Published in final edited form as:

Expert Opin Biol Ther. 2015 July ; 15(7): 1015–1021. doi:10.1517/14712598.2015.1042362.

Oligoclonal Antibodies to Target the ErbB Family

Jimson W. D'Souza and Matthew K. Robinson

Molecular Therapeutics Program, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111

Matthew K. Robinson: matthew.robinson@fccc.edu

Abstract

Introduction—Use of monoclonal antibodies to inhibit signaling through the ErbB receptor tyrosine kinase family has proven to be an effective strategy for treating ErbB-driven cancers. Advances in the field of antibody engineering and manufacturing now allow us to more effectively mimic the natural immune response by generating oligoclonal mixtures of antibodies against desired targets of interest.

Areas Covered—In this review we examine the literature describing the development of oligoclonal mixtures of antibodies against ErbB family members and the impact of those mixtures on preclinical and clinical efficacy.

Expert Opinion—Oligoclonal antibodies, facilitated by the improved antibody engineering and manufacturing techniques, hold the promise of improving patient outcomes. Through the use of empirical methods, oligoclonal mixtures with enhanced capacity to block signaling through ErbB family members can be identified. The intrinsic mechanisms associated with each of the component monoclonal antibodies provide an opportunity to block signaling via multiple mechanisms of action. In addition, combinations of antibodies targeting multiple ErbB family members provides the capacity to downregulate signaling through multiple components of this critical pathway.

2. Introduction

Over the past two decades advances in field of antibody engineering have overcome hurdles associated with first generation antibody-based therapies. First generation monoclonal antibody (mAbs) therapies were murine in origin. These agents exhibited limited efficacy due to the generation of human anti-mouse antibody (HAMA) responses against the therapeutic agents, which both resulted in rapid clearance and prevented readministration.¹⁻³ The advent of strategies to generate chimeric and humanized mAbs from murine antibodies and methods to isolate fully human mAbs led to dramatic improvements in the utility of this class of agents. Monoclonal antibodies are now one of the fastest growing categories of pharmaceuticals and represent a mainstay for the treatment of a variety of diseases, including cancer and autoimmunity. With six FDA-approvals in 2014 the number of antibody-based therapeutics currently being marketed now stands at 36. Of those, 15 are used for the treatment of cancer (http://antibodysociety.org/news/approved_mabs.php).

The ErbB receptor tyrosine kinase family (EGFR/ErbB1, HER2/ErbB2, HER3/ErbB3, and HER4/ErbB4) play essential roles in normal development and tissue homeostasis. Abnormal signaling through EGFR and ErbB2, either through protein overexpression or mutations that make the kinases refractory to normal regulatory mechanisms, is directly implicated in formation and progression of many cancer types.⁴ While initially overlooked as a drug target due to its inactive kinase domain, ErbB3 is now recognized as an integral component of ErbB-driven cancers.⁵ The role of ErbB4 remains to be fully elucidated. Because of their roles in driving disease the ErbB family represents a high-value set of targets for mAb-based cancer therapy. The effectiveness of mAb-based therapies at disrupting ErbB signaling is supported by the fact that five of the 10 mAbs that are FDA-approved to treat solid malignancies target either EGFR or ErbB2 (Table 1). Many additional antibody-based therapies targeting the ErbB family are at various stages of development.

The promise and limitations associated with ErbB-targeted mAbs are exemplified by trastuzumab, an anti-ErbB2/HER2 mAb approved for the treatment of ErbB2+ breast and gastric cancer. Trastuzumab has dramatically altered both the rates of disease progression and overall survival for ErbB2+ patients.⁶⁻¹⁰ However, both intrinsic and acquired resistance to trastuzumab therapy is a clinical reality. Even patients that initially respond to trastuzumab therapy will almost invariably relapse. Despite all of the successes associated with mAb-based therapies, significant room for improving patient outcomes still exists. Similar clinical outcomes are associated with the anti-EGFR mAbs in the settings of head and neck, colorectal, and non-small cell lung cancer cancers.¹¹⁻¹³ Thus, improving the efficacy of antibody-based therapies has been the subject of intense investigation and led to the development of a series of different approaches to address this shortcoming.

Antibody-drug conjugates (ADCs) represent one such approach. In recent years improvements to linker strategies have allowed for more effective conjugation of highly potent cytotoxics to mAbs to generate ADCs. ADCs enhance the therapeutic window of these cytotoxics by promoting delivery to the tumor microenvironment. Aspects of ADC development are reviewed elsewhere.^{14,15} The ErbB2-targeted trastuzumab emtansine (T-DM1) is a representative of this expanding class of cancer therapies.¹⁶ Results from a recent phase III trial (EMILIA) led to its FDA-approval for use in the setting of progressive metastatic breast cancer.¹⁷ Findings demonstrated that T-DM1 significantly prolongs both progression-free and overall survival as compared to a regimen of lapatinib plus capecitabine. The overall safety profile also favored the T-DM1 arm where the number of grade three and above adverse events were lower (200, 40.8%) as compared to the control arm (278, 57%). Similar findings were also observed in a second phase III trial (TH3RESA) that compared T-DM1 to physician's choice.¹⁸ A second approach to improve the efficacy of antibody-based therapies is focused on generation of bispecific, or multispecific, molecules capable of blocking signaling through two or more proteins at one time. This approach is exemplified by MM-111^{19,20} and MEHD7945A^{21,22} that block signaling through ErbB2/ErbB3 and EGFR/ErbB3 heterodimers, respectively. Clinical trials are ongoing with these agents to determine the potential benefit to patient outcomes.

A third approach, that is generating interest in both the cancer and infectious disease space, is an attempt to more closely mimic the natural polyclonal immune response by generating

mixtures of recombinant antibodies, the components of which are each selected based upon defined criteria, such as epitope specificity, affinity, and intrinsic activity. It is hypothesized that antibody mixtures will be more effective at both blocking signaling through the ErbB RTKs and at redirecting the patients immune system through an antibody dependent cellular cytotoxicity (ADCC) mechanism. As described in more detail below, evidence from both the preclinical and clinical settings suggest that targeting the ErbB family with either oligoclonal mixtures (defined here as 2 mAbs against non-overlapping epitopes on the same target antigen) or recombinant polyclonal mixtures (rpAb, defined here as 3 mAbs against more than one target antigen), examples of which are listed in Table 2, holds significant promise for improving patient outcomes.

3. Production of Antibody Mixtures

Robust manufacturing processes utilizing CHO cell expression platforms have been established for mAbs and yields of purified material on the order of g/L can be readily achieved. In principle, such yields can facilitate generation of antibody mixtures by expressing and purifying the component mAbs in parallel, followed by either mixing or simultaneously administering them at defined ratios. As described below, this approach has proven feasible for combination therapies based upon two previously approved mAbs.²³ However, estimates suggest that production of antibody mixtures in this manner may be cost prohibitive for combinations of three or more mAbs²⁴. To circumvent this issue, single-batch production strategies, such as the Sympress technologies (Symphogen, Denmark) described by Tolstrup and colleagues^{25,26}, have been developed to facilitate the cost effective manufacture of antibody mixtures. The Sympress technologies rely upon either site-directed (Sympress I) or random (Sympress II) insertion of antibody expression cassettes into appropriate engineered cell lines to generate clones, each with defined growth and mAb expression properties. The clones are then mixed to create polyclonal master cell banks, which are later used to express product and/or generate polyclonal working cell banks following a traditional two-tiered cell banking approach. A major hurdle in producing antibody mixtures in this manner, which has been circumvented by Sympress and other platforms, is consistent lot-to-lot characteristics of the final antibody mixture.^{26,27}

4. Oligoclonal Targeting of EGFR

The FDA-approved mAbs cetuximab and panitumumab inhibit signaling through the EGFR by competing ligand binding to domain III of EGFR extracellular domain. Preclinical data from the Yarden^{28,29} and Wittrup³⁰ groups demonstrated that a combination of two anti-EGFR mAbs that bind to non-overlapping epitopes on domain III increased rates of receptor downregulation from the cell surface than was seen with the individual mAbs. This downregulation led to more pronounced inhibition of EGFR-dependent signaling. Koefoed et al³¹ extended this approach by determining the intrinsic activity of 88 unique anti-EGFR mAbs and selected 24 for investigation of all possible pairwise combinations. Not all combinations resulted in similar levels of enhanced efficacy, pointing to the current need to empirically determine the optimal combination of antibodies. A panel of 10 mAbs associated with high anti-proliferative activity in all combinations tested was investigated further and their binding was mapped to one of four different epitope bins, one on domain I

and three non-overlapping bins on domain III, including one bin that included the cetuximab and panitumumab epitopes. A 1:1 mixture of two mAbs, 992 and 1024, that both bind to domain III were identified as the combination with the highest level of activity. These molecules have subsequently been used to generate Sym-004. *In vitro* Sym-004 acts synergistically as compared to either constituent mAb and leads to efficient internalization of EGFR. A comparison to cetuximab and panitumab suggests that the Sym-004 is more effective at counteracting the tumor promoting activity of EGFR in preclinical models.³² Phase II trials to examine the clinical activity of Sym-004 activity are currently underway. A second anti-EGFR oligoclonal antibody, MM-151, comprises two domain III and one domain I binding antibodies. They bind non-overlapping epitopes and each is capable of antagonizing ligand binding. Preclinical data supports a model where the combination of these three high affinity antibodies is more effective than individual mAbs at blocking ligand-dependent signaling, downregulating receptor expression and improving ADCC response (*Gerami-Moayed et al EORTC 2014, abstract#152*). Recently reported phase I data (*Lieu, et al ASCO GI 2015, abstract#647*) is consistent with MM-151 having objective clinical activity alone and in combination with irinotecan in both cetuximab-naïve and cetuximab-refractory patients.

5. Oligoclonal targeting of ErbB2

Overexpression of ErbB2, as seen in the settings of ErbB2+ breast and gastric cancers, leads to non-canonical, ligand-independent signaling through the ErbB2/ErbB3 heterodimer in addition to standard ligand-dependent signaling. The use of antibodies, including mixtures of non-competitive antibodies,^{33,34} to inhibit ErbB2 has long been recognized to have a therapeutic effect. This led to the development of the anti-ErbB2 mAbs trastuzumab³⁵ and pertuzumab³⁶ that block the ligand-independent and -dependent signaling events, respectively. While both mAbs demonstrated preclinical efficacy against models of ErbB2+ breast cancer, only trastuzumab exhibited sufficient clinical activity to warrant FDA-approval in combination with chemotherapy. Subsequent preclinical data suggested that blockade of both ligand-dependent and independent signaling improved the preclinical efficacy of ErbB2 blockade both *in vitro* and *in vivo*³⁷. The Yarden group, using a pair of mAbs distinct from pertuzumab and trastuzumab, demonstrated that the oligoclonal mixture, as compared to its components, was more effective at blocking ErbB2-dependent signaling. Consistent with the proposed mechanism for EGFR oligoclonal antibodies, the improved activity was due in part to increased downregulation of ErbB2 from the cell surface.^{28,38}

This type of data led, in part, to a series of pivotal clinical trials (CLEOPATRA, TRYPHAENA, NeoSphere) to evaluate the efficacy of an oligoclonal mixture of trastuzumab plus pertuzumab in combination with docetaxel for front-line therapy for various stages of breast cancer. Results from the Phase III CLEOPATRA trial demonstrated that the combination of trastuzumab plus pertuzumab, as compared to trastuzumab alone improved progression free survival of metastatic patients from 12.4 to 18.5 months.²³ Subsequent follow-up demonstrated that addition of pertuzumab, versus placebo control, in this intention-to-treat Phase III trial led to a statistically significant increase ($P < 0.001$, hazard ratio 0.68; 95% CI, 0.56 – 0.84) in overall survival from 40.8 months (95% CI, 35.8 – 48.3) to 56.5 months (95% CI, 49.3 to not reached). Addition of pertuzumab did not result

in increased toxicities.³⁹ Results from the Phase II NeoSphere demonstrated that the addition of pertuzumab to standard regimen of trastuzumab plus docetaxel in the neoadjuvant setting led to improvement in pathologic complete response (pCR) in the breast and lymph nodes. The addition of pertuzumab resulted in a 45.8 % pCR (95% CI, 36.1-55.7) that was statistically significant (P=0.014) versus the 29.0% pCR obtained with trastuzumab plus docetaxel (95% CI, 20.6-38.5). As seen with the other trials the addition of pertuzumab did not dramatically alter the toxicity profile.⁴⁰ The combination of the NeoSphere, TRYPHAENA and CLEOPATRA data led to FDA-approval of this regimen as front-line therapy. Importantly, and as noted by the authors of the CLEOPATRA trial, although the mixture of anti-ErbB2 antibodies leads to significantly better clinical outcomes, the majority of deaths in the study were due to disease. This supports the need for additional therapies to improve overall survival.

6. Oligoclonal targeting of ErbB3

In the setting of ErbB2-positive breast cancer, preclinical data suggests that complete blockade of ErbB2 is insufficient to inhibit ErbB3 signaling; ErbB2 blockade leads to both transcriptional upregulation and mobilization of cytoplasmic stores of ErbB3 that promote activation of downstream signaling and circumvent ErbB2 inhibition.⁴¹⁻⁴³ Thus, ErbB3-dependent signaling is a critical target for overcoming drug resistance mechanisms and suggests that direct targeting of ErbB3 will be critical for optimizing efficacy of ErbB-targeted therapeutics. As stated above, clinical development of agents designed to disrupt signaling through ErbB3 lag behind those targeting EGFR and ErbB2. Although at least six anti-ErbB3 mAbs have entered clinical trials, currently none have progressed to the point of FDA-approval. Patritumab (U3-1287/AMG888), being evaluated in a Phase III trial in combination with erlotinib for treatment of lung cancer, is the most advanced.

The improved efficacy of oligoclonal antibodies targeting EGFR and ErbB2, as compared to the constituent mAbs, suggests that an oligoclonal approach to inhibit ErbB3 will also improve blockade of ErbB3-dependent signaling. This was observed with a mixture of two anti-ErbB3 mAbs that target domain I and domain III of the ErbB3 extracellular domain.⁴⁴ The oligoclonal combination was more effective at blocking both ligand-dependent and ligand-independent signaling through the ErbB3 receptor *in vitro*. This oligoclonal mixture of anti-ErbB3 antibodies also inhibited the growth inhibition of ErbB2-positive tumor xenografts. Subsequent data generated with a different panel of mAbs again suggests that a combination of two non-competitive mAbs is more effective at inhibiting cancer cell growth both *in vitro* and *in vivo*, albeit in this case with a modest increase in potency.⁴⁵ Interestingly, a single, highly efficacious anti-ErbB3 mAb (NG33) was identified as part of this work. Oligoclones containing this mAb exhibited only modest increases in efficacy over NG33 alone. The authors speculate that because of ErbB3's lack of kinase activity, its different mechanism of internalization, and level of expression on the cell surface compared to other members of the ErbB family might make it less subjected to the enhanced effects of oligoclonal targeting.

Perhaps more relevant to targeting of ErbB3 is the use of anti-ErbB3 oligoclones in combination with mAbs targeting either EGFR or ErbB2, depending upon the disease

setting. D'Souza et al observed that the anti-ErbB3 oligoclonal, enhanced both erlotinib and trastuzumab efficacy and was capable of preventing the compensatory upregulation of signaling through ErbB3 that is known to occur in response to trastuzumab treatment. To this end, the recombinant pAb mixture SYM-013 is being developed as a 6 mAb cocktail targeting EGFR/ErbB2/ErbB3.

7. Expert Opinion

The immune system has naturally evolved to elicit a polyclonal response, rather than the mAbs we currently use as therapeutics. Improvements in our ability to express and purify oligoclonal antibody mixtures have now opened up the possibility of the mimicking this approach for generating therapeutic agents. Data obtained to date with polyclonal antibodies targeting members of the ErbB RTK family suggest that using such an approach might improve overall response. A hypothesis that is yet to be fully explored is whether the enhanced activity of oligoclones can allow for administration of lower doses of individual mAbs. In addition to more effectively down regulating signaling through the receptors it has the potential to be more effective at eliciting ADCC against the tumor. Oligoclones against ErbB family members may also circumvent resistance that can arise due to mutations within the extracellular domains that disrupt antibody binding. Recombinant pAbs can also be evaluated for blocking signaling through more than one family member at a time, much like is being done with bispecific antibodies. In addition to incorporating the benefits associated with oligoclonal antibodies, pAb may have a benefit over bispecific antibodies due to characteristics such as their more native structure, greater stability, and possibility of being less prone to induce an immunogenic response.

Prospective testing of two FDA-approved mAbs against the same target, such as was done with pertuzumab in combination with trastuzumab, has had a defined regulatory path. It needs to be stressed that despite the clinical improvements seen by combining these two FDA-approved mAb, preclinical data generated during development of Sym-004 study suggests that not all combinations of mAbs are effective to a similar extent and that the number of component antibodies in a mixture does not necessarily translate to the efficacy of the mixture. Analogous results were seen with anti-ErbB3 mAbs. Therefore room for improvement over trastuzumab plus pertuzumab may exist and should be explored. Pioneering efforts of companies such as Symphogen and Merrimack, with the clinical development of Sym-004 and MM-151, have started to break down the regulatory hurdles initially perceived to exist for clinical testing of oligoclonal reagents. This should allow the perceived cost saving advantage of the multiple cell line production approach to be realized in this setting. However, the hurdles to be faced by testing of recombinant pAbs targeting multiple receptors, such as Sym-013, remain to be seen. In addition, how newly designed oligoclonal/polyclonal mixtures versus combinations of FDA-approved mAbs will behave in the clinic remains to be investigated in phase III studies.

In summary, the approach of developing oligoclonal and/or recombinant pAbs to treat ErbB-driven cancers is a promising strategy and is one that is potentially applicable to other cancer-relevant targets.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Badger CC, Anasetti C, Davis J, et al. Treatment of malignancy with unmodified antibody. *Pathol Immunopathol Res.* 1987; 6:419–434. [PubMed: 3333188]
2. Khazaeli MB, Conry RM, Lobuglio AF. Human Immune-Response to Monoclonal-Antibodies. *Journal of Immunotherapy.* 1994; 15:42–52. [PubMed: 8110730]
3. Lee J, Fenton BM, Koch CJ, et al. Interleukin 2 expression by tumor cells alters both the immune response and the tumor microenvironment. *Cancer Research.* 1998; 58:1478–1485. [PubMed: 9537251]
4. Hynes NE, MacDonald G. ErbB receptors and signaling pathways in cancer. *Curr Opin Cell Biol.* 2009; 21:177–184. [PubMed: 19208461]
5. Hsieh AC, Moasser MM. Targeting HER proteins in cancer therapy and the role of the non-target HER3. *Br J Cancer.* 2007; 97:453–457. [PubMed: 17667926]
6. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet.* 2010; 376:687–697. [PubMed: 20728210]
7. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med.* 2001; 344:783–792. [PubMed: 11248153]
8. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after Adjuvant Chemotherapy in HER2-Positive Breast Cancer. *N Engl J Med.* 2005; 353:1659–1672. [PubMed: 16236737]
9. Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus Adjuvant Chemotherapy for Operable HER2-Positive Breast Cancer. *N Engl J Med.* 2005; 353:1673–1684. [PubMed: 16236738]
10. Smith I, Procter M, Gelber RD, et al. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. *The Lancet.* 2007; 369:29–36.
11. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med.* 2006; 354:567–578. [PubMed: 16467544]
12. Vermorken JB, Mesia R, Rivera F, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. *The New England journal of medicine.* 2008; 359:1116–1127. [PubMed: 18784101]
13. Pirker R, Pereira JR, Szczesna A, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. *Lancet.* 2009; 373:1525–1531. [PubMed: 19410716]
14. Carter PJ. Potent antibody therapeutics by design. *Nature Reviews Immunology.* 2006; 6:343–357.
15. Carter PJ, Senter PD. Antibody-drug conjugates for cancer therapy. *Cancer J.* 2008; 14:154–169. [PubMed: 18536555]
16. Lewis Phillips GD, Li G, Dugger DL, et al. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Res.* 2008; 68:9280–9290. [PubMed: 19010901]
17. Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *The New England journal of medicine.* 2012; 367:1783–1791. [PubMed: 23020162]
18. Krop IE, Kim SB, Gonzalez-Martin A, et al. Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA): a randomised, open-label, phase 3 trial. *The Lancet Oncology.* 2014; 15:689–699. [PubMed: 24793816]
19. McDonagh CF, Huhalov A, Harms BD, et al. Antitumor activity of a novel bispecific antibody that targets the ErbB2/ErbB3 oncogenic unit and inhibits heregulin-induced activation of ErbB3. *Molecular Cancer Therapeutics.* 2012; 11:582–593. [PubMed: 22248472]
20. Huhalov A, Adams S, Paragas V, et al. *Proceedings AACR.* :3485.

21. Huang S, Li C, Armstrong EA, et al. Dual targeting of EGFR and HER3 with MEHD7945A overcomes acquired resistance to EGFR inhibitors and radiation. *Cancer Research*. 2013; 73:824–833. [PubMed: 23172311]
22. Schaefer G, Haber L, Crocker LM, et al. A two-in-one antibody against HER3 and EGFR has superior inhibitory activity compared with monospecific antibodies. *Cancer Cell*. 2011; 20:472–486. [PubMed: 22014573]
- 23**. Baselga J, Cortes J, Kim SB, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *The New England journal of medicine*. 2012; 366:109–119. Describes the first phase III clinical trial (CLEOPATRA) designed to evaluate the efficacy of trastuzumab plus pertuzumab for treatment of metastatic breast cancer. [PubMed: 22149875]
24. Wang XZ, Coljee VW, Maynard JA. Back to the future: recombinant polyclonal antibody therapeutics. *Curr Opin Chem Eng*. 2013; 2:405–415. [PubMed: 24443710]
- 25**. Nielsen LS, Baer A, Muller C, et al. Single-batch production of recombinant human polyclonal antibodies. *Mol Biotechnol*. 2010; 45:257–266. Production methods for producing oligoclonal antibodies from mixed cell cultures. [PubMed: 20306237]
- 26**. Frandsen TP, Naested H, Rasmussen SK, et al. Consistent manufacturing and quality control of a highly complex recombinant polyclonal antibody product for human therapeutic use. *Biotechnology and Bioengineering*. 2011; 108:2171–2181. Describes approaches to generate oligoclonal antibody mixtures via a mixed cell method. [PubMed: 21495017]
27. de Kruif J, Kramer A, Nijhuis R, et al. Generation of stable cell clones expressing mixtures of human antibodies. *Biotechnology and Bioengineering*. 2010; 106:741–750. [PubMed: 20564612]
28. Friedman LM, Rinon A, Schechter B, et al. Synergistic down-regulation of receptor tyrosine kinases by combinations of mAbs: implications for cancer immunotherapy. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:1915–1920. [PubMed: 15684082]
29. Ferraro DA, Gaborit N, Maron R, et al. Inhibition of triple-negative breast cancer models by combinations of antibodies to EGFR. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:1815–1820. [PubMed: 23319610]
- 30*. Spangler JB, Neil JR, Abramovitch S, et al. Combination antibody treatment down-regulates epidermal growth factor receptor by inhibiting endosomal recycling. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:13252–13257. Impact of targeting EGFR with oligoclonal mixture. [PubMed: 20616078]
- 31**. Koefoed K, Steinaa L, Soderberg JN, et al. Rational identification of an optimal antibody mixture for targeting the epidermal growth factor receptor. *mAbs*. 2011; 3:584–595. Manuscript detailing the efforts necessary to generate a clinically relevant optimized anti-EGFR oligoclonal. [PubMed: 22123060]
32. Pedersen MW, Jacobsen HJ, Koefoed K, et al. Sym004: a novel synergistic anti-epidermal growth factor receptor antibody mixture with superior anticancer efficacy. *Cancer Res*. 2010; 70:588–597. [PubMed: 20068188]
33. Drebin JA, Link VC, Greene MI. Monoclonal antibodies reactive with distinct domains of the neu oncogene-encoded p185 molecule exert synergistic anti-tumor effects in vivo. *Oncogene*. 1988; 2:273–277. [PubMed: 2451200]
34. Kasprzyk PG, Song SU, Di Fiore PP, et al. Therapy of an animal model of human gastric cancer using a combination of anti-erbB-2 monoclonal antibodies. *Cancer Research*. 1992; 52:2771–2776. [PubMed: 1349849]
35. Junttila TT, Akita RW, Parsons K, et al. Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. *Cancer Cell*. 2009; 15:429–440. [PubMed: 19411071]
36. Agus DB, Akita RW, Fox WD, et al. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. *Cancer Cell*. 2002; 2:127–137. [PubMed: 12204533]
- 37*. Nahta R, Hung MC, Esteva FJ. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. *Cancer Research*. 2004; 64:2343–2346. Preclinical efficacy of combining Trastuzumab and pertuzumab. [PubMed: 15059883]

38. Ben-Kasus T, Schechter B, Lavi S, et al. Persistent elimination of ErbB-2/HER2-overexpressing tumors using combinations of monoclonal antibodies: relevance of receptor endocytosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:3294–3299. [PubMed: 19218427]
39. Swain SM, Baselga J, Kim SB, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *The New England journal of medicine*. 2015; 372:724–734. [PubMed: 25693012]
40. Gianni L, Pienkowski T, Im YH, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *The Lancet Oncology*. 2012; 13:25–32. [PubMed: 22153890]
41. Garrett JT, Olivares MG, Rinehart C, et al. Transcriptional and posttranslational up-regulation of HER3 (ErbB3) compensates for inhibition of the HER2 tyrosine kinase. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:5021–5026. [PubMed: 21385943]
42. Garrett JT, Sutton CR, Kuba MG, et al. Dual Blockade of HER2 in HER2-Overexpressing Tumor Cells Does Not Completely Eliminate HER3 Function. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2013; 19:610–619. [PubMed: 23224399]
43. Chandarlapaty S, Sawai A, Scaltriti M, et al. AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. *Cancer Cell*. 2011; 19:58–71. [PubMed: 21215704]
- 44*. D'Souza JW, Reddy S, Goldsmith LE, et al. Combining Anti-ERBB3 Antibodies Specific for Domain I and Domain III Enhances the Anti-Tumor Activity over the Individual Monoclonal Antibodies. *PLoS One*. 2014; 9:e112376. Impact of targeting ERBB3 with an oligoclonal antibody. [PubMed: 25386657]
- 45*. Gaborit N, Abdul-Hai A, Mancini M, et al. Examination of HER3 targeting in cancer using monoclonal antibodies. *Proceedings of the National Academy of Sciences of the United States of America*. 2015; 112:839–844. Impact of targeting ERBB3 with an oligoclonal antibody. [PubMed: 25564668]
46. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *The New England journal of medicine*. 2004; 351:337–345. [PubMed: 15269313]
47. Van Cutsem E, Peeters M, Siena S, 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. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2007; 25:1658–1664. [PubMed: 17470858]
48. Scheuer W, Friess T, Burtscher H, et al. Strongly enhanced antitumor activity of trastuzumab and pertuzumab combination treatment on HER2-positive human xenograft tumor models. *Cancer Res*. 2009; 69:9330–9336. [PubMed: 19934333]

Highlights Box

- ErbB family members represent validated targets for antibody-based treatment of a variety of cancers
- Preclinical data supports the hypothesis that combinations of antibodies that bind non-overlapping epitopes on an ErbB family member will increase efficacy over the individual mAbs. This was confirmed clinically based on data from trials testing trastuzumab in combination with pertuzumab
- Combinations of antibodies can result in unique mechanisms of action
- Manufacturing methods have been developed to allow for cost effective production of oligoclonal mixtures of antibodies
- Trials are ongoing with oligoclonal mixtures against single ErbB family members.

Table 1
FDA-approved ERBB-targeted monoclonal antibodies for cancer therapy

Non-proprietary (Trade) name	Target (Domain)	Format	Indication	Reference
Cetuximab (Erbix)	EGFR(III)	IgG1(Chimeric)	CRC, ANSCLC, MSCCHN	Cunningham, et al. (2004) ⁴⁶
Panitumumab (Vectibix)	EGFR(III)	IgG2(Human)	CRC	Van Cutsem, et al. (2007) ⁴⁷
Trastuzumab (Herceptin)	HER2(IV)	IgG1(Humanized)	BC, GA	Romond, et al (2005) ⁹ Piccart-Gebhart, et al (2005) ⁸
Pertuzumab (Perjeta)	HER2(II)	IgG1(Humanized)	MBC	Baselga, et al. (2012) ²³

CRC: colorectal cancer; ANSCLC: advanced non-small cell lung cancer; MSCCHN: metastatic head and neck squamous cell carcinoma; BC: HER2+ breast cancer; GA: HER2+ gastric cancer; MBC: metastatic HER2+ breast cancer

Table 2
Oligoclonal Antibody Mixtures

Oligoclonal	Component antibodies	Target (Domain)	Production strategy	Reference
111+565	111	EGFR(III)	Post-production mixture	Spangler, et al. (2010) ³⁰
	565	EGFR(III)		
225+H11	225	EGFR(III)	Post-production mixture	Spangler, et al. (2010) ³⁰
	H11	EGFR(III)		
MM151	P2X	EGFR(I)	Not specified	Merrimack Pharmaceuticals
	P1X	EGFR(III)		
	P3X	EGFR(III)		
Sym004	992	EGFR(IIIB)	Mixed cell lines	Koefoed, et al. (2011) ³¹
	1024	EGFR(IIIC)		
Trastuzumab + Pertuzumab	Trastuzumab	HER2(IV)	Post-production mixture	Scheuer, et al. (2009) ⁴⁸
	Pertuzumab	HER2(II)		
L26+Trastuzumab	L26	HER2(unknown)	Post-production mixture	Friedman, et al. (2005) ²⁸
	Trastuzumab	HER2(IV)		
A5+F4	A5	HER3(III)	Post-production mixture	D'Souza, et al. (2014) ⁴⁴
	F4	HER3(I)		
NG33+NG140 / NG33+NG83	NG140	HER3(unknown)	Post-production mixture	Gaborit, et al. (2015) ⁴⁵
	NG33	HER3(unknown)		
	NG83	HER3(unknown)		
Sym013 (six antibody cocktail)	Unknown	EGFR(undisclosed)	Mixed cell lines	Symphogen
	Unknown	EGFR(undisclosed)		
	Unknown	HER2(undisclosed)		
	Unknown	HER2(undisclosed)		
	Unknown	HER3(undisclosed)		
	Unknown	HER3(undisclosed)		