Loss of *p53* impedes the antileukemic response to BCR-ABL inhibition

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Targeted cancer therapies exploit the continued dependence of cancer cells on oncogenic mutations. Such agents can have remarkable activity against some cancers, although antitumor responses are often heterogeneous, and resistance remains a clinical problem. To gain insight into factors that influence the action of a prototypical targeted drug, we studied the action of imatinib (STI-571, Gleevec) against murine cells and leukemias expressing BCR-ABL, an imatinib target and the initiating oncogene for human chronic myelogenous leukemia (CML). We show that the tumor suppressor p53 is selectively activated by imatinib in BCR-ABL-expressing cells as a result of BCR-ABL kinase inhibition. Inactivation of p53, which can accompany disease progression in human CML, impedes the response to imatinib *in vitro* and *in vivo* without preventing BCR-ABL kinase inhibition. Concordantly, p53 mutations are associated with progression to imatinib resistance in some human CMLs. Our results identify p53 as a determinant of the response to oncogene inhibition and suggest one way in which resistance to targeted therapy can emerge during the course of tumor evolution.

imatinib | mouse model | targeted therapy | drug resistance | tumor-suppressor gene

Most conventional cancer therapies were identified through empirical screens for agents that preferentially kill tumor cells compared with normal tissues. Although these agents are effective in treating some human cancers, many tumors are nonresponsive or evolve to a resistant state. More recently, insights into the molecular basis of cancer have enabled the development of more rational drugs that attack activities involved in the oncogenic process (1). These "targeted" therapeutics are often less toxic than conventional drugs and have remarkable activity against some cancers. The effectiveness of such agents may reflect the cancer cell's continued dependency on an oncogenic lesion such that it cannot tolerate the absence of its signal, a phenomenon sometimes referred to as "oncogene addiction" (2, 3). However, the molecular determinants that contribute to the sensitivity and resistance of tumor cells to targeted therapies are poorly understood.

Imatinib (STI-571, Gleevec) is a small-molecule inhibitor of BCR-ABL whose effectiveness against CML makes it the paradigm for targeted cancer therapy. Clinical resistance to imatinib is most often caused by point mutations in the BCR-ABL kinase that prevent the drug–target interaction and subsequent kinase inhibition (4), an observation that provides formal proof that the BCR-ABL kinase is essential for imatinib sensitivity and that has produced rational strategies to circumvent drug resistance (5, 6). However, imatinib is generally less effective against Ph^+ (Philadelphia chromosome) acute lymphoblastic leukemia or CML that has progressed to a more genetically complex phase known as "blast crisis," a response pattern that cannot be entirely explained by the appearance of BCR-ABL mutants (7–9). Presumably, other factors associated with disease progression can impede imatinib action.

The *p53* tumor suppressor provides a potent barrier to tumorigenesis by triggering cell-cycle checkpoints, cellular senescence, or apoptosis in response to DNA damage and aberrant proliferative signals (10, 11). Because conventional chemotherapeutic agents can exploit the same signaling networks influenced by *p53* for their optimal antitumor effects, *p53* mutations acquired during tumorigenesis can promote drug resistance as a byproduct of tumor evolution (12). However, how *p53* influences the response of tumors to targeted cancer therapy is not known. Interestingly, *p53* mutations can accompany disease progression in human CML (13–17), and there is substantial cross-talk between the *BCR-ABL* and *p53* signaling networks (18–22). Here, we examine the impact of *p53* on the cellular response to imatinib in several well characterized models of *BCR-ABL*-induced malignant transformation. Our results illustrate how factors linked to malignant progression can modulate the response of tumor cells to targeted cancer therapy and have implications for understanding the heterogeneous responses to these therapies in the clinic.

Results

BCR-ABL Kinase Inhibition Induces *p53*. To examine the impact of *p53* on imatinib responses *in vitro*, we first examined Ba/F3 cells, a murine Pro-B cell line that has been used extensively to study *BCR-ABL* transformation and imatinib response (5). Cells were infected with retroviruses expressing a control vector, BCR-ABL (p210), or a BCR-ABL mutant that is insensitive to imatinib (p210/T315I), and the resulting populations were assessed for p53 levels and BCR-ABL kinase activity 8 h after imatinib addition.

As expected, p53 was expressed at low levels in the parental cells and was not induced after imatinib treatment. In contrast, upon imatinib treatment, p53 was induced in BCR-ABL (p210)-expressing cells but not those expressing the BCR-ABL mutant (T315I). Phosphorylation of $p56^{DOK\text{-}2}$, which is a downstream target of BCR-ABL signaling, was high in both BCR-ABL-expressing populations and was repressed in treated cells expressing p210 but not the p210/T315I mutant (Fig. 1*a*). Similar results were observed in 32D cells and after treatment with PD166326, a dual BCR-ABL/SRC kinase inhibitor with a distinct chemical structure (6) (Fig. 5, which is published as supporting information on the PNAS web site, and data not

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Abbreviations: CML, chronic myelogenous leukemia; HSC, hematopoietic stem cell; RNAi, RNA interference.

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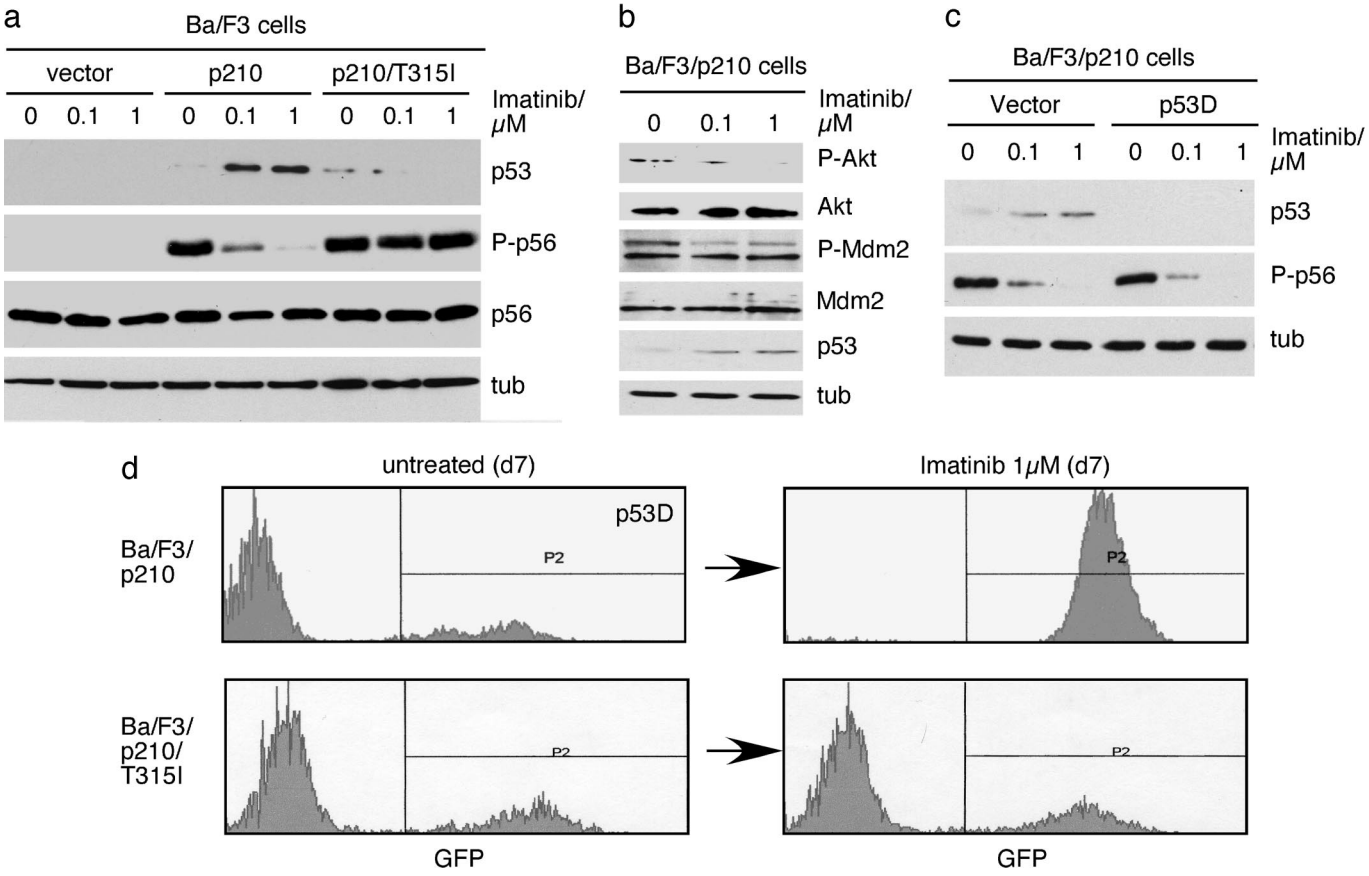

Fig. 1. *p53* modulates sensitivity to imatinib *in vitro*. (*a*) Immunoblot analysis of lysates prepared from Ba/F3 cells stably transduced with empty vector, BCR-ABL (p210), or mutant BCR-ABL (p210/T315I) treated with imatinib for 8 h as indicated were probed for p53, phosphorylated; and total levels of $p56^{Dok\text{-}2}$ protein (P-p56 and p56, respectively), and tubulin (tub) as loading control. (*b*) Lysates of imatinib-treated Ba/F3/p210 cells were probed with antibodies against p53, total and phosphorylated (Ser-473) Akt (Akt and P-Akt), and total and phosphorylated (Ser-166) Mdm2 (Mdm2 and P-Mdm2), with tub as loading control. (*c*) Immunoblot of Ba/F3/p210 cells lysates expressing either an RNAi vector targeting *p53* (p53D) or control vector (Vector) treated as indicated and probed for p53, phosphorylated (P-p56) and total (p56) p56 protein, and tub. (*d*) *In vitro* competition assay. Populations of Ba/F3 cells stably expressing either BCR-ABL (p210; *Upper*) or the T315I mutant (p210/T315I; *Lower*) were partially transduced with an RNAi vector against *p53* (p53D) and propagated in the presence or absence of 1 μM imatinib for 1 week and then subjected to flow cytometry to determine the fraction of cells containing the RNAi vector (high GFP expression).

shown). However, in a T cell leukemia line expressing BCR-ABL (BV173), significantly higher concentrations of kinase inhibitor were needed to induce p53 levels (e.g., 500 nM PD166326, data not shown), suggesting that cell-line-specific differences can exist (22).

BCR-ABL promotes oncogenesis through several downstream effectors, including the Akt/PKB kinase (23). Akt also phosphorylates Mdm2 on Ser-166, which acts to prevent Mdm2 from efficiently targeting p53 for degradation (24). Interestingly, phosphorylation of Akt/PKB was reduced in response to imatinib in p210-expressing BaF/3 cells, coinciding with a parallel decrease in Ser-166-phosphorylated Mdm2 (Fig. 1*b*). Together, these data imply that imatinib activates p53, at least in part, through inhibition of BCR-ABL signaling.

***p53* Contributes to Imatinib Action *in Vitro*.** To evaluate the impact of *p53* on imatinib action, we used RNA interference (RNAi) to suppress *p53* and determined how this RNAi impacted cellular responses to the drug. We introduced a retroviral vector coexpressing one of two different *p53* short hairpin (sh)RNAs [p53C (25) and p53D (26)] with GFP into Ba/F3 cells expressing p210 or p210/T315I, and the percentage of GFP positive cells was assessed by flow cytometry. FACS-purified GFP-positive cells expressing the *p53* shRNA showed substantial p53 knockdown and prevented p53 induction after imatinib treatment, although BCR-ABL kinase activity was inhibited as indicated by the reduction in phosphorylated $p56^{Dok\text{-}2}$ protein, a BCR-ABL target (Fig. 1*c*). Treatment of the mixed populations with 1 μM imatinib revealed that p53 knockdown with either shRNA conferred a selective advantage to Ba/F3 cells expressing p210, because the fraction of RNAi/GFP-expressing cells increased substantially (Fig. 1*d Upper*; and see Fig. 6, which is published as supporting information on the PNAS web site). By contrast, the RNAi vector targeting *p53* conferred no advantage to Ba/F3 cells expressing the imatinib-resistant p210/T315I mutant and neither did a control vector (Figs. 1*d Lower* and 6). Similar results were observed in BCR-ABL-expressing derivatives of the 32D myeloid cell line (see Fig. 7, which is published as supporting information on the PNAS web site). Thus, *p53* can contribute to the antileukemic effects of imatinib *in vitro*.

We next examined the relationship between *p53* and imatinib action in primary cultures enriched for hematopoietic stem cells (HSCs). To this end, we isolated and expanded fetal liver cells derived from crosses of $p53^{+/-}$ mice. We transduced these cells with a retroviral vector encoding BCR-ABL and measured p53 expression after imatinib treatment. Similar to our findings in the established Ba/F3 cell line, we found that *in vitro* treatment of $p53^{+/+}$-enriched HSCs caused decreased activation of Akt and Mdm2 and induction of p53 across a range of imatinib concentrations (Fig. 2*a*).

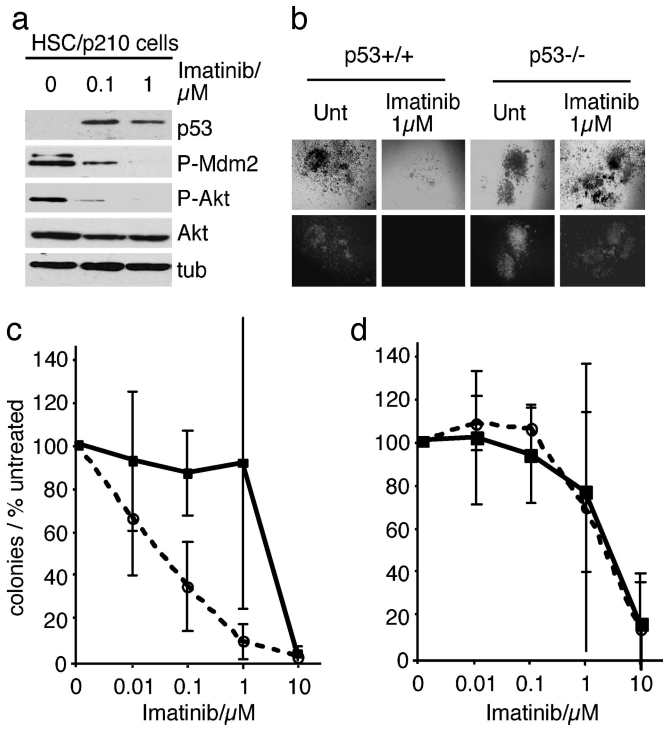

Fig. 2. BCR-ABL sensitization of primary HSCs to imatinib depends on *p53*. (*a*) Immunoblot analysis of $p53^{+/+}$ HSCs expressing BCR-ABL before therapy (Untr) or 8 h after treatment with different doses of imatinib, as indicated. Lysates were immunoblotted for p53, total and phosphorylated (Ser-473) Akt (Akt and P-Akt), phosphorylated (Ser-166) Mdm2 (P-Mdm2), and tubulin (tub). (*b*) Representative microphotographs of colonies formed by $p53^{+/+}$ or $p53^{-/-}$ HSCs in methylcellulose untreated or treated with 1 μM imatinib (*Lower*) fluorescence detection of GFP expression in *BCR-ABL*-transformed colonies. (*c* and *d*) Results of methylcellulose colony-formation assays, $p53^{+/+}$ (circles) and $p53^{-/-}$ (squares) HSC expressing BCR-ABL (*c*) or control (*d*) were incubated with imatinib at the indicated concentrations and colony-forming units counted after 10 days (mean ± SD, $n = 7$; $P = 0.016$ and $P = 0.4$ for IC_{50} ($p53^{+/+}$ vs. $p53^{-/-}$) in *c* and *d*, respectively).

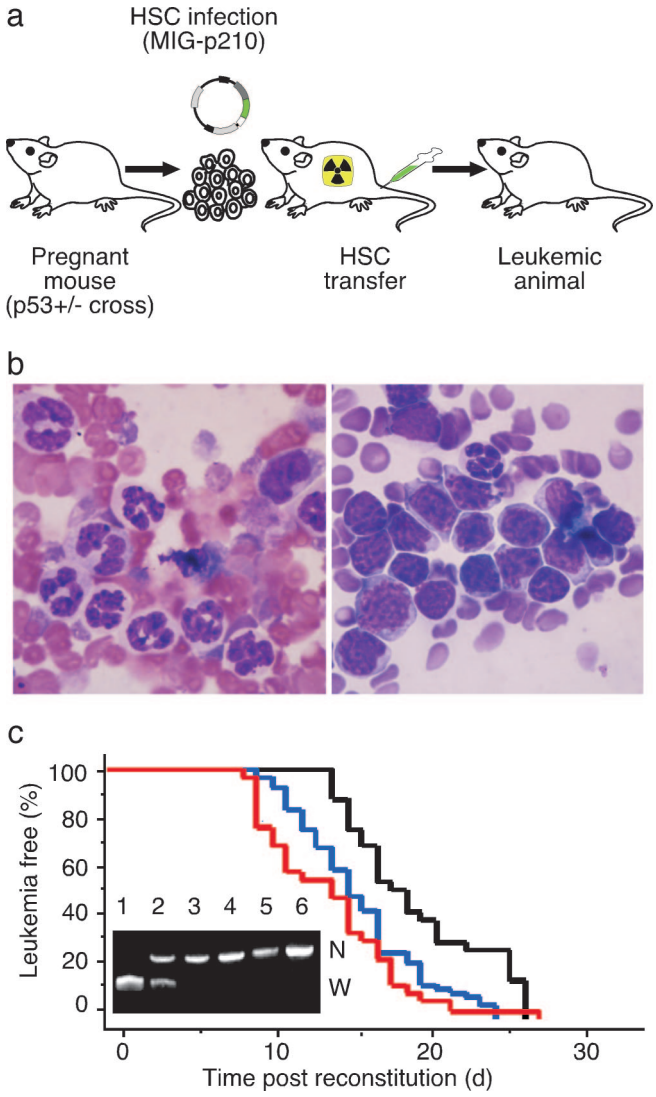

Fig. 3. BCR-ABL induced leukemia *in vivo*. (*a*) Schematic of the generation of mice harboring leukemias of defined *p53* status; MIG-p210: MSCV-p210-IRES-GFP. (*b*) Representative microphotographs of blood smears illustrating the resulting pathologies. Most animals develop a CML-like myeloproliferative disease (*Left*), whereas some have acute leukemias (*Right*). (*c*) Latency to leukemia onset after transplantation (day 0) of *BCR-ABL*-transduced HSCs of these genotypes: $p53^{+/+}$ (black, $n = 28$), $p53^{+/-}$ (blue, $n = 57$), and $p53^{-/-}$ (red, $n = 30$); $P = 0.0008$ ($p53^{+/+}$ vs. $p53^{+/-}$); $P = 0.0005$ ($p53^{+/+}$ vs. $p53^{-/-}$); $P = 0.18$ ($p53^{+/-}$ vs. $p53^{-/-}$). (*Inset*) PCR to detect loss of heterozygosity in the *p53* locus. N, the knockout allele; W, the wild-type allele. Lane 1, $p53^{+/+}$ control; lane 2, $p53^{+/-}$ control; lane 3–6, CML samples derived from $p53^{+/-}$ HSCs.

To test the impact of imatinib and *p53* on the proliferative capacity of these BCR-ABL-expressing hematopoietic progenitors, we performed colony assays in methylcellulose. Here, cells derived from $p53^{+/+}$ and $p53^{-/-}$ fetal livers were transduced with a BCR-ABL retrovirus that coexpresses GFP such that 15–30% of the population was infected and, thus, expressed both BCR-ABL and GFP. Non-GFP-expressing cells served as an internal control to assess the contribution of BCR-ABL to drug action. Cells were plated in methylcellulose in the presence of various concentrations of imatinib, and colonies were quantified and inspected for GFP fluorescence 10 days later. Consistent with a role for *p53* in suppressing BCR-ABL transformation (18), $p53^{-/-}$ cells produced ≈10-fold more BCR-ABL-expressing colonies than the $p53^{+/+}$ controls (data not shown). As expected, imatinib potently suppressed colony formation by $p53^{+/+}$ HSCs expressing BCR-ABL with an IC_{50} of ≈0.05 μM. By contrast, $p53^{-/-}$ HSC-expressing BCR-ABL were less sensitive to BCR-ABL inhibition, displaying an increase in IC_{50} ($IC_{50} = 2.1$ μM) (Fig. 2 *b* and *c*). Interestingly, colony formation by non-BCR-ABL-expressing cells was not substantially influenced by *p53* status (IC_{50} ($p53^{+/+}$) = 3.2 μM, IC_{50} ($p53^{-/-}$) = 3.8 μM) (Fig. 2*d*). Therefore, *p53* can modulate the cellular effects of oncogene inhibition and contribute to the therapeutic index of imatinib therapy.

***p53* Loss Reduces Leukemic Cell Clearance and Survival in Mice Harboring BCR-ABL Leukemias.** We generated mice bearing leukemias of defined *p53* status by infecting HSCs derived from $p53^{+/+}$, $p53^{+/-}$, and $p53^{-/-}$ fetal livers with a BCR-ABL/GFP retrovirus transferred into lethally irradiated recipients (Fig. 3*a*). Consistent with previous reports (27), these animals developed a CML-like myeloproliferative disease and, occasionally, an acute leukemia. Although *p53* loss did not impact the range of pathologies we observed, the onset of leukemias in mice reconstituted with $p53^{-/-}$ cells was more rapid than controls, and leukemias arising from $p53^{+/-}$ cells invariably lost the wild-type *p53* allele (Fig. 3 *b*, *c*, and *Inset*). Still, BCR-ABL-expressing leukemias arising in $p53^{+/+}$ cells retained an intact *p53* pathway, because p53 levels and activity (as measured by expression of its transcriptional target p21) were increased after treatment of leukemia-bearing mice with doxorubicin, a conventional chemotherapeutic agent known to activate *p53* (see Fig. 8, which is

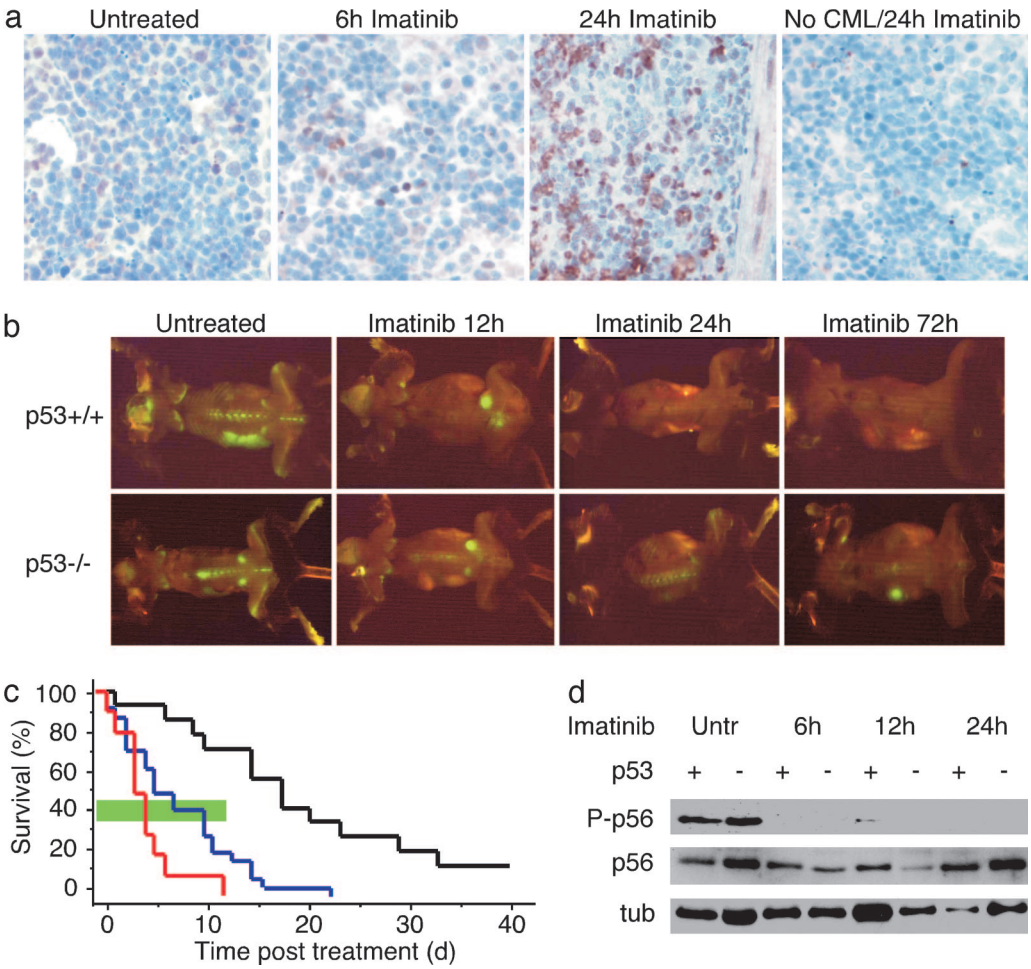

Fig. 4. *p53* and targeted therapy in murine CML. (*a*) Representative immunohistochemical stains to assess p53 expression in the spleens of healthy vs. leukemic mice treated as indicated. (*b*) Fluorescence imaging of a cohort of $p53^{+/+}$ and $p53^{-/-}$ leukemia-bearing mice killed at various times after imatinib treatment. Representative examples are shown. (*c*) Kaplan–Meier plot detailing survival times of leukemic mice grouped by genotype upon imatinib treatment; imatinib was started at the onset of leukemia (day 0), and a green bar indicates the treatment interval. Leukemias are derived from *BCR-ABL*-transduced $p53^{+/+}$ HSCs (black, $n = 15$), $p53^{+/-}$ HSCs (blue, $n = 24$), or $p53^{-/-}$ (red, $n = 10$); $P = 0.0002$ ($p53^{+/+}$ vs. $p53^{+/-}$); $P < 0.0001$ ($p53^{+/+}$ vs. $p53^{-/-}$); $P = 0.08$ ($p53^{+/-}$ vs. $p53^{-/-}$). (*d*) Bone marrow lysates of $p53^{+/+}$ (+) or $p53^{-/-}$ (−) leukemias were prepared from untreated animals (Untr) or at various times after a single treatment with imatinib and subjected to immunoblotting with antibodies against phosphorylated and total p56 (P-p56, p56) and tubulin (tub).

published as supporting information on the PNAS web site). Thus, as occurs in other mouse models of BCR-ABL-induced leukemia (28) and in human CML, *p53* loss conferred an advantage to BCR-ABL-expressing cells during leukemogenesis.

To test the impact of *p53* on imatinib responses *in vivo*, animals were treated upon leukemia manifestation with a 2-week twice-daily schedule of 100 mg/kg of body weight imatinib (29). One cohort of mice was killed at various times after treatment to assess p53 induction and BCR-ABL kinase inhibition, whereas others were monitored for treatment responses by fluorescence imaging or peripheral blood analysis. Consistent with *in vitro* results, we found an induction of p53 in spleens of leukemic animals but not in normal, nonleukemic mice (Fig. 4*a*; Fig. 9*a*, which is published as supporting information on the PNAS web site; and data not shown). Furthermore, whereas $p53^{+/+}$ leukemias showed substantial apoptosis in the peripheral blood and were completely cleared, *p53* mutant leukemias underwent less apoptosis and only partial responses to imatinib therapy, as determined by blood counts and fluorescence imaging (Figs. 4*b* and 9*b*).

A cumulative analysis of the treatment data for survival (Fig. 4*c*) and time to relapse (see Fig. 10, which is published as supporting information on the PNAS web site) confirmed a modifying effect of *p53* on imatinib response. Although all animals bearing *p53*-deficient leukemias, generated from either $p53^{-/-}$ or $p53^{+/-}$ HSCs, succumbed to their disease by day 25 after the start of imatinib therapy; nearly 40% of animals with $p53^{+/+}$ leukemias lived beyond this point (Fig. 4*c*). Although imatinib clearly has *p53*-independent activities, these results demonstrate that *p53* loss can impede the response to imatinib therapy *in vivo*.

Imatinib Efficiently Inhibits BCR-ABL Kinase Activity in p53-Deficient Cells. *p53* mutations produce drastic changes in cell physiology and can promote genomic instability (30, 31). Although it is possible that such secondary changes contribute to the decreased imatinib sensitivity we observe in *p53*-deficient leukemias, our *in vitro* data imply that *p53* can directly influence cellular responses to imatinib (see Figs. 1 and 2). To determine whether *p53* loss impairs imatinib action by preventing effective BCR-ABL kinase inhibition (as occurs in cells harboring p210/T315I) or through a downstream mechanism, we monitored BCR-ABL kinase activity in leukemic bone marrow isolates from imatinib-treated mice. Here, phosphorylation of $p56^{Dok\text{-}2}$ (a surrogate marker for kinase activity) was repressed within 6 h after a single dose of imatinib, and this repression was sustained for up to 24 h. More

importantly, *p53*-expressing and -deficient leukemias showed similar reductions in $p56^{Dok\text{-}2}$ phosphorylation after imatinib treatment (Fig. 4*D*). Therefore, *p53* loss does not interfere with the ability of imatinib to inhibit BCR-ABL kinase activity but, instead, attenuates the cellular response to BCR-ABL inhibition.

***p53* Mutations Can Accompany Progression to Imatinib Resistance in Some CML Patients.** Our studies use several well characterized models of *BCR-ABL* transformation to establish that parallel pathways independent from those emanating from the primary oncogenic signal can influence the response to targeted cancer therapy. Although our focus on BCR-ABL and imatinib reflected our desire to study a well characterized model of targeted therapy, our results raise the possibility that these observations may be relevant to human CML. We therefore surveyed a heterogeneous set of 50 specimens from CML patients, most of whom started imatinib in late disease stages and developed hematologic resistance to the drug (8). Ten resistant samples (20%) had evidence of cytogenetic alterations on chromosome 17, where *p53* resides, and 29 (58%) had point mutations in the BCR-ABL kinase domain. Only two resistant specimens showed both *BCR-ABL* mutations and chromosome 17 abnormalities. However, of the resistant cases that displayed chromosome 17 alterations without *BCR-ABL* mutations, three of five cases examined by DNA sequence analysis showed *p53* mutations that were not present in the preimatinib samples (Table 1, which is published as supporting information on the PNAS web site). Thus, *p53* mutations can accompany progression to imatinib resistance in human CML.

Discussion

We show that disruption of *p53* in *BCR-ABL*-transformed cells can impede sensitivity to imatinib. In contrast to the complete resistance conferred by mutations in the BCR-ABL kinase, *p53* loss does not prevent BCR-ABL kinase inhibition but attenuates its antiproliferative effects and modifies imatinib responses *in vivo*. As a consequence, *p53* loss impedes the clearance of leukemic cells, which may increase the population of surviving cells prone to additional progression- and resistance-promoting mutations. In this manner, *p53* mutations, or other mutations affecting the *p53* network, may contribute to the increase in refractory cases in blast crisis CML or in Ph^+ (Philadelphia chromosome) acute lymphoblastic leukemia. Such a possibility is consistent with our limited analysis of matched patient samples and clinical data linking *p53* mutations to blast crisis and poor outcome and linking an apoptosis-defective *p53* variant (Pro-72) to imatinib failure in patients (15, 32).

Studies in cultured cell lines have noted variable effects of BCR-ABL inhibition on p53 levels and activity (19–22). Using BCR-ABL-transformed BaF/3 cells, primary fetal liver HSCs, and a murine model of CML, we see that, in all cases, p53 is induced in response to imatinib treatment, and, at least *in vitro*, this finding correlates with a decreased phosphorylation of Akt and Mdm2. Other mechanisms may also contribute to p53 activation after imatinib treatment, including effects of BCR-ABL on Mdm2 translation and checkpoint pathways involved in DNA damage responses (19, 33). Paradoxically, whereas BCR-ABL kinase inhibition can induce p53 in transformed cells, v-Abl or BCR-ABL expression can induce p53 via the ARF pathway to restrain transformation (ref. 18; data not shown). Presumably, BCR-ABL simultaneously induces both pro- and antisurvival signals that impact p53 regulation such that acute ablation of BCR-ABL kinase activity has a greater impact on those signals that restrain p53 action, revealing their antiproliferative potential. Other parallel signaling networks may also impact how a cell responds to BCR-ABL inhibition, thus producing heterogeneity in treatment responses.

The treatment of BCR-ABL-transformed cells with imatinib represents the paradigm of targeted cancer therapy. Irrespective of the overall impact of *p53* mutations on response to imatinib therapy in CML patients, our results have implications for the use of targeted therapies in the clinic. For example, we demonstrate that *p53* contributes to the dependence of cancer cells on the continued activity of an initiating oncogene, sometimes called oncogene addiction (2), such that *p53* loss attenuates the cellular response to oncogene inactivation. These findings suggest that *p53* might contribute to the antitumor activity of other targeted therapeutics, and it is noteworthy that *p53* loss facilitates oncogene-independence and the eventual outgrowth of tumors in conditional transgenic mice upon inactivation of the *wnt* (34) oncogene. Our results also illustrate how mutations that accompany disease progression can reduce the efficacy of targeted therapy, presumably because drug action relies, in part, on genes and processes that normally limit disease progression (e.g., *p53*). In this view, the response of tumor cells to targeted therapy displays some parallels to conventional therapy, which can also depend on *p53* or other stress-response networks for best efficacy. Together, these results may help explain the heterogeneous response of tumors to targeted therapeutics and provide clues for their optimal use.

Materials and Methods

Cell Lines and Gene Transfer. Ba/F3 cells, 32D cells, and primary HSCs were stably transduced with MSCV-IRES-GFP vectors expressing BCR-ABL or an imatinib-resistant and kinase-active T315I mutant (a generous gift from C. L. Sawyers, University of California, Los Angeles). Ba/F3/p210, Ba/F3/T315I, or 32D cells expressing BCR-ABL were also transduced with two RNAi vectors against *p53* [shp53C (25) shp53D/1224 (26)] or an MSCV control vector. Retroviral gene transfer was performed as described in ref. 35.

Western Blot Analysis. Immunoblots were performed from whole-cell lysates (36). Antibodies against α-tubulin (1:5,000, B-5–1-2; Sigma), p56 and phosphorylated p56 (1:1,000, Cat#s 3914 and 3911; Cell Signaling Technology, Beverly, MA), p53 (1:500, p53–505; NovoCastra, Newcastle upon Tyne, U.K.), p21 (C19, SC-397; Santa Cruz Biotechnology), phosphorylated (Ser-473) Akt (1:1,000, Cat# 4051; Cell Signaling Technology), total Akt (1:1000, Cat# 2966; Cell Signaling Technology), total Mdm2 (clones 2A10 and 4B11 each at 1:50, a gift from A. Levine, Institute for Advanced Study, Princeton) (37), and phosphorylated (ser166) Mdm2 (1:1,000, Cat# 3521; Cell Signaling Technology) were used as probes and detected by using enhanced chemiluminescence.

***In Vitro* Drug-Response Assays.** For "competition" assays, Ba/F3/p210 or Ba/F3/T315I cells were partially transduced with an RNAi vector (p53C or p53D) or a control vector and propagated for 1 week in the presence of absence of 1 μM imatinib in standard media. The percentage of GFP-expressing cells was determined by flow cytometry. For methylcellulose assays, fetal liver $p53^{+/+}$ and $p53^{-/-}$ HSCs (embryonic day 13–15) were derived and retrovirally transduced as described in ref. 35. Viability was determined by Trypan blue exclusion, and 1×10^3 viable cells per well were plated in Methocult GF media (Cat# 3534; StemCell Technologies, Vancouver) in the presence or absence of imatininb. The total number and the number of BCR-ABL/GFP-expressing colony-forming units were determined 10 days after plating.

Generation of Mice. The murine model of a retrovirally induced CML-like disease has been described in ref. 27. We modified the model using fetal liver HSCs (embryonic day 13–15) from a cross of $p53^{+/-}$ C57BL/6 mice. Determination of *p53* status was by

allele-specific PCR (12). After reconstitution, the mice were monitored by blood counts. Leukemic mice (counts >50,000 per μl) were treated or bone marrow and spleen were harvested for pathology or *in vitro* studies. Preparation and staining of tissue samples and cytospin preparations were according to published recommendations (38). Mice of both $p53^{+/+}$ and $p53^{-/-}$ genotypes were later diagnosed with a CML-like myeloproliferative disease in two of three cases and an acute (mostly lymphocytic) leukemia in one of three cases.

Treatment Studies. Leukemic mice were treated with 100 mg/kg of body weight imatinib twice daily i.p. over 2 weeks. Mice were monitored for response by blood counts. A complete remission was defined as absence of leukemia, and leukemia-free survival was defined as the duration of a complete remission between treatment and relapse. Whole-body fluorescence imaging was performed as described in ref. 12. Disease onset and treatment data were analyzed in Kaplan–Meier format and logrank (Mantel–Cox) for statistical significance. IC_{50} data from separate and duplicate experiments ($n = 7$) were compared by using a t test and are shown as mean ± SD.

***p53* Sequencing.** Patient selection, response criteria, cytogenetics, and analysis of *BCR-ABL* were as described in ref. 8. Generation of cDNAs and sequencing of the *p53* sequences corresponding to *p53* exons 2–11 were performed by using published primers and an established protocol (39).

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