The recent regulatory approval in the United States and Europe of imatinib mesylate (Gleevec®) for patients with bcr/abl translocation positive chronic myelogenous leukemia (Fig. 14.1) and the subsequent approval for gastrointestinal stromal tumors featuring an activating c-kit growth factor receptor mutation infused the oncology drug development community with enthusiasm for anticancer targeted therapy.1,2 Recent major news magazines have headlined the considerable public interest in new anticancer drugs that exploit disease-specific genetic defects as the target of their mechanism of action.3,4 Many scientists, clinicians, and pharmaceutical company executives now believe that in the next 5 to 10 years, the integration of molecular oncology and molecular diagnostics will further revolutionize oncology drug discovery and development; customize the selection, dosing, and route of administration of both previously approved traditional agents and new therapeutics in clinical trials; and truly personalize medical care for the cancer patient.5–8

Targeted Therapies for Cancer: Definitions

During the last several years, multiple definitions for the term “targeted therapy” have emerged. From the regulatory perspective, targeted therapy has been defined as a drug in whose approval label there is a specific reference to a simultaneously or previously approved diagnostic test that must be performed before the patient can be considered eligible to receive the drug. The classic example of this definition for targeted therapy is the coapprovals of the anti-breast cancer antibody trastuzumab (Herceptin®) and the eligibility tests (Herceptest®, Pathway®, and Pathvysion®) (see Chapter 16 and below). For many scientists and oncologists, targeted therapy is defined as a drug with a focused mechanism that specifically acts on a well-defined target or biologic pathway that, when inactivated, causes regression or destruction of the malignant process. Examples of this type of targeted therapy include hormonal-based therapies (see Chapter 15), inhibitors of the epidermal growth factor receptor (EGFR) pathway (see Chapter 17), blockers of invasion and metastasis enabling proteins and enzymes (see Chapter 18), antiangiogenesis agents (see Chapter 19), proapoptotic drugs (see Chapter 21), and proteasome inhibitors (see Chapter 24). In addition, most scientists and oncologists consider anticancer antibody therapeutics that seek out and kill malignant cells bearing the target antigen as another type of targeted therapy.
The Ideal Target

The ideal cancer target (Table 14.1) can be defined as a macromolecule that is crucial to the malignant phenotype and is not significantly expressed in vital organs and tissues; that has biologic relevance that can be reproducibly measured in readily obtained clinical samples; that is definably correlated with clinical outcome; and that interruption, interference, or inhibition of such a macromolecule yields a clinical response in a significant proportion of patients whose tumors express the target with minimal to absent responses in patients whose tumors do not express the target. For antibody therapeutics, additional important criteria include the use of cell surface targets that when complexed with the therapeutic naked or conjugated antibody, internalize the antigen–antibody complex by reverse pinocytosis, thus facilitating tumor cell killing.

The Original Targeted Therapy: Antiestrogens for Breast Cancer

Arguably the first type of targeted therapy in oncology was the development of antiestrogen therapies for patients with breast cancer that expressed the estrogen receptor (ER) protein (see Chapter 15) (Fig. 14.2).9 Originally developed as a competitive binding bioassay performed on fresh tumor protein extracts and used to select for hormone production ablation by surgery (oophorectomy, adrenalectomy, and hypophysectomy), the ER and progesterone receptor test format converted to an immunohistochemistry (IHC) platform when the decreased size of primary tumors enabled by mass screening programs produced insufficient material for creating enough fresh tissue to perform the assay.10 The drug tamoxifen (Nolvadex®), which has both hormonal and nonhormonal mechanisms of action, has been the most widely prescribed antiestrogen for the treatment of metastatic breast cancer and chemoprevention of the disease in high risk women.11,12 Although, ER and progesterone receptor testing is the front line for predicting tamoxifen response, additional biomarkers, including HER-2/neu (HER-2) and cathepsin D testing, have been used to further refine therapy selection.13 The introductions of specific estrogen response modulators and aromatase inhibitors such as anastrozole (Arimidex®), letrozole (Femara®), and the combination chemotherapeutic, estramustine (Emcyt®)14–18 have added new strategies for evaluating tumors for hormonal therapy.

Leukemia and Lymphoma Lead the Way

The introduction of immunophenotyping for leukemia and lymphoma was followed by the first applications of DNA based assays, the polymerase chain reaction, and RNA based molecular technologies in these diseases that complemented continuing advances in tumor cytogenetics.19,20 In addition to the imatinib (Gleevec®) targeted therapy for chronic myelogenous leukemia, other molecular targeted therapy in hematologic malignancies includes the use of all-trans-retinoic acid (ATRA) for the treatment of acute promyelocytic leukemia21; anti-CD20 antibody therapeutics targeting non-Hodgkin lymphomas, including rituximab (Rituxan®)22; and the emerging Flt-3 target for a subset of acute myelogenous leukemia patients (see below).23

Table 14.1  Features of the Ideal Anticancer Target

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crucial to the malignant phenotype</td>
<td></td>
</tr>
<tr>
<td>Not significantly expressed in vital organs and tissues</td>
<td></td>
</tr>
<tr>
<td>Biologically relevant molecular feature</td>
<td></td>
</tr>
<tr>
<td>Reproducibly measurable in readily obtained clinical samples</td>
<td></td>
</tr>
<tr>
<td>Correlated with clinical outcome</td>
<td></td>
</tr>
<tr>
<td>When interrupted, interfered with, or inhibited, the result is a clinical response in a significant proportion of patients whose tumors express the target</td>
<td></td>
</tr>
<tr>
<td>Responses in patients whose tumors do not express the target are minimal</td>
<td></td>
</tr>
</tbody>
</table>

Thirty Years Later: HER-2 and Trastuzumab (Herceptin®)

After the introduction of hormone receptor testing, some 30 years then elapsed before the next major targeted cancer chemotherapy program for a solid tumor was developed. In the mid-1980s, the discovery of the HER-2 (c-erbB2) gene and protein and subsequent association with an adverse outcome in breast cancer provided clinicians with a new biomarker that could be used to guide adjuvant chemotherapy.24 The development of trastuzumab (Herceptin®), a humanized monoclonal
antibody designed to treat advanced metastatic breast cancer that had failed first- and second-line chemotherapy, caused a rapid wide adoption of HER-2 testing of the patients' primary tumors. However, soon after its approval, widespread confusion concerning the most appropriate diagnostic test to determine HER-2 status in formalin-fixed paraffin-embedded breast cancer tissues (see Chapter 16) substantially impacted trastuzumab use. Since its launch in 1998, trastuzumab has become an important therapeutic option for patients with HER-2–positive breast cancer.

Reports that fluorescence in situ hybridization (FISH) could out-perform IHC in predicting trastuzumab response and the well-documented lower response rates of intermediate (2+) IHC staining versus intense (3+) staining tumors resulted in a variety of approaches, including IHC as a primary screen with follow-on FISH testing of either 1+ cases, 2+ cases, or both 1+ and 2+ cases or primary FISH based testing. In a recently published study where trastuzumab was used as a single agent, the response rates in 111 assessable patients with 3+ IHC staining was 35% and the response rates for 2+ cases was 0%; the response rates in patients with and without HER-2 gene amplification detected by FISH were 34% and 7%, respectively. In another study of HER-2–positive breast cancer treated with trastuzumab plus paclitaxel,
response rates ranged from 67% to 81% compared with 41% to 46% in patients with normal expression of HER-2. Interestingly, although FISH based testing is more expensive to perform and is not as widely available as IHC, a recent published review from New York and Italy suggested that FISH was actually the most cost-effective option. With trastuzumab achieving excellent results in the treatment of HER-2–positive advanced metastatic disease and under extensive evaluation in major clinical trials for its potential efficacy when used in earlier stages, the potential role(s) for HER-2 testing as a predictor(s) of responses to other therapies being resolved by large prospective clinical outcome studies and the more convenient gene-based chromogenic in situ hybridization technique “waiting in the wings,” the story of HER-2 testing in breast cancer will continue to unfold over the next several years.

### Targeted Anticancer Therapies Using Antibodies

An unprecedented number and variety of targeted small molecule and antibody based therapeutics are currently in early development and clinical trials for the treatment of cancer. Therapeutic antibodies have become a major strategy in clinical oncology because of their ability to specifically bind to primary and metastatic cancer cells with high affinity and create antitumor effects by complement-mediated cytolysis and antibody-dependent cell-mediated cytotoxicity (naked antibodies) or by the focused delivery of radiation or cellular toxins (conjugated antibodies). Currently, there are six anticancer therapeutic antibodies approved by the US Food and Drug Administration (FDA) for sale in the United States. Therapeutic monoclonal antibodies are typically of the IgG class containing two heavy and two light chains. The heavy chains form a fused “Y” structure with two light chains running in parallel to the open portion of the heavy chain. The tips of the heavy-light chain pairs form the antigen binding sites, with the primary antigen recognition regions known as the complementarity determining regions.

The early promise of mouse monoclonal antibodies for the treatment of human cancers was not realized because (1) unfocused target selection led to the identification of target antigens that were not critical for cancer cell survival and progression, (2) there was a low overall potency of naked mouse antibodies as anticancer drugs, (3) antibodies penetrated tumor cells poorly, (4) there was limited success in producing radioisotope and toxin conjugates, and (5) the development of human antimouse antibodies (HAMA) prevented the use of multiple dosing schedules.

The next advance in antibody therapeutics began in the early 1980s when recombinant DNA technology was applied to antibody design to reduce the antigenicity of murine and other rodent-derived monoclonal antibodies. Chimeric antibodies were developed where the constant domains of the human IgG molecule were combined with the murine variable regions by transgenic fusion of the immunoglobulin genes; the chimeric monoclonal antibodies were produced from engineered hybridomas and CHO cells. The use of chimeric antibodies significantly reduced the HAMA responses but did not completely eliminate them. Although several chimeric antibodies achieved regulatory approval, certain targets required humanized antibodies to achieve appropriate dosing. Partially humanized antibodies were then developed where the six complementarity determining regions of the heavy and light chains and a limited number of structural amino acids of the murine monoclonal antibody were grafted by recombinant technology to the complementarity determining region depleted human IgG scaffold. Although this process further reduced or eliminated the HAMA responses, in many cases significant further antibody design procedures were needed to reestablish the required specificity and affinity of the original murine antibody.

A second approach to reducing the immunogenicity of monoclonal antibodies has been to replace immunogenic epitopes in the murine variable domains with benign amino acid sequences, resulting in a deimmunized variable domain. The deimmunized variable domains are genetically linked to human IgG constant domains to yield a deimmunized antibody (Biovation, Aberdeen, Scotland). Additionally, primatized antibodies were subsequently developed that featured a chimeric antibody structure of human and monkey that, as a near exact copy of a human antibody, further reduced immunogenicity and enabled the capability for continuous repeat dosing and chronic therapy. Finally, fully human antibodies have now been developed using murine sources and transgenic techniques.
Using modern antibody design and deimmunization technologies, scientists and clinicians have attempted to improve the efficacy and reduce the toxicity of anticancer antibody therapeutics. The bacteriophage antibody design system has facilitated the development of high affinity antibodies by increasing antigen binding rates and reducing corresponding detachment rates. Increased antigen binding is also achieved in bivalent antibodies with multiple attachment sites, a feature known as avidity. Modern antibody design has endeavored to create small antibodies that can penetrate to cancerous sites but maintain their affinity and avidity. A variety of approaches has been used to increase antibody efficacy. Clinical trials have recently combined anticancer antibodies with conventional cytotoxic drugs, yielding promising results.

### Table 14.2 Targeted Anticancer Antibody Therapeutics

<table>
<thead>
<tr>
<th>Name</th>
<th>FDA Approval</th>
<th>FDA Source and Partners</th>
<th>Type</th>
<th>Target</th>
<th>Indication(s) (both approved and investigational)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alemtuzumab (Campath®)</td>
<td>05/01</td>
<td>BTG Schering AG</td>
<td>Monoclonal antibody, humanized</td>
<td>CD52</td>
<td>Cancer, leukemia, chronic lymphocytic Cancer, leukemia, chronic myelogenous Multiple sclerosis, chronic progressive</td>
</tr>
<tr>
<td>Daclizumab (Zenapax®)</td>
<td>03/02</td>
<td>Protein Design Labs Hoffmann-La Roche</td>
<td>Monoclonal IgG, Chimeric Immunosuppressant</td>
<td>Transplant rejection, general Transplant rejection, bone marrow Uveitis Multiple sclerosis, relapsing-remitting Multiple sclerosis, chronic progressive Cancer, leukemia, general Psoriasis Diabetes, type I Asthma Colitis, ulcerative</td>
<td></td>
</tr>
<tr>
<td>Rituximab (Rituxan®)</td>
<td>11/97</td>
<td>IDEC Hoffmann-La Roche Zenyaku Kogyo</td>
<td>Monoclonal IgG, Chimeric Anticancer, immunologic Antiarthritic, immunologic Immunosuppressant</td>
<td>CD20</td>
<td>Cancer, lymphoma, non-Hodgkin Cancer, lymphoma, B cell Arthritis, rheumatoid Cancer, leukemia, chronic lymphocytic Thrombocytopenic purpura</td>
</tr>
<tr>
<td>Trastuzumab (Herceptin®)</td>
<td>09/98</td>
<td>Genentech Hoffmann-La Roche Immunogen</td>
<td>Monoclonal IgG, Humanized Anticancer, immunologic</td>
<td>p185neu</td>
<td>Cancer, breast Cancer, lung, non–small cell Cancer, pancreatic</td>
</tr>
<tr>
<td>Gemtuzumab (Mylotarg®)</td>
<td>05/00</td>
<td>Wyeth/AHP</td>
<td>Monoclonal IgG, Humanized CD33/coleacheamycin</td>
<td>CD20</td>
<td>Cancer, leukemia, AML patients older than 60 years</td>
</tr>
<tr>
<td>Ibritumomab (Zevalin®)</td>
<td>02/02</td>
<td>IDEC</td>
<td>Monoclonal IgG, Murine Anticancer</td>
<td>CD20/131I</td>
<td>Cancer, lymphoma, low grade, follicular, transformed non-Hodgkin (relapsed or refractory)</td>
</tr>
<tr>
<td>Edrecolomab (Panorex™)</td>
<td>01/95</td>
<td>Glaxo-Smith-Kline</td>
<td>Monoclonal IgG, Murine Anticancer</td>
<td>Epithelial cell adhesion molecule (Ep-CAM)</td>
<td>Cancer, colorectal</td>
</tr>
<tr>
<td>Tositumomab (Bexxar®)</td>
<td>06/03</td>
<td>Corixa</td>
<td>Anti-CD 20 Murine Monoclonal antibody with 131I conjugation</td>
<td>CD20</td>
<td>Cancer, lymphoma, non-Hodgkin</td>
</tr>
</tbody>
</table>

AML, acute myelogenous leukemia.
Unconjugated or naked antibodies include a variety of targeting molecules both on the market and in early and late clinical development. A variety of mechanisms has been cited to explain the therapeutic benefit of these drugs, including enhanced immune effector functions and direct inactivation of the targeted pathways as seen in the antibodies directed at surface receptors such as HER-1 (EGFR) and HER-2.\textsuperscript{2–5} Surface receptor targeting can reduce intracellular signaling, resulting in decreased cell growth and increased apoptosis.\textsuperscript{56}

As seen in Table 14.2, of the seven anticancer antibodies on the market, one is conjugated with a radioisotope Y\textsuperscript{90}-ibritumomab tiuxetan (Zevalin\textsuperscript{80}) and one is conjugated to a complex natural product toxin gemtuzumab ozogamicin (Mylotarg\textsuperscript{81}). Conjugation procedures have been designed to improve antibody therapy efficacy and have used a variety of methods to complex the isotope, toxin, or cytotoxic agent to the antibody.\textsuperscript{42,43} Cytotoxic small molecule drug conjugates have been widely tested, but enthusiasm for this approach has been limited by the relatively low potency of these compounds.\textsuperscript{42} Fungal derived potent toxins have yielded greater success with the calicheamicin conjugated anti-CD33 antibody gemtuzumab ozogamicin approved for the treatment of acute myelogenous leukemia and a variety of antibodies conjugated with the fungal toxin maytansanoid (DM-1) in preclinical development and early clinical trials. The interest in radioimmunotherapy increased significantly in 2001 with the FDA approvals of the 90Y-conjugated anti-CD20 antibody Y\textsuperscript{90}-ibritumomab tiuxetan and the 131I-conjugated anti-CD20 antibody I\textsuperscript{131}-tositumomab. A variety of isotopes is under investigation in addition to 90Y as potential conjugates for anticancer antibodies.\textsuperscript{43} Radioimmunotherapy features the phenomenon of the bystander effect, in which if antigen expression is heterogeneous, extensive tumor cell killing can still take place, even on nonexpressing cells, but can also lead to significant toxicity when the neighboring cells are vital non-neoplastic tissues such as the bone marrow and liver.
By taking advantage of improved recombinant technologies generating more specific and higher affinity monoclonal antibodies with reduced immunogenicity after humanization or deimmunization and the emerging conjugation capabilities, antibody therapeutics have become a major weapon in the treatment of leukemias and lymphomas.60–63

Approved in 1997, rituximab (Rituxan®) is arguably the most commercially successful anticancer drug of any type since the introduction of taxanes. Rituximab sales exceeded $700 million in sales in the United States in 2001.44 Targeting the CD20 surface receptor common to many B cell non-Hodgkin lymphoma subtypes, rituximab is a chimeric monoclonal IgG1 antibody that induces apoptosis, antibody-dependent cell cytotoxicity, and complement-mediated cytotoxicity56 and has achieved significantly improved disease-free survival rates compared with patients receiving cytotoxic agents alone.64–67

Y90-ibritumomab tiuxetan (Zevalin®) consists of the murine version of the anti-CD20 chimeric monoclonal antibody, rituximab, which has been covalently linked to the metal chelator, MD-DTPA, permitting stable binding of 111In when used for radionucleotide tumor imaging and 90Y when used to produce enhanced targeted cytotoxicity.68–71 In early 2002, Y90-ibritumomab tiuxetan became the first radioconjugated antibody therapeutic for cancer approved by the FDA. Since its FDA approval, numerous patients who have received Y90-ibritumomab tiuxetan after becoming refractory to a rituximab-based regimen have achieved significant responses.69,70

The approval of gemtuzumab ozogamicin (Mylotarg®) by the FDA in 2000 marked the first introduction of a plant toxin conjugated antibody therapeutic.72–76 Gemtuzumab ozogamicin is targeted against CD33, a surface marker expressed by 90% of myeloid leukemic blasts but absent from stem cells, armed with calicheamicin, a potent cytotoxic antibiotic that inhibits DNA synthesis and induces apoptosis.72 The current indication for use of gemtuzumab ozogamicin is in acute myelogenous leukemia patients older than 60 years with the recommendation that before the initiation of therapy, the leukemic blast count is below 30,000/mL.73–75

Alemtuzumab (Campath®), a humanized monoclonal antibody, was approved in mid-2001 for the treatment of B-cell chronic lymphocytic leukemia in patients who have been treated with alkylating agents and who have failed fludarabine therapy.77,78 Daclizumab (Zenapax®) is a chimeric monoclonal antibody that targets the interleukin-2 receptor. This antibody is primarily used to prevent and treat patients with organ transplant rejection but has also been used in a wide variety of chronic inflammatory conditions, including psoriasis, multiple sclerosis, ulcerative colitis, asthma, type I diabetes mellitus, uveitis, and also in a variety of leukemias.79,80 I131-tositumomab (Bexxar®) is a radiolabeled anti-CD20 murine monoclonal antibody approved in 2003 for the treatment of relapsed and refractory follicular/low grade and transformed non-Hodgkin lymphoma.81,82

**Antibody Therapeutics for Solid Tumors**

Interest in the development of antibody therapeutics for solid tumors among many commercial organizations and universities has been significantly impacted by the technologic advances in antibody engineering and the approval and recent clinical and commercial success of trastuzumab, the only therapeutic antibody approved by the FDA for the treatment of solid tumors (edrecolomab is approved in Germany, but not in the United States). Trastuzumab has been described extensively above.

During the 4 years since the FDA approval of trastuzumab, there have been no additional antibodies approved for the treatment of solid tumors. Nonetheless, significant progress has been made in this field, and a number of both late stage and early stage products show substantial promise.

**Cetuximab (Erbitux®)**

The EGFR (HER-1) is the target of a variety of small molecule drugs and the late stage antibody cetuximab.83 Cetuximab, a chimeric monoclonal antibody, binds to the EGFR with high affinity, blocking growth factor binding, receptor activation, and subsequent signal transduction events and leading to cell proliferation.84 Cetuximab enhanced the antitumor effects of chemotherapy and radiotherapy in preclinical models by inhibiting cell proliferation, angiogenesis, and metastasis and by promoting apoptosis.84 Cetuximab has been evaluated both alone and in combination with radiotherapy and various cytotoxic chemotherapeutic
agents in a series of phase II/III studies that primarily treated patients with either head and neck or colorectal cancer.\textsuperscript{84,85} Breast cancer trials are also underway.\textsuperscript{86} Although the FDA approval process for cetuximab was initially slowed because of concerns over clinical trial design and outcome data management,\textsuperscript{87} the antibody was approved for use in the treatment of advanced metastatic colorectal cancer in February 2004. Similar to trastuzumab, the development of cetuximab also included an immunohistochemical test for determining EGFR overexpression to define patient eligibility to receive the antibody. Thus, cetuximab has joined trastuzumab as an FDA-approved targeted therapy featuring an unconjugated antibody. Although anti-EGFR small molecule drugs (see below) are under continuing clinical trial evaluation in breast cancer,\textsuperscript{88} cetuximab is not currently projected as a potential therapeutic for this disease.

**Bevacizumab (Avastin®)**

Bevacizumab (rhuMAb-VEGF) is a humanized murine monoclonal antibody targeting the vascular endothelial growth factor (VEGF) ligand. VEGF regulates both vascular proliferation and permeability and functions as an antiapoptotic factor for newly formed blood vessels.\textsuperscript{89,91} Patients treated with bevacizumab alone have shown significant tumor responses. Patients treated with bevacizumab in combination with conventional chemotherapy have had greater responses.\textsuperscript{89,91} In clinical trials for advanced metastatic breast cancer, the initial results of the combination treatment of bevacizumab and paclitaxel showed antitumor activity,\textsuperscript{92} but follow-on studies were not convincing that the targeting of VEGF in this clinical setting would be effective. Bevacizumab has also been combined with trastuzumab in a two antibody therapeutic strategy for HER-2 overexpressing breast cancer.\textsuperscript{93} The phase II study evaluating bevacizumab in metastatic renal cell carcinoma reached its prespecified efficacy end point earlier than expected. Finally, late-stage clinical trials using bevacizumab with 5-fluorouracil, leucovorin, and CPT-11 (Irinotecan\textsuperscript{94}) in advanced colorectal cancer are currently underway,\textsuperscript{94} with recently released results indicating a major prolongation in the time to disease progression and overall survival in patients who received the angiogenesis inhibitor versus those who did not.\textsuperscript{95} These data led to an approval by the FDA of bevacizumab for the treatment of metastatic colorectal cancer in February 2004.

Unlike cetuximab, the development of bevacizumab has not included a diagnostic eligibility test. Neither direct measurement of VEGF expression or assessment of tumor microvessel density has been incorporated into the clinical trials or linked to the response rates to the antibody. Thus, bevacizumab cannot be considered a true targeted therapy, and further focusing of this agent for colorectal, breast, lung, and other cancers will likely be inhibited by the inability to individually select patients with a diagnostic test who will be more likely to benefit from its use, either alone or in combination with other known and novel drugs.

**Edrecolomab (Panorex®)**

Edrecolomab is a murine IgG 2A monoclonal antibody that targets the human tumor-associated antigen Ep-CAM (17-1A). Edrecolomab has been approved in Europe (Germany) since 1995, but to date has not been approved by the FDA. In a study of 189 patients with resected stage III colorectal cancer, treatment with edrecolomab resulted in a 32% increase in overall survival compared with no treatment (\(P < 0.01\)).\textsuperscript{96} Edrecolomab’s antitumor effects are mediated through antibody-dependent cellular cytotoxicity, complement-mediated cytolysis, and the induction of an anti-idiotypic network.\textsuperscript{97} Edrecolomab is also currently being tested in large multicenter adjuvant phase III studies in stage II/III rectal cancer and stage II colon cancer. Edrecolomab was well tolerated when used as monotherapy and added little to chemotherapy-related side effects when used in combination. Sequential treatment of patients with metastatic breast cancer with edrecolomab after adjuvant chemotherapy reduced levels of disseminated tumor cells in the bone marrow and eliminated Ep-CAM-positive micrometastases.\textsuperscript{98}

**huJ-591 (Anti-PSMA\textsubscript{\textsuperscript{EX}})**

Prostate-specific membrane antigen (PSMA) is a membrane-bound glycoprotein restricted to normal prostatic epithelial cells, prostate cancer, and the endothelium of the neovascularature of a wide variety of nonprostatic carcinomas and other solid tumors.\textsuperscript{99–101} PSMA expression per cell progressively increases in primary prostate cancer, metastatic hormone
sensitive prostate cancer, and hormone refractory metastatic disease.\textsuperscript{96-101} PSMA expression is increased further in association with clinically advanced prostate cancer, particularly in hormone refractory disease, and is an ideal sentinel molecule for use in targeting prostatic cancer cells. Increasing expression levels of PSMA in resected primary prostate cancer is associated with increased rates of subsequent disease recurrence.\textsuperscript{100} In addition, significant PSMA expression has been identified in the tumor vasculature of a variety of nonprostate tumors, including breast cancer (Fig. 14.3).\textsuperscript{101} Humanized and fully human antibodies specific for the extracellular domain of PSMA have been developed. A phase I clinical trial of one of these antibodies, huJ-591 conjugated with \textsuperscript{90}Y, has yielded promising results.\textsuperscript{102} Programs using toxin conjugates with anti-PSMA antibodies have completed preclinical development\textsuperscript{102} and are currently in early stage clinical trials for hormone-refractory advanced metastatic prostate cancer. Finally, antibodies to PSMA have been used as diagnostic imaging agents (Fig. 14.3), including the commercially available Prostascint\textsuperscript{104}.

\textbf{Selected Targeted Anticancer Therapies Using Small Molecules}

\textbf{ATRA}

Arguably the first truly targeted therapy after the development of hormonal therapy for breast cancer was the development of ATRA for the treatment of acute promyelocytic leukemia, a subset of acute nonlymphocytic leukemia featuring a disease-defining retinoic acid receptor activating t(15:17) reciprocal translocation.\textsuperscript{105,106} For these selected patients, direct targeting of the retinoic acid receptor with ATRA has resulted in very high response rates, delay in disease progression, and long-term cures for these patients.\textsuperscript{105,106}

\textbf{Imatinib (Gleevec\textsuperscript{\textregistered})}

The development of imatinib for patients with chronic myelogenous leukemia in 2001 ushered in a new excitement both in the scientific and public communities for targeted anticancer therapy. Imatinib received fast-track approval by the FDA as an ATP-competitive selective inhibitor of \textit{bcr-abl} and has unprecedented efficacy for the treatment of early stage chronic myelogenous leukemia typically achieving durable complete hematologic and complete cytogenetic remissions, with minimal toxicity.\textsuperscript{107-109} Imatinib is a true targeted therapy for leukemia in that a test for the \textit{bcr/abl} translocation must be performed before a patient will be considered as eligible to receive the drug.

Imatinib has also achieved regulatory approval for the treatment of relapsed and metastatic gastrointestinal stromal tumors (GISTs), which characteristically feature an activating point mutation in the \textit{c-kit} receptor tyrosine kinase gene.\textsuperscript{110} For GISTs, the response to imatinib treatment appears to be predictable based on the location of the \textit{c-kit} mutation.\textsuperscript{111} The use of imatinib in GIST is also an example of targeted therapy as a measurement of \textit{c-kit} expression usually performed by IHC, required to confirm the diagnosis and render the patient eligible for treatment. Interestingly, most commercially available antibodies for \textit{c-kit} recognize the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{psma_expression.png}
\caption{PSMA expression in non-prostate cancer. (Top) Traditional bone scan demonstrating bilateral activity in the femur indirectly indicating the presence of metastatic renal cell carcinoma. (Bottom) \textsuperscript{111}huJ591 EXT diagnostic immunoscintiscan of the same patient showing direct localization of the anti-PSMA antibody conjugate to the sites of metastatic renal cell carcinoma that feature PSMA expression in the tumor neovasculature.}
\end{figure}
an internal tandem duplication that creates an abnormal FLT-3 receptor that promotes the growth and survival of the leukemic cells.\textsuperscript{113–115}

Three small molecule compounds are in clinical trials for the treatment of acute myelogenous leukemia by targeting the \textit{flt}-3 internal tandem duplication (ITD). These drugs are also examples of potential true targeted therapies in that a test for detecting an internal tandem duplication that causes the \textit{flt}-3 gene activation will likely be required and incorporated into the FDA drug approval label should these agents be successful in future clinical trials.

**Gefitinib (Iressa\textsuperscript{®})**

Gefitinib was approved by the FDA in 2003 as monotherapy for the treatment of patients with locally advanced or metastatic non–small cell lung cancer after failure of both platinum based and docetaxel chemotherapies.\textsuperscript{116,117}
Gefitinib is a small molecule drug that targets the EGFR. In contrast with the approval of trastuzumab, the approval of gefitinib did not include an eligibility requirement reference to a specific tumor diagnostic test designed to select patients that were more likely to respond to the drug. Overexpression of EGFR typically identified by IHC is extremely common in both lung and breast cancers, but in contrast with HER-2 overexpression, which is virtually limited to cases with gene amplification, multiple mechanisms of dysregulation of EGFR and associated activation of signaling pathways have been described for both of these tumors (see Chapter 17). Thus, it has been difficult to develop this drug for expanded indications or combination therapies in the absence of a well-defined efficacy test. However, more recently, two independent groups reported their similar discovery of a specific activating mutation in the tyrosine kinase domain of the EGFR receptor that was associated with a high response of patients with non-small cell lung cancer to gefitinib. The clinical significance of this important finding for the development of gefitinib in breast cancer is currently unknown. Preclinical studies of gefitinib have demonstrated activity on breast cancer cell lines, and clinical trials using the drug in combination with standard cytotoxic drugs are underway (see Chapter 17).

Erlotinib (Tarceva®)

Erlotinib is another targeted inhibitor of EGFR currently in late stage clinical trials for the treatment of non–small cell lung cancer and pancreatic cancer. Clinical studies of erlotinib in breast cancer are ongoing (see Chapter 17). Erlotinib has shown efficacy in preclinical models of brain tumors and has recently shown promising results in the treatment of high grade malignant gliomas. To date, similar to gefitinib, the clinical trials for erlotinib have not included an assessment of the EGFR status or other diagnostic test for eligibility to receive the drug.

Antiangiogenesis (SU5416, Thalidomide, Endostatin/Angiostatin, and Marimastat)

A variety of small molecule drugs is currently in clinical trials for breast cancer and other malignancies that target the establishment and growth of tumor blood vessels. Additional compounds that target matrix metalloproteases, such as the drug marimastat, are also considered to be angiogenesis inhibitors. To date, none of these compounds has linked a diagnostic test such as tumor microvessel density or the expression of an angiogenesis promoting gene or protein in their clinical development plans. Matrix metalloprotease inhibitors are discussed in Chapter 18, and antiangiogenesis therapies are discussed in detail in Chapter 19.

G3139 (Genasense®)

Another emerging strategy in anticancer therapy is the targeting of chemotherapy resistance by overcoming the antiapoptosis mechanisms of cancer cells. An example of this approach is the novel antisense oligonucleotide G3139, which targets the antiapoptotic gene bcl-2. This agent has been tested mostly in hematologic malignancies and has not been used widely in either preclinical experiments or clinical trials in breast cancer. Therapeutic strategies targeting apoptosis are covered in depth in Chapter 21.

Bortezomib (Velcade®)

Recently, drugs targeting the proteasome have been developed that are designed to impact downstream pathways regulating angiogenesis, tumor growth, adhesion, and resistance to apoptosis. One of these agents, bortezomib (PS-341), has recently been approved for the treatment of advanced refractory multiple myeloma. Bortezomib has shown preclinical activity in breast cancer as a single agent and is being tested in combination therapy strategies in multiple clinical trials. Proteasome biology and proteasome inhibitors are discussed in detail in Chapter 24.

The Future Is Now: Pharmacogenomics and Personalized Medicine

Targeted therapy in oncology has been a major stimulus for the evolving field of pharmacogenomics (see Chapter 28). In its broadest definition, pharmacogenomics can encompass both germline and somatic (disease) gene and protein measurements used to predict the likelihood that a patient will respond to a specific single or multiagent chemotherapy regimen.
and to predict the risk of toxic side effects.136,137 During the next several years, the field of oncology drug development will see numerous products pass through the approval process and enter the market accompanied by diagnostic tests designed to “personalize” their use, dosage, route of administration, and length of treatment for each patient, one at a time. Only time will tell whether this new approach to anticancer pharmaceuticals will yield breakthrough results, reducing morbidity and mortality and improving outcomes for all who will be afflicted with the disease.

References

Chapter 14  Targeted Therapy for Cancer

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