Commentary

The future of cytotoxic therapy: selective cytotoxicity based on biology is the key

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Abstract

Although mortality from breast cancer is decreasing, 15% or more of all patients ultimately develop incurable metastatic disease. It is hoped that new classes of target-based cytotoxic therapeutics will significantly improve the outcome for these patients. Many of these novel agents have displayed cytotoxic activity in preclinical and clinical evaluations, with little toxicity. Such preferential cytotoxicity against malignant tissues will remain tantamount to the Holy Grail in oncologic therapeutics because this portends improved patient tolerance and overall quality of life, and the capacity to deliver combination therapy. Combinations of such rationally designed target-based therapies are likely to be increasingly important in treating patients with breast carcinoma. The anticancer efficacy of these agents will, however, remain dependent on the involvement of the targets of these agents in the biology of the individual patient's disease. Results of DNA microarray analyses have raised high hopes that the analyses of RNA expression levels can successfully predict patient prognosis, and indicate that the ability to rapidly 'fingerprint' the oncogenic profile of a patient's tumor is now possible. It is hoped that these studies will support the identification of the molecules driving a tumor's growth, and the selection of the appropriate combination of targeted agents in the near future.

Keywords: breast cancer, preferential cytotoxicity, target-based

Introduction

Until recently, anticancer drug development has mainly involved the screening of libraries of frequently unselected compounds against tumor cell lines *in vitro* [1]. Active agents in this screen were then assessed preclinically before their assessment in clinical trials. This nonspecific process proved expensive, lengthy and inefficient, with paclitaxel taking three decades to progress from bench to bedside [2]. The earliest exception to this drug development paradigm was the successful development of hormone therapy after the discovery by Sir George Beatson that mammary carcinomas regressed after bilateral oophorectomy [3]. This led to the use of tamoxifen, the development of aromatase inhibitors and the selective estrogen receptor (ER) modulators, which remain arguably the most successful therapeutics for the treatment of breast cancer. Another exception was the successful development of the monoclonal antibody trastuzumab (Herceptin) [4]. This followed the identification of erbB2 (HER2) amplification in a subgroup of breast cancers and the recognition that erbB2 signaling has an important role in driving the proliferation of this variant of the disease

These rationally designed and target-based agents are characterized by low toxicity, clinical efficacy and wide therapeutic indices. This is due to their ability to induce selective tumor cell cytotoxicity, inducing disease regression in cancers by targeting aberrations that contribute to the tumor's proliferative advantage, while sparing normal tissue. Preferential cytotoxicity against malignant tissues remains tantamount to the Holy Grail in oncologic therapeutics because it portends improved patient tolerance and overall quality of life. This would result in selective killing of tumor cells, affecting the equilibrium between

ER = estrogen receptor; MEK = MAP kinase/ERK kinase; mTOR = mammalian target of rapamycin; VEGF = vascular endothelial growth factor.

tumor cell proliferation and cell death and leading to disease regression, the patient's symptomatic improvement, and a survival advantage, while sparing normal tissues and inducing minimal toxicity. It also allows more 'breathing room' or capacity to develop combinations of these agents. This is critically important: effective anticancer treatment is likely to involve combinations of multiple agents because carcinogenesis is a multi-step process involving several genes and pathways [5]. The primary thesis of this commentary is that the future of cytotoxic anticancer therapy lies with the development of biology-based combinations of molecularly targeted agents that can induce selective tumor cell death.

Non-toxic target-based cytotoxics

The successful development of these selectively cytotoxic, rationally targeted, antitumor agents resulted directly from observations demonstrating the critical biological relevance of ER and erbB2 in subgroups of breast cancers. The development of similar anticancer therapeutics targeted to specific oncogenic molecules is becoming the norm, with drug discovery efforts being increasingly focused on such therapies. This has accelerated the preclinical discovery of target-based compounds with demonstrable activity against their target, and has rapidly increased the number of antitumor agents in clinical development. These include monoclonal antibodies, small molecules, and synthetic nucleic acid sequence-based approaches targeting a variety of pathways associated with cancer (see Table 1).

A large, and rapidly increasing, number of potential molecular targets have been described. Mechanisms being exploited by these agents include the modulation of cellular signaling, programmed cell death, the cell cycle, and angiogenesis. Although the specific roles of many of these potential molecular targets in driving tumor growth in breast cancers remains frequently unknown, drugs targeting an increasingly large number of putative oncoproteins are now available for clinical evaluation. The attraction of developing agents against these targets is the potential to induce selective tumor cytotoxicity, sparing normal tissues and therefore resulting in little toxicity, with their success being largely dependent on the magnitude of this specificity. Agents targeting for example the signaling proteins erbB1, Ras, Raf, MAP kinase/ERK kinase (MEK), Akt, mTOR (mammalian target of rapamycin), the nuclear factor NF-κB, apoptotic transducers such as bcl-2 and TRAIL, and angiogenic factors such as vascular endothelial growth factor (VEGF) are currently being evaluated (Fig. 1). Promising tolerability has already been demonstrated in many early clinical trials with agents targeting the erbB receptors, Ras, Raf, MEK, mTOR, bcl-2, and both VEGF and its receptor (VEGFR) signaling; many of these studies have confirmed effective clinical target blockade [6–16].

Table 1

Targeted therapies in clinical development

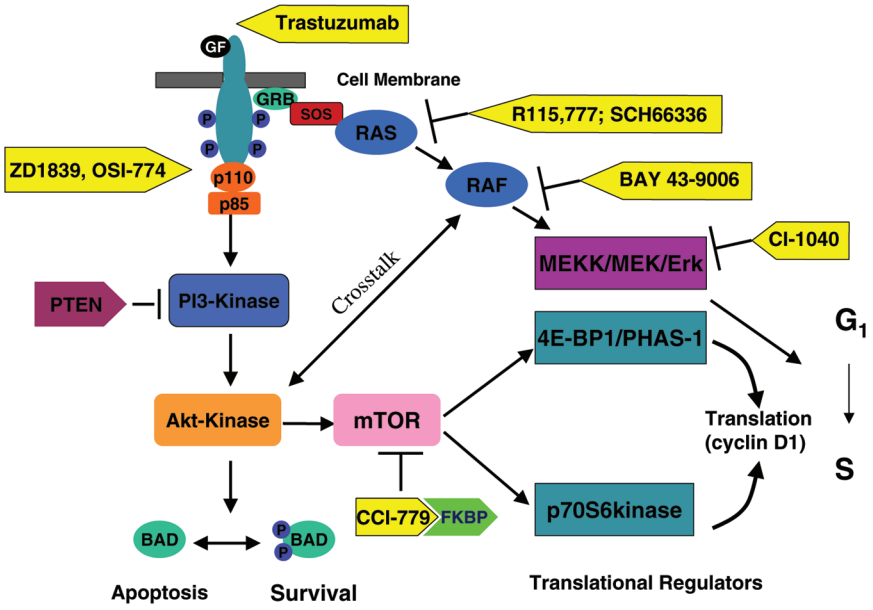
Target	Agent	Agent class
EGFR	IMC-C225 Cetuximab, Erbitux (Imclone)	Monoclonal antibody
	ABX-EGF (Abgenix)	Monoclonal antibody
	EMD 72000 (Merck KgaA Darmstadt)	Monoclonal antibody
	ZD 1839 gefitinib, Iressa (AstraZeneca)	Small molecule kinase inhibitor
	OSI-774, erlotinib, Tarceva (OSI-Pharmaceuticals)	Small molecule kinase inhibitor
	CI-1033/PD183805 (Pfizer)	Small molecule kinase inhibitor
	EKB-569 (Wyeth Ayerst)	Small molecule kinase inhibitor
	GW2016/572016 (GlaxoSmithKline)	Small molecule kinase inhibitor
HER2/neu	Trastuzumab, Herceptin (Genentech)	Monoclonal antibody
	2C4 (Genentech)	Monoclonal antibody
	17-AAG	Geldanamycin derivative inhibits HSP90
	TAK-165 (Takeda Pharmaceuticals)	Small molecule inhibitor
	GW2016/572016 (GlaxoSmithKline)	Small molecule kinase inhibitor
	CP 724, 714 (Pfizer)	Small molecule inhibitor
Ras	R115777 (Johnson and Johnson)	Farnesyl transferase inhibitor
	SCH66336 (Schering-Plough)	Farnesyl transferase inhibitor
	BMS214662 (Bristol Myers Squibb)	Farnesyl transferase inhibitor
	CT-2584HMS (Cell Therapeutics)	Farnesyl transferase inhibitor
Raf	BAY 43-9006 (Onyx/Bayer)	Small molecule kinase inhibitor
MEK	PD 184352/CI-1040 (Pfizer)	Small molecule kinase inhibitor
PKC-α	ISIS 3521/LY900003 Affinitak (ISIS Pharmaceuticals)	Antisense oligonucleotide
	CGP41251/PKC412 (Novartis)	Staurosporine analogue
	Bryostatin-1	Small molecule kinase inhibitor
	UCN-01 (Kyowa Hakko Kogyo)	Staurosporine analogue
PKC-β	LY333531 (Eli Lilly)	Small molecule kinase inhibitor
Akt	17-AAG	Inhibitor of HSP90
	Perifosine (Zenataris)	Alkylphospholipid
mTOR	CCI-779 (Wyeth)	Inhibits mTOR kinase by binding to FKBP
	RAD001 (Novartis)	Inhibits mTOR kinase by binding to FKBP

EGFR, epidermal growth factor receptor; MEK, MAP kinase/ERK kinase; mTOR, mammalian target of rapamycin; PKC, protein kinase C.

Optimal efficacy requires drug combinations

Our clinical expectations with these targeted compounds remain similar to those associated with the development

Figure 1

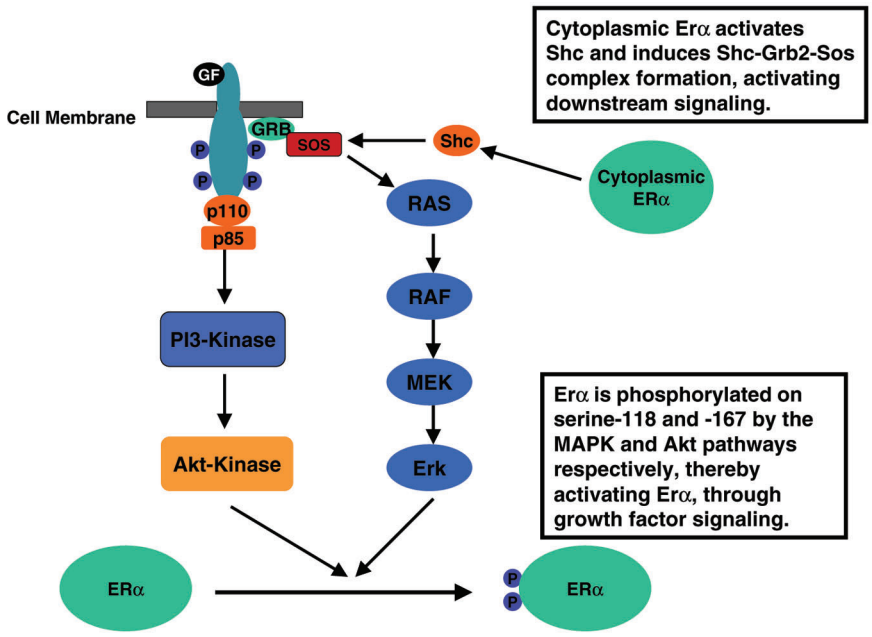


Clinical trials evaluating combinations of trastuzumab and other signal transduction inhibitors targeting erbB growth factor receptor tyrosine kinases (for example OSI-774, ZD-1839, GW572016, CI-1033) or downstream kinases (R115,777, BAY 43-9006, CI-1040) are needed to enhance the anticancer activity of trastuzumab and reverse trastuzumab resistance in HER2-positive disease. Erk, extracellular signal-regulated protein kinase; MEKK, MAP kinase/Erk kinase kinase; PI3-kinase, phosphoinositide 3-kinase.

of nonspecific therapeutics. These relevant therapeutic endpoints include increasing overall survival, regressing tumor lesions in association with clinical benefit, and/or palliating disease-related symptoms [17]. However, it is likely that the clinical benefit from these agents used individually as single agents will be of low magnitude because most cancers have multiple defects driving tumor cell growth. This has in fact already been observed in efficacy studies with many targeted agents. Low-level response rates in patients with unselected metastatic breast carcinoma have been documented with agents targeting erbB1 (ZD1839; Astra-Zeneca Pharmaceuticals), Ras (R115,777; Johnson and Johnson Pharmaceuticals), mTOR (CCI-779; Wyeth Pharmaceuticals) and VEGF (bevacizumab; Genentech) [8,13,16,18]. Nevertheless, the low incidence of severe nonspecific toxic effects of these agents enhances their overall appeal and supports a rationale for preferentially focusing on the development of these agents. However, it is likely that maximal benefit from these agents will not be attained until they are used in combinations that can, overall, reverse the malignant drive of the tumor cell.

Realistic expectations from early clinical trials with these agents, based on an understanding of cancer biology, are required to avoid the rejection of valuable agents due to perceived inefficacy in single-agent efficacy trials. To maximize the clinical benefit from these agents they will need to be administered in combination to patients with tumors with the appropriate molecular signatures. For example, preclinical data indicate that trastuzumab resistance in erbB2-positive breast carcinoma might be due in part to signaling by the insulin growth factor-I receptor [19]. Blockade of downstream receptor signaling by, for example, a farnesyltransferase inhibitor might therefore potentiate the anticancer activity of trastuzumab (see Fig. 1). Combination therapy with trastuzumab and the farnesyltransferase inhibitor R115,777 is being investigated in the clinic; initial clinical studies indicate that full doses of both agents can be concurrently administered in the clinic with minimal toxicity [20]. Growth factor signaling has also been demonstrated to have a role in the development of endocrine-resistant breast carcinoma. Preclinical studies suggest that growth factor receptor signaling can activate the estrogen receptor in the absence of estrogen ligand, thereby mediating hormone resistance (Fig. 2) [21–23]. It has therefore been postulated that growth factor signaling blockade might enhance the antitumor activity of hormone therapy, and could potentially reverse hormone resistance in patients with estrogen-receptor-positive disease. Combination studies with erbB receptor tyrosine kinase inhibitors are therefore being pursued, including Phase II studies of ZD1839 and anastrazole, and GW572016 and letrozole, in patients that have previously failed aromatase inhibition. Preclinical data also support combination clinical studies of hormonal agents and

Figure 2



Estrogen receptor-α (ERα) can be directly activated, by serine phosphorylation at Ser-118 and Ser-167, by mitogen-activated protein kinase (MAPK) and Akt respectively, through growth factor signaling. This can result in the ligandless activation of ERα and hormone resistance. Clinical trials of combinations of signaling inhibitors and hormonal agents are needed to investigate whether signaling blockade can enhance hormone resistance.

downstream signaling inhibitors such as the farnesyltransferase inhibitors or the mTOR inhibitors in treatment-naïve, estrogen-receptor-positive, disease. These preclinical studies indicate that cytoplasmic estrogen receptor activates Shc directly, generating Shc–Grb2–Sos complex formation and downstream signaling through activated Src and Ras (see Fig. 2) [24]. Clinical data from these combination studies are likely to become available in the very near future.

Optimal efficacy requires patient selection

Inherent in the development of target-based therapeutics is also the notion that this anticancer activity may be maximized by selectively treating patients whose tumors are particularly driven by the target aberration and would therefore be expected to respond most profoundly. This can be achieved by screening tumors for the relevant target, or targets, and either structural or functional determinants that can predict antitumor activity. For trastuzumab, the study of tumor cell HER2 gene amplification, based on screening by fluorescence *in situ* hybridization (FISH) or immunohistochemistry, allows the selection of the patients most likely to benefit from therapy [25]. For other rationally designed target-based therapeutics such as the erbB1 inhibitors, the farnesyltransferase inhibitors, and the mTOR kinase inhibitors, similar screening test determinants predicting anticancer activity, thereby allowing patient selection, have not yet been refined. It is envisaged, for these inhibitors of kinase signaling, that immunohistochemical studies of tumor tissue, perhaps using phosphorylation-specific antibodies for the respective signaling targets, might allow the oncologist to select the optimal rationally designed targeted agent for the individual patient. For example, studies are needed to evaluate whether screening for phosphorylated tumor-cell Akt expression could portend benefit from mTOR inhibitors such as CCI-779 [12].

Although these simple screening approaches could optimize the clinical benefits imparted from a single agent, a more comprehensive understanding of the critically important differences between breast cancer cells and normal cells may be essential to affect patient outcome substantially. In particular, understanding target function, and the impact of target blockade, in the overall molecular framework of normal and cancer cells, may be crucial in selecting the most clinically relevant molecular targets for the individual patient.

Target selection must be based on tumor biology

Overall, it is envisaged that the future successful development of these target-based cytotoxic agents will depend on a detailed understanding of breast cancer biology.

These target-based drugs might not achieve their full therapeutic potential until the oncogenic role of their targets, in the biology of a specific variant of breast cancer, is ascertained. The dissection of the biology of breast cancer remains a major challenge, particularly because of the considerable interpatient and intrapatient molecular heterogeneity in tumor cells. Nevertheless, major inroads into precise molecular profiling of this disease using DNA microarrays are being rapidly made through the study of the entire set of genes expressed in these tumor cells. These studies indicate that gene expression signatures can distinguish between good prognosis and poor prognosis patients through the analyses of a small subset of 70 predictor genes, with the majority of genes not influencing clinical outcome [26,27].

These encouraging results have identified that a small number of genes regulating the cell cycle, invasion, metastasis, and angiogenesis predict poor clinical outcome. They raise high hopes that the analyses of RNA expression levels by DNA microarray can successfully predict patient prognosis, and suggest that the ability to rapidly 'fingerprint' the oncogenic profile of a patient's tumor might soon become reality. It is probable that these studies will support the identification of the molecular aberrations contributing to the tumor's proliferative advantage, and the selection of the appropriate combination of targeted agents in the near future. This would then direct the future successful clinical application of the rapidly increasing numbers of targeted therapeutics being developed.

Conclusion

It is hoped that the development of target-based therapeutics, coupled with an increased understanding of tumor biology, will allow the delivery of tailored and highly efficacious tolerable combinations of these agents. These combinations, selected for the individual patient through the molecular profiling of the individual tumor, might then be able to maximize tumor cell kill, tumor regression, and patient benefit. The earlier evaluation of combinations of well-tolerated target-based compounds that have not demonstrated sufficient anticancer efficacy as single agents to warrant regulatory approval must be encouraged. This will help to ensure that potentially valuable agents are not discarded because of the inefficacy of a single agent, when these agents could impart clinically significant benefit when used in combination.

Competing interests

None declared.

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