The New Era of Molecular-Targeted Therapy: Where Bench Meets Bedside in the War Against Cancer and Hematologic Malignancies

Internal Medicine Grand Rounds

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November 8, 2001

This is to acknowledge that Robert Ilaria, Jr., M.D. has not disclosed any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Ilaria will be discussing off-label uses in his presentation.

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Research interests: Our laboratory studies chimeric oncogenes implicated in cancer and leukemia, with a focus on the development of novel model systems for the discovery and evaluation of novel therapeutic agents. Current projects include translational mouse models of Bcr/Abl-induced leukemia; the study of signal transduction inhibitors such as STI571 (Gleevec), CI-1040, Herceptin, and ZD1839 (Iressa); and the molecular biology of adult and childhood sarcomas, including Ewing's sarcoma, rhabdomyosarcoma, and synovial cell sarcoma.

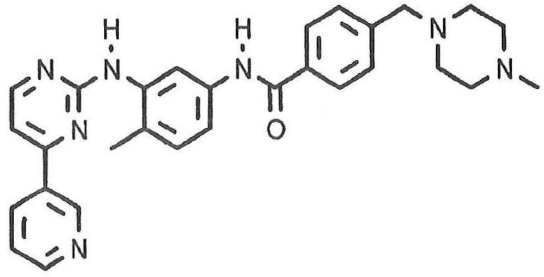
Introduction

Over the last several years there has been a tremendous increase in the number of novel targeted therapeutic agents for patients with cancer and hematologic malignancies. The clinical success of several of these agents validates the decades of basic science investigation that led to their discovery and development. Since there are almost 100 molecularly-targeted compounds in pre-clinical or clinical development, this review will focus on a representative sample of anti-neoplastic agents, each illustrating some of the basic translational approaches to the development of more specific and less toxic therapy. The products to be discussed will include the tyrosine kinase inhibitors, such as STI571 (Gleevec) and ZD1839 (Iressa); antibody-based inhibitors of growth factor signaling pathways, such as Herceptin; and immunotherapeutic agents that target specific cell surface molecules, such as Rituximab (Rituxan) and Edrecolomab (Panorex). The field of rational cancer therapeutics is an exciting and rapidly changing field, and as will become evident, it is also a discipline that must take on the daunting task of synthesizing basic science observations into clinical decision making.

Protein Tyrosine Kinase Inhibitors

STI571 (Gleevec): CML and Philadelphia chromosome positive ALL

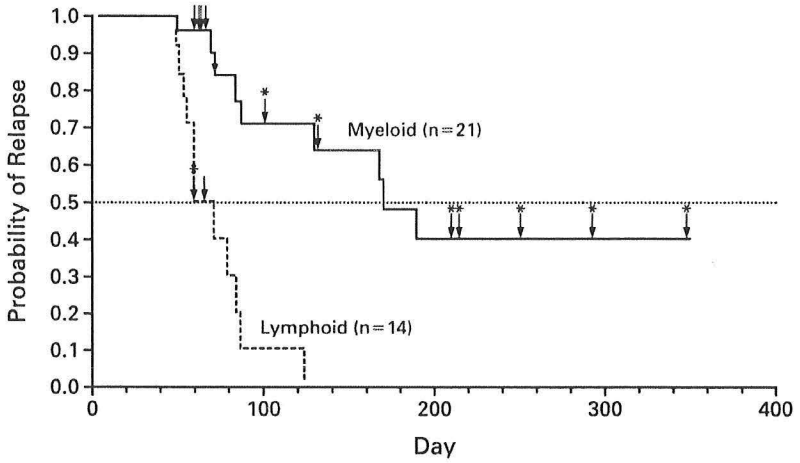
Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder in which the malignant cells contain the chimeric gene *BCR/ABL*, the product of a reciprocal translocation between the Abl gene on chromosome 9 and the Bcr gene of chromosome 22 (1-3). Patients with CML exhibit pronounced granulocyte expansion with preserved myeloid differentiation in the initial stages of the disease, generally referred to as chronic or stable phase CML. Although this chronic phase may persist for 3-5 years or longer with standard therapy, the disease inevitably progresses to an acute leukemia (blastic phase or blast crisis) generally resistant to treatment. Allogeneic bone marrow transplantation (BMT) during the early phases of the disease remains the only curative treatment for CML at this time; however, only a relatively modest proportion of patients has an appropriately HLA-matched donor. Fortunately, the successful development of mouse models for CML provided considerable support for the hypothesis that *BCR/ABL* played a causative agent role in CML (4-8). This led investigators to therapeutically target the Bcr/Abl fusion protein, a non-receptor tyrosine kinase. The result of this effort is a compound called STI571 (Gleevec®, Novartis), a rationally designed 2-phenylaminopyrimidine compound that potently inhibits the Bcr/Abl tyrosine kinase (9, 10).



Chemical structure of STI571. Figure reprinted from (11).

Generally, tyrosine kinase inhibitors target the region of the enzyme that binds ATP, which is required for its catalytic activity. Specificity lies in developing inhibitors that recognize nuances in the amino acid composition in the neighborhood of the ATP binding region. Recently, the crystal structure of STI571 bound to the c-Abl catalytic domain has been reported, and it confirms that indeed the drug binds in the ATP-binding pocket (12). In pre-clinical studies, STI571 induced profound apoptosis and growth suppression of Bcr/Abl-expressing cells and tumor xenografts, with little effect on cells expressing other oncogenes (9, 10). These findings demonstrated that STI571 had a remarkably specific effect against Bcr/Abl-expressing cells, and provided solid rationale for next studying this compound in humans with *BCR/ABL*-positive leukemia.

Initial human clinical trial experience with STI571 has also been equally impressive. Patients with CML that had failed standard interferon-α therapy were given STI571, administered orally at an average dose of 400 mg daily. Almost all patients achieved a complete hematologic response, characterized by normalization of peripheral white blood count (WBC) and platelet count (13). Approximately one third of patients experienced a major cytogenetic response-defined as a decrease in *BCR/ABL* positive metaphases to 35% or less-and 13% patients achieved normal cytogenetics. The results of STI571 in the treatment of advanced and therapy-resistant CML was less effective, but still remarkable given the limited therapeutic options normally available to such patients. Fifty-five percent of patients with CML in myeloid blast crisis responded to STI571 treatment, 19% of them achieving a complete hematopoietic response (14). In patients with CML lymphoid blast crisis or with acute lymphoblastic leukemia containing BCR/ABL (Philadelphia chromosome positive ALL), 70% had some degree of hematopoietic response, including 20% with complete hematopoietic responses. About one third of the patients with myeloid blast crisis had responses durable for almost one year, but almost all patients with lymphoid blast crisis and Philadelphia chromosome positive ALL failed STI571 therapy within 3-4 months (14). At present it is unknown why the durability of response to STI571 differs between myeloid and lymphoid blast crisis patients.



Time to Relapse in Patients with Myeloid or Lymphoid Blast Crisis Who Had a Response to STI571.

Arrows with asterisks indicate patients still enrolled in the study and in remission at the time of the last follow-up; arrows without asterisks indicate the day on which patients were removed from the study.

Figure reprinted from (14).

STI571 therapy was generally very well tolerated in these studies. The most commonly observed side effects from STI571 were nausea, edema, liver function test elevations, myalgia, thrombocytopenia, and neutropenia (13, 14). Generally, however, these side effects were mild and could be managed with some adjustment of the STI571 dose. This side effect profile compares quite favorably to standard interferon-α therapy, which is associated with intolerable constitutional side effects such as fatigue, nausea, myalgia, and anorexia, in 30-35% of CML patients.

DRUG-RELATED ADVERSE EVENTS ACCORDING TO THE DAILY DOSE OF STI571.*

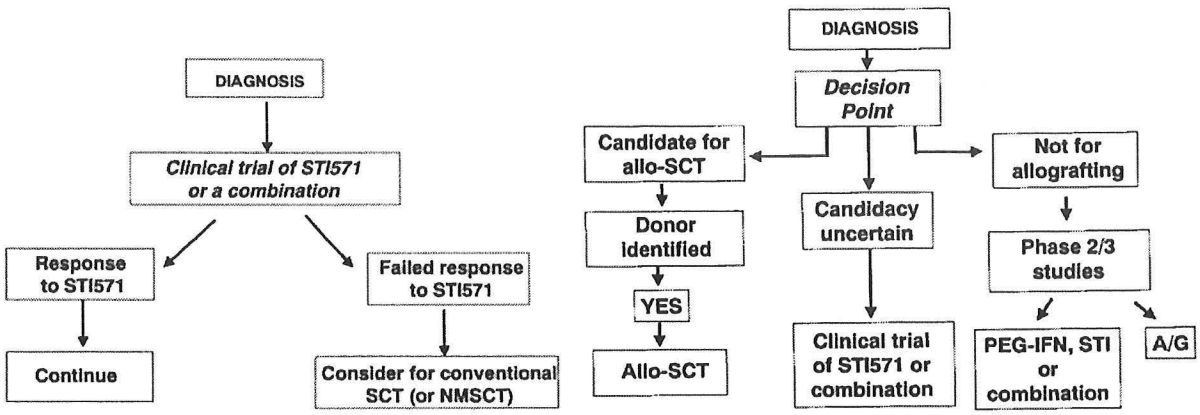
ADVERSE EVENT	25–140 mg (N=14)		200–300 mg (N=23)		350–500 mg (N=18)		600–1000 mg (N=28)		TOTAL (N=83)
	GRADE 1 OR 2	GRADE 3 OR 4	GRADE 1 OR 2	GRADE 3 OR 4	GRADE 1 OR 2	GRADE 3 OR 4	GRADE 1 OR 2	GRADE 3 OR 4	GRADES 1–4
	% of patients								no. (%)
Nausea	21	0	30	0	50	0	59	0	36 (43)
Myalgias	21	0	52	0	33	6	28	14	34 (41)
Edema	21	0	22	0	33	0	55	7	32 (39)
Diarrhea	14	0	4	0	33	0	38	3	21 (25)
Fatigue	14	0	22	0	11	0	24	3	17 (20)
Rash	7	0	17	0	11	0	28	3	16 (19)
Dyspepsia	14	0	13	0	28	0	17	0	15 (18)
Vomiting	0	0	13	0	11	0	34	0	15 (18)
Thrombocytopenia	0	0	4	0	11	6	7	24	13 (16)
Neutropenia	0	0	9	4	6	6	0	24	12 (14)
Arthralgias	0	0	4	0	6	0	28	3	11 (13)

*The adverse events listed here were considered to be related to STI571 and were reported in more than 10 percent of patients. A grade of 1 indicates a mild adverse effect, a grade of 2 a moderate effect, a grade of 3 a severe effect, and a grade of 4 a life-threatening effect.

Table reprinted from (13).

Therefore, the initial results of clinical studies of STI571 in *BCR/ABL* positive hematologic are quite encouraging, and suggest that STI571 might be as effective as standard interferon therapy, but with a more favorable side-effect profile. However, many questions remain about the implications of STI571 therapy, particularly whether the impressive results of STI571 observed so far will remain durable, and whether any CML patients will be cured with STI571. Recently, bone marrow cells from CML patients resistant to STI571 treatment were analyzed to determine possible mechanisms of resistance. In 3 of 9 patients, there was evidence of BCR/ABL gene amplification (15), consistent with what had been observed when Bcr/Abl-expressing cell lines had been exposed to sub-therapeutic concentrations of STI571 for prolonged periods (16-18). In the other 6 patients a single mutation was found in the *BCR/ABL* gene causing a single amino acid change in a region of the ATP binding pocket previously identified interacting with the STI571 compound (15). Whether such a mechanism of resistance will remain a relatively rare phenomenon or an inevitable one is currently unknown. However, these results suggest that Bcr/Abl may still remain a viable therapeutic target, providing some impetus to consider STI571 in combination with other therapy in future clinical trials.

The rational development of the kinase inhibitor STI571 for CML represents an important therapeutic advance and also serves as a paradigm for what can be achieved when basic science insights into a disease are applied towards a translational goal. What lies ahead is learning how STI571 will affect the natural history of CML, and how it should be integrated into decisions regarding BMT. As discussed earlier, allogeneic BMT remains the only proven curative treatment for patients with CML, and the best outcome is obtained with patients are transplanted within the first year of diagnosis (19). This suggests the possibility that like with interferon-α, treatment of patients with STI571 beyond one year in the hope that such therapy might be curative may carry the risk of decreased cure with allogeneic BMT. The current dilemma regarding how best to integrate STI571 into the treatment plan for CML patients is depicted on the following page.



Different treatment approaches for patients with newly diagnosed CML. Figure reprinted from (19).

Here the authors depict two different treatment strategies in which a trial of STI571 is given first priority (left), or an approach in which the established curative potential role of allogeneic BMT is emphasized (right), with STI571, interferon (INF), and/or auto BMT (A/G) reserved for non-BMT candidates (19). This lack of consensus, even among experts in the field, illustrates the current uncertainty in formulating treatment plans for patients with available bone marrow transplant donors. Therefore, until more mature data is available from current STI571-based clinical trials, the formulation of treatment strategies for patients with CML will remain a complex challenge for both physician and patient.

STI571: other solid tumors

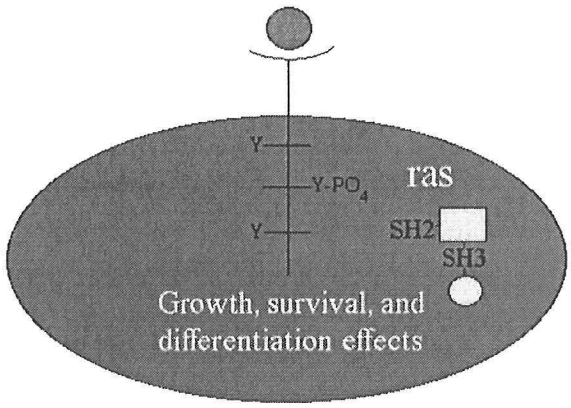
Although STI571 is a highly specific tyrosine kinase inhibitor, this compound is also a potent inhibitor of some other cellular tyrosine kinases besides Bcr/Abl and c-Abl. Platelet-derived growth factor receptor (PDGFR), c-kit, and ARG are protein tyrosine kinases have also been demonstrated to be inhibited by this compound (9, 20-22). This observation has recently led investigators to explore STI571 treatment in malignancies associated with constitutive activation of these signaling pathways. One recent success story has been the use of STI571 in patients with the sarcomas of the gastrointestinal stromal tumor (GIST) class. Mutations in c-kit had been identified in most of these tumors, which result in the constitutive activation of c-kit in a ligand-independent fashion (23, 24). When patients with locally advanced and/or metastatic GIST have been treated with therapeutic doses of STI571, marked tumor reduction has occurred, a significant achievement considering the extremely poor outcome expected with standard chemotherapy (25). Although it is not expected that STI571 alone will be curable in advanced GIST patients, the clinical success of this compound in targeting a signaling pathway (c-kit) implicated in GIST pathogenesis is another validation of the rational, targeted approach to cancer therapy. Research is ongoing to determine whether interference with c-kit signaling will have a therapeutic effect in other tumors known to express c-kit.

Because STI571 has also been demonstrated to inhibit the PDGFR tyrosine kinase (9), investigators have also begun to explore whether interference with this signaling pathway might have biologic significance in cancer. Sarcomas of intermediate malignant potential such as dermatofibrosarcoma protuberans and giant cell fibroblastoma have been demonstrated to have a translocation resulting in the juxtaposition of the *COLIA1* and *PDGFB* genes (26), which leads to constitutive PDGFR activation by an autocrine mechanism (27). STI571 has been shown to inhibit the growth of cells containing the *COLIA1/PDGFB* fusion gene (28), and induce apoptosis of dermatofibrosarcoma protuberans tumors in mouse tumor xenografts (29). Dysregulated PDGFR signaling has been implicated in the pathogenesis of glioblastoma multiforme and tumors of the malignant astrocytoma class (30-34), tumors which generally are highly resistant to current therapy. STI571 treatment has been shown to inhibit the proliferation of human brain tumor cell lines in a mouse xenograft model (35), suggesting the possibility of a therapeutic role for this compound. It will be of considerable interest to learn

whether patients with tumors dependent on the PDGFR for growth or viability will experience the same success with STI571 treatment as those with Bcr/Abl or c-kit-related malignancy.

Inhibition of the EGFR tyrosine kinase: ZD1839 (Iressa)

The epidermal growth factor receptor (EGFR, Erb1) is the first and best-studied member of the EGFR family of protein tyrosine kinases. The EGFR is a classic example of a receptor tyrosine kinase, which coveys extracellular signals to the nucleus by activating a cascade of intracellular signaling molecules. Upon binding its ligand EGF, the EGFR tyrosine kinase becomes activated and forms dimers with another EGFR molecule, or other EGFR family member. Kinase activation leads to autophosphorylation of specific tyrosine residues on the cytoplasmic region of the EGFR, and these tyrosine phosphorylated residues then become binding sites for specific signaling molecules and adapter proteins that contain src homology two (SH2) protein domains. Once bound to the activated EGFR receptor, these molecules in turn become tyrosine phosphorylated by the EGFR, leave the receptor, and mediate a variety of downstream signaling events. The end result of receptor activation is the induction of genes important for proliferation, adhesion, differentiation, and survival. Ras, an important early target for EGFR-dependent signaling, is localized on the cytoplasmic side of the cell surface, distant from many cytoplasmic signaling molecules (36). Ras becomes activated when adapter molecules, drawn to the activated EGFR, act as shuttles for ras regulatory proteins (via src homology 3 (SH3) domain interactions) (37-40).



The EGFR is expressed on a wide variety of cell types and increased expression and activation of this receptor has been reported in many human tumors, particularly those of epithelial origin. In some cases, dysregulated EGFR activation occurs by an autocrine mechanism, in which tumors secrete EGF ligand. Other tumors have been shown to express constitutively active EGFR mutants or contain multiple copies of the EGFR gene (41). Extensive basic science and pre-clinical data using EGFR mutants and anti-EGFR neutralizing antibodies have demonstrated that interference with EGFR-dependent signaling impairs the growth and survival of a variety of human tumor cell lines and xenografts (reviewed in (42)). These findings, and the observation that altered EGFR signaling in tumors was often associated with sub-optimal treatment outcomes and a poorer prognosis (43), made inhibitors of the EGFR tyrosine kinase an attractive therapeutic target. Although many EGFR tyrosine kinase inhibitors are in various stages of pre-clinical and clinical development, probably the best studied so far is the EGFR kinase inhibitor ZD1839 (Iressa).

ZD1839 (Iressa): 4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy) quinazoline

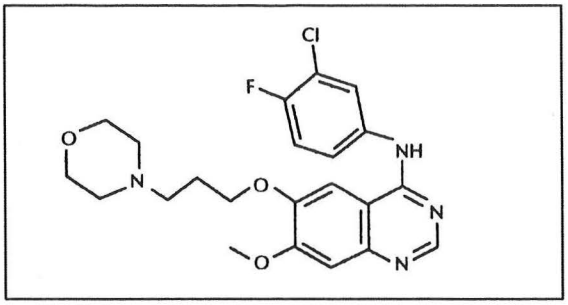
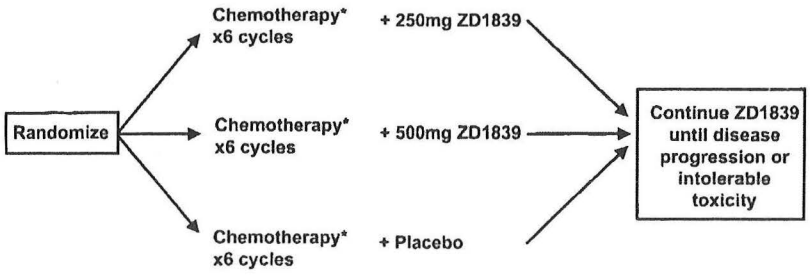


Figure reprinted from (44).

ZD1839 is in the anilinoquinazoline class of kinase inhibitors, designed to inhibit EGFR by interfering with ATP binding (45). This orally administered drug has shown fairly specific inhibition of the EGFR, although a recent study reported ZD1839 may also inhibit the activation of other EGFR family members (46), perhaps by an indirect, EGFR-dependent mechanism. In limited clinical trial experience thus far, ZD1839 has shown some activity as a single agent, particularly in non-small cell lung cancer (NSCLC), where responses have ranged from 10-25% (42, 47). Several groups have also reported stable disease in about another one third of patients, although the significance of this is hard to interpret from phase 1 trials. Generally, Iressa is well tolerated, although side-effects such as diarrhea, abnormal liver function tests, and a fairly characteristic skin rash have been observed, and are often dose related (47). Because of pre-clinical data suggesting a synergistic effect of EGFR inhibitors with chemotherapy or radiation (reviewed in (42, 44)), Iressa has also been combined with chemotherapy in patients with solid tumors. In NSCLC, addition of ZD1839 to Carboplatin/Taxol chemotherapy resulted in a 25% response rate, and some additional patients were felt to have stable disease (48). Another EGFR tyrosine kinase inhibitor, OSI774, has shown some activity in advanced head and neck cancer and Cisplatin-refractory NCSLC patients, with a response rate of approximately 6% and 10%, respectively, with another one third of patients experiencing stable disease (49, 50). The side effect profile of OSI774 appears to be quite similar to ZD1839, including the associated skin rash. Other EGFR-specific tyrosine kinase inhibitors are in pre-clinical and phase 1 development, including CI-1033, PKI-166, and GW2016. Much remains to be understood about the best dose schedule of the EGFR kinase inhibitors, and whether these drugs will prove synergistic with standard chemotherapy in a clinically significant way.

The results of randomized trials currently in progress, such as the one outlined below, will play a crucial role in defining the role of EGFR kinase inhibitors in the treatment of solid tumors. In this trial, patients with inoperable stage 3 or stage 4 NSCLC have been randomized to receive a standard chemotherapeutic regimen alone, or the same chemotherapy with one of two maintenance doses of ZD1839.



Randomized trial schema for patients with inoperable stage 3 or stage 4 NSCLC testing efficacy of ZD1839. Figure reprinted from (42).

This trial has already met accrual, and an initial analysis should be available within the next year. Since patients were not required to have EGFR positive tumors for study entry, the results of this trial will help establish whether EGFR inhibition alone or in combination will have broader application than just in certain select high EGFR-expressing tumors. It is likely that the EGFR is only one of several non-essential pathways active in epithelial cancers, suggesting that treatment strategies for these tumors may grow ever more complex as additional rational targets are identified.

Antibodies Targeting Specific Growth Factor Signaling Pathways

Herceptin and the human growth factor receptor 2 (HER2) in breast cancer

The human growth factor receptor 2 (HER2, also known as Erb2) is a protein tyrosine kinase in the EGFR family that is overexpressed in 20-30% cases of breast cancer (51). The most common mechanism leading to HER2 protein overexpression is amplification of the HER2 gene, although there are rare examples of HER2 gene amplification without protein overexpression, and HER2 protein overexpression without gene amplification. Increased HER2 expression leads to constitutive HER2 activation in a ligand-independent fashion, perhaps secondary to auto-receptor activation due to excessive receptor crowding (52). In cell culture systems, dysregulated HER2 expression leads to cellular transformation, resistance to apoptotic signals, and enhanced proliferative capacity. Breast cancers that overexpress HER2 tend to have an increased proliferative index, chromosomal aneuploidy, and are more often negative for progesterone and estrogen receptor (53). As might be expected, HER2 positive breast cancers also have an increased risk of lymph node involvement and distant metastasis (53, 54). The biologic features of HER2 overexpression have made HER2 an important negative prognostic indicator, with several studies demonstrating a decreased disease-free and overall survival of patients with HER2-positive breast cancer (55-57). There is also some evidence that HER2-positive breast cancers might respond differently to chemotherapy and hormonal manipulation than HER2 negative tumors (58), although this is controversial.

It not surprising that HER2 expression status has now become a standard part of the clinical workup of patients with newly diagnosed breast cancer, although healthcare institutions differ in their methods to determine HER2 status. Some assess HER2 expression directly by immunohistochemistry (IHC), using antibodies to HER2 protein, while others use fluorescent in-situ hybridization (FISH), looking for evidence of HER2 gene amplification. Generally these assays give similar results, but the best correlation is usually at the extremes of HER2 expression, as seen in the table below. Of particular interest is the observation that only about a quarter of 2+ IHC positive breast cancers are positive for HER2 gene amplification by FISH.

IHC	#cases	% FISH+
0	214	3
1+	30	7
2+	88	24
3+	197	89

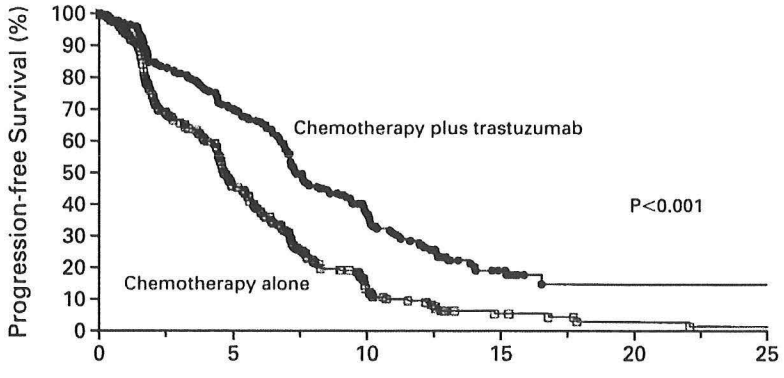
Comparison of HER2 status as determined by IHC and FISH. Table reprinted from Buehler et al, ASCO 5/2000, abstr. #294.

Given the important role of the HER2 in the pathophysiology of breast cancer, a considerable effort was undertaken to develop inhibitors of this signaling pathway. Pre-clinical studies of anti-HER2 antibodies showed considerable activity against human breast cancer cell lines and tumor xenografts (59-61). The culmination of this investigation was the release of Herceptin® (Trastuzumab), a genetically-engineered, humanized anti-HER2 monoclonal antibody. In human breast cancer clinical trials, Herceptin has shown

activity in patients with heavily pretreated HER2-positive metastatic breast cancer, with a response rate of approximately 12-15% (62, 63). Additional patients have also experienced some disease stabilization. The duration of response to Herceptin has ranged between 5-8 months. Of note, a reasonable proportion of patients with 2+ HER2 IHC staining were included in some of these trials, and those patients had a response rate of 6% with Herceptin, compared to 18% for the 3+ HER2 patients (63). These results suggest that HER2 analysis by FISH may be a better predictor of response to Herceptin treatment.

Although the response rate with Herceptin monotherapy has been modest, it compares favorably with standard chemotherapy salvage rates in heavily pre-treated patients, without the customary chemotherapy-associated side effects. Herceptin is generally quite well tolerated. Fever and chills are the most common adverse effects, most commonly seen during the first infusion. These adverse effects generally respond to Tylenol and Benadryl, which are usually given prophylactically as well. Unexpectedly, cardiac dysfunction has been observed with Herceptin therapy, occurring in about 5% of patients receiving Herceptin as a single agent and up to 27% of patients receiving Herceptin in combination with an anthracycline, like Adriamycin (64). Because this humanized antibody contains only about 5% mouse-derived protein sequence (65), the development of human anti-mouse antibodies during the course of Herceptin treatment has been quite uncommon. The mechanism of Herceptin's anti-tumor effect is incompletely understood, but appears to be multifactorial. Besides interfering with HER2 activation, Herceptin has also been shown to accelerate the degradation of HER2 protein, induce expression of cell cycle inhibitors, and activate immune-mediated cytotoxic pathways (reviewed in (66)).

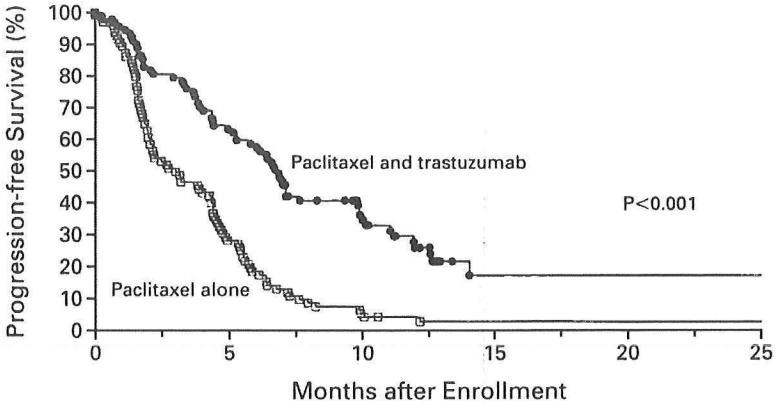
Herceptin has shown its most impressive efficacy when it has been used in combination with chemotherapy. This was first appreciated when Herceptin was combined with cisplatin chemotherapy in heavily pre-treated metastatic breast cancer patients. In this phase 2 trial, almost one third of patients responded to this therapeutic combination, more than would be expected from single-agent Cisplatin salvage chemotherapy in this setting (67). Furthermore, the response rate was greater than expected with Herceptin monotherapy, and was without additional toxicity over that expected with Cisplatin alone. The improved efficacy of Trastuzumab in combination with chemotherapy led to a phase 3 trial in which patients with previously untreated metastatic breast were randomized to receive chemotherapy alone or chemotherapy plus Trastuzumab.



No. at Risk				
Chemotherapy plus trastuzumab	235	152	63	15
Chemotherapy alone	234	103	25	6

Progression-free survival of patients with HER2+ breast cancer randomized to receive Herceptin (Trastuzumab) plus chemotherapy, or chemotherapy alone. Figure reprinted from (64).

Addition of Herceptin to chemotherapy improved overall response rate (50% vs. 32%), duration of response (9.1 vs. 6.1 months), and resulted in longer overall survival (25 vs. 20 months) (64). Of the chemotherapeutic agents studied, the most synergy was observed with Taxol (Paclitaxel).



No. at Risk			
Paclitaxel and trastuzumab	92	54	23
Paclitaxel alone	96	26	5

Progression-free survival of patients with HER2+ breast cancer randomized to receive Herceptin (Trastuzumab) plus Taxol (Paclitaxel), or chemotherapy alone. Figure reprinted from (64).

As in the single agent studies, Herceptin was associated with increased cardiac toxicity, worse in those patients receiving Herceptin in combination an anthracycline (64). Exactly how Herceptin acts synergistically with chemotherapy is unknown. However, one likely mechanism is that Herceptin blocks constitutive HER2 activation, a pathway normally important for HER2 overexpressing tumors to resist chemotherapy-induced apoptotic signals. The encouraging results achieved with Herceptin will likely lead to numerous additional studies of Herceptin in HER2 overexpressing breast cancer patients, particularly in the adjuvant setting.

Targeted antibody therapy for the epidermal growth factor receptor (EGFR)

Besides specific tyrosine kinase inhibitors such as ZD1839 discussed previously, there are also antibody-based approaches to target the EGFR signaling pathway. Antibodies blocking EGFR activation showed considerable promise in pre-clinical studies of human tumor cell lines and tumor xenografts (68, 69), leading to the release of a human/mouse chimeric antibody called C225 (Cetuximab) for human cancer clinical trials. Although C225 has activity in some epithelial cancers as a single agent, much of the recent investigation has focused on combining this agent with chemotherapy. In a phase I trial of patients with recurrent head and neck cancer receiving C225 in combination with Cisplatin, several tumor responses were observed, including some in patients that had failed prior Cisplatin-based therapy (70). Recent studies have suggested that C225 may also act synergistically with radiation in head and neck cancer (71, 72). Although incompletely understood at present, the mechanism of C225 anti-tumor activity will likely be similar to that reported with Herceptin, although a recent study suggests C225 may also interfere with tumor-associated angiogenesis (73). Compared to Herceptin, less is presently known about the clinical future of targeted EGFR antibody therapy, but additional anti-EGFR products, such as ICR62 and E7.6.3, are also in pre-clinical and early clinical development, suggesting this area will be the subject of even more intense clinical investigation in the future.

Antibodies Targeting Specific Cell Surface Molecules: Hematologic Malignancies

Rituxan (Rituximab)

About three-quarters of non-Hodgkin's lymphomas (NHL), and most cases of chronic lymphocytic leukemia (CLL) and Waldenstrom's macroglobulinemia, are of B-lymphocyte origin, making a B cell-targeted antibody an attractive treatment strategy for hematologic malignancies. Rituxan, the first FDA-approved monoclonal antibody therapy for lymphoma, is a mouse/human chimeric antibody against the B-cell surface antigen CD20, a molecule important for B-cell growth and differentiation (74). CD20 is expressed in approximately 95% of NHL cases, so initial trials of Rituxan focused on the treatment of patients with recurrent low-grade (indolent) NHL. The first large phase 3 trial of this compound demonstrated impressive activity in low-grade NHL, with almost 50% of patients responding to Rituxan, administered weekly at a dose of 375 mg/m^2 for a total of four doses (75). The mean duration of response was almost one year. The results were particularly impressive since many of the patients had extensive and heavily pre-treated disease. Since the initial success of this study, numerous other trials of Rituxan alone or in combination with chemotherapy in NHL have been conducted or are in progress. One recent study investigated whether Rituxan had a role in the treatment of low-grade NHL patients relapsing after an initial response to this drug, and found some surprising results. In this study, 40% of patients had a response to Rituxan re-treatment, including 11% with a complete response.

Clinical Response in Rituximab Re-Treatment NHL Patients

	Patients						
	Total	Complete Response		Partial Response		Overall Response		95% CI for CR
Patient Group	No.	No.	%	No.	%	No.	%	and PR (%)
Intent-to-treat	60	6	10	17	28	23	38	26-51
Assessable	57	6	11	17	30	23	40	28-53

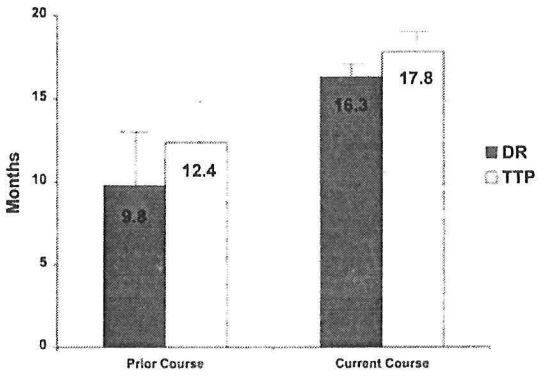
Figure reprinted from (76).

Interestingly, the median duration of response (DR) and time to progression (TTP) of Rituxan re-treated patients were longer than the patients had experienced during their first course of Rituxan (76). Again, most of these patients had received considerable prior therapy, including some who had received bone marrow transplantation.

Prior Lymphoma Therapy in Rituximab Re-Treatment NHL Patients

	No. of	No. of Courses of Therapy	
Type of Prior Therapy	Patients	Median	Range
Chemotherapy	58	2	1-6
Rituximab	60	1	1-2
Other bioimmunotherapy	18	1	1-1
Radiotherapy	18	2	1-5
ABMT	9	1	1-1
ABMT, PSC	5	1	1-1
ABMT, BM	4	1	1-1
All therapies	60	4	2-11

Abbreviations: ABMT, autologous bone marrow transplant; PSC, peripheral-blood stem cells; BM, bone marrow.



DR and TTP (with upper 95% confidence interval stems) in the current (Kaplan-Meier estimates) and previous course of rituximab in patients responding to rituximab therapy.

Clinical features of low-grade NHL patients re-treated with Rituxan (left), and clinical outcome (right). Figures reprinted from (76).

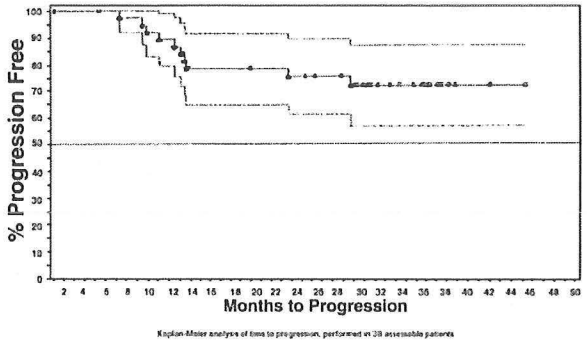
Studies of Rituxan in combination with traditional chemotherapeutic regimens in low-grade NHL have also been encouraging. In a recent trial investigating Rituxan in combination with standard CHOP chemotherapy, the overall response rate was 95%, with 55% experiencing a complete response (77). The median duration of response had not yet been reached, but responses were quite durable with 74% of patients in remission at 29+ months. Importantly, this particular study included patients with negative prognostic factors such as bulky disease, elevated LDH, age greater than 60, and involvement of extranodal sites, including bone marrow.

Response to Therapy

		Response					
		CR		PR		CR + PR	
Patient Group	No.	No.	%	No.	%	No.	%
Intent-to-treat	40	22	55	16	40	38	95
Extranodal disease	29	15	52	14	48	29	100
Elevated LDH	11	5	45	6	55	11	100
Bone marrow involvement	22	11	50	11	50	22	100
Age ≥ 60 years	11	5	45	6	55	11	100
Bulky disease*	14	4	29	10	71	14	100

Abbreviation: LDH, lactate dehydrogenase.

*Patients who had a lesion measuring at least 5 cm (but no more than 10 cm) in the largest diameter.



Kaplan-Meier analysis of time to progression, performed in 28 assessable patients

Clinical features of patients with low-grade NHL receiving CHOP/Rituxan therapy (right), and their progression-free survival (left). Figures reprinted from (77). The lighter curves (at right) represent the confidence intervals for the progression-free survival curve.

It should be noted that similar complete response rates have been reported in studies using CHOP chemotherapy alone in low grade NHL (78, 79). Whether the addition of Rituxan will prove superior to CHOP will require a randomized trial with lengthy follow-up, since the median survival of patients with low-grade NHL is 8-10 years. Of particular interest in this and other Rituxan-based studies is that a substantial proportion of patients became polymerase chain reaction (PCR) negative for the t(14;18) chromosomal translocation (77, 80, 81). Translocations involving t(14;18) are detectable in over three-quarters of patients with low-grade NHL, and are associated with rearrangement of the proto-oncogene, *bcl-2*. Approximately 50% of patients treated with Rituxan alone or CHOP/Rituxan became negative for the *bcl-2* rearrangement by PCR, and those patients experienced a higher response rate than patients who did not become *bcl-2* negative (81). Bcl-2 negativity has not been seen in association with standard CHOP therapy (82). Whether a survival benefit will be seen in low-grade NHL patients rendered *bcl-2* negative with Rituxan-based therapy will require longer follow up. The issue of *bcl-2* negativity in low-grade NHL is of particular importance in the context of autologous bone marrow transplantation (BMT), which has been extensively used in this disease, but is generally not felt to be curative. In studies of autologous BMT for low-grade NHL in which anti-B cell antibodies were used to purge the BM autograft ex vivo, improved survival was seen in those patients whose BM product could be purged to *bcl-2* negativity (83). Whether Rituxan can be used alone or in combination to accomplish "in vivo purging" of patients with low-grade NHL is unknown, but this question will likely be addressed in future Rituxan clinical trials.

Rituxan has also been used in the treatment of high-grade (aggressive) NHL. In a large phase 2 trial, Rituxan was combined with CHOP chemotherapy, resulting in an overall response rate of 94% (84). Approximately 60% of patients achieved a complete remission and about one third achieved a partial response, at least as good as expected from CHOP therapy alone. At the time of publication the median duration of response still had not been reached, with a median follow up of 26 months. To determine whether CHOP/Rituxan is superior to CHOP alone in aggressive NHL, the French clinical trial group, GELA, recently reported an initial analysis of a randomized trial comparing these two regimens in previously untreated elderly patients with large cell NHL (85). Patients receiving CHOP/Rituxan experienced a higher rate of complete

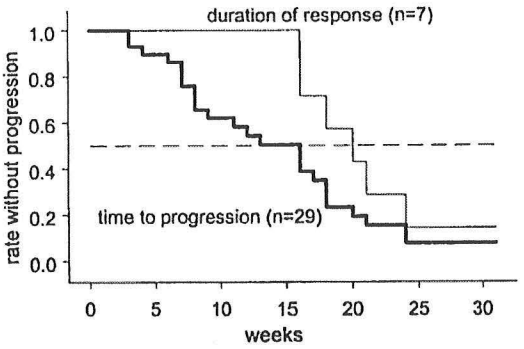
response (76% vs. 60%), and one-year survival (83% vs. 68%) than CHOP alone. Although these are encouraging results, definitive conclusions about the efficacy of CHOP/Rituxan will require longer follow-up, and appropriate confirmation from other randomized trials already in progress.

Given the encouraging results of Rituxan in low-grade and aggressive lymphoma, its utility in other B-cell hematologic malignancies is currently being investigated. Rituxan as a single agent has been demonstrated to have variable activity in previously treated patients with chronic lymphocytic leukemia (CLL). In one study only one of seven patients with fludarabine-resistant CLL had a partial response to Rituxan (86), while in a more recent study approximately 25% patients achieving a partial response (87). In this latter study, almost half the patients experienced a 50% reduction in lymphocytosis that was durable for at least one month. The overall response to Rituxan was relatively short, with a median remission duration of about 5 months, likely because this patient cohort included many extensively treated patients.

Patient demographics and pretreatment characteristics

Characteristic	No.	%
Median age, y (range)	60 (47-75)	
Sex, male/female	20/10	67/33
Karnofsky index		
Less than 80%	6	20
Between 80% and 100%	24	80
Median time since first CLL diagnosis, y (range)	5 (1-19)	
Histological type		
B-CLL	29	97
B-PLL	1	3
Binet stage at study entry		
B	11	37
C	19	63
B symptoms	14	47
Bone marrow infiltration (n = 28)		
Nodular	13	46
Mixed	6	21
Diffuse	9	32
No. of previous treatments for CLL		
1	1	3
2	5	17
3	8	27
4	4	13
More than 4	12	40
Time since end of last CLL treatment		
No more than 12 wk	10	33
3-6 mo	6	20
6-12 mo	8	27
1-2 y	3	10
More than 2 y	3	10

PLL indicates prolymphocytic leukemia.



Response duration and time to disease progression (Kaplan-Meier estimation).

Clinical features of previously treated CLL patients treated with Rituxan (left) and outcome (right). Figures reprinted from (87).

These results suggest that prolonged treatment with Rituxan may be required to achieve a more durable response in CLL, or that Rituxan will need to be used in combination with standard chemotherapeutic agents.

Rituxan has also demonstrated significant activity in another CD20 positive B-cell hematologic malignancy, Waldenstrom's macroglobulinemia. Approximately 30% of Waldenstrom's patients have achieved a partial response with Rituxan (88, 89), and about 50% of patients had improvement in hematocrit and platelet counts, resulting in a decrease in transfusion requirement (89). Durability of response has generally been about 7-8 months after four weekly doses of Rituxan. Given these encouraging results, studies of Rituxan in combination with chemotherapy in Waldenstrom's are currently underway. Rituxan has also been tried in

mantle cell NHL, an intermediate grade NHL not curable with standard chemotherapy. Rituxan as a single agent has resulted in a response rate of 22-38% (90-92), but with only modest durability, about one year. Trials are currently under way to determine if Rituxan might be added to standard chemotherapy or BMT strategies to improve the outlook for this otherwise unfortunate group of patients.

Rituxan is generally well tolerated, with the most common side effects associated with the initial infusion. Side effects such as fever, pruritis, and chills are usually easily managed and/or prevented by agents such as Tylenol and Benadryl. Alterations in blood pressure, conjunctivitis, bronchospasm, and cardiac arrhythmias have also been reported (93). Tumor lysis-like syndromes are uncommon, but have been reported, leading some physicians to use allopurinol and intravenous fluid hydration prior to administering Rituxan to patients with heavy tumor burdens. Another syndrome that appears to be distinct from classic tumor lysis is the "cytokine release syndrome." This syndrome has been reported in CLL patients with extensive peripheral blood lymphocytosis at the time of the Rituxan infusion, and in NHL patients with a peripheral blood "leukemic" phase (86). Affected patients develop a sepsis-like syndrome, with elevated liver function tests, and coagulation abnormalities resembling diffuse intravascular coagulation (DIC), accompanied by marked elevations of serum tumor necrosis factor-α and interleukin-6. Because these symptoms tend to occur in patients with pronounced lymphocytosis, it has been recommended that patients with peripheral lymphocytosis greater than 50 X $10^6/\mu l$ receive Rituxan at small initial doses, or receive prior chemotherapy for better immediate control of their disease (86).

Rituxan has been demonstrated to exert a broad range of effects, both on normal and malignant CD-20 positive cells. Rituxan has an inhibitory effect on B-cell proliferation and survival, and is also capable of eradicating B-lymphocytes through complement-mediated and antibody-dependent cell-mediated cytotoxicity. Rituxan has also been demonstrated to enhance chemosensitivity (94). Therefore, it is not surprising that normal CD-20 positive B-lymphocytes are depleted in most Rituxan-treated patients. Although B cell levels may not return to normal for almost a year after treatment, serum immunoglobulin levels are not affected (95), and like in animal models (96), patients receiving Rituxan do not appear to incur additional risk of infection. Interestingly, despite patients receiving multiple courses of Rituxan therapy, the development of antibodies against Rituxan have been rare.

Campath-1H

Campath-1H is a humanized monoclonal antibody against the cell surface membrane glycoprotein CD52, which is found on most lymphocytes and cells of the monocyte/macrophage lineage (97). Over the last several years, Campath has been increasingly used in a variety of clinical applications, including the treatment of autoimmune disease (97), modulation of the immune system for bone marrow and solid organ transplantation (98), and as therapy for certain hematologic malignancies. Campath-1H has been shown to have activity in CLL, T-cell prolymphocytic leukemia (T-PLL), NHL, and mycosis fungoides/Sezary syndrome (99). There have been several reported trials of Campath-1H in CLL patients, most of which have had advanced disease. A fairly wide range of response rates has been reported, probably because CLL patients with predominantly lymph node disease are less likely to respond to Campath-1H than those patients whose disease is mostly manifested in the peripheral blood and bone marrow (101). The reason for this difference is unknown, but could conceivably be related to accessibility of the CD52 antigen. In the largest series, about one third of fludarabine-resistant CLL patients responded to Campath-1H, two of the patients experiencing a complete response (102).

Campath-1H in chronic lymphocytic leukemia

Study	Patients, *n*	Schedule	Response (CR/PR)	Prior therapy
Osterborg *et al.*	9	5 IV / 4 SQ: 3 mg initial with escalation to 30 mg unitl CR or 18 wk	89% (3/5)	No previous therapy
Dyer *et al.*	6	10–20 mg test dose, then 30 mg tiw for 6–12 wk	83% (5/0)	Fludarabine, chlorambucil, prednisolone/prednisone, cyclophosphamide, pentostatin, doxorubicin, vincristine, epirubicin
Bowen *et al.*	6	SQ: 10 mg test dose, then 30 mg tiw for 6–12 wk	50% (0/3)	Fludarabine
Ginaldi *et al.*	12	Not specified	75% (4/5)	Not identified
Osterborg *et al.*	29	2-h IV infusion for 12 wk, inital dose 3 or 10 mg	42% (1/11)	Fludarabine, chlorambucil, prednisolone/prednisone, cyclophosphamide, doxorubicin, vincristine, epirubicin, interferon
Rawstron *et al.*	10	30 mg IV tiw × 6 weeks	70% (5/2)	Refractory disease
Keating *et al.*	92	30 mg IV over 2 h tiw for 4–12 wk	33% (2/29)	Refractory to fludarabine, alkylator exposed
Rawstron *et al.*	15	30 mg IV tiw × 6 weeks	–	Fludarabine, chlorambucil, prednisolone/prednisone, cyclophosphamide, doxorubicin, vincristine, pentostatin
Kennedy *et al.*	29	30 mg IV tiw until maximum response	59% (10/7)	Refractory to therapy including purine analogues
Ferrajoli *et al.*	30	IV 3 mg day 1, 10 mg day 2, 30 mg day 3, and 30 mg tiw for 12 wk thereafter	20% (1/5)	Refractory disease
Rai *et al.*	13	30 mg IV tiw for 16 wk	69% (3/6)	Not identified

CR, complete response; IV, intravenous; PR, partial response; SQ, subcutaneous; tiw, 3 times/wk.

Studies of Campath-1H in CLL. Table reprinted from (100)

It is likely that future study of Campath-1H in CLL will focus on patients with earlier stage CLL, and explore the efficacy of Campath-1H in combination with chemotherapy or other lymphocyte-targeted therapy such as Rituxan. Probably the most impressive responses to Campath-1H have been in patients with T-cell PLL, which is a fairly rare lymphoid malignancy that tends to be quite clinically aggressive and generally resistant to standard chemotherapeutic agents.

Campath-1H in T-cell prolymphocytic leukemia

Study	Patients, *n*	Date	Overall response	Prior therapy
Cazin *et al.*	9 T-PLL	1999	67% (6 CR)	Multiple prior regimens
Ginaldi *et al.*	12 T-PLL	1998	75% (7 CR/2 PR)	8 patients had disease refractory to prior therapy
Pawson *et al.*	15 T-PLL	1997	73% (9 CR/ 2 PR)	All failed to enter a CR with prior therapies

CR, comple response; PR, partial response; T-PLL, T-cell prolymphocytic leukemia.

Studies of Campath-1H in patients with T-cell PLL. Table reprinted from (100).

Approximately 70% of T-cell PLL patients responded to Campath-1H, many with complete responses, which is uncommon with standard therapies. Some of the patients in these studies were able to take advantage of their complete responses and were consolidated with BMT. Whether any T-cell PLL patients are cured with this approach will require longer follow up. Campath-1H also has activity in NHL, but response rates have tended to be lower than those reported for CLL, T-cell PLL, and mycosis fungoides. In a couple of recent studies the response rates in NHL ranged between 14-19%, likely due to the decreased efficacy of Campath-1H when lymphocytes are aggregated within lymph nodes compared to when they reside in the bone marrow and or peripheral blood (99, 103). In one study, Campath-1H was much more effective in clearing NHL cells from the bone marrow and peripheral blood (32% and 94% of patients, respectively) (99), suggesting a possible role for Campath-1H in depleting malignant cells from these sites prior to peripheral blood stem cell harvest and autologous BMT.

Since this drug depletes normal B- and T-lymphocytes, sometimes for up to 18 months after treatment (104), infectious complications associated with Campath-1H have been significant, even with appropriate

antimicrobial prophylaxis. Infections with CMV, HSV, pneumocystis, candida, aspergillus, and encapsulated bacteria have been reported in association with Campath-1H therapy (99). Although some of the increased infection risk is likely secondary to the patients' advanced disease at time of therapy, use of Campath-1H requires careful antibiotic prophylaxis and heightened clinical vigilance for early signs of infection. Apart from the infectious disease concerns, most of the remaining adverse effects with Campath-1H are associated with the infusion itself, including fevers, chills, rigors, and rash, which are usually managed with appropriate pre-medication and symptomatic treatment.

Other antibody-based therapy for NHL

The success of targeted therapies such as Rituxan and Campath-1H has led to an explosion of additional antibody-based products, now in various stages of clinical investigation (reviewed in (105)). In addition to anti-CD20-based antibody products, antibodies against lymphoid antigens CD22, CD37, CD19, and others are currently under study. In many cases investigators have begun to conjugate some of these targeted agents to toxins or isotopes in attempts to enhance anti-tumor activity.

Antibodies conjugated to isotopes and immunotoxins for NHL therapy

Antibody	Antigen	Isotope or toxin
Lym-1	HLA Class II	^{131}I, ^{67}Cu
LL2	Anti-CD22	^{131}I, ^{90}Y
RFBR	Anti-CD22	Deglycosylated ricin A chain
MB-1	Anti-CD37	^{131}I
Anti-idiotype	B-cell surface Ig	^{90}Y
Anti-B4-blocked ricin	Anti-CD19	Blocked ricin
IF5	Anti-CD20	^{131}I
B-1	Anti-CD20	^{131}I
IDEC-2B8 (ibritumomab)	Anti-CD20	^{90}Y

Conjugated antibodies for patients with lymphoid malignancy. Table reprinted from (106).

All of the radioimmunotherapy-based NHL-targeted therapies have an increased risk for bone marrow suppression, with thrombocytopenia being a dose-limiting factor for several of the agents. As might be expected, patients heavily pre-treated with chemotherapy and those with significant bone marrow involvement by NHL are at particular risk for myelosuppression from radiolabeled antibody treatment. One radioconjugated product, IDEC-Y2B8 (Zevalin®, Ibritumomab) has been investigated in several recent trials, some still in progress. IDEC-Y2B8 is a murine antibody that recognizes the same CD20 determinant as Rituxan, but is labeled with ^{90}Y, a beta-emitting isotope, amenable to outpatient therapy. This product has been recently studied in patients with quite advanced and refractory NHL, with some encouraging results.

In a recent phase 1/2 trial of previously-treated patients with NHL, IDEC-Y2B8 had a response rate of 82% (26% complete responses) in low-grade NHL, and 43% in intermediate NHL (107). Although there were only three patients with mantle cell NHL, none of them responded to IDEC-Y2B8. The overall response rate for the entire cohort (67%) was encouraging since many of the patients had extranodal involvement, and some of the patients had bulky disease. Durability was over one year in the responding patients. Generally IDEC-Y2B8 was well tolerated, and hematopoietic toxicity was manageable and seemed to correlate with the degree of bone marrow involvement by NHL. The development of human anti-mouse antibody was uncommon in this trial (2%), which the authors speculated might be due to the patients receiving unlabeled Rituxan before the IDEC-Y2B8 infusion, to improve tissue delivery (107). Interim analysis of a few other IDEC-Y2B8 trials have also shown encouraging results. Approximately 80% of low-grade NHL patients responded to IDEC-Y2B8,

compared to 44% of Rituxan-treated patients (108). In another study, IDEC-Y2B8 had a 46% overall response rate in patients with Rituxan-resistant low-grade NHL.

Radiolabeled antibodies: IDEC-Y2B8 clinical studies

Indication	Treatment	N[a]	Response rate (%)	TTP[b]
Relapsed or refractory B-cell NHL	Phase I/II IDEC-Y2B8 (pretreatment with unlabeled murine anti-CD20)	17 (14 single-dose)	ORR = 64 CR = 28 PR = 36	median 8 months (6–13 months)
Relapsed or refractory NHL	Phase I/II IDEC-Y2B8 (pretreatment with unlabeled rituximab)	51 total: 34 LG	ORR = 67 CR = 26 PR = 41 ORR = 82 CR = 27 PR = 56	Estimated median 11.7+ months
Relapsed or refractory, LG/F, or transformed	Phase III IDEC-Y2B8 vs. rituximab	90 total: 46 IDEC-Y2B8	ORR = 80 CR: N/A PR: N/A	N/A
Rituximab-refractory, follicular NHL	Phase III IDEC-Y2B8	24	ORR = 64 CR: N/A PR: N/A	N/A
Relapsed or refractory, LG/F, or transformed thrombocytopenic patients	Phase II IDEC-Y2B8	24 total: 22 evaluable	ORR = 64 CR: N/A PR: N/A	N/A

Note: ORR, overall response rate; CR, complete response; PR, partial response; LG/F = low-grade or follicular; NA = not available.

[a]*N* is the number of patients who received the treatment.

[b]TTP, time to progression among patients who responded to the treatment.

Summary of recent trials of IDEC-Y2B8 in patients with NHL. Table reprinted from (106).

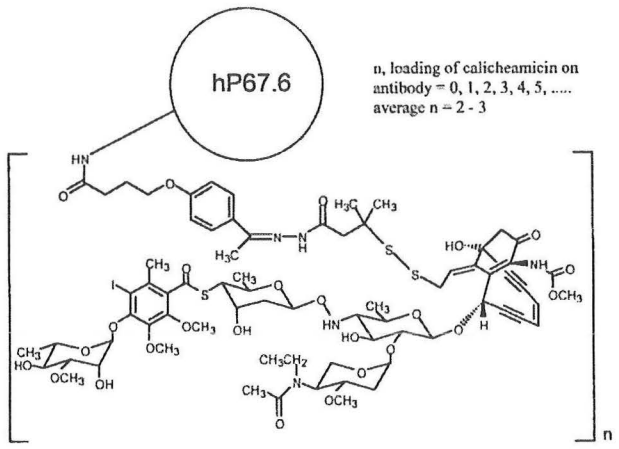
It will be interesting to see if these promising results are maintained during subsequent analyses of these trials, and whether IDEC-Y2B8 responses will be more durable than Rituxan, or result in any survival differences. Extensively-treated NHL patients, especially those who have had autologous BMT, are at increased risk for the development of myelodysplasia and secondary acute myelogenous leukemia (AML). Whether IDEC-Y2B8-treated NHL patients will also be at increased risk for the development of MDS/AML is currently unknown.

Over the next several years, there will be a vast amount of data generated from numerous single-agent and combination trials of antibody-based therapy for lymphoid malignancies. Although the choice of therapy will grow ever more complex, it is anticipated that advances in targeted therapy for these diseases will result in improved patient outcome, perhaps with less toxicity.

Targeted therapy for acute myelogenous leukemia (AML)

For AML relapsing after standard chemotherapy, traditional salvage options have been quite limited. Recently, an antibody targeting the cell surface glycoprotein CD33 has been developed, called Mylotarg (Gemtuzumab ozogamicin), which has demonstrated encouraging activity in relapsed or refractory AML. The rationale for developing a targeted antibody against CD33 is based on the observation that CD33 expression in myeloid cells tends to be inversely correlated with differentiation. That is, CD33 expression tends to be highest in immature myeloid cells, including most AML blasts, and lowest in maturing granulocytes (109). Importantly, CD33 is

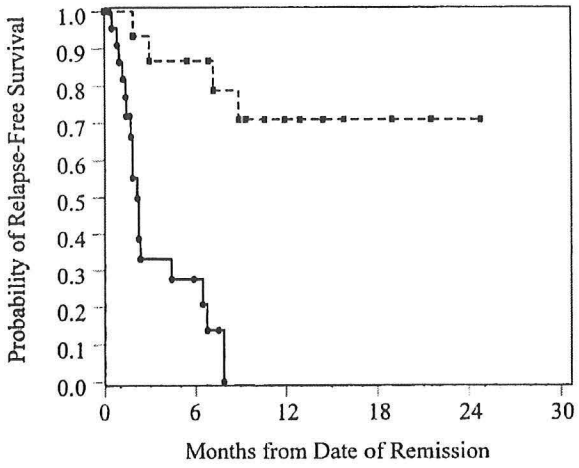
not expressed on CD34 + hematopoietic stem cells (109, 110). Mylotarg is a humanized antibody against CD33 that is conjugated to the highly cytotoxic antibiotic, Calicheamicin, originally isolated from a screen of marine-derived actinomycetes for compounds with anti-tumor properties.



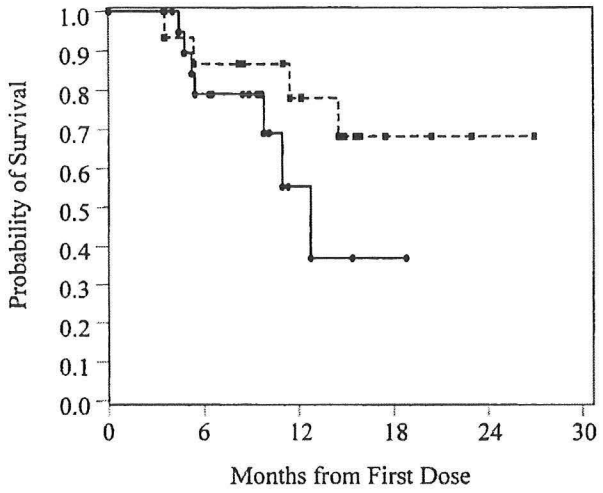
Structure of gemtuzumab ozogamicin. HP67.6 represents the humanized monoclonal antibody directed against CD33. Calicheamicin is linked to the antibody in variable molar ratios; approximately 50% of the antibody is unconjugated.

Figure reprinted from (111).

Calicheamicin belongs to a class of enediyene antibiotics that target specific oligopyrimidine/oligopurine regions of DNA, and cause double-strand DNA breaks through the formation of a highly reactive intermediate (112,113). A recent report described the results of the largest published trial of Mylotarg, which studied this drug in patients with CD33-positive AML in first relapse. The median age of the cohort was 61 years old. Thirty percent of patients attained a remission, which in this study deviated slightly from the standard, allowing patients with incomplete count recovery to be considered in remission as long as they had fewer than 5% bone marrow blasts (114). Interestingly, the remission rate for Mylotarg-treated patients with a first remission less than 12 months was similar to those with longer remissions, and elderly patients (older than 60 years) tended to fare as well as younger patients. The durability of response was approximately 7 months for all patients, but this included the one third of patients were underwent BMT after a Mylotarg-induced remission. In fact, the median relapse-free survival was only 2.1 months for those patients who received no further therapy after Mylotarg and at least 8.9 months for those patients able to be consolidated with BMT (114).



Relapse-free survival for OR patients who received HSCT (■) and for OR patients who received no further therapy (●) as postremission therapy. Fifteen OR patients received HSCT (median, > 8.9 months), and 23 OR patients received no further therapy (median, 2.1 months).



Overall survival for OR patients who received HSCT(■) and for OR patients who received no further therapy (●) as postremission therapy. Fifteen OR patients received HSCT (median, > 14.5 months), and 23 OR patients received no further therapy (median, 12.8 months).

Clinical outcome for patients with CD33+ AML receiving Mylotarg. Figures reprinted from (114). HSCT denotes hematopoietic stem cell transplantation and OR is overall responder (total achieving complete remission).

Median overall survival was at least 14.5 months in the Mylotarg patients able to undergo BMT, and 12.8 months in those patients receiving no post-Mylotarg therapy. In contrast, patients who did not respond to Mylotarg had a median survival of only 2.5 months without subsequent therapy, and only 4.2 months with bone marrow transplantation.

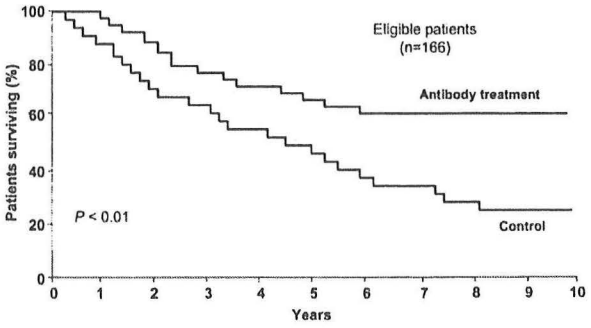
Mylotarg is generally well tolerated, with approximately 10% of patients experiencing infusion related symptoms such as fever, chills, and transient hypotension. Mylotarg is generally administered as two separate two-hour infusions (9 mg/m^2), spaced two weeks apart, potentially allowing a significant proportion of the treatment cycle to be performed as an outpatient. However, since the infusions are separated by 2 weeks, and Mylotarg induces significant myelosuppression, the duration of neutropenia can be quite prolonged (median 40 days), although in a recent study the incidence of grade 3/4 infections with Mylotarg compared favorably to standard chemotherapy (114). Nausea, vomiting, and oral stomatitis, are commonly associated with standard AML chemotherapy, but only 4% of patients experienced grade 3/4 mucositis with Mylotarg. Whether this accounts for why Mylotarg was associated with a relatively modest risk of infection despite prolonged neutropenia is unknown. Probably the most concerning adverse effect seen with Mylotarg therapy is hepatotoxicity, occurring in about one third of patients (111), usually manifested by modest hyperbilirubinemia. In some cases it can progress to hepatic failure and a veno-occlusive disease-like (VOD) syndrome. Although Mylotarg-associated VOD is more common in patients who have had a prior bone marrow transplant (115), it also can occur in 5-12% of non-transplant patients (114, 116, 117).

Mylotarg represents an encouraging advance in the therapy of relapsed AML. The patients experiencing the most benefit will probably be elderly patients with AML relapsing less than one year after diagnosis (115). However, the best responses to Mylotarg appear to be those who are able to undergo additional consolidative therapy, especially BMT. Future studies will address whether Mylotarg can safely combined with chemotherapy, or whether inhibition of multi-drug resistance genes, which may play a role in Mylotarg resistance (118), could improve its efficacy.

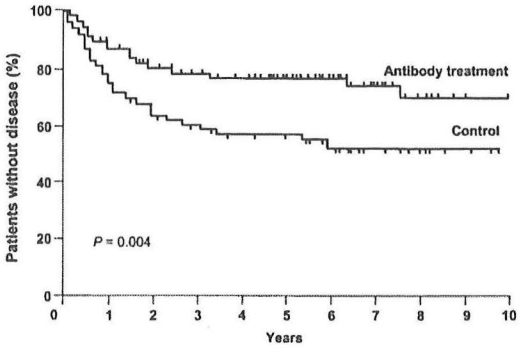
Antibodies targeting cell surface molecules: solid tumors

Edrecolomab (17-1A, Panorex®)

Edrecolomab (Panorex®) is a mouse monoclonal antibody that recognizes a non-secreted cell surface antigen called CD-17A, expressed on normal and malignant epithelial cells (119). The precise function of CD-17A, also called Ep-CAM, is unknown, but it does appear to play a role in adhesion (120). Edrecolomab was originally identified and characterized as an antibody with cytotoxic and anti-tumor activity against human colon cancer lines (121). There appears to be multiple possible mechanisms by which Edrecolomab exerts its cytotoxic effect, including antibody-dependent cell cytotoxicity, complement-mediated cytolysis, and formation of an anti-idiotype network (122, 123). In humans, Edrecolomab has been chiefly studied in colon cancer, tumors that generally stably express CD-17A. Edrecolomab has shown only very modest activity in metastatic colon cancer, either as a single agent or when combined with chemotherapy (122). In contrast, when used in the adjuvant setting, Edrecolomab has generated some encouraging results. In a trial initiated several years ago, 189 patients with stage C colon cancer were randomized to Edrecolomab or observation alone after curative resection. Patients could have no evidence of residual disease, and those randomized to the Edrecolomab arm received 500 mg postoperatively, and four monthly infusions of 100 mg each (124). The results of this trial were updated in 1998, at which time the vast majority of patients had been followed for at least five years. The Edrecolomab-treated patients experienced a 23% decrease in recurrence rate and a 32% reduction in overall mortality, all highly statistically significant (125). Interestingly, Edrecolomab therapy appeared to have its most pronounced inhibitory effect on occult visceral metastases, because Edrecolomab-treated patients had a significant reduction in distant failure rate, but no decrease in the rate of local recurrence.



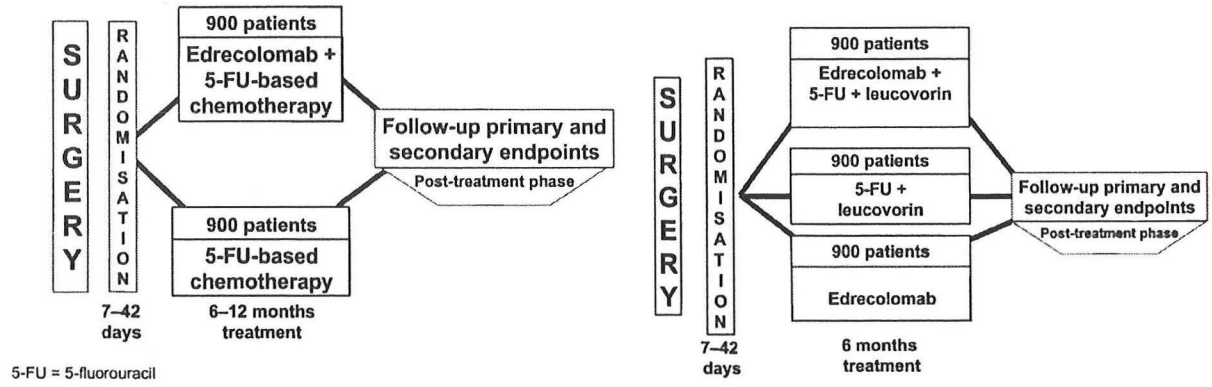
Overall survival of eligible patients randomized to treatment with edrecolomab ($n = 90$) or observation alone ($n = 76$) after 7 years of follow-up



Kaplan–Meier analysis of distant metastatic recurrences as the first event in eligible patients randomized to treatment with edrecolomab ($n = 90$) or observation alone ($n = 76$)

Clinical outcomes of patients with stage C colon cancer randomized to Edrecolomab or observation. Figures reprinted from (125).

Edrecolomab was generally well tolerated, with the most common side effects being diarrhea and nausea. Adverse reactions to the infusion were relatively uncommon, and manageable with appropriate pharmacological treatment. Many patients developed positive human anti-mouse antibody titers after Edrecolomab therapy, but there was no evidence that these antibodies impaired the efficacy of Edrecolomab, and their presence or absence did not correlate with clinical outcome (123). These promising results have led to several large confirmatory and combination clinical trials.



Ongoing randomized clinical research trials examining Edrecolomab alone or in combination with chemotherapy in patients with stage III (C) colon cancer. Schemas reprinted from (122).

The trial to the left has been open in North and South America, and seeks to determine whether addition of Edrecolomab will improve the efficacy of 5-FU-based adjuvant chemotherapy in stage 3 (C) colon cancer. The trial to the right has been conducted mainly in Europe, and employs a similar schema, but with the addition of a third Edrecolomab-alone arm. Both of the trials have already met accrual, and although there is no efficacy or survival data yet available, there have been some early reports on the toxicity profiles of combination Edrecolomab/chemotherapy. Thus far it appears that the addition of Edrecolomab to 5-FU-based chemotherapy has not resulted in any additive toxicity. Specifically, the incidence of side effects commonly experienced with 5-FU, such as stomatitis, diarrhea, and nausea and vomiting, were similar in the Edrecolomab/chemotherapy and chemotherapy alone arms (122). Other large cooperative group clinical trials still in progress include a trial comparing Edrecolomab to placebo (standard) as adjuvant therapy for stage 2 (B) colon cancer, and another trial in stage 2/3 resectable rectal cancer that will compare standard chemotherapy to combination chemotherapy/Edrecolomab. The outcomes of these trials will provide important insights into the role of Edrecolomab in adjuvant colorectal therapy, and will be eagerly awaited. The effectiveness of Edrecolomab in the adjuvant setting, in contrast to its limited activity in advanced disease, suggests this drug acts by immunologically targeting micrometastatic disease. If confirmed, this finding would suggest that the future of solid tumor-targeted immunotherapy might lie in early-stage disease, and that more advanced tumors will require multimodality therapy, including rationally targeted agents.

Conclusions

The rational, molecular-targeted approach to cancer therapy is an exciting and rapidly evolving field. Kinase inhibitors and antibody-based therapeutics have already made a substantial impact on the traditional practice of oncology. Additional therapeutic agents are being developed that will target cellular processes such as DNA replication, cell cycle regulation, gene transcription, and angiogenesis. An important challenge for the future will be to define the strengths and limitations of these new compounds, and to decipher how they might be incorporated into traditional therapeutic approaches. As cancer therapeutics grows more complex, there will be a growing need to study these drugs in the context of clinical trials, and to use sophisticated molecular diagnostic techniques to identify those patients most likely to benefit from specific molecular-targeted agents.

References

1. Groffen, J., J.R. Stephenson, N. Heisterkamp, A. de Klein, C.R. Bartram, and G. Grosveld, *Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22.* Cell, 1984. **36**: p. 93-99.
2. Shtivelman, E., B. Lifshitz, R.P. Gale, and E. Canaani, *Fused transcript of abl and bcr genes in chronic myelogenous leukaemia.* Nature, 1985. **315**: p. 550-554.
3. Heisterkamp, N., K. Stam, and J. Groffen, *Structural organization of the bcr gene and its role in the Ph' translocation.* Nature, 1985. **315**: p. 758-761.
4. Daley, G.Q., R.A. Van Etten, and D. Baltimore, *Induction of chronic myelogenous leukemia in mice by the P210$^{bcr/abl}$ gene of the Philadelphia chromosome.* Science, 1990. **247**: p. 824-830.
5. Elefanty, A.G., I.K. Hariharan, and S. Cory, *bcr-abl, the hallmark of chronic myeloid leukemia in man, induces multiple hematopoietic neoplasms in mice.* EMBO J., 1990. **9**(4): p. 1069-1078.
6. Kelliher, M.A., J. McLaughlin, O.N. Witte, and N. Rosenberg, *Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and bcr/abl.* Proc. Natl. Acad. Sci. USA, 1990. **87**: p. 6649-6653.
7. Gishizky, M.I., J. Johnson-White, and O.N. Witte, *Efficient transplantation of BCR-ABL-induced chronic myelogenous leukemia-like syndrome in mice.* Proc. Natl. Acad. Sci. USA, 1993. **90**: p. 3755-3759.
8. Pear, W.S., J.P. Miller, L. Xu, J.C. Pui, B. Soffer, R.C. Quackenbush, A.M. Pendergast, R. Bronson, J.C. Aster, M.L. Scott, and D. Baltimore, *Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow.* Blood, 1998. **92**(10): p. 3780-92.
9. Buchdunger, E., J. Zimmermann, H. Mett, T. Meyer, M. Muller, B.J. Druker, and N.B. Lydon, *Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative.* Cancer Res, 1996. **56**(1): p. 100-4.
10. Druker, B.J., S. Tamura, E. Buchdunger, S. Ohno, G.M. Segal, S. Fanning, J. Zimmermann, and N.B. Lydon, *Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells.* Nat Med, 1996. **2**(5): p. 561-6.
11. Carroll, M., S. Ohno-Jones, S. Tamura, E. Buchdunger, J. Zimmermann, N.B. Lydon, D.G. Gilliland, and B.J. Druker, *CGP 57148, a tyrosine kinase inhibitor, inhibits the growth of cells expressing BCR-ABL, TEL-ABL, and TEL-PDGFR fusion proteins.* Blood, 1997. **90**(12): p. 4947-52.
12. Schindler, T., W. Bornmann, P. Pellicena, W.T. Miller, B. Clarkson, and J. Kuriyan, *Structural mechanism for STI-571 inhibition of abelson tyrosine kinase.* Science, 2000. **289**(5486): p. 1938-42.
13. Druker, B.J., M. Talpaz, D.J. Resta, B. Peng, E. Buchdunger, J.M. Ford, N.B. Lydon, H. Kantarjian, R. Capdeville, S. Ohno-Jones, and C.L. Sawyers, *Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia.* N Engl J Med, 2001. **344**(14): p. 1031-7.
14. Druker, B.J., C.L. Sawyers, H. Kantarjian, D.J. Resta, S.F. Reese, J.M. Ford, R. Capdeville, and M. Talpaz, *Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome.* N Engl J Med, 2001. **344**(14): p. 1038-42.
15. Gorre, M.E., M. Mohammed, K. Ellwood, N. Hsu, R. Paquette, P.N. Rao, and C.L. Sawyers, *Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification.* Science, 2001. **293**(5531): p. 876-80.
16. Weisberg, E. and J.D. Griffin, *Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines.* Blood, 2000. **95**(11): p. 3498-505.
17. le Coutre, P., E. Tassi, M. Varella-Garcia, R. Barni, L. Mologni, G. Cabrita, E. Marchesi, R. Supino, and C. Gambacorti-Passerini, *Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification.* Blood, 2000. **95**(5): p. 1758-66.

18. Mahon, F.X., M.W. Deininger, B. Schultheis, J. Chabrol, J. Reiffers, J.M. Goldman, and J.V. Melo, *Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance.* Blood, 2000. **96**(3): p. 1070-9.
19. Goldman, J.M. and B.J. Druker, *Chronic myeloid leukemia: current treatment options.* Blood, 2001. **98**(7): p. 2039-42.
20. Heinrich, M.C., D.J. Griffith, B.J. Druker, C.L. Wait, K.A. Ott, and A.J. Zigler, *Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor.* Blood, 2000. **96**(3): p. 925-32.
21. Buchdunger, E., C.L. Cioffi, N. Law, D. Stover, S. Ohno-Jones, B.J. Druker, and N.B. Lydon, *Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors.* J Pharmacol Exp Ther, 2000. **295**(1): p. 139-45.
22. Okuda, K., E. Weisberg, D.G. Gilliland, and J.D. Griffin, *ARG tyrosine kinase activity is inhibited by STI571.* Blood, 2001. **97**(8): p. 2440-8.
23. Hirota, S., K. Isozaki, Y. Moriyama, K. Hashimoto, T. Nishida, S. Ishiguro, K. Kawano, M. Hanada, A. Kurata, M. Takeda, G. Muhammad Tunio, Y. Matsuzawa, Y. Kanakura, Y. Shinomura, and Y. Kitamura, *Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors.* Science, 1998. **279**(5350): p. 577-80.
24. Lux, M.L., B.P. Rubin, T.L. Biase, C.J. Chen, T. Maclure, G. Demetri, S. Xiao, S. Singer, C.D. Fletcher, and J.A. Fletcher, *KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors.* Am J Pathol, 2000. **156**(3): p. 791-5.
25. Joensuu, H., P.J. Roberts, M. Sarlomo-Rikala, L.C. Andersson, P. Tervahartiala, D. Tuveson, S. Silberman, R. Capdeville, S. Dimitrijevic, B. Druker, and G.D. Demetri, *Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor.* N Engl J Med, 2001. **344**(14): p. 1052-6.
26. Simon, M.P., F. Pedeutour, N. Sirvent, J. Grosgeorge, F. Minoletti, J.M. Coindre, M.J. Terrier-Lacombe, N. Mandahl, R.D. Craver, N. Blin, G. Sozzi, C. Turc-Carel, K.P. O'Brien, D. Kedra, I. Fransson, C. Guilbaud, and J.P. Dumanski, *Deregulation of the platelet-derived growth factor B-chain gene via fusion with collagen gene COL1A1 in dermatofibrosarcoma protuberans and giant-cell fibroblastoma.* Nat Genet, 1997. **15**(1): p. 95-8.
27. Greco, A., L. Fusetti, R. Villa, G. Sozzi, F. Minoletti, P. Mauri, and M.A. Pierotti, *Transforming activity of the chimeric sequence formed by the fusion of collagen gene COL1A1 and the platelet derived growth factor b-chain gene in dermatofibrosarcoma protuberans.* Oncogene, 1998. **17**(10): p. 1313-9.
28. Greco, A., E. Roccato, C. Miranda, L. Cleris, F. Formelli, and M.A. Pierotti, *Growth-inhibitory effect of STI571 on cells transformed by the COL1A1/PDGFB rearrangement.* Int J Cancer, 2001. **92**(3): p. 354-60.
29. Sjoblom, T., A. Shimizu, K.P. O'Brien, K. Pietras, P. Dal Cin, E. Buchdunger, J.P. Dumanski, A. Ostman, and C.H. Heldin, *Growth inhibition of dermatofibrosarcoma protuberans tumors by the platelet-derived growth factor receptor antagonist STI571 through induction of apoptosis.* Cancer Res, 2001. **61**(15): p. 5778-83.
30. Westphal, M., E. Ackermann, J. Hoppe, and H.D. Herrmann, *Receptors for platelet derived growth factor in human glioma cell lines and influence of suramin on cell proliferation.* J Neurooncol, 1991. **11**(3): p. 207-13.
31. Vassbotn, F.S., M. Andersson, B. Westermark, C.H. Heldin, and A. Ostman, *Reversion of autocrine transformation by a dominant negative platelet- derived growth factor mutant.* Mol Cell Biol, 1993. **13**(7): p. 4066-76.
32. Shamah, S.M., C.D. Stiles, and A. Guha, *Dominant-negative mutants of platelet-derived growth factor revert the transformed phenotype of human astrocytoma cells.* Mol Cell Biol, 1993. **13**(12): p. 7203-12.

33. Strawn, L.M., E. Mann, S.S. Elliger, L.M. Chu, L.L. Germain, G. Niederfellner, A. Ullrich, and L.K. Shawver, *Inhibition of glioma cell growth by a truncated platelet-derived growth factor-beta receptor.* J Biol Chem, 1994. **269**(33): p. 21215-22.
34. Potapova, O., H. Fakhrai, S. Baird, and D. Mercola, *Platelet-derived growth factor-B/v-sis confers a tumorigenic and metastatic phenotype to human T98G glioblastoma cells.* Cancer Res, 1996. **56**(2): p. 280-6.
35. Kilic, T., J.A. Alberta, P.R. Zdunek, M. Acar, P. Iannarelli, T. O'Reilly, E. Buchdunger, P.M. Black, and C.D. Stiles, *Intracranial inhibition of platelet-derived growth factor-mediated glioblastoma cell growth by an orally active kinase inhibitor of the 2-phenylaminopyrimidine class.* Cancer Res, 2000. **60**(18): p. 5143-50.
36. Buday, L. and J. Downward, *Epidermal growth factor regulates p21ras through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor.* Cell, 1993. **73**(3): p. 611-20.
37. Lowenstein, E.J., R.J. Daly, A.G. Batzer, W. Li, B. Margolis, R. Lammers, A. Ullrich, E.Y. Skolnik, D. Bar-Sagi, and J. Schlessinger, *The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling.* Cell, 1992. **70**(3): p. 431-42.
38. Egan, S.E., B.W. Giddings, M.W. Brooks, L. Buday, A.M. Sizeland, and R.A. Weinberg, *Association of Sos Ras exchange protein with Grb2 is implicated in tyrosine kinase signal transduction and transformation [see comments].* Nature, 1993. **363**(6424): p. 45-51.
39. Rozakis-Adcock, M., R. Fernley, J. Wade, T. Pawson, and D. Bowtell, *The SH2 and SH3 domains of mammalian Grb2 couple the EGF receptor to the Ras activator mSos1 [see comments].* Nature, 1993. **363**(6424): p. 83-5.
40. Gale, N.W., S. Kaplan, E.J. Lowenstein, J. Schlessinger, and D. Bar-Sagi, *Grb2 mediates the EGF-dependent activation of guanine nucleotide exchange on Ras [see comments].* Nature, 1993. **363**(6424): p. 88-92.
41. Wilks, A.F., *Protein tyrosine kinase growth factor receptors and their ligands in development, differentiation, and cancer.* Adv. Cancer Res., 1993. **60**: p. 43-73.
42. Ciardiello, F. and G. Tortora, *A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor.* Clin Cancer Res, 2001. **7**(10): p. 2958-70.
43. Grandis, J.R., M.F. Melhem, W.E. Gooding, R. Day, V.A. Holst, M.M. Wagener, S.D. Drenning, and D.J. Tweardy, *Levels of TGF-α and EGFR protein in head and neck squamous cell carcinoma and patient survival.* J. Natl. Cancer Inst., 1998. **90**(824-832).
44. Arteaga, C.L. and D.H. Johnson, *Tyrosine kinase inhibitors-ZD1839 (Iressa).* Curr. Opin. Oncol., 2001. **13**: p. 491-498.
45. Barker, A.J., K.H. Gibson, W. Grundy, A.A. Godfrey, J.J. Barlow, M.P. Healy, J.R. Woodburn, S.E. Ashton, B.J. Curry, L. Scarlett, L. Henthorn, and L. Richards, *Studies leading to the identification of ZD1839 (IRESSA): an orally active, selective epidermal growth factor receptor tyrosine kinase inhibitor targeted to the treatment of cancer.* Bioorg Med Chem Lett, 2001. **11**(14): p. 1911-4.
46. Moasser, M.M., A. Basso, S.D. Averbuch, and N. Rosen, *The tyrosine kinase inhibitor ZD1839 ("Iressa") inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells.* Cancer Res, 2001. **61**(19): p. 7184-8.
47. Kris, M.G., R. Herbst, D. Rischin, P. LoRusso, J. Baselga, L. Hammond, A. Feyereislova, O. Ochs, and S. Averbuch, *Objective regression in non-small cell lung cancer patients treated in Phase I trials of oral ZD1839 (Iressa), a selective tyrosine kinase inhibitor that blocks the epidermal growth factor receptor (EGFR).* Lung Cancer, 2000. **29 (suppl. 1)**: p. 72.
48. Miller, V.A., D. Johnson, R.T. Heelan, B.A. Pizzo, W.J. Perez, A. Bass, M.G. Kris, J. Ochs, and S. Averbuch, *A pilot trial demonstrates the safety of ZD1839 (Iressa), an oral epiderma growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), in combination with carboplatin (C) and paclitaxel (P)*

in previously untreated advanced non-small cell lung cancer (NSCLC). Proc. Am. Soc. Clin. Oncol., 2001. **21**: p. 326.

49. Senzer, N.N., D. Soulieres, L. Siu, S. Agarwala, E. Vokes, M. Higalgo, S. Silberman, L. Allen, K. Ferrante, D. Fisher, C. Marsolais, and P. Nadler, *Phase 2 evaluation of OSI-774, a potent oral antagonist of the EGFR-TK in patients with advanced squamous cell carcinoma of the head and neck.* Proc. Am. Soc. Clin. Oncol., 2001. **21**: p. 2.
50. Perez-Soler, R., A. Chachoua, M. Huberman, D. Karp, J. Rigas, L. Hammond, E. Rowinski, G. Preston, K.J. Ferrante, L.F. Allen, P.I. Nadler, and P.A. Bonomi, *A phase II trial of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor OSI-774, following platinum-based chemotherapy, in patients (pts) with advanced, EGFR-expressing, non-small cell lung cancer (NSCLC).* Proc. Am. Soc. Clin. Oncol., 2001. **21**: p. 310.
51. Slamon, D.J., G.M. Clark, S.G. Wong, W.J. Levin, A. Ullrich, and W.L. McGuire, *Human breast cancer: correlation of relapse and survival with amplification of Her2-2/neu oncogene gene.* Science, 1987. **235**: p. 177-182.
52. Reese, D.M. and D.J. Slamon, *HER-2/neu signal transduction in human breast and ovarian cancer.* Stem Cells, 1997. **15**(1): p. 1-8.
53. Kallioniemi, O.P., K. Holli, T. Visakorpi, T. Koivula, H.H. Helin, and J.J. Isola, *Association of c-erbB-2 protein over-expression with high rate of cell proliferation, increased risk of visceral metastasis and poor long-term survival in breast cancer.* Int J Cancer, 1991. **49**(5): p. 650-5.
54. Tiwari, R.K., P.I. Borgen, G.Y. Wong, C. Cordon-Cardo, and M.P. Osborne, *HER-2/neu amplification and overexpression in primary human breast cancer is associated with early metastasis.* Anticancer Res, 1992. **12**(2): p. 419-25.
55. Tandon, A.K., G.M. Clark, G.C. Chamness, A. Ullrich, and W.L. McGuire, *HER-2/neu oncogene protein and prognosis in breast cancer.* J Clin Oncol, 1989. **7**(8): p. 1120-8.
56. Paik, S., R. Hazan, E.R. Fisher, R.E. Sass, B. Fisher, C. Redmond, J. Schlessinger, M.E. Lippman, and C.R. King, *Pathologic findings from the National Surgical Adjuvant Breast and Bowel Project: prognostic significance of erbB-2 protein overexpression in primary breast cancer.* J Clin Oncol, 1990. **8**(1): p. 103-12.
57. Seshadri, R., F.A. Firgaira, D.J. Horsfall, K. McCaul, V. Setlur, and P. Kitchen, *Clinical significance of HER-2/neu oncogene amplification in primary breast cancer. The South Australian Breast Cancer Study Group.* J Clin Oncol, 1993. **11**(10): p. 1936-42.
58. Ellis, M.J., A. Coop, B. Singh, L. Mauriac, A. Llombert-Cussac, F. Janicke, W.R. Miller, D.B. Evans, M. Dugan, C. Brady, E. Quebe-Fehling, and M. Borgs, *Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor- positive primary breast cancer: evidence from a phase III randomized trial.* J Clin Oncol, 2001. **19**(18): p. 3808-16.
59. Drebin, J.A., V.C. Link, R.A. Weinberg, and M.I. Greene, *Inhibition of tumor growth by a monoclonal antibody reactive with an oncogene-encoded tumor antigen.* Proc Natl Acad Sci U S A, 1986. **83**(23): p. 9129-33.
60. Hudziak, R.M., G.D. Lewis, M. Winget, B.M. Fendly, H.M. Shepard, and A. Ullrich, *p185HER2 monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor.* Mol Cell Biol, 1989. **9**(3): p. 1165-72.
61. Sarup, J.C., R.M. Johnson, K.L. King, B.M. Fendly, M.T. Lipari, M.A. Napier, A. Ullrich, and H.M. Shepard, *Characterization of an anti-p185HER2 monoclonal antibody that stimulates receptor function and inhibits tumor cell growth.* Growth Regul, 1991. **1**(2): p. 72-82.
62. Baselga, J., D. Tripathy, J. Mendelsohn, S. Baughman, C.C. Benz, L. Dantis, N.T. Sklarin, A.D. Seidman, C.A. Hudis, J. Moore, P.P. Rosen, T. Twaddell, I.C. Henderson, and L. Norton, *Phase II study of weekly intravenous recombinant humanized anti- p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer.* J Clin Oncol, 1996. **14**(3): p. 737-44.

63. Cobleigh, M.A., C.L. Vogel, D. Tripathy, N.J. Robert, S. Scholl, L. Fehrenbacher, J.M. Wolter, V. Paton, S. Shak, G. Lieberman, and D.J. Slamon, *Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease.* J Clin Oncol, 1999. **17**(9): p. 2639-48.
64. Slamon, D.J., B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, J. Baselga, and L. Norton, *Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2.* N Engl J Med, 2001. **344**(11): p. 783-92.
65. Carter, P., L. Presta, C.M. Gorman, J.B. Ridgway, D. Henner, W.L. Wong, A.M. Rowland, C. Kotts, M.E. Carver, and H.M. Shepard, *Humanization of an anti-p185HER2 antibody for human cancer therapy.* Proc Natl Acad Sci U S A, 1992. **89**(10): p. 4285-9.
66. Baselga, J. and J. Albanell, *Mechanism of action of anti-HER2 monoclonal antibodies.* Ann Oncol, 2001. **12**(Suppl 1): p. S35-41.
67. Pegram, M.D., A. Lipton, D.F. Hayes, B.L. Weber, J.M. Baselga, D. Tripathy, D. Baly, S.A. Baughman, T. Twaddell, J.A. Glaspy, and D.J. Slamon, *Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment.* J Clin Oncol, 1998. **16**(8): p. 2659-71.
68. Goldstein, N.I., M. Prewett, K. Zuklys, P. Rockwell, and J. Mendelsohn, *Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model.* Clin Cancer Res, 1995. **1**(11): p. 1311-8.
69. Prewett, M., P. Rockwell, R.F. Rockwell, N.A. Giorgio, J. Mendelsohn, H.I. Scher, and N.I. Goldstein, *The biologic effects of C225, a chimeric monoclonal antibody to the EGFR, on human prostate carcinoma.* J Immunother Emphasis Tumor Immunol, 1996. **19**(6): p. 419-27.
70. Shin, D.M., N.J. Donato, R. Perez-Soler, H.J. Shin, J.Y. Wu, P. Zhang, K. Lawhorn, F.R. Khuri, B.S. Glisson, J. Myers, G. Clayman, D. Pfister, J. Falcey, H. Waksal, J. Mendelsohn, and W.K. Hong, *Epidermal growth factor receptor-targeted therapy with C225 and cisplatin in patients with head and neck cancer.* Clin Cancer Res, 2001. **7**(5): p. 1204-13.
71. Huang, S.M., J.M. Bock, and P.M. Harari, *Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck.* Cancer Res, 1999. **59**(8): p. 1935-40.
72. Robert, F., M.P. Ezekiel, S.A. Spencer, R.F. Meredith, J.A. Bonner, M.B. Khazaeli, M.N. Saleh, D. Carey, A.F. LoBuglio, R.H. Wheeler, M.R. Cooper, and H.W. Waksal, *Phase I study of anti--epidermal growth factor receptor antibody cetuximab in combination with radiation therapy in patients with advanced head and neck cancer.* J Clin Oncol, 2001. **19**(13): p. 3234-43.
73. Perrotte, P., T. Matsumoto, K. Inoue, H. Kuniyasu, B.Y. Eve, D.J. Hicklin, R. Radinsky, and C.P. Dinney, *Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice.* Clin Cancer Res, 1999. **5**(2): p. 257-65.
74. Tedder, T.F. and P. Engel, *CD20: a regulator of cell-cycle progression of B lymphocytes.* Immunol Today, 1994. **15**(9): p. 450-4.
75. McLaughlin, P., A.J. Grillo-Lopez, B.K. Link, R. Levy, M.S. Czuczman, M.E. Williams, M.R. Heyman, I. Bence-Bruckler, C.A. White, F. Cabanillas, V. Jain, A.D. Ho, J. Lister, K. Wey, D. Shen, and B.K. Dallaire, *Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program.* J Clin Oncol, 1998. **16**(8): p. 2825-33.
76. Davis, T.A., A.J. Grillo-Lopez, C.A. White, P. McLaughlin, M.S. Czuczman, B.K. Link, D.G. Maloney, R.L. Weaver, J. Rosenberg, and R. Levy, *Rituximab anti-CD20 monoclonal antibody therapy in non-Hodgkin's lymphoma: safety and efficacy of re-treatment.* J Clin Oncol, 2000. **18**(17): p. 3135-43.

77. Czuczman, M.S., A.J. Grillo-Lopez, C.A. White, M. Saleh, L. Gordon, A.F. LoBuglio, C. Jonas, D. Klippenstein, B. Dallaire, and C. Varns, *Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy.* J Clin Oncol, 1999. **17**(1): p. 268-76.
78. Jones, S.E., P.N. Grozea, T.P. Miller, E.J. Van Slyck, S.P. Balcerzak, J.J. Costanzi, F.S. Morrison, H.J. Eyre, C.J. Fabian, L. Dabich, and et al., *Chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisone alone or with levamisole or with levamisole plus BCG for malignant lymphoma: a Southwest Oncology Group Study.* J Clin Oncol, 1985. **3**(10): p. 1318-24.
79. Dana, B.W., S. Dahlberg, B.N. Nathwani, E. Chase, C. Coltman, T.P. Miller, and R.I. Fisher, *Long-term follow-up of patients with low-grade malignant lymphomas treated with doxorubicin-based chemotherapy or chemoimmunotherapy.* J Clin Oncol, 1993. **11**(4): p. 644-51.
80. Foran, J.M., R.K. Gupta, D. Cunningham, R.A. Popescu, A.H. Goldstone, J.W. Sweetenham, R. Pettengell, P.W. Johnson, E. Bessell, B. Hancock, K. Summers, J. Hughes, A.Z. Rohatiner, and T.A. Lister, *A UK multicentre phase II study of rituximab (chimaeric anti-CD20 monoclonal antibody) in patients with follicular lymphoma, with PCR monitoring of molecular response.* Br J Haematol, 2000. **109**(1): p. 81-8.
81. Czuczman, M.S., A.J. Grillo-Lopez, P. McLaughlin, C.A. White, M. Saleh, L. Gordon, A.F. LoBuglio, J. Rosenberg, B. Alkuzweny, and D. Maloney, *Clearing of cells bearing the bcl-2 [t(14;18)] translocation from blood and marrow of patients treated with rituximab alone or in combination with CHOP chemotherapy.* Ann Oncol, 2001. **12**(1): p. 109-14.
82. Gribben, J.G., D. Neuberg, A.S. Freedman, C.D. Gimmi, K.W. Pesek, M. Barber, L. Saporito, S.D. Woo, F. Coral, N. Spector, and et al., *Detection by polymerase chain reaction of residual cells with the bcl-2 translocation is associated with increased risk of relapse after autologous bone marrow transplantation for B-cell lymphoma.* Blood, 1993. **81**(12): p. 3449-57.
83. Freedman, A.S., D. Neuberg, P. Mauch, R.J. Soiffer, K.C. Anderson, D.C. Fisher, R. Schlossman, E.P. Alyea, T. Takvorian, H. Jallow, C. Kuhlman, J. Ritz, L.M. Nadler, and J.G. Gribben, *Long-term follow-up of autologous bone marrow transplantation in patients with relapsed follicular lymphoma.* Blood, 1999. **94**(10): p. 3325-33.
84. Vose, J.M., B.K. Link, M.L. Grossbard, M. Czuczman, A. Grillo-Lopez, P. Gilman, A. Lowe, L.A. Kunkel, and R.I. Fisher, *Phase II study of rituximab in combination with chop chemotherapy in patients with previously untreated, aggressive non-Hodgkin's lymphoma.* J Clin Oncol, 2001. **19**(2): p. 389-97.
85. Coiffier, B., E. Lepage, R. Herbrecht, H. Tilly, P. Solal-Celigny, J.N. Munck, R. Bouabdallah, P. Lederlin, C. Sebban, P. Morel, C. Haioun, G. Salles, T. Molina, and C. Gisselbrecht, *MAbthera (rituximab) plus CHOP is superior to CHOP alone in elderly patients with diffuse large B-cell lymphoma (DLCL): interim results of a randomized GELA trial.* Blood, 2000. **96**(11): p. 223a (abstract 950).
86. Winkler, U., M. Jensen, O. Manzke, H. Schulz, V. Diehl, and A. Engert, *Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8).* Blood, 1999. **94**(7): p. 2217-24.
87. Huhn, D., C. von Schilling, M. Wilhelm, A.D. Ho, M. Hallek, R. Kuse, W. Knauf, U. Riedel, A. Hinke, S. Srock, S. Serke, C. Peschel, and B. Emmerich, *Rituximab therapy of patients with B-cell chronic lymphocytic leukemia.* Blood, 2001. **98**(5): p. 1326-31.
88. Byrd, J.C., C.A. White, B. Link, M.S. Lucas, W.S. Velasquez, J. Rosenberg, and A.J. Grillo-Lopez, *Rituximab therapy in Waldenstrom's macroglobulinemia: preliminary evidence of clinical activity.* Ann Oncol, 1999. **10**(12): p. 1525-7.
89. Treon, S.P., D.B. Agus, B. Link, G. Rodrigues, A. Molina, M.Q. Lacy, D.C. Fisher, C. Emmanouilides, A.I. Richards, B. Clark, M.S. Lucas, R. Schlossman, D. Schenkein, B. Lin, E. Kimby, K.C. Anderson, and J.C. Byrd, *CD20-Directed Antibody-Mediated Immunotherapy Induces Responses and Facilitates*

Hematologic Recovery in Patients With Waldenstrom's Macroglobulinemia. J Immunother, 2001. **24**(3): p. 272-279.

90. Coiffier, B., C. Haioun, N. Ketterer, A. Engert, H. Tilly, D. Ma, P. Johnson, A. Lister, M. Feuring-Buske, J.A. Radford, R. Capdeville, V. Diehl, and F. Reyes, *Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study.* Blood, 1998. **92**(6): p. 1927-32.
91. Ghielmini, M., S.F. Schmitz, K. Burki, G. Pichert, D.C. Betticher, R. Stupp, M. Wernli, A. Lohri, D. Schmitter, F. Bertoni, and T. Cerny, *The effect of Rituximab on patients with follicular and mantle-cell lymphoma. Swiss Group for Clinical Cancer Research (SAKK).* Ann Oncol, 2000. **11**(Suppl 1): p. 123-6.
92. Foran, J.M., D. Cunningham, B. Coiffier, P. Solal-Celigny, F. Reyes, M. Ghielmini, P.W. Johnson, C. Gisselbrecht, M. Bradburn, J. Matthews, and T.A. Lister, *Treatment of mantle-cell lymphoma with Rituximab (chimeric monoclonal anti-CD20 antibody): analysis of factors associated with response.* Ann Oncol, 2000. **11**(Suppl 1): p. 117-21.
93. Foran, J.M., A.Z. Rohatiner, D. Cunningham, R.A. Popescu, P. Solal-Celigny, M. Ghielmini, B. Coiffier, P.W. Johnson, C. Gisselbrecht, F. Reyes, J.A. Radford, E.M. Bessell, B. Souleau, A. Benzohra, and T.A. Lister, *European phase II study of rituximab (chimeric anti-CD20 monoclonal antibody) for patients with newly diagnosed mantle-cell lymphoma and previously treated mantle-cell lymphoma, immunocytoma, and small B-cell lymphocytic lymphoma.* J Clin Oncol, 2000. **18**(2): p. 317-24.
94. Maloney, D.G., *Mechanism of action of rituximab.* Anticancer Drugs, 2001. **12**(Suppl 2): p. S1-4.
95. McLaughlin, P., F.B. Hagemeister, and A.J. Grillo-Lopez, *Rituximab in indolent lymphoma: the single-agent pivotal trial.* Semin Oncol, 1999. **26**(5 Suppl 14): p. 79-87.
96. Reff, M.E., K. Carner, K.S. Chambers, P.C. Chinn, J.E. Leonard, R. Raab, R.A. Newman, N. Hanna, and D.R. Anderson, *Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20.* Blood, 1994. **83**(2): p. 435-45.
97. Gilleece, M.H. and T.M. Dexter, *Effect of Campath-1H antibody on human hematopoietic progenitors in vitro.* Blood, 1993. **82**(3): p. 807-12.
98. Hale, G., S. Cobbold, and H. Waldmann, *T cell depletion with CAMPATH-1 in allogeneic bone marrow transplantation.* Transplantation, 1988. **45**(4): p. 753-9.
99. Lundin, J., A. Osterborg, G. Brittinger, D. Crowther, H. Dombret, A. Engert, A. Epenetos, C. Gisselbrecht, D. Huhn, U. Jaeger, J. Thomas, R. Marcus, N. Nissen, C. Poynton, E. Rankin, R. Stahel, M. Uppenkamp, R. Willemze, and H. Mellstedt, *CAMPATH-1H monoclonal antibody in therapy for previously treated low-grade non-Hodgkin's lymphomas: a phase II multicenter study. European Study Group of CAMPATH-1H Treatment in Low-Grade Non-Hodgkin's Lymphoma.* J Clin Oncol, 1998. **16**(10): p. 3257-63.
100. Flynn, J.M. and J.C. Byrd, *Campath-1H monoclonal antibody therapy.* Curr Opin Oncol, 2000. **12**(6): p. 574-81.
101. Osterborg, A., M.J. Dyer, D. Bunjes, G.A. Pangalis, Y. Bastion, D. Catovsky, and H. Mellstedt, *Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. European Study Group of CAMPATH-1H Treatment in Chronic Lymphocytic Leukemia.* J Clin Oncol, 1997. **15**(4): p. 1567-74.
102. Keating, M.J., J.C. Byrd, K. Rai, I. Flinn, V. Jain, J.L. Binet, R. Bolin, P. Hillmen, and M. Hutchinson, *Multicenter study of CAMPATH-1H in patients with chronic lymphocytic leukemia (B-CLL) refractory to fludarabine.* Blood, 1999. **94**: p. 705a, abstr.
103. Khorana, A., P. Bunn, P. McLaughlin, J. Vose, C. Stewart, and M.S. Czuczman, *A phase II multicenter study of CAMPATH-1H antibody in previously treated patients with nonbulky non-Hodgkin's lymphoma.* Leuk Lymphoma, 2001. **41**(1-2): p. 77-87.
104. Brett, S., G. Baxter, H. Cooper, J.M. Johnston, J. Tite, and N. Rapson, *Repopulation of blood lymphocyte sub-populations in rheumatoid arthritis patients treated with the depleting humanized monoclonal antibody, CAMPATH-1H.* Immunology, 1996. **88**(1): p. 13-9.

105. Rosen, S.T.E., *Recent advances and future directions using monoclonal antibodies for B-cell malignancies. Proceedings of a conference. Kaupulehu, Hawaii, USA. January 1999.* Semin Oncol, 1999. **26**(5 Suppl 14): p. 1-122.
106. White, C.A., R.L. Weaver, and A.J. Grillo-Lopez, *Antibody-targeted immunotherapy for treatment of malignancy.* Annu Rev Med, 2001. **52**: p. 125-45.
107. Witzig, T.E., C.A. White, G.A. Wiseman, L.I. Gordon, C. Emmanouilides, A. Raubitschek, N. Janakiraman, J. Gutheil, R.J. Schilder, S. Spies, D.H. Silverman, E. Parker, and A.J. Grillo-Lopez, *Phase I/II trial of IDEC-Y2B8 radioimmunotherapy for treatment of relapsed or refractory CD20(+) B-cell non-Hodgkin's lymphoma.* J Clin Oncol, 1999. **17**(12): p. 3793-803.
108. Witzig, T.E., *The use of ibritumomab tiuxetan radioimmunotherapy for patients with relapsed B-cell non-Hodgkin's lymphoma.* Semin Oncol, 2000. **27**(6 Suppl 12): p. 74-8.
109. Pierelli, L., L. Teofili, G. Menichella, C. Rumi, A. Paoloni, S. Iovino, P.L. Puggioni, G. Leone, and B. Bizzi, *Further investigations on the expression of HLA-DR, CD33 and CD13 surface antigens in purified bone marrow and peripheral blood CD34+ haematopoietic progenitor cells.* Br J Haematol, 1993. **84**(1): p. 24-30.
110. Dinndorf, P.A., R.G. Andrews, D. Benjamin, D. Ridgway, L. Wolff, and I.D. Bernstein, *Expression of normal myeloid-associated antigens by acute leukemia cells.* Blood, 1986. **67**(4): p. 1048-53.
111. Bross, P.F., J. Beitz, G. Chen, X.H. Chen, E. Duffy, L. Kieffer, S. Roy, R. Sridhara, A. Rahman, G. Williams, and R. Pazdur, *Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia.* Clin Cancer Res, 2001. **7**(6): p. 1490-6.
112. Zein, N., A.M. Sinha, W.J. McGahren, and G.A. Ellestad, *Calicheamicin gamma 1I: an antitumor antibiotic that cleaves double-stranded DNA site specifically.* Science, 1988. **240**(4856): p. 1198-201.
113. Ikemoto, N., R.A. Kumar, T.T. Ling, G.A. Ellestad, S.J. Danishefsky, and D.J. Patel, *Calicheamicin-DNA complexes: warhead alignment and saccharide recognition of the minor groove.* Proc Natl Acad Sci U S A, 1995. **92**(23): p. 10506-10.
114. Sievers, E.L., R.A. Larson, E.A. Stadtmauer, E. Estey, B. Lowenberg, H. Dombret, C. Karanes, M. Theobald, J.M. Bennett, M.L. Sherman, M.S. Berger, C.B. Eten, M.R. Loken, J.J. van Dongen, I.D. Bernstein, and F.R. Appelbaum, *Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse.* J Clin Oncol, 2001. **19**(13): p. 3244-54.
115. Sievers, E.L. and M. Linenberger, *Mylotarg: antibody-targeted chemotherapy comes of age.* Curr Opin Oncol, 2001. **13**(6): p. 522-7.
116. Neumeister, P., M. Eibl, W. Zinke-Cerwenka, M. Scarpatetti, H. Sill, and W. Linkesch, *Hepatic veno-occlusive disease in two patients with relapsed acute myeloid leukemia treated with anti-CD33 calicheamicin (CMA-676) immunoconjugate.* Ann Hematol, 2001. **80**(2): p. 119-20.
117. Giles, F.J., H.M. Kantarjian, S.M. Kornblau, D.A. Thomas, G. Garcia-Manero, T.A. Waddelow, C.L. David, A.T. Phan, D.E. Colburn, A. Rashid, and E.H. Estey, *Mylotarg (gemtuzumab ozogamicin) therapy is associated with hepatic venoocclusive disease in patients who have not received stem cell transplantation.* Cancer, 2001. **92**(2): p. 406-13.
118. Linenberger, M.L., T. Hong, D. Flowers, E.L. Sievers, T.A. Gooley, J.M. Bennett, M.S. Berger, L.H. Leopold, F.R. Appelbaum, and I.D. Bernstein, *Multidrug-resistance phenotype and clinical responses to gemtuzumab ozogamicin.* Blood, 2001. **98**(4): p. 988-94.
119. Gottlinger, H.G., I. Funke, J.P. Johnson, J.M. Gokel, and G. Riethmuller, *The epithelial cell surface antigen 17-1A, a target for antibody- mediated tumor therapy: its biochemical nature, tissue distribution and recognition by different monoclonal antibodies.* Int J Cancer, 1986. **38**(1): p. 47-53.
120. Litvinov, S.V., M.P. Velders, H.A. Bakker, G.J. Fleuren, and S.O. Warnaar, *Ep-CAM: a human epithelial antigen is a homophilic cell-cell adhesion molecule.* J Cell Biol, 1994. **125**(2): p. 437-46.
121. Herlyn, D.M., Z. Steplewski, M.F. Herlyn, and H. Koprowski, *Inhibition of growth of colorectal carcinoma in nude mice by monoclonal antibody.* Cancer Res, 1980. **40**(3): p. 717-21.

122. Schwartzberg, L.S., *Clinical experience with edrecolomab: a monoclonal antibody therapy for colorectal carcinoma.* Crit Rev Oncol Hematol, 2001. **40**(1): p. 17-24.
123. Gruber, R., L.J.M. van Haarlem, S.O. Warnaar, E. Holz, and G. Riethmuller, *The human antimouse immunoglobulin response and the anti-idiotype network have no influence on clinical outcome in patients with minimal residual colorectal cancer treated with monoclonal antibody CO17-1A.* Cancer Res., 2001. **60**: p. 1921-1926.
124. Riethmuller, G., E. Schneider-Gadicke, G. Schlimok, W. Schmiegel, R. Raab, K. Hoffken, R. Gruber, H. Pichlmaier, H. Hirche, R. Pichlmayr, and et al., *Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma. German Cancer Aid 17-1A Study Group.* Lancet, 1994. **343**(8907): p. 1177-83.
125. Riethmuller, G., E. Holz, G. Schlimok, W. Schmiegel, R. Raab, K. Hoffken, R. Gruber, I. Funke, H. Pichlmaier, H. Hirche, P. Buggisch, J. Witte, and R. Pichlmayr, *Monoclonal antibody therapy for resected Dukes' C colorectal cancer: seven-year outcome of a multicenter randomized trial.* J Clin Oncol, 1998. **16**(5): p. 1788-94.