# A Distinct p53 Protein Isoform Signature Reflects the Onset of Induction Chemotherapy for Acute Myeloid Leukemia

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**Abstract**

**Purpose:** The antioncogene protein product p53 has not been studied previously in cancer patients during *in vivo* chemotherapy. This study examined the early p53 protein and gene expression during induction chemotherapy in acute myeloid leukemia (AML).

**Experimental Design:** Leukemic cells were collected from five AML patients during their first 18 hours of induction chemotherapy and examined for p53 protein and gene expression by one- and two-dimensional gel immunoblot and high-density gene expression arrays.

**Results:** Up-regulation of p53 protein expression was detected in AML patients posttreatment *in vivo*. One- and two-dimensional gel immunoblots showed two main forms of p53, denominated αp53 and Δp53, both recognized by various $NH_2$-terminal directed antibodies. As a response to treatment, we detected rapid accumulation of αp53, with significantly altered protein expression levels already after 2 hours. The accumulation of αp53 was accompanied by increased transcription of putative p53 target genes and subsequent cytopenia in the patients.

**Conclusion:** Up-regulation of the p53 protein and target genes seems to be a prominent feature in induction chemotherapy of AML. The rapid shift from a shorter p53 protein form (Δ) toward the full-length protein (α) underscores the complexity of p53 protein modulation in patients undergoing chemotherapy.

Acute myeloid leukemia (AML) is a rapidly developing malignancy characterized by proliferation of immature cells that suppress normal bone marrow function (1–3). The overall long-term disease-free survival rarely exceeds 50% even for younger patients who can receive the most intensive treatment (4, 5). Current therapy of AML often consists of an induction regimen with anthracycline (e.g., daunorubicine and idarubicine) and cytarabine followed by consolidation therapies determined by prognostic classification (5). However, most patients are ages >60 years and therefore unable to undergo this intensive chemotherapy due to high risk of severe treatment-related toxicity. A constant challenge is therefore to offer effective therapy to the older patients where low toxicity therapy is needed to obtain disease control (6–8). The development of molecular targeted therapy with attenuated toxicity may be the most effective way of obtaining increased overall survival for AML patients (9, 10).

The p53 protein is a sequence-specific transcription factor that can halt progression through the cell cycle or initiate apoptosis on activation by genotoxic stimuli (11). In contrast to other malignancies, where as many as 70% of cases may comprise mutations in TP53 (12), >90% of AML cases have the wild-type gene (13, 14). Development of AML is a result of combined genetic defects, often including constitutive kinase activation together with alterations of transcription factors (15, 16). Gene expression profiling has shown varying kinase and phosphatase expression (17), resulting in altered responsiveness in signaling pathways (18). This may be a possible mechanism for AML blast survival even in the presence of wild-type p53.

The p53 protein is regulated by multiple post-translational modifications in response to chemotherapy as well as other cellular stress inducers (19). Post-translational modifications will contribute to p53 stabilization and accumulation leading to nuclear localization of the protein and specific gene transactivation (19). As many as 1,500 genes have been reported to respond to p53 activation (20) directly or indirectly, including the extensively studied genes p21/cyclin-dependent kinase inhibitor 1A (21), BAX (22), MDM2 (23), and GADD45 (24).

Major variants of the p53 protein may be formed through alternative splicing of mRNA or protein cleavage. To date, the human p53 gene has been reported to encode for 10 isoforms through variously spliced mRNA (25, 26). These are the full-length proteins, three $NH_2$-terminally truncated isoforms translated from an alternative point of initiation at codon 40

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**Table 1.** Clinical and biological characteristics of AML patients

| Patients | Sex | Age | FAB | Membrane molecules | | | | | | | WBC before therapy | Time to cytopenia | Remission after first induction | Survival (mo) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | CD13 | CD14 | CD15 | CD33 | CD34 | Karyotype | Flt3 | | | | |
| 1 | M | 30 | $M_3$ | + | – | – | + | + | t(15;17) | ITD | 29 | 3 | ND | 0 |
| 2 | F | 58 | $M_1$ | + | – | – | + | + | Normal | WT | 18 | 6 | – | 5 |
| 3 | M | 46 | $M_{4e}$ | + | – | – | + | + | inv16 | WT | 50 | 4 | + | >8 |
| 4 | M | 60 | $M_{4/5}$ | + | – | + | + | – | Normal | ITD | 71 | 6 | + | >23 |
| 5 | M | 55 | Atypical | + | – | + | + | – | Multiple | WT | 6 | 5 | + | >23 |

NOTE: Five consecutive patients were sampled during chemotherapy. Patient 1 had platelets $<5 \times 10^9$/L, lactate dehydrogenase 815 units/L, and fragment D-dimer >20 mg/L before start of chemotherapy and developed sepsis and multiorgan failure within 2 weeks. Patient 1 died before determination of hematologic remission (ND). Patient 4 had previously diagnosed prostate cancer not treated with chemotherapy. The class III receptor tyrosine kinase Flt3 was analyzed for juxtamembranous length mutation (ITD) as described previously (18). Time to cytopenia is defined as days before $<0.5 \times 10^9$/L WBC in peripheral blood.



or, by alternative splicing of intron 2 (25, 27, 28), two COOH-terminally truncated isoforms produced by alternative splicing of intron 9 (25, 29), three isoforms produced from an internal promoter in intron 4 (25), and one isoform produced by alternative splicing within exons 7 to 9 (26). In addition, truncated p53 protein products are known produced as a result of protease action most likely through an autoproteolysis mechanism (30, 31). Based on these observations, one would expect the presence of multiple isoforms of p53 protein that could disclose nuances in the cellular response *in vivo*.

We explored p53 protein modulation *in vivo* within the first 18 hours after start of chemotherapy. The p53 response was analyzed using information dense methodology like two-dimensional p53 protein analysis and gene arrays with high number of probes (32). A panel of $NH_2$-terminally directed p53 antibodies showed a dynamic protein response with a shift in p53 isoform distribution and accumulation of the full-length protein. This effect on p53 protein expression was accompanied by putative p53 target gene activation as a response to *in vivo* chemotherapy in primary AML.

## Materials and Methods

***Patients.*** The study was approved by the local ethics committee and samples were collected after informed consent. We collected peripheral blood AML blasts from five consecutive patients during chemotherapy by antecubital vein puncture. The patients were treated with i.v. infusion of idarubicine (12 mg/m$^2$ during 30 minutes on days 1-3) and cytarabine (190 mg/m$^2$ daily as a continuous infusion days 1-7) through a central venous catheter. The clinical and biological characteristics of the patients are presented in Table 1. Analysis of 20 AML patients showed that the p53 protein pattern shown in the present study was associated with wild-type TP53.[5]

***Isolation of cells.*** Cells were isolated from peripheral venous blood and collected before treatment and at 2, 4, and 18 hours after start of chemotherapy. Cells were prepared by density gradient separation (Ficoll-Hypaque, NyCoMed, Oslo, Norway; specific density, 1.077). The percentage of blasts exceeded 95% for all samples. Peripheral blood mononuclear cells were collected from healthy donors, separated as described for AML cells, and used fresh.

***Sample preparation for protein and gene expression analysis.*** Material for protein analysis was collected as described previously in detail (33, 34). Briefly, cells were washed in NaCl (9 mg/mL) and then lysed in 7% TCA. Protein precipitate was then washed in 5% TCA and water saturated ether to remove salts. The protein pellet was suspended in sample buffer for two-dimensional gel electrophoresis (7 mol/L urea, 2 mol/L thiourea, 100 mmol/L DTT, 1.5% ampholyte 3-10, 0.5% ampholyte 5-6, 0.5% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate). RNA was isolated from AML blasts as described previously (17). Aminoallyl-UTP (Ambion, Cambridgeshire, United Kingdom) was incorporated into cRNA followed by cross-coupling of Cy5 and Cy3 with reactive Cy-NHS compounds (Amersham Biosciences AB, Uppsala, Sweden) to generate fluorochrome-labeled targets for DNA microarray analysis.

***Gene expression analysis.*** The Agilent Human Whole Genome Oligonucleotide Microarray (44K; Agilent Technologies, Inc., Palo Alto, CA) was used for gene expression analysis of patients 1, 4, and 5, and the Human 1 cDNA Microarray (Agilent Technologies) containing 12,814 cDNA clones was used for validation purposes (17). The procedure was done according to the manufacturer's protocols, except for a more stringent wash (0.1 × SSC at 35°C for 10 minutes for the oligonucleotide array). The oligonucleotide microarrays were scanned and features were automatically extracted, recorded, and analyzed using the Agilent Microarray Scanner Bundle. Normalization, flooring, or filtration of data was done according to previously described in-house bioinformatic procedures (17, 35). Rank product analysis (36) was used to detect differentially expressed genes in the oligonucleotide microarray experiments. The data were formatted in a J-Express file suitable for additional data mining.[6] Selected genes and samples were clustered by hierarchical clustering using average linkage and Pearson correlation as similarity metrics.

For further confirmation of Array data, mRNA level of p21, BAX, PUMA, GADD45, PCNA, and MDM2 was analyzed using Taqman low-density arrays (LDA), 384-well customizable microfluidic cards for real-time PCR (Applied Biosystems, Foster City, CA). Each LDA card was configured for genes in duplicates, including Celera gene ID. Hexamer-primed single-stranded cDNA corresponding to 5 ng AML blast total RNA was diluted in Taqman universal buffer (Applied Biosystems) and added to each loading well. The samples were distributed to the microwells by centrifugation for 1 minutes at 343 × $g$. The cards were sealed and placed in the ABI 7900 Sequence Detection System using the following cycling variables: 2 minutes at 50°C, 10 minutes at 95°C, and 40 alternate cycles of 15 seconds at 95°C and 60 seconds at 60°C. LDA

[5] N. Anensen and B.T. Gjertsen, submitted for publication.

[6] http://www.molmine.com/.

analysis used GAPDH as normalizer and one sample as calibrator. GAPDH was used for normalization of cDNA microarray values. The SDS2.2 software was used for qualitative analysis and data were exported to Excel spreadsheet (Microsoft, Inc., Seattle, WA) for further exploration and visualization. The correlation coefficients between Array and LDA gene expression values were calculated using XL Statistical Correlation Analysis.

Detection of p53 isoform mRNA expression was done as described previously by Bourdon et al. (25).

***Gel electrophoresis and immunoblotting.*** Protein separation was done by SDS-PAGE following standard procedures. Two-dimensional gel electrophoresis was done using 7-cm pH 3 to 10 (Zoom Strip, Invitrogen Corp., Carlsbad, CA) isoelectric focusing gel strips following the manufacturer's instructions. The focusing strips were incubated with sample rehydration buffer (8 mol/L urea, 1% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate, 20 mmol/L DTT, 1.5% ampholyte 3-10, 0.5% ampholyte 5-6, traces of bromphenol blue) overnight at room temperature to rehydrate the strips. Protein was added directly to the rehydration buffer. Isoelectric focusing was done at 200 V for 40 minutes, 450 V for 30 minutes, 750 V for 30 minutes, and 2,000 V for 60 minutes. Following isoelectric focusing, the strips were either stored at −80°C until further use or equilibrated directly for 15 minutes in LDS sample buffer (Invitrogen) containing 100 mmol/L DTT and then 15 minutes in LDS sample buffer containing 125 mmol/L iodacetamide. For the second dimension, the Zoom strip was aligned in a 0.5% agarose solution added into the isoelectric focusing well of a NuPAGE Novex 4% to 12% Bis-Tris Zoom Gel (Invitrogen). Electrophoresis was done at 200 V for 60 minutes, after which the proteins were transferred to polyvinylidene fluoride membrane by standard electroblotting.

The p53 protein was detected using Bp53-12 monoclonal antibody, recognizing a $NH_2$-terminal epitope of p53 (epitope mapping amino acids 20-25). Bp53-12 visualized p53 identical to the antibody DO-1 (epitope mapping amino acids 21-25) and Ab-2/PAb1801 in two-dimensional immunoblot (data not shown). p63 protein was detected using primary D-9 antibody, BAX protein was detected using primary 2D2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA), and p21 protein was detected using primary SX118 antibody (BD Biosciences, CA). Actin was detected using primary AC-15 antibody from Abcam (Cambridge, United Kingdom). Secondary horseradish peroxidase–conjugated mouse antibody (Jackson ImmunoResearch, West Grove, PA) was visualized using the SuperSignal West Pico or SuperSignal West Femto Chemoluminescent Substrate (Pierce Biotechnology, Inc., Rockford, IL). The immunoblots were imaged using a Kodak Image Station 2000R (Eastman Kodak Co., Lake Avenue, Rochester, NY), and spots were quantified using the Kodak analysis software. Data were exported to Excel spreadsheet, and a Student's two-tailed $t$ test was used for statistics of the ratio between αp53 and Δp53.

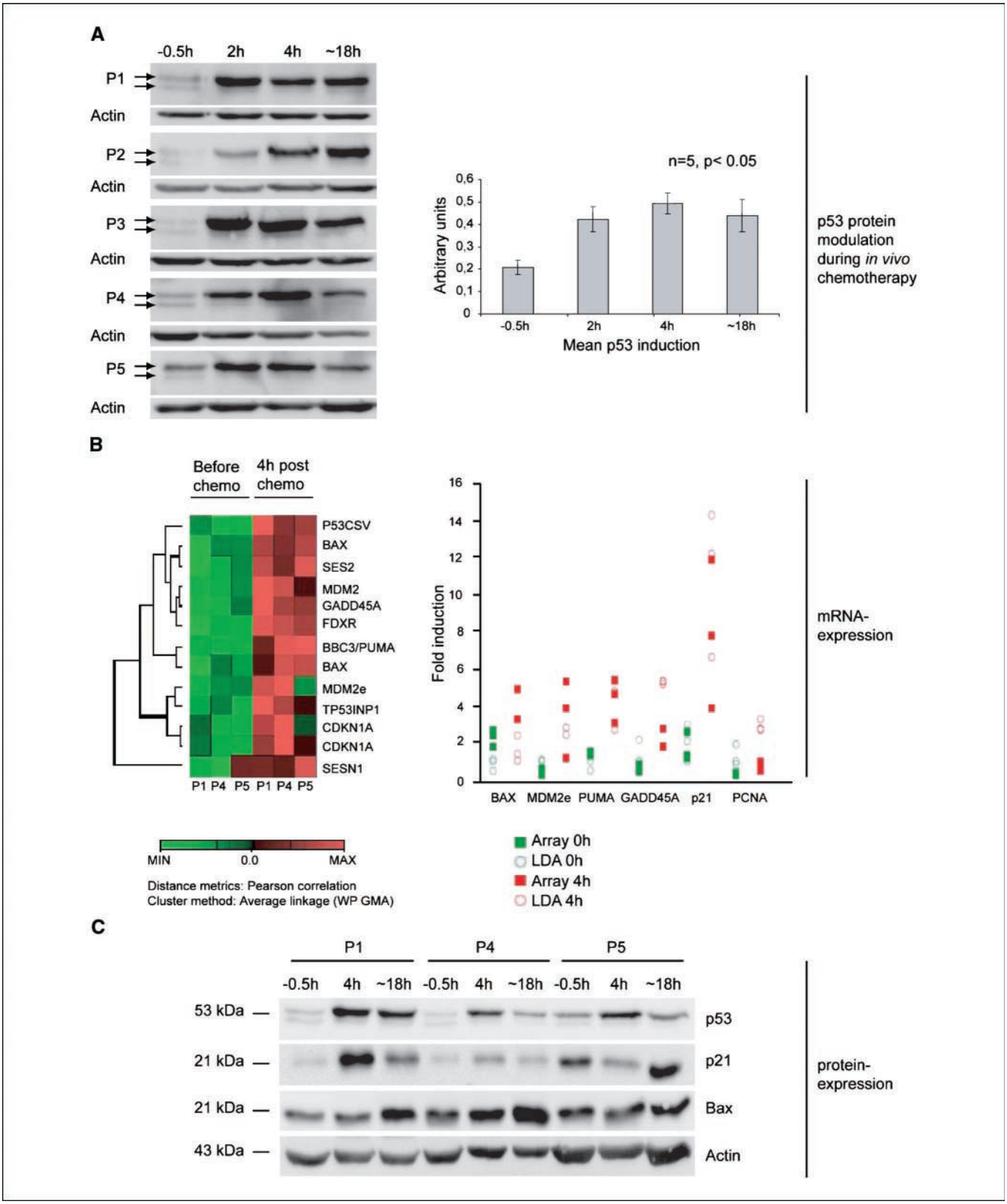
## Results

***p53 and gene expression in human AML cells during* in vivo *exposure to chemotherapy.*** In cell protein extract from five AML patients, we found a significant up-regulation of p53 protein in response to induction chemotherapy (Fig. 1A). Interestingly, p53 protein was present in two isoforms before treatment induction. Only the higher molecular form was up-regulated in response to chemotherapy, whereas the lower molecular form seemed to be down-regulated (Fig. 1A). To determine whether this p53 response lead to the activation of p53-targeted genes, we randomly selected three patients (Table 1, patients 1, 4, and 5) for gene expression analysis (Fig. 1B). mRNA isolated from AML cells before and after 4 hours of chemotherapy was analyzed by Agilent Human Whole Genome Oligonucleotide Microarray and Taqman LDA (Fig. 1B), and the 10 most up-regulated mRNA signals were all putative p53-inducible genes: p53CSV/p53-inducible cell survival factor (37), BAX/BCL2-associated X protein variant β (22), SES2/hypoxia-induced gene 95 (38, 39), MDM2 (40), GADD45A/growth arrest and DNA damage inducible α (24), FDXR/ferridoxin reductase variant 2 (41), BBC3/PUMA/BCL2-binding component 3 (42), TP53INP1/p53-inducible nuclear protein 1 (43), CDKN1A/cyclin-dependent kinase inhibitor 1A (p21; refs. 21, 44), and SESN1/p53-regulated PA26 nuclear protein (45). The increased expression of p53-induced genes was reproduced by mRNA expression analysis of BAX, MDM2, PUMA, GADD45, p21, and PCNA using cDNA microarray (Fig. 1B). The correlation coefficient values in the mRNA expression analysis were 0.95 for BAX, 0.98 for MDM2e, 0.91 for PUMA, 0.92 for GADD45A, 0.86 for p21, and 0.72 for PCNA. For each of the genes, we calculated the fold change (mean value) for Array and LDA gene expression 4 hours after chemotherapy induction. BAX mRNA expression 4 hours following induction was increased 1.7-fold according to Array (■) and 2.0 times according to LDA (○) values. The average fold changes 4 hours following induction were 2.1 (■) and 2.4 (○) for MDM2e, 3.1 (■) and 3.7 (○) for PUMA, 3.9 (■) and 4.1 (○) for GADD45A, 4.6 (■) and 5.2 (○) for p21, 2.5 (■) and 2.4 (○) for PCNA. The false discovery rate of the presented genes varied from <0.05% to 0.1%, and the correlation between the gene expression data obtained from oligonucleotide microarray and cDNA microarray analysis ranged between 85% and 97%. The patients analyzed by microarrays were also investigated by immunoblotting to see whether the results could be confirmed on protein level. Patients 1, 4, and 5 were tested before (−0.5 hour) and after (4 and 18 hours) chemotherapy and all three patients showed induction of p53 protein and the p53-targeted proteins p21 and BAX (Fig. 1C). Taken together, our protein and gene expression analysis in AML cells from patients undergoing chemotherapy suggest that p53 is functional and causes appropriate induction of p53-regulated genes (Fig. 1).

***Mapping of p53 protein patterns by two-dimensional immunoblots.*** To better distinguish between the two p53 isoforms detected in Fig. 1A, we used two-dimensional electrophoresis and immunoblots. This method revealed several p53-reactive spots (Fig. 2A), including an ~63-kDa spot (pI 5.2), the putative full-length p53 (~53 kDa, pI 5.0-6.5), denominated αp53, and a group of spots below 50 kDa (~45 kDa, pI 4.8-5.3), denominated Δp53. The observed pattern was detected using the Bp53-12 antibody directed toward the extreme $NH_2$ terminus of p53 (amino acids 20-25). This part of the protein contains several phosphorylation sites modified by stress-related kinases, such as Chk1, Chk2, ATM, and p38, and is a highly important domain for p53 activity. Using an antibody directed toward this part of the protein ensures the detection of transcriptional active p53 and might also detect changes in isoform distribution caused by changes in expression of p53-targeting kinases and phosphatases. Other $NH_2$-terminal antibodies (DO-1 and PAb1801) revealed the same isoform distribution (data not shown). According to a recent publication by Bourdon et al. (25), p53 may transcribe into several protein isoforms. To see if the isoforms detected could correspond to these, we tested whether the three known p53 isoforms with an intact $NH_2$ terminus were present in our

material (Fig. 2B). Full-length p53, p53β, and p53γ were all present in patients 1, 4, and 5. p53β is identical to Δp53 in size and we therefore tested a p53β-specific antibody (KJC8; ref. 25), but no signal was detected (data not shown).

The two-dimensional immunoblot variability was examined in normal peripheral blood mononuclear cells (Fig. 2C). This showed that the 63-kDa spot could be visualized using a p63-specific antibody, suggesting that Bp53-12 recognized this



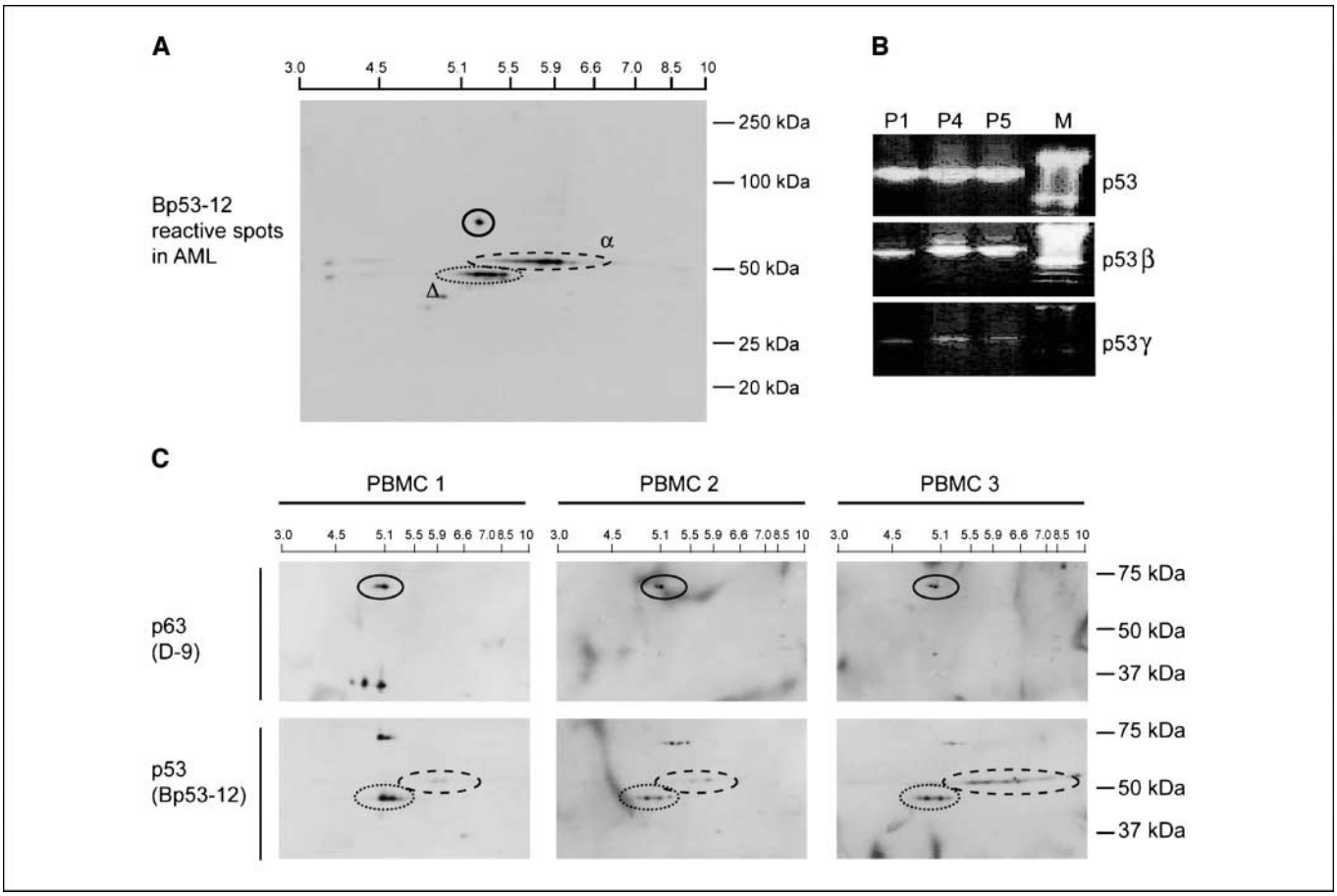



**Fig. 2.** Mapping of the p53 protein by two-dimensional gel electrophoresis and immunoblot and expression of mRNA splice forms. *A,* our analysis facilitated mapping of total p53 in AML cells detected by antibody Bp53-12, identifying putative p63 (*circled top spot*), a nonidentified putative p53 cleavage product or short form (*bottom dotted spot*), and a spot group corresponding to full-length p53 (*top dotted spot*). *B,* mRNA of p53 isoforms with intact $NH_2$ terminus (p53, p53β, and p53γ) were detected as described previously (25). All three isoforms were detected in patients 1, 4, and 5. *C,* reproducibility of two-dimensional immunoblot was tested by examining three healthy donors of peripheral blood mononuclear cells (*PBMC*) in three separate runs of two-dimensional immunoblot, examining total p63 (D-9) and p53 (Bp53-12). Note that the putative p63 is detected by both Bp53-12 and D-9. The estimation of molecular weight (kDa) and isoelectric point (pI) of different p53 family members is described in Materials and Methods.

distinct member of the p53 family (Fig. 2C). The p63-specific antibody was also reactive with other spots showing interindividual variance (Fig. 2C). The p53 family member p73 was visualized in the same peripheral blood mononuclear cell extract but not by the Bp53-12 antibody (data not shown).

***Modulation of p53 protein patterns by chemotherapy.*** For examination of p53 modulation after chemotherapy, AML cells derived from five patients sampled *in vivo* was analyzed using two-dimensional immunoblot. After only 4 hours of treatment, we observed a marked increase in αp53 and the αp53/Δp53 ratio (Fig. 3). In untreated cells, the ratio usually is Δp53 > αp53, but after cell stress this balance was disturbed and a shift toward αp53 was observed. The increase in αp53 most likely reflects stabilization of the wild-type, full-length p53 protein. Later during treatment, the increase in αp53 was halted possibly through HDM2 feedback down-regulation of p53 (46).

The ratio of αp53/Δp53 was determined for the five *in vivo* treated patients (Fig. 3) and the αp53/Δp53 ratios in pre-chemotherapy samples (mean, 1.0; SD, 0.09; $n = 5$) were found to be significantly increased after 4 hours of chemotherapy (mean, 2.02; SD, 0.51; $n = 5$; $P = 0.002$).

## Discussion

In our analysis of p53 protein in cell samples collected from patients undergoing chemotherapy, we observed a p53 protein

**Fig. 1.** p53 response in *de novo* AML patients treated with standard chemotherapy. *A,* a significant p53 protein response after chemotherapy induction with daunorubicine and cytarabine was detected in five AML patients treated *in vivo* (see Table 1). Cells were collected as described in Materials and Methods before start of induction and at three different time points. The p53 signal was quantified (*A, right*) and the induction of p53 was found to be significant ($P < 0.05$). *B,* relative mRNA abundance in patients 1, 4, and 5 was determined by Agilent 44K DNA Oligonucleotide Microarray (■) and Taqman quantitative PCR LDA (○) as described in Materials and Methods. The hierarchical cluster analysis (*B, left*) shows the 10 highest ranked p53-inducible genes activated by *in vivo* chemotherapy examined in patients 1, 4, and 5 by oligonucleotide microarray. The heat diagram illustrates changes in mRNA expression in AML blasts from the three patients before treatment and 4 hours after induction chemotherapy. False discovery rates for the 10 genes in the dendrogram were 0.0% to 0.1%, and *P*s ranged from $3.9 \times 10^{-11}$ to $4.1 \times 10^{-8}$. The relative mRNA abundance measured by microarray and LDA was compared (*B, right*) in AML blasts before induction therapy (*green*) and 4 hours after induction (*red*). The correlation coefficients (*CF*) between microarray and LDA gene expression values were between 0.72 and 0.98. *C,* patients 1, 4, and 5 were also subject to protein analysis using standard immunoblotting of p53, p21/CDKN1A, and BAX.

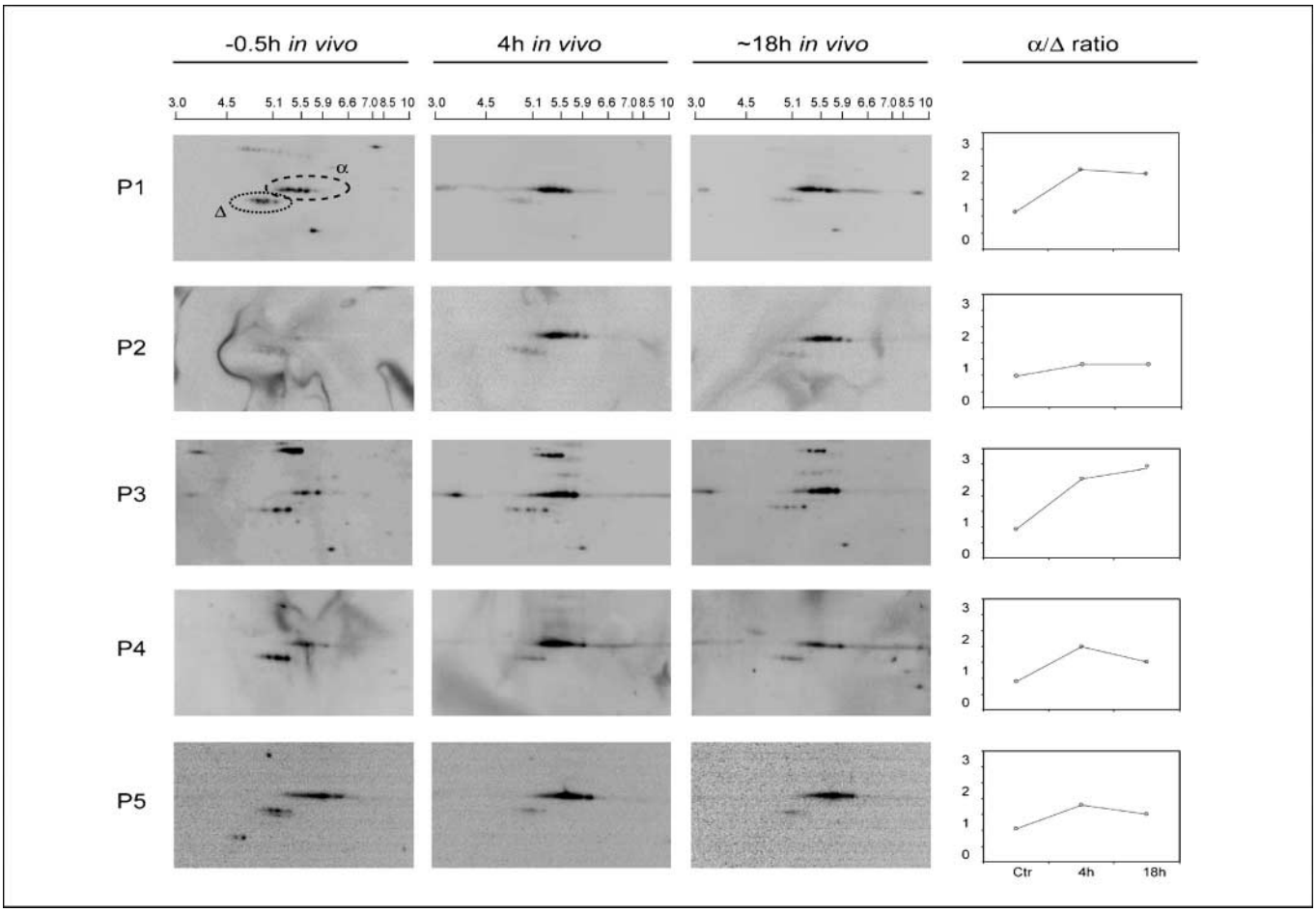



**Fig. 3.** p53 isoform modulation during chemotherapy *in vivo.* AML cells from patients treated with chemotherapy *in vivo* were analyzed by two-dimensional immunoblot. Note the shift in p53 from the Δp53 toward the full-length αp53 protein. Control cells were collected 0.5 hours before start of treatment (−0.5 hour). *Right,* changes in αp53/Δp53 protein ratio in arbitrary units. Representative for two to three independent immunoblots.

induction as would be expected from previous *in vitro* studies (ref. 47; Fig. 1A). Up-regulation of putative p53 target genes was also observed after chemotherapy (Fig. 1B) and up-regulation of two of these was also verified on the protein level (Fig. 1C). During AML induction, therapy apoptosis was not seen in the peripheral circulation up to 18 hours,[7] and previous studies have only been able to detect apoptotic AML cells in bone marrow after 24 to 48 hours (48, 49). At the same time, cytopenia developed within 3 to 6 days in our patients (Table 1) with significant decline in peripheral leukemic cells during the first 18 hours. The lack of apoptotic cells in the circulation during induction chemotherapy is probably due to the organism's high capacity of apoptotic cell eradication from peripheral blood by the spleen and liver (50). We therefore suggest that the gene induction observed in this study represents an early chemotherapy-induced event and exhibits the importance of p53-inducible genes *in vivo*. The majority of the most intensely induced genes (e.g., BAX, PUMA, and TP53INP1) are reported to induce apoptosis if their expression is enforced one by one *in vitro* (22, 42, 51). On the other hand, p53-inducible cell survival factor (p53CSV) and HDM2 are known to protect against cell death (37, 40), suggesting that this p53 transcriptional induction comprises genes that may improve the efficiency of therapy if targeted.

The p53 protein is extensively regulated post-translationally by phosphorylation, acetylation, sumoylation, neddylation, and ubiquitination (11). Many signaling enzymes known to modify p53 are themselves mutated in cancers, thereby mimicking disease with p53 mutation (52). One of the enzymes of particular interest over the recent years has been Chk2, which has been reported to be mutated in a Li-Fraumeni syndrome family with normal p53 (53). As p53 mutations are rare in AML, kinase or phosphatase mutations may contribute to the AML pathogenesis and be a contributing factor causing incomplete remission. In this study, we used a p53 antibody directed toward the extreme $NH_2$ terminus of p53 (amino acids 20-25), a region that contains phosphorylation sites modified by Chk2 as well as other cell stress-related kinases (19, 54). The p53 protein isoform distribution could reflect altered expression and/or activity of these kinases in AML, suggestive of a study of the p53 protein in a more extensive AML material. In our study, the two-dimensional immunoblot methodology failed to map the distribution of selected phosphorylated residues in p53 isoforms in resting cells (data not shown). However, it has been shown previously that most of the p53

[7] B.T. Gjertsen, unpublished data.

forms visualized on a two-dimensional map are phosphorylated (55), and future efforts are planned to elucidate the nature of post-translational modifications behind the p53 isoform map.

The known p53 isoforms have been found to possess different functions. Bourdon et al. recently reported that p53 isoforms are expressed in normal human tissue in a tissue-dependent manner (25). They also report differential binding of these isoforms to p53-responsive promoters and alternate apoptotic responses. Detection of a shift toward full-length p53 accompanied by p53 target gene induction in our material suggests that the full-length isoform is the most important isoform in the p53 response to cell stress in AML. The second main isoform found in AML, Δp53, resembles p53β and p53γ isoforms (25) in size (~45 kDa) and pI (~5.5; theoretical pI calculated using the Expasy protparam tool[8]). An antibody specific for the p53β isoform (KJC8; ref. 25) did not, however, reveal any signal resembling Δp53. The Δp53 isoform reported by Rohaly et al. (26) is also similar to the Δp53 found in our material in size and pI. Further experiments are, however, needed to elucidate the exact nature of the Δp53 isoform.

This work shows that the rapid *in vivo* activation of the p53 network in response to induction chemotherapy in AML is an important event in leukemic cell debulkment. The complex regulation of the p53 protein during *in vivo* chemotherapy underscores the feasibility for further characterization of the p53 network in AML.

[8] http://ca.expasy.org/tools/protparam.html.

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