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**Original Article**

# PROGNOSTIC VALUES OF LUNG RESISTANCE PROTEIN (LRP) AND P53 IN PATIENTS WITH MULTIPLE MYELOMA (MM)

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## ABSTRACT

**Purpose:** This study was undertaken to assess the significance of lung-resistance related protein (LRP) expression and p53 mutations in plasma cells from untreated multiple myeloma (MM) patients and to determine whether LRP expression and p53 deletions was associated with a poor response and survival in patients treated with a conventional dose of melphalan.

**Patients and Methods:** We have studied LRP expression by immunocytochemistry and p53 deletions by interphase fluorescence in situ hybridization (FISH) with a DNA probe specific for the p53 locus at 17p13 in bone marrow plasma cells from 38 untreated patients with MM received conventional oral dose melphalan (0.25 mg/kg, day 1 to 4) combined with prednisone (MP). We also studied their association with both response to chemotherapy and survival of the patients.

**Results:** By FISH, deletions of p53, were detected in 31.5% of patients and LRP was found positive in 36.8%. Patients with a p53 deletion were predominantly at stage III (p53 deletion in 83.4% of stage III patients vs. 8.3% of patients at stages I and II respectively; P = 0.04). Both p53 deletion and LRP expression were correlated with other laboratory and clinical parameters. The overall response rate of all of the evaluable patients was 63.2%. The response rate was 79.2% for patients without LRP expression but only 35.7% for patients with LRP expression (P = 0.01). Among the 12 patients with a p53 deletion by FISH, 25% responded compared with 75% of patients without p53 deletion (P = 0.001). With the exception of LRP expression and p53 deletions, there was no other prognostic variable sharing any correlation with response. The median survival of LRP-positive patients was 11 months (95%-Cl: 9 to 24), whereas the median survival duration of LRP negative patients was not reached (Cl: 26 to 82; P < .002; hazard ratio [HR] = 2.9 (1.4-5.7). Patients with and without p53 deletions had significantly different overall survival times (median 13.9 months, 95%-CI: 8 to 26 and median not reached, CI: 24 to 84, respectively; P < .0001; hazard ratio [HR] = 2.6).

**Conclusion:** For patients with MM who were treated with conventional-dose chemotherapy, LRP expression and p53 deletions provides prognostically relevant information in addition to that provided by standard prognostic factors.

**Key Words:** Multiple myeloma, chemotherapy, lung resistance protein immunohistochemistry, P53, FISH.

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## INTRODUCTION

Alkylating agents and corticosteroids are still the mainstay of therapy for patients with multiple myeloma (MM).[1,2] Multiple drug resistance (MDR) has been identified as an important path of drug resistance in MM. MDR is the phenomenon of cancer cells developing cross-resistance to a variety of structurally unrelated chemotherapeutic compounds such as vinca-alkaloids, anthracyclines, and epipodophyllotoxins.[3] MDR is associated with the expression of the drug transport mediating proteins P-glycoprotein (PgP) and the multidrug resistance-related protein (MRP)[4]. The increasing evidence of additional mechanisms of MDR led to the identification of a novel protein associated with MDR, termed the lung-resistance protein (LRP).[4]

The LRP gene has been cloned and identified as the human p110 major vault protein[5]. Vaults are novel cellular organelles first described by Kedersha and Rome in[6] which are thought to mediate intracellular transport of a wide variety of substrates. LRP has been found to be widely distributed in human normal tissues and in tumors, closely reflecting the susceptibility to chemotherapy of different tumor types[7]. Importantly, studies in myeloma and other human cancer cell lines relate LRP expression to resistance against the alkylating agent melphalan.[8,9]

The p53 tumor suppressor gene, which maps to 17p13,[10] has been shown to be implicated in the control of normal cellular proliferation, differentiation, and

apoptosis[11]. Inactivation of p53 due to mutation or allelic loss has been observed in many solid tumors, and results suggested a relation between presence of a p53 mutation and tumor development or progression[12,13]. In addition, alterations of the p53 gene have been reported to be of prognostic relevance in non-small cell lung cancer[14] as well as in breast cancer[15]. In hematological malignancies, mutations of p53 generally occur at a lower frequency compared with solid tumors[16], but when present they appear to identify prognostically relevant subgroups of patients. Recent evidence suggests that alterations in apoptosis are involved in the drug resistance of MM[17,18]. MM patients with p53 deletions had shorter overall survival than those without such a deletion.[17]

This study was undertaken to assess the significance of lung-resistance related protein (LRP) expression and p53 mutations in plasma cells from untreated multiple myeloma (MM) patients and to determine whether LRP expression by immunocytochemistry and p53 deletions was associated with a poor response and survival in patients treated with a conventional dose of melphalan.

## MATERIALS AND METHODS

### Patients:

The study included thirty-eight patients with multiple myeloma diagnosed between May 2001 and February 2002. Clinical staging was defined according to the criteria proposed by Salmon and Durie[19]. Median age of patients treated was 55 years. The patient characteristics are summarized in table (1).

Chemotherapy regimen and response evaluation: Patients received melphalan in combination with prednisone. The intermittent MP regimen consisted of oral melphalan 0.25 mg/kg/d and prednisone 2 mg/kg/d administered for 4 days. Courses were repeated every 6 weeks. Response was determined by standard criteria for myeloma response. A partial response was defined as a reduction of at least 50% in serum M protein or urinary light chain concentration with no progression of lytic bone lesions, without increase of bone pain or anaemia. A complete response (CR) was defined as complete disappearance of myeloma proteins from serum and urine and normalization of the bone marrow. Response was determined after 3 courses, or earlier when progression was obvious. When a partial response (>50% reduction in M protein) was achieved, therapy was continued for at least 1 year. Patients with a minimal response (between 25% and 50% reduction in M protein) received another four courses. Patients unresponsive after three courses (less than 25% reduction in M-protein concentration) and patients with a minimal response after three courses but no further improvement of response after six courses, continued with second-line chemotherapy, usually a combination of vincristine 0.4 mg/day and doxorubicin 9 mg/m² administered by continuous infusion for 4 days with oral dexamethasone 40 mg/day for days 1-4, 9-12 and 17-20 for the first cycle only to be repeated every 28 days (VAD regimen).

Cells: BM aspirates were obtained from the posterior iliac crest or sternum during standard diagnostic procedures and were collected in a heparinized syringe. After dilution with phosphate-buffered saline (PBS), mononuclear BM cells were separated by density gradient centrifugation over Ficoll-Hypaque (density = 1.077; Sigma Inc, St Louis, MO). Mononuclear cells were washed twice with PBS, treated with Carnoy's fixative (methanol/glacial acetic acid 3:1 [vol/vol]), and stored at -20°c.

Specimens that were used as controls consisted of peripheral blood samples (n =10) of healthy individuals and of bone marrow samples from patients with non-Hodgkin's lymphoma without histologic evidence of bone marrow involvement (n =6).

Interphase FISH studies: BM cells were consecutively assessed for cytomorphology and interphase FISH using a modification of the technique published by Drach et al.[17] Fixed BM cells were stained with Wright-Giemsa, and cells with the cytomorphologic appearance of plasma cells were documented. After hybridization, the same cells were relocalized, and hybridization signals were specifically enumerated in plasma cells.

For FISH, DNA probes specific for the p53-locus on 17p13 (directly conjugated with SPECTRUM-Orange), for the Rb-1 locus on 13q14 (directly conjugated with SPECTRUM-Orange), and for the centromeric region of chromosome 17 (directly conjugated with SPECTRUM-Green) were purchased from Vysis, Inc.

For pre-hybridization, slides were immersed in 0.1 N HCl/0.05% Triton-X-100, washed two times in saline sodium citrate (SSC) and PBS, and treated with formaldehyde (1% in PBS). After washes with PBS and 2X SSC, cells were dehydrated through 70%, 85%, and 100% ethanol. DNA was denatured by incubation with formamide (70% in 2X SSC) at 70°c for 5 minutes. Cells were again dehydrated through ethanol.

Hybridization mixture (10 pL) was then applied to each slide, which was cover slipped and sealed with rubber cement. Hybridization solution contained formamide (65%; Sigma), 2X SSC, dextran sulfate (10%, Vysis), salmon sperm DNA (100 pg/mL; Sigma), and the specific probe (2 pglmL). Hybridization was performed overnight at 37°c in a humidified chamber.

Post-hybridization washes consisted of three rinses in 50% for mamidel 2x SSC at 45°c and two rinses in 2X SSC at 37°c. Finally, nuclei were counterstained

with DAPI. Cells were analyzed under a fluorescence microscope equipped with a triple band-pass filter to simultaneously visualize DAPI, FITC/Spectrum-green and Spectrum-orange.

Since none of the myeloma specimens was characterized by loss of the chromosome 17 centromere, presence of ≥2 signals with this probe in more than 90% of nuclei was considered as evidence for high hybridization efficiency. Only slides fulfilling these criteria were scored, and at least 200 cells with the appearance of plasma cells were evaluated.

### Immunocytochemical staining of lung resistance protein (LRP):

LRP expression was determined by an alkaline phosphatase immunocytochemical detection method[20] using the specific murine monoclonal antibody (MoAb) LRP-56 (IgG2b) (Dako, Glostrup, Denmark). Bone marrow cells were separated by Ficoll-Hypaque, washed twice with minimal essential medium (MEM; GIBCO, Grand Island, NY) and stored at -20°c until use. Cytocentrifuged slides were air dried overnight and fixed in acetone for 10 minutes.

After pre-incubation for 20 minutes with 10% rabbit serum in phosphate-buffered saline plus 1% bovine serum albumin (PBS/BSA; Sigma Chemical Co, St Louis, MO), cytospins were incubated with LRP-56 (diluted 1:500 in 1% BSA) or with idiotype matched control (non-specific mouse IgG-1; Cappel: Organon Teknica 50327/36345) for 1.5 hours. Next, rabbit anti-mouse immunoglobulin (RAM; Dakopatts Z 259, DAKO Corp, Glastrup, Denmark) diluted 1:25 for 1 hour was added followed by incubation with alkaline phosphatase substrate (APAAP; Dakopatts D 651, DAKO), diluted 1:50 for 1 hour. Incubations with RAM and APAAP were repeated for 0.5 hour. The color reaction was produced using a Neufuchsin (Merck 4041; Merck, Darmstadt, Germany) substrate incubating for 40 minutes. All incubations were performed at room temperature. Between incubation steps, slides were washed thoroughly in PBS for 10 minutes.

All slides were examined and scored. Plasma cells were identified on morphological criteria. At least 250 plasma cells were evaluated. A sample was considered to be LRP-positive if >10% of the plasma cells stained with the LRP-56 antibody. These criteria were based on previous experience of Izquierdo et al.[7,21]

### Determination of prognostic factors:

The serum B2-microglobulin level was determined by means of a competitive enzyme immunoassay (Phadezym; Pharmacia, Uppsala, Sweden). Serum levels of lactate dehydrogenase (LDH) were measured according to standard methods.

### Statistical analysis:

Data analysis was performed using the EPI-INFO (version 6.1) statistical software package[22]. Statistical evaluation of results included Fisher's exact test, $\chi^2$-test, t-test, and the nonparametric Kruskal-Wallis test. Odds ratios were determined according to Mantel-Haenszel. Kendall's tau coefficients were used in testing correlations. Survival time, measured both from the date of diagnosis and the date of start of induction treatment, was calculated using Kaplan-Meier estimates. Differences between survival curves were analyzed by means of Breslow and Mantel-Cox tests. Multivariate analysis of survival time data was performed using the proportional hazards regression model of Cox. Stepwise evaluation of maximum partial likelihood ratio was used to identify prognostic factors.

## RESULTS

### Control hybridizations:

Hybridizations were performed with peripheral blood (PB) (n = 10) of healthy individuals and bone marrow (BM) specimens (n = 6) from patients with non-Hodgkin's lymphoma without histological evidence of bone marrow involvement to determine the cut-off level for presence of a p53 deletion by interphase FISH.

As with the myeloma specimens, simultaneous hybridizations with probes specific for the chromosome 17 centromere and the p53 locus were performed. In normal specimens, less than two hybridization signals per nucleus were observed with the p53 probe in 3.24% ± 1.79% (mean ± standard deviation [SD]) of nuclei. There was no significant difference between normal PB and BM samples. Thus, the cut-off level for the presence of a p53 deletion was set at 8.6% (mean + 3 SD of the frequency of control cells with only one p53 hybridization signal).

### Deletions of p53 in MM by interphase FISH:

In 12 of 38 BM samples (31.5%) from patients with MM, interphase FISH analysis of plasma cells provided evidence for a p53 gene deletion (Figure1 A and B). The percentage of plasma cells exhibiting a p53 deletion ranged between 16.6% and 94.0% (median, 48.7%).

### LRP expression:

According to the percentage of staining plasma cells, 4 (10.5%) samples had no LRP staining cells, 20 (52.6%) samples contained <10% staining cells and 14 (36.8%) showed ≥10% staining plasma cells. (Table 1) The intensity of LRP staining was low, intermediate, and high in 11 (28.9%), 15 (39.5%), and 8 (21.1%) samples,

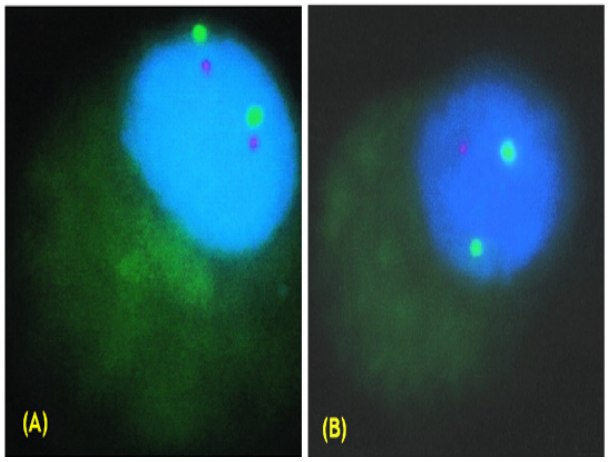


**Fig. 1:** Interphase FISH analysis of the p53 gene in MM: (A) Plasma cell from a patient without p53 deletion: Two hybridization signals with both probes (chromosome 17 centromere, green signals; p53, red signals) can be recognized. (B) Plasma cells exhibiting a monoallelic deletion of p53 (probes as in A).

respectively. In all of the samples with a high staining intensity, ≥10% staining plasma cells were observed. For additional analyses, samples were divided into positive (≥10% staining plasma cells) and negative (<10% staining plasma cells). According to this division, LRP was found positive in 14 (36.8%) of 38 bone marrow samples at diagnosis (Figure 2).

**Table 1:** LRP expression in MM before chemotherapy.

| Intensity of staining | Percentage of staining plasma cells | | | Total |
|---|---|---|---|---|
| | 0% (n =4) | <10% (n =20) | ≥10 (n =14) | |
| - | 4 | - | - | 4 |
| + | - | 9 | 2 | 11 |
| ++ | - | 11 | 4 | 15 |
| +++ | - | - | 8 | 8 |

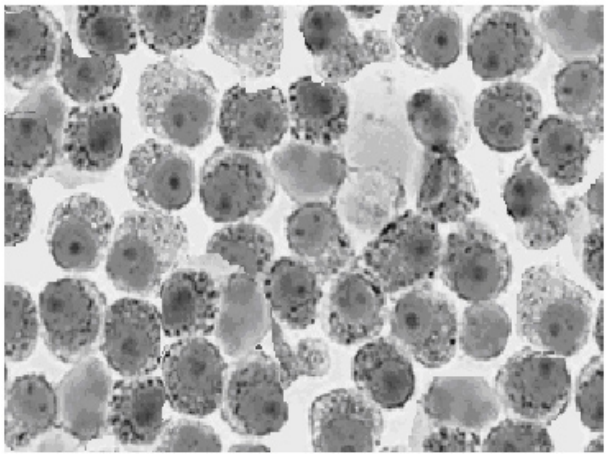

**Fig. 2:** Plasma cells from a patient with multiple myeloma showing cytoplasmic staining with LRP-56 antibody. (× 500)

## Correlation of LRP expression with other prognostic factors:

Using previously defined cut-off levels the distribution of serum B2-microglobulin level, and serum LDH in relation to plasma cell LRP expression was studied. The cut-off level for B2-microglobulin was >4 µg/mL[23], and for LDH >300 U/L[23]. LRP did not correlate with age (P = 0.9), sex (P = 0.065), M-component (P = 0.86), stage of disease (P=0.71), serum creatinine (P=0.63), serum B2-microglobulin (B2M) (P = 0.74), serum LDH (P=0.07) or C-reactive protein (P=0.066). However, LRP expression was more frequently observed in patients with a p53 deletion detected by means of FISH than in those without a deletion of p53. p53 deletions were detected in 71.4% (10/14) of patients with LRP positive immunostaining (P = 0.001; Table 2).

**Table 2:** LRP expression and patient's characteristics.

| | No. of patients | LRP +ve patients n (%) | LRP -ve patients n (%) | P |
|---|---|---|---|---|
| No. of patients | 38 | 14 (37) | 24 (63) | |
| Age >60 yr | 18 | 7 (39) | 11 (61) | 0.9 |
| Gender | | | | |
| Male<br>Female | 26<br>12 | 10 (71.4)<br>4 (28.6) | 16 (66.7)<br>8 (33.3) | 0.065b |
| M-component | | | | |
| Ig G<br>Ig A<br>Ig D<br>LCD | 22<br>9<br>1<br>6 | 7 (50)<br>3 (21.4)<br>1 (7.2)<br>3 (21.4 | 15 (62.5)<br>6 (25)<br>0<br>3 (12.5) | 0.86b |
| Stage (Durie and Salmon) | | | | |
| I<br>II<br>III | 8<br>14<br>16 | 4 (28.6)<br>5 (35.7)<br>5 (35.7 | 4 (16.7)<br>9 (37.5)<br>11 (45.8) | 0.71b |
| Creatinine >2 mg/dl | 8 | 3 (21.4) | 5 (20.8) | 0.63c |
| B2M ≥4 mg/L | 26 | 10 (71.4) | 16 (66.7) | 0.74b |
| LDH ≥ 300 | 20 | 6 (42.9) | 14 (58.3) | 0.07b |
| CRP >6 mg/L | 16 | 5 (35.7) | 11(45.8) | 0.66b |
| Deletion of p53 | 12 | 10 (71.4) | 2 (8.3) | 0.01b |

LRP: lung-resistance protein, LDH: lactate dehydrogenase, B2M: B2-microglobulin CRP: C-reactive protein. bP of ×2 test, cP of Fisher's exact test

Correlation of p53 with other prognostic factors: Analysing data from all 38 patients with newly diagnosed MM, regarding the Durie & Salmon stage, patients with a p53 deletion were predominantly at stage III (p53 deletion in 83.4% of stage III patients vs. 8.3% of patients at stages I and II respectively; P = 0.04). Also, LRP expression was more frequently observed in patients with a p53 deletion.

LRP was positive in 83.3% of patients (10/12) with a p53 deletion and only in 15.4% of patients (4/26) without a p53 deletion (P = 0.001; Table 3). However, there was no significant correlation of p53 status with age (P = 0.7), sex (P = 0.4), M-component (P = 0.076), serum creatinine (P = 0.66), B2-microglobulin (B2M) (P = 0.08), serum LDH (P = 0.9) or C-reactive protein (CRP) (P = 0.08) (Table 3).

**Table 3:** p53 and patient's characteristics.

| | No. of patients | Normal p53 | Deletion of p53 | p |
|---|---|---|---|---|
| No. of patients | 38 | 26 (68) | 12 (32) | |
| Age >60 yr | 18 | 14 (78) | 4 (22) | 0.7b |
| **Gender** | | | | |
| Male | 26 | 19 (73.1) | 7 (58.3) | 0.4b |
| Female | 12 | 7 (26.9) | 5 (41.7) | |
| **M-component** | | | | |
| Ig G | 22 | 17 (57.7) | 5 (41.7) | 0.076b |
| Ig A | 9 | 5 (19.2) | 4 (33.4) | |
| Ig D | 1 | - | 1 (8.3) | |
| LCD | 6 | 4 (15.4) | 2 (16.7) | |
| **Stage (Durie and Salmon)** | | | | |
| I | 8 | 7(26.9) | 1 (8.3) | 0.04b |
| II | 14 | 13 (50) | 1 (8.3) | |
| III | 16 | 6 (23.1) | 10 (83.4) | |
| Creatinine >2 mg/dl | 8 | 4 (15.4) | 4 (33.3) | 0.66c |
| B2M 4 mg/L | 26 | 18 (69.2) | 8 (66.7) | 0.08b |
| LDH 300 | 20 | 13 (50) | 7 (58.3) | 0.9b |
| CRP >6 mg/L | 16 | 8 (30.7) | 8 (66.7) | 0.08b |
| **LRP-expression** | | | | |
| +ve | 14 | 4 (15.4) | 10 (83.3) | 0.001b |
| -Ve | 24 | 22 (84.6) | 2 (16.7) | |

LRP: Lung-resistance protein, LDH: lactate dehydrogenase, B2M: B2-microglobulin CRP: C-reactive protein. bP of ×2 test, cP of Fisher's exact test.

## Response to induction chemotherapy and prognostic factors:

Thirty-eight patients were evaluable for response to induction chemotherapy. The overall response rate of all of the evaluable patients was 63.2%. The response rate was 79.2% for patients without LRP expression but only 35.7% for patients with LRP expression (P = 0.01; Table 4). Among the 12 patients with a p53 deletion by FISH, 25% responded compared with 75% of patients without p53 deletion (P = 0.001). With the exception of LRP expression and p53 deletions, there was no other prognostic variable sharing any correlation with response (Table 4).

### LRP, p53 and survival:

Kaplan-Meier survival curves of LRP-positive and LRP-negative patients are presented in figure (3). An inverse correlation was found between LRP expression and survival duration. The median survival of LRP-positive patients was 11 months (95%-Cl: 9 to 24), whereas the median survival duration of LRP negative patients was not reached (95%-Cl: 26 to 82; P < .002; hazard ratio [HR] = 2.9 (1.4-5.7); Figure 3).

**Table 4:** Outcome of induction chemotherapy.

| | No. of patients | Response n (%) | No response n (%) | P |
|---|---|---|---|---|
| **LRP** | | | | |
| Negative | 24 | 19 (79.2) | 5 (20.8) | 0.01a |
| Positive | 14 | 5 (35.7) | 9 (64.3) | |
| **P53** | | | | |
| Normal | 26 | 21 (80.8) | 5 (19.2) | 0.001a |
| Deletion | 12 | 3 (25) | 9 (75) | |
| **Age** | | | | |
| ≤60 | 20 | 14 (70) | 6 (30) | 0.07a |
| >60 | 18 | 10 (55.6) | 18 (44.4) | |
| **Sex** | | | | |
| Male | 26 | 19 (73.1) | 7 (26.9) | 0.3 a |
| Female | 12 | 5 (41.7) | 7 (58.3) | |
| **Immunoglobulin subtype** | | | | |
| IgG | 22 | 16 (72.7) | 6 (27.3) | 0.7 a |
| Non-IgG | 16 | 8 (50) | 8 (50) | |
| **Stage** | | | | |
| I and II | 22 | 17 (77.3) | 5 (22.7) | 0.08 a |
| III | 16 | 7 (43.8) | 9 (56.2) | |
| **Creatinine** | | | | |
| ≤2 mg/dl | 30 | 21 (70) | 9 (30) | 0.06 c |
| >2 mg/dl | 8 | 3 (37.5) | 5 (62.5) | |
| **ß2-microglobulin** | | | | |
| ≤4 mg/liter | 12 | 8 (66.7) | 4 (33.3) | 0.08 a |
| >4 mg/liter | 26 | 16 (61.5) | 10 (36.5) | |
| **LDH** | | | | |
| ≤300 units/liter | 18 | 12 (66.7) | 6 (33.3) | 0.06 a |
| >300 units/liter | 20 | 12 (60) | 8 (40) | |
| **CRP** | | | | |
| ≤6 mg/liter | 22 | 13 (59.1) | 9 (40.9) | 0.4 a |
| >6 mg/liter | 16 | 11 (68.8) | 5 (31.2) | |

a P of 2 test. c P of Fisher's exact test.

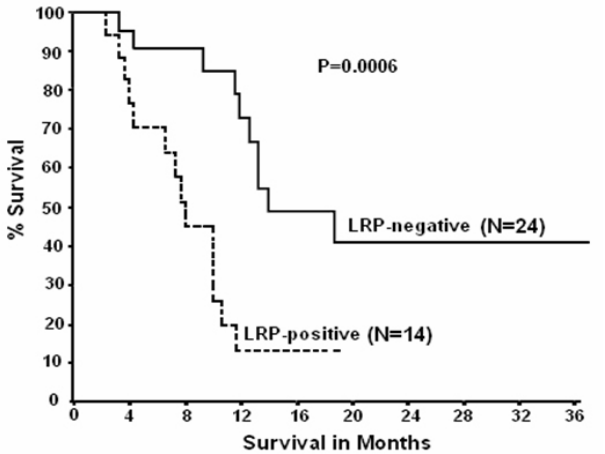


**Fig. 3:** The survival probabilities in patients with and without LRP expression.

The survival probability was also estimated according to Kaplan-Meier for patients with and without p53 deletions: Patients with and without p53 deletions had significantly different overall survival times (median 13.9 months, 95%-CI: 8 to 26 and median not reached, CI: 24 to 84, respectively; P < .0001; hazard ratio [HR] = 2.6) (Figure 4).

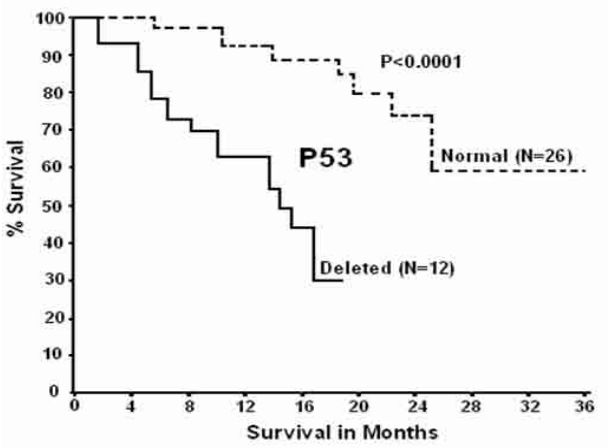


**Fig. 4:** shows the survival probabilities in patients with and without p53 deletions.

## DISCUSSION

Clinical resistance to chemotherapy results from the interaction of numerous biological variables. Several clinical studies provided evidence for the relationship of LRP with chemoresistance to both classical and MDR-related drugs. In this study, we found that over expression of the major vault transporter protein, LRP, has prognostic significance in MM. Our results revealed that LRP is expressed in untreated MM. LRP positivity was found in 36.8% of the patients with newly diagnosed MM. This figure is consistent with data indicating the widespread expression of LRP in untreated human malignancies.[7] LRP expression was associated with poor response to chemotherapy and shorter overall survival of MM patients. Similar results have been reported by Raaijmakers et al.[24], who found LRP expression as a predictive and prognostic factor with regard to chemotherapy response and overall survival in patients treated with conventional-dose melphalan. A similar predictive and prognostic value of LRP expression has previously been reported for acute myeloid leukemia[25-27], acute lymphoblastic leukemia[28], and advanced ovarian cancer.[21]

In present study, deletions of the p53 gene were detectable by interphase FISH in a significant proportion of patients with MM. Specifically, plasma cells from 31.5% of patients with newly diagnosed MM carry a p53 deletion. Our results revealed that deletions of 17p involving the p53 gene locus are more common than previously reported based on findings by metaphase cytogenetics. Evidence that 17p may be deleted in MM was also obtained in a previous study by means of comparative genomic hybridization, under representation of region 17p11.2-p13 was a frequent finding[29]. The higher frequency of p53 gene deletions by FISH compared with banding analysis can only in part be explained by the fact that metaphase cytogenetics remains normal or non-informative in the majority of patients with newly diagnosed MM despite the finding of chromosomal aneuploidy by interphase FISH[30]. The most likely explanation for the failure of detecting this deletion by banding studies is the presence of a physical deletion of 17p on the submicroscopical level. In agreement with this, there is increasing evidence that FISH is a more sensitive method than banding analysis to detect deletions of narrow chromosomal regions. For example, FISH was shown to identify deletions of 9p involving the CDKN2 gene (p16INK4a/MTS1) in acute lymphoblastic leukemia,[31,32] and of 12p in acute leukemias[33-37] both in the absence of microscopically visible deletions on metaphase chromosomes. Our results demonstrated that presence of a p53 deletion in plasma cells from patients with MM was associated with significantly poor response to chemotherapy and shortened survival. The main reason for short survival of myeloma patients with a p53 deletion by FISH was poor response to chemotherapy, many cytotoxic drugs act through induction of apoptosis, and apoptosis requires a functional p53, it has been suggested that in tumor cells with impaired p53 function, apoptosis may not occur leading to resistance to chemotherapeutic agents.[38] Alternatively, an altered p53 gene may lose its function as `guardian of the genome[39,40] and, as a consequence, complex cytogenetic abnormalities may develop. These genetic changes could then be the true reason for resistance to treatment.

According to the clinical correlations, the present study shows that presence of a p53 deletion and expression of LRP in plasma cells from patients with MM was associated with significantly shortened survival. Cox regression analysis revealed that LRP expression and deletion of p53 were the two most important independent parameters that were predictive for shortened survival. Other known indicators of poor prognosis, like elevated B2M, stage of disease, elevated LDH, elevated CRP, impaired renal function, type of paraprotein, gender and advanced age, could not influence the prognostic power of these two factors.

Results presented in this study have been obtained in a patient population that was treated by conventional-dose chemotherapy. Considering results from recent clinical trials that demonstrated improved response rates and prolonged survival time after high-dose chemotherapy with autotransplantation, it will be of particular clinical significance to determine the outcome of the different risk groups after high-dose chemotherapy. LRP and p53 may help to determine such groups but further studies on the expression of LRP, p53 deletions and other mechanisms of drug resistance seem warranted to confirm these

results and to clarify the functional characterization and the biological role of LRP and p53 in myeloma.

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