Pharmacokinetics, Clinical Indications, and Resistance Mechanisms in Molecular Targeted Therapies in Cancer

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I. Introduction

A. The Transition from Cytotoxic to Targeted Drugs

The strategy for discovery and development of cancer drugs has undergone a remarkable transformation over the past two decades. The discovery of cytotoxic chemotherapy began with the testing of nitrogen mustard at Yale in the 1940s (Gilman, 1963). For the next four decades, cancer drug discovery relied on efforts to inhibit DNA synthesis or function. One important approach aimed at blocking purine and pyrimidine nucleotide synthesis through the design of antifolates and base or nucleoside analogs such as 6-mercaptopurine or 5-fluorouracil. A second successful approach identified cytotoxic molecules from random natural product collections or from reactive synthetic chemicals (Chabner and Roberts, 2005). These studies did not have specific molecular targets in mind, but used tumor cell death or the inhibition of DNA synthesis as their endpoint. Often the drug’s specific mode of action was not revealed until years after the drug had been widely used in the treatment of human disease. Thus, the enzymatic target of methotrexate, an antifolate, was shown to be dihydrofolate reductase more than a decade after its initial clinical trials against childhood leukemia (Osborn et al., 1958). The target of anthracyclines, etoposide, and other inhibitors of topoisomerase II was discovered many years after these drugs were shown to be active and were approved for clinical use (Liu, 1989).

The transition to rationally designed, molecularly targeted drugs occurred in the period from 1990 to the present, following a dramatic expansion of knowledge of the molecular drivers of cell transformation and the identification of specific signaling pathways that controlled proliferation, cell death, angiogenesis, and metabolism. Based on this new knowledge, and in rapid succession, two different classes of molecules proved the value of the targeted drug discovery approach. Imatinib was the first rationally designed, low molecular weight inhibitor to achieve clinical success. Its target is the intracellular breakpoint cluster region (BCR–ABL) tyrosine kinase that drives cell proliferation in chronic myeloid leukemia. Imatinib produced

ABBREVIATIONS: ADCC, antibody-dependent cellular cytotoxicity; AKT, protein kinase B; ALK, anaplastic lymphoma kinase; AP26113, 5-chloro-N2-(4-bromo-2-chlorophenyl)amino]-7-fluoro-4-(dimethylamino)-2-butenamide; BIBW2992, N-[4-(3-chloro-4-fluorophenyl)amino]-7-fluoro-N-(2-hydroxyethoxy)-3-methylbenzimidazol-5-carboxamide; BCR, breakpoint cluster region; BIM, Bcl-2-interacting mediator of cell death; CDC, complement-dependent cytotoxicity; CLL, chronic lymphocytic leukemia; CRC, colorectal cancer; CRKL, V-Crk sarcoma virus CT10 oncogene homolog-like; CSF, cerebrospinal fluid; EC, endothelial cell; EGFR, epidermal growth factor receptor; EML, echinoderm microtubule-associated protein-like gene; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; FKB, FK506-binding; GBM, glioblastoma multiforme; GIST, gastrointestinal stromal tumor; HER2, human epidermal growth factor receptor 2; HGF, hepatic growth factor; HIF-1a, hypoxia-inducible factor a; HKI-272, (2E)-N-[4-(3-chloro-4-([pyridin-2-yl)methoxy]phenyl)amino]-3-cyano-7-ethoxyquinolin-6-yl]-4-[dimethylamino]but-2-enamide; IGF-1R, insulin-like growth factor 1 receptor; IGF-IR, insulin-like growth factor 1 receptor; LDK378, 5-chloro-N2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N4-(2-isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine; mAb, monoclonal antibody; MAP, mitogen-activated protein; MAPK, MAP kinase; mCRC, metastatic colorectal cancer; MEGK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; NSCLC, non-small-cell lung cancer; OCT-1, organic cation transporter 1; pB1BRAF, 61-kDa splicing product of BRAF in resistant cell lines; OS, overall survival; PD-1, programmed death-1; PDGFR, platelet-derived growth factor receptor; PDGFRa, platelet-derived growth factor receptor alpha; PF-00299804, (E)-N-[4-(3-chloro-4-fluoro-anilino)-7-methoxy-quinazolin-6-yl]-4-41-piperidyl]but-2-enamide hydrate; PFS, progression free survival; PDK, phosphotyrosinol 3-kinase; PiG, placental growth factor; PTEN, phosphatase and tensin homolog; RCC, renal cell carcinoma; TKI, tyrosine-kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; WT, wild type; WZ4002, N-3-(3,5-chloro-2-[2-methoxy-4-(4-methylpiperazin-1-yl)phenyl]iminod-4-oxo)phenyl)acylamide; X-396, (E)-6-amino-5-(1-[2,6-dichloro-5-fluorophenylethoxy]-N-4-(4-methylpiperazin-1-yl)carbonylphenyl)pyridazine-3-carboxamide; XL184, N-[4-(6,7-dimethoxyquinolin-5-yl)oxy]phenyl]-N’-[4-(4-fluorophenyl)cylopropane-1,1-dicarboxamide.
long-lasting molecular remissions in this disease in its earliest trials in 1998, and later proved effective against c-KIT–driven gastrointestinal stromal tumors (Druker et al., 2001). The epidermal growth factor receptor (EGFR) inhibitors gefitinib and erlotinib were used with variable success in a small number of patients before the identification of sensitizing EGFR mutations. Identification of this oncogenic driver provided the molecular basis for the stratification of patients with non–small-cell lung cancer (NSCLC) with sensitizing EGFR mutations. Treatment of this patient population with EGFR inhibitors produced consistent responses. At the same time, trastuzumab, a monoclonal antibody against the human epidermal growth factor receptor 2 (HER2), which is amplified in 25% of breast cancers, dramatically enhanced the efficacy of taxanes and other chemotherapy in both metastatic and adjuvant therapy. Other antibodies, including rituximab, which targets CD20 and is effective in the treatment of non–Hodgkin’s lymphoma, showed similar enhancement of chemotherapy. Bevacizumab, a monoclonal antibody that binds the vascular endothelial growth factor (VEGF) and blocks tumor angiogenesis, prolonged cytotoxic drug-induced responses in colon cancer and NSCLC (Hurwitz et al., 2004).

Following these fundamental discoveries, a series of more than 30 new drugs, all targeted to specific receptors or enzymes, found useful application in specific subsets of human cancers. Table 1 provides a summary of key clinical trials that led to approval of U.S. Food and Drug Administration–approved targeted therapies. At the same time, interest in the discovery and development of new cytotoxic molecules has waned. Perhaps the only significant new cytotoxics in the past decade have been bendamustine, a unique alkylating agent highly active in lymphoid malignancies and available in Europe since the 1980s (Hoy, 2012); eribulin, a marine natural product active in breast cancer (Scarpace, 2012); and trabectedin, also a marine natural product useful in second-line treatment of sarcomas (Samuels et al., 2013).

Although much attention has turned to understanding the molecular interaction of targeted drugs and the pathways they inhibit in cancer cells, less consideration has been paid to the clinical pharmacological properties of these carefully synthesized molecules. For the first time, oncologists now routinely use monoclonal antibodies and oral agents that are largely free of myelosuppression and other serious host toxicity that complicate cytotoxic chemotherapy.

An important difference between targeted therapy and conventional chemotherapy with curative intent is the continuous treatment with targeted therapy after treatment response. In the treatment of some malignancies, maintenance therapy with targeted therapies is even continued in cases of progression to prevent uncontrolled tumor growth. Cytotoxic chemotherapy cannot be used in this way, mostly due to intolerability, except in maintenance therapy of acute leukemia with 6-mercaptopurine and methotrexate, where continuous treatment is curative.

Common themes have emerged regarding the pharmacokinetics, toxicities, drug interactions, and mechanisms of tumor cell resistance of these unique agents. In particular, in contrast to natural products or alkylating agents, the synthetic targeted inhibitors incorporate a number of favorable properties not found in most cytotoxics. As a class, they have good bioavailability, a slow rate of metabolism, and favorable distribution, all properties that are engineered into the clinical candidate in the preclinical phase of development. However, unfavorable effects in humans are difficult to predict at the preclinical stage. Off-target toxicities and drug interactions emerge during clinical testing and may present serious hurdles to further clinical development. Understanding and dealing with these pharmacological properties is a critical step in successful drug development.

B. General Properties of Synthetic Targeted Inhibitor Cancer Drugs. The discovery of a new targeted agent begins with validation of the target and construction of a high-throughput assay system of identifying inhibitors. Systems for validating the target and establishing high-throughput assays are covered in detail in other articles (Garnett et al., 2012). In most instances, a “hit,” a compound that inhibits the target enzyme or receptor at low micromolar concentrations, is found by screening compounds from an appropriate library, which is often chosen because it contains a collection of synthetic chemicals enriched for structures likely to mimic substrates of the target enzyme. For example, the search for inhibitors of histone methyltransferases centered on analogs of the key substrate, S-adenosyl methionine (Copeland et al., 2013). Through further chemical modification of the lead compound, and often with the guidance of X-ray crystallographic pictures of the inhibitor–enzyme complex, the molecule can be altered to increase ionic and hydrophobic bonding within the substrate pocket and to improve steric conformation, resulting in an inhibitor with low nanomolar potency. The physicochemical properties are further refined to enhance uptake and stability in cell culture systems, to improve pharmacokinetics and bioavailability in vivo, to decrease susceptibility to multidrug resistance, and to produce relative metabolic stability in the presence of human microsomes. Through in vitro testing against human microsomes and studies of metabolism in rodents, it is possible to develop a reasonable prediction of metabolic behavior of the compound in humans, and to further modify the molecule to improve its stability. However, all predictions regarding the properties in humans require confirmation through detailed studies in early clinical trials, as there are often
TABLE 1
FDA–approved targeted therapies with specific FDA-labeled indications
This table gives an overview of clinical trials that led to approval of each drug and extension of the FDA label for particular indications following initial approval. Important key trials were added at the discretion of the authors. Accelerated approval of drugs for particular indications is highlighted in the indications column.

<table>
<thead>
<tr>
<th>Drug, U.S. FDA-Labeled Approval(s)</th>
<th>Study Name</th>
<th>Phase</th>
<th>Study Population, Size</th>
<th>Intervention(s)</th>
<th>Primary Outcome(s)</th>
<th>Selected Secondary Outcomes</th>
<th>Reference(s)</th>
</tr>
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<tbody>
<tr>
<td>Alemtuzumab</td>
<td></td>
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<tr>
<td>B-CLL, previously untreated</td>
<td></td>
<td>3</td>
<td>N = 297</td>
<td>Alemtuzumab versus chlorambucil</td>
<td>PFS: 14.6 versus 11.7 months*</td>
<td>RR: 83.2% versus 55.4% * PR: 21%–31% CR: 0%–2%</td>
<td>(Hillmen et al., 2007)</td>
</tr>
<tr>
<td>Initial accelerated approval</td>
<td></td>
<td>N/A</td>
<td>N = 149; 3 single-arm studies</td>
<td>Alemtuzumab</td>
<td></td>
<td></td>
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<tr>
<td>Axitinib</td>
<td>AXIS</td>
<td>3</td>
<td>N = 723</td>
<td>Axitinib versus sorafenib</td>
<td>PFS: 6.7 versus 4.7 months*</td>
<td>ORR: 19.4% versus 9.4%*</td>
<td>(Rini et al., 2011)</td>
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<tr>
<td>Advanced RCC after failure of prior systemic therapy</td>
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<td>Metastatic CRC, with 5-FU, first- or second-line therapy</td>
<td>Study 2107</td>
<td>3</td>
<td>Previously untreated, N = 813</td>
<td>Bevacizumab versus placebo, with IFL</td>
<td>OS: 20.3 versus 15.6 months*</td>
<td>PFS: 10.6 versus 6.2 months* ORR: 44.8% versus 34.8%*</td>
<td>(Hurwitz et al., 2004)</td>
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<tr>
<td>E3200</td>
<td></td>
<td>3</td>
<td>Previously treated, N = 829</td>
<td>FOLFOX4, bevacizumab, versus both</td>
<td>OS: 12.9 (combination) versus 10.8 months (FOLFOX)*</td>
<td>PFS: 7.3 versus 4.7 months* ORR: 22.7% versus 8.6%*</td>
<td>(Giantonio et al., 2007)</td>
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<tr>
<td>Metastatic CRC, second-line after first progression on bevacizumab-based chemotherapy</td>
<td>ML18147</td>
<td>3</td>
<td>N = 820</td>
<td>Bevacizumab with chemotherapy (oxaliplatin- or irinotecan-based) versus chemotherapy alone</td>
<td>OS: 11.2 versus 9.8 months*</td>
<td>PFS: 5.7 versus 4.1 months*</td>
<td>(Bennouna et al., 2013)</td>
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<tr>
<td>Advanced NSCLC, nonsquamous, with carboplatin/paclitaxel, first-line</td>
<td>E4599</td>
<td>3</td>
<td>N = 878</td>
<td>Paclitaxel and carboplatin +/- bevacizumab</td>
<td>OS: 12.3 versus 10.3 months*</td>
<td>PFS: 6.2 versus 4.5 months* ORR: 33% versus 15%*</td>
<td>(Sandler et al., 2006)</td>
</tr>
<tr>
<td>AVAiL</td>
<td></td>
<td>3</td>
<td>N = 1043</td>
<td>Cisplatin/gemcitabine with bevacizumab 7.5 mg/kg versus bevacizumab 15 mg/kg versus placebo</td>
<td>PFS: 6.7 (7.5 mg/kg) versus 6.5 (15 mg/kg) versus 6.1 months (chemotherapy) *</td>
<td>OS: 13.1 (placebo) versus 13.6 months (7.5 mg/kg) versus 13.4 months (15 mg/kg)</td>
<td>(Reck et al., 2009, 2010)</td>
</tr>
<tr>
<td>Glioblastoma, progressive after prior therapy Accelerated approval</td>
<td>NCI-06-C-0064E</td>
<td>2</td>
<td>N = 48</td>
<td>Bevacizumab</td>
<td>Radiographic response rate: 71% PFS at 6 months: 42.6% bevacizumab versus 50.3% combination*</td>
<td>OS: 31 weeks</td>
<td>(Kreial et al., 2009)</td>
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<tr>
<td>BRAIN</td>
<td></td>
<td>2</td>
<td>First or second relapse, N = 167</td>
<td>Bevacizumab +/- irinotecan</td>
<td>PFS at 6 months: 42.6% bevacizumab versus 50.3% combination*</td>
<td>OS: 9.2 versus 8.7 months (significance testing not provided)</td>
<td>(Friedman et al., 2009)</td>
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<td>Ovarian cancer, advanced newly diagnosed and stage IV</td>
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<tr>
<td>Metastatic RCC, with INFalpha</td>
<td>AVOREN Trial</td>
<td>3</td>
<td>Previously untreated, N = 649</td>
<td>IFN-α2a with bevacizumab versus IFN with placebo</td>
<td>OS: 23.3 versus 21.3 months</td>
<td>PFS: 10.2 versus 5.4 months*</td>
<td>(Escudier et al., 2007a, 2010)</td>
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</tbody>
</table>

Bortezomib (continued)
<table>
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<tr>
<th>Drug, U.S. FDA-Labeled Approval(s)</th>
<th>Study Name</th>
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<th>Primary Outcome(s)</th>
<th>Selected Secondary Outcomes</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Multiple myeloma, first-line</strong></td>
<td>VISTA</td>
<td>3</td>
<td>Ineligible for stem cell transplant, N = 682</td>
<td>Melphalan/prednisone +/- bortezomib</td>
<td>TTP: 24 versus 16.6 months*</td>
<td>CR: 30% versus 4%* OS, median: 56.4 versus 43.1 months* ORR: 38% versus 18%* OS: 29.8 versus 23 months*</td>
<td>(San Miguel et al., 2008)</td>
</tr>
<tr>
<td><strong>Multiple myeloma, relapsed/refractory</strong></td>
<td>APEX</td>
<td>3</td>
<td>Relapsed after 1-3 prior therapies, N = 669</td>
<td>Bortezomib versus high-dose dexamethasone</td>
<td>TTP: 6.22 versus 3.49 months*</td>
<td>ORR: 35%</td>
<td>(Richardson et al., 2005, 2007)</td>
</tr>
<tr>
<td><strong>SUMMIT</strong></td>
<td>2</td>
<td>N = 202</td>
<td>Bortezomib (dexamethasone if poor response)</td>
<td>ORR: 50% (1.3 mg/m²), 33% (1 mg/m²)</td>
<td>TTP: 11 months (1.3 mg/m²), 7 months (1 mg/m²) OS: median not reached (1.3 mg/m²) and 26.7 months (1 mg/m²)</td>
<td>ORR: 33%</td>
<td>(Fisher et al., 2006)</td>
</tr>
<tr>
<td><strong>CREST</strong></td>
<td>2</td>
<td>N = 54</td>
<td>Bortezomib (two dosages, with dexamethasone if poor response)</td>
<td>TTP: 11 months (1.3 mg/m²), 7 months (1 mg/m²) OS: median not reached (1.3 mg/m²) and 26.7 months (1 mg/m²)</td>
<td>ORR: 33%</td>
<td>(Jagannath et al., 2004)</td>
<td></td>
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<tr>
<td><strong>Multiple myeloma, SQ administration</strong></td>
<td>3</td>
<td>Relapsed after 1-3 prior therapies, N = 222</td>
<td>Bortezomib SQ versus i.v.</td>
<td>ORR at 4 months: 42% in both groups</td>
<td>TTP: 19.4 versus 11.7 months OS, 1 year: 72.6% versus 76.7% ORR: 33% DR: 9.2 months TTP: 6.2 months</td>
<td>(Moreau et al., 2011)</td>
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<tr>
<td><strong>Mantle cell lymphoma, previously treated</strong></td>
<td>PINNACLE</td>
<td>2</td>
<td>N = 155</td>
<td>Bortezomib</td>
<td>TTP versus historical controls: insufficient controls</td>
<td>ORR: 34% OS: 22.4 months DR: 12.6 months PI</td>
<td>(Fisher et al., 2012)</td>
</tr>
<tr>
<td><strong>Brentuximab vedotin</strong></td>
<td>Hodgkin's lymphoma, relapsed/refractory after auto SCT or at least two chemotherapy regimens</td>
<td>2</td>
<td>N = 102</td>
<td>Brentuximab vedotin</td>
<td>ORR: 75%</td>
<td>PFS: 5.6 months</td>
<td>(Younes et al., 2012)</td>
</tr>
<tr>
<td><strong>Accelerated approval</strong></td>
<td>sALCL, after failed systemic therapy</td>
<td>2</td>
<td>N = 58</td>
<td>Brentuximab vedotin</td>
<td>ORR: 86%</td>
<td>OS: 22.4 months DR: 12.6 months</td>
<td>(PI)</td>
</tr>
<tr>
<td><strong>Carfilzomib</strong></td>
<td>Multiple myeloma, relapsed/refractory</td>
<td>2</td>
<td>N = 266</td>
<td>Carfilzomib</td>
<td>ORR: 23.7%</td>
<td>DR: 7.8 months</td>
<td>(Siegel et al., 2012)</td>
</tr>
<tr>
<td><strong>Cetuximab</strong></td>
<td>Metastatic CRC, K-RAS WT, EGFR+, with FOLFIRI, first-line</td>
<td>3</td>
<td>N = 1198</td>
<td>FOLFIRI with versus without cetuximab</td>
<td>PFS: 8.9 versus 8.0 months*</td>
<td>OS (K-RAS WT): 23.5 versus 20 months* PFS (K-RAS WT): 9.9 versus 8.4 months* ORR (K-RAS WT): 59.3 versus 43.2%* TTP: 4.1 versus 1.5 months* OS: 8.6 versus 6.9 months</td>
<td>(Van Cutsem et al., 2009, 2011)</td>
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<tr>
<td><strong>Metastatic CRC, K-RAS WT, EGFR+, with irinotecan, in patients refractory to irinotecan-based chemotherapy</strong></td>
<td>BOND</td>
<td>2</td>
<td>N = 329</td>
<td>Cetuximab with versus without irinotecan</td>
<td>ORR: 22.9% (combination) versus 10.8% (cetuximab)*</td>
<td>TTP: 4.1 versus 1.5 months* OS: 8.6 versus 6.9 months</td>
<td>(Cunningham et al., 2004)</td>
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<tr>
<td><strong>Metastatic CRC, K-RAS WT, EGFR+, single agent, in patients who have failed oxaliplatin and irinotecan-</strong></td>
<td>CRYSTAL</td>
<td>3</td>
<td>N = 572</td>
<td>Cetuximab versus BSC</td>
<td>OS: 6.1 months versus 4.6 months*</td>
<td>PFS: HR 0.68*</td>
<td>(Jonker et al., 2007)</td>
</tr>
<tr>
<td>Drug, U.S. FDA-Labeled Approval(s)</td>
<td>Study Name</td>
<td>Phase</td>
<td>Study Population, Size</td>
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<td>Locoregionally advanced SCCHN, with radiation therapy or are intolerant to irinotecan</td>
<td>Locoregionally advanced SCCHN, with radiation therapy</td>
<td>3</td>
<td>N = 424</td>
<td>Radiotherapy with versus without cetuximab</td>
<td>Duration of disease control: 24.4 versus 14.9 months*</td>
<td>OS: 49 versus 29.3 months* PFS: 17.1 versus 12.4 months*</td>
<td>(Bonner et al., 2006)</td>
</tr>
<tr>
<td>Recurrent or metastatic SCCHN, with platinum-based therapy/5-FU</td>
<td>EXTREME</td>
<td>3</td>
<td>N = 442</td>
<td>Platinum-based chemotherapy with versus without cetuximab</td>
<td>OS: 10.1 versus 7.4 months*</td>
<td>PFS: 5.6 versus 3.3 months* ORR: 35% versus 20%*</td>
<td>(Vermorken et al., 2008)</td>
</tr>
<tr>
<td>Recurrent locoregional/metastatic SCCHN, single agent, after failure of platinum-based chemotherapy</td>
<td>N = 103</td>
<td>2</td>
<td>Cetuximab, with platinum-based chemotherapy if poor response</td>
<td>ORR: 13%</td>
<td>TTP: 70 days</td>
<td>OS: 178 days</td>
<td>(Vermorken et al., 2007)</td>
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<tr>
<td>Crizotinib</td>
<td>NSCLC, locally advanced or metastatic, ALK positive</td>
<td>Study A</td>
<td>2</td>
<td>N = 136</td>
<td>Crizotinib</td>
<td>ORR: 50%</td>
<td>DR: 41.9 weeks</td>
</tr>
<tr>
<td>Accelerated approval</td>
<td>Study B</td>
<td>1</td>
<td>N = 119</td>
<td>Crizotinib</td>
<td>ORR: 61%</td>
<td>DR: 48 weeks</td>
<td>PFS: 10 months</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>CML-CP, Ph+, newly diagnosed</td>
<td>DASISION</td>
<td>3</td>
<td>CML-CP, N = 519</td>
<td>Dasatinib (once daily) versus imatinib</td>
<td>CCyR at 12 months: 77% versus 66%*</td>
<td>MMR: 52% versus 34%*</td>
</tr>
<tr>
<td>Accelerated approval</td>
<td>CML-CP, resistant or intolerant to imatinib, once daily dosing</td>
<td>Accelerated approval for daily dosing</td>
<td>3</td>
<td>CML-CP, N = 670</td>
<td>Dasatinib dose optimization</td>
<td>MCyR:</td>
<td>OS, estimated at 24 months</td>
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<tr>
<td>START-C</td>
<td>CML-CP, N = 186</td>
<td>Dasatinib BID</td>
<td>MCyR at 24 months: 53% versus 33%*</td>
<td>CHR: 93% versus 82%*</td>
<td>TTP: not reached versus 3.5 months*</td>
<td></td>
<td>(Kantarjian et al., 2007a)</td>
</tr>
<tr>
<td>START-R</td>
<td>CML-CP, N = 150</td>
<td>Dasatinib BID (70 mg PO BID) versus imatinib</td>
<td>MCyR at 24 months: 53% versus 33%*</td>
<td>CHR: 93% versus 82%*</td>
<td>TTP: not reached versus 3.5 months*</td>
<td></td>
<td>(Kantarjian et al., 2007a)</td>
</tr>
<tr>
<td>CML advanced phase or Ph+ ALL, resistant or intolerant to imatinib</td>
<td>Accelerated approval</td>
<td>3</td>
<td>CML-AP, MB, LB, Ph+ ALL, N = 611</td>
<td>Dasatinib 140 mg daily versus 70 mg BID</td>
<td>MaHR: Daily versus BID</td>
<td>OS: 63 vs. 72% (no significant difference) FFS: HR 0.14*</td>
<td>(Kantarjian et al., 2009; Lilly et al., 2010; Saglio et al., 2010a)</td>
</tr>
<tr>
<td>CML-AP: 66% versus 68%</td>
<td>CML-MB: 28% versus 28%</td>
<td>CML-LB: 42% versus 32%</td>
<td>Ph+ ALL: 38% vs 32%</td>
<td>Ph+ ALL: 6.5 months, median</td>
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<tr>
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<tbody>
<tr>
<td>START-A</td>
<td>2 CML-AP, N = 174</td>
<td>Dasatinib BID</td>
<td>MaHR: 64%</td>
<td>PFS, at 12 months: 66%</td>
<td>OS, at 12 months: 82%</td>
<td>(Apperley et al., 2009)</td>
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<tr>
<td>START-B and START-L</td>
<td>2 CML-MB, N = 74</td>
<td>Dasatinib BID</td>
<td>MaHR: 34% (CML-MB), 31% (CML-LB)</td>
<td>PFS: 6.7 months (CML-MB), 3.0 months (CML-LB)</td>
<td>OS: 11.8 months (CML-MB), 5.3 months (CML-LB)</td>
<td>(Cortes et al., 2007)</td>
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<tr>
<td>CML-LB, N = 42</td>
<td></td>
<td>Dasatinib BID</td>
<td>MaHR: 34% (CML-MB), 31% (CML-LB)</td>
<td>PFS: 6.7 months (CML-MB), 3.0 months (CML-LB)</td>
<td>OS: 11.8 months (CML-MB), 5.3 months (CML-LB)</td>
<td>(Cortes et al., 2007)</td>
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<tr>
<td>START-L</td>
<td>2 Ph+ ALL, N = 36</td>
<td>Dasatinib BID</td>
<td>MaHR: 42%</td>
<td>PFS: 6.7 months (CML-MB), 3.0 months (CML-LB)</td>
<td>OS: 11.8 months (CML-MB), 5.3 months (CML-LB)</td>
<td>(Cortes et al., 2007)</td>
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<tr>
<td>Denileukin diftitox</td>
<td>3 CTCL, stage IA to III, N = 144</td>
<td>Denileukin diftitox</td>
<td>ORR: 49.1% (18 μg/kg) vs 37.8% (9 μg/kg) vs 15.9% (placebo)</td>
<td>PFS: 971 days (18 μg/kg) vs 794 days (9 μg/kg) vs 124 days (placebo)</td>
<td>(Prince et al., 2010)</td>
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<tr>
<td>Erlotinib</td>
<td>3 CTCL, stage Ib to III, N = 71</td>
<td>Denileukin diftitox</td>
<td>ORR: 30%</td>
<td>DR: 6.9 months</td>
<td>(Olsen et al., 2001)</td>
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<tr>
<td>SATURN</td>
<td>3 N = 889</td>
<td>Erlotinib versus placebo</td>
<td>PFS: 12.3 versus 11.1 weeks*</td>
<td>OS: 12.0 vs 11.0 months*</td>
<td>(Cappuzzo et al., 2010; Shepherd et al., 2005).</td>
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<tr>
<td>Locally advanced or metastatic NSCLC, maintenance after first-line therapy</td>
<td>3 N = 731</td>
<td>Erlotinib</td>
<td>OS: 6.7 versus 4.7 months*</td>
<td>ORR: 8.9% versus &lt;1%*</td>
<td>PFS: 2.2 versus 1.8 months*</td>
<td>(Olsen et al., 2001)</td>
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<tr>
<td>Locally advanced or metastatic pancreatic cancer, with gemcitabine, first-line therapy</td>
<td>3 N = 569</td>
<td>Gemcitabine with erlotinib</td>
<td>OS: 6.24 versus 5.91 months*</td>
<td>One year survival: 23% versus 17%*</td>
<td>PFS: 3.75 versus 3.55 months*</td>
<td>(Moore et al., 2007)</td>
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<tr>
<td>Everolimus</td>
<td>3 N = 724</td>
<td>Exemestane, with everolimus versus placebo</td>
<td>PFS: 10.6 versus 4.1 months*</td>
<td>ORR: 9.5% versus 0.4%*</td>
<td>(Baselga et al., 2012a)</td>
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<tr>
<td>Advanced HER2(-), HR(+) breast cancer, postmenopausal women, with exemestane, after failed letrozole or anastrozole</td>
<td>3 N = 410</td>
<td>Everolimus versus placebo</td>
<td>PFS: 11.0 versus 4.6 months*</td>
<td>(Yao et al., 2011)</td>
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<tr>
<td>Progressive PNET, locally advanced/metastatic disease</td>
<td>3 N = 410</td>
<td>Everolimus versus placebo</td>
<td>PFS: 4.0 versus 1.9 months*</td>
<td>OS: no significant difference</td>
<td>(Motzer et al., 2008)</td>
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<tr>
<td>Advanced RCC, after failure of sunitinib or sorafenib</td>
<td>3 N = 118</td>
<td>Everolimus versus placebo</td>
<td>ORR: 42% versus 0%*</td>
<td>(Bissler et al., 2013)</td>
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<tr>
<td>Renal angiomylipoma with TSC, not requiring immediate surgery</td>
<td>3 N = 117</td>
<td>Everolimus (suspension) versus placebo</td>
<td>50%+ reduction of target lesion volume: 35% versus 0%*</td>
<td>Est PFS at 6 months: 100% versus 86%*</td>
<td>(Franz et al., 2013)</td>
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<tr>
<td>SEGA, TSC-associated, unresectable</td>
<td>3 N = 117</td>
<td>Everolimus (suspension) versus placebo</td>
<td>50%+ reduction of target lesion volume: 35% versus 0%*</td>
<td>Est PFS at 6 months: 100% versus 86%*</td>
<td>(Franz et al., 2013)</td>
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<tbody>
<tr>
<td><em>Ibritumomab tiuxetan</em></td>
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<tr>
<td>Follicular lymphoma, consolidation therapy after response to first-line therapy</td>
<td>1-2</td>
<td>N = 28</td>
<td>Everolimus (tablets)</td>
<td>Median reduction in primary lesion volume at 6 months: 0.80 cm^3*</td>
<td>Median one fewer seizure over 6 months*</td>
<td>(Krueger et al., 2010)</td>
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<tr>
<td>Relapsed/refractory low-grade or follicular non–Hodgkin’s lymphoma</td>
<td>3</td>
<td>Stage III/IV, N = 414</td>
<td>Ibritumomab regimen versus observation</td>
<td>PFS: 36.5 versus 13.3 months*</td>
<td>CR/CRu: 87.5% versus 53.3% (significance testing not provided)</td>
<td>(Morschhauser et al., 2008)</td>
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<tr>
<td>Accelerated approval</td>
<td>3</td>
<td>N = 130</td>
<td>Rituximab naive</td>
<td>Ibritumomab regimen versus rituximab</td>
<td>ORR: 83% versus 55%*</td>
<td>TTP: 12.1 versus 10.1 months</td>
<td>(Witzig et al., 2002a)</td>
</tr>
<tr>
<td><em>Imatinib</em></td>
<td>3</td>
<td>N = 1106</td>
<td>Ibritumomab regimen versus rituximab</td>
<td>ORR: 74%</td>
<td>CR: 15%</td>
<td>TTP: 6.8 months</td>
<td>(Witzig et al., 2002b)</td>
</tr>
<tr>
<td>Ph+ CML, chronic phase, newly diagnosed</td>
<td>2</td>
<td>CML, late chronic phase, N = 532</td>
<td>Imatinib</td>
<td>ORR: 83%</td>
<td>CR 37%</td>
<td>DR: 6.4 months</td>
<td>(Wiseman et al., 2002)</td>
</tr>
<tr>
<td>Initial accelerated approval</td>
<td>2</td>
<td>CML-AP, N = 235</td>
<td>Imatinib</td>
<td>ORR: 55.6%</td>
<td>MCyR: 16.8%</td>
<td>OS: 6 year estimated: 76%</td>
<td>(Hochhaus et al., 2008; Kantarjian et al., 2002)</td>
</tr>
<tr>
<td>Ph+ CML (CP, AP, or BC), after failed IFN-α therapy</td>
<td>2</td>
<td>CML-blast crisis, N = 260</td>
<td>Imatinib</td>
<td>Sustained hematological response, lasting at least 4 weeks: 30.6%</td>
<td></td>
<td></td>
<td>(Sawyers et al., 2002)</td>
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<tr>
<td>Initial accelerated approval</td>
<td>2</td>
<td>Pediatric CML-CP, N = 51</td>
<td>Imatinib</td>
<td>CHR 78% at 8 weeks</td>
<td>CCyR 71%</td>
<td></td>
<td>PI</td>
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<tr>
<td>CML, pediatric (initial) accelerated approval</td>
<td>1</td>
<td>Pediatric CML-CP, recurrent, N = 14</td>
<td>Imatinib</td>
<td>CCyR 71%</td>
<td></td>
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<td>PI</td>
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<tr>
<td></td>
<td>1</td>
<td>CML-CP, resistant to IFN-α, N = 3</td>
<td>Imatinib</td>
<td>CCyR: 2 of 3 patients</td>
<td></td>
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<td>PI</td>
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<tr>
<td>KIT+ GIST, adjuvant treatment after resection</td>
<td>3</td>
<td>N = 397</td>
<td>Imatinib for 12 versus 36 months</td>
<td>5-year RFS: 65.6% versus 47.9%*</td>
<td>5-year OS: 92% versus 81.7%*</td>
<td>(Joensuu et al., 2012)</td>
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<tr>
<td>Initial accelerated approval</td>
<td>3</td>
<td>N = 713</td>
<td>Imatinib versus placebo</td>
<td>1-year RFS: 98% versus 83%*</td>
<td>1-year OS: No significant difference</td>
<td>(Dematteo et al., 2009)</td>
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<tr>
<td>KIT+ GIST, unresectable/ metastatic</td>
<td>3</td>
<td>N = 746</td>
<td>Imatinib daily versus BID</td>
<td>PFS: 18 months (daily) versus 20 months (BID)</td>
<td>OS: 55 months (daily) versus 51 months (BID)</td>
<td>(Blanke et al., 2008)</td>
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</tr>
<tr>
<td>Initial accelerated approval</td>
<td>3</td>
<td>N = 946</td>
<td>Imatinib daily versus BID</td>
<td>PFS at median 760 day follow-up: 56% (one daily) versus 50% (twice daily)*</td>
<td>CR: 5%, no significant difference between groups</td>
<td>(Verweij et al., 2004)</td>
<td></td>
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<tr>
<td>Ph+ ALL, relapsed/refractory</td>
<td>2</td>
<td>N = 48</td>
<td>Imatinib</td>
<td>CHR: 19%</td>
<td></td>
<td>TTP 2.2 months</td>
<td>(continued)</td>
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<tr>
<td>Drug, U.S. FDA-Labeled Approval(s)</td>
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<tr>
<td>MDS/MPD, with PDGFR rearrangement</td>
<td>NA</td>
<td>Phase 2/case reports, N = 31</td>
<td>Imatinib</td>
<td>OS: 4.9 months</td>
<td>CHR 45%</td>
<td>PI</td>
<td>Ottmann et al., 2002</td>
</tr>
<tr>
<td>ASM without c-Kit mutation/unknown c-Kit status</td>
<td>NA</td>
<td>Phase 2/case reports, N = 28</td>
<td>Imatinib</td>
<td>OS: 10.0 months (combination) * versus 10.1 months (ipilimumab)* versus 6.4 months (gp100)</td>
<td>ORR: 10.9% (ipilimumab)* versus 5.7% (combination)* versus 1.5% (gp 100 alone)</td>
<td>(Hodi et al., 2010)</td>
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<tr>
<td>HES/CEL, with FIP1L1-PDGFalpha or unknown status</td>
<td>NA</td>
<td>Phase 2/case reports, N = 176</td>
<td>Imatinib</td>
<td>OS, adjusted for crossover: 75 versus 56.4 weeks*</td>
<td>ORR (HER2+): 27.9 versus 14.3%*</td>
<td>(Schwartzberg et al., 2010), PI</td>
<td></td>
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<tr>
<td>DFSP, unresectable/recurrent/metastatic</td>
<td>NA</td>
<td>Phase 2/case reports, N = 18</td>
<td>Imatinib</td>
<td>OS at 3 years: no significant difference</td>
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<tr>
<td>Ipilimumab</td>
<td>3</td>
<td>N = 676</td>
<td>Ipilimumab with gp100 vaccine, versus ipilimumab vaccine</td>
<td>OS: 10.0 months (combination) * versus 10.1 months (ipilimumab)* versus 6.4 months (gp100)</td>
<td>ORR: 10.9% (ipilimumab)* versus 5.7% (combination)* versus 1.5% (gp 100 alone)</td>
<td>(Hodi et al., 2010)</td>
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<tr>
<td>Lapatinib</td>
<td>N = 399</td>
<td>Capecitabine with versus without lapatinib</td>
<td>TTP: 8.4 versus 4.4 months*</td>
<td>ORR: 22% versus 14%.*</td>
<td>OS, adjusted for crossover: 75 versus 56.4 weeks*</td>
<td>(Cameron et al., 2010; Geyer et al., 2006)</td>
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<tr>
<td>HER2+, HR+ breast cancer, metastatic, with letrozole, for postmenopausal women in whom hormonal therapy is indicated</td>
<td>3</td>
<td>N = 1286 (219 HER2+ patients)</td>
<td>Letrozole with versus without lapatinib</td>
<td>PFS (HER2+): 35.4 versus 13.0 weeks*</td>
<td>ORR (HER2+): 27.9 versus 14.3%*</td>
<td>(Schwartzberg et al., 2010), PI</td>
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<tr>
<td>Accelerated approval</td>
<td>Nilotinib</td>
<td>ENESTnd</td>
<td>3</td>
<td>N = 846</td>
<td>Nilotinib 300 or 400 mg PO BID versus imatinib</td>
<td>12 month MMR: 44% (300 mg nilotinib)* versus 43% (400 mg nilotinib)* versus 22% (imatinib)</td>
<td>(Larson et al., 2012; Saglio et al., 2010b)</td>
</tr>
<tr>
<td>Accelerated approval</td>
<td>Ofatumumab</td>
<td>CLL, relapsed/refractory</td>
<td>NS</td>
<td>N = 154</td>
<td>Ofatumumab</td>
<td>ORR: 42%</td>
<td>DR: 6.5 months</td>
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<tr>
<td>Accelerated approval</td>
<td>Ofatumumab</td>
<td>dose escalation</td>
<td>1/2</td>
<td>N = 33</td>
<td>Ofatumumab dose escalation</td>
<td>ORR: 44%</td>
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<tr>
<td>Ponatinib</td>
<td>PACE</td>
<td>2</td>
<td>N = 444</td>
<td>Ponatinib</td>
<td>MCyR</td>
<td>Time to MCyR:</td>
<td>(Cortes et al., 2012)</td>
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<tr>
<td>CML or Ph+ ALL, resistant or intolerant to tyrosine kinase inhibitor therapy</td>
<td>Patient-Centric Evaluation of the Effectiveness of Ponatinib (PACE)</td>
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<tr>
<td>Accelerated approval</td>
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<td></td>
<td>CML-CP: 54%</td>
<td>CML-CP: 84 days</td>
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<td>MaHR:</td>
<td>Duration of MaHR:</td>
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<td>CML-AP: 52%</td>
<td>CML-AP: 9.5 months</td>
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<td>CML-BP: 31%</td>
<td>CML-BP: 4.7 months</td>
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<td>Ph+ ALL: 41%</td>
<td>Ph+ ALL: 3.2 months</td>
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<tr>
<td>Panitumumab</td>
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<td>Panitumomab</td>
<td>PFS: 8 weeks versus 7.3 weeks*</td>
<td>ORR: 10% versus 0%*</td>
<td>(Amado et al., 2008; Van Cutsem et al., 2007)</td>
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<tr>
<td>Metastatic CRC, K-RAS WT, single agent, after progression on fluoropyrimidine, oxaliplatin, and irinotecan</td>
<td>PALETTE</td>
<td>3</td>
<td>N = 463</td>
<td>Panitumomab versus BSC</td>
<td>PFS: 8 weeks versus 7.3 weeks*</td>
<td>OS, K-RAS WT: 8.1 versus 7.6 months*</td>
<td>(Amado et al., 2008; Van Cutsem et al., 2007)</td>
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<td>Retrospective:</td>
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<td>K-RAS WT: 12.3 versus 7.3 weeks*</td>
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<td>K-RAS+: 7.4 versus 7.3 weeks*</td>
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<tr>
<td>Pazopanib</td>
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<td>Pazopanib versus placebo</td>
<td>PFS: 9.2 versus 4.2 months*</td>
<td>ORR: 30% versus 3%*</td>
<td>(Sternberg et al., 2010)</td>
</tr>
<tr>
<td>Advanced RCC</td>
<td>PALETTE</td>
<td>3</td>
<td>First-line or cytokine refractory, N = 395</td>
<td>Pazopanib versus placebo</td>
<td>PFS: 9.2 versus 4.2 months*</td>
<td>ORR: 30% versus 3%*</td>
<td>(Sternberg et al., 2010)</td>
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<tr>
<td>Advanced soft tissue sarcoma, after prior chemotherapy</td>
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<tr>
<td>Pertuzumab</td>
<td>PALETTE</td>
<td>3</td>
<td>N = 369</td>
<td>Pazopanib versus placebo</td>
<td>PFS: 9.2 versus 4.2 months*</td>
<td>ORR: 30% versus 3%*</td>
<td>(van der Graaf et al., 2012)</td>
</tr>
<tr>
<td>Metastatic breast cancer, HER2+, no prior HER2 therapy or chemotherapy for metastatic disease, with trastuzumab/docetaxel</td>
<td>CLEOPATRA</td>
<td>3</td>
<td>N = 808</td>
<td>Trastuzumab plus docetaxel, with versus without pertuzumab</td>
<td>PFS: 18.5 months versus 12.4 months*</td>
<td>OS (median 19.3 months follow-up: 23.6% versus 17.2%*</td>
<td>(Baselga et al., 2012b)</td>
</tr>
<tr>
<td>Rituximab</td>
<td>PRIMA</td>
<td>3</td>
<td>After response to rituximab chemotherapy, N = 1018</td>
<td>Rituximab maintenance versus observation</td>
<td>PFS at mean 36 months follow-up: 74.9% versus 57.6%*</td>
<td>OS: no significant difference</td>
<td>(Salles et al., 2011)</td>
</tr>
<tr>
<td>Low-grade/follicular NHL, after response to first-line induction</td>
<td>ECOG 1496</td>
<td>3</td>
<td>N = 311</td>
<td>CVP followed by rituximab versus observation</td>
<td>PFS: 4.3 versus 1.3 years*</td>
<td>3-year OS: 92% versus 86%*</td>
<td>(Hochster et al., 2009)</td>
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<tr>
<td>Follicular lymphoma, previously untreated</td>
<td>CVP chemotherapy with versus without rituximab</td>
<td></td>
<td></td>
<td></td>
<td>CR: 30% versus 8%*</td>
<td>Estimated TTP: 32 versus 15 months*</td>
<td>(Marcus et al., 2005)</td>
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<tr>
<td>Follicular/low-grade NHL, relapsed/refractory</td>
<td>NS</td>
<td>2</td>
<td>N = 166</td>
<td>Rituximab</td>
<td>ORR 45%</td>
<td>Estimated TTP: 12.5 months</td>
<td>(McLaughlin et al., 1998)</td>
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<tr>
<td>DLBCL, in combination with CHOP or anthracycline-based chemotherapy</td>
<td>MInT Study</td>
<td>3</td>
<td>Age 18-60 years, N = 824</td>
<td>CHOP-like chemotherapy/− rituximab</td>
<td>3-year event-free survival: 79% versus 59%*</td>
<td>OS, at 3 years: 93% versus 84%*</td>
<td>(Pfreundschuh et al., 2006)</td>
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<tr>
<td></td>
<td>ECOG 4494 trial</td>
<td>3</td>
<td>Age 60−80, N = 632</td>
<td>CHOP with versus without rituximab</td>
<td>3-year FFS: 53% versus 46%*</td>
<td>PFS, adjusted: 3.1 versus 1.6 years*</td>
<td>(Habermann et al., 2006, PI)</td>
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<tr>
<td>Drug, U.S. FDA-Labeled Approval(s)</td>
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<tr>
<td>GELA/LNH-98.5</td>
<td>OS, 2 year, adjusted: 74% versus 63%*</td>
<td>3 Age 60–80, N = 399</td>
<td>CHOP with versus without rituximab</td>
<td>EFS, at median 24 months: 57% versus 49%*</td>
<td>OS: 8.4 versus 3.5 years*</td>
<td>(Coiffier et al., 2002)</td>
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<tr>
<td>Previously untreated follicular NHL or DLBCL, as 90-minute infusion CLL</td>
<td>RATE</td>
<td>NS</td>
<td>N = 363</td>
<td>Follicular lymphoma: R- CVP DLBCL: R-CHOP Fludarabine/cyclophosphamide with versus without rituximab</td>
<td>Grade 3–4 infusion reactions: 2.8% starting at cycle 2 PFS at 3 years: 65% versus 45%*</td>
<td>OS at 3 years: 87% versus 83%</td>
<td>(Hallek et al., 2010)</td>
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<tr>
<td>REACH</td>
<td>OS: No significant difference, crossover design ORR: 69.9% versus 58%*</td>
<td>3 CLL, previously untreated, N = 817</td>
<td>Fludarabine/cyclophosphamide with versus without rituximab</td>
<td>PFS: 27 versus 21.9 months*</td>
<td>OS at median 24 months: 74% versus 63%*</td>
<td>(Roak et al., 2010)</td>
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<tr>
<td>Regorafenib</td>
<td>OS: 6.4 versus 5.0 months*</td>
<td>3</td>
<td>N = 760</td>
<td>Regorafenib versus placebo</td>
<td>PFS: 2.0 versus 1.7 months* ORR: 1% versus 0.4%</td>
<td>(Grothey et al., 2013), PI</td>
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<td>Metastatic CRC, previously treated with fluoropyrimidine/oxaliplatin/irinotecan-based chemotherapy, VEGF therapy and anti-EGFR therapy if K-RAS WT</td>
<td>CORRECT</td>
<td>2 N = 96</td>
<td>Romidepsin</td>
<td>ORR: 34%</td>
<td>DR: 15 months Improved pruritus: 43%, median duration 6 months ORR: 34% CRR 7% DR: 13.7 months ORR: 25.4%</td>
<td>(Whittaker et al., 2010)</td>
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<tr>
<td>2 N = 71</td>
<td>Romidepsin</td>
<td></td>
<td></td>
<td></td>
<td>TTR: 1.9 months DR: 16.6 months DR: 8.9 months</td>
<td>(Piekarz et al., 2009)</td>
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<tr>
<td>PTCL, after at least one prior therapy Accelerated approval</td>
<td>2 N = 130</td>
<td>Romidepsin</td>
<td>CR+CRu: 14.6% (95% CI 9%–21.9%)</td>
<td>ORR: 38% (95% CI 24%–53%) CR: 18%</td>
<td></td>
<td>(Coiffier et al., 2012)</td>
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<tr>
<td>2 N = 47</td>
<td>Romidepsin</td>
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<td></td>
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<td>(Piekarz et al., 2011)</td>
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<tr>
<td>Ruxolitinib</td>
<td>Symptom improvement: 45.9% versus 5.3%* OS at median 51 weeks: 91.6% versus 84.4%*</td>
<td>Intermediate or high-risk myelofibrosis</td>
<td>COMFORT-I</td>
<td>3 N = 309</td>
<td>Ruxolitinib versus placebo</td>
<td>Spleen volume decreased by &gt;35% at 24 weeks: 41.9% versus 0.7%*</td>
<td>(Verstovsek et al., 2012)</td>
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<td>No significant difference in TTP or OS at</td>
<td>COMFORT-II</td>
<td>3 N = 219</td>
<td>Ruxolitinib versus best available therapy</td>
<td>Spleen volume decreased by &gt;35%, at 48 weeks: 28 versus 0%*</td>
<td>(Harrison et al., 2012)</td>
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<tr>
<td>Drug, U.S. FDA-Labeled Approval(s)</td>
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<td>Sorafenib</td>
<td>Advanced RCC</td>
<td>TARGET</td>
<td>3 Refractory to standard therapy, ( N = 903 )</td>
<td>Sorafenib versus placebo</td>
<td>OS: 14.7 months versus not reached*</td>
<td>48-week follow-up</td>
<td>(Escudier et al., 2007b)</td>
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<td>PFS: 5.5 versus 2.8 months*</td>
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<td>Unresectable HCC</td>
<td>SHARP</td>
<td>3 No prior systemic treatment, ( N = 602 )</td>
<td>Sorafenib versus placebo</td>
<td>OS: 10.7 versus 7.9 months*</td>
<td>PFS: 10.7 versus 7.9 months*</td>
<td>(Llovet et al., 2008)</td>
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<td>Time to symptomatic progression: 4.9 versus 4.1 months</td>
<td>Time to radiological progression: 5.5 versus 2.8 months*</td>
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<tr>
<td>Sunitinib</td>
<td>GIST, after imatinib resistance/intolerance</td>
<td>3 ( N = 312 )</td>
<td>Sunitinib versus placebo</td>
<td>TTP: 27.3 versus 6.4 weeks*</td>
<td>PFS: 24.1 versus 6 weeks*</td>
<td>(Demetri et al., 2006)</td>
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<tr>
<td></td>
<td></td>
<td>NS</td>
<td>( N = 55 )</td>
<td>Sunitinib dose escalation</td>
<td>ORR: 9.1%</td>
<td>PR: 9.1%</td>
<td>PI</td>
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<td></td>
<td>Advanced RCC</td>
<td>Initial accelerated approval</td>
<td>3 No prior treatment, ( N = 750 )</td>
<td>Sunitinib versus IFN-( \alpha )</td>
<td>PFS: 11 versus 5 months*</td>
<td>ORR: 31% versus 6%*</td>
<td>(Motzer et al., 2007)</td>
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<td>PNET, unresectable or metastatic</td>
<td>3 ( N = 171 )</td>
<td>Sunitinib versus placebo</td>
<td>PFS: 11.4 versus 5.5 months*</td>
<td>ORR: 9.3% versus 0%*</td>
<td>OS at 6 months: 92.6% versus 85.2%*</td>
<td>(Raymond et al., 2011)</td>
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<td>Temsirolimus</td>
<td>Advanced RCC</td>
<td>3 Metastatic RCC, ( N = 626 )</td>
<td>Temsirolimus versus IFN-( \alpha ) versus combination</td>
<td>OS: 10.9 months (temsirolimus)* versus 7.3 months (IFN) versus 8.4 months (combination)</td>
<td>ORR: 8.6% (temsirolimus)* versus 4.8% (IFN) versus 8.1% (combination)</td>
<td>(Hudes et al., 2007)</td>
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<tr>
<td>Tositumomab</td>
<td>Relapsed/refractory CD20+ NHL, progression after/on rituximab</td>
<td>2 ( N = 40 )</td>
<td>Tositumomab therapeutic regimen</td>
<td>ORR: 68%</td>
<td>PFS: 10.4 months CR: 33%</td>
<td>DR: 16 months</td>
<td>(Horning et al., 2005)</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Metastatic gastric or GE junction adenocarcinoma, HER2+</td>
<td>ToGA</td>
<td>3 Previously untreated for metastatic disease, ( N = 594 )</td>
<td>Capecitabine or fluouracil, with cisplatin, with versus without trastuzumab</td>
<td>OS: 13.8 versus 11.1 months*</td>
<td>PFS: 6.7 versus 5.5 months*</td>
<td>(Bang et al., 2010)</td>
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<tr>
<td>Breast cancer, HER2+, adjuvant</td>
<td>NCCTG N8831; NSABP B-31</td>
<td>3 ( N = 3351 )</td>
<td>Dxorubicin/ cyclophosphamide with paclitaxel, with versus without trastuzumab</td>
<td>Disease-free survival, median 4-month follow-up: 65.2% versus 67.1%*</td>
<td>OS at 4 years: 91.4% versus 86.6%*</td>
<td>(Romond et al., 2005)</td>
<td></td>
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<tr>
<td>Drug, U.S. FDA-Labeled Approval(s)</td>
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<tr>
<td>HERA</td>
<td>HERA</td>
<td>3</td>
<td>N = 3401</td>
<td>Trastuzumab versus observation</td>
<td>DFS at 4 years: 78.6% versus 72.2%*</td>
<td>OS at 4 years: 89.3% versus 87.7%</td>
<td>(Gianni et al., 2011; Piccart-Gebhart et al., 2005)</td>
</tr>
<tr>
<td>BCIRG 006</td>
<td>BCIRG 006</td>
<td>3</td>
<td>N = 3222</td>
<td>AC-T (doxorubicin + cyclophosphamide and docetaxel q3 weeks) versus AC-T with trastuzumab for 52 weeks versus doxorubicin and carboplatin with trastuzumab for 52 weeks (TCH)</td>
<td>DFS at 5 years (estimated): 75% (AC-T), 84% (AC-T + trastuzumab) * versus 81% (TCH) *</td>
<td>OS at 5 years (estimated): 87% (AC-T) versus 92% (AC-T + trastuzumab) versus 91% (TCH) *</td>
<td>(Slamon et al., 2011)</td>
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<tr>
<td>HER2+ breast cancer, metastatic</td>
<td>H0648g</td>
<td>3</td>
<td>Previously untreated, N = 469</td>
<td>Paclitaxel or AC with versus without trastuzumab</td>
<td>TTP: 7.2 versus 4.5 months*</td>
<td>ORR: 45% versus 29%*</td>
<td>(Eiermann, 2001; Slamon et al., 2001)</td>
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<tr>
<td>HER2+ breast cancer, metastatic</td>
<td>H0649g</td>
<td>2</td>
<td>Progressive despite 1–2 prior chemotherapy regimens, N = 222</td>
<td>Trastuzumab</td>
<td>ORR: 15%</td>
<td>DR: 9.1 months</td>
<td>(Cobleigh et al., 1999)</td>
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<tr>
<td>Trastuzumab-DM1</td>
<td>EMILIA</td>
<td>3</td>
<td>N = 991</td>
<td>Trastuzumab-DM1 versus lapatinib with capecitabine</td>
<td>PFS: 9.6 versus 6.4 months*</td>
<td>OS: 30.9 versus 25.1 months*</td>
<td>(Verma et al., 2012)</td>
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<tr>
<td>Metastatic breast cancer, HER2 +, second-line after prior treatment with trastuzumab and a taxane</td>
<td>ZETA</td>
<td>3</td>
<td>N = 331</td>
<td>Vandetanib versus placebo</td>
<td>PFS: 30.5 versus 19.3 months*</td>
<td>ORR: 45% versus 13%*</td>
<td>(Wells et al., 2010, 2012)</td>
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<tr>
<td>Medullary thyroid cancer, symptomatic or progressive, and unresectable/metastatic</td>
<td>Vemurafenib</td>
<td>BRIM3</td>
<td>Previously untreated, N = 675</td>
<td>Vemurafenib versus dacarbazine</td>
<td>OS at 6 months: 84% versus 64%*</td>
<td>PFS: 5.3 versus 1.6 months*</td>
<td>(Chapman et al., 2011; Flaherty et al., 2010)</td>
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<tr>
<td>Melanoma, with the BRAF V600E mutation, unresectable or metastatic</td>
<td>BRIM2</td>
<td>2</td>
<td>Previously treated, N = 132</td>
<td>Vemurafenib</td>
<td>ORR at 12.9-month follow-up: 53%</td>
<td>DR: 6.7 months</td>
<td>(Sosman et al., 2012)</td>
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<tr>
<td>Vismodegib</td>
<td>BRIM2</td>
<td>2</td>
<td>N = 96</td>
<td>Vismodegib</td>
<td>ORR: 30% (metastatic), 43% (locally advanced)</td>
<td>DR: 7.6 months</td>
<td>(sekulic et al., 2012)</td>
</tr>
<tr>
<td>BCC, metastatic or recurrent locally advanced, not surgical or radiation therapy candidate</td>
<td>ERIVANCE</td>
<td>2</td>
<td>N = 96</td>
<td>Vismodegib</td>
<td>ORR: 30% (metastatic), 43% (locally advanced)</td>
<td>DR: 7.6 months</td>
<td>(sekulic et al., 2012)</td>
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<tr>
<td>vorinostat</td>
<td>CTCL</td>
<td>2B</td>
<td>N = 74</td>
<td>Vorinostat</td>
<td>ORR: 29.7%</td>
<td>DR: Est 185+ days</td>
<td>(Olsen et al., 2007)</td>
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<tr>
<td>CTCL, cutaneous manifestations, despite two prior systemic therapies</td>
<td>2</td>
<td>N = 33</td>
<td>Vorinostat</td>
<td>ORR: 24.2%</td>
<td>DR: 15.1 weeks</td>
<td>(Duvic et al., 2007)</td>
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TABLE 1—Continued

<table>
<thead>
<tr>
<th>Drug, U.S. FDA-Labeled Approval(s)</th>
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<th>Selected Secondary Outcomes</th>
<th>Reference(s)</th>
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<tr>
<td>Ziv-aflibercept</td>
<td>VELOUR</td>
<td>3</td>
<td>N = 1226</td>
<td>FOLFIRI with Ziv-aflibercept versus placebo</td>
<td>OS: 13.5 versus 12.06 months*</td>
<td>PFS: 6.90 versus 4.67 months*</td>
<td>ORR: 19.8% versus 11.1%*</td>
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</table>

Key: AC, anthracycline plus cyclophosphamide; AC-T, anthracycline, cyclophosphamide, taxol; ALL, acute lymphoblastic leukemia; AP, accelerated phase; ASM, aggressive systemic mastocytosis; BCC, basal cell carcinoma; B-CLL, B cell CLL; BID, twice daily; BP, blast phase; BSC, best supportive care; CCyR, complete cytogenetic response; CEL, chronic eosinophilic leukemia; CHOP, cyclophosphamide, hydroxyldaunorubicin, oncovin, and prednisone; CHR, complete hematological response; CP, chronic phase; CR, complete response; CRu, unconfirmed complete response; CTCL, cutaneous T-cell lymphoma; CVP, cyclophosphamide, vincristine, and prednisone; DFS, disease-free survival; DFSP, dermatofibrosarcoma protuberans; DLBCL, diffuse large B cell lymphoma; DR, disease recurrence; EFS, event-free survival; EPO, erythropoietin; ER, estrogen receptor; ERK, extracellular signal-regulated kinase; ES-SCLC, extensive small-cell lung cancer; ETV6, breakpoint cluster region; FOLFIRI, 5-fluorouracil, leucovorin, irinotecan; GG, glycogen storage disease; GIST, gastrointestinal stromal tumor; GPC-3, glypican-3; HCC, hepatocellular carcinoma; HES, hypereosinophilic syndrome; HR, hazard ratio; IFN-α, interferon-α; IL-6, interleukin-6; IRS, isoform-related sialosugars; LB, lymphoid blast; LV, leucovorin; MaHR, major hematological response; MB, myeloid blast; MDR, multidrug resistance; MDS, myelodysplastic syndromes; MMR, major molecular response; MPD, myeloproliferative disease; N/A, not applicable; NHL, non–Hodgkin’s lymphoma; NS, not significant; OHR, overall hematological response; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PI, package information, package insert; PO, by mouth; PR, partial response; PTCL, peripheral T cell lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; SCLC, small-cell lung cancer; SD, stable disease; SCLC, small-cell lung cancer; SOR, sorafenib; STS, soft tissue sarcoma; TAE, tyrosine kinase inhibitor; TAE-318, sorafenib; TAGE, tyrosine kinase inhibitor; TAE-789, sorafenib; TAE-900, sorafenib; TSC, tuberous sclerosis complex; TTF, time to treatment failure; TTP, time to progression; TTR, time to recurrence. |
<table>
<thead>
<tr>
<th>Agent</th>
<th>Target of Inhibition</th>
<th>Plasma T1/2</th>
<th>Primary Metabolism</th>
<th>Renal Excretion</th>
<th>Dose Adjustment (% Reduction of Recommended Dose if Available)</th>
<th>Drug Interactions</th>
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<td>Imatinib</td>
<td>BCR-ABL, KIT, PDGFR</td>
<td>18 h (aM = 40 h)</td>
<td>CYP3A4, IA2, 2D6, 2C9 and 2C19</td>
<td>f405 Other Total Par. Comp.</td>
<td>Hap Inc Neutropenia, thrombocytopenia</td>
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<td>Neutropenia, thrombocytopenia</td>
<td>Yes Yes Yes Tylenol: increased hepatotoxicity. Hypothyroidism: increase levothyroxine dose</td>
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<td>Dasatinib</td>
<td>BCR-ABL, KIT, PDGFR</td>
<td>3-5 h</td>
<td>CYP3A4</td>
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<td>None Neutropenia, thrombocytopenia</td>
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<td>Neutropenia, thrombocytopenia</td>
<td>Yes Yes No QT prolonging agents. PPIs/antacids - decr. absorption</td>
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<td>Nilotinib</td>
<td>BCR-ABL, KIT, PDGFR</td>
<td>17 h</td>
<td>CYP3A4</td>
<td>5</td>
<td>Mild, mod or severe: 25%-35% Neutropenia, thrombocytopenia</td>
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<td>Neutropenia, thrombocytopenia, QT prolongation, lipase/amylase elev</td>
<td>Yes Yes No QT prolonging agents. PPIs/antacids - decr. absorption</td>
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<tr>
<td>Ponatinib</td>
<td>BCR-ABL, (including</td>
<td>24 h</td>
<td>CYP3A4 and 2C8, 2D6 and 3A5</td>
<td>5</td>
<td>Not studied Arterial thrombosis, hepatotoxicity, GI perforation, wound healing complications, hemorrhage, cytopenias, cardiac arrhythmias, pancreatitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T315I, VEGFR, PDGFR, FGF, SRC, KIT, RET, TIE-2, FLT-3</td>
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<td></td>
<td></td>
<td>Neutropenia, thrombocytopenia, QT prolongation, hepatotoxicity</td>
<td>Yes Yes No Ponatinib inhibits ABCG2 and Pgp</td>
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<tr>
<td>Bosutinib</td>
<td>BCR-ABL, SRC, LYN, HCK</td>
<td>23.5 h</td>
<td>CYP3A4</td>
<td>3</td>
<td>Mild, mod or severe: 60% Neutropenia, thrombocytopenia</td>
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<td></td>
<td>Neutropenia, thrombocytopenia, QT prolongation, hepatotoxicity</td>
<td>Yes Yes No PPi/antacids - decr. absorption</td>
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<td>Axitinib</td>
<td>VEGFR1-3</td>
<td>2.54-1 h</td>
<td>CYP3A4, 3C19, and A2</td>
<td>23</td>
<td>Med: 50% Neutropenia, thrombocytopenia, QT prolongation,</td>
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<td></td>
<td></td>
<td>hepatotoxicity, HTN, associated St, proptosis, bleeding, surgery</td>
<td>Yes Yes No None reported</td>
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<tr>
<td>Pazopanib</td>
<td>VEGFR1-3, PDGFR, FGF1 AND 3, s-KIT, ITK, LCK, C-10, C-FMS</td>
<td>31 h</td>
<td>CYP3A4, IA2, and 2C8</td>
<td>&lt;4</td>
<td>Sev: not studied Med: 75% Sev: do not use None</td>
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<td>Hepatotoxicity, HTN, proptosis, surgery</td>
<td>Yes Yes Yes (weak) QT prolonging agents</td>
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<tr>
<td>Sorafenib</td>
<td>VEGFR2, VEGFR3, PDGFR-A, FLT3, s-KIT, p85-alpha, B-raf</td>
<td>1-2 d</td>
<td>CYP3A4, ald, pyrazino-n-oxide</td>
<td>19</td>
<td>None None None None</td>
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<td></td>
<td>Neutropenia, persistent skin toxicity, high-grade HTN, dyspea and rash, desquamation</td>
<td>Yes Yes No QT prolonging agents</td>
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<td>Plasma T1/2</td>
<td>Primary Metabolism</td>
<td>Renal Excretion</td>
<td>Dose Adjustment %</td>
<td>Drug Interactions</td>
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<td>Reduction of Recommended Dose if Available</td>
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<td>Dose of Available</td>
<td>P450 Inh.</td>
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(continued)
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<th>Agent</th>
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<th>Plasma T1/2</th>
<th>Primary Metabolism</th>
<th>Renal Excretion</th>
<th>Dose Adjustment (% Reduction of Recommended Dose if Available)</th>
<th>Drug Interactions</th>
</tr>
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<tbody>
<tr>
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<td>P450: Total, Par. Comp.</td>
<td>P450 Inh.</td>
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<tr>
<td>Ruxolitinib</td>
<td>Dysregulated JAK-1 and JAK-2</td>
<td>2.85-5.8 h</td>
<td>CYP3A4</td>
<td>74</td>
<td>Mild, mod, severe + platelets 300-150: 33%-50%.</td>
<td>Yes</td>
</tr>
<tr>
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<td>Mild, mod, severe + platelets &lt; 100: do not use</td>
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<td>CrCl &lt; 15: do not use dialysis + platelets &gt; 200: 31%-50%</td>
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<td>CrCl 15-59 + platelets 300-150: 30%-50%</td>
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<td>CrCl 15-59 + platelets &lt; 100: do not use</td>
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<td></td>
<td></td>
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<td>Thrombocytopenia, anemia</td>
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<tr>
<td>Vandetanib</td>
<td>EGFR and VEGF</td>
<td>19 d</td>
<td>CYP3A4, aM= N-desmethyl-vandetanib and vandetanib-N-oxide</td>
<td>25</td>
<td>None</td>
<td>Yes</td>
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<td>CrCl &lt; 50: 31%.</td>
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<td></td>
<td>QT prolongation, torsade de pointes, sudden death</td>
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<tr>
<td>Vemurafenib</td>
<td>B-raf</td>
<td>57 h</td>
<td>unknown</td>
<td>1</td>
<td>None</td>
<td>Yes</td>
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<td></td>
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<td>CrCl &lt; 30: not sufficiently studied</td>
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<td></td>
<td>QT prolongation</td>
<td></td>
</tr>
<tr>
<td>Vemodogli</td>
<td>Hedgehog pathway via inhibition</td>
<td>4-12 d</td>
<td>likely CYP3A9 and 3A45</td>
<td>44</td>
<td>Not sufficiently studied</td>
<td>No</td>
</tr>
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</tbody>
</table>

ABC2, ATP-binding cassette subfamily G member 2; aM, active metabolites; AXL, axr1 receptor tyrosine kinase; BBW, Black Box Warning; C-FMS, colony-stimulating factor-1 receptor; DDR2, discoidin domain receptor tyrosine kinase; FKBP, FK506-binding protein; FLT-3, fms-like tyrosine kinase-3; FMO, Flavin-containing monooxygenase; GI, gastrointestinal; HCK, hematopoietic cell kinase; Hep Ins, hepatic insufficiency; HTN, hypertension; ILD, interstitial lung disease; ITK, interleukin-2-inducible T-cell kinase; JAK, Janus kinase; LCK, lymphocyte-specific protein tyrosine kinase; LVEF, left ventricular ejection fraction; LYN, V-Yes-1 Yamaguchi sarcoma viral related oncogene homolog; mod, moderate; NSAID, nonsteroidal anti-inflammatory drug; P450, cytochrome P450; Par, Comp., parent compound; P-gp, P-glycoprotein; PPAR, palmar-plantar erythrodysesthesia syndrome; PPI, proton-pump inhibitor; PRES, posterior reversible encephalopathy syndrome; PTK, protein tyrosine-kinase; RBT, Ret Proto Oncogene; rol-1, c-Ros oncogene 1, receptor tyrosine kinase; SAPK, stress-activated protein kinase; sev, severe; S2a, seizure; TIE2, tunica interna endothelial cell kinase; TRKB, tyrosine kinase receptor B; UGT, UDP-glucuronosyltransferases.

*P450 inhibitors resulting in increased concentrations of drug.

Drug inhibits P450, leading to increased concentration of drugs metabolized by P450.

BBW.
<table>
<thead>
<tr>
<th>Agent</th>
<th>Target of Inhibition</th>
<th>Plasma T1/2</th>
<th>Pharmacokinetics</th>
<th>Dose Adjustment (% Reduction of Recommended Dose if Available)</th>
<th>Drug Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monoclonal antibodies</strong></td>
<td></td>
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</tr>
<tr>
<td>Trastuzumab</td>
<td>HER2</td>
<td>5.8 d</td>
<td>Likely eliminated via RES</td>
<td>None None</td>
<td>Cardiotoxicity, reduced LVEF, serious infusion reactions, pulmonary toxicities, nephrotic syndrome (rare)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No No No Anthracyclines, paclitaxel, and cyclophosphamide - increased cardiotoxicity. Paclitaxel - increased levels</td>
</tr>
<tr>
<td>Pertuzumab</td>
<td>HER2</td>
<td>18 d</td>
<td>None (not studied)</td>
<td>CrCl&gt; 30: none CrCl&lt; 30: none (not studied)</td>
<td>Cardiotoxicity, reduced LVEF, serious infusion reactions, embryo-fetal toxicity d</td>
</tr>
<tr>
<td>Ibritumomab tuixetan Y-90</td>
<td>CD20. Tiuxetan links the antibody and Y-90, a high-energy beta emission</td>
<td>27–30 h</td>
<td>6%–11% renal excretion, 7.2% parent compound</td>
<td>None None</td>
<td>Serious infusion reactions, severe cytopenias, cut. and mucocut. reactions, radiation injury</td>
</tr>
<tr>
<td>Tositumomab I131</td>
<td>CD20. Binding of the I131 loaded antibody and beta emission</td>
<td>8 d</td>
<td>None</td>
<td>None</td>
<td>Infusion-related reactions, prolonged and severe cytopenias, anaphylactic reactions</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>5–78 d</td>
<td>None</td>
<td>None</td>
<td>Serious infusion reactions, TLS, severe mucocut. reactions, PML d</td>
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<tr>
<td>Alemtuzumab</td>
<td>CD52 on several immune cells</td>
<td>12 d</td>
<td>None</td>
<td>None</td>
<td>Cardiotoxicity, serious infusion reactions, infections, cytopenias d</td>
</tr>
<tr>
<td>Ofatumumab</td>
<td>CD20</td>
<td>12–14 d</td>
<td>None</td>
<td>None</td>
<td>Infusion-related reactions</td>
</tr>
<tr>
<td>Brentuximab vedotin</td>
<td>CD30. Internalization and disruption of microtubules by MMAE inhibiting</td>
<td>4–6 d</td>
<td>Metabolized by CYP3A4/5; 24% renal excretion</td>
<td>None (not determined for MMAE)</td>
<td>Neutropenia, PNP, PML d:</td>
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</tbody>
</table>

*CrCl in ml/min*
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<tr>
<th>Agent</th>
<th>Target of Inhibition</th>
<th>Plasma T1/2</th>
<th>Pharmacokinetics</th>
<th>Dose Adjustment (% Reduction of Recommended Dose if Available)</th>
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<td>Hep Ins</td>
<td>Renal Ins</td>
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<tr>
<td>Ipilimumab</td>
<td>cancer cell growth</td>
<td>14.7 d</td>
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<td>None</td>
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<tr>
<td>Bevacizumab</td>
<td>VEGF</td>
<td>20 d</td>
<td>None</td>
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<tr>
<td>Cetuximab</td>
<td>EGFR</td>
<td>3–7 d</td>
<td>Internalization in liver and skin</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Panitumumab</td>
<td>EGFR</td>
<td>7.5 d</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<tr>
<td>VEGFR-1 and 2 fusion</td>
<td>Binds VEGFR-1 and 2 ligands</td>
<td>6 d</td>
<td>Mild-mod: none, severe (not studied)</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Denileukin difitox</td>
<td>Cells that express high affinity for IL-2 receptor (CD25, CD122, CD152)</td>
<td>70–80 min</td>
<td>None</td>
<td>None</td>
<td>Severe infusion reactions, capillary leak syndrome, loss of visual acuity, avoid in hypoalbuminemia &lt; 3g/dl</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>26S proteasome</td>
<td>9–15 h</td>
<td>Metabolized by CYP3A4, 2D6, 2C19, 2C9, 1A2;</td>
<td>Mild: none</td>
<td>Mod-sev: −45%</td>
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<td>Agent</td>
<td>Target of Inhibition</td>
<td>Plasma T1/2</td>
<td>Pharmacokinetics</td>
<td>Dose Adjustment (% Reduction of Recommended Dose if Available)</td>
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<tr>
<td>Carfilzomib</td>
<td>Similar to bortezomib</td>
<td>&lt;1 h</td>
<td>extent of renal excretion unclear Possibly metabolized by CYP3A4/5, metabolized by P-glycoprotein; extent of renal excretion unclear</td>
<td>Not studied</td>
<td>PAH, hepatotoxicity, cardiotoxicity, thrombocytopenia</td>
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<tr>
<td>Histone deacetylase inhibitors</td>
<td></td>
<td></td>
<td></td>
<td>n/a</td>
<td>n/a n/a n/a None reported</td>
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<tr>
<td>Vorinostat</td>
<td>Histone deacetylases that are overexpressed in some cancer cells</td>
<td>2 h</td>
<td>Metabolism through glucuronidation, hydrolysis, and beta-oxidation; 52% renal excretion, less than 1% parent compound</td>
<td>Mild-mod: not sufficiently studied, caution</td>
<td>Severe thrombocytopenia and leukopenia</td>
</tr>
<tr>
<td>Romidepsin</td>
<td>Same as vorinostat</td>
<td>3 h</td>
<td>Metabolized by CYP3A4</td>
<td>Mild: none</td>
<td>Neutropenia, thrombocytopenia</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Mod-sev: not studied, caution</td>
<td>Yes Yes No None reported</td>
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</tbody>
</table>

**Histone deacetylase inhibitors**

Histone deacetylase inhibitors

Vorinostat: Histone deacetylases that are overexpressed in some cancer cells

Romidepsin: Same as vorinostat

BBW, Black Box Warning; CTLA4, cytotoxic T lymphocyte–associated antigen 4; cut., cutaneous; ESRD, end-stage renal disease; GI, gastrointestinal; Hep Ins, hepatic insufficiency; HTN, hypertension; IL-2, interleukin-2; ILD, interstitial lung disease; LVEF, left ventricular ejection fraction; MMAE, monomethyl auristatin E; mod, moderate; mucocut., mucocutaneous; n/a, not applicable; NSAID, nonsteroidal anti-inflammatory drug; P450, cytochrome P450; PAH, pulmonary artery hypertension; PML, progressive multifocal leukoencephalopathy; PNP, peripheral neuropathy; PPI, proton-pump inhibitor; PRES, posterior reversible encephalopathy syndrome; Renal Ins, renal insufficiency; sev, severe; St., seizure; TEN, total epidermal necrolysis; TLS, tumor lysis syndrome.

*P450 inhibitors resulting in increased concentrations of drug.

*P450 inducers resulting in decreased drug concentration.

*Drug inhibits P450, leading to increased concentration of drugs metabolized by P450.

*BBW.
inactivated in their initial metabolic transformation. Many of the orally administered drugs have significant toxicity to intestinal epithelium and frequently cause mild hepatocellular toxicity (where the highest drug concentrations are found). Idiosyncratic severe or fatal hepatic toxicity and pulmonary toxicity occur infrequently, but have been reported with many agents. Other drugs, including the second- and third-generation BCR-ABL inhibitors, may prolong the QT interval and cause serious cardiac arrhythmias.

Because most synthetic targeted inhibitors are administered continuously over weeks to months, there exists the possibility for changes in drug clearance due to induction of metabolism or transporters, buildup of an inhibitory metabolite, changes in hepatic function, or other pharmacokinetics factors. For example, a large population-based pharmacokinetics study of imatinib suggested an increase of 33% in drug clearance in the first month of drug administration, a finding that could explain decreased toxicity of the drug over time (Judson et al., 2005). A similar finding, a 25% decrease in drug exposure after 3 months of treatment, was reported for sorafenib in patients with hepatocellular cancer, and exposure was almost 50% lower at the time of disease progression as compared with the exposure after 1 month of treatment (Arrondeau et al., 2012). The authors suggest that disease progression could be related to changes in drug pharmacokinetics over time.

Drug interactions are common for the small molecules and result from their susceptibility to changes in cytochrome P450 isoenzyme activity, which may be induced or inhibited by nononcological drugs. Dose adjustment is usually necessary when the oral synthetic targeted inhibitors are used with inhibitors of cytochrome P450 metabolism such as calcium channel blockers, antibiotics (erythromycin, antifungal imidazoles), herbal medications (St. John’s wort), or antiretroviral drugs (ritonavir), or with inducers of metabolism such as glucocorticoids, phenytoin, or proton-pump inhibitors. The issue of drug interactions becomes increasingly important as combinations of targeted agents are used to overcome or circumvent drug resistance (Kwak et al., 2007).

Studies of pharmacokinetics and toxicity of targeted drugs in patients with hepatic or renal dysfunction have yielded important insights. In general, doses of targeted drugs do not have to be reduced in patients with mild hepatic dysfunction and normal bilirubin levels (Child-Pugh A), the exceptions being bosutinib and ruxolitinib (Tables 1 and 2), which do require dose reduction in this circumstance. Higher levels of hepatic dysfunction (Child-Pugh B and C), with increasingly abnormal serum bilirubin levels, in general require that the dose be decreased by 50–75%. Renal dysfunction does not directly affect most targeted drugs since renal excretion plays a minor role in elimination of these agents. Dose adjustment is necessary for ruxolitinib, vandetanib, and imatinib when the creatinine clearance falls below 30 ml/min.

Central nervous system penetration of most targeted drugs is quite limited. In the few examples where simultaneous blood and cerebrospinal fluid (CSF) samples were analyzed, agents such as gefitinib and crizotinib do penetrate at rates that produce responses in brain metastases (Falk et al., 2012; Kuiper and Smit, 2013) but are not sufficient enough to prevent progression of central lesions. The occasional dramatic response to targeted therapies, such as crizotinib in the treatment of anaplastic lymphoma kinase (ALK)-positive NSCLC, may be due to disruption of the blood-brain barrier, allowing for higher concentrations of the compound.

C. Clinical Pharmacology of Monoclonal Antibodies.

As clinical agents, monoclonal antibodies have had a major impact on cancer treatment, primarily as components of combination therapy. They possess many favorable properties, which are summarized as follows:

1. Monoclonal antibodies have few drug interactions, as they do not affect hepatic microsomal metabolism. Their pharmacokinetics are unaffected by renal or hepatic dysfunction.

2. They have prolonged half-lives in plasma, due to their slow elimination by the reticuloendothelial system, thus allowing intermittent weekly or monthly dosing. Half-lives are typically 7–23 days. IgG1, IgG2, and IgG4 have the longest survival in plasma, whereas IgG3 has the shortest (7 days). Antibodies are removed through interaction with the FcRB (Brambell) receptor on the vascular endothelium, a process that may accelerate in the presence of inflammation, fever, and cytokine release.

3. They have highly specific drug action, with few off-target side effects. The least toxic are antibodies that target unique receptors on tumor cells, but other target molecules such as HER2, CD20, EGFR, and VEGF are found on normal tissues, leading to significant toxicities when antibodies are administered. For example, HER2 is found on cardiac myocytes and participates in repair and remodeling of cardiac tissue. Trastuzumab, an anti-HER2 antibody, causes myocardial injury (Cote et al., 2012). Rituximab, an anti-CD20 antibody, depletes both malignant and normal B-lymphocytes and increases the risk of opportunistic infection. Gemtuzumab ozogamicin, an anti-CD33/toxin conjugate, potently kills myeloid leukemic cells but produces prolonged depletion of platelet precursors (Ravandi et al., 2012). The radioconjugate Y-90 Zevalin (ibritumomab) produces severe myelosuppression in patients with lymphoma and involvement of bone marrow.

4. Because of their ability to target specific cell types, monoclonal antibodies can be used to carry extremely potent cytotoxins with minimal toxicity.
to the host. Toxins such as bacterial peptides (pseudomonas exotoxin), radionuclides (yttrium-90 or iodine-131), and potent small molecules (maytansine and aurestatin antimitotic derivatives) have been linked to antibodies. The resulting conjugates are highly effective in clinical use (see Table 1).

5. They have the ability to recruit T cells, macrophages, and complement to targeted cells, thus amplifying their action.

6. They may potentiate the action of cytotoxic drugs with minimal overlapping toxicity. This compatibility of antibodies with cytotoxics has allowed the addition of antibodies to combination chemotherapy regimens, without dose reductions and with significant enhancement of activity in breast cancer (trastuzumab with taxanes), colon cancer (cetuximab with fluorouracil/irinotecan, bevacizumab with fluorouracil/oxaliplatin), and lung cancer (bevacizumab with platinum-based combinations) and in non-Hodgkin’s lymphoma (rituximab with cyclophosphamide, hydroxydaunorubicin, oncovin, and prednisone) (see Table 3 for references).

The penetration of solid malignancies by monoclonal antibodies is in part determined by the size of the antibody. In many cases, such as bevacizumab, antibodies are used in conjunction with chemotherapeutics. Antibodies can potentiate the response to cytotoxic therapy significantly. Synergistic effects may be due to drug interactions with vessels or interference with survival pathways of cancer cells, making them more susceptible to DNA damaging agents.

Despite their obvious advantages, monoclonal antibodies have a few negative pharmacological features:

1. The molecules are more complex than synthetic targeted inhibitors, making them difficult to manufacture, and require humanization to avoid allergic responses. Thus, they tend to be costly.

2. Despite their “humanization” (the insertion of human sequences in place of mouse antibody peptides), they can provoke febrile and systemic toxicity, which usually decreases in succeeding cycles of therapy. Partially humanized antibodies, such as rituximab, or mouse antibodies, such as Bexxar (tositumomab), are more immunogenic than fully humanized molecules.

3. Because of their high molecular weight, they do not penetrate well into the central nervous system.

4. Most require intravenous administration, an inconvenient and expensive restriction on their use.

The reader is referred to primary texts (Laurent et al., 2011) for a more complete discussion of the mechanism of action and clinical applications of monoclonal antibodies.

II. Resistance

Despite considerable success of targeted treatments, resistance emerges after several months of treatment in most patients. The only exception is the experience with BCR-ABL–targeted drugs in chronic myeloid leukemia (CML), a setting in which long-term stable molecular remissions have been achieved with imatinib and the second- and third-generation inhibitors (Table 1). The investigation of resistance mechanisms allows further understanding of the adaptability of malignancies on a molecular level and leads to insights crucial for the development of treatment alternatives, including new drug development and multiagent approaches.

In general, resistance is characterized as either intrinsic (i.e., primary) or acquired (i.e., secondary) resistance. Intrinsic resistance describes a de novo lack of response to therapy, whereas secondary resistance emerges following drug exposure and manifests after a period of response. Often, the mechanisms of primary and secondary resistance are the same, although the understanding of both is far from complete. Well-established mechanisms of acquired resistance include 1) clonal selection of pre-existing resistant cells present at low allelic frequencies and selected to grow out through the pressure of drug, or 2) genetic events acquired during the course of therapy. It is notable that resistance mechanisms to targeted therapies are similar to mechanisms of resistance to chemotherapeutic agents. In general, mechanisms of resistance to targeted drugs have been much more carefully studied in the clinic through rebiopsy of patients at the time of progression. Distinct mechanisms of resistance, such as activation of alternative pathways that are less frequently seen in resistance to cytotoxic agents, seem to predominate with targeted therapies.

In the following section, we will provide an overview of specific mechanisms of resistance to targeted therapy. We will emphasize resistance mechanisms identified through analysis of patient-derived specimens. Where clinical evidence is lacking, we will provide an overview of preclinical data on resistance mechanisms.

An examination of mechanisms of resistance to various agents across malignancies of diverse molecular backgrounds and treatment experience may provide a more global view that will inform strategies for overcoming resistance. Despite the diversity of specific resistance mechanisms across human malignancies, a number of common principles have emerged. These general mechanisms include genetic alterations that alter the level of expression of the target or the conformation of its catalytic pocket (e.g., BCR-ABL-1 in CML, EGFR and EML4/ALK in NSCLC) and activation of pathways that bypass the inhibited target and lead to downstream signaling cascades (e.g., N- and K-RAS activation in BRAF-positive melanoma or MET amplification...
in EGFR-mutated NSCLC). Often, secondary mutations of the primary oncogenic driver lead to decreased binding affinity of the inhibitor to the catalytic pocket of the enzyme, restrict access to the pocket, or change the configuration of the enzyme. Additional factors, such as failure of apoptotic mechanisms (for example, low BIM (Bel-2-interacting mediator of cell death) expression), pharmacokinetics factors, and major histological changes that likely reflect altered gene expression (epithelial-to-mesenchymal transformation), may contribute to resistance and will be discussed in the appropriate section. We will consider resistance affecting specific agents in the following sections.

A. Resistance to Synthetic Targeted Inhibitors

1. Drugs Targeting Epidermal Growth Factor Receptor Mutations in Non–Small-Cell Lung Cancer

NSCLC constitutes approximately 80% of all lung cancers and is associated with an overall 5-year mortality rate of 85% (Jemal et al., 2010). The majority of NSCLC cases present with locally advanced or metastatic disease. Chemotherapy has been modestly effective in prolonging survival to approximately 1 year in these patients, but at the cost of significant toxicity. An important insight into tumor biology occurred with the observation that 10% of U.S. patients with NSCLC, primarily nonsmokers, express a mutated form of the EGFR encoded by the EGFR (ErbB1) gene. In its physiological state, EGFR is a transmembrane protein with intracellular tyrosine kinase activity that transduces growth and survival signals via the mitogen-activated protein (MAP) kinase and phosphatidylinositol kinase (PI3K) pathways. In NSCLC, oncogenic mutations are found in the EGFR receptor’s catalytic domain, which extends from exon 18 through 21. The most common alterations are exon 19 deletions (45% of cases), exon 21 point mutation L858R (42%), rare substitutions in exon 18 (3%), and insertions or substitutions in exon 20 (4%) (Lynch et al., 2004; Paez et al., 2004; Shepherd et al., 2005). Activating EGFR mutations are transforming in vitro and in vivo, and affected cells become dependent on EGFR signaling for survival and proliferation. Loss of signaling causes the “addicted” cell to undergo apoptosis, although there is unexplained heterogeneity in the ability to induce apoptosis in similar cells with the same oncogenic driver (Faber et al., 2011) (see “Failure to Induce Proapoptotic Signalling and Response to Targeted Therapy”). The concept of “oncogene addiction” provides a rationale for targeted therapies in oncogene-driven malignancies, such as EGFR- and ALK-mutated NSCLC, c-KIT–mutated gastrointestinal stromal tumor (GIST), CML, and B-RAF–mutated melanoma.

The reversible EGFR inhibitors gefitinib and erlotinib are effective in the management of EGFR mutant tumors. Approximately 70% of patients with advanced NSCLC with EGFR mutation initially respond to treatment with these tyrosine-kinase inhibitors (TKIs), which improve progression-free survival and tolerability. These drugs cause mild toxicity when compared with conventional management with standard chemotherapy (Shepherd et al., 2005; Mok et al., 2009; Rosell et al., 2012). “Classic” exon 19 and 21 mutations are associated with marked sensitivity to the EGFR TKIs (Lynch et al., 2004; Paez et al., 2004). However, NSCLC patients relapse after a median of 10 months following TKI treatment initiation.

2. Primary Resistance to Epidermal Growth Factor Receptor Tyrosine-Kinase Inhibitors

Thirty percent of patients show primary resistance to TKI treatment (defined as less than 30% objective response by Response Evaluation Criteria In Solid Tumors criteria) (Therasse et al., 2000). At presentation, tumors with exon 20 mutations or the T790M mutation (also a common mutation associated with acquired resistance) have a low response rate to EGFR TKI (Yasuda et al., 2012). In a compilation of published data, only one of 20 patients with an exon 20 mutation had a response to EGFR inhibitor therapy (Yasuda et al., 2012). In vitro studies likewise demonstrated resistance of cells with exon 20 mutations to multiple reversible and irreversible EGFR TKIs (Yasuda et al., 2012).

3. Acquired Resistance to Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors and Strategies to Overcome Resistance

Rebiopsy of tumor at the time of progression has disclosed that T790M is the most frequent genetic change conferring acquired resistance to EGFR TKIs, accounting for 30–68% of such cases (Kobayashi et al., 2005; Arcila et al., 2011; Sequist et al., 2011). This point mutation, which results in a threonine-to-methionine amino acid change, affects the “gatekeeper” residue in the catalytic domain of the kinase and restricts access to the pocket. Most oncogenic EGFR mutations reduce the enzyme’s affinity for ATP. T790M may also contribute to resistance by restoring the ATP affinity of mutant EGFR back to wild-type levels, an action not duplicated by gatekeeper mutations of other oncogenic tyrosine kinases (Yun et al., 2008).

When present as the initial and sole mutation in EGFR, T790M enhances kinase activity and oncogenicity, resulting in resistance. In combination with other classic activating mutations, T790M confers resistance to EGFR TKIs (Godin-Heymann et al., 2007; Mulloy et al., 2007; Vikis et al., 2007). However, in recent in vitro studies, T790M, as a secondary mutation, was associated with a growth disadvantage relative to parental cells (Chmielecki et al., 2011). In line with this observation, patients with acquired T790M mutation seem to have a more indolent clinical course when maintained on treatment despite progression (Chmielecki et al., 2011; Oxnard et al., 2011). When EGFR inhibitors are discontinued in drug-resistant NSCLC, sensitivity to TKIs may be restored, as the original cell...
line re-emerges and the T790M mutation disappears. It is uncertain whether continued TKIs in patients with resistant tumors, either with chemotherapy or in combination with new TKI inhibitors, will delay disease progression (Chmielecki et al., 2011). Other mutations besides T790M, such as D761Y (Balak et al., 2006), L747S (Costa et al., 2007), and T854A (Bean et al., 2008), confer secondary resistance and together account for fewer than 5% of cases.

In an attempt to overcome resistance, second-generation EGFR TKIs with greater potency against T790M-resistant cells are being evaluated. These are irreversible inhibitors, and many block other members of the ErbB family (Kwak et al., 2005; Shimamura et al., 2008). Some of these second-generation inhibitors, including the dual EGFR/HER2 inhibitors HKI-272 [(2E)-N-[4-[(3-chloro-4-yl)methoxy]phenyl]amino]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethylamino)but-2-enamide (Yap et al., 2010) and BIBW 2992 [N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[[3S]-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(dimethylamino)-2-butenamide] (Engelman et al., 2007a) and the multi-TKI PF-02998044 [[(E)-N-[4-(3-chloro-4-fluoro-anilino)-7-methoxy-quinazolin-6-yl]-4-(1-piperidyl)but-2-enamide hydrate] (Engelman et al., 2007a), have completed phase II clinical trials, but results were disappointing. These inhibitors have a high affinity to wild-type (WT) EGFR and, therefore, a lower therapeutic index. Given that the T790M mutation restores ATP affinity to a level comparable to that of WT EGFR (Yun et al., 2010), concentrations of second-generation TKIs necessary to inhibit T790M-mutated tumors in patients may result in side effects that ultimately limit their use. Third-generation TKIs, such as WZ4002 [N-[(3-chloro-2-[2-methoxy-4-(4-methylpiperazin-1-yl)phenyl]amino)-pyrimidin-4-yloxy)phenyl]acrylamide] and CO-1686, may be more promising. They are irreversible inhibitors with a different structural scaffold (and an allosteric binding site). They are highly selective for T790M-mutated cells and have marginal activity against WT EGFR (Zhou et al., 2009; Walter et al., 2011), which should decrease host toxicity and allow dose escalation.

A mouse model in which the tumor carries the compound Del19-T790M or L858R-T790M mutation may allow testing of the efficacy of different compounds and combination therapies against T790M tumors in vivo (Xu et al., 2012).

Amplification of the MET oncogene that encodes for a transmembrane TK receptor is the second most common mechanism of acquired resistance to EGFR TKIs, accounting for 5–20% of acquired resistance (Engelman et al., 2007b; Arcila et al., 2011; Sequist et al., 2011). In the presence of EGFR TKIs, amplification of MET leads to resistance by dimerizing with and transactivating ErbB3, which subsequently activates PI3KAKT and thereby bypasses EGFR signaling (Engelman et al., 2007b). In addition to MET amplification, the same pathway may be activated by overexpression of its ligand, hepatic growth factor (HGF), a change that confers both primary and secondary resistance to EGFR inhibitors in experimental systems. Yano et al. (2008) demonstrated that HGF induces gefitinib resistance in PC-9 and HCC827 cells through MET/PI3K activation, independent from ErbB3/PI3K signaling, and may also confer primary resistance to gefitinib and erlotinib. High expression of HGF was found in tumor specimens from patients with primary resistance and in patients with acquired resistance, whereas responding tumors exhibited low expression levels of HGF (Yano et al., 2008; Turke et al., 2010). These findings were confirmed in a study by Engelman et al. (2007a,b). The authors also examined 16 patient-derived resistant specimens. Four of these patients showed MET amplification in pretreatment tumor specimens, and all four subsequently developed MET amplification as their sole resistance mechanism following treatment, supporting the idea that resistance arises due to a pre-existing MET-amplified clone that expands under gefitinib/erlotinib selection pressure (Turke et al., 2010).

Less frequent mechanisms that confer resistance via bypass tract activation include CRKL amplification (Cheung et al., 2011), loss of phosphatase and tensin homolog (PTEN) (Sos et al., 2009), BRAF mutations (Ohashi et al., 2012), HER2 amplification (Takezawa et al., 2012), PI3KCA mutations (Sequist et al., 2011), and less well understood mechanisms, such as epithelial-to-mesenchymal transformation. In a minority of resistant tumors, a remarkable transformation from NSCLC to small-cell lung cancer (Sequist et al., 2011) is seen in the resistant tumor, which retains the original EGFR mutation, suggesting a major reprogramming of gene expression.

4. Resistance to ALK Inhibitors in Non–Small-Cell Lung Cancer: A subset of NSCLC carries a chromosomal translocation that activates the ALK. Approximately 3–5% of NSCLC patients (~8000 patients in the United States every year) have tumors that harbor a translocation that connects the 5’ end of the ALK gene to the echinoderm microtubule-associated protein-like 4 gene (EML4); although this is the most common activating mutation, other partners can produce transformation with ALK (Chiarle et al., 2008). Based on retrospective observations, resulting malignancies are associated with advanced stage at presentation and with a more aggressive phenotype (Rodig et al., 2009; Shaw et al., 2009; Doebele et al., 2012a). Lung cancers with ALK translocations rely on ALK kinase activity as their oncogenic driver, and most are exquisitely sensitive to ALK inhibition. The ALK inhibitor crizotinib was granted accelerated U.S. Food and Drug Administration approval based on phase 1 and phase 2 trials, both of which resulted in a response rate of ~60% and a long progression-free survival of ~10 months (Kwak et al., 2010). However, development of resistance to crizotinib is a major limiting factor.
The first report on clinically relevant acquired mechanisms of resistance described point mutations 4374G→A and 4493C→A, resulting in nonoverlapping C1156Y and L1196M mutations, respectively (Choi et al., 2010). Interestingly, L1196M corresponds to T790M in EGFR mutant NSCLC and T315I in the ABL oncogene, and represents the “gatekeeper” position. In each case, these amino acid changes lead to steric interference with inhibitor entry into the active site of the enzyme. Other clinically relevant mutations are listed in Table 4 and confer variable levels of resistance. S1206Y and G1202R (which is analogous to the G340W mutation in imatinib-resistant CML) were first identified as conferring resistance in a random mutagenesis screen, likely because of their decreased binding affinity for crizotinib (Katayama et al., 2012). Aside from secondary mutations in the EML4-ALK kinase, less frequent mechanisms that contribute to the spectrum of crizotinib resistance include ALK amplification, c-KIT amplification, EGFR activation (Katayama et al., 2012; Sasaki et al., 2011), loss of the ALK fusion gene, and activating mutations of K-RAS (Doebele et al., 2012b).

Despite the great diversity of ALK secondary mutations discovered thus far, only 30% of clinically resistant tumors have clearly defined mutations. The absence of mutations may imply inadequate crizotinib potency or unfavorable pharmacokinetics. Addressing this possibility, five next-generation ALK inhibitors are currently in phase I trials (LDK378, AP26113, CH5424802, ASP-3026, and X-396) and have more potent inhibitory activity in vitro and worse clinical outcomes in patients with NSCLC and CML when compared with patients with the BIM wild-type allele (Ng et al., 2012). This variability in apoptotic potential may contribute to variable resistance in malignancies with the same oncogenic driver.

Interestingly, BIM levels were not predictive of response to cytotoxic chemotherapy in EGFR-mutant cancer cell lines. Further studies are required to determine whether BIM plays a role in acquired resistance to targeted therapy.

C. Resistance to BRAF-Targeted Therapy

1. BRAF Mutations in Melanoma. Melanoma is an aggressive skin cancer with a yearly incidence of 180,000 cases worldwide. It causes 48,000 deaths a year. It is the second most common malignancy in patients aged <40 years (Jemal et al., 2010). Treatment of patients with advanced melanoma has been ineffective until development of targeted therapies, including the monoclonal antibody ipilimumab, which inactivates the cytotoxic T lymphocyte–associated antigen 4 (Hodi et al., 2010; Robert et al., 2011), and vemurafenib (Flaherty et al., 2010, 2012), a selective, low-molecular-weight BRAF inhibitor. A second, highly promising set of antibodies targeting the PD-1/PDL-1 axis, which downregulates T cell autoimmunity, is early in development, but clearly active in melanoma and lesser autoimmune adverse effects observed with BRAF inhibitors (hypophysitis, thyroiditis) (Brahmer et al., 2010; Topalian et al., 2012). Among melanomas of advanced stage, 50% harbor an activating mutation of the gene encoding the serine-threonine protein kinase BRAF, most commonly due to V600E and V600K mutations (Davies et al., 2002). Treatment of patients with BRAFV600E positive advanced melanoma with vemurafenib led to an unparalleled ~80% antitumor response rate (Flaherty et al., 2010).

BRAF is part of the RAS/RAF/MAP kinase (MAPK) pathway, which is activated through ligand binding to a TK receptor, such as EGFR and platelet-derived growth factor receptor (PDGFR) (Fig. 1). This binding results in interaction of transducer proteins with the RAS protein, ultimately leading to RAS cleavage, methylation, and activation through its translocation to the cell membrane. Downstream from RAS, three RAF isoforms

The circumvention of apoptosis by dysregulation of endogenous mechanisms is a hallmark of cancer. Essential to the group of proapoptotic regulators are Bcl-2–like proteins. One member of this group, BIM, an activator of apoptosis, has been repeatedly implicated as playing an essential role in the induction of apoptosis by TKIs in both hematological and solid tumor cell lines (Kuroda et al., 2006; Cragg et al., 2007; Gong et al., 2007; Faber et al., 2011; Ng et al., 2012). Activation of the PI3K/AKT pathway results in inhibition of BIM. For reasons that are yet to be identified, human tumors with the same oncogenic driver express different levels of BIM at baseline and show a variable response of BIM levels to targeted treatment. Lower pretreatment levels of BIM are associated with worse response to TKIs, such as gefitinib in NSCLC, imatinib in CML, and trastuzumab in HER2-positive breast cancer, and confer intrinsic resistance to these therapies (Faber et al., 2011; Ng et al., 2012). Lower BIM mRNA levels in 24 NSCLC specimens derived from patients receiving gefitinib/erlotinib treatment were associated with worse progression-free survival compared with patients with higher BIM transcript levels. A common BIM deletion polymorphism that lacks the proapoptotic BCL-2–homology domain 3 was found to be associated with impaired ability to induce apoptosis in vitro and worse clinical outcomes in patients with NSCLC and CML when compared with patients with the BIM wild-type allele (Ng et al., 2012). This variability in apoptotic potential may contribute to variable resistance in malignancies with the same oncogenic driver.

Interestingly, BIM levels were not predictive of response to cytotoxic chemotherapy in EGFR-mutant cancer cell lines. Further studies are required to determine whether BIM plays a role in acquired resistance to targeted therapy.
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<th>Drug</th>
<th>Cancer</th>
<th>Primary Target(s)</th>
<th>Primary Resistance</th>
<th>Acquired Resistance Due to Genetic Alteration of Target(s)</th>
<th>Acquired Resistance Due to Bypass Tracts and Other Mechanism(s)</th>
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<td>Gefitinib, erlotinib</td>
<td>NSCLC</td>
<td>EGFR</td>
<td>MET amplification (Turke et al., 2010) Primary T790M mutation (Maheswaran et al., 2008)</td>
<td>T790M (Kobayashi et al., 2005) L747S (Costa et al., 2007)</td>
<td>MET amplification (Engelman et al., 2007b) HGF induced MET activation (Yano et al., 2008) CRKL amplification (Cheung et al., 2011)</td>
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<td>(Shih et al., 2005) EGFR exon 20 mutations (Yasuda et al., 2012)</td>
<td>D761Y (Balak et al., 2006) T854A (Bean et al., 2008)</td>
<td>Loss of PTEN (Sos et al., 2009) BRAF (G469A and V600E) (Ohashi et al., 2012) PIK3CA (Sequist et al., 2011)</td>
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<td>K-RAS mutations (Pao et al., 2005) Low BIM expression and BIM polymorphism (Faber et al., 2011; Ng et al., 2012)</td>
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<td>HER2 amplification (Tuckelawa et al., 2012) Transformation to SCLC (Sequist et al., 2011)</td>
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<td>Crizotinib</td>
<td>NSCLC</td>
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<td>EML4-ALK amplification (Katayama et al., 2012) Loss of ALK (Doebel et al., 2012b)</td>
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<td>KIT amplification (Katayama et al., 2012) EGFR activation or mutation (Doebel et al., 2012b; Katayama et al., 2012)</td>
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<td>Cetuximab</td>
<td>CRC</td>
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<td>K-RAS mutation (Lièvre et al., 2006; Bardelli and Siena, 2010) BRAFV600E (Loupakis et al., 2009)</td>
<td>EGFR S492R (Montagut et al., 2012)</td>
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<td>Panitumumab</td>
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<td>Dasatinib</td>
<td>CML</td>
<td>BCR-ABL, Kit, PDGFR</td>
<td>T315I (Müller et al., 2009)</td>
<td>BRAF amplification (Shi et al., 2012) MEK1P124L and MEK1C121S (Emery et al., 2009; Wagle et al., 2011) N-RAS Q61R/N-RAS Q61K (Nazarian et al., 2010) PDGFRB overexpression IGF-1 overexpression (Villanueva et al., 2010)</td>
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<td>Nilotinib</td>
<td>CML</td>
<td>BCR-ABL, Kit, PDGFR</td>
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<td>BRAF splicing variants (Poulikakos et al., 2011)</td>
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<td>Vemurafenib</td>
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<td>BRAF</td>
<td>Multiple (BRAF V600E)</td>
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(continued)
(ARAF, BRAF, and CRAF) form a family of serine/threonine kinases. Upon RAS activation, RAF proteins are phosphorylated, dimerize, and phosphorylate the downstream proteins MEK and ERK1 or ERK2. The ERK proteins ultimately control multiple nuclear transcription factors and kinases that alter gene expression.

2. Mitogen-Activated Protein Kinase–Dependent Mechanisms of Acquired Resistance to BRAF-Inhibitors. Acquired resistance to vemurafenib develops within a median of 6 months. Sequencing of all BRAF exons in 16 patients with acquired vemurafenib resistance failed to show mutations of BRAF beyond the initial BRAFV600E mutation. There were no gatekeeper mutations (Nazarian et al., 2010). Unlike other malignancies, drug resistance in melanoma relies on mechanisms other than secondary mutations in the BRAF gene.

Amplification of BRAF was found to be one mechanism of acquired resistance in four out of 20 patients who had an initial response to vemurafenib and subsequently relapsed on treatment (Shi et al., 2012) (Fig. 2). Alternative splicing of the BRAF message may account for other cases of resistance. Poulikakos et al. (2011) reported a 61-kDa splicing product of BRAFV600E in resistant cell lines, herein called p61BRAFV600E. This variant results from a deletion of exons 4–8 and lacks the RAS binding domain, which is critical for RAS-dependent BRAF activation. This truncated protein activates ERK independently from RAS (Poulikakos et al., 2011). Furthermore, p61BRAFV600E shows enhanced dimerization properties compared with full-length enzyme. This results in p61BRAFV600E dimerization and signaling at BRAF inhibitor concentrations that effectively inhibit BRAFV600E catalytic function, thus leading to resistance to vemurafenib.

To investigate the clinical role of splicing variants as a mechanism of vemurafenib resistance, 19 patient-derived specimens were analyzed. Five of 19 vemurafenib-resistant tumors harbored BRAFV600E splicing variants. One patient had the truncated protein that was found in vitro (deletion of exons 4–8), and four patients had other splicing products. All products resulted in a protein that lacked the RAS binding domain, whereas the kinase domain that carries the BRAFV600E mutation in exon 15 remained intact. Pretreatment tumors from these 19 patients did not contain splicing variants of BRAF, nor did pretreatment tumors from 27 additional melanomas. These findings suggest that variant splicing may be a mechanism of acquired rather than primary resistance.

In the same study, four of the 19 vemurafenib-resistant patients had tumors with activating N-RAS mutations (Q61K and Q61R) that occurred mutually exclusive from BRAF splicing variants. N-RAS activation has been confirmed as a mechanism of resistance
in other patient specimens and in preclinical experiments (Nazarian et al., 2010; Poulikakos et al., 2011).

The ability of N-RAS to maintain ERK activation despite BRAF inhibition may be explained by alternative RAS-dependent activation of wild-type A- and CRAF isoforms (Poulikakos et al., 2010). In vitro studies showed that wild-type A- and/or CRAF isoforms dimerize and maintain ERK phosphorylation despite concomitant inhibition of BRAF. In the presence of upstream activation of RAS oncoproteins, only simultaneous knockdown of all three isoforms prevents downstream activation of ERK (Dumaz et al., 2006; Hatzivassiliou et al., 2010; Heidorn et al., 2010; Poulikakos et al., 2010; Villanueva et al., 2010). A recent study also demonstrated the ability of ARAF to bind to BRAFV600E in vitro (Rebocho and Marais, 2013). Consistent with these observations, increased CRAF overexpression was validated as a clinically relevant resistance mechanism in an independent study in a patient specimen (Montagut et al., 2008).

Further proof of the ability of RAS to signal through the inhibited BRAF comes from a study of the combination of a BRAF and a MEK inhibitor. Activated K-RAS in premalignant skin lesions leads to squamous cell carcinomas when patients receive vemurafenib, a side effect that is almost completely abolished by cotreatment with a MEK (downstream) inhibitor (Johannessen et al., 2010). A C to T transition in MEK1 exon 3 at codon 124 encodes an activating proline-to-leucine substitution (MEK1P124L), and was identified in a patient with relapsed metastatic melanoma following AZD6244 (a MEK1 inhibitor) treatment. In a subsequent study, a cysteine-to-serine substitution at codon 121 (MEK1C121S) was confirmed in a vemurafenib-resistant patient specimen.

BRAF-independent activation of MEK1 by COT (Cancer Osaka Thyroid Oncogene) overexpression, which encodes for a MAPK downstream of BRAF, was also established as a clinically relevant resistance mechanism (Johannessen et al., 2010).

### 3. Mitogen-Activated Protein Kinase–Independent Mechanisms of Acquired Resistance to Inhibition of BRAFV600E in Melanoma

Other mechanisms of resistance that originate upstream of BRAF and bypass its blockade confer resistance to BRAF inhibition. These mechanisms include platelet-derived growth receptor β activation and insulin-like growth factor-1 receptor (IGF-1R)–mediated activation of AKT (Villanueva et al., 2010). Loss of PTEN function (Villanueva et al., 2010), an important inhibitor of the PI3K/ AKT pathway, has also been identified in resistant tumors.

In an effort to overcome resistance through mutations upstream from BRAF, dabrafenib, a BRAF inhibitor, was combined with trametinib, a MEK inhibitor. The combination has produced a longer duration of freedom from progression as well as an improved toxicity profile (fewer squamous-cell skin cancers) in the first
reported combination trial in melanoma patients (Flaherty et al., 2012). This study highlights a promising strategy in overcoming resistance to single-agent treatment: combination of targeted therapies. Unlike other approaches, such as designing and developing next-generation TKIs with improved drug characteristics, the successful rationale combines drugs that target the same pathway on multiple levels and does this before mutations emerge clinically.

4. Resistance to BRAF Inhibitors in Colorectal Cancer. Although BRAF mutations in melanoma create marked sensitivity to vemurafenib, colorectal cancers (CRCs) with the same BRAF mutations are unresponsive to BRAF inhibition. In cell culture experiments, this insensitivity to BRAF inhibition was linked to a rapid feedback activation of EGFR, which in turn maintains a strong oncogenic signal in the presence of BRAF inhibition (Prahallad et al., 2012). Ectopic expression of EGFR in BRAF-mutant melanoma cells rendered these cells resistant to BRAF inhibition, supporting the notion that EGFR expression in BRAF inhibitor–resistant tumors is responsible for resistance. Combined inhibition of EGFR with a monoclonal antibody (mAb) or an EGFR TKI and a BRAF inhibitor resulted in sensitivity in BRAF-mutated CRC cells in experimental models (Corcoran et al., 2012).

D. Resistance to Breakpoint Cluster Region–ABL1, KIT, and Platelet-Derived Growth Factor Receptor–Targeted Therapy

1. Breakpoint Cluster Region–ABL1 Mutations in Chronic Myeloid Leukemia. Targeted therapy was pioneered in the 1990s with the introduction of imatinib for the treatment of CML. CML is a myeloproliferative disorder that is characterized by the Philadelphia chromosome, created by a translocation t(9;22) (q34;q11), resulting in the fusion gene BCR-ABL1 with activation of the ABL kinase function. This gene lacks
feedback regulation and results in uncontrolled cell proliferation. BCR-ABL1 is essential to the pathogenesis of CML and therefore became a major focus of initial efforts at targeted small-molecule drug development. First approved in 2001, the BCR-ABL1 inhibitor imatinib yielded response rates approaching 100% in its initial trials, and revolutionized the treatment of this disease (Druker et al., 2001). Despite the remarkable initial success of imatinib, resistance was recognized as a limiting factor, developing in about 2–3% of patients per year of observation (Baccarani et al., 2009). Early identification of resistance mechanisms has led to successful development of more potent and non–cross-resistant second-generation drugs (dasatinib, nilotinib, and bosutinib) and most recently the very promising third-generation TKI ponatinib.

2. Breakpoint Cluster Region-ABL1-Dependent Resistance Mechanisms in Chronic Myeloid Leukemia. The most common and best-characterized resistance mechanism to imatinib is attributable to mutations of the primary target gene BCR-ABL1. Overall, the appearance of resistance-associated BCR-ABL1 mutations correlates with the approaching onset of advanced disease state (accelerated or blast-phase CML) and worse clinical outcomes. More than 50 different resistance-associated mutations of BCR-ABL1 have been identified. They cause either conformational changes in the enzyme or disrupt contact points in the catalytic pocket of imatinib, thereby leading to insensitivity to the drug. The majority of these mutations hold the enzyme in an active conformation. Mutations can be found in pretreatment specimens and, under the pressure of drug exposure, emerge to become the major tumor population during treatment.

Mutations of the P (phosphate-binding) loop of the kinase domain are associated with imatinib resistance (Shah et al., 2002). Imatinib is an ATP mimic compound that only binds to the closed or inactive conformation of the enzyme. Mutations that hold the kinase domain in its active configuration result in suboptimal binding of the drug and loss of potent inhibition. In contrast to imatinib, second-generation TKIs, such as dasatinib or nilotinib, bind potently and inhibit the active enzyme conformation and overcome imatinib resistance due to P-loop mutations.

The gatekeeper T315I mutation directly impairs binding of both imatinib and the second-generation inhibitors and leads to high-level resistance. A third-generation TKI, ponatinib, was designed to bypass the T315I mutation and exhibits a high level of activity in patients refractory to imatinib, including those with T315I mutations (Cortes et al., 2012). Ponatinib is the first example of TKI targeted therapy that overcomes resistance mediated by the gatekeeper mutation, serves as a model for overcoming other gatekeeper mutant TKs, and has received accelerated approval for treatment of T315I-resistant CML.

Although target mutations are the most frequent findings in resistant CML, other mechanisms have been implicated, albeit less frequently. Amplification of BCR-ABL1 has been found in resistant CML cells, but is uncommon (Gorre et al., 2001).

Transport of drug across the intestinal epithelial barrier may also influence response. The organic cation transporter 1 (OCT-1) is the major transmembrane channel responsible for influx transport of imatinib. Low OCT-1 activity, related to polymorphisms in the OCT-1 gene, has been associated with worse clinical outcomes and treatment resistance (White and Hughes, 2009; Bazeos et al., 2010). Dose escalation of imatinib from 400 to 600–800 mg/day converts 30% of poorly responding patients to a meaningful clinical response. One potential explanation for treatment success in these patients is achieving higher plasma and intracellular drug levels due to low activity of transporters, such as OCT-1 (White et al., 2010). Switching to a second-generation TKI is an alternative option in patients with low OCT-1 activity, since neither dasatinib nor nilotinib transport depends on OCT-1 (Hiwase et al., 2008).

Resistance to second-generation TKIs, such as dasatinib and nilotinib, is mechanistically similar to that of imatinib. In addition to T315I, other mutations (F317L, E255K) have been implicated, albeit less frequently. Amplification of PDGFRA, which is essential to the pathogenesis of GISTs, has not been clearly characterized. Virtually all GISTs expresses c-KIT, and particular P-loop mutations such as E255K/V or Q252H also confer resistance to dasatinib and nilotinib (Müller et al., 2009). At this point, ponatinib resistance has not been clearly characterized.

3. Resistance to KIT/Platelet-Derived Growth Factor Receptor α-Targeted Therapy in Gastrointestinal Stromal Tumor. In addition to its activity against BCR-ABL1, imatinib is highly active against c-KIT and the structurally related platelet-derived growth factor receptor α (PDGFRα) kinase, either of which may act as an oncogenic driver in the pathogenesis of GISTs. Virtually all GISTs expresses c-KIT, 80% with an activating c-KIT mutation, 5–10% have an activating PDGFRα mutation, and only 10–15% lack mutations in either KIT or PDGFRα (Heinrich et al., 2003a; Debiec-Rychter et al., 2006). Imatinib produced a 50%–75% objective response rate in its early clinical trials (van Oosterom et al., 2001; Demetri et al., 2002; Blanke et al., 2008). The degree of responsiveness to imatinib varies depending upon the underlying mutation. The best clinical responses are found in patients with exon 11 mutations. These mutations are associated with dysregulation of the autoinhibitory region of c-KIT, resulting in a hyperactive kinase, which is effectively inhibited by imatinib (Heinrich et al., 2003b).

4. Primary Imatinib Resistance in Gastrointestinal Stromal Tumor. Primary resistance to imatinib (progression within 6 months of treatment initiation) occurs in 10–15% of patients and is associated with emergence of tumors with acquired mutations in the
catalytic domain of c-KIT or PDGFRA (Demetri et al., 2002; Verweij et al., 2004). Primary resistance mutations of c-KIT are most frequently found in exon 9 (Heinrich et al., 2003b) (Fig. 3). The most common PDGFRA mutations that underlie primary imatinib resistance are D842V and, less frequently, D846V (Antonescu et al., 2005; Miselli et al., 2007; Liegl et al., 2008).

5. Secondary Imatinib Resistance in Gastrointestinal Stromal Tumor and Response to Next-Generation Tyrosine-Kinase Inhibitors. Acquired resistance to imatinib in GISTs occurs after a median of 2 years of treatment and most frequently due to a second c-KIT mutation in tumors that carry a primary exon 11 mutation. Secondary mutations mostly involve exons 17, 13, and, less frequently, 14. Some of these mutations, such as V654A, cause intrinsic resistance to imatinib when introduced into wild-type protein. Other mutations, such as N822K, are associated with greater sensitivity to imatinib when occurring alone, but in combination with an underlying exon 11 mutation confer imatinib resistance, possibly due to combined structural changes in the protein (Heinrich et al., 2006).

It remains unclear why exon 9 mutations confer primary imatinib resistance. Understanding resistance is further complicated by the genetic inter- and intraindividual heterogeneity of this disease, the interplay of different mutations, and the resulting dysregulation of the oncoprotein (Antonescu et al., 2005; Wardelmann et al., 2005).

6. Resistance to Sunitinib in Gastrointestinal Stromal Tumor. Sunitinib is the agent of choice in patients with imatinib-resistant GISTs. In addition to its inhibition of KIT and PDGFRA, sunitinib inhibits VEGF receptors (VEGFRs) 1–3, FLT3 (Fms-related tyrosine kinase 3) and RET (Ret proto-oncogene), all of which have well defined roles in carcinogenesis. Similar to imatinib, sunitinib competes with ATP for binding to the kinase domain of KIT and PDGFRA. Its ability to block tumor growth through VEGFR inhibition may also contribute to its antitumor activity (Demetri et al., 2006; Heinrich et al., 2008; George et al., 2009). Tumors with primary (preimatinib) exon 9 mutations that developed imatinib resistance were more responsive to sunitinib than those with primary exon 11 mutation, and had improved PFS and OS on sunitinib, as compared with the exon 11–mutated tumors. Furthermore, tumors with secondary exon 13 or 14 mutations had better outcomes on sunitinib compared with those with secondary exon 17– or 18–mutated GISTs (Prenen et al., 2006; Heinrich et al., 2008). These findings

![Fig. 3. Exon sequence of exons 9–18 of the c-KIT gene. The table denotes primary mutations of the c-KIT and PDGFR genes with associated frequency in GISTs. The associated clinical response rate to imatinib is given for each exon. Frequency and location of secondary mutations of the c-KIT and PDGFR gene are given, and associated treatment response to second-line treatment (i.e., sunitinib) are shown for each exon. Figure references: Demetri et al., 2006, 2013; Dewaele et al., 2008; Nishida et al., 2008; Schöffski et al., 2010; Benjamin et al., 2011; Reichardt and Montemurro, 2011; Sawaki et al., 2011; Montemurro et al., 2013. JM, juxtamembrane domain; CB, clinical benefit.](image-url)
differ from the genetic patterns that underlie imatinib responsiveness, and may suggest the possible importance of the ability of sunitinib to inhibit multiple pathways and to inhibit KIT kinase associated with the gatekeeper mutation T670I (Guo et al., 2007).

Resistance to sunitinib develops after a median of 12 months. The mechanisms are poorly understood (Demetri et al., 2006). Recently, the novel multikinase inhibitor regorafenib was approved for patients whose tumors progress on imatinib and sunitinib. Regorafenib produced a median PFS of 4.8 months compared with approximately 1 month in the placebo group (Demetri et al., 2013).

E. Resistance to Mammalian Target of Rapamycin Inhibitors

The serine/threonine kinase mammalian target of rapamycin (mTOR) controls cell survival, proliferation, and intermediary metabolism in both normal and malignant cell types. It is positioned at a central point in the PI3K/AKT pathway, receiving upstream signals from a number of cell-surface TK receptors such as the IGF-1R and insulin receptor, as well as cross-talk from adjacent pathways (Fig. 4).

mTOR participates in two multiprotein complexes, mTORC1 and mTORC2, each of which has unique components and which carry out different biochemical functions. mTORC1 activates protein synthesis (and proliferation) via activation of S6K1 (P70 S6 kinase, alpha) and by inhibition of 4E-BP1 (eukaryotic translation initiation factor 4E-binding protein 1), a translation repressor. mTORC2 activates PI3K as well as other downstream targets. PI3K phosphorylation leads to activation of mTORC1 and to cell proliferation, and its stimulation by growth factor receptors may overcome inhibition by selective inhibitors of the mTORC1 complex.

The PI3K/AKT pathway is activated by mutations at any one of several sites (PI3K, AKT) in at least 50% of human tumors (Rodon et al., 2013). Alternatively, the pathway may be activated by loss of function or deletion of the PI3K antagonist PTEN, which dephosphorylates phosphoinositols triphosphate, or by loss of function of tuberous sclerosis complex 1, a negative regulator of mTOR. Specific pathway-activating mutations of mTOR pathway components (PI3K, AKT, PTEN, and S6K1) are found in subsets of many epithelial cancers, including endometrial, pancreatic, neuroendocrine, colon, lung, and breast cancers, and may occur in conjunction with other mutations. However, no clear relationship between specific pathway mutations, or PTEN loss, and response to mTOR inhibition has been established in clinical trials.

Fig. 4. (A) Physiological PI3K/AKT/mTOR pathway and inhibition by mTORi. (B) Mechanisms of resistance to mTOR inhibitors (refer to text for explanation). FKBP12, FK506-binding protein 1A, 12kDa; GBL, mTOR-associated protein, LST8 homolog (S. cerevisiae); PDK1, 3-phosphoinositide-dependent protein kinase 1; PRB5, prline rich 5 (renal); Sin1, mitogen-activated protein kinase-associated protein 1. TSC, tuberous sclerosis complex.
Two allosteric mTOR inhibitors, everolimus and temsirolimus, are currently approved for cancer indications. Both drugs are useful for renal cell cancer treatment, whereas everolimus has additional activity against pancreatic neuroendocrine tumors, breast cancer, and astrocytoma. Both agents are selectively inhibitory to mTORC1 activity, sparing mTORC2. They bind to FKBP12, and the complex then binds to and inhibits mTORC1.

A number of mechanisms of resistance to mTORC1 complex inhibition have been identified in preclinical studies, and one prominent mechanism, compensatory AKT activation, has been verified in studies of human tumors during treatment. Selective mTORC1 inhibition activates multiple feedback loops, leading to activation of receptor tyrosine kinases and phosphorylation/activation of AKT at Thr308 (via PI3K) and Ser473 (via mTORC2). These changes tend to override mTORC1 inhibition.

In clinical specimens from different malignancies, including breast cancer, cervical cancer, leukemia, and lymphoma, increased AKT activity correlated with poor response to mTOR inhibitors (Carracedo et al., 2008; Gupta et al., 2009; Ihle et al., 2009; Janes et al., 2010; Janku et al., 2012). On the other hand, constitutive PI3K activation, due to activating PI3K mutations or due to loss of PTEN, has been proposed as a marker for sensitivity to mTOR inhibition in melanoma and glioblastoma multiforme (Galanis et al., 2005; Margolin et al., 2005). In a phase I trial of 78 patients with refractory metastatic CRC treated with a combined PI3K/AKT/mTOR inhibitor, 18 (23%) responded (partial response, complete response, or stable disease >6 months).

Activation of alternative pathways may contribute to resistance to mTOR inhibition. Mutations of either K-RAS or BRAF were associated with a worse survival in this analysis, indicating that activation of alternative pathways may contribute to resistance to inhibitors of mTOR. Consistent with this observation, patients with metastatic colorectal cancer with PI3K and concomitant K-RAS mutation seem to have a lower response rate to mTOR inhibition (Garrido-Laguna et al., 2012). Other mutations that modify mTOR activity or cell death responses may influence the efficacy of mTOR inhibitors. In metastatic bladder cancer patients, loss of function mutation in tuberous sclerosis complex 1, an mTOR regulatory protein, was identified in 8% of 109 patients and was associated with sensitivity to everolimus (Iyer et al., 2012).

Preclinical experiments have implicated other resistance mechanisms, as yet not validated in clinical studies. These alternative mechanisms include hyperactivity of PIM [Pim oncogene (proviral integration site)] kinases as found in hematopoietic cells (Hamerman et al., 2005), pyruvate dehydrogenase kinase 1–dependent myelocytomatosis phosphorylation (Liu et al., 2011), overexpression of the mTORC1 substrate eukaryotic translation initiation factor 4E (Dilling et al., 2002), imbalance of pro- and antiapoptotic proteins [BIM and BCL-2 or BLC-2 homologous proteins (inhibitor of apoptosis protein, IAPs)] (Gyrd-Hansen and Meier, 2010; Mahalingam et al., 2010), and mutation of FKBP-12 or the FKB domain of mTOR (Feldman and Meier, 2010; Mahalingam et al., 2010), and mutation of the FKB domain of mTOR (Feldman and Meier, 2010; Mahalingam et al., 2010), and mutation of FKBP-12 or the FKB domain of mTOR (Feldman and Meier, 2010; Mahalingam et al., 2010), and mutation of FKBP-12 or the FKB domain of mTOR (Feldman and Meier, 2010; Mahalingam et al., 2010). Alternative mechanisms include hyperactivity of PIM [Pim oncogene (proviral integration site)] kinases as found in hematopoietic cells (Hamerman et al., 2005), pyruvate dehydrogenase kinase 1–dependent myelocytomatosis phosphorylation (Liu et al., 2011), overexpression of the mTORC1 substrate eukaryotic translation initiation factor 4E (Dilling et al., 2002), imbalance of pro- and antiapoptotic proteins [BIM and BCL-2 or BLC-2 homologous proteins (inhibitor of apoptosis protein, IAPs)] (Gyrd-Hansen and Meier, 2010; Mahalingam et al., 2010), and mutation of FKBP-12 or the FKB domain of mTOR (Feldman and Meier, 2010; Mahalingam et al., 2010).

Strategies to overcome resistance to mTOR inhibitors have reached the stage of clinical development. Novel inhibitors that block both mTORC complexes (1 and 2) have entered clinical trials, as have single agents with combined mTORC1 and PI3K inhibition. Results are thus far inconclusive. Combinations of a drug that inhibits mTOR and a second agent that blocks upstream kinases (AKT or PI3K) or receptors (IGFR) are also under investigation.

There are clear opportunities to expand the clinical application of the mTOR inhibitor class of drugs. Of great interest is the finding that activation of the PI3K pathway may underlie resistance to hormonal therapy and to inhibitors of cell-surface receptors such as HER2. One successful trial has shown that everolimus significantly enhances the response to second-line antiestrogen therapy in breast cancer (Baselga et al., 2012a).

F. Resistance to Monoclonal Antibody Therapies

1. Drugs Targeting Epidermal Growth Factor Receptor in Colorectal Cancer. Colorectal cancer remains the third most common cancer worldwide, and nearly half of newly diagnosed patients have metastatic disease that is associated with a poor median overall survival of 18–21 months (Ferlay et al., 2007; Jemal et al., 2010). In refractory metastatic CRC (mCRC), two monoclonal antibodies directed against EGFR, cetuximab and panitumumab, were found to be effective when combined with conventional chemotherapy or used as monotherapy (Cunningham et al., 2004; Saltz et al., 2004; Chung et al., 2005). In contrast to NSCLC, somatic mutations of the EGFR gene in CRC are exceedingly rare and do not explain the sensitivity of CRCs to these antibodies (Cunningham et al., 2004; Chung et al., 2005). Modestly increased EGFR copy number (3- to 5-fold) correlates with improved response to cetuximab and panitumumab. However, the increased gene dose does not result in increased expression of EGFR, and, for reasons that are unclear, increased EGFR expression does not correlate with response to these antibodies (Moroni et al., 2005). Expression of other biomarkers, such as amphiregulin and epiregulin, may be more useful in predicting response to monoclonal antibodies directed against EGFR (Moroni et al., 2005; Kambata-Ford et al., 2007).

2. Primary Resistance to Monoclonal Antibodies against Epidermal Growth Factor Receptor. Only 10–20% of patients with mCRC respond to EGFR-mAb monotherapy or combination therapy with chemotherapy
(Cunningham et al., 2004; Saltz et al., 2004; Chung et al., 2005). Intrinsic or primary resistance, therefore, is a major limitation of the use of mAbs directed against EGFR. Mutations of K-RAS have been identified as strong negative predictive markers and may explain resistance in about 40% of the treatment-resistant population (Linardou et al., 2008). This observation was initially made in retrospective studies (Lièvre et al., 2006) and later confirmed in several prospective trials (Bardelli and Siena, 2010). This finding resulted in limiting the use of cetuximab and panitumumab to only K-RAS wild-type tumors. A retrospective study of patients with K-RAS G13D (n = 32) treated with cetuximab alone or in combination with chemotherapy found that these patients had a higher response rate (9.1 vs. 1%) and a longer PFS (4.0 vs. 1.9 months) and OS (7.6 vs. 5.7 months) compared with patients with other K-RAS mutations. The authors concluded that, despite the presence of a K-RAS mutation, these patients should receive anti-EGFR–targeted treatment (De Roock et al., 2010). However, this observation contradicts results from two prospective trials (Andreyev et al., 2001; Roth et al., 2010) and needs further investigation.

A second important oncogenic driver of resistance to EGFR is mutated BRAF (V600E being the most common), which is found mutated in 5–15% of CRC patients. This mutation in CRC is mutually exclusive from K-RAS mutations (Di Nicolantonio et al., 2008; Loupakis et al., 2009; Safaee Ardekani et al., 2012). Together, mutations in the K-RAS and BRAF pathways account for ~50% of primary resistance to EGFR–mAb therapy. Other mutated drivers that may impair responsiveness to EGFR-targeted therapy in CRC include PTEN and PI3K, although data are conflicting and the association with resistance is not as strong as for K-RAS or BRAF mutations (Jhawer et al., 2008; Perrone et al., 2009; Prenen et al., 2009). PI3K mutations, with or without concomitant loss of PTEN, occur in ~15% of resistant tumors. Although mutations of K-RAS and BRAF are mutually exclusive, they can each occur in conjunction with either PTEN or PI3K mutations. These combined mutations of BRAF or K-RAS with mutations in the PI3K pathway account for 5–10% of all resistance mutations (Bardelli and Siena, 2010).

3. Secondary Resistance to Epidermal Growth Factor Receptor Monoclonal Antibody. Although patients who respond to cetuximab or panitumumab may show a dramatic regression of their disease, tumor recurs in 9–18 months (Cunningham et al., 2004; Diaz et al., 2012; Misale et al., 2012). In contrast to the extensive clinical evidence for drivers of primary resistance, little was known about mechanisms of secondary resistance to EGFR-mAb in colorectal cancer until recent studies shed some light on this important issue (Bertotti et al., 2011; Diaz et al., 2012; Misale et al., 2012; Montagut et al., 2012). Montagut et al. (2012) first identified a missense mutation in the extracellular domain of EGFR. This mutation involves a substitution of serine to arginine at amino acid 492 (S492R). In 2 of 10 patients with CRC who developed resistance to cetuximab, biopsies postcetuximab treatment carried this mutation, whereas 156 specimens from treatment-naive patients were free of this mutation.

Other mechanisms of secondary resistance may involve K-RAS. These include 1) expansion of a pre-existing K-RAS mutant clone, 2) de novo K-RAS mutations in tumors that were K-RAS wild type prior to treatment, and 3) amplification of K-RAS post-treatment. In the most extensive clinical study, investigators found new K-RAS mutations post-treatment in 6 of 10 patients who, pre-treatment, had K-RAS wild-type disease (Misale et al., 2012). Four of the 6 patients had tumors harboring the point mutation G13D, 1 patient had a Q61H mutation, and 1 patient developed a combined mutation (G12D and G13D) in post-treatment samples. A seventh patient had K-RAS amplification. Furthermore, by analyzing circulating tumor DNA, it was possible to detect mutations in K-RAS in another group months prior to radiographic recurrence (Diaz et al., 2012). These investigators took monthly serum samples from 24 patients with K-RAS wild-type disease during panitumumab treatment and found that 9 patients had K-RAS mutations, all on exon 12. Using a mathematical model that incorporates the initial tumor burden, doubling time, and circulating tumor DNA levels of post-treatment K-RAS-mutant patients, the authors predicted that a population of K-RAS-mutant cells must have been present prior to treatment.

Using an elegant method that involves transplantation of patient-derived tumors (metastasis) of patients treated with cetuximab, Bertotti and colleagues (2011) identified amplification of HER2 as a resistance mechanism independent from K-RAS mutation status. Patient-derived tumors with this resistance mechanism treated with a combination of lapatinib and cetuximab or pertuzumab had long-lasting response compared with monotherapy with either drug in the same patient-derived tumor, indicating that combination therapy may provide clinical benefit (Bertotti et al., 2011).

4. Resistance to Human Epidermal Growth Factor Receptor 2–Targeted Therapy in Human Epidermal Growth Factor Receptor 2–Positive Breast Cancer. Approximately 25% of invasive breast cancers have amplified HER2. This finding is associated with aggressive clinical behavior, chemotherapy resistance, and a worse clinical outcome than HER2-negative tumors. HER2 is a member of the EGFR family, but lacks intrinsic ligand-induced signaling. Its signaling requires formation of heterodimers with any of the
other three ligand binding members of the EGFR-like receptor family (HER1, 3, or 4). The HER2 heterodimers activate intracellular pathways (MAP kinase and PI3K/AKT), resulting in cancer cell survival and proliferation. The HER2/HER3 dimer strongly phosphorylates and activates PI3K. Trastuzumab, a humanized recombinant monoclonal antibody, targets the extracellular domain of the HER2 protein, and significantly inhibits growth of HER2-positive breast cancer (Slamon et al., 2001; Romond et al., 2005). Although most HER2-positive patients have no response to treatment with trastuzumab as a single agent, the drug significantly enhances effectiveness of taxanes and other chemotherapy in both the adjuvant setting and in the treatment of metastatic disease. Virtually all patients with metastatic disease will develop resistance.

5. Primary Resistance to Trastuzumab. Approximately 75% of patients with HER2-positive breast cancer do not respond to trastuzumab. However, the clinical endpoint of primary resistance to trastuzumab monotherapy is difficult to recognize in the clinical setting, since a significant portion of patients respond to treatment with trastuzumab when given with cytotoxic therapy. The latter is indicated by the major benefit of trastuzumab in combination with cytotoxic therapy in metastatic and adjuvant therapy, settings in which it produces a doubling of response rates and prolonged disease-free survival. The biological effect of trastuzumab may be due to slowing tumor growth and sensitizing the cells to DNA damage by cytotoxic chemotherapy. Even with patients who progress on trastuzumab, the drug is usually continued for the life of the patient as clinical studies have defined benefit (slower tumor growth) in this setting. A minor subgroup of HER2-positive breast cancers express a truncated 95–100 kDa receptor, p95HER2, which lacks the ectodomain of the full-length protein. Indeed, in some tumors, these fragments can be the predominant form of HER2 (Molina et al., 2002). p95HER fragments have preserved kinase activity and are associated with an aggressive trastuzumab-resistant course (Sáez et al., 2006; Scaltriti et al., 2007). The lack of response to trastuzumab in these tumors is likely due to absence of a critical portion of the extracellular domain of HER2, the primary target of trastuzumab.

6. Secondary Resistance to Trastuzumab. With the knowledge that HER2 signals as a dimer through the PI3K pathway, it was logical to examine activation of the latter pathway as a cause of resistance. Attention has focused on PTEN (Nagata et al., 2004), which inactivates PI3K function. Reduced expression or loss of PTEN in 47 HER2-positive tumors in patients treated with trastuzumab plus taxane (paclitaxel or docetaxel) correlated with a worse response compared with the outcome in patients with PTEN-positive tumors. In another group of patients with HER2-negative tumors (n = 37), levels of PTEN expression did not influence outcome of taxane-based therapy. These results are limited by the lack of sequential biopsy data showing a loss of PTEN expression as tumors become resistant during treatment, but overall, the study suggests that a lack of PTEN expression results in increased trastuzumab resistance, presumably via release of PI3K/AKT signaling. Inhibition of PI3K inhibits cells depleted of PTEN in vitro, supporting this idea. Furthermore, mutations of PI3K have been found in tumors resistant to trastuzumab (Berns et al., 2007).

Alternative TK receptor signaling may also contribute to resistance to trastuzumab. Trastuzumab lacks activity against other EGFR family growth factor receptors, such as EGFR(HER1) and HER3. Homodimerization (EGFR/EGFR) or heterodimerization (EGFR/HER3) of these alternative receptors, with subsequent downstream activation of the PI3K/AKT pathway, has been demonstrated in cells exposed to trastuzumab and may contribute to resistance (Motoyama et al., 2002).

Additional studies in the preclinical setting implicate increased levels of IGF-1R and its ability to build heterodimers with HER2, despite treatment with trastuzumab (Lu et al., 2001; Nahta et al., 2005). The clinical role of IGF-1R in trastuzumab-resistant HER2-positive breast cancer is uncertain. Although some studies failed to show a significant correlation of IGF-1R expression with trastuzumab response (Smith et al., 2004; Köstler et al., 2006), others indicate that IGF-1R expression is associated with poor clinical outcomes, particularly when associated with concomitant activation of downstream effectors such as mTOR (Harris et al., 2007; Gallardo et al., 2012; Yerushalmi et al., 2012). Finally, MET was found to be overexpressed in HER2 breast cancer cell lines treated with trastuzumab. MET expression led to decreased treatment response, and MET short hairpin RNA interference was able to restore sensitivity to trastuzumab (Shattuck et al., 2008). Further potential mechanisms have been suggested, but not clinically validated (Nahta and Esteva, 2006).

Newer anti-HER2 antibodies, which differ in their mechanism of cell killing and which may overcome resistance, have been approved for clinical use. Pertuzumab prevents HER2/HER3 dimerization and enhances trastuzumab activity when the two antibodies are given in combination (Baselga et al., 2012b). Herceptin emtansine is a covalent conjugate of the antibody with a maytansine derivative. It delivers a potent cytotoxin through its binding to the HER2 receptor, as the antibody-toxin conjugate is internalized and the toxin is released intracellularly in lysosomes. It retains activity in patients resistant to trastuzumab (Baselga et al., 2010). The mechanisms of resistance to these newer products are unknown.
G. Resistance to Anti-CD20 Antibodies

Two anti-CD20 antibodies, rituximab (1997) and ofatumumab (2011), have been approved for treatment of lymphoid tumors (see Table 1). They engage different epitopes on CD20, differ in their level of activation of complement-dependent cytotoxicity, and are at least partially non–cross-resistant clinically, ofatumumab having clinical activity in rituximab refractory follicular lymphoma (Czuczman et al., 2012). Rituximab is extensively used with chemotherapy for treatment of chronic lymphocytic leukemia, follicular lymphoma, mantle cell lymphoma, diffuse large B cell lymphoma, and has additional indications for a variety of autoimmune disorders. It has multiple potential mechanisms of antitumor activity (Alduaj and Illidge, 2011; Rezvani and Maloney, 2011). Its engagement of CD20 leads to complement fixation through the antibody’s Fc arm, which binds to the C1q component of complement, mediating complement-dependent cytotoxicity (CDC) of the lymphoma cell and normal B cells. Rapid depletion of complement follows infusion of rituximab, perhaps the byproduct of complement fixation accompanying infusion reactions (fever, chills, rash, urticaria). The same Fc arm can bind to the Fc gamma receptor of macrophages, activating antibody-dependent cellular cytotoxicity (ADCC). Engagement of CD20 by antibody may cause a nonapoptotic cell death in its own right. Finally, the anti-CD20 antibodies may promote dendritic cell appreciation of B cell antigens and lead to adoptive cellular immunity. Of these mechanisms, ADCC seems most important for rituximab, whereas ofatumumab may have stronger CDC activity (Teeling et al., 2006).

Resistance to rituximab is incompletely understood (Rezvani and Maloney, 2011). Animal models suggest that the predominant mechanism of cytotoxicity is ADCC, but clinical studies are inconclusive, and its efficacy may depend heavily on the interaction of rituximab with cells exposed simultaneously to chemotherapy. The level of expression of CD20, which is highest in follicular lymphoma and lowest in chronic lymphocytic leukemia (CLL), influences the response to monotherapy. Loss of CD20 expression, either through internalization of the complex or “shaving” of the complex by macrophages, has been implicated in resistant clinical tumors. Specific polymorphisms of the Fcy RIIIA (the valine/valine variant at position 158) and the Fcy RIIA receptors with higher affinity for IgG antibodies confer a better therapeutic response to rituximab in patients with follicular lymphoma (Weng and Levy, 2003), but this has not been a consistent finding in CLL (Rezvani and Maloney, 2011). In CLL, CDC may play a more important role, as resistant cells may express high levels of inhibitors of complement fixation (CD55 and CD59).

Pharmacokinetic factors may play a role in resistance. Higher sustained levels of rituximab and slower clearance are associated with response, as is a low tumor burden. A large tumor burden leads to depletion of antibody during monotherapy with rituximab, although the efficacy of rituximab in combination with chemotherapy is less affected by tumor burden. There has been no prospective study of individualizing dose or schedule of either rituximab or ofatumumab to adjust to interindividual differences in pharmacokinetics and drug exposure.

H. Resistance to Anti-Angiogenic Drugs

Angiogenesis and the formation of an extensive vascular bed are required for growth of solid malignancies beyond a few millimeters in size. The construction of a vascular network for tumors requires proliferation of vascular endothelial cells (ECs) and the recruitment of supporting pericytes, a process known as the angiogenic switch (Folkman, 1971). A number of growth factors are responsible for the early differentiation of mesodermal precursor cells to ECs and for their sprouting. These factors include the VEGF, which interacts with three receptor isoforms (VEGFRs 1–3). Of these VEGFRs, VEGFR2 (or kinase insert domain receptor) has been the most consistent target of successfully developed agents. Other factors, including matrix metalloproteinases, PDGFB, its receptor PDGFR-B, transforming growth factor B, and angiopoietins with their tie-1 (tyrosine kinase with immunoglobulin and epidermal growth factor homology domain 1) receptor, facilitate vascular invasion of extracellular matrix, induce pericyte recruitment, stabilize new vessels, and promote tumor angiogenesis (Risau, 1997). Gene expression of PDGFB and VEGF is regulated by the hypoxia-inducible factor alpha (HIF-alpha). Multiple stimuli and signaling pathways activate HIF, including local hypoxia, mTORC1 activation, and loss of the tumor suppressor Von Hippel–Lindau, and all of these factors activate transcription of proangiogenic genes.

There are three clinically successful approaches to antiangiogenic therapy for cancer: 1) antibody-mediated binding of VEGF by bevacizumab; 2) ziv-afliborect, a humanized chimeric protein that contains the extracellular domains of VEGFR1 and VEGFR2 and acts as a decoy receptor; and 3) small molecules that directly inhibit VEGFR(s) and PDGFR-B signaling (sunitinib, sorafenib, axitinib, pazopanib, and vandetanib).

Antiangiogenic drugs are approved as single agents for various solid tumors, including renal cell carcinoma (RCC), sarcomas, GIST, PNET, HCC, glioblastoma multiforme (GBM), and medullary thyroid carcinoma. They are further approved for use in combination with cytotoxic chemotherapy for colorectal cancer, NSCLC, and ovarian cancer. The benefits of sunitinib against GIST and vandetanib against medullary thyroid carcinoma are likely related to off-target inhibition of c-KIT and RET, respectively.
Unlike resistance to treatment directed against other targets, such as BCR-ABL, KIT, and EGFR, resistance to antiangiogenic therapy does not rely on mutation of the target cell-surface TK (VEGFR), which is situated on normal endothelial cells. The mechanism is poorly understood, but likely to be rather complex, perhaps transient, and even reversible, as evidenced by recent studies. Mice bearing RCC xenografts were exposed to sorafenib until resistance developed, while a second group did not receive any treatment (untreated group). Resistant tumors were then transplanted into naive mice, followed by a treatment break from sorafenib. The gene expression profiles of reimplanted tumors, after a treatment break, were very similar to the profiles of untreated tumors, but were distinct from profiles of tumors examined at the height of resistance. Accordingly, reimplanted tumors responded when re-exposed to sorafenib (Zhang et al., 2011). Translating this observation into clinic, one could hypothesize that continued administration or readministration of antiangiogenic agents, even in the case of prior development of resistance, may be beneficial. In the clinic, reversible/transient resistance was observed in a small study that included patients with RCC who progressed on sunitinib treatment. There is anecdotal evidence that readministration of sunitinib after tumor progression may be beneficial. Two of five patients with metastatic RCC relapsed at the site of prior metastasis 3 and 8 months after sunitinib discontinuation, respectively. Readministration of sunitinib resulted in major responses in both patients (Johannsen et al., 2009). Similar responses were observed in 5 of 23 patients with RCC who had previously progressed on sunitinib and were rechallenged, after a treatment break, with the same agent (Zama et al., 2010).

Tumors thought to be resistant to one anti-VEGF(R) therapy appear to remain dependent on VEGF signaling. This was further evidenced by objective response in clinical trials of patients with RCC undergoing sequential antiangiogenic therapy with sunitinib and axitinib after disease progression on bevacizumab-based therapy and sorafenib, respectively (Rini et al., 2008, 2009). A similar observation was made in patients with mCRC who progressed on a bevacizumab-based regimen. Continued inclusion of bevacizumab plus chemotherapy produced improved survival when compared with chemotherapy alone (Bennouna et al., 2013).

Overall, data on specific mechanisms of resistance to antiangiogenic treatment are mostly limited to preclinical observations. Clinical evidence is scarce and fragmented. Research on mechanisms of resistance to angiogenesis has focused on two factors: 1) VEGF-signaling pathways and 2) VEGF-independent restoration of angiogenesis.

Experimentally, blockade of VEGF signaling leads to a drastic decrease in blood flow to the tumor, but reperfusion of the tumor occurs months after treatment and corresponds to tumor regrowth (Schor-Bardach et al., 2009). Cessation of blood flow results in hypoxia and necrosis of tumor tissue. A potential resistance mechanism in this setting could be hypoxia-induced HIF upregulation and target gene transcription, including VEGF and other proangiogenic factors, ultimately resulting in revascularization and reperfusion of tumor tissue. Additionally, tumor cells that are more resistant to hypoxia could be selected to grow in this constricted microenvironment. In fact, it has been hypothesized that disintegration of the primary tumor by VEGF blockade may lead to selection of more invasive, hypoxia-resistant tumor cells that have increased potential to invade and metastasize (Ebos et al., 2009; Pàez-Ribes et al., 2009).

Evidence for MET as an important hypoxia-induced factor was proposed in clinical specimens from 22 GBM patients with disease recurrence on bevacizumab treatment. In a xenograft model with bevacizumab-resistant GBMs, increased MET expression and phosphorylation of its downstream targets were associated with the development of anti-VEGF therapy resistance. Treatment with the MET inhibitor XL184 led to tumor shrinkage and prolonged survival of mice with bevacizumab-resistant GBM, and short hairpin RNA-mediated blockade of MET-abrogated resistance to bevacizumab (Jahangiri et al., 2013). In addition, the same study also found compensatory upregulation of VEGF, VEGFR, and basic fibroblast growth factor (FGF) in resistant GBMs, indicating that angiogenesis continues to be, at least in part, dependent on VEGF signaling. Thus, relative underdosing of antiangiogenic therapy at that particular state of disease development may be an additional factor that is currently not recognized due to lack of clinical specimens from resistant tumors.

Increased expression of alternate proangiogenic factors and endothelial receptors is able to promote angiogenesis independent of VEGF signaling. These angiogenic factors include FGF-1 and FGF-2, ephrin-A1 and -A2, and angiopoietin-1 in pancreatic island cells (Casanovas et al., 2005); FGF-2 and stem cell–derived factor-1 in GBM (Batchelor et al., 2007); placental growth factor (PIGF) in various tumor cells (Fischer et al., 2007); interleukin-8 in colon cancer cells (Mizukami et al., 2005); PDGF-C (Crawford et al., 2009); and PIGF, basic FGF, and HGF from sera of patients with colon cancer recurrent on a bevacizumab-based treatment regimen (Kopetz et al., 2010). Notably, some proangiogenic factors, including PIGF, osteopontin, and granulocyte colony-stimulating factor are induced by sunitinib in a manner independent of tumor type (Ebos et al., 2007).

Furthermore, various circulating host cells and cells of the tumor microenvironment have been implicated in angiogenesis of resistant tumors. These include bone
marrow–derived cells, such as CD11b+/Gr1+ myeloid suppressor-type cells activated through granulocyte colony-stimulating factor and Bv8–dependent mechanisms, and endothelial TEK tyrosine kinase (Tie2)–expressing monocytes via angiopeptien-2–dependent signaling, tumor-associated macrophages, circulating endothelial precursors, and circulating ECs and tumor fibroblasts (Lewis et al., 2007; Shojaei et al., 2007, 2009; Ferrara, 2010). These cells may be recruited to the tumor microenvironment and contribute to VEGF-independent angiogenesis in anti-VEGF–resistant tumors. Tumor-associated macrophages also contribute to local PDGF-C expression (Crawford et al., 2009). Pericytes may facilitate revascularization by retaining a vascular scaffold that supports EC adhesion and proliferation (Mancuso et al., 2006).

Finally, the utilization of pre-existing healthy vasculature by tumor growth around already present vessels, a process called vascular co-option, has been observed in glioma and NSCLC models, may provide nutrition and oxygen delivery to the tumor without the need for de novo angiogenesis, and may support anti-VEGF treatment resistance (Kunkel et al., 2001; Offersten et al., 2001).

In conclusion, the biology behind resistance to antiangiogenic therapies is poorly understood at both the experimental and the clinical level. Although many factors are known to contribute to tumor angiogenesis in model systems, none has been clearly implicated in the response to currently used small molecules or antiangiogenic antibodies. The identification of a biomarker that predicts response could lead to the development of new agents with different targets in the angiogenic cascade.

### III. Conclusion

Mechanisms of resistance to targeted therapies have been identified for BRAF+, EGFR+, ALK+, and BCR-ABL–driven tumors, and have led to new and, in some cases, successful strategies that use new drugs or new drug combinations to restore sensitivity to treatment. However, resistance in even the best understood tumors is poorly understood, and major areas of research into drug resistance, such as antiangiogenic therapies, are fragmentary. Virtually unexplored is the relationship of individual pharmacokinetics with response, the impact of polymorphisms on drug receptors and metabolic pathways, and the differences in tumor response that are governed by the tissue of origin (as seen in the variability of response to inhibitors of BRAFV600E in melanoma, colon, lung, and thyroid malignancies). A better understanding of drug resistance to targeted agents will require carefully planned and executed prospective trials in which patients’ tumors are sequentally biopsied before treatment and at the time of progression. This seems like a simple mandate, but one that is extremely difficult to pursue clinically. With a detailed knowledge of mutations and their interaction with specific drugs, it is possible to design new molecules that overcome specific forms of resistance, as illustrated by the success of ponatinib in CML and LDK378 in NSCLC. Activation of alternative pathways presents a more complex and challenging problem, in that it will be necessary to inhibit the initial activating mutation as well as the new contributing pathway with a combination of drugs.

### Authorship Contributions

Wrote or contributed to the writing of the manuscript: Izar, Chahner, Rotow, Gainor, Clark.

### References


