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ALL-TRANS RETINOIC ACID IN COMBINATION WITH α-INTERFERON AND DEXAMETHASONE FOR ADVANCED MULTIPLE MYELOMA

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ABSTRACT

The *in vitro* inhibitory effect of all-trans retinoic acid (ATRA) on myeloma cell growth may be synergistically potentiated by the activity of dexamethasone (DEX) and α-interferon (IFN). We treated 10 patients with advanced, refractory multiple myeloma (MM) using a combination of ATRA (100 mg p.o., once a day for two weeks every month), DEX (40 mg i.v., for 4 days every 4 weeks) and IFN (3 MU s.c., three times a week). Eight patients completed at least three months of treatment and were evaluable for response. Two of them showed a partial response which persists after 15 to 17 months. Three patients experienced a stable *plateau* phase of 4 to +11 months, with a significant improvement in the performance status and bone pain. Progressive disease was seen in the remaining three patients. We conclude that the association of ATRA, DEX and IFN warrants further consideration in MM patients.

Key words: multiple myeloma, all-trans retinoic acid, dexamethasone, interferon, interleukin-6

All-trans retinoic acid (ATRA) has a pivotal role in the treatment of acute promyelocytic leukemia. It has been demonstrated recently that ATRA also exerts inhibitory effects on the *in vitro* cell growth of human IL-6-dependent myeloma cells by lowering the expression of the IL-6 receptor (IL-6R) and/or its signal transducer, gp-130, and by inhibiting IL-6 production by both myelomatous and stromal cells.[1-3] In addition, ATRA is a growth inhibitor of various IL- 6 independent myelomatous human cell lines, thus suggesting the possibility of mechanisms other than IL-6 modulation.[4] Despite the promising results obtained *in vitro*, we and other investigators have failed to demonstrate the usefulness of ATRA alone in patients with advanced multiple myeloma (MM) in three small phase I/II clinical trials.[5-7]

α-interferon (IFN) and high-dose dexamethasone (DEX) are effective drugs for MM patients. Recent studies suggest that IFN may modulate ATRA pharmacokinetics by down-regulating catabolic mechanisms of ATRA clearance (i.e. by inhibition of cytochrome P-450 enzymes)[8] and that ATRA and IFN synergistically inhibit myeloma cell growth *in vitro*.[4,9] Furthermore, ATRA and DEX exert synergistic antiproliferative activity on cultured myeloma cells through modulation of the IL-6 autocrine loop at the level of both the cytokine and its receptor.[10] Moreover, ATRA appeared to sensitize myelomatous cell lines to the apoptotic effect of DEX.[11]

Based on these data, we started a pilot study to evaluate the *in vivo* effects of the combination of ATRA, IFN and DEX in patients with advanced MM.

Patients and Methods

Ten patients were selected for this study following informed, written consent. Their main clinical and laboratory characteristics are depicted in Table 1. All patients suffered from advanced, stage III A MM and were resistant (as defined by no reduction or even an increase in bone marrow plasma cell infiltration and M-component) after at least three cycles of conventional treatment. Eight of them had previously received 2 to 4 lines of chemotherapy, while two subjects were unresponsive to first-line treatment. Mean time from diagnosis was 38.1 months (range 7-68).

The treatment schedule was the following: ATRA (Italian Institute of Vitamins) 100 mg p.o., once a day, for 2 weeks a month; DEX (Soldesam F, Laboratorio Farmacologico Italiano, Saronno, Italy) 40 mg i.v., for 4 days every 4 weeks; IFN (Intron-A, Schering-Plough, Milan, Italy) 3 mega-units s.c., three times a week. Serum levels of IL-6 (Biokine IL-6 Test Kit, T-cell Diagnostics, Woburn, USA) and soluble IL-6R (Quantikine IL-6 sR, R&D Systems, Abingdon, UK) were also monitored monthly by means of immunoenzymatic assays, according to the manifacturers' instructions.

Results

Table 2 summarizes the results of the study.

One patient interrupted the trial early (after two weeks) because of nausea and abdominal pain. Mental depression and severe stomatitis occurred in 2 female patients after 3 and 4 cycles, respectively. Another patient refused further treatment after the first cycle, in the absence of relevant side effects. Hematological toxicity higher than grade 1

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Table 1. Clinical characteristics of 10 MM patients treated with the combination ATRA/DEX/IFN.

Pt.	Sex	Age	M-COMP (g/dL)	% BMPC	Lines CHT (last CHT)	Time from diagnosis (mos.)
1) FR	M	63	IgGk (4)	65	3 (DEX)	44
2) MMG	F	67	IgGk (4.2)	15	3 (VCR/DEX/IFN)	34
3) DG	F	63	IgGk (4.5)	55	4 (DEX/IFN)	36
4) MP	M	62	IgGλ (2.5)	20	4 (IDC/DEX)	48
5) PA	F	79	IgGk (3.1)	45	2 (MPH/PDN)	59
6) FD	M	62	IgGk (3.9)	5	3 (M-90)	28
7) DA	F	46	λ (n.a.)	35	4 (MPH/PDN)	24
8) MA	F	84	IgGk (3.7)	15	1 (MPH/PDN)	33
9) DG	F	67	IgAk (1.7)	40	1 (IDC/DEX)	7
10) CG	M	74	IgGk (7.1)	62	3 (VMD)	68

BMPC: bone marrow plasma cells; CHT: chemotherapy; DEX: high-dose dexamethasone; IFN: alpha-interferon; VCR: vincristine; IDC: intermediate-dose cyclophosphamide; MPH: melphalan; PDN: prednisone; M-90: rotation of IDC/DEX, modified EDAP and VAD; VMD: vincristine, mitoxantrone, dexamethasone; n.a. not available.

Table 2. Results of treatment with the combination ATRA/DEX/IFN in the 10 advanced MM patients.

Pt.	N. cycles ATRA/IFN/DEX	Side effects	Response
1. FR	6	no	Plateau phase maintained for 4 months, then progression
2. MMG	8	no	25% reduction of M-component; reduction of BMPC from 15% to 2%; stable at + 17 months
3. DG	1	Nausea and abdominal pain	Early interruption due to side effects
4. MP	8	no	32% reduction of M-component; reduction of BMPC from 20% to 7%; stable at+ 15 months
5. PA	3	Stomatitis	no
6. FD	3	no	no
7. DA	1	no	Refusal of further treatment
8. MA	8	no	Stable plateau phase at + 11 months
9. DG	4	Mental depression	Plateau phase maintained for 7 months, then progression
10. CG	4	no	no

WHO was never seen (data not shown).

Eight patients completed at least three months of treatment and were evaluated for response (M-component and bone marrow plasma cell percentage at bone biopsy) at that time. However, for those patients who continued the treatment, the final assessment of response was made after six cycles.

Two patients showed M-component reduction of 25% and 32%, respectively, with a concomitant decrease of bone marrow plasma cells. These partial responses still persist after 15 and 17 months, respectively. Three subjects experienced a stable plateau phase for at least 4 months, with significant improvement in the performance status and bone pain, as evaluated by WHO scores (data not shown). No response, on the other hand, was seen in the remaining three patients, who received three cycles of ATRA/DEX/IFN.

No evidence of hypercalcemia was observed. Baseline serum levels of IL-6 and soluble IL-6R were found to be within the normal range in 5 patients and increased in the remaining 3 subjects. No significant change was observed in these molecules during the ATRA/DEX/IFN treatment (data not shown).

Discussion

The first trials exploring the *in vivo* activity of ATRA in patients affected by MM, including our own experience, were disappointing.[5-7] Indeed in these clinical studies relevant side effects, progression of the disease, development of hypercalcemia and an increase of circulating IL- 6 serum levels were frequently observed. Thus, though the explanation of this discrepancy with *in vitro* results is still not known and the number of patients treated in limited, it seems that ATRA alone is not only ineffective in advanced MM but also potentially dangerous.

By contrast, in keeping with *in vitro* studies that demonstrate a possible synergistic effect of ATRA in combination with DEX[10,11] or IFN,[4,8,9] our preliminary *in vivo* results suggest that this combination of drugs may be of some benefit in a certain proportion of patients with advanced MM resistant to conventional chemotherapy schemes. It is noteworthy that good, though often temporary, control

of the disease was obtained in 4 out of 7 patients refractory to or in progression after treatments including DEX (or even DEX plus IFN in one case) (Table 1). Furthermore, episodes of hypercalcemia, possibly caused by the increased production of IL-6 observed under ATRA alone,[6,7] were not seen during our study. This suggests that the adjunct of DEX and IFN may positively influence the *cytokine balance* in MM patients treated with ATRA.[11]

The relevant side effects observed in three patients pose some considerations about the feasibility and tolerability of this therapeutic program. Indeed one would expect that undesired effects due to different drugs (such as mental depression for IFN, or gastro-intestinal troubles related to ATRA) might accumulate in a trial based on biological rather than cytostatic agents. Whether this fact actually represents a limit in the clinical setting remains to be established.

Long-term ATRA treatment induces a decrease in plasma drug levels.[12] This fact could explain the acquired resistance to ATRA in acute promyelocytic leukemia, despite retention of sensitivity *in vivo*. Intermittent dosing of ATRA, as was adopted in the present study, affects ATRA pharmacokinetics thereby allowing prolonged effective plasma drug concentrations.[8] Together with the postulated synergistic effect of DEX and IFN, this may have contributed to the more favorable results obtained in these MM patients with respect to others treated with ATRA alone.[7]

In conclusion, we believe that the combination of ATRA with DEX and IFN warrants further consideration in larger, possibly randomized studies in patients affected by MM. Moreover, on the basis of recent evidence,[3] the role of cis-retinoic acid, alone or in combination with other cytokines (i.e. γ-interferon), also needs to be investigated in MM patients.

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