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Feature Articles

Treatment of Advanced Colorectal Cancer with 5-Fluorouracil and Interferon-α: An Overview of Clinical Trials

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5-Fluorouracil (5-FU) is the most active single agent for treatment of advanced colorectal cancer, although objective responses occur in only 20% of patients, and there seems to be no impact on overall survival. Experimental findings suggesting that interferon-α (IFN-α) enhances 5-FU cytotoxicity have stimulated an increasing number of clinical trials to evaluate the therapeutic potential of this combination. This article summarises the possible mechanisms of interaction of 5-FU and IFN-α, and provides an overview of the current status of this approach in advanced colorectal cancer. A computerised (Medline) and manual search were performed to identify all trials using 5-FU and IFN-α for the treatment of advanced colorectal cancer published in the English literature between 1960 and 1994. Information abstracted included treatment regimen, number of patients, pretreatment status, complete and partial remissions, remission duration, overall survival, and toxicity. A total of 417 patients were enrolled in 16 trials using different regimens of 5-FU and IFN-α, and double modulation of 5-FU with leucovorin (LV) and IFN-α was investigated in nine trials involving 332 patients. The mean overall response rate in these phase II trials was only 31% (range 3–76) and 35% (range 0–54), respectively. Early results of six prospectively randomised studies of 5-FU or 5-FU/LV ± IFN-α also did not suggest a significant enhancement of the antitumour effectiveness with the addition of IFN-α. There is increasing evidence, however, that administration of IFN-α along with 5-FU enhances toxicity. Because of their modest therapeutic index, currently employed regimens of 5-FU ± LV plus IFN-α cannot be recommended for routine use at the present time. The combination of 5-FU plus LV represents an equally effective and less expensive alternative. Nevertheless, there is still hope that further attempts to elucidate the complex mechanisms of this potentially synergistic drug combination will allow the rational design of regimens with a superior therapeutic index.

Key words: colorectal cancer, chemotherapy, biochemical modulation, 5-fluorouracil, interferon-α
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INTRODUCTION

SINCE THE introduction of 5-fluorouracil (5-FU) more than three decades ago, there have been only little therapeutic advances in the treatment of metastatic colorectal cancer. Attempts to enhance the antimetabolic effect of 5-FU have included the use of sequential methotrexate with 5-FU [1, 2], leucovorin combined with 5-FU [3–7], and thymidine, uridine or *N*-(phosphonacetyl)-L-aspartate (PALA) [8–10] with 5-FU. Despite attractive biochemical rationales and *in vitro* evidence of improved anticancer activity, these various treatment strategies have had only minor effects upon the clinical response rate in metastatic colorectal cancer, without significantly enhancing complete response (CR) or survival duration. New approaches are clearly needed to improve these results.

Based upon preclinical data suggesting that interferon-α (IFN-α) and 5-FU have a synergistic cytostatic effect upon cultured colon cancer cells [11], Wadler and associates [12, 13] and numerous other investigators soon started to investigate the potential benefits of this combination in the clinical setting. In this article, after briefly reviewing the mechanisms of action that have been postulated to explain the synergism of 5-FU and IFN-α, available clinical data including preliminary results of randomised controlled studies of this combination will be reviewed and discussed.

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METHODS

We review all trials using 5-FU plus IFN-α in the treatment of advanced colorectal cancer reported in the English literature. Using a computerised (Medline) and manual search, we were able to identify 25 phase II studies and six randomised phase III studies published between 1989 and 1994. Eligibility criteria included histological proof of colorectal cancer and radiological evidence of recurrence. Only studies requesting measurable disease were included. Studies with previous chemotherapy for advanced disease were not excluded, but are clearly labelled as such. No effort was made to search for unpublished trials, thus publication bias cannot be excluded. Information abstracted included treatment regimen, number of patients, pretreatment status, objective (complete and partial) remissions, remission duration, overall survival, and toxicity. Tumour responses were analysed as reported by the investigators. The response criteria were essentially identical in all trials; a CR was defined as the disappearance of all detectable tumour, and a partial response (PR) was defined as a ≥50% reduction in the sum of the products of the largest perpendicular diameters of measurable disease without new lesions. In this analysis, minimal response, stable disease and progressive disease were classified as no response. Survival was defined as the time from study entry/randomisation to the date of death, whatever its cause.

MECHANISMS OF INTERACTION OF 5-FU AND IFN-α

The interferons are a family of proteins that possess potent antiviral, immunomodulatory and antiproliferative effects [14]. Whereas clinical trials with interferons as single agents have remained disappointing in most solid tumours, including colorectal cancer [15, 16], several laboratories have demonstrated that IFN-α may interact with 5-FU or fluorodeoxyuridine in a greater than additive manner to produce cytotoxicity in a variety of cell lines [17–20]. Because of the ability of thymidine to rescue cells from the additive effects of IFN-α, several investigators have concluded that IFN-α enhances the DNA-directed actions of 5-FU [21, 22]. In HL-60 leukaemia cells and HT-29 cells, IFN-α selectively augmented anabolism of 5-FU to its active metabolite, fluorodeoxyuridylate (FdUMP), by inducing an 8-fold increase in the activity of thymidine phosphorylase (the initial enzyme responsible for 5-FU anabolism) [23].

These *in vitro* data suggesting IFN-α mediated enhanced intratumoral 5-FU anabolism have been supported by *in vivo* ^{19}F magnetic resonance spectroscopy measurements in hepatic metastases [24]. Increased levels of FdUMP resulted in augmented competition with the endogenous nucleotide, deoxyuridylate (dUMP), for access to thymidylate synthase (TS), the target enzyme for 5-FU. In HT-29 and SW 480 cells, IFN-α treatment also resulted in significantly decreased deoxythymidine triphosphate (dTTP) pools as compared with cells treated with 5-FU alone, producing an increase in DNA double-strand breaks [25]. In other experimental model systems, Houghton and associates [26] reported that although IFN-α did not modulate 5-FU metabolism, it did enhance DNA damage resulting from fluoropyrimidine exposure.

Apart from these IFN-α effects at the cellular level, another mechanism which might explain the synergistic effect with 5-FU is a pharmacokinetic interaction between the two drugs, at least with certain regimens. Serial measurements of 5-FU levels by high pressure liquid chromatography [16] suggested that after a single bolus injection of IFN-α2b, the level of 5-FU rises 16-fold within 1 h, and that this elevation persists for hours. Grem [27] and other investigators [28] have reported dose-dependent inhibition of bolus 5-FU clearance by IFN-α, resulting in an increase in the 5-FU area under the curve (AUC). Yee and associates [29] have shown that IFN-α inhibits the activity of dihydropyrimidine dehydrogenase (DPD), the hepatic enzyme responsible for the catabolism of 5-FU to its inactive metabolites, thereby providing one potential explanation for this phenomenon. Whereas Danhauser and associates [30] and Lindley and associates [31] found that IFN-α increases plasma 5-FU concentrations when administered in a continuous intravenous (i.v.) infusion schedule, subsequent analysis of 5-FU concentrations by a method that adjusts for its known diurnal variation [32], failed to detect such significant effects of IFN-α on 5-FU kinetics for the continuous administration schedule [33].

A third interpretation of the interaction of 5-FU and IFN-α involves the immunomodulatory effects of IFN [34–38]. IFN-α augments the activity of various classes of cytotoxic effector cells both *in vitro* and *in vivo*. Also, by increasing the expression of human leucocyte class I antigens, IFN-α reduces the sensitivity of tumour cell lines to cell-mediated killing, an effect termed resistance. It has been demonstrated that 5-FU reverses the resistance in a time- and dose-dependent manner [39]. This effect of 5-FU is not blocked by thymidine and is generated more readily by the ribosylated than by the deoxyribosylated analogue of 5-FU. The effect of 5-FU in the resistance system may be mediated through modulation of HLA class I antigens.

The combination of 5-FU with IFN-α thus seems to represent a promising and logical therapeutic strategy to potentiate 5-FU cytotoxicity, although the biochemical basis and the complex interplay of the two drugs may not be fully understood.

PHASE II TRIALS OF 5-FU AND IFN-α

Preclinical evidence suggested that IFN-α should be administered concurrently with 5-FU for optimal potentiation. Based on demonstrations of a dose- and schedule-dependent enhancement of the cytotoxicity of the antimetabolite by IFN-α2 in human tumour cell lines [40], a pilot phase II trial in 30 patients with advanced colorectal cancer was initiated at the Albert Einstein Medical Center in New York in 1989. The treatment regimen consisted of 750 mg/m^2/day 5-FU administered as a loading course by continuous i.v. infusion over 5 consecutive days, followed by a week of rest and weekly bolus therapy thereafter. Recombinant IFN-α was given subcutaneously three times per week at a dose of 9 million units starting with day 1 of the protocol. Wadler and colleages [12] reported 13 partial remissions, all of which were noted among a subgroup of 17 patients who had received no prior chemotherapy for their colorectal cancer. Responses were noted in liver and lung metastases, in patients with bulky disease as well as with lower tumour burden. Extension of this trial to 32 untreated patients, published in 1990, again demonstrated an objective response in 63% [13]. These impressive therapeutic results led to confirmatory trials at the University of Texas M.D. Anderson Cancer Center and the Memorial Sloan-Kettering Cancer Center that were similar in patient eligibility criteria, treatment regimen and response criteria. Objective response rates for these trials were 36% (1 CR, 15 PR out of 45 patients) and 26% (9 PR out of 35 patients), respectively [41, 42]. Additional phase II studies that were initiated in an attempt to further evaluate the therapeutic potential of this combination are provided in Table 1. The majority of these trials, whether using the empirically designed regimen of Wadler and colleagues [43–47] or other modified 5-FU/IFN-α dose schedules [16, 48–53], have also failed to

Table 1. Results of trials of 5-fluorouracil (5-FU) plus interferon-α (IFN-α) for treatment of colorectal cancer

Reference	5-FU dose	IFN-α dose	No. of patients	Responses CR	PR	%OR
Wadler [12]	750 mg/m²/day CI days 1–5, then weekly bolus	9 MU 3 times per week	17*	1	12	76
			13†	—	—	—
Wadler [13]	As above	As above	32*	—	20	63
Pazdur [41]	As above	As above	45*	1	15	36
Kemeny [42]	As above	As above	35*	—	9	26
Huberman [43]	As above	As above	8*	—	3	36
Wadler [44]	As above	As above	36*	1	14	41
Weh [45]	As above	As above	55*	—	17	31
Rubio [46]	As above	As above	35*	3	5	23
Aiba [47]	As above	6 MU 3 times per week	25*†	—	6	24
Meadows [16]	300 mg/m²/day CI for 8 weeks	2–10 MU/day	17*†	2	2	23
Kreuser [48]	10 mg/kg/day 2 times per week	2 MU 2 times per week	6†	—	1	17
De Vecchis [49]	450 mg/m²/day, days 1–5	3 MU day 0, 0.5 MU days 1–8	12*	1	1	17
Pavlick [50]	225 mg/m² CI	3.4 MU 3 times per week	11†	—	4	36
John [51]	250 mg/m² CI	3 MU 3 times per week	24*†	3	6	38
Meehan [52]	400 mg/m²/day, days 1–5	3 MU 3 times per week	17*†	—	3	18
Clark [53]	750 mg/m²/days, days 1–5	20 MU i.v. days 1–5, or 5 MU s.c./day	29†	—	1	3
Total			417	12	119	31

CR, complete response; PR, partial response; %OR, overall response rate in percent. *Untreated patients. †Patients who had received previous 5-FU based chemotherapy for advanced disease. CI, continuous infusion; i.v., intravenous, s.c., subcutaneous.

reproduce the initial results. There was evidence from these studies, however, including the original trial by Wadler and associates, that IFN-α significantly enhances systemic toxicities of 5-FU. Some authors noted that the toxicity profile of the combination regimen was different from the toxicities seen with either agent alone. Major side-effects associated with 5-FU/IFN-α included mucositis, diarrhoea, neutropenia and neurotoxicity. A novel clinical syndrome described with this therapy was the occurrence of watery diarrhoea followed by life-threatening septic complications. In the Memorial Sloan-Kettering investigators' trial, a particularly high incidence of neurological adverse reactions was noted [26]: 34% of the patients treated in this study developed CNS toxicity consisting of dizziness, gait disturbances and amnesia, and there was even one case with irreversible dementia. Eighty-four per cent of the patients in this trial required a dose reduction of IFN-α2a by 50%, and 63% required a ≥25% decrease in the 5-FU dose.

PHASE II TRIALS WITH 5-FU, LEUCOVORIN AND IFN-α

A number of other strategies to modulate the cytotoxicity of 5-FU have been investigated in an effort to improve its antitumour activity. One approach has focused on the use of the reduced folate leucovorin in combination with 5-FU to stabilise the binding of the active metabolite, fluorodeoxyuridine monophosphate, to its target enzyme, TS. The 5-FU/LV combination produced statistically significant improvements in response rates compared with 5-FU alone in several randomised trials in metastatic colorectal cancer [54–58], although an improvement in survival was noted in only two trials [7, 59]. Inhibition of thymidine incorporation into DNA is likely to be enhanced by IFN-α as a result of its inhibitory effect on thymidine kinase. Because resistance to 5-FU/LV has been correlated with the latter salvage pathway of thymidine, synergy might be expected by combining these two agents with IFN-α. Based on this biochemical rationale of inhibiting two key enzymes of 5-FU metabolism, a number of trials have been planned and performed in order to investigate the possibility of biochemical double modulation and to assess the toxicity profile of this combination. The first clinical results with this three-drug-combination were published in 1990 by Yalavarthi and coworkers [60]. The treatment schedule consisted of 1–20 MU IFN-α/day for 5 days and 370–425 mg/m²/day 5-FU also given on 5 consecutive days, initiated 1 day after IFN-α administration. To further enhance the antitumour activity, leucovorin 500 mg/m²/day was given over 5 days with 5-FU. Only 7 of 31 patients (23%) entered into this trial demonstrated PR, which was disappointing. In the next 2 years, several investigators tested the potential value of 5-FU double modulation with IFN-α plus LV [61–68], and various modes of drug administration were studied in order to define the optimal therapeutic approach (Table 2).

In a study conducted by Grem and associates [61], 44 patients received 370 mg/m²/day 5-FU along with 500 mg/m²/day LV. Both drugs were given on days 2–6 in 4-weekly intervals. IFN-α was administered subcutaneously on days 1–7 of each treatment course using a daily dose of 5 MU/m². Three complete and 21 partial responses were noted with this schedule yielding an overall response rate of 54%. In this trial, 27% of the patients required dose reductions of 5-FU, and 26% of IFN-α because of severe side-effects. Grade 3–4 mucositis and diarrhoea were experienced by 37 and 40% of the patients, and 13% developed granulocytopenia grade 3 or 4. Pharmacokinetic analysis suggested that IFN-α altered the AUC of 5-FU; patients experiencing grade 3–4 diarrhoea or mucositis showed a statistically significant correlation with elevated AUCs when compared with patients experiencing less severe side-effects [69]. Further efforts

Table 2. Results of trials of 5-fluorouracil (5-FU), leucovorin (LV) plus interferon-α (IFN-α) for treatment of colorectal cancer

Reference	5-FU/LV regimen	IFN-α dose	No. of patients	Responses CR	PR	%OR
Yalavarthi [60]	370–425 mg/m²/day 5-FU + 500 mg/m²/day LV, days 2–6	1–20 MU days 1–5	31*	—	7	23
Grem [61]	As above	5–10 MU days 1–7 or 3 MU days 1–14	44*	3	21	54
Kreuser [62]	500 mg/m²/day 5-FU (4 h) + 200 mg/m²/day LV, days 1–7	5 MU days 1–7	45*	1	13	31
Löffler [63]	2000 mg/m² 5-FU (24 h) + 500 mg/m² LV (1 h)	3 MU ×3/week	54*†	4	23	50
Bernhard [64]	500 mg/m²/day 5-FU + 500 mg/m²/day LV (2 h) × 1/week	9 MU ×3/week	15†	—	—	0
Punt [65]	60 mg/kg 5-FU (48 h) + 90 mg LV, ×8 orally	3–10 MU	10*	—	3	30
Schmoll [66]	500 mg/m²/day 5-FU + 200 mg/m²/day LV, days 1–5	5 MU days 1–5	32*	—	3	9
Cascinu [67]	370 mg/m²/day 5-FU + 200 mg/m²/day LV, days 2–6	3 MU days 1–7	45*	6	17	51
Labianca [68]	400 mg/m²/day 5-FU +200 mg/m²/day LV, days 1–5	3 MU on alternate days, daily along with 5-FU	56*	2	13	24
Total			332	16	100	35

CR, complete response; PR, partial response; %OR, overall response rate in percent. *Untreated patients. †Patients who had received previous 5-FU based chemotherapy for advanced disease.

were made in an attempt to optimise the sequence, administration schedule and dose of the three therapeutic components (Table 2). Although encouraging results were reported in some of these studies, their relevance seems hampered by the small numbers of patients and the considerable interstudy variations in terms of treatment effects, despite the use of almost identical drug regimens.

In summary, although the majority of phase II studies with 5-FU, IFN-α ± LV have demonstrated objective responses in approximately 30% of patients with advanced colorectal cancer, there is a wide range between different studies (Tables 1 and 2). The small number of patients in some of these trials and differences in pretreatment characteristics, such as inclusion of patients refractory to 5-FU monotherapy, may partially account for these divergent therapeutic results.

RANDOMISED PHASE III TRIALS

To date, preliminary results of six randomised trials have been published using various approaches of immunomodulation of 5-FU by IFN-α (Table 3). An Italian trial published by Recchia and associates [70] was designed to clarify whether IFN-α could further potentiate the therapeutic potential of 5-FU in combination with high-dose leucovorin. 83 patients were entered to this trial. Therapy consisted of 200 mg/m²/day i.v. bolus leucovorin followed by 370 mg/m²/day 5-FU on days 1–5. In the second treatment arm, 3 MU IFN-α three times a week administered subcutaneously for the entire duration of the study was added to the same chemotherapeutic regimen. Courses were repeated every 4 weeks. 72 patients were evaluable for response, survival and toxicity, and patient characteristics appeared to be well balanced in both arms. The response rate in the 5-FU/LV arm was 45% (6 CR, 12 PR out of 40 patients) and 22% (2 CR, 5 PR out of 32 patients) in the experimental arm using IFN-α. The authors concluded that no benefit could be achieved with the addition of IFN-α in this dose and schedule. Two other controlled trials utilising somewhat different IFN-α doses support these findings. A group of investigators from Buenos Aires [71] randomised 55 patients between treatment with 5-FU/leucovorin with or without IFN-α administered at a daily dose of 5 MU. Their preliminary results do not suggest a statistically significant difference in terms of treatment outcome between the two arms. Disappointing results have also been reported by the Hellenic Cooperative Oncology Group [72]. To date, a total of 95 patients have been enrolled in this trial. The response rates were 19% in the arm without, and 6% in the arm with coadministration of IFN-α.

The aim of a French multicentre trial initiated in September 1990 was to compare the therapeutic index of the treatment regimen originally designed by Wadler and colleages [12, 13] with that of 5-FU monotherapy using the same dose schedule. 28 patients with colonic and 15 patients with rectal adenocarcinoma were thus randomised to treatment with 5-FU given as continuous infusion for 5 days, followed by weekly bolus administration or to the same regimen plus 9 MU IFN-α three times a week [73]. Interim results indicate an overall response activity of 24% (1 CR, 4 PR in 21 patients) in the IFN-α arm and 18% (4 PR in 22 patients) in the 5-FU control arm. Mean duration of response was similar in both patient cohorts. A higher rate of adverse

Table 3. Results of randomised trials of 5-FU (± LV) versus 5-FU (± LV) plus IFN-α in the treatment of colorectal cancer

Reference	No. of evaluable patients	Response rate 5-FU CR	PR	%OR	Response rate 5-FU/IFN-α CR	PR	%OR
Recchia [70]	72	6	12	45 (18/40)	2	5	22 (7/32)
5-FU: 370 mg/m²/day, days 1–5, leucovorin: 200 mg/m²/day, days 1–5, IFN-α: 3 MU ×3/week							
Pensel [71]	48	3	5	33 (8/24)	4	6	41 (10/24)
5-FU: 600 mg/m²/day, leucovorin: 100 mg/m²/day, days 1–5, IFN-α: 5 MU day, days 1–5							
Kosmidis [72]	90	2	7	19 (9/44)	1	2	6 (3/46)
5-FU: 450 mg/m² weekly for 6 weeks, leucovorin: 200 mg/m² weekly, IFN-α: 5 MU ×3/week							
Dufour [73]	43	0	4	18 (4/22)	1	4	24 (5/21)
5-FU: 750 mg/m²/day CI, days 1–5, then weekly bolus, IFN-α: 9 MU ×3/week							
York [74]	146	ns	ns	19	ns	ns	31
5-FU: as above, IFN-α: as above							
Kocha [75]	486	5	40	19* (45/238)	7	43	21† (50/240)
*5-FU: 370 mg/m²/day, days 1–5, leucovorin 200 mg/m²/day, days 1–5; †5-FU: as above, IFN-α: as above							

CR, complete response; PR, partial response; %OR, overall response rate in per cent; CI, continuous infusion; ns, not specified. All six randomised trials have been published in abstract form only.

reactions was observed in the IFN-α arm: the most prominent toxicities associated with IFN-α were fever (7 versus 1 grade 1; 11 versus 1 grade 2) and leucopenia (5 versus 0 grade 1; 4 versus 3 grade 2; 4 versus 1 grade 3). Gastrointestinal side-effects were comparable between the two treatment groups. Another multicentre trial evaluating the Wadler regimen versus 5-FU [74] demonstrated no difference in overall toxicity, although again fever and fatigue were more commonly noticed in patients receiving IFN-α plus 5-FU. In this French–American study involving 146 evaluable patients, the IFN-α combination regimen was found to be more active than 5-FU. The difference between the response rates (31 versus 19%), however, did not reach the level of statistical significance, and the duration of response was similar.

The Corfu-A Collaborative Group [75] has initiated a study comparing bolus 5-FU/leucovorin with infusional 5-FU plus IFN-α. Between January 1990 and April 1991, 486 patients were accrued in 42 centres in Europe, Australia, Canada and South Africa. They were randomised between combined immunochemotherapy according to Wadler and colleagues, and chemotherapy consisting of 200/mg/m²/day leucovorin plus 370 mg/m²/day 5-FU, both given by i.v. bolus injection for 5 days every 4 weeks. Overall toxicity was mild to moderate in both arms. Severe gastrointestinal side-effects including diarrhoea, stomatitis and nausea, were more frequently associated with 5-FU/leucovorin, while fatigue, somnolence and fever occurred more often in the 5-FU/IFN-α arm. This study demonstrated an equal response rate (21 versus 19%), response duration and a median survival of 11 months in both treatment arms.

In summary, none of these six randomised trials provided evidence for a significant enhancement of the antitumour effectiveness of 5-FU ± LV with the addition of IFN-α. Mature results of these trials, however, are not yet available.

CONCLUSIONS

The focus of current clinical research efforts in colorectal cancer remains the development of novel therapeutic strategies to improve response rates, time to progression and survival. Experimental and pharmacokinetic studies have provided a compelling rationale for clinical evaluation of biochemical modulation of 5-FU by IFN-α. Despite evidence of drug synergism in various human cancer cell lines and murine tumour models [17–23], published results of trials involving more than 1000 patients with advanced colorectal cancer have not provided sufficient evidence that chemoimmunomodulation of 5-FU by IFN-α results in enhanced antitumour effectiveness compared to 5-FU alone. According to nine recent phase II studies and preliminary results of three randomised trials, the concept of 5-FU double modulation using IFN-α and leucovorin does not seem to fulfil its promise either. Therefore, for cost considerations and because combined chemoimmunotherapy with 5-FU and IFN-α ± leucovorin seems to result in enhanced systemic toxicity, currently used dose regimens of this approach cannot be recommended for routine use at present. It should be emphasised, however, that definite conclusions must await mature results of several, still ongoing, prospective randomised trials.

Further research efforts are required to determine the optimal dose and schedule of 5-FU and IFN-α, to determine whether the addition of other modulating agents offers any therapeutic advantage, and to understand more precisely the biochemical basis for their interaction. Clinical studies of drug combinations should not be solely based on *in vitro* results, particularly if a complex immunomodulatory agent, such as IFN-α, constitutes a part of this combination. Apart from overlooking possible pharmacokinetic perturbations, there may also exist important differences between *in vitro* and *in vivo* therapeutic effects. In contrast to experimental data indicating a dose-dependent drug synergism for 5-FU and IFN-α, in a recent phase I study, objective responses were noticed only with lower doses of IFN-α [69]. This observation could be one of several leads to be followed in subsequent clinical investigations of the complex interaction between 5-FU and IFN-α.

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