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Phase I/II trial of intravesical methotrexate for superficial bladder tumors*

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Summary. Twenty-one patients with superficial transitional cell carcinoma of the bladder received a total of 121 doses of intravesical methotrexate (MTX) at 11 different concentrations of drug, ranging from 40 mg/m^2 (mean concentration of $2.9 \times 10^{-3} M$) to 500 mg/m^2 ($4.9 \times 10^{-2} M$). Biochemical evidence of absorption was minimal in all cases. The maximum serum level was observed within 0.5–2 h in all patients and ranged from 1.8×10^{-8} *M* to 5.0×10^{-7} *M*. By 24 h the serum levels were negligible and ranged from 5.5×10^{-9} *M* (the lowest limit detectable by the assay) to 4.4×10^{-8} *M* in the patient who received the highest dosage of 500 mg/m^2. Biologic evidence of absorption was minimal. Myelosuppression, mucositis, and nausea were not observed. Eighteen patients received six consecutive weekly doses ranging from 40 to 500 mg/m^2. All patients had repeat cytoscopy performed within 2–4 weeks after six consecutive doses to evaluate local toxicity and efficacy. Flow cytometry was performed on the bladder washings of 22 patients, illustrating the use of flow cytometry, in conjunction with conventional cytology, as an additional means of objectively quantifying results. Despite MTX's established activity in systemic treatment of advanced bladder carcinoma, this study failed to demonstrate any clinical response to intravesically administered MTX, in doses of up to 500 mg/m^2, and in concentrations of up to $4.9 \times 10^{-2} M$.

Introduction

Superficial bladder tumors, which have a variable biological potential for recurrence and progression to muscle invasion with subsequent metastases, include a spectrum from carcinoma in situ (CIS), to low-grade, low-stage papillary tumors (T_a, T_{1-2}, stages 0–A).[1] Transurethral resection (TUR) often fails to control tumor recurrence; this may be due to implantation of tumor cells at surgery, incomplete resection, or multifocal carcinogenesis. Thus, intravesical chemotherapy and immunotherapy has assumed increasing importance, by virtue of ease of administration, minimal systemic toxicity, and high drug concentrations at the target area. Systemic absorption of chemotherapeutic agents is dependet upon alterations in the urothelial surface and upon the drug's molecular weight, pH, concentration, polarity, and duration of exposure to the bladder mucosa. In both randomized and nonrandomized trials, intravesical chemotherapy or immunotherapy, particularly thiophosphoramide, mitomycin C, epodyl, doxorubicin, and BCG, has been shown to be variably efficacious in eliminating established disease and in preventing tumor recurrence [6, 8, 11].

Methotrexate (MTX) is one of the most active agents against locoregional and metastatic transitional cell carcinoma of the urinary bladder, with an objective response rate (complete and partial remission) of 29% in 236 cases (95% confidence limits 23%–35%) [13]. Although Abbassian and Wallace found no benefit in three patients given intravesical MTX 30 mg for 12 concecutive days followed by 3 mg citrovorum factor IM 1 h after instillation, Hall et al. reported responses in 16/17 patients with recurring multiple superficial carcinoma given oral MTX 50 mg QW for 18 months [1, 5].

Methotrexate is an excellent candidate for intravesical therapy because its high molecular weight (454) and polarity should limit drug absorption through the bladder mucosa, thereby permitting high intravesical concentrations with minimal systemic toxicity. Thus, a Phase I/II study of intravesically administered MTX was undertaken at Memorial Hospital. In addition, thiophosphoramide was given concomitantly with MTX in three cases to evaluate its influence on systemic absorption of MTX.

Materials and methods

Patient entry required a history of recurrent, superficial bladder tumors, Karnofsky Performance Status 50%, WBC 3500 cell/mm^3, platelet count 150000 cells/mm^3, BUN 30 mg/dl, serum creatinine 1.5 mg/dl, and informed consent. Pretreatment evaluation included complete history and physical examination, urinalysis, cystoscopy with endoscopic resection and fulgeraton of all recognized tumors, conventional urinary cytology, and urinary flow cytometry. Two to four weeks after confirmation of patient eligibility, specimens for urinalysis and conventional cytology were obtained by means of a Foley catheter prior to MTX administration.

Flow cytometry was carried out on bladder irrigation specimens also obtained via catheter by rapidly injecting 100 ml NS, using a Tooney syringe. The effluent was con-

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centrated by centrifugation, resuspended in saline at approximately 10^6 cells/ml, and stained with Darzynkiewicz and Traganos two-step acridine orange stain [4, 7]. With this technique, which is carried out on fresh, unfixed specimens within 24 h of collection, nuclear DNA and cellular RNA are differentially stained. The cells in suspension are then made to pass single file through the glass flow channel of the flow cytometer, where they are measured at rates of several hundred cells per second. A beam of blue argon ion laser light is narrowly focused on the glass flow channel and as each cell intersects the beam it emits a fluorescent flash that is separated optically into green and red components, representing dye bound stoichiometrically to DNA and RNA respectively. Five thousand cells per sample are measured, and the measurements recorded, analyzed, and later displayed by computer.

The initial intravesical dose of MTX was 40 mg/m^2/50 ml NS with dose escalation by 40 mg/m^2 until either local toxicity or a serum 24-h level of 1×10^{-5}–1.5×10^{-6} was reached. Two patients per dosage level were treated with intravesical MTX within 2–4 weeks after cystoscopy. Serum MTX levels were measured by a dihydrofolate reductase inhibition procedure prior to therapy and at 0.5, 1, 2, 3, 4, 8, and 24 h post therapy [9].

A Foley catheter was inserted and residual urine for urinalysis and conventional cytology was obtained. After intravesical drug administration, the catheter was clamped. Two hours later, the catheter was released and urine removed for urinalysis, cytology, pH, MTX concentration, and residual volume. The bladder was then lavaged with 100 ml NS for measurement of MTX levels and volume. The bladder was vigorously irrigated with an additional 100 ml NS to obtain a suitable specimen for flow cytometry. Urinalysis and cytology, and in three cases flow cytometry, were obtained again at 24 h.

Patient characteristics in 21 patients included: 16 males, 5 females; median Karnofsky Performance Status 100%, range 50%–100%; median age 69 years, range 37–86. All patients had transitional cell carcinoma (TCC) of the bladder on histological examination after cystoscopic examination. Eight patients had CIS, which was widespread and symptomatic in three patients. Six patients had papillary TCC, three had CIS plus papillary TCC or lamina propria involvement. Three patients were found on review to have muscle-infiltrating disease, and one patient had only cystititis in conjunction with a positive cytology.

Eighteen patients with superficial disease received six consecutive weekly doses ranging from 40 to 500 mg/m^2. Of these patients, seven had no prior treatment, six had had intravesical cytotoxic drugs (thiophosphoramide, mitomycin C, adriamycin, and epodyl) plus BCG, and five had had BCG alone. All patients had repeat cystoscopy performed within 2–4 weeks after six consecutive doses to evaluate local toxicity and efficay. In two cases after initial MTX administration, and in one additional case, MTX 200–240 mg/m^2 was administered in a volume of 30 ml NS with the addition of 60 mg thiophosphoramide in 20 ml sterile water for six doses.

Results

Twenty-one patients received a total of 121 doses of intravesical MTX at 11 different concentrations of drug. The initial dose of 40 mg/m^2 corresponded to a mean concentration of 2.9×10^{-3} *M* in two patients. The final dose given was 500 mg/m^2 or 4.9×10^{-2} *M* (Table 1). Biochemical evidence of absorption was minimal in all cases. The maximum serum level was observed within 0.5–2 h in all patients and ranged from 1.8×10^{-8} *M* to 5.0×10^{-7} *M*. By 24 h the serum levels were negligible and ranged from 5.5×10^{-9} *M* (the lowest limit detectable by the assay) to 4.4×10^{-8} *M* in the patient who received the highest dosage of 500 mg/m^2.

Table 1. Serum levels after intravesical administration of MTX

No. of patients	MTX (mg/m^2)	MTX (M)	Maximum mean serum level, $^1/_2$–2 h (M)	Mean serum level, 24 h (M)
2	40	2.9×10^{-3}	3.7×10^{-8}	5.5×10^{-9}
2	80	5.9×10^{-3}	1.8×10^{-8}	5.6×10^{-9}
2	120	9.2×10^{-3}	1.6×10^{-7}	6.3×10^{-9}
2	160	1.3×10^{-2}	2.3×10^{-7}	7.7×10^{-9}
2	200	1.7×10^{-2}	3.9×10^{-7}	9.6×10^{-9}
2[a]	200	1.4×10^{-2}	7.8×10^{-8}	6.3×10^{-9}
3	240	2.1×10^{-2}	1.1×10^{-7}	6.7×10^{-9}
1[a]	240	2.2×10^{-2}	4.5×10^{-8}	5.5×10^{-9}
2	280	2.3×10^{-2}	2.9×10^{-7}	2.0×10^{-8}
2	320	2.7×10^{-2}	1.2×10^{-7}	1.0×10^{-8}
2	360	2.9×10^{-2}	1.3×10^{-7}	9.5×10^{-9}
2	400	3.4×10^{-2}	1.3×10^{-7}	1.8×10^{-8}
1	500	4.9×10^{-2}	5.0×10^{-7}	4.4×10^{-8}

[a] MTX dose plus 60 mg thiophosphoramide

Biological evidence of absorption was also minimal. Myelosuppression, mucositis, and nausea were not observed. One patient who received MTX in a concentration of 4.9×10^{-2} *M* had an improvement of symptoms secondary to rheumatoid arthritis while on weekly intravesical therapy. Arthritic symptoms worsened when therapy was discontinued and were subsequently alleviated with low-dose oral MTX. Absorption of thiophosphoramide probably was responsible for thrombocytopenia (platelets 84000 cells/mm^3) in one of three patients given the two drugs concomitantly for 6 weeks.

Irritative symptoms occured in four patients without evidence of local reaction by urinalysis at 2 or 24 h. There was an increase in local symptoms in one of the three patients who received multiple doses of the combination of MTX and thiophosphoramide.

Flow cytometry was performed on the bladder washings of 22 patients, with adequate specimens in 18. Seven of the 18 had distinct aneuploid peaks prior to MTX therapy, and six others had more than 15% hyperdiploid cells without a distinct peak; by these criteria, 13 of the 18 had flow cytrometry evidence of carcinoma. Cytology was postive in six of the seven with aneuploid peaks and in one of the patients with an increase in hyperdiploid cells (Table 2). At 2 h post MTX therapy, there were changes in DNA distribution in at least five cases (5, 10, 15, 21, 22), but the changes were not consistent and they were believed due to sampling differences. The modal value for RNA was either increased (11 cases) or unchanged (six cases) at 2 h, and flow cytometry was normal in three of the six cases with unchanged RNA. At 24 h, in two of three cases examined there was evidence of unbalanced cell growth, with an increase in RNA content unmatched by DNA synthesis

Table 2. Flow cytometry and cytology results after intravesical administration of MTX

Patient no.	MTX (mg/m^2)	Flow cytometry			Cytology		
		DNA % hyperdiploid (peak)		RNA			
		0 h	2 h	2 h	0 h	2 h	24 h
1.	40	33	33	I	−	−	−
2.	40	60 (2.6 C)	72 (2.6 C)	I	+	+	+
3.	80	29	20	NC	−	−	−
4.	80	42	21	I	−	−	−
5.	120	39 (3.9/6.0 C)	72 (3.9/6.0 C)	I	−	+	+
6.	120	(−)	(−)	(−)	+	+	+
7.	160	30 (4.0 C)	20 (3.9 C)	I	+	−	−
8.	160	56.5 (3.7 C)	71 (3.7 C)	I	+	−	−
9.	200	16	13	I	−	−	−
10.	200	19 (3.3 C)	54 (3.7)	I	+	+	+
11.	240	1.6	1.4	NC	−	−	+
12.	240	12	8	NC	−	−	−
13.	240	7.4	5.6	NC	−	−	−
14.	280	24 (2.9 C)	24.7 (3 C)	I	+	+	−
15.	280	2.6	28.6 (3.7 C)	NC	−	−	+
16.	320	2.0	2.0	I	+	+	+
17.	320	(−)	(−)	(−)	−	−	−
18.	360	17	7	NC	−	−	−
19.	360	(−)	(−)	(−)	−	−	−
20.	400	(−)	(−)	(−)	−	−	+
21.	400	20	2.8	I	−	−	−
22.	500	29 (4.3 C)	66 (4.0 C)	I	−	+	+

Abnormal flow cytometry is defined by the presence of an aneuploid DNA peak greater than 15% hyperdiploid cells. DNA peak of benign epithelium is diploid, or 2c. Aneuploid DNA values in parenthesis are relative to 2.0 C diploid. I, increased; NC, no change; (−), inadequate or no specimen; −, negative for malignant cells; +, positive for malignant cells

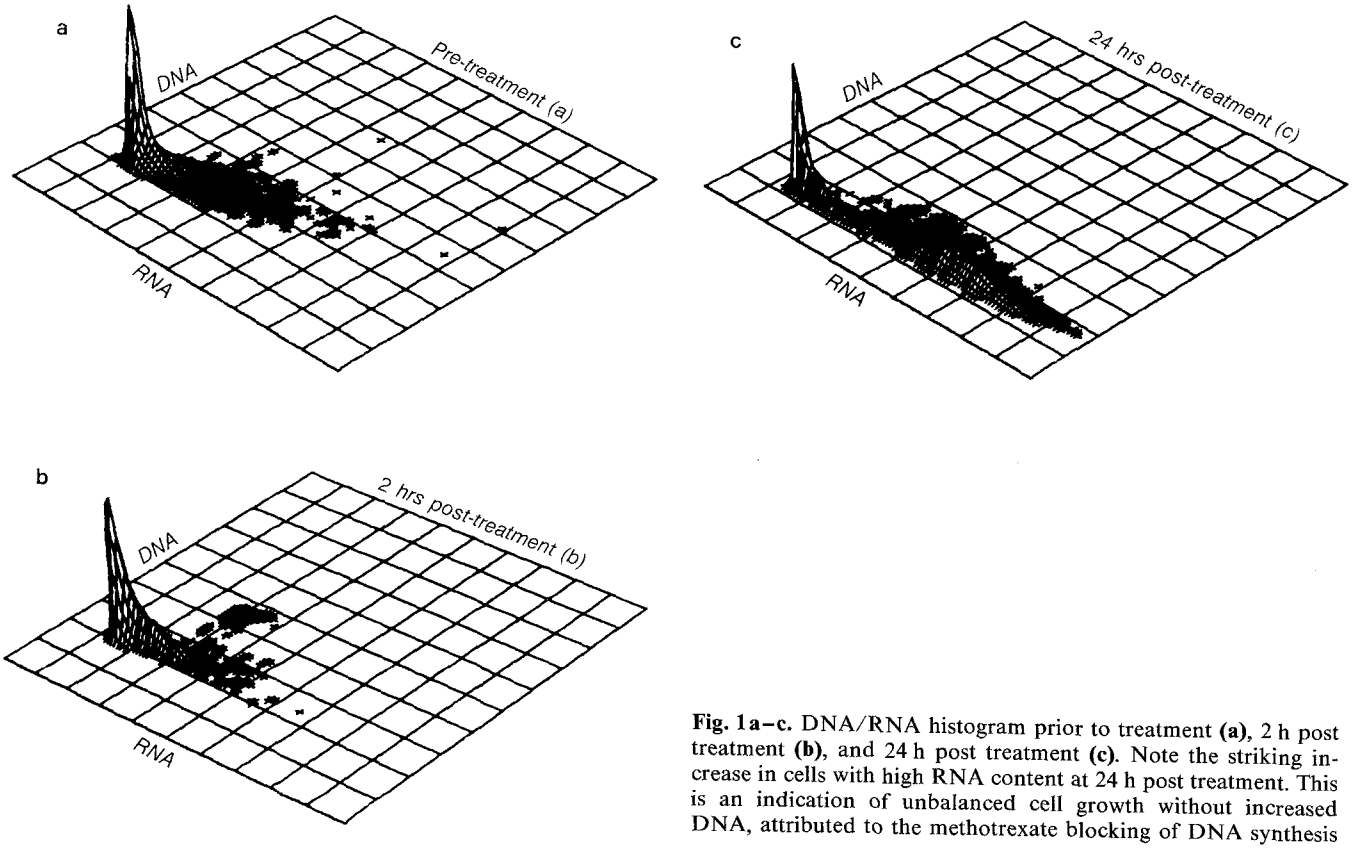

Fig. 1a–c. DNA/RNA histogram prior to treatment **(a)**, 2 h post treatment **(b)**, and 24 h post treatment **(c)**. Note the striking increase in cells with high RNA content at 24 h post treatment. This is an indication of unbalanced cell growth without increased DNA, attributed to the methotrexate blocking of DNA synthesis

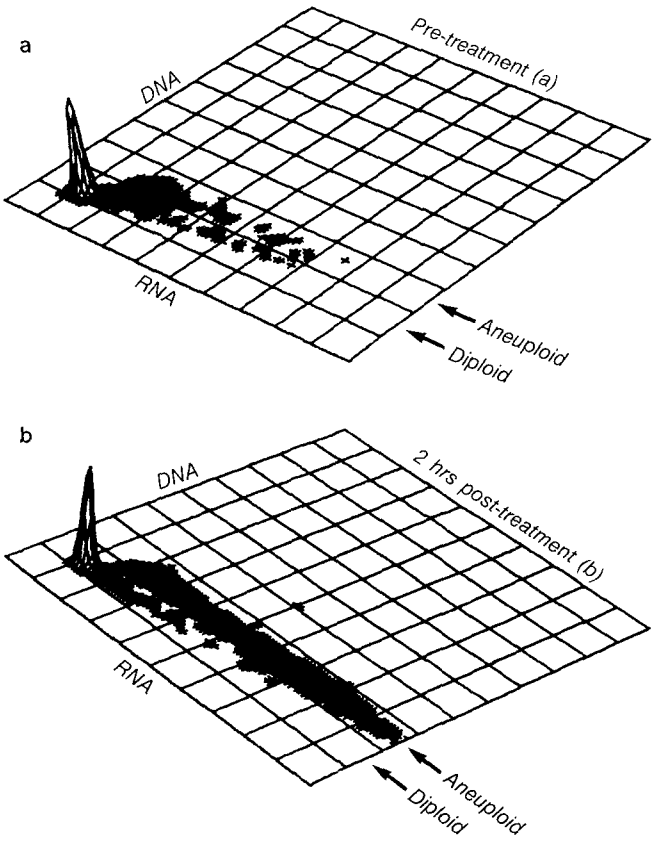

Fig. 2a, b. DNA/RNA histogram in this case shows a marked increase in the number of aneuploid cells with high RNA content at 2 h post treatment **(b)**, compared with prior to treatment **(a)**. While this could be due in part to sampling variation, the fact that no such increase in RNA content of diploid cells is seen mitigates against sampling variation as the sole explanation

(Fig. 1, 2). In one patient, DNA synthesis appeared partially blocked at G_2M, and in the other, in early S phase.

No concistent change or drug effect was noted by conventional cytological examinations at 2 and 24 h. Despite atraumatic catheterizations, seven urinalysis specimens had from 15 to 50 red blood cells/hpf; resolution of microscopic hematuria occurred in six of the 24-h specimens. A slight increase in the number of white blood cells was noted in two urines at 2 h and in five at 24 h. In the 19 courses of weekly intravesical MTX, no significant antitumor activity was demonstrated cystoscopically, even at concentrations of 4.9×10^{-2} *M*. At repeat cystoscopy within 2–4 weeks after six consecutive doses, all 18 patients with CIS, papillary TCC, or the combination of CIS and TCC had exactly the same pathology as reported prior to institution of methotrexate. Only one patient, who had diffuse CIS, and received 160 mg/m^2, was thought initially to demonstrate some improvement and underwent a second course of therapy. No antitumor activity was observed with the combination of intravesical MTX and thiophosphoramide.

Discussion

Despite the established activity of MTX in systemic treatment of advanced bladder carcinoma, this study failed to demonstrate any clinical response to intravesically administered MTX in concentrations of up to 4.9×10^{-2} *M* and doses of up to 500 mg/m^2. Without any demonstration of efficacy, and the potential cost of weekly administration of higher doses, dose escalation above 500 mg/m^2 was not considered feasible. Minimal systemic absorption was observed in one patient who had improvement of arthritic symptoms, further corroborating recent reports of benefit from low-dose MTX treatment in refractory rheumatoid arthritis [3, 12].

In the initial observations of Hall et al. [5], oral MTX was used as primary cytotoxic treatment of superficial bladder tumors, with 5 of 14 patients attaining reduction in the total number and size of tumors by more than 50%. Irrespective of the response to MTX at cystoscopy, bladders were rendered tumor-free by transurethral resection, which was repeated after a few weeks in an unspecified number of cases to ensure absence of tumor. Patients were then maintained on oral MTX for 18 months to assess its value as a prophylactic agent against recurrence. For the 12-month period prior to MTX, 17 patients underwent 63 cystoscopies, with 61 revealing tumor. After MTX, 36 cystoscopies found tumor in 21 cases; 15 were free of tumor. Although serum MTX levels were not obtained, urinary concentrations, measured in 12 patients, exceeded 1×10^{-6} *M* for 24 h after oral administration. At 4 h levels of 5×10^{-5} were obtained, and at 8 h 3×10^{-5}, with a mean of 41% (range 10%–79%) of the oral dose retrieved in the urine. Although the majority of drug was excreted in the urine within 8 h, the longer exposure of the bladder mucosa to drug achieved with oral administration may account for differences in efficacy.

Smith et al. gave MTX intravesically in concentrations as high as 1×10^{-2} *M*. Efficacy when used prophylactically was noted in 11 of 35 patients; the authors did note that most patients had a short history of bladder tumors. However, in 10 of 14 courses of definitive therapy (tumor was not resected), tumors were unchanged or worse. Some absorption was noted: plasma levels rose above 0.1 μmol/l, the highest being 0.58 μmol/l, in only 9/40 instillations as measured by an EMIT method [10]. The authors did not recommend intravesical use of MTX for the therapy of superficial bladder cancer.

The present study illustrates the use of flow cytometry, in conjunction with conventional cytology, as an additional means of objectively quantifying results. There was a suggestion of unbalanced cell growth in bladder epithelial cells as early as 2 h after treatment, manifested by a disproportionate increase in cellular RNA. However, this may be explained by variations in sampling or by effects of the lavage technique itself, which may dislodge the most fragile, superficial cells in the first irrigation, these being the cells most likely to be stripped of cytoplasm [4, 7]. In the few cases evaluated at 24 h, a block in DNA synthesis was suggested, consistent with MTX inhibition of thymidine synthesis. No consistent change in cytology was demonstrated to indicate an MTX effect.

Folic acid antagonists are extremely attractive antitumor agents because of the critical role in folate metabolism in the synthesis of DNA precursors. Limitations in their use arise due to poor membrane permeability and the need for carrier-mediated active transport. 10-Deaza-aminopterin, an analog with more selective transport into tumor cells than MTX, when administered systemically, produced objective tumor regression in three patients with

advanced measurable urothelial carcinoma who had initially responded and subsequently failed to respond to MTX [2]. Lipid-soluble antifolates not requiring active transport, such as BW 301U and trimetrexate, and other antifolates which use selective transport systems offer promise in circumventing resistance. Such agents also need to be evaluated for intravesical therapy for superficial bladder cancer.

References

1. Abbassian A, Wallace DM (1966) Intracavitary chemotherapy of diffuse non-infiltrating papillary carcinoma of the bladder. J Urol 86: 461–465
2. Ahmed T, Yagoda A, Scher HI, Sternberg CN, Watson RC (1986) Phase II trial of 10 deaza-aminopterin in patients with bladder cancer. Invest New Drugs 4: 171–174
3. Anderson PA, West SG, O'Dell Jr, Via CS, Claypool RG, Kotzin BL (1985) Weekly pulse methotrexate in rheumatoid arthritis: clinical and immunologic effects in a randomized double-blind study. Ann Intern Med 103: 489–496
4. Coico-Staiano L, Huffman J, Wolf R, Pinsky CM, Herr HW, Whitmore WF, Jr, Oettgen HF, Darzyniewicz Z, Melamed MR (1985) Monitoring intravesical bacillus Calmette-Guerin treatment of bladder carcinoma with flow cytometry. J Urol 133: 786–788
5. Hall RR, Herring DW, McGill AC, Gibb I (1981) Oral methotrexate for multiple superficial bladder carcinomata. Cancer Treat Rep 65 [Suppl 1]: 175–178
6. Herr HW, Pinsky CM, Whitmore WF, Jr, Oettingen HF, Melamed MR (1983) Effect of intravesical bacillus Calmette-Guerin (BCG) on carcinoma-in-situ of the bladder. Cancer 51: 1323–1326
7. Klein FA, Herr HW, Sogani PC, Whitmore WF, Jr, Melamed MR (1982) Detection and follow-up of carcinoma of the urinary bladder by flow cytometry. Cancer 50: 389–395
8. Scher HI, Sternberg CN (1985) Chemotherapy of genitourinary malignancy. Semin Urol 3 (4): 239–280
9. Schwartz MK, Mehta B, Fleisher M (1980) The application of centrifugal analysis to methotrexate determination compared to existing methodology. Clin Chem 26: 969
10. Smith G, Theodoorou C, Field G, Hargreave TB, Chisholm CD (1984) Intravesical methotrexate in the treatment of superficial bladder cancer. Br J Urol 56: 663–667
11. Soloway MS (1980) Rationale for intensive intravesical chemotherapy for superficial bladder cancer. J Urol 123: 461–466
12. Weinblatt ME, Coblyn JS, Fox DA, Fraser PA, Holdsworth DE, Glass DN, Trentham DE (1985) Efficacy of low-dose methotrexate in rheumatoid arthritis. New Engl J Med 312: 818–822
13. Yagoda A (1983) Chemotherapy for advanced urothelial cancer. Semin Urol 1: 60–74

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