

Expert Opinion

1. Introduction
2. Some ligands for ErbB RTKs function as partial agonists
3. ErbB receptor partial agonists function antagonize ErbB receptor signaling
4. Conclusion
5. Expert opinion

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Ligand-based receptor tyrosine kinase partial agonists: new paradigm for cancer drug discovery?

David J Riese II

Auburn University Harrison School of Pharmacy, 2316 Walker Building, Auburn, AL, USA

Introduction: Receptor tyrosine kinases (RTKs) are validated targets for oncology drug discovery and RTK antagonists are available to treat human malignancies. Nonetheless, the development of RTK antagonists has lagged behind the development of agents that target GPCRs. In part, this is because it has been difficult to discover analogs of RTK agonists that function as antagonists.

Areas covered: In this review the author describes ligands of ErbB receptors that function as partial agonists for these receptors and antagonize the activity of full agonists for these receptors. The author provides insights into the mechanisms by which these ligands function as antagonists and discuss how these insights can be translated into screens for novel small molecule- and antibody-based antagonists of ErbB receptors that hold potential as targeted cancer chemotherapeutics.

Expert opinion: While there have been numerous key findings in this field, the identification of the structural basis of ligand functional specificity is still of the greatest importance. While it is true that, with some notable exceptions, peptide hormones and growth factors have not been good platforms for oncology drug discovery, addressing the fundamental issues of antagonistic partial agonists for RTKs has the potential of steering oncology drug discovery in new directions. Mechanism-based approaches are emerging to enable the discovery of small molecule and antibody RTK partial agonists that may antagonize RTK signaling and may hold promise as targeted cancer chemotherapeutics.

Keywords: ErbB/EGF receptors, ligand-based antagonists, receptor partial agonists and antagonists, receptor tyrosine kinases, targeted cancer chemotherapeutics

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1. Introduction

1.1 Introduction to RTKs and their functional probes

Deregulated signaling by receptor tyrosine kinases (RTKs) plays critical roles in numerous disease states, particularly in various human malignancies. Indeed, numerous RTKs are validated targets for oncology drug discovery. Paradigms exist for targeting these proteins, including small molecule tyrosine kinase inhibitors (TKIs), antagonistic antibodies and ligand sinks. Nonetheless, the discovery and development of functional probes and lead antagonists of these receptors have occurred at a pace dwarfed by the pace at which molecules that target many GPCRs have been discovered and developed. In part, this disparity reflects the relative ease

Article highlights.

- Many receptor tyrosine kinases (RTKs), including the VEGFR, the EGFR (EGFR/ErbB2) and ErbB2 (HER2/Neu), are validated targets for oncology drug discovery.
- Existing strategies for targeting RTKs include antibodies that function as ligand sinks, antibodies that bind to an RTK and antagonize ligand binding to that of RTK, antibodies that inhibit RTK dimerization and signaling, antibodies that may function as RTK partial agonists, and small molecule tyrosine kinase inhibitors.
- Some naturally occurring ligands for ErbB RTKs possess less efficacy (intrinsic activity) than others. These partial agonists can competitively inhibit (antagonize) the activity of more efficacious agonists at the same receptor. In the case of partial agonists that stimulate ErbB receptor turnover, the antagonistic effects may be irreversible.
- Some cases of differences in ErbB receptor ligand efficacy appear to be due to differences in the sites of ErbB receptor tyrosine phosphorylation and differences in ErbB receptor coupling to signaling effectors and biological responses. Generalization of this paradigm to other RTKs may lead to straightforward strategies for generating novel ligand-based small molecule or antibody RTK antagonists.

This box summarizes key points contained in the article.

in discovering analogs of endogenous GPCR ligands that possess antagonistic activity. However, here I discuss the discovery of analogs of endogenous RTK ligands that possess partial agonist activity and herald the possibility of clinically relevant ligand-based RTK antagonists.

1.2 Principles of ligand-induced RTK signaling

RTKs are single-pass transmembrane proteins that possess an extracellular ligand-binding motif, an intracellular tyrosine kinase domain and intracellular tyrosine residues whose phosphorylation creates docking sites for the intracellular signaling effectors that mediate receptor coupling to biological responses. The fundamental effect of agonist binding to RTKs is RTK tyrosine phosphorylation as a consequence of agonist-induced RTK dimerization. There are multiple mechanistic paradigms for agonist-induced RTK dimerization [1]: i) a multivalent, monomeric agonist bridges two RTK monomers; ii) a dimer of agonists (frequently monovalent) bridges two RTK monomers and iii) two monomeric agonists each induce or stabilize a conformational change in an RTK monomer that enables RTK dimerization. A general paradigm for the mechanism by which RTK dimerization promotes RTK tyrosine phosphorylation has yet to be established. It has been postulated that agonist-induced dimerization of the EGFR extracellular domains induces asymmetric dimerization of the EGFR intracellular domains. This causes the tyrosine kinase domain of one of the EGFR monomers to adopt an active conformation and juxtaposes the cytoplasmic tyrosine

residues of the other EGFR monomer with that active tyrosine kinase domain. Thus, at least in the case of EGFR and related ErbB family RTKs, agonist binding may not induce autophosphorylation by itself; rather, agonist binding causes tyrosine phosphorylation in *trans* across the receptor dimer [2-5]. It should be noted that some data indicate that tyrosine phosphorylation is due to autophosphorylation in a manner somewhat reminiscent of Src family kinase autophosphorylation [6,7].

1.3 Common strategies for antagonizing ligand-induced RTK signaling

Small molecules and antibodies that target and antagonize RTK signaling have entered clinical practice. Emerging paradigms for targeting RTK signaling include RTK fragments and agonist fragments and analogs. Here, I briefly review these paradigms and highlight the challenges associated with their development into clinical agents.

1.3.1 Small molecule TKIs

Small molecule TKIs target the ATP binding pocket of RTKs. TKIs antagonize RTK coupling to biological responses by inhibiting RTK tyrosine kinase activity and phosphorylation-dependent RTK coupling to signaling effectors. The discovery and development of RTK TKIs has been spurred in part by the success of the Abl/c-Kit TKI imatinib (Gleevec[®], Novartis, East Hanover, NJ, USA) in treating Philadelphia chromosome-positive chronic myelogenous leukemia and c-Kit-positive gastrointestinal stromal tumors [8-15]. However, this advance has not translated into widespread successful targeting of RTKs with TKIs, in part due to the frequency of RTK kinase domain mutations that abrogate TKI activity. For example, the EGFR TKIs gefitinib (Iressa[™], AstraZeneca, Wilmington, NC, USA) and erlotinib (Tarceva[®], Genentech, South San Francisco, CA, USA) are effective against only the small fraction of NSCLCs that harbor kinase domain mutations that render the tumor cells dependent on EGFR. Moreover, this efficacy is frequently abrogated by a second site mutation that reduces TKI affinity for the EGFR kinase domain [16,17].

1.3.2 Antibodies

There are numerous therapeutic mAbs that target extracellular epitopes of cell surface proteins whose expression is associated with a pathologic state. In some cases, these antibodies appear to function primarily by eliciting an immune response specific for the cells that express the targeted cell surface antigen. For example, the mAb rituximab (Rituxan[®], Genentech) is effective against many B-cell lymphomas by targeting the CD20 antigen, which is overexpressed by these tumor cells [18-23]. A thorough discussion of this class of agents lies outside the scope of this review.

In addition, there are several antibodies that elicit their therapeutic effects by disrupting RTK signaling. These antibodies can be grouped according to their mechanism of

action. These groups include ligand sinks, inhibitors of ligand binding, inhibitors of receptor dimerization and agents with other mechanisms of action.

1.3.2.1 Ligand sinks

Ligand sinks antagonize RTK signaling by binding the RTK agonist and preventing the agonist from binding to the RTK and stimulating its signaling. One example is the mAb bevacizumab (Avastin[®], Genentech), which binds to VEGF. This prevents VEGF from binding to the VEGF receptor and prevents VEGF stimulation of VEGF receptor signaling. Bevacizumab is approved as part of combination therapies for the treatment of NCSLC, as well as metastatic breast, kidney and colorectal cancers [24-31].

1.3.2.2 Inhibitors of ligand binding

Other mAbs bind to an RTK and prevent agonist binding to the RTK and agonist stimulation of RTK signaling. Theoretically, two mechanisms of action are possible. mAbs could directly compete with agonists for binding to a common or overlapping binding site on the RTK. Cetuximab (Erbix[®], Bristol-Myers Squibb, Princeton, NJ, USA) is an example of this class of agents; it competes with EGF and other EGFR agonists for binding to EGFR, thereby inhibiting agonist-induced EGFR signaling [32,33]. mAbs could theoretically inhibit agonist-induced RTK signaling by inducing the RTK to adopt a conformation with lower affinity for agonist (allosteric inhibition). However, the challenges associated with generating such agents may be part of the reason why this mechanistic paradigm has yet to be widely exploited.

1.3.2.3 Inhibitors of receptor dimerization

Pertuzumab (formerly known as omnitarg) is an antibody specific for ErbB2 (HER2/Neu) RTK that inhibits ErbB2 heterodimerization with other ErbB family receptors, including EGFR and ErbB3 (HER3) [34,35]. Because ErbB2 lacks a specific soluble agonist, agonist binding to an ErbB receptor other than ErbB2 and consequent heterodimerization and cross-talk with ErbB2 are a common mechanism by which ErbB2 signaling can be regulated. Clinical trials (MARIANNE: NCT01120184 and CLEOPATRA: NCT00567190) that assess the efficacy of pertuzumab in combination chemotherapy regimens for the treatment of ErbB2-positive breast tumors are ongoing.

1.3.2.4 Other mechanisms of action

Trastuzumab (Herceptin[®], Genentech) is perhaps the most famous therapeutic antibody used in oncology. This antibody is specific for ErbB2 and is used to target breast tumors that overexpress ErbB2. A number of mechanisms, including antibody-dependent cellular cytotoxicity, may account for the antitumor activities of trastuzumab [36-39]. However, 4D5, the mouse mAb from which trastuzumab is derived, stimulates ErbB2 tyrosine phosphorylation and internalization [40]. This mechanism may also account for some of the antitumor activities displayed by trastuzumab.

1.3.3 Other agents

RTK fragments that include the agonist-binding domain(s) may serve as decoy receptors for agonists (agonist sinks). For example, a recombinant soluble protein containing the extracellular subdomains I – III of ErbB4 antagonizes agonist-induced signaling by ErbB4 [41]. Proteins that are not derived from RTKs may also function as agonist sinks. Perhaps the best known is the drosophila Argos protein, which binds to the drosophila EGF homolog Spitz and antagonizes stimulation of drosophila EGFR (DER) signaling by preventing Spitz binding to DER [42,43]. Finally, a fragment of an RTK agonist that retains the site of binding to the RTK may competitively antagonize agonist-induced signaling by that RTK. For example, a fragment corresponding to residues 33 – 42 of murine EGF inhibits EGF stimulation of endothelial cell motility and EGF stimulation of chicken egg angiogenesis [44]. Nonetheless, these three mechanisms have not been common approaches for developing therapeutic RTK antagonists.

2. Some ligands for ErbB RTKs function as partial agonists

The ErbB receptor signaling network is quite complex and features multiple naturally occurring peptide growth factor ligands for EGFR, ErbB3 and ErbB4. An emergent attribute of this signaling network is that different EGF family ligands for the same ErbB family receptor are functionally distinct, with some of these ligands functioning as partial agonists and others functioning as full agonists at the same receptor [45]. For example, a saturating concentration of the EGFR ligand amphiregulin (AREG) stimulates greater EGFR coupling to cell proliferation, motility and metastatic activities than does a saturating concentration of EGF, another EGFR ligand. Thus, with respect to these outputs, AREG is a full agonist for EGFR whereas EGF is a partial agonist (Figure 1A) [45-47].

Published and unpublished data indicate that the functional difference appears to be due to the fact that EGF stimulates greater EGFR tyrosine phosphorylation at EGFR Tyr1045 than does AREG, resulting in greater EGFR coupling to the ubiquitin ligase c-Cbl and greater EGFR ubiquitination and more rapid EGFR turnover. Thus, AREG stimulates EGFR signaling of greater duration than does EGF. This appears to permit greater EGFR coupling to phospholipase C γ through phosphorylation of EGFR Tyr992 [45-47]. It has been postulated this specificity in ligand-induced EGFR signaling is due to differences in the geometry of the EGFR dimer, resulting in differences in the presentation of tyrosine residues of one monomer to the catalytic domain of the other monomer and differences in the sites of EGFR tyrosine phosphorylation [45,48].

More compelling results have been obtained through analyses of ErbB4 ligands. The ErbB4 ligand Neuregulin 2 β (NRG2 β) potently stimulates ErbB4 coupling to cell proliferation, whereas the ErbB4 ligand Neuregulin 2 α

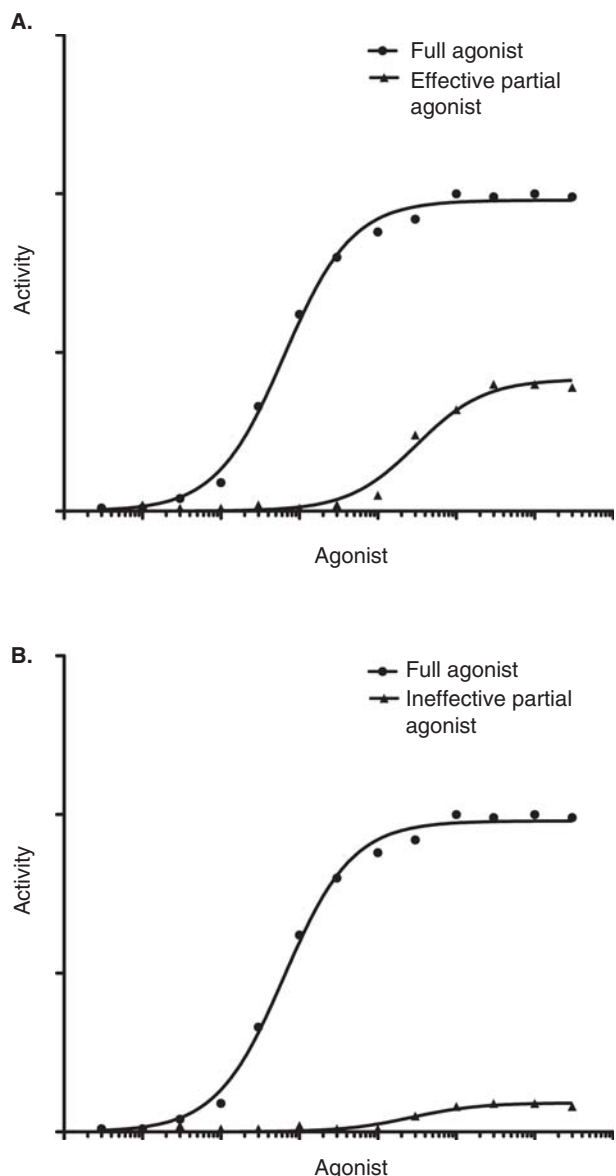


Figure 1. A saturating concentration of a theoretical full agonist elicits a greater response than a saturating concentration of a theoretical partial agonist. A. A theoretical full agonist vs a relatively effective theoretical partial agonist. The activity of these ligands resembles the activity of AREG (full agonist) and EGF (partial agonist). **B.** A theoretical full agonist vs a relatively ineffective theoretical partial agonist. The activity of these ligands resembles the activity of wild-type NRG2 β (full agonist) and NRG2 β /Q43L (partial agonist).

AREG: Amphiregulin; NRG2 β : Neuregulin 2 β .

(NRG2 α) does not. Dose–response experiments suggest that the failure of NRG2 α to stimulate ErbB4 coupling to cell proliferation is not due inadequate affinity of NRG2 α for ErbB4. Moreover, the NRG2 β /F45K mutant stimulates ErbB4 coupling to cell proliferation, but the NRG2 α /K45F

mutant still does not. This is despite the fact that the NRG2 α /K45F mutant and the NRG2 β /F45K mutant have roughly equivalent affinity for ErbB4 and despite the fact that the two ligands stimulate ErbB4 tyrosine phosphorylation with roughly equivalent potency [49–51].

A genetic approach can resolve the functional distinction between NRG2 α and NRG2 β . The NRG2 β /Q43L mutant fails to stimulate ErbB4 coupling to cell proliferation despite the fact that the Q43L mutation does not markedly reduce the affinity of NRG2 β for ErbB4 and does not markedly reduce the potency of NRG2 β stimulation of ErbB4 tyrosine phosphorylation. Moreover, the corresponding NRG2 α /L43Q mutant stimulates ErbB4 coupling to cell proliferation despite the fact that the L43Q mutation does not markedly increase the affinity of NRG2 α for ErbB4 and does not markedly increase the potency of NRG2 α stimulation of ErbB4 tyrosine phosphorylation [52]. Thus, with respect to ErbB4 coupling to cell proliferation, wild-type NRG2 α and the NRG2 β /Q43L mutant function as very weak partial agonists at ErbB4 whereas wild-type NRG2 β and the NRG2 α /L43Q mutant function as full agonists (Figure 1B).

Unpublished data indicate that NRG2 α stimulates much less ErbB4 coupling to Akt (protein kinase B) phosphorylation than does NRG2 β . Furthermore, the signaling pathway comprised in part by PI3K and Akt is required for NRG2 β stimulation of ErbB4 coupling to cell proliferation. Given the paradigm for ligand-specific EGFR signaling, it seems reasonable to postulate that ErbB4 full agonists stimulate greater ErbB4 tyrosine phosphorylation on a site that is coupled to the PI3K/Akt pathway than do ErbB4 partial agonists. Another possibility is that ErbB4 partial agonists stimulate greater ErbB4 tyrosine phosphorylation on a site that is coupled to ErbB4 ubiquitination and/or turnover than do ErbB4 full agonists. Experimentation is required to address these two possibilities.

3. ErbB receptor partial agonists function antagonize ErbB receptor signaling

The full and partial agonists for a given ErbB receptor share a common binding site on that receptor. Thus, full and partial agonists are expected to compete with each other for receptor binding and partial agonists are expected to function as competitive antagonists of agonist-induced ErbB receptor signaling. Indeed, preliminary data indicate that increasing concentrations of EGF partially antagonize stimulation of EGFR coupling to cell proliferation by a fixed concentration of AREG (as in Figure 2A). Because EGF possesses some ability to stimulate EGFR coupling to cell proliferation, EGF does not fully antagonize EGFR coupling to cell proliferation. This effect is mediated by EGFR, as increasing concentrations of AREG overcome partial antagonism of EGFR signaling by a fixed concentration of EGF; the rightward shift of the AREG dose–response curve in the presence of a fixed concentration of EGF indicates

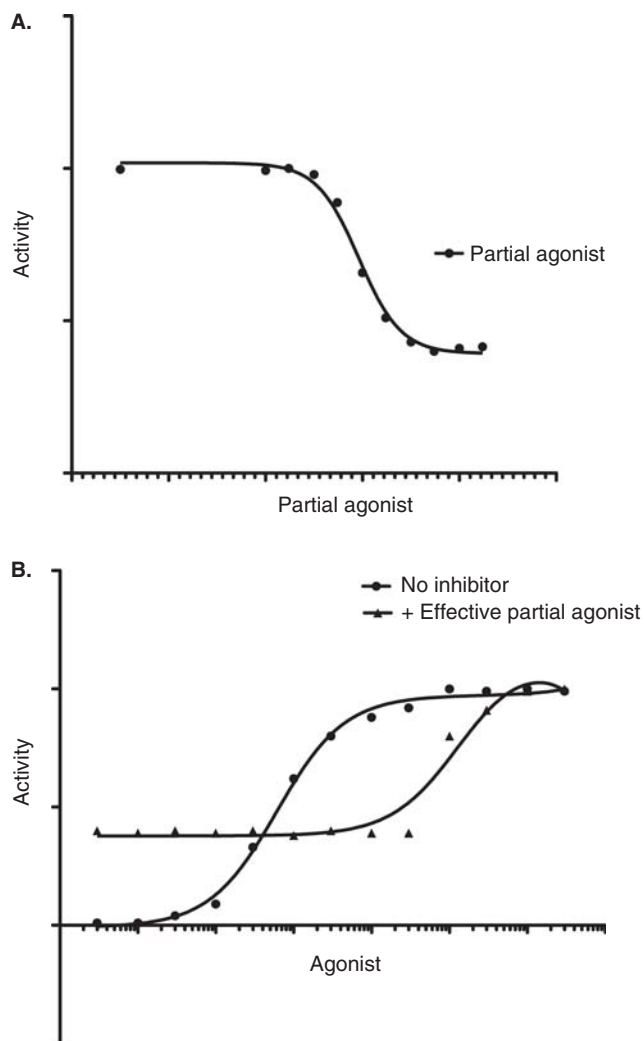


Figure 2. A theoretical partial agonist can antagonize the activity of a theoretical full agonist. The activity of these ligands resembles the activity of AREG (full agonist) and EGF (partial agonist). **A.** Increasing concentrations of a relatively effective theoretical partial agonist inhibit the activity of a fixed concentration of a theoretical full agonist. **B.** The antagonistic effects of a fixed concentration of a relatively effective theoretical partial agonist can be overcome by increasing concentrations of a theoretical full agonist. The resulting rightward shift in the full agonist dose–response curve in the presence of the partial agonist is indicative of competitive inhibition of full agonist activity by the partial agonist.

AREG: Amphiregulin.

that EGF competitively antagonizes AREG stimulation of EGFR signaling, thereby, reducing the potency (but not efficacy) of AREG (as in Figure 2B).

Preliminary data reveal that ErbB4 ligands tell a more compelling story. The NRG2 β /Q43L mutant fully antagonizes stimulation of ErbB4 coupling to cell proliferation by a fixed concentration of NRG2 β (as in Figure 3A). This effect is

mediated by ErbB4, as increasing concentrations of NRG2 β overcome antagonism of ErbB4 signaling by a fixed concentration of NRG2 β /Q43L; the rightward shift of the NRG2 β dose–response curve in the presence of a fixed concentration of NRG2 β /Q43L indicates that NRG2 β /Q43L competitively antagonizes NRG2 β stimulation of ErbB4 signaling, thereby, reducing the potency (but not efficacy) of NRG2 β (as in Figure 3B). NRG2 β is also an agonist for EGFR and the ErbB2/ErbB3 heterodimer [50,53]. Consequently, it is not entirely surprising that NRG2 β /Q43L competitively antagonizes agonist stimulation of EGFR signaling and agonist stimulation of ErbB2/ErbB3 signaling. Thus, NRG2 β /Q43L functions as a pan-ErbB receptor antagonist.

Preliminary data indicate two additional findings of some note. Preincubation of cells that express EGFR with NRG2 β /Q43L irreversibly partially block EGF binding to EGFR, suggesting that NRG2 β /Q43L antagonizes EGFR signaling in part by stimulating rapid EGFR ubiquitination and turnover. Consistent with this hypothesis, expression of EGF in cells irreversibly blocks AREG stimulation of EGFR coupling to cell proliferation.

4. Conclusion

Some naturally occurring ErbB family receptor peptide growth factor ligands function as partial agonists for their cognate receptor. Partial and full agonism are mutable; ligand mutations can convert partial agonists into full agonists and vice versa. The mechanistic basis for the difference in efficacy displayed by full and partial ErbB receptor agonists appears to be differences in the individual sites of receptor tyrosine phosphorylation. Thus, screens based on the absence or presence of particular sites of RTK tyrosine phosphorylation could be used to identify novel RTK partial agonists that possess RTK antagonist activities.

5. Expert opinion

5.1 Key findings and weaknesses

One key finding is that some naturally occurring ErbB receptor ligands function as a partial agonist for their cognate receptor(s). The rationale for the existence of full and partial agonists remains to be elucidated. Partial agonists may allow for spatiotemporal fine-tuning of ErbB receptor signaling. Partial agonists may also serve to prevent pathologic levels of ErbB receptor signaling.

Another key finding is that functional specificity of ligands for a given ErbB receptor appears to reflect differences in the sites of tyrosine phosphorylation, resulting in differences in ErbB receptor coupling to effectors and/or differences in ErbB receptor turnover. However, in most cases, the mechanism underlying ligand functional specificity has yet to be definitively established or adequately explored. In particular, identifying the structural basis of ligand functional specificity is of

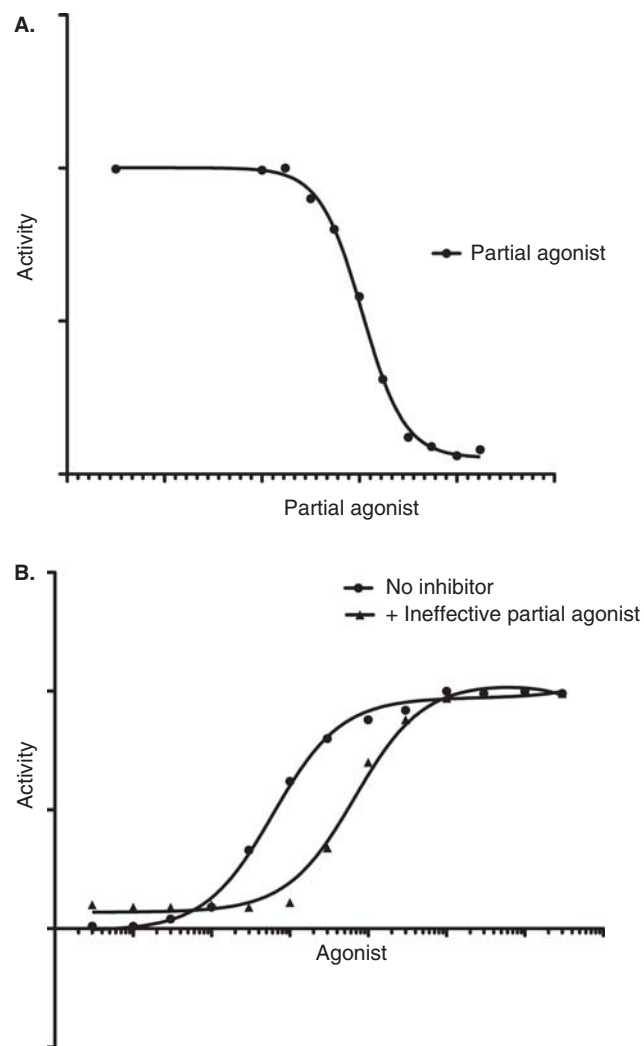


Figure 3. A relatively ineffective theoretical partial agonist can have a profound effect on the activity of a theoretical full agonist. The activity of these ligands resembles the activity of wild-type NRG2 β (full agonist) and NRG2 β /Q43L (partial agonist). **A.** Increasing concentrations of a relatively ineffective theoretical partial agonist almost completely abrogate the activity of a fixed concentration of a theoretical full agonist. **B.** The antagonistic effects of a fixed concentration of a relatively ineffective theoretical partial agonist can be overcome by increasing concentrations of a theoretical full agonist. The resulting rightward shift in the full agonist dose-response curve in the presence of the partial agonist is indicative of competitive inhibition of full agonist activity by the partial agonist.

NRG2 β : Neuregulin 2 β .

paramount importance, as such information will yield new insights into the development of partial agonists with therapeutic potential.

Partial agonists for a given ErbB receptor appear to antagonize the activity of full agonists at that ErbB receptor. In part, the partial agonists competitively antagonize the activity

of the full agonists, as an increasing concentration of agonist can overcome the antagonistic activity of a fixed concentration of a partial agonist. However, these experiments have been performed using heterologous, artificial systems; antagonism of ErbB receptor signaling in tumor cells by partial agonists has yet to be observed.

Finally, preincubation of target cells with a partial agonist or expression of a partial agonist by target cells results in irreversible antagonism of agonist-induced ErbB receptor signaling. Presumably, this irreversible inhibition reflects partial agonist stimulation of receptor ubiquitination and/or turnover. However, these mechanistic details have yet to be definitively established.

5.2 Future topics of interest and potential of this research

With some notable exceptions, peptide hormones and growth factors have not proven to be good platforms for oncology drug discovery. Nonetheless, once some fundamental issues are addressed, antagonistic partial agonists for RTKs could drive oncology drug discovery in new directions.

First of all, the antagonistic activity of ErbB receptor partial agonists in human tumor cell lines must be established. It is anticipated that partial agonists will antagonize the effects of soluble full agonists added to the culture medium. However, it is not apparent whether partial agonists will antagonize full agonist/receptor autocrine loops. Given that these autocrine loops may feature receptor signaling prior to the appearance of the receptor at the cell surface, it is likely that soluble partial agonists will not disrupt autocrine signaling but expression of the partial agonists by the target cells will disrupt autocrine signaling. Moreover, it is not apparent whether partial agonists will antagonize ligand-independent receptor signaling. Given that partial agonists may function by causing receptor ubiquitination and/or turnover, it is possible that partial agonists may antagonize pathologic, ligand-independent receptor signaling caused by overexpression or by those activating mutations of the receptor that do not disrupt antagonist binding [54]. However, ErbB receptor truncation/deletion mutants that feature reduced agonist binding (such as EGFR vIII [54]) are predicted to be refractory to the antagonistic action of ErbB receptor partial agonists.

Next, the mechanism by which partial agonists antagonize agonist-induced (or agonist-independent) ErbB receptor signaling must be definitively established. A focal point will be the identification of agonist- and antagonist-specific sites of receptor tyrosine phosphorylation and the validation of their relevance to ErbB receptor coupling. As stated elsewhere, two hypotheses will be explored. i) Agonists stimulate greater phosphorylation of tyrosine residues that enable receptor coupling to mitogenic and other pro-malignant responses than do antagonists. ii) Antagonists stimulate greater phosphorylation of tyrosine residues that trigger receptor ubiquitination and/or turnover than do agonists.

The identification of agonist- and antagonist-specific sites of ErbB receptor tyrosine phosphorylation will permit the development of screening strategies for novel ErbB receptor antagonists. It may be possible that such screening strategies will take advantage of the numerous commercial antibodies specific for individual sites of EGFR and ErbB2 tyrosine phosphorylation. However, in many cases it may be necessary to develop novel tools for assessing phosphorylation of specific ErbB receptor tyrosine residues.

The approaches could be deployed to identify novel mutants of naturally occurring ligands that possess partial agonist and antagonist activities. However, as noted elsewhere, efforts to exploit peptide growth factors and hormones as platforms for oncology drug discovery have not been particularly successful. Part of the reason may be due to the pleiotropic effects of peptide growth factors and hormones, particularly when administered systemically. Thus, screens for small molecule and antibody partial agonists (that possess antagonistic activity) may yield molecules with greater therapeutic potential than low-efficacy naturally occurring peptide growth factors or growth factor mutants. The fact that trastuzumab possesses partial agonist activity [38] is validation for such a screening strategy.

Targeting the extracellular domain with partial agonists that possess antagonistic activities may be a more effective general paradigm than targeting the intracellular tyrosine kinase domain with small molecule, ATP-competitive inhibitors. Partial agonists do not need to fulfill the design constraints necessary to ensure cell penetrance. Moreover, the fact that partial agonists are likely to share the same binding sites on RTKs as the naturally occurring RTK agonists suggests that mutations that abrogate the binding of partial

agonists will not be selected for, as these mutations are likely to also disrupt the binding of the naturally occurring agonists. Indeed, there have been no descriptions of acquired ErbB2 mutations that disrupt the binding of the trastuzumab partial agonist; likewise, there have been no descriptions of acquired EGFR mutations that disrupt the binding of cetuximab, which shares its binding site on EGFR with the naturally occurring EGFR agonists. In contrast, there are numerous examples of acquired tyrosine kinase domain mutations that disrupt the activity of small molecule TKIs.

Finally, the partial agonist/antagonist paradigm may be generally applicable to all RTKs. Screening for competitive antibody or small molecule partial agonists that function as antagonists would be performed in two steps. The first would be to assess whether candidate molecules inhibit the binding of naturally occurring agonists to the RTK. The second would be to assess whether candidate molecules fail to stimulate RTK phosphorylation on those tyrosine residues that enable RTK coupling to a malignant phenotype. Screening for irreversible antibody or small molecule partial agonists that function as antagonists would also be performed in two steps. Again, the first would be to assess whether candidate molecules inhibit the binding of naturally occurring agonists to the RTK. The second would be to assess whether candidate molecules stimulate RTK phosphorylation on those tyrosine residues that enable RTK ubiquitination and/or turnover.

Declaration of interest

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Affiliation

David J Riese II PhD
 George Fulton Gilliland & Olga Hooser
 Gilliland Franklin Professor,
 Associate Dean for Research and Graduate
 Programs,
 Auburn University Harrison School of Pharmacy,
 2316 Walker Building,
 Auburn, AL 36849-5501, USA
 Tel: +1 334 844 8358; Fax: +1 334 844 8353;
 E-mail: driese@auburn.edu