

Developing Biomarkers for PI3K-targeted Cancer Therapies

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Phosphatidylinositol 3-kinases (PI3Ks) are intracellular lipid kinases that phosphorylate the 3'-hydroxyl group of the inositol ring in phosphatidylinositol and phosphoinositides. In response to growth factors, cytokines or other environmental cues, PI3Ks generate lipid second messengers that create binding sites for specific lipid-binding domains on many intracellular signaling proteins and thereby regulate a variety of cellular processes, including cell cycle progression, cell survival, growth, migration, and vesicular trafficking [1, 2]. Hyper activation of PI3K signaling is one of the most common features of many human cancers [2, 3], making this class of enzymes a prime drug target. Tremendous efforts have been made to develop effective PI3K inhibitors for cancer therapy, including both pan-PI3K inhibitors and isoform-selective PI3K inhibitors. A significant need has emerged for analytically validated and clinically qualified biomarkers that can assess the pharmacodynamic effects of the drug, guide patient selection, and understand mechanisms of resistance.

Quantification of the lipid products of PI3K (eg. PIP3) has been challenging in clinical setting. To date, the inhibition of PI3K pathway has been primarily assessed through evaluating the inhibition of phosphorylation in downstream proteins. The most frequently evaluated readouts include phosphorylation of AKT at Thr308 and Ser473, phosphorylation of the AKT substrate PRAS40 at Thr246, phosphorylation of 4E-BP1 at Ser65 and Thr70, and phosphorylation of RPS6 at Ser240 and Ser244. It is noteworthy that 4E-BP1 and RPS6 can also be regulated by RAS/RAF/ERK/mTORC1 pathway, and thereby potentially mask pharmacodynamic effects.

Recent studies taking advantage of a metabolomic analysis identified a panel of 22 circulating metabolite biomarker candidates exhibited significant dose- and time-dependent changes in response to PI3K inhibition in a dose-escalation phase I clinical trial of pictilisib (GDC-0941) (Ang et al. 2016). This study provides a link between modulation of the PI3K activity and changes in the plasma metabolome, suggesting that plasma metabolomics is a feasible and promising strategy for non-invasive biomarker development.

Preclinical studies indicated that activating PIK3CA mutations or PTEN loss could predict sensitivities to PI3K inhibitors. However, correlative studies in the clinical trials have been inconsistent. For instance, the pan-class I PI3K inhibitor BKM120 had significant effects on cells carrying PIK3CA mutations, whereas cells with PTEN loss or KRAS aberrations are not sensitive to BKM120 [4]. The lack of response in PTEN null tumors may be explained by insufficient inhibition of p110 β , the other ubiquitously expressed PI3K isoform encoded by PIK3CB gene. It was reported that PTEN deficient tumors rely on p110 β [5] and BKM120 is slightly less potent against p110 β than p110 α [6]. The co-occurrence of KRAS activating mutations reduces the sensitivity to a PI3K inhibitor, probably by activating alternate KRAS-dependent signaling pathways such as the ERK pathway. Notably, the combination of BKM120 and a MAP/ERK kinase (MEK) inhibitor induced cell death in KRAS mutant/PIK3CA mutant HCT116 cells when single-agent treatment was essentially ineffective [4], highlighting the complexity of signaling pathways and the therapeutic potential of rational combination regimens. In addition to the ERK pathway, ribosomal S6 kinases RPS6KA2 (RSK3) and RPS6KA6 (RSK4), NOTCH, and c-myc activation can also render cells resistant to PI3K inhibitors. The combination of PI3K inhibitors with MEK or RSK inhibitors may help to overcome this resistance [7-10].

Another potential predictive biomarker is INPP4B, a tumor suppressor that inhibits PI3K/AKT signaling. Loss of INPP4B was frequently observed in PTEN null tumors and it correlates with poor prognosis. Therefore tumors with INPP4B loss may also benefit from PI3K inhibitor treatment [11].

Recent advances in the sensitivity and accuracy of DNA sequencing technologies have allowed for genotyping of somatic genomic alterations in circulating cell-free DNA. The feasibility and potential utility of circulating tumor DNA for detection of PI3K mutations have been highlighted in breast cancer patients by using droplet digital PCR [12] and a BEAMing approach [13]. These results, although preliminary, provide a potential less invasive tool for predictive biomarker development.

It should be noted that targeting PI3K also has effects on tumor angiogenesis, immune cells and other tumor micro environmental interactions. Moving forward, molecular profiling of clinical tumor specimens and the correlation with therapeutic response and clinical outcome to PI3K inhibitors will provide critical information for future treatment optimization.

We are entering into a new era of precision cancer medicine. With the development of different PI3K inhibitors for use either as mono therapy or in combination regimens, robust predictive biomarkers/signatures for efficacy and resistance together with the comprehensive evaluation of pharmacodynamics will become crucial for successful clinical application of PI3K inhibitors and maximization of the clinical benefit in patients.

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