Persistent inhibition of telomerase reprograms adult T-cell leukemia to p53-dependent senescence

Abhik Datta, Marcia Bellon, Uma Sinha-Datta, Ali Bazarbachi, Yves Lepelletier, Danielle Canioni, Thomas A. Waldmann, Olivier Hermine, and Christophe Nicot

Introduction

Adult T-cell leukemia (ATL) is etiologically linked to the human T-cell leukemia virus type I (HTLV-I). HTLV-I–mediated T-cell transformation presumably arises from a multistep oncogenic process in which the virus induces chronic T-cell proliferation, resulting in an accumulation of genetic defects and the deregulated growth of infected cells. Since its discovery, many aspects of HTLV-I biology have been uncovered; however, treatment of the disease remains disappointing, with minimal improvement in the overall survival of patients. The prognosis of ATL patients is associated with the resistance of neoplastic cells to the conventional combination of high-dose chemotherapy and radiotherapy. Recently, a higher response rate following azidothymidine/interferon α (AZT/IFN) treatment of ATL patients has been reported in several human trials. However, the mechanism of action of AZT/IFN remains unknown and, therefore, predictive markers for prognostic therapy are not available. Several human cancers have been treated using antiretroviral AZT, including AIDS-related Kaposi sarcoma, Kaposi sarcoma–associated primary effusion lymphoma (PEL), Epstein-Barr (EBV)–associated lymphoma, and primary central nervous system lymphoma (PCNL). In vitro treatment of PEL cell lines with AZT resulted in TNF-related apoptosis-inducing ligand (TRAIL)–dependent apoptosis alongside NF-κB inhibition and apoptosis in EBV-associated Burkitt lymphoma. In contrast, remission of ATL patients treated with AZT follows a slow kinetic over several months, suggesting that a nonapoptotic pathway may be involved. These observations prompted us to investigate the long-term potential effects of AZT on telomerase and telomere functions. Since DNA polymerase is unable to replicate the end of linear DNA, every replication cycle leads to a progressive shortening of the telomeric ends and to the limited proliferative capacity of normal cells, termed “replicative senescence.” Activation of human telomerase, an RNA-dependent DNA polymerase that elongates telomeres, has been proposed as a mechanism for avoiding telomere shortening. Consistent with this model, most cancer cells, including HTLV-I–infected cells, have detectable telomerase activity, as opposed to normal somatic cells. In some cases, immortalized cells do not have any detectable telomerase activity in spite of long telomere length, suggesting the existence of an alternative mechanism, referred to as ALT and characterized by specific makers.

Here we report that enduring treatment of HTLV-I–infected cells with AZT result in telomere attrition and reactivation of p53 transcriptional activities leading to senescence of tumor cells. Importantly, in vivo–treated ATL patients responded to therapy only when p53 was wild type in sequence, and conversely disease relapse or absence of response to treatment was associated with mutation and inactive p53. Our results indicate that p53 is a predictive marker and that a response to AZT therapy requires a functional p53 gene.

Patients, materials, and methods

Cell culture

HTLV-I cell lines were maintained in RPMI 1640-10% fetal bovine serum with and without interleukin-2 (IL-2). Cells were treated with 50 μM AZT or 10 μM 2′,3′-dideoxyguanosine, ddG (CalBiochem, La Jolla, CA).
Jolla, CA). Medium containing AZT or ddG was replaced every 3 days until growth arrest.

**Patients**

Samples were obtained after informed consent was provided and in agreement with regulations for the protection of human subjects according to National Institutes of Health guidelines. Treatment regimens with AZT and IFN were previously reported. Patients’ clinical status and response to AZT treatment are reported in Table 2. Patients 2 and 7 were initially treated with chemotherapy (cyclophosphamide + vincristine + prednisone), while patients 13 and 14 received yttrium-90 monoclonal antibody therapy. All other patients received AZT therapy combined with IFN-α as a first line of treatment. PR (partial remission) refers to when ATL cells were still detectable by fluorescence-activated cell sorter scanner (FACS) analysis (>5%), while CR (complete remission) means there were no ATL cells detectable by FACS in the peripheral blood.

**TRAP assay**

Telomerase activity was measured by telomerase repeat amplification protocol (TRAP) assay using Trapeze Telomerase detection kit (Chemicon, Temecula, CA) followed by SYBR-green staining (Molecular Probes, Eugene, OR) and was quantified as previously reported. An equal amount of protein lysates in CHAPS (3-[3-cholamidopropyl]dimethylammonio)-1-propanesulfonic acid) buffer was used. Results are representative of 3 experiments.

**Telomere length analysis**

Genomic DNA was isolated, and telomere length was determined by Southern blot analysis using TeloloTAGGG telomere length assay kit (Roche, Indianapolis, IN) according to manufacturer’s instructions.

**Quantitative flow–fluorescence in situ hybridization (Q-FISH)**

Peripheral CD257 T cells were isolated from HTLV-I–infected ATL patients using anti-CD25 magnetic Dynabeads (Dynal Biotech, Lake Success, NY). The telomere length of CD257 T cells from different patients were quantitatively determined by hybridization of Telomere peptide nucleic acid (PNA)/FITC (fluorescein isothiocyanate) probe by flow cytometry (FACS-Calibur, Becton Dickinson, San Jose, CA) using Telomere PNA/FITC kit (DakoCytomation, Carpinteria, CA) as reported and according to manufacturer’s instructions.

**Western blots**

Equal amounts of proteins from untreated and long-term AZT-treated MT2 cells were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. All primary and secondary horseradish peroxidase–conjugated antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Bax (N-20), Bcl-xL (H-62), Mcl-1 (S-19), Bcl-2 (N-19), PARP (H-250), caspase-3 (H-277), p14ARF (FL-132), p53 (FL-393), MDM2 CA). Bax (N-20), Bcl-xL (H-62), Mcl-1 (S-19), Bcl-2 (N-19), PARP antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) followed by SYBR-green staining (Molecular Probes, Eugene, OR) and was quantified as previously reported. An equal amount of proteins from untreated and long-term AZT-treated MT2 cells were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Equal amounts of proteins from untreated and long-term AZT-treated MT2 cells were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. All primary and secondary horseradish peroxidase–conjugated antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Bax (N-20), Bcl-xL (H-62), Mcl-1 (S-19), Bcl-2 (N-19), PARP (H-250), caspase-3 (H-277), p14ARF (FL-132), p53 (FL-393), MDM2 (SMP-14), MDMX (D-19), p21WAF (C-19), p27KIP (C-19G), and β-tubulin (D-10) were used as loading controls.

**Senescence β-gal (SA-β-gal) assay**

Untreated and long-term AZT-treated MT2 cells were fixed in 2% formaldehyde-0.2% glutaraldehyde for 3 minutes, washed with phosphate-buffered saline, and incubated at 37°C with SA-β-gal staining solution, pH 6.0. Images were captured using a Nikon EFD3 microscope (Boyce Scientific, St Louis, MO) and Nikon camera with an Eplan 100x (160x/0.17) objective. Acquisition software, Image-ProExpress version IV, was from Media Cybernetics (Silver Spring, MD).

**PCR and semiquantitative RT-PCR**

Reverse transcriptase–polymerase chain reaction (RT-PCR) primers were as follows: p53-forward primer F: (5’-GTCCCCGGACGATATTG-3’), reverse primer R: (5’-CCAGAATGCAAGAAGCCCAG-3’); p27WAF F: (5’-GAAGAAGGTTAGCTGGGCATT-3’), R: (5’-CTCTAAGGTTGGCACGGGTG-3’); p27KIP F: (5’-TGGCCCGATTCTACTACAGC-3’); p14ARF exon1 F: (5’-CTGGAAGGGCCGGAAGACG-3’), R: (5’-GGCCTTCCTACCTGCTT-3’); p14ARF exon2 F: (5’-GTCTACACAAGCTCTTTCTTGCG-3’), and GAPDH F: (5’-GAAGGGAAGCTGGGAAGCT-3’), R: (5’-GAATGGTGATGGATGGATTT-3’). Total RNA was isolated by Trizol reagent (Invitrogen, Carlsbad, CA), and RT-PCR was performed using the One-Step RT PCR kit (Quigen, Valencia, CA).

**Inverse LTR-PCR**

Proviral integration site was determined by digesting genomic DNA with Smal, ligation, and inverse PCR with HTLV-I long-terminal-repeat (LTR) primers F: (5’-CCCCAAATATTCCCGGGG-3’), R: (5’-GGGCGTTATT-GGCTTAGT-G3’). The amplified products were cloned into pCR2.1 TA vector (Invitrogen) and sequenced.

**p53 status and functional assay**

Total RNA was isolated from ATL patients, and the p53 hot spot region amplified by RT-PCR using the following primers: F: 5’-CCAGAAAAAAN-CTACCAAGGGC-3’, R: 5’-GTCGCTTTAGTGCTCCCTGG-3’, cloned, and sequenced. For p53 full-length RT-PCR and cloning we used F: 5’-CGAGATTTGAGGAGGACGCCCTGCACT-3’ and R: 5’-CCGCTCGAGTCACTGCTAGTCAGCCCTCTTG-3’. For the p53 functional assay, the p53 from MT-2 (wild type) was cloned into pcDNA3.1Zeo vector (Invitrogen), and the hot spot region from pcDNA5p3MT2 was substituted by respective ATL p53 hot spot regions using Drdl-Bsal restriction enzymes. Luciferase assays were performed with Luciferase Reporter Assay kit (Promega, Madison, WI) by transfecting Jurkat E-6 cells with p53-responsive pG13Luc and respective ATL pcDNA3.1Zeo-p53.

**Results**

Inhibition of telomerase induces telomere attrition and cell death in HTLV-I–transformed cell lines

We and others have reported that HTLV-I infection is associated with increased telomerase activity in transformed cells in vitro and in vivo. As expected, treatment of HTLV-I–transformed cell lines with telomerase inhibitors AZT or ddG resulted in a marked reduction of telomerase activity in HTLV-I–infected cells (Figure 1A). As telomerase activity is required for continual proliferation and avoidance of replicative senescence of tumor cells, we evaluated the long-term effects of telomerase inhibition by maintaining HTLV-I cell lines in medium supplemented with AZT or ddG. While short-term treatment had no effect on proliferation or survival (data not shown and Bazarbachi et al), all HTLV-I cell lines stopped growing (Figure 1B), and died after several weeks of culture in the presence of AZT or ddG, as observed by cell counts and trypan blue exclusion (data not shown). Longer survival, as observed in MT-2 and HUT-102, appeared to be associated with a lower telomerase activity compared with C10MJ and LAF (Figure 1A). In contrast, the Jurkat T-cell line, treated under the same conditions, did not enter growth arrest (data not shown), in spite of significant telomere shortening as previously reported. HTLV-I–transformed cells do not acquire resistance to AZT-mediated inhibition of telomerase activity, even after a prolonged period of treatment, because residual activity present in long-term–treated cells (Figure 1C, lane 3) can be further inhibited in MT-2 as well as in other HTLV-I cell lines by addition of AZT for 48 hours (Figure 1C, lane 4, and data not shown). Analysis of telomere length by
Southern blot revealed a progressive shortening in all HTLV-I cell lines cultured in continuous presence of AZT (Figure 1D).

**AZT induces senescence of HTLV-I–infected cells**

As telomere shortening has been associated with chromosomal instability, causing either apoptosis or senescence,23,24 we investigated the mechanism of tumor cell death in long-term AZT-treated cells. Previous studies reported that EBV-positive Burkitt lymphoma cells from AIDS patients respond in vitro to AZT by undergoing caspase-dependent apoptosis. HTLV-I–infected cells are protected from apoptosis in part through high levels of Bcl-2 and antiapoptotic proteins remaining unchanged in long-term AZT-treated MT-2 cells (Figure 2A).

Absence of procaspase-3 and poly(ADP-ribose) polymerase (PARP) cleavage (Figure 2B), along with negative annexin V staining (data not shown), further confirmed the absence of apoptosis in AZT-treated HTLV-I cells. Instead, we found that HTLV-I cells undergo senescence as shown by the senescence β-galactosidase assay, which was strongly positive in long-term AZT-treated MT-2 cells but not in MT-2 control cells (Figure 2C). This also was confirmed in other treated HTLV-I cell lines (C10MJ, HUT102, and LAF) (data not shown).

Analysis of samples collected at different intervals in MT-2 cells cultured in the presence of AZT showed no significant differences in the levels of expression of apoptotic markers (Figure 2D). In contrast to previous observations made in HTLV-I–infected cells treated with arsenic trioxide and IFN,26,27 the levels of the viral oncoprotein Tax remain unchanged during AZT treatment (Figure 2E).

**Continuing inhibition of telomerase by AZT in HTLV-I–infected cells induces posttranscriptional stabilization of p53**

To confirm these findings and to gain further insights into the mechanisms involved, the expression level of p14ARF, a well-known senescence marker,28 was analyzed. We found significant increased expression of p14ARF in long-term AZT-treated MT-2 cells (Figure 3A). Consistent with its ability to prevent Mdm2-mediated degradation of p53,29 levels of p53 expression were considerably increased in AZT-treated cells when compared with control MT-2 cells (Figure 3A). The underlying mechanism of increased p53 expression was posttranscriptional, because the levels of p53 mRNA in MT-2 and AZT-treated MT-2 cells remained similar (Figure 3B). In addition, the effect on p53 protein stabilization was detected only after long-term treatment with AZT, and treatment with AZT for 24 hours did not result in any significant increase in the levels of p53 expression (Figure 3C).

These results exclude a potential direct effect of AZT on p53 and suggest that telomere attrition plays an essential role in increasing the levels of p14ARF and p53 expression. Importantly, long-term effects of AZT on cell cycle arrest and increased expression of p53 were reproduced in 2 independent experiments using MT-2 and C10MJ cell lines and were further confirmed in 3 other HTLV-I cell lines, C10MJ, 1185, and LAF (Figure 3D), suggesting a general mechanism rather than an observation limited to MT-2 cells. Increased expression of p14ARF and p53 following long-term AZT treatment was accompanied by an increase in Mdm2 expression (Figure 3A), and consistent with the previous findings that Mdm2 promotes ubiquitination and proteasome degradation of MdmX,30 our results also revealed a significant...
expression significantly increased in MT-2 but not in MT-2 AZT-treated cells (Figure 4D). These findings suggest that the p27kip degradation pathway is hampered in long-term AZT-treated cells, which allows for its stabilization. Our results are consistent with previous observations that limiting amounts of p27kip correlate with constitutive activation of the cyclin E-CDK2 complex and increased expression of p27kip triggers cell cycle arrest. We next confirmed that in the original MT-2 cells, p53 was wild type but transcriptionally inactive. To this end, p53 cDNA was amplified, cloned, and sequenced. As shown in Figure 4E, we found 3 nucleotides changed in the p53 sequence from MT-2 cells.

However, these mutations were silent and did not affect the amino acid sequence, which was wild type in MT-2 (Figure 4F). To confirm that p53 transcription was impaired, we gamma-irradiated MT-2 cells and analyzed expression of p53-responsive genes bax and p21waf as reported in Figure 4A. In clear contrast to AZT-treated MT-2 cells, our results demonstrate that p53 is transcriptionally inactivated in untreated MT-2 cells (Figure 4G).

Overall our results suggest that prolonged AZT treatment and inhibition of telomerase activity leads to telomere attrition, restores the functions of p53, and increases expression of senescence markers p14ARF and p21Waf in long-term AZT-treated MT-2 cells.

**AZT-based treatment induces telomerase inhibition and telomere shortening in vivo in ATL patients**

The effect of AZT in vitro requires several weeks of treatment, which parallels the slow kinetics observed in ATL patients treated with AZT. Strikingly, the estimated 30% failure rate of AZT treatment in HTLV-I–infected ATL patients coincides with the approximate percentage of ATL patients carrying a mutated inactive p53 gene. These observations prompted us to investigate whether the effect of AZT in treatment of ATL patients also relied on telomerase inhibition, telomere attrition, and p53 functions.

Samples were obtained from 18 ATL patients at different clinical stages, lymphomatous, smoldering, chronic, and acute from 2 different origins (Necker Hospital, Paris, France, and National Institutes of Health, Bethesda, MD). All samples were blind-tested, and in each case the response to therapy was provided only after analysis. In the case of 3 ATL patients (ATL3, ATL4, ATL5) a paired sample, diagnostic and remission/relapse, could be obtained. Due to the intrinsic limitation in the amounts of each of the patient samples available, telomere sizes were measured by Q-FISH before and after in vivo AZT treatment. To avoid potential variations between patient’s sample origins, we cell-sorted HTLV-I–infected from noninfected cells of 3 uncultured ATL patient samples collected prior to and after several weeks of AZT treatment and compared the relative telomere size in each cellular fraction. As HTLV-I–infected ATL cells overexpress the CD25 activation marker on their surface, sorted cells correspond to HTLV-I–infected and CD25− cells isolated from peripheral blood mononuclear cells (PBMCs) of a healthy individual and those of an ATL patient were tested by TRAP assay. Successful sorting was confirmed by staining each sorted cell population with an anti-CD25 FITC-conjugated antibody, and FACS analysis showed at least 95% purity (Figure 5A). To ensure that CD25+ sorted cells correspond to HTLV-I–infected cells and not uninfected CD25+ regulatory T cells (Tregs), CD25+ and CD25− cells isolated from peripheral blood mononuclear cells (PBMCs) of a healthy individual and those of an ATL patient were tested by TRAP assay. Significant telomerase activity was detected only in the CD25+ population isolated from the ATL patient, and no or very low telomerase activity was detectable in either population isolated from the non–HTLV-I–infected donor (Figure 5B), suggesting that Tregs are mostly telomerase negative and that the CD25+ cell fraction...
isolated from the ATL patients indeed corresponds to HTLV-I–infected cells.

In fact, integrated HTLV-I provirus was detected by PCR in CD25+/H11001 cells isolated from the ATL patient only (Figure 5C). Specific hybridization of the FITC-labeled probe to the telomeric ends was confirmed by microscopic observation of a punctuated pattern consistent with telomere labeling (Figure 5D,E).

Telomerase activity was readily detected by TRAP assay in all tested ATL patients (data not shown). Treatment with AZT reduced telomerase activity in vivo (Figure 6A). While average telomere size from CD25– cells was not affected by treatment with AZT, a 14% to 30% reduction in telomere length occurred in HTLV-I–infected CD25+/H11001 cells after treatment with AZT (Figure 6B and Table 1). Since p14ARF is an important player in p53 functions and overlaps the p16ink locus, a gene frequently methylated, deleted, or mutated in ATL patients, we analyzed the integrity of the p14ARF gene. At the time of diagnosis p14ARF exons 1 and 2 were present in an ATL5 patient, but p14ARF exon 2 was deleted in ATL4 patient (Figure 6C). However, a recent study showed that exon 2 is dispensable for p14ARF function and that the amino-terminal 29 residues of p14ARF are sufficient for stabilization of p53.43 Interestingly, analysis of the ATL4 patient’s DNA revealed the presence of both p14ARF exons after the course of AZT treatment and patient disease relapse (Figure 6C). These findings suggest that in patient ATL4, treatment with AZT eradicated the initial tumor clone but resulted in the outgrowth of a different tumor clone at relapse. In fact, inverse PCR experiments and sequencing demonstrated a different provirus integration site in ATL4 patient’s DNA samples collected at diagnostic and after disease relapse (Figure 6D).

Figure 4. AZT-mediated reactivation of p53 functions and stabilization of CDKI p21WAF and p27KIP. (A) Expression of p21WAF and Bax mRNA before and after ionizing radiation in AZT-treated MT-2 cells (18 weeks). GAPDH was used as internal control for amplification. (B) Western blot analysis for expression of p21WAF and p27KIP in untreated MT-2 or after culture with AZT for 18 weeks. Equal amounts (50 μg) of each extract were used and confirmed by β-tubulin. (C) Analysis of p21WAF and p27KIP mRNA expression by RT-PCR in MT-2 and after culture with AZT for 18 weeks. GAPDH was used as internal control for amplification. (D) Western blot analysis for expression of p27KIP in untreated MT-2 or after culture with AZT for 18 weeks, in absence or presence of proteosome inhibitor lactacystin. Equal amounts of each extract (50 μg) were used and confirmed by β-tubulin. (E) MT-2–derived p53 cDNA nucleotide sequence. (F) p53 amino acid sequence from MT-2 cells compared with wild-type p53. (G) RT-PCR for p53 responsive genes before and after gamma irradiation of MT-2 cells.
Response of ATL patients to AZT-based therapy correlates with their p53 transcriptional status

As most mutations that inactivate p53-dependent transcription lead to protein stabilization, we investigated p53 stabilization by immunochemistry in 4 ATL patients. Many studies have reported stabilization and increased expression of transcriptionally inactive mutated p53. In 3 patients who did not respond to AZT treatment, p53 expression was readily detectable by immunohistochemistry (Figure 7A). In contrast, in patients responding to AZT, p53 expression was very weak, consistent with a wild-type p53 sequence (Figure 7A). To establish a correlation between the p53 status and the outcome of ATL patients who received AZT therapy, the p53 hot spot region, exons 4 through 9, was cloned by RT-PCR. In each case, we sequenced 2 p53 hot spot clones from 14 ATL patients. For ATL3, 4, and 5, two p53 hot spot clones were sequenced both from before treatment and after remission/disease relapse. Whenever the p53 hot spot region was found to be wild type in sequence, the whole p53 cDNA was then cloned, and 2 additional clones were sequenced to confirm the phenotype.

Our results demonstrate that all ATL patients carrying a wild-type p53 gene responded to AZT treatment and went into partial or complete remission (Table 2). In contrast, all patients carrying a mutated p53 at diagnosis did not respond to AZT treatment and died within a short period of time (Table 2). Thus, a total correlation between p53 and AZT response was found and makes the results highly significant.

The biological relevance of our finding is further supported in the case of ATL4, in which the initial response to treatment was associated with the presence of a wild-type p53, but as indicated in Table 2, AZT treatment led to the selection of another tumor clone and disease relapse. Strikingly, the clone responsible for the relapse carried a p53 mutated at R273H, a well-characterized mutation that abrogates p53 transcriptional activities. All p53 mutations of unknown function were further tested in a luciferase functional reporter assay after transfection in Jurkat T cells (Figure 7B). Results confirmed an absolute correlation between the p53 transcriptional status and the response of ATL patients to AZT treatment (Table 2 and Figure 7B).

Discussion

Results described here provide a novel link between telomerase targeting, telomere attrition, and reactivation of p53-dependent senescence pathways in HTLV-I–infected cells. While it is established that p53 is inactive in HTLV-I–infected cells, the molecular mechanism of p53 transcriptional inactivation remains a matter of
Our results clearly indicate that the p53 inactivation mechanism is reversible and could therefore be used to develop novel therapeutic interventions. Previous studies have shown that localization and DNA-binding activity of p53 is not altered in HTLV-I cells, and it is more likely that posttranscriptional modification and/or association with specific cellular partners dictates the inhibition. Whether prolonged action of AZT modulates the phosphorylation or the acetylation status of p53 or its interaction with cellular partners warrants further studies.

Results obtained from patient ATL 4 further underscore the importance of a functional p53 (Figure 6 and Table 2). Initial responses to AZT treatment coincides with the presence of a major tumor clone that is wild-type p53 in sequence. Eradication of that tumor clone, however, led to the emergence of a minor clone carrying a mutated and inactive p53. This selection also coincides with disease relapse and death of the patient. Analyses of the p14ARF locus and the provirus integration sites, at the initiation of treatment and at disease relapse, clearly indicate that the 2 tumor clones present in this patient are distinct.

Several studies have found that transforming growth factor–β (TGF-β) signaling is impaired in HTLV-I–infected cells in vitro and in ex vivo ATL patient samples. Several studies have shown that the majority of ATL cases are wild-type p53 and die of their disease despite AZT/IFN or other treatments. In this study, we did not use IFN-α, because AZT has been shown to have some effect on its own in several virus-associated hematologic disorders and to limit the number of variable parameters and facilitate analysis. However, synergies previously observed in vivo between AZT and IFN-α may in part result from the transcriptional inhibition of the hTERT promoter by IFN-α, as significant down-regulation of hTERT mRNA expression and telomerase activity has been reported in leukemic cell lines as well as in primary

### Table 2. Correlation between AZT treatment response and p53 status in ATL patients

<table>
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<tr>
<th>Diagnosis</th>
<th>p53 status before initiation of AZT treatment</th>
<th>Patient response to AZT treatment</th>
<th>p53 status after AZT treatment</th>
<th>p53 transcriptional activity</th>
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Status of patients before AZT treatment, response to treatment, and status of p53 gene and its transcriptional activity. The criteria for clinical therapeutic response were as follows: complete remission was defined as the disappearance of all measurable and assessable disease, lasting more than 3 months; partial remission was defined as reduction of leukemic cell count, lasting more than 1 month but less than 3 months; and not responding was defined as increase in leukemic cell count. CR indicates complete remission; PR, partial remission; NR, not responding; R, relapse; NA, not available; and ND, not determined.
leukemic cells.\textsuperscript{36} It is also possible that additional functions of IFN-α may be involved.

Our study identifies p53 status as an essential predictive marker for the response of HTLV-I–infected ATL patients to AZT/IFN treatment and suggests that such therapy may not have any clinical benefits in patients carrying a mutated, inactive p53 gene for which alternative therapies such as allogeneic bone marrow transplant, almentuzumab, or radio-immunotherapy for IL-2R\textsuperscript{60,61} should be considered. Patients treated with chemotherapy or radiotherapy usually select for tumor clones with mutated p53 and become nonresponders to AZT when used in the second line of treatment.\textsuperscript{35} We propose that for patients carrying wild-type p53, AZT/IFN should be considered as the first line of treatment. Whether the effect of AZT/IFN on AIDS-related Kaposi sarcoma, Kaposi sarcoma–associated primary effusion lymphoma, and AIDS-related primary central nervous system lymphoma in vivo is also p53 dependent is of significant importance and warrants further investigations. In addition, it is possible that other human cancers, such as human papillomavirus (HPV)–associated cervical cancer or breast carcinomas, for which p53 remains wild type, may benefit from AZT/IFN therapy and deserve to be considered.

References


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Persistent inhibition of telomerase reprograms adult T-cell leukemia to p53-dependent senescence

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