In this issue of *Clinical Cancer Research* (CCR), Jacobsen and colleagues (1) describe a novel strategy to inhibit solid tumors by using multiple antibodies. Attempts to precisely interrupt pathogenic agents date back to 1906 and the “Magic Bullet” concept of Paul Ehrlich, which has inspired generations of physicians and drug developers. Drugs and dyes alike might selectively home at disease-causing agents, while ignoring neighboring healthy tissues. Seventy years later, César Milstein and Georges Köhler developed the hybridoma technique for the production of monoclonal antibodies (mAb). The first anticancer mAbs were approved for patient treatment around the end of the 20th century. They included trastuzumab (Herceptin), an anti-HER2 mAb, and cetuximab (Erbitux), which binds with EGFR. The turn of the millennium has witnessed an unprecedented avalanche of anticancer agents, such that mAbs are becoming one of the best exemplifications of Ehrlich’s visionary “magic bullets”. They are available in practically unlimited amounts and they might be engineered to evade antibody responses in patients. In addition, their remarkable homogeneity makes the pharmacology and clinical approval of mAbs relatively straightforward. However, unlike vaccination, which represents a form of active immunotherapy, treatment of patients with recombinant antibodies (passive immunotherapy) avoids essential features of the natural immune response, such as gradually increasing strength of antigen recognition (affinity maturation), immunoglobulin class switching from pentavalent IgM to bivalent IgG molecules, and memory conferred by antibody-producing B lymphocytes. In addition, unlike the uniform mAbs used in cancer therapy, the human immune response often generates antibodies to multiple, sometimes homologous antigens presented by the invader, and each antigen is decorated by many different antibodies, each recognizing a distinct epitope (polyclonal antiserum). As demonstrated by Jacobsen and colleagues (1), adopting the polyclonal nature of the B-cell immune response, as well as simultaneously engaging several closely-related and collabo-rating antigens, might enhance antitumor efficacy of the presently applied mAbs.

The antigens selected by Jacobsen and colleagues are three receptors for growth factors, namely EGFR, HER2 (a coreceptor), and HER3, which binds ligands called neuregulins. Because the fourth member of the human EGF receptor family (HER4, also called ERBB4) is also expressed in cardiac and in nerve cells, it was not targeted. Physiologic activation of these receptors by their ligands absolutely depends on the formation of dimers, either heterodimers or the less-active homodimers. Especially active heterodimers are EGFR-HER2, which use the MAPK pathway, and HER2–HER3 dimers, which recruit the PI3K cascade. Accordingly, anticancer therapeutic antibodies might block growth factor binding (e.g., cetuximab and panitumumab), or they can paralyze the receptor’s ability to assist other, ligand-occupied receptors (e.g., trastuzumab and pertuzumab; ref. 2). Besides their activation by growth factors, constitutively active, aberrant forms of HER proteins are frequently detected in cancer. Examples include receptors harboring activating mutations or internal deletions, and overexpressed receptors. However, due to their extensive cross-talk and functional redundancy, clinically meaningful and long-lasting blockade of HER proteins, both mutant and wild-type forms, has turned out to be an immense pharmacologic challenge (3). For example, patients with breast cancer treated with trastuzumab often develop resistance that might be due to upregulation of EGFR (4) or one of its ligands. Likewise, resistance of colorectal cancer patients to cetuximab is attributable, in many cases, to the emergence of KRAS mutations (5) or, less frequently, to HER2 amplification (6).

To circumvent both structural plasticity and the functional redundancy of the HER network, Jacobsen and colleagues undertook a dual pronged attack (see Fig. 1): First, they simultaneously targeted EGFR, HER2, and HER3, such that evasion mechanisms were blocked. Second, because pairs of mAbs engaging mutually nonoverlapping epitopes robustly eliminate the target (7, 8), and a similar HER2-targeting pair has been approved for the treatment of mammary tumors (9), the authors used pairs rather than single mAbs. This strategy translated to application of a mixture of six mAbs, which they denoted Pan-HER. As expected, each of the selected pairs synergistically inhibited metabolic activity of epithelial cells,
in vitro, and more strongly sorted their targets for degradation than did the respective single mAbs. When combined, the three mAb pairs comprising Pan-HER exhibited even stronger and wider inhibitory effects on a surprisingly wide spectrum of tumor cell lines, including some expressers of mutant forms of TP53 and KRAS. In support of an ability to overcome emergent (secondary) resistance, Pan-HER retained potency in the presence of ligands of EGFR (EGF) and HER3 (neuregulin 1). Likewise, Pan-HER prevented compensatory HER upregulation, which often accompanies responses to single receptor blockers. Similarly, Pan-HER arrested cells that acquired, in vitro, resistance to the inhibitory effects of cetuximab, trastuzumab, or pertuzumab. Ultimately, the authors tested Pan-HER on several hard-to-treat cancer models, in animals, including xenografts derived from ovary, colorectal, lung, and pancreas cancer patients (PDX models). In all models, Pan-HER out-performed cetuximab and trastuzumab, and in some models the inhibitory action was not only sustained but also persisted after withdrawal of the mixture of six antibodies.

What could possibly underlie the relatively high potency of Pan-HER in animal models? One clue is the requirement that none of the six mAbs of Pan-HER shares target epitopes with the other five mAbs. Because the study made use of athymic nude mice, it is possible that Pan-HER was able to recruit six times more natural killer lymphocytes to cancer cells, as compared with any single mAb. In support of this scenario, the effector domains (called Fc) of anti-HER2 monoclonals are required for optimal in vivo activity of antibody mixtures (10). Because immunohistochemical analysis of some xenografts revealed marked downregulation of all three receptors following treatment with Pan-HER, the authors raise the possibility that Pan-HER effectively eliminates receptors for EGF family survival factors. Yet another open issue relates to the safety of co-applying six different antibodies. It is conceivable that skin, gastrointestinal, or other adverse effects will limit clinical application of Pan-HER. Hence, it would be worthwhile testing less complex mixtures of two to four mAbs, which are expected to be less toxic and, presumably, as effective as Pan-HER. This option has been supported by pancreatic cancer studies that utilized mixtures comprising anti-HER2 and anti-EGFR antibodies (11, 12). A similar option is offered by recombinant bispecific antibodies able to simultaneously bind two HER proteins. Clearly, more studies in animal models and in patients are needed in order to unravel the true therapeutic potential of Pan-HER and similar oligoclonal approaches. Such future endeavors seem worthy, because they might open a new era in cancer immunotherapy.

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Synergistic Mixtures of Anticancer Antibodies

Figure 1. Moving away from monoclonal anticancer antibodies to synergistic polyclonal mixtures of antibodies? Many currently approved protocols of cancer therapy make use of a single mAb, in combination with a chemotherapeutic agent (not shown). However, due to adaptive biochemical mechanisms, including stimulation of alternative receptor dimers and downstream pathways, cancer cells might evade the inhibitory effect of singly applied mAbs. Jacobsen and colleagues (1) propose in this issue of CCR an alternative protocol: By applying a mixture of three pairs of mAbs, each pair directed against a distinct growth factor receptor, the strategy they propose might overcome patient resistance and also enhance antitumor effects. Note that the tradeoffs in implementing the proposed protocol would involve relatively complicated approval procedures and higher costs of manufacturing.
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