AACR Meeting Report:

Targeting PI3K/mTOR Signaling in Cancer

Brooke M. Emerling¹ and Argun Akcakanat²

¹Department of Systems Biology, Harvard Medical School; Division of Signal Transduction, Beth Israel Deaconess Medical Center, Boston, MA; ²Department of Surgical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX.

ABSTRACT

The American Association for Cancer Research (AACR) Special Conference on Targeting PI3K/mTOR Signaling in Cancer was held in San Francisco, California on February 24-27, 2011. The meeting was co-chaired by Drs. Lewis C. Cantley, David M. Sabatini, and Funda Meric-Bernstam. The main focus of this event was the therapeutic potential of drugs targeting the PI3K/mTOR signaling pathway for the treatment of cancer. This report summarizes the recent discoveries in the field, with particular emphasis on the major themes of the conference.

Introduction

The Phosphoinositide 3-kinase (PI3K) family of lipid kinases phosphorylate the 3'-hydroxyl group of phosphoinositides (1). There are three classes of PI3K, each with distinct lipid products and substrate specificity (1). To date, the class IA PI3Ks are the most widely implicated class in human cancers (2). PI3K activation initiates a signal transduction pathway that promotes cancer cell growth, metabolism, and survival (Figure 1). Akt, a serine-threonine kinase that is directly activated in response to PI3K, is a crucial effector of PI3K in tumorigenesis. Activation of Akt leads to increased cellular growth and survival. One of the key downstream effectors of Akt is mTOR complex 1 (mTORC1). The regulation of mTORC1 is complex in that the PI3K-Akt signaling is not the only means of regulating mTORC1. mTORC1 is under the control of many pathways, including growth factor signaling, nutrient and oxygen availability, and the energy state of the cell. Therefore, from a therapeutic standpoint, the regulation of mTORC1 is crucial as some PI3K inhibitors will block both PI3K-mTOR signaling, whereas others will only target PI3K itself. The importance of targeting the PI3K/mTOR for cancer therapy was highlighted at the recent AACR Special Conference on “Targeting PI3K/mTOR Signaling in Cancer” in San Francisco, California on February 24-27, 2011. This meeting underscored the challenge that those in the PI3K/mTOR field face in developing dual PI3K-mTOR inhibitors and emphasized this exciting era of targeted therapies for cancer. In this short report, we review some of the key presentations from this meeting.

PI3K/mTOR Signaling

It is well recognized that the PI3K/mTOR signaling pathway is vital for the growth and survival of cancer cells. With the rapid pace that PI3K/mTOR inhibitors are entering the
clinic, there was no shortage of scientific research and clinical data to go through at this meeting. To start off the meeting the keystone address was given by Jose Baselga (Massachusetts General Hospital, Charlestown, MA) who presented PI3K mutations in cancer and their contribution to resistance to hormonal therapy. The first agents targeting PI3K pathway were rapamycin analogues, and breast cancer patients who received RAD001 in combination with endocrine therapy showed a clinical benefit. Recently, new inhibitors are under development, such as BEZ235 (PI3K, mTORC1/2 inhibitor), BKM120 (PI3K inhibitor), XL147 (selective class I PI3K inhibitor), and BYL719 (alpha isoform specific PI3K inhibitor). He emphasized the need to change the way clinical trials are done. Trials need to be smaller, smarter, and combinational approaches need to be taken. Additionally, screening for mechanisms of resistance to PI3K inhibition via shRNA screens, etc is needed. Moreover, novel therapies need to be incorporated earlier into disease treatment. Jose Baselga gave an informative introduction to targeting the PI3K pathway and elucidated the future of targeted therapies to PI3K/mTOR, particularly combination treatments.

Lewis C. Cantley (Beth Israel Deaconess Medical Center, Boston, MA) started the morning addressing PI3K and cancer cell metabolism. Lewis C. Cantley underscored the need for co-clinical trials with mouse models. Even when agents are already in clinical trials, mouse models give invaluable insights. For example, in animal models, antitumor effects of PI3K inhibitors correlate very well with PET response; suggesting FDG PET may be an important early marker of response in the clinic. Although, when PI3K is shut off with drug but glucose uptake is not completely shut off as measured by PET, these tumors will rebound aggressively. He showed convincing PET images of mice to highlight this point. Also, he showed that PTEN mutant prostate mice tumors respond to the Glaxo Smith Kline PI3K/mTOR inhibitor, but the tumor does not go way completely. Combination therapy with a MEK inhibitor is needed to achieve complete tumor regression and complete inhibition of FDG uptake. Further, one of the best predictors of relapse was reemergence of PET signal. Of importance also is to utilize mice with known mutational backgrounds and see what therapy will be successful and to uncover resistance mechanisms. Furthermore, Lewis C. Cantley discussed that the heterogeneity in PI3K activity is beneficial to normal tissues by restricting PI3K activation to only a subset of cells. This work was published recently by Yuan TL et al. in Current Biology, which elegantly showed that this heterogeneity might serve to protect the population as a whole from over-activation of the PI3K pathway, which ultimately can lead to cellular senescence or cancer (3).

Bart Vanhaesebroeck (Queen Mary University of London Institute of Cancer, London, United Kingdom) introduced the eight isoforms of PI3K in mammals and showed that by using genetic and pharmacological approaches his lab has uncovered isoform-selective roles of the PI3K isoforms. Interestingly, Foukas et al. derived primary cell lines from their PI3K-dead-knock-in (KI) mice, which carry a mutation within the ATP binding site leading to inactivation of the kinase, and showed that in reality cells can proliferate effectively with greater than 90% of p85-associated PI3K activity gone (4). This implies that by inhibiting PI3K alone may not be effective to cancer cells. Therefore, there may be a role for using inhibitors that target the tumor stroma. p110δ null KI mice have
reduced growth and metastasis of solid tumors in syngeneic B16 and 4T1 xenograft models. Moreover, the δ inhibitor PI-3065 in the 4T1 model in the KI mice, the main effect is on adaptive immunity (B/T cells), less on innate regression (macrophages, neutrophils). Bart Vanhaesebroeck’s take home message really was that p110δ inhibition is not immunosuppressive and is a potential target in cancer inflammation and immunity. Langdon Miller (Calistoga Pharmaceuticals Inc., Seattle, WA) underscored the importance of constitutive PI3Kδ pathway in driving the overgrowth of malignant B-cells and showed striking evidence that selective inhibition of this pathway in the clinic can safely abrogate aberrant PI3Kδ signaling with the CAL-101 drug, which is an oral, small-molecule PI3Kδ inhibitor with a low nano-molar EC₅₀ and greater than 200-fold selectively for PI3Kδ relative to the other PI3K isofoms. Phase I trials demonstrated high levels of durable antitumor activity in mantle cell lymphoma, indolent Non-Hodgkin lymphoma (iNHL), and chronic lymphocytic lymphoma (CLL) patients who have received extensive prior chemo-immunotherapy. Reversible lymphoid depletion was observed as a side effect. These findings from Calistoga Pharmaceuticals demonstrated that CAL-101 holds significant promise as a therapeutic option for patients with lymphoid malignancies. Phase II/III trials of CAL-101 are planned for iNHL and CLL.

The first talk of the meeting specifically addressing mTOR was by David M. Sabatini (Whitehead Institute for Biomedical Research, Cambridge, MA). mTOR is the target of the immunosuppressive drug rapamycin and is a vital player in nutrient and growth factor signaling. Recently, the Rag family of GTPases was shown to be amino acid specific regulators of the mTORC1 pathway (5, 6). The laboratory of David Sabatini identified a new trimeric protein complex, which they termed Ragulator (7). The Ragulator is encoded by the MAPKSP1, ROBLD3, and c11orf59 genes and interacts with the Rag GTPases. They further showed that the Ragulator recruits the Rag GTPases to the lysosomes, which also induces translocation of mTORC1 to lysosomes and is crucial for the activation of mTORC1 by amino acids through Rheb (7). Interestingly, individuals with partial loss of function of a component of the Ragulator are small, immunosuppressed, and very young-looking. Ragulator null cells thus have very low mTOR signaling and are absolutely sensitive to rapamycin. Sancak et al. showed that you could rescue this effect by forcing mTOR to the lysosome, where proteins are degraded and amino acids are made. It thus makes sense that mTOR needs to be around the storage centers of amino acids. Moreover, RagA<sub>GTP/GTP</sub> mice die perinatally with slightly decreased size, which very nicely phenocopies the Atg5<sup>−/−</sup> autophagy deficient mice, who die of starvation (8). So it appears like there is a loss of amino acid sensing in these mice. David Sabatini further introduced the identification of the mTOR dependent phosphoproteome. They defined the mTOR phosphoproteome by looking at cells under starvation, insulin stimulated, rapamycin and Torin (mTORC1/2 inhibitor) treated conditions. Based on global phospho profile, mTOR inhibition mimics serum starvation. Rapamycin resistant Torin sensitive sites seem to be downstream of mTOR signaling. Additionally, they identified Grb10 as a novel mTORC1 substrate. Grb10 is a known negative regulator of insulin IGF signaling. They propose an intriguing model in which mTOR activates Grb10, which negatively regulates the pathway by acting on receptor tyrosine kinases (RTKs) and IRS1, possibly embellishing the classical negative feedback through S6K loop.
Next Brendan D. Manning (Harvard School of Public Health, Boston, MA) focused on the biological effects of mTORC signaling. They investigated the downstream targets and functions in cancer and in human diseases. To investigate this they performed a simple, clean genetic and metabolomics assessment of wild-type, Tsc1/2−/− MEFs, and Tsc1/2−/− MEFs treated with rapamycin, which stimulated transcription factors controlling metabolic pathways. They found that mTOR seems to be exerting its biggest effects on metabolism through influencing the oxidative branch of the pentose phosphate pathway through sterol regulatory element-binding protein 1 (SREBP1) to upregulate lipid and sterol biosynthesis (9). SREBP traffics to the Golgi where it is cleaved and translocates to the nucleus in order to function as a transcription factor, activating genes such as fatty acid synthase. Duvel et al. find that mTORC1 acts in the processing of SREBP1 and SREBP2 through S6K1 (9). Furthermore, they proposed that mTORC1 has two major roles: through Hypoxia Inducible Factor (HIF) to induce glucose uptake and glycolysis and through SREBP to ramp up the pentose phosphate pathway. In addition to playing a role in protein synthesis, mTORC1 promotes specific bioenergetics and anabolic cellular processes. TSC1 knockout mice are protected from age and diet induced hepatic steatosis. In hepatocytes, mTORC1 activation is necessary but not enough to activate lipogenesis, SREBP1/2 are required for mTORC1 driven proliferation.

Akt activates SREBPs and regulates expression of enzymes involved in cholesterol and lipid biosynthesis. Caroline A. Lewis (CRUK London Research Institute, London, United Kingdom) presented that mTORC1 inhibitor rapamycin blocked induction of SREBP target genes in immortalized human retinal pigment cells, whereas activated mTORC1 increased the level of nuclear SREBP1 and also regulated the transcriptional activity of SREBP1. Glioblastoma multiforme is associated with mTOR pathway activation and hypoxia. In U87 glioblastoma cells, in response to hypoxia, expression of a subset of SREBP target genes, as well as, SREBP1 protein decrease, which results in a decrease in de novo lipid synthesis. However, another subset of genes involved in lipid uptake and transport are increased, such as SCD. The mechanism of differential expression is not known yet.

Cheryl Lyn Walker (University of Texas MD Anderson Cancer Center, Smithville, TX) presented progress in the field of reactive oxygen species (ROS)-induced ATM signaling. ATM is the gene mutated in the genetic disease ataxia telangiectasia (AT) and is the cellular damage sensor that plays a crucial role in signaling to DNA repair machinery and the cell cycle checkpoints. Recently, Alexander et al identified a cytoplasmic pathway for oxidative stress induced ATM activation of tumor suppressor TSC2 to regulate mTORC1 signaling and autophagy (10, 11). ROS plays an important role in many physiological and pathophysiological processes, including cancer. Upon elevated ROS, ATM activates TSC2 through the LKB1/AMPK metabolic sensing pathway in the cytoplasm to repress mTORC1 and induce autophagy. She showed that TSC signaling is regulated by ROS at the peroxisome. TSC1/2 both contain PTS1 sequences to be transferred to the peroxisome and PTS1 mutations result in TSC disease. mTOR suppression by TSC2 requires localization to peroxisome. ATM acts at the peroxisome as a local sensor of ROS. This also explains the induction of autophagy after exposure to H2O2 or etoposide via
ATM/AMPK/TSC2/mTORC1 pathway, because of mTOR suppression. Elucidation of this stress pathway offers a molecular role for ATM in the cytoplasm and demonstrates that ATM is more than a DNA repair protein, it functions in different cellular compartments.

Alexandra Newton (University of California, San Diego, La Jolla, CA) discussed the balance that is needed between kinases and phosphatases in cancer. Lipid second messenger pathways are strictly controlled by the kinases protein kinase C (PKC) and Akt and the Ser/Thr phosphatase PH domain leucine-rich repeat protein phosphatase (PHLPP). Basically PHLPP provides “the brakes” for Akt and PKC signaling, whereas PDK1 and mTORC2 phosphorylate them. Both are central players in cancer cell proliferation. PHLPP controls cellular levels of PKC and in cells PKC is almost always phosphorylated. PHLPP also suppresses EGFR and controls amplitude of agonist-stimulated EGFR kinase activity. PHLPP knockdown increases p-Akt, p-ERK and EGFR. Unlike the well-known phosphatase and tensin homologue (PTEN), which is somatically mutated in cancer, the PHLPP phosphatases are actually functional in many cancer cell lines examined thus far. There is a delicate balance between phosphorylation and dephosphorylation states which may lead to cancer. Consequently, PHLPP phosphatase activity could be modulated for the treatment of cancer. Specific inhibitors that activate PHLPP may inhibit multiple cancer signaling pathways that are activated by PKC and Akt. Alexandra Newton’s laboratory currently focuses on the function of the PHLPP phosphatases in modulating signaling via Akt and PKC with the hope of developing small molecule inhibitors for the treatment of cancer.

Jean J. Zhao (Dana-Farber Cancer Institute, Boston, MA) presented elegant work using genetic mouse models in targeting PI3K in cancer. The two commonly expressed class Ia catalytic isoforms of PI3K are p110α and p110β. To date in most cell types studied p110α is the major player in the PI3K signaling through RTKs, while p110β is necessary for signaling via G protein coupled receptors (GPCRs). In the mouse, p110α and p110β have been shown to be more essential in some processes and dispensable for others. Unexpectedly, Jean Zhao’s laboratory discovered that p110α and p110β actually play opposing roles in the normal development and pathology of mammary tissue. The absence of p110α during breast development impaired mammary duct outgrowth, while mice deficient in p110β in mammary tissue triggered developmental hypertrophy. Thus, p110 isoform specific inhibition may potentially be a therapeutic strategy in the future. Jean Zhao also presented a mouse model of breast cancer that conditionally expressed an H1047R oncogenic allele of PIK3CA, in order to address resistance mechanisms to PI3K-targeted therapy. This model initiates hyperplasia followed by palpable tumors and metastasis. If the mutation is removed, tumors sometimes show partial regression but may keep on growing. If recurrent tumor relies on high p-Akt levels, then it responds to PI3K inhibitor treatment. Interestingly, genomic analysis revealed Met, c-Myc and Mdm2 amplification in recurrence, thus tumors recur via both PI3K pathway dependent and independent mechanisms, highlighting the challenges for the development of therapeutic agents that target the PI3K pathway in cancer.
To wrap up the PI3K/mTOR signaling part of the meeting, Davide Ruggero (UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA) discussed the pharmacogenetic targeting of the translational machinery downstream of oncogenic mTOR signaling. He provided insight into the post-genomic mechanisms of prostate cancer development regulated by mTOR signaling and showed data that ATP active site inhibitors of mTOR perturb the aberrant translation program in prostate cancer. For example, the potent and selective TORC1/2 inhibitor, INK128 (Intellikine, Inc.) decreased prostate tumors in mice and was more effective than various rapalogs, which only interfere with TORC1 activity. By employing a new technology, Ribosome Profiling, translational state of prostate cancer was assessed. Inhibition of mTOR regulated 148 target genes and YB-1 was identified as a major post-translational regulon, which was regulated both by rapamycin and PP242 (mTORC1/2 inhibitor). YB-1 is involved in cancer cell invasion and PI3K/mTOR/eIF4E/YB1 axis regulates epithelial-mesenchymal transition genes. Interestingly, YB-1 expression is reduced at the translational level by mTORC1/2 in vivo.

**PI3K pathway aberrations in human cancer**

When cancer cell lines are addicted to RTKs, usually PI3K or MEK pathway inhibitors inhibit growth. However, it is not clear if PI3K inhibitors alone will be successful in the clinic. Jeffrey A. Engelman (Massachusetts General Hospital) reported on strategies for targeting PI3K when employing combination therapy. He stressed that the need to inhibit both the PI3K and MEK signaling hinges on the key concept of inducing apoptosis, not just inducing cytostasis. MEK regulates BIM and PI3K regulates MCL-1, a key factor in inducing tumor regression. KRAS tumors are inherently difficult because they activate both PI3K and MEK. In the KRAS mutant lung cancer model, the PI3K inhibitor BEZ235 failed to shrink the tumor but the combination with MEK1/2 inhibitor AZD6244 (ARRY-142886) inhibited both pathways, leading to an apoptotic response. Another approach would be to inhibit both pathways indirectly, so to avoid toxicities to patients. For example, for EGFR mutations, hitting the RTK is an indirect method to inhibit both signaling events. The combination IGF1R and MEK inhibition may be a good strategy for colorectal KRAS mutant tumors. He showed data that in a large panel of patient samples, p85 is immunoprecipitated consistently with IRS1, indicating that this is a commonly engaged network in colorectal cancers to activate PI3K signaling. The data support that in KRAS mutated tumors, PI3K signaling is critical for KRAS-driven tumor growth in the early stages, but as the tumor progresses, its’ reliance on signaling comes more from the RTKs. He further emphasized the dynamics of resistance mechanisms and showed very intriguing and impressive patient data (12). In non-small cell lung cancer (NSCLC) patients who are originally responsive to the EGFR inhibitors gefinitib or erlotinib but their disease starts to progress, through multiple biopsies it can be shown that there is acquisition of other mutations such as EGFR T790M or PIK3CA mutations. These patients are taken off the targeted therapy, put on just chemotherapy for one year, and as mutations disappear, they can become sensitive to EGFR-targeted therapy again. It remains unclear whether resistant mechanisms are acquired or whether they pre-exist, affecting a small fraction of cells. Resistance is probably due to both and is a major challenge in designing treatment strategies for targeting the PI3K pathway.
Ramon Parsons (Columbia University, Manhasset, NY) outlined the history of PTEN, the most commonly mutated tumor suppressor to date, which was discovered in 1997. They found that adenoviral expression of PTEN could lead to apoptosis in cancer cells. Furthermore, they screened for PTEN-regulated genes (that were FOXO-regulated in a PI3K-dependent manner) and identified NFIL3 (nuclear factor, interleukin 3 regulated)/E4BP4. NFIL3 binds to the endogenous TRAIL promoter and represses transcription. It also has effects on numerous other FOXO target genes like FasL and GADD45. Increased expression of NFIL3 correlates with poor prognosis in breast cancer, glioblastoma multiforme, and ovarian cancer, and high NFIL3 mRNA correlates with PTEN negative status in breast cancer. Ramon Parsons underscored the following model: PI3K activates Akt, which in turn activates NFIL3, a transcriptional repressor. NFIL3, in association with HDAC2 can inhibit FOXO transcription of its target genes by the remaining pool of nuclear FOXO. Thus, correlating with Jeff Engelman’s data, the goal of cancer therapy should be to induce cell death.

Eric C. Holland (Memorial Sloan-Kettering Cancer Center, New York, NY) further emphasized that the loss of PTEN in gliomas induces transcriptional changes (13). In normal brain, ABCG2 transporter activity generates “side population” phenotype and this phenotype marks stem-like cells. Loss of PTEN, increased PI3K/Akt (but not mTOR) induces ABCG2 activity which in turn increases tumorigenicity and chemoresistance (14). He presented a new technology, TRAP (Translating Ribosome Affinity Purification), which allows isolation of ribosome bound mRNA and measures translational efficiency under different conditions or treatments. Six hours of radiation exposure of glioma enriched for p53 and E2F1 transcriptional targets. At 2 hours, p53 targets are activated and at 6 hours E2F1 targets are lost. A following experiment with PTEN loss, downregulated 255 genes and upregulated 98 genes, however, differential gene expression profiles of radiation and PTEN loss did not show overlapping genes. Radiation and mTOR blockage showed overlaps but at the transcriptional level only.

Carlos L. Arteaga (Vanderbilt-Ingram Cancer Center, Nashville, TN) discussed the importance of signaling feedback upon inhibition of PI3K and the implications this has for clinical trials. He presented data on XL147 (an ATP-competitive reversible PI3K inhibitor from Exelixis, Inc.) against a panel of breast cancer cell lines harboring alterations, such as HER2 gene amplification. XL147 decreases Akt activity, however leads to the up-regulation of the HER3 transcript. In HER2+ cells, PI3K inhibition is followed by an increase in expression and phosphorylation of RTKs, including HER3. This increase is suppressed by knocking down FoxO1 and FoxO3a. In HER2+ cells, HER3 siRNA knockdown or HER2 inhibition by trastuzumab or lapatinib combined with XL147 show synergistic effect in vitro and in vivo. p-HER3 and p-Akt are lower compared to tumors treated with single agents. It appears that in HER2+ breast tumors, PI3K inhibitors used in combination with HER2/3 inhibitors can stop compensatory feedback mechanisms, which may limit therapeutic efficacy of PI3K inhibitors alone. Overall in HER2+ cancers, PI3K inhibitors will have limited clinical activity if used as single agents, and in HER2- cancers, activated pathways in response to PI3K inhibitor therapy is not clear yet.
Lloyd C. Trotman (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) noted that $Pten^{-/-}$ $P hlpp^{-/-}$ compound mutants cause a worse outcome than signal deficiencies in mouse prostate models. Moreover, that PHLPP gene loss (approx. 40%) is about as frequent as PTEN and p53 in patient prostate samples. Interestingly, the compound PTEN/PHLPP mutant acquires massive Akt activation late in tumor development, correlating to loss of p53. PTEN and PHLPP combined loss triggers p53 activation and senescence arrest. Consistent with this, human metastatic prostate tumors are the only samples that show the PTEN+PHLPP+p53 triple deletion, which rarely occurs in primary tumors. Both PHLPP proteins (1 and 2) compensate for PTEN loss initially, and this feedback response depends on PI3K pathway activation.

Gordon Mills (University of Texas MD Anderson Cancer Center, Houston, TX) introduced the endometrial cancer mutation program and demonstrated that PIK3CA mutations and PTEN loss are the driving mutations in endometrial cancer. Unlike most other cancers, loss of PTEN and PIK3CA mutation may coexist or at least one of them is detected in 80% of cancers. PI3K pathway is frequently hyperactivated in this disease and he focused on somatic mutations of PI3K regulatory subunit 1 (p85$\alpha$; PIK3R1). p85 homodimerizes when there is an excess of p110 and binds to PTEN via the RhoGAP domain of p85 and the C2 domain of PTEN. p85 gain of function mutations in endometrial cancers are unable to bind PTEN, thereby leading to PTEN degradation and pathway activation.

**Development of Novel PI3K/mTOR Pathway Inhibitors**

Kevan Shokat (University of California, San Francisco, CA) began the session on the development of novel PI3K/mTOR pathway inhibitors by introducing chemical genetic investigations of protein and lipid kinase signaling. They tested PP242 (ATP site mTORC1/2 inhibitor) in a xenotransplantation model of human liver tumors serially passaged in mice. This model maintains the integrity of the tumor model quite nicely. They found that cell lines that had wild-type KRAS/PI3K responded to the drug, yet tumor lines with mutant KRAS and wild-type or mutant PI3K responded poorly. Even more specifically, wild-type PI3K was less responsive than lines with H1047K mutant PI3K. From this, Kevan Skokat proposed the model that mutant KRAS drives some mTOR activity, yet when there is a PI3K mutation as well, mTOR activity is rewired to a strong PI3K-driven route, thereby making it more acutely sensitive to PP242. He also presented the kinase suppressor of Ras (KSR), which is a scaffolding protein and assembles Raf-MEK-ERK complexes, facilitating MEK phosphorylation (15). Furthermore, mass spectrometry identified MEK1 Ser24 and Ser72 as KSR dependent phosphorylation sites.

After a summary of PI3K pathway regulation, William R. Sellers (Novartis Institutes for BioMedical Research, Cambridge, MA) presented new inhibitors: BEZ235, BKM120, and BYL719. He described an exciting research initiative, The Cell Line Encyclopedia. Novartis screened 1000 cancer cell lines (characterized their expression profiles, genetics, and SNPs), and their sensitivity/resistance to various compounds. Amazingly, they can
profile approximately 500 cell lines in three months and 1300 compounds in greater than 500 cell lines. This will reveal biomarkers to sensitivity of particular compounds. Additionally, one can ask for a specific genotype and select what is the best compound to treat it with. However, this system is only screening cell growth inhibition by cell counts, not taking into account migration, invasion, and other characteristics of tumor cells. The next step will be the generation of an encyclopedia of human tumors that have been serially passaged and maintained in mice. To date Novartis has received 1281 human tumors and have established almost 300 xenografts of the tumors in mice. The goal of this project will be to make this information available online to the science community.

Robert T. Abraham (Pfizer Biopharmaceuticals, Pearl River, NY) discussed current inhibitors and dosing strategies. Rapamycin, in addition to inhibiting cell growth, inhibits HIF-1 and VEGF expression. Rapamycin-resistant mTOR mutants does not bind to rapamycin in head and neck squamous cell carcinoma cell lines and are relatively insensitive to rapamycin’s growth inhibitory effect in vitro (16). Further, in xenograft models, rapamycin treatment does not inhibit the in vivo tumor growth of rapamycin-resistant mTOR mutant models, suggesting that the primary tumor effect of rapamycin is through its direct anti-tumor effect, rather than its effects on the vasculature Dr Abraham also introduced WYE-112132 (WYE-132), an ATP-competitive inhibitor of mTOR. WYE-132 is potent and against a panel of cell lines its’ IC$_{50}$ is in the nanomolar range (17).

Joel Greshock (GlaxoSmithKline, Collegeville, PA) presented a pan-PI3K pyridylsulfonamide inhibitor, GSK2126458. This is a reversible, ATP-competitive inhibitor of wild-type and mutated forms of p110α. In a wide panel of human cancer cell lines, bladder, ovarian and particularly PIK3CA activating mutations harboring breast cancer cell lines were responsive to growth inhibition. Colon cancer cell lines show moderate level sensitivity and KRAS mutations predict decreased sensitivity. Combination with a MEK inhibitor shows strong synergy. Yi Liu (Intellikine, Inc., La Jolla, CA) introduced INK1117, a potent and selective PI3Kα inhibitor that demonstrates a greater than 100-fold selectivity relative to other class 1 PI3K family members and mTOR. Through structure-guided drug design they discovered INK1117, which is proving to be an efficacious PI3Kα inhibitor. In addition, in vitro and in vivo, INK1117 blocks VEGF signaling and angiogenesis but shows much less activity in PTEN-deficient tumors, which have constitutive activation of PI3K/Akt/mTOR pathway independent of PI3Kα. This drug does not impair glucose homeostasis or B and T cell functions. This new therapeutic approach of selectively targeting PI3Kα offers a novel strategy for the treatment of cancers. Moreover, pre-clinical data implicates that isoform-selective inhibitors may be more effective in cancers with PIK3CA activation along with a better safety profile.

**Targeting mTOR**

Funda Meric-Bernstam (University of Texas MD Anderson Cancer Center, Houston) presented some of the ongoing studies to determine the mechanism of rapamycin action and markers for rapamycin response/resistance. Preclinical studies have suggested that
activation of Akt/mTOR signaling may be a predictor of response. However there have been challenges in validating these markers as predictors of response clinically. By immunohistochemistry, comparison of p-Akt and p-4E-BP1 expression in primary and matched distant metastases of breast tumors showed poor concordance, which might reflect tumor heterogeneity or challenges in doing immunohistochemistry on archived tissue (18). She presented modulation of downstream signaling by rapamycin and novel pharmacodynamic markers of response. Rapamycin–regulated transcriptome predicts survival in breast cancer patients, showing importance of the pathway in human cancer biology (19). Rapamycin also was shown to regulate the expression of stearoyl-CoA desaturase 1 (SCD1), a critical enzyme in fatty acid metabolism SCD1 knockdown decreases, and SCD1 overexpression increases breast cancer cell growth, thus rapamycin-mediated downregulation of SCD1 may play an important role in its antitumor effect (20). Studies are ongoing to determine the role SCD1 in cancer biology and as a pharmacodynamic marker. Dr Meric-Bernstam also presented work in functional proteomics approaches to identify predictors and pharmacodynamic markers of response. These studies demonstrated that baseline Akt pathway activation is associated with response, but interestingly, feedback loop activation of Akt, is also associated with response. Similar data is emerging from clinical trial specimens. These studies highlight that there is a great need for additional work on the mechanism of action of rapamycin and its analogs, as well as, predictors and pharmacodynamic markers of response to these agents.

Robert J. Schneider (New York University Cancer Institute, New York, NY) has shown that inflammatory breast cancer (IBC) is radiation-resistant, in part because of overexpression of eIF4GI and constitutively active mTOR pathway. In response to ionizing radiation (IR); expression of DNA-damage response (DDR) proteins increase and protect IBC cells. In vitro, reducing high levels of eIF4GI sensitizes non-transformed breast epithelial cells to IR, but surprisingly increases cancer stem cell (CSC) population. Overall, there is no enhancement in radiation sensitivity. Next, using an IBC cell line, SUM149, they compared everolimus (allosteric mTOR inhibitor), PP242 (mTORC1/2 inhibitor) and eIF4GI silencing. PP242 and eIF4GI siRNA knockdown, but not everolimus, prevented growth of IBC xenografts, enhanced radiosensitivity, decreased clonogenic survival, inhibited Akt activation, more efficiently inhibited downstream targets and translation of mRNAs involved in DDR. In vivo, treatment of SUM149 xenografts with PP242 and IR lead to better tumor control and longer survival. He concluded that eIF4G silencing or mTORC1/2 inhibition might also sensitize CSC to IR.

Sylvie M. Guichard (AstraZeneca R&D, Macclesfield, United Kingdom) introduced AZD8055, an ATP-competitive inhibitor of mTOR kinase activity (21). Cell line experiments show that AZD8055 dephosphorylates p-4E-BP1 Thr37/46 completely and compared to rapamycin has a greater effect on inhibition of cap-dependent translation, affects major cyclins as well as p27 and p21, and results in a profound G1 arrest. Animal experiments show that AZD8055 primarily target tumor growth, has a limited effect on tumor vasculature, and inhibits tumor growth more than rapamycin. As Akt is inhibited, glucose uptake by tumor cells decrease. Uptake of glucose analog FDG observed by PET scanning is correlated with tumor growth, and used as a biomarker. She justified
combination treatment by two examples: in HER2 amplified breast cancer cell lines, AZD8055 increases p-HER3, and combination of AZD8055 and HER2 inhibitor lapatinib is synergistic. Secondly, AZD8055 results in a temporary increase in p-MEK. Combination of AZD8055 and the MEK inhibitor selumetinib (AZD6244) produces cell death and tumor regression in NSCLC xenografts.

Neil Rosen (Memorial Sloan-Kettering Cancer Center) classified PI3K signaling pathway as “pathway activation without ERK dysregulation (prostate, breast cancer), with coexisting ERK dysregulation (colorectal cancer, melanoma), and harboring multiple mutations (endometrial).” Next, he analyzed the failures in clinical trials as “not good patient selection, suboptimal drugs, inadequate inhibition, and adaption-release of feedback inhibition.” He focused on feedback inhibition, mainly use of a non-ATP-competitive Akt1 and Akt2 selective inhibitor, Akti-1/2 (22). In multiple cell lines, not only in breast cancer cell lines with HER2 overexpression, Akti-1/2 treatment activates p-HER3, p-INSR (insulin receptor), p-IGF-1R, whereas expression of HER2 does not change of decrease. Several other receptor tyrosine kinases, including HER4, show an increase, which is postulated to be lack of inhibition of FOXO proteins because of Akt inhibition.

Late-Breaking Research/Hot Topics

This session of the meeting was filled with short talks that were proffered from their abstract submission. To start, William C. Cobb (University of North Carolina, Chapel Hill, NC) presented his research on IKK-dependent phosphorylation of PI3K, which induces nutrient deprivation-induced autophagy. IKK phosphorylates p85α in response to starvation in vivo, which leads to feedback inhibition of PI3K/Akt/mTOR pathway, promoting autophagy. This crosstalk between IKK/NF-kB and PI3K/AKT probably will have significant implications for inflammation-associated pathologies, such as cancer.

Next, Jonathan Ross Hart (The Scripps Research Institute, La Jolla, CA) described an elegant SILAC screen where he identified STAT-3 targets as a major group of proteins that are overexpressed during PI3K-mediated oncogenic transformation. PI3K activates Tec family of tyrosine kinases, which in turn phosphorylates and activates STAT3. Inhibition of Tec kinases/STAT3 interferes with oncogenic transformation induced by p110α H1047R.

Nathan T. Ihle (University of Texas MD Anderson Cancer Center, Houston) showed that specific amino acid substitutions in mutant KRAS effect PI3K signaling and may predict patient survival and their response to targeted therapies. NSCLC KRAS G12C and colon or pancreas cancer G12D amino acid substitutions are common. Analysis of NSCLC cell lines with G12D showed activation of the PI3K/Akt and MEK pathways, whereas G12C mutants have a weak activation of PI3K but strong activation of RAL signaling. In NSCLC patients, G12C mutations reveal a worse progression-free survival compared to other mutations, including G12D mutation, which shows that different mutations may require different inhibitors.
Devin T. Worster (Harvard Medical School, Boston, MA) introduced that the loss of p57Kip2 increases the PI3K/AKT oncogenic signaling pathway. p57Kip2 is a cyclin-dependent kinase inhibitor which is induced under conditions such as IGF-1 or insulin stimulation. Interestingly, p57Kip2 is downregulated in response to EGF and EGF induces proliferation in the mammary epithelial cell line, MCF10a. Also, knockdown of p57Kip2 releases acini from standard growth arrest during morphogenesis of mammary epithelia cell in three-dimensional culture. p57Kip2 is activated by Akt but inhibited by ERK, sensitive to Akt/ERK ratio, resulting in cell cycle arrest if the ratio is high. Remarkably, the p57Kip2 locus is silenced in many breast cancers, which frequently show hyperactivation of the PI3K pathway. p57Kip2 level may help to decide targeted therapy against ERK and PI3K pathways. This interesting hypothesis needs to be prospectively validated in the clinic.

Both the PI3K and MEK pathways contribute to PHLPP-dependent proliferation. Matt J. Niederst (University of California, San Diego) showed that not only do the PHLPP family phosphatases regulate the PI3K pathway via their effect on Akt, but they also regulate growth factor receptor signaling. PHLPP suppresses Ras activation and PHLPP knockdown increases EGFR protein levels and activity, as well as, transcription of other growth factors such as VEGFR1, MET, PDGFRα, and IGFR2. Many cancers overexpress growth factor receptors, such as EGFR, thereby making regulation of PHLPP an attractive therapeutic strategy.

APC loss results in WNT signaling activation and WNT activation is important in intestinal regeneration, particularly crypt growth. William J. Faller (Beatson Institute for Cancer Research, Glasgow, United Kingdom) presented in mice that raptor knockout or rapamycin treatment inhibits tumor growth induced by APC and PTEN deletion within LGR5-positive stem cell population. This growth effect is mediated through S6K1 and indicates that mTOR pathway mediates effects of WNT activation in intestine.

Olga K. Mirzoeva (University of California, San Francisco) showed that in pancreatic ductal adenocarcinoma, inhibiting PI3K or mTOR induces growth inhibition whereas inhibiting both induces cell death. Use of PI103, XL765 (dual inhibitors), or combination of PIK90, XL147 (p110α inhibitors) with rapamycin or with PP242 (mTOR inhibitors) resulted in apoptosis but induced autophagy. Inhibiting autophagy by chloroquine enhanced apoptosis. Thus, combining PI3K/mTOR inhibitors with autophagic inhibitors may promote pancreatic cancer treatment.

**Novel PI3K/mTOR Pathway Inhibitors in Clinical Trials**

Ana Maria Gonzalez-Angulo (University of Texas MD Anderson Cancer Center, Houston) discussed molecular marker-based clinical trial designs and once again emphasized a general theme of the meeting- that clinical trials need to be smaller, shorter, cheaper, and individualized. Evidence based medicine is generally practiced today and this is best for the average population but not specific for individuals. Treatments for individual patients should be personalized based on their tumor’s molecular characteristics and the patient’s genetic map. For selection of correct markers, she
stressed importance of identifying pathway drivers, understanding limitations of marker research, and the fact that pathway signatures may be superior to single markers. Even if a marker is technically valid and scientifically reasonable, clinical validation may come later, even in the trial. Thereby, carefully matching patients and specific single agents or combination therapies will improve our likelihood of accomplishment. Furthermore, she discussed molecular marker-based clinical trials targeting PI3K/Akt/mTOR pathway and promising results of combination therapies, and specific challenges to clinical trials such as time from sample acquisition to processing, additional time needed for patient enrollment, high cost and extensive regulations.

Li Yan (Merck & Co., North Wales, PA) presented data concerning MK-2206, an oral allosteric AKT inhibitor. In a Phase I study, it is pretty well tolerated with widespread rash as the major toxicity and resulted in a decrease in AKT signaling but increase in p-ERK and p-MEK. Rationale combination trials are currently ongoing with MK-2206 in combination with chemotherapies as well as targeted agents, such as MEK inhibitors, in order to make the most of the overall clinical outcome.

Razelle Kurzrock (University of Texas MD Anderson Cancer Center, Houston) presented the PREDICT program, a program where all patients are screened for common oncogenic mutations (PIK3CA, KRAS, NRAS, BRAF, EGFR, PTEN loss, etc.) with the goal of enhancing efficacy of personalized targeted agents tested in Phase I trials.

Mark R. Lackner (Genentech, Inc., South San Francisco, CA) further emphasized this point and highlighted that we need to follow tumor genetics in order to optimize therapy for cancer patients. Genentech has several clinical clinical trials in progress that strive to identify patients likely to benefit from targeted therapy where they are testing putative predictive signatures.

Joanne J. Lager (Sanofi-Aventis, Cambridge, MA) described early clinical studies of XL147 (pan-PI3K inhibitor) and XL765 (pan PI3K/mTORC1/2 inhibitor). Interestingly, XL147 inhibits not only p-Akt, but also p-MEK and this is evident across many tumor types with no change in total MEK or ERK. Additionally as a single agent there are anti-proliferative effects but no apoptosis. XL765 passes the blood-brain barrier and also inhibits p-Akt and p-ERK with no change in total MEK or ERK levels. She postulates that the p-ERK effect is not unique to these PI3K/mTOR pathway inhibitors. Furthermore, that p-ERK inhibition likely lags behind the Akt inhibition, implicating that the combination with a MEK inhibitor may be more effective than these compounds as monotherapies.

Ryan J. O. Dowling (Ontario Cancer Institute, Toronto, ON, Canada) presented the biological effects of metformin in early stage breast cancer. Metformin is a frontline therapy for type II diabetes that reduces circulating insulin and glucose levels. Metformin has emerged as a potential anti-cancer agent because it can activate AMPK and decrease mTOR signaling, leading to a decrease in proliferation in cancer cells. Dowling and colleagues conducted a neoadjuvant, single arm, “window of opportunity” clinical trial to treat breast cancer patients with metformin prior to breast surgery. Their interim analysis
demonstrated that metformin decreased proliferation; there were no changes in p-AMPK, but there was a decrease in p-Akt. Together, suggesting that there may there be an indirect mechanism of metformin that provides its anti-tumor effects.

Summary

This Special Conference highlighted the fact that although there has been a large amount of research performed on the PI3K/mTOR signaling pathway over the years, there is still a great deal of work to do to better understand how to effectively use the inhibitors of the this pathway in the clinic. Moreover, we need to intimately understand the oncogenic mechanisms of individual tumors, allowing us to treat patients more effectively, with the least toxicity. These are exciting times in cancer research, particularly in the PI3K/mTOR signaling field. We look forward to the next AACR special Conference on Targeting PI3K/mTOR Signaling in Cancer.

References

Figure Legends

**Figure 1.** The PI3K/Akt/mTOR signaling pathway. Arrows represent activation and bars represent inhibition. PI3K is induced by receptor tyrosine kinases (RTKs) which are receptors for growth factors and insulin. Nutrient status and energy regulates mTOR signaling. Abbreviations: 4EBP1 = 4E-binding protein 1; AMPK = adenosine monophosphate-activated protein kinase; BAD = Bcl-2-associated death promoter; FOXO = forkhead box 1; GSK3β = glycogen synthase kinase 3 beta; IRS1 = insulin receptor substrate 1; MDM2 = p53 binding protein homolog; mLST8 = mTOR associated protein, LST8 homolog; mTOR = mammalian target of rapamycin; mTORC1 = mTOR complex 1; mTORC2 = mTOR complex 2; PDK1 = phosphoinositide-dependent kinase 1; PHLPP = PH domain and leucine rich repeat protein phosphatase 1; PI3K = phosphatidylinositol 3-kinase; PIP2 = phosphatidylinositol (4,5) biphosphate; PIP3 = phosphatidylinositol (3,4,5) triphosphate; PRAS40 = proline rich Akt substrate 40; PTEN = phosphatase and tensin homolog deleted on chromosome 10; Raptor = regulatory associated protein of mTOR; Rheb = Ras homolog enriched in brain; Rictor = rapamycin-insensitive companion of mTOR; RTK = receptor tyrosine kinase; S6K = ribosomal S6 kinase; SIN1 = mitogen-activated protein kinase associated protein 1; TSC1 = tuberous sclerosis complex 1; TSC2 = tuberous sclerosis complex 2.
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Brooke M Emerling and Argun Akcakanat

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