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EFFECTS OF RECOMBINANT HUMAN INTERFERON-ALPHA, BETA AND GAMMA ON THE ANTIPROLIFERATIVE ACTIVITY OF CYTARABINE IN K562 HUMAN MYELOID LEUKEMIA CLONOGENIC CELLS

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SUMMARY

The effects of recombinant human interferon-alpha, beta and gamma (IFN) on the antiproliferative activity of cytarabine (ara-C) in K562 human myeloid leukemia clonogenic cells were studied in an agar capillary microassay. The addition of IFN-alpha did not affect the antiproliferative activity of ara-C in K562 cultures treated with low concentrations of ara-C: 10–50 nM, whereas in those treated with high concentrations of ara-C: 100 500 nM, IFN-alpha significantly reduced the antiproliferative activity of ara-C. The addition of 3×10^4 U/ml IFN-alpha to ara-C-treated K562 cultures increased the IC_{50} of ara-C on day 5 from 102 to 214 nM, i.e. ara-C + IFN-alpha was about twofold less potent than ara-C alone. Low concentrations of IFN-beta and IFN-gamma did not affect the antiproliferative activity of ara-C on K562 colonies, but high concentrations of these two interferons reduced the antiproliferative activity of ara-C. The addition of 4×10^3 U/ml IFN-beta or 10^4 U/ml IFN-gamma increased the IC_{50} of ara-C on day 5 to 304 nM or to 316 nM, respectively, i.e. ara-C + IFN-beta or IFN-gamma was about threefold less potent than ara-C alone. The significant reduction of the desired antiproliferative activity of ara-C by the three interferons was reproduced in liquid suspension cultures of K562 cells on day 4 in the following order: IFN-gamma > IFN-beta > IFN-alpha. The present negative interactions of the three interferons with ara-C particularly at high concentrations, may caution against the clinical use of the combination of ara-C and interferon in the treatment of myeloid leukemia patients.

KEY WORDS Cytarabine Interferon Myelocytic leukemia

INTRODUCTION

The human myeloid leukemia cell line K562, which was established from the pleural effusion of a chronic myeloid leukemia patient in blastic crisis,[1] provides a unique population of primitive myeloid leukemia cells.[2] Cytarabine is not only the most widely used drug in the treatment of myeloid leukemia,[3] but also the most effective agent in K562 human myeloid leukemia cells.[4] Several biological agents including interleukin-3, granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor and all-*trans* retinoic acid enhanced cytarabine activity in human myeloid leukemia cells.[5–7] Interferons (IFN) showed synergistic interactions with several chemotherapeutic agents in numerous non-leukemic tumour cell lines.[8] Also, mouse semi-purified alpha/beta-interferon was effective alone in increasing the survival time of mice inoculated

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intravenously with Friend erythroleukemia cells[9] and showed synergism with cisplatin in P388 murine leukemia in CDF1 mice.[10] Moreover, IFN-alpha[11] and IFN-gamma[12] showed synergistic antiproliferative effect with 5-fluorouracil in HL-60 human myeloid leukemia cells.

Recombinant human interferon-alpha 2b alone has achieved about 70 per cent complete sustained hematologic remission and 20 per cent complete sustained cytogenetic remission in patients with chronic myeloid leukemia.[13] Also, IFN-alpha has achieved objective responses in chronic myelomonocytic leukemia patients.[14,15] In addition, treatment with recombinant human IFN-gamma alone induced clinical response in two of four smoldering myeloid leukemia patients[16] and in 10 of 26 chronic myeloid leukemia patients.[17]

Therefore, we have investigated the effects of the combinations of IFNs: alpha, beta and gamma with cytarabine in K562 human myeloid leukemia clonogenic cells, in an effort to explore any synergistic or additive interactions which could provide the basis for an improved therapeutic benefit in myeloid leukemia patients.

MATERIALS AND METHODS

Materials

Agar, ara-C, L-glutamine, RPMI 1640 culture medium and trypan blue were purchased from Sigma Chemicals. Fetal calf serum (FCS) was purchased from Boehringer. Recombinant human IFN-alpha 2b (specific activity of 42×10^6 units per mg protein) was purchased from Hoffmann-La Roche. Recombinant human IFN-beta 2 (specific activity of 10×10^6 units per mg protein) and recombinant human IFN-gamma (specific activity of 25×10^6 units per mg protein) were purchased from Genzyme Corporation. Ara-C was diluted with RPMI 1640 medium to 1 mM stock solution and kept at $-20°C$. Prior to incubation, ara-C was freshly diluted with RPMI 1640 medium to working concentrations. IFNs were diluted with RPMI 1640 medium containing 10 per cent fetal calf serum to $0{\cdot}5 \times 10^6$ units per ml stock solution and kept at $-50°C$. Prior to incubation, IFNs were freshly diluted with RPMI 1640 medium containing 10 per cent FCS to the final concentrations.

Cells

K562 cells were grown in suspension in RPMI 1640 medium containing 10 per cent FCS in 10 ml in 25 cm^2 tissue culture flasks at 37°C in a humidified incubator under 7·5 per cent CO_2. The cultures were diluted to 5×10^4 cells per ml in fresh medium twice weekly. Cell concentrations and viability were routinely assessed by trypan blue exclusion test.

Clonogenic agar capillary microassay

The clonogenic microassay for K562 cells was carried out in agar-containing capillaries as described before.[18] Briefly, K562 cells were cultured at a concentration of 24×10^3 cells per ml in glass capillaries containing RPMI 1640 medium supplemented with L-glutamine, 10 per cent FCS and 0·18 per cent agar in a final volume of 30 µl. The low concentration of agar (0·18 per cent) used in the present study was well-established in our laboratory as the optimal for colony formation in glass capillaries which are very different from other tissue culture plates in which 0·3 per cent of agar is usually used.[19,20] Each of the three recombinant human IFNs was added at graded concentrations ($10–3 \times 10^4$ units per ml) either alone or in combinations with ara-C at graded concentrations ($10–10^3$ nM). Also, cultures containing only ara-C at the same graded concentrations and control cultures containing neither cytarabine nor IFNs were set up in conditions otherwise identical. Triplicate capillaries of each of the culture conditions in three separate experiments were incubated

for 5 days at 37°C in a humidified incubator under 7·5 per cent CO_2. The time point was selected to allow adequate time for colony formation but to avoid density arrest in more rapidly growing colonies and to avoid problems with the limited *in vitro* stability of IFNs. K562 colonies which were aggregates of eight or more cells, were counted on day 5 in each capillary using a special dissecting microscope DZ-240 (NSK Co., Tokyo).

Liquid suspension culture

K562 cells were cultured at a concentration of 100×10^3 cells per ml in 96-flat bottomed well tissue culture plates containing RPMI 1640 medium supplemented with L-glutamine and 10 per cent FCS in a final volume of 100 µl. Each of the three recombinant human IFNs was added at graded concentrations ($10–3 \times 10^4$ units per ml) either alone or in combinations with cytarabine at graded concentrations ($10–10^3$ nM). Also, cultures containing only cytarabine at the same graded concentrations and control cultures containing neither cytarabine nor IFNs were set up in conditions otherwise identical. Triplicate plates of each of the culture conditions in three separate experiments were incubated for 4 days at 37°C in a humidified incubator under 7·5 per cent CO^2. The time point was selected to allow adequate time for growth inhibition but to avoid density arrest in more rapidly growing wells and to avoid problems with the limited *in vitro* stability of recombinant human IFNs.

Assessment of K562 colony inhibition

The K562 colony inhibition was expressed as percentage of control cultures and determined as follows: Percentage K562 colony inhibition = [(number of K562 colonies in control culture − number of K562 colonies in treated culture) ÷ (number of K562 colonies in control culture)] × 100. Dose response curves of the percentage of K562 colony inhibition in relation to control cultures on day 5 were plotted versus the logarithm of concentrations of cytarabine and IFNs when used alone or in combinations with each other. IC_{20}, IC_{50} and IC_{80} values which are the concentrations of each agent inducing 20 per cent, 50 per cent and 80 per cent, respectively, inhibition of K562 colony formation on day 5 compared with control cultures containing neither cytarabine nor IFNs were calculated from the logarithmic regression analysis of each dose response curve as described before.[18]

Assessment of K562 cell growth inhibition

After 4 days of incubation, the viable cell numbers in each well of the triplicate plates were counted using the trypan blue dye exclusion test rather than other bromide dyes which were shown to underestimate the growth inhibitory effects of interferons.[21] The K562 cell growth inhibition was expressed as a percentage of control cultures and dose response curves of the percentage of K562 cell growth inhibition in relation to control cultures on day 4 were plotted versus the logarithm of concentrations of cytarabine and interferons when used alone or in combinations with each other. IC_{20}, IC_{50} and IC_{80} values which are the concentrations of each agent inducing 20 per cent, 50 per cent and 80 per cent, respectively, inhibition of K562 cell growth on day 4 compared with control cultures containing neither cytarabine nor IFNs were calculated from the logarithmic regression analysis of each dose response curve as described before.[18]

Statistical analysis

The results were represented as mean ± standard deviation of nine determinations of triplicate cultures from three separate experiments and the statistical significance was determined using the two-tailed Student *t*-distribution test.

Table 1. Effect of IFN-alpha 2b on the antiproliferative activity of ara-C in K562 human myeloid leukemia clonogenic cells

Molar concentration (nM) of ara-C	K562 colonies* (percentage of control) Plus IFN-alpha 2b (units/ml)				
	Alone	30	300	3000	30 000
0	100	79·4 ± 5·2	79·5 ± 5·6	80·3 ± 3·8	99·2 ± 1
10	92·3 ± 8	83·2 ± 7·3	83·5 ± 4·7	84·4 ± 1·8	91·3 ± 2
50	74·0 ± 6	71·9 ± 1·3	73·5 ± 5·3	77·4 ± 1·4	80·3 ± 2
100	50·5 ± 4	56·8 ± 2·1	60·9 ± 3·8	70·6 ± 6·1†	72·6 ± 1
500	16·0 ± 5	28·5 ± 5·0	32·2 ± 3·0†	37·4 ± 3·8†	50·0 ± 1
1000	0	0	2·1 ± 0·5	3·9 ± 1·6	5·0 ± 2
IC_{50} (nM)	102	106·5	123	157†	214†
IC_{20} (nM)	24	19·5	21·6	27	35†
IC_{80} (nM)	431	582†	702†	904†	1280†

*K562 colonies are aggregates of eight or more cells on day 5.
†Significant with $p < 0·01$ using two-tailed Student t-distribution test.

RESULTS

In control cultures, K562 cells formed 37·2 ± 4·6 colonies. The addition of ara-C alone to K562 cultures decreased the number of colonies on day 5 in a dose-dependent manner with an IC_{50} of 102 nM (Table 1).

Effect of IFN-alpha on cytarabine activity

The addition of 30–3000 U/ml IFN-alpha alone to K562 cultures decreased the number of colonies by about 20 per cent compared to control cultures (Table 1). However, at the high concentration of 3×10^4 U/ml IFN-alpha alone did not affect K562 colony formation (Table 1). The addition of IFN-alpha to K562 cultures treated with low concentrations of ara-C: 10–50 nM did not affect the antiproliferative activity of ara-C (Table 1). On the other hand, in K562 cultures treated with high concentrations of ara-C: 100–500 nM, IFN-alpha significantly reduced the antiproliferative activity of ara-C (Table 1). The addition of 3×10^4 U/ml IFN-alpha to ara-C treated K562 cultures increased the IC_{50} of ara-C on day 5 from 102 to 214 nM (Table 1). Also, the addition of 3×10^3 U/ml IFN-alpha to ara-C-treated K562 cultures increased the IC_{80} of ara-C on day 5 from 431 to 904 nM (Table 1). Thus, ara-C plus a high concentration of IFN-alpha was about twofold less potent than ara-C alone.

In liquid suspension culture, the addition of 3×10^4 U/ml IFN-alpha to 100 nM and 500 nM ara-C-treated K562 cultures significantly reduced the antiproliferative activity of ara-C from 90·5 ± 1·7 per cent and 97·1 ± 2·8 per cent to 66·7 ± 3·9 per cent and 86·2 ± 3·4 per cent, respectively, $p < 0·01$.

Effect of IFN-beta on cytarabine activity

The addition of 10–100 U/ml IFN-beta alone did not affect K562 colony formation (Table 2). However, at the high concentrations of 1000 U/ml and 4000 U/ml IFN-beta alone increased the number of K562 colonies by 30·1–50·4 per cent compared to control cultures (Table 2). The addition of low concentrations of IFN-beta: 10 U/ml and 100 U/ml did not affect the antiproliferative activity of ara-C (Table 2). On the other hand, high concentrations of IFN-beta: 1000 U/ml

Table 2. Effect of IFN-beta 2 on the antiproliferative activity of ara-C in K562 human myeloid leukemia clonogenic cells

Molar concentration (nM) of ara-C	K562 colonies* (percentage of control) Plus IFN-beta 2 (units/ml)				
	Alone	10	100	1000	4000
0	100	94·4±4·7	107·5±3·1	132·8±2·7	148·9±1
10	92·3±8	100±2·3	103·8±2·6	109·1±5·5	112·9±1
50	74·0±6	82·6±3·0	82·9±5·5	85·5±3·7	93·0±4†
100	50·5±4	54·5±1·6	56·1±3·6	80·6±2·6†	81·7±1†
500	16·0±5	16·8±1·9	16·9±2·6	35·5±2·5†	43·1±2†
1000	0	0	3·8±1·0	7·0±1·6†	14·5±2†
IC_{50} (nM)	102	118	167†	242†	304†
IC_{20} (nM)	24	32	56†	78.5†	94†
IC_{80} (nM)	431	431	502†	748†	982†

*K562 colonies are aggregates of eight or more cells on day 5.
†Significant with $p < 0{\cdot}01$ using two tailed Student t-distribution test.

and 4000 U/ml significantly reduced the antiproliferative activity of ara-C (Table 2). The addition of 1000 U/ml and 4000 U/ml IFN-beta to ara-C K562 cultures increased the IC_{50} of ara-C from 102 to 242 nM and 304 nM, respectively, and the IC_{20} of ara-C of ara-C from 24 to 78·5 nM and 94 nM, respectively, on day 5 (Table 2). Thus, ara-C plus a high concentration of IFN-beta was about 2–4-fold less potent than ara-C alone. In liquid suspension culture, the addition of 4000 U/ml IFN-beta to 100 nM and 500 nM ara-C-treated K562 cultures significantly reduced the antiproliferative activity of ara-C from 90·5 ± 1·7 per cent and 97·1 ± 2·8 per cent to 25·3 ± 4·7 per cent and 34·6 ± 5·9 per cent, respectively, $p < 0{\cdot}001$.

Effect of IFN-gamma on cytarabine activity

The addition of 10 U/ml IFN-gamma alone did not affect K562 colony formation (Table 3). However, at the high concentrations of IFN-gamma: 10^2–10^4 U/ml alone increased the number of K562 colonies by 9·7–64·5 per cent compared to control cultures (Table 3). Similar stimulation of the proliferation of K562 cells by IFN-gamma was previously reported.[22] Whereas the low concentrations of IFN-gamma: 10–10^2 U/ml did not affect the antiproliferative activity of ara-C (Table 3).

On the other hand, the high concentration of 10^4 U/ml IFN-gamma significantly reduced the antiproliferative activity of ara-C (Table 3). The addition of 10^4 U/ml IFN-gamma to ara-C-treated K562 cultures increased the IC_{50} and IC_{20} of ara-C from 102 and 24 nM to 316 and 60 nM, respectively, on day 5 (Table 3). Therefore, ara-C plus a high concentration of IFN-gamma was about 2·5–3-fold less potent than ara-C alone.

In liquid suspension culture, the addition of 1000 U/ml IFN-gamma to 100 nM and 500 nM ara-C-treated K562 cultures significantly reduced the antiproliferative activity of ara-C from 90·5 ± 1·7 per cent and 97·1 ± 2·8 per cent to only 22·9 ± 5·7 per cent and 25·1 ± 5·4 per cent, respectively, $p < 0{\cdot}001$.

DISCUSSION

The primary objective of combination therapeutic regimens is to increase the patient's response rate through the synergistic or additive effects of different biological and/or chemotherapeutic

Table 3. Effect of IFN-gamma on the antiproliferative activity of ara-C in K562 human myeloid leukemia clonogenic cells

Molar concentration (nM) of ara-C	K562 colonies* (percentage of control) Plus IFN-gamma (units/ml)				
	Alone	10	100	1000	10 000
0	100	90·5±9·2	111·4±1·7	139·2±2·9	158·4±6
10	92·3±8	92·7±3·8	93·8±4·0	105·2±4·5	137·3±7†
50	74·0±6	81·7±1·9	83·1±3·2	85·6±5·3	88·4±1†
100	50·5±4	51·1±2·1	52·5±1·2	56·8±2·7	61·2±3†
500	16·0±5	16·7±2·0	19·9±1·5	21·9±2·1	34·6±3†
1000	0	0	0	5·1±1·5	12·5±1†
IC_{50} (nM)	102	97	118	166†	316†
IC_{20} (nM)	24	23	28·5	52†	60†
IC_{80} (nM)	431	408	485	533†	780†

*K562 colonies are aggregates of eight or more cells on day 5.
†Significant with $p < 0·01$ using two-tailed Student t-distribution test.

agents, while at the same time avoiding any decreased therapeutic efficacy caused by antagonistic effects. The major finding of the present study is the negative interactions of the three interferons: alpha, beta and gamma with ara-C particularly at high concentrations. The combination of ara-C and a high concentration of interferon was about 2–4-fold less potent than ara-C alone. The significant reduction of the desired antiproliferative activity of ara-C by the three interferons was observed *in vitro* in both clonogenic agar capillary microassay and liquid suspension cultures. The present results are in line with the previous results of the interaction between interferon-alpha and ara-C *in vivo* in L1210 murine leukemia.[23]

The enthusiastic preclinical reports of synergy between interferons and chemotherapeutic agents in non-leukemic tumour cell lines were not translated into an important benefit in clinical trials for patients with solid tumours.[8] In general, clinical trials for the treatment of patients with non-haematological malignancies combining interferon with chemotherapy have not resulted in significantly improved response rates.[8] Also, clinical trials for the treatment of patients with myelodysplastic syndromes with various dosages of recombinant human interferon-alpha 2b[14,15,24] and interferon-gamma[25,26] administered alone by different routes revealed no significant therapeutic benefit. Since a definite response to these interferons was achieved only in patients with early stages of chronic myeloid leukemia[27] and of chronic lymphocytic leukemia,[28] a low tumour burden may thus be a prerequisite for a successful interferon therapy. Also, the administration of a high dose of interferon was important in achieving complete remissions in these leukemia patients.[27]

In K562 cells which were established from a chronic myeloid leukemia patient in accelerated blastic phase,[1] combining ara-C with a high concentration of interferon reduced its efficacy to about 2–4-fold that of ara-C alone (Tables 1–3). Therefore, given the present negative interactions of interferons with ara-C in these K562 cells particularly at high concentrations together with the two apparent prerequisites for successful interferon treatment: a low tumour burden in an early stage of disease and a high dose of interferon, caution may be exercised against the clinical use of the combination of cytarabine and interferon in the treatment of myeloid leukemia patients.

However, limits exist to the comparability of blast cells from myeloid leukemia patients with cultured cell lines such as K562. Whereas each cell line represents a single population of myeloid leukemia cells, cells from myeloid leukemia patients are heterogenous.[29] Also, such cell lines also

their growth properties and change their response to agents and drugs after continuous passage for extended durations.[29] Moreover, the predictive value of *in vitro* results in these cell lines is undermined by the complex multiple cascade of the *in vivo* agent/cell and cell/cell interactions.[30] Therefore, further evaluation of the present negative interactions between cytarabine and the three IFNs are warranted in myeloid leukemic cells from patients in primary culture.

The mechanisms of action of the present negative interactions between interferons and cytarabine remain to be elucidated. Studies of the effects of interferons on the pharmacokinetics and metabolism of cytarabine in human myeloid leukemia cells both *in vitro* and *in vivo* are warranted to explore the biochemical and kinetic interactions of these agents. The present results suggest that the effect on cytarabine activity is universal for the three IFNs and is less likely to be specific for a certain IFN. The IFN anti-antiproliferative effect could also be due to direct stimulation of K562 cell proliferation or indirect effect via producing other growth-promoting cytokines which subsequently stimulate K562 cell proliferation or both. Addressing this question could further our understanding of the mechanisms of the negative interactions between IFNs and cytarabine.

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REFERENCES

1. Lozzio, C. B., Lozzio, B. B. Human chronic myelogenous leukaemia cell line with positive philadelphia chromosome. *Blood* 1975, **45,** 321–334.
2. Sutherland, J. A., Turner, A. R., Mannoni, P., McGann, L. E., Ture, J. M. Differentiation of K562 leukaemic cells along erythroid, macrophage, granulocyte and megakaryocyte lineages. *J. Biol. Response Modifiers* 1986, **5,** 250–262.
3. Büchner, T. H., Hiddemann, W. Treatment strategies in acute myeloid leukemia (AML): A first line chemotherapy. *Blut* 1990, **60,** 61–67.
4. Rowley, P. T., Wilhelm, B. M., Farley, B. A., Le Balla, S. Inducers of erythroid differentiation in K562 human leukemia cells. *Exp. Hematol.* 1981, **9,** 32–37.
5. Tafuri, A., Andreeff, M. Kinetic rationale for cytokine-induced recruitment of myeloblastic leukemia followed by cycle specific chemotherapy *in vitro*. *Leukemia* 1990, **4,** 826–834.
6. Hassan, H. T., Rees, J. K. H. Retinoic acid alone and in combination with cytosine arabinoside induces differentiation of human myelomonocytic and monoblastic leukemic cells. *Hematol. Oncol.* 1988, **6,** 39–45.
7. Hassan, H. T., Maurer, H. R. Synergistic combinations for differentiation therapy of myeloid leukaemia patients. *Med. Sci. Res.* 1991, **19,** 195–198.
8. Wadler, S., Schwartz, E. L. Antineoplastic activity of the combination of interferon and cytotoxic agents against experimental and human malignancies. *Cancer Res.* 1990, **50,** 3473–3486.
9. Gresser, I., Maury, C., Belardelli, F., Maunoury, M.-T., Machover, D. Effectiveness of mouse interferon alpha-beta compared to single agent chemotherapy in increasing survival time of mice after intravenous inoculation of Friend erythroleukaemia cells. *J. Natl. Cancer Inst.* 1988, **80,** 126–131.
10. Mowshowitz, S. L., Chin-Bow, S. T., Smith, G. D. Interferon and cis-DDP: combination chemotherapy for P388 leukaemia in CDF1 mice. *J. Interferon Res.* 1982, **2,** 587–591.
11. Elias, L., Crissman, H. A. Interferon effects upon the adenocarcinoma 38 and HL-60 cell lines: antiproliferative responses and synergistic interactions with halogenated pyrimidine antimetabolites. *Cancer Res.* 1988, **48,** 4868–4873.
12. Kafka, M., Dvilansky, A., Nathan, I. Mechanism of interaction between gamma interferon and antineoplastic agent on the differentiation of HL-60 promyelocyte leukemia cells. *Exp. Hematol.* 1990, **18,** 153–158.
13. Talpaz, M., Kantarjian, H., Kurzrock, R., Trujillo, J. M., Gutterman, J. U. Interferon-alpha produces sustained cytogenetic responses in chronic myeloid leukaemia philadelphia chromosome positive patients. *Ann. Intern. Med.* 1991, **114,** 532–537.

14. Catalano, L., Majolino, I., Musto, P., Magrin, S., Rotoli, B. A trial with alpha interferon in CMML (abstr). *Blut* 1988, **56,** C16.
15. Aul, C., Gattermann, N., Schneider, W. Treatment of advanced myelodysplastic syndromes with recombinant interferon-alpha 2b. *Eur. J. Haematol.* 1991, **46,** 11–16.
16. Beran, M., Andersson, B., Kantarijian, N., *et al.* Hematologic response of four patients with smoldering acute myelogenous leukemia to partially pure gamma interferon. *Leukemia* 1987, **1,** 52–57.
17. Kurzrock, R., Talpaz, M., Kantarijian, H., *et al.* Therapy of chronic myelogenous leukemia with recombinant interferon gamma. *Blood* 1987, **70,** 943–947.
18. Hassan, H. T., Veit, A., Maurer, H. R. Synergistic interactions between differentiation induction agents in inhibiting the proliferation of HL-60 human myeloid leukaemia cells in clonogenic microassay. *J. Cancer Res. Clin. Oncol.* 1991, **117,** 227–231.
19. Maurer, H. R. Cell culture techniques for testing of biologically active peptides and drugs: clonogenic assays using agar containing glass capillaries. In: Tschesche, H. ed. *Modern Techniques in Protein Chemistry*. Berlin: Walter de Gruyter, 1988: 335–357.
20. Maurer, H. R., Echarti, C. Clonogenic assays for haemopoietic and tumour cells using agar containing capillaries. In: Pollard, J. W. and Walker, M., eds. *Methods in Molecular Biology*, Vol. 5. *Animal Cell Culture*. New York: The Humana Press, 1990, 379–394.
21. Jabbar, S. A. B., Twentyman, P. R., Watson, J. V. The MTT assay underestimates the growth inhibitory effects of interferons. *Brit. J. Cancer* 1989, **60,** 523–528.
22. Hosoi, T., Ozawa, K., Ohta, M., Okabe, T., Urabe, A., Takaku, F. Recombinant immune interferon inhibits leukemic cell growth by a monocyte-macrophage-mediated mechanism. *Exp. Hematol.* 1985, **13,** 597–602.
23. Slater, L. M., Wetzel, M. W., Cesario, T. Combined interferon-antimetabolite therapy of murine L1210 leukemia. *Cancer (Phila.)* 1981, **48,** 5–9.
24. Elias, L., Hoffman, R., Boswell, S., Tensen, L., Bonnem, E. M. A trial of recombinant alpha-interferon in the myelodysplastic syndromes. *Leukemia* 1987, **1,** 105–110.
25. Ogawa, M., Yoshida, Y., Moriyama, Y. Phase II clinical trial of recombinant human interferon gamma on myelodysplastic syndromes. Third Int. Congress of Myelodysplasia. *Blut* 1988, **56,** c21, 12A.
26. Maiolo, A. T., Cortelezzi, A., Calori, R., Polli, E. E. Recombinant human gamma interferon as first line therapy for high risk myelodysplastic syndromes. *Leukemia,* 1990, **4,** 480–485.
27. Ozer, H. Biotherapy of chronic myelogenous leukaemia with interferon. *Semin. Oncol.* 1988, **15,** 14–20.
28. Rozman, C., Montserrat, E., Vinolas, N. Recombinant human alpha-interferon in the treatment of B chronic lymphocytic leukaemia in early stages. *Blood* 1988, **71,** 1295–1298.
29. Hassan, H. T. *In vitro* differentiation induction of human normal and leukaemic myeloid progenitor cells. Doctorate (Ph.D.) Thesis 1990 in Haematology, University of Cambridge Clinical School, Cambridge, England, U.K.
30. Hassan, H. T. *In vitro* interaction between retinoids and cytokines in human myeloid leukaemia cells. *Eur. J. Cancer* 1991, **27,** 1182–1183.