# On the Development of Gemcitabine-Based Chemoradiotherapy Regimens in Pancreatic Cancer

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The use of chemotherapy with concurrent radiation therapy remains a standard treatment option for patients with unresectable or resected adenocarcinoma of the pancreas. This treatment strategy is based in large part on data from serial Gastrointestinal Tumor Study Group trials that have included 5-fluorouracil. Unfortunately, the majority of patients continue to succumb to the disease process. Recently, there has been a resurgence in clinical trials utilizing gemcitabine as a single agent, in combination chemotherapy regimens, and with concurrent radiation therapy. Use with concurrent radiation therapy is based in part on laboratory studies investigating mechanisms of radiosensitization and strategies that might increase the therapeutic index. In the current review, the authors summarize the preclinical data that support the use of gemcitabine as a radiosensitizing agent and the clinical trials that have been conducted to date. Issues regarding the use of gemcitabine in concurrent radiotherapy regimens need to be viewed in the context of both local and distant disease control, given the radiosensitizing and systemic activity of this agent. *Cancer* 2002;95:933–40. © 2002 American Cancer Society.
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**D**rugs that affect nucleoside and nucleotide metabolism are among the most effective and most widely used agents to sensitize tumor cells to radiation treatment (i.e., radiosensitizers). These include fluoropyrimidines (e.g., 5-FU) and the thymidine analogs (e.g., bromodeoxyuridine [BrdUrd] and iododeoxyuridine [IdUrd]). Among these, 5-FU remains the predominant agent. The deoxycytidine analog gemcitabine (2′,2′-difluoro-2′-deoxycytidine, or dFdCyd) has recently been shown to be a potent radiosensitizer in preclinical studies. It has now been approved for clinical use as a single agent and in combination chemotherapy regimens.

The integration of gemcitabine in chemoradiotherapy regimens is based on this information and the accepted role of chemotherapy with concurrent radiation therapy in the management of patients with pancreatic cancer. This treatment strategy is based in large part on data from serial GITSG trials that investigated the value of 5-FU based chemoradiotherapy. A marginal benefit associated with this therapy has been reported in patients with unresectable disease and in patients who have undergone surgical resection. Unfortunately, the annual mortality from pancreatic cancer continues nearly to equal the annual incidence, and few significant advances have been made in the nonoperative management of these patients.

Advances in the technical delivery of radiation therapy have further stimulated the renewed interest in alternative chemoradiother-

apy regimens for patients with pancreatic cancer. The integration of new systemic agents such as gemcitabine with advanced radiation therapy techniques in combined modality regimens holds great promise.

## Preclinical Studies

As an analog of deoxycytidine, gemcitabine closely resembles cytarabine (ara-C). However, unlike ara-C, gemcitabine has exhibited activity against solid tumors in several murine tumor models.[1] The cytotoxic 5'-diphosphate (dFdCDP) and triphosphate (dFdCTP) forms are produced following initial phosphorylation by deoxycytidine kinase.[2,3] These metabolites interfere with DNA synthesis through several mechanisms. Reduction of deoxyribonucleotide pools occurs following dFdCDP inhibition of ribonucleotide reductase.[4] DNA polymerases necessary for replication are inhibited by dFdCTP, which competes with dCTP.[5,6] The monophosphate can be incorporated into DNA as well, resulting in termination of DNA elongation and decreased fidelity of DNA replication.[6–8] Several other "self-potentiating" actions of gemcitabine have been shown, including the inhibition of dCMP deaminase activity, resulting in decreased dFdCTP catabolism.[9]

Investigation of the radiosensitizing potential of gemcitabine was prompted by the observation that intracellular changes produced by gemcitabine are similar to those seen following exposure to fluoropyrimidines and thymidine analogs and by recognition of the activity observed in malignancies in which radiation therapy is commonly used.[10–12] Studies in human solid tumor cell lines were supported further by demonstration of at least additive interaction in the rodent EMT6 tumor cell line.[13]

## In Vitro Studies

In the initial studies using human colorectal carcinoma cell lines (HT-29), metabolism and cytotoxicity were investigated in addition to radiosensitization.[14] Significant enhancement of radiation-induced cell killing at both noncytotoxic (10 nM) and cytotoxic (30 nM) concentrations was noted. The importance of the timing of irradiation prior to or during a 24 hour exposure to the noncytotoxic concentration was also studied. There was no evidence of radiosensitization when the cells were irradiated prior to gemcitabine exposure, while the greatest enhancement ratio was observed when cells were incubated for the full 24 hours prior to irradiation. Subsequent studies focused on short term exposure to higher concentrations, which would more closely approximate the clinical administration of once weekly infusions. Radiosensitization equivalent to or greater than that resulting from a 24 hour continuous incubation with a low concentration of gemcitabine occurred 24 to 48 hours after a 2 hour exposure to 100 nM (noncytotoxic) or 3 $\mu$M (cytotoxic) concentrations. The enhancement ratios were 1.6 and 2.9 24 hours after exposure to these conditions, respectively.[15] Since plasma levels greater than 10 $\mu$M can be attained following clinical infusions, these findings suggested that gemcitabine could be a clinical radiosensitizer.[16,17] Additional studies have revealed that cells derived from pancreatic cancer, head and neck cancers, adrenal cancer, and breast cancer are all sensitized by clinically achievable concentrations of gemcitabine.[18–20]

The initial efforts to investigate mechanisms of radiosensitization focused on the mechanisms involved following exposure to other nucleoside analogs. Incorporation of the thymidine analogs (BrdUrd, IdUrd) into DNA has been associated with increased induction and decreased rates of repair of radiation induced DNA damage.[21] Studies using similar techniques (pulsed-field gel electrophoresis) have found no effect on radiation induced DNA damage or repair following exposure to gemcitabine under conditions known to produce radiosensitization.[15,22] The relationship between incorporation and radiosensitization was studied further by evaluation of the time course of dFdCTP accumulation and radiosensitization during a 24 hour gemcitabine exposure. Evaluation of dFdCTP levels is relevant, since analog incorporation is proportional to the level of gemcitabine triphosphate.[6] Accumulation of dFdCTP occurred rapidly and plateaued within six hours. Yet maximal radiosensitization did not occur until 16 to 24 hours into the exposure.[18] Furthermore, increasing concentrations of gemcitabine (from 0.1 to 10 $\mu$M) in a 4 hour exposure, resulting in a 10-fold increase in concentration of the triphosphate, resulted in no additional increase in radiosensitization.[14] This, and other evidence, suggests that dFdCTP accumulation is not closely associated with radiosensitization.

In contrast, data tend to support the hypothesis that gemcitabine mediated radiosensitization is related to concurrent disruption of deoxyribonucleotide pools and redistribution of cells into S phase of the cell cycle. As noted above, the diphosphate metabolite inhibits ribonucleotide reductase, with a selective effect on dATP levels in solid tumors. This dATP pool depletion after gemcitabine occurs with a time course that correlates with radiosensitization for both colon and pancreatic cancer cells.[14,18] In addition, the reduction in dATP associated with radiosensitization in the two pancreatic cancer cell lines (to 1 $\mu$M) was similar, despite the fact that Panc-1 cells have a 10-fold higher endogenous dATP level compared to the BxPC-3 cells. In all cell lines investigated, dATP pool

depletion is required to obtain sensitization. Taken together, these data provide compelling evidence for dATP depletion as the important mediator of radiosensitization in these cell lines.

Although dATP pool depletion appears to be necessary, it is not sufficient for sensitization to occur. For instance, treatment of HT-29 cells with high concentrations of gemcitabine for a short time (< 4 hours) produces only moderate sensitization despite near maximal depletion of dATP pools. Maximum sensitization appears to require simultaneous redistribution into S phase along with dATP pool depletion.[23] It has also been proposed that radiosensitization of log phase cells occurs via selective sensitization of radioresistant S phase cells.[24]

More recently, the role of apoptosis in gemcitabine mediated radiosensitization has been investigated, since this mechanism of cell death has been shown to be the pathway by which the drug exerts its cytotoxic action.[7] Cell lines that differ substantially in their ability to undergo radiation induced apoptosis were evaluated to test the hypothesis that gemcitabine produced radiosensitization by potentiating preexisting death pathways. Indeed, radiosensitization of apoptotic prone HT-29 cells (enhancement ratio 1.81 ± 0.16) was accompanied by an increase in apoptosis.[25] However, apoptotic resistant UMSCC-6 cells and A549 cells were modestly radiosensitized (enhancement ratio 1.47 ± 0.24 and 1.31 ± 0.04, respectively) via a nonapoptotic mechanism. These findings suggest that although apoptosis can contribute significantly to gemcitabine mediated radiosensitization, the role of apoptosis in radiosensitization depends on the cell line rather than representing a general property of the drug.

### In Vivo Studies

In vivo studies have confirmed radiosensitization by gemcitabine and provided insight into the timing of gemcitabine administration, relative to radiation exposure, which may optimize the therapeutic index. In one study, Milas et al. gave tumor-bearing mice a single 50 mg/kg intraperitoneal dose of gemcitabine at various times before or after a single 25 Gy fraction.[26] The combination of gemcitabine and radiation resulted in regrowth delays greater than the sum of the individual treatments when gemcitabine was delivered 3–96 hours before and 1–24 hours after irradiation. The longest regrowth delays occurred when gemcitabine was administered 24–60 hours prior to irradiation. It is important to note that a single 5 mg/kg intraperitoneal dose of gemcitabine, which alone had minimal effect on tumor regrowth, enhanced tumor radioresponse.[27] This confirms the in

vitro finding that minimally cytotoxic concentrations of gemcitabine can radiosensitize. Gemcitabine reduced the dose of radiation required for tumor cure in 50% of animals ($TCD_{50}$) at all time intervals, with the largest effect observed when gemcitabine was delivered one day before irradiation ($TCD_{50}$ of 33.8 Gy compared to 51.9 Gy with radiation alone). Again, this confirms the in vitro data, in which the maximum enhancement ratios occurred 24 hours after a brief exposure to gemcitabine.[15]

A subsequent study investigated radiosensitization of both tumor and normal tissue in vivo in an attempt to determine timing and frequency of gemcitabine administration that would optimize the therapeutic index.[27] In this study, gemcitabine was given once prior to a course of fractionated radiation, twice during the course of irradiation, or daily during irradiation (total dose, 25 mg/kg). Tumor regrowth delay was similar for each schedule of gemcitabine administration over a range of total radiation doses. However, the radiosensitizing effect on jejunal mucosa was dependent on the schedule of gemcitabine administration. The highest therapeutic gain was achieved by giving a single dose prior to the start of fractionated radiotherapy. Similar findings have been observed in studies of bromodeoxyuridine radiosensitization based on more rapid elimination of analog in normal tissues.

We have investigated the therapeutic index by defining an equitoxic regimen utilizing once weekly or twice weekly gemcitabine during fractionated radiation, and then using these in murine tumor regrowth delay studies.[28] A once weekly administration was selected, since gemcitabine is generally delivered once weekly in the clinic. A twice weekly delivery was selected in an attempt to maximize radiosensitization, given the in vitro data indicating radiosensitization for only 48–72 hours following drug exposure. Intraperitoneal delivery of 800 mg/kg once weekly with concurrent radiation (27.5 Gy in five daily fractions) resulted in a similar level of normal tissue toxicity when compared to 100 mg/kg twice weekly during the same radiation treatment, based on acute lip reaction and weight loss. Tumors treated with twice weekly gemcitabine were significantly smaller than tumors treated with once weekly drug plus radiation, suggesting a greater therapeutic index as predicted by the in vitro studies.

### Clinical Trials of Gemcitabine Dose Escalation

The use of gemcitabine with concurrent radiotherapy may represent an approach to improve the outcome of pancreatic cancer patients considering these preclinical data and clinical reports indicating that gemcit-
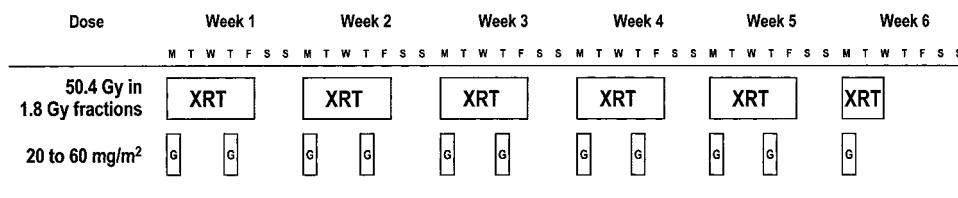
**FIGURE 1.** Treatment schema utilizing twice weekly gemcitabine (G) with concurrent radiation therapy, which was tested further in a Phase II Cancer and Leukemia Group B trial. Reproduced with permission from Reference 43.
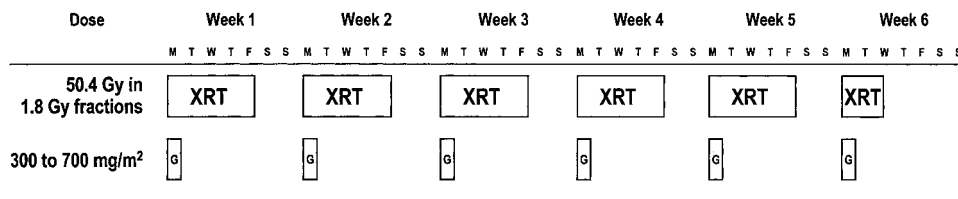


**FIGURE 2.** Gemcitabine (G) dose escalation schema investigated in patients with unresectable and potentially resectable pancreatic cancer. Reproduced with permission from Reference 43.

abine provides a survival advantage over 5-FU in patients with locally advanced (unresectable) or metastatic pancreatic cancer.[10] It has also been shown to provide symptomatic relief to patients with metastatic pancreatic cancer who have failed prior treatment with 5-FU.[29]

Scalliet et al. conducted one of the earliest clinical trials utilizing gemcitabine with concurrent radiation therapy in a Phase II trial in patients with Stage III nonsmall-cell lung cancer.[30] In this trial, a conventional course of radiation therapy (60 Gy in six weeks) was delivered with a weekly intravenous infusion of gemcitabine at 1000 mg/m$^2$. The trial was closed after eight patients were accrued, secondary to unacceptable pulmonary toxicity (four of eight patients with Grade 4/5 pulmonary toxicity). The toxicity was attributed, in part, to large radiation treatment volumes, but also provided evidence of substantial normal tissue radiosensitization. It thus highlighted the need for Phase I trials to investigate gemcitabine dose escalation with concurrent radiation therapy.

A variety of Phase I studies have now been conducted in patients with pancreatic cancer in an effort to define a tolerable regimen utilizing radiation therapy with concurrent gemcitabine. Blackstock et al. have investigated twice-weekly gemcitabine during a course of conventional radiotherapy (50.4 Gy) in 19 patients in an attempt to maximize radiosensitization (Fig. 1).[31] Both hematologic and gastrointestinal toxicity were dose limiting. Hematologic toxicity noted in this trial at 60 mg/m$^2$ is near the maximum tolerated dose of 65 mg/m$^2$ when gemcitabine alone is delivered twice weekly.[32] Gastrointestinal toxicity may have been influenced by the schedule of gemcitabine administration, with increased radiosensitization of normal tissues. However, the inclusion of prophylactic nodal basins in the treatment volume, resulting in a large volume of normal tissue irradiated, may have

been a more critical factor. A dose of 40 mg/m$^2$ (Monday and Thursday) concurrent with 50.4 Gy has now been investigated in a Phase II Cancer and Leukemia Group B (CALGB) 89805 trial, based on this work.[33] The toxicity was judged to be manageable, although only 26% of patients completed therapy without treatment breaks or dose reductions. The median overall survival for 38 patients treated on this trial was 7.9 months.

In 1996, multicenter Phase I trials were initiated in patients with locally advanced unresectable disease or with potentially resectable disease.[34,35] Both trials attempted to determine the maximum tolerable dose of gemcitabine when delivered once weekly, concurrent with 50.4 Gy (1.8 Gy fractions; Fig. 2). A margin of 3 cm around the gross target volume was required for the initial field (39.6 Gy). This margin was reduced to 2 cm for the final boost (10.8 Gy). The starting dose of gemcitabine was 300 mg/m$^2$. Hematologic and gastrointestinal toxicity have been found to be dose limiting at 700 mg/m$^2$. Late toxicity remains a concern, as two of six patients treated at 600 mg/m$^2$ on the trial for unresectable tumors developed duodenal strictures three months within completion, with one requiring a duodenal stent. Objective partial responses were not observed at doses ranging from 300–500 mg/m$^2$. However, three out of six patients treated with 600 mg/m$^2$ experienced an objective partial response. The final reports describing these trials have not yet been published.

Significant gastrointestinal toxicity was encountered when weekly gemcitabine was delivered at doses $\geq$ 400 mg/m$^2$ with concurrent rapid fractionation (30 Gy in three Gy fractions).[36] In this study, gemcitabine was delivered on the Friday prior to initiation of radiation, and continued weekly during and following the course of radiation (Fig. 3). This schedule was based on in vivo data indicating more selective tumor radio-

**FIGURE 3.** Gemcitabine (G) dose escalation schema with concurrent rapid fractionation. Reproduced with permission from Reference 43.
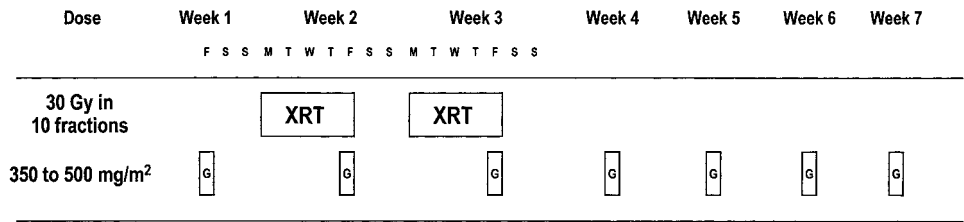


**FIGURE 4.** Gemcitabine (G) dose escalation schema investigated in the Eastern Cooperative Oncology Group, with concurrent protracted venous infusion 5-fluorouracil (PVI 5-FU) and radiation therapy. Fx: fraction. Reproduced with permission from Reference 43.
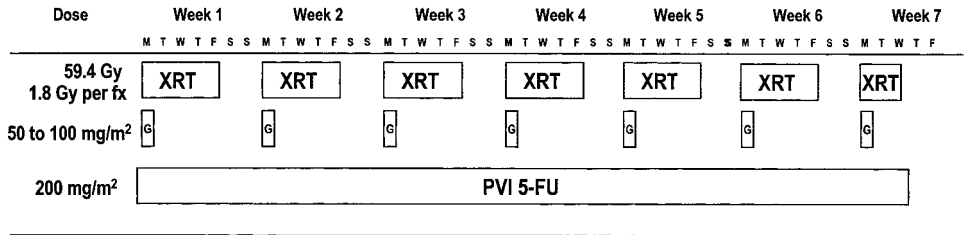


**FIGURE 5.** Radiation dose escalation schema with concurrent full dose gemcitabine (G) investigated at the University of Michigan. Reproduced with permission from Reference 43.
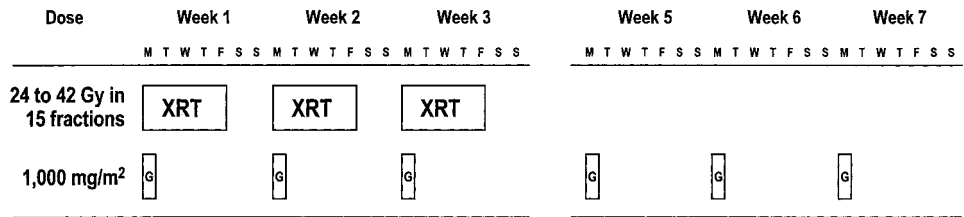
sensitization with exposure at least 24 hours prior to radiation.[27] The treatment volumes covered the primary tumor with a 3 to 5 cm margin, as well as porta hepatis and celiac axis lymph nodes. Field sizes ranged from 10 cm × 10 cm to 15 cm × 15 cm, and certainly may be implicated in the toxicity encountered. It is unclear if the schedule of gemcitabine administration influenced toxicity.

In a recent Eastern Cooperative Oncology Group trial, the use of gemcitabine with concurrent protracted venous infusion (PVI) 5-FU and radiation therapy was investigated, with weekly gemcitabine doses ranging from 50 to 100 mg/m$^2$ (Fig. 4).[37] The radiation treatment volume included nodes at risk for occult metastases. Three of seven patients on the trial experienced gastrointestinal dose limiting toxicity (DLT) at low weekly doses of gemcitabine. In two patients, this occurred following completion of radiotherapy (59.4 Gy in 1.8 Gy fractions). In this trial, therefore, the delivery of concurrent PVI 5-FU, a relatively high dose of radiation therapy, and the treatment of volumes including lymph nodes at risk may have contributed to the toxicity observed.

## Clinical Trials of Radiation Dose Escalation

In each of these above-mentioned trials, the emphasis was on the delivery of radiotherapy with dose escala-

tion of gemcitabine. An alternative strategy has been investigated at the University of Michigan using standard doses of gemcitabine, in light of the clinical benefit associated with its use as a systemic agent.[10] The goal was to maximize systemic drug effect while providing local control through sensitization of a modest radiation dose. The use of a standard dose (1000 mg/m$^2$/wk) is also consistent with our laboratory data that show maximum radiosensitization when cytotoxic concentrations of drug are used.[18] Considering prior clinical experience, it was clear that the use of full dose gemcitabine required reduction and investigation of the radiation dose and modification of treatment volumes. Radiation dose escalation in this Phase I trial was achieved by increasing the fraction size, thus keeping the duration of radiation at three weeks. The radiation fields were planned with three dimensional radiation treatment planning (3D RTP) to cover only the gross tumor volume (i.e., no elective nodal irradiation). Doses in the range of 24–42 Gy (1.6–2.8 Gy fractions) have been investigated. A second cycle of gemcitabine alone was intended following a one week rest (Fig. 5).

Thirty seven patients with unresectable (n = 34) or incompletely resected pancreatic cancer (n = 3) were treated.[38] Suspected or confirmed metastatic disease was identified at the time of enrollment in 14

patients. Three patients experienced DLT, two with Grade 4 vomiting (one at 30 Gy and one at 42 Gy dose levels), and one with gastric/duodenal ulceration at the 42 Gy dose level. Thus, at the final planned dose level of the trial (42 Gy in 2.8 Gy fractions), DLT was noted in two of six evaluable patients. An additional patient at this dose level experienced late gastrointestinal toxicity that required surgical repair. The occurrence of gastrointestinal DLT in two patients at this final dose level suggests that further dose escalation may result in intolerable toxicity. We have elected not to investigate a higher dose based on this observation and the potential for late toxicity. The concern for late toxicity is based on radiobiologic data that indicate an increased risk for late toxicity as the fraction size increases. Application of the linear quadratic model indicates that 42 Gy in 2.8 Gy fractions is biologically equivalent (with regard to late effects) to 50.4 Gy in 1.8 Gy fractions, a relatively standard dose and fractionation schedule used in the management of patients with unresectable pancreatic cancer.

With a median potential followup of 22 months, local progression was noted in 7 of 37 patients, regional progression in 3 of 37, and distant progression in 25 of 37. These data represent failure at any site, rather than the first site of failure. Only one patient has developed local or regional progression in the absence of distant progression. This suggests that the reduction in radiation dose and field size did not result in excess failures at these sites. Median survival was 11.6 months (95% confidence interval 9.9 to 19.2 months). The presence of metastatic disease at study entry did not have a significant impact on survival ($P = .69$). This approach is currently being incorporated into a multi-institutional Phase II trial.

The relative lack of gastrointestinal toxicity in our experience, using a more conformal approach and exclusion of prophylactic nodal irradiation, suggests that the radiation treatment volume is perhaps the most critical variable influencing gastrointestinal toxicity in gemcitabine based chemoradiotherapy regimens.

### Radiation Therapy Considerations

Traditional radiotherapy treatment volumes have included the primary tumor, as defined by chemotherapy (CT) and/or surgical clips placed at the time of surgery, as well as pancreaticoduodenal, porta hepatis, and celiac nodes that may be at risk.[39] A margin of normal tissue around these structures is included as well, given the uncertainties of target definition and variation in daily patient setup. As a result, a substantial volume of small bowel is included within the treatment fields. The use of 3D RTP is now becoming more

common and may reduce the toxicity associated with radiotherapy.[40,41] This technology enables more accurate definition of target volumes, as identified on a dedicated radiation treatment planning CT scan, thus limiting the amount of adjacent normal tissue irradiated. In addition, nonaxial beam arrangements can be utilized that may permit further reduction in the radiation dose to normal tissue.[42] While the impact of this technology has not been fully assessed at the present time, 3D RTP may become critically important as more effective systemic therapies are developed. In this setting, prophylactic irradiation of regional nodes may not be required, while accurate targeting of the gross tumor volume, with a minimal margin of normal tissue, may be essential to optimize local control.

The issue of radiation treatment volume needs to be critically addressed in all chemoradiation studies utilizing gemcitabine, since it is likely to be a potent radiosensitizer of normal tissues. Comparison of local toxicity (both acute and late) between trials of gemcitabine dose escalation and full dose gemcitabine with radiation dose escalation may be informative, as the volumes of normal tissue irradiated (as indicated by the margins of expansion) are dramatically different.

### Future Considerations

When considering the therapeutic options for patients with pancreatic cancer, both locoregional control and systemic failure must be addressed, particularly in situations in which the radiosensitizer also has systemic activity as a cytotoxic agent and distant failure is relevant. This is certainly the case in the setting of pancreatic cancer, where additional improvements in local control will not likely result in a survival advantage. However, the need for local control is obvious and may certainly be augmented by the use of gemcitabine concurrent with radiation. The radiation dose escalation trial described above, in which full dose gemcitabine is delivered, is an example of such consideration. The regimen is also based on available preclinical data, and may serve as a new paradigm of regimes using gemcitabine with concurrent radiation therapy. It is also apparent that radiation treatment volumes will need to be critically assessed and controlled in subsequent Phase II or Phase III trials. As these trials are designed, the role of gemcitabine based chemoradiotherapy regimens in the adjuvant setting will need to be considered as well.

The sequencing of multimodality therapy in pancreatic cancer deserves further study as well. Treatment algorithms generally begin with determination of resectability followed by surgery in patients who

appear to have operable disease. Surgical treatment alone rarely cures patients with this disease. A substantial fraction of patients are unable to receive postoperative adjuvant therapy or have treatment delayed due to postoperative recovery (following resection or exploration without resection). Preoperative chemodiotherapy can shorten the course of treatment and increase the fraction of patients receiving all modalities of therapy. As distant disease is a component of failure in a majority of cases, earlier application of systemic treatment may be a better strategy for control of micrometastasis. This potential would be maximized with the use of chemoradiotherapy regimens that emphasize the systemic component of therapy. Preoperative radiotherapy may also be associated with less toxicity due to radiation treatment volume considerations, since the target remains in situ. Issues of sequencing must also be considered in patients with unresectable disease based on imaging, particularly when the combined modality therapy is designed to optimize local control. In this setting, delivery of systemic therapy preceding the measures for local control should be emphasized.

The considerations discussed above may, if appropriately applied, provide some incremental benefit to patients with pancreatic cancer. The development of novel agents may improve results as well. As newer agents are integrated into more conventional combined modality regimes, these considerations become even more critical, such that the novel agents can be incorporated to maximize their potential.

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