Intrinsic and Extrinsic Heterogeneity in the Responses of Parent and Clonal Human Colon Carcinoma Xenografts to Photon Irradiation

John T. Leith, Sarah F. Bliven, Eun Sun Lee, Arvin S. Glicksman, and Daniel L. Dexter

Department of Radiation Oncology, Rhode Island Hospital [J. T. L., S. F. B., E. S. L., A. S. G.], and Departments of Radiation Medicine [J. T. L., A. S. G.] and Medicine [D. L. D.], Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912, and Pharmaceuticals Research and Development, Cancer Chemotherapy, E. I. DuPont De Nemours & Co., Inc., Wilmington, Delaware 19898 [D. L. D.]

ABSTRACT

Responses to photon irradiation of xenografted human colon tumors derived from the heterogeneous DLD-1 line or its derivative A and D subpopulations were determined using excision assay and tumor regrowth delay assays. Differential responses among the three xenografted carcinomas were demonstrated. Clone A tumors treated with up to 17.5 Gy showed no actual regression below pretreatment volume. In contrast, clone D tumors were sensitive to doses as low as 3.5 Gy, and tumor volumes were reduced by 65% with a dose of 17.5 Gy. The responses of DLD-1 tumors were intermediate between the clone A and clone D tumor responses. The survival parameters obtained in the excision assay studies for the DLD-1, clone A, and clone D tumors were, respectively: n = 3.3, 1.4, and 1.0; D0 (Gy) = 2.1, 2.2, and 2.7; and D0 (Gy) = 2.6, 0.6, and 0.0. These data indicate that the DLD-1 tumors were the most resistant, with clone A of intermediate sensitivity, clone D being the most sensitive tumor. In addition to the interclonal diversity among xenograft lines, intraclonal variation was also observed with clone A (but not clone D or DLD-1) tumors. A biphasic survival curve of cells from clone A xenografts irradiated in air-breathing hosts clearly indicated a minority (~3%) subpopulation of hypoxic cells. Similar results indicating a small percentage of hypoxic cells in clone A solid tumors were obtained from the tumor regrowth delay studies. Also, excision assay data from experiments in which the heterografted carcinomas were irradiated under anoxic conditions support the interpretation that clone A tumors contain a small fraction of hypoxic cells. This study indicates that: (a) heterogeneity in vivo to ionizing radiation exists in the DLD-1 system; and (b) intraclonal variation occurs in vivo due to extrinsic (e.g., environmental hypoxia) factors, such that the intrinsic radiosensitivity of a subpopulation (clone A) of a heterogeneous human tumor can be further increased.

INTRODUCTION

The existence of phenotypic heterogeneity within single mouse and human solid tumors has been compellingly documented (1, 4–6, 9, 11–15, 19, 20, 26, 27, 29, 30, 35, 36, 41, 43). Data have been obtained from sublines cloned from heterogeneous human parent cancer cell lines (9, 11, 15, 35), from in vivo selection of variant subpopulations (14, 41), and from direct sampling of murine and human neoplasms (1, 36, 43). Furthermore, the clinical implications of this intraneoplastic diversity have been discussed by several investigators (4, 5, 19, 24).

However, there remain a number of unanswered questions that arise as one considers the phenomenology associated with intratumor heterogeneity. For example, do results obtained with cultured parent and subpopulation cell lines necessarily correlate with in vivo data obtained from the same heterogeneous system? What are the clinical implications for different patterns of heterogeneity demonstrated in vitro and in vivo for the same phenotypic characteristic (i.e., differential responses to cytotoxic agents)? Finally, could a differential in vivo response between parent and subpopulations to a given treatment modality, which may rank sensitive versus resistant lines quite differently from results of in vitro testing, indicate the existence of other levels of variability within solid tumors? Clearly, such levels of additional complexity may not be demonstrable in experiments with cultured lines.

Our laboratory has previously reported differential responses in vitro to X-irradiation among the heterogeneous parent human colon carcinoma cell line DLD-1 and its clonal A and D subpopulations (23, 24). The purposes of the study described here were to determine: (a) whether a heterogeneous response to irradiation also occurs in vivo; (b) whether the ranking of DLD-1, A, and D in terms of sensitivity to ionizing radiation is the same for cultured cells versus xenograft tumors; (c) whether one can explain any observed ranking differences on the basis of a new level of heterogeneity (complexity) associated with the 3-dimensional architecture of the tumor compared to the 2-dimensional monolayer culture; and (d) what clinical implications are indicated by these findings? In this regard, we have addressed these questions using 2 distinct experimental methods for assessment of solid tumor responses, excision assay (e.g., 8, 22, 31–34) and tumor regrowth delay (2, 28, 38, 39).

MATERIALS AND METHODS

Tumor Lines. The DLD-1 tumor system used in this investigation has been extensively characterized and studied (4, 10, 13, 23, 24, 37). The original DLD-1 human colon carcinoma removed from the patient was histologically heterogeneous, and the cell line established from it was shown to be morphologically and karyotypically heterogeneous. Two distinct subpopulations, A and D, were cloned from an agar culture of the parent DLD-1 line. Clones A and D in vitro are different in their karyotypes, morphologies, cloning efficiencies in soft agar, and responses to several antineoplastic drugs, ionizing radiation, and hyperthermia (4, 11, 23–26). Clone A cells and clone D cells produce poorly differentiated and moderately differentiated colon cancers, respectively, in nude mice; DLD-1 cells produce moderately to poorly differentiated colon tumors in athymic hosts (11). Importantly, our laboratory has recently reported preliminary findings that demonstrate that clone A has greater metastatic potential than clone D (37). Our results strongly

SEPTEMBER 1984

3757
indicate that the 2 subpopulations are responsible for the heterogeneity observed in the parent neoplasm and the cell line established from it (11). The 3 lines are maintained in tissue culture according to methods reported previously from our laboratory (11, 23–26).

Production of Xenograft Tumors. Mice bearing the nu/nu gene on an outbred Swiss background were bred and maintained in the Roger Williams Cancer Center Animal Care Facility. Nude mice of both sexes, 4 to 6 weeks of age, were used in these studies. Animals were each inoculated in the upper hip region with 1 × 10^2 cultured human colon cancer cells; xenograft tumors appeared in 4 to 7 days. After tumors had reached a size of about 5 × 5 mm, mice were carefully matched into groups of 10 animals each and were ear-tagged so that host mice could be followed individually. Tumors were irradiated when they had attained a size of approximately 8 × 8 mm.

Measurement of Tumor Size. The solid tumors were measured by calipers in 2 orthogonal diameters, and the tumor volumes were calculated using the formula for a prolate ellipsoid:

\[ V = \frac{4}{3} \pi a \times b \times c \]

where \( L \) and \( W \) are the major and minor diameters (in mm), respectively. The average tumor volumes for each irradiation group were then plotted as a function of time postirradiation to obtain tumor growth curves.

Irradiation of Solid Tumors. Tumors were irradiated using a Siemens's 6 MeV linear accelerator at the Rhode Island Hospital Department of Radiation Oncology. The mice were lightly anesthetized with methoxyflurane (Metofane; Pitman-Moore, Inc., Washington Crossing, NJ) and then placed on a 1.5-mm-thick slab of Lucite. Mice were restrained in place by tape and allowed to recover from the anesthesia. Two mice were irradiated simultaneously and were positioned with the flank tumors in a 3 × 3-cm irradiation field, with the photon beam traversing the Lucite slab from beneath to ensure proper electronic equilibrium. A source to target distance of 100 cm was used, and the mice were irradiated at ambient temperature at a dose rate of 2.0 Gy/min. This procedure was used for the irradiation of the solid tumors for the tumor regrowth delay studies. However, for the oxic and hypoxic tumor cell survival curve work, due to the fact that the tumors were excised immediately after irradiation, the field size was increased to 10 × 10 cm. This allowed us to irradiate each group of mice per dose point in a short period of time, which facilitated rapid excision of the tumors.

Clonogenic Cell Survival Studies Using Excision Assay. Excision assays is an accepted method for determining the effect of ionizing radiation or other cytotoxic agents on solid tumors as measured by the survival of clonogenic cells (3, 22, 31–34). In our experiments, the basic protocol was to irradiate solid tumors in vivo which were then immediately excised, placed into ice-cold Hank's balanced salt solution (Grand Island Biological Co., Grand Island, NY), and weighed. Tumors were then minced into approximately 1-cm fragments using a scalpel blade and subsequently treated with enzyme solution to prepare single-cell suspensions. Detailed studies of 0.5% trypsin, 0.2% trypsin, or an enzyme cocktail containing pronase (Grand Island Biological Co.), collagenase (Worthington Biochemical Co., Freehold, NJ), and DNase I (Sigma Chemical Co., St. Louis, MO) were performed in order to establish which treatment would be optimal in terms of cell yield (cells/mg/min) and CFE (data not shown). In all of this work, 0.5% solutions of trypsin [prepared from 2 to 5% trypsin stock (Grand Island Biological Co.) in Hank's balanced salt solution] were used.

Generation of Survival Curves. As discussed previously, the entire tumor-bearing mouse was irradiated, and groups of restrained but unanesthetized or nitrogen gas-asphyxiated mice (10 min) were used. Tumors from the air-breathing mice yield survival curves representative of cells from normally oxygenated tumors, whereas tumors from the nitrogen gas-asphyxiated mice yield survival curves representative of tumors that were 100% anoxic. These 2 sets of data were necessary to calculate any possible hypoxic fractions present in the 3 solid tumor lines. The solid tumors were handled exactly as in the protocol described above. After obtaining a single-cell suspension of tumor cells in complete fresh RPMI 1640 medium, appropriate numbers of tumor cells were seeded into 60-mm plastic dishes to produce an adequate number of visible colonies at 10 to 14 days of incubation in vitro in a 5% CO2/95% air humidified 37°C environment. Only colonies containing more than 50 cells were counted. Colonies were gently washed with 0.9% NaCl solution and were then fixed and stained using a 0.5% solution of crystal violet in absolute methanol. Survival data were calculated according to a standard radiobiological formalism. To describe the data, we used the single-hit, multitarget (SHMT) equation in which 3 parameters can be identified: \( n \) (extrapolation number), \( D_0 \) (the slope of the linear region of the inactivation curve), and \( D_0 \) (the quasithreshold dose given by the intersection of the back extrapolation of the linear region of the survival curve to 100% survival). Standard statistical methods were used to calculate the error limits on \( n \) and \( D_0 \) (16).

Tumor Regrowth Delay Study. After photon treatment, mice were followed for radiation effects on tumor size. Tumor dimensions were obtained at least 3 times weekly for a period of about 1 month. Tumor sizes in cm were estimated using the formula given previously.

As an index of the sensitivities of the xenografted tumor lines to irradiation, we calculated the time (in days) needed for the tumors to grow to twice their volumes at the time of irradiation. As this time will increase with increasing radiation dose, it provides dose-response curves for the 3 different solid tumors which can be used to intercompare their relative radiation sensitivities.

RESULTS

Oxic Survival Parameters from Excision Assay Experiments. In Table 1, we list the CFE values of control studies on oxic and hypoxic tumors and the average tumor weights found in these excision assay studies. Neither the CFE values nor the tumor weights are significantly different among groups.

The survival data obtained from hypoxic and oxic tumors for the 3 tumor lines are shown in Chart 1. It may be seen that the responses are qualitatively similar, with the exception of the biphasic curve seen for the clone A tumor cells obtained from tumors irradiated in air-breathing hosts. Also, in Tables 2 and 3, we have summarized the survival data obtained from these in vivo irradiations of the solid tumors as assessed by excision assay. In Table 2, survival parameters obtained from irradiating tumors in the restrained, air-breathing mice are listed. In terms of the inactivation of the 3 cell lines indicated by the \( D_0 \) value (excluding the data in the "tail" region of the clone A oxic survival curve), there is no significant difference among the lines. An average \( D_0 \) value of 2.36 Gy would adequately describe the survival responses of all 3 lines (as the 95% confidence limits on the individual values overlap). Both clone A and clone D appear to have extrapolation numbers of approximately 1.0, whereas DLD-1 has an \( n \) value significantly greater than 1 (3.34).

Hypoxic Survival Parameters from Excision Assay Experiments. The radiation survival parameters for the cells obtained from hypoxic tumors are listed in Table 3. The major change is the increase in the \( D_0 \) value caused by the presence of complete hypoxia. Indeed, in this case, the dose-modifying action of oxygen can be calculated (the OER) from the ratios of the \( D_0 \) values for the oxic and survival curves. These OER values are 2.42 ± 0.30 (S.E.), 3.10 ± 0.45, and 2.49 ± 0.70 for the DLD-1, clone A, and clone D tumors, respectively. The OER for clone A appears to be higher than that for the DLD-1 or clone D tumors,
Carcinoma Xenograft Responses to Photon Irradiation

Table 1

<table>
<thead>
<tr>
<th>Subline</th>
<th>DLD-1 (parent line)</th>
<th>Clone A</th>
<th>Clone D</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFE (%)</td>
<td>Av. wt (mg)</td>
<td>CFE (%)</td>
<td>Av. wt (mg)</td>
</tr>
<tr>
<td>Oxic controls</td>
<td>27.9 ± 8.4(4)b</td>
<td>603.1 ± 68.9</td>
<td>33.4 ± 14.3(4)</td>
</tr>
<tr>
<td>Hypoxic controls</td>
<td>35.0 ± 4.7(5)</td>
<td>685.4 ± 91.5</td>
<td>29.6 ± 11.3(5)</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Subline</th>
<th>Extrapolation no.</th>
<th>D0 (Gy)</th>
<th>D0 (Gy)</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLD-1 (parent line)</td>
<td>3.34 (1.77-6.33)</td>
<td>2.12 (±0.21)</td>
<td>2.56</td>
<td>0.990d (12)c</td>
</tr>
<tr>
<td>Clone A</td>
<td>1.35 (1.26-1.39)</td>
<td>6.67 (±0.53)</td>
<td>0.9899 (17)</td>
<td></td>
</tr>
<tr>
<td>Clone A (tail)d</td>
<td>0.89 (0.49-1.60)</td>
<td>2.66 (±0.31)</td>
<td>0.9786 (17)</td>
<td></td>
</tr>
<tr>
<td>Clone D</td>
<td>2.50 (1.56-3.99)</td>
<td>5.14 (±0.38)</td>
<td>4.70</td>
<td>0.990d (16)c</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Subline</th>
<th>Extrapolation no.</th>
<th>D0 (Gy)</th>
<th>D0 (Gy)</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLD-1 (parent line)</td>
<td>2.50 (1.56-3.99)</td>
<td>6.68 (±0.83)</td>
<td>1.16</td>
<td>0.976d (17)</td>
</tr>
<tr>
<td>Clone A</td>
<td>0.92 (0.34-2.50)</td>
<td>6.62 (±1.71)</td>
<td>0.930 (16)</td>
<td></td>
</tr>
</tbody>
</table>

Although it appears that the radiation responses of these 3 tumor lines are essentially similar, there is one important exception. The exception is the clear indication in the clone A tumor responses of a biphasic curve (Chart 1B), seen at doses greater than about 10 Gy. In Table 2, we have supplied the inactivation parameter for this region of the survival curve ("tail"), which has a D0 value of 6.87 Gy. This may be interpreted as indicating the presence of a small percentage of hypoxic cells in the original solid tumor which consequently possess a different radiosensitivity. The hypoxic fraction in clone A solid tumors may be calculated, and it is approximately 3.3 ± 0.6% (95% confidence limits) (16). As the 95% confidence limits on this value do not include 0, we feel that a true hypoxic fraction exists in clone A solid tumors. This presumption can be verified by inspection of data presented in Table 3 for hypoxic survival curve responses. For clone A, the D0 value for the hypoxic cells from 100% hypoxic tumors is 6.68 Gy. This is not statistically different from the value of 6.87 Gy obtained from fitting of the tail region of the oxic tumor responses (Chart 1B). This result fits the criterion of the so-called parallel line hypoxic cell bioassay as described by other investigators (21, 42).

Therefore, while the sensitivities of the 3 solid tumors appear roughly similar using the excision assay approach, clone A exhibits another feature of tumor heterogeneity, that of extrinsic heterogeneity in cell survival produced by in situ hypoxia.

Tumor Regrowth Studies. The effects of graded photon doses on tumor growth are shown in Charts 2 to 4 for clone A, clone D, and DLD-1 tumors, respectively. Normalized tumor volumes as estimated from caliper measurements are plotted against time on these graphs. Clone A was the most resistant tumor; no actual regression of clone A neoplasms (i.e., below the tumor volume at the time of irradiation) was observed, even at a dose of 17.5 Gy. Clone D tumors showed the greatest sensitivity to irradiation, with tumor volumes being reduced 65% by Day 13 at a dose of 17.5 Gy compared to tumor volumes at the initiation of treatment. Clone D was the only tumor to exhibit
a radiation response to the lowest dose tested (3.5 Gy). DLD-1 tumors displayed an intermediate sensitivity to radiation. The tumor volumes of these neoplasms were reduced by 35% by Day 16 postirradiation at 17.5 Gy as compared to tumors just prior to irradiation.

**Radiation-induced Tumor Growth Delay.** In Chart 5, we have plotted the radiation-induced delay demonstrated in the 3 solid tumor lines as a function of photon dose. These values are taken from the data presented in Charts 2 to 4, and we have used the time (days) needed for tumors to grow to twice their volume at the time of irradiation as our index of differential radiation response among the 3 tumor types. To define the "excess" delay produced by ionizing radiation in the 3 tumors, the doubling time found for the unirradiated control solid tumors was subtracted from the doubling time seen in irradiated tumors. This procedure essentially normalizes the responses of the 3 tumor lines by separating radiation effects from inherent tumor kinetic effects, and it produces a set of dose-response curves that can be directly compared. For the unirradiated control tumors, the time needed to grow to twice the volume at the time of irradiation was calculated to be 5.4 days for the DLD-1 tumors, 3.8 days for the clone A tumors, and 4.5 days for the clone D tumors.

**DISCUSSION**

The results obtained from this investigation show that DLD-1, clone A, and clone D xenograft tumors exhibit different sensitivities to ionizing radiation. The data obtained with 2 different methodologies, the excision assay and the tumor regrowth delay analysis, are quite consistent and demonstrate that, in vivo, clone A is the most radioresistant subpopulation, clone D is most sensitive, and DLD-1 is intermediate in its sensitivity. This is in contrast to our earlier findings with cultured cells, which indicated that DLD-1 cells were approximately as resistant to ionizing radiation as were clone A cells (24), although clone D cells were still the most radiosensitive. Therefore, although heterogeneity within the DLD-1 system in response to ionizing radiation can be demonstrated with either in vitro or in vivo tumor cell studies, the ranking of the lines is somewhat different, depending on whether one compares cultured cells or xenografted carcinomas.

It is important to point out the stability of these tumor populations in the interpretation of the intrinsic and extrinsic responses described in this publication. If these tumor populations were not stable, it would not be possible to relate our current findings to
Carcinoma Xenograft Responses to Photon Irradiation

There was no effect produced by 3.5 Gy of photons in either clone A or DLD-1 tumors compared to control tumors. This supports our earlier finding on the effect of ionizing radiation against cultured clone A and DLD-1 cells; both of these lines were also resistant in vitro to low doses of X-irradiation (24). In contrast, clone D was sensitive to in vivo irradiation at 3.5 Gy. A different picture is observed in the high-dose region. In agreement with the excision assay results, DLD-1 and clone D tumors continue to show a linear response to increasing doses of photons. However, a possible break in the clone A survival curve is indicated by the responses seen at the high dose of 17.5 Gy. Although the data are limited, this is in good agreement with the results obtained with clone A tumors using the excision assay (Chart 1B). Clone A tumors therefore appear to be radioresistant both in the low-dose (3.5 Gy) (Chart 1B) and higher-dose (>13 Gy) regions, as indicated by the tumor growth delay data (Chart 5).

Therefore, one interpretation of our results is that yet another level of heterogeneity exists in clone A tumors but not in DLD-1 or clone D neoplasms. A small (around 3%) yet significant (in terms of radiocurability) percentage of cells in clone A tumors differs in its response to X-irradiation compared to the majority of cells in clone A xenografts. This small subset of cells manifests a radiosensitivity phenotype in the high-dose region that is distinct from the majority of clone A cells. Our analysis of clone A cells surviving high doses of X-irradiation (15 to 20 Gy) and recovered in excision assays indicates that the morphology, karyotype, and growth properties of these "resistant" cells are essentially indistinguishable from the more sensitive clone A cells obtained from unirradiated tumors or cell cultures. Therefore, the hypoxic fraction occurring in vivo is due to extrinsic (environmental) rather than intrinsic (cellular) factors. These could include variable delivery of oxygen to cells in different regions of the tumor, unequal distribution of nutrients to all cells, and failure to remove waste products and dead cells with equal efficiency from all portions of the neoplasm (3, 21, 34, 42).

In this regard, Guichard et al. (17) have recently studied the radiobiological characteristics of 1 rectal and 2 colonic human adenocarcinomas (HRT18, HCT8, and HT29, respectively) using excision assay techniques. It was determined that the hypoxic fractions of these tumors were approximately 14, 85, and 17%, respectively. Therefore, from the data of Guichard et al. (17) and the data presented in this paper, it is apparent that the hypoxic fractions of solid gastrointestinal tumors can vary appreciably, either between or within a single neoplasm. These data and the finding that one of our tumors (clone D) apparently contains essentially no hypoxic cells points up the need for additional studies on human tumor xenografts.

However, there are several other important correlates of radiocurability that are suggested, if not demonstrated, by this investigation. These correlates would not be apparent from an evaluation of data obtained from studies with cultured cells and, to our knowledge, they have not been assessed from the perspective of heterogeneity within human carcinomas. (a) Differential resistance to ionizing radiation can arise within a clone, as well as among clones within the heterogeneous parent tumor. In this study, interclonal heterogeneity for response to X-irradiation in the low-dose region is probably due to intrinsic cellular factors such as differences in repair enzymes levels. The relative radiodose resistance for A compared to D is incremented in the high-dose region, due probably to extrinsic factors causing intraclonal
heterogeneity for response to X-irradiation within the A subpopulation itself. This intracranial variation could result from the presence of hypoxic conditions within a portion of the clone A tumor. (b) This environmental creation of a new level of heterogeneity within clone A tumors cannot be predicted from in vitro radiation studies. (c) The appearance of a hypoxic clone A fraction, shown by the break in the high-dose region of the survival curve, indicates that it will be quite difficult to cure this tumor with high doses of radiation without exceeding the radiotoxicity of host tissues in the beam volume. Furthermore, treatment with a fractionated dose scheme may not work either, because of the resistance demonstrated in the low-dose region. We conclude that the clone A subpopulation would be extremely difficult to eradicate in vivo.

In summary, a clone intrinsically resistant to radiation can become even less sensitive because of the appearance of a hypoxic subset of cells within that clone. This hypoxic fraction would probably appear because of extrinsic, that is, host-related factors rather than intrinsic factors. Thus, a neoplastic clone resistant to radiation due to a relatively greater ability to repair DNA damage could become even more radioresistant due to tumor tissue geography. The major conclusion of this study is that tumor heterogeneity due to extrinsic factors can enhance resistance due to intrinsic tumor diversity. The clinical implications of resistance in neoplasms arising from extrinsic, host-related events should be considered by investigators, regardless of the therapeutic modality utilized.

REFERENCES


15. Hart, I. R., and Fidler, I. J. Clinical implications of resistance in neoplasms arising from extrinsic, host-related factors rather than intrinsic factors. Thus, a neoplastic clone resistant to radiation due to a relatively greater ability to repair DNA damage could become even more radioresistant due to tumor tissue geography. The major conclusion of this study is that tumor heterogeneity due to extrinsic factors can enhance resistance due to intrinsic tumor diversity. The clinical implications of resistance in neoplasms arising from extrinsic, host-related events should be considered by investigators, regardless of the therapeutic modality utilized.
Intrinsic and Extrinsic Heterogeneity in the Responses of Parent and Clonal Human Colon Carcinoma Xenografts to Photon Irradiation


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/9/3757

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.