The pharmacology of cell differentiation
R A Rifkind, editor


# SYNERGISM BETWEEN DIFFERENTIATION AGENTS : PRECLINICAL AND CLINICAL STUDIES IN MYELOID LEUKAEMIA

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Since the late the 1940s leukaemia therapy has focused on the development of cytotoxic and cytostatic agents . In recent years , the large number of publications describing the induction of differentiation of cultured leukaemia cells that has appeared , underscores the growing importance of this area of leukaemia research and offers the prospect of a novel therapeutic strategy for human leukaemia . The treatment of human leukaemia is usually approached by the combined use of two or more antileukaemic agents . Also , with the exception of all-trans retinoic acid in patients with acute promyelocytic leukaemia , the therapeutic effects of differentiation agents when used singly are rather weak . The present article reviews the preclinical and clinical studies of synergism between differentiation agents in myeloid leukaemia and summarises the recent useful informations contributing to the progress at this new frontier of leukaemia therapy .

## Introduction

Although the major clinical trials have achieved more than 75% remission rate in younger acute myeloid leukaemia (AML) patients with conventional cytotoxic chemotherapy , elderly patients receive little or no treatment because such intensive therapy is unsuitable [1] . Recovery from myelotoxicity , an almost universal complication of antineoplastic chemotherapy , is impaired in elderly humans due to depletion of normal haemopoietic stem cells , reduced production of haemopoietic growth factors , impaired response to humoral or stromal stimulation and/or microenvironmental dysfunctions [2] . As the majority of AML patients are over 65 years of age , there is a pressing need for another form of treatment with could effectively control the disease in this age group [3] .

Differentiation therapy provides a suitable alternative that does not incur an unreasonable risk of shortening the elderly's life or make it unbearable[2] . The identification of synergistic combinations of differentiation inducing agents which can induce maximum differentiation of human myeloid leukaemic cells *in vitro* , could provide effective theraputic regimens for these elderly AML patients .

**Spontaneous *in vitro* differentiation and complete remissions of myeloid leukaemia**

Several studies showed some AML cases with spontaneous *in vitro* differentiation in various primary cultures [4-11] . The mechanisms that brought about this spontaneous *in vitro* differentiation include : (1) intrinsic : involving the autocrine and paracrine production of haemopoietic growth factors by AML cells and/or (2) extrinsic : involving the haemopoietic growth factors present in the various conditioned media added to the primary cultures [11] . These haemopoietic growth factors can induce directly and/or indirectly the spontaneous on *vitro* differentiation through stimulating commitment divisions . Also , a positive correlation between the spontaneous in *vitro* differentiation of AML cells and the maintenance of viability in primary cultures was demonstrated , suggesting that AML cells undergo this spontaneous in *vitro* differentiation as a means of surviving in an adverse environment [10] .

Evidence for the leukaemic origin of the differentiated cells in primary cultures included the retention of Auer bodies and other dysplastic features in the differentiated cells and the correlation of specific types of mature cells appearing in primary cultures with the FAB subtype of their AML cells [4-11] . Spontaneous complete remission up to 34 months was observed in very few AML patients following infections and/or blood transfusions [12-17] and also after termination of pregnancy [18,19] . The AML cells in these patients showed a weak proliferative activity [15] and therefore could differentiate *in vivo* more easily in response to the haemopoietic growth factors produced by leucocyte transfusion and/or mycobacteria . This phenomenon was supported by Metcalf's findings that the sera from AML patients who had infections , induced in *vitro* differentiation of HL-60 human myeloid leukaemic cells [20] .

These spontaneous *in vitro* differentiation and complete remissions of myeloid leukaemia imply that the differentiation process in myeloid leukaemia patients is not absolutely blocked but simply insufficient .

**Synergism in inducing differentation of human myeloid leukaemic cell lines**

Numerous studies have identified many synergistic combinations in inducing differentiation of human myeloid leukaemic cell lines including HL-60 , U937 , ML-1 , KG-1 and K562 (Table 1) . However , limits exist to the comparability of myeloid leukaemic cells from patients with these cultured cell lines . After continuous passage for extended durations, cell lines develop additional chromosomal changes, alter thier growth properties and gene expression, and change their response to differentiation inducing agents [21 , 22] . Also , each cell line represents a single population of myeloid leukaemic cells, whereas cells from AML patients are heterogeneous . Moreover , such cell lines are at a more maturational stage than myeloid leukaemic cells from patients and may be primed for differentiation in culture . Therefore caution should be exercised when extrapolating results from these cell lines for prospective clinical trials of differentiation therapy in myeloid leukaemic cells .

**Synergism in inducing differentation of myeloid leukaemic cell from patients in primary culture**

Several studies have demonstrated synergy between all-trans retinoic acid and other agents in inducing differentiation of myeloid leukaemic cells from most AML patients except those from patients with the FAB subtype M1 in primary culture (Table 1) . Also , the combinations of cytarabine with polar-planar compounds were synergistic in inducing differentiation of cells from many AML patients in primary culture [11] . Moreover , the time of transit along the differentiation pathway of AML cells become shorter with the increase in the concentration of and/or the number of differentiation inducing agents in primary culture [11] . The induced differentiation was related not only to the concentration of and/or the number of these agents but also to the karyotype of AML patients [11] . Cells from AML patients with the karyotypes : inv (3) and + 22 , which are usually associated with resistance to

**Table 1**
Synergistic combinations of differentiating agents in human myeloid leukaemic cells .

| Human myeloid leukaemic cells | Synergistic combination | Reference |
|---|---|---|
| -HL-60 cells : | Retinoic acid+Interferons | 31 |
| | Retinoic acid+Tumour necrosis factor-alpha | 32 |
| | Retinoic acid+Prostaglandin E | 31 |
| | Retinoic acid+Dibutyryl cAMP | 31 |
| | Retinoic acid+Aphidicolin | 33 |
| | Retinoic acid+Verapamil | 34 |
| | Retinoic acid+Trypsin | 35 |
| | Retinoic acid+Phorbol-myristate acetate (TPA) | 36 |
| | Retinoic acid+Hydroxyurea | 37 |
| | Retinoic acid+Cytarabine | 38 |
| | Retinoic acid+Ouabain | 39 |
| | Retinoic acid+Sodium butyrate | 40 |
| | Retinoic acid+Dimethylsulphoxide | 40 |
| | Retinoic acid+Hexamethylene bisacetamide (HMBA) | 40 |
| | Retinoic acid+Interleukin-6 | 41 |
| | Retinoic acid+Dimethylsulphoxide+5 - azacytidine | 42 |
| | Gamma interferon+Tumour necrosis factor-alpha | 43 |
| | Gama Interferon+ 5-Fluorouracil | 44 |
| | Vitammin-D3+Transforming growth factor-beta | 45 |
| | Cytarabine+5-azacytidine | 46 |
| | Cytarabine+Hexamethylene bisacetamide | 47 |
| -U937 cells : | Retinoic acid+Interferons | 48 |
| | Retinoic acid+Tumour necrosis factor-alpha | 32 |
| | Retinoic acid+Trypsin | 38 |
| | Retinoic acid+Interleukin-6 | 49 |
| | Vitamin-D3+Interferons | 48 |
| | Vitamin-D3+Tumour necrosis factor-alpha | 43 |
| | Vitamin-D3+Recombinant human GM-CSF | 50 |
| | Gamma interferon+Interleukin-6 | 51 |
| | Interleukin-1+Interleukin-6 | 52 |
| | Prostaglandin E+Tumour necrosis factor-alpha | 53 |
| -ML-1 cells : | Tumour necrosis factor-alpha+Gamma interferon | 54 |
| | Tumour necrosis factor-alpha+TPA | 55 |
| | Tumour necrosis factor-alpha+Interleukin-6 | 56 |
| | Transforming growth factor-beta+TPA | 55 |

**Table 1**
Synergistic combinations of differentiating agents in human myeloid leukaemic cells . **(continued)**

| Human myeloid leukaemic cells | Synergistic combination | Reference |
|---|---|---|
| -KG1 cells : | Recombinant human GM-CSF+Cytarabine | 57 |
| | Recombinant human G-CSF +Cytarabine | 57 |
| -K562 cells : | Recombinant human GM-CSF+Erythropoietin | 58 |
| | Cytarabine+Hemin | 59 |
| -AML cells in primary culture : | Retinoic acid+6-thioguanine | 60 |
| | Retinoic acid+Cytarabine | 61 |
| | Retinoic acid+Dimethylsulphoxide | 11 |
| | Retinoic acid+Dimethylformamide | 62 |
| | Retinoic acid+HMBA | 63 |
| | Retinoic acid+Alpha-interferon | 64 |
| | Retinoic acid+Gamma interferon | 65 |
| | Retinoic acid+Vincristine | 66 |
| | Retinoic acid+Recombinant human G-CSF | 67 |
| | Retinoic acid+Cytarabine+Dimethylsulphoxide | 11 |
| | Retinoic acid+Cytarabine+Dimethylformamide | 11 |
| | Retinoic acid+Cytarabine+HMBA | 63 |
| | Gamma interferon+Tumour necrosis factor-alpha | 68 |
| | Cytarabine+Dimethylsulphoxide | 11 |
| | Cytarabine+Dimethylformamide | 11 |
| | Cytarabine+Hexamethylene bisacetamide | 11 |

HL-60 : human promyelocytic leukaemia cells, U937: human monoblastic leukaemia cells, ML-1: human myeloblastic leukaemia cells, KG-1: human myeloblastic leukaemia cells, K562: human erythroleukaemia cells.

treatment with conventional cytotoxic chemotherapy [23] , were resistant to the various combinations of differentiation inducing agents in primary culture [11] .

The combinations of differentiation inducing agents with all-trans retinoic acid resulted in marked reductions in the doses of each agent that were required in combinations to achieve the same differentiation effect as single agents [11] (Table 2) . The decreases were reflected in dose reduction index values of 10.7 for cytarabine , 5.0 for dimethylformamide , 4.5 for hexamethylene bisacetamide and 3.7 for dimethylsulphoxide [11] (Table 2) . The dose reduction index indicates how many folds of dose reduction are required to achieve a given effect in combination compared with each agent alone and is calculated by dividing the $ED_{50}$ value for a single differentiation agent by the dose of the same agent required in combination with another agent to produce the same effect [24] .

The doses of hexamethylene bisacetamide that were required in triple combinations with all-trans retinoic acid plus cytarabine to achieve the same differentiation as single agent , were reduced about 8 fold (Table 2) . The doses of cytarabine that were required in triple combinations with all-trans retinoic acid plus a polar-planar compound to achieve the same differentiation as single agent , were markedly reduced about 20-fold (Table 2) .

**Potential clinical benefits of synergistic differentiation therapy for myeloid leukaemic patients**

The potential clinical benefits of synergistic differentiation therapy in myeloid leukaemic patients include :

**1-** Synergistic combinations of differentiation inducing agents could overcome the inherent heterogeneity in myeloid leukaemic patients . Heterogeneity , which is a striking feature of myeloid leukaemias has three dimensions : a) patient-to- patient variation that is seen conspicuously in the survival curves for AML patients treated with chemotherapy in clinical trials, b) Cellular heterogeneity in AML clones which develop during the expression of leukaemic clones, and c) Self-renewal heterogeneity in AML clones: whereas self-renewal capacity is almost absent in progenitor cells from most AML patients, only in few patients is the self-renewal extensive [25]. The synergistic differentiating agents, by impinging on the

differentiation process via different routes at once, can cater for the insufficient activity in a complementary fashion [26] .

**2** - Synergistic combinations of differentiation inducing agents could clinically not only increase the duration of effective drug levels *in vivo* but also limit the toxicity by providing shorter and less toxic courses of treatment in elderly myeloid leukaemic patients .

**3** - Synergistic combinations of differentiation inducing agents could provide an effective maintenance therapy which could increase the remission duration in myeloid leukaemic patients by preventing the regrowth of the residual leukaemic cells remaining after remission induction therapy .

**4** - Synergistic combinations of differentiation inducing agents could provide an effective post bone marrow transplantation therapy which could eliminate the residual myeloid leukaemic cells remaining after transplantation by inducing their differentiation into non-dividing mature cells [27] .

**5** - Synergistic combinations of differentiation inducing agents could overcome the emergence of resistant myeloid leukaemic cells. The combination of low concentration of an antileukaemic drug with differentiation inducing agents caused the resistant myeloid leukaemic cells to become sensitive to several differentiation inducing agents *in vitro* and enhanced their therapeutic efficacy *in vivo* [28] .

**6** - Several differentiation inducing agents including polar planar compounds and low concentration of antileukaemic drugs stimulate normal human myeloid cell differentiation [29, 30]. Therefore, synergistic combinations of these agents could reduce the duration of post-chemotherapy neutropenia and accelerate the restoration of normal myelopoiesis by stimulating normal human myeloid cell differentiation.

**7** -Synergistic combinations of cytarabine with polar planar compounds such as dimethylsulphoxide, dimethylformamide or hexamethylene bisacetamide which can cross the blood-brain barrier, could provide an effective therapy for myeloid leukaemic patients with CNS relapse by acting as a vehicle for cytarabine.

**Table 2**
Dose reductions by combinations of all-trans retinoic acid with cytarabine and/or polar-planar compounds

| Combination of differentiation-inducing agents | Dose reduction index* (folds) | | | | |
|---|---|---|---|---|---|
| | RA | Ara-C | HMBA | DMF | DMSO |
| RA+DMSO | 1.7** | | | | 4.5 |
| RA+DMF | 1.8 | | | 5.0 | |
| RA+HMBA | 1.6 | | 3.7 | | |
| RA+Ara-C | 1.5 | 10.7 | | | |
| Ara-C+DMSO | | 2.9 | | | 5.0 |
| Ara-C+DMF | | 3.4 | | 2.6 | |
| Ara-C+HMBA | | 2.6 | 3.1 | | |
| RA+Ara-C+DMSO | 2.3 | 20.2 | | | 8.8 |
| RA+Ara-C+DMF | 2.4 | 19.3 | | 4.6 | |
| RA+Ara-C+HMBA | 2.3 | 25.7 | 8.1 | | |

RA: all-trans retinoic acid, Ara-C: cytarabine, HMBA:hexamethylene bisacetamide, DMSO: dimethylsulphoxide, DMF:dimethylformamide.

*The number of folds of dose reduction needed to achieve 50% differentiation in combination compared with each differentiation inducing agent alone. This was calculated by dividing the $ED_{50}$ value for a single differentiation inducing agent by the dose of the same agent required in combination with another agent (s) to produce the same effect.

** Mean of triplicate determinations of dose reduction indices for morphological, cytochemical and functional differentiation of AML cells in primary culture with the standard deviation for each value not exceeding 8% of the mean [11] .

**Synergistic Differentiation Therapy with Interferon-alpha plus low dose Cytarabine in Acute Monoblastic Leukaemia patients :**

Acute monoblastic leukaemia (AMoL) represents a unique subtype of acute myeloid leukaemias with a frequency of occurrence in childhood of about 20% [69]. Hyperleucocytosis, extramedullary organ involvement, haemorrhage, early deaths, high remission induction failure rates, high relapse rates and karyotypic abnormalities at the q23 band of the long arm of chromosome no.11 are common features in children with AMoL [69] . Gradual rather than sudden leukaemic cell reduction using cytostatic treatment with hydroxyurea or low dose cytarabine is usually recommended [70] .

Interferon secretion appeared to be important in monocyte differentiation, induced synergistically with cytarabine in vitro differentiation of human monoblastic leukaemia cells [71,72] and also the juxtaposition of the interferon type 1 and c-ets-1 genes may be involved in the pathogenesis of the differentiation defect in AMoL patients with t (9; 11) (p22;q23) [73]. In view of these experimental results and because of the sustained complete haematologic and cytogenetic remissions achieved in about 75% and 20%, respectively of recombinant human interferon-alpha-treated chronic myeloid leukaemia patients [74-78] we performed a clinical pilot study to examine the efficacy of recombinant human interferon-alpha plus low dose cytarabine with or without hydroxyurea in children with acute monoblastic leukaemia.

Six children with untreated primary acute monoblastic leukaemia (three boys and three girls) were included in the present study after informed consents had been obtained from their parents. Their median age was 9.8 years (range, 7.5 - 13). All patients had normal cardiac, liver and renal functions and were diagnosed according to the French-American-British (FAB) criteria [79] usmg the following stains: leishman, sudan black B and dual chloroacetate and butyrate esterases as described before [80] . The haematological characteristics of the patients before and after treatment are shown in Table 3 .

Patients were treated with 3 million Units/ $m^2$/ day recombinant human interferon-alpha 2b (Intron A) subcutaneously three times per week plus 10 mg / $m^2$/ day cytarabine (Cytosar) subcutaneously daily for 21 days in the Department of Haematology of Medical Research Institute, University of Alexandria. In addition, 50 mg/ kg/ day Hydroxyurea (Hydra) orally daily for the first 10 days was prescribed for patients with initial leucocytic counts of 50 x $10^9$/L or more. Prophylactic paracetamol was given orally daily to suppress concomitant influenzal symptoms and fever. Also, prophylactic antibiotics including neomycin and cephalosporins as well as allopurine were given orally daily to prevent infections and urate nephropathy. Platelets or packed red blood cells transfusions were administered to maintain the platelet counts above 20 x $10^9$/L and haemoglobin above 8.0 g/d, respectively. An electrocardiogram, chest x-ray and neurological examination were performed weekly. Side effects were documented for each patient and toxicity was

graded according to the World Health Organisation (WHO) criteria [81] . On day 28, bone marrow aspiration samples were evaluated for cellularity and blast cell count and the complete and partial remissions were defined as described before [82]. In responding cases, a second 21-day course of interferon-alpha plus low dose cytarabine with or without hydroxyurea was administered until complete remission was achieved. Upon the achievement of complete remission, central nervous system prophylaxis with cranial irradiation was administered as described before [83] followed by maintenance therapy with 30 mg / $m^2$/ day low dose 6-thioguanine orally daily plus 50 mg/ kg/ day hydroxyurea orally daily for 7 days every three weeks. Regular evaluations of complete blood and platelets counts were done weekly and of bone marrow picture were done every 2 months. Patients who did not achieve complete remission after two 21-day treatment courses were taken off this therapy and received instead the established treatment regimen utilizing epodophyllotoxins (VP -16 plus VM-26) and intrathecal cytarabine as described before [84] .

Patients received two 21-day treatment courses except one patient (no.4) who achieved complete remission after one treatment course with hydroxyurea (Table 3) Complete remission was also achieved in two patients (no. 1 and 2) after two treatment courses with hydroxyurea and in one patient (no.6) after two treatment courses without hydroxyurea (Table 3) . At the present time, the four patients (no. 1,2,4 and 6) have sustained ongoing complete remission for a follow-up durations of 8 - 12 months. Two patients (no. 3 and 5) achieved only partial remission after two treatment courses (Table 1) and consequently were taken off this therapy and received instead the established treatment regimen utilizing epipodophyllotoxins (VP-16 plus VM-26) and intrathecal cytarabine as described before [84] which unfortunately also failed to achieve complete remission. In patient no.3, the second treatment course was given without hydroxyurea due to the worsening of thrombocytopenia during the first treatment course (less than 20 x $10^9$/L).

Treatment-associated side effects consisted predominantly of thrombocytopenia which required repeated platelet transfusions [median : 4 units per treatment course, range: 2- 7] (Table 4) . During the first week of therapy, four patients (no. 1, 2, 3 and 5) developed transient influenzal symptoms and mild fever (WHO I-II)

**Table 3**
Haematological characteristics of acute monoblastic leukaemia patients before and after treatment .

| Patient | BEFORE TREATMENT | | | | No.of treatment | AFTER TREATMENT | | | | **Treatment** |
|---|---|---|---|---|---|---|---|---|---|---|
| no. Age/Sex | Hb (g/dl) | WBC ($x10^9$/L) | Platelets ($x10^9$/L) | BM [FAB] blast cell% | Courses administered | Hb (g/dl) | WBC ($x10^9$/L) | Platelets ($x10^9$/L) | BM blast cell% | **Outcome** |
| **1.** 7/F | 12.1 | 168.0 | 41.7 | 93 [M5a] | 2 with HU | 13.7 | 4.9 | 169 | 3 | CR |
| **2.** 8/M | 7.0 | 135.1 | 12.1 | 79 [M5b] | 2 with HU | 11.4 | 2.7 | 187 | 2 | CR |
| **3.** 11/M | 9.2 | 92.0 | 11.2 | 83 [M5b] | 1 with HU + 1 without HU | 10.8 | 2.4 | 82 | 18 | PR |
| **4.** 9/F | 8.2 | 74.3 | 25.3 | 70 [M5b] | 1 with HU | 12.5 | 8.2 | 211 | 3 | CR |
| **5.** 10/F | 9.6 | 26.0 | 37.1 | 98 [M5a] | 2 without HU | 9.2 | 2.2 | 92 | 21 | PR |
| **6.** 13/M | 7.1 | 20.0 | 14.0 | 69 [M5b] | 2 without HU | 13.6 | 3.1 | 243 | 1 | CR |

Hb : haemoglobin, WBC:total leucocytic count, BM: bone marrow, FAB: French-American-British classification, HU: hydroxyurea, CR: complete remission, PR: partial remission .

(Table 4) . Also, in two patients (no. 3 and 6) a body weight loss of about 10% of pretreatment levels (WHO I-II) was observed, whereas one patient (no.4) had no side effects at all (Table 4) .

**Table 4**
Transfusion requirements and Side effects during therapy in acute monoblastic leukaemia patients .

| Patient no. | TRANSFUSION REQUIREMENTS (No. of Units per treatment course) | | TREATMENT-ASSOCIATED SIDE EFFECTS (WHO Grading System) | | |
|---|---|---|---|---|---|
| | Packed RBCs | Platelets | Fever* | Infleunzal Symptoms* | Weight Loss |
| **1.** | 1 | 3 | II | I | 0 |
| **2.** | 2 | 4 | I | I | 0 |
| **3.** | 2 | 5 | II | I | II |
| **4.** | 2 | 2 | 0 | 0 | 0 |
| **5.** | 2 | 7 | II | I | 0 |
| **6.** | 2 | 4 | 0 | 0 | I |

RBCs: red blood cells. * mainly during the first week of therapy .

The efficacy of recombinant human interferon-alpha 2b alone and in combination with chemotherapeutic drugs has been established in the treatment of patients with both chronic myeloid leukaemia (CML) [74-78] and chronic lymphoid leukaemia [85] . As acute monoblastic leukaemia is a malignant disease in which neither conventional intensive therapy with a multitude of chemotherapeutic agents nor bone marrow transplantation was beneficial [69] , it is justified in this disease to evaluate the efficacy of interferon-alpha that seems promising on the basis of a possible role for its juxtapositioned genes in the pathogenesis of the differentiation defect in some AMoL patients[73] , its regulatory effect in monocyte differentiation [71,72] and of its success in the treatment of chronic leukaemias [74 -78,85] .

The present results demonstrated the efficacy of interferon-alpha in combination with low dose cytarabine in inducing complete sustained haematologic remission in children with untreated primary acute monoblastic leukaemia (Table 3) . Also, hydroxyurea was effective in reducing the leukaemic mass and preventing leukostasis in acute monoblastic leukaemia patients with high leucocytic count of 50 $x10^9$/L or more, in line with previous experience with acute leukaemia patients [86] . Moreover, recently treatment with interferon-alpha plus hydroxyurea with or without low dose cytarabine was reported to achieve complete sustained haematologic and cytogenetic remissions in CML patients [77,78] . On the other hand, interferon-alpha showed only limited activity in relapsed and previously treated acute myeloid leukaemia patients using short courses of extremely high dose interferon ( more than 50 million units) [87-89], a setting where treatment failure is the rule.

Treatment with the triple combination of interferon alpha, low dose cytarabine and hydroxyurea was well tolerated, with thrombocytopenia comprising the main side effect which required repeated platelet transfusions particularly during the second treatment course. In the present study, thrombocytopenia in acute monoblastic leukaemia could be due to the disease's suppression of normal bone marrow megakaryopoiesis [11] and/or interferon-alpha which has been shown to suppress in vitro the proliferation of human bone marrow megakaryocyte progenitor cells [90] .

A large randomised clinical trial would be necessary to evaluate the beneficial effect of the present treatment regimen in comparison with established standard regimens in acute monoblastic leukaemia patients. In our ongoing clinical study, the recruitment of more untreated acute monoblastic leukaemia patients (only children aged 6 years or more and adults) and a follow-up for longer period would determine any survival advantage for treatment with interferon-alpha plus low dose cytarabine with or without hydroxyurea .

Interferon-alpha plus low dose cytarabine induced synergistic in vitro differentiation of human acute monoblastic leukaemia cells [72] ,therefore it would be interesting to investigate any possible in vivo differentiation in response to the treatment with their combination in acute monoblastic leukaemia patients using Bromodeoxyuridine labelling [91] and/or premature condensed chromosome [92] analyses. Also, cytogenetic and molecular biology studies are required to determine

whether or not the response to treatment with interferon-alpha is related to the presence of karyotypic abnormalities in the long arm of chromosome 11 at the q23 band and/or the involvement of c-ets-l and interferon type I genes in certain translocations in acute monoblastic leukaemia patients. Recently, treatment with interferon alpha has been shown to generate immunocytotoxic activities against leukaemic cells in chronic myeloid leukaemia patients [93,94], in whom these activities are usually defective [95,96].Thus, immunocytotoxic studies are warranted to examine any similar immunomodulatory effect of interferon-alpha in generating natural killer (NK) and/or lymphokine-activated killer (LAK) cells activities in acute monoblastic leukaemia patients,in whom these activities are also defective [96-98] .

In conclusion, the present study provides the basis for a prospective clinical trial of synergistic differentiation therapy comparing the recombinant human interferon alpha plus low dose cytarabine with or without hydroxyurea regimen with the established standard treatment regimens in acute monoblastic leukaemia patients.

**Conclusion**

The reversal of malignancy in myeloid leukaemia, while an attractive goal, is one that is rarely attainable in its entirety in clinical practice. What is more feasible and has proved to be attainable, is the removal of the differentiation block in the myeloid leukaemic clone, with the resulting development of varying features of lineage-specific differentiation. Synergistic combinations of differentiation-inducing agents, while falling short in their ability to reverse malignancy, can nevertheless be highly effective in suppressing the myeloid leukaemic cells capacity of self-renewal by inducing death by differentiation to a post-mitotic state. The superiority of a missionary (differentiating myeloid leukaemic cells to functional mature cells) over the crusadel approach (destroying leukaemic together with many normal cells) appeals to clinicians treating myeloid leukaemia patients.

More preclinical studies are required to identify the effect of the sequence of administration (simultaneous versus sequential) in the synergistic combinations of differentiation inducing agents on AML cells in primary culture. Also, future clinical trials designed to exploit the preclinical experience with these synergistic combinations are warranted.

**Acknowledgements**

Dr.H.T.Hassan is supported by a research fellowship from the Alexander von Humboldt Stiftung ,Bonn, Germany. The author dedicates this chapter to his loving wife Christina for her care and support .

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## Discussion - SYNERGISM BETWEEN DIFFERENTIATION AGENTS: PRECLINICAL AND CLINICAL STUDIES IN MYELOID LEUKAEMIA

M. Crescenzi

Have you attempted to establish estimates of reduction of tumour mass to see whether there is any correlation with protracted remissions or even cure in the long run?

H.T. Hassan

As far as the leukemic mass is concerned, in acute monoblastic leukemia it tends to be a bit greater than in the other subtypes of myeloblastic leukemias, so it became a sort of tradition for hematologists using conventional chemotherapy to try to reduce the leukemic cell mass. This is the reason why in this protocol of treatment which, hopefully, I could prove in vivo that it is based on differentiation therapy like the picture was in vitro, we adopted the same approach by trying to reduce the leukemic cell mass with hydroxyurea. Of course, hydroxyurea works as a cytotoxic agent in this case, not as a differentiating agent. In terms of cure or survival advantages, it is too early because the follow up of these children is not long enough, even to compare it with historical controls with other established chemotherapeutic protocols in previous trials.