Targeted Agents: The Rules of Combination

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Abstract The success of molecularly targeted agents (MTA) in the treatment of cancer has led to the investigation of their use in combination with other MTAs and with conventional chemotherapies. An overview of the MTAs that have emerged as Food and Drug Administration – approved drugs is presented, along with a framework for the consideration of how MTAs can best be combined to maximize therapeutic effect.

During the past decade, molecularly targeted agents (MTA) have become the primary focus of therapeutic cancer research. The lure of molecular targets is derived from several factors: the strong rationale of a target that represents a selective advantage for tumor killing as compared with normal tissue toxicity, the relative ease of high-throughput screening against molecular entities, and the potential for refinement of leads through screening against molecular, cellular, and animal models. Further support for this effort has come from the demonstration that certain tumors in humans are addicted to signals from mutant or amplified receptors (1), such as the epidermal growth factor receptor (EGFR; ref. 2), and undergo rapid apoptosis upon pathway inhibition. Even when the MTA has no discernible effect on tumor size, it may drastically alter tumor metabolism, block survival signals from receptor-linked pathways such as phosphatidylinositol 3-kinase (PI3K), and lower the threshold for apoptosis (3). The value of MTAs may thus increase dramatically when they are used with cytotoxic agents.

Although there have been active investigation of MTAs as monotherapy, there have been fewer explorations of MTA combinations in human clinical trials. From a biological standpoint, solid tumors often contain mutations in multiple genes (4), but the significance of these "accessory" mutations for survival and proliferation remains unproven. In addition, because the widespread development and use of MTAs has been a relatively recent event, hormonal therapy notwithstanding, there are few MTAs whose properties are understood well enough as single agents to have proceeded on to development as combination therapy. In this report, we will explore additional challenges for MTA combinations in human tumor therapy. We will first summarize the clinical successes of single-agent MTAs, and then examine the rationale, strategies, and experience to date for MTA combinations. From this discussion, we will address the "rules" that seem logical for further development of MTA combinations.

Single-Agent Experience with Molecularly Targeted Drugs

A growing number of MTAs have gained Food and Drug Administration approval and have become standard of care for specific tumors (Table 1). These agents have ranged from antibodies with a high degree of selectivity for their targets to small-molecule kinase inhibitors with broader spectrums of target-inhibitory activity. The targets of these agents may reside in the tumor cell itself, or may exist in the tumor's microenvironment. Although some of these drugs are used in combination with cytotoxic drugs, they have each shown activity as single agents.

As a generalization, none of the drugs, with the possible exception of imatinib, are curative as single agents. Furthermore as single agents, the small molecules, such as sunitinib, the EGFR inhibitors, and imatinib, have greater activity than monoclonal antibodies in the more common solid tumors. Many such agents are available or in development, with varying degrees of specificity for their targets. The most striking responses have been seen in cancers with "oncogene addiction." That is, the growth and survival of the cancer is completely dependent on the signal that is inhibited by the targeted agent (1). However, even in the most successful example of an oncogene-addicted tumor, chronic myelogenous leukemia (CML), whereas the majority of patients experience a long-lasting hematologic and cytogenetic remission, eradication of BCR-ABL – positive cells occurs in <20% of patients, and there is a constant overall relapse rate of 1% to 2% per year (5).

Single addicting mutations are rare in solid tumors. Non – small cell lung cancer with activating mutations in exons 19 or 21 of the EGFR (6), exon 11 mutations in C-KIT – positive gastrointestinal stromal tumor (GIST; ref. 7), and C-MET-mutant hereditary papillary renal cell carcinoma (RCC; ref. 8) are the rare exceptions. Although therapy with erlotinib or imatinib can produce previously unimagined responses in EGFR-mutant non – small cell lung cancers or GIST, respectively, monotherapy with these targeted agents produces few complete remissions, and these are usually of 3 to 6 months in duration. Similarly, in the case of clear cell RCC, in which von Hippel-Lindau mutations are responsible for the up-regulation

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of proangiogenic signals such as vascular endothelial growth factor and platelet-derived growth factor, inhibition of angiogenesis with agents such as sunitinib is only partially effective (9).

Therefore, it is likely that the broadest potential of targeted therapies in solid tumors will not be as single agents in the rare addicted cancers, but in combinations that allow rational inhibition of the multiple pathways that contribute to tumorigenesis. Up to this point, the advanced development of MTAs has largely been devoted to their combination with well-established cytotoxic drug combinations in advanced disease, such as bevacizumab with fluorouracil/leucovorin/oxaliplatin (FOLFOX) in metastatic colon cancer. The next step will be the exploitation of rational combinations of MTAs in advanced disease. Furthermore, the principle of MTA effectiveness in combination with cytotoxics has been established in the adjuvant setting, as shown by trastuzumab with taxol-based combinations in breast cancer. Meanwhile, the role of MTA combinations in the adjuvant setting, in which there may be a more realistic opportunity for cure, will likely become an active area of investigation.

Rules for Trials of Combinations of Targeted Agents

The dearth of clinical experience with combinations of MTAs makes it impossible to offer any definitive rules for combination therapy. A variety of rationales for constructing combinations can be proposed, and these can be assigned a priority based on biological evidence and preclinical experiments in model systems. It must be remembered, however, that no preclinical system, whether it be human tumor cells in culture or human xenografts in mice, or genetically engineered tumors in mice, has a track record for predicting success in humans (10), and that model tumors simply represent models of their own specific biology, no more and no less. With that caveat, the following rules are proposed.

Based on the single-agent experience described above, the best chances of success will be realized when both agents in a combination inhibit separate pathways known to be critical to the survival of the tumor. Combinations might be based on knowledge that activation of an alternative pathway confers resistance to hormonal therapy or signal inhibition. Cell and animal tumor experiments suggest that activation of alternative pathways may circumvent the inhibition of a primary signaling receptor such as EGFR or HER2/neu (11, 12). For example, resistance to estrogen-targeted therapy in breast cancer can be mediated by activation of the PI3K pathway in human breast cancer cells (13). Trastuzumab- or tamoxifen-resistant cells in culture regain sensitivity in the presence of inhibitors of the PI3K pathway (14), such as the direct PI3K inhibitor LY 294002 or mTOR inhibitors of the rapamycin class.

An example of collateral pathway activation as a mechanism of resistance has recently come to light in the studies of EGFR inhibitors. Engelman and colleagues found that amplification of C-MET circumvents the antitumor effects of EGFR inhibition in otherwise sensitive lung cancer cells carrying an activating mutation in EGFR (11). They found examples of C-MET amplification in gefitinib-resistant human tumors, and showed that sensitivity to gefitinib could be restored by exposing cells concurrently to a C-MET inhibitor and gefitinib, thus providing an example of concurrent dependence on two distinct targets. Although this activating C-MET amplification was found in only 4 of 18 resistant tumor samples, it may herald the discovery of a generalizable finding in other solid tumors. The combination of EGFR and C-MET inhibitors could represent a logical approach to overcoming resistance in carefully selected patients.

Another example in which inhibition of separate pathways resulted in enhanced antitumor activity was shown in glioblastoma cell lines in which the PI3K pathway and Ras/mitogen-activated protein kinase pathways were targeted in combination. PI3K-regulated integrin-linked kinase was inhibited using integrin-linked kinase antisense oligonucleotides or small interfering RNA, whereas Ras/mitogen-activated protein kinase signaling was targeted with small molecule inhibitors of either Raf or MEK. Inhibition of both pathways resulted in synergistic decreases in colony formation and increases in apoptosis (15). Thus, the clinical use of agents targeting the PI3K pathway used in conjunction with Ras pathway inhibition could lead to improved outcomes in glioblastoma or other diseases whose growth and survival are dependent on multiple pathways.

Mutations conferring drug resistance may produce new versions of the same target resulting in reduced sensitivity to a given MTA's inhibitory effect: combination therapy may inhibit multiple alternate forms of the target. Perhaps the most straightforward case will be a combination of agents based on an understanding of resistance to a single agent. Thus, a clear and strong case can be made for combining imatinib and dasatinib, either given together or in sequence, as the primary treatment of CML. The mutations in BCR-ABL that confer resistance to imatinib have been carefully defined by molecular studies of patients failing imatinib treatment. All but two of these mutations are susceptible to dasatinib (16). Interestingly, according to preliminary results, the T315I mutation which confers resistance to both imatinib and dasatinib is sensitive to another class of drugs, the Merck aurora kinase inhibitor (17). Should all three drugs be given together, or is dasatinib or the aurora kinase inhibitor alone sufficient? At this point, it is unclear that either of the newer drugs has equal activity to imatinib in nonmutated BCR-ABL–driven disease. If one were to extrapolate the success of multiple reverse transcriptase inhibitors in AIDS, the combination strategy would be worth evaluating in CML. The alternative of using imatinib first and waiting for resistance to develop before using the other drugs is less likely to be curative, as one would be facing a resistant tumor with a single agent, or two agents, rather than three. The overlapping toxicities of the three drugs, however, might present a problem for their concurrent use. Thus, sequential use of two or three drugs against CML might be a more feasible strategy. Similar preliminary evidence of C-KIT kinase mutations causing resistance to imatinib therapy in GIST might lead to combination therapies, although the case is less certain in this tumor because sunitinib, which is active in imatinib-resistant GIST (18), may be acting through a second, unrelated mechanism (angiogenesis).

Despite the appeal of a combination therapy approach in preventing the emergence of resistance mutations in CML, it is unclear whether the above strategy would yield more cures. There is clinical and *in vitro* evidence that the leukemic stem cell population is relatively resistant to imatinib and dasatinib,

Table 1. Food and Drug Administration–approved molecularly targeted drugs

Name of compound	Target(s)	Class of molecule	Approved indications (References)	Date of first approval
Imatinib (Gleevec, Novartis)	ABL BCR-ABL PDGFR C-KIT	Small-molecule inhibitor	CML: chronic phase, newly diagnosed; chronic phase after IFN-α failure; blast crisis, accelerated phase (34) Ph+ALL: relapsed or refractory MDS/MPD ASM HES/CEL DP GIST: C-KIT+, unresectable or metastatic (35)	May 2001
Dasatinib (Sprycel, Bristol-Myers Squibb)	BCR-ABL SRC family (SRC, LCK, YES, FYN) C-KIT EPHA2 PDFGRβ	Small-molecule inhibitor	CML: chronic, accelerated, blast phase, with intolerance or resistance to imatinib (16) Ph+ALL: refractory to imatinib (36)	June 2006
Sunitinib (Sutent, Pfizer)	VEGFR1, VEGFR2, and VEGFR3 PDGFRα and β C-KIT FLT3 Colony-stimulating factor receptor type 1 (CSF-1R) Glial cell line–derived neurotrophic factor receptor (RET)	Small-molecule inhibitor	GIST: refractory or intolerant to imatinib (18) RCC: first-line metastatic (9)	January 2006
Sorafenib (Nexavar, Onyx/Bayer)	VEGFR-2,VEGFR-3 PDGFR-β FLT-3 RET C-Raf, B-raf	Small-molecule inhibitor	RCC: second line (37) HCC: advanced disease (38)	December 2005
Bevacizumab (Avastin, Genentech)	VEGF	Antibody	CRC: metastatic, first-or second-line with 5-fluorouracil-based chemotherapy (39) NSCLC (nonsquamous): unresectable or metastatic with carboplatin and paclitaxel (40)	February 2004
Rituximab (Rituxan, Genentech)	CD20	Antibody	NHL, B cell: CD20 + relapsed or refractory low-grade or follicular; diffuse with anthracycline-based chemotherapy regimen (41)	November 1997
Bortezomib (Velcade, Millenium Pharmaceuticals)	Proteasome inhibitor	Small-molecule inhibitor	Multiple myeloma: relapsed disease after 2 prior treatments, with resistance to last prior treatment (42)	May 2003
Trastuzumab (Herceptin, Genentech)	HER2	Antibody	BrCA: HER2-overexpressing metastatic, prior chemotherapy; adjuvant in HER2-overexpressing node-positive in combination with a regimen containing doxorubicin, cyclophosphamide, paclitaxel; metastatic in combination with paclitaxel (43)	October 1998
Cetuximab (Erbitux, Bristol-Meyers Squibb)	EGFR	Antibody	CRC: metastatic, single agent or in combination with irinotecan (44) SCC head and neck: locally advanced disease in combination with radiation therapy; recurrent or metastatic disease after platinum failure (45)	February 2004
Panitumumab (Vectibix, Amgen)	EGFR	Antibody	CRC: metastatic, chemotherapy refractory (46)	September 2006

(*Continued on the following page*)

Table 1. Food and Drug Administration–approved molecularly targeted drugs (Cont'd)

Name of compound	Target(s)	Class of molecule	Approved indications (References)	Date of first approval
Gefitinib (Iressa, Astra-Zeneca)	EGFR	Small-molecule inhibitor	NSCLC: advanced, chemotherapy-refractory (47)	May 2003
Erlotinib (Tarceva, Genentech)	EGFR	Small-molecule inhibitor	NSCLC: locally advanced or metastatic chemotherapy-refractory Pancreatic cancer: unresectable or metastatic first-line (48)	November 2004
Lapatinib (Tykerb, GlaxoSmithKline)	EGFR HER2	Small-molecule inhibitor	BrCA: advanced or metastatic HER2 overexpressing breast cancer after chemotherapy and trastuzumab failure (26)	March 2007
Temsirolimus (Torisel, Wyeth)	mTOR	Rapamycin analogue	RCC: advanced disease (49)	May 2007

Abbreviations: ASM, aggressive systemic mastocytosis; HES/CEL, hypereosinophilic syndrome/chronic eosinophilic leukemia; DP, dermatofibrosarcoma protuberans; MDS/MPD, myelodysplastic syndrome/myeloproliferative disease; Ph+ALL, Philadelphia chromosome-positive acute lymphocytic leukemia; NHL, non–Hodgkin's lymphoma; CRC, colorectal cancer; NSCLC, non–small cell lung cancer; BrCA, breast cancer; SCC, squamous cell carcinoma; HCC, hepatocellular carcinoma. PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

and therefore, even concurrent use of multiple agents targeting the same molecule may not be adequate to counteract the mechanisms responsible for the intrinsic resistance of the stem cell population (19, 20).

Combinations of inhibitors might be designed that attack sequential steps in a single pathway. This concept has a rational basis in experiments with antimetabolite chemotherapy, in which sequential inhibitors of purine and pyrimidine biosynthesis were synergistic in selected animal tumors (21). There is no rigorous proof of this concept in human trials, but the availability of multiple inhibitors of a single pathway raises the possibility of exploring this strategy in the clinic. Indeed, the concept of sequential inhibition underlies many recent studies of the PI3K pathway, which mediates survival and antiapoptotic signals generated from activation of cell surface receptors such as HER2, EGFR, C-MET, or the insulin-like growth factor receptor (12, 22, 23). Blocking both the receptor and the downstream PI3K pathway may produce supra-additive effects. Even downstream within the PI3K pathway, there is experimental evidence that would support the simultaneous use of inhibitors at multiple points. For instance, treatment of glioma cells *in vitro* with rapamycin alone resulted in AKT activation, likely through the disruption of negative feedback signals. Simultaneous inhibition of both PI3K and mTOR was able to prevent the AKT activation and overcome resistance to mTOR inhibition alone (24).

Another interesting possibility for "sequential" therapy is presented by lapatinib and trastuzumab. In preclinical experiments with breast cancer cells, these drugs together were more potent in decreasing cell survival than either drug alone (25). Lapatinib recently received Food and Drug Administration approval on the basis of results of a phase III trial of capecitabine alone versus capecitabine plus lapatinib in patients with HER2-positive metastatic breast cancer that had progressed on previous trastuzumab therapy. Of the approximately 400 patients on the study, the half who received lapatinib and capecitabine experienced a significantly longer time-to-progression and higher response rate than those who received capecitabine alone (27.1 versus 18.6 weeks; 23.7% versus 13.9%, respectively; ref. 26). Why lapatinib is active in patients resistant to trastuzumab is currently unclear. It may be that trastuzumab-resistant cells signal through alternative HER1 or HER3 receptor activation, independent of HER2, but these positive clinical findings suggest that the optimal use of other receptor-directed antibodies may require the blockade of ancillary mechanisms.

There are negative aspects to the blockade of sequential steps in a pathway. Sequential combinations are unlikely to overcome resistance related to the activation of alternative pathways, and could have additive toxicities, as was observed for bevacizumab and sorafenib, both inhibitors of the vascular endothelial growth factor pathway (27).

The combination of a targeted agent and a second drug that modulates the target function has strong appeal. A pertinent example is the combined use of trastuzumab or imatinib and 17-AAG, a HSP90 inhibitor. HSP90 serves a chaperone function, protecting key intracellular proteins against degradation and inducing the folding of mutant proteins. Preclinical experiments have shown that 17-AAG blocks HSP90 interaction with its client proteins and induces the rapid turnover of the clients, such as HER2 (28) and other oncogenic tyrosine kinases (29), including BCR-ABL. Clinical trials are ongoing to test the ability of 17-AAG and related compounds to enhance response and overcome resistance to trastuzumab in breast cancer, and imatinib in CML.

The combination of agents that target different functional pathways (e.g., survival and angiogenesis) might have additive or synergistic activity. The biotechnology industry has now discovered inhibitors for a variety of other functions that contribute to the survival of most tumor cells. These targets include proteins involved in angiogenesis and antiapoptotic proteins of the BH3 family, including BCL-2 and survivin (30, 31). Antiapoptotic drugs and antiangiogenic agents are of general interest as modulators of the response to both cytotoxic and targeted agents. The rationale for adding an antiangiogenic agent can be generalized to virtually any tumor and any targeted drug. One notable trial in RCC combined bevacizumab and erlotinib, an EGFR inhibitor

with minimal activity in this disease. The preliminary trial of the combination showed an improvement in response rate over bevacizumab alone (32), but final results of the randomized trial are still pending.

The ongoing development of multitargeted small molecule inhibitors allows one molecule to provide inhibition of several pathways, for instance, proapoptotic/antiproliferative signals along with antiangiogenic activity. This prompts the question of the relative benefits of treatment with multiple drugs hitting single pathways (e.g., erlotinib and bevacizumab) versus single drugs hitting multiple pathways (e.g., ZD6474, a combined EGFR and vascular endothelial growth factor receptor inhibitor; ref. 33). Although there is no data comparing the two approaches head-to-head, one can imagine that the potential for advantages in single multitargeted agents will lie in the potency and specificity of a particular agent for the proteins that it inhibits. If the MTA is too "dirty" in its inhibitory spectrum, increased side effects may result, and this may prevent treatment with therapeutic doses of drug, thus compromising target inhibition. Broad-spectrum activity may also limit the ability to further combine a given multitargeted agent with additional chemotherapeutic or targeted agents. Nonetheless, the clinical care of patients would be simplified if therapy could be administered as a single drug effectively inhibiting multiple relevant targets with minimal additional toxicities.

Challenges to Combination Therapy with MTAs

Clearly, the potential for MTA combinations designed to modulate different aspects of the same or different targets brings tremendous promise for the improved therapy of cancer. There are, however, a number of challenges that need to be overcome for this to become a reality. For instance, the targeted agents attractive for combination therapy are frequently produced by different sponsors. There often is little financial incentive for one pharmaceutical sponsor to support clinical trials combining its agent with an agent(s) from another sponsor, particularly in early phase trials. Even if a compelling scientific rationale for the combination prevails, prolonged negotiations are required to reach an agreement between the different sponsors and academic partners. Measures proposed to facilitate these types of collaborations have included assigning a central role to the National Cancer Institute/Cancer Therapy Evaluation Program in fostering combination trials of targeted agents from different sponsors. Another solution is the inclusion of such potential combination clinical trials in master agreements between sponsors and academic centers. Although not insurmountable, continued efforts encouraging cooperation between pharmaceutical companies to facilitate these studies are needed.

Another challenge to combination therapy with MTAs is the heightened potential for pharmacokinetic interactions between small molecule kinase inhibitors. This is due to the high percentage of these agents that are substrates for the p450 system, especially CYP3A4, a major player in the hepatic metabolism of MTAs. The potential for adverse pharmacokinetic interactions is further compounded by the large number of other drugs that are either inhibitors or inducers of these enzymes. Given the relative newness of MTAs, there have been few examples of careful clinical evaluation of pharmacokinetic interactions and how these interactions affect the administration of combination therapy to patients.

Combinations of MTAs also raise issues about the optimal design of clinical trials so as to obtain the most information about the relative roles of each agent separately as well as in combination. Some of the guideposts in combining chemotherapeutic agents (e.g., avoidance of overlapping toxicities or response rate end points as indicators of activity) might not be available when combining targeted agents that are cytostatic rather than cytocidal. For instance, the criteria for MTA activity may be stable disease rather than disease shrinkage; however, this will be complicated by variable growth rates of tumors and may require that patients are followed longer on study, increasing the overall duration and cost of clinical trials.

Given the difficulty in evaluating clinical activity, pharmacodynamic studies to assess the effect of an agent on its target are essential components of MTA trials. Although such studies increase in complexity when attempting to evaluate two or more targets, pharmacodynamic end points in combination trials become particularly important in assessing the minimal effective doses needed for target modulation. This information is especially useful if the agents under study are well-tolerated because there may not be a maximum tolerated dose in the traditional sense to guide "optimal" dosing. Unfortunately, the acquisition of pharmacodynamic data is not only associated with increased complexity, but increased costs and potentially increased risks to the patient. For instance, repeat biopsies of tumor tissue raise both ethical and patient acceptance issues that must be considered. Even if pretreatment and posttreatment biopsies of tumors could be obtained, the optimal use of limited tissue to address the effect of MTAs on multiple targets must be determined. Furthermore, routine sample processing and preservation protocols may be inadequate for pharmacodynamic studies on pretreatment and posttreatment tumor specimens. For example, many small molecule inhibitors lead to changes in protein phosphorylation, thus, phosphorylation status may be an important pharmacodynamic assay. However, because the phosphorylation of proteins can change very rapidly upon removal from the patient, biopsy tissue may need immediate and tailored processing.

Finally, in evaluating the effect of targeting specific genes or proteins, ideally, one would stratify tumors for study on the basis of presence/activation or absence of the target rather than the histology of the tumor. This, however, would require patient recruitment and physician collaboration across different academic groups representing different cancer types, thus increasing the complexity of trial implementation.

Conclusion

This is an exciting time in cancer drug development, and the promise of combination therapy using MTAs is only beginning to emerge. The most informative clinical trials will be designed by careful consideration of cellular targets and preclinical models of their function. Nonetheless, unanticipated results are bound to surface in the clinical setting, and we should look on these as opportunities to expand our understanding of the basic biology of cancer.

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