Molecular Therapeutics: Is One Promiscuous Drug against Multiple Targets Better than Combinations of Molecule-specific Drugs?  

Carlos L. Arteaga  
Departments of Medicine and Cancer Biology, Vanderbilt University School of Medicine, and Breast Cancer Program, Vanderbilt-Ingram Comprehensive Cancer Center, Nashville, Tennessee 37232  

Two manuscripts from the same group in this issue illustrate important aspects relevant to the discovery and development of molecule-targeted therapeutics. In the first paper, Ciardiello et al. (1) report the ability of the VEGFR-2 (Flk-1 and KDR) tyrosine kinase inhibitor ZD6474 to also block activation of the EGFR. ZD6474 inhibited the growth in soft agar of several EGFR-expressing human cancer cell lines that lack VEGFR-2 expression. Although there is no direct demonstration in the paper that ZD6474 inhibited the tumor cell EGFR tyrosine kinase in situ nor how the IC50 against this receptor compared with that against VEGFR-2, these data clearly imply that ZD6474 is not specific for the VEGFR-2 kinase. Second, as stated by the authors, ZD6474 can target not only tumors that use VEGF signaling for the promotion of neoangiogenesis but also EGFR-dependent tumors, thus potentially expanding possible future indications of ZD6474. 

The ability of ATP competitive small molecule tyrosine kinase inhibitors to block more than one catalytic pocket is not unique to ZD6474, e.g., the small molecule SU6668 is a potent inhibitor of VEGFR-2, PDGFR, and fibroblast growth factor receptor (2), three transmembrane tyrosine kinases involved in different phases of tumor angiogenesis (3). New vessel formation in the adult is mainly observed in neoplastic processes. Therefore, multitargeted drugs like SU6668 that block multiple steps in neoangiogenesis may potentially exhibit better antivascular activity while still remaining cancer selective and relatively nontoxic to host tissues. Another example is the Ab1 tyrosine kinase inhibitor STI-571 (imatinib mesylate and Gleevec), currently approved for the treatment of chronic myelogenous leukemia (4). STI-571 also inhibits the c-kit (stem cell factor receptor) and PDGFR tyrosine kinases (5). It has already shown remarkable clinical activity against gastrointestinal stromal tumors, where activating mutations of c-kit are a pathogenetic event (6). Kit is also expressed in acute myeloid leukemia, small cell lung carcinoma, germ cell tumors, melanoma, myeloma, and neuroblastomas (7). Pending clinical trials in these disorders, these molecules ‘promiscuity’ of STI-571 may serve to expand its repertoire of disease targets. Furthermore, its activity against the PDGFR tyrosine kinase may suggest an additional antiangiogenic effect. One final example is the development of bifunctional inhibitors of EGFR and its homologous tyrosine kinase HER2 (erbB2; reviewed in Ref. 8). In this case, the development of small molecules that directly recognize each ATP-binding pocket of both EGFR and HER2 is not surprising, considering the high homology in the secondary structure of the kinase domain of these receptors. 

On the basis of these data, it would appear that the lack of tight molecular specificity of tyrosine kinase inhibitors may serve a good therapeutic purpose and that the bifunctionality of ZD6474 is a good feature of this small molecule. In fairness, however, it is likely that the ability of ZD6474 to inhibit the EGFR tyrosine kinase was a fortuitous finding but not the search result of a bifunctional VEGFR-2/EGFR inhibitor in which the tri-dimensional structure of the kinase domain of both receptors was used as a template for drug design. Therefore, as more ATP competitive inhibitors are developed, it is possible that some of these may hit other kinases whose inactivation may result in undue host toxicity. This possibility cannot be minimized and should be carefully evaluated in Phase I trials of novel tyrosine kinase inhibitors that will also validate their molecular targets in situ. 

The second paper by Tortora et al. (9) reports the synergistic antitumor activity of a combination of three antisignaling molecules: (a) the EGFR tyrosine kinase inhibitor ZD1839 (‘Iressa’; Ref. 10); (b) the COX-2 inhibitor SC-236; and (c) a DNA/RNA mixed backbone antisense oligonucleotide targeted against the Rho regulatory subunit of PKA (AS-PKA1; Ref. 11). Activation of the EGFR induces PKA function (12), as well as COX-2, by a mechanism regulated by cAMP and PKA (13), thus providing a biochemical rationale for the therapeutic combination used in this report. A cooperative effect of the COX-2 inhibitor with either ZD1839 or AS-PKA1, as well as all three agents together, was observed against breast and colon cancer cells. This synergistic effect was observed against cells in culture, implying that it was tumor cell autonomous. The combination also exhibited an additive antitumor effect against GEO colon cancer xenografts established in nude mice. The expression of COX-2 and VEGF and density of tumor microvessels were all synergistically reduced by the combination, indicating a suppression of tumor angiogenesis. The combination did not induce apparent toxicity, and a large proportion of mice remained tumor free several weeks after discontinuation of treatment, supporting a sustained cytotoxic antitumor effect. This result confirms other studies that support the synergistic effect of COX-2 and PKA, and also suggests that combinations of antisignaling drugs targeted against tumor cell endogenous mechanisms, as well as the tumor microenvironment, are better than single agents. 

Although one could speculate on elegant biochemical/molecular explanations for the observed supra-additive effect of the combination, the better efficacy of it compared to single drugs may mainly reflect the inability of short half-life, molecule-specific drugs to inhibit their target persistently and completely. Another possibility is that in late cancers, as those represented by the cell lines and xenografts used by Tortora et al., the redundancy of aberrant signaling pathways and additional genetic alterations counteract the effect of a single molecule-specific inhibitor, e.g., SC-236 may not completely block COX-2 in a tumor cell with simultaneous amplification of EGFR and/or PKA signaling. It is also possible that by using antisignaling combinations, acquired...
mechanisms of drug resistance can be abrogated. e.g., it has been shown that EGFR and HER2 inhibitors alone reduce immunohistochemical levels of VEGF and microvessel density in drug-sensitive xenografts (16–19). Interestingly, A431 tumor cells with acquired resistance to the EGFR antibody C225 exhibit increased expression and secretion of VEGF (20). Forced expression of VEGF in C225-sensitive A431 cells renders them resistant to EGFR antibodies in vivo (20). These data imply that: (a) subversion of EGFR-dependent tumor neoangiogenesis is required for the antitumor effect of EGFR inhibitors, such as ZD1839; and (b) enhanced angiogenesis can endow tumors with resistance to EGFR blockade. These results also provide a strong rationale for combinations of anti-EGFR agents with inhibitors of angiogenesis.

One aspect not highlighted by Tortora et al. is the possible additive toxicity of the combination used in this study. It is not clear if the mouse or another animal model would be the most appropriate to test this possibility preclinically. It is not inconceivable though that rational and not-so-rational combinations of molecular therapies may eventually lead to severe side effects. Therefore, there is an increasing need for the development of animal models that would predict the toxicity of these combinations. Another suggestion for future experiments of the type shown in the second paper is the inclusion of a chemotherapy control arm. If combinations like the one used above are as effective as chemotherapy against tumors but better tolerated, they should become robust therapeutic alternatives to current chemotherapy standards.

So, is a promiscuous drug, like ZD6474, that targets multiple molecules conceptually a better therapeutic approach than one that uses combinations of (presumed) molecule-specific drugs? Unfortunately, this question was not directly addressed by the studies discussed above. Nonetheless, the data presented in these two manuscripts illustrate highly relevant aspects to the discovery and development of novel agents that are destined to become the mainstream of the anticancer therapeutic and prevention portfolio over the next several years.

Note added in proof:


References


Molecular Therapeutics: Is One Promiscuous Drug against Multiple Targets Better than Combinations of Molecule-specific Drugs?

Carlos L. Arteaga