

REVIEW

Adaptive stress signaling in targeted cancer therapy resistance

E Pazarentzos^{1,2} and TG Bivona^{1,2}

The identification of specific genetic alterations that drive the initiation and progression of cancer and the development of targeted drugs that act against these driver alterations has revolutionized the treatment of many human cancers. Although substantial progress has been achieved with the use of such targeted cancer therapies, resistance remains a major challenge that limits the overall clinical impact. Hence, despite progress, new strategies are needed to enhance response and eliminate resistance to targeted cancer therapies in order to achieve durable or curative responses in patients. To date, efforts to characterize mechanisms of resistance have primarily focused on molecular events that mediate primary or secondary resistance in patients. Less is known about the initial molecular response and adaptation that may occur in tumor cells early upon exposure to a targeted agent. Although understudied, emerging evidence indicates that the early adaptive changes by which tumor cells respond to the stress of a targeted therapy may be crucial for tumor cell survival during treatment and the development of resistance. Here we review recent data illuminating the molecular architecture underlying adaptive stress signaling in tumor cells. We highlight how leveraging this knowledge could catalyze novel strategies to minimize or eliminate targeted therapy resistance, thereby unleashing the full potential of targeted therapies to transform many cancers from lethal to chronic or curable conditions.

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INTRODUCTION

The identification of specific somatic oncogenic alterations that drive tumor growth^{1–4} and the development of targeted therapies that act against these oncogenic drivers have transformed the treatment of many cancer patients. Common oncogenic signaling components and pathways are depicted in Figure 1. Targeted therapies often elicit substantial initial tumor responses in patients with advanced-stage cancers in which conventional cytotoxic chemotherapy is largely inactive (Table 1). Paradigm-defining examples of this approach include the use of BRAF inhibitors in BRAF V600E mutant melanoma patients⁵ and the use of EGFR or anaplastic lymphoma kinase tyrosine kinase inhibitors in EGFR mutant and anaplastic lymphoma kinase fusion positive non-small cell lung cancer (NSCLC) patients,^{6–9} respectively. However, these targeted agents do not induce durable or curative responses in patients, with a few notable exceptions, because of therapy resistance that emerges after an initial response (secondary or acquired resistance^{10–12}). Furthermore, many patients whose tumors harbor a genetic driver of tumor growth fail to respond initially to the relevant targeted agent and exhibit primary (or innate) resistance.^{11,13,14}

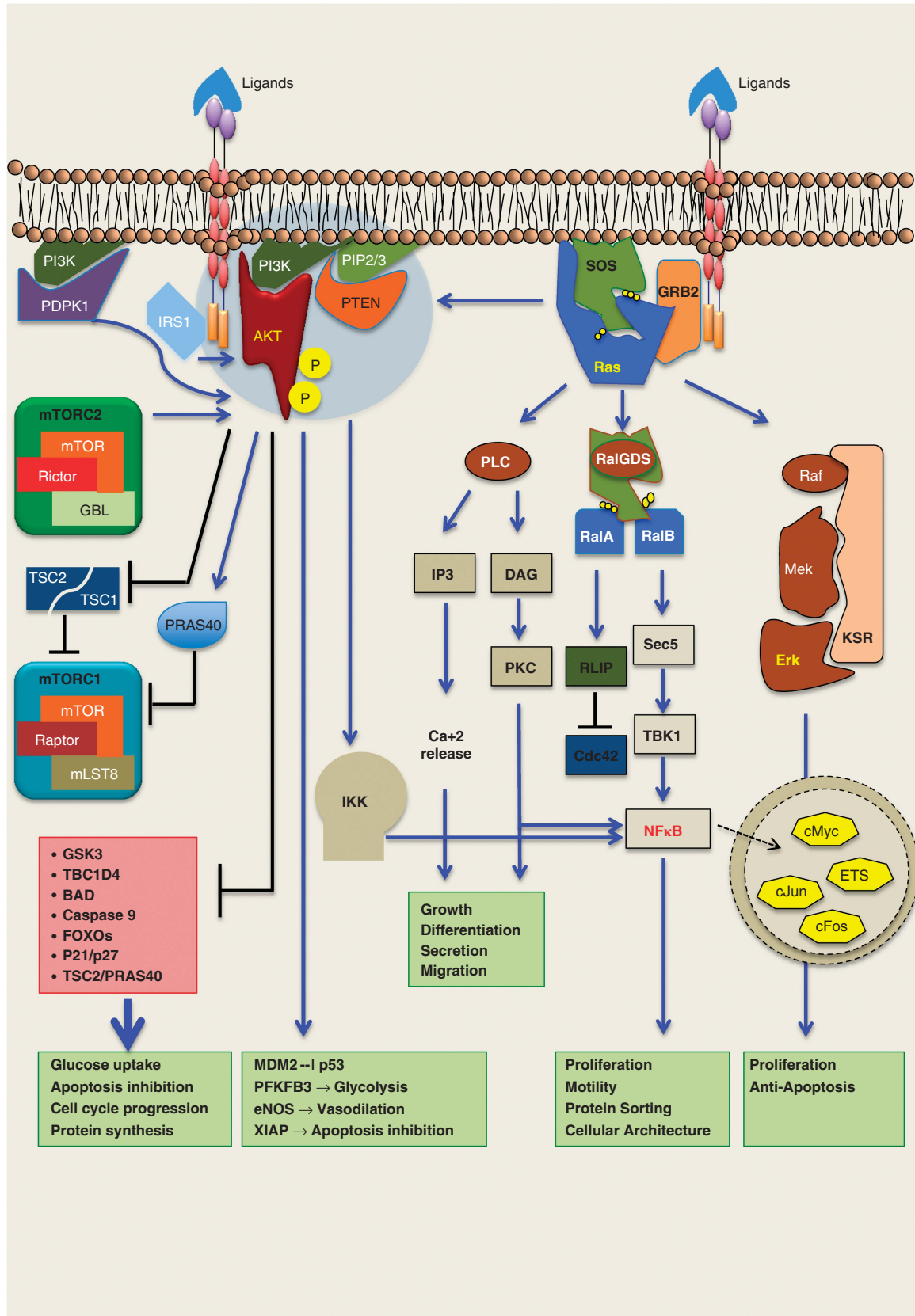
To date, efforts to understand the basis of therapy resistance have largely focused on uncovering mechanisms of this secondary or primary resistance many of which consist of genetic or epigenetic events that pre-exist before treatment (recently extensively reviewed elsewhere^{15,16}). These mechanisms are broadly categorized into three main groups: (a) on-target mutations that compromise binding or inhibition of the drug to the target (for example, the EGFR T790M resistance mutation in EGFR mutant lung cancer^{17–19}); (b) bypass signaling, in which the

target remains inhibited by the targeted drug but compensatory engagement (or disengagement) of other critical signaling components rescues the tumor cells from death and enables proliferation and survival (for example, upregulation of MET or AXL receptor kinase signaling in resistance to EGFR-targeted therapy^{20–23}); (c) phenotypic transformation from one histology or morphology to another (for example, lung adenocarcinoma-to-small cell lineage transformation or prostate adenocarcinoma to neuroendocrine small-cell morphology^{24–26}). In contrast, the signaling events that occur dynamically and immediately in tumor cells in response to initial therapy that may adaptively enable tumor-cell survival and drive resistance are less well understood. To unleash the full potential of targeted cancer therapy to transform cancers from lethal to chronic or curable conditions, it is essential to understand the biological mechanisms by which tumor cells adapt and survive the stress of initial therapy that enable initial or eventual escape from death. Recent studies have begun to fill this critical knowledge gap, and herein we categorize these adaptive signaling events as ‘adaptive stress signaling’. We review emerging findings that unveil how tumor cells respond dynamically and adapt to the stress of targeted therapy, emphasizing the promise this new knowledge holds for enabling truly transformative advances in cancer patient survival. Critically, we focus here not on mechanisms of signaling crosstalk or primary or secondary resistance to targeted therapy but rather more specifically on summarizing the biological events that have been shown to constitute an adaptive stress response in tumor cells treated with targeted therapy.

¹Division of Hematology-Oncology, Department of Medicine, San Francisco, CA, USA and ²Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA, USA. Correspondence: Dr TG Bivona, Division of Hematology-Oncology, Department of Medicine, University of California San Francisco, 600 16th Street Genentech Hall, N212D, San Francisco, CA 94158, USA.

E-mail: Trever.Bivona@ucsf.edu

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ADAPTIVE STRESS RESPONSE TO TARGETED INHIBITION OF ONCOGENIC RECEPTORS

In this section, we discuss important findings that demonstrate how tumor cells with a particular oncogenic receptor can rewire intracellular signaling pathways as a stress response to survive initial targeted therapy. Oncogenic alterations in EGFR drive the growth of several tumor types, most notably NSCLC^{27–29} and glioblastoma multiforme³⁰ (GBM). Inhibition of oncogenic EGFR can elicit compensatory signaling events that contribute to tumor cell survival. Inhibition of oncogenic EGFR (EGFRvIII) in GBM cells

resulted in derepression of the platelet-derived growth factor receptor, beta polypeptide (PDGFRB). This EGFR inhibitor induced upregulation of PDGFRB occurred via relief of mammalian target of rapamycin complex 1 (mTORC1) and ERK mediated suppression of PDGFRB expression. PDGFRB, when upregulated, provided survival signaling that limited the antitumor effects of EGFR oncogene inhibition in GBM cells.³¹ The data raise the possibility that co-inhibition of EGFR and PDGFR using potent and selective targeted agents that cross the blood–brain barrier could enhance response in GBM patients.

Table 1. Targeted genetic events, targeted agents in clinical use and adaptive response mechanisms identified in tumor cells

Oncogenic driver	Alteration type	Targeted therapy	Cancer type	Adaptive response
EGFR	Mutation or Amplification	Erlotinib, afatinib, gefitinib, lapatinib, neratinib, rociletinib, dacomitinib, sapitinib, cetuximab, panitumumab,	NSCLC, colorectal, glioblastoma	PDGFR, STAT3, NFκB
ALK	Translocation	Crizotinib, ceritinib	NSCLC	Non-receptor kinase, STAT3
ERBB2	Mutation or Amplification	Lapatinib, sapitinib, dacomitinib, neratinib, afatinib, trastuzumab, pertuzumab	Breast, gastric	ERBB3, NFκB, mTORC1/2, AKT
ERBB3	Mutation	Anti-ERBB, MM-121, AV-203	Colorectal, gastric	Unknown
ERBB4	Mutation or translocation	Dacomitinib	Melanoma	Unknown
ABL	Translocation	Imatinib, ponatinib, nilotinib, dasatinib, bosutinib	CLL	Unknown
KIT	Mutation	Sunitinib, imatinib, dasatinib, nilotinib	Melanoma, AML, GIST	Unknown
MET	Mutation or amplification	Crizotinib, tivantinib, foretinib, volitinib, MK-8083, INC280, cabozantinib, MGCD-265	NSCLC, gastric	Unknown
FGFR1	Amplification	BGJ398, AZD4547, dovitinib, E-3810, PD173074, lucitanib, ponatinib, BAY1163877	NSCLC (sq) breast	Unknown
FGFR2	Mutation or amplification	BGJ398, AZD4547, dovitinib, ponatinib, BAY1163877	Gastric, breast, endometrial	Unknown
FGFR3	Mutation or translocation	BGJ398, AZD4547, dovitinib, ponatinib, BAY1163877	NSCLC, bladder	Unknown
FGFR4	Mutation or amplification	Ponatinib	Rhabdomyosarcoma	Unknown
NTRK1	Translocation	LOXO-101, ARRY-470, MGCD-516, RXDX-101, crizotinib, TSR-011	NSCLC, GBM	Unknown
BRAF	Mutation	Vemurafenib, dabrafenib, encorafenib, sorafenib	colorectal, thyroid	EGFR, IGF1R, AXL, PDGFRB, DDR1/2, KDR, metabolic, phenotype switching
JAK2	Mutation	Ruxolitinib, fedratinib, AZD1480, pacritinib, momelotinib, baricitinib,	Myelofibrosis	Unknown
FLT3	Mutation	Lestaurtinib, quizartinib, sunitinib, sorafenib	AML	Unknown
PIK3CA	Mutation or amplification	GDC-941, GDC-0890, BKM-120, BYL719, XL147, XL765, dactolisib	Colorectal, GBM	IRS-1, mTORC2, EGFR, IGF1R, AXL, ERBB2, NFκB, STAT3/6
AKT1/2/3	Mutation, amplification, translocation	MK-2206, AZD5363, GDC-0068, perifosine, GSK690693	NSCLC, breast	ERBB3, IGF1R, IR, ERK, mTORC
VEGFR2	Amplification or mutation	Bevacuzimab, sorafenib, sunitinib, cabozantinib, pazopanib, cetiranib, dovitinib, linifanib, MGCD-265, ramucirumab	RCC, colorectal, HCC, gastric, thyroid	Unknown
ROS1/RET	Mutation or translocation	Cabozantinib, crizotinib, sorafenib, regorafenib,	NSCLC, thyroid	Unknown
AR	Amplification	Enzalutamide, bicalutamide	Prostate	PI3K, GR, β-catenin

Abbreviations: ALK, anaplastic lymphoma kinase; AML, acute lymphoblastic leukemia; CLL, chronic lymphoblastic leukemia; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; GBM, glioblastoma; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma; mTORC, mammalian target of rapamycin complex; NSCLC, non-small cell lung cancer; PDGFR, platelet-derived growth factor receptor; RCC, renal cell carcinoma. Shown are the major genetic alterations that drive the growth of many human cancers as well as the cognate targeted therapies approved or in clinical development and, where known, specific stress signaling adaptations that occur in response to targeted therapy.

Figure 1. Oncogenic signaling in tumor cells. Shown are major signaling pathways involved in the initiation and progression of many tumors. Pathway crosstalk can occur at multiple levels from signaling emanating from the plasma membrane to the mitochondrial and nuclear events. Thus, there is significant potential for stress response signaling such that the inhibition of one pathway results in the engagement of a distinct pathway that maintains tumor cell homeostasis and promotes escape from therapy. This profound robustness is a critical feature of the evolution of tumors both in the absence and the presence of therapy, consistently enhancing tumor survival in response to various stresses. AKT, v-akt murine thymoma viral oncogene homolog; Erk, extracellular signal related kinase; GRB2, growth factor receptor-bound protein; IKKα/β, inhibitor of nuclear factor kappa-B kinase subunit alpha/beta; IRS-1, insulin receptor substrate; Mek, mitogen-activated protein kinase kinase; mTORC1/2, mammalian target of rapamycin; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K, phosphoinositide 3-kinase; PDPK1, 3-phosphoinositide-dependent protein kinase 1; PLC, phospholipase C; PTEN, phosphatase and tensin homolog; RalGDS, ral guanine nucleotide dissociation stimulator; Raf, Raf proto-oncogene serine/threonine kinase; SOS, son of sevenless homolog; TSC1/2, tuberous sclerosis.

More recently, inhibition of oncogenic EGFR was shown to increase STAT3 signaling and thereby rescue tumor cells from death upon EGFR targeted therapy in NSCLC cells.³² These effects occurred via inhibition of MEK downstream of EGFR that led to activation of STAT3 and downstream IL6 signaling to promote cell survival and eventual (acquired) resistance.^{32,33} This signaling axis may be a more general stress response, as some tumor cells with oncogenic forms of the anaplastic lymphoma kinase or MET exhibited similar upregulation of STAT3 upon oncogene inhibition.^{32,34}

Additional work by our group has revealed another layer of complexity in the adaptive response to EGFR inhibitor treatment. We found that EGFR targeted therapy elicits immediate activation of NFκB survival signaling via an NFκB activating biochemical complex in NSCLC cells that increased IL6-STAT signaling (Blakely, Pazarentzos & Bivona, manuscript under revision). This NFκB driven stress response enforced a cell survival circuit that was required for the development of acquired EGFR inhibitor resistance. NFκB activation occurred as a consequence of oncogene inhibitor induced ubiquitination of TRAF2, which in turn activates RIP1. Subsequently, RIP1 activates NEMO which provides the scaffolding for the activation of IKKβ and ultimately the phosphorylation and degradation of IκBα. Within minutes of EGFR inactivation, the RelA subunit of NFκB translocates to the nucleus and initiates an extensive transcriptional survival program. In summary, the oncogene-driven cell rewires the signaling to compensate immediately for inhibition of the oncogene and assembles a TRAF2-RIP1-IKK-EGFR complex to activate anti-apoptotic and prosurvival NFκB targets. These studies provide new insight into the resiliency in tumor cells with oncogenic EGFR and reveal an interesting role for dynamic modulation of ubiquitination as a molecular switch in this context. Together these findings indicate that targeting NFκB or IL6-STAT signaling in combination with oncogenic EGFR initially may deprive tumor cells the opportunity to adapt and survive primary therapy and thereby eliminate the eventual emergence of drug resistance.

The lessons learned through the study of oncogenic EGFR also extend to other oncogenic ERBB family members. Overexpression of ERBB2 by genomic amplification occurs in ~15% of breast cancers and ERBB2 targeted therapies such as lapatinib are approved for use in patients.³⁵ However, patients almost inevitably develop resistance during therapy. Recently mechanisms of adaptive stress response have been identified that contribute to the development of acquired resistance. For example, inhibition of amplified ERBB2 can lead to transcription upregulation of ERBB3.³⁶ ERBB3 upregulation is caused by ERBB2 inhibitor treatment, which leads to derepression of ERBB3 expression that occurs via PI3K-AKT signaling operating downstream of ERBB2 in breast cancer cells. ERBB3 upregulation is dependent on FOXO3A that is activated upon PI3K-AKT signaling inhibition and leads to compensatory activation of ERBB3 signaling and tumor cell survival.^{37,38} Thus, dual inhibition of ERBB2 and ERBB3 may subvert this adaptive survival circuit and enhance response in patients. Another example of such adaptive stress response mechanisms is hyperactivation of NFκB.^{39,40} Treatment of ERBB2 positive tumors led to activation of NFκB through NIBP upregulation. The findings were also validated in ERBB2 expressing esophageal cancers.⁴⁰ Interestingly, lapatinib-induced NFκB activation created a dependency on NFκB signaling that was exploited therapeutically using proteasome inhibitors, which were effective against these tumor cells.⁴¹

Beyond oncogenic receptor kinases, inhibition of hormone receptors that drive the growth of endocrine cancers can lead to adaptive signaling events that promote tumor cell survival and thereby limit targeted therapy efficacy. The proliferation and progression of prostate cells to a malignant state is mainly driven by the stimulatory effects of the androgen receptor (AR). Recent evidence revealed that inhibition of the AR with small molecule

AR-targeted agents led to rapid activation of PI3K-AKT signaling in prostate adenocarcinomas.⁴² This effect of AR inhibition occurred via downregulation of the AKT phosphatase PHLPP whose expression is controlled, in part, by AR. This dynamic activation of PI3K-AKT limited response to AR blockade and provided rationale for co-targeting AR and PI3K-AKT signaling in prostate adenocarcinoma patients. Recently, another mechanism that seems to appear acutely following AR inhibition is the immediate upregulation of the glucocorticoid receptor, which in turn leads to a transcriptional program that promotes resistance to anti-androgen therapy.⁴³ Activation of WNT-β catenin signaling is another mechanism of adaptive response to androgen deprivation therapy that promotes reactivation of AR output.⁴⁴ This study revealed that prostate-specific antigen is re-expressed via transcriptional upregulation by β-catenin which binds to prostate-specific antigen promoter.

Nearly 75% of breast cancers are positive for the estrogen receptor (ER) and tamoxifen has revolutionized the treatment of ER-positive tumors by antagonizing ligand binding. In ER-positive breast cancer, activation of the PI3K-AKT-mTOR pathway appears to be an important mechanism of resistance that occurs early after tamoxifen therapy.⁴⁵ In addition, IGF1R, which activates PI3K-AKT-mTOR signaling, has been shown to provide an immediate escape mechanism from tamoxifen therapy via the upregulation of an IGF1R transcription program and the ultimate development of resistance in ER-positive breast cancers.⁴⁶ Altogether, these emerging findings provide further impetus to explore the dynamic response to the stress induced by both receptor and non-receptor-targeted therapies across a broad range of tumor types.

ADAPTIVE STRESS RESPONSE TO TARGETED INHIBITION OF CANCER-DRIVING NON-RECEPTOR KINASES

Here we review important findings that reveal how tumor cells with a particular cancer-promoting non-receptor kinase can rewire intracellular signaling pathways as a stress response to survive initial targeted therapy. The RAS-RAF-MEK-ERK signaling pathway is critical for the initiation and progression of a wide spectrum of human cancers.^{2,3} This pathway is activated in tumor cells either through somatic alterations in pathway components, most commonly RAS and RAF, or via oncogenic activation of an upstream receptor kinase such as EGFR. Although direct inhibitors of RAS remain under investigation, to date no direct inhibitor of RAS has been clinically effective.⁴⁷⁻⁴⁹ However, RAF and MEK inhibitors⁵⁰ are approved for use in patients with advanced-stage BRAF^{V600E} mutant melanoma although therapy resistance remains a major challenge that limits the overall clinical impact of these agents.

Rapid activation of upstream receptor kinases has recently been shown to limit response to targeted inhibition of BRAF or MEK in different tumor types. Primary resistance to BRAF inhibition in BRAF^{V600E} colon cancer cells has been attributed to compensatory activation of EGFR.⁵¹ EGFR was activated as a consequence of RAF inhibitor induced suppression of ERK and CDC25C, a phosphatase that negatively regulates EGFR.⁵² Importantly, EGFR inhibition together with BRAF inhibitor treatment counteracted this stress response and enhanced efficacy, providing rationale for combination therapy trials in colon cancer patients. Recent studies have extended this paradigm, indicating that BRAF or MEK inhibition leads to activation of multiple receptor kinases including EGFR in melanoma.⁵³ In this context, increased receptor kinase expression and signaling was a consequence of suppression of the transcription factor sex determining region Y-box10 following BRAF- or MEK-targeted therapy.⁵³ Interestingly, the supraphysiologic activation of MEK signaling that occurs as a consequence of this receptor kinase activation was detrimental to tumor cell growth. However, this signaling adaptation impaired tumor cell proliferation but not survival. Therefore, this study revealed that

this adaptive compensatory activation may provide a context-specific survival advantage in melanoma, in that a subpopulation of tumor cells may survive RAF-MEK-targeted therapy at a fitness cost that nevertheless enables the emergence of a drug-resistant tumor over time.

Parallel findings have been observed in various cancer cell lines treated with a MEK inhibitor. Indeed, the inhibition of MEK can induce rapid dephosphorylation of EGFR and ERBB2 on inhibitory sites phosphorylated by ERK.^{54,55} Increased EGFR and ERBB2 activation, in turn, promotes ERBB3 upregulation and signaling that buffers the cells against the effects of MEK inhibition. Similarly, MEK inhibition has been shown to induce activation of multiple receptor kinases beyond those in the ERBB family by a variety of adaptive, rapid transcriptional and posttranslational mechanisms in breast cancer and other tumor types. These receptor kinases induced upon MEK inhibition include IGF1R,⁵⁶ AXL,⁵⁷ DDR1/2,⁵⁷ PDGFRB,^{53,57} and KDR. Interestingly, the molecular basis of this stress response included MYC-driven upregulation of these receptor kinases in triple negative breast cancer, colorectal and NSCLC cells.⁵⁸ Combined MEK plus receptor kinase inhibition could abolish compensatory survival signaling that occurred through both ERK and AKT,⁵⁷⁻⁵⁹ providing rationale for potential combination therapies in the relevant patient subsets.

Recent work has highlighted a role for energy metabolism in the adaptive response to RAF-MEK-targeted therapy in some tumors. The primary molecular circuit to produce ATP in normal cells resides at mitochondria where pyruvate enters and is converted to ATP. The process is called oxidative phosphorylation (OXPHOS) and utilizes glucose and oxygen.⁶⁰ Otto Warburg in 1956 described an alternative process by which oxygen is not used even if present and glucose is instead converted to lactate, generating ATP albeit inefficiently.⁶¹ The exact reason that cancer cell switch to this inefficient process of ATP generation is currently not completely understood and is not likely to be due to loss of OXPHOS.⁶² However, recent reports demonstrate that Warburg glycolysis is required to overcome oncogene-induced senescence and this has been clearly shown in cells with mutant KRAS or mutant BRAF.⁶³ Recently RAF inhibition was shown to promote a rapid switch from Warburg glycolysis to OXPHOS in melanoma.⁶⁴ This adaptive response involved metabolic reprogramming that enabled mutant BRAF melanoma cells to survive anti-RAF therapy. Patients treated with vemurafenib showed increase ATP production and expression of an OXPHOS genetic program. This metabolic reprogramming was mediated through EKR1/2 inhibition, which promoted upregulation of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α). Specifically in melanoma cells, PGC1α upregulation coincided with stabilization and increase in the expression of the microphthalmia-associated transcription factor (MITF). The upregulation of PGC1α and MITF resulted in decrease in the glycolytic flux with concurrent enhancement of OXPHOS. The data suggest that ERK1/2 inhibition either by RAF or MEK inhibitors forced the cells to induce an adaptive mechanism that at first glance seems unfavorable for cancer cells. However, melanoma cells shift their dependency from glycolysis to OXPHOS to dynamically enable tumor initiation by an oncogene or adapt to inhibition of that oncogene. Another adaptive response mechanism involving a metabolic switch to OXPHOS was utilized by BRAF mutant melanoma cells, namely the upregulation of lysine-specific demethylase 5B (JARID1B).⁶⁵ JARID1B upregulation occurred within hours of RAF inhibition or chemotherapy, demonstrating that cells can quickly engage adaptive mechanisms to resist the lethal consequences of oncogene inhibition or DNA damage. PGC1α-mediated MITF upregulation and overexpression of JARID1B seem to have the same consequences to the cells, indicating that metabolic adaptation is a mechanism of adaptive response that is possibly utilized by other tumor types where MITF is not involved. Interestingly in EGFR mutant NSCLC

cells, increased histone demethylase function, specifically that of JARID1A, has been observed in a subpopulation of cells that are treated with gefitinib.⁶⁵ Although in that study, the effects of JARID1B were attributed to IGF1R and not OXPHOS, it is possible that that energy regulation is a more general means by which tumor cells of distinct lineages adapt to therapy and survive. Inhibitors of OXPHOS in combination with RAF inhibitors, or perhaps other targeted therapies, may be a useful and successful strategy to decrease tumor burden and suppress drug resistance.

Beyond the RAS-RAF-MEK-ERK pathway, resiliency in the face of targeted inhibition of PI3K-AKT-mTOR signaling has also been observed in some tumor cells. Indeed, pharmacologic suppression of mTORC1 with rapamycin relieves inhibition of the upstream adaptor protein IRS-1,⁶⁶ resulting in mTORC2-mediated AKT activation in breast cancer and multiple myeloma cells.^{67,68} This rapid activation of the pathway occurs because mTORC1 inhibition decreases phosphorylation of the downstream target S6K which, when active, phosphorylates and inhibits IRS-1. Dual inhibition of mTORC1 and mTORC2 can overcome this adaptive signaling circuit. However, this dual suppression of mTORC1-2 then led to upstream activation of receptor kinases via their transcriptional upregulation.⁶⁹⁻⁷⁴ Thus, the combined inhibition of PI3K-AKT-mTOR and receptor kinase signaling may be effective clinically if a sufficient therapeutic window can be achieved in patients.

Additional adaptive responses to inhibition of PI3K were recently described and, interestingly, involved regulation of structural components of tumors, including the extracellular matrix and basement membrane.⁷² In studies in ovarian and breast cancer cells, adaptive protection from the suppressive effects of PI3K-AKT-mTOR inhibition involved upregulation of several receptor kinases including EGFR, ERBB2, IGF1R, AXL and alternative signaling pathways such as JAK-STAT3/6 signaling.⁷² Interestingly Bcl-2 was also found to be upregulated in this context. As Bcl-2 is an established NFκB target gene, these findings suggest another potential role for NFκB in the adaptive remodeling of the extracellular matrix during adaptation to therapy. The combination of PI3K-AKT-mTOR targeted drugs together with BH3 mimetic agents targeting anti-apoptotic proteins such as Bcl-2 may overcome this adaptive response and enhance antitumor efficacy.

Hence, intra and interpathway crosstalk in the RAS-RAF-MEK-ERK and PI3K-AKT-mTOR signaling pathways enables dynamic rewiring and adaptation to targeted therapy in cancer cells. Leveraging this knowledge clinically to enhance therapy efficacy will require not only knowledge of the tumor-type selective and context-specific signaling network features that enable tumor cell escape from treatment but also the appropriate use of combinatorial drug strategies that are safe and well tolerated in patients.

ADAPTIVE STRESS RESPONSE TO TARGETED THERAPY BY PHENOTYPE SWITCHING

Here we review intriguing recent evidence linking drug response in cancer to cellular phenotype switching. Phenotype switching can be observed after the inhibition of receptor or non-receptor kinases as well as in response to chemotherapy and represents an additional manifestation of adaptive stress signaling, and one that may be functionally related to the adaptive signaling events discussed above in oncogene-driven tumor cells. Lineage-specific factors that regulate cellular phenotype and oncogenesis have been identified in certain cancers, including melanoma. The microphthalmia-associated transcription factor is one such factor that has been shown to contribute to the pathogenesis of melanoma.^{75,76} Interestingly, suppression of MITF has been shown to not only decrease cell cycle progression but also simultaneously promote a stem cell-like invasive phenotype characterized by loss of differentiation and tyrosinase expression.⁷⁷ These findings suggested that agents that decrease MITF levels may have dual,

opposing effects on tumor growth and that activation of MITF may be beneficial in some contexts. In a recent study, treatment with the chemotherapy agent methotrexate adaptively and rapidly induced MITF expression to suppress invasiveness and promote differentiation of melanoma cells regardless of the genetic status of BRAF or other common somatic genetic alterations present in melanoma. These effects were accompanied by increased tyrosinase expression and consequent tumor cell specific hypersensitivity to a tyrosinase-processed antifolate drug.⁷⁷ Together the data indicate understanding context-specific modulation of therapy-induced, adaptive signaling changes that mediate cellular phenotype switching can unveil therapy strategies to enhance responses and limit systemic toxicity in patients.

Beyond melanoma, phenotype switching has been associated with drug resistance in several epithelial cancers. Indeed, the epithelial–mesenchymal transition and transformation to small cell histology has been associated with resistance to targeted therapies against EGFR in NSCLC, AR in prostate adenocarcinoma, and targeted agents in different tumor types. In the case of epithelial–mesenchymal transition, the drug-resistant cells are often hyper-invasive and acquire other features associated with increased metastasis such as stem-like molecular profiles. The extent to which this phenotype switching is triggered early and dynamically in response to targeted therapy and the underlying molecular basis remains to be deeply explored. Interestingly, several studies have implicated increased expression of the receptor kinase AXL in phenotype switching in NSCLC, breast cancer and melanoma. This finding raises the possibility the stress response to targeted therapy may involve a conserved molecular pathway involving AXL and the

acquisition of stem-like molecular properties that culminates in a cellular phenotype switch coupling metastasis and drug resistance. Future studies are needed to shed light in this important area, as the knowledge gained could provide tumor-type selective and context-specific strategies to subvert both drug resistance and metastatic tumor progression simultaneously.

CONCLUSIONS AND FUTURE PERSPECTIVES

As targeted cancer therapy gains a broader foothold in the clinic, it will become increasingly important to define the immediate molecular and cellular responses by which tumor cells adapt and buffer against these potentially lethal insults. The genetic and epigenetic evolution of tumor cells endows them with substantial resiliency, confounding even our most potent targeted therapy attacks. To eliminate the presence of innate and the emergence of acquired resistance to treatment, therapies that pre-empt the stress response enabling tumor cell survival early during therapy are needed (Figure 2). A systematic approach, perhaps through the use of coupled genetic and proteomic profiling together with functional genomics screens, to define these molecular escape routes is critical for progress. In addition, access to tumor specimens from patients not only before treatment and after the development of resistance but also early during therapy is necessary to define and validate the most clinically relevant molecular and cellular adaptations for subsequent therapeutic targeting.

Although this review focuses on the adaptive stress responses induced by targeted therapeutics, it is worth noting that another interesting area of adaptive stress signaling that has been

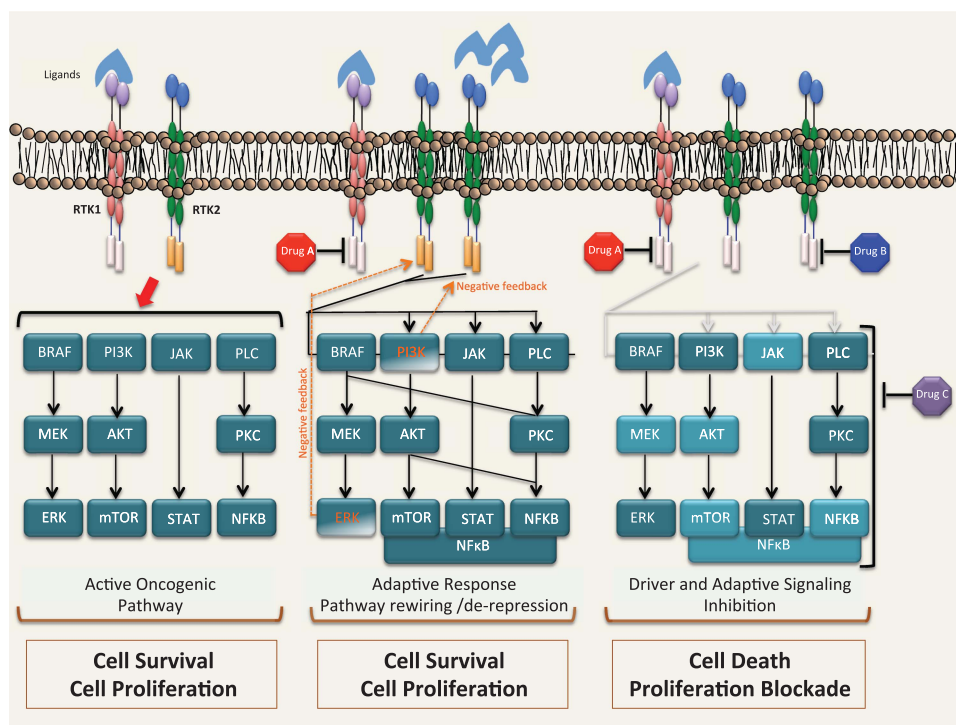


Figure 2. Model for blocking primary and adaptive molecular events underpinning tumor cell survival in order to induce profound and durable responses in patients. Ligand stimulation, mutation, amplification or crosstalk between receptor tyrosine kinases (RTK1, RTK2) leads to their constitutive activation, which in turn activates downstream effector pathways. Inhibition of the driver oncogene leads to an immediate, adaptive stress response. Subsequently, signaling pathways are rewired in order to adapt to the new condition in which signaling from the oncogene is inhibited and sustain cell proliferation and survival. A new paradigm for cancer treatment would be the use of upfront combination therapies along with the oncogenic driver inhibition. Whereas Drug A is normally used for inhibition of the driver oncogene, Drug B or Drug C can be used in combination upfront to prevent cancer cell adaptation. The outcome of this combination therapy approach would be enhanced killing of tumor cells and delay or prevention of therapy resistance. AKT, v-akt murine thymoma viral oncogene homolog; Erk, extracellular signal related kinase; Mek, mitogen-activated protein kinase kinase; mTORC1/2, mammalian target of rapamycin; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K, phosphoinositide 3-kinase; PLC, phospholipase C; Raf, Raf proto-oncogene serine/threonine kinase.

relatively unexplored is the response to radiotherapy. Interestingly, the activation of a HER2-NFκB signaling axis that induced additional HER2 in a feedback loop manner was observed in cancer stem cells in response to radiotherapy in breast cancer cells.⁷⁸ This work is particularly interesting because many breast cancers respond initially to radiation therapy but relapse occurs and may be linked to residual or emergent stem cells resident within the tumor. In this recent work, radiotherapy rapidly induced NFκB activation, which in turn promoted the expression of HER2.⁷⁹ Another interesting example of radiation-induced adaptive-stress response is the activation and cytosolic sequestering of cyclin D1 as well as activation of NFκB. In keratinocytes treated with low-dose radiation therapy NFκB was rapidly activated and induced radioresistance.⁸⁰ In a similar model of keratinocytes, radiotherapy induced cytosolic levels of cyclin D1 that interacted with the pro-apoptotic Bax and prevented apoptosis.⁸¹ Induction of cyclin D1 levels was rapid and did not coincide with translocation of cyclin D1 into the nucleus. Instead, a complex between cyclin D1 and BAX occurred that prevented each protein from translocating to the appropriate cellular locale, namely the mitochondria for Bax and nucleus for cyclin D1.⁸¹ More work is required to understand the adaptive stress response to radiotherapy and the role of stem cells in adaptive stress responses more generally.

It is tempting to speculate that immediate adaptations to therapy occur not only in tumor cells but also in tumor microenvironmental cells that impact tumor growth, drug response and metastatic progression. Hence, it is critical to extend studies of the dynamic response to therapy to stromal and immune cells that reside within the broader tumor ecosystem. Indeed, recent data indicate that the inhibition of MEK may lead to activation of cytotoxic T cells in some melanomas, providing rationale to further explore this largely uncharted role of the adaptive response to therapy in cancer. Such investigations could yield novel combinatorial treatment strategies that suppress adaptive survival signaling in tumor cells while simultaneously engaging the host antitumor response to enhance the magnitude and duration of response in patients.

In summary, a deeper understanding of the survival mechanisms engaged rapidly in response to the stress of targeted therapy promises to offer not only increased fundamental knowledge but also improved treatment strategies capable of unleashing the full potential of targeted cancer therapy to transform cancer patient survival.

CONFLICT OF INTEREST

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REFERENCES

- Weinstein IB. Cancer. Addiction to oncogenes—the Achilles heel of cancer. *Science* 2002; **297**: 63–64.
- Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature* 2001; **411**: 355–365.
- Dhillon AS, Hagan S, Rath O, Kolch W. MAP kinase signalling pathways in cancer. *Oncogene* 2007; **26**: 3279–3290.
- Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2000; **103**: 211–225.
- Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J *et al*. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011; **364**: 2507–2516.
- Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H *et al*. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* 2010; **362**: 2380–2388.
- Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E *et al*. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EORTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012; **13**: 239–246.
- Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T *et al*. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* 2013; **31**: 3327–3334.
- Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ *et al*. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* 2013; **368**: 2385–2394.
- Diaz Jr LA, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J *et al*. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature* 2012; **486**: 537–540.
- Misale S, Di Nicolantonio F, Sartore-Bianchi A, Siena S, Bardelli A. Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. *Cancer Discov* 2014; **4**: 1269–8.
- Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D *et al*. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* 2012; **486**: 532–536.
- Lovly CM, Shaw AT. Molecular pathways: resistance to kinase inhibitors and implications for therapeutic strategies. *Clinical Cancer Res* 2014; **20**: 2249–2256.
- Goltsov A, Langdon SP, Goltsov G, Harrison DJ, Bown J. Customizing the therapeutic response of signaling networks to promote antitumor responses by drug combinations. *Front Oncol* 2014; **4**: 13.
- Garraway LA, Janne PA. Circumventing cancer drug resistance in the era of personalized medicine. *Cancer Discov* 2012; **2**: 214–226.
- Glickman MS, Sawyers CL. Converting cancer therapies into cures: lessons from infectious diseases. *Cell* 2012; **148**: 1089–1098.
- Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M *et al*. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005; **352**: 786–792.
- Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF *et al*. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005; **2**: e73.
- Godin-Heymann N, Ulkus L, Brannigan BW, McDermott U, Lamb J, Maheswaran S *et al*. The T790M "gatekeeper" mutation in EGFR mediates resistance to low concentrations of an irreversible EGFR inhibitor. *Mol Cancer Ther* 2008; **7**: 874–879.
- Bardelli A, Corso S, Bertotti A, Hobor S, Valtorta E, Siravegna G *et al*. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. *Cancer Discov* 2013; **3**: 658–673.
- Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L *et al*. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci USA* 2007; **104**: 20932–20937.
- Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO *et al*. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007; **316**: 1039–1043.
- Zhang Z, Lee JC, Lin L, Olivas V, Au V, LaFramboise T *et al*. Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. *Nat Genet* 2012; **44**: 852–860.
- Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidler I *et al*. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011; **3**: 75ra26.
- Watanabe S, Sone T, Matsui T, Yamamura K, Tani M, Okazaki A *et al*. Transformation to small-cell lung cancer following treatment with EGFR tyrosine kinase inhibitors in a patient with lung adenocarcinoma. *Lung Cancer* 2013; **82**: 370–372.
- Aparicio A, Logothetis CJ, Maity SN. Understanding the lethal variant of prostate cancer: power of examining extremes. *Cancer Discov* 2011; **1**: 466–468.
- Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW *et al*. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004; **350**: 2129–2139.
- Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S *et al*. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; **304**: 1497–1500.
- Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I *et al*. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA* 2004; **101**: 13306–13311.
- Huang PH, Xu AM, White FM. Oncogenic EGFR signaling networks in glioma. *Sci Signal* 2009; **2**: re6.

- 31 Akhavan D, Pourzia AL, Nourian AA, Williams KJ, Nathanson D, Babic I *et al.* De-repression of PDGFR β transcription promotes acquired resistance to EGFR tyrosine kinase inhibitors in glioblastoma patients. *Cancer Discov* 2013; **3**: 534–547.
- 32 Lee HJ, Zhuang G, Cao Y, Du P, Kim HJ, Settleman J. Drug resistance via feedback activation of Stat3 in oncogene-addicted cancer cells. *Cancer Cell* 2014; **26**: 207–221.
- 33 Ishiguro Y, Ishiguro H, Miyamoto H. Epidermal growth factor receptor tyrosine kinase inhibition up-regulates interleukin-6 in cancer cells and induces subsequent development of interstitial pneumonia. *Oncotarget* 2013; **4**: 550–559.
- 34 Haber DA, Gray NS, Baselga J. The evolving war on cancer. *Cell* 2011; **145**: 19–24.
- 35 Scaltriti M, Verma C, Guzman M, Jimenez J, Parra JL, Pedersen K *et al.* Lapatinib, a HER2 tyrosine kinase inhibitor, induces stabilization and accumulation of HER2 and potentiates trastuzumab-dependent cell cytotoxicity. *Oncogene* 2009; **28**: 803–814.
- 36 Garrett JT, Olivares MG, Rinehart C, Granja-Ingram ND, Sanchez V, Chakrabarty A *et al.* Transcriptional and posttranslational up-regulation of HER3 (ErbB3) compensates for inhibition of the HER2 tyrosine kinase. *Proc Natl Acad Sci USA* 2011; **108**: 5021–5026.
- 37 Kirouac DC, Du JY, Lahdenranta J, Overland R, Yarar D, Paragas V *et al.* Computational modeling of ERBB2-amplified breast cancer identifies combined ErbB2/3 blockade as superior to the combination of MEK and AKT inhibitors. *Sci Signal* 2013; **6**: ra68.
- 38 McDonagh CF, Huhlov A, Harms BD, Adams S, Paragas V, Oyama S *et al.* Antitumor activity of a novel bispecific antibody that targets the ErbB2/ErbB3 oncogenic unit and inhibits heregulin-induced activation of ErbB3. *Mol Cancer Ther* 2012; **11**: 582–593.
- 39 Wetterskog D, Shiu KK, Chong I, Meijer T, Mackay A, Lambros M *et al.* Identification of novel determinants of resistance to lapatinib in ERBB2-amplified cancers. *Oncogene* 2014; **33**: 966–976.
- 40 Bailey ST, Miron PL, Choi YJ, Kochupurakkal B, Maulik G, Rodig SJ *et al.* NF-kappaB activation-induced anti-apoptosis renders HER2-positive cells drug resistant and accelerates tumor growth. *Mol Cancer Res* 2014; **12**: 408–420.
- 41 Chen YJ, Yeh MH, Yu MC, Wei YL, Chen WS, Chen JY *et al.* Lapatinib-induced NF-kappaB activation sensitizes triple-negative breast cancer cells to proteasome inhibitors. *Breast Cancer Res* 2013; **15**: R108.
- 42 Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandralapaty S *et al.* Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. *Cancer Cell* 2011; **19**: 575–586.
- 43 Arora VK, Schenkein E, Murali R, Subudhi SK, Wongvipat J, Balbas MD *et al.* Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell* 2013; **155**: 1309–1322.
- 44 Schweizer L, Rizzo CA, Spires TE, Platero JS, Wu Q, Lin TA *et al.* The androgen receptor can signal through Wnt/beta-Catenin in prostate cancer cells as an adaptation mechanism to castration levels of androgens. *BMC Cell Biol* 2008; **9**: 4.
- 45 Miller TW, Balko JM, Arteaga CL. Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer. *J Clin Oncol* 2011; **29**: 4452–4461.
- 46 Fox EM, Miller TW, Balko JM, Kuba MG, Sanchez V, Smith RA *et al.* A kinase-wide screen identifies the insulin/IGF-1 receptor pathway as a mechanism of escape from hormone dependence in breast cancer. *Cancer Res* 2011; **71**: 6773–6784.
- 47 Baker NM, Der CJ. Cancer: drug for an 'undruggable' protein. *Nature* 2013; **497**: 577–578.
- 48 Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature* 2013; **503**: 548–551.
- 49 Zimmermann G, Papke B, Ismail S, Vartak N, Chandra A, Hoffmann M *et al.* Small molecule inhibition of the KRAS-PDEdelta interaction impairs oncogenic KRAS signalling. *Nature* 2013; **497**: 638–642.
- 50 Zhao Y, Adjei AA. The clinical development of MEK inhibitors. *Nat Rev Clin Oncol* 2014; **11**: 385–400.
- 51 Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D *et al.* Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 2012; **483**: 100–103.
- 52 Corcoran RB, Ebi H, Turke AB, Coffee EM, Nishino M, Cogdill AP *et al.* EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. *Cancer Discov* 2012; **2**: 227–235.
- 53 Sun C, Wang L, Huang S, Heynen GJ, Prahallad A, Robert C *et al.* Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. *Nature* 2014; **508**: 118–122.
- 54 Li X, Huang Y, Jiang J, Frank SJ. ERK-dependent threonine phosphorylation of EGF receptor modulates receptor downregulation and signaling. *Cell Signal* 2008; **20**: 2145–2155.
- 55 Turke AB, Song Y, Costa C, Cook R, Arteaga CL, Asara JM *et al.* MEK inhibition leads to PI3K/AKT activation by relieving a negative feedback on ERBB receptors. *Cancer Res* 2012; **72**: 3228–3237.
- 56 Flanigan SA, Pitts TM, Newton TP, Kulikowski GN, Tan AC, McManus MC *et al.* Overcoming IGF1/IR resistance through inhibition of MEK signaling in colorectal cancer models. *Clin Cancer Res* 2013; **19**: 6219–6229.
- 57 Duncan JS, Whittle MC, Nakamura K, Abell AN, Midland AA, Zawistowski JS *et al.* Dynamic reprogramming of the kinase in response to targeted MEK inhibition in triple-negative breast cancer. *Cell* 2012; **149**: 307–321.
- 58 Sun C, Hobor S, Bertotti A, Zecchin D, Huang S, Galimi F *et al.* Intrinsic resistance to MEK inhibition in KRAS mutant lung and colon cancer through transcriptional induction of ERBB3. *Cell Rep* 2014; **7**: 86–93.
- 59 Lito P, Saborowski A, Yue J, Solomon M, Joseph E, Gadal S *et al.* Disruption of CRAF-mediated MEK activation is required for effective MEK inhibition in KRAS mutant tumors. *Cancer Cell* 2014; **25**: 697–710.
- 60 Papa S, Martino PL, Capitanio G, Gaballo A, De Rasmio D, Signorile A *et al.* The oxidative phosphorylation system in mammalian mitochondria. *Adv Exp Med Biol* 2012; **942**: 3–37.
- 61 Warburg O. On the origin of cancer cells. *Science* 1956; **123**: 309–314.
- 62 Moreno-Sanchez R, Rodriguez-Enriquez S, Marin-Hernandez A, Saavedra E. Energy metabolism in tumor cells. *FEBS J* 2007; **274**: 1393–1418.
- 63 Kaplon J, Zheng L, Meissl K, Chaneton B, Selivanov VA, Mackay G *et al.* A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. *Nature* 2013; **498**: 109–112.
- 64 Haq R, Shoag J, Andreu-Perez P, Yokoyama S, Edelman H, Rowe GC *et al.* Oncogenic BRAF regulates oxidative metabolism via PGC1alpha and MITF. *Cancer Cell* 2013; **23**: 302–315.
- 65 Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S *et al.* A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* 2010; **141**: 69–80.
- 66 Haruta T, Uno T, Kawahara J, Takano A, Egawa K, Sharma PM *et al.* A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. *Mol Endocrinol* 2000; **14**: 783–794.
- 67 O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D *et al.* mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 2006; **66**: 1500–1508.
- 68 Shi Y, Yan H, Frost P, Gera J, Lichtenstein A. 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* 2005; **4**: 1533–1540.
- 69 Chandralapaty S. Negative feedback and adaptive resistance to the targeted therapy of cancer. *Cancer Discov* 2012; **2**: 311–319.
- 70 Chandralapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V *et al.* AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. *Cancer Cell* 2011; **19**: 58–71.
- 71 Ducker GS, Atreya CE, Simko JP, Hom YK, Matli MR, Benes CH *et al.* Incomplete inhibition of phosphorylation of 4E-BP1 as a mechanism of primary resistance to ATP-competitive mTOR inhibitors. *Oncogene* 2014; **33**: 1590–1600.
- 72 Muranen T, Selfors LM, Worster DT, Iwanicki MP, Song L, Morales FC *et al.* Inhibition of PI3K/mTOR leads to adaptive resistance in matrix-attached cancer cells. *Cancer Cell* 2012; **21**: 227–239.
- 73 Rodrik-Outmezguine VS, Chandralapaty S, Pagano NC, Poulikakos PI, Scaltriti M, Moskatel E *et al.* mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. *Cancer Discov* 2011; **1**: 248–259.
- 74 Serra V, Scaltriti M, Prudkin L, Eichhorn PJ, Ibrahim YH, Chandralapaty S *et al.* PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. *Oncogene* 2011; **30**: 2547–2557.
- 75 Carreira S, Goodall J, Denat L, Rodriguez M, Nuciforo P, Hoek KS *et al.* Mitf regulation of Dia1 controls melanoma proliferation and invasiveness. *Genes Dev* 2006; **20**: 3426–3439.
- 76 Cheli Y, Giuliano S, Botton T, Rocchi S, Hofman V, Hofman P *et al.* Mitf is the key molecular switch between mouse or human melanoma initiating cells and their differentiated progeny. *Oncogene* 2011; **30**: 2307–2318.
- 77 Saez-Ayala M, Montenegro MF, Sanchez-Del-Campo L, Fernandez-Perez MP, Chazarra S, Freter R *et al.* Directed phenotype switching as an effective antimelanoma strategy. *Cancer Cell* 2013; **24**: 105–119.
- 78 Duru N, Candas D, Jiang G, Li JJ. Breast cancer adaptive resistance: HER2 and cancer stem cell repopulation in a heterogeneous tumor society. *J Cancer Res Clin Oncol* 2014; **140**: 1–14.
- 79 Duru N, Fan M, Candas D, Menaa C, Liu HC, Nantajit D *et al.* HER2-associated radioresistance of breast cancer stem cells isolated from HER2-negative breast cancer cells. *Clin Cancer Res* 2012; **18**: 6634–6647.
- 80 Chen X, Shen B, Xia L, Khaletskiy A, Chu D, Wong JY *et al.* Activation of nuclear factor kappaB in radioresistance of TP53-inactive human keratinocytes. *Cancer Res* 2002; **62**: 1213–1221.
- 81 Ahmed KM, Fan M, Nantajit D, Cao N, Li JJ. Cyclin D1 in low-dose radiation-induced adaptive resistance. *Oncogene* 2008; **27**: 6738–6748.