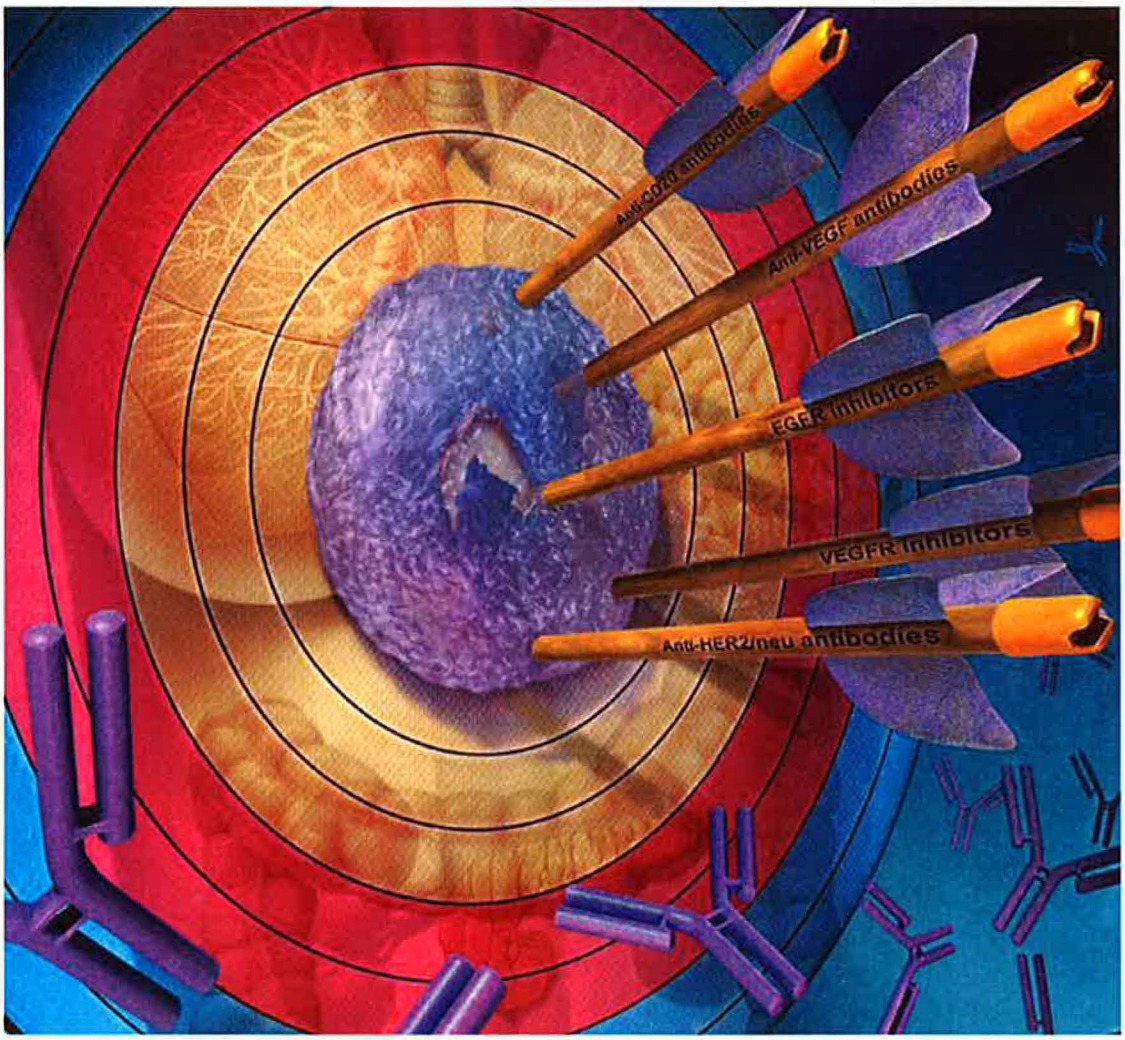
Targeted Therapy 101: A Primer for Clinicians



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- Lung cancer clinical research and drug development
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Introduction: the complexities of conventional (cytotoxic) chemotherapy

Molecularly targeted therapies have revolutionized the treatment of cancer. To understand the impact of these new classes of drugs, one must place them in clinical context and compare them to conventional cancer therapies. For decades, oncologists have employed cytotoxic chemotherapy for the definitive treatment or palliation of hematologic malignancies and solid tumors. The underlying premise of conventional, cytotoxic chemotherapy is the interruption of cell division. Because tumors grow more rapidly—and cancer cells divide more frequently—than most normal tissues, a therapeutic index is achieved.

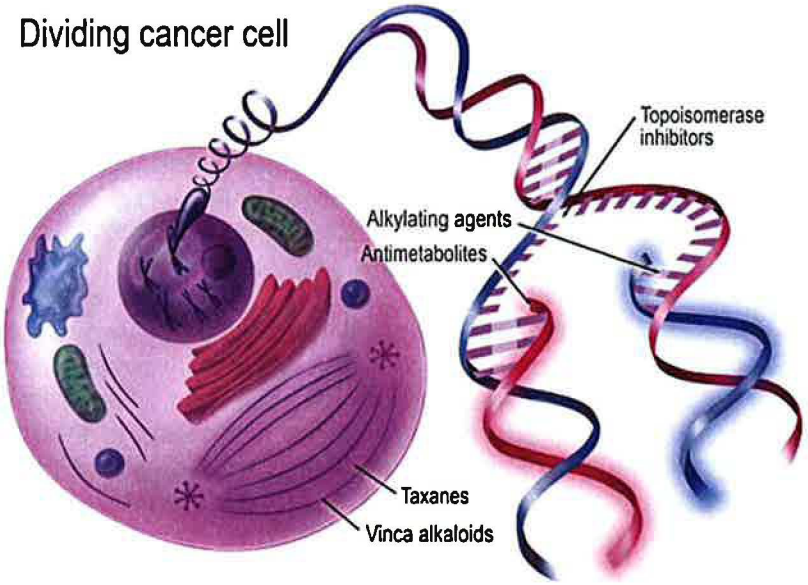

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Figure 1. Mechanisms of conventional (cytotoxic) chemotherapy.

Figure 1 displays the main classes of conventional, cytotoxic chemotherapy. Alkylating agents (eg, cyclophosphamide, cisplatin) interfere with DNA base pairing. Topoisomerase inhibitors (eg, etoposide, irinotecan) prevent DNA strand uncoiling. Antimetabolites (eg, 5-fluorouracil, cytarabine) prevent the synthesis and incorporation of nucleotides into the growing DNA strand. Taxanes (eg, paclitaxel, docetaxel) and vinca alkaloids (eg, vincristine, vinblastine) interfere with microtubule function, which is essential for cellular mitosis. The classic toxicities of chemotherapy occur because, in addition to cancer, certain normal tissues undergo rapid growth and frequent cell division. These include hair, gastrointestinal epithelium, and bone marrow, resulting in alopecia, nausea and mucositis, and myelosuppression, respectively. In some instances, the cellular targets of chemotherapy drugs are involved in biologic processes beyond cell division. For instance, microtubule function is a key component of axonal transport. Consequently, the microtubule-inhibiting taxanes and vinca alkaloids frequently cause peripheral neuropathy.

The administration of conventional chemotherapy is a complex task. These drugs are typically administered intravenously. In many instances, frequent drug dosing, blood draws, and chemotherapy-associated vessel damage necessitate placement of an indwelling vascular catheter such as a mediport. Routine monitoring includes assessing hematologic parameters (total white blood cell count, neutrophils, hemoglobin/hematocrit, platelets), renal function, and liver function prior to each chemotherapy dose. Doxorubicin, an anthracycline used for the treatment of breast cancer, leukemia, and sarcomas, must be protected from light. The taxanes paclitaxel and docetaxel are not soluble in saline or dextrose. Instead, they are dissolved in the carriers cremaphor and Tween-20, which may precipitate allergic reactions. Patients receiving these drugs therefore require premedication with corticosteroids and antihistamines (both H1 and H2 blockers). One of the greatest recent advances in cancer care has been the ability to prevent nausea and vomiting in the majority of treated patients. However, doing so requires a complex regimen, which may include 5-HT_3 antagonists (eg, ondansetron), substance P inhibitors (eg, aprepitant), corticosteroids (eg, dexamethasone), phenothiazines (eg, prochlorperazine), and benzodiazepines (eg, lorazepam). While conventional chemotherapy may be given only once every 2-3 weeks, treatment days are long. In most instances, patients receive more than one drug. Paclitaxel is infused over three hours. The administration of cisplatin—which requires extensive pre- and post-hydration, mannitol diuresis, and electrolyte repletion—takes approximately six hours.

In contrast to conventional chemotherapy, some targeted therapy regimens are quite simple. Many of these drugs are taken orally, at home, on a daily basis. Many of these drugs are also better tolerated than conventional chemotherapy. Nevertheless, these drugs do have significant toxicities, and many of them are prone to interactions with other medications. Accordingly, it is important that primary care physicians and other clinicians caring for patients with cancer have a basic understanding of these new medications.

The field of oncology has truly entered the era of targeted therapy. Since the year 2000, only about five new conventional chemotherapy drugs have been approved by the U.S. Food and Drug Administration (FDA), compared to over 15 targeted therapies.[1] While conventional chemotherapy remains the backbone of medical cancer treatment for the majority of malignancies, targeted therapies are now employed in the treatment of most common cancers, including breast, colorectal, lung, and pancreatic cancers, as well as lymphoma, leukemia, and multiple myeloma.

The biology of targeted therapy

The term "targeted therapy" generally refers to two classes of drugs: monoclonal antibodies and small molecule inhibitors. These drugs interfere with specific cell markers and pathways, which are depicted in *Figure 2*. A therapeutic index is achieved when these cell markers and pathways are unique to, over-expressed in, or mutated in cancer cells as compared to normal tissues. However, the biologic distribution of these molecular targets is rarely clear-cut. For instance, cluster of differentiation 20 (CD20) is present on the cells of non-Hodgkin's lymphoma (NHL), but also on normal B lymphoid cells. Epidermal growth factor receptor (EGFR) is present in multiple carcinomas, but also normal epithelial tissues. The presence of molecular targets on normal cells accounts for most of the toxicities of targeted therapies.

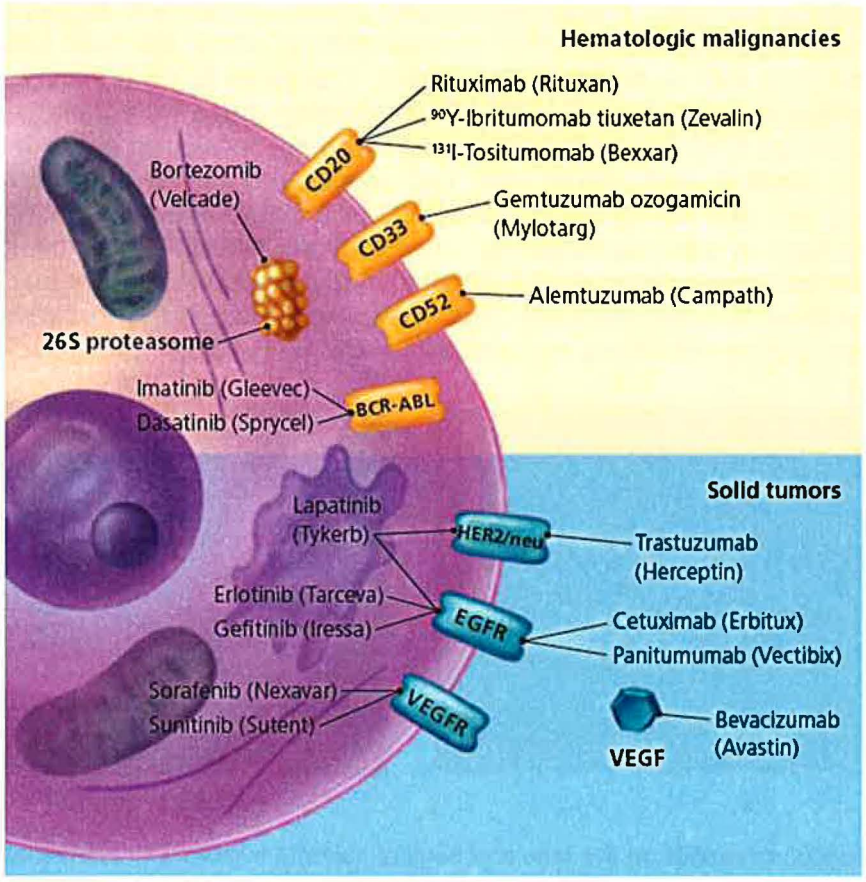

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Figure 2. Mechanisms of targeted therapies. Note that the molecular targets in the figure are not actually expressed in a single cell type, but in various malignant and normal tissues. BCR-ABL, breakpoint cluster region-Abelson; CD, cluster of differentiation; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

Epidermal Growth Factor Receptor (EGFR)

Two of the most frequently targeted molecules in oncology are EGFR and vascular endothelial growth factor (VEGF). EGFR belongs to a four-member family of transmembrane receptor tyrosine kinases. This group of molecules is known alternatively as the HER (human epidermal growth factor receptor) or ErbB (so named for homology to a virus associated with erythroblastosis) family. In both normal and malignant tissues, circulating ligand (including EGF, transforming growth factor [TGF], and others) binds to the extracellular domain of EGFR. This induces receptor subunit dimerization and activation of an intracellular tyrosine kinase. As the name implies, the tyrosine kinase is composed of tyrosine amino acid residues that undergo autophosphorylation and then transfer phosphate groups to other intracellular molecules. These downstream molecules in turn mediate signal transduction that results in cell proliferation, resistance to apoptosis, migration, and angiogenesis.[2-6]

Because EGFR is also present on normal epithelial tissues, such as skin and gastrointestinal mucosa, the main side effects of EGFR inhibition are an acneiform rash (see *Figure 3*) and diarrhea. The diarrhea, which is rarely severe, can usually be

managed with loperamide. The acneiform rash can be treated with topical and systemic antibiotics and corticosteroids. What is most striking about the rash associated with EGFR inhibition is that, in multiple tumor types, it appears to be a surrogate marker of treatment efficacy. In clinical trials of EGFR inhibiting drugs for colorectal, pancreatic, and non-small cell lung cancer (NSCLC), patients who developed a Grade 2 or greater (ie, requiring medical intervention) rash had significantly longer survival than patients who did not.[7-10] It is not clear if this association is due to individual patient differences in EGFR biology or pharmacokinetics. Associations between treatment toxicity and efficacy have long been observed for conventional chemotherapy. For instance, severe myelosuppression is associated with higher response rates in the treatment of multiple malignancies, among them lung and ovarian cancers.[11, 12]

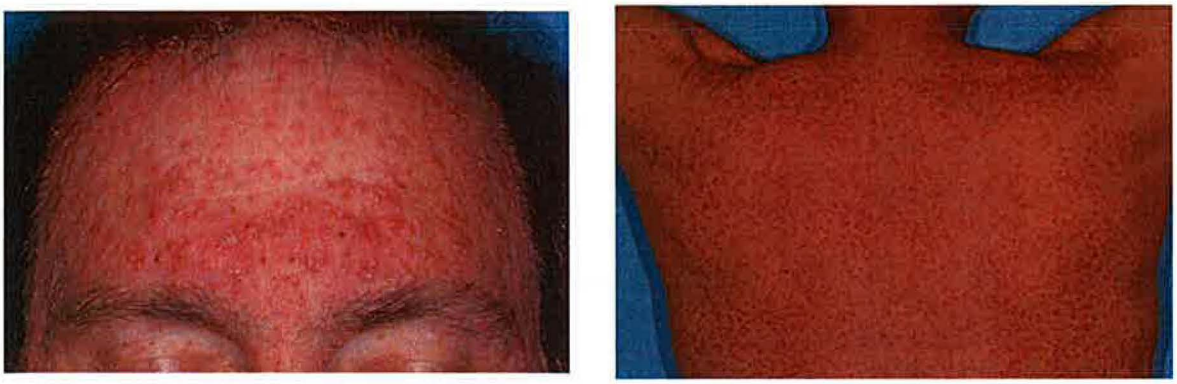

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Figure 3. Acneiform rashes on the face and back of patients treated with cetuximab (Erbitux), a monoclonal antibody targeting epidermal growth factor receptor (EGFR).

Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF) is the principal mediator of angiogenesis. Angiogenesis is the development of new blood vessels from pre-existing vasculature. Its relevance to the initiation and promotion of cancer was pioneered by Judah Folkman. It took over a decade for the scientific community to embrace the theories of Dr. Folkman, who died in early 2008. It is now understood that cells cannot survive more than 2-3 mm from their blood supply, so the ingrowth of new blood vessels is essential for tumor development.[13-15] The inhibition of angiogenesis—which can be accomplished by targeting VEGF or its receptor, VEGFR—prevents new blood vessel formation. It also leads to vessel normalization. Within a tumor, the vascular network is often convoluted, dilated, and porous. With anti-angiogenic therapy, these vessels become less tortuous and less permeable, a process that may improve the delivery of other drugs to the tumor.[16, 17] Because anti-angiogenic therapies affect normal blood vessels in addition to tumor vasculature, these drugs are associated with a distinct and extensive toxicity profile. Adverse effects include bleeding, clotting (primarily arterial), hypertension, gastrointestinal perforation, wound healing complications, and—through effects on glomerular capillaries—proteinuria. It is generally recommended that patients undergoing major surgical procedures not receive the anti-VEGF antibody bevacizumab for several weeks before or after the operation.[18] Bevacizumab was recently approved for the treatment of NSCLC, but its use is restricted to patients with non-squamous cell histology.[19] In phase 2 clinical trials, patients with squamous cell NSCLC treated with bevacizumab experienced unacceptably high levels of severe hemoptysis.[20] To date,

brain metastases and concomitant anticoagulation have also been considered contraindications to bevacizumab, although recent data suggests that, in certain circumstances, bevacizumab may be tolerated by such patients.[21] Bevacizumab and other antiangiogenic therapies should be distinguished from vascular disrupting agents (VDAs). These drugs, which remain in clinical development, target pre-existing tumor blood vessels.

Tailoring therapy

The holy grail of targeted therapy is the identification of individuals most likely to benefit from specific drugs. This provides optimal cancer treatment, while sparing patients unnecessary toxicities and costs. Despite years of effort, oncologists remain largely incapable of predicting the effect of conventional chemotherapy on an individual tumor. By contrast, in the field of infectious diseases, physicians not only identify the causative organism but also a panel of antimicrobial drug sensitivities. Recent genomic-based approaches notwithstanding, similar attempts to characterize a tumor's chemotherapy sensitivity have not proven clinically useful. One of the earliest and most clinically robust of tailoring cancer therapy individually is the hormone receptor tamoxifen. Early on, it became clear that tamoxifen provided a benefit only in breast tumors that were hormone dependent. For decades, oncologists have therefore limited the use of this drug to the two-thirds of breast cancer patients with tumors that express the estrogen receptor and/or the progesterone receptor.[22]

A more recent example of tailored cancer therapy is HER2/neu, a cell surface tyrosine kinase related to EGFR. HER2/neu, which is present in approximately 25% of breast cancer cases, conveys biologic aggressiveness and a worse overall prognosis.[23] HER2/neu targeting drugs, including both small molecule inhibitors and monoclonal antibodies, are used only if HER/neu is demonstrated in a tumor specimen, either by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH).[24-26]

Tailoring therapy is less straightforward for most other cancers. For the treatment of NSCLC, the use of EGFR small molecule inhibitors (eg, erlotinib, gefitinib) results in the most dramatic radiographic responses in individuals with mutations in exons 19 and 21 of the *EGFR* gene.[27] These mutations render the cancer dependent on the EGFR pathway for survival (a concept known as "oncogene addiction") and thus exquisitely sensitive to EGFR inhibition. Although EGFR mutation testing of tumor specimens is commercially available, its applicability to an American population has been questioned. EGFR mutations occur most frequently in women, never-smokers, East Asians, and adenocarcinoma histology (particularly bronchioloalveolar carcinoma [BAC]).[28-31] In Japan and Korea, it is estimated that over 30% of NSCLC cases feature EGFR mutations, but only about 10% of cases in the United States do.[32-35] In Western populations, other EGFR parameters may be more useful. EGFR gene amplification and increased gene copy number, which may be ascertained using FISH, occur in approximately 30% of NSCLC in the United States and Europe.[33, 36, 37] Furthermore, recent research has suggested that these molecular features may be better predictors of overall survival (in contrast to radiographic response) than EGFR gene mutations.[38]

In colorectal cancer, early studies suggested that the presence of EGFR in tumor specimens, as determined by IHC, did not predict efficacy of EGFR inhibitors. Instead, it has emerged that mutations in K-ras, a downstream intracellular proto-oncogenic

mediator of EGFR signal transduction, conveys resistance to EGFR inhibiting drugs. K-ras mutations, which occur in over 30% of colorectal cancer cases, render K-ras constitutively active, independent of upstream signaling from EGFR.[39] This observation has resulted in formal recommendations that K-ras mutation status be assessed in all colorectal cancer patients prior to starting anti-EGFR therapy.

The individual tailoring of anti-angiogenic therapies represents a particular challenge. The VEGF pathway involves both tumor and patient biology. VEGF may be secreted by tumor cells or by host pericytes. VEGFR may be present on tumor cells or on host endothelial cells. Consequently, the factors that predict response to anti-VEGF or anti-VEGFR treatments remain largely unclear. Proposed indicators include the nature and number of myeloid cells infiltrating a tumor, and germline polymorphisms in the VEGF gene. [40, 41] Baseline and post-treatment serum VEGF levels do not appear to predict or indicate a therapeutic response.[42, 43]

Classes of targeted therapy

Currently, there are nine monoclonal antibodies and ten small molecule inhibitors approved by the FDA for cancer treatment. In many instances, monoclonal antibodies and small molecule inhibitors target the same cell molecule or pathway (see *Figure 2*). However, these two classes of drugs differ in several ways. *Table 1* compares monoclonal antibodies and small molecule inhibitors.

	Monoclonal antibodies	**Small molecule inhibitors**
Size	+++++	++
Specificity	+++++	+++
Administration	IV	PO (usually)
Cost	+++++	+++
Half-life	Days	Hours
Targets	Extracellular	Intracellular
Drug interactions	Minimal	Many (CYP450)
Infusion reactions	Yes	No
Suffix	-ab	-ib (usually)

Table 1. Characteristics of monoclonal antibodies and small molecule inhibitors.

The name of a drug indicates its class. Monoclonal antibodies have names ending in "-ab." In almost all instances, the name of a small molecule inhibitor ends in "-ib." Small molecule inhibitors typically have a molecular weight in the range of 400-500 daltons (which is actually larger than conventional chemotherapy drugs, with MW usually 100-250 daltons). Monoclonal antibodies are intact IgG molecules and have a MW of approximately 150,000 daltons. Because of their large size, monoclonal antibodies do

not cross the cell membrane and exert their effects extracellularly. Small molecule inhibitors act intracellularly. All monoclonal antibodies are administered intravenously. As proteins, they would be denatured in the gastrointestinal tract. Eight of the ten approved small molecule inhibitors are given orally. The oral administration of small molecule inhibitors is complicated by potential drug-drug interactions. Most of these drugs are metabolized by cytochrome P450 enzymes, requiring caution in patients who are also taking azole antifungals, macrolide antibiotics, certain anticonvulsants, warfarin, and other drugs.[44] Monoclonal antibodies typically target a single molecule, while small molecule inhibitors are somewhat less specific. This lack of specificity may enhance anti-tumor effects, but it also expands toxicities. Even in instances where a monoclonal antibody and a small molecule inhibitor share a target, their mechanisms differ. Consider erlotinib, a small molecule inhibitor of EGFR, and cetuximab, an anti-EGFR monoclonal antibody. Erlotinib binds to the intracellular tyrosine kinase, preventing downstream signal transduction. Cetuximab binds extracellularly, blocking ligand binding and preventing downstream signal transduction. However, cetuximab is also thought to recruit host immune cells to attack the targeted cancer cell and to promote EGFR internalization, degradation, and long-term downregulation.[45, 46] Neither of these effects occurs with erlotinib. These biologic distinctions may explain some of the clinical differences between these two drugs.

Monoclonal antibodies (mAbs)

Few cancer therapies have attracted the level of interest given to monoclonal antibodies. These drugs, first approved for cancer treatment in the late 1990s, provide unprecedented target specificity. In so doing, they have begun to fulfill the concept put forth by Paul Ehrlich over one hundred years earlier—a "magic bullet" that kills cancer, but does not harm normal tissues.[47] The production of monoclonal antibodies has captured the public's awe and appreciation. The hybridoma technique, which entails the fusion of mouse and human cells into antibody "factories," exemplifies the clinical benefits of biologic research.[48]

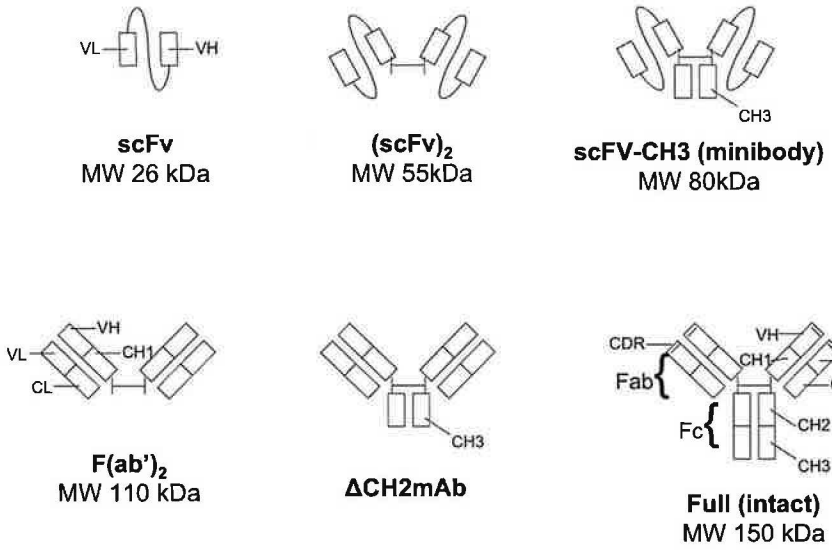
Antibody production

Understanding the production of monoclonal antibodies helps explain their structure, function, and toxicities. In the hybridoma technique, mice are inoculated with a specific protein, the desired antigenic target, over several months. The mouse is then sacrificed, and splenic lymphocytes are harvested. The murine splenic cells are co-incubated and fused with immortalized human myeloma cells *in vitro*.[49] The fused cells grow into colonies that effectively serve as biologic factories for antibody production. Each colony produces molecularly identical antibodies arising from the same original mouse lymphocyte—hence the term monoclonal. Antibodies are then tested for specificity or desired immune effect.

The major limitation of the hybridoma technique is that the resulting antibodies are composed entirely of murine (mouse) proteins. Consequently, they may be recognized by the patient receiving them as foreign substances. This may result in acute anaphylactic-type infusion reactions and in human anti-mouse antibodies (HAMA), which can neutralize the therapeutic effect of the exogenous antibody. To limit these toxicities, monoclonal antibodies are often administered with antihistamine premedication, with an available supply of corticosteroids, epinephrine, and equipment for airway management. More recently, it has become possible to incorporate human protein sequences into

antibody structures, resulting in decreased immunogenicity. Chimeric antibodies are approximately 2/3 human, 1/3 mouse protein. Humanized antibodies are 95% human, 5% mouse. Fully human antibodies are 100% human. The suffix of an antibody name indicates antibody species: "-momab"=murine; "-ximab"=chimeric; "-zumab"=humanized; "-mumab"=human. This rule can be applied to monoclonal antibodies used outside of oncology. Infliximab (Remicaid), which targets tumor necrosis factor-α (TNFα) and is approved for the treatment of certain autoimmune diseases, is a chimeric antibody. Abciximab (ReoPro), which targets glycoprotein IIB/IIIA and is used during interventional cardiology procedures, is also chimeric.

Genetic engineering has permitted the synthesis of fully human antibodies. One approach involves the inactivation of murine immunoglobulin gene loci and replacement by human genes encoding the desired heavy and light chains. An alternative approach involves the use of phage (bacterial virus) display libraries.[50] These libraries contain millions of ligands fused to the gene encoding the phage (virus) coat protein. The resulting phage particles express the desired ligand on their surface, to which antibodies are applied. Genes encoding bound antibodies are cloned into an expression vector in transformed cells, resulting in antibody-producing colonies.



Axelson MD and Gerber DE. Current Therapeutic Uses of Monoclonal Antibodies. In: Pharmaceutical Perspectives of Cancer Therapies. AAPS-Springer, in press.

Figure 4. Antibody structure and constructs. C, constant; CDR, complementarity-determining region; Fab, fragment antigen binding; Fc, fragment crystallizable; H, heavy chain; L, light chain; MW, molecular weight; V, variable

Antibody structure

Whether produced via hybridoma or recombinant techniques, monoclonal antibodies share a common structure (see *Figure 4*). These Y-shaped molecules contain an Fab (fragment antigen binding), which recognizes and binds to antigen, and an Fc (fragment crystallizable). The large size (approx 150,000 daltons) of monoclonal antibodies results in issues of drug delivery, particularly related to the central nervous system. This observation has been most pronounced with the use of the anti-HER2 antibody

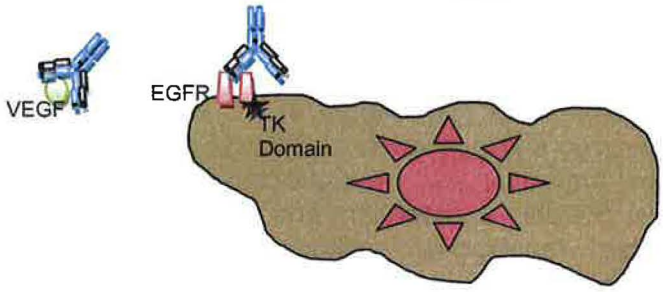
trastuzumab (Herceptin) in patients with HER2-positive breast cancer. In some series, up to 25-50% of patients develop "sanctuary" brain metastases, a finding that has been attributed to trastuzumab's enhanced control of systemic disease but apparent failure to cross the blood-brain barrier.[51] To address this and similar issues, smaller antibody constructs and fragments have been developed (see *Figure 4*). Compared to intact antibodies, these molecules have improved delivery, enhanced tumor penetration, and decreased immunogenicity. However, they may also be characterized by decreased antigen binding, more rapid clearance, possible aggregation, and lack of Fc-dependent functions.[52-54] Currently, only intact monoclonal antibodies are approved for therapeutic use in oncology.

Non-therapeutic (diagnostic) uses of monoclonal antibodies

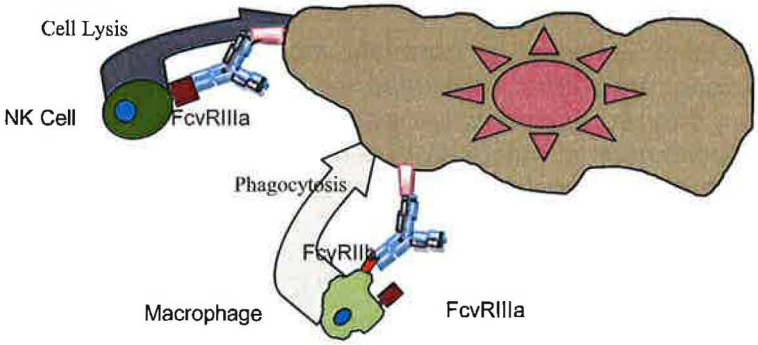
Although the term "monoclonal antibody" is currently most closely associated with therapeutic agents, monoclonal antibodies were used for diagnostic purposes, both *in vitro* and *in vivo*, well before they were used as anti-cancer drugs. Their high degree of specificity often provides confirmation in pathology cases that are not straightforward by microscopic appearance alone. Immunohistochemistry (IHC) is the process of tissue antigen recognition of pathology samples though the binding of antibodies. The antibodies can either be labeled with a dye [e.g., fluorescein isothiocyanate (FITC)] or be counterstained with a second, dye-labeled antibody. IHC may contribute to tissue diagnosis. For example, tissue staining with anti-cytokeratin (CK)-7 and anti-thyroid transcription factor (TTF)-1 antibodies may support a diagnosis of non-small cell lung cancer; tissue staining with anti-CK20 antibodies may support a diagnosis of colorectal cancer. IHC may also guide treatment planning. For example, breast cancer tissue staining with anti-estrogen receptor (ER) or anti-progesterone receptor (PR) antibodies may result in the use of the hormone receptor modulator tamoxifen; breast cancer tissue staining with anti-HER2 antibodies may lead to the use of the therapeutic monoclonal antibody trastuzumab or the small molecule tyrosine kinase inhibitor lapatinib. Flow cytometry, a technique first developed in the late 1960s, applies similar principles to particles suspended in a stream of fluid, such as blood and other body fluids. Fluorescence-activated cell sorting (FACS), a specialized form of flow cytometry, provides rapid and objective cell counting and sorting.

Since the early 1990s, murine radioconjugates have been employed for diagnostic imaging studies. Satumomab pendetide (OncoScint), an Indium-111 (^{111}In) labeled anti-tumor-associated glycoprotein (TAG)-72 IgG1, targets antigens on colorectal and ovarian cancers and is used to image these malignancies. Other approved imaging antibodies include arcitumomab (CEA-Scan) for colorectal cancer, nofetumomab merpentan (Verluma) for small cell lung cancer, and capromab pendetide (ProstaScint) for prostate cancer.[55] Despite the promise of these diagnostic antibodies, their slow biodistribution and systemic clearance, high liver uptake, and concerns over immunogenicity limit their use. They have been largely replaced by positron emission tomography (PET) scans.[56]

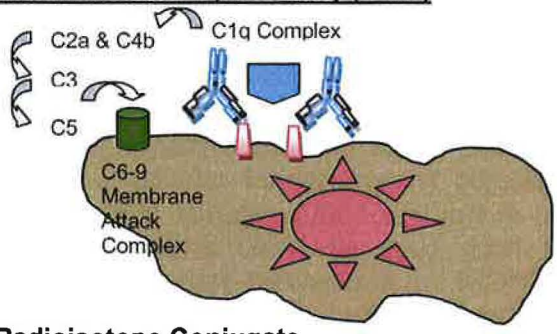
Interference with Receptor or Ligand Function



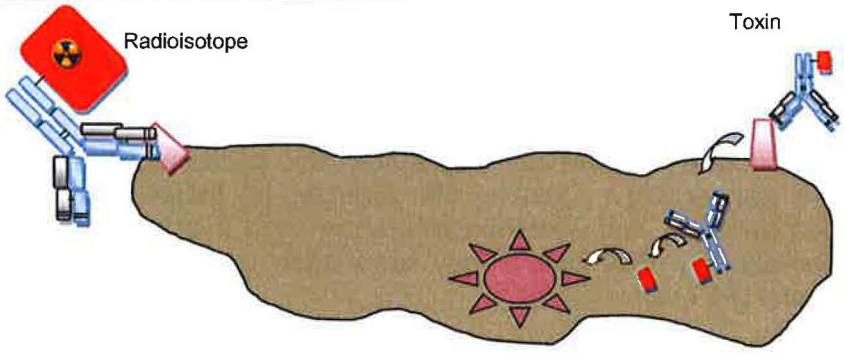
Antibody Dependent Cellular Cytotoxicity (ADCC)



Complement Mediated Cytotoxicity (CMC)



Toxin / Radioisotope Conjugate



Axelson MD and Gerber DE. Current Therapeutic Uses of Monoclonal Antibodies. In: Pharmaceutical Perspectives of Cancer Therapies. AAPS-Springer, in press.

Figure 5. Mechanisms of cancer cell killing by monoclonal antibodies.

Mechanisms of tumor cell killing

Monoclonal antibodies kill cancer cells via a number of mechanisms (see *Figure 5*). First, an antibody may interrupt a process essential for cancer cell survival. The most prominent examples are anti-EGFR and anti-VEGF antibodies. Anti-EGFR antibodies bind to the extracellular domain of EGFR, preventing ligand binding, receptor subunit dimerization, and activation of the intracellular tyrosine kinase.[57, 58] Anti-VEGF antibodies bind to circulating VEGF ligand, preventing VEGF binding to its receptor, VEGFR, and inhibiting angiogenesis.

Second, certain monoclonal antibodies recruit host effector (immune) functions to the targeted tumor. This process resembles the means by which endogenous antibodies kill bacteria or viruses. The effector functions include antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity (CMC).[59] In ADCC, patient immune cells, including neutrophils, natural killer cells and macrophages, interact with the Fc of the exogenous therapeutic antibody bound to the tumor.[60] The recruitment of immune cells results in phagocytosis, cytokine release, and tumor cell lysis. The degree to which ADCC and CMC contribute to tumor cell killing depends on both antibody and host characteristics. Antibodies with IgG1 isotype recruit ADCC more effectively than do IgG2 antibodies. Host immune cells bind to therapeutic antibodies via Fc gamma receptors (FcγR): FcγRI (CD64), FcγRII (CD32), and FcγRIII. Polymorphisms in these molecules appear to impact an individual patient's immune response and the anti-tumor effect of the antibody.[61, 62] In CMC, C1q binding sites become available on the therapeutic antibody, thereby activating the complement cascade. IgM antibodies are the most effective for this mechanism, followed by IgG3 and IgG1. [63]

Third, lethal payloads (including radioisotopes, toxins, enzymes, and drugs) may be conjugated to monoclonal antibodies, which provide precise, specific targeting. To date, the FDA has approved three conjugated antibodies, two of which incorporate radioisotopes (^{90}Y Ibritumomab tiuxetan [Zevalin] and ^{131}I Tositumomab [Bexxar]) and one that incorporates the calicheamicin toxin, a DNA poison (Gemtuzumab ozogamicin [Mylotarg]). A major obstacle to further use of conjugated antibodies is that the process of conjugation may alter properties of the antibody itself and of the conjugated payload. Antibody-directed enzyme prodrug therapy (ADEPT), first proposed in 1987, is a multi-step process that begins with the infusion of a monoclonal antibody conjugated to a drug-activating enzyme. After binding of antibody to the target tumor antigen, a prodrug is administered and converted by the enzyme to its active moiety. Ideally, this approach results in a high concentration of active drug at the site of the target tumor, with relatively low systemic exposure.[64]

Approved therapeutic antibodies

Currently, there are nine monoclonal antibodies FDA approved for the treatment of cancer: five for the treatment of hematologic malignancies, four for the treatment of solid tumors (see *Table 2*). Their structure is distributed as follows: unconjugated (6), radioisotope conjugate (2), toxin conjugate (1). Their species: murine (2), chimeric (2), humanized (4), fully human (1). Their isotypes: IgG1 (6), IgG2 (2), IgG4 (1). Their targets: CD20 (3), CD52 (1), CD33 (1), HER2/neu (1), EGFR (2), VEGF (1). For the treatment of both hematologic malignancies and solid tumors, antibodies are employed as monotherapy and also in combination with other agents. To date, it appears that the

use of monoclonal antibodies as single agents may be more effective against hematologic malignancies, which are generally characterized by fewer molecular aberrations and greater responsiveness to medical therapies than are solid tumors. For solid tumors, monoclonal antibodies may be most effective when combined with either conventional chemotherapy or radiotherapy.

Drug	Target	Type	Indications	Toxicities
Alemtuzumab (Campath)	CD52	Humanized, unconjugated	CLL	Hematologic, infections
Bevacizumab (Avastin)	VEGF	Humanized, unconjugated	Colorectal cancer, NSCLC (nonsquamous), breast cancer	GI perforation; wound healing complications; bleeding; clotting; proteinuria; hypertension
Cetuximab (Erbitux)	EGFR	Chimeric, unconjugated	Colorectal cancer, H+N cancer	Rash; diarrhea; nausea
Gemtuzumab ozogamicin (Mylotarg)	CD33	Humanized, toxin conjugate (calicheamicin)	AML	Hematologic; hepatic
^{90}Y-Ibritumomab tiuxetan (Zevalin)	CD20	Murine, radioisotope conjugate	NHL	Hematologic; radiation; immune reaction
Panitumumab (Vectibix)	EGFR	Human, unconjugated	Colorectal cancer	Rash; diarrhea; nausea
Rituximab (Rituxan)	CD20	Chimeric, unconjugated	NHL, Rheumatoid arthritis	Lymphopenia
^{131}I-Tositumomab (Bexxar)	CD20	Murine, radioisotope conjugate	NHL	Hematologic; thyroid; radiation; immune reaction
Trastuzumab (Herceptin)	HER2/neu	Humanized, unconjugated	Breast cancer with HER2/neu overexpression	Cardiac

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Table 2. Monoclonal antibodies for cancer treatment. AML, acute myeloid leukemia; CD, cluster of differentiation; CLL, chronic lymphocytic leukemia; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; NHL, non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer; VEGF, vascular endothelial growth factor

Small molecule inhibitors

Small molecule inhibitors most commonly interrupt cellular processes by interfering with intracellular kinase activity. In clinical and research parlance, these drugs are alternatively referred to as small molecule tyrosine kinase inhibitors, small molecule kinase inhibitors, tyrosine kinase inhibitors, or small molecules. These drugs are chemically manufactured, a process that is often far less expensive than the bioengineering required for monoclonal antibodies.[65]

Currently, there are ten FDA approved small molecule inhibitors for the treatment of cancer (see *Table 3*). As mentioned previously, these drugs generally are less specific than monoclonal antibodies, and many of them have multiple molecular targets. The toxicities of a small molecule inhibitor are often directly attributable to the drug's molecular targets. For instance, dasatinib (Sprycel) inhibits BCR-ABL, the src family of proteins, c-KIT, and platelet-derived growth factor receptor (PDGFR). PDGFR is present not only on certain tumor cells, but also on normal pericytes, which support vascular endothelium and control vessel permeability. Consequently, the inhibition of PDGFR by dasatinib may result in effusions, edema, and weight gain. c-KIT (CD117) is present on certain sarcoma and leukemia cells, but also on hematopoietic cells. Consequently, the inhibition of c-KIT by dasatinib may result in hematologic toxicity. Of the ten approved small molecule inhibitors, two (bortezomib [Velcade] and temsirolimus [Torisel]) are administered intravenously. Temsirolimus is also the only approved small molecule inhibitor with a name that does not end in "-ib."

Imatinib (Gleevec) was the first approved small molecule inhibitor. To date, it remains arguably the most effective drug in this class. Approved in 2002 for the treatment of chronic myeloid leukemia (CML), imatinib inhibits BCR-ABL (breakpoint cluster region-Abelson). BCR-ABL is a constitutively active intracellular tyrosine kinase that results from the translocation of chromosomes 9 and 22 (the Philadelphia chromosome). Prior to imatinib, there were few effective therapies for CML. For the minority of patients sufficiently medically fit, bone marrow transplantation offered the only chance for long-term remission, albeit at a cost of substantial morbidity. With imatinib, 98 percent of patients achieve a complete hematologic response.[66, 67] After more than 5 years of follow-up, more than 90 percent of patients remain progression free.[68]

However, as seen with the use of other small molecule inhibitors, resistance may develop to imatinib. Point mutations in the BCR-ABL gene may result in three-dimensional conformational changes that prevent imatinib binding. While later generation BCR-ABL inhibitors such as dasatinib and nilotinib (Tasigna) are effective against most of these mutations, T315I (a substitution of isoleucine for threonine at codon 315) conveys resistance to high levels of these newer drugs as well.[69] Similarly, the most common acquired mechanism of resistance to the EGFR small molecule inhibitor erlotinib in the treatment of NSCLC is T790M (a substitution of methionine for threonine at codon 790). In this case, the bulkier methionine amino acid residue results in steric hindrance to erlotinib binding.[70, 71]

Drug	Target	Indications	Toxicities
Bortezomib (Velcade)	26S proteasome	Multiple myeloma, mantle cell lymphoma (a subtype of NHL)	Peripheral neuropathy; hematologic; rash; diarrhea; edema; nausea
Dasatinib (Sprycel)	BCR-ABL, SRC family, c-KIT, PDGFR	CML, ALL	Rash; diarrhea; pleural effusion; fluid retention; mucositis; hematologic; QT prolongation
Erlotinib (Tarceva)	EGFR	NSCLC, pancreatic cancer	Rash; diarrhea; nausea; fatigue; conjunctivitis; hepatic
Gefitinib (Iressa)	EGFR	NSCLC	Rash; diarrhea; hepatic
Imatinib (Gleevec)	BCR-ABL, c-KIT, PDGFR	CML, ALL, GIST, mastocytosis, hypereosinophilic syndrome	Rash; weight gain; edema; pleural effusion; cardiac; nausea; arthralgias and myalgias; hematologic
Lapatinib (Tykerb)	HER2/neu, EGFR	Breast cancer with HER2 overexpression	Cardiac; rash; hand-foot syndrome; diarrhea; nausea; hepatic
Nilotinib (Tasigna)	BCR-ABL, PDGRF, c-KIT	CML	Rash; diarrhea; nausea; edema; arthalgias, myalgias
Sorafenib (Nexavar)	BRAF, VEGFR, EGFR, PDGFR	RCC, hepatocellular carcinoma	HTN; alopecia; bleeding; rash; hand-foot syndrome; hypophosphatemia; diarrhea; nausea; elevated amylase, lipase; hematologic; wound healing complications
Sunitinib (Sutent)	VEGFR, PDGFR, c-KIT, FLT3	RCC, GIST	Nausea; yellow discoloration of skin; thyroid; cardiac; adrenal; diarrhea; hematologic; mucositis; hepatic; renal
Temsirolimus (Torisel)	mTOR	RCC	Rash; edema; increased glucose, lipids; nausea; hepatic; renal

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Table 2. Small molecule inhibitors for cancer treatment. ALL, acute lymphocytic leukemia; BCR-ABL, breakpoint cluster region-Abelson; CML, chronic myeloid leukemia; EGFR, epidermal growth factor receptor; flt3, FMS-like tyrosine kinase 3; GIST, gastrointestinal stromal tumor; HER2, human epidermal growth factor receptor 2; mTOR, mammalian target of rapamycin; NHL, non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer; PDGFR, platelet-derived growth factor receptor; RCC, renal cell carcinoma; VEGF, vascular endothelial growth factor receptor; VEGFR, VEGF receptor.

The impact and implications of targeted therapy

Clinical outcomes and treatment populations

In addition to the dramatic success of imatinib for the treatment of CML, a number of targeted therapies have markedly changed disease outcomes. When added to conventional CHOP chemotherapy, the anti-CD20 antibody rituximab (Rituxan) increases 2-year overall survival by 15 percent, without adding clinically significant toxicities.[72] For patients with resected early-stage HER2-positive breast cancer, the addition of the anti-HER2 antibody trastuzumab (Herceptin) to conventional chemotherapy increases 4-year disease-free survival by 18 percent and 4-year overall survival by 5 percent.[25] This additional degree of benefit is similar to that achieved by chemotherapy itself. It led to early stopping of the clinical trial and resulted in accelerated FDA approval.

In other instances, the degree of clinical benefit achieved by targeted therapy is more modest. In patients with advanced pancreatic cancer, the addition of erlotinib to standard chemotherapy increases median survival by only three weeks.[8] Although the hazard ratio in the study was statistically significant and led to FDA approval of the combined regimen, few medical oncologists would argue that this is a major advance for this challenging disease. For patients with NSCLC harboring EGFR kinase mutations, treatment with EGFR inhibitors may result in dramatic radiographic responses (see *Figure 6*). However, such tumors inevitably go on to develop resistance, with disease often progressing after 6-12 months.[73]

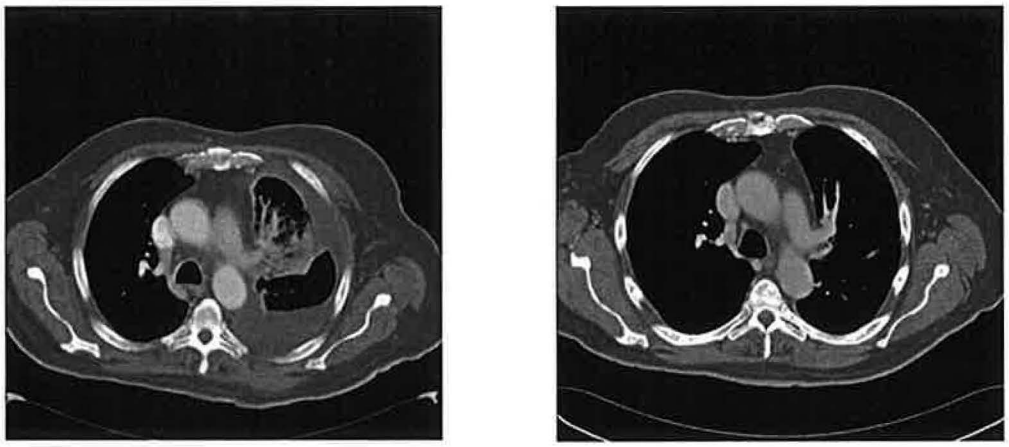

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Figure 6. Chest CT scans demonstrating a dramatic response to targeted therapy in a patient with NSCLC. *Left,* Baseline scan showing a left hilar tumor, lymphangitic spread, and a large pleural effusion. *Right,* Follow-up scan after two months of treatment with erlotinib (Tarceva), an EGFR small molecule inhibitor.

The effect of targeted therapy on the field of oncology is not limited to radiographic response rates and survival curves. Because targeted therapies generally are less toxic than conventional chemotherapy, these drugs have expanded the pool of patients eligible for cancer treatment. For instance, the median age at diagnosis of NSCLC is 70 years. Due to either frailty or medical comorbidities, many of these patients are not candidates for conventional chemotherapy. However, these individuals may tolerate the orally administered EGFR inhibitor erlotinib. Similarly, the median age at diagnosis of

non-Hodgkin's lymphoma is almost 65 years. Certain NHL histologic subtypes may be treated with the anti-CD20 antibody rituximab alone, a regimen considerably less toxic than combination chemotherapy.

Assessing drug dosing and efficacy

Targeted therapy has introduced issues affecting the design and interpretation of clinical trials. In early-phase clinical studies of conventional, cytotoxic chemotherapy, drug dose is escalated until a maximum tolerated dose (MTD) is achieved. Most often, hematologic toxicity is the limiting factor. Targeted therapies, however, often do not cause significant myelosuppression. Determining the MTD becomes a true challenge for drugs that, as monotherapy, may not be associated with clinically significant toxicities. In such cases, some researchers are instead seeking to identify the optimal biologic dose, an endpoint that may depend on levels of circulating biomarkers, changes in tumor genetics and protein expression, or novel imaging techniques.

Assessment of treatment efficacy, in the setting of a clinical trial or routine clinical practice, also may require a paradigm shift. When conventional chemotherapy is effective, reduction in tumor volume is anticipated on serial radiographic studies. In contrast, targeted therapies—which often have cytostatic rather than cytotoxic properties—may impart a clinical benefit by stabilizing rather than shrinking tumors. As described above, cancer researchers and clinicians increasingly are turning to pharmacodynamic endpoints, such as tumor metabolic activity on PET scans, levels of circulating tumor cells, and serial levels of target molecules in tumor tissue.[74, 75] These studies add complexity and cost to clinical trials. Additionally, repeat biopsies of tumor tissue may be inconvenient for patients and unacceptable to institutional review boards. Although these studies may initially increase research time and expense, they may ultimately improve the long-term cost-effectiveness of therapy by identifying the subset of patients most likely to benefit from specific drugs.

Adherence

Conventional chemotherapy is typically administered intravenously in an observed infusion area. Patient adherence to treatment regimens is thus readily assessed. Delayed or missed chemotherapy doses—whether due to patient preference or treatment-related toxicities—are recognized and documented immediately. In contrast, most small molecule inhibitors are taken at home on a long-term daily basis. The task of assessing patient adherence to these regimens more closely resembles that encountered with therapies for chronic diseases such as diabetes or hypertension. The few studies performed to date reveal that patient adherence to oral cancer treatment regimens is highly variable and somewhat unpredictable.[76]

Cost

As the national spotlight again turns to the cost of health care, the economic considerations of targeted therapy are likely to come under scrutiny. Substituting oral small molecule inhibitors for conventional chemotherapy eliminates some treatment costs, such as those associated with vascular access and intravenous infusions. However, targeted therapy is often administered in addition to, rather than in place of, conventional chemotherapy. If monoclonal antibodies are employed, costs may escalate exponentially. Trends in the treatment of metastatic colorectal cancer provide a

noteworthy example. Until the mid-1990s, the standard therapy for this disease was 5-fluorouracil and leucovorin, with an adjusted cost of $63 for eight weeks of therapy. Current state-of-the-art regimens containing cytotoxic agents plus a monoclonal antibody such as bevacizumab or cetuximab may cost over $30,000 for an eight-week regimen.[77-79] These treatments have effectively doubled the median survival of patients with metastatic colorectal cancer, a clinically meaningful benefit that, to date, has made it difficult to consider limiting the use of these drugs.

For other diseases, cost considerations have already impacted patient care. Radioimmunotherapy (ie, ^{90}Y-ibritumomab tiuxetan or ^{131}I-tositumomab) is a highly effective treatment for refractory NHL. However, current Medicare reimbursement for these therapies falls below drug and administration costs, limiting the use of these treatments in the United States. Although patent expiration typically results in the availability of less costly generic medications, this process may be less straightforward for monoclonal antibodies. The design and production of these drugs often include multiple patents. Furthermore, the FDA has yet to establish clear guidelines for the development of follow-along biologic agents or bio-similar agents.[80] It will be several years before this transition is tested. The oldest monoclonal antibodies, rituximab and trastuzumab, which were approved in the late 1990s, are not expected to come off patent protection until 2015.

References

1. Gerber, D.E., *Targeted therapies: a new generation of cancer treatments.* Am Fam Physician, 2008. **77**(3): p. 311-9.
2. Schreiber, A.B., M.E. Winkler, and R. Derynck, *Transforming growth factor-alpha: a more potent angiogenic mediator than epidermal growth factor.* Science, 1986. **232**(4755): p. 1250-3.
3. Petit, A.M., et al., *Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells in vitro and in vivo: angiogenic implications for signal transduction therapy of solid tumors.* Am J Pathol, 1997. **151**(6): p. 1523-30.
4. Gibson, S., et al., *Epidermal growth factor protects epithelial cells against Fas-induced apoptosis. Requirement for Akt activation.* J Biol Chem, 1999. **274**(25): p. 17612-8.
5. O-charnoenrat P, et al., *Epidermal growth factor-like ligands differentially up-regulate matrix metalloproteinase 9 in head and neck squamous carcinoma cells.* Cancer Res, 2000. **60**(4): p. 1121-8.
6. Harari, P.M., G.W. Allen, and J.A. Bonner, *Biology of interactions: antiepidermal growth factor receptor agents.* J Clin Oncol, 2007. **25**(26): p. 4057-65.
7. Jonker, D.J., et al., *Cetuximab for the treatment of colorectal cancer.* N Engl J Med, 2007. **357**(20): p. 2040-8.
8. Moore, M.J., et al., *Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group.* J Clin Oncol, 2007. **25**(15): p. 1960-6.
9. Wacker, B., et al., *Correlation between development of rash and efficacy in patients treated with the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib in two large phase III studies.* Clin Cancer Res, 2007. **13**(13): p. 3913-21.

10. Van Cutsem, E., et al., *Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer.* J Clin Oncol, 2007. **25**(13): p. 1658-64.
11. Banerji, U., et al., *The association of chemotherapy induced neutropenia on treatment outcomes in small cell lung cancer.* Lung Cancer, 2006. **54**(3): p. 371-7.
12. Rocconi, R.P., et al., *Chemotherapy-related myelosuppression as a marker of survival in epithelial ovarian cancer patients.* Gynecol Oncol, 2008. **108**(2): p. 336-41.
13. Weidner, N., et al., *Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma.* N Engl J Med, 1991. **324**(1): p. 1-8.
14. Folkman, J., *Angiogenesis.* Annu Rev Med, 2006. **57**: p. 1-18.
15. Cardones, A.R. and L.L. Banez, *VEGF inhibitors in cancer therapy.* Curr Pharm Des, 2006. **12**(3): p. 387-94.
16. Willett, C.G., et al., *Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer.* Nat Med, 2004. **10**(2): p. 145-7.
17. Jain, R.K., *Molecular regulation of vessel maturation.* Nat Med, 2003. **9**(6): p. 685-93.
18. Hurwitz, H. and S. Saini, *Bevacizumab in the treatment of metastatic colorectal cancer: safety profile and management of adverse events.* Semin Oncol, 2006. **33**(5 Suppl 10): p. S26-34.
19. Sandler, A., et al., *Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer.* N Engl J Med, 2006. **355**(24): p. 2542-50.
20. Johnson, D.H., et al., *Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer.* J Clin Oncol, 2004. **22**(11): p. 2184-91.
21. Akerley, W.L. *Acceptable safety of bevacizumab therapy in patients with brain metastases due to non-small cell lung cancer.* American Society of Clinical Oncology 2008 Annual Meeting. Abstract 8043.
22. Early Breast Cancer Trialists' Collaborative Group. *Tamoxifen for early breast cancer: an overview of the randomised trials.* Lancet, 1998. **351**(9114): p. 1451-67.
23. Slamon, D.J., et al., *Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene.* Science, 1987. **235**(4785): p. 177-82.
24. Bast, R.C., Jr., et al., *2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology.* J Clin Oncol, 2001. **19**(6): p. 1865-78.
25. Romond, E.H., et al., *Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer.* N Engl J Med, 2005. **353**(16): p. 1673-84.
26. Piccart-Gebhart, M.J., et al., *Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer.* N Engl J Med, 2005. **353**(16): p. 1659-72.
27. Scagliotti, G.V., et al., *The biology of epidermal growth factor receptor in lung cancer.* Clin Cancer Res, 2004. **10**(12 Pt 2): p. 4227s-4232s.
28. Fukuoka, M., et al., *Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected].* J Clin Oncol, 2003. **21**(12): p. 2237-46.

29. Kris, M.G., et al., *Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial.* JAMA, 2003. **290**(16): p. 2149-58.
30. Janne, P.A., et al., *Outcomes of patients with advanced non-small cell lung cancer treated with gefitinib (ZD1839, "Iressa") on an expanded access study.* Lung Cancer, 2004. **44**(2): p. 221-30.
31. Perez-Soler, R., et al., *Determinants of tumor response and survival with erlotinib in patients with non--small-cell lung cancer.* J Clin Oncol, 2004. **22**(16): p. 3238-47.
32. Kosaka, T., et al., *Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications.* Cancer Res, 2004. **64**(24): p. 8919-23.
33. Cappuzzo, F., et al., *Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer.* J Natl Cancer Inst, 2005. **97**(9): p. 643-55.
34. Chou, T.Y., et al., *Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with non-small cell lung cancer.* Clin Cancer Res, 2005. **11**(10): p. 3750-7.
35. Han, S.W., et al., *Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib.* J Clin Oncol, 2005. **23**(11): p. 2493-501.
36. Hirsch, F.R., et al., *Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study.* J Clin Oncol, 2005. **23**(28): p. 6838-45.
37. Tsao, M.S., et al., *Erlotinib in lung cancer - molecular and clinical predictors of outcome.* N Engl J Med, 2005. **353**(2): p. 133-44.
38. Hirsch, F.R., et al., *Combination of EGFR gene copy number and protein expression predicts outcome for advanced non-small-cell lung cancer patients treated with gefitinib.* Ann Oncol, 2007. **18**(4): p. 752-60.
39. Khambata-Ford, S., et al., *Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab.* J Clin Oncol, 2007. **25**(22): p. 3230-7.
40. Schneider, B.P., et al., *Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100.* J Clin Oncol, 2008. **26**(28): p. 4672-8.
41. Shojaei, F., et al., *Role of myeloid cells in tumor angiogenesis and growth.* Trends Cell Biol, 2008. **18**(8): p. 372-8.
42. Golshayan, A.R., A.J. Brick, and T.K. Choueiri, *Predicting outcome to VEGF-targeted therapy in metastatic clear-cell renal cell carcinoma: data from recent studies.* Future Oncol, 2008. **4**(1): p. 85-92.
43. Dowlati, A., et al., *Cell adhesion molecules, vascular endothelial growth factor, and basic fibroblast growth factor in patients with non-small cell lung cancer treated with chemotherapy with or without bevacizumab--an Eastern Cooperative Oncology Group Study.* Clin Cancer Res, 2008. **14**(5): p. 1407-12.
44. Micromedex healthcare series. http://www.thomsonhc.com/hcs/librarian.
45. Lilenbaum, R.C., *The evolving role of cetuximab in non-small cell lung cancer.* Clin Cancer Res, 2006. **12**(14 Pt 2): p. 4432s-4435s.

46. Chen, D.J. and C.S. Nirodi, *The epidermal growth factor receptor: a role in repair of radiation-induced DNA damage.* Clin Cancer Res, 2007. **13**(22 Pt 1): p. 6555-60.
47. Strebhardt, K. and A. Ullrich, *Paul Ehrlich's magic bullet concept: 100 years of progress.* Nat Rev Cancer, 2008. **8**(6): p. 473-80.
48. Kohler, G. and C. Milstein, *Continuous cultures of fused cells secreting antibody of predefined specificity.* Nature, 1975. **256**(5517): p. 495-7.
49. Mechetner, E., *Development and characterization of mouse hybridomas.* Methods Mol Biol, 2007. **378**: p. 1-13.
50. Hoogenboom, H.R., et al., *Antibody phage display technology and its applications.* Immunotechnology, 1998. **4**(1): p. 1-20.
51. Cheng, X. and M.C. Hung, *Breast cancer brain metastases.* Cancer Metastasis Rev, 2007. **26**(3-4): p. 635-43.
52. Wu, A.M. and P.D. Senter, *Arming antibodies: prospects and challenges for immunoconjugates.* Nat Biotechnol, 2005. **23**(9): p. 1137-46.
53. Colcher, D., et al., *Pharmacokinetics and biodistribution of genetically-engineered antibodies.* Q J Nucl Med, 1998. **42**(4): p. 225-41.
54. Wochner, R.D., W. Strober, and T.A. Waldmann, *The role of the kidney in the catabolism of Bence Jones proteins and immunoglobulin fragments.* J Exp Med, 1967. **126**(2): p. 207-21.
55. Maguire, R.T., et al., *Immunoscintigraphy in patients with colorectal, ovarian, and prostate cancer. Results with site-specific immunoconjugates.* Cancer, 1993. **72**(11 Suppl): p. 3453-62.
56. Frangioni, J.V., *New technologies for human cancer imaging.* J Clin Oncol, 2008. **26**(24): p. 4012-21.
57. Masui, H., T. Moroyama, and J. Mendelsohn, *Mechanism of antitumor activity in mice for anti-epidermal growth factor receptor monoclonal antibodies with different isotypes.* Cancer Res, 1986. **46**(11): p. 5592-8.
58. Sunada, H., et al., *Monoclonal antibody against epidermal growth factor receptor is internalized without stimulating receptor phosphorylation.* Proc Natl Acad Sci U S A, 1986. **83**(11): p. 3825-9.
59. Iannello, A. and A. Ahmad, *Role of antibody-dependent cell-mediated cytotoxicity in the efficacy of therapeutic anti-cancer monoclonal antibodies.* Cancer Metastasis Rev, 2005. **24**(4): p. 487-99.
60. Clynes, R.A., et al., *Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets.* Nat Med, 2000. **6**(4): p. 443-6.
61. Taylor, R.J., et al., *FcgammaRIIIa polymorphisms and cetuximab induced cytotoxicity in squamous cell carcinoma of the head and neck.* Cancer Immunol Immunother, 2008.
62. Zhang, W., et al., *FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab.* J Clin Oncol, 2007. **25**(24): p. 3712-8.
63. Strome, S.E., E.A. Sausville, and D. Mann, *A mechanistic perspective of monoclonal antibodies in cancer therapy beyond target-related effects.* Oncologist, 2007. **12**(9): p. 1084-95.
64. Senter, P.D., et al., *Anti-tumor effects of antibody-alkaline phosphatase conjugates in combination with etoposide phosphate.* Proc Natl Acad Sci U S A, 1988. **85**(13): p. 4842-6.
65. Tanner, J.E., *Designing antibodies for oncology.* Cancer Metastasis Rev, 2005. **24**(4): p. 585-98.

66. Kantarjian, H., et al., *Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia.* N Engl J Med, 2002. **346**(9): p. 645-52.
67. Kantarjian, H.M., et al., *Imatinib mesylate therapy in newly diagnosed patients with Philadelphia chromosome-positive chronic myelogenous leukemia: high incidence of early complete and major cytogenetic responses.* Blood, 2003. **101**(1): p. 97-100.
68. Druker, B.J., et al., *Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia.* N Engl J Med, 2006. **355**(23): p. 2408-17.
69. Tanaka, R. and S. Kimura, *Abl tyrosine kinase inhibitors for overriding Bcr-Abl/T315I: from the second to third generation.* Expert Rev Anticancer Ther, 2008. **8**(9): p. 1387-98.
70. Pao, W., et al., *Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain.* PLoS Med, 2005. **2**(3): p. e73.
71. Kosaka, T., et al., *Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib.* Clin Cancer Res, 2006. **12**(19): p. 5764-9.
72. Coiffier, B., et al., *CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma.* N Engl J Med, 2002. **346**(4): p. 235-42.
73. Sharma, S.V., et al., *Epidermal growth factor receptor mutations in lung cancer.* Nat Rev Cancer, 2007. **7**(3): p. 169-81.
74. Weber, W.A., *Positron emission tomography as an imaging biomarker.* J Clin Oncol, 2006. **24**(20): p. 3282-92.
75. Park, J.W., et al., *Rationale for biomarkers and surrogate end points in mechanism-driven oncology drug development.* Clin Cancer Res, 2004. **10**(11): p. 3885-96.
76. Partridge, A.H., et al., *Adherence to therapy with oral antineoplastic agents.* J Natl Cancer Inst, 2002. **94**(9): p. 652-61.
77. Schrag, D., *The price tag on progress--chemotherapy for colorectal cancer.* N Engl J Med, 2004. **351**(4): p. 317-9.
78. Hurwitz, H., et al., *Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer.* N Engl J Med, 2004. **350**(23): p. 2335-42.
79. Cunningham, D., et al., *Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer.* N Engl J Med, 2004. **351**(4): p. 337-45.
80. Grabowski, H., *Follow-on biologics: data exclusivity and the balance between innovation and competition.* Nat Rev Drug Discov, 2008. **7**(6): p. 479-88.