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TARGETED CANCER THERAPY

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Humana Press
Dedication

This book is dedicated to our courageous patients and their families engaged in the war against cancer.
Based on experience with a small number of cancers, it is now apparent that remarkable responses (without serious side effects) can be achieved by targeted therapy—that is, by using drugs that directly affect the molecular abnormality that characterizes a tumor. The era of personalized medicine in cancer has begun, and we believe that these early therapeutic successes represent just the tip of the iceberg. No longer a “one-size-fits-all” approach, the treatment of cancer is now increasingly being individualized based on an understanding of the underlying biologic mechanisms.

The limitations of chemotherapy, radiotherapy, and surgery are apparent and have been the impetus for developing targeted therapies. Novel emerging technologies in target identification, drug discovery, molecular markers, and imaging are rapidly changing the face of cancer. Personalized health planning, early diagnosis, and selecting optimal drugs for each patient with predictable side effects are developments that are directly in our sights. It is also becoming increasingly important to develop combinations of therapies and therapeutic modalities for treating cancer, as many cancers are complex and driven by more than one aberration. A primary objective is to design drug combinations that can overcome drug resistance. Cross talk among signaling pathways and parallel pathways that contribute to tumor pathogenesis is thought to contribute to single-agent resistance.

We are poised to change the landscape in oncology. The purpose of Targeted Cancer Therapy is to provide a state-of-the-art overview of where we are now in this process. We hope that the book will provide a foundation of knowledge in targeted cancer therapeutics that is useful to practicing and academic physicians, fellows, residents, and students, as well as basic scientists who are interested in the cancer field.

Razelle Kurzrock, MD, FACP
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Abstract

Translational medicine has opened the gateway to the era of personalized medicine. No longer a “one size fits all” approach, the treatment of cancer is now based on an understanding of underlying biologic mechanisms and is increasingly being tailored to the molecular specificity of a tumor. Although oncology still functions within broad disease categories, the future will see an increasing shift to treatment based on the characteristics of an individual’s tumor type. Interestingly, one of the first targeted therapies, all-trans retinoic acid in acute promyelocytic leukemia, was first demonstrated at the bedside with a subsequent return to the bench to elucidate its underlying basis. This was a translocation resulting in disruption of the retinoic acid receptor-α gene. Since then, a rigorous translational approach has led to other success stories, such as imatinib and dasatinib in Philadelphia-positive leukemias. With the discovery of novel biologic agents, future challenges lie in the investigation of optimal combinations and the identification of biomarkers that can provide both predictive and prognostic information. Genomics, proteomics, and the application of mathematical modeling are leading the way to biomarker discovery. Further elucidation of the cancer “stem cell hypothesis” will lead to treatment with combinations of agents used to
target both early epigenetic mechanisms and downstream molecular events. With targeted agents that demonstrate increased efficacy and decreased toxicity, we are now approaching cancer as a chronic disease model. Personalized medicine, with a “bench to bedside and back” paradigm is poised to permanently alter the landscape for cancer management.

Key Words: Targeted therapy, Translational medicine, Bioinformatics, All-trans retinoic acid, GIST, Imatinib, Dasatinib, Epigenetics, Decitabine, Stem cell hypothesis

1. INTRODUCTION

We are in the midst of a revolution in the treatment of cancer. Translational medicine is leading the way into a new era of personalized medicine. Traditionally, there has been a gap between basic science and clinical medicine. Translational medicine fills this gap with a bidirectional approach linking the two. Scientific discovery is taken from the laboratory to the clinical setting, and clinical data are taken back to the laboratory. This model has been successfully adopted to identify targets in the preclinical setting, develop compounds based on these targets, and treat patients in clinical trials. Less acknowledged but just as important is the art of taking clinical observation back to the laboratory to clarify molecular pathways, resistance mechanisms, and genetic alterations.

During the past 20 years, this translational paradigm of “bench to bedside and back again” has enabled rapid progress in the elucidation of complex biologic mechanisms and the development of rational, targeted therapies. Key molecular processes—growth factor binding, signal transduction, gene transcription control, cell cycle checkpoints, apoptosis, angiogenesis—have emerged as potential targets. The development and regulatory approval of drugs such as rituximab, trastuzumab, imatinib, erlotinib, lapatinib, bevacizumab, and cetuximab have provided clinical validation for this molecularly targeted approach. These discoveries have radically changed the way cancer treatment is conceptualized. The historic “one size fits all” approach of treating cancer in the setting of broad tumor categories is being supplanted by the identification of specific targets and molecular subtypes, leading to a personalized approach to treatment based on a patient’s unique tumor characteristics.

Targeted therapy is personalized therapy. To appreciate the novelty and success of the bench to bedside and back paradigm and the exponential growth in the field of translational medicine, it is helpful to contextualize this progress within a larger historical perspective. In the past, cancer treatments evolved from empiric observation and, not infrequently, chance observation. In 1942, the sinking of a U.S. battleship led to the recognition that mustard gas causes profound lymphoid and myeloid suppression. Following this accidental discovery, mustine (the prototype nitrogen mustard) was used to treat non-Hodgkin's lymphoma (1). Since then, a majority of the more than 200 currently available cancer drugs were identified serendipitously from plants or fungi.

Translational medicine, fueled by the human genome project, has led to a bench to bedside approach to drug discovery. In revealing a vast amount of information about normal and malignant cells, the human genome project has provided a gateway to the current era of molecular medicine. The emerging fields of proteomics and genomics, bioinformatics and systems biology, and nuclear imaging and nanotechnology (among others) are providing the tools to mine and navigate these data. The development of rational drug therapy is now based on an understanding of the molecular genotypes and phenotypes of disease.

The histories behind the development of all-trans retinoic acid (ATRA), imatinib (Gleevac; Novartis, Cambridge, MA, USA), and decitabine (Dacogen; MGI Pharma/SuperGen, Minneapolis, MN, USA) are different versions of the bench to bedside and back
paradigm. Each story illustrates the importance and potential of a rigorous translational approach. The story of cancer stem cells is just beginning; however, it is already clear that the cancer stem cell hypothesis will further accelerate our understanding of carcinogenesis and lead to additional targeted therapies.

Translational medicine is the language that delineates and clarifies discoveries made at the bench so they can evolve into stories of clinical efficacy. We are currently authoring what might be conceptualized as the first chapter of personalized medicine. Subsequent chapters will describe combination therapies that target both the cancer stem cell for disease eradication and the downstream pathways for disease control and stabilization.

The bench to bedside and back paradigm of translational medicine has led to the discovery of therapies that have transformed the way cancer patients live. Although the ultimate goal is to prevent cancer entirely, a more immediate milestone is to transform cancer into a manageable disease. The language of translational medicine has enabled open communication and understanding between basic scientists and clinical researchers that will further refine targeted therapies to support a chronic disease model.

2. DEVELOPMENT OF ONE OF THE FIRST TARGETED THERAPIES: ALL-TRANS RETINOIC ACID AND THE BEDSIDE TO BENCH PARADIGM

With the exception of hormonal therapy for breast cancer, all-trans retinoic acid (ATRA) is one of the earliest examples of a successful targeted drug and serves as a model for developing novel biologic agents tailored to various other malignant tumors. The introduction of ATRA was a major breakthrough in the treatment of acute promyelocytic leukemia (APL). The molecular mechanism underlying ATRA-induced APL cell differentiation was not discovered until after the introduction of ATRA to the clinic. This discovery launched the first molecular marker used to diagnose and monitor minimal residual disease. ATRA exemplifies the success that can come from returning a compound from the clinical setting to the laboratory (bedside to bench) to enhance the understanding of disease and of a drug’s activity. In recent years, ATRA in combination treatment has become an example of how systems-based synergistic targeted therapy is used to improve clinical outcomes.

Leukemia as a model for other disease types is being emulated in terms of molecular identification of subtypes and individualized treatment approaches. The responses of leukemia to therapies differ from one subtype to another; therefore, therapeutic strategies are disease pathogenesis-based and individualized. APL is a distinct subset of acute myeloid leukemia (AML), initially described in 1957 with case studies and characterized as the most malignant form of acute leukemia (2). APL constitutes approximately 10% to 15% of all cases of AML.

ATRA was taken to the clinic before its specific mechanism, related to circumventing the impact of APL’s aberrant PML-RAR (retinoic acid receptor) genotype was understood. ATRA was tested clinically based on the hypothesis that it could lead to the differentiation of immature leukemic cells. Until the late 1980s, the only effective treatment for APL was intensive chemotherapy, typically combining an anthracycline with cytosine arabinoside (AraC). During the late 1980s, investigators in China demonstrated that the vitamin A derivative ATRA could induce differentiation of HL-60, a cell line with promyelocytic features, as well as in fresh leukemic cells from patients. These preclinical studies led to the first clinical trial in 1986. This study of ATRA in 24 patients with APL demonstrated complete remission in all but one of the patients (3). The remaining patient achieved a complete response (CR) when chemotherapy was added. A significant observation was the
gradual terminal differentiation of malignant cells in the bone marrow. A second (French) study resulted in a CR in 14 of 22 patients through a differentiation effect of treatment (4).

Consistent with our current paradigm of targeted therapy being more gentle and less toxic than past therapies, ATRA demonstrated less toxicity than conventional chemotherapy. The most severe associated side effect, retinoic acid syndrome (RAS), was rare. By its association with increased numbers of differentiated neutrophils secreting inflammatory cytokines, the RAS caused fever and respiratory distress followed by interstitial pulmonary infiltrates, weight gain, pleural or pericardial effusion, and renal failure. The incidence of RAS toxicity was minimized by the addition of cytotoxic therapy to ATRA.

Many subsequent randomized studies, internationally, confirmed the efficacy of ATRA for the treatment of APL. Studies demonstrated improved CR rates, decreased severe adverse effects compared to cytotoxic chemotherapy alone, and increased duration of remission (5–7). Most patients treated with ATRA alone after demonstrating CR ultimately relapse, so the combination of ATRA and cytotoxic chemotherapy now constitutes front-line therapy for newly diagnosed APL.

The development of treatment approaches for APL serves as a paradigm for the design of rational therapies based on an oncogene-dependent pathway to PML-RARα (8). APL is characterized by a distinct morphology of blast cells, an expansion and accumulation of leukemic cells that are thwarted at the promyelocytic stage of myelopoiesis. APL is cytogenetically associated in 95% of patients with reciprocal chromosomal translocations involving the retinoic acid receptor-α (RARα) gene on chromosome 17 [chromosomal translocation t(15;17)(q22;q21)]. The PML gene fuses with the RARα gene to form PML-RARα and RARα-PMIL chimeric genes with corresponding fusion oncoproteins. RARα is vital for granulocyte development (9); and PML-RARα, the dysregulated RARα, plays a key role in the pathogenesis of APL. RARα−/− mice demonstrated accelerated granulopoiesis, indicating that this pathway can also function as a negative controller of granulocytic proliferation (10). Heterodimerization of PML-RARα with PML confers a growth advantage, apoptosis resistance, and differentiation arrest of APL cells.

Further correlative investigations after these early clinical trials led to the discovery of one of the earliest validated biomarkers to predict response to treatment and as a harbinger of residual disease. Warrell and colleagues (11) showed that clinical response to ATRA in APL was associated with the expression of aberrant RARα nuclear receptor. The detection of this receptor demonstrated accuracy in its ability to detect residual leukemia in these patients. After entering CR, 75% to 90% of patients still have the PML-RARα fusion gene detected by reverse transcription polymerase chain reaction (RT-PCR) (12).

Dependence on aberrant RARα oncoprotein expression for APL pathogenesis was subsequently further elucidated. RARα is a member of the nuclear hormone receptor superfamily and acts as a ligand-inducible transcriptional regulator. RARα requires heterodimerization with retinoid X receptor-α (RXRα) to bind retinoic acid response element (RARE) located in the promoter regions of the target genes. In APL cells, PML-RARα binds RARE through the DNA binding domain of RARα in a dominant manner. PML-RARα mediates transcriptional repression of RARα target genes by recruitment of co-repressors (CoR), histone deacetylase (HDAC), and DNA methyltransferase. This leads to a maturational block in myeloid differentiation, thought to be a first step of leukemogenesis.

By contrast, ATRA triggers the dissociation of the HDAC complex and recruitment of coactivators. ATRA also induces degradation of PML-RARα and activates normal RARα and PML, resulting in differentiation of APL cells. ATRA works by dissociating CoR from
the PML-RARα oncoprotein and recruiting CoR to open the chromatin structure. The result is a conversion of PML-RARα from a transcription repressor to transcription activator (13). ATRA also degrades the PML-RARα oncoprotein through a caspase-mediated cleavage pathway and proteosome-dependent degradation pathway. Simultaneously, RXR is released to reheterodimerize with RARα, leading to recovery of the RA pathway and granulocytic differentiation (14,15). Degradation of PML-RARα also leads to relocalization of PML and the recovery of PML functions with subsequent growth arrest and apoptosis induction (16,17).

In recent years, arsenic compounds, particularly arsenic trioxide (AS$_2$O$_3$), have shown success in the treatment of both primary cases and in relapsed and ATRA-resistant patients. During the 1990s, investigators in China reported that arsenic trioxide could induce a CR in patients with APL (18). Functioning by a mechanism different from that of ATRA, arsenic can induce a CR rate of more than 80% in relapsed patients after treatment with ATRA. Trials in the United States showed molecular remission in more than 80% of relapsed APL patients, as measured by RT-PCR of marrow specimens for PML-RARα transcript. Currently, arsenic is considered a first-line therapy for relapsed APL patients. Because arsenic does not completely eradicate leukemic cells, the optimal postremission therapy remains undefined. Outcomes of autologous stem cell transplantation in patients with a second molecular remission after arsenic therapy have demonstrated a good success rate.

Elucidation of molecular mechanisms in the pathogenesis of APL and in the pharmacologic approaches has led to high CR rates with ATRA in combination with chemotherapy and as postremission consolidation treatment. The ATRA story introduced the concept of molecular target-based cancer treatment, which has been further developed by the tyrosine kinase inhibitor imatinib to treat chronic myelogenous leukemia (CML) successfully. Lessons learned from APL treatment inspire and increase our understanding of the complex biology of tumors and their mechanism-based diagnosis and treatment.

### 3. A MODEL OF BENCH TO BEDSIDE TARGETED DRUG DEVELOPMENT: THE STORY OF IMATINIB IN PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA, CHRONIC MYELOGENOUS LEUKEMIA, AND GASTROINTESTINAL STROMAL TUMORS

The success of imatinib in chronic myelogenous leukemia (CML) validated the hypothesis that a specific molecular understanding of cancer can directly affect cancer therapy. Imatinib demonstrated proof of the concept that major clinical benefit can be gained by targeting an oncogenic abnormality that drives a particular type of cancer in a particular subject population. It represents a notable example of how research at the “bench” translated to the “bedside” and then back again, can lead to the development of a successful molecular targeted therapy. The path leading to the U.S. Food and Drug Administration’s (FDA) approval of imatinib for CML in 2001 and its subsequent development in gastrointestinal stromal tumors (GISTs) provided significant insight into the challenges of effectively translating targeted agents into use in the clinic.

The story behind the development of imatinib and its subsequent use in CML and GISTs illustrates fully the paradigm of bench to bedside and back. Whereas the mechanism of action of ATRA was discovered only after clinical efficacy was demonstrated in patients with APL, the mechanism of imatinib and its specific target were elucidated before it was transferred to the clinic. The theme of “kinase-dependent” cancers explains cancers whose growth is driven
by a specific set of kinases. The distinct molecular abnormality underlying Philadelphia-positive leukemia is activation of ABL tyrosine kinase by a chromosome translocation (19,20). Imatinib, a small-molecule tyrosine kinase inhibitor, is a direct inhibitor of BCR-ABL, KIT, and the platelet-derived growth factor receptor (PDGFR) alpha and beta. Imatinib binds competitively with ATP to BCR-ABL, blocking abnormal signaling and selectively inhibiting proliferation of BCR-ABL-positive cells.

The observation that imatinib inhibits other kinases, notably the oncoprotein c-Kit, led to another successful clinical translation of the drug for the treatment of GISTs. The basic science and subsequent clinical application followed by the return to the laboratory and reemergence in other treatment models serves as an example of how distinct molecular features of cancers can be used to select appropriate treatment. Both the successes and limitations of imatinib, notably de novo and acquired resistance patterns, provide guidance for the development of other molecular targeted therapies in cancer.

Just as APL demonstrates oncogene dependence on PML-RARα, CML requires the BCR-ABL oncogene for pathogenesis. CML is characterized by an overabundance of granulocytes, erythrocytes, and platelets in peripheral blood. The molecular hallmark of CML is the Philadelphia (Ph) chromosome, a reciprocal 9;22 translocation that generates the fusion oncogene bcr-abl. Since its identification in 1960, the Ph chromosome is now known to be present in more than 90% of CML cases (21). The presence of the Ph chromosome in a hematopoietic stem cell leads to cytokine-independent cell growth and survival. Imatinib created a revolution in the treatment of CML. CML typically has three progressive and distinct phases: a benign chronic phase, an accelerated phase, and a rapidly fatal blast crisis. Efficacy with imatinib treatment has been demonstrated in all three phases of the disease. Philadelphia-positive acute leukemias, which are notoriously resistant to chemotherapy, also bear a Bcr-Abl abnormality (p190Bcr−Abl) (19) and respond to imatinib, albeit not as well as CML, which is characterized by a slightly different Bcr-Abl aberration (p210Bcr−Abl). Before imatinib’s discovery, treatment options were relatively limited, even for CML. Allogeneic bone marrow transplantation had good success rates; however, the need for a matched donor limited patient eligibility. Treatment for most patients initially consisted of hydroxyurea, busulfan or interferon-α. Intensive chemotherapy regimens also were used but were harsh and had little impact on survival.

3.1. Imatinib as a Model for Discovery of Approaches to Overcome Resistance

Resistance patterns significantly complicate the successful application of kinase inhibitors and other targeted therapies. The identification of CML resistance to imatinib is an example of how genomic and proteomic approaches can help identify both de novo and acquired resistance and help predict response to therapy. Investigation of resistance patterns to imatinib in CML led to the development of a successful second-generation tyrosine kinase inhibitor that overcomes resistance in some patients.

Defined as a relapse of disease in a patient who has been on continuous therapy after an initial response and first recognized in advanced-stage CML patients, acquired resistance also occurs in chronic-phase CML and GISTs. Some relapsed patients with CML have mutations in the ABL kinase domain that affects drug sensitivity (22,23). In ABL, three mutations are most common. The emergence of imatinib-resistant kinase mutants in patients with CML prompted the development of second-generation inhibitors, such as dasatinib. Dasatinib targets the more conserved active form of the kinase and inhibits production of many of the imatinib-resistant mutants as well as other targets such as the nonreceptor
tyrosine kinase Src (24). Refinement of technologies, genotyping in particular, have led to the identification of resistant mutations.

Dasatinib is a compound that can successfully overcome acquired resistance in most CML patients. It was first reported to induce hematologic and cytogenetic remissions in a high percentage of imatinib-resistant CML patients in a Phase I trial (25,26). With a potency of more than 300 times that of imatinib, dasatinib is an orally available, selective kinase inhibitor. It differs from imatinib in that it can bind to both the active and inactive conformations of the ABL kinase domain. Dasatinib also inhibits kinases such as Src, KIT, PDGFR, and others. There is only one known mutation of BCR-ABL against which dasatinib is not effective.

Results from a Phase I dose-escalation trial of dasatinib (27) demonstrated a complete hematologic response in 37 of 40 patients with chronic-phase CML. Major hematologic responses were seen in 31 of 44 patients with accelerated-phase CML, CML with blast crisis, or Ph-positive ALL. In these two phases, the rates of major cytogenetic response were 45% and 25%, respectively. At a median follow-up of more than 12 months, 95% of patients with chronic-phase disease had a durable response. At a median follow-up of 5 months, 82% of patients with accelerated-phase disease maintained this response. Consistent with preclinical data, all BCR-ABL genotypes with the exception of the T3151 mutation, demonstrated a response. The investigators concluded that dasatinib induces hematologic and cytogenetic responses in patients with CML or Ph-positive ALL who cannot tolerate or are resistant to imatinib.

Based on these results and on initial Phase II data, the FDA granted accelerated approval for dasatinib in June 2006 for patients with CML who demonstrated resistance or intolerance to prior therapy (including imatinib). Full approval was also granted for the treatment of adults with Ph+ ALL (Ph+ALL) with resistance or intolerance to prior therapy.

Recently published Phase II study results confirmed the beneficial effect of dasatinib in imatinib-resistant patients. Results of a randomized Phase II trial of dasatinib or high-dose imatinib for CML after failure of first-line imatinib demonstrated that, with a median follow-up of 15 months, complete hematologic responses were seen in 93% of patients receiving dasatinib versus 82% of patients receiving high-dose imatinib (28). This difference was statistically significant \( (p = 0.034) \). Also significant was the improvement in cytogenetic response rate (RR) in patients treated with dasatinib (52%) versus those treated with high-dose imatinib (33%) \( (p = 0.023) \). Patients treated with dasatinib had significantly better molecular RRs and progression-free survival (PFS).

Findings from these studies highlight the importance of genotyping BCR-ABL to predict which patients will respond to treatment. The effectiveness of dasatinib in patients with imatinib-resistant disease also underscores the persistent role of BCR-ABL in disease activity and the need to define further the mechanisms of resistance and the therapies that target this pathway. In the future, genotyping will likely be performed on all CML patients. Genotyping allows the identification, a priori, of patients who will not respond to conventional treatment.

Further studies are needed to determine the role of other abnormalities in resistance. For instance, recent data from our laboratory suggest that some patients with advanced CML demonstrate down-regulation of the loss of Bcr-Abl expression (29).

### 3.2. Mathematical Modeling as a Tool for Discovery

A quantitative understanding of cancer biology requires devising a mathematical framework that can describe fundamental principles underlying tumor initiation and
progression. Tumorigenesis is governed by principles that explain evolution: mutation and selection. An example of the application of mathematical modeling to help streamline the acquisition of knowledge about molecular mechanisms is one developed for evaluating resistant populations in leukemia. A formula has been derived to calculate the probability of resistance by considering an exponentially growing leukemic stem cell population that produces resistant leukemic cells (30). These calculations might be helpful for determining when imatinib therapy ought to be combined with other therapies, provide an accurate prognosis for the disease, and direct future efforts. This model may offer predictive power regarding the course the disease is likely to take.

The interactions between targeted agents and cancer cells is still not well understood. A mathematical approach can help augment molecular biology discoveries. For example, imatinib can be studied using a mathematical model of signaling events in CML cells. Models can elucidate the effects of imatinib on autophosphorylation of the BCR-ABL oncprotein and subsequent signaling to predict a minimal concentration for drug efficacy. One such model demonstrates that cellular drug clearance mechanisms decrease the value of imatinib in blast crisis cells (31). Michor and colleagues also designed a mathematical model to evaluate the kinetics of CML during treatment with imatinib (32).

A mathematical approach can be applied to the complex interactions among molecular pathways. The possible combinations and permutations are so vast that the traditional methodical, labor-intensive approach of in vitro and in vivo experimentation cannot solve the problems of optimal drug targeting, delivery, and finding effective combinations in an efficient manner. In the future, mathematical modeling and other systems biology approaches will likely be the most feasible way to identify hypotheses that can then be efficiently tested preclinically and entered into early trial design.

3.3. GIST and the Development of Targeted Therapy

The discovery that the c-kit gene drives malignant activity in GISTs was exploitable by treatment with imatinib. The success of the treatment of GIST with imatinib provides another example of how biomarker identification predicts clinical response. Once again, the theme of “oncogene dependence” explains an underlying pathogenic mechanism and rationale for clinical evaluation of a targeted drug.

In February 2002, imatinib received accelerated approval by the FDA for use in patients with KIT-positive unresectable or metastatic GISTs. The approval was based on the RR observed in open-label, multinational studies (33,34). Imatinib was subsequently demonstrated to prolong PFS in patients with GISTs (35).

GISTs were rarely diagnosed until around the year 2000, when they became a focus of research. Prefiguring this increased interest was the finding during the late 1990s that gain-of-function mutations of the receptor tyrosine kinase KIT play a major role in the pathogenesis of GISTs. Previously classified as leiomyomas, leiomyosarcomas, and leiomyoblastomas, the term GIST was first used in 1983 (36) to describe gastrointestinal nonepithelial neoplasms that lacked the characteristics of smooth muscle cells. Historically, GISTs have been associated with a poor prognosis. The rates of recurrence after surgical resection are high as 90% (37), with a 5-year overall survival (OS) ranging between 28% and 42% (38). GISTs are highly radiation-resistant and chemoresistant. RRs to single-agent or combination chemotherapy have ranged from none to 9% in the presence of metastatic disease (39–43). A combination trial using mesna, doxorubicin, ifosfamide, and dacarbazine reported an objective RR of 22% (44), but these results have been questioned.
Preclinical evidence showed that most GISTs demonstrated gain-of-function mutations in the KIT (c-kit) proto-oncogene (45). Constitutive activation of KIT in GISTs leads to uncontrolled cell proliferation and resistance to apoptosis (46). GISTs also commonly demonstrate mutations of PDGFRα kinase. Imatinib’s known mechanism of inhibition of the tyrosine kinases KIT and PDGFRα provided a rationale for its clinical evaluation in GIST.

In 2000, imatinib was administered for the first time to a GIST patient in a compassionate use setting. In this patient with advanced, heavily pretreated GIST, a dramatic radiographic, metabolic, and symptomatic response was observed at a dose of 400 mg daily (47). The choice of dose was based on evidence of efficacy at this dose level in patients with CML as well as laboratory prediction of effective levels. The patient’s liver lesions decreased to less than 25% of the initial tumor burden after 8 months of therapy. Toxicities were minimal. This patient remained in remission for several years before the disease recurred.

Based on the dramatic response in this GIST patient and a solid preclinical rationale (as well as a dearth of effective treatment options), several clinical trials were undertaken. Studies demonstrated that 75% to 90% of patients with advanced GISTs treated with imatinib had a clinical benefit (33,35,48). Several large, randomized, Phase III studies demonstrated prolonged PFS and OS in patients with advanced GISTs (49,51). Imatinib is now the standard of care in patients with metastatic or unresectable disease and has led to prolonged disease-free and OS.

The KIT gene encodes the KIT protein, which is the transmembrane receptor for the cytokine stem cell factor. KIT’s intracytoplasmic domain functions as a tyrosine kinase. The c-kit proto-oncogene product CD117 (KIT) is a transmembrane protein receptor for the growth factor known as stem cell factor (SCF, or Steel factor) (52). CD117 is a more specific marker for GIST than CD34, and it is expressed on 85% to 100% of GIST samples (53). KIT is both a diagnostic marker and a therapeutic target. The malignant behavior of most GISTs can be explained by constitutive activation of KIT. After Hirota and colleagues initially identified the c-kit gain-of-function mutations (45), another c-kit gene mutation was identified that causes constitutive activation of the ligand (54). More than 100 gain-of-function mutations for c-kit have been identified in GISTs (55,56).

These mutations have subsequently been evaluated as both prognostic and predictive markers. The presence of any kit mutation has been demonstrated to be an independent prognostic factor, indicating poor prognosis for patients with a localized GIST (57). For example, patients with exon 11 mutations were found to have a 5-year PFS rate of 89% compared to 40% for patients with mutations at other exon sites (p = 0.03) (58), thus establishing the presence of an exon 11 mutation as an independent predictor of survival. In the largest series to date, Heinrich and associates (59) evaluated 324 tumors of patients enrolled in a Phase II study for kit mutations and corresponding response to imatinib. Kit mutations were identified in 280 (86.4%) tumors. The presence of exon 11 mutations corresponded to a 67% objective RR, whereas exon 9 mutations corresponded to a 40% objective RR; the absence of any identifiable mutation was associated with a 39% partial RR (p = 0.0022.) Time-to-treatment failure was significantly longer for patients with exon 11 mutations compared to any other mutational status (576 days for exon 11 mutations, 308 days for exon 9 mutations, 251 days for no mutation; p = 0.00112). Patients with exon 11 mutations also demonstrated a trend toward increased OS compared to other mutations. The authors of this significant study concluded that kit mutational status could be used as a predictor of clinical response in patients with GISTs.
Despite high RRs and long duration of response in patients with GISTs treated with imatinib, resistance typically develops after a year of treatment. This acquired resistance is characterized by secondary mutations in the KIT or PDGFRα kinases, gene amplification, or loss of target kinase expression \((49,60,61)\). Approximately 20% of GIST patients have de novo resistance to imatinib.

In January 2006, sunitinib (Suitent) received approval for the treatment of patients with imatinib-refractory (or intolerant) GISTs. Sunitinib is a small-molecule tyrosine kinase inhibitor. It inhibits vascular endothelial growth factor receptors (VEGFRs) types 1 and 2, PDGFRα and PDGFRβ, c-Kit, and the FLT3 and RET kinases. Compared to imatinib, sunitinib demonstrates greater potency against the c-Kit and PDGF kinases and additional inhibition of VEGFR2 and PDGFRβ. This activity may explain its efficacy in imatinib-resistant disease \((62,63)\).

FDA approval was based on an international, randomized, double-blind, placebo-controlled trial of sunitinib in patients with GIST who had disease progression during prior imatinib treatment or who were intolerant of imatinib \((64)\). The study randomized patients to receive sunitinib or placebo, and all patients received best supportive care. Patients who were randomized to placebo were allowed to cross over to receive sunitinib at the time of progression. At the time of the prespecified interim analysis, there was a statistically significant advantage for sunitinib over placebo regarding both time to progression (TTP) and PFS. The median TTP was 27 weeks for patients treated with sunitinib compared to 6 weeks for patients receiving placebo.

This study of sunitinib in GISTs is a good example of how novel targeted therapies may have a cytostatic rather than a cytotoxic effect. Whereas a significant improvement in TTP was noted in patients receiving sunitinib versus placebo, the RR for patients treated with sunitinib was only 7%. Biologic therapies may delay progression (and stabilize disease) without inducing any significant shrinkage in the tumor burden. This underscores a few key issues in the drug development process. Likely due in large part to the high cost associated with the clinical trials, compounds that fail to demonstrate significant activity, defined as an objective response, in the Phase I setting often do not progress further in development to Phase II trials. RR continues, therefore, to be an important endpoint, in a realistic sense, even if it is not necessarily stated as a primary objective in the Phase I trials. Despite a modest objective response, the approval of sunitinib for GISTs was enabled because of its orphan tumor status. For a lack of better therapies, sunitinib was approved because it improved PFS. In fact, because of the crossover design of the study, a valid OS endpoint will never be realized. Similarly, when many of the novel biologic agents are administered as single agents they may be effective only in terms of disease stabilization. For these agents, the challenge will be finding the right combination to further increase efficacy.

4. WHAT IS OLD IS NEW AGAIN: DECITABINE AND THE DAWN OF EPIGENETICS

Increasingly, attention is being focused on epigenetic changes to explain the pathogenesis of cancer. Epigenetic changes are important in both the primary growth of a tumor and the metastatic dissemination and survival of tumor cells \((65)\). Although still in its infancy, epigenetics is an area of intense research. In contrast to genetic mutations that result in permanent alterations in primary DNA sequences, epigenetic mechanisms of gene expression involve reversible alterations in chromatin structure without changes in the primary nucleotide
sequence (66). The epigenetic mechanisms that are currently best understood are DNA methylation and histone acetylation, which lead to inactivation of transcription and silencing of tumor suppressor genes.

Hypermethylation inhibits the activation of promoter regions on DNA and subsequently the transcription of tumor suppressor genes. This area presents a rational target for therapy. Epigenetic therapy consists of reversing the silencing of these critical genes. The azacitidine class of drugs, including 5-azacitidine and 5-aza-2-deoxycytidine (decitabine), reverses DNA methylation and therefore prevents inactivation of tumor suppressor genes. The story of the development of these drugs spans more than 40 years and illustrates how the bench to bedside and back paradigm can be successfully implemented. First synthesized during the 1960s (67), the azacitidine family was shown to have activity in hematologic malignancies. It was not until more than 20 years later, however, that the mechanism of action was understood.

Epigenetic changes caused by DNA methylation lead to gene silencing and subsequent malignant transformation. Promoter hypermethylation can inactivate transcription, and genome-wide hypomethylation leads to genomic instability. Both epigenetic phenomena are present in cancer. The potential reversibility of DNA methylation makes this a logical target for cancer treatment. When the promoter region of DNA is methylated, gene expression is inhibited. Transcriptional methylation is mediated by the recruitment of transcription repressors that are part of a large complex, HDACs (68,69). Histone lysine methylation can result in either activation or repression of transcription. Histone modifications therefore promote or prevent binding of proteins and protein complexes that drive particular regions of the genome into active transcription or repression.

The azacitidine and the HDAC inhibitor classes of molecules exploit the epigenetic mechanisms of DNA methylation and histone modification. During the early 1990s, studies of 5-azacitidine in myelodysplastic syndrome (MDS), compared to best standard of care, resulted in improved response, quality of life, and OS (70–72). In 2004, 5-azacitidine received FDA approval for the treatment of MDS.

Decitabine has demonstrated efficacy in leukemias and other hematologic malignancies and has had a low RR in solid tumors (73). Initially shown to have antileukemic activity (74), decitabine was subsequently studied in MDS, AML, and CML patients. Responses were seen for each of these diseases (75–78). In May 2006, the FDA approved decitabine for the treatment of MDS. The approval was based in part on a Phase III trial of MDS patients at medium to high risk of developing AML. Results showed that the addition of decitabine to supportive therapy yielded a significant increase in overall RR compared with supportive therapy alone (17% vs. 0%; \( p < 0.001; \text{CR, 9%; PR, 8%} \)). The median time to response was 93 days, with a median duration of 288 days. Among decitabine-treated patients who had undergone two or more treatment cycles, the overall RR was 21%. Benefit in the form of hematologic improvement was observed in an additional 13% of patients compared with 7% in the supportive care group. Treatment with decitabine did not significantly delay the median time to AML or death.

The uncertainties that remain in terms of decitabine’s exact mechanism of action highlight the need for ongoing translational studies from the bedside back to the bench. Although preventing DNA promoter hypermethylation is a rational strategy, it is possible that drugs that prevent methylation could also produce unwanted effects. Decitabine is a cytosine analogue that inhibits DNA methyltransferases (DNMTs), reverses methylation, and can reactivate silenced genes (79). DNA methylation status is determined by a complex mechanism.
involving interaction among various DNMTs and other enzymes (e.g., HDACs). Decitabine becomes incorporated into DNA and inhibits DNA methylation. It may, however, have more than one mechanism of action. It leads to reactivation of genes that were previously silenced through its demethylation activity, and it may also exert an effect through a cytotoxic pathway. High concentrations of decitabine incorporated into DNA can inhibit DNA synthesis and lead to cell cycle arrest. Low concentrations of decitabine incorporated into DNA in place of cytosine can trap DNA methyltransferases and then lead to DNMT degradation without cell cycle arrest. DNA replication without DNMTs leads to global and gene-specific hypomethylation. Because global hypomethylation can increase the activation of specific genes (e.g., oncogenes), the off-target effects remain controversial and uncertain.

Over the years, decitabine has been studied in escalating doses to the maximum tolerated dose even though in vitro studies demonstrated that optimal methylation occurred at lower doses (80). Not until 2004 was decitabine shown to be more efficacious at a lower dose of 15 mg/m², although no correlation was made with DNA methylation after therapy (81). Other recent clinical trials also showed that lower doses have a better response with less toxicity (82,83). In solid tumors, however, higher doses may be desirable to produce a cytostatic effect.

Analysis of molecular response in tumor tissues following exposure to chromatin remodelling agents may enable us to identify novel mechanisms pertaining to cancer epigenetics and design more efficacious regimens. Discoveries in laboratory techniques and assays since the early 1990s have refined the quantification of DNA methylation. Better assays have enabled measurement of global hypomethylation changes before and after administration of decitabine. Hypermethylation of repetitive sequences called long interspersed nucleotide elements (LINE) can be a good pharmacodynamic surrogate of decitabine’s hypomethylating activity but not necessarily a biologic surrogate of clinical activity. Results from correlative studies in clinical trials, taken together, provide proof-of-concept that decitabine acts by hypomethylation in hematologic malignancies. Further studies will be undertaken to assess the possibility that decitabine may induce responses in ways other than demethylation.

In contrast to the significant activity of decitabine demonstrated in hematologic malignancies, it produced only limited activity in early Phase II trials of solid tumors (including melanoma, colorectal, head and neck, renal cell, testicular, and ovarian cancers) (84). The difference in clinical activity between hematologic and solid tumors may be explained by several discrete factors, including differences in drug penetration, drug conversion, and the speed of cell cycles. Most of the early clinical trials of decitabine in solid tumors probably did not use high enough dose levels of the drug, and responses were typically evaluated too soon (after only two courses). Subsequent studies showed activity between two to five courses of treatment. Future studies in solid tumors need to incorporate pharmacodynamic endpoints and correlative epigenetic analyses, and they must wait until after completion of three cycles before evaluating patients for response (to assess the objective response accurately).

Further preclinical research since these initial studies has demonstrated a potential benefit of decitabine in solid tumors. The study of epigenetic mechanisms in thoracic oncology, for example, is an area of intense interest and research. In preclinical studies, decitabine is being evaluated for a possible role in increasing the immunogenicity of tumor cells, Decitabine leads to increased expression of the cancer testis antigens (CTAs) NY-ESO-1 and MAGE-3 CTA and restored p16, RASSF1A, and TFPI-2 expression in cultured tissue from thoracic malignancies. CTAs comprise a group of immunogenic tumor-associated antigens
that are not expressed in normal tissue (except in male germ line cells and placenta). After treatment with decitabine, cancer cells, but not normal human bronchial epithelial cells, can be recognized by cytotoxic T lymphocytes (CTLs) specific for NY-ESO-1 (85).

These preclinical data led to a Phase I proof of concept study (86) designed to demonstrate that decitabine exposure can result in CTAs and tumor suppression gene modulation. Although there were no objective clinical responses, disease stabilization was noted in 4 of 25 evaluable patients in this study. One patient remained on study for 10 months, and one remained on study for 1 year before disease progression. Results from correlative studies on tissue biopsies from these patients confirmed previous in vitro studies and the initial hypothesis. Up-regulated mRNA and protein expression of the CTAs NY-ESO-1 and MAGE-3 and p16 were shown in a significant number of patients. The immune response to CTAs is limited in thoracic oncology patients. Conceivably, the up-regulated expression of these antigens could lead to the development of effective immunotherapy strategies.

Induction/repression of gene expression were also studied in an exploratory analysis with decitabine. Although the sample size was limited and did not permit robust statistical outcomes, the feasibility of comprehensive gene expression profiling was demonstrated. The use of RNA amplified from laser-captured tumor cells (from fine-needle aspirations) indicated that exposure to decitabine modulates global gene expression patterns in these thoracic malignancies. Notably, the genes that were induced by decitabine were generally repressed in lung cancers compared to their expression in normal epithelial cells. Conversely, genes that were repressed by decitabine treatment were generally overexpressed by lung cancer cells compared to cells in the normal adjacent tissue.

The rationale for evaluating combinations of epigenetic mechanisms is exemplified by combining DNMT inhibitors with HDAC inhibitors, which leads to a synergistic activation of gene expression. In vitro studies have explored this hypothesis (87), and clinical studies are underway. The DNMT inhibitor azacitidine was evaluated in combination with phenylbutyrate, an HDAC inhibitor, and showed efficacy in myeloid neoplasms (88). Combining decitabine with valproic acid in patients with AML and MDS resulted in an RR of 22% (89). These studies support further trials of chromatin remodeling agents for cancer immunotherapy, including administration with HDAC inhibitors or recombinant NY-ESO-1 or MAGE-3 protein vaccines and/or infusion of tumor antigen-recognizing CTLs (90).

Decitabine has been in development for four decades. A rigorous translational approach, with studies that continually return from the clinic to the laboratory, has led to the discovery of several key mechanisms of action, including the reactivation of silenced genes and the activation of immunomodulatory genes. Further studies are needed to elucidate other downstream mechanisms that are not yet completely understood. Decitabine’s use in solid tumors was initially disappointing, but it is increasingly apparent that it has possible benefit, particularly in combination treatment. Translational medicine continues to generate a greater understanding of its potential use. Epigenetic mechanisms present the hope of being an even more robust source of therapeutic targets than those provided by genetic permutations.

5. NEW MODEL OF CARCINOGENESIS: CANCER STEM CELL HYPOTHESIS

Research in both hematologic and solid malignancies has demonstrated that only a small percentage of cancer cells have the capacity to form new tumors. Despite a clonal origin, evidence shows that the tumor cell population is, in fact, heterogeneous in terms of capacity
for proliferation and differentiation (91). The cancer stem cell hypothesis could explain this heterogeneity. This hypothesis speculates that only a small percentage of cells in the tumor, called cancer stem cells, are able to proliferate and self-renew. Prior to consideration of the role of stem cells, the cancer model was based on unregulated growth attributed to acquired genetic events. Such events result in activation of genes promoting proliferation, silencing of genes that inhibit proliferation, and altered function of genes responsible for programmed cell death.

It was first suggested more than 40 years ago that CML arises from a transformed hematopoietic stem cell. This theory was based on the observation that granulocytes and red blood cells (RBCs) from patients with CML had a common cell of origin (91). Stem cells were isolated a decade and a half ago (with isolated CML stem cells showing expansion ex vivo). More than a decade ago, hematopoietic stem cells from patients with AML or CML generated leukemia in vivo when injected into NOD/SCID mice. More recently, cancer stem cells that are distinct from differentiated cells comprising the bulk of the tumor have been identified in MDS, multiple myeloma, and brain, breast, and lung cancers. The significance of these cancer stem cells is not yet known.

The stem cell hypothesis may help explain the failure of imatinib therapy to cure CML. Whereas imatinib is toxic to differentiated CML progenitor cells, CML stem cells may be resistant to the drug. Hematopoietic stem cells are largely quiescent and typically express high levels of ATP-binding cassette transporters, including the multidrug resistance genes. In CML stem cells, the BCR-ABL gene may be silent. It is possible that CML stem cells survive for years even if BCR-ABL activity is completely inhibited. The rapid responses seen in patients with CML when imatinib is administered may be explained by imatinib’s activity against differentiated CML progenitor cells that constitute most of the leukemia. Not being able to cure CML even with such potent activity is consistent with this hypothesis of stem cell resistance to imatinib. By contrast, the slow, durable responses that were seen occasionally with CML patients treated with interferon (IFN) are explained by data showing activity of IFN against the rare CML stem cells.

Therapy aimed at BCR-ABL activity in CML is an example of a target-directed approach for anticancer therapy. However, drugs that target cancer-specific pathways may not be the ultimate answer. Many cancers, even at the initial diagnosis, have probably already acquired multiple oncogenic mutations that are capable of driving tumor growth. Targeting only one mechanism may have a limited effect. Even a response to therapy may not ultimately predict OS because the response reflects only the targeting of differentiated cells versus stem cells with clonogenic potential.

Properties that are shared with normal stem cells seem to be responsible for cancer stem cell resistance to many therapeutic agents. Targets that share properties with normal stem cells, therefore, may have particularly strong anticancer potential. Examples of these targets include signaling pathways such as Notch, Wnt, and Hedgehog, which are important for the generation and maintenance of normal stem cells during embryonic development. Considering pathways that are involved in the development of normal stem cells raises the question of how it would be possible to develop agents without significant toxicity profiles. Several hypotheses have been offered to explain how normal stem cells may be protected. Normal stem cells have cell cycle checkpoints that can protect them from cellular damage. The stage of differentiation may also constitute a therapeutic ratio; or in other words, the stem cells that engender cancer might not be the most primitive tissue stem cells (92).
Logical too is the possibility of further progress by using therapeutic combinations that lead to both disease stabilization and eradication of cancer stem cells (for potential cure). This paradigm has not been explored adequately in combination studies; however, the emergence in recent years of a rich literature on cancer stem cells, as well as the elucidation of pathways involved with stem cell differentiation will likely lead to future combination trials.

Traditional response criteria measure tumor bulk and may not reflect changes in cancer stem cells. Dramatic responses may occur, but they are unlikely to be maintained over the long term if rare cancer stem cells responsible for maintaining disease are not targeted as well. Standard response criteria may potentially overestimate or underestimate treatment effect on cancer stem cells. It is important to develop validated biomarkers so the potential benefit of biologic agents can be realized before they are abandoned for lack of single-agent, cytotoxic activity.

6. LOOKING FORWARD: THE FUTURE OF TRANSLATIONAL MEDICINE

Personalized medicine has arrived, but this is just the beginning. Further innovation in technologies will contribute to this revolution in drug development. Among the most important aspects of improved drug development will be the identification of pharmacodynamic biomarkers, validating effective early surrogates of tumor response, and the predictive reclassification of disease. Molecular phenotyping prior to selecting a drug is now in widespread use.

The realization of a vision of personalized medicine is complex. Targeted therapies are emerging from our knowledge of the human genome and the use of sophisticated bioinformatics. Targeted imaging agents will be used to deliver therapy. Drug resistance will become more predictable. Newly identified biomarkers will allow earlier measurement of drug effects. Traditional disease categories will continue to be eroded as genetic profiling enables more targeted therapy. Treatment with more selective and less toxic therapies will further redefine the paradigm of cancer as one of a chronic disease model.

It is likely that during the next decade we will focus on eliciting more information from smaller and smaller pieces of tumor tissue. Molecular histopathology will be a core discipline. Eventually, developments in functional imaging and perhaps serum proteomics will drive non-tissue-based methods of obtaining the same information.

Translational medicine has created a language that is used for effective communication between clinical researchers and basic scientists. No longer perceived as a gap, this space between the bench and the bedside is now a robust, interdisciplinary meeting place. The stories behind the development of early targeted therapies—including ATRA, imatinib, and decitabine—provide models and lessons for ongoing discoveries. As the language of translational medicine evolves, the bench to bedside and back paradigm will transform cancer medicine.

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Targeted Therapy in Acute Myelogenous Leukemia

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Abstract

This chapter explores the antiacute myelogenous leukemia effects of various targeted therapies. It discusses combinations of targeted therapies with each other and particularly with chemotherapy. The extent to which a “targeted therapy’s” target is known a priori and the adequacy of relevant trial designs are discussed along with the relevance of new criteria for response, which patients are candidates for targeted therapy, and the appropriateness of conventional statistical methodology. The utility of identifying the “maximum tolerated” dose and emphasis on single-arm, Phase II trials followed by large randomized trials versus alternative methods such as a focus on the “optimal biologic” dose, more adaptive single-arm designs, and smaller randomized trials are also covered.

Key Words: Acute myelogenous leukemia, Targeted therapy, ATRA and arsenic trioxide gemtuzumab, FLT-3 inhibitors, Farnesyl transferase inhibitors, Epigenetic-acting agents, Clinical trial design

1. INTRODUCTION

The only therapy known to produce long-term survival of patients with acute myelogenous leukemia (AML) is “chemotherapy,” generally containing cytosine arabinoside (ara-C). Such therapy is itself targeted; that is, if there were no selectivity of a given chemotherapy for