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# Drug-Resistant Phosphatidylinositol 3-Kinase: Guidance for the Preemptive Strike

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**In this issue of *Cancer Cell*, Zunder et al. (2008) describe surprising findings from investigating inhibitor-resistant mutations in the affinity pocket of p110α of phosphatidylinositol 3-kinase (PI3K). Information on these critical residues provides a road map for generating novel PI3K inhibitors that can overcome the anticipated resistance mutations.**

Small-molecule inhibitors targeting the tyrosine kinases Abl and EGFR have been spectacularly successful as cancer drugs (Druker, 2004). However, these successes have been curtailed by the appearance of kinase mutants that are resistant to the inhibitors (Gorre et al., 2001; Pao et al., 2005). The inhibitors typically bind to a conserved structural motif, referred to as an affinity pocket, that is located in the immediate vicinity of the ATP binding site. They compete with ATP for binding to the kinases. Most of the resistance mutations block inhibitor binding. A particularly effective and commonly encountered mutation occurs at a position referred to as the ''gatekeeper'' that controls access of inhibitors to the affinity pocket. The resistance mutations have now led to the development of second-generation inhibitors that are effective against many of the mutant kinases (Burgess and Sawyers, 2006; Druker, 2006; Kwak et al., 2005). However, targeting gatekeeper mutant kinases remains a significant challenge. This situation illustrates the general problem of mutation-induced drug resistance that needs to be anticipated in all therapeutic strategies.

In recent years, phosphatidylinositol 3-kinase (PI3K) has emerged as an exceedingly attractive and promising drug target. The PI3K signaling pathway is upregulated in most cancers as a result of various genetic and epigenetic changes. *PIK3CA*, the gene encoding the catalytic subunit p110α of PI3K, is frequently mutated in cancers of the breast, colon, endometrium, and prostate (Samuels et al., 2004). About 80% of these mutations map to one of three hot spots in the gene. They induce a gain of function in enzymatic and signaling activity. The mutant p110α is also oncogenic in cell culture and in animal model systems, strongly suggesting that it contributes to the oncogenic cellular phenotype in human cancers. Academic and industrial laboratories have responded to this development by generating PI3K inhibitors, some of which are entering clinical trials (Marone et al., 2008). Mutations resulting in inhibitor resistance will surely arise. Can we apply the lessons learned from the protein kinases to the lipid kinase PI3K? This is the question asked in a study published in the current issue of *Cancer Cell* (Zunder et al., 2008).

The recently determined structure of p110α shows some broad similarities to that of protein kinases, notably a hydrophobic cavity that corresponds to the affinity pocket of protein kinases (Huang et al., 2007). Several PI3K inhibitors target this structural motif and therefore could be affected by resistance mutations similar to those found in protein kinases. These basic similarities guided Zunder and colleagues (2008) in their study of drug resistance in PI3K. The researchers mutagenized selected residues lining the p110α affinity pocket, including the homolog of the gatekeeper, I848. The mutants were tested against several PI3K inhibitors using an ingenious yeast lethality test. The assay is based on the fact that *Saccharomyces cerevisiae* does not contain class I PI3Ks and that expression of p110α depletes its essential stores of phosphatidylinositol 4,5-bisphosphate, thus interfering with cell replication. Inhibition of p110α activity restores growth and viability of p110α-expressing *S. cerevisiae* (Figure 1). The simplicity and rapidity of the yeast screen allowed coverage of a sizable number of mutant/inhibitor combinations, including saturation mutagenesis and diverse inhibitor chemotypes.

The results of these mutagenesis studies are both unexpected and instructive. In contrast to the affinity pockets of protein kinases, which can accommodate diverse mutations, the pocket of p110α is relatively intolerant to change. Most mutations led to a loss of enzymatic activity. Gatekeeper mutations were another surprise: not only did they fail to induce resistance, most of them were also catalytically inactive or retained only minimal enzymatic activity. A single residue in the affinity pocket gave rise to resistant

mutants (I800M and I800L). In addition, the yeast assay identified mutations that convey enhanced sensitivity to inhibitors; one example is L814C. The mutation I800L is unique in that it induces a split phenotype: resistance against some inhibitors and enhanced sensitivity against others. The results obtained with yeast were validated in mammalian systems: the mutation-induced changes in catalytic and signaling activities and in resistance or sensitivity to inhibitors could be faithfully reproduced in human cells, in which the catalytically active mutants retained oncogenic potential.

We can derive several lessons from this study. The resistance mutations identified in the affinity pocket can guide a preemptive strike. It is probably not too early to start generating small-molecule inhibitors that are effective against the I800L and I800M mutants. The L814C mutant, showing increased sensitivity to inhibitors, is a potentially useful tool for the study of isoform-specific functions of p110α. There are currently no isoform-specific inhibitors available for p110α. Therefore, cells carrying a knockin-sensitizing mutation could be used with available compounds at low enough inhibitor concentrations to analyze the selective effects on p110α.

Despite the general structural similarities between kinases, there are sharp differences between protein and lipid kinases. A reflection of these differences is the pronounced intolerance of the PI3K affinity pocket to mutation. A functional explanation of this remarkable inflexibility remains an important goal for future research.

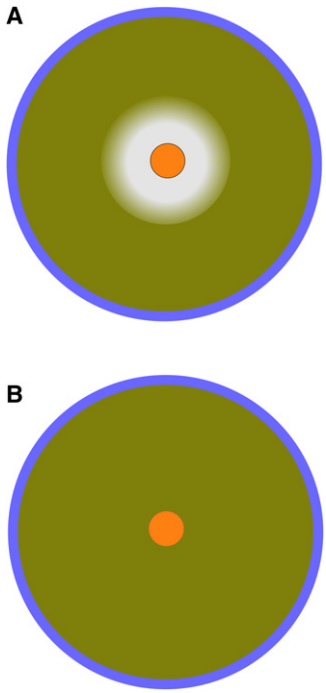


**Figure 1. Testing for PI3K Inhibitors in Yeast: The "Reverse Halo" Assay**
The two plates contain a lawn of yeast cells that express p110α and hence fail to grow. A PI3K inhibitor spotted on a cellulose disk (orange) diffuses into the surrounding lawn, inhibits p110α, and restores cell growth (A). A control disk with DMSO has no effect (B).

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# The Ever-Lengthening Arm of p53

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p53 is a pivotal bulwark against cancer, but exactly how it suppresses tumors remains elusive, in part because it modulates such diverse biological processes via so many downstream pathways. In a recent issue of *Cell*, Godar et al. (2008) now identify another string to p53's anticancer bow—repression of the CD44 cell-surface glycoproteins that coordinate many attributes of tumor progression.

As principal cell factotum of stress responses, sentinel of damage, guardian of the genome, and scourge of all cancers, the p53 protein has assumed an almost mythological status. Such remarkable attributes have garnered p53 much attention from the scientific community, and, some 46,581 scientific publications later (as of July 21, 2008), one might be forgiven for thinking that we know all there is to know about this eldritch protein. Well, actually, no. There are just a few questions that remain