Only 53% (16/30) of *de novo* AML patients in complete response (CR) and with an overall survival exceeding 1 year were Hsp70-positive, whereas 93% (14/15) of the therapy refractory, non-responders were Hsp70-positive. Functionally, Hsp70-membrane localization correlates with an increased sensitivity to lysis mediated by non-MHC restricted allogeneic natural killer (NK) cells.[5] Incubation of isolated NK cells with either Hsp70-protein or an Hsp70-peptide termed *TKD* ($Hsp70_{aa450-463}$ TKDNNLLGRFELSG, Bachem Bubendorf, Switzerland) plus low dose interleukin-2 (100 IU/mL) further enhances cytolytic activity.[6,7] In the present study, the stimulatory capacity of *TKD* was investigated using patient-derived peripheral blood mononuclear cells (PBMC) as effectors and autologous leukemic blasts derived from bone marrow as target cells. The percentage of immunologically determined Hsp70-positive cells varied between 4% and 70%. As shown in the upper panel of Figure 1A, neither interleukin-2 (IL-2) alone nor IL-2 in combination with *TKD* stimulated a cytolytic response against Hsp70-membrane-negative bone marrow cells. However, the cytolytic response (n=6) against *Hsp70-positive* blasts (range: 20% - 70%) was significantly greater when autologous PBMC were stimulated with *TKD* plus low dose IL-2 rather than IL-2 alone. These data indicate that membrane-bound Hsp70 provides a target structure for *TKD*-activated PBMC. To prove that NK-cells but not T-cells are responsible for the cytolytic activity, patient-derived PBMC were separated into CD3-negative NK-cell and CD3-positive T cell populations by a negative bead separation method using the CD3 specific antibody OKT3 for T-cell depletion and the CD56 specific antibody for NK cell depletion. As shown in Figure 1B, T cells, whether stimulated with IL-2 or IL-2 plus *TKD,* did not exhibit any significant cytolytic activity against Hsp70-positive (50%) autologous leukemic blasts. In contrast, NK-cells stimulated with IL-2 plus *TKD* revealed significant lysis against autologous bone marrow cells. Low dose IL-2 alone was less efficient in activating autologous NK cells against Hsp70-positive leukemic blasts. This result was further confirmed by antibody-blocking studies. The MHC class I specific antibody did not affect the cytolytic effect of *TKD* plus IL-2-stimulated cells against Hsp70-positive (40%) blasts, no cytolytic response was observed with IL-2 stimulated PBMC (Figure 1C). Recurrent chronic myelogenous leukemia (CML) can be successfully treated by transfusion of donor lymphocytes after marrow transplantation.[8] However, this strategy is effective in only some patients with acute leukemias and lymphoma. Major complications are graft-versus-host disease and myelosuppression. In order to modify graft-versus-host disease without suppressing the graft-versus-leukemia effect, studies are ongoing using NK cells for adoptive transfer. Recently, Velardi's group has convincingly demonstrated the beneficial effects of allogeneic NK-cells in the treatment of AML.[9] In our approach we intend to trigger the anti-leukemic effect of autologous NK-cells by exploiting the immunostimulatory capacity of the Hsp70-peptide *TKD* against prognostically unfavorable, Hsp70-positive AML blasts.

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Different prognostic values of $p15^{INK4b}$ and $p16^{INK4a}$ gene methylation in multiple myeloma

We analyzed 61 multiple myeloma (MM) samples by methylation-specific polymerase chain reaction (PCR). Survival was significantly lower in patients with methylated $p16^{INK4a}$ gene, but was not different between patients with methylated and unmethylated $p15^{INK4b}$ gene, suggesting that $p15^{INK4b}$ and $p16^{INK4a}$ genes have a distinct influence on the outcome of MM.

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$p15^{INK4b}$ and $p16^{INK4a}$ proteins are cell cycle regulators involved in the inhibition of G1 phase progression.[1] Frequencies of $p16^{INK4a}$ or $p15^{INK4b}$ gene methylation of up to 75% have been reported in multiple myeloma (MM).[2,3] Recent reports have shown that methylation of the $p16^{INK4a}$ gene is significantly

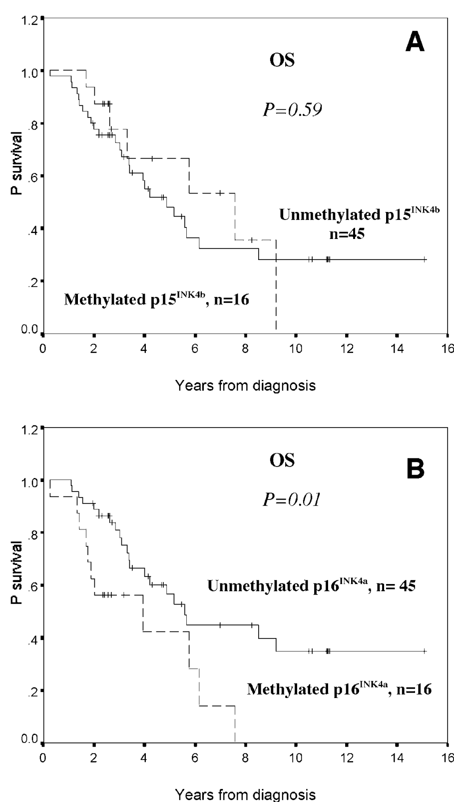

Figure 1. Overall survival of 61 patients with multiple myeloma according to the methylation status of the (A) $p15^{INK4b}$ and (B) $p16^{INK4a}$ gene. Dotted line: methylated cases; solid line: unmethylated cases.

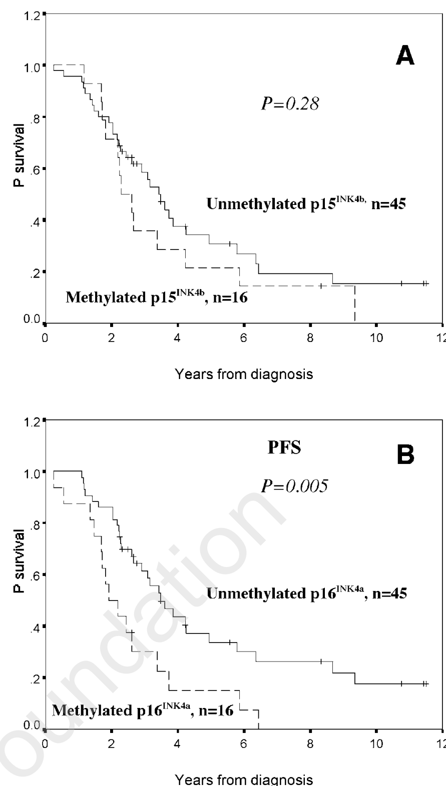

Figure 2. Progression-free survival of 61 patients with multiple myeloma according to the methylation status of the (A) $p15^{INK4b}$ and (B) $p16^{INK4a}$ gene. Dotted line: methylated cases; solid line: unmethylated cases.

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associated with short survival.[4,5] Expression of the $p15^{INK4b}$ gene is induced by TGF-β and inactivation of $p15^{INK4b}$ by methylation suggests a possible mechanism of escape from regulatory signals provided by the bone marrow micro-environment.[6] However, the role of $p15^{INK4b}$ methylation in the development of MM and progression of the disease remains unknown. In order to investigate the relative distinct influences of $p15^{INK4b}$ and $p16^{INK4a}$ methylation on the clinical outcome of MM, we analyzed $p16^{INK4a}$ and $p15^{INK4b}$ gene methylation using methylation-specific PCR (MSP) in 61 previously untreated MM patients.[7] The median age of the patients was 65 years (range: 39-83). Clinical staging was: stage I, 11 (18%); II, 8 (13%); III, 42 (69%) and B in 5 (8.2%). M-component was IgG in 36 cases (59%), IgA in 16 (26%), and Bence Jones in 9 (14.8%). The median percentage of BM plasma cells was 20% (SD 23.8; range: 6-96). Thirty-three patients (54.1%) were treated with melphalan-prednisone, eighteen (29.5%) with intensive protocols, and nine (14.8%) were not treated.

The median time of follow-up was 8.6 years (range 3.5-16.1). Methylation of $p16^{INK4a}$ was found in 16 cases (26.2%), of $p15^{INK4b}$ in 16 (26.2%), of $p16^{INK4a}$ or $p15^{INK4b}$ in 25 (41%), and of both genes in 7 (11.5%). $p15^{INK4b}$ and $p16^{INK4b}$ methylation was found to be independent (χ^2=2.3, *p*= 0.13). No correlation could be made between methylation of either $p16^{INK4a}$ or $p15^{INK4b}$ and gender, age, isotype, level of M-component, percentage of bone marrow plasmacytosis, stage of disease, renal insufficiency, serum levels of lactate dehydrogenase, albumin, calcium, β_2-microglobulin, C-reactive protein, or response to treatment. However, patients with methylated $p16^{INK4a}$ gene showed significantly poorer overall survival (OS) (*p*= 0.01, log-rank test) and progression-free survival (PFS) (*p*=0.005)(Figures 1 and 2). The only other initial characteristic which affected OS in univariate analysis was β2-microglobulin (*p*= 0.006). Final models of multivariate analysis showed that methylation of the $p16^{INK4a}$ gene remained an independent prognostic factor for OS (*p*= 0.035, relative risk = 2.86, 1.076-7.60) with β_2-microglobulin (*p*= 0.003, relative risk = 1.18, 1.08-1.3). In contrast, OS and PFS were not significantly different between patients with methylated and unmethylated $p15^{INK4b}$ gene (*p*= 0.59 and 0.28, respectively) (Figures 1 and 2). When OS and PFS were compared between patients with either $p15^{INK4b}$ or $p16^{INK4a}$ gene methylation (n=25) and patients with both genes unmethylated (n=36), the difference was significant for PFS (*p*= 0.029) but not for OS (*p*= 0.24). Incorporation of $p15^{INK4b}$ methylation in the multivariate model with $p16^{INK4a}$ methylation and β_2-microglobulin did not modify the results. Thus, the poor outcome of MM patients with methylation of CDK inhibitors seems entirely related to $p16^{INK4a}$ gene methylation. The prognostic impact of $p16^{INK4a}$ methylation has been previously described by other groups and recently Mateos *et al.* found a correlation between $p16^{INK4a}$ methylation in MM patients and the percentage of S-phase plasma cells.[4] This group also reported a significant association between $p16^{INK4a}$ methylation and the stage of disease,

β_2-microglobulin serum levels, and high C-reactive protein values. We did not observe any correlation between $p16^{INK4a}$ methylation and the initial characteristics of the patients. However we observed almost the same differences in OS and PFS as Mateos *et al.* did. Ng *et al.* reported similar incidences of $p16^{INK4a}$ gene methylation in pre-treated and post-treated MM patients.[3] These findings suggest that in spite of possible variations between techniques used, heterogeneity of patients, and other unknown factors, $p16^{INK4a}$ methylation analysis in MM might provide interesting prognostic information and warrants future prospective studies.

The absence of prognostic impact of $p15^{INK4b}$ gene methylation is in marked contrast with the prognostic value of $p16^{INK4a}$ methylation. We previously reported frequent methylation of $p15^{INK4b}$ and $p16^{INK4a}$ genes in CD138-purified plasma cells from patients with monoclonal gammopathy of undetermined significance, suggesting that methylation of $p15^{INK4b}$ and $p16^{INK4a}$ might be an early event in the course of MM.[7] Combined with our current findings, these data suggest that both $p15^{INK4b}$ and $p16^{INK4a}$ methylation might play a role in the initial transformation of plasma cells. However, $p15^{INK4b}$ methylation might exert a lesser influence on subsequent tumor progression.

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Non-myeloablative stem cell transplantation with low-dose total body irradiation and fludarabine for metastatic renal cell carcinoma

We evaluated the feasibility of non-myeloablative stem cell transplantation for metastatic renal cell carcinoma after a non-myeloablative conditioning regimen combining low-dose TBI and fludarabine. Seven consecutive patients were included. Initial engraftment occurred in all patients and 6/6 evaluable patients achieved sustained donor chimerism. One patient experienced a partial response but the other 6 progressed.

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Metastatic renal cell carcinoma (RCC) is largely insensitive to chemotherapy. In 2000, Childs *et al.* published the results of non-myeloablative stem cell transplantation (NMSCT) combining cyclophosphamide and fludarabine in 19 patients with metastatic RCC.[1] Ten of the 19 patients enjoyed objective responses, including 3 with sustained CR. Although the conditioning regimen was non-myeloablative, the neutrophil count fell to less than 0.1×10^9/L in all patients.[1] The Seattle team has recently proposed an original approach to NMSCT with a conditioning regimen based on 2 Gy TBI $\pm$ fludarabine, followed by post-transplant immunosuppression with cyclosporine A (CyA) and mycophenolate mofetil (MMF) that permitted the transplant to be performed in an ambulatory care setting.[2] In the present study, we report our experience with 7 patients with RCC.

Seven consecutive patients with metastatic RCC, were included (Table 1). Written informed consent was obtained from patients and donors and our institution's Ethical Committee approved the protocol. Four patients had HLA-identical siblings and three had alternative donors. Conditioning consisted in 90 mg/m² fludarabine combined with 2 Gy TBI.[2-4] The whole post-transplant procedure was carried out as outpatient except in the haemodialyzed patient. Post-transplant immunosuppression consisted in CyA and MMF.[3] Disease responses were defined using the criteria of Childs *et al.*[1] Stem cell mobilization and collection were carried out as previously reported.[5] The protocol involved a prospective comparison of graft manipulation, so that patients #1-3 received unmanipulated PBSC, patients #4-6 CD8-depleted PBSC and patient #7 CD34-selected PBSC.[3] Three patients without GVHD received additional DLI (per protocol) on days 40 and 80. Per protocol, DLI were unmanipulated in patient 2 and CD8-depleted in patients #5 and 7. Chimerism[6,8] was assessed as previously reported.[3]

None of the patients developed grade >2 regimen-related toxicity.[7] The neutrophil nadir occurred on day 7 and was 0.97×10^9/L (0.12-1.67). Two patients did not require hospitalization within the first 30 days following NMSCT, and the other five were hospitalized for a median of 9 (6-22) days. Total white blood cell (WBC) and CD3+ cell chimerisms were 91% (90-95) and 67% (20-89) on day 28 and 95 (95-96) and 83 (32-96) on day 100, respectively (Figures 1A and 1B).

We observed only 1 partial response. This response occurred in patient #1 who had extensive lung metastases. The disease remained stable the first 150 days after transplantation (Figure 1C) but the tumor mass was markedly (> 50%) reduced on day 240. This patient experienced both acute and chronic GVHD. Response persisted until day 389 when a chest CT-scan showed elimination or major reduction of 80% of the metastases with stabilization of the others, with the exception of two lesions that progressed (Figure 1D). Unfortunately the patient subsequently relapsed in the liver and died of disease progression. All other patients progressed (Table 1). We show here that engraftment can be achieved in RCC patients with this low-intensity

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