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Polyvalent Tumor Vaccines and Malignant Melanoma

By Perry Shen, MD, and Donald Morton, MD

In recent years, increased understanding of the immunobiology of tumors has renewed interest in an immunotherapeutic approach to malignancies, especially melanoma. Melanoma is considered an immunogenic tumor; the well-documented phenomenon of spontaneous regression in cutaneous malignant melanoma is thought to be due to anti-melanoma antibodies against tumor antigens. Laboratory investigators have found a significantly higher serum titer of anti-melanoma antibodies in patients with localized disease and in those who have experienced spontaneous regression than in patients with metastatic melanoma. Additionally, cytotoxic T-lymphocytes, which have demonstrated in-vitro tumor cell killing, have been isolated from melanoma patients. These findings seem to indicate that melanoma tumors express tumor-associated antigens (TAAs) that can serve as targets for immunotherapy.[1] In addition, unlike other solid tumors such as those found in the breast or colon, there are no standard forms of adjuvant therapy that have proved effective against melanoma, thus providing more impetus for alternative treatments.

Theoretical Advantages of Polyvalent Vaccines

Polyvalent vaccines can be allogeneic or autologous. Some of the problems with preparations developed from the patient's own (autologous) cells are the limited number of TAAs, tolerance of the patient to these TAAs, and the logistical difficulty and expense of harvesting tumor cells and preparing a customized vaccine for each patient. By contrast, an allogeneic vaccine is not patient-specific. Instead, it can be produced from selected cells lines that are known to express multiple TAAs and a broad range of human leukocyte antigen (HLA) expression. Because polyvalent vaccines possess foreign HLA antigens, they induce a stronger immune response to cross-reacting TAAs than do autologous cells.[2-4] Moreover, the same polyvalent vaccine can be used to treat different individuals; thus, its manufacturing methods and quality control can be standardized, reducing its cost.

Some allogeneic vaccines may consist only of a purified TAA. Unfortunately, preparations with a limited profile of purified TAA also have a limited number of potential immune targets. Because of

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the variable expression of each tumor antigen in melanoma, each patient's melanoma and HLA type must be matched to antigens in the vaccine. This is complicated by the fact that a cancer cell is genetically unstable and becomes heterogenous in antigen expression over time. Thus, tumor clones can escape an immune attack that targets only a few, specific TAAs. By contrast, a broad-spectrum polyvalent vaccine will generate a cross-reacting immune response with nearly all melanomas.[2,5,6]

Whole Cell Vaccine

Animal studies have shown that whole cells induce a stronger cytotoxic cellular and humoral immune response than do tumor cell lysates or shed antigens. Whole cells may also be able to directly present their tumor cells to host T-cells if there is a defect in the patient's antigen processing and presentation system.[1] In 1978, Morton and associates reported a randomized, clinical trial of an allogeneic melanoma vaccine in patients with metastatic melanoma.[7] Their efforts eventually led to the development of CancerVax (C-Vax), a polyvalent "antigen-enriched" whole-cell melanoma vaccine. C-Vax is a live-cell preparation of three allogeneic melanoma cell lines chosen for their high expression of immunogenic TAAs and melanoma-associated antigens. Initial phase II trials of C-Vax in patients with distant metastatic melanoma (American Joint Committee on Cancer [AJCC] Stage IV) demonstrated a five-year overall survival rate of 25% for 157 patients treated with vaccine, compared with only 6% for 1,521 historic controls treated with nonvaccine therapies (P = 0.0001). The median survival of the vaccine patients and historical controls was 23 months and 7.5 months, respectively.[8] A subsequent matched-pair analysis was undertaken to compare the outcome of AJCC Stage IV patients receiving C-Vax vs. non-C-Vax adjuvant therapy after complete metastasectomy. Rates of five-year overall survival were 35.6% and 17.1% for C-Vax and non-C-Vax groups, respectively, and the median overall survival was 37.2 and 14.3 months, respectively (P = 0.0005).[9]

Phase II studies in patients with melanoma metastatic to the regional lymph nodes (AJCC Stage III) also showed a significant difference (P < 0.0002) in 5-year overall survival rates between 283 C-Vax patients and 1474 historical controls: 53% and 39%, respectively.[10] Most recently, a retrospective matched-pair analysis demonstrated a significantly (P = 0.0071) higher rate of five-year overall survival in 165 patients receiving C-Vax than in 165 patients undergoing observation after resection of intermediate/thick primary (AJCC Stage II) melanoma (80% vs 68%, respectively).[11] Based on these findings, the John Wayne Cancer Institute began multicenter phase III studies of C-Vax in patients undergoing complete resection of AJCC Stage III and IV melanoma.

The immunologic basis for the improved survival of melanoma patients treated with C-Vax was analyzed in a study of 77 AJCC Stage IV patients who received C-Vax after complete resection of all disease.[12] Patients (n = 29) who exhibited elevated levels of IgM antibody against a glycoprotein tumor-associated antigen (TA90) and a strong delayed-type-hypersensitivity (DTH) skin response had a five-year overall survival rate of 75%, while those (n = 35) who exhibited only one of those parameters had a five-year overall survival of 36%, and patients (n = 13) who had neither elevated antibody levels nor a strong DTH response had a five-year overall survival of 8% (P < 0.001). This study provides evidence that humoral and cellular immune responses strongly correlate with clinical outcome in melanoma.

Cell Lysate Vaccines

Lysate vaccines are based on the idea that immunogenic anti-tumor responses can be generated by attaching a foreign component, such as a bacterial or viral derivative, to membrane TAA. Mitchell and associates developed a lysate of membrane-associated tumor antigens from two melanoma cell lines. This allogeneic homogenate was combined with a nonspecific adjuvant DETOX (a combination of detoxified bacterial endotox-

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in and mycobacterial cell wall skeletons; Ribi ImmunoChem Research, Hamilton, MT), to form a vaccine (Melacine, Ribi ImmunoChem Research) that possessed both TAAs and melanoma-specific antigens.[13]

Phase II studies have been performed using Melacine in AJCC Stage IV, III, and II melanoma. One hundred six patients receiving Melacine for treatment of metastatic melanoma (AJCC Stage IV) had a 20% response rate, with 5% complete responses and 15% partial responses. The median survival time of the entire group was 12.2 months. Eight percent of patients had a long-term response which resulted in a median overall survival of 46 months.[14] In another phase II study, Melacine was administered with low-dose cyclophosphamide (to reduce suppressor T-cell activity) to 23 patients with resected AJCC Stage II disease and 44 patients with resected AJCC Stage III disease. Although clinical response strongly correlated with an increase in cytotoxic T-lymphocytes, only 30% of those with increased T-cells had a remission of more than one year.[13]

One phase III trial with Melacine vs. standard chemotherapy in AJCC Stage IV melanoma produced no significant difference in overall survival.[14] However, a significant percentage of patients who failed to respond to a least one treatment cycle of Melacine developed major clinical responses to salvage therapy with interferon-alfa-2b.[15] Two other phase III trials have been initiated to compare Melacine plus interferon-alfa vs. interferon-alfa alone in AJCC Stage III and IV melanoma. Also, the Southwest Oncology Group (SWOG) has completed a prospective, randomized trial evaluating Melacine vs. observation in intermediate thickness (1.5-4.0 mm) melanomas. The final results await further observation and data analysis.[13]

The first multicenter phase III trial of postoperative adjuvant active specific immunotherapy for AJCC Stage III melanoma was performed by Wallack and associates in 1988. They used a polyvalent vaccine derived from four human allogeneic melanoma cell lines, which were then infected with a lytic vaccinia virus. Viral-induced oncolysis can induce strong antitumor immune responses by combining immunogenic viral antigens with weak TAAs. The combined lysates of all four melanoma cell lines formed the vaccinia melanoma oncosylate (VMO) vaccine.[13]

In his phase III trial, 250 patients with surgically resected AJCC Stage III melanoma were randomized to treatment with VMO or vaccinia virus alone as a control. With a median follow-up of 46.3 months, there was no statistically significant increase in disease-free or overall survival in patients treated with VMO compared with vaccinia virus alone. A retrospective subset analysis showed a statistically significant improvement in survival of 21.3% at five years for male patients aged 44-57 years (n = 20) with one to five positive nodes after treatment with VMO compared to the vaccinia virus alone group (P = 0.046). None of the melanoma cell lines used in the vaccine expressed tumor peptide antigens associated with HLA-A2. It is possible the VMO vaccine may not have been able to induce a proper cellular immune response to HLA-A2-restricted tumor antigens. Approximately 50% of individuals in the North American population are HLA-A2-positive.[16]

Table

Polyvalent Melanoma Vaccines

Vaccine (Investigator)	Preparation	Number of cell lines	Completed clinical trials		
			AJCC Stages tested	Eligible Patients	Results
CancerVax (Morton)	Whole irradiated live cells	3 human	AJCC Stage IV (phase II)	157	OS: P = 0.0001
			AJCC Stage IV (phase II)	116 surgically resected	DFS: P = 0.005
					OS: P = 0.0005
			AJCC Stage III (phase II)	283 surgically resected	OS: P ≤ 0.0002
			AJCC Stage II (phase II)	330 surgically resected	OS: P = 0.0071
Melacine (Mitchell)	Mechanically disrupted lysate	2 human	AJCC Stage IV (phase II)	106	Median OS 12.2 months
			AJCC Stage III (phase II)	44 surgically resected	5-year OS 66%
			AJCC Stage II (phase II)	23 surgically resected	5-year OS 78%
			AJCC Stage IV (phase III)	140	OS: NS
Polyvalent Shed Antigen (Bystryn)	Partially purified shed antigens	3 human and 1 hamster	AJCC Stage III (phase II)	94 surgically resected	OS: 5-year OS 50%
			AJCC Stage III (phase III)	38 surgically resected	2-year OS 77%
Vaccinia Melanoma Oncosylate (Wallack)	Viral lysate	4 human	AJCC Stage III (phase III)	217 surgically resected	DFS: NS; OS: NS
Vaccinia Virus Melanoma Lysate (Hersey)	Viral lysate	1 human	AJCC Stage III (phase II)	182 surgically resected	5-year OS 50-60%

AJCC = American Joint Committee on Cancer; **DFS** = disease-free survival; **OS** = overall survival; **NS** = not significant

Hersey and associates have also developed a polyvalent vaccine using a vaccinia virus melanoma cell lysate (VMCL). The preparation method is similar to that used by Wallack et al, except this vaccine only uses one allogeneic melanoma cell line. In a phase II study,[17] 80 patients with AJCC Stage III melanoma were treated with VMCL after surgical resection of disease. The five-year overall survival of the VMCL group (60%) was superior to that of a historical control group of 151 patients (34%) and a concurrent, nonrandomized control group of 55 patients (35%). A prospective, randomized phase III trial of postoperative adjuvant therapy with VMCL vs. observation was initiated in patients with thick primary melanomas (T4 lesions) and regional nodal melanoma.[18] The trial has accrued 569 patients so far, with a median survival of 84 and 65 months in the VMCL and observation groups, respectively. The results have not yet reached statistical significance, but the trial is planning to accrue patients for another three years.

Polyvalent Shed Antigen Vaccine

A polyvalent preparation of purified antigens lacks HLA antigens and thus does not induce anti-HLA antibodies, which can make it difficult to measure the cellular immune response to vaccine treatment.[19] Bystryn and associates developed a polyvalent vaccine consisting of partially purified shed antigens prepared from four melanoma cell lines (3 human and 1 hamster). Ninety-four AJCC Stage III patients treated with this vaccine after surgical resection demonstrated a five-year overall survival of 50%, vs. 33% for historical controls. A strong correlation was found between immune response and prognosis. Of the vaccine-treated patients, those who developed a cellular immune response (as measured by DTH skin reaction) to the vaccine had a median disease-free survival 4.7 years longer and an overall survival 3.7 years longer than those who did not ($p \leq 0.02$). Among vaccine-treated patients, the five-year overall survival of antibody responders and nonresponders was 71% and 44%, respectively ($p \leq 0.01$). Based on these results, a phase III, double-blinded, randomized trial was initiated in resected AJCC Stage III melanoma. Unfortunately, the trial was interrupted early because of slow patient accrual, and only 38 patients were randomized to treatment with the shed polyvalent vaccine or a placebo. Median follow-up was 39 months, and the median disease-free survival of the vaccine ($n = 24$) and placebo ($n = 14$) groups was 18.6 and 7.1 months, respectively. Mortality at two years was 23% for the vaccine group and 40% for the placebo group. These results suggest efficacy, but the number of patients is too small for definitive conclusions.[20-22]

Commentary and Future Directions

This brief review of polyvalent vaccines for melanoma offers cause for both optimism and caution. Polyvalent active specific immunotherapy, one of the older vaccine technologies, finally is starting to produce objective clinical results. The Table summarizes clinical trials using the polyvalent vaccines presented in this article. Phase II trials of CancerVax (Morton's group) and VMCL (Hersey's group) have shown significant efficacy, and these vaccines are currently being evaluated in prospective, multicenter, randomized phase III studies. On the other hand, completed phase III trials of vaccine formulations developed by Mitchell's, Bystryn's, and Wallack's groups have not demonstrated a conclusive clinical benefit in patients with regional and distant metastatic melanoma.

Polyvalent allogeneic cell-based vaccines are but one of myriad approaches to immunotherapy in melanoma. Two new advances in vaccine development are the in-situ modification of autologous tumor cells with immunostimulatory genes and the use of dendritic cells to stimulate cytotoxic T-lymphocyte responses. The concept of genetically modifying tumor cells to be more immunogenic by introducing genes that encode immunomodulatory proteins has stimulated much interest. Also, the priming of cytotoxic T-cells is a critical component of the immune response to tumors and is dependent on the presentation of the relevant antigen by professional antigen-presenting cells. Dendritic cells are considered to be the most potent of all antigen-presenting cells and have been proposed as ideal candidates for the induction of anti-tumor immunity in the vaccine treatment paradigm. Both of these immunotherapeutic modalities are still in the early phases of study, but there have been encouraging clinical trials in patients with metastatic melanoma.[13]

With the failure of the most recent Eastern Cooperative Oncology Group (ECOG) trial to demonstrate any significant improvement in overall survival using interferon-alfa-2b in high risk primary and regionally metastatic melanoma,[23] it can be stated that there is currently no effective standard adjuvant therapy available for patients with high-risk melanoma. Any patients with advanced disease should be enrolled in a clinical trial testing investigational therapy. Active specific immunotherapy, based on our increased understanding of tumor-host immune interactions and whose clinical efficacy has been successfully tested in prospective randomized studies, eventually will significantly improve the quality and duration of life for patients with malignant melanoma. *(Dr. Shen is a Senior Fellow, Training Program in Surgical Oncology; Dr. Morton is Medical Director and Surgeon-in-Chief at the John Wayne Cancer Institute, Saint John's Health Center, Santa Monica, CA.)* ❖

References

1. Chan AD, Morton DL. *Semin Oncol* 1998;25:611-622.
2. Ollila DW, Kelley MC, Gammon G, et al. *Semin Surg Oncol* 1998;14:328-336.

3. Toes RE, Blom RFF, van der Voort E, et al. *Cancer Res* 1996;56:3782-3787.
4. Knight BC, Souberbielle BE, Rizzardi GP, et al. *Melanoma Res* 1996;4:299-306.
5. Morton DL, Foshag LJ, Hoon DSB, et al. *Ann Surg* 1992;216:463-482.
6. Albino AP, Lloyd KO, Houghton AN, et al. *J Exp Med* 1981;154:1764-1778.
7. Morton DL, Eilber FR, Holmes EC, et al. *Aust NZ J Surg* 1978;48:49-52.
8. Morton DL, Barth A. *CA Cancer J Clin* 1996;46:225-244.
9. Hsueh EC, Nizze A, Essner R, et al. *Proc Am Soc Clin Oncol* 1997;16:492.
10. Barth A, Hoon DSB, Foshag L, et al. *Cancer Res* 1994;54:3342-3345.
11. Shen P, Foshag LJ, Essner R, et al. *Am Soc Clin Oncol* Annual Mtg (1999); poster #2059.
12. Hsueh EC, Gupta RK, Qi K, et al. *J Clin Oncol* 1998;16:2913-2920.
13. Hemmila MR, Chang AE. *J Surg Oncol* 1999;70:263-274.
14. Mitchell MS. *Semin Oncol* 1998;25:623-635.
15. Mitchell MS, Jakowatz J, Harel W, et al. *J Clin Oncol* 1994;12:402-411.
16. Wallack MK, Sivanandham M, Balch CM, et al. *J Am Coll Surg* 1998;187:69-77.
17. Hersey P. *World J Surg* 1992;16:251-260.
18. Hersey P, Coates A, Tyndall L. *Mel Res* 1997;7(S):S22-S23.
19. Ravindranath MH, Morton DL. *Cancer Medicine.* Philadelphia: Lea & Febiger, 1998.
20. Bystryn JC, Oratz R, Henn M, et al. *Cancer* 1992;69:1157-1164.
21. Miller K, Abeles G, Oratz R, et al. *Cancer* 1995;75:495-502.
22. Bystryn JC, Oratz R, Shkapiro RL, et al. *Proc Am Soc Clin Oncol* 1998;17:434a(abstr).
23. Kirkwood JM, Ibrahim J, Sondak V, et al. *Proc Am Soc Clin Oncol* 1999;537a(abstr).

Clinical Trial of MGI-114 in Prostate Cancer

By Neil N. Senzer, MD

This year, it is estimated that approximately 179,300 men in the United States will develop clinical prostate cancer and that approximately 37,000 deaths will be attributable to this malignancy. The incidence rates for prostate cancer increased 65% between 1980 and 1990, in large part due to the aging of the population, greater awareness of prostate cancer, and prostate specific antigen (PSA) screening. However, since 1996 when an estimated 317,000 men were diagnosed, the trend has reversed. This most likely represents a cull effect as prevalent cases are removed from the population. Yet, while often thought of as a "disease that people die with, not of," prostate cancer remains the second most common organ-specific neoplastic cause of death in men.

Unfortunately, in spite of a high probability of response to initial androgen deprivation, all men with metastatic disease will eventually develop hormone-refractory disease (HRPC). With PSA monitoring, a higher percentage of patients are asymptomatic with recurrent metastatic prostate cancer and, although no more than one-third have measurable disease, almost all have rising PSA values. PSA end points were first reported based on data from a study at the Memorial Sloan-Kettering Institute. That data were confirmed by an NCI study which demonstrated that baseline-to-post-treatment declines of 75% in PSA reliably predicted for survival. Given that single-agent chemotherapy regimens have invariably demonstrated less than 30% response rates in patients with HRPC, when used with reservation,[1] PSA analysis in conjunction with measurable disease, bone-only disease, and quality-of-life markers allows for a more rapid and, with hope, accurate assessment of responsiveness to new treatments prior to embarking on longer term and expensive phase III survival end-point studies.

MGI-114 (6-hydroxymethylacylfulvene: HMAF), is a semi-synthetic analog derived from the naturally occurring sesquiterpene, illudin S, isolated from mushrooms of the genus *Omphalotus*. Accumulation in sensitive tumor cell types is rapid and, following metabolism to a reactive intermediate, it is covalently bound to macromolecules. The degree of DNA binding is closely linked to the cytotoxic potential of MGI-114. DNA synthesis is rapidly and potently inhibited causing cell cycle arrest in S phase. DNA strand breaks and adducts are not readily repaired. Induction and progression of apoptosis is time and concentration-dependent and was demonstrated to decrease with androgen dependency in six human prostate cancer cell lines.[2] Of note, MGI-114-induced apoptosis is not affected by caspase-3 inhibitors but is blocked by the broad-spectrum caspase inhibitor Z-VAD-fmk. MGI-114 is effective against both MDR1/gp 170- and MRP/gp 180-positive tumor xenografts[3,4] and demonstrates cytotoxicity independent of p53 and p21 status.[5] All tested ERCC1-6 (excision repair cross complementing) deficient cell lines retain sensitivity to acylfulvene analogues in contrast to other drugs that covalently bind to DNA which show no more than a two-fold greater sensitivity to ERCC2 or ERCC3 helicase deficient cells.[6] Previous studies have demonstrated that ERCC1 upregulation may contribute to the drug resis-

tance observed against standard DNA-binding anticancer drugs.[7] Neither ERCC2 nor ERCC3 are known to be upregulated and the apparent requirement for their action in the repair of MGI-114-induced DNA lesions may contribute to the maintenance of drug sensitivity in drug-resistant tumor cell lines.

MGI-114 has demonstrated substantial antitumor activity in two human prostate tumor xenograft models. At the maximum tolerated dose (MTD), 10 of 10 mice showed partial tumor shrinkage (mean shrinkage, 72.5%) in the PC-3 prostate xenograft model. In the DU-145 prostate xenograft model five of eight animals had a complete response at the MTD. Animals that received one-half MTD showed mean rumor shrinkage of 35% with maximum shrinkage (96%) occurring on day 11. At maximally tolerated doses, daily dosing for five days produced significantly better antitumor responses than single doses with responses better than or equal to other multiple dose administration schedules in different xenograft models.

Dosage administrations in phase I studies include five minutes, 30 minutes, and one hour intravenous infusions. All have been daily × 5 and repeated every 28 days. The greatest experience has been with the five-minute infusion. The most common drug-related toxicities reported with this schedule are nausea (78%), vomiting (41%), fatigue (50%), facial erythema (41%), and lymphocytopenia (56%). Grade 3,4 nonhematologic toxicities include nausea and vomiting, fatigue, hyperglycemia, and increased alkaline phosphatase. Myelosuppression has been consistently observed at 14.15 mg/m^2. Patients with compromised renal function may be at risk for developing renal tubular acidosis. The recommended dose for phase II evaluation was 10.6 mg/m^2 over five minutes.

Preliminary results of a Phase II Trial of MGI-114 in Patients with Hormone-Refractory Prostate Cancer were presented at the 35th Annual American Society of Clinical Oncology meeting.[8] The study was designed to assess the single-agent antitumor activity of MGI-114 in patients with stage D2 (metastatic) HRPC using a PSA surrogate. Secondary objectives were: 1) to determine the objective response rate in patients with measurable disease (excluding bone); 2) to determine the duration of response and time-to-progression; and 3) to describe and quantify drug related toxicity. MGI-114 at 10.6 mg/m^2, daily × 5, every 28 days was administered in a five-minute infusion. A PICC line and prophylactic 5-HT3/steroid antiemetics were required. At the time of the report, 31 patients were registered with documented disease progression having received no more than two hormone-suppression regimens. Twenty-one of the patients (mean age, 68; range, 52-84) are evaluable. The mean pretreatment PSA is 306 ng/mL (range, 11.6-2294 ng/mL). At study entry, patients had ECOG scores of 0 (6 patients), 1 (14 patients), and 2 (1 patient). Prior endocrine treatments include orchiectomy in eight, LH/RH agonist in 14, and antiandrogen (discontinued 4-6 weeks prior to initiation of therapy) in 18 patients. Six patients received primary radiation therapy and nine, palliative. Sixteen patients exhibited metastatic disease to bone, six to lymph node, two to liver, and three in multiple sites.

Grade 3 asthenia and nausea occurred in three patients each. Grade 3 thrombocytopenia and neutropenia each occurred in six patients, with prolonged thrombocytopenia emerging as the primary cause of treatment delay. PSA responses included three patients with greater than a 50% reduction for a median 23 weeks duration (range, 2-26+) and 13 patients with stable PSA for a median 13 weeks duration (range, 9-33+) for an overall response rate of 76%. To date, one patient has experienced a complete soft-tissue response (retroperitoneal lymph nodes) and, a second patient, a partial soft-tissue response (iliac nodes). The study is continuing with a targeted accrual of 30 evaluable patients.

MGI-114 is reasonably well tolerated in this "chemonaïve" group of patients. Nausea and vomiting, usually emerging on the fourth to fifth day of infusion, is controlled with a prophylactic HT3/steroid combination, which should be continued orally. MGI-114 appears to have sufficient clinical activity in patients with hormone-refractory prostate cancer to warrant further assessment, especially in combination with other compounds and/or radiation. Britten and colleagues[9] have recently described a supra-additive interaction between MGI-114 and CPT-11 in the HT29 human colon tumor xenograft. Although there is no in vitro evidence that a topoisomerase is required for nucleotide excision repair, mismatch repair, or repair by DNA synthesis, inhibiting topoisomerase could alter the level of supercoiling in a chromosomal domain, which could lead to the recruitment of other enzymes acting on the damaged site. In the MVI-522 human lung xenograft model, Mangold[10] was able to show significant enhancement of response to the combination of MGI-114 with the topoisomerase inhibitor topotecan and paclitaxel. DNA helicase inhibitors (e.g., CI-958, distamycin, and mithramycin) have also been shown to act synergistically with MGI-114 in a series of pediatric tumor cell lines.[11] Finally, given the recognition of the role of transcription coupled nucleotide excision repair in radiation ROI mediated damage and the role of the Ku80 helicase in homology dependent DNA double-strand break repair, the potential for coordinated delivery of MGI-114 and radiation is a reasonable combination to investigate. *(Dr. Senzer is Director of Radiation Oncology Research, Co-Director Urologic Oncology, Sammons Cancer Center, Dallas, TX.)* ❖

References

1. Dawson NA. Response criteria in prostatic carcinoma. *Sem Oncology* 1999;26:174-184.
2. Woynarowska BA, et al. *Proc Am Assoc Cancer Res 1999;*40:488.
3. Kelner MJ,et al. *Proc Am Assoc Cancer Res* 1999;40:300.
4. Kelner MJ, et al. *Eur J Cancer* 1998;34:908.
5. Izbicka E, et al. *Proc Am Assoc Cancer Res* 1999;40:300.
6. Kelner MJ, et al. *Cancer Res* 1995;55:4936-4940.
7. Dabholker M, et al. *J Natl Cancer Inst* 1992;84: 1512-1517.
8. Senzer NN, et al. ASCO Program/Proceedings 1999;18:321a.
9. Britten, et al. *Cancer Res* 1999;59:1049-1053.
10. 10th NCI-EORTC Symposium on New Drugs in Cancer Therapy, 1998.
11. Moore R, et al. *Proc Am Assoc Cancer Res* 1999;40: 590-591.

Prevention of Colitis-Associated Colon Cancer

By M.L.Clapper, PhD; H. S. Cooper, MD; and S. Murthy, PhD

Inflammatory bowel disease (ibd) continues to affect approximately one million individuals in the United States.[1] Although the high incidence of colorectal cancer among these IBD patients has been well established, the molecular basis for this increased risk remains unknown. The animal model of dextran sulfate sodium (DSS)-induced colitis provides a unique opportunity to evaluate the sequence of biochemical and histological changes that lead to dysplasia and cancer. The similarity of DSS-induced colitis to human disease makes this model a valuable resource with which to develop strategies for the chemoprevention of colitis-associated colon cancer.

The incidence of colorectal cancer in the United States is among the highest in the world, with an estimated 129,400 new cases diagnosed each year. Patients with inflammatory bowel disease (specifically ulcerative colitis) face a significantly increased risk of developing colonic malignancies. This risk begins approximately 10 years after the onset of colitis and increases with each decade of life, reaching as high as 25-40% after 40 years in patients who have not had a prophylactic colectomy.[2] Duration of disease and extent of tissue involvement are the most significant determinants of colon cancer risk in this population. Use of conventional screening endoscopy for the early detection of colitis-associated neoplasms has been compromised by the characteristic flat morphology of both precancerous and cancerous lesions. It is unfortunate that colectomy remains the only definitive treatment for the prevention of colorectal cancer in patients with ulcerative colitis.

The molecular basis for the colonic dysplasias and cancers among ulcerative colitis patients remains unknown. Much attention has focused on the contribution of inflammation and highly reactive oxygen species to cellular damage and neoplasia, including the anticipated increased incidence of mutations under these conditions. Although several common colon cancer genes (APC, p53, DCC, MCC, Rb) are mutated in colitis-associated neoplasms,[3,4] neither a defined sequence of genetic events nor germline mutations have been identified to explain their development. These data, when combined with additional supportive observations: 1) suggest that colonic dysplasias progress to colon cancers via a pathway that is distinct from that of both sporadic and heritable colon cancers; and 2) provide little insight into the identity of potential risk factors and molecular targets for intervention.

Preclinical Animal Models

Relevant and reproducible animal models of colitis-associated neoplasms are required to elucidate the molecular pathways involved in the progression of colitis to dysplasia. Subsequent evaluation of agents with known chemopreventive activity (i.e., DFMO [difluoromethylornithine], oltipraz, sulindac sulfone) in these preclinical models should facilitate the establishment of a nontoxic, efficacious regimen for the prevention of colitis-associated colon cancer. Although many animal models of colitis have been established, few are appropriate to study the dysplasia-cancer sequence. Cotton top tamarins spontaneously develop colitis and colon cancer when maintained in captivity. However, cancers do not arise from dysplasias in these animals and take several years to develop.[5] More recently, Interleukin-10[6] and G protein[7] knockout mice have been generated which develop colitis and adenocarcinomas. The relevance of these mouse models to human disease has not been established.

Adaptation of the hamster model of DSS-induced colitis[8] to mice by this group[9] and others[10] has provided a unique opportunity to evaluate the spectrum of biochemical and histological changes that lead to colitis and subsequent dysplasia/cancer. In this model, Swiss Webster mice are administered DSS (4%) in the drinking water for seven days. Recovery for 14 days on

untreated drinking water results in the establishment of acute colitis and the development of histological ulcerations and erosions. Clinical symptoms of colitis are preceded by progressive loss of the epithelial crypts. Inflammation is secondary to loss of the entire crypt on Day 5, with subsequent erosions.

Chronic colitis is induced by repeating the administration of DSS and water (one cycle = 7 days DSS and 14 days water) for two to four cycles. Animals with chronic disease exhibit lymphocyte and plasma cell infiltration, crypt distortion, and focal erosions similar to those found in human patients with ulcerative colitis. By the end of the fourth cycle, approximately 15% of the animals develop neoplastic lesions that are morphologically identical to dysplasias and/or cancers in humans. Non-neoplastic background mucosa show changes of classic chronic ulcerative colitis. Inflammation is more extensive in animals with dysplasia and cancer as compared to those without lesions.

A detailed characterization of the pathology of DSS-induced colitis suggests that this is a reliable and relevant model for the future development of strategies for the chemoprevention of human colitis-associated colon cancer. Similar to humans with ulcerative colitis, DSS-treated animals: 1) experience periods of clinical activity and inactivity; 2) possess varying degrees of inflammation many months after DSS;[9] and 3) develop both flat and polypoid dysplasias and flat and polypoid cancers. The resulting dysplasias and carcinomas exhibit patterns of immunohistochemical staining for lectins and β-catenin that are similar to those of human colon tumors.[11]

Chemoprevention

Recent advances in the area of cancer chemoprevention have provided new opportunities for the development of therapeutic regimens for the prevention of colitis-associated colon cancer. Chemoprevention refers to the use of natural or synthetic agents to delay the formation of precancerous lesions or inhibit their progression to invasive cancers. Although several clinical chemoprevention trials have been performed in individuals at increased risk for sporadic colorectal cancer, none have targeted patients with IBD. Retrospective analyses in patients with long-standing ulcerative colitis suggest that daily folate supplementation may inhibit tumor formation.[12,13] The ability of routine maintenance therapy for colitis (sulphasalazine or 5-aminosalicylic acid) to delay the development of colon cancer remains equivocal. The long-term benefit of treatment with nonsteroidal anti-inflammatory agents has not been assessed in this population. Prospective, randomized, clinical trials in patients with ulcerative colitis are needed to definitively assess the chemopreventive activity of each of these agents.

Preclinical development of chemopreventive regimens for individuals with ulcerative colitis continues to be challenged by our inability to identify a target population at increased risk of disease. Treatment of high-risk individuals with chemopreventive agents prior to the establishment of disease, a conventional approach to chemoprevention, is thus not practical and dictates the need to identify early biomarkers of risk for colon cancer. Previous studies have suggested that the activity of the Phase II detoxication enzyme glutathione S-transferase (GST) is significantly decreased in blood lymphocytes from individuals at increased risk for colorectal cancer (individuals without IBD, but with a personal or family history of colon cancer or personal history of polyps) as compared to cancer-free, healthy controls.[14] A direct correlation between the GST activity of blood lymphocytes and colonic mucosa from the same individual was also established. Application of these findings to individuals with IBD has been precluded by the potential effect of routine colitis therapy on detoxication enzyme activity. The mouse model of DSS-induced colitis represents a relevant system in which to assess colon cancer risk.

We have recently characterized Phase II detoxication enzyme expression during acute and chronic colitis in the DSS mouse model.[15] Examination of colon tissue after each cycle of DSS revealed significant reductions in GST, γ-glutamylcysteine synthetase, the rate-limiting enzyme of glutathione synthesis, and glutathione levels after two cycles of treatment. Levels continued to decrease with each subsequent cycle of DSS exposure. In the case of GST, reductions in enzymatic activity were confirmed at both the protein and RNA level. Immunohistochemical analyses revealed that the loss of GST was not specific to any particular cell type, but instead was present in all cellular compartments (epithelium, smooth muscle, and endothelial cells) of the colon. These data suggest that Phase II detoxication enzyme inducers may be efficacious in the prevention of colitis-associated colon cancer.

Oltipraz is a Phase II enzyme inducer that was marketed previously for the treatment of schistosomiasis. Its observed ability to significantly elevate the detoxication potential of the host while depleting glutathione levels within the schistosome provided the first evidence that oltipraz may be effective in increasing cellular protection. One of the most exciting attributes of this compound is its ability to protect numerous target organs from a variety of structurally diverse carcinogens. Its effectiveness in inhibiting colon carcinogenesis in animal models[16] and inducing the transcription of Phase II detoxication

enzymes within the colonic mucosa of high-risk individuals (non-IBD)[17] has been reported. Based upon these findings, we have initiated an evaluation of the effect of oltipraz on the formation of colitis-associated neoplasms. Preliminary studies in the DSS model of induced colitis indicate that oltipraz can inhibit the development of carcinomas in this model.[18]

Commentary

Several opportunities currently exist for developing clinical chemopreventive interventions for colitis patients at increased risk for colorectal cancer. The mouse model of DSS-induced colitis exhibits many characteristics of the human disease and represents a valuable system in which to examine both the molecular events associated with colitis and the inhibition of them by chemopreventive agents. Translation of these preclinical findings to a clinical setting will be facilitated by focusing on promising agents such as oltipraz for which the optimal dosage and schedule for administration have been established. *(Dr. Clapper is Member, Division of Population Science, and Dr. Cooper is Senior Member, Department of Pathology, Fox Chase Cancer Center; Dr. Murthy is Professor of Medicine, Associate Vice President for Research, Krancer Center for Inflammatory Bowel Disease Research, MCP Hahnemann University, Philadelphia.)* ❖

References

1. Landis SH, Murray T, Bolden S, et al. *CA Cancer J Clin* 1999;49:8-31.
2. Bansal P, Sonnenberg A. *Am J Gastroenterol* 1996;91:44-48.
3. Fogt F, Vortmyer AO, Goldman H, et al. *Hum Pathol* 1998;29:131-136.
4. Greenwald BD, Harpaz N, Yin J, et al. *Cancer Res* 1992;52:741-745.
5. Johnson RD, Ausman LM, Sehgal PK, et al. *Gastroenterology* 1996;110:102-115.
6. Berg DJ, Davidson N, Kuhn R, et al. *J Clin Invest* 1995;98:1010-1020.
7. Rudolph U, Finegold MJ, RIdge SS, et al. *Nat Genet* 1995;10:143-150.
8. Ohkusa T. Jpn J *Gasteroenterol* 1985;82:1327-1336.
9. Cooper HS, Murthy SNS, Shah RS, et al. *Lab Invest* 1993;69;238-249.
10. Okayasu I, Hatekayama S, Yamada M, et al. *Gastroenterology* 1990;98:694-702.
11. Cooper HS, Murthy S, Kido K, et al. *Am J Pathol* (Submitted) 1999.
12. Lashner BA, Hanuer SB, Silverstein MD. *Ann Intern Med* 1988;108:274-278.
13. Mouzas IA, Papavassiliou E, Koutroubakis I. *Ital J Gastroenterol Hepatol* 1998;30:421-425.
14. Szarka CE, Pfeiffer GR, Hum ST. *Cancer Res* 1995;55:2789-2793.
15. Clapper ML, Adrian RH, Pfeiffer GR, et al. *Aliment Pharmacol Ther* 1999;13:389-396.
16. Clapper ML. *Pharmacol Ther* 1998;78:17-27.
17. O'Dwyer PJ, Szarka CE, Yao KS, et al. *J Clin Invest* 1996;98:1210-1217.
18. Clapper ML, Cooper HS, Flanigan A, et al. *Gastroenterology* 1999;116:A390.

Role of Copper in the Genesis of Liver Cancer

By Kalipatnapu N. Rao, PhD, and Patricia K. Eagon, PhD

Many chemicals are carcinogenic to liver in rodents, some of which are also suspected to cause liver cancer in humans. These chemicals include common household and industrial agents as well as agricultural chemicals, pesticides, herbicides, and several therapeutic drugs that have wide human exposure and use. Experimental rodent models of liver cancer developed in our laboratory, as well as human liver specimens, demonstrate alterations in copper metabolism and accumulation of excess copper. Copper is an essential element and its levels are tightly regulated in the body. However, excess copper levels are toxic and may contribute to the development of liver cancer.[1] Removal of excess copper by chelating agents or by zinc treatment inhibits the development of liver tumors. Human liver tumors also show accumulation of excess copper, similar to that seen in rodent models. Thus, it may be possible to reduce the incidence of human liver cancer by the use of copper-chelating agents or by a diet high in zinc.

Background

Liver cancer is one of the most common cancers world-wide and is known to affect several million individuals. In the United States, it is less common than in other areas of the world. However, recent evidence suggests that the incidence of this cancer in this country is increasing. The reason for this increased incidence is not known. Liver cancer takes several years to develop and the exact mechanism(s) by which liver cancer develops is not understood, even though several causative agents are suspected in the genesis of this cancer. There is no successful curative treatment for advanced liver cancer. Although liver transplantation, radiation, and chemotherapy are often attempted, these efforts usually

meet with limited success. Unless we understand the mechanisms of this disease, the ideal therapy will continue to be elusive. Experiments conducted with rodents suggest that exposure to certain chemicals through the environment such as food, water, and air might initiate liver cancer. Once initiated, diet, as well as other physiological and environmental factors, is likely to influence the development of tumors further.

Environmental Chemicals and Liver Cancer

Several man-made and industrial chemicals that have wide human exposure are inducers of liver cancer in rodents. These chemicals include pesticides, herbicides, plasticizers, hypolipidemic agents such as clofibrate and gemfibrozil, and several others. These compounds, when given at high doses, are known to cause liver cancer in rodents. One theory regarding the mechanisms by which these agents cause liver cancer has received recent attention. It is known that these chemicals do not damage DNA, so identification of their specific actions was perplexing. It appears that one mechanism of action is the promotion of a high and sustained level of cell division. In addition, these chemicals change pathways of metabolism by altering enzyme activities and levels of chemicals, which results in metabolic imbalance. Sooner or later, an error will occur, and one or more cells will be altered. Each of these actions appears to inhibit the growth of normal liver cells and favor the growth of cancer cells, leading to the development of tumors.

It is also interesting to note that some of these chemicals mimic estrogens and alter sex steroid hormone levels in the blood of experimental rats. Therefore, these chemicals may also be classified as endocrine disrupters. Elevated sex steroid hormones such as estrogen may result in the transformation of a normal liver cell to a cancer cell and promote its growth to tumor.

The question becomes whether it is possible to reverse the actions of these chemicals and retard the growth of cancer cells. There may be several ways to achieve this goal. Of course, the primary goal would be to understand the metabolic changes and develop a strategy to reverse these processes in order to prevent malignant transformation. Our recent research has shown that in rats, exposure to these agents results in an accumulation of copper in the livers of experimental animals. As outlined below, copper has been shown to have a significant role in cellular damage and in the genesis of tumors in certain situations. Our studies confirmed that during the progression of liver cancer, the genes that precisely regulate copper levels in the body are suppressed, resulting in abnormal accumulation of copper in liver, liver tumors (if present), and possibly in other tissues as well. Copper is an essential element required for normal body functions; however, copper in excess is quite toxic to the liver cell. But somehow the cancer cell is able to overcome the toxic effects of excess copper and exhibit malignant growth and progression. These findings suggest that one strategy to reverse this process might be the removal of excess copper by copper chelators such as trientine or penicillamine, or by zinc administration—agents that appear to inhibit the development of liver tumors. Although these studies have only been performed to date in animal models, we find these results exciting and see potential for the development of preventive and therapeutic strategies.

Copper Toxicity

As stated above, copper is an essential element, which must be generally supplied through the diet. The recommended daily copper intake for humans is 1.5-3.0 mg. Copper is a cofactor for many enzymes. Copper deficiency is associated with reduced hemoglobin production and reduced elastin formation. Copper does not usually accumulate in excess in healthy individuals. The development of chronic effects occurs very infrequently because acute toxic episodes usually limit the excess intake of copper. However, copper toxicity can result from accidental or intentional exposure. Excessive copper intake causes intestinal disturbances, dizziness, headache, and a metallic taste in the mouth, and, if excessive, death. Respiratory collapse, hemolytic anemia, hemoglobinemia, and hepatic and renal failure were also documented in cases of excessive copper intake. Copper poisoning by drinking water is very uncommon.

Copper Disorders

Genetic disorders of copper metabolism include Wilson's disease and Menkes' disease.[2] Wilson's disease is an autosomal recessive disease and its prevalence rate is high. In this disease, copper accumulates in many tissues. Clinical manifestations include neurologic and liver disorders. Characteristic findings are Kayser-Fleischer rings in the eye and low serum ceruloplasmin, a copper-containing protein. Copper accumulation in the liver is caused by disturbance in biliary excretion of copper. A decrease in expression of the Wilson's disease gene, which encodes a protein P-type ATPase, causes a block in the incorporation of copper into ceruloplasmin and also limits its excretion through the bile. Hepatic sequelae of Wilson's disease demonstrate a broad spectrum of chronic, chronic active, and acute liver diseases, cirrhosis, and fulminant hepatic failure. Copper also plays an important role in iron metabolism as well. Copper deficiency impairs iron absorption, resulting in anemia. Ceruloplasmin has peroxidase activity that oxidizes ferrous iron to the ferric state prior to its binding by plasma transferrin. Elevated copper levels in the liver sometimes coincide with elevated iron in the liver. Zinc treatment prevents the absorption of copper

from the intestine. If they are not treated, patients with Wilson's disease die either due to progressive liver failure or neurological disease. Patients are treated with a copper-chelating agent such as penicillamine or trientine, zinc, and/or a copper-restricted diet. Patients should avoid foods high in copper such as shellfish, chocolate, mushrooms, nuts, and liver. Occasionally, liver transplantation is necessary for selected patients. Orally administered zinc inhibits copper absorption through the intestine. Therefore, Wilson's disease patients are treated with zinc to prevent copper absorption from the intestine and prevent excess copper deposition in the body. If excess copper is not removed by the above treatments the accumulated copper causes cirrhosis of the liver, neurological degeneration and ultimately death. The defect in copper excretion is attributed to a lack of Wilson's disease gene expression. The Wilson Disease gene has recently been cloned. This discovery holds promise to cure this disease as well as liver cancer. Only 11 cases of hepatocellular carcinoma have been reported in patients with Wilson's disease. This low rate may be due to death from hepatic failure precluding the development of liver cancer or to the success of treatments to reduce copper levels.

Copper and Liver Cancer

Does excess copper cause liver cancer? If so, is it possible to prevent the development of liver cancer by removing the excess copper? The high incidence of cancer among coppersmiths suggests a primary carcinogenic role for the copper ion. A higher incidence of stomach cancers are noted in regions where soil zinc-to-copper ratios are found to be reduced. There are several experimental animal models available to study the role of copper in the genesis of liver cancer. Long-Evans rats with cinnamon-like coat (LEC), Bedlington terriers, and toxic milk mutation mice are known to have increased copper accumulation in the liver. LEC rats appear to closely mimic human Wilson's disease. LEC rats suffer from spontaneous hepatitis with hemolytic anemia and jaundice, developing around 4 months after birth, which results in death in 50% of rats due to fulminant hepatitis. The rats that recover from fulminant hepatitis develop chronic hepatitis and a high rate of liver cancer (95%). LEC rats accumulate copper in liver and have a decreased serum ceruloplasmin. In LEC rats, the gene which is homologous with Wilson's disease gene for humans decreases. Copper-restricted or high-zinc diets or treatment with copper-removing agents inhibits the growth of liver tumors in LEC rats. Thus, the role of copper as a causative agent of liver cancer is established. However, the mechanism by which copper induces liver cancer is not known. It is suggested that copper overload inhibits the growth of normal liver cells, giving a growth advantage to cancer cells that subsequently progress to tumors. It is also known that copper causes a variety of damages to liver cells, and, thus, may provoke the liver cells to carcinogenic changes or to increased cell growth.

During the course of our studies with liver cancer induced in rats by several hypolipidemic agents, we observed a significant alteration in copper metabolism.[3] These animals showed accumulation of copper in liver tumors, and a significant decrease in serum ceruloplasmin. Expression of the genes that control copper metabolism such as Wilson's disease gene and ceruloplasmin gene also decreased in liver tumors, confirming our contention that the induction of liver cancer in these rats by hypolipidemic chemicals causes alterations in copper metabolism as seen in patients with Wilson's disease and LEC rats. In our initial experiments with rats treated with hypolipidemic agents, we observed that removal of excess copper by trientine or zinc diet partially prevented the liver pathology that leads to the progression of liver cancer and prevented deposition of copper. These results clearly underscored our contention that an essential element like copper, when deposited in excess, can cause cancer.

We next asked several important questions with respect to human liver cancer. Does human liver cancer tissue also demonstrate accumulation of excess copper? Does excess copper in the liver cause liver cancer? Concomitant with accumulation of excess copper, does the tumor show a significant decrease in zinc levels? Does the suppression of Wilson's disease gene and ceruloplasmin gene expression precede the development of human liver tumors? To answer these important questions, we examined several human liver tumors, the tissue around the tumors, and normal liver tissues to determine levels of copper, zinc, and iron. The human liver cancer also possessed a several-fold accumulation of copper and a significant decrease in zinc levels, as was seen in the rats treated in our laboratory with hypolipidemic agents. Currently, experiments are under way to determine whether human liver tumors also exhibit suppression of Wilson's disease and ceruloplasmin genes. If indeed copper plays an important role in the development of human liver cancer, then it may be possible to ameliorate the growth of liver cancer by copper-chelating agents or by zinc supplementation, as demonstrated unequivocally in LEC rats and in our experimental rats with liver cancer. The results obtained in these studies have potentially far-reaching clinical implications in the management of patients with liver cancer. *(Dr. Rao is Professor of Pathology and Chief, Toxicology Laboratory; Dr. Eagon is Associate Professor of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA.)* ❖

References

1. Toxicity of Metals. LW Chang, ed.; L. Magos, T. Suzuki, Associate Eds. 1996; CRC Press, Inc.
2. Harris ZL, Gitlin JD. *Am J Clin Nutr* 1996;63:836S-841S.
3. Eagon PK, Teepe AG, et al. *Carcinogenesis* 1999;20:1091-1096.

Funding News

The American Digestive Health Foundation ([ADHF], Web site: www.gastro.org/adhf.html) was established in 1995, as the unified voice of three leading gastroenterology and hepatology societies: the American Gastroenterological Association, the American Society for Gastrointestinal Endoscopy, and the American Association for the Study of Liver Diseases. ADHF's mission is to provide financial support for research and education in the cause, prevention, diagnosis, treatment, and cure of digestive and liver diseases. The ADHF offers **research awards** that are directed toward furthering the understanding of the development of malignancies of the gastrointestinal tract as well as nonmalignant diseases. These grants support a broad spectrum of research ranging from basic science to purely clinical projects.

The R. Robert & Sally D. Funderburg Research Scholar Award in Gastric Biology Related to Cancer is offered to established investigators working on novel approaches to the understanding and treatment of gastric cancer. Awards are provided at $25,000 per year for two years for salary support, equipment, and supplies. Finalists will be selected and interviewed by a selection committee.

The Astra Fellowship/Faculty Transition Award is offered to physicians currently in a gastroenterology-related fellowship who are committed to academic careers and have completed two years of research training at the start of the award. Applicants may not currently hold similar awards. The award consists of $36,000 per year for two years to be used as a salary supplement. Applicants must be sponsored by a member of one of the ADHF organizations. Awardees will be selected by a committee at a study section in February of the year of the award.

The Student Research Fellowship Awards are provided to high school, undergraduate, medical, or graduate students not yet engaged in thesis research who do not have similar salary support from other agencies. Up to 35 awards, ranging from $1,500 to $2,500 each, are offered for students performing research under the direction of a preceptor for a minimum of 12 weeks.

Other awards offered through ADHF include the Research Training and Career Development Awards, Innovative Seed Grants in Basic Research in Liver Diseases, the Jan Albrecht Commitment to Clinical Research Award in Liver Diseases, Sponsored Research Symposium Awards, the Olympus Endoscopic Career Enhancement Awards, the Wilson-Cook Endoscopic Research Career Development Awards, the Miles & Shirley Fiterman Foundation Basic Research Awards, and the Miles & Shirley Fiterman Foundation Clinical Research in Gastroenterology or Hepatology/Nutrition Awards.

Applications for ADHF grants are available at: www.gastro.org/adhf/1999-2000ra.pdf ❖

CME Questions

11. The cytotoxicity of MGI-114 results from:
- A. disruption of microtubules.
- B. disruption of nuclear matrix.
- C. covalent binding of a reactive intermediate to DNA.
- D. generation of reactive oxygen free radicals.

12. Cell lysate vaccines:
- A. use the tumor cell as an antigen presenting cell.
- B. utilize adjuvants derived from bacteria or viruses.
- C. lack tumor-associated antigens.
- D. utilize only a single antigen.

13. Oltipraz:
- A. causes increased expression of Phase II detoxication enzymes.
- B. may have a broad protective effect against carcinogens in multiple organs.
- C. is an anti-schistosomal agent.
- D. All of the above.

14. Copper toxicity may be treated with copper-chelating agents or with:
- A. sodium.
- B. zinc.
- C. potassium.
- D. iron.

15. The same polyvalent vaccine may be used to treat different individuals, thus potentially lowering the cost of its production.
- A. True
- B. False

16. The risk of colitis-associated colon cancer is most closely associated with:
- A. red meat intake.
- B. saturated fat intake.
- C. duration of disease and extent of tissue involvement.
- D. fistula formation.

In Future Issues: Telomerase