Clinical Cancer Research

Molecular Pathways: Sensitivity and Resistance to Anti-EGFR Antibodies

Andrea Bertotti[1,2,3] and Francesco Sassi[2]

Abstract

Monoclonal antibodies targeting the EGF receptor (EGFR) tyrosine kinase, such as cetuximab and panitumumab, achieve clinically meaningful responses in patients affected by head and neck and colorectal cancers. Despite this evidence of efficacy, no genomic abnormalities that robustly predict sensitivity to EGFR blockade have been yet identified. This suggests that, in some tumor contexts, EGFR dependency is not acquired during neoplastic transformation and rather reflects an aberrant declination of physiologic traits typical of normal tissue counterparts. Indeed, EGFR signals are crucial for the reconstitution of damaged mucosa in the context of acute inflammation, and their sustained activation is likely to turn into a pro-oncogenic cue during chronic inflammation. Although positive predictors of response to anti-EGFR antibodies remain unknown, multiple determinants of resistance have been described, including alterations interfering with antibody–receptor interaction, deregulation of parallel signaling pathways, and mutations in downstream transducers. These findings provide new opportunities for the optimization of therapeutic strategies based on drug combinations. However, the emerging notion that genetic interactions and compensatory mechanisms may affect—both positively and negatively—the efficacy of targeted therapies complicates the rational design of combinatorial approaches and implies a rethinking of the criteria required to prioritize laboratory findings for clinical validation in investigational trials. *Clin Cancer Res; 21(15); 3377–83. ©2015 AACR.*

Background

EGFR belongs to the ERBB family of receptor tyrosine kinases (RTK). Besides EGFR itself (also known as ERBB1/HER1), the ERBB receptor family comprises ERBB2 (neu, HER2), ERBB3 (HER3), and ERBB4 (HER4; refs. 1, 2). EGFR is activated through autocrine or paracrine stimulation by ligands that either bind specifically to the receptor [EGF, TGFα, and amphiregulin (AREG)] or bind to both EGFR and ERBB4 [betacellulin (BTC), heparin-binding growth factor (HB-EGF), and epiregulin (EREG); refs. 2, 3]. Ligand binding triggers receptors' homo- or heterodimerization, which is followed by the activation of mitogenic and antiapoptotic signaling cascades, mainly via the RAS–RAF–MEK–ERK and the PI3K–AKT–mTOR axes (3, 4). In addition to interacting with the other members of the family, EGFR has also been reported to crosstalk with other cancer-relevant RTKs, such as the hepatocyte growth factor receptor MET and the insulin-like growth factor receptor 1 (IGF1R; refs. 5, 6), whose signaling converges on downstream pathways similar to those activated by EGFR (Fig. 1). Besides these core signaling interactions, alternative networks have been proposed, including kinase-independent signaling activities related to receptor internalization (7, 8). A more detailed picture of EGFR-related signals is shown in Fig. 1 and is comprehensively reviewed elsewhere (2).

EGFR was originally proposed as a target in cancer due to generic biologic indication (i.e., its frequent overexpression in tumors; ref. 9), and the inclusion of anti-EGFR inhibitors into the toolbox of clinical oncologists was primarily based on evidence of efficacy (10). Two strategies for EGFR inhibition led to the development of agents that are approved for clinical use either as monotherapies or in combination with chemotherapeutic agents (11): anti-EGFR antibodies and small-molecule EGFR tyrosine kinase inhibitors. Anti-EGFR antibodies, such as cetuximab and panitumumab, bind to the extracellular portion of the receptor, causing its internalization and displacing ligand–receptor interactions, thereby interfering with EGFR activation (12); small-molecule kinase inhibitors (such as erlotinib, gefinitib, and afatinib) inhibit EGFR autophosphorylation and downstream signaling by competing with ATP (12).

Interestingly, the two classes of inhibitors are endowed with largely nonoverlapping clinical activities. On one side, small molecules have been approved for treatment of non–small cell lung cancer (NSCLC). In line with the concept of oncogene addiction, response to EGFR inhibitors in NSCLC is strongly associated with the presence of activating mutations in the target gene (13). On the other hand, anti-EGFR antibodies are used for the treatment of colorectal cancer and head and neck cancer (HNC; ref. 14), for which no univocal genetic determinants of sensitivity to EGFR inhibition have been yet identified.

The dependency of colorectal cancer and HNC on EGFR signals is reminiscent of an aberrant declination of physiologic or paraphysiologic traits typical of normal tissues. In vertebrates, EGFR is a crucial regulator of cellular homeostasis and controls the organogenesis of multiple epithelial tissues, including lining epithelia of endodermal origin (15, 16). It is tempting to speculate that the reason for the functional dependency on EGFR activity in HNC

[1]Department of Oncology, University of Torino Medical School, Candiolo (Torino), Italy. [2]Laboratory of Translational Cancer Medicine, Candiolo Cancer Institute—FPO IRCCS, Candiolo (Torino), Italy. [3]Istituto Nazionale di Biostrutture e Biosistemi, INBB, Rome, Italy.

Corresponding Author: Andrea Bertotti, Candiolo Cancer Institute—FPO, IRCCS, University of Torino, IRCC - SP142 km3.95, 10060 Candiolo (Torino), Italy. Phone: 39-011-993-32-42; Fax: 39-011-993-32-25; E-mail: andrea.bertotti@unito.it

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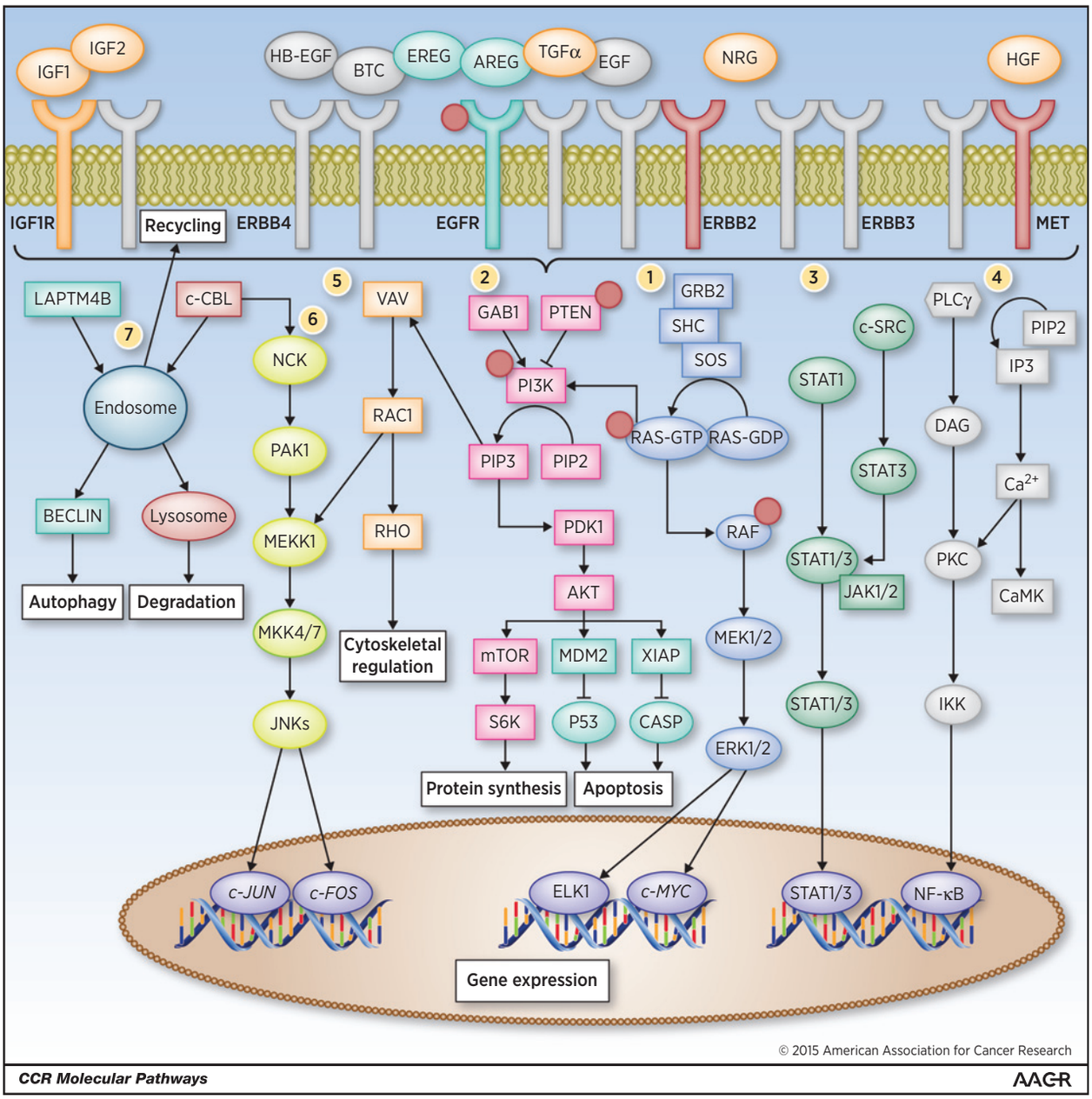

Figure 1.
EGFR signaling pathways and causes of resistance to anti-EGFR antibodies. Ligand binding to EGFR is followed by the activation of several downstream signaling cascades, which are categorized by colored outlines. Color fills represent the correlations between gene overexpression or genetic alterations and sensitivity to cetuximab treatment. Mutations causing resistance to cetuximab are represented as red circles; similarly, the genes that cause resistance when amplified are filled in red; genes that, when overexpressed, cause desensitization to treatment are depicted in yellow; finally, genes whose upregulation correlates with sensitivity to treatment are represented in green. **1**.) The RAS–RAF–MEK–ERK pathway (blue): GRB2, through SHC, recruits SOS to the cell membrane. SOS induces activation, which, in turn, triggers an RAF-dependent phosphorylation cascade involving MEK and causing ERK translocation to the nucleus, where transcription factors, such as ELK1, are phosphorylated/stabilized to transactivate immediate early genes, for example, *MYC*. **2**.) The PI3K–AKT–mTOR cascade (pink): PI3K is recruited to active EGFR through mediators, such as GAB1, and subsequently mediates the conversion of phosphatidylinositol (3,4)-bisphosphate (PIP2) lipids to phosphatidylinositol (3,4,5)-trisphosphate (PIP3). This allows PDK1 to phosphorylate AKT, which, in turn, impinges on mTOR, MDM2, and XIAP. On the one hand, mTOR promotes protein synthesis through the phosphorylation of the ribosomal protein S6. On the other hand, MDM2 and XIAP act as antiapoptotic cues through inhibition of P53 and CASP, respectively. **3**.) The JAK–STAT pathway (green): Recruitment of JAKs upon EGFR activation induces c-SRC binding, which is then responsible for STAT phosphorylation. Phosphorylated STATs translocate to the nucleus and initiate the transcription of specific target genes. **4**.) The PLCγ–PKC cascade (gray): phospholipase C gamma (PLCγ) catalyzes the production of DAG and IP3; the latter binds to the endoplasmic reticulum, causing the release of calcium. These events induce the activation of CaMK and PKC, leading to NF-κB transcription factor activation via IKK. **5**.) The Rho family GTPase pathway (orange): VAV mediates the activation of RHO and RAC, which control actin/myosin filament contraction and lamellipodia protrusion, promoting cell motility and invasion. (*Continued on the following page.*)

and colorectal cancer may be related to the close interaction of their epithelium of origin with mucosa-associated lymphoid tissues (MALT), which are large-capacity receptacles of inflammatory cells. Indeed, in the adult intestine, EGFR signaling has a key function in mediating mucosal regeneration and inflammatory responses (17, 18). The abrogation of the EGFR signaling axis impairs or reduces the regenerative potential of the intestine (18) and decreases the propensity of epithelial cells to undergo neoplastic transformation in the presence of chronic inflammation (19). In line with the assumption that cancer is a wound that never heals (20), it is likely that the upregulation of EGFR activity—normally involved in tissue repair in the context of acute inflammation—is turned into a pro-oncogenic cue during chronic inflammation. This would select for cancer cells relying on EGFR-driven signals for their growth, explaining why a fraction of colorectal cancers are strictly dependent on EGFR activity even in the absence of genetic alterations of the receptor. Accordingly, the autocrine production of selected ligands (namely, AREG and EREG) and the upregulation of EGFR itself are not only key mediators of intestinal regeneration during inflammation (21), but also positive predictors of sensitivity to EGFR inhibition in colorectal cancer (22–25). Although fewer data are available, a similar scenario seems to apply also to HNC. Indeed, a strict cross-talk between inflammatory cytokines and EGFR signaling to foster tissue regeneration has been repeatedly reported for epithelial tissues in general (26), and for squamous epithelia in particular (27–29).

Clinical–Translational Advances

Resistance to anti-EGFR antibodies: causes and therapeutic opportunities

If EGFR signaling is a physiologic cue for epithelial repair that is usurped in cancer for growth and survival, then any tumor would be in principle sensitive to anti-EGFR antibodies, unless some specific event happens to cause resistance, either by interfering with the pharmacologic activity of the drugs or by subsidizing alternative ways to activate equivalent downstream effectors. Unfortunately, too little information is available about the potential determinants of resistance to anti-EGFR antibodies in HNCs. Instead, more data exist for colorectal cancer, and these findings are consistent with the proposed model. The known mechanisms of primary and acquired resistance to approved anti-EGFR agents in colorectal cancer are largely superimposable (30) and can be categorized into three classes (see Fig. 1 and Table 1): (i) reduced antibody–receptor interaction; (ii) activation of parallel substitute pathways; and (iii) constitutive activation of downstream signals.

Class I—multiple EGFR inhibition. The most frequent cause of resistance to first-generation small molecules targeting EGFR in NSCLC is a mutation in *EGFR* (T790M), which reduces the affinity between the ATP-binding domain of the kinase and the inhibitor, impairing the ATP-competitive activity of the drug (31, 32). In such a case, cancer cells remain dependent on EGFR signaling yet escape the therapeutic activity of the drug by modifying its pharmacodynamics. Similarly, a mutation in *EGFR* extracellular domain (S492R) causes resistance to cetuximab by impeding binding of the antibody to the receptor (33). Notably, cells bearing the S492R mutation in *EGFR* are still sensitive to treatment with panitumumab, which recognizes different receptor epitopes (33, 34).

Some EGFR ligands, such as EGF and TGFα, can compete with cetuximab for binding to EGFR. In particular, strong overexpression of TGFα has been shown to correlate with reduced sensitivity to cetuximab (24). Interestingly, TGFα-overexpressing tumors are not fully resistant to cetuximab; instead, they show an intermediate phenotype, characterized by partial desensitization to treatment (25). It is likely that, similar to mutations of the extracellular domain, TGFα does not intrinsically release dependency on EGFR, but reduces cetuximab binding efficacy (35). This suggests that more powerful inhibition of EGFR by combining multiple EGFR-directed drugs might be efficacious in tumors in which EGFR signaling is necessary for proliferation, but cetuximab activity *per se* is weaker for pharmacodynamic reasons. This is suggested by the observation that, in patient-derived xenografts (PDX) of colorectal cancer tumors, addition of the EGFR small-molecule inhibitors lapatinib or erlotinib to cetuximab can turn tumor growth inhibition into overt tumor shrinkage (>75% reduction in tumor volume upon treatment) in more than 25% of the cases that respond to cetuximab alone with disease stabilization (25). At the clinical level, a recent study has demonstrated encouraging activity of combined treatment with cetuximab and erlotinib in 37 chemorefractory patients with *KRAS* wild-type metastatic colorectal cancer tumors (36): the response rate (41%) was definitely higher than that achieved by single-agent anti-EGFR antibodies, in which the response rate is normally around 15% to 17% (2, 37, 38). An analogous improvement in efficacy has been described when combining the dual HER1/HER2 inhibitor afatinib with cetuximab in *EGFR*-mutant NSCLCs resistant to erlotinib monotherapy (30% overall response rate; ref. 39), suggesting a wide range of applicability of "on-target" combination strategies for EGFR inhibition. A similar "diversification" strategy can also be pursued by designing *ad hoc* antibody mixtures targeting multiple epitopes of the same receptor. A successful example of this approach is represented by Sym004 (40), which is currently being tested in several phase II trials (NCT01117428).

Class II—cotargeting parallel pathways. Another mechanism adopted by cancer cells to escape oncogene dependency is the activation of parallel signaling cascades impinging on similar downstream effectors. Some such pathways have been clinically validated to trigger resistance to EGFR antibodies in colorectal cancer, including hyperactivation of HER2 and MET (22, 41, 42). In both cases, preclinical evidence indicates that combined

(*Continued.*) **6**.) JNK pathway (light yellow): PAK1, following its recruitment to EGFR through the adaptor NCK, activates a signaling cascade involving MAP2K family, i.e., MEKK1 and MAP3Ks MKK4 and MKK7. The cascade converges to JNKs, which, in turn, modulate the transcription of target genes, such as *c-JUN* and *c-FOS*, regulating cell proliferation, differentiation, and survival. **7**.) Receptor internalization, degradation, and recycling (light green and red): LAPTM4B can induce the endosomal accumulation of kinase-inactive EGFR, which drives BECLIN-dependent initiation of autophagy, a mechanism of resistance to starvation (light green). Alternatively, the phosphorylated receptor undergoes ubiquitination and degradation in the presence of c-CBL (red), or recycling in its absence.

Table 1. Resistance to anti-EGFR antibodies and strategies to overcome it: preclinical evidence and clinical trials

Resistance mechanisms		Strategy	Preclinical evidence	Clinical evidence
Reduced antibody–receptor interaction	*EGFR* S492R (ref. 33), TGFα overexpression (refs. 24, 25)	Multiple EGFR inhibition	Sym004 (ref. 40) Cetuximab + lapatinib/erlotinib (ref. 25)	Panitumumab (approved, ref. 33) Erlotinib + cetuximab (DUX, NCT00784667, ref. 36) Sym004 (NCT01117428) MM151 (NCT01520389) Gefitinib + cetuximab (NCT00820417) Sym004 (NCT01417936)
Activation of parallel substitute pathways	*HER2* amplification (ref. 41), *MET* amplification (ref. 42), IGF1R/IGF1/IGF2 overexpression (refs. 25, 45, 46)	Cotargeting parallel pathways	Cetuximab + pertuzumab/trastuzumab/lapatinib (ref. 41) Lapatinib + cetuxumab/cetuximab (ref. 22)	Lapatinib/pertuzumab + trastuzumab (HERACLES, EUDRACT 2012-002128-33, clinicaltrialsregister.eu)
			AZD8931 (ref. 53)	Capmatinib (INC280) + cetuximab (NCT02205398)
			Cetuximab + JNJ-38877605/crizotinib (ref. 42)	Cetuximab + BMS754807 (NCT00908024)
			Cetuximab + BMS754807 (ref. 25)	Lapatinib + cetuximab (TYKERB-ITUX 1, NCT01184482) BMS-599626 (NCT00093730) Panitumumab + rilotumumab (AMG 102)/ganitumab (AMG479) (NCT00788957, ref. 48) Cixutumumab (IMCA12) + cetuximab (NCT00503685, ref. 47) Tivantinib (ARQ 197) + cetuximab (NCT01892527)
Activation of downstream signals	KRAS pathway mutations (ref. 30)	Inhibition of downstream effectors	Dabrafenib (GSK436) + trametinib (GSK212) (ref. 62)	Cetuximab + selumetinib (AZD6244) (NCT01287130, ref. 51)
			Selumetinib (AZD6244) + BEZ235 (ref. 52)	Cetuximab + encorafenib (LGX818) + alpelisib (BYL719) (ref. 54) Vemurafenib + panitumumab (NCT01791309) Dacomitinib (PF-00299804) + PD-0325901 (NCT02039336) MK-2206+ selumetinib (AZD6244) (NCT01333475)

inhibition of the resistance-conferring pathway and EGFR itself can induce objective responses or prolonged disease control in most instances (22, 41, 42). For the specific case of *HER2* amplification causing *de novo* resistance to cetuximab and panitumumab, a phase II trial is ongoing to test a combination of the anti-HER2 antibody trastuzumab and lapatinib (EUDRACT 2012-002128-33, clinicaltrialsregister.eu). Interim results are promising, with more than 30% objective response rates among heavily pretreated patients (median, 5 prior therapies; ref. 43).

Besides causing overt drug resistance, parallel signals can also induce partial desensitization to treatment. For example, insulin-like growth factor 2 (IGF2) is highly overexpressed in a fraction of colorectal cancers (44); however, the frequency of IGF2 overexpressors among cetuximab responders is null or very low (25), pointing to IGF2-mediated activation of the cognate receptor IGF1R as a means to desensitize colorectal cancer cells to EGFR inhibition. Indeed, increased IGF1R signaling has been repeatedly documented to be associated with low sensitivity to EGFR blockade (45, 46). Of note, IGF2 overexpressors are highly enriched among cases responding to cetuximab treatment with disease stabilization ($P < 0.001$ by Fisher exact test), but not among those that are fully resistant to treatment (25). Thus, similar to the autocrine production of TGFα, IGF2 overexpression behaves as a response modifier, reducing but not abating EGFR dependency. Accordingly, addition of an IGF1R kinase inhibitor potentiated the antitumor effects of cetuximab in IGF2-overexpressing PDXs, suggesting that these tumors can be targeted successfully by a combined treatment against IGF1R and EGFR.

Despite this promising preclinical evidence, recent clinical trials in patients with *KRAS* wild-type colorectal cancer have shown that combined therapy with anti-EGFR and anti-IGF1R antibodies did not improve response compared with EGFR-targeted monotherapy (47, 48). The reasons for these discrepancies are currently not fully understood, and there is still considerable discussion about the validity of IGFs and IGF1R as viable therapeutic targets in cancer. On the one hand, the apparent lack of efficacy shown by IGF/IGF1R inhibitors in the clinic could be due to the fact that no attempts were made to select for potential responders through molecular stratification based on predictive biomarkers (49). On the other hand, preclinical studies could not adequately represent the clinical situation: differences could be ascribed to (i) the use of different pharmacologic modalities to inhibit IGF1R in PDX experiments versus clinical trials and/or to (ii) host-related modifiers, because the tumor microenvironment of xenografts is of mouse origin. Whatever the case, dedicated retrospective and prospective clinical studies are necessary to rigorously address this issue.

Class III—inhibition of downstream effectors. Most of the genetic alterations causing *de novo* and secondary resistance to anti-EGFR

agents trigger constitutive activation of downstream signaling pathways. These include mutations in *KRAS* and *NRAS* (exon 2 to 4), *BRAF* (exon 15), and *PIK3CA* (exon 20), as well as gene amplification of *KRAS* (30). Results of randomized phase III studies on chemorefractory metastatic colorectal cancers treated with cetuximab or panitumumab monotherapy led to the limitation in the use of anti-EGFR antibodies, together with chemotherapy, to patients bearing *KRAS* wild-type tumors (50).

KRAS is the most sought-after target for new therapies, being alone responsible for resistance in more than 40% of the overall cases. However, given the lack of robust approaches for direct inhibition of KRAS signaling activity, different strategies based on blockade of downstream effectors, such as MEK and ERK, have been proposed. Disappointingly, results obtained in preclinical models and then confirmed in the clinical setting have been fairly modest (51, 52), possibly because of feedback activation of subsidiary pathways, which sustain cancer cell viability during treatment. These compensatory mechanisms have been reported in colorectal cancer as well as in other cancer types, and involve upregulation of diverse RTKs, including EGFR, HER2, HER3, and MET (53). Based on such evidence, drug combinations have been tested preclinically, in which MEK or ERK inhibition is complemented by cotargeting the RTK responsible for the feedback activation (53). Some of these combination therapies are currently undergoing clinical evaluation (53), with two phase I trials recently completed (NCT01287130; ref. 54).

A further layer of complexity emerged from the observation that most genetic causes of resistance to EGFR inhibitors lead to hyperactivity of the MEK–ERK axis, a phenomenon recalling the Darwinian definition of "convergent evolution" (30). For this reason, it has been proposed that the MEK–ERK hub represents a culprit signaling node, whose inhibition can be exploited to prevent or retard the emergence of resistance. Consequently, clinical trials have been designed to test the concomitant upfront inhibition of EGFR and MEK–ERK, with the aim to extend the time to progression by anticipating the blockade of the most probable funnel of resistance-related signals.

Conclusions

The treatment paradigm for a patient should not be decided based solely on the mutational status of a single gene with potential cancer-causing function, but rather on the basis of the heterogeneous context in which that mutation is found. Due to this complexity, drug combinations rather than monotherapies are likely required to provide long-lasting benefit. However, the amount of conceivable treatment hypotheses based on drug permutations largely exceeds the number of clinically testable choices, due to costs, ethical implications, and logistical restraints. This situation calls for efficacious preclinical models for evidence-based prioritization of clinical experimentation. In this context, evidence is emerging that comparative testing of multiple combination strategies in PDXs can be exploited to select for the most effective therapeutic strategy among different options based on a similar rationale (22). For example, the observation obtained in PDXs that a combination of trastuzumab and lapatinib, but not treatment with either drug alone, can induce effective tumor shrinkage of *HER2*-amplified colorectal cancer is being confirmed in the clinical setting (43); again in line with observations obtained in PDX, trials have failed to demonstrate clinical efficacy of trastuzumab-based regimens in colorectal cancer (55). Similarly, the efficacy of the afatinib/cetuximab combination was effectively predicted by genetically modified mouse models of NSCLC and then confirmed by clinical data (39, 56). Another relevant example of effective anticipation of clinical findings by preclinical studies is the lack of efficacy against *KRAS*- and *BRAF*-mutant colorectal cancers demonstrated by MEK and PI3K inhibitors, a notion that was anticipated by PDX-based data (52) and has been then supported by clinical evidence (51). Analogously, the efficacy of combined MEK–ERK inhibition in treating *BRAF*-mutated melanomas and preventing reactive squamous cell carcinomas was proposed by preclinical studies (57) and has been now validated clinically (58, 59). This suggests that preclinical studies can effectively guide clinical experimentation when properly designed. However, discrepancies have also been observed between preclinical and clinical data, which raising questions about the general validity of this principle. We believe this disconnect between preclinical and clinical findings is ascribable to two main reasons: (i) the lack of a population-based contextualization of the findings obtained in most preclinical studies; and (ii) the interference in treatment outcome produced by host-related modifiers of therapeutic response (immune system, species-specific ligand–receptor interactions, etc.) that are not properly recapitulated by traditional xenografts. Tackling such issues will require significant efforts and investments to achieve a better representation of the complexity and heterogeneity of cancer at the preclinical level. In particular, the progressive humanization of mouse models is generating great expectations. Humanization is a time- and labor-intensive process that will require several technical challenges to be met before being widely exploited. However, encouraging preliminary results (60) suggest the feasibility and effectiveness of this approach. Finally, a critical step will be the adoption of stringent criteria for clinical translation of preclinical findings (61), so that only those hypotheses endowed with high probability of success will enter clinical investigation.

Disclosure of Potential Conflicts of Interest

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Authors' Contributions

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References

1. Ferguson KM, Berger MB, Mendrola JM, Cho HS, Leahy DJ, Lemmon MA. EGF activates its receptor by removing interactions that autoinhibit ectodomain dimerization. Mol Cell 2003;11:507–17.
2. Yarden Y. The EGFR family and its ligands in human cancer. signalling mechanisms and therapeutic opportunities. Eur J Cancer 2001;37 Suppl 4: S3–8.
3. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001;2:127–37.
4. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 2005;5:341–54.
5. Jo M, Stolz DB, Esplen JE, Dorko K, Michalopoulos GK, Strom SC. Cross-talk between epidermal growth factor receptor and c-Met signal pathways in transformed cells. J Biol Chem 2000;275:8806–11.
6. Morgillo F, Woo JK, Kim ES, Hong WK, Lee HY. Heterodimerization of insulin-like growth factor receptor/epidermal growth factor receptor and induction of survivin expression counteract the antitumor action of erlotinib. Cancer Res 2006;66:10100–11.
7. Tan X, Thapa N, Sun Y, Anderson RA. A kinase-independent role for EGF receptor in autophagy initiation. Cell 2015;160:145–60.
8. Weihua Z, Tsan R, Huang WC, Wu Q, Chiu CH, Fidler IJ, et al. Survival of cancer cells is maintained by EGFR independent of its kinase activity. Cancer Cell 2008;13:385–93.
9. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med 2008;358:1160–74.
10. Baselga J. The EGFR as a target for anticancer therapy–focus on cetuximab. Eur J Cancer 2001;37 Suppl 4:S16–22.
11. Mahipal A, Kothari N, Gupta S. Epidermal growth factor receptor inhibitors: coming of age. Cancer Control 2014;21:74–9.
12. Arteaga CL. ErbB-targeted therapeutic approaches in human cancer. Exp Cell Res 2003;284:122–30.
13. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.
14. Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. J Clin Oncol 2003; 21:2787–99.
15. Avraham R, Yarden Y. Feedback regulation of EGFR signalling: decision making by early and delayed loops. Nat Rev Mol Cell Biol 2011;12:104–17.
16. Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Biol 2006;7:505–16.
17. Egger B, Büchler MW, Lakshmanan J, Moore P, Eysselein VE. Mice harboring a defective epidermal growth factor receptor (waved-2) have an increased susceptibility to acute dextran sulfate-induced colitis. Scand J Gastroenterol 2000;35:1181–7.
18. Egger B, Procaccino F, Lakshmanan J, Reinshagen M, Hoffmann P, Patel A, et al. Mice lacking transforming growth factor alpha have an increased susceptibility to dextran sulfate-induced colitis. Gastroenterology 1997; 113:825–32.
19. Fukata M, Chen A, Vamadevan AS, Cohen J, Breglio K, Krishnareddy S, et al. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. Gastroenterology 2007;133:1869–81.
20. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986;315: 1650–9.
21. Brandl K, Sun L, Neppl C, Siggs OM, Le Gall SM, Tomisato W, et al. MyD88 signaling in nonhematopoietic cells protects mice against induced colitis by regulating specific EGF receptor ligands. Proc Natl Acad Sci U S A 2010;107:19967–72.
22. Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov 2011;1:508–23.
23. Khambata-Ford S, Garrett CR, Meropol NJ, Basik M, Harbison CT, Wu S, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 2007;25:3230–7.
24. Tabernero J, Cervantes A, Rivera F, Martinelli E, Rojo F, von Heydebreck A, et al. Pharmacogenomic and pharmacoproteomic studies of cetuximab in metastatic colorectal cancer: biomarker analysis of a phase I dose-escalation study. J Clin Oncol 2010;28:1181–9.
25. Zanella ER, Galimi F, Sassi F, Migliardi G, Cottino F, Leto SM, et al. IGF2 is an actionable target that identifies a distinct subpopulation of colorectal cancer patients with marginal response to anti-EGFR therapies. Sci Transl Med 2015;7:272ra12.
26. Georgopoulos NT, Kirkwood LA, Southgate J. A novel bidirectional positive-feedback loop between Wnt-β-catenin and EGFR-ERK plays a role in context-specific modulation of epithelial tissue regeneration. J Cell Sci 2014;127:2967–82.
27. Campbell P, Morton PE, Takeichi T, Salam A, Roberts N, Proudfoot LE, et al. Epithelial inflammation resulting from an inherited loss-of-function mutation in EGFR. J Invest Dermatol 2014;134:2570–8.
28. Licitra L, Perrone F, Tamborini E, Bertola L, Ghirelli C, Negri T, et al. Role of EGFR family receptors in proliferation of squamous carcinoma cells induced by wound healing fluids of head and neck cancer patients. Ann Oncol 2011;22:1886–93.
29. Repertinger SK, Campagnaro E, Fuhrman J, El-Abaseri T, Yuspa SH, Hansen LA. EGFR enhances early healing after cutaneous incisional wounding. J Invest Dermatol 2004;123:982–9.
30. Misale S, Di Nicolantonio F, Sartore-Bianchi A, Siena S, Bardelli A. Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. Cancer Discov 2014;4:1269–80.
31. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:e73.
32. Kobayashi S, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 2005;352:786–92.
33. Montagut C, Dalmases A, Bellosillo B, Crespo M, Pairet S, Iglesias M, et al. Identification of a mutation in the extracellular domain of the Epidermal Growth Factor Receptor conferring cetuximab resistance in colorectal cancer. Nat Med 2012;18:221–3.
34. Voigt M, Braig F, Göthel M, Schulte A, Lamszus K, Bokemeyer C, et al. Functional dissection of the epidermal growth factor receptor epitopes targeted by panitumumab and cetuximab. Neoplasia 2012;14:1023–31.
35. Li S, Schmitz KR, Jeffrey PD, Wiltzius JJ, Kussie P, Ferguson KM. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. Cancer Cell 2005;7:301–11.
36. Weickhardt AJ, Price TJ, Chong G, Gebski V, Pavlakis N, Johns TG, et al. Dual targeting of the epidermal growth factor receptor using the combination of cetuximab and erlotinib: preclinical evaluation and results of the phase II DUX study in chemotherapy-refractory, advanced colorectal cancer. J Clin Oncol 2012;30:1505–12.
37. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351:337–45.
38. Van Cutsem E, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol 2007;25:1658–64.
39. Gibbons DL, Byers LA. A HER 1-2 punch: dual EGFR targeting deals resistance a deadly blow. Cancer Discov 2014;4:991–4.
40. Skartved NJ, Jacobsen HJ, Pedersen MW, Jensen PF, Sen JW, Jørgensen TK, et al. Preclinical pharmacokinetics and safety of Sym004: a synergistic antibody mixture directed against epidermal growth factor receptor. Clin Cancer Res 2011;17:5962–72.
41. Yonesaka K, Zejnullahu K, Okamoto I, Satoh T, Cappuzzo F, Souglakos J, et al. Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. Sci Transl Med 2011;3:99ra86.
42. Bardelli A, Corso S, Bertotti A, Hobor S, Valtorta E, Siravegna G, et al. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov 2013;3:658–73.
43. Siena S, Sartore-Bianchi A, Trusolino L, Martino C, Bencardino K, Lonardi S, et al. Therapeutic dual inhibition of HER2 pathway for metastatic colorectal cancer (mCRC): The HERACLES trial. J Clin Oncol 33, 2015 (suppl 3; abstr 565).
44. Network CGA. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012;487:330–7.
45. Guix M, Faber AC, Wang SE, Olivares MG, Song Y, Qu S, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. J Clin Invest 2008;118:2609–19.

46. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 2010;141:69–80.
47. Reidy DL, Vakiani E, Fakih MG, Saif MW, Hecht JR, Goodman-Davis N, et al. Randomized, phase II study of the insulin-like growth factor-1 receptor inhibitor IMC-A12, with or without cetuximab, in patients with cetuximab- or panitumumab-refractory metastatic colorectal cancer. J Clin Oncol 2010;28:4240–6.
48. Van Cutsem E, Eng C, Nowara E, Swieboda-Sadlej A, Tebbutt NC, Mitchell E, et al. Randomized phase Ib/II trial of rilotumumab or ganitumab with panitumumab versus panitumumab alone in patients with wild-type KRAS metastatic colorectal cancer. Clin Cancer Res 2014; 20:4240–50.
49. Pollak M. The insulin and insulin-like growth factor receptor family in neoplasia: an update. Nat Rev Cancer 2012;12:159–69.
50. Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 2008;359:1757–65.
51. Adjei AA, Cohen RB, Franklin W, Morris C, Wilson D, Molina JR, et al. Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. J Clin Oncol 2008; 26:2139–46.
52. Migliardi G, Sassi F, Torti D, Galimi F, Zanella ER, Buscarino M, et al. Inhibition of MEK and PI3K/mTOR suppresses tumor growth but does not cause tumor regression in patient-derived xenografts of RAS-mutant colorectal carcinomas. Clin Cancer Res 2012;18:2515–25.
53. Sun C, Bernards R. Feedback and redundancy in receptor tyrosine kinase signaling: relevance to cancer therapies. Trends Biochem Sci 2014;39: 465–74.
54. Drug combo beneficial in *BRAF*-mutant colorectal cancer. Cancer Discov 2015;5:102.
55. Ramanathan RK, Hwang JJ, Zamboni WC, Sinicrope FA, Safran H, Wong MK, et al. Low overexpression of HER-2/neu in advanced colorectal cancer limits the usefulness of trastuzumab (Herceptin) and irinotecan as therapy. A phase II trial. Cancer Invest 2004;22:858–65.
56. Regales L, Gong Y, Shen R, de Stanchina E, Vivanco I, Goel A, et al. Dual targeting of EGFR can overcome a major drug resistance mutation in mouse models of EGFR mutant lung cancer. J Clin Invest 2009;119:3000–10.
57. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, et al. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. N Engl J Med 2012;366:207–15.
58. Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med 2015;372:30–9.
59. Larkin J, Ascierto PA, Dréno B, Atkinson V, Liszkay G, Maio M, et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med 2014;371:1867–76.
60. Werner-Klein M, Proske J, Werno C, Schneider K, Hofmann HS, Rack B, et al. Immune humanization of immunodeficient mice using diagnostic bone marrow aspirates from carcinoma patients. PLoS One 2014;9: e97860.
61. Bertotti A, Trusolino L. From bench to bedside: does preclinical practice in translational oncology need some rebuilding? J Natl Cancer Inst 2013; 105:1426–7.
62. Corcoran RB, Falchook GS, Infante JR, Hamid O, Messersmith WA, Daud A, et al. Pharmacodynamic and efficacy analysis of the BRAF inhibitor dabrafenib (GSK436) in combination with the MEK inhibitor trametinib (GSK212) in patients with BRAFV600 mutant colorectal cancer (CRC). J Clin Oncol 31, 2013 (suppl; abstr 3507).