In vivo Therapeutic Responses Contingent on Fanconi Anemia BRCA2 Status of the Tumor

Michiel S. van der Heijden,1,4 Jonathan R. Brody,1 David A. Dezentje,1 Eike Gallmeier,1 Steven C. Cunningham,1 Michael J. Swartz,3 Angelo M. DeMarzo,2 G. Johan A. Offerhaus,5 William H. Isaconf,6 Ralph H. Hruban,1,2 and Scott E. Kern1,2

Abstract Purpose: BRCA2, FANCC, and FANCG gene mutations are present in a subset of pancreatic cancer. Defects in these genes could lead to hypersensitivity to interstrand cross-linkers in vivo and a more optimal treatment of pancreatic cancer patients based on the genetic profile of the tumor. Experimental Design: Two retrovirally complemented pancreatic cancer cell lines having defects in the Fanconi anemia pathway, PL11 (FANCC-mutated) and Hs766T (FANCG-mutated), as well as several parental pancreatic cancer cell lines with or without mutations in the Fanconi anemia/BRCA2 pathway, were assayed for in vitro and in vivo sensitivities to various chemotherapeutic agents. Results: A distinct dichotomy of drug responses was observed. Fanconi anemia–defective cancer cells were hypersensitive to the cross-linking agents mitomycin C (MMC), cisplatin, chlorambucil, and melphalan but not to 5-fluorouracil, gemcitabine, doxorubicin, etoposide, vincristine, or paclitaxel. Hypersensitivity to cross-linking agents was confirmed in vivo; FANCC-deficient xenografts of PL11 and BRCA2-deficient xenografts of CAPAN1 regressed on treatment with two different regimens of MMC whereas Fanconi anemia–proficient xenografts did not. The MMC response comprised cell cycle arrest, apoptosis, and necrosis. Xenografts of PL11 also regressed after a single dose of cyclophosphamide whereas xenografts of genetically complemented PL11 FANCC did not. Conclusions: MMC or other cross-linking agents as a clinical therapy for pancreatic cancer patients with tumors harboring defects in the Fanconi anemia/BRCA2 pathway should be specifically investigated.

In 2004, 31,860 Americans were expected to be diagnosed with pancreatic cancer and 31,270 were expected to die from this disease, making pancreatic cancer the fourth leading cause of cancer death in the United States (1). The only effective treatment is surgical resection, which is done in <20% of cases. Marginally effective therapies include irradiation, gemcitabine, and 5-fluorouracil (5-FU; refs. 2, 3). Regimens combining various chemotherapeutic drugs are being investigated and a rare complete response is achieved on treatment with mitomycin C (MMC) or cisplatin-containing regimens (4–8). Clearly, new therapeutic approaches are necessary. The emerging field of cancer pharmacogenomics offers an attractive new therapeutic approach in which treatment is based on the genetic profile of a patient’s cancer. Although successful examples exist, opportunities to translate this approach into the clinic are not often encountered (9–12).

Proteins encoded by the BRCA2, FANCC, and FANCG genes function in the repair of damage caused by DNA-interstrand cross-linking agents. One of these three genes is inactivated in 5% to 10% of apparently sporadic pancreatic cancers (13–16). Although present in only a minority of pancreatic cancers, mutations in the BRCA2 gene and in genes that code for proteins in the proximal Fanconi anemia pathway (collectively referred to as the Fanconi anemia/BRCA2 pathway) could provide a rational target for treatment with chemotherapeutic agents. Nonneoplastic cells taken from Fanconi anemia patients have a striking hypersensitivity in vitro to interstrand cross-linking agents, such as MMC and cisplatin. This hypersensitivity was confirmed in vivo in pancreatic cancer cells with mutations in the BRCA2, FANCC, or FANCG genes as compared with several Fanconi anemia–proficient pancreatic cancer cell lines (14). The sensitivity of Fanconi anemia–deficient cancer cells to other chemotherapeutic agents has not yet been assessed. It also remains...
unknown whether Fanconi anemia–deficient pancreatic cancer cells are hypersensitive to standard doses of cross-linking agents in vivo. In this study, we use retrovirally corrected pancreatic cancer cell lines to study the effect of defects in the FANCC and FANCG genes on the sensitivities to a panel of antineoplastic agents (14). We find that Fanconi anemia–defective pancreatic cancer cells are selectively hypersensitive to DNA-interstrand cross-linking agents in vivo.

Materials and Methods

Samples. Pancreatic cancer cell lines MiaPaCa2, AsPc1, Su86.86, CFPAC, CAPAN1, and Hs766T were obtained from American Type Culture Collection (Manassas, VA); PL11 (Panc3.27) was kindly provided by Dr. E.M. Jaffee (Department of Oncology, Johns Hopkins University, Baltimore, MD) and is also available from American Type Culture Collection. Cells were grown in conventional tissue medium supplemented with 10% fetal bovine serum, penicillin/streptomycin, and L-glutamine. PL11 and Hs766T were infected with retroviral Moloney murine leukemia virus/vesicular stomatitis virus-G protein vectors containing the puromycin resistance gene and the cDNA of FANCC or FANCG, respectively (kind gift of Dr. A. d’Andrea), or infected with only the puromycin resistance gene (empty vector) as previously described (14, 17, 18). A pooled population of stable clones was selected with G418 (Invitrogen, Carlsbad, CA). Institutional Review Board guidelines of Johns Hopkins University were followed for commercially available cell lines.

Immunofluorescence and immunohistochemistry. For Fancd2 immunofluorescence, cells were grown on slides and treated with MMC (Sigma, St. Louis, MO) at 100 nmol/L for 18 hours. Slides were fixed in 2% paraformaldehyde, permeabilized in 0.3% Triton X-100 for 10 minutes, blocked in 1% serum for 20 minutes, and labeled using mouse anti-Fancd2 primary antibody (diluted 1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA) and Cy3-conjugated goat anti-mouse secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA). For immunobLOTS, cells were lysed and boiled, then proteins were separated on 3% to 8% Tris-acetate polyacrylamide gels (Invitrogen), transferred onto a polyvinylidene difluoride membrane, blocked for 1 hour in TBS-Tween 20-5% milk, and incubated overnight.
with anti-Fancd2 antibody. Binding was detected using SuperSignal West Pico chemiluminescence substrate (Pierce Biotechnology, Rockford, IL). For immunohistochemistry, xenografts were fixed and labeled using the Envision+ kit (DAKO Corp., Carpertania, CA) as previously described (13). Slides were washed and stained with primary antibody (1:400 dilution for cleaved caspase 3; Cell Signaling, Beverly, MA). Positive cells were counted by a blinded study and by random sampling of 500 cells per slide (19).

Survival assay. After the appropriate dose range was determined, 1.1 × 10^6 cancer cells per well were incubated in 96-well plates with various concentrations of MMC, doxorubicin, etoposide, vinblastine, cisplatin, paclitaxel, melphalan, 5-FU (Sigma), or gemcitabine (Lilly, Indianapolis, IN). Cells were incubated long enough to allow nontreated cells to reach at least a 3-fold increase in fluorescence as compared with day 1 (4-6 days). Medium was replaced by drug-free medium after 72 hours. Cells were washed with PBS and lysed in 100 μL sterile water. After 1 hour, 100 μL of 0.5% PicoGreen (Molecular Probes, Eugene, OR) in Tris-EDTA buffer were added to each well. After 45 minutes, wells were read in a fluorometer. Survival was averaged from four identical wells per experiment and converted to a percentage; the wells without drugs were considered as 100%. At least six experiments were done per cell line per concentration on at least three separate occasions.

Apoptosis assay. For the Annexin V/propidium iodide assay, cells were plated, allowed to adhere overnight, and incubated with 0 or 25 μmM MMC for 72 hours. Cells were then harvested, washed with PBS, stained with Annexin V and propidium iodide (Molecular Probes) as indicated by the manufacturer, and measured in a flow cytometer. Caspase inhibitor I (50 μmM; Calbiochem) was added to the medium 1 hour before treatment with MMC.

Xenograft establishment and treatment. Pancreatic cancer cell lines were injected s.c. in the flank of female athymic nude mice of ages 5 to 9 weeks. Xenografts were established and measured with an electronic caliper every 2 days; tumor volume was estimated with the formula (width × length^2) / 2. Xenografts were grown to a size of at least 280 mm^3 before treatment was initiated. MMC, cyclophosphamide (Sigma), or gemcitabine were injected i.p. in accordance with our institutional guidelines, mice bearing xenografts over 2,500 mm^3 in size were sacrificed.

### Results

Pancreatic cancer cells mutated in FANCC or FANCG are hypersensitive to DNA-interstrand cross-linking agents in vitro. In previous work, the pancreatic cancer cell line Hs766T was found to harbor a germ line truncating mutation in the FANCC gene (15); another pancreatic cancer cell line, PL11, was found to have a homozygous deletion of the FANCC gene (14). These mutations lead to a defect in Fancd2 monoubiquitination that can be corrected by retroviral complementation with FANCC and FANCG cDNAs (14). In the current work, we found that Fancd2 nuclear focus formation in Hs766T was restored by retroviral complementation (Fig. 1A). Empty vector–transfected, Fanconi anemia–defective cancer cells had an 8- to 10-fold greater sensitivity to MMC as compared with the appropriately complemented cell lines (Fig. 1B; Table 1). The parental Fanconi anemia–defective cell lines Hs766T and PL11 and the BRC-A2-mutated pancreatic cancer cell line CAPAN1 are also hypersensitive to MMC as compared with the Fanconi anemia–proficient pancreatic cancer cell lines Su66.86, CFPAC, AsPC1, and MiaPaCa2 (ref. 15; Fig. 1C).

To establish whether Fanconi anemia–defective cancer cells were hypersensitive exclusively to MMC and other DNA-interstrand cross-linking agents, the sensitivity of a panel of cell lines to several commonly used drugs from different classes of antineoplastic agents was determined. Two cell line pairs were used: the retrovirally corrected PL11 cell line (PL11FANCC) and the empty vector–transduced, FANCC-mutated PL11 cell line (PL11EV); and the corrected Hs766T cell line (Hs766TFANCC) and the FANCC-mutated Hs766T cell line transduced with an empty vector (Hs766TEV). Both FANCC- and FANCG-deficient cancer cells were hypersensitive to the DNA-interstrand cross-linking agents cisplatin, chlorambucil, and melphalan (Fig. 2A-C; Table 1). These results were consistent over multiple experiments. The functional Fanconi anemia status of the cell lines was confirmed periodically throughout the study by Fancd2 immunoblot (data not shown).

There was no difference in sensitivity to the microtubule inhibitors paclitaxel and vinblastine or to the topoisoeraser inhibitors etoposide and doxorubicin when comparing the Fanconi anemia–defective cancer cells to their genetically corrected counterparts (Fig. 2D-G; Table 1). Unexpectedly, the complemented cell line PL11FANCC was more sensitive than PL11EV to the two antimetabolites tested, 5-FU and gemcitabine (Fig. 2H and I); Hs766TEV and Hs766T FANCC were equally sensitive to these drugs.

Pancreatic cancer cells mutated in FANCC or FANCG are hypersensitive to DNA-interstrand cross-linkers in vivo. To establish whether the observed drug hypersensitivity of Fanconi anemia–deficient cancer cells in vitro can be extended to an in vivo model, we transplanted various human pancreatic cancer cell lines s.c. into athymic nude mice. The s.c. placement of xenografts precluded a local effect on the tumor by potential inflammatory processes caused by i.p. chemotherapeutic injection. The xenografts were measured every 2 days and were grown to a size of at least 280 mm^3 (average, 395 mm^3; SD, 84) before treatment was initiated. In the first MMC treatment regimen, we aimed to induce a maximum response using an intensive regimen of repeated doses. Xenografts of the pancreatic cancer cell lines Su66.86 (n = 11), CFPAC (n = 11), MiaPaCa2 (n = 11), CAPAN1 (BRC-A2-mutated, n = 12), PL11 (FANCC-mutated, n = 9), and PL11 FANCC (n = 12) were treated with 2.5 mg/kg MMC i.p. every 4 days. Due to differing xenograft growth rates, the two Hs766T cell lines (Hs766TEV and Hs766T FANCC) could not be analyzed. Xenografts were followed for at least 40 days, up

### Table 1. Sensitivity of Fanconi anemia–deficient cell lines to various chemotherapeutic agents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ratio of IC_{50} values determined for retrovirally corrected cells and empty vector–infected cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hs766T (FANCG)</td>
</tr>
<tr>
<td>MMC</td>
<td>7.6</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>5.1</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>4.0</td>
</tr>
<tr>
<td>Melphalan</td>
<td>6.4</td>
</tr>
<tr>
<td>5-FU</td>
<td>0.9</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>0.8</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>1.2</td>
</tr>
<tr>
<td>Etoposide</td>
<td>1.5</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>0.7</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>1.2</td>
</tr>
</tbody>
</table>
to a maximum of 60 days (Fig. 3A and data not shown). Xenografts of CAPAN1 and PL11, harboring mutations in the BRCA2 and FANCC genes, respectively, regressed (a decline in volume) soon after treatment was initiated whereas the Fanconi anemia–proficient xenografts, including the genetically corrected PL11FANCC, progressed. After several weeks of treatment with repeated doses of MMC, growth inhibition of Fanconi anemia–proficient xenografts became apparent.

Substantial toxicity after treatment was observed with the regimen of repetitive doses of MMC in both mice with Fanconi anemia–deficient and Fanconi anemia–proficient xenografts. Mice frequently became anemic after ~ 30 days of treatment. The mortality rate with this treatment regimen was 43%, with mice dying, on average, 51 days (range 45-59) after the treatment was initiated. Death often occurred several days after petechiae initially appeared, eventually progressing to widespread hemorrhage.

Separately, we treated a panel of pancreatic cancer xenografts with a single dose of MMC (5 mg/kg; Fig. 3B and C). This treatment regimen is generally well tolerated and is more similar to clinical practice, where patients are usually treated using large intervals between MMC treatments. Fanconi anemia–defective xenografts (PL11EV, n = 12) regressed remarkably after a single dose of MMC (Fig. 3B). In contrast, genetically corrected xenografts (PL11FANCC, n = 10) progressed. PL11EV xenografts were followed for 60 days after treatment with a single dose of MMC. Through 50 days after treatment, all tumors remained smaller than they were at the initiation of treatment. At 60 days after a single dose of MMC, two tumors had reached a size larger than at the start of treatment; the average size at 60 days was 32% (SE, 22%) of the size at treatment initiation (data not shown). Eight of eleven (73%) CAPAN1 (BRCA2-mutated) xenografts regressed after treatment with a single dose of MMC; a substantial growth inhibition was also seen when all observations were averaged. Two of nine (22%) CFPAC (Fanconi anemia/BRCA2–proficient) xenografts regressed after MMC treatment; the other xenografts progressed almost uninhibited (Fig. 3C).

PL11EV xenografts (n = 7) also regressed on a single-dose treatment with cyclophosphamide (280 mg/kg), another

to a maximum of 60 days (Fig. 3A and data not shown). Xenografts of CAPAN1 and PL11, harboring mutations in the BRCA2 and FANCC genes, respectively, regressed (a decline in volume) soon after treatment was initiated whereas the Fanconi anemia–proficient xenografts, including the genetically corrected PL11FANCC, progressed. After several weeks of treatment with repeated doses of MMC, growth inhibition of Fanconi anemia–proficient xenografts became apparent.

Substantial toxicity after treatment was observed with the regimen of repetitive doses of MMC in both mice with Fanconi anemia–deficient and Fanconi anemia–proficient xenografts. Mice frequently became anemic after ~ 30 days of treatment. The mortality rate with this treatment regimen was 43%, with mice dying, on average, 51 days (range 45-59) after the treatment was initiated. Death often occurred several days after petechiae initially appeared, eventually progressing to widespread hemorrhage.

Separately, we treated a panel of pancreatic cancer xenografts with a single dose of MMC (5 mg/kg; Fig. 3B and C). This treatment regimen is generally well tolerated and is more similar to clinical practice, where patients are usually treated using large intervals between MMC treatments. Fanconi anemia–defective xenografts (PL11EV, n = 12) regressed remarkably after a single dose of MMC (Fig. 3B). In contrast, genetically corrected xenografts (PL11FANCC, n = 10) progressed. PL11EV xenografts were followed for 60 days after treatment with a single dose of MMC. Through 50 days after treatment, all tumors remained smaller than they were at the initiation of treatment. At 60 days after a single dose of MMC, two tumors had reached a size larger than at the start of treatment; the average size at 60 days was 32% (SE, 22%) of the size at treatment initiation (data not shown). Eight of eleven (73%) CAPAN1 (BRCA2-mutated) xenografts regressed after treatment with a single dose of MMC; a substantial growth inhibition was also seen when all observations were averaged. Two of nine (22%) CFPAC (Fanconi anemia/BRCA2–proficient) xenografts regressed after MMC treatment; the other xenografts progressed almost uninhibited (Fig. 3C).

PL11EV xenografts (n = 7) also regressed on a single-dose treatment with cyclophosphamide (280 mg/kg), another
DNA-interstrand cross-linking agent, whereas PL11\textsuperscript{FANCC} xenografts (n = 10) did not (Fig. 3D). The growth rate of PL11\textsuperscript{EV} xenografts was slower than that of PL11\textsuperscript{FANCC} xenografts.

In vivo treatment of Fanconi anemia–defective cancer cells with gemcitabine. The genetically corrected PL11\textsuperscript{FANCC} cells had an unexpected in vitro hypersensitivity to gemcitabine as compared with PL11\textsuperscript{EV}. We extended these studies in vivo and observed that PL11\textsuperscript{FANCC} (n = 10) regressed measurably for a period after a single dose of gemcitabine (100 mg/kg) whereas PL11\textsuperscript{EV} xenografts (n = 9) were not growth inhibited (Fig. 3E).

Cell cycle arrest contributes to mitomycin C hypersensitivity. An arrest in late S or G\textsubscript{2}-M phase of the cell division cycle is known to parallel the MMC hypersensitivity (20, 21); the role of apoptosis is less certain in Fanconi anemia–deficient cancer cells. We examined cell cycle progression in Hs766T\textsuperscript{EV}, Hs766T\textsuperscript{FANCG}, PL11\textsuperscript{EV}, and PL11\textsuperscript{FANCC} cells at 48 and 72 hours after in vitro treatment with 25 nmol/L MMC (Fig. 4A), at which time a majority of the viable PL11\textsuperscript{EV} and Hs766T\textsuperscript{EV} cells had a 4N DNA content, reflective of an arrest in late S or G\textsubscript{2}-M phase of the cell cycle. Most PL11\textsuperscript{FANCC} and Hs766T\textsuperscript{FANCG} cells had a cell cycle distribution approximately equal to untreated cells. The difference in cell cycle distribution between Fanconi anemia–deficient and Fanconi anemia–proficient cells was more obvious for the PL11 cells than for the Hs766T cells.

Mitomycin C toxicity is in part attributable to caspase-dependent apoptosis. Hs766T\textsuperscript{EV}, Hs766T\textsuperscript{FANCG}, PL11\textsuperscript{EV}, and PL11\textsuperscript{FANCC} cells were treated in vitro with 25 nmol/L MMC for 72 hours; proportions of Annexin V (an early marker for apoptosis)– and propidium iodide–stained cells were measured and corrected for the levels of “background” (untreated) apoptosis (Fig. 4B). Annexin V stained 16.5% of the MMC-treated PL11\textsuperscript{EV} population but only 4.2% of the PL11\textsuperscript{FANCC} cells (P = 0.009). Likewise, 17.3% of Hs766T\textsuperscript{EV} cells, but only 6.4% of Hs766T\textsuperscript{FANCG} cells, was positive for Annexin V (P = 0.013). MMC treatment of parental PL11 cells in vitro increased the percentage of Annexin V–positive cells as assayed by flow cytometry assay. PL11 cells treated with MMC were 19.9% positive (SE, ±1.98) for Annexin V as compared to untreated PL11 cells with 11.4% positive (SE, ±1.83). The addition of a caspase inhibitor cocktail resulted in a reduction in apoptosis. PL11 cells treated with MMC and caspase inhibitor cocktail were 12.7% positive for Annexin V; untreated...
Fanconi Defects: Chemosensitivity In vivo

PL11 cells also exhibited less apoptosis on caspase inhibition (4.1% positive; SE, ±0.9%) presumably because the background apoptosis was also a caspase-dependent event. PL11 cells treated with MMC acquired caspase-2 and -9 cleavage products as assayed by immunoblot (data not shown). Xenografts treated with MMC and then examined by caspase-3 immunohistochemistry (a marker for apoptosis) had a greater increase in apoptosis in PL11 tumors than did Fanconi anemia–corrected tumors at day 7 (Fig. 5B).

Morphology of mitomycin C–treated Fanconi anemia–deficient xenografts. Fanconi anemia–proficient and Fanconi anemia–deficient xenografts were treated with MMC, harvested, and processed for routine histopathologic examination (Fig. 5). Specifically, PL11 EV and PL11 FANCC xenografts were treated with a single dose of MMC (5 mg/kg) and harvested at days 0, 3, and 7 (each, n = 4). Xenografts of PL11 EV and PL11 FANCC treated with MMC had islands with morphologically atypical cells. These atypical cells had pleomorphic nuclei, a prominent cytoplasm, and large nucleoli (Fig. 5A). Areas with atypical cells were larger and more prevalent at 7 days after treatment as compared with the appearance at day 3. All tumors examined had some individual necrotic cells but only the Fanconi anemia–deficient PL11 EV xenografts had confluent areas of necrosis (Fig. 5A), which increased from ~10% at 3 days after treatment to 30% to 40% of the tumor as assessed in histologic slides at day 7. After a single dose of MMC, xenografts regressed for ~40 to 50 days, after which some of the xenografts started to regain size. Four xenografts were excised 40 days after a single dose of MMC: within the tumors were bands and large areas of fibrosis with islands of viable cancer cells (Fig. 5B).

The histologic effects of repeated dosing were also surveyed. Xenografts of CAPAN1, PL11, and Su86.86, treated once every 4 days with 2.5 mg/kg MMC for 28 days, were harvested at day 30. The Fanconi anemia/BRCA2–deficient CAPAN1 and PL11 cells had large areas of dramatically altered cells 30 days after treatment (Fig. 5C). These cells were characterized by a greatly increased cell mass, syncytial change, and large polymorphic and hyperchromatic nuclei with abnormal mitotic figures and prominent nucleoli. Morphologic alterations were much less pronounced in the Fanconi anemia/BRCA2–proficient Su86.86 xenografts (Fig. 5C).

Discussion

Recent years have brought an impressive increase in the understanding of the genetic basis of pancreatic cancer (22) but, thus far, it has not been possible to translate this improved knowledge of the genetic alterations in pancreatic cancer into significant advancements in clinical treatment. In this study, we show that pancreatic cancer cells having defects in the Fanconi anemia/BRCA2 pathway are remarkably sensitive to DNA-interstrand cross-linking agents both in culture and as xenografts in mice. The hypersensitivity of Fanconi anemia–defective cancer cells to DNA-interstrand cross-linking agents echoes the observed hypersensitivity of nonneoplastic Fanconi anemia cells (23–26). The current study shows for the first time that the hypersensitivity to cross-linking agents of pancreatic cancer cells having defects in the Fanconi anemia/BRCA2 pathway can be extended to the in vivo setting: Fanconi anemia–defective xenografts PL11 and CAPAN1 regressed after a variety of treatment schedules of MMC whereas Fanconi anemia–proficient xenografts did not. The use of syngeneic matched cells and the gene dependence of the MMC hypersensitivity essentially exclude the possibility that unintended drug effects could predominate in our model system. The i.p. MMC doses used in this study are higher (per kilogram) than the i.v. doses used in humans. However, these doses are likely to have been appropriate based on the tumor response in Fanconi anemia–proficient tumors. These responses were partial and were less than in Fanconi anemia–deficient tumors. In human pancreatic cancer patients, whose majority of tumors are Fanconi anemia proficient, single therapy with MMC also often produces a partial response.

Fanconi anemia cells have an exaggerated arrest in the late S or G2-M cell cycle compartment in response to low doses of MMC (21). A report by Akkari et al. (20) suggested that MMC could induce long-term, but reversible, cell cycle arrest in
nonneoplastic Fanconi anemia–deficient cells in the absence of significant cell death. In a recent study, we found that Fanconi anemia–defective pancreatic cancer cells tended to arrest in late S or G2-M phase 48 hours after an ~10-fold lower pulsed dose of MMC than needed for Fanconi anemia–proficient cancer cells (14). To determine whether treated xenografts remained in cell cycle arrest, eventually resumed cycling, or underwent apoptosis, we assayed MMC-treated cells by Annexin V/propidium iodide cytometry and did histopathologic examination and immunohistochemistry for activated caspase 3 on resected tumors. The tumor regression observed in vivo argued (almost by definition) for a significant contribution from apoptosis and necrosis, which was confirmed by the Annexin V/propidium iodide assay and histopathologic and immunohistochemical assessment of xenografts. We conclude that the observed toxic effect of MMC on Fanconi anemia–deficient cancer cells is multifaceted, including cell cycle arrest, apoptosis, and confluent necrosis.

Unexpectedly, the complemented PL11 FANCC cells were 3- to 4-fold more sensitive to gemcitabine and were also more sensitive to 5-FU as compared with the paired Fanconi anemia–defective PL11 EV cells. This hypersensitivity of PL11 FANCC cells to gemcitabine was also seen in vivo. An explanation is not readily available; further studies will be needed to validate whether this FANCC-induced hypersensitivity is unique to this cell line. If this were a general finding, it would argue against using a combination of MMC and gemcitabine in a clinical trial investigating the connection between Fanconi anemia defects and sensitivity to MMC or other cross-linking agents. Unfortunately, Hs766T cell lines were difficult to grow as comparable xenografts and could not be used for in vivo studies. Follow-up studies are

Fig. 5. Morphology of MMC-treated xenografts. A, histopathology of PL11 FANCC and PL11 EV xenografts 0, 3, and 7 days after treatment with a single dose of MMC. Necrosis was noted in PL11 EV xenografts as early as day 3 and was quite extensive by day 7 (right). B, PL11 EV xenografts. Left, histopathology at day 40 after a single dose of MMC; middle, activated caspase 3 at day 0; right, activated caspase 3 at day 7. By day 40, only scattered neoplastic glands remained embedded in a densely fibrotic stroma. Xenografts labeled for cleaved caspase 3 were evaluated by counting of positive cells on day 7 [PL11 EV tumors, 8.25% (SE, ±1.6%), compared with PL11 FANCC tumors, 3.9% (SE, ±1.0%)]. C, xenografts 30 days after treatment with 2.5 mg/kg MMC every 4 days. Dramatic nuclear enlargement and pleomorphism were present in all three cell lines 30 days after treatment. Numerous individual necrotic cells were present in PL11 tumors.
currently limited by a lack of genetically appropriate cancer cell lines. These results suggest a clinical trial in which DNA-interstrand cross-linking agents, particularly MMC or cisplatin, could be studied in patients with cancers harboring mutations in one of the members of the Fanconi anemia/BRCA2 pathway. Because our results suggest that hypersensitivity of Fanconi anemia/BRCA2 defective tumors applies to all cross-linking agents, treatment options could also include other effective cross-linkers that may be better tolerated, such as oxaliplatin (27). The known germline origin of most pancreatic cancer BRCA2 defects and the ready availability of reliable and rapid BRCA2 testing allow for the design of directed studies in either resected or unresectable cases of pancreatic cancer. One could envision that in the treatment of pancreatic cancer, after surgical excision (which most readily permits the full genetic analysis envisioned), MMC (or another cross-linking agent) could be a curatively intended adjuvant treatment used selectively for tumor defects in the Fanconi anemia/BRCA2 pathway.

References
4. Fanconi anemia/BRCA2 defective tumors applies to all cross- pathway. Because our results suggest that hypersensitivity of Fanconi anemia/BRCA2 defective tumors applies to all cross-linking agents, treatment options could also include other effective cross-linkers that may be better tolerated, such as oxaliplatin (27). The known germline origin of most pancreatic cancer BRCA2 defects and the ready availability of reliable and rapid BRCA2 testing allow for the design of directed studies in either resected or unresectable cases of pancreatic cancer. One could envision that in the treatment of pancreatic cancer, after surgical excision (which most readily permits the full genetic analysis envisioned), MMC (or another cross-linking agent) could be a curatively intended adjuvant treatment used selectively for tumor defects in the Fanconi anemia/BRCA2 pathway.

References
In vivo Therapeutic Responses Contingent on Fanconi Anemia/BRCA2 Status of the Tumor


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/11/20/7508

Cited articles
This article cites 26 articles, 12 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/11/20/7508.full.html#ref-list-1

Citing articles
This article has been cited by 24 HighWire-hosted articles. Access the articles at:
/content/11/20/7508.full.html#related-urls

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.