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Chemotherapy Resistant Transitional Cell Carcinoma as a Target for Chemoprevention

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Abstract α-Interferon combined with 5-fluorouracil results in significant antitumoral activity in metastatic bladder carcinoma refractory to standard MVAC chemotherapy.

As a single agent, α-interferon is ineffective for invasive or metastatic disease, but appears to contribute to the increased response rate of patients with invasive chemotherapy-refractory disease. Although most patients with superficial bladder carcinoma will not develop invasive disease, patients in complete remission from invasive disease are at high risk for relapse.

In vitro assays indicate that fenretinide (4-HPR), α-interferon, and 5-fluorouracil possess significant antitumoral activity in human transitional cell carcinoma (TCC) lines. Some features of postchemotherapy-refractory TCC are similar to those of initial superficial disease (sensitivity to biological therapy). The biological study of patients with residual postchemotherapy disease may permit the development of strategies which will prevent the recurrence of malignancy within the bladder following an initial complete remission, in addition to developing strategies for the selection and treatment of patients with high risk superficial disease. © 1992 Wiley-Liss, Inc.

Key words: bladder carcinoma, chemoprevention, chemotherapy

Transitional cell carcinoma (TCC) of the bladder has been widely studied as a target for chemoprevention. The study of superficial bladder carcinoma as a target for chemoprevention is based on the belief that effective therapy for superficial disease will reduce the frequency of invasive TCC and result in improved survival. Because of the long natural history of superficial TCC and the accessibility of the organ for repeated inspection, this seems both logical and feasible. A minority of patients with invasive TCC will have a previous history of superficial disease. Almost half of patients with TCC will initially present with invasive cancer. In addition, only a small portion of patients with invasive TCC have a history of previously treated superficial TCC. Therefore, the beneficial impact of an effective chemoprevention strategy for superficial TCC will be on morbidity rather than survival. Our experience mirrors previously reported results. In three consecutive trials for the treatment of invasive bladder carcinoma, only 7% of patients had a history of superficial disease. Although a chemoprevention trial directed toward *de novo* superficial disease will have only a modest effect on survival, the chemoprevention of persistent superficial disease following therapy for invasive cancer may result in a greater impact.

Combination chemotherapy for invasive or metastatic urothelial tumors is effective in achieving a high rate of response. While cytotoxic agents have significant antitumor activity in invasive disease, they are less effective in superficial disease. Although intravesical chemotherapies influence the time to recurrence, they do not influence the frequency of progression to invasive disease [1]. In contrast to the lack of efficacy of intravesical chemotherapy, Bacillus Calmette-Guerin (BCG) may result in a significant reduction in the frequency of progression to invasive disease [2,3]. The mechanism of action of intravesical BCG is thought to be immune-mediated. Other biologic response modifiers [interleukin-2 (IL-2), α-interferon

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(α-IFN)] achieve responses when delivered intravesically for superficial disease [4,5].

The use of intravesical BCG does not result in responses in co-existent invasive disease or metastatic sites [6]. While α-IFN possesses antitumor activity in patients with superficial disease when given intravesically, it is ineffective in the treatment of patients with invasive disease when delivered systemically [7].

A phase II clinical trial was performed at the University of Texas M.D. Anderson Cancer Center (UTMDACC) using IL-2 in patients with chemotherapy-refractory and metastatic urothelial tumors. Fourteen patients were treated, nine had cystectomies, and all progressed following methotrexate and cisplatin-based combination chemotherapy. None of the metastatic sites responded to IL-2, but disappearance of the co-existent superficial disease occurred even as the metastatic sites progressed in two of five patients who had persistent superficial disease at the time of therapy with systemic IL-2.

These data imply that the superficial phenotype of bladder cancer is characterized by a differential sensitivity to biologic response modifiers and a relative resistance to cytotoxic chemotherapy, while progression to the invasive phenotype disease results in sensitivity to cytotoxic chemotherapy and resistance to biologic agents. This potential similarity in the response phenotype of superficial disease and persistent TCC following chemotherapy has led us to search for other similarities. We hypothesize that the study of tumors which are persistent following chemotherapy may result in the development of predictive variables and therapy for patients with *de novo* superficial disease in addition to strategies for the treatment of chemotherapy-resistant disease. The validity of such an approach will be based on the ability to confirm the existence of the hypothesized similarities in the two categories. This manuscript will outline the results of treatment for, and characteristics of, chemotherapy-refractory TCC which supports the existence of such similarities.

Recent clinical trials at the UTMDACC have evaluated the combination of 5-fluorouracil (5-FU) and α-IFN in the therapy of bladder cancer in various schedules and in combination with 13-*cis*-retinoic acid (13-CRA). In a series of clinical trials, we were able to confirm the existence of significant antitumor activity with this combination when compared to a combination regimen using 5-FU with mitomycin-C (MMC). In the 5-FU and MMC study, the 5-FU was optimally delivered (continuous infusion at 21 day intervals). Despite the use of a higher dose of 5-FU in that study compared to the studies combining 5-FU and α-IFN (Table I), the response rate was significantly lower (Table II). We concluded that optimally delivered 5-FU is an ineffective agent in chemotherapy of refractory bladder cancer.

The addition of 13-CRA with α-IFN and 5-FU in a phase I trial failed to result in an obvious benefit. The results of that trial need to be interpreted critically in view of the three dosage levels used and the small numbers of patients in the study.

In vitro studies performed in the Section of Genitourinary Oncology evaluated the contribution of each of the studied drugs to the antiproliferative effect of therapy. The studies were performed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay [5] against a variety of established human TCC lines. The tumor lines studied span the spectrum of disease from high grade (T24) to the low grade papillary TCC (Table III).

These studies confirmed the value of adding α-IFN to 5-FU. The addition of α-IFN resulted in a 50% reduction in proliferation (IC50) with a reduced dose of 5-FU. Fenretinide (4-HPR) is a synthetic retinol which may possess unique antitumoral activity in TCC and breast cancer. 4-HPR also has a more favorable toxicity profile than 13-CRA. The IC50 of both 5-FU and 4-HPR) in each of the tumor lines studied was within the therapeutically achievable dose (Fig. 1). The individual cytotoxicity of α-IFN and 13-CRA was minimal. 4-HPR was the agent that had the most favorable influence on the IC50 of 5-FU (data not shown).

Based on these clinical and preclinical data, we believe the study of combinations of biological and cytotoxic agents may be of therapeutic benefit in TCC. Although our initial clinical experience with retinoid combinations failed to identify an obvious benefit with the addition of 13-CRA, this does not preclude benefit from the use of other retinoids in combination. The preclinical trial predicted the relative ineffectiveness of 13-CRA compared to 4-HPR. In addi-

TABLE I. Chemotherapy Regimens

Regimen	Agent	Dosage Schedule
Chemotherapy A:	5-FU	1,000 mg/m^2 BSA ci day × 5 repeated every 21 days.
	MMC	14 mg/m^2 BSA iv × 1 repeated at 36 days.
Chemotherapy B:	5-FU	750 mg/m^2 BSA ci day × 5 repeated every 36 days.
	5-FU	Bolus 750 mg/m^2 weekly.
	α-IFN	9 mIU sq daily × 5 (day 1 & day 36) repeated M-W-F.
Chemotherapy C:	5-FU	500 mg/m^2 ci day × 5 repeated every 28 days.
	α-IFN	5 mIU/m^2 sq daily × 5 (day 1 & day 28) repeated every M-W-F for 21 days.
	13-CRA	10 mg/m^2 po q daily.

BSA: body surface area
ci: continuous infusion
mIU: milli International Units

TABLE II. Response Rates Per Regimen*

Response Category	Chemo A	Chemo B	Chemo C
P.R.	1 (6%)	9 (30%)	8 (27%)
C.R.	0 (0%)	0 (0%)	0 (0%)
P.D.	16 (94%)	21 (70%)	22 (73%)
Total	17	30	30

* **Chemo A:** 5-FU+ MMC; **Chemo B:** 5-FU + α-IFN; **Chemo C:** 5-FU + α-IFN + 13-CRA; **P.D.** = Progressive Disease; **P.R.** = Partial Response; **C.R.** = Complete Response

TABLE III. Median Effect Dose (IC50) for 5-FU, α-IFN, 13-CRA, and 4-HPR on T24 and RT4 Cell Lines

Agent	T24	RT4
5-FU	1.72 ± 0.12 μg/ml	2.07 ± 0.26 μg/ml
α-IFN	9683 ± 1094 IU/ml	3420 ± 631 IU/ml
13-CRA	65.52 ± 2.26 μg/ml	205.01 ± 18.5 μg/ml
4-HPR	3.65 ± 0.10 μg/ml	3.96 ± 0.21 μg/ml

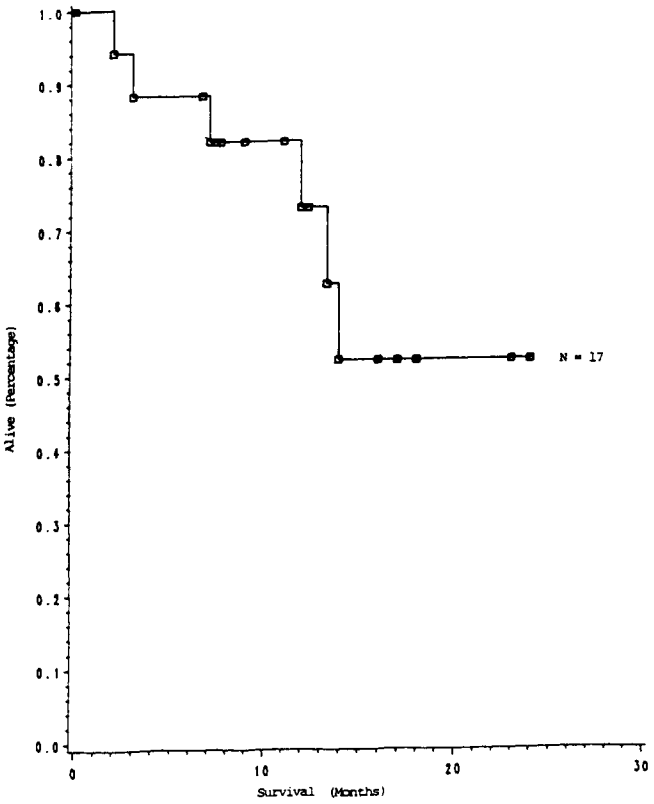

Fig. 1. Median survival of responding patients treated with 5-FU + α-IFN combination regimens.

tion, the reported minimal toxicity with 4-HPR makes it a more attractive retinoid for combination therapy. Based on existing epidemiologic data, the successful reduction in the frequency of second primary urothelial tumors following cytotoxic therapy will benefit more patients than the chemoprevention of *de novo* superficial disease.

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