Cancer Drug Discovery and Development

Nandini Dey
Pradip De
Brian Leyland-Jones *Editors*

PI3K-mTOR in Cancer and Cancer Therapy

Foreword by Matthew J. Ellis

Humana Press

Cancer Drug Discovery and Development

Series editor

Beverly A. Teicher
Bethesda, Maryland, USA

Cancer Drug Discovery and Development, the Springer series headed by Beverly A. Teicher, is the definitive book series in cancer research and oncology. Volumes cover the process of drug discovery, preclinical models in cancer research, specific drug target groups, and experimental and approved therapeutic agents. The volumes are current and timely, anticipating areas where experimental agents are reaching FDA approval. Each volume is edited by an expert in the field covered, and chapters are authored by renowned scientists and physicians in their fields of interest.

More information about this series at http://www.springer.com/series/7625

Nandini Dey · Pradip De
Brian Leyland-Jones
Editors

PI3K-mTOR in Cancer and Cancer Therapy

Foreword by Matthew J. Ellis

Humana Press

Editors
Nandini Dey
Avera Cancer Institute Center for Precision Oncology
Sioux Falls, SD
USA

Pradip De
Avera Cancer Institute Center for Precision Oncology
Sioux Falls, SD
USA

Brian Leyland-Jones
Avera Cancer Institute Center for Precision Oncology
Sioux Falls, SD
USA

ISSN 2196-9906 ISSN 2196-9914 (electronic)
Cancer Drug Discovery and Development
ISBN 978-3-319-34209-2 ISBN 978-3-319-34211-5 (eBook)
DOI 10.1007/978-3-319-34211-5

Library of Congress Control Number: 2016939046

Printed on acid-free paper

This Humana Press imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland

To the past, present and future patients of Avera Cancer Institute

Foreword

Analysis of the genomic landscape of cancer over the last half decade through next generation sequencing (NGS) studies has revealed a diverse array of somatic mutations that activate the phosphoinositol-3-kinase (PI3 kinase) pathway. These include loss of negative regulators (PTEN, INPP4B) that critically tip the membrane phospholipid balance in favor of active signaling. Additionally, gain of function mutations in downstream PI3 kinase components, most particularly in the phosphoinositol-3-kinase alpha subunit (PIK3CA); are among the most common somatic events in epithelial malignancies. Interruption of signaling flux through the phospho-inositol-3-kinase pathway is therefore considered key to successful treatment and, ultimately, the cure of a very wide range of cancer types. There has been a strenuous effort to develop pharmacological inhibitors of the PI3 kinase pathway, spurred by the initial success for analogs of the natural compound rapamycin, which inhibits the mTOR kinase. Rapamycin analogs are now approved for the treatment of renal cell carcinoma, breast cancer, pancreatic neuroendocrine tumors and tuberous sclerosis related malignancies. A PI3 kinase delta inhibitor, idelalisib, has been recently approved in chronic lymphocytic leukemia/lymphoma and follicular lymphoma. In these indications, PI3 kinase inhibitors are useful palliative treatments but resistance is essentially inevitable. This suggests that rational combinations based on an understanding of resistance pathways are a critical next step. Another barrier to drug development is the difficulty in predicting response. NGS analysis of samples from an everolimus study in breast cancer as well as smaller studies of selective inhibitors of PI3 kinase has failed to develop a convincing genomic profile associated with the response. Newer approaches based on proteomics or perhaps integrated proteogenomic approaches are therefore under study. This book provides a critical summary of these important areas of

investigation. With clinical trials reporting out new results from multiple classes of inhibitors targeting the PI3 kinase pathway at multiple levels, the summaries provided by the authors provide timely and critical context for the interpretation of these investigations and will help drive the design of the next generation of clinical trials.

Matthew J. Ellis MB., BChir., Ph.D., FRCP
Director, Lester and Sue Smith Breast Center,
Baylor College of Medicine, Houston TX

Preface

Our effort to edit this book is aimed to present readers who sincerely endeavor to examine the role of the PI3K-AKT-mTOR signaling pathway in the context of the emerging challenges of oncology, including energy metabolism, genomic instability, signaling-driven combination therapies, drug response, and drug resistance. Through our effort, we seek to engage translational researchers, clinicians, and clinical researchers who aspire to develop therapies for the management of cancer patients. The book is a reference textbook intended for readers who are actively perusing basic, translational and clinical studies in the course of their graduate classes and fellowship trainings as well as researchers from academia, hospitals, and pharmaceutical industries.

We were fortunate to have Prof. Lewis C. Cantley, who had kindly agreed to author the opening chapter of the book. It is any editor's dream to have an introductory chapter of a book entitled "*PI3K-mTOR in Cancer and Cancer Therapy*" to be written by none other than Prof. Lewis C. Cantley. We feel privileged and grateful to Prof. Lewis C. Cantley. The topic of "*PI3K-mTOR in Cancer and Cancer Therapy*" is too diverse and extensive to be covered in one book with less than ten chapters. We decided to present the book in two parts, Part I entitled "PI3K-mTOR Pathway in Cancers" containing four Chapters (2–5) and Part II entitled "PI3K-mTOR Pathway in Cancer Medicine" containing four Chapters (6–9). The first part comprises of four basic and translational topics emerging in the field of PI3K-mTOR signals, including cancer cell metabolism, DNA damage repair, drug response prediction and prognostic signatures and resistance to anti PI3K-mTOR pathway related drugs.

Keeping in mind the central role of the PI3K-mTOR pathway in sensing tumor cells' nutrient and energy need and rewiring it with the growth signals, the Chap. 2 of the first part of the book is written by Prof. Estela Jacinto and his colleagues. The review focuses on the role of mTOR complex 1 and mTOR complex 2 in different metabolic and biosynthetic processes with special emphasis on the role of mTORCs in the reprogramming of cancer metabolism. The chapter also discusses the role of mTOR in the metabolic processes of tumor cells and how oncogenic mutations can

trigger metabolic reprogramming. The chapter encourages readers to study the clinical relevance of targeting mTOR and metabolic pathways for cancer therapy. In reading this chapter, a reader will get the basic knowledge to understand how mTORCs reprogram metabolic and biosynthetic pathways under specific oncogenic mutations and how mTORCs signal will influence the sensitivity to chemotherapeutic agents especially towards the development of resistance to mTOR/PI3K inhibition due to induction of alternative pathways. As wisely pointed out by Prof. Estela Jacinto that "*identifying synthetic lethal interactions and drug resistance mechanisms inherent to metabolic or growth signaling pathways upon mTOR inhibition will be important to develop more effective cancer therapy*".

The PI3K-AKT-mTOR pathway interacts with the DNA damage repair pathway in solid tumors. In Chap. 3 of the first part of the book, we have tried to review how the PI3K-AKT-mTOR pathway cooperates with the DNA damage repair pathway toward oncogenesis of Triple Negative Breast Cancers in the light of the translational relevance of a combination of PARP inhibitors with PI3K-AKT-mTOR inhibitors. Delving into this chapter, a reader will learn that alteration(s) of the PI3K-AKT-mTOR pathway in breast cancers and its subtypes are contextual and DNA damage response is one of such important contexts in Triple Negative Breast Cancers. We intended to focus on the recent development in the field of a combination of PARP inhibitor(s) and PI3K-AKT-mTOR pathway inhibitor(s) in the light of drug-sensitivity and development of resistance.

Chapter 4 of the first part of the book is written by Prof. Mariaelena Pierobon and his colleagues on "*The AKT-mTOR signaling pathway for drug response prediction and prognostic signatures*". This chapter provides "*an overview of the role of the PI3K-AKT-mTOR signaling network as a predictive and prognostic biomarker across different tumors along with a panel of high throughput and multiplex technologies used to broadly investigate functional changes*".

Chapter 5 of the first part of the book is written by Prof. Sarat Chandarlapaty and his colleagues on "Resistance to PI3K pathway inhibition". The chapter describes the basic circuitry of the PI3K-AKT-mTOR pathway and its mechanism of reaction to inhibitors in bringing the "*drug-induced relief-of-feedback results in pathway reactivation*" and "*drug-induced adaptive/compensatory activation of parallel signaling pathways in the network*". Built on this, the chapter then elegantly introduces the concept of development of resistance to the PI3K-AKT-mTOR pathway inhibitors and discusses the "*coordinated activation of RAS/RAF signaling in resistance," "feedback regulation of nuclear hormone signaling," "Wnt-β-catenin cooperation with PI3K signaling," "Myc amplification,"* and *"JAK2/STAT5 inhibition."* We are convinced that in reading this chapter, a reader will comprehend "*mechanisms of resistance common to cellular signaling pathways*".

In the second part of the book, we have compiled four chapters describing the state-of-art of the PI3K-mTOR pathway based cancer therapies in selected solid and liquid tumors. Keeping in mind that the PI3K-mTOR pathway is one of the most genetically altered pathways in cancers and a vast intellectual wealth that is vested on exploiting this pathway towards the development of therapies to manage cancers, Chap. 6 is written by Prof. Funda Meric-Bernstam and Prof. Gordon Mills. In this

chapter authors have eloquently described the signaling basis of "*combination therapies targeting the PI3K/AKT/mTOR pathways*" including "chemotherapy," "hormonal agents," "immunotherapy," "biological therapy," "proximal/distal inhibition," "parallel signaling," "biomarkers," and "pharmacodynamic markers of response."

Inhibitors targeting the pathway are entering clinical trials at a rapid pace. Recently isoform specific (p110 delta) inhibitor has been approved by FDA in hematological malignancies. Chapter 7, the second chapter of the second part of the book is written by Dr. Chan and her colleague on "*phospho-inositol-3-kinase activity and dysregulation in pediatric leukemia and lymphoma*" highlighting the studies that have defined the role of PI3K regulatory and catalytic subunits in childhood hematologic malignancies, and addressing how these findings are now being translated into murine preclinical and human clinical trials.

In Chap. 8, the third chapter of the second part of the book we have reviewed the "*HER2 signaling network in advanced breast cancers as a basis for combination therapies*." A reader of this chapter will have an opportunity to learn in-depth signaling of the pathway and how the response to HER2/HER-family inhibitors in combination with isoform-specific/pan PI3K/dual inhibitors has shaped the current status of the clinical practice of oncology.

Chapter 9, the fourth chapter of the second part of the book is written by Prof. Leland W.K. Chung and his colleague on "biological significance and therapeutic opportunities" of the PI3K/AKT/mTOR pathway inhibitors in prostate cancers. The chapter discusses the development as well as the use of pathway inhibitors as single or combined therapies highlighting ongoing clinical trials for the treatment of prostate cancers.

Nandini Dey
Pradip De
Brian Leyland-Jones

Acknowledgments

We sincerely acknowledge the Avera Cancer Institute Center for Precision Oncology, Sioux Falls, SD, the USA for providing us the opportunity for research. We wish to thank Avera Cancer Institute in Sioux Falls, South Dakota for supporting our effort to edit the book. Our wholehearted thanks go to the series editor Dr. Beverly A. Teicher for giving us the opportunity for edit this book. We sincerely acknowledge all the chapter writers and their respective Institutes for willingly participate in this project. We earnestly acknowledge Springer Science for making this project a success. We thank Ms. Hannah Koble for being associated with this project from the beginning and for her outstanding secretarial assistance in the preparation of this book. We also acknowledge the Department of internal medicine, SSOM, USD, SD, USA.

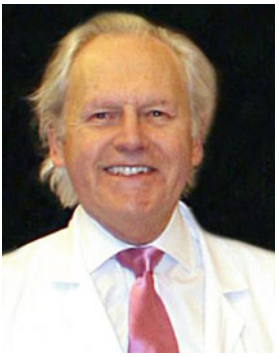
Contents

Editors and Contributors

About the Editors

Nandini Dey M.S., Ph.D. is a Senior Scientist Head of Cancer Biology, Avera Cancer Institute Center for Precision Oncology. She is also Assistant Professor of the Department of Internal Medicine at The University of South Dakota Sanford School of Medicine. Dey has more than 20 years of experience in translational research and has authored or coauthored more than 80 publications.

Pradip De M.S., Ph.D. is a Senior Scientist Head of Translational Oncology, Avera Cancer Institute Center for Precision Oncology. He is also Assistant Professor of the Department of Internal Medicine at The University of South Dakota Sanford School of Medicine. De has more than 20 years of experience in translational research in academics and industry and has authored or coauthored more than 90 publications.



Brian Leyland-Jones MBBS, Ph.D. is Vice President of Avera Cancer Institute Center for Precision Oncology. Dr. Leyland-Jones is a consulting professor at the Stanford University School of Medicine. He is an internationally renowned breast cancer expert with more than 35 years experience in patient care and research, has published over 170 papers and has received over 11,000 citations in his scientific career.

Contributors

Elisa Baldelli Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA, USA; Medical Oncology Division, S. Maria Della Misericordia Hospital, Perugia, Italy

Lewis C. Cantley Sandra and Edward Meyer Cancer Center, Department of Medicine, Weill Cornell Medical College, New York, NY, USA

Jennifer H. Carlson Avera Cancer Institute Center for Precision Oncology, Sioux Falls, SD, USA

Rebecca J. Chan Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, USA; Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indianapolis, IN, USA; Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA

Sarat Chandarlapaty Department of Medicine and Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Sameer S. Chopra Laboratory for Systems Pharmacology, Department of Systems Biology, Harvard Medical School, Boston, MA, USA

Leland W.K. Chung Cedars-Sinai Medical Center, Los Angeles, CA, USA

Pradip De Avera Cancer Institute Center for Precision Oncology, Sioux Falls, SD, USA; Departmental of Internal Medicine, SSOM, University of South Dakota, Sioux Falls, SD, USA

Nandini Dey Avera Cancer Institute Center for Precision Oncology, Sioux Falls, SD, USA; Departmental of Internal Medicine, SSOM, University of South Dakota, Sioux Falls, SD, USA

Charles B. Goodwin Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA

Estela Jacinto Department of Biochemistry and Molecular Biology, Rutgers-Robert Wood Johnson Medical School, Piscataway, NJ, USA

Brian Leyland-Jones Avera Cancer Institute Center for Precision Oncology, Sioux Falls, SD, USA; Departmental of Internal Medicine, SSOM, University of South Dakota, Sioux Falls, SD, USA

Thomas Lynch Department of Biochemistry and Molecular Biology, Rutgers-Robert Wood Johnson Medical School, Piscataway, NJ, USA

Funda Meric-Bernstam Department of Investigational Cancer Therapeutics (Phase I Clinical Trials Program), The University of Texas MD Anderson Cancer Center, Houston, TX, USA; Institute of Personalized Cancer Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Gordon B. Mills Institute of Personalized Cancer Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Joseph G. Moloughney Department of Biochemistry and Molecular Biology, Rutgers-Robert Wood Johnson Medical School, Piscataway, NJ, USA

John Conor Moran Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA, USA

Aung Naing Department of Investigational Cancer Therapeutics (Phase I Clinical Trials Program), The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Emanuel F. Petricoin Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA, USA

Mariaelena Pierobon Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA, USA

Payal D. Shah Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Jason Boyang Wu Cedars-Sinai Medical Center, Los Angeles, CA, USA

Introduction

We began to learn from the collective work of pioneers such as Mariano Barbacid, Michael J. Bishop, Harold Eliot Varmus, Robert Allan Weinberg, Alex Matter, and Bert Vogelstein that identifying cancer-causing genes in humans was opening the door to a new era of the targeted anticancer research. It was also understood that genomic alteration(s) alters signals of different cancer-promoting pathways. In the post "human-genome project" era, cancer-specific genomic maps started redesigning the tumor taxonomy by transcending it from histopathology to molecular pathology. The success of a cancer drug today is thus fundamentally based on the success of the identification of the target gene(s) that is responsible for the control of the beneficiary pathway. Today as a scientific community we recognize that cancer being a genetic disease, modern oncology demands a gene-based rationale for the precision medicine in clinics to be complemented by translational research. Cancer research has seen tremendous technology-based modernization over the past decade. The advent and progress of sequencing technology have gifted us with the identification of landmark genetic alterations in different organ-type cancers, which revolutionized clinical science and practice by pointing to new kinase targets, such as phosphatidylinositol-3-kinases (PI3K), the EGF receptor, or BRAF. For the past few decades, cancer researchers have been paying attention to the central importance of the small GTPase, RAS—the first identified oncogene—in a neoplastic transformation. Extensive biochemical and genetic studies of the signaling components upstream and downstream of RAS in model organisms led to the understanding of mitogenic signaling by receptor tyrosine kinases (RTKs) through RAS and its downstream MEK-ERK signaling components. Conserved through evolution from flies to mammals, the central importance of this pathway in neoplastic cell proliferation has been confirmed by the clinical success of therapeutics that targets tyrosine kinases, such as erlotinib, lapatinib and imatinib.

In last 25 years, a second pathway downstream of receptor tyrosine kinases, RTKs (and also via RAS) that involves PI3K, AKT, or its further downstream target mammalian target of rapamycin (mTOR) has been under intense focus as an important regulator of cell proliferation, survival, and angiogenesis. The growth factor stimulated PI3K pathway which controls cell survival/proliferation and

integrates nutrient signals has emerged as a unique candidate pathway in oncology because its dysfunctional upregulation by both oncogenic activations, as well as loss of tumor suppressor, has been documented in all forms of cancers. Oncogenic signaling in the majority of the solid tumors' is sustained via the PI3K-AKT-mTOR pathway. TCGA studies indicated that numerous components of the PI3K-AKT-mTOR pathway are upregulated more frequently than any other pathway in cancer patients by amplification(s), translocation(s), DNA methylation, loss and mutation(s), with resultant activation of the pathway. Somatic or germline mutations frequently occur in tumor suppressor genes (PTEN, TSC1/2, and LKB1) and oncogenes (PIK3CA, PIK3R1, AKT) in the PI3K-mTOR pathway. The strategy of "drugging the cancer kinome" has led to the successful development and regulatory approval of several novel molecular targeted agents. The spotlight is now shifting to the PI3K–AKT–mTOR pathway as a potential key target. Strategies for horizontal/vertical blockade of the pathway as well as the use of biomarkers to stratify appropriate patients and to justify target modulation in response to ever-evolving drug resistance are critical for the clinical management of the disease.

The fact that the PI3K-mTOR pathway is deregulated in a large number of human malignancies, and its importance for different cellular functions critical for tumorigenesis, makes it an attractive drug target. Significant progress has been made in recent years in elucidating the cellular/molecular mechanism of cancer cell proliferation/survival, angiogenesis and drug resistance in regards to PI3K-mTOR pathway for cancer drug discovery. Currently, a wide range of selective PI3K inhibitors (pan-PI3K inhibitor, isoform-specific PI3K inhibitor, dual PI3K-mTOR inhibitor, and kinase mTORC1/C2 inhibitor) have been tested in preclinical studies. Based on the preclinical data several multicentre clinical trials conducted by different institutes, and participated by a large number of patients led to the knowledge that cancer in patients undergoes evolution as well as progression by selection through natural process and drug exposure. Riding on successful clinical trials, delta-isoform-specific PI3K inhibitor got the FDA approval for hematologic malignancy.

By learning everything so far about the PI3K-mTOR signaling pathway, we have learned that PI3K is much a difficult pathway to target and more so in "smart" solid tumors. Why is PI3K such a difficult target? The PI3K pathway controls several cellular functions. Firstly as a flawlessly preserved survival and pro-proliferation pathway in organisms, it is rightly connected to the extracellular growth factor signals, GPCR signals, autocrine–paracrine cytokines signals and steroid functions. As a pro-proliferation pathway, the PI3K-mTOR signals are also in a tight loop with the transcriptional signals from the RAS-RAF-MEK-ERK pathway. Secondly to continue a sustainable cell proliferation in an anabolic way, the PI3K-mTOR pathway acts as a default energy sensor and it processes major nutrient signals controlling both intracellular metabolism as well as the extracellular availability of the rate-limiting amino acids, glucose, and fatty acids. This function

of the PI3K-mTOR pathway works in unison with its role in mRNA translation/cap-dependent protein synthesis. Third as the pathway supports sustained cell proliferation, it is strategically placed to inhibit both mitochondrial and extra-mitochondrial apoptosis signals. Finally, in the face of continuous growth, the signal of the PI3K-mTOR pathway is tied to the hypoxic challenge to cells. *How does a single pathway orchestrate all of the above cellular functions flawlessly?* The answer to this riddle is ingrained not only in the multilayered horizontal and vertical connections of the PI3K-mTOR pathway to other pathways but also to the bidirectional feeding loops and cross-talk connections with the help of which the PI3K-mTOR pathway connects to relevant upstream effectors as well as downstream effector molecules. Rightfully enough the PI3K-mTOR pathway is placed upstream of the cell cycle pathway and downstream of the RAS-MAPK pathway. In an ideal PI3K-mTOR world, when a pro-growth signal is received in the PI3K-mTOR pathway it is streamlined through various independent yet interacting compartments of cells toward a seamless execution of proliferation. Thus, we think that PI3K-mTOR pathway is an enterprise by its own right controlling every aspect of cell survival and proliferation. The above logic justifies why a single dysfunctional change in any protein component of the PI3K-mTOR pathway as a result of genetic alteration(s) like PTEN loss or PIK3CA activating mutation can culminate into a tumorigenic event. *How the challenge of targeting PI3K-mTOR pathway is inbuilt to the opportunity it provides to target itself?* PI3K-mTOR pathway is primarily a cell survival pathway with immense responsibilities in the life of a cell. In the course of tumorigenesis its deregulation is caused by the alteration(s) of gene(s) whose altered protein product(s) fails to control the critical rate-limiting and regulatory nodes of the pathway. Thus, when a particular node of the pathway is disabled by the genetic alteration, the rest of its default intricate wiring becomes unstable and dysfunctional. When targeted by drugs the resistance and the toxicity ensues as a rebound phenomenon as well as a bypass mechanism. Due to the complexity of the PI3K-mTOR signaling pathway, developing an effective anticancer therapy remains a challenge. Till date, no PI3K inhibitor has been approved by FDA in solid tumors. The emerging challenges of oncology are energy metabolism, genomic instability, signaling-driven combination therapies, drug response, and drug resistance. Thus, a PTEN loss or a PIK3CA activating mutation is so easy to identify, so easy to target yet so difficult to achieve. In trying to target the PI3K-mTOR pathway what we have observed thus far is the difficulty in achieving a complete drug response. The prime question remaining in the context of the PI3K-mTOR pathway-based drug development is whether or not the efficacy of PI3K pathway inhibitors can be predicted based on the activation status of the pathway members. The mixed responses from various clinical trials to date reflect the complexity of cancers as a disease and the complexity of the PI3K-mTOR signaling network which contains multiple signaling nodes including parallel regulatory pathways and feedback loops. This multilayered complexity causes various cellular responses to the PI3K-mTOR pathway inhibitors in a context-dependent manner.

A better understanding of PI3K-mTOR pathway, its biochemistry, and its signaling network is likely to lead to better ways of targeting the pathway and defeating PI3K-mTOR driven cancers. In the era of genome-driven precision medicine in cancer, understanding the role of PI3K-mTOR signaling provides opportunities in clinical practice and challenges our collective intellect to overcome limitations of the clinical management of the disease.

Chapter 1
PI3K-Akt-mTOR Signaling in Cancer and Cancer Therapeutics

Sameer S. Chopra and Lewis C. Cantley

Introduction

Nearly three decades ago, a previously unrecognized kinase activity was identified that modifies membrane phosphatidylinositols at the D-3 position of the inositol ring and is associated with the transforming effects of the polyomavirus middle T antigen [115, 116]. The PI3K signaling pathway is now known to subserve a wide range of normal physiological functions in addition to contributing to pathophysiological states such as cancer. Perhaps most well described is the activation of PI3K by insulin receptors, which couples signals regarding systemic nutrient availability with the regulation of intracellular metabolism, growth, and proliferation. In part due to the sequencing and analysis of human cancer genomes, the PI3K-Akt-mTOR signaling pathway is also known to be commonly dysregulated in cancers arising from diverse tissues of origin. Somatic mutations and/or copy number changes have been identified in numerous genes including *PIK3CA*, *PTEN*, *Akt1*, and others, many of which have been clearly demonstrated to play important functional roles in cancer cell proliferation and survival. The ubiquity of PI3K-Akt-mTOR pathway activation in cancer has prompted significant interest in the development of small molecule inhibitors of various components of the signaling pathway. Multiple compounds are presently being evaluated in human clinical trials and may enter the armamentarium of standard cancer treatments in the future. Key translational challenges of the next decade will include the development of strategies to inhibit oncogenic PI3K-Akt-mTOR signaling while sparing

S.S. Chopra
Laboratory for Systems Pharmacology, Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA

L.C. Cantley (✉)
Sandra and Edward Meyer Cancer Center, Department of Medicine, Weill Cornell Medical College, New York, NY 10065, USA
e-mail: LCantley@med.cornell.edu

N. Dey et al. (eds.), *PI3K-mTOR in Cancer and Cancer Therapy*,
Cancer Drug Discovery and Development, DOI 10.1007/978-3-319-34211-5_1

physiologic signaling; the identification of improved treatment response biomarkers for patient selection; and the formulation of effective combinations of PI3K-directed therapies with other targeted agents, cytotoxic chemotherapy or immunotherapy to boost clinical response rates, and the durability of therapy.

The PI3K-Akt-mTOR Signaling Pathway

Of the three major classes of PI3K enzymes, class I enzymes are the most commonly dysregulated in cancer [105]. Class IA PI3K enzymes are heterodimers that include an 85, 55, or 50 kiloDalton (kDa) regulatory subunit (p85α, p55α, p50α, p85β, p55γ) and one of the three 110-kDa catalytic kinases (p110α, p110β, p110δ). Class IB enzymes are heterodimers that include a p101 regulatory subunit and the p110γ catalytic kinase. The p110 kinases are each encoded by a different gene (*PIK3CA*, *PIK3CB*, *PIK3CD*, and *PIK3CG*). The p85α, p55α, and p50α regulatory subunits are all derived from the same locus (*PIK3R1*), while p85β and p55γ are encoded by different genes (*PIK3R2* and *PIK3R3*, respectively).

Activation of PI3K is one mechanism by which extracellular cues such as growth factors and nutrients generate intracellular signals that regulate anabolic metabolism, cell cycle progression, protein synthesis, and other important aspects of normal cellular physiology [105]. PI3K signaling is commonly initiated with the binding of a mitogenic ligand to a cell-surface receptor tyrosine kinase (RTK), resulting in the phosphorylation of tyrosine residues on the intracellular C-terminus of the receptor. These phosphorylated tyrosines create binding sites for Src homology 2 (SH2) domain-containing proteins such as the p85 regulatory subunit. While p85 normally inhibits the function of the p110 kinase subunit in the cytoplasm via direct protein–protein interaction, the binding of the p85/p110 heterodimer to phosphorylated tyrosine residues induces a conformational change that results in the activation of the p110 kinase subunit. The p110 kinase modifies membrane phospholipids by catalyzing the transfer of a phosphate group from ATP to the 3 prime-hydroxyl position of phosphatidylinositol (4,5)-bisphosphate (PIP2) to produce phosphatidylinositol (3,4,5)-trisphosphate (PIP3). PIP3 acts as a second messenger by creating binding sites for pleckstrin homology (PH) domain-containing proteins, whose activation requires relocalization from the cytosol to the cell membrane. Protein kinase B (Akt), a serine/threonine kinase that was identified as the oncogene in the murine transforming retrovirus Akt8, is the most well-studied PH domain-containing protein in the PI3K signaling pathway [6]. PI3K-dependent signaling may also be transduced by PH domain-containing TEC family protein-Tyr kinases and by a subset of PH domain-containing proteins that regulate RAC and ARF family GTP binding proteins [118]. At the cell membrane, Akt is activated by phosphorylation at two key sites, threonine 308 (T308) and serine 473 (S473). While PDK1 catalyzes the transfer of a phosphate group to T308, a protein complex that includes mTOR, Rictor, GβL, and mSin1 (TORC2) phosphorylates S473 [46]. While the phosphorylation of Akt at T308 is sufficient

to increase kinase activity and propagate downstream signals, the full activation of Akt requires concomitant phosphorylation at S473 [50]. Akt has numerous downstream targets whose functions are regulated by posttranslational modification, resulting in the modulation of diverse physiological processes including metabolism, proliferation, growth, survival, and autophagy, among others [71].

Among the most important targets of Akt signaling is TORC1, a protein complex composed of mTOR kinase, Raptor, GβL, PRAS40, and Deptor. TORC1 directly regulates protein synthesis/degradation, ribosome biogenesis, *de novo* pyrimidine biosynthesis, autophagy, and cell cycle progression, among other key aspects of cellular physiology [62]. Analysis of the mTOR phosphoproteome by mass spectrometry identified substrates that also implicate mTOR signaling in regulation of RNA splicing, mRNA stability, and vesicle-mediated transport [47].

The function of TORC1 is regulated by numerous cellular and environmental cues, including both mitogenic signals (i.e., PI3K-Akt) and amino acids such as leucine, arginine, and lysine [31, 53]. Both types of inputs are ultimately required for TORC1 activation, with spatial integration occurring on the surface of the lysosome. Amino acids signal to TORC1 via Rag GTPases, which are regulated by GATOR1, GATOR2, and Sestrin2 ("Ragulator"), and the v-ATPase to promote the translocation of mTORC1 from the cytoplasm to the lysosomal surface [3, 95]. On the lysosome, mTORC1 can be activated by the Rheb GTPase. Mitogenic inputs regulate mTORC1 through the tuberous sclerosis complex (TSC), comprised of the proteins TSC1, TSC2, and TBC1D7 [31]. TSC functions as the GTPase activating protein (GAP) for Rheb, accelerating Rheb-GTP hydrolysis on the lysosome surface to the inactive Rheb-GDP state. Akt phosphorylation of TSC2 causes the dissociation of TSC from the lysosome, therein favoring the active state of Rheb (i.e. Rheb-GTP) [75]. PI3K-Akt signaling can also modulate the function of TORC1 through the direct phosphorylation of the inhibitory subunit PRAS40, causing its dissociation from mTOR kinase and augmenting mTORC1 activity [94].

The activation of PI3K-mTOR signaling is normally opposed at numerous levels. Activated RTKs are dephosphorylated by protein tyrosine phosphatases. Phosphatidylinositol second messengers at the membrane are dephosphorylated by lipid phosphatases, including PTEN, INPP4B, synaptojanin, and SHIP1/2 [15, 117]. PTEN is a dual-specificity phosphatase that selectively removes phosphate from the 3′-hydroxyl group of PI(3,4,5)P3 to produce PI(4,5)P2. Synaptojanins and SHIPs dephosphorylate the 5′-hydroxyl group of PI(3,4,5)P3 to produce PI(3,4)P2. INPP4B removes a phosphate group from the 4′ position of PI(3,4)P2 to produce PI(3)P. With some evidence suggesting that both PI(3,4,5)P3 and PI(3,4)P2 can bind to and promote activation of Akt, all three phosphatases have been shown to play important roles in terminating PI3K signaling in different contexts. The phosphorylation and activation of Akt is also countered by multiple phosphatases including PP2A, PHLPP1 and PHLPP2 [12]. Akt phosphorylation is also inhibited by carboxyl-terminal modulator protein (CTMP), which binds to the Akt carboxyl-terminal regulatory domain [70].

PI3K-Akt-mTOR Signaling in Human Physiology: Anabolic Metabolism

As a key component of the insulin signaling pathway, PI3K plays an integral role in regulating normal physiological responses to nutrient availability. Upon activation by the insulin receptor, PI3K-Akt signaling in peripheral tissues such as skeletal muscle causes increased glucose uptake by promoting translocation of GLUT4 to the cell membrane via the phosphorylation of AS160 [96]. Akt signaling also augments the expression and function of the glycolytic enzymes hexokinase II and phosphofructokinase II, the latter of which generates an allosteric activator of phosphofructokinase I [30, 89]. In the liver, Akt promotes the storage of excess glucose as glycogen by phosphorylating and inhibiting GSK3, which normally inhibits the enzymatic activity of glycogen synthase [24]. Simultaneously, Akt signaling suppresses hepatic gluconeogenesis by phosphorylating and inhibiting the transcriptional factor FOXO1 and activating the serine/threonine kinase SIK2, which phosphorylates CRTC2 (CREB Regulated Transcription Coactivator 2) and leads to its degradation [29, 79, 104]. In the fasting state, FOXO1 and CRTC2/CREB normally function to regulate the expression of gluconeogenic enzymes in the liver.

In the setting of nutrient excess, PI3K/Akt signaling also stimulates fatty acid biosynthesis and suppresses fatty acid breakdown. Citrate, which is produced in the citric acid cycle from the condensation of oxaloacetate and acetyl-CoA, can be exported from the mitochondria to the cytosol where the Akt-regulated enzyme ATP citrate lyase regenerates both metabolites [5]. Acetyl-CoA can then act as a substrate for fatty acid biosynthesis. Acetyl-CoA may also enter the isoprenoid pathway, which ultimately produces sterols, cholesterol, and the farnesyl and geranyl-geranyl moieties used to prenylate proteins. Notably, Akt also has an indirect role (i.e., via TORC1 and S6K1) in the regulation of SREBP1, a transcription factor that augments both fatty acid and cholesterol biosynthesis by regulating the expression of key enzymes in both pathways [87].

Through its regulation of TORC1, Akt also regulates protein synthesis and degradation. TORC1 phosphorylates and activates ribosomal S6 kinases and inhibits the eukaryotic initiation factor (eIF) 4E-binding proteins 4E-BP1 and 4E-BP2, which determine the availability of the mRNA cap-binding protein eIF4E [41]. TORC1 also regulates the expression of NFE2L1, which increases the transcription of genes encoding proteasome subunits, the number of intact proteasomes, and overall rates of protein degradation [122]. This newly discovered role for TORC1 is suspected to both serve as a quality control mechanism and contribute to maintaining adequate supplies of free intracellular amino acids for new protein synthesis. However, it is yet unclear how these functions are physiologically coordinated with the role of TORC1 in inhibiting protein and organelle catabolism via autophagy, through the phosphorylation of ULK1/2 and ATG13 [54].

Concurrent with increasing the activity of anabolic pathways and new protein synthesis, PI3K/Akt signaling also inhibits the β-oxidation of fatty acids in the

mitochondria. This is achieved by suppressing the expression of carnitine palmitoyltransferase IA (CPT1A), an enzyme located in the mitochondrial outer membrane that catalyzes the transfer of an acyl group from long-chain fatty acyl-CoA to carnitine, generating an acylcarnitine that can be transported from the cytosol into the mitochondrial matrix [28]. Finally, Akt directly inhibits autophagy via phosphorylation of Beclin1 and indirectly inhibits autophagy via its regulation of TORC1, as discussed above [113].

Although Akt is the major mediator of PI3K-dependent regulation of metabolism, recent studies have shown that PI3K-dependent activation of Rac also contributes to regulation of glycolysis. Several enzymes involved in glycolysis, most notably aldolase, are sequestered in inactive states on f-actin and Rac-dependent severing of f-actin releases these enzymes and thereby accelerates glycolysis [48].

PI3K-Akt-mTOR Signaling in Human Physiology: Regulation of Cell Cycle Progression and Survival

In addition to its role in anabolic metabolism, PI3K-Akt-mTOR signaling promotes proliferation by phosphorylating and inhibiting multiple negative regulators of cell cycle progression at the transition from G1 to S phase [64]. These effects ultimately result in increased phosphorylation and inhibition of the retinoblastoma protein (Rb) by cyclin-dependent kinase 4/6 (CDK4/6), which in turn permits E2F-mediated transcription of genes that promote S-phase entry. Several studies indicate that PI3K/Akt signaling activity can augment the levels of cyclin D1, a protein whose primary function is to bind and activate CDK4/6. This may occur by Akt-mediated phosphorylation and inhibition of GSK3β, which was shown to phosphorylate cyclin D1 at threonine 286 and promote its nuclear export, ubiquitination, and proteasome-mediated degradation in the cytoplasm. Akt-mediated phosphorylation and inhibition of GSK3β therefore may result in the accumulation of nuclear cyclin D1. However, the relationship between Akt, GSK3β, and cyclin D1 levels may be context dependent. Others have found that while PI3K inhibition does indeed diminish cyclin D1 levels in G1 and G2, this effect may not require inhibition of GSK3β [119].

PI3K-Akt signaling was also found to regulate cell cycle progression via TORC1. Progression from G1 to S phase was found to require both TORC1-mediated activation of S6K1 signaling and inhibition of 4E-BP1 [38]. Translation of messenger RNAs encoding cyclin D and cyclin E appears to be sensitive to the activity of eIF4E [1]. In addition to these TORC1-mediated effects, TORC2 was also found to independently augment cyclin D1 stability via a mechanism that requires the ubiquitin E3 ligase FBX4 [59]. This newly identified role for TORC2 appears to be independent of its known contribution to the activation of Akt, which as discussed above, was previously shown to regulate cyclin D1 levels via phosphorylation and inhibition of GSK3β.

Akt may also promote the G1- to S-phase transition by regulating the activity of proteins that normally act to inhibit cell cycle progression. Akt-mediated phosphorylation of the nuclear localization signal of $p27^{Kip1}$ (*CDKN1B*), an inhibitor of cyclin–cyclin-dependent kinase (CDK) complexes including cyclin D-CDK4 and cyclin E-CDK2, causes its cytoplasmic sequestration [65]. Akt also phosphorylates and inhibits $p21^{Cip1/Waf1}$, a second inhibitor of cyclin-CDK complexes whose expression is regulated by the p53 tumor suppressor [124]. Akt phosphorylation of p21 disrupts its inhibitory interaction with PCNA, thereby accelerating DNA replication. Similar to p27, phosphorylation of p21 by Akt also promotes a change in subcellular localization from the nucleus to the cytoplasm. These effects of Akt on cell cycle progression are reinforced by its phosphorylation and inhibition of forkhead box (FOXO) transcription factors, which have been shown to regulate not only the expression of p21 and p27 but also members of a second class of cell cycle inhibitors, p15 (*INK4B*) and p19 (*INK4D*) [11, 33, 74, 106].

The antiapoptotic effects of Akt signaling arise from several mechanisms including phosphorylation of Bcl-2-associated death promoter (BAD), Mouse double-minute 2 homolog (MDM2), and Forkhead box O3 (FOXO3) proteins [13, 16, 26, 73]. BAD is a proapoptotic, BH3-only family member that binds to and inhibits the prosurvival proteins Bcl-2 and Bcl-xL and is itself inhibited by Akt. MDM2 is a negative regulator of the p53 tumor suppressor that both represses p53-mediated transcriptional activity and acts as an E3 ubiquitin-protein ligase targeting p53 to the proteasome for degradation; phosphorylation of MDM2 by Akt augments this process. Caspase-9 is an intracellular protease inhibited by Akt that cleaves caspase-3 and is activated upon Apaf-1/cytochrome c release from the mitochondria during apoptosis. FOXO3 has been shown in various contexts to upregulate the expression of proapoptotic proteins including Bim, FasL, and PUMA [40, 121]. Akt regulates the activity of FOXO3 by altering its subcellular localization; when phosphorylated in the nucleus, FOXO3 binds 14-3-3 proteins and the complex is exported from the nucleus to the cytoplasm where FOXO3 is unable to regulate gene expression [14, 32] Additional mechanisms of apoptosis suppression have also been described. In primary chronic lymphocytic leukemia (CLL) B cells, introduction of a constitutively active Akt resulted in increased expression of Mcl-1, XIAP, and Bcl-xL proteins, all of which act to inhibit apoptosis [68]. In rodent fibroblast (Rat-1) cells, Akt inhibits p53 through the inhibition of GSK3; GSK3 was found to phosphorylate and activate the histone acetyltransferase TIP60, which in turn acetylates and activates p53 [80].

mTOR kinase activity may also promote cell survival. In the *Eμ-myc* murine lymphoma model, the level of the antiapoptotic bcl-2 family member Mcl-1 appears to be sensitive to inhibition of TORC1-regulated translation [76]. TORC2 was also found to promote the stability of Mcl-1; knockdown of rictor or pharmacologic inhibition of TORC2 resulted in the degradation of Mcl-1 via a pathway requiring GSK3 and the SCF-FBXW7 complex [60].

PI3K-Akt-mTOR in Human Disease: Germline Disorders of Overgrowth and Cancer Susceptibility

The critical roles of PI3K-Akt-mTOR signaling in regulating normal metabolism, growth, proliferation, and survival are underscored by the discovery of mutations in genes encoding key pathway regulators in human disease. In the germline, rare mutations that dysregulate PI3K-Akt-mTOR signaling cause several well-described, autosomal dominant disorders of growth and cancer susceptibility. Inherited loss-of-function mutations in the tumor suppressor and lipid phosphatase *PTEN* cause both Cowden syndrome (CS) and Bannayan–Riley–Ruvalcaba syndrome (BRRS) [44]. Patients with CS develop both benign and malignant tumors of the breast, thyroid, and endometrium, and have an 85 % lifetime risk of developing invasive breast cancer [103]. Germline *PTEN* mutations also appear to increase the lifetime risk for renal cell carcinoma (RCC), colorectal cancer, and melanoma [103]. Adult-onset Lhermitte–Duclos disease, or dysplastic gangliocytoma of the cerebellum, is also a feature of CS. Individuals with CS may additionally exhibit macrocephaly, autism, and a variety of benign cutaneous neoplasms including trichilemmomas, acral keratoses, and papillomatous papules. More recently, subsets of patients with CS were found to have germline mutations in *PIK3CA* and *Akt1* [86]. BRRS, a second congenital disorder that arises from inherited *PTEN* mutations, presents with macrocephaly, hamartomatous polyps of the gastrointestinal tract, and distinctive pigmented macules of the glans penis [44]. The term "hamartomatous" refers to benign growths that contain the normal cellular components of the tissue of origin but markedly abnormal architecture.

TSC is another germline overgrowth syndrome caused by dysregulated PI3K-Akt-mTOR signaling [23]. Inherited in an autosomal dominant manner, TSC is caused by loss-of-function mutations in the tumor suppressor genes *TSC1* (*hamartin*) and *TSC2* (*tuberin*) that result in increased activity of mTOR kinase. Clinically, TSC presents with benign tumors of multiple organ systems including the skin, central nervous system, kidney, and lungs. Cutaneous manifestations are found in nearly all individuals with TSC and include angiofibromas, shagreen patches, hypomelanotic macules, and periungual fibromas. Tumors of the central nervous system, the major source of morbidity in TSC patients, include cortical tubers, subependymal nodules, and subependymal giant cell astrocytomas (SEGAs). In the kidney, patients with TSC develop benign angiomyolipomas, epithelial cysts, and oncocytomas. In the heart and lungs, TSC causes rhabdomyomas and lymphangioleiomyomatosis (LAM), respectively. While noninvasive, these tumors cause significant morbidity by disrupting normal organ function, inducing seizures, arrhythmias, and other consequences. Although rare, malignant angiomyolipomas and RCC are associated with TSC.

Inherited genetic variation in PI3K signaling may also adversely affect the immune system. Germline activating mutations in *PIK3CD* encoding the p110 PI3K delta isoform have been described in autosomal dominant disorders of human immunity [69]. Patients with such mutations were found to have deficiencies of

naïve T cells but an excess of senescent T effector cells. Ex vivo, these cells were found to have increased activation of both Akt and mTOR.

PI3K-Akt-mTOR in Human Disease: Somatic Genetic Mutations in Syndromes of Segmental Overgrowth and Cancer

Somatic genetic dysregulation of PI3K-Akt-mTOR signaling is now well established as a cause of both rare clinical disorders as well as more common conditions such as cancer. The Proteus syndrome (PS) presents with progressive, disfiguring, segmental overgrowth of skin, connective tissue, nerve, and other tissues derived from all germ layers. PS is primarily caused by somatic mosaicism involving gain-of-function mutations in *Akt1* [66]. Somatic mosaicism of activating mutations in *PIK3CA* are found in a similar segmental overgrowth syndrome, congenital lipomatous asymmetric overgrowth of the trunk, lymphatic, capillary, venous, and combined-type vascular malformations, epidermal nevi, skeletal, and spinal anomalies (CLOVES) [61]. Somatic dysregulation of *PIK3CA*, *Akt3,* and *MTOR* have also been identified in a spectrum of congenital brain malformations ranging from hemimegalencephaly to focal cortical dysplasia [52, 63, 88]. Clinically, these conditions typically present with intractable seizures in children.

In part due to large-scale next-generation sequencing projects such as the cancer genome atlas (TCGA), somatic alterations in genes encoding various components of the PI3K-Akt-mTOR signaling pathway have been commonly observed in cancers of diverse tissues of origin. Mutations in *PIK3CA* were first identified into occur in a high frequency of colon cancers but not in premalignant colorectal tumors, suggesting that these genetic alterations arise late in tumorigenesis in parallel with the development of invasiveness [93]. PI3K-Akt-mTOR signaling can be constitutively activated as the result of a spectrum of different types of somatic changes identified in cancer genomes. These mechanisms include amplification of RTK genes such as *HER2* in breast cancer; activating mutations and copy number changes in the gene encoding the PI3K p110 alpha subunit *PIK3CA*; inactivating mutations in the p85 alpha regulatory subunit gene *PIK3R1*; inactivating mutations in the genes encoding the lipid phosphatases *PTEN* and *INPP4B*, which dephosphorylate phosphotidylinositol second messengers; activating mutations in the serine/threonine kinase genes *Akt1-3*; inactivating mutations in the genes encoding mTOR regulators such as *TSC1* and *TSC2*; and activating mutations in *MTOR* itself, among other changes [36, 92, 101]. PI3K p110β, p110γ, and p110δ isoforms have also been shown to be oncogenic as wild-type proteins [56]. With an abundance of basic evidence supporting roles for PI3K-Akt-mTOR in the regulation of proliferation, growth, survival, and metabolism, such genetic changes are widely considered to be “driver” rather than “passenger” mutations when identified in cancer genomes. As further evidence of functional importance, specific mutations

in the helical or kinase domains of *PIK3CA* appear across tumors with relatively high frequency. Such genetic alterations are sufficient to transform chick embryo fibroblasts in vitro, albeit by different mechanisms [2, 123]. PI3K signaling can also be induced as a consequence of other known driver mutations. In mouse models, for example, PI3K signaling was found to be required for the transforming effects of oncogenic mutations in *Ras*, the small GTPase that is dysregulated in a number of different cancers [35, 42].

Targeting Oncogenic PI3K-Akt-mTOR Signaling for Cancer Therapy

The prevalence of PI3K-Akt-mTOR pathway mutations in cancer, as well as abundant evidence supporting a pro-tumorigenic role for many of these genetic events, has generated intense interest in the development of novel targeted therapeutics. Over 30 compounds directed against different proteins in the pathway have undergone varying degrees of clinical evaluation over the past decade. The rapalogs, which are allosteric inhibitors of TORC1, were among the first drugs targeting the pathway to enter clinical trials for the treatment of cancer. Temsirolimus was approved by the FDA in 2007 as a first-line treatment of advanced RCC after demonstrating an overall survival benefit compared to interferon alpha in a large phase III clinical trial. Everolimus is currently FDA approved as a treatment for advanced hormone receptor-positive breast cancer in combination with exemestane, a steroidal aromatase inhibitor. Everolimus is also approved for advanced RCC after progression on either sunitinib or sorafenib, and for the treatment of progressive pancreatic neuroendocrine tumors (PNET). Despite these important milestones, the clinical activity of rapalogs for the treatment of more common cancers has been modest. With the hope that targeting other proteins in the pathway with improved drug molecules might boost clinical response rates, a large collection of inhibitors of PI3K-Akt-mTOR signaling have been developed and entered clinical trials over the past five to seven years. Among the earliest compounds to enter clinical development were the derivatives of wortmannin, a steroid fungal metabolite that was the first described inhibitor of PI3K. Like wortmannin, the "pan" PI3K inhibitors antagonize all of the individual PI3K kinase isoforms to varying degrees. In phase I/II trials, these agents commonly caused hyperglycemia, rash, nausea, vomiting, diarrhea, stomatitis, and anorexia, among other adverse effects, but were nonetheless reasonably well tolerated compared to chemotherapy. These agents are currently being studied alone or in combination with other therapies in a number of different clinical settings including breast cancer, head and neck cancer, glioblastoma, non-small cell lung cancer, endometrial cancer, gastrointestinal stromal cancer, RCC, prostate cancer, and others. The pan-PI3K inhibitor BKM120 (Buparlisib) has advanced the furthest in trials and is now being

studied in combination with fulvestrant in a phase III randomized, double-blind clinical trial (BELLE-3) for women with hormone-positive breast cancer who were previously treated with an aromatase inhibitor and subsequently progressed on the combination of exemestane and everolimus or equivalent.

Emerging from the class of pan-PI3K isoform inhibitors was a group of small molecules discovered to also directly inhibit mTOR kinase due to shared similarities in the ATP binding site of PI3Ks and mTOR. This class of dual inhibitors includes the molecules PI-103, GDC0980, and BEZ235. These compounds inhibit not only multiple PI3K p110 isoforms but also the TORC1 and TORC2 complexes. Inhibition of the active site of mTOR kinase was found to more completely inhibit TORC1 signaling than allosteric inhibitors such as rapamycin and everolimus, which do not effectively inhibit 4eBP1 phosphorylation and cap-dependent translation. Direct inhibition of mTOR kinase also inhibits the activity of TORC2, which prevents the full activation of Akt and may independently regulate the stability of cyclin D1, among other favorable antitumorigenic properties. In phase I clinical trials, the toxicity profiles for dual PI3K-mTOR inhibitors appeared similar to pan-isoform PI3K inhibitors. Selective inhibitors of mTOR kinase were also developed and referred to as "TORKinhibs" to distinguish them from the rapalogs [37, 72]. A number of these molecules such as AZD8055 and AZD2014 were previously studied in clinical trials. The drug MLN0128 is presently undergoing evaluation for the treatment of a range of different cancers including anaplastic thyroid cancer, merkel cell carcinoma, and hepatocellular carcinoma.

In part to improve the therapeutic window for targeting PI3K and thereby facilitate dosing schedules that result in more complete pathway inhibition, isoform-specific PI3K inhibitors have also been developed [21, 105]. While PI3K p110 alpha and beta kinases and mTOR are ubiquitously expressed, the PI3K delta and gamma isoforms are primarily expressed in hematopoietic cells. In genetically engineered mouse models, knockout of *Pik3ca* or *Pik3cb* are embryonic lethal at E10.5 and E3.5, respectively. Germline inactivation of *Pik3cd* or *Pik3cg* do not affect viability but compromise immune cells; loss of p110 delta impairs the normal function of lymphocytes, neutrophils, and mast cells, and loss of p110 gamma perturbs T cell development and activation as well as the migration of neutrophils and macrophages.

PI3K p110 alpha-selective inhibitors such as BYL719 are being studied in patients whose tumors harbor *PIK3CA* activating mutations and in other settings where preclinical data have demonstrated a preferential functional role for the p110 alpha kinase. Several studies suggest that the subset of breast cancers that harbor *HER2* amplification preferentially signal through the PI3K p110 alpha subunit. BYL719 is therefore being studied in women with *HER2*-amplified breast cancer who have progressed after prior trastuzumab and taxane chemotherapy.

Agents that preferentially inhibit the p110 beta kinase have also been developed. Several preclinical studies presented evidence that the loss of *PTEN* might preferentially activate PI3K signaling via the p110 beta subunit, especially in the setting of prostate cancer [108, 114]. The mechanistic basis of this association has not been fully elucidated. Moreover, a study of 422 cell lines in vitro demonstrated only a

modest association between *PTEN* mutation and sensitivity to selective pharmacologic p110 beta inhibition: drug sensitivity was observed in only 35 % of cell lines harboring *PTEN* mutations but also in 16 % of cell lines with wild-type *PTEN* [81]. Emerging evidence suggests that the genetic context in which *PTEN* loss occurs and the tissue of origin may strongly influence the functional importance of PI3K p110 alpha and beta isoforms [8, 97, 112]. Selective inhibitors of the p110 beta isoform including AZD8186 have entered clinical trials and are being studied as monotherapy in advanced castrate-resistant prostate cancer, squamous non-small cell lung cancer, triple-negative breast cancer and other solid tumors with PTEN deficiency and/or mutation/amplification of *PIK3CB*. The p110 beta inhibitor GSK2636771 is similarly being studied in prostate cancer but is also being evaluated in the treatment of advanced gastric adenocarcinoma with PTEN deficiency.

The most successful example of selective PI3K isoform targeting exploited the relatively restricted expression of *PIK3CD* and the important functional role of the PI3K p110 delta isoform in B-lymphocytes [85, 120]. Idelalisib (formerly CAL-101) is the first PI3K inhibitor of any type to be approved for clinical use by the FDA and European regulatory agencies. PI3K p110 delta plays a critical role in transducing intracellular signals from the B cell receptor and cytokine receptors to promote lymphocyte survival, proliferation, and chemokine secretion. Moreover, pathologic PI3K-Akt signaling mediated by p110 delta has been identified in a number B cell malignancies such as CLL. In the US, Idelalisib was granted full approval for the treatment of recurrent CLL in conjunction with rituximab. The drug is relatively well tolerated and can be given even to elderly patients who cannot tolerate chemotherapy. Idelalisib also received accelerated approved as a third-line systemic treatment for two types of recurrent indolent non-Hodgkin lymphoma, follicular B cell non-Hodgkin lymphoma and small lymphocytic lymphoma. Confirmatory trials are presently being conducted for full approval. A novel PI3K delta/gamma dual inhibitor (IPI-145, Duvelisib) has also entered phase III clinical trials for CLL and is similarly being studied for activity in follicular lymphoma, T cell lymphoma, SLL, and marginal zone lymphoma [84].

Clinical Responses to PI3K-Akt-mTOR Inhibitors

Contrary to the expectations of many in the field, PI3K-Akt-mTOR inhibitors used as monotherapy have provided only modest benefits to patients with most solid tumors, with objective response rates in clinical trials often falling below 10 % [39, 90]. In the era of precision oncology, the efficacy of PI3K-Akt-mTOR inhibitors in patients preselected based on tumor genotype has not matched the response rates of other molecular therapies that have become the standard of care in clinical oncology. These treatments include vemurafenib (and more recently the combination of dabrafenib/trametinib) for *BRAF* V600E-mutant melanoma, EGFR inhibitors for *EGFR*-mutant NSCLC, crizotinib for *ALK*- and *ROS*-rearranged NSCLC, the combination of trastuzumab/pertuzumab for *HER2*-amplified breast cancer, and

various forms of hormone therapy for prostate and breast cancer, respectively. Collectively, these therapies have relatively high clinical response rates when administered to patients whose tumors harbor specific molecular/genetic biomarkers. Moreover, phase III clinical trials have demonstrated measurable improvements in overall survival.

In addition to low clinical response rates for the drugs evaluated to date, the field of PI3K-Akt-mTOR therapeutics has been challenged to identify patients who are most likely to benefit from therapy. In multiple completed clinical trials, genetic biomarkers such as *PIK3CA* mutation and *PTEN* deletion have limited utility as treatment response biomarkers. In the phase I trial of the pan-PI3K inhibitor BKM120, the presence of tumor molecular status (i.e. *PIK3CA* and/or *PTEN* alterations) did not predict clinical benefit in the patients who demonstrated partial responses [7]. Similarly, in a phase I trial of the dual PI3K/mTOR inhibitor GSK458, durable responses were observed across several different tumor types (overall response rate 5 %) but there was no significant association between the presence of *PIK3CA* mutations and the likelihood of response [77]. In a clinical trial studying the efficacy of PI3K-Akt-mTOR inhibitors for patients with breast, cervical, endometrial, or ovarian cancers whose tumors harbor *PIK3CA* mutations, a partial response was observed in only 30 % of patients [51]. However, approximately 10 % of patients with wild-type *PIK3CA* also responded to treatment. A recent study of archival tumor specimens from BOLERO-2 demonstrated that progression-free survival benefit from the addition of everolimus to exemestane was present regardless of the presence of *PIK3CA* alterations [45]. When taken together, these results from clinical trials of PI3K-Akt-mTOR therapeutics suggest that tumor mutational status is uncommonly a faithful predictor of drug response. Similar results are apparent when one considers PI3K pathway activation as a biomarker of response to other therapies. It was hypothesized, for example, that *PTEN* deletion and/or *PIK3CA* mutation would identify patients who would not benefit from anti-HER2 directed therapies, as the PI3K signaling pathway is "downstream" from this RTK. In vitro, both types of genetic alterations were found to confer resistance to trastuzumab [9]. However, conflicting data have emerged from studies in patients. Analysis of 429 biopsies of patients treated in the neoadjuvant setting with trastuzumab, lapatinib, or the combination in addition to taxane chemotherapy (Neo-ALTTO trial) found that neither *PIK3CA* mutation nor *PTEN* deficiency was predictive of any meaningful measure of clinical efficacy [82]. In contrast, *PIK3CA* mutations in other patient cohorts (GeparQuatrro, GeparQuinto, GeparSixto trials) were indeed found to be associated with lower rates of pathologic complete response to neoadjuvant anthracycline/taxane chemotherapy with anti-HER2 directed therapy [67]. These conflicting results are difficult to interpret. The future success of PI3K-pathway directed therapy might in part depend on the successful identification of novel biomarkers—mutational, transcriptional, protein-based, or combinations of these—that are more predictive of treatment response. Adding a layer of complexity, response biomarkers may ultimately be found to be specific to certain classes of drugs (i.e., pan-PI3K inhibitor,

PI3K isoform-selective inhibitor, PI3K/mTOR inhibitor) or tumors (i.e., *PTEN*-deficient endometrial versus triple-negative breast cancer).

Putative Mechanisms Underlying Suboptimal Drug Responses: Incomplete Pathway Suppression

The discordance between extensive preclinical evidence supporting a pathological role for PI3K-Akt-mTOR signaling in cancer and the objective performance of this class of therapeutics in clinical trials merits further analysis. One hypothesis is that the importance of PI3K-Akt-mTOR signaling pathway activity in normal tissues, coupled with the lack of mutant protein-specific inhibitors, limits the therapeutic window for targeting this pathway for cancer therapy. In other words, suppression of the pathway to the extent required for sustained tumor regression in vivo simply may not be achievable in most patients for many of the current agents administered at their maximum tolerated doses. A rigorous test of this hypothesis will require detailed analysis of on-treatment biopsies from patients in clinical trials of PI3K-Akt-mTOR inhibitors, which were not routinely performed in the trials that have been completed and reported to date. Although serial on-treatment biopsies are often difficult to obtain and may place patients at risk, such data would improve our understanding of how varying degrees of pathway suppression over time relate to tumor response. At present, it is unclear how this relationship varies among patients and among tumors of different tissues of origin. While response biomarkers such as ribosomal S6 phosphorylation in skin biopsies or hyperglycemia/insulin/C-peptide levels are useful measures of target engagement, it uncertain to what extent these surrogates reflect posttreatment changes in PI3K-Akt-mTOR signaling in actual tumors.

Such on-treatment studies will be particularly important for newer agents in clinical trials such as PI3K p110 isoform-selective inhibitors. While there is optimism that these molecules will have a greater therapeutic window than pan-PI3K inhibitors or dual PI3K/mTOR inhibitors, it is yet unclear whether the restricted pharmacology of these agents will lead to improved clinical response rates. In one recent study, for example, *de novo* resistance of *PIK3CA*-mutant breast cancers to the PI3K p110 alpha-selective inhibitor BYL719 appeared to result from incomplete suppression of mTOR activity [34]. Therapeutic effectiveness was improved with the addition of the allosteric mTOR inhibitor RAD001.

Putative Mechanisms Underlying Suboptimal Drug Responses: PI3K Inhibitors Cause Hyperglycemia and Hyperinsulinemia

A related hypothesis is that interpatient variability in treatment response may in part arise from differential metabolic responses to drug therapy. PI3K p110 alpha inhibitors and pan PI3K inhibitors cause dramatic increases in serum insulin and can also cause elevation in serum IGF1 and/or suppress IGF1 binding proteins. These changes are physiological consequences of prolonged elevation in serum glucose due to suppression of insulin signaling in muscle, fat and liver. Since many solid tumors express high levels of insulin receptor or IGF1 receptor or both, the elevation in these growth factors may cause activation of PI3K in the tumor, countering the effects of the drugs. It is possible and plausible that variable responses to PI3K p110 alpha and pan PI3K inhibitors among patients whose tumors harbor similar pathway-activating mutations result from differences in the elevation of serum insulin and/or IGF1 during therapy. Although metformin is typically given to reduce serum glucose during therapy, serum insulin and IGF1 levels are not routinely monitored. Patients who consume sugary drinks and sugar-laden foods during therapy are likely to have extremely high levels of insulin and IGF1 while those on ketogenic diets are likely to have very low levels of these hormones. Of course, if drugs are developed that only target mutant forms of p110 alpha, the elevation in serum glucose and insulin would not occur and therapy would be predicted to be more effective.

Putative Mechanisms Underlying Suboptimal Drug Responses: Drug-Induced Signaling Adaptation and Feedback

Another emerging hypothesis is that interpatient variability in treatment response may arise from differences in how cancer cells respond to drug perturbation. A number of preclinical studies have uncovered adaptive phenomena in cancer cells that may act to counter the intended effects of targeted drug therapy. These studies collectively suggest that signaling pathways in cancer cells are commonly resilient to the perturbation of individual proteins. Such findings have been associated with sensitivity/resistance to targeted therapeutics in preclinical studies but are more difficult to assess in the context of actual human clinical trials, where the ability to monitor dynamic signaling responses in tumors remains challenging. Nonetheless, such discoveries may give rise to combinatorial strategies that more completely and durably inhibit oncogenic signaling networks and may be vetted in vitro and in animal models of human disease such as patient-derived xenografts.

Within the PI3K-Akt-mTOR signaling network, a number of feedback mechanisms elicited by targeted therapeutics have now been well defined. Pharmacologic inhibition of TORC1 causes increased signaling through RTKs by at least 2 known mechanisms. The first is mediated by S6 kinase, whose activation by TORC1 normally results in feedback inhibition of insulin and insulin-like growth factor receptor signaling through the phosphorylation and degradation of IRS-1, a substrate of the insulin receptor (IR) [43, 83, 100, 109, 111]. Pharmacologic inhibition of TORC1 by rapamycin results in decreased activity of S6 kinase, reduced phosphorylation/inhibition of IRS-1, and increased activation of PI3K and ERK through RTK signaling [17, 109]. TORC1 signaling also directly phosphorylates and stabilizes Grb10, which results in inhibition of growth factor signaling [47]. Pharmacologic inhibition of TORC1 therefore results in the loss of Grb10-mediated inhibition and enhanced signaling through RTKs. These feedback mechanisms are hypothesized to contribute to the modest clinical efficacy of rapalogs as anticancer therapeutics. However, pertinent to the question of how signaling networks might vary in their organization across different tissues, rapalogs demonstrate significant clinical benefit in patients with advanced RCC and are FDA approved for this indication. It is unclear whether the strength of feedback activation of RTKs is less marked in RCC than in other cancer types.

A number of additional drugs targeting the PI3K-Akt-mTOR signaling pathway induce various forms of feedback adaptation through similar mechanisms. Inhibitors of mTOR kinase such as AZD8055 were found to have a biphasic effect with respect to Akt signaling [91]. Inhibition of TORC2 results in dephosphorylation of Akt at serine 473 and transient inhibition of phosphorylation at Akt threonine 308. However, because TORC1 inhibition relieves negative feedback of multiple RTKs, PI3K is reactivated in the presence of drug and causes increased phosphorylation of Akt at T308. This partial activation state of Akt was found to be sufficient to signal to effector proteins in the pathway. Complete inhibition of Akt signaling and maximal cell death and tumor regression in vivo required both inhibition of mTOR kinase (TORC1/2) as well as induced RTKs.

In *HER2*-amplified breast cancer, inhibition of PI3K was found to cause increased *HER3* expression, ERBB receptor dimerization and phosphorylation, binding of adaptor molecules, ultimately activation of the MAPK signaling pathway [99]. In this setting, combined administration of ERBB2 antagonists, PI3K inhibitors, and MEK inhibitors were found to be more efficacious than any individual treatment alone. A second study similarly revealed that inhibition of PI3K signaling in *HER2*-amplified breast cancers resulted in increased expression of *HER3*, *InsR*, *IGF1R*, and *FGFR2* mRNA, and that these changes could be ameliorated by knockdown of FOXO1 and FOXO3a transcription factors [19]. Co-drugging with a PI3K inhibitor and trastuzumab or lapatinib was synergistic in established xenografts. Additional studies confirmed that an Akt-FOXO signaling axis might more broadly mediate feedback activation following inhibition of PI3K signaling. Direct pharmacologic antagonism of Akt was found to induce the expression and/or phosphorylation of multiple RTKs including EGFR, HER3, HER4, IGF-1R, and IR due to the combined effects of TORC1 inhibition and the activation of forkhead (FOXO) transcription

factors [20]. The latter mechanism was found to regulate the transcription of *HER3*, *IGF-1R*, and *IR* and in all tissue lineages evaluated including breast, prostate, ovary, lung, and melanoma. In xenografts, these effects resulted in a small recovery of Akt activation but a more significant augmentation of MAPK signaling. Combined inhibition of Akt and ERBB receptors was found to be effective in vivo. Activation of adaptive Akt-FOXO signaling demonstrates cell-to-cell variability: in ovarian cancer spheroids, dual inhibition of PI3K/mTOR lead to death of inner matrix-deprived cells while matrix-attached cells were resistant to the effects of therapy by FOXO–regulated transcription of RTKs and other prosurvival factors [78].

Inhibition of PI3K by isoform-selective drugs may also result in feedback-mediated signal reactivation. Pharmacologic antagonism of PI3K p110 beta in a *PTEN*-deficient model of prostate cancer led only to transient inhibition of PI3K signaling, with rebound signaling to Akt mediated by relief of negative feedback of IGF-IR and other RTKs that primarily signal through the PI3K p110 alpha subunit [98]. Previously it was found that antagonism of PI3K in prostate cancer causes the activation of androgen receptors [18]; in this study, combined antagonism of PI3K p110 alpha, p110 beta, and the androgen receptor resulted in greater tumor regressions than either treatment alone. Similarly, it was previously found that combining inhibitors of PI3K p110 alpha and beta with endocrine therapy was highly effective for hormone-positive breast cancer [25]. Finally, selective inhibition of PI3K p110 alpha was found to only transiently reduce the PIP3s messenger in several *PIK3CA* mutant luminal and *HER2*-amplified breast cancer models due to a rebound in signaling at the membrane mediated by PI3K p110 beta [22]. The combination of selective PI3K p110 alpha and beta inhibitors prevented this rebound and produced greater efficacy than either treatment alone in breast cancer models in vitro and in vivo.

Future Directions: Identifying Effective Drug Combinations

While compounds targeting mutant PI3K or mutant Akt might have a broader therapeutic window than the drugs that are presently undergoing clinical evaluation, it is unlikely that inhibiting these enzymes alone will be sufficient to cure patients: rational drug combinations may prove more efficacious. Historically, anti-HER2 directed therapy followed a similar path in development. In the pivotal phase II trial of trastuzumab for the treatment of *HER2*-amplified metastatic breast cancer, the intention-to-treat clinical response rate was 15 % [4]. Response rates in this subset of breast cancer increased when trastuzumab was combined with effective chemotherapy. More recently, the combination of dual-HER2 blockade with trastuzumab and pertuzumab, when combined with taxane chemotherapy, became the new standard of care for metastatic *HER2*-amplified breast cancer. When used as adjuvant therapy following surgery, the combination of trastuzumab and chemotherapy has proven to be extremely effective in preventing recurrence, and therefore is considered curative for patients with early stage *HER2*-amplified breast cancers.

Beyond targeting the specific signaling mechanisms that mediate feedback activation, several preclinical studies have identified novel strategies for co-drugging inspired by an improved understanding of how cells respond to PI3K inhibition. With the availability of a broad armamentarium of targeted therapies, some of the most promising combinatorial strategies can be rapidly translated into clinical trials. In hormone-driven cancers, for example, crosstalk was observed between PI3K signaling and hormone receptor-mediated transcriptional activity. In prostate cancer, antagonism of PI3K results in increased androgen receptor (AR) stability and activity and increased expression of AR-regulated genes [18]. In hormone-positive breast cancer, antagonism of PI3K signaling was analogously observed to augment estrogen receptor activity [10]. In each case, concomitant administration of PI3K inhibitors with the appropriate endocrine therapy resulted in greater antitumor activity than the individual treatments administered alone.

PI3K signaling was also found to contribute to the regulation of DNA repair. In *BRCA*-mutant breast cancer mouse model and cell lines, PI3K inhibition increased markers of DNA damage such as poly-ADP-ribosylation and γH2AX and perturbed the recruitment of RAD51 to DNA damage foci [55]. Inhibitors of PARP had previously demonstrated efficacy in *BRCA*-mutant breast cancers. In this study, the combination of the PARP inhibitor olaparib and the pan-PI3K inhibitor BKM120 was significantly more effective than either agent alone. A second study also revealed enhanced benefit from the sequential use of PI3K and PARP inhibitors in *BRCA1*-mutant breast cancer cell lines [58]. Intriguingly, in TNBC tumor models with wild-type *BRCA1*, dual PI3K/mTOR inhibition with GDC-0980 also increased DNA damage and sensitized cells to the combination of a PARP inhibitor (ABT888) and carboplatin [27]. PI3K inhibition was found to increase DNA damage response signaling in TNBC cell lines with wild-type *BRCA1* by regulating the expression of *BRCA1* and *BRCA2*, thereby sensitizing cell lines to PARP inhibition [49]. The downregulation of *BRCA1/2* in this study appeared to be secondary to increased MAPK signaling and activation of the ETS1 transcription factor. These findings have led to an ongoing clinical trial studying the combination of the pan-PI3K inhibitor BKM120 or the p110 alpha-selective inhibitor BYL719 with the PARP inhibitor olaparib in patients with recurrent triple-negative breast cancer or high grade serous ovarian cancer.

Antagonism of transcriptional regulators may also act synergistically with PI3K inhibitors. In a model of metastatic breast cancer driven by PI3K and MYC, adding an inhibitor of BET bromodomains to PI3K inhibition resulted in greater cell death and tumor regression [102]. In this model, BET bromodomain inhibition was found to cause the dissociation of the transcriptional coactivator BRD4 from chromatin at regions that regulate the expression of both insulin and ERBB receptor tyrosine kinases, thereby interrupting a critical feedback circuit that normally results in reactivation of signaling after inhibition of PI3K. Synergy between PI3K and BET bromodomain inhibition was also observed in cell line models of hematologic malignancies including acute myeloid leukemia, T-cell acute lymphoblastic leukemia, multiple myeloma, and Burkitt lymphoma [107]. The combination of a dual

mTOR inhibitor and a BET bromodomain inhibitor was also found to be highly effective in merkel cell carcinoma, and aggressive neuroendocrine skin cancer [57].

Lastly, the co-targeting of PI3K and cell cycle regulators also appears to enhance efficacy over either agent used alone. In a combinatorial drug screen performed on multiple *PIK3CA* mutant cancers with relative insensitivity to PI3K inhibitors, the combination of PI3K p110 alpha (BYL719) and CDK4/6 inhibition (LEE001) resulted in greater suppression of phosphorylated Rb and increased tumor regressions in *PIK3CA* mutant xenografts [110].

Conclusion

PI3K-Akt-mTOR signaling is commonly dysregulated in cancers arising from numerous tissues of origin. As a result, an important opportunity remains to effectively target the pathway for the possible benefit of many patients suffering from cancer. To realize the full potential of PI3K-Akt-mTOR therapeutics, a number of key advances will be required. First, the therapeutic window for PI3K pathway inhibition must be improved. Newer isoform-selective inhibitors, or even better, inhibitors that preferentially target the mutant protein, may permit dosing regimens that more completely inhibit oncogenic PI3K-Akt-mTOR signaling while sparing normal tissues. Second, the identification of improved response biomarkers will be needed to facilitate precision trials that enroll only the patients who are most likely to respond to therapy. It is yet unclear whether genetic biomarkers like *PTEN* or *INPP4B* deletion confer similar therapeutic vulnerabilities when they occur in different tissues of origin. Advancing our understanding of the context dependence of specific genetic mutations is particularly critical for "basket" clinical trial designs that prioritize patient enrollment based on mutation/molecular status rather than cancer type. Third, a comprehensive understanding of the most common mechanisms that underlie recovery of signaling during drug treatment may provide important leads for new therapeutic combinations. Finally, it also appears that strategies for co-drugging may arise from an improved understanding of how inhibition of PI3K signaling—even when cell cycle arrest and/or apoptosis are insufficient for clinical benefit—creates new vulnerabilities that may be targeted by other available therapies.

Acknowledgments This contribution was supported by NIH grants P50-GM107618 and T32-CA009172, as well as by the Dana-Farber Leadership Council.

References

1. Averous J, Fonseca B, Proud C (2007) Regulation of cyclin D1 expression by mTORC1 signaling requires eukaryotic initiation factor 4E-binding protein 1. Oncogene 27:1106–1113
2. Bader A, Kang S, Vogt P (2006) Cancer-specific mutations in PIK3CA are oncogenic in vivo. Proc Natl Acad Sci USA 103:1475–1479

3. Bar-Peled L, Chantranupong L, Cherniack A, Chen W, Ottina K, Grabiner B, Spear E, Carter S, Meyerson M, Sabatini D (2013) A Tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. Science (New York, N. Y.) 340:1100–1106
4. Baselga J (2001) Phase I and II clinical trials of trastuzumab. Ann Oncol 12(Suppl 1):S49–S55
5. Bauer D, Hatzivassiliou G, Zhao F, Andreadis C, Thompson C (2005) ATP citrate lyase is an important component of cell growth and transformation. Oncogene 24:6314–6322
6. Bellacosa A, Testa J, Staal S, Tsichlis P (1991) A retroviral oncogene, Akt, encoding a serine-threonine kinase containing an SH2-like region. Science (New York, N.Y.) 254:274–277
7. Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, Demanse D, De Buck SS, Ru QC, Peters M et al (2012) Phase I, dose-escalation study of BKM120, an oral pan-Class I PI3K inhibitor, in patients with advanced solid tumors. J Clin Oncol Off J Am Soc Clin Oncol 30:282–290
8. Berenjeno I, Guillermet-Guibert J, Pearce W, Gray A, Fleming S, Vanhaesebroeck B (2012) Both p110α and p110β isoforms of PI3K can modulate the impact of loss-of-function of the PTEN tumour suppressor. Biochem J 442:151–159
9. Berns K, Horlings H, Hennessy B, Madiredjo M, Hijmans EM, Beelen K, Linn S, Gonzalez-Angulo A, Stemke-Hale K, Hauptmann M et al (2007) A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 12:395–402
10. Bosch, Li, Bergamaschi, Ellis (2015) PI3K inhibition results in enhanced estrogen receptor function and dependence in hormone receptor–positive breast cancer. Sci Transl Med 7:283ra51
11. Bouchard C, Lee S, Paulus-Hock V, Loddenkemper C, Eilers M, Schmitt C (2007) FoxO transcription factors suppress Myc-driven lymphomagenesis via direct activation of Arf. Genes Dev 21:2775–2787
12. Brognard J, Sierecki E, Gao T, Newton A (2007) PHLPP and a second isoform, PHLPP2, differentially attenuate the amplitude of Akt signaling by regulating distinct Akt isoforms. Mol Cell 25:917–931
13. Brunet A, Bonni A, Zigmond M, Lin M, Juo P, Hu L, Anderson M, Arden K, Blenis J, Greenberg M (1999) Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96:857–868
14. Brunet A, Kanai F, Stehn J, Xu J, Sarbassova D, Frangioni J, Dalal S, DeCaprio J, Greenberg M, Yaffe M (2002) 14-3-3 transits to the nucleus and participates in dynamic nucleocytoplasmic transport. J Cell Biol 156:817–828
15. Bunney T, Katan M (2010) Phosphoinositide signalling in cancer: beyond PI3K and PTEN. Nat Rev Cancer 10:342–352
16. Cardone M, Roy N, Stennicke H, Salvesen G, Franke T, Stanbridge E, Frisch S, Reed J (1998) Regulation of cell death protease caspase-9 by phosphorylation. Science (New York, N.Y.) 282:1318–1321
17. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, Egia A, Sasaki A, Thomas G, Kozma S et al (2008) Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J Clin Investig 118:3065–3074
18. Carver B, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S, Arora V, Le C, Koutcher J, Scher H et al (2011) Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell 19:575–586
19. Chakrabarty A, Sánchez V, Kuba M, Rinehart C, Arteaga C (2012) Feedback upregulation of HER3 (ErbB3) expression and activity attenuates antitumor effect of PI3K inhibitors. Proc Natl Acad Sci USA 109:2718–2723
20. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, Majumder P, Baselga J, Rosen N (2011) Akt inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer Cell 19:58–71

21. Clarke P, Workman P (2012) Phosphatidylinositide-3-kinase inhibitors: addressing questions of isoform selectivity and pharmacodynamic/predictive biomarkers in early clinical trials. J Clin Oncol Official J Am Soc Clin Oncol 30:331–333
22. Costa C, Ebi H, Martini M, Beausoleil S, Faber A, Jakubik C, Huang A, Wang Y, Nishtala M, Hall B et al (2015) Measurement of PIP3 levels reveals an unexpected role for p110β in early adaptive responses to p110α-specific inhibitors in luminal breast cancer. Cancer Cell 27:97–108
23. Crino P, Nathanson K, Henske E (2006) The tuberous sclerosis complex. N Engl J Med 355:1345–1356
24. Cross D, Alessi D, Cohen P, Andjelkovich M, Hemmings B (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378:785–789
25. Crowder R, Phommaly C, Tao Y, Hoog J, Luo J, Perou C, Parker J, Miller M, Huntsman D, Lin L et al (2009) PIK3CA and PIK3CB inhibition produce synthetic lethality when combined with estrogen deprivation in estrogen receptor-positive breast cancer. Cancer Res 69:3955–3962
26. Datta S, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg M (1997) Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91:231–241
27. De P, Sun Y, Carlson J, Friedman L, Leyland-Jones B, Dey N (2014) Doubling down on the PI3K-Akt-mTOR pathway enhances the antitumor efficacy of PARP inhibitor in triple negative breast cancer model beyond BRCA-ness. Neoplasia (New York, N.Y.) 16:43–72
28. Deberardinis R, Lum J, Thompson C (2006) Phosphatidylinositol 3-kinase-dependent modulation of carnitine palmitoyltransferase 1A expression regulates lipid metabolism during hematopoietic cell growth. J Biol Chem 281:37372–37380
29. Dentin R, Liu Y, Koo S, Hedrick S, Vargas T (2007) Insulin modulates gluconeogenesis by inhibition of the coactivator TORC2. Nature 449:366–369
30. Deprez J, Vertommen D, Alessi D, Hue L, Rider M (1997) Phosphorylation and activation of heart 6-phosphofructo-2-kinase by protein kinase B and other protein kinases of the insulin signaling cascades. J Biol Chem 272:17269–17275
31. Dibble C, Manning B (2013) Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. Nat Cell Biol 15:555–564
32. Dobson M, Ramakrishnan G, Ma S, Kaplun L, Balan V, Fridman R, Tzivion G (2011) Bimodal regulation of FoxO3 by Akt and 14-3-3. Biochim Biophys Acta 1813:1453–1464
33. Eijkelenboom A, Burgering B (2013) FOXOs: signalling integrators for homeostasis maintenance. Nat Rev Mol Cell Biol 14:83–97
34. Elkabets M, Vora S, Juric D, Morse N, Mino-Kenudson M, Muranen T, Tao J, Campos A, Rodon J, Ibrahim Y et al (2013) mTORC1 inhibition is required for sensitivity to PI3K p110α inhibitors in PIK3CA-mutant breast cancer. Sci Transl Med 5:196ra99
35. Eser S, Reiff N, Messer M, Seidler B, Gottschalk K, Dobler M, Hieber M, Arbeiter A, Klein S, Kong B et al (2013) Selective requirement of PI3K/PDK1 signaling for Kras oncogene-driven pancreatic cell plasticity and cancer. Cancer Cell 23:406–420
36. Fedele C, Ooms L, Ho M, Vieusseux J, O'Toole S, Millar E, Lopez-Knowles E, Sriratana A, Gurung R, Baglietto L et al (2010) Inositol polyphosphate 4-phosphatase II regulates PI3K/Akt signaling and is lost in human basal-like breast cancers. Proc Natl Acad Sci USA 107:22231–22236
37. Feldman M, Apsel B, Uotila A, Loewith R, Knight Z, Ruggero D, Shokat K (2009) Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol 7(2):e38
38. Fingar D, Richardson C, Tee A, Cheatham L, Tsou C, Blenis J (2004) mTOR controls cell cycle progression through its cell growth effectors S6K1 and 4E-BP1/eukaryotic translation initiation factor 4E. Mol Cell Biol 24:200–216
39. Fruman D, Rommel C (2014) PI3K and cancer: lessons, challenges and opportunities. Nat Rev Drug Discov 13:140–156
40. Fu Z, Tindall D (2008) FOXOs, cancer and regulation of apoptosis. Oncogene 27:2312–2319

41. Gingras A, Kennedy S, O'Leary M, Sonenberg N, Hay N (1998) 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt(PKB) signaling pathway. Genes Dev 12:502–513
42. Gupta S, Ramjaun A, Haiko P, Wang Y, Warne P, Nicke B, Nye E, Stamp G, Alitalo K, Downward J (2007) Binding of ras to phosphoinositide 3-kinase p110alpha is required for ras-driven tumorigenesis in mice. Cell 129:957–968
43. Harrington L, Findlay G, Gray A, Tolkacheva T, Wigfield S, Rebholz H, Barnett J, Leslie N, Cheng S, Shepherd P et al (2004) The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. J Cell Biol 166:213–223
44. Hobert J, Eng C (2009) PTEN hamartoma tumor syndrome: an overview. Genet Med Official J Am Coll Med Genet 11:687–694
45. Hortobagyi G, Chen D, Piccart M, Rugo H, Burris H, Pritchard K, Campone M, Noguchi S, Perez A, Deleu I, Shtivelband M, Masuda N, Dakhil S, Anderson I, Robinson D, He W, Garg A, McDonald E, Bitter H, Huang A, Taran T, Bachelot T, Lebrun F, Lebwohl D, Baselga J (2015) Correlative analysis of genetic alterations and everolimus benefit in hormone receptor–positive, human epidermal growth factor receptor 2–negative advanced breast cancer: results from BOLERO-2. J Clin Oncol 34:419–426
46. Hresko R, Mueckler M (2005) mTOR.RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1 adipocytes. J Biol Chem 280:40406–40416
47. Hsu P, Kang S, Rameseder J, Zhang Y, Ottina K, Lim D, Peterson T, Choi Y, Gray N, Yaffe M et al (2011) The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. Science (New York, N.Y.) 332:1317–1322
48. Hu H, Juvekar A, Lyssiotis CA, Lien EC, Albeck JG, Oh D, Varma G, Hung YP, Ullas S, Lauring J et al (2016) Phosphoinositide 3-kinase regulates glycolysis through mobilization of aldolase from the actin cytoskeleton. Cell 164:433–446
49. Ibrahim Y, García-García C, Serra V, He L, Torres-Lockhart K, Prat A, Anton P, Cozar P, Guzmán M, Grueso J et al (2012) PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. Cancer Discov 2:1036–1047
50. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung S, Huang Q, Qin J, Su B (2006) SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. Cell 127:125–137
51. Janku F, Wheler J, Westin S, Moulder S, Naing A, Tsimberidou A, Fu S, Falchook G, Hong D, Garrido-Laguna I et al (2012) PI3K/Akt/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations. J Clin Oncol Official J Am Soc Clin Oncol 30:777–782
52. Jansen, Mirzaa, Ishak, O'Roak, Hiatt (2015) PI3K/Akt pathway mutations cause a spectrum of brain malformations from megalencephaly to focal cortical dysplasia. Brain 138:613–628
53. Jewell J, Kim Y, Russell R, Yu F-X, Park H, Plouffe S, Tagliabracci V, Guan K-L (2015) Differential regulation of mTORC1 by leucine and glutamine. Science 347:194–198
54. Jung C, Ro S, Cao J, Otto N, Kim D (2010) mTOR regulation of autophagy. FEBS Lett 584:1287–1295
55. Juvekar A, Burga L, Hu H, Lunsford E, Ibrahim Y, Balmañà J, Rajendran A, Papa A, Spencer K, Lyssiotis C et al (2012) Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for BRCA1-related breast cancer. Cancer Discov 2:1048–1063
56. Kang S, Denley A, Vanhaesebroeck B, Vogt P (2006) Oncogenic transformation induced by the p110β, -γ, and -δ isoforms of class I phosphoinositide 3-kinase. Proc Natl Acad Sci USA 103:1289–1294
57. Kannan A, Lin Z, Shao Q, Zhao S, Fang B, Moreno M, Vural E, Stack B, Suen J, Kannan K, Gao L (2015) Dual mTOR inhibitor MLN0128 suppresses Merkel cell carcinoma (MCC) xenograft tumor growth. Oncotarget (epub ahead of print)
58. Kimbung S, Biskup E, Johansson I, Aaltonen K, Ottosson-Wadlund A, Gruvberger-Saal S, Cunliffe H, Fadeel B, Loman N, Berglund P et al (2012) Co-targeting of the PI3K pathway

improves the response of BRCA1 deficient breast cancer cells to PARP1 inhibition. Cancer Lett 319:232–241
59. Koo J, Yue P, Gal A, Khuri F, Sun S-Y (2014) Maintaining glycogen synthase kinase-3 activity is critical for mTOR kinase inhibitors to inhibit cancer cell growth. Cancer Res 74:2555–2568
60. Koo J, Yue P, Deng X, Khuri F, Sun S-Y (2015) mTOR complex 2 stabilizes Mcl-1 protein by suppressing its glycogen synthase kinase 3-dependent and SCF-FBXW7-mediated degradation. Mol Cell Biol 35:2344–2355
61. Kurek KC, Luks VL, Ayturk UM, Alomari AI, Fishman SJ, Spencer SA, Mulliken JB, Bowen ME, Yamamoto GL, Kozakewich HP et al (2012) Somatic mosaic activating mutations in PIK3CA cause CLOVES syndrome. Am J Hum Genet 90:1108–1115
62. Laplante M, Sabatini D (2012) mTOR signaling in growth control and disease. Cell 149:274–293
63. Lee J, Huynh M, Silhavy J, Kim S, Dixon-Salazar T, Heiberg A, Scott E, Bafna V, Hill K, Collazo A, Funari V, Russ C, Gabriel S, Mathern G, Gleeson J (2012) De novo somatic mutations in components of the PI3K-Akt3-mTOR pathway cause hemimegalencephaly. Nat Genet 44:941–945
64. Liang J, Slingerland J (2003) Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression. Cell Cycle (Georgetown, Tex.) 2:339–345
65. Liang J, Zubovitz J, Petrocelli T, Kotchetkov R, Connor M, Han K, Lee J-H, Ciarallo S, Catzavelos C, Beniston R et al (2002) PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest. Nat Med 8:1153–1160
66. Lindhurst M, Sapp J, Teer J, Johnston J, Finn E, Peters K, Turner J, Cannons J, Bick D, Blakemore L et al (2011) A mosaic activating mutation in Akt1 associated with the Proteus syndrome. N Engl J Med 365:611–619
67. Loibl S, Minckwitz V, Schneeweiss A (2014) PIK3CA mutations are associated with lower rates of pathologic complete response to anti-human epidermal growth factor receptor 2 (her2) therapy in primary HER2-overexpressing breast cancer. J Clin Oncol 32:3212–3220
68. Longo P, Laurenti L, Gobessi S, Sica S, Leone G, Efremov D (2008) The Akt/Mcl-1 pathway plays a prominent role in mediating antiapoptotic signals downstream of the B-cell receptor in chronic lymphocytic leukemia B cells. Blood 111:846–855
69. Lucas C, Kuehn H, Zhao F, Niemela J, Deenick E, Palendira U, Avery D, Moens L, Cannons J, Biancalana M et al (2014) Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. Nat Immunol 15:88–97
70. Maira S, Galetic I, Brazil D, Kaech S, Ingley E, Thelen M, Hemmings B (2001) Carboxyl-terminal modulator protein (CTMP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane. Science (New York, N.Y.) 294:374–380
71. Manning B, Cantley L (2007) Akt/PKB signaling: navigating downstream. Cell 129:1261–1274
72. Marshall G, Howard Z, Dry J, Fenton S, Heathcote D, Gray N, Keen H, Logie A, Holt S, Smith P et al (2011) Benefits of mTOR kinase targeting in oncology: pre-clinical evidence with AZD8055. Biochem Soc Trans 39:456–459
73. Mayo L, Donner D (2001) A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. Proc Natl Acad Sci USA 98:11598–11603
74. Medema R, Kops G, Bos J, Burgering B (2000) AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1. Nature 404:782–787
75. Menon S, Dibble C, Talbott G, Hoxhaj G, Valvezan A, Takahashi H, Cantley L, Manning B (2014) Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. Cell 156:771–785
76. Mills J, Hippo Y, Robert F, Chen S, Malina A, Lin CJ, Trojahn U, Wendel H, Charest A, Bronson R, Kogan S, Nadon R, Housman D, Lowe S, Pelletier J (2008) mTORC1

promotes survival through translational control of Mcl-1. Proc Natl Acad Sci USA 105:10853–10858
77. Munster PN, Aggarwal R, Hong D, Schellens J (2015) First-in-human phase I study of GSK2126458, an oral pan-class I phosphatidylinositol-3-kinase inhibitor, in patients with advanced solid tumor malignancies. Clin Cancer Res [Epub ahead of print]
78. Muranen T, Selfors L, Worster D, Iwanicki M, Song L, Morales F, Gao S, Mills G, Brugge J (2012) Inhibition of PI3K/mTOR leads to adaptive resistance in matrix-attached cancer cells. Cancer Cell 21:227–239
79. Nakae J, Park B, Accili D (1999) Insulin stimulates phosphorylation of the forkhead transcription factor FKHR on serine 253 through a Wortmannin-sensitive pathway. J Biol Chem 274:15982–15985
80. Nayak G, Cooper G (2012) p53 is a major component of the transcriptional and apoptotic program regulated by PI 3-kinase/Akt/GSK3 signaling. Cell Death Dis 3:e400
81. Ni J, Liu Q, Xie S, Carlson C, Von T, Vogel K, Riddle S, Benes C, Eck M, Roberts T et al (2012) Functional characterization of an isoform-selective inhibitor of PI3K-p110β as a potential anticancer agent. Cancer Discov 2:425–433
82. Nuciforo P, Aura C, Holmes E, Prudkin L, Jimenez J, Martinez P, Ameels H, de la Peña L, Ellis C, Eidtmann H, Piccart-Gebhart M, Scaltriti M, Baselga J (2015) Benefit to neoadjuvant anti-human epidermal growth factor receptor 2 (HER2)-targeted therapies in HER2-positive primary breast cancer is independent of phosphatase and tensin homolog deleted from chromosome 10 (PTEN) status. Ann Oncol 26:1494–1500
83. O'Reilly K, Rojo F, She Q-B, Solit D, Mills G, Smith D, Lane H, Hofmann F, Hicklin D, Ludwig D et al (2006) mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 66:1500–1508
84. Okkenhaug K (2013) Two Birds with one stone: dual p110δ and p110γ inhibition. Chem Biol 20:1309–1310
85. Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, Meek S, Salpekar A, Waterfield M et al (2002) Impaired B and T cell antigen receptor signaling in p110δ PI 3-kinase mutant mice. Science 297:1031–1034
86. Orloff M, He X, Peterson C, Chen F, Chen J-L, Mester J, Eng C (2013) Germline PIK3CA and Akt1 mutations in Cowden and Cowden-like syndromes. Am J Hum Genet 92:76–80
87. Porstmann T, Santos C, Griffiths B, Cully M, Wu M, Leevers S, Griffiths J, Chung Y-L, Schulze A (2008) SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab 8:224–236
88. Rivière J-B, Mirzaa G, O'Roak B, Beddaoui M, Alcantara D, Conway R, St-Onge J, Schwartzentruber J, Gripp K, Nikkel S et al (2012) De novo germline and postzygotic mutations in Akt3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. Nat Genet 44:934–940
89. Robey RB, Hay N (2009) Is Akt the "Warburg kinase"?-Akt-energy metabolism interactions and oncogenesis. Semin Cancer Biol 19:25–31
90. Rodon J, Dienstmann R, Serra V, Tabernero J (2013) Development of PI3K inhibitors: lessons learned from early clinical trials. Nat Rev Clin Oncol 10:143–153
91. Rodrik-Outmezguine V, Chandarlapaty S, Pagano N, Poulikakos P, Scaltriti M, Moskatel E, Baselga J, Guichard S, Rosen N (2011) mTOR kinase inhibition causes feedback-dependent biphasic regulation of Akt signaling. Cancer Discov 1:248–259
92. Saal L, Gruvberger-Saal S, Persson C, Lövgren K, Jumppanen M, Staaf J, Jönsson G, Pires M, Maurer M, Holm K et al (2008) Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. Nat Genet 40:102–107
93. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell S, Riggins G et al (2004) High frequency of mutations of the PIK3CA gene in human cancers. Science (New York, N.Y.) 304:554
94. Sancak Y, Thoreen C, Peterson T, Lindquist R, Kang S, Spooner E, Carr S, Sabatini D (2007) PRAS40 Is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol Cell 25:903–915

95. Sancak Y, Peterson T, Shaul Y, Lindquist R, Thoreen C, Bar-Peled L, Sabatini D (2008) The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science (New York, N.Y.) 320:1496–1501
96. Sano H, Kane S, Sano E, Mîinea C, Asara J, Lane W, Garner C, Lienhard G (2003) Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. J Biol Chem 278:14599–14602
97. Schmit F, Utermark T, Zhang S, Wang Q, Von T, Roberts T, Zhao J (2014) PI3K isoform dependence of PTEN-deficient tumors can be altered by the genetic context. Proc Natl Acad Sci USA 111:6395–6400
98. Schwartz S, Wongvipat J, Trigwell C, Hancox U, Carver B, Rodrik-Outmezguine V, Will M, Yellen P, de Stanchina E, Baselga J, Scher H, Barry S, Sawyers C, Chandarlapaty S, Rosen N (2015) Feedback suppression of PI3Kα signaling in PTEN-mutated tumors is relieved by selective inhibition of PI3Kβ. Cancer Cell 27:109–122
99. Serra V, Scaltriti M, Prudkin L, Eichhorn P, Ibrahim Y, Chandarlapaty S, Markman B, Rodriguez O, Guzman M, Rodriguez S, Gili M, Russillo M, Parra J, Singh S, Arribas J, Rosen N, Baselga J (2011) PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. Oncogene 30:2547–2557
100. Shi Y, Yan H, Frost P, Gera J, Lichtenstein A (2005) Mammalian target of rapamycin inhibitors activate the Akt kinase in multiple myeloma cells by up-regulating the insulin-like growth factor receptor/insulin receptor substrate-1/phosphatidylinositol 3-kinase cascade. Mol Cancer Ther 4:1533–1540
101. Stemke-Hale K, Gonzalez-Angulo A, Lluch A, Neve R, Kuo W-L, Davies M, Carey M, Hu Z, Guan Y, Sahin A et al (2008) An integrative genomic and proteomic analysis of PIK3CA, PTEN, and Akt mutations in breast cancer. Cancer Res 68:6084–6091
102. Stratikopoulos E, Dendy M, Szabolcs M, Khaykin A, Lefebvre C, Zhou M, Parsons R (2015) Kinase and BET inhibitors together clamp inhibition of PI3K signaling and overcome resistance to therapy. Cancer Cell 27:837–851
103. Tan M-H, Mester J, Ngeow J, Rybicki L, Orloff M, Eng C (2012) Lifetime cancer risks in individuals with germline PTEN mutations. Clin Cancer Res Official J Am Assoc Cancer Res 18:400–407
104. Taniguchi C, Kondo T, Sajan M, Luo J, Bronson R, Asano T, Farese R, Cantley L, Kahn CR (2006) Divergent regulation of hepatic glucose and lipid metabolism by phosphoinositide 3-kinase via Akt and PKClambda/zeta. Cell Metab 3:343–353
105. Thorpe L, Yuzugullu H, Zhao J (2015) PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. Nat Rev Cancer 15:7–24
106. Tinkum K, White L, Marpegan L, Herzog E, Piwnica-Worms D, Piwnica-Worms H (2013) Forkhead box O1 (FOXO1) protein, but not p53, contributes to robust induction of p21 expression in fasted mice. J Biol Chem 288:27999–28008
107. Tinsley S, Meja K, Shepherd C, Khwaja A (2014) Synergistic induction of cell death in haematological malignancies by combined phosphoinositide-3-kinase and BET bromodomain inhibition. Br J Haematol 170:275–278
108. Torbett N, Luna-Moran A, Knight Z, Houk A, Moasser M, Weiss W, Shokat K, Stokoe D (2008) A chemical screen in diverse breast cancer cell lines reveals genetic enhancers and suppressors of sensitivity to PI3K isoform-selective inhibition. Biochem J 415:97–110
109. Tremblay F, Brûlé S, Um S, Li Y, Masuda K, Roden M, Sun X, Krebs M, Polakiewicz R, Thomas G et al (2007) Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance. Proc Natl Acad Sci 104:14056–14061
110. Vora SR, Juric D, Kim N, Mino-Kenudson M, Huynh T (2014) CDK 4/6 inhibitors sensitize PIK3CA mutant breast cancer to PI3K inhibitors. Cancer Cell 26:136–149
111. Wan X, Harkavy B, Shen N, Grohar P, Helman L (2006) Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. Oncogene 26:1932–1940

112. Wang Q, Liu P, Spangle JM, Von T, Roberts TM, Lin NU, Krop IE, Winer EP, Zhao JJ (2015) PI3K-p110α mediates resistance to HER2-targeted therapy in HER2+, PTEN-deficient breast cancers. Oncogene [Epub ahead of print]
113. Wang R, Wei Y, An Z, Zou Z, Xiao G, Bhagat G, White M, Reichelt J, Levine B (2012) Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. Science (New York, N.Y.) 338:956–959
114. Wee S, Wiederschain D, Maira S-M, Loo A, Miller C, deBeaumont R, Stegmeier F, Yao Y-M, Lengauer C (2008) PTEN-deficient cancers depend on PIK3CB. Proc Natl Acad Sci USA 105:13057–13062
115. Whitman M, Kaplan D, Schaffhausen B, Cantley L, Roberts T (1985) Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation. Nature 315:239–242
116. Whitman M, Downes C, Keeler M, Keller T, Cantley L (1988) Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. Nature 332:644–646
117. Woscholski R, Finan P, Radley E, Totty N, Sterling A, Hsuan J, Waterfield M, Parker P (1997) Synaptojanin Is the major constitutively active phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase in rodent brain. J Biol Chem 272:9625–9628
118. Yang W, Ching K, Tsoukas C, Berg L (2001) Tec kinase signaling in T cells is regulated by phosphatidylinositol 3-kinase and the Tec pleckstrin homology domain. J Immunol 166 387–395
119. Yang K, Guo Y, Stacey WC (2006) Glycogen synthase kinase 3 has a limited role in cell cycle regulation of cyclin D1 levels. BMC Cell Biol 7:33
120. Yang Q, Modi P, Newcomb T, Quéva C, Gandhi V (2015) Idelalisib: first-in-class PI3K delta inhibitor for the treatment of chronic lymphocytic leukemia, small lymphocytic leukemia, and follicular lymphoma. Clin Cancer Res Official J Am Assoc Cancer Res 21:1537–1542
121. Zhang X, Tang N, Hadden T, Rishi A (2011) Akt, FoxO and regulation of apoptosis. Biochim Biophys Acta (BBA)—Mol Cell Res 1813:1978–1986
122. Zhang Y, Nicholatos J, Dreier J, Ricoult S, Widenmaier S, Hotamisligil G, Kwiatkowski D, Manning B (2014) Coordinated regulation of protein synthesis and degradation by mTORC1. Nature 513:440–443
123. Zhao L, Vogt P (2008) Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. Proc Natl Acad Sci USA 105:2652–2657
124. Zhou B, Liao Y, Xia W, Spohn B, Lee M, Hung M (2001) Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. Nature Cell Biol 3 245–252

Part I
PI3K-mTOR Pathway in Cancers

Chapter 2
The mTOR Complexes in Cancer Cell Metabolism

Thomas Lynch, Joseph G. Moloughney and Estela Jacinto

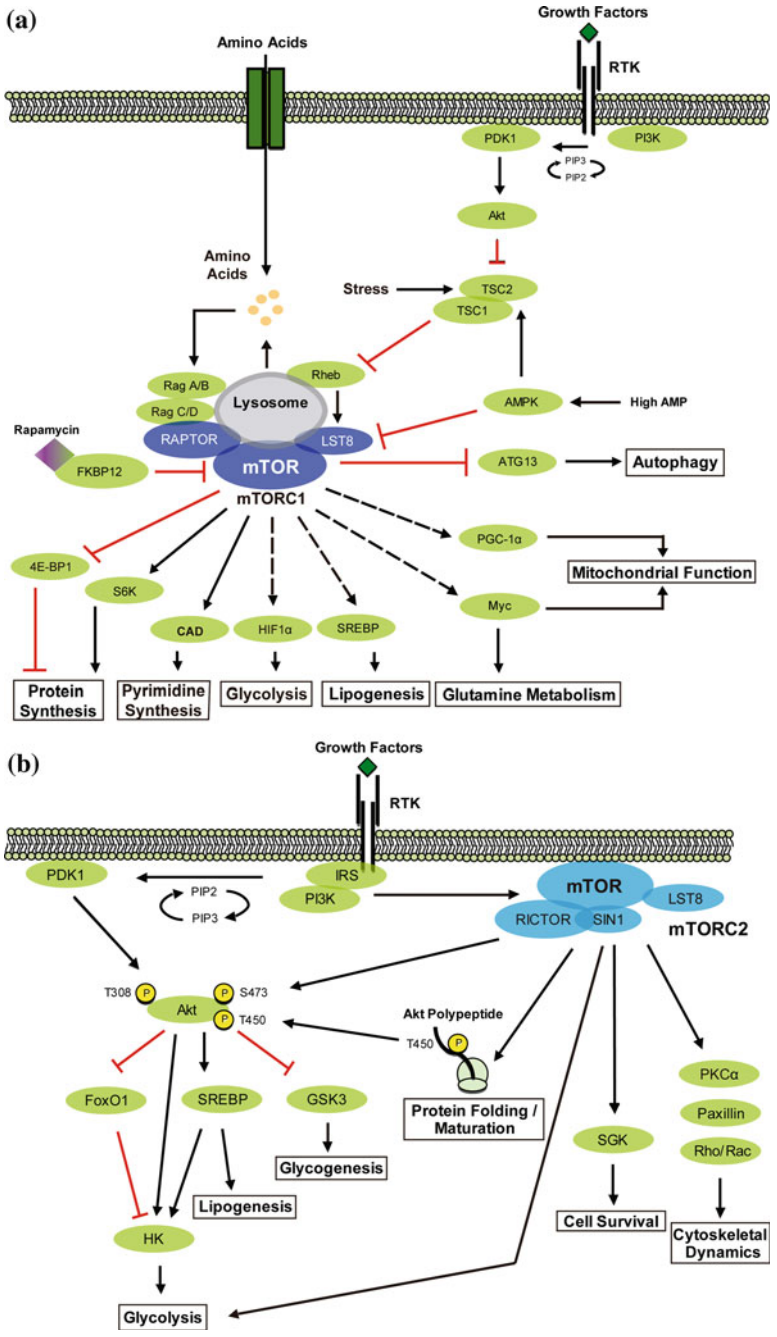
Introduction

Our understanding of the intersection between cellular signaling pathways, nutrient sensing, and metabolic functions is rapidly growing, and at the center of this intersection is mTOR. mTOR is an atypical protein kinase, that is conserved from yeast to man, and is allosterically inhibited by a complex formed by the natural compound rapamycin and the prolyl isomerase FKBP12 [15, 61, 62, 85, 125]. Using rapamycin and genetic studies in yeast, it was shown that TOR promotes protein synthesis when nutrient conditions are favorable [3]. Its inhibition by rapamycin arrests cells in G1 phase of the cell cycle, eliciting a phenotype characteristic of starved cells [3, 15, 18, 59]. Moreover, rapamycin induces autophagy, a starvation response that degrades and recycles cellular components [14, 110]. In mammals, rapamycin blocks the activation of the translation regulator p70 S6K [22, 86, 118]. The major target of S6K is the 40S ribosomal protein S6, which is highly phosphorylated during G1 [148]. Around this time, the phosphorylation of S6 was discovered to be sensitive to amino acids, and that rapamycin and amino acids have opposite effects on autophagy and protein synthesis [14]. Another translation regulator, 4E-BP1 is also sensitive to rapamycin [9, 52]. Furthermore, phosphorylation of S6K diminishes upon amino acid withdrawal, resembling rapamycin treatment [58]. These observations, along with the early studies in yeast and in *Drosophila*, led to the notion that TOR/mTOR participates in nutrient sensing [3, 59, 114, 177].

Subsequent studies demonstrating that yeast TOR and mTOR play a role in the expression and trafficking of nutrient transporters, and that mTOR can sense levels of ATP, further reinforced the idea that mTOR is part of a nutrient signaling cascade [5, 34, 39, 133]. Moreover, studies on different model organisms have

T. Lynch · J.G. Moloughney · E. Jacinto (✉)
Department of Biochemistry and Molecular Biology, Rutgers-Robert Wood Johnson Medical School, 683 Hoes Lane, Piscataway, NJ 08854, USA
e-mail: jacintes@rwjms.rutgers.edu

N. Dey et al. (eds.), *PI3K-mTOR in Cancer and Cancer Therapy*,
Cancer Drug Discovery and Development, DOI 10.1007/978-3-319-34211-5_2


(a)
Growth Factors
Amino Acids
RTK
PDK1
PI3K
PIP3
PIP2
Akt
Amino Acids
Stress
TSC2
TSC1
Rag A/B
Rag C/D
Rheb
Lysosome
AMPK
High AMP
Rapamycin
RAPTOR
LST8
FKBP12
mTOR
ATG13
Autophagy
mTORC1
PGC-1α
Mitochondrial Function
4E-BP1
S6K
Myc
CAD
HIF1α
SREBP
Protein Synthesis
Pyrimidine Synthesis
Glycolysis
Lipogenesis
Glutamine Metabolism
(b)
Growth Factors
RTK
PDK1
PIP2
PIP3
IRS
PI3K
mTOR
LST8
RICTOR
SIN1
mTORC2
T308
S473
Akt
T450
Akt Polypeptide
T450
FoxO1
SREBP
GSK3
Protein Folding / Maturation
PKCα
Paxillin
Rho/Rac
SGK
Glycogenesis
Lipogenesis
Cell Survival
Cytoskeletal Dynamics
HK
Glycolysis

◀ **Fig. 2.1** mTOR forms two distinct protein complexes. **a** mTOR association with LST8 and RAPTOR characterizes the rapamycin-sensitive mTORC1. This complex responds to amino acids and its activity is enhanced by inputs from growth factor signaling via the PI3K/AKT pathway. mTORC1 is negatively regulated by TSC1/2 and AMPK, in response to nutrient/energy availability. Together, these opposing signaling pathways provide modulation for mTORC1 signaling to various downstream effectors that positively regulate anabolic metabolism and protein synthesis while negatively regulating catabolic processes including autophagy. **b**. mTOR association with LST8, RICTOR, and SIN1 characterizes mTORC2, the rapamycin-insensitive mTOR complex. mTORC2 is activated by growth factor/PI3K signaling and association with translating ribosomes. mTORC2 regulates protein maturation by a cotranslational phosphorylation mechanism, whereby nascent peptide stability is enhanced. AKT is stabilized in such a manner and is further activated by additional mTORC2 phosphorylation, leading to the upregulation of several metabolic pathways. mTORC2 activity regulates additional cellular processes through additional downstream effectors. Dashed lines refer to indirect regulation. Abbreviations used here include 4E-BP1: eukaryotic translation initiation factor 4E-binding protein 1, AMP: adenosine monophosphate, AMPK: AMP-activated protein kinase, ATG13: autophagy-related 13, CAD: carbamoyl phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase, FoxO1: forkhead box O1, FKBP12: 12-kDa FK506-binding protein, GSK3: glycogen synthase kinase 3, HIF1α: hypoxia inducible factor 1α subunit, HK: hexokinase, IRS: insulin receptor substrate, LKB1: liver kinase B1, LST8: lethal with SEC13 protein 8, mTOR: mammalian target of rapamycin, PDK1: phosphoinositide-dependent kinase-1, PGC-1α: peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PI3K: phosphatidylinositol-3-kinase, PIP2: phosphatidylinositol-4,5-bisphosphate, PIP3: phosphatidylinositol-(3,4,5)-trisphosphate, PKCα: protein kinase Cα, Rag: Ras-related GTP-binding protein, RAPTOR: Regulatory-associated protein of mTOR, RHEB: Ras-homolog enriched in brain, RICTOR: rapamycin-insensitive companion of mammalian target of rapamycin, RTK: receptor tyrosine kinase, S6K: ribosomal protein S6 kinase, SGK1: serum and glucocorticoid-regulated kinase 1, SIN1: stress-activated protein kinase-interacting 1, SREBP: sterol regulatory element-binding proteins, and TSC: tuberous sclerosis complex

unraveled that TOR controls cell growth or increases in cell mass, as opposed to cell proliferation or cell cycle progression [132]. Genome-wide screening has further uncovered the effect of rapamycin on metabolic genes, revealing that TOR/mTOR mediates the expression of genes involved in nutrient metabolism [59, 114]. Together, these early studies have laid the groundwork that paved the way for understanding how nutrient signaling is linked to growth and metabolic signaling pathways via mTOR.

In multicellular organisms, cell growth is coordinated with tissue and organismal growth. Thus, in addition to nutrients, other extracellular inputs, such as growth factors and hormones, signal cell growth processes. The insulin and insulin-like growth factors play central roles in anabolic metabolism. The phosphatidylinositol-3 kinase (PI3K)/AKT signaling pathway couples signals from the insulin receptor to the control of gene expression and cellular growth responses [99]. In the presence of amino acids, insulin enhances S6K and 4E-BP1 phosphorylation, which can be inhibited by PI3K inhibitors or rapamycin, thus placing mTOR as a downstream effector of insulin signals [89]. The insulin/PI3K signals are wired to mTOR via the tuberous sclerosis protein complex (TSC1/TSC2) [67] (Fig. 2.1a). TSC1/2 are tumor suppressors and mutation of either one leads to hyperactivation of the mTOR/S6K1 branch. Numerous signals, including AKT, and stress signals impinge on TSC1/2,

modulating mTOR signaling. TSC1/2 negatively regulates mTOR (as part of mTOR complex 1/mTORC1, see below) via inactivation of Rheb. In its GTP-bound form, Rheb directly interacts and stimulates mTORC1. mTORC1 responds to amino acid signals via Rag GTPases [126, 127]. Amino acids promote GTP loading of RagA/B and enable the Rag heterodimers to interact with raptor and facilitate translocation of mTORC1 to the lysosomal surface [126]. Additionally, the presence of insulin or growth factors acutely dissociates TSC and thereby excludes this negative regulator of mTORC1 from this compartment [33, 103]. On the lysosomal surface, mTORC1 becomes activated possibly via Rheb, which is found throughout the endomembrane system. Thus, nutrient and growth factor signals converge to regulate the activation and subcellular localization of mTORC1.

In addition to compartmental regulation, mTOR is also highly regulated by its protein partners. Previous studies in yeast revealed a rapamycin-insensitive function of TOR [134, 179]. Unlike mammals wherein only one gene encodes mTOR, there are two in yeast, namely *TOR1* and *TOR2*. TOR2 performs a function involving actin cytoskeleton polarization that is insensitive to rapamycin treatment [134]. Biochemical purification has led to the identification of two structurally distinct TOR complexes in both yeast and mammals [46, 57, 73, 74, 81, 94, 128, 167]. The rapamycin-sensitive mTORC1 forms a complex with raptor and mLST8 (Fig. 2.1a), whereas the rapamycin-insensitive mTORC2 forms a complex with rictor (mAVO3), SIN1, and mLST8 (Fig. 2.1b). In addition to these conserved partners, the mTORCs also associate with other distinct, less well-conserved proteins that could regulate its activity and function [164]. The well-characterized function of mTOR in regulating the translation regulators S6K1 and 4E-BP1 is mediated by mTORC1. On the other hand, mTORC2, but not mTORC1, can phosphorylate AKT, and this phosphorylation which leads to optimal activation of AKT, is not acutely sensitive to rapamycin [64, 130]. Although AKT phosphorylation at its conserved hydrophobic motif site is used as a hallmark of mTORC2 activity, other direct substrates of mTORC2, such as SGK and PKC, are emerging from recent studies [164]. mTORC2 has also been linked to other cellular functions, such as actin cytoskeleton reorganization, translation, and protein maturation/folding [111]. The latter function entails cotranslational phosphorylation of AKT and PKC at a conserved turn motif that is critical for folding and stabilization of the kinase domain [43, 71, 112]. Consistent with this function of mTORC2, it was found to associate with ribosomes [112, 181]. Thus, both mTORCs function during translation, albeit by distinct mechanisms. Whereas mTORC1 promotes translation in response to amino acids, it remains unclear what activates mTORC2. The association with ribosomes enhances its activity toward AKT but precisely how mTORC2 gets activated remains elusive [181]. ATP depletion and glucose withdrawal can prevent optimal phosphorylation of AKT at the turn motif in vitro by mTORC2, suggesting that these signals could regulate mTORC2 [19].

AKT has long been studied as a central regulator of the response to insulin [99]. Among its many functions, it mediates the increase in glucose transporters on the plasma membrane upon insulin stimulation. Furthermore, it regulates numerous

enzymes in the glycolytic pathway, emphasizing its pivotal role in cell metabolism [122]. Thus, the finding that mTORC2 controls AKT and that both mTOR complexes feed back to regulate insulin/insulin receptor substrate (IRS) signals attest to a broader role of mTOR in the control of cellular metabolism [65, 82, 173]. However, much of the recent studies on the role of mTOR in cell metabolism highlight the involvement of mTORC1. This is partly due to the availability of rapamycin and TSC knockout models, which have expanded our knowledge on the functions of mTORC1. Availability of mTOR inhibitors (MTI) that block both mTORC1 and mTORC2, along with genetic models containing deficiencies in mTORC1 or mTORC2 components are now providing more insights on the role of these complexes in cancer cell metabolism.

A hallmark of cancer cells is increased aerobic glycolysis despite the presence of oxygen, also known as the Warburg effect [152]. The uncontrolled proliferation necessitates reprogramming of energy metabolism in order to sustain cell growth and division. Not only do cancer cells augment their uptake of nutrients, they also rewire signaling circuits in order to route nutrient catabolism toward macromolecular synthesis. The increased uptake of glucose enhances flux through glycolysis. When glycolytic flux is high, this leads not only to production of abundant ATP, but also produces intermediates that are required for biosynthetic pathways [31]. In this review, we will discuss evidence supporting the role of mTORC1 and mTORC2 in cell metabolism. We will discuss different metabolic pathways including glycolysis, mitochondrial, and biosynthetic pathways that become rewired in proliferating cancer cells and consider the role of the mTOR complexes in metabolic reprogramming.

Glucose Metabolism (Glycolysis)

In normal differentiated cells, glucose is metabolized to pyruvate via glycolysis. This process yields a net production of 2 mol of ATP/mol of glucose while reducing the cofactor NAD^+ to NADH. Cancer cells enhance their rate of glucose uptake and produce pyruvate at a higher rate than can be metabolized by the mitochondria. Under this condition, the excess pyruvate is diverted from being metabolized in the mitochondria and converted to lactate in the cytosol. This glycolytic switch can occur under aerobic conditions. Genetic mutations that lead to enhanced growth factor-PI3K/AKT/mTOR pathway signaling can drive and/or maintain this switch [152]. Indeed, multiple points along the glycolytic pathway are influenced by mTOR via regulation of two critical transcription factors HIF1α and Myc (Fig. 2.2).

A crucial signaling protein that is involved in the glycolytic switch is HIF1 (hypoxia inducible factor 1). HIF1 is a heterodimer, consisting of an O_2-regulated HIF1α subunit and a constitutively expressed HIF1β subunit [136]. Increased HIF1α expression is sufficient to induce expression of genes whose products increase glycolytic flux [66]. While HIF1α expression is normally elevated under

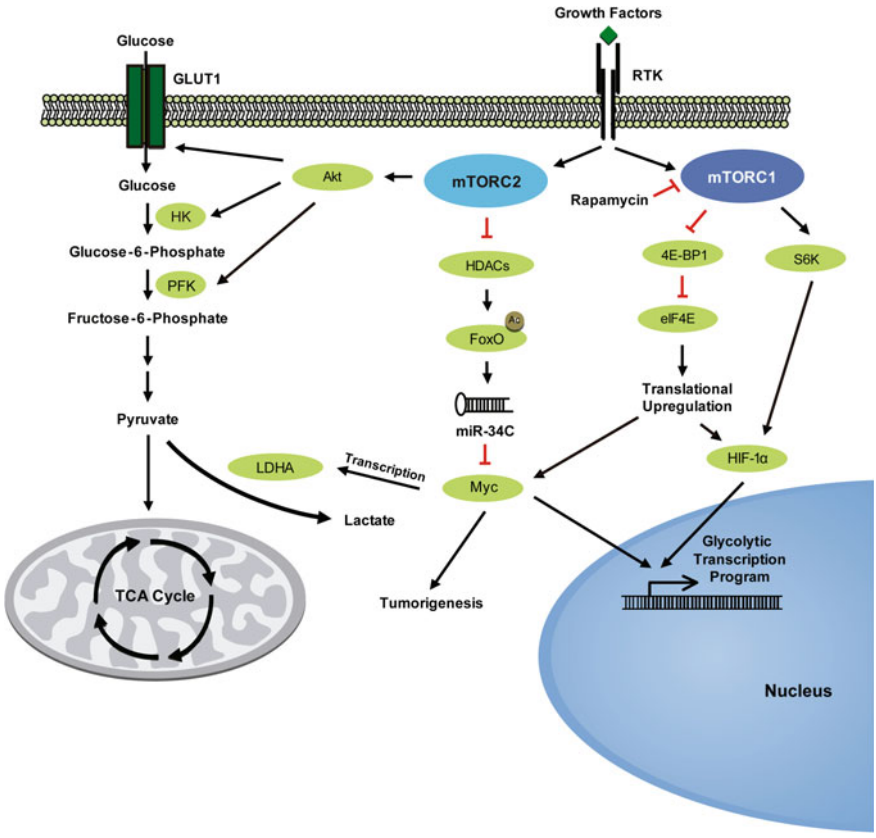


Fig. 2.2 mTORCs and glucose metabolism. mTORC1 contributes to the upregulation of the transcription factors HIF-1α and Myc, both of which drive a pro-glycolytic transcriptional program. This activity can be inhibited by rapamycin. mTORC2, through regulation of AKT activity, can enhance the activity of various glycolytic enzymes, and through the stabilization of Myc, can additionally promote transcription of glycolytic enzymes. Myc has been associated with tumorigenesis. Additional abbreviations used here include GLUT1: glucose transporter 1, HDACs: histone deacetylases, HIF-1α: hypoxia inducible factor 1α, HK: hexokinase, LDHA: lactate dehydrogenase A, miR-34C: microRNA 34c, PFK: phosphofructokinase, RTK: receptor tyrosine kinase, S6K: ribosomal protein S6 kinase, and TCA Cycle: tricarboxylic acid cycle

hypoxia, deregulated mechanisms in cancer cells boost HIF1α levels despite aerobic conditions. Its expression is upregulated in many primary and metastatic human tumors [136]. Early studies in prostate cancer cells have shown that inhibiting mTOR by rapamycin blocks the growth factor- and mitogen-induced HIF1α expression [180]. Rapamycin also decreases HIF1α stabilization and transcriptional activity under hypoxic conditions [69]. Elevated mTORC1 activation that occurs in $TSC2^{-/-}$ cells also increases translation of HIF1α mRNA [17] while rapamycin decreases its mRNA levels [90, 149]. The control of HIF1α translation

involves the mTORC1 target, 4E-BP1 [38]. Thus, mTORC1 can regulate HIF1α expression via translational and posttranslational mechanisms.

mTORC1 is also linked to regulation of genes that are controlled by HIF1α. Transcriptional profiling of rapamycin-treated lymphocytes revealed altered glycolytic gene expression in these cells [53, 114]. In prostate epithelial cells of transgenic mice expressing active AKT, rapamycin diminishes the levels of glycolytic enzyme genes controlled by HIF1α [98]. A combination of genomic, metabolomics, and bioinformatics approaches further confirmed the involvement of mTORC1 in inducing a HIF1α-dependent transcriptional program to promote glycolysis [38]. Among the HIF1α-regulated genes that are transcriptionally upregulated in an mTORC1-dependent manner include glycolytic enzymes and VEGF. The expression of the rate-limiting glycolytic enzyme pyruvate kinase M2 (PKM2), which is exclusively expressed in proliferating and tumor cells, is also regulated transcriptionally by mTORC1 via HIF1α [145]. Hence, mTORC1 also regulates HIF1α-target genes at the level of transcription.

Other genes that become upregulated during glycolysis are those encoding nutrient transporters. mTOR has been shown to play a role in regulating uptake of nutrients via control of gene expression or membrane trafficking of their transporters. Overexpression of kinase-inactive mTOR perturbs amino acid transporter trafficking while rapamycin diminishes glycolytic activity [39]. In differentiating T lymphocytes, rapamycin inhibits expression of HIF1α and genes involved in glucose transport and metabolism [139]. Rapamycin treatment in vivo also reduces fluorodeoxyglucose (FDG) uptake in kidney cancers with loss of the tumor suppressor von Hippel Landau (VHL1), supporting a role for mTORC1 in glucose uptake [149]. The sensitivity to mTOR inhibition is attributed to a block in translation of mRNA encoding HIF1α, a target of VHL. However, in another study using liver-specific *Tsc1* mutant mice, increased mTORC1 activation is accompanied by reduced glucose uptake [77]. This is likely due to the mTORC1/S6K1-mediated negative feedback loop that downregulates PI3K/AKT pathway, which plays a role in glucose transport [60]. The reduced glucose uptake under elevated mTORC1 activity is also in line with the findings that TSC-deficient cells are hypersensitive to glucose withdrawal [72]. Together, these findings suggest that under normal conditions, mTOR couples uptake of nutrients with the metabolic demands.

Another effector of mTORC1 that promotes glycolytic gene expression is the transcription factor Myc [49, 159]. Using a bioinformatics approach, Manning and coworkers have identified cis-regulatory elements among rapamycin-sensitive genes to be regulated by Myc [38]. In T cells, mTORC1 inhibition also diminishes the T cell receptor (TCR)-induced Myc expression, which is accompanied by reduction of glycolytic activity [155]. HIF1 and Myc have overlapping metabolic gene targets. One example of a common target gene is that encoding lactate dehydrogenase (LDH) [29, 135]. LDH is a tetrameric enzyme composed of a combination of the subunits LDHA and LDHB which converts pyruvate to lactate. Rapamycin treatment of prostate cancer cell lines downregulates LDHA expression among other metabolic effectors [151]. On the other hand, the expression of LDHB

is upregulated in an mTOR-dependent manner in murine embryonic fibroblasts that have deficiency in TSC1, TSC2, or PTEN and with activated AKT. The enhanced LDHB levels are critical for hyperactive mTOR-mediated tumorigenesis [176]. Another common target of HIF1α and Myc is PKM2. Unlike transcriptional activation of the PKM2 gene by HIF1α, Myc appears to regulate PKM2 expression in an mTORC1-dependent manner via the alternative splicing repressors, hnRNPs [145]. How mTORC1 contributes to the regulation of LDH and other common target genes of HIF1α and Myc awaits further investigation. In addition, how mTORC1 can regulate Myc remains to be characterized.

Studies using raptor knockout models are also beginning to reveal more insights on specific roles of mTORC1 in cellular metabolism. More recently, Yang et al. showed that the metabolic programming that drives the exit of T cells from quiescence is dependent on mTORC1. TCR stimulation of raptor-deficient $CD4^+$ T cells triggers reduced glycolytic activity compared to wild type cells [166]. This defect is accompanied by attenuated mRNA expression of glycolytic enzymes and protein expression of Myc. Furthermore, mTORC1 links glucose metabolism to cytokine receptor expression and responsiveness [166]. There is also evidence that downstream mTORC1 substrates mediate glycolytic metabolism. Knockdown of S6K1 in PTEN-deficient cells decreases HIF1α expression and glycolysis [146]. In these studies, targeting the mTORC1 substrate S6K1 in PTEN-deficient mouse model of leukemia delays leukemogenesis. Additionally, pharmacological or genetic inhibition of another mTORC1 target, 4E-BP1, is also sufficient to block Myc-driven tumorigenesis [117]. Whether other substrates of mTORC1 in addition to S6K1 and 4E-BP1 mediate the function of mTORC1 in glycolysis will need to be addressed.

mTORC2 also plays a role in glycolytic metabolism. Early on, the mTORC2 substrate AKT was shown to couple growth factor signaling to glucose metabolism [122]. Using an experimental leukemia model, Elstrom et al. [41] demonstrated that AKT activation was sufficient in increasing the rate of glucose metabolism. They also found increased rates of aerobic glycolysis in glioblastoma cells harboring constitutive AKT activity. In prostate epithelial cells, activated AKT induces glycolytic genes via HIF1α [98]. On the other hand, AKT deficiency is sufficient to suppress tumor development in PTEN± mice [20]. Thus, AKT plays a crucial role in enhanced aerobic glycolysis. The most compelling evidence demonstrating a role for mTORC2 in glycolytic metabolism was presented using liver-specific rictor knockout mice. In the liver of these mice, AKT phosphorylation is abrogated, glycolysis is impaired and the activity of glucokinase is reduced. Expression of a constitutively active AKT or glucokinase rescues glucose flux in these mice [55]. Thus, mTORC2 regulates glycolysis in the liver via AKT. Recently, mTORC2 was shown to control glycolytic metabolism in glioblastoma independently of AKT [101]. In this cancer model, mTORC2 controls the acetylation of FoxO through inhibition of Class IIa HDAC phosphorylation [101]. These findings support that mTORC2 has functions in glycolytic metabolism distinct from AKT.

Oxidative and Mitochondrial Metabolism

The maximum production of ATP under normal conditions requires metabolism of pyruvate in the mitochondria. Pyruvate enters the mitochondria and is converted to acetyl coenzyme A (acetyl CoA), which is further metabolized via the tricarboxylic acid (TCA) cycle. Whereas the TCA cycle serves to produce maximal ATP production in nonproliferating cells, it primarily generates intermediates that are utilized as biosynthetic precursors in highly proliferating cells [152]. mTOR has been linked to these processes occurring in the mitochondria (Fig. 2.3). Inhibition of mTOR by rapamycin reduces mitochondrial membrane potential, oxygen consumption, ATP

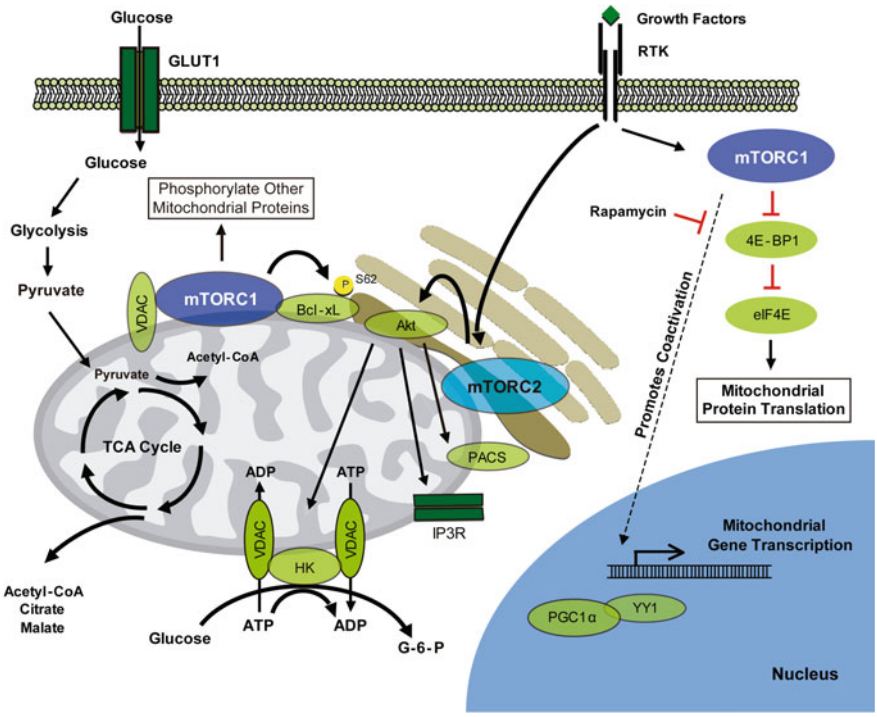


Fig. 2.3 mTORCs in oxidative and mitochondrial metabolism. Mitochondrial gene transcription is upregulated in an mTORC1-dependent manner, through the stimulation of PGC1α/YY1 coactivation, and mitochondrial gene translation is upregulated through eIF4E activity. Additionally, mTORC1 promotes phosphorylation of mitochondrial proteins and associates with VDAC and Bcl-xL, regulating mitochondrial substrate availability and apoptosis, respectively. mTORC2 modulates mitochondrial metabolism and integrity through AKT activity. Additional abbreviations used here include ADP: adenosine diphosphate, ATP: adenosine triphosphate, Bcl-xL: B-cell lymphoma-extra large, G-6-P: glucose-6-phosphate, IP3R: inositol trisphosphate receptor, PACS: phosphofurin acidic cluster sorting protein, PGC-1α: peroxisome proliferator-activated receptor gamma coactivator 1-alpha, VDAC: voltage-dependent anion channel, and YY1: yin yang 1

synthetic capacity and alters the mitochondrial phosphoproteome [131]. Interestingly, these effects of rapamycin do not seem to be mediated by S6K or 4E-BP1. In rapamycin-treated skeletal muscle tissue and cells, the expression of mitochondrial transcriptional regulators such as PGC-1α is decreased along with mitochondrial gene transcription [27]. By bioinformatics analysis, the transcription factor YY1 was identified as a target of mTOR and PGC1-α in these cells. Knockdown of YY1 decreases mitochondrial gene expression and YY1 is required for the rapamycin-dependent repression of these genes. In TCR-stimulated raptor-deficient CD4+ T cells, reduced oxygen consumption rate and diminished expression of genes involved in oxidative phosphorylation are also observed [166]. Thus, mTORC1 could control mitochondrial metabolism via transcriptional mechanisms.

Other studies have shown a more direct control of mitochondrial function by mTOR [119]. mTOR can be found at the outer membrane of the mitochondria [35] and associates with the outer mitochondrial membrane proteins Bcl-xL and VDAC1, proteins that are involved in cellular apoptosis and substrate transport, respectively [119]. mTOR can phosphorylate Bcl-xL in vitro at Ser62, a site that regulates Bcl-xL activity. Although phosphorylation of VDAC has not been demonstrated, inhibition of VDAC2 in Jurkat cells generated some overlapping metabolic profile as rapamycin treatment including increased lactate, glycerol and upstream glycolytic intermediates [119]. Decreased levels of TCA cycle intermediates are also found. These findings suggest that mTORC1 inhibition could limit mitochondrial substrate availability and thus promotes diversion from mitochondrial respiration to aerobic glycolysis.

mTORC1 also controls genes involved in mitochondrial function at the level of translation. Raptor knockdown reduces mitochondrial respiration and the amounts of TCA cycle intermediates. mTORC1, but not mTORC2, induces the expression of nucleus-encoded mitochondrial proteins [105]. mTORC1 performs this function via inhibition of 4E-BP. S6K1 does not appear to affect mitochondrial respiration and glucose flux to pyruvate and lactate [105]. Knockdown of S6K1 also does not decrease expression of mitochondrial genes [27]. Thus, the mTORC1 effector 4E-BP plays a more significant role in mitochondrial metabolism.

The role of mTORC2 in oxidative and mitochondrial metabolism is poorly understood. Transformed cells that are mTORC2 addicted are highly dependent on mitochondrial functions [23]. Knockdown of rictor stimulates mitochondrial respiration while diminishing the amounts of pyruvate and lactate, suggesting a negative regulatory role in mitochondrial respiration [105]. mTORC2 localizes to mitochondria-associated endoplasmic reticulum (ER) membrane (MAM) upon growth factor stimulation [12]. In this compartment, mTORC2 controls growth factor-mediated MAM integrity, calcium flux, and mitochondrial membrane potential. This function of mTORC2 is mediated by AKT, which regulates hexokinase II, along with other proteins involved in MAM integrity. AKT can also regulate hexokinase-VDAC interaction at the outer mitochondrial membrane [50]. It was proposed that AKT increases coupling of glucose metabolism to oxidative

phosphorylation. mTORC2 at MAM is associated with ribosomes and while this suggests it is active in this compartment [12, 181], it remains unclear whether there are AKT-independent functions of mTORC2 in this compartment.

Glutamine Metabolism

Glutamine is the most abundant nonessential amino acid in the plasma and is avidly used by proliferating tumor cells. Glutamine is a versatile molecule, as it serves as a carbon source for energy production and its carbon and nitrogen are also used for biosynthetic reactions [30]. Glutamine is a precursor for α-ketoglutarate (αKG) and is used to replenish TCA intermediates (anaplerosis) in proliferating cells. Incorporation of αKG into the TCA is the major anaplerotic step in proliferating cells and important for production of oxaloacetate which reacts with acetyl CoA to produce citrate. αKG and glutamine are also precursors for nucleotides and other amino acids. Glutamine is also used for the production of UDP-GlcNAc, a metabolite produced by the hexosamine biosynthesis pathway, which, in turn is used for protein glycosylation. Glutamine is metabolized via glutaminolysis, which consists of two steps: the first is catalyzed by glutaminase (GLS) and converts glutamine to glutamate. The second is catalyzed by glutamate dehydrogenase (GDH) and converts glutamate to αKG. Oncogenic signals such as elevated levels of Myc increase glutamine uptake and metabolism through a transcriptional program that includes enhancement of expression of mitochondrial glutaminase [47, 161]. As with glucose metabolism, mTOR signaling impinges on multiple aspects of glutamine metabolism (Fig. 2.4).

mTORC1 is sensitive to glutamine levels. Glutamine, in combination with leucine activates mTORC1 by enhancing glutaminolysis and αKG production. Glutaminolysis correlates with increased mTORC1 activity and is necessary for GTP loading of RagB and activation of mTORC1 signaling. It also promotes cell growth and inhibits autophagy via regulation of mTORC1 [37]. The uptake of glutamine by the transporter SLC1A5 has also been suggested to be the rate-limiting step that activates mTOR. The heterodimeric glutamine antiporter SLC7A5/SLC3A2 (CD98) uses intracellular glutamine as an efflux substrate to regulate the uptake of leucine. This in turn leads to activation of mTORC1 [109]. On the other hand, glutamine depletion that can occur as an off target effect of using asparaginase, which has glutaminase activity, indirectly inhibits mTOR activity via decreased leucine uptake in AML [160]. Thus, although mTORC1 may not directly sense these amino acids, the above findings suggest an indirect mechanism whereby glutaminolysis activates mTORC1. Since glutaminase uses glutamine as the substrate and GDH is allosterically activated by leucine [137], the enzymes catalyzing glutaminolysis themselves instead sense glutamine and leucine directly [37].

mTORC1, via Myc, can also stimulate glutamine metabolism via regulation of transcription factors involved in expression of glutaminolysis-related genes. Deletion of Myc in T cells markedly inhibits T cell activation-induced

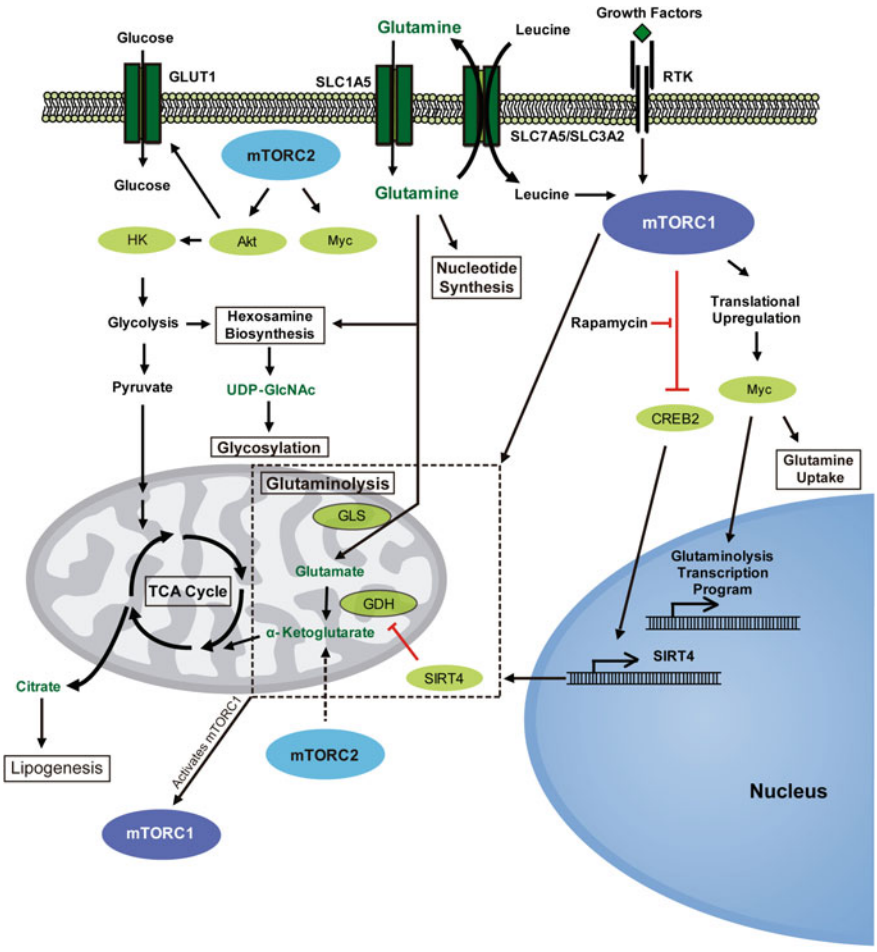


Fig. 2.4 mTORCs and glutamine metabolism. Glutamine serves as a precursor to generate molecules necessary for biosynthesis. mTORC1 itself is sensitive to levels of glutamine and leucine, both of which can drive glutaminolysis and α-ketoglutarate production, activating mTORC1. Through the translational upregulation of Myc, mTORC1 can drive the transcription of genes involved in glutaminolysis (indicated by dashed box). Further, mTORC1 directly inhibits CREB2, which drives the transcription of the GDH inhibitor, SIRT4. mTORC2 contributes to glycolytic and TCA cycle flux. The expression of Myc can be mTORC2-dependent but the mTORC2 role in glutaminolysis remains to be investigated. Additional abbreviations used here include CREB2: cAMP response element-binding protein 2, GDH: glutamine dehydrogenase, GLS: glutaminase, SIRT4: sirtuin 4, SLC1A5: solute carrier family 1 member 5, SLC7A5/SLC3A2: solute carrier family 7 member 5/solute carrier family 3 member 2 (CD98), and UDP-GlcNAc: uridine diphosphate *N*-acetylglucosamine

glutaminolysis and decreases phosphorylation of mTORC1 substrates [155]. In the Myc-deleted T cells, the transcription and translation of glutaminase 2, along with other enzymes in the glutamine catabolic pathway are downregulated [155]. Since

mTORC1 mediates translational upregulation of Myc [159], these findings further highlight a regulatory loop wherein mTORC1 regulates Myc, which in turn promotes glutaminolysis that further activates mTORC1. Another transcription factor that is regulated by mTORC1 to promote glutaminolysis is CREB2. Using bioinformatics, Csibi et al. identified a CREB2 (cAMP-responsive element-binding 2) recognition motif in the promoter region of *SIRT4*. CREB2 is a transcription factor that plays a role in metabolic processes. mTORC1 promotes the proteasome-mediated degradation of CREB2 and represses SIRT4 transcription [26]. SIRT4, which is localized in the mitochondria, is a member of the sirtuin family of nicotinamide adenine dinucleotide (NAD)-dependent enzymes that have been implicated in metabolism and longevity [56]. SIRT4 negatively regulates GDH by ADP-ribosylation. Thus, repression of SIRT4 by rapamycin treatment decreases GDH activity. These findings indicate that mTORC1 promotes glutamine metabolism via negative regulation of CREB2, which ultimately leads to activation of GDH.

Whether mTORC2 plays a role in glutamine metabolism is obscure. The expression of Myc in glioblastoma is mTORC2-dependent [101]. However, previous studies have shown that activation of PI3K and AKT is not required for glutaminolysis in Myc-expressing cells [161]. Inhibition of GDH or glutaminase does not affect phosphorylation of AKT, suggesting mTORC2 activity is intact under such conditions [37]. However, knockdown of rictor decreases levels of αKG that are likely derived from glutaminolysis, suggesting that mTORC2 could regulate this process as well [105].

Amino Acid Metabolism

Amino acids are the building blocks for protein synthesis and also serve as metabolic precursors. During nutrient-limiting conditions, protein synthesis is downregulated, autophagy is induced, and amino acid biosynthesis is enhanced via mTOR-dependent mechanisms. Several recent reviews have discussed the role of mTOR in protein synthesis and autophagy in response to the presence of amino acids [76, 96]. Much work has been done characterizing the role that amino acids play in stimulating mTOR signaling, however, there has been comparatively less work done on describing the role mTOR plays in the regulation of amino acid synthesis. Here, we will discuss its implicated role in amino acid biosynthesis.

For humans, essential amino acids must be supplied through the diet; however, nonessential amino acids can be synthesized intracellularly. An abundant supply of amino acids, that are utilized for enhanced protein synthesis and as metabolic precursors, are required by cancer cells. Despite the apparent diversity of amino acids, there is a common source of precursor molecules for their synthesis. The importance of amino acid biosynthesis in metabolic reprogramming is underscored by the use of amino acid depleting enzymes such as asparaginase as an anticancer drug. Asparaginase has been used for the treatment of pediatric and adult acute

lymphocytic leukemia as well as pediatric AML [108]. Asparaginase catalyzes the hydrolysis of the nonessential amino acid asparagine into aspartic acid and ammonia, thereby depleting the serum of asparagine. Malignant cells that are auxotrophic for asparagine (due to lower asparagine synthetase activity than normal cells) have impaired protein synthesis due to limiting amounts of this amino acid. Rapamycin treatment of cells decreases asparagine levels and gene expression of asparagine synthetase, suggesting a role for mTOR in regulating amino acid biosynthesis [114, 119]. In contrast, argininosuccinate synthetase-1 (ASS1), the rate-limiting enzyme for arginine biosynthesis, is increased by rapamycin treatment [114]. The mechanism for this is not clear, but it is interesting to note that arginine-auxotrophic tumors, such as melanoma and hepatocellular carcinoma [87], develop resistance to therapy using arginine deiminase, which degrades extracellular arginine. This resistance to the deiminase is due to elevation of ASS and these tumors become particularly sensitive to PI3K/AKT inhibitors [95]. Future studies should reveal how the mTORCs can regulate amino acid synthetases.

The mTORCs may also regulate amino acid metabolism at the level of their transporters. Genomic studies identified neutral amino acid transporters to be decreased upon rapamycin treatment [114]. In contrast, metabolic profiling of rapamycin-treated cells revealed intracellular upregulation of specific amino acids due to increased uptake via transporters rather than anabolic processes [119]. Lastly, mTORCs are indirectly involved in amino acid biosynthesis based on their role in other metabolic pathways. Intermediates from glycolysis, the pentose phosphate pathway (PPP), and the citric acid cycle supply the necessary building blocks used for synthesizing the nonessential amino acids in human cells. Since mTOR is known to regulate various steps along these pathways [38, 55, 171], it can be deduced that mTOR regulates amino acid synthesis indirectly via different mechanisms.

Pentose Phosphate Pathway and Nucleotide Synthesis

Cells utilize the PPP for two critical functions; to generate reducing equivalents in the form of NADPH and ribose-5-phosphate for nucleic acid synthesis (Fig. 2.5). About 5–30 % of glucose is metabolized via the PPP. Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the rate-limiting reaction in the PPP [143]. PPP is divided into an oxidative and a nonoxidative branch, which are irreversible and reversible, respectively. In the oxidative branch glucose-6-phosphate (G6P) is oxidized by G6PD to produce NADPH. In contrast, the nonoxidative branch is a series of reversible reactions, converting glycolytic intermediates into ribose-5-phosphate. Both pathways ultimately generate phosphoribosyl pyrophosphate, the precursor for nucleotide synthesis. Rapidly dividing cells have increased PPP activity. In addition to the requirement for pentose phosphates in nucleotide production, NADPH is also used as a reducing agent in several synthetic steps of fatty acid, cholesterol, and steroid hormones, along with detoxification reactions.

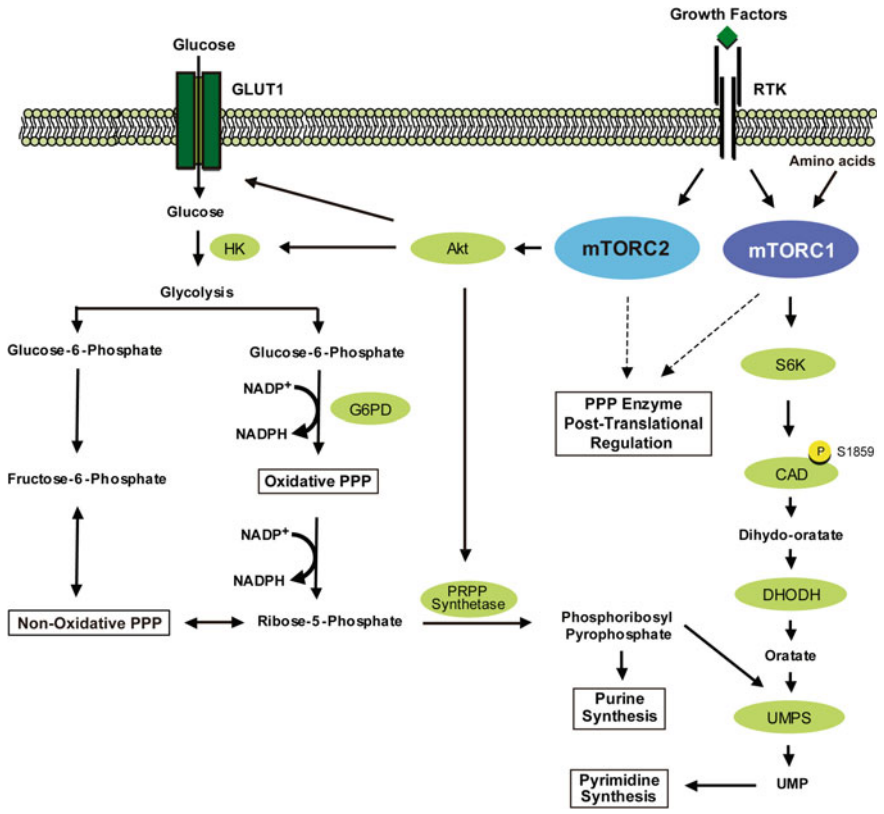


Fig. 2.5 mTORCs in the pentose phosphate pathway and nucleotide synthesis. Both mTORCs enhance transcription of enzymes involved in the PPP. mTORC2 has also been linked to posttranslational regulation of PPP enzymes. mTORC2, through AKT, promotes flux through the PPP and enhances levels of PRPP, the precursor for nucleotide synthesis. mTORC1, through S6K activity, can stimulate nucleotide synthesis through CAD, which catalyzes the initial steps of de novo synthesis. Additional abbreviations used here include: CAD: carbamoyl phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase, DHODH: dihydroorotase dehydrogenase, G6PD: glucose-6-phosphate dehydrogenase, PPP: pentose phosphate pathway, PRPP: phosphoribosyl pyrophosphate, UMP: uridine monophosphate, and UMPS: uridine monophosphate synthetase

mTORC1 can stimulate flux through the oxidative branch [38, 172]. mTORC1 is involved in this pathway via transcription of genes encoding enzymes that drive the PPP. By regulating expression of these genes, mTORC1 promotes production of ribose-5-phosphates, which are used in purine and pyrimidine nucleotide synthesis and production of NADPH.

Rapidly proliferating cells require an ample pool of nucleotides which are critical for cellular processes such as ribosome biogenesis. These pools are synthesized through two pathways: the salvage pathway, which generates nucleotides

from degradation intermediates and the de novo synthesis pathway, which assembles complex nucleotides from basic molecules. Utilizing phosphoproteomic and metabolomic profiling approaches, mTORC1 has been linked to production of pyrimidines via de novo pathways [7, 123]. mTORC1, via phosphorylation of S6K1, promotes activation of CAD (carbamoyl phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase). S6K1 directly phosphorylates CAD on Ser1859. In raptor-, but not rictor-depleted MEFs, CAD phosphorylation is abrogated, demonstrating that this phosphorylation is mTORC1-specific [123]. CAD catalyzes the initial steps of pyrimidine synthesis by utilizing glutamine, bicarbonate, and aspartic acid to generate pyrimidine rings. mTORC1 and S6K1-mediated phosphorylation of CAD enhances its oligomerization [123]. De novo pyrimidine synthesis is enhanced by mTORC1 and S6K in response to growth factor or amino acid stimulation, although they are not essential for de novo synthesis per se [7].

mTORC2 also plays a role in the PPP. Hyperactive mTORC2 can result in increased flux through the PPP. AKT phosphorylates hexokinase and drives its association with the mitochondria, where hexokinase can phosphorylate glucose, and thus elevate the levels of G6P, the substrate for the PPP [144]. Moreover, in an insulin-driven model of hepatocellular carcinoma cells, Evert et al. [42] have shown that AKT drives the upregulation of the PPP through several mechanisms, including via increase of phosphate dehydrogenase and ribose 5-phosphate isomerase A expression and activity, as well as through driving glycolysis. A significant role for mTORC2 in regulating the PPP was recently demonstrated using a chemical genetic screen. The yeast TORC2 specifically interacts with the PPP [83]. Proteins that play a role in the PPP physically associate with TORC2. Furthermore, metabolic intermediates such as 6-phospho-D-gluconate (6PG) and ribose-5-phosphate are strongly downregulated in response to TOR2 inhibition. Since the decrease in PPP metabolite levels is rapid, it was proposed that TORC2 likely plays a posttranslational role in the regulation of the PPP.

So far, the role of mTORC2 in nucleotide metabolism is likely via stabilization of AKT, which regulates purine synthesis [156]. The PI3K/AKT signaling axis regulates the early steps of the nonoxidative PPP at the level of phosphoribosylpyrophosphate (PRPP) synthesis and later steps by modulating the activity of aminoimidazole-carboxamide ribonucleotide transformylase IMP cyclohydrolase [156]. Whether mTORC2 has a more direct role in regulating the expression or activity of the enzymes involved in nucleotide synthesis remains to be elucidated.

Lipid Metabolism

Cancer cells undergo increased de novo lipid synthesis. Production of lipids and fatty acids are enhanced for biosynthesis of membranes and signaling molecules. Cell membrane lipids including phospholipids, sterols, sphingolipids, and lyso-phospholipids are derived in part from acetyl CoA. The enhanced glutamine

metabolism that occurs in cancer cells leads to elevated citrate production. Citrate, in turn, is exported from the mitochondria to the cytosol. Cytosolic citrate is processed by ATP citrate lyase (ACL) to generate cytosolic acetyl CoA, the building block for endogenous synthesis of acyl groups and sterols. Indeed, ACL expression is found upregulated in a number of cancers [6, 104, 157].

Early studies on the use of rapamycin in transplantation have revealed that an adverse side effect of mTOR inhibition is hyperlipidemia [162], underscoring the role of mTOR in lipid metabolism (Fig. 2.6). Although the in vivo studies imply a negative regulatory role for mTOR in lipid biosynthesis, cellular studies have revealed the opposite. Rapamycin reduces the expression of acetyl CoA carboxylase, fatty acid synthase and stearoyl CoA desaturase, which are lipogenic enzymes whose transcription is targeted by the transcription factor SREBP (sterol regulatory element-binding protein) [16, 102, 114]. Rapamycin also elevates levels of glycerol

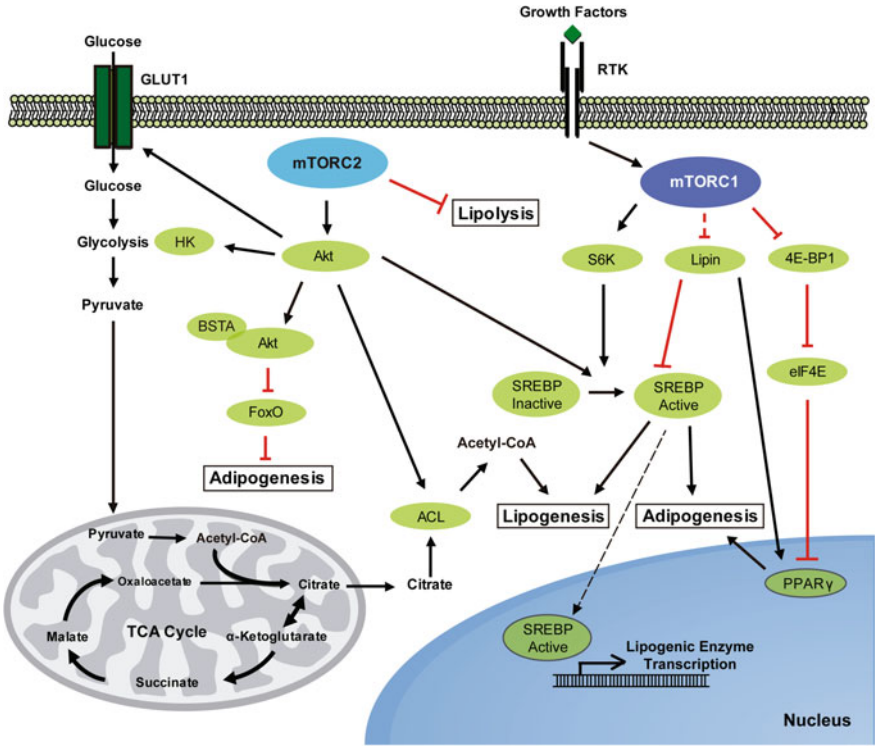


Fig. 2.6 mTORCs and lipid metabolism. mTORC1, through regulation of downstream effectors such as SREBP and PPARγ, modulates lipogenesis and adipogenesis. mTORC2 regulates lipogenesis via an AKT-dependent and -independent manner. mTORC2, via AKT can stimulate lipogenesis by enhancing glycolytic and TCA flux and promotes adipogenesis by enhancing Acetyl CoA levels and FoxO inhibition. Additional abbreviations include: ACL: ATP citrate lyase, PDH: pyruvate dehydrogenase, PPARγ: peroxisome proliferator-activated receptor γ, SREBP: sterol regulatory element-binding proteins

and promotes accumulation of acetyl CoA and malonyl CoA, which are substrates for lipid synthesis [119]. These findings suggest that the hyperlipidemia observed upon rapamycin treatment in vivo is probably not due to increased synthesis in the liver but is due to delayed peripheral clearance [16]. Indeed, numerous studies support a positive role for mTOR in lipid biosynthesis [88, 121].

Several studies have uncovered a role for mTORC1 in lipogenesis [38, 116, 166]. In cells expressing active AKT, the induction of lipid synthesis is dependent on mTORC1 [116]. In TSC2-deficient fibroblasts where mTORC1 activity is upregulated, there is a rapamycin-sensitive increase in de novo lipid biosynthesis [38]. A more direct analysis in liver-specific *raptor* knockout confirmed the requirement for mTORC1 in lipogenesis [154]. Similarly, raptor-deficient $CD4^+$ T cells have defective de novo lipid synthesis and fails to induce genes involved in lipogenic pathways upon TCR stimulation [166]. In T regulatory cells (T_{regs}), mTORC1 promotes cholesterol and lipid metabolism, with the mevalonate pathway being particularly important for T_{reg} proliferation and upregulation of mediators important for suppressive function [175]. Several studies support that the mTORC1 function in lipid metabolism is mediated via SREBP. An enrichment in DNA binding elements that recognize the transcription factor SREBP is found in rapamycin-sensitive genes identified in microarray studies using TSC-deficient cells [38]. There are three isoforms of SREBP in mammalian cells, SREBP1a, SREBP1c, and SREBP-2 that regulate distinct, yet overlapping transcriptional programs governing lipid synthesis. In raptor-deficient T cells, the protein expression of both SREBP1 and SREBP2 is attenuated [166]. In hepatocytes, the insulin-mediated increase in SREBP-1c mRNA is mTORC1-dependent but S6K-independent [113, 154]. Thus, mTORC1 can regulate SREBP at the level of transcription and protein expression to control lipogenesis.

SREBPs are synthesized as inactive precursors that reside in the ER and translocate to the nucleus after processing from the Golgi. This active processed form induces transcription of SRE-containing target genes. The processing step is thus sensitive to sterol levels and controlled by mTORC1 signaling. An enhancement of processed forms of SREBP1 has been observed in the TSC-deficient cells and the mTORC1 target S6K1 has been shown to regulate SREBP processing [38, 113]. The stability of its processed active form has also been linked to AKT regulation [116]. mTORC1 also regulates SREBP by controlling the nuclear localization of lipin 1, a phosphatidic acid phosphatase that represses SREBP activity [115]. Thus, mTORC1 signals promote accumulation of the active form of SREBP, serving as another mode of regulation of lipogenesis by mTORC1.

In addition to SREBP, mTORC1 has been linked to the regulation of peroxisome proliferator-activated receptor γ (PPARγ). Upregulation of the mTORC1 pathway, such as by inactivation of TSC2, promotes adipocyte differentiation, in which PPARγ plays a central role [178]. The mTORC1 effectors 4E-BP1 and S6K can in part mediate this function of mTORC1 in adipogenesis. mTORC1 can also increase the activity of lipin 1, which promotes triglyceride synthesis and enhances the PPARγ adipogenic activity [70]. How this function of mTORC1 in adipogenesis becomes deregulated in cancer is not clear but reducing fatty acid availability to

cancer cells by diverting them to storage pathways or blocking their release from storage could be promising as therapeutic targets [28].

The role of mTORC2 in lipid metabolism is revealed in studies from knockout models using different organisms. In yeast, the TORC2 component, Avo3 (rictor orthologue) regulates ceramide and downstream sphingolipid synthesis [2]. Sphingolipids are a major component of cell membranes and are required for cell growth. In *C. elegans,* a high body fat phenotype occurs in Rictor null worms, which is independent of AKT, but rather dependent on SGK1 [79, 142]. In mice, knockout of *rictor* specifically in the liver decreases lipogenesis [55, 174]. Hagiwara et al. have shown that in these mice, there is a loss of SREBP1c activity and in turn, loss of lipogenesis, suggesting that there is an mTORC2 specific role for the regulation of lipogenesis, as these mice have functional mTORC1. Further, expression of a constitutively active AKT is able to rescue de novo lipogenesis [55]. In glucose stimulated hematopoietic cells, knockdown of ACL impairs AKT-induced tumorigenesis [4]. ACL is itself phosphorylated by AKT [11]. The requirement for AKT in lipogenesis is strengthened by the findings that mTORC1 activation is not sufficient to stimulate lipogenesis under conditions wherein AKT signaling is downregulated [154, 172]. On the other hand, Yuan et al. [174] reported that active AKT is unable to drive hepatic lipogenesis in the absence of mTORC2. Thus, mTORC2 plays an additional role in lipogenesis that is independent of AKT.

mTORC2 also plays a role in adipogenesis. The BSD domain containing protein, BSTA, is targeted by mTORC2 and its interaction with AKT enhances its activation and suppression of FoxC2, driving adipogenesis [169]. Given the link between obesity and cancer, the impact of mTOR signaling in adipogenesis and cancer progression needs further investigation.

Acetylation

Protein modifications such as acetylation are dependent on the generation of metabolites. In addition to its use in lipogenesis, acetyl CoA is a key metabolite for acetylation and is also used to generate metabolites required for glycosylation [158]. Protein acetylation is a critical posttranslation modification in the regulation of various cellular processes. Analogous to phosphorylation, acetylation can modify protein function and interactions. Histone acetylation is the most common example of protein acetylation, in which the acetylation and deacetylation of lysine residues alter the interaction of histones with DNA, providing access for or inhibiting transcription of specific genes. Thus, levels of acetyl CoA could have profound effects on epigenetic regulation. As discussed above under lipid metabolism, the PI3K/AKT pathway plays a role in the production of acetyl CoA via regulation of ACL. Whether mTORC2 functions in regulating ACL remains unclear. Nevertheless, mTORC2 has a role in the indirect regulation of acetylation. Recent work has shown that mTORC2 regulates class IIa histone deacetylases [101]. Using a model of glioblastoma, this regulation of histone deacetylases occurs via

phosphorylation of histone deacetylases, which suppresses acetylation of FoxO, ultimately resulting in an increase in c-Myc expression and metabolic reprogramming [101]. Histone acetylation changes that precede tumor development are also stimulated by AKT and that this effect of AKT is mediated via ACL [91]. Since the mTORC2-mediated AKT phosphorylation at Ser473 correlated with histone acetylation marks in gliomas and prostate tumors, these findings also support a role for mTORC2 in acetylation. Interestingly, multiple sites of acetylation have been identified on the mTORC2 subunit Rictor [48]. Glidden et al. [48] identified a region along Rictor that is the target of acetylation and that the consequence of this acetylation is increased mTORC2 activity, as determined by stimulation of AKT activity. These findings reveal a regulatory loop wherein mTORC2 can control and is controlled by acetylation.

Clinical Relevance

mTOR sits within a key regulatory node, integrating extracellular cues such as growth and stress signals to control cell growth, metabolism and proliferation. This role makes mTOR a viable target for cancer therapeutic intervention (Table 2.1). Deregulated activation of the mTOR pathway is prevalent in many types of cancer. Mutations in mTOR itself that increase its activity have been found in solid tumors [51, 153]. Furthermore, mutations in upstream regulators of mTOR occur frequently in human tumors, including the oncogene *PIK3CA* (encoding PI3K) and the tumor suppressor *PTEN* [163]. There are also other genetic lesions that induce activation of the PI3K/mTOR pathway in cancer cells such as those encoding Ras, AKT, TSC1/2, Notch1, and receptor tyrosine kinases [10, 138]. Mutations and genomic alterations in metabolic enzymes and other key regulators of metabolic pathways of which mTOR has been linked are also common in cancers [54, 136]. Altogether, these mutations reprogram metabolism to favor biosynthetic processes crucial for the growth and proliferation of cancer cells. Hence, there is substantial effort to test MTI in the clinic and utilize existing drugs that target the pathways that become deregulated due to the above mutations (Table 2.1). More importantly, research efforts are geared toward improving cancer therapeutic strategies via better understanding of the growth and metabolic signaling network and identifying predictive biomarkers. This would allow more specific targeting using single agent therapies and/or more efficacious approach by combined therapeutic approaches. A more targeted approach holds promise particularly when specific mutations driving the malignancy are identified and the gene products are druggable. In support of this notion, a recent Phase I study wherein a urothelial cancer patient had an exceptional response to the mTOR inhibitor, everolimus, turned out to have activating mutations in mTOR [153]. In another study that analyzed gene expression patterns of patients with different types of gastric cancer, it was found that tumors of the mesenchymal subtype are particularly sensitive to MTI [92]. Whether common mutations in the mTOR/PI3K pathway in this cancer subtype occur

Table 2.1 Compounds currently being evaluated as anticancer agents targeting mTOR, as well as other aspects of cellular metabolism

Drug	Target	Mechanism of action	Clinical trial/status
mTOR inhibitors			
AZD8055	mTORC1/2	Inhibits mTOR signaling	NCT01316809, NCT00973076, NCT00999882, NCT00731263
Everolimus	mTORC1	Inhibits mTOR signaling	NCT00510068, NCT00912340, NCT00410124
MLN0128	mTORC1/2	Inhibits mTOR signaling	NCT01058707
OSI-027	mTORC1/2	Inhibits mTOR signaling	NCT00698243
Ridaforolimus	mTORC1	Inhibits mTOR signaling	NCT00770185, NCT00736970, NCT01234857, NCT01605396
Temsirolimus	mTORC1	Inhibits mTOR signaling	NCT01111825, NCT00909831
Glucose metabolism (glycolysis)			
2-deoxyglucose	Hexokinase	Inhibits glucose flux	NCT00096707, NCT00633087, NCT00247403
AZD-3965	MCT1	Inhibits lactate transport	NCT01791595
Dichloroacetate	PDK	Promotes oxidative metabolism	NCT00566410, NCT00703859, NCT00540176, NCT01163487
Metformin	AMPK	Impairs glucose metabolism	NCT02149459, NCT2145559, NCT02048384
PX-478	HIF1α	Decrease HIF1α levels	NCT00522652
TLN-232	PKM2	Inhibits anaerobic glycolysis	NCT00422786
Oxidative and mitochondrial metabolism			
AG-120	IDH1/2	Inhibits mutant IDH1/2	NCT02073994, NCT02074839
Dichloroacetate	PDK	Modulates mitochondrial metabolism	NCT00566410, NCT00703859, NCT00540176, NCT01163487
Metformin	Mitochondrial complex I	Inhibits oxidative metabolism	NCT02145559, NCT02048384
Glutamine metabolism			
L-asparaginase	Asparagine	Impairs glutamine uptake	Approved agent
Amino acid metabolism			
L-asparaginase	Asparagine	Depletes asparagine	Approved agent
Methotrexate	DHFR	Impairs folate metabolism	Approved agent
Pentose phosphate pathway and nucleotide synthesis			
5-fluorouracil			Approved agent

(continued)

Table 2.1 (continued)

Drug	Target	Mechanism of action	Clinical trial/status
	Thymidylate synthase	Irreversibly inhibits thymidine synthesis	
Gemcitabine	Nucleoside analog	Impedes nucleotide incorporation	Approved agent
Hydroxyurea	Ribonucleotide reductase	Impairs deoxyribonucleotide production	Approved agent

remains to be examined. Nevertheless, these studies support the concept that more effective therapy could be achieved as we gain better understanding of the molecular basis of tumor heterogeneity.

The clinical use of rapamycin is limited due to poor water solubility and stability. Thus, several pharmaceutical companies have developed rapamycin analogs (rapalogues) with improved pharmacokinetic properties. Rapalogues are already being used for the treatment of specific types of cancers and are also undergoing clinical trials for a number of different types of malignancies. We refer the reader to previous excellent reviews on rapamycin clinical trials and we focus our discussion here on more recent findings [8, 36]. Everolimus (Afinitor, RAD001; Novartis) has been efficacious for the treatment of renal cell carcinoma [106], subependymal giant cell astrocytoma and angiomyolipoma in tuberous sclerosis [13, 84]. Temsirolimus (Torisel, CCI779; Wyeth) is currently approved for advanced renal cell carcinoma and refractory mantle cell lymphoma [63]. Everolimus significantly prolonged progression-free survival (PFS) among patients with advanced pancreatic neuroendocrine tumors in a Phase III study [168], making everolimus the first effective treatment in prolonging the life of patients with this type of cancer. Ridaforolimus (A23573, deforolimus; Merck/Ariad) delayed tumor progression albeit modestly in patients with metastatic sarcoma [32]. It also has antitumor activity in advanced endometrial cancer patients in a Phase II clinical trial [24]. However, in most cancer types, rapalogues only stabilize the disease. Studies using cell lines have provided clues on possible molecular basis for this. Rapamycin and its analogs do not inhibit all the functions of mTORC1 and can inhibit mTORC2 indirectly only in certain cell types [21, 45, 129, 150]. Furthermore, inhibiting mTORC1 can also trigger a feedback loop that activates the PI3K/AKT pathway [60]. In addition, there are likely other bypass mechanisms or alternative pathways that tumor cells employ to acquire resistance against mTOR inhibition and evade cell death [68]. Thus, identifying such mechanisms would provide additional viable targets.

Indeed, although rapalogues as a single agent therapy have not lived up to expectations, combination therapy is yielding more promising clinical results. Everolimus in combination with the aromatase inhibitor, exemestane, is now approved for the treatment of ER-positive, HER2-negative advanced breast cancer resistant to nonsteroidal aromatase inhibitors based on extended median PFS of patients treated with everolimus + exemestane compared to placebo + exemestane

in the BOLERO-2 Phase III trial [170]. In the BOLERO-3 trial, use of everolimus in addition to trastuzumab and vinorelbine also significantly prolonged the PFS of patients with trastuzumab-resistant, taxane pretreated, HER2-positive advanced breast cancer [1]. Several clinical trials are ongoing to evaluate rapalogues in different breast cancer types either as combination or adjuvant/neoadjuvant therapy [75]. Temsirolimus in combination with the autophagy inhibitor hydroxychloroquine gave significant antitumor activity in melanoma patients in a Phase I clinical trial [120].

When combined with conventional chemotherapeutic agents (such as doxorubicin, camptothecin, paclitaxel, carboplatin, cisplatin, and vinorelbine), preclinical studies have revealed that rapamycin and rapalogues have enhanced antitumor activity. Thus, rapalogues in combination with chemotherapeutic agents are undergoing clinical trials for a number of different cancers. Phase I clinical trials have been conducted for use of everolimus with conventional chemotherapeutic agents for the treatment of advanced/metastatic pancreatic cancer [78], recurrent/metastatic squamous cell carcinoma of the head and neck [124], cholangiocarcinoma [25]. Phase II clinical trials of everolimus with carboplatin for the treatment of patients with triple negative breast cancer demonstrated efficacy [141].

While the use of rapalogs have mainly cytostatic effects, use of pan-mTOR kinase inhibitors either by itself or in combination with other targeted therapies and chemotherapeutic agents have been more successful in inducing cytotoxicity in preclinical studies. Unlike rapamycin, which allosterically inhibits mTOR and blocks some of mTORC1 functions, the ATP-competitive or active site inhibitors selectively target the ATP-binding pocket of mTOR and thus inhibit the catalytic activity of both mTORC1 and mTORC2 [45, 150]. The pan-mTOR inhibitors are currently undergoing a number of preclinical and early clinical trials. In U87-MG glioma xenografts, treatment with AZD8055 (AstraZeneca) led to a rapid decrease in uptake of labeled glucose, suggesting that this response can be used as an early biomarker for the metabolic changes that occur upon mTOR inhibition [80]. The mTOR kinase domain is structurally related to PI3K. Thus, some PI3K inhibitors can block both mTOR and PI3K activity. Dual mTOR/PI3K inhibitors are also undergoing early clinical trials. A clinical trial investigating the combination of mTOR and p100α-specific PI3K inhibition in non-hematological cancers is ongoing (NCT01899053) based on preclinical studies demonstrating that mTORC1 inhibition was required for PI3K p100α inhibitor sensitivity in breast cancer cells harboring *PIK3CA* mutations [40].

The activation of the mTOR pathway in cancers leads to altered expression and/or activity of a number of metabolic enzymes. This represents another attractive strategy particularly since antimetabolic agents have been in clinical use to effectively treat various cancers. For example, folate analogs were one of the first agents to cure liquid and solid tumors and remain in use as adjuvant therapy and for the management of several cancers [44, 93]. In preclinical studies, combined methotrexate (an antifolate) and MTI were synergistically effective for treatment of ALL in xenograft mouse models [147]. Gemcitabine, a nucleoside analog, is used in various cancers. Phase II clinical trials are ongoing that combine gemcitabine

with MTI based on promising results from a Phase I study on patients with advanced solid tumors and xenografts of sarcoma and leiomyosarcoma [100]. The antidiabetic drug, metformin, which acts by inhibiting the mitochondrial complex I and suppressing glucose production in the liver, has also been combined with MTI in several clinical studies. In a phase I clinical trial combining metformin and temsirolimus, one of 11 enrolled patients experienced partial response and five of the 11 patients experienced stable disease for 22 months [97]. Additional studies are ongoing investigating the combination of metformin and sirolimus in advanced solid tumors (NCT02145559), as well as metformin and rapamycin in pancreatic cancer (NCT02048384) (Table 2.1).

As we continue to gain insights from current preclinical and clinical studies about the role of mTOR in cancer, we can better design agents and combinatorial strategies to combat oncogenesis and tumor progression. Further understanding of the unbalanced metabolic processes that uniquely occur in different cancer subtypes and perhaps even at the individual level would pave the way for more rational therapeutic strategies with better treatment specificity and efficacy while having minimal toxicity.

Conclusions and Future Perspectives

The mTORC complexes control metabolic pathways at different levels, from transcription, translation, and posttranslational mechanisms. Most of the mTORC functions in cellular metabolism that have been uncovered point to the regulation of metabolic enzymes, transcription factors, and other effectors that ultimately modulate metabolite production and/or flux through a metabolic and biosynthetic pathways. While direct regulation of the mTORCs by nutrients remains elusive, most of the metabolic enzymes and effectors that are mTORC-dependent are allosterically regulated by nutrients or metabolites or utilize nutrients as substrates. Localization of mTORCs and association with specific regulators and metabolic enzymes in cellular compartments could serve to acutely modulate mTORC activity while triggering a cascade of events that ultimately induce gene expression of metabolic effectors.

An outstanding question is how the two mTOR complexes can overlap or diverge in the regulation of metabolic pathways. A number of studies using rapamycin, particularly in vivo models, have employed prolonged treatment of this drug, which can also inhibit mTORC2 under such conditions [129]. Furthermore, studies on mTORC2 and its function in metabolism, in comparison to mTORC1, are lagging behind. Thus, the function of mTORC2 on metabolism is so far underestimated. Knockout mouse models are shedding light on the distinct functions of these two complexes. Combined metabolomics and genomics using these mouse models along with cancer models would further enhance our understanding on the function of these two complexes. Development of specific inhibitors for each complex would also accelerate our analysis of their metabolic functions.

Phosphoproteomic studies have identified a number of mTOR targets, both directly and indirectly [65, 123, 173]. A number of these targets are involved in metabolic pathways and protein synthesis. Most of the mTORC metabolic targets that have been characterized and described herein appear to be indirectly regulated by mTOR. Although a number of transcriptional targets involved in metabolism have been pulled out, it remains unclear how mTORCs can regulate transcription in a more direct manner, i.e., whether its protein kinase activity is required. In some cases, it has been shown that the function of mTORCs is mediated by their canonical substrates such as AKT and S6K1. Given the predominant role of both mTORCs in control of protein synthesis, future studies should address precisely how metabolic enzymes and effectors could be regulated at the level of translation. Subcellular compartmentalization of mTORCs is emerging to play a key role in how mTOR integrate nutrient signals with growth and metabolic pathways. Thus, how mTORCs become recruited to membrane compartments and whether they control protein synthesis in such compartments would provide important clues on its regulation and functions in response to a specific nutrient. Further understanding of the structure of mTOR in complex with its partners would also shed light on its activation mechanisms and development of more specific MTI [165].

Lastly, we are just beginning to understand how oncogenic mutations can trigger metabolic reprogramming and the role mTOR plays in these metabolic processes. Future studies should further delineate how the mTORCs reprogram metabolic and biosynthetic pathways under specific oncogenic mutations. It has been recognized early on that although rapamycin by itself does not induce apoptosis, it augmented apoptosis and increased sensitivity to chemotherapeutic agents [140]. Nevertheless, tumors develop resistance to mTOR/PI3K inhibition due to induction of alternative pathways [107]. Identifying synthetic–lethal interactions and drug resistance mechanisms inherent to metabolic or growth signaling pathways upon mTOR inhibition will be important in order to develop more effective cancer therapy.

Acknowledgments The authors gratefully acknowledge funding from the NJ Commission on Cancer Research (TL), NIH (GM079176 and CA154674) and AACR/Stand Up to Cancer-Innovative Research Grant (IRG0311) (EJ). Stand Up to Cancer is a program of the Entertainment Industry Foundation administered by the American Association for Cancer Research.

References

1. Andre F, O'Regan R, Ozguroglu M, Toi M, Xu B, Jerusalem G, Masuda N, Wilks S, Arena F, Isaacs C et al (2014) Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Oncol 15:580–591
2. Aronova S, Wedaman K, Aronov PA, Fontes K, Ramos K, Hammock BD, Powers T (2008) Regulation of ceramide biosynthesis by TOR complex 2. Cell Metab 7:148–158
3. Barbet NC, Schneider U, Helliwell SB, Stansfield I, Tuite MF, Hall MN (1996) TOR controls translation initiation and early G1 progression in yeast. Mol Biol Cell 7:25–42

4. Bauer DE, Hatzivassiliou G, Zhao F, Andreadis C, Thompson CB (2005) ATP citrate lyase is an important component of cell growth and transformation. Oncogene 24:6314–6322
5. Beck T, Hall MN (1999) The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. Nature 402:689–692
6. Beckner ME, Fellows-Mayle W, Zhang Z, Agostino NR, Kant JA, Day BW, Pollack IF (2010) Identification of ATP citrate lyase as a positive regulator of glycolytic function in glioblastomas. Int J Cancer (Journal international du cancer) 126:2282–2295
7. Ben-Sahra I, Howell JJ, Asara JM, Manning BD (2013) Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. Science 339:1323–1328
8. Benjamin D, Colombi M, Moroni C, Hall MN (2011) Rapamycin passes the torch: a new generation of mTOR inhibitors. Nat Rev Drug Discov 10:868–880
9. Beretta L, Gingras AC, Svitkin YV, Hall MN, Sonenberg N (1996) Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. EMBO J 15:658–664
10. Bertrand FE, McCubrey JA, Angus CW, Nutter JM, Sigounas G (2014) NOTCH and PTEN in prostate cancer. Adv Biol Regul 56:51–65
11. Berwick DC, Hers I, Heesom KJ, Moule SK, Tavare JM (2002) The identification of ATP-citrate lyase as a protein kinase B (AKT) substrate in primary adipocytes. J Biol Chem 277:33895–33900
12. Betz C, Stracka D, Prescianotto-Baschong C, Frieden M, Demaurex N, Hall MN (2013) Feature Article: mTOR complex 2-AKT signaling at mitochondria-associated endoplasmic reticulum membranes (MAM) regulates mitochondrial physiology. Proc Natl Acad Sci USA 110:12526–12534
13. Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E, Sauter M, Nonomura N, Brakemeier S, de Vries PJ et al (2013) Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet 381:817–824
14. Blommaart EF, Luiken JJ, Blommaart PJ, van Woerkom GM, Meijer AJ (1995) Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes. J Biol Chem 270:2320–2326
15. Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL (1994) A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Nature 369:756–758
16. Brown NF, Stefanovic-Racic M, Sipula IJ, Perdomo G (2007) The mammalian target of rapamycin regulates lipid metabolism in primary cultures of rat hepatocytes. Metabolism 56:1500–1507
17. Brugarolas JB, Vazquez F, Reddy A, Sellers WR, Kaelin WG Jr (2003) TSC2 regulates VEGF through mTOR-dependent and -independent pathways. Cancer Cell 4:147–158
18. Cardenas ME, Cutler NS, Lorenz MC, Di Como CJ, Heitman J (1999) The TOR signaling cascade regulates gene expression in response to nutrients. Genes Dev 13:3271–3279
19. Chen CH, Kiyan V, Zhylkibayev AA, Kazyken D, Bulgakova O, Page KE, Bersimbaev RI, Spooner E, dos Sarbassov D (2013) Autoregulation of the mechanistic target of rapamycin (mTOR) complex 2 integrity is controlled by an ATP-dependent mechanism. J Biol Chem 288:27019–27030
20. Chen ML, Xu PZ, Peng XD, Chen WS, Guzman G, Yang X, Di Cristofano A, Pandolfi PP, Hay N (2006) The deficiency of AKT1 is sufficient to suppress tumor development in Pten± mice. Genes Dev 20:1569–1574
21. Choo AY, Yoon SO, Kim SG, Roux PP, Blenis J (2008) Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. Proc Natl Acad Sci USA 105:17414–17419
22. Chung J, Kuo CJ, Crabtree GR, Blenis J (1992) Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. Cell 69:1227–1236

23. Colombi M, Molle KD, Benjamin D, Rattenbacher-Kiser K, Schaefer C, Betz C, Thiemeyer A, Regenass U, Hall MN, Moroni C (2011) Genome-wide shRNA screen reveals increased mitochondrial dependence upon mTORC2 addiction. Oncogene 30:1551–1565
24. Colombo N, McMeekin DS, Schwartz PE, Sessa C, Gehrig PA, Holloway R, Braly P, Matei D, Morosky A, Dodion PF et al (2013) Ridaforolimus as a single agent in advanced endometrial cancer: results of a single-arm, phase 2 trial. Br J Cancer 108:1021–1026
25. Costello BA, Borad MJ, Qi Y, Kim GP, Northfelt DW, Erlichman C, Alberts SR (2014) Phase I trial of everolimus, gemcitabine and cisplatin in patients with solid tumors. Invest New Drugs 32:710–716
26. Csibi A, Fendt SM, Li C, Poulogiannis G, Choo AY, Chapski DJ, Jeong SM, Dempsey JM, Parkhitko A, Morrison T et al (2013) The mTORC1 pathway stimulates glutamine metabolism and cell proliferation by repressing SIRT4. Cell 153:840–854
27. Cunningham JT, Rodgers JT, Arlow DH, Vazquez F, Mootha VK, Puigserver P (2007) mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. Nature 450:736–740
28. Currie E, Schulze A, Zechner R, Walther TC, Farese RV Jr (2013) Cellular fatty acid metabolism and cancer. Cell Metab 18:153–161
29. Dang CV, O'Donnell KA, Zeller KI, Nguyen T, Osthus RC, Li F (2006) The c-Myc target gene network. Semin Cancer Biol 16:253–264
30. Daye D, Wellen KE (2012) Metabolic reprogramming in cancer: unraveling the role of glutamine in tumorigenesis. Semin Cell Dev Biol 23:362–369
31. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB (2008) The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab 7:11–20
32. Demetri GD, Chawla SP, Ray-Coquard I, Le Cesne A, Staddon AP, Milhem MM, Penel N, Riedel RF, Bui-Nguyen B, Cranmer LD et al (2013) Results of an international randomized phase III trial of the mammalian target of rapamycin inhibitor ridaforolimus versus placebo to control metastatic sarcomas in patients after benefit from prior chemotherapy. J Clin Oncol 31:2485–2492
33. Demetriades C, Doumpas N, Teleman AA (2014) Regulation of TORC1 in response to amino acid starvation via lysosomal recruitment of TSC2. Cell 156:786–799
34. Dennis PB, Jaeschke A, Saitoh M, Fowler B, Kozma SC, Thomas G (2001) Mammalian TOR: a homeostatic ATP sensor. Science 294:1102–1105
35. Desai BN, Myers BR, Schreiber SL (2002) FKBP12-rapamycin-associated protein associates with mitochondria and senses osmotic stress via mitochondrial dysfunction. Proc Natl Acad Sci USA 99:4319–4324
36. Don AS, Zheng XF (2011) Recent clinical trials of mTOR-targeted cancer therapies. Rev Recent Clin Trials 6:24–35
37. Duran RV, Oppliger W, Robitaille AM, Heiserich L, Skendaj R, Gottlieb E, Hall MN (2012) Glutaminolysis activates Rag-mTORC1 signaling. Mol Cell 47:349–358
38. Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, Triantafellow E, Ma Q, Gorski R, Cleaver S et al (2010) Activation of a metabolic gene regulatory network downstream of mTOR complex 1. Mol Cell 39:171–183
39. Edinger AL, Linardic CM, Chiang GG, Thompson CB, Abraham RT (2003) Differential effects of rapamycin on mammalian target of rapamycin signaling functions in mammalian cells. Cancer Res 63:8451–8460
40. Elkabets M, Vora S, Juric D, Morse N, Mino-Kenudson M, Muranen T, Tao J, Campos AB, Rodon J, Ibrahim YH et al (2013) mTORC1 inhibition is required for sensitivity to PI3K p110alpha inhibitors in PIK3CA-mutant breast cancer. Sci Transl Med 5:196ra199
41. Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR, Zhuang H, Cinalli RM, Alavi A, Rudin CM et al (2004) AKT stimulates aerobic glycolysis in cancer cells. Cancer Res 64:3892–3899
42. Evert M, Calvisi DF, Evert K, De Murtas V, Gasparetti G, Mattu S, Destefanis G, Ladu S, Zimmermann A, Delogu S et al (2012) V-AKT murine thymoma viral oncogene

homolog/mammalian target of rapamycin activation induces a module of metabolic changes contributing to growth in insulin-induced hepatocarcinogenesis. Hepatology 55:1473–1484
43. Facchinetti V, Ouyang W, Wei H, Soto N, Lazorchak A, Gould C, Lowry C, Newton AC, Mao Y, Miao RQ et al (2008) The mammalian target of rapamycin complex 2 controls folding and stability of AKT and protein kinase C. EMBO J 27:1932–1943
44. Farber S, Diamond LK (1948) Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid. New Engl J Med 238:787–793
45. Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, Shokat KM (2009) Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol 7:e38
46. Frias MA, Thoreen CC, Jaffe JD, Schroder W, Sculley T, Carr SA, Sabatini DM (2006) mSin1 is necessary for AKT/PKB phosphorylation, and its isoforms define three distinct mTORC2s. Curr Biol 16:1865–1870
47. Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT et al (2009) c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature 458:762–765
48. Glidden EJ, Gray LG, Vemuru S, Li D, Harris TE, Mayo MW (2012) Multiple site acetylation of Rictor stimulates mammalian target of rapamycin complex 2 (mTORC2)-dependent phosphorylation of AKT protein. J Biol Chem 287:581–588
49. Gordan JD, Thompson CB, Simon MC (2007) HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. Cancer Cell 12:108–113
50. Gottlob K, Majewski N, Kennedy S, Kandel E, Robey RB, Hay N (2001) Inhibition of early apoptotic events by AKT/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. Genes Dev 15:1406–1418
51. Grabiner BC, Nardi V, Birsoy K, Possemato R, Shen K, Sinha S, Jordan A, Beck AH, Sabatini DM (2014) A diverse array of cancer-associated MTOR mutations are hyperactivating and can predict rapamycin sensitivity. Cancer Discov 4:554–563
52. Graves LM, Bornfeldt KE, Argast GM, Krebs EG, Kong X, Lin TA, Lawrence JC Jr (1995) cAMP- and rapamycin-sensitive regulation of the association of eukaryotic initiation factor 4E and the translational regulator PHAS-I in aortic smooth muscle cells. Proc Natl Acad Sci USA 92:7222–7226
53. Grolleau A, Bowman J, Pradet-Balade B, Puravs E, Hanash S, Garcia-Sanz JA, Beretta L (2002) Global and specific translational control by rapamycin in T cells uncovered by microarrays and proteomics. J Biol Chem 9:9
54. Gustafson WC, Weiss WA (2010) Myc proteins as therapeutic targets. Oncogene 29:1249–1259
55. Hagiwara A, Cornu M, Cybulski N, Polak P, Betz C, Trapani F, Terracciano L, Heim MH, Ruegg MA, Hall MN (2012) Hepatic mTORC2 activates glycolysis and lipogenesis through AKT, glucokinase, and SREBP1c. Cell Metab 15:725–738
56. Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G et al (2006) SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. Cell 126:941–954
57. Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J, Yonezawa K (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell 110:177–189
58. Hara K, Yonezawa K, Weng QP, Kozlowski MT, Belham C, Avruch J (1998) Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. J Biol Chem 273:14484–14494
59. Hardwick JS, Kuruvilla FG, Tong JK, Shamji AF, Schreiber SL (1999) Rapamycin-modulated transcription defines the subset of nutrient- sensitive signaling pathways directly controlled by the Tor proteins. Proc Natl Acad Sci USA 96:14866–14870
60. Harrington LS, Findlay GM, Lamb RF (2005) Restraining PI3K: mTOR signalling goes back to the membrane. Trends Biochem Sci 30:35–42

61. Heitman J, Movva NR, Hall MN (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science 253:905–909
62. Helliwell SB, Wagner P, Kunz J, Deuter-Reinhard M, Henriquez R, Hall MN (1994) TOR1 and TOR2 are structurally and functionally similar but not identical phosphatidylinositol kinase homologues in yeast. Mol Biol Cell 5:105–118
63. Hess G, Herbrecht R, Romaguera J, Verhoef G, Crump M, Gisselbrecht C, Laurell A, Offner F, Strahs A, Berkenblit A et al (2009) Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. J Clin Oncol 27:3822–3829
64. Hresko RC, Mueckler M (2005) mTOR•RICTOR is the Ser473 kinase for AKT/protein kinase B in 3T3-L1 adipocytes. J Biol Chem 280:40406–40416
65. Hsu PP, Kang SA, Rameseder J, Zhang Y, Ottina KA, Lim D, Peterson TR, Choi Y, Gray NS, Yaffe MB et al (2011) The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. Science 332:1317–1322
66. Hu CJ, Wang LY, Chodosh LA, Keith B, Simon MC (2003) Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. Mol Cell Biol 23:9361–9374
67. Huang J, Manning BD (2008) The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. Biochem J 412:179–190
68. Huang S, Houghton PJ (2001) Mechanisms of resistance to rapamycins. Drug Resist Updates 4:378–391 (Reviews and commentaries in antimicrobial and anticancer chemotherapy)
69. Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, Giaccia AJ, Abraham RT (2002) Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol Cell Biol 22:7004–7014
70. Huffman TA, Mothe-Satney I, Lawrence JC Jr (2002) Insulin-stimulated phosphorylation of lipin mediated by the mammalian target of rapamycin. Proc Natl Acad Sci USA 99:1047–1052
71. Ikenoue T, Inoki K, Yang Q, Zhou X, Guan KL (2008) Essential function of TORC2 in PKC and AKT turn motif phosphorylation, maturation and signalling. EMBO J 27:1919–1931
72. Inoki K, Zhu T, Guan KL (2003) TSC2 mediates cellular energy response to control cell growth and survival. Cell 115:577–590
73. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, Huang Q, Qin J, Su B (2006) SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates AKT phosphorylation and substrate specificity. Cell 127:125–137
74. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, Hall MN (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biol 6:1122–1128
75. Jerusalem G, Rorive A, Collignon J (2014) Use of mTOR inhibitors in the treatment of breast cancer: an evaluation of factors that influence patient outcomes. Breast cancer 6:43–57
76. Jewell JL, Russell RC, Guan KL (2013) Amino acid signalling upstream of mTOR. Nat Rev Mol Cell Biol 14:133–139
77. Jiang X, Kenerson H, Aicher L, Miyaoka R, Eary J, Bissler J, Yeung RS (2008) The tuberous sclerosis complex regulates trafficking of glucose transporters and glucose uptake. Am J Pathol 172:1748–1756
78. Joka M, Boeck S, Zech CJ, Seufferlein T, Wichert G, Licht T, Krause A, Jauch KW, Heinemann V, Bruns CJ (2014) Combination of antiangiogenic therapy using the mTOR-inhibitor everolimus and low-dose chemotherapy for locally advanced and/or metastatic pancreatic cancer: a dose-finding study. Anticancer Drugs 25:1095–1101
79. Jones KT, Greer ER, Pearce D, Ashrafi K (2009) Rictor/TORC2 regulates *Caenorhabditis elegans* fat storage, body size, and development through sgk-1. PLoS Biol 7:e60
80. Keen HG, Ricketts SA, Maynard J, Logie A, Odedra R, Shannon AM, Wedge SR, Guichard SM (2014) Examining changes in [^{18} F]FDG and [^{18} F]FLT uptake in U87-MG glioma xenografts as early response biomarkers to treatment with the dual mTOR1/2

inhibitor AZD8055. Mol Imaging Biol 16:421–430 (MIB: the official publication of the academy of molecular imaging)
81. Kim DH, dos Sarbassov D, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM (2002) mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell 110:163–175
82. Kim SJ, DeStefano MA, Oh WJ, Wu CC, Vega-Cotto NM, Finlan M, Liu D, Su B, Jacinto E (2012) mTOR complex 2 regulates proper turnover of insulin receptor substrate-1 via the ubiquitin ligase subunit Fbw8. Mol Cell 48:875–887
83. Kliegman JI, Fiedler D, Ryan CJ, Xu YF, Su XY, Thomas D, Caccese MC, Cheng A, Shales M, Rabinowitz JD et al (2013) Chemical genetics of rapamycin-insensitive TORC2 in *S. cerevisiae*. Cell Rep 5:1725–1736
84. Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P, Wilson KA, Byars A, Sahmoud T, Franz DN (2010) Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. New Engl J Med 363:1801–1811
85. Kunz J, Henriquez R, Schneider U, Deuter-Reinhard M, Movva NR, Hall MN (1993) Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. Cell 73:585–596
86. Kuo CJ, Chung J, Fiorentino DF, Flanagan WM, Blenis J, Crabtree GR (1992) Rapamycin selectively inhibits interleukin-2 activation of p70 S6 kinase. Nature 358:70–73
87. Kuo MT, Savaraj N, Feun LG (2010) Targeted cellular metabolism for cancer chemotherapy with recombinant arginine-degrading enzymes. Oncotarget 1:246–251
88. Laplante M, Sabatini DM (2009) An emerging role of mTOR in lipid biosynthesis. Curr Biol 19:R1046–R1052
89. Laplante M, Sabatini DM (2012) mTOR signaling in growth control and disease. Cell 149:274–293
90. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL (2001) HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 21:3995–4004
91. Lee JV, Carrer A, Shah S, Snyder NW, Wei S, Venneti S, Worth AJ, Yuan ZF, Lim HW, Liu S et al (2014) AKT-dependent metabolic reprogramming regulates tumor cell histone acetylation. Cell Metab 20:306–319
92. Lei Z, Tan IB, Das K, Deng N, Zouridis H, Pattison S, Chua C, Feng Z, Guan YK, Ooi CH et al (2013) Identification of molecular subtypes of gastric cancer with different responses to PI3-kinase inhibitors and 5-fluorouracil. Gastroenterology 145:554–565
93. Li MC, Hertz R, Bergenstal DM (1958) Therapy of choriocarcinoma and related trophoblastic tumors with folic acid and purine antagonists. New Engl J Med 259:66–74
94. Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P, Hall MN (2002) Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol Cell 10:457–468
95. Long Y, Tsai WB, Wangpaichitr M, Tsukamoto T, Savaraj N, Feun LG, Kuo MT (2013) Arginine deiminase resistance in melanoma cells is associated with metabolic reprogramming, glucose dependence, and glutamine addiction. Mol Cancer Ther 12:2581–2590
96. Ma XM, Blenis J (2009) Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol 10:307–318
97. MacKenzie MJ, Ernst S, Johnson C, Winquist E (2012) A phase I study of temsirolimus and metformin in advanced solid tumours. Invest New Drugs 30:647–652
98. Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, Manola J, Brugarolas J, McDonnell TJ, Golub TR et al (2004) mTOR inhibition reverses AKT-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. Nat Med 10:594–601
99. Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. Cell 129:1261–1274

100. Martin-Liberal J, Gil-Martin M, Sainz-Jaspeado M, Gonzalo N, Rigo R, Colom H, Munoz C, Tirado OM, Garcia Del Muro X (2014) Phase I study and preclinical efficacy evaluation of the mTOR inhibitor sirolimus plus gemcitabine in patients with advanced solid tumours. Br J Cancer 111:858–865
101. Masui K, Tanaka K, Akhavan D, Babic I, Gini B, Matsutani T, Iwanami A, Liu F, Villa GR, Gu Y et al (2013) mTOR complex 2 controls glycolytic metabolism in glioblastoma through FoxO acetylation and upregulation of c-Myc. Cell Metab 18:726–739
102. Mauvoisin D, Rocque G, Arfa O, Radenne A, Boissier P, Mounier C (2007) Role of the PI3-kinase/mTor pathway in the regulation of the stearoyl CoA desaturase (SCD1) gene expression by insulin in liver. J Cell Commun Signal 1:113–125
103. Menon S, Dibble CC, Talbott G, Hoxhaj G, Valvezan AJ, Takahashi H, Cantley LC, Manning BD (2014) Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. Cell 156:771–785
104. Migita T, Narita T, Nomura K, Miyagi E, Inazuka F, Matsuura M, Ushijima M, Mashima T, Seimiya H, Satoh Y et al (2008) ATP citrate lyase: activation and therapeutic implications in non-small cell lung cancer. Cancer Res 68:8547–8554
105. Morita M, Gravel SP, Chenard V, Sikstrom K, Zheng L, Alain T, Gandin V, Avizonis D, Arguello M, Zakaria C et al (2013) mTORC1 controls mitochondrial activity and biogenesis through 4E-BP-dependent translational regulation. Cell Metab 18:698–711
106. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grunwald V, Thompson JA, Figlin RA, Hollaender N et al (2008) Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 372:449–456
107. Muellner MK, Uras IZ, Gapp BV, Kerzendorfer C, Smida M, Lechtermann H, Craig-Mueller N, Colinge J, Duernberger G, Nijman SM (2011) A chemical-genetic screen reveals a mechanism of resistance to PI3K inhibitors in cancer. Nat Chem Biol 7:787–793
108. Muller HJ, Boos J (1998) Use of L-asparaginase in childhood ALL. Crit Rev Oncol Hematol 28:97–113
109. Nicklin P, Bergman P, Zhang B, Triantafellow E, Wang H, Nyfeler B, Yang H, Hild M, Kung C, Wilson C et al (2009) Bidirectional transport of amino acids regulates mTOR and autophagy. Cell 136:521–534
110. Noda T, Ohsumi Y (1998) Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. J Biol Chem 273:3963–3966
111. Oh WJ, Jacinto E (2011) mTOR complex 2 signaling and functions. Cell Cycle 10:2305–2316
112. Oh WJ, Wu CC, Kim SJ, Facchinetti V, Julien LA, Finlan M, Roux PP, Su B, Jacinto E (2010) mTORC2 can associate with ribosomes to promote cotranslational phosphorylation and stability of nascent AKT polypeptide. EMBO J 29:3939–3951
113. Owen JL, Zhang Y, Bae SH, Farooqi MS, Liang G, Hammer RE, Goldstein JL, Brown MS (2012) Insulin stimulation of SREBP-1c processing in transgenic rat hepatocytes requires p70 S6-kinase. Proc Natl Acad Sci USA 109:16184–16189
114. Peng T, Golub TR, Sabatini DM (2002) The immunosuppressant rapamycin mimics a starvation-like signal distinct from amino acid and glucose deprivation. Mol Cell Biol 22:5575–5584
115. Peterson TR, Sengupta SS, Harris TE, Carmack AE, Kang SA, Balderas E, Guertin DA, Madden KL, Carpenter AE, Finck BN et al (2011) mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. Cell 146:408–420
116. Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, Griffiths JR, Chung YL, Schulze A (2008) SREBP activity is regulated by mTORC1 and contributes to AKT-dependent cell growth. Cell Metab 8:224–236
117. Pourdehnad M, Truitt ML, Siddiqi IN, Ducker GS, Shokat KM, Ruggero D (2013) Myc and mTOR converge on a common node in protein synthesis control that confers synthetic lethality in Myc-driven cancers. Proc Natl Acad Sci USA 110:11988–11993

118. Price DJ, Grove JR, Calvo V, Avruch J, Bierer BE (1992) Rapamycin-induced inhibition of the 70-kilodalton S6 protein kinase. Science 257:973–977
119. Ramanathan A, Schreiber SL (2009) Direct control of mitochondrial function by mTOR. Proc Natl Acad Sci USA 106:22229–22232
120. Rangwala R, Chang YC, Hu J, Algazy KM, Evans TL, Fecher LA, Schuchter LM, Torigian DA, Panosian JT, Troxel AB et al (2014) Combined MTOR and autophagy inhibition: phase I trial of hydroxychloroquine and temsirolimus in patients with advanced solid tumors and melanoma. Autophagy 10:1391–1402
121. Ricoult SJ, Manning BD (2013) The multifaceted role of mTORC1 in the control of lipid metabolism. EMBO Rep 14:242–251
122. Robey RB, Hay N (2009) Is AKT the "Warburg kinase"?-AKT-energy metabolism interactions and oncogenesis. Semin Cancer Biol 19:25–31
123. Robitaille AM, Christen S, Shimobayashi M, Cornu M, Fava LL, Moes S, Prescianotto-Baschong C, Sauer U, Jenoe P, Hall MN (2013) Quantitative phosphoproteomics reveal mTORC1 activates de novo pyrimidine synthesis. Science 339:1320–1323
124. Saba NF, Hurwitz SJ, Magliocca K, Kim S, Owonikoko TK, Harvey D, Ramalingam SS, Chen Z, Rogerio J, Mendel J et al (2014) Phase 1 and pharmacokinetic study of everolimus in combination with cetuximab and carboplatin for recurrent/metastatic squamous cell carcinoma of the head and neck. Cancer 120(24):3940–3951
125. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH (1994) RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell 78:35–43
126. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM (2010) Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell 141:290–303
127. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM (2008) The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 320:1496–1501
128. Sarbassov D, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol 14:1296–1302
129. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and AKT/PKB. Mol Cell 22:159–168
130. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of AKT/PKB by the rictor-mTOR complex. Science 307:1098–1101
131. Schieke SM, Phillips D, McCoy JP Jr, Aponte AM, Shen RF, Balaban RS, Finkel T (2006) The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity. J Biol Chem 281:27643–27652
132. Schmelzle T, Hall MN (2000) TOR, a central controller of cell growth. Cell 103:253–262
133. Schmidt A, Beck T, Koller A, Kunz J, Hall MN (1998) The TOR nutrient signalling pathway phosphorylates NPR1 and inhibits turnover of the tryptophan permease. EMBO J 17:6924–6931
134. Schmidt A, Kunz J, Hall MN (1996) TOR2 is required for organization of the actin cytoskeleton in yeast. Proc Natl Acad Sci USA 93:13780–13785
135. Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3:721–732
136. Semenza GL (2013) HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Invest 123:3664–3671
137. Sener A, Malaisse WJ (1980) L-leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. Nature 288:187–189
138. Shaw RJ, Cantley LC (2006) Ras, PI(3)K and mTOR signalling controls tumour cell growth. Nature 441:424–430

139. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, Chi H (2011) HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. J Exp Med 208:1367–1376
140. Shi Y, Frankel A, Radvanyi LG, Penn LZ, Miller RG, Mills GB (1995) Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro. Cancer Res 55:1982–1988
141. Singh J, Novik Y, Stein S, Volm M, Meyers M, Smith J, Omene C, Speyer J, Schneider R, Jhaveri K et al (2014) Phase 2 trial of everolimus and carboplatin combination in patients with triple negative metastatic breast cancer. Breast Cancer Res 16:R32
142. Soukas AA, Kane EA, Carr CE, Melo JA, Ruvkun G (2009) Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in *Caenorhabditis elegans*. Genes Dev 23:496–511
143. Stanton RC (2012) Glucose-6-phosphate dehydrogenase, NADPH, and cell survival. IUBMB Life 64:362–369
144. Stiles BL (2009) PI-3-K and AKT: onto the mitochondria. Adv Drug Deliv Rev 61:1276–1282
145. Sun Q, Chen X, Ma J, Peng H, Wang F, Zha X, Wang Y, Jing Y, Yang H, Chen R et al (2011) Mammalian target of rapamycin up-regulation of pyruvate kinase isoenzyme type M2 is critical for aerobic glycolysis and tumor growth. Proc Natl Acad Sci USA 108:4129–4134
146. Tandon P, Gallo CA, Khatri S, Barger JF, Yepiskoposyan H, Plas DR (2011) Requirement for ribosomal protein S6 kinase 1 to mediate glycolysis and apoptosis resistance induced by Pten deficiency. Proc Natl Acad Sci USA 108:2361–2365
147. Teachey DT, Sheen C, Hall J, Ryan T, Brown VI, Fish J, Reid GS, Seif AE, Norris R, Chang YJ et al (2008) mTOR inhibitors are synergistic with methotrexate: an effective combination to treat acute lymphoblastic leukemia. Blood 112:2020–2023
148. Thomas G (1993) $p70^{s6k}/p85^{s6k}$: mechanism of activation, effects of rapamycin and role in mitogenesis. Biochem Soc Trans 21:901–904
149. Thomas GV, Tran C, Mellinghoff IK, Welsbie DS, Chan E, Fueger B, Czernin J, Sawyers CL (2006) Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. Nat Med 12:122–127
150. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM, Gray NS (2009) An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. J Biol Chem 284:8023–8032
151. van der Poel HG, Hanrahan C, Zhong H, Simons JW (2003) Rapamycin induces Smad activity in prostate cancer cell lines. Urol Res 30:380–386
152. Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324:1029–1033
153. Wagle N, Grabiner BC, Van Allen EM, Hodis E, Jacobus S, Supko JG, Stewart M, Choueiri TK, Gandhi L, Cleary JM et al (2014) Activating mTOR mutations in a patient with an extraordinary response on a phase I trial of everolimus and pazopanib. Cancer Discov 4:546–553
154. Wan M, Leavens KF, Saleh D, Easton RM, Guertin DA, Peterson TR, Kaestner KH, Sabatini DM, Birnbaum MJ (2011) Postprandial hepatic lipid metabolism requires signaling through AKT2 independent of the transcription factors FoxA2, FoxO1, and SREBP1c. Cell Metab 14:516–527
155. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, McCormick LL, Fitzgerald P, Chi H, Munger J et al (2011) The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity 35:871–882
156. Wang W, Fridman A, Blackledge W, Connelly S, Wilson IA, Pilz RB, Boss GR (2009) The phosphatidylinositol 3-kinase/AKT cassette regulates purine nucleotide synthesis. J Biol Chem 284:3521–3528
157. Wang Y, Wang Y, Shen L, Pang Y, Qiao Z, Liu P (2012) Prognostic and therapeutic implications of increased ATP citrate lyase expression in human epithelial ovarian cancer. Oncol Rep 27:1156–1162

158. Wellen KE, Thompson CB (2012) A two-way street: reciprocal regulation of metabolism and signalling. Nat Rev Mol Cell Biol 13:270–276
159. West MJ, Stoneley M, Willis AE (1998) Translational induction of the c-myc oncogene via activation of the FRAP/TOR signalling pathway. Oncogene 17:769–780
160. Willems L, Jacque N, Jacquel A, Neveux N, Maciel TT, Lambert M, Schmitt A, Poulain L, Green AS, Uzunov M et al (2013) Inhibiting glutamine uptake represents an attractive new strategy for treating acute myeloid leukemia. Blood 122:3521–3532
161. Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB et al (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci USA 105:18782–18787
162. Wlodarczyk Z, Vitko S, Salmela K, Czajkowski Z, Margreiter R, Group TS (2005) Lipid metabolism in renal transplant patients receiving tacrolimus/sirolimus combination therapy. Transpl Proc 37:1871–1873
163. Wong KK, Engelman JA, Cantley LC (2010) Targeting the PI3K signaling pathway in cancer. Curr Opin Genet Dev 20:87–90
164. Wu CC, Chou P, Jacinto E (2012) The target of rapamycin: structure and functions. In: Da Silva Xavier G (ed) Protein kinases. Intech, Rijeka, pp 1–40
165. Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ, Pavletich NP (2013) mTOR kinase structure, mechanism and regulation. Nature 497:217–223
166. Yang K, Shrestha S, Zeng H, Karmaus PW, Neale G, Vogel P, Guertin DA, Lamb RF, Chi H (2013) T cell exit from quiescence and differentiation into Th2 cells depend on Raptor-mTORC1-mediated metabolic reprogramming. Immunity 39:1043–1056
167. Yang Q, Inoki K, Ikenoue T, Guan KL (2006) Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity. Genes Dev 20:2820–2832
168. Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG et al (2011) Everolimus for advanced pancreatic neuroendocrine tumors. New Engl J Med 364:514–523
169. Yao Y, Suraokar M, Darnay BG, Hollier BG, Shaiken TE, Asano T, Chen CH, Chang BH, Lu Y, Mills GB et al (2013) BSTA promotes mTORC2-mediated phosphorylation of AKT1 to suppress expression of FoxC2 and stimulate adipocyte differentiation. Sci Sig 6:ra2
170. Yardley DA, Noguchi S, Pritchard KI, Burris HA 3rd, Baselga J, Gnant M, Hortobagyi GN, Campone M, Pistilli B, Piccart M et al (2013) Everolimus plus exemestane in postmenopausal patients with HR(+) breast cancer: BOLERO-2 final progression-free survival analysis. Adv Ther 30:870–884
171. Yecies JL, Manning BD (2011) Transcriptional control of cellular metabolism by mTOR signaling. Cancer Res 71:2815–2820
172. Yecies JL, Zhang HH, Menon S, Liu S, Yecies D, Lipovsky AI, Gorgun C, Kwiatkowski DJ, Hotamisligil GS, Lee CH et al (2011) AKT stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways. Cell Metab 14:21–32
173. Yu Y, Yoon SO, Poulogiannis G, Yang Q, Ma XM, Villen J, Kubica N, Hoffman GR, Cantley LC, Gygi SP et al (2011) Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling. Science 332:1322–1326
174. Yuan M, Pino E, Wu L, Kacergis M, Soukas AA (2012) Identification of AKT-independent regulation of hepatic lipogenesis by mammalian target of rapamycin (mTOR) complex 2. J Biol Chem 287:29579–29588
175. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H (2013) mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. Nature 499:485–490
176. Zha X, Wang F, Wang Y, He S, Jing Y, Wu X, Zhang H (2011) Lactate dehydrogenase B is critical for hyperactive mTOR-mediated tumorigenesis. Cancer Res 71:13–18
177. Zhang H, Stallock JP, Ng JC, Reinhard C, Neufeld TP (2000) Regulation of cellular growth by the *Drosophila* target of rapamycin dTOR. Genes Dev 14:2712–2724

178. Zhang HH, Huang J, Duvel K, Boback B, Wu S, Squillace RM, Wu CL, Manning BD (2009) Insulin stimulates adipogenesis through the AKT-TSC2-mTORC1 pathway. PLoS ONE 4: e6189
179. Zheng XF, Florentino D, Chen J, Crabtree GR, Schreiber SL (1995) TOR kinase domains are required for two distinct functions, only one of which is inhibited by rapamycin. Cell 82:121–130
180. Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW, Semenza GL (2000) Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res 60:1541–1545
181. Zinzalla V, Stracka D, Oppliger W, Hall MN (2011) Activation of mTORC2 by association with the ribosome. Cell 144:757–768

Chapter 3
PI3K-AKT-mTOR Pathway Cooperates with the DNA Damage Repair Pathway: Carcinogenesis in Triple-Negative Breast Cancers and Beyond

Pradip De, Jennifer H. Carlson, Brian Leyland-Jones and Nandini Dey

Introduction

Understanding of the biology of breast cancers (BC) like most of other solid tumors has undergone an evolutionary change at the molecular level with the advent of microarray technology. BC gene expression patterns derived from cDNA microarrays identified four major intrinsic gene signatures of luminal (which was later classified as luminal A and luminal B subgroups), HER2-enriched, basal-like and normal breast-like subtype [126]. The paramount importance of classification was soon exemplified by a correlation between the gene expression patterns of the individual group/subgroup and survival, disease relapse, site of metastasis, and chemotherapy response [77, 123, 134, 144]. The revelation of BC intrinsic subtypes and its importance promptly led to the development of five novel gene expression

Review Criteria The information for this chapter is compiled in part by searching the PubMed database for articles those are published before January 2016. Electronic early release publications listed in these databases are also included. Only articles published in English are considered. The search terms used included "Breast cancer" in association with the following search terms: "Apoptosis," "proliferation," "prognosis," "cell signaling pathways," "resistance," "senescence," "biomarkers," "DNA Damage," "DNA Damage Repair pathway," "Triple-Negative Breast Cancer," "HRD signature," "PI3K-AKT-mTOR inhibitors," "Basal-type Breast Cancer," "PI3K-mTOR Pathway," "chemotherapy," "PARP inhibitor," "BRCA1/BRCA2," and "therapeutics." These search terms are also used to search the abstracts from annual meetings and symposia.

P. De · J.H. Carlson · B. Leyland-Jones · N. Dey (✉)
Avera Cancer Institute Center for Precision Oncology, Sioux Falls, SD 57105, USA
e-mail: nandini.dey@avera.org

P. De · B. Leyland-Jones · N. Dey
Departmental of Internal Medicine, SSOM, University of South Dakota, Sioux Falls, SD, USA

N. Dey et al. (eds.), *PI3K-mTOR in Cancer and Cancer Therapy*,
Cancer Drug Discovery and Development, DOI 10.1007/978-3-319-34211-5_3

(multigene based) prognostic tests for BC including 70-gene MammaPrint assay (first commercially available and FDA-approved signatures; stratifies patients into low or high risk of distant metastases at 5 years), MapQuant Dx, 21-gene Oncotype DX assay (estimates the risk of relapse in ER+, node-negative BC and their chemosensitivity and divides patients into three groups on the basis of their recurrence score), PAM50 (defined the four major intrinsic subtypes of BC through the analysis of 50 classifier genes and five control genes), and Theros Breast Cancer Index (for distant recurrence and overall survival in comparison to adjuvant). These multigene-based prognostic tests eventually added prognostic and predictive information to conventional biomarkers and extended more reliable and reproducible techniques than the subjective interpretation associated with the IHC assays. Gene expression profile tests in the context of BC subtypes and the significance and utility of these signatures on a comparative basis (evaluation and the comparison between the predictive value of PAM50 and Oncotype DX) are discussed in detail by Angela Toss and Massimo Cristofanilli in their recent review [152]. In today's "clinic to laboratory and back" culture of medical practice, this genetic alteration-driven (biomarker) approach has not only provided a better justification for the treatment of BC but also added an invaluable tool for the sequential diagnosis of cancer.

Alterations of PI3K-AKT-mTOR Pathway in BC

Genomic aberrations in breast carcinogenesis act as the fundamental basis of the target(s)-guided therapeutic approach in personalized medicine. The "Introductory/Opening Chapter" of this textbook by Prof. Lewis Cantley elegantly elucidated the basic signaling of the PI3K-AKT-mTOR pathway in the context of cancers. His original work pioneered our understanding of the signaling cascade of the PI3K-AKT-mTOR pathway, its components, its downstream effectors, its associated transcription factors and its feedback loops as well as its cross-talks with the RAS-RAF-MAPK-ERK pathway which has collectively presented us the opportunity to revolutionize our therapeutic regime in clinics. Growth factor-mediated upregulation of the PI3K-AKT-mTOR pathway is the most commonly upregulated oncogenic signaling pathway in BC and the predominant mechanisms of the pathway upregulation is attributed to PIK3CA mutations and protein loss of PTEN [23, 31, 37]. Alterations of PI3K-AKT-mTOR pathway are the most widely reported in BC [20]. The alteration is not only common but also subtype specific and contextual in character.

We have obtained oncoprints showing percentage of the alterations (amplification, homozygous deletion mutation, mRNA upregulation, mRNA downregulation, RPPA upregulation, RPPA downregulation) in 17 key genes of PI3K-AKT-mTOR signaling pathway in breast invasive carcinomas (TCGA Provisional and TCGA, Nature 2012) and the subtypes (Luminal A,TCGA, Nature 2012; Luminal B TCGA, Nature 2012; HER2 enriched, TCGA, Nature 2012; Basal-like, TCGA, Nature 2012) using cBioPortal (Fig. 3.1a–g). Data mining was carried out using

cBioPortal for Cancer Genomics, a data portal (cBioPortal for Cancer Genomics), available at http://www.cbioportal.org to measure the alterations in the PI3K-AKT-mTOR pathway genes as per the criteria mentioned in the legends of the respective figure (Fig. 3.1). The database query was based on deregulation (mutant, copy-number alterations and altered expression) of the pathway genes. We have prioritized "Mutation and CNA" data type (We acknowledge the cBioPortal for Cancer Genomics site (http://cbioportal.org) which provides a Web resource for exploring, visualizing, and analyzing multidimensional cancer genomics data. We also acknowledge the TCGA Research Network for generating TCGA datasets. This in-depth exercise demonstrated that in Breast Invasive Carcinoma (TCGA, Provisional; case set of all 1062 tumor samples), the PIK3CA gene is altered to the greatest extent (37 %) as compared to the alterations in 16 others genes (Fig. 3.1a). Interestingly, we have observed a wide range of mutations in PIK3CA gene in samples of tumors from our Avera breast cancer patients (unpublished observations). Although this percentage is a little lower in the oncoprint obtained from the Breast Invasive Carcinoma (TCGA, Nature 2012; case set of all 825 tumor samples) the alteration of this gene remains the highest altered gene in these samples and mutation remains the predominant type of alteration (Fig. 3.1b).

Alterations of PI3K-AKT-mTOR Pathway in Subtypes of BC

Although the PI3K-AKT-mTOR pathway is the most common and predominant oncogenic pathway altered in BC, the alteration of an individual component of the pathway which eventually leads to the overall upregulation of the pathway varies between BC subtypes and is characteristic of that particular subtype. The alterations of genes belonging to the PI3K-AKT-mTOR pathway characterize different subtypes of BC [20, 38]. The PI3K-AKT-mTOR pathway contributes to the complex control of cellular energy, glucose metabolism, senescence, and angiogenesis, and more specifically ER-positive BC cells bring out ER transcriptional activity. The most common mutations or amplifications in the PI3K-AKT-mTOR pathway affect the genes encoding the PI3K catalytic subunits (*PIK3CA, PIK3CB*), PI3K regulatory subunit (*PIK3R1*), PI3K effectors (*AKT1, AKT2, PDK1*), and loss of *PTEN* and *INPP4B*. The activation of this parallel pathway is known to provide alternative proliferation and survival stimuli to cancer cells, even in the presence of ER pathway inhibition following targeted therapy against estrogen.

We extended our analysis of alterations of 17 genes of PI3K-AKT-mTOR pathway in subtypes of BC. In the luminal A (TCGA, Nature 2012; case set of all 235 tumor samples), the alteration reaches a striking 49 % as compared to 37 % in the luminal B (TCGA, Nature 2012; case set of all 131 tumor samples) (Fig. 3.1c–d). A composite table (Fig. 3.1g) of a simultaneous comparison of alterations in all 17 genes in all subtypes showed that PIK3CA was highly altered in luminal A and HER2-enriched tumors.

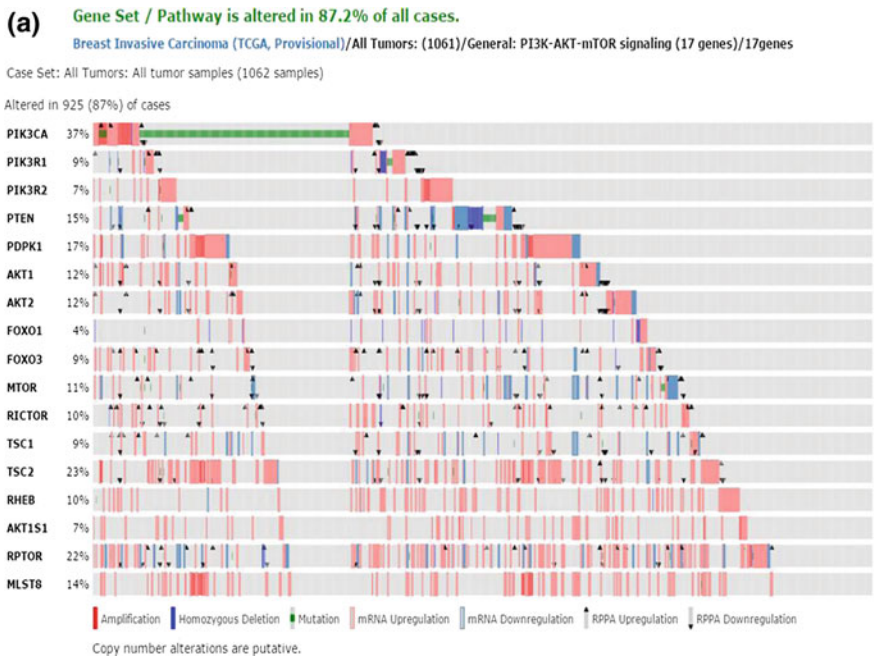


Fig. 3.1 Oncoprints showing the percentage of the alterations (amplification, homozygous deletion mutation, mRNA upregulation, mRNA downregulation, RPPA upregulation, RPPA downregulation) in 17 key genes of PI3K-AKT-mTOR signaling pathway in: **a** Breast Invasive Carcinoma (TCGA, Provisional) case set (all 1062 tumor samples), **b** Breast Invasive Carcinoma (TCGA, Nature 2012) case set (all 825 tumor samples), **c** Luminal A (TCGA, Nature 2012) case set (all 235 tumor samples), **d** Luminal B (TCGA, Nature 2012) case set (all 131 tumor samples), **e** HER2-enriched (TCGA, Nature 2012) case set (all 58 tumor samples), **f** Basal-like(TCGA, Nature 2012) case set (all 80 tumor samples). **g** Summary of alterations of 17 key genes of the PI3K-AKT-mTOR signaling pathway in luminal A, luminal B, HER2-enriched and basal-like tumor samples of Invasive Carcinoma of breast from cBioPortal (nature 2012). cBioPortal data is subjected to scheduled updates. Genomic study selected were (1) mutations, (2) putative copy-number alteration from GISTIC, (3) mRNA expression Z-scores (microarray) with Z-score thresholds ±2.0, and (4) protein/phosphoprotein level (RPPA) with Z-score thresholds ±2.0. *Gray bars* represent unaltered cases. Advanced cancer genomic data visualization is obtained with the help of "The Onco Query Language (OQL)". We used the Onco Query Language (OQL) to select and define genetic alterations for all the oncoprint outputs on the cBioPortal for Cancer Genomics. Oncoprints (different levels of zoom) have been generated using cBioPortal. Tumor types (tumor data sets) are chosen in accordance with the publication guidelines (last updated on January 17, 2014) of TCGA (tcga@mail.nih.gov). *cBioPortal data is subjected to scheduled updates.* We acknowledge the cBioPortal for Cancer Genomics site (http://cbioportal.org) which provides a Web resource for exploring, visualizing, and analyzing multidimensional cancer genomics data. The portal reduces molecular profiling data from cancer tissues and cell lines into readily understandable genetic, epigenetic, gene expression, and proteomic events [59]. We acknowledge works of Cerami et al. [24] and Gao et al. [59]. We acknowledge the TCGA Research Network for generating TCGA datasets

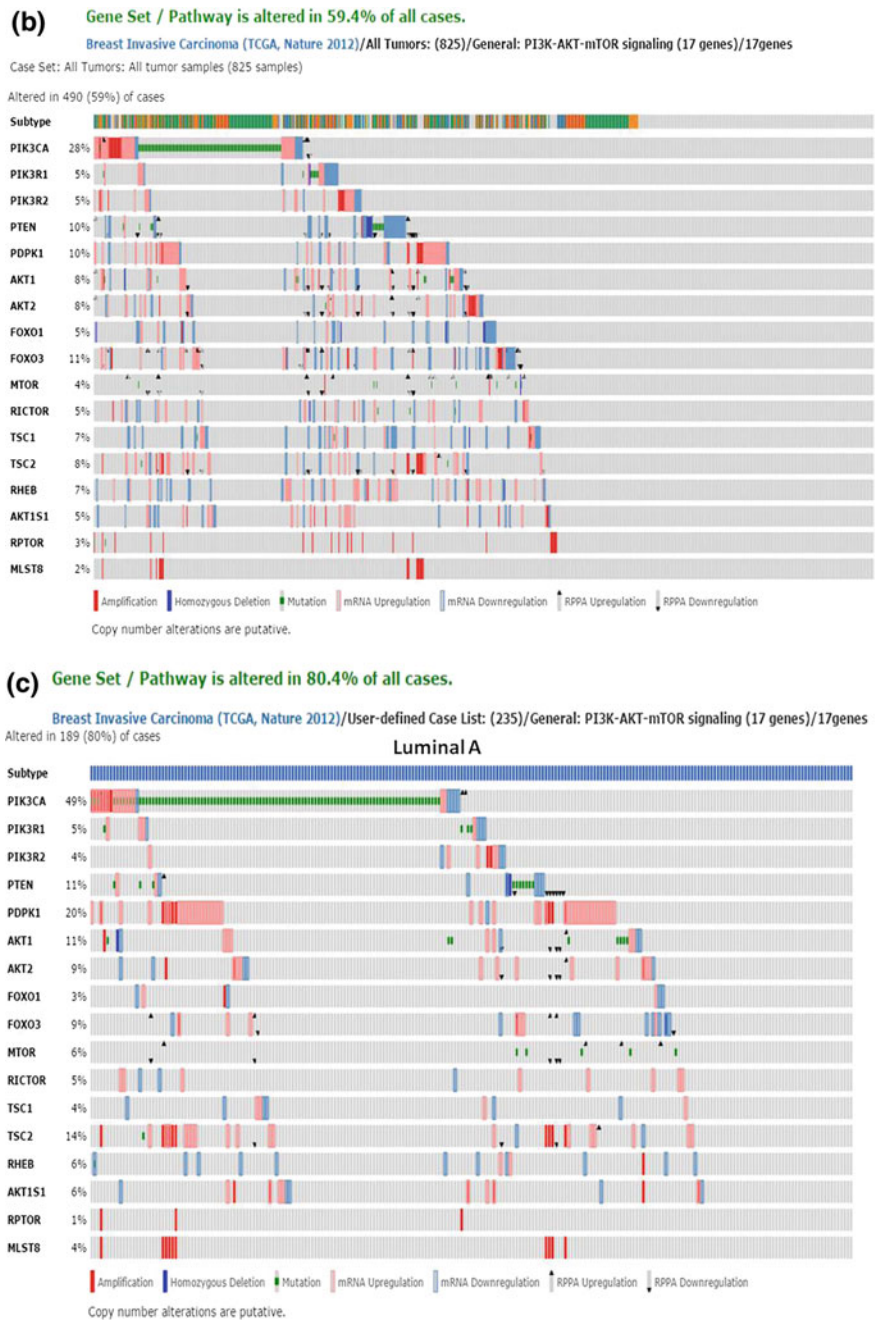


Fig. 3.1 (continued)

Fig. 3.1 (continued)

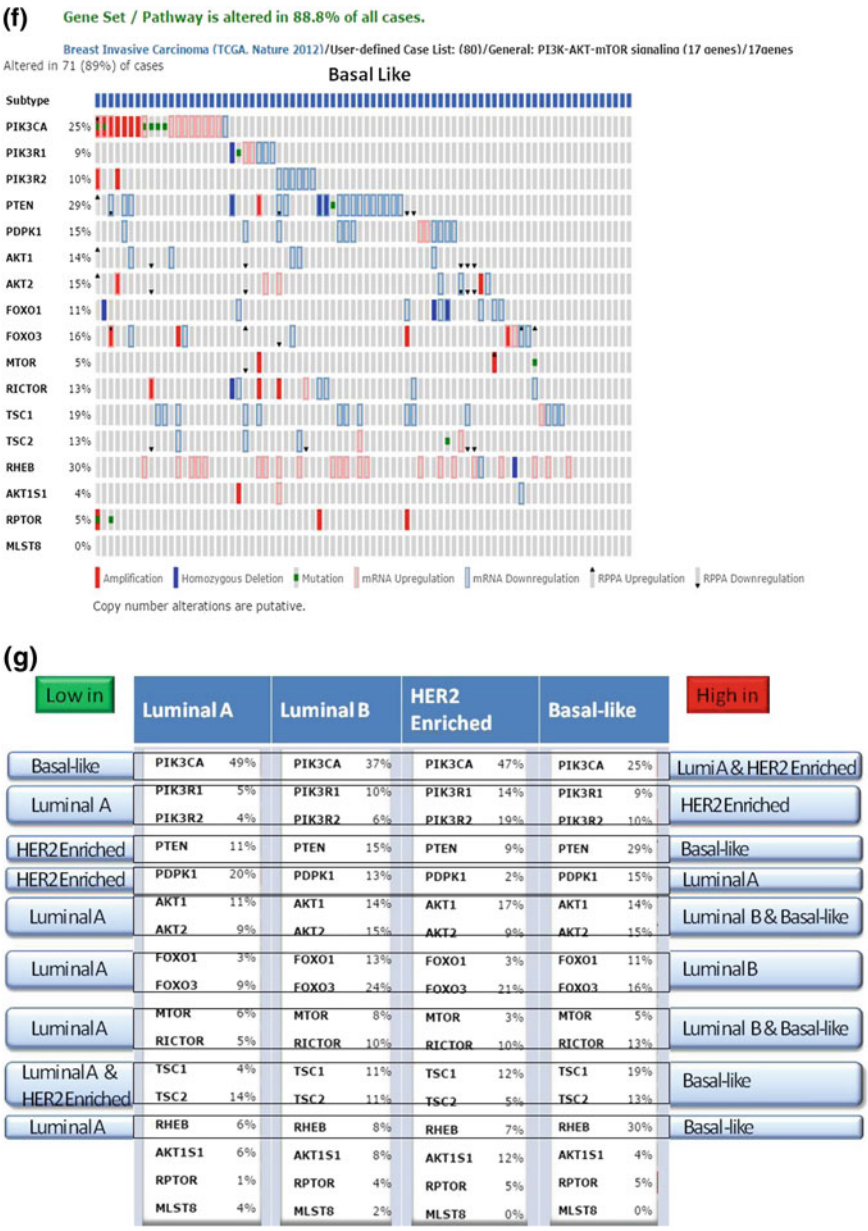


Fig. 3.1 (continued)

Numerous preclinical and clinical studies demonstrated the fundamental role of the PI3K-AKT-mTOR pathway in the onset and the progression of ER + breast tumors, and its intrinsic cross talk with ER signaling [27, 73, 139, 150]. The prognostic and therapeutic implications of PI3K mutations in breast cancer have been reviewed in detail by several scientists [48, 115]. The staggering wealth of knowledge about the biology and therapeutic potential of PI3K-AKT-mTOR signaling in ER + BC [58, 93, 115, 136, 157] has been the fundamental basis for the development of ongoing strategies to combine PI3K-AKT-mTOR pathway inhibitors (rapalogues) with the standard endocrine therapy toward obtaining improved clinical outcomes [10, 64]. Neoadjuvant trial (RAD2222) and trails in the metastatic setting (TAMRAD, BOLERO-2) have reported improved clinical outcome of patients with unselected luminal BC following the addition of mTOR inhibitors to standard endocrine treatment. The success of these clinical studies (Breast Cancer Trials of Oral Everolimus, *BOLERO 1 Trial, BOLERO 2 Trial, BOLERO 3 Trial*) proved the importance of selecting patient populations that would benefit most from this combination treatment. Therapeutic targeting of the PI3K pathway showed promise toward improved clinical outcomes for patients with luminal breast cancer [160].

Luminal B BC has early relapse following endocrine therapy and exhibits a poor prognosis similar to that of the aggressive basal-like BC. Several groups have identified phosphatidylinositol 3-kinase (PI3K) pathway activation as a frequent event in luminal B cancers with poor outcomes [27] (*Belle 2 Trial, Belle 3 Trial*). A composite table (Fig. 3.1g) shows that AKT1 (14 %) and AKT2 (15 %) were altered mostly in luminal B and basal-like tumors. In identifying a genetic mechanism that adds to the luminal B endocrine-resistant phenotype Fu, colleagues have designed a human model of the luminal B subtype and were able to control the expression of phosphatase and tensin homolog (PTEN) using inducible short hairpin RNAs. Through varying the expression of PTEN, the authors were successful in conferring endocrine resistance as well as recapitulating the luminal B gene expression signature. Their findings demonstrated the importance of PTEN expression levels in the development and progression of endocrine resistance in luminal B BC indicating that patients with PTEN-low estrogen receptor-positive tumors might benefit from combined endocrine and PI3K pathway therapies as even a moderate reduction in PTEN was sufficient to activate the PI3K pathway toward endocrine resistance [57]. Figure 3.1g shows that alterations in PTEN were second highest in luminal B (15 %) which was mainly comprised of mRNA downregulation and RPPA downregulation. Luminal B also had high alterations of FOXO1 (11 %) and 3 (16 %) while the basal-like had high alterations of TSC1 (19 %), TCS2 (13 %), and RHEB (30 %) (Fig. 3.1g). Cornen et al. studied candidate luminal B breast cancer genes identified by the genome, gene expression, and DNA methylation profiling [29]. In their study a total of 100 candidate oncogenes were validated in a public series of 5,765 BCs and the overexpression of 67 of these was associated with poor survival in luminal tumors. They reported that FOXO3, PIK3CA, and TP53 were the most frequently mutated genes among the nine tested. Interestingly, they also observed that 24 genes presented a deregulated expression in relation with a high DNA methylation level.

The HER2 signaling network in BC is one of the most intriguing and most studied signaling networks in oncology [45]. Despite advances in targeted therapy for this subtype of BC, drug resistance and recurrence of disease are still challenging for clinicians to overcome. Since PTEN phosphatase loss or activating mutations of the phosphoinositol-3 (PI3) kinase (PIK3CA) are known to be connected to trastuzumab resistance, the prognostic and predictive significance of PI3K-AKT-mTOR pathway has been extensively studied [38, 113, 145]. Although PTEN status determination was not found to be a useful biomarker to predict resistance to trastuzumab and lapatinib-based therapies and the benefit to neoadjuvant anti-human epidermal growth factor receptor 2 (HER2)-targeted therapies in HER2-positive primary breast cancer was independent of PTEN status [118], the importance of this pathway in mediating trastuzumab resistance remains critical. A systematic review of dual targeting in HER2-positive breast cancer described efficacy and safety of lapatinib, pertuzumab, or trastuzumab-DM1 in combination with trastuzumab in the (neo)adjuvant and metastatic settings as well as combinations of trastuzumab with drugs targeting the downstream pathway [83]. In an HER2 enriched group (TCGA, Nature 2012; case set of all 58 tumor samples) the PIK3CA alteration was comparable to that of in the luminal B subtype as 47 % (Fig. 3.1e). PIK3CA genotype has been reported to determine treatment decisions in HER2 BC in studies reported by Majewski et al. [95] wherein they observed that PIK3CA mutations are associated with decreased benefit to neoadjuvant human epidermal growth factor receptor 2-targeted therapies. Patients treated with a combination of trastuzumab and lapatinib having a wild-type PIK3CA obtained a total pathologic complete response (pCR) rate of 53.1 %, which decreased to 28.6 % in patients with tumors that carried PIK3CA activating mutations. In their study, activating mutations in PIK3CA predicted poor pCR in patients with HER2-positive breast cancer treated with neoadjuvant therapies targeting HER2. The results called for the obvious combination of anti-HER2 agents and PI3K inhibitors. Similarly, PIK3CA mutations are associated with lower rates of pathologic complete response to antihuman epidermal growth factor receptor 2 (HER2) therapy in primary HER2-overexpressing breast cancer [94] and these HER2-positive breast carcinomas with a PIK3CA mutation were found less likely to achieve a pCR after neoadjuvant anthracycline–taxane-based chemotherapy plus anti-HER2 treatment, even if a dual anti-HER2 treatment is given. A preclinical evaluation of the PI3K alpha/delta dominant inhibitor BAY 80-6946 in HER2-positive breast cancer models with acquired resistance to the HER2-targeted therapies trastuzumab and lapatinib demonstrated that the combination of HER2-targeted therapies and BAY 80-6946 inhibited growth more effectively than either therapy used alone (with clear synergism in many cases) and can restore sensitivity to trastuzumab and lapatinib in cells with acquired resistance to either trastuzumab and/or lapatinib [51].

Terms TNBC and basal-like BC are not synonymous. Determination of clonal genotype of an individual tumor is important to understand the biology and hence establish the treatment regime [3, 8, 12, 42, 140]. TNBC and basal-like BC remain a therapeutic challenge as a translation of the molecular message(s) of TNBC and

basal-like BC into targeted therapy is daunting [6, 30, 65, 96, 151, 155]. TNBC and basal-like BC are inherently heterogeneous forms of BC. In 2011 Lehmann et al. identified six different subtypes within TNBC with the help of RNA microarray analyses [87] and a differential response to neoadjuvant chemotherapy has been subsequently reported among these seven molecular subtypes of TNBC [1, 84, 103]. Mayer et al. in a review of new strategies for triple-negative breast cancer based on deciphering its heterogeneity commented on the identified six distinct TNBC subtypes, each of which is known to display a unique biology. The study to establish a novel-targeted therapy in this breast cancer subtype is of critical importance because, (1) less than 30 % of women with metastatic breast cancer survive 5 years and virtually all women with metastatic TNBC die of their disease despite systemic therapy, and (2) not a single targeted therapy has been approved for the treatment of TNBC and cytotoxic chemotherapy remains the standard treatment [105, 106]. The mechanism of activation of PI3K-AKT-mTOR pathway in basal-like BC is largely contributed by the frequent genomic alteration of PTEN and INPP4B phosphatases [49]. Microarray phosphatome profiling of breast cancer patients showed that a complex phosphatase regulatory role of the MAPK and PI3K pathways exist for estrogen receptor-negative BC [97]. In the basal-like (TCGA, Nature 2012; case set of all 80 tumor samples) subtype, the predominant alteration was observed in PTEN (29 %) which comprised of most mRNA downregulation and homozygous deletion (Fig. 3.1f). In a comprehensive analysis of PTEN status in breast carcinomas Jones et al. demonstrated that the PTEN complete loss status was significantly associated with estrogen receptor (ER) negativity ($p = 0.006$) and, in particular, the basal-like phenotype [71]. Studies by Marty et al. reported that basal-like BC expressed significantly lower levels of PTEN and PTEN levels were negatively correlated with AKT activity within that population. PTEN protein expression correlated significantly with PTEN DNA copy number and more importantly, reduced PTEN DNA copy numbers were observed specifically in basal-like BC [102]. Thus, frequent genomic alterations of PTEN directly contribute to the activated phosphatidylinositol 3-kinase pathway in basal-like breast cancer cells. In addition to PTEN phosphatase, the activation of PI3K-AKT-mTOR pathway in basal-like BC is also contributed by inositol polyphosphate 4-phosphatase II (INPP4B), a phosphatase which regulates PI3K/AKT signaling and is lost in basal-like BC. Fedele et al. reported that INPP4B protein expression was frequently lost in primary human breast carcinomas that are associated with high clinical grades, tumor sizes, loss of hormone receptors and was lost most commonly in aggressive basal-like breast carcinomas. INPP4B protein loss was also frequently observed in PTEN-null tumors and that loss of INPP4B protein is a marker of aggressive basal-like breast carcinomas [54]. Although alteration of PIK3CA is found to be the lowest (25 %) as compared to other subtypes (luminal A 49 %, luminal B 37 % and HER2 enriched 47 %) (Fig. 3.1g), a recent study by Young et al. reported that activating PIK3CA mutations induce an EGFR/ERK paracrine signaling axis in basal-like breast cancer [158].

Alteration(S) of PI3K-AKT-mTOR Pathway in BC Is Contextual

It is clear from the above discussion that the most important characteristic feature of alterations of PI3K-AKT-mTOR pathway in BC is that the oncogenic effect of alteration of a particular tumor suppressor or an oncogene in a particular BC subtype is contextual. A good example is that almost all basal-like tumors have *TP53* mutations (80 %) or the loss of TP53 functions (through gene mutations or dysfunctions in theTP53 pathway). As a tumor suppressor gene, TP53 following the activation by oncogenic stress signals promotes either cell cycle arrest along with DNA repair or cell apoptosis. This activity of TP53 is achieved by downstream targets (p21), indirect targets (PTEN), cell cycle regulation proteins (Chk1 and Chk2), and DNA repair proteins (PARP-1 and BRCA-1). BL1 and BL2 subtypes of TNBC express high cell cycle genes and DNA damage response genes (ATR/BRCA) and representative cell lines respond particularly to antimitotic and DNA-damaging agents, such as platinum agents. Hence, the loss of TP53 function in BL1 and BL2 tumor cells will have characteristically different oncogenic driver effect than in a different subset of TNBC, for example, immune modulatory or mesenchymal type of TNBC wherein tumor cells do not necessarily express high cell cycle genes and DNA damage response genes (ATR/BRCA). Since genetic alterations that cause the upregulation of the PI3K-AKT-mTOR pathway is contextual, the resulting characteristic features of the deregulated signaling (caused by the alteration of a particular tumor suppressor or an oncogene about the PI3K-AKT-mTOR pathway) of the PI3K-AKT-mTOR pathway is also contextual. For example, almost all BC have some alterations in one or more genes corresponding to the proteins of the PI3K-AKT-mTOR pathway. However, the effect of these alterations is manifested in the context of other genetic alterations which eventually affects the process of oncogenesis, malignant progression, and the response of the tumor to a drug as well as the development of the resistance to that drug. The other examples include the oncogenes *MYC* and *PIK3CA*, the products of which are well-established oncoproteins that contribute to the development of resistance to that particular drug in many solid tumors including breast oncogenesis [36, 41]. However, their similarities outnumber their dissimilarities in the context of their specific oncogenic cellular signals and MYC overexpression is sufficient to confer resistance to PI3K and mTOR inhibitors. We observed an overlap between alterations of human MYC and PIK3CA genes in Breast Invasive Carcinoma (TCGA, Provisional; cBioPortal) and differential alterations of human MYC and PIK3CA genes in subtypes of Breast Invasive Carcinoma (TCGA, Nature 2012; cBioPortal) (See Fig. 2 of [41]. We have described that the specific cellular signals initiated the following alteration in the MYC gene and PIK3CA gene in BC and interrogated how MYC gene alterations influence the action of PI3K pathway-targeted drugs in the context of PIK3CA mutation toward the development of PI3K inhibitor-induced drug resistance in BC [41]. Others have also reported

alterations in the PI3K-AKT-mTOR pathway in the context of another pathway alteration which eventually affects the response of the tumor to a drug as well as the development of resistance to that drug in different subsets of BC.

PI3K-AKT-mTOR Pathway Alterations in the Context of DNA Damage Response

In the light of results from studies undertaken by several scientists and data from various clinical trials instituted in different centers, it is becoming increasingly clear that alterations in HRD (Homologous Recombination Defect) genes are one of the important contextual events of the upregulation of the PI3K-AKT-mTOR pathway. The PI3K family includes (three distal homologs of PI3K, DNA-PKcs, ATM, and ATR, as key DDR regulators; DNA-PKcs controls NHEJ; ATM and ATR control HR) ataxia telangiectasia mutated (ATM), ataxia telangiectasia and RAD3-related (ATR), and DNA-dependent protein kinase (DNA-PKcs) [44, 86, 91, 138]. These kinases are all activated following DNA damage [2]. Since the nucleus provides a unique compartmental environment for protein–protein and protein–DNA/RNA interactions required for cell survival, growth, and proliferation, it is important to understand the nuclear PI3K signaling in cell growth and tumorigenesis and its relationship to alterations of HRD genes/DNA damage (DDR) response genes. For PI3K signaling to regulate DDR and affect the outcome of treatment in BC in the context of alterations of HRD genes, PI3K signaling should be functional in the nucleus. The existence of a nuclear phosphatidylinositol (PtdIns) cycle was presented by Manzoli and his group [98, 99]. Kumar et al. reported that the p85β/p110β complex localizes to the nucleus in several cell types [82]. Among the four class I PI3Ks (alpha, beta, gamma, and delta) that are implicated in cancer, the p110b catalytic isoform has been found in the nucleus as described by Kumar et al. [82] where it may regulate DNA double-strand break (DSB) repair and p110beta deletion induced radiation sensitivity and genomic instability [81]. Their study concluded that p110bata activity (PIP3) facilitates DNA repair and p110beta expression is required for this process, supporting the concept that p110beta contribution in DDR is mainly kinase-independent [81]. A recent and an elegant review on nuclear PI3K signaling in cell growth and tumorigenesis describes the spatial and temporal localization of the major nuclear kinases having PI3K activities and the counteracting phosphatases as well as the role of nuclear PI3K/AKT signaling in many nuclear functions including DNA replication and repair in the context of cell survival and tumorigenesis [34].

On the flip side of the coin, PTEN tumor suppressor, the master negative regulator of the PI3K-AKT-mTOR pathway which is lost in all subtypes of BC predominantly TNBC, acts as a guardian of genome integrity and actively participates in DDR. Shen et al. demonstrated that PTEN acts on chromatin and regulated expression of RAD51, which reduces the incidence of spontaneous DSBs and thus

(a)

Cell Signaling Pathway	Genes	Breast Invasive Carcinoma The Cancer Genome Atlas (TCGA) Breast Invasive Carcinoma project. Total 825 cases. Raw data via the TCGA Data Portal. TCGA, Nature 2012 PAM50 Basal
DNA Damage Response (12 Genes)	*CHEK1 CHEK2 RAD51 BRCA1 BRCA2 MLH1 MSH2 ATM ATR MDC1 PARP1 FANCF*	Gene Set / Pathway is altered in 90.1% of all 81 cases
PI3K-AKT-mTOR signaling (17 Genes)	*PIK3CA PIK3R1 PIK3R2 PTEN PDPK1 AKT1 AKT2 FOXO1 FOXO3 MTOR RICTOR TSC1 TSC2 RHEB AKT1S1 RPTOR MLST8*	Gene Set / Pathway is altered in 88.9% of all 81 cases

(b)

Patient / Case Set	DNA Damage Response Pathway (12 Genes) *CHEK1 CHEK2 RAD51 BRCA1 BRCA2 MLH1 MSH2 ATM ATR MDC1 PARP1 FANCF*	The Cancer Genome Atlas (TCGA) Breast Invasive Carcinoma project. 825 cases. Nature 2012. Raw data via the TCGA Data Portal. TCGA, Nature 2012 Breast Invasive Carcinoma
PAM50 Basal	DNA Damage Response	Gene Set / Pathway is altered in 90.1% of all 81 cases
Basal-Like	DNA Damage Response	Gene Set / Pathway is altered in 88.8% of all 80 cases
Basal-Like Recurred/ Progressed	DNA Damage Response	Gene Set / Pathway is altered in 100% of all 6 cases
Basal-Like Disease Free	DNA Damage Response	Gene Set / Pathway is altered in 87.1% of all 70 cases

regulated control of DNA repair. PTEN dephosphorylates PIP3 and also controls DSB repair by regulating RAD51 expression [142]. Liu et al. reported that PTEN also associates with the centromeric protein CENP-C to maintain centromere integrity and suppresses chromosomal instability from DNA DSBs via transcriptional regulation of RAD51 (radiosensitive yeast mutant 51) [92]. Nuclear PTEN is involved in chromosome stability, DNA repair, cell cycle arrest, and cellular stability [127]. Bassi et al. demonstrated that nuclear PTEN controls DNA repair [11]. Localized and retained in the nucleus following sumoylation at K254, PTEN efficiently carries out genotoxic stress-induced DSB repair. Nuclear localization

◀ **Fig. 3.2** Percent change in the alterations in 17 PI3K-AKT-mTOR signaling genes and12 DNA damage response genes in BC. **a** Changes in 12 DNA damage response genes and 17 PI3K-AKT-mTOR signaling genes in PAM50 Basal case set of Breast Invasive Carcinoma (The Cancer Genome Atlas (TCGA; Raw data via the TCGA Data Portal. TCGA, Nature 2012). A custom case set was built for the number of matching cases of PAM50 basal breast cancers using cBioPortal (TCGA, Nature 2012 data; raw data at the NCI.). Following Genomic Profiles were selected: (1) mutations, (2) putative copy-number alteration (CNA) from GISTIC, (3) mRNA expression Z-scores (RNA-Seq V2 RSEM) with Z-score thresholds ±2.0, and (4) protein/phosphoprotein level (RPPA) with Z-score thresholds ±2.0. (Breast Invasive Carcinoma project. 825 cases. Nature 2012). *cBioPortal data is subjected to scheduled updates.* **b** Changes in 12 DNA damage response genes in PAM50 Basal case set and Basal-like Breast Invasive Carcinoma (The Cancer Genome Atlas (TCGA; Raw data via the TCGA Data Portal. TCGA, Nature 2012). A custom case set was built for the number of matching cases of PAM50 basal and basal-like breast cancers using cBioPortal (TCGA, Nature 2012 data; raw data at the NCI.). Following Genomic Profiles were selected: (1) mutations, (2) putative copy-number alteration (CNA) from GISTIC, (3) mRNA expression Z-scores (RNA-Seq V2 RSEM) with Z-score thresholds ±2.0, and (4) protein/phosphoprotein level (RPPA) with Z-score thresholds ±2.0. (Breast Invasive Carcinoma project. 825 cases. Nature 2012). A custom case set was built for Disease Free Status and progressed/recurred status of basal-like cases. *cBioPortal data is subjected to scheduled updates.* **c** Changes in BRCA1 and BRCA2 in PAM50 Basal case set and Basal-like Breast Invasive Carcinoma (The Cancer Genome Atlas (TCGA; Raw data via the TCGA Data Portal. TCGA, Nature 2012). A custom case set was built for the number of matching cases of PAM50 basal and basal-like breast cancers using cBioPortal (TCGA, Nature 2012 data; raw data at the NCI.). Following Genomic Profiles were selected: (1) mutations, (2) putative copy-number alteration (CNA) from GISTIC, (3) mRNA expression Z-scores (RNA-Seq V2 RSEM) with Z-score thresholds ±2.0, and (4) protein/phosphoprotein level (RPPA) with Z-score thresholds ±2.0. (Breast Invasive Carcinoma project. 825 cases. Nature 2012). A custom case set was built for Disease Free Status and progressed/recurred status of basal-like cases. *cBioPortal data is subjected to scheduled updates.* **d** Oncoprint of changes in 12 DNA damage response genes in PAM50 Basal case set of Breast Invasive Carcinoma (The Cancer Genome Atlas (TCGA; Raw data via the TCGA Data Portal. TCGA, Nature 2012). A custom case set was built for the number of matching cases of PAM50 basal breast cancers using cBioPortal (TCGA, Nature 2012 data; raw data at the NCI.). Following Genomic Profiles were selected: (1) mutations, (2) putative copy-number alteration (CNA) from GISTIC, (3) mRNA expression Z-scores (RNA-Seq V2 RSEM) with Z-score thresholds ±2.0, and (4) protein/phosphoprotein level (RPPA) with Z-score thresholds ±2.0. (Breast Invasive Carcinoma project. 825 cases. Nature 2012). Genetic alterations were amplification, deep deletion, mRNA Downregulation, mRNA upregulation, RPPA Downregulation, RPPA upregulation, missense mutation, and truncating mutation. 81 patient/81 samples were used. *cBioPortal data is subjected to scheduled updates*

(c)

Breast Invasive Carcinoma The Cancer Genome Atlas (TCGA) Breast Invasive Carcinoma project. Total 825 cases. Raw data via the TCGA Data Portal. TCGA, Nature 2012 Patient / Case Set	BRCA1 & BRCA2
All Breast Cancer Tumors	Gene Set / Pathway is altered in 12.7% of all 825 cases
PAM50 Basal	Gene Set / Pathway is altered in 38.3% of all 81 cases
Basal-Like	Gene Set / Pathway is altered in 38.3% of all 80 cases
Basal-Like Recurred/Progressed	Gene Set / Pathway is altered in 16.7% of all 6 cases
Basal-Like Disease Free	Gene Set / Pathway is altered in 42.9% of all 70 cases

(d)

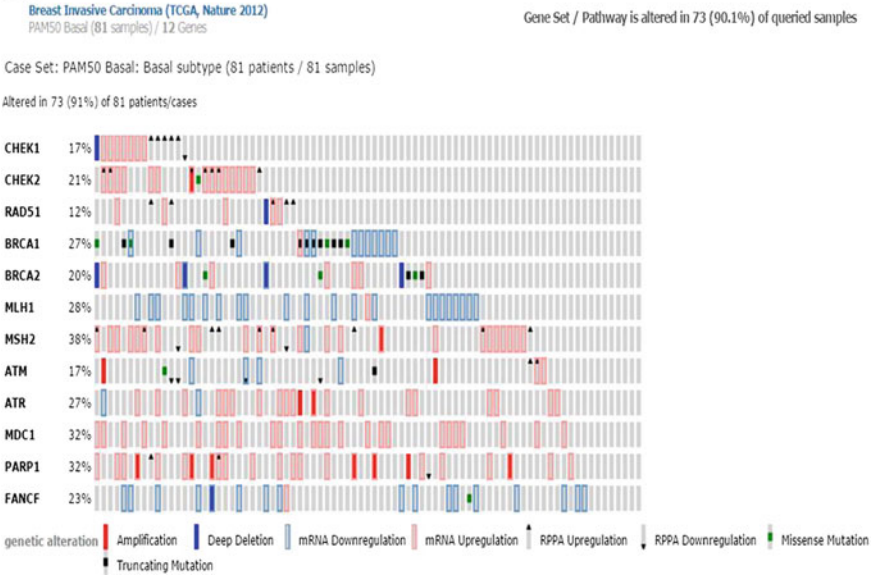


Fig. 3.2 (continued)

without functional sumoylation of PTEN was not sufficient to recruit the recombinase RAD51 to sites of DNA damage to initiate DNA repair [11]. PTEN's functional role in DNA damage repair (especially in DSB repair and nucleotide excision repair) and DNA damage response (through its interaction with the Chk1 and p53 pathways) included (1) to the ability of PTEN to suppress the formation of the γ-H2AX foci, a marker of DNA damage, which alternatively suggests that PTEN decreases DSB levels, (2) PTEN's ability to promote NER, and (3) PTEN's interaction with Chk1, p53 [111].

Thus, both PI3K enzyme and PTEN phosphatase directly regulate DDR and strongly indicate that in a tumor cell PI3K-AKT-mTOR pathway alterations can be contextually connected to DNA damage response elements and alterations in HRD genes especially in the specific subset of BC like TNBC wherein PTEN gene and HRD genes are most commonly altered.

DNA Damage in Invasive Carcinoma of Breast: Alterations of DDR Pathway Genes and HRD Genes in Basal-Like BC

An elevated load of DNA damage has been significantly associated with BC risk. It is logical to propose that a higher level of DNA damage in the context of a deficient DNA damage repair system may predispose individuals to BC. Logically, it suggests that the accumulation of DNA damage may contribute to breast carcinogenesis. In order to understand the above proposition we extended our analysis of alterations of 12 genes of DNA damage response pathway genes in PAM50 basal subtypes of BC and compared it with the changes in the alterations of 17 genes of PI3K-AKT-mTOR pathway in the same subtypes of BC (Fig. 3.2a) (TCGA, Nature 2012; 81 cases) using cBioPortal. Table (Fig. 3.2) shows that 12 of DNA damage response pathway genes (*CHEK1, CHEK2, RAD51, BRCA1, BRCA2, MLH1, MSH2, ATM, ATR, MDC1, PARP1, FANCF*) are altered in 90.1 % of all 81 cases while 17 PI3K-AKT-mTOR signaling genes are altered in 88.9 % of all 81 cases. Figure 3.2b shows changes in 12 DNA damage response genes in PAM50 Basal case set and Basal-like Breast Invasive Carcinoma (The Cancer Genome Atlas (TCGA; Raw data via the TCGA Data Portal. TCGA, Nature 2012; [20]). A custom case set was build for the number of matching cases of PAM50 basal and basal-like BC using cBioPortal (TCGA, Nature 2012 data; raw data at the NCI.). The custom case set was built for disease-free status and progressed/recurred status of basal-like cases. Figure 3.2c shows changes in BRCA1 and BRCA2 in PAM50 Basal case set and Basal-like Breast Invasive Carcinoma (The Cancer Genome Atlas (TCGA; Raw data via the TCGA Data Portal. TCGA, Nature 2012; [20]). A custom case set was build for the number of matching cases of PAM50 basal and basal-like BC using cBioPortal (TCGA, Nature 2012 data; raw data at the NCI.). The custom case set was built for disease-free status and progressed/recurred status of basal-like cases. Figure 3.2d presents oncoprint of changes in 12 DNA damage response genes in

PAM50 Basal case set of Breast Invasive Carcinoma (The Cancer Genome Atlas (TCGA; Raw data via the TCGA Data Portal. TCGA, Nature 2012; [20]). A custom case set was build for the number of matching cases of PAM50 basal BC using cBioPortal (TCGA, Nature 2012 data; raw data at the NCI.). Genetic alterations were amplification, deep deletion, mRNA Downregulation, mRNA upregulation, RPPA Downregulation, RPPA upregulation, missense mutation, and truncating mutation (81 patient/81 samples were used).

PI3K-AKT-mTOR Pathway and DDR Pathway in Basal-like BC: Protein–Protein Interactions

Interaction between these two pathways was evaluated using 13 oncogenes and tumor suppressor genes which were altered most frequently in basal-like cancers (Nature 2012 of cBioPortal) including *PTEN, AKT1, AKT2, TSC1, TSC2, mTOR, RICTOR, RHEB, BRCA1, BRCA2, ATM, ATR, FANCF* were used as input to STRING10 in order to test the association at the two confidence views, 0.900 (highest confidence) and 0.700 (high confidence). The active prediction methods included databases and Text mining. The **P**rotein–**P**rotein **I**nteraction **N**etwork (PPIN) was built from PPIs in the STRING database. STRING is a comprehensive PPI database, and the PPIs are experimentally derived or predicted by comparative genomics and Text mining. The subnetwork was built by hits in the original screen, at highest confidence interactions in the STRING network (confidence score > 0.9 as otherwise mentioned).

Proteins corresponding to the 17 PI3K-AKT-mTOR signaling pathway genes (highlighted in red) as well as 12 DNA Damage Repair pathway genes (highlighted in green) and their cellular functions used in the PPIN using STRING database were presented below.

17 PI3K-AKT-mTOR Signaling Pathway Genes: Proteins (Amino Acid; *Aa*) and Their Cellular Functions

PIK3CA—Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*1068 aa*); Phosphoinositide-3-kinase (PI3K) enzyme phosphorylates PtdIns (Phosphatidylinositol), PtdIns4P (Phosphatidylinositol 4-phosphate), and PtdIns (4,5)P2 (Phosphatidylinositol 4,5-bisphosphate) to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3) as the product. PIP3 plays a cardinal role in signaling the cell growth, survival, proliferation, motility, and morphology by recruiting PH domain-containing proteins to the membrane, including AKT1 and PDPK1. The membrane recruitment of AKT1 and PDPK1 initiates an activating signaling cascade which culminates into a complex and interactive chain of reactions comprising

of (1) activation of enzymes/transcription factors/structural proteins that facilitates cell growth, survival, proliferation, motility, and morphology and (2) inhibition of enzymes/transcription factors/structural proteins that facilitates cell death, apoptosis, and necrosis. It participates in cellular signaling (1) in response to various external agents including growth factors, cytokines, hormones, nutrients, energy, stress signals, and drugs and (2) following alterations in the specific genes in the nucleus due to external (physical and chemical) and internal (ROS and DNA repair failure). By acting as a transducer of signals from both outsides and insides of the cell, PIK3CA acts as a key component of PI3K-AKT-mTOR pathway, a master signal processor for survival and growth in a cell. Mutation (activation) of this gene has a high oncogenic potential.

PIK3R1—Phosphoinositide-3-kinase, regulatory subunit 1 (alpha) (*724 aa*)

PIK3R2—Phosphoinositide-3-kinase, regulatory subunit 2 (beta); PIK3R1 protein (*728 aa*) is an adaptor protein. It binds to activated (phosphorylated) protein-tyrosine kinases, through its SH2 domain, and acts as an adapter in mediating the association of the p110 catalytic unit to the plasma membrane.

PTEN—Phosphatase and tensin homolog; It is a tumor suppressor gene. PTEN protein (*403 aa*) acts as a dual-specificity protein phosphatase, dephosphorylating tyrosine-, serine-, and threonine-phosphorylated proteins and a lipid phosphatase, eliminating the phosphate in the D3 position of the inositol ring from phosphatidylinositol 3,4,5-trisphosphate, phosphatidylinositol 3,4-diphosphate, phosphatidylinositol 3-phosphate, and inositol 1,3,4,5-tetrakisphosphate with order of substrate preference in vitro PtdIns(3,4,5)P3 > PtdIns(3,4)P2 > PtdIns3P > Ins (1,3,4,5)P4. The lipid phosphatase activity of PTEN is critical for its tumor suppressor function and serves as a "virtual break" in downregulating an activated PI3K-AKT-mTOR pathway.

PDPK1—3-Phosphoinositide dependent protein kinase-1; serine/threonine kinase; PDPK1 protein (*556 aa*) acts as a master kinase, phosphorylating and activating a subgroup of the AGC family of protein kinases. Its targets are protein kinase B (PKB/AKT1, PKB/AKT2, PKB/AKT3), p70 ribosomal protein S6 kinase (RPS6KB1), p90 ribosomal protein S6 kinase (RPS6KA1, RPS6KA2 and RPS6KA3), cyclic AMP-dependent protein kinase (PRKACA), protein kinase C (PRKCD and PRKCZ), serum and glucocorticoid-inducible kinase (SGK1, SGK2, and SGK3), p21-activated kinase-1 (PAK1), protein kinase PKN (PKN1 and PKN2).

AKT1—v-akt murine thymoma viral oncogene homolog 1; AKT1 protein (*480 aa*) is one of the three closely related serine/threonine protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase. AKT protein regulates cellular processes including metabolism, proliferation, cell survival, growth, protein synthesis, apoptosis, and angiogenesis. The action of AKT is mediated through its serine and/or threonine phosphorylation of a wide range of downstream substrates (over 100 substrate candidates have been reported so far) which in turn controls or initiates these functions.

AKT2—v-akt murine thymoma viral oncogene homolog 1; AKT2 protein *(481 aa)* is one of the three closely related serine/threonine protein kinases (AKT1, AKT2

and AKT3) called the AKT kinase. No isoform specificity for the substrates of AKT has been reported so far.

FOXO1—Forkhead box O1; FOXO1 protein(*655 aa*) is a transcription factor that acts as the main target of insulin signaling and regulates metabolic homeostasis specifically in response to oxidative stress. FOXO1 binds to the insulin response element (IRE) with consensus sequence 5'-TT[G/A]TTTTG-3' and the related Daf-16 family binding element (DBE) with consensus sequence 5'-TT[G/A] TTTAC-3'. Activity is suppressed by insulin. FOXO1 is the main regulator of redox balance and the bone-specific endocrine function via regulating glucose metabolism.

FOXO3—Forkhead box O3; FOXO3 protein (*673 aa*) is a transcriptional activator which triggers apoptosis in the absence of survival factors, including neuronal cell death upon oxidative stress. FOXO3 protein recognizes and binds to the DNA sequence 5'-[AG]TAAA[TC]A-3' as a transcriptional factor and participates in posttranscriptional regulation of MYC: following phosphorylation by MAPKAPK5, it promotes induction of miR-34b and miR-34c expression, two posttranscriptional regulators of MYC that bind to the 3'UTR of MYC transcript and prevents its translation.

AKT1S1—AKT1 substrate 1 (proline-rich); Subunit of mTORC1; AKT1S1 protein (*256 aa*) regulates cell growth and survival in response to nutrient and hormonal signals. When growth factor stimulates mTORC1 activation, it involves an AKT1-mediated phosphorylation of TSC1-TSC2 that leads to the activation of the RHEB GTPase which potently activates the protein kinase activity of mTORC1. AKT1S1 acts as an amino acid sensor to mTORC1 which requires its relocalization to the lysosomes, mediated by the Ragulator complex and the Rag GTPases.

mTOR—Mechanistic target of rapamycin (serine/threonine kinase); mTOR protein (*2549 aa*) is a serine/threonine protein kinase which acts as a central regulator of cellular metabolism, growth, cap-dependent protein synthesis, autophagy and survival in response to hormones, drugs, growth factors, nutrients, energy, PI3K pathway activation, and stress signals. mTOR protein functions as part of two structurally and functionally distinct signaling complexes mTORC1 and mTORC2 (mTOR complex 1 and 2).

RICTOR—RPTOR independent companion of MTOR, complex 2; RICTOR protein (*1708 aa*) is a subunit of mTORC2, which regulates cell growth and survival in response to hormonal signals. mTORC2 is activated by growth factors, but, in contrast to mTORC1, appears to be nutrient-insensitive. mTORC2 function upstream of Rho GTPases to regulate the actin cytoskeleton, probably by activating one or more Rho-type guanine nucleotide exchange factors. mTORC2 promotes the serum-induced formation of stress fibers or F-actin. mTORC2 plays a critical role in AKT1 'Ser-473' phosphorylation, which may facilitate a feedback loop in the PI3K-pathway.

TSC1—Tuberous sclerosis 1; TSC1 protein (*1164 aa*) acts as a tumor suppressor. In complex with TSC2, TSC1 protein inhibits nutrient-mediated or growth factor-stimulated phosphorylation of S6K1 and EIF4EBP1 by negatively regulating mTORC1 signaling. However, it appears not to be required for TSC2 GAP activity

toward RHEB. TSC1 controls microtubule-mediated protein transport following deregulation of mTOR signaling.
TSC2—Tuberous sclerosis 2; TSC2 protein (*1807 aa*) in complex with TSC1, inhibits the nutrient-mediated or growth factor-stimulated phosphorylation of S6K1 and EIF4EBP1 by negatively regulating mTORC1 signaling. TSC2 protein acts as a GTPase-activating protein (GAP) for the small GTPase RHEB, and thus a direct activator of the protein kinase activity of mTORC1. TSC2 protein is a tumor suppressor. TSC2 protein weakly stimulates the intrinsic GTPase activity of the Ras-related proteins RAP1A and RAB5 in vitro.
RHEB—Ras homolog enriched in brain; RHEB protein (*184 aa*) has a low intrinsic GTPase activity. RHEB protein stimulates the phosphorylation of S6K1 and EIF4EBP1 via activation of mTORC1 signaling. RHEB protein activates the protein kinase activity of mTORC1.
RPTOR—Regulatory associated protein of mTOR, complex 1; RPTOR (RAPTOR) protein (*1335 aa*) functions as a scaffold for recruiting mTORC1 substrates. RPTOR (RAPTOR) protein controls the mammalian target of rapamycin complex 1 (mTORC1). mTORC1 is activated in response to growth factors or amino acids. Growth factor-stimulated mTORC1 activation occurs via AKT1-mediated phosphorylation of TSC1-TSC2 leading to the activation of the RHEB GTPase which activates the protein kinase activity of mTORC1.
MLST8—mTOR-associated protein, LST8 homolog (*S. cerevisiae*).

12 DNA Damage Repair Pathway Genes: Proteins and Their Cellular Functions

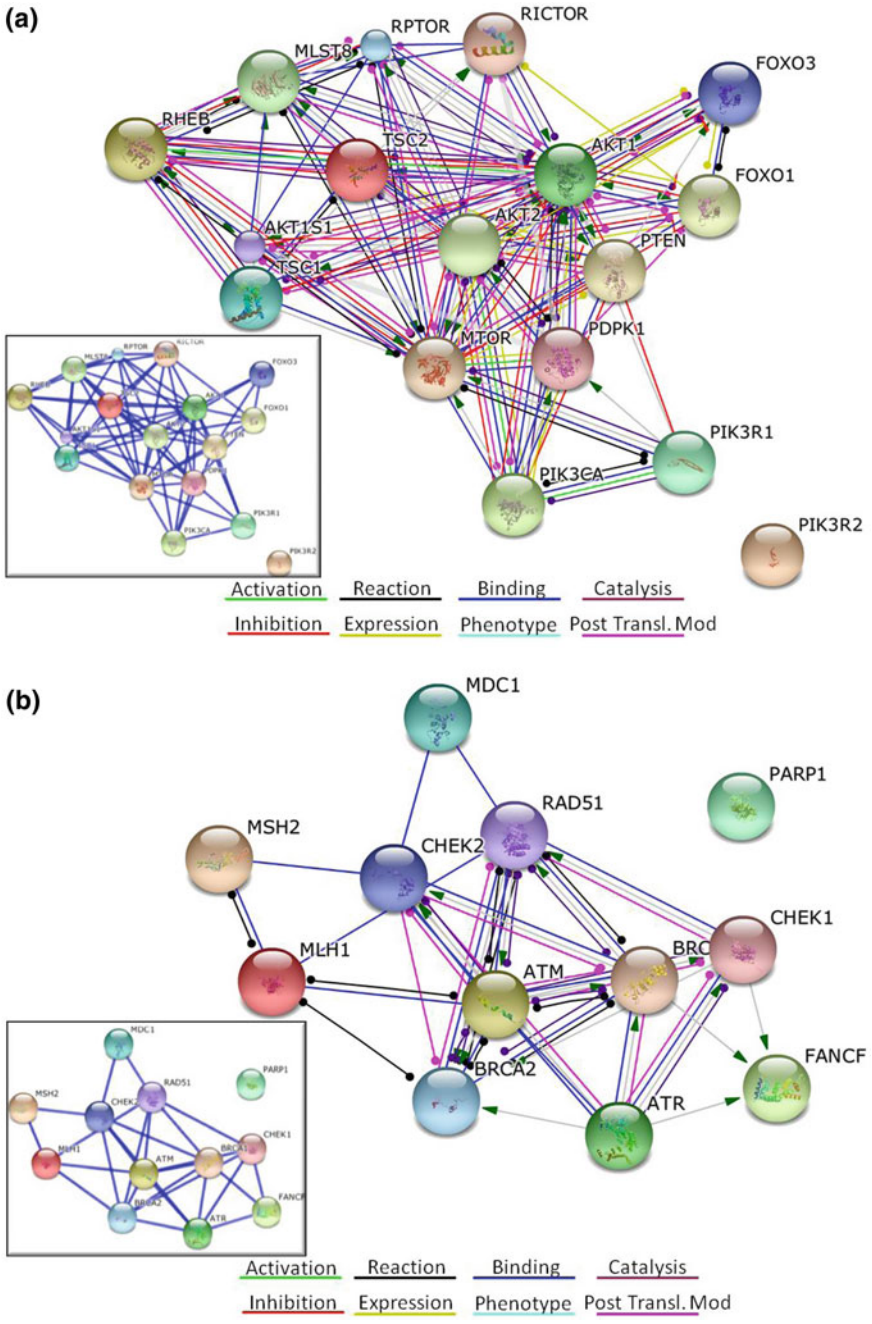
CHEK1—Checkpoint kinase 1; CHEK1 protein preserves the integrity of the genome. CHEK1 protein (*476 aa*) is a serine/threonine protein kinase which is required for checkpoint-mediated cell cycle arrest and activation of DNA repair in response to the presence of DNA damage or unreplicated DNA. CHEK1 protein also negatively regulates cell cycle progression during unperturbed cell cycles. CHEK1 protein preserves the integrity of the genome. CHEK1 protein recognizes the substrate consensus sequence [R-X-X-S/T] and binds to and phosphorylates CDC25A, CDC25B, and CDC25C.
CHEK2—Checkpoint kinase 2 (*586 aa*)
MLH1—MutL homolog 1, colon cancer, nonpolyposis type 2 (*E. coli*); MLH1 protein (*756 aa*) heterodimerizes with PMS2 to form MutL alpha, a component of the postreplicative DNA mismatch repair system (MMR).
MSH2—MutS homolog 2, colon cancer, nonpolyposis type 1 (*E. coli*); MLH1 protein (*934 aa*) is a component of the postreplicative DNA mismatch repair system (MMR). MLH1 protein forms two different heterodimers-MutS alpha (MSH2-MSH6 heterodimer) and MutS beta (MSH2-MSH3 heterodimer) which bind to DNA mismatches thereby initiating DNA repair.

ATM—Ataxia telangiectasia mutated; ATM protein (*3056 aa*) is a DNA damage sensor. ATM is a serine/threonine protein kinase which activates checkpoint signaling upon double-strand breaks (DSBs), apoptosis and genotoxic stresses such as ionizing ultraviolet A light (UVA). ATM recognizes the substrate consensus sequence [ST]-Q and phosphorylates 'Ser-139' of histone variant H2AX/H2AFX at DSBs and thus initiates and regulates DNA damage response mechanism.
ATR—*A*taxia telangiectasia and RAD3 related; ATR protein (*2644 aa*) is a DNA damage sensor for ionizing radiation (IR), ultraviolet light (UV), or DNA replication stalling. ATR protein is a serine/threonine protein kinase which activates checkpoint signaling upon genotoxic stresses such as IR, UV, or DNA replication stalling. Recognizes the substrate consensus sequence [ST]-Q and phosphorylates BRCA1, CHEK1, MCM2, RAD17, RPA2, SMC1, and p53/TP53, which collectively inhibit DNA replication and mitosis and promote DNA repair, recombination, and apoptosis. ATR protein phosphorylates 'Ser-139' of histone variant H2AX/H2AFX at sites of DNA damage and initiates the complex repair process.
FANCF—Fanconi anemia, complementation group F; FANCF protein (*374 aa*) is a DNA repair protein that operates in a postreplication repair or a cell cycle checkpoint function. FANCF protein is implicated in interstrand DNA cross-link repair and in the maintenance of normal chromosome stability.
PARP1—Poly (ADP-ribose) polymerase 1; PARP1 is a nick-sensor. PARP1 protein (*1014 aa*) is involved in the base excision repair (BER) pathway. It catalyzes the poly(ADP-ribosyl)-ation of a number of acceptor proteins involved in chromatin architecture, in damage repair, and in DNA metabolism. PARylation initiates DNA damage repair and appears as a mandatory step in detection/signaling pathway leading to the reparation of DNA strand breaks. PARP1 protein mediates the poly (ADP-ribosyl)-ation of APLF and CHFR. PARP1 protein positively regulates the transcription of MTUS1 and negatively regulates the transcription of MTUS2/TIP150. PARP1 also PARylates itself.
MDC1—*M*ediator of DNA damage checkpoint 1 (*2089 aa*)
BRCA1—Breast cancer 1, early onset; BRCA1 protein (*1884 aa*) is an E3 ubiquitin-protein ligase that specifically mediates the formation of 'Lys-6'-linked polyubiquitin chains and plays a central role in DNA repair by facilitating cellular responses to DNA damage. The E3 ubiquitin-protein ligase activity of BRCA1 protein is required for its tumor suppressor function. The BRCA1-BARD1 heterodimer coordinates a diverse range of cellular pathways such as DNA damage repair, ubiquitination, and transcriptional regulation to maintain genomic stability.
BRCA2—Breast cancer 2, early onset; BRCA2 protein (*3418 aa*) is involved in DSB repair and/or homologous recombination. BRCA2 protein binds RAD51 and potentiates recombinational DNA repair by promoting assembly of RAD51 onto single-stranded DNA (ssDNA). BRCA2 protein acts by targeting RAD51 to ssDNA over double-stranded DNA, enabling RAD51 to displace replication protein A (RPA) from ssDNA and stabilizing RAD51-ssDNA filaments by blocking ATP hydrolysis. BRCA2 protein participates in S phase checkpoint activation. BRCA2 protein binds selectively to ssDNA, and to ssDNA in tailed duplexes and replication

fork structures. BRCA2 mutation (germline and sporadic are strongly associated with cancer predisposition including ovary and breast).
RAD51—RAD51 homolog (S. cerevisiae); RAD51protein (*340 aa*) participates in a common DNA damage response pathway associated with the activation of homologous recombination and DSB repair. RAD51 protein binds to single- and double-stranded DNA and exhibits DNA-dependent ATPase activity. RAD51 protein reorients duplex DNA and forms helical nucleoprotein filaments. RAD51protein regulates mitochondrial DNA copy number under conditions of oxidative stress in the presence of RAD51C and XRCC3.

We used the network image of the "Action View" of the network summary of 17 PI3K-AKT-mTOR signaling pathway genes which are frequently altered in cancers (cBioPortal) as obtained using STRING 10. The network is displayed in Fig. 3.3a. Using STRING 10 we obtained the "Action View" of the network summary of 12 DNA damage repair genes those are identified in cBioPortal as genes "known" to be frequently altered in cancers Fig. 3.3b. Figure 3.3c presents interaction between these two pathways, PI3K-AKT-mTOR and DNA damage repair pathways which included 13 oncogenes and tumor suppressor genes that were highly altered in basal-like cancers as shown in Fig. 3.1g (data from cBioPortal; Nature 2012 of cBioPortal) including *PTEN, AKT1, AKT2, TSC1, TSC2, mTOR, RICTOR, RHEB, BRCA1, BRCA2, ATM, ATR, FANCF* which were used as "input" to STRING10 in order to find the association at the two "Confidence Views", 0.900 (highest confidence). "Action View" is presented with "Confidence View" as the inset. The active prediction methods included databases and Text mining. The customs limit was chosen for the interactors. Figure 3.3d presents interaction between these two pathways, PI3K-AKT-mTOR and DNA damage repair pathways which included 13 oncogenes and tumor suppressor genes that were highly altered in basal-like cancers (data from cBioPortal; Nature 2012 of cBioPortal) including *PTEN, AKT1, AKT2, TSC1, TSC2, mTOR, RICTOR, RHEB, BRCA1, BRCA2, ATM, ATR, FANCF* which were used as "input" to STRING10 in order to find the association at the two "Confidence Views", 0.700 (high confidence). "Action View" is presented with "Confidence View" as the inset. The active prediction methods included databases and Text mining. The customs limit was chosen for the interactors.

16 genes of the PI3K-AKT-mTOR signaling pathway appear to be closely associated both at the level of "Confidence Views" and "Action Views" (Fig. 3.3a). A similar close association was observed among 11 genes of the DNA Damage Repair pathway (Fig. 3.3b). These close and intricate associations in between genes of both the pathways clearly indicate the overall complexity of the pathways and also form the basis of the tremendous amount of plasticity of the signals originating from the respective pathways. This plasticity is by far the best way to explain the development of inevitable resistance to the drug in the tumor cells. When the "Action Views" of 16 genes of the PI3K-AKT-mTOR signaling pathway is compared with the "Action Views" of 11 genes of the DNA Damage Repair pathway it is clearly evident that the associations between the individual gene of the PI3K-AKT-mTOR signaling pathway is much tighter and intricate than the association of the DNA Damage Repair


(a)
RPTOR
RICTOR
MLST8
FOXO3
RHEB
TSC2
AKT1
FOXO1
AKT1S1
AKT2
PTEN
TSC1
MTOR
PDPK1
PIK3R1
PIK3CA
PIK3R2
Activation
Reaction
Binding
Catalysis
Inhibition
Expression
Phenotype
Post Transl. Mod
(b)
MDC1
PARP1
RAD51
MSH2
CHEK2
CHEK1
MLH1
BRC
ATM
FANCF
BRCA2
ATR
Activation
Reaction
Binding
Catalysis
Inhibition
Expression
Phenotype
Post Transl. Mod

◀ **Fig. 3.3** **a** The network image of the "Action View" and "Confidence View" of association of 17 PI3K-AKT-mTOR signaling pathway genes that were frequently altered in cancers (cBioPortal) is obtained using STRING 10. The network is displayed as Nodes which are either colored (as they are directly linked to the input of 17 genes). Edges, i.e., predicted functional links, consist of up to eight lines: one color for each type of evidence (as shown in the picture). Active prediction methods included Databases and Text mining. Confidence view of the association is presented as an inset. Stronger associations are represented by thicker lines. The confidence score presented is at the highest confidence level (0.900). The confidence score is the approximate probability that a predicted link exists between two enzymes in the same metabolic map in the KEGG database. **b** "Action View" of the network summary of 12 DNA damage repair genes those are frequently altered in cancers as obtained using STRING 10. The network is displayed as Nodes which are either colored (as they are directly linked to the input of 12 genes). Edges, i.e., predicted functional links, consist of up to eight lines: one color for each type of evidence (as shown in the picture). Active prediction methods included Databases and Text mining. "Confidence View" of the association is presented as an inset. Stronger associations are represented by thicker lines. The confidence score presented is at the highest confidence level (0.900). **c** Interaction between these two pathways, PI3K-AKT-mTOR and DNA damage repair pathways which included 13 oncogenes and tumor suppressor genes that were highly altered in basal-like cancers (data from cBioPortal; Nature 2012 of cBioPortal) including *PTEN, AKT1, AKT2, TSC1, TSC2, mTOR, RICTOR, RHEB, BRCA1, BRCA2, ATM, ATR, FANCF* were used as "input" to STRING10 in order to find the association at the two "Confidence Views", 0.900 (highest confidence). "Action View" is presented with "Confidence View" as the inset. The active prediction methods included databases and Text mining. The customs limit was chosen for the interactors. **d** Interaction between these two pathways, PI3K-AKT-mTOR and DNA damage repair pathways which included 13 oncogenes and tumor suppressor genes that were highly altered in basal-like cancers (data from cBioPortal; Nature 2012 of cBioPortal) including *PTEN, AKT1, AKT2, TSC1, TSC2, mTOR, RICTOR, RHEB, BRCA1, BRCA2, ATM, ATR, FANCF* were used as "input" to STRING10 in order to find the association at the two confidence views, 0.700 (high confidence). "Action View" is presented with "Confidence View" as the inset. The active prediction methods included databases and Text mining. The customs limit was chosen for the interactors. STRING *(Search Tool for the Retrieval of Interacting Genes/Proteins)* is being developed at CPR (NNF Center for Protein Research), EMBL (European Molecular Biology Laboratory), SIB (Swiss Institute of Bioinformatics), KU (**SUND-KU,** University of Copenhagen), TUD (Technical University Dresden, Biotec), and UZH (University of Zurich). The version 10 of STRING covers more than 2000 organisms and is equipped with improved prediction algorithms. We acknowledge the version 10 of STRING and authorities/institutions/organizations/Universities/resources which institutionally and financially support the STRING

pathway genes indicating a higher level of plasticity in the PI3K-AKT-mTOR signaling pathway. Interestingly, we find that among 17 PI3K-AKT-mTOR signaling pathway genes, PIK3R2 was not associated with other genes as shown in the network image of both "Confidence Views" and "Action Views" (Fig. 3.3a). Similarly among 12 DNA Damage Repair pathway genes, PARP1 was not associated with other genes as shown in the network image of both "Confidence Views" and "Action Views" (Fig. 3.3b). At the high level of confidence, PTEN gene of the PI3K-AKT-mTOR signaling pathway was observed to be associated with BRCA1/2 genes of the DDR pathway both in "Confidence Views" and "Action Views" (Fig. 3.3d) in contrast to the highest confidence level (Fig. 3.3c).

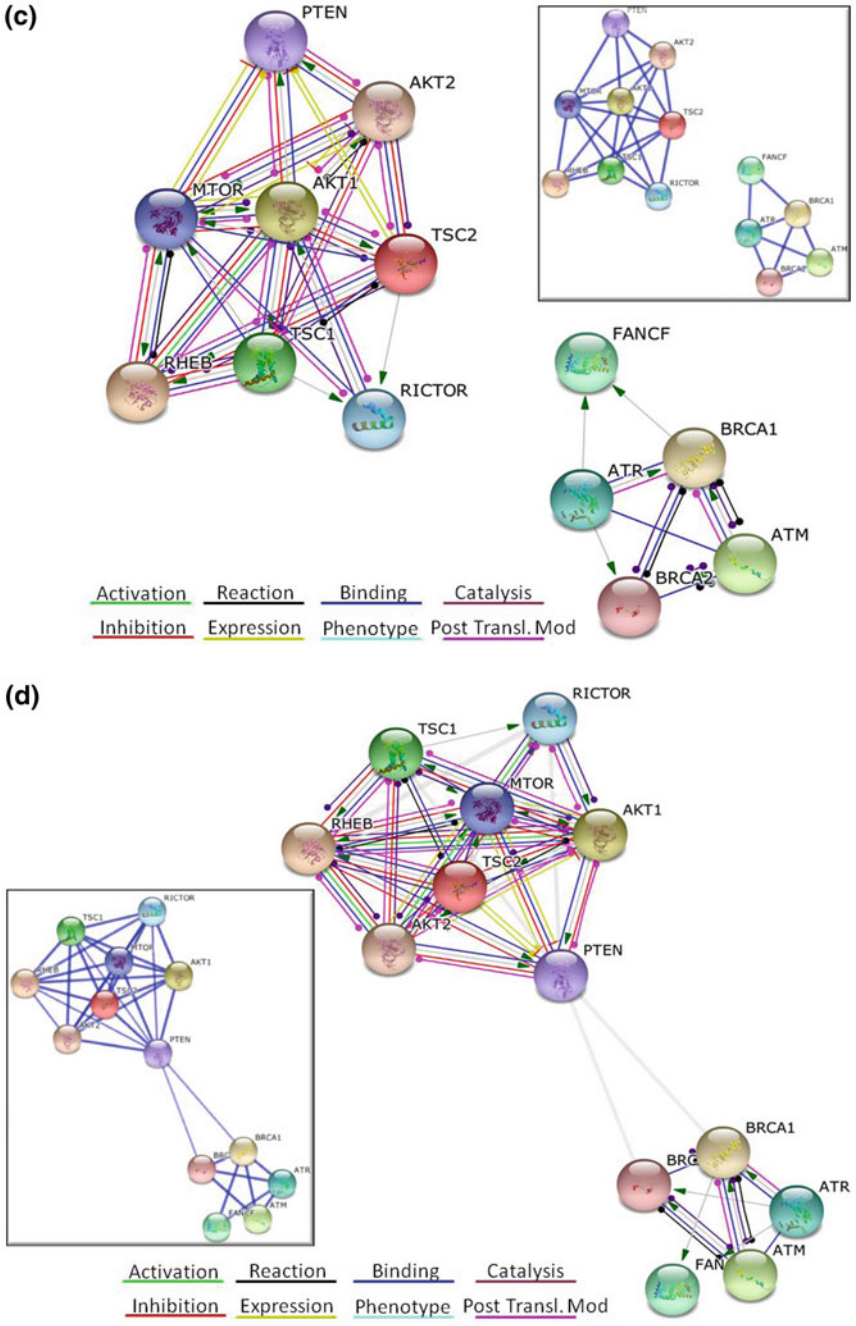


Fig. 3.3 (continued)

PI3K-AKT-mTOR Pathway Inhibition in the Context of DDR: PARP Inhibitors in BC

The outcome of DDR is an integrated response of both proapoptotic and prosurvival signaling routes. A proapoptotic response which initiates the death of a cell will ensue depending on the inferiority/failure/surrender of the prosurvival signaling (to the proapoptotic signaling) which would have facilitated the survival via initiating the repair. Thus, it is obvious that DDR is linked with the cell prosurvival pathway like the PI3K-AKT-mTOR pathway. Recently, a review by Paull highlighted functional similarities between the activation mechanisms of ATM, phosphatidylinositol 3-kinases (PI3Ks), and the other PI3K-like kinases, as well as the cellular mode of ATM kinase activation following DNA damage/oxidative stress and structural insights into their regulation [125]. DNA-PK, ATM, and ATR members of PI3K family are involved in the H2AX phosphorylation, a marker of DNA damage [62]. Evidence suggests that there is a close functional relationship between the DDR and PI3K-AKT-mTOR pathway. PI3K/AKT and MDM2 activation were reported to be associated with the inhibitory effect of cAMP increasing agents on DNA damage-induced cell death in cells [60].

PI3K-AKT-mTOR pathway's involvement in DDR and PI3K-AKT-mTOR pathway's cross-talk to the components of DDR are the fundamental bases for the testing of the combination of these drugs in tumor cells. As the details about the signaling landscape of these two pathways are being identified explaining their interactions at different functional levels, the rationale behind the combination of inhibitors of these two pathways are becoming more logical in the treatment of cancers and the management of the resistance to the anticancer drugs in clinics. The example of the cross talk between the components of DDR and the PI3K-AKT pathway was clearly evident from effects of DNA-PKcs deficiency on the chemosensitivity of human hepatoma HepG2 cells to cisplatin (CDDP) and 5-fluorouracil (5-Fu). Recently, DNA-PKcs deficiency has been reported to sensitize the human hepatoma HepG2 cells to DNA-damaging cytotoxic drugs including cisplatin and 5-fluorouracil through suppression of the PI3K-AKT-NF-κB pathway [52].

In addition to the cross-talk between the PI3K-AKT-mTOR pathway and DDR, there exists a direct functional interplay between two predominant tumor suppressors of each pathway *BRCA1* and *PTEN* as reported in BC [109]. BRCA1 regulates the PI3K-AKT pathway by acting on upstream kinases of AKT and some of the BC with *BRCA1*mutations have high frequencies of *PTEN* mutations [135]. PTEN acts on chromatin and regulates expression of RAD51, which repairs spontaneous DSBs [142, 143]. It is possible that nuclear PTEN participates in a variety of nuclear functions like DNA repair, cell cycle arrest, and genome stability and these functions are brought out in cooperation with BRCA1. A connection between tumor suppressor BRCA1 and PTEN in damaged DNA repair has been reviewed by Minami, Nakanishi et al. PTEN and BRCA1 genes are now recognized as one of the most frequently deleted and/or mutated in many human cancers and PTEN as well as BRCA1 is known to play critical role in DNA damage responses. The PI3K/AKT

pathway has been found to be constitutively active in BRCA1-defective human cancer cells. Loss or decrease of PTEN or BRCA1 function, either by mutation or following reduced expression contributed to the development of various tumors. The literature showed that the *BRCA1* gene product can functionally cooperate with PTEN and might be an essential blockage in the development of several tumors [109]. Their review summarized recent findings regarding the functions of BRCA1 and PTEN which were involved in genomic stability and cancer cell signaling. It can be argued here that this cooperation can be mediated by the effect of both PTEN and BRCA1 on genome stability. From PTEN's side of the story, a loss of PTEN would increase cell survival and reduce DNA repair, which would lead to genomic instability. Resistance to cytotoxic chemotherapy drugs/radiation which causes alterations in the DNA to induce genotoxic stress to the tumor cells although poses the most unsurpassable obstacle in clinics provides an opportunity to study the drug interactions in tumor cells on a real-time basis. Hence, the other evidence regarding the close interaction between the PI3K-AKT-mTOR pathway and DDR is presented from the radioresistance and chemoresistance in cancers. Several reports have indicated that reduced levels of PTEN are associated with radioresistance, which can be suppressed by ectopic PTEN expression [74, 89]. AKT is the direct downstream effector of PTEN and a signaling readout of PTEN function (lipid phosphatase) as a direct inverse relationship exists between phosphorylated AKT (S473) and lipid phosphatase function of PTEN in cells. A study by Toker et al., on doxorubicin resistance in BC reported that AKT substrate MERIT40 helps in the resolution of DNA damage induced by chemotherapy [15]. MERIT40, a component of the BRCA1-A DNA damage repair complex has been demonstrated to be phosphorylated following doxorubicin treatment which facilitates assembly of the BRCA1-A complex in response to DNA damage and contributes to doxorubicin resistance via DNA repair and cell survival (following doxorubicin treatment).

Thus, an understanding of the connection between BRCA1 and PTEN would be a key to appreciating the cross talk between the PI3K-AKT-mTOR pathway and DDR pathway toward evolving to a therapeutically beneficial regime. A similar functional relationship was reported in the case of INPP4B, the lipid phosphatase inositol polyphosphate 4-phosphatase type II has been described as a tumor suppressor in the PI3K-Akt pathway with the loss of expression found most commonly in the breast (TNBC), ovarian cancer, and melanoma. In a recent report, Ip et al. demonstrated the DNA repair defect in INPP4B-deficient cells by comet assays and quantification of γH2AX, RAD51, and 53BP1 foci formation. INPP4B loss resulted in significantly increased PARP sensitivity in vitro models, as well as in vivo xenograft models. INPP4B forms a protein complex with ATR and BRCA1, in GST pull-down and 293T overexpression assays, and INPP4B loss affected BRCA1, ATM and ATR protein stability resulting in the observed DNA repair defect [67]. Thus, a defect in the repair of DNA damage due to a loss of a phosphatase belonging to the PI3K pathway has been demonstrated to be associated with the loss of BRCA1, ATM, and ATR and was targeted by PARP inhibitor.

PARP Inhibitors as Chemo/Radiopotentiating Agents: TNBC and Beyond

TNBC with all its clinical distinctiveness including (1) discordance of definition between TNBC and basal-like BC, (2) diverse and heterogeneous molecular features, (3) conspicuous absence of actionable targets, (4) BRCA-ness, and (5) yet-to-define biomarker status is the most challenging BC subtype at the clinical setting. Although several studies [1, 17, 18, 26, 42, 56, 84, 87, 88, 107, 122, 129, 144, 153, 159] have been undertaken to resolve the differences TNBC and basal-like BC, their inherent discordance, and the intratumor heterogeneity in order to define the molecular subtypes of this BC subtype, the results of those studies and their associated clinical trials have added more questions/controversies than answers.

One of the most agreeable conclusions amidst of different controversies regarding the usefulness of PARP inhibitor(s) is the possibility that PARP inhibitors can target the BRCA-ness in order to inhibit DDR which in turn induces apoptosis [7, 17, 35, 72, 85, 128, 133, 147]. The premise of the use of PARP inhibitors in BRCA1/2-associated and sporadic cancers have come a long way based on the concept of "synthetic lethality" [16, 46, 53, 55]. Following the "proof-of-concept trial" by Andrew Tutt et al. using oral poly(ADP-ribose) polymerase (PARP) inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer [154], Iniparib plus chemotherapy was soon tested in metastatic TNBC [119]. Although the pathway of the development of PARP inhibitor was rough [50, 104, 119, 120], PARP inhibition is projected as the "targeted" therapy for advanced breast cancer (both TNBC and BRCA deficient) [5, 9, 22]. In a comprehensive overview of the distinct levels of the polypharmacology of PARP-1 inhibitors, (interfamily polypharmacology, intrafamily polypharmacology, and multisignaling polypharmacology) Passeri et al. have recently reviewed the progress made in gaining insight into the molecular basis of the multiple target-independent and target-dependent activities of PARP-1 inhibitors [124]. Regulation of FANCD2 by the mTOR pathway has been shown to contribute to the resistance of cancer cells to DNA DSBs by Shen et al., as inhibition of mTOR resulted in sensitization to DNA-damaging agents. In their study, it was demonstrated that AZD8055, an mTOR-selective kinase inhibitor significantly enhanced the sensitivity of a pediatric rhabdomyosarcoma xenograft to radiotherapy and sensitized rhabdomyosarcoma cells to the DNA interstrand cross-linked (ICL) melphalan. Sensitization correlated with drug-induced downregulation of a key component of the Fanconi anemia pathway, FANCD2 through mTOR regulation of FANCD2 gene transcripts via mTORC1-S6K1 [141]. Interestingly in a study delineating large deletion causing von Hippel–Lindau disease and hereditary breast cancer syndrome, a deletion of FANCD2 gene has been reported to be an important gene in the DNA repair pathway that might be associated with an increased risk of breast cancer wherein large VHL deletions (patients with intragenic mutations of the VHL gene) that remove the FANCD2 gene occur [80]. In a study similar to Shen et al., Zang et al. reported that targeting the PI3K-AKT-mTOR pathway signaling can sensitize cancer cells to radiotherapy and

chemotherapy [156]. Mechanistically, PI3K-AKT-mTOR signaling contributes to DDR via its control on FANCD2 and ribonucleotide reductase (RNR) which helps in the entire process of DDR at different nodes including ATM-Chk2 and ATR-Chk1 activation [141]. FANCD2 is involved in the development of resistance of cells to DNA damage agents and the activation of DNA damage checkpoints while RNR is critical for the completion of DNA replication and repair in response to DNA damage and replication stress. Thus the regulation of FANCD2 and RNR by PI3K-AKT-mTOR signaling advocates that cancer cells depend on PI3K-AKT-mTOR pathway for survival in response to DNA damage, indicating that the PI3K-AKT-mTOR pathway promotes resistance to chemotherapy and radiotherapy by enhancing DNA damage repair, thus linking PI3K-AKT-mTOR pathway to DDR pathway in tumor cells and strengthening the premise for a therapeutic combination of PARP inhibitor and PI3K-AKT-mTOR pathway inhibitor.

Because genomic instability marks cancer, several chemotherapeutic drugs and radiotherapy induce DNA damage to prevent cancer cell replication. Mechanistically, cancer cells being treated with chemotherapeutic drugs or radiotherapy, in turn, activate different DNA damage response pathways to either repair the damage or induce cell death. DDR pathways also elicit metabolic alterations and thus the PI3K-AKT-mTOR signaling can logically contribute to DDR via its control of cellular metabolism. To further strengthen this concept, we refer to a recent study by Bhute and Palecek who studied metabolic effects resulting from different types of DNA damage and repair mechanisms in BC cell lines [13]. Using NMR metabolomics to identify metabolic pathways they studied altered cellular metabolism in MCF7 cells in response to different DNA-damaging agents. Their findings point out that cancer cell metabolic responses depend on the type of DNA damage responses and can also be used to classify the type of DNA damage suggesting an application of metabolomics to classify the types of DNA damage responses. Interestingly enough, the suppression of homologous recombination by Insulin-like growth factor-1 inhibition has been reported to sensitize cancer cells to PARP inhibitors [4]. The study demonstrated that cells with mutated/methylated BRCA1 had an impaired HR function, and had an overactivation of the IGF-1R pathway. These cells were found to be more sensitive to IGF-1R inhibition compared to HR-proficient cells. The IGF-IR inhibitor reduced RAD51 expression at mRNA and protein levels in HR-proficient cells and sensitized these cells to PARP inhibitor. Targeting IGF-1R might lead to improved personalized therapeutic approaches in cancer patients with HR deficiency. These results indicate that targeting both PARP and IGF-1R might be beneficial in obtaining better clinical efficacy in HR-deficient patients and expand the use of PARP inhibitors to the population of patients who have indications to target IGF-1R. The most direct mode of interaction between DDR and the PI3K-mTOR pathway can be understood from the studies of Prof. G Mills and his colleagues [112] who reported that mTOR inhibitors suppress homologous recombination repair and synergize with PARP inhibitors in BRCA-proficient triple-negative breast cancer which strongly suggest that combination of mTOR inhibitors and PARP inhibitors would be clinically effective to treat BRCA-proficient TNBC patients.

The wealth of information we have regarding the merits of the clinical use of Poly (ADP-ribose) polymerase inhibitors in metastatic breast cancer and TNBC is staggering [28, 42, 68, 85, 132, 148]. It has also been suggested that the treatment combination of a PARP inhibitor with DNA-damaging chemotherapy may be an effective strategy for some of these tumors [50]. The initial reports regarding the chemopotentiating effect of temozolomide, irinotecan, and cisplatin activity by a PARP inhibitor was presented by Miknyoczki et al., using CEP-6800, a PARP inhibitor [108]. A number of articles described the chemo/radiopotentiating property of PARP inhibitors in a wide variety of solid tumors [14, 19, 32, 40, 114, 117, 121, 149] and many clinical trials were initiated based on the results of these studies. A phase II trial of the PARP inhibitor veliparib (ABT888) and temozolomide was instituted for metastatic breast cancer [69]. Doxorubicin has been reported to enhance Snail/LSD1-mediated PTEN suppression in a PARP1-dependent manner [90]. The PARP inhibitor ABT-888 has been reported to synergize irinotecan treatment of colon cancer cell lines indicating that ABT-888 may be a clinically effective adjuvant to current colon cancer therapies that include the use of irinotecan and/or oxaliplatin [33]. Initial reports regarding the chemo potentiation of temozolomide, irinotecan, and cisplatin activity by a PARP inhibitor was presented by Miknyoczki et al., using CEP-6800, a PARP inhibitor [108]. The results of these studies describing the chemo/radiopotentiating property of PARP inhibitors in a wide variety of solid tumors [14, 19, 32, 40, 117, 137, 149] have generated an increased interest toward the employment of PARP inhibitors as chemotherapeutic adjuvants. Murai et al. put forward the rationale for PARP inhibitors in combination therapy with camptothecins or temozolomide based on PARP trapping versus catalytic inhibition [116]. The results showed that catalytic PARP inhibitors are highly effective in combination with camptothecins, whereas PARP inhibitors capable of PARP trapping are more effective with temozolomide and provided insights in combination treatment rationales for different PARP inhibitors. A synergistic effect of olaparib with a combination of cisplatin was observed in PTEN-deficient lung cancer cells wherein the mechanistic investigations revealed that PTEN deficiency caused reductions in nuclear RAD51 and RPA focus formation and phosphorylated Chk1 and Mre11, which also indicated that genetic inactivation of PTEN might lead to the suppression of DNA repair in the tumor cells [110]. Efficacy of Carboplatin Alone and in Combination with ABT888 in intracranial murine models of BRCA-Mutated and BRCA–wild-type TNBC has been reported [76]. A recent study by Mariano et al. demonstrated that the PARP inhibitor ABT-888 modulated the MDA-MB-231 cell response to doxorubicin, leading to an increase in the rate of apoptosis. Mechanistically, PARP-1 controlled Snail expression at transcriptional level in cells exposed to doxorubicin. Their results suggested that one of the mechanisms through which PARP inhibitions can chemosensitize cancer cells is targeting Snail expression and thus promoting apoptosis [100]. Synergistic effect of PARP inhibitor with c-Met inhibitor has been observed in BC. The combination of c-Met and PARP1 inhibitors synergized to suppress the growth of breast cancer cells in vitro and xenograft tumor models. In their report, Du et al. demonstrated that blocking c-Met-mediated PARP1

phosphorylation enhances antitumor effects of PARP inhibitors suggesting that the abundance of PARP1 pY907 may predict tumor resistance to PARP inhibitors and that treatment with a combination of c-Met and PARP inhibitors may benefit patients with high c-Met expression and insensitive to PARP inhibition alone [47].

A Combination of PARP Inhibitors and PI3K-AKT-mTOR Pathway Inhibitors: Drug Sensitivity and Drug Resistance

The first experimental evidence in favor of the potential to combine inhibition of PI3K and PARP in cancer therapy [130] was provided by LC Cantley's group in BRCA1-related breast cancer [75]. Responding to need to improve treatments for metastatic breast cancer, Juvekar et al. used a MMTV-CreBrca1(f/f)Trp53(±) mouse model of BC with the activation of the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways [75]. Treatment with the pan-class IA PI3K inhibitor NVP-BKM120 delayed the tumor doubling from 5 to 26 days. Mechanistically, PI3K inhibition increased indicators of DNA damage, poly-ADP-ribosylation (PAR), and γ-H2AX and decreased RAD51 focus formation suggesting a role of PI3K activity for RAD51 recruitment. When combined with NVP-BKM120 and olaparib tumor doubling was delayed to more than 70 days in the mouse model and more than 50 days in xenotransplants from human BRCA1-related tumors indicating that the combination of a PI3K inhibitor with a PARP inhibitor provides a new trend of combination to be tested in clinics for BRCA1-related BC. In BRCA1-deficient breast cancer cells co-targeting of the PI3K pathway was reported to improve the response of to PARP1 inhibition by Kimbung et al. [78]. In their study, a sequential combination of PARP and PI3K inhibitors interacted synergistically to significantly decrease growth compared to PARP inhibition alone. Global transcriptional profiling showed that this decrease in growth was associated with downregulation of macromolecule biosynthesis and the induction of apoptosis. During the same time (same publication issue), J Baselga's group put forward experimental evidence that inhibition of PI3K following BKM120 impaired BRCA1/2 expression and sensitized BRCA-proficient TNBC to PARP inhibition [66]. Their study demonstrated that in TNBC cells PI3K inhibition leads to DNA damage, downregulation of BRCA1/2, the gain in poly-ADP-ribosylation, and subsequent sensitization to PARP inhibition. In line with this observation a dual PI3K and PARP inhibition with BKM120 and olaparib reduced the growth of patient-derived primary TNBC xenografts and displaying BRCA1/2 downregulation following PI3K inhibition. PI3K-mediated BRCA downregulation was mediated by ERK-dependent ETS1 transcription factor. Considering the limited therapy options for TNBC patients and more so for the BRCA-proficient TNBC patients (PARP inhibitors have clinical activity restricted to a small subgroup of patients with BRCA mutations) their study demonstrated that PI3K blockade-mediated HR impairment can effectively sensitize PARP inhibition

in BRCA-proficient TNBC patients [66]. Building on their study in the following years, we hypothesized that a node-specific inhibition of the PI3K pathway by GDC-0980 in the presence of carboplatin would result in (1) an enhanced impairment of DSB repair and (2) a subsequent sensitization to PARPi. This effect occurring simultaneously with the inhibition of classic PI3K-mTOR survival signal (s) would induce a robust antiproliferative/proapoptotic signal(s) even in BRCA-competent TNBC cells (a BC-subtype model in which PARPi are not active). We reported for the *first time* that a node-specific dual inhibition of the PI3K-mTOR pathway by GDC-0980 caused an impairment of DSB repair and resulted in a consequent sensitization to ABT888 plus carboplatin treatment in a BRCA-competent TNBC model. We demonstrated that in a BRCA-competent model, GDC-0980 enhanced the antitumor activity of ABT888 plus carboplatin by inhibiting both tumor cell proliferation and tumor-induced angiogenesis along with an increase in the tumor cell apoptosis [39]. This is the first mechanism-based study to demonstrate that a dual-node inhibition of the PI3K-AKT-mTOR pathway can potentiate the antitumor efficacy of PARP inhibitor in combination with a chemotherapy drug in BRCA-proficient TNBC model. Our data identified the inhibition of DDR as another mode of action of GDC-0980 and demonstrate that when combined with carboplatin plus ABT888, GDC-0980 sensitized BRCA-competent TNBC cells to PARP inhibitor to induce an effective antitumor effect. This study demonstrates that an additional mechanism of action of GDC-0980 is the inhibition of the PI3K pathway-dependent DNA damage response, which is augmented by PARP inhibition owing to an inability to respond to additional DNA damage induced by carboplatin. The latter provides additional support that PI3K and mTOR regulate DNA damage responses both in vitro and in vivo and provides a strong mechanistic rationale for the combination of the PI3K-mTOR pathway inhibitors with PARPi in TNBC that is BRCA proficient [39]. Interestingly, Cardnell et al. demonstrated that proteomic markers of DNA repair ("DNA repair protein score") and PI3K pathway activation predicted response to the PARP inhibitor BMN 673 in SCLC xenograft models which complemented the report regarding the cooperation between DNA repair and PI3K pathways [21].

In the phase II TBCRC009 trial reported in the Journal of Clinical Oncology, Isakoff et al. found that platinum monotherapy was active in treatment of metastatic triple-negative breast cancer, particularly in cases with BRCA1/2 mutation, and that an assay of genomic instability characteristic of BRCA1/2 deficiency may predict better outcome in patients without BRCA1/2 mutation (*TBCRC009: A Multicenter Phase II Clinical Trial of Platinum Monotherapy With Biomarker Assessment in Metastatic Triple-Negative Breast Cancer; Steven J. Isakoff, Erica L. Mayer, Lei He, Tiffany A. Traina, Lisa A. Carey, Karen J. Krag, Hope S. Rugo, Minetta C. Liu, Vered Stearns, Steven E. Come, Kirsten M. Timms, Anne-Renee Hartman, Darrel R. Borger, Dianne M. Finkelstein, Judy E. Garber, Paula D. Ryan, Eric P. Winer, Paul E. Goss and Leif W. Ellisen; Presented in part at the 47th Annual Meeting of the American Society of Clinical Oncology (ASCO), Chicago, IL, June 3–7, 2011; the 50th Annual Meeting of the ASCO, Chicago, IL, May 30–June 3, 2014; and the*

Clinical and Translational Research Center-American Association for Cancer Research 35th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 4–8, 2012.). The investigators concluded: "Platinum agents are active in [metastatic triple-negative breast cancer], especially in patients with germline BRCA1/2 mutations. A measure of tumor DNA repair function may identify patients without mutations who could benefit from platinum therapy agents. Prospective controlled confirmatory trials are warranted" (ASCO Post; By Matthew Stenger, Posted: 4/14/2015 12:17:00 PM; Last Updated: 4/14/2015 12:17:00 PM). Our report indicated that an additional load of DNA damage by carboplatin leads to an enhanced impairment of DSB repair effective in obtaining an antitumor effect of PARP inhibitor and PI3K-mTOR pathway inhibitor [39]. Similarly, radiation was used by Jang et al., to induce the burden of DNA DSBs. They reported that radiosensitization with combined use of olaparib and PI-103 has found to be efficacious in a xenograft model of TNBC [70]. Efficacy of carboplatin alone and in combination with ABT888 in Intracranial murine models of BRCA-mutated and BRCA–wild-type TNBC is also reported by Karginova et al. [76].

Subsequently, we demonstrated that following a dual inhibition of PI3K-mTOR, S6RP/4EBP1 dephosphorylation tracks more consistently with the drug's tumor growth inhibitory response rather than the upstream state of AKT activation. Unlike mTORC1 inhibitor RAD001, GDC-0980 potently eliminates (in vitro and in vivo) feedback reactivation of the pathway as, (1) it targets PI3K, reactivation of AKT^{T308} is blocked and (2) inhibition of the mTORC2 complex blocks the reactivation of AKT^{S473}. TNBC tumors with PTEN-independent RAS/RAF mutation-mediated activation of PI3K-mTOR pathway can be controlled by dual node blockade of the PI3K-mTOR pathway when combined with a PARP inhibitor and carboplatin. In contrast, blocking a single nodal point of PI3K by GDC-0941 failed to inhibit significantly the growth of preestablished tumors (>20 %) even in combination with A and C in MDA-MB231 xenografts, while GDC-0980 potentiated an antitumor effect by inhibiting tumor growth by 90 %. Data indicated that the nullness of PTEN synergized with the drug effects [43]. The role of PTEN was reported in prostate cancer model by González-Billalabeitia et al., wherein they demonstrated that PARP and PI3K inhibitors effectively synergize to suppress tumorigenesis in human prostate cancer cell lines and in a *PTEN/TP53*-deficient mouse model of advanced prostate cancer. Their study identified a combinatorial treatment with PARP and PI3K inhibitors as an effective option for PTEN-deficient prostate cancer [61]. Interestingly, PTEN's role in the nucleus in the context of DNA damage response is also reported to be connected to its role in autophagy. PTEN phosphorylation by ATM was found to be essential for PTEN nuclear translocation and the subsequent induction of autophagy in response to DNA damage [25]. On the flip side of it, overexpression of INPP4B which encodes the inositol polyphosphate 4-phosphatase type II, one of the other phosphatase involved in phosphatidylinositol signaling pathways (removes the phosphate group at position 4 of the inositol ring from inositol 3,4-bisphosphate) has been shown to enhance the antitumor efficacy of PARP inhibitor AG014699 in MDA-MB-231 TNBC cells [146]. Loss of INPP4B

has been shown to cause a DNA repair defect through loss of BRCA1, ATM and ATR and can be targeted with PARP inhibitor treatment [67].

The sensitivity of a tumor cell to a rational drug combination is obvious and the development of resistance in the tumor cell to the drug combination in due course of time is inevitable. The biology of the signal transduction in the tumor cell is responsible for determining its sensitivity to a drug and is critical to understand and or predict the development of the resistance to the drug combination. Thus, sensitivity to the drug is the "action" of the drug on the cellular signals while the resistance to the drug is the "opposite reaction" of the cell signals to the drug; this "opposite reaction" ensues with time and is far more than equal. Thus, resistance to PARP inhibitors is inevitable. Our success in the targeted therapies in TNBC [101] with PARP inhibitor will have to depend on our collective ability to manage the resistance to PARP inhibitors. In the latest study by J Jonkers group, a selective resistance to the PARP inhibitor olaparib in a mouse model for BRCA1-deficient metaplastic breast cancer (MBC) was studied and their results indicate that patients with BRCA1-associated MBC show poor response to olaparib and illustrate the value of GEMM-ESC models of human cancer for evaluation of novel therapeutics [63]. The successes and challenges of PARP inhibitors are one of the crucial avenues of cancer therapy [131]. In-depth mechanisms of resistance to targeting the PI3K pathway have been elegantly reviewed by Prof. L Cantley [79]. Future holds the knowledge regarding the prospect of drug action and the puzzle of drug resistance of a combination of PARP inhibitors and PI3K-AKT-mTOR pathway inhibitors in BC.

Acknowledgments The authors also acknowledge Avera Cancer Institute Center for Precision Oncology, Sioux Falls, SD. The authors acknowledge the cBioPortal and STRING10. *Cancer Discovery*. May 2012 2; 401 (Cerami et al. [24] and Gao et al. [59]). We acknowledge the TCGA Research Network for generating TCGA datasets. We acknowledge the version 10 of STRING and authorities/institutions/organizations/Universities/resources which institutionally and financially support the STRING. The authors also acknowledge the Department of Internal Medicine, USD (Vermilion, SD). Authors acknowledge the secretarial help of Ms. Hannah Koble.

Disclosure

Authors have no declared conflict of interest.

References

1. Abramson VG, Lehmann BD, Ballinger TJ, Pietenpol JA (2015) Subtyping of triple-negative breast cancer: implications for therapy. Cancer 121(1):8–16
2. Al-Ejeh F, Kumar R, Wiegmans A, Lakhani SR, Brown MP, Khanna KK (2010) Harnessing the complexity of DNA-damage response pathways to improve cancer treatment outcomes. Oncogene 29(46):6085–6098
3. Alluri P, Newman LA (2014) Basal-like and triple-negative breast cancers: searching for positives among many negatives. Surg Oncol Clin N Am 23(3):567–577

4. Amin O, Beauchamp MC, Nader PA, Laskov I, Iqbal S, Philip CA, Yasmeen A, Gotlieb WH (2015) Suppression of Homologous Recombination by insulin-like growth factor-1 inhibition sensitizes cancer cells to PARP inhibitors. BMC Cancer 15:817
5. Anders CK, Winer EP, Ford JM, Dent R, Silver DP, Sledge GW, Carey LA (2010) Poly (ADP-Ribose) polymerase inhibition: "targeted" therapy for triple-negative breast cancer. Clin Cancer Res 16(19):4702–4710
6. Andre F, Zielinski CC (2012) Optimal strategies for the treatment of metastatic triple-negative breast cancer with currently approved agents. Ann Oncol 23(Suppl 6): vi46–vi51
7. Ang JE, Gourley C, Powell CB, High H, Shapira-Frommer R, Castonguay V, De Greve J, Atkinson T, Yap TA, Sandhu S, Banerjee S, Chen LM, Friedlander ML, Kaufman B, Oza AM, Matulonis U, Barber LJ, Kozarewa I, Fenwick K, Assiotis I, Campbell J, Chen L, de Bono JS, Gore ME, Lord CJ, Ashworth A, Kaye SB (2013) Efficacy of chemotherapy in BRCA1/2 mutation carrier ovarian cancer in the setting of PARP inhibitor resistance: a multi-institutional study. Clin Cancer Res 19(19):5485–5493
8. Arnedos M, Bihan C, Delaloge S, Andre F (2012) Triple-negative breast cancer: are we making headway at least? Ther Adv Med Oncol 4(4):195–210
9. Bajrami I, Kigozi A, Van Weverwijk A, Brough R, Frankum J, Lord CJ, Ashworth A (2012) Synthetic lethality of PARP and NAMPT inhibition in triple-negative breast cancer cells. EMBO Mol Med 4(10):1087–1096
10. Baselga J, Campone M, Piccart M, Burris HA 3rd, Rugo HS, Sahmoud T, Noguchi S, Gnant M, Pritchard KI, Lebrun F, Beck JT, Ito Y, Yardley D, Deleu I, Perez A, Bachelot T, Vittori L, Xu Z, Mukhopadhyay P, Lebwohl D, Hortobagyi GN (2012) Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 366 (6):520–529
11. Bassi C, Ho J, Srikumar T, Dowling RJ, Gorrini C, Miller SJ, Mak TW, Neel BG, Raught B, Stambolic V (2013) Nuclear PTEN controls DNA repair and sensitivity to genotoxic stress. Science 341(6144):395–399
12. Bernardi R, Gianni L (2014) Hallmarks of triple negative breast cancer emerging at last? Cell Res 24(8):904–905
13. Bhute VJ, Palecek SP (2015) Metabolic responses induced by DNA damage and poly (ADP-ribose) polymerase (PARP) inhibition in MCF-7 cells. Metabolomics 11(6):1779–1791
14. Bridges KA, Toniatti C, Buser CA, Liu H, Buchholz TA, Meyn RE (2014) Niraparib (MK-4827), a novel poly(ADP-Ribose) polymerase inhibitor, radiosensitizes human lung and breast cancer cells. Oncotarget 5(13):5076–5086
15. Brown KK, Montaser-Kouhsari L, Beck AH, Toker A (2015) MERIT40 Is an Akt substrate that promotes resolution of DNA damage induced by chemotherapy. Cell Rep 11(9):1358–1366
16. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 434(7035):913–917
17. Burgess M, Puhalla S (2014) BRCA 1/2-mutation related and sporadic breast and ovarian cancers: more alike than different. Front Oncol 4:19
18. Burstein MD, Tsimelzon A, Poage GM, Covington KR, Contreras A, Fuqua SA, Savage MI, Osborne CK, Hilsenbeck SG, Chang JC, Mills GB, Lau CC, Brown PH (2015) Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. Clin Cancer Res 21(7):1688–1698
19. Calabrese CR, Batey MA, Thomas HD, Durkacz BW, Wang LZ, Kyle S, Skalitzky D, Li J, Zhang C, Boritzki T, Maegley K, Calvert AH, Hostomsky Z, Newell DR, Curtin NJ (2003) Identification of potent nontoxic poly(ADP-Ribose) polymerase-1 inhibitors: chemopotentiation and pharmacological studies. Clin Cancer Res 9(7):2711–2718
20. Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490(7418):61–70

21. Cardnell RJ, Feng Y, Diao L, Fan YH, Masrorpour F, Wang J, Shen Y, Mills GB, Minna JD, Heymach JV, Byers LA (2013) Proteomic markers of DNA repair and PI3K pathway activation predict response to the PARP inhibitor BMN 673 in small cell lung cancer. Clin Cancer Res 19(22):6322–6328
22. Carey LA (2011) Novel targets for triple-negative breast cancer. Clin Adv Hematol Oncol 9 (9):678–680
23. Carnero A, Blanco-Aparicio C, Renner O, Link W, Leal JF (2008) The PTEN/PI3K/AKT signalling pathway in cancer, therapeutic implications. Curr Cancer Drug Targets 8(3):187–198
24. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C, Schultz N (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2(5):401–404
25. Chen JH, Zhang P, Chen WD, Li DD, Wu XQ, Deng R, Jiao L, Li X, Ji J, Feng GK, Zeng YX, Jiang JW, Zhu XF (2015) ATM-mediated PTEN phosphorylation promotes PTEN nuclear translocation and autophagy in response to DNA-damaging agents in cancer cells. Autophagy 11(2):239–252
26. Chen X, Li J, Gray WH, Lehmann BD, Bauer JA, Shyr Y, Pietenpol JA (2012) TNBCtype: a subtyping tool for triple-negative breast cancer. Cancer Inform 11:147–156
27. Ciruelos Gil EM (2014) Targeting the PI3K/AKT/mTOR pathway in estrogen receptor-positive breast cancer. Cancer Treat Rev 40(7):862–871
28. Comen EA, Robson M (2010) Poly(ADP-ribose) polymerase inhibitors in triple-negative breast cancer. Cancer J 16(1):48–52
29. Cornen S, Guille A, Adelaide J, Addou-Klouche L, Finetti P, Saade MR, Manai M, Carbuccia N, Bekhouche I, Letessier A, Raynaud S, Charafe-Jauffret E, Jacquemier J, Spicuglia S, de The H, Viens P, Bertucci F, Birnbaum D, Chaffanet M (2014) Candidate luminal B breast cancer genes identified by genome, gene expression and DNA methylation profiling. PLoS ONE 9(1):e81843
30. Crown J, O'Shaughnessy J, Gullo G (2012) Emerging targeted therapies in triple-negative breast cancer. Ann Oncol 23(Suppl 6):vi56–vi65
31. Cully M, You H, Levine AJ, Mak TW (2006) Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nat Rev Cancer 6(3):184–192
32. Curtin NJ, Wang LZ, Yiakouvaki A, Kyle S, Arris CA, Canan-Koch S, Webber SE, Durkacz BW, Calvert HA, Hostomsky Z, Newell DR (2004) Novel poly(ADP-ribose) polymerase-1 inhibitor, AG14361, restores sensitivity to temozolomide in mismatch repair-deficient cells. Clin Cancer Res 10(3):881–889
33. Davidson D, Wang Y, Aloyz R, Panasci L (2013) The PARP inhibitor ABT-888 synergizes irinotecan treatment of colon cancer cell lines. Invest New Drugs 31(2):461–468
34. Davis WJ, Lehmann PZ, Li W (2015) Nuclear PI3K signaling in cell growth and tumorigenesis. Front Cell Dev Biol 3:24
35. De Lorenzo SB, Patel AG, Hurley RM, Kaufmann SH (2013) The elephant and the blind men: making sense of PARP inhibitors in homologous recombination deficient tumor cells. Front Oncol 3:228
36. De P, Carlson J, Leyland-Jones B, Dey N (2014) Oncogenic nexus of cancerous inhibitor of protein phosphatase 2A (CIP2A): an oncoprotein with many hands. Oncotarget 5(13):4581–4602
37. De P, Dey N, Leyland-Jones B (2013) Growth factor and signaling networks. Brenner's encyclopedia of genetics. Elsevier Inc, Philadelphia
38. De P, Miskimins K, Dey N, Leyland-Jones B (2013) Promise of rapalogues versus mTOR kinase inhibitors in subset specific breast cancer: old targets new hope. Cancer Treat Rev 39 (5):403–412
39. De P, Sun Y, Carlson JH, Friedman LS, Leyland-Jones BR, Dey N (2014) Doubling down on the PI3K-AKT-mTOR pathway enhances the antitumor efficacy of PARP inhibitor in triple negative breast cancer model beyond BRCA-ness. Neoplasia 16(1):43–72

40. Delaney CA, Wang LZ, Kyle S, White AW, Calvert AH, Curtin NJ, Durkacz BW, Hostomsky Z, Newell DR (2000) Potentiation of temozolomide and topotecan growth inhibition and cytotoxicity by novel poly(adenosine diphosphoribose) polymerase inhibitors in a panel of human tumor cell lines. Clin Cancer Res 6(7):2860–2867
41. Dey N, Leyland-Jones B, De P (2015) MYC-xing it up with PIK3CA mutation and resistance to PI3K inhibitors: summit of two giants in breast cancers. Am J Cancer Res 5(1):1–19
42. Dey N, Smith BR, Leyland-Jones B (2012) Targeting basal-like breast cancers. Curr Drug Targets 13(12):1510–1524
43. Dey N, Sun Y, Carlson J, Friedman L, De P, Leyland-Jones B (2014) Anti-tumor efficacy of PI3K-mTOR pathway inhibitors in combination with PARP inhibitor plus carboplatin in BRCA1-competent TNBC, beyond PTEN: a proof of concept study. Thirty-Seventh Annual CTRC-AACR San Antonio Breast Cancer Symposium, San Antonio, TX, AACR
44. Difilippantonio S, Nussenzweig A (2007) The NBS1-ATM connection revisited. Cell Cycle 6(19):2366–2370
45. Dittrich A, Gautrey H, Browell D, Tyson-Capper A (2014) The HER2 signaling network in breast cancer-like a spider in its web. J Mammary Gland Biol Neoplasia
46. Dizdar O, Arslan C, Altundag K (2015) Advances in PARP inhibitors for the treatment of breast cancer. Expert Opin Pharmacother 16(18):2751–2758
47. Du Y, Yamaguchi H, Wei Y, Hsu JL, Wang HL, Hsu YH, Lin WC, Yu WH, Leonard PG, Lee GRT, Chen MK, Nakai K, Hsu MC, Chen CT, Sun Y, Wu Y, Chang WC, Huang WC, Liu CL, Chang YC, Chen CH, Park M, Jones P, Hortobagyi GN, Hung MC (2016) Blocking c-Met-mediated PARP1 phosphorylation enhances anti-tumor effects of PARP inhibitors. Nat Med
48. Dumont AG, Dumont SN, Trent JC (2012) The favorable impact of PIK3CA mutations on survival: an analysis of 2587 patients with breast cancer. Chin J Cancer 31(7):327–334
49. Ellis MJ, Perou CM (2013) The genomic landscape of breast cancer as a therapeutic roadmap. Cancer Discov 3(1):27–34
50. Ellisen LW (2011) PARP inhibitors in cancer therapy: promise, progress, and puzzles. Cancer Cell 19(2):165–167
51. Elster N, Cremona M, Morgan C, Toomey S, Carr A, O'Grady A, Hennessy BT, Eustace AJ (2015) A preclinical evaluation of the PI3K alpha/delta dominant inhibitor BAY 80-6946 in HER2-positive breast cancer models with acquired resistance to the HER2-targeted therapies trastuzumab and lapatinib. Breast Cancer Res Treat 149(2):373–383
52. Fang Y, Chai Z, Wang D, Kuang T, Wu W, Lou W (2015) DNA-PKcs deficiency sensitizes the human hepatoma HepG2 cells to cisplatin and 5-fluorouracil through suppression of the PI3K/Akt/NF-kappaB pathway. Mol Cell Biochem 399(1–2):269–278
53. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, Martin NM, Jackson SP, Smith GC, Ashworth A (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434 (7035):917–921
54. Fedele CG, Ooms LM, Ho M, Vieusseux J, O'Toole SA, Millar EK, Lopez-Knowles E, Sriratana A, Gurung R, Baglietto L, Giles GG, Bailey CG, Rasko JE, Shields BJ, Price JT, Majerus PW, Sutherland RL, Tiganis T, McLean CA, Mitchell CA (2010) Inositol polyphosphate 4-phosphatase II regulates PI3K/Akt signaling and is lost in human basal-like breast cancers. Proc Natl Acad Sci USA 107(51):22231–22236
55. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, Ashworth A, Carmichael J, Kaye SB, Schellens JH, de Bono JS (2009) Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 361(2):123–134
56. Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. N Engl J Med 363(20):1938–1948
57. Fu X, Creighton CJ, Biswal NC, Kumar V, Shea M, Herrera S, Contreras A, Gutierrez C, Wang T, Nanda S, Giuliano M, Morrison G, Nardone A, Karlin KL, Westbrook TF, Heiser LM, Anur P, Spellman P, Guichard SM, Smith PD, Davies BR, Klinowska T,

Lee AV, Mills GB, Rimawi MF, Hilsenbeck SG, Gray JW, Joshi A, Osborne CK, Schiff R (2014) Overcoming endocrine resistance due to reduced PTEN levels in estrogen receptor-positive breast cancer by co-targeting mammalian target of rapamycin, protein kinase B, or mitogen-activated protein kinase kinase. Breast Cancer Res 16(5):430

58. Fu X, Osborne CK, Schiff R (2013) Biology and therapeutic potential of PI3K signaling in ER+/HER2-negative breast cancer. Breast 22(Suppl 2):S12–S18
59. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C, Schultz N (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6(269):pl1.doi:10.1126/scisignal.2004088
60. Ghorbani A, Jeddi-Tehrani M, Saidpour A, Safa M, Bayat AA, Zand H (2015) PI3K/AKT and Mdm2 activation are associated with inhibitory effect of cAMP increasing agents on DNA damage-induced cell death in human pre-B NALM-6 cells. Arch Biochem Biophys 566:58–66
61. Gonzalez-Billalabeitia E, Seitzer N, Song SJ, Song MS, Patnaik A, Liu XS, Epping MT, Papa A, Hobbs RM, Chen M, Lunardi A, Ng C, Webster KA, Signoretti S, Loda M, Asara JM, Nardella C, Clohessy JG, Cantley LC, Pandolfi PP (2014) Vulnerabilities of PTEN-TP53-deficient prostate cancers to compound PARP-PI3K inhibition. Cancer Discov 4(8):896–904
62. Guo Z, Zhao J, Song L, Ma JX, Wang CJ, Pei SY, Jiang C, Li SB (2014) Induction of H2AX phosphorylation in tumor cells by gossypol acetic acid is mediated by phosphatidylinositol 3-kinase (PI3K) family. Cancer Cell Int 14(1):141
63. Henneman L, van Miltenburg MH, Michalak EM, Braumuller TM, Jaspers JE, Drenth AP, de Korte-Grimmerink R, Gogola E, Szuhai K, Schlicker A, Bin Ali R, Pritchard C, Huijbers IJ, Berns A, Rottenberg S, Jonkers J (2015) Selective resistance to the PARP inhibitor olaparib in a mouse model for BRCA1-deficient metaplastic breast cancer. Proc Natl Acad Sci USA 112:8409–8414
64. Hortobagyi GN (2015) Everolimus plus exemestane for the treatment of advanced breast cancer: a review of subanalyses from BOLERO-2. Neoplasia 17(3):279–288
65. Hudis CA, Gianni L (2011) Triple-negative breast cancer: an unmet medical need. Oncologist 16(Suppl 1):1–11
66. Ibrahim YH, Garcia-Garcia C, Serra V, He L, Torres-Lockhart K, Prat A, Anton P, Cozar P, Guzman M, Grueso J, Rodriguez O, Calvo MT, Aura C, Diez O, Rubio IT, Perez J, Rodon J, Cortes J, Ellisen LW, Scaltriti M, Baselga J (2012) PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. Cancer Discov 2(11):1036–1047
67. Ip LR, Poulogiannis G, Viciano FC, Sasaki J, Kofuji S, Spanswick VJ, Hochhauser D, Hartley JA, Sasaki T, Gewinner CA (2015) Loss of INPP4B causes a DNA repair defect through loss of BRCA1, ATM and ATR and can be targeted with PARP inhibitor treatment. Oncotarget 6(12):10548–10562
68. Irshad S, Ashworth A, Tutt A (2011) Therapeutic potential of PARP inhibitors for metastatic breast cancer. Expert Rev Anticancer Ther 11(8):1243–1251
69. Isakoff SJ, Overmoyer B, Tung NM, Gelman RS, Giranda VL, Bernhard KM, Habin KR, Ellisen LW, Winer EP, Goss PE (2010) A phase II trial of the PARP inhibitor veliparib (ABT888) and temozolomide for metastatic breast cancer. J Clin, Oncol
70. Jang NY, Kim DH, Cho BJ, Choi EJ, Lee JS, Wu HG, Chie EK, Kim IA (2015) Radiosensitization with combined use of olaparib and PI-103 in triple-negative breast cancer. BMC Cancer 15:89
71. Jones N, Bonnet F, Sfar S, Lafitte M, Lafon D, Sierankowski G, Brouste V, Banneau G, Tunon de Lara C, Debled M, MacGrogan G, Longy M, Sevenet N (2013) Comprehensive analysis of PTEN status in breast carcinomas. Int J Cancer 133(2):323–334
72. Jones P, Wilcoxen K, Rowley M, Toniatti C (2015) Niraparib: a poly(ADP-ribose) polymerase (PARP) inhibitor for the treatment of tumors with defective homologous recombination. J Med Chem 58(8):3302–3314

73. Jordan NJ, Dutkowski CM, Barrow D, Mottram HJ, Hutcheson IR, Nicholson RI, Guichard SM, Gee JM (2014) Impact of dual mTORC1/2 mTOR kinase inhibitor AZD8055 on acquired endocrine resistance in breast cancer in vitro. Breast Cancer Res 16(1):R12
74. Jung IL, Kang HJ, Kim KC, Kim IG (2010) PTEN/pAkt/p53 signaling pathway correlates with the radioresponse of non-small cell lung cancer. Int J Mol Med 25(4):517–523
75. Juvekar A, Burga LN, Hu H, Lunsford EP, Ibrahim YH, Balmana J, Rajendran A, Papa A, Spencer K, Lyssiotis CA, Nardella C, Pandolfi PP, Baselga J, Scully R, Asara JM, Cantley LC, Wulf GM (2012) Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for BRCA1-related breast cancer. Cancer Discov 2(11):1048–1063
76. Karginova O, Siegel MB, Van Swearingen AE, Deal AM, Adamo B, Sambade MJ, Bazyar S, Nikolaishvili-Feinberg N, Bash R, O'Neal S, Sandison K, Parker JS, Santos C, Darr D, Zamboni W, Lee YZ, Miller CR, Anders CK (2015) Efficacy of carboplatin alone and in combination with ABT888 in intracranial murine models of BRCA-mutated and BRCA-wild-type triple-negative breast cancer. Mol Cancer Ther 14(4):920–930
77. Kennecke H, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, Nielsen TO, Gelmon K (2010) Metastatic behavior of breast cancer subtypes. J Clin Oncol 28(20):3271–3277
78. Kimbung S, Biskup E, Johansson I, Aaltonen K, Ottosson-Wadlund A, Gruvberger-Saal S, Cunliffe H, Fadeel B, Loman N, Berglund P, Hedenfalk I (2012) Co-targeting of the PI3K pathway improves the response of BRCA1 deficient breast cancer cells to PARP1 inhibition. Cancer Lett 319(2):232–241
79. Klempner SJ, Myers AP, Cantley LC (2013) What a tangled web we weave: emerging resistance mechanisms to inhibition of the phosphoinositide 3-kinase pathway. Cancer Discov 3(12):1345–1354
80. Krzystolik K, Jakubowska A, Gronwald J, Krawczynski MR, Drobek-Slowik M, Sagan L, Cyrylowski L, Lubinski W, Lubinski J, Cybulski C (2014) Large deletion causing von Hippel-Lindau disease and hereditary breast cancer syndrome. Hered Cancer Clin Pract 12 (1):16
81. Kumar A, Fernandez-Capetillo O, Carrera AC (2010) Nuclear phosphoinositide 3-kinase beta controls double-strand break DNA repair. Proc Natl Acad Sci USA 107(16):7491–7496
82. Kumar A, Redondo-Munoz J, Perez-Garcia V, Cortes I, Chagoyen M, Carrera AC (2011) Nuclear but not cytosolic phosphoinositide 3-kinase beta has an essential function in cell survival. Mol Cell Biol 31(10):2122–2133
83. Kumler I, Tuxen MK, Nielsen DL (2014) A systematic review of dual targeting in HER2-positive breast cancer. Cancer Treat Rev 40(2):259–270
84. Le Du F, Eckhardt BL, Lim B, Litton JK, Moulder S, Meric-Bernstam F, Gonzalez-Angulo AM, Ueno NT (2015) Is the future of personalized therapy in triple-negative breast cancer based on molecular subtype? Oncotarget 6(15):12890–12908
85. Lee JM, Ledermann JA, Kohn EC (2014) PARP Inhibitors for BRCA1/2 mutation-associated and BRCA-like malignancies. Ann Oncol 25(1):32–40
86. Lees-Miller SP, Meek K (2003) Repair of DNA double strand breaks by non-homologous end joining. Biochimie 85(11):1161–1173
87. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA (2011) Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 121(7):2750–2767
88. Lehmann BD, Pietenpol JA (2014) Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes. J Pathol 232(2):142–150
89. Li HF, Kim JS, Waldman T (2009) Radiation-induced Akt activation modulates radioresistance in human glioblastoma cells. Radiat Oncol 4:43
90. Lin Y, Kang T, Zhou BP (2014) Doxorubicin enhances Snail/LSD1-mediated PTEN suppression in a PARP1-dependent manner. Cell Cycle 13(11):1708–1716
91. Lisby M, Rothstein R (2004) DNA damage checkpoint and repair centers. Curr Opin Cell Biol 16(3):328–334

92. Liu W, Zhou Y, Reske SN, Shen C (2008) PTEN mutation: many birds with one stone in tumorigenesis. Anticancer Res 28(6A):3613–3619
93. Loi S, Haibe-Kains B, Majjaj S, Lallemand F, Durbecq V, Larsimont D, Gonzalez-Angulo AM, Pusztai L, Symmans WF, Bardelli A, Ellis P, Tutt AN, Gillett CE, Hennessy BT, Mills GB, Phillips WA, Piccart MJ, Speed TP, McArthur GA, Sotiriou C (2010) PIK3CA mutations associated with gene signature of low mTORC1 signaling and better outcomes in estrogen receptor-positive breast cancer. Proc Natl Acad Sci USA 107(22):10208–10213
94. Loibl S, von Minckwitz G, Schneeweiss A, Paepke S, Lehmann A, Rezai M, Zahm DM, Sinn P, Khandan F, Eidtmann H, Dohnal K, Heinrichs C, Huober J, Pfitzner B, Fasching PA, Andre F, Lindner JL, Sotiriou C, Dykgers A, Guo S, Gade S, Nekljudova V, Loi S, Untch M, Denkert C (2014) PIK3CA mutations are associated with lower rates of pathologic complete response to anti-human epidermal growth factor receptor 2 (her2) therapy in primary HER2-overexpressing breast cancer. J Clin Oncol 32(29):3212–3220
95. Majewski IJ, Nuciforo P, Mittempergher L, Bosma AJ, Eidtmann H, Holmes E, Sotiriou C, Fumagalli D, Jimenez J, Aura C, Prudkin L, Diaz-Delgado MC, de la Pena L, Loi S, Ellis C, Schultz N, de Azambuja E, Harbeck N, Piccart-Gebhart M, Bernards R, Baselga J (2015) PIK3CA mutations are associated with decreased benefit to neoadjuvant human epidermal growth factor receptor 2-targeted therapies in breast cancer. J Clin Oncol 33(12):1334–1339
96. Mancini P, Angeloni A, Risi E, Orsi E, Mezi S (2014) Standard of care and promising new agents for triple negative metastatic breast cancer. Cancers (Basel) 6(4):2187–2223
97. Manzano RG, Martinez-Navarro EM, Forteza J, Brugarolas A (2014) Microarray phosphatome profiling of breast cancer patients unveils a complex phosphatase regulatory role of the MAPK and PI3K pathways in estrogen receptor-negative breast cancers. Int J Oncol 45(6):2250–2266
98. Manzoli FA, Capitani S, Maraldi NM, Cocco L, Barnabei O (1978) Chromatin lipids and their possible role in gene expression. A study in normal and neoplastic cells. Adv Enzyme Regul 17:175–194
99. Manzoli FA, Capitani S, Mazzotti G, Barnabei O, Maraldi NM (1982) Role of chromatin phospholipids on template availability and ultrastructure of isolated nuclei. Adv Enzyme Regul 20:247–262
100. Mariano G, Ricciardi MR, Trisciuoglio D, Zampieri M, Ciccarone F, Guastafierro T, Calabrese R, Valentini E, Tafuri A, Del Bufalo D, Caiafa P, Reale A (2015) PARP inhibitor ABT-888 affects response of MDA-MB-231 cells to doxorubicin treatment, targeting Snail expression. Oncotarget 6:15008
101. Marme F, Schneeweiss A (2015) Targeted therapies in triple-negative breast cancer. Breast Care (Basel) 10(3):159–166
102. Marty B, Maire V, Gravier E, Rigaill G, Vincent-Salomon A, Kappler M, Lebigot I, Djelti F, Tourdes A, Gestraud P, Hupe P, Barillot E, Cruzalegui F, Tucker GC, Stern MH, Thiery JP, Hickman JA, Dubois T (2008) Frequent PTEN genomic alterations and activated phosphatidylinositol 3-kinase pathway in basal-like breast cancer cells. Breast Cancer Res 10(6):R101
103. Masuda H, Baggerly KA, Wang Y, Zhang Y, Gonzalez-Angulo AM, Meric-Bernstam F, Valero V, Lehmann BD, Pietenpol JA, Hortobagyi GN, Symmans WF, Ueno NT (2013) Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. Clin Cancer Res 19(19):5533–5540
104. Mateo J, Ong M, Tan DS, Gonzalez MA, de Bono JS (2013) Appraising iniparib, the PARP inhibitor that never was–what must we learn? Nat Rev Clin Oncol 10(12):688–696
105. Mayer IA (2013) Subtyping of triple-negative breast cancer. Clin Adv Hematol Oncol 11 (11):731–732
106. Mayer IA, Abramson VG, Lehmann BD, Pietenpol JA (2014) New strategies for triple-negative breast cancer–deciphering the heterogeneity. Clin Cancer Res 20(4):782–790

107. Metzger-Filho O, Tutt A, de Azambuja E, Saini KS, Viale G, Loi S, Bradbury I, Bliss JM, Azim HA Jr, Ellis P, Di Leo A, Baselga J, Sotiriou C, Piccart-Gebhart M (2012) Dissecting the heterogeneity of triple-negative breast cancer. J Clin Oncol 30(15):1879–1887
108. Miknyoczki SJ, Jones-Bolin S, Pritchard S, Hunter K, Zhao H, Wan W, Ator M, Bihovsky R, Hudkins R, Chatterjee S, Klein-Szanto A, Dionne C, Ruggeri B (2003) Chemopotentiation of temozolomide, irinotecan, and cisplatin activity by CEP-6800, a poly(ADP-ribose) polymerase inhibitor. Mol Cancer Ther 2(4):371–382
109. Minami A, Nakanishi A, Ogura Y, Kitagishi Y, Matsuda S (2014) Connection between tumor suppressor BRCA1 and PTEN in damaged DNA repair. Front Oncol 4:318
110. Minami D, Takigawa N, Takeda H, Takata M, Ochi N, Ichihara E, Hisamoto A, Hotta K, Tanimoto M, Kiura K (2013) Synergistic effect of olaparib with combination of cisplatin on PTEN-deficient lung cancer cells. Mol Cancer Res 11(2):140–148
111. Ming M, He YY (2012) PTEN in DNA damage repair. Cancer Lett 319(2):125–129
112. Mo W, Liu Q, Lin CC, Dai H, Peng Y, Liang Y, Peng G, Meric-Bernstam F, Mills GB, Li K, Lin SY (2016) mTOR inhibitors suppress homologous recombination repair and synergize with PARP inhibitors via regulating SUV39H1 in BRCA-proficient triple-negative breast cancer. Clin Cancer Res 22(7):1699–712. doi: 10.1158/1078-0432. CCR-15-1772. Epub 6 Nov 2015. PMID: 26546619, 1 Apr 2016
113. Monteiro Ide P, Madureira P, de Vasconscelos A, Pozza DH, de Mello RA (2015) Targeting HER family in HER2-positive metastatic breast cancer: potential biomarkers and novel targeted therapies. Pharmacogenomics 16(3):257–271
114. Morales J, Li L, Fattah FJ, Dong Y, Bey EA, Patel M, Gao J, Boothman DA (2014) Review of poly (ADP-ribose) polymerase (PARP) mechanisms of action and rationale for targeting in cancer and other diseases. Crit Rev Eukaryot Gene Expr 24(1):15–28
115. Mukohara T (2015) PI3K mutations in breast cancer: prognostic and therapeutic implications. Breast Cancer (Dove Med Press) 7:111–123
116. Murai J, Zhang Y, Morris J, Ji J, Takeda S, Doroshow JH, Pommier Y (2014) Rationale for poly(ADP-ribose) polymerase (PARP) inhibitors in combination therapy with camptothecins or temozolomide based on PARP trapping versus catalytic inhibition. J Pharmacol Exp Ther 349(3):408–416
117. Norris RE, Adamson PC, Nguyen VT, Fox E (2014) Preclinical evaluation of the PARP inhibitor, olaparib, in combination with cytotoxic chemotherapy in pediatric solid tumors. Pediatr Blood Cancer 61(1):145–150
118. Nuciforo PG, Aura C, Holmes E, Prudkin L, Jimenez J, Martinez P, Ameels H, de la Pena L, Ellis C, Eidtmann H, Piccart-Gebhart MJ, Scaltriti M, Baselga J (2015) Benefit to neoadjuvant anti-human epidermal growth factor receptor 2 (HER2)-targeted therapies in HER2-positive primary breast cancer is independent of phosphatase and tensin homolog deleted from chromosome 10 (PTEN) status. Ann Oncol 26(7):1494–1500
119. O'Shaughnessy J, Osborne C, Pippen JE, Yoffe M, Patt D, Rocha C, Koo IC, Sherman BM, Bradley C (2011) Iniparib plus chemotherapy in metastatic triple-negative breast cancer. N Engl J Med 364(3):205–214
120. O'Shaughnessy J, Schwartzberg L, Danso MA, Miller KD, Rugo HS, Neubauer M, Robert N, Hellerstedt B, Saleh M, Richards P, Specht JM, Yardley DA, Carlson RW, Finn RS, Charpentier E, Garcia-Ribas I, Winer EP (2014) Phase III study of iniparib plus gemcitabine and carboplatin versus gemcitabine and carboplatin in patients with metastatic triple-negative breast cancer. J Clin Oncol 32(34):3840–3847
121. Orlando L, Schiavone P, Fedele P, Calvani N, Nacci A, Cinefra M, D'Amico M, Mazzoni E, Marino A, Sponziello F, Morelli F, Lombardi L, Silvestris N, Cinieri S (2012) Poly (ADP-ribose) polymerase (PARP): rationale, preclinical and clinical evidences of its inhibition as breast cancer treatment. Expert Opin Ther Targets 16(Suppl 2):S83–S89

122. Pal SK, Childs BH, Pegram M (2011) Triple negative breast cancer: unmet medical needs. Breast Cancer Res Treat 125(3):627–636
123. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, Quackenbush JF, Stijleman IJ, Palazzo J, Marron JS, Nobel AB, Mardis E, Nielsen TO, Ellis MJ, Perou CM, Bernard PS (2009) Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 27(8):1160–1167
124. Passeri D, Camaioni E, Liscio P, Sabbatini P, Ferri M, Carotti A, Giacche N, Pellicciari R, Gioiello A, Macchiarulo A (2015) Concepts and molecular aspects in the polypharmacology of PARP-1 inhibitors. ChemMedChem. doi: 10.1002/cmdc.201500391. [Epub ahead of print] PMID: 26424664, 1 Oct 2015
125. Paull TT (2015) Mechanisms of ATM activation. Annu Rev Biochem 84:711–738
126. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D (2000) Molecular portraits of human breast tumours. Nature 406(6797):747–752
127. Planchon SM, Waite KA, Eng C (2008) The nuclear affairs of PTEN. J Cell Sci 121(Pt 3):249–253
128. Plummer R (2011) Poly(ADP-ribose) polymerase inhibition: a new direction for BRCA and triple-negative breast cancer? Breast Cancer Res 13(4):218
129. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, He X, Perou CM (2010) Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res 12(5):R68
130. Rehman FL, Lord CJ, Ashworth A (2012) The promise of combining inhibition of PI3K and PARP as cancer therapy. Cancer Discov 2(11):982–984
131. Ricks TK, Chiu HJ, Ison G, Kim G, McKee AE, Kluetz P, Pazdur R (2015) Successes and Challenges of PARP Inhibitors in Cancer Therapy. Front Oncol 5:222
132. Rios J, Puhalla S (2011) PARP inhibitors in breast cancer: BRCA and beyond. Oncology (Williston Park) 25(11):1014–1025
133. Rosen EM, Pishvaian MJ (2014) Targeting the BRCA1/2 tumor suppressors. Curr Drug Targets 15(1):17–31
134. Rouzier R, Perou CM, Symmans WF, Ibrahim N, Cristofanilli M, Anderson K, Hess KR, Stec J, Ayers M, Wagner P, Morandi P, Fan C, Rabiul I, Ross JS, Hortobagyi GN, Pusztai L (2005) Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 11(16):5678–5685
135. Saal LH, Gruvberger-Saal SK, Persson C, Lovgren K, Jumppanen M, Staaf J, Jonsson G, Pires MM, Maurer M, Holm K, Koujak S, Subramaniyam S, Vallon-Christersson J, Olsson H, Su T, Memeo L, Ludwig T, Ethier SP, Krogh M, Szabolcs M, Murty VV, Isola J, Hibshoosh H, Parsons R, Borg A (2008) Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. Nat Genet 40(1):102–107
136. Sabine VS, Crozier C, Brookes CL, Drake C, Piper T, van de Velde CJ, Hasenburg A, Kieback DG, Markopoulos C, Dirix L, Seynaeve C, Rea DW, Bartlett JM (2014) Mutational analysis of PI3K/AKT signaling pathway in tamoxifen exemestane adjuvant multinational pathology study. J Clin Oncol 32(27):2951–2958
137. Samol J, Ranson M, Scott E, Macpherson E, Carmichael J, Thomas A, Cassidy J (2012) Safety and tolerability of the poly(ADP-ribose) polymerase (PARP) inhibitor, olaparib (AZD2281) in combination with topotecan for the treatment of patients with advanced solid tumors: a phase I study. Invest New Drugs 30(4):1493–1500
138. Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S (2004) Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 73:39–85
139. Sanchez CG, Ma CX, Crowder RJ, Guintoli T, Phommaly C, Gao F, Lin L, Ellis MJ (2011) Preclinical modeling of combined phosphatidylinositol-3-kinase inhibition with endocrine therapy for estrogen receptor-positive breast cancer. Breast Cancer Res 13(2):R21
140. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, Turashvili G, Ding J, Tse K, Haffari G, Bashashati A, Prentice LM, Khattra J, Burleigh A, Yap D, Bernard V, McPherson A,

Shumansky K, Crisan A, Giuliany R, Heravi-Moussavi A, Rosner J, Lai D, Birol I, Varhol R, Tam A, Dhalla N, Zeng T, Ma K, Chan SK, Griffith M, Moradian A, Cheng SW, Morin GB, Watson P, Gelmon K, Chia S, Chin SF, Curtis C, Rueda OM, Pharoah PD, Damaraju S, Mackey J, Hoon K, Harkins T, Tadigotla V, Sigaroudinia M, Gascard P, Tlsty T, Costello JF, Meyer IM, Eaves CJ, Wasserman WW, Jones S, Huntsman D, Hirst M, Caldas C, Marra MA, Aparicio S (2012) The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 486(7403):395–399

141. Shen C, Oswald D, Phelps D, Cam H, Pelloski CE, Pang Q, Houghton PJ (2013) Regulation of FANCD2 by the mTOR pathway contributes to the resistance of cancer cells to DNA double-strand breaks. Cancer Res 73(11):3393–3401
142. Shen WH, Balajee AS, Wang J, Wu H, Eng C, Pandolfi PP, Yin Y (2007) Essential role for nuclear PTEN in maintaining chromosomal integrity. Cell 128(1):157–170
143. Shen Z, Huhn S (2013) PTEN sumo-wrestles human RAD52 to mystery land. Cell Cycle 12 (23):3585
144. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lonning PE, Borresen-Dale AL (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 98(19):10869–10874
145. Sueta A, Yamamoto Y, Yamamoto-Ibusuki M, Hayashi M, Takeshita T, Yamamoto S, Iwase H (2014) An integrative analysis of PIK3CA mutation, PTEN, and INPP4B expression in terms of trastuzumab efficacy in HER2-positive breast cancer. PLoS ONE 9(12):e116054
146. Sun Y, Ding H, Liu X, Li X, Li L (2014) INPP4B overexpression enhances the antitumor efficacy of PARP inhibitor AG014699 in MDA-MB-231 triple-negative breast cancer cells. Tumour Biol 35(5):4469–4477
147. Tangutoori S, Baldwin P, Sridhar S (2015) PARP inhibitors: a new era of targeted therapy. Maturitas 81(1):5–9
148. Telli ML, Ford JM (2010) PARP inhibitors in breast cancer. Clin Adv Hematol Oncol 8 (9):629–635
149. Tentori L, Graziani G (2005) Chemopotentiation by PARP inhibitors in cancer therapy. Pharmacol Res 52(1):25–33
150. Tokunaga E, Hisamatsu Y, Tanaka K, Yamashita N, Saeki H, Oki E, Kitao H, Maehara Y (2014) Molecular mechanisms regulating the hormone sensitivity of breast cancer. Cancer Sci 105(11):1377–1383
151. Tomao F, Papa A, Zaccarelli E, Rossi L, Caruso D, Minozzi M, Vici P, Frati L, Tomao S (2015) Triple-negative breast cancer: new perspectives for targeted therapies. Onco Targets Ther 8:177–193
152. Toss A, Cristofanilli M (2015) Molecular characterization and targeted therapeutic approaches in breast cancer. Breast Cancer Res 17:60
153. Turner N, Tutt A, Ashworth A (2004) Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer 4(10):814–819
154. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, Friedlander M, Arun B, Loman N, Schmutzler RK, Wardley A, Mitchell G, Earl H, Wickens M, Carmichael J (2010) Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet 376(9737):235–244
155. Vidula N, Rugo HS (2015) Translating the molecular message of triple-negative breast cancer into targeted therapy. Clin Cancer Res 21(7):1511–1513
156. Wang Z, Huang Y, Zhang J (2014) Molecularly targeting the PI3K-Akt-mTOR pathway can sensitize cancer cells to radiotherapy and chemotherapy. Cell Mol Biol Lett 19(2):233–242
157. Yamamoto-Ibusuki M, Arnedos M, Andre F (2015) Targeted therapies for ER +/ HER2-metastatic breast cancer. BMC Med 13:137
158. Young CD, Zimmerman LJ, Hoshino D, Formisano L, Hanker AB, Gatza ML, Morrison MM, Moore PD, Whitwell CA, Dave B, Stricker T, Bhola NE, Silva GO, Patel P, Brantley-Sieders DM, Levin M, Horiates M, Palma NA, Wang K, Stephens PJ,

Perou CM, Weaver AM, O'Shaughnessy JA, Chang JC, Park BH, Liebler DC, Cook RS, Arteaga CL (2015) Activating PIK3CA mutations induce an EGFR/ERK paracrine signaling axis in basal-like breast cancer. Mol Cell Proteomics 14:1959–1976

159. Yu KD, Zhu R, Zhan M, Rodriguez AA, Yang W, Wong S, Makris A, Lehmann BD, Chen X, Mayer I, Pietenpol JA, Shao ZM, Symmans WF, Chang JC (2013) Identification of prognosis-relevant subgroups in patients with chemoresistant triple-negative breast cancer. Clin Cancer Res 19(10):2723–2733
160. Zardavas D, Fumagalli D, Loi S (2012) Phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin pathway inhibition: a breakthrough in the management of luminal (ER+/HER2−) breast cancers? Curr Opin Oncol 24(6):623–634

Chapter 4
The AKT-mTOR Signaling Pathway for Drug Response Prediction and Prognostic Signatures

John Conor Moran, Elisa Baldelli, Emanuel F. Petricoin and Mariaelena Pierobon

The PI3K-AKT-mTOR Signaling Pathway in Cancer: An Overview

The PI3K-AKT-mTOR signaling pathway is a well-known and central contributor to tumorigenesis and metastasis and orchestrates a number of cellular functions including cell survival, proliferation, and migration as well as regulation of a number of metabolic functions [1–3]. Genomic analyses of tumors originated from different organs have shown that different members of this pathway are often mutated in human malignancies [4]. For this reason, efforts have increasingly been focused on developing therapeutic compounds targeting directly the products of

J.C. Moran
Center for Applied Proteomics and Molecular Medicine, George Mason University, 10920 George Mason Circle, Manassas, VA 20110, USA
e-mail: jmoran9@masonlive.gmu.edu

E. Baldelli
Center for Applied Proteomics and Molecular Medicine, George Mason University, 10920 George Mason Circle Room 2019, Manassas, VA 20110, USA
e-mail: ebaldell@gmu.edu

E.F. Petricoin (✉)
Center for Applied Proteomics and Molecular Medicine, George Mason University, 10920 George Mason Circle Room 2006, Manassas, VA 20110, USA
e-mail: epetrico@gmu.edu

M. Pierobon
Center for Applied Proteomics and Molecular Medicine, George Mason University, 10920 George Mason Circle Room 2016, Manassas, VA 20110, USA
e-mail: mpierobo@gmu.edu

E. Baldelli
Medical Oncology Division, S. Maria Della Misericordia Hospital, Perugia, Italy

N. Dey et al. (eds.), *PI3K-mTOR in Cancer and Cancer Therapy*,
Cancer Drug Discovery and Development, DOI 10.1007/978-3-319-34211-5_4

these genes (e.g. PI3K, AKT, and mTOR) to modulate the aberrant signaling network of this key pathway.

Although derangements at the genomic level (e.g. gene mutation/deletion/amplification and epigenetic modification) are the underlying cause of cancer, these alterations lead to biochemical dysfunctions at the protein level: functionally cancer is a proteomic disease. Exploring the functional impacts these derangements have on the signaling network has become a new paradigm for understanding the molecular mechanisms driving tumor onset and progression and for identifying "real drivers" within the malignant lesions. Because most targeted therapeutics, especially those that concentrate on AKT-mTOR signaling, work by modulating protein enzymatic (e.g. kinase) activity, focusing on the functional protein signaling network may represent a more direct approach to predictive and prognostic biomarker analysis and implementation. Moreover, rather than concentrating on the identification of new proteins or on single molecules, functional proteomic studies focus instead on protein expression and post-translational modifications that lead to the activation of biochemical networks driving all major cell functions [5]. Among others, protein kinases, a large family of enzymes that are known for being constitutively activated in malignant lesions, are considered major players of the signaling network and as a consequence are highly involved in tumorigenesis and cancer progression [6].

The PI3K-AKT-mTOR Pathway: Kinase Activation and Signaling Cascades

Kinases regulate most cellular functions by activating downstream substrates via phosphorylation, a post-translational modification that controls signaling transduction, protein localization, and formation of dynamic and transient protein complexes. These heterotypic complexes, or "signaling hubs", which are responsible for malignant transformations, contain the direct targets for therapeutic interventions. For all of the aforementioned reasons, the vast majority of the new generation of anti-cancer compounds do not target specifically mutated genes, but rather interfere with the activation of key membrane or intracellular kinases and the signaling network in which they are involved.

Most of the PI3K-AKT-mTOR pathway members and their downstream substrates are protein kinases that are highly regulated via changes in their phosphorylation status (Fig. 4.1). The PI3K itself is a lipid kinase usually located on the plasma membrane able to phosphorylate the 3'OH group of phosphatidylinositol-4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP3), a secondary messenger involved in recruitment of signaling proteins (e.g. PDK1 and AKT). Recruitment and activation of these kinases leads to the initiation of complex AKT-dependent or -independent signal transduction cascades ultimately leading to cell proliferation and survival [2, 7].

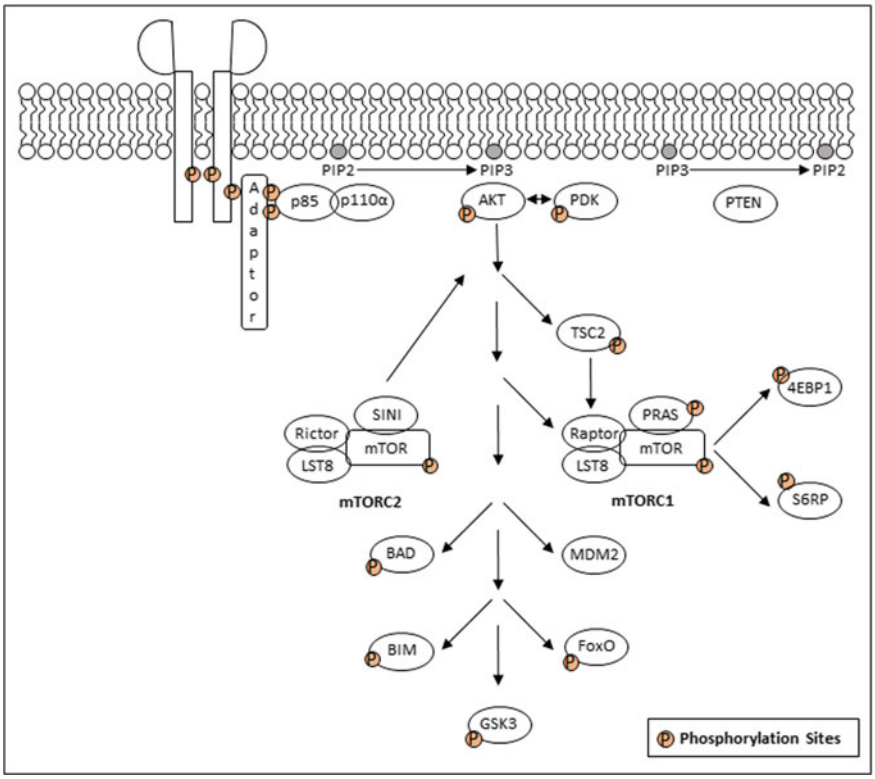


Fig. 4.1 Simplified schematic representation of the kinase interactions within the PI3K-AKT-mTOR signaling network

AKT, a serine/threonine kinase, regulates a number of cellular functions via direct phosphorylation of its downstream substrates; glycogen synthase kinase 3α (GSK3α), GSK3β, Forkhead box O transcription factors (FoxO), MDM2, and a number of proteins involved in the regulation of programmed cell death. In addition, AKT modulates the activation of the mTOR complex 1 (mTORC1), a "hub" where a number of different signaling pathways are integrated. Indeed, the activation of mTORC1, via phosphorylation and consequent inhibition of tuberous sclerosis 2 (TSC2) and activation of the AKT1 substrate 1 (PRAS40) leads to the phosphorylation of downstream substrates like p70S6 Kinase, 4E-binding protein 1 (4E-BP1), 4E-BP2 and 4E-BP3, and the S6 Ribosomal protein. All of these biochemical changes within the signaling network translate into increased cell survival and metabolic adaptation to nutrient and O_2 availability and overall cellular energy level [7]. A number of different feedback mechanisms and tumor suppressors are responsible for controlling the PI3K signaling in physiological conditions. Among others, it is worth mentioning the dual protein/lipid phosphatase and tensin

homolog (PTEN), an oncosuppressor able to inactivate the PI3K signaling at its origin via dephosphorylation of PIP3.

The PI3K-AKT-mTOR Pathway: Association Between PIK3CA Mutation and Activation of the PI3K-AKT-mTOR Signaling Network

As recently shown by the analysis of *The Cancer Genome Atlas* (TCGA) dataset, mutations of the PI3K-AKT-mTOR axis are overall relatively frequent in tumors [8]. Although differences in term of incidence have been detected across tumors of different anatomical origin, *PIK3CA* mutations appear to be one of the most frequent genetic abnormalities in tumors, second only the mutation of the *TP53* oncosuppressor with an overall incidence across all tumors of 17.8 % [8]. Most of the *PIK3CA* mutations affect either the kinase domain of p110α, which results in constitutive activation of PI3K, or the helical domain causing a change in the intermolecular interaction between p110α and the regulatory subunit p85 [9, 10]. Additional major abnormalities affecting the PI3K-AKT-mTOR pathways are: loss of the *PTEN* oncosuppressor (overall incidence: 9 %), *PIK3R1* mutation (overall incidence: 4.4 %), and somatic mutation of *AKT1* (overall incidence <1 %) [4, 8, 11].

Because PI3K-AKT-mTOR is one of the most important pathways in tumor progression and mutations of its components are relatively frequent in malignant lesions, a number of studies have evaluated whether the identification of a mutation on one or more of the members of the pathway is sufficient for predicting the activation status of the PI3K signaling network in cancerous cells. While pre-clinical models have shown significant correlation between *PIK3CA* mutation leading to PI3K gain-of-function and activation, via phosphorylation, of the downstream substrates like AKT [12, 13], analysis of clinical specimens evaluating this correlation have shown controversial results [14]. Stemke-Hale et al. [15] have evaluated the association between *PTEN* and *PIK3CA* mutation and phosphorylation of a number PI3K of downstream substrates including AKT (S473 and T308), GSK3 (S21), mTOR (S2448), and p70S6K (T389) in over 300 breast cancers. While increased phosphorylation of AKT, mTOR, and p70S6 was detected in tumors affected by a *PTEN* loss, there was no difference regarding activation of AKT, mTOR, GSK3, and p70S6 in tumors harboring a *PIK3CA* mutation versus *PIK3CA* wild-type lesions. Similarly, Hashimoto et al. as well as Lazaridis et al. showed that the activation of AKT in breast cancer samples is not necessarily associated with the presence of a *PIK3CA* mutation [16, 17]. Taken together, these findings indicate that the presence of a *PIK3CA* mutation in human specimens may not be sufficient for identifying patients with hyper-activated downstream effectors. This observation becomes particularly important when molecular information are

used as predictive markers of response to therapy or as therapeutic biomarkers for stratifying cancer patients to different targeted treatments.

Because signaling pathways are regulated by a number of different mechanisms, to identify the "real" molecular drivers of individual tumors it is essential to combined genomic characterization with functional studies of the signaling network. While *PIK3CA* mutations leading to gain-of-function can undoubtedly result in the activation of the PI3K-AKT-mTOR pathway, they are not sufficient in predicting the activation of the PI3K signaling network. Indeed, different molecular mechanisms can induce activation of PI3K and its downstream substrates independently from the mutation. For example, upstream activation of receptor tyrosine kinases (RTK) can lead to the direct hyper-activation of the PI3K-AKT-mTOR pathway in human cancer regardless of *PIK3CA* mutation status [7, 18, 19]. Similarly, pathway cross-talk can also deeply affect the activation level of PI3K and its downstream substrates. An example is given by the interplay between PI3K-AKT-mTOR and the MAPK pathway, especially through Ras, a small GTPase that can directly activate PI3K [7, 20, 21]. Finally, the establishment of feedback loops within or between different pathways can also deeply influence the activation level of PI3K and its downstream effectors [22, 23]. For example, phosphorylated mTOR can activate negative feedback mechanisms via S6 Ribosomal protein which can lead to down-regulation of PI3K and AKT [22]. As a consequence, inhibition of mTOR using rapalouges can ultimately result in increased activation of AKT and consequent increased tumor growth [24, 25]. Similarly, negative feedback loops involving collateral networks can induce PI3K independent activation of AKT and downstream substrates [22, 23].

Activation of the PI3K-AKT-mTOR Signaling Pathway as Prognostic and Predictive Biomarker

Numerous studies have explored the prognostic role of the PI3K-AKT-mTOR pathway across different cancers, but results between individual studies are inconsistent probably due to heterogeneous patient populations, sample size, and other confounding factors [26–29]. To further explore these discrepancies, a number of systematic reviews of the literature and meta-analyses have recently been conducted to explore the role of the PI3K/AKT/mTOR pathway comprehensively as prognostic factor for patient overall survival (OS) as well as progression free survival (PFS).

Ocana et al. [30] have evaluated the overall association between genomic and functional alterations of the PI3K-AKT-mTOR pathway and patient OS across a panel of tumors. Pooled analysis of 17 studies indicated that overall *PIK3CA* mutations are not associated with OS. On the other hand, phosphorylation of AKT and mTOR along with loss of PTEN were identified as molecular predictors of poor OS indicating that the activation of PI3K downstream effectors may be more

clinically relevant in identifying patients with less favorable prognosis than the *PIK3CA* mutational status itself. As discussed by the author, a major limitation of the analysis was given by the lack of distinction between mutations affecting the catalytic versus the helical domain of *PIK3CA* and the exclusion from the analysis of other molecular parameters (e.g. ER and ErbB2 expression) relevant to PI3K activation.

Two distinct analyses have evaluated the role of AKT activation in non-small cell lung carcinoma (NSCLC) [31, 32]. Unadjusted univariate analysis presented by Qiu et al. found an HR of 1.49 (CI: 1.01–2.20) for patients with increased activation of AKT compared to individuals with low AKT phosphorylation. Yang et al. [32] have confirmed the prognostic role of AKT activation in lung cancer. In addition, this second analysis found AKT activation to be a prognostic factor especially for early stage tumors (HR: 1.35, CI: 1.08–1.69 for stage I–II versus HR 1.22 CI: 0.64–2.33 for stage III–IV). This association was stronger in surgically treated patients, but lost in patients treated with RTK inhibitors possibly suggesting that RTK targeted compounds indirectly affect the activation of the PI3K-AKT-mTOR axis [32].

A meta-analysis of eleven epithelial ovarian cancer studies has also shown a strong correlation between PTEN expression and OS. While the prognostic value of PI3K expression was significant only in multivariate analysis, AKT phosphorylation was the only marker to be an indicator not only of poor OS, but also worse PFS [33]. Finally, Yang and colleagues evaluated the role of phosphorylated AKT across twenty breast cancer studies. The data suggested that patients with high activation of AKT have approximately 50 % increase in risk of dying from the disease than patients with low activation of AKT. This analysis also identified phosphorylated AKT as a molecular marker associated with lower disease free survival interval [34].

The role of the PI3K-AKT-mTOR axis has also been amply evaluated as a predictive factor for response to therapy. Significant evidence, for example, has been collected on the role of this pathway in predicting response to endocrine therapy, HER targeted agents, and cytotoxic compounds, especially in breast cancer [35, 36]. It is well established that in estrogen receptor (ER) positive breast cancers the PI3K-AKT-mTOR pathway mediates response to ER-targeted agents like tamoxifen. In addition, recent studies have shown that response to adjuvant endocrine therapy is associated with increased activation of PI3K substrates like phosphorylated AKT, mTOR, p70S6K and 4E-BP1 [37–41]. As a consequence, these biomarkers may be informative in predicting resistance to treatment with tamoxifen and should be kept into consideration when treating ER positive breast cancer patients.

Similarly, activation of the PI3K pathway can predict response rate to RTK inhibitors targeting the HER family. Using in vitro breast cancer models, O'Brien et al. [42] have reported an association between activation of AKT at the residue S473 and resistance to Trastuzumab, but not lapatinib. Haas-Kogan et al. [43] have shown that in glioblastomas treated with erlotinib +/− temozolomide phosphorylation of AKT may be the strongest biomarker of response prediction where low

response rate may be associated with increased AKT phosphorylation. On the contrary, pAKT seems to be associated with response to anti-EGFR targeted treatment in NSCLC. Emery et al. [44] have found a significant association between activation of AKT and response rate to Erlotinib and/or Gefitinib. Independent studies have confirmed the association between longer survival in NSCLC patient treated with Gefitinib and pre-treatment hyper activation of AKT [45–47].

Finally, the therapeutic efficacy and safety, both as single agent or in combination therapies, of targeted compound against the PI3K-AKT-mTOR have been tested. A large panel of Pan-PI3K, dual mTOR-PI3K, selected p110α, AKT, and mTOR inhibitors are currently used in pre-clinical and clinical studies across different types of solid tumors and hematological malignancies [2]. Unlike most compounds targeting upstream receptors and substrates like EGFR, BRAF or EML4/ALK translocation, compounds targeting PI3K signaling network have shown overall limited efficacy as single therapeutic agents most likely due to an ineffective upfront selection of patients that may benefit from this type of treatment [48]. For example, Li et al. [49] have explored the role of PI3K downstream substrates, and in particular phosphorylation of AKT, mTOR, eIF4E, 4E-BP1, and S6 Ribosomal Protein as a read out for identifying renal cancer patients that may benefit from treatment with the mTOR inhibitor everolimus. This analysis showed that 71.4 % of the patients with high activation of mTOR at the residue S2448 had clinical benefit from the administration of everolimus. Conversely, none of the patients with low activation of mTOR S2448 benefited from the mTOR targeted agent. Similar observations were also reported for the downstream effector S6RP S235/236, but not for pAKT S473 and p4E-BP1 T37/46 [49].

High Throughput Multiplexed Platforms for Functional Analysis of the PI3K-AKT-mTOR Pathway of Clinical Samples

Although encouraging results are emerging from functional proteomic studies on the ability to identify accurate predictive and prognostic biomarkers as well as "real drivers" within the complex cellular network, findings are often inconsistent between studies [50, 51]. A major issue associated with these conflicting results is given by the lack of consensus within the scientific community on how to consistently measure the activation of the PI3K-AKT-mTOR axis. Indeed, most proteomic-based diagnostic tests used in the clinic for stratifying patients to targeted treatments are based on conventional immunohistochemistry and are focused on the measurement of the overall expression of a given protein in its unmodified form (e.g. expression of ER or HER2 in breast cancer). Similar scoring systems still need to be developed for quantifying post-translational modifications and their effect on the cellular network. In addition, because these protein networks are extremely complex and dynamic there is an urgent need to develop high throughput and

multiplex clinical tests able to capture functional changes of a number of targeted analytes and effectors starting from little biological material like a fine needle aspirate or a core needle biopsy. This chapter will briefly describe a few platforms available for exploring the human kinome and its activation. Two major classes of technologies will be described: Antibody-based technologies and Non-antibody-based platforms.

Antibody-Based Technologies

Like for conventional IHC, protein recognition and quantification in antibody-based assays is achieved by using a single or multiple antibodies able to selectively recognize and efficiently bind to the epitope(s) of interest. These technologies allow for the identification of proteins in their unmodified form as well as specific post-translation modifications, including phosphorylation. Multiplex Immunoassays including planar or suspension arrays, Reverse Phase Protein Microarray (RPPA), Phosphoflow, and Tissue Microarrays (TMA) will be briefly described and compared in terms of their ability to measure changes of the phosphoproteome (Figs. 4.2 and 4.3).

Overall, antibody specificity strongly impacts the accuracy by which these platforms can detect any analyte of interest as well as the ability to be multiplexed.

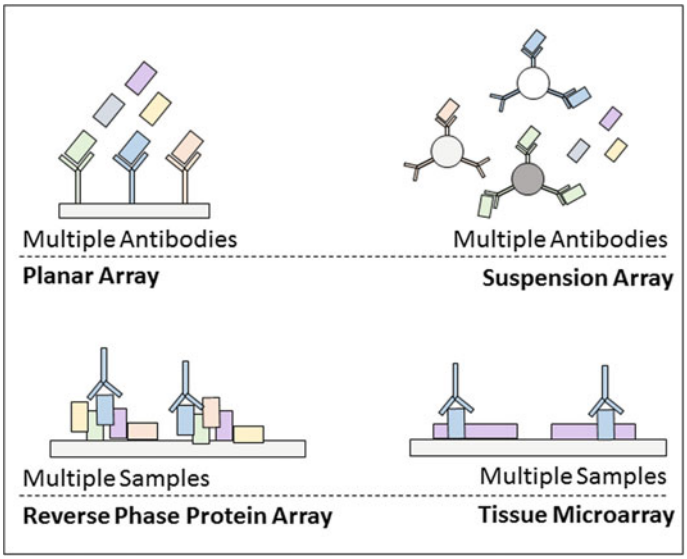


Fig. 4.2 Schematic representation of four different antibody based platforms that can be used for multiplex high throughput analysis: planar and suspension immunoassay, Reverse Phase Protein Microarray, and Tissue Microarrays

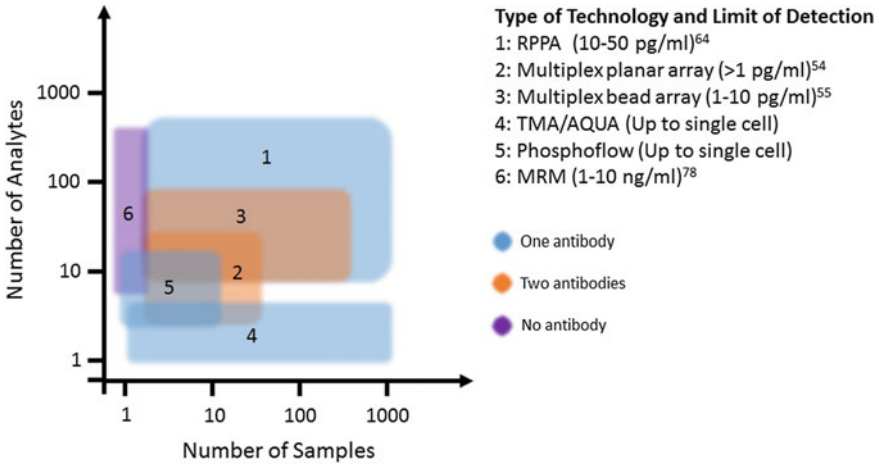


Fig. 4.3 Representation of number of samples (*x* axis) and analytes (*y* axis) for six different proteomic platforms that can be used for functional analysis

For these reasons increasing efforts have been focused on developing highly specific antibodies along with databases to track their performance. An important example in the field is offered by the Human Protein Atlas, a database that is publicly available and that contains validation images for thousands of antibodies using different technologies (e.g. protein assay, western blot, immunohistochemistry, and immunofluorescence) along with tissue distribution of each protein [52].

Multiplex Immunoassays

These technologies are sandwich-based immunoassays able to concomitantly measure multiple analytes within the same samples. Signal detection of sandwich-based assays, by definition, requires two antibodies targeting different epitopes of the same protein. The first antibody is usually immobilized either on a solid substrate (planar assay) or on the surface of beads (suspension assay) [53–55]. After incubation of the immobilized antibody with a complex protein solution (e.g. biological fluid, cell/tissue lysate) only proteins selectively captured by the antibodies are bound and retained on substrate. A second antibody targeting a different epitope of the same protein is then used to detect the protein of interest. Standard curves prepared according to international guidelines are usually run along site with experimental samples. Because of their ability of concomitantly measuring a relatively large number of analytes across multiple samples, multiplex immunoassays are considered high throughput and multiplex technologies able to generate quantitative or semi-quantitative data starting from relatively little biological material (detection range from ng to pg/mL).

FAST Quant®, A2™ Protein MicroArray System, Cira™ immunoassay platform, and Meso-scale discovery (MSD) are a few examples of planar multiplex immunoassay [56, 57]. Of interest, the MSD platform incorporates a unique electrochemiluminescence-based detection system which, compared to other options on the market, makes the platform highly sensitive while protecting the signal from a time-dependent degradation. Example of commercially available multiplex suspension bead arrays are Luminex or xMAP and FlowCytomix [53]. Suspension beads array allow for the concomitant measurement of a greater number of analytes compared to the planar platforms (less than ten vs. hundreds).

Reverse Phase Protein Microarray

The RPPA platform is a high throughput proteomic technology capable of quantitatively measuring the activation level of hundreds of kinases in clinical samples as well as in in vitro and in vivo models. Because of its ability to measure the activation level of hundreds of kinases across a large number of samples, this technology has been widely used to perform signaling network analysis of human cancers [58–61].

Hundreds of denatured cell lysates are immobilized onto nitrocellulose coated slides using automated contact or non-contact systems (e.g. Aushon 2740 arrayer, Arrayjet Marathon systems) [62–64]. Protein quantification is usually achieved by using a single antibody targeting one protein of interest. Colorimetric or fluorescent detection systems can be employed for the detection of the signal. Both detection methods have been successfully coupled with tyramide-based amplification systems to further increase the limit of detection of the RPPA platform [65]. A major advantage of the RPPA technology is its ability to concomitantly measure large panels of analytes across hundreds of samples starting from very little biological material (e.g. fine needle aspirates and core biopsies) [63]. Indeed, hundreds of arrays can be prepared starting from only 5,000 to 20,000 cells. For this reason, this platform has been used not only in predictive clinical studies, but also as a companion to diagnostics in precision medicine clinical trials [60, 66, 67].

Phosphoflow

Phosphoflow, or the use of flow cytometry to monitor intracellular phosphorylation events, is another antibody-based method that can be used to investigate the phosphoproteome [68]. Similar to the multiplex immunoassays previously described, this platform allows for the measurement of multiple analytes within a sample, and multiple samples can be analyzed concomitantly when coupled with upfront barcoding [69]. Like the RPPA, it has been utilized for signaling network analysis of human cancers, although its use is limited to liquid biopsies, blood, or in vitro

models [70, 71]. Because it uses a flow cytometry-based approach, this techniques is suitable for analyzing heterogeneous samples in terms of cell composition and for single cells analysis. Nonetheless, to effectively measure membrane and intracellular protein this technique requires upfront membrane permeabilization which can affect antibody's epitope binding capacity [70, 71]. Finally, because denaturing agents cannot be used and proteins need to be detected in their native form, the panel of analytes that can be measured using this technique is inferior compare to the other platforms previously described [72].

Tissue Microarrays

The last antibody-based method described in this chapter is tissue microarrays (TMA), an extension of conventional immunohistochemistry (IHC) that allow for the direct visualization of the subcellular localization of the analyte(s) of interest. Similar to the RPPA, protein identification is based on a single antibody targeting the protein of interest. In the TMA platform small sections (as little as a few mm) derived from up to one thousand samples are mounted on the same glass slide and can be concomitantly probed with the same antibody [73]. The main advantage of this configuration is the requirement of a lower amount of biological material and high sample throughput, although, like most of the multiplex immunoassay, this platform has relatively low multiplex capability (≤ 10 analytes per field). Similar to the phosphoflow, membrane permeabilization and antigen retrieval procedures required for effective antibody staining can also cause loss of the phospho-epitope of interest or ineffective antibody-epitope recognition.

Scoring systems conventionally used for IHC and TMA are semi-quantitative and operator-dependent. For this reason inter-operator reproducibility can be highly variable [74]. To overcome this limitation, IHC and TMA evaluation have recently been coupled with Automated Quantitative Analysis (AQUA) and automated system that provides objective and quantitative measurement of the analyte of interest [73, 74].

Non-antibody-Based Technologies

Mass spectrometry represents a valid alternative for qualitative and quantitative measurement of proteins in their unmodified form as well as for post-translational modification. A major advantage of mass spectrometry over the antibody based assays is its ability to provide absolute quantification of a large panel of analytes without the need for highly regulated reference standards. Nonetheless, although measuring abundant phosphoproteins in human specimens is relatively easy to achieve, most of the proteins involved in the signaling networks are present in sub-stoichiometric quantities and as a consequence are challenging to detect even

by the new generation of mass spectrometers. There is still an urgent need to develop mass spectrometry platforms with analytical sensitivity similar to the immunoassays routinely used not only for clinical studies, but also for stratifying patients to targeted treatments. To overcome this challenge a number of affinity-based upfront enrichment methods have been developed and are routinely coupled with MS for the analysis of low abundance phosphoproteins (e.g. immobilized metal affinity chromatography (IMAC), metal oxide affinity chromatography, kinase-selective affinity purification) [75–77].

Multiple reaction monitoring (MRM) has also been used to measure low abundance phospho-peptides in human specimens [78]. MRM utilizes a triple quadrupole (QQQ) technology, whereby peptides are selectively filtered by charge to mass ratio which allows for investigation specific peptides selected a priori [79]. While this method is extremely precise, it is time consuming and has overall low throughput, two characteristics that make it hard to incorporate in clinical practice.

Concluding Remarks

The use of functional proteomics offers unique opportunities for understanding the role of the PI3K-AKT-mTOR axis in tumor progression. The ability of measuring not only individual proteins affected by specific mutations, but also to evaluate the interaction and activation/phosphorylation level of downstream effectors (e.g. AKT, mTOR, 4E-BP1, S6RP) provides a window into the dynamic molecular mechanisms that drive tumor growth and progression. A number of high throughput multiplex platforms are available on the market for performing broad protein pathway activation based profiling at the signaling network level starting with little biological material, a characteristic that renders these platforms particularly attractive for clinical studies. Because of their high throughput multiplex format, these platforms allow for exploring the signaling network at a true network level basis (as opposed to a one signaling molecule at a time level) and the post-translational modifications responsible for the activation of these networks. These new technologies have opened new opportunities for identifying not only prognostic and predictive biomarkers, but also therapeutic targets within the signaling network driving individual tumors. Finally, the combination of genomic and proteomic technologies are able to generate a more comprehensive pictures of the molecular events driving individual lesions and is providing an entirely new and more comprehensive approach towards the realization of precision medicine for cancer patients. This, in turn, has led to the integration of multi-omics analysis into clinical trials for more effective stratification of cancer patients to targeted treatments.

References

1. Thorpe LM, Yuzugullu H, Zhao JJ (2015) PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. Nat Rev Cancer 15(1):7–24
2. Fruman DA, Rommel C (2014) PI3K and cancer: lessons, challenges and opportunities. Nat Rev Drug Discov 13(2):140–156
3. Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 7:606–619
4. Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490(7418):61–70
5. Kolch W, Pitt A (2010) Functional proteomics to dissect tyrosine kinase signalling pathways in cancer. Nat Rev Cancer 10(9):618–629
6. Manash KP, Anup KM (2004) Tyrosine kinase—role and significance in cancer. Int J Med Sci. 1(2):101–115
7. Engelman JA (2009) Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 9(8):550–562
8. Kandoth C, McLellan MD, Vandin F et al (2013) Mutational landscape and significance across 12 major cancer types. Nature 502(7471):333–339
9. Miled N, Yan Y, Hon WC et al (2007) Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. Science 317(5835):239–242
10. Huang CH, Mandelker D, Schmidt-Kittler O et al (2007) The structure of a human p110α/p85α complex elucidates the effects of oncogenic PI3Kα mutations. Science 318 (5857):1744–1748
11. Cizkova M, Vacher S, Meseure D et al (2013) PIK3R1 underexpression is an independent prognostic marker in breast cancer. BMC Cancer 13:545
12. Isakoff SJ, Engelman JA, Irie H et al (2005) Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells. Cancer Res 65(23):10992–11000
13. Zhao JJ, Liu Z, Wang L et al (2005) The oncogenic properties of mutant p110α and p110β phosphatidylinositol 3-kinases in human mammary epithelial cells. Proc Natl Acad Sci U S A 102(51):18443–18448
14. Mukohara T (2015) PI3K mutations in breast cancer: prognostic and therapeutic implications. Breast Cancer (Dove Med Press) 7:111–123
15. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A et al (2008) An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. Cancer Res 68 (15):6084–6091
16. Hashimoto K, Tsuda H, Koizumi F et al (2014) Activated PI3K/AKT and MAPK pathways are potential good prognostic markers in node-positive, triple-negative breast cancer. Ann Oncol 25(10):1973–1979
17. Lazaridis G, Lambaki S, Karayannopoulou G et al (2014) Prognostic and predictive value of p-Akt, EGFR, and p-mTOR in early breast cancer. Strahlenther Onkol 190(7):636–638, 640–645
18. Courtney KD, Corcoran RB, Engelman JA (2010) The PI3K pathway as drug target in human cancer. J Clin Oncol 28(6):1075–1083
19. Carracedo A, Pandolfi PP (2008) The PTEN-PI3K pathway: of feedbacks and cross-talks. Oncogene 27(41):5527–5541
20. Rodriguez-Viciana P, Warne PH, Dhand R et al (1994) Phosphatidylinositol 3-OH kinase as a direct target of Ras. Nature 370:527–532
21. Mendoza MC, Er EE, Blenis J (2011) The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. Trends Biochem Sci 36:320–328
22. Liu P, Cheng H, Roberts TM et al (2009) Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov 8(8):627–644
23. Lee YH, Giraud J, Davis RJ et al (2003) c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. J Biol Chem 278:2896–2902

24. O'Reilly KE, Rojo F, She QB et al (2006) mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 66(3):1500–1508
25. Chen J, Zhao KN, Li R et al (2014) Activation of PI3K/Akt/mTOR pathway and dual inhibitors of PI3K and mTOR in endometrial cancer. Curr Med Chem 21(26):3070–3080
26. Nakanishi K, Sakamoto M, Yamasaki S et al (2005) Akt phosphorylation is a risk factor for early disease recurrence and poor prognosis in hepatocellular carcinoma. Cancer 103(2): 307–312
27. Nam SY, Lee HS, Jung GA et al (2003) Akt/PKB activation in gastric carcinomas correlates with clinicopathologic variables and prognosis. APMIS 111(12):1105–1113
28. Malinowsky K, Nitsche U, Janssen KP et al (2014) Activation of the PI3K/AKT pathway correlates with prognosis in stage II colon cancer. Br J Cancer 110(8):2081–2089
29. Shah A, Swain WA, Richardson D et al (2005) Phospho-Akt expression is associated with a favorable outcome in non-small cell lung cancer. Clin Cancer Res 11:2930–2936
30. Ocana A, Vera-Badillo F, Al-Mubarak M et al (2014) Activation of the PI3K/mTOR/AKT pathway and survival in solid tumors: systematic review and meta-analysis. PLoS ONE 9(4): e95219
31. Qiu ZX, Zhang K, Qiu XS et al (2013) The prognostic value of phosphorylated AKT expression in non-small cell lung cancer: a meta-analysis. PLoS ONE 8(12):e81451
32. Yang Y, Luo J, Zhai X et al (2014) Prognostic value of phospho-Akt in patients with non-small cell lung carcinoma: a meta-analysis. Int J Cancer 135(6):1417–1424
33. Cai J, Xu L, Tang H et al (2014) The role of the PTEN/PI3K/Akt pathway on prognosis in epithelial ovarian cancer: a meta-analysis. Oncologist 19(5):528–535
34. Yang ZY, Di MY, Yuan JQ et al (2015) The prognostic value of phosphorylated Akt in breast cancer: a systematic review. Sci Rep 5:7758
35. Paplomata E, O'Regan R (2014) The PI3K/AKT/mTOR pathway in breast cancer: targets, trials and biomarkers. Ther Adv Med Oncol 6(4):154–166
36. Clark AS, West K, Streicher S et al (2002) Constitutive and inducible Akt activity promotes resistance to chemotherapy, trastuzumab, or tamoxifen in breast cancer cells. Mol Cancer Ther 1:707–717
37. Bostner J, Karlsson E, Pandiyan MJ et al (2013) Activation of Akt, mTOR, and the estrogen receptor as a signature to predict tamoxifen treatment benefit. Breast Cancer Res Treat 137 (2):397–406
38. Kim EK, Kim HA, Koh JS et al (2011) Phosphorylated S6K1 is a possible marker for endocrine therapy resistance in hormone receptor-positive breast cancer. Breast Cancer Res Treat 126(1):93–99
39. Kirkegaard T, Witton CJ, McGlynn LM et al (2005) AKT activation predicts outcome in breast cancer patients treated with tamoxifen. J Pathol 207(2):139–146
40. Karlsson E, Pérez-Tenorio G, Amin R et al (2013) The mTOR effectors 4EBP1 and S6K2 are frequently coexpressed, and associated with a poor prognosis and endocrine resistance in breast cancer: a retrospective study including patients from the randomised Stockholm tamoxifen trials. Breast Cancer Res 15(5):R96
41. Beelen K, Opdam M, Severson TM et al (2014) Phosphorylated p-70S6K predicts tamoxifen resistance in postmenopausal breast cancer patients randomized between adjuvant tamoxifen versus no systemic treatment. Breast Cancer Res 16(1):R6
42. O'Brien NA, Browne BC, Chow L et al (2010) Activated phosphoinositide 3-kinase/AKT signaling confers resistance to trastuzumab but not lapatinib. Mol Cancer Ther 9(6): 1489–1502
43. Haas-Kogan DA, Prados MD, Tihan T et al (2005) Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib. J Natl Cancer Inst 97:880–887
44. Emery IF, Battelli C, Auclair PL et al (2009) Response to gefitinib and erlotinib in non-small cell lung cancer: a restrospective study. BMC Cancer 9:333
45. Villaflor VM, Buckingham L, Gale M et al (2005) EGFR mutations and pAKT expression as potential predictors of gefitinib efficacy in non-small cell lung cancer (NSCLC) patients (pts). J Clin Oncol 23(16_suppl) (Meeting Abstracts)

46. Cappuzzo F, Magrini E, Ceresoli GL et al (2004) Akt phosphorylation and gefitinib efficacy in patients with advanced non-small-cell lung cancer. J Natl Cancer Inst 96(15):1133–1141
47. Cappuzzo F, Hirsch FR, Rossi E et al (2005) Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst 97(9): 643–655
48. Polivka J Jr, Janku F (2014) Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. Pharmacol Ther 142(2):164–175
49. Li S, Kong Y, Si L et al (2014) Phosphorylation of mTOR and S6RP predicts the efficacy of everolimus in patients with metastatic renal cell carcinoma. BMC Cancer 28(14):376
50. Trédan O, Treilleux I, Wang Q et al (2013) Predicting everolimus treatment efficacy in patients with advanced endometrial carcinoma: a GINECO group study. Target Oncol 8(4):243–251
51. Reis H, Herold T, Ting S et al (2015) HER2 expression and markers of phosphoinositide-3-kinase pathway activation define a favorable subgroup of metastatic pulmonary adenocarcinomas. Lung Cancer 88(1):34–41
52. Uhlén M, Fagerberg L, Hallström BM et al (2015) Proteomics. Tissue-based map of the human proteome. Science 347(6220):1260419
53. Houser B (2012) Bio-Rad's Bio-Plex® suspension array system, xMAP technology overview. Arch Physiol Biochem 118(4):192–196
54. Fu Q, Zhu J, Van Eyk JE (2010) Comparison of multiplex immunoassay platforms. Clin Chem 56(2):314–318
55. de Jager W, Rijkers GT (2006) Solid-phase and bead-based cytokine immunoassay: a comparison. Methods 38(4):294–303
56. Gowan SM, Hardcastle A, Hallsworth AE et al (2007) Application of meso scale technology for the measurement of phosphoproteins in human tumor xenografts. Assay Drug Dev Technol 5(3):391–401
57. Sourial S, Marcusson-Ståhl M, Cederbrant K (2009) Meso scale discovery and luminex comparative analysis of calbindin D28K. J Biomed Biotechnol 187426:1–6
58. Baldelli E, Bellezza G, Haura EB et al (2015) Functional signaling pathway analysis of lung adenocarcinomas identifies novel therapeutic targets for KRAS mutant tumors. Oncotarget. doi:10.18632/oncotarget.5941
59. Silvestri A, Calvert V, Belluco C et al (2013) Protein pathway activation mapping of colorectal metastatic progression reveals metastasis-specific network alterations. Clin Exp Metastasis 30 (3):309–316
60. Wulfkuhle JD, Berg D, Wolff C et al (2012) Molecular analysis of HER2 signaling in human breast cancer by functional protein pathway activation mapping. Clin Cancer Res 18 (23):6426–6435
61. Li J, Lu Y, Akbani R et al (2013) TCPA: a resource for cancer functional proteomics data. Nat Methods 10(11):1046–1047
62. Masuda M, Yamada T (2015) Signaling pathway profiling by reverse-phase protein array for personalized cancer medicine. Biochim Biophys Acta 1854(6):651–657
63. VanMeter A, Petricoin EF III, Signore M et al (2007) Reverse-phase protein microarrays: application to biomarker discovery and translational medicine. Expert Rev Mol Diagn 7 (5):625–633
64. Paweletz CP, Charboneau L, Bichsel VE et al (2001) Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. Oncogene 20(16):1981–1989
65. Pin E, Federici G, Petricoin EF 3rd (2014) Preparation and use of reverse protein microarrays. Curr Protoc Protein Sci 75:Unit 27.7
66. Pierobon M, Silvestri A, Spira A et al (2014) Pilot phase I/II personalized therapy trial for metastatic colorectal cancer: evaluating the feasibility of protein pathway activation mapping for stratifying patients to therapy with imatinib and panitumumab. J Proteome Res 13 (6):2846–2855

67. Jameson GS, Petricoin EF, Sachdev J et al (2014) A pilot study utilizing multi-omic molecular profiling to find potential targets and select individualized treatments for patients with previously treated metastatic breast cancer. Breast Cancer Res Treat 147(3):579–588
68. Rahman M (2014) Introduction to flow cytometry. AbD Serotec, A Bio-Rad Company
69. Krutzik PO, Nolan GP (2006) Fluorescent cell barcoding in flow cytometry allows high-throughput drug screening and signaling profiling. Nat Methods 3(5):361–368
70. Firaguay G, Nunès JA (2009) Analysis of signaling events by dynamic phosphoflow cytometry. Sci Signal 2(86):pl3
71. Coppin E, Malergue F, Thibult ML et al (2015) Flow cytometric analysis of intracellular phosphoproteins in human monocytes. Cytometry B Clin Cytom. doi:10.1002/cyto.b.21207
72. Wu S, Jin L, Vence L et al (2010) Development and application of 'phosphoflow' as a tool for immunomonitoring. Expert Rev Vaccines 9(6):631–643
73. Elfiky AA, Aziz SA, Conrad PJ et al (2011) Characterization and targeting of phosphatidylinositol-3 kinase (PI3K) and mammalian target of rapamycin (mTOR) in renal cell cancer. J Transl Med 9(1):133
74. Camp RL, Chung GG, Rimm DL (2002) Automated subcellular localization and quantification of protein expression in tissue microarrays. Nat Med 8(11):1323–1327
75. Stasyk T, Huber LA (2012) Mapping in vivo signal transduction defects by phosphoproteomics. Trends Mol Med 18(1):43–51
76. Daub H, Olsen JV, Bairlein M et al (2008) Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. Mol Cell 31(3):438–448
77. Shi T, Fillmore TL, Sun X et al (2012) Antibody-free, targeted mass-spectrometric approach for quantification of proteins at low picogram per milliliter levels in human plasma/serum. Proc Natl Acad Sci U S A 109(38):15395–15400
78. Keshishian H, Addona T, Burgess M et al (2009) Quantification of cardiovascular biomarkers in patient plasma by targeted mass spectrometry and stable isotope dilution. Mol Cell Proteomics 8(10):2339–2349
79. Lange V, Picotti P, Domon B et al (2008) Selected reaction monitoring for quantitative proteomics: a tutorial. Mol Sys Biol 4(222):222

Chapter 5
Resistance to PI3K Pathway Inhibition

Payal D. Shah and Sarat Chandarlapaty

Introduction

The phosphatidylinositol 3-kinase (PI3K) pathway, which includes the PI3K holoenzyme and the downstream effector kinases, AKT and the mammalian target of rapamycin (mTOR), is critical for cancer cell growth, proliferation, and survival. Aberrant activation of this pathway promotes transformation [1] and is frequently observed through various mechanisms including activation of upstream receptor tyrosine kinases (e.g., *HER2* amplification), activating mutations in pathway components (e.g., accessory and kinase domain mutations in *PIK3CA*) and loss of function of regulatory components (e.g., inactivation of *PTEN*). Among the PI3Kinases, the class I family has been most implicated in cancer. Class IA PI3K is composed of a catalytic (p110) and a regulatory (p85) subunit.

PIK3CA, the gene which encodes the alpha-isoform of p110, is among the most frequently mutated genes in human cancer. The finding of frequent activation of this pathway and demonstration of its key functions in transformation and tumor maintenance has spawned an enormous effort to pharmacologically target the pathway for cancer therapy. For the earliest generation of inhibitors, a significant disconnect was observed between antitumor effects in laboratory models and clinical benefit. This was due in part to the lack of selectivity and poor pharmacologic properties of several of these drugs. More recently, highly selective and potent inhibitors of class I PI3Ks, AKT, and mTOR have been developed. While these drugs have not overcome all issues related to effective inhibition of the target

P.D. Shah
Department of Medicine, Memorial Sloan Kettering Cancer Center,
New York, NY 10065, USA

S. Chandarlapaty (✉)
Department of Medicine and Human Oncology and Pathogenesis Program,
Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA
e-mail: chandars@mskcc.org

N. Dey et al. (eds.), *PI3K-mTOR in Cancer and Cancer Therapy*,
Cancer Drug Discovery and Development, DOI 10.1007/978-3-319-34211-5_5

kinase, they have allowed a more detailed study of biologic reasons for intrinsic and acquired resistance to inhibition of the pathway in tumors where the pathway is aberrantly activated. The present chapter aims to summarize our current understanding of key biologic mechanisms of resistance to PI3K–AKT–mTOR inhibition in tumors featuring genetic activation of the pathway.

The PI3K–AKT–mTOR Pathway

A simplified cartoon illustration of key members of the PI3K pathway is presented in Fig. 5.1 and provides a framework for understanding some of the relationships of the components. PI3K is a cytoplasmic lipid kinase activated by membrane-bound receptor tyrosine kinases (RTKs) such as HER3 and IGFR1 often via SH2 domain containing docking molecules such as IRS1. The lipid kinase activity of PI3K is induced upon binding of the regulatory subunit (p85) to the receptor complex, relieving negative interactions with the kinase domain containing subunit (p110). Upon activation of PI3K, PIP2 is phosphorylated to generate the second messenger PIP3. Phosphatase and tensin homolog (PTEN) is a phosphatase, dephosphorylating PIP3 to PIP2 to inhibit signaling through the PI3K pathway. PIP3 binds to the pleckstrin homology domain (PHD) of AKT, thereby recruiting this downstream effector to the membrane. Interaction with PIP3 provides access to key residues, T308 and S473, which can then be phosphorylated by the PDK1 and mTORC2

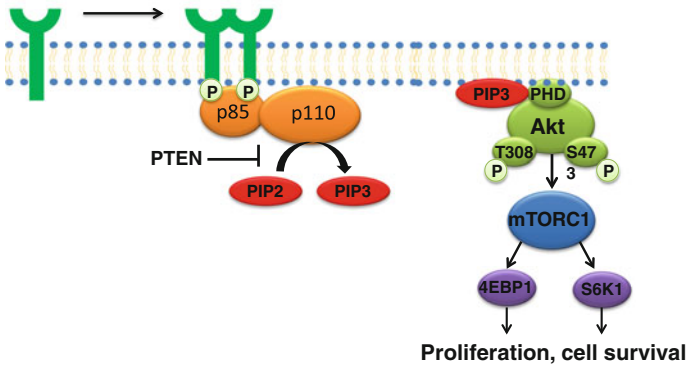


Fig. 5.1 The PI3K-Akt-mTOR pathway. Key components of the PI3K pathway are depicted here. Upon activation, for example by dimerization, membrane-bound RTKs activate PI3K by binding to the PI3K regulatory subunit, p85. This relieves inhibitory interactions with the kinase domain subunit, p110 and results in PI3K activation. PI3K phosphorylates PIP2 to generate PIP3. PTEN serves as a phosphatase, dephosphorylating PIP3 to PIP2 to inhibit signaling through the PI3K pathway. PIP3 binds to the pleckstrin homology domain of AKT, recruiting this effector to the membrane. AKT is phosphorylated by PDK1 and mTORC2 at T308 and S473, respectively, resulting in full AKT activation. AKT activation results in mTORC1 activation, and cell survival and proliferation ultimately results

kinases respectively. These phosphorylations facilitate the full activation of the Ser/Thr directed AKT kinase toward its many substrates. Among its substrates are several members that can promote activation of the mTOR kinase. Activated AKT directly phosphorylates PRAS40, which otherwise binds to and inhibits mTORC1. AKT is also shown to phosphorylate TSC2, which otherwise negatively regulates the RHEB GTPase that activates mTORC1. Finally, AKT has been shown to directly phosphorylate and promote mTOR activity. Thus, PIP3-mediated activation of AKT provides multiple routes for activation of mTORC1. Downstream effectors of mTORC1, including the ribosomal protein S6 kinase 1 (S6K1) and the translational repressor protein eukaryotic initiation factor 4E (elF-4E) binding protein 1 (4EBP1), are involved in control of cell size and protein translation among other processes key for cell proliferation.

The pathway as schematized depicts a relatively simple set of processive steps to allow signal transmission. However, physiologic activation, as with all signaling systems, is expected to involve numerous components whose function is to attenuate or downregulate the signal. Such negative feedback regulation is pervasive and selected for in complex systems [2] (see Fig. 5.2). In tumors, the pathway is hyperactivated through mutational events. We and others have observed that the negative feedback is similarly hyperactivated in most of these systems [3–6]. This presents a potential hurdle to nearly any drug designed to inhibit oncoprotein activated signaling. Inhibition of the single node will result in activation of upstream signaling through the loss of this hyperactivated negative feedback. This set of newly active signals represents a means for the cancer cell to adapt to the drug and ultimately resist its effects. This form of resistance appears to play a major role in modulating the effects of PI3K-directed therapies.

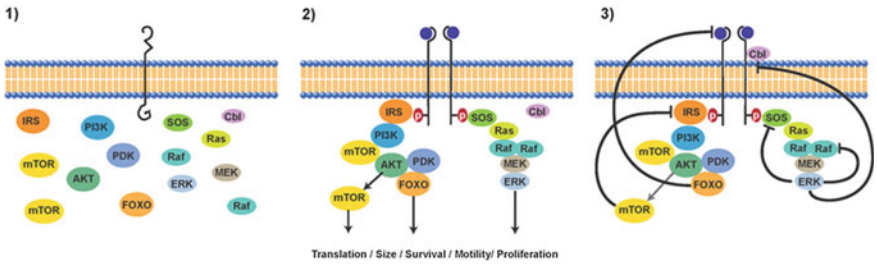


Fig. 5.2 Growth factor activation of signaling and negative feedback. The *first panel* depicts major elements of the EGFR signaling transduction apparatus in a disassembled state in the absence of growth factor stimulation. In the *second panel*, addition of growth factor ligand triggers conformational change, dimerization, and transphosphorylation of the receptor. Binding of adaptor proteins follows, resulting in activation of kinase cascades, and stimulation of cellular programs involved in transformation (cell cycle progression, evasion from apoptosis, motility and invasion, increases in cell size, stimulation of protein translation). In the *third panel*, negative feedback programs are depicted including FOXO mediated repression of expression of receptor tyrosine kinases such as HER3, and mTOR mediated destabilization of the IRS1 adaptor protein via S6K activation

Drug-Induced Relief of Feedback Results in Pathway Reactivation

mTORC1 Inhibition Promotes AKT Activation

During physiologic activation such as IGF stimulation, mTORC1 phosphorylates and activates S6K1. Phosphorylated S6K1 in turn phosphorylates the insulin receptor substrate-1 (IRS1) adaptor protein, inducing its degradation and thereby allowing signal attenuation [7–9] (see Fig. 5.3). O'Reilly et al. examined the significance of this negative feedback pathway in tumors, studying the effect of the allosteric mTORC1 inhibitor, rapamycin on upstream signaling. They demonstrated that treatment of PI3K-hyperactivated cell lines with rapamycin caused an increase in S473 phosphorylation and AKT kinase activity [8]. They further demonstrated that this AKT kinase activation was associated with increased phosphorylation of endogenous AKT substrates including the FoxO1a, FoxO3a, and FoxO4 transcription factors, confirming functional activation of AKT kinase by mTOR

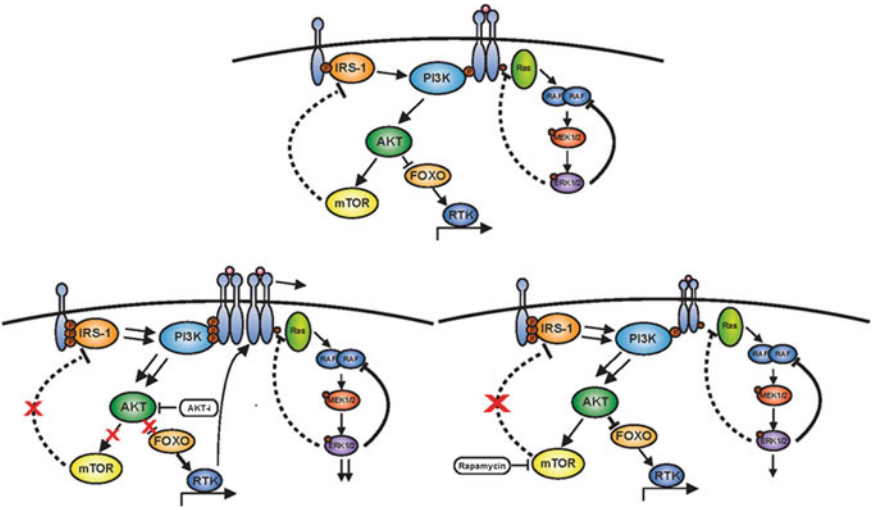


Fig. 5.3 Negative feedback regulation of PI3K/AKT/mTOR signaling. Depicted in the *top panel* is negative feedback regulation of PI3K/AKT/mTOR signaling through two major pathways from mTOR and AKT. mTOR regulates adaptor proteins such as IRS1 while AKT regulates the expression of receptor tyrosine kinases (RTK) through the FOXO transcription factors. The consequence of drug inhibition of AKT is shown in the *bottom left panel* with inhibition of AKT causing loss of negative feedback on RTK expression and so inducing RTK expression. In addition, AKT in many cells activates mTOR and so drug inhibition of AKT leads to inhibition of mTOR leading to loss of negative feedback on IRS1. The sum consequence of AKT inhibition is to activate RTK function through adaptors and increases in RTK expression. Depicted in the *right panel* is mTORC1 inhibition with rapamycin which predominantly impacts RTK function through the effects on adaptor proteins without the effects on RTK expression

inhibition. Using quantitative immunohistochemical assessments of paired pre-treatment and on-treatment tumor biopsies, increased pAKT following treatment with the mTOR inhibitor everolimus was confirmed in patients. Furthermore, using a human monoclonal inhibitory antibody to the IGF1R, they demonstrated the significance of this induction of AKT activity. They showed that in cells dependent on IGF1R for proliferation, the induction of AKT activity was also IGF1R dependent and IGF1R inhibition could sensitize cells to the antiproliferative effects of rapamycin, underscoring the relevance of the feedback induction of PI3K/AKT signaling. These findings were corroborated in a study by Shi et al. in a myeloma model where rapamycin resulted in enhanced AKT activity, phosphorylation, and PI3K activity that were IGF1R dependent [9]. Li et al. proposed an alternative mechanism for rapamycin-induced AKT phosphorylation in addition to feedback mediated alterations in IRS1 and GRB10 adaptors [10]. Utilizing small molecule inhibitors, viral inactivators, and siRNA cell-based models, they demonstrated that rapamycin treatment was associated with inhibition of protein phosphatase 2A (PP2A) and this caused reduced activation of DNA-dependent protein kinase (DNA-PK), and consequently reduced phosphorylation of AKT. While mTORC2 is thought to be the predominant kinase for AKT S473 phosphorylation, DNA-PK might also play a role in certain contexts and thus this represents yet another means by which mTOR ultimately feedback regulates AKT signaling.

The clinical significance of this finding has been further highlighted in recent years. In a phase I study of single-agent everolimus in patients with advanced solid tumors, immunohistochemical analysis of paired pre-therapy and on-therapy tumor and skin biopsies demonstrated that while downstream markers of mTOR inhibition including pS6 and p4EBP1 were reduced with everolimus, there was an overall statistically significant increase in AKT phosphorylation in tumors and in skin. This increase in pAKT was not attributable to changes in protein expression [11]. A neoadjuvant study of everolimus in PTEN-deficient glioblastoma similarly showed that rapamycin treatment resulted in AKT activation in seven of ten treated patients, and this activation was significantly associated with shorter time to progression during postsurgical maintenance rapamycin therapy [12], evidencing the clinical relevance of this inhibition-induced relief of negative feedback.

mTORC1 Inhibition Relieves GRB10-Mediated PI3K Suppression

In addition to the effect of S6K on IRS1 stability, two simultaneously published reports demonstrated an additional link between mTOR activation and PI3K activity via the GRB10 adaptor protein [13, 14]. The groups found that GRB10 was directly phosphorylated by mTORC1 and thereby promoted its stability. GRB10 functions as a negative regulator of PI3K activity and thus, similar net results would be expected as with IRS1 with mTORC1 inhibition resulting in PI3K activation.

These studies illustrate the complexity of feedback regulation of signaling in having redundant means of downregulating the same node.

mTOR Kinase Inhibition Causes Feedback-Dependent Biphasic Regulation of AKT Signaling

Evidence discussed above demonstrates that mTORC1 inhibition causes relief of negative feedback mechanisms and thus an increase in upstream PI3K signaling [8, 9, 11–14]. In the presence of mTORC1 inhibition, this signaling promotes a mTORC2-dependent increase in AKT, with accordant attenuation of therapeutic effects. Therefore, Rodrik-Outmezguine and colleagues studied an mTOR kinase inhibitor which, by binding to the ATP pocket of mTOR, blocks both mTORC1 and mTORC2 [15]. They found that mTOR kinase inhibition blocked mTORC2-mediated phosphorylation of AKT-S473 leading to destabilization of AKT-T308 phosphorylation in cell lines. However, surprisingly, the effect on T308 was transient. Levels of T308 phosphorylation increased within several hours of drug treatment even while S473 phosphorylation remained suppressed. As a result of T308 phosphorylation, AKT activity was induced as evidenced by phosphorylation of its downstream products including FOXO. This effect was not dependent on drug concentration and occurred despite potent mTORC1 inhibition. The basis for these effects appeared to again return to the concepts of relief of feedback. The reactivation of T308 was shown to be coincident and dependent on an induction of RTK signaling.

Active site mTOR kinase inhibitors, initially designed to inhibit mTORC2 in addition to mTORC1, were found to inhibit phosphorylation of 4EBP more completely than the mTORC1 inhibitor rapamycin [16]. Thus, the ATP competitive inhibitors of mTOR appear to be more potent inhibitors of mTORC1 complex than rapalogs. In addition to inhibition-induced relief of negative feedback demonstrated by AKT upregulation, the limited efficacy of rapamycin may in part be due to inherent pharmacologic limitations of FKBP12-dependent allosteric inhibition.

AKT Feedback Regulation of RTK Expression

To understand mechanistically how the PI3K pathway feedback regulates upstream signaling, Chandarlapaty et al. examined the effects of selective AKT and mTORC1 inhibition on RTK signaling [17]. They found that AKT inhibition, but not mTORC1 inhibition, induced the RNA expression of a conserved set of RTKs, including HER3, IGF1R, and insulin receptor, all of which are well-known direct activators of PI3K. The link between RTK expression and AKT activation was found in the FOXO transcription factors that are direct AKT substrates thus explaining the specificity of

the effect for AKT and not mTORC1 inhibition (see Fig. 5.3). The induction of RTK expression was shown to have major implications for the tumor cells as monotherapy with the AKT inhibitor was shown to only cause tumor stasis or tumor growth delay, whereas combined inhibition of AKT and the feedback induced RTKs promoted tumor regressions. It was expected and indeed observed that direct inhibitors of PI3K, to the extent that they result in AKT inhibition, also result in a similar induction of RTK expression [17, 18]. The details of the survival signals provided by the induced RTKs are still being examined. The authors show or imply that the induced receptors can both activate other pathways like the RAF/MEK/ERK pathway or simply reactivate the PI3K/AKT pathway and in effect make the drug less potent. The implications of these results are profound and have led to several studies examining combination therapy to more effectively antagonize activated PI3K signaling in tumors. Indeed, such combined targeting has met with early clinical success [19, 20].

Drug-Induced Adaptive/Compensatory Activation of Parallel Signaling Pathways in the Network

Activation of ERK Signaling

Many of the signaling components upstream of PI3K are multivalent and can activate other signaling pathways upon induction. For instance, receptors like IGF1R and HER3 bind to adaptor proteins that can then activate RAS signaling. RAS itself is able to activate both PI3K and ERK signaling. Given the findings that upstream signaling is induced by inhibitors of the PI3K pathway, it was of interest to determine whether ERK signaling was altered by any of these drugs, in what contexts, and to what biologic impact. Among the first studies, Carracedo and colleagues reported their finding of activation of ERK signaling in response to mTORC1 inhibition and demonstrated a benefit in antitumor activity of combined blockade of mTORC1 and MAPK signaling in vitro and in vivo [21]. The authors established the dependency for the induction of MAPK by RAD001 (everolimus) on the activity of both PI3K and RAS as inhibitors of PI3K and dominant negative RAS could abrogate the effects. The data suggested a role for the known S6K-IRS feedback mechanism as this serves as one route (among several) to activate PI3K. Importantly, the authors examined paired tumor biopsies from patients receiving RAD001 in the Phase I setting and corroborated their results showing a marked induction of pERK in the on-treatment biopsies compared to the pretreatment. These data confirmed the clinical relevance of the findings and led them to investigate combined MEK and mTORC1 inhibition and find a marked increase in apoptosis for the combination.

More recently, as several groups have identified profound effects of inhibition of mTOR and AKT on RTK signaling, the consequences of this on the RAS pathway have been investigated. In agreement with the findings by Carracedo, inhibition of

both mTOR and AKT in tumors caused a marked upregulation in ERK signaling [15, 17, 22]. In cancer models featuring ErbB signaling activation, the induction was shown to be dependent on EGFR/HER2 as kinase inhibitors could prevent the induction and, like MEK inhibitors, promote apoptosis when given in combination. Taken together, the data do not point to a singular mechanism whereby inhibitors of AKT/mTOR induce ERK signaling, but suggest multiple routes of feedback activation of RTK signaling that could ultimately induce ERK and promote resistance. The data on the effects of direct PI3K inhibitors on the ERK pathway appear to be less clear as some studies have pointed to the requirement for PI3K activity for the ERK induction while others have pointed to the opposite with inhibitors of PI3K causing the ERK induction. A more detailed study using selective PI3K inhibitors will be needed to fully establish the relationship between PI3K activity and ERK activation.

Coordinated Activation of RAS/RAF Signaling in Resistance

As noted earlier, alterations in the PI3K pathway are frequently coincident with lesions that alter the RAS/RAF/MEK/ERK pathway. The specific aspects of transformation that activation of each pathway confers vary considerably based on cell lineage and type of alteration. We and others have observed that in one RAS driven model, MEK inhibition alone may be sufficient to impair tumor growth and even cause regressions, while in another, PI3K pathway inhibition is necessary for full antitumor effects [23]. A general conclusion from these studies is that in many tumor types featuring concurrent RAS or BRAF activation with PI3K pathway alteration, PI3K pathway inhibition alone is insufficient to cause tumor regressions. In most cases, coordinate downregulation of both pathways is necessary but in select ones only one pathway is essential. It has been more commonly observed that such tumors are dominantly dependent on the RAS/RAF/MEK/ERK pathway for survival and thus it has often been considered that PI3K activation serves as a marker of resistance to RAF or MEK inhibition [23]. Conversely, Kras activation may serve as a marker for resistance to PI3K inhibition. In a mouse model of Kras-driven lung tumors, PI3K inhibition did not cause tumor regression despite effective PI3K inhibition [24]. Consistent with this, De Nicolantonio and colleagues demonstrated that *KRAS* mutations are a negative prognostic marker for sensitivity to mTORC1 inhibition with rapalogs [25]. The authors studied a panel of cell lines derived from multiple disease types including glioblastoma and carcinomas of the breast, ovary, prostate, colon, and uterus known to have *PIK3CA* or *PTEN* genetic alterations. They found that everolimus-resistant tumor cells displayed mutations in both *PIK3CA* and *KRAS/BRAF*, while everolimus-sensitive cell lines had PI3K pathway mutations but wild-type *KRAS/BRAF*. Examination of the sensitivity of isogenic cells was consistent with this result with *RAS/BRAF* mutant models being resistant to rapamycin.

In human tumor xenografts, Ihle and colleagues observed that *RAS* mutations were associated with marked resistance to PI3K inhibition [26]. Going further, this group examined *PIK3CA, KRAS* and *BRAF* mutations in a cohort of cancer patients who had received single-agent everolimus as part of single-institution phase I and phase II studies. They demonstrated with statistical significance that cancer patients whose tumors harbored *PIK3CA* kinase domain mutations or *PTEN* loss of function could benefit from everolimus treatment, except in the presence of concomitant *KRAS/BRAF* mutations. Furthermore, *KRAS* mutations negatively and significantly affected clinical benefit of everolimus when examined in univariate analysis.

Overall, the above studies seem to corroborate an assumption that the main set of signals downstream of mutant *KRAS* and *BRAF* is the MEK/ERK pathway and thus targeted inhibition of PI3K pathway components alone is unlikely to be effective for most of these tumors.

Feedback Regulation of Nuclear Hormone Signaling

For many years it has been known that breast and prostate cancers characterized by nuclear hormone dependence demonstrate coincident mutational activation of the PI3K pathway [27–30]. This finding led to investigations into how the pathways might participate in cross talk. A major question Carver and colleagues sought to understand was how activation of the PI3K pathway affected androgen receptor (AR) signaling. To study this type of crosstalk, they utilized murine and cell line models as well as analyses of human prostate cancers [31]. They focused attention on tumors characterized by activation of the PI3K pathway via loss of PTEN that is frequently seen in castrate resistant prostate cancer.

An analysis of one hundred and six primary prostate tumor specimens for gene expression showed that a signature of AR activation was highly enriched in those tumors with wild-type *PTEN* status compared to those with *PTEN* deficiency. Then, examining the effect of acute pathway inhibition using PI3K pathway directed drugs, they found the inverse held as inactivation of PI3K signaling in laboratory models of prostate cancer resulted in increases in AR and AR target gene expression. Given the findings on how PI3K and AKT feedback regulate RTK expression, they examined the role of RTK induction in this phenomenon and observed that increases in RTK activity were necessary for the induction in AR signaling. The mechanisms through which the induced RTKs altered AR activity are yet to be fully elucidated, but it has long been known that activation of ErbB signaling can hyperstimulate AR signaling [32]. Interestingly, the authors further show that there is feedback in the opposite direction as well with inhibition of AR signaling causing an activation of PI3K signaling through an increase in AKT activity [31]. These data provide a strong logic for testing of combinations of inhibitors of the PI3K pathway with AR antagonists that are now under way.

In further support of these ideas, the estrogen receptor (ER) seems to show similar patterns in its relationship to PI3K signaling in breast cancer. Creighton and

colleagues used gene expression and proteomic profiling data to create molecular signatures of PI3K activity and found an inverse correlation between PI3K activity and ER protein expression [33]. In breast cancer cell lines, PI3K pathway stimulation resulted in reduction in ER mRNA in a dose-responsive manner, and pathway inhibition increased expression of ER and ER-inducible target genes. Similar to the study by Carver and colleagues [31], this study suggested a role for the PI3K pathway in escape from endocrine therapy in ER driven disease again supporting combination therapy currently under clinical testing.

Wnt-β-Catenin Cooperation with PI3K Signaling

One of the major downstream effects of activated PI3K signaling is to suppress the FOXO transcription factors that play key roles in cell cycle control and survival. The FOXO family of transcription factors has additionally been implicated in Wnt-β-catenin signaling as β-catenin interacts with the FOXO family of transcription factors and acts as a transcriptional co-activator, enhancing expression of FOXO-target genes [34].

Tenbaum and colleagues investigated the physiologic significance of this Wnt and PI3K pathway interaction in colorectal tumor maintenance and progression [35]. They found that when β-catenin and FOXO co-localized in the nucleus through Wnt activation and PI3K pathway inhibition, respectively, metastatic outgrowth was induced. They demonstrated that activation of β-catenin conferred resistance to AKT inhibitor-induced apoptosis. Moreover, they demonstrated that activation of β-catenin or inhibition of AKT had minimal effect on finding metastasis, but together sharply increased the frequency of metastases from multiple organ sites. Moreover, the authors demonstrated that reduction of β-catenin content could potentially reverse this form of resistance by restoring the apoptotic response to PI3K/AKT inhibition. This study has clear implications for the potential efficacy of PI3K/AKT inhibitors in tumors with activated Wnt signaling and the phenomenon will be followed closely as drugs targeting the PI3K pathways are examined in PI3K activated tumors.

Myc *Amplification*

Myc amplification is among the most common alterations promoting tumor formation in all of cancer and has been specifically identified in many of the same malignancies that also feature PI3K activation. Liu et al. examined the question of mechanisms of resistance to PI3K utilizing a transgenic murine models of *PIK3CA* driven mammary tumors [36]. In this model, enforced overexpression of mutant

PIK3CA resulted in tumor formation. They examined resistance by taking established tumors and removing mutant expression by doxycycline withdrawal. This was shown to cause sustained tumor regression followed by approximately two-thirds of tumors resuming growth in the absence of mutant *PIK3CA* expression. The tumors were analyzed and showed *Myc* amplification in 2 of the tumors and concurrent *c-Met* overexpression in another set of tumors. The *Myc*-amplified tumors were shown to be Myc dependent using knockdown and PI3K-independent using pharmacologic studies.

In further support, indirect data from a synthetic lethal screen identified NOTCH pathway activation with consequent Myc activation as potentially of relevance in PI3K pathway inhibitor resistance [37]. In addition, activation of Myc was further shown by a group in response to rapalogs through an effect of inducing PDK activity [38]. The relevance of these different routes of activating Myc in PI3K activated and driven tumors has yet to be fully ascertained in human tumors but represent important areas for investigation among patients being treated with PI3K directed therapies. Specifically, looking for *Myc* concurrent amplification and examining tumoral *Myc* levels on drug would be of great interest.

JAK2/STAT5 Inhibition Circumvents PI3K Resistance

The Janus family of kinases (JAKs) and the associated signal transducers and activators of transcription factors (STATs) are well-described inducers of oncogenic phenotypes in numerous lineages. JAK kinases are activated upon binding of ligands (including hormones and cytokines) to their receptors, or by mutational activation, as in the *JAK2* V617F mutation in myeloproliferative disorders. In malignant states, activating ligands can be secreted by cancer cells and/or may be present in the tumor microenvironment. There is particular interest in the possible roles of activated JAK/STAT signaling in inflammatory and aggressive triple negative breast cancers which also feature activation of the PI3K pathway via loss of PTEN. Britschgi et al. investigated the effects of PI3K pathway inhibition on JAK/STAT signaling in triple-negative breast cancer [39]. In cell line and murine models, they identified upregulation of JAK2 and STAT5 in response to both PI3K and mTOR inhibitors. The basis for this induction was found to involve both the already described feedback induction of IGF1R signaling and its interaction with JAK/STAT signaling as well a secondary mechanism involving upregulation of IL8-CXCR1. They determined the significance of this upregulation utilizing the JAK2 selective inhibitor, NVP-BSK805 and showed this promoted apoptosis when given in combination with the PI3K pathway inhibitor. This ultimately translated to improved survival for murine models administered the combination over the single agents.

Additional Implicated Mechanisms of PI3K Resistance, Mechanisms not yet Elucidated

mTORC1 Inhibition Is Required for Sensitivity to PI3K-Alpha Inhibition

Many of the mechanisms described above involve induction of upstream or parallel signaling pathways that ultimately cause activation of new oncogenic signals, while a few involve pathway reactivation. The latter type of phenomenon has been more commonly implicated in resistance to targeted therapy of cancer and consistent with this was a recent report demonstrating that mTORC1 inhibition is required for sensitivity to PI3K p110α inhibitors in a panel of *PIK3CA*-mutant breast cancer cell lines [40]. In this report, the authors examined biomarkers of sensitivity to PI3K alpha inhibition among *PIK3CA* mutant cell lines. They identified degree of inhibition of phosphorylation of residues 240/4 of S6 as the best indicator of sensitivity to BYL719, a highly selective PI3K alpha inhibitor. Among the sensitive models, resistance to PI3K inhibition was induced by mTORC1 activation, while among the resistant models, increased apoptosis was seen in cells treated with combination mTORC1 and p110α inhibition. Most provocatively, the authors studied paired tumor biopsies of patients treated with a p110α inhibitor on a phase I clinical trial and found that the magnitude of inhibition of S6 phosphorylation correlated with clinical response, and that reactivation of pS6 correlated with development of acquired resistance. This study emphasized the critical role of mTORC1 inhibition in conferring and maintaining sensitivity to PI3K pathway inhibitors and suggested the possibility that addition of rapamycin to selective PI3K inhibitors may be rational while also raising questions as to what factors cause uncoupling of mTORC1 from *PIK3CA*.

Inactivating PTEN *Mutations Result in PI3K-Beta Hyperactivation*

As mentioned previously, PTEN is a tumor suppressor that negatively regulates the PI3K pathway by dephosphorylating PIP3 to PIP2 as well as serving as a phosphatase for IRS-1 [41], thereby inhibiting AKT activation and downstream pathway activation. Loss of function mutations in *PTEN* comprise a known mechanism of constitutive PI3K pathway activation. Interestingly, PTEN deficient cancers have been previously shown to depend largely on pathway activation via the PI3K beta isoform [42–44]. It logically follows that inactivating mutations in *PTEN* may serve as a mechanism of intrinsic and acquired resistance to PI3K inhibitors that selectively antagonize PI3K alpha. Indeed emerging data is consistent with this [45]. Castel, Baselga and colleagues sequenced the DNA of multiple metastatic tumors from a patient with a *PIK3CA* mutant breast tumor who initially responded to an

alpha-selective PI3K inhibitor with subsequent progression of disease in several metastatic sites. Unlike the pretreatment tumor sample, *PTEN* loss and a missense mutation were identified in multiple progressing metastases, while a periaortic lesion that was responding at the time of death did not show evidence of *PTEN* loss. The authors went on to generate a patient derived xenograft from one of the non-responding sites and showed that addition of a beta-selective PI3K inhibitor to the alpha-selective inhibitor could cause tumor growth inhibition unlike the alpha-selective inhibitor alone. These data powerfully illustrate the dynamic nature of therapeutic resistance and argue for genomic evaluation of tissue at progression as an invaluable correlate of ongoing clinical trials.

Mechanisms of Resistance Common to Cellular Signaling Pathways

Ligand-Mediated Activation of Distinct, Non-inhibited Kinases with Shared Downstream Targets

Wilson et al. discussed a broadly relevant mechanism of resistance to inhibitors of growth factor-driven kinases. They described that in the presence of an inhibitor to a specific kinase, RTK ligands are able to mediate activation of distinct, non-inhibited kinases that share critical downstream targets with the kinase being inhibited [46]. To demonstrate this, the authors exposed kinase-"addicted" and inhibited human cancer cell lines to various, widely expressed RTK ligands including hepatocyte growth factor (HGF) and epidermal growth factor (EGF). They found that exposure of these kinase-addicted and inhibited cells to ligands could "rescue" them from the effect of kinase inhibition. Co-targeting the secondary activated kinase blocked ligand rescue, whereas targeting the secondary activated kinases alone did not impact cell proliferation. These findings indicated that the "rescue" effect seen was in fact associated with ligand-mediated activation of other RTKs which stimulated redundant, pro-survival signaling pathways, on which tumor cells became reliant in the setting of inhibition of a primary growth pathway. Additionally, the RTK profile of tumor cells prior to treatment correlated with ligand-induced rescue, generating the hypothesis that the RTK profile of tumors prior to treatment could have relevance in determining optimal therapeutic combinations in a given patient.

As a specific example of this phenomenon, the authors demonstrated that HGF conferred resistance to the BRAF inhibitor vemurafenib in *BRAF*-mutant melanoma cells. This effect correlated with MET expression, was delayed by concomitant MET inhibition, and involved MAPK reactivation. After xenograft confirmation, the authors next investigated these findings in a clinical context. Pretreatment HGF levels were assessed in 126 patients with BRAF-mutant metastatic melanoma treated with vemurafenib on the BRIM2 clinical trial. Plasma HGF levels correlated with

outcome in a continuous manner, and suggested that HGF may have a role in the response to BRAF inhibition in patients with this disease. Overall, this concept of ligand-mediated resistance fits in well with the finding of the signaling network being adapted to the inhibitor by negative feedback and thus very susceptible to reactivation by such mechanisms. Indeed, this idea that the network has increased "signalability," or transduction of signals from activated RTKs, was further demonstrated by Lito et al. again examining BRAF inhibition in melanoma [3]. They demonstrated that in *BRAF*-mutant melanomas, high levels of ERK-dependent feedback reduce signaling from activated RTKs, but that ERK inhibition relieves this feedback and as an adaptation to inhibition, enhances the ability of ligands to activate signaling. This resultant ligand-mediated signaling may attenuate the anti-tumor effects of the inhibitor.

Elkabets et al. examined the role of ligands in resistance to PI3K-alpha inhibition more specifically. They used a high-throughput platform to screen for secreted ligands that opposed antiproliferative effects of a p110α inhibitor in otherwise sensitive cell lines [40]. They identified insulin-like growth factor 1 (IGF1) and neuregulin 1 (NRG1) as ligands which were able to reverse the effects of the p110α inhibitor and promote growth. They then validated these findings through the exogenous supplementation of IGF1 and NRG1 to *PIK3CA*-mutant cell lines treated with p110α inhibition. They found that with supplementation of IGF1 and NRG1 to these cell lines, an increase in downstream PI3K pathway effectors, including pS6, was seen. This effect was reversed by the addition of an mTORC1 inhibitor, suggesting that the resistance-conferring ligands activated mTORC1 in a p110α-independent manner, again emphasizing the importance of redundancy and feedback within signaling networks.

Tumor Microenvironment May Confer Targeted Therapy Resistance

Although the majority of studies investigating targeted therapy resistance focus on mechanisms of resistance relating to molecular events within a cell, Straussman and colleagues described the significance of the tumor microenvironment [47]. The authors developed a coculture system utilizing green fluorescent protein (GFP)-labeled tumor cells in culture with stromal cells. When they examined interactions between cancer cells, stromal cells and anticancer drugs, they found that drugs that induce tumor apoptosis are sometimes rendered ineffective in the presence of stromal cells. Furthermore, this effect was significantly more evident when the drugs used were targeted agents as opposed to cytotoxic chemotherapy. In further support of the importance of ligand activation of signaling, they showed that stromal cell secretion of HGF resulted in activation of the HGF receptor MET, MAPK, and PI3K signaling, and resistance to RAF inhibition. This resistance to RAF inhibition correlated with HGF expression. Dual inhibition, either with RAF

and HGF neutralizing antibodies, or RAF and MET inhibitors, was able to overcome this drug resistance. Although there was not a specific discussion of PI3K inhibitor resistance, this study generates an important and understudied concept relating to targeted therapy resistance: elements, such as secreted ligands, of a tumor microenvironment can confer therapy resistance. A fuller understanding the mechanisms behind specific resistance-conferring microenvironments may inform rational drug combinations.

Drug-Resistance-Conferring Oncogene Alterations

Intrinsic and acquired point mutations in protein kinase inhibitors have been noted to confer resistance to targeted therapy, for example, as in the case of the *EGFR* T790M mutation conferring resistance to erlotinib. Zunder and colleagues studied whether or not point mutations in *PIK3CA* confer resistance to PI3K inhibitors [48]. They used an *Saccharomyces cerevisiae* screen, comprised of mutagenized residues lining the affinity pocket, and multiple PI3K inhibitors. A potential hotspot for resistance mutations, I800, was identified with two potential resistance-conferring mutations: I800L and I800M. However, resistance mutations at the "gatekeeper" residue, which controls access to a large hydrophobic pocket in which most kinase inhibitors bind, were not identified. Following the publication of this study in 2008, to our knowledge, no definitive literature has highlighted the clinical importance of inhibitor resistance-conferring mutations though assessing for resistance mutations has not either been a major component of correlative studies accompanying the clinical investigation of PI3K pathway inhibitors.

Huw and colleagues examined *PIK3CA* mutant, *HER2*-amplified cell pools and single cell clones that were able to grow in the presence of high concentrations of the pan-class I PI3K inhibitor GDC-0941 [49]. Using genome-wide copy number analyses they found high-level amplification of the *PIK3CA* locus. Knockdown of *PIK3CA* in the resistant cells decreased pathway activation and restored sensitivity to PI3K inhibitor, confirming that resistance to PI3K inhibition in these cells was likely due to this specific amplification event. Whether overexpression or amplification of PI3K mediates resistance in patients is unknown, however it has been previously seen with resistance to other kinase inhibitors such as in the case of *BRAF* amplification in *BRAF* V600E mutant tumors [50].

Conclusion and Future Directions

The frequent deregulation of the PI3K signaling pathway in human malignancy reflects its crucial role in cell growth, survival and proliferation. Despite the strong scientific rationale supporting therapeutic inhibition of components of this pathway, early studies on pharmacologic agents targeting this pathway among patients with

activated tumors have met with less success than has been seen for other selective inhibitors of mutant oncoprotein drivers. Above, we have reviewed the substantial and expanding body of literature investigating reasons why this might be the case. First, it is evident that under physiologic conditions, the PI3K pathway is regulated by numerous, homeostatic negative feedback loops; under conditions of oncogenic activation, negative feedback is similarly hyperactivated. Pharmacologic inhibition as a therapy for such cancers relieves this hyperactive feedback and thus results in activation of upstream signaling. The consequence of this induced signaling is that it can transmit oncogenic signals and thus promote drug resistance. The two major ways by which this can occur is through (1) reactivation of the same signaling network—that is, making the drug less effective at inhibiting its target, and (2) induction of other signaling network that deliver new oncogenic signals. These feedback signals make the case for combination therapy directed specifically at the most relevant/dominant feedback pathway. Complicating such efforts is the layers of feedback present and thus the difficulty of determining which is most important to target.

A second and related means of resistance relates to coincident alternate pathway activation. Tumors may harbor hits in both the PI3K pathway and, for instance, the RAS/RAF pathway and thus have a more limited dependence on either alone. This similarly argues for genotype-directed combination therapy. Complicating these efforts has been the challenge of trying to combine inhibitors of such fundamental cell signaling pathways; this ultimately results in compromises in drug dosing which likely limit efficacy. Despite these challenges, the need for such complexity in therapeutic decision-making is obvious. The PI3K pathway is a key driver of transformation, tumor maintenance, and tumor progression in a large fraction of cancers. PI3K pathway inhibitors alone are unlikely to be sufficiently effective or to produce sustained responses, but combinations do hold the possibility of durable efficacy in the metastatic setting and curative therapy in the adjuvant setting. Ongoing preclinical and clinical efforts are thus being aimed at detecting, anticipating, and therapeutically manipulating pathways that promote PI3K inhibitor resistance [20, 51] (Table 5.1). Currently, the majority of clinical trials examining pharmacologic PI3K inhibition involve not only a PI3K inhibitor, but also chemotherapeutic, targeted, or endocrine therapy. Correlative analyses of these trials are increasingly employing phosphoproteomic studies to identify biomarkers indicative of pathway inhibition and activation (Table 5.1). With the continued incorporation of next generation sequencing techniques, genetic predictors of therapeutic resistance and sensitivity are being increasingly explored as well. We thus anticipate a major landscape change in the coming years for incorporation of PI3K pathway inhibitors into clinical practice—we simply do not anticipate it to be typically as a single agent.

Table 5.1 Clinical trials based on mechanisms of resistance to PI3K/mTOR/AKT inhibition

	Clinicaltrials.gov identifier	Title (Phase)	Agents	**Status**
mTOR/endocrine therapy	NCT00863655	Everolimus in combination with exemestane in the treatment of postmenopausal women with estrogen receptor-positive, locally advanced, or metastatic breast cancer who are refractory to letrozole or anastrozole (phase III, BOLERO-2)	Everolimus, exemestane	Active, not recruiting, has results [51]
PI3K/endocrine therapy	NCT01610284	Phase III study of BKM120/placebo with fulvestrant in postmenopausal patients with hormone receptor positive HER2-negative locally advanced or metastatic breast cancer refractory to aromatase inhibitor (phase III; BELLE-2)	BKM120, fulvestrant	Recruiting
	NCT01633060	A Phase III Study of BKM120 With Fulvestrant in Patients With HR+, HER2-, AI Treated, Locally Advanced or Metastatic Breast Cancer Who Progressed on or After mTORi (Phase III; BELLE-3)	BKM120, fulvestrant	Recruiting
	NCT01296555	A dose escalation study evaluating the safety and tolerability of GDC-0032 in patients with locally advanced or metastatic solid tumors and in combination with endocrine therapy in patients with locally advanced or metastatic hormone receptor-positive breast cancer (phase I/II)	GDC-0032, endocrine therapy	Recruiting

(continued)

Table 5.1 (continued)

	Clinicaltrials.gov identifier	Title (Phase)	Agents	**Status**
	NCT01870505	BYL719 plus letrozole or exemestane for patients with hormone receptor positive locally advanced unresectable or metastatic breast cancer (Phase I)	BYL719, letrozole or exemestane	Recruiting
mTOR/HER2 targeted	NCT01111825	Temsirolimus plus neratinib for patients with metastatic HER2-amplified or triple negative breast cancer (phase I/II)	Temsirolimus, neratinib	Recruiting
	NCT00317720	Trastuzumab and RAD001 in patients with human epidermal growth factor receptor 2 (HER-2) overexpressing breast cancer (phase I/II)	Everolimus, trastuzumab	Completed, has results [20]
PI3K/HER2 targeted	NCT02167854	Open-label study evaluating the safety and tolerability of LJM716, BYL719 and trastuzumab in patients with metastatic HER2 + breast cancer (phase I)	LJM716, BYL719, trastuzumab	Recruiting
PI3K/mTOR/MEK targeted	NCT01390818	Trial of MEK inhibitor and PI3K/mTOR inhibitor in subjects with locally advanced or metastatic solid tumors (phase I)	MSC1936369B, SAR245409	Completed
	NCT00996892	A study evaluating the safety, tolerability and pharmacokinetics of GDC-0973 in combination with GDC-0941 when administered in patients with locally advanced or metastatic solid tumors (phase I)	GDC-0973, GDC-0941	Completed

(continued)

Table 5.1 (continued)

	Clinicaltrials.gov identifier	Title (Phase)	Agents	**Status**
AKT/MEK targeted	NCT01476137	A study of the safety and activity of the MEK inhibitor given together with the AKT inhibitor to patients with multiple myeloma or solid tumor cancers (phase I)	GSK1120212, GSK2110183	Completed
mTOR/IGFR1 targeted	NCT01154335	Everolimus and OSI-906 for patients with refractory metastatic colorectal cancer (phase I)	Everolimus, OSI-906	Completed
	NCT01234857	A study of ridaforolimus (MK-8669) in combination With dalotuzumab (MK-0646) compared to standard of care treatment in estrogen receptor positive breast cancer patients (phase II)	Ridaforolimus (MK-8669), dalotuzumab (MK-0646)	Completed
	NCT00880282	Cixutumumab and Temsirolimus in treating younger patients with solid tumors that have recurred or not responded to treatment (phase I)	Cixutumumab, temsirolimus	Completed

A number of clinical trials exploit our current understanding of mechanisms of PI3K/mTOR/AKT inhibitor resistance. This table includes clinical trials compiled from the NCI database (clinicaltrials.gov: date of search July 28, 2014) which are felt to include therapeutic combinations based on this understanding. Due to the volume of pertinent studies, the following table is not a comprehensive listing but rather, includes trials that are representative of ongoing investigative efforts

References

1. Vivanco I, Sawyers CL (2002) The phosphatidylinositol 3-kinase AKT pathway in human cancer. Nat Rev Cancer 2(7):489–501. doi:10.1038/nrc839
2. Chandarlapaty S (2012) Negative feedback and adaptive resistance to the targeted therapy of cancer. Cancer Discovery 2(4):311–319. doi:10.1158/2159-8290.CD-12-0018
3. Lito P, Pratilas CA, Joseph EW, Tadi M, Halilovic E, Zubrowski M, Huang A, Wong WL, Callahan MK, Merghoub T, Wolchok JD, de Stanchina E, Chandarlapaty S, Poulikakos PI, Fagin JA, Rosen N (2012) Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. Cancer Cell 22(5):668–682. doi:10.1016/j.ccr.2012.10.009
4. Montero-Conde C, Ruiz-Llorente S, Dominguez JM, Knauf JA, Viale A, Sherman EJ, Ryder M, Ghossein RA, Rosen N, Fagin JA (2013) Relief of feedback inhibition of HER3 transcription by RAF and MEK inhibitors attenuates their antitumor effects in BRAF-mutant thyroid carcinomas. Cancer Discovery 3(5):520–533. doi:10.1158/2159-8290.CD-12-0531
5. Pratilas CA, Taylor BS, Ye Q, Viale A, Sander C, Solit DB, Rosen N (2009) (V600E)BRAF is associated with disabled feedback inhibition of RAF-MEK signaling and elevated transcriptional output of the pathway. Proc Natl Acad Sci USA 106(11):4519–4524. doi:10.1073/pnas.0900780106
6. Ercan D, Xu C, Yanagita M, Monast CS, Pratilas CA, Montero J, Butaney M, Shimamura T, Sholl L, Ivanova EV, Tadi M, Rogers A, Repellin C, Capelletti M, Maertens O, Goetz EM, Letai A, Garraway LA, Lazzara MJ, Rosen N, Gray NS, Wong KK, Janne PA (2012) Reactivation of ERK signaling causes resistance to EGFR kinase inhibitors. Cancer Discovery 2(10):934–947. doi:10.1158/2159-8290.CD-12-0103
7. Haruta T, Uno T, Kawahara J, Takano A, Egawa K, Sharma PM, Olefsky JM, Kobayashi M (2000) A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. Mol Endocrinol 14(6):783–794
8. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin DJ, Ludwig DL, Baselga J, Rosen N (2006) mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates AKT. Cancer Res 66(3):1500–1508. doi:10.1158/0008-5472.CAN-05-2925
9. Shi Y, Yan H, Frost P, Gera J, Lichtenstein A (2005) Mammalian target of rapamycin inhibitors activate the AKT kinase in multiple myeloma cells by up-regulating the insulin-like growth factor receptor/insulin receptor substrate-1/phosphatidylinositol 3-kinase cascade. Mol Cancer Ther 4(10):1533–1540. doi:10.1158/1535-7163.MCT-05-0068
10. Li Y, Wang X, Yue P, Tao H, Ramalingam SS, Owonikoko TK, Deng X, Wang Y, Fu H, Khuri FR, Sun SY (2013) Protein phosphatase 2A and DNA-dependent protein kinase are involved in mediating rapamycin-induced AKT phosphorylation. J Biol Chem 288(19):13215–13224. doi:10.1074/jbc.M113.463679
11. Tabernero J, Rojo F, Calvo E, Burris H, Judson I, Hazell K, Martinelli E, Ramon Y, Cajal S, Jones S, Vidal L, Shand N, Macarulla T, Ramos FJ, Dimitrijevic S, Zoellner U, Tang P, Stumm M, Lane HA, Lebwohl D, Baselga J (2008) Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: a phase I tumor pharmacodynamic study in patients with advanced solid tumors. Journal of clinical oncology. Off J Am Soc Clin Oncol 26(10):1603–1610. doi:10.1200/JCO.2007.14.5482
12. Cloughesy TF, Yoshimoto K, Nghiemphu P, Brown K, Dang J, Zhu S, Hsueh T, Chen Y, Wang W, Youngkin D, Liau L, Martin N, Becker D, Bergsneider M, Lai A, Green R, Oglesby T, Koleto M, Trent J, Horvath S, Mischel PS, Mellinghoff IK, Sawyers CL (2008) Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN-deficient glioblastoma. PLoS Med 5(1):e8. doi:10.1371/journal.pmed.0050008

13. Hsu PP, Kang SA, Rameseder J, Zhang Y, Ottina KA, Lim D, Peterson TR, Choi Y, Gray NS, Yaffe MB, Marto JA, Sabatini DM (2011) The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. Science 332 (6035):1317–1322. doi:10.1126/science.1199498
14. Yu Y, Yoon SO, Poulogiannis G, Yang Q, Ma XM, Villen J, Kubica N, Hoffman GR, Cantley LC, Gygi SP, Blenis J (2011) Phosphoproteomic analysis identifies GRB10 as an mTORC1 substrate that negatively regulates insulin signaling. Science 332(6035):1322–1326. doi:10.1126/science.1199484
15. Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, Poulikakos PI, Scaltriti M, Moskatel E, Baselga J, Guichard S, Rosen N (2011) mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. Cancer Discovery 1(3):248–259. doi:10.1158/2159-8290.CD-11-0085
16. Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, Shokat KM (2009) Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol 7(2):e38. doi:10.1371/journal.pbio.1000038
17. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, Majumder PK, Baselga J, Rosen N (2011) AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer Cell 19(1):58–71. doi:10.1016/j.ccr.2010.10.031
18. Chakrabarty A, Sanchez V, Kuba MG, Rinehart C, Arteaga CL (2012) Feedback upregulation of HER3 (ErbB3) expression and activity attenuates antitumor effect of PI3K inhibitors. Proc Natl Acad Sci USA 109(8):2718–2723. doi:10.1073/pnas.1018001108
19. Gajria D, King T, Pannu H, Sakr R, Modi S, Drullinsky P, Syldor A, Patil S, Seidman A, Norton L, Rosen N, Hudis C, Chandarlapaty S (2012) Tolerability and efficacy of targeting both mTOR and HER2 signaling in trastuzumab-refractory HER2 + metastatic breast cancer. Cancer Res 72(24 Suppl 3). doi:10.1158/0008-5472.SABCS12-P5-18-04
20. Morrow PK, Wulf GM, Ensor J, Booser DJ, Moore JA, Flores PR, Xiong Y, Zhang S, Krop IE, Winer EP, Kindelberger DW, Coviello J, Sahin AA, Nunez R, Hortobagyi GN, Yu D, Esteva FJ (2011) Phase I/II study of trastuzumab in combination with everolimus (RAD001) in patients with HER2-overexpressing metastatic breast cancer who progressed on trastuzumab-based therapy. J Clin Oncol Off J Am Soc Clin Oncol 29(23):3126–3132. doi:10.1200/JCO.2010.32.2321
21. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, Egia A, Sasaki AT, Thomas G, Kozma SC, Papa A, Nardella C, Cantley LC, Baselga J, Pandolfi PP (2008) Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J Clin Invest 118(9):3065–3074. doi:10.1172/JCI34739
22. Serra V, Scaltriti M, Prudkin L, Eichhorn PJ, Ibrahim YH, Chandarlapaty S, Markman B, Rodriguez O, Guzman M, Rodriguez S, Gili M, Russillo M, Parra JL, Singh S, Arribas J, Rosen N, Baselga J (2011) PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. Oncogene 30(22):2547–2557. doi:10.1038/onc.2010.626
23. Halilovic E, She QB, Ye Q, Pagliarini R, Sellers WR, Solit DB, Rosen N (2010) PIK3CA mutation uncouples tumor growth and cyclin D1 regulation from MEK/ERK and mutant KRAS signaling. Cancer Res 70(17):6804–6814. doi:10.1158/0008-5472.CAN-10-0409
24. Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, Maira M, McNamara K, Perera SA, Song Y, Chirieac LR, Kaur R, Lightbown A, Simendinger J, Li T, Padera RF, Garcia-Echeverria C, Weissleder R, Mahmood U, Cantley LC, Wong KK (2008) Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med 14(12):1351–1356. doi:10.1038/nm.1890
25. Di Nicolantonio F, Arena S, Tabernero J, Grosso S, Molinari F, Macarulla T, Russo M, Cancelliere C, Zecchin D, Mazzucchelli L, Sasazuki T, Shirasawa S, Geuna M, Frattini M, Baselga J, Gallicchio M, Biffo S, Bardelli A (2010) Deregulation of the PI3K and KRAS signaling pathways in human cancer cells determines their response to everolimus. J Clin Invest 120(8):2858–2866. doi:10.1172/JCI37539

26. Ihle NT, Lemos R Jr, Wipf P, Yacoub A, Mitchell C, Siwak D, Mills GB, Dent P, Kirkpatrick DL, Powis G (2009) Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic ras is a dominant predictor for resistance. Cancer Res 69(1):143–150. doi:10.1158/0008-5472.CAN-07-6656
27. Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, Konishi H, Karakas B, Blair BG, Lin C, Peters BA, Velculescu VE, Park BH (2004) The PIK3CA gene is mutated with high frequency in human breast cancers. Cancer Biol Ther 3(8):772–775
28. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) High frequency of mutations of the PIK3CA gene in human cancers. Science 304(5670):554. doi:10.1126/science.1096502
29. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A, Symmans WF, Pusztai L, Nolden LK, Horlings H, Berns K, Hung MC, van de Vijver MJ, Valero V, Gray JW, Bernards R, Mills GB, Hennessy BT (2008) An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. Cancer Res 68(15):6084–6091. doi:10.1158/0008-5472.CAN-07-6854
30. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, Antipin Y, Mitsiades N, Landers T, Dolgalev I, Major JE, Wilson M, Socci ND, Lash AE, Heguy A, Eastham JA, Scher HI, Reuter VE, Scardino PT, Sander C, Sawyers CL, Gerald WL (2010) Integrative genomic profiling of human prostate cancer. Cancer Cell 18(1):11–22. doi:10.1016/j.ccr.2010.05.026
31. Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S, Arora VK, Le C, Koutcher J, Scher H, Scardino PT, Rosen N, Sawyers CL (2011) Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell 19(5):575–586. doi:10.1016/j.ccr.2011.04.008
32. Mellinghoff IK, Vivanco I, Kwon A, Tran C, Wongvipat J, Sawyers CL (2004) HER2/neu kinase-dependent modulation of androgen receptor function through effects on DNA binding and stability. Cancer Cell 6(5):517–527. doi:10.1016/j.ccr.2004.09.031
33. Creighton CJ, Fu X, Hennessy BT, Casa AJ, Zhang Y, Gonzalez-Angulo AM, Lluch A, Gray JW, Brown PH, Hilsenbeck SG, Osborne CK, Mills GB, Lee AV, Schiff R (2010) Proteomic and transcriptomic profiling reveals a link between the PI3K pathway and lower estrogen-receptor (ER) levels and activity in ER + breast cancer. Breast Cancer Res BCR 12 (3):R40. doi:10.1186/bcr2594
34. Essers MA, de Vries-Smits LM, Barker N, Polderman PE, Burgering BM, Korswagen HC (2005) Functional interaction between beta-catenin and FOXO in oxidative stress signaling. Science 308(5725):1181–1184. doi:10.1126/science.1109083
35. Tenbaum SP, Ordonez-Moran P, Puig I, Chicote I, Arques O, Landolfi S, Fernandez Y, Herance JR, Gispert JD, Mendizabal L, Aguilar S, Ramon Y, Cajal S, Schwartz S, Jr., Vivancos A, Espin E, Rojas S, Baselga J, Tabernero J, Munoz A, Palmer HG (2012) Beta-catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer. Nat Med 18(6):892–901. doi:10.1038/nm.2772
36. Liu P, Cheng H, Santiago S, Raeder M, Zhang F, Isabella A, Yang J, Semaan DJ, Chen C, Fox EA, Gray NS, Monahan J, Schlegel R, Beroukhim R, Mills GB, Zhao JJ (2011) Oncogenic PIK3CA-driven mammary tumors frequently recur via PI3K pathway-dependent and PI3K pathway-independent mechanisms. Nat Med 17(9):1116–1120. doi:10.1038/nm.2402
37. Muellner MK, Uras IZ, Gapp BV, Kerzendorfer C, Smida M, Lechtermann H, Craig-Mueller N, Colinge J, Duernberger G, Nijman SM (2011) A chemical-genetic screen reveals a mechanism of resistance to PI3K inhibitors in cancer. Nat Chem Biol 7(11):787–793. doi:10.1038/nchembio.695
38. Tan J, Li Z, Lee PL, Guan P, Aau M, Lee ST, Feng M, Lim CZ, Lee EY, Wee ZN, Lim YC, Karuturi RK, Yu Q (2013) PDK1 signaling towards PLK1-Myc activation confers oncogenic transformation and tumor initiating cell activation and resistance to mTOR-targeted therapy. Cancer Discovery. doi:10.1158/2159-8290.CD-12-0595

39. Britschgi A, Andraos R, Brinkhaus H, Klebba I, Romanet V, Muller U, Murakami M, Radimerski T, Bentires-Alj M (2012) JAK2/STAT5 inhibition circumvents resistance to PI3K/mTOR blockade: a rationale for cotargeting these pathways in metastatic breast cancer. Cancer Cell 22(6):796–811. doi:10.1016/j.ccr.2012.10.023
40. Elkabets M, Vora S, Juric D, Morse N, Mino-Kenudson M, Muranen T, Tao J, Campos AB, Rodon J, Ibrahim YH, Serra V, Rodrik-Outmezguine V, Hazra S, Singh S, Kim P, Quadt C, Liu M, Huang A, Rosen N, Engelman JA, Scaltriti M, Baselga J (2013) mTORC1 inhibition is required for sensitivity to PI3K p110α inhibitors in PIK3CA-mutant breast cancer. Sci Trans Med 5(196):196–199. doi:10.1126/scitranslmed.3005747
41. Shi Y, Wang J, Chandarlapaty S, Cross J, Thompson C, Rosen N, Jiang X (2014) PTEN is a protein tyrosine phosphatase for IRS1. Nat Struct Mol Biol 21(6):522–527. doi:10.1038/nsmb.2828
42. Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH, Zhang J, Signoretti S, Loda M, Roberts TM, Zhao JJ (2008) Essential roles of PI(3)K-p110beta in cell growth, metabolism and tumorigenesis. Nature 454(7205):776–779. doi:10.1038/nature07091
43. Torbett NE, Luna-Moran A, Knight ZA, Houk A, Moasser M, Weiss W, Shokat KM, Stokoe D (2008) A chemical screen in diverse breast cancer cell lines reveals genetic enhancers and suppressors of sensitivity to PI3K isoform-selective inhibition. Biochem J 415 (1):97–110. doi:10.1042/BJ20080639
44. Wee S, Wiederschain D, Maira SM, Loo A, Miller C, deBeaumont R, Stegmeier F, Yao YM, Lengauer C (2008) PTEN-deficient cancers depend on PIK3CB. Proc Natl Acad Sci USA 105 (35):13057–13062. doi:10.1073/pnas.0802655105
45. Castel P, Juric D, Won H, Ainscough B, Ellis H, Ebbesen S, Griffith M, Griffith O, Iyer G, Sgroi D, Isakoff S, Mardis E, Solit D, Lowe S, Quadt C, Peters M, Berger M, Scaltriti M, Baselga J (2014) Loss of PTEN leads to clinical resistance to the PI3Kα inhibitor BYL719 and provides evidence of convergent evolution under selective therapeutic pressure (abstract) abstract LB-327. In: Proceedings of the annual meeting of the American Association for Cancer Research, 8 April 2014
46. Wilson TR, Fridlyand J, Yan Y, Penuel E, Burton L, Chan E, Peng J, Lin E, Wang Y, Sosman J, Ribas A, Li J, Moffat J, Sutherlin DP, Koeppen H, Merchant M, Neve R, Settleman J (2012) Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. Nature 487(7408):505–509. doi:10.1038/nature11249
47. Straussman R, Morikawa T, Shee K, Barzily-Rokni M, Qian ZR, Du J, Davis A, Mongare MM, Gould J, Frederick DT, Cooper ZA, Chapman PB, Solit DB, Ribas A, Lo RS, Flaherty KT, Ogino S, Wargo JA, Golub TR (2012) Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. Nature 487(7408):500–504. doi:10.1038/nature11183
48. Zunder ER, Knight ZA, Houseman BT, Apsel B, Shokat KM (2008) Discovery of drug-resistant and drug-sensitizing mutations in the oncogenic PI3K isoform p110α. Cancer Cell 14(2):180–192. doi:10.1016/j.ccr.2008.06.014
49. Huw LY, O'Brien C, Pandita A, Mohan S, Spoerke JM, Lu S, Wang Y, Hampton GM, Wilson TR, Lackner MR (2013) Acquired PIK3CA amplification causes resistance to selective phosphoinositide 3-kinase inhibitors in breast cancer. Oncogenesis 2:e83. doi:10.1038/oncsis.2013.46
50. Shi H, Moriceau G, Kong X, Lee MK, Lee H, Koya RC, Ng C, Chodon T, Scolyer RA, Dahlman KB, Sosman JA, Kefford RF, Long GV, Nelson SF, Ribas A, Lo RS (2012) Melanoma whole-exome sequencing identifies (V600E)B-RAF amplification-mediated acquired B-RAF inhibitor resistance. Nat Commun 3:724. doi:10.1038/ncomms1727
51. Baselga J, Campone M, Piccart M, Burris HA 3rd, Rugo HS, Sahmoud T, Noguchi S, Gnant M, Pritchard KI, Lebrun F, Beck JT, Ito Y, Yardley D, Deleu I, Perez A, Bachelot T, Vittori L, Xu Z, Mukhopadhyay P, Lebwohl D, Hortobagyi GN (2012) Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 366 (6):520–529. doi:10.1056/NEJMoa1109653

Part II
PI3K-mTOR Pathway in Cancer Medicine

Chapter 6
Combination Therapies Targeting the PI3K/AKT/mTOR Pathways

Aung Naing, Gordon B. Mills and Funda Meric-Bernstam

Introduction

The phosphoinositide 3-kinase (PI3K) pathway was first described by Lewis C. Cantley's group in 1998 [1, 2]. Upon activation, PI3K signaling can act on diverse downstream substrates [2, 3]. The PI3K pathway that includes AKT and mTOR contributes to many events that are critical for normal and pathophysiological cell metabolism, regulation of gene expression, cytoskeletal rearrangement, and cell survival (Fig. 6.1). These pathways play a role in tumor biology; in addition, triggering the PI3K/AKT/mTOR pathways is vital for the initiation, activation, and subsequent proliferation of effector T cells and for arresting the development of T regulatory cells [4]. In normal physiology, regulatory mechanisms tightly control the activity and homeostasis of the PI3K/AKT/mTOR pathway in parallel with the pathway activation dictated by extracellular signals. However, the pathway may become activated by amplification or mutation of *PI3K*, *AKT*, and other pathway members, functional activation of growth factor receptors (such as insulin-like

A. Naing (✉) · F. Meric-Bernstam
Department of Investigational Cancer Therapeutics (Phase I Clinical Trials Program), The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
e-mail: anaing@mdanderson.org

G.B. Mills · F. Meric-Bernstam
Institute of Personalized Cancer Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

F. Meric-Bernstam
Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

G.B. Mills
Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

N. Dey et al. (eds.), *PI3K-mTOR in Cancer and Cancer Therapy*,
Cancer Drug Discovery and Development, DOI 10.1007/978-3-319-34211-5_6

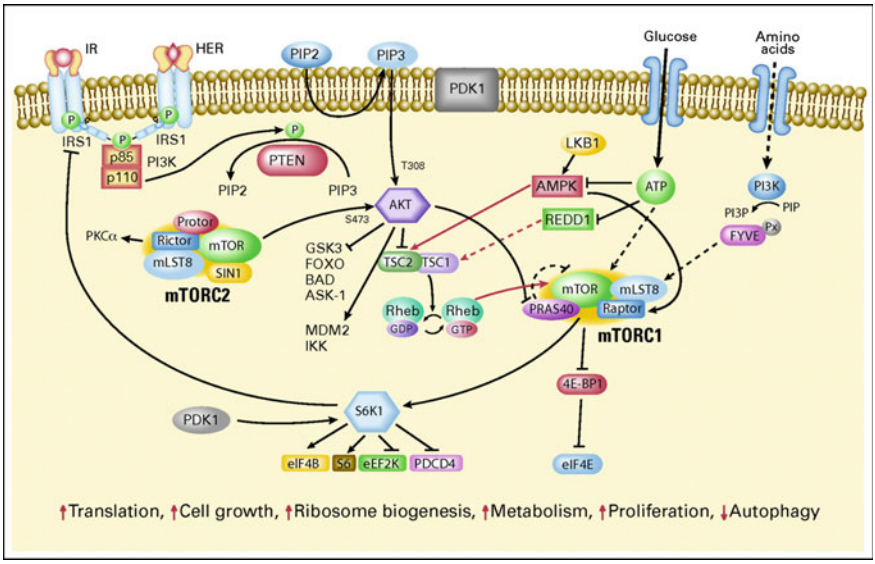


Fig. 6.1 PI3K/AKT/mTOR signaling network. *Arrows* represent activation, and inhibition is depicted by *bars*. Reprinted with permission from JCO [190]

growth factor-1 [*IGF-1*]), mutation or overexpression of growth factor receptors (epidermal growth factor receptor 1 [*EGFR*] or 2 [*HER2*, *erbB2*]), loss of function of key tumor suppressors (phosphatase and tensin homolog [*PTEN*] or inositol polyphosphate-4 phosphatase type II [*INPP4B*]), and tuberous sclerosis complex 1 and 2 (*TSC1* and *TSC2*). Mutations, amplifications, and rearrangements of components in this pathway are associated with human diseases including cancer, overgrowth syndromes such as tuberous sclerosis, and diabetes [2, 5].

The PI3K pathway is best thought of as having/being composed of two parts: an initiator arm and an effector arm. The initiator arm is initiated by activation of cell surface receptors and integrates with signals from the AMPK and MAPK pathway [s?] at the *TSC1/2* tumor suppressor. The effector arm, downstream of *TSC1/2*, initiates a number of downstream effects that either promote protein synthesis or inhibit protein catabolism. In addition to these two canonical arms, PIK3CA (a class IA PI3K; see below), AKT, and other upstream components of the pathway such as PDK1 (pyruvate dehydrogenase kinase, isozyme 1) and serine/threonine-protein kinases, activate a suite of effectors that contribute to many aspects of cell biology. In many cancers, aspects of the pathway are overactive, and it is this overactivity that is critical to prevent apoptosis and promote unchecked proliferation [6–9]. Indeed, there are more activating mutations in the PI3K/AKT/mTOR pathway members in a wider variety of cancers than occur in any other signaling pathway. Consequently, a large number of experimental cancer drugs designed to inhibit the signaling cascade at some point are in or near to entering clinical trials [10].

Additionally, the PI3K/AKT/mTOR pathways may be overactive because *TSC1/2*, *PTEN*, or *INPP4B* is mutated, genomically deleted, or not expressed due to promoter methylation or the action of micro-RNAs [11] (Fig. 6.1).

The critical role that the PI3K pathway, which is highly targetable, plays in cancer suggests that this pathway should be an optimal target. Indeed, monotherapy with PI3K pathway inhibitors does demonstrate activity in a subset of patients. However, so far the PI3K pathway inhibitors used as single agents have failed to fulfill their promise. Furthermore, biomarkers such as pathway mutations that were expected to predict response have generally been unreliable. Whether the lack of broad activity represents a narrow therapeutic index and inadequate coverage or inhibition of the pathway, adaptive resistance or the emergence of other forms of resistance remains to be fully elicited. Nevertheless, it is now clear that the promise of inhibition of the PI3K pathway is likely to only be manifest in rational drug combinations. Indeed, preliminary evidence already demonstrates the promising activity of combination therapies with hormonal manipulation in breast cancer and with poly (ADP-ribose) polymerase (PARP) inhibitors in ovarian and breast cancers. In this chapter, we discuss the different members of the PI3K/AKT/mTOR pathways that may be targeted to block the signaling cascade, singly and in combination with other agents, with the goal of reducing disease processes such as unchecked proliferation and, importantly, to sensitize tumor cells to death induced by other agents.

We first discuss *PI3K*, *AKT*, and *mTOR*, which are positive regulators of the PI3K/AKT/mTOR pathway, and then examine *PTEN*, *TSC1*, *TSC2*, and adenosine monophosphate (AMP)-activated protein kinase (*AMPK*), which are negative regulators of the PI3K/AKT/mTOR pathways. In both sections, we focus on the potential utility of combination therapies targeting the PI3K/AKT/mTOR pathways.

Positive Regulators of PI3K/AKT/mTOR Pathways

PI3K

There are three classes (I, II, and III) of PI3K. Only the class I and III PI3Ks are known to be involved in cancer. Class I PI3Ks are heterodimers, and they are further divided into two subfamilies, IA and IB.

Class IA PI3Ks comprise a p110 catalytic subunit and a regulatory subunit from 50 to 85 kDa [12]. The class IA PI3Ks (PIK3CA, PIK3CB, and PIK3CD) and the class IB PI3K (PIK3CG) encode the highly homologous p110 catalytic subunit isoforms p110 alpha, p110 beta, p110 delta, and p110 gamma, respectively. While *PIK3CA* and *PIK3CB* are expressed ubiquitously, *PIK3CD* and *PIK3CG* are predominantly expressed in leukocytes [1, 13, 14]. Gain-of-function, hotspot mutation, and gene amplification is primarily restricted to *PIK3CA*, frequently resulting in human cancer [15, 16].

Class II PI3Ks are monomers of high molecular weight; this class has three members, PI3KC2 alpha, beta, and gamma [17, 18]. A single class III PI3K, PI3K Vps34 (PIK3C3), is an essential regulator of autophagy and is critical for normal heart and liver function [19].

Mutations, deletions (rare), amplification, and multiple alterations of *PIK3CA* occur at different frequencies across cancer lineages, ranging from over 55 % in endometrioid endometrial cancers to less than 1 % in some leukemias (Fig. 6.1) [20].

Agents Targeting PI3K Isoforms

Several agents targeting the PI3K complex are being tested in cancer clinical trials, including AMG 319, BAY 80-6946, BKM120, BYL719, idelalisib, pictilisib, GDC0032, GDC-0980, GSK2269557, GS-9820, IPI-145, LY294002, MLN1117, PA799, PX-866, SAR260301, SF1126, XL147 (SAR245408), VS-5584, WX-037, wortmannin, and ZSTK474 [21]. These agents have differential specificity for PI3K isoforms as well as selectivity for PI3K as compared to other PI3K family members such as mTOR, ATR, and ATM. Idelalisib/Cal 101 was recently approved by FDA for chronic lymphocytic leukemia (CLL), relapsed follicular B-cell non-Hodgkin's lymphomas (FL) and relapsed small lymphocytic lymphoma (SLL) (Fulman NEJM 2014).

AKT

AKT directly regulates key effector molecules involved in apoptosis, anoikis, and cell cycle progression and thus have been shown to be involved in the genesis and/or progression of numerous human tumor lineages. AKT is recruited to the membrane by phosphatidylinositol 3 phosphates, the products of PI3K activity [22, 23], where it is activated by sequential phosphorylation by PDK1 and the TORC2 complex. Three human isoforms of AKT (AKT-1, -2, and -3) have been identified [24–28]. AKT-1 and -2 are broadly expressed across cell lineages. AKT-3 expression is much more limited [25–27, 29, 30]. *AKT-1* is mutationally activated in about 2 % of breast cancers and 1 % of cancers overall [20]. *AKT-2* is rarely mutated but is amplified or overexpressed in a number of lineages; in particular, it is overexpressed in ovarian cancer [29] and perhaps as many as 15 % of uterine carcinosarcomas [20]. *AKT-3* is amplified in a number of tumor lineages such as breast and liver cancer and is mutated at a low frequency in a subset of tumors [31].

Agents Targeting AKT

Several AKT inhibitors, including ARQ 092, AZD5363, GDC-0068, GSK690693, GSK2110183, GSK2141795, MK-2206, and perifosine, are being tested in clinical

trials [21]. None of the AKT inhibitors has yet been approved by the FDA for use against any cancer. Inhibition of mTOR as a consequence of a feedback loop initiated by S6K results in AKT activation [32]. High phosphorylated AKT levels can associated with in vitro sensitivity to the mTOR inhibitor rapamycin [33]. Although feedback loop activation is more prominent in rapamycin-sensitive models, it may still limit the antitumor activity of mTOR inhibition with rapalogs [33].

mTOR Complex

The mTOR pathway has been extensively studied [34, 35]. Activation of the mTOR complex downstream of the PI3K initiator arm, as well as MAPK and p90RSK signaling, plays a crucial role in deregulating proliferation, angiogenesis, and resistance to apoptosis, contributing to the development of multiple human tumor types and resistance to anticancer agents [32, 35–40]. In contrast, AMPK activation due to metabolic stress results in inhibition of mTOR activity. Furthermore, independent of regulatory kinases, mTOR acts as a sensor of amino acid levels and the ability of cells to support protein synthesis and cell cycle progression. mTOR exists in two complexes, one with Raptor, which is rapamycin sensitive, and one with Rictor, which was initially proposed to be rapamycin insensitive, although Rictor phosphorylation is regulated by rapamycin [41]. Rapamycin-sensitive mTOR complex 1 (mTORC1) phosphorylates 4EBP1 and S6 kinase; rapamycin-insensitive mTOR complex 2 (mTORC2) has been shown to directly phosphorylate and activate the upstream kinase AKT at serine 473 [42]. The two mTOR complexes appear compartmentalized in the cell, and mTOR does not appear to cycle between them.

Agents Targeting mTORC1

Currently, there are no mTORC2 selective inhibitors, but there are four mTORC1 allosteric inhibitors—sirolimus, temsirolimus, everolimus, and ridaforolimus—commonly called rapalogs, with three being FDA approved for various indications [43–45]. All of the rapalogs have similar structures and exhibit high binding affinity to intracellular FK 506-binding proteins (FKBPs), resulting in selective inhibition of a subset of functions of TORC1, including reducing the activity of the downstream effectors S6K1 and 4E-BP [46–49]. The protein-drug complex inhibits the kinase activity of mTOR [50] as a true neomorphic activity, as FKBPs do not normally interact with mTOR. Sirolimus is a hydrophobic macrocyclic lactone isolated from *Streptomyces hygroscopius* originally identified from extracts from Easter Island [51]. Sirolimus is FDA approved as an immunosuppressive agent for patients with renal transplants [52]. Temsirolimus is an orally available rapamycin ester that is rapidly converted to rapamycin by serum esterases. Temsirolimus is FDA approved for used in renal cell carcinoma [44, 53]. Everolimus is a

hydroxyethyl derivative of rapamycin. Everolimus in combination with exemestane has been FDA approved for hormone receptor-positive metastatic [54] breast cancer [45, 55]. Everolimus is also FDA approved for pediatric and adult patients with subependymal giant cell astrocytoma (SEGA), SEGA associated with tuberous sclerosis, advanced renal cell carcinoma, and pancreatic neuroendocrine tumors [56–59]. Ridaforolimus (also known as AP23573 and MK-8669, and formerly known as deforolimus) is a potent, orally available small-molecule inhibitor of mTORC [60, 61]. Ridaforolimus has not yet been approved by the FDA; however, it has been observed to have activity in sarcoma [62, 63].

Dual Kinase Inhibitors

To improve the efficacy of single-target agents and overcome the resistance associated with their use, investigators are evaluating dual kinase inhibitors in clinical trials. BEZ235, BGT226, DS-7423, GSK1059615, GSK2126458, LY3023414, PF-04691502, PF-05212384, PWT33597, SAR245409 (XL765), and VS-5584 block both PI3K and mTOR due to similarities between these two PI3K family members. MSC2363318A and XL418 are dual AKT and S6K1 inhibitors. mTOR kinase inhibitors such as AZD2014, INK128, OSI-027, and Palomid 529 that inhibit TORC1 and TORC2 are also being tested in clinical trials [21].

Negative Regulators of PI3K/AKT/mTOR Pathways

PTEN

Located on chromosome 10q23, a genomic region known for loss of heterozygosity, the *PTEN* tumor-suppressor gene, also known as *MMAC1* (mutated in multiple advanced cancers) or *TEP1* (TGFβ-regulated and epithelial cell–enriched phosphatase), negatively regulates the PI3K signaling pathway [64]. *PTEN* expression is frequently altered in human cancers as a result of mutation, deficiency, or promoter methylation silencing [1, 10, 20]. *PTEN* is the key negative regulator of the PI3K pathway. Indeed, *PTEN* knockout is sufficient to sensitize murine models to tumor development. Importantly, *PTEN* loss can be an early event in cancer development and is the germline cause of the Cowden's cancer predisposition syndrome. *PTEN* loss is associated with a high level of activation of the PI3K pathway and, indeed, much higher than that which is observed with mutation or amplification of other pathway members.

Tuberous Sclerosis Complex (TSC) and the TSC1–TSC2 Complex

The multisystemic tumor syndrome TSC arises from mutations in two tumor suppressor genes, *TSC1* and *TSC2* [65]. *TSC1* and *TSC2* form a complex that functions as a central signal-integrating node within the cell; it is inactivated by several oncogenes (e.g., *RTKs*, *PI3K*, *AKT*, *ERK*, *p90RSK*, and *Ras*) and activated by other tumor suppressors (e.g., *PTEN*, *LKB1*, and *NF1*) [66].

TSC1 stabilizes TSC2, while TSC2 acts as a GTPase-activating protein for the small GTPase Rheb (Ras homolog enriched in brain) [67]. Consequently, GTP-bound Rheb potently activates the mTORC1 complex [68].

Alterations of *TSC* genes are associated with many human cancers. Interestingly, recent studies have suggested that mutations in *TSC1* can signal responsiveness to mTOR inhibitors [69].

AMPK

The AMPK serine/threonine protein kinase is an established metabolic stress sensor [70]. *AMPK* is a key effector of the tumor suppressor liver kinase B1 (*LKB1*), which in turn inhibits mTOR through activation of the TSC1/2 complex. Additionally, *AMPK* activates checkpoint mediators such as p53 and the cyclin-dependent kinase inhibitors p21cip1 and p27kip1 [71]. In vitro, the antidiabetes agent metformin activates *AMPK*, albeit indirectly likely as a consequence of inhibition of Complex II in the mitochondria, resulting in phosphorylation and activation of *TSC1* and *TSC2*. This leads to inhibition of mTOR, which prevents mRNA activation [72–74].

Toxicity of PI3K/AKT/mTOR Inhibitors

Mucositis is one of the most common side effects of mTOR inhibitor-based therapy and can be dose limiting, is dose related, and tends to occur in earlier cycles [75–78]. Other side effects include asthenia, maculopapular rash, gastrointestinal intolerance, and pneumonitis. Laboratory abnormalities such as hyperglycemia, hyperlipidemia, and anemia also occur with PI3K/AKT/mTOR inhibitors [44, 79–87]. Rash with AKT inhibitors and neurologic effects (including depression) with blood-brain barrier—penetrating PI3K inhibitors have been regarded as dose-limiting toxicities [80, 88]. Combining these targeted agents with others can increase the toxic effects, depending on the mechanisms of action of the second drug. For example, our group has reported mucositis and endocrine complications in patients who received temsirolimus and an IGF-1 receptor (IGF-1R) inhibitor [78, 82, 84, 89]. Toxicities of targeted agents for PI3K/AKT/mTOR pathways are generally acceptable and

manageable with supportive medication. Although PI3K/AKT/mTOR-targeting agents can cause endocrine-related side effects, patients with diabetes and high cholesterol should be included in clinical trials utilizing these agents, provided their blood sugar and lipid levels are well controlled. The dose-limiting toxicity with PI3K and AKT targeting agents appear to occur at or below doses required to completely inhibit the PI3K pathway. Indeed, this may account at least in part for the limited efficacy of monotherapy. Drugs that enhance PI3K/AKT/mTOR inhibitors' toxic effects due to a likely narrow therapeutic index or that decrease the inhibitors' efficacy should be avoided. For example, CYP3A4 is the primary enzyme responsible for metabolizing a number of PI3K/AKT/mTOR inhibitors, so strong CYP3A4 modifiers should be avoided when using those agents [90, 91].

PI3K/AKT/mTOR Combination Strategies

Understanding the mechanisms of resistance to PI3K/AKT/mTOR inhibition is critical. Various pathways have been suggested to be responsible for resistance to such inhibition and multiple strategies devised to overcome this resistance (Fig. 6.2).

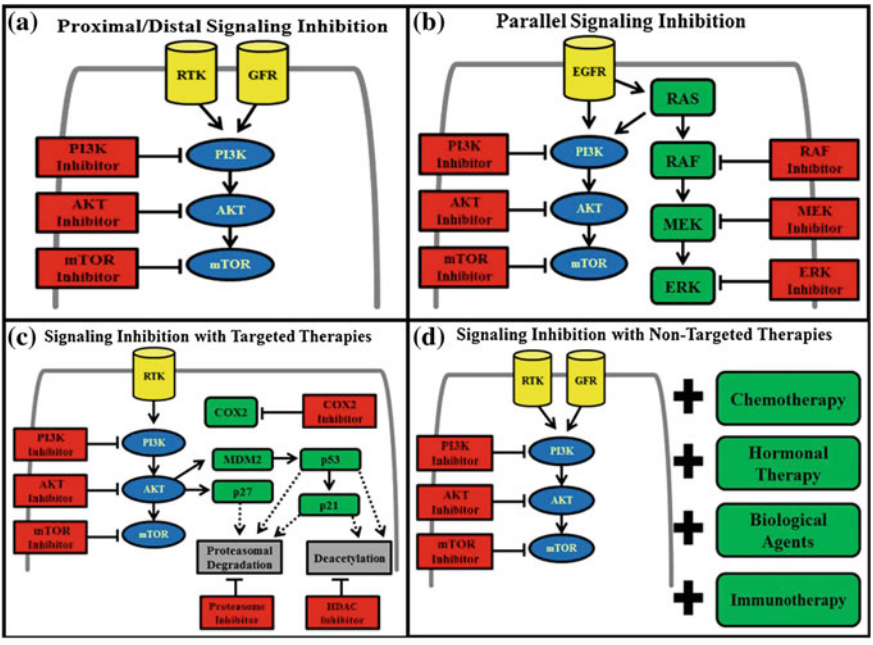


Fig. 6.2 **a** Proximal/distal signaling inhibition; **b** inhibition of two or more signaling pathways in parallel; **c** signaling inhibition and other targeted therapies; and **d** signaling inhibition and combination with nontargeted therapies. Modified from LoPiccolo et al. [84, 92] and used with permission

Here, we discuss seven strategies for overcoming resistance to PI3K/AKT/mTOR inhibition: (1) combination of a targeted agent with chemotherapy, (2) combination of a targeted agent with hormonal therapy, (3) combination of a targeted agent with immunotherapy, (4) combination of a targeted agent with biological therapy, (5) inhibition of both proximal and distal components of the signaling pathway, (6) inhibition of two or more signaling pathways in parallel, and (7) combination of a targeted agent with other targeted therapies [92].

PI3K/AKT/mTOR Inhibitor and Chemotherapy

In vitro, rapamycin and its analogues are synergistic with a variety of chemotherapeutic agents, including paclitaxel, carboplatin, and vinorelbine [93]. In vivo models have shown enhanced antitumor efficacy with the combination of rapalogs and paclitaxel [93]. In a neoadjuvant chemotherapy trial, breast cancer patients with operable triple-negative breast cancer received paclitaxel alone or with everolimus, followed by anthracyclines [94]. Although everolimus treatment indeed decreased mTOR signaling, it did not increase the pathological complete response rate. Other combination trials with rapalogs are ongoing.

Several studies are combining a PI3K/AKT/mTOR pathway signaling inhibitor with cytotoxic chemotherapy, such as XL147 with carboplatin and paclitaxel [21]. Paclitaxel and carboplatin in combination with oral XL147 was well tolerated, with no major pharmacokinetic interactions or emergent toxic effects. Partial responses were observed in patients with prior platinum exposure [95]. Several combinations such as AKT inhibitors and PI3K inhibitors in combination with paclitaxel have shown preliminary evidence of activity and are undergoing further study [96, 97].

PI3K/AKT/mTOR Inhibitor and Hormonal Agents

Estrogen receptor is expressed in approximately 30 and 70 % of premenopausal and postmenopausal patients with breast cancer, respectively, and blocking the estrogen receptor is important in controlling cancer cell proliferation and metastasis [98–100]. Intrinsic resistance to hormonal therapy is seen in approximately 30 % of these patients; the remainder eventually acquires resistance to hormonal therapy, and such resistance is associated with dysregulated PI3K/AKT/mTOR signaling. In fact, hormone resistance can be a consequence of cross-talk between estrogen receptor signaling and the PI3K/AKT/mTOR pathways [54, 101–106]. The estrogen receptor activates tyrosine kinase receptors, activating additional downstream resistance pathways, such as extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) and PI3K/AKT. Adding an mTOR inhibitor may overcome resistance to endocrine therapy and restore antitumor response [107, 108]. Endocrine

therapy-resistant cells become dependent on PI3K/AKT/mTOR signaling for growth and thus become extremely sensitive to PI3/AKT/mTOR inhibition [54, 102, 103]. Moreover, PI3K/AKT/mTOR inhibition restores the sensitivity of endocrine-resistant breast cancer cells to endocrine therapy [109–111]. Letrozole (a nonsteroidal aromatase inhibitor) and everolimus (an mTOR inhibitor) independently inhibit the androstenedione-induced proliferation of aromatase-expressing MCF7/Aro and T47D/Aro breast cancer cells; combined, the effect is significantly augmented. The increased antitumor activity of combined hormonal therapy and PI3K/AKT/mTOR inhibition is associated with more intense effects on G1 progression and a significant decrease in cell viability [110].

Exemestane in combination with everolimus has been FDA approved for hormone receptor-positive metastatic breast cancer based on results from the BOLERO-2 clinical trial [45, 55]. Notably, everolimus also enhanced the antitumor efficacy of tamoxifen in the TAM-RAD trial [112]. Several other PI3K/AKT/mTOR inhibitors are in clinical trials in combination with aromatase inhibitors or fulvestrant. Other possibilities for combining PI3K/AKT/mTOR inhibitors with androgen receptor inhibitors are being considered based on emerging data from experiments showing prolonged hormone sensitivity in prostate cancer xenografts [113] as well as in triple-negative breast cancer.

PI3K/AKT/mTOR Inhibitor and Immunotherapy

Cancer immunotherapy comprises a number of approaches that are intended to activate the immune system to induce objective responses and stabilize the disease [114]. The most promising strategy to boost the patient's natural antitumor response consists of blocking the immunoregulatory mechanisms that brake host responses to tumor; for instance, checkpoint inhibitors such as anti-CTLA-4 and anti-PD-1/anti-PD-L1 decrease T cell tolerance and increase host immune responses to tumor.

Inhibition of PI3K/AKT/mTOR has been used an immunosuppressant for many years [115]. However, mTOR inhibition may have a stimulatory effect on T cells under certain conditions [116]. Temsirolimus boosted the formation of CD8 memory cells following administration of a heat shock protein-based cancer vaccine targeting renal cell carcinoma (RENCA) and melanoma cells. In murine models, a greater cytotoxic T cell response and more interferon gamma were produced by CD8 T cells from mice treated with temsirolimus than by those from mice treated with a heat shock protein-based antitumor vaccine alone [117, 118]. Additionally, PI3K/AKT/mTOR inhibition eliminates tumor pro-survival signals so cytotoxic T lymphocytes can execute tumor lysis via perforin and granzymes. While PI3K/AKT/mTOR inhibitors exert antitumor responses by targeting PI3K pathway activation in tumor cells, they also undesirably exert their effects on normal functioning T cells, resulting in immunosuppression, which may affect the host response

to tumor. Sequencing of therapy may be critical, as inhibition of PI3K/AKT/mTOR pathways expands the population of T regulatory cells if the inhibitor is given prior to an immune stimulus, whereas continuous mTOR inhibition after immune stimulation can impede both T regulatory cells and effector T cells equally [119, 120]. mTOR inhibition promotes T cell anergy under conditions that would normally induce priming in vivo [121]. Notably, PI3K/AKT/mTOR signaling itself may increase expression of PD-L1, and inhibitors of the pathway may downregulate PD-L1 expression [122]. An intriguing possibility is that combining a PI3K/AKT/mTOR inhibitor with immunotherapy could maximize the host response to tumor and antitumor activity while minimizing the autoimmune phenomenon frequently seen with immunotherapy. To maximize antitumor effects, the timing, sequence of administration, and cycling of PI3K/AKT/mTOR inhibitors with immunotherapy need to be explored [121].

The combination of temsirolimus with interferon alpha has been tested in patients with advanced renal cell carcinoma. Although temsirolimus alone improved overall survival in patients with advanced renal cell carcinoma with a poor prognosis compared to patients who received interferon alpha alone, the combination treatment did not further improve survival, and the patients in the combination group experienced more frequent grade 3 or 4 adverse events and subsequent dose delays and reductions [53]. Further understanding of how the interactions between PI3K/AKT/mTOR signaling inhibitors and immunotherapeutic agents affect immunosuppression should be pursued.

PI3K/AKT/mTOR Inhibitor and Biological Therapy

Although the immunotherapies just discussed are one form of biological therapy, in this section we focus on the group of biological therapies that includes therapeutic vaccines, adoptive T cell transfer cytokines, monoclonal antibodies, cancer-killing viruses, and gene therapy. Several clinical trials have combined PI3K/AKT/mTOR signaling inhibitors with biological agents such as bevacizumab. The combination of temsirolimus and bevacizumab has been studied extensively, and the emerging data have yielded various outcomes. In a small phase II study, the combination of temsirolimus and bevacizumab had clinical activity in BRAF wild-type melanoma [123]. However, the combination of temsirolimus and bevacizumab failed to produce meaningful clinical activity in progressive glioblastoma multiforme beyond that of bevacizumab alone [124]. There also have been concerns about toxic effects associated with this combination. In patients with recurrent or persistent endometrial cancer, the combination of temsirolimus and bevacizumab elicited an objective tumor response in 24.5 % of patients and progression-free survival at 6 months in 46.9 % of patients, but this combination was also associated with significant toxic effects [125].

Proximal/Distal Inhibition (mTOR Inhibitor and IGF Receptor [IGFR], PI3K, or AKT Inhibitor)

AKT activation plays a critical role in the PI3K/AKT/mTOR pathways and their downstream effects, such as cancer cell survival, proliferation, and growth [23]. Induction of phosphorylated AKT is thus an unfavorable consequence of mTOR inhibition, potentially limiting antitumor activity with mTOR inhibition [23]. mTOR inhibition induces insulin receptor substrate-1 (IRS-1) expression that can increase sensitivity to IGF pathway signaling and activates AKT in tumors from patients treated with everolimus [126]. An IGF-1R inhibitor can reduce AKT phosphorylation induced by an mTOR inhibitor. Such resistance to mTOR inhibition could potentially be overcome by combining an mTOR inhibitor with an IGFR, PI3K, or AKT inhibitor; a number of these combinations are being explored in clinical trials.

The combination of a novel, potent, and selective inhibitor of the IGF-1R kinase (NVP-AEW541) and an mTOR inhibitor (rapamycin) resulted in additive antiproliferative effects in DU-145, MCF-7, and MDA-MB-468 cancer cells [32]. In patients with Ewing sarcoma and adrenocortical carcinoma, combinations ofmTOR and IGF-1R inhibitors yielded antitumor effects, manifesting as significant tumor reduction, and/or prolonged stable disease [82, 84, 89].

PI3K pathway activation through *PIK3CA* mutations and *PTEN* loss has been associated with trastuzumab resistance [127, 128]. Preclinically, combinations of HER2 and PI3K/AKT/mTOR inhibitors have shown increased antitumor efficacy [129]. BOLERO-3 has demonstrated that the addition of everolimus to vinorelbine and trastuzumab improved progression-free survival compared to vinorelbine and trastuzumab alone; however, although statistically significant, the difference in progression-free survival was modest, limiting the clinical relevance of this effect [130]. Additional studies are ongoing with everolimus in combination with endocrine therapy and trastuzumab, as well as with new PI3K/AKT/mTOR inhibitors in combination with HER2-targeted therapy. The AKT inhibitor MK-2206 augments the antiproliferative effect of everolimus in cholangiocarcinoma cell lines and causes a concomitant reduction in pGSK3β (S9) and pS6 (S240/244), which are signals of AKT activation. The combination of everolimus and MK-2206 was synergistic in vivo. This combination resulted in a significantly greater accumulation of cholangiocarcinoma cells in G0/G1 phase than did either drug alone; however, the combination did not induce apoptosis [131].

AKT inhibitors (ArQ092, AZD5363, BAY1125976, GDC-0068, GSK2141795, LY 2780301, MK-2206, perifosine, RX-0201, PBI-05204, and triciribine) are currently being investigated in clinical trials as single agents or in combination with other mTOR inhibitors or chemotherapy [21].

Ongoing research on dual PI3K/mTOR inhibitors is of interest because of their potential to block PI3K concurrently with signaling downstream of mTORC1. In a panel of six glioma cell lines varying in *PTEN* or *p53* mutational status, inhibition of p110 alpha or p110 beta blocked the activation of AKT; however, only inhibition

of p110 alpha, not p110 beta, blocked the proliferation of glioma cells. Simultaneous inhibition of PI3K alpha and mTORC1 and mTORC2 by PI-103 blocked proliferation without inducing apoptosis [132–135]. Daily treatment with BEZ235 resulted in a dose-dependent reduction in the tumoral expression of p-S6K1 and p-4EBP1 levels in nasopharyngeal cancer xenografts [136]. New agents such as DS-7423, GSK2126458, PF-04691502, PF-05212384, SAR245409 (XL765), and VS-5584 possess dual PI3K- and mTOR-inhibiting properties and are currently being tested as single agents or in combination with other targeted agents or chemotherapy in several clinical trials [21]. Other agents that that target both AKT and S6K1 complexes, including XL418 and MSC2363318A, are being tested in the clinical setting [21].

Catalytic mTOR kinase inhibitors are referred to by some as "dual" mTOR inhibitors because they inhibit mTORC1, the complex containing Raptor, and mTORC2, the complex containing Rictor [137]. These are not identical to the group of proximal/distal inhibitors; however, in some preclinical tests, dual mTOR inhibitors have demonstrated potential to overcome resistance to rapalogs. On one hand, mTORC1 phosphorylates 4EBP1 and S6 kinase, resulting in cell cycle progression. On the other hand, mTORC2 directly phosphorylates and activates the upstream kinase AKT [137, 138]. The negative feedback loop between S6K1 and IRS-1 is impeded by inhibition of mTORC1, resulting in increased PI3K and AKT activity, further limiting the activity of mTORC1 inhibitors [137]. A dual kinase inhibits both the mTORC1 and mTORC2 complexes, and a dual kinase inhibitor controls both S6K1 and AKT; thus, dual kinase inhibitors theoretically provide a broader spectrum of activity than does an mTORC1 inhibitor alone [137, 139, 140]. Several dual kinase inhibitors, such as AZD2014, CC-223, DS-3078a, MLN0128, and Palomid 529, are being tested in clinical trials.

Parallel Signaling Inhibition

Parallel signaling inhibition consists of inhibiting the MAPK pathway and the PI3K pathway simultaneously. The MAPK pathway (also known as the RAS/RAF/MEK/ERK signaling pathway) is one of the best categorized kinase cascades in cancer cell biology [141]. Growth factors and activating mutations of oncogenic kinases are responsible for activation and deregulation of the MAPK pathway, which are common in several cancers [141]. Therefore, kinases of this pathway are promising targets for many antineoplastic therapies, and several agents are being developed to target such mutations. It is important to note, however that the PI3K and RAS/RAF/MEK/ERK signaling pathways interact at multiple levels. For example, mutant *RAS* activates PI3K, *erk* and p90RSK inhibit TSC2, and *AKT* inhibits RAF. Thus, inhibition of the PI3K pathway results in MAPK pathway activation and vice versa [142].

In a wide variety of tumors, *PIK3CA* mutations coexist with *RAS/RAF/MEK/ERK* mutations [143]. Receptor tyrosine kinases and the downstream effector RAS

activate both pathways [144, 145]. The PI3K pathway and the MAPK pathway share common upstream activators in transmembrane tyrosine kinase receptors. However, these receptors have a marked preference for IGF1 and insulin receptors, selectively activating the PI3K, EGFR, and RAS/RAF/MEK/ERK pathways. Thus, the mTOR and MAPK pathways are interconnected by feedback loops, leading to compensatory activation of one in response to inhibition of the other. The inactivation of either the MAPK or PI3K pathway can lead to incomplete tumor growth inhibition. Therefore, simultaneous inhibition of the PI3K/AKT/mTOR and MAPK pathways can theoretically produce more antitumor activity than inhibition of either pathway alone.

In vitro evidence supports dual inhibition of mTOR and MAPK pathways. For example, dual activation of the PI3K and MAPK pathways in some rhabdomyosarcoma cell lines yielded a synergistic antiproliferative effect. Specifically, the combination of the mTOR dual kinase inhibitor AZD8055 and the MEK1/2 inhibitor selumetinib was synergistic in the RH-30, RD, and RMS-YM rhabdomyosarcoma cell lines [146].

In both *PTEN*-mutant and wild-type cells, parallel inhibition of the PI3K/AKT/mTOR and MAPK pathways can lead to synergistic increases in apoptosis [92, 147]. Several clinical trials are combining the Raf (multikinase) inhibitor sorafenib with temsirolimus. Some trials have yielded disappointing results; for example, in patients with glioblastoma or gliosarcoma, increased toxic effects and minimal clinical activities were observed [148]. Furthermore, patients with hepatocellular carcinoma had a lower maximum tolerated dose than patients with other types of cancer. In addition, further delineation of the superiority of the combination over single-agent sorafenib is needed in different subsets of the hepatocellular carcinoma population [149]. In vivo studies have shown marked synergies when PI3K/AKT/mTOR inhibitors are combined with MEK inhibitors in *KRAS*-mutant cancers [150]. While the addition of a MEK inhibitor is appropriate for enhancing the efficacy of mTOR-targeted anticancer therapy, the combination's toxicity may be a barrier to combining effective doses of each drug [151]. However, there are several trials ongoing to test the combination of PI3K/AKT/mTOR and MEK inhibitors, testing a variety of combinations, doses, and schedules.

PI3K/AKT/mTOR Inhibitor and Other Targeted Therapies

PI3K/AKT/mTOR inhibitors can be combined with several different targeted agents to enhance efficacy and overcome resistance to single-agent therapy.

Metformin is the most commonly prescribed antidiabetic oral agent. Combining metformin with an mTOR inhibitor is attractive because metformin appears to inhibit the mTOR pathway by a different mechanism than that used by the mTOR inhibitors developed so far [152, 153]. That is, in preclinical models, metformin inhibits the mTOR pathway through upstream, albeit indirect, activation of *AMPK* [153]. This activation causes an inhibitory site of IRS-1 to be phosphorylated,

subsequently decreasing *AKT* activation, *mTOR* activation, and feedback inhibition [153]. In addition to acting through *AMPK*-dependent mechanisms, metformin enhances the effect of PI3K/AKT/mTOR signaling inhibition via *AMPK*-independent mechanisms. Metformin's ability to induce mTOR inhibition and cell cycle arrest through *REDD1* (regulated in development and DNA damage responses 1, a negative regulator of mTOR) in androgen-sensitive human prostate adenocarcinoma cells is independent of *AMPK* [154]. In fact, metformin upregulates *REDD1*, leading to an antiproliferative effect. However, metformin's action via *TSC2* of exerting an inhibitory effect on the PI3K/AKT/mTOR pathway is *AMPK* dependent [155]. Metformin improves hyperglycemia caused by mTOR inhibitors [73, 156]. Another example of metformin's potential is in endometrial cancers. Progesterone normally limits the proliferation and growth of endometrial cells. IGF-1 inhibits the progesterone receptor via the PI3K/AKT/mTOR pathway [157, 158]. Metformin promotes progestin receptor expression via inhibition of mTOR in endometrial cell lines; therefore, the combination of metformin with an mTOR inhibitor is being investigated as a potential therapeutic option for endometrial carcinoma [73, 159].

mTOR inhibitors may also play an important role in overcoming resistance to other drugs, such as anti-epidermal growth factor receptor (EGFR) and anti-vascular endothelial growth factor (VEGF) agents [91].

The inhibition of the mTOR pathway has been proven to exhibit antiangiogenic effects, further strengthening the capabilities of angiogenesis inhibitors, such as bevacizumab and aflibercept [160]. The angiogenesis signaling pathway might be triggered by the release of angiogenic promoters such as VEGF from tumor cells into the local microenvironment [125]. VEGF is largely upregulated by the presence of hypoxia-inducible factor 1α (HIF-1α). HIF-1α mediates adaptive responses to hypoxic conditions. The increase in HIF-1α that occurs in response to a hypoxic environment is critical to the establishment and progression of many common cancers. The HIF-1-dependent activation of genes allows cancer cells to survive and metastasize. Increased HIF-1α is associated with increased expression of VEGF, aggressive tumor growth, and poor patient prognosis. Thus, HIF-1α inhibition in combination with antiangiogenic therapy is a promising strategy for targeting tumor resistance. Agents that inhibit the mTOR pathway have been shown to inhibit the activity of several angiogenic factors, including HIF-1α, which in turn results in decreased VEGF and then decreased angiogenic activity [161]. In a phase III study of everolimus in pancreatic neuroendocrine tumors, it was demonstrated that mTOR inhibition may reduce circulating levels of soluble VEGF receptor-1, placental growth factor, and basic fibroblast growth factor [162]. mTOR inhibitors can improve the antiangiogenic activity of anti-VEGF therapies through inhibiting endothelial cell function in neoangiogenesis and inhibiting VEGF production induced by HIF-1α [163]. Our group recently reported that 37 % of heavily pretreated patients with gynecologic malignancies achieved stable disease for more than 6 months or a partial response with the combination of bevacizumab and temsirolimus [164].

EGFR inhibitors may be able to potentiate PI3K/AKT/mTOR signaling; combining PI3K/AKT/mTOR and EGFR inhibitors has the reciprocal benefit of

improving each agent's resistance to the other. Cancer cell lines exposed to mTOR inhibition have demonstrated increased EGFR/RAS/RAF/ERK/MEK pathway signaling [165]. In return, increased activity of the IGF pathway and of its downstream effectors—PI3K, AKT, and mTOR—was observed in glioma cell lines resistant to EGFR tyrosine kinase inhibitors [166]. Therefore, it was logical to find that co-inhibition of the EGFR and PI3K/AKT/mTOR pathways repressed tumor cells that were resistant to small-molecule inhibitors of EGFR [166–168]. In a similar analysis, everolimus caused an efficient dose-dependent inhibition of in vitro growth of human colon cancer GEO cell lines, with IC_{50} values ranging between 1 and 5 mM, regardless of the cells' degree of sensitivity to EGFR inhibitors and *PTEN* status [169]. The combination of PI3K/AKT/mTOR and EGFR inhibitors thus offers a tactic for overcoming cellular resistance to either drug. In a pilot study of the combination of erlotinib and sirolimus in recurrent malignant glioma, the regimen was well tolerated, and 19 % of patients had a partial response, with 25 % experiencing 6 month progression-free survival [170].

A recent study has shown that cell lines that are sensitive to PI3K inhibitors demonstrate suppression of Rb phosphorylation upon treatment with single-agent inhibitors, while those resistant to PI3K inhibitors do not [171]. Drug screens in *PIK3CA*-mutant cancer cell lines demonstrated synergy between PI3K inhibitors and cyclin-dependent kinase 4/6 inhibitors in vitro. Furthermore, the combination of PI3K inhibitors and cyclin-dependent kinase 4/6 inhibitors had antitumor efficacy in several in vivo xenograft models. The combination of PI3K/AKT/mTOR inhibitors and cyclin-dependent kinase 4/6 inhibitors is now being pursued in clinical trials.

There has been increasing interest in leveraging homologous recombination defects for cancer therapy; this has already led to several clinical trials with PARP inhibitors, especially in breast and ovarian cancer patients who carry deleterious *BRCA1/2* mutations. Interestingly, transgenic models of *BRCA*-mutant mice demonstrated that the combination of PI3K inhibitors with PARP inhibitors showed enhanced antitumor efficacy [171]. Also notably, patient-derived xenografts from patients with triple-negative breast cancer without deleterious germline *BRCA* mutations responded more to the combination of PI3K inhibitors and PARP inhibitors than to either agent alone [172, 173]. Recently, a phase I trial of the combination of the PI3K inhibitor BKM120 and the PARP inhibitor olaparib was shown to be tolerable and to have promising antitumor efficacy [174].

Triple Combinations

In addition to combinations of two agents, combinations of three agents have been actively studied. Our group previously reported a study of a combination of liposomal doxorubicin, bevacizumab, and temsirolimus in patients with advanced gynecologic and breast malignancies [175]. This combination was well tolerated, and a greater number of responses were seen in patients with *PIK3CA* mutations or

PTEN loss than in patients without these mutations. Furthermore, this combination was effective in metaplastic breast cancer [176], which has a high frequency of *PIK3CA* mutations. Our group has also reported results of the combination of sorafenib, temsirolimus, and bevacizumab in patients with advanced cancer. The combination was well tolerated, and antitumor activity was seen in various patients with ovarian cancer, colorectal cancer, endometrial cancer, leiomyosarcoma, and squamous lung cancer [177].

Biomarkers

Predictors of response: Currently, there are no validated biomarkers that can predict responses to PI3/AKT/mTOR inhibitors in clinical settings. One of the difficulties to establish biomarker is mutations of PI3/AKT/mTOR pathways are observed in heterogeneous tumor types. In addition, mutations of PI3K/AKT/mTOR coexist with other pathways mutations [178, 179].

In vitro studies have found associations between PIK3CA/PTEN mutations and rapamycin sensitivity [33]. However, *PIK3CA* mutations did not have a significant association with sensitivity to everolimus plus exemestane in the randomized BOLERO-2 trial [180]. In FERGI trial, addition of PI3K inhibitor pictilisib (Genentech, Inc) to the endocrine therapy fulvestrant (Faslodex, AstraZeneca Pharmaceuticals LP) restored sensitivity to fulvestrant and modestly improved progression-free survival. However, it did not show enhanced efficacy in PIK3CA mutant patients [181].

There have been reports of inactivating mutations in TSC1 and TSC2 in exceptional responders to everolimus in bladder cancer and anaplastic thyroid cancer, and activating mutations in mTOR in a patient with urothelial carcinoma. With an exceptional response to everolimus and pazopanib [69, 182, 183]. Both TSC1 and mTOR mutations are relatively rare thus unlikely to account for most of the rapalog sensitivity. However, these potential associations may allow for future personalized treatment selections in patients when these alterations are identified.

Proteomic predictors of response: A few immunohostochemical biomarkers have been investigated such as PTEN loss, p-AKT, and p-S6K. PTEN loss has been extensively studied [184]. Loss of tumor suppressor *PTEN* subsequently results in activation of downstream signaling *AKT* and *mTOR*. While poor prognosis is associated with PTEN loss in patients with renal cell carcinoma, there was increased sensitivity to temsirolimus in patients with *PTEN* loss in a small study [185–187]. In the BOLERO 3 trial which is a randomized, double-blind, placebo-controlled, phase 3 trial, for women with HER2-positive, trastuzumab-resistant, advanced breast carcinoma with everolimus vs placebo combined with trastuzumab and vinorelbine, PTEN loss by immunohistochemistry was significantly associated with benefit from the addition of everolimus to chemotherapy (HR 0,4, CI 0.2–0.82, $p = 0.01$) [130].

One compelling thought is to identify a proteomic marker of pathway activation as a predictor of response. In vitro, baseline phospho AKT may be a marker of sensitivity but this is a biomarker that has been clinically difficult to implement [33]. This is at least in part due the fact that phospho-residues are relatively unstable, and there are differences even between phospho-AKT status between in vivo biopsies and surgical excisions of the same samples. Thus obtaining fresh biopsies, and identifying markers which are more stable may be necessary to better implement these markers. Notably, in the BOLERO-3 trial, patients with higher pS6 levels in archival tissue had greater benefit from the addition on everolimus to trastuzumab and vinorelbine (HR 0.48, CI 0.24–0.96; $p = 0.04$) [130],

Pharmacodynamic markers of response: Inhibition of p-4E-BP1, p-S6K, and PS6 has been monitored in many studies to ensure pathway inhibition. Although it seems intuitive that the pathway needs to be inhibited for antitumor efficacy, it is yet to proven that reduction in phospho 4EBP1 and phospho s6K is associated with improved clinical outcome. [178]

Since mTOR inhibitions can result in upregulation of *AKT* phosphorylation in the tumors, this has been proposed as a mechanism that limits the antitumor efficacy of rapalogs. [7, 9] However, in vitro cell lines that are rapamycin sensitive have greater increase in p-AKT levels with treatment, suggesting this feedback loop activation is not of value as a predictor of resistance. increased phospho AKT [33].

Mechanisms of Acquired Resistance: Temsirolimus and everolimus have been in clinical use for several years and there is also much known about mechanisms of acquired resistance. However, continuous treatment of rapamycin sensitive breast cancer cell lines was associated with an acquired mTOR mutation (S2035F) in the FKBP12-rapamycin binding domain. In this cell line with acquired resistance to rapamycin, mTORC1 signaling was not inhibited by rapalogs but was inhibited with catalytic inhibitors [188]. Similarly an anaplastic thyroid cancer patient was found to harbor a mutation in MTOR that confers resistance to allosteric mTOR inhibition upon development of resistance to everolimus [182, 183]. Thus suggests that novel genomic alterations may be at least one mechanism of resistance to raplogs and genomic profiling may help select additional therapeutic options which may include other PI3K/AKT/mTOR inhibitors.

Conclusion

Mutation, amplification, and rearrangement of genes within the PI3K/AKT/mTOR pathways are frequently seen in multiple cancer lineages. Targeting mutant *PI3K*, *AKT*, and/or *mTOR* is an area of active research. While significant clinical responses have been obtained by targeting PI3K/AKT/mTOR signaling, there is still a need to improve the efficacy of PI3K/AKT/mTOR signaling inhibition. Combining PI3K/AKT/mTOR inhibitors with different agents is required to achieve this goal. Furthermore, PI3K/AKT/mTOR inhibition can improve the therapeutic effect of other antineoplastic agents, such as anti-EGFR and anti-VEGF therapies.

By evaluating biomarkers of response, the efficacy of PI3K/AKT/mTOR inhibitors themselves and their rational combination with other therapeutic agents should be improved over time.

While combination therapies theoretically offer better efficacy than single-inhibitor treatments, they come hand-in-hand with the risk of greater toxic effects. Optimization of dose and schedule and maintenance of an appropriate sequence of drug administration are critical. Optimizing the management of side effects is absolutely necessary [87]. Whenever possible, conducting early trials with new agents or new combinations that include tumor biopsies at baseline, during treatment, and at the time of progression can give insights into target engagement, relative pathway inhibition, and adaptive responses. High-throughput molecular profiling including Next-generation gene sequencing at baseline and at progression can give insights into the mechanism(s) of intrinsic sensitivity/resistance and mechanisms of acquired resistance to PI3K/AKT/mTOR inhibitors [189]. Further, refinement in patient selection and better understanding of rational combinations are needed to optimally incorporate PI3K/Akt/mTOR inhibitors into our clinical armamentarium.

References

1. Fruman DA, Meyers RE, Cantley LC (1998) Phosphoinositide kinases. Annu Rev Biochem 67:481–507
2. Cantley LC (2002) The phosphoinositide 3-kinase pathway. Science 296(5573):1655–1657
3. Laplante M, Sabatini DM (2012) mTOR signaling in growth control and disease. Cell 149 (2):274–293
4. Weichhart T, Saemann MD (2009) The multiple facets of mTOR in immunity. Trends Immunol 30(5):218–226
5. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB (2005) Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat Rev Drug Discovery 4(12):988–1004
6. Meric-Bernstam F, Mills GB (2004) Mammalian target of rapamycin. Semin Oncol 31(6 Suppl 16):10-7; discussion 33
7. Wan X, Harkavy B, Shen N, Grohar P, Helman LJ (2007) Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. Oncogene 26 (13):1932–1940
8. Liu P, Cheng H, Roberts TM, Zhao JJ (2009) Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov 8(8):627–644
9. Shi Y, Yan H, Frost P, Gera J, Lichtenstein A (2005) Mammalian target of rapamycin inhibitors activate the AKT kinase in multiple myeloma cells by up-regulating the insulin-like growth factor receptor/insulin receptor substrate-1/phosphatidylinositol 3-kinase cascade. Mol Cancer Ther 4(10):1533–1540
10. Yap TA, Garrett MD, Walton MI, Raynaud F, de Bono JS, Workman P (2008) Targeting the PI3K-AKT-mTOR pathway: progress, pitfalls, and promises. Curr Opin Pharmacol 8 (4):393–412
11. Carnero A, Blanco-Aparicio C, Renner O, Link W, Leal JF (2008) The PTEN/PI3K/AKT signalling pathway in cancer, therapeutic implications. Curr Cancer Drug Targets 8 (3):187–198

12. Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 7(8):606–619
13. Lee JW, Soung YH, Kim SY, Lee HW, Park WS, Nam SW, Kim SH, Lee JY, Yoo NJ, Lee SH (2005) PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. Oncogene 24(8):1477–1480
14. Geering B, Cutillas PR, Vanhaesebroeck B (2007) Regulation of class IA PI3Ks: is there a role for monomeric PI3K subunits? Biochem Soc Trans 35(Pt 2):199–203
15. Samuels Y, Velculescu VE (2004) Oncogenic mutations of PIK3CA in human cancers. Cell Cycle 3(10):1221–1224
16. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) High frequency of mutations of the PIK3CA gene in human cancers. Science 304(5670):554
17. Falasca M, Maffucci T (2012) Regulation and cellular functions of class II phosphoinositide 3-kinases. Biochem J 443(3):587–601
18. Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, Bilanges B (2010) The emerging mechanisms of isoform-specific PI3K signalling. Nat Rev Mol Cell Biol 11(5):329–341
19. Jaber N, Dou Z, Chen JS, Catanzaro J, Jiang YP, Ballou LM, Selinger E, Ouyang X, Lin RZ, Zhang J, Zong WX (2012) Class III PI3K Vps34 plays an essential role in autophagy and in heart and liver function. Proc Natl Acad Sci USA 109(6):2003–2008
20. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C, Schultz N (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2(5):401–404
21. ClinicalTrials.gov. Available from URL http://clinicaltrials.gov/
22. Brugge J, Hung MC, Mills GB (2007) A new mutational AKTivation in the PI3K pathway. Cancer Cell 12(2):104–107
23. Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. Cell 129 (7):1261–1274
24. Nakatani K, Sakaue H, Thompson DA, Weigel RJ, Roth RA (1999) Identification of a human Akt3 (protein kinase B gamma) which contains the regulatory serine phosphorylation site. Biochem Biophys Res Commun 257(3):906–910
25. Nakatani K, Thompson DA, Barthel A, Sakaue H, Liu W, Weigel RJ, Roth RA (1999) Up-regulation of Akt3 in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer lines. J Biol Chem 274(31):21528–21532
26. Miwa W, Yasuda J, Murakami Y, Yashima K, Sugano K, Sekine T, Kono A, Egawa S, Yamaguchi K, Hayashizaki Y, Sekiya T (1996) Isolation of DNA sequences amplified at chromosome 19q13.1-q13.2 including the AKT2 locus in human pancreatic cancer. Biochem Biophys Res Commun 225(3):968–974
27. Bellacosa A, de Feo D, Godwin AK, Bell DW, Cheng JQ, Altomare DA, Wan M, Dubeau L, Scambia G, Masciullo V, Ferrandina G, Benedetti Panici P, Mancuso S, Neri G, Testa JR (1995) Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. Int J Cancer 64(4):280–285
28. Coffer PJ, Jin J, Woodgett JR (1998) Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. Biochem J 335(Pt 1):1–13
29. Cheng JQ, Godwin AK, Bellacosa A, Taguchi T, Franke TF, Hamilton TC, Tsichlis PN, Testa JR (1992) AKT2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. Proc Natl Acad Sci USA 89(19):9267–9271
30. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB (2005) Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat Rev Drug Discov 4(12):988–1004
31. Davies MA, Stemke-Hale K, Tellez C, Calderone TL, Deng W, Prieto VG, Lazar AJ, Gershenwald JE, Mills GB (2008) A novel AKT3 mutation in melanoma tumours and cell lines. Br J Cancer 99(8):1265–1268

32. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin DJ, Ludwig DL, Baselga J, Rosen N (2006) mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 66(3):1500–1508
33. Meric-Bernstam F, Akcakanat A, Chen H, Do KA, Sangai T, Adkins F, Gonzalez-Angulo AM, Rashid A, Crosby K, Dong M, Phan AT, Wolff RA, Gupta S, Mills GB, Yao J (2012) PIK3CA/PTEN mutations and Akt activation as markers of sensitivity to allosteric mTOR inhibitors. Clin Cancer Res 18(6):1777–1789
34. Harris TE, Lawrence JC Jr (2003) TOR signaling. Sci Signal 2003(212):re15
35. Hassan B, Akcakanat A, Holder AM, Meric-Bernstam F (2013) Targeting the PI3-kinase/Akt/mTOR signaling pathway. Surg Oncol Clin N Am 22(4):641–664
36. Lemmon MA, Schlessinger J (2010) Cell signaling by receptor tyrosine kinases. Cell 141 (7):1117–1134
37. Vivanco I, Sawyers CL (2002) The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2(7):489–501
38. Vignot S, Faivre S, Aguirre D, Raymond E (2005) mTOR-targeted therapy of cancer with rapamycin derivatives. Ann Oncol 16(4):525–537
39. Strimpakos AS, Karapanagiotou EM, Saif MW, Syrigos KN (2009) The role of mTOR in the management of solid tumors: an overview. Cancer Treat Rev 35(2):148–159
40. Hay N (2005) The Akt-mTOR tango and its relevance to cancer. Cancer Cell 8(3):179–183
41. Akcakanat A, Singh G, Hung MC, Meric-Bernstam F (2007) Rapamycin regulates the phosphorylation of rictor. Biochem Biophys Res Commun 362(2):330–333
42. Huang J, Manning BD (2009) A complex interplay between Akt, TSC2 and the two mTOR complexes. Biochem Soc Trans 37(Pt 1):217–222
43. MacDonald AS (2001) A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. Transplantation 71(2):271–280
44. Kwitkowski VE, Prowell TM, Ibrahim A, Farrell AT, Justice R, Mitchell SS, Sridhara R, Pazdur R (2010) FDA approval summary: temsirolimus as treatment for advanced renal cell carcinoma. Oncologist 15(4):428–435
45. Beaver JA, Park BH (2012) The BOLERO-2 trial: the addition of everolimus to exemestane in the treatment of postmenopausal hormone receptor-positive advanced breast cancer. Future Oncol 8(6):651–657
46. Faivre S, Kroemer G, Raymond E (2006) Current development of mTOR inhibitors as anticancer agents. Nat Rev Drug Discov 5(8):671–688
47. Sedrani R, Cottens S, Kallen J, Schuler W (1998) Chemical modification of rapamycin: the discovery of SDZ RAD. Transplant Proc 30(5):2192–2194
48. Schuurman HJ, Cottens S, Fuchs S, Joergensen J, Meerloo T, Sedrani R, Tanner M, Zenke G, Schuler W (1997) SDZ RAD, a new rapamycin derivative: synergism with cyclosporine. Transplantation 64(1):32–35
49. Schuler W, Sedrani R, Cottens S, Haberlin B, Schulz M, Schuurman HJ, Zenke G, Zerwes HG, Schreier MH (1997) SDZ RAD, a new rapamycin derivative: pharmacological properties in vitro and in vivo. Transplantation 64(1):36–42
50. Staehler M, Haseke N, Khoder W, Stief CG (2010) Profile of temsirolimus in the treatment of advanced renal cell carcinoma. Onco Targets Ther 3:191–196
51. Sehgal SN, Molnar-Kimber K, Ocain TD, Weichman BM (1994) Rapamycin: a novel immunosuppressive macrolide. Med Res Rev 14(1):1–22
52. Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS (1997) A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. FK506 Kidney Transplant Study Group. Transplantation 63(7):977–983
53. Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, Staroslawska E, Sosman J, McDermott D, Bodrogi I, Kovacevic Z, Lesovoy V, Schmidt-Wolf IG, Barbarash O, Gokmen E, O'Toole T, Lustgarten S, Moore L, Motzer RJ (2007) Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 356(22):2271–2281

54. Santen RJ, Song RX, Zhang Z, Kumar R, Jeng MH, Masamura S, Lawrence J Jr, MacMahon LP, Yue W, Berstein L (2005) Adaptive hypersensitivity to estrogen: mechanisms and clinical relevance to aromatase inhibitor therapy in breast cancer treatment. J Steroid Biochem Mol Biol 95(1–5):155–165
55. Baselga J, Campone M, Piccart M, Burris HA 3rd, Rugo HS, Sahmoud T, Noguchi S, Gnant M, Pritchard KI, Lebrun F, Beck JT, Ito Y, Yardley D, Deleu I, Perez A, Bachelot T, Vittori L, Xu Z, Mukhopadhyay P, Lebwohl D, Hortobagyi GN (2012) Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 366 (6):520–529
56. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, Grunwald V, Thompson JA, Figlin RA, Hollaender N, Urbanowitz G, Berg WJ, Kay A, Lebwohl D, Ravaud A (2008) Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 372(9637):449–456
57. Yao JC, Phan AT, Chang DZ, Wolff RA, Hess K, Gupta S, Jacobs C, Mares JE, Landgraf AN, Rashid A, Meric-Bernstam F (2008) Efficacy of RAD001 (everolimus) and octreotide LAR in advanced low- to intermediate-grade neuroendocrine tumors: results of a phase II study. J Clin Oncol 26(26):4311–4318
58. Yao JC, Lombard-Bohas C, Baudin E, Kvols LK, Rougier P, Ruszniewski P, Hoosen S, Peter JS, Haas T, Lebwohl D (2010) Daily oral everolimus activity in patients with metastatic pancreatic neuroendocrine tumors after failure of cytotoxic chemotherapy: a phase II trial. J Clin Oncol 28(1):69–76
59. Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, Tomassetti P, Pavel ME, Hoosen S, Haas T, Lincy J, Lebwohl D, Oberg K (2011) Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med 364(6):514–523
60. Rivera VM, Squillace RM, Miller D, Berk L, Wardwell SD, Ning Y, Pollock R, Narasimhan NI, Iuliucci JD, Wang F, Clackson T (2011) Ridaforolimus (AP23573; MK-8669), a potent mTOR inhibitor, has broad antitumor activity and can be optimally administered using intermittent dosing regimens. Mol Cancer Ther 10(6):1059–1071
61. Berk L, Mita MM, Kreisberg J, Bedrosian CL, Tolcher AW, Clackson T, Rivera VM (2012) Analysis of the pharmacodynamic activity of the mTOR inhibitor ridaforolimus (AP23573, MK-8669) in a phase 1 clinical trial. Cancer Chemother Pharmacol 69(5):1369–1377
62. Mita M, Sankhala K, Abdel-Karim I, Mita A, Giles F (2008) Deforolimus (AP23573) a novel mTOR inhibitor in clinical development. Expert Opin Investig Drugs 17(12):1947–1954
63. Mita MM, Poplin E, Britten CD, Tap WD, Rubin EH, Scott BB, Berk L, Rivera VM, Loewy JW, Dodion P, Haluska F, Sarantopoulos J, Mita A, Tolcher A (2013) Phase I/IIa trial of the mammalian target of rapamycin inhibitor ridaforolimus (AP23573; MK-8669) administered orally in patients with refractory or advanced malignancies and sarcoma. Ann Oncol 24(4):1104–1111
64. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DH, Tavtigian SV (1997) Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 15(4):356–362
65. Crino PB, Nathanson KL, Henske EP (2006) The tuberous sclerosis complex. N Engl J Med 355(13):1345–1356
66. Inoki K, Corradetti MN, Guan KL (2005) Dysregulation of the TSC-mTOR pathway in human disease. Nat Genet 37(1):19–24
67. Chong-Kopera H, Inoki K, Li Y, Zhu T, Garcia-Gonzalo FR, Rosa JL, Guan KL (2006) TSC1 stabilizes TSC2 by inhibiting the interaction between TSC2 and the HERC1 ubiquitin ligase. J Biol Chem 281(13):8313–8316
68. Inoki K, Li Y, Xu T, Guan KL (2003) Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev 17(15):1829–1834
69. Iyer G, Hanrahan AJ, Milowsky MI, Al-Ahmadie H, Scott SN, Janakiraman M, Pirun M, Sander C, Socci ND, Ostrovnaya I, Viale A, Heguy A, Peng L, Chan TA, Bochner B,

Bajorin DF, Berger MF, Taylor BS, Solit DB (2012) Genome sequencing identifies a basis for everolimus sensitivity. Science 338(6104):221
70. Hardie DG (2007) AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat Rev Mol Cell Biol 8(10):774–785
71. Zhuang Y, Miskimins WK (2008) Cell cycle arrest in Metformin treated breast cancer cells involves activation of AMPK, downregulation of cyclin D1, and requires p27Kip1 or p21Cip1. J Mol Signal 3:18
72. Rozengurt E, Sinnett-Smith J, Kisfalvi K (2010) Crosstalk between insulin/insulin-like growth factor-1 receptors and G protein-coupled receptor signaling systems: a novel target for the antidiabetic drug metformin in pancreatic cancer. Clin Cancer Res 16(9):2505–2511
73. Hajjar J, Habra MA, Naing A (2013) Metformin: an old drug with new potential. Expert Opin Investig Drugs 22(12):1511–1517
74. Dancey JE (2002) Agents targeting ras signaling pathway. Curr Pharm Des 8(25):2259–2267
75. Gridelli C, Maione P, Rossi A (2008) The potential role of mTOR inhibitors in non-small cell lung cancer. Oncologist 13(2):139–147
76. Soria JC, Shepherd FA, Douillard JY, Wolf J, Giaccone G, Crino L, Cappuzzo F, Sharma S, Gross SH, Dimitrijevic S, Di Scala L, Gardner H, Nogova L, Papadimitrakopoulou V (2009) Efficacy of everolimus (RAD001) in patients with advanced NSCLC previously treated with chemotherapy alone or with chemotherapy and EGFR inhibitors. Ann Oncol 20(10): 1674–1681
77. Sonis S, Treister N, Chawla S, Demetri G, Haluska F (2010) Preliminary characterization of oral lesions associated with inhibitors of mammalian target of rapamycin in cancer patients. Cancer 116(1):210–215
78. Liu X, Lorusso P, Mita M, Piha-Paul S, Hong DS, Fu S, McQuinn L, Asatiani E, Doyle LA, Chen HX, Hess KR, Kurzrock R, Naing A (2014) Incidence of mucositis in patients treated with temsirolimus-based regimens and correlation to treatment response. Oncologist 19 (4):426–428
79. Yap TA, Yan L, Patnaik A, Fearen I, Olmos D, Papadopoulos K, Baird RD, Delgado L, Taylor A, Lupinacci L, Riisnaes R, Pope LL, Heaton SP, Thomas G, Garrett MD, Sullivan DM, de Bono JS, Tolcher AW (2011) First-in-man clinical trial of the oral pan-AKT inhibitor MK-2206 in patients with advanced solid tumors. J Clin Oncol 29(35):4688–4695
80. Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, Demanse D, De Buck SS, Ru QC, Peters M, Goldbrunner M, Baselga J (2012) Phase I, dose-escalation study of BKM120, an oral pan-Class I PI3K inhibitor, in patients with advanced solid tumors. J Clin Oncol 30(3):282–290
81. Naing A, Lorusso P, Fu S, Hong D, Chen HX, Doyle LA, Phan AT, Habra MA, Kurzrock R (2013) Insulin growth factor receptor (IGF-1R) antibody cixutumumab combined with the mTOR inhibitor temsirolimus in patients with metastatic adrenocortical carcinoma. Br J Cancer 108(4):826–830
82. Naing A, LoRusso P, Fu S, Hong DS, Anderson P, Benjamin RS, Ludwig J, Chen HX, Doyle LA, Kurzrock R (2012) Insulin growth factor-receptor (IGF-1R) antibody cixutumumab combined with the mTOR inhibitor temsirolimus in patients with refractory Ewing's sarcoma family tumors. Clin Cancer Res 18(9):2625–2631
83. Busaidy NL, Farooki A, Dowlati A, Perentesis JP, Dancey JE, Doyle LA, Brell JM, Siu LL (2012) Management of metabolic effects associated with anticancer agents targeting the PI3K-Akt-mTOR pathway. J Clin Oncol 30(23):2919–2928
84. Naing A, Kurzrock R, Burger A, Gupta S, Lei X, Busaidy N, Hong D, Chen HX, Doyle LA, Heilbrun LK, Rohren E, Ng C, Chandhasin C, LoRusso P (2011) Phase I trial of cixutumumab combined with temsirolimus in patients with advanced cancer. Clin Cancer Res 17(18):6052–6060
85. Hidalgo M, Buckner JC, Erlichman C, Pollack MS, Boni JP, Dukart G, Marshall B, Speicher L, Moore L, Rowinsky EK (2006) A phase I and pharmacokinetic study of temsirolimus (CCI-779) administered intravenously daily for 5 days every 2 weeks to patients with advanced cancer. Clin Cancer Res 12(19):5755–5763

86. Iacovelli R, Palazzo A, Mezi S, Morano F, Naso G, Cortesi E (2012) Incidence and risk of pulmonary toxicity in patients treated with mTOR inhibitors for malignancy. A meta-analysis of published trials. Acta Oncol 51(7):873–879
87. Naing A (2013) Overcoming resistance to mTOR inhibition for enhanced strategies in clinical trials. Expert Opin Investig Drugs
88. Fouladi M, Perentesis JP, Phillips CL, Leary S, Reid JM, McGovern RM, Ingle AM, Ahern CH, Ames MM, Houghton P, Doyle LA, Weigel B, Blaney SM (2014) A phase I trial of MK-2206 in children with refractory malignancies: a Children's Oncology Group study. Pediatr Blood Cancer 61(7):1246–1251
89. Naing A, LoRusso P, Fu S, Hong D, Chen HX, Doyle LA, Phan AT, Habra MA, Kurzrock R (2013) Insulin growth factor receptor (IGF-1R) antibody cixutumumab combined with the mTOR inhibitor temsirolimus in patients with metastatic adrenocortical carcinoma. Br J Cancer 108(4):826–830
90. Dancey J (2010) mTOR signaling and drug development in cancer. Nat Rev Clin Oncol 7 (4):209–219
91. Naing A (2013) Overcoming resistance to mTOR inhibition for enhanced strategies in clinical trials. Expert Opin Investig Drugs 22(6):679–685
92. LoPiccolo J, Blumenthal GM, Bernstein WB, Dennis PA (2008) Targeting the PI3K/Akt/mTOR pathway: effective combinations and clinical considerations. Drug resistance updates: reviews and commentaries in antimicrobial and anticancer chemotherapy 11(1–2):32
93. Mondesire WH, Jian W, Zhang H, Ensor J, Hung MC, Mills GB, Meric-Bernstam F (2004) Targeting mammalian target of rapamycin synergistically enhances chemotherapy-induced cytotoxicity in breast cancer cells. Clin Cancer Res 10(20):7031–7042
94. Gonzalez-Angulo AM, Akcakanat A, Liu S, Green MC, Murray JL, Chen H, Palla SL, Koenig KB, Brewster AM, Valero V, Ibrahim NK, Moulder-Thompson S, Litton JK, Tarco E, Moore J, Flores P, Crawford D, Dryden MJ, Symmans WF, Sahin A, Giordano SH, Pusztai L, Do KA, Mills GB, Hortobagyi GN, Meric-Bernstam F (2014) Open-label randomized clinical trial of standard neoadjuvant chemotherapy with paclitaxel followed by FEC versus the combination of paclitaxel and everolimus followed by FEC in women with triple receptor-negative breast cancerdagger. Ann Oncol 25(6):1122–1127
95. Traynor AM KR, Bailey HH, Attia S, Scheffold C, van Leeuwen B, Wu B, Falchook GS, Moulder SL, Wheler J (2010) A phase I safety and pharmacokinetic (PK) study of the PI3K inhibitor XL147 (SAR245408) in combination with paclitaxel (P) and carboplatin (C) in patients (pts) with advanced solid tumors. J Clin Oncol 28(suppl):abstr 3078
96. Sangai T, Akcakanat A, Chen H, Tarco E, Wu Y, Do KA, Miller TW, Arteaga CL, Mills GB, Gonzalez-Angulo AM, Meric-Bernstam F (2012) Biomarkers of response to Akt inhibitor MK-2206 in breast cancer. Clin Cancer Res 18(20):5816–5828
97. Bradford LS, Rauh-Hain A, Clark RM, Groeneweg JW, Zhang L, Borger D, Zukerberg LR, Growdon WB, Foster R, Rueda BR (2014) Assessing the efficacy of targeting the phosphatidylinositol 3-kinase/AKT/mTOR signaling pathway in endometrial cancer. Gynecol Oncol 133(2):346–352
98. Cotterchio M, Kreiger N, Theis B, Sloan M, Bahl S (2003) Hormonal factors and the risk of breast cancer according to estrogen- and progesterone-receptor subgroup. Cancer Epidemiol Biomarkers Prev 12(10):1053–1060
99. Fuqua SA (2001) The role of estrogen receptors in breast cancer metastasis. J Mammary Gland Biol Neoplasia 6(4):407–417
100. Fuqua SA, Russo J, Shackney SE, Stearns ME (2001) Selective estrogen receptor modulators. An aid in unraveling the links between estrogen and breast cancer. Postgrad Med 2001:Spec No: 3–10
101. Lee C, Dhillon J, Wang MY, Gao Y, Hu K, Park E, Astanehe A, Hung MC, Eirew P, Eaves CJ, Dunn SE (2008) Targeting YB-1 in HER-2 overexpressing breast cancer cells induces apoptosis via the mTOR/STAT3 pathway and suppresses tumor growth in mice. Cancer Res 68(21):8661–8666

102. Yue W, Fan P, Wang J, Li Y, Santen RJ (2007) Mechanisms of acquired resistance to endocrine therapy in hormone-dependent breast cancer cells. J Steroid Biochem Mol Biol 106(1–5):102–110
103. Miller TW, Hennessy BT, Gonzalez-Angulo AM, Fox EM, Mills GB, Chen H, Higham C, Garcia-Echeverria C, Shyr Y, Arteaga CL (2010) Hyperactivation of phosphatidylinositol-3Kinase promotes escape from hormone dependence in estrogen receptor-positive human breast cancer. J Clin Invest 120(7):2406–2413
104. Tokunaga E, Kataoka A, Kimura Y, Oki E, Mashino K, Nishida K, Koga T, Morita M, Kakeji Y, Baba H, Ohno S, Maehara Y (2006) The association between Akt activation and resistance to hormone therapy in metastatic breast cancer. Eur J Cancer 42(5):629–635
105. Tokunaga E, Kimura Y, Mashino K, Oki E, Kataoka A, Ohno S, Morita M, Kakeji Y, Baba H, Maehara Y (2006) Activation of PI3K/Akt signaling and hormone resistance in breast cancer. Breast Cancer 13(2):137–144
106. Tokunaga E, Kimura Y, Oki E, Ueda N, Futatsugi M, Mashino K, Yamamoto M, Ikebe M, Kakeji Y, Baba H, Maehara Y (2006) Akt is frequently activated in HER2/neu-positive breast cancers and associated with poor prognosis among hormone-treated patients. Int J Cancer 118(2):284–289
107. Ghayad SE, Vendrell JA (2010) Ben Larbi S, Dumontet C, Bieche I, Cohen PA. Endocrine resistance associated with activated ErbB system in breast cancer cells is reversed by inhibiting MAPK or PI3K/Akt signaling pathways. Int J Cancer 126(2):545–562
108. Ghayad SE, Bieche I, Vendrell JA, Keime C, Lidereau R, Dumontet C, Cohen PA (2008) mTOR inhibition reverses acquired endocrine therapy resistance of breast cancer cells at the cell proliferation and gene-expression levels. Cancer Sci 99(10):1992–2003
109. deGraffenried LA, Friedrichs WE, Russell DH, Donzis EJ, Middleton AK, Silva JM, Roth RA, Hidalgo M (2004) Inhibition of mTOR activity restores tamoxifen response in breast cancer cells with aberrant Akt Activity. Clin Cancer Res 10(23):8059–8067
110. Boulay A, Rudloff J, Ye J, Zumstein-Mecker S, O'Reilly T, Evans DB, Chen S, Lane HA (2005) Dual inhibition of mTOR and estrogen receptor signaling in vitro induces cell death in models of breast cancer. Clin Cancer Res 11(14):5319–5328
111. Beeram M, Tan QT, Tekmal RR, Russell D, Middleton A, DeGraffenried LA (2007) Akt-induced endocrine therapy resistance is reversed by inhibition of mTOR signaling. Ann Oncol 18(8):1323–1328
112. Villarreal-Garza C, Cortes J, Andre F, Verma S (2012) mTOR inhibitors in the management of hormone receptor-positive breast cancer: the latest evidence and future directions. Ann Oncol 23(10):2526–2535
113. Schayowitz A, Sabnis G, Goloubeva O, Njar VC, Brodie AM (2010) Prolonging hormone sensitivity in prostate cancer xenografts through dual inhibition of AR and mTOR. Br J Cancer 103(7):1001–1007
114. Mellman I, Coukos G, Dranoff G (2011) Cancer immunotherapy comes of age. Nature 480 (7378):480–489
115. Araki K, Ellebedy AH, Ahmed R (2011) TOR in the immune system. Curr Opin Cell Biol 23 (6):707–715
116. Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, Larsen CP, Ahmed R (2009) mTOR regulates memory CD8 T-cell differentiation. Nature 460 (7251):108–112
117. Wang Y, Wang XY, Subjeck JR, Shrikant PA, Kim HL (2011) Temsirolimus, an mTOR inhibitor, enhances anti-tumour effects of heat shock protein cancer vaccines. Br J Cancer 104(4):643–652
118. Jiang Q, Weiss JM, Back T, Chan T, Ortaldo JR, Guichard S, Wiltrout RH (2011) mTOR kinase inhibitor AZD8055 enhances the immunotherapeutic activity of an agonist CD40 antibody in cancer treatment. Cancer Res 71(12):4074–4084
119. Procaccini C, De Rosa V, Galgani M, Abanni L, Cali G, Porcellini A, Carbone F, Fontana S, Horvath TL, La Cava A, Matarese G (2010) An oscillatory switch in mTOR kinase activity sets regulatory T cell responsiveness. Immunity 33(6):929–941

120. Wang Y, Camirand G, Lin Y, Froicu M, Deng S, Shlomchik WD, Lakkis FG, Rothstein DM (2011) Regulatory T cells require mammalian target of rapamycin signaling to maintain both homeostasis and alloantigen-driven proliferation in lymphocyte-replete mice. J Immunol 186 (5):2809–2818
121. Zheng Y, Collins SL, Lutz MA, Allen AN, Kole TP, Zarek PE, Powell JD (2007) A role for mammalian target of rapamycin in regulating T cell activation versus anergy. J Immunol 178 (4):2163–2170
122. Mittendorf EA, Philips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, Su X, Wang Y, Gonzalez-Angulo AM, Akcakanat A, Chawla A, Curran M, Hwu P, Sharma P, Litton JK, Molldrem JJ, Alatrash G (2014) PD-L1 expression in triple-negative breast cancer. Cancer Immunol Res 2(4):361–370
123. Slingluff CL Jr, Petroni GR, Molhoek KR, Brautigan DL, Chianese-Bullock KA, Shada AL, Smolkin ME, Olson WC, Gaucher A, Chase CM, Grosh WW, Weiss GR, Wagenseller AG, Olszanski AJ, Martin L, Shea SM, Erdag G, Ram P, Gershenwald JE, Weber MJ (2013) Clinical activity and safety of combination therapy with temsirolimus and bevacizumab for advanced melanoma: a phase II trial (CTEP 7190/Mel47). Clin Cancer Res 19(13): 3611–3620
124. Lassen U, Sorensen M, Gaziel TB, Hasselbalch B, Poulsen HS (2013) Phase II study of bevacizumab and temsirolimus combination therapy for recurrent glioblastoma multiforme. Anticancer Res 33(4):1657–1660
125. Alvarez EA, Brady WE, Walker JL, Rotmensch J, Zhou XC, Kendrick JE, Yamada SD, Schilder JM, Cohn DE, Harrison CR, Moore KN, Aghajanian C (2013) Phase II trial of combination bevacizumab and temsirolimus in the treatment of recurrent or persistent endometrial carcinoma: a Gynecologic Oncology Group study. Gynecol Oncol 129(1):22–27
126. Tabernero J, Rojo F, Calvo E, Burris H, Judson I, Hazell K, Martinelli E, Ramon y Cajal S, Jones S, Vidal L, Shand N, Macarulla T, Ramos FJ, Dimitrijevic S, Zoellner U, Tang P, Stumm M, Lane HA, Lebwohl D, Baselga J (2008) Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: a phase I tumor pharmacodynamic study in patients with advanced solid tumors. J Clin Oncol 26(10): 1603–1610
127. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, Linn SC, Gonzalez-Angulo AM, Stemke-Hale K, Hauptmann M, Beijersbergen RL, Mills GB, van de Vijver MJ, Bernards R (2007) A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 12(4):395–402
128. Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung MC, Yu D (2004) PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 6(2):117–127
129. Lu CH, Wyszomierski SL, Tseng LM, Sun MH, Lan KH, Neal CL, Mills GB, Hortobagyi GN, Esteva FJ, Yu D (2007) Preclinical testing of clinically applicable strategies for overcoming trastuzumab resistance caused by PTEN deficiency. Clin Cancer Res 13(19):5883–5888
130. Andre F, O'Regan R, Ozguroglu M, Toi M, Xu B, Jerusalem G, Masuda N, Wilks S, Arena F, Isaacs C, Yap YS, Papai Z, Lang I, Armstrong A, Lerzo G, White M, Shen K, Litton J, Chen D, Zhang Y, Ali S, Taran T, Gianni L (2014) Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Oncol 15(6):580–591
131. Ewald F, Grabinski N, Grottke A, Windhorst S, Norz D, Carstensen L, Staufer K, Hofmann BT, Diehl F, David K, Schumacher U, Nashan B, Jucker M (2013) Combined targeting of AKT and mTOR using MK-2206 and RAD001 is synergistic in the treatment of cholangiocarcinoma. Int J Cancer 133(9):2065–2076
132. Breslin EM, White PC, Shore AM, Clement M, Brennan P (2005) LY294002 and rapamycin co-operate to inhibit T-cell proliferation. Br J Pharmacol 144(6):791–800

133. Sun SY, Rosenberg LM, Wang X, Zhou Z, Yue P, Fu H, Khuri FR (2005) Activation of Akt and eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition. Cancer Res 65(16):7052–7058
134. Takeuchi H, Kondo Y, Fujiwara K, Kanzawa T, Aoki H, Mills GB, Kondo S (2005) Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors. Cancer Res 65(8):3336–3346
135. Fan QW, Knight ZA, Goldenberg DD, Yu W, Mostov KE, Stokoe D, Shokat KM, Weiss WA (2006) A dual PI3Kinase/mTOR inhibitor reveals emergent efficacy in glioma. Cancer Cell 9(5):341–349
136. Ma BB, Lui VW, Hui CW, Lau CP, Wong CH, Hui EP, Ng MH, Cheng SH, Tsao SW, Tsang CM, Cheung CS, Ho K, Chan AT (2014) Preclinical evaluation of the mTOR-PI3K inhibitor BEZ235 in nasopharyngeal cancer models. Cancer Lett 343(1):24–32
137. Petroulakis E, Mamane Y, Le Bacquer O, Shahbazian D, Sonenberg N (2005) mTOR signaling: implications for cancer and anticancer therapy. Br J Cancer 94(2):195–199
138. Guertin DA, Sabatini DM (2007) Defining the role of mTOR in cancer. Cancer Cell 12(1): 9–22
139. Chresta CM, Davies BR, Hickson I, Harding T, Cosulich S, Critchlow SE, Vincent JP, Ellston R, Jones D, Sini P (2010) AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. Cancer Res 70(1):288–298
140. Naing A, Aghajanian C, Raymond E, Olmos D, Schwartz G, Oelmann E, Grinsted L, Burke W, Taylor R, Kaye S, Kurzrock R, Banerji U (2012) Safety, tolerability, pharmacokinetics and pharmacodynamics of AZD8055 in advanced solid tumours and lymphoma. Br J Cancer 107(7):1093–1099
141. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EW, Chang F, Lehmann B, Terrian DM, Milella M, Tafuri A, Stivala F, Libra M, Basecke J, Evangelisti C, Martelli AM, Franklin RA (2007) Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta 1773(8):1263–1284
142. Mirzoeva OK, Das D, Heiser LM, Bhattacharya S, Siwak D, Gendelman R, Bayani N, Wang NJ, Neve RM, Guan Y, Hu Z, Knight Z, Feiler HS, Gascard P, Parvin B, Spellman PT, Shokat KM, Wyrobek AJ, Bissell MJ, McCormick F, Kuo WL, Mills GB, Gray JW, Korn WM (2009) Basal subtype and MAPK/ERK kinase (MEK)-phosphoinositide 3-kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. Cancer Res 69(2):565–572
143. Janku F, Lee JJ, Tsimberidou AM, Hong DS, Naing A, Falchook GS, Fu S, Luthra R, Garrido-Laguna I, Kurzrock R (2011) PIK3CA mutations frequently coexist with RAS and BRAF mutations in patients with advanced cancers. PLoS ONE 6(7):e22769
144. Shimizu T, Tolcher AW, Papadopoulos KP, Beeram M, Rasco DW, Smith LS, Gunn S, Smetzer L, Mays TA, Kaiser B (2012) The clinical effect of the dual-targeting strategy involving PI3K/AKT/mTOR and RAS/MEK/ERK pathways in patients with advanced cancer. Clin Cancer Res 18(8):2316–2325
145. Carracedo A, Pandolfi P (2008) The PTEN–PI3K pathway: of feedbacks and cross-talks. Oncogene 27(41):5527–5541
146. Renshaw J, Taylor KR, Bishop R, Valenti M, De Haven Brandon A, Gowan S, Eccles SA, Ruddle RR, Johnson LD, Raynaud FI, Selfe JL, Thway K, Pietsch T, Pearson AD, Shipley J (2013) Dual blockade of the PI3K/AKT/mTOR (AZD8055) and RAS/MEK/ERK (AZD6244) pathways synergistically inhibits rhabdomyosarcoma cell growth in vitro and in vivo. Clin Cancer Res 19(21):5940–5951
147. She QB, Solit DB, Ye Q, O'Reilly KE, Lobo J, Rosen N (2005) The BAD protein integrates survival signaling by EGFR/MAPK and PI3K/Akt kinase pathways in PTEN-deficient tumor cells. Cancer Cell 8(4):287–297
148. Lee EQ, Kuhn J, Lamborn KR, Abrey L, DeAngelis LM, Lieberman F, Robins HI, Chang SM, Yung WK, Drappatz J, Mehta MP, Levin VA, Aldape K, Dancey JE, Wright JJ, Prados MD, Cloughesy TF, Gilbert MR, Wen PY (2012) Phase I/II study of sorafenib in

combination with temsirolimus for recurrent glioblastoma or gliosarcoma: North American Brain Tumor Consortium study 05-02. Neuro Oncol 14(12):1511–1518
149. Kelley RK, Nimeiri HS, Munster PN, Vergo MT, Huang Y, Li CM, Hwang J, Mulcahy MF, Yeh BM, Kuhn P, Luttgen MS, Grabowsky JA, Stucky-Marshall L, Korn WM, Ko AH, Bergsland EK, Benson AB 3rd, Venook AP (2013) Temsirolimus combined with sorafenib in hepatocellular carcinoma: a phase I dose-finding trial with pharmacokinetic and biomarker correlates. Ann Oncol 24(7):1900–1907
150. Wee S, Jagani Z, Xiang KX, Loo A, Dorsch M, Yao Y-M, Sellers WR, Lengauer C, Stegmeier F (2009) PI3K pathway activation mediates resistance to MEK inhibitors in KRAS mutant cancers. Cancer Res 69(10):4286–4293
151. Naing A, Mita M, Komarnitsky P, Milner A, von Richter O, Ogden J, Piha-Paul S, Fu S, Asatiani E, Kurzrock R (2012) 608 phase I dose-escalation trial of a selective oral MEK1/2 inhibitor, pimasertib (MSC1936369B), combined with an mTOR inhibitor, temsirolimus, in patients with advanced solid tumors. Eur J Cancer 48(Suppl 6):187
152. Gonzalez-Angulo AM, Meric-Bernstam F (2010) Metformin: a therapeutic opportunity in breast cancer. Clin Cancer Res 16(6):1695–1700
153. Zakikhani M, Blouin MJ, Piura E, Pollak MN (2010) Metformin and rapamycin have distinct effects on the AKT pathway and proliferation in breast cancer cells. Breast Cancer Res Treat 123(1):271–279
154. Ben Sahra I, Regazzetti C, Robert G, Laurent K, Le Marchand-Brustel Y, Auberger P, Tanti JF, Giorgetti-Peraldi S, Bost F (2011) Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. Cancer Res 71(13):4366–4372
155. Goodwin PJ, Ligibel JA, Stambolic V (2009) Metformin in breast cancer: time for action. J Clin Oncol 27(20):3271–3273
156. Tomic T, Botton T, Cerezo M, Robert G, Luciano F, Puissant A, Gounon P, Allegra M, Bertolotto C, Bereder JM, Tartare-Deckert S, Bahadoran P, Auberger P, Ballotti R, Rocchi S (2011) Metformin inhibits melanoma development through autophagy and apoptosis mechanisms. Cell Death Dis 2:e199
157. Cui X, Zhang P, Deng W, Oesterreich S, Lu Y, Mills GB, Lee AV (2003) Insulin-like growth factor-I inhibits progesterone receptor expression in breast cancer cells via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway: progesterone receptor as a potential indicator of growth factor activity in breast cancer. Mol Endocrinol 17 (4):575–588
158. Cui X, Lazard Z, Zhang P, Hopp TA, Lee AV (2003) Progesterone crosstalks with insulin-like growth factor signaling in breast cancer cells via induction of insulin receptor substrate-2. Oncogene 22(44):6937–6941
159. Xie Y, Wang YL, Yu L, Hu Q, Ji L, Zhang Y, Liao QP (2011) Metformin promotes progesterone receptor expression via inhibition of mammalian target of rapamycin (mTOR) in endometrial cancer cells. J Steroid Biochem Mol Biol 126(3–5):113–120
160. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5): 646–674
161. Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, Manola J, Brugarolas J, McDonnell TJ, Golub TR, Loda M, Lane HA, Sellers WR (2004) mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. Nat Med 10(6):594–601
162. Yao J TZ, Paneerselvam A, Winkler R, Bugarini R, Pavel M (2011) Effect of everolimus treatment on markers of angiogenesis in patients with advanced pancreatic neuroendocrine tumors (pNET): results from the phase III RADIANT-3 study. Ann Oncol
163. Del Bufalo D, Ciuffreda L, Trisciuoglio D, Desideri M, Cognetti F, Zupi G, Milella M (2006) Antiangiogenic potential of the mammalian target of rapamycin inhibitor temsirolimus. Cancer Res 66(11):5549–5554
164. Piha-Paul SA, Wheler JJ, Fu S, Levenback C, Lu K, Falchook GS, Naing A, Hong DS, Tsimberidou AM, Kurzrock R (2014) Advanced gynecologic malignancies treated with a

combination of the VEGF inhibitor bevacizumab and the mTOR inhibitor temsirolimus. Oncotarget
165. Chaturvedi D, Gao X, Cohen MS, Taunton J, Patel TB (2009) Rapamycin induces transactivation of the EGFR and increases cell survival. Oncogene 28(9):1187–1196
166. Goudar RK, Shi Q, Hjelmeland MD, Keir ST, McLendon RE, Wikstrand CJ, Reese ED, Conrad CA, Traxler P, Lane HA (2005) Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma tumor growth inhibition. Mol Cancer Ther 4(1):101–112
167. Fan Q-W, Specht KM, Zhang C, Goldenberg DD, Shokat KM, Weiss WA (2003) Combinatorial efficacy achieved through two-point blockade within a signaling pathway—a chemical genetic approach. Cancer Res 63(24):8930–8938
168. Li B, Chang C-M, Yuan M, McKenna WG (2003) Shu H-KG. Resistance to small molecule inhibitors of epidermal growth factor receptor in malignant gliomas. Cancer Res 63 (21):7443–7450
169. Bianco R, Garofalo S, Rosa R, Damiano V, Gelardi T, Daniele G, Marciano R, Ciardiello F, Tortora G (2008) Inhibition of mTOR pathway by everolimus cooperates with EGFR inhibitors in human tumours sensitive and resistant to anti-EGFR drugs. Br J Cancer 98 (5):923–930
170. Doherty L, Gigas DC, Kesari S, Drappatz J, Kim R, Zimmerman J, Ostrowsky L, Wen PY (2006) Pilot study of the combination of EGFR and mTOR inhibitors in recurrent malignant gliomas. Neurology 67(1):156–158
171. Vora SR, Juric D, Kim N, Mino-Kenudson M, Huynh T, Costa C, Lockerman EL, Pollack SF, Liu M, Li X, Lehar J, Wiesmann M, Wartmann M, Chen Y, Cao ZA, Pinzon-Ortiz M, Kim S, Schlegel R, Huang A, Engelman JA (2014) CDK 4/6 inhibitors sensitize PIK3CA mutant breast cancer to PI3K inhibitors. Cancer Cell 26(1):136–149
172. Ibrahim YH, Garcia-Garcia C, Serra V, He L, Torres-Lockhart K, Prat A, Anton P, Cozar P, Guzman M, Grueso J, Rodriguez O, Calvo MT, Aura C, Diez O, Rubio IT, Perez J, Rodon J, Cortes J, Ellisen LW, Scaltriti M, Baselga J (2012) PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. Cancer Discov 2(11):1036–1047
173. Juvekar A, Burga LN, Hu H, Lunsford EP, Ibrahim YH, Balmana J, Rajendran A, Papa A, Spencer K, Lyssiotis CA, Nardella C, Pandolfi PP, Baselga J, Scully R, Asara JM, Cantley LC, Wulf GM (2012) Combining a PI3K inhibitor with a PARP inhibitor provides an effective therapy for BRCA1-related breast cancer. Cancer Discov 2(11):1048–1063
174. Matulonis U, Wulf GM, Birrer MJ, Westin SN, Quy P, Bell-McGuinn KM, Lasonde B, Whalen C, Aghajanian C, Solit DB, Mills GB, Cantley L, Winer EP (2014) Phase I study of oral BKM120 and oral olaparib for high-grade serous ovarian cancer (HGSC) or triple-negative breast cancer (TNBC) J Clin Oncol 32:5s (suppl; abstr 2510)
175. Moroney JW, Schlumbrecht MP, Helgason T, Coleman RL, Moulder S, Naing A, Bodurka DC, Janku F, Hong DS, Kurzrock R (2011) A phase I trial of liposomal doxorubicin, bevacizumab, and temsirolimus in patients with advanced gynecologic and breast malignancies. Clin Cancer Res 17(21):6840–6846
176. Moulder S, Moroney J, Helgason T, Wheler J, Booser D, Albarracin C, Morrow PK, Koenig K, Kurzrock R (2011) Responses to liposomal doxorubicin, bevacizumab, and temsirolimus in metaplastic carcinoma of the breast: biologic rationale and implications for stem-cell research in breast cancer. J Clin Oncol 29(19):e572–e575
177. Westin SN, Smart ML, Pal N, Urbauer DL, Janku F, Wheler JJ, Piha-Paul SA, Naing A, Tsimberidou AM, Fu S, Falchook GS, Hong DS, Zinner R, Subbiah V, Culotta KS, Lu KH, Coleman RL, Kurzrock R (2013) Phase I trial of sorafenib, bevacizumab, and temsirolimus in advanced solid tumors. J Clin Oncol 31(Abstract 2611)
178. O'Reilly T, McSheehy PM (2010) Biomarker development for the clinical activity of the mTOR inhibitor everolimus (RAD001): processes, limitations, and further proposals. Transl Oncol 3(2):65–79

179. Pantuck AJ, Seligson DB, Klatte T, Yu H, Leppert JT, Moore L, O'Toole T, Gibbons J, Belldegrun AS, Figlin RA (2007) Prognostic relevance of the mTOR pathway in renal cell carcinoma: implications for molecular patient selection for targeted therapy. Cancer 109 (11):2257–2267
180. Hortobagyi GN, Piccart-Gebhart MJ, Rugo HS, Burris HA, Campone M, Noguchi S, Perez AT, Deleu I, Shtivelband M, Provencher L, Masuda N, Dakhil SR, Anderson I, Chen D, Damask A, Huang A, McDonald R, Taran T, Sahmoud T, Baselga J (2013) Correlation of molecular alterations with efficacy of everolimus in hormone receptor–positive, HER2-negative advanced breast cancer: results from BOLERO-2. J Clin Oncol 31 (suppl):abstr LBA509
181. Krop I JS, Mayer IA, Dickler M, Ganju V, Forero-Torres A, Melichar B, Morales S, de Boer R, Gendreau S, Derynck M, Lackner M, Spoerke J, Yeh R-F, Levy G, Ng V, O'Brien C, Savage H, Xiao Y, Wilson T, Lee SC, Petrakova K, Vallentin S, Yardley D, Ellis M, Piccart M, Perez EA, Winer E, Schmid P (2014) The FERGI phase II study of the PI3K inhibitor pictilisib (GDC-0941) plus fulvestrant vs fulvestrant plus placebo in patients with ER+, aromatase inhibitor (AI)-resistant advanced or metastatic breast cancer—part I results. In: San Antonio breast cancer symposium (S2-02)
182. Wagle N, Grabiner BC, Van Allen EM, Amin-Mansour A, Taylor-Weiner A, Rosenberg M, Gray N, Barletta JA, Guo Y, Swanson SJ, Ruan DT, Hanna GJ, Haddad RI, Getz G, Kwiatkowski DJ, Carter SL, Sabatini DM, Janne PA, Garraway LA, Lorch JH (2014) Response and acquired resistance to everolimus in anaplastic thyroid cancer. N Engl J Med 371(15):1426–1433
183. Wagle N, Grabiner BC, Van Allen EM, Hodis E, Jacobus S, Supko JG, Stewart M, Choueiri TK, Gandhi L, Cleary JM, Elfiky AA, Taplin ME, Stack EC, Signoretti S, Loda M, Shapiro GI, Sabatini DM, Lander ES, Gabriel SB, Kantoff PW, Garraway LA, Rosenberg JE (2014) Activating mTOR mutations in a patient with an extraordinary response on a phase I trial of everolimus and pazopanib. Cancer Discov 4(5):546–553
184. Gonzalez-Angulo AM, Blumenschein GR Jr (2013) Defining biomarkers to predict sensitivity to PI3K/Akt/mTOR pathway inhibitors in breast cancer. Cancer Treat Rev 39 (4):313–320
185. Cho D, Signoretti S, Dabora S, Regan M, Seeley A, Mariotti M, Youmans A, Polivy A, Mandato L, McDermott D, Stanbridge E, Atkins M (2007) Potential histologic and molecular predictors of response to temsirolimus in patients with advanced renal cell carcinoma. Clin Genitourin Cancer 5(6):379–385
186. Velickovic M, Delahunt B, McIver B, Grebe SK (2002) Intragenic PTEN/MMAC1 loss of heterozygosity in conventional (clear-cell) renal cell carcinoma is associated with poor patient prognosis. Mod Pathol 15(5):479–485
187. Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, Petersen R, Frost P, Gibbons JJ, Wu H, Sawyers CL (2001) Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. Proc Natl Acad Sci USA 98(18):10314–10319
188. Hassan B, Akcakanat A, Sangai T, Evans KW, Adkins F, Eterovic AK, Zhao H, Chen K, Chen H, Do KA, Xie SM, Holder AM, Naing A, Mills GB, Meric-Bernstam F (2014) Catalytic mTOR inhibitors can overcome intrinsic and acquired resistance to allosteric mTOR inhibitors. Oncotarget 5(18):8544–8557
189. Meric-Bernstam F, Mills GB (2012) Overcoming implementation challenges of personalized cancer therapy. Nat Rev Clin Oncol 9(9):542–548
190. Meric-Bernstam F, Gonzalez-Angulo AM (2009) Targeting the mTOR signaling network for cancer therapy. J Clin Oncol 27(13):2278–2287

Chapter 7
Phospho-Inositol-3-Kinase Activity and Dysregulation in Pediatric Leukemia and Lymphoma

Charles B. Goodwin and Rebecca J. Chan

Introduction

Leukemias and lymphomas are the most common malignancies diagnosed in children, accounting for 34 and 12 % of pediatric cancers, respectively [1]. Rather than a single disease, pediatric leukemia represents a diverse group of hematologic conditions in which the patient's bone marrow produces excessive amounts of dysfunctional and abnormal white blood cells. Leukemias can be categorized broadly as myeloid or lymphocytic, depending on whether the affected lineage is myeloid or lymphoid. Lymphocytic leukemia is more common in children, while myeloid leukemias tend to affect adults. Furthermore, leukemias can be divided into acute forms and chronic forms, with acute forms generally resulting in the rapid overproduction of immature or blast forms of the affected lineage, while chronic forms are characterized by a more protracted and indolent course, resulting in gradual excessive production of relatively mature cells of the affected lineage. Closely related to lymphocytic leukemias are lymphomas, which typically present as a solid tumors of lymphoid cells either in lymph nodes or in a variety of extranodal tissue sites. Finally, Juvenile Myelomonocytic Leukemia (JMML) is rare hematologic disorder of young children that has features of both myeloid leukemia

R.J. Chan
Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, USA

R.J. Chan (✉)
Department of Pediatrics, Herman B Wells Center for Pediatric Research, 1044 W. Walnut Street, R4-W170, 46202 Indianapolis, IN, USA
e-mail: rchan@iu.edu

C.B. Goodwin · R.J. Chan
Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA

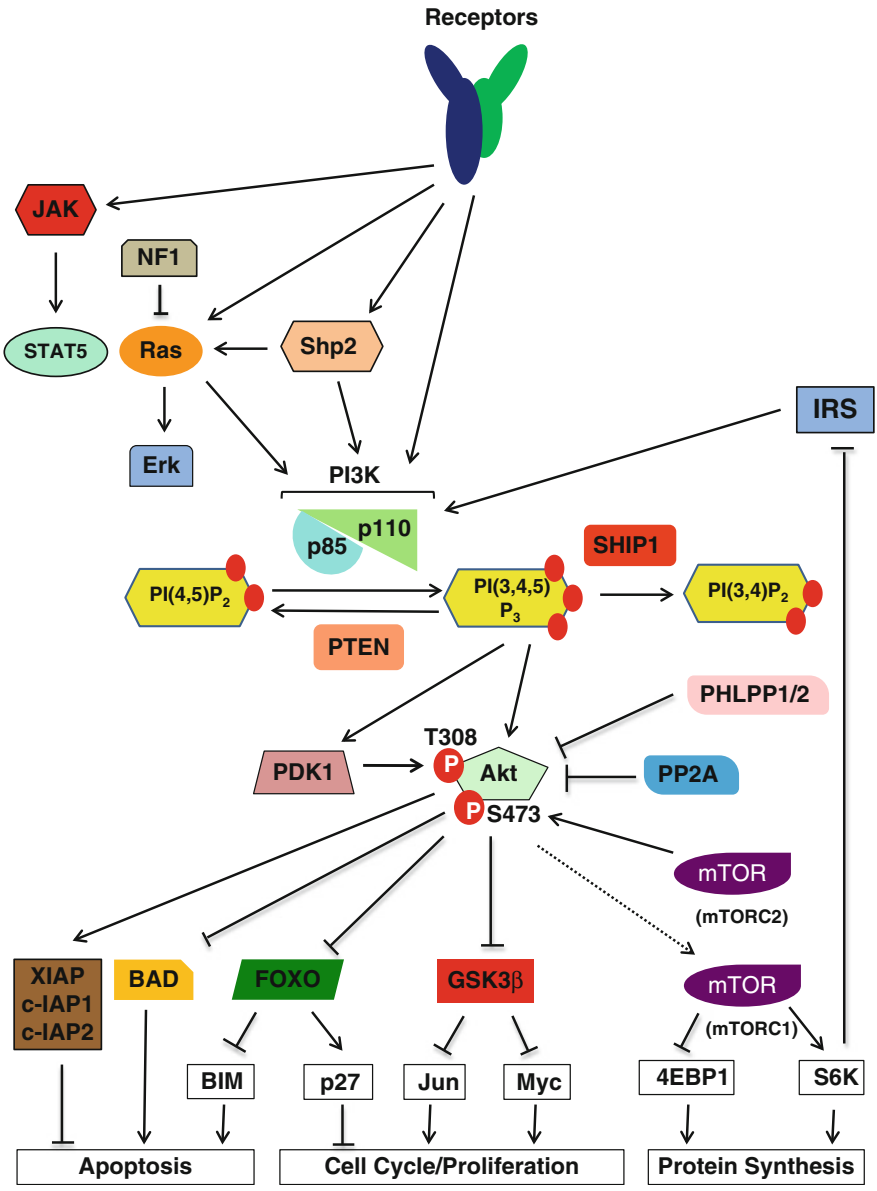
N. Dey et al. (eds.), *PI3K-mTOR in Cancer and Cancer Therapy*,
Cancer Drug Discovery and Development, DOI 10.1007/978-3-319-34211-5_7

as well as myelodysplastic syndrome (MDS), a hematologic condition characterized by defective production of myeloid lineage cells.

Although a lot of progress has been made in treating pediatric hematologic malignancies in recent decades using modalities such as chemotherapy, radiation, and hematopoietic stem cell transplantation (HSCT), relapsed and refractory disease continue to be problems, as do the treatment-related morbidity and mortality inherent in these modalities, which can cause great distress to the vulnerable pediatric leukemia patient and his or her family. Consequently, significant research is being dedicated to understanding the underlying molecular mechanisms of these diseases with the goal of developing targeted therapies, which ideally promise increased tolerability and improved therapeutic efficacy.

Phosphatidylinositol-3-Kinase (PI3K)-AKT-mTOR Signaling

Phosphatidylinositol-3-kinase (PI3K) signaling is a crucial regulator of proliferation, growth, survival, and numerous other important cellular processes. Not surprisingly, PI3K activity is dysregulated in many malignancies, including pediatric hematologic malignancies. Class I PI3Ks are lipid kinases, which phosphorylate the 3 position of the plasma membrane-associated lipid, phosphatidylinositol (4,5) bisphosphate ($PI(4,5)P_2$), to generate $PI(3,4,5)P_3$. $PI(3,4,5)P_3$ serves to recruit Pleckstrin Homology (PH)-domain-containing signaling kinases to the plasma membrane, especially PDK1, and its downstream effector, the serine-threonine kinase AKT, which it phosphorylates at Threonine 308 (Fig. 7.1) [2–5]. Activated AKT, by phosphorylating several downstream effectors, regulates multiple cellular processes, including survival, proliferation, and protein synthesis, the latter of which is mediated by the mammalian target of rapamycin (mTOR) as part of the multi-protein subunit mTOR Complex 1 (mTORC1) (Fig. 7.1) [2]. Through a complex positive feedback loop, AKT is further activated by mTOR-mediated phosphorylation of Serine 473; in this latter case, mTOR is part of a different multi-protein subunit mTOR complex 2 (mTORC2) (Fig. 7.1) [6]. Additionally, mTORC1 negatively regulates AKT via a negative feedback loop involving S6K-mediated phosphorylation and inactivation of the Insulin Receptor Substrate-1 (IRS-1), which contributes to the activation of PI3K. As a consequence, mTOR inhibitors which primarily target only mTORC1 can interrupt this negative feedback loop and promote paradoxical hyperactivation of AKT, thus suggesting a need for either dual mTORC1/mTORC2 inhibitors or inhibitors that target more than one step in the PI3K-AKT-mTOR pathway (e.g. dual PI3K/mTOR inhibitors) to achieve full blockade of oncogenic hyperactivated signaling of this pathway [7]. Furthermore, many examples of cross-talk between the PI3K-AKT-mTOR pathway and other mitogenic pathways such as the RAS-MEK-ERK (MAP Kinase) pathway and the JAK-STAT pathway have been demonstrated, and as a consequence,


Receptors
JAK
NF1
STAT5
Ras
Shp2
IRS
Erk
PI3K
p85
p110
SHIP1
PI(4,5)P2
PI(3,4,5)P3
PI(3,4)P2
PTEN
PHLPP1/2
T308
PDK1
P
Akt
PP2A
P
S473
mTOR
(mTORC2)
XIAP
c-IAP1
c-IAP2
BAD
FOXO
GSK3β
mTOR
(mTORC1)
BIM
p27
Jun
Myc
4EBP1
S6K
Apoptosis
Cell Cycle/Proliferation
Protein Synthesis

◀ **Fig. 7.1** Detailed overview of PI3K-AKT signaling cascade. Diagram showing the PI3K-AKT-mTOR signaling cascade, including RAS, a direct activator of PI3K activity, the major downstream effectors of PI3K, PDK1 and AKT, and the major downstream effectors of AKT, including BAD, FOXO, GSK3β, and mTOR, which together regulate cellular processes including proliferation, survival, and protein synthesis. Notably, full activation of AKT requires phosphorylation at the Threonine 308 site, which is mediated by PDK1 and at the Serine 473 site, which is mediated by mTOR, while in the mTORC2 complex. mTOR, as part of the mTORC1 complex, is also positively regulated by AKT via several intermediate mediators not shown in this figure (represented by the *dotted arrow* from AKT to mTOR). The downstream effectors of the mTORC1 complex, notably 4EBP1 and S6K, regulate protein synthesis. S6K activity also negatively regulates IRS, a positive regulator of PI3K, creating a negative feedback loop that can be interrupted with pharmacologic inhibition of mTORC1-associated mTOR, leading to enhanced AKT activity. Also shown is the tumor suppressor lipid phosphatase, PTEN, which catalyzes the dephosphorylation of $PI(3,4,5)P_3$ to generate $PI(4,5)P_2$, thereby antagonizing the activity of PI3K. Additionally, the lipid phosphatase SHIP1 can also negatively regulate PI3K by dephosphorylating the 5′ phosphate group of $PI(3,4,5)P_3$, generating the inactive moiety $PI(3,4)P_2$. AKT itself can be inactivated by dephosphorylation via the activity of the protein phosphatases such as PHLPP1/2 and PP2A. Also shown are additional important mitogenic signaling pathways, JAK-STAT and ERK (MAPK), which often cooperate with the PI3K-AKT-mTOR pathway in promoting leukemogenesis

blockade of PI3K-AKT signaling may result in either down-regulation or compensatory hyperactivation of one of these parallel pathways. Likely, effective pharmacologic inhibition of PI3K may be best achieved with simultaneous inhibition of one or more of these collaborating pathways.

There are two subclasses of Class I PI3K, and both classes are heterodimers consisting of a regulatory and a catalytic subunit. The three catalytic subunits for Class IA PI3K include p110α (encoded by *PIK3CA*), p110β (encoded by *PIK3CB*), and p110δ (encoded by *PIK3CD*) [2]. These three catalytic subunits can bind to any Class IA regulatory subunit, including p85α, and its splice variants p55α and p50α (encoded by *PIK3R1*), p85β (encoded by *PIK3R2*), or p55γ (encoded by *PIK3R3*) via an interaction between the adapter binding domain (ABD) of the catalytic subunit and the inter-SH2 (iSH2) domain of the regulatory subunit (Fig. 7.2) [2, 8]. Class IB PI3K consists of catalytic subunit p110γ (encoded by *PIK3CG*), and its unique regulatory subunits, p84/87 and p101 [2]. The catalytic subunits p110α and p110β are expressed ubiquitously, while p110δ and p110γ are found predominantly in hematopoietic cells [2].

The Class IA regulatory subunits (p85) help promote PI3K activity through two important mechanisms: (1) stabilizing the p110 catalytic subunits, thereby preventing their rapid degradation, and (2) recruiting the p110 catalytic subunits to tyrosine phosphorylated signaling and adapter molecules via its N-SH2 and C-SH2 domains (Fig. 7.2) [2, 9]. Conversely, p85 also negatively regulates PI3K activity through an inhibitory interaction between its N-SH2 domain and the helical domain of the p110 catalytic subunit, which is relieved upon N-SH2 domain binding to a phospho-tyrosine residue [2, 10–12]. In addition, GTP-bound RAS promotes PI3K activation via an interaction with the RAS-binding domain (RBD) of the catalytic subunit (Fig. 7.2) [2, 13–17].

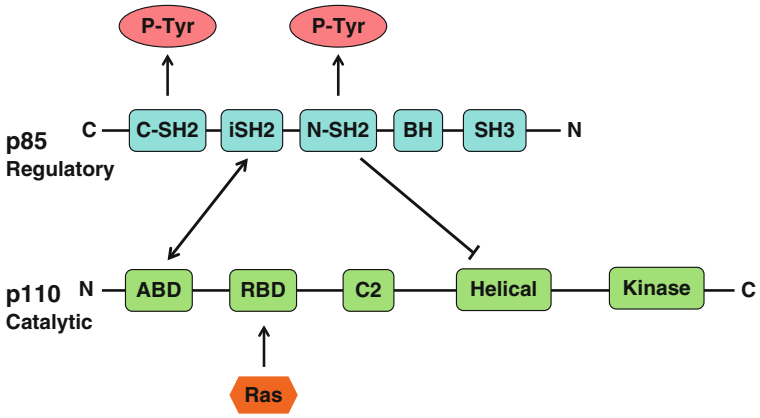


Fig. 7.2 Schematic diagram of the functional domains and the inter-molecular interactions between PI3K catalytic subunit and regulatory subunit. Structurally, PI3K is a heterodimer, consisting of a regulatory subunit (p85) and a catalytic subunit (p110). The regulatory subunit has two Src Homology-2 (SH2) domains, which interact with phosphorylated tyrosine residues; a Src Homology-3 (SH3) domain, which interacts with proline-rich domains; a Breakpoint clustered Homology (BH) domain; and an inter-SH2 (iSH2), which is the principal interacting domain with the catalytic subunit. The catalytic subunit contains an Adapter-Binding Domain (ABD), which mediates the major interaction with iSH2 in the regulatory subunit; a RAS-Binding Domain (RBD), to which RAS binds, promoting activation of PI3K; a protein-kinase-C homology-2 (C2) domain, which mediates interactions with lipid membranes; and the Kinase domain, which mediates the lipid phosphorylation activity

PI3K and Cancer

Hyperactivation of PI3K signaling is a common feature among a wide variety of human malignancies, which is not surprising given the role of PI3K in regulating proliferation, growth, and survival [18]. Frequently, hyperactivation of PI3K is the result of oncogenic transformation of receptor tyrosine kinases or signaling proteins upstream of PI3K. Alternatively, PI3K hyperactivation results from loss of expression or function of the lipid phosphatase, PTEN, which removes the phosphate group from PI(3,4,5)P_3 at the 3 position, thereby converting the active lipid moiety to PI(4,5)P_2, the inactive lipid moiety (Fig. 7.1) [18–20].

Likewise, cancer-causing mutations in genes encoding the Class I catalytic or regulatory subunits have also been found. Of the four catalytic subunits, only *PIK3CA* (encoding p110α) has been found to have cancer-causing mutations [21]. Mutations in *PIK3R1* (encoding p85α) have been identified in certain cancers and promote increased PI3K activity as the mutant p85α loses its inhibitory effect on the p110 catalytic subunit. Interestingly, overexpression of p110δ and p110γ has also been observed in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), respectively [22–24]. However, very recently two studies described germline gain-of-function mutations in *PIK3CD*, which encodes p110δ, resulting in

a primary immunodeficiency condition with an increased predisposition to respiratory infections as the principal phenotype [25, 26]. One study reported that patients exhibited lymphopenia [25], while the other reported a lymphoproliferative phenotype in multiple patients, including diffuse lymphadenopathy and hepatosplenomegaly with two patients diagnosed with B-cell lymphoma and a third patient with a strong family history of B-cell lymphoma [26]. Although more work needs to be done to evaluate the role of these germline mutations in lymphoma development, somatic activating mutations in *PIK3CD* do not appear to be a common leukemia-causing genetic lesions.

The Catalytic Subunit p110δ as a Potential Target in Hematologic Malignancies

In recent years, there has been significant progress in identifying the roles of individual PI3K catalytic subunits in particular physiologic processes and disease states, especially cancer. This research has been complemented by simultaneous efforts to develop improved PI3K inhibitors that target specific PI3K catalytic subunits with several fold specificity by taking advantage of subtle structural differences between the various catalytic subunits [27]. The goal of this work is to inhibit only the activity of the relevant p110 catalytic subunit contributing to the pathogenesis of a disease, while sparing the activity of the other catalytic subunits, thus reducing the risk for off-target and potentially toxic side effects.

The Class IA PI3K catalytic subunit, p110δ, like the sole Class IB PI3K catalytic subunit, p110γ, is expressed predominantly in hematopoietic cells, in contrast to p110α and p110β, which are ubiquitously expressed, suggesting that p110δ and p110γ might be ideal pharmacologic targets in diseases involving the hematopoietic system such as allergic disorders, autoimmune disorders, and leukemias [28–30]. While p110γ is associated with signaling mediated by G-Protein-Coupled Receptors (GPCRs), p110δ is typically found promoting activation of PI3K signaling downstream of receptor tyrosine kinases and cytokine receptors, indicating that p110δ and p110γ bear unique functions, though much work has recently demonstrated their potential cooperation in many diseases processes [30].

A knockin mouse model has been generated bearing a point mutation in the *Pik3cd* gene, resulting in endogenous expression of a kinase-dead mutant p110δ D910A [28]. This knockin model, rather than a knockout model, is useful for studies as it circumvents the potential compensatory upregulation and recruitment of the remaining Class IA catalytic subunits, p110α and p110β. Although this genetically inactivated *Pik3cd* gene is constitutively expressed, homozygous mutant mice are born at normal Mendelian ratios and do not exhibit any overt phenotype except some impairment of B- and T-cell maturation and function and the development of a mild inflammatory bowel disease. The fact that $p110\delta^{D910A/D910A}$ mice are completely viable with only modest health effects suggests that p110δ would be

an ideal therapeutic target for hematologic malignancies, since pharmacologic inhibition of its activity would be expected to have modest side effects [28].

Because the effects of genetic inactivation of p110δ were mostly observed in B- and T-cells, much of the research on the role of p110δ in health and disease has focused on processes involving the lymphoid lineage, such a multiple myeloma, T-cell leukemia, lymphoma, and a variety of inflammatory conditions [29, 31–34]. However, p110δ has been found to be overexpressed in AML [22], and to mediate proliferation and migration of macrophages [35], suggesting p110δ may also be relevant for myeloid leukemias such as AML, CML, and JMML.

Accordingly, there has been much interest in particular for developing inhibitors with high specificity for the p110δ catalytic subunit [36]. IC87114 was the first such compound to be developed, but its low potency precluded it from entering clinical trials, though it still remains an important research tool [37]. More recently, GS-1101 (idelalisib, formerly known as CAL-101), also an isoquinolinone derivative, has been developed, has greatly enhanced potency against p110δ, and has entered several clinical trials for lymphoid malignancies, and recently gained FDA approval for patients with relapsed in CLL in combination with rituximab, as well as relapsed follicular B-cell lymphoma and relapsed small lymphocytic lymphoma [31, 38–43].

Another interesting aspect of p110δ is its apparent capacity to induce malignant transformation independent of RAS [44]. The conserved structure of all Class I PI3K catalytic subunits contains a RBD (Fig. 7.2) [2], to which RAS can bind in order to promote full activation of PI3K signaling. The common oncogenic mutation in p110α, H1047A, along with other nearby mutations in the kinase domain, results in a conformational change similar to that induced by p110α binding to RAS, thereby promoting its constitutive activation [45]. These kinase domain mutations of p110α have been shown to promote RAS-independent PI3K hyperactivation, as mutating a conserved lysine residue in the RBD (and, thus, abrogating p110α interaction with RAS) does not reduce AKT phosphorylation or cellular transforming ability [45]. Similarly, p110δ bearing an analogous RBD mutation retains its cellular transforming ability even in the absence of additional activating mutations, suggesting that p110δ can mediate its signaling function independent of RAS interaction [44].

Because p110γ, like p110δ, has a hematopoietic-specific expression profile, it likewise is being evaluated as a potential therapeutic target for hematologic malignancies, both alone and in conjunction with p110δ, particularly in conditions such as T-Cell Acute Lymphoblastic Leukemia (T-ALL) [46]. In addition to efforts to target PI3K directly with catalytic subunit-specific inhibitors, significant work has been dedicated to exploring various downstream effectors of PI3K activity as possible therapeutic targets; this is particularly true for AKT and mTOR, for which several highly potent inhibitors have been recently developed [47–50].

Acute Lymphoblastic Leukemia (ALL)

Acute Lymphoblastic Leukemia (ALL) is the most common type of leukemia found in children, accounting for approximately 70–80 % of childhood leukemias with an annual incidence of about 3000 cases per year in the United States. It was one of the first cancers cured with chemotherapy, and now has an impressive survival rate approaching 90 % [51]. ALL can affect either the B-cell lineage or T-cell lineage, though the pre-B-cell type is the most common. Hyperactivation of the PI3K-AKT-mTOR pathway is a particularly common feature in T-cell ALL (T-ALL), which tends to have a poorer prognosis compared to pre-B-cell ALL, and several molecular mechanisms for activation of this pathway have been reported in the literature. For example, Notch1 activating mutations, which are found in about 50 % of T-ALL cases, can promote hyperactivation of PI3K-AKT signaling, either by inactivating the tumor suppressor lipid phosphatase PTEN, which directly antagonizes the activity of PI3K, or by decreasing the activity of the serine/threonine protein phosphatase 2A (PP2A), which negatively regulates AKT (Fig. 7.3) [52–55]. Additionally, increased Notch1 activity can increase the expression of the interleukin-7 receptor α (IL7Rα) and the insulin-like growth factor-1 receptor (IGF1R), both of which can promote activation of the PI3K-AKT-mTOR signaling pathway [56–58]. As a consequence, γ-secretase inhibitors (GSIs) demonstrate efficacy in reducing hyperactivated PI3K-AKT-mTOR signaling in T-ALL cells, though resistance commonly ensues due to numerous mechanisms, including inactivating mutations in or deletions of *PTEN*, activating mutations in *AKT1*, or activating mutations in *IL7R* (IL7Rα) [53, 59–62].

In addition to targeting hyperactivated PI3K-AKT-mTOR signaling in T-ALL indirectly with Notch inhibitors (GSIs), efficacy has also been demonstrated by targeting this pathway directly with multiple inhibitors including dual PI3K/PDK1 inhibitors such as NVP-BAG956, pan-Class I PI3K inhibitors such as GDC-0941, AKT inhibitors such as MK-2206 and GSK690693, mTOR inhibitors such as RAD-001 and KU-63794, and dual PI3K/mTOR inhibitors such as NVP-BEZ235, either alone or cooperatively in combination [63–66]. Not surprisingly, targeting hyperactivated Notch1 and PI3K-AKT-mTOR signaling simultaneously with a GSI and rapamycin has demonstrated a cooperative effect [67]. Additionally, effective combination of targeted signaling pathway inhibitors and standard anti-leukemic chemotherapeutic agents has been reported, specifically, the mTOR inhibitor rapamycin combined with idarubicin as well as the AKT inhibitor triciribine combined with vincristine [68, 69]. Taking advantage of the newer classes of catalytic subunit-specific inhibitors, it has also been shown that hyperactivated PI3K activity in PTEN-null T-ALL is mediated by the hematopoietic-specific catalytic subunits p110δ and p110γ, which can be effectively targeted with CAL-130, a highly potent and specific dual p110δ/p110γ inhibitor [46].

Although the mechanisms for dysregulated PI3K-AKT-mTOR signaling in pre-B-cell ALL are not as clearly worked out as they are in T-ALL, hyperactivation of AKT in patient samples has been linked with poorer prognosis, including

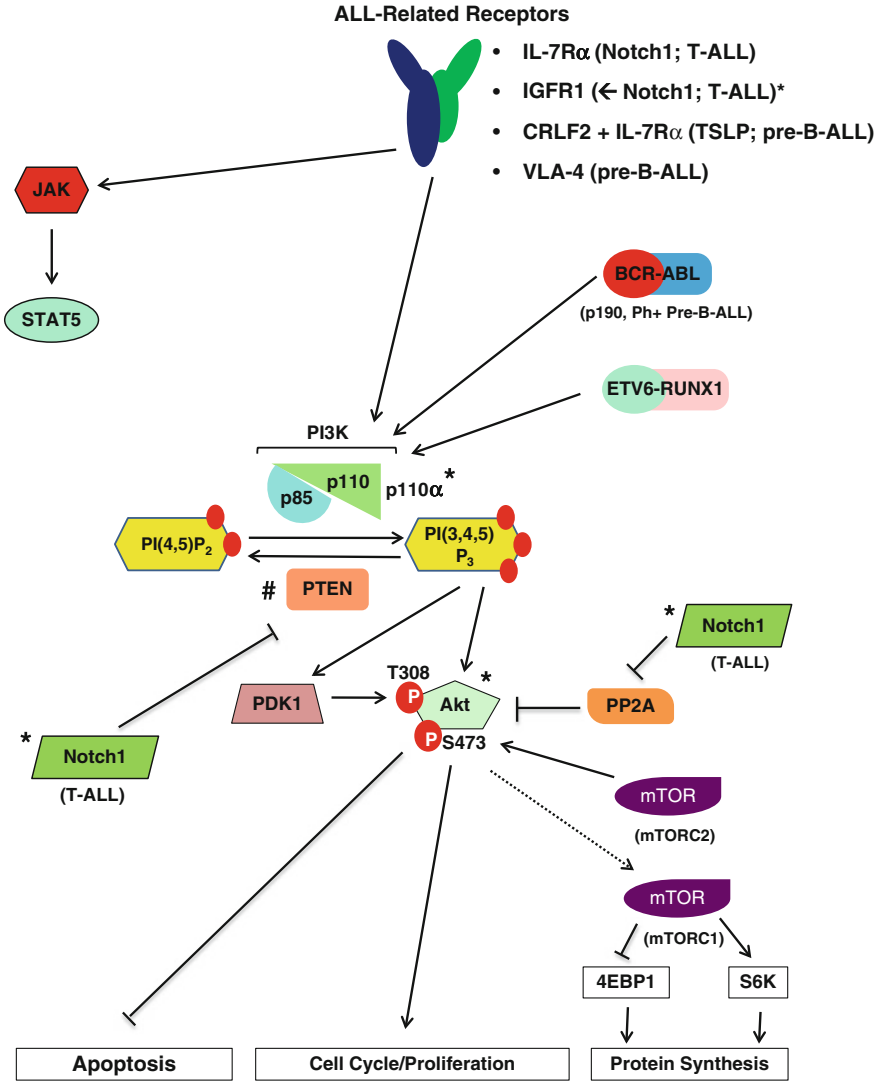


Fig. 7.3 Diagram of the PI3K-AKT-mTOR pathway highlighting important mediators of dysregulated signaling in the development of pediatric Acute Lymphoblastic Leukemia (ALL). Diagram of the PI3K-AKT-mTOR pathway and key associated receptors known to contribute to hyperactivated PI3K signaling described in different forms of pediatric Acute Lymphoblastic Leukemia (ALL), including IL-7Rα and IGFR1, both of which have roles in T-lymphoblastic ALL (associated with gain-of-function mutations in Notch1), the thymic stromal lymphopoietin (TSLP) receptor, a heterodimer formed from CRLF2 and IL-7Rα, and the integrin receptor VLA-4, both of which are associated with pre-B-ALL. Also indicated is the p190 BCR-ABL and ETV6-RUNX1 fusion proteins found in some cases of ALL. Proteins indicated with *asterisk* have gain-of-function mutations reported and those indicated with *hash* have loss-of-function mutations reported

resistance to chemotherapy and reduced survival. This same study also found that exogenously expressing constitutively active AKT in a Pre-B-cell ALL cell line reduces apoptotic response to chemotherapeutic agents suggesting that hyperactivated PI3K-AKT signaling imparts chemoresistance in B-cell leukemic cells [70].

Pre-B-cell ALL is often classified based on characteristic chromosomal abnormalities and translocations, which may provoke hyperactivation of the PI3K-AKT-mTOR signaling pathway through a variety of molecular mechanisms. For example, three percent of pre-B-cell ALL is positive for the Philadelphia Chromosome (Ph+) defined by the translocation 9;22 [t(9;22)], which typically is found in CML and results in the constitutively active fusion protein BCR-ABL [51]. However, while the p210 BCR-ABL variant is found in CML, Ph+ ALL is characterized by the smaller p190 BCR-ABL variant (Fig. 7.3). Both result in constitutive ABL activity, though p190 BCR-ABL is reported to have a stronger transforming potential [71]. Furthermore, it has been observed that Ph+ ALL does not respond as well to the ABL kinase inhibitor imatinib as CML, possibly due to hyperactivation of downstream effectors of ABL activity, including PI3K, which is being investigated as a potential alternative or cooperative therapeutic target. One possible explanation for the hyperactivated PI3K-AKT signaling observed in Ph+ ALL is decreased expression of PTEN as a result of hypermethylation of the *PTEN* gene promoter. This study found that treatment of Ph+ ALL cells with the PI3K inhibitor LY294002 could restore imatinib sensitivity, thereby increasing apoptosis [72]. Another study screening Ph+ pre-B-cell ALL lines found activating mutations in *PIK3CA*, which encodes the catalytic subunit p110α, providing another possible mechanism for hyperactivated PI3K-AKT signaling and imatinib resistance [73]. Furthermore, genetic disruption of PI3K signaling by deletion of *Pik3r1* and *Pik3r2*, which encodes the major Class IA PI3K regulatory subunits p85α and p85β, respectively, significantly decreased BCR-ABL-mediated oncogenic transformation of murine B-cell progenitors and increased imatinib sensitivity. The same study also found that treatment of BCR-ABL-transformed B-cells with the dual PI3K/mTOR inhibitor PI-103 reduced survival both alone and synergistically in combination with imatinib [74]. Finally, the mTOR inhibitor MLN0128 significantly reduced proliferation of Ph+ ALL cells in vitro and cooperated with dasatinib to reduce leukemia burden in an in vivo xenograft mouse model of Ph+ ALL; additionally, MLN0128 was also effective in a xenograft mouse model of Ph− ALL [75]. Similar results were found using the dual mTORC1/mTORC2 inhibitor PP242 in both in vitro and in vivo models of Ph+ ALL [76].

The most common genetic aberration found in Pre-B-cell ALL is the *ETV6/RUNX1* (or *TEL/AML1*) fusion gene t(12;21), which is found in approximately 25 % of patients (Fig. 7.3). One study found that the protein product of this fusion gene regulates PI3K-AKT-mTOR signaling, as evidenced by the fact that knocking it down with shRNA resulted in reduced AKT and ribosomal protein S6 (rpS6) phosphorylation—two mTOR-mediated events—while at the same time increasing apoptosis. Furthermore, pharmacologic inhibition of the PI3K-AKT-mTOR pathway with either the PI3K inhibitor LY294002 or the dual PI3K/mTOR inhibitor PI-103 induced

apoptosis in ALL cells and sensitized glucocorticoid-resistant leukemia cells to the apoptotic effect of prednisolone [77].

Very recently, genomic analysis of MLL-rearranged pre-B-cell ALL revealed a potential role for targeting PI3K signaling. This type of ALL, which accounts for approximately 8 % of cases, affects infants and portends a poor prognosis because of its characteristic glucocorticoid resistance [51, 78]. However, studies have shown that treating MLL-rearranged ALL cells with the PI3K inhibitor LY294002 restored sensitivity to the glucocorticoid prednisolone, through an undefined mechanism that involves down-regulating expression of the *FCRG1B* gene, which encodes the high affinity receptor for IgG (CD64) [78].

Additionally, overexpression of the fibronectin adhesion molecule, very late antigen-4 (VLA-4) is associated with relapse in pediatric ALL and was found to be associated with aberrant expression of several PI3K-AKT-regulated genes, consistent with VLA-4's known role of positively regulating PI3K activity through its α_4 integrin subunit [79]. This again suggests that dysregulated PI3K-AKT-mTOR signaling may be a crucial mediator of chemoresistance, and thus a potentially effective target for relapse or refractory cases of Pre-B-cell ALL.

More recently mutations resulting in hyperactivation or overexpression of the *CRLF2* (cytokine receptor-like factor 2) gene have been described in 6–8 % of pre-B-cell ALL cases [51, 80–82]. CRLF2 heterodimerizes with IL-7Rα to form a receptor for thymic stromal lymphopoietin (TSLP), which mediates normal lymphopoiesis. Aberrant *CRLF2* expression promotes hyperactivated PI3K-AKT-mTOR (as well as JAK-STAT) signaling, particularly in response to TSLP stimulation, which can be reduced with PI3K and mTOR inhibitors such as rapamycin, PI-103, and PP242 [82].

With time, future studies will continue to unravel the variety of molecular mechanisms that give rise to the heterogeneous forms of acute lymphocytic leukemia, especially the forms that are prone to relapse or are refractory to standard chemotherapy and radiation, and will better define which ones may respond to targeted inhibition of the PI3K-AKT-mTOR signaling pathway. This work will also help determine how best to target this pathway, be it with mTOR inhibitors, catalytic subunit-specific PI3K inhibitors, or perhaps even a combination of these inhibitors with a number of other pharmacologic modalities including standard chemotherapy or other targeted agents such as γ-secretase inhibitors.

Lymphoma

Lymphomas are among the most common malignancies of childhood and include Hodgkin Lymphoma and a variety of non-Hodgkin lymphomas, including diffuse large B-cell lymphoma, Burkitt lymphoma, and anaplastic large cell lymphoma [83, 84]. Hodgkin lymphoma, derived from the B-cell lineage, is characterized by Reed-Sternberg cells, is the most common malignancy of adolescence, and currently has a five-year survival greater than 90 % with current chemo- and

radiotherapy protocols. The five year survival of non-Hodgkin lymphoma is lower at 70–90 %, depending on subtype and stage [83, 84]. Despite the overall high cure rate in pediatric lymphoma treatment, there remains concern about the long-term consequences of chemotherapy and radiotherapy in children, including therapy-related secondary malignancies, cardiac dysfunction secondary to thoracic irradiation, and infertility; thus, there continues to be a great need to develop better targeted therapies for these patients.

Hodgkin lymphoma, particularly in young children, the elderly, and immunocompromised patients, is frequently associated with Epstein–Barr virus, whose viral protein latent membrane protein 1 (LMP1) can promote expression of the anti-apoptotic protein BCL-2 and increased NFκB activity [85]. Several studies, however, have reported that LMP1, and the related protein LMP2a, can also promote activation of PI3K-AKT signaling in lymphocytes (Fig. 7.4) [86–88]. Not surprisingly, hyperactivation of PI3K-AKT-mTOR signaling has been demonstrated in several Hodgkin lymphoma-derived cell lines, though not through a loss of PTEN expression. Likewise, these cell lines exhibited reduced proliferation in response to LY294002 and demonstrated a cooperative reduction in cell survival when treated with a combination of rapamycin and doxorubicin [89, 90]. Further studies have suggested that this enhanced PI3K activity, particularly in response to signals from the microenvironment, may be largely mediated by the hematopoietic-specific catalytic subunit p110δ, as efficient apoptosis could be induced with GS-1101, which has shown promise in non-Hodgkin lymphoma as well [34, 91]. These results are interesting particularly in light of the recent findings of EBV+ Hodgkin lymphoma and diffuse B-cell lymphoma in some patients bearing germline gain-of-function mutations in *PIK3CD*, suggesting an important role for p110δ in lymphoma pathogenesis [26].

Most types of non-Hodgkin lymphoma encountered in children, including Burkitt lymphoma and diffuse large B-cell lymphoma, are derived from B-cells, except for anaplastic large cell lymphoma, which is derived from T-cells [92]. Burkitt lymphoma, as with many cases of Hodgkin lymphoma, is associated with Epstein–Barr virus infection, but is also characterized by translocations that result in the juxtaposition of the gene encoding c-Myc with one of the immunoglobulin promoters leading to overexpression of this pro-growth transcription factor, which is normally regulated in part by AKT (Fig. 7.4) [92]. Overexpression of c-Myc and constitutive activation of PI3K signaling have been shown to be crucial events in the pathogenesis of Burkitt lymphoma [93]. The therapeutic potential of rapamycin in Burkitt lymphoma has been demonstrated in a mouse model bearing transgenic expression of c-Myc and LMP2a [94]. Similarly, combined treatment with dual PI3K/mTOR inhibitor PI-103 and the Bcl-XL inhibitor ABT-737, also effectively induced apoptosis in Burkitt lymphoma cell lines [95].

Hyperphosphorylation of AKT has been reported in 52 % of diffuse large B-cell lymphomas tested and correlated with shorter survival [96]. This study found that diffuse large B-cell lymphoma cell lines expressed elevated levels of the anti-apoptotic protein XIAP (Fig. 7.4), which was reduced upon treatment with LY294002 in sensitive cells [96]. Hyperactivation of PI3K has been also linked to

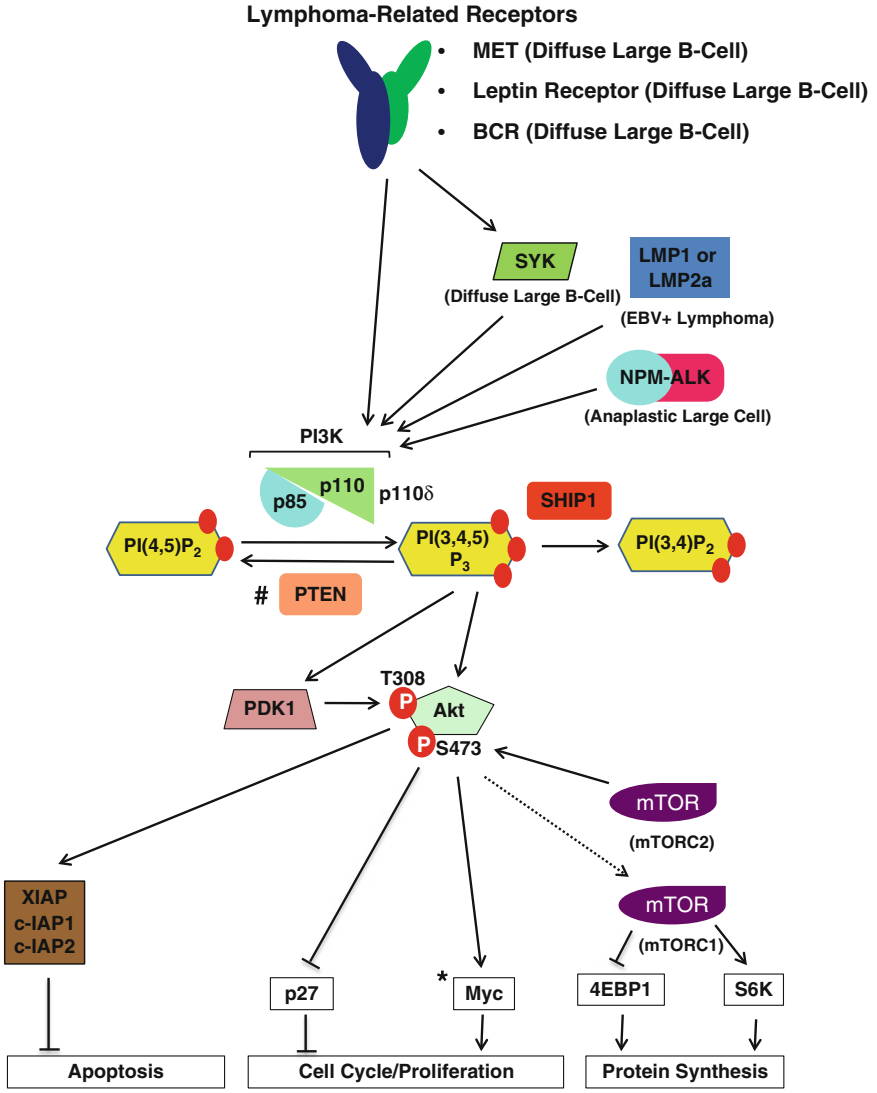


Fig. 7.4 Diagram of the PI3K-AKT-mTOR pathway highlighting important mediators of dysregulated signaling in the development of pediatric Lymphoma. Diagram of the PI3K-AKT-mTOR pathway and key associated receptors known to contribute to hyperactivated PI3K signaling described in different forms of pediatric Lymphoma, including MET, the Leptin receptor, and the B cell receptor (BCR), all three of which are associated with Diffuse Large B-Cell Lymphomas. Additional proteins including Syk in Diffuse Large B-Cell Lymphoma, the viral-encoded protein LMP in EBV+ lymphomas, and the NPM-ALK fusion protein in Anaplastic Large Cell lymphoma have been shown to participate in PI3K signaling in lymphoma. Proteins indicated with *asterisk* have gain-of-function mutations reported and those indicated with *hash* have loss-of-function mutations reported

dysregulated hepatocyte growth factor (HGF)-MET signaling in one study and overexpression of the leptin receptor in another, while the mTOR effector p70S6K and the microRNA miR155 have also been identified as potential therapeutic targets in diffuse large B-cell lymphoma [97–100]. In a recent study, the spleen tyrosine kinase (SYK) downstream of the B-cell receptor (BCR) was identified as another activator of PI3K-AKT signaling in diffuse large B-cell lymphoma. Accordingly, several studies have demonstrated the potential effectiveness of targeted inhibitors of the PI3K-AKT-mTOR signaling pathway in diffuse large B-cell lymphoma, including rapamycin in combination with rituximab, the pan-PI3K inhibitor NVP-BKM120, or the dual PI3K/mTOR inhibitor NVP-BEZ235 [101–103].

Anaplastic large cell lymphoma is associated with the translocation t(2;5), which leads to expression of the chimeric protein NPM-ALK (nucleophosmin-anaplastic lymphoma kinase), bearing constitutive tyrosine kinase activity [92, 104]. One study found that two-thirds of anaplastic large cell lymphoma patient samples show evidence of hyperactivated PI3K-AKT signaling and many of these samples show post-translational inactivation of PTEN by increased phosphorylation [105]. Furthermore, ALK has been reported to promote activation of PI3K signaling by directly binding the p85 regulatory subunit [106–108]. Increased AKT phosphorylation in anaplastic large cell lymphoma has been correlated with decreased expression of the cell cycle inhibitor p27, which can be reversed with pharmacologic inhibition of AKT [109]. Hyperactivated mTOR has also been described as potential therapeutic target in anaplastic large cell lymphoma [110, 111].

Acute Myeloid Leukemia (AML)

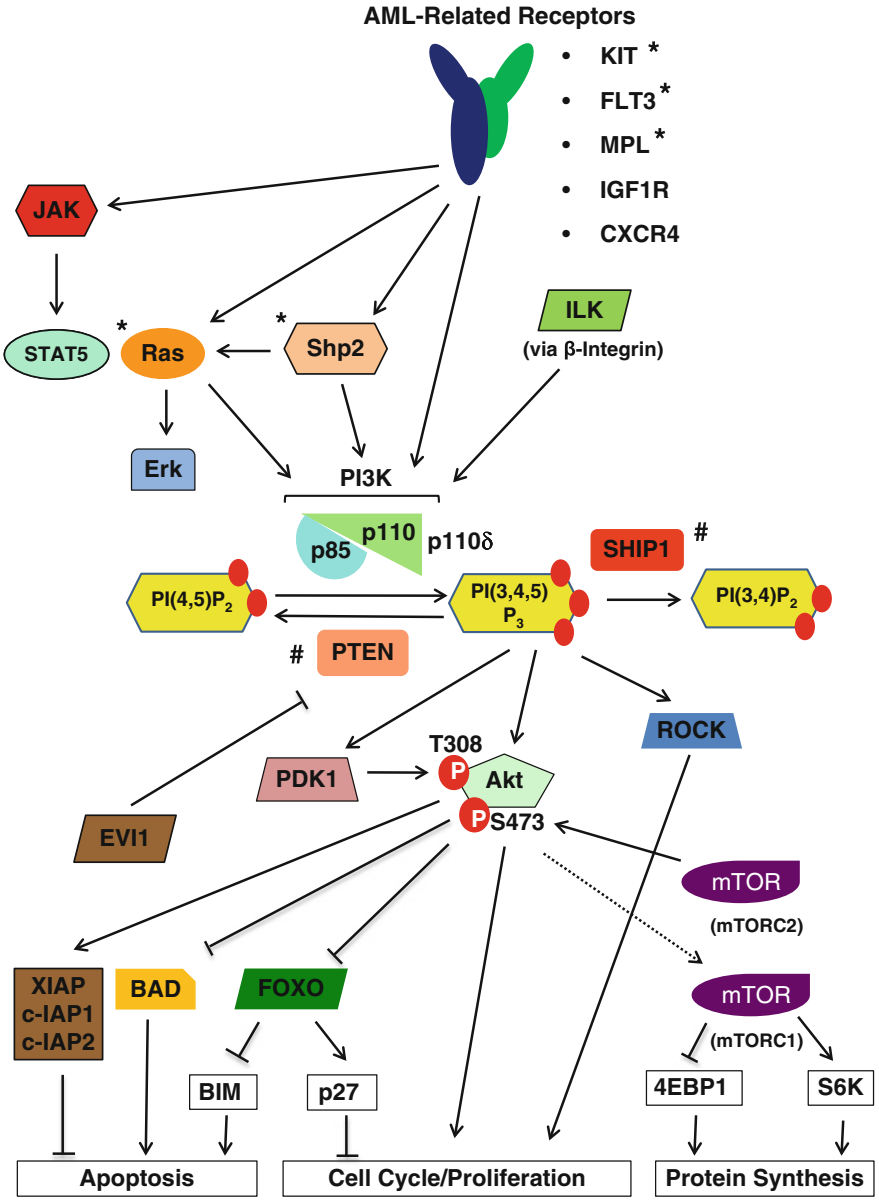
Acute myeloid leukemia (AML) represents a clonal hyperproliferation of immature myeloid lineage progenitor cells, which is relatively uncommon in children (about 15–20 % of childhood leukemias), but is much more prevalent in adults. Because AML is predominantly a disease of elderly adults, there are only a few studies that specifically address pediatric AML and the role of PI3K-AKT signaling in its pathogenesis; however, presumably much of what is learned from studying adult AML can be applied to pediatric AML. Like ALL, pediatric AML is a very heterogeneous disease in terms of underlying genetic and cytogenetic lesions, but unlike ALL, it has a more dismal prognosis with the current overall survival being approximately 60–70 % [112]. As with ALL, the goal for improving outcomes in pediatric AML is to develop a better understanding of the molecular pathogenesis associated with each genetic and phenotypic subtype and to identify and implement targeted therapeutic strategies accordingly.

A number of recent studies have shown that hyperactivated PI3K-AKT-mTOR signaling represents a potentially relevant therapeutic target for many AML subtypes, as over-expressed and/or hyperphosphorylated AKT has been reported in 50–80 % of adult AML cases and has been linked with decreased survival [50, 113, 114]. However, contrary to AKT's traditionally understood role in cancer

development, at least one study found that constitutive activation of PI3K-AKT signaling was associated with improved overall and relapse-free survival in de novo adult AML, consistent with another study that found that reduced AKT activity and elevated FOXO activation promoted AML pathogenesis by inhibiting differentiation of leukemia-initiating cells [115, 116]. In both pediatric and adult AML, hyperactivated PI3K-AKT-mTOR signaling may result as a consequence of a number of commonly encountered leukemogenic genetic lesions, including mutations in receptors such as KIT and FLT3 and upstream signaling molecules such as RAS (Fig. 7.5), though other molecular mechanisms have been reported.

One of the most commonly encountered mutated genes in AML resulting in PI3K hyperactivation is the Class III receptor tyrosine kinase *KIT*, which is found to be mutated in a variety of other non-hematologic malignancies as well, including gastrointestinal stromal tumors (GISTs) [117, 118]. One study found *KIT* mutations in 11.3 % of pediatric AML samples [119]. Notably, mutant *KIT* bearing point mutations in the activation loop (KIT D816V), which is found commonly in both AML and the myeloproliferative neoplasm, systemic mastocytosis, is insensitive to imatinib inhibition [120–125]. In contrast, mutant *KIT* bearing mutations in the extracellular domain (typically complex in-frame deletion plus insertion mutations in exon 8 affecting the conserved D419 amino acid) or in the transmembrane domain (V530I) exhibit hyperactivated PI3K-AKT signaling which is sensitive to pharmacologic inhibition with imatinib [126].

The activation loop mutations in *KIT*, as well as other *KIT* mutations, are frequently associated with core binding factor (CBF) AML, an AML subset characterized by the cytogenetic aberrations, t(8;21) or inversion of chromosome 16 [inv (16)], that disrupt the function of the core binding factors, CBFα (also known as AML1) and CBFβ, by generating the fusion genes, *AML1-ETO* and *CBFβ-MYH11*, respectively. Interestingly, CBF AML typically has a better prognosis than normal karyotype AML [127]. A possible explanation for this observation is that CBFα positively regulates expression of PI3K catalytic subunit p110δ, so that loss of CBFα by t(8;21) might decrease p110δ expression, resulting in reduced PI3K signaling and, as a result, enhanced chemosensitivity [128]. Conversely, the addition of a *KIT* mutation to a CBF cytogenetic aberration actually portends a poorer prognosis in AML [129–135]. A study analyzing common mutations in pediatric AML found that 37 % of pediatric CBF AML had a concurrent *KIT* mutation [119]. The Kasumi-1 murine AML cell line, which is characterized by t(8;21) and a *Kit* activating loop mutation (N822K) and which exhibits hyperactivated PI3K-AKT signaling, is a frequently used research tool to investigate the molecular pathogenesis of this type of leukemia [136]. Because leukemias bearing *KIT* D816V mutations cannot be effectively treated with imatinib, hyperactivated downstream signaling pathways such as PI3K-AKT are being investigated as potential therapeutic targets. For example, it has been reported that p85α-dependent PI3K signaling is hyperactivated in the presence of *Kit* D814V (the murine form of *KIT* D816V), contributing to the ligand-independent hyperproliferative phenotype, which can be reversed by genetic disruption of *Pik3r1* (encoding p85α) or by pharmacologic inhibition of mTOR with rapamycin [122]. Furthermore, genetic


AML-Related Receptors
KIT *
FLT3 *
MPL *
IGF1R
CXCR4
JAK
STAT5
Ras
Shp2
ILK
(via β-Integrin)
Erk
PI3K
p85
p110
p110δ
SHIP1
#
PI(4,5)P2
PI(3,4,5)P3
PI(3,4)P2
PTEN
ROCK
T308
PDK1
Akt
S473
EVI1
mTOR
(mTORC2)
XIAP
c-IAP1
c-IAP2
BAD
FOXO
mTOR
(mTORC1)
BIM
p27
4EBP1
S6K
Apoptosis
Cell Cycle/Proliferation
Protein Synthesis

◀ **Fig. 7.5** Diagram of the PI3K-AKT-mTOR pathway highlighting important mediators of dysregulated signaling in the development of pediatric Acute Myeloid Leukemia (AML). Diagram of the PI3K-AKT-mTOR pathway and key associated receptors known to contribute to hyperactivated PI3K signaling described in different forms of pediatric Acute Myeloid Leukemia (AML), including KIT, FLT3, MPL, IGF1R, and CXCR4. The intracellular kinase, integrin linked kinase (ILK), associated with β-Integrin signaling has also been reported to play a role in dysregulated PI3K signaling in AML. Proteins indicated with *asterisk* have gain-of-function mutations reported and those indicated with *hash* have loss-of-function mutations reported

disruption of p85α improves survival in a murine model of *Kit* D814V-induced myeloproliferative disease, suggesting that targeting hyperactivated PI3K represents a potential therapeutic option for mutant *KIT*-associated AML and mastocytosis [137]. Additional work has shown that dysregulated PI3K signaling promotes hyperactivation of Rho Kinase (ROCK), which is an important mediator of not only mutant KIT-mediated leukemogenesis, but also of enhanced proliferation and survival induced by other common leukemia-associated mutations, including FLT3 internal tandem duplications (FLT3-ITD, also commonly found in AML) and BCR-ABL (pathognomonic for CML) [138]. Activating loop mutations in *KIT* can dysregulate several signaling pathways along with PI3K-AKT, including STAT5, which along with PI3K-AKT has been reported to promote rapid cell cycling, and SHP2-RAS-ERK, which has been reported to promote enhanced survival [139]. Thus, it is becoming more and more apparent that additional signaling pathways may need to be targeted alongside hyperactivated PI3K-AKT to effectively treat hematologic malignancies such as AML. Finally, in addition to point mutations in *KIT*, internal tandem duplications (ITD) involving the juxtamembrane domain (exons 11 and 12) have also been reported to affect 7 % of pediatric AML cases. *KIT*-ITD mutations similarly promote hyperactivation of PI3K-AKT-mTOR signaling, but are sensitive to imatinib treatment, such that dual inhibition with imatinib and rapamycin synergistically suppresses ligand-independent hyperproliferation [140].

Accounting for approximately one-third of adult AML cases, the most frequently mutated gene in AML is *FLT3* (FMS-like tyrosine kinase 3), encoding a Class III Receptor Tyrosine Kinase like *KIT*. The most frequently encountered mutations in *FLT3* are internal tandem duplications (*FLT3*-ITD) and point mutations in the activating loop of the second tyrosine kinase domain, most often affecting D835 [141]. When stimulated by its ligand, FLT3 activates a number of signaling pathways and molecules including STAT5, RAS-ERK, and PI3K-AKT, the latter of which is mediated by interactions with the protein tyrosine phosphatase SHP2 or the lipid phosphatase SHIP [141–143]. Expression of both *FLT3*-ITD and *FLT3* with tyrosine kinase domain mutations induces constitutive activation of AKT as well as SHP2, ERK, and STAT5 [144, 145].

FLT3-ITD mutations are found in approximately 5–13 % of pediatric AML cases, and are associated with CBF aberrations much less frequently than *KIT* or *RAS* mutations [119, 146–148]. Expression of *FLT3*-ITD in the murine-derived Ba/F3 cell line has been observed to promote activation of AKT, which can

phosphorylate and inactivate the Forkhead transcription factor family member, Foxo3a, leading to downregulation of the cell cycle inhibitor p27 and the pro-apoptotic mediator, Bim, resulting in enhanced proliferation and survival (Fig. 7.5) [149]. In another study, investigators found that treatment of *FLT3*-ITD-positive cell lines and primary patient samples with KP372-1, a multi-kinase inhibitor that targets PDK1, AKT, and FLT3, reduced phosphorylation of AKT as well as FOXO3a and BAD, two direct targets of AKT kinase activity, and induced apoptosis [150]. BAD, a positive regulator of apoptosis, is inhibited by phosphorylation by not only AKT, but also by PIM1, another signaling kinase that is hyperactivated downstream of FLT3-ITD [150, 151]. Accordingly, simultaneous targeting of PI3K with GDC-0941 and PIM1 with the highly potent and selective inhibitor EVP-45299 synergistically induced apoptosis in MV411 cells, which displays hyperactivated PI3K-AKT signaling as a result of *FLT3*-ITD [152]. Furthermore, dual inhibition of PDK1 and PI3K with the compound BAG956 reduced proliferation in Ba/F3 cells exogenously expressing FLT3-ITD both alone and in combination with rapamycin, in addition to synergizing with the tyrosine kinase inhibitor PKC412, which previously had been shown to reduce FLT3-ITD-induced hyperphosphorylation of AKT [149, 153]. Targeting AKT alone with an inhibitor such as perifosine has also been shown to induce apoptosis and chemosensitivity in two AML cell lines including MV411 and THP-1, the latter of which displays hyperactivated PI3K-AKT (as well as elevated ERK activity) as a consequence of p110δ hyperactivity [154, 155]. Furthermore, mTOR and its key downstream effectors, 4EBP1 and p70S6K (Fig. 7.5), have been shown to be hyperactivated in the presence of *FLT3*-ITD as well, suggesting that these too might be druggable targets in AML [156].

Mutations in the *RAS* proto-oncogenes (*NRAS*, *KRAS*, and *HRAS*) are among the most common genetic lesions found in human malignancies and account for 10–25 % of adult AML cases, with *NRAS* being the most common; the presence of *RAS* mutations appears to either have a positive or equivocal effect on disease prognosis [157–162]. Specifically in pediatric AML, two independent studies found *RAS* mutations in 18 % of cases, making them the most commonly mutated genes in pediatric AML [119, 163]. Furthermore, *RAS* mutations, like *KIT* mutations, are frequently associated with pediatric CBF AML, and have been reported to be present in about 30 % of pediatric CBF AML samples [119]. As PI3K is a known direct effector of RAS, it is not surprising that hyperactivated PI3K-AKT-mTOR signaling in addition to hyperactivated MAPK signaling is found in the presence of activating *RAS* mutations [13–17, 157].

Numerous other less common genetic lesions and molecular aberrations that promote dysregulation of the PI3K-AKT-mTOR signaling pathway have been identified, and their frequency in patients and relevance to disease progression are just beginning to be understood. For instance, one study found that a small subset of *AML1-ETO*-positive AML exhibited overexpression of the thrombopoietin receptor, MPL, which resulted in increased PI3K activity [164]. A series of recent reports have described loss of function mutations in the tumor suppressor *SHIP1* in about 3 % of AML cases. The lipid phosphatase SHIP1, similar to PTEN, antagonizes

PI3K activity, except by removing the 5' phosphate group of $PI(3,4,5)P_3$ rather than the 3′ phosphate (Fig. 7.5). These mutations have been reported to interrupt either the phosphatase activity of SHIP1 or its ability to interact with other signaling molecules, suggesting that it has scaffolding function as well as an enzymatic function [165–167]. Although frequently associated with JMML, activating mutations in the protein tyrosine phosphatase SHP2, a known positive regulator of RAS and PI3K activity, have also been reported in AML [168]. At the same time, given the importance of hyperactivated PI3K-AKT signaling in the development and progression in AML, it is surprising that some expected mutations such as activating mutations in the PH domain of *AKT1*, which have been described in some solid tumors and which can induce leukemia in mice, have not been found in adult or pediatric AML patients [169, 170].

Efforts to identify pathologic functions for individual PI3K catalytic subunits have also been applied to AML. Gain-of-function mutations in the helical domain or the kinase domain of p110α (encoded by *PIK3CA*) are frequently encountered in solid tumors, but are rare in hematologic malignancies, though the leukemogenic potential of constitutively active mutant p110α (but not p85α) has been demonstrated in vitro [171, 172]. Sujobert et al. [22] analyzed AML patient samples and found that p110δ, in contrast to the other Class I PI3K catalytic subunits, was consistently highly expressed in leukemic blasts, and that treatment with the p110δ-specific inhibitor IC87114 efficiently reduced proliferation and AKT hyperphosphorylation. Additionally, IC87114 has been reported to enhance the cytotoxic effects of the chemotherapeutic agent VP16, demonstrating the potential utility of p110δ-specific inhibitors as a valuable adjunct to standard chemotherapy [155]. Similarly, another group found that targeting p110δ specifically with PCN5603 (either alone or in synergistic combination with the MEK inhibitor U0126) reduced AKT phosphorylation and promoted cell killing in AML patient samples, lending further support for the role of p110δ. However, these investigators also found similar results blocking p110α with PI3Kα inhibitor, suggesting that p110α may also be a reasonable target [173, 174].

The role of PTEN in adult or pediatric AML is not quite clear. One study did not find a consistent decrease in PTEN expression in patient samples, while other studies found that inactivating mutations in *PTEN* were rare [175, 176]. C-terminal phosphorylation of PTEN, which is associated with decreased PTEN activity, was found in one study to be present in almost 75 % of AML patient samples and to correlate with increased AKT activation and decreased survival [177]. Overexpression of the transcription factor EVI1 is found in 10 % of AML cases and has been reported to downregulate *PTEN* [178, 179]. A more recent study found that the microRNA miR-193a is epigenetically silenced in the presence of *AML1-ETO*, leading to downregulation of PTEN [180]. Thus, the role of aberrant PTEN expression or function in promoting hyperactivation of PI3K-AKT signaling in pediatric and adult AML appears limited, but more studies will be required to resolve this question definitively.

Besides pathologically enhancing survival and proliferation, hyperactivation of PI3K signaling has been associated with promoting chemoresistance in AML. For

example, one study found that hyperactivated PI3K-AKT signaling in AML promotes MDM2-mediated downregulation of p53, resulting in derepression of the multidrug resistance-associated protein 1 (MRP1) [181]. Additionally, a number of studies have linked constitutively active PI3K-AKT signaling and chemoresistance to autocrine insulin-like growth factor-1 (IGF-1) signaling, which can be interrupted by pharmacologically inhibiting PI3K or blocking IGF-1 receptor with a neutralizing antibody [182, 183]. Investigators in one study found that hyperactivated PI3K-AKT signaling due to autocrine IGF-1 signaling decreased sensitivity to the chemotherapeutic agents camptothecin and etoposide and the differentiation-inducing agent all-trans retinoic acid (ATRA) in a chemoresistant clone of the HL-60 cell line, which could be reversed with the PI3K inhibitors LY294002 or Wortmannin [184]. It should also be noted that this autocrine IGF-1 signaling loop can be disinhibited by pharmacologic blockade of mTORC1/mTORC2 with RAD001 as an example of the complex regulatory networks that govern PI3K-AKT signaling in AML [185]. Another group found that this autocrine IGF-1-stimulated PI3K activity was largely mediated by p110δ and p110β, as evidenced by shRNA knockdown of those two isoforms or by pharmacologic inhibition of p110δ with IC87114 or p110β with TGX-221 [182].

In acute promyelocytic leukemia (APL), associated with t(15;17) and expression of the PML-RARα fusion protein, ATRA is frequently used to induce differentiation of accumulating immature leukemic blasts, though use of this agent is also associated with promoting survival and counteracting the cytotoxic effects of chemotherapeutic agents such as doxorubicin and arsenic trioxide, possibly through a PI3K-AKT-mediated survival mechanism [186–190]. However, it has been demonstrated that targeting PI3K, particularly the p110β and p110δ isoforms, can reverse the pro-survival effects of ATRA, while sparing its pro-differentiation effects [190].

A number of other PI3K-AKT-mTOR pathway inhibitors such as deguelin, a naturally occurring compound that inhibits PI3K, the AKT inhibitor MK-2206, and the dual PI3K/mTOR inhibitors PI-103 and NVP-BEZ235 have also been shown to induce apoptosis and enhance chemosensitivity [191–195]. Furthermore, simultaneous inhibition of the anti-apoptotic proteins Bcl-2 and Bcl-xL with ABT-737 enhanced the apoptosis-inducing effects of PI-103 and NVP-BEZ235, as well as the pan-PI3K inhibitor GDC-0941 [196, 197]. PI3K-mediated resistance to chemotherapy-induced apoptosis in AML may be due to AKT's negative regulation of pro-apoptotic proteins like BAD and BIM, discussed above, but increased expression of anti-apoptotic proteins such as c-IAP1, c-IAP2, and XIAP in the presence of hyperactivated AKT have also been reported (Fig. 7.5) [184, 198, 199]. Furthermore, AKT-mediated overexpression of these anti-apoptotic proteins may be in cooperation with other hyperactivated signaling pathways such as MAPK and NF-κB, suggesting that targeting additional pathways may be necessary to effectively overcome evasion of apoptosis and chemoresistance in AML cells [198, 200–202].

Besides causing dysregulation of pro- and anti-apoptotic proteins, others have reported additional mechanisms by which PI3K-AKT signaling might result in chemoresistance, especially in regard to transducing signals involved in bone marrow microenvironment interactions. For example, co-culturing HL-60 with

bone marrow-stromal cells resulted in a PI3K-dependent upregulation of the anti-apoptotic protein XIAP [203]. Specifically, one proposed mechanism for bone marrow microenvironment-mediated activation of PI3K-AKT involves direct activation of integrin-linked kinase (ILK) by β-integrins [204]. In addition to PI3K playing a role in integrin "outside-in" signaling, another study found that *FLT3*-ITD-mediated hyperactivation of PI3K positively regulated $\alpha_4\beta_1$ integrin (VLA4) affinity via a Pyk2-dependent "inside-out" signaling mechanism, which might contribute to enhanced bone marrow environment interactions of leukemic stem cells, resulting in quiescence and chemoresistance [205]. Another group found that chemoresistance in *FLT3*-ITD expressing cells mediated by hypoxia-induced downregulation of PIM1 and MCL-1 could be reversed with combined treatment with sorafenib, a tyrosine kinase inhibitor with high specificity for FLT3, and the highly potent pan-PI3K inhibitor GDC-0941 [206]. Expression of the CXC chemokine receptor type 4 (CXCR4), which promotes stromal-mediated survival of hematopoietic and leukemic stem and progenitor cells when bound by its ligand SDF-1α, has also been found to be positively regulated by PI3K-AKT-mTOR signaling, and can be repressed with mTORC1/mTORC2 inhibitor PP242 [207]. Thus, in addition to serving as a primary therapeutic target for AML, inhibiting PI3K may also play a crucial role in overcoming resistance to standard chemotherapy in refractory cases.

Although provoked by a wide variety of molecular mechanisms, hyperactivated PI3K-AKT-mTOR signaling is clearly present and plays a key pathophysiologic role in the majority of AML cases, and thus it represents a potentially important molecular target for this disease. As AML is primarily a condition of the elderly, most of the research focus has been directed towards adult AML with few studies specifically addressing pediatric AML. Thus, it is not clear what differences if any exist between the pediatric and adult forms of the disease in regard to the potential therapeutic efficacy of targeting PI3K-AKT signaling or any other molecular aberrations. Presumably, much of what is learned in adult AML can be effectively applied to the treatment of pediatric AML patients, though certainly more work is needed to understand each as a distinct entity.

Chronic Myeloid Leukemia (CML)

Chronic myeloid leukemia (CML) is extremely rare in children and is, as in adult patients, classically defined by the translocation t(9;22) resulting in the Philadelphia Chromosome and the p210 BCR-ABL fusion protein (Fig. 7.6). CML is characterized by three phases: an indolent chronic phase, during which most pediatric and adult patients present; a more aggressive accelerated phase, exhibiting less than 20 % blasts; and the rapidly fatal blast crisis, exhibiting more than 20 % blasts and resembling AML [208]. Previously only curable with HSCT, with the introduction of the ABL kinase-specific tyrosine kinase inhibitor (TKI) imatinib, CML has become the paradigm of translating the fundamental understanding of the molecular

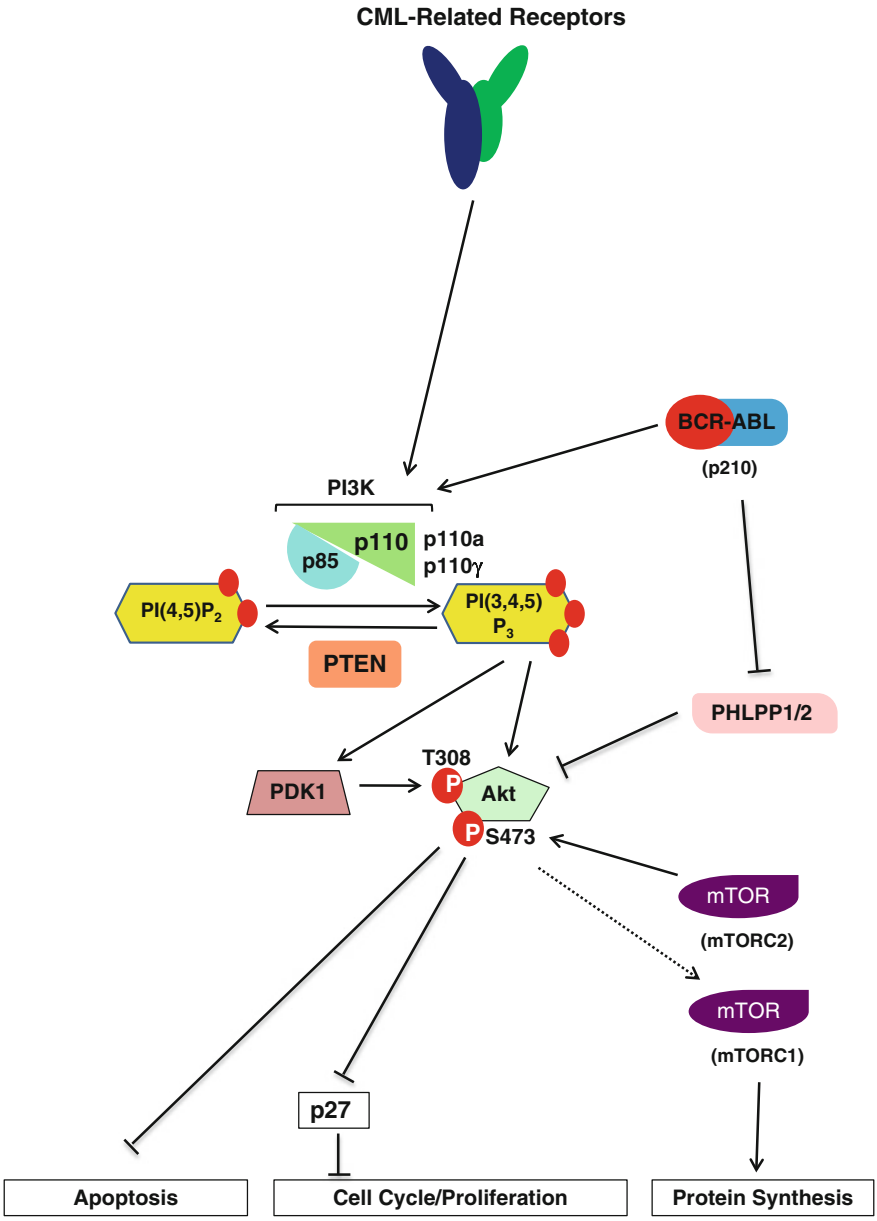


Fig. 7.6 Diagram of the PI3K-AKT-mTOR pathway highlighting important mediators of dysregulated signaling in the development of pediatric Chronic Myelogenous Leukemia. Diagram of the PI3K-AKT-mTOR pathway in pediatric Chronic Myelogenous Leukemia (CML), which is characterized by the intracellular kinase fusion protein BCR-ABL (typically the p210 form)

pathogenesis of a disease into rational drug design [209, 210]. Like adults, children with CML are primarily treated with imatinib or second generation TKIs such as dasatinib, with HSCT reserved for cases in which TKI resistance develops [211–213]. However, there have been reports that long-term treatment of children with TKIs has resulted in growth retardation and bone defects, so it is appropriate to explore other potential therapeutic targets as alternative therapies or in combination with currently used TKIs for the treatment of childhood CML [214–218]. Furthermore, the development of imatinib resistance due mutations in or amplifications of *BCR-ABL* or other mechanisms have also prompted the investigation of other potential therapeutic strategies for both adult and childhood CML [219–222].

BCR-ABL, as a consequence of its constitutive tyrosine kinase activity, is believed to promote dysregulation of a number oncogenic signaling pathways that control proliferation and survival, including the PI3K-AKT-mTOR signaling pathway; accordingly, this pathway is being investigated as a potential alternative or cooperative target for the treatment of CML (and Ph+ B-ALL as described above). For instance, treatment with the PI3K inhibitor LY294002 in one study reduced hyperproliferation of CML cells both alone and cooperatively with imatinib, and it has also been reported that imatinib can reduce hyperphosphorylation of both AKT and ERK in chronic phase CML patient samples. Notably, AKT hyperphosphorylation was not altered by imatinib treatment of blast crisis patient samples, suggesting that progression of CML in part may depend on acquired mechanisms of dysregulated PI3K signaling [223, 224].

A number of mechanisms for BCR-ABL-mediated activation of PI3K have been proposed, including one study that found the p85 regulatory subunit interacts directly with the BCR-ABL fusion protein, while another study found that activation of PI3K-AKT and RAS-ERK signaling was mediated by the adapter protein Gab2, which forms a complex with another adapter protein Grb2 and binds to the phosphorylated Tyr177 of BCR-ABL [225, 226]. Additional work demonstrated that the serine-threonine phosphatases PHLPP1 and PHLPP2, which inactivate AKT by dephosphorylation of Ser473, were downregulated in the presence of BCR-ABL (Fig. 7.6) [227]. BCR-ABL-mediated increases in reactive oxygen species have also been reported to promote hyperactivation of AKT [228]. Finally, Hickey and colleagues showed that BCR-ABL can promote overexpression of the hematopoietic-specific Class IB PI3K catalytic subunit p110γ, suggesting that CML-mediated PI3K hyperactivation might also be targeted with a catalytic subunit-specific pharmacologic approach [24].

As with other leukemias, the consequence of BCR-ABL-induced hyperactivation of PI3K-AKT signaling is enhanced proliferation, survival, and chemoresistance. For example, several groups have found that BCR-ABL promotes uncontrolled proliferation through AKT-mediated inactivation of cell cycle inhibitors such as p21 and p27, possibly through upregulation of the E3 ubiquitin ligase, SKP2 [229–231]. Reported mechanisms for PI3K-mediated enhanced survival in the presence of BCR-ABL include downregulation of the HOXA10 gene and c-Myc-mediated upregulation of the anti-apoptosis protein survivin [232, 233].

In addition to promoting BCR-ABL-induced leukemogenesis, additional dysregulation of PI3K-AKT-mTOR signaling in response to imatinib therapy has also been reported to contribute to the development of imatinib resistance in CML, which can be blocked by the dual mTORC1/mTORC2 inhibitor, RAD001 [234, 235]. Hyperphosphorylation of the adapter molecule Gab2 at Tyr452, a known binding site for the p85 regulatory subunit of PI3K, has also been linked to TKI resistance, which can be overcome with combined treatment with dasatinib and the dual PI3K/mTOR inhibitor NVP-BEZ235 [236]. Beside TKI resistance, a role for dysregulated p110α-mediated PI3K signaling has also been described in decreasing sensitivity to the standard chemotherapeutic agent doxorubicin in CML [237].

Thus, while the original hope for CML was to cure it with TKIs such as imatinib, the development of resistance has prompted a search for secondary molecular targets that can be taken advantage of once resistance has developed or to prevent resistance from developing in the first place. As an important mediator of BCR-ABL's pathologic growth and survival promoting activity, the PI3K-AKT-mTOR signaling pathway has become one of the most widely studied and promising candidates.

Juvenile Myelomonocytic Leukemia (JMML)

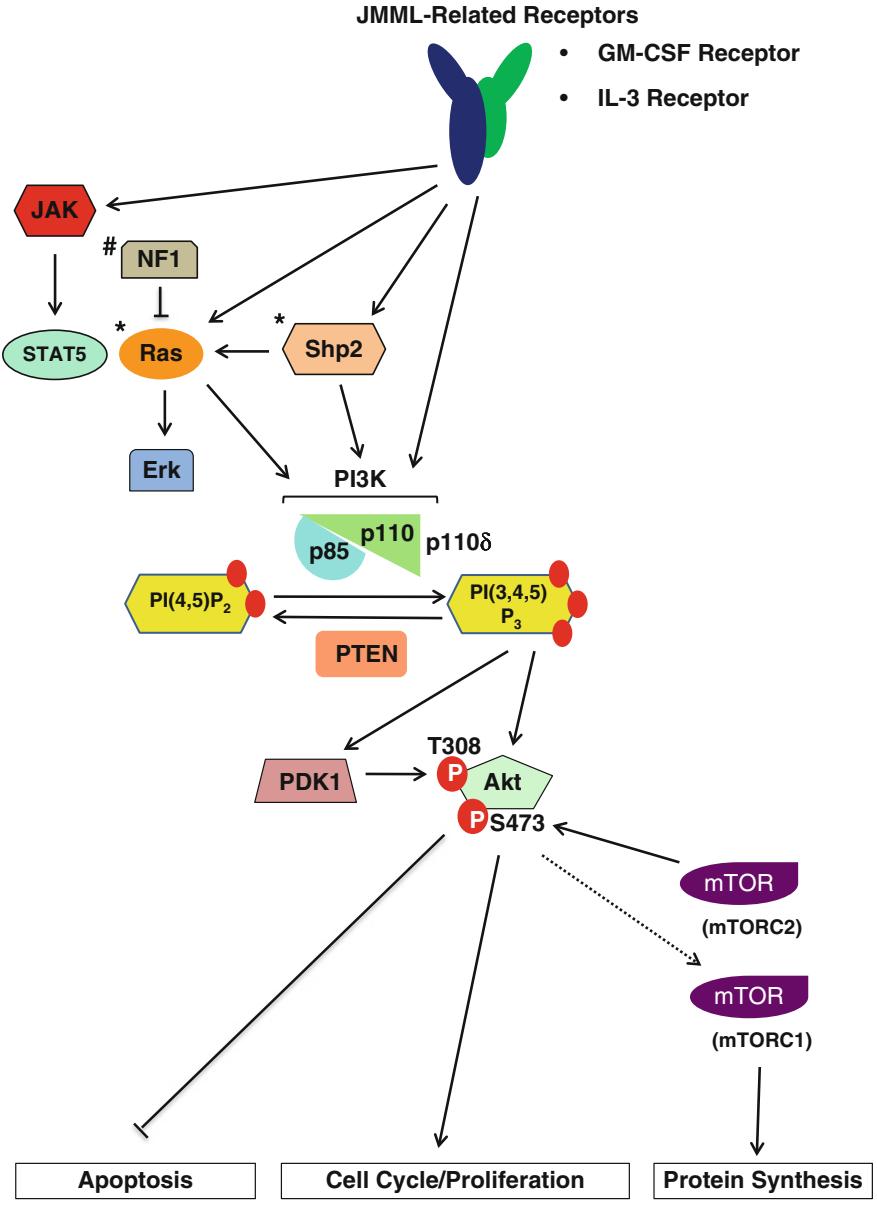
Juvenile Myelomonocytic Leukemia (JMML) is a rare and often fatal myeloproliferative disease (MPD) of early childhood, typically affecting infants and toddlers. The World Health Organization (WHO) categorizes JMML as an overlap myeloproliferative neoplasm/myelodysplastic syndrome (MPN/MDS), accounting for 2–3 % of childhood leukemias with an incidence of 1–2 cases per million per year and approximately 25–50 cases per year in the United States [238]. The typical initial presentation of a child with JMML is non-specific, with signs and symptoms that include fever, failure to thrive, lymphadenopathy, splenomegaly, and elevated white blood cell counts with monocytosis. Due to the somewhat non-specific presentation of JMML, it can be difficult to distinguish from bacterial or viral infections, causing a delay in the proper diagnosis [238, 239]. WHO diagnostic criteria for JMML include absence of the *BCR-ABL* fusion gene, which characterizes CML, circulating monocyte count greater than 1×10^9/L, and less than 20 % blasts observed in a bone marrow biopsy [240]. In addition, almost all patients develop splenomegaly at some point during the course of their disease, and for most patients it has already developed by the time of presentation [238]. Other common clinical features that may assist in the diagnosis of JMML include elevated Hemoglobin F, the presence of a cytogenetic abnormality (most commonly monosomy 7), and hypersensitivity of myeloid progenitor cells to Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) [240–243].

At this time, the only curative therapy for JMML is allogeneic HSCT, which under current protocols has around a 50 % overall survival rate [242, 244, 245]. HSCT is plagued with many challenges including relatively high rates of relapse

and complications such as infection, bleeding, and graft-versus-host disease (GVHD); therefore better treatment modalities for JMML are badly needed [244]. To that end, much work has been done over the past several years to understand the molecular mechanisms underlying JMML. Patients with JMML frequently exhibit mutations in genes encoding proteins involved in the RAS-ERK signaling pathway, suggesting that RAS hyperactivation is a common etiologic feature in JMML. These mutations include gain-of-function mutations in *KRAS* and *NRAS* (together accounting for approximately 20 % of cases) [246, 247], as well as loss-of-function mutations in the RAS GTPase Activating Protein (GAP), *NF1* (in 15 % of cases) [248], which negatively regulates RAS activity. Most recently, loss-of-function mutations in the E3 ubiquitin ligase, *CBL*, have been described (in 10–15 % of cases) [249]. The most common mutations in JMML patients, however, are gain-of-function (GOF) mutations in *PTPN11*, which encodes the protein tyrosine phosphatase, SHP2, accounting for slightly more than one-third of JMML cases [250]. SHP2 is known to be a positive regulator of RAS activity, but the precise mechanism is still not completely understood. Despite these advances, causative mutations have not been identified in approximately 10–15 % of JMML patients, though progress continues to be made as very recently secondary mutations in two new genes—*SETBP1* and *JAK3*—have been described [251–253].

Of particular importance to JMML is hyperactivation of mitogenic signaling pathways such as PI3K-AKT downstream of the GM-CSF receptor (Fig. 7.7), since a common clinical diagnostic feature of JMML is hypersensitivity to GM-CSF, in which myeloid progenitors from JMML patients exhibit an enhanced proliferative response following GM-CSF stimulation [243]. SHP2 positively regulates PI3K activation in response to GM-CSF stimulation (Fig. 7.7), and we and others have shown that expression of GOF SHP2, such as E76K or D61Y, in myeloid progenitor cells is sufficient to induce the GM-CSF hypersensitivity phenotype in these cells [254–257]. Additionally, we have shown that cells expressing GOF mutant SHP2 exhibit substantially increased signaling through both the RAS-ERK and PI3K-AKT pathways, as demonstrated by increased phosphorylation of ERK and AKT, respectively, compared to WT SHP2-expressing cells in response to GM-CSF stimulation [258, 259]. Furthermore, SHP2 has been shown to positively regulate PI3K activity downstream of the IL-3 receptor, which shares its β_c subunit with the GM-CSF receptor, and, therefore, is presumed to promote and regulate signal transduction pathways similarly to the GM-CSF receptor [255, 260]. This suggests a model in which activating SHP2 mutations result in enhanced RAS-ERK and PI3K-AKT signaling that regulate downstream effectors, which promote enhanced proliferation, growth, and survival leading to pathologic expansion of myeloid-lineage cells and development of JMML.

To date, much of the work on mutant SHP2-induced aberrant signaling and the pathogenesis of JMML has focused on the RAS-ERK pathway. However, we have shown that genetic disruption of *Pik3r1*, which encodes the Class IA PI3K regulatory subunit p85α and its splice variants p55α and p50α, can significantly reduce GM-CSF-stimulated hyperproliferation and can decrease not only AKT hyperphosphorylation, but ERK hyperphosphorylation as well, suggesting that dysregulated


JMML-Related Receptors
• GM-CSF Receptor
• IL-3 Receptor
JAK
#
NF1
STAT5
*
Ras
*
Shp2
Erk
PI3K
p85
p110
p110δ
PI(4,5)P2
PI(3,4,5)P3
PTEN
PDK1
T308
P
Akt
P
S473
mTOR
(mTORC2)
mTOR
(mTORC1)
Apoptosis
Cell Cycle/Proliferation
Protein Synthesis

◀ **Fig. 7.7** Diagram of the PI3K-AKT-mTOR pathway highlighting important mediators of dysregulated signaling in the development of pediatric Juvenile Myelomonocytic Leukemia (JMML). Diagram of the PI3K-AKT-mTOR pathway and key associated receptors known to contribute to hyperactivated PI3K signaling described in different forms of Juvenile Myelomonocytic Leukemia (JMML), including the GM-CSF and IL-3 receptors. The intracellular protein tyrosine phosphatase SHP2 is associated with dysregulated signaling, including RAS-ERK and PI3K-AKT signaling in JMML. Proteins indicated with *asterisk* have gain-of-function mutations reported and those indicated with *hash* have loss-of-function mutations reported

PI3K signaling can reinforce mutant SHP2-induced ERK hyperactivation [259]. Additionally, we have shown that mutant SHP2-induced GM-CSF hypersensitivity can be reduced by pharmacologic inhibition of PI3K with either GDC-0941 or with the p110δ-specific inhibitor IC87114, suggesting that PI3K represents a potential therapeutic target for JMML and that hyperactivated PI3K may be largely mediated by p110δ [259]. In subsequent work, we found the genetic disruption of p110δ, but not p110α, reduces mutant SHP2-induced GM-CSF hypersensitivity in vitro as well as correcting features of JMML in an in vivo mouse model such as splenomegaly and aberrant distribution of myeloid precursor populations [261]. Furthermore, we showed that the highly potent p110δ-specific inhibitor GS-9820 and the related compound idelalisib, which has recently been FDA-approved for several lymphoid malignancies, significantly reduced gain-of-function mutant Shp2-induced GM-CSF hypersensitivity, both alone and cooperatively in combination with the MEK inhibitor PD0325901 [261]. Gritsman et al. [262] found that pharmacologic inhibition of either p110δ with idelalisib or p110α with BYL719 reduced gain-of-function mutant KRAS-induced GM-CSF hypersensitivity, and that, similarly, pharmacologic inhibition of p110α had a cooperative effect with the MEK inhibitor MEK162.

The mechanisms by which SHP2 regulates PI3K remain to be fully elucidated. It is possible that SHP2 regulates PI3K indirectly through RAS, since it is well known that SHP2 promotes activation of RAS, and that PI3K is a direct downstream effector of RAS (Fig. 7.7) [263]. Alternatively, studies demonstrate that SHP2 forms a complex with the scaffolding molecules Grb2 and Gab2, and the p85 Class IA PI3K regulatory subunit, suggesting that SHP2 promotes PI3K activation by participating in this complex [260, 263, 264]. We have shown that RAS inhibition with the farnesyltransferase inhibitor, tipifarnib, cooperatively reduces GM-CSF hypersensitivity with either genetic or pharmacologic disruption of PI3K, suggesting that mutant SHP2 can regulate PI3K activity at least in part independently of RAS and that, as a result, targeting the RAS-ERK pathway alone likely will not adequately reduce all dysregulated oncogenic signaling pathways promoted by mutant SHP2 [259]. More work still needs to be done to elucidate the mechanisms of SHP2-mediated PI3K activation, particularly in the context of JMML, but it is significant that mutant SHP2 appears to promote hyperactivation of p110δ-mediated PI3K-AKT signaling, as p110δ has been reported to transform cells independently of RAS [44]. Additional work is also needed to evaluate the role of PI3K activity in other JMML-associated mutations such as gain-of-function mutations in *RAS* and loss-of-function mutations in *NF1* and *CBL* to determine its suitability as a therapeutic target for the majority of JMML patients.

Clinical Trials

Despite the promise of the numerous pre-clinical studies described above, only a few small studies have been conducted to evaluate the efficacy of PI3K-AKT-mTOR pathway inhibitors in patients with pediatric hematologic malignancies. For example one small study of rapamycin (sirolimus) in pediatric ALL patients found that the addition of sirolimus to the standard tacrolimus/methotrexate treatment regimen modestly reduced acute graft-versus-host disease, but had no effect on survival [265]. Notably, none of the newer classes of highly specific and potent inhibitors of PI3K (both pan-PI3K and catalytic subunit-specific), AKT, mTOR, or combination targets, have been assessed in children clinically.

Similarly, relatively few clinical studies of PI3K-AKT-mTOR pathway inhibitors have been completed in adult patients with hematologic malignancies, though there are now several trials for many inhibitors open and enrolling patients (www.clinicaltrials.gov). For example, a small phase I study of a combination of two AKT inhibitors, UCN-01 and perifosine in patients with relapsed or refractory AML found that these compounds were tolerated but did not improve clinical outcomes [266], though a phase II study of eight relapsed and refractory CLL patients found that perifosine resulted in a partial response in one patient, and stable disease in another six [267].

By far, the greatest success has come with the p110δ-specific inhibitor idelalisib (formerly known as GS-1101 and CAL-101), which has recently been approved by the FDA for the treatment of patients with relapsed in chronic lymphocytic leukemia in combination with rituximab, as well as relapsed follicular B-cell lymphoma and relapsed small lymphocytic lymphoma [38–42]. It is also been investigated for other lymphoid malignancies such as mantle cell lymphoma [43], but so far has not been studied in either pediatric patients or patients with myeloid malignancies, though as can be seen from the pre-clinical studies above, there is significant rationale to do so.

Likely, idelalisib is the first of many of PI3K-AKT-mTOR pathway inhibitors to be evaluated clinically and approved for the treatment of hematologic malignancies in both adult and pediatric patients, which will usher in a new era of improved outcomes in this patient population.

Conclusions

Over the last two decades, there has been great progress in understanding the molecular pathogenesis of the many types of hematologic malignancies that afflict children and, as with many other forms of cancer, the role that dysregulated PI3K-AKT-mTOR signaling plays in these diseases is becoming increasingly more appreciated. Preclinical researchers have made great progress identifying the

mechanisms by which this dysregulated signaling pathway promotes uncontrolled growth and proliferation, enhanced survival, and chemoresistance, which has been paralleled by the development of evermore potent and specific inhibitors of many of its components, including PI3K itself, AKT, and mTOR. Particularly exciting for the field of hematologic malignancies are the development of PI3K inhibitors specific for the p110δ and p110γ catalytic subunits, which because of their hematopoietic-specific expression profile, present themselves as ideal molecular targets for hematologic diseases with reduced risk of toxic side effects that might occur with pharmacologic inhibition of more ubiquitously expressed targets.

Clinical trials using PI3K-AKT-mTOR inhibitors have already begun in certain solid tumors and hematologic malignancies in adult patients, and if these are successful, there is hope that they may make their way into clinical trials for pediatric leukemias and lymphomas. Despite the great successes that have been made in treating pediatric leukemias and lymphomas with chemotherapy, radiotherapy, and HSCT, there are still unacceptably high numbers of relapses and refractory cases. Furthermore, these treatment modalities carry significant risks of opportunistic infections, graft-versus-host disease, the development of future secondary malignancies, and other complications, not to mention their inherent morbidity, which can be quite distressing for patients and their families.

The recent success of imatinib and second generation tyrosine kinase inhibitors has ushered in the hopeful era of rationally designed molecularly targeted therapies, but the development of resistance to these compounds is a sobering reminder that such targeted monotherapy approaches will probably not be sufficient to offer a definitive cure. Most likely, targeting two or more aberrant, cancer-causing molecular pathways or processes will bring us closer to that goal, as combination polytherapy reduces the risk of developing molecular resistance to targeted inhibitors and potentially offers the possibility of using lower doses of each inhibitor to achieve more tolerable side effect profiles. Almost certainly, small molecule inhibitors of the PI3K-AKT-mTOR signaling pathway will have a prominent place in this expanding armamentarium of targeted therapeutic agents that will increase survival and reduce morbidity and complications in children suffering from leukemia and lymphoma.

Review Criteria

Publications were searched for on PubMed using the following search terms: “pediatric leukemia,” “juvenile leukemia,” “acute lymphoblastic leukemia,” “lymphoma,” “acute myeloid leukemia,” “chronic myelogenous leukemia,” “juvenile myelomonocytic leukemia,” “PI3K,” “AKT,” and “mTOR.” The most recent articles cited are from June 2014. Manuscripts cited were full-length, contained abstracts, were written in English, and included both original articles and reviews.

Acknowledgements This work was supported by and the U.S. National Institutes of Health (HL104867, CBG; CA134777, RJC), the Riley Children's Foundation, and the IUPUI Office of the Vice Chancellor for Research. The authors gratefully acknowledge the administrative assistance of Marilyn L. Wales.

References

1. Kaatsch P (2010) Epidemiology of childhood cancer. Cancer Treat Rev 36(4):277–285. doi:10.1016/j.ctrv.2010.02.003
2. Zhao L, Vogt PK (2008) Class I PI3K in oncogenic cellular transformation. Oncogene 27 (41):5486–5496. doi:10.1038/onc.2008.244
3. Cantley LC (2002) The phosphoinositide 3-kinase pathway. Science 296(5573):1655–1657. doi:10.1126/science.296.5573.1655
4. Corvera S, Czech MP (1998) Direct targets of phosphoinositide 3-kinase products in membrane traffic and signal transduction. Trends Cell Biol 8(11):442–446
5. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P (1997) Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Curr Biol 7(4):261–269
6. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of AKT/PKB by the rictor-mTOR complex. Science 307(5712):1098–1101. doi:10.1126/science.1106148
7. Shi Y, Yan H, Frost P, Gera J, Lichtenstein A (2005) Mammalian target of rapamycin inhibitors activate the AKT kinase in multiple myeloma cells by up-regulating the insulin-like growth factor receptor/insulin receptor substrate-1/phosphatidylinositol 3-kinase cascade. Mol Cancer Ther 4(10):1533–1540. doi:10.1158/1535-7163.MCT-05-0068
8. Walker EH, Perisic O, Ried C, Stephens L, Williams RL (1999) Structural insights into phosphoinositide 3-kinase catalysis and signalling. Nature 402(6759):313–320. doi:10.1038/46319
9. Fruman DA (2010) Regulatory subunits of class IA PI3K. Curr Top Microbiol Immunol 346:225–244. doi:10.1007/82_2010_39
10. Yu J, Zhang Y, McIlroy J, Rordorf-Nikolic T, Orr GA, Backer JM (1998) Regulation of the p85/p110 phosphatidylinositol 3′-kinase: stabilization and inhibition of the p110alpha catalytic subunit by the p85 regulatory subunit. Mol Cell Biol 18(3):1379–1387
11. Luo J, Field SJ, Lee JY, Engelman JA, Cantley LC (2005) The p85 regulatory subunit of phosphoinositide 3-kinase down-regulates IRS-1 signaling via the formation of a sequestration complex. J Cell Biol 170(3):455–464. doi:10.1083/jcb.200503088
12. Luo J, Cantley LC (2005) The negative regulation of phosphoinositide 3-kinase signaling by p85 and it's implication in cancer. Cell Cycle 4(10):1309–1312
13. Rodriguez-Viciana P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ, Waterfield MD, Downward J (1994) Phosphatidylinositol-3-OH kinase as a direct target of Ras. Nature 370(6490):527–532. doi:10.1038/370527a0
14. Rodriguez-Viciana P, Warne PH, Vanhaesebroeck B, Waterfield MD, Downward J (1996) Activation of phosphoinositide 3-kinase by interaction with Ras and by point mutation. EMBO J 15(10):2442–2451
15. Chan TO, Rodeck U, Chan AM, Kimmelman AC, Rittenhouse SE, Panayotou G, Tsichlis PN (2002) Small GTPases and tyrosine kinases coregulate a molecular switch in the phosphoinositide 3-kinase regulatory subunit. Cancer Cell 1(2):181–191
16. Pacold ME, Suire S, Perisic O, Lara-Gonzalez S, Davis CT, Walker EH, Hawkins PT, Stephens L, Eccleston JF, Williams RL (2000) Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma. Cell 103(6):931–943

17. Rodriguez-Viciana P, Marte BM, Warne PH, Downward J (1996) Phosphatidylinositol 3′ kinase: one of the effectors of Ras. Philos Trans R Soc Lond B, Biol Sci 351(1336):225–231. doi:10.1098/rstb.1996.0020 (discussion 231–222)
18. Luo J, Manning BD, Cantley LC (2003) Targeting the PI3K-AKT pathway in human cancer: rationale and promise. Cancer Cell 4(4):257–262
19. Vivanco I, Sawyers CL (2002) The phosphatidylinositol 3-kinase AKT pathway in human cancer. Nat Rev Cancer 2(7):489–501. doi:10.1038/nrc839
20. Salmena L, Carracedo A, Pandolfi PP (2008) Tenets of PTEN tumor suppression. Cell 133 (3):403–414. doi:10.1016/j.cell.2008.04.013
21. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) High frequency of mutations of the PIK3CA gene in human cancers. Science 304(5670):554. doi:10.1126/science.1096502
22. Sujobert P, Bardet V, Cornillet-Lefebvre P, Hayflick JS, Prie N, Verdier F, Vanhaesebroeck B, Muller O, Pesce F, Ifrah N, Hunault-Berger M, Berthou C, Villemagne B, Jourdan E, Audhuy B, Solary E, Witz B, Harousseau JL, Himberlin C, Lamy T, Lioure B, Cahn JY, Dreyfus F, Mayeux P, Lacombe C, Bouscary D (2005) Essential role for the p110delta isoform in phosphoinositide 3-kinase activation and cell proliferation in acute myeloid leukemia. Blood 106(3):1063–1066. doi:10.1182/blood-2004-08-3225
23. Skorski T, Bellacosa A, Nieborowska-Skorska M, Majewski M, Martinez R, Choi JK, Trotta R, Wlodarski P, Perrotti D, Chan TO, Wasik MA, Tsichlis PN, Calabretta B (1997) Transformation of hematopoietic cells by BCR/ABL requires activation of a PI3k/AKT-dependent pathway. EMBO J 16(20):6151–6161. doi:10.1093/emboj/16.20.6151
24. Hickey FB, Cotter TG (2006) BCR-ABL regulates phosphatidylinositol 3-kinase-p110gamma transcription and activation and is required for proliferation and drug resistance. J Biol Chem 281 (5):2441–2450. doi:10.1074/jbc.M511173200
25. Angulo I, Vadas O, Garcon F, Banham-Hall E, Plagnol V, Leahy TR, Baxendale H, Coulter T, Curtis J, Wu C, Blake-Palmer K, Perisic O, Smyth D, Maes M, Fiddler C, Juss J, Cilliers D, Markelj G, Chandra A, Farmer G, Kielkowska A, Clark J, Kracker S, Debre M, Picard C, Pellier I, Jabado N, Morris JA, Barcenas-Morales G, Fischer A, Stephens L, Hawkins P, Barrett JC, Abinun M, Clatworthy M, Durandy A, Doffinger R, Chilvers ER, Cant AJ, Kumararatne D, Okkenhaug K, Williams RL, Condliffe A, Nejentsev S (2013) Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. Science 342(6160):866–871. doi:10.1126/science.1243292
26. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, Avery DT, Moens L, Cannons JL, Biancalana M, Stoddard J, Ouyang W, Frucht DM, Rao VK, Atkinson TP, Agharahimi A, Hussey AA, Folio LR, Olivier KN, Fleisher TA, Pittaluga S, Holland SM, Cohen JI, Oliveira JB, Tangye SG, Schwartzberg PL, Lenardo MJ, Uzel G (2013) Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. Nat Immunol. doi:10.1038/ni.2771
27. Marone R, Cmiljanovic V, Giese B, Wymann MP (2008) Targeting phosphoinositide 3-kinase: moving towards therapy. Biochim Biophys Acta 1784(1):159–185. doi:10.1016/j.bbapap.2007.10.003
28. Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, Meek SE, Salpekar A, Waterfield MD, Smith AJ, Vanhaesebroeck B (2002) Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. Science 297(5583):1031–1034. doi:10.1126/science.1073560
29. Rommel C (2010) Taking PI3Kdelta and PI3Kgamma one step ahead: dual active PI3Kdelta/gamma inhibitors for the treatment of immune-mediated inflammatory diseases. Curr Top Microbiol Immunol 346:279–299. doi:10.1007/82_2010_79

30. Cushing TD, Metz DP, Whittington DA, McGee LR (2012) PI3Kdelta and PI3Kgamma as targets for autoimmune and inflammatory diseases. J Med Chem 55(20):8559–8581. doi:10.1021/jm300847w
31. Castillo JJ, Furman M, Winer ES (2012) CAL-101: a phosphatidylinositol-3-kinase p110-delta inhibitor for the treatment of lymphoid malignancies. Expert Opin Investig Drugs 21(1):15–22. doi:10.1517/13543784.2012.640318
32. Ikeda H, Hideshima T, Fulciniti M, Perrone G, Miura N, Yasui H, Okawa Y, Kiziltepe T, Santo L, Vallet S, Cristea D, Calabrese E, Gorgun G, Raje NS, Richardson P, Munshi NC, Lannutti BJ, Puri KD, Giese NA, Anderson KC (2010) PI3K/p110{delta} is a novel therapeutic target in multiple myeloma. Blood 116(9):1460–1468. doi:10.1182/blood-2009-06-222943
33. Herman SE, Gordon AL, Wagner AJ, Heerema NA, Zhao W, Flynn JM, Jones J, Andritsos L, Puri KD, Lannutti BJ, Giese NA, Zhang X, Wei L, Byrd JC, Johnson AJ (2010) Phosphatidylinositol 3-kinase-delta inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. Blood 116(12):2078–2088. doi:10.1182/blood-2010-02-271171
34. Lannutti BJ, Meadows SA, Herman SE, Kashishian A, Steiner B, Johnson AJ, Byrd JC, Tyner JW, Loriaux MM, Deininger M, Druker BJ, Puri KD, Ulrich RG, Giese NA (2011) CAL-101, a p110delta selective phosphatidylinositol-3-kinase inhibitor for the treatment of B-cell malignancies, inhibits PI3K signaling and cellular viability. Blood 117(2):591–594. doi:10.1182/blood-2010-03-275305
35. Papakonstanti EA, Zwaenepoel O, Bilancio A, Burns E, Nock GE, Houseman B, Shokat K, Ridley AJ, Vanhaesebroeck B (2008) Distinct roles of class IA PI3K isoforms in primary and immortalised macrophages. J Cell Sci 121(Pt 24):4124–4133. doi:10.1242/jcs.032763
36. Fruman DA, Rommel C (2011) PI3Kdelta inhibitors in cancer: rationale and serendipity merge in the clinic. Cancer Discovery 1(7):562–572. doi:10.1158/2159-8290.CD-11-0249
37. Berndt A, Miller S, Williams O, Le DD, Houseman BT, Pacold JI, Gorrec F, Hon WC, Liu Y, Rommel C, Gaillard P, Ruckle T, Schwarz MK, Shokat KM, Shaw JP, Williams RL (2010) The p110delta structure: mechanisms for selectivity and potency of new PI(3)K inhibitors. Nat Chem Biol 6(3):244. doi:10.1038/nchembio0310-244b
38. Brown JR, Byrd JC, Coutre SE, Benson DM, Flinn IW, Wagner-Johnston ND, Spurgeon SE, Kahl BS, Bello C, Webb HK, Johnson DM, Peterman S, Li D, Jahn TM, Lannutti BJ, Ulrich RG, Yu AS, Miller LL, Furman RR (2014) Idelalisib, an inhibitor of phosphatidylinositol 3-kinase p110delta, for relapsed/refractory chronic lymphocytic leukemia. Blood 123(22):3390–3397. doi:10.1182/blood-2013-11-535047
39. Flinn IW, Kahl BS, Leonard JP, Furman RR, Brown JR, Byrd JC, Wagner-Johnston ND, Coutre SE, Benson DM, Peterman S, Cho Y, Webb HK, Johnson DM, Yu AS, Ulrich RG, Godfrey WR, Miller LL, Spurgeon SE (2014) Idelalisib, a selective inhibitor of phosphatidylinositol 3-kinase-delta, as therapy for previously treated indolent non-Hodgkin lymphoma. Blood 123(22):3406–3413. doi:10.1182/blood-2013-11-538546
40. Gopal AK, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ, Flinn IW, Flowers CR, Martin P, Viardot A, Blum KA, Goy AH, Davies AJ, Zinzani PL, Dreyling M, Johnson D, Miller LL, Holes L, Li D, Dansey RD, Godfrey WR, Salles GA (2014) PI3Kdelta inhibition by idelalisib in patients with relapsed indolent lymphoma. N Engl J Med 370(11):1008–1018. doi:10.1056/NEJMoa1314583
41. Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, Barrientos JC, Zelenetz AD, Kipps TJ, Flinn I, Ghia P, Eradat H, Ervin T, Lamanna N, Coiffier B, Pettitt AR, Ma S, Stilgenbauer S, Cramer P, Aiello M, Johnson DM, Miller LL, Li D, Jahn TM, Dansey RD, Hallek M, O'Brien SM (2014) Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med 370(11):997–1007. doi:10.1056/NEJMoa1315226
42. Fruman DA, Cantley LC (2014) Idelalisib–a PI3Kdelta inhibitor for B-cell cancers. N Engl J Med 370(11):1061–1062. doi:10.1056/NEJMe1400055

43. Kahl BS, Spurgeon SE, Furman RR, Flinn IW, Coutre SE, Brown JR, Benson DM, Byrd JC, Peterman S, Cho Y, Yu A, Godfrey WR, Wagner-Johnston ND (2014) A phase 1 study of the PI3Kdelta inhibitor idelalisib in patients with relapsed/refractory mantle cell lymphoma (MCL). Blood 123(22):3398–3405. doi:10.1182/blood-2013-11-537555
44. Denley A, Kang S, Karst U, Vogt PK (2008) Oncogenic signaling of class I PI3K isoforms. Oncogene 27(18):2561–2574. doi:10.1038/sj.onc.1210918
45. Zhao L, Vogt PK (2008) Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. Proc Natl Acad Sci USA 105(7):2652–2657. doi:10.1073/pnas.0712169105
46. Subramaniam PS, Whye DW, Efimenko E, Chen J, Tosello V, De Keersmaecker K, Kashishian A, Thompson MA, Castillo M, Cordon-Cardo C, Dave UP, Ferrando A, Lannutti BJ, Diacovo TG (2012) Targeting nonclassical oncogenes for therapy in T-ALL. Cancer Cell 21(4):459–472. doi:10.1016/j.ccr.2012.02.029
47. Younes A, Samad N (2011) Utility of mTOR inhibition in hematologic malignancies. Oncologist 16(6):730–741. doi:10.1634/theoncologist.2010-0318
48. Chapuis N, Tamburini J, Green AS, Willems L, Bardet V, Park S, Lacombe C, Mayeux P, Bouscary D (2010) Perspectives on inhibiting mTOR as a future treatment strategy for hematological malignancies. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 24 (10):1686–1699. doi:10.1038/leu.2010.170
49. Barrett D, Brown VI, Grupp SA, Teachey DT (2012) Targeting the PI3K/AKT/mTOR signaling axis in children with hematologic malignancies. Paediatr Drugs 14(5):299–316. doi:10.2165/11594740-000000000-00000
50. Martelli AM, Evangelisti C, Chiarini F, McCubrey JA (2010) The phosphatidylinositol 3-kinase/AKT/mTOR signaling network as a therapeutic target in acute myelogenous leukemia patients. Oncotarget 1(2):89–103
51. Lo Nigro L (2013) Biology of childhood acute lymphoblastic leukemia. J Pediatr Hematol Oncol 35(4):245–252. doi:10.1097/MPH.0b013e31828f8746
52. Hales EC, Taub JW, Matherly LH (2013) New insights into Notch1 regulation of the PI3K-AKT-mTOR1 signaling axis: Targeted therapy of gamma-secretase inhibitor resistant T-cell acute lymphoblastic leukemia. Cell Signal. doi:10.1016/j.cellsig.2013.09.021
53. Palomero T, Sulis ML, Cortina M, Real PJ, Barnes K, Ciofani M, Caparros E, Buteau J, Brown K, Perkins SL, Bhagat G, Agarwal AM, Basso G, Castillo M, Nagase S, Cordon-Cardo C, Parsons R, Zuniga-Pflucker JC, Dominguez M, Ferrando AA (2007) Mutational loss of PTEN induces resistance to Notch1 inhibition in T-cell leukemia. Nat Med 13(10):1203–1210. doi:10.1038/nm1636
54. Hales EC, Orr SM, Larson Gedman A, Taub JW, Matherly LH (2013) Notch1 receptor regulates AKT protein activation loop (Thr308) dephosphorylation through modulation of the PP2A phosphatase in phosphatase and tensin homolog (PTEN)-null T-cell acute lymphoblastic leukemia cells. J Biol Chem 288(31):22836–22848. doi:10.1074/jbc.M113.451625
55. Sanda T, Li X, Gutierrez A, Ahn Y, Neuberg DS, O'Neil J, Strack PR, Winter CG, Winter SS, Larson RS, von Boehmer H, Look AT (2010) Interconnecting molecular pathways in the pathogenesis and drug sensitivity of T-cell acute lymphoblastic leukemia. Blood 115(9):1735–1745. doi:10.1182/blood-2009-07-235143
56. Gonzalez-Garcia S, Garcia-Peydro M, Martin-Gayo E, Ballestar E, Esteller M, Bornstein R, de la Pompa JL, Ferrando AA, Toribio ML (2009) CSL-MAML-dependent Notch1 signaling controls T lineage-specific IL-7R{alpha} gene expression in early human thymopoiesis and leukemia. J Exp Med 206(4):779–791. doi:10.1084/jem.20081922
57. Barata JT, Silva A, Brandao JG, Nadler LM, Cardoso AA, Boussiotis VA (2004) Activation of PI3K is indispensable for interleukin 7-mediated viability, proliferation, glucose use, and growth of T cell acute lymphoblastic leukemia cells. J Exp Med 200(5):659–669. doi:10.1084/jem.20040789
58. Medyouf H, Gusscott S, Wang H, Tseng JC, Wai C, Nemirovsky O, Trumpp A, Pflumio F, Carboni J, Gottardis M, Pollak M, Kung AL, Aster JC, Holzenberger M, Weng AP (2011)

High-level IGF1R expression is required for leukemia-initiating cell activity in T-ALL and is supported by Notch signaling. J Exp Med 208(9):1809–1822. doi:10.1084/jem.20110121
59. Zenatti PP, Ribeiro D, Li W, Zuurbier L, Silva MC, Paganin M, Tritapoe J, Hixon JA, Silveira AB, Cardoso BA, Sarmento LM, Correia N, Toribio ML, Kobarg J, Horstmann M, Pieters R, Brandalise SR, Ferrando AA, Meijerink JP, Durum SK, Yunes JA, Barata JT (2011) Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat Genet 43(10):932–939. doi:10.1038/ng.924
60. Shochat C, Tal N, Bandapalli OR, Palmi C, Ganmore I, te Kronnie G, Cario G, Cazzaniga G, Kulozik AE, Stanulla M, Schrappe M, Biondi A, Basso G, Bercovich D, Muckenthaler MU, Izraeli S (2011) Gain-of-function mutations in interleukin-7 receptor-alpha (IL7R) in childhood acute lymphoblastic leukemias. J Exp Med 208(5):901–908. doi:10.1084/jem.20110580
61. Zuurbier L, Petricoin EF 3rd, Vuerhard MJ, Calvert V, Kooi C, Buijs-Gladdines JG, Smits WK, Sonneveld E, Veerman AJ, Kamps WA, Horstmann M, Pieters R, Meijerink JP (2012) The significance of PTEN and AKT aberrations in pediatric T-cell acute lymphoblastic leukemia. Haematologica 97(9):1405–1413. doi:10.3324/haematol.2011.059030
62. Gutierrez A, Sanda T, Grebliunaite R, Carracedo A, Salmena L, Ahn Y, Dahlberg S, Neuberg D, Moreau LA, Winter SS, Larson R, Zhang J, Protopopov A, Chin L, Pandolfi PP, Silverman LB, Hunger SP, Sallan SE, Look AT (2009) High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. Blood 114(3):647–650. doi:10.1182/blood-2009-02-206722
63. Bressanin D, Evangelisti C, Ricci F, Tabellini G, Chiarini F, Tazzari PL, Melchionda F, Buontempo F, Pagliaro P, Pession A, McCubrey JA, Martelli AM (2012) Harnessing the PI3K/AKT/mTOR pathway in T-cell acute lymphoblastic leukemia: eliminating activity by targeting at different levels. Oncotarget 3(8):811–823
64. Schult C, Dahlhaus M, Glass A, Fischer K, Lange S, Freund M, Junghanss C (2012) The dual kinase inhibitor NVP-BEZ235 in combination with cytotoxic drugs exerts anti-proliferative activity towards acute lymphoblastic leukemia cells. Anticancer Res 32(2):463–474
65. Chiarini F, Grimaldi C, Ricci F, Tazzari PL, Evangelisti C, Ognibene A, Battistelli M, Falcieri E, Melchionda F, Pession A, Pagliaro P, McCubrey JA, Martelli AM (2010) Activity of the novel dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235 against T-cell acute lymphoblastic leukemia. Cancer Res 70(20):8097–8107. doi:10.1158/0008-5472.CAN-10-1814
66. Levy DS, Kahana JA, Kumar R (2009) AKT inhibitor, GSK690693, induces growth inhibition and apoptosis in acute lymphoblastic leukemia cell lines. Blood 113(8):1723–1729. doi:10.1182/blood-2008-02-137737
67. Cullion K, Draheim KM, Hermance N, Tammam J, Sharma VM, Ware C, Nikov G, Krishnamoorthy V, Majumder PK, Kelliher MA (2009) Targeting the Notch1 and mTOR pathways in a mouse T-ALL model. Blood 113(24):6172–6181. doi:10.1182/blood-2008-02-136762
68. Wu KN, Zhao YM, He Y, Wang BS, Du KL, Fu S, Hu KM, Zhang LF, Liu LZ, Hu YX, Wang YJ, Huang H (2013) Rapamycin interacts synergistically with idarubicin to induce T-leukemia cell apoptosis in vitro and in a mesenchymal stem cell simulated drug-resistant microenvironment via AKT/mammalian target of rapamycin and extracellular signal-related kinase signaling pathways. Leuk Lymphoma. doi:10.3109/10428194.2013.811579
69. Evangelisti C, Ricci F, Tazzari P, Chiarini F, Battistelli M, Falcieri E, Ognibene A, Pagliaro P, Cocco L, McCubrey JA, Martelli AM (2011) Preclinical testing of the AKT inhibitor triciribine in T-cell acute lymphoblastic leukemia. J Cell Physiol 226(3):822–831. doi:10.1002/jcp.22407
70. Morishita N, Tsukahara H, Chayama K, Ishida T, Washio K, Miyamura T, Yamashita N, Oda M, Morishima T (2012) Activation of AKT is associated with poor prognosis and

chemotherapeutic resistance in pediatric B-precursor acute lymphoblastic leukemia. Pediatr Blood Cancer 59(1):83–89. doi:10.1002/pbc.24034
71. Hunger SP (2011) Tyrosine kinase inhibitor use in pediatric Philadelphia chromosome-positive acute lymphoblastic anemia. Hematol Educ Prog Am Soc Hematol Am Soc Hematol Educ Prog 2011:361–365. doi:10.1182/asheducation-2011.1.361
72. Montiel-Duarte C, Cordeu L, Agirre X, Roman-Gomez J, Jimenez-Velasco A, Jose-Eneriz ES, Garate L, Andreu EJ, Calasanz MJ, Heiniger A, Torres A, Prosper F (2008) Resistance to Imatinib Mesylate-induced apoptosis in acute lymphoblastic leukemia is associated with PTEN down-regulation due to promoter hypermethylation. Leuk Res 32(5):709–716. doi:10.1016/j.leukres.2007.09.005
73. Quentmeier H, Eberth S, Romani J, Zaborski M, Drexler HG (2011) BCR-ABL1-independent PI3Kinase activation causing imatinib-resistance. J Hematol Oncol 4:6. doi:10.1186/1756-8722-4-6
74. Kharas MG, Janes MR, Scarfone VM, Lilly MB, Knight ZA, Shokat KM, Fruman DA (2008) Ablation of PI3K blocks BCR-ABL leukemogenesis in mice, and a dual PI3K/mTOR inhibitor prevents expansion of human BCR-ABL+ leukemia cells. J Clin Investig 118 (9):3038–3050. doi:10.1172/JCI33337
75. Janes MR, Vu C, Mallya S, Shieh MP, Limon JJ, Li LS, Jessen KA, Martin MB, Ren P, Lilly MB, Sender LS, Liu Y, Rommel C, Fruman DA (2013) Efficacy of the investigational mTOR kinase inhibitor MLN0128/INK128 in models of B-cell acute lymphoblastic leukemia. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 27(3):586–594. doi:10.1038/leu.2012.276
76. Janes MR, Limon JJ, So L, Chen J, Lim RJ, Chavez MA, Vu C, Lilly MB, Mallya S, Ong ST, Konopleva M, Martin MB, Ren P, Liu Y, Rommel C, Fruman DA (2010) Effective and selective targeting of leukemia cells using a TORC1/2 kinase inhibitor. Nat Med 16 (2):205–213. doi:10.1038/nm.2091
77. Fuka G, Kantner HP, Grausenburger R, Inthal A, Bauer E, Krapf G, Kaindl U, Kauer M, Dworzak MN, Stoiber D, Haas OA, Panzer-Grumayer R (2012) Silencing of ETV6/RUNX1 abrogates PI3K/AKT/mTOR signaling and impairs reconstitution of leukemia in xenografts. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 26(5):927–933. doi:10.1038/leu.2011.322
78. Spijkers-Hagelstein JA, Pinhancos SS, Schneider P, Pieters R, Stam RW (2013) Chemical genomic screening identifies LY294002 as a modulator of glucocorticoid resistance in MLL-rearranged infant ALL. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK. doi:10.1038/leu.2013.245
79. Shalapour S, Hof J, Kirschner-Schwabe R, Bastian L, Eckert C, Prada J, Henze G, von Stackelberg A, Seeger K (2011) High VLA-4 expression is associated with adverse outcome and distinct gene expression changes in childhood B-cell precursor acute lymphoblastic leukemia at first relapse. Haematologica 96(11):1627–1635. doi:10.3324/haematol.2011.047993
80. Mullighan CG, Collins-Underwood JR, Phillips LA, Loudin MG, Liu W, Zhang J, Ma J, Coustan-Smith E, Harvey RC, Willman CL, Mikhail FM, Meyer J, Carroll AJ, Williams RT, Cheng J, Heerema NA, Basso G, Pession A, Pui CH, Raimondi SC, Hunger SP, Downing JR, Carroll WL, Rabin KR (2009) Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet 41(11):1243–1246. doi:10.1038/ng.469
81. Harvey RC, Mullighan CG, Chen IM, Wharton W, Mikhail FM, Carroll AJ, Kang H, Liu W, Dobbin KK, Smith MA, Carroll WL, Devidas M, Bowman WP, Camitta BM, Reaman GH, Hunger SP, Downing JR, Willman CL (2010) Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, hispanic/latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. Blood 115(26):5312–5321. doi:10.1182/blood-2009-09-245944
82. Tasian SK, Doral MY, Borowitz MJ, Wood BL, Chen IM, Harvey RC, Gastier-Foster JM, Willman CL, Hunger SP, Mullighan CG, Loh ML (2012) Aberrant STAT5 and PI3K/mTOR

pathway signaling occurs in human CRLF2-rearranged B-precursor acute lymphoblastic leukemia. Blood 120(4):833–842. doi:10.1182/blood-2011-12-389932
83. Kelly KM, Hodgson D, Appel B, Chen L, Cole PD, Horton T, Keller FG (2013) Children's Oncology Group's 2013 blueprint for research: Hodgkin lymphoma. Pediatr Blood Cancer 60(6):972–978. doi:10.1002/pbc.24423
84. Bollard CM, Lim MS, Gross TG (2013) Children's Oncology Group's 2013 blueprint for research: non-Hodgkin lymphoma. Pediatr Blood Cancer 60(6):979–984. doi:10.1002/pbc.24416
85. Yung L, Linch D (2003) Hodgkin's lymphoma. Lancet 361(9361):943–951. doi:10.1016/S0140-6736(03)12777-8
86. Dawson CW, Tramountanis G, Eliopoulos AG, Young LS (2003) Epstein–Barr virus latent membrane protein 1 (LMP1) activates the phosphatidylinositol 3-kinase/AKT pathway to promote cell survival and induce actin filament remodeling. J Biol Chem 278(6):3694–3704. doi:10.1074/jbc.M209840200
87. Swart R, Ruf IK, Sample J, Longnecker R (2000) Latent membrane protein 2A-mediated effects on the phosphatidylinositol 3-Kinase/AKT pathway. J Virol 74(22):10838–10845
88. Morrison JA, Gulley ML, Pathmanathan R, Raab-Traub N (2004) Differential signaling pathways are activated in the Epstein–Barr virus-associated malignancies nasopharyngeal carcinoma and Hodgkin lymphoma. Cancer Res 64(15):5251–5260. doi:10.1158/0008-5472.CAN-04-0538
89. Dutton A, Reynolds GM, Dawson CW, Young LS, Murray PG (2005) Constitutive activation of phosphatidyl-inositide 3 kinase contributes to the survival of Hodgkin's lymphoma cells through a mechanism involving AKT kinase and mTOR. J Pathol 205 (4):498–506. doi:10.1002/path.1725
90. Georgakis GV, Li Y, Rassidakis GZ, Medeiros LJ, Mills GB, Younes A (2006) Inhibition of the phosphatidylinositol-3 kinase/AKT promotes G1 cell cycle arrest and apoptosis in Hodgkin lymphoma. Br J Haematol 132(4):503–511. doi:10.1111/j.1365-2141.2005.05881.x
91. Meadows SA, Vega F, Kashishian A, Johnson D, Diehl V, Miller LL, Younes A, Lannutti BJ (2012) PI3Kdelta inhibitor, GS-1101 (CAL-101), attenuates pathway signaling, induces apoptosis, and overcomes signals from the microenvironment in cellular models of Hodgkin lymphoma. Blood 119(8):1897–1900. doi:10.1182/blood-2011-10-386763
92. Evans LS, Hancock BW (2003) Non-Hodgkin lymphoma. Lancet 362(9378):139–146. doi:10.1016/S0140-6736(03)13868-8
93. Sander S, Calado DP, Srinivasan L, Kochert K, Zhang B, Rosolowski M, Rodig SJ, Holzmann K, Stilgenbauer S, Siebert R, Bullinger L, Rajewsky K (2012) Synergy between PI3K signaling and MYC in Burkitt lymphomagenesis. Cancer Cell 22(2):167–179. doi:10.1016/j.ccr.2012.06.012
94. Cen O, Longnecker R (2011) Rapamycin reverses splenomegaly and inhibits tumor development in a transgenic model of Epstein–Barr virus-related Burkitt's lymphoma. Mol Cancer Ther 10(4):679–686. doi:10.1158/1535-7163.MCT-10-0833
95. Spender LC, Inman GJ (2012) Phosphoinositide 3-kinase/AKT/mTORC1/2 signaling determines sensitivity of Burkitt's lymphoma cells to BH3 mimetics. Mol Cancer Res MCR 10(3):347–359. doi:10.1158/1541-7786.MCR-11-0394
96. Uddin S, Hussain AR, Siraj AK, Manogaran PS, Al-Jomah NA, Moorji A, Atizado V, Al-Dayel F, Belgaumi A, El-Solh H, Ezzat A, Bavi P, Al-Kuraya KS (2006) Role of phosphatidylinositol 3′-kinase/AKT pathway in diffuse large B-cell lymphoma survival. Blood 108(13):4178–4186. doi:10.1182/blood-2006-04-016907
97. Zhao MY, Auerbach A, D'Costa AM, Rapoport AP, Burger AM, Sausville EA, Stass SA, Jiang F, Sands AM, Aguilera N, Zhao XF (2009) Phospho-p70S6K/p85S6K and cdc2/cdk1 are novel targets for diffuse large B-cell lymphoma combination therapy. Clin Cancer Res Official J Am Assoc Cancer Res 15(5):1708–1720. doi:10.1158/1078-0432.CCR-08-1543
98. Tjin EP, Groen RW, Vogelzang I, Derksen PW, Klok MD, Meijer HP, van Eeden S, Pals ST, Spaargaren M (2006) Functional analysis of HGF/MET signaling and aberrant HGF-activator

expression in diffuse large B-cell lymphoma. Blood 107(2):760–768. doi:10.1182/blood-2005-05-1929
99. Uddin S, Bu R, Ahmed M, Hussain AR, Ajarim D, Al-Dayel F, Bavi P, Al-kuraya KS (2010) Leptin receptor expression and its association with PI3K/AKT signaling pathway in diffuse large B-cell lymphoma. Leuk Lymphoma 51(7):1305–1314. doi:10.3109/10428191003802365
100. Huang X, Shen Y, Liu M, Bi C, Jiang C, Iqbal J, McKeithan TW, Chan WC, Ding SJ, Fu K (2012) Quantitative proteomics reveals that miR-155 regulates the PI3K-AKT pathway in diffuse large B-cell lymphoma. Am J Pathol 181(1):26–33. doi:10.1016/j.ajpath.2012.03.013
101. Xu ZZ, Xia ZG, Wang AH, Wang WF, Liu ZY, Chen LY, Li JM (2013) Activation of the PI3K/AKT/mTOR pathway in diffuse large B cell lymphoma: clinical significance and inhibitory effect of rituximab. Ann Hematol 92(10):1351–1358. doi:10.1007/s00277-013-1770-9
102. Zang C, Eucker J, Liu H, Muller A, Possinger K, Scholz CW (2013) Concurrent inhibition of PI3-kinase and mTOR induces cell death in diffuse large B cell lymphomas, a mechanism involving down regulation of Mcl-1. Cancer Lett 339(2):288–297. doi:10.1016/j.canlet.2012.11.013
103. Zang C, Eucker J, Liu H, Coordes A, Lenarz M, Possinger K, Scholz CW (2013) Inhibition of pan-class I phosphatidyl-inositol-3-kinase by NVP-BKM120 effectively blocks proliferation and induces cell death in diffuse large B-cell lymphoma. Leuk Lymphoma. doi:10.3109/10428194.2013.806800
104. Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, Look AT (1994) Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 263(5151):1281–1284
105. Thakral C, Hutchison RE, Shrimpton A, Barrett D, Laver J, Link M, Halleran DR, Hudson S (2012) ALK+ anaplastic large cell lymphoma exhibits phosphatidylinositol-3 kinase/AKT activity with retained but inactivated PTEN–a report from the Children's Oncology Group. Pediatr Blood Cancer 59(3):440–447. doi:10.1002/pbc.24153
106. Bai RY, Ouyang T, Miething C, Morris SW, Peschel C, Duyster J (2000) Nucleophosmin-anaplastic lymphoma kinase associated with anaplastic large-cell lymphoma activates the phosphatidylinositol 3-kinase/AKT antiapoptotic signaling pathway. Blood 96(13):4319–4327
107. Slupianek A, Nieborowska-Skorska M, Hoser G, Morrione A, Majewski M, Xue L, Morris SW, Wasik MA, Skorski T (2001) Role of phosphatidylinositol 3-kinase-AKT pathway in nucleophosmin/anaplastic lymphoma kinase-mediated lymphomagenesis. Cancer Res 61(5):2194–2199
108. Polgar D, Leisser C, Maier S, Strasser S, Ruger B, Dettke M, Khorchide M, Simonitsch I, Cerni C, Krupitza G (2005) Truncated ALK derived from chromosomal translocation t(2;5)(p23;q35) binds to the SH3 domain of p85-PI3K. Mutat Res 570(1):9–15. doi:10.1016/j.mrfmmm.2004.09.011
109. Rassidakis GZ, Feretzaki M, Atwell C, Grammatikakis I, Lin Q, Lai R, Claret FX, Medeiros LJ, Amin HM (2005) Inhibition of AKT increases p27Kip1 levels and induces cell cycle arrest in anaplastic large cell lymphoma. Blood 105(2):827–829. doi:10.1182/blood-2004-06-2125
110. Vega F, Medeiros LJ, Leventaki V, Atwell C, Cho-Vega JH, Tian L, Claret FX, Rassidakis GZ (2006) Activation of mammalian target of rapamycin signaling pathway contributes to tumor cell survival in anaplastic lymphoma kinase-positive anaplastic large cell lymphoma. Cancer Res 66(13):6589–6597. doi:10.1158/0008-5472.CAN-05-3018
111. Marzec M, Kasprzycka M, Liu X, El-Salem M, Halasa K, Raghunath PN, Bucki R, Wlodarski P, Wasik MA (2007) Oncogenic tyrosine kinase NPM/ALK induces activation of the rapamycin-sensitive mTOR signaling pathway. Oncogene 26(38):5606–5614. doi:10.1038/sj.onc.1210346

112. Moore AS, Kearns PR, Knapper S, Pearson AD, Zwaan CM (2013) Novel therapies for children with acute myeloid leukaemia. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 27(7):1451–1460. doi:10.1038/leu.2013.106
113. Min YH, Eom JI, Cheong JW, Maeng HO, Kim JY, Jeung HK, Lee ST, Lee MH, Hahn JS, Ko YW (2003) Constitutive phosphorylation of AKT/PKB protein in acute myeloid leukemia: its significance as a prognostic variable. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 17(5):995–997. doi:10.1038/sj.leu.2402874
114. Bardet V, Tamburini J, Ifrah N, Dreyfus F, Mayeux P, Bouscary D, Lacombe C (2006) Single cell analysis of phosphoinositide 3-kinase/AKT and ERK activation in acute myeloid leukemia by flow cytometry. Haematologica 91(6):757–764
115. Tamburini J, Elie C, Bardet V, Chapuis N, Park S, Broet P, Cornillet-Lefebvre P, Lioure B, Ugo V, Blanchet O, Ifrah N, Witz F, Dreyfus F, Mayeux P, Lacombe C, Bouscary D (2007) Constitutive phosphoinositide 3-kinase/AKT activation represents a favorable prognostic factor in de novo acute myelogenous leukemia patients. Blood 110(3):1025–1028. doi:10.1182/blood-2006-12-061283
116. Sykes SM, Lane SW, Bullinger L, Kalaitzidis D, Yusuf R, Saez B, Ferraro F, Mercier F, Singh H, Brumme KM, Acharya SS, Scholl C, Tothova Z, Attar EC, Frohling S, DePinho RA, Armstrong SA, Gilliland DG, Scadden DT (2011) AKT/FOXO signaling enforces reversible differentiation blockade in myeloid leukemias. Cell 146(5):697–708. doi:10.1016/j.cell.2011.07.032
117. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 279(5350):577–580
118. Beghini A, Peterlongo P, Ripamonti CB, Larizza L, Cairoli R, Morra E, Mecucci C (2000) C-kit mutations in core binding factor leukemias. Blood 95(2):726–727
119. Goemans BF, Zwaan CM, Miller M, Zimmermann M, Harlow A, Meshinchi S, Loonen AH, Hahlen K, Reinhardt D, Creutzig U, Kaspers GJ, Heinrich MC (2005) Mutations in KIT and RAS are frequent events in pediatric core-binding factor acute myeloid leukemia. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 19(9):1536–1542. doi:10.1038/sj.leu.2403870
120. Nagata H, Worobec AS, Oh CK, Chowdhury BA, Tannenbaum S, Suzuki Y, Metcalfe DD (1995) Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. Proc Natl Acad Sci USA 92(23):10560–10564
121. Feger F, Ribadeau Dumas A, Leriche L, Valent P, Arock M (2002) Kit and c-kit mutations in mastocytosis: a short overview with special reference to novel molecular and diagnostic concepts. Int Arch Allergy Immunol 127(2):110–114. doi:10.1159/000048179
122. Munugalavadla V, Sims EC, Borneo J, Chan RJ, Kapur R (2007) Genetic and pharmacologic evidence implicating the p85 alpha, but not p85 beta, regulatory subunit of PI3K and Rac2 GTPase in regulating oncogenic KIT-induced transformation in acute myeloid leukemia and systemic mastocytosis. Blood 110(5):1612–1620. doi:10.1182/blood-2006-10-053058
123. Ma Y, Zeng S, Metcalfe DD, Akin C, Dimitrijevic S, Butterfield JH, McMahon G, Longley BJ (2002) The c-KIT mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors; kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. Blood 99(5):1741–1744
124. Akin C, Brockow K, D'Ambrosio C, Kirshenbaum AS, Ma Y, Longley BJ, Metcalfe DD (2003) Effects of tyrosine kinase inhibitor STI571 on human mast cells bearing wild-type or mutated c-kit. Exp Hematol 31(8):686–692
125. Pardanani A, Elliott M, Reeder T, Li CY, Baxter EJ, Cross NC, Tefferi A (2003) Imatinib for systemic mast-cell disease. Lancet 362(9383):535–536
126. Cammenga J, Horn S, Bergholz U, Sommer G, Besmer P, Fiedler W, Stocking C (2005) Extracellular KIT receptor mutants, commonly found in core binding factor AML, are

constitutively active and respond to imatinib mesylate. Blood 106(12):3958–3961. doi:10.1182/blood-2005-02-0583
127. Raimondi SC, Chang MN, Ravindranath Y, Behm FG, Gresik MV, Steuber CP, Weinstein HJ, Carroll AJ (1999) Chromosomal abnormalities in 478 children with acute myeloid leukemia: clinical characteristics and treatment outcome in a cooperative pediatric oncology group study-POG 8821. Blood 94(11):3707–3716
128. Edwards H, Xie C, LaFiura KM, Dombkowski AA, Buck SA, Boerner JL, Taub JW, Matherly LH, Ge Y (2009) RUNX1 regulates phosphoinositide 3-kinase/AKT pathway: role in chemotherapy sensitivity in acute megakaryocytic leukemia. Blood 114(13):2744–2752. doi:10.1182/blood-2008-09-179812
129. Care RS, Valk PJ, Goodeve AC, Abu-Duhier FM, Geertsma-Kleinekoort WM, Wilson GA, Gari MA, Peake IR, Lowenberg B, Reilly JT (2003) Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias. Br J Haematol 121 (5):775–777
130. Nanri T, Matsuno N, Kawakita T, Suzushima H, Kawano F, Mitsuya H, Asou N (2005) Mutations in the receptor tyrosine kinase pathway are associated with clinical outcome in patients with acute myeloblastic leukemia harboring t(8;21)(q22;q22). Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 19(8):1361–1366. doi:10.1038/sj.leu.2403803
131. Cairoli R, Beghini A, Grillo G, Nadali G, Elice F, Ripamonti CB, Colapietro P, Nichelatti M, Pezzetti L, Lunghi M, Cuneo A, Viola A, Ferrara F, Lazzarino M, Rodeghiero F, Pizzolo G, Larizza L, Morra E (2006) Prognostic impact of c-KIT mutations in core binding factor leukemias: an Italian retrospective study. Blood 107(9):3463–3468. doi:10.1182/blood-2005-09-3640
132. Shimada A, Taki T, Tabuchi K, Tawa A, Horibe K, Tsuchida M, Hanada R, Tsukimoto I, Hayashi Y (2006) KIT mutations, and not FLT3 internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group. Blood 107(5):1806–1809. doi:10.1182/blood-2005-08-3408
133. Gari M, Goodeve A, Wilson G, Winship P, Langabeer S, Linch D, Vandenberghe E, Peake I, Reilly J (1999) c-kit proto-oncogene exon 8 in-frame deletion plus insertion mutations in acute myeloid leukaemia. Br J Haematol 105(4):894–900
134. Wang YY, Zhou GB, Yin T, Chen B, Shi JY, Liang WX, Jin XL, You JH, Yang G, Shen ZX, Chen J, Xiong SM, Chen GQ, Xu F, Liu YW, Chen Z, Chen SJ (2005) AML1-ETO and C-KIT mutation/overexpression in t(8;21) leukemia: implication in stepwise leukemogenesis and response to Gleevec. Proc Natl Acad Sci USA 102(4):1104–1109. doi:10.1073/pnas.0408831102
135. Beghini A, Ripamonti CB, Cairoli R, Cazzaniga G, Colapietro P, Elice F, Nadali G, Grillo G, Haas OA, Biondi A, Morra E, Larizza L (2004) KIT activating mutations: incidence in adult and pediatric acute myeloid leukemia, and identification of an internal tandem duplication. Haematologica 89(8):920–925
136. Larizza L, Magnani I, Beghini A (2005) The Kasumi-1 cell line: a t(8;21)-kit mutant model for acute myeloid leukemia. Leuk Lymphoma 46(2):247–255. doi:10.1080/10428190400007565
137. Munugalavadla V, Sims EC, Chan RJ, Lenz SD, Kapur R (2008) Requirement for p85alpha regulatory subunit of class IA PI3K in myeloproliferative disease driven by an activation loop mutant of KIT. Exp Hematol 36(3):301–308. doi:10.1016/j.exphem.2007.11.008
138. Mali RS, Ramdas B, Ma P, Shi J, Munugalavadla V, Sims E, Wei L, Vemula S, Nabinger SC, Goodwin CB, Chan RJ, Traina F, Visconte V, Tiu RV, Lewis TA, Stern AM, Wen Q, Crispino JD, Boswell HS, Kapur R (2011) Rho kinase regulates the survival and transformation of cells bearing oncogenic forms of KIT, FLT3, and BCR-ABL. Cancer Cell 20(3):357–369. doi:10.1016/j.ccr.2011.07.016
139. Buet D, Gallais I, Lauret E, Denis N, Lombard B, Guillonneau F, Kosmider O, Loew D, Dusanter-Fourt I, Guillouf C, Mayeux P, Moreau-Gachelin F (2012) Cotargeting signaling

pathways driving survival and cell cycle circumvents resistance to Kit inhibitors in leukemia. Blood 119(18):4228–4241. doi:10.1182/blood-2011-07-368316
140. Corbacioglu S, Kilic M, Westhoff MA, Reinhardt D, Fulda S, Debatin KM (2006) Newly identified c-KIT receptor tyrosine kinase ITD in childhood AML induces ligand-independent growth and is responsive to a synergistic effect of imatinib and rapamycin. Blood 108 (10):3504–3513. doi:10.1182/blood-2006-05-021691
141. Gilliland DG, Griffin JD (2002) Role of FLT3 in leukemia. Curr Opin Hematol 9(4):274–281
142. Zhang S, Mantel C, Broxmeyer HE (1999) Flt3 signaling involves tyrosyl-phosphorylation of SHP-2 and SHIP and their association with Grb2 and Shc in Baf3/Flt3 cells. J Leukoc Biol 65(3):372–380
143. Zhang S, Broxmeyer HE (1999) p85 subunit of PI3 kinase does not bind to human Flt3 receptor, but associates with SHP2, SHIP, and a tyrosine-phosphorylated 100-kDa protein in Flt3 ligand-stimulated hematopoietic cells. Biochem Biophys Res Commun 254(2):440–445. doi:10.1006/bbrc.1998.9959
144. Grundler R, Miething C, Thiede C, Peschel C, Duyster J (2005) FLT3-ITD and tyrosine kinase domain mutants induce 2 distinct phenotypes in a murine bone marrow transplantation model. Blood 105(12):4792–4799. doi:10.1182/blood-2004-11-4430
145. Nabinger SC, Li XJ, Ramdas B, He Y, Zhang X, Zeng L, Richine B, Bowling JD, Fukuda S, Goenka S, Liu Z, Feng GS, Yu M, Sandusky GE, Boswell HS, Zhang ZY, Kapur R, Chan RJ (2013) The protein tyrosine phosphatase, Shp2, positively contributes to FLT3-ITD-induced hematopoietic progenitor hyperproliferation and malignant disease in vivo. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 27(2):398–408. doi:10.1038/leu.2012.308
146. Xu F, Taki T, Yang HW, Hanada R, Hongo T, Ohnishi H, Kobayashi M, Bessho F, Yanagisawa M, Hayashi Y (1999) Tandem duplication of the FLT3 gene is found in acute lymphoblastic leukaemia as well as acute myeloid leukaemia but not in myelodysplastic syndrome or juvenile chronic myelogenous leukaemia in children. Br J Haematol 105 (1):155–162
147. Iwai T, Yokota S, Nakao M, Okamoto T, Taniwaki M, Onodera N, Watanabe A, Kikuta A, Tanaka A, Asami K, Sekine I, Mugishima H, Nishimura Y, Koizumi S, Horikoshi Y, Mimaya J, Ohta S, Nishikawa K, Iwai A, Shimokawa T, Nakayama M, Kawakami K, Gushiken T, Hyakuna N, Fujimoto T et al (1999) Internal tandem duplication of the FLT3 gene and clinical evaluation in childhood acute myeloid leukemia. The Children's Cancer and Leukemia Study Group, Japan. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 13 (1):38–43
148. Kondo M, Horibe K, Takahashi Y, Matsumoto K, Fukuda M, Inaba J, Kato K, Kojima S, Matsuyama T (1999) Prognostic value of internal tandem duplication of the FLT3 gene in childhood acute myelogenous leukemia. Med Pediatr Oncol 33(6):525–529
149. Scheijen B, Ngo HT, Kang H, Griffin JD (2004) FLT3 receptors with internal tandem duplications promote cell viability and proliferation by signaling through Foxo proteins. Oncogene 23(19):3338–3349. doi:10.1038/sj.onc.1207456
150. Zeng Z, Samudio IJ, Zhang W, Estrov Z, Pelicano H, Harris D, Frolova O, Hail N Jr, Chen W, Kornblau SM, Huang P, Lu Y, Mills GB, Andreeff M, Konopleva M (2006) Simultaneous inhibition of PDK1/AKT and Fms-like tyrosine kinase 3 signaling by a small-molecule KP372-1 induces mitochondrial dysfunction and apoptosis in acute myelogenous leukemia. Cancer Res 66(7):3737–3746. doi:10.1158/0008-5472.CAN-05-1278
151. Kim KT, Levis M, Small D (2006) Constitutively activated FLT3 phosphorylates BAD partially through pim-1. Br J Haematol 134(5):500–509. doi:10.1111/j.1365-2141.2006.06225.x
152. Blanco-Aparicio C, Collazo AM, Oyarzabal J, Leal JF, Albaran MI, Lima FR, Pequeno B, Ajenjo N, Becerra M, Alfonso P, Reymundo MI, Palacios I, Mateos G, Quinones H, Corrionero A, Carnero A, Pevarello P, Lopez AR, Fominaya J, Pastor J, Bischoff JR (2011) Pim 1 kinase inhibitor ETP-45299 suppresses cellular proliferation and synergizes with PI3K inhibition. Cancer Lett 300(2):145–153. doi:10.1016/j.canlet.2010.09.016

153. Weisberg E, Banerji L, Wright RD, Barrett R, Ray A, Moreno D, Catley L, Jiang J, Hall-Meyers E, Sauveur-Michel M, Stone R, Galinsky I, Fox E, Kung AL, Griffin JD (2008) Potentiation of antileukemic therapies by the dual PI3K/PDK-1 inhibitor, BAG956: effects on BCR-ABL- and mutant FLT3-expressing cells. Blood 111(7):3723–3734. doi:10.1182/blood-2007-09-114454
154. Papa V, Tazzari PL, Chiarini F, Cappellini A, Ricci F, Billi AM, Evangelisti C, Ottaviani E, Martinelli G, Testoni N, McCubrey JA, Martelli AM (2008) Proapoptotic activity and chemosensitizing effect of the novel AKT inhibitor perifosine in acute myelogenous leukemia cells. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 22(1):147–160. doi:10.1038/sj.leu.2404980
155. Billottet C, Grandage VL, Gale RE, Quattropani A, Rommel C, Vanhaesebroeck B, Khwaja A (2006) A selective inhibitor of the p110delta isoform of PI3-kinase inhibits AML cell proliferation and survival and increases the cytotoxic effects of VP16. Oncogene 25 (50):6648–6659. doi:10.1038/sj.onc.1209670
156. Chen W, Drakos E, Grammatikakis I, Schlette EJ, Li J, Leventaki V, Staikou-Drakopoulou E, Patsouris E, Panayiotidis P, Medeiros LJ, Rassidakis GZ (2010) mTOR signaling is activated by FLT3 kinase and promotes survival of FLT3-mutated acute myeloid leukemia cells. Mol Cancer 9:292. doi:10.1186/1476-4598-9-292
157. Kadia TM, Kantarjian H, Kornblau S, Borthakur G, Faderl S, Freireich EJ, Luthra R, Garcia-Manero G, Pierce S, Cortes J, Ravandi F (2012) Clinical and proteomic characterization of acute myeloid leukemia with mutated RAS. Cancer 118(22):5550–5559. doi:10.1002/cncr.27596
158. Bacher U, Haferlach T, Schoch C, Kern W, Schnittger S (2006) Implications of NRAS mutations in AML: a study of 2502 patients. Blood 107(10):3847–3853. doi:10.1182/blood-2005-08-3522
159. Bos JL, Verlaan-de Vries M, van der Eb AJ, Janssen JW, Delwel R, Lowenberg B, Colly LP (1987) Mutations in N-ras predominate in acute myeloid leukemia. Blood 69(4):1237–1241
160. Bowen DT, Frew ME, Hills R, Gale RE, Wheatley K, Groves MJ, Langabeer SE, Kottaridis PD, Moorman AV, Burnett AK, Linch DC (2005) RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. Blood 106(6):2113–2119. doi:10.1182/blood-2005-03-0867
161. Kiyoi H, Naoe T, Nakano Y, Yokota S, Minami S, Miyawaki S, Asou N, Kuriyama K, Jinnai I, Shimazaki C, Akiyama H, Saito K, Oh H, Motoji T, Omoto E, Saito H, Ohno R, Ueda R (1999) Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood 93(9):3074–3080
162. Neubauer A, Dodge RK, George SL, Davey FR, Silver RT, Schiffer CA, Mayer RJ, Ball ED, Wurster-Hill D, Bloomfield CD et al (1994) Prognostic importance of mutations in the ras proto-oncogenes in de novo acute myeloid leukemia. Blood 83(6):1603–1611
163. Liang DC, Shih LY, Fu JF, Li HY, Wang HI, Hung IJ, Yang CP, Jaing TH, Chen SH, Liu HC (2006) K-Ras mutations and N-Ras mutations in childhood acute leukemias with or without mixed-lineage leukemia gene rearrangements. Cancer 106(4):950–956. doi:10.1002/cncr.21687
164. Pulikkan JA, Madera D, Xue L, Bradley P, Landrette SF, Kuo YH, Abbas S, Zhu LJ, Valk P, Castilla LH (2012) Thrombopoietin/MPL participates in initiating and maintaining RUNX1-ETO acute myeloid leukemia via PI3K/AKT signaling. Blood 120(4):868–879. doi:10.1182/blood-2012-03-414649
165. Zhang SJ, Shi JY, Zhu YM, Shi ZZ, Yan S, Gu BW, Bai XT, Shen ZX, Li JY (2006) The investigation of mutation and single nucleotide polymorphism of receptor tyrosine kinases and downstream scaffold molecules in acute myeloid leukemia. Leuk Lymphoma 47 (12):2610–2616. doi:10.1080/10428190600948048
166. Gilby DC, Goodeve AC, Winship PR, Valk PJ, Delwel R, Reilly JT (2007) Gene structure, expression profiling and mutation analysis of the tumour suppressor SHIP1 in Caucasian acute myeloid leukaemia. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 21(11):2390–2393. doi:10.1038/sj.leu.2404864

167. Brauer H, Strauss J, Wegner W, Muller-Tidow C, Horstmann M, Jucker M (2012) Leukemia-associated mutations in SHIP1 inhibit its enzymatic activity, interaction with the GM-CSF receptor and Grb2, and its ability to inactivate PI3K/AKT signaling. Cell Signal 24 (11):2095–2101. doi:10.1016/j.cellsig.2012.07.017
168. Bentires-Alj M, Paez JG, David FS, Keilhack H, Halmos B, Naoki K, Maris JM, Richardson A, Bardelli A, Sugarbaker DJ, Richards WG, Du J, Girard L, Minna JD, Loh ML, Fisher DE, Velculescu VE, Vogelstein B, Meyerson M, Sellers WR, Neel BG (2004) Activating mutations of the noonan syndrome-associated SHP2/PTPN11 gene in human solid tumors and adult acute myelogenous leukemia. Cancer Res 64(24):8816–8820
169. Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, Uhlik M, Lin A, Du J, Qian YW, Zeckner DJ, Tucker-Kellogg G, Touchman J, Patel K, Mousses S, Bittner M, Schevitz R, Lai MH, Blanchard KL, Thomas JE (2007) A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 448(7152):439–444. doi:10.1038/nature05933
170. Tibes R, Kornblau SM, Qiu Y, Mousses SM, Robbins C, Moses T, Carpten JD (2008) PI3K/AKT pathway activation in acute myeloid leukaemias is not associated with AKT1 pleckstrin homology domain mutation. Br J Haematol 140(3):344–347. doi:10.1111/j.1365-2141.2007.06920.x
171. Lee JW, Soung YH, Kim SY, Lee HW, Park WS, Nam SW, Kim SH, Lee JY, Yoo NJ, Lee SH (2005) PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. Oncogene 24(8):1477–1480. doi:10.1038/sj.onc.1208304
172. Horn S, Bergholz U, Jucker M, McCubrey JA, Trumper L, Stocking C, Basecke J (2008) Mutations in the catalytic subunit of class IA PI3K confer leukemogenic potential to hematopoietic cells. Oncogene 27(29):4096–4106. doi:10.1038/onc.2008.40
173. Xing Y, Gerhard B, Hogge DE (2012) Selective small molecule inhibitors of p110alpha and delta isoforms of phosphoinosityl-3-kinase are cytotoxic to human acute myeloid leukemia progenitors. Exp Hematol 40(11):922–933. doi:10.1016/j.exphem.2012.07.006
174. Xing Y, Hogge DE (2013) Combined inhibition of the phosphoinosityl-3-kinase (PI3Kinase) P110delta subunit and mitogen-extracellular activated protein kinase (MEKinase) shows synergistic cytotoxicity against human acute myeloid leukemia progenitors. Leuk Res 37 (6):697–704. doi:10.1016/j.leukres.2013.03.003
175. Liu TC, Lin PM, Chang JG, Lee JP, Chen TP, Lin SF (2000) Mutation analysis of PTEN/MMAC1 in acute myeloid leukemia. Am J Hematol 63(4):170–175
176. Aggerholm A, Gronbaek K, Guldberg P, Hokland P (2000) Mutational analysis of the tumour suppressor gene MMAC1/PTEN in malignant myeloid disorders. Eur J Haematol 65 (2):109–113
177. Cheong JW, Eom JI, Maeng HY, Lee ST, Hahn JS, Ko YW, Min YH (2003) Phosphatase and tensin homologue phosphorylation in the C-terminal regulatory domain is frequently observed in acute myeloid leukaemia and associated with poor clinical outcome. Br J Haematol 122(3):454–456
178. Valk PJ, Verhaak RG, Beijen MA, Erpelinck CA, van Waalwijk Barjesteh, van Doorn-Khosrovani S, Boer JM, Beverloo HB, Moorhouse MJ, van der Spek PJ, Lowenberg B, Delwel R (2004) Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med 350(16):1617–1628. doi:10.1056/NEJMoa040465
179. Yoshimi A, Goyama S, Watanabe-Okochi N, Yoshiki Y, Nannya Y, Nitta E, Arai S, Sato T, Shimabe M, Nakagawa M, Imai Y, Kitamura T, Kurokawa M (2011) Evi1 represses PTEN expression and activates PI3K/AKT/mTOR via interactions with polycomb proteins. Blood 117(13):3617–3628. doi:10.1182/blood-2009-12-261602
180. Li Y, Gao L, Luo X, Wang L, Gao X, Wang W, Sun J, Dou L, Li J, Xu C, Zhou M, Jiang M, Zhou J, Caligiuri MA, Nervi C, Bloomfield CD, Marcucci G, Yu L (2013) Epigenetic silencing of microRNA-193a contributes to leukemogenesis in t(8;21) acute myeloid leukemia by activating the PTEN/PI3K signal pathway. Blood 121(3):499–509. doi:10.1182/blood-2012-07-444729

181. Tazzari PL, Cappellini A, Ricci F, Evangelisti C, Papa V, Grafone T, Martinelli G, Conte R, Cocco L, McCubrey JA, Martelli AM (2007) Multidrug resistance-associated protein 1 expression is under the control of the phosphoinositide 3 kinase/AKT signal transduction network in human acute myelogenous leukemia blasts. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 21(3):427–438. doi:10.1038/sj.leu.2404523
182. Doepfner KT, Spertini O, Arcaro A (2007) Autocrine insulin-like growth factor-I signaling promotes growth and survival of human acute myeloid leukemia cells via the phosphoinositide 3-kinase/AKT pathway. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 21(9):1921–1930. doi:10.1038/sj.leu.2404813
183. Chapuis N, Tamburini J, Cornillet-Lefebvre P, Gillot L, Bardet V, Willems L, Park S, Green AS, Ifrah N, Dreyfus F, Mayeux P, Lacombe C, Bouscary D (2010) Autocrine IGF-1/IGF-1R signaling is responsible for constitutive PI3K/AKT activation in acute myeloid leukemia: therapeutic value of neutralizing anti-IGF-1R antibody. Haematologica 95 (3):415–423. doi:10.3324/haematol.2009.010785
184. Neri LM, Borgatti P, Tazzari PL, Bortul R, Cappellini A, Tabellini G, Bellacosa A, Capitani S, Martelli AM (2003) The phosphoinositide 3-kinase/AKT1 pathway involvement in drug and all-trans-retinoic acid resistance of leukemia cells. Mol Cancer Res: MCR 1 (3):234–246
185. Tamburini J, Chapuis N, Bardet V, Park S, Sujobert P, Willems L, Ifrah N, Dreyfus F, Mayeux P, Lacombe C, Bouscary D (2008) Mammalian target of rapamycin (mTOR) inhibition activates phosphatidylinositol 3-kinase/AKT by up-regulating insulin-like growth factor-1 receptor signaling in acute myeloid leukemia: rationale for therapeutic inhibition of both pathways. Blood 111(1):379–382. doi:10.1182/blood-2007-03-080796
186. Xia L, Wurmbach E, Waxman S, Jing Y (2006) Upregulation of Bfl-1/A1 in leukemia cells undergoing differentiation by all-trans retinoic acid treatment attenuates chemotherapeutic agent-induced apoptosis. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 20(6):1009–1016. doi:10.1038/sj.leu.2404198
187. Chomienne C, Ballerini P, Balitrand N, Daniel MT, Fenaux P, Castaigne S, Degos L (1990) All-trans retinoic acid in acute promyelocytic leukemias. II. In vitro studies: structure-function relationship. Blood 76(9):1710–1717
188. Drach J, Lopez-Berestein G, McQueen T, Andreeff M, Mehta K (1993) Induction of differentiation in myeloid leukemia cell lines and acute promyelocytic leukemia cells by liposomal all-trans-retinoic acid. Cancer Res 53(9):2100–2104
189. Lal L, Li Y, Smith J, Sassano A, Uddin S, Parmar S, Tallman MS, Minucci S, Hay N, Platanias LC (2005) Activation of the p70 S6 kinase by all-trans-retinoic acid in acute promyelocytic leukemia cells. Blood 105(4):1669–1677. doi:10.1182/blood-2004-06-2078
190. Billottet C, Banerjee L, Vanhaesebroeck B, Khwaja A (2009) Inhibition of class I phosphoinositide 3-kinase activity impairs proliferation and triggers apoptosis in acute promyelocytic leukemia without affecting atra-induced differentiation. Cancer Res 69 (3):1027–1036. doi:10.1158/0008-5472.CAN-08-2608
191. Bortul R, Tazzari PL, Billi AM, Tabellini G, Mantovani I, Cappellini A, Grafone T, Martinelli G, Conte R, Martelli AM (2005) Deguelin, A PI3K/AKT inhibitor, enhances chemosensitivity of leukaemia cells with an active PI3K/AKT pathway. Br J Haematol 129 (5):677–686. doi:10.1111/j.1365-2141.2005.05504.x
192. Kojima K, Shimanuki M, Shikami M, Samudio IJ, Ruvolo V, Corn P, Hanaoka N, Konopleva M, Andreeff M, Nakakuma H (2008) The dual PI3 kinase/mTOR inhibitor PI-103 prevents p53 induction by Mdm2 inhibition but enhances p53-mediated mitochondrial apoptosis in p53 wild-type AML. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 22 (9):1728–1736. doi:10.1038/leu.2008.158
193. Park S, Chapuis N, Bardet V, Tamburini J, Gallay N, Willems L, Knight ZA, Shokat KM, Azar N, Viguie F, Ifrah N, Dreyfus F, Mayeux P, Lacombe C, Bouscary D (2008) PI-103, a dual inhibitor of Class IA phosphatidylinositide 3-kinase and mTOR, has antileukemic activity in AML. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 22(9):1698–1706. doi:10.1038/leu.2008.144

194. Chapuis N, Tamburini J, Green AS, Vignon C, Bardet V, Neyret A, Pannetier M, Willems L, Park S, Macone A, Maira SM, Ifrah N, Dreyfus F, Herault O, Lacombe C, Mayeux P, Bouscary D (2010) Dual inhibition of PI3K and mTORC1/2 signaling by NVP-BEZ235 as a new therapeutic strategy for acute myeloid leukemia. Clin Cancer Res Official J Am Assoc Cancer Res 16(22):5424–5435. doi:10.1158/1078-0432.CCR-10-1102
195. Rosen DB, Harrington KH, Cordeiro JA, Leung LY, Putta S, Lacayo N, Laszlo GS, Gudgeon CJ, Hogge DE, Hawtin RE, Cesano A, Walter RB (2013) AKT signaling as a novel factor associated with in vitro resistance of human AML to gemtuzumab ozogamicin. PLoS ONE 8(1):e53518. doi:10.1371/journal.pone.0053518
196. Rahmani M, Aust MM, Attkisson E, Williams DC Jr, Ferreira-Gonzalez A, Grant S (2013) Dual inhibition of Bcl-2 and Bcl-xL strikingly enhances PI3K inhibition-induced apoptosis in human myeloid leukemia cells through a GSK3- and Bim-dependent mechanism. Cancer Res 73(4):1340–1351. doi:10.1158/0008-5472.CAN-12-1365
197. Jin L, Tabe Y, Kojima K, Shikami M, Benito J, Ruvolo V, Wang RY, McQueen T, Ciurea SO, Miida T, Andreeff M, Konopleva M (2013) PI3K inhibitor GDC-0941 enhances apoptotic effects of BH-3 mimetic ABT-737 in AML cells in the hypoxic bone marrow microenvironment. J Mol Med (Berl) 91(12):1383–1397. doi:10.1007/s00109-013-1076-3
198. Carter BZ, Milella M, Tsao T, McQueen T, Schober WD, Hu W, Dean NM, Steelman L, McCubrey JA, Andreeff M (2003) Regulation and targeting of antiapoptotic XIAP in acute myeloid leukemia. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 17(11):2081–2089. doi:10.1038/sj.leu.2403113
199. Zhao S, Konopleva M, Cabreira-Hansen M, Xie Z, Hu W, Milella M, Estrov Z, Mills GB, Andreeff M (2004) Inhibition of phosphatidylinositol 3-kinase dephosphorylates BAD and promotes apoptosis in myeloid leukemias. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 18(2):267–275. doi:10.1038/sj.leu.2403220
200. Tabellini G, Cappellini A, Tazzari PL, Fala F, Billi AM, Manzoli L, Cocco L, Martelli AM (2005) Phosphoinositide 3-kinase/AKT involvement in arsenic trioxide resistance of human leukemia cells. J Cell Physiol 202(2):623–634. doi:10.1002/jcp.20153
201. Rahmani M, Anderson A, Habibi JR, Crabtree TR, Mayo M, Harada H, Ferreira-Gonzalez A, Dent P, Grant S (2009) The BH3-only protein Bim plays a critical role in leukemia cell death triggered by concomitant inhibition of the PI3K/AKT and MEK/ERK1/2 pathways. Blood 114(20):4507–4516. doi:10.1182/blood-2008-09-177881
202. Abrams SL, Steelman LS, Shelton JG, Wong EW, Chappell WH, Basecke J, Stivala F, Donia M, Nicoletti F, Libra M, Martelli AM, McCubrey JA (2010) The Raf/MEK/ERK pathway can govern drug resistance, apoptosis and sensitivity to targeted therapy. Cell Cycle 9(9):1781–1791
203. Wang X, Wang C, Yan SK, Gao YR (2007) XIAP is upregulated in HL-60 cells cocultured with stromal cells by direct cell contact. Leuk Res 31(8):1125–1129. doi:10.1016/j.leukres.2006.09.006
204. Tabe Y, Jin L, Tsutsumi-Ishii Y, Xu Y, McQueen T, Priebe W, Mills GB, Ohsaka A, Nagaoka I, Andreeff M, Konopleva M (2007) Activation of integrin-linked kinase is a critical prosurvival pathway induced in leukemic cells by bone marrow-derived stromal cells. Cancer Res 67(2):684–694. doi:10.1158/0008-5472.CAN-06-3166
205. Katsumi A, Kiyoi H, Abe A, Tanizaki R, Iwasaki T, Kobayashi M, Matsushita T, Kaibuchi K, Senga T, Kojima T, Kohno T, Hamaguchi M, Naoe T (2011) FLT3/ ITD regulates leukaemia cell adhesion through alpha4beta1 integrin and Pyk2 signalling. Eur J Haematol 86(3):191–198. doi:10.1111/j.1600-0609.2010.01556.x
206. Jin L, Tabe Y, Lu H, Borthakur G, Miida T, Kantarjian H, Andreeff M, Konopleva M (2013) Mechanisms of apoptosis induction by simultaneous inhibition of PI3K and FLT3-ITD in AML cells in the hypoxic bone marrow microenvironment. Cancer Lett 329(1):45–58. doi:10.1016/j.canlet.2012.09.020
207. Zeng Z, Shi YX, Tsao T, Qiu Y, Kornblau SM, Baggerly KA, Liu W, Jessen K, Liu Y, Kantarjian H, Rommel C, Fruman DA, Andreeff M, Konopleva M (2012) Targeting of mTORC1/2 by the mTOR kinase inhibitor PP242 induces apoptosis in AML cells under

conditions mimicking the bone marrow microenvironment. Blood 120(13):2679–2689. doi:10.1182/blood-2011-11-393934
208. Maloney KW, Giller R, Hunger SP (2012) Recent advances in the understanding and treatment of pediatric leukemias. Adv Pediatr 59(1):329–358. doi:10.1016/j.yapd.2012.04.010
209. Druker BJ (2008) Translation of the Philadelphia chromosome into therapy for CML. Blood 112(13):4808–4817. doi:10.1182/blood-2008-07-077958
210. Deininger M, Buchdunger E, Druker BJ (2005) The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood 105(7):2640–2653. doi:10.1182/blood-2004-08-3097
211. Suttorp M, Millot F (2010) Treatment of pediatric chronic myeloid leukemia in the year 2010: use of tyrosine kinase inhibitors and stem-cell transplantation. Hematol Educ Prog Am Soc Hematol Am Soc Educ Prog 2010:368–376. doi:10.1182/asheducation-2010.1.368
212. Andolina JR, Neudorf SM, Corey SJ (2012) How I treat childhood CML. Blood 119(8):1821–1830. doi:10.1182/blood-2011-10-380774
213. Muramatsu H, Takahashi Y, Sakaguchi H, Shimada A, Nishio N, Hama A, Doisaki S, Yagasaki H, Matsumoto K, Kato K, Kojima S (2011) Excellent outcomes of children with CML treated with imatinib mesylate compared to that in pre-imatinib era. Int J Hematol 93(2):186–191. doi:10.1007/s12185-010-0764-9
214. Mariani S, Giona F, Basciani S, Brama M, Gnessi L (2008) Low bone density and decreased inhibin-B/FSH ratio in a boy treated with imatinib during puberty. Lancet 372(9633):111–112. doi:10.1016/S0140-6736(08)61023-5
215. Schmid H, Jaeger BA, Lohse J, Suttorp M (2009) Longitudinal growth retardation in a prepuberal girl with chronic myeloid leukemia on long-term treatment with imatinib. Haematologica 94(8):1177–1179. doi:10.3324/haematol.2009.008359
216. Kimoto T, Inoue M, Kawa K (2009) Growth deceleration in a girl treated with imatinib. Int J Hematol 89(2):251–252. doi:10.1007/s12185-008-0251-8
217. Narayanan KR, Bansal D, Walia R, Sachdeva N, Bhansali A, Varma N, Marwaha RK (2013) Growth failure in children with chronic myeloid leukemia receiving imatinib is due to disruption of GH/IGF-1 axis. Pediatr Blood Cancer 60(7):1148–1153. doi:10.1002/pbc.24397
218. Bansal D, Shava U, Varma N, Trehan A, Marwaha RK (2012) Imatinib has adverse effect on growth in children with chronic myeloid leukemia. Pediatr Blood Cancer 59(3):481–484. doi:10.1002/pbc.23389
219. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL (2001) Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 293(5531):876–880. doi:10.1126/science.1062538
220. Branford S, Rudzki Z, Walsh S, Grigg A, Arthur C, Taylor K, Herrmann R, Lynch KP, Hughes TP (2002) High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood 99(9):3472–3475
221. von Bubnoff N, Schneller F, Peschel C, Duyster J (2002) BCR-ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. Lancet 359(9305):487–491. doi:10.1016/S0140-6736(02)07679-1
222. Hochhaus A, Kreil S, Corbin AS, La Rosee P, Muller MC, Lahaye T, Hanfstein B, Schoch C, Cross NC, Berger U, Gschaidmeier H, Druker BJ, Hehlmann R (2002) Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 16(11):2190–2196. doi:10.1038/sj.leu.2402741
223. Kawauchi K, Ogasawara T, Yasuyama M, Ohkawa S (2003) Involvement of AKT kinase in the action of STI571 on chronic myelogenous leukemia cells. Blood Cells Mol Dis 31(1):11–17
224. Klejman A, Rushen L, Morrione A, Slupianek A, Skorski T (2002) Phosphatidylinositol-3 kinase inhibitors enhance the anti-leukemia effect of STI571. Oncogene 21(38):5868–5876. doi:10.1038/sj.onc.1205724

225. Sattler M, Salgia R, Okuda K, Uemura N, Durstin MA, Pisick E, Xu G, Li JL, Prasad KV, Griffin JD (1996) The proto-oncogene product p120CBL and the adaptor proteins CRKL and c-CRK link c-ABL, p190BCR/ABL and p210BCR/ABL to the phosphatidylinositol-3′ kinase pathway. Oncogene 12(4):839–846
226. Sattler M, Mohi MG, Pride YB, Quinnan LR, Malouf NA, Podar K, Gesbert F, Iwasaki H, Li S, Van Etten RA, Gu H, Griffin JD, Neel BG (2002) Critical role for Gab2 in transformation by BCR/ABL. Cancer Cell 1(5):479–492
227. Hirano I, Nakamura S, Yokota D, Ono T, Shigeno K, Fujisawa S, Shinjo K, Ohnishi K (2009) Depletion of Pleckstrin homology domain leucine-rich repeat protein phosphatases 1 and 2 by Bcr-Abl promotes chronic myelogenous leukemia cell proliferation through continuous phosphorylation of AKT isoforms. J Biol Chem 284(33):22155–22165. doi:10.1074/jbc.M808182200
228. Naughton R, Quiney C, Turner SD, Cotter TG (2009) Bcr-Abl-mediated redox regulation of the PI3K/AKT pathway. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 23(8):1432–1440. doi:10.1038/leu.2009.49
229. Keeshan K, Cotter TG, McKenna SL (2003) Bcr-Abl upregulates cytosolic p21WAF-1/CIP-1 by a phosphoinositide-3-kinase (PI3K)-independent pathway. Br J Haematol 123(1):34–44
230. Andreu EJ, Lledo E, Poch E, Ivorra C, Albero MP, Martinez-Climent JA, Montiel-Duarte C, Rifon J, Perez-Calvo J, Arbona C, Prosper F, Perez-Roger I (2005) BCR-ABL induces the expression of Skp2 through the PI3K pathway to promote p27Kip1 degradation and proliferation of chronic myelogenous leukemia cells. Cancer Res 65(8):3264–3272. doi:10.1158/0008-5472.CAN-04-1357
231. Chen JY, Wang MC, Hung WC (2009) Transcriptional activation of Skp2 by BCR-ABL in K562 chronic myeloid leukemia cells. Leuk Res 33(11):1520–1524. doi:10.1016/j.leukres.2009.03.007
232. Sugimoto Y, Nakamura S, Okinaka K, Hirano I, Ono T, Shigeno K, Shinjo K, Ohnishi K (2008) HOXA10 expression induced by Abl kinase inhibitors enhanced apoptosis through PI3K pathway in CML cells. Leuk Res 32(6):962–971. doi:10.1016/j.leukres.2007.11.034
233. Fang ZH, Dong CL, Chen Z, Zhou B, Liu N, Lan HF, Liang L, Liao WB, Zhang L, Han ZC (2009) Transcriptional regulation of survivin by c-Myc in BCR/ABL-transformed cells: implications in anti-leukaemic strategy. J Cell Mol Med 13(8B):2039–2052. doi:10.1111/j.1582-4934.2008.00549.x
234. Burchert A, Wang Y, Cai D, von Bubnoff N, Paschka P, Muller-Brusselbach S, Ottmann OG, Duyster J, Hochhaus A, Neubauer A (2005) Compensatory PI3-kinase/AKT/mTor activation regulates imatinib resistance development. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 19(10):1774–1782. doi:10.1038/sj.leu.2403898
235. Mancini M, Corradi V, Petta S, Martinelli G, Barbieri E, Santucci MA (2010) mTOR inhibitor RAD001 (Everolimus) enhances the effects of imatinib in chronic myeloid leukemia by raising the nuclear expression of c-ABL protein. Leuk Res 34(5):641–648. doi:10.1016/j.leukres.2009.07.012
236. Wohrle FU, Halbach S, Aumann K, Schwemmers S, Braun S, Auberger P, Schramek D, Penninger JM, Lassmann S, Werner M, Waller CF, Pahl HL, Zeiser R, Daly RJ, Brummer T (2013) Gab2 signaling in chronic myeloid leukemia cells confers resistance to multiple Bcr-Abl inhibitors. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 27(1):118–129. doi:10.1038/leu.2012.222
237. Hui RC, Gomes AR, Constantinidou D, Costa JR, Karadedou CT, Fernandez de Mattos S, Wymann MP, Brosens JJ, Schulze A, Lam EW (2008) The forkhead transcription factor FOXO3a increases phosphoinositide-3 kinase/AKT activity in drug-resistant leukemic cells through induction of PIK3CA expression. Mol Cell Biol 28(19):5886–5898. doi:10.1128/MCB.01265-07
238. Loh ML (2011) Recent advances in the pathogenesis and treatment of juvenile myelomonocytic leukaemia. Br J Haematol 152(6):677–687. doi:10.1111/j.1365-2141.2010.08525.x

239. Emanuel PD (2008) Juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 22(7):1335–1342. doi:10.1038/leu.2008.162
240. Chan RJ, Cooper T, Kratz CP, Weiss B, Loh ML (2009) Juvenile myelomonocytic leukemia: a report from the 2nd international JMML symposium. Leuk Res 33(3):355–362. doi:10.1016/j.leukres.2008.08.022
241. Niemeyer CM, Kratz CP (2008) Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia: molecular classification and treatment options. Br J Haematol 140(6):610–624. doi:10.1111/j.1365-2141.2007.06958.x
242. Bergstraesser E, Hasle H, Rogge T, Fischer A, Zimmermann M, Noellke P, Niemeyer CM (2007) Non-hematopoietic stem cell transplantation treatment of juvenile myelomonocytic leukemia: a retrospective analysis and definition of response criteria. Pediatr Blood Cancer 49(5):629–633
243. Emanuel PD, Bates LJ, Castleberry RP, Gualtieri RJ, Zuckerman KS (1991) Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. Blood 77(5):925–929
244. Locatelli F, Nollke P, Zecca M, Korthof E, Lanino E, Peters C, Pession A, Kabisch H, Uderzo C, Bonfim CS, Bader P, Dilloo D, Stary J, Fischer A, Revesz T, Fuhrer M, Hasle H, Trebo M, van den Heuvel-Eibrink MM, Fenu S, Strahm B, Giorgiani G, Bonora MR, Duffner U, Niemeyer CM (2005) Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. Blood 105(1):410–419
245. Yoshimi A, Mohamed M, Bierings M, Urban C, Korthof E, Zecca M, Sykora KW, Duffner U, Trebo M, Matthes-Martin S, Sedlacek P, Klingebiel T, Lang P, Fuhrer M, Claviez A, Wossmann W, Pession A, Arvidson J, O'Marcaigh AS, van den Heuvel-Eibrink MM, Stary J, Hasle H, Nollke P, Locatelli F, Niemeyer CM (2007) Second allogeneic hematopoietic stem cell transplantation (HSCT) results in outcome similar to that of first HSCT for patients with juvenile myelomonocytic leukemia. Leuk Official J Leuk Soc Am, Leuk Res Fund, UK 21(3):556–560. doi:10.1038/sj.leu.2404537
246. Kalra R, Paderanga DC, Olson K, Shannon KM (1994) Genetic analysis is consistent with the hypothesis that NF1 limits myeloid cell growth through p21ras. Blood 84(10):3435–3439
247. Miyauchi J, Asada M, Sasaki M, Tsunematsu Y, Kojima S, Mizutani S (1994) Mutations of the N-ras gene in juvenile chronic myelogenous leukemia. Blood 83(8):2248–2254
248. Shannon KM, O'Connell P, Martin GA, Paderanga D, Olson K, Dinndorf P, McCormick F (1994) Loss of the normal NF1 allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders. N Engl J Med 330(9):597–601. doi:10.1056/NEJM199403033300903
249. Loh ML, Sakai DS, Flotho C, Kang M, Fliegauf M, Archambeault S, Mullighan CG, Chen L, Bergstraesser E, Bueso-Ramos CE, Emanuel PD, Hasle H, Issa JP, van den Heuvel-Eibrink MM, Locatelli F, Stary J, Trebo M, Wlodarski M, Zecca M, Shannon KM, Niemeyer CM (2009) Mutations in CBL occur frequently in juvenile myelomonocytic leukemia. Blood 114(9):1859–1863. doi:10.1182/blood-2009-01-198416
250. Tartaglia M, Niemeyer CM, Fragale A, Song X, Buechner J, Jung A, Hahlen K, Hasle H, Licht JD, Gelb BD (2003) Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet 34(2):148–150
251. Shiba N, Ohki K, Park MJ, Sotomatsu M, Kudo K, Ito E, Sako M, Arakawa H, Hayashi Y (2013) SETBP1 mutations in juvenile myelomonocytic leukaemia and myelodysplastic syndrome but not in paediatric acute myeloid leukaemia. Br J Haematol. doi:10.1111/bjh.12595
252. Sakaguchi H, Okuno Y, Muramatsu H, Yoshida K, Shiraishi Y, Takahashi M, Kon A, Sanada M, Chiba K, Tanaka H, Makishima H, Wang X, Xu Y, Doisaki S, Hama A, Nakanishi K, Takahashi Y, Yoshida N, Maciejewski JP, Miyano S, Ogawa S, Kojima S

(2013) Exome sequencing identifies secondary mutations of SETBP1 and JAK3 in juvenile myelomonocytic leukemia. Nat Genet 45(8):937–941. doi:10.1038/ng.2698
253. Stieglitz E, Troup CB, Gelston LC, Haliburton J, Chow ED, Yu KB, Akutagawa J, Taylor-Weiner AN, Liu YL, Wang YD, Beckman K, Emanuel PD, Braun BS, Abate A, Gerbing RB, Alonzo TA, Loh ML (2015) Subclonal mutations in SETBP1 confer a poor prognosis in juvenile myelomonocytic leukemia. Blood 125(3):516–524. doi:10.1182/blood-2014-09-601690
254. Chan RJ, Leedy MB, Munugalavadla V, Voorhorst CS, Li Y, Yu M, Kapur R (2005) Human somatic PTPN11 mutations induce hematopoietic-cell hypersensitivity to granulocyte-macrophage colony-stimulating factor. Blood 105(9):3737–3742. doi:10.1182/blood-2004-10-4002
255. Mohi MG, Williams IR, Dearolf CR, Chan G, Kutok JL, Cohen S, Morgan K, Boulton C, Shigematsu H, Keilhack H, Akashi K, Gilliland DG, Neel BG (2005) Prognostic, therapeutic, and mechanistic implications of a mouse model of leukemia evoked by Shp2 (PTPN11) mutations. Cancer Cell 7(2):179–191. doi:10.1016/j.ccr.2005.01.010
256. Schubbert S, Lieuw K, Rowe SL, Lee CM, Li X, Loh ML, Clapp DW, Shannon KM (2005) Functional analysis of leukemia-associated PTPN11 mutations in primary hematopoietic cells. Blood 106(1):311–317
257. Perugini M, Brown AL, Salerno DG, Booker GW, Stojkoski C, Hercus TR, Lopez AF, Hibbs ML, Gonda TJ, D'Andrea RJ (2010) Alternative modes of GM-CSF receptor activation revealed using activated mutants of the common beta-subunit. Blood 115 (16):3346–3353. doi:10.1182/blood-2009-08-235846
258. Yang Z, Li Y, Yin F, Chan RJ (2008) Activating PTPN11 mutants promote hematopoietic progenitor cell-cycle progression and survival. Exp Hematol 36(10):1285–1296. doi:10.1016/j.exphem.2008.04.016
259. Goodwin CB, Yang Z, Yin F, Yu M, Chan RJ (2012) Genetic disruption of the PI3K regulatory subunits, p85alpha, p55alpha, and p50alpha, normalizes mutant PTPN11-induced hypersensitivity to GM-CSF. Haematologica 97(7):1042–1047. doi:10.3324/haematol.2011.046896
260. Yu WM, Daino H, Chen J, Bunting KD, Qu CK (2006) Effects of a leukemia-associated gain-of-function mutation of SHP-2 phosphatase on interleukin-3 signaling. J Biol Chem 281 (9):5426–5434. doi:10.1074/jbc.M507622200
261. Goodwin CB, Li XJ, Mali RS, Chan G, Kang M, Liu Z, Vanhaesebroeck B, Neel BG, Loh ML, Lannutti BJ, Kapur R, Chan RJ (2014) PI3K p110delta uniquely promotes gain-of-function Shp2-induced GM-CSF hypersensitivity in a model of JMML. Blood 123 (18):2838–2842. doi:10.1182/blood-2013-10-535104
262. Gritsman K, Yuzugullu H, Von T, Yan H, Clayton L, Fritsch C, Maira SM, Hollingworth G, Choi C, Khandan T, PAKTinat M, Okabe RO, Roberts TM, Zhao JJ (2014) Hematopoiesis and RAS-driven myeloid leukemia differentially require PI3K isoform p110alpha. J Clin Investig 124(4):1794–1809. doi:10.1172/JCI69927
263. Neel BG, Gu H, Pao L (2003) The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. Trends Biochem Sci 28(6):284–293. doi:10.1016/S0968-0004(03)00091-4
264. Chan G, Kalaitzidis D, Neel BG (2008) The tyrosine phosphatase Shp2 (PTPN11) in cancer. Cancer Metastasis Rev 27(2):179–192. doi:10.1007/s10555-008-9126-y
265. Pulsipher MA, Langholz B, Wall DA, Schultz KR, Bunin N, Carroll WL, Raetz E, Gardner S, Gastier-Foster JM, Howrie D, Goyal RK, Douglas JG, Borowitz M, Barnes Y, Teachey DT, Taylor C, Grupp SA (2014) The addition of sirolimus to tacrolimus/methotrexate GVHD prophylaxis in children with ALL: a phase 3 Children's Oncology Group/Pediatric Blood and Marrow Transplant Consortium trial. Blood 123 (13):2017–2025. doi:10.1182/blood-2013-10-534297
266. Gojo I, Perl A, Luger S, Baer MR, Norsworthy KJ, Bauer KS, Tidwell M, Fleckinger S, Carroll M, Sausville EA (2013) Phase I study of UCN-01 and perifosine in patients with

relapsed and refractory acute leukemias and high-risk myelodysplastic syndrome. Invest New Drugs 31(5):1217–1227. doi:10.1007/s10637-013-9937-8

267. Friedman DR, Lanasa MC, Davis PH, Allgood SD, Matta KM, Brander DM, Chen Y, Davis ED, Volkheimer AD, Moore JO, Gockerman JP, Sportelli P, Weinberg JB (2014) Perifosine treatment in chronic lymphocytic leukemia: results of a phase II clinical trial and in vitro studies. Leuk Lymphoma 55(5):1067–1075. doi:10.3109/10428194.2013.824080

Chapter 8
HER2 Signaling Network in Advanced Breast Cancer: Opportunities for Combination Therapies

Nandini Dey, Brian Leyland-Jones and Pradip De

Background

Fifteen to twenty percent of breast cancer patients with *HER2* amplification and they might benefit from anti-HER2 therapies. Although anti-HER2 therapy has notably evolved during the past two to five years (development of a dual HER2: HER3 specific antibody, pertuzumab; an antibody drug conjugate, T-DM1 and pan-HER kinase inhibitor, neratinib or afatinib), the invariable appearance of anti-HER2 therapy resistance, either de novo or acquire, remains an important issue in the clinic. The improvement of understanding the cancer genome has identified some promising targets that might be responsible or linked to anti-HER2 therapy resistance including alterations affecting main signaling pathways like PI3K-AKT-mTOR and CYCLINE-CDK2 as well as the identification of new HER2 somatic mutations, leading to array of new targeted therapies that might circumvent or prevent anti-HER2 therapy resistance (Fig. 8.1).

HER2 belongs to a family of receptor tyrosine kinases regulating diverse biologic processes, including growth and proliferation [1]. HER2 is a member of the epidermal growth factor receptor (EGFR/HER) family of receptor tyrosine kinases, which includes EGFR (HER1), HER2, HER3, and HER4. These receptors contain a glycosylated extracellular domain (ECD), a single hydrophobic trans-membrane segment, and an intracellular portion with a juxta-membrane portion, a protein kinase domain, and a carboxy-terminal tail (C-terminal domain). Homo- or heterodimer-

N. Dey · B. Leyland-Jones · P. De
Avera Cancer Institute Center for Precision Oncology, Sioux Falls, SD 57105, USA

N. Dey · B. Leyland-Jones · P. De (✉)
Departmental of Internal Medicine, SSOM, University of South Dakota, Sioux Falls, SD, USA
e-mail: pradip.de@avera.org

N. Dey et al. (eds.), *PI3K-mTOR in Cancer and Cancer Therapy*,
Cancer Drug Discovery and Development, DOI 10.1007/978-3-319-34211-5_8

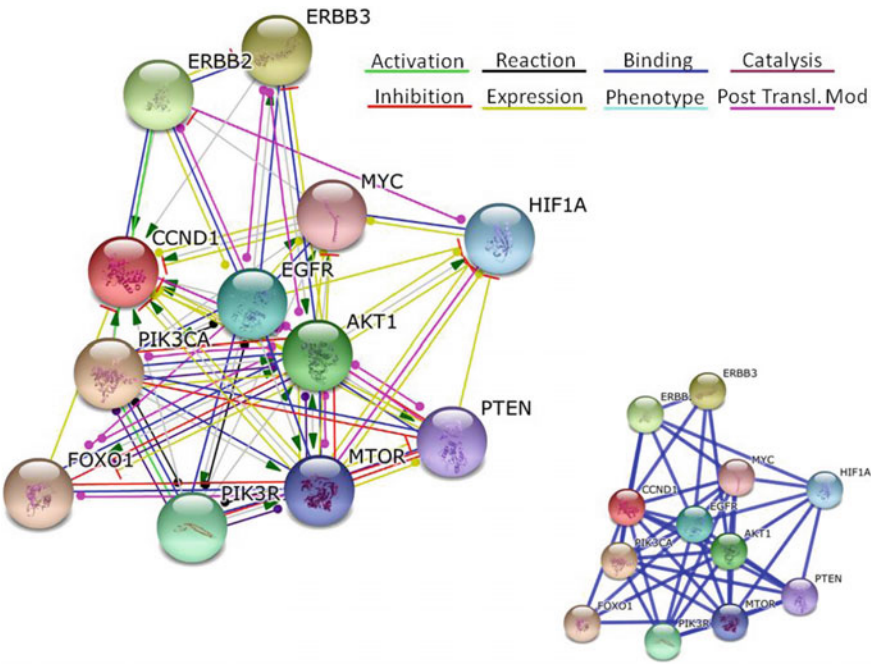


Fig. 8.1 The network image of the "action view" of the network summary of the PI3K-AKT-mTOR signaling pathway genes (*HER1, HER2, HER3, PIK3CA, PIK3R1, PTEN, AKT1, mTOR, CCND1, MYC, HIF1A,* and *FOXO1*) as obtained using STRING 10. The network is displayed as Nodes which are either colored (as they are directly linked to the input of the PI3K-AKT-mTOR signaling pathway genes). Edges, i.e., predicted functional links, consist of up to eight lines: one color for each type of evidence (as shown in the picture). Active prediction methods included databases and text mining. Confidence view of the association is presented as an inset. Stronger associations are represented by *thicker* lines. The confidence score presented is at the highest confidence level (0.900). The confidence score is the approximate probability that a predicted link exists between two enzymes in the same metabolic map in the KEGG database

ization of these receptors results in phosphorylation of tyrosine residues in the intracellular domain and resulting recruitment of adapter molecules responsible for the initiation of downstream signaling pathways involved in cell proliferation and survival [2, 3]. The heterodimer consists of ERBB2, which lacks a ligand, and ERBB3, which is kinase impaired, is the most robust signaling complex of this receptor family. Downstream ERBB signaling cascades include the phosphatidylinositol 3-kinase-AKT (PI3K) pathway, the RAS-RAF-MEK-ERK1/2 pathway, and the phospholipase C (PLCγ) pathway. Approximately 20 % of breast cancers exhibit *HER2* gene amplification/overexpression, leading to an aggressive tumor phenotype and reduced survival [4–6]. The development of a monoclonal antibody targeting HER2, trastuzumab (Herceptin), has had a huge impact on the survival of this population of patients. Currently, *HER2* gene amplification guides clinical decision-making in breast cancer and HER2-targeted drugs are only approved for use in patients

designated HER2-positive. Yet new research suggests that these drugs may actually have much broader applications, benefiting patients who are not designated HER2-positive by routine testing, with far-reaching implications not just for the diagnosis and treatment of breast cancer, but for the understanding of the molecular drivers of cancers as a whole and the development of targeted therapies in general. Fortunately, *HER2* amplification/overexpression in breast cancer (HER2+ breast cancer) is associated with high benefit from anti-HER2 therapies in combination with chemotherapy even in metastatic settings [7–10]. In addition, dual HER targeting without chemotherapy (trastuzumab + HER1 and HER2 dual tyrosine kinase inhibitor, lapatinib) [11] and trastuzumab plus pertuzumab (HER2:HER3 anti-dimerize antibody) with or without chemotherapy are showing good activities in a subset of HER2+ tumors [12, 13]. In 1998, trastuzumab became the first HER2-targeted therapy to be approved by the FDA for the treatment of advanced, metastatic, HER2-overexpressing breast cancer in combination with chemotherapy or as a single agent, based on pivotal clinical trials showing overall response rates of 45 and 14 %, respectively. This was followed in 2006 by approval to treat early-stage HER2-positive breast cancer in combination with a chemotherapeutic regimen containing doxorubicin, cyclophosphamide, and paclitaxel, following studies demonstrating greater than 50 % reduction in the risk of recurrence, second primary cancer, or death among patients treated with trastuzumab plus chemotherapy, compared with chemotherapy alone. In the years that followed its approval, trastuzumab has been joined by several other FDA-approved HER2-targeted agents (Table 8.1). The clinical trials that were instrumental in gaining approval for these HER2-targeted agents indicated that their benefits were restricted to patients whose tumors exhibited overexpression of the HER2 protein or amplification of the *HER2* gene. As such, administration of these drugs is dependent on the demonstration of a patient's HER2-positive status, typically using the standard FDA-approved assays—fluorescence in situ hybridization, to evaluate the number of *HER2* genes in a cancer cell (gene amplification), and immunohistochemistry, to examine the level of HER2 protein present on the cancer cell surface (protein overexpression). A third method, chromogenic in situ hybridization, also has been approved to evaluate gene expression.

To date, HER2+ breast cancer has been considered as a single disease entity, since the dominant role of the HER2 receptor itself as well as the availability of the anti-HER2 agent trastuzumab, it is now increasingly apparent that HER2+ is clinically and biologically heterogeneous [14–18]. Gene expression profiling has identified four main intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched [HER2E], and Basal-like/triple negative) with different outcomes and responses to therapy [19–24]. Among the different subtypes, the HER2E subtype is characterized by the high expression of HER2-regulated genes and low expression of luminal-related [14, 25, 26]. Surrogate pathology-based definitions of the intrinsic subtypes are an integral part of the St. Gallen Expert Consensus Guidelines for the recommendation of chemotherapy, endocrine therapy, and/or anti-HER2 therapy in early breast cancer. However, St. Gallen's criteria that divide HER2+ disease into two groups (i.e., HER2+/ER+ and HER2+/ER−)

Table 8.1 Approved and late stage of clinical development in HER2-targeting therapies for breast cancer

Agent	Breast cancer indications	Company	Initial FDA approval
Trastuzumab (Herceptin)	• Adjuvant treatment with chemotherapy or as single agent after multimodality therapy • MBC with chemotherapy in first line or as single agent after chemotherapy	Genentech	1998
Lapatinib (Tykerb)	• Advanced BC or MBC with capecitabine after prior therapy including trastuzumab • Postmenopausal women with HR + MBC in combination with letrozole	GlaxoSmith-Kline	2007
Pertuzumab (Perjeta)	• MBC in combination with trastuzumab and docetaxel in patients with no prior anti-HER2 or chemotherapy for metastatic disease	Genentech	2012
Ado-trastuzumab emtansine, or T-DM1 (Kadcyla)	• MBC as single agent after prior trastuzumab and taxane-based therapy, or disease recurrence within 6 months of adjuvant therapy	Genentech	2013
Afatinib (Gilotrif)	• For the first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test • Currently in different phases of clinical trials in HER2+ breast cancer	Boehringer Ingelheim	2013
Neratinib (HKI-272)	• It is in development for the treatment of early- and late-stage HER2+ breast cancer	Puma Biotechnology	Not yet approved

are driven by treatment considerations mostly based on the necessity to recommend endocrine therapy for patients with ER+, while chemotherapy and anti-HER2 therapy is recommended for both [27]. Following three studies have shown that hormone receptor status determined by IHC identifies two main groups of HER2+

Table 8.2 Role of upregulation of the PI3K-AKT-mTOR pathway in anti-HER2 therapy and efficacy of mTOR allosteric inhibitor, everolimus in *HER2*+ breast cancer patients

NeoALTTO	*Lapatinib* pCR (%)	*Trastuzumab* pCR (%)	Lapatinib + trastuzumab pCR (%)
All patients	27.7	29.5	51.3
PIK3CA wild type	20.4	28.4	55.8
PIK3CA mutant	14.8	20	28.6

Gepar (Quattro/Quinoto/Sixtro)	pCR *PIK3CA* mutant (%)	pCR *PIK3CA* wild type (%)	OR/*p* value
All patients	19.4	32.8	OR: 0.34; $p = 0.008$
HR-positive patients	11.3	27.5	OR: 0.34; $p = 0.011$
HR-negative patients	30.4	40.1	OR: 0.65; $p = 0.292$

CLEOPATRA	PFS *PIK3CA* mutant (months)	PFS *PIK3CA* wild type (months)
Pertuzumab + trastuzumab + docetaxel	12.5	21.8
Placebo + trastuzumab + docetaxel	8.6	13.8

BOLERO1**	*Everolimus* PFS (months)	*Placebo* PFS (months)	HR/*p* Value
Total patients	14.95	14.49	HR: 0.89; $p = 0.1166$
HR-negative patients	20.27	13.08	HR: 0.66; $p = 0.0049$

BOLERO3**	*Everolimus* median PFS (months)	*Placebo* median PFS (months)	HR/*p* value
Total patients	7	5.78	HR: 0.78; $p = 0.0067$
Low PTEN H-score cut point <20th percentile	9.6	5.2	HR: 0.40
Normal PTEN			HR: 1.05
High pS6K H-score cut point >75th percentile			HR: 0.48
Low pS6K			HR: 1.14

**for recent update please see *Andre Fabrice, Sara Hurvitz et al 2016 JCO Apr 18. pii: JCO639161; PMID: 27091708.*

tumors with different survival outcomes. In the 4-year follow-up of the joint N9831 and National Surgical Adjuvant Breast and Bowel Project B-31 adjuvant trials of trastuzumab in HER2+ disease (n = 4045), hormone receptor-positive disease was found statistically significantly associated with approximately 40 % increased DFS (disease-free survival) and OS (overall survival), compared to hormone receptor-negative disease [28]. Similar data was observed in a prospective cohort study of 3394 patients with stage I to III HER2+ breast cancer from National Comprehensive Cancer Network centers [29]. In NeoALTTO study, the pCR (pathological complete response) rates after paclitaxel in combination with one of the three anti-HER2 regimens (lapatinib, trastuzumab, and lapatinib combined with trastuzumab) were higher in hormone receptor-negative disease compared to hormone receptor-positive disease (please see detail in Table 8.2) [30].

Researchers from the Siteman Cancer Center and The Genome Institute at Washington University in St. Louis, Missouri, analyzed data from eight breast cancer genome-sequencing studies (a total of 1500 patients) to further examine HER2 status. They identified a group of patients who did not have *HER2* gene amplification (thus giving them a HER2-negative designation, meaning they would not receive HER2-targeted therapy according to current guidelines) but whose tumors displayed mutations in the *HER2* gene that resulted in the excess activity of the HER2 protein. They then went on to show that these activating mutations (G309A, D769H, D769Y, V777L, P780ins, V842I, and R896C) rendered the tumors susceptible to the approved HER2 agents trastuzumab and lapatinib (Tykerb), as well as neratinib, a pan-HER family inhibitor [31]. All of the activating mutations described in this study were sensitive to neratinib. Importantly, however, a non-activating mutation, L755S, was discovered in 25 % of patients with somatic mutations; it likely drives resistance to lapatinib, and was also sensitive to neratinib. This suggests that neratinib could be an important agent in helping to overcome resistance to other HER2-targeted agents. Lead author, Ron Bose, MD, PhD, said in an email interview that the findings about the L755S mutation prompted investigators to make changes to a phase II trial of lapatinib with trastuzumab in HER2-negative patients with stage IV breast cancer that had been launched to examine their *HER2* genes for mutations and assessed their response to anti-HER2 therapy. Now, neratinib has replaced lapatinib (NCT01670877). Bose and colleagues concluded that these newly identified mutations represented “an alternative mechanism to activate HER2 in breast cancer” and valid drug targets for breast cancer treatment. It is estimated that these mutations could be responsible for tumor growth in approximately 1.6 % of cases, translating into some 4000 cases of breast cancer per year in the United States alone.

The PI3K-AKT-mTOR Pathway in HER2 Positive Breast Cancer

An Overview of the Pathway

The PI3K-AKT-mTOR signaling pathway involves many nodal points and several important signaling effector molecules. In addition to PI3K (phosphatidylinositol 3-kinase), there are two important signaling nodes and they are directly involved in cancer progression, e.g., AKT (also known as protein kinase B, PKB), and mTOR (mammalian target of rapamycin). The pathway also includes the following: raptor, rictor, GDL protein, P70 S6 kinase (P70S6K), ribosomal S6 protein kinase (S6K), eukaryotic initiating factor 4E binding protein 1 (4EBP1), tuberous sclerosis complex (TSC)-1 and 2, the small GTPase Rheb (Ras-homolog enriched in brain), cyclin D1, hypoxia-inducible factor-1 (HIF-1), and phosphatase and tensin homolog (PTEN) located on chromosome 10 (Fig. 8.2). Although activated to different degrees in tumors, all of these proteins are known tumor-related factors. The PTEN gene has been observed to be lost in a variety of tumors, and PTEN gene deletion in mice often leads to the development of multiple tumors [32, 33]. Rheb is a highly conserved protein with abnormal expression in several tumor types and can promote the malignant transformation of mouse fibroblasts [34]. The TSC1 and TSC2 complexes are considered as tumor suppressors and are often abnormally expressed in tumor tissue [35]. The PI3K-AKT-mTOR pathway is sensitive to a variety of exogenous growth factors, cytokines, hormones, and nutrients (ATP, glucose, and amino acids). After membrane receptor tyrosine kinases bind extracellular growth factor ligands, tyrosine residues are phosphorylated and activated. Subsequently, the receptor tyrosine kinases activate PI3K via adapter protein(s). Activated PI3K phosphorylates 3,4-bisphosphate phosphatidylinositol (PIP2), to convert it into 3,4,5-triphosphate phosphatidylinositol (PIP3). AKT binds to PIP3 through its PH domain and is activated by PDK1 phosphorylation on threonine 308 (Thr308). Ser473 is also phosphorylated and activated in the presence of mTORC2 [36]. Under normal circumstances, TSC-1 and TSC-2 form a dimer that functions as an inhibitor of the small GTPase Rheb. Rheb is necessary for mTORC1 activation, and therefore, TSC-1/TSC-2 inhibits mTORC1 function and acts as a tumor suppressor. In addition, activated AKT is able to phosphorylate TSC-2 Ser939 and Thr1462, thereby inhibiting the formation of the TSC-1/TSC-2 complex and abolishing the inhibition of Rheb to promote mTOR activation [34]. The carboxyl terminal end of mTOR is highly homologous to the PI3K catalytic domain; therefore, mTOR is considered a PI3K-related protein kinase family member. Downstream effectors of mTORC1 (which contains the binding protein, raptor) are P70S6K and 4EBP1, and hence control 5 TOP mRNA and cap-dependent mRNA translation of HIF1α (major transcription factor for synthesis of VEGF, a master controller of tumor-induced angiogenesis), cell cycle regulating proteins, c-MYC, cyclin D1, ODC1, and cell survival/proliferation, SURVIVIN, XIAP, BCL2. In addition, mTORC2 complex (which contains the novel protein rictor) is functionally distinct

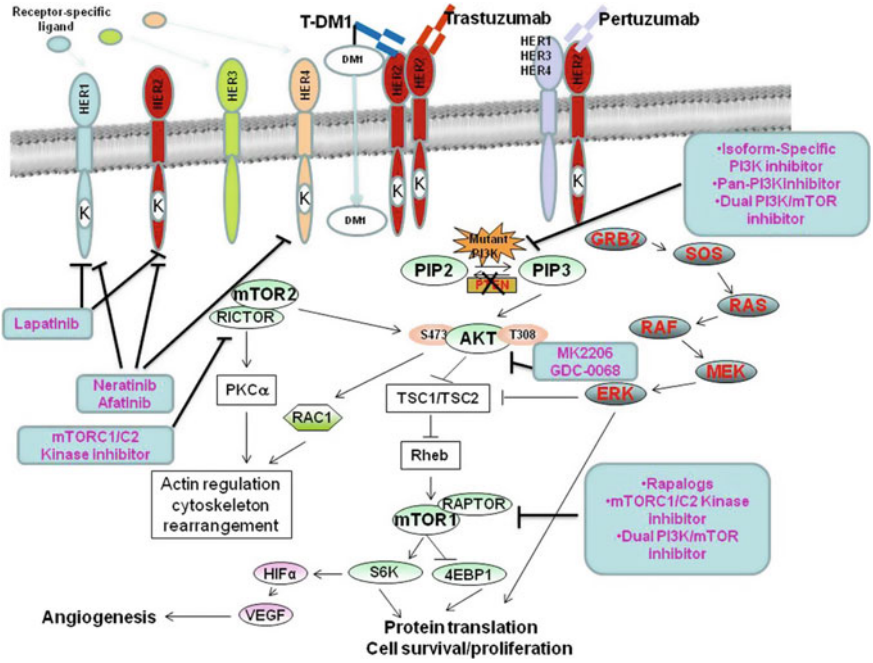


Fig. 8.2 Diagrammatic representation of the HER2, HER3, their downstream signaling pathways and relevant drugs that target each of the critical components of the pathway

from the mTORC1 complex. mTORC2 phosphorylates AKT at serine 473, PKCα and SGK1 [37, 38]. The activation of mTORC2 leads to actin regulation, cytoskeleton rearrangement, and cell survival [39], however, the precise function and specifically the regulation of mTORC2 has not yet been fully clarified. Collectively, mammalian target of rapamycin (mTOR) constitutes the most important node of the PI3K-mTOR pathway as it controls, (1) growth factor-induced survival and proliferative signals, (2) hypoxic stress response, (3) metabolic response to nutrients, (4) cellular energy levels, (5) ribosome biogenesis, (6) transcription/translation, (7) autophagy, and (8) actin organization [36].

Involvement of the PI3K Pathway HER2+ Breast Cancer

In *HER2*-amplified breast cancers, the heterodimer of HER2 with kinase-impair HER3 is a major activator of PI3K-AKT signaling, and HER3, when phosphorylated, can directly couple to the p85 subunit of PI3K [40]. HER2:HERB3 heterodimers are powerful oncogenic units, in part, because phospho-HER3 augments signaling through the PI3K-AKT-mTOR pathway [40–43]. The p85 regulatory subunit of PI3K interacts directly with phospho-HER3 at 6 consensus PY

p85-binding motifs (YXXM) within HER3 [44]. In contrast, HER2 cannot directly engage p85. It has been established that spontaneous formation of HER2:HER3 heterodimer can occur in tumors where HER2 expression on the cell surface is dramatically increased as a consequence of gene amplification [45]. Therefore, it is contemplated that HER3 functions primarily to drive HER2-mediated PI3K signaling [46, 47] which is important for tumor cells survival, proliferation, invasion, migration, angiogenesis, and cellular metabolism. It has been recently shown by others that HER2/HER3 co-overexpression is significantly associated with impaired overall survival (OS) from diagnosis of metastatic disease in patients with HER2-positive metastatic breast cancer [48]. Similar to HER2, the IGF-1R, which can form homodimers or heterotrimers with HER2 [49] activates the PI3K-AKT pathway and this mechanism is thought to be an important source of trastuzumab resistance [50–52]. In HER2-overexpressing cells following ligand receptor interaction, the signal is transduced via the PI3K-AKT-mTOR pathway (Fig. 8.2). Several laboratory-based results implicate that upregulation of HER3 expression by inhibition of the RTKs (receptor tyrosine kinases)-PI3K-AKT pathway is prerequisite for neuregulin (NRG) ligand-mediated activation of HER3 that might be important in the context of tumor microenvironment and autocrine/paracrine resistance to receptor tyrosine kinase inhibitors. Breast cancer cells often express high levels of HER-family receptor-activating ligands, and this plays an important role in developing anti-HER2 therapy resistance including trastuzumab [46, 53–56]. The overexpression of neuregulin has also been shown to be a driver of breast cancer progression. It is known that growth factor ligands EGF, betacellulin, and neuregulin reduce the growth inhibitory effect of trastuzumab in a preclinical model by 57–90 % [57]. Autocrine or paracrine-derived HER3 ligand neuregulin can trigger the formation of HER2:HER3 heterodimers, (propagate downstream PI3K-AKT-mTOR signal) which are blocked by pertuzumab, but not by trastuzumab [58]. HER2-amplified tumors show significant reliance on PI3K-AKT-mTOR signaling [59, 60]. Importantly, inhibition of PI3K-AKT signaling is believed to be an essential component of the antitumor effect of anti-HER2 therapies [61, 62].

Activation of PTEN-PI3K-AKT-mTOR Pathway and Anti-HER2 Therapy Resistance

Alterations of signal transducers lying downstream of HER2, which facilitate signaling independently of the HER2 kinase, have been extensively studied as potential mechanisms of HER2-directed therapy resistance. Oncogenic activation of the PI3K-AKT-mTOR pathway alterations frequently co-occurs in breast cancer, suggesting that they confer advantages to cancer cells by different mechanisms. Aberrant activation of this pathway promotes transformation and is frequently observed through various mechanisms including activation of upstream receptor

tyrosine kinases (e.g., *HER*2 amplification, HER2:HER3 heterodimerization), activating mutations in pathway components (e.g., helical and kinase domain mutations in *PIK3CA*) and loss of function of regulatory components (e.g., inactivation of *PTEN*). Among the PI3 kinases, the class I family has been most implicated in cancer. Class IA PI3K is composed of a catalytic (p110) and a regulatory (p85) subunit. Of particular interest are activating "hotspot" mutations in the gene encoding the PI3K catalytic subunit (*PIK3CA*), which affect around 25 % of breast cancers [63] and can overlap with *HER2* amplification (30 % of somatic mutation of *PIK3CA* was observed in *HER2* amplified breast cancer) [64]. Approximately 90 % of these mutations are localized in 3 major hotspots concentrated in the helical (E542K and E545K) and kinase (H1047R) domains [65]. We (unpublished observation) and others have found that both helical and catalytic domain mutations of *PIK3CA* can confer resistance to HER2 inhibitors [59, 66, 67]. Similarly, around 40 % of breast cancers show loss of expression of PTEN, a negative regulator of PI3K. Activating *PIK3CA* mutation or PTEN loss are each sufficient to confer HER2-directed therapy resistance in preclinical models [61, 68–70] and in patients in some studies [68, 69, 71]. Chandarlapaty et al. revealed that the incidence of PTEN loss and/or *PIK3CA* mutation in trastuzumab-refractory tumors was 71 % compared with 44 % in primary tumors from an untreated cohort [71]. However, it has been reported from prospective NeoALTTO study that benefit to neoadjuvant anti-human HER2-targeted therapies (trastuzumab and lapatinib-based therapies) in HER2+ primary breast cancer is independent of PTEN status. Interestingly, they also observed that PTEN loss (by IHC staining by two different anti-PTEN monoclonal antibodies from CST and DAKO) was more frequently observed in hormone receptor (HR)-negative (33 and 36 % with CST and DAKO, respectively) compared with HR-positive tumors (20 and 22 % with CST and DAKO, respectively) [72]. In the same line, Stem and colleagues also reported that in the HER2+ patient population, absence of tumor cell PTEN staining occurred at a rate of 5.4 % compared to 15.9 % of HER2-negative patients exhibited absence of PTEN staining. They also reported from two adjuvant breast cancer trials (BCIRG-006 and BCIRG-005) using a PTEN immunohistochemical assay that PTEN loss was associated with worse outcomes (decrease in disease-free survival and overall survival) in *HER2* amplified breast cancer patients but was not significantly associated with trastuzumab resistance [73]. The tumor suppressor gene PTEN, which is often deleted or expressed at low levels in breast cancer, negatively regulates PI3K signaling by dephosphorylating PIP3 at the D3 position to form PIP2. In addition, 5 % of *PIK3R1* somatic mutations were observed in HER2+ breast cancer [64]. On the contrary Sueta et al. [74] examined PTEN as one of the biomarkers to trastuzumab efficacy in HER2+ disease in neoadjuvent setting using digital PCR and conventional sequencing methodology and they found that Low PTEN expression was associated with less pathologic complete response compared to high expression (33 % vs. 72 %, $P = 0.034$). Similar to Sueta's group, Park and group also reported from their biomarker study in HER2+ metastatic breast cancer that patient with higher PTEN showed longer PFS ($P = 0.006$; median PFS, 13 months vs. 9 months) and longer OS ($P = 0.005$; median OS,

48 months vs. 25 months) than did those with a low PTEN. Interestingly, patients who had negative HER3 staining (62.4 %) (upstream activator of the PI3K pathway) had a better progression-free survival (PFS) than did those who had positive HER3 staining ($P = 0.001$; median PFS, 21 months vs. 11 months) [75]. In early breast cancer (EBC), quantitative analysis of tissue microarrays from 122 trastuzumab-treated patients demonstrated a significant correlation between decreasing PTEN expression and mortality. Compared with high PTEN expression, PTEN deficiency was associated with a threefold increase in the risk of death (relative risk [RR] 3.0; 95 % CI: 1.6–5.5; $p < 0.0001$) and a mean reduction in OS of 21.6 months [76].

Other retrospective studies in trastuzumab-treated MBC suggest that the predictive power of PI3K/PTEN-related biomarkers might be improved by considering tumors with either PTEN loss and/or *PIK3CA* mutation together (i.e., those with PI3K pathway activation). In one study, although neither PTEN deficiency nor *PIK3CA* mutation alone was significantly associated with PFS in their cohort ($n = 55$), patients with either or both of these biomarkers had significantly shorter PFS compared with patients with neither ($p = 0.007$) [68]. PTEN loss and/or *PIK3CA* mutation independently predicted progression after adjustment for covariates (HR: 1.9; 95 % CI: 1.0–3.6; p: 0.048). Biomarker analyses from a prospective trial of adjuvant trastuzumab plus chemotherapy for HER2-positive EBC ($n = 240$), *PIK3CA* mutation, but not PTEN deficiency, independently predicted OS [77]. Patients whose tumors showed either or both of these features had a 2.35-fold increased mortality risk in multivariate analyses (HR: 2.35; 95 % CI: 1.10–5.04; $p = 0.03$). Another small trial ($n = 35$) showed that pCR rates with neoadjuvant trastuzumab plus docetaxel were numerically lower in tumors with low versus high PTEN expression (15.4 % vs. 44.4 %; $p = 0.13$) and in patients with mutated versus wild-type PIK3CA (20.0 % vs. 38.1 %; $p = 0.43$) [78]. A significant reduction in pCR was found in tumors with either or both of these characteristics versus those with neither (18.2 % vs. 66.7 %; $p = 0.02$).

The correlation of *PIK3CA* mutations with HER2 directed therapy remains controversial; some groups report that *PIK3CA* mutations are related to negative HER2 expression [79] whereas others report showed that *PIK3CA* mutations are overlapped with *HER2* amplification [80, 81], and critical for HER2-directed therapies. Studies on the relationship between *PIK3CA* mutations and the prognosis of breast cancer demonstrated that *PIK3CA* is a carcinogenic marker and that its mutation leads to overactivation of the PI3K pathway, tumor formation, and poor prognosis [79, 80, 82]. Mutant *PIK3CA* enhances HER2-mediated transformation of MCF710A breast epithelial cells in vitro [46]. HER2 and *PIK3CA* (kinase domain mutation H1047R) cooperated to promote the transformation of the mammary epithelium, cancer establishment, and metastasis [67]. Preclinical data also suggest that HER2+ breast cancers harboring *PIK3CA* mutations exhibit a more virulent behavior than HER2+ tumors with wild-type *PIK3CA* gene [67]. However, researchers at the Memorial Sloan Kettering Cancer Center [79] studied 590 cases of primary invasive breast cancers and found that *PIK3CA* mutation was correlated with late-onset, positive estrogen receptor status, negative HER2

expression, early clinical staging, and negative lymph node status and that patient with *PIK3CA* mutations have a longer overall survival. Several studies suggest that *PIK3CA* mutations confer resistance to anti-HER2 therapies [68, 71, 83] but confirmations of a causal relationship between activating mutation of *PIK3CA* and failure to HER2-directed therapies in the clinic is not established yet. Two different groups separately reported that dual-targeting of HER2 is not sufficient to overcome resistance to HER2 inhibition, particularly in the case of *HER2*-amplified cancer with a *PIK3CA* mutation [66, 67]. Arteaga and group have previously shown that, once resistance to HER2 inhibitors is established, inhibition of the PI3K pathway added to continued HER2 inhibition can overcome this resistance [84]. Rexer and group elegantly showed that acquisition of a hotspot *PIK3CA* mutation (either in helical or in kinase domain) is a mechanism of acquired resistance to lapatinib and that *PIK3CA* mutations partially uncouple PI3K from HER2 to permit the development and maintenance of resistance. Moreover, targeting of PI3K itself, in combination with maximal HER2 blockade with both an antibody and a TKI, is more effective than HER2 targeting alone for HER2+ breast tumors without *PIK3CA* mutations and this combination is definitely required for HER2+ breast tumors with *PIK3CA* mutations [85].

The presence of *PIK3CA* mutations has been shown to determine clinical outcomes in different trials. Women bearing HER2+/HR+ tumors with *PIK3CA* mutations respond poorly to neoadjuvent therapy in the German GeparSixto study (*reported in* [86]). The combined analysis from GeparQuattro, GeparQuino, and GeparSixto studies demonstrated that in HER2+ patients, pathological complete response rates after dual HER2 blockade were significantly lower in the *PIK3CA* mutant group compared to patients with wild-type *PIK3CA* (19.4 % with *PIK3CA* mutations vs. 32.8 % with *PIK3CA* wild type). In HER2+/HR+ patients who harbored *PIK3CA* mutations, only 11.3 % achieved pathological complete response (pCR)compared with 27.8 % for those without a *PIK3CA* mutation and in patients with HER2+/HR− tumors pCR rate was 30.4 % with *PIK3CA* mutations and 40.1 % without mutations [87] (see Table 8.2). Similarly, *PIK3CA* mutations are associated with decreased benefit to neoadjuvant HER2+ breast cancer (NeoALTTO) treated with lapatinib, trastuzumab, or both. Patients treated with a combination of lapatinib plus trastuzumab who had wild-type *PIK3CA* obtained a total pCR rate of 53.1 % which decreased to 28.6 % in patients with tumors that carried *PIK3CA* activating mutations ($p = 0.12$) [88] (see details in Table 8.2). Biomarker analysis from the randomized NeoSphere study (pertuzumab plus trastuzumab plus docetaxel) also associated *PIK3CA* mutations with reduced pCR following neoadjuvant therapy across all four treatment arms [89]. Furthermore, in a large biomarker analysis from a phase III randomized CLEOPATRA trial with HER2+ metastatic breast cancer patients of pertuzumab plus trastuzumab plus docetaxel versus placebo plus trastuzumab plus docetaxel, *PIK3CA* mutations (mostly affecting the kinase or helical domains) were of adverse prognostic significance in both treatment arms. In the pertuzumab-containing group, median progression-free survival (PFS) was 21.8 months for patients with wild-type *PIK3CA* versus 12.5 months for patients with *PIK3CA* mutation. Corresponding

values for the placebo arm were 13.8 and 8.6 months, respectively [90] (see Table 8.2). However, *PIK3CA* mutational status did not predict benefit from pertuzumab, which significantly improved PFS in both the mutant and wild-type PI3K subgroups. However, in adjuvant setting of HER2-enriched breast cancer patients in the NSABP B-31 trial efficacy of trastuzumab treatment do not depend on the *PIK3CA* mutation status [91]. These data indicate that results from the metastatic and neoadjuvant setting may not be always applicable to the adjuvant setting. Recently, it has been reported by other that p85 (a regulatory subunit of PI3K) protein expression is associated with poor survival in HER2+ patients with advanced breast cancer treated with trastuzumab [92].

Clinical trials data from a combination of trastuzumab plus PI3K-mTOR pathway-specific inhibitor (everolimus, an allosteric inhibitor of mTOR) in HER2+ breast cancer is also encouraging. The BOLERO-1 trial randomized 719 patients with locally advanced or metastatic HER2+ breast cancer in a 2:1 ratio of either 10 mg of oral everolimus daily with 80 mg/m^2 of weekly paclitaxel and trastuzumab at a 4 mg/kg loading dose followed by 2 mg/kg weekly (n = 480), or placebo plus paclitaxel and trastuzumab at the same doses (n = 239). At baseline 43.3 % of patients were HR-negative, 70.5 % had visceral metastasis, 24.9 % had received a taxane and 10.8 % had prior trastuzumab. In the full study population, PFS was 14.95 months in the everolimus arm versus 14.49 months for placebo and there was no statistical significant difference between these two arms. However, among HR-negative patients, PFS was 20.27 months with everolimus compared with 13.08 months with placebo (HR: 0.68, p = 0.049) and there was a 7 months improvement in the everolimus arm [93] (see Table 8.2). Similarly, the BOLERO-3 trial (NCT01007942) randomized, double-blind, placebo-controlled, phase 3 trial, with HER2+, trastuzumab-resistant, advanced breast carcinoma (who had previously received taxane therapy) were treated with everolimus (5 mg/day) plus weekly trastuzumab (2 mg/kg) and vinorelbine [25 mg/m(2)] or placebo plus trastuzumab plus vinorelbine, in 3-week cycles. Median PFS was 7.00 months with everolimus and 5.78 months with placebo (HR: 0.78 p = 0.0067). Interestingly, patients with low PTEN level obtained more benefit with everolimus than placebo (everolimus 9.6 months vs. placebo 5.2 months). Similarly, patients with high pS6K level obtained more benefit with everolimus (HR: 0.48). In contrast, patients with low pS6K or normal PTEN did not appear to obtain any benefit from addition of everolimus (HR: 1.14 and HR: 1.05 respectively). A tread of enhanced everolimustreatment benefit was observed in patients with *PIK3CA* mutations [94, 95] (see Table 8.2). Data from above mentioned studies indicate that identifying the right subgroup is the key for right treatment decision.

PI3K-AKT-mTOR Pathway-Specific Inhibitors Either Approve or Active in Clinical Trial

Experiments/clinical trials across HER2+ breast tumors have found association between maintained PI3K-mTOR signaling and resistance to HER2-directed therapies. Multitude of several agents under preclinical or clinical investigations presents different challenges for drug development. Several drugs targeting multiple levels of the PI3K network (that is PI3K, AKT, mTOR) have been developed (Fig. 8.3). Several drugs targeting multiple levels of the PI3K network (that is PI3K, AKT, mTOR) have been developed. A number of ATP-mimetics that bind competitively and reversibly to the ATP-binding pocket of p110 are in early clinical development. These include the pan-PI3K inhibitors (BKM120, XL-147, PX-866, PKI-587, and GDC-0941), the p110α-specific inhibitors (BYL719, GDC-0032, and MLN1117), p110β-specific inhibitors (GSK-2636771, SAR260301), the p110δ-specific inhibitor (CAL-101, Idelalisib; very recently approved by FDA for CLL patients [96], the dual PI3K/mTOR inhibitors (BEZ235, BGT226, PF-4691502, GDC-0980, and XL-765), the allosteric inhibitors of mTOR (FDA-approved everolimus, temsirolimus, and ridaforolimus) and the mTORC1/2 kinase inhibitors (MLN0128, AZD-8055 and OSI-027). The pan-PI3K and p110α-specific inhibitors are equally potent against oncogenic p110α mutants. The

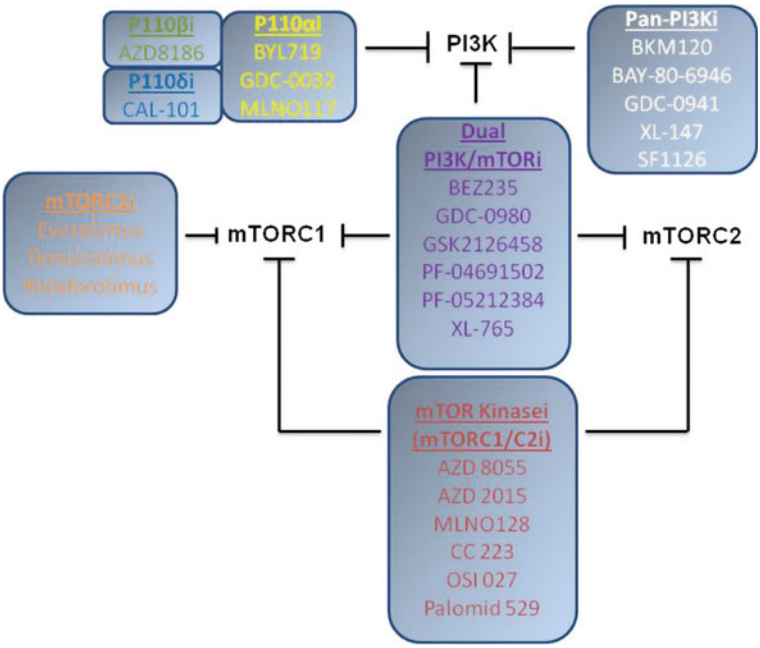


Fig. 8.3 Inhibitors of the PI3K-mTORC1/C2 pathway: active in preclinical and clinical settings

development of isozyme-specific antagonists has permitted the delivery of significantly higher doses of the drugs (anti-p110α and anti-p110β) with appreciably lower side effects as compared to pan-PI3K inhibitors. A rational approach or a biomarker-driven rule must be developed to identify the optimal clinical and genomic contexts in which each class of inhibitors should be used. Inhibitors of the PI3K pathway have shown modest activity in the clinic and PI3K pathway inhibitors have shown most promise when given in combination with other therapies. The most convincing argument for using PI3K-mTOR pathway inhibitors as part of the combination therapy comes from phase III trials with everolimus. In BOLERO 2 trial, the combination of everolimus and exemestane significantly improved PFS to 7.8 months compared with 3.2 months with exemestane alone, in postmenopausal women with advanced ER+ breast cancer [97]. In the BOLERO-3 trail the combination of everolimus, trastuzumab, and vinorelbine, improved PFS significantly in HER2+ patients with low PTEN expression than the placebo group (everolimus 9.6 months vs. 5.2 months) [94, 95].

Several studies have investigated the potential importance of PI3K inhibition as a therapeutic strategy in *HER2*-amplified breast cancer. Catalytic inhibition of p110α subunit is an emerging therapeutic option, with a number of inhibitors currently in preclinical and clinical development [98]. A PI3K inhibitor, GDC-0941, inhibited the growth of *HER2*-amplified cells in culture, but the combination of GDC-0941 with trastuzumab was required for in vivo tumor growth inhibition in mice [61]. Furthermore, the combination of GDC-0941 with antibody inhibitors of HER2 (trastuzumab and pertuzumab) appeared to be more effective than GDC-0941 alone in combination with a TKI [99]. In HER2+ cells with *PIK3CA* mutations, low doses of lapatinib is ineffective, but the cells are susceptible to dual PI3K-mTOR inhibitors such as BEZ235 [66] or mTORC1/C2 kinase inhibitor MLN0128 [100, 101]. Additionally, in a transgenic HER2+/*PIK3CA* H1047R mutant mouse model, tumors that were resistant to the combinations of trastuzumab and lapatinib or trastuzumab and pertuzumab could be inhibited by BKM120 (a pan PI3K inhibitor from Novartis) alone or in combination with HER2 inhibitors [67]. Interestingly, alpha-selective inhibitors are very potent inhibitors of the alpha subunit, a key component of PI3K activity in cancer. Phase I studies using alpha-selective PI3K inhibitors have shown extremely encouraging results in patients who with *PIK3CA* mutations indicating that *PIKCA* mutation could be a relevant target in metastatic breast cancers [102, 103]. Interestingly, it has been also reported by others that dual blocking of the HER2 signaling network with an HER3 antibody that inhibits HER2:HER3 heterodimers in combination with a p110 alpha-specific inhibitor (BYL719) in the absence of direct HER2 antagonist is also efficacious against HER2 overexpressing breast cancer [104]. In a dose-escalation phase I/II study, patients with trastuzumab-resistant, locally advanced or metastatic HER2+ breast cancer were treated oral doses of BKM120 (a pan-PI3K inhibitor) and weekly intravenous trastuzumab. The pharmacokinetic profile of BKM120 was not affected by its combination with trastuzumab. At the recommended phase II dose, 17 % patients had partial response, 58 % had stable disease (more than 6 weeks) and the disease control rate was 75 % [105]. Several preclinical data

including our xenograft data have demonstrated that continued HER2 blockade is required for potent tumor regression in response to PI3K inhibition even after development of trastuzumab resistance [106, 107]. From both preclinical and clinical data, we can assume that the clinical activity observed in the combination (BKM120 plus trastuzumab) study may be due to the results of the combined activities of both BKM120 and trastuzumab and that the PI3K pathway inhibitor can restore sensitivity to trastuzumab. It has been also noted that BYL719 (alpha-selective inhibitor) used to inhibit the PI3K α-isoform in human breast cancer cells with *HER2* amplification or *PIK3CA* activating mutations. BYL719 initially abrogated PI3K signaling as evidenced by their low AKT activation status. After 24 h, the BYL719 treated cells showed a rebound increase in the PIP3 level. Moreover, in *PIK3CA* mutated cells there was an increase in the level of PIP3 even in the absence of AKT activation. Further analysis revealed that the elevation in PIP3 levels was due to increase in the recruitment of PIK3β isoform to the HER3 receptor. These data suggest that co-treatment of breast cancer cells with both α and β isoform-specific inhibitors will get the fullest response [108].

Diversity for 8q24 (this chromosomal region harbors several important oncogenes including *MYC*) was consistently higher in HER2+ tumors compared to other subtype. MYC overexpression is sufficient to confer resistance to PI3K and mTOR inhibitors [109]. It was an initial general consensus that mutations in the oncogenic PI3K, possibly in the kinase domain (inhibitor binding domain), would yield resistance to catalytic PI3K inhibition. But the success of the PI3K pathway-specific inhibitors at least in preclinical settings and also in some extent in clinical settings with other targeted inhibitors, translational scientists thought and found that acquired drug resistance may involve alterations that deregulated signaling component acting downstream of the targeted protein. Using an integrated copy number and expression-based-approach within the dual PI3K/mTOR inhibitor (BEZ235) resistant cells, Thomas M. Roberts and colleagues determined that *MYC*, a commonly deregulated breast cancer oncogene, was responsible for the acquired BEZ235 resistance [110]. Pixu Liu and group have demonstrated using transgenic mouse models and a series of elegant experiments that *MYC* contributed to *PIK3CA* independent of tumor growth and resistance to PI3K-catalytic inhibition [111]. However, the detail discussion regarding the resistance mechanism of isoform-specific PI3K inhibitor is beyond the scope in this review article. Multiple clinical trials are currently evaluating the efficacy of over 30 drugs targeting different nodes in the PI3K pathway in breast including HER2+ breast cancer and other cancers (https://clinicaltrials.gov) (see Table 8.3).

Pan HER Inhibitors in HER2+ Breast Cancer

HER-family receptor tyrosine kinases are implicated in many cancers and several anti-HER treatments are now approved. In recent years, a new group of compounds that bind irreversibly to ATP-binding pocket of HER-family receptors has been

Table 8.3 Ongoing clinical trials of the PI3K-AKT-mTOR pathway inhibitors for the treatment of advanced and metastatic *HER2* amplified breast cancers

Ongoing trial	Trail design	Comments
mTOR inhibitors		
NC00876395 (BOLERO-1)	Everohmus, trastuzumab and paclitaxel	Phase 1/2, safety and efficacy of BKM120 and lapatinib in HER2 +/PI3K-activated (loss of expression of PTEN and/or activating mutation or overexpression of PIK3CA) trastuzumab-resistant advanced breast cancer
NCT01007942 (BOLERO-3)	Everolimus, trastuzumab and vinorelbine	Phase 3, in HER2+ women with locally advanced or metastatic breast cancer who are resistant to trastuzumab and have been pretreated with a taxane
NCT01305941	Everohmus, trastuzumab and vinorelbine	Phase 2, in the treatment of progressive HER2+ breast cancer brain metastases
NCT01111825	Temsirolimus and neratinib	Phase 1/2, for patients with metastatic *HER2*-amplified or triple negative breast cancer
NCT00411788	Sirolinius (rap amyein) and trastuzumab	Phase 2, patients with HER2+ metastatic breast cancer
NCT00458237	RAD001 and trastuzumab	Phase 2, in HER2+ metastatic breast cancer
NCT01283789	Lapatinib and RAD001	Phase 2, in HER2 positive metastatic breast cancer
NCT02152943	Everolimus, letrozole and trastuzumab	Phase 1, in HR− and HER2+ patients
NCT00736970	Deforolimus and trastuzumab	Phase 2, in patients with HER2+ trastuzumab-refractory metastatic breast cancer

(continued)

Table 8.3 (continued)

Ongoing trial	Trail design	Comments
Pan-PI3K inhibitors		
NCT01589861	BKM120 and lapatinib	Phase 1/2, treatment of HKR2+ locally advanced or metastatic breast cancer
NCT01132664	BKM120 and trastuzumab	Phase 1, in patients with relapsing HER2 over expressing breast cancer who have previously failed trastuzumab
NCT01816594 (NeoPHOEBE)	BKM120, trastuzumab and weekly paclitaxel	Phase 2, (neoadjuvant) in HER2+ primary breast cancer
NCT01285466	BKM120, BEZ235 and weekly paclitaxel with or without trastuzumab	Phase lb, in patients with HER2+ metastatic breast cancer
NCT00928330	Trastuzumab T-DM1 and GDC-0941	Phase 1, in patients with HER2+ metastatic breast cancer who have progressed on previous trastuzumab-based therapy
NCT01042925	XL147 trastuzumab and paclitaxel plus trastuzumab	Phase 1/2, in subjects with metastatic breast cancer who have progressed on a previous trastuzumab-based regimen
AKT inhibitors		
NCT01245205	MK2206 and lapatinib	Phase 1, for HER2+ advanced breast cancer
NCT01277757	MK2206	Phase 2, in patients with advanced breast cancer who have tumors with a PIK3CA mutation, or an AKT mutation, and/or PTEN loss/PTEN mutation

(continued)

Table 8.3 (continued)

Ongoing trial	Trail design	Comments
NCT00567879	Panobinostat and trastuzumab	Phase 1, for adult female patients with HER2+ metastatic breast cancer whose disease has progressed on or after trastuzumab
Alpha isoform-specific inhibitors		
NCT02038010	BYL719 and T-DM1	Phase 1, in HER2+ metastatic breast cancer patients who progress on prior trastuzumab and taxane treatment
NCT01300962	BYL719 or BKM120	Phase 1, a four part, dose-escalation study of the combinations of concurrent BKM20 and capecitabine, or concurrent BYL719 and capecitabine, or concurrent BKM120 and capecitabine and trastuzumab, or concurrent BKM120 and capecitabine and lapatinib in patients with metastatic breast cancer
Dual PI3K-mTOR inhibitors		
NCT01471847	BEZ235 and trastuzumab	Phase lb/2, in patients with HER2+ breast cancer who failed prior to trastuzumab
NCT01285466	BEZ235 and	
BKM120 + paclitaxel ± trastuzumabPhase 1b, multicenter, open-label, 4-arm dose-escalation study of oral BEZ235 and BKM120 in combination with weekly paclitaxel in patients with advanced solid tumors and weekly paclitaxel/trastuzumab in patients with HER2+ metastatic breast cancer		

developed. Several oral small molecules, tyrosine kinase inhibitors are also in development. Afatinib (BIBW2992) is an oral an irreversible covalent inhibitor of HER family that targets epidermal growth factor receptor (EGFR), HER2, and HER4. It has received regulatory approval for use as a treatment for non-small cell lung cancer (*"GILOTRIF (afatinib) tablet, film coated [Boehringer Ingelheim Pharmaceuticals, Inc.]". DailyMed. Boehringer Ingelheim Pharmaceuticals, Inc. November 2013. Retrieved 28 January 2014; "GIOTRIF® Afatinib (as afatinib dimaleate)" (PDF). TGA eBusiness Services. Boehringer Ingelheim Pty Limited.*

Table 8.4 Ongoing clinical trials with pan-HER tyrosine kinase inhibitors in HER2+ advanced or metastatic breast cancers

Ongoing trial	Trail design	Details
HER-family tyrosine kinase receptor inhibitors (afatinib and neratinib)		
NCT01125566 (LUX-breast 1)	Afatinib, vinorelbine and trastuzumab	Phase 3, comparing afatinib plus vinorelbine (experimental arm) with trastuzumab plus vinorelbine in patients with HER2-positive metastatic breast cancer after failure of prior trastuzumab-based therapy
NCT01271725 (LUX-breast 2)	Afatinib, vinorelbine and pacitaxel	Phase 2, to investigate the efficacy and safety of afatinib alone and in combination with weekly paclitaxel or weekly vinorelbine (in patients who progress on afatinib monotherapy within this trial) as treatment in patients with HER2-overexpressing, metastatic breast cancer, who failed HER2-targeted treatment in the neoadjuvant or adjuvant setting
NCT01441596 (LUX-breast 3)	Afatinib and vinorelbine	Phase 2, in patients with HER2+ breast cancer progressing with brain metastases, the three-armed trial compared afatinib, afatinib plus vinorelbine and investigator's choice of therapy, after prior trastuzumab and or lapatinib-based therapy
NCT01594177	Afatinib and trastuzumab	Phase 2, as neoadjuvant treatment for patients with locally advanced or operable breast cancer receiving taxane-anthracycline containing chemotherapy
NCT01325428	Afatinib and vinorelbine	Phase 2, in HER2-overexpressing inflammatory breast cancer
NCT00431067	Afatinib	Phase 2, in patients with HER2+ metastatic breast cancer alter failure of trastuzumab therapy
NCT00826267	Afatinib, lapatinib or trastuzumab	Phase 2, to explore the efficacy of aftinib as a single agent versus lapatinib versus trastuzumab in patients with HER2+ treatment-naïve Stage IIIa locally advanced breast cancer
NCT01649271	Afatinib and trastuzumab	Phase 1, to determine the MTD of afatinib in combination with 3-weekly trastuzumab inHER2 overexpressing cancer
NCT01423123	Neratinib and trastuzumab	Phase 1, combination of weekly paclitaxel with neratinib and trastuzumab in women with metastatic HER2+ breast cancer
NCT02236000	Neratinib and T-DM1	Phase l/2, Evaluating the combination of T-DM1 with neratinib in women with metastatic HER2+ breast cancer
NCT01008150	Neratinib and trastuzumab	Phase 2, evaluating neoadjuvant therapy with neratinib and/or trastuzumab followed by postoperative trastuzumab in women with locally advanced HER2+ breast cancer
NCT01494662	Neratinib	Phase 2, for HER2-positive breast cancer and brain metastases

(continued)

Table 8.4 (continued)

Ongoing trial	Trail design	Details
NCT00878709 (ExteNET)	Neratinib	Phase 3, evaluating the effects of neratinib after adjuvant trastuzumab in women with early-stage breast cancer
NCT00777101	Neratinib, lapatinib plus capecitabine	Phase 2, evaluating neratinib versus lapatinib plus capecitabine for ErbB2 positive advanced breast cancer
NCT00915018 (NEFERTT)	Neratinib trastuzumab and paclitaxel	Phase 2, evaluating neratinib plus paclitaxel versus trastuzumab plus paclitaxel in ErbB-2+ advanced breast cancer
NCT00146172	Neratinib (HKI-272)	Phase 1, evaluating HKI-272 in HER-2/NEU or HER-1/EGFR-positive tumors
NCT00741260	Neratinib and capecitabine	Phase 1/2, evaluating the combination of neratinib and capecitabine in solid tumors and breast cancer including HER2+ breast cancer
NCT00706030	Neratinib and vinorelbine	Phase 1/2, evaluating neratinib (HKI-272) in combination with vinorelbine in subjects with solid tumors and HER2+ metastatic breast cancer
NCT01111825	Neratinib and temsirolimus	Phase 1/2, for patients with metastatic HER2-amplified or triple negative breast cancer

7 November 2013. Retrieved 28 January 2014; "Giotrif 20 mg film-coated tablets —Summary of Product Characteristics (SPC)". Electronic Medicines Compendium. Boehringer Ingelheim Limited. 20 January 2014. Retrieved 28 January 2014), although there is an emerging evidence to support its use in other cancers such as breast cancer [112]. In a phase II study, afatinib monotherapy in heavily pretreated HER2+ metastatic breast cancer demonstrated partial response in 4 patients (10 % of 41) and stable disease in 11 patients (37 % of 41) [112]. It has also demonstrated efficacy in early-phase trials of advanced solid tumors and trastuzumab-refractory HER2-positive breast cancer [113–115]. Ring and colleagues combined afatinib with trstuzumab in a Phase1 trial with confirmed advanced/metastatic HER2+ breast cancer. Overall, objective response and disease control rates were 11 and 39 % of patients who received afatinib 20 and 30 mg with standard dose of trastuzumab respectively. Median progression-free survival was 111.0 days (95 % confidence interval [116]). Preclinical data showed low nanomolar potency for EGFR, HER2, and HER4 kinases [114] as well as anti-proliferative effects in HER2-dependent models [117].

Neratinib (HKI-272; Pfizer, New York, NY, USA) is a potent irreversible tyrosine kinase inhibitor that disrupts signal transduction via EGFR, HER2, and HER4 [118]. Neratinib has passed preclinical phases and is currently undergoing various clinical trials (Table 8.4). Neratinib has been shown to be effective against HER2 overexpressing or *HER2* mutant tumors in vitro and in vivo [31]. In a phase-I dose-escalation study of neratinib in advanced solid malignancies, partial response was seen in 8 out of 25 (32 %) in HER2+ breast cancer patients who were previously treated with trastuzumab, anthracyclines, and taxanes [119]. An open-label,

phase-II multicenter trial of single agent neratinib in advanced HER2-positive breast cancer, which enrolled both trastuzumab-refractory ($n = 66$) and trastuzumab-sensitive ($n = 70$) patients, demonstrated modest clinical activity in both cohorts. Objective response rates of 24 and 56 % were seen in the trastuzumab-refractory and trastuzumab-sensitive groups, respectively, with a median PFS of 22.3 and 39.6 weeks [120].

Currently, studies of single agent afatinib or neratinib and combinations with other HER2-targeted agent are under evaluation in HER2+ breast cancer (see Table 8.4).

Conclusion

An extensive literature search was carried out to critically evaluate the current knowledge of HER-family signaling in HER2+ breast cancer and response to anti-HER2-therapies. Understanding mechanisms of resistance in individual patients remains a challenge and trials that incorporate on-treatment biopsies (including liquid biopsies) and biomarker analysis will be needed in the new world of precision medicine in cancer therapy. Emerging strategies to circumvent resistance to HER2-targeted therapies in *HER2*-amplified breast cancer include dual HER2 therapy, novel therapies such as T-DM1, pan-HER family inhibition, and co-targeting HER2 plus either isoform-specific PI3K inhibition or pan-PI3K inhibition. There is evidence that immunity plays a critical role in the efficacy of HER2-targeted therapy, and attempts are being made to utilize the immune system in order to improve the efficacy of current anti-HER2 therapies. With our rapidly expanding knowledge of HER2 and its downstream signaling mechanisms along with the repertoire of HER family and other pathway-specific targeted therapies, it is expected that the near future holds further vivid improvements for the prognosis of women with HER2+ breast cancer.

Acknowledgments Authors acknowledge Avera Cancer Institute Center for Precision Oncology, Sioux Falls, SD and Dept. of Internal Medicine, USD, Vermilion, SD. Authors thank Ms. Sanyogita Chandra for proof editing of the MS. Authors acknowledge the secretarial help of Ms. Hannah Koble.

Disclosure

The authors have no declared conflict of interest.

References

1. Spector NL, Blackwell KL (2009) Understanding the mechanisms behind trastuzumab therapy for human epidermal growth factor receptor 2-positive breast cancer. J Clin Oncol 27(34):5838–5847
2. Citri A, Yarden Y (2006) EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Biol 7(7):505–516

3. Olayioye MA, Neve RM, Lane HA, Hynes NE (2000) The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J 19(13):3159–3167
4. Scaltriti M, Eichhorn PJ, Cortes J, Prudkin L, Aura C, Jimenez J, Chandarlapaty S, Serra V, Prat A, Ibrahim YH, Guzman M, Gili M, Rodriguez O, Rodriguez S, Perez J, Green SR, Mai S, Rosen N, Hudis C, Baselga J (2011) Cyclin E amplification/overexpression is a mechanism of trastuzumab resistance in HER2+ breast cancer patients. Proc Natl Acad Sci USA 108(9):3761–3766
5. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235(4785):177–182
6. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A et al (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244(4905):707–712
7. Burstein HJ, Harris LN, Marcom PK, Lambert-Falls R, Havlin K, Overmoyer B, Friedlander RJ Jr, Gargiulo J, Strenger R, Vogel CL, Ryan PD, Ellis MJ, Nunes RA, Bunnell CA, Campos SM, Hallor M, Gelman R, Winer EP (2003) Trastuzumab and vinorelbine as first-line therapy for HER2-overexpressing metastatic breast cancer: multicenter phase II trial with clinical outcomes, analysis of serum tumor markers as predictive factors, and cardiac surveillance algorithm. J Clin Oncol 21(15):2889–2895
8. Joensuu H, Kellokumpu-Lehtinen PL, Bono P, Alanko T, Kataja V, Asola R, Utriainen T, Kokko R, Hemminki A, Tarkkanen M, Turpeenniemi-Hujanen T, Jyrkkio S, Flander M, Helle L, Ingalsuo S, Johansson K, Jaaskelainen AS, Pajunen M, Rauhala M, Kaleva-Kerola J, Salminen T, Leinonen M, Elomaa I, Isola J, FinHer Study I (2006) Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. N Engl J Med 354(8):809–820
9. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C, Cameron D, Dowsett M, Barrios CH, Steger G, Huang CS, Andersson M, Inbar M, Lichinitser M, Lang I, Nitz U, Iwata H, Thomssen C, Lohrisch C, Suter TM, Ruschoff J, Suto T, Greatorex V, Ward C, Straehle C, McFadden E, Dolci MS, Gelber RD, Herceptin Adjuvant Trial Study T (2005) Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 353(16):1659–1672
10. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344(11):783–792
11. Rimawi MF, Mayer IA, Forero A, Nanda R, Goetz MP, Rodriguez AA, Pavlick AC, Wang T, Hilsenbeck SG, Gutierrez C, Schiff R, Osborne CK, Chang JC (2013) Multicenter phase II study of neoadjuvant lapatinib and trastuzumab with hormonal therapy and without chemotherapy in patients with human epidermal growth factor receptor 2-overexpressing breast cancer: TBCRC 006. J Clin Oncol 31(14):1726–1731
12. Baselga J, Cortes J, Kim SB, Im SA, Hegg R, Im YH, Roman L, Pedrini JL, Pienkowski T, Knott A, Clark E, Benyunes MC, Ross G, Swain SM, Group CS (2012) Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 366(2):109–119
13. Gianni L, Pienkowski T, Im YH, Roman L, Tseng LM, Liu MC, Lluch A, Staroslawska E, de la Haba-Rodriguez J, Im SA, Pedrini JL, Poirier B, Morandi P, Semiglazov V, Srimuninnimit V, Bianchi G, Szado T, Ratnayake J, Ross G, Valagussa P (2012) Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 13(1):25–32
14. Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490(7418):61–70
15. Ellis MJ (2014) HER2-positive breast cancer, intrinsic subtypes, and tailoring therapy. J Natl Cancer Inst 106(8)

16. Prat A, Baselga J (2008) The role of hormonal therapy in the management of hormonal-receptor-positive breast cancer with co-expression of HER2. Nat Clin Pract Oncol 5(9):531–542
17. Prat A, Baselga J (2013) Dual human epidermal growth factor receptor 2 (HER2) blockade and hormonal therapy for the treatment of primary HER2-positive breast cancer: one more step toward chemotherapy-free therapy. J Clin Oncol 31(14):1703–1706
18. Prat A, Carey LA, Adamo B, Vidal M, Tabernero J, Cortes J, Parker JS, Perou CM, Baselga J (2014) Molecular features and survival outcomes of the intrinsic subtypes within HER2-positive breast cancer. J Natl Cancer Inst 106(8)
19. Dowsett M, Sestak I, Lopez-Knowles E, Sidhu K, Dunbier AK, Cowens JW, Ferree S, Storhoff J, Schaper C, Cuzick J (2013) Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. J Clin Oncol 31(22):2783–2790
20. Ellis MJ, Suman VJ, Hoog J, Lin L, Snider J, Prat A, Parker JS, Luo J, DeSchryver K, Allred DC, Esserman LJ, Unzeitig GW, Margenthaler J, Babiera GV, Marcom PK, Guenther JM, Watson MA, Leitch M, Hunt K, Olson JA (2011) Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor-rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype—ACOSOG Z103. J Clin Oncol 29(17):2342–2349
21. Esserman LJ, Berry DA, Cheang MC, Yau C, Perou CM, Carey L, DeMichele A, Gray JW, Conway-Dorsey K, Lenburg ME, Buxton MB, Davis SE, van't Veer LJ, Hudis C, Chin K, Wolf D, Krontiras H, Montgomery L, Tripathy D, Lehman C, Liu MC, Olopade OI, Rugo HS, Carpenter JT, Livasy C, Dressler L, Chhieng D, Singh B, Mies C, Rabban J, Chen YY, Giri D, Au A, Hylton N, Investigators IST (2012) Chemotherapy response and recurrence-free survival in neoadjuvant breast cancer depends on biomarker profiles: results from the I-SPY 1 TRIAL (CALGB 150007/150012; ACRIN 6657). Breast Cancer Res Treat 132(3):1049–1062
22. Nielsen TO, Parker JS, Leung S, Voduc D, Ebbert M, Vickery T, Davies SR, Snider J, Stijleman IJ, Reed J, Cheang MC, Mardis ER, Perou CM, Bernard PS, Ellis MJ (2010) A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. Clin Cancer Res 16(21):5222–5232
23. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D (2000) Molecular portraits of human breast tumours. Nature 406(6797):747–752
24. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lonning PE, Brown PO, Borresen-Dale AL, Botstein D (2003) Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA 100(14):8418–8423
25. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, Quackenbush JF, Stijleman IJ, Palazzo J, Marron JS, Nobel AB, Mardis E, Nielsen TO, Ellis MJ, Perou CM, Bernard PS (2009) Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 27(8):1160–1167
26. Prat A, Perou CM (2011) Deconstructing the molecular portraits of breast cancer. Mol Oncol 5(1):5–23
27. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thurlimann B, Senn HJ, Panel M (2013) Personalizing the treatment of women with early breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 24(9):2206–2223
28. Perez EA, Romond EH, Suman VJ, Jeong JH, Davidson NE, Geyer CE Jr, Martino S, Mamounas EP, Kaufman PA, Wolmark N (2011) Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive

breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. J Clin Oncol 29(25):3366–3373
29. Vaz-Luis I, Ottesen RA, Hughes ME, Marcom PK, Moy B, Rugo HS, Theriault RL, Wilson J, Niland JC, Weeks JC, Lin NU (2012) Impact of hormone receptor status on patterns of recurrence and clinical outcomes among patients with human epidermal growth factor-2-positive breast cancer in the National Comprehensive Cancer Network: a prospective cohort study. Breast Cancer Res 14(5):R129
30. Baselga J, Bradbury I, Eidtmann H, Di Cosimo S, de Azambuja E, Aura C, Gomez H, Dinh P, Fauria K, Van Dooren V, Aktan G, Goldhirsch A, Chang TW, Horvath Z, Coccia-Portugal M, Domont J, Tseng LM, Kunz G, Sohn JH, Semiglazov V, Lerzo G, Palacova M, Probachai V, Pusztai L, Untch M, Gelber RD, Piccart-Gebhart M, Neo AST (2012) Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. Lancet 379(9816):633–640
31. Bose R, Kavuri SM, Searleman AC, Shen W, Shen D, Koboldt DC, Monsey J, Goel N, Aronson AB, Li S, Ma CX, Ding L, Mardis ER, Ellis MJ (2013) Activating HER2 mutations in HER2 gene amplification negative breast cancer. Cancer Discov 3(2):224–237
32. Lopez-Knowles E, O'Toole SA, McNeil CM, Millar EK, Qiu MR, Crea P, Daly RJ, Musgrove EA, Sutherland RL (2010) PI3K pathway activation in breast cancer is associated with the basal-like phenotype and cancer-specific mortality. Int J Cancer 126(5):1121–1131
33. Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, Wigler MH, Downes CP, Tonks NK (1998) The lipid phosphatase activity of PTEN is critical for its tumor supressor function. Proc Natl Acad Sci USA 95(23):13513–13518
34. Zhang X, Li XR, Zhang J (2013) Current status and future perspectives of PI3K and mTOR inhibitor as anticancer drugs in breast cancer. Curr Cancer Drug Targets 13(2):175–187
35. Inoki K, Zhu T, Guan KL (2003) TSC2 mediates cellular energy response to control cell growth and survival. Cell 115(5):577–590
36. De P, Miskimins K, Dey N, Leyland-Jones B (2013) Promise of rapalogues versus mTOR kinase inhibitors in subset specific breast cancer: old targets new hope. Cancer Treat Rev 39(5):403–412
37. Dowling RJ, Topisirovic I, Fonseca BD, Sonenberg N (2010) Dissecting the role of mTOR: lessons from mTOR inhibitors. Biochim Biophys Acta 1804(3):433–439
38. Guertin DA, Sabatini DM (2007) Defining the role of mTOR in cancer. Cancer Cell 12(1): 9–22
39. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM (2004) Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol 14(14):1296–1302
40. Dey N, Williams C, Leyland-Jones B, De P (2015) A critical role for HER3 in HER2-amplified and non-amplified breast cancers: function of a kinase-dead RTK. Am J Transl Res 7(4):733–750
41. De P, Leyland-Jones B (2010) Whither HER2-related therapeutics? J Clin Oncol 28(7):1091–1096
42. Holbro T, Beerli RR, Maurer F, Koziczak M, Barbas CF 3rd, Hynes NE (2003) The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. Proc Natl Acad Sci USA 100(15):8933–8938
43. Vaught DB, Stanford JC, Young C, Hicks DJ, Wheeler F, Rinehart C, Sanchez V, Koland J, Muller WJ, Arteaga CL, Cook RS (2012) HER3 is required for HER2-induced preneoplastic changes to the breast epithelium and tumor formation. Cancer Res 72(10):2672–2682
44. Stern DF (2008) ERBB3/HER3 and ERBB2/HER2 duet in mammary development and breast cancer. J Mammary Gland Biol Neoplasia 13(2):215–223
45. Gwin WR, Spector NL (2014) Pertuzumab protects the achilles' heel of trastuzumab—emtansine. Clin Cancer Res 20(2):278–280

46. Chakrabarty A, Rexer BN, Wang SE, Cook RS, Engelman JA, Arteaga CL (2010) H1047R phosphatidylinositol 3-kinase mutant enhances HER2-mediated transformation by heregulin production and activation of HER3. Oncogene 29(37):5193–5203
47. Lee-Hoeflich ST, Crocker L, Yao E, Pham T, Munroe X, Hoeflich KP, Sliwkowski MX, Stern HM (2008) A central role for HER3 in HER2-amplified breast cancer: implications for targeted therapy. Cancer Res 68(14):5878–5887
48. Berghoff AS, Bartsch R, Preusser M, Ricken G, Steger GG, Bago-Horvath Z, Rudas M, Streubel B, Dubsky P, Gnant M, Fitzal F, Zielinski CC, Birner P (2014) Co-overexpression of HER2/HER3 is a predictor of impaired survival in breast cancer patients. Breast 23 (5):637–643
49. Huang X, Gao L, Wang S, McManaman JL, Thor AD, Yang X, Esteva FJ, Liu B (2010) Heterotrimerization of the growth factor receptors erbB2, erbB3, and insulin-like growth factor-i receptor in breast cancer cells resistant to herceptin. Cancer Res 70(3):1204–1214
50. Nahta R, Esteva FJ (2006) HER2 therapy: molecular mechanisms of trastuzumab resistance. Breast Cancer Res 8(6):215
51. Nahta R, Shabaya S, Ozbay T, Rowe DL (2009) Personalizing HER2-targeted therapy in metastatic breast cancer beyond HER2 status: what we have learned from clinical specimens. Curr Pharmacogenomics Pers Med 7(4):263–274
52. Nahta R, Yuan LX, Zhang B, Kobayashi R, Esteva FJ (2005) Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. Cancer Res 65(23):11118–11128
53. Revillion F, Lhotellier V, Hornez L, Bonneterre J, Peyrat JP (2008) ErbB/HER ligands in human breast cancer, and relationships with their receptors, the bio-pathological features and prognosis. Ann Oncol 19(1):73–80
54. Ritter CA, Perez-Torres M, Rinehart C, Guix M, Dugger T, Engelman JA, Arteaga CL (2007) Human breast cancer cells selected for resistance to trastuzumab in vivo overexpress epidermal growth factor receptor and ErbB ligands and remain dependent on the ErbB receptor network. Clin Cancer Res 13(16):4909–4919
55. Sun Y, Dey N, Brammer M, De P, Leyland-Jones B (2013) Bevacizumab confers additional advantage to the combination of trastuzumab plus pertuzumab in trastuzumabrefractory breast cancer model. Cancer Chemother Pharmacol 72(4):733–745
56. Wilson TR, Fridlyand J, Yan Y, Penuel E, Burton L, Chan E, Peng J, Lin E, Wang Y, Sosman J, Ribas A, Li J, Moffat J, Sutherlin DP, Koeppen H, Merchant M, Neve R, Settleman J (2012) Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. Nature 487(7408):505–509
57. Motoyama AB, Hynes NE, Lane HA (2002) The efficacy of ErbB receptor-targeted anticancer therapeutics is influenced by the availability of epidermal growth factor-related peptides. Cancer Res 62(11):3151–3158
58. Hellyer NJ, Kim MS, Koland JG (2001) Heregulin-dependent activation of phosphoinositide 3-kinase and Akt via the ErbB2/ErbB3 co-receptor. J Biol Chem 276(45):42153–42161
59. Brachmann SM, Hofmann I, Schnell C, Fritsch C, Wee S, Lane H, Wang S, Garcia-Echeverria C, Maira SM (2009) Specific apoptosis induction by the dual PI3K/mTor inhibitor NVP-BEZ235 in HER2 amplified and PIK3CA mutant breast cancer cells. Proc Natl Acad Sci USA 106(52):22299–22304
60. O'Brien C, Wallin JJ, Sampath D, GuhaThakurta D, Savage H, Punnoose EA, Guan J, Berry L, Prior WW, Amler LC, Belvin M, Friedman LS, Lackner MR (2010) Predictive biomarkers of sensitivity to the phosphatidylinositol 3′ kinase inhibitor GDC-0941 in breast cancer preclinical models. Clin Cancer Res 16(14):3670–3683
61. Junttila TT, Akita RW, Parsons K, Fields C, Lewis Phillips GD, Friedman LS, Sampath D, Sliwkowski MX (2009) Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. Cancer Cell 15 (5):429–440
62. Yakes FM, Chinratanalab W, Ritter CA, King W, Seelig S, Arteaga CL (2002) Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt Is required for

antibody-mediated effects on p27, cyclin D1, and antitumor action. Cancer Res 62(14): 4132–4141
63. Perez-Tenorio G, Alkhori L, Olsson B, Waltersson MA, Nordenskjold B, Rutqvist LE, Skoog L, Stal O (2007) PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. Clin Cancer Res 13(12):3577–3584
64. Ellis MJ, Perou CM (2013) The genomic landscape of breast cancer as a therapeutic roadmap. Cancer Discov 3(1):27–34
65. Loi S, Michiels S, Lambrechts D, Fumagalli D, Claes B, Kellokumpu-Lehtinen PL, Bono P, Kataja V, Piccart MJ, Joensuu H, Sotiriou C (2013) Somatic mutation profiling and associations with prognosis and trastuzumab benefit in early breast cancer. J Natl Cancer Inst 105(13):960–967
66. Eichhorn PJ, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, Beijersbergen RL, Valero V, Seoane J, Bernards R, Baselga J (2008) Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. Cancer Res 68(22):9221–9230
67. Hanker AB, Pfefferle AD, Balko JM, Kuba MG, Young CD, Sanchez V, Sutton CR, Cheng H, Perou CM, Zhao JJ, Cook RS, Arteaga CL (2013) Mutant PIK3CA accelerates HER2-driven transgenic mammary tumors and induces resistance to combinations of anti-HER2 therapies. Proc Natl Acad Sci USA 110(35):14372–14377
68. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, Linn SC, Gonzalez-Angulo AM, Stemke-Hale K, Hauptmann M, Beijersbergen RL, Mills GB, van de Vijver MJ, Bernards R (2007) A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 12(4):395–402
69. Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung MC, Yu D (2004) PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 6(2):117–127
70. Serra V, Markman B, Scaltriti M, Eichhorn PJ, Valero V, Guzman M, Botero ML, Llonch E, Atzori F, Di Cosimo S, Maira M, Garcia-Echeverria C, Parra JL, Arribas J, Baselga J (2008) NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. Cancer Res 68(19):8022–8030
71. Chandarlapaty S, Sakr RA, Giri D, Patil S, Heguy A, Morrow M, Modi S, Norton L, Rosen N, Hudis C, King TA (2012) Frequent mutational activation of the PI3K-AKT pathway in trastuzumab-resistant breast cancer. Clin Cancer Res 18(24):6784–6791
72. Nuciforo PG, Aura C, Holmes E, Prudkin L, Jimenez J, Martinez P, Ameels H, de la Pena L, Ellis C, Eidtmann H, Piccart-Gebhart MJ, Scaltriti M, Baselga J (2015) Benefit to neoadjuvant anti-human epidermal growth factor receptor 2 (HER2)-targeted therapies in HER2-positive primary breast cancer is independent of phosphatase and tensin homolog deleted from chromosome 10 (PTEN) status. Ann Oncol 26(7):1494–1500
73. Stern HM, Gardner H, Burzykowski T, Elatre W, O'Brien C, Lackner MR, Pestano GA, Santiago A, Villalobos I, Eiermann W, Pienkowski T, Martin M, Robert N, Crown J, Nuciforo P, Bee V, Mackey J, Slamon DJ, Press MF (2015) PTEN loss is associated with worse outcome in HER2-amplified breast cancer patients but is not associated with trastuzumab resistance. Clin Cancer Res 21(9):2065–2074
74. Sueta A, Yamamoto Y, Yamamoto-Ibusuki M, Hayashi M, Takeshita T, Yamamoto S, Iwase H (2014) An integrative analysis of PIK3CA mutation, PTEN, and INPP4B expression in terms of trastuzumab efficacy in HER2-positive breast cancer. PLoS ONE 9(12):e116054
75. Park YH, Jung HA, Choi MK, Chang W, Choi YL, Do IG, Ahn JS, Im YH (2014) Role of HER3 expression and PTEN loss in patients with HER2-overexpressing metastatic breast cancer (MBC) who received taxane plus trastuzumab treatment. Br J Cancer 110(2):384–391
76. Faratian D, Goltsov A, Lebedeva G, Sorokin A, Moodie S, Mullen P, Kay C, Um IH, Langdon S, Goryanin I, Harrison DJ (2009) Systems biology reveals new strategies for personalizing cancer medicine and confirms the role of PTEN in resistance to trastuzumab. Cancer Res 69(16):6713–6720

77. Jensen JD, Knoop A, Laenkholm AV, Grauslund M, Jensen MB, Santoni-Rugiu E, Andersson M, Ewertz M (2012) PIK3CA mutations, PTEN, and pHER2 expression and impact on outcome in HER2-positive early-stage breast cancer patients treated with adjuvant chemotherapy and trastuzumab. Ann Oncol 23(8):2034–2042
78. Dave B, Migliaccio I, Gutierrez MC, Wu MF, Chamness GC, Wong H, Narasanna A, Chakrabarty A, Hilsenbeck SG, Huang J, Rimawi M, Schiff R, Arteaga C, Osborne CK, Chang JC (2011) Loss of phosphatase and tensin homolog or phosphoinositol-3 kinase activation and response to trastuzumab or lapatinib in human epidermal growth factor receptor 2-overexpressing locally advanced breast cancers. J Clin Oncol 29(2):166–173
79. Kalinsky K, Jacks LM, Heguy A, Patil S, Drobnjak M, Bhanot UK, Hedvat CV, Traina TA, Solit D, Gerald W, Moynahan ME (2009) PIK3CA mutation associates with improved outcome in breast cancer. Clin Cancer Res 15(16):5049–5059
80. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmstrom PO, Mansukhani M, Enoksson J, Hibshoosh H, Borg A, Parsons R (2005) PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res 65(7):2554–2559
81. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A, Symmans WF, Pusztai L, Nolden LK, Horlings H, Berns K, Hung MC, van de Vijver MJ, Valero V, Gray JW, Bernards R, Mills GB, Hennessy BT (2008) An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. Cancer Res 68(15):6084–6091
82. Li SY, Rong M, Grieu F, Iacopetta B (2006) PIK3CA mutations in breast cancer are associated with poor outcome. Breast Cancer Res Treat 96(1):91–95
83. Esteva FJ, Guo H, Zhang S, Santa-Maria C, Stone S, Lanchbury JS, Sahin AA, Hortobagyi GN, Yu D (2010) PTEN, PIK3CA, p-AKT, and p-p70S6K status: association with trastuzumab response and survival in patients with HER2-positive metastatic breast cancer. Am J Pathol 177(4):1647–1656
84. Rexer BN, Ham AJ, Rinehart C, Hill S, Granja-Ingram Nde M, Gonzalez-Angulo AM, Mills GB, Dave B, Chang JC, Liebler DC, Arteaga CL (2011) Phosphoproteomic mass spectrometry profiling links Src family kinases to escape from HER2 tyrosine kinase inhibition. Oncogene 30(40):4163–4174
85. Rexer BN, Chanthaphaychith S, Dahlman K, Arteaga CL (2014) Direct inhibition of PI3K in combination with dual HER2 inhibitors is required for optimal antitumor activity in HER2+ breast cancer cells. Breast Cancer Res 16(1):R9
86. San Antonio Breast Cancer Symposium (2013) San Antonio, TX
87. Loibl S, von Minckwitz G, Schneeweiss A, Paepke S, Lehmann A, Rezai M, Zahm DM, Sinn P, Khandan F, Eidtmann H, Dohnal K, Heinrichs C, Huober J, Pfitzner B, Fasching PA, Andre F, Lindner JL, Sotiriou C, Dykgers A, Guo S, Gade S, Nekljudova V, Loi S, Untch M, Denkert C (2014) PIK3CA mutations are associated with lower rates of pathologic complete response to anti-human epidermal growth factor receptor 2 (her2) therapy in primary HER2-overexpressing breast cancer. J Clin Oncol 32(29):3212–3220
88. Majewski IJ, Nuciforo P, Mittempergher L, Bosma AJ, Eidtmann H, Holmes E, Sotiriou C, Fumagalli D, Jimenez J, Aura C, Prudkin L, Diaz-Delgado MC, de la Pena L, Loi S, Ellis C, Schultz N, de Azambuja E, Harbeck N, Piccart-Gebhart M, Bernards R, Baselga J (2015) PIK3CA mutations are associated with decreased benefit to neoadjuvant human epidermal growth factor receptor 2-targeted therapies in breast cancer. J Clin Oncol 33(12):1334–1339
89. Gianni L, Bianchini G, Kiermaier A, Bianchi G, Im YH, Pienkowski T, Roman L (2011) Neoadjuvant pertuzumab (P) and trastuzumab (H): biomarker analyses of a 4-arm randomized phase II study (NeoSphere) in patients (pts) with HER2-positive breast cancer (BC)
90. Baselga J, Cortes J, Im SA, Clark E, Ross G, Kiermaier A, Swain SM (2014) Biomarker analyses in CLEOPATRA: a phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2-positive, first-line metastatic breast cancer. J Clin Oncol 32(33):3753–3761

91. Pogue-Geile KL, Song N, Jeong JH, Gavin PG, Kim SR, Blackmon NL, Finnigan M, Rastogi P, Fehrenbacher L, Mamounas EP, Swain SM, Wickerham DL, Geyer CE Jr, Costantino JP, Wolmark N, Paik S (2015) Intrinsic subtypes, PIK3CA mutation, and the degree of benefit from adjuvant trastuzumab in the NSABP B-31 trial. J Clin Oncol 33(12):1340–1347
92. Pavlakis K, Bobos M, Batistatou A, Kotoula V, Eleftheraki AG, Stofas A, Timotheadou E, Pentheroudakis G, Psyrri A, Koutras A, Pectasides D, Papakostas P, Razis E, Christodoulou C, Kalogeras KT, Fountzilas G (2015) p85 protein expression is associated with poor survival in HER2-positive patients with advanced breast cancer treated with trastuzumab. Pathol Oncol Res 21(2):273–282
93. Hurvitz SA, Andre F, Jiang Z, Shao Z, Mano MS, Neciosup SP, Tseng LM, Zhang Q, Shen K, Liu D, Dreosti LM, Burris HA, Toi M, Buyse ME, Cabaribere D, Lindsay MA, Rao S, Pacaud LB, Taran T, Slamon D (2015) Combination of everolimus with trastuzumab plus paclitaxel as first-line treatment for patients with HER2-positive advanced breast cancer (BOLERO-1): a phase 3, randomised, double-blind, multicentre trial. Lancet Oncol
94. Andre F, O'Regan R, Ozguroglu M, Toi M, Xu B, Jerusalem G, Masuda N, Wilks S, Arena F, Isaacs C, Yap YS, Papai Z, Lang I, Armstrong A, Lerzo G, White M, Shen K, Litton J, Chen D, Zhang Y, Ali S, Taran T, Gianni L (2014) Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Oncol 15(6):580–591
95. Jerusalem G, Andre F, Chen D (2013) Evaluation of everolimus (EVE) in HER2+ advanced breast cancer (BC) with activated PI3K/mTOR pathway: Exploratory biomarker observations from the BOLERO-3 trial. European Cancer Congress Amsterdam, Netherlands
96. Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, Barrientos JC, Zelenetz AD, Kipps TJ, Flinn I, Ghia P, Eradat H, Ervin T, Lamanna N, Coiffier B, Pettitt AR, Ma S, Stilgenbauer S, Cramer P, Aiello M, Johnson DM, Miller LL, Li D, Jahn TM, Dansey RD, Hallek M, O'Brien SM (2014) Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med 370(11):997–1007
97. Yardley DA, Noguchi S, Pritchard KI, Burris HA 3rd, Baselga J, Gnant M, Hortobagyi GN, Campone M, Pistilli B, Piccart M, Melichar B, Petrakova K, Arena FP, Erdkamp F, Harb WA, Feng W, Cahana A, Taran T, Lebwohl D, Rugo HS (2013) Everolimus plus exemestane in postmenopausal patients with HR(+) breast cancer: BOLERO-2 final progression-free survival analysis. Adv Ther 30(10):870–884
98. Miller TW, Rexer BN, Garrett JT, Arteaga CL (2011) Mutations in the phosphatidylinositol 3-kinase pathway: role in tumor progression and therapeutic implications in breast cancer. Breast Cancer Res 13(6):224
99. Yao E, Zhou W, Lee-Hoeflich ST, Truong T, Haverty PM, Eastham-Anderson J, Lewin-Koh N, Gunter B, Belvin M, Murray LJ, Friedman LS, Sliwkowski MX, Hoeflich KP (2009) Suppression of HER2/HER3-mediated growth of breast cancer cells with combinations of GDC-0941 PI3K inhibitor, trastuzumab, and pertuzumab. Clin Cancer Res 15(12): 4147–4156
100. Garcia-Garcia C, Ibrahim YH, Serra V, Calvo MT, Guzman M, Grueso J, Aura C, Perez J, Jessen K, Liu Y, Rommel C, Tabernero J, Baselga J, Scaltriti M (2012) Dual mTORC1/2 and HER2 blockade results in antitumor activity in preclinical models of breast cancer resistant to anti-HER2 therapy. Clin Cancer Res 18(9):2603–2612
101. De P, Sun Y, Wu H, Rommel C, Liu Y, Dey N, Leyland-Jones B (2012) Efficacy of INK128, an mTORC1/mTORC2 kinase inhibitor, in breast cancer models driven by HER2-PI3K-AKT-mTOR pathway, ASCO annual meeting Chicago, IL
102. Saura C, Sachdev J, MR P, Cervantes A, Juric D, Infante JR, Richards D, Sanabria S, Lu X, Ware J, Wilson TR, Parmar H, Hsu JY, Oliveira M, Winer EP, Von Hoff DD, Baselga J, Krop IE (2014) Ph1b study of the PI3K inhibitor taselisib (GDC-0032) in combination with letrozole in patients with hormone receptor-positive advanced breast cancer
103. Shah PD, Moynahan ME, Modi S, Caravella BA, Datko FM, Zamora S, Comen E, Gilewski T, Sugarman SM, D'Andrea G, Lake DE, Goldfarb SB, Patil S, Covey A,

Berger MF, Lacouture ME, Norton L, Hudis CA, Baselga J, Chandarlapaty S, Dickler MN (2014) Phase I trial: PI3Kα inhibitor BYL719 plus aromatase inhibitor (AI) for patients with hormone receptor-positive (HR +) metastatic breast cancer (MBC)

104. Garrett JT, Sutton CR, Kurupi R, Bialucha CU, Ettenberg SA, Collins SD, Sheng Q, Wallweber J, Defazio-Eli L, Arteaga CL (2013) Combination of antibody that inhibits ligand-independent HER3 dimerization and a p110alpha inhibitor potently blocks PI3K signaling and growth of HER2+ breast cancers. Cancer Res 73(19):6013–6023
105. Saura C, Bendell J, Jerusalem G, Su S, Ru Q, De Buck S, Mills D, Ruquet S, Bosch A, Urruticoechea A, Beck JT, Di Tomaso E, Sternberg DW, Massacesi C, Hirawat S, Dirix L, Baselga J (2014) Phase Ib study of Buparlisib plus Trastuzumab in patients with HER2-positive advanced or metastatic breast cancer that has progressed on Trastuzumab-based therapy. Clin Cancer Res 20(7):1935–1945
106. O'Brien NA, McDonald K, Tong L et al (2012) PI3K/mTOR inhibition overcomes in vitro and in vivo trastuzumab resistance independent of feedback activation of pAKT
107. De P, Sun Y, Carlson J, Dey N, Leyland-Jones B (2015) Dual inhibition of mTORC1/C2 and HER2 results in maximal antitumor efficacy in preclinical model of HER2+ breast cancer resistant to trastuzumab therapy AACR annual meeting, Philadelphia, PA
108. Schwartz S, Wongvipat J, Trigwell CB, Hancox U, Carver BS, Rodrik-Outmezguine V, Will M, Yellen P, de Stanchina E, Baselga J, Scher HI, Barry ST, Sawyers CL, Chandarlapaty S, Rosen N (2015) Feedback suppression of PI3Kalpha signaling in PTEN-mutated tumors is relieved by selective inhibition of PI3Kbeta. Cancer Cell 27(1):109–122
109. Dey N, Leyland-Jones B, De P (2015) MYC-xing it up with PIK3CA mutation and resistance to PI3K inhibitors: summit of two giants in breast cancers. Am J Cancer Res 5(1):1–19
110. Ilic N, Utermark T, Widlund HR, Roberts TM (2011) PI3K-targeted therapy can be evaded by gene amplification along the MYC-eukaryotic translation initiation factor 4E (eIF4E) axis. Proc Natl Acad Sci USA 108(37):E699–E708
111. Liu P, Cheng H, Santiago S, Raeder M, Zhang F, Isabella A, Yang J, Semaan DJ, Chen C, Fox EA, Gray NS, Monahan J, Schlegel R, Beroukhim R, Mills GB, Zhao JJ (2011) Oncogenic PIK3CA-driven mammary tumors frequently recur via PI3K pathway-dependent and PI3K pathway-independent mechanisms. Nat Med 17(9):1116–1120
112. Lin NU, Winer EP, Wheatley D, Carey LA, Houston S, Mendelson D, Munster P, Frakes L, Kelly S, Garcia AA, Cleator S, Uttenreuther-Fischer M, Jones H, Wind S, Vinisko R, Hickish T (2012) A phase II study of afatinib (BIBW 2992), an irreversible ErbB family blocker, in patients with HER2-positive metastatic breast cancer progressing after trastuzumab. Breast Cancer Res Treat 133(3):1057–1065
113. Hickish T, Wheatley D, Lin N, Carey LA, Houston S, Mendelson DS, Solca F, Uttenreuther-Fischer M, Winer E (2009) Use of BIBW 2992, a novel irreversible EGFR/HER1 and HER2 tyrosine kinase inhibitor to treat patients with HER2-positive metastatic breast cancer after failure of treatment with trastuzumab. Cancer Res 69:5060
114. Yamamoto N, Katakami N, Atagi S, Hida T, Goto K, Horai T, Inoue A, Ichinose Y, Kobayashi K, Takeda K, Kiura K, Saka H, Tamura T, Okamoto I, Nogami N, Moringa R, Nishio K, Seki Y, Lorence R, Shahidi M (2011) A phase II trial of afatinib (BIBW 2992) in patients (pts) with advanced nonsmall cell lung cancer previously treated with erlotinib or gefitinib, annual meeting of the American Society of Clinical Oncology, Chicago, IL
115. Yap TA, Vidal L, Adam J, Stephens P, Spicer J, Shaw H, Ang J, Temple G, Bell S, Shahidi M, Uttenreuther-Fischer M, Stopfer P, Futreal A, Calvert H, de Bono JS, Plummer R (2010) Phase I trial of the irreversible EGFR and HER2 kinase inhibitor BIBW 2992 in patients with advanced solid tumors. J Clin Oncol 28(25):3965–3972
116. Ring A, Wheatley D, Hatcher H, Laing R, Plummer R, Uttenreuther-Fischer M, Temple G, Pelling K, Schnell D (2015) Phase I study to assess the combination of afatinib with trastuzumab in patients with advanced or metastatic HER2-positive breast cancer. Clin Cancer Res 21(12):2737–2744

117. Li D, Ambrogio L, Shimamura T, Kubo S, Takahashi M, Chirieac LR, Padera RF, Shapiro GI, Baum A, Himmelsbach F, Rettig WJ, Meyerson M, Solca F, Greulich H, Wong KK (2008) BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene 27(34):4702–4711
118. Rabindran SK, Discafani CM, Rosfjord EC, Baxter M, Floyd MB, Golas J, Hallett WA, Johnson BD, Nilakantan R, Overbeek E, Reich MF, Shen R, Shi X, Tsou HR, Wang YF, Wissner A (2004) Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. Cancer Res 64(11):3958–3965
119. Wong KK, Fracasso PM, Bukowski RM, Lynch TJ, Munster PN, Shapiro GI, Janne PA, Eder JP, Naughton MJ, Ellis MJ, Jones SF, Mekhail T, Zacharchuk C, Vermette J, Abbas R, Quinn S, Powell C, Burris HA (2009) A phase I study with neratinib (HKI-272), an irreversible pan ErbB receptor tyrosine kinase inhibitor, in patients with solid tumors. Clin Cancer Res 15(7):2552–2558
120. Burstein HJ, Sun Y, Dirix LY, Jiang Z, Paridaens R, Tan AR, Awada A, Ranade A, Jiao S, Schwartz G, Abbas R, Powell C, Turnbull K, Vermette J, Zacharchuk C, Badwe R (2010) Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. J Clin Oncol 28(8):1301–1307
121. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136(5):E359–E386

Chapter 9
The PI3K-mTOR Pathway in Prostate Cancer: Biological Significance and Therapeutic Opportunities

Jason Boyang Wu and Leland W.K. Chung

Introduction

Prostate cancer (PCa) is the second most common malignancy and fifth leading cause of cancer-related mortality in men worldwide, accounting for 1,111,700 newly diagnosed cancer cases and 307,500 cancer deaths in 2014 [1]. Owing to advances in widespread screening for prostate specific antigen (PSA), most PCa cases are diagnosed with localized or regional disease at an early stage, which can be cured, with a good prognosis, by active surveillance, surgery, radiation therapy, chemotherapy and/or hormone therapy (androgen deprivation therapy, ADT) [2]. However, after an initial response most patients develop therapy resistance. For instance, patients treated by ADT experience disease recurrence as castration-resistant prostate cancer (CRPC). CRPC and associated metastases to nearby and distant organs are a highly aggressive, incurable and terminal disease affecting the quality of life of PCa patients [3, 4]. In the United States, the 5-year survival rate for patients who develop metastatic disease is only 29 % compared to nearly 100 % in patients with localized early disease [2]. In recent years, the therapeutic arena in PCa has greatly expanded with the introduction of new therapeutic agents, including hormonal agents, the androgen synthesis inhibitor abiraterone acetate and the androgen receptor (AR) inhibitor enzalutamide which revolutionized the treatment of metastatic CRPC, the novel taxane chemotherapeutic cabazitaxel, and the bone microenvironment-targeted radiopharmaceutical alpharadin (Radium-223). Several other treatments targeting the androgenic pathway (TAK-700, ARN-509, ODM-201, TOK-001), DNA repair pathway (olaparib, veliparib, BMN 673, MK4827), and immune system (sipuleucel-T, ipilimumab, PROSTVAC-V/F, tasquinimod), are also on trial [5–7]. While these

J.B. Wu · L.W.K. Chung (✉)
Cedars-Sinai Medical Center, Los Angeles, CA, USA
e-mail: Leland.Chung@cshs.org

N. Dey et al. (eds.), *PI3K-mTOR in Cancer and Cancer Therapy*,
Cancer Drug Discovery and Development, DOI 10.1007/978-3-319-34211-5_9

therapies have provided clinical relief to men with late-stage PCa, the survival benefit in patients with metastatic CRPC remains brief, and additional therapeutic approaches are needed.

The phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway has emerged as a key node coordinating a number of upstream inputs as well as downstream signaling components with important roles in cancer development and progression in a wide spectrum of cancer types [8, 9]. In PCa, activation of the PI3K/AKT/mTOR pathway is strongly associated with cancer progression and exhibits alterations at the genomic and transcriptional level in nearly all advanced PCa [10]. By integrating intra- and extracellular growth signals with many crucial cellular processes, this pathway allows cancer cells to survive stress conditions and develop resistance to therapies [11–13]. Therefore, targeting the PI3K/AKT/mTOR pathway offers great potential for treatment of PCa, especially advanced PCa, further encouraged by the fact that a variety of drugs specifically targeting this pathway are currently in clinical development [14, 15].

PI3K-mTOR Signaling and Function

The PI3K/AKT/mTOR pathway exhibits evolutionary conservation from worms to humans and regulates a variety of cellular processes, including protein synthesis, proliferation, survival, differentiation, migration, stem cell-like properties, metabolism and angiogenesis. The diverse array of functions underlying this pathway is achieved by a complex signaling network connecting upstream inputs from nutrients and growth factors with a number of effectors that mediate the phosphorylation, transcription and translation of downstream target genes [11–13]. The PI3K family of lipid kinase is capable of phosphorylating the 3′-hydroxyl group of the inositol ring of phosphatidylinositol. There are three classes (I–III) of PI3K categorized by their substrate preferences and sequence similarities with the class IA PI3K most frequently implicated in cancer. Class IA PI3Ks are heterodimeric molecules composed of a p110 catalytic subunit and a p85 regulatory subunit. There are five isoforms of p85 (p85α, p55α, p50α, p85β and p55γ), encoded by the genes *PIK3R1* (p85α, p55α and p50α), *PIK3R2* (p85β) and *PIK3R3* (p55γ). There are also three isoforms of p110 (α, β and δ) encoded by the genes *PIK3CA*, *PIK3CB* and *PIK3CD* respectively [8]. PI3Ks are activated primarily by receptor tyrosine kinases (RTKs) but also through G-protein-coupled receptors and oncogenes such as rat sarcoma oncogene (RAS) [16–19]. Upon activation, the catalytic subunit of PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] to phosphatidylinositol 3,4,5-trisphosphate [$PI(3,4,5)P_3$], leading to subsequent recruitment of a variety of pleckstrin homology domain-containing proteins such as AKT to the cell membrane [8]. This process is negatively regulated by tumor suppressor phosphatase and tensin homolog (PTEN) and inositol polyphosphate-

4-phosphatase, type II (INPP4B), which convert PI(3,4,5)P_3 to PI(4,5)P_2 and PI(3,4)P_2 to PI(3)P respectively. As a major target recruited by PI3K, the AKT family of serine/threonine protein kinases is activated by phosphorylation, which subsequently phosphorylates a spectrum of downstream effectors, including glycogen synthase kinase 3 (GSK3), forkhead box O (FOXO) transcription factors, and tuberous sclerosis complex 2 (TSC2), to regulate a variety of processes that coordinate cell proliferation, survival, metabolism and angiogenesis [13, 20].

mTOR is a serine/threonine kinase forming the catalytic subunit of two structurally distinct complexes, mTORC1 and mTORC2. mTORC1 is composed of mTOR, Raptor, mLST8, PRAS40, Deptor and TTI1/TEL2 and assembles following AKT phosphorylation of TSC2 whereby TSC2 loses its GAP activity and stabilizes Rheb-GTP, an mTORC1 activator. Additionally, phosphorylation of PRAS40 by AKT and by mTORC1 itself leads to dissociation of PRAS40 from mTORC1 and relieves an inhibitory constraint on mTORC1 activity. In response to a number of upstream signals, such as insulin, growth factors, amino acids and oxidative stress, mTORC1 classically functions as a nutrient/energy/redox sensor and controls protein synthesis through phosphorylation of S6K1 and 4E-BP1. mTORC1 also participates in a plethora of cellular processes, such as autophagy, lipid synthesis and mitochondrial metabolism and biogenesis, through interaction with associated regulators and protein complexes. The mTORC2 complex consists of mTOR, Rictor, mSIN1, mLST8, Deptor, Protor 1/2 and TTI1/TEL2. mTORC2 is regulated by insulin, growth factors, serum and nutrients through shared and distinct mechanisms compared to mTORC1, and is considered in general resistant to rapamycin. mTORC2 activates AKT, SGK1, RhoA, Rac1, Cdc42 and PKCα and regulates cellular metabolism as well as cytoskeletal organization. The distinct downstream substrate specificities and cellular functions of mTORC1 and mTORC2 are believed to depend in part on the different composition of each mTOR complex [12, 21–23].

PI3K-mTOR Signaling in Prostate Cancer

The PI3K/AKT/mTOR pathway is frequently deregulated with genetic alterations found in nearly every major node in a spectrum of cancers [24]. In PCa, dysregulation of this pathway, including mutations, copy number amplifications, and altered mRNA expression, has been reported in 42 % of primary prostate tumors and 100 % of metastatic tumors [10]. Similar observations have also been made in four independent clinical PCa data sets recently analyzed by The Cancer Genome Atlas project (Table 9.1). Moreover, these alterations show significant correlation with PCa patient outcomes. For example, genomic deletion of *PTEN* with a loss of protein expression, which is a negative regulator of the PI3K/AKT/mTOR pathway, is associated with high Gleason score and an early onset of biochemical recurrence and

Table 9.1 Molecular alterations of select key components in the PI3K/AKT/mTOR pathway from 4 independent prostate cancer clinical data sets deposited at the cBioportal for Cancer Genomics

	Primary		Metastatic	
Gene	TCGA-1 (*N* = 258) (%)	TCGA-2 (*N* = 333) (%)	Michigan (*N* = 59) (%)	SU2C/PCF (*N* = 150) (%)
PTEN	24	17	51	40
PIK3R1	6	7	10	5
PIK3R2	1	0.9	2	3
PIK3R3	2	0.6	2	3
PIK3CA	9	5	10	5
PIK3CB	4	3	7	7
PIK3CD	None	1	2	1
AKT1	2	2	None	3
AKT2	0.8	0.3	2	1
AKT3	1	2	2	1
RICTOR	0.4	0.3	5	3
RPTOR	0.8	0.9	2	3

metastasis in PCa patients [25–27]. Furthermore, elevated p-4E-BP1 and p-S6K expression levels, two common downstream effectors of the pathway, are significantly correlated with clinical stage and distant metastasis in PCa patients [28]. These clinical observations collectively indicate the functional importance of this pathway in PCa development and progression. The PI3K/AKT/mTOR signaling pathway and its crosstalk with other pathways in PCa are discussed below (Fig. 9.1).

The PI3K-mTOR Pathway and Epigenetic Programming in Prostate Cancer Pathogenesis

PCa is a heterogeneous disease which develops through a dynamic process of genetic mutation, clonal evolution and/or epigenetic programming [29–31]. During this process, an initial specific genetic mutation could influence the subsequent behaviors of cancer cells through progressive expansion of the genetically mutated cells or accumulation of additional genetic alterations in the clonally evoluted cancer cells [30, 31]. Moreover, recent studies from our laboratory suggest a third epigenetic mechanism, where a small cluster of metastasis-initiating cells (MICs) reprogram indolent resident cancer cells at metastatic sites toward an aggressive and metastatic phenotype contributing to PCa bone metastasis [29, 32–34]. Remarkably, we documented permanent genetic and behavioral changes conferred to indolent resident cancer cells by MICs through in vitro 3-D co-culture and in vivo tumor growth in mice [29, 32]. Collectively, genetic or epigenetic pathways

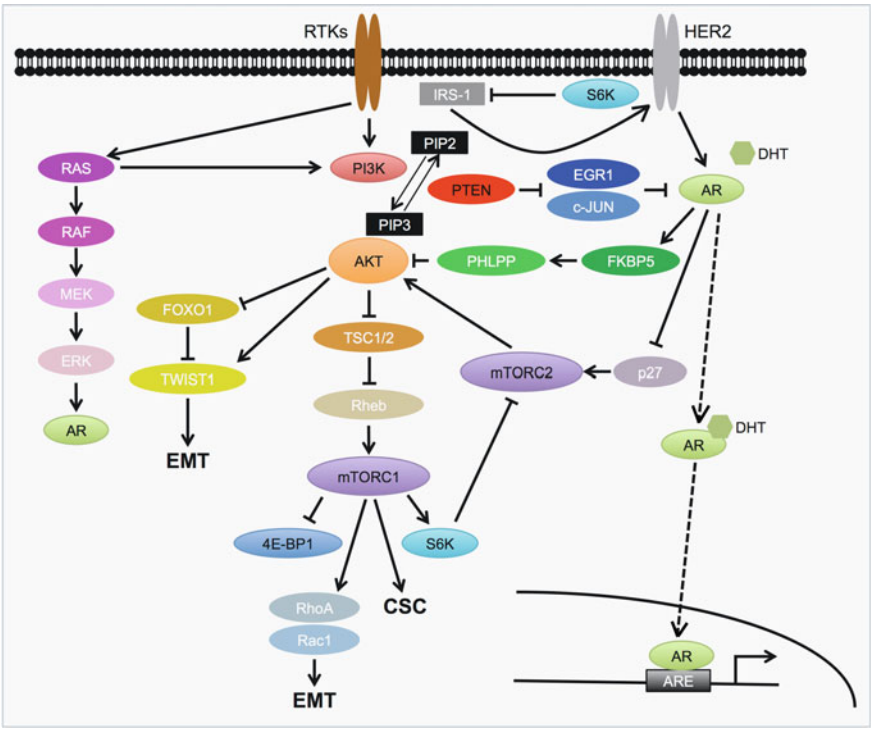


Fig. 9.1 The PI3K/AKT/mTOR signaling pathway and its crosstalk with other pathways in prostate cancer [14, 15]. *AR* androgen receptor; *ARE* androgen response element; *CSC* cancer stem cell; *DHT* dihydrotestosterone; *EMT* epithelial-mesenchymal transition; *PI3K* phosphoinostide-3-kinase; *RTK* receptor tyrosin kinase

are believed to be highly coordinated and may confer a common selective growth advantage on cancer cells to promote prostate tumorigenesis [30–32, 34]. Due to the lack of additional data on how the PI3K/AKT/mTOR and associated pathways may be altered specifically by MICs-driven epigenetic programming in PCa, only genetic perturbation directly relevant to the PI3K/AKT/mTOR pathway components will be discussed in this section.

In the last two decades, much study has been focused on characterizing the roles of genetic dysregulation in PI3K/AKT/mTOR pathway components in PCa development. These studies have clearly demonstrated the importance of PI3K/AKT/mTOR pathway components in a series of transgenic mouse models. Prostate-specific overexpression of AKT, a central target recruited by PI3K, leads to S6K activation, prostatic intraepithelial neoplasia (PIN), and bladder obstruction in transgenic mice (the MPAKT model), which also show a similar mRNA expression profile from the ventral prostate to human PCa [35]. Inactivation of PTEN, a negative regulator of the PI3K/AKT/mTOR pathway, in prostate epithelial cells in both heterozygous and homozygous forms confirms PTEN as a bona fide tumor

suppressor in PCa. *Pten*$^{+/-}$ mice show a broad spectrum of spontaneous tumor development but a long latency for PIN and a lack of metastatic disease [36, 37]. Prostate-specific biallelic deletion of *Pten* in mice recapitulates the disease progression seen in humans by a shortened latency of PIN formation followed by progression to invasive adenocarcinoma and metastatic stage [38]. Moreover, temporal control of PTEN deletion in the post-pubertal prostate in several inducible knockout mouse models suggest PTEN's autonomous role in dictating the pace of PIN development and prostate tumor formation [39, 40]. These studies suggest that hyperactivation of the PI3K/AKT/mTOR pathway by genetic alteration of a single component gene, such as *PTEN*, is sufficient to initiate PCa in vivo.

AKT-activated or PTEN-deficient prostate tumor growth depends on mTOR and its associated complex activity. Conditional inactivation of mTOR in the mouse prostate leads to a marked suppression of Pten loss-induced prostate tumor initiation and progression in mice, with more profound effects than those elicited by the sole pharmacological abrogation of mTORC1, suggesting a requisite role of both mTORC1 and mTORC2 activities in growth and maintenance of Pten-null prostate tumors [41]. Moreover, Rheb-GTP, the upstream activator of mTORC1, is amplified in human PCa. Rheb overexpression induces hyperplasia and a low-grade neoplastic phenotype in the mouse prostate, and further promotes prostate tumorigenesis when cooperating with *Pten* haploinsufficiency in mice [42]. mTORC2 is also required for the development of PCa caused by Pten loss in mice, since concurrent deletion of PTEN and Rictor, a key regulatory subunit of the mTORC2 complex, protects mice from PCa. Interestingly, mTORC2 activity is not essential for normal prostate epithelial cells [43]. Pharmacologically, rapamycin treatment in *Pten*$^{+/-}$ mice reduced prostate lesion growth along with a reduction in both proliferative index and phospho-S6 levels, an mTOR downstream effector [44]. Treatment of PTEN-null PCa cells with NVP-BEZ235, a dual PI3K/mTOR inhibitor, also induced autophagic cell death [45]. On the other hand, pharmacological inhibition of mTOR reverses AKT-dependent PIN phenotype in the MPAKT mouse model expressing human AKT1 in the ventral prostate through induced apoptosis of epithelial cells and downregulation of HIF1α activity [46]. Furthermore, co-targeting AKT and mTOR delivered additive antitumor effects in vivo when compared to a single agent in a prostate-specific Pten-deficient mouse model associated with AKT activation [47].

Although elevated expression of p-mTOR is commonly detected during PCa development across different progressive stages, and also shows positive correlation with a trend toward worse prognosis in PCa patients, its activity varies depending on the subtype [48]. For example, loss of p(Ser2448)-mTOR expression is linked to adverse prognosis and prostate tumor progression in a small (4 %) subset of PCa patients that show PTEN deletion and ERG-fusion positivity [49], which suggests that potential epigenetic gene-microenvironment interactions may play a role. These studies nevertheless demonstrate that the PI3K/AKT/mTOR pathway mediates coordinative interactions among components in both common and subtype-specific manners to drive prostate tumorigenesis and development.

Crosstalk with AR and Other Signaling in Castration-Resistant Prostate Cancer

In addition to its direct tumor-initiating/-promoting ability, the PI3K/AKT/mTOR pathway contributes to the development and progression of PCa through signaling crosstalk with other pathways that are also implicated in PCa. Among all regulatory mechanisms underlying PCa, the AR signaling pathway is essential for normal prostate growth and differentiation and also for PCa initiation and progression [50, 51]. It functions through the AR and its ligands, testosterone and 5α-dihydrotestosterone (DHT) [52, 53]. Upon activation by ligand binding, AR is translocated to the nucleus and binds to androgen response elements at the promoter of target genes to induce transcription [51]. AR is critical for maintaining PCa cell proliferation in most early-stage PCa, considered as androgen-dependent, which constitutes the rationale for using ADT to achieve cancer cell apoptosis [50, 51]. *AR* gene amplification, mutations in the AR ligand-binding domain, induction of *AR* splice variants, and increased sensitivity of AR for activation appear frequently in post ADT PCa samples, marking the disease progression to a CRPC state, the aggressive and lethal phenotype of PCa [3, 54–56]. Recent global gene expression profiling further indicates AR is one of several genes constitutively upregulated in CRPC [57, 58]. These facts all show the importance of the androgenic pathway in disease progression.

It has been shown that PI3K/AKT/mTOR pathway deregulation by PTEN loss is associated with CRPC development after ADT intervention. Pten-null mouse prostate tumors regress after androgen ablation and develop androgen-independent characteristics for cell proliferation [38]. At the cellular level, genetic knockdown of *PTEN* converted the androgen-dependent PCa cells to androgen independence without prior exposure to ADT, suggesting an intrinsic role of PTEN in the control of androgen responsiveness [59]. PTEN re-expression in a PTEN-negative background upregulates AR function by negatively regulating the expression of transcription factors EGR1 and c-JUN, which physically interact with AR and downregulate AR targeted gene expression [60–62]. Conversely, knockdown of AR in PTEN-null cells reversed androgen-independent growth in vitro and partially inhibited tumorigenesis in vivo, which indicates the AR dependence of PTEN-mediated prostate tumorigenesis [59]. These studies taking advantage of transgenic mouse models clearly suggest a potential reciprocal interaction between the PI3K/AKT/mTOR and AR pathways. However, future investigations should also examine how genetic changes in cancer cells per se may alter the tumor microenvironment and affect epigenetic programming of cancer cell behaviors including growth, invasion, migration and metastasis [32, 63, 64].

A series of studies in both mouse models and cell lines of PCa have further explored the mechanisms by which these two pathways regulate each other to promote CRPC. In a PCa mouse model driven by Pten loss, PI3K pathway inhibition with a PI3K inhibitor activated AR signaling through upregulation of HER3 kinase, which enhances both AR stability and transcriptional activity [65, 66].

On the other hand, AR inhibition in the background of PTEN loss, which was achieved by both surgical castration plus enzalutamide treatment for blockade of AR nuclear translocation and DNA binding, promoted PI3K activity and AKT signaling by downregulation of FKBP5 to reduce levels of the AKT phosphatase PHLPP [60, 65]. Androgen ablation also activated AKT via INPP4B downregulation, a negative regulator of PI3K signaling, which mitigates the antitumor effects of ADT [67]. In addition, a recent study points out the roles of different isoforms of PI3Ks in prostate tumor growth and AR activation. In *PTEN*-mutated tumors, inhibition of PI3Kβ that drives PI3K signaling activated pre-silenced PI3Kα by relieving feedback inhibition of IGF1R and other receptors, and effective combined inhibition of PI3Kα and PI3Kβ caused marked activation of AR activity, suggesting again a requirement of combined inhibition of both PI3K isoforms and AR to achieve major tumor regressions [68]. In line with observations made in transgenic mouse models, biallelic loss of *PTEN* in a cohort of hormone refractory PCa clinical samples is correlated with activated p-AKT and AR protein expression, further associated with poor outcomes including disease-specific mortality [69]. These studies demonstrate a compensatory crosstalk between the PI3K and AR pathways associated with relief of feedback inhibition following PI3K or AR inhibition, which provides a mechanistic rationale for co-targeting both pathways in CRPC. This concept has been supported by several preclinical studies where synergistic targeting of PI3K/AKT and AR signaling axes significantly delayed CRPC progression in vivo [70].

Constitutively active AR isoforms, such as the C-terminally truncated AR variants derived from alternative splicing of cryptic exons, including AR-V7, have been identified under conditions of androgen depletion [71–73]. AR-V7 mRNA is expressed in 24 % of metastatic lesions in CRPC patients, with its protein level overexpressed in CRPC compared with benign prostate or hormone-naïve PCa [73, 74]. AR-V7 expression also increases in response to the most current therapies developed for CRPC [55]. The PI3K/AKT pathway contributes to increased AR-V7 constitutive activity in PCa cells by suppression of FOXO1, a downstream effector phosphorylated by AKT, under conditions of PTEN inactivation [75]. Besides AR itself, AR target genes such as *PLZF* also reciprocally interact with PI3K/AKT signaling. PTEN rescue or PI3K inhibition increases PLZF expression in PCa cells through a direct binding of FOXO3a, a transcription factor phosphorylated by PI3K/AKT, to the promoter of *PLZF* gene, which exerts inhibitory effects on prostate tumorigenesis in vivo [76, 77].

The signaling crosstalk between AR and mTOR and associated complexes is also significantly involved in PCa in general and CRPC specifically. In regulation of cell cycle progression, AR enhances the degradation of the p27 cyclin-dependent kinase inhibitor through rapid and selective mTORC2 activation followed by subsequent activation of AKT and phosphorylation of a discrete set of AKT substrates that control cellular proliferation and survival [78]. In addition to mTORC2, *KLK4* and *PLZF*, two androgen-regulated genes, integrate androgen and mTOR signaling by mTORC1 activation, mediated through downregulation of REDD1, an inhibitor of mTORC1, to promote prostate tumor growth in mice [79]. On the other

hand, Rheb-GTP, an mTORC1 activator, potentiates proliferation of several aggressive PCa cell lines by regulation of AR transactivity, which was blocked by rapamycin treatment with a reduction in p-S6K level [80]. These findings suggest a reciprocal relationship between AR- and mTOR-mediated pathways and also provide a mechanistic rationale for co-targeting AR and mTOR in PCa, which has been supported by a pharmacologic study showing that dual inhibition of AR and mTOR restores tumor sensitivity to anti-androgen therapy in a LNCaP CRPC xenograft model [81].

Cancer cells require increases in nutrients such as amino acids to maintain a growth advantage. Several groups have provided evidence of a regulatory system combining androgen response and nutrient stress pathways working in concert to promote PCa growth and progression. In response to DHT treatment, the proliferation of PTEN-deficient LNCaP PCa cells was induced by post-transcriptional increase in D-type cyclin proteins, which is mediated by mTOR activation through AR-stimulated mRNA synthesis but not the PI3K/AKT or other kinase-mediated pathways. Oligonucleotide microarrays further showed DHT-stimulated increases in an array of genes responsible for nutrient availability, including transporters for amino acids. This suggests a critical function of AR to support the pathologic activation of mTOR in a PTEN-null background, possibly by increasing the expression of proteins that enhance nutrient availability and thereby prevent feedback inhibition of mTOR [82]. In line with this observation, other evidence showed that expression of the L-type amino acid transporter LAT3 is activated directly by AR in the presence of androgen while LAT1 expression, controlled by ATF4, is induced upon androgen deprivation, which promotes mTORC1 signaling activity and PCa cell growth by facilitating the uptake of essential amino acids [83]. In CRPC, the signaling convergence between AR and mTOR may also impact the transition of PCa from androgen dependence to ADT resistance, which selectively allows cancer cells to survive conditions of low androgen and suboptimal nutrients produced by ADT. AR increases mTOR activity possibly by downregulation of TSC1 and TSC2, two negative regulators of mTOR, in both low and high testosterone levels. A sub-baseline mTOR level in turn stimulates AR protein expression only with low testosterone to compensate for decreased availability of testosterone. This loop benefits both molecular partners and enhances cell survival under ADT-induced cellular stress, and disrupting this loop at the beginning of ADT may delay or prevent the development of castration resistance and recurrence of PCa [84].

In addition to interacting with androgenic signaling, the PI3K/AKT/mTOR pathway also cooperates with other signaling in PCa progression. In a Pten-deficient mouse model of PCa, ADT induced phenotypic plasticity to promote therapeutic escape. Unlike castration-naïve tumors which depend on AR and PI3K/AKT activation for growth and survival, castration-resistant tumors attain increased heterogeneity, which is characterized by loss of their dependence on PI3K signaling and activation of alternative pathways, such as MAPK and JNK/STAT, for cell growth and survival [85]. RTKs, which activate both the PI3K/AKT/mTOR and other kinase pathways, are in turn regulated by mTORC1 activity through feedback loops [86]. Inhibition of mTOR induced IRS-1 expression, a major

substrate for multiple cell surface receptors, including IGF-1, HER2/3 and EGFR, and abrogated feedback inhibition of the pathway, leading to compensatory reactivation of PI3K and AKT [65, 87, 88]. These studies in aggregate suggest a potential combination therapy with PI3K, cell surface RTKs and/or other kinase(s) to improve treatment outcomes in PCa.

The PI3K-mTOR Pathway in Prostate Cancer Metastasis

Advanced PCa is frequently accompanied by the development of metastases to distant organs, such as bone, lymph nodes, lungs, liver and brain, which significantly compromises quality of life and can lead to death [7, 89]. PI3K/AKT/mTOR pathway deregulation has been strongly implicated in PCa metastasis, since all metastatic prostate tumors harbor genetic alterations of this pathway [10]. PTEN loss or PI3K/AKT signaling pathway activation can mediate prostate tumor metastatic progression through induction of an epithelial-mesenchymal transition (EMT) program in cancer cells, considered an initiating step in metastasis that confers increased motility and invasiveness to cancer cells for their dissociation from the epithelial layer [90]. Activation of PI3K/mTOR signaling increases phosphorylation of TWIST1, an EMT-inducing transcription factor, to promote its DNA-binding ability [91]. Alternatively, PI3K/AKT pathway activation stabilizes TWIST1 expression to induce EMT by downregulation of FOXO1 activity, a downstream effector phosphorylated by AKT as well as a transcription repressor of TWIST1 [92]. The activated PI3K/AKT pathway also inhibits metastasis suppressor FOXO4, another FOXO family protein, by direct phosphorylation to prevent its translocation to the nucleus [93]. Moreover, by activating AKT-dependent transcription factor NFκB, the PI3K/AKT/NFκB axis promotes PCa bone metastasis by transcriptional activation of BMP-2 and induced phosphorylation of associated Smad1/5/8 [94].

In addition to its direct role in initiating and/or promoting metastasis, the PI3K/AKT pathway regulates metastatic progression through signaling crosstalk with other pathways. A recent clinic survey of human PCa tissue microarrays identified an elevated RAS/MAPK pathway in both primary and metastatic lesions, which may cooperate with PTEN loss in PCa progression. In a preclinical mouse model of PCa, conditional activation of K-ras and Pten deletion in a prostate-specific manner significantly accelerated prostate tumor progression caused by Pten loss, accompanied by EMT and macrometastases with 100 % penetrance, which mimics the metastatic burden seen in human disease. Moreover, inhibition of the RAS/MAPK pathway by a MEK inhibitor dramatically reduced metastatic progression initiated by PTEN-deficient and K-RAS-activated stem/progenitor cells [95]. These findings indicate that both signaling axes collaborate with each other to drive metastatic progression and also suggest a co-targeting strategy for effective blockade of metastasis.

Recently, a translational landscape of the PCa genome controlled by oncogenic mTOR signaling has been delineated using ribosome profiling. A specific repertoire of genes involved in invasion and metastasis, such as YB1, VIM, MTA1 and CD44, downstream of mTOR signaling was revealed, which suggests the functional role of mTOR signaling in regulating metastatic progression of PCa [96]. On the other hand, several studies have demonstrated that mTOR-associated mTORC1 and mTORC2 play a crucial role in the regulation of cell motility and invasion [97, 98]. Mechanistically, mTOR activation with increased expression of Raptor and Rictor is found to induce EMT through upregulation of the RhoA and Rac1 pathway in PCa cells. Inhibition of mTORC1 or mTORC2 by knockdown of Raptor or Rictor respectively attenuated PCa cell migration and invasion [99]. In addition, the mTOR pathway also promotes PCa metastasis by integrating other signaling. Activated mTOR pathway mediates TGFβ-promoting PCa bone metastasis through Smad-dependent induction of miR-96, an oncomir and metastamir [100]. These studies collectively demonstrate the mTOR pathway as a promising target for therapeutic intervention in PCa metastasis.

Emerging evidence indicates the importance of cancer stem cells (CSCs), a subpopulation of tumor cells endowed with self-renewal and multi-lineage differentiation capability, in the initiation of tumor metastasis [101]. Indeed, the $CD44^+$ prostate CSC-enriched cells are highly metastatic [102]. In another study, an 11 stemness-gene signature, including BMI-1, was identified in highly metastatic PCa, which also predicts poor outcomes in PCa patients [103, 104]. The link between the PI3K/AKT/mTOR pathway and prostate CSCs has been evidently established in a PCa progenitor cell model in which preferential activation of PI3K/AKT signaling was enriched in the $CD133^+/CD44^+$ cell population with increased PI3K p110α/β protein expression. *PTEN* knockdown increased the sphere-forming ability and tumorigenic potential of PCa progenitor cells, whereas inhibition of PI3K activity by a dual PI3K-mTOR inhibitor BEZ235 resulted in growth inhibition of PCa progenitor cells, suggesting that the PI3K/AKT/mTOR pathway is crucial for maintenance of PCa stem-like properties [105]. In addition, in a TRAMP mouse model of PCa, upregulation of AKT mediated by an mTOR/S6K/IRS-1 feedback loop mechanism was demonstrated in isolated $Sca\text{-}1^+/CD49f^+$ mouse prostate CSCs, which was stimulated by hypoxia [106]. These findings suggest that targeting PI3K/AKT/mTOR signaling may have therapeutic benefit in PCa metastasis by eliminating prostate CSCs as a source of metastasis.

Targeting the PI3K-mTOR Pathway in Prostate Cancer

Given the importance of the PI3K/AKT/mTOR pathway in regulating the development and progression of PCa and the highly prevalent alterations of this pathway in PCa, inhibitors of this pathway offer great potential for clinical benefit in men with advanced stage PCa. A number of agents are under development today and multiple ongoing clinical trials aim to determine the efficacy of specific pathway

inhibitors as monotherapy or in combination for PCa treatment. The therapeutic profiles and potential use of a select group of promising PI3K/AKT/mTOR pathway inhibitors currently being tested in the clinic for PCa are discussed below (Table 9.2).

Table 9.2 Currently active clinical trials with PI3K/AKT/mTOR pathway inhibitors in prostate cancer

Agent	Phase	Regimen	Population	Registry
Pan-PI3K inhibitors				
BKM120 (Novartis)	I	+Abiraterone acetate	CRPC	NCT01634061
	I	+Abiraterone acetate	Metastatic CRPC	NCT01741753
	II	Monotherapy	Metastatic PCa	NCT01385293
	II	Monotherapy	High-risk PCa	NCT01695473
PX-866 (Oncothyreon)	II	Monotherapy	Metastatic PCa	NCT01331083
Allosteric AKT inhibitors				
MK-2206 (Merck)	I	+Hydroxychloroquine	Advanced solid tumors including PCa	NCT01480154
	I	+Ridaforolimus	Advanced cancer, including PCa	NCT01295632
	II	+Bicalutamide	Previously treated PCa	NCT01251861
ATP-competitive AKT inhibitors				
GDC-0068 (Genentech)	II	+Abiraterone acetate	CRPC previously treated with docetaxel chemotherapy	NCT01485861
AZD5363	I	Monotherapy	PCa	NCT01692262
	II	+DP chemotherapy	Metastatic PCa	NCT02121639
Allosteric mTOR inhibitors				
Everolimus (Novartis)	I	+Enzalutamide	Metastatic PCa	NCT02125084
	I	+ARN 509	Metastatic CRPC after treatment with abiraterone acetate	NCT02106507
	I	+Radiation therapy	Recurrent PCa	NCT01548807
	I/II	+Docetaxel	Advanced PCa	NCT00574769
	II	+Gefitinib	Metastatic PCa	NCT00085566
	II	+Pasireotide	Hormone resistant, chemotherapy naïve PCa	NCT01313559
	II	+Bicalutamide	Recurrent or metastatic PCa	NCT00814788
Temsirolimus (Wyeth)	I	+Vorinostat	Metastatic PCa	NCT01174199
	I	+Vinorelbine ditartrate	Metastatic solid tumors including PCa	NCT01155258
	I	+Docetaxel	Resistant solid tumors including PCa	NCT00703625
	I/II	+Cixutumumab	Metastatic PCa	NCT01026623
	I/II	+Bevacizumab	Hormone-resistant metastatic PCa that did not respond to chemotherapy	NCT01083368

(continued)

Table 9.2 (continued)

Agent	Phase	Regimen	Population	Registry
Ridaforolimus (Merck and ARIAD)	I	+MK-2206 or MK-0752	Advanced cancer including PCa	NCT01295632
	II	+Bicalutamide	PCa	NCT00777959
ATP-competitive mTOR inhibitors				
MLN0128 (Takara)	II	Monotherapy	CRPC	NCT02091531
AZD2014 (AstraZeneca)	I	Monotherapy	High risk PCa	NCT02064608
Dual PI3K-mTOR inhibitors				
BEZ235 (Novartis)	I	+Abiraterone acetate	CRPC	NCT01634061
GDC-0980 (Genentech)	II	+Abiraterone acetate	CRPC previously treated with docetaxel chemotherapy	NCT01485861

PI3K Inhibitors

Two types of PI3K inhibitors are currently available: pan-PI3K and isoform-specific inhibitors. The pan-PI3K inhibitors target the catalytic subunits of all three isoforms of class IA PI3K (p110α, β and δ) and class IB PI3K catalytic subunit (p110γ). In preclinical studies, pan-PI3K inhibitor BKM120 inhibited proliferation of an androgen-independent metastatic PC-3M cell line and blocked PC-3M tumor growth in xenograft mice [107]. In a phase I first-in-human study of BKM120, the maximum tolerated dose is defined as 100 mg/day. Overall, treatment with BKM120 is well tolerated with observable frequent treatment-related adverse events including rash, hyperglycemia, diarrhea, anorexia, mood alteration (37 %), nausea (31 %), fatigue (26 %), pruritus (23 %), and mucositis (23 %). Of the 31 evaluable patients, one patient with triple-negative breast cancer had a confirmed partial response, and seven patients, including one patient with PCa, remained on the study for ≥ 8 months [108]. Neuropsychiatric adverse events, including reversible mood alterations, have been seen during BKM120 treatment, which suggests the potential ability of BKM120 to penetrate the blood-brain barrier and intervene in the PI3K/AKT/mTOR pathway in the brain [108]. This is supported by observations made in mice that PI3K deficiency increases anxiety and decreases neurotransmitter GABA and serotonin levels in the amygdala [109]. Therefore, further investigations monitoring neurologic and psychiatric symptoms in patients receiving BKM120 treatment are warranted. Other pan-PI3K inhibitors, including GDC-0941, PX-866 and SAR245408, have also shown good tolerability in patients with common adverse events including nausea, diarrhea, vomiting, fatigue, decreased appetite, and dysgeusia. These inhibitors show a partial response in PCa patients [110–112]. One limitation of pan-PI3K inhibitor use is the possibility of compensatory increases in AR, RAS and cell surface RTKs activities by stringent inhibition of the PI3K/AKT/mTOR pathway, which has been seen in

preclinical models [65, 87, 88]. Therefore, it will be beneficial to develop combination therapies with existing agents such as second-generation antiandrogens for complete blockade. Currently, BKM120 in combination with abiraterone acetate is in phase I clinical trials for CRPC (NCT01634061) and metastatic CRPC (NCT01741753) patients, and a phase II clinical trial combining BKM120 and enzalutamide for men with metastatic CRPC (NCT01385293) is also in progress.

In contrast to pan-PI3K inhibitors, isoform-specific PI3K inhibitors target a single p110 isoform, which provides greater specificity with the possibility of an improved safety profile. Given that each isoform of p110 may have a distinct role in tumorigenesis, as seen in preclinical models, patients may benefit from isoform-selective PI3K inhibitors as a precision medicine aimed at rational treatment choices tailored to individual patients based on genomic data [68, 113–116]. *PIK3CA*, which encodes p110α, exhibits genetic alterations in 6 % of primary and 16 % of metastatic PCa [10]. *PIK3CA* mutation H104R may further predict sensitivity to treatment with pan-PI3K inhibitors in multiple types of advanced cancer [117]. In preclinical studies, p110α isoform-specific PI3K inhibitors BYL719 and MLN1117 demonstrated significant antiproliferative and antitumor efficacy in cell lines and tumor xenografts harboring *PIK3CA* mutations with good tolerability [118, 119]. In a phase I clinical trial in patients with advanced solid tumors carrying *PIK3CA* mutations, BYL719 was well-tolerated at up to 400 mg/day, with adverse events including hyperglycemia (49 %), nausea (45 %), diarrhea (40 %), decreased appetite (38 %), vomiting (30 %), and fatigue (27 %). Of the 39 patients receiving the maximum tolerated dose on trial, partial responses were seen in 7 patients, none of whom were PCa patients, with 17 patients remaining on study for >24 weeks [120]. Despite these results, these inhibitors are not efficacious in PTEN-deficient tumor models; preclinical data demonstrate that PI3Kβ but not PI3Kα is the dominant form to drive PI3K signaling and prostate tumorigenesis in Pten-null mouse models [113, 114, 118, 119]. As such, inhibitors of p110β, such as GSK2636771, are in clinical development, and a phase I/II clinical trial is currently underway for patients with PTEN-deficient advanced solid tumors including PCa (NCT01458067). One expected limitation of isoform-selective inhibitor use is the potential development of a compensatory effect among isoforms particularly in response to tumor heterogeneity and therapeutic resistance, which may be overcome by the development of targeted combination strategies.

AKT Inhibitors

As a central regulator of the PI3K/AKT/mTOR pathway, AKT has long been considered an attractive target for blocking PI3K signaling as a therapeutic intervention. Although AKT-selective inhibitor development is difficult due to the homology between AKT and other kinases, a number of allosteric and ATP-competitive inhibitors have been developed to date. Preclinical studies demonstrated that the allosteric AKT inhibitor perifosine induced differentiation

and cell death in PTEN-defective PCa cells including PC-3 cells [121, 122]. However, the drug showed no clinical benefit, including no evidence of radiographic response or PSA >50 % decline, in two independent phase II clinical trials in men with CRPC [123, 124]. MK-2206, an allosteric AKT inhibitor, enhanced antitumor efficacy in combination with chemotherapeutic agent docetaxel in a PC-3 xenograft model, whereas either MK-2206 or docetaxel alone showed moderate antitumor activity, which suggests that rational combination therapies with MK-2206 may maximize the therapeutic benefit by AKT signaling blockade [125]. In a phase I first-in-human clinical trial in patients with advanced solid tumors, MK-2206 showed good tolerability up to 60 mg on alternate days, with drug-related toxicities including skin rash (51.5 %), nausea (36.4 %), pruritus (24.2 %), hyperglycemia (21.2 %), and diarrhea (21.2 %). Of the 33 patients receiving different doses, a patient with PTEN-loss pancreatic cancer treated at 60 mg every other day exhibited a partial response of 23 % shrinkage in tumor measurements [126]. A phase II clinical trial that permits concomitant treatment with bicalutamide and MK-2206 is currently underway for previously treated PCa patients (NCT01251861).

Recently, ATP-competitive AKT inhibitors, such as GDC-0068 and AZD5363, have shown antitumor activity in both prostate tumor cell lines and xenograft models. Moreover, AZD5363 in combination with the antiandrogen bicalutamide delayed CRPC progression in a LNCaP xenograft model [70]. In another recent study, AZD5363 showed proapoptotic and antiproliferative activity as monotherapy in enzalutamide-resistant PCa cell lines and significantly decreased the growth of enzalutamide-resistant tumor xenografts. Importantly, combination of AZD5363 and enzalutamide either at or after castration resulted in significant regression of tumors and suppression of PSA secretion [127]. In two phase I clinical trials of AZD5363 in patients with advanced solid tumors, conducted in Europe and Japan, AZD5363 administered at 480 mg twice a day was generally well tolerated with the most common adverse events including hyperglycemia, rash, and diarrhea. In addition, partial responses were seen in patients with mutations driving the PI3K pathway [128]. It remains to be seen whether these ATP-competitive AKT inhibitors will eventually show clinical benefit. Currently, GDC-0068 is in a phase I/II clinical trial in combination with abiraterone acetate in CRPC patients who received prior docetaxel chemotherapy (NCT01485861). Despite the promising results seen in preclinical studies and early clinical trials, further investigation of optimal dosing and resistance mechanisms in patients, such as therapeutic escape by activation of AKT-independent PI3K signaling [129], is still needed to ensure clinical efficacy.

mTOR Inhibitors

Given the frequent genetic alterations of the mTOR pathway in clinical samples as well as the significant positive correlation between mTOR hyperactivation and poor PCa patient outcomes, the mTOR pathway has been identified as an optimal

therapeutic target. The allosteric mTORC1 inhibitor rapamycin and its analogs (rapalogs), including everolimus, temsirolimus, and ridaforolimus, were developed as first-generation inhibitors of the mTOR pathway in the clinical arena. In preclinical studies, these early allosteric inhibitors of mTOR exhibited both reversal of the AKT-dependent PIN phenotype in the MPAKT mouse model and antitumor activity on Pten loss-induced prostate tumorigenesis and progression [41, 46]. Despite initial optimism about their potential efficacy, the clinical experience with rapalog inhibition of mTORC1 in CRPC is dismal, with few patients exhibiting PSA decline and radiographic response as reported in 2 independent clinical trials [130, 131]. Moreover, in a pharmacodynamics study of rapamycin given to men with intermediate to high-risk localized PCa before radical prostatectomy, inhibition of p-S6, a target of mTORC1, was demonstrated concurrently with no change in Ki-67 or caspase-3 cleavage in PCa samples [132]. There are several explanations for the contrast in rapalog efficacy between preclinical and clinical studies. On reason is that rapalogs do not inhibit mTORC2, which allows mTORC2 phosphorylation of its downstream substrate AKT at Ser473 for full activation in PCa cells [21, 133]. Moreover, rapalogs do no directly bind to and inhibit the catalytic core of mTOR kinase but rather act in complex with their intracellular receptor FKBP12 to bind to the FRB domain of mTOR to allosterically inhibit mTORC1 activity [134]. As a result, rapalogs only inhibit the phosphorylation of a limited subset of mTORC1 substrates, leading to insufficient negative regulation of mTORC2 by S6K, a downstream effector of mTORC1, which further activates AKT by elevated mTORC2 activity [135]. Furthermore, paradoxical activation of cell surface RTKs, such as HER2/3 and AR, by rapalog inhibition of mTOR may also contribute to treatment resistance [65, 87, 88]. In addition, several mTORC downstream targets, such as 4E-BP1 and associated eIF4E, may escape inhibition by these agents and promote prostate tumorigenesis by upregulation of a number of oncogenic pathway genes [96, 136]. These analyses provide a mechanistic rationale for poor clinical performance of rapalogs and also suggest requirements of combined mTORC1/2 inhibition for improved efficacy.

In line with various limitations seen in rapalogs, second-generation ATP-competitive mTOR inhibitors have been recently highlighted for their potential clinical prospect of directly targeting the ATP binding site to achieve complete inhibition of mTOR kinase activity. These new agents include MLN0128, Torin1/2, CC-223, OSI-027, AZD8055, AZD2014, DS-3078a, and Palomid 529 [137]. These dual mTORC1/2 inhibitors potently inhibit full mTOR kinase activity and show superior antitumor efficacy over allosteric mTOR inhibitors in preclinical studies. For example, MLN0128 treatment exhibited a more significant reduction in prostate tumor burden than everolimus in Pten-deficient mouse models, associated with decreased proliferation and a 10-fold elevation in apoptosis as compared with marginal changes achieved by rapalog treatment. MLN0128 but not rapamycin decreased the invasive potential of PC-3 cells and also downregulated expression of a signature of metastasis-promoting genes. Importantly, mechanistic exploration demonstrated that reprogrammed expression of these pro-invasive genes depends

on activation of the 4E-BP1-eIF4E axis, which was sensitive to inhibition by MLN0128 but not rapamycin [96]. This is supported by observations that dual mTORC1/2 inhibitors more completely inhibit 4E-BP1 phosphorylation by mTORC1 than rapamycin, suggesting that the improved efficacy of dual mTORC1/2 inhibitors may not be solely due to their enhanced inhibitory potency on mTOR but also their more complete inhibition of TORC1 downstream effectors, such as the 4E-BP1-eIF4E complex [138, 139]. As such, these pharmacologic applications also provide new insights into the role of the PI3K/AKT/mTOR pathway in PCa development and progression. Currently, several mTORC1/2 inhibitors, including MLN0128 (NCT01058707), CC-223 (NCT01177397), AZD2014 (NCT01026402), and DS-3078a (NCT01588678), are in early-stage clinical trials in patients with advanced solid tumors, including prostate tumors.

Dual PI3K-mTOR Inhibitors

Dual PI3K-mTOR inhibitors target the ATP site of all p110 isoforms of PI3K and both mTOR complexes, which are expected to more completely inhibit the PI3K/AKT/mTOR pathway and better overcome treatment resistance caused by potential relief from feedback pathways. In preclinical studies, dual PI3K-mTOR inhibitors BEZ235 and GDC-0980 potently inhibited signal transduction downstream of both PI3K and mTOR across a panel of cancer cell lines, with the greatest potency in breast, prostate, and lung cancer cells [107, 140]. Treatment of cancer cell lines with both agents resulted in G1 cell-cycle arrest. Moreover, GDC-0980 induced cell apoptosis in contrast to mTOR inhibitors, while BEZ235 promoted cell death through an apoptotic or autophagic route depending on the *PTEN* genotype [45, 107, 140]. These inhibitors further exhibited robust antitumor activity in mouse models with activated PI3K or Pten loss. BEZ235 significantly reduced tumor burden in a prostate-specific *Pten*-knockout mouse model of PCa as well as in a PTEN-null PC-3M xenograft model. GDC-0980 also effectively suppressed growth of PTEN-null PC-3 tumor xenografts with a dose proportional response [65, 107, 140]. Furthermore, combination BEZ235 and enzalutamide led to more profound tumor regressions in both a PTEN-deficient LNCaP xenograft model and conditional *Pten*-knockout mouse model of PCa than single agent use [65]. These studies demonstrate potential synergy by co-targeting the PI3K/mTOR and AR signaling pathways for better therapeutic outcomes in PCa. In phase I clinical trials of patients with advanced solid tumors, both BEZ235 and GDC-0980 were generally well tolerated with adverse events including fatigue, diarrhea, decreased appetite, nausea, rash, mucositis, hyperglycemia, vomiting, and constipation [141, 142]. However, in a pilot phase I/II clinical trial of abiraterone acetate plus BEZ235 in patients with metastatic CRPC, of the 6 evaluable patients on trial, three patients experienced dose-limiting toxicity when administered with BEZ235 at a starting dose of 200 mg twice a day, leading to termination of the trial, which suggests

appropriate dose adjustments coupled with treatment stages are necessary for future trials [143]. Currently, BEZ235 (NCT01634061) and GDC-0980 (NCT01485861) are both in phase I and II clinical trials respectively for patients with CRPC as monotherapy or in combination with abiraterone acetate.

Future Perspectives

The PI3K/AKT/mTOR pathway is undoubtedly emerging as a vital and viable therapeutic target in PCa, which is evidenced by its frequent genomic aberrations in clinical samples as well as encouraging results from preclinical studies and early clinical data on pathway inhibition. Given the complexity of the PI3K/AKT/mTOR pathway and its crosstalk with other signaling networks, which contribute to development of treatment resistance by awakening different feedback and compensatory mechanisms among signaling nodes, combination approaches co-targeting several pathways may be worthy of further investigation to enhance the therapeutic activities of the PI3K/AKT/mTOR pathway inhibitors. A couple of important considerations are suggested. First, to improve the understanding of the PI3K/AKT/mTOR pathway in the context of tumor cells and their microenvironment, we suggest designing a rational approach co-targeting both cancer cells and their supportive microenvironment. In our experience, blocking the epigenetic programing of MICs or the secretion of critical factors by cells in the tumor microenvironment to recruit neighboring bystander cells by employing potent inhibitors disrupting the activities of transcription factors can potentially revert the phenotype of the programmed cancer cells to normalcy [29, 32–34]. Second, to improve the delivery of targeted agents to cancer cells without enhancing toxicity to normal organs, we have developed a platform technology based on organic anion-transporting polypeptide-based drug delivery vehicles demonstrating specific in vivo anti-tumor effects without interfering with normal tissues [144–147].

Another important clinical need is predictive surrogate PI3K/AKT/mTOR pathway biomarkers to identify patients who will respond to these types of therapies. Despite a growing body of preclinical work recording the responses of PI3K/AKT/mTOR and associated pathways to different treatments, the clinical validation of the predictive nature of these pathways has achieved limited success, in part due to the impracticability of obtaining serial longitudinal tumor tissues, leading to insufficient sample analysis prior to treatment initiation or upon treatment with a new therapeutic agent, combined with the problem of tumor heterogeneity and rapid additional genetic alterations during tumor progression. To this end, circulating tumor cells (CTCs) and other circulating biomarkers, such as DNA, microRNA, long non-coding RNA, may provide alternative tools for the relatively noninvasive molecular analysis of a patient's tumor across different progressive stages before, during and after treatment courses. Indeed, the examination of distinct molecular phenotypes in CTCs shows their advantage of retaining dynamic

changes in oncogenic events, such as EMT, further correlated with disease progression, which is not commonly seen in conventional tumor biopsies [148]. On the other hand, patient-derived tumor xenograft models also provide an opportunity for preliminary assessment of treatment response. These approaches are believed to better address the increased need for precision medicine on an individualized basis in therapeutically manipulated PCa patients. With the introduction of next-generation antiandrogens including enzalutamide and abiraterone acetate, which is expected to shape the CRPC treatment landscape for the next decade, it is also anticipated that new phenotypes of PCa will emerge in some patients, selectively induced by highly potent AR-targeted agents, such as the expression of a treatment-related neuroendocrine PCa phenotype [149]. The mechanisms underlying the development of such phenotypes remain to be determined, but it is interesting to speculate on their relationship with the PI3K/AKT/mTOR pathway considering the central role this pathway plays in the emergence of castration resistance and developed resistance to hormonal or chemotherapeutic agents. A number of clinical trials are currently underway to evaluate the clinical efficacy of PI3K/AKT/mTOR pathway inhibitors as either individual or combined therapies to treat PCa, especially advanced stages of the disease. These clinical explorations and associated preclinical experiences will not only determine the therapeutic benefit of these agents, leading to the transition of novel PI3K/AKT/mTOR pathway inhibitors from the bench to the bedside of PCa patients, but also significantly enhance our current understanding of PCa and drive clinically oriented scientific breakthroughs in the near future, ultimately improving the quality of life and lifespan of patients with PCa.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics, 2012. CA Cancer J Clin 65:87–108
2. Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T et al (2012) Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin 62:220–241
3. Schrecengost R, Knudsen KE (2013) Molecular pathogenesis and progression of prostate cancer. Semin Oncol 40:244–258
4. Katsogiannou M, Ziouziou H, Karaki S, Andrieu C, Henry de Villeneuve M, Rocchi P (2015) The hallmarks of castration-resistant prostate cancers. Cancer Treat Rev
5. Thibault C, Massard C (2015) New therapies in metastatic castration resistant prostate cancer. Bulletin du cancer
6. Helsen C, Van den Broeck T, Voet A, Prekovic S, Van Poppel H, Joniau S et al (2014) Androgen receptor antagonists for prostate cancer therapy. Endocr Relat Cancer 21: T105–T118
7. Bayne CE, Williams SB, Cooperberg MR, Gleave ME, Graefen M, Montorsi F et al. (2015) Treatment of the primary tumor in metastatic prostate cancer: current concepts and future perspectives. Eur Urol
8. Courtney KD, Corcoran RB, Engelman JA (2010) The PI3K pathway as drug target in human cancer. J Clin Oncol Off J Am Soc Clin Oncol 28:1075–1083

9. Porta C, Paglino C, Mosca A (2014) Targeting PI3K/Akt/mTOR signaling in cancer. Front Oncol 4:64
10. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS et al (2010) Integrative genomic profiling of human prostate cancer. Cancer Cell 18:11–22
11. Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 7:606–619
12. Laplante M, Sabatini DM (2012) mTOR signaling in growth control and disease. Cell 149:274–293
13. Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. Cell 129:1261–1274
14. Bitting RL, Armstrong AJ (2013) Targeting the PI3K/Akt/mTOR pathway in castration-resistant prostate cancer. Endocr Relat Cancer 20:R83–R99
15. Edlind MP, Hsieh AC (2014) PI3K-AKT-mTOR signaling in prostate cancer progression and androgen deprivation therapy resistance. Asian J Androl 16:378–386
16. Carpenter CL, Auger KR, Chanudhuri M, Yoakim M, Schaffhausen B, Shoelson S et al (1993) Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85-kDa subunit. J Biol Chem 268:9478–9483
17. Rodriguez-Viciana P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ et al (1994) Phosphatidylinositol-3-OH kinase as a direct target of Ras. Nature 370:527–532
18. Skolnik EY, Margolis B, Mohammadi M, Lowenstein E, Fischer R, Drepps A et al (1991) Cloning of PI3Kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. Cell 65:83–90
19. Stephens L, Smrcka A, Cooke FT, Jackson TR, Sternweis PC, Hawkins PT (1994) A novel phosphoinositide 3 kinase activity in myeloid-derived cells is activated by G protein beta gamma subunits. Cell 77:83–93
20. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB et al (1997) Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Curr Biol CB 7:261–269
21. Laplante M, Sabatini DM (2009) mTOR signaling at a glance. J Cell Sci 122:3589–3594
22. Wiza C, Nascimento EB, Ouwens DM (2012) Role of PRAS40 in Akt and mTOR signaling in health and disease. Am J Physiol Endocrinol Metab 302:E1453–E1460
23. Johnson SC, Rabinovitch PS, Kaeberlein M (2013) mTOR is a key modulator of ageing and age-related disease. Nature 493:338–345
24. Yuan TL, Cantley LC (2008) PI3K pathway alterations in cancer: variations on a theme. Oncogene 27:5497–5510
25. McMenamin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR (1999) Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. Cancer Res 59:4291–4296
26. Yoshimoto M, Cunha IW, Coudry RA, Fonseca FP, Torres CH, Soares FA et al (2007) FISH analysis of 107 prostate cancers shows that PTEN genomic deletion is associated with poor clinical outcome. Br J Cancer 97:678–685
27. Lotan TL, Gurel B, Sutcliffe S, Esopi D, Liu W, Xu J et al (2011) PTEN protein loss by immunostaining: analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. Clin Cancer Res Off J Am Assoc Cancer Res 17:6563–6573
28. Dai B, Kong YY, Ye DW, Ma CG, Zhou X, Yao XD (2009) Activation of the mammalian target of rapamycin signalling pathway in prostate cancer and its association with patient clinicopathological characteristics. BJU Int 104:1009–1016
29. Chu GC, Zhau HE, Wang R, Rogatko A, Feng X, Zayzafoon M et al (2014) RANK- and c-Met-mediated signal network promotes prostate cancer metastatic colonization. Endocr Relat Cancer 21:311–326
30. Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61:759–767

31. Kim J, Roh M, Doubinskaia I, Algarroba GN, Eltoum IE, Abdulkadir SA (2012) A mouse model of heterogeneous, c-MYC-initiated prostate cancer with loss of Pten and p53. Oncogene 31:322–332
32. Chu GC, Chung LW (2014) RANK-mediated signaling network and cancer metastasis. Cancer Metastasis Rev 33:497–509
33. Li Q, Li Q, Nuccio J, Liu C, Duan P, Wang R, et al (2015) Metastasis initiating cells in primary prostate cancer tissues from transurethral resection of the prostate (TURP) predicts castration-resistant progression and survival of prostate cancer patients. Prostate 75 (12):1312–1321
34. Ziaee S, Chu GY, Huang J, Sieh S, Chung LW (2015) Prostate cancer metastasis: roles of recruitment and reprogramming, cell signal network and three-dimensional growth characteristics. Transl Androl Urol
35. Majumder PK, Yeh JJ, George DJ, Febbo PG, Kum J, Xue Q et al (2003) Prostate intraepithelial neoplasia induced by prostate restricted Akt activation: the MPAKT model. Proc Natl Acad Sci USA 100:7841–7846
36. Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP (1998) Pten is essential for embryonic development and tumour suppression. Nat Genet 19:348–355
37. Freeman DJ, Li AG, Wei G, Li HH, Kertesz N, Lesche R et al (2003) PTEN tumor suppressor regulates p53 protein levels and activity through phosphatase-dependent and -independent mechanisms. Cancer Cell 3:117–130
38. Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J et al (2003) Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell 4:209–221
39. Mirantes C, Eritja N, Dosil MA, Santacana M, Pallares J, Gatius S et al (2013) An inducible knockout mouse to model the cell-autonomous role of PTEN in initiating endometrial, prostate and thyroid neoplasias. Dis Models Mech 6:710–720
40. Luchman HA, Benediktsson H, Villemaire ML, Peterson AC, Jirik FR (2008) The pace of prostatic intraepithelial neoplasia development is determined by the timing of Pten tumor suppressor gene excision. PLoS ONE 3:e3940
41. Nardella C, Carracedo A, Alimonti A, Hobbs RM, Clohessy JG, Chen Z et al (2009) Differential requirement of mTOR in postmitotic tissues and tumorigenesis. Sci Signal 2:ra2
42. Nardella C, Chen Z, Salmena L, Carracedo A, Alimonti A, Egia A et al (2008) Aberrant Rheb-mediated mTORC1 activation and Pten haploinsufficiency are cooperative oncogenic events. Genes Dev 22:2172–2177
43. Guertin DA, Stevens DM, Saitoh M, Kinkel S, Crosby K, Sheen JH et al (2009) mTOR complex 2 is required for the development of prostate cancer induced by Pten loss in mice. Cancer Cell 15:148–159
44. Blando J, Portis M, Benavides F, Alexander A, Mills G, Dave B et al (2009) PTEN deficiency is fully penetrant for prostate adenocarcinoma in C57BL/6 mice via mTOR-dependent growth. Am J Pathol 174:1869–1879
45. Hong SW, Shin JS, Moon JH, Kim YS, Lee J, Choi EK et al (2014) NVP-BEZ235, a dual PI3K/mTOR inhibitor, induces cell death through alternate routes in prostate cancer cells depending on the PTEN genotype. Apoptosis Int J Programmed Cell Death 19:895–904
46. Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM et al (2004) mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. Nat Med 10:594–601
47. Zhang W, Haines BB, Efferson C, Zhu J, Ware C, Kunii K et al (2012) Evidence of mTOR activation by an AKT-independent mechanism provides support for the combined treatment of PTEN-deficient prostate tumors with mTOR and AKT inhibitors. Transl Oncol 5:422–429
48. Sutherland SI, Pe Benito R, Henshall SM, Horvath LG, Kench JG (2014) Expression of phosphorylated-mTOR during the development of prostate cancer. Prostate 74:1231–1239
49. Muller J, Ehlers A, Burkhardt L, Sirma H, Steuber T, Graefen M et al (2013) Loss of pSer2448-mTOR expression is linked to adverse prognosis and tumor progression in ERG-fusion-positive cancers. Int J Cancer 132:1333–1340

50. Balk SP (2002) Androgen receptor as a target in androgen-independent prostate cancer. Urology 60:132–138 (discussion 8–9)
51. Gelmann EP (2002) Molecular biology of the androgen receptor. J Clinical Oncol Off J Am Soc Clin Oncol 20:3001–3015
52. Chang CS, Kokontis J, Liao ST (1988) Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science 240:324–326
53. Lubahn DB, Joseph DR, Sar M, Tan J, Higgs HN, Larson RE et al (1988) The human androgen receptor: complementary deoxyribonucleic acid cloning, sequence analysis and gene expression in prostate. Mol Endocrinol 2:1265–1275
54. Koivisto P, Kononen J, Palmberg C, Tammela T, Hyytinen E, Isola J et al (1997) Androgen receptor gene amplification: a possible molecular mechanism for androgen deprivation therapy failure in prostate cancer. Cancer Res 57:314–319
55. Mostaghel EA, Marck BT, Plymate SR, Vessella RL, Balk S, Matsumoto AM et al (2011) Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants. Clin Cancer Res Off J Am Assoc Cancer Res 17:5913–5925
56. Watson PA, Chen YF, Balbas MD, Wongvipat J, Socci ND, Viale A et al (2010) Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. Proc Natlional Acad Sci USA 107:16759–16765
57. Baca SC, Prandi D, Lawrence MS, Mosquera JM, Romanel A, Drier Y et al (2013) Punctuated evolution of prostate cancer genomes. Cell 153:666–677
58. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R et al (2004) Molecular determinants of resistance to antiandrogen therapy. Nat Med 10:33–39
59. Jiao J, Wang S, Qiao R, Vivanco I, Watson PA, Sawyers CL et al (2007) Murine cell lines derived from Pten null prostate cancer show the critical role of PTEN in hormone refractory prostate cancer development. Cancer Res 67:6083–6091
60. Mulholland DJ, Tran LM, Li Y, Cai H, Morim A, Wang S et al (2011) Cell autonomous role of PTEN in regulating castration-resistant prostate cancer growth. Cancer Cell 19:792–804
61. Sato N, Sadar MD, Bruchovsky N, Saatcioglu F, Rennie PS, Sato S et al (1997) Androgenic induction of prostate-specific antigen gene is repressed by protein-protein interaction between the androgen receptor and AP-1/c-Jun in the human prostate cancer cell line LNCaP. J Biol Chem 272:17485–17494
62. Gitenay D, Baron VT (2009) Is EGR1 a potential target for prostate cancer therapy? Future Oncol 5:993–1003
63. Alberti C (2008) Genetic and microenvironmental implications in prostate cancer progression and metastasis. Eur Rev Med Pharmacol Sci 12:167–175
64. Taddei ML, Giannoni E, Comito G, Chiarugi P (2013) Microenvironment and tumor cell plasticity: an easy way out. Cancer Lett 341:80–96
65. Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S et al (2011) Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell 19:575–586
66. Mellinghoff IK, Vivanco I, Kwon A, Tran C, Wongvipat J, Sawyers CL (2004) HER2/neu kinase-dependent modulation of androgen receptor function through effects on DNA binding and stability. Cancer Cell 6:517–527
67. Hodgson MC, Shao LJ, Frolov A, Li R, Peterson LE, Ayala G et al (2011) Decreased expression and androgen regulation of the tumor suppressor gene INPP4B in prostate cancer. Cancer Res 71:572–582
68. Schwartz S, Wongvipat J, Trigwell CB, Hancox U, Carver BS, Rodrik-Outmezguine V et al (2015) Feedback suppression of PI3Kα signaling in PTEN-mutated tumors is relieved by selective inhibition of PI3Kβ. Cancer Cell 27:109–122
69. Sircar K, Yoshimoto M, Monzon FA, Koumakpayi IH, Katz RL, Khanna A et al (2009) PTEN genomic deletion is associated with p-Akt and AR signalling in poorer outcome, hormone refractory prostate cancer. J Pathol 218:505–513

70. Thomas C, Lamoureux F, Crafter C, Davies BR, Beraldi E, Fazli L et al (2013) Synergistic targeting of PI3K/AKT pathway and androgen receptor axis significantly delays castration-resistant prostate cancer progression in vivo. Mol Cancer Ther 12:2342–2355
71. Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ (2008) Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. Cancer Res 68:5469–5477
72. Hu R, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E et al (2009) Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res 69:16–22
73. Guo Z, Yang X, Sun F, Jiang R, Linn DE, Chen H et al (2009) A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. Cancer Res 69:2305–2313
74. Sun S, Sprenger CC, Vessella RL, Haugk K, Soriano K, Mostaghel EA et al (2010) Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. J Clin Invest 120:2715–2730
75. Mediwala SN, Sun H, Szafran AT, Hartig SM, Sonpavde G, Hayes TG et al (2013) The activity of the androgen receptor variant AR-V7 is regulated by FOXO1 in a PTEN-PI3K-AKT-dependent way. Prostate 73:267–277
76. Jiang F, Wang Z (2004) Identification and characterization of PLZF as a prostatic androgen-responsive gene. Prostate 59:426–435
77. Cao J, Zhu S, Zhou W, Li J, Liu C, Xuan H et al (2013) PLZF mediates the PTEN/AKT/FOXO3a signaling in suppression of prostate tumorigenesis. PLoS ONE 8: e77922
78. Fang Z, Zhang T, Dizeyi N, Chen S, Wang H, Swanson KD et al (2012) Androgen receptor enhances p27 degradation in prostate cancer cells through rapid and selective TORC2 activation. J Biol Chem 287:2090–2098
79. Jin Y, Qu S, Tesikova M, Wang L, Kristian A, Maelandsmo GM et al (2013) Molecular circuit involving KLK4 integrates androgen and mTOR signaling in prostate cancer. Proc Natl Acad Sci USA 110:E2572–E2581
80. Kobayashi T, Shimizu Y, Terada N, Yamasaki T, Nakamura E, Toda Y et al (2010) Regulation of androgen receptor transactivity and mTOR-S6 kinase pathway by Rheb in prostate cancer cell proliferation. Prostate 70:866–874
81. Schayowitz A, Sabnis G, Goloubeva O, Njar VC, Brodie AM (2010) Prolonging hormone sensitivity in prostate cancer xenografts through dual inhibition of AR and mTOR. Br J Cancer 103:1001–1007
82. Xu Y, Chen SY, Ross KN, Balk SP (2006) Androgens induce prostate cancer cell proliferation through mammalian target of rapamycin activation and post-transcriptional increases in cyclin D proteins. Cancer Res 66:7783–7792
83. Wang Q, Bailey CG, Ng C, Tiffen J, Thoeng A, Minhas V et al (2011) Androgen receptor and nutrient signaling pathways coordinate the demand for increased amino acid transport during prostate cancer progression. Cancer Res 71:7525–7536
84. Wu Y, Chhipa RR, Cheng J, Zhang H, Mohler JL, Ip C (2010) Androgen receptor-mTOR crosstalk is regulated by testosterone availability: implication for prostate cancer cell survival. Anticancer Res 30:3895–3901
85. De Velasco MA, Tanaka M, Yamamoto Y, Hatanaka Y, Koike H, Nishio K et al (2014) Androgen deprivation induces phenotypic plasticity and promotes resistance to molecular targeted therapy in a PTEN-deficient mouse model of prostate cancer. Carcinogenesis 35:2142–2153
86. Chandarlapaty S (2012) Negative feedback and adaptive resistance to the targeted therapy of cancer. Cancer Discov 2:311–319
87. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D et al (2006) mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 66:1500–1508

88. Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, Poulikakos PI, Scaltriti M, Moskatel E et al (2011) mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. Cancer Discov 1:248–259
89. Nandana S, Chung LW (2014) Prostate cancer progression and metastasis: potential regulatory pathways for therapeutic targeting. Am J Clin Exp Urol 2:92–101
90. Tsai JH, Yang J (2013) Epithelial-mesenchymal plasticity in carcinoma metastasis. Genes Dev 27:2192–2206
91. Gajula RP, Chettiar ST, Williams RD, Nugent K, Kato Y, Wang H et al (2015) Structure-function studies of the bHLH phosphorylation domain of TWIST1 in prostate cancer cells. Neoplasia 17:16–31
92. Wu JB, Shao C, Li X, Li Q, Hu P, Shi C et al (2014) Monoamine oxidase A mediates prostate tumorigenesis and cancer metastasis. J Clin Invest 124:2891–2908
93. Su B, Gao L, Baranowski C, Gillard B, Wang J, Ransom R et al (2014) A genome-wide RNAi screen identifies FOXO4 as a metastasis-suppressor through counteracting PI3K/AKT signal pathway in prostate cancer. PLoS ONE 9:e101411
94. Graham TR, Odero-Marah VA, Chung LW, Agrawal KC, Davis R, Abdel-Mageed AB (2009) PI3K/Akt-dependent transcriptional regulation and activation of BMP-2-Smad signaling by NF-κB in metastatic prostate cancer cells. Prostate 69:168–180
95. Mulholland DJ, Kobayashi N, Ruscetti M, Zhi A, Tran LM, Huang J et al (2012) Pten loss and RAS/MAPK activation cooperate to promote EMT and metastasis initiated from prostate cancer stem/progenitor cells. Cancer Res 72:1878–1889
96. Hsieh AC, Liu Y, Edlind MP, Ingolia NT, Janes MR, Sher A et al (2012) The translational landscape of mTOR signalling steers cancer initiation and metastasis. Nature 485:55–61
97. Gulhati P, Bowen KA, Liu J, Stevens PD, Rychahou PG, Chen M et al (2011) mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. Cancer Res 71:3246–3256
98. Liu L, Li F, Cardelli JA, Martin KA, Blenis J, Huang S (2006) Rapamycin inhibits cell motility by suppression of mTOR-mediated S6K1 and 4E-BP1 pathways. Oncogene 25:7029–7040
99. Chen X, Cheng H, Pan T, Liu Y, Su Y, Ren C et al (2014) mTOR regulate EMT through RhoA and Rac1 pathway in prostate cancer. Mol Carcinog
100. Siu MK, Tsai YC, Chang YS, Yin JJ, Suau F, Chen WY et al (2014) Transforming growth factor-β promotes prostate bone metastasis through induction of microRNA-96 and activation of the mTOR pathway. Oncogene
101. Li H, Tang DG (2011) Prostate cancer stem cells and their potential roles in metastasis. J Surg Oncol 103:558–562
102. Patrawala L, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S et al (2006) Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. Oncogene 25:1696–1708
103. Glinsky GV (2005) Death-from-cancer signatures and stem cell contribution to metastatic cancer. Cell Cycle 4:1171–1175
104. Glinsky GV, Berezovska O, Glinskii AB (2005) Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer. J Clin Invest 115:1503–1521
105. Dubrovska A, Kim S, Salamone RJ, Walker JR, Maira SM, Garcia-Echeverria C et al (2009) The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. Proc Natl Acad Sci USA 106:268–273
106. Marhold M, Tomasich E, El-Gazzar A, Heller G, Spittler A, Horvat R et al (2015) HIF1α Regulates mTOR signaling and viability of prostate cancer stem cells. Mol Cancer Res MCR 13:556–564
107. Maira SM, Pecchi S, Huang A, Burger M, Knapp M, Sterker D et al (2012) Identification and characterization of NVP-BKM120, an orally available pan-class I PI3-kinase inhibitor. Mol Cancer Ther 11:317–328

108. Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D et al (2012) Phase I, dose-escalation study of BKM120, an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors. J Clin Oncol Off J Am Soc Clin Oncol 30:282–290
109. Ackermann TF, Hortnagl H, Wolfer DP, Colacicco G, Sohr R, Lang F et al (2008) Phosphatidylinositide dependent kinase deficiency increases anxiety and decreases GABA and serotonin abundance in the amygdala. Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol 22:735–744
110. Shapiro GI, Rodon J, Bedell C, Kwak EL, Baselga J, Brana I et al (2014) Phase I safety, pharmacokinetic, and pharmacodynamic study of SAR245408 (XL147), an oral pan-class I PI3K inhibitor, in patients with advanced solid tumors. Clin Cancer Res Off J Am Assoc Cancer Res 20:233–245
111. Hong DS, Bowles DW, Falchook GS, Messersmith WA, George GC, O'Bryant CL et al (2012) A multicenter phase I trial of PX-866, an oral irreversible phosphatidylinositol 3-kinase inhibitor, in patients with advanced solid tumors. Clin Cancer Res Off J Am Assoc Cancer Res 18:4173–4182
112. Wagner AJ, Von Hoff DH, LoRusso PM, Tibes R, Mazina KE, Ware JA et al (2009) A first-in-human phase I study to evaluate the pan-PI3K inhibitor GDC-0941 administered QD or BID in patients with advanced solid tumors. In: 2009 ASCO annual meeting. South San Francisco 2009, p abstract 3501
113. Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH et al (2008) Essential roles of PI(3)K-p110beta in cell growth, metabolism and tumorigenesis. Nature 454:776–779
114. Wee S, Wiederschain D, Maira SM, Loo A, Miller C, deBeaumont R et al (2008) PTEN-deficient cancers depend on PIK3CB. Proc Natl Acad Sci USA 105:13057–13062
115. Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O et al (2006) A pharmacological map of the PI3-K family defines a role for p110α in insulin signaling. Cell 125:733–747
116. Galazi M, Rodriguez-Vida A, Ng T, Mason M, Chowdhury S (2014) Precision medicine for prostate cancer. Expert Rev Anticancer Ther 14:1305–1315
117. Janku F, Wheler JJ, Naing A, Falchook GS, Hong DS, Stepanek VM et al (2013) PIK3CA mutation H1047R is associated with response to PI3K/AKT/mTOR signaling pathway inhibitors in early-phase clinical trials. Cancer Res 73:276–284
118. Fritsch CM, Schnell C, Chatenay-Rivauday C, Guthy DA, De Pover A, Wartmann M et al (2012) NVP-BYL719, a novel PI3Kalpha selective inhibitor with all the characteristics required for clinical development as an anti-cancer agent. In: The 103rd annual meeting of the American Association for Cancer Research. Chicago, IL, p abstract 3748
119. Jessen K, Kessler L, Kucharski J, Guo X, Staunton J, Janes M et al (2011) A potent and selective PI3K inhibitor, INK1117, targets human cancers harboring oncogenic PIK3CA mutations. In: The AACR-NCI-EORTC international conference: molecular targets and cancer therapeutics. San Francisco, CA 2011. p abstract A171
120. Gonzalez-Angulo AM, Juric D, Argiles G, Schellens JH, Burris HA, Berlin J et al (2013) Safety, pharmacokinetics, and preliminary activity of the alpha-specific PI3K inhibitor BYL719: Results from the first-in-human study. In: 2013 ASCO annual meeting. New York, NY, p abstract 2531
121. Festuccia C, Gravina GL, Muzi P, Millimaggi D, Dolo V, Vicentini C et al (2008) Akt down-modulation induces apoptosis of human prostate cancer cells and synergizes with EGFR tyrosine kinase inhibitors. Prostate 68:965–974
122. Floryk D, Thompson TC (2008) Perifosine induces differentiation and cell death in prostate cancer cells. Cancer Lett 266:216–226
123. Chee KG, Longmate J, Quinn DI, Chatta G, Pinski J, Twardowski P et al (2007) The AKT inhibitor perifosine in biochemically recurrent prostate cancer: a phase II California/Pittsburgh cancer consortium trial. Clinical Genitourin Cancer 5:433–437
124. Posadas EM, Gulley J, Arlen PM, Trout A, Parnes HL, Wright J et al (2005) A phase II study of perifosine in androgen independent prostate cancer. Cancer Biol Ther 4:1133–1137

125. Hirai H, Sootome H, Nakatsuru Y, Miyama K, Taguchi S, Tsujioka K et al (2010) MK-2206, an allosteric Akt inhibitor, enhances antitumor efficacy by standard chemotherapeutic agents or molecular targeted drugs in vitro and in vivo. Mol Cancer Ther 9:1956–1967
126. Yap TA, Yan L, Patnaik A, Fearen I, Olmos D, Papadopoulos K et al (2011) First-in-man clinical trial of the oral pan-AKT inhibitor MK-2206 in patients with advanced solid tumors. J Clin Oncol Off J Am Soc Clin Oncol 29:4688–4695
127. Toren P, Kim S, Cordonnier T, Crafter C, Davies BR, Fazli L et al (2015) Combination AZD5363 with enzalutamide significantly delays enzalutamide-resistant prostate cancer in preclinical models. Eur Urol 67:986–990
128. Banerji U, Ranson M, Schellens JH, Esaki T, Dean E, Zivi A et al (2013) Results of two phase I multicenter trials of AZD5363, an inhibitor of AKT1, 2 and 3: biomarker and early clinical evaluation in Western and Japanese patients with advanced solid tumors. In: The 104th annual meeting of the American Association of Cancer Research. Washington, DC, p abstract LB-66
129. Vasudevan KM, Barbie DA, Davies MA, Rabinovsky R, McNear CJ, Kim JJ et al (2009) AKT-independent signaling downstream of oncogenic PIK3CA mutations in human cancer. Cancer Cell 16:21–32
130. Amato RJ, Jac J, Mohammad T, Saxena S (2008) Pilot study of rapamycin in patients with hormone-refractory prostate cancer. Clin Genitourin Cancer 6:97–102
131. Templeton A, Rothermundt C, Cathomas R, Baertschi D, Droege C, Gautschi O et al (2011) Everolimus as first-line therapy in non-rapidly progressive metastatic castration resistant prostate cancer (mCRPC): a multicenter phase II trial (SAKK 08/08). In: 2011 ASCO annual meeting. Chicago, IL, p abstract 4588
132. Armstrong AJ, Netto GJ, Rudek MA, Halabi S, Wood DP, Creel PA et al (2010) A pharmacodynamic study of rapamycin in men with intermediate- to high-risk localized prostate cancer. Clin Cancer Res Off J Am Assoc Cancer Res 16:3057–3066
133. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307:1098–1101
134. Sarbassov DD, Ali SM, Sabatini DM (2005) Growing roles for the mTOR pathway. Curr Opin Cell Biol 17:596–603
135. Dibble CC, Asara JM, Manning BD (2009) Characterization of Rictor phosphorylation sites reveals direct regulation of mTOR complex 2 by S6K1. Mol Cell Biol 29:5657–5670
136. Furic L, Rong L, Larsson O, Koumakpayi IH, Yoshida K, Brueschke A et al (2010) eIF4E phosphorylation promotes tumorigenesis and is associated with prostate cancer progression. Proc Natl Acad Sci USA 107:14134–14139
137. Benjamin D, Colombi M, Moroni C, Hall MN (2011) Rapamycin passes the torch: a new generation of mTOR inhibitors. Nat Rev Drug Discov 10:868–880
138. Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D et al (2009) Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol 7:e38
139. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y et al (2009) An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. J Biol Chem 284:8023–8032
140. Wallin JJ, Edgar KA, Guan J, Berry M, Prior WW, Lee L et al (2011) GDC-0980 is a novel class I PI3K/mTOR kinase inhibitor with robust activity in cancer models driven by the PI3K pathway. Mol Cancer Ther 10:2426–2436
141. Wagner AJ, Bendell JC, Dolly JA, Morgan JA, Ware JA, Fredrickson J et al (2011) A first-in-human phase I study to evaluate GDC-0980, an oral PI3K/mTOR inhibitor, administered QD in patients with advanced solid tumors. In: 2011 ASCO annual meeting. Chicago, IL, p abstract 3020
142. Burris H, Rodon J, Sharma S, Herbst RS, Tabernero J, Infante JR et al (2010) First-in-human phase I study of the oral PI3K inhibitor BEZ235 in patients (pts) with advanced solid tumors. In: 2010 ASCO annual meeting. Chicago, IL, p abstract 3005

143. Siegel AP, Bryce AH, Lin AM, Friedlander TW, Hsieh AC, Hang E et al (2014) Results of a multicenter phase I/II trial of abiraterone acetate plus BEZ235 in metastatic, castration-resistant prostate cancer (mCRPC). In: 2014 ASCO annual meeting. Chicago, IL, p abstract e16042
144. Wu J, Pan D, Chung LW (2013) Near-infrared fluoresence and nuclear imaging and targeting of prostate cancer. Transl Androl Urol 2:254–264
145. Wu JB, Lin TP, Gallagher JD, Kushal S, Chung LW, Zhau HE et al (2015) Monoamine oxidase a inhibitor-near-infrared dye conjugate reduces prostate tumor growth. J Am Chem Soc 137:2366–2374
146. Wu JB, Shao C, Li X, Shi C, Li Q, Hu P et al (2014) Near-infrared fluorescence imaging of cancer mediated by tumor hypoxia and HIF1alpha/OATPs signaling axis. Biomaterials 35:8175–8185
147. Yang X, Shi C, Tong R, Qian W, Zhau HE, Wang R et al (2010) Near IR heptamethine cyanine dye-mediated cancer imaging. Clin Cancer Res Off J Am Assoc Cancer Res 16:2833–2844
148. Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT et al (2013) Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science 339:580–584
149. Beltran H, Tagawa ST, Park K, MacDonald T, Milowsky MI, Mosquera JM et al (2012) Challenges in recognizing treatment-related neuroendocrine prostate cancer. J Clin Oncol Off J Am Soc Clin Oncol 30:e386–e389

Index

N. Dey et al. (eds.), *PI3K-mTOR in Cancer and Cancer Therapy*,
Cancer Drug Discovery and Development, DOI 10.1007/978-3-319-34211-5