Up-front fludarabine impairs stem cell harvest in multiple myeloma: report from an interim analysis of the NMSG 13/03 randomized placebo controlled phase II trial

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Abstract

The impact of chemotherapy resistant B cells in multiple myeloma (MM) needs to be evaluated by *in vivo* targeted therapy. Here we report the conclusions from a phase II randomized, placebo controlled trial adding fludarabine to the induction with cyclophosphamide-dexamethasone. Based on an interim toxicity and safety analysis, the trial was stopped following inclusion of 34 of a planned 80 patients due to a reduced number of patients (4/17) actually harvested in the experimental arm compared to the control arm (11/17; $p<0.05$). In conclusion, the scheduled fludarabine dosage in 2 cycles combined with alkylating therapy impairs stem cell mobilization and standard therapy in young MM patients and should not be administrated up-front.

Introduction

Due to a range of new drugs there has been a continuous progress in the treatment of multiple myeloma (MM). However, only a few patients are considered cured so far, most likely due to the nature of the disease.[1-3] Recent data have indicated that the myeloma cell hierarchy includes resistant circulating clonal memory B cells, which differ considerably from the classical end stage plasma cells, infiltrating the bone marrow. The pathophysiological significance of these cells is unknown, but hypothetically they may serve as "sleeping" myeloma stem cells responsible for and "feeding" post-treatment relapse and disease progression.[4-5] The clinical impact of these cells needs to be evaluated by *in vivo* targeted therapy. Therefore, we initiated a randomized phase II multicenter trial comparing induction therapy by cyclophosphamide plus dexamethasone with and without fludarabine, a DNA repair inhibitor. Fludarabine, 9-β-D-arabinofuranosyl-2-fluoroadenine, is an analog to adenosine cytotoxic against dividing and resting cells.[6-7] *In vivo*, the combination of a DNA damaging agent, e.g. adriamycine or cyclophosphamide combined with fludarabine is clinically active against B cells in CLL and low-grade follicular lymphomas.[8-10] Although it has been documented active against leukemia and lymphoma, only recent data has suggested efficacy in MM. In a previous open phase II pilot study, we have documented that addition of fludarabine to induction therapy is clinically feasible with only minor toxicity. A beneficial clinical outcome was suggested including a reduction of minimal residual disease (MRD) following the addition of fludarabine.[11] However, one concern during the trial design discussion was the adverse impact of fludarabine on stem cell harvest experienced in advanced CLL,[13] which, however, was not considered in untreated patient treatment.

The main objective of the subsequent NMSG n.13/03 phase II trial was to generate data on toxicity, safety and efficacy by adding fludarabine to standard induction therapy.[12] In the close follow-up of the patient cohort it was decided to perform an interim analysis, which concluded that fludarabine in the experimental arm inhibits stem cell mobilization capacity and reduced the number of patients reaching high-dose therapy and the trial was stopped. Consequently, fludarabine in combination with alkylating agents should not be administrated as up-front therapy, if high-dose therapy supported by autologous transplantation is standard care.

Materials and Methods

Approval and patient eligibility

The scientific protocols were reviewed and approved by the regional ethics committees in Denmark and the Danish Drug Agency (Sagsnr. KA 03103 ms) and all patients gave written informed consent before study entry. All patients were over 18 years of age and were referred to the departments for diagnostic evaluation. Patients under 60 years of age who had Durie-Salmon stage I with at least one bone lesion, II, or III myeloma were eligible. The criteria for exclusion were prior treatment for myeloma, another cancer, abnormal cardiac function, chronic respiratory disease, abnormal liver function or psychiatric disease.

Trial design

This was a randomized, placebo controlled, single blinded, phase II study evaluating toxicity and safety of fludarabine added to cyclophosphamide and dexamethasone (CyDex) as induction therapy in younger newly diagnosed symptomatic multiple myeloma requiring therapy. The treatment regimen CyDex as standard induction therapy was documented in NMSG trial n.11/01.[12] Patients were randomized at diagnosis either to CyDex + placebo (control Arm A) or CyDex + fludarabine (experimental Arm B).

Treatment procedure

Fludarabine was considered as the only investigational drug in this study administrated in induction phase I.

Phase I

Arm A (conventional arm): CyDex + placebo, two (three) cycles in Phase I: two courses of CyDex: cyclophosphamide 1000 mg/m² IV day 1 and dexamethasone 40 mg/day PO on day 1-4, and 9-12 + placebo PO; repeated once

day 21. The third cycle of CyDex (without placebo) was only given if the phase II treatment could not be initiated within six weeks after the start of CyDex II. Other steroids in equipotent dose could be used instead of dexamethasone.[12]

Arm B (experimental arm): CyDex plus fludarabine, two (three) cycles in Phase I: two courses of CyDex: cyclophosphamide 1000 mg/m² day 1 IV and dexamethasone 40 mg/d (or other steroids in equipotent dose) PO on days 1-4, and 9-12, combined with fludarabine 40 mg/m² PO day 1-3 each cycle; repeated once day 21. The third cycle of CyDex (without fludarabine) was only given if the phase II treatment could not be initiated within six weeks after the start of the second CyDex plus fludarabine course.

Common trunk (phases II–IV)

This was as described in previous reports from NMSG.[3,12] In brief, the priming and apheresis phase II included cyclophosphamide 2 g/m² given as a single dose intravenously during 60 minutes. Uroprotection with Mesna 160% of the cyclophosphamide dose divided in four doses (before 3, 6 and 9 h after start of cyclophosphamide) and diuresis of at least 2.5 L/m² the following 24 hours. Granulocyte colony-stimulating factor (G-CSF) was initiated day 4 after cyclophosphamide as Neupo-gen® 5-10 ug/kg daily adjusted to appropriate vial size. Peripheral blood stem cell leukapheresis were performed during mobilization, guided by CD34 blood levels, by harvest of a minimum of 2×10⁶ CD34+ cells per kilogram body weight.

Following harvest of a sufficient graft, the patients passed to phase III: high-dose therapy with melphalan 200 mg/m² given as a single dose intravenously, followed by stem cell infusion 48 hours later, and G-CSF (Neupo-gen® 5 µg/kg daily or Neulasta® 12 mg) one injection from day 4 after graft reinfusion, until the absolute neutrophil count is more than 1.0×10⁹/L for three consecutive days.

The patients were followed as outpatients during phase IV.

Statistical analysis

The proportions of patients with a given characteristic were compared using Fisher's exact test for binary data. The distributions of continuous quantities were examined to control that they followed Gaussian distributions with good approximation using Kolmogorow-Smirnow's test supplemented by Q-Q plots.

If they did, either directly or following transformation (square root or logarithmic), the mean values of the two groups were compared using a *t*-test. If not the two groups were compared using Mann Whitney's test.

Results and Discussion

Treatment cycles given during induction phase I

All patients in the conventional arm received the scheduled cycles of therapy. However, in the experimental arm this was only the case for 11/17 patients as 6 patients were stopped before or following the first cycle of therapy (Table 1). Three of these patients did not start therapy, suggesting bias from the clinician, for whom the therapy arm was not blinded. Such a bias may be the result from the relative intensive dosage of fludarabine administrated in this trial of 40 mg/m² PO for three days in each of two cycles, compared to our previous trial[2] where we administered a dose of 25 mg/m² intravenously for five days. Following discussions within NMSG the protocol group selected oral administration over three days attempting to reach an equivalent total dose of fludarabine as used previously. Our previous experience[2] administrating a total dosage of 125 mg/m² fludarabine over five days was moderate neutropenia, no thrombocytopenia or severe infectious episodes. We observed all 9 of the fludarabine-treated patients responding to treatment with 2 complete remissions and 7 partial remissions, compared to 5 responders (all PR) in the control-arm. Furthermore, the effect on the blood circulating myeloma compartments identified a significant reduction of CD19+ B cells and myeloma plasma cells in the fludarabine-arm, concluding that fludarabine therapy in MM was feasible with a potential clinical efficacy. However, in the current trial, unexpected side effects were initially observed which may explain the drop-out of the 6 patients (Table 1) before the end of induction therapy.

Toxicity and adverse events following induction phase I

In accordance with the CTC criteria, no difference in severe toxicity was found. However, analysis of laboratory quantities following the second treatment showed a borderline reduction of blood lymphocytes from mean 1.12 (SD 0.4) to 0.73 (SD 0.6; p=0.055) and an increased plasma creatinine level from mean 57.8 (SD 14.2) to mean 124.2 (SD 28.8; p=0.035). All other variables registered showed no difference including performance score. Many clinical trials in CLL have shown the combination of fludarabine and cyclophosphamide to have tolerable toxicity,[13-15] however, the observed significant reduction in renal function may be due to latent myeloma specific kidney impairment. All serious adverse events were reported to The Trial Secretariat within one working day of discovery or notification of the event. Initial serious adverse event information and all amendments or addi-

Table 1. Number of treatment cycles during induction therapy (phase I).

Variable	Placebo (n) %	Intervention (n) %	p
No treatment	(0) 0.0	(3) 17.6	0.054
			(Mann Whitney test)
1 treatment	(0) 0.0	(3) 17.6	
2 treatments	(15) 88.2	(9) 52.9	
3 treatments	(2) 11.8	(2) 11.8	

Table 2. Fraction of patients subjected to harvest of an autograft (phase II).

Variable	Placebo (n) %	Intervention (n) %	p
Number of patients subjected to at least one harvest	(11) 64.7 %	(4) 23.5 %	0.037
Mean total harvest of CD34+ cells ×10⁶ ± SD	(11) 736±465	(4) 416±248	0.12
Mean average number of CD34+ cells ×10⁶ per harvest ± SD	(11) 584±501	(4) 251.2 (152.6)	0.071

Number of apheresis to collect >5×10⁶ CD 34+ cells/kg	Placebo (n)	Intervention (n)	p
No harvest session/mobilization failure	(6) 35.3%	(13) 76.5%	0.11
1 harvest session	(7) 41.2%	(02) 11.8%	
2 harvest sessions	(2) 11.8%	(01) 05.9%	
3 harvest sessions	(2) 11.8%	(01) 05.9%	

tions were recorded on the Adverse Event Form. This was reviewed and documented that CMV-reactivating was seen in one patient in the standard arm and 3 patients in the experimental arm.

Priming for stem cell mobilization and harvest during phase II

Fifteen of 17 patients and 12/17 were primed with standard care cyclophosphamide and rhG-CSF in arms A and B, respectively. Successful mobilization to reach the level of >10 CD34⁺ cells per microliter blood triggered leukapheresis and was obtained in 11/17 patients in arm A but only in 4/17 patients in arm B (Table 2). This difference was significant at the interim analysis performed by an independent group of experts and the decision was taken to stop the trial. Comparison of the total and average number of CD34⁺ cells harvested did not reveal differences in patients actually undergoing apheresis. The situation concerning published data about the adverse impact of fludarabine on stem cell harvest is now clearer. In a survey of advanced CLL from 122 centers of the European Group of Blood and Marrow Transplantation (EBMT), it was concluded that attention should be given to the timing of mobilization with respect to the last dose of fludarabine.[13] This has been further supported by a study of B-CLL after front-line treatment with fludarabine (30 mg/m² per day) and cyclophosphamide (200 mg/m² per day) both given orally for five consecutive days in six monthly courses. After evaluation performed two months after the last course, responding patients were considered for PBPC collection. Following conventional rhG-CSF, priming until adequate blood CD34 circulation was achieved and a harvest procedure was initiated successfully in only 12 of the 32 CLL patients.[14]

The present report supports this observation and further adds to our knowledge that as few as one to two series of fludarabine may result in poor mobilization and impair standard therapy. Recently, the stem cell toxicity has been supported by the observation that the risk for sMDS/AML was correlated to the use of fludarabine based on an unknown mechanism.[15]

Response evaluation following phase II

Response evaluation performed following phase I-II prior to high-dose therapy revealed 7/13 and 5/7 patients obtained a partial remission. The trend towards more patients achieving CR in the intervention arm observed in our previous phase II study could not be confirmed in this trial (*results not shown*). There was no difference in graft quality, evaluated by time to neutrophil and platelet recovery. One patient died from treatment complication in the experimental arm due to protocol violation: the patient had impaired renal function and

received a full dose of fludarabine. This was reported and reviewed by the Danish Drug Agency. Follow-up in December 2008 revealed that 12/13 and 9/13 patients had responded to therapy with 4/13 and 3/13 obtaining CR at any time during follow-up. The number of patients dying from complications or progressive disease was 2/17 and 5/16, respectively, with no significant difference between the two arms.

Conclusions

In conclusion, the scheduled fludarabine dosage in two cycles combined with alkylating therapy impairs stem cell mobilization and standard therapy in young MM patients and should not be administrated up-front. This observation is in accordance with recent data from up-front therapy in CLL.

We are now left with the challenge of understanding the mechanisms of fludarabine responsible for the negative side effect on mobilization of normal hematopoietic progenitors, as well as the potential therapeutic effect on marrow and blood B cells in MM and other B-cell malignancies. This is of special interest as the myeloma cell hierarchy includes resistant circulating clonal memory B cells, which differ considerably from the classical end stage plasma cells, infiltrating the bone marrow. The pathophysiological significance of these cells is at present unknown, but hypothetically they may serve as "sleeping" myeloma stem cells responsible for and "feeding" post-treatment relapse and disease progression,[4,5] as studied by the Myeloma Stem Cell Network supported by the 6ᵗʰ FP from the EU.

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