PREFACE

As the treatment of cancer continues to evolve, clinicians are constantly seeking new and innovative strategies to expand the use of currently available treatment modalities. Among the different strategies to improve therapy, the combining of chemotherapeutic drugs with radiation has perhaps had the strongest impact on current solid tumor treatment practice. This combination has been in use for many decades, but now has become a common treatment option in many clinical settings. This is particularly true for concurrent chemoradiotherapy, which in many recent clinical trials has been shown to be superior to radiotherapy alone in controlling local–regional disease and in improving patient survival. Combining chemotherapeutic drugs with radiotherapy has a strong biologic rationale. Such agents reduce the number of cells in tumors undergoing radiotherapy by their independent cytotoxic action and by rendering tumor cells more susceptible to killing by ionizing radiation. An additional benefit of combined treatment is that chemotherapeutic drugs, by virtue of their systemic activity, may also act on metastatic disease. Most drugs have been chosen for combination with radiotherapy based on their known clinical activity in particular disease sites. Alternatively, agents that are effective in overcoming resistance mechanisms associated with radiotherapy could be chosen. There have been recent clinical successes of concurrent chemoradiotherapy using traditional drugs, such as cisplatin and 5-FU, but these studies have led to extensive research on exploring newer chemotherapeutic agents for their interactions with radiation. A number of new potent chemotherapeutic agents, including taxanes, nucleoside analogs, and topoisomerase inhibitors, have entered clinical trial or practice. Preclinical testing has shown that they are potent enhancers of radiation response and thus might further improve the therapeutic outcome of chemoradiotherapy. Also, there are rapidly emerging molecular targeting strategies aimed at improving the efficacy of chemoradiotherapy. All these important aspects of combined modality therapy in solid tumors are discussed in this book, particularly for tumors that historically have had a poor prognosis and few treatment options.

Curry and Curran review the literature on the combined modality treatment of patients with malignant glioma, focusing on the data from prospective randomized trials, and discuss future directions in research to improve outcome for patients affected by this disease. It is clear that any one systemic agent or multiagent regimen will not have substantial effects on altering the natural history of malignant glioma. A significant improvement in survival will be realized only when improvements in local–regional control are combined with progress in the systemic management of the disease. Specific opportunities to improve surgical and radiotherapy approaches to this disease need to be explored concurrently with development of novel agents targeted to modify the biologic response of these tumors to chemotherapy and radiation. However, novel approaches when combining standard cytotoxic chemotherapy agents with new cytotoxic and cytostatic agents and improved radiotherapy techniques are promising in promoting

decreased radioresistance, toxicities, and possibly increased overall survival of head and neck cancer. Outside of an academic setting, cisplatin and 5-FU still remain the standard of treatment. Though more aggressive, as mentioned in Drs. Eng and Vokes' chapter, these drugs have overall demonstrated improved response rates in locally advanced and recurrent disease. Newer agents will continue to be discovered and provide a basis for further consideration in the treatment of head and neck cancer.

It is apparent that significant progress has been made in improving the outcome of treatment for stage III nonsmall-cell lung cancer, even though there is still a long way to go before victory can be declared. It is clear that radiation alone and surgery alone are inadequate for most stage III disease. Preoperative radiation therapy alone is of limited benefit. Postoperative radiation is controversial, but there may be a limited role in resected N2 patients. For selected stage III cases (N2), there may be a role for surgery after chemoradiation, but this conclusion awaits the outcome of a major phase III study. For inoperable stage III disease, combined modality now appears to be the new standard of care. Concurrent chemoradiation seems to be superior to sequential chemoradiation, but combined sequential followed by concurrent chemoradiation remains under investigation as does consolidative chemotherapy after concurrent chemoradiation. The best results combining chemotherapy with radiation therapy were also seen in limited-stage small-cell lung cancer. At this time, standard treatment for patients with limited-stage small-cell lung cancer is early concurrent twice-daily radiation therapy of 1.8 Gy fractions for a total dose of 45 Gy and platinum-based chemotherapy.

As discussed by Brahmer et al., newer chemotherapy regimens emerge for the treatment of small-cell lung cancer, and these regimens are currently undergoing evaluation for combining chemo- and radiation therapy. As far as esophageal cancer goes, results from surgery alone or primary chemoradiation are equivalent, and both can be offered as options for patients with locally advanced esophageal cancer. The optimal treatment may be based on individual patient selection criteria such as the ability to undergo major surgery, histology, and the location of the tumor. The fact that local recurrence is high despite primary chemoradiation, provides a rationale for tri-modality therapy that includes surgery following preoperative chemoradiation.

The major advance in the treatment of local–regional gastric carcinoma had been the new standard of adjuvant chemoradiotherapy following a curative resection. Laparoscopy is more or less established as a staging procedure prior to surgery. Staging with endoscopic ultrasonography has improved. New strategies will include the use of preoperative approaches and incorporation of new agents. Similar to the carcinoma of the esophagus, the use of molecular markers to predict response and survival is needed. Investigative efforts are underway to further improve the results of multimodality therapy of colorectal carcinoma. In addition to phase III trials discussed in Chapter 14, other studies are incorporating novel chemotherapeutic agents to improve systemic control and radiosensitization, to optimize physical delivery of radiation, and to perform risk stratification with current molecular and genetic techniques. Chronomodulation may have a role in combined modality therapy for colorectal cancer by affecting higher response rates and less stomatitis and neuropathy in metastatic colorectal carcinoma and may become a viable option for treatment of primary disease.

The inferior results with radiotherapy alone compared to cystectomy in patients with muscle-invasive disease have prompted a large number of trials adding systemic chemotherapy to radiotherapy in an attempt to increase local control and eliminate micrometastatic disease frequently present at the time of diagnosis of muscle-invasive disease.

As discussed by Dr. Roth, it is not easy to directly compare surgical series with trials of bladder-sparing approaches. A number of confounding factors can potentially complicate the interpretation of trials of chemoradiotherapy, including the effect of the TURBT on the natural history of this disease, the errors of clinical staging both before and after chemotherapy/radiotherapy, and the endpoints utilized to determine efficacy. Nonetheless, his approach can certainly be offered to patients who are not surgical candidates because of medical co-morbidities, or the occasional patient who refuses surgical intervention. Recent studies in a variety of gynecologic malignancies have convincingly demonstrated that concurrent chemotherapy can significantly improve the outcome of some patients who require radiation therapy for treatment of their disease. Despite the fact that controversies persist about the indications for chemoradiation and ideal drug regimens, the fundamental value in patients with loco–regionally advanced cervical cancer has been established.

The chapter by Dr. Eifel reviews trials of chemoradiation in cervical cancer, including the recent trials that established the value of this approach, and discusses several questions that remain to be resolved regarding this treatment, including the ideal dose and schedule and the effect of chemoradiation on compliance and complications.

One of the most exciting areas of combined modality therapy is the specific molecular targeted therapy in combination with radiation. Over the past decade there has been a quantum increase in the understanding of molecular mechanisms that underlie the process of tumor development, proliferation, invasion, and metastasis.

This has led to a growing awareness of mechanisms by which tumors and normal tissue are able to overcome damage from radiation injury. This knowledge has resulted in a vast amount of preclinical study of ways that these molecular abnormalities may be specifically targeted to result in clinical benefit, not only by potentially impacting on systemic disease, but by enhancing radiosensitivity. The last part of this book describes some of these agents and pathways.

Although we have made significant progress in our understanding of the role of combined modality therapy, much remains to be accomplished. Current and future research may provide exciting opportunities to improve response and survival for patients with tumors previously associated with a dismal prognosis.

ACKNOWLEDGMENT

I would like to thank my assistant, Laura Jewell Tyree, for her attention to detail and her many hours devoted to editing.

Hak Choy, ***MD***

2 Fluoropyrimidines as Radiation Sensitizers

Muhammad Wasif Saif, MD
and Robert B. Diasio, MD

CONTENTS

1. INTRODUCTION

Radiation sensitization with concurrent chemotherapy with an aim to improve radioresponse has long been a focus of investigation. As a result of these efforts, the concomitant use of cytostatic drugs and radiation has become the standard approach for many tumors including head and neck, rectal, anal, esophageal, pancreatic, and gastric cancers. The combined chemoradiation offers many potential advantages vs single modality treatment, such as reduction in local failure rates, eradication of micrometastases to enhance distant control, preservation of organ function, and decrease in tumor bulk prior to surgery to make complete resection possible and improve survival.

From: *Chemoradiation in Cancer Therapy*
Edited by: H. Choy

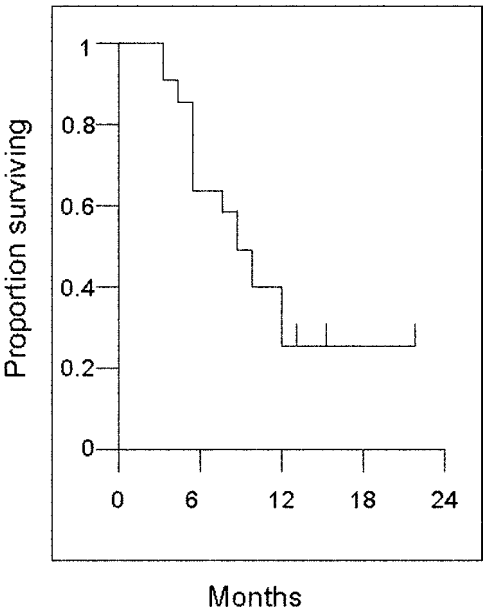

Fig. 1. Floxuridine radiosensitization—long-term freedom from liver progression for patients with nondiffuse primary hepatobiliary cancer treated with combined radiation therapy and hepatic artery infusion of floxuridine.

An ideal radiosensitizer would be the one that can maximize radiation therapy benefit, can be easily administered, can be optimally sequenced with radiation therapy for best effects, and have no overlapping toxicities with radiation. Although falling short in certain of these characteristics, 5-fluorouracil (5-FU) has become the most promising clinical radiosensitizer in combined chemoradiotherapy regimens.

Randomized trials have demonstrated that a combination of fluorouracil (5-FU)-based chemotherapy and radiotherapy significantly improves the survival of patients with both pancreatic *(1)* and rectal cancers *(2,3)* when compared with the administration of radiation alone. Furthermore, the improved response rates with use of biomodulators of 5-FU, such as lecovorin (LV) in colorectal cancers, led to the use of this biomodulator also in the combined chemoradiotherapy regimens involving 5-FU *(4)*. FdUrd is a related nucleoside, which has also been used with radiation. FdUrd is actively metabolized by the liver, and results in high regional drug concentrations with minimal systemic toxicity when administered via hepatic artery infusion *(5)*. FdUrd has also been tested with concurrent whole-liver radiation for colon carcinoma metastatic to the liver *(6,7)* (Fig. 1).

5-FU is an analog of uracil in which the hydrogen in the 5 position is replaced by fluorine, whereas FdUrd is an analog of deoxyuridine (Fig. 2). Its cytotoxicity is achieved through several mechanisms. In vitro studies have demonstrated enhanced cytotoxicity of radiation by fluorouracil. The combined effects of radiation and fluorouracil in controlling tumor growth are better than the additive effects of the two modalities given independently. The radiosensitizing efficacy of 5-FU depends on continuous exposure of tumor cells to 5-FU for 8 h or more following radiation *(8)*. Because of the short half-life of 5-FU, the drug must be administered as a continuous infusion (CI) to achieve prolonged tumor cell exposure to effective levels of 5-FU. This schedule of administration

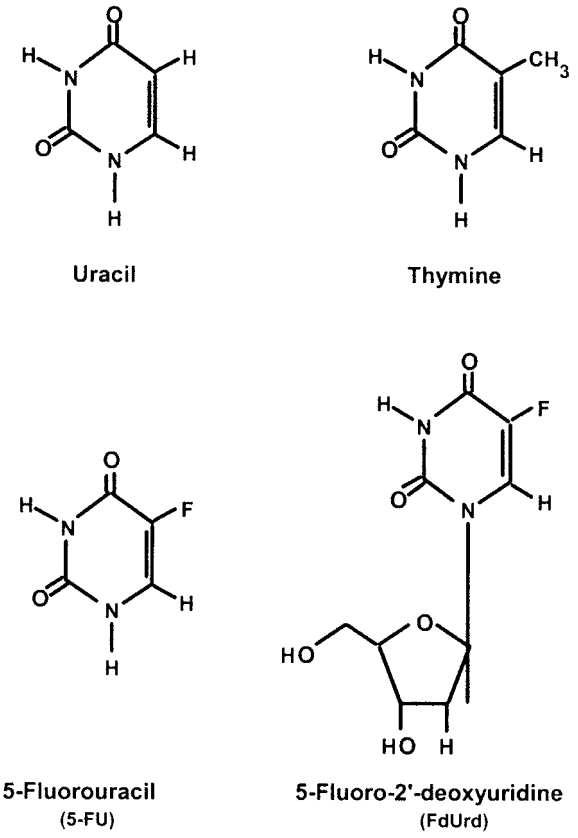

Fig. 2. Structure of fluorouracil (5-FU) and floxuridine (5-fluoro-2'-deoxyuridine, FdUrd).

is also associated with less myelotoxicity. Use of CI 5-FU infusion regimens, however, has been limited by the need for an indwelling venous catheter and a portable infusion pump. These catheters are associated with development of complications including thrombosis and infection *(9)*.

The recent availability of oral formulations of 5-FU, may provide not only an improvement in the ease of administration and the efficacy of fluoropyrimidine therapy, but also alleviate complications related to the catheters. Such agents include uracil:tegafur (UFT) and capecitabine (Xeloda).

The mechanisms of interaction between fluorouracil and radiation are not clearly understood. Different hypotheses have been postulated to explain the synergistic or potentiated effect of 5-FU with radiation including redistribution of cells to a more radiosensitive cell cycle phase, deranged pyrimidine pools with reduced capacity for repair of DNA damage, and activation of apoptosis. The effect of 5-FU on radiation damage also appears to vary in different cell lines, thus complicating the extrapolation of laboratory results into clinical practice.

2. PHARMACOLOGY OF FLUOROPYRIMIDINES

The fluoropyrimidines as a group can affect the synthesis and function of both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and both of these two mechanisms

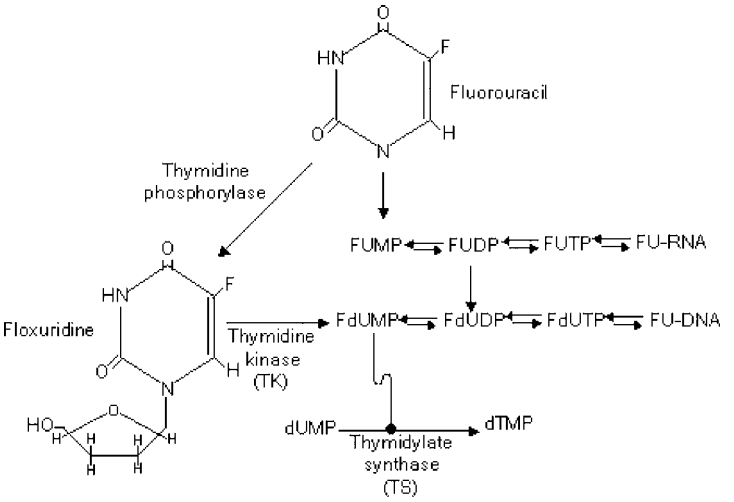

Fig. 3. Metabolism of the fluoropyrimidines: dTMP = deoxythymidine monophosphate, dUMP = deoxyuridine monophosphate, FdUDP = fluorodeoxyuridine diphosphate, FdUMP - fluorodeoxyuridine monophosphate, FdUTP = fluorodeoxyuridine triphosphate, FU-DNA= fluorouracil-deoxyribonucleic acid, FUDP = fluorouracil diphosphate, FUMP = fluorouracil monophosphate, FU-RNA = fluorouracil-ribonucleic acid, FUTP = fluorouracil triphosphate.

have lead to different consequences. Some cell lines are more sensitive to 5-FU's DNA-directed pathways, whereas RNA-medicated cytotoxicity predominates in other cell lines *(10,11)*.

2.1. DNA-Directed Effects of Fluropyrimidines

5-FU can be metabolized to form fluorodeoxyuridine monophosphate (FdUMP), which finally affects DNA. FdUrd is phosphorylated to FdUMP is (via thymidine kinase). For 5-FU to be converted into FdUMP, it involves at least two steps (Fig. 3). FdUMP is a potent inhibitor of the enzyme thymidylate synthase (TS), which is responsible for converting deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). TS inhibition leads to depletion of thymidine nucleotides and accumulation of deoxyurindine nucleotides, which leads to several events, including perturbations in other nucleotide pools, arrest in S phase of the cell cycle (growth arrest), and, ultimately, to DNA fragmentation and loss of clonogenicity *(12,13)*. In addition to inhibition of TS activity, FdUMP can be converted to fluorodeoxyuridine triphosphate (FdUTP) and become incorporated into DNA. The relative importance of TS inhibition and FdUTP incorporation into DNA on FdUrd-mediated DNA damage is not yet clear *(14,15)*.

2.2. RNA-Directed Effects of Fluropyrimidines

Although FdUrd produces only DNA-medicated cyotoxicity, 5-FU can also be metabolized to fluorouracil monophosphate (FUMP) and ultimately to fluorouracil triphosphate (FUTP), which can be incorporated into RNA in place of uridine triphosphate (UTP). In other words, incorporation of 5-FU into RNA mimics uracil *de novo* synthesis and affects the production of ribosomal RNAs (rRNAs) *(16,17)*. 5-FU also affects several aspects of messenger RNA (mRNA) function, including transcription *(18)*, translation *(19)*, and slicing *(20)*.

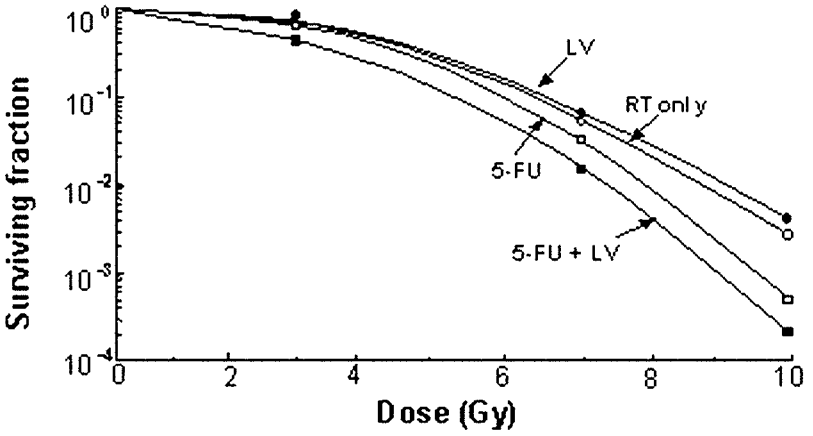

Fig. 4. 5-FU-induced radiosensitization of HT29 human colon cells is potentiated by LV. HT29 cells were exposed for 14 h to: median alone (○), 10 umol/L LV (●), 1 umol/L 5-FU (■). Cells were assessed and data are expressed as described in Fig. 4.

3. BIOMODULATION

The DNA-mediated effects of fluropyrimidines can be modulated by a number of agents, such as leucovorin (LV), levamisole, and interferon-alpha (IFN-alpha). LV prolongs TS inhibition by increasing the availability of the reduced folate cofactor necessary for formation of the inactive TS–FdUMP complex *(21)* (Fig. 4). Studies show alpha-interferon can potentiate 5-FU-mediated cytotoxicity, but the mechanisms are not yet defined *(22,23)*. Another approach to modulate the activity of fluoropyrimidines is the use of the nucleoside transport inhibitor dipyridamole. Dipyridamole probably permits 5-FU to enter the cell, and may trap intracellular nucleoside metabolites, hence increasing cytotoxicity *(24,25)*, but the exact mechanism underlying the selective cytotoxicity against tumor cells compared with normal tissues still needs to be determined.

4. FLUOROPYRIMIDINE-RADIATION INTERACTIONS

Fluoropyrimidine-radiation interactions can best be understood in terms of the fundamental mechanisms by which fluoropyrimidines lead to DNA damage and ultimately cell death. Two such mechanisms have been described as:

1. Futile repair.
2. Endonuclease activation.

4.1. Futile-Repair Hypothesis

The futile-repair hypothesis is based on the concept that "FdUrd treatment lethally deranges normal mechanisms of the cells for removing low levels of dUTP that become misincorporated in DNA." dUTP is a good substrate for DNA polymerases (alpha and beta). A high dUTP/thymidine triphosphate (dTTP) ratio occurs after treatment with sufficient concentrations of FdUrd, which subsequently leads to misincorporation of dUTP into DNA. Uracil-*N*-glycosylase, an enzyme that recognizes uracil misincorporation and cleaves the glycosidic bond, producing an apyrimidinic site. This site is recognized by an apurinic/apyrimidinic nuclease, producing a DNA break. The cell has

several mechanisms for repairing DNA single-strand breaks *(26)*. One such repair mechanism under normal conditions is as follows: neighboring nucleotides are cleaved, DNA polymerase fills in the gap, and DNA ligase seals the new, correct bases (including dTTP) into place. On the other hand, in the case of a high dUTP/dTTP ratio, such as following administration of FdUrd, dUTP will still be favored over dTTP, and this futile cycle of excision and repair would repeat itself. As mentioned earlier, FdUTP can also be incorporated into DNA in place of dTTP, which would be expected to produce a similar futile cycle to that produced by dUTP incorporation. It has been hypothesized that larger gaps would occur over time that would be sensitive to other cellular nucleases, resulting in a potentially lethal DNA double-strand break and DNA fragmentation *(27)*. This is called "futile-repair hypothesis."

4.2. Endonuclease Activation

Hirota et al. *(28)* observed that treatment of FM3A murine mammary carcinoma cells with FdUrd produced cytotoxicity that was accompanied by the generation of DNA fragments having an unusually discrete size distribution (ranging from 50 to 200 kb). The lack of incorporated uracil in these cells and the finding that this damage could also be caused by agents that did not cause dUTP elevation and dTTP depletion argued against the futile repair hypothesis *(28)*. It was found that the fragment size range noted in these cells is similar to the estimated size of an individual replication unit, and it was proposed that the observed pattern of damage resulted from selective digestion of actively replicating DNA by an induced endonuclease activity. This hypothesis was further supported by findings that lysates prepared from FdUrd-treated FM3A cells contained an endonuclease activity that was absent from control cells, and that the appearance of this endonuclease activity could be prevented by inhibiting protein synthesis with cyclohexamide treatment *(29)*.

In brief, it is postulated that futile repair and endonuclease activation may both lead to DNA fragmentation after FdUrd treatment, with the dominant process depending on a cell-line-specific factor. Neither of the two observed fragmentation patterns resembles the random damage produced by ionizing radiation.

5. MECHANISMS OF RADIOSENSITIZATION BY FLUOROPYRIMIDINES

There are four hypotheses that underline the combined effect of chemoradiation therapy employing fluoropyrimidines:

1. Fluoropyrimidines cause changes in nucleotide pools that alone increase the cytotoxicity of radiation (i.e., by depleting substrates used in the repair of radiation-induced DNA damage).
2. Fluoropyrimidines do radiosensitize by causing redistribution of cells into a relatively sensitive phase of the cell cycle (early S).
3. Radiosensitization depends on the incorporation of FdUTP into DNA.
4. Radiation as a potentiation for fluoropyrimidine-mediated cytotoxicity.

5.1. Nucleotide Pool Perturbations

One hypothesis for fluoropyrimidine-induced radiosensitization is that fluoropyrimidines induce changes in nucleotide pools including the ability of polymerases to

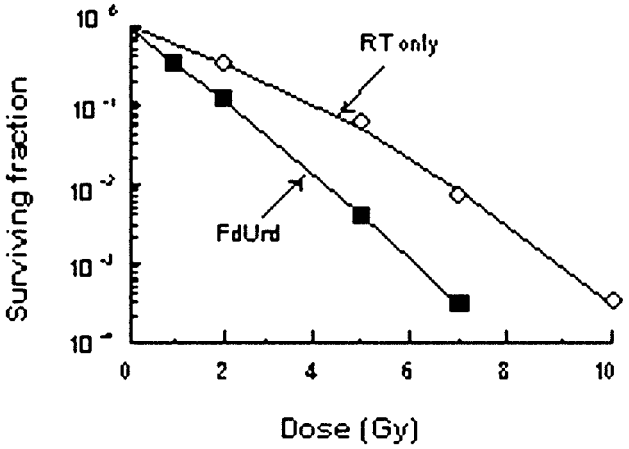

Fig. 5. HuTu80 human colon cancer cells are radiosensitized by FddUrd. HuTu80 cells were irradiated under control conditions (O) or after a 14-h exposure to 100 nmol L FdUrd (■). They were then assessed doe survival using a clonogenic assay. Data are expresses as the mean (point) ± SE (bar), which is within the symbol unless indicated.

find the correct base required for DNA repair. This alteration leads to either misrepaired or unrepaired DNA double-strand breaks, which is consistent with the decrease in sublethal damage repair and DNA double-strand break repair. Studies have indicated that both 5-FU *(30)* and FdUrd *(12)* deplete dTTP pools in human colorectal cancer cells within 1–2 h of drug exposure, whereas radiosensitization takes many hours to ensue. This suggests that nucleotide pool perturbations may result in radiosensitization under some circumstances, but pool changes alone do not seem to be the sole mechanism responsible to fluropyrimidine-induced radiosensitization.

5.2. Cell Cycle Redistribution

Another proposal is that radiosensitization embarks from 5-FU-induced cell cycle redistribution. Both 5-FU and FdUrd result in arrest of S phase cells and block cells that are not in S phase at the G1/S interface. Studies in rodent *(31)* and HeLa cells *(32)* have revealed that early S phase is a relatively sensitive phase of the cell cycle, thereby suggesting that fluoropyrimidine-mediated radiosensitization may result from redistribution into a more radiosensitization phase of the cell cycle. Investigators also tried to evaluate dependence on the timing of exposure to fluoropyrimidines in relation to radiation on the resultant radiosensitization, such as to correlate the enhancement ratio with the fraction of cells in early S phase *(12)* (Fig. 5).

Cell cycle redistribution may not be the sole factor if cells are irradiated before drug exposure, but it has been shown that 5-FU can sensitize even when cells are irradiated before drug exposure. Byfield et al. found that 5-FU radiosensitizes HeLa cells only when the drug exposure followed radiation (the cells were treated with 5-FU, either before or after radiation, for up to 8 d). A similar finding was observed on HT29 human colon cancer cells, except that in these experiments cells were exposed to 5-FU for a maximum of only 30 min before radiation *(33)*. These observations demonstrate that radiosensitization can be produced in the absence of cell cycle redistribution.

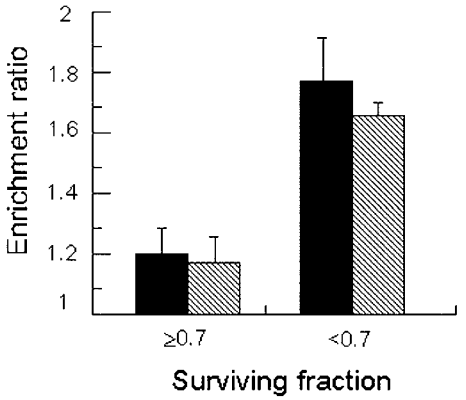

Fig. 6. FdUrd-induced radiosensitization is greater under treatment conditions that produce cytotoxicity. Data from Miller and Kinsella et al. and the data from Bruso et al. and Lawrence et al., the average radiation enhancement ratio is shown for a variety of FdUrd treatment conditions.

Investigations were also focused to assess cell cycle redistribution when cells are exposed to fluoropyrimidines before radiation. Lawrence et al. used a mechanical technique called centrifugal elutriation to obtain populations enriched in various phases of the cell cycle and found large differences in radiation sensitivity during different phases of the cell cycle, HT29 cells evidenced no significant differences in radiation sensitivity during different phases of cell cycle *(34,35)*. These findings suggest that although cell cycle redistribution accompanies fluoropyrimidine treatment, it does not appear to cause the increase in radiation sensitivity observed.

5.3. Incorporation of FdUTP into DNA

CB3717 is a TS inhibitor and does not incorporate into DNA. CB3717 is also a potent radiosensitizer of HT29 cells (under the same conditions as FdUrd) *(35)*. This finding reflects that incorporation of FdUTP into DNA may not be a prerequisite for radiosensitization.

5.4. Radiation as a Potentiation for Fluoropyrimidine-Mediated Cytotoxicity

It has been postulated that radiation may act as a potentiator of fluoropyrimidine-medicated cytotoxicity. This possibility is borne out of the observation that fluoropyrimidine-induced radiosensitization of human colon cancer cells tends to occur under conditions that produce at least some cytotoxicity by the drug alone *(15,33,36,37)*. This hypothesis is further supported by the data employing FdUrd treatment in HT29 cells, which showed that enhancement ratio was significantly greater when surviving fraction was <0.7 than when the surviving fraction was ≥0.7 ($p < 0.05$ by t test) (Fig. 6).

6. PHARMACOLOGICAL AND SCHEDULING REQUIREMENTS FOR OBTAINING EFFECTIVE RADIOSENSITIZATION WITH FLUOROPYRIMIDINES

6.1. Effect of Pharmacology of 5-FU on Radiosensitization

5-FU has a short half-life (10–15 min). Bolus doses of 5-FU disappear from the blood stream rapidly because of hepatic degradation of drug. Since radiosensitization requires

constant drug exposure, bolus drug dosing cannot achieve effective radiosensitization *(38)*. Pharmacologic studies have also shown that 5-FU has nonlinear pharmacokinetics, which is another pharmacologic factor as with its radiosensitizing effect *(39,40)*. Nonlinear pharmacokinetics may occur due to the presence of two mechanisms competing for drug removal: removal of drug by proliferating body tissues, largely through incorporation into RNA, and hepatic degradation *(40)*. It is postulated that these two mechanisms are antagonistic because hepatic degradation eliminates the drug whereas incoporation of the drug into RNA is one of the mechanisms of action responsible for the cytoxicity of 5-FU *(41)*. Clinically, these two phenomena interact and result in essentially no drug demonstrable when 5-FU is infused at doses below 15 mg/kg/24 h. The investigators hypothesized that under such conditions, 5-FU "clearance" equals the cardiac output, i.e., the drug is totally cleared during a single passage (subject to minor differences in tissue bed uptake). However, at dosages higher than about 15mg/kg/24 h, a linear relationship between infusion dose and mean plasma level have been noted (>15–65 mg/kg/24 h). The plasma 5-FU levels obtained during continuous infusions between dose levels of 15 and 65 mg/kg/d provide the concentrations necessary to cause significant cytotoxicity in human tumor cells in tissue culture and to induce radiosensitization. This observation suggested a quantitative relationship between tissue culture data and 5-FU levels required for in vivo cytotoxic radiosensitization of tumor cells *(42)*.

6.2. Effect of Scheduling of 5-FU on Radiosensitization

The studies have also suggested the "scheduling" requirements for obtaining radiosensitization induced by 5-FU, and that 5-FU must be present for at least 24 h after each (and every) radiation fraction to achieve maximum radiosensitization. Investigators also tried to determine the optimum duration of the infusion. Moertel et al. studied CI up to 24 h in duration and found no significant difference in toxicity vs bolus therapy *(43)*. On the other hand, Seifert et al. showed that 5-FU infusions for 5 d resulted in a marked shift in limiting toxicity *(44)*. Byfield et al. examined 72-h infusions of 5-FU and found central nervous system (CNS) toxicity, previously not seen with shorter infusions (where marrow suppression is the main toxicity) *(40)*. Lokich et al. studied "protracted" infusions in which 5-FU is essentially infused constantly until toxicity, tumor progression, or mechanical complications affected the therapy *(45)*. The studies proposed that infusion between 96 h and infinite hours (PI: protracted infusion) can be used for radiosensitizing regimens provided the drug is infused to limiting toxicity. In almost all patients this toxicity will involve some components of each patient's squamous cell renewal system.

6.3. Integration of 5-FU and Radiation

Byfield proposed certain principles that may govern the development of radiosensitization produced by 5-FU *(23,46,47)*.

6.3.1. Duration of Schedule

The infusion should be at least 96 h and preferably be protracted infusion. The schedule is based on the degree of radiosensitization as a function of primary 5-FU cytotoxicity. Although radiosensitization can be achieved with infusions shorter than 96 h, neurological side-effects become a limiting toxicity *(40)*. Therefore, probably 96 h is the "shortest" infusion that is both safe and effective in inducing radiosensitization (Figs. 7 and 8).

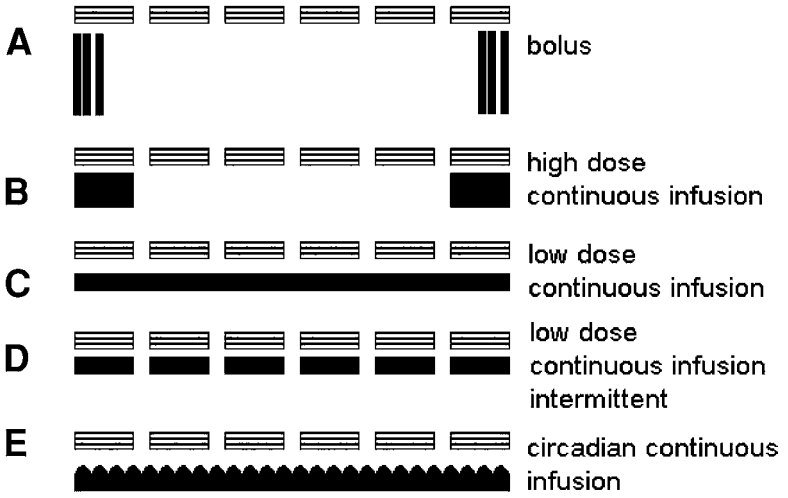

Fig. 7. A schematic representation of treatment of 5-FU and external irradiation. The stippled rectangles represent weekly irradiation (9 Gy total/wk, given in five doses of 1.8 Gy). Concurrent 5-FU is represented by the solid bars beneath the irradiation, and the height of the bars represents the peak level of radiosensitizing chemotherapy. By using protracted infusional schedules of 5-FU, radiosensitizing chemotherapy can be given with each daily dose of irradiation (from 5 to 35 d). Newer schedules using continuous intermittent and circadian schedules have achieved high tumor activity with acceptable toxicity in recent trials.

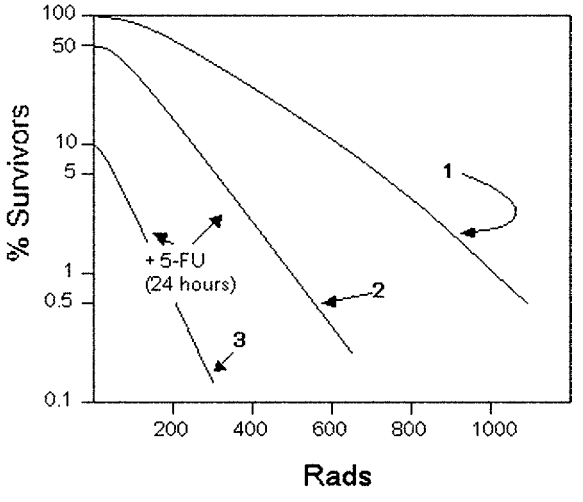

Fig. 8. Diagram of 5-FU radiosensitization. Curve 1: Radiation survival curve for cells not treated with 5-FU. Curve 2: Radiation survival curve for cells treated postradiation with sufficient 5-FU to kill 50% of the cells without radiation (partial response equivalent). Curve 3: Radiation survival curve with 5-FU killing to 10% (typical of cell system very sensitive to 5-FU).

Table 1
Cancers Sensitive to 5-FU Radiosensitization

Cancers
Esophagus
Anus
Larynx
Vulva
Penis
Bladder
Rectum
Head and neck
Pancreas
Gastric

6.3.2. Fractionation Scheme of Radiation

Investigators have employed all schemes inducing conventional fractionation (180–200 rad/d), hyperfractionation, and hypofractionation. There is no convincing evidence that infused 5-FU affects the late effects of radiation, which are a function, primarily, of the daily treatment fraction size. However, the capacity to combine radiosensitizing infused 5-FU with hyperfractionated radiation should be considered, especially in patients requiring retreatment where tolerance is an issue and can be increased by hyperfractionation.

6.3.3. Cyclicity of Administration of 5-FU

Cyclicity of administration is vital in the use of 5-FU as a radiosensitizer. The concept of cyclical treatment has been well established in cancer chemotherapy and alien to classical radiation therapy (where it is termed "split-course" therapy). 5-FU radiosensitizes tumor tissue as well as normal cells. However, this normal tissue radiosensitization is limited to the irradiated field. Suitable fractionation (i.e., cyclical therapy) can permit rapid normal tissue recovery *(23)*. The results of infused 5-FU and radiation in head and neck cancer supports the principle that cyclical treatment with 5-FU does not suffer from the limitations apparent in split-course radiation treatments.

The above described "principles" imply that optimal therapy should include tumors that are 5-FU-sensitive or, in other words, are derived from normal tissues sensitive to infused 5-FU. Tumors insensitive to 5-FU cannot be radiosensitized using this approach *(42)*.

Practically speaking, 5-FU infused at 25–30 mg/kg/d continuously for 5 d will radiosensitize virtually all 5-FU-sensitive tumors listed in Table 1. Although the "optimal" regimen has yet to be established, the 5-d schedule of 5-FU currently appears close to an ideal regimen.

7. ROLE OF *p53* IN 5-FU-INDUCED RADIOSENSITIZATION

The role of *p53* in flouropyrimidine-radiation interaction remains controversial. Some investigators have suggested that cells which are *p53* mutated are more resistant to radiation *(48)*, whereas others have found that *p53* status is unrelated to radiation sensitization *(49–51)*. Studies on SW620 cells (which are not sensitized) and HT29 cells

(which are sensitized) that have the identical *p53* mutations, revealed that *p53* status does not play an independent role in fluoropyrimidine-mediated radiosensitization *(52)*. It has also been found that RKO cells, which are mutant, wild-type (wt), or effectively null (through E6 transfection) in *p53*, are equally radiosensitized by FdUrd. Interestingly enough, fluoropyrimidines lead to elevation of *p53* levels in cells with wt *p53 (53)*. It is also known that *p53* elevation produced by TS inhibition occurs when cells enter S (i.e., after having passed through the G_1 checkpoint) *(54)*. This all leads to a model in which fluoropyrimidine-treated cells progress through the classic G_1 checkpoint and into S phase for several hours before arresting or progressing (slowly); the latter condition is associated with radiosensitization.

8. ROLE OF 5-FU RADIOSENSITIZATION IN GENE THERAPY

Although it is beyond the scope of this chapter to delve deeply into gene therapy, it is worth mentioning that 5-FU could have a potential role as a radiosensitizer in this complex area as well. It has been revealed that the bacterial enzyme cytosine deaminase (CD) can be introduced into cells, so that they become capable of converting 5-flucytosine (nontoxic antifungal agent) into 5-FU *(55)*. 5-FU produced from 5-flucytosine in cells containing CD can also radiosensitize *(56)*. Another possibility is that the activation of this agent under the control of a carcinoembryonic antigen (CEA) promoter could allow nonspecificity of the introduction of the CD gene, relying on the presence of CEA for the tumor specificity. However, only a fraction of cells are transduced in vivo and these cells must be capable of killing the rest of the tumor (the bystander effect), thereby disqualifying this concept. In the case of intracellular CD, high levels of intracellular 5-FU are generated from 5-flucytosine, which tends to kill the "factory" before the bystander *(57)*. It underlines the basis to develop gene therapy strategies for both direct cytotoxicity and radiosensitization that maximize the bystander effect.

9. ORAL FORMS OF 5-FU

The recent availability of oral formulations of 5-FU involving the ability to modulate the anabolic and catabolic metabolism of 5-FU with LV and dihydropyrimidine dehydrogenase (DPD) inhibitors has provided a substantial improvement in the ease of administration and may probably improve the efficacy of fluoropyrimidine-induced radiosensitization. Such oral fluoropyrimidines include UFT (uracil:tegafur) plus oral LV (Orzel™), an oral DPD-inhibitory fluoropyrimidine (DIF), and capecitabine (Xeloda; Roche).

With daily administration, Orzel results in similar concentrations of 5-FU as those achieved with CI 5-FU, without the necessity for indwelling catheters and infusion pumps. The predominant toxicity of Orzel is gastrointestinal and myelosuppression *(58)*. Capecitabine is an oral prodrug of 5-FU. Capecitabine is an approved agent (by FDA) that offers potential for simulating an intermittent continuous infusion of 5-FU without the inconvenience and morbidity associated with indwelling catheters. Clinical data suggest a favorable safety profile when given alone. Another compelling reason to evaluate capecitabine in combination with radiotherapy is that some tumors have high levels of thymidine phosphorylase (dThdPase). In the clinical studies, high tumor levels of this enzyme correlated with low likelihood of benefit to 5-FU. In contrast, high intratumoral dThdPase levels in preclinical models are associated with enhanced sensitivity

to capecitabine. Moreover, combining capecitabine with radiotherapy may mimic the radiosensitizing effect of 5-FU when given intravenously *(59)*.

9.1. Orzel in Combination with Radiation Therapy

The regimes combining Orzel and capecitabine with radiation therapy have become the focus of increasing interest in the management of patients' various malignancies including rectal, anal, locally advanced head and neck, esophageal, and pancreatic cancers.

9.2. Head and Neck Cancer

Takahashi et al. *(60)* found that UFT with concurrent radiotherapy was both effective and well tolerated, with a response rate approaching 94%. Fujii et al. *(61)* performed a phase I study and reported that UFT 300 mg/m^2 daily and carboplatin AUC: 5.0 d 1 every 8 wk was well tolerated with local radiation and recommended this dose schedule for phase II evaluation. Rivera et al. *(62)* in a phase II study in patients with stage III and IV squamous cell cancer of the head and neck involving UFT (6 mg/kg on d 1–21), vinorelbine (25 mg/m^2 on d 1 and 8) and CDDP (100 mg/m^2 on d 1), the combination being repeated every 21 d for four cycles followed by UFT (5 mg/kg/d) and carboplatin (100 mg/m^2/wk) administered concurrently with radiation found encouraging results. Gonzalez-Larriba et al. *(63)* performed a phase III study comparing UFT (300 mg/m^2 d 2 to 20) and CDDP (100 mg/m^2 d 1) with CI 5-FU (100 mg/m^2 d 2–6) and CDDP (100 mg/m^2 d 1) (both regimens repeated every 21 d for four cycles) as neoadjuvant therapy prior to radiation therapy (50–70 Gy) in patients with stage III and IV head and neck cancer. The overall response rates of the two arms were 79% vs 73%, overall survival of 37 vs 15 wk, and time to progression of 14.5 and 8.5 wk, respectively. However, the trend in each efficacy parameter favored the UFT/CDDP arm.

9.3. Nonsmall-Cell Lung Cancer

Takeda et al. *(64)* performed a phase I/II study consisting of low-dose CDDP (6–10 mg/m^2/d) and UFT (600 mg/d) combined with radiotherapy (50 Gy/25 fractions) as postoperative adjuvant therapy following curative resection for patients with nonsmall-cell lung cancer (NSCLC). The combined therapy was well tolerated and resulted in a disease-free survival rate of 78% at 2 yr. Another study in a small number of patients with unresectable stage III nonsmall-cell lung cancer, UFT (400 mg/m^2 on d 1–52) and CDDP (80 mg/m^2 on d 8, 29, and 50) were administered with radiation therapy (total dose of 60.8 Gy in 38 fractions on d 1–52). Among 17 evaluable patients, 94% (16 patients) achieved partial responses with median time to tumor progression of 30 wk, and the 1-yr survival rate of 80% *(65)*.

9.4. Gastric Cancer

UFT was studied in combination with radiation therapy in patients with locally advanced, inoperable gastric carcinoma. Tsukiyama et al. *(66)* evaluated combined modality therapy (CMT) consisting of UFT and mitomycin-C administered together with radiation therapy, and reported local control in 70% of patients with advanced inoperable gastric cancer.

9.5. Pancreatic Cancer

Robert et al. *(67)* conducted a phase I trial in patients with pancreatic cancer, consisting of UFT/LV (starting dose of UFT was 150 mg/m^2/d, escalated in increments of 50 mg/m^2/d with three patients per cohort to a current level of 300 mg/m^2/d), LV 90 mg/d, both

in three divided doses for 35 d starting on d 1 of radiation, and concurrent radiation therapy (45 Gy; 1.8 Gy/d). The preliminary results revealed minimal hematologic toxicity (except for one episode of grade 4 neutropenia on d 38) and infrequent and reversible nonhematologic toxicity, median survival of 12.5 mo (range 4–19 mo) and the median time to progression of 9 mo. Patient accrual was stopped because an MTD had not been reached at the 300 mg/m^2/d dose of UFT. A second phase of the study is planned incorporating changes in patient selection criteria and in treatment schedule.

9.6. Rectal Cancer

Hoff et al. *(68)* performed a phase I study in patients with clinical stage II and III rectal carcinoma involving preoperative chemoradiotherapy consisting of UFT starting at 250 mg/m^2/d (escalated by 50 mg/m^2/d for subsequent levels) plus LV (90 mg/d) combined with radiotherapy (45.0 Gy) followed later by surgery. Then postoperatively, patients received adjuvant UFT/LV (UFT 300 mg/m^2/d and LV 90 mg/d) in a 28-d schedule every 35 d for four cycles. The recommended dose level of UFT with radiation was 350 mg/m^2/d with 90 mg/d of LV. de la Torre et al. *(69)* conducted a phase II study of UFT/LV (300 mg/m^2/d UFT and 30 mg/d LV on days 8–35) administered with concurrent pelvic radiation (total dose of 35 Gy) in patients with unresectable or recurrent rectal cancer, and found that 13% of patients had a complete response, 69% a partial response, and complete pathologic response was observed in 9%. Studies aiming at postoperative UFT/LV plus radiotherapy are ongoing at present.

10. CAPECITABINE (XELODA) IN COMBINATION WITH RADIATION THERAPY

Xeloda mimics continuous FU infusion with a more convenient administration schedule. In addition, it has been demonstrated in experimental models that radiation upregulated dThdPase activity in tumor tissue. It has also been demonstrated that Xeloda given in combination with radiation therapy was associated with superior activity when compared to either given alone, whereas FU and XRT in combination did not show clear evidence of an additive effect *(70)*.

A phase I study of Xeloda in combination with XRT in rectal cancer is in the adjuvant, neoadjuvant, and palliative settings *(71–73)*. The DLT of the combination is hand-foot syndrome and mild to moderate leukopenia, diarrhea, and local skin reaction *(71–73)*. The recommended dose for phase 2 studies is Xeloda 825 mg/m^2 twice daily without interruption in combination with standard dose of radiation. Promising activity has been demonstrated in neoadjuvant therapy with six objective responses in seven evaluable patients including one pathological confirmed CR.

11. CLINICAL INDICATIONS OF FLOUROPYRIMIDINE-INDUCED RADIOSENSITIZATION

The use of 5-FU in combination with radiotherapy has shown improved survival in various malignancies including unresectable pancreatic cancer, resectable pancreatic cancer, Dukes B2 and C rectal cancer, esophageal cancer, and hepatobiliary cancer (Table 2). Similarly, 5-FU with concurrent radiation has also been used for organ preservation in different tumors involving bladder cancer, anal cancer, and laryngeal cancer (Table 3).

Table 2
Malignancies in which Fluoropyrimidines and Radiation Therapy Appear to Improve Survival

Ref.	*Disease*	*Group*	*Treatment*	*Survival*
1	Unresectable pancreatic cancer	GITSG	Radiation (40 Gy) + 5-FU	42.2 wk[a]
			Radiation alone (60 Gy)	22.9 wk[a]
78	Resectable pancreatic cancer	GITSG	Radiation alone (40 Gy) + 5-FU	21.0 mo[a]
			No adjustment treatment	10.9 mo[a]
2	Dukes B2 and C rectal cancer	GITSG	No adjuvant therapy	35%[b]
			Radiation (40–44 Gy + 5-FU + semustine	55%[b]
3	Dukes B2 and C rectal cancer	NCCTG	Radiation (45-50.4 Gy) alone	35%[c]
			Radiation (45–50.4 Gy)+ 5-FU +/- semustine	55%[c]
80	Dukes B2 and C rectal cancer	NCCTG	Radiation (54 Gy) + bolus 5-FU +/- semustine	60%[d]
			Radiation (54 Gy) + infusion 5-FU +/- semustine	70%[d]
76	Esophageal cancer	RTOG	Radiation (64 Gy) alone	8.9 mo[a]
			Radiation (50 Gy)+5-FU + cisplatin	12.5 mo[a]
85	Primary hepatobiliary cancer	University of Michigan	Radiation + hepatic arterial floxuridine	16 mo

[a]Median survival.
[b]5-yr survival.
[c]7-yr survival.
[d]4-yr survival.
[e]Phase I/II study.

Table 3
Malignancies in which 5-FU and Radiation Therapy Have Been Used for Organ Preservation

Ref.	*Disease*	*Treatment*	*Number of patients*	*Percentage of organ sparing*
86	Bladder cancer	Radiation (60 Gy) + 5-FU	34	70%
87		Radiation (60–65 Gy)	19	89%
81	Anal cancer	Radiation (30 Gy) + 5-FU + mitomycin	45	76%
82		Radiation (30–45 Gy) + 5-FU + mitomycin	22	100%
88	Laryngeal cancer	5-FU + cisplatin + radiation (66–76 Gy)[a]	332	64%

[a]Sequential delivery of chemotherapy and radiation.

11.1. Esophageal Cancer

The feasibility of concomitant chemoradiotherapy has been evaluated in numerous studies in esophageal cancer, given either as preoperative treatment or as primary therapy. In most of these studies, fluorouracil was an integral part of the chemotherapy regimen. Byfield et al. *(74)* evaluated the efficacy of 5-FU infusion (for 5 d) and 10 Gy of radiation

(in four fractions given every 2 wk) for a total of six cycles. Five of six patients achieved complete responses and were alive at the time of the report (range: 1–22 mo). Coia et al. *(75)* reported the results of 57 patients with stage I or II esophageal cancer who received fluorouracil infusion (for 4 d × two cycles, starting on d 2 and 29), and CMT (on d 2) with radiation (60 Gy in 30 fractions). The 3- and 5-yr actuarial survivals were 29% and 18%, respectively. The disease-specific survival was 41% and 30% at 3 and 5 yr, respectively. In a randomized intergroup trial, 121 patients with localized esophageal cancer were administered either 64 Gy of radiation alone or four cycles of fluorouracil and cisplatin plus 50 Gy of radiation. The results showed 24-mo survival of 38% in the chemoradiotherapy arm vs 10% in the radiation-alone arm ($p = 0.001$). An additional follow-up revealed a 3-yr survival of 31% in the chemoradiotherapy arm vs no 3-yr survival in the radiation-only arm *(77)*. The local failure rate was 44% in the combined-modality arm vs 65% in the radiation-only arm ($p < 0.01$). Within 12 mo, the rate of distant metastasis was 22% in the combined-modality arm vs 38% in the radiation-only arm ($p = 0.005$), and more toxicity was associated with the combined-modality treatment. In addition, 44% of patients in the combined-modality arm had severe side effects and 20% had life-threatening side effects vs 25% and 3%, respectively, in the radiation-alone arm.

The results of the intergroup trial compound the concept of radiosensitization, since a lower dose of radiation in the combined-modality group improved local control compared with the radiation-alone group. The chemotherapy also decreased the risk of micrometastasis. Despite the better outcome with chemoradiotherapy, the overall prognosis of these patients remains poor. No randomized trials comparing chemoradiotherapy with esophagectomy have been performed; it seems that chemoradiotherapy provides a reasonable alternative to esophagectomy in selected patients.

11.2. Pancreatic Cancer

In a Gastrointestinal Tumor Study Group (GITSG) trial *(78)*, patients were randomized to adjuvant chemoradiotherapy or to observation alone after complete resection. Radiation was administered in two courses of 20 Gy each, separated by an interval of 2 wk, for a total dose of 40 Gy. Fluorouracil was given for three consecutive days at the beginning of each radiation course and was then continued once weekly for 2 yr. Although only a small number of patients were evaluated, the study suggested a significant survival benefit for the combined treatment arm vs the control arm (median survival: 20 mo vs 11 mo, respectively). However, 71% of the treatment arm and 86% of the control arm developed recurrent disease.

The GITSG then randomized 194 patients with locally advanced, unresectable pancreatic cancer to receive 60 Gy of radiation alone, 40 Gy of radiation plus fluorouracil, or 60 Gy radiation plus fluorouracil *(1)*. The median time to progression was 12.6 wk for the radiation group vs 30–34 wk for the combined-modality groups. The median survival for radiation-alone group was 5.5 mo vs 10 mo for the combined-modality group. Although the median survival of patients receiving 60 Gy was slightly better than those who received 40 Gy, the difference was not significant.

In another GITSG study, patients with locally unresectable pancreatic cancer were randomized to multidrug chemotherapy (streptozocin, CMT, and fluorouracil) or to 54 Gy of radiation plus fluorouracil followed by the same three-drug chemotherapy regimen. Overall survival for the combined chemoradiotherapy group was superior, i.e., 41% at one year vs 19% for the chemotherapy group *(79)*.

11.3. Rectal Cancer

The GITSG randomized patients with resected Dukes B2 or C rectal cancer to one of four arms: no adjuvant therapy, postoperative radiotherapy of 40–48 Gy, postoperative chemotherapy with fluorouracil (500 mg/m^2 on the first 3 d and the last 3 d of radiotherapy) and semustine, or combined chemotherapy and radiotherapy *(2)*. Two hundred and two evaluable patients of the original 227 patients showed at 7 yr, the survival of 56% for the combined treatment group compared with 32% for the control group ($p = 0.005$). Both locoregional and distant recurrence decreased, but the main advantage was improved locoregional control. The chemotherapy alone or radiotherapy alone groups did not improve local control or survival significantly.

The Mayo Clinic/North Central Cancer Treatment Group (NCCTG) randomized patients with resected rectal cancer and tumor penetration through the rectal wall or with metastatically involved lymph nodes were assigned to postoperative radiation alone (45–50.4 Gy) or to two courses of chemotherapy with fluorouracil and semustine followed by concomitant fluorouracil and radiotherapy and two additional courses of chemotherapy *(3)*. At 5 yr, the recurrence-free survival was 37% for the radiation group vs 58% for the combined-modality group ($p = 0.0016$). The overall survival was 44% for the radiation group vs 57% for the combined-modality group ($p = 0.025$). The incidence of severe late complications was similar between the two treatment groups. An intergroup trial demonstrated the benefit of protracted fluorouracil infusion when given concomitantly with radiation as indicated by a significant decrease in recurrence (from 47% to 37%) and distant metastasis (from 40% to 31%) compared with those who received bolus fluorouracil *(80)*. Survival was better in patients treated with protracted venous infusion of fluorouracil. Patients in the protracted fluorouracil group had a higher incidence of severe diarrhea, whereas the bolus fluorouracil group had more severe myelosuppression.

11.4. Anal Cancer

The Wayne State regimen consisted of two cycles of fluorouracil infusion (1000 mg/m^2/d d 1–4 and on d 29–32), and CMT (15 mg/m^2 on d 1) with radiation (total dose of 30 Gy) *(81)*. The results revealed overall 5-yr survival rates of 70–90% and preservation of anal sphincter function in more than two-thirds of the patients. The main toxicities of CMT may include severe, life-threatening hematologic and pulmonary toxicity, and hemolytic uremic syndrome. Cummings et al. *(82)* evaluated its contribution to the efficacy of the combined modality therapy in a series of nonrandomized protocols in which patients were treated with radiation plus fluorouracil and CMT, radiation plus fluorouracil, or radiation only. The local control and cause-specific survival for patients receiving radiation and fluorouracil plus CMT were significantly superior compared with those receiving radiation and fluorouracil or radiation alone. In a randomized trial by the Radiation Therapy Oncology Group (Study 87-04), patients were treated with 45 Gy of radiation and two courses of fluorouracil (1000 mg/m^2/d for 4 d on wk 1 and 4) *(82)*. They were randomized to receive or not receive CMT (10 mg/m^2 on d 1 and 29). Preliminary results suggested significant improvements in the four-year locoregional control (82% vs 64%, respectively), colostomy-free survival (71% vs 59%, respectively), and survival without evidence of disease (73% vs 51%, respectively) for patients who received both fluorouracil and CMT vs those who received fluorouracil alone. The difference in overall survival was not statistically significant (76% vs 67%, respectively). Patients receiving

fluorouracil and CMT were also noted to have a higher incidence of severe toxicities than those receiving fluorouracil alone. This study confirmed that CMT constitutes an important component of the combined chemoradiotherapy modality at the cost of increased toxicity. In a study that included 20 patients with locally recurrent and/or metastatic anal cancer, fluorouracil and cisplatin achieved a 55% response rate *(83)*. The preliminary result of a phase II trial by the Eastern Cooperative Oncology Group (ECOG) using fluorouracil, cisplatin, and 59.4 Gy of radiation showed an overall response rate of 92% and a complete response rate of 74% *(84)*. An intergroup randomized study is underway to determine whether a high dose (59.4 Gy) is more effective than a moderate dose (45 Gy) of radiation and whether fluorouracil plus cisplatin is a more effective chemotherapy regimen than fluorouracil plus CMT.

12. SUMMARY

The pharmacologic studies indicated that intrinsic pharmacokinetics of 5-FU hinder their ability to reproduce the conditions required for radiosensitization. Indeed, the short half-life of the drug (from hepatic removal) preclude anything other than addictive effects when bolus drug is added to any variety of radiation fractionation scheme. These two sets of requirements together demonstrated that a continuous infusion (in which drug is made present for at least 24 h after each radiation fraction) would be optimal. In summary, 5-FU is a potent radiosensitizer under the following defined circumstances.

1. 5-FU has to be present for at least 24 h after each radiation exposure in order to establish the radiosensitive state. Prior exposure to the drug (with its removal after X-ray exposure) has no effect on radiation survival.
2. In order for 5-FU to render cells sensitive to radiation, a demonstrable degree of cell killing by 5-FU has to occur. In other words, some effectiveness of drug alone must be seen (equivalent quantitatively to killing slightly short of a clinical partial response).
3. 5-FU-insensitive human cancers probably cannot be radiosensitized by 5-FU.

Although, there are theoretical advantages of combining chemotherapy and radiotherapy to enhance cytotoxicity, 5-FU combined with concurrent radiation has been shown to improve treatment outcome in several malignancies, in particular, gastrointestinal cancers. Despite this progress, further efforts are required to improve the current results by well-designed randomized clinical trials, especially involving newer/oral agents. Basic laboratory research is also indicated to elucidate the underlying mechanisms of interaction between 5-FU and radiation, to facilitate the development of more effective drugs, and to provide the framework for future clinical trials.

REFERENCES

1. Moertel CG, Frytak S, Hahn RG, et al. Therapy of locally unresectable pancreatic carcinoma: A randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and high dose radiation +5-fluorouracil. *Cancer* 1981; 48:1705–1710.
2. Gastrointestinal Tumor Study Group. Prolongation of the disease-free interval in surgically treated rectal carcinoma. *N Eng J Med* 1985; 312:1465–1472.
3. Krook JE, Moertel CG, Gunderson LL, et al. Effective surgical adjuvant therapy for high-risk rectal carcinoma. *N Eng J Med* 1991; 324:709–715.
4. Minsky BD, Cohen AM, Kemeny N, et al. Enhancement of radiation-induced downstaging of rectal cancer by fluorouracil and high-dose leucovorin chemotherapy. *J Clin Oncol* 1992; 10:79–84.

5. Ensminger WD, Rosowsky A, Raso V, et al. A clinical pharmacological evaluation of hepatic arterial infusions of 5-fluoro-2'-deoxyuridine and 5-fluorouracil. *Cancer Res* 1978; 38:3784–3792.
6. Byfield JE, Barone RM, Frankel SS, et al. Treatment with combined intra-arterial 5-FUdR infusion and whole-liver radiation for colon carcinoma metastatic to the liver. *Am J Clin Oncol* 1984; 7:319–325.
7. Raju PI, Maruyama Y, DeSimone P, et al. Treatment of liver metastases with a combination of chemotherapy and hyperfractionated external radiation therapy. *Am J Clin Oncol* 1987; 10:41–43.
8. Lawrence TS, Tepper JE, Blackstock AW. Flouropyrimidine-radiation interaction in cells and tumors. *Semin Radiat Oncol* 1997; 7:260–266.
9. Monreal M, Davant E. Thrombotic complications of central venous catheters in cancer patients. *Acta Haematol* 2001; 106(1–2):69–72.
10. Daher GC, Harris BE, Diasio RB. Metabolism of pyrimidine analogues and their nucleosides. *Pharmacol Ther* 1990; 48:189–222.
11. Parker WB, Cheg YC. Metabolism and mechanism of action of 5-fluorouracil. *Pharmacol Ther* 1990; 48:381–395.
12. Miller EM, Kinsella TJ. Radiosensitization by fluorodeoxyuridine: Effects of thymidylate synthase inhibition and cell synchronization. *Cancer Res* 1992; 52:1687–1694.
13. Heimburger DK, Shewach DS, Lawrence TS. The effect of fluorodeoxyuridine on sublethal damage repair in human colon cancer cells. *Int J Radiat Oncol Biol Phys* 1991; 21:983–987.
14. Kufe DW, Scott P, Fram R, et al. Biologic effect of 5-fluoro-2'-deoxyuridine incorporation in L1210 deoxyribonucleic acid. *Biochem Pharmcol* 1983; 32:1337–1340.
15. Danenberg PV, Heidelberger C, Mulkins MA, et al. The incorporation of 5-fluoro-2'-deoxyuridine into DNA of mammalian tumor cells. *Biochem Biophys Res Commun* 1981; 102:654–658.
16. Goel R, Cleary SM, Horton C, et al. Selective intraperitoneal biochemical modulation of methotrexate by dipyridamole. *J Clin Oncol* 1989; 7:262–269.
17. Wilkinson DS, Pitot HC. Inhibition of ribosomal ribonucleic acid maturation in Novikoff hepatoma cells by 5-fluorouracil and 5-fluorouridine. *J Biol Chem* 1973; 248:63–68.
18. Greenhalgh DA, Parish JH. Effect of 5-fluorouracil combination therapy on RNA processing in human colonic carcinoma cells. *Br J Cancer* 1990; 61:415–419.
19. Iwata T, Watanabe T, Kufe DW. Effects of 5-fluorouracil on globin mRNA synthesis in murine erythroleukemia cells. *Biochemistry* 1986; 25:2703–2707.
20. Dolnick BJ, Pink JJ. Effects of 5-fluorouracil in dihydrofolate reductase and dihydrofolate reductase mRNA from methotrexate-resistant KB cells. *J Biol Chem* 1985; 260:3006–3014.
21. Houghton JA, Houghton P, Wooten RS. Mechanism of induction of gastrointestinal toxicity in the mouse by 5-fluorouracil, 5-fluorourdine, and 5-fluoro-2'-deoxyuridine. *Cancer Res* 1979; 39:2406–2413.
22. Chu E, Zinn S. Boarman D, et al. Interaction of gamma interferon and 5-fluorouracil in the H630 human colon carinoma cell line. *Cancer Res* 1990; 50:5834–5840.
23. Wadler S, Wersto R, Weinberg V, et al. Interaction of fluorouracil and interferon in human colon cancer cell lines: Cytotoxic and cytokinetic effects. *Cancer Res* 1990; 50:5735–5739.
24. Grem, JL, Mulcahy T, Miller EM, et al. Interaction of deoxyuridine with fluorouracil and dipyridamole in a human colon cancer cell line. *Biochem Pharmacol* 1989; 38:51–59.
25. Curtin NJ, Harris AL, Aherne GW. Mechanism of cell death following thymidylate synthase inhibition: 2-deoxyuridine-5-triphosphate accumulation, DNA damage, and growth inhibition following exposure to CB3717 and dipyridamole. *Cancer Res* 1991; 51:2346–2352.
26. Friedberg EC, Hanawalt PC (eds). DNA Repair, New York; Marcel Dekker, 1988.
27. Ingraham H. Dickey L, Goulian M. DNA fragmentation and cytotoxicity from increased cellular deoxyuridylate. *Biochemistry* 1986; 25:3225–3230.
28. Hirota Y, Yoshioka A, Tanaka S, et al. Imbalance of deoxyribonucleoside triphosphates, DNA double-strand breaks and cell death caused by 2-chlorodeoxyadenosine in mouse FM3A cells. *Cancer Res* 1989; 49:915–919.
29. Yoshioka A. Tanaka S, Hiraoka O, et al. Deoxyribonucleoside triphosphate imbalance. 5-fluorodeoxyuridine-induced DNA double strand breaks in mouse FM3A cells and the mechanism of cell death. *J Biol Chem* 1987; 262:8235–8241.
30. Berger SH, Hakala MT. Relationship of dUMP and free FdUMP pools to inhibition of thymidylate synthase by 5-fluorouracil. *Mol Pharmacol* 1984; 25:303–309.
31. Sinclair WK. Cyclic X-ray responses in mammalian cells in vitro. *Radiat Res* 1968; 33:620–643.
32. Terasima T, Tolmach LJ. Variation in several responses of Hela cells to x-irradiation during the division cycle. *Biophys J* 1963; 3:11–33.

33. Byfield JE, Sharp TR, Tang S, et al. Phase I and II trial of cyclical 5-day infused 5-fluorouracil and coincident radiation in advanced cancer of the head of neck. *J Clin Oncol* 1983; 2:406–413.
34. Smalley SR, Kimler BF, Evans RG. 5-fluorouracil modulation of radiosensitivity in cultured human carcinoma cells. *Int J Radiat Oncol Biol Phys* 1991; 20:207–211.
35. Lawrence TS, Davis M, Tang HY, et al. Fluorodeoxyuridine-mediated radiosensitization of human colon cancer cells is not caused by cell cycle redistribution. *Proc AACR* 1992; 33:502, (abstr 3002).
36. Bruso CE, Shewach DS, Lawrence TS. Fluorodeoxyuridine-induced radiosensitization and inhibition of DNA double strand break repair in human colon cancer cells. *Int J Radiat Oncol Biol Phys* 1990; 19:1411–1417.
37. Lawrence TS, Davis MA, Maybaum J, et al. The potential superiority of bromodeoxyuridine to iododeoxyuridine as a radiation sensitizer in the treatment of colorectal cancer. *Cancer Res* 1992; 52:3698–3704.
38. Chadwick M, Chang C. Comparative physiologic dispositions of 5-fluoro-2'-deoxyuridine and 5-fluorouracil in mice bearing solid L1210 lymphocytic leukemia. *Cancer Treat Rep* 1976; 60(7): 845–855.
39. Myers CE. The pharmacology of the fluoropyrimidines. *Pharmacol Rev* 1981; 33:1–15.
40. Byfield JE, Sharp TR, Frankel SS, et al. Phase I and pharmacologic study of 72-hour infused 5-fluorouracil in man. *Amer J Clin Oncol* 1985; 8:429–440.
41. Valerote F, Santelli G. 5-fluorouracil (FUra). *Pharmacol Ther* 1984; 24:107–132.
42. Byfield JE. 5-FU radiation sensitization. *Invest New Drugs* 1989; 7:111–116.
43. Moertel CG, Schutt AJ, Reitemeier RJ, et al. A comparison of 5-fluorouracil administered by slow infusion and rapid injection. *Cancer Res* 1972; 32:2717–2719.
44. Seifert P, Baker LH, Reed ML, et al. A comparison of 5-fluorouracil administered by slow infusion and rapid injection. *Cancer Res* 1972; 32:2717–2719.
45. Lokich J. Bothe A, Fine N, et al. Phase I study of protracted venous infusion of 5-fluorouracil. *Cancer* 1981; 48:2565–2568.
46. Byfield JE. Theoretical basis and clinical applications of 5-fluorouracil used as a radiosensitizer. In: Rosenthal CJ, Rothman M (eds) Clinical Applications of Continuous Infusion Chemotherapy and Concomitant Radiation Therapy, Plenum Publishing Corp. New York, 1986, pp. 113–125.
47. Byfield JE. The clinical use of 5-fluorouracil and other halopyrimidines as radiosensitizers in man. In: Lokich J (ed) Cancer Chemotherapy by Infusion. Precept Press, Chicago, 1987, pp. 479–501.
48. Lowe SW, Schmitt EM, Smith SW, et al. p53 is required for radiation-inducted apoptosis in mouse thymocytes. *Nature* 1993; 362:847–849.
49. Brachman DG, Beckett M, Graves D, et al. p53 mutation does not correlate with radiosensitivity n 24 head and neck cancer cell lines. *Cancer Res* 1993; 53:3667–3669.
50. Slichenmyer WJ, Nelson WG, Slebos RJ, et al. Loss of p53-associated G1 checkpoint does not decrease cell survival following DNA damage. *Cancer Res* 1993; 53:4164–4168.
51. Zellars R, Naida J, Davis MA, et al. The effect of p53 overexpression on radiation sensitivity of human colon cancer cells. *Radiat Oncol Investing* 1997; 5:43–49.
52. Rodrigues NR, Rowan A, Smith MEF, et al. p53 mutations in colorectal cancer. *Proc Natl Acad Sci USA* 1990; 87:7555–7559.
53. Tishler RB, Calderwood SK, Coleman CN, et al. Increases in sequence specific DNA binding by p53 following treatment with chemotherapeutic and DNA damaging agents. *Cancer Res* 1993; 53:2212–2216, (suppl).
54. Matsui S, Arrendondo A, Wrzosek C, et al. DNA damage and p53 induction do not cause ZD1694-induced cell cycle arrest in human colon carcinoma cells. *Cancer Res* 1996; 56:4715–4723.
55. Huber BE, Austin EA, Richards CA, et al. Metabolism of 5-fluorocytosine to 5-fluorouracil in human colorectal tumor cells transduced with the cytosine deaminase gene: Significant antitumor effects when only a small percentage of tumor cells express cytosine deaminase. *Proc Natl Acad Sci USA* 1994; 91:8302–8306.
56. Khil MS, Kim JH, Mullen CA, et al. Radiosensitization by 5-fluorocytosine of human colorectal carcinoma cells in culture transduced with cytosine deaminase gene. *Clin Cancer Res* 1996; 2:53–57.
57. Ng EY, Rehemtulla A, Laurence TS, et al. Preferential cytotoxicity of cells transduced with cytosine deaminase compared to bystander cells after treatment with 5-flucytosine. *Radiat Res Soc Proc* 1977.
58. Hoff PM, Pazdur R, Benner SE, Canetta R. UFT and leucovorin: a review of its clinical development and therapeutic potential in the oral treatment of cancer. *Anticancer Drugs* 1998; 9:479–490.
59. Budman DR, Meropol NJ, Reigner B, et al. Preliminary studies of a novel oral fluoropyrimidine carbamate: capecitabine. *J Clin Oncol* 1998; 16:1795–1802.

60. Takahashi H, Yao K, Sawaki S, Kubota A, Tsukuda M, Miyake H, Horiuchi M, Takeyama I, Kikuchihara M, Kubota T, et al. Investigation of UFT and radiation combination therapy of head and neck cancer mainly in laryngeal cancer. *Gan To Kagaku Ryoho* 1990; 17:2037–2042.
61. Fujii M, Nameki H, Kawaura M, et al. A dose finding study of carboplatin (CBDCA) + UFT in head and neck cancer. *Proc Am Soc Clin Oncol* 1998; 18:406a (abstr 1564).
62. Rivera F, Lopez-Brea M, Lopez-Vega J, Pascual C, Rubio A, et al. High activity of UFT vinorelbine and cisplatin (UFTVP) as induction chemotherapy for locally advanced squamous cell head and neck carcinoma (SCHNC). *Proc Am Soc Clin Oncol* 1997; 16:386a (abstr 1376).
63. Gonzalez-Larriba J, Garcia Carbonero I, Sastre Valera J, Perez Segura P, Diaz-Rubino E. Neoadjuvant therapy with cisplatin/fluorouracil vs cisplatin/UFT in locally advanced squamous cell head and neck cancer. *Oncology (Huntingt)* 1997; 11(9 Suppl 10): 90–97.
64. Ito H, Takeda Y, Uno T, et al. A Phase I/II trial of UFT and low-dose cisplatin plus concurrent thoracic radiotherapy (TRT) for resected P-N2 non-small cell lung cancer. *Proc Am Soc Clin Oncol* 1999; 18:510a (abstr 1966).
65. Ichinose Y, Yano T, Asoh H, et al. UFT plus cisplatin with concurrent radiotherapy for locally advanced non-small-cell lung cancer. *Oncology (Huntingt)* 1999; 13(7 Suppl 3): 98–101.
66. Tsukiyama I, Akine Y, Kajiura Y, et al. Radiation therapy for advanced gastric cancer. *Int J Radiat Oncol Biol Phys* 1988; 15:123–127.
67. Robert F, Raben D, Spencer S. UFT/oral calcium folinate plus radiation in pancreatic cancer. *Oncology (Huntingt)* 1999; 13(7 Suppl 3):127–128.
68. Hoff PM, Janjan N, Saad Ed, et al. Phase I study of preoperative oral uracil and tegafur plus leucovorin and radiation therapy in rectal cancer. *J Clin Oncol* 2000; 18:3529–3534.
69. de la Torre A, Ramos S, Valcarcel FJ, et al. Phase II study of radiochemotherapy with UFT and low-dose oral leucovorin in patients with unresectable rectal cancer. *Int J Radiat Oncol Biol Phys* 1999; 45:629–634.
70. Sawada N, Ishikawa T, Sekiguchi F, Tanaka Y, Ishitsuka H. X-ray irradiation induces thymidine phosphorylase and enhances the efficacy of capecitabine in human xenografts. *Clin Cancer Res* 1999; 5(10):2948–2953.
71. Dunst J, Reese T, Frings S. Phase I Study of Capecitabine Combined with Simultaneous Radiotherapy Rectal Cancer. ASCO 2001, 149a.
72. Yoon W-H, Choi J-H, Kim J-R, et al. Enhanced Tumoricidal Effect of Preoperative Chemoradiation Using Capecitabine for Locally Advanced Rectal Cancer. ASCO 2001, 104b.
73. Ben-Josef E, Vaishampayan UN, Vaitkevicius VK, et al. A Single Institution Experience with Concurrent Capecitabine and Radiation Therapy in Gastrointestinal Malignancies. ASCO 2001, 1436.
74. Byfield JE, Barone R, Mendelsohn J, et al. Infusional 5-fluorouracil and X-ray therapy for non-resectable esophageal cancer. *Cancer* 1980; 45:703–708.
75. Coia LR, Engstrom PF, Paul AR, et al. Long-term results of infusional 5-FU, mitomycin-C, and radiation as primary management of esophageal carcinoma. *Int J Radiat Oncol Biol Phys* 1991; 20:29–36.
76. Herskovic A, Martz K, al-Sarraf M, et al. Combined chemotherapy and radiotherapy compared with radiotherapy alone in patients with cancer of the esophagus. *N Engl J Med* 1992; 326:1593–1598.
77. Al-Sarraf M, Pajak T, Herskovic A, et al. Progress report of combined chemo-radiotherapy (CT-RT) vs radiotherapy (RT) alone in patients with esophageal cancer. An intergroup study. *Proc Annu Meet Am Soc Clin Oncol* 1993; 12:197.
78. Kalser MH, Ellenberg SS. Pancreatic cancer: adjuvant combined radiation and chemotherapy following curative resection. *Arch Surg* 1985; 120:899–903.
79. Gastrointestinal Tumor Study Group. Treatment of locally unresectable carcinoma of the pancreas: comparison of combined-modality therapy (chemotherapy plus radiotherapy) to chemotherapy alone. *J Natl Cancer Inst* 1988; 80:751–755.
80. O'Connell MJ, Martenson JA, Wieand HS, et al. Improving adjuvant therapy for rectal cancer by combining protracted-infusion fluorouracil with radiation therapy after curative surgery. *N Engl J Med* 1994; 331:502–507.
81. Nigro ND, Vaitkevicius VK, Considine B. Combined therapy for cancer of the anal canal: a preliminary report. *Dis Colon Rectum* 1974; 17:354–356.
82. Flam MS, John JM, Pajak T, et al. Radiation (RT) and 5-fluorouracil (5-FU) vs radiation, 5-FU, mitomycin-C (MMC) in the treatment of anal carcinoma: results of a phase III randomized RTOG/ECOG intergroup trial. *Proc Annu Meet Am Soc Clin Oncol* 1995; 14:191. Abstract A443.

83. Mahjoubi M, Sadek H, Francois E, et al. Epidermoid anal canal carcinoma (EACC): activity of cisplatin and continuous 5-fluorouracil in metastatic and/or local recurrent disease. *Proc Annu Meet Am Soc Clin Oncol* 1990; 9:114.
84. Martenson JA, Lipitz S, Wagner H, et al. Phase II trial of radiation therapy, 5-fluorouracil and cisplatin in patients with anal cancer. *Int J Radiat Oncol Biol Phys* 1995; 32(suppl 1):158.
85. Robertson JM, Lawrence TS, Walker S. The treatment of colorectal liver metastases with conformal radiation therapy and regional chemotherapy. *Int J Rad Oncol Biol Phys* 1995; 32:445–450.
86. Russel KJ, Bioleau MA, Higano C. Combined 5-FU and irradiation for transitional cell carcinoma of the urinary bladder. *Int J Rad Oncol Biol Phys* 1990; 19:693–699.
87. Rotman M, Macchia R, Silverstein M. Treatment of advanced bladder carcinoma with irradiation and concomitant 5-FU infusion. *Cancer* 1987; 59:710–714.
88. Induction chemotherapy plus radiation compared with surgery plus radiation in patients with advanced laryngeal cancer: The Department of Veterans Affairs Laryngeal Cancer Study Group. *N Eng J Med* 1991; 324:1685–1690.