

# Phase I Trial of Intraperitoneal Injection of the E1B-55-kd-Gene-Deleted Adenovirus ONYX-015 (dl1520) Given on Days 1 Through 5 Every 3 Weeks in Patients With Recurrent/Refractory Epithelial Ovarian Cancer

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**Purpose:** Resistance to chemotherapy in ovarian cancer is frequently associated with mutations in the *p53* gene. The adenovirus dl1520 (ONYX-015) with the E1B 55-kd gene deleted, allowing selective replication in and lysis of *p53*-deficient tumor cells, has shown preclinical efficacy against *p53*-deficient nude mouse-human ovarian carcinomatosis xenografts.

**Patients and Methods:** We undertook a phase I trial of intraperitoneal dl1520 in patients with recurrent ovarian cancer. Sixteen women with recurrent/refractory ovarian cancer received 35 cycles (median, two cycles) of dl1520 delivered on days 1 through 5 in four dose cohorts:  $1 \times 10^9$  plaque forming units (pfu),  $1 \times 10^{10}$  pfu,  $3 \times 10^{10}$  pfu, and  $1 \times 10^{11}$  pfu.

**Results:** The most common significant toxicities related to virus administration were flu-like symptoms, emesis, and abdominal pain. One patient receiving  $1 \times$

$10^{10}$  pfu developed common toxicity criteria grade 3 abdominal pain and diarrhea, which was dose-limiting. The maximum-tolerated dose was not reached at  $10^{11}$  pfu, and at this dose level patients did not experience significant toxicity. There was no clear-cut evidence of clinical or radiologic response in any patient. Blood samples were taken for adenovirus DNA and neutralizing antibodies. Polymerase chain reaction data indicating presence of virus up to 10 days after the final (day 5) infusion of dl1520 are suggestive of continuing viral replication.

**Conclusion:** This article therefore describes the first clinical experience with the intraperitoneal delivery of any replication-competent/-selective virus in cancer patients.

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EPITHELIAL OVARIAN cancer is the fourth most frequent cause of cancer death among women in both the United States and the United Kingdom, and it is the most frequent cause of death from gynecologic cancer in the developed world.<sup>1</sup> It is considered one of the most chemosensitive cancers, with response rates to platinum-containing regimens of greater than 60%.<sup>2</sup> However, fewer than 25% of patients with advanced disease survive 5 years, and treatment of recurrent disease is complicated by the emergence of drug resistance. The probability of response to second-line or salvage chemotherapy is related to the interval between primary chemotherapy and relapse, al-

though other factors, such as tumor burden and histology, are also important.<sup>3</sup> Patients who relapse after 6 months are termed "platinum-sensitive" and are usually treated with platinum-based salvage therapy. Patients who either progress on first-line therapy or relapse within 6 months of completion are termed "platinum-resistant," have a poor prognosis, and are suitable candidates for experimental therapy approaches.

In normal cells, the tumor suppressor gene *p53* mediates cell cycle arrest and apoptosis in response to DNA damage induced by radiotherapy, DNA alkylating chemotherapy agents, or foreign DNA synthesis.<sup>4-6</sup> Correlations have been established in laboratory studies between the emergence of cisplatin-resistant ovarian cancer cells and the presence of mutations in the *p53* gene, and it is probable that a substantial number of drug-resistant tumor cells at the time of clinical relapse in ovarian cancer lack functional *p53*.<sup>7</sup> dl1520 (ONYX-015) is an adenovirus made from human group C adenovirus (serotypes 2 and 5) that has several small mutations within the serotype 5 portion of the virus. It has been attenuated by deletion of the E1B 55-kd gene region, the protein product of which is known to bind and inactivate *p53* and allow continued DNA synthesis and viral replication. Mutants such as dl1520 that lack this early gene product are severely deficient in their ability to replicate in normal cells.<sup>8,9</sup> In vitro studies have demonstrated that

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dl1520 is capable of efficient, selective replication and cytopathogenicity via cytolysis in *p53*-deficient human tumor cells, whereas replication was abrogated in cells containing wild-type *p53*.<sup>10</sup> In addition, laboratory studies in Glasgow have demonstrated that selective replication of dl1520 in cells with nonfunctioning *p53* is due to the induction of apoptosis in cells with wild-type *p53*.<sup>11</sup> Cells with nonfunctioning *p53* that are therefore resistant to apoptosis are permissive for replication, leading to virus spread and subsequent cytolysis of the cell population. There is some controversy because *p53* gene sequence alone does not predict for the antitumor efficacy of dl1520<sup>12-14</sup>; however, *p53* function can be lost via many mechanisms. Furthermore, *in vivo* correlation of *p53* function and dl1520 efficacy was demonstrated in three nude mouse-human ovarian carcinomatosis xenograft models, A2780/Cp70, OVCAR3 (mutant *p53*), and A2780 (wild-type *p53*).<sup>15</sup> In these experiments, both antitumor efficacy and improved survival were demonstrated for the mice carrying mutant *p53* xenografts, whereas no such effects were evident for the wild-type *p53* xenografts. Clinical studies of dl1520 have been undertaken in patients with recurrent head and neck cancer, a disease in which *p53* gene mutations or deletions are present in up to 70% of patients.<sup>16</sup> Significant activity has been documented in a dose-finding phase I study, with evidence on magnetic resonance imaging of tumor necrosis at the site of viral injection in five of 32 patients (four of whom had mutant *p53* tumors).<sup>17</sup> Dose-limiting toxicity (DLT) was not reached at  $10^{10}$  plaque-forming units (pfu)/d by five consecutive daily doses every 4 weeks.

Direct administration of cytotoxic drugs into the peritoneal cavity is a strategy designed to enhance locoregional drug delivery while abrogating systemic toxicities. Furthermore, this route allows concentrations to be attained at the site of the tumor that are many times higher than would be tolerated in the systemic circulation; these can easily exceed concentrations shown *in vitro* to be required to overcome clinical drug resistance.<sup>18</sup> This method of administration is particularly relevant in ovarian cancer, since the disease at presentation is confined to the abdominal cavity in approximately three quarters of patients and subsequent relapses also tend to remain thus compartmentalized. This model system of metastatic ovarian cancer growing within a clearly defined anatomic space bathed in free fluid has many potential advantages in delivery, safety, and efficacy of novel therapies. This study therefore seeks to exploit the molecular differences between normal and malignant cells, utilizing the specificity of dysfunctional *p53*-dependent cytotoxicity. The primary objective was therefore to determine the safety of intraperitoneal administration with dl1520 daily for 5 days and to determine the maximum-

tolerated dose (MTD). Secondary objectives included the determination of dl1520 propensity to replicate in ovarian carcinoma cells within the ascitic fluid, and the evaluation of the humoral immune response (antibody development) to dl1520 in both blood and ascitic fluid.

## PATIENTS AND METHODS

### *Patient Selection*

This study was conducted at four cancer centers in the United States and the United Kingdom. Eligible patients had histologically or cytologically confirmed primary epithelial ovarian cancer, with recurrent disease suspected either radiologically or by rising CA125 levels. All patients had received previous chemotherapy with platinum-containing regimens and had relapsed within the previous 6 months. Karnofsky performance status of  $\geq 70\%$ , age of at least 18 years, and life expectancy of at least 3 months were required, as was written informed consent. Patients were, in the opinion of the investigators, suitable candidates for either a laparotomy or laparoscopy. Patients in whom laparoscopy was contraindicated were those with known dense adhesions, periumbilical infection, peritonitis, intestinal obstruction, ileostomy, or gross obesity. Normal organ function was required, as evidenced by the following: neutrophil count more than  $2 \times 10^9/L$ , hemoglobin concentration more than 10 g/L, serum creatinine level less than 150  $\mu\text{mol/L}$ , aminotransferase levels less than 2.5 times the upper limit of normal, prothrombin time or international normalized ratio  $\leq 2$ , and partial thromboplastin time within normal limits. Patients were excluded from study entry if they had known chronic liver dysfunction before the development of ovarian cancer or had either cirrhosis evident on gross examination during laparotomy/laparoscopy or more than 50% liver replaced by tumor. Other exclusion criteria included ongoing, active infection including human immunodeficiency virus, recent viral syndrome, recent chemotherapy or radiotherapy, concomitant hematologic malignancy, ongoing requirement for immunosuppressive medication including glucocorticoids or cyclosporine, and pregnancy/lactation. Patients treated on any other investigational program within the previous 6 weeks were also excluded from participation, as were patients previously treated on a research protocol involving the administration of adenovirus-based therapies. The protocol was reviewed and approved by the institutional review boards, the biosafety committees, and in the United States by the Food and Drug Administration before patient accrual.

### *Pretreatment Studies*

Before treatment, patients had routine hematologic and biochemical analyses performed, in addition to undergoing chest radiography, urinalysis, ECG, CA125 marker level assessment, and a complete physical examination. A baseline computed tomography scan of the abdomen with documentation of any measurable tumor was carried out. In addition, blood was drawn for serum antibody to group C adenovirus, adenovirus DNA (by polymerase chain reaction [PCR]), immune function evaluation (CD3, CD4, CD8, total lymphocyte count, delayed-type hypersensitivity skin test). Finally, a test for pregnancy was performed if appropriate.

### *Surgery and Virus Administration*

For placement of the intraperitoneal catheter, all patients underwent either a laparoscopy or laparotomy, the choice depending on the

presence of ascites and the size and site of recurrent tumor masses. Generally, laparoscopy was performed (following paracentesis in the presence of ascites) if there were no visible or resectable lesions, whereas laparotomy was preferred if there was an option to resect disease. In the case of laparoscopy, contingency plans were made to proceed to laparotomy if there were complications or to confirm relapse if laparoscopy results were negative. The catheter (Tenckhoff or Port-a-Cath [Pharmacia Deltec, St Paul, MN]) was placed intraoperatively, and a tumor biopsy was performed to assess *p53* status by gene sequencing. Any adhesions in the peritoneum were divided if technically possible. To prevent catheter leakage, a minimum 7-day period was allowed to elapse before usage, and therefore heparinized saline was instilled into the tubing during this period.

dl1520 was grown and "titered" on the human embryonic kidney cell line HEK293,<sup>19</sup> formulated in a sterile viral solution in TRIS buffer (10 mmol/L [pH 7.4], MgCl<sub>2</sub> 1 mmol/L, NaCl 150 mmol/L, and 10% glycerol), and supplied frozen in single-use plastic screw-cap vials containing 0.5 mL of virus at a specified concentration. dl1520 was produced and supplied by Magenta Corp (Rockville, MD). These vials were stored below -20°C and were thawed and initially diluted to the appropriate titer for a particular dose level. Thawed virus was maintained at 2°C to 8°C during dilution and handling, before being warmed to room temperature for intraperitoneal administration. Dilutions to a final volume of 500 mL were performed immediately before infusion. dl1520 adenovirus infusion through the catheter proceeded over 15 minutes suspended in 500 mL of physiologic saline. After infusion, the patient was rotated on all sides over a 30-minute period to maximize peritoneal exposure. Vital signs were taken every 15 minutes before and at the start of treatment and at 60, 90, and 120 minutes afterward. Repeat infusions were carried out daily for 5 days.

#### Follow-Up Studies

On day 5, blood was taken to assess liver function and to measure virus DNA by PCR. Before dl1520 infusion, peritoneal fluid was drawn for cytologic evaluation. Patients were then reviewed in the clinic on days 8, 15, and 22 for each cycle of dl1520 treatment. At each visit, general status and toxicity assessment was carried out by examination, adverse event reporting, and scoring of Karnofsky performance status. Blood was also drawn for hematology, serum chemistry, CA125, and adenovirus DNA and antibody tests, and a urinalysis was performed. In addition, on day 15, peritoneal fluid was withdrawn, if present. If this was not possible, 1,000 mL of 0.9% saline was infused through the catheter, allowed to distribute throughout the peritoneal cavity for 15 to 20 minutes, and then drained. On day 22, repeat hematologic, biochemical, and CA125 analyses were performed and patients received chest radiograms and ECGs. Every second cycle, patients had a repeat computed tomography scan performed.

#### Biologic Studies

Tumor biopsies were performed at laparotomy when possible, and immunohistochemistry was performed on formalin-fixed paraffin-embedded tumors for *p53* status. In addition, Oncormed Corp (Gaithersburg, MD) sequenced exons 5 to 9 of the *p53* gene on pretreatment tumor biopsy samples. This analysis was carried out retrospectively and was not a prerequisite for entry onto the study. Ascitic fluid samples were obtained from eight patients at the time of day 5 and 15 samples/washes. The fluid was spun to pellet any cells, and the supernatant was frozen. The presence of adenovirus in plasma samples or the cell-free fraction of patients' peritoneal fluid was determined by PCR using primers of the E1A region of the adenoviral genome. The

cell pellet was smeared and fixed onto slides before in situ hybridization (ISH) for adenovirus DNA to determine the ability of dl1520 to replicate in ovarian cancer cells within the ascitic fluid in vivo. Certified pathologists examined slides to characterize any infected cells. Finally, determination of neutralizing antibody titers was carried out on plasma samples.

#### Dose Escalation

DLT was defined as National Cancer Institute of Canada common toxicity criteria (CTC) grade 4 flu-like symptoms (eg, fever, fatigue, myalgia) or any other grade 3 toxicity attributed to ONYX-015 administration. The MTD was defined as the dosage at which two of six patients experienced a DLT after the first treatment cycle with ONYX-015. Patients were enrolled sequentially onto treatment cohorts of increasing dose level as follows. Three patients were entered onto cohort 1, and if no DLT occurred in the first two patients over the first 3 weeks and no DLT occurred in the first week after infusion for the third patient, recruitment to the next dose level proceeded. If one of three patients in a cohort experienced a DLT, up to three additional patients were enrolled at the same dose. If one or more of the additional patients had a DLT (ie,  $\geq$  two of six), the MTD was defined and no further escalation took place. Patients were eligible for repeat dosage cycles of dl1520 at the same dose if they experienced no DLT or no disease progression. A maximum of six cycles was allowed.

## RESULTS

Sixteen patients, all white women, received 35 cycles of dl1520 in four dose cohorts:  $1 \times 10^9$  pfu,  $1 \times 10^{10}$  pfu,  $3 \times 10^{10}$  pfu, and  $1 \times 10^{11}$  pfu. Baseline characteristics of these patients are shown in Table 1. Most patients had platinum-resistant ovarian cancer and bulky residual tumor masses (defined as disease volume  $> 2$  cm). The median number of prior therapies was four, and all patients had received platinum-based chemotherapy. Ten patients (62.5%) had been optimally cytoreduced at their initial operation for ovarian cancer. The three patients in cohort 4 were selected specifically for nonbulky disease volume and minimal prior therapy. One patient in cohort 2 had further surgical cytoreduction to less than 2 cm residuum at the time

Table 1. Baseline Patient Characteristics (N = 16)

Age, years	
Mean	56
Range	38-72
KPS, no. of patients	
90%-100%	7
70%-80%	9
Tumor volume $> 2$ cm, no. of patients	11 (69%)
Previous chemotherapy regimens, n	
Median	4
Range	1-6
Platinum resistant,* no. of patients	11 (68%)
Mutant <i>p53</i> ,† no. of patients	5 (71%)

\*Defined as relapsing within 6 months or progressing on therapy.

†Of seven assessable samples obtained.

**Table 2. Treatment Delivery**

Cohort	Dose (pfu)	No. of Patients	Cycles*
1	10 <sup>9</sup>	4	2, 1, 2, 2
2	10 <sup>10</sup>	6	4, 2, 2, 1, 4, 2
3	3 × 10 <sup>10</sup>	3	3, 2, 1
4†	10 <sup>11</sup>	3	1, 4, 2

\*Median number of cycles was two for all cohorts.

†Selected for nonbulky disease.

of catheter insertion. Treatment delivery is shown in Table 2. Most patients received more than one cycle of dl1520, and three patients received four cycles (median, two cycles).

### Toxicity

Side effects from the administration of dl1520 were common, but CTC grade 4 toxicity was not reported. The most common toxicities were related to the acute administration of dl1520 and are readily grouped together as “viremic” or “flu-like.” These toxicities, CTC grades 2 and 3, are shown in Table 3. Other toxicities considered to be at least possibly related to the administration of ONYX-015 and reaching CTC grades 2 and 3 are shown in Table 4. There were no cumulative nonhematologic toxicities noted, and no evidence of hematologic toxicity was demonstrated.

Most patients reported flu-like symptoms after administration of dl1520, and eight patients (50%) described at least grade 2 severity. Symptoms generally started after the first daily infusion of dl1520 and consisted of any or all of the following: generalized malaise, headaches, nausea, myalgias, pyrexias, and rhinorrhea. Three patients, one in cohort 2 and two in cohort 3, reported CTC grade 3 viremic symptoms, but they were not considered to be dose-limiting. Acetaminophen (up to 4 g daily) and antiemetics (eg, cyclizine hydrochloride 50 mg three times daily) were given as prophylaxis and as treatment of these symptoms, with some amelioration on subsequent cycles demonstrated. Two patients reported the nausea associated with other flu-like symptoms to be precipitated by head movement in a similar manner to that seen with viral labyrinthitis.

Additionally, abdominal pain was commonly observed after dl1520 administration. This had features consistent

with peritonism and was associated with diarrhea and/or increased stoma effluence in some patients. Other associated symptoms included heartburn and vomiting. One patient in cohort 2 experienced grade 3 abdominal pain with grade 3 diarrhea after 1 cycle of dl1520. The diarrhea and pain developed during the 5 days of viral administration and eventually required the administration of loperamide hydrochloride and parenteral opiates. She was admitted for symptom control and required a nasogastric tube insertion for palliation. This toxicity profile was considered to be dose-limiting, and three additional patients were recruited at this dose level. Four of the other five patients in this cohort experienced grade 2 abdominal pain but no associated severe diarrhea or other features that would have stopped dose escalation. There were no other dose-limiting toxicities described in any of the other cohorts.

During the study, there had been an impression that patients in cohorts 1 to 3 with known bulky intra-abdominal disease were experiencing more severe symptoms of viremia and/or abdominal pain. As a consequence, cohort 4 patients were recruited specifically with nonbulky residual tumor volume and good performance status and were less heavily pretreated (although all three were considered to be platinum-resistant). Although dl1520-related pyrexias were documented, there were no significant flu-like symptoms and only one patient described grade 2 abdominal pain. There were no toxicities higher than grade 2 in these patients, which suggests a better tolerance for patients with low volumes of tumor. However, the two patients with nonbulky disease in cohorts 2 and 3 both experienced the flu-like syndrome and either abdominal pain or diarrhea; therefore, no conclusions can be made regarding this association. The MTD was therefore not reached in any of the four cohorts. Further dose escalation was not pursued because the limit of virus manufacturing capacity had been attained.

### Biologic Studies

In seven patients, the *p53* status of pretreatment tumor biopsy specimens was evaluated retrospectively by gene sequencing. Five patients (71%) were found to have mutations in the *p53* gene on sequencing. Twenty-eight perito-

**Table 3. Viremic/Flu-Like Toxicity**

Cohort	No. Patients	Pyrexia/Sweats		Headaches		Myalgias		Malaise	
		Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 3
1	4	1			1				
2	6	2	1	2			1		1
3	3		2		1				
4	3	2							

NOTE. Grade was based on National Cancer Institute of Canada common toxicity criteria.

**Table 4. Other Significant Toxicities (NCIC-CTC)**

Cohort	No. Patients	Nausea		Vomiting		Diarrhea/Ileitis		Abdominal Pain	
		Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 3
1	4	1	1		1	2		1	
2	6	1		1			1*	4	1*
3	3		2		1	2			
4	3	1						1	

NOTE. Grade was based on National Cancer Institute of Canada common toxicity criteria.

\*Dose-limiting toxicity (both in same patient).

neal washings sampled at day 5 and day 15 (and later if possible; timings were not specified in the protocol) after dl1520 administration were obtained from eight patients. Using PCR, the presence of dl1520 viral DNA in the cell-free fraction was assessed in 25 of 26 of these washings. Seven of these eight patients had evidence of viral presence at day 5, and five of these had additional evidence of viral presence on day 15. In one patient, dl1520 DNA was detected in the peritoneal fluid up to 354 days after the fourth cycle of treatment. Sixty-two percent (16 of 26) of the cytology specimens prepared from the cell fraction of the peritoneal washings were not assessable due to either low cellularity or no evidence of malignancy in the specimens. Of the remaining 10 cytology specimens, adenovirus DNA was only detected in one patient specimen by ISH. However, the positive cells in this specimen did not appear to be malignant based primarily on size and nuclear morphology assessment. Because of the small number of assessable specimens, it is not possible to conclude if dl1520 is able to replicate in tumor cells in vivo. In addition, eight patients from cohorts 1 to 3 had blood samples collected after the first infusion of dl1520 at 15 minutes, 1 hour, 6 hours, 12 hours, and 24 hours in order to examine plasma for adenovirus by quantitative PCR. Circulating levels of dl1520 were not detected in any of these samples. Finally, six (46%) of 13 patients had positive titers of neutralizing immunoglobulin G antibodies to adenovirus dl1520 at the start of treatment. Of these, all six developed increased levels of antibody titers during treatment, and six of the seven patients without neutralizing antibody titers at baseline developed high titers of neutralizing antibodies after dl1520 treatment, indicating a significant humoral response. There was no clear correlation between the presence or absence of neutralizing antibody titers at baseline and the severity of the flu-like symptoms.

#### *Efficacy*

There was no clear-cut evidence of clinical or radiologic response in any patient. Stable disease was demonstrated, with four patients receiving more than two cycles of dl1520

before progressive disease developed. One patient did experience a fall in CA125 from 1,585 kilounits/L to 692 kilounits/L during two cycles of dl1520 but was not classified as having a true CA125 response<sup>20</sup> because of the transperitoneal fluid shifts induced by the day 5 and day 15 peritoneal aspirations and washes. In addition, this patient subsequently developed progressive disease with rising markers and new sites of disease requiring radiotherapy during her third cycle. The median survival time for all patients was 165 days (range, 15 to 528 days). Of the 16 patients on study, 15 stopped ONYX-015 because they eventually developed progressive disease and one was taken off-protocol because of DLT.

#### DISCUSSION

This phase I study successfully achieved the primary aim of defining safety and evaluating dose-related toxicity associated with intraperitoneal delivery of dl1520. Proof of principle for viral replication, antibody response, and evidence of an antitumor effect were secondary objectives, but they were important nevertheless in delineating the possible role of such novel therapies in the future. The viremic/flu-like symptoms seen after infusion could suggest that active viral replication is occurring, as shown in previous studies with oncolytic viruses. One of the first such illustrative studies was performed at the National Cancer Institute, wherein 30 cervical cancer patients were treated with direct tumoral infusion of wild-type human adenoviruses of 10 different serotypes.<sup>21</sup> In this trial, all patients developed a humoral response with neutralizing antibodies within 7 days, viral replication was documented for up to 17 days in one patient, and a transient viral syndrome lasting 2 to 7 days was reported. In the study reported by Ganly et al,<sup>17</sup> 21 of 22 patients with advanced head and neck cancer showed an increase in neutralizing antibody after intratumoral injection of dl1520 adenovirus, and viral replication was demonstrated by ISH in four patients, all known to have mutant *p53* tumors. In the current study, PCR data indicating that, generally, viral DNA can be detected up to 10 days after the final (day 5) infusion of dl1520 (and much longer

in one patient) provides evidence consistent with viral infection and possibly replication. However, this is not conclusive proof of ongoing viral replication, because levels were generally lower than on day 5 and not enough is known about the peritoneal clearance of dl1520. In addition, the relatively acute onset of the flu-like syndrome may also suggest that this is due merely to viral particles, as there may not have been enough time for replication to have taken place. Furthermore, no conclusive evidence of viral replication was demonstrated in cells distilled from the ascitic fluid of eight patients, although many samples were obtained after a peritoneal wash; therefore, a dilutional effect is inevitable. For future studies, PCR for E4 or hexon protein mRNA expression could be used as other surrogate indicators for replication, as these studies may be more sensitive for detecting gene expression than immunohistochemistry for viral proteins or ISH. However, it should be noted that gene expression alone cannot be used as definitive evidence for completed replication and release of infectious virus.

Although *in vivo* studies have clearly demonstrated efficacy and improved survival for mice bearing mutant *p53* peritoneal tumours,<sup>15</sup> no clear-cut antitumor efficacy could be demonstrated in the current study. Tumor response was not a primary objective of this study, but it is relevant to discuss the factors potentially influencing the clinical activity of oncolytic viruses in patients with peritoneal carcinomatosis. Despite the stated pharmacologic advantage for the delivery of intraperitoneal chemotherapy, proof is still lacking as to the clinical relevance of such data, and little is known about the biokinetics of distribution for intravenous and intraperitoneal adenovirus. Delivery of any drug to the innermost core of tumor nodules depends on vascular delivery rather than regional administration. Using rat peritoneal tumor nodules, Los et al<sup>22</sup> compared the concentration of cisplatin at the periphery of the tumor (< 1.5 mm from tumor surface) with the concentration at the center of the tumor, after both intraperitoneal and intravenous administration. Increased concentration of cisplatin was demonstrated at the periphery with intraperitoneal administration, but there was no difference in the tumor core. This suggested that any major therapeutic benefit was likely to be restricted to small tumor nodules, and therefore surgical tumor debulking is likely to facilitate activity. In concordance with this, Heise et al<sup>23</sup> hypothesized that the tumor mass at the time of treatment might be an important determinant of the antitumoral efficacy of dl1520. Two mutant *p53* xenografts, OVCAR3 and A2780/Cp70, were treated with intraperitoneal dl1520 at three time points following intraperitoneal inoculation with tumor cells. At the earlier time points (day +3 and day +17 after inocula-

tion), when tumor masses were either microscopic or less than 2 mm in diameter, all treated mice were rendered tumor-free and had significantly improved survival. In contrast, no abrogation of tumor growth was demonstrated in mice treated with dl1520 at day +31, ie, when tumor nodules were more than 2 mm in size. Such data strongly suggest that unless penetration of dl1520 into tumor nodules can be improved, efficacy is likely to be limited to patients with low tumor burdens. Furthermore, adequate coverage of peritoneal surfaces by intraperitoneally administered fluids is affected by adhesion formation, produced via an inflammatory reaction induced by both the initial surgery and some cytotoxic agents. Anticancer agents administered in small fluid volumes are unlikely to achieve adequate intra-abdominal dispersion, even with multiple positional changes as achieved in this study. Dilutions in 2 L of fluid have been advocated as the minimum volume required to achieve uniform coverage of all peritoneal surfaces.<sup>24</sup>

As delivery of intraperitoneally administered anticancer agents is unlikely to penetrate bulky tumor nodules, vascular delivery to tumor centers may be important. Although clearly not the planned primary modality of access to tumor in this study—the intention was to retain high concentrations of dl1520 in the peritoneal cavity—this may have an effect on efficacy. However, the reticuloendothelial cell uptake of systemic virus particles is likely to abrogate distribution via the circulation to other metastatic tumor sites. Intraperitoneal administration of dl1520 is unlikely to escape this effect, as solutes vacate the peritoneal cavity into the systemic circulation either by diffusing through the parietal/visceral peritoneum or by absorption through the lymphatics. The plasma-peritoneal barrier has been described as having unidirectional transport characteristics, with intraperitoneally administered substances appearing rapidly in the systemic circulation, whereas intravenous administration of the same substance causes it to appear more slowly in the peritoneal fluid.<sup>25</sup>

The presence of neutralizing antibodies, indicating an active antiviral immune response, is likely to be a major factor in limiting the efficacy of oncolytic viruses such as dl1520. Binding of antibody to virus will affect infection,<sup>26</sup> and the development of virus-specific cytotoxic T lymphocytes could potentially result in the lysis of infected cells before successful virus replication.<sup>27</sup> However, Khuri et al<sup>28</sup> treated head and neck cancer patients with intratumoral dl1520 combined with systemic chemotherapy and found that the presence of baseline neutralizing antibodies did not prevent tumor responses from occurring. One postulated reason for maintaining the efficacy of intratumoral dl1520 injections is the inefficient penetration of solid tumors by the such antibodies.<sup>29,30</sup>

In vitro studies using nude mice engrafted with the human tumor xenograft model HlaC strongly support the combination of dl1520 with chemotherapeutic agents, particularly cisplatin, and these data suggest that additive or potentially synergistic effects can be demonstrated.<sup>19</sup> This significant antitumor activity for intratumoral dl1520 in combination with cisplatin and fluorouracil was confirmed in a phase II trial in squamous carcinoma of the head and neck,<sup>28</sup> and interestingly, response rates were not related to *p53* status. Furthermore, the sequence of agents in combination may have therapeutic relevance.<sup>23</sup> The mechanism(s) for this dl1520/cisplatin interaction is unclear, but these preclinical and clinical trials suggest that dl1520 may be able to sensitize both infected and uninfected cells to killing by chemotherapy. *E1A* gene expression has been shown to increase cellular sensitivity to chemotherapy in a *p53*-independent manner.<sup>6,31,32</sup> As dl1520 expresses *E1A* when infecting both *p53* wild-type and *p53* dysfunctional tumors, this may account for this chemosensitization. In addition, the induction of apoptosis by platinum drugs is enhanced by wild-type *p53* expression,<sup>33</sup> and recent work has suggested that the therapeutic success of platinum and paclitaxel combinations (paclitaxel does not require the presence of functional *p53* to induce apoptosis<sup>34</sup>) may reflect the efficacy of the agents on different cellular populations with different genetic backgrounds.<sup>35</sup> It is relevant to note in this context that two patients with platinum-resistant disease, having developed progressive disease on dl1520 therapy, subsequently demonstrated falling CA125 after further carboplatin chemotherapy. One of these patients had a confirmed CA125 response after three cycles, and although her disease subsequently progressed, this supports the hypothesis that eradication or attenuation of a population of platinum-resistant, mutant *p53*-expressing clones by dl1520 may have allowed further sensitivity to platinum.

This article describes the first clinical experience with the intraperitoneal delivery of any replication-competent/-selective virus in cancer patients. Multiple doses can be safely administered to patients after a mini-laparotomy, cytoreductive surgery, and installation of a peritoneal catheter. The main side effects were the flu-like syndrome, nausea/vomiting, and abdominal pain/peritonitis. This latter symptom was occasionally severe and reproducible, perhaps signifying an inflammatory process initiated by viral infection. The MTD, as defined, was not reached at  $10^{11}$  pfu, and at this dose level, patients with good performance status and nonbulky disease did not experience significant toxicity. However, intraperitoneal administration of dl1520 was associated with greater systemic toxicity, compared with that seen in the studies utilizing intratumoral injections in head and neck cancer.<sup>17,28</sup> This is likely to reflect greater sys-

tem exposure of virus. Local toxicity (abdominal pain/peritonism) secondary to an inflammatory process was also common and troublesome in this patient population and correlates with the injection site pain reported by over 50% of the head and neck cancer patients in the previous studies.

In the head and neck cancer trial by Khuri et al,<sup>28</sup> dl1520 replication was demonstrated within tumor tissue in four of six posttreatment biopsy specimens, with necrotic tumor tissue present in an additional three. In the current study, it was not possible to conclusively demonstrate that intraperitoneally administered dl1520 can gain access to and replicate in mutant *p53* tumor cells. One patient had material suggestive of ISH-positive malignant cells, but this was not confirmed and therefore not classified as a true-positive result because of the poor quality of the cellular material obtained and questionable assay specificity. However, one must remember that intratumoral injections of dl1520 in the head and neck clinical trials consisted of only a few milliliters of 0.9% saline containing up to  $10^{11}$  pfu of virus. It is therefore not surprising that comparable doses of virus diluted in 500 mL of saline within the peritoneal cavity result in a much lower pickup rate for demonstrating active viral replication. Furthermore, anatomic barriers to infectivity and spread (eg, adhesions) and the presence of neutralizing antibodies in the peritoneal fluid would further mitigate against viral replication capability and, consequently, our ability with currently available assays to demonstrate this irrefutably.

In conclusion, replication-selective oncolytic adenoviruses such as dl1520 may offer a novel approach to the treatment of ovarian cancer. Preclinical studies suggest that they may be most effective when used in conjunction with conventional cytotoxic agents, such as cisplatin or carboplatin. The optimal sequencing of these agents are being examined in preclinical models, and further trials will be required to carry these "proof of principle" experiments forward. The effect of antiviral immunity dl1520 antitumor activity is still incompletely understood, and further investigation is needed in order to optimize treatment. Better delivery of virus to intraperitoneal tumor nodules is required, particularly with respect to longer retention times within the abdominal cavity. Modification of dl1520 and other second-generation viral constructs will be necessary to enhance replication and virulence against the target tumor cell population; however, the systemic toxicities and abdominal pain associated with the intraperitoneal administration will need to be carefully monitored. Highest efficacy may be seen dl1520 when administered to patients with low tumor burden. Therefore, the ideal scenario for dl1520 therapy as a single agent may be as consolidation treatment in patients with minimal residual disease following conventional chemotherapy. However, it

will be important to observe evidence of clear-cut clinical efficacy or a biologic effect (eg, tumor cell infection) before proceeding with combination studies of intraperitoneal dl1520 and chemotherapy in ovarian cancer.

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## REFERENCES

1. Bray F, Sankila R, Ferlay J, et al: Estimates of cancer incidence and mortality in Europe in 1995. *Eur J Cancer* 38:99-166, 2002
2. Conte PF, Cianci C, Gadducci A: Update in the management of advanced ovarian carcinoma. *Crit Rev Oncol Hematol* 32:49-58, 1999
3. Eisenhauer EA, Vermorken JB, Van Glabbeke M: Predictors of response to subsequent chemotherapy in platinum pre-treated ovarian cancer: A multivariate analysis of 704 patients. *Ann Oncol* 8:963-968, 1997
4. Debbas M, White E: Wild type p53 mediates apoptosis by E1A, which is inhibited by E1B. *Genes Dev* 7:546-554, 1993
5. Grand RJA, Grant ML, Gallimore PH: Enhanced expression of p53 in human cells infected with mutant adenoviruses. *Virology* 203:229-240, 1994
6. Lowe SW, Ruley HE, Jacks T, et al: p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* 74:957-967, 1993
7. Kaye SB: Ovarian cancer, from the laboratory to the clinic: Challenges for the future. *Ann Oncol* 7:9-13, 1996
8. Yew PR, Kao CC, Berk AJ: Dissection of functional domains in the adenovirus 2 early 1B 55K polypeptide by suppressor-linker insertional mutagenesis. *Virology* 179:795-805, 1990
9. Kao CC, Yew PR, Berk AJ: Domains required for in vitro association between the cellular p53 and the adenovirus 2 E1B 55K proteins. *Virology* 179:806-814, 1990
10. Bischoff JR, Kim DH, Williams A, et al: A mutant adenovirus which selectively replicates in tumour cells with non-functional p53. *Science* 274:373-376, 1996
11. Ganly I, Kim YT, Hann B, et al: Replication and cytolysis of an E1B-attenuated adenovirus in drug resistant ovarian tumour cells is associated with reduced apoptosis. *Gene Therapy* (in press)
12. Hall AR, Dix BR, O'Carroll SJ, et al: p53-dependent cell death/apoptosis is required for a productive adenovirus infection. *Nat Med* 4:1068-1072, 1998
13. Harada J, Berk A: p53-independent and -dependent requirements for E1B-55kD in adenovirus type 5 replication. *J Virol* 73:5333-5344, 1999
14. Goodrum FD, Ornelles DA: p53 status does not determine outcome of E1B 55kilodalton mutant adenovirus lytic infection. *J Virol* 72:9479-9490, 1998
15. Heise C, Ganley I, Kim YT, et al: Efficacy of a replication-sensitive adenovirus against ovarian carcinomatosis is dependent on tumour burden, viral replication and p53 status. *Gene Ther* 7:1925-1929, 2000
16. Boyle JO, Koch W, Hruban RH, et al: The incidence of p53 mutations increases with progression of head and neck cancer. *Cancer Res* 53:4477-4480, 1993
17. Ganly I, Eckhardt SG, Rodriguez GI, et al: A phase I study of ONYX-015, and E1B attenuated adenovirus, administered intratumorally to patients with recurrent head and neck cancer. *Clin Cancer Res* 6:798-806, 2000
18. Vasey PA: Rationale for and complications of intraperitoneal chemotherapy. *CME J Gynaecol Oncol* 3:83-89, 1999
19. Heise C, Sampson-Johannes A, Williams A, et al: ONYX-015, an E1B gene-attenuated adenovirus, causes tumour-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. *Nat Med* 3:639-645, 1997
20. Rustin GJS, Nelstrop AE, McClean P, et al: Defining response of ovarian carcinoma to initial chemotherapy according to serum CA 125. *J Clin Oncol* 14:1545-1551, 1996
21. Smith R: Studies on the use of viruses in the treatment of carcinoma of the cervix. *Cancer* 9:1211-1218, 1956
22. Los G, Mutsaers PHA, van der Vijgh WJF, et al: Direct diffusion of cis-diamminedichloroplatinum(II) in intraperitoneal rat tumours after intraperitoneal chemotherapy: A comparison with systemic chemotherapy. *Cancer Res* 49:3380-3384, 1989
23. Heise C, Lemmon M, Kirn D: Efficacy with a replication-selective adenovirus plus cisplatin-based chemotherapy: Dependence on sequencing but not p53 functional status or route of administration. *Clin Cancer Res* 4:4908-4914, 2000
24. Rosenshein N, Blake D, McIntyre PA, et al: The effect of volume on the distribution of substances instilled into the peritoneal cavity. *Gynaecol Oncol* 6:106-110, 1978
25. Gross ML, Somani P, Ribner BS, et al: Ceftizoxime elimination kinetics in continuous ambulatory peritoneal dialysis. *Clin Pharmacol Ther* 34:673-680, 1983
26. Zinkernagel RM: Immunology taught by viruses. *Science* 271:173-178, 1996
27. Yang Y, Nunes FA, Berencsi K, et al: Cellular immunity to viral antigens limits E1B-deleted adenoviruses for gene therapy. *Proc Natl Acad Sci U S A* 91:4407-4411, 1994
28. Khuri FR, Nemunaitis J, Ganly I, et al: A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat Med* 6:879-885, 2000
29. Baxter LT, Zhu H, Mackensen DG, et al: Physiologically based pharmacokinetic model for specific and non-specific monoclonal antibodies and fragments in normal tissues and human tumour xenografts in nude mice. *Cancer Res* 54:1517-1528, 1994
30. Shisler J, Duerksen HP, Hermiston TM, et al: Induction of susceptibility to tumour necrosis factor by E1A is dependent on binding to either p300 or p105-Rb and induction of DNA synthesis. *J Virol* 70:68-77, 1996
31. Lowe SW, Bodis S, McClatchey A, et al: p53 status and the efficacy of cancer therapy in vivo. *Science* 266:807-810, 1994
32. Sanchez-Prieto R, Quintanilla M, Cano A, et al: Carcinoma cells become sensitive to DNA-damaging agents by the expression of the adenovirus E1A gene. *Oncogene* 13:1083-1092, 1996
33. Harris CC: Structure and function of the p53 tumour suppressor gene: Clues for rational cancer therapeutic strategies. *J Natl Cancer Inst* 88:1442-1455, 1996
34. Vasey PA, Jones NA, Jenkins C, et al: Cisplatin, camptothecin and Taxol sensitivities of cells with p53-associated multidrug resistance. *Mol Pharmacol* 50:1536-1540, 1996
35. Lavarino C, Pilotti S, Oggionni M, et al: p53 gene status and response to platinum/paclitaxel-based chemotherapy in advanced ovarian carcinoma. *J Clin Oncol* 18:3936-3945, 2000