wild-type mice, 4T1 tumours were detected by day 10 and grew progressively until day 30, at which point the mice became moribund (Fig. 1f). In some δD910A mice, 4T1 tumours grew initially, but then started to regress from days 15–20 onwards (Fig. 1f). Across ten independent experiments, 97% (71/73) of wild-type mice had an observable cancer mass at the end of study, compared to 65% (43/66) of δD910A mice, with a median survival time of 23 and 40 days in wild-type and δD910A mice, respectively (Fig. 1g).

Effective tumour immunity is limited by regulatory T cell (Treg)–mediated immune suppression. δD910A mice show enhanced FOXP3+ CD4+ Treg in the thymus but impaired subsequent Treg maintenance and functionality in the periphery. δD910A Treg also produce less interleukin (IL)-10 and express lower levels of CD38, but show normal expression of most Treg–signature genes, including FOXP3, CD25 (also known as IL2RA), CTLA4 and ICOS. We therefore considered that reduced Treg function in δD910A mice might lead to enhanced tumour resistance. FOXP3+ CD4+ Treg in the draining lymph nodes of 4T1 tumour-bearing δD910A mice did not expand as robustly as in wild-type mice (Fig. 2a); however, no consistent differences in Treg expansion were observed in the B16 or EL4 tumour models between naive and tumour-bearing mice of either genotype (not shown). To assess Treg function, we carried out adoptive Treg transfer experiments in EL4 tumour-bearing mice in wild-type or δD910A recipients. 

Figure 1 | Impact of genetic inactivation of p110δ on tumour growth and metastasis. a. Percentage of mice with visible B16 ear tumours (left) or lymph nodes metastasis (right). Photographs show B16 metastases in cervical lymph node (left) and lung (right) of WT (a) and δD910A (b) recipient mice. b–d. Primary tumour burden of the indicated tumour lines. e. 4T1 metastasis as detected by luciferase activity (left and middle) or histology (right), expressed as a percentage of the total number of tumour-bearing animals per group. f. Growth of primary 4T1 tumours. g. Survival of 4T1 tumour-bearing mice. *P<0.05, **P<0.01 (non-parametric Mann–Whitney t-test). Numbers in brackets represent number of mice used per experiment. Each dot represents an individual mouse. Shown are the mean± standard error of mean (s.e.m.) from at least two independent experiments in which statistical significance was demonstrated.
These data show that CD8+ splenocytes from tumour-bearing mice, incubated in vitro with mitomycin C-treated 4T1 cells, generated equivalent cytotoxic activity against 4T1, with no specific lysis of LLC (Fig. 3c). Compared to wild-type cultures, D910A cultures contained similar proportions of CD4 and CD8 T-cell subsets (Extended Data Fig. 1b), with a reduced frequency of activated/memory CD44hi CD4+ cells (Fig. 3d) and unaffected frequency of CD44hi CD8+ cells (Fig. 3d). Interestingly, despite this reduced proportion of D910A CD44hi CD4+ cells, the frequency of interferon (IFN)-γ+ CD4+ cells in phorbol myristate acetate (PMA)/ionomycin-stimulated cultures of splenocytes from 4T1 tumour-bearing mice was unaffected by p110δ inactivation (Fig. 3e), with the frequency of IFN-γ+ CD8+ cells even enhanced upon p110δ inactivation (Fig. 3e). Upon inoculation with LLC cells expressing ovalbumin (LLC-OVA), wild-type and D910A mice generated similar levels of tumour-infiltrating OVA-specific CD8+ T cells (Fig. 3f), showing that systemic in vivo inactivation of p110δ does not impede the development or recruitment of antigen-specific anti-tumour CD8+ cells.

To test the intrinsic ability of D910A CD8 T cells to eliminate tumours, we crossed D910A mice to OT-I transgenic mice, which carry an OVA-specific MHC class I-restricted T-cell receptor transgene. In vitro-generated D910A OT-I cytotoxic T lymphocytes (CTLs) were less efficient than wild-type OT-I CTLs at EL4-OVA killing (Fig. 3g) and produced lower levels of cytotoxic mediators (Extended Data Fig. 1c). Pharmacological inactivation of p110δ during the in vitro CTL expansion of wild-type OT-I cells partially suppressed CTL function, in a manner indistinguishable from genetic inactivation of p110δ (Fig. 3g), whereas p110δ blockade during the killing phase itself did not affect CTL function (Fig. 3g). Despite these in vitro effects in D910A OT-I CTLs, adoptive transfer of these cells in wild-type mice before challenge with EL4-OVA provided equal cancer protection to inoculation of wild-type OT-I T cells (Fig. 3h), showing that in vivo CTL responses can remain competent in the absence of CD8 T-cell-intrinsic p110δ activity. Taken together, these data indicate that p110δ inhibition impairs differentiation of CD8 T cells to become fully competent CTLs; however, fully differentiated CTLs do not seem to require p110δ activity to kill target cells and on balance, in the context of reduced Treg function in D910A mice, can mediate effective anti-tumour activity.

CD4 T cells can also contribute to tumour elimination by promoting the activation of macrophages and natural killer cells or by direct lysis of MHC class II+ tumour cells. Indeed, CD4+ T cells with enhanced PI(3)K activity are superior in their capacity to reject tumour growth, probably as a consequence of their increased production of IFN-γ. Conversely, D910A OT-II CD4 cells were less effective than wild-type OT-II cells in preventing EL4-OVA tumour growth (Fig. 3h), consistent with our previous finding that D910A OT-II T cells produce less IFN-γ in vitro and in vivo. Therefore, in the context of an otherwise normal immune system, D910A CD4+ cells show inferior anti-tumour immunity. However, the production of IFN-γ by CD4 and CD8 T cells from 4T1 tumour-bearing D910A mice, in which Treg are also defective, appeared to be intact (Fig. 3e), suggesting that p110δ inhibition can affect the balance between regulatory and effector CD4+ T cells such that the effector cells prevail in the context of anti-tumour responses.

A salient feature of CD4 and CD8 T cells is the ability to raise a more potent and rapid immune response to subsequent exposure to cognate antigen. Upon surgical removal of 4T1 primary tumours when they had reached 9 mm in diameter and established metastatic foci, wild-type mice all succumbed to regrowth of the primary tumour and metastatic disease. By contrast, >50% of post-surgical D910A mice showed survival extension beyond 100 days (Fig. 3i), demonstrating that p110δ inhibition can suppress cancer relapse and presumably metastatic cancer after surgery. D910A mice which had remained tumour-free for >200 days after surgery were cancer-resistant upon rechallenge with a higher 4T1 dose (Fig. 3j), suggesting that surgical intervention in D910A mice supports the development of an effective memory anti-tumour response.
To assess the potential importance of p110δ in myeloid cells in cancer, we tested the impact of p110δ inactivation on Rag\(^{−/−}\) mice. Rag\(^{−/−}\) mice, which lack mature B and T cells, showed enhanced primary 4T1 tumour size and metastasis (Fig. 4a) compared to wild-type mice. Rag\(^{−/−} \delta^{910}\) mice showed a similar 4T1 primary tumour burden to Rag\(^{−/−}\) mice (Fig. 4a) but had fewer metastatic lesions in lung (Fig. 4a) and liver (not shown), indicating that p110δ inactivation in a non-B/T-cell lineage delays 4T1 tumour progression but is not sufficient to instigate tumour rejection. We next assessed the impact of p110δ inactivation on myeloid-derived suppressor cells (MDSCs), a heterogeneous population of bone marrow-derived myeloid cells that co-express the CD11b and Gr1 surface markers and which have a prominent role in immune suppression in cancer.\(^{15,16}\) Neutrophils are also CD11b\(^{+}\) Gr1\(^{+}\) but are thought not to be immune-suppressive\(^{17}\). Upon inoculation with 4T1 cells, known to be potent MDSC inducers\(^{17}\), CD11b\(^{+}\) Gr1\(^{+}\) cells accumulated in the spleens of both wild-type and \(\delta^{910}\) mice, even before tumours were palpable, and continued to differentially accumulate in both genotypes as tumours grew, correlating with tumour size (Fig. 4b). The Ly6C and Ly6G surface markers, which are both recognized by the Gr1 antibody, have been used to subdivide MDSCs into two CD11b\(^{+}\) subpopulations, namely monocytic (M)-MDSCs (Ly6ChiLy6Glow) and polymorphonuclear (PMN)-MDSCs (Ly6C\(^{−}\)LoLy6Ghi)\(^{17}\). Although neutrophils are difficult to differentiate from PMN-MDSCs, here we designated the neutrophil population as Ly6G\(^{−}\) cells with intermediate/high Ly6C expression (Fig. 4c and Extended Data Fig. 2a). PMN-MDSCs, predominant in 4T1 tumour-bearing wild-type mice (Fig. 4c), were substantially reduced in \(\delta^{910}\) mice, correlating with a relative increase in neutrophils in the latter (Fig. 4c). Interestingly, the number of PMN-MDSCs in spleens from 4T1 tumour-bearing mice correlated with the number of T\(_{reg}\) (Fig. 4d). Depletion of CD8\(^{+}\) cells in 4T1 tumour-bearing \(\delta^{910}\) mice, which led to enhanced tumour growth (Fig. 3a, b), also led to increased PMN-MDSC numbers and reduced neutrophil numbers (Fig. 4e). It was therefore difficult to ascertain whether the reduced PMN-MDSC numbers in \(\delta^{910}\) mice are a consequence of an intrinsic role for p110δ in these cells or an indirect consequence of a reduced tumour burden in \(\delta^{910}\) mice (Fig. 4b). In support of the former, wild-type PMN-MDSCs suppressed T-cell proliferation \textit{in vitro}, whereas MDSCs from \(\delta^{910}\) mice with regressing tumours did not (Fig. 4f and Extended Data Fig. 2b). Neutrophils from both genotypes did not suppress T-cell responses (Fig. 4f). Moreover, splenocytes from tumour-bearing \(\delta^{910}\) mice showed reduced \textit{in vitro} production of transforming growth factor-\(\beta\), vascular endothelial growth factor and IL-6 (Fig. 4g), each of which can contribute to immune suppression and/or tumour growth\(^{15,16}\).

Administration of PI-3065, a small molecule inhibitor with selectivity for p110δ (Extended Data Fig. 3a, b and Extended Data Table 1), also suppressed 4T1 tumour growth and metastasis, to a similar extent as genetic inactivation of p110δ, marked by initial tumour progression, followed by tumour regression (Fig. 5a and Extended Data Fig. 3c, d). Of interest, 4T1 cells do not express detectable levels of p110δ (Extended Data Fig. 3e) and are not growth-inhibited \textit{in vitro} by PI-3065 (Extended Data Fig. 3f). Long-term administration of PI-3065 to mice was well-tolerated and did not induce weight loss (Extended Data Fig. 3g).

We next tested the impact of PI-3065 in the LSL.Kras\(^{G12D}\)/R172H;\(p53^{R172H}\) (or KPC) model of pancreatic ductal adenocarcinoma, which expresses endogenous mutant KRAS\(^{G12D}\) and \(p53^{R172H}\) in PDX1\(^{+}\) pancreatic cells. KPC mice were left to develop palpable disease before treatment with vehicle or PI-3065 was commenced. Under these therapeutic conditions, PI-3065 prolonged survival and reduced the incidence of macroscopic metastases and other disease-associated pathologies (Fig. 5b). The relative abundance of peripheral T\(_{reg}\) in lymph nodes after 7 days...
Figure 4 | Impact of p110δ inactivation on myeloid cells in 4T1 tumour-bearing mice. a. 4T1 primary tumour growth and lung metastasis in wild-type, δ9910A, Rag-/- and Rag-/- × D910A mice. b, 4T1 tumour growth and total numbers of splenic CD11b+ Gr1high myeloid cells in wild-type and δ9910A mice. c. Gating strategy used to identify myeloid cell subsets and frequency of splenic PMN-MDSCs and neutrophils of naive and 4T1 tumour-bearing wild-type and δ9910A mice. d. Spearman correlation between accumulation of splenic PMN-MDSCs and Treg in wild-type or δ9910A mice.
e. Impact of depleting CD8+ T cells in δ9910A mice on 4T1 tumour burden and presence of splenic myeloid cell populations. f. Impact of purified splenic myeloid cells on proliferation of anti-CD3-stimulated wild-type T cells.
g. Cytokine production by splenocytes from 4T1 tumour-bearing (30 days after inoculation) cells from wild-type or δ9910A mice, individually cultured for 4 days. Statistics are as described in the legend to Fig. 2.

of treatment was reduced (Fig. 5c), correlating with higher levels of CD44highCD8+ lymphocytes in the draining lymph nodes (Fig. 5d) and relatively higher levels of infiltrating CD8+ T cells in pancreatic lesions 14 days after treatment (Fig. 5e). These data indicate that therapeutic targeting of p110δ can promote immune-mediated elimination of cancer.

Concerns have been raised about inhibiting p110δ in cancer as this might impair CTLs and negatively impact on cancer immune surveillance. Our data show that although p110δ blockade reduces the effectiveness of CTLs, it also overrides Treg- and probably also MDSC-mediated suppression of anti-tumour immune responses, enabling even weakened CTLs to successfully attack tumours. Thus, p110δ is apparently more essential for regulatory rather than effector T-cell responses against cancer cells. In addition, inhibition of the PI(3)K pathway in CD8+ T cells may help maintain them in a stem-cell-like state with enhanced potential for generating durable anti-tumour responses. Consistent with this notion, δ9910A mice resisted tumour rechallenge following surgical removal of the first tumour. The p110δ inhibitor Idelalisib has shown impressive therapeutic impact in chronic lymphocytic leukaemia (CLL) and non-Hodgkin’s lymphoma. In CLL, p110δ blockade interferes with stroma-derived survival and adhesion signals supporting the tumour cells, but it is unclear if this fully explains the effectiveness of p110δ inhibition. Our finding that p110δ inhibition can unlock adaptive anti-tumour responses provides a potential additional mechanism for the efficacy of p110δ blockade in CLL, and adds to the emerging rationale for targeting PI(3)K in the tumour stroma, to dampen inflammation (p110y)20 and angiogenesis (p110α)21.

Tumour-induced immune suppression constitutes an important barrier for effective anti-tumour immunity and immunotherapy in cancer. Our work suggests that p110δ inhibitors, by disrupting the function of Treg and possibly of MDSCs, have the potential to shift the balance from immune tolerance towards effective anti-tumour immunity. This provides a rationale for p110δ inhibition both in solid and haematological cancers, possibly as an adjuvant to cancer vaccines, adoptive cell therapy, or other strategies that promote tumour-specific immune responses.
Figure 5 | Impact of pharmacological inactivation of p110δ on tumour growth and T-cell responses. a, Mice, dosed with vehicle or PI-3065 (75 mg kg\(^{-1}\), daily) for 36 days and inoculated with \(10^5\) 4T1 cells 12 h post first dosing, were assessed for tumour growth by luciferase imaging (first panel), tumour weight (second panel) or luciferase activity in tumours excised 35 days after inoculation (third panel). Incidence of 4T1 metastasis (fourth panel), as detected by haematoxylin and eosin (H&E) staining and histology, expressed as percentage of the total number of tumour-bearing animals per group. b, Impact of PI-3065 (75 mg kg\(^{-1}\)) on KPC mouse survival (left) and macrophages and cancer-associated pathology (right). c, Proportion of Treg (percentage of CD4\(^+\)) in the draining lymph nodes of KPC mice administered vehicle or PI-3065. d, Proportion of CD4\(^{+}\) T cells (percentage of CD3\(^+\)) in the draining lymph nodes of KPC mice administered vehicle or PI-3065. e, Relative numbers of CD8\(^+\) T cells (percentage of CD45\(^+\)) in normal pancreas and PDAC lesions of KPC mice treated or not with PI-3065. Statistics are as described in the legend to Fig. 2.

METHODS SUMMARY

All animal procedures were in compliance with institutional animal care and use committee guidelines. Details of procedures and reagents are described in Supplementary Information.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.
Extended Data Figure 1 | Impact of p110δ inactivation on CD4 and CD8 T cells in mice with 4T1 or EL4 tumours. a, Levels of CD44high CD4+ and CD44high CD8+ T cells in the indicated immune compartments of naive and 4T1 tumour-bearing on day 26 after inoculation in wild-type or δD910A mice. b, Distribution of cells on day 5 of culture of splenocytes, isolated from 4T1 tumour-bearing wild-type and δD910A mice 21 days after inoculation, in the presence of mitomycin-treated 4T1 cells. c, Gene expression in CTLs derived from splenocytes from wild-type and δD910A OT-I mice, cultured in the presence of SIINFEKL OVA peptide and IL-2. GzmA, granzyme A; GzmB, granzyme B; Prf1, perforin and (FasL or CD95L) Fas ligand. Expression levels are presented relative to β2-microglobulin. *P < 0.05, **P < 0.01, ***P < 0.001 (non-parametric Mann–Whitney t-test). Numbers in brackets indicate the number of mice used per experiment. Each dot represents an individual mouse.
Extended Data Figure 2 | Impact of p110δ inactivation on myeloid cells in 4T1 tumours. a, Gating strategy used to identify myeloid cell subsets. Splenic cells were gated on CD11bhigh cells followed by Ly6C and Ly6G gating. FSC, forward scatter; SSC, side scatter (top). Frequency of CD11b+ cells in the spleen of wild-type and δD910A naive mice and in 4T1 tumour-bearing mice on day 21 after inoculation (bottom). b, [3H]-Thymidine incorporation in co-cultures of splenocytes and purified myeloid cells, in combinations as indicated, with or without stimulation with anti-CD3 antibodies. Cultures were made using cells derived from individual mice. Error bars represent standard deviation from the mean of biological replicates. *P < 0.05, **P < 0.01 (non-parametric Mann–Whitney U-test). Numbers in brackets indicate the number of mice used per experiment. Each dot represents an individual mouse.
Extended Data Figure 3 | Characterization of the p110δ-selective inhibitor PI-3065. a, PI-3065 structure and in vitro IC_{50} on selected PI3K family members. No significant activity against 72 protein kinases was observed at \( \leq 10 \mu M \) in a KinaseProfiler assay (Millipore). b, Pharmacokinetic parameters of PI-3065. Mean (± s.d.) plasma concentration profile of PI-3065 following a single oral dose (75 mg kg\(^{-1}\)) administered per os (po) to female BALB/c mice. AUC\(_{\text{inf}}\), area under the curve, extrapolated to infinity; \( C_{\text{max}} \), highest observed plasma concentration; \( t_{\text{max}} \), time at which \( C_{\text{max}} \) occurred, QD, quaque die (every day). c, Growth of primary 4T1 tumours, inoculated in the breast fat pad, measured by calipers and expressed as tumour volume. Mice were dosed per os with vehicle or PI-3065 (75 mg kg\(^{-1}\), daily) for 36 days. 10^5 tumour cells were inoculated 12 h post first dosing. d, Percentage of tumour-free mice upon continuous per os treatment of mice with vehicle or PI-3065 (25 mg kg\(^{-1}\), twice daily) for 37 days, with tumour cells inoculated on day 7 of PI-3065 dosing. 15 mice were used for each genotype. e, Class I PI3K isoform expression in 4T1 cells. f, Proliferation of 4T1 cells following a 4-h treatment with the indicated p110δ inhibitors, washing and (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy phenyl)-2-(4-sulphophenyl)-2H-tetrazolium salt (MTS) staining after 48 h culture. g, Percentage body weight change (from day 0) of 4T1 tumour-bearing mice upon daily per os administration of PI-3065 (75 mg kg\(^{-1}\)) or vehicle for 36 consecutive days. *\( P < 0.05 \), **\( P < 0.01 \) (non-parametric Mann–Whitney \( U \) test). Numbers in brackets indicate the number of mice used per experiment.
Extended Data Table 1 | Comparison of PI-3065 with Idelalisib (formerly called GS-1101 or CAL-101) and IC87114

| Compound | p110α | p110α | p110α | p110α | IC50 (nM) | anti-IgM-stimulated whole blood | anti-IgM-stimulated human B cell lymphoma | CD69 expression IC50 (nM) | pAkt IC50 (nM) |
|----------|-------|-------|-------|-------|----------|-----------------------------|-----------------------------------|----------------|-------------|
| PI-3065  | 1.5   | 110   | 130   | 940   |          | >10000                      | >10000                            | 1300           | >7800       |
| Idelalisib| 1.1   | 270   | 121   | 16    |          | >10000                      | >10000                            | 60             | 41          |
| IC87114  | 34    | >2100 | >2100 | 370   |          | >10000                      | >10000                            | 3500           | >7800       |

Human whole blood was stimulated with anti-IgM followed by FACS for CD69 as described22,23. Human B-cell lymphoma Ri-1 cells were pre-incubated for 30 min with vehicle or compound before stimulation with anti-IgM for 1 h at 37 °C, followed by determination of Akt-Ser 473 phosphorylation, as described22,23.

22. Murray, J. M. et al. Potent and highly selective benzimidazole inhibitors of PI3-kinase delta. J. Med. Chem. 55, 7686–7695 (2012).
23. Safina, B. S. et al. Discovery of novel PI3-kinase δ-specific inhibitors for the treatment of rheumatoid arthritis: taming CYP3A4 time-dependent inhibition. J. Med. Chem. 55, 5887–5900 (2012).
Corrigendum: Inactivation of PI(3)K p110δ breaks regulatory T-cell-mediated immune tolerance to cancer

Khaled Ali, Dalya R. Soond, Roberto Piñeiro, Thorsten Hagemann, Wayne Pearce, Ee Lyn Lim, Hicham Bouabe, Cheryl L. Scudamore, Timothy Hancox, Heather Maeccker, Lori Friedman, Martin Turner, Klaus Okkenhaug & Bart Vanhaesebroeck

Nature 510, 407–411 (2014); doi:10.1038/nature13444

Queen Mary University London notified Nature and University College London that there is reason to question the provenance of the data for Fig. 5b, d, e of this Letter (Fig. 5a, c data are unaffected). Ongoing studies are investigating the reported effect of p110δ inhibition in the pancreatic cancer mouse model. We therefore wish to withdraw these figure panels and associated text from the published paper. This does not affect the overall conclusion of the manuscript or any of the other experiments performed for this study as they repeat effects shown using other model systems in the paper (see Supplementary Information for raw data of experiments). We apologise for the inconvenience that this may have caused.

Supplementary Information is available in the online version of the Corrigendum.