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Acquired resistance to drugs targeting receptor tyrosine kinases

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ABSTRACT

Development of resistance to chemotherapeutic drugs represents a significant hindrance to the effective treatment of cancer patients. The molecular mechanisms responsible have been investigated for over half a century and have revealed the lack of a single cause. Rather, a multitude of mechanisms have been delineated ranging from induction and expression of membrane transporters that pump drugs out of cells (multidrug resistance (MDR) phenotype), changes in the glutathione system and altered metabolism to name a few. Treatment of cancer patients/cancer cells with chemotherapeutic agents and/or molecularly targeted drugs is accompanied by acquisition of resistance to the treatment administered. Chemotherapeutic agent resistance was initially assumed to be due to induction of mutations leading to a resistant phenotype. This has also been true for molecularly targeted drugs. Considerable experience has been gained from the study of agents targeting the Bcr-Abl tyrosine kinase including imatinib, dasatinib and sunitinib. It is clear that mutations alone are not responsible for the many resistance mechanisms in play. Rather, additional mechanisms are involved, ranging from epigenetic changes, alternative splicing and the induction of alternative/compensatory signaling pathways. In this review, resistance to receptor tyrosine kinase inhibitors (RTKIs), RTK-directed antibodies and antibodies that inactivate ligands for RTKs are discussed. New approaches and concepts aimed at avoiding the generation of drug resistance will be examined. The recent observation that many RTKs, including the IGF-1R, are dependence receptors that induce apoptosis in a ligand-independent manner will be discussed and the implications this signaling paradigm has on therapeutic strategies will be considered.

Contents

Abbreviations: Akt, Ak (mouse strain) – thymoma; Axl, a receptor tyrosine kinase; Bcr-Abl, breakpoint cluster-Abelson tyrosine kinase; CML, chronic myelogenous Leukemia; CrkL, v-crk sarcoma virus CT10 oncogene homolog (avian)-like; DACH1, Dachshund homolog 1 (Drosophila); Dok, docking protein (downstream of tyrosine kinase 1); DTP, drug-tolerant persisters; DTEP, drug-tolerant expanded persisters; ECD, extracellular domain; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; Erk, extracellular-regulated kinase; FGFR, fibroblast growth factor receptor; FIT3, FMS-like tyrosine kinase 3; GIST, gastrointestinal stromal tumor; Grb2, growth factor receptor bound-2; HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IGF-F1-1, cyclic hexadecapeptide, IGF antagonist; IGF-1R, insulin-like growth factor-1 receptor; IGFBP, insulin-like growth factor binding protein; IQGAP1, Ras GTPase-activating-like protein; Jak, Janus kinase; KD, kinase domain; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; MDR, multidrug resistance; MEK, map kinase kinase; Met, MNNG HOS Transforming gene; mTOR, mammalian target of rapamycin; NSCLC, non-small cell lung cancer; OCT1, organic cation transporter 1; PDGFR, platelet-derived growth factor; PDK1, phosphoinositide-dependent kinase 1; PH, pleckstrin homology; PI3K, phosphoinositide 3-kinase; PTB, phosphotyrosine binding domain; PTEN, phosphatase and tensin homolog; Raf, Ras family member; Ras, rat sarcoma; RTK, receptor tyrosine kinase; SH2, src homology 2 domain; Shc, SH2 domain containing; SHIP, SH2 domain-containing inositol phosphatase; Sos, son of sevenless; c-Src, cellular-sarcoma; Stat, signal transducer and activator of transcription; TKI, tyrosine kinase inhibitor; Vav, guanine nucleotide exchange factor, vertical line, pillar (Hebrew); VEGFR, vascular endothelial growth factor receptor.

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1. Introduction

The ability of cancer cells to resist the growth inhibitory and cytotoxic actions of chemotherapeutic agents reflects their capacity to undergo the equivalent of molecular evolution and develop survival strategies. Multiple mechanism(s) have been identified as being responsible for cancer cell chemo resistance/drug tolerance, these range from acquisition of survival-enhancing mutations in key signaling molecules to "switching" between different receptor-driven signaling pathways, to the induction of transporter protein expression enabling efflux of drug. As we probe deeper into the processes involved in drug resistance, it is becoming clear that additional mechanisms are at work. In this review, the basis for resistance to tyrosine kinase inhibitors (TKIs) will be discussed. These mechanisms will be compared and contrasted to resistance to receptor TKIs (RTKIs) and how these differ from what has been observed for monoclonal antibodies (mAbs) that target RTKs. In the latter case, we will consider the role of the IGF-1R as a dependence receptor and how this may impact the response to TKIs *vs.* mAbs to yield resistance or therapeutic efficacy. It is important to remember that the cells populating any given tumor are heterogeneous and that natural selection by drug dosing is a key mechanism in this process.

2. Cellular signaling pathways regulated by receptor and non-receptor tyrosine kinases

Receptor and non-receptor tyrosine kinases utilize a number of common effector proteins to mediate their downstream effects in normal and cancer cells. As shown in Fig. 1, activation of the EGFR tyrosine kinase leads to stimulation of multiple downstream signaling pathways including Ras-MAPK (Erk), PI3K/Akt and Stat activation downstream of the Jak non-receptor tyrosine kinase. Moreover, activation of the IGF-1R can result in "receptor cross-talk" as a result to protease activation and shedding of EGFR ligands or activation of the HIF-1 transcription factor resulting VEGF expression, in turn activating the EGFR and VEGFR, respectively (Fig. 1; [1–4]). Fig. 2 illustrates signaling pathways regulated by Bcr-Abl underscoring that common pathways to those regulated by RTKs are activated by this non-receptor tyrosine kinase leading to enhanced cell proliferation, tumorigenesis, invasion and metastasis [5]. The existence of overlapping or

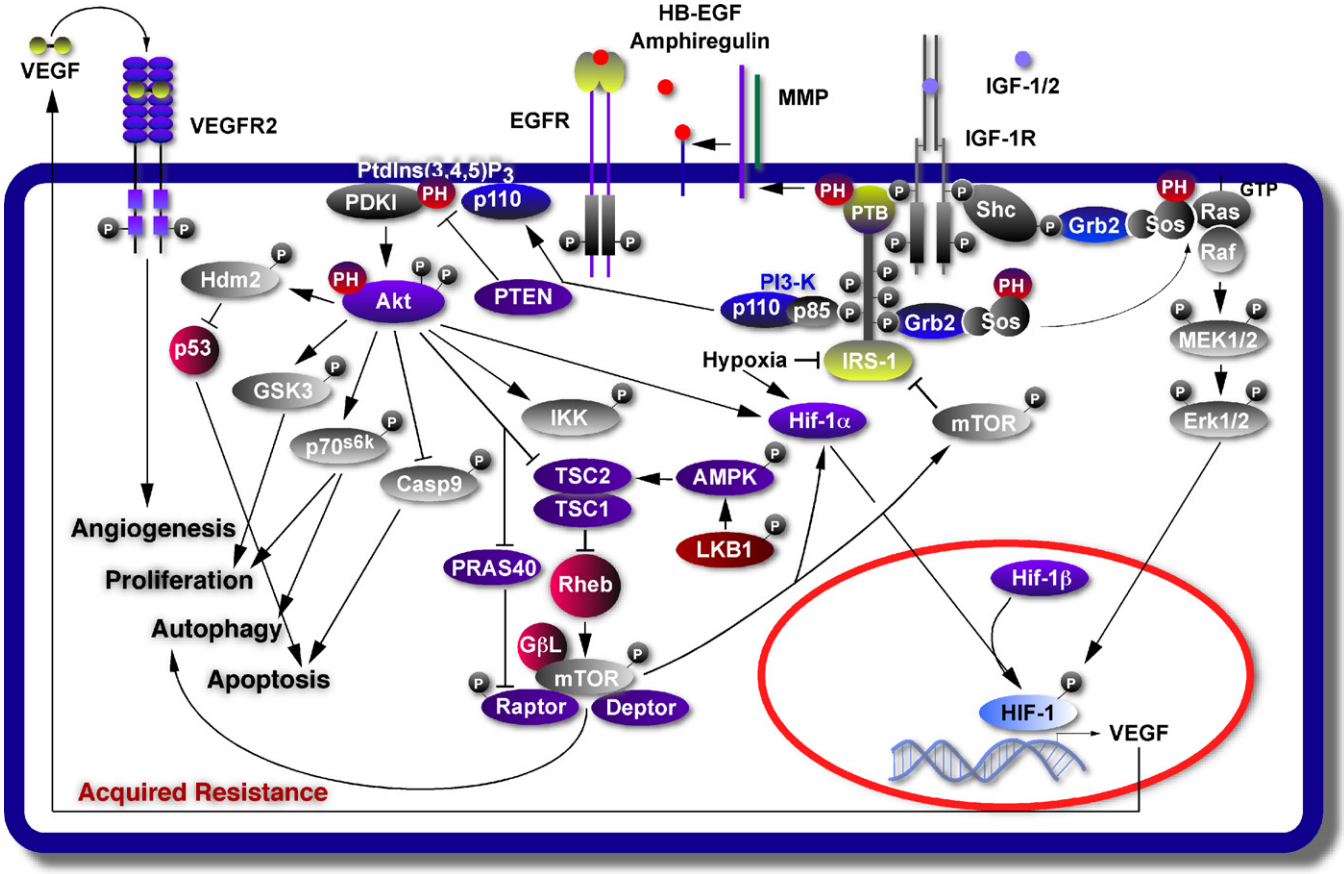

Fig. 1. Receptor tyrosine kinase signaling pathways. Following ligand-induced receptor transphosphorylation, growth factor receptor tyrosine kinases such as the EGFR and IGF-1R recruit effector molecules containing SH2 or PTB domains to initiate a downstream cascade activating the Ras–Erk or PI3-K/Akt pathways, which impinge upon a number of additional pathways and activities including mTOR regulation.

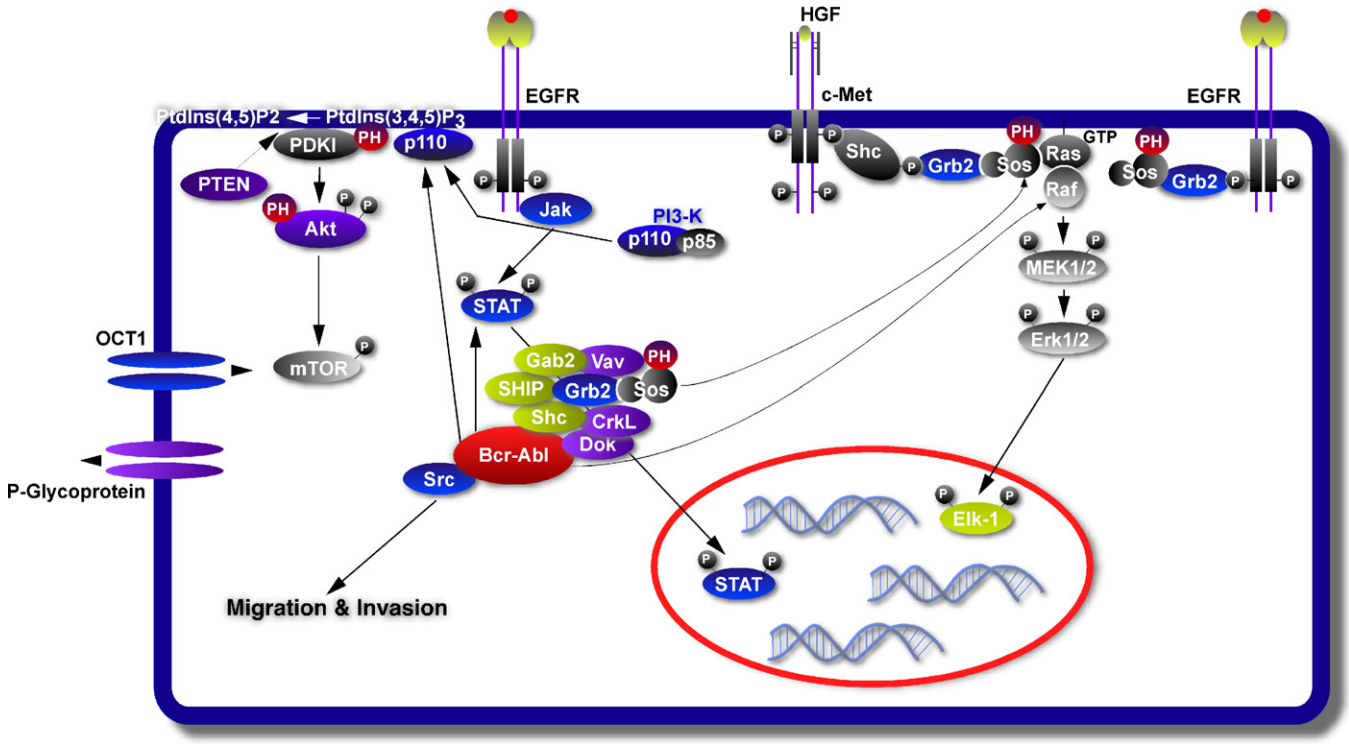

Fig. 2. Bcr-Abl signaling pathways. Formation of the Bcr-Abl fusion protein results in its mis-localization within the cell. This, in turn, leads to the phosphorylation and activation of a number of pathways common to receptor tyrosine kinases.

"redundant" pathways across receptor and non-receptor kinases provides insight as to how compensatory signaling pathways take the place of those RTK pathways inhibited by a given molecularly targeted RTKI. These mechanisms, in addition to kinase mutations, represent important ways in which cancer cells become resistant to targeted therapeutics and will be reviewed below starting with Bcr-Abl, TKIs and extending to a discussion of EGF and IGF-1 receptors. While this review is focused on receptor and non-receptor tyrosine kinase inhibitors and mechanisms of acquired resistance, it should be kept in mind that there are currently inhibitors being evaluated or in clinical trials that target one or more of the kinases depicted in Figs. 1 and 2 [4,6].

Table 1
Drugs discussed.

Drug name	Mechanism of action
Imatinib (Gleevec; STI-571)	Bcr-Abl TKI, ATP competitive
Nilotinib (Tasigna; AMN107)	Bcr-Abl TKI, ATP competitive; 2nd generation
Dasatinib (Sprycel; BMS-354825)	Bcr-Abl and Src TKI, ATP competitive; 2nd generation
Erlotinib (Tarceva)	EGFR (HER1) TKI
Gefitinib (Iressa)	EGFR (HER1) TKI
NVP-AEW541	IGF-1R/IR TKI
BMS-754807	IGF-1R/IR TKI
MAB391	IGF-1R mAb
Herceptin (Trastuzumab)	HER2/ErbB2/EGFR2 TKI
Vorinostat (Zolinza)	HDAC inhibitor
Panobinostat (LBH-589)	Non-selective HDAC inhibitor
Trichostatin A (TSA)	Class I and II HDAC inhibitor; antifungal antibiotic
Rapamycin (Sirolimus)	mTOR inhibitor

3. Inhibition of Bcr-Abl and non-receptor tyrosine kinases

Historically, Gleevec (STI-571; imatinib) an Abl kinase inhibitor was the first therapeutically successful treatment for chronic myeloid leukemia (CML) and has served as an instructional model for rational drug design of receptor and non-receptor TKIs since its FDA approval in 2001. For patients taking imatinib, the primary cause for relapse is reactivation of Bcr-Abl kinase due to point mutation(s) in the kinase domain (KD; [7]). Importantly, these mutations alter imatinib action without significantly reducing ATP binding or kinase function [8]. Identification of the sites of point mutations in Bcr-Abl resulting from imatinib, and the second-line Abl-kinase inhibitors dasatinib and nilotinib and there impact on kinase function have been well characterized by a number of investigative teams [9].

A number of kinase domain point mutations have been identified and characterized for their effects on Bcr-Abl function *in vitro* and sensitivity to dasatinib and nilotinib; these analyses have recently been reviewed [9]. Based on elegant crystallographic studies of Abl kinase in the presence of imatinib (then referred to as STI-571 or CGP 57148) a clear mechanism of inhibition was defined with imatinib binding to the inactive conformation of the Abl activation loop thereby locking it in the off position. [10]. The natural evolution of KD mutations is typified by alterations at residue T315, a key contact site for imatinib. Mutations here block imatinib access to the activation loop or block the necessary hydrogen binding to form a stable enzyme:inhibitor complex. Additional point mutations located within the ATP binding loop disallow Abl to assume a high affinity conformation capable of binding imatinib. Activation loop mutations are thought to stabilize the active conformation, which imatinib is incapable of binding to. Significantly, many of these mutations were inhibitable with newer Bcr-Abl kinase inhibitors such as nilotinib [11] and dasatinib, a dual Src/Abl inhibitor [12], resulting from their increased affinity for Abl kinase. Besides having a 300-fold greater potency than imatinib, dasatinib binds to the catalytically active

conformation of Abl, further enabling its capability to inhibit imatinib resistant mutants [12] (Table 1).

The point mutations identified in the Bcr-Abl KD result in resistance to imatinib as a result of reduced KD flexibility, limiting its ability to form an inactive conformation necessary for imatinib binding and inhibition [13]. On this basis, second generation inhibitors were developed with the goal of increased potency above that of imatinib. Indeed, mutations found to be resistant to dasatinib are present within contact sites [13] while nilotinib point mutations were also resistant to imatinib. [14].

In contrast, *in vitro* induction of imatinib resistance is typically associated with Bcr-Abl mRNA and protein overexpression, which is not always associated with gene amplification. Elevated P-glycoprotein expression and multidrug resistance-based drug efflux, as seen with many chemotherapeutics, has also been observed for imatinib [15], and the activation of integrin and/or growth factor receptor signaling pathways have been described as mechanisms responsible for imatinib refractoriness [8].

4. Receptor tyrosine kinase inhibitors and the epidermal growth factor receptor (EGFR) family

As observed with chemotherapeutic agents that lack targeting specificity, rationally designed drugs (TKIs and mAbs) that selectively target receptor and non-receptor tyrosine kinases can also result in acquired resistance. Considerable experience has been gained in the study of drugs that target the EGFR family both in terms of acquired resistance and in defining drug sensitivities. It was determined early on in the experience with sensitivity to gefitinib and erlotinib (TKIs that target the EGFR), that drug-sensitive patient populations could be selected for therapy based on the presence of an activating mutation in the EGFR [16–18]. For example, ~10% of all non-small cell lung cancer (NSCLC) patients in the United States – with a higher percentage in East Asia – exhibit gain of function mutations within the EGFR KD. These are all attributable to a single amino acid substitution of arginine (R) for leucine (L) at position 858 (nucleotide 2573 T to G in exon 21) or an exon 19 in-frame deletion, removing the tetrapeptide Leu–Arg–Glu–Ala [19]. Despite early positive responses to therapy, most of these patients became resistant to erlotinib and gefitinib as seen in acquired resistance to imatinib treatment in CML. The underlying cause for resistance was eventually shown to be due to secondary mutations as observed in the Abl KD [20]. These "loss of inhibition" mutations were found in over half of the patients exhibiting acquired resistance to imatinib, clustering in the ATP binding and activation loops of the Abl KD resulting in blocking imatinib binding to Abl [20]. A single nucleotide change C to T in the EGFR resulting in replacing a threonine with isoleucine at residue 315 (T315I) is typically observed. It is notable that analogous mutations, T670I in c-Kit and T674I in PDGFR-α, are responsible for acquired resistance to imatinib in gastrointestinal stromal tumors (GIST) [19,21].

Based on the previous experience with acquired resistance to imatinib, a number of investigators [16–18] examined the EGFR kinase domain, spanning exons 18–24 in patients who were initially responsive to RTKI treatment, but whose tumors progressed over time. Pao et al. [19] examined the EGFR KD in 5 patients with acquired resistance to EGFR TKIs and found the presence of a second mutation in exon 20 at residue 790 (T790M). The net effect of replacing threonine with the bulkier and more hydrophobic methionine residue is loss of the TKI binding cleft created by the threonine residue thereby eliminating this druggable site. This mechanism is common to multiple kinases including Abl, Src, FIT3 (FMS-like tyrosine kinase 3), platelet-derived growth factor-β, (PDGFR-β) and the fibroblast growth factor receptor (FGFR) (reviewed in [22]). Moreover, this substitution located within the ATP binding pocket, results in a greater affinity of the EGFR for ATP, reducing the potency of ATP-competitive drugs [23]. Significantly, this mutation was not detected in tumor tissue from untreated patients, underscoring the selection for this somatic mutation by TKI treatment [22]. These findings underscore both the desire and need to carry out genomic studies on patients, which provides an advantage in screening patients for their drug-sensitivities as well as their potential and/or eventual drug resistance [22].

In addition to the acquired resistance in TKI-sensitive tumors stemming from the generation of secondary mutation(s) in the EGFR, additional mechanisms of acquired resistance have been observed. Two such examples are overexpression of the Met receptor or of its ligand, hepatocyte growth factor (HGF), accounting for acquired resistance in a small percentage of tumors [24,25]. Additional studies using cell culture models of EGFR acquired resistance confirm that Met overexpression and phosphorylation compensate for loss of EGFR [26]. In this case, it was shown that Met served as a co-receptor for the EGFR and that the physical link between these two proteins resulted in Met activation in the absence of HGF, but in the presence of c-Src kinase activity [26]. A study of gefitinib-resistant cell lines and human lung adenocarcinoma specimens showed that HGF overexpression (coupled with Met activation) leads to PI-3 kinase pathway restoration in the absence of Met amplification or T790M mutation of the EGFR [27]. An important observation was that HGF expressed by tumor stromal cells affects gefitinib resistance in mutant EGFR expressing tumor cells [27], underscoring the role the tumor microenvironment plays in what is referred to as non-cell autonomous drug resistance mechanisms *vs.* cell autonomous mechanisms; the latter occurring independent of cells in the tumor microenvironment, alterations in drug metabolism, angiogenesis, epigenetic changes or other considerations [22,28].

4.1. Epigenetic mechanisms of resistance

Epigenetic alterations have been shown to affect resistance mechanisms in addition to their well known effects on tumor induction and development. Histone acetyltransferases acetylate histone N-terminal lysine residues promoting chromatin expansion and transcription factor access to promoter regions. Histone deacetylases (HDACs) catalyze the removal of acetyl groups from histone lysines resulting in DNA/histone complex compaction that blocks transcription factor access to binding sites decreasing gene transcription. Blockade of this modification with HDAC inhibitors favors growth arrest, differentiation, and apoptosis [29]. Accordingly, HDAC inhibitors such as vorinostat have anti-tumor activity and are effective in cancer therapy [30].

Epigenetic mechanisms may further play into RTKI resistance mechanisms. For example, the EGFR like many RTKs requires the chaperone protein heat shock protein 90 (Hsp90) for proper folding and function. The HDAC inhibitor LBH589 (panobinostat) increases Hsp90 acetylation thereby reducing its association with EGFRs causing down-regulation of survival signaling proteins and inducing apoptosis [31]. The EGFR is therefore, sensitive to the actions of HDAC inhibitors. However, in cells lacking EGFR-dependence, LBH589 has a negligible effect on apoptosis causing cell cycle arrest instead. A 10-fold increase in LBH589 dose was required to deplete EGFR and Akt in cells lacking EGFR mutations. Co-treatment of cells with erlotinib and LBH589 resulted in synergistic effects on lung cancer cells dependent on EGFRs for growth and/or survival suggesting that EGFR mutation status may be predictive of a positive response to LBH589 and other HDAC inhibitors [31].

Taken together, these observations reinforce the notion that drug resistant cell populations may be selected *via* multiple

mechanisms ranging from drug efflux, modulation of drug metabolism, secondary mutation of the target protein, induction of alternate signaling pathways and the induction of epigenetic mechanisms [32]. An additional mechanism is the selection of drug refractory cancer stem cell populations or cancer-initiating cells; their existence also underscores the cellular heterogeneity within a tumor that enhances a tumor's ability to adapt to a changing environment [33]. In keeping with the idea that cancer cell populations within a tumor are heterogeneous, Sharma et al. [34] recently described a subpopulation of PC9 cells (EGFR mutant NSCLC cell line) that were reversibly drug-tolerant and labeled as "drug-tolerant persisters" (DTPs) that remained viable under conditions that killed-off the majority cell populations. DTPs were detected following expansion of single drug-sensitive cells and their phenotype was reversible. Because DTPs occurred at frequencies higher than what would be expected as a result of mutation, epigenetic regulatory mechanisms were implicated [34]. While DTPs are quiescent cells, a small percentage (~20%) exhibited normal proliferation over time in the presence of drug and were termed "drug-tolerant expanded persisters": (DTEPs).

In defining the underlying mechanisms of the drug tolerant state, Sharma et al. [34] determined that these cells retained the sensitizing EGFR mutation and did not acquire the T790M mutation or MET gene amplification suggesting an alternative modification. Based on genome-wide gene expression analysis of parental PC9, DTP and DTEP cells it was noted that significant expression differences existed across the three cell lines. Examination of genes elevated in DTPs and DTEPs revealed a single gene, KDM5A/RBP2/Jarid1A (KDM5A) a histone H3KA-demethylase [35,36]. Importantly, KDM5A silencing in PC9 cells reduced the number of DTEPs generated in response to cisplatin challenge without affecting PC9 cell proliferation, enabling the conclusion that KDM5A expression was necessary for induction of reversible drug tolerance [34]. Although there are no current KDM5A inhibitors, KDM5A is known to interact with HDACs [36]. Accordingly, HDAC inhibition was tested for its ability to phenocopy KDM5A knockdown in PC9 cells. Addition of the HDACI/II inhibitor, trichostatin A caused the rapid death of DTPs and DTEPs without having an effect on parental PC9 cells and this was verified by demonstrating HDAC inhibitor co-treatment of PC-9 cells in the presence of an EGFR TKI eliminated the emergence of DTEPs, suggesting that drug-tolerant cell populations are susceptible to HDAC inhibition. In addition to HDAC inhibitors, the IGF-1R TKI, NVP-AEW541 [4] was capable of inhibiting the emergence of DTEPs, potentially suggesting that IGF-1R signaling results in chromatin modifications resulting from altered KDM5A activity or expression. A small percentage of DTEPs harboring the T790M EGFR mutation arose during treatment of PC9 cells with NVP-AEW541 and erlotinib, suggesting that mutational mechanisms mediate the pathway to drug resistance under these conditions.

4.2. Resistance to receptor tyrosine kinase inhibitors vs. receptor targeted antibodies: IGF-1R

While Abl kinase and the EGFR provide cogent examples of how KD mutations can influence drug sensitivity and resistance to small molecule TKIs, other mechanisms are also in play for these and other RTKs and non-receptor TKs. A case in point is the insulin-like growth factor-1 receptor (IGF-1R), which has become a major focus of targeted therapeutic strategies, with a large number of TKIs and antibodies being developed to target this receptor in cancer (reviewed in [4]). The IGF-1R is a prosurvival anti-apoptotic signaling growth factor receptor tyrosine kinase that is frequently overexpressed in cancer, but lacks a profile of mutations, SNPs or gene amplification. The small molecule, dual-kinase IGF-1R/insulin receptor (IR) TKI, BMS-754807 has been reported to inhibit IGF-1R signaling *in vitro* and in *in vivo* animal models [37]. In an effort to define the mechanisms of acquired resistance to TKIs and mAbs targeting the IGF-1R, Huang et al. [37] generated two drug resistant rhabdomyosarcoma cell lines from parental Rh41 cells: Rh41-807R with acquired resistance to BMS-754807 and Rh41-MAB391R cells with acquired resistance to an IGF-1R blocking antibody, MAB391. Based on gene expression profiling and DNA copy analyses both unique and common mechanisms were identified. In common, both cell lines up-regulated alternate signaling pathways, but the pathways induced differed in each case. PDGFR-α was amplified, overexpressed and constitutively activated in Rh41-807R cells with knockdown of PDGFR-α resulting in re-sensitization of the cells to BMS-754807. Axl expression levels were increased in Rh41-MAB391R cells, and this pathway was down regulated in Rh41-807R cells. Although both inhibitors target the IGF-1R, their mechanisms of action are significantly different and likely to contribute to the observed differing mechanisms of acquired resistance. Whether these mechanisms utilize mutational or epigenetic mechanisms remains to be established. What is clear is that small molecule TKIs have access to all intracellular compartments, unlike mAbs, enabling them to bind to and potentially influence multiple proteins besides the RTK to which they are targeted. Specific to the IGF-1R, which typically lacks mutations or amplification in cancer, induction of alternate compensatory pathways over mutational changes may be the more expected outcome. Acquired resistance to herceptin occurs whether it is administered as monotherapy or as the more common combination therapy with standard chemotherapeutics [38].

4.3. Other mAbs and acquired resistance: herceptin

The human EGF receptor-2 (HER-2, erbB2/neu) is overexpressed in 20–25% of metastatic breast cancers [39]. Herceptin (trastuzumab) is a humanized mAb directed against the HER2 extracellular domain (ECD), that is in current use as a targeted therapy in cases where HER2 is shown to be overexpressed [40]. While the mechanism by which herceptin action leads to tumor regression is not completely known, treatment of tumor cells with herceptin results in reduced HER2 signaling, cell cycle arrest, reduced proliferation, HER2 endocytosis and down regulation [40]. Whether used as monotherapy or in combination therapy, patients who initially exhibited a positive response to herceptin eventually exhibit acquired resistance [38]. A number of mechanisms may be responsible for acquired herceptin resistance. An obvious possibility is mutation of the HER2 ECD, precluding herceptin binding to the HER2 ECD, similar to mutational events seen in response to EGFR TKIs (see above). Alternatively, elevated EGFR:HER3 heterodimers, EGFR homodimers or loss of HER2 could be responsible for a loss of herceptin sensitivity. Akt and/or PI3K activation [41] or loss of PTEN activity [42] can also lead to herceptin resistance. Induction of alternate signaling pathways has been observed in herceptin resistance, in particular, elevation of IGF-1R signaling [43]. This is similar to the induction of the redundant Met pathway [22,24,25] in EGFR TKI resistance. Indeed, IGF-1R levels were found to be increased in herceptin-resistant breast cancer cell lines; treatment with the IGF-1R TKI, NVP-AEW541 restored sensitivity to herceptin [44]. It has also been reported that trastuzumab treatment of trastuzumab-sensitive SKBR3 breast cancer cells induces insulin-like growth factor binding protein-3 (IGFBP-3) secretion which blocks autocrine and paracrine expressed IGF-1/2 access to the IGF-1R to cause growth inhibition [45].

Induction of IGF-1R signaling has also been implicated in acquired resistance to EGFR TKIs. Generation of gefitinib-resistant A431 squamous cancer cells was associated with the loss of IGFBP-3and IGFBP-4 expression leading to increased IGF access to the IGF-1R [46]. Treatment of cells with recombinant IGFBP-3 restored

Table 2
Dependence receptors.

Receptors	Ligands
P75NTR	NGF, BDNF, NT-3, NT-4/5, β-amyloid, prion
DCC	netrin-1, netrin-4
Neogenin	netrin-1, RGMa, RGMb, RGMc, netrin-3, netrin-4
Unc5's	netrin-1, netrin-4
RET	GDNF, neuturin, artemin, persephin
Ptc	Shh
TrkC	NT-3
EphA4	ephrinB3, ephrinA1, ephrinA4
ALK	pleiotrophin, midkin, jeb
MET	HGF
IGF-1R	IGF-1, IGF-2, insulin
InsulinR	insulin, IGF-1
AR	Androgens
Integrin αvβ3 and α5β1	extracellular matrix

Adapted from Reference []
In the presence of cognate ligand, dependence receptors signal to cell growth, survival, differentiation, migration, *etc*. In the absence of ligand they stimulate apoptosis.

gefitinib sensitivity and co-treatment of mice bearing A431 xenografts with gefitinib and an IGF-1R targeting mAb blocked tumor growth, whereas either treatment alone had no effect on tumor growth [46].

The scaffold protein IQGAP1 was recently shown to interact with HER2 to regulate resistance to herceptin [47]. Herceptin resistant human breast epithelial cells were shown to overexpress IQGAP1, with reduction of IQGAP1 levels restoring herceptin sensitivity [47]. The tumor suppressor DACH1 which is known to down regulate EGFRs and cyclin D1 exhibited loss of its suppressor activity in response to IGF-1stimulation suggesting that IGF-dependent cancer cells are capable of escaping the tumor suppressive effects of DACH1 [48].

5. IGF-1R and dependence receptors in drug resistance

Over the last few years the IGF-1R has become the focus of a number of therapeutic strategies for the treatment of solid tumors [4]. The IGF-1R is an important regulator of prosurvival, anti-apoptotic signaling that has surfaced as a significant target in multiple cancers. To accomplish this, the IGF-1R is a potent activator of Akt which fits with the findings that inhibition of mTOR signaling by rapamycin frequently results in the loss of feedback inhibition of IGF-1R signaling, in turn, resulting in Akt activation [49]. These findings have been observed by a number of laboratories and support the co-treatment of patients with rapamycin analogs plus an IGF-1R targeting TKI or mAb [50]. In addition to its involvement in the acquired resistance to EGFR TKIs and herceptin ([51] and described above), IGF-1R signaling was reported to regulate RON receptor activation by direct physical interaction in pancreatic cancer cells suggesting that RON activation may be involved in acquired resistance to IGF-1R therapies [52]. IGF-1Rs have been found to be downstream of RTKs [53] and G-protein coupled receptors with cross talk occurring at the receptor level, as well as *via* downstream effectors [54]. For example, cross talk between IGF-1Rs and neurotensin receptors was shown to be Src-dependent, providing evidence for IGF-1R dependent regulation of inflammatory signaling in human colonic epithelial cells [55].

As indicated, the IGF-1R is well known for its prosurvival anti-apoptotic signaling paradigm mediated by PI3K/Akt signaling. The IGF-1R, along with the insulin receptor (IR) was recently shown to be a member of the "dependence receptor" family [56]. Dependence receptors derive their name from the fact that when unliganded, they promote apoptosis; therefore cells expressing them are dependent upon ligands for survival [57]. There are more than 12 members of this family, which includes a wide variety of membrane receptor proteins including p75 neurotrophin receptor, MET, RET, ALK, EphA4, integrin α5β1 and the androgen receptor (Table 2; [57]). They lack specific homology domains, however many dependence receptors contain caspase cleavage sites enabling them to recruit and bind to caspases, which may be a part of their mechanism of action [58].

Recent observations showed that double knockout (DKO) cells lacking both IGF-1Rs and IRs were resistant to apoptosis *via* intrinsic or extrinsic pathway stimulation [56]. The dependence-pathway is receptor-dependent, ligand-independent and required for cells to undergo apoptosis. Thus, if one of these receptors is re-expressed cells can undergo apoptosis in the absence of ligand; re-expression of a kinase-dead mutant also re-establishes the ability of cells to undergo apoptosis [56]. The ramifications of this pathway to cancer therapeutics are significant. If the IGF-1R is required/permissive to cell death, then mechanisms that down regulate IGF-1Rs or remove them from the cell surface may have the unwanted effect of promoting cancer cell survival. IGF-1R TKIs, on the other hand, have no effect on receptor expression, but may promote a TK-independent cell death signaling paradigm. Other reports of the TK-independent activation of IGF-1R signaling have also appeared. IGF-1 treatment of smooth muscle cells was reported to result in extracellular-regulated kinase 1/2 phosphorylation/activity in the presence of IGF-1R TKIs [59]. This observation further supports the concept that this activity was independent of IGF-1R tyrosine kinase signaling.

In a similar example for the IGF-1R, we tested the effects of IGF-F1-1, a cyclic hexadecapeptide developed by phage display screening technology to be an IGFBP-mimetic [60] based on its ability to block IGF-1 action. Surprisingly, we found that IGF-F1-1, which has binding affinity for the IGFBP-binding domain on IGF-1, blocked IGF-1 binding to MCF-7 cells, but also increased Akt activation, S-phase transition and thymidine incorporation into DNA without stimulating IGF-1R tyrosine phosphorylation. Paradoxically, these activities could be blocked by the IGF-1R/IR TKI NVP-AEW541 [60]. The signaling mechanisms responsible for these effects, as well as the TK-independent apoptotic signaling of the IGF-1R, although not well understood, could shed light on future cancer therapeutic strategies. Given that the IGF-1R is a dependence receptor, novel approaches to cancer therapeutics that promote apoptosis *via* the unliganded receptor may be developed. This suggests that combination therapy comprised of inactivating RTKs in conjunction with either an antagonist that blocks endogenous ligand binding, the use of a decoy receptor [61] or an alternative method for ligand inactivation/removal may have merit as a future therapeutic strategy. These observations provide a rationale for targeting RTKs in a way that does not induce their endocytosis and down-regulation in future therapeutic strategies.

6. Conclusions and future perspective

With the experience obtained in administering receptor and non-receptor TKI therapeutics has come the realization that selecting patient populations sensitive to a particular inhibitor – based on the presence of a specific mutation or the existence of oncogene addiction – provides a key therapeutic advantage. Conversely, there have been attempts to predict patient populations that may become resistant to targeted therapeutics such as erlotinib [62,63], with women, Asian patients with adenocarcinoma and never-smokers, being more likely to positively respond to erlotinib and gefitinib treatment due to EGFR TK domain mutations or EGFR amplification [64]. Unfortunately, while erlotinib and gefitinib sensitivity may predict responsiveness, this does not necessarily equate to survival. The same unpredictability has been seen with IGF-1R TKIs. Here, acquired resistance to NVP-AEW541

in a mouse model of metastatic alveolar rhabdomyosarcoma was due to ERK reactivation and HER2 overexpression instead of the predicted induction of PDGFR-α [65]. This may be the result of HER2:IGF-1R heterodimerization and receptor cross-phosphorylation by alternate ligands; in this case, the combined treatment of lapatinib and an IGF-1R TKI was more effective than either drug alone. The physical association of heterologous receptors adds a new dimension to future therapeutic strategies. In addition to the identification of RTK heterodimerization the future clearly holds promise for the development of new RTKIs, mAbs and the identification of new cancer-related receptors belonging to the dependence receptor family. While autocrine/paracrine signaling by these receptors maintains cell and tissue growth and ligand overexpression assures tumor survival, future therapies may emphasize targeting their ligands in order to enhance apoptotic signaling.

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