Precision targeting of mutant PI3Kα in cancer by selective degradation

Bart Vanhaesebroeck[1,*], John E. Burke[2.3] and Ralitsa R. Madsen[1]

[1]University College London Cancer Institute, Paul O'Gorman Building, University College London, London, UK.
[2]Department of Biochemistry and Microbiology, University of Victoria, Victoria, Canada.
[3]Department of Biochemistry and Molecular Biology, The University of British Columbia, Vancouver, Canada.

[*]Corresponding author: Bart Vanhaesebroeck, Cancer Institute, Paul O'Gorman Building, University College London, 72 Huntley Street, London WC1E 6BT, United Kingdom. Email: bart.vanh@ucl.ac.uk – Tel. 00-44-(0)751-5851-130

Authors' Disclosures

B.V. reports personal fees from iOnctura (Geneva, Switzerland), Venthera (Palo Alto, CA, USA), Olema Pharmaceuticals (San Francisco, USA) and Pharming (Leiden, The Netherlands). J.E.B. reports personal fees from Olema Pharmaceuticals (San Francisco, USA) and Scorpion Therapeutics (Boston, USA).

Summary: *PIK3CA*, which encodes the p110α catalytic subunit of PI 3-kinase alpha (PI3Kα), is one of the most frequently genetically-activated kinases in solid tumors. Song *et al.* report that the related PI3Kα inhibitors taselisib and inavolisib trigger receptor-tyrosine kinase (RTK)-dependent degradation of the mutant p110α protein in breast cancer cells that are positive for the human epidermal growth factor receptor 2 (HER2) RTK, limiting feedback-mediated drug resistance and potentially widening the therapeutic index of PI3Kα inhibition.

Activating mutations in *PIK3CA* are frequent across human cancers, particularly breast. The last decade has therefore seen considerable investment in the development of PI3Kα-specific inhibitors, culminating in the recent FDA approval of alpelisib (BYL719; Novartis) for use with the estrogen receptor (ER) degrader fulvestrant in HER2-negative, ER-positive breast cancers (1). Despite such progress, major challenges remain, due to the essential function of PI3Kα in cellular and organismal homeostasis, with induction of cell-intrinsic and organismal negative feedback loops that act to oppose pharmacological PI3Kα inhibition. PI3Kα is especially important for insulin-mediated blood glucose control, with systemic PI3Kα suppression causing hyperglycemia and a compensatory increase in pancreatic insulin secretion which reactivates PI3K signaling in tumor cells (2). A potential solution to this problem would be to spare the wild-type PI3Kα enzyme by developing PI3Kα mutant-selective inhibitors, thereby widening the therapeutic index. Work presented in this issue of *Cancer Discovery* shows this goal to be within reach.

In their manuscript, Song *et al.* (3) report that a range of cancer cells, particularly the HER2-amplified breast cancer cell subtype, exhibit selective degradation of mutant p110α upon treatment with the chemically-related ATP-competitive inhibitors taselisib (GDC-0032; a dual PI3Kα/δ inhibitor) and inavolisib (GDC-0077, RG6114; a PI3Kα-selective inhibitor), both generated by Genentech/Roche. This was unexpected, given that these compounds inhibit recombinant wild-type and mutant p110α to the same extent *in vitro*, hinting at the existence of a specific cellular mechanism of action. Evidence is emerging that this role of a PI3K inhibitor as a monomeric small molecule protein degrader might be a more widespread function of some small molecule inhibitors (Mullard A. On the hunt for monomeric degraders https://cen.acs.org/biological-chemistry/proteomics/hunt-monomeric-degraders/99/i40).

PI3Kα is a heterodimer of the p110α catalytic subunit and a p85 regulatory subunit, of which there are 5 species: p85α, p55α and p50α (encoded by the *PIK3R1* gene), p85β (encoded by *PIK3R2*) and p55γ (encoded by *PIK3R3*). In unstimulated cells, the p110α protein is kept in an inactive and stable cytosolic configuration due to its interactions with the regulatory subunit. De-inhibition occurs upon recruitment of the enzyme complex to phosphorylated RTKs or associated adapter proteins at the plasma membrane (Fig. 1), as well as upon binding to the small GTPase RAS (4). The resulting conformational changes in PI3Kα lead to membrane recruitment and catalytic activity towards its lipid substrate $PI(4,5)P_2$ (4). These changes can also be mimicked by activating *PIK3CA* mutations, of which the most common occur in so-called hotspot regions – either in the helical domain (E542K, E545K) or the kinase domain (H1047R, H1047L) (4).

Song *et al.* (3) observed that the combined presence of a hotspot *PIK3CA* mutation and high RTK activity was common to breast cancer cells with potent drug-induced degradation of mutant p110α. Further biochemical experiments suggest that conformational changes in PI3Kα, facilitated by recruitment of PI3Kα to receptor complexes, exposes sites in p110α for ubiquitination at the membrane, leading to mutant-selective p110α degradation by the proteasome (Fig. 1). The p110α-degradation effect was mainly observed for p110α in complex with p85β, possibly due to preferential recruitment of p85β/p110α over p85α/p110α to activated RTKs such as HER2 and HER3 (3).

From a drug development perspective, the finding of Song *et al.* (3) was serendipitous, and it is not clear at present why the structurally-related taselisib and inavolisib lead to mutant p110α degradation while other compounds such as alpelisib (BYL719) and pictilisib (GDC-0941) do not. Taselisib/inavolisib, alpelisib and pictilisib all have different chemical scaffolds, and future structural studies may provide a structure-function insight into how to convey PI3Kα-degrading capacity to small molecule inhibitors. An meeting report (5) indicated that alpelisib may also induce preferential degradation of mutant p110α in some cellular contexts, yet this was not apparent under the conditions tested by Song *et al*. (3).

The recent cryo-EM structural report of p110α-p85α (6) may add some insight into possible mutant-specific effects of PI3Kα inhibitors. This work reported two distinct conformational changes that may be relevant to understanding how some PI3K inhibitors may make hotspot mutants more accessible to degradation. Upon RTK activation, there is disengagement of the p85 regulatory subunit from the catalytic core of p110α, with this likely representing the activated membrane-bound state, which will be more frequent for hotspot mutants. There was also a major rearrangement of the N- and C-terminal domains of p85α upon alpelisib binding to p110α. If a similar conformational change were to occur in p85β, it is possible that when PI3K inhibitors bind, along with the enhanced membrane binding found in mutated p110α (4), this leads to a specific conformation more accessible to E3 ligases. This hypothesis will require further study into the exact mechanism of p110α ubiquitination and degradation, the role of the regulatory subunits in the recruitment of E3 ligases, and the identity of the E3 ligase that targets p110α.

Due to their ability to promote mutant-selective PI3Kα degradation, taselisib and inavolisib could also reduce or fully block the cell-intrinsic, negative feedback-mediated reactivation of the PI3K pathway in HER2-amplified breast cancer cells, unlike alpelisib (3). As a result, both taselisib and inavolisib had a stronger growth inhibitory effect in cell-based and tumor xenograft studies (3). Nevertheless, a key challenge remains. Despite lack of wild-type p110α protein degradation, taselisib and inavolisib still inhibit its enzymatic activity and are thus expected to trigger the systemic glucose-mediated insulin feedback loop, similar to other PI3Kα inhibitors. Indeed, this has already been shown for taselisib in both mice and humans. The hope is nevertheless that inavolisib's improved selectivity for p110α over p110δ will reduce the additional immune-related toxicity that negated the benefits of taselisib in breast cancer trials (1).

Overall, the study by Song *et al.* (3) offers the first evidence of preferential targeting of mutant p110α. It remains to be determined whether this mechanism of taselisib/inavolisib will extend to non-hotspot p110α mutations. Interestingly, additional mutant-specific inhibitors of p110α were presented at the October 2021 AACR-NCI-EORTC Molecular Targets Conference. These are LOXO-783 (LOX-22783), an allosteric PI3Kα-H1047R inhibitor from Petra Pharmaceuticals (now acquired by Loxo Oncology at Lilly) and RLY-2608, an allosteric pan-mutant-selective PI3Kα inhibitor from Relay Therapeutics. Evidence was presented that these allosteric inhibitors do not induce metabolic dysregulation in mice, clearly setting the scene for widening of the therapeutic window of PI3Kα inhibitors. The impact of these compounds on cellular p110α degradation was not reported. Moving forward, such mutant-selective PI3Kα inhibitors may also benefit patients with PROS (*PIK3CA*-related overgrowth spectrum), a group of benign but highly debilitating diseases caused by developmental acquisition of mosaic *PIK3CA* mutations, including the same hotspot variants seen in cancer (7).

Song *et al.*'s work (3) also adds to our understanding of the biochemical mechanisms of PI3Kα turnover, and further reinforces how little is understood about the biological differences of the distinct p85 regulatory subunit isoforms (8,9). It is speculated that p85α provides stronger basal inhibition of p110α relative to p85β due to subtle but important structural differences. This is consistent with the notion of p85α as a tumor suppressor, and the relatively frequent pan-cancer occurrence of *PIK3R1* mutations that lead to increased PI3K activity. On the other hand, p85β acts as a *bona fide* oncogene: oncogenic mutations in *PIK3R2* have mainly been reported in endometrial cancer, and in benign overgrowth disorders with brain abnormalities; *PIK3R2* is also frequently

amplified in lymphoma, breast and colorectal cancers (8,9). The putative weaker inhibitory interface between p85β with p110α compared to p85α may therefore contribute to the preferential inhibitor-induced, mutant-selective degradation of p85β/p110α over p85α/p110α in Song *et al.*'s study. It is generally unclear, however, what factors determine the involvement of p85α *versus* p85β in a given cellular context, including any differences in their ability to interact with specific RTKs – an area that warrants further study in light of Song *et al.*'s data. It will also be important to determine the sensitivity towards degradation of different oncogenic mutants in both the p110α and p85α/p85β subunits, as this may identify further mutations that are either sensitive or resistant to degradation, which will be important in understanding possible mechanisms of acquired inhibitor resistance.

Last but not least, the study of Song *et al*. has opened a new area for mechanism-based therapeutic exploitation of PI3Kα inhibition, by uncovering *HER2*-driven breast cancers with *PIK3CA* mutations as a clinical setting for mutant-selective p110α degraders. Anti-HER2 antibodies represent the standard of care for *HER2*-amplified breast cancers, and *PIK3CA*-mutant tumors are known to be less responsive to such HER2-targeted therapy. Based on this, in 2019, Novartis had already started a phase III randomized trial comparing maintenance anti-HER2 therapy with or without alpelisib in *PIK3CA*-mutated *ERBB2*-amplified breast cancer (NCT04208178). In the wake of Song *et al.*'s data (3), Roche is now testing inavolisib in HER2-positive breast cancer, in combination with a range of agents, including endocrine therapies, CDK4/6 inhibition, HER2-targeting antibodies or metformin (https://clinicaltrials.gov NCT04191499, NCT03006172, NCT04802759).

Together with the success of PI3Kδ inhibitors in some B-cell leukemias and their emerging potential in immunotherapy of solid tumors (1), the development of mutant-specific PI3Kα inhibitors is likely to usher a new and more productive era of PI3K targeting in cancer.

Grant Support

R.R.M. is supported by a Sir Henry Wellcome Fellowship (220464/Z/20/Z). Work in the laboratory of B.V. is supported by Cancer Research UK (C23338, A25722) and PTEN Research. J.E.B. is supported by CIHR (Project grant 168998), the Cancer Research Society (843232), NSERC (Discovery grant NSERC-2020-04241), and the Michael Smith foundation for health research (17686).

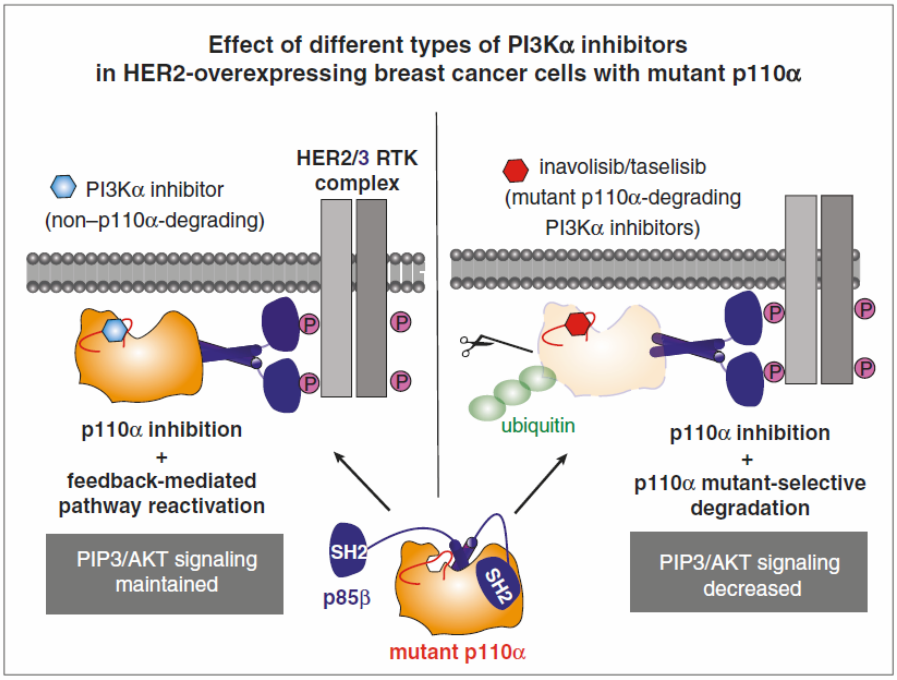

Figure 1. Mutant specific targeting of p110α/p85β through inhibitor specific degradation

The PI3Kα complex is recruited to the plasma membrane by phosphorylated RTKs. Inhibition of PI3Kα by a non-mutant selective PI3K inhibitor (left panel) causes initial inhibition of PI3K pathway activity, however, feedback leads to increased RTK expression, which results in maintained PI3K signaling. For the p110α-mutant selective-degrading PI3K inhibitors inavolisib and taselisib (right panel), binding of the inhibitor to mutant p110α/p85β causes proteasome-mediated degradation downstream of activation by HER2/3. Degradation of p110α prevents feedback-mediated reactivation of the pathway, leading to sustained inhibition of mutant PI3K signalling. Shown is the binding of p85 to the HER2/HER3 RTK dimer, with the pYXXM motifs in HER3 functioning as docking sites for the SH2 domains of p85 (note that HER2 lacks such pYXXM motifs).

REFERENCES

1. Vanhaesebroeck B, Perry MWD, Brown JR, Andre F, Okkenhaug K. PI3K inhibitors are finally coming of age. Nat Rev Drug Discov **2021**;20(10):741-69 doi 10.1038/s41573-021-00209-1.
2. Hopkins BD, Pauli C, Du X, Wang DG, Li X, Wu D*, et al.* Suppression of insulin feedback enhances the efficacy of PI3K inhibitors. Nature **2018**;560(7719):499-503 doi 10.1038/s41586-018-0343-4.
3. Song KW, Edgar KA, Hanan EJ, Hafner M, Oeh J, Merchant M*, et al.* RTK-dependent inducible degradation of mutant PI3Kalpha drives GDC-0077 (Inavolisib) efficacy. Cancer discovery **2021** doi 10.1158/2159-8290.CD-21-0072.
4. Burke JE, Perisic O, Masson GR, Vadas O, Williams RL. Oncogenic mutations mimic and enhance dynamic events in the natural activation of phosphoinositide 3-kinase p110alpha (PIK3CA). Proc Natl Acad Sci U S A **2012**;109(38):15259-64 doi 10.1073/pnas.1205508109.
5. Fritsch C, Pfister E, Ebel N, Guthy D, Schnell C, Hofmann F. Abstract 3934: Determination of the PI3Kα selective inhibitor alpelisib mechanism of action and efficacy in ER+/ PIK3CA mutant breast cancer preclinical models. Cancer Research **2018**;78(13 Supplement):3934- doi 10.1158/1538-7445.Am2018-3934.
6. Liu X, Yang S, Hart JR, Xu Y, Zou X, Zhang H*, et al.* Cryo-EM structures of PI3Kα reveal conformational changes during inhibition and activation. Proceedings of the National Academy of Sciences **2021**;118(45):e2109327118 doi 10.1073/pnas.2109327118.
7. Madsen RR, Vanhaesebroeck B, Semple RK. Cancer-Associated PIK3CA Mutations in Overgrowth Disorders. Trends Mol Med **2018**;24(10):856-70 doi 10.1016/j.molmed.2018.08.003.
8. Thorpe LM, Yuzugullu H, Zhao JJ. PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. Nat Rev Cancer **2015**;15(1):7-24 doi 10.1038/nrc3860.
9. Vallejo-Diaz J, Chagoyen M, Olazabal-Moran M, Gonzalez-Garcia A, Carrera AC. The Opposing Roles of PIK3R1/p85alpha and PIK3R2/p85beta in Cancer. Trends in cancer **2019**;5(4):233-44 doi 10.1016/j.trecan.2019.02.009.