

High, intermittent dose of all-trans retinoic acid in combination with  $\alpha$ -interferon for advanced multiple myeloma

**Retinoids appear to have anti-proliferative effects on multiple myeloma lines. All trans retinoic acid (ATRA) given at high dose according to an intermittent schedule has better pharmacokinetically sustained levels. We studied 10 patients with relapsed/refractory MM who were treated with ATRA and interferon (IFN). We failed to demonstrate that this combination has clinically significant activity. Interleukin-6 (IL-6) receptor was not downregulated by ATRA in the patients we studied. Significant hematologic and neurotoxicity were encountered. This regimen of intermittent ATRA and IFN is ineffective and poorly tolerated.**

Multiple myeloma is a fatal, progressive neoplasm characterized by growth and accumulation of malignant plasma cells. IL-6 has been shown to regulate growth of myeloma cells<sup>3,6,7</sup> and hence IL-6 signaling pathways are potential targets for new therapies. Sidell *et al.*<sup>11</sup> identified the anti-proliferative action of all trans retinoic acid (ATRA) on a myeloma cell line Af-10, as a result of down regulation of IL-6 receptor (IL-6R) and subsequent inhibition of IL-6 mediated autocrine growth. Ogata *et al.*<sup>10</sup> demonstrated dose-dependent growth inhibition of freshly isolated myeloma cells by ATRA. Pharmacokinetic studies have revealed a rapid and sustained decrease in plasma drug concentrations when ATRA is administered on a continuous daily schedule.<sup>12</sup> An intermittent schedule of ATRA administration results in repetitive periods of exposure to concentrations of ATRA normally only observed on the first day of treatment with a continuous schedule.<sup>10,12,1</sup> Interferon- $\alpha$  (IFN- $\alpha$ ), retinoids, and steroids have been noted to have *in vitro* anti-proliferative synergistic effects on different cancer cell lines, including myeloma.<sup>13</sup> We therefore initiated a phase II trial to 1) evaluate the biological and clinical roles of high intermittent dose ATRA in refractory multiple myeloma patients, and 2) determine whether there is a role for IFN- $\alpha$  in combination with ATRA in the management of this disease.

The study was conducted between November 1996 and August 1997 at the Cleveland Clinic Taussig Cancer Center; ten patients were enrolled. Multiple myeloma patients who failed to respond to at least two prior regimens and were experiencing progressive disease were eligible. Responses were based on SWOG criteria. ATRA was given as a one time dose of 190 mg/m<sup>2</sup> orally  $\times$  7 days on odd weeks; this dose was selected because it was the maximum tolerated dose (MTD) in a phase I adjuvant trial conducted in breast cancer patients at our institution.<sup>4</sup> Since IFN- $\alpha$  appears to be most effective against dormant myeloma cells as shown by *in vitro* studies,<sup>13</sup> our objective was to achieve a dormant phase by administering two cycles of ATRA (study weeks 1, 3) then adding IFN- $\alpha$  at a dose of 3 mU/m<sup>2</sup> SQ TIW in combination with ATRA at study week 4.

ATRA was given over a 6-month period (12 cycles) then patients were maintained on IFN- $\alpha$  alone until disease progression or unacceptable toxicity. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed on mononuclear cells from the peripheral blood and the bone marrow for expression of the GAPDH gene (housekeeping gene), IL-6R and IL-6 genes before initiating ATRA and on day 5 of the first week of ATRA therapy. This timing was based on pharmacokinetics of ATRA used in this schedule.<sup>4</sup> Toxicity was assessed every 4 weeks and graded using NCI Common Toxicity Criteria

This study was designed as a two-stage phase II trial, in which an objective response of  $>20\%$  would justify further study of this regimen and a response of  $<5\%$  would indicate lack of efficacy. Accrual to the first stage was stopped early due to unacceptable toxicity and lack of meaningful response.

**Table 1. Patients' characteristics.**

Gender	N	Mean	Range
Male	6		
Female	4		
Performance status			
0	2		
1	8		
Age	10	54 (Y)	32-77
Time on study	10	116.9 (D)	11-260

**Table 2. Toxicity of the two regimens.**

	Grade 1	Grade 2	Grade 3	Grade 4
GI	5	1	1	0
Hematologic	0	3	4	3
Bleeding	6	0	1	0
Neurologic	2	6	1	1

Ten patients were treated (six males, four females). Two patients had ECOG grade 0 performance status and the other 8 patients had ECOG grade 1 performance status. One patient had non-secretory MM; the other nine patients secreted the following proteins:  $\lambda$  light chain (n=1), IgG $\kappa$  (n=2), IgG $\lambda$  (n=3) and IgA $\kappa$  (n=3) (Table 1).

Eight patients completed the ATRA alone part of the schedule. Two patients did not complete the ATRA alone part of this study (4 courses); these patients withdrew consent because of toxic side effects after receiving one and two courses of ATRA. Two patients were removed from the study because of disease progression. The remaining six went on to receive at least one week of IFN- $\alpha$  (median 16 courses, range 4-26). Of these six only two remained in the study long enough to receive IFN- $\alpha$  alone as maintenance therapy. The median treatment duration of all ten patients was 3.5 months (range 0.4 to 8.6 months).

Nine patients required dose reductions because of ATRA toxicity. Seven required dose reduction during the initial ATRA alone phase of the study (25% reduction in five, 50% reduction in two patients). The remaining two patients required a 25% dose reduction 8 and 9 weeks after the initiation of IFN- $\alpha$  therapy.

All patients were evaluable for toxicity. Grade 3-4 toxicities seen during these four courses of ATRA were neurologic, hematologic, and gastrointestinal (Table 2). Eight of the ten patients experienced some degree of thrombocytopenia, which was severe in four of them. Severe thrombocytopenia occurred during the first four courses of ATRA in three of these four patients, and in one patient after six months of therapy; all four had a baseline platelet count of less than 125,000  $\mu$ l. No evidence of coagulopathy or marrow failure was noted.

All patients were evaluable for response. Two patients progressed quickly (11 and 16 days after starting treatment). One patient had a transient response three days after adding IFN- $\alpha$  to the regimen, only to have recurrent disease in three weeks. The remaining seven patients remained stable for a median of 4.7 months before their disease progressed.

All patient samples that expressed GAPDH also expressed IL-

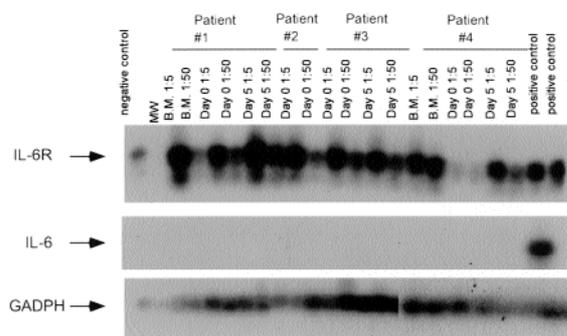


Figure 1.

6R, but not IL-6, both before and five days after the initiation of ATRA. We failed to demonstrate down regulation of IL-6R by ATRA on the peripheral blood or bone marrow mononuclear cells (Figure 1).

We have shown in this study that although ATRA has been effective *in vitro* against myeloma cells,<sup>10,13</sup> there were no significant responses in our group of patients with refractory/advanced disease. ATRA has been tested at low continuous doses in myeloma patients without response.<sup>15,14</sup> According to the pharmacodynamics of ATRA, the low dose continuous therapy could have been responsible for the lack of efficacy noted in these studies.

Despite our design of high, intermittent dose ATRA, the best result of therapy was stable disease for a median of 3.5 months and a short-lived response in a patient with multiple plasmacytomas. The addition of IFN- $\alpha$  has been shown to have activity in the management of myeloma.<sup>9</sup> Musto *et al*<sup>8</sup> treated 10 patients with refractory multiple myeloma with IFN- $\alpha$ , ATRA, and dexamethasone. Their results are in agreement with the responses noted with high dose Decadron as a single agent.<sup>5</sup> The contribution of ATRA to these results is difficult to judge. At full ATRA dose, significantly more toxicity was noted in our myeloma patient than in breast cancer patients in whom the intent of therapy was adjuvant. We could not demonstrate any added benefit derived from adding IFN- $\alpha$  to ATRA, although IFN- $\alpha$  did not add significant toxicity to the regimen. Following 5 days of therapy with ATRA, RT-PCR of peripheral lymphocytes and the mononuclear bone marrow cell population showed persistent IL-6R expression in all patients. We therefore failed to confirm that this dose and schedule of ATRA achieve biologic effects *in vivo*, despite the reported *in vitro* ones.<sup>11</sup>

Considering the significant incidence of hematologic toxicity, especially symptomatic thrombocytopenia, in our trial, we recommend that future studies in this field carefully monitor patients with platelet counts <125,000/ $\mu$ L. We conclude that high intermittent dose ATRA in advanced myeloma patients was not effective but was toxic. Adding IFN- $\alpha$  did not confer any advantage in terms of response.

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