26. Cooper DN. Eukaryotic DNA methylation. *H-im Genet* 1983, **64**, 315–333.
27. Holliday R. Strong effects of 5-azacytidine on the *in vitro* lifespan of human diploid fibroblasts. *Exp Cell Res* 1986, **166**, 543–552.

Acknowledgements—We would like to thank Grace Chojnowski for flow cytometric analysis of DNA content and Irene Perkic for help in preparation of the manuscript.
S.D. was supported by the Myra DeGroot Foundation.

Eur J Cancer, Vol. 28, No. 2/3, pp. 368–372, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00

Interactions of Interferon-α_{2a} with 5′-Deoxy-5-fluorouridine in Colorectal Cancer Cells *in vitro*

H.T. Tevaearai, P.L. Laurent, L. Suardet, J.F. Eliason, J-C. Givel and N. Odartchenko

The biological activity of 5′-deoxy-5-fluorouridine (5′-dFUrd) depends upon intracellular enzymatic cleavage by pyrimidine phosphorylase to form 5-fluorouracil (5-FU). Interferon-α_{2a} (IFN-α) effect was analysed alone and combined with 5-FU or 5′-dFUrd, on proliferation inhibition of eight human colorectal cancer cell lines. The toxicity of 5-FU was enhanced by IFN-α in only one line (SW-480). In contrast, interactive enhancement of IFN-α was observed with 5′-dFUrd in five lines (WiDr, HT-29, 513, SW-480 and Co-115). In each of the lines showing potention by IFN/5′dFUrd but not by IFN/5-FU, cytoplasmic pyrimidine phosphorylase activity was increased after 5 days' incubation with IFN-α in a dose-dependent manner. Two lines (LISP-1 and SW-620) showed no potentiation of either 5-FU or 5′-dFUrd toxicity by IFN-α, and no change in pyrimidine phosphorylase activity. Potentiation of 5′-dFUrd effect by IFN-α may thus be explained by an enhancement of its conversion to 5-FU through stimulation of pyrimidine phosphorylase activity.
Eur J Cancer, Vol. 28, No. 2/3, pp. 368–372, 1992.

INTRODUCTION

COLORECTAL CARCINOMAS are remarkably resistant to chemotherapy and, therefore, are mainly left to surgical treatment. Despite significant improvement in diagnosis and surgical techniques, decreased operative mortality and development of several chemotherapy and radiotherapy protocols, overall prognosis has not improved over the past 30 years. Large studies show survival advantage for adjuvant 5-fluorouracil (5-FU) and levamisole, and 5-FU, lomustine and radiotherapy [1, 2]. Clearly, new forms of effective systemic adjuvant therapy should be developed.

The anticancer activity of interferons (IFN) is presently beyond doubt for a limited number of cancers [3], but IFN therapy alone does not have much activity against colorectal cancer[4]. One of the most promising aspects of these cytokines is their ability to modulate the cytotoxic effects *in vitro* of chemotherapeutic drugs, as shown for vinblastine, cisplatin [5] or doxorubicin[6]. Synergistic effects of IFN with fluoropyrimidines *in vitro* have also been reported [7].

Correspondence to H.T. Tevaearai.
H.T. Tevaearai and J.C. Givel are at the Department of Surgery, CHUV, CH-1011 Lausanne; P.L. Laurent, L. Suardet, J.F. Eliason and N. Odartchenko are at the Department of Cellular Biology, ISREC, Epalinges s/Lausanne; and J.F. Eliason is also at Pharma Research, Department of Experimental Dermatology/Oncology, F. Hoffmann-La Roche, Basel, Switzerland.
Revised 3 Sep. 1991; accepted 19 Sep. 1991.

Fluoropyrimidines are commonly used in clinical oncology, but their toxicity limits therapeutic application. The synthetic derivative 5′-deoxy-5-fluoridine (5′-dFUrd) [8] has raised marked interest due to higher therapeutic index as derived from *in vitro* and animal data and as compared to 5-FU or other related compounds [9]. It is also less potent in inducing leukopenia [9, 10] and is not as strongly immunosuppressive as other fluoropyrimidines [11, 12]. The biological activity of this prodrug is dependent upon its enzymatic cleavage to 5-FU by a pyrimidine phosphorylase [13–17], levels of which are markedly increased in human and animal tumours compared to normal tissues, with the exception of the intestinal tract [16, 18–20].

Recently, the combination of interferon α (IFN-α) plus 5-FU has been shown to give objective responses in 20 of 32 previously untreated patients with advanced colorectal carcinoma [21, 22]. This prompted us to analyse the *in vitro* effects of IFN-α on the cytotoxicity of 5-FU and of the prodrug 5′-dFUrd with human colon carcinoma cell lines.

MATERIALS AND METHODS

Cell lines

We used a panel of eight human colon cell lines, derived from primary tumours: WiDr (ATCC: CCL 218), HT-29 (ATCC: HTB 38), SW-480 (ATCC: CCL 228), SW-620 (ATCC: CCL 227), LISP-1 (obtained from Dr D. Lopez, Ludwig Institute for Cancer Research, Sao Paulo, Brazil), Co-115 [23], 513 and 411 (both established in our own laboratory). Cells were cultured

in a 1:1 mixture of EMED (enriched Dulbecco's modified Eagle's medium) and FMED (modified Ham's F-12 nutrient mixture)[24] supplemented with 10% fetal calf serum (Seromed, Biochrom, Berlin). The cell lines were tested periodically for the presence of mycoplasma using the Myco Test assay system (Gibco/BRL, Paisley, UK) and consistently found to be free from contamination.

Drugs

Recombinant human IFN-α_{2a} (Roferon-A, F. Hoffmann–La Roche, Basel) was used in the following concentrations: 100, 1000 and 10000 U/ml. 5-FU and its prodrug 5'-dFUrd were also provided by F.Hoffmann–La Roche.

Cell proliferation assay

The modified colorimetric assay described by Twentyman *et al.* [25] was used to measure the growth inhibiting properties of the drug combinations. This assay is based on the capacity of viable cells to reduce (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) to the blue formazan product. Cells were plated in 96-well flat bottom microtitre plates (Falcon, Becton Dickinson). After 3 days of incubation at 37°C in a fully humidified atmosphere of 5% CO_2 in air, drugs were added to the appropriate wells in triplicate. Preliminary experiments were performed with the cytotoxic drugs alone with each cell line to determine the best concentrations to use for the combination studies. The plates were incubated with drugs for a further 5 days period before 20 μl of MTT stock (5 mg/ml) were added. The plates were returned to the incubator for 4 (WiDr, HT-29, SW-480 SW-620 and LISP-1) or 12 more hours (513, 411 and Co-115). The medium was then removed. The formazan crystals were dissolved in 100 μl of dimethyl sulphoxide. Optical density at 570 nm was measured spectrophotometrically (Easy Reader, EAR 400, Tecan, Hombrechtikon, Switzerland) with a reference reading at 690 nm.

The overall design of the combination studies was as previously described[26]. Briefly, the controls consisted of 4 different cell dilutions (1000, 500, 250 and 100 cells/well) treated with each concentration of IFN-α (0, 100, 1000 and 10000 U/ml). These controls were used to determine the relationship between numbers of cells plated and optical density. This standard cell titration curve was used to convert the absorbance values obtained in wells containing 1000 and incubated with the cytotoxic drugs in combination with each concentration of IFN-α. Four dilutions of 5-FU or 5'-dFUrd were tested in each case.

In essence, this procedure normalises the drug dose response curves, empirically correcting for the effects of IFN-α alone. The doses of cytotoxic drug giving 50% survival (IC_{50}), calculated from the least squares regression lines for log(survival) vs. drug concentration, can then be compared directly. If there is no interaction between IFN-α and the cytotoxic drug, the IC_{50}s for the cytotoxic drug remains the same (often referred to as an additive effect of the two drugs). If there is a synergistic interaction, the IC_{50}s decrease. Statistical significance of the effect of IFN-α on the IC_{50}s was determined using Student's *t* test.

Pyrimidine phosphorylase activity

The activity was measured by the method of Choong and Lee[27] which is based on differences in UV absorption spectra of 5'-dFUrd and 5-FU in alkaline conditions. For each cell line, cells were cultured in 175 cm^2 flasks (Falcon). To reproduce cell culture conditions used for growth inhibitory tests, IFN-α was added at day 3, at the same concentrations as mentioned above. At day 8, cells were scraped away into Ca^{2+}, Mg^{2+}-free phosphate buffered saline, and homogenised with a Teflon pestle for 2 min. Homogenates were centrifuged at 15,000 *g* for 10 min and the supernatants were stored at −70°C.

For the enzymatic assay, cell extracts were incubated for 12 hr at 37°C with 4 μmol 5'-dFUrd. The optical density was measured at 305 nm, which corresponds to the maximum absorption of 5-FU. The production of 5-FU was quantitated using a standard 5-FU curve. The results were normalised to the amounts of protein present in the cellular extracts, using the BioRad (Richmond, California) protein assay, and to minutes of incubation.

Table 1. Concentrations of INF-α and fluoropyrimidines inhibiting proliferation by 50% (IC_{50}s) of eight colorectal cell lines

	IFN-α (103 U/ml)		5-FUra (μmol/l)		5'-dFUrd (μmol/l)	
Cell line	*n*	IC_{50}	*n*	IC_{50}	*n*	IC_{50}
WiDr	3	22 (8)	4	9.6 (3.1)	4	380 (129)
HT-29	3	58 (12)	3	7.0 (2.2)	3	380 (130)
513	3	38 (8)	3	4.9 (1.8)	4	86 (30)
411	3	18 (5)	2	9.8 (0.5)	2	10 (1)
Co-115	3	37 (12)	3	8.9 (2.2)	3	9 (1)
LISP-1	3	24 (6)	4	6.1 (2.1)	4	46 (8)
SW-620	3	147 (27)	2	6.0 (1.8)	2	94 (22)
SW-480	3	14 (5)	2	5.8 (1.7)	2	231 (97)

Mean (S.D.).

RESULTS

The effects of IFN-α, 5-FU and 5'-dFUrd as single agents on proliferation of eight colorectal cell lines are shown in Table 1. None of the cell lines was particularly sensitive to the anti-proliferative effects of IFN-α, since the highest dose tested, 10000 IU/ml, was below the IC_{50} for each. The IC_{50} for 5-FUra fall in a narrow range between 4.9 mol/l for line 513 and 9.8 μmol/l for line 411. In contrast, the IC_{50} for 5'-dFUrd vary approximately 40-fold. The lines Co-115 (9 μmol/l) and 411 (10 μmol/l) were the most sensitive to this drug whereas WiDr and its parental line HT-29 were the least sensitive with identical IC_{50}s of 380 μmol/l.

In order to determine if IFN-α enhances the toxicity of 5-FU and 5'-dFUrd, drug titrations were performed at each concentration of IFN-α and the IC_{50}s for 5-FU and 5'-dFUrd were determined from dose-response curves such as those shown in Fig. 1. As can be seen in Fig. 1a for one representative experiment wiht WiDr cells, the presence of IFN-α had no significant effect on the dose-response curves for 5-FU. On the other hand, the cells were increasingly sensitive to 5'-dFUrd with increasing concentrations of IFN-α (Fig. 1b).

Summaries of effect of IFN-α on the IC_{50}s of 5-FU and 5'-dFUrd in the eight colorectal cell lines are given in Tables 2 and 3, respectively.

The IC_{50}s for 5-Fu in the presence of IFN-α (corrected for the effect of IFN-α alone) are also decreased to a reasonable extent by IFN-α in a dose-dependent way only in one line, SW-480. With the other lines, the combination of IFN-α with 5-FU resulted in only additive effects as seen by the fact that IC_{50}

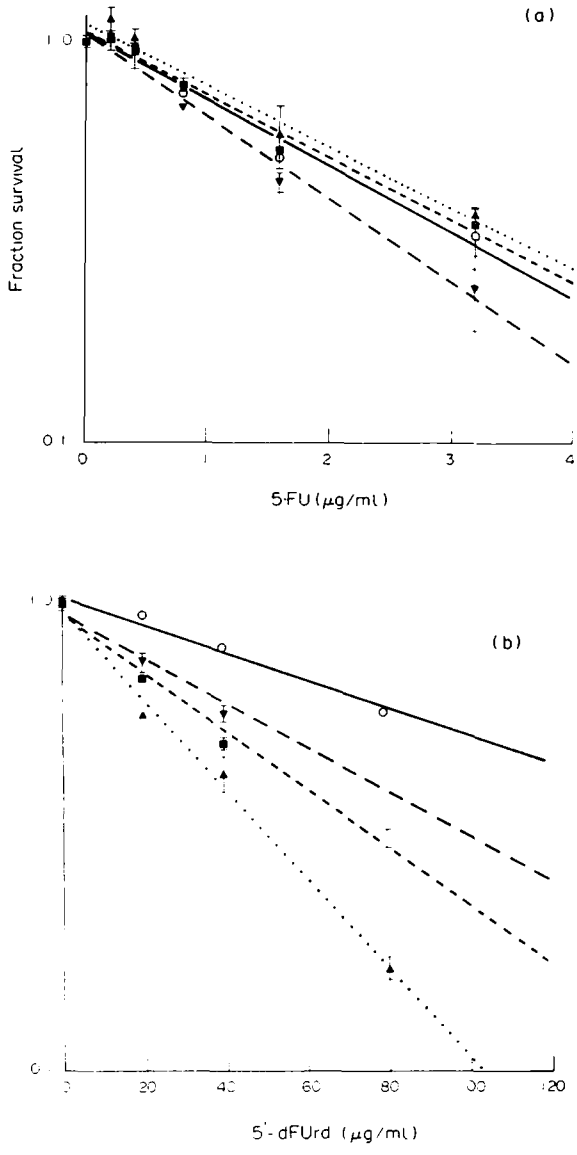

Fig. 1. Drug dose-fraction survival curves for WiDr cells treated with 5-Fu (a) or 5'-dFUrd (b) in combination with 0 (o), 100 (▼), 1000 (■) or 10 000 U/ml IFN-α (▲). The points represent mean values from four replicate wells from one representative experiment. The horizontal bars represent S.D.

Table 2. Effect of IFN-α on IC_{50} of 5-FUra in human colorectal cell lines

		IFN-α (% of control)*		
Cell line	*n*	10^2 U/ml	10^3 U/ml	10^4 U/ml
WiDr	4	90 (14)	110 (24)	102 (16)
HT-29	3	85 (7)	105 (7)	97 (1)
513	3	95 (4)	104 (8)	104 (9)
411	2	106 (1)	96 (4)	127 (10)
Co-115	3	99 (2)	80 (9)	109 (3)
LISP-1	4	110 (20)	102 (18)	102 (13)
SW-620	2	98 (6)	128 (22)	129 (26)
SW-480	2	69 (3)	51 (8)	43 (9)

Mean (S.D.). *Per cent of control IC_{50} in cultures without IFN-α.

Table 3. Effect of IFN-α on IC_{50} of 5'-dFUrd in human colorectal cell lines

		IFN-α (% of control)*		
Cell line	*n*	10^2 U/ml	10^3 U/ml	10^4 U/ml
WiDr	4	59 (15)	50 (14)	43 (15)
HT-29	3	80 (10)	84 (3)	60 (12)
513	4	70 (9)	66 (6)	61 (6)
411	2	72 (2)	71 (4)	57 (2)
Co-115	3	75 (10)	65 (9)	50 (15)
LISP-1	4	106 (18)	102 (12)	94 (12)
SW-620	2	95 (1)	90 (2)	104 (9)
SW-480	2	96 (3)	84 (4)	64 (4)

Mean (S.D.). *Per cent of control IC_{50} in cultures without IFN-α.

values remain virtually the same for all concentrations of IFN-α.

The results presented in Table 3 show that the IC_{50}s of 5'-dFUrd were decreased with higher concentrations of IFN-α for 6 of the cell lines. This indicates an interactive enhancement between the two drugs. Only the lines LISP-1 and SW-620 appeared to be insensitive to the potentiating effect of IFN-α. With 3 of the lines (WiDr, 513 and 411) as little as 100 IU/ml of IFN-α was sufficient to give a statistically significant decrease in IC_{50}s of 5'-dFUrd, and the decrease observed for HT-29 and Co-115 at higher interferon concentrations were statistically significant as well.

One possible explanation for the enhanced interaction between IFN-α and 5'-dFUrd but not with 5-FU is that IFN-α might increase pyrimidine phosphorylase activities in the treated cells, thereby increasing the conversion of the prodrug to the active principle. To examine this possibility, pyrimidine phosphorylase activities of cells incubated under the same conditions as used for the survival studies were determined. The results (Table 4) show that the baseline levels for the different cell lines are roughly correlated with the sensitivites of the lines to 5'-dFUrd, with Co-115 having the highest enzyme activity and WiDr and HT-29 having the lowest activities.

The pyrimidine phosphorylase activities in cytoplasmic extracts from cells treated with IFN-α (Table 4) increased with increasing IFN-α concentration in those cell lines where synergy was seen with 5'-dFUrd but not with 5-FU. This increase was particularly striking in lines 513 and Co-115. There was no

Table 4. Effect of INF-α on pyrimidine phosphorylase activity in human colorectal cell lines

IFN-α	Control	10^2 U/ml	10^3 U/ml	10^4 U/ml
WiDr	0.84 (0.13)	1.05 (0.29)	1.60 (0.24)	1.84 (0.09)
HT-29	0.71 (0.03)	1.03 (0.09)	1.07 (0.10)	1.91 (0.32)
513	1.42 (0.16)	1.77 (0.26)	2.31 (0.39)	3.09 (0.39)
411	1.54 (0.01)	1.54 (0.06)	2.00 (0.30)	2.18 (0.35)
Co-115	2.46 (0.17)	3.04 (0.53)	4.41 (0.32)	5.98 (0.23)
LISP-1	1.63 (0.48)	1.84 (0.60)	1.71 (0.55)	1.81 (0.55)
SW-620	1.65 (0.32)	2.09 (0.03)	1.83 (0.15)	1.80 (0.32)
SW-480	0.93 (0.15)	0.99 (0.23)	1.01 (0.21)	1.19 (0.15)

Mean (S.D.)

change of enzymatic activity in the two lines that showed no enhancement between IFN-α and 5'-dFUrd (LISP-1 and SW-620). There appeared to be an increase as well in the line SW-480 that had IFN-α induced decreases in IC_{50}s for both drugs, but the magnitude (30%) was quite small. Statistical analysis showed that the increase for WiDr, HT-29, 513 and Co-115 lines was significant.

DISCUSSION

The effect of fluoropyrimidines on cellular metabolism depends on the ability of the cells to form the active nucleotides 5'-fluorouridine 5'-triphosphate (FUTP), 5'-fluorodeoxyuridine 5'-triphosphate (FdUTP) or 5-fluorodeoxyuridine 5'-monophosphate (FdUMP). The first two compounds compete with their normal counterparts for incorporation into RNA and DNA, respectively. The last nucleotide, FdUMP, prevents DNA synthesis by inhibiting thymidilate synthetase. The activity of orotate phosphoribosyl transferase is also a determinant for cell response to 5-FU therapy [28] because it catalyses the first conversion to 5-fluorouridine 5'-monophosphate (FUMP). The prodrug 5'-dFUrd must be cleaved enzymatically to 5-FU to be biologically active. Pyrimidine phosphorylase, which is responsible for that conversion, appears then essential for efficacity of 5'-dFUrd.

In our study, the sensitivity of the eight different cell lines to 5-FU differed only 2-fold whereas the sensitivity to the prodrug 5'-dFUrd varied widely. We therefore, analysed the activity of pyrimidine phosphorylase, using an assay that measures the conversion of 5'-dFUrd to 5-FU directly. Our results show that the enzyme level is related to the sensitivity of cells to the prodrug 5'-dFUrd: HT-29, WiDr and SW-480 cell lines possessed the lowest enzyme activity and were the less sensitive to effect of 5'-dFUrd. Our results with the HT-29 line differ from those of Wadler *et al* [29]. This disparity might be answered by examining the time course of the IFN-induced changes in pyrimidine phosphorylase activity and/or by using cytometry. The enzymatic activity was high in 513, LISP-1 and Co-115 cell lines, which were the cells most sensitive to the prodrug.

Several groups have reported that IFN-α enhances the cytotoxic effects or 5-FU *in vitro* [7, 30], although in most studies it is difficult to determine precisely if the effects are truly synergistic or merely additive. The method we have used was developed specifically to examine synergistic interactions between two drugs [24]. We found some evidence of an enhancement beween IFN-α and 5-FU with only one of the lines that we tested (SW-480). The exact mechanism by which IFN-α might potentiate the action of 5-FU in this line is not known, but results of studies with HL-60 leukaemia cells suggest that it could be by increasing the accumulation of FdUMP in cells which had been treated with IFN, thus causing increased thymidilate synthetase inhibition [31].

In five of the seven other lines (WiDr, HT-29, 513, 411 and Co-115), IFN-α had a dose-dependent effect to increase the sensitivity of the cells to the prodrug 5'-dFUrd. The fact that such an effect was seen with 5'-dFUrd and not 5-FU suggests that IFN-α might act by increasing the activity of pyrimidine phosphorylase, thereby increasing the quantity of 5-FU in the tumour cells. Therefore, this enzyme activity was determined in cells previously treated with IFN-α. The results confirm that cell lines showing an enhanced effect between 5'-dFUrd and IFN-α (WiDr, HT-29, 513, 411 and Co-115) exhibit enhanced pyrimidine phosphorylase activity, while no change is noted in two cell lines (LISP-1 and SW-620) where no enhancement occurred. Induction of pyrimidine phosphorylase activity thus appears to be a possible mechanism through which IFN-α can potentiate the effect of 5'-dFUrd.

Encouraging preliminary clinical findings show objective responses with the combination of IFN-α and 5-FU [21, 22] in 20 of 32 patients with advanced colorectal cancer. These may not be entirely due to direct effects of IFN-α on colorectal tumour cells. Other possible factors may well be important, such as enhancement of host immune responses as it has been demonstrated for IFN-γ [32], or as protection of the haematopoietic system in mice [33]. Taken together, our results suggest that additional benefit might be expected. If the increased level of pyrimidine phosphorylase activity that we have observed *in vitro* also occurs in patient tumours, the use of 5'-dFUrd in combination with IFN-α could indeed reveal to be even more effective and less toxic than its combination with 5-FU.

1. Carbone PP. Progress in the systemic treatment of Cancer. Concepts, trials, drugs and biologics. *Cancer* 1990, **65**, 625–633.
2. Wadler S, Schwartz EL. Antineoplastic activity of the combination of interferon and cytotoxic agents against experimental and human malignancies, a review. *Cancer Res* 1990, **50**, 3473–3486.
3. Balkwill FR. Interferons. In: Balkwill FR, ed. *Cytokines in Cancer Therapy*. Oxford, Oxford University Press, 1989, 8–53.
4. Krown SE, Mintzer D, Cunningham-Rundles S, *et al*. High-dose human lymphoblastoid interferon in metastatic colorectal cancer: clinical results and modification of biological responses. *Cancer Treat Rep* 1987, **71**, 39–45.
5. Aapro MS, Alberts DS, Salmon SE. Interactions of human leucocyte interferon with vinca alkaloids and other chemotherapeutic agents against human tumors in clonogenic assay. *Cancer Chemother Pharmacol* 1983, **10**, 161–166.
6. Welander CE, Morgan TM, Homesley HD, Trotta PP, Spiegel RJ. Combined recombination human interferon alpha2 and cytotoxic agents studied in a clonogenic assay. *Int J Cancer* 1985, **35**, 721–729.
7. Elias L, Crissman HA. 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.
8. Cook AF, Holman MJ, Kramer MJ, Trown PW. Fluorinated pyrimidine nucleosides. III. Synthesis and antitumor activity of a series of 5'-deoxy-5-fluoropyrimidine nucleosides. *J Med Chem* 1979, **22**, 1330–1335.
9. Bollag W, Hartmann HR. Tumor inhibitory effects of a new fluorouracil derivative: 5'-deoxy-5-fluorouridine. *Eur J Cancer Clin Oncol*, 1980, **16**, 427–432.
10. Kramer MJ, Trown PW, Cleeland R, Cook AF, Grunberg E. 5'-deoxy-5-fluorouridine, a new orally active antitumor agent. Comparative activity with 5-fluorouracil, 2'-deoxy-5-fluorouridine and ftorafur against transplantable tumors in mice and rats. *Proc Am Assoc Cancer Res* 1979, **20**, 79.
11. Connolly KM, Diasio RB, Armstrong RD, Kaplan AM. Decreasd immunosuppression associated with antitumor activity of 5-deoxy-5-fluorouridine compared to 5-fluorouracil and 5-fluorouridine. *Cancer Res* 1983, **43**, 2529–2535.
12. Ohta Y, Sueki K, Kitta K, Takemoto K, Ishitsuka H, Yagi Y. Comparative studies on the immunosuppressive effect among 5'-deoxy-5-fluorouridine, ftorafur, and 5-fluorouracil. *Jpn J Cancer Res* 1980, **71**, 190–196.
13. Armstrong RD, Diasio RB. 5'-deoxy-5-fluorouridine: cellular metabolism of a new fluoropyrimidine with antineoplastic activity. *Proc Am Assoc Cancer Res* 1979, **260**, 1054.
14. Armstrong RD, Diasio RB. Metabolism and biological activity of 5'-deoxy-5-fluorouridine, a novel fluoropyrimidine. *Cancer Res* 1980, **40**, 3333–3338.
15. Ishitsuka H, Miwa M, Takemoto K, Fukuoka K, Itoga A, Maruyama HB. Role of uridine phosphorylase for antitumor activity of 5'-deoxy-5-fluorouridine. *Jpn J Cancer Res* 1980, **71**, 112–123.
16. Kono A, Hara Y, Sugata S, Karube Y, Matsushima Y, Ishitsuka H. Activation of 5'-deoxy-5-fluorouridine by thymidine phos-

phorylase in human tumors. *Chem. Pharmacol Bull* 1983, **31**, 175–178.
17. Armstrong RD, Gesmonde J, Wu T, Cadman E. Cytotoxic activity of 5'-deoxy-5-fluorouridine in cultured human tumors. *Cancer Treat Rep* 1983, **67**, 541–545.
18. Zimmerman M, Seidenberg J. Deoxyribosyl transfer. I. Thymidine phosphorylase and nucleoside deoxyribosyltransferase in normal and malignant tissues. *J Biol Chem* 1964, **239**, 2618–2627.
19. Suzuki S, Hongu Y, Fukazawa H, Ichihara S, Shimizu H. Tissue distribution of 5'-deoxy-5-fluorouridine and derived 5-fluorouracil in tumor-bearing mice and rats. *Jpn J Cancer Res* 1980, **71**, 238–245.
20. Choong YS, Lee SP, Alley PA. Comparison of the pyrimidine nucleoside phosphorylase activity in human tumors and normal tissues. *Exp Pathol* 1988, **33**, 23–25.
21. Wadler S, Schwartz EL, Goldman M, *et al.* Fluorouracil and recombinant alpha-2a-interferon: an active regimen against advanced colorectal carcinoma. *J Clin Oncol* 1989, 7, 1769–1775.
22. Wadler S, Wiernik PH. Clinical update on the role of fluorouracil and recombinant interferon alpha-2a in the treatment of colorectal carcinoma. *Semin Oncol* 1990, **17**, 16–20.
23. Carrel S, Sordat B, Merenda C. Establishment of a cell line (Co-115) from a human colon carcinoma transplanted into nude mice. *Cancer Res* 1976, **36**, 3978–3984.
24. Eliason JF, Aapro MS, Decrey D, Brink-Petersen M. Non-linearity of colony formation by human tumor cells from biopsy samples. *Br J Cancer* 1985, **52**, 311–318.
25. Twentyman PR, Luscombe M. A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. *Br J Cancer* 1987, **56**, 279–285.
26. Eliason JF, Ramuz H, Kaufmann F. Human multi-drug-resistant cancer cells exhibit a high degree of selectivity for stereoisomers of verapamil and quinidine. *Int J Cancer* 1990, **46**, 113–117.
27. Choong YS, Lee SP. The degradation of 5'-deoxy-5-fluorouridine by pyrimidine nucleoside phosphorylase in normal and cancer tissues. *Clin Chim Acta* 1985, **149**, 175–183.
28. Peters GJ, Laurensse E, Leyva A, Lankelma J, Pinedo HM. Sensitivity of human, murine, and rat cells to 5-fluorouracil and 5'-deoxy-5-fluorouridine in relation to drug-metabolizing enzymes. *Cancer Res* 1986, **46**, 20–28.
29. Wadler S, Wersto R, Weinberg V, Thompson D, Schwartz DL. Interaction of fluorouracil and interferon in human colon cancer cell lines: cytotoxic and cytokinetic effects. *Cancer Res* 1990, **50**, 5735–5739.
30. Namba M, Miyoshi T, Kanamori T, Nobuhara M, Kimoto T, Ogawa S. Combined effects of 5-fluorouracil and interferon on proliferation of human neoplastic cells in cultures. *Jpn J Cancer Res* 1982, **73**, 819–824.
31. Elias L, Sandoval JM. Interferon effects upon fluorouracil metabolism by HL-60 cells. *Biochem Biophys Res Commun.*, 1989, **163**, 867–874.
32. Morikawa K, Fan D, Denkins YM, *et al.* Mechanisms of combined effects of γ-interferon and 5-fluorouracil on human colon cancers implanted into nude mice. *Cancer Res* 1989, **49**, 799–805.
33. Stolfi RL, Martin DS, Sawyer RC, Spiegelman S. Modulation of 5-fluorouracil-induced toxicity in mice with interferon or with the interferon inducer, polyinosinic-polycytidylic acid. *Cancer Res* 1983, **43**, 561–566.

Acknowledgements—This work was supported by the Swiss National Foundation for Scientific Research, F. Hoffmann–La Roche Ltd and the Swiss Cancer League. We are deeply indebted to Dr B. Sordat for kindly providing the HT-29, Co-115, SW-480 and SW-620 cell lines, and to Mrs M. Lorenzoni for technical assistance.

Eur J Cancer, Vol. 28, No. 2/3, pp. 372–377, 1992.
Printed in Great Britain

0964–1947/92 $5.00 + 0.00
Pergamon Press plc

Expression of pp60$^{c\text{-}src}$ in Human Small Cell and Non-small Cell Lung Carcinomas

Natalia N. Mazurenko, Eugenia A. Kogan, Irina B. Zborovskaya and Fjodor L. Kisseljov

c-*src* protein was found in 60% of lung carcinomas (20 of 33 cases or primary tumours) by immunoblotting with a monoclonal antibody (Mab 327) and immunohistochemistry with serum from rabbits bearing tumours induced by Rous sarcoma virus. *src* protein expression was assessed in 4 small cell lung carcinomas and in an atypical carcinoid of neuroendocrine origin. However, pp60$^{c\text{-}src}$ was also found in non-small cell lung carcinomas: in 60–80% of adenocarcinomas and bronchiolo-alveolar cancers and in 50% of squamous cell carcinomas. In the squamous cell carcinomas, *src* protein was expressed more frequently in poorly differentiated than in well and moderately differentiated carcinomas. Expression of pp60$^{c\text{-}src}$ was not found in epithelial cells of histologically unchanged lung tissues. These results show that pp60$^{c\text{-}src}$ may be activated in human lung carcinomas of different histopathological types.
Eur J Cancer, Vol. 28, No. 2/3, pp. 372–377, 1992.

INTRODUCTION

THE PROTOONCOGENE c-*src* is the normal cellular homologue of the Rous sarcoma virus transforming gene v-*src*, which codes pp60$^{c\text{-}src}$ membrane-associated phosphoprotein with endogenous tyrosine-specific protein kinase activity [1]. The highest levels of pp60$^{c\text{-}src}$ have been found in the brain [2], platelets [3], and peripheral blood lymphocytes [3]. A study on differentiation and development of the neural retina showed elevated pp60$^{c\text{-}src}$, with high c-*src* kinase activity levels [4]. Whereas some oncogenes (*myc, fos, ras*) are widely expressed in human tumours, mRNA analysis [5, 6] shows that c-*src* activation is restricted to tumours of neuroendocrine origin, especially neuroblastomas [7, 8]. pp60$^{c\text{-}src}$ kinase activity was also found in mammary [9] and colon [10] carcinomas.