Eur J Cancer Clin Oncol, Vol. 19, No. 11, pp. 1625–1632, 1983.
Printed in Great Britain.

0277-5379/83$3.00+0.00

Tumor Growth Stimulation *in Vitro* by Interferons*

CHRISTIAN U. LUDWIG,†‡ BRIAN G. M. DURIE,‡§ SYDNEY E. SALMON‡ and THOMAS E. MOON‡
‡*Section of Hematology-Oncology and the Cancer Center, University of Arizona College of Medicine, Tucson, AZ 85724, U.S.A.*

Abstract—*Interferons (IFNs) are a family of polypeptides originally identified as antiviral substances. Subsequently, other properties of interferons were recognized, including inhibition of cell proliferation, and effects on the immune response and on expression of surface antigens. In this paper we present evidence that interferons, even the highly purified cloned IFNs, can stimulate clonogenic tumor growth* in vitro. *Of 225 human tumor (HT) samples tested with IFN in a clonogenic assay (HTCA), 30 (13.3%) showed growth stimulation (>2 S.E. above control). The phenomenon was observed most frequently with acute myeloid leukemia (6/22 samples, 27.3%), and renal (2/10, 20%) and breast cancer (4/21, 19%), but significantly less frequent in melanomas (2/34, 5.9%). As an independent assessment of proliferation, tritiated thymidine uptake by tumor cells was measured autoradiographically in 21 patients with multiple myeloma. A significant increase of the thymidine labeling index was seen in 4 (19%) of the samples. Since this growth stimulatory effect was also observed with cell lines which lack any contaminating immunoreactive cells, there is strong evidence that interferons can directly stimulate the proliferation of clonogenic tumor cells* in vitro. *Growth stimulation by interferons occurred preferentially with lower dosages. It is important to be cognizant of potential clinical implications of tumor growth stimulation by interferons.*

INTRODUCTION

INTERFERONS (IFNs) are a family of polypeptides produced by eukaryotic cells in response to a variety of stimulating agents. Interferon was originally defined as an antiviral substance [1]. However, subsequently, interferons were reported to inhibit multiplication of L cells [2], stimulate lymphocytes [3] and also inhibit the growth of a variety of normal and transformed cells [4]. No specific block in cell cycle phase has been demonstrated, but, rather, delayed transit through G_1 and $S+G_2$ [5]. It has further been shown that effects other than direct inhibition of cell growth can be produced. For example, interferons have profound effects on the immune response [3, 6] and can also affect the expression of surface antigens [7–9].

The molecular mechanisms of interferons' actions seem to be reflected in part by the induction of several enzyme systems including protein kinase PK-i, (2′-5′)oligo-A synthetase E, and phosphodiesterase 2′-PDi, which function primarily to regulate protein synthesis [10]. The activity of these enzymes has been correlated with the antiviral and the antiproliferative effects of interferons [10].

In animal systems interferons have antitumor activity both in virus induced and in spontaneous tumors *in vivo* [8]. Clinical data suggesting antitumor activity of interferon type I have been reported for osteosarcoma [11], myeloma [12] and breast carcinoma [13].

Accepted 19th July 1983.
*These studies were supported by a grant from the Swiss Cancer League (C.U.L.) and grants from the USPHS (CA-21839, CA-17094, and CA-21074), and from Hoffmann-La Roche Inc., Nutley, NJ, U.S.A.
†Current address: Onkologische Poliklinik, Kantonsspital, Petersgraben, 4031 Basel, Switzerland.
§To whom requests for reprints should be addressed.
Abbreviations: HTCA: human tumor clonogenic assay; IFN: interferon; IFN-α: leukocyte interferon (Cantell method); IFN-αA: cloned leukocyte interferon (clone A, prepared by recombinant DNA technology); IFN-αD: cloned leukocyte interferon (clone D, prepared by recombinant DNA technology).

Recently the recombinant DNA technology has been successfully applied to interferon production [14–16]. This has made possible the examination of the growth regulatory properties of these much more highly purified interferons. In an effort to document the spectrum of activity of the various interferons against human cancers, our group initiated a screening program using the *in vitro* human tumor clonogenic assay (HTCA). In these studies about 36% of human tumors manifest greater than 50% inhibition of colony formation after exposure to pharmacologically achievable dosages of IFN-αA [17]. However, some of the fresh tumor specimens exhibited growth stimulation on exposure to IFN as manifested by a significant increase in the number of tumor colonies as compared to the controls. In this report we present the details of *in vitro* stimulation produced by IFNs on tumor colony formation. The effect of IFNs on cell proliferation has also been assessed independently using [^{3}H]-thymidine exposure.

MATERIALS AND METHODS

Fresh human tumor samples

The procedure for collecting, preparing and culturing single cell suspensions of human tumor cells has been described [18, 19]. In all samples the diagnosis of cancer was histologically confirmed. Tumor cells were obtained either from solid tumor specimens, bone marrow, or ascitic or pleural fluid. An aliquot of the tumor cell suspension was cytologically examined to confirm the malignant histology.

Human tumor cell lines

The human myeloma cell line RPMI 8226 was obtained from the American Type Culture Collection, Rockville, MD. The cells were harvested 24–48 hr after splitting the cultures and adding fresh media.

Type of interferons used

INF-α, prepared by the Cantell method, was kindly provided by Dr. J. Gutterman [20]. Recombinant leukocyte interferon clone A (IFN-αA) and clone D (IFN-αD) were kindly provided by Dr. P. Trown (Hoffmann-La Roche). They were produced in *Escherichia coli* transformed with the plasmid LeIFA25 [21] and LeIFD3 [22] respectively. All dilutions were made in McCoy's 5A medium containing 10% heat-inactivated fetal bovine serum (FBS).

Human tumor clonogenic assays (HTCA)

The HTCA used for solid tumors and multiple myeloma has been described in detail [18, 19]. Briefly, underlayers of augmented McCoy's medium A and 0.5% Difco-Agar were prepared in 35-mm Petri dishes. Cells (5×10^5) of a tumor cell suspension were plated in 0.3% agar in enriched CMRL 1066 over a 0.5% agar underlayer. For multiple myeloma details were as per Hamburger and Salmon [18]. The human myeloma cell line RPMI 8226 was plated at a cell concentration of 8×10^4/plate in RPMI 1640 with 10% FCS. For leukemias the methylcellulose assay described by Buick *et al.* was used [23]. All specimens were plated in triplicate. The interferons to be tested were incorporated into the medium at final concentrations of 0.4–4.0 ng/ml for the cloned interferons (for IFN-αA, these dosages were the equivalent of 80 and 800 units/ml of interferon activity). The non-cloned leukocyte IFN (IFN-α) was used in concentrations of 50, 100, 250 and 500 U/ml. The interferons were added to the plating mixture just prior to plating. The plates were incubated at 37°C in a humidified atmosphere of 7% CO_2. All plates were monitored for aggregation on the morning after plating and discarded if significant aggregation was present. Cultures were subsequently reviewed by inverted microscopy every 3 days. Leukemic colonies were counted on days 5–7 by inverted microscopy, while solid tumor colonies were counted between days 14 and 17 using a Bausch and Lomb FAS II image analyzer specially equipped and programmed for tumor colony counting [24]. At the time of counting the control plates were also compared with additional control plates fixed in 3% glutaraldehyde kept at 4°C for the incubation period (to prevent proliferation). If not otherwise stated, cell aggregates of >60 μm were counted as colonies. Results reported are for tumors tested which gave rise to at least 30 colonies per control plate (500,000 cells plated). A median of 80 colonies per control plate was obtained in these studies.

In vitro *incorporation of* [^{3}H]-*thymidine* (*LI %*) *after short-term culture*

The method for the tritiated thymidine labeling index (LI %) in myeloma specimens has been described previously [25–27]. The use of measuring thymidine uptake as a marker of drug efficacy has also been described [28, 29]. A modification of this method was used in the experiments described in this paper. Briefly, bone marrow cells from patients with multiple myeloma were incubated for 3 hr at 37°C, with and without interferons, at the same concentrations as used for the human tumor clonogenic assay. [^{3}H]-TdR (5 μCi/ml) was then added and the cell suspension incubated for a further 1 hr at 37°C. After washing free the cells of unincorporated [^{3}H]-TdR, slides for autoradiography and microscopic examina-

tion were prepared with a cytocentrifuge. After methanol fixation autoradiographs were prepared for exposure by dipping Kodak NTB-3 emulsion and further processed using our previously published method of high-speed scintillation autoradiography (HSARG) [25]. Slides used in this study were incubated for 24 hr. Standard developing techniques were used and the slides were stained with acid Giemsa stain. All marrow cells that were morphologically in the lymphoid-plasma cell series were defined as myeloma cells.

Plasma cells containing five grains over the nucleus were considered labeled. One thousand cells were counted to determine the LI %, which was then expressed as a percentage.

Statistical methods

The results of the HTCA are all reported as a percentage of the control number of tumor colonies (percentage of control). The mean coefficient of variation of the control plates was 4.8%. As an operational definition, growth stimulation in the HTCA was defined as tumor colony growth more than two standard errors above control colony growth (>2 S.E. above 100%).

For the [^{3}H]-thymidine uptake, duplicate autoradiographs were counted and compared using the method of Livingston *et al.* [30]. A two-fold increase or an increase from 0 to >1% was considered as stimulation of proliferation.

RESULTS

A total of 500 human tumor cloning assays (HTCA) were carried out with the samples of 225 patients. In 30 (13.3%) of these patient samples, the HTCA showed growth stimulation (>2 S.E. above control) with one or more of the different types of IFN (Table 1). Considered in terms of numbers of assays, 34/500, or 6.8%, showed evidence of growth stimulation with one or another of the interferons (Table 2). With the IFN-α there was a clear dose dependency. At 50 U/ml, the number of samples with evidence of growth stimulation was 5.7% (8/140), but at 100 U/ml, 26.3% (5/19). At 250 and 500 U/ml no stimulation was seen (0/38 and 0/18) (Table 2). There was also a trend towards a similar dose response with IFN-αA. At the lowest dose tested (4×10^{-4} μg/ml) 6.6% (6/90) of the samples showed evidence of growth stimulation; at 8×10^{-4} μg/ml, 22.2% (2/9); and at 4×10^{-3} μg/ml, 6.5% (6/93). So far the numbers are too small to draw final conclusions about a potential dose response with these fresh human tumor samples.

The frequency of growth stimulation varied with tumor cell type. Growth stimulation with IFN-α was most frequent with AML samples; 6 of 19 assays performed on AML samples with doses of IFN-α of 50 to 100 U/ml showed significant growth stimulation in the HTCA. Figure 1a shows the dose response of a bone marrow sample from a patient with acute myelogenous leukemia to IFN-αA. Growth stimulation with IFN-αA was mainly observed in breast carcinoma (5/27 assays) and in ovarian carcinoma (5/47 assays). In contrast, growth stimulation was not observed with IFN-αA in melanoma (0/39 assays). However, stimulation was observed in melanoma with IFN-αD. Figure 1b depicts the response of a melanoma sample to IFN-αD.

Figure 2 gives the dose responses of the human myeloma cell line 8226 to IFN-αA. At the lower concentrations the 8826 cell line shows growth stimulation; at higher concentrations, growth inhibition. In evaluation of colony size ($\geq$60, $\geq$86 and $\geq$148 μm), growth stimulation with the lower doses of interferon was most clearly evident for the larger colonies. This suggested an effect upon proliferation of clonogenic cells rather than recruitment into the clonogenic compartment. No significant change in the size of individual myeloma cells was noted.

In our studies of tritiated thymidine uptake after short-term culture (measuring LI %)

Table 1. *Overall growth stimulation in fresh human tumor samples by interferons*

Tumor type	No. of samples tested*	Samples with growth stimulation No. (%)†
AML	22	6 (27.3)
Renal ca.	10	2 (20.0)
Breast ca.	21	4 (19.0)
Ovarian ca.	43	5 (11.6)
Lung ca.	18	2 (11.0)
Unknown primary	12	1 (8.3)
Melanoma	34	2 (5.9)
Myeloma	23	1 (4.3)
Sarcoma	8	3
Bladder	3	1
NHL	4	1
Carcinoid	3	0
Corpus uteri ca.	5	1
CML	7	1
Colon ca.	3	0
Pancreatic ca.	2	0
Mesothelioma	1	0
Prostate ca.	2	0
Stomach ca.	1	0
Testicular ca.	2	0
Thyroid ca.	1	0
Total	225	30 (13.3%)

*A total of 500 HTCAs were performed with the various leukocyte IFNs at different concentrations.

†Percentage calculated only for tumor types where at least 10 samples have been tested.

Table 2. Growth stimulation by IFNs in the HTCA: dose response relationships

Type of interferon	Concentration of interferon	No. of HTCAs performed*	No. of tests with percentage of survival >2 S.E.above control (= 100%)	Percentage of tests exhibiting stimulation
IFN-α	50 U/ml	140	8	15.7
	100 U/ml	19	5	26.3
	250 U/ml	38	0	0.0
	500 U/ml	18	0	0.0
IFN-αA	4×10^{-4} μg/ml	90	6	6.6
	8×10^{-4} μg/ml	9	2	22.2†
	4×10^{-3} μg/ml	93	6	6.5
IFN-αD	4×10^{-4} μg/ml	45	4	8.9
	4×10^{-3} μg/ml	48	3	6.3
Total		500	34	Mean · 6.8

*A total of 225 tumors were tested against three different types of interferons in a total of 500 assays.

†Only acute leukemias were tested at 8×10^{-4} μg/ml.

samples from 21 patients with multiple myeloma were tested with IFN-α or IFN-αA. A total of 41 assays were performed with the two IFNs at different dosages. In four patients a significant increase of the LI was observed (19%). An increase of the LI was found in 14% (3/21) using the IFN-α (Table 3). This stimulatory effect was only seen at the concentration of 100 U/ml. Analyzing only the samples tested at 100 U/ml, the percentage of growth stimulation was 18.8% (3/16). With IFN-αA, 15% (3/20) showed an increase of the LI. With IFN-αA, stimulation was observed at all concentrations tested. The number of assays performed measuring the thymidine uptake are too small to draw final conclusions about frequency of proliferation stimulation in this system. However, they do corroborate the observed clonal growth stimulatory effect of IFNs in the HTCA. With the 8226 cell line, IFN-α also produced an increase of the LI from 14 (control) to 24% at 1000 U/ml.

DISCUSSION

Interferons (IFN) have been shown to have several properties, including antiviral activity, inhibitory effects upon cell division and enhancement of certain specialized cell functions including cell surface effects. Based on the antiproliferative effect, IFN type I has been evaluated as treatment for tumors in animals [8] and in humans [12, 13, 31]. Our data indicate that IFN type I, even in its pure form as recombinant-IFN, can, in a percentage of cases, stimulate rather than inhibit the growth of some human tumors *in vitro*. This stimulation has been observed in two different *in vitro* systems: clonal growth in

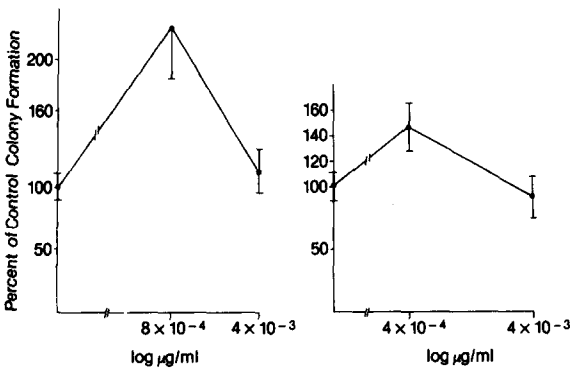

Fig. 1. (a) Dose response to IFN-αA in an acute myelogenous leukemia using blast cell assay in methyl cellulose [23]. *(b) Dose response to IFN-αD in a melanoma using the human tumor clonogenic assay* [18].

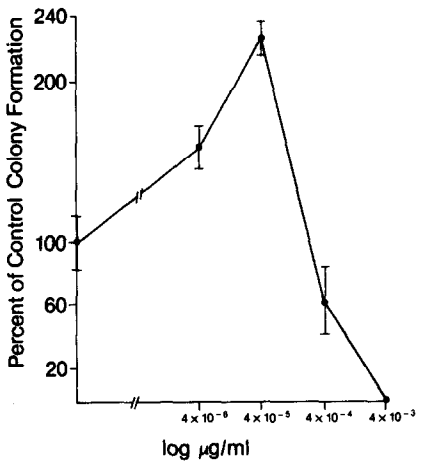

Fig. 2. Effect of IFN-αA on colony formation by human myeloma cell line 8226 (149-μm *colonies) using the human tumor clonogenic assay* [18].

Table 3. Increased tritiated thymidine uptake (LI %) induced by interferons in multiple myeloma (41 assays)

Leuk IFN†	21 (16)*	3 (3)*	14.2 (18.8)*
IFN-αA‡	20	3	15
Total	41	6	14.6

*Only samples tested at 100 U/ml.
†Tested at 100/500 and 1000 μm.
‡Tested at 4×10^{-4}, 8×10^{-4} and 4×10^{-3} μg/ml.

semisolid medium (agar or methylcellulose) and thymidine incorporation after short-term culture. Our findings support a recent report by Bradley and Ruscetti showing a stimulatory effect on human tumor proliferation *in vitro* using fibroblast IFN [32]. Others have also observed growth stimulation with IFNs using MDBK cells [Stebbing and Czarwick, personal communication]. Enhancement of tumor growth *in vivo* has also been reported in transplantable murine tumors using interferon inducers [33].

In reviewing the overall effects of interferons in the clonogenic assay system two major populations can be identified: (1) those samples in which there is significant inhibition of colony growth (previously published, [17]); and (2) those samples, currently reported, demonstrating increased growth using the >2 S.E.M. (standard error of the mean) increase as the lower cutoff. The distribution of samples in this population has a Gaussian type distribution, as does the population of samples demonstrating growth inhibition. This therefore suggests two discrete biologic situations: one facilitating growth inhibition and the other growth stimulation.

The growth stimulatory effect of interferons observed in the HTCA could be mediated directly via an antiproliferative action on tumor cells or indirectly through an effect on host immunoreactive cells present in the HTCA [19, 34, 35]. The fact that both Bradley and Ruscetti's [32] and our results show a growth stimulatory effect in cell lines which lack any contaminating immunoreactive cells strongly suggests that interferons can directly stimulate proliferation of tumor cells.

In considering potential mechanisms by which this stimulatory effect might be mediated, it is helpful to review the enzyme systems involved in the previously documented antiproliferative activity of IFN. The antiproliferative and antiviral effects of IFN have been correlated with the induction of several enzymes by IFN, especially the (2′-5′)oligoisoadenyl synthetase E referred to as 2′-5′-A synthetase (Fig. 3) [10]. In lymphocytes stimulated by Con-A the accumulation of this enzyme is decreased. If IFN is added to the Con-A-stimulated cells or to unstimulated resting cells, the activity of the 2′-5′-A synthetase is increased [36]. The growth regulatory function of the 2′-5′-A synthetase has also been shown in other systems [37]. On the other hand, Wood and Hovonessian measured the 2′-5′-A synthetase level in mouse embryonal carcinoma cell lines and found that IFN increased the 2′-5′-A synthetase level in these undifferentiated cells, but without any antiproliferative or antiviral effect [38].

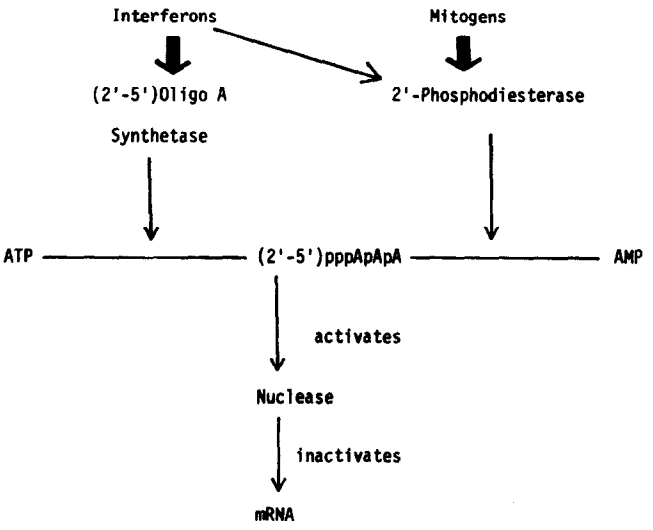

Fig. 3. Regulatory effect of interferons on cellular enzymes and mRNA (Modified from Revel et al. [10]).

However, regulation of cell growth appears to be a function both of the level of the 2'-5'-A synthetase and the amount of 2'-phosphodiesterase responsible for degradation of 2'-5'-pppApAp into AMP (Fig. 3). It is known that this later enzyme is elevated in Con-A-stimulated lymphocytes and also in interferon-treated L cells [10]. The ratio of 2'-5'-A synthetase to 2'-phosphodiesterase may be the critical factor regulating cell proliferation: 2'-5'-A synthetase inhibiting protein synthesis and finally cell proliferation; 2'-phosphodiesterase degrading the 2'-5'-A synthetase and leading to cell proliferation [10]. In L cells the ratio of the 2'-5'-A synthetase to 2'-phosphodiesterase is about 0.16 in untreated cells; with low doses of IFN the ratio decreases to about 0.07; with a further increase of the IFN dose the ratio increases again to about 1.0 [10]. It is of interest that this dose-dependent effect of interferon parallels the dose effects upon clonogenic growth which we have observed in the HTCA, with more growth stimulation at lower dosages of IFNs favoring induction of more phosphodiesterase than 2'-5'-A synthetase and a low ratio. Whether these two effects of IFN are related remains to be determined.

The growth stimulatory effect of IFN in the HTCA was observed mainly at the relatively lower concentrations (50–100 U/ml). If IFN is given i.m. to patients, serum peak levels of 55 U/ml (after the first injection and up to 330 U/ml after repeated injections) have been reported [39]. The concentrations of IFN which we have observed to produce growth stimulation *in vitro* fall within this clinically achievable range of serum concentrations.

One possible concern is whether IFN-induced growth stimulation of tumor cells *in vitro* in any way relates to a similar phenomenon in the clinical situation. It is well documented *in vitro* that IFN can stimulate cytotoxic lymphocytes, stimulate or inhibit natural killer cells and modulate phagocytosis by macrophages [34]). Modulation of the immune response by IFN administered *in vivo* has also been demonstrated in different animal systems [36]. Such immunoregulatory effects might counterbalance the growth stimulatory effect on tumor cells which we have observed *in vitro*. *In vitro–in vivo* correlations for tumor growth stimulation will be difficult to document. Spontaneous tumor progression and tumor growth stimulation by the treatment cannot easily be distinguished clinically. In animals, however, growth stimulation has been described using interferon inducers [33].

Although *in vitro* growth stimulation was observed with one or more of the IFNs only in a minority of patients (13.3%) tested, it would seem reasonable to recommend use of the HTCA when feasible to identify such patients. This might prove relevant when achievable IFN doses result in relatively low blood levels comparable to those shown *in vitro* to potentially enhance tumor proliferation.

Awareness of potential tumor growth stimulation by IFNs is important for the clinicians; patients on IFNs should be carefully monitored and if rapid tumor progression occurs, prompt interruption of the IFN treatment considered.

The dose response observed with IFNs resulting in more growth stimulation *in vitro* at lower dosages may also suggest the preferential use of high-dose intermittent IFN schedules in clinical studies.

Acknowledgements—The generous gifts of IFN-α from Dr. J. Gutterman (material from K. Cantell) and IFN-αA and IFN-αD from P. Trown, Ph.D. (Hoffmann-La Roche) are gratefully acknowledged. We thank L. Young, B. Soehnlen, R. Liu and L. Vaught for excellent technical assistance and Mr. J. Isaman for his skill in computer programming.

REFERENCES

1. ISAACS A, LINDENMANN J. Virus interference I. The interferon. *R Soc Lond Proc Ser B, Biol Sci* 1957, **147**, 258–267.
2. PAUCKER K, CANTELL K, HENLE W. Quantitative studies on viral interference in suspended L cells. III. Effect of interfering viruses and interferon on the growth rate of cells. *Virology* 1962, **17**, 324–334.
3. LINDAHL-MAGNUSSEN P, LEARY P, GRESSER I. Interferon inhibits DNA synthesis induced in mouse lymphocyte suspensions by phytohemagglutinin or by allogeneic cells. *Nature (New Biol)* 1972, **237**, 120–121.
4. GRESSER I. On the varied biologic effects of interferon. *Cell Immunol* 1977, **34**, 406–415.
5. BALKWILL F, WATLING D, TAYLOR-PAPADIMITRIOU J. Inhibition by lymphoblastoid interferon of growth of cells derived from the human breast. *Int J Cancer* 1978, **22**, 258–265.
6. LINDAHL P, LEARY P, GRESSER I. Enhancement by interferon of the specific cytotoxicity of sensitized lymphocytes. *Proc Natl Acad Sci USA* 1972, **69**, 721–725.
7. FRIDMAN WH, GRESSER I, BANDER MT *et al.* Interferon enhances the expression of Fcγ receptors. *J Immunol* 1980, **124**, 2436.

8. KRIM M. Towards tumor therapy with interferons, Part I. Interferons: production and properties. *Blood* 1980, **55**, 711; Part II. Interferons: *in vivo* effects. *Blood* 1980, **55**, 875.
9. FELLOUS M, BORO R, HYAFIL F *et al.* Interferon enhances the amount of membrane bound β_2 microglobulin and its release from human Burkitt cells. *Eur J Immunol* 1980, **11**, 524.
10. REVEL M, KIMCHI A, SHULMAN L *et al.* Role of interferon induced enzymes in the antiviral and antimitogenic effects of interferon. *Ann NY Acad Sci* 1980, **350**, 459.
11. STRANDER H. Interferons: antineoplastic drugs. *Blut* 1977, **35**, 277.
12. MELLSTEDT H, AHRE A, BJORKHOLM M *et al.* Interferon therapy in myelomatosis. *Lancet* 1979, **i**, 245–247.
13. GUTTERMAN JU, BLUMENSCHEIN GR, ALEXANIAN R *et al.* Leukocyte interferon-induced tumor regression in human metastatic breast cancer, multiple myeloma and malignant lymphoma. *Ann Intern Med* 1980, **93**, 399–406.
14. GOEDDEL DV, SHEPARD HM, YELVERTON E *et al.* Synthesis of human fibroblast interferon by *E. coli*. *Nucleic Acids Res* 1980, **8**, 4057–4074.
15. NAGATA S, BRACK C, HENCO K *et al.* Partial mapping of ten genes in the human interferon-α family. *J Interferon Res* 1980, **1**, 333–336.
16. GOEDDEL DV, LEUNG DW, DULL TJ *et al.* The structure of eight distinct cloned human leukocyte interferon cDNAs. *Nature* 1981, **290**, 20–26.
17. SALMON SE, DURIE BGM, YOUNG L *et al.* Effects of cloned human leukocyte interferons in the human tumor stem cell assay. *J Clin Oncol* 1983, **1**, 217–225.
18. HAMBURGER AW, SALMON SE. Primary bioassay of human tumor stem cells. *Science* 1977, **197**, 461–463.
19. HAMBURGER AW, SALMON SE, ALBERTS DS. Development of a bioassay for ovarian carcinoma colony forming cells. In: SALMON SE, ed. *Cloning of Human Tumor Stem Cells*. New York, Alan R. Liss, 1980, Ch. 6.
20. CANTELL K, HIRVONEN S, MOGENSEN KE *et al.* In: WAYMOUTH C. eds. *The Production and Use of Interferon for the Treatment and Prevention of Human Virus Infections*. Rockville, MD, Tissue Culture Association, *In vitro* Monograph 1974, Vol. 3, 35–38.
21. GOEDDEL DV, YELVERTON E, ULLRICH A *et al.* Human leukocyte interferon produced by *Escherichia coli* is biologically active. *Nature* 1980, **287**, 411–416.
22. WECK PK, APPERSON S, STEBBING N *et al.* Antiviral activities of hybrids of two major human leukocyte interferons. *Nucleic Acids Res* 1981, **9**, 6153–6166.
23. BUICK RN, TILL JE, MCCULLOCH EA. Colony assay for proliferative blast cells circulating in myeloblastic leukemia. *Lancet* 1977, **i**, 862–863.
24. KRESSNER BE, MORTON RRA, MARTENS AE *et al.* Use of an image analysis system to count colonies in stem cell assays of human tumors. In: SALMON SE, ed. *Cloning of Human Tumor Stem Cells*. New York, Alan R. Liss, 1980, Ch. 15.
25. DURIE BGM, SALMON SE. High speed scintillation autoradiography. *Science* 1975, **190**, 1093–1095.
26. DURIE BGM, SALMON SE, MOON TE. Pretreatment tumor mass, cell kinetics and prognosis in multiple myeloma. *Blood* 1980, **55**, 364–372.
27. HOFFMAN V, SALMON SE, DURIE BGM. Drug resistance in multiple myeloma associated with high *in vitro* incorporation of ^{3}H-thymidine. *Blood* 1981, **58**, 471.
28. DURIE BGM, VAUGHT L, SOEHNLEN B *et al.* Sensitivity to interferons and bisantrene in refractory multiple myeloma; comparison between thymidine suppression, myeloma stem cell culture and clinical results. *Proc Am Soc Clin Oncol* 1982, abstract No. 455.
29. LIVINGSTON RB, TITUS GA, HEILBURN LK. *In vitro* effects on DNA synthesis as a predictor of biological effect from chemotherapy. *Cancer Res* 1980, **40**, 2209–2212.
30. LIVINGSTON RB, AMBUS U, GEORGE SL *et al.* *In vitro* determination of thymidine ^{3}H labeling index in human solid tumors. *Cancer Res* 1974, **34**, 1376–1380.
31. MERRIGAN TC, SIKORA K, BREEDEN JH *et al.* Preliminary observations on the effect of human leukocyte interferon in non-Hodgkin's lymphoma. *N Engl J Med* 1978, **299**, 1449–1453.
32. BRADLEY EC, RUSCETTI FW. Effect of fibroblast lymphoid and myeloid interferons on human tumor colony formation *in vitro*. *Cancer Res* 1981, **41**, 244.
33. GAZDAR AF. Enhancement of tumor growth rate by interferon inducers. *JNCI* 1972, **49**, 1435.
34. EPSTEIN LB. The comparative biology of immune and classical interferons. In: COHEN S, PICK E, OPPERHEIM JL, eds. *Biology of Lymphokines*. New York, Academic Press, 1979, 443.
35. BUICK RN, FRY SE, SALMON SE. Effect of host cell interactions in clonogenic carcinoma cells in human malignant effusions. *Br J Cancer* 1980, **41**, 695–704.

36. KIMCHI A, SKULMAN L, SCHMIDT A *et al.* Kinetics of the induction of three translation-regulatory enzymes by interferon. *Proc Natl Acad Sci USA* 1979, **76,** 3208.
37. STARK GR, DOWER WT, SOBIMKE RT *et al.* 2'-5'-A synthetase assay distribution and variation with growth hormone studies. *Nature* 1979, **278,** 471.
38. WOOD JN, HOVONESSIAN AG. Interferon enhances 2'-5'-A synthetase in enbryonal carcinoma cells. *Nature* 1979, **282,** 74.
39. GREENBERG SB, HARMON MW, COUCH RB. Exogenous interferon: stability and pharmacokinetics. In: STRINGFELLOW DA, ed. *Interferon and Interferon Inducers.* New York, Marcel Dekker, 1980, 57.