Potentiation of Radiation-induced Regrowth Delay in Murine Tumors by Fludarabine

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ABSTRACT

Fludarabine (9-/3-o-arabinofuranosyl-2-fluoroarabinosine-5'-monophosphate), an adenosine nucleoside analogue, has previously been shown to inhibit the repair of radiation-induced chromosome damage. Thus, fludarabine may have therapeutic utility in combination with photon irradiation. The purpose of this study was to determine whether fludarabine could enhance radiation-induced murine tumor regrowth delay and to determine the most effective dose and schedule of the combination. A significant (P < 0.05) absolute regrowth delay enhancement was observed in three murine tumor models (SA-NH, a sarcoma; and MCA-K and MCA-4, mammary carcinomas) when fludarabine (800 mg/kg) was given 1 h prior to 25 Gy γ-irradiation. While fludarabine enhanced radiation-induced tumor regrowth delay when given between −36 h and +6 h of radiation (SA-NH tumor), the greatest enhancement was observed when fludarabine was given at −24 h prior to irradiation (radiation dose modification factor of 1.82 at −24 h compared to 1.57 at −3 h prior to radiation). The degree of fludarabine enhancement (at −3 or −24 h) was dose dependent at doses above 200 mg/kg. While fludarabine and radiation were administered on a fractionated schedule (fludarabine given 3 h prior to radiation each day for 4 days), the dose modification factor increased to 2.14 (1.63 if the effect of fludarabine alone is subtracted). These results suggest that fludarabine enhances radiation-induced tumor regrowth delay in a more than additive fashion after both single and fractionated treatments, and the degree of enhancement is dependent on the sequence and timing of administration, the fludarabine dose, and the tumor type. Thus, fludarabine may have clinical potential as a radiation enhancer in the treatment of solid tumors.

INTRODUCTION

Intrinsic cellular radiosensitivity is an important determinant of tissue response to ionizing radiation (1) such that radiotherapeutic treatment failure has been correlated with the presence of intrinsically radioresistant cells (2–6). The molecular basis for cellular radiosensitivity has been suggested to be dependent on a number of factors which predominate in different cells, including the initial level of induced DNA (7, 8) or chromosome damage (9, 10), the ability of the cell to repair DNA or chromosome damage (11–15), the ability of the cell to regulate its cell cycle after radiation insult (16–19), and the intrinsic sensitivity of the cell to induced cell loss mechanisms such as apoptosis (20, 21). Current strategies for improving the outcome of radiotherapy are targeted toward modulating these cellular processes selectively in tumors or normal tissues.

In the case where tumors are radioresistant because of an intrinsically competent mechanism for repairing radiation-induced DNA or chromosome damage, one potential therapeutic strategy is to combine radiotherapy with repair inhibitors. Since DNA repair and DNA synthesis have been suggested to involve some common enzymatic components (22), inhibitors of DNA synthesis might also serve as repair inhibitors. Along this line, nucleoside analogues such as ara-A and ara-C have been reported to inhibit DNA and chromosome repair after ionization radiation, increase the residual levels of chromosome damage (23–26), and enhance in vitro radiation-induced cell lethality (27–29).

However, particular characteristics of some of these agents limit their translation to the in vivo setting. For example, ara-A is rapidly inactivated by deamination in vivo.

F-ara-A is a fluorinated nucleoside analogue of ara-A that is relatively resistant to deamination (30). When given to cells, F-ara-A is transported into the cell, where it is phosphorylated to its active triphosphate F-ara-ATP form (31); it inhibits DNA synthesis through the inhibition of DNA polymerases α and ε, DNA primase, ribonucleotide reductase, and DNA ligase I (32–35). In addition, it can act as an efficient chain terminator when incorporated into DNA. F-ara-A has also been shown to inhibit the repair of ionizing radiation-induced chromosome damage in human peripheral blood cells in a dose-dependent fashion (36). Moreover, F-ara-A enhances radiation-induced cell lethality in plateau phase Chinese hamster ovary cells.5 Because of the relative insolubility of F-ara-A, its 5′ monophosphate form, designated fludarabine, has been synthesized for in vivo use and has been shown to exhibit significant activity against several hematological malignancies (37–39).

Because of the activity of F-ara-A as a repair inhibitor and its demonstrated bioavailability in vivo, the possibility existed that fludarabine would act as a radiosensitizing agent and potentiate tumor response in vivo. Indeed, it has been shown that fludarabine potentiates the tumor response of a murine sarcoma in vivo after single and fractionated irradiation (40). While this latter study demonstrated the promise of this therapeutic strategy, Kim and colleagues did not address important considerations such as the comparative effect on different tumors, the optimal dose and schedule of fludarabine administration, or the mechanisms underlying the positive interaction between fludarabine and radiation. In the present study, we sought to determine whether fludarabine can enhance the radiation-induced tumor regrowth delay of additional murine syngeneic tumors and to determine the optimal dose and schedule of fludarabine administration in relation to irradiation. In addition, we attempted to identify possible mechanisms underlying the potentiation of tumor response by fludarabine.

MATERIALS AND METHODS

Mice and Tumors. Inbred 3–3.5-month-old male C3Hf/Kam mice were housed 5/cage and maintained in a specific pathogen-free mouse colony for the duration of the experiments.

5 The abbreviations used are: ara-A, 9-/3-o-arabinofuranosyladenine; ara-C, 1-/3-o-arabinofuranosylcytosine; F-ara-A, 9-/3-o-arabinofuranosyl-2-fluoroarabinosine; fludarabine, F-ara-AMP; 9-/3-o-arabinofuranosyl-2-fluoroarabinosine-5′-monophosphate; F-ara-ATP, 9-/3-o-arabinofuranosyl-2-fluoroarabinosine-5′-triphosphate; AGD, absolute growth delay; NGD, normalized growth delay; DMF, dose modification factor.

1 The abbreviations used are: ara-A, 9-/3-o-arabinofuranosyladenine; ara-C, 1-/3-o-arabinofuranosylcytosine; F-ara-A, 9-/3-o-arabinofuranosyl-2-fluoroarabinosine; fludarabine, F-ara-AMP; 9-/3-o-arabinofuranosyl-2-fluoroarabinosine-5′-monophosphate; F-ara-ATP, 9-/3-o-arabinofuranosyl-2-fluoroarabinosine-5′-triphosphate; AGD, absolute growth delay; NGD, normalized growth delay; DMF, dose modification factor.

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Three murine tumors of spontaneous origin, syngeneic to C3H/Kam mice, were used for these experiments: a sarcoma designated SA-NH and two mammary carcinomas designated MCA-4 and MCA-K, respectively. SA-NH and MCA-4 are nonimmunogenic tumors, and MCA-K is immunogenic (41).

Tumor cells of the second, third, and seventh isotransplant generations for MCA-4, SA-NH, and MCA-K tumors, respectively, were injected into the mouse flank to generate tumor sources. Single cell suspensions of excised tumors were used for these experiments: a sarcoma designated SA-NH and two mammary carcinomas designated MCA-4, MCA-K, and SA-NH tumors, respectively. Tumors for regrowth delay experiments were generated in the right thigh by i.m. injection of 5 × 10^6 tumor cells in 10 μl of Hsu’s medium.

**Irradiation.** The tumor-bearing leg was locally irradiated under air-breathing conditions with a dual-source 137Cs γ-ray unit. Unanesthetized mice were immobilized in a specially designed jig, and the tumor was centered in a 3-cm-diameter circular irradiation field. During the course of the experiments, the dose rate varied from 7.35 to 7.22 Gy min⁻¹.

**Fludarabine.** Fludarabine was generously supplied by Berlex Laboratories (Richmond, CA). Before each experiment, the drug was reconstituted in phosphate-buffered saline, pH adjusted to 6.8–7.0, filtered through a 0.45-μm Millipore filter, and stored at 4°C. The concentrations of fludarabine in solution were adjusted to inject 0.01 ml/g mouse body weight. Fludarabine was injected i.p. at room temperature.

**Tumor Regrowth Delay Assay.** When tumors reached 8.0 ± 0.3 mm in diameter, the mice were randomly assigned to a treatment group. After treatment, the tumors were measured every other day until they reached a diameter of 15–16 mm, at which time the mice were sacrificed. For each tumor, cranio-caudal, antero-posterior, and transversal measurements were obtained. An average tumor diameter was then calculated. Within an average diameter of 7–8 mm to 15–16 mm, the relationship between the average diameter and time after treatment could be fitted to a straight line; from this, the time to reach a particular tumor size was calculated for each mouse. For all calculations, the irradiation day was defined as day 0.

The quantitative effect of the treatments on tumor regrowth was expressed both as AGD and NGD. The AGD was defined as the time in days for the tumors to reach 10 mm in a treated mouse minus the mean time to reach 10 mm in the untreated control group. An end point diameter of 10 mm was chosen because it is close to the initial tumor size at the time of treatment; this choice minimizes the contribution of the tumor bed effect phenomenon in the determination of the regrowth delay (43). The NGD was defined as the time to reach 10 mm in a mouse treated by the combination of fludarabine and radiation, minus the mean time to reach 10 mm in the group treated by fludarabine alone. The NGD provides an estimate of the tumor regrowth delay induced by the combined treatment but without the contribution of the drug alone. Thus, the NGD provides a measure of synergy for antitumor response of fludarabine combined with radiation.

For each experiment, the mean (±SE) regrowth delay was computed from data obtained with 8–10 mice. A two-tailed Student’s t-test was used to test the statistical significance of differences between mean regrowth delay values.

**RESULTS**

**Effect of Fludarabine on Radiation-induced Regrowth Delay.** To determine whether the promising results of Kim et al. (40) could be generalized to other mouse tumor model systems, feasibility studies were initiated to assess whether fludarabine could enhance radiation-induced tumor regrowth delay in three murine tumors of spontaneous origin, SA-NH, MCA-K, and MCA-4. Tumor-bearing mice were treated with irradiation alone, fludarabine alone, or the combination of fludarabine and radiation, and the tumor regrowth delays were measured. To avoid tumor cure but still achieve a measurable tumor regrowth delay, a single radiation dose of 25 Gy was chosen for all three tumors (44). To avoid drug toxicity, a fludarabine dose of 800 mg/kg was chosen. This dose is approximately one-half the 10% lethal dose reported in BALB/c mice (1600 mg/kg) (40) and in BD2F1 mice (1485 mg/kg) (45). For the initial experiments, to ensure that inhibitory F-ara-ATP levels would be present in the tumor cells at the time of irradiation, fludarabine was injected 1 h prior to irradiation. This time was chosen based on the finding that intracellular F-ara-ATP reaches its maximum level after 1 h in mice bearing P388 leukemia cells (45).

A single radiation dose of 25 Gy induced a significant regrowth delay in all three tumors without any tumor cure, while a single dose of fludarabine alone had only a limited effect (Table 1 and Fig. 1). After combined fludarabine and radiation treatment, the AGD was significantly (P < 0.05) increased in all three tumors compared to radiation alone (Table 1 and Fig. 1). However, the magnitude of this effect strongly varied with the tumor type. It increased by a factor of 1.5 in SA-NH (17.6 versus 11.9 days), but only by 1.2 in MCA-K (39.2 versus 32.9 days) and MCA-4 (17.2 versus 14.3 days) tumors. However, it should be noted that 4 of the 10 mice bearing MCA-K tumors were tumor free at 100 days in the combined fludarabine and radiation treatment. Since calculation of the regrowth delays excluded the four cured mice, the value is likely to be an underestimate of the enhanced effect of the combined treatment. Therefore, to get a better estimate of the differential effect between the combined treatment group and the group treated by radiation alone, a Kaplan-Meier analysis was performed to determine the 10-mm recurrent tumor-free survival in the two groups. A statistically significant longer (P < 0.01) tumor-free survival was observed in the combined treatment group when AGDs were used as duration of disease-free survival.

To detect synergism between fludarabine and radiation, the data from the combined treatments were expressed as NGD as defined in "Materials and Methods." If the effect of the combined fludarabine and radiation treatment were only additive (i.e., equaled the delay induced by radiation alone plus the delay induced by fludarabine alone), the NGD after combined treatment would be identical to the AGD after radiation alone. As shown in Table 1, the NGDs after combined treatment were higher than the AGDs for radiation alone in all three tumor models, and it reached significance (P < 0.05) in both SA-NH and MCA-K (Kaplan-Meier analysis) tumors.

**Timing of Fludarabine Administration in Relation to Irradiation.** The working hypothesis of this project was that fludarabine acts as a repair inhibitor and thus would have its greatest impact on radiosensitization if it were given proximal in time to radiation such that the intracellular F-ara-ATP level would be high during the repair period. To test this hypothesis, we administered fludarabine (800 mg/kg) to mice bearing SA-NH tumors at various times before irradiation (−36, −24, −18, −12, −6, −3, or −1 h), within 2 min of irradiation, or after irradiation (1 or 6 h), and determined the radiation-induced regrowth delays.

As shown in Fig. 2, fludarabine induced an increase in radiation-induced regrowth delay at all times of administration before and after irradiation (i.e., the regrowth delay enhancement, defined as the ratio of the AGD for the combined fludarabine and radiation treatment over AGD for radiation alone).
that for radiation alone, was \( \approx 1.29 \) for all sequence time intervals). Contrary to expectation, however, the greatest regrowth delay enhancement (1.76) was observed when fludarabine was administered 24 h prior to irradiation. Nevertheless, when fludarabine was administered between 18 and 1 h prior to irradiation, the enhancement ratio ranged from 1.35 to 1.52, and enhancement was still observed when fludarabine was administered after irradiation. These results suggest that the interaction between radiation and fludarabine must involve mechanisms in addition to repair inhibition.

The Relationship between Fludarabine Dose and Radiation Growth Delay Enhancement. Previous experiments using cells in vitro had demonstrated that the ability of F-ara-A to inhibit chromosome repair was dose dependent: the slow component of chromosome repair was inhibited at an intracellular F-ara-ATP concentration of approximately 30 \( \mu \)M and the fast component at approximately 75–100 \( \mu \)M (36). Thus we expected that the degree of enhancement of radiation-induced tumor regrowth delay might also be dependent on fludarabine dose. Similarly, an important component of fludarabine cytotoxic effect on cycling cells is the duration of exposure to inhibitory levels of the active component (i.e., intracellular F-ara-ATP concentration time interval). Therefore, mice bearing SA-NH tumors were treated with various doses of fludarabine (100, 200, 400, 800, and 1200 mg/kg) at 3 or 24 h prior to 25 Gy irradiation, and the effect on tumor regrowth delay was determined. For each dose level, the control animals received either that fludarabine dose alone, radiation alone, or no treatment.

No detectable regrowth delay was induced by fludarabine alone at doses of 100 and 200 mg/kg, whereas it progressively increased for higher doses (Table 2). A fludarabine dose of 1200 mg/kg was considered toxic since 2 of 18 mice died within 36 h of fludarabine administration. As a consequence, no further experiments were performed at this highest fludarabine dose level. Lower doses, however, caused no mouse mortality. A significant (\( P < 0.01 \)) dose-dependent increase in the AGD in comparison with radiation alone was found at fludarabine doses of 200 mg/kg and above. This was true at both the 3-h and 24-h intervals (Fig. 3A). However, the differences in AGD between 400 and 800 mg/kg or between 800 and 1200 mg/kg were not significant (\( P > 0.1 \)). When the regrowth delay induced by fludarabine alone was subtracted from that induced by the combined treatment (Fig. 3B), the dose-dependent enhancement of radiation-induced tumor regrowth delay was still significant (\( P < 0.01 \)) at both the 3-h and 24-h intervals (Fig. 3B). Interestingly, the NGD did not increase for

![Graph](https://example.com/graph.png)
doses above 400 mg/kg. This finding suggests that the interactive mechanisms between fludarabine and irradiation are saturated at 400 mg/kg and that the additional regrowth delay observed at doses above 400 mg/kg is due to the increased antitumor effect at higher fludarabine doses.

Determination of the Radiation Dose Modification Factor after Single Doses of Irradiation. In the previous experiments, the effect of combined fludarabine and radiation treatment was quantified by its effect on tumor regrowth delay beyond that of each treatment alone. An alternative way to measure the effect of the combination is to determine how much the radiation dose could be reduced in combination with fludarabine to obtain the same biological effect as irradiation alone. The ratio of the two radiation doses is the DMF. To determine the DMF associated with fludarabine addition, mice with SA-NH tumors were irradiated with single radiation doses (ranging from 16 to 28 Gy) in the presence or absence of fludarabine (800 mg/kg) given either 3 or 24 h prior to irradiation. For each treatment arm, the relationship between radiation dose and tumour regrowth delay was fitted to a straight line, and the radiation DMFs were calculated for a regrowth delay end point of 13 days. The r values were greater than 0.97.

Determination of the Radiation Dose Modification Factor after Fractionated Irradiation. The initial studies described above were designed to investigate the potential synergism between fludarabine and irradiation; only single doses of fludarabine and radiation were given. In clinical radiotherapy, however, radiation is delivered on a fractionated schedule at lower doses per fraction (usually 2 Gy/fraction) over a protracted treatment period. We therefore wanted to determine whether fludarabine potentiation could still be observed after fractionated irradiation at doses closer to those used in the clinic. Besides choosing the daily radiation dose per fraction as close as possible to those of standard radiotherapy regimens, we took into account the requirement that for SA-NH tumors, at doses below 10 Gy, the overall treatment time should not exceed 4 days. Otherwise, rapid tumor regrowth between fractions would overpower the cytotoxic effect of each fraction. Pilot studies of 4-day overall treatment time showed that the maximal tolerable daily dose of fludarabine was 400 mg/kg.

In a feasibility experiment, SA-NH tumors were irradiated daily with 4.5 Gy for 4 days in the absence or presence of fludarabine. The drug was given either 3 or 24 h prior to each radiation dose. As shown in Fig. 5, with radiation treatment alone, the tumors continued growing; only a small AGD of 5.3 ± 0.5 days was observed. Fludarabine...
The results presented here suggest that fludarabine can potentiate radiation-induced tumor regrowth delay in three murine tumor models. When irradiation and fludarabine were given as single doses, the degree of potentiation was dependent upon the dose, sequence, and timing of fludarabine administration, as well as the tumor type. The magnitude of the potentiating effect of fludarabine on a single irradiation dose was substantial (DMFs of 1.57–1.82). Moreover, the potentiating effect was enhanced when the irradiation was given on a fractionated schedule. These results suggest that the combination deserves further investigation as a strategy for improving radiotherapeutic intervention.

The data indicated that the combined effect of radiation and fludarabine was more than additive. While the interaction could be called synergistic, in a strict sense based on the terminology and criteria for quantifying the association of radiation and drugs proposed by Steel (46), the interaction might be better described as an enhancement of the radiation response.

Only a few in vivo studies on the combination of purine or pyrimidine nucleoside analogues and radiation in tumor models have been published, and a comparison with the data presented here is limited. A DMF of 1.6 was reported when fludarabine (400 mg/kg) was given 1 h prior to irradiation in a mouse fibrosarcoma using tumor cure as an end point (40). On the other hand, in a RIF-1 mouse tumor and a SCCVII human tumor model, ara-A showed no potentiation of radiation response (47). These differences may reflect the rapid inactivation of ara-A by deamination in vivo. Thus one advantage of fludarabine is its relative insensitivity to deamination in vivo. The combination of radiation and ara-C for murine leukemia and osteosarcoma also produced potentiation when ara-C was administered proximal to radiation (48). However, no analysis regarding a DMF of radiation effect was reported.

The exact mechanisms underlying the positive therapeutic interaction of fludarabine and radiation are not known. The initial working hypothesis underlying the present studies was that fludarabine might radiosensitize cells by interfering with the repair of radiation-induced damage. With other nucleoside analogues such as ara-A and ara-C, in vitro studies have demonstrated an effect on potentially lethal damage repair (25–29). High ara-A concentrations (≥500 μM) have been shown to nearly eliminate the shoulder on the cell survival curve after a single radiation dose, although the effect of ara-C was not so dramatic (26). Since the shoulder region of the cell survival curve is thought to reflect a repair component, these results are consistent with the finding that ara-A (and to a lesser extent ara-C) can inhibit the repair of DNA double-strand breaks (measured by neutral filter elu-

**DISCUSSION**

The results presented here suggest that fludarabine can potentiate radiation-induced tumor regrowth delay in three murine tumor models. When irradiation and fludarabine were given as single doses, the degree of potentiation was dependent upon the dose, sequence, and timing of fludarabine administration, as well as the tumor type.
tion) and of chromosome breaks (measured by premature chromosome condensation) (25, 26). There have been no reports of the repair of DNA double-strand breaks after F-ara-A treatment, but our previous studies indicated that F-ara-A could completely block chromosome repair in irradiated peripheral blood cells at high intracellular concentrations of F-Ara-ATP (which have been measured in lymphocytes of patients treated with fludarabine) and inhibit the slow component of chromosome repair at lower intracellular concentrations (36). An alternative molecular mechanism is that some nucleoside analogues could inhibit base excision repair after irradiation and create new DNA lesions, which might then interact with unrepair DNA breaks to create chromosome exchange events. Indeed, ara-C treatment following irradiation has been shown to increase the frequency of radiation-induced dicentric chromosomes (49-51).

In any event, our report that fludarabine can prolong radiation-induced tumor regrowth delay when administered just prior to or after irradiation is consistent with an interference with the repair of radiation-induced DNA damage by fludarabine as well as a minor antitumor effect of fludarabine alone. Interestingly, in another study, no enhancement of radiation-induced local tumor cure was observed when fludarabine was injected 1 h after the irradiation of a methylcholanthrene-induced mouse fibrosarcoma (40). This would suggest that the repair kinetics and repair capabilities might differ from tumor to tumor.

That the greatest enhancement of radiation-induced tumor regrowth delay was obtained when fludarabine was given 24 h prior to irradiation is somewhat puzzling. It is known that the important determinant of fludarabine’s effect on DNA synthesis and other cellular processes is the level of F-ara-ATP in the cells (52). It is also known that, depending on the particular cell type, both the accumulation and fall in level of intracellular F-ara-ATP is a time-dependent process such that effective intracellular levels may exist for defined periods of time after a single fludarabine administration. While tumor F-ara-ATP pharmacokinetics were not determined in our experiments, it is unlikely that inhibitory F-ara-ATP levels still remained in the tumor cells 24 h after fludarabine administration. First, after i.v. injection of fludarabine in BDF1 mice, the elimination half-life of F-ara-A in serum has been found to be on the order of 3 h (53). Second, the degree of tumor regrowth delay enhancement at -12, -6, -3, -1, or -24 h (Fig. 2). These observations therefore suggest that additional mechanisms need to be considered to explain the high degree of tumor regrowth delay enhancement offered by fludarabine when given 24 h prior to irradiation.

One possible mechanism for the radiosensitizing effect of fludarabine when given 24 h prior to irradiation is an effect on cell cycle redistribution. Fludarabine, when given in vivo, and F-ara-A, when given in vitro, are known inhibitors of DNA synthesis and block cells at the G1-S phase boundary in a dose- and time-dependent manner (54). Thus, it is possible that at the doses used, fludarabine treatment caused tumor cells to temporarily accumulate in late G1 or early S phase which, when F-ara-ATP levels had fallen to noninhibitory levels, then proceeded in a synchronized wave to a more radiosensitive phase of the cell cycle (G2). Indeed, the data shown in Table 2 indicated that for tumor-bearing mice treated with fludarabine alone, the magnitude of the tumor regrowth delay was dependent upon fludarabine dose. Similarly, the enhancement by fludarabine of radiation-induced regrowth delay was also fludarabine dose related (Fig. 3), and the most significant levels of enhancement were seen at doses that caused detectable regrowth delay due to drug alone. Preliminary in vivo data in our laboratory offer further evidence that fludarabine-induced DNA synthesis inhibition and a subsequent cell cycle redistribution resulted in the synchronization of tumor cells at a more radiosensitive phase of the cell cycle 24 h after fludarabine administration (55).

As shown in Fig. 3, while the degree of absolute regrowth delay increased with increased fludarabine dose, when the effect of fludarabine alone was subtracted, the enhancing effect of fludarabine remained constant at doses of 400 mg/kg and above. This effect was independent of the timing of fludarabine administration. This result suggests that one component of the fludarabine-radiation interaction mechanism reached a maximum at 400 mg/kg. While the dose-related positive interaction observed when fludarabine was given 3 h prior to radiation might be related to repair inhibition (i.e., sufficient levels of F-ara-ATP present at the time of irradiation to inhibit the repair process), the additional effects seen at 24 h might be related to cell cycle redistribution (i.e., synchronized tumor cells in a sensitive phase at the time of irradiation).

The data presented here indicate that the degree to which fludarabine enhances radiation-induced regrowth delay is tumor type dependent (Fig. 1 and Table 1). While the degree of fludarabine enhancement in the MCA-K tumor was probably underestimated owing to the exclusion of cured animals from the quantitation of regrowth delay, the MCA-4 tumor appeared to be less sensitive to fludarabine’s potentiating activity. The mechanisms underlying the differential sensitivity between cell lines are not well understood. Two possible mechanisms need to be considered. First the tumors may differ in their pharmacokinetics of F-ara-ATP. Second, the success of repair inhibition may depend on the repair capacity of the particular tumor. For example, this strategy would be expected to have the greatest effect on those tumors that are radioresistant due to a high repair capacity. Both SA-NH and MCA-K tumors exhibit sublethal damage repair after split-dose experiments, but the relative amount of repair between these two tumors cannot be accurately estimated. A direct measurement of the chromosome repair kinetics using the technique of premature chromosome condensation might help clarify this issue and might further serve as a selection assay to identify tumors most likely to benefit from combined radiation and fludarabine treatment.

In conclusion, the data presented here and elsewhere suggest that fludarabine is a potent enhancer of radiation-induced tumor regrowth delay and may have some advantage over other nucleoside analogues. However, further investigations are needed before its potential clinical benefit can be predicted. One important aspect is the validation of the results reported here using tumor cure as an end point after single or fractionated irradiation. A second consideration is whether the drug levels achieved in animals can be achieved in patients. While pharmacological experiments were not part of these studies, levels of DNA synthesis inhibition were achieved in the treated mice that were similar to those achieved when fludarabine was used in the clinical setting (data not shown and Ref. 31). Another important consideration is the effect of the combination on early and late-reacting normal tissues. Indeed, a clinical advantage will be seen from the combined modality treatment only if the tumor response is greater than the added toxicity to normal tissue included in the radiation field. Such studies are ongoing in our laboratory.

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Luka Milas, unpublished data.
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