Oncogene addiction: pathways of therapeutic response, resistance, and road maps toward a cure

Raymond Pagliarini, Wenlin Shao & William R Sellers*

Abstract

A key goal of cancer therapeutics is to selectively target the genetic lesions that initiate and maintain cancer cell proliferation and survival. While most cancers harbor multiple oncogenic mutations, a wealth of preclinical and clinical data supports that many cancers are sensitive to inhibition of single oncogenes, a concept referred to as ‘oncogene addiction’. Herein, we describe the clinical evidence supporting oncogene addiction and discuss common mechanistic themes emerging from the response and acquired resistance to oncogene-targeted therapies. Finally, we suggest several opportunities toward exploiting oncogene addiction to achieve curative cancer therapies.

Keywords feedback; oncogene addiction; oncogenic shock; targeted therapy

Clinical evidence for oncogene addiction

As we focus herein on the clinical evidence for oncogene addiction, we direct the reader to excellent reviews of the preclinical data supporting oncogene and non-oncogene addiction [3,4]. A prime clinical example of oncogene addiction is in CML. CML is driven by the BCR-ABL mutant oncogene, produced as a result of chromosome 9:22 translocation, otherwise known as the ‘Philadelphia’ chromosome [5,6]. While preclinical studies provided evidence that BCR-ABL was a bona fide oncogene both in vitro and in vivo [7,8], addition of CML to BCR-ABL was demonstrated in patients through the profound clinical responses attained with the kinase inhibitor imatinib, which targets BCR-ABL. This addiction was further reinforced by the description of genetic mechanisms of resistance that largely led to reactivation of BCR-ABL kinase activity (described in more detail below). These observations in aggregate provided a transformative proof-of-concept for oncogene-targeted cancer therapy [9]. As summarized in Table 1 (and referenced therein), the strategy of targeting mutant oncogenic kinases has now been repeated many times over in a variety of cancer types. The common theme from these studies is a marked improvement in initial patient responses when oncogene-targeted therapies, tested in the correct oncogene-mutated patient population, are compared head-to-head with prior standard of care therapeutics.

The oncogene-addicted phenotype is not unique to mutated kinases. One of the earliest examples of targeted therapy (albeit one where clinical efficacy was established prior to molecular cloning of the causative oncogene) was the use of ATRA in APL. APL bears characteristic translocations affecting the retinoic acid receptor, generating fusion proteins such as PML-RARA that interfere with normal cell differentiation [10]. ATRA binds to the ligand-binding domain of PML-RARA, which inhibits its oncogenic function [11]. An additional example is the use of antiandrogens for the treatment of prostate cancers, which are ‘lineage-addicted’ [12] to AR and bear recurrent AR amplifications or mutations upon resistance to first-line therapies [13,14]. Finally, recent cancer genome sequencing has revealed a prevalent novel class of mutated oncogenes involved in the regulation of epigenetic states [15]. Examples include oncogenic
point mutations or chromosomal translocations affecting EZH2, NSD2, BRD4, IDH1, and IDH2. Given the aforementioned history of cancer addiction to mutated driver oncogenes, as well as emerging preclinical studies demonstrating the dependence on these oncogenes for tumor maintenance, drugs targeting these lesions have been rapidly developed [16–20]. Several of these have already entered early clinical investigation, with encouraging initial responses [21–24]. Together, these striking results demonstrate that the concept of oncogene addiction indeed translates into clinical responses.

### Therapeutic resistance reveals oncogenic pathway addiction

Despite the robust initial clinical responses described above, chronic exposure to most targeted therapeutics often gives way to relapse, and cures remain elusive. Does this argue against oncogene addiction? Answers lie in the observed clinical mechanisms of resistance to oncogene-targeted therapeutics. As detailed below, three common themes emerge upon resistance to many oncogene-targeted therapies; these themes demonstrate that most cancers retain an underlying addiction to oncogene-induced signaling pathways, if not a monolithic addiction to the originally mutated oncogene.

#### Secondary alterations of the oncogene drug target

Single-agent BCR-ABL inhibition often results in cancer cell apoptosis and profound long-term responses [9,25]; however, a significant fraction of patients show resistance to existing therapies. The main observed mechanism of resistance to BCR-ABL inhibition is the acquisition of second-site mutations in BCR-ABL itself [26]. Predominant among these is mutation of the ATP-binding pocket at the ‘gatekeeper’ residue threonine 315. Mutation at this site prevents optimal binding of imatinib and other inhibitors, while still allowing gating addiction to oncogene-induced signaling pathways, if not a monolithic addiction to the originally mutated oncogene.

#### Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADC</td>
<td>antibody-drug conjugate</td>
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<tr>
<td>AKT</td>
<td>Akt thymoma kinase, key member of the PI3K pathway</td>
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<td>ALK</td>
<td>anaplastic lymphoma kinase, activated by gene translation in cancer</td>
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<tr>
<td>APL</td>
<td>acute promyelocytic leukemia</td>
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<tr>
<td>AR</td>
<td>androgen receptor, lineage driver of prostate cancer</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<tr>
<td>ATRA</td>
<td>all-trans retinoic acid</td>
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<tr>
<td>BCR-ABL</td>
<td>B-cell receptor–Abelson kinase, oncogenic fusion protein that drives CML</td>
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<tr>
<td>BRAF</td>
<td>v-Raf murine sarcoma viral oncogene homolog B, activated by point mutations in cancer</td>
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<tr>
<td>BRD4</td>
<td>bromodomain containing 4, chromatin modulator activated by translocation in cancer</td>
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<tr>
<td>BTK</td>
<td>Bruton’s tyrosine kinase, key member of oncogenic B-cell receptor signaling</td>
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<td>CCLE</td>
<td>cancer cell line encyclopedia</td>
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<td>CGP</td>
<td>cancer genome project</td>
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<tr>
<td>CML</td>
<td>chronic myelogenous leukemia</td>
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<tr>
<td>CRAF</td>
<td>v-Raf murine sarcoma viral oncogene homolog B</td>
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<tr>
<td>CRISPR/CAS9</td>
<td>clustered regularly interspaced short palindromic repeats/Cas9 nuclease; eukaryotic gene editing technology derived from a prokaryotic viral immune editing system</td>
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<td>DM1</td>
<td>maytansine derivative, toxic payload often linked to ADCs</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>DUSP</td>
<td>dual specificity phosphatase, negative regulator of MAPK pathway</td>
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<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor, activated by point mutation and small deletions in cancer</td>
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<tr>
<td>ER</td>
<td>estrogen receptor, lineage driver for breast and other cancers</td>
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<tr>
<td>ERK</td>
<td>extracellular signal-regulated kinase, member of MAPK pathway</td>
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<tr>
<td>EZH2</td>
<td>enhancer of zeste homolog 2, activated by point mutation in cancer</td>
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<tr>
<td>FGFR</td>
<td>fibroblast growth factor receptor, activated by gene fusion and point mutation in cancer</td>
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<tr>
<td>FLT3</td>
<td>Fms-like tyrosine kinase 3, activated by ITD and point mutation in cancer</td>
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<tr>
<td>GIST</td>
<td>gastrointestinal stromal tumor</td>
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<tr>
<td>HER2</td>
<td>human epidermal growth factor receptor 2</td>
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<tr>
<td>HER3</td>
<td>human epidermal growth factor receptor 3</td>
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<tr>
<td>IDH1</td>
<td>isocitrate dehydrogenase 1, activated by point mutation in cancer</td>
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<tr>
<td>IDH2</td>
<td>isocitrate dehydrogenase 2, activated by point mutation in cancer</td>
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<tr>
<td>IGF-1R</td>
<td>insulin-like growth factor 1 receptor, feedback activator of oncogenic signaling</td>
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<td>IL-6</td>
<td>interleukin 6 cytokine</td>
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<td>ITD</td>
<td>internal tandem duplication, a common mutation of the FLT3 oncogene</td>
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<tr>
<td>KIT</td>
<td>v-kit Hardy–Zuckerman 4 feline sarcoma viral oncogene homolog</td>
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<tr>
<td>KRAS</td>
<td>Kirsten Ras viral oncogene homolog, activated by point mutation in cancer</td>
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<tr>
<td>MAP2K1</td>
<td>mitogen-activated protein kinase kinase 1</td>
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<td>MAP2K2</td>
<td>mitogen-activated protein kinase kinase 2</td>
</tr>
<tr>
<td>MEK</td>
<td>MAPK/ERK kinase, alternate common name for MAPK2K1, MAPK2K2, and/or MAPK pathway</td>
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<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
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<tr>
<td>MITF</td>
<td>microphthalmia-associated transcription factor, lineage driver for melanoma</td>
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<tr>
<td>MYB</td>
<td>v-myc avian myeloblastosis viral oncogene homolog</td>
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<tr>
<td>NRAS</td>
<td>neuroblastoma Ras viral oncogene homolog, activated by point mutation in cancer</td>
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<tr>
<td>NSCLC</td>
<td>non-small cell lung cancer</td>
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<td>NSD2</td>
<td>Wolf–Hirschhorn syndrome candidate 1</td>
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<tr>
<td>PI3K</td>
<td>phosphoinositide 3-kinase pathway</td>
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<tr>
<td>PIK3CA</td>
<td>phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha, activated by point mutation in cancer</td>
</tr>
<tr>
<td>PTEN</td>
<td>phosphatase and tensin homolog, tumor suppressor and negative regulator of PI3K pathway</td>
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<tr>
<td>RAS</td>
<td>rat sarcoma viral oncogene; generic name for KRAS, NRAS, HRAS oncogenes, and/or the signaling pathway</td>
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<tr>
<td>RNAi</td>
<td>RNA interference</td>
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<tr>
<td>ROS1</td>
<td>ROS proto-oncogene 1</td>
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<tr>
<td>RTK</td>
<td>receptor tyrosine kinase</td>
</tr>
<tr>
<td>SPRY</td>
<td>Sprouty homologs (e.g., SPRY1, SPRY2), negative regulators of RTK signaling</td>
</tr>
<tr>
<td>TERT</td>
<td>telomerase reverse transcriptase, activated by promoter point mutations in cancer</td>
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Table 1. Examples of approved oncogene-targeted therapies and observed resistance mechanisms in patients.

<table>
<thead>
<tr>
<th>Target/Indication</th>
<th>Inhibitor(s)</th>
<th>Observed clinical responses</th>
<th>Resistance mechanisms</th>
<th>Secondary oncogene mutation</th>
<th>Pathway mutations</th>
<th>Bypass</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIT mutant GIST</td>
<td>Imatinib</td>
<td>53.7% partial response in patients with refractory disease [170]</td>
<td>KIT mutations (e.g., V654A, T670I) or amplification [171,172]</td>
<td>PDGFR mutation [172]</td>
<td>Rhabdomyosarcomatous differentiation [109]</td>
<td></td>
</tr>
<tr>
<td>BRAF mutant melanoma</td>
<td>Vemurafenib, dabrafenib</td>
<td>45–51% response rate; benefits observed versus prior standard of care [38,59,175]</td>
<td>P61-BRAF splice variant [28]; BRAF amplification [174]</td>
<td>NRAS, NF1, MAP2K1, MAPK2K4 mutation</td>
<td>MITF amplification [31–33,175,176]</td>
<td></td>
</tr>
<tr>
<td>EGFR mutant NSCLC</td>
<td>Gefitinib, erlotinib, afatinib</td>
<td>9–13 months progression-free survival; 73.7% response rate for gefitinib; benefit versus standard chemotherapy [178–180,181,182]</td>
<td>T970M mutation (+/− gene amplification −40–65%); other EGFR point mutations (−1–2%) [105,183–185]</td>
<td>PIK3CA mutation, BRAF mutation (−1%) [105,185]</td>
<td>MET or HER2 amplification; histologic transformation (EMT, SCLC ~12–14%) [37,38,105,185,186]</td>
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</tr>
<tr>
<td>EGFR-amplified colorectal cancer</td>
<td>Cetuximab, panitumumab</td>
<td>Improvements in progression-free survival versus best supportive care [187]</td>
<td>Kras, BRAF, PIK3CA, PTEN mutation [187]</td>
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<tr>
<td>ROS1-translocated NSCLC</td>
<td>Crizotinib</td>
<td>72% objective response rate [197]</td>
<td>G2032R mutation [198]</td>
<td></td>
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<tr>
<td>RET mutant medullary thyroid carcinoma (MTC)</td>
<td>Vandetanib</td>
<td>46% objective response rate in patients with hereditary MTC harboring RET mutation [199]</td>
<td></td>
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</tr>
<tr>
<td>Retinoic acid receptor (RAR)-translocated APL</td>
<td>ATRA</td>
<td>Complete response rates of &gt; 90% superior to prior chemotherapy regimens [200]</td>
<td>Ligand-binding domain mutation (−40%) [11,201]</td>
<td></td>
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<tr>
<td>AR-positive castration-resistant prostate cancer</td>
<td>Enzalutamide</td>
<td>18.4-month overall survival, 54% PSA reduction [202]</td>
<td>F876L mutation [99–101]; AR-V7 ligand-binding domain truncation [203]</td>
<td></td>
<td>CR upregulation [203]</td>
<td></td>
</tr>
<tr>
<td>ER-positive metastatic breast cancer</td>
<td>Tamoxifen, toremifene, fulvestrant, letrozole, anastrozole, exemestane</td>
<td>Tamoxifen: approximately 50% drop in mortality with 10 years of treatment [204]</td>
<td>Ligand-binding domain mutation [30,205]</td>
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fraction of resistant disease bearing second-site oncogene mutations that restore oncogene function in the presence of drug. These acquired mutations often occur within the highly conserved gatekeeper residue (Table 1). The HER2 oncogene commonly develops resistance to the humanized HER2 antibody trastuzumab in a slightly different fashion; in this case, the trastuzumab-binding epitope is lost, while oncogene function is retained [27]. BRAF obtains resistance to kinase inhibitors, at least in part, through either kinase amplification or truncations that further activate kinase activity [28,29]. Outside of kinases, AR, PML-RAR, and ER (a lineage driver for many breast cancers) acquire mutations in their ligand-binding domains that reduce or abrogate drug efficacy (Table 1 [30]) while restoring oncogene function. The common theme of treatment-acquired secondary oncogene alterations is that they provide resistance to therapy while reinstating oncogene function—this clinically observed resistance mechanism makes the most compelling argument for oncogene addiction.

**Activating mutations in oncogenic pathway components**

Acquired resistance to oncogene-targeted drugs also occurs via mutation of alternate components of oncogene-induced signaling pathways. For example, mutant BRAF signals through the MAPK signaling pathway to promote melanoma growth. As such, one key resistance mechanism to BRAF inhibitors such as vemurafenib is the acquisition of activating mutations in other known MAPK signaling pathway components such as NRAS [31], or more rarely MAP2K1, and MAP2K2 [31,32]; loss of function mutations in the negative MAPK pathway regulator NF1 [31,33]; or amplification and activation of the MAPK pathway target gene MITF, a lineage driver of melanoma [31]. All of these mutations restore MAPK oncogenic pathway signals despite continued pharmacological inhibition of mutant BRAF (Fig 1B). This theme recurs in NSCLC, where activation of RTKs such as EGFR and ALK are key driver events. RTKs signal through several intracellular pathways, including the MAPK and PI3K pathways. As such, acquired resistance to HER2-, EGFR-, and ALK-targeted therapies includes selection for activating mutations in the MAPK or PI3K pathways (Table 1 and references therein). As with second-site mutation of the oncogene itself, resistance mutations in key members of an oncogenic signaling pathway highlights that many cancers retain dependence upon specific oncogenic pathways, if not always the oncogene itself.

**Induction of bypass pathway signaling**

The third common theme in acquired resistance is the induction of bypass signaling pathways. A key example is observed in resistance to BRAF inhibitors. RAF family proteins normally function as dimers; common oncogenic mutations in BRAF (e.g., V600E) allow monomeric BRAF proteins to activate downstream signaling pathways in the absence of upstream activating signals [28]. This activity is blocked by selective BRAF kinase inhibitors. However, CRAF,
another key RAF isoform, can still activate the MAPK pathway in the presence of upstream pathway signals. This commonly occurs through the induction of growth factor-dependent and/or RAS-dependent signals [34]. Such upstream activating signals are often paradoxically induced by oncogene inhibition, as discussed in more detail below. Despite mutant BRAF inhibition, upstream pathway activation can still signal through CRAF to reinstate MAPK pathway signaling. This is facilitated through the activation of CRAF homodimers, or through CRAF:BRAF heterodimers that are stabilized in the presence of some BRAF inhibitors [34–36].

Induction of oncogene bypass signaling is not unique to BRAF inhibitors. As discussed in more detail below, acquired resistance to EGFR-, HER2-, and ALK-targeted therapeutics commonly occurs through the upregulation or amplification of alternate RTK’s (Table 1). This bypasses the need for the mutated oncogene, but often reinstates the original downstream signaling pathways. A key example is the selection for activated MET signaling as a resistance mechanism to EGFR-targeted therapies [37,38] (Fig 1C). As another recent example, resistance to the AR inhibitor enzalutamide can be caused by glucocorticoid receptor (GR)-dependent bypass of AR signaling [39]. While many bypass resistance mechanisms reinstate the same signaling pathways originally activated by the oncogene, this is not always the case. For acquired resistance to BRAF inhibitors in melanoma, mutational activation of the PI3K-PTEN-AKT pathway has been identified as a prevalent mechanism in bypassing tumor dependence on MAPK signaling [29,40].

In summary, despite the apparent mechanistic diversity of acquired resistance to oncogene-targeted therapies, the three major themes of resistance outlined above demonstrate that most cancers retain addiction to specific oncogene-activated pathway signals. This suggests that the key dependencies of cancer cells remain tractable despite acquired resistance and that a better knowledge of resistance mechanisms can lead to rational therapeutic strategies that reduce or prevent resistance in the clinic.

'Oncogenic shock' as a model to understand response versus resistance to therapy

While many oncogene-addicted cancers show striking initial responses to targeted therapies, the heterogeneity of response within and across cancers must be noted. Why is the proportion of response and resistance so different between different oncogenes—for example, why are durable single-agent responses often seen with BCR-ABL inhibition in CML, while inhibitors of FLT3-ITD in AML appear to only provide transient benefits [41,42]? Similarly, why does inhibition of the same oncogene have divergent responses in different cancer types—as exemplified by a ~50% response rate to BRAF inhibitors in BRAF mutant melanoma, but a less than 5% response rate in BRAF mutant colorectal cancers [43]? Such inconsistencies could be explained by an inability to achieve complete and sustained target inhibition in different tumor types, due to pharmacological limitations across different drugs and among different patient populations [44]. However, emerging data demonstrate that intrinsic biological differences across oncogenes and tumor types also exist.

A useful paradigm to understand the biological diversity of responses to oncogene inhibition is that of ‘oncogenic shock’ [45,46]. This hypothesis builds on the knowledge that activated oncogenes promote proliferation and survival, but at the same time paradoxically activate signals that promote arrest or apoptosis [47–51]. Upon acute inactivation of oncogene signaling, the timing of how these two pathways respond may differ for different oncogenes, or in different contexts. If the oncogenic pathway is quickly blocked by a drug, while the paradoxical oncogene-activated growth inhibitory pathway is slow to turn off, then apoptosis, or oncogenic shock, prevails. Conversely, if the paradoxical growth inhibitory signals from the oncogene can quickly reset, this provides a scenario where cells may survive to become resistant to oncogene inhibition. This differential in pathway response, presumably due to differences in the turnover of signaling proteins such as phosphatases that negatively regulate discreet prosurvival or proapoptotic pathways [46], may explain why some oncogenes show more profound responses than others upon acute inhibition. What are the mechanisms that allow oncogenic shock phenotypes to occur—or to be bypassed—and are they common among cancers?

An early view into the mechanism of paradoxical oncogene-induced growth inhibitory pathways in cancer cells was afforded by a study of the differential sensitivity of BRAF mutant versus RTK-activated cancer cells to MAPK pathway inhibition [52]. This study suggested that mutant BRAF activates a unique ERK-dependent transcriptional output, including the upregulation of DUSP phosphatases and the SPRY family of secreted RTK inhibitory proteins, both of which negatively regulate MAPK pathway signaling [53,54]. BRAF inhibition blocks MAPK-dependent growth signaling, but also shuts off MAPK-dependent SPRY expression, which relinquishes SPRY-dependent inhibition of HER-family-, FGFR-, and/or IGF-1R-dependent responsiveness to exogenous growth factors [55,56]. Such feedback appears to be particularly active in BRAF mutant colorectal cancers via the rapid activation of EGFR upon BRAF inhibition [57]. While this explains the limited efficacy of BRAF inhibitors in this indication, it also provides the rationale for dual inhibition of BRAF and EGFR in BRAF mutant colorectal cancers [43,57]. In melanoma, paradoxical feedback pathway activation (as well as most other clinically observed resistance mechanisms) reinstates MAPK signaling, providing the rationale for dual BRAF/MEK inhibition [58,59].

While BRAF signaling inhibition has become a paradigm for paradoxical feedback pathway activation, oncogenic alterations in EGFR, HER2, ALK, and MET also function through a MAPK-dependent feedback pathway to block IL-6-facilitated activation of STAT3 and PI3K pathway-mediated survival signals [60]. Also like BRAF, BCR-ABL and FLT3-ITD oncogenes can block the expression of growth factor receptors via a MAPK pathway-dependent feedback mechanism [61]. The contrasting behavior of BCR-ABL- and FLT3-ITD-dependent feedback responses noted in an isogenic cell background [61] is of particular interest for the oncogenic shock hypothesis: pulsed inhibition of BCR-ABL rapidly shuts down BCR-ABL-dependent downstream survival signaling (including MAPK pathway signaling), but BCR-ABL- and MAPK pathway-dependent inhibition of normal growth factor-dependent signaling is slow to revert back to its basal state. This creates a window of time where no prosurvival signals are present, resulting in apoptosis. While pulsed FLT3 inhibition in FLT3-ITD mutant expressing cells similarly inhibits oncogenic signaling, MAPK pathway-dependent negative feedback is rapidly lost and growth factor-dependent
signaling pathway signaling is quickly restored. In this setting, there is not enough time for apoptosis to be induced before the FLT3-ITD-inhibited cells restore functional growth factor receptor-dependent signaling.

Together, these data provide evidence that oncogene-dependent feedback inhibition of growth factor-dependent signaling may be pervasive across many cancers (even BCR-ABL mutant CML). These studies furthermore suggest that the turnover rate of these feedback mechanisms in different cancers dictates the fine line between oncogenic shock versus the activation of bypass resistance mechanisms (e.g., BCR-ABL inhibition in CML versus BRAF inhibition in colorectal cancer). Finally, the data implicate the MAPK pathway as a key node regulating the oncogene-induced feedback inactivation of growth factor receptor signaling (Fig 2).

While MAPK pathway-dependent feedback may commonly attenuate oncogenic shock responses across cancers, alternate mechanisms have been reported. First, altered epigenetic regulation can generate ‘drug-tolerant’ states that allow for the survival of small subpopulations within otherwise treatment-sensitive cancer cells; despite this alternate mechanism of resistance, survival is still generated through the upregulation of growth factor receptors such as IGF-1R [62]. Second, PI3K-AKT-dependent feedback pathways have also been reported [63,64]—once again, resistance is driven through relief of oncogene-induced negative feedback regulation of growth factor receptors. A particularly interesting case is in prostate cancer—oncogenic PI3K and AR activation exists in a vicious cycle where inhibition of either pathway results in feedback upregulation of the other, via a mechanism that induces EGFR family RTK signaling [65]. As above, knowledge of these feedback pathways sheds light onto rationally designed therapeutic combinations to prevent resistance—in this case, dual inhibition of AR and PI3K signaling pathways shuts down paradoxical bypass signaling and achieves remarkable efficacy in preclinical models.

A Road map for targeting oncogene addiction

The clinical benefits observed with agents targeting mutated oncogenes provide hope that the ‘one-step remedy’ to oncogene addiction initially proposed by Weinstein [2] may be attainable for some cancers. However, the common patterns of resistance to oncogene-targeted therapies must be anticipated and intercepted in order to achieve deep and sustained clinical benefit, and ultimately cures. The road map to curative therapy will require a rationally designed ‘one-two punch’ with combinations of targeted agents, rather than a one-step remedy. Below, we offer some suggestions to reach this goal.

Genetically define all cancers

Classical characterization of cancer subtypes include distinctions based on tissue type, histology, pathology, and level of differentiation, characteristics that are open to biased interpretation [66]. Targeted therapies require a genetic definition for the patient populations most likely to respond. The most straightforward example is that of CML, where the BCR-ABL fusion defines the disease better than any histologic distinction. This is precisely because the genetic lesion directly impacts the therapeutic strategy (i.e., sensitivity to BCR-ABL inhibitors). This paradigm extends to other tumor types with more heterogeneous genetic etiology. Clinical trials of the EGFR inhibitor gefitinib in otherwise non-selected NSCLC patients originally failed to show strong differences in overall survival. It was not until the ‘outlier’ patients who responded to therapy [67] were retrospectively analyzed for EGFR mutations that the patterns of response in NSCLC could be rationalized [68–70]. EGFR mutant NSCLC is therefore a key clinical subtype. This initial finding now extends to other mutations in NSCLC that are paired with targeted therapeutics (e.g., ALK translocations and ceritinib). An initially homogeneous histological subtype of lung cancer is therefore
redefined into a mutation-stratified spectrum of diseases that dictates different therapeutic options [71]. Many other tumor types such as glioma and glioblastoma are being similarly redefined based on molecular subtypes [72].

An extreme version of the mutation-driven definition of cancer is apparent in so-called basket trials (e.g., NCI-MATCH and the Novartis Signature trial), where both the histological and even lineage definitions of tumors break down. Patients with the mutation that predicts sensitivity to the disease are entered into a trial regardless of tumor type or lineage [73]. The utility of such studies requires the sequencing of large panels of actionable oncogenes across all tumor types, with the affordability and rapid turnaround to routinely impact clinical decision-making.

To best pair the mutant oncogenes with targeted therapies, we need to complete our understanding of the genetic underpinnings of all cancers. Our knowledge of the key driver mutations across many cancers is approaching saturation for genes mutated at a frequency > 10%. However, our knowledge of important driver mutations is rapidly expanding for oncogenes mutated at lower frequency [74]. Elucidating lower frequency oncogenes will uncover new molecular driver events and could highlight common signaling pathways that can be exploited therapeutically. In some cancers however, it has been challenging to define key oncogenic driver mutations. An extreme example of this is in pediatric ependymomas of the posterior fossa, where zero recurrent driver mutations in either oncogenes or tumor suppressor genes have been identified [75]. Identifying driver mutations could be aided by improving our ability to sequence difficult regions of the genome, or by improving the analysis of existing cancer genome sequences [76,77]. In addition, improvements can be made in the analysis of genetic or epigenetic alterations in the ‘dark matter’ of noncoding DNA sequence [1]. Regulatory sequence mutations can drive oncogenic function, as has been recently shown for TERT promoter mutations [78,79], as well as somatic enhancer mutations that activate MYB oncogene binding [80]. Similar mutations or epigenetic alterations that exist in other DNA regulatory sequences remain to be uncovered, as the characterization of these dark regions of the genome is only starting to come to light [81,82].

Address tumor heterogeneity

A key consideration for proper molecular diagnosis of cancers is to account for inter- and intratumor heterogeneity. Issues with correctly diagnosing oncogene alterations from patient to patient could be influenced by the particular test used, as has been noted for expression-based tests for HER2 amplification [83]—diagnostics focused on the specific detection of somatic cancer mutations will likely prove superior. Tumor biopsies can be biased by analyses of a small portion of the tumor that does not appropriately reflect intra-tumor heterogeneity, as has been documented in glioblastoma [84–86], or that does not capture the mutational heterogeneity of disseminated disease [1,31]. More systematic methods of identifying oncogene mutations and acquired resistance mutations, such as with the analysis of circulating tumor DNA [87], may provide a more holistic view of actionable mutations in a patient’s disease, particularly in the case of treatment resistance.

Understanding intratumor heterogeneity is critical not just for diagnosis, but also for choosing the best targets for durable antitumor responses. The acquisition of mutations upon the progression of individual tumors resembles a phylogenetic tree, where ‘trunk’ mutations are initiating mutations present in every cancer cell, ‘branch’ mutations occur later during tumor progression and are present in distinct subregions, and ‘private’ mutations occur only in small individual regions of the disease. Patterns of trunk versus branch mutations are seen across a wide variety of cancer types [88–91]. Targeting trunk mutations, while unfortunately reducing the universe of possible drug targets, may provide key benefit in that it reduces the contribution of intratumor mutational heterogeneity in engendering resistance to treatment. Understanding branch mutations in parallel may allow one to recognize independently arising but convergent resistance mechanisms.

Address therapeutic resistance early

Due to the widespread heterogeneity of most cancers, the alterations promoting ‘acquired’ therapeutic resistance are almost certainly preexisting in most cancers [1]. Therefore, resistance should be expected to arise as a by-product of any single-agent-targeted therapy, and as such should be addressed up-front. Resistance may be minimized by using multiple drugs targeting the same oncogene or oncogenic pathway in combination, as the chance any one tumor cell bears resistance mutations to multiple agents is small (discussed in more detail below). Resistance may also be minimized by expediting the use of novel targeted therapies in treating earlier stage cancers versus advanced metastatic disease. The chance of earlier stage tumors bearing resistance mutations is likely reduced, and therefore, responses may be more durable. To this point, the movement of trastuzumab into the adjuvant and neoadjuvant settings for early-stage HER2-positive breast cancer has demonstrated significant benefits [92,93]. On the other hand, the durable responses and long-term improvements in overall survival seen in chronic-phase CML upon BCR-ABL inhibition [94] are much less apparent in CML that has progressed to the accelerated phase or blast crisis [95,96].

Two key areas of research are essential. First, it is critical that wherever feasible, serial biopsies from untreated tumors and resulting treatment-refractory tumors are analyzed for mutations and other alterations in oncogenic signaling pathways. Such studies have already revealed a broader picture of resistance mechanisms [31]. As mentioned above for initial cancer diagnoses, systemic analyses of resistance in patients through methods such as circulating tumor DNA analyses [97] may aid in rapidly identifying clinically relevant resistance mutations. Second, prospective studies to identify acquired drug resistance mechanisms should be undertaken in the laboratory [98], even before resistance is seen in the clinic. Where performed comprehensively, such studies can guide subsequent analyses in clinical samples, as has been seen for enzalutamide-resistant prostate cancers [99–101] and BRAF and MEK inhibitor-resistant melanoma [102]. Similarly, comprehensive functional genomic screens to identify novel combination targets with existing therapeutics, or screens to identify novel nodes to target treatment-resistant disease, should be actively explored [103,104].

While, as described above, most cancers acquire therapeutic resistance through common themes that retain addiction to core oncogene pathways, an insidious emerging exception must be noted. Upon treatment with EGFR inhibitors, a substantial proportion of EGFR mutant lung cancers makes histological transformations to a small cell/neuroendocrine or mesenchymal phenotypes...
[105–107]. This is not unique to NSCLC; prostate cancers transform to small cell/neuroendocrine phenotypes in order to evade androgen deprivation therapies, and there is growing concern that this may be an increasing issue for antiandrogen therapies such as enzalutamide and abiraterone [108]. GISTs can also transdifferentiate, in this case to a rhabdomyoblastic state, upon imatinib resistance [109]. Recently, a change in melanoma cell state has also been suggested as a novel resistance mechanism for MAPK pathway inhibition [110]. Changes in cell state can completely abrogate dependencies on the signaling pathways present in the original tumor, negating most previously known therapeutic options. As targeted inhibitors (and combinations thereof) become more potent and selective, the transformation of cancers to a different cell type may become a more commonly observed phenomenon to bypass oncogene addiction. A better understanding of this phenomenon, and how to prevent it, is highly warranted.

Make better inhibitors

For many oncogenes, incomplete antitumor response and/or resistance can be facilitated simply by incomplete target inhibition. Incomplete inhibition could be due to insufficient drug potency, suboptimal pharmacokinetics, or poorly tolerated side effects that either preclude dosing to maximum efficacy or limit patient compliance. There are several clinical examples of more potent and selective ‘next-generation’ inhibitors showing responses superior to those of their predecessors. These include the efficacy of nilotinib and other next-generation BCR-ABL inhibitors in imatinib-pretreated CML [111–113], and the benefit of vemurafenib and dabrafenib in BRAF mutant melanoma, where the RAF/multikinase inhibitor sorafenib had previously failed [58,114]. Enzalutamide, a more potent androgen receptor (AR) antagonist, has shown clinical efficacy in castration-resistant prostate cancer, further validating continued addiction of these cancers to AR-dependent signaling [115]. The more potent and selective ALK inhibitors ceritinib and alectinib show efficacy in crizotinib-resistant NSCLC [116,117]. It is anticipated that next-generation PI3K inhibitors with an optimized selectivity profile will show benefit in PIK3CA mutant cancers, where many earlier generation drugs have not yet shown durable responses [118,119]. Well-optimized next-generation inhibitors therefore are not merely ‘me too’ drugs, but an essential part of our cancer therapeutic armamentarium.

As inhibitors become more potent and selective for their intended targets, an upper limit can be reached where the dose-limiting toxicity is due to inhibition of the normal physiological function of the wild-type proto-oncogene. For example, earlier generation EGFR inhibitors are associated with diarrhea, skin rash, and other likely ‘on-target’ toxicities due to inhibition of physiological EGFR-dependent functions in epithelia [120,121]. Acquired resistance mutations such as those at the T790M gatekeeper residue increase the affinity of the mutant enzyme for ATP and further tip the balance of early generation EGFR inhibitors in favor of blocking wild-type over mutant oncogenic EGFR function [122]. To address this issue, third-generation EGFR inhibitors have been designed to enhance the targeting of mutant EGFR isoforms, including the T790M gatekeeper mutation. These inhibitors show remarkable selectivity for mutant EGFR versus wild-type and therefore can be dosed more optimally to fully inhibit mutant EGFR signaling with a reduced liability of affecting physiological EGFR function in normal tissues [123,124]. This reduction in on-target side effects is a landmark step toward improving the efficacy of oncogene-selective drugs. Mutant-selective inhibition, wherever possible, should be a goal. Evidence suggests that this can be feasible for other oncogenes, as has been reported with the discovery of mutant-selective inhibitors of the IDH1 and IDH2 oncogenes [18,19].

Develop different modes (and nodes) of target inhibition

For many oncogenes, it is likely that there is more than one way to pharmacologically inhibit protein function—all potential therapeutic options should be explored, as there is no a priori notion of which mode of target inhibition is best. For example, most BCR-ABL inhibitors work through similar mechanisms, and therefore, all eventually engender resistance through kinase domain mutations such as the gatekeeper T315I alteration. Recently, allosteric BCR-ABL inhibitors have been discovered, which show a different spectrum of secondary resistance mutation versus ATP-binding site inhibitors [125]. Therefore, the combination of two different modes of inhibition targeting the same protein in parallel could dramatically reduce the occurrence of any one type of resistance mechanism. Allosteric kinase inhibition is not unique to BCR-ABL, as allosteric inhibition of other kinases such as MEK and AKT is attainable [126,127]. Indeed, allosteric MEK inhibitors that lock RAF:MEK protein complexes in an inactive state may provide a novel opportunity for more effective inhibition of BRAF- and MEK-dependent MAPK pathway signaling [128].

An alternate example of combining different therapeutic strategies against the same target is with the HER2 oncogene in breast cancer. Combination of HER2 targeting using both trastuzumab (targeting the extracellular domain) and lapatinib (targeting kinase activity) has shown significant improvements in pathological complete responses versus either agent alone [129], presumably because each drug targets a different part of the HER2 oncogene. Similarly, the HER2 antibody pertuzumab, which works in a mechanism complementary to trastuzumab, also shows combination benefit [130]. In addition, the observation that HER2 expression is maintained in trastuzumab-resistant breast cancer has spurred the use of T-DM1, an ADC that delivers the cytotoxic microtubule inhibitor DM1 to cancers via HER2-dependent internalization. This modality has shown remarkable efficacy in patients previously treated with trastuzumab and taxanes [131]—therefore, a combination of functional antibody and ADC approaches could reduce resistance.

Insight into oncogene-addicted signaling pathways affords the opportunity to discover key signaling nodes that can be exploited to more potently kill tumor cells. As mentioned above, most of the treatment-acquired resistance mechanisms to BRAF inhibitors in melanoma occur via restoration of MAPK pathway signaling. Thus, ‘vertical’ combination therapies simultaneously targeting different signaling nodes of the MAPK pathway (e.g., BRAF-, MEK-, ERK-, RTK-dependent signals) could overcome mutational or feedback-mediated resistance mechanisms and increase response to therapies. Indeed, results from clinical studies comparing single-agent RAF inhibitors versus RAF plus MEK inhibitor combinations demonstrate an increased rate of progression-free survival with the combination treatments [58,59]. Attacking multiple nodes in the HER2 signaling pathway may also provide benefit [132,133]. Some cancers may require ‘parallel’ combinations targeting different
oncogenes in order to see any benefit; this may be due either to comutation, rapid feedback upregulation, or other mechanisms. As examples, combined up-front BRAF and EGFR inhibition may blunt the rampant feedback pathway signaling present in BRAF mutant colorectal cancers [57]; co-inhibition of the MAPK and PI3K pathways may be required to see clinical benefit in many cancers with co-activation of these pathways [134]; and intrinsic resistance to EGFR-targeted therapies may occur through baseline upregulation of potentially targetable factors such as CRIP1T01 [135]. Finally, improved knowledge of oncogenic signaling in B-cell malignancies provides the rationale for BTK inhibition, which has shown dramatic clinical benefit; analysis of resulting resistance mutations further suggests vertical combinations within the same pathway that could address or hopefully prevent resistance [136].

**Make data-driven decisions on optimal dosing regimens**
While complete inhibition of oncogene pathways is a key goal, this does not always warrant continuous exposure to a drug, particularly if complete pathway inhibition engenders an oncogenic shock response. In these cases, intermittent dosing might effectively kill tumor cells while also reducing any on- or off-target side effects that could occur through continuous dosing. To this point, the BCR-ABL inhibitor dasatinib can induce apoptosis upon pulse dosing in BCR-ABL mutant CML cells, both in preclinical models and in CML patients [137]. These findings in CML were also extended, at least preclinically, to EGFR mutant cells treated with the EGFR inhibitor erlotinib [137]. Together, these results suggest high pulse doses of inhibitors could indeed unleash oncogenic shock phenotypes in cancer cells. Intermittent dosing could also prevent the induction of bypass resistance mechanisms. A recently reported primary melanoma xenograft model of vemurafenib resistance shows that in some instances, melanomas can become vemurafenib dependent. Vemurafenib withdrawal causes regression of such tumors, and an intermittent dosing strategy keeps both the drug-sensitive and drug-resistant cancer cell populations in check, leading to extended efficacy [138]. This finding is not unique to BRAF or the preclinical setting—resistance mutations in AR cause antagonists such as flutamide to become partial agonists [139]. Also, patients with acquired EGFR T790M resistance mutations who are taken off drug demonstrate loss of this mutation in their tumors, which may explain why these patients respond upon retreatment with EGFR inhibitors that do not target the T790M mutation [105,140]. Similar retreatment effects are also seen with crizotinib [141,142]. Such findings must be interpreted carefully, as drug holidays prior to the acquisition of resistance may actually speed the resistance process [143]; however, in total, these results suggest the optimization of dosing regimens for oncogene-targeted therapeutics is never ‘one size fits all.’ Optimal dosing schedules should be actively explored in preclinical models and inform the clinical testing of distinct dosing hypotheses in patients.

**Challenge our notions of what is druggable**
Cancer drug discovery has been rightly focused on kinases due to their clear genetic links to cancer, as well as their “druggability”—which simply means a proven ability to be inhibited by small molecules or antibodies. However, without the perspective of history, kinase druggability is not self-evident—the kinase pocket is highly conserved in paralogous proteins and the high intracellular concentration of ATP substrate could raise concerns for at least ATP-competitive inhibitors. Similar questions can be raised for virtually any novel target, and *a priori* notions of druggability should be avoided. While new classes of oncogenes will certainly not be easy to target, challenging mechanisms of target inhibition such as protein–protein interactions have yielded important breakthroughs with the discovery of p53/Mdm2 interaction inhibitors [144], BCL2 inhibitors [145], and SMAC mimetics [146], among others. The discovery of allosteric or induced-fit pockets on a protein may be a path forward for challenging cancer targets such as phosphatases [147] and metabolic enzymes [148]. This warrants a fresh look at well-validated but historically undruggable oncogene targets such as Ras oncogenes [149] and transcription factors [150,151]. If a target truly proves undruggable, systematic efforts to find potential synthetic lethal signaling nodes, as described below, are highly warranted.

**Comprehensively identify all liabilities of oncogene-addicted cells**
While a plethora of functional genomics screens have reported novel synthetic lethal targets to exploit oncogene (and non-oncogene) addiction, most studies rely on addressing single hypothesis in only one or a few cell lines. It is not uncommon that many such discoveries do not pan out in additional cell line or tumor models. In addition, many functional genomics studies rely on an RNAi approach—due to the known off-target effects of this method, it is essential that studies are sufficiently powered with multiple RNAi constructs per gene to avoid false-positive results. Most studies are underpowered in this regard. As such, the industry validation rate of many novel targets identified through ‘one-off’ functional approaches has been poor [152]. A few studies have begun to address such issues by either increasing the number of cell lines used [153] or increasing the number of RNAi constructs used per gene [154]. Studies that combine both, while labor-intensive, will be better powered to identify the best targets within and across cancers. In addition, novel approaches such as gene editing via the CRISPR/CAS9 system [155] could and should be explored as target identification methods, as the off-target profile of such approaches may be reduced or at least different from RNAi, and complete genetic knockout may yield novel targets that require a higher level of inhibition than partial RNAi gene knockdown can afford. Such approaches, performed at scale, will be critical for the identification of robust synthetic lethal or ‘non-oncogene’ targets for oncogene and tumor suppressor gene mutations [3,156].

**Make well-characterized disease-relevant models, and make them available**
Robust validation of genetic cancer dependencies in cell line and animal models is critical to progressing therapeutics toward the clinic. To facilitate this work, it is essential to have detailed characterization of available cell line and primary patient-derived xenograft models with respect to features including mutation, copy number, gene expression, and compound vulnerability, and to make such data publicly available to the research community, as has been done for the CCLE, CGP, and other cell line collections [157–159]. One limit to available cell line datasets is that some tumor lineages (e.g., prostate cancer) and genotypes (e.g., IDH1/2 mutation) are underrepresented. It will be critical for the research community to
identify these gaps in currently available models, and work to develop and characterize new in vitro and in vivo models for disease. Most importantly, the models should be readily available to the research community for use. With the increased use of PDX models [160], and the advent of alternate cell culture techniques that could facilitate the outgrowth of traditionally difficult tumor types such as glioma and prostate [161–163], comprehensive screens for oncogene vulnerabilities in currently underrepresented tumor types may soon be feasible.

Conclusions

Oncogene addiction is readily apparent from the remarkable responses seen in patients treated with drugs that target key mutated oncogenic drivers of their cancers. Potent inhibition of oncogenic signaling pathways can result in oncogenic shock, a robust apoptotic response that results in sustained remissions, not just transient growth inhibition. While acquired resistance to therapy remains a key challenge, clinically observed resistance mutations largely demonstrate that most cancers retain addiction to their original oncogenic signaling pathways, if not always the mutated oncogene itself. In addition to acquired resistance mutations, the induction of paradoxical bypass pathway activation that reactivates growth factor-dependent signaling (commonly through relief of MEK- or PI3K-dependent feedback inhibition) upon oncogene inhibition is likely pervasive across cancers and should be anticipated. Together, these findings underscore that many resistance mechanisms fall into predictable and therapeutically tractable themes, and can be effectively targeted with rationally designed combination therapies. Recognizing that this work is not completed until patients are cured of their disease, we have outlined some key items that must be addressed to take advantage of our growing knowledge of oncogene addiction. Not discussed in this review, but also essential to our ability to optimally kill cancers, are at least two additional approaches that should be combined with therapies that exploit oncogene addiction. First is the exploitation of somatic loss-of-function tumor suppressor gene mutations through the synthetic lethal targeting of ‘non-oncogene-addicted’ signaling nodes [3]. Second is the utilization of our emerging ability to reactivate immune responses to tumors [164,165]. As we learn to effectively combine these emerging therapeutic options, the road toward a cure is becoming clear.

Sidebar A: In need of answers

- What therapeutic combinations can best exploit oncogene addiction and result in oncogenic shock (versus a transient response)?
- How can therapeutics be optimized and/or combined to prevent resistance?
- As paradoxical bypass pathway activation is a pervasive response to many therapies, what accounts for the differences in the activation of these pathways (e.g., oncogenic shock for BCR-ABL inhibition vs. common bypass pathway activation for BRAF inhibition)? Can bypass signaling mechanisms be anticipated and therapeutically exploited?
- What actionable oncogenic mutations and pathways remain to be found across all cancers?
- What are the best ways to tackle inter- and intratumor heterogeneity?

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Author contributions

RAP, WS, and WRS all contributed to the concept and writing of this manuscript.

Conflict of interest

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