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STATE-OF-THE-ART IN THE MANAGEMENT OF CANCER

BLADDER CANCER — PART III

TREATMENT APPROACHES IN CURRENT PRACTICE AND IN EVALUATION

MEETING COVERAGE

GENE THERAPY OF CANCER — PART I

GENE TRANSFER STRATEGIES FROM THE 9TH INTERNATIONAL CONFERENCE ON GENE THERAPY OF CANCER, SPONSORED BY SIDNEY KIMMEL CANCER CENTER, IN SAN DIEGO, CA, ON DECEMBER 7-9, 2000

STATE-OF-THE-ART IN THE MANAGEMENT OF CANCER

BLADDER CANCER — PART III

TREATMENT APPROACHES IN CURRENT PRACTICE AND IN EVALUATION

This article, Part III of a series, describes various approaches used to treat bladder cancer, mostly transitional cell carcinoma (TCC). Part IV of this series will discuss in detail the application of these approaches to tumor stage and grade, and also describe prognostic factors associated with this disease. Generally, for treatment purposes, bladder cancer is classified as superficial (noninvasive), carcinoma *in situ* (CIS), muscle-invasive, or metastatic. However, better understanding of graduations of tumor aggressiveness within these broad categories allows fine tuning of treatment options. The advent of prognostic markers and sensitive disease monitoring approaches is also changing standard therapy protocols.

SURGERY

Surgery is the mainstay curative approach for all stages of bladder cancer except metastatic disease, although it may also be used to excise metastatic tumors when feasible.

Transurethral Resection (TUR)

Transurethral resection (TUR) of the bladder may be used both as a diagnostic and therapeutic procedure. In addition, chemotherapy can be directly delivered into the bladder at the time of TUR. As a surgical intervention, TUR of the bladder is an organ-sparing approach for treatment of bladder cancer. In TUR, a cystoscope inserted into the bladder through the urethra, is used to either excise or fulgurate the tumor. TUR is mostly used as a curative intervention in superficial TCC. One major challenge in the use of TUR in this setting is to ensure that it is complete, removing all of the involved tissue area. TUR may also be combined with adjuvant systemic or intravesical chemotherapy and/or immunotherapy to prevent recurrence as well as repeated for recurring superficial tumors.

When used in the treatment of superficial (Ta or T1) TCC, a beneficial intervention during TUR is postoperative irrigation with glycine or saline for a minimum of 18 hours after TUR. According to preliminary results of a multicenter, randomized, controlled trial (Whelan P, etal, ASCO01, Abs. 708:178a), among 866 patients the hazard ratio was 0.83 in favor of postoperative irrigation, which translated to an absolute improvement in 2-year recurrence-free rate of 6%, 51% with irrigation and 45% with no irrigation.

In muscle-invasive bladder cancer TUR is used primarily to establish the diagnosis and local extent of the disease. However, repeated TUR may be the only choice for some patients who are too fragile to have a total cystectomy and, most likely, are also not fit for systemic chemotherapy. Currently, TUR is also being investigated as a bladder-sparing procedure in muscle-invasive disease with some success. Use of TUR for definitive treatment of muscle-invasive bladder cancer requires careful patient selection, predicated on tumor volume, multifocality, and associated CIS.

Selected patients with muscle-invasive bladder cancer who undergo bladder-sparing treatment may achieve comparable long-term survival to those undergoing radical cystectomy. One problem arises from understaging the depth of tumor involvement, which occurs in up to 40% of cases. Despite the challenges associated with TUR in this setting, several series have shown that it provides disease control, particularly in patients with lower clinical disease stages. Clinical trials have demonstrated that TUR, in selected patients with muscle-invasive bladder cancer, can produce 5-year survival rates comparable to that of radical cystectomy (Herr HW, J Urol, Nov 1987;138(5):1162-3, and Solsona E, etal, J Urol, Jun 1992;147(6):1513-7).

Best results with radical TUR alone in muscle-invasive disease have been reported in patients with no residual tumor during a repeat vigorous resection of the primary tumor site. Among 99 patients treated with TUR as definitive therapy (57% with bladder preserved), the 10-year disease-specific survival was 76%, compared with 71% of 52 patients who underwent immediate cystectomy. Among the 99 patients treated with TUR, 82% of 73 with T0 disease on restaging TUR survived versus 57% of the 26 patients with residual T1 tumor on restaging TUR. Among 34 (34%) patients who relapsed with a new muscle-invasive tumor in the bladder, 18 (53%) were successfully treated with salvage cystectomy. Overall, 16 (16%) patients died from their disease (Herr HW, J Clin Oncol, 1 Jan 2001;19(1):89-93).

In a prospective study involving 133 candidates for conservative treatment, radical TUR was justified when the tumor was clinically limited to the muscular layer and when all biopsies of the periphery and depth of the tumor bed were free of tumor cells. At 5 and 10 years of follow-up, cause-specific survival rates were 80.5% and 74.5%, respectively, and bladder preservation rates were 82.7% and 79.6%, respectively. Patients with initial CIS need not be excluded from this treatment, but endovesical Bacillus Calmette-Guérin (BCG) immunotherapy should be administered and a closer follow-up is recommended (Solsona E, etal, J Urol, Jan 1998;159(1):95-8; discussion 98-9).

Although long-term results appear promising, physicians are still hesitant to use TUR as definitive treatment for muscle-invasive bladder cancer because optimal treatment and surveillance schedules as well as patient selection criteria, have not yet been established, and there are no definitive guidelines regarding this issue. Eventually, bladder preservation in muscle-invasive bladder cancer will probably require a multimodality approach involving conservative surgery, chemotherapy and radiation therapy (Dunst J, etal, Semin Surg Oncol, Jan-Feb 2001;20(1):24-32).

Partial (Segmental) Cystectomy

In segmental cystectomy the part of the bladder where the cancer is found is removed, preserving the remaining organ. Partial cystectomy permits complete pathologic staging of the tumor and pelvic lymph nodes while preserving both bladder and sexual function. However, because bladder cancer often occurs in more than one part of the bladder, this operation is used only in selected cases where the cancer is located in only one area. As with any bladder preservation technique, appropriate patient selection is important to achieve adequate survival rates. Partial cystectomy is an infrequently used technique that has not been fully evaluated in the treatment of bladder cancer. In muscle-invasive disease this technique may be considered for patients with a tumor that is primary, solitary, and amenable to removal with 2-cm surgical margins. At the time of partial cystectomy, a frozen section is taken for evaluation of the margins by a uropathologist. Also, a biopsy must be performed on the remaining urothelium to ensure that it is normal.

Radical Cystectomy

In radical cystectomy the bladder, surrounding tissue and various organs such as the uterus, ovaries, fallopian tubes, and part of the vagina, in women, and the prostate and seminal vesicles, in men, are removed. Preserving the urethra allows for bladder reconstruction and normal urine diversion. The lymph nodes in the pelvis may also be dissected. Currently, radical cystectomy is the definitive treatment option for long-term curative intervention in muscle-invasive disease. However, it is also preferable even in early disease because of the substantial risk of progression to muscle-invasive cancer in patients with CIS or high-grade superficial tumors. Currently available bladder reconstruction approaches that usually result in a well functioning bladder without incontinence or significant hematuria, have revived the cystectomy option in this setting.

The cost of radical cystectomy with urine diversion is approximately $25,000. Other cost factors include adjuvant chemotherapy for the treatment of extravesical disease, and prolonged hospitalization or reoperations that occur in up to 25% of patients following radical cystectomy and urinary diversion.

Radical cystectomy provides excellent control of the primary tumor and is superior to either radiation therapy (RT) alone, or organ-conserving surgery. Operative mortality is less than 2%. However, approximately 50% of all cystectomy candidates with high-grade tumors have unrecognized distant metastasis at the time of surgery, and they die of disseminated disease within 2 years of presentation. Radical cystectomy has no role in metastatic disease. Nonetheless, it is unfortunately often performed in patients deemed to have only regional disease at diagnosis but whose cancer has already metastasized. According to autopsies of 367 patients with T2-T4 muscle-invasive bladder carcinomas, metastases were found in 251 (68%). The most frequent sites of metastases were regional lymph nodes (90%), liver (47%), lung (45%), bone (32%), peritoneum (19%), pleura (16%), kidney (14%), adrenal gland (14%), and the intestine (13%). There was no difference in the frequency and location of metastases between 308 TCC and 38 squamous cell carcinoma cases, dispelling the notion of clinical differences between histologic tumor types. The frequency of metastases tracked local tumor extension; among patients treated with cystectomy, 36% with T2, 45% with T3a, 69% with T3b, and 79% with T4 disease had metastases at autopsy. The high frequency of metastases in patients having undergone cystectomy indicates that metastasis often occurs before the time of diagnosis, emphasizing the need for a better prediction of the metastatic capability of these tumors at presentation (Wallmeroth A, etal, Urol Int 1999;62(2):69-75). Presence of undiagnosed metastases at the time of organ-preserving interventions also makes comparisons difficult. The overall staging error for bladder cancer is 73%, with 20.3% overstaging and 52.3% understaging.

Even after cystectomy, patients run the risk of developing upper tract malignancies, although the incidence of such occurrences is low (3%). A retrospective study analyzed data related to upper tract recurrence among 529 patients who underwent radical cystectomy and urinary diversion at Memorial Sloan-Kettering Cancer Center (NY, NY) between July 1989 and June 1997. Upper tract recurrence occurred in 16/529 (3%) patients within a median follow-up of 16.9 months for the entire group and 49.1 months for patients with upper tract recurrence, with a median time to recurrence of 37.2 months. Of 12 upper tract recurrences, 7 (58%) were locally advanced at surgery (≥T3a with or without lymph-node metastasis) and 5/16 (31.3%) patients with recurrence presented with bilateral tumors (2 synchronous and 3 metachronous). Overall survival from the time of diagnosis of upper tract recurrence after radical cystectomy was poor, with a median of 10 months (Balaji KC, etal, J Urol, Nov 1999;162(5):1603-6).

Bladder reconstruction surgery (urinary diversion) provides an effective means of eliminating the quality of life (QoL) issues associated with radical cystectomy. Reconstruction of the lower urinary tract using a variety of approaches (ileal conduit, continent cutaneous diversion, or orthotopic neobladder) is a standard option for those undergoing radical cystectomy. In orthotopic reconstruction, a pouch (neobladder) is connected to the ureters at one end and to the urethra at the other end, allowing urine to pass out of the body, obviating the use of a stoma. In a retrospective study, the majority of 224 patients (ileal conduit=25, cutaneous Kock pouch=93, and urethral Kock pouch=103), regardless of type of urinary diversion, reported good overall QoL, little emotional distress and few problems with social, physical or functional activities (Hart S, etal, J Urol, Jul 1999;162(1):77-81). Similar results were also obtained from patients having undergone radical cystectomy and urinary diversion procedures in Japan (Kitamura H, etal, Int J Urol, Aug 1999;6(8):393-9). In addi-

tion, it has been shown that continent diversion can safely be offered to patients at high risk for local recurrence. Availability of this procedure enhances survival by decreasing the interval to cystectomy and favorably impacts QoL of patients undergoing cystectomy for invasive bladder cancer (Clark PE and Klein EA, Curr Opin Urol, Sep 1999;9(5):413-8).

The most common orthotopic approach involves all-ileum neobladders but segments of the large bowel have also been used. Researchers at Medical School Mainz (Wuppertal, Germany) used a large bowel segment in the Mainz-pouch technique of orthotopic bladder substitution that provided good reservoir capacity and continence rates, with less ileum used than in all-ileum pouches. This surgical technique is simple and reproducible, and in particular the antireflux ureteric implantation into the caecum protects the upper urinary tracts (Leissner J, etal, BJU Int, Jun 1999;83(9):964-70). Similarly, surgeons at Brady Urological Institute at Johns Hopkins University (Baltimore, MD) used a composite ileocolic segment for neobladder reconstruction in patients desiring orthotopic reconstruction of the lower urinary tract. Long-term experience with the ileocolic neobladder suggests that this composite segment provides excellent results for lower urinary tract reconstruction after radical cystectomy (Eisenberger CF, etal, Urol Clin North Am, Feb 1999;26(1):149-56, ix).

Patients at high risk of pelvic recurrence need not be excluded from orthotopic urinary diversion. In a retrospective review of 201 consecutive cases of radical cystectomy for bladder cancer, performed at Wayne State University School of Medicine and Barbara Ann Karmanos Cancer Institute (Detroit, MI), between March 1991 and March 1996, there were 33 patients (16.4%) with disease recurrence in the pelvis with or without systemic metastasis. Urinary diversion in patients with tumor recurrence was an ileal conduit (n=19), continent cutaneous diversion (n=3), or orthotopic neobladder (n=11). Mean follow-up for all patients undergoing cystectomy was 25.9 months and mean time to diagnosis of local disease recurrence after cystectomy was 13.9 months. In 21/33 (63.6%) patients, pelvic recurrence and systemic metastasis were present simultaneously. Disease recurrence was associated with poor outcome, with only 8 patients (24.2%) being alive and disease free, 7 of whom had isolated local recurrence without evidence of systemic metastasis.

There was no difference between patients with an orthotopic neobladder and those with a cutaneous (continent or incontinent) urinary diversion, in overall survival or type of therapy delivered once disease recurrence was diagnosed. The only diversion-related complication resulting from pelvic recurrence was one case of tumor invasion into an orthotopic neobladder, requiring conversion to an ileal conduit. The type of urinary diversion did not impact a patient's risk of complications, the ability to be treated by a salvage regimen, or overall survival once pelvic recurrence was diagnosed (Tefilli MV, etal, Urology, May 1999; 53(5):999-1004).

RADIOTHERAPY (RT)

Radiotherapy (RT) with or without single-agent cisplatin has been the major therapeutic modality in patients with locally advanced bladder cancer, or in those medically unfit for surgery. In muscle-invasive disease, external beam RT (EBRT) is an essential component of a multimodality bladder-preservation therapy consisting of cytoreductive surgery, such as TUR, followed by a planned combination of RT and chemotherapy. However, EBRT alone, or preoperative RT, are used infrequently and only in carefully selected patients (Petrovich Z, etal, Am J Clin Oncol, Feb 2001;24(1):1-9). RT alone, or in combination with chemotherapy, remains an important and effective palliative therapy for patients with recurrent and/or metastatic bladder cancer.

EBRT is the primary treatment for invasive bladder cancer in some European countries. In North America, EBRT is regarded as inferior to cystectomy and, thus, is rarely recommended as a primary treatment because it does not provide survival rates comparable to radical cystectomy, even when combined with salvage cystectomy. In patients who fail to respond to RT, the survival rates are lower than those achieved with primary radical cystectomy.

The exact value of radical RT is difficult to establish because changes in treatment techniques and selection of patients have biased results. A standard RT regimen involves a conventional dose and fractionation schedule to deliver a total dose of 60-66 Gy with a 3- or 4-field technique covering the bladder and tumor. Several other factors, like performance status and hemoglobin level, influence outcome. Morbidity of radical RT depends on several treatment and patient related factors, but 50-75% experience acute intestinal or urologic symptoms, and 10-20% may develop severe late toxicity. The importance of field size or overall treatment time cannot be established from available data. Hyperfractionation with dose escalation has proven effective in one study. Preoperative RT with cystectomy has not proven better than cystectomy alone or better than RT alone. The addition of systemic chemotherapy has increased disease-free survival (DFS) but has not significantly reduced the rate of distant metastases or improved overall survival. Additional irradiation of regional lymph nodes is of questionable efficacy. New treatment possibilities with advanced techniques of RT, hyperfractionation and dose escalation and/or the addition of systemic chemotherapy may improve outcome (Sengelov L and von der Maase H, Radiother Oncol, Jul 1999;52(1):1-14).

In a population-based study of the use and outcome of radical RT for invasive bladder cancer, electronic records of invasive bladder cancer (ICD code 188) from the Ontario Cancer Registry were linked to surgical records from all Ontario hospitals and RT records from all Ontario

cancer centers. From the 20,906 new cases of bladder cancer diagnosed in Ontario from 1982 to 1994, 1,372 cases treated by radical RT were identified. The median interval to start of radical RT from diagnosis was 13.4 weeks. Nearly all (93%) patients were treated on high-energy linacs, and the most common dose/fractionation scheme (31% of cases) was 60 Gy/30. Cause-specific for bladder cancer 5-year survival rates were 41%, 28% overall, 25% cystectomy-free, 36% bladder cancer cause-specific following salvage cystectomy, and 28% overall following salvage cystectomy. Histology (squamous or nonpapillary TCC) and advanced age were associated with a higher risk of death and a poorer cystectomy-free survival. This population-based study confirms previous institutional studies and clinical trials, and shows that radical RT has a curative role in the management of invasive bladder cancer, allowing about one-quarter of patients treated by RT to survive 5 years while retaining the bladder. Salvage cystectomy following RT provides a chance of cure at the time of bladder relapse (Hayter CR, etal, Int J Radiat Oncol Biol Phys, 1 Dec 1999;45(5):1239-45).

Radiosensitization

Radiosensitizers may improve RT outcomes. Various chemotherapeutics, including cisplatin, paclitaxel and gemcitabine, have been used in chemoradiotherapy regimens with considerable success (see multimodality therapy below). In animal models carbogen (normobaric 95% oxygen, 5% carbon dioxide) provided significant enhancement of local tumor control with fractionated RT. This approach to radiosensitization has been evaluated in the treatment of patients with bladder carcinoma using radical RT.

In a phase III clinical trial, conducted at Mount Vernon Hospital (Northwood, Middlesex, UK), 61 patients with locally advanced bladder cancer were treated with RT (50-55 Gy in 20 daily fractions over 4 weeks) to the bladder with inhalation of carbogen alone in 30 patients, and the addition of oral nicotinamide (80 mg/kg) prior to RT with carbogen in 31 patients. Results from this trial were then compared with those from two earlier attempts at hypoxic sensitization, the second Medical Research Council (MRC) hyperbaric oxygen trial in patients with bladder carcinoma, and a phase III trial of misonidazole with RT in patients with bladder cancer performed at Mount Vernon Hospital. Although there was no difference between the hyperbaric oxygen and misonidazole trials, when compared with the two earlier series, there was a large, statistically significant difference in favor of administration at carbogen, with or without nicotinamide, for local control, progression-free survival (PFS), and overall survival. Although the advantage for the carbogen group may be explained in part by changes in RT practice over the period of the three studies, the improvement in local control is significant enough to support the hypothesis that hypoxia is important in modifying the control of bladder carcinoma treated with RT (Hoskin PJ, etal, Cancer, 1 Oct 1999;86(7):1322-8).

Brachytherapy

Combination of EBRT and interstitial RT with the aim of bladder preservation is standard treatment in a selected group of patients with muscle-infiltrating bladder cancer in several European cancer centers. In a multimodality regimen involving preoperative EBRT, delivered at a dose depending on tumor stage, in patients who subsequently underwent surgical exploration with or without partial cystectomy and insertion of source carrier tubes for afterloading with iridium-192 (^{192}Ir), the technique was shown to be safe and effective (Van Poppel H, etal, Eur Urol, May 2000;37(5):605-8). However, this approach is rarely used in North America.

Outcomes of bladder-preservation brachytherapy are on par with those reported in similar patient populations treated with radical cystectomy. The main benefit in conservatively treated patients is a functioning bladder in about 50%. Local control rates at 5 years vary from 64% to 88% and the 5-year overall survival and DFS range from 47% to 66% and from 62% to 81%, respectively. Therefore, this approach is successful in preserving the bladder when performed in carefully selected patients in up-to-date facilities (Wijnmaalen A, etal, Semin Urol Oncol, Nov 2000;18(4):308-12).

Brachytherapy achieves excellent results in selected patients with solitary bladder cancer, maintaining a fully functional bladder. In 120 selected patients with a solitary tumor of a maximum diameter of 5 cm, and Stage T1 Grade 3, T2, or T3a disease, treated with brachytherapy at two Dutch centers, an extensive TUR was performed before implantation of ^{192}Ir wires. The administered tumor dose was 60 Gy at a dose rate of 60-90 cGy/hour. The 5-year overall survival was 67% and DFS was 73%. The 5-year DFS was 100% in T1, 80% in T2 and 67% in T3. Local failure was observed in 15 patients (13%); 6 of these relapses were salvaged by cystectomy. Ten patients had a superficial tumor Ta-T1 in bladder sites other than the original tumor site. Acute and late complications were unusual (Gonzalez Gonzalez D, etal, Arch Esp Urol, Jul-Aug 1999;52(6):655-61).

Interstitial brachytherapy for infiltrative vesical carcinomas also yields both high local control and satisfying results in regard to patient wellbeing. From 1975 to 1996, 98 patients with infiltrative vesical carcinomas (Tis=3, T1=28, T2=38, T3a=24, T3b=4, and Tx=1) were treated at the Centre Alexis Vautrin (Vandaeuvre-les-Nancy, France) by conservative surgery and interstitial ^{192}Ir brachytherapy in a retrospective non-randomized clinical trial. Mean follow-up was about 8 years. The protocol consisted of pelvic RT (3 fractions of 3.5 Gy) immediately followed by lymphadenectomy for stage T3 tumors, and by cystotomy or partial cystectomy during which brachytherapy plastic tubes were inserted. The delivered dose was 50 Gy for superficially infiltrative and 30 Gy for deeply infiltrative tumors; at the lowest dose, the treatment ended with EBRT. At 5 years the control rate was 72%, the specific

survival 80% and the global survival 71%. Local recurrence was seen in 29 patients; of these, 7 underwent total cystectomy. Regarding complications, 37 patients developed 43 complications; 35 were intravesical, with 10 (28%) estimated to be >Grade 2 because of technical problems that led to technique modification (Hoffstetter S, etal, Cancer Radiother, Apr 1998;2 Suppl 1:54s-61s).

CHEMOTHERAPY

In the past, chemotherapy was traditionally confined to metastatic bladder cancer. However, because TCC of the urothelium is a highly chemosensitive tumor, this treatment option is currently being revisited in most stages of bladder cancer, such as in the adjuvant setting to prevent recurrence, and in the neoadjuvant setting as a means of tumor downstaging before surgical excision. Both systemic and intravesical chemotherapy offer unique contributions to the treatment of bladder cancer. Results from chemotherapy and multimodality regimens, evaluated in all stages of bladder cancer, are summarized in Exhibit 1.

Systemic Chemotherapy

Systemic chemotherapy is being used in the treatment of metastatic disease, mostly in the palliative setting, and is being evaluated in the treatment of noninvasive and invasive bladder cancer in the adjuvant and neoadjuvant setting. Traditional agents considered active against TCC include cisplatin, methotrexate, vinblastine, mitomycin C, and doxorubicin, with the first two being the most active and most widely studied. These are being joined by newer cytotoxics such as carboplatin (Paraplatin; Bristol-Myers Squibb), oxaliplatin (Eloxatin; Sanofi-Synthelabo), gemcitabine (Gemzar; Lilly), epirubicin (Ellence; Pharmacia), paclitaxel, docetaxel (Taxotere; Aventis), and arsenic trioxide (Arsenox; Cell Therapeutics), among others. Also, older cytotoxics, such as cyclophosphamide and 5-fluorouracil (5-FU), that exhibit a lower level of activity when used as single agents in metastatic TCC, are being used in combination therapies. As monotherapy, most agents yield objective responses in only 10% to 20% of cases, including CR in 5% to 10%. In addition, duration of response is short at <4 to 6 months.

Among currently ongoing monotherapy studies is a multicenter phase II clinical trial of arsenic trioxide which was initiated in February 2001, in patients with recurrent cancer of the bladder or urinary tract. According to the protocol, arsenic trioxide is infused IV over 1 hour on days 1-5, with treatment repeating every 28 days for a minimum of 2 courses in the absence of disease progression or unacceptable toxicity. Patients who achieve CR are treated with 2 additional courses. Patients are followed every 2 months for 1 year after registration and then every 6 months for 1 year or until disease progression or relapse. A total of 12-35 patients will be accrued for this study within 12-18 months. Dean F. Bajorin of the Cancer and Leukemia Group B is the study's chair.

Combination chemotherapy can provide both palliation and a modest survival advantage in patients with advanced disease. Use of combination cytotoxic chemotherapy regimens in treating patients with metastatic bladder cancer has nearly doubled MST to 12 months, with a 3-year survival rate of approximately 20% to 25%.

Platinum-based chemotherapy relies on combinations of chemotherapeutics in which cisplatin is most often a component. The overall response rates for two-drug regimens of cisplatin/paclitaxel, carboplatin/paclitaxel and cisplatin/gemcitabine range from 63% to 72%, 14% to 65% and 42% to 66%, respectively. In phase II clinical trials, ORR for platinum/paclitaxel/gemcitabine 3-drug regimens ranged from 58% to 80%, but their clinical benefit in the treatment of TCC needs to be tested in phase III clinical trials (Bellmunt J, etal, Eur J Cancer 2000 Jul;36 Suppl 2:17-25).

The current standard MVAC chemotherapy regimen for metastatic bladder cancer consists of methotrexate (30 mg/m^2) on days 1, 15 and 22, vinblastine (3 mg/m^2) on days 2, 15 and 22, doxorubicin (30 mg/m^2) on day 2, and cisplatin (70 mg/m^2) on day 2. Developed at Memorial Sloan-Kettering Cancer Center, it has been in use for over 10 years (Sternberg CN, etal, J Urol, Mar 1985;133(3):403-7). Long-term survival has been recorded in 20% of patients treated with MVAC. When compared to single-agent cisplatin therapy, MVAC increased MST from 8 months to 12 months (Loehrer PJ Sr, etal, J Clin Oncol, Jul 1992;10(7):1066-73). MVAC has also proven superior to a regimen consisting of cisplatin, cyclophosphamide, and doxorubicin (CISCA), increasing ORR from 46% to 65% and MST from 36 weeks to 48 weeks (Logothetis CJ, etal, J Clin Oncol, Jun 1990;8(6):1050-5), further validating its role as a new standard. Another regimen, cisplatin, methotrexate, and vinblastine (CMV), developed at Stanford University (Palo Alto, CA), has also produced significant improvements in remission rates.

Although MVAC increases ORR and MST compared to single-agent cisplatin or CISCA therapy, toxicities associated with this treatment, including myelosuppression, granulocytopenic fever, and mucositis, and a 3% drug-related mortality, temper enthusiasm for this regimen. Consequently, in many patients, MVAC chemotherapy cycles are administered at 5- rather than the classic 4-week intervals. Recent clinical trials have indicated that MVAC's hematologic toxicities, such as neutropenia and fever, may be mitigated through the inclusion of granulocyte colony-stimulating factor (G-CSF) used prophylactically in all patients treated with this regimen, allowing more patients to be administered full-dose therapy. Response rates of MVAC, when used in combination with G-CSF, range up to 60%, and MVAC can be administered every 2 weeks as opposed to 4-week intervals. However, the regimen is still associated with a 3% to 5% drug-related death rate.

Attempts to escalate and/or dose intensify the above combinations have not been productive. The Genitourinary Group of the European Organization for Research and Treatment of Cancer (EORTC) conducted a

Exhibit 1
Selected Chemotherapy and Multimodality Regimens Under Evaluation in the Treatment of Bladder Cancer

Treatment	Outcome	References/Comments
Systemic Chemotherapy-Monotherapy		
Docetaxel (100 mg/m²) over 1 hour, *q* 21 days	Among 30 evaluable previously treated patients there were 4 (13%) PR with durations of response ranging from 3 to 8 months; MST was 9 months	Phase II ❑ McCaffrey JA, etal, J Clin Oncol, May 1997;15(5): 1853-7
Paclitaxel (250 mg/m²) by 24-hour continuous IV, *q* 3 weeks + rhG-CSF (5 mg/kg), daily, for at least 10 days during each cycle, until progression or intolerance	Among 26 previously untreated patients 11 (42%) experienced an objective response with 7 (27%) CR; median duration of response was 7+ months	Phase II ❑ Roth BJ, etal, J Clin Oncol, Nov 1994;12(11): 2264-70
Paclitaxel (60 mg/m²) weekly x 4 for a 4-week cycle until disease progression or prohibitive toxicity	Among 30 patients with advanced urothelial cancer who had failed prior chemotherapy, there were 2 (7%) PR and disease stabilized in 6 (20%) after 5 cycles of therapy; PR occurred in patients who had previously responded to chemotherapy; MTP was 66 days	Phase II ❑ Broome CM, etal, ASCO00, Abs. 1381:351a
Gemcitabine (1200 mg/m²) as a 30-minute IV infusion on days 1, 8 and 15, repeated *q* 28 days	Among 31 evaluable patients with TCC of the urinary tract, previously treated with cisplatin, ORR was 22.5%, with 4 (12.9%) CR and 3 (9.6%) PR; disease stabilized in 13 (42%); MST was 5 months with a decrease in cancer-related symptoms, as assessed by use of analgesics and improved performance status	Phase II ❑ Lorusso V, etal, Eur J Cancer, Jul 1998;34(8): 1208-12
Ifosfamide (3750 mg/m²) + mesna (2250 mg/m²) IV as a 2-day schedule in 26 patients, or ifosfamide (1500 mg/m²) IV + mesna (750 mg/m²), as a 5-day regimen in 30 patients	Among 56 refractory patients, there were 11 (20%) OR, with CR in 5 (9%) patients, 4 with soft-tissue/ lymph node-only disease and 1 with skeletal and pulmonary metastasis, and 6 (11%) PR; renal and CNS toxicities were severe (Grade 3 and 4) on the 2-day schedule, which necessitated a change to the 5-day regimen	Phase II (EST-3889) ❑ Witte RS, etal, J Clin Oncol, Feb 1997;15(2):589-93
Topotecan (1.5 mg/m²) IV, daily, for 5 days, *q* 3 weeks for 6 cycles (dose was modified for leukopenic fever, thrombocytopenic bleeding, and any Grade 3/4 toxicity)	Among 44 patients with progressive advanced urothelial carcinoma following prior systemic chemotherapy (32 had been treated previously treated with cisplatin-based chemotherapy), there were 4 PR for an ORR of 9.1%; major toxicities were gastrointestinal and myelosuppression	Phase I (ECOG 58) ❑ Witte RS, etal, Invest New Drugs 1998;16(2):191-5
Systemic Chemotherapy-Combination Therapy		
Carboplatin (AUC=4 or 5) + gemcitabine (1000 mg/m²) on days 1, 8	Among 16 evaluable "unfit" patients with advanced bladder cancer, the response rate was 43%	Phase I ❑ Lladó A, etal, ASCO00, Abs. 1354:344a
Carboplatin (AUC=5) + gemcitabine (1000 mg/m²) on days 1, 8	Among 25 evaluable chemonaive patients with advanced TCC, the response rate was 60%	Phase II ❑ Nogué M, etal, ASCO00, Abs. 1359:345a
Carboplatin (AUC=6) + paclitaxel (225 mg/m²) *q* 3 hours	Among 34 evaluable patients with advanced urothelial cancer and renal insufficiency, the response rate was 21%	Phase II (E-2896) ❑ Vaughn DJ, etal, ASCO00, Abs. 1351:343a
Carboplatin (AUC=2) weekly x 6 + paclitaxel (135 mg/m²) weekly	Among 33 chemonaive patients with advanced urothelial cancer, ORR was 29%	Phase II ❑ Friedland DM, etal, ASCO00, Abs. 1386:352a
Cisplatin (75 mg/m²) + docetaxel (75 mg/m²) *q* 3 weeks	Among 26 evaluable chemonaive patients, there were 6 CR and 10 PR for an ORR of 62%; disease stabilized in 2 and progressed in 8; after a median follow-up period of 132 days, MTP was 209 days and median overall survival 232 days; main toxicity was manageable hematologic events	Phase II ❑ del Muro G, etal, ASCO00, Abs. 1356:345a
Docetaxel (75 mg/m²) + cisplatin (75 mg/m²) *q* 3 weeks	Among 25 previously untreated patients (metastatic disease=20 and locoregional disease=5), there were 15 (60%) responders, including 7 (26%) CR; MST was 13.6 months; toxicities were tolerable	Phase II ❑ Sengelov L, etal, J Clin Oncol, Oct 1998;16(10): 3392-7

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Paclitaxel (135 mg/m^2) over 3 hours, followed by cisplatin (70 mg/m^2) over 2 hours, *q* 3 weeks for a maximum of 6 cycles	Among 34 chemonaive patients, the CR rate was 32%, the PR rate was 38%, ORR was 70%, and MST was 13 months; this combination, administered in an outpatient setting, was adequately tolerated	Phase II ❑ Burch PA, etal, J Urol, Nov 2000;164(5):1538-42
Paclitaxel (175 mg/m^2), over 3 hours, on day 1, followed by cisplatin (75 mg/m^2), *q* 21 days	Among 52 patients with advanced urothelial carcinoma, there were 26 objective responses, for an ORR of 50%; there were 4 CR; MST was 10.6 months; toxicity was moderate, with granulocytopenia and neurotoxicity being the most common side effects	Phase II (E-2895) ❑ Dreicer R, etal, J Clin Oncol 2000; 18(5):1058-61, and ASCO98, Abs. 1233:320a
Paclitaxel (150 mg/m^2) over 3 hours, on days 1, 8, 15 + gemcitabine (3000 mg/m^2) *q* 2 weeks, on days 1, 8, 15	Among 31 evaluable chemonaive patients with locally advanced or metastatic urothelial cancer, ORR was 39%	Phase II ❑ Kaufman DS, etal, ASCO00, Abs. 1341:341a
Paclitaxel (110 mg/m^2) + gemcitabine (1000 mg/m^2) on days 1, 8 and 15 *q* 4 weeks for a maximum of 6 cycles	Among 24 chemonaive patients with locally unresectable or metastatic pure TCC of the bladder (14 patients had visceral metastases) evaluable for toxicity, Grade 3 and 4 granulocytopenia was seen in 4 (17%) and 5 (21%), respectively; there was no episode of neutropenic fever; there were 2 cases of Grade 3 thrombocytopenia and 3 (12.5%) of adult respiratory distress syndrome (ARDS) resulting in 1 death; among 23 patients evaluable for response and survival, there were 9 (39%) CR with a median duration of 5 months and 5 (22%) PR with a median duration of 4 months; ORR was 61% (14/23); there were 11 deaths	Phase II ❑ Parameswaran R, etal, ASCO01, Abs. 798:200a
Paclitaxel (200 mg/m^2) on day 1 + gemcitabine (800 mg/m^2) on days 1, 8, 15	Among 34 evaluable previously untreated patients with advanced TCC of the bladder, the response rate was 59%	Phase II ❑ Meluch AA, etal, ASCO00, Abs. 1338:340a
Paclitaxel (175 mg/m^2) as a 3-hour infusion + carboplatin (AUC=5) as a 30-minute IV infusion immediately after paclitaxel	Among 32 patients with metastatic TCC, ORR was 23 (72%), with 10 (31%) CR and 13 (41%) PR; disease stabilized in 4 and progressed in 5; MTP after CR was 7 months; there was no nephrotoxicity	Phase II ❑ Pycha A, etal, Urology, Mar 1999;53(3):510-5
Paclitaxel (200 mg/m^2), followed by carboplatin (AUC=5) *q* 21 days	Among 35 evaluable chemonaive patients with advanced cancer of the urothelium, there were 7 (20%) CR and 11 (31%) PR with an MST of 9.5 months	Phase II ❑ Redman BG, etal, J Clin Oncol, May 1998;16(5): 1844-8
Paclitaxel (200 mg/m^2) on day 1 + carboplatin (AUC=5) on day 1 + gemcitabine (800 mg/m^2) on days 1, 8	Among 43 chemonaive patients, the response rate was 66%, including 15/21 (71%) patients with 1 disease site and 12/22 (55%) with 2 or more sites	Phase II ❑ Vayshampayan UN, etal, ASCO00, Abs. 1343:341a
Paclitaxel (200 mg/m^2) as a 3-hour infusion + carboplatin (AUC=6) + methotrexate (10 mg/m^2), increasing in 10 mg/m^2 increments, on day 1, *q* 21 days and thereafter with G-CSF and leucovorin support (phase I); carboplatin (AUC=5) was used without G-CSF (phase II)	Among 33 patients, no DLT were seen in phase I portion despite escalation of methotrexate to 60 mg/m^2; principal toxicities were myelosuppression and neuropathy; ORR (phase I and II) was 56%, MST was 15.5 months; 88% of patients overexpressed p53 at the primary site	Phase I/II ❑ Edelman MJ, etal, Urology, Apr 2000;55(4):521-5
Paclitaxel (200 mg/m^2) over 3 hours + methotrexate (30 mg/m^2) + cisplatin (70 mg/m^2)	Among 25 previously treated patients, there were 10 (40%) PR, including 3/7 patients with liver metastases	Phase I ❑ Tu SM, etal, J Urol, Nov 1995;154(5):1719-22
Doxorubicin (50 mg/m^2) + gemcitabine (2000 mg/m^2) *q* 2 weeks for 6 cycles, followed by 4 cycles of ifosfamide (1500 mg/m^2) on days 1-3 + paclitaxel (200 mg/m^2) over 3 hours, on day 1 + cisplatin (70 mg/m^2) on day 1, in a dose-dense rather than a dose-intense fashion; ancillary medications included mesna and G-CSF	Among 21 patients with metastatic disease, toxicities included Grade 3/4 neutropenia (57%), anemia (33%), thrombocytopenia (24%), neutropenic fever (10%), syncope (10%), fatigue (10%), neuropathy (10%) and 5% incidence of emesis, mucositis, pneumonitis, and depression; ORR was 86% with CR in 43%	Phase II ❑ Maluf FC, etal, ASCO00, Abs. 1344:342a
Gemcitabine (1,000 mg/m^2) on days 1, 8 and 15 + cisplatin (70 mg/m^2) on day 2 (GC), for a maximum of 6 cycles	Among 203 patients, the CR rate was 12%, the PR rate was 37%, for an ORR of 50%; the toxicity profile was more favorable with GC than MVAC	Phase II ❑ von der Maase H, etal, J Clin Oncol, Sep 2000; 18(17):3068-77, and von der Masse H, etal, ASCO00, Abs. 1293:329a

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Gemcitabine (1000 mg/m^2) as a 30-minute infusion on days 1, 8, and 15, followed by cisplatin (70 mg/m^2) on day 1, *q* 28 days	Among 27 elderly patients with Stage IV (T4b, N2, N3, M1) TCC, Grade 3/4 neutropenia occurred in 16 patients (59.3%), febrile neutropenia in 3 (11%), Grade 3/4 thrombocytopenia in 15 (55.5%), and Grade 3 anemia in 6 (22%); among; 24/27 patients were assessable for response, 3 were too early to evaluate; there were 3 (12.5%) CR, 9 (37.5%) PR, for an ORR of 12 (50%), and disease stabilized in 8 (33%) and progressed in 4 (17%); MTP was 7 months	Phase II ❑ Ventriglia MO, etal, ASCO01, Abs. 793:199a
Gemcitabine (1000 mg/m^2) on days 1, 8 and 15 + cisplatin (70 mg/m^2), *q* 28 days	Among 28 evaluable chemonaive patients with advanced TCC, there were 6 CR and 10 PR, for an ORR of 57%	Phase II ❑ Moore MJ, etal, J Clin Oncol, Sep 1999;17(9): 2876-81
Gemcitabine (1000 mg/m^2) on days 1, 8, 15, *q* 28 days + epirubicin (20 mg/m^2) on days 1, 8, 15	Among 26 evaluable chemonaive patients, the response rate was 46%	Phase II ❑ Neri B, etal, ASCO00, Abs. 1380:351a
Methotrexate (30 mg/m^2) on day 1 + vinblastine (3 mg/m^2) on day 2 + doxorubicin (30 mg/m^2) on day 2 + cisplatin (70 mg/m^2) on day 2, with G-CSF on days 4-11, repeated *q* 14 days (HD-MVAC)	Response rate was 73% with HD-MVAC, with a CR rate of 25%, PFS of 24.7%, and MST of 14.1 months	Phase III ❑ Sternberg CN, etal, ASCO00, Abs. 1292:329a
Methotrexate (30 mg/m^2) + vinblastine (4 mg/m^2) on days 1 and 8 + cisplatin (100 mg/m^2) on day 2 + folinic acid (15 mg) for 6 hours x 4 on days 2 and 9; 3 cycles of CMV were delivered over 3 weeks	Among 38 patients with advanced (Stage T3-T4) squamous cell carcinoma (SCC) of the urinary tract, there were 5 (13%) CR, 10 (26%) PR, and disease stabilized in 9 (24%) and progressed in 6 (16%); 8 (21%) were not assessable; among 29 patients with pure SCC, at 9 weeks, there were 4 (14%) CR, 8 (28%) PR, and disease stabilized in 7 (24%), and progressed in 4 (14%); 6 (21%) were not assessable; 29 patients died, 76% (n=22) from SCC, 3% (n=1) from the treatment and 21% (n=6) from other reasons	Phase II ❑ Russell JM, etal, ASCO01, Abs. 762:191a
Methotrexate (60 mg/m^2) on day 1 + cisplatin (25 mg/m^2), by continuous IV, on days 2-6 + 5-FU (800 mg/m^2,), by continuous IV, on days 3-6 + leucovorin (500 mg/m^2), by continuous IV, on days 3-6	Among 24 patients with muscle-invasive (T2-T4=9) and metastatic (n=15) urothelial cancer, ORR was 63%; MST was 65 months in the muscle-invasive group and 17 months in the metastatic group, with an MDR of 6 months in the latter group; however, significant hematologic toxicity was seen, with 80% of patients developing Grade 3/4 myelosuppression	Phase II ❑ Oh WK, etal, Cancer, 1 Oct 1999;86(7): 1329-34
Vinblastine (0.11 mg/kg) on days 1 and 2 + ifosfamide (1.2 g/m^2) on days 1-5, with mesna uroprotection + gallium nitrate (300 mg/m^2) as a continuous infusion on days 1-5	Among 45 patients, there 20 major responses with 6 (13%) CR; MDR was 47 weeks and MST for all patients was 10 months	Phase II (E-5892) ❑ Dreicer R, etal, Cancer, 1 Jan 1997;79(1): 110-4
Intravesical Chemotherapy		
Valrubicin (800 mg) administered intravesically at 6 weekly instillations	Among 90 patients with refractory recurrent CIS, there were 19 (21%) CR, including 7 who remained disease-free at a median follow-up of 30 months; 14 patients who did not meet the strict protocol definition of CR had superficial Ta disease only; median time to failure and/or last follow-up for complete responders was >18 months; recurrence occurred in 79 patients, and 44 (56%), 4 responders and 40 nonresponders underwent radical cystectomy; 4 nonresponders who did not undergo cystectomy following valrubicin died of bladder cancer	Phase II ❑ Steinberg G, etal, J Urol, Mar 2000;163(3):761-7
Mitoxantrone (10 mg diluted in 50 ml normal saline), administered weekly for 6 weeks after TUR	Among 25 patients with Ta/T1 TCC of the bladder, after a mean follow-up of 12 months, 76% remained relapse-free (69% of newly diagnosed and 89% of previously relapsed patients); therapy was well tolerated, with only two cases of Grade 3 local toxicity	Phase II ❑ Chiang PH and Chiang CP, Kaohsiung J Med Sci, Feb 2000;16(2):91-4

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Epirubicin (1 mg/ml or 2 mg/ml in 50 or 100 mg/50 ml solution) as a 1-hour treatment	Among 122 patients with superficial (Ta/T1) bladder cancer, there was no difference in the marker tumor response rate in 24/52 patients treated with the 1 mg/ml dose compared with 21/50 treated with the higher dose; similarly, the higher dose was not superior in regard to TFR, with a hazard ratio of 1.46	
Multimodality Regimens		
Cisplatin (75 mg/m^2) on day 1 + 5-FU (1 gm/m^2), daily, on days 1 to 4 and definitive RT; chemotherapy was repeated *q* 28 days, twice during and twice after RT	Among 53 evaluable patients (response was not determined for 18) with muscle-invasive bladder cancer (Stage T2-T4) with nodal involvement at or below the level of bifurcation of the iliac vessels, ORR was 51% based on intent to treat with a CR rate of 49%; estimated MST of 56 patients was 27 months with an overall 5-year survival of 32%; the 5-year survival of 25 patients who refused surgery was 45%	Phase II ❑ Hussain MH, etal, J Urol, Jan 2001;165(1):56-60, discussion 60-1, comment 65-6
Paclitaxel (175 mg/m^2) over 3 hours, for 3 cycles *q* 3 weeks, followed by cisplatin (75 mg/m^2); patients were restaged by CT, cytoscopy and multiple biopsies; cystectomy was not performed in case of pathologic CR but patients underwent RT; those with PR, SD or PD underwent radical cystectomy	Among 44 patients with Stage II (n=9, 20.5%), Stage III (n=35, 79.5%), T2 (n=2, 4.5%), T3a (n=7, 15.9%), T3b (n=27, 61.4%), T4b (n=8,18.2%) disease, neutropenia Grade 3/4 was the main hematologic toxicity seen in 31.8%; there were no toxic deaths or febrile neutropenia; there were 24 (54.5%) pathologic CR, 11 (25%) PR, 6 (11.4%) SD and 4 (9.1%) PD; after a median follow-up of 15.5 months, there were 4 local and 4 distant relapses	Mel JR, etal, ASCO01, Abs. 792:199a
Gemcitabine (1000 mg/m^2) on day 1 and 8 for 4 courses at 28 day intervals; the first 8 patients were also treated with carboplatin (200 mg/m^2) on day 1; concurrently a radical 60 Gy dose of RT was administered in 30 fractions, in 2 phases	Among 19 consecutive patients with T2-T4 TCC of the bladder (2 with metastases), treatment was generally well tolerated with the principal adverse effects being hematologic toxicity; at one year, 16/19 patients were free of disease with no long-term toxicity	Phase I ❑ Madavan KS, etal, ASCO01, Abs. 788:198a
Radical RT (median 64 Gy) + concurrent 5-FU (200-250 mg/m^2), daily, by continuous IV for 7 days during all RT courses	Among 18 elderly patients with Grade III muscle-invasive bladder cancer, no hematologic, renal or hepatic toxicity was observed during continuous 5-FU; when matched with 21 RT only controls, among 15 evaluable patients the CR rate was 67% and 65% in the 5-FU + RT group, respectively; after a median follow-up of 14 months, the 2-year distant metastases DFS was significantly better with concurrent treatment (100% versus 73.7%), while no significant differences were observed in terms of overall survival, disease-related survival, or local relapse DFS, although higher rates were observed in the patients treated with 5-FU	Caffo O, etal, ASCO01, Abs. 2444
TUR + cisplatin (15 mg/m^2) IV and 5-FU (400 mg/m^2) IV on day 1, 2, 3, 15, 16, and 17; RT immediately following chemotherapy on day 1, 3, 15, and 17, using twice-a-day 3 Gy per fraction cores to the pelvis for a total RT dose of 24 Gy; CR patients were treated by consolidation therapy with the same drugs and doses on day 1, 2, 3, 15, 16, and 17 combined with twice-daily RT (2.5 Gy per fraction) for a total consolidation dose of 20 Gy and a total induction plus consolidation dose of 44 Gy	Among 34 patients with muscle invasive (Stage T2-T4a) bladder cancer without hydronephrosis, at a median follow of 29 months, 26/34 had a visibly complete TUR; after induction treatment, 22 (67%) of the 33 patients had no tumor detectable on urine cytology or rebiopsy; overall 6 patients died of bladder cancer and the actuarial overall survival at 3 years was 83% with a 66% 3-year survival with an intact bladder	Phase I/II ❑ Kaufman DS, etal, Oncologist 2000;5(6):471-6

Legend: ***CR**=complete response* ***DFS**=disease-free survival* ***MDR**=median duration of response* ***MST**=median survival time* ***MTP**=median time to progression* ***OR**=objective response* ***ORR**=overall response rate* ***PD**=progressive disease* ***PFS**=progression-free survival* ***PR**=partial response* ***SD**=stable disease* ***TFR**=time to first recurrence*

phase III, randomized, international trial (protocol ID: EORTC-30924) to evaluate whether high-dose MVAC (HD-MVAC) with growth factor support could improve MST, by comparing the classic MVAC regimen (n=129) to a HD-MVAC regimen (n=134) consisting of methotrexate (30 mg/m^2) on day 1, vinblastine (3 mg/m^2) on day 2, doxorubicin (30 mg/m^2) on day 2, and cisplatin (70 mg/m^2) on day 2, with G-CSF on days 4-11, repeated every 14 days. Standard MVAC was administered every 28 days without G-CSF. With standard MVAC, the ORR was 58%, with a CR rate of 11%, and with HD-MVAC, the ORR was 73%, with a CR rate of 25%. However, after a median follow-up of 38 months, the overall survival for MVAC-and HD-MVAC-treated patients was 25.4% and 35.3%, respectively, which was not statistically significant; nor was PFS of 11.6% and 24.7%, and MST of 14.5 months and 14.1 months, respectively. There was no difference in the incidence of non-hematologic toxicities between the two regimens, but there was significantly more neutropenia and neutropenic fever in the MVAC group (26% versus 10%). This can be explained by the fact that only 19% of MVAC-treated patients were administered G-CSF, compared to 94% in the HD-MVAC group. The drug-related death rate was 3% in the MVAC group and 5% in the HD-MVAC group. Although the HD-MVAC regimen with G-CSF support was feasible and associated with an improvement in the CR rate, it did not improve MST. Hoewever, it may have improved 2-year DFS but the trial was not powered to detect an improvement in this parameter (Sternberg CN, etal, ASCO00, Abs. 1292:329a, and Sternberg CN, etal, J Clin Oncol, 15 May 2001;19(10):2638-46).

An attractive alternative regimen to MVAC in the treatment of bladder cancer consists of the combination of gemcitabine and cisplatin (GC); its lower toxicity profile makes it ideal for elderly patients or patients with comorbidities (Sternberg CN, etal, ASCO00, Abs. 1292:329a). Many patients with advanced disease are not candidates for MVAC therapy because of pre-existing cardiac and renal disease that contraindicate use of doxorubicin and cisplatin, respectively. Switching from MVAC to the GC regimen provides a better risk:benefit ratio.

In the largest randomized, multinational (19 countries), multicenter, phase III clinical trial ever performed, under lead investigator Hans von der Maase, MD, of Aarhus University Hospital in Denmark, the GC regimen was compared with MVAC in 405 chemotherapy-naive patients with locally advanced or metastatic TCC of the urothelium. Half of the patients in each treatment group had visceral metastases, and 90% in each group had measurable disease. This trial, conducted between 1996 and 1998 in centers in Europe, the USA, and Canada, was supported by the results from 3 multicenter phase II trials with GC that produced response rates of 35% to 50% in TCC. The phase III trial had an 80% power to detect a 4-month improvement in MST, allowing for detection of a 33% difference in survival between the two treatment groups; other endpoints included response rate, QoL, and toxicity.

Patients with Stage IV TCC and no prior systemic chemotherapy were randomized so that 203 were treated with gemcitabine (1000 mg/m^2) on days 1, 8 and 15, and cisplatin (70 mg/m^2) on day 2, and 202 patients were treated by standard MVAC every 28 days for a maximum of six cycles. The groups were well balanced with respect to prognostic factors. In terms of response, none of the differences in results were statistically significant. The CR rate was 12% in both arms while the PR rate was 37% with GC and 34% with MVAC, for an ORR of 50% and 46%, respectively; MST was 13.8 months with GC and 14.8 months with MVAC.

However, the GC regimen was more tolerable, with more GC patients completing six cycles of therapy (the maximum allowed), with fewer dose adjustments. The drug-related death rate was 1% on the GC arm and 3% on the MVAC arm. Significant anemia (27% versus 18%) and thrombocytopenia (58% versus 21%) were more common with the GC regimen compared to the MVAC regimen, but there were few clinical consequences, such as transfusions and bleeding, from these toxicities. On both arms, the RBC transfusion rate was 13 patients per 100 cycles, and Grade 3/4 hemorrhage or hematuria was 2%; the platelet transfusion rate was 4 patients per 100 cycles and 2 patients per 100 cycles on GC and MVAC, respectively. More MVAC patients, compared with GC patients, experienced Grade 3/4 neutropenia (82% versus 71%), neutropenic fever (14% versus 2%), neutropenic sepsis (12% versus 1%), mucositis (22% versus 1%), and alopecia (55% versus 11%). Patients on the GC treatment arm spent an average of 33 days in the hospital for drug-related neutropenic fever compared to 272 days for patients on the MVAC regimen. QoL was maintained during treatment on both arms; however, when compared to a baseline measure, more patients on the GC arm had sustained improvements in performance status, weight and level of fatigue. Therefore, GC provides a survival advantage similar to MVAC but with a better safety profile and improved tolerability. This better risk:benefit ratio suggests that GC can be considered an alternative to MVAC in the treatment of locally advanced and metastatic TCC, although the cost and availability of gemcitabine may prevent GC from completely replacing MVAC (von der Maase H, etal, J Clin Oncol, Sep 2000;18(17):3068-77, and von der Masse H, etal, ASCO00, Abs. 1293:329a). Eli Lilly has submitted these findings to the FDA to determine if GC should be approved to treat advanced TCC of the bladder

Despite its high activity, cisplatin is associated with serious toxicities that are even more pronounced in the elderly population that make up the majority of those treated for metastatic TCC. Numerous trials have been conducted to see if cisplatin could be replaced by carboplatin because of the latter's milder toxicity profile (Exhibit 1). Overall response rates ranging from 37% to 84% have been reported in trials with carboplatin-based multidrug combinations. However, in a randomized clinical trial comparing the combination of methotrexate, carboplatin, and

vinblastine (MCAVI) to cisplatin-based MVAC in the treatment of surgically incurable bladder carcinoma, no therapeutic advantage was ascribable to carboplatin (Bellmunt J, etal, Cancer, 15 Nov 1997;80(10):1966-72). A total of 47 patients, of whom 17 had lymph node disease and 30 had distant metastases, were randomized to either MCAVI or MVAC treatment arms, with similar patient characteristics in each arm. The ORR was 39% for the MCAVI arm and 52% for the MVAC arm, with 3 CR among patients treated with MVAC and no CR in the MCAVI group. While it is unknown whether the absence of doxorubicin in the carboplatin-based regimen may have reduced therapeutic efficacy or whether the applied carboplatin dose (AUC=5) might have been inadequate in polychemotherapy, this study suggests that response rates to carboplatin may be lower than those for cisplatin and indicates that carboplatin cannot be routinely substituted for cisplatin without the risk of compromising response. Nevertheless, carboplatin has been occasionally substituted in combination regimens for patients whose medical conditions contraindicate use of cisplatin.

Paclitaxel (Taxol; Bristol-Myers Squibb) demonstrated one of the highest single-agent response rates (42%) in previously untreated patients with advanced urothelial carcinoma, and was well tolerated when administered with hematopoietic growth factor support, prompting its extensive evaluation in combination regimens, including paclitaxel/platinum-based doublets or triplets. In a phase II clinical trial (protocol ID: E-2895), paclitaxel plus cisplatin produced an ORR of 70% (38% PR and 32% CR) in patients with advanced urothelial cancer (Exhibit 1). In this trial, sites of metastases correlated with the pattern of response rates which is in accordance with that seen with other conventional single agents or combination regimens; no responses were observed in hepatic metastases compared with a 62% response rate in nodal and soft-tissue disease, and a 38% response rate in nonhepatic visceral metastasis (Burch PA, etal, ASCO99, Abs. 1266:329a, and Burch PA, etal, J Urol, Nov 2000;164(5):1538-42).

When carboplatin was substituted for cisplatin in phase II clinical trials, the results were similar in efficacy to cisplatin, with response rates ranging from 14 to 65%, and generally carboplatin was better tolerated (Exhibit 1). Given paclitaxel's relatively low renal excretion, a paclitaxel/carboplatin regime may be considered in patients with advanced urothelial cancer and renal insufficiency, who are unable to tolerate standard cisplatin-based regimens; a phase II clinical trial (protocol ID: E-2896) has investigated this regimen specifically in this patient population (Exhibit 1). Ongoing ECOG trials are comparing paclitaxel/carboplatin with MVAC in both the advanced disease and adjuvant settings. In a phase III randomized clinical trial (protocol IDs: 199/14006; E-1897), initiated in March 1999, standard MVAC is being compared to paclitaxel and carboplatin as postoperative adjuvant therapy in patients with muscle- invasive TCC of the bladder at high risk for relapse. A total of 490 patients are being accrued for this study over a 2.6-year period. In another phase III randomized clinical trial (protocol ID: 199/13573; E-4897; CTSU), initiated in September 1998, MVAC versus carboplatin and paclitaxel is being evaluated in patients with progressing regional or metastatic TCC or mixed histologies with a component of TCC of the urothelium. A total of 330 patients will be accrued for this study within 3.3 years. Both these studies are being conducted by ECOG, under Bruce J. Roth, MD, as Study Chair.

An ongoing phase II/III clinical trial (protocol ID: EORTC-GU-30986) is comparing a paclitaxel and carboplatin regimen with a methotrexate, carboplatin, and vinblastine regimen to clarify the role of paclitaxel-based combinations. The combination of paclitaxel, carboplatin, and methotrexate in advanced TCC of the urothelium does not appear to be related to p53 status. This regimen was well tolerated and active in advanced TCC despite frequent p53 mutation. This is consistent with the p53-independent mechanism of paclitaxel. Whether this regimen is superior to MVAC, other paclitaxel-based regimens, or to paclitaxel alone will require comparative trials (Edelman MJ, etal, Urology, Apr 2000;55(4):521-5). Such combinations are also under evaluation in the adjuvant setting, and future trials may assess their potential role as neoadjuvant therapy or in combination with RT (Bajorin DF, Oncology (Huntingt), Jan 2000;14(1):43-52, 57; discussion 58, 61-2).

Paclitaxel/ifosfamide (Ifex; Bristol-Myers Squibb)/cisplatin is another promising alternative regimen for patients who can tolerate cisplatin-based regimens. In a phase II clinical trial, in a heavily cisplatin-pretreated cohort with advanced urothelial carcinoma, single-agent ifosfamide demonstrated significant activity but also major toxicity (Exhibit 1), prompting investigators to suggest a modification of dose and/or schedule for future trials. A phase II clinical trial assessed the ITP regimen, consisting of a combination of ifosfamide (1.5 g/m^2), per day, for 3 days, cisplatin (70 mg/m^2) on day 1, and paclitaxel (200 mg/m^2) on day 1, administered to previously untreated patients with unresectable or metastatic TCC. The regimen was recycled either every 4 weeks (n=30) or 3 weeks (n=15) with G-CSF administered during each cycle. Among 44 assessable patients, 30 (68%) experienced a major response with 10 (23%) CR and 20 (45%) PR, with durations of response ranging from 4 to 36 months. At a median follow-up of 28 months, the MST was 20 months and 11 (25%) patients were disease free at last follow-up. Overall toxicity for the 15 patients whose treatment was recycled at 3 weeks was similar to that for patients treated every 4 weeks. Hematologic toxicity included anemia, thrombocytopenia, and febrile neutropenia which was observed in 7 (16%) patients and in 3.3% of cycles of therapy. No Grade 4 nonhematologic toxicity was observed. Grade 3 nonhematologic toxicity included alopecia, renal insufficiency (11%), and neuropathy (9%). ITP recycled at 3-week intervals was an active and well-tolerated regimen in this setting (Bajorin DF, etal, Cancer, 1 Apr 2000;88(7):1671-8).

A phase I/II clinical trial (protocol ID: MSKCC-97095, NCI-G97-1339) of sequential doxorubicin/gemcitabine and ifosfamide, paclitaxel, and cisplatin chemotherapy for metastatic or locally advanced TCC of the urothelium was initiated in September 1997. This regimen has substantial activity with an encouraging CR proportion. However, toxicity is substantial. Therefore, the phase II clinical trial will continue with modifications to reduce toxicity including deletion of a sixth doxorubicin/gemcitabine dose and the addition of erythropoietin. The trial continues patient accrual to further delineate the CR proportion and median survival (Maluf FC, etal, ASCO00, Abs. 1344:342a).

A phase III randomized clinical trial (protocol ID: 199/15783; MSKCC-00138; NCI-G01-1935), involving adjuvant doxorubicin and gemcitabine followed by ifosfamide, paclitaxel, and cisplatin, versus adjuvant cisplatin and gemcitabine in completely resected locally advanced TCC, was initiated in December 2000, at Memorial Sloan-Kettering Cancer Center under the direction of Dean F. Bajorin, MD. Patients are also administered G-CSF SC daily on days 6-17. Treatment repeats every 21 days for a total of 4 courses in the absence of disease progression or unacceptable toxicity. A total of 276 patients will be accrued for this study within 4 years.

Docetaxel is also proving effective in the treatment of bladder cancer, both alone and in combination with other chemotherapeutics. Based on studies in other cancers, docetaxel has documented activity in cisplatin-resistant disease, and the combination of docetaxel and cisplatin has also been shown to be effective with a manageable safety profile. Larger randomized studies of docetaxel and cisplatin against standard MVAC are needed to test the validity of these significant preliminary results.

Gemcitabine is also emerging as an important drug in the treatment of TCC of the bladder. Phase I and II gemcitabine monotherapy trials have shown promising outcomes with regard to convenience in administration, acceptable toxicity, and activity in elderly patients. Also, encouraging results regarding gemcitabine's effectiveness against bladder cancer with hepatic metastases, led to the initiation of several phase II clinical trials in TCC in Europe, the USA, and Canada. Data from these trials suggested that cisplatin resistance in TCC does not necessarily imply resistance to gemcitabine. When administered as a single agent, gemcitabine produces response rates of 23% to 28% in both pretreated and chemonaive patients with an excellent toxicity profile. When combined with cisplatin, overall response rates increase to 66% with maintained tolerability (Sternberg CN, Semin Oncol, Feb 2000;27(1 Suppl 2):31-9). An international phase III clinical trial is being planned to compare GC to GC plus paclitaxel.

Other cisplatin- and carboplatin-based combination regimens have been tested, or are currently evaluated in clinical trials (Exhibit 1). Also, based on the activity of cisplatin, other metallic compounds have been evaluated, including gallium and second- to fourth-generation platinum complexes. In a phase II clinical trial (protocol ID: EORTC-30931), lobaplatin, a third-generation water soluble and stable platinum complex developed by Asta Medica (Frankfurt am Main, Germany), produced an ORR of 10% in patients refractory to platinum-based regimens. Although nonhematologic side effects were mild, hematological toxicity was significant, with 89% of patients demonstrating Grade 3-4 thrombocytopenia and 61% of patients exhibiting Grade 3-4 neutropenia (De Mulder P, etal, ASCO97, Abs. 1158:325a, and Sternberg CN, etal, Ann Oncol, Jul 1997;8(7):695-6). European pilot trials of oxaliplatin, a fourth-generation platinum analog, against TCC have also been encouraging.

Gallium nitrate, however, when administered by continuous infusion to patients with refractory bladder cancer did not prove effective and was quite toxic. An evaluation by the Southwest Oncology Group (SWOG) confirmed the relative lack of activity of this agent. The combination of gallium nitrate and 5-FU was compared with the MVAC regimen in a phase II randomized trial (protocol ID: MSKCC-92079A1, NCI-V92-0139) in chemonaive patients with advanced urothelial cancer. Only 2/17 (12%) patients on the gallium/5-FU arm experienced a PR lasting 4 and 12 months while 13/15 (94%) on MVAC experienced a major response consisting of 2 CR and 11 PR lasting a median duration of 7.5 months (range=3.5-21+ months). Among 12 patients who crossed over to MVAC after failing gallium/5-FU, there were 3 CR lasting 17+, 22+, 24+ months, and 2 PR lasting 5.5 and 14+ months (McCaffrey J, etal, ASCO95, Abs. 600).

Other combination regimens are also being attempted (Exhibit 1). One promising regimen involves gemcitabine and paclitaxel. In an *in vitro* evaluation, 4 bladder cancer cell lines (RT4, RT112, T24, SUP) were exposed to either single, simultaneous or four sequential combinations of gemcitabine and paclitaxel in various concentrations. Gemcitabine and paclitaxel inhibited cell proliferation dose-dependently. According to the cell-cycle experiments, either simultaneous or sequential application of gemcitabine administered 24 hours before paclitaxel was effective and should be evaluated in phase II trials (Lindner H, etal, ASCO00, Abs. 1389:353a). A phase II clinical trial (protocol ID: E-5899)of paclitaxel and gemcitabine is ongoing in advanced TCC of the urothelium in patients with renal insufficiency.

A phase II clinical trial (protocol ID: E-2899), evaluating the combination of docetaxel and gemcitabine in progressive regional or metastatic carcinoma of the urothelium, is ongoing, as well as one (protocol ID: AMGEN-GCSF-990125, NCI-V00-1594) with this combination and G-CSF in locally recurrent or advanced TCC of the urothelial tract.

Intravesical Chemotherapy

Intravesical chemotherapy permits direct contact between tumor(s) and drug and is associated with reduced systemic exposure compared with IV administration.

Although most intravesical interventions involve immunotherapy, intravesical chemotherapy appears to have a role in the adjuvant treatment of superficial TCC. Currently, in the USA, the most commonly used intravesical agent is mitomycin C. Valrubicin (Valstar; Anthra Pharmaceuticals) has also been approved for the treatment of BCG-refractory CIS. An older drug, thiotepa, the first intravesical agent used for the management of superficial bladder cancer, is seldom used in the USA as most of the reported studies showed marginal or no benefit when compared with controls. Various other chemotherapeutics such as epirubicin and mitoxantrone (Novantrone: Immunex) have been evaluated intravesically and trials are ongoing with gemcitabine (protocol ID: PCI-99039, NCI-G01-1926, LILLY-PCI-99039, PCI-IRB-990814), and paclitaxel (protocol ID: WVU-13707-OSP-97-092, NCI-V97-1114).

Intravesical chemotherapy has been traditionally considered to be of little value in preventing recurrences, with efficacy on the order of a 14% reduction in recurrence. However, according to a meta-analysis of 11 randomized trials involving 3,703 patients, administration of intravesical chemotherapy after complete TUR in patients with newly diagnosed bladder cancer significantly reduces the risk of tumor recurrence. The odds ratio for 1-year recurrence was 0.56, representing a 44% reduction among patients administered adjuvant intravesical chemotherapy compared with TUR alone. Sensitivity analyses pointed to the chemotherapy treatment schedule as the reason for the heterogeneity in tumor recurrence rates across studies. In addition, the researchers found that the rate of tumor recurrence fell by 30% to 80% after intravesical chemotherapy, depending on whether recurrence was examined at 1, 2, or 3 years post-TUR. According to the findings of this study, intravesical chemotherapy appears to have a major impact on decreasing the chance of recurrence of superficial TCC of the bladder (Huncharek M, etal, J Clin Epidemiol, Jul 2000;53(7):676-80). Optimal dose and treatment schedules for adjuvant intravesical chemotherapy have not been established but a 6- to 8-week course of therapy is usually recommended. Patients who fail primary treatment with one agent may be treated again with a different agent.

Mitomycin C, an alkylating agent, is used in doses ranging from 20 mg to 60 mg diluted in water at concentrations ranging from 0.5 mg/ml to 2.0 mg/ml. Mitomycin C is administered weekly for 6 to 8 weeks as induction therapy with or without maintenance therapy for 1 year. The most common side effects associated with intravesical mitomycin C are chemical cystitis and allergic skin reactions. The effect of single and multiple instillations of intravesical mitomycin C on recurrence in newly diagnosed superficial bladder cancer was investigated in a multicenter randomized clinical trial involving 502 patients. After complete TUR, patients were randomized into 1 of 3 treatment arms, no further treatment, 1 instillation of mitomycin C (40 mg/40 ml water) at resection, or 1 instillation at resection followed by instillations at 3-month intervals for 1 year for a total of 5 instillations. After median follow-up of 7 years, 1 and 5 instillations of mitomycin C resulted in decreased recurrence rates and increased recurrence-free interval in patients at low, medium and high risk for subsequent recurrence. There was suggestive but not conclusive evidence that 5 instillations of mitomycin C offered a slight advantage over 1 instillation (Tolley DA, etal, J Urol, Apr 1996;155(4):1233-8).

The impact of a single mitomycin C instillation in patients with low-risk superficial bladder cancer with short- and long-term follow-up, was also investigated in 131 patients in a prospective randomized controlled trial conducted at the Instituto Valenciano de Oncologia (Valencia, Spain). All patients had a ≤3 cm single, papillary, primary, or recurrent tumor, and were disease-free for more than 1 year. Patients with muscular invasion, Grade III tumors or bladder CIS on pathologic examination were excluded from the study. After complete tumor excision, patients were randomized into 2 arms of either no further treatment (control group), or a single immediate instillation of mitomycin C (30 mg). Recurrences were considered to be early if they occurred within the first 2 years of follow-up. At 24-month follow-up the recurrence-free interval was significantly increased, and tumor per year recurrence rates were decreased in the mitomycin C group compared to controls. However, at long-term follow-up these differences were not statistically significant and the recurrence-free interval curves were parallel. Shorter hospital stay and catheterization periods were noted in the mitomycin C group which were also not significant. However, early recurrences were concentrated in the first year in the control but not in the mitomycin C group. A significant relationship between early and late recurrences was found in the mitomycin C but not in the control group. This analysis confirms the positive effect of a single immediate mitomycin C instillation in patients with low-risk superficial bladder cancer. This benefit is limited to early recurrence and is not maintained with long-term follow-up. Results of this study also imply that cell implantation as a mechanism of early recurrence may be controllable with a single mitomycin C instillation (Solsona E, etal, J Urol, Apr 1999;161(4):1120-3).

Weekly instillations of intravesical mitomycin C (40 mg) after TUR were administered at Ospedali Riuniti di Bergamo in Italy, to 242 consecutive patients with superficial bladder cancer at high-risk of recurrence (Ta, Grade I/III, or T1, Grade I/II, primary multiple or recurrent tumor). Tumor-free patients were administered a maintenance course of monthly instillations for 3 months. Overall, during a median follow-up time of 57 (range=10-114) months, the recurrence rate was 4.9%. The progression rate was 5.8% (14/242), with a mean time-to-progression of 34 months. The crude survival rate was 86.4%, disease-specific mortality 2.5%, and non-disease-specific mortality 6.6%. Patients with multiple tumors seemed to bene-

fit the least from this treatment (Hurle R, etal, Urol Int 1998;61(4):220-6).

A pharmacologically optimized intravesical mitomycin C treatment produced statistically significant enhanced efficacy in a prospective, two-arm, randomized, multicenter phase III trial in which patients with histologically proven TCC at high risk for recurrence were randomized to a treatment arm (n=119) involving administration of mitomycin C (40 mg) and pharmacokinetic manipulations to increase drug concentration by decreasing urine volume, and urine alkalinization to stabilize the drug. Patients in the standard-treatment arm (n=111) were administered mitomycin C (20 mg) without any additional intervention. Both regimens were administered weekly for 6 weeks. Dysuria occurred more frequently in the optimized arm but did not lead to more frequent treatment termination. In an intent-to-treat analysis, patients in the optimized arm exhibited a longer median time-to-recurrence (29.1 months) and a greater recurrence-free fraction (41.0%) at 5 years than those in the standard arm (11.8 months and 24.6%, respectively). In the optimized arm, improvements were encountered in all risk groups defined by tumor stage, grade, focality, and recurrence (Au JL, etal, JNCI, 18 Apr 2001;93(8):597-604).

Intravesical instillation of mitomycin C, in combination with ara-C, as a means of preventing recurrent bladder tumors after surgery for superficial TCC of the upper urinary tract, was investigated in a prospective randomized multicenter clinical trial. Patients were randomized into a treatment group involving postoperative intravesical instillation of mitomycin C (20 mg) and ara-C (200 mg) 28 times over a period of 2 years, or a non-instillation group. Of the 27 enrolled patients, 25 (13 with instillation and 12 without instillation) were evaluable, with a median follow-up period of 45 months. Despite a strong trend indicating that bladder tumors recurred less frequently in the treated group, the difference was not statistically significant (Sakamoto N, etal, Int J Urol, May 2001;8(5):212-216).

Valrubicin is an anthracycline related to doxorubicin. In animals, valrubicin is less toxic than doxorubicin on contact with the bladder urothelium, and does not cause cardiotoxicity when administered systemically. Valrubicin, developed by Anthra Pharmaceuticals (Princeton, NJ), was approved in the USA in September 1998. It is currently marketed domestically by Celltech Medeva (Leatherhead, Surrey, UK). Paladin Labs (Montreal, Quebec, Canada) is marketing valrubicin in Canada under the brand name Valtaxin. Valstar is manufactured for Anthra by Ben Venue (Cleveland, OH). Valstar is administered for six weeks at a dose of 800 mg per week. Valstar is available in cartons of 4 or 24 vials. Currently, the AWP of 4 vials of 200 mg each is $1,782 and of 24 vials $10,692. Valstar costs about $11,000 for a 6-course treatment.

In a pilot study (Patterson AL, etal, Urology, 1 Aug 2000;56(2):232-5), intravesical administration of valrubicin was shown to be feasible with the main toxicity being bladder irritation leading to dysuria (77%), hematuria (59%), and urgency/frequency of urination (23%). Based on results from several clinical trials, valrubicin is recommended for patients with CIS who have failed BCG and in whom immediate cystectomy is contraindicated. In a study to identify the expected clinical costs associated with intravesical valrubicin therapy for patients with BCG-refractory CIS, a decision analytic model was used to estimate first- and second-year expected costs for valrubicin therapy at $19,912 and $23,496, respectively (Marchetti A, etal, Clin Ther, Apr 2000;22(4):422-38).

Many other chemotherapeutics administered intravesically have also been investigated. A multicenter randomized phase III clinical trial, conducted by the Japanese Urological Cancer Research Group (JUCRG), evaluated the efficacy of intravesical epirubicin for chemoprophylaxis in the prevention of tumor recurrence, disease-free interval and toxicity post-TUR for superficial bladder cancer. Patients with recurrent bladder cancer or multiple primary tumors were randomized to one of three groups after complete resection of the original tumors. Group A was treated with 17 intravesical instillations of epirubicin (20 mg/40 ml saline) for 1 year after TUR, group B was administered 12 intravesical instillations of epirubicin (30 mg/40 ml) for 7 months, and group C was administered 9 intravesical instillations of epirubicin (40 mg/40 ml) for 4 months. Among 622 enrolled patients with similar disease characteristics, 614 were evaluable. Median non-recurrence periods were 688 days in group A, 1,007 days in group B, and 1,186 days in group C. Incidence of pollakiuria and pain on urination was dose-dependant. Intravesical chemoprophylaxis using epirubicin for 4 months (group C) was effective and feasible in patients with recurrent bladder cancer or multiple primary tumors (Kuroda M, etal, ASCO00, Abs. 1347:342a).

Situs (Solana Beach, CA) has developed the UROS Infusor, designed for the treatment of local and systemic conditions, that can deliver drugs via the bladder over an extended period of time. Situs is in phase I/II clinical testing for delivery of I-OXY, a new formulation of oxybutynin, that is currently administered orally for the treatment of overactive bladder. Other applications include chemotherapeutic agents for bladder cancer, hormones for genitourinary conditions, and analgesics for pain.

IMMUNOTHERAPY

Immunotherapy, using intravesical BCG, interferon α (IFN-α), or interleukin-2 (IL-2), is considered effective in prophylaxis against recurrence, and in the treatment of superficial TCC of the bladder. Systemic immunotherapy, using such biological response modifiers, alone or in combination with chemotherapy, has also been evaluated in clinical trials.

Intravesical Immunotherapy

The most common prophylactic approach in superficial TCC of the bladder is intravesical administration of BCG.

Bacillus Calmette-Guérin (BCG) is a live attenuated form of *Mycobacterium bovis* used in the treatment of superficial bladder cancer and as a vaccine against tuberculosis. The exact mechanism by which BCG produces its antitumor effect is unknown, although it appears that intravesical BCG induces both local and systemic immunomodulatory effects (Elsasser-Beile U, etal, J Urol, Jan 2000;163(1):296-9). The beneficial effect of local instillation of BCG on maintenance of the relapse-free state in superficial bladder cancer may be attributable to local generation of BCG-activated killer cells (Brandau S, etal, Clin Infect Dis, Sep 2000;31 Suppl 3:S94-S100). Using a human *in vitro* system, investigators analyzed the role of NK cells in BCG-induced cellular cytotoxicity. After stimulation of mononuclear cells with BCG for 7 days, these BCG-activated killer cells displayed substantial cytotoxicity against bladder tumor cells. Also, BCG therapy was completely ineffective in NK-deficient beige mice and in mice treated with anti-NK 1.1 monoclonal antibody. These findings suggest a key role for NK cells during BCG immunotherapy (Brandau S, etal, Int J Cancer, 1 Jun 2001;92(5):697-702).

BCG is considered the most active agent in the treatment of superficial bladder cancer, especially for CIS. It has been shown in many clinical trials that BCG is superior to intravesical chemotherapy and to other immune response modifiers and is standard first-line regimen in high-grade superficial TCC. In standard BCG induction therapy for an initial or recurrent episode of superficial bladder cancer, one 120 mg-dose is instilled intravesically in the bladder weekly for 6 weeks. This standard course of intravesical BCG therapy, however, is associated with a high level of toxicity.

Use of BCG for the treatment of superficial bladder cancer was originally described in 1976 (Morales A, J Urol, Aug 1976;116(2):180-3). Since then, multiple studies have confirmed the efficacy of BCG in decreasing tumor recurrences from 83% to 44% and tumor progression from 35% to 7% when compared to TUR alone. A recent study also demonstrated an improved long-term PFS among patients with high-risk T1G3 bladder tumors treated with TUR and BCG therapy, and followed for a minimum of 15 years (Herr HW, Br J Urol, Nov 1997;80(5):762-5; comment in Br J Urol, Oct 1998;82(4):608-9). The median PFS time was 151 months. However, of concern was the finding that 35% of treated patients eventually died of bladder cancer within 15 years. In another study, 78 (25%) of 307 patients with multiple recurrent papillary bladder cancer and CIS, treated with TUR and intravesical BCG, and monitored for a median of 12 years (range=10 to 18), developed upper urinary tract tumors (UTT). Among the 251 men treated in this study, 61 (24%) had tumors detected in the prostatic urethra or ducts (T4). Median times to detection of an UTT or prostatic epithelial tumor were 56 months and 11 months, respectively, and 32% of the UTT and 44% of the T4 relapses were lethal (Herr HW, J Clin Oncol, Mar 1998;16(3):1099-102).

These findings indicate that, when treated conservatively, patients with high-risk superficial bladder tumors are at life-long risk of tumor relapse that involves extravesical mucosa or muscle, and dying from bladder cancer. Therefore, surveillance of all patients with a diagnosis of bladder cancer is mandatory. Evaluation consists of cystoscopy and urine cytology with the frequency of examinations depending on risk. Patients with low-grade and low-stage tumors who fail BCG are candidates for a subsequent treatment with other intravesical agents. These patients have a low risk of progression with multiple recurrences being the major clinical challenge. Among those with high-grade tumors or CIS who fail a first course of BCG, 50% will respond to a second course of BCG. Patients who fail this second course of treatment should undergo cystectomy as they are at a high likelihood (30%-60%) of developing invasive or metastatic disease. IFN-α and valrubicin may be used in patients who decline cystectomy or are not candidates for surgery.

A recent study (protocol ID: SWOG 8507) suggests that a BCG maintenance program consisting of 3 weekly treatments at 3 months, 6 months, and every 6 months thereafter for 3 years, following standard BCG therapy, significantly reduces tumor recurrence and progression compared to induction therapy alone (Lamm DL, Eur Urol 2000;37 Suppl 1:9-15). In this study, additional instillations increased the CR rate in CIS from 68% to 84%, and reduced long-term (7 years) tumor recurrence in high-risk patients from 52% to 25%. In 391 randomized patients, the 86% survival rate at 4 years observed with induction therapy only, was improved to 92% in patients treated with maintenance BCG. Unfortunately, these results were accompanied by Grade 3/4 toxicity in 26% of patients, and only 16% in the maintenance arm were administered the 7 prescribed BCG courses because of dose-limiting toxicity (DLT).

At Loyola University Medical Center (Maywood, IL), researchers have attempted to reduce toxicity secondary to BCG therapy by using a monthly maintenance program administered over one year. In a prospective, non-randomized trial, patients with high risk superficial bladder cancer were treated with monthly instillations of BCG for 12 months following one or two 6-week induction courses of intravesical BCG. Among 37 patients, 28 (75.7%) remained recurrence-free at a median of 40.7 (range=13-101) months, and only one patient progressed to muscle-invasive disease. Only 1/37 (2.7%) patients experienced Grade 3/4 toxicity, a rate dramatically lower than reported in SWOG 8507 (Flanigan RC, etal, Urol. Oncol, 15 Dec 2000;6(1):16-19). Intravesical chemoimmunotherapy using various chemotherapeutics (epirubicin, mitomysin C) and immunotherapeutics (BCG, IFN), administered in various dose and sequence regimens, has also been investigated as prophylaxis in superficial TCC. A randomized phase III clinical trial (protocol ID: EORTC 30993) of sequential chemoimmunotherapy versus immunotherapy alone in CIS is planned to begin in July 2001. Approximately

602 patients will be recruited for this multicenter effort by many urological groups throughout Europe, to be coordinated by A.V. Bono, MD, at Ospedale di Circolo Fondazione Macchi (Varese, Italy).

Many different strains of BCG are available worldwide, all derived from the original Calmette-Guérin strain identified at the Institut Pasteur (Paris, France). However, these strains may differ in their ability to induce an immune response to tuberculin. BCG strains currently available for clinical use in the USA include the Connaught, Armand-Frappier, and Tice strains.

The Connaught strain, supplied as TheraCys Intravesical by Aventis Pasteur (Swiftwater, PA), was transferred from a strain maintained at the University of Montreal in Canada. It was approved in the USA in November 1999, for prophylaxis of primary and recurrent Ta and/or T1 papillary tumors following TUR.

TICE BCG, developed at the University of Illinois (Chicago, IL), is manufactured by Organon Teknika (Boxtel, The Netherlands and Durham, NC), and marketed by Organon (West Orange, NJ). TICE BCG was approved by the FDA in August 1998 for the treatment of recurrent (Stage Ta or T1) papillary bladder carcinoma.

The Armand-Frappier strain is a substrain of the Montreal strain, supplied as Pacis by BioChem Pharma (Laval, Quebec, Canada), formerly IAF BioVac. It was approved in Canada in September 1994 for intravesical treatment of the initial, or a recurrent episode of bladder cancer. It is also indicated for the treatment of papillary tumors of the bladder following surgical resection, and in cases in which the tumors have failed to respond to other treatments. In the USA, Pacis was approved for these indications and for CIS in February 2000, and launched in June 2000 by its USA distributor, UroCor (Oklahoma City, OK). By late October 2000, UroCor's sales of Pacis had reached BioChem Pharma's capacity to supply product, and in February 2001, UroCor and BioChem Pharma agreed to terminate the USA distribution agreement that had been signed in December 1994. Pursuant to the termination, BioChem Pharma paid UroCor $7 million. In July 1999, Paladin Labs entered into an exclusive Canadian distribution agreement with BioChem Pharma for Pacis.

Interferon α (IFN-α), is the most commonly studied interferon in the treatment of superficial bladder cancer. In a randomized control study comparing 10 and 100 million units of IFN-α2b (Intron A; Schering-Plough), administered weekly for 12 weeks and then monthly for 1 year in the treatment of CIS, the CR rate in the high-dose group was 43% and 5% in low-dose group; 6/9 patients (67%) who failed prior BCG therapy experienced a CR with IFN-α. Toxicity was low, and only 17% of patients had flu-like symptoms at the higher IFN dose (Sarosdy H, Anticancer Drugs, May 1992;3 Suppl 1:13-7). In a another clinical trial, 12 patients were treated with a combination of low-dose BCG and IFN-α2b; 4 groups of 3 patients each were treated with IFN-α (10, 30, 60, or 100 million units) and with weekly BCG (60 mg). Although no tumor progressions were observed for up to 12 months after treatment, solitary tumor recurrences occurred in 2 patients. The treatment was safe and well tolerated (Stricker P, etal, Urology, Dec 1996:48(6):957-61, discussion 961-2).

In a recently completed NCI-sponsored multicenter phase II clinical trial (protocol IDs: RPCI-DS-99-07, NCI-G99-1605), patients with superficial TCC of the bladder were treated with intravesical BCG and IFN-α2b. Patients were stratified in 3 groups according to prior BCG exposure and BCG tolerance (no prior BCG exposure versus prior BCG exposure, BCG tolerant versus prior BCG, and IFN-α treatment failure, BCG intolerant). The trial compared the efficacy of BCG plus IFN-α2b, and assessesed the relative local and systemic toxicities of this regimen and its effect on quality of life. Also, an evalution was made regarding the effect of BCG dose reduction during therapy on symptom tolerance and the ability to maintain an extended treatment plan in these patients. Patients were treated by induction therapy consisting of varying strengths of BCG plus IFN-α2b intravesically, weekly, for 6 weeks. Those with disease recurrence which is resectable and/or amenable to intravesical therapy following the first induction course could be tretaed with an additional course of induction therapy. At 3 months, patients underwent evaluatory cystoscopy and cytology. At 4 months, those with no evidence of disease were treated with varying strengths of maintenance therapy consisting of BCG and IFN-α2b, intravesically, weekly, for 3 weeks. Treatment was repeated every 6 months for 3 courses. QoL was assessed within 1 week following the last induction and maintenance treatment and prior to cystoscopy. Patients are followed every 3 months for 6 months, every 6 months for 1.5 years, and then annually thereafter. Approximately 660 pateints were to be accrued over 2.5 years. David A. Corral of Roswell Park Cancer Institute (Buffalo, NY) is the study's Chair.

Interleukin-2 (IL-2) is also being evaluated for intravesical immunotherapy in bladder cancer. Among 27 patients with TCC of the bladder (Stage Ta-T1/Grade II/III), treated with neoadjuvant intravesical instillation of recombinant IL-2 prior to TUR, no effect was observed on the neoplasms but the recurrence rate was lower than expected. It is possible that treatment of bladder carcinoma with intravesical instillation of IL-2 may promote immunoprophylaxis (Grasso M, etal, J Immunother, Mar-Apr 2001;24(2):184-7). An *in situ* intravesical IL-2 gene delivery system for the treatment of high-risk superficial bladder tumors that may obviate the need for tumor procurement and *ex vivo* gene transfer has been evaluated in preclinical trials (Horiguchi Y, etal, Gene Ther, May 2000;7(10):844-51).

Systemic Immunotherapy

The potential for synergy from *in vivo* modulation of cisplatin by IFN-α2b, that has been demonstrated in patients

with advanced melanoma, was explored in patients with advanced TCC treated in a phase II clinical trial (Parnis FX, etal, Am J Clin Oncol, Jun 1997;20(3): 319-21). However, among 20 evaluable patients only 6 were able to complete the full planned six cycles of treatment. There were 7 (35%) PR. Cisplatin in combination with IFN-α2b did not demonstrate any advantage over using cisplatin alone in terms of response rates, duration of responses, and overall survival of patients.

Systemic IL-2 therapy was investigated in the treatment of metastatic urothelial cancer. In a phase II trial, conducted at M. D. Anderson Cancer Center (Houston, TX), patients with measurable refractory metastatic urothelial carcinoma, previously exposure to no more than one cisplatin-containing chemotherapy regimen, were treated with IL-2 (3×10^6 IU) by continuous IV infusion on days 1-4 of each week for 4 weeks. Cycles were repeated every 6 weeks until disease progression. Of 17 patients enrolled in the study, 16 were assessable for toxic effects and 14 were evaluable for response. Grade 3/4 toxic effects included anemia (n=5), thrombocytopenia (n=4), granulocytopenia (n=3), hypotension (n=12), hyperbilirubinemia (n=2), rise in creatinine (n=1), cardiac toxicity (n=1), and bowel perforation (n=1). There was one sudden death that was assumed to be treatment-related. Because of the lack of objective response in this first group of patients, the trial was terminated early. However, despite the lack of objective antitumor activity with IL-2, MST of 16 patients was 45.5 weeks. Further clinical investigation using IL-2 in urothelial carcinoma is under way (Jeri Kim, ASCO00, Abs. 1388:353a).

PHOTODYNAMIC THERAPY (PDT)

Superficial bladder cancer was one of the first indications for photodynamic therapy (PDT), and Photofrin, currently marketed by Axcan Pharma (Mont-Saint-Hilaire, Quebec, Canada), was approved in Canada in 1993 for this indication. Unfortunately, early results as first-line therapy of superficial TCC were disappointing because of tumor recurrence. Subsequently, PDT was approved for several other cancer indications and, today, it is employed by various centers to treat recurrent or refractory superficial urothelial tumors on an experimental basis.

Results from various clinical studies and a small number of sites that offer PDT as an option to patients who are facing cystectomy for superficial bladder cancer that no longer responds to medical treatment, indicate that PDT may have a role in preserving the bladder, at least for a period of time. Investigators at West Virginia University (Morgantown, WV) retrospectively reviewed the cases of 58 patients to assess the long-term role of PDT in the management of resistant superficial TCC, including Ta or T1 of any grade, and refractory CIS of the urinary bladder. All patients had failed at least one course of standard intravesical therapy or were not suitable such therapy. Patients were confirmed to be tumor-free by cystoscopy and cytology after complete TUR, before prophylactic PDT. The PDT protocol involved a single PDT treatment with 2.0 mg/kg or 1.5 mg/kg of Photofrin, and 10-60 J/cm^2 of red light (630 nm). Post-PDT evaluations included weekly telephone contact to assess acute adverse reactions, and assessment of efficacy and bladder toxicity at 3 months and quarterly thereafter. At 3 months, CR rates were 84% and 75% for residual resistant papillary TCC and refractory CIS, respectively; 90% of patients treated prophylactically had not experienced a recurrence. At a median follow-up of 50 (range=9 to 110) months, 59% (34/58) of the responders were alive, with 91.2% (31/34) being disease-free (Nseyo UO, etal, J Clin Laser Med Surg, Feb 1998;16(1):61-8).

In a phase I clinical trial, 20 patients with recurrent superficial TCC of the bladder despite a mean of 2.6 courses of intravesical therapy, were treated with PDT using Photofrin II (1.5 or 2.0 mg/kg). Following 21 treatments, complications included asymptomatic reflux in 4 patients, and bladder contraction and fibrosis in one other patient treated at the highest total light dose. At 3 months follow-up, no tumor was evident by cystoscopy, random biopsy, or urinary cytology in 9 (45%) patients; disease did not recur in 4 patients over periods of 23 months to 56 months. However, 16/20 (80%) patients experienced recurrence, and 8/16 underwent cystectomy (Walther MM, etal, Urology, Aug 1997;50(2):199-206).

PDT may be particularly suitable for the treatment of refractory diffuse superficial bladder cancer. Investigators at George Washington University Medical Center (Washington, DC) believe that patients with this type of tumor should be given the benefit of whole bladder PDT before resorting to cystectomy (Manyak MJ, Tech Urol, Summer 1995;1(2):84-93). Whole bladder PDT may also be effective in patients for whom prior intravesical therapy for CIS has failed. In a study conducted by the Bladder Photofrin Study Group, 36 patients with CIS who had failed at least one course of BCG, were treated with whole bladder PDT using Photofrin (2 mg/kg), as an alternative to cystectomy. At 3 months post-treatment, 58% of the patients experienced CR while treatment failed in 42%. At a mean follow-up of 12 (range=9 to 48) months, disease recurred in 10/21 complete responders for an overall durable response rate of 31%; 14 patients subsequently underwent cystectomy for persistent CIS (n=12) and CIS recurrence (n=2). Among 36 patients treated, 7 experienced bladder contracture (Nseyo UO, etal, J Urol, Jul 1998;160(1):39-44).

Originally developed by QLT (Vancouver, Canada), all commercial rights to Photofrin were sold to Axcan Pharma in May 2000 for about $CDN60 million, including initial cash and deferred payments, equi- ty in Axcan, and future milestone payments. Grupo Ferrer (Barcelona, Spain) distributes Photofrin in Spain, Portugal, and Greece as well as in all Central and South American countries, under a sublicense agreement with Axcan. In August 2000, Diomed (Andover, MA) agreed to supply Axcan with its 630 PDT diode laser and optical delivery fibers for use in conjunction with Photofrin.

HYPERTHERMIA

In clinical trials, microwave-induced local hyperthermia, combined with intravesical mitomycin C, was shown to be a feasible, safe and elective approach for conservative treatment of multifocal and recurrent superficial bladder tumors when other treatment strategies fail, and as a bladder-sparing approach in locally advanced disease. In a preclinical evaluation, potentiation of the action of mitomycin C by hyperthermia was observed that prevented recurrence of superficial bladder tumors, achieving increased efficacy and/or a decreased number of instillations (Mauroy B, etal, Prog Urol, Feb 1999;9(1):69-80).

In 19 patients with multifocal, superficial Grade I/III bladder tumors that recurred after intravesical chemoimmunoprophylaxis, extensive superficial involvement of the bladder walls precluded complete TUR of all tumors, making radical cystectomy the treatment of choice. Microwave-induced hyperthermia and intravesical chemotherapy with mitomycin C was then offered as a debulking approach. Endovesical hyperthermia at 42.5°C to 46°C was delivered using a microwave transurethral applicator that irradiates the bladder filled with a circulating solution of mitomycin C. Patients underwent 8 weekly 1-hour sessions on an outpatient basis without anesthesia. After hyperthermia, TUR of residual tumors and all suspicious areas was feasible and curative in 16 patients (84%). Histology revealed 9 (47%) CR and 7 (37%) PR. Because of extensive residual tumors, radical cystectomy was performed in 3 (16%) patients. At a median 33-month follow-up, 8 superficial TCC recurrences were documented and easily eradicated by TUR, or laser therapy (Colombo R, etal, J Urol, Mar 1998;159(3):783-7).

Thermochemotherapy and administration of mitomycin C using the electromotive drug-assisted (EMDA) approach, was evaluated in 80 patients with single, recurrent, low-stage, low-grade superficial bladder tumors; 36 patients were treated with mitomycin C instillation as a standard procedure, 29 patients were administered mitomycin C in combination with local microwave-induced hyperthermia, and mitomycin C was administered by the EMDA procedure in 15 patients. The treatment was scheduled as a short-term neoadjuvant regimen prior to TUR. All treatments were feasible and safe. Local toxicity induced by thermochemotherapy was more severe than that that with EMDA and standard intravesical chemotherapy, but was always short and self-healing without early or delayed major complications. A higher CR rate on marker lesion was observed after thermochemotherapy compared to other administration methods (Colombo R, etal, Eur Urol, Jan 2001;39(1):95-100).

Unlike thermochemotherapy, hyperthermia combined with RT did not produce lasting results in locally advanced bladder cancer. Hyperthermia was added to RT in a prospective, randomized, multicenter clinical trial conducted at cancer centers in The Netherlands, that between 1990 and 1996, enrolled 358 patients diagnosed with muscle-invasive (T2, T3, or T4, N0, M0) bladder cancer among other pelvic tumors. Patients were randomly assigned to RT (median total dose=65 Gy) alone (n=176) or to RT in combination with hyperthermia (n=182). Although treatment effect did not differ significantly by tumor site, the addition of hyperthermia seemed to be most important for cervical cancer. In bladder cancer, an initial difference in local control disappeared during follow-up (van der Zee J, etal, Lancet, 1 Apr 2000;355(9210):1119-25).

MULTIMODALITY THERAPY

Almost all treatment approaches, in all stages of bladder cancer, involve multimodality regimens, including chemoradiotherapy, chemoimmunotherapy, adjuvant and neoadjuvant chemotherapy, and trimodality approaches as described above. For instance, in superficial TCC and CIS, TUR is often accompanied with intravesical chemotherapy or immunotherapy. In muscle-invasive disease, adjuvant chemotherapy may become the treatment of choice in dealing with occult metastases, and in also preventing cancer spread. In organ-sparing approaches in this setting, TUR may be combined with chemotherapy and RT in a trimodality regimen.

A most promising approach appears to be neoadjuvant chemotherapy. The role of neoadjuvant chemotherapy was questioned in the past because of lack of definitive information concerning efficacy. In a meta-analysis, to evaluate the role of platinum-based chemotherapy administered either before, or during local treatment, individual data on 479 patients from 4 randomized trials was obtained as well as data from one other trial involving 325 patients that was used in a supplementary analysis. Although a newer meta-analysis is planned that will include data not available in this initial effort, based on the data at hand, investigators concluded that there was insufficient information to obtain a definitive answer to the question of whether neoadjuvant cisplatin-based chemotherapy improves the survival of patients with locally advanced bladder cancer (Meta-analysis Group, MRC Clinical Trials Unit, Cochrane Database Syst Rev 2000;(2):CD001426, Scher HI, J Urol, Nov 1995;154(5): 1970, and Advanced Bladder Cancer Overview Collaboration, Br J Urol, Feb 1995;75(2):206-13).

However, results from more recent clinical trials indicate that neoadjuvant chemotherapy may improve survival of patients in whom the probability of occult distant metastases is high, by not delaying systemic treatment with cytostatic drugs, and by enabling bladder preservation in patients who respond well to chemotherapy. Improvement in 3-year survival rates with neoadjuvant chemotherapy, vary between 0% and 11%, but increased DFS strengthens the evidence that this approach improves outcome. Regimens used in the neoadjuvant setting include multimodality approaches involving RT, surgery and various combination chemotherapies.

In a randomized phase III clinical trial (protocol IDs: SWOG 8710, INT-0080) of neoadjuvant MVAC and cystec-

tomy versus cystectomy alone, 3 cycles of MVAC chemotherapy was used prior to cystectomy to improve survival of patients with locally advanced bladder cancer. The trial randomized patients with T2-T4a, N0, or M0 TCC to either MVAC plus cystectomy (n=126) or cystectomy alone (n=125); 48/126 (38%) MVAC-treated cystectomy patients had no pathologic evidence of disease at cystectomy. With a median follow-up time of 7.1 years, 128 patients remained alive with 85 and 94 deaths, respectively, in the MVAC and no-MVAC arms. Survival in the MVAC arm was significantly superior to survival in the no-MVAC arm with a hazard ratio of 0.74 and estimated MST of 6.2 and 3.8 years, respectively. This trial provides strong evidence of a survival benefit for neoadjuvant MVAC in locally advanced bladder cancer (Natale RB, etal, ASCO01, Abs. 3:2a).

A multicenter, multinational prospective randomized trial, initiated in November 1989, compared local radical treatment alone (cystectomy, full-dose EBRT, or preoperative RT and cystectomy) with local radical treatment preceded by three cycles of neoadjuvant chemotherapy in patients with advanced TCC of the bladder (T2/Grade III, T3, or T4a). The trial enrolled 976 patients from 106 institutions in 20 countries who were randomly assigned to combined chemotherapy in addition to RT or surgery or no chemotherapy. The chemotherapy protocol included methotrexate (30 mg/m^2) and vinblastine (4 mg/m^2) IV bolus on day 1, cisplatin (100 mg/m^2) by IV infusion before hydration on day 2, and after hydration, folinic acid (15 mg), oral or IV, every 6 hours (total 4 doses), started 24 hours after methotrexate on day 1, and methotrexate (30 mg/m^2) and vinblastine (4 mg/m^2) IV bolus on day 8, and oral folinic acid (15 mg) every 6 hours (total 4 doses) on day 9, started 24 hours after methotrexate on day 8. This schedule was repeated every 21 days for 3 cycles. The RT protocol involved 2 Gy per day, 5 days per week, for a total dose of 60 Gy to 64 Gy. Serious side-effects from chemotherapy were uncommon but 5 patients (1%) assigned chemotherapy died from toxic effects during treatment. Overall, 518 patients developed metastases or died, for a hazard ratio of 0.79, which demonstrated a 21% decrease in the risk of metastases, or death, with chemotherapy, and an absolute difference in 3-year metastasis-free survival of 8%. Median metastasis-free survival was 32 months for the chemotherapy group and 25 months for controls, a difference of 7 months. There was no evidence of a difference between treatments for locoregional control. This trial of neoadjuvant cisplatin-based chemotherapy for muscle-invasive bladder cancer, the largest reported, was powered to detect a 10% improvement in survival. However, results showed no evidence of a survival benefit, with only a possible 5.5% difference in the 3-year survival between the groups. To reliably confirm this benefit, which was smaller than expected, would require a trial of about 3,500 patients (Lancet, 14 August 1999;354(9178):533-40, and Bartelink H, Lancet, 14 Aug 1999;354(9178):526-7).

A trimodality approach combines TUR, EBRT, and concurrent chemotherapy for bladder preservation in patients with invasive bladder cancer, based on clinical evidence that such a combination could be effective in carefully selected patients who are deemed medically unfit for cystectomy, or as an alternative to radical cystectomy. In this setting, EBRT is used to achieve improved local control, while systemic chemotherapy addresses the issue of micrometastases. However, concurrent systemic chemotherapy and RT may cause acute morbidity. The ideal bladder preservation case involves a Stage T2 tumor, no associated ureteral obstruction, visibly complete TUR, and complete response after induction chemoradiation based on endoscopic evaluation including rebiopsy and cytology (Shipley WU, etal, J Urol, Aug 1999;162(2):445-50; discussion 450-1).

Chemoradiotherapy using paclitaxel/carboplatin for muscle invasive TCC of the bladder is a well-tolerated and effective combination. Among 8 patients treated with RT (39.60-41.40 Gy) to the pelvis, followed by a boost to the initial site of disease, and concomitant paclitaxel (150 mg/m^2) and carboplatin (AUC=7) at 3-week cycles during the RT, none required treatment interruption. With a median follow-up of 27 months, 3 patients remained free of local and distant disease at follow-up intervals of 24, 25, and 31 months. All patients with a visibly complete TUR prior to RT achieved local disease control. For this group of patients, the absolute 2-year pelvic tumor control rate was 57%. The 2-year disease-specific survival was 43%. This regimen may be of particular value in elderly patients or those with renal impairment (Nichols RC Jr, etal, Int J Cancer, 20 Oct 2000;90(5):281-6).

MEETING COVERAGE

GENE THERAPY OF CANCER — PART I

GENE TRANSFER STRATEGIES FROM THE 9TH INTERNATIONAL CONFERENCE ON GENE THERAPY OF CANCER, SPONSORED BY SIDNEY KIMMEL CANCER CENTER, SAN DIEGO, CA, DECEMBER 7-9, 2000

Although initially gene therapy was viewed as an approach to correct single-gene defects in inherited genetic disease, it has been most widely employed in the treatment of cancer. Since the clinical introduction of gene marking technology in 1989 (Wolff JA, etal, PNAS USA, Nov 1989;86(22):9011-4), over 5,000 patients have participated in gene therapy clinical trials worldwide, with more than 70% treated by anticancer regimens. The first clinical use of gene therapy in cancer was initiated in 1991 in patients with melanoma (Gershon D, Nature, 7 Feb 1991; 349(6309):445); since then, researchers and clinicians have sought to modulate disease on several levels, including:

- compensation for losses of tumor suppressor gene activity
- inactivation/modulation of oncogene expression
- direct killing of target cells
- alteration of the malignant phenotype to enhance immunologic recognition and destruction
- suicide gene approaches
- enhancement of drug sensitivity of tumor cells
- bolstering resistance of critical host tissues to chemotherapy

Gene therapy may represent one of the most important developments in oncology with numerous active clinical protocols reported. Yet, although early data from these studies appears promising, with side effects being typically rare and usually mild, nearly 90% of clinical trials are still only in phase I or II; just two reported clinical protocols in the USA are in phase II/III and III stages. According to NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), residing at oncologyknowledgebase.com, more than 157 gene therapy/transfer constructs have been or are being actively pursued in preclinical or clinical trials for the treatment of human cancer.

However, the technical complexities surrounding gene manipulation in human cancer are clearly greater than had been originally anticipated, and significant barriers remain to be overcome before gene therapy achieves widespread clinical application in oncology. This article reviews current directions and recent advances which attempt to overcome these obstacles, presented at the Ninth International Conference on Gene Therapy of Cancer (ICGTC00), that took place under the sponsorship of Sidney Kimmel Cancer Center, in San Diego, CA, on December 7-9, 2000.

Although transgene expression has been demonstrated in patients *in vivo*, a principal reason for the only marginal clinical success achieved so far with gene therapy in cancer has been the difficulty of securing safe, efficient and accurate gene delivery into cancer cells *in vivo*. At present, both viral and nonviral methods of gene transfer are being used *in vivo* as well as *ex vivo/in vitro*; these two approaches have different advantages with regard to transduction and expression efficiency, ease of production, and safety.

VIRAL VECTORS

Viral vectors are created from specific viruses, modified to carry a functional gene to cellular targets. In general, viral vectors have a higher efficiency of gene transfer than nonviral gene delivery methods. The most important viral vectors in clinical development are adenoviruses, adeno-associated viruses, retroviruses, and herpesviruses, although other viral vectors based on lentiviruses or vaccinia virus as well as pseudovirion vectors based on simian virus 40 (SV40), are also being considered for cancer gene therapy applications.

Adenovirus

Adenoviral vectors are probably the most efficient vehicles to deliver long (about 8 kb) segments of genetic information. The adenovirus is a double-stranded DNA virus approximately 36 kb in length, which is capable of infecting both dividing and nondividing cells. The adenovirus life cycle does not require integration into the host cell genome; following receptor-mediated entry into a target cell, the foreign genes encoded by adenovirus vectors are expressed episomally and, therefore, have low genotoxicity to host cells.

Adenoviruses are not associated with any significant pathologies, appearing to be linked to mild forms of disease, and there are no known human malignancies associated with adenovirus infection. To avoid potential problems associated with toxicity and immunogenicity of adenovirus, certain viral genes are commonly deleted, thereby reducing or eliminating expression of adenoviral proteins in the host. Clearly, if the gene is essential to viral replication, the function must be complemented, and this is accomplished by providing a "helper" or "producer" cell line that is transformed with a copy of the deleted viral gene. When the helper cell is infected, the gene produces its essential product, allowing the virus to replicate, but in a cell not transformed, the virus can infect but will not replicate.

According to nm|OK, approximately 44 different adenoviral gene transfer constructs are in preclinical or clinical development by commercial entities. Numerous academic groups are also working in this area. However, none of the adenoviral vectors in current use represent an ideal gene-delivery or gene-therapeutic system. Fundamental to the ultimate success of cancer gene therapy with adenoviral vectors or oncolytic viruses is a better understanding of the factors influencing tumor-specific replication competency, the direction of vector-mediated therapy to tissues associated with a tumor, and engineering of the proteins the vector uses to bind to and enter target cells.

At the New York University School of Medicine (NY, NY), researchers have found that replicating adenoviral vectors can persist in tumor xenografts for up to 100 days after viral infection, but that while cell-to-cell spread may be sufficient to prevent tumor establishment or growth, barriers within established tumors, such as connective tissue and tumor matrix, may limit virus spread to the extent that the vector will be unable to eradicate established tumors (Sauthoff H, etal, ICGTC00, Abs. PD-45:S13). For instance, intravesical adenovirus-mediated gene therapy for superficial bladder cancer has been hampered a glycosaminoglycan (GAG) layer secreted on the luminal surface of the bladder; GAG is a hydrophilic, polyanionic barrier that prevents viruses from adhering to epithelial surfaces. At Canji (San Diego, CA), researchers have identified a polyamide compound, Syn3, that significantly enhances adenoviral-mediated transgene expression in the urothelium, with little or no toxicity to normal tissue (Yamashita M, etal, ICGTC00, Abs. PD-104:36).

In other work to potentially enhance viral spread through established tumors, scientists at Canji, in collaboration with ML Laboratories (St. Albans, Hertfordshire, UK), are investigating the glucose polymer, icodextrin, as a carrier to improve adenovirus-mediated transgene expression when the vector is administered intraperitoneally (Engler H, etal, ICGTC00, Abs. PD-44:S13). Clinical and animal studies have demonstrated significantly increased vector residence time and maintained volume with icodextrin compared to a carrier such as phosphate buffered saline solution (PBS), features that may provide a prolonged and large surface area of exposure to promote adenoviral vector-mediated gene delivery. When compared to PBS, use of an icodextrin formulation as a carrier solution for a recombinant adenovirus encoding beta-galactosidase resulted in increased transgene expression in the peritoneal wall of normal rabbits and tumor-bearing mice after intraperitoneal administration, and increased expression in the peritoneal tumors of mice; transgene expression was low and variable with PBS.

There has been some concern that adenoviral shedding in gene therapy protocols might allow the replication-deficient viral vector to interact with wild-type virus that can provide the missing promoter function in trans. This has raised the issue of potential viral safety in treated patients, their family, and hospital staff. It is for this reason, in France, it is required that patients remain in isolation until virus is undetectable in body fluids. However, in reporting on two consecutive gene therapy trials for lung cancer, Bernard J. Escudier, MD, of the Insitut Gustave Roussy (Villejuif, France), noted that although presence of the adenoviral genome was detectable by PCR for up to 90 days after a single injection of recombinant virus, infectious particles were found in the blood, sputum and stools only during the first two days following injection, and then only in patients treated with the maximum virus dose of 10^9 pfu (Escudier B, etal, ICGTC00, Abs. O-28:S9).

Introgen Therapeutics (Houston, TX), in collaboration with researchers at the University of Texas M. D. Anderson Cancer Center (Houston, TX), generated adenoviral vectors engineered to contain a synthetic inducible promoter that controls at least one essential gene. Specifically, the recombinant adenoviral vector was constructed using human adenovirus 5 (Ad5) in which the essential early genes E1 and E3 are under control of a cytomegalovirus (CMV) promoter. The vectors are propagated in helper cells that express the inducing factor, permitting the virus to replicate to high titer, but lack of the inducing factor in target cells precludes viral replication, avoiding vector toxicity or immunogenicity. The expectation is that this vector approach will provide for higher levels of transgene expression over longer periods of time than achievable with other viral vectors. This technology is the subject of USA patent # 6,110,744 issued in August 2000 to Dr. Bingliang Fang and Dr. Jack A. Roth of M. D. Anderson, and exclusively licensed to Introgen by the University of Texas. Introgen has used this technology to construct adenoviral vectors to deliver the p53 and mda-7 tumor suppressor genes (Roth JA, etal, ICGTC00, Abs. O-1:S1, and Mhashilkar AM, etal, ICGTC00, Abs. O-6:S2).

Cell Genesys (Foster City, CA) is using adenoviral vectors in its GVAX vaccine approach to modify tumor cells *ex vivo* (Maples P, etal, ICGTC00, Abs. PD-93:S28). Adenovirus is rendered replication-deficient through deletion of two lethal early region genes, E1 and E4, that normally transcribe virus early proteins; the E1 or E4 regions are replaced by a transgene or therapeutic gene. A packaging cell line that provides the E1 as well as the E4 gene functions of Ad5 is established by introduction of the full-length Ad5 E4 region into murine 293 cells. To avoid E1A transactivation of the E4 gene expression, the E4 promoter is replaced by the mouse α-inhibin promoter containing a cAMP response element. This cell line is used to generate E1/E4-deleted adenovirus vectors.

Canji, the gene therapy discovery center for the Schering-Plough Research Institute (Kenilworth, NJ), has constructed SCH58500, a recombinant, E1-deleted, replication-deficient Ad5 vector expressing human wildtype p53 tumor suppressor under the control of a cytomegalovirus (CMV) promoter (Buller RE, etal, ICGTC00, Abs. O-3:S1, Schuler M, etal, ICGTC00, Abs. O-4:S1, and Wen SF, etal, ICGTC00, Abs. P-25:S7). In preclinical studies, SCH58500 has shown efficacy against many tumor types with nonfunctional p53; this activity arises from both p53-mediated and adenovirus vector-mediated mechanisms, the latter involving natural killer (NK) cell activity (Nielsen LL, Oncol Rep, Jan-Feb 2000;7(1):151-5).

Genphar (Mt. Pleasant, SC), in collaboration with the Medical University of South Carolina (Charleston, SC), in an effort to develop a replication-defective adenoviral vector having tissue specificity in addition to high transgene expression levels and the ability to regulate such expression, created "double recombinant" Ad5 vectors, deleted in the E1 and E3 regions, in which the transgene and a tissue-specific promoter are under the control of a tetracycline (tet)-regulated gene expression system (Rubinchik S, etal, Gene Ther, May 2000;7(10):875-85, Dong J-Y, etal, ICGTC00, Abs. O-35:S10). The tissue-specific promoter is placed under the control of a tet-transactivator gene and the transgene is placed under the control of a tet-responsive element; the tet-responsive and tet-transactivator elements are built into the opposite ends of the same vector to avoid enhancer interference, and gene expression is regulated by tet or its derivatives in a dose-dependent manner.

Onyx Pharmaceuticals (Richmond, CA), in collaboration with Pfizer, is investigating E1B-deleted adenoviruses for their selective cytolytic effect on p53-deficient cancer cells. Although gene-deleted adenoviruses have been widely

used as transgene-delivery vectors, and have even been engineered for tumor selectivity, gene deletions also reduce the anticancer potency of the viruses themselves. To take advantage of the cytolytic properties of replication-competent adenovirus, adenoviral vectors have been constructed in which partial deletions in the E1 gene and/or tumor-specific transcriptional promoters are used to confer conditional replication competency upon the targeted tumor cells. Onyx's mutants are engineered to be completely defective for the 55 kD protein from the E1B region of Ad5; this protein binds to and inactivates p53, and E1B-deleted adenoviruses are able to replicate in and lyse p53-deficient cells due to a requirement of p53 inactivation for efficient viral replication (Bischoff JR, etal, Science, 18 Oct 1996;274(5286):373-6, Heise C and Kirn DH, J Clin Invest, Apr 2000;105(7):847-51). Onyx's E1B-deleted *dl*1520 (ONYX-015 or CI-1042, Pfizer) adenovirus was the first genetically engineered, replication-selective adenovirus to enter human clinical trials (Neumunaitis J, etal, ICGTC00, Abs. O-27:S8). However, lack of significant single agent efficacy has lead Onyx to evaluate *dl*1520 as a neoadjuvant treatment in combination with radiation therapy and chemotherapy (Khuri FR, etal, Nat Med, Aug 2000;6(8):879-85).

A potential drawback to the use of E1B-deleted adenoviruses as anticancer agents has been the recent observation that constructs of this type are capable of replicating in cells independent of p53 genotype (Harada JN and Berk AJ, J Virol, Jul 1999;73(7):5333-44). The true implications of this finding are unclear, for although these viruses can replicate in cells incorporating either p53 wild-type or mutant genes *in vitro*, normal human cells have been shown to be highly resistant to E1B-deleted adenovirus-mediated, replication-dependent cytolysis and, *in vivo*, the virus demonstrates significantly greater antitumor activity against p53-mutant or –negative tumors than p53-positive tumors (Heise CC, etal, Cancer Gene Ther, Nov-Dec 1999;6(6):499-504 and Rogulski KR, etal, Cancer Res, 1 Mar 2000;60(5):1193-6).

Another approach to producing replication-selective oncolytic adenoviruses has been to introduce deletions into the E1A gene that leave intact its ability to transactivate adenovirus genes but make it incapable of interacting with p300 and CREB-binding protein (CBP), coactivators that influence the activity of a wide variety of transcription factors implicated in cell differentiation, growth and homeostasis, and with retinoblastoma tumor suppressor protein (pRb) family members. Interrupting this interaction prevents E1A from deregulating the cell cycle and forcing cells into S-phase, making the vectors incapable of replicating well in human primary or quiescent cells, but allowing them to replicate in cancer cells, which already have a deregulated cell cycle. Onyx is collaborating with Chiron (Emeryville, CA) on the development of an E1A mutant adenovirus (*dl*922-947) that replicates in and lyses a wide range of cancer cells having abnormalities in cell-cycle checkpoints (Heise C, etal, Nat Med, Oct 2000;6(10):1134-9, Kirn D, ICGTC00, Abs. O-26:S8); this mutant has demonstrated reduced S-phase induction and replication in nonproliferating normal cells, and superior *in vivo* potency relative to the E1B-deleted adenovirus *dl*1520.

Canji scientists, to avoid concerns associated with the host range specificity of E1B-55 kD-deleted adenoviruses while retaining the cytolytic efficacy of a replication-competent virus in a tumor-specific construct, have constructed recombinant Ad5 vectors in which the p53-dependent expression of a fusion protein, consisting of the DNA-binding domain of E2F and the growth suppression domain of pRb, is used to selectively attenuate viral replication in normal cells (Ramachandra M, etal, ICGTC00, Abs. O-30:S9). Lack of E2F-pRb expression in tumor cells having defects in the p53 pathway allows for tumor-specific adenovirus replication and cell lysis. To further enhance virus spread and tumor cell killing, the virus' major late promoter (MLP), that becomes active after the onset of DNA replication, is introduced into the virus' E3 region to drive overexpression of the Ad5-E3 10.5 kD protein. When p53-regulated E2F-pRb and MLP-controlled 10.5 kD are combined, tumor cell/normal cell cytopathicity is increased/decreased relative to E2F-pRb modified adenovirus alone.

St. Louis University School of Medicine (St. Louis, MO) researchers have also constructed E1A-mutant adenoviruses, but have additionally replaced the E4 promoter with a tissue-specific promoter, to produce replication-competent, cancer-specific oncolytic adenoviral agents (Doronin K, etal, ICGTC00, Abs. P-51:S15).

Yale University School of Medicine (New Haven, CT) scientists have created an E1A-mutant recombinant adenoviral vector incorporating the human L-plastin promoter (LP-P). This vector is capable of conferring transgene expression in malignant epithelial cells, which constitutively express LP at high levels, but not in normal tissues, which do not express LP (Zhang L, etal, ICGTC00, Abs. O-31:S9). When compared to an adenoviral vector incorporating a CMV promoter, equivalent reporter transgene expression was observed in ovarian cancer cells transfected with either vector; however, in normal fibroblasts, transgene expression was seen only with the CMV vector. Similarly, reporter gene activity was observed in fresh ascitic ovarian cancer cells after infection with either the LP-P or CMV vector, but transgene activity was only detected in a biopsy specimen of normal peritoneum when the tissues were exposed to the CMV vector.

These results suggest that the transcription of therapeutic genes in cells infected by the LP-P vectors would be restricted to LP-positive ovarian carcinoma cells, avoiding the normal mesothelial cells of the peritoneal cavity, and giving such vectors potential value in the intracavitary treatment of ovarian cancer.

University of Arizona (Tucson, AZ) scientists have developed a technique to amplify transgene expression that may allow production of a new generation of high-level vectors for gene therapy, including adenoviral vectors as well as other viral vectors (Tang TC, etal, ICGTC00, Abs. PD-47:S14). This amplifier strategy consists of a constitu-tive, inducible or tissue-specific promoter controlling the expression of a transcription factor that transactivates a second promoter (or promoters) located on the same construct, with the second promoter(s) driving the gene(s) of interest.

Ontario Cancer Institute and University of Toronto (Toronto, Canada) researchers, to improve targeting of adenoviral vector-mediated transgene expression to tumor cells, have used hyperthermia to take advantage of the strong transcriptional response exhibited by heat-shock genes (Brade AM, etal, ICGTC00, Abs. P-108:40). Heat-shock gene expression is controlled largely by the interaction of heat-shock factor 1 (HSF-1) with specific heat-shock elements (HSE) found in the promoters of heat-inducible genes; when HSE sequences are added to the wild-type heat-shock protein 70 (HSP70) promoter within the context of an adenoviral vector, heat responsiveness is enhanced. Infection of a panel of breast cancer cell lines with an adenoviral vector incorporating a beta-galactosidase reporter gene under the control of an HSE-modified HSP70 gene promoter resulted in 200- to 950-fold increases in reporter gene expression following heating to 43°C for 30 minutes. In contrast, the parental HSP70 promoter provided 50- to 800-fold increases under the same conditions.

GenVec (Gaithersburg, MD) identified and used a conserved receptor binding site on the fibers of human coxsackie and adenovirus receptor (CAR)-binding adenovirus to construct modified Ad5 vectors that bypass CAR requirements (Marini F, etal, ICGTC00, Abs. PD-40:S12, Roelvink PW, etal, ICGTC00, Abs. P-109:41). The development of more target-selective adenoviral vectors requires the ablation of native receptor binding and the addition of tissue-specific ligands. The recently identified CAR represents the primary cellular site of virus attachment during infection (Anders M, etal, ICGTC00, Abs. PD-41:S12, Jee YS, etal, ICGTC00, Abs. PD-42:S12, Srivastava SC, etal, ICGTC00, Abs. PD-43:S13), and has been an obstacle to the construction of targetable adenoviral vectors. Mutation of the Ad5 fiber knob AB loop, and introduction of an HA tag into the HI loop, results in a vector that no longer binds CAR and that can be redirected to a new receptor. However, even in the complete absence of fiber protein, some residual transduction of cell lines is observed. Experimental data has confirmed that continued interaction of the arginine-glycine-aspartic acid (RGD) motif on the viral penton base with cellular alpha V integrins offers a surrogate route of entry into tissues *in vivo*. To better prevent the infection of nontarget tissue, the adenoviral vectors were further modified by removal of the RGD sequence from the penton base protein, resulting in a prototype "doubly ablated" vector that has no affinity for either the fiber CAR receptor or the alpha V integrins.

Adeno-associated Virus (AAV)

Adeno-associated virus (AAV) is a nonpathogenic human parvovirus, 4.7 kb long, containing a "rep" gene, which directs production of the proteins that enable the virus to replicate its genome and integrate into the AAVSI locus of human chromosome 19, and a "cap" gene, which directs production, through differential splicing, of the three proteins that make up the viral genome's protein coat. AAV depends for growth on coinfection with a helper adenovirus or herpesvirus. In contrast to other gene delivery systems, recombinant AAV (rAAV) vectors lack all viral genes, exhibiting the unique ability to be stably maintained both *in vitro* and *in vivo* in dividing as well as non-dividing host cells as an integrated provirus at the AAVS1 locus of human chromosome 19, without immune response or toxicity. Reliable transgene expression using rAAV vectors has been observed in a number of tumor models (Enger PO, etal, ICGTC00, Abs. PD-46:S14).

Retrovirus

Retroviruses are a frequently used vector system in clinical gene therapy protocols, with most retroviral vectors used in humans derived from the Maloney murine leukemia virus (MoMLV). Although retroviral vectors are limited by their ability to infect only dividing cells and only moderate transgene capacity (approximately 8 kb), retroviral cell transduction efficiencies approaching 100% *in vitro* have been demonstrated. Retroviral vectors also integrate stably and permanently into the genome of the infected cell, with persistence of transgene expression observed for over five years in canines (Schuening FG, etal, Blood, 15 Nov 1991;78(10):2568-76, Kiem HP, etal, Hum Gene Ther, Jan 1997;7(1):89-96).

Retroviral-mediated gene transfer has attracted interest for the introduction of transgenes into hematopoietic stem cells. However, MoMLV vectors typically exhibit low transduction efficiencies (0.01% to 0.1%) in these rarely dividing cell populations, especially in large animals and humans (Kiem HP, etal, Curr Opin Oncol, Mar 1995;7(2):107-14).

Vanderbilt University Medical Center (Nashville, TN) scientists have developed HIV-1-based packaging constructs that encode one or more accessory proteins (Vif, Vpr, Vpu) in addition to the HIV-1 Gag/Pol proteins (Schuening FG, ICGTC00, Abs. O-52:S15), to improve transgene expression in hematopoietic stem cells. Virus stocks produced with these packaging constructs can transduce growth-arrested cells, with the HIV-1 LTR promoter producing higher levels of transgene expression than vectors with either simian CMV or SV40 promoters. HIV-1 vectors pseudotyped with the VSV-G envelope were also more efficient than MoMLV vectors for gene transfer into hematopoietic progenitor cells, transducing canine marrow cells even in the absence of cytokine prestimulation.

King's College London (UK) researchers, to address the problem of low titer in retroviral vectors, have developed strategies based on the attachment of retrovirus vectors to particulate substrates that have produced 1,000- to 10,000-fold increases in vector concentration with 100% recovery. An added advantage of magnetic field-directed targeting of host cells is achieved by using paramagnetic beads in the substrate (Gaeken J, etal, ICGTC00, Abs. PD-106:38).

Lentivirus

Lentiviruses represent second-generation retroviral vectors which have demonstrated efficient and long-lasting gene transfer into both dividing and nondividing human cells (Tai C-K, etal, ICGTC00, Abs. P-50:S15). Lentiviruses have the capability to insert approximately 9 kb of genetic information directly into the host cell genome.

Herpes Simplex Virus (HSV)

Herpes simplex virus (HSV) is a double-stranded, linear DNA virus of 152 kb, which has the ability to target nerve cells, HSV's natural host. As a vector for gene therapy, HSV has a transgene capacity of approximately 30 kb. HSV is also naturally cytopathic, and HSV mutants are being evaluated as direct oncolytic agents and adjuvants in conventional therapy (Advani SJ, etal, Gene Ther, Feb 1998;5(2):160-5, Bradley JD, etal, Clin Cancer Res, Jun 1999;5(6):1517-22, Coukos G, etal, Clin Cancer Res, Aug 2000;6(8):3342-53). However, to ensure an acceptable safety profile, HSV requires mutation to eliminate killing of normal, nondividing nerve cells. An example of this is HSV-R3616, a recombinant HSV-1 mutant lacking both copies of the neurovirulence gamma(1)34.5 gene (Chou J, etal, Science, 30 Nov 1990;250(4985):1262-6, Andreansky S, etal, Cancer Res, 15 Apr 1997;57(8):1502-9, Cassady KY, etal, J Virol, Sep 1998;72(9):7005-11).

University of Calgary (Calgary, Alberta, Canada) researchers have found that transformed cells with an activated Ras pathway are particularly sensitive to HSV-R3616 as well as wild-type HSV-1; further investigation has revealed that the Erk (extracellular-regulated protein kinase) pathway, but not the p38 kinase pathway, is involved in this preferential infection (Farassati F, etal, ICGTC00, Abs. O-57:S17). These findings suggest that Ras activity might serve as a potential marker for tumor response in HSV-mediated oncolytic therapy.

Live Poxvirus

Live poxvirus vectors (vaccinia or cowpox virus and fowlpox virus) are also being developed for anticancer gene therapy applications. Live poxvirus is typically genetically engineered to incorporate genes expressing tumor suppressor proteins, such as p53, or tumor-associated proteins recognized by the immune system. These constructs may be administered independently, or as part of a combined regimen in which one antigen- or tumor suppressor-expressing poxvirus vector is delivered in combination with either an immunologically unrelated poxvirus expressing the same antigen, or the same poxvirus vector expressing a different tumor suppressor gene or tumor-associated antigen and/or cytokines, or T-cell co-stimulatory factors, to boost the tumor killing effect. Poxvirus vectors may also be used to deliver genes for cytokines or T-cell stimulatory factors alone.

Loma Linda University School of Medicine (Loma Linda, CA) scientists are studying combination antitumor therapy with recombinant vaccinia viruses (rVV) expressing p53, IL-2, and IL-12 (Fodor I, etal, ICGTC00, Abs. PD-20:S6).

Thomas Jefferson University (Philadelphia, PA) investigators are conducting phase I clinical testing of a fully-replicating rVV vector expressing GM-CSF for intratumoral gene therapy in patients with cutaneous melanoma (Lattime EC and Mastrangelo MJ, ICGTC00, Abs. O-75:S22).

Pseudovirion

Pseudovirion vectors based on the simian virus SV40 have also attracted attention for gene transfer (Kimchi-Sarfaty C, etal, ICGTC00, Abs. PD-49:S14). Pseudovirions incorporate the SV40 capsid but not the virus' large T-transforming antigen region. Infectious particles are produced when the capsid proteins are mixed with supercoiled DNA. In a manner analogous to retroviral vectors, pseudovirion SV40 vectors are capable of infecting a wide range of host cells. Also, exogenous DNA plasmids can be introduced into host cell genomes without destabilizing introns flanking the DNA sequence. However, gene integration is random and vector carrying capacity is limited to approximately 5 kb of genetic material.

NONVIRAL GENE TRANSFER

Nonviral gene transfer is a promising area of development and, for practical purposes, can broadly be divided into three categories:

- delivery of naked DNA by mechanical methods, such as intracellular microinjection or electroporation
- molecular conjugates, which can include complexation of plasmid DNA with proteins/peptides, lipids, synthetic polymers or phage
- carrier encapsulation using liposomes or synthetic microspheres

Mechanical methods of gene transfer are simple but are also typically slow, inefficient, cell damaging, and limited to *ex vivo* applications. Gene transfer techniques relying on molecular conjugations, or carrier encapsulation, generally have higher insertion efficiencies and can be used *in vivo* as well as *in vitro*, although like mechanical techniques, these methods result in random insertion of the transgene. Carrier encapsulation and molecular complexation were the subject of several papers presented at the conference.

Polycationic Polymers

Polycationic polymers have been extensively used for nonviral gene delivery in transfection experiments.

Baylor College of Medicine (Houston, TX) scientists complexed plasmid DNA with branched polyethyleneimine (PEI), a polycationic synthetic polymer, to form a robust vehicle for transfection in the lung by aerosol gene delivery (Densmore CL, etal, Mol Ther, Feb 2000;1(2):180-8, Gautam A, etal, Mol Ther, Jul 2000;2(1):63-70, Densmore CL, etal, ICGTC00, Abs. O-5:S2). Aerosol delivery represents a noninvasive means of targeting gene preparations to pulmonary surfaces for treating lung cancer and a variety of genetic lung disorders. However, rapid degradation of naked DNA, viral vectors and many lipid-based transgene formulations occurs during the process of jet nebulization. Complexation of DNA with PEI yields stability during nebulization and a high level of pulmonary transfection, e.g., some 10- to 100-fold greater than that observed with many cationic lipids. These PEI-DNA formulations also exhibit a high degree of lung tissue specificity, and time-course studies reveal significant levels of reporter gene expression to be detectable after one month following a single treatment.

The manner in which PEI functions during transfection is still unclear. There is general agreement that PEI-DNA complexes move from endocytosis to nuclear entry without significant cellular obstacles, but while some investigators have suggested that lysosomes are the site of action (Lecocq M, etal, Biochem Biophys Res Commun, 19 Nov 2000;278(2):414-8), others have concluded that lysosomal involvement is absent in PEI-mediated transfection (Godbey WT, etal, J Biomed Mater Res, 5 Sep 2000;51(3):321-8).

Valentis (The Woodlands, TX), uses polyvinylpyrrolidone (PVP), an amphiphilic hydrogel, as a polymeric interactive noncondensing (PINC) modifier for bioconjugation of cytokine plasmids to increase cytokine antitumor potency *in vivo* (Mendiratta SK, etal, ICGTC00, Abs. O-79:S23). The small size and hydrophilic nature of PVP conjugates significantly reduces their uptake by macrophages in the liver and spleen, allowing cytotoxic agents or immune modifiers to be targeted to tumors while minimizing the likelihood of toxicity to the reticuloendothelial system.

Supratek Pharma (Laval, Quebec, Canada) has constructed polycations having graft-copolymer architectures by grafting a nonionic soluble polymer, polyethylene oxide (PEO), onto a PEI backbone (Lemieux P, etal, J Drug Target 2000;8(2):91-105). These constructs spontaneously form electrostatic micelles with plasmid DNA, resulting in complexes with a hydrophobic core from neutralized DNA/polycation complex and a hydrophilic shell from the non-ionic soluble polymer component. Although these copolymer-DNA complexes are stable, they have low surface charge and are cleared from the bloodstream relatively quickly; they are also practically inactive in cell transfection studies.

To improve cellular accumulation of these complexes, Supratek scientists have replaced the PEO-corona by two membrane-active amphiphilic block copolymers, pluronic L61 and pluronic F127, also known as poloxamers, to produce a nonionic carrier, SP1017, that has been shown to increase gene expression of plasmid DNA by 10- to 20-fold in skeletal muscle, dermal, and tumor tissues (Lemieux P, etal, Gene Ther, Jun 2000; 7(11):986-91). In addition to improving the efficacy of DNA vaccination by increasing gene expression in target tissues, SP1017-formulated DNA produces a strong immune-mediated anticancer effect compared to nonformulated DNA (Lemieux P, etal, ICGTC00, Abs. O-85:S25).

Cationic Lipids

Cationic lipids can also be formulated to facilitate the entry of DNA through the cell membrane in a nonspecific fashion. Lipid facilitators possess a positive electrostatic charge, naturally complexing with negatively charged DNA; the overall complex has a general affinity for cell membranes, which are also negatively charged. Cationic lipid-mediated gene transfer has been shown to be a safe and effective means of delivering potent immunomodulatory cytokines directly into tumors.

Vical (San Diego, CA) uses cationic lipid-mediated transfer in its Allovectin-7 and Leuvectin plasmid DNA-based immunotherapies for cancer (Hersh EM, etal, ICGTC00, Abs. O-78:S23). Both these formulations are in clinical testing and incorporate the cationic lipid N-(1-(2,3-dimyristyloxypropyl)-N,N-dimethyl-(2-hydroxyethyl) ammonium bromide/dioleoyl phosphatidylethanolamine (DMRIE/DOPE), with Allovectin-7 replacing absent or defective MHC class I molecules, and Leuvectin coding for the IL-2 gene. Both agents have demonstrated excellent safety profiles with broad therapeutic index and no dose-limiting toxicity (DLT).

Genzyme Molecular Oncology (Framingham, MA) has also taken a cationic-lipid mediated gene transfer approach to the inhibition of tumor growth by complexing lipid with noncoding plasmid DNA (pNull), obtaining a tumor-specific cytolytic immune response with this formulation (Siders WM, etal, ICGTC00, Abs. O-86:S26).

Peptide Conjugates

Original research on histidylated polylysine as a synthetic vector for gene transfer (Midoux P and Monsigny M, Bioconjug Chem, May-Jun 1999;10(3):406-11, Pichon C, etal, Nucleic Acids Res, 15 Jan 2000;28(2):504-12) and integrin-mediated gene delivery using RGD-oligolysine cyclic peptides (Harbottle RP, etal, Hum Gene Ther, 1 May 1998;9(7):1037-47) has been conducted at the University of Orleans in France, and the Imperial College School of Medicine at St. Mary's (London, UK), respectively.

Shinshu University School of Medicine (Matsumoto, Japan) has used peptide conjugates for integrin-mediated gene delivery (Aoki Y, etal, ICGTC00, Abs. O-38:S11) by constructing an RGD-histidylated oligolysine gene transfer vector for systemic and repeated administration to patients with advanced solid cancers. The vector incorporates an RGD peptide in a cyclic motif to target alpha V integrin-expressing tumor tissues, and a cationic polymer, polylysine, that forms complexes with plasmid DNA and mediates the transfection of various cell lines in the absence of membrane-disrupting reagents such as chloroquine or fusogenic peptides; the polymer is partially substituted with histidyl residues which become cationic upon protonation of the imidazole groups at slightly acidic pH, and which facilitate the escape of plasmid DNA from endosomes, increasing their nuclear accumulation. Using a luciferase expression plasmid as a reporter, this vector was observed to efficiently transduce alpha V integrin-expressing hepatoma cell lines (PLC and HepG2) and pancreatic cancer cell lines (Hs700T and MIAPaCa-2) *in vitro*. When the vector-plasmid was tested in nude mice bearing PLC and MIAPaCa-2 xenografts, luciferase activity was detected in the tumor tissues, and was significantly higher than in the lung, kidney, and spleen, but only slightly higher than activity in the liver.

Filamentous Bacteriophage

Selective Genetics (San Diego, CA) has adapted filamentous bacteriophage for gene delivery to mammalian cells. Phage-mediated gene transfer offers an alternative to more conventional strategies for gene therapy as phage do not possess a natural tropism for mammalian cells and vectors can be produced at high titer in bacteria. In Selective Genetics' approach, phage libraries are screened to identify functional ligands capable of delivering DNA to cells (Kassner PD, etal, Biochem Biophys Res Commun, 2 Nov 1999;264(3):921-8). Company scientists have engineered phage vectors for targeted EGF transgene delivery by fusing the EGF gene to the coat protein of M13 bacteriophage (Burg MA, etal, ICGTC00, Abs. O-39:S12). Transduction efficiencies of 5% to 10% were obtained with a multivalent phage-plasmid vector in human prostate and lung carcinoma cell lines. Phage contains single-stranded DNA genomes, and because DNA-damaging agents have been shown to increase the transduction efficiency of AAV vectors, particularly in nondividing cells (Russell DW, etal, PNAS USA, 6 Jun 1995;92(12):5719-23), Selective Genetics has investigated the effect of genotoxic treatments on phage-mediated gene transfer. Genotoxic treatment was found to significantly increase not only the percentage of cells transfected but the levels of transgene expression; transduction efficiencies of 30% to 45% were obtained in two carcinoma cell lines in combination with camptothecin, a topoisomerase I inhibitor.

Liposomal Vesicles

Liposomal vesicles or liposomes represent assemblies of lipid molecules into hollow structures that can be used to stably encapsulate DNA molecules. Formulations of liposomes may be varied to alter size as well as charge properties. Also, their limited permeability protects the contents from enzymatic degradation. Liposomes have a good safety profile and can be coupled to various ligands to increase cell-specific targeting. Upon accumulation at the cell surface, liposomes fuse with the plasma membrane or are taken up by endocytosis, resulting in the intracellular delivery of the encapsulated DNA molecules.

Teikyo University School of Medicine (Tokyo, Japan) researchers have found liposomal-mediated plasmid delivery to be superior to recombinant adenoviral vector-mediated plasmid delivery in prolonging the survival of leukemic mice treated with vectors expressing mitochondrial antisense RNA for cytochrome c oxidase (MARCO). MARCO has been shown to induce cell death in various human and murine leukemia cell lines (Shirafuji N, ICGTC00, Abs. PD-12:S4).

Immunoprotective Microcapsules

University of Bergen (Bergen, Norway) scientists used immunoprotective microcapsules for local delivery of an angiostatic protein (endostatin) to experimental gliomas (Read TA, etal, Int J Dev Neurosci, Aug-Oct 1999;17(5-6):653-63, Read T-A, etal, ICGTC00, Abs. O-10:S3). In this approach, cells transfected with the gene for human endostatin are encapsulated in alginate, a semipermeable, immuno-isolating hydrogel extracted from brown seaweed (Klock G, etal, Appl Microbiol Biotechnol, Jan 1994;40(5):638-43), which offers an optimal geometry for transmembrane protein diffusion (Hasse C, etal, World J Surg, Jul 1998;22(7):659-65, and Zimmermann U, etal, Biotechniques, Sep 2000;29(3):564-72,574,576). Morphologic studies have shown that the encapsulated cells proliferated and continued to deliver therapeutic amounts of endostatin for several months following implantation.

Combined Nonviral and Viral Gene Delivery

Genetic Therapy (Gaithersburg, MD), in collaboration with scientists at the University of Southern California (USC; Los Angeles, CA), have used combined nonviral and viral gene delivery techniques in an attempt to secure the advantages of these two approaches in gene transfer while reducing their respective disadvantages (Medvedkin V, etal, ICGTC00, Abs. O-37:S11). These investigators have developed a TAGD (targeted alternative gene delivery) particle through polymeric linkage of synthesized functional units to the surface of virus-derived cores. The integrity of the viral core is maintained, allowing DNA plasmid complexed with the core to efficiently enter host cells and concentrate within the nucleolus. The particle avoids problems associated with viral envelope proteins, which are unstable and cause vector-related toxicities because of nonspecific tropism. At the same time, the particle's surface is modified to contain targeting ligands, fusion proteins, and immuno-decoy functional elements.

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