MONOCLONAL ANTIBODY PRODUCTION FOR THE IDENTIFICATION OF HUMAN TRANSITIONAL CELL CARCINOMA

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CHAPTER 7

Discussion

There is an ongoing search for tumor identification techniques that may be of prognostic significance in making therapeutic decisions in patients with bladder cancer e.g., cystectomy vs. endoscopic management or in choosing the most appropriate chemotherapy. DNA banding studies have demonstrated certain chromosomes that identify low grade tumors (102) and abnormal chromosome patterns associated with invasive well differentiated tumors (103). Studies of cell surface expression found that the loss of the ABO isoantigens are associated with tumor invasiveness (104, 105). Research using lectins for characterizing the cell surface is under way (106). None of these techniques has so far proven to be the ideal test.

Three promising techniques for tumor study were evaluated here. The first explored the use of the soft agar clonogenic assay as a tool to identify the most effective response to chemotherapy and to evaluate drugs which have theoretical anti-tumor potential. The use of anti-CEA antibody as a tumor marker to determine prognosis was the second to be evaluated. Finally, a family of monoclonal antibodies was produced in an attempt to identify different bladder tumor cell types. Each of these techniques are discussed under their own heading later in this chapter.

[illegible] unexplained plateaus in survival curves (71). This may all be standardized in the future by the use of an automated image analyzer system (110) which could store and compare the change in size of each colony on each plate.

The rationale for the use of drugs in the treatment of cancer is to achieve the selective killing of tumor cells. This is based on the "cell kill hypothesis" elucidated by Skipper et al (30). For most drugs, a clear relationship exists between the dose of drug and its ability to eradicate tumor cells within the limits of toxicity to the host. A given dose of a drug kills a constant fraction of cells, not a constant number, regardless of the number of cells present at the time of therapy. Thus, cell destruction follows first order kinetics, and at least a one log response is required in order to

MONOCLONAL ANTIBODIES AND BLADDER CANCER

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ABSTRACT

The soft agar clonogenic assay was used to produce survival curves for several human bladder transitional cell carcinomas when exposed to mitomycin C, adriamycin, thiotepa, DMSO, retinoic acid and sulphapentosan sodium. These curves indicated in vitro growth inhibition with mitomycin C, adriamycin, and thiotepa, but not with DMSO, retinoic acid and sulphapentosan sodium. A resistant subpopulation of cells was documented with some tumors after exposure to mitomycin C and adriamycin. Growth of human bladder tumor stem cells in soft agar was not associated statistically with the grade of the tumor. The soft agar clonogenic assay was found to have potential as a research tool for the study of tumor responses to standard chemotherapeutic agents as well as an aid in evaluating new drugs as neoplastic agents in a phase II study.

A monoclonal antibody reactive to carcinoembryonic antigen (CEA) was used to stain thirty three human transitional cell bladder tumors. There was no statistical correlation between this antigen and tumor grade or growth in vitro. CEA was a poor indicator of tumor prognosis for transitional cell carcinoma of the bladder.

A family of monoclonal antibodies against human transitional cell carcinoma of the bladder was produced in mice by inoculating membrane preparations or whole cells derived from well differentiated human bladder tumor tissue. Two fusions produced 145 hybridomas which were initially screened by an enzyme linked immunosorbent assay (ELISA). Thirty two hybridomas were positive to a human bladder cancer membrane preparation and negative to a human liver membrane preparation. These thirty two hybridomas were further characterized using an avidin / biotin linked immunoperoxidase assay. Antibodies reactive to urothelium in a cell specific pattern were identified as well as grade specific antibodies. One antibody was found to be epithelial cell specific. Further characterization is needed to fully define the properties of these monoclonal antibodies.

RÉSUMÉ

Le dosage clonogénique sur gélose semi-solide a été utilisé pour produire des courbes de survie dans plusieurs cas de carcinome urothélial chez l'humain, qu'on a exposés à la mitomycine C, à Adriamycin, au thiotépa, au diméthylsulfoxyde (DMSO), à l'acide rétinoïque et au sulfapentosane sodique. Ces courbes indiquent une inhibition de la croissance in vitro avec la mitomycine C, Adriamycin et le thiotépa, mais pas avec le DMSO, l'acide rétinoïque ni le sulfapentosane sodique. Une sous-population résistante de cellules a été établie avec certaines tumeurs après exposition à la mitomycine C et Adriamycin. La croissance des cellules souches du cancer de la vessie chez l'humain dans la gélose semi-solide n'a pas été associée, sur le plan statistique, au stade de la tumeur. Le dosage clonogénique sur gélose semi-solide s'est avéré prometteur comme instrument de recherche pour l'étude des réponses des tumeurs aux agents chimiothérapeutiques standard et utile pour l'évaluation de nouveaux médicaments comme agents antinéoplasiques dans une étude de phase II.

Un anticorps monoclonal réagissant à un antigène carcino-embryonnaire (ACE) a été utilisé pour colorer trente-trois carcinomes urothéliaux chez l'humain. Il n'y a pas de corrélation entre cet antigène et le stade de la tumeur ou sa croissance in vitro. L'ACE s'est révélé un piètre indicateur de pronostic pour le carcinome urothélial chez l'humain.

Une famille d'anticorps monoclonaux contre un carcinome urothélial humain a été créée chez une souris par l'inoculation de préparations membranaires ou de cellules entières dérivées de tissu tumoral bien différencié provenant de la vessie humaine. Deux fusions ont produit 145 hybridomes qui ont d'abord été détectés par un dosage enzymo-immunologique (ELISA). Trente-deux hybridomes se sont révélés positifs à une préparation membranaire d'un cancer de vessie humaine et négatifs à une préparation membranaire de cancer du foie humain. Ces trente-deux hybridomes ont plus tard été caractérisés au moyen d'un test à l'immunoperoxydase à liaison avidine-biotine. Des anticorps réagissant à l'urothélium d'une façon dépendante des cellules ont été identifiés de même que des anticorps dépendant du stade. Un anticorps s'est révélé dépendant de la cellule épithéliale. Une caractérisation plus poussée est nécessaire pour définir tout à fait les propriétés de ces anticorps monoclonaux.

CHAPTER 1
Introduction

Transitional cell carcinoma of the bladder has been and is a therapeutic challenge to the urological oncologist. It is apparent that this heterogenous tumor has two distinct natural histories (1). The first consists of low grade, low stage superficial tumors that may be managed with conservative treatment. The second encompasses high grade, high stage invasive tumors that require intensive treatment; they metastasize early and have a guarded prognosis. Unfortunately, the prognosis is difficult to ascertain for an individual patient since the clinical presentation, findings and histology do not always correlate with the tumor's aggressiveness.

This thesis will approach these problems by focusing on two in vitro techniques that assist in identifying properties useful for the prognosis, treatment and characterization of human transitional cell carcinoma of the bladder. The first is the soft agar clonogenic assay. This short term project describes the growth of transitional cell carcinoma in agar and the response in growth of various transitional cell tumors when exposed to traditional chemotherapy as well as other chemical agents which have theoretical therapeutic potential. The formidable task of finding the optimum response to a specific drug treatment as well as the search for new drugs which may effectively be used in the treatment of this tumor will be addressed by using the soft agar clonogenic assay.

The second in vitro technique is the use of monoclonal antibodies. This thesis evaluates the ability of a reference mouse monoclonal antibody against CEA (carcinoembryonic antigen) to perform as a tumor marker in human transitional cell carcinoma. It describes the initial characterization of a number of new mouse monoclonal antibodies which were developed against human transitional cell carcinoma using hybridoma technology. The clinical enigma of placing a patient into a specific prognostic group in order to avoid over or under treatment is addressed by studying monoclonal antibodies as cell markers.

The work reported herein is separated into 4 divisions (8 chapters). The first division introduces background concepts and consists of one chapter with 3 sections. In the first section of this chapter (section 2.1), the historical aspects and possible

applications in the solution of therapeutic problems of the soft agar clonogenic assay are discussed and current and experimental chemotherapeutic agents are introduced. The last section of this chapter (section 2.2) discusses the concepts and importance of monoclonal antibody production using the hybridoma technology developed by Kohler and Milstein (2). The use of a reference monoclonal anti CEA antibody as a transitional cell bladder cancer cell marker is discussed in section 2.21. The development of new monoclonal antibodies is outlined in section 2.22.

In the second division, consisting of chapter 3, the methods and materials used in the experiments are presented.

Division 3 comprises three chapters and presents the results of this thesis. The first, chapter 4, deals with the results of the experiments using the soft agar clonogenic assay to characterize human transitional cell carcinoma and to study drug response and resistance. The second, chapter 5, deals with the use of a mouse monoclonal antibody against CEA as a tumor marker of human transitional cell carcinoma. The final chapter of this section, chapter 6, discusses the development and initial characterization of a group of monoclonal antibodies developed against fresh human transitional cell carcinoma of the bladder using hybridoma technology.

The last division (chapter 7 and 8) discusses and summarizes the findings of this thesis and presents some of the future applications and investigations initiated by this work.

CHAPTER 2
Review of the Literature

Section 2.1: The Soft Agar Clonogenic Assay

The need for further study and characterization of transitional cell carcinoma of the human bladder and its treatment becomes apparent when reviewing its effects on the population at large. Its incidence is 20 per 100,000 for the over 40 years old age group (3), and it accounts for 2 per cent of all malignant cancers. Twenty percent of all cases present with an advanced stage (4) and in spite of radical surgery, radiotherapy and chemotherapy, is infrequently cured, and only seldom adequately palliated. The low stage or superficial tumors can be controlled with local resection, but require careful follow up since there is a 50 to 70 percent recurrence rate of which 10-20 percent will become invasive (5). In both advanced and local disease, there is need for new treatment modalities, new chemotherapeutic agents or more accurate indicators of response to the use of current chemotherapy. The soft agar clonogenic assay has the potential to fill these needs.

The soft agar clonogenic assay is an in vitro technique which allows growth of tumor cells that have the ability to divide indefinitely and are known as stem cells (6). These stem cells form colonies in agar and can be identified and counted (7, 8). They are of particular interest to the oncologist because they are the target of all cancercidal therapy and as such are potentially useful in monitoring the response to therapy.

The development of in vitro testing of chemotherapeutic agents started in the 1904's shortly after chemotherapy was first introduced. Each method studied the decrease in the number of cells with proliferative potential in response to drug treatment. The protocols included biochemical assays of thymine synthesis for 5FU and inhibition of folate reductase by methotrexate for measurement of nonspecific effects such as morphological changes, oxygen consumption, or cell wall integrity in vitro. Unfortunately, these techniques were limited by insensitivity, expense, and highly specialized technology. Other tests such as radioactive "labeling uptake" have shown promise in reflecting clinical sensitivities for hematological tumors, but are

not useful for solid tumors. Animal systems such as the nude mouse or implants beneath the renal capsule in normal mice have also been used successfully. However, cost and manpower requirements make these techniques prohibitive. Ideally, one should test each drug for activity against the individual patient's tumor and plan chemotherapy on the basis of sensitivity tests just as one does with antibiotic therapy against bacterial infections.

One approach in solving the problems of the afore mentioned chemotherapy testing evolved from the early work of Costachel and associates. They found that agar beneath growth media inhibited the growth of fibroblasts. About the same time, Park and associates (9) developed a soft agar system for assaying mouse myeloma cells using a feeder layer of irradiated, tumor inoculated spleen cells. In 1977, Hamburger and Salmon (10) reported a method using a double layer of soft agar in which myeloma stem cells grew. The underlayer utilized a media incorporating mice spleen cells. Later, they were able to grow many non-hematologic human tumors using an enriched standard culture media with 20% horse serum. This human clonogenic tumor assay is commercially available, inexpensive, and easy to use (see figure #1).

The malignant nature of the colonies which grow in the clonogenic soft agar assay was confirmed in several ways. First, colony histology between parent tumors and clones was shown to be the same. Second, using electron microscopy, malignant ultrastructures were found in clonal colony cells. Karyotypic examinations of clone colonies were the same as the original tumor. Finally, injection of the clone into nude mice produced histologically similar tumors to the original.

Clinical correlation of the clonogenic assay with results of therapy was reported by several researchers for myeloma, ovarian cancer, breast cancer, lung cancer, and neuroblastoma (11-13). The major finding indicated that if the clonogenic cell of the tumor are resistant to a given cytotoxic drug in the in vitro assay, then there was a greater than 90 per cent chance that the tumor itself would be unresponsive when the patient was treated with the same drug. It was also suggested that the behavior of a tumor in the clonogenic assay might provide insight into its behavior in the patient and in certain settings have the potential to be helpful for staging or following the cancer (14).

To date, approximately 25% of all reported clinical cases tested by the

Soft Agar Clonogenic Assay

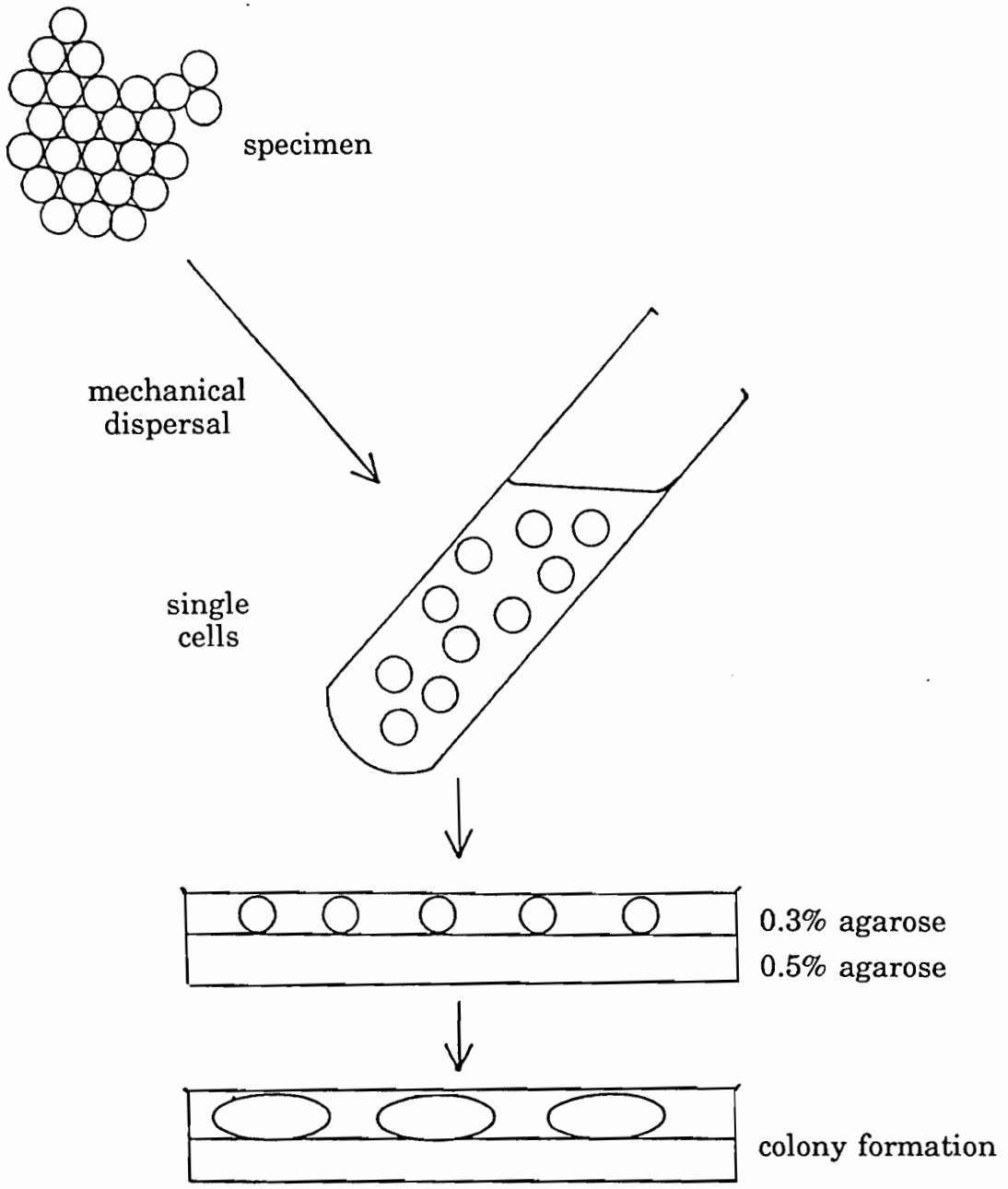


Figure #1: Preparation of the soft agar clonogenic assay.

clonogenic assay technique generate clinically relevant information for use of specific chemotherapeutic agents (15). The problems that limit the application of this test are low plating efficiencies, small numbers of tumor cells supplied for the test, difficulty in preparing single cell suspensions, and a general lack of knowledge of chemotherapy concentrations, exposure times, and cell survival curves for each type of tumor.

The use of the soft agar clonogenic assay for studying transitional carcinoma of the bladder was reported in several recent papers (14, 16-23). Studies were used to assess cell growth in vitro, identify tumor cells in bladder washings, isolate and give prognosis for varying histological types of tumors, and test various drugs against tumors. These studies are preliminary and several are based on 10 patients or less.

There are no published studies of transitional bladder cancer response curves to various concentrations of chemotherapeutic agents, though Lieber has grown cells in the soft agar assay with continuous exposure to one concentration of drug (24, 25). In some tests using the soft agar assay single cell suspensions of tumor are placed in a growth medium containing a concentration of the chemotherapeutic agent to be studied. After one hour in this medium, the cells are washed and plated. This concentration is usually based on a peak blood level obtained clinically during patient treatment. It is obvious that this arbitrary protocol will be inaccurate in many instances, for it does not take into account tissue uptake of the drug, fraction of cell population in drug sensitive phases of the mitotic cycle, or drug induction of enzymatic activity in tumor tissue. Production of response curves for specific tumor types with continuous drug exposure would be useful in evaluating these factors as well as indicating the best concentration and time of exposure required for correlating in vitro with in vivo response.

The agent used in intravesical chemotherapy should have certain specific properties. It should have direct activity against transitional carcinoma, act by contact over a short period of time, not be phase specific, and not be susceptible to resistance. It should not be toxic, and should demonstrate minimal local side effects. The latter two properties can only be determined by clinical studies. The other properties should be known prior to clinical use and could possibly be evaluated using the soft agar clonogenic assay as a screening procedure (26).

The use of the soft agar clonogenic assay in determining response curves and

sensitivities of transitional cell carcinoma of the bladder to chemotherapy may increase the future efficiency of the phase II trial and decrease the morbidity of patients from unselected and unproven new drugs.

Currently there are several intravesical chemotherapeutic drugs which may be evaluated for their potential in treating a given case of transitional cell carcinoma of the bladder using the soft agar clonogenic assay technique. These are thiotepa, adriamycin and mitomycin-C. In addition, cell differentiating agents such as retinoic acid and DMSO, and a GAG membrane stabilizing agent sulphopentosan (27) that may also be beneficial in the treatment of bladder tumor may be evaluated using a similar assay.

Thiotepa was the first intravesical chemotherapy agent and is still the most widely utilized agent today. This drug is a non phase specific alkylator of purine and pyrimidine bases which cause cross linking between DNA, RNA and proteins. This usually blocks the cell cycle at the G2 premitotic phase where the affected cells have a double compliment of DNA and continue to produce cellular elements resulting in unbalanced growth and eventual cell death. It is usually administered in doses of 30-60 milligrams at a concentration of 1 milligram per milliliter. The first dose is given between 48 hours and 7 days after tumor resection, and then 4 weekly doses, followed by monthly doses for 1 year. The treatment is terminated if there is no evidence of tumor or further tumor growth. From the data collected from 11 reported series (28-40), this drug has a complete response rate of 29%, and a partial response rate of 26%. Thiotepa was noted to perform best when used against low grade tumors, but with little effect against high grade tumors (41). Thiotepa is absorbed through the intact urothelium, and the amount absorbed increases in the presence of inflammation, tumor, or urothelial disruption. Myelosuppression is a noted side effect that is dependent on the amount of thiotepa absorbed systemically (42, 43) and occurs in 2-25% of patients treated (44).

Adriamycin is a non phase specific intercalator for which the mechanism of action is not yet specifically known. This drug was first used in Italy between 1967 and 1969. It is a product of Streptomyces peucetius, variety Caesius with a molecular weight of 579.9. The high binding affinity of adriamycin to DNA was demonstrated in vitro. Its maximal effect occurs during S phase (45, 46). Adriamycin has an 11%

absorption when administered as an intravesical agent. Its use as a topical intravesical agent was first reported in 1972 by Pavone-Macaluso and Caramia (47). Twelve other reports have since been published. From these, the complete response rate is 38% while the partial response rate is 35% (42-59).

Mitomycin-C is a non phase specific antitumor antibiotic. It is minimally absorbed when given intravesically, probably due to its molecular weight of 329. The drug was isolated from the Streptomyces casepitosus fungus twenty years ago but has only recently been used as an anti neoplastic agent. It inhibits DNA synthesis by inducing interstrand and intrastrand DNA cross-links, and degrades DNA (60, 61). Its maximum action is expressed in cells in the late G1 phase or S phase of the cell cycle. The usual dose ranges from 20-60 milligrams, usually in a concentration of 1 milligram per milliliter. The treatment schedule varies widely, 1-2 times per week for 21 weeks to once every two weeks for several years. From the five published studies, the complete response rate is 48% and the partial response rate 26% (62-66). Two recent studies have documented a reduction in the death rate from bladder cancer when intravesical mitomycin C was used (41, 67).

Both retinoic acid and DMSO have been described as differentiating agents. Retinoic acid is used in superficial epithelial tumors as an anticancer agent (68). It was shown that vitamin A deficient rats will develop bladder tumors earlier than normal animals (69). Retinoic acid appears to have an adjuvant effect when combined with BCG and applied to soft tissue sarcoma (70). A number of animal studies utilizing analogs of retinoic acid have indicated an inhibition of tumor growth (71, 72). Retinoic acid is required for normal differentiation and one could hypothesize its usefulness in the topical treatment of human transitional cell carcinoma of the bladder.

DMSO has been shown to induce morphological differentiation in Friend leukemic cells, rabdomyosarcoma cells, neuroblastoma cells, and human promyelocytic leukemia cells (80-84). This effect may occur with human bladder tumors, making it potentially useful in topical bladder cancer treatment.

Sulphopentosan sodium has been used in interstitial cystitis to renew the GAG layer of the bladder wall. This effect may be potentially useful as a topical therapy for bladder cancer as it may protect against the development of further malignancy in

response to urinary carcinogens.

Several transitional cell carcinomas of the human bladder of various stages and grades will be tested using the soft agar clonogenic assay. The treatment response of these cancers to the above drugs will be examined by analyzing the drug response curves generated.

Section 2.2: Monoclonal Antibodies

The struggle to discover better predictors of biological behavior for transitional cell carcinoma of the bladder has gone on for many years. Clinical tests such as endoscopy, CT scans, ultrasound, cytology and histology of biopsied lesions have not been found to be adequate to date. Research has turned to utilizing new techniques for prognosis such as blood group ABH antigens markers, Thomsen-Fredenreich antigen markers and karyotyping of the initial tumor (78, 79). In fact, recently, with the development of sensitive and specific monoclonal antibodies, detecting and using cell markers for prognosis has proved very promising.

The exploitation of antibodies directed against tumor cell surface markers for diagnosis and treatment began at the turn of the century (80). Despite great hopes and extensive experimental attempts (81), antibody use as a therapeutic modality has not been successful in humans. The lack of tumor specific antigens, with the exception of idiotypes on B-cell tumors (82), which distinguish absolutely between tumor and normal cells, may be the prime cause of this failure. However, there are a number of tumor related antigens (in melanoma, colon carcinoma, neuroblastoma, leukemia, lymphoma, lung cancer, glioma, and mammary carcinoma) which are more or less restricted to tumor cells and their cells of origin, and which have been used to aid in the diagnosis and prognosis of disease (82).

Initially, conventional antisera containing the products of hundreds of thousands of different antibody secreting clones were used in the isolation of a specific tumor antigen. Many of these polyclonal antibodies were directed against undesirable antigens or impurities. This antibody sera could only be produced in limited quantities and wide variations between batches was common. Standardization between laboratories was impossible. Only recently, due to a major break-

through, have these drawbacks been overcome.

Kohler and Milstein (2) described a procedure for long term production of monoclonal antibodies that combined the use of cultured plasmacytoma cells for long term production of monoclonal antibodies with a selected clone of spleen cells making an antibody of predetermined specificity. Specifically, the antibodies, mouse anti-sheep red blood cells, were made by fusing together spleen cells from a line of mouse myeloma cells that lack a purine salvage enzyme, hypoxanthine phosphoribosyl transferase, and thus die in a selective medium containing hypoxanthine, aminopterin and thymidine (first described by Littlefield). At best, only about one myeloma cell in a thousand fuses to a spleen cell to give a viable hybrid. This hybrid will be selected as the unfused spleen cells will naturally die after a few weeks. The surviving hybrid cells can then be screened for the designed antibody activity. Since the advent of this hybridoma technology in 1975, the technique has become a routine as well as an essential tool in many research laboratories for the production of specific antibodies in the characterization of a variety of biological systems.

Section 2.2.1: CEA Antibody Reactivity To TCC

Carcinoembryonic antigens (CEA) are glycoproteins with a molecular weight of 200,000. They were first described by Gold and Freeman in 1965 (83, 84). Originally, CEA was believed to be a specific antigen in colonic adenocarcinoma. However, they have been found in higher concentrations in the serum or the urine of patients with various types of carcinoma. Though reports have been presented that CEA is present in urogenital carcinomas (85-86), CEA blood levels have not been found to be a reliable tumor marker of transitional cell carcinoma of the bladder because of high false positive or false negative rates.

The idea that CEA may be a tumor marker for human transitional cell bladder cancer developed from the work of Goldenberg et al (87) who showed CEA to be present in 4 out of 35 patients with transitional cell carcinoma of the bladder by the triple layer immunoperoxidase method. Goldenberg and Wahren reported that the CEA content in cancer tissue does not always correlate with levels in the serum or the urine because plasma and urine levels depend on a number of factors, such as CEA

biosynthesis, release into the blood or urine, and host metabolism (88). Furthermore, Leung et al reported that membrane bound CEA are different from those found in the serum or urine (89). This would indicate a possible role of tissue CEA in determining the malignant potential of a specific tumor.

Nakatsu et al also studied the CEA content in transitional cell carcinoma of the bladder (90). They found that CEA contents correlated well with histological grade, stage and survival rate of patients with bladder cancer. This conclusion was based on 54 patients of which 15 had tumors positive for CEA. However, they discarded 15 of the tumors studied due to the difficulty they had in obtaining good positive stains with their immunoperoxidase technique. This introduces a large margin of error in their data. Even so, this use of a known tumor specific marker as a grade specific marker merits further consideration.

The promise of increased sensitivity in technique by the use of a multiplier step such as that found in the avidin/ biotin technique used in the method of this thesis, as well as the availability of an anti-tissue CEA monoclonal antibody whose specificity has been well proved (91) provided a incentive for the research here to test this tumor marker for possible prognostic significance for transitional cell carcinoma of the bladder.

Section 2.2.2: The Production of New Monoclonal Antibodies

Currently, monoclonal antibodies are produced in three systems; mouse, rat, and human. Each has advantages and disadvantages. Xenogenic immunizations respond to a wide variety of antigens, but this may include many normal antigens expressed on tumor cells (e.g. histocompatibility antigens, blood group substances, etc.). Work with the human system is limited, in many cases, by the lack of readily obtainable B cells. Peripheral blood may not contain sufficient tumor specific lymphocytes in the right stage of differentiation to yield productive fusions. Lymph nodes draining tumors are easily obtained for some tumors, but impracticable for others: e.g. bladder cancer. Thus, the mouse or rat system are most productive for bladder tumor studies (see figure #2).

Detergents, enzymes, mechanical lysis and separation of cells have all been

Monoclonal Antibody Production

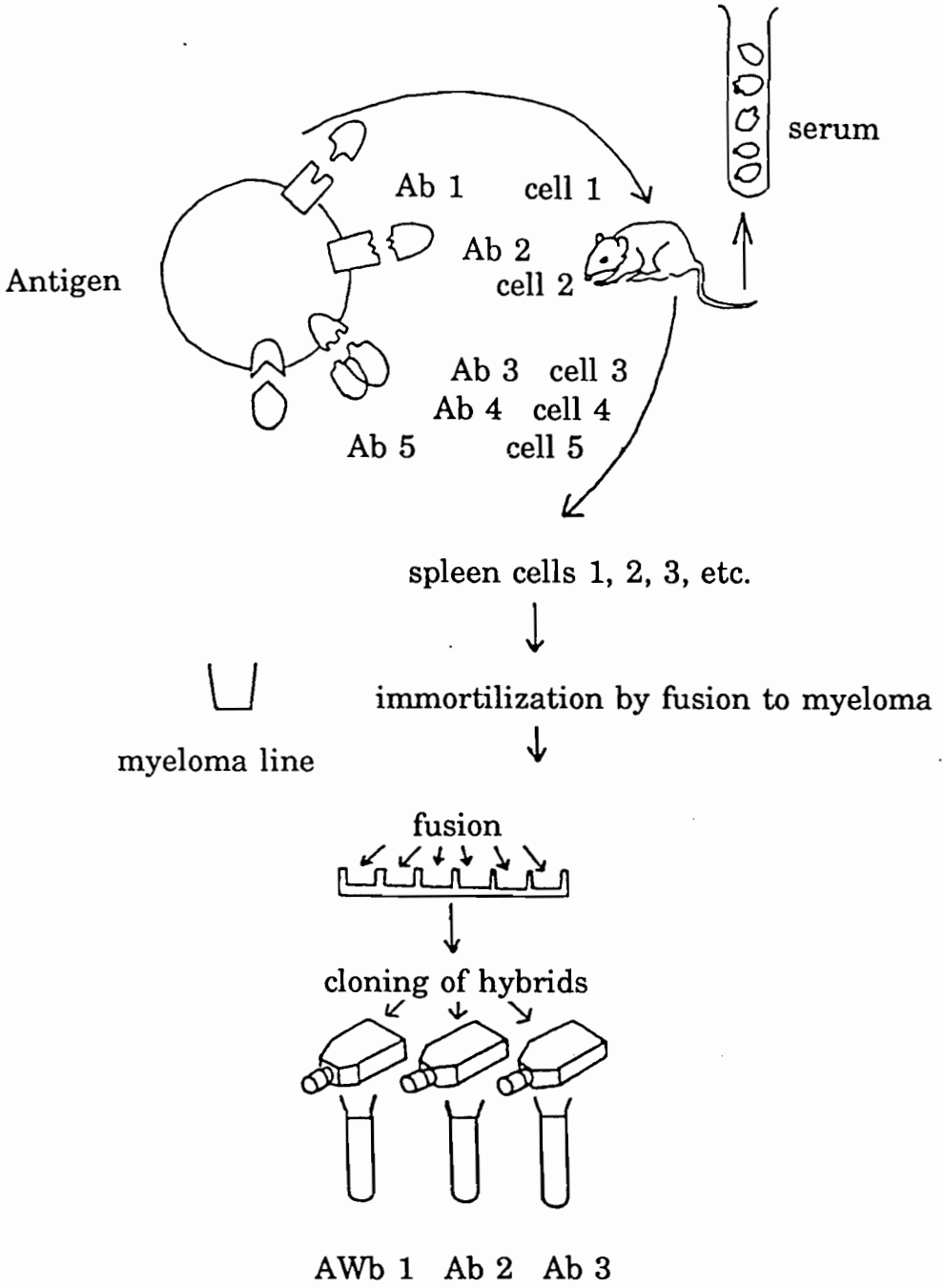


Figure #2: The production of pure antibodies from pure antigens.

used to prepare material for immunization. Since there apparently are no obvious advantages of one method over another, the mechanical lysis and separation of cell wall from the remaining cellular material is utilized here.

Most bladder cancer researchers have utilized cell lines as immunogens due to the ease in obtaining material (92-97). This may be an inadequate model of real human tumors, as some antigens may be lost or poorly expressed on the surface of in vitro cell lines (98). The monoclonal antibodies to human bladder cancer produced to date support this theory. Thus far, four laboratories have reported the pattern of reactivity of their reagents with cell lines (92-95). Two (95-97) tested their monoclonal antibodies against fresh human material. Fradet (97) described one group of monoclonal antibodies that appear to have the potential of distinguishing among tumors of varying malignancy. This group of monoclonal antibodies was the only one produced using fresh human transitional cell carcinoma as the immunogen. For this reason, primary human material was used in the immunization, screening and characterization described here.

Immunization protocols have varied considerably from one laboratory to another. All protocols are designed to expose the desired antigens maximally to the animal immune system. Timing of injections are based on the ability of the animal's immune system to recognize and produce B cells tot he immunogen, and is standard for the rat or mouse system used. Adjuvants are frequently used to enhance antibody production. The system which is used here makes use of Freund's complete and incomplete adjuvant mixed with the immunogen.

The screening procedure is the key to successful hybridoma production. There are two basic approaches. One is to select all cell hybrids producing antibodies that bind to the cell used as the immunogen, and subsequently study their specificity. The alternate approach is to design a selective assay for screening the hybrids so that only those making the antibody of the desired specificity are selected. The latter produces antibodies which are immediately useful and will be used here for the selection of antibodies which are 1) prognosis specific. 2) bladder tumor cell type specific. 3) tumor specific, or 4) urothelial specific.

There are many assays which may be used to detect the presence of antibodies (99). These include assays ranging from precipitation reactions and radioimmunoassays

ABC Immunoperoxidase Staining Method

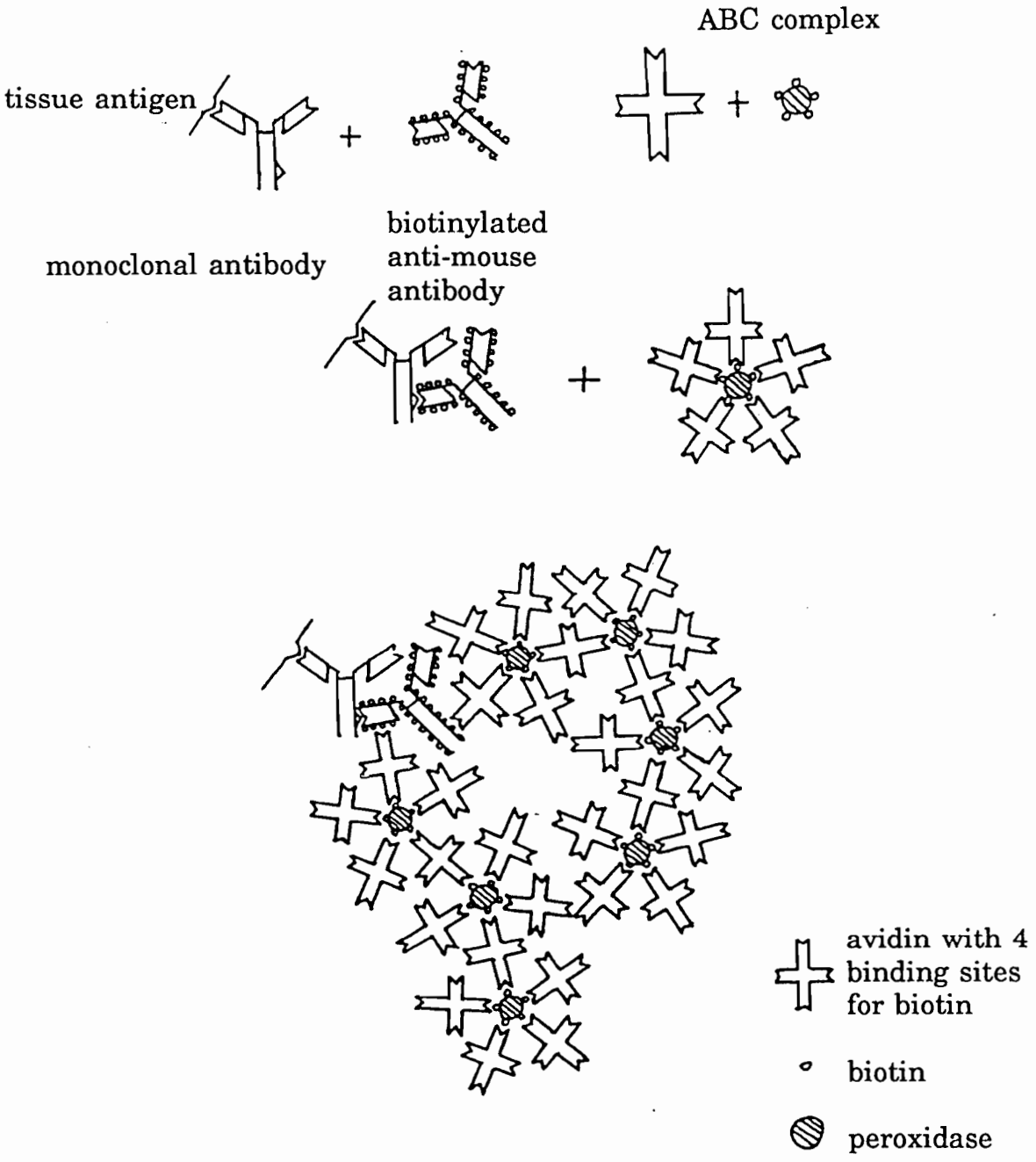


Figure #3: Multiplying effect of the ABC immunoperoxidase staining method.

to assays based on the biological activities of recognized antigens. However, not all of these may be applied to monoclonal antibodies because the concentration of the antibody in the tissue culture supernatant is usually much lower than that of a hyperimmune serum, and traditional immunoassays often rely on the polyvalent recognition of antigens typically obtained with polyclonal antisera.

One popular assay is the enzyme linked immunosorbent assay (ELISA) (99). This has the advantage of providing a specific and highly sensitive method for identification and quantitation of a wide range of substances (see Methods and Materials section 3.11 for details of procedure). The enzyme label used here was horse radish peroxidase. This could be obtained in large pure amounts, has high specific activity, is stable and soluble, is absent from biological fluids, is capable of amplifying the reaction locally and is easily attached to an anti-mouse antibody. The ELISA procedure is inexpensive and relatively easy to perform and is used here for the preliminary screening of all monoclonal antibodies produced from fusions.

The preliminary screening of the monoclonal antibodies produced by the hybridomas will be against a human liver membrane preparation using an ELISA technique (100). This will select antibodies which are generally reactive to human cells, and therefore can be discarded. The next screen involves a membrane preparation made from a mixture of bladder tumors of several grades and stages. Only hybridomas which react very positively are kept for further study. These human bladder tumor reactive hybridomas are then cloned and grown in tissue culture until sufficient numbers are available for preservation by freezing. The procedure is similar to that described by Kennett (81).

Once the preliminary screening is completed, the remaining antibodies are screened using an avidin-biotin immunoperoxidase staining technique (see figure #3). This method of screening is many times more sensitive than the ELISA, but is also more time consuming and difficult to perform (see Methods and Materials section 3.13 for details of procedure). The immunoperoxidase technique produces a permanent record and allows for a qualitative as well as quantitative pattern of antibody uptake. This method has the advantage of making use of only one labeled antibody for the activation of a large number of antibodies, and uses the avidin-biotin system as a multiplier for more sensitivity.

To evaluate the bladder specificity of the antibodies, normal human tissue, including bladder, are used in the secondary screening. In the final screening for antibodies against bladder cancers of varying grades, tumor specificity can be evaluated. The ultimate assessment is made by examining differential staining on normal bladder or bladder cancer slides.

Chapter 3
Methods And Materials

Section 3.1: Human Tissue

Eighty-four specimens of human bladder tumor tissue were obtained from the operating room immediately after removal either trans urethrally (70) or by open resection (14). The material used for study was selected by the surgeon and pathologist directly involved with the cases, so that a representative portion of the study material was histologically examined. There were 39 well differentiated, 18 intermediate and 27 undifferentiated tumor specimens. The tumor specimen destined for in vitro studies was placed in sterile saline at 4^0 C, and transported immediately to the laboratory. Other types of tissue were obtained from autopsy specimens less than 6 hours old, surgical pathological specimens, and cadaver kidney donors; these were transported to the laboratory in a similar fashion.

Section 3.2: Flash Freezing Technique

Upon arrival, a representative portion of the specimen was placed in gelatin capsules for molding purposes, embedded with O.C.T. media, and flash frozen in an isopentane solution cooled by suspension in an isopentane/ dry ice slurry. The gelatin capsules containing the frozen tissue were then placed in a minus 80 degree centigrade freezer for antibody testing.

Section 3.3: Single Cell Suspension Preparation

A portion of the bladder tumor specimen was transferred into alpha growth media (Flow Laboratories) with 10% fetal calf serum (Flow Laboratories) and penicillin and streptomycin (Flow Laboratories). Tumor dispersion was achieved by using a scalpel to reduce the tumor to small pieces before putting the tumor specimen through a coarse mesh (500 micron), and passing the resulting suspension gently through an 18 gauge needle. Enzymes were not used in tumor preparation. Prior to

plating, the viability of the tumor specimen was assessed by staining a small portion with trypan blue dye (Gibco Laboratories). Cells that excluded the dye were counted as viable. The total and viable cell counts were performed using a hemocytometer.

Section 3.4: Plating

Tumors were plated in triplicate in 6 well dishes 3.5 cm in diameter and 1 cm deep (Linbro). The bottom of each well was covered with 1.5 millimeters of alpha growth media containing 5% agarose (type 7 Sigma). this underlayer was allowed to congeal prior to plating. The cell suspension was then examined to ensure the absence of numerous clumps. $5x10^5$ cells suspended in 1 ml of alpha growth media containing 10% fetal calf serum, 10% penicillin and streptomycin, and 3% agarose were then placed into each well. This was allowed to set, and the plates were transferred into a 37 degree centigrade incubator containing 5% carbon dioxide.

Section 3.5: Scoring Growth After Plating

Within 24 hours of plating, the plates were counted for clumps with he use of an inverted microscope. Aggregates of cells larger than 100 microns in diameter were considered to be clumps, and this count was subtracted from the initial colony count 2 weeks after plating. Aggregates of cells that were symmetrical and larger than 100 microns in diameter were counted as colonies at the time of the initial colony count. This measurement criteria was used since the average cell size was found to be 12 microns, and a 100 micron diameter aggregate would represent approximately 200 cells. This would represent a doubling time of 1.75 days if a 100 micron diameter aggregate originated from a single cell. This rate of growth was acceptable as a lower limit of human bladder cell replication (115). In addition, very few clumps larger than 100 microns in diameter were seen within 24 hours of plating, further supporting this technique of scoring growth after plating.

Section 3.6: Drug Sensitivity Assay In Soft Agar

Drug sensitivity assays were done using 6 agents: thiotepa (Lederle), adriamycin (Adria Lab.), mitomycin C (Bristol Lab.), retinoic Acid (Sigma), DMSO (Fisher), and sulphopentosan sodium (Pharmacia Inc.). In each drug sensitivity assay, the tumor cells were plated as described above. Each drug concentration was plated in triplicate and included a control. The drugs were mixed immediately before use, and added in the appropriate concentrations to the cell suspension immediately prior to plating. Continuous exposure of drugs was used in our assay to facilitate the plating of the tumors and to keep the trauma of the tumor cells to a minimum (we have noted clump formation when the tumors are centrifuged indicating that further separation was required).

Section 3.7: Membrane Preparation

Low grade tumor cells from a single patient were washed three times with PBS, and the final cell pellet was suspended in 0.01 M TRIS/ 0.2 mM CaCl2 (1:6.6 weight of the pellet in grams to volume of buffer). The cells in the suspension were sheared 5 times using a Polytron Homogenizer (Brinkman) (homogenized 15 seconds, cooled in an ice bath 15 seconds). The homogenized suspension was examined microscopically for the number of intact clumps of tissue, and if there were many clumps of cells, the homogenization was repeated. A stirring rod was placed into the homogenate of cells, followed by immersion in a nitrogen bomb which was immersed in an ice bath. During continuous agitation, the tumor suspension was exposed to 1000 PSI for 30 minutes, then decompressed. The suspension was then examined for the number of intact cell membranes, and the bombing was repeated until less than 10% of the cells remained intact. After bombing, the suspension was centrifuged at 1000 G for five minutes, and the supernatant was removed. The pellet was resuspended in 0.01 M TRIS/ 0.2 nM CaCl2 buffer (0.5 ml the volume used to suspend the cells initially), and centrifuged again for five minutes at 1000 G. The supernatant from this was added to the previous supernatant, and the pellet discarded. The supernatant was then layered on the interface of 20% / 40% sucrose dissolved in

0.01M TRIS and 2.0 mM CaCl2 solution, and centrifuged at 100,000 G for 22 hours. After centrifugation, the band between the 20% and 40% sucrose solutions was removed, diluted with 5 volumes of 0.01 M TRIS, and centrifuged at 100,000 G for 2 hours. The pellet was resuspended with 500 microliters of PBS, and assayed for protein content using the Biorad assay. PBS was added to the membrane preparation to adjust the concentration to 1 milligram of protein per milliliter. The preparation was then aliquoted into 100 micron tubes (Linbro) and stored at minus 80 degrees centigrade.

Section 3.8: Membrane Preparation Protein Assay

The BIORAD assay was used to estimate the protein content of the membrane preparations. Seven 3 ml plastic tubes (Linbro) were filled with 0.8 ml of PBS containing Ca and Mg. One tube was used as a control, 4 others had 2, 4, 8, and 12 microliters of 1 mg/ ml bovine serum albumen (BSA) added to them. The remaining 3 tubes had 2, 4, and 8 microliters of the membrane preparation added respectively. 0.2 ml of BIORAD dye was added to each tube and the contents of each tube was placed into a cuvet and read on a spectrometer at 595 Angstroms. The control tube was used as a reference. PBS was added appropriately to the membrane preparation to decrease the protein content to 1 mg/ ml.

Section 3.9: Hybridoma Immunization

Two BALB/ c female mice 8 weeks old were used for two fusions, #48 and #51. Both mice were injected using the same protocol for the first 5 months. This protocol consisted of washing $5X10^7$ well differentiated grade 2/ 3 cells three times in PBS, and resuspending the cells in 0.2 ml of PBS, which were then injected intraperitoneally. This protocol was repeated each month for each animal.

The mouse used for fusion (#48) was injected with a total of 100 micrograms of membrane preparation #4 (a well differentiated tumor) suspended in Freund's incomplete adjuvant (.1 volume to volume) in two different sites on its flanks during the sixth month. In addition, $2x10^7$ cells from another well differentiated grade 2/ 3

tumor were washed three times in PBS, and resuspended in 0.2 ml of PBS, and injected intraperitoneally. In the seventh month, the mouse was injected with 300 micrograms of membrane preparation #7 (a well differentiated tumor grade 2/ 3) suspended in Freund's incomplete adjuvant (1.1 volume to volume) divided equally between two subcutaneous sites, and one intraperitoneal site. One week later, 15 micrograms of membrane preparation #7 suspended in 0.1 ml PBS was injected intavenously. Three days later, the fusion was performed.

The mouse used for fusion #51 was injected in the seventh month in each of 3 subcutaneous sites on the flanks, with $5x10^7$ cells of a well differentiated tumor after washing three times in PBS and resuspending in 0.2 ml of PBS and $2x10^6$ cells of the same tumor washed three times in PBS and resuspended in 0.2 ml of PBS intraperitoneally. In the eighth month, 300 micrograms of membrane preparation #7 were suspended in an equal volume of Freund's complete adjuvant and one quarter injected in each of 3 subcutaneous sites and one intraperitoneal site. In the ninth month, the same procedure as used in the eighth month was repeated substituting Freund's incomplete adjuvant for the Freund's complete adjuvant. Two weeks later, 15 micrograms of membrane preparation #7 suspended in 0.1 ml of PBS was injected intravenously, and the fusion performed three days later.

Section 3.10: Hybridoma Fusion

The mouse used for the fusion was sacrificed and immediately wet down with 70% ethanol. The spleen was removed aseptically and placed in a P60 plate (Limbro) containing 3 ml of IFM (incomplete feeding media lacking fetal calf serum) and gentamicin (Flow Laboratories). The fat was trimmed from the spleen, and the spleen was transferred to a second P60 plate containing 3 ml of IFM and gentamicin. The spleen was cut in half, and the spleen cells were teased out using forceps. The cell suspension was transferred to a 15 ml conical tube (Limbro), and the large pieces of tissue allowed to settle for 2 minutes. The suspension was removed to another conical 15 ml tube leaving the tissue debris in the bottom of the first tube to be discarded. The suspension was then centrifuged at 100 G for 7 minutes. The supernatant media was aspirated off and discarded. The pellet was resuspended by gently tapping the

tube. 3 ml of ACT (Gibco Laboratories) were added to the pellet, and the pellet was gently resuspended. After 10 minutes at room temperature, 5 ml of IFM and gentamicin were added, and the suspension was centrifuged at 100 G for 7 minutes. The supernatant was discarded and the pellet resuspended in 4 ml of IFM and gentamicin. 0.9 ml of 0.5% trypan blue dye was added to 0.1 ml of cell suspension, and the viable cells were counted.

SP2/ 0-Ag14 myeloma cells in log phase growth were pelleted in a 50 ml conical tube (Sorvall RC3B) at 100 G for 10 minutes. The growth media was aspirated off and discarded. The pellet of cells was resuspended by gently tapping the tube and then adding 25 ml of IFM and gentamicin, inverting the tube to wash the cells. One half ml SP2 suspension was added to 0.5 ml of 2% trypan blue dye, and a visible count was performed. The cell suspension was again centrifuged at 100 G for 10 minutes and the pellet resuspended in IFM and gentamicin as before, so that a final viable cell count of $5x10^7$ cells per ml was obtained.

A 30% polyethylene glycol (PEG) (BDH Chemicals) solution was prepared prior to fusion. A microwave oven was used to liquefy an aliquot of PEG 1000, and after cooling somewhat, 1.5 ml was transferred to a tube containing 3.5 ml of IMF and gentamicin. After mixing, the pH was adjusted to the range of 7.2-7.4.

The SP2 cells and spleen cells were combined in a ratio of 1:5 viable cells in a 15 ml round bottom sterile tube (Limbro). The cells were centrifuged at 100 G for 10 minutes, and the medium supernatant was aspirated off. The pellet was loosened from the bottom of the tube by flicking the tube. Prepared PEG solution (0.3 ml) was added and the cells were mixed by flicking the tube for 1 minute. The mixture was then centrifuged for 6 minutes at 100 G. Eight minutes after the PEG was added to the cells, 10 ml of IFM in 1 ml aliquots were slowly added to the pellet with a pasteur pipet, lifting the pellet from the bottom of the tube. The tube was then centrifuged at 100 G for 10 minutes and the media aspirated off. Five to ten ml of CFM (complete feeding media) and gentamicin were slowly added in 1 ml aliquots, removing the pellet from the bottom of the tube. The media and pellet were poured into a P60 plate and placed inside a P100 dish for ease of handling. The plates were incubated at 37 degrees for 1 hour. The pellet was then gently triterated, and the suspension transferred to the P100 plate. CFM and gentamicin was then added so that the final

cell concentration was $2.5x10^6$ cells per ml. This was then incubated at 37 degrees in a 5% CO2 incubator overnight. the following day, 100 microliters of the cell suspension were placed in each well of a 96 well plate (Fisher). One hundred microliters of culture media containing 1.6 micromoles of aminopterin was added to each well.

After 7 days of incubation, half of the supernatant in each well was removed and replaced with fresh culture media without aminopterin. A second feeding was done 2 to 4 days later. Two to 4 days after the second feeding, wells showing growth were sampled and assayed for reactivity against membrane preparations.

Selected hybridomas were subcloned twice by the technique of limiting dilutions. The cells in the chosen well (less than 1000) were suspended in 1 ml of complete feeding solution. One microliter of this suspension was then placed in each well of a 96 well plate (Fisher) and 100 microliters of complete feeding solution was added to each well. This was repeated for the second subcloning. This technique ensured that, at most, only one cell was present in each well.

Section 3.11: ELISA (enzyme linked immunosorbent assay) For Initial Hybridoma Supernatant Screening

All assay steps were performed at room temperature. The polyvinyl chloride plates (Dynatech) were coated by dispensing 1 microgram of antigen in 50 micrograms of PBS in each well. the plates were then dried in a vacuum dryer overnight. The plates were blocked by filling each well with PBS/ 0.5% bovine albumin (BSA)/ azide, and incubated for 30 minutes. The liquid was then aspirated off and washed twice with Tween buffer (0.1% tween 20, 0.05 molar NaCl, pH 8.0). Human liver membrane preparation was used as a positive antigen control, BSA as a negative antigen control and the immunogen (membrane preparation #7) as the test antigen.

Supernatants from the hybridoma were tested for reactive antibodies by adding 50 microliters into each of the three coated wells (BSA, normal human liver, and immunogen antigen membrane preparation #7). A positive control (12-2H3 antibody) which reacts non-specifically to all tissues and a negative control (P3 antibody) which does not react to any tissue were tested at the same time to ensure

the validity of the results.

The supernatant being tested was left incubating at room temperature for 1-2 hours, then aspirated off. The wells were washed twice with Tween buffer. One hundred microliters of dilute conjugate (gam-hrp conjugate 1:2000 in tween/ BSA) were added to each well and incubated for 1 hour before aspiration and washing 4 times with Tween buffer. One hundred and fifty microliters of substrate (0.33% tetra-methylbenzidine, 0.02% H202 in methanol/ citrate phosphate buffer) were then added to each well and incubated for 30 minutes. The plates were then read on a Dynatech ELISA reader.

Section 3.12: Hybridoma Culture

Hybridomas which were reactive to the immunogen, but negative to human liver when compared to the positive and negative controls were selected for further study. the cells from the 96 well plate wee expanded several times over several weeks and finally to a 50 ml culture flask; 10 ml of supernatant containing antibody was collected and retested by the ELISA assay again. Only the hybridomas which were reactive to the immunizing antigen were screened by the avidin/ biotin immunoperoxidase staining of frozen sections of selected tissues.

Section 3.13: Avidin/ Biotin Immunoperoxidase Frozen Tissue Staining

Tissues which had been flash frozen in OCT compound were cut 6 microns thick and mounted on gelatin coated (tissue-grip Fisher) glass microscope slides. The slides were air dried for 30 minutes, then fixed in acetone for 5 minutes. After a 5 minute wash with water, the slides were immersed in 3% hydrogen peroxide solution for 5 minutes to neutralize endogenous peroxidase activity. This step and the following steps were done in a humidity chamber. They were then washed for 5 minutes in PBS and incubated for 20 minutes with avidin (Vectastain Kit, Cedarlane Laboratories, Toronto) to block endogenous biotin. The slides were washed with PBS, incubated with biotin for 15 minutes, and washed with PBS for 5 minutes. They were then incubated with 5% horse serum for 20 minutes and the excess shaken off. The

slides were then incubated for 30 minutes with an undiluted monoclonal IgG antibody and washed once with PBS. They were incubated in diluted biotinylated antibody solution (Vectastain Kit) for 30 minutes and washed twice with PBS. They were then treated with the ABC reagent (Vectastain Kit) for 45 minutes and washed three times with PBS. The slides were then flooded with the peroxidase substrate solution, 6 mg of 3, 4, 3', 4' -tetra -amino -biphenyl -hydrochloride (BDH #13033) with 30 microliters of 30% hydrogen peroxide in 11.5 milliliters of PBS for five minutes and washed in running water for 5 minutes. They were counterstained with Harris hematoxilin (BDH #RO-3312). The slides were then washed in water for 2 minutes and in lithium water briefly. The slides were dehydrated by immersion in the following baths for 1 minute each: 90% ethyl alchohol. 100% ethyl alchohol, and 3 separate baths of toluene. They were then mounted with a coverslip using Perma-mount (Fisher).

Section 3.14: CEA Antibody Testing

Frozen cells of 33 transitional cell carcinomas were thawed rapidly and washed three times in PBS. The cells suspended in PBS were diluted to $5x10^5$ cells per milliliter. One milliliter of this solution was then evenly distributed over a slide using a cytospin centrifuge. The cells were then stained using the avidin/ biotin immunoperoxidase tissue staining technique (see section 3.13) and the anti-CEA antibody D6.17.7 developed by Dr. A. Fuks (91). The stained slides were then scored for any cells which were stained.

Section 3.15: Tissue Selection And Scoring For Monoclonal Antibodies

Each antibody that was tested against frozen tissue sections was initially tested against a panel of 10 tissues to check for bladder tissue specificity. These tissues were colon, fallopian tube, heart, liver, lung, ovary, skin, small bowel, smooth muscle, and stomach. The antibodies were then screened against 3 normal bladders followed by screening against 8 bladder cancers. Two of these were high grade, three low grade, and the remainder intermediate grade. For some antibodies, extra sections

of normal bladder and bladder cancer were tested because the antibodies were questionable and required further screening or the initial tissues had been completely used and the additional sections were used as substitutes.

The slides were scored by comparing the results to a positive (2H3 antibody which stained all tissues) and a negative control (P3 antibody which stained no tissues) prepared for each batch of slides processed. Each stained tissue section was independently scored by a pathologist and two researchers. If the individual scorings were not identical for the slide, it was repeated and rescored.

CHAPTER 4

Results Of Soft Agar Clonogenic Assay Experiments

Thirty-four different tumors ranging from low grade to high grade were grown in the soft agar clonogenic assay (see table #1). Thirty percent of all tumors grew in the assay as measured by our criteria. Twenty percent of the tumors plated had bacterial or fungal overgrowth independent of the tumor grade and in spite of the addition of penicillin and gentamicin to the growth media. These plates could not be assessed for growth and were therefore considered to have no growth. No identifiable factor could be isolated that would account for the lack of growth in the remainder of the tumors plated.

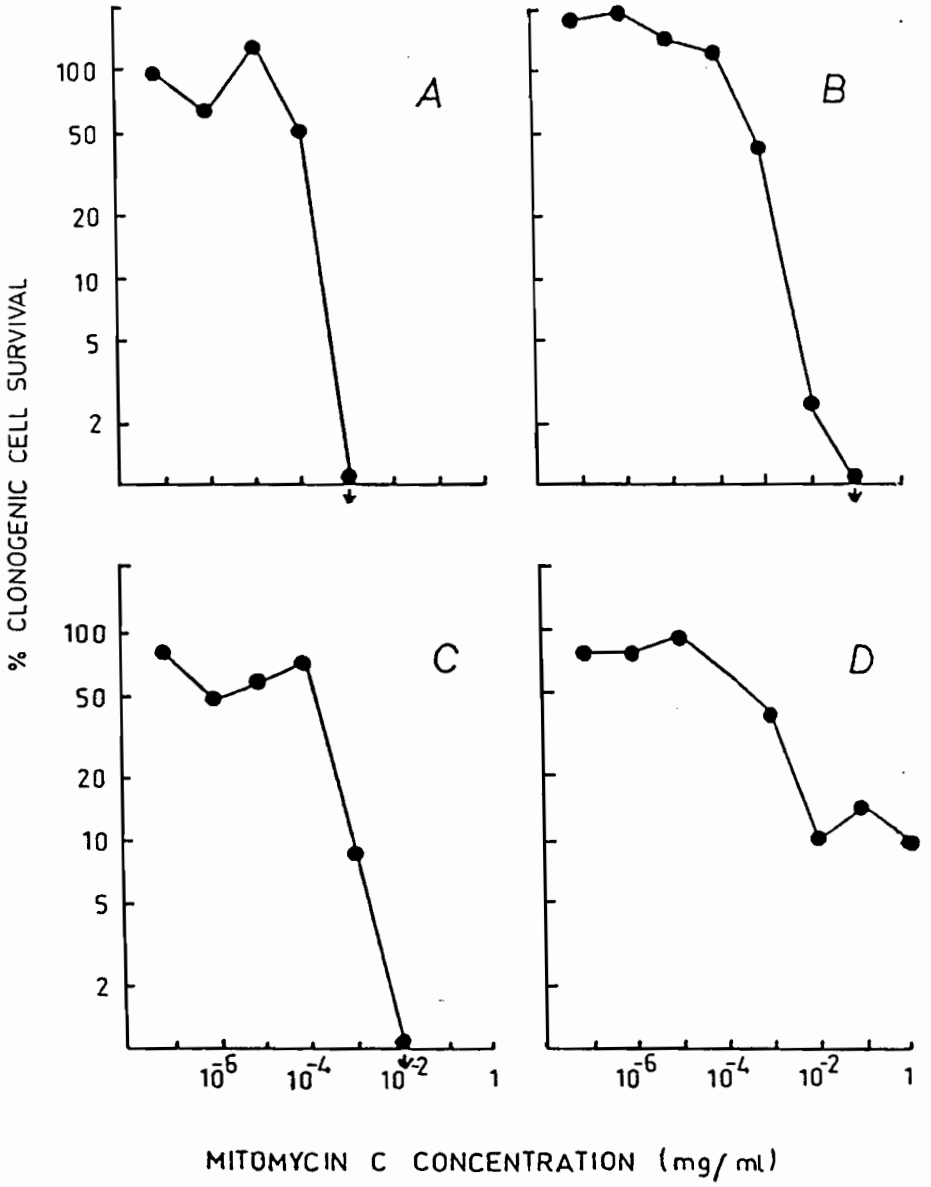
The mean plating efficiencies of low to moderate grade (1-2/ 3) and high grade tumors (3/ 3) were 0.011% ± 0.017% and 0.014% ± 0.15%, respectively. There was no statistical difference between these two groups ($P \geq 0.05$ using Chi squared tests with one degree of freedom).

The low rate of colony formation was due to our strict adherence to colony size criteria. It has been demonstrated that not all tumor cells which form colonies are stem cells (101), and though the correct colony size cut off point is not clear, cells forming large colonies are more likely to be stem cells and are the ideal target of cancer therapy (6,8). This important point will be discussed further in chapter 7.

Drug chemosensitivity curves (see graphs #1-#5) were constructed to study resistance for three established intravesical chemotherapy drugs, mitomycin C, adriamycin and thiotepa, and three drugs with chemotherapeutic potential, DMSO, retinoic acid and sulphapentosan sodium. Twelve human bladder tumors which produced unequivocal growth were used in these tests.

Graph #1 shows the independent chemosensitivity curves for mitomycin C obtained for two low grade bladder cancers, and two high-grade bladder cancers. Three tumors show a similar sensitivity to this drug. Each curve has an initial plateau for cell survival up to a concentration of 1 nanogram per milliliter of mitomycin C. A one log kill is seen at drug concentrations of greater than 0.01 mg/ ml for all three tumors. Cell survival was near zero for drug concentrations greater than 0.01 mg/ ml as measured by this assay system, and there was no evidence of any

Mitomycin C



Graph # 1: Panels A and B represent the mitomycin C sensitivity of 2 well differentiated transitional cell carcinomas. Panels C and D give the same information for 2 poorly differentiated transitional cell carcinomas. All data points represent means of triplicate samples.

resistant subpopulation. The fourth tumor, poorly differentiated in grade, shows a similar initial plateau, but has a distinct subpopulation of cells which are relatively resistant to higher doses of mitomycin C. This subpopulation is approximately 10 per cent of the colonies that grew.

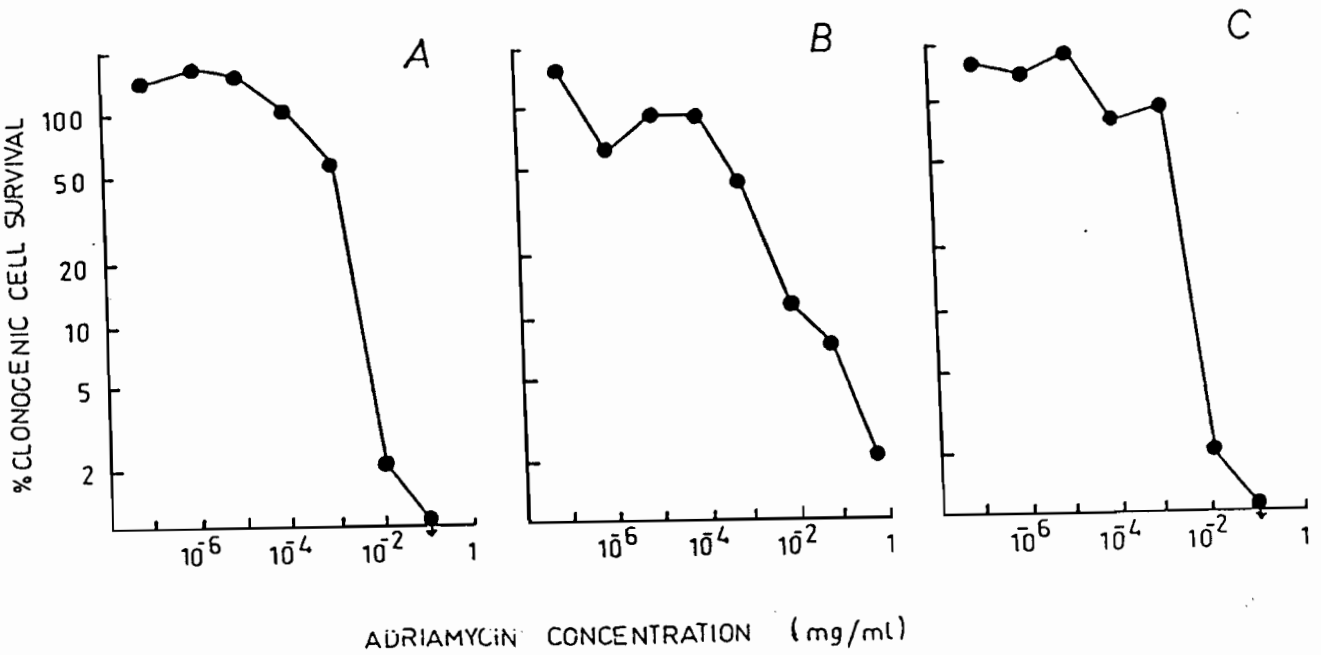
The drug response of two low grade and one high grade tumor to adriamycin is seen in graph #2. As for mitomycin C, two low grade tumors show sensitivity to this drug at concentrations greater than 0.1 mg/ ml, with no evidence of resistant subpopulations, and a near zero survival for drug concentrations greater than 0.1 mg/ ml. The third case, a poorly differentiated tumor, seen in panel B shows initial response to low concentration of adriamycin, but has a shoulder at higher concentration suggesting a resistant subpopulation of cells. The curves of the data point to an initial enhancement of clonogenic cell survival for drug concentrations less than 1 nanogram per milliliter. There is a one log kill at concentrations greater than 0.1 mg/ ml for each of the tumors and a near zero survival for drug concentrations greater than 0.1 mg/ ml.

Composite graphs were made from each individual drug sensitivity curve and are displayed in graph #3 for three cytotoxic drugs. The tumors exposed to the three drugs show definite sensitivity. Case to case variations, as noted above, are obscured when presented in this form, but it is a useful manner of presenting a large volume of data in comprehensible form.

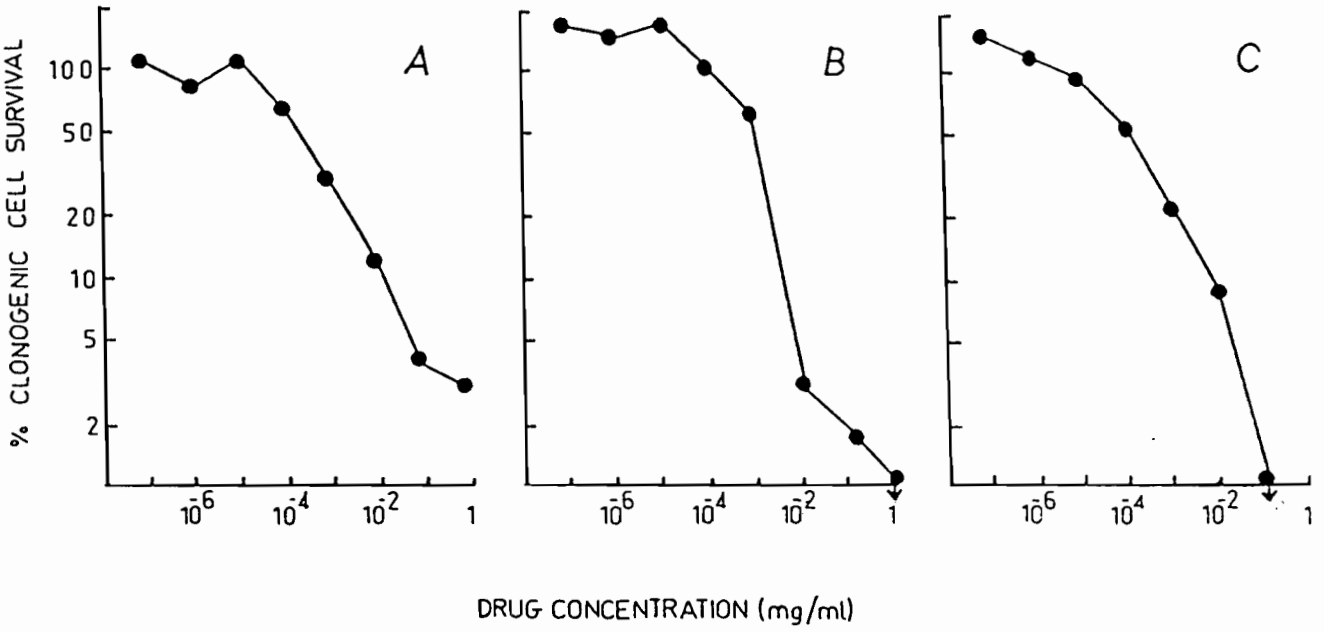
The composite drug curves for both adriamycin and thiotepa show similar results to that for mitomycin C, but the drug concentrations required for a one log kill are slightly higher than for mitomycin C. This may explain why the clinical response rates for these two drugs are not as high as those reported for mitomycin C.

There is one low grade tumor (see panel A, graph #4) which showed sensitivity to retinoic acid; the other low grade and two high grade tumors displayed no definite response at the drug concentrations studied. The one responsive tumor shows only a slow decrease in clonogenic cell survival as the concentration increases, having a one log kill at $1x10^5$ mg/ ml concentration.

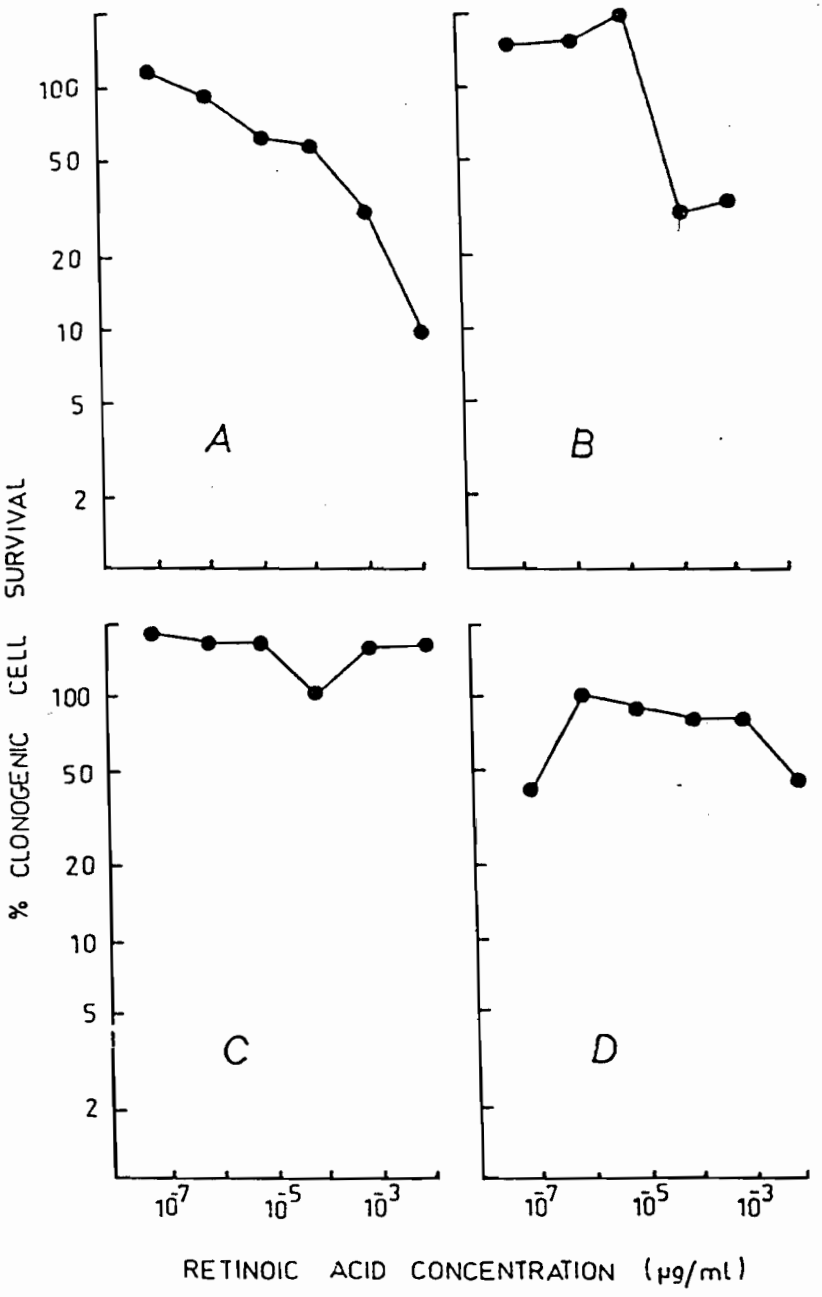
Graph #5 shows the composite graphs for retinoic acid and two other drugs which have not been used as anti-neoplastic agents against transitional cell carcinoma of the bladder. The assay results of retinoic acid, dimethylsulfoxide (DMSO),



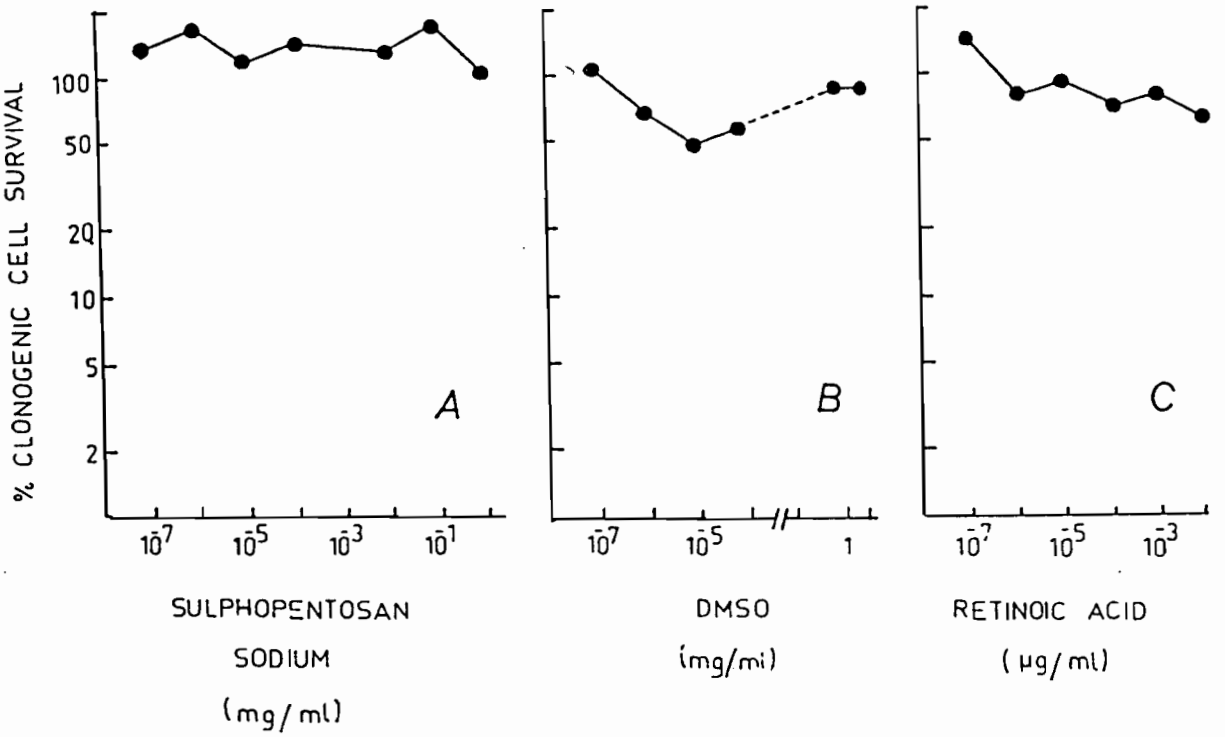
Graph #2: Panels A and B represent the adriamycin sensitivity of 2 well differentiated transitional cell carcinomas. Panel C gives the same information for a single poorly differentiated transitional carcinoma.



Graph #3: Panel A shows the mitomycin C sensitivity of a series of 6 transitional cell carcinomas (the 4 cases shown in Graph #1 plus 2 others). Panel B gives the same information for 4 cases treated with thiotepa. Panel C represents the composite adriamycin sensitivity of 3 transitional cell carcinomas studied independently.



Graph #4: Panels A and B show the effect of retinoic acid on the growth of 2 well differentiated transitional cell carcinomas. Panels C and D give similar information for 2 poorly differentiated tumors.



Graph #5: Panel A is a composite showing the sensitivity of 3 transitional cell carcinomas to sulphopentosan. Panel B shows similar composite information based on studies of 3 cases treated with DMSO. Panel C is a composite based on the 4 cases treated with retinoic acid illustrated in graph #4 plus 2 other cases.

and sulphapentosan sodium all exhibit natural drug resistance, as there is little variation in clonogenic cell survival for the concentrations studied. The two tumors exposed to sulphapentosan sodium had near identical responses to each drug concentration. However there was some minor variation in responses to retinoic acid and DMSO. Compared to the composite curves in graph #3, there is little drug activity present.

CHAPTER 5
Results of CEA Testing

A monoclonal antibody (D6.17.7) against CEA developed by Dr. A. Fuks (91) using the hybridoma technique was used here to stain single cells obtained from 33 human transitional tumors of varying grades to test for a correlation between tumor grade or an ability to predict tumor growth in vitro.

Three low grade and one high grade tumor did not have enough cells for proper staining evaluation. Eleven out of 20 (55%) low grade tumors were found to be positive for CEA, while 7 out of 13 (54%) high grade tumors were positive and 2 other high grade tumors had some borderline reactivity (see table #1). There was no statistical difference between the incidence of CEA on low grade or high grade transitional cell carcinoma of the bladder.

The use of this monoclonal anti CEA antibody as a tumor specific antibody has a sensitivity of only 55%. The specificity could not be calculated from this experiment as no normal bladder tissues were stained.

This monoclonal antibody against CEA could not be used to predict in vitro tumor growth as there was no statistical correlation between tumor binding and in vitro growth.

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Chapter 6
Monoclonal Antibody Production And Initial Screening

Cell surface antigens were classified by Old (114) into three categories. Class 1 antigens are tumor specific and cannot be demonstrated on any other normal or malignant cells. Class 2 antigens are shared tumor antigens found on autologous as well as on allogenic tumors of similar and dissimilar origin. Finally, class 3 antigens are widely distributed on normal and malignant cells, both autologous and xenogenic. This classification works well for polyclonal antibodies, but for practical purposes, monoclonal antibodies should be classed in a more exacting fashion since the monoclonal antibodies react to one marker only and their use will be restricted to humans and not against xenogenic materials.

To date, there is no universally accepted categorization of monoclonal antibodies. Systems offered have included cell specificity patterns, distribution of reactions to a panel of tissues, biochemical characterization, antigen of reactivity description, and others. The best system will be one that will most likely describe the criteria of a useful antibody for a given situation.

The criteria of importance here is the isolation of a monoclonal antibody which may be used as an indicator of the natural history of a specific transitional cell carcinoma of the human bladder.

Thus, monoclonal antibodies produced using human transitional cell carcinoma as an antigen could be categorized into 1 of 7 possible groups. The first group is prognostic specific antibodies. These antibodies would not necessarily have to urothelial specific, but would distinguish between benign and malignant transitional bladder tumors. The second group is specific for different cells contained in human transitional cell carcinoma of the bladder. These antibodies would distinguish between stem cells and specialized cells, or superficial and basal cells. Again, this group of antibodies need not be urothelial specific. The third group is tumor specific. This group distinguishes between tumor and normal epithelium and need not be urothelial specific. The fourth group is urothelial specific. The fifth group is cell type specific. This group would identify epithelial cells, connective tissue cells or other elemental types of cells independent of the organ of origin. The sixth group is non-

Results of Selected Monoclonal Antibodies

TISSUE	GRADE	3.48	5.48	6.48	39.48	2.51	49.51	70.48	49.48
Bladder Ca #1	3/ 3	–	+						–
Bladder Ca #2	3/ 3	–							
Bladder Ca #4	3/ 3	–							
Bladder Ca #5	3/ 3	+	+						–
Bladder Ca #7	3/ 3	–							–
Bladder Ca #9	3/ 3	–							–
Bladder Ca #10	3/ 3	+							
Bladder Ca #11	3/ 3	–	–	–	–	S≥O	–		–
Bladder Ca #13	3/ 3				S≥B	+			
Bladder Ca #14	3/ 3	–	–	–	–	+	–	–	
Bladder Ca #15	3/ 3	+	+	+	+	+		–	–
Bladder Ca #16	2 3/ 3			–					
Bladder Ca #17	2 3/ 3		+	+	+	+		+	+
Bladder Ca #18	2/ 3		+	+	+	+	–	+	+
Bladder Ca #19	2/ 3		S						
Bladder Ca #20	2/ 3	S≥O							
Bladder Ca #21	2/ 3	–	B≥S	+	+	+	–	–	–
Bladder Ca #22	2/ 3	+	+						
Bladder Ca #23	2/ 3		S≥B						
Bladder Ca #24	2/ 3	±	S≥B	+	S≥B	+	+	–	–
Bladder Ca #25	2/ 3		+						
Bladder Ca #27	2/ 3	+	+						
Bladder Ca #28	2/ 3				+	+		+	
Bladder Ca #29	1 2/ 3	–	+	–	+	+	–	–	–
Bladder Ca #30	1 2/ 3							+	
Bladder Ca #31	1/ 3			+	–	–	B	–	
Bladder Ca #32	1/ 3	+	–	–	–	+			–
Bladder Ca #33	1/ 3	+					–	–	

Legend:

+ active reaction to tissue

– no reaction to tissue

B reaction to basal mucosa

S≥O reaction to some cells, but not specific

Table #2: Results of initial antibody screening of selected antibodies against human transitional cell carcinoma of the bladder.

Results of Selected Monoclonal Antibodies

TISSUE	3.48	5.48	6.48	39.48	2.51	49.51	70.48	49.48
Colon	+	+	+	+	+	–	+	–
Endothelium	–	–	–	–	+	–	–	–
Fallopian Tube	–	–	–	+	+	–	+	–
Heart Muscle	–	–	–	–	–	+	+	–
Liver	–	–	–	–	–	–	+	–
Lung	–	–	–	–	+	–	–	–
Ovary	–	–	+	–	+	–	+	–
Skin	–	–	+	+	–	–	+	–
Small Bowel	–	–	–	+	+	–	–	–
Smooth Muscle	–	–	–	–	–	–	–	–
Stomach	+	+	+	+	+	–	+	–
Bladder #1	S	S≥B	S	S≥B	B≥S	–	+	–
Bladder #2	S	+	+	+	B≥S	–	S	–
Bladder #3	+	S≥B	+	+	+		+	–
Bladder #4	S		+	+			–	
Bladder #5	+	+	+	B≥S	+		+	–
Bladder #6	S≥B	S≥B	S≥B	S≥B	+	–	S	–

Legend:

+ active reaction to tissue
– no reaction to tissue
B reaction to basal mucosa
S reaction to superficial mucosa
S≥O reaction to some cells, but not specific
B≥S stains basal more than superficial
S≥B stains superficial more than basal

Table #3: Results of initial antibody screening of selected antibodies against normal human tissues.

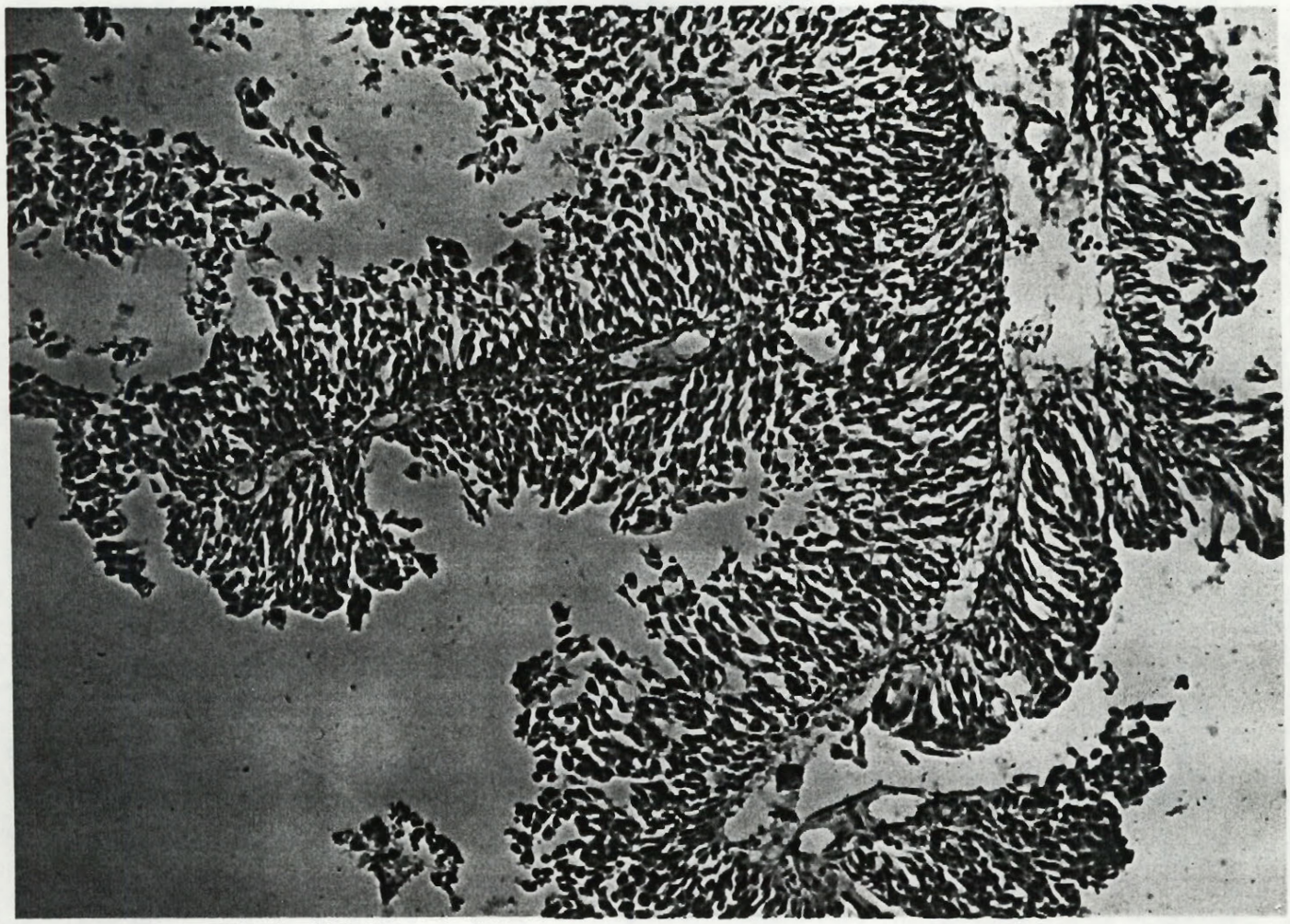
specific. This group could not be used reliably to distinguish between cells of different organs and would probably be active against an antigen such as an ABO surface antigen. This antibody would find uses outside the study of transitional cell carcinoma. The last group is non-reactive. An antibody which would fit into any of the first five groups would be extremely useful either clinically or in research. This classification will be applied to the monoclonal antibodies produced here.

The hybridomas produced from two fusions were screened and partially characterized. The first fusion produced 85 hybridomas. Fifty-nine hybridomas were eliminated by preliminary screening with the ELISA due to cross reactivity with normal human liver. The second fusion formed 60 hybridomas of which 50 were eliminated in a like manner. The remaining 36 hybridomas produced monoclonal antibodies which were tested against several normal human tissues and several transitional cell carcinomas of the human bladder of various grades using the avidin / biotin immunoperoxidase staining technique.

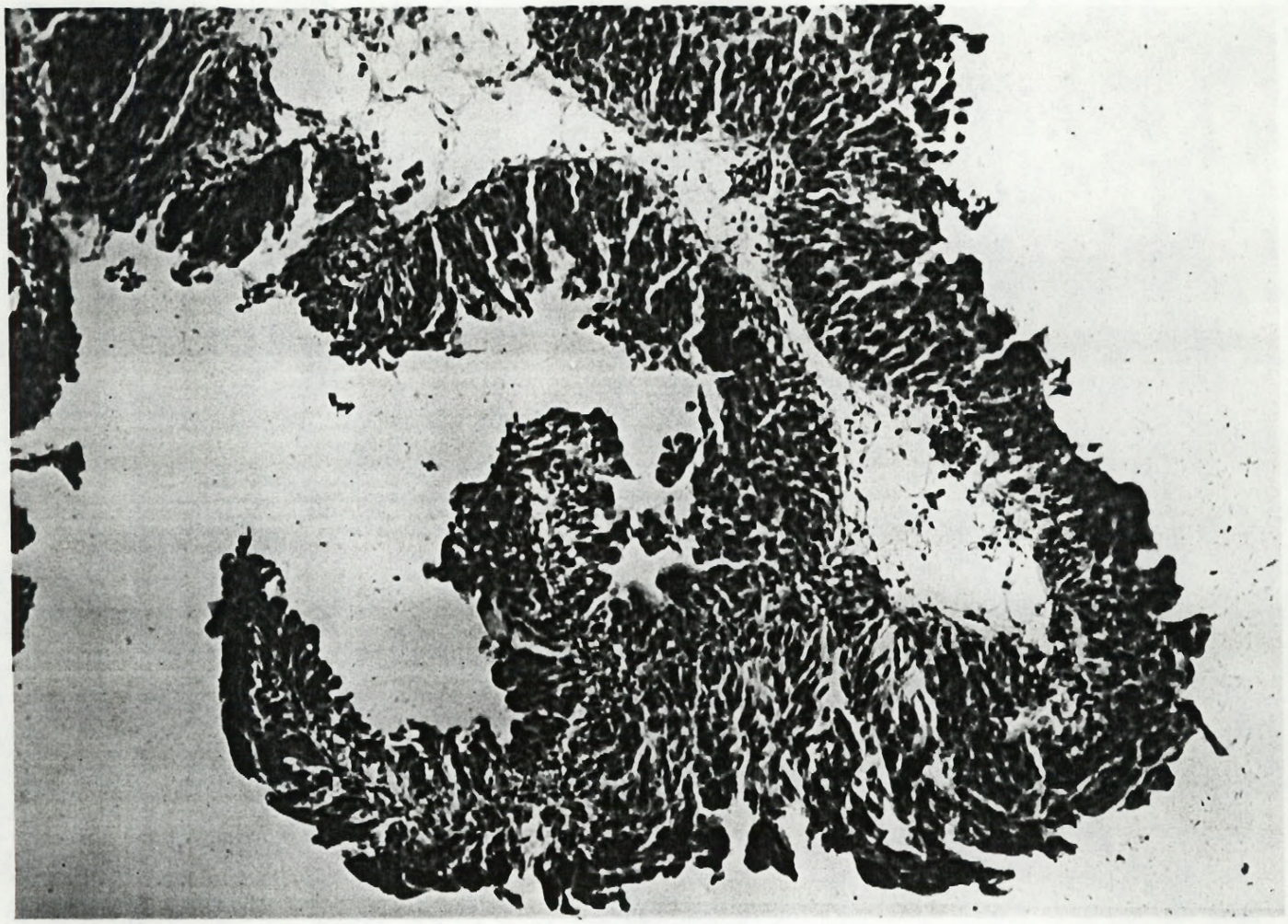
Thirty-three human transitional cell carcinomas of the bladder were snap frozen for the avidin / biotin immunoperoxidase screening tests. Eight of these had enough material to produce slides for almost every antibody tested. Slides were often redone due to the fragility of the frozen tissue. The remaining tumors were cut and used as often as possible so that a panel with representative tumor grades could be made for each antibody. Six normal bladders were likewise tested, three of which had adequate material for the majority of the antibodies. Various representative normal tissues were also studied (see detailed tables in Appendix A).

Most of the family of 36 antibodies produced were found to stain the panel of tissues in a non-specific fashion. They stained mucosa of all grades, but did not consistently stain every bladder mucosal surface. In addition, the monoclonal antibodies in this non-discriminating class stained various structures of normal tissues in no distinct pattern. Their potential use as a prognostic indicator or as a research tool for transitional cell carcinoma is very limited. Thus, they were classed as non specific (group 6) and excluded from further study (for detailed analysis see Appendix A).

Photographs of some tissue sections can be seen in photographs #1-5. Photographs #1 and #2 show typical non-binding and binding, respectively, to low



Photograph #1: Low grade transitional cell carcinoma stained with the non-binding control antibody.



Photograph #2: Low grade transitional cell carcinoma stained with the test antibody which binds to all cells.

grade transitional cell carcinoma.

Three antibodies, 7.51, 18.51 and 72.48, were produced by their hybridomas a week later than all the other antibodies. Because of this, there was insufficient time to expose them to the tissues needed to adequately characterize their nature. These will be studied more thoroughly at a later date.

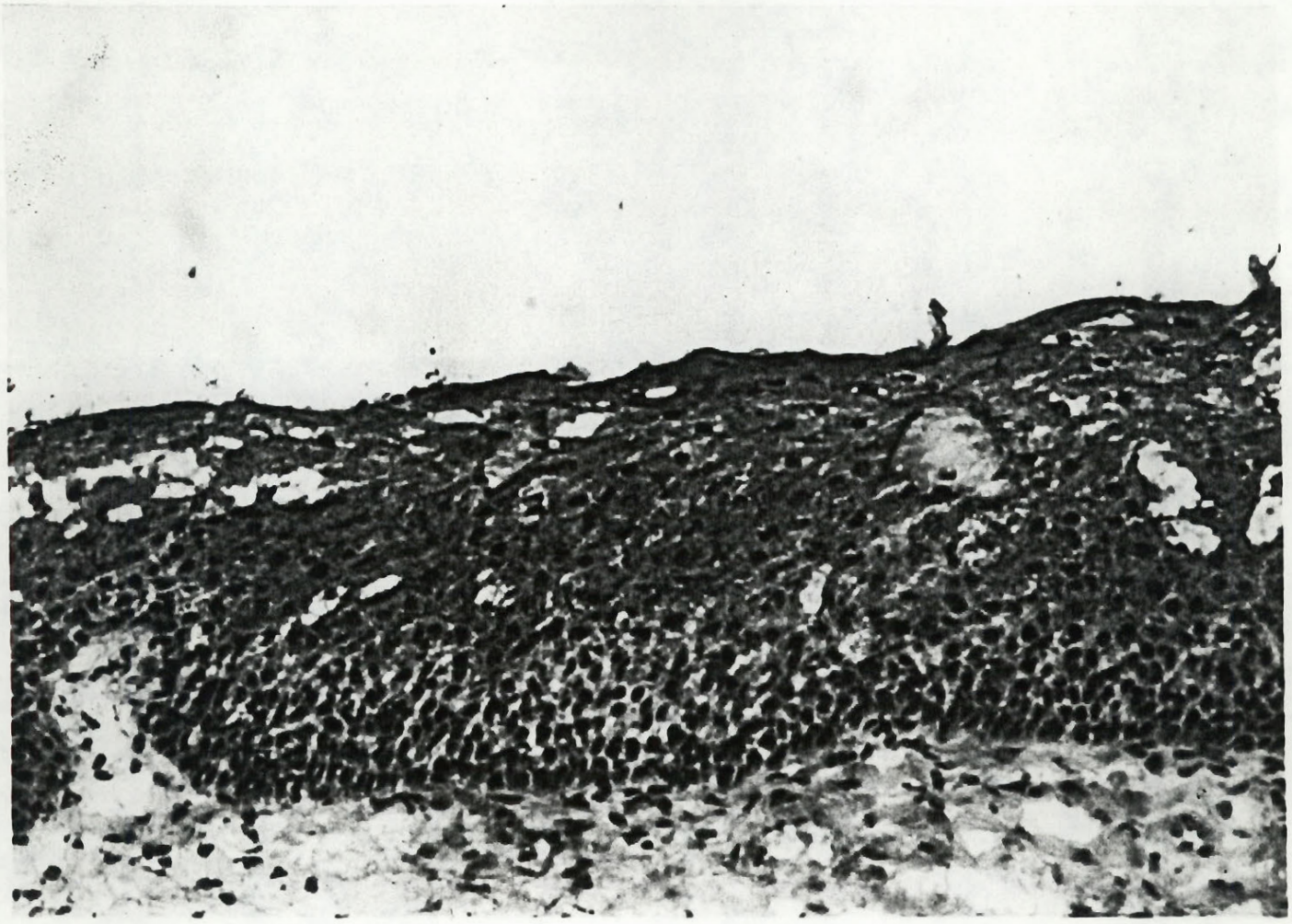
The antibodies which were produced could be placed into each of the seven proposed groups of antibodies after the screening against frozen tissues. Group 1 is represented by antibody 46.48, 49.51 and 70.48. Antibody 49.51 and 70.48 both tended to stain lower grade transitional cell carcinoma mucosa, while 49.48 stained intermediate grade only. Further screening against larger tissue panels will be required to verify these initial findings.

Antibody 3.48, 5.48, 6.48 and 39.48 could all be placed into group 2. They initially stained urothelium in a cell specific pattern, often staining the superficial mucosa layer more than the basal cell layer (see photograph #3). This staining pattern was present in sections 2/ 16, 6/ 18, 2/ 13 and 4/ 14 times, respectively: all were positive. Each of the four antibodies stained tissues other than bladder, but would not inhibit use restricted to bladder tissue. Antibody 3.48 showed a cell specific staining pattern which could not be defined as either superficial or basal epithelial (see photograph #5). Monoclonal antibody 5.48 seems to be the most valuable of the group since it preferentially stained superficial mucosal cells more frequently than the other three antibodies. These antibodies may be used to separate and concentrate specific cells for further in vitro studies.

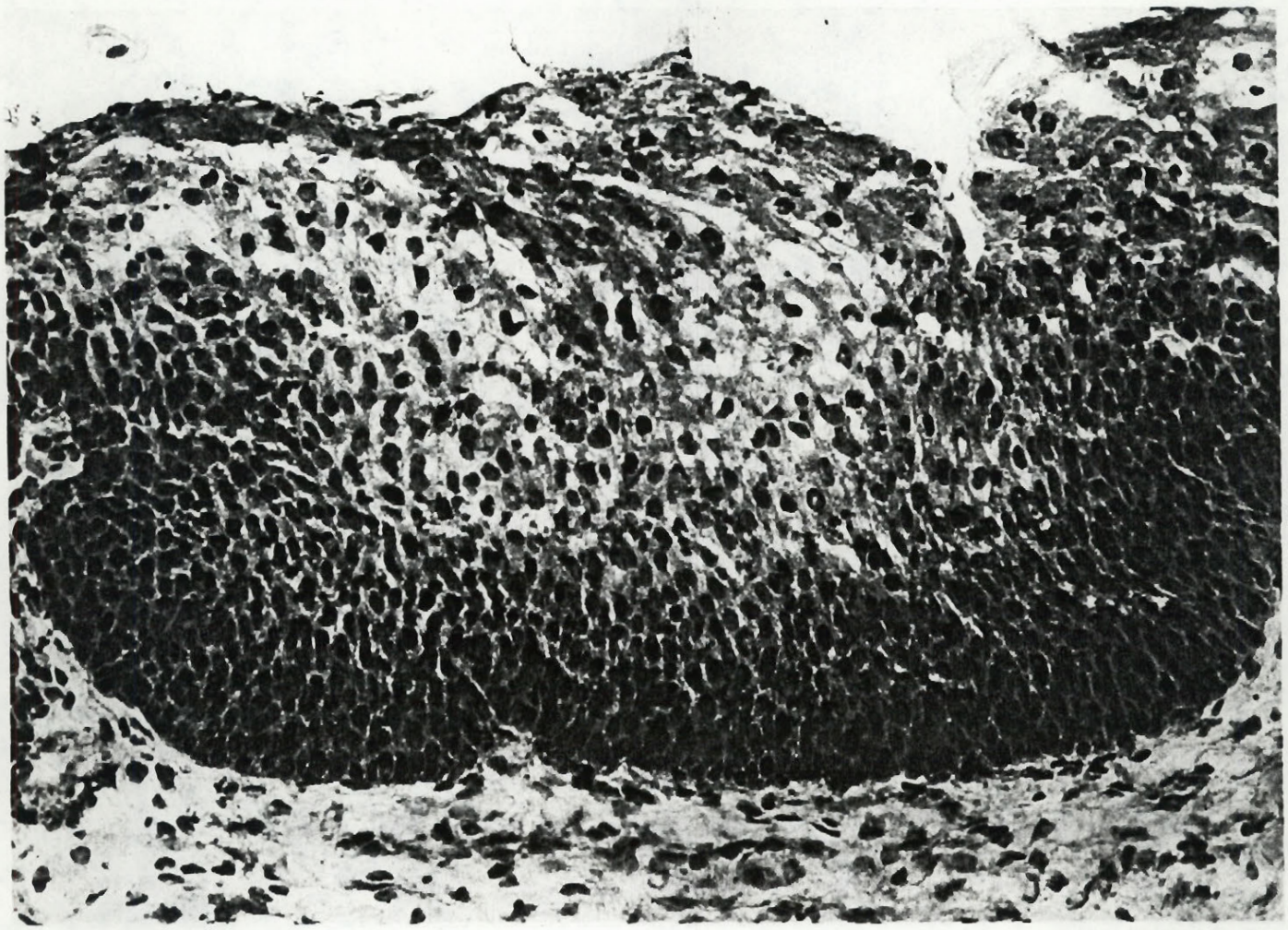
Antibody 2.51 was similar to the previous group of antibodies in expression (group 2 antibody), except that it preferentially stains the basal mucosal cell layer (see photograph #4). It therefore provides an excellent companion to 5.48. Antibody 2.51 also stains mucosal surfaces on other tissues.

Antibody 49.48 was the only one that was bladder tumor specific and hence an example of group 3. This group would be very useful in diagnosis and treatment. However, as 49.48 did not react to very many bladder tumors, its usage would be severely limited.

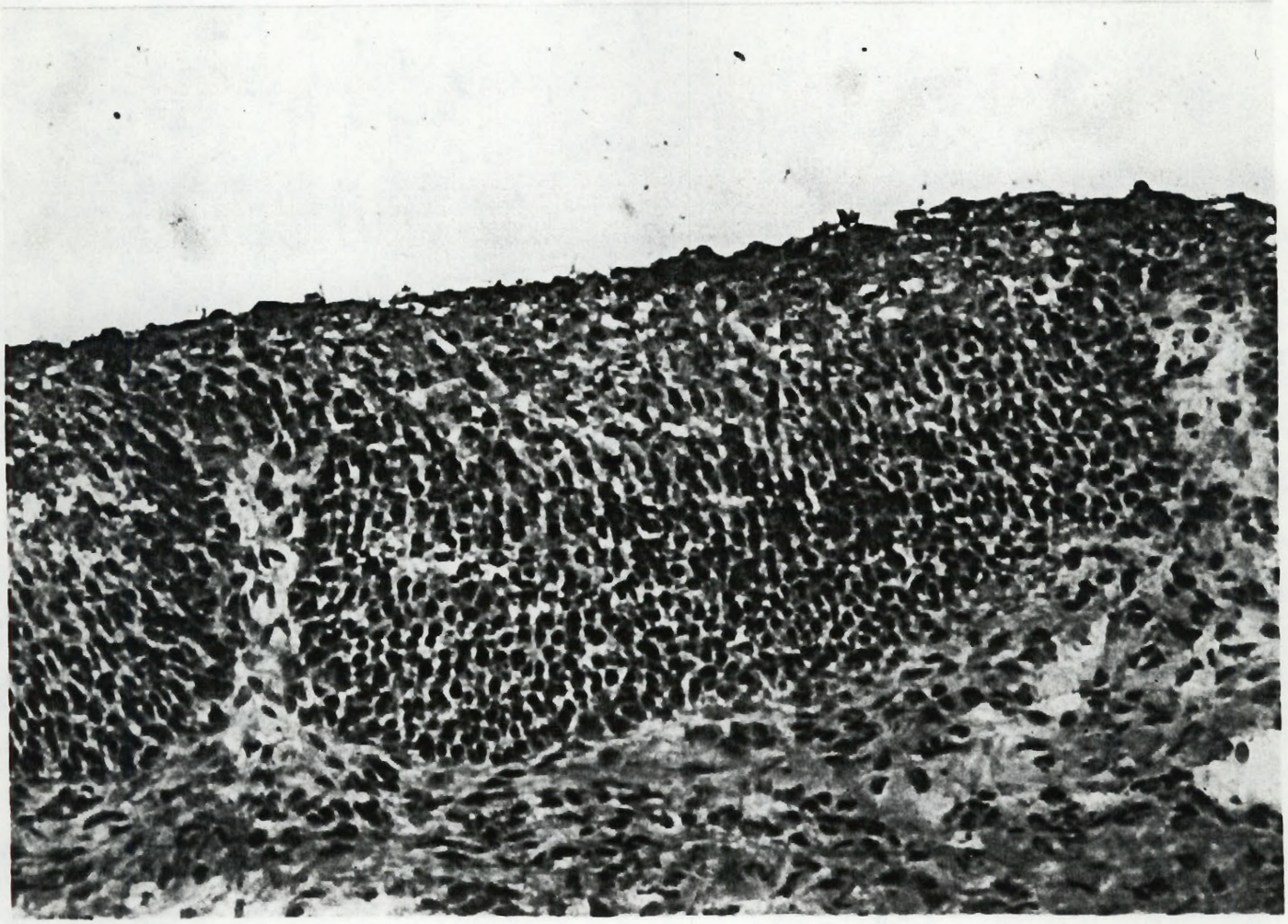
Antibody 46.48 and 49.48 only stained mucosa and were bladder specific and thus members of group 4. This group (if it binds antigen found on all urothelial tissue)



Photograph #3: Low grade transitional cell carcinoma demonstrating antibody binding to superficial cells.



Photograph #4: Low grade transitional cell carcinoma demonstrating antibody binding to basal cells.



Photograph #5: Low grade transition cell carcinoma demonstrating an unexplainable pattern of antibody binding to some cells and not to other cells.

has great potential for determining the source of a metastatic lesion of unknown primary.

Antibody 12.48 was reactive to epithelial surfaces in both malignant and benign urothelial tissues and normal human tissues (group 5 antibody). While not of practical use in identifying or characterizing transitional bladder cell carcinoma, it may have a place in the study of epithelial tumors.

Antibodies 3.48, 9.51, 14.48, 41.48 and 78.48 are of special note. All were reactive only to bladder tissue and gastrointestinal tract tissue. The antigen they detect may be from their common embryological origin. These antibodies may be of use in studying fetal development.

Group 6 antibodies make up the remaining antibodies in appendix A. There were no non reactive antibodies listed in this thesis as they would have been discarded after the ELISA screening.

CHAPTER 7

Discussion

There is an ongoing search for tumor identification techniques that may be of prognostic significance in making therapeutic decisions in patients with bladder cancer e.g., cystectomy vs. endoscopic management or in choosing the most appropriate chemotherapy. DNA banding studies have demonstrated certain chromosomes that identify low grade tumors (102) and abnormal chromosome patterns associated with invasive well differentiated tumors (103). Studies of cell surface expression found that the loss of the ABO isoantigens are associated with tumor invasiveness (104, 105). Research using lectins for characterizing the cell surface is under way (106). None of these techniques has so far proven to be the ideal test.

Three promising techniques for tumor study were evaluated here. The first explored the use of the soft agar clonogenic assay as a tool to identify the most effective response to chemotherapy and to evaluate drugs which have theoretical anti-tumor potential. The use of anti-CEA antibody as a tumor marker to determine prognosis was the second to be evaluated. Finally, a family of monoclonal antibodies was produced in an attempt to identify different bladder tumor cell types. Each of these techniques are discussed under their own heading later in this chapter.

The theoretical ability of the soft agar clonogenic assay to identify the malignant potential of plated tumors was not verified. The low growth rate of tumors plated would make this assay a poor clinical test. In addition, the lack of statistical correlation between plating efficiency and histology makes it highly improbably that growth in this assay reflects the aggressiveness of the tumor in the patient. It is unlikely, therefore, that growth alone will be useful as a prognostic tool.

Assessing the colony size, and thus the growth of plated tumor cells, is the most important technical problem relating to this assay. This critical feature of colony size is masked by the problem of clump formation. Transitional cell carcinoma of the bladder is a difficult tumor to suspend as a single cell preparation but even in a single cell form, it tends to aggregate rapidly and therefore may be considered as a very "clusterogenic" tumor. Emendation of this feature has been achieved by using a large diameter cut off and by counting only aggregates above this diameter as clumps. The clumps, noted 24 hours after plating, were subtracted from the final colony count. As a consequence, small clumps that formed colonies were counted instead of being discarded. It was noted by Lieber et al (107) that most colony formations originate from these smaller clumps rather than from single cells. Other studies which examined human transitional cell carcinoma in soft agar assays fail to distinguish between aggregates at the time of plating and final colony counts (108, 109). This may readily account for the reported resistances of tumors to chemotherapy and unexplained plateaus in survival curves (71). This may all be standardized in the future by the use of an automated image analyzer system (110) which could store and compare the change in size of each colony on each plate.

The rationale for the use of drugs in the treatment of cancer is to achieve the selective killing of tumor cells. This is based on the "cell kill hypothesis" elucidated by Skipper et at (30). For most drugs, a clear relationship exists between the dose of drug and its ability to eradicate tumor cells within the limits of toxicity to the host. A given dose of a drug kills a constant fraction of cells, not a constant number, regardless of the number of cells present at the time of therapy. Thus, cell destruction follows first order kinetics, and at least a one log response is required in order to

indicate drug response.

The growth of tumors exposed to various concentrations of drugs in the assay system resulted in three distinct types of clonogenic cell survival curves. The first showed little initial change in survival at low drug concentrations, but consistently demonstrated a rapid decrease in survival at exposures above a specific drug concentration. This was considered as a positive type of response curve (see Graphs #1 - #2). The second type of clonogenic cell survival curve showed little change in cell survival for all concentrations of the study drug. This type of curve was considered as representing the natural resistance of the tumor to the study drug (see Graphs #4 - #5). The curves obtained for positive drug response make it highly improbable that the apparent resistance to other agents is due to an artifact. The third type of curve noted was one in which there was an initial response to the drug, but because of a distinct subpopulation of cells was relatively resistant to higher doses (see Graphs #1 and #2). This may represent an acquired resistance.

It is of interest that three out of four tumors that grew in the soft agar assay when exposed to mitomycin C underwent growth inhibition at a drug level of 0.01mg/ ml and that one tumor exhibited a resistant subpopulation. From the many published series of clinical trials using this drug for intravesical chemotherapy (see chapter 2.3), it is known that only 75% of the tumors treated will respond. This correlation between in vitro and in vivo responses infers that this assay may be of use in predicting clinical responses to chemotherapy for individual patients if the assay system will support growth of their tumor.

Two poorly differentiated tumors were shown to be resistant to standard chemotherapeutic agents using this assay. Such refractiveness would not have been perceived if the tumors had been subjected to only one randomly chosen concentration of drug as has been the practice by most researchers when using this assay. The drug curves may, in future, be used to identify a critical concentration of chemotherapeutic drug which will best identify resistant or sensitive tumors.

Tumor growth in the soft agar clonogenic assay only occurred in one third of all tumors plated. When growth did occur, the number of colonies was invariably small and only a 2 log kill could be demonstrated. Growth may be enhanced in the future by using low levels of oxygen (less than 20%) in the incubators (111) or by adding

epidermal growth factor to the growth media (112). If plating efficiency can be improved, then perhaps screening tests for individual tumors similar to bacterial antibiotic testing may be done.

Even with the low plating efficiency found here, resistant subpopulations were detected and resistance and susceptibility to drugs could be assessed. The results from the exposure of tumor cells to retinoic acid, DMSO and sulphapentosan sodium in the soft agar clonogenic assay suggest that these drugs do not warrant any further clinical study. This study establishes the use of the soft agar clonogenic assay as a possible phase II test which would spare patients' morbidity from test drugs.

The usual clinical dose for intravesical therapy has been determined empirically. These doses may be adjusted to higher or lower levels depending on the sensitivity of the tumor stem cells to the chemotherapeutic agent as evidenced in the soft agar clonogenic assay. Dose changes should be assessed in controlled studies, as there are drug tumor interactions in vivo which cannot be rigidly controlled or even documented in this assay. Tumor stem cells may be protected from drug contact by tissue depth; drug concentration may be decreased by urinary dilution, and drug activity may be neutralized by components in the urine or mucosal membrane. These factors, representing bioavailability, may be clinically compensated for in part by increasing the intravesical drug concentration or time of exposure. Also, the clonogenic stem cell assay reflects inhibition of growth, not necessarily stem cell death. Stem cells, in vivo, may undergo inhibition of growth for a time, but regain their ability to proliferate when they are no longer exposed to chemotherapy. This latter point has been minimized by the use of constant chemotherapy exposure of tumor cells so that stem cells not cycling during the period of in vitro short term drug exposure would not be scored as a resistant plateau. Further study will be required to verify these points.

Monoclonal antibody techniques are readily adaptable to the use in immunological assays. Assays for differentiation antigens such as alpha fetoprotein, beta human chorionic gonadotropin and prostatic acid phosphatase have become standard tools in monitoring neoplastic disease. CEA is also a non-tumor specific antigen which is found on bladder tumors (1117) and has available reference monoclonal antibodies, and thus has potential as the basis for an immunological assay to determine the prognosis for bladder cancer.

The use of a reference monoclonal anti-CEA antibody to identify transitional cell carcinoma with a high malignant potential was not verified in our experiment. There was no statistical difference between the incidence of CEA on low grade or high grade transitional cell carcinoma of the bladder. This finding is contrary to that of Nakatsu et al (90). Their conclusion was based on 54 patients of which 15 had tumors positive for CEA. However, they discarded 15 of the tumors due to the difficulty in obtaining good positive stains with their immunoperoxidase technique, thus introducing a large margin of error that could have been eliminated by the use of a multiplier step such as that found in the avidin/ biotin technique used in the method of this thesis. If this is taken into account, the data from their experiment and that presented in this thesis are comparable, although the conclusions are the antithesis of theirs.

The experimental data here shows a low specificity of only 54% for the use of this monoclonal anti-CEA antibody as a tumor specific marker. Thus, the use of this antibody as a clinical tumor marker cannot be justified. However, it may have a place in adding to the accuracy of prognostication as it does for colon cancer when it is used to manage patients who have already undergone surgery and have had pre-operative CEA antigens present.

In this report fusion #48 produced 30% more hybridomas than fusion #51. In addition, fusion #48 produced double the number of potential hybridomas after the first screening and 4 times the number of useful hybridomas after the second screening when compared to fusion #51. This difference may be due to the additional exposure to different antigens which fusion #48 received.

The monoclonal antibodies produced by the hybridoma technique are classified into one or more of the seven proposed groups. The design of the screening procedure is the key to identification. There are two basic approaches. One is the selection of cell hybridomas producing antibodies that bind to the cell used as the immunogen, and subsequently studied for specificity. The alternate approach is to design a selective assay for screening the hybrids so that only those secreting antibody of the desired specificity are selected. The latter procedure was used to facilitate placement in one of the above groups because of the anticipation that a large number of non-specific or non-reactive hybridomas would be produced. In retrospect, this was justified, as 95% of the hybridomas did fall into these 2 unwanted groups.

Preliminary screening of hybridomas against a human liver membrane preparation using an ELISA technique provided a specific, rapid, inexpensive, relatively easy to perform and highly sensitive screening method. These antibodies were generally reactive to all human cells and therefore could be discarded as non-specific (group 6). The next screen employed a membrane preparation made from a mixture of the bladder tumors of several grades and stages, including those used as inoculants. Only hybridomas which reacted very positively were kept for further study, ensuring they were not in the non-reactive group (group 7).

The tissues used in the secondary screening were selected in order to allocate the antibodies to their proper group. The urothelial specificity of the antibodies was evaluated by screening against normal human tissue, including bladder. Tumor specificity and, to some extent prognostic specificity, were evaluated by screening against bladder cancers of varying grades. The ultimate assessment of prognostic specificity could not be made here, as the patients from which the tumor material was removed would have to be adequately followed. Likewise, bladder tumor cell type specificity can only be indicated by distribution of reactivity on bladder tumor material.

The group 1 antibody, which is prognostic, would be useful in routine pathological examination of tissue. Immunopathology would allow diagnosis on a molecular level, instead of the current cytostructural level. Antibodies 49.51 and 70.48 both bound preferentially to low and intermediate grade tumors, but did not do this consistently. Antibody 49.48 bound to intermediate grade tumors. This group of

antibodies would best function as a panel to enhance accuracy.

There are several potentially useful antibodies in group 2 which may be used to explore the heterogeneous nature of transitional cell carcinoma of the bladder. These include antibodies 2.51, 3.48, 5.48, 6.48 and 39.48. The most interesting of this group is antibody 5.48. This antibody bound preferentially to the superficial cells of both normal urothelium and well differentiated transitional cell carcinoma in which the architecture was sufficiently preserved to distinguish superficial from basal layers. This antibody may be reactive against a differentiation antigen which is produced or uncovered as a cell becomes more differentiated. Antibody 5.48 may be useful in purifying cell types. It has been shown that only a limited subpopulation of cells is capable of forming colonies in vitro and that these cells can be partially purified by physical separation techniques. Morphologically and histochemically this putative stem cell population resembles the basal layer of normal epithelium (101). The isolation of these stem cells may be enhanced using a combination of cell sorting along with this cell specific group of antibodies as markers. Continued research, using such cells, may help in revealing the key to tumor prognosis and identification.

Tumor specific antibodies (group 3) such as 49.51 would allow screening and early detection of bladder tumors by testing a patient's urine or serum. This would greatly improve early management and improve long term prognosis. Detecting the levels of antigen in the serum may serve as an indication of tumor progression or may be used to monitor therapeutic agents as they are administered. Finally, they may be used on stored serum or paraffin sections permitting retrospective studies. If an antibody of this group also belonged to group 4 as does 49.48, it could be radioactively labeled and used to localize a tumor, or may be coupled with toxic substances and used to target these agents to the tumor.

Two antibodies, 49.48 and 46.48, were urothelial specific and thus belong to group 4. If further testing against more tissues confirms this initial evaluation, these could play an important role in identifying the bladder as the primary source of metastasis.

CHAPTER 8

Summary

The need for further reliable, reproducible, and non-invasive tests for tumor prognosis and treatment response is addressed in this thesis. The soft agar clonogenic assay was found to be useful as a phase II test for new chemotherapeutic agents, permitting the usefulness of the drug to be explored without patient morbidity. Due to technical problems associated with the assay, it is not practical to use the assay to perform routine in vitro sensitivity testing for routine clinical specimens at this time. More accurate techniques such as thymidine incorporation to quantitate the endpoint of the soft agar colony formation assay or propagating the tumor suspension in small capillary tubes in order to facilitate more tests per sample of tumor are required. With continued research, standard methodology may be developed to further increase the scope of the soft agar clonogenic assay.

The monoclonal antibodies examined here, including the already developed monoclonal anti-CEA antibody, show no evidence of being useful in directly distinguishing between those transitional cell carcinomas which have a benign natural history and those which do not. However, there are several potentially useful antibodies that may be of benefit in exploring the heterogeneous nature of this tumor by their cell selecting abilities. The search for stem cells may be enhanced using a combination of cell sorting along with these antibodies as markers. Continued research, using such cells, may help in revealing the key to tumor prognosis and identification.

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APPENDIX A

Antibody 2.51: This antibody reacts with tissues including bladder. It is basal mucosal cell specific on some bladder specimens. This can be classified as a group 6 antibody.

Antibody 3.48: This antibody reacts with tissues including bladder. It is superficial mucosal cell specific on some bladder specimens and mucosal cell specific in an undetermined pattern on some bladder cancer specimens. This can be classified as a group 2 antibody.

Antibody 5:48: This antibody reacts with tissues including bladder. It is superficial mucosal cell specific on some bladder specimens and some bladder tumor specimens. This can be classified as group 2 antibody.

Antibody 6.48: This antibody reacts with many tissues including bladder. It is superficial mucosal cell specific on some bladder specimens. This can be classified as a group 2 antibody.

Antibody 6.51: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder tissues. This can be classified as a group 6 antibody.

Antibody 7.51: This antibody was produced later than the others tested. Therefore its screening is incomplete.

Antibody 9.51: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 12.48: This antibody reacts with tissues including bladder It exhibits no specific staining patterns for bladder but reacts only to epithelial surfaces. This can be classified as a group 5 antibody.

Antibody 14.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 17.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 17.51: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 18.51: This antibody was produced later than the others tested. Therefore its screening is incomplete. On partial screening it reacts to liver and to superficial bladder mucosa.

Antibody 21.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues This can be classified as a group 6 antibody.

Antibody 22.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patters for bladder or other tissues, but does only react to epithelial surfaces. This can be classified as a group 5 antibody.

Antibody 23.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 24.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 27.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 35.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 37.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 39.48: This antibody reacts with tissues including bladder. It is superficial mucosal cell specific on some bladder specimens and some bladder tumor specimens. This can be classified as a group 2 antibody.

Antibody 41.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 43.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 45.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder or other tissues. This can be classified as a group 6 antibody.

Antibody 46.48: This antibody is bladder tissues specific. It reacts to some normal bladder tissue and some tumor tissues. This can be classified as a group 4 antibody.

Antibody 49.48: This antibody reacts preferentially to low and intermediate grade bladder tumors. It is also bladder specific. This can be classified as belonging to groups 1, 3 and 4.

Antibody 49.51: This antibody reacts with heart and bladder cancer. It exhibits preferential reactivity with low grade bladder cancer. This can be classified as belonging to groups 1 and 3 and possibly 2.

Antibody 51.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder of other tissues. This can be classified as a group 6 antibody.

Antibody 52.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder of other tissues. This can be classified as a group 6 antibody.

Antibody 55.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder of other tissues. This can be classified as a group 6 antibody.

Antibody 57.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder of other tissues. This can be classified as a group 6 antibody.

Antibody 57.51: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder of other tissues. This can be classified as a group 6 antibody.

Antibody 63.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder of other tissues. This can be classified as a group 6 antibody.

Antibody 70.48: This antibody reacts with tissues including bladder. It exhibits preferential reactivity to low grade bladder tumor mucosa and superficial mucosa of normal bladder. This can be classified as belonging to group 1 and 2.

Antibody 72.48: This antibody was produced later than the others tested. Therefore its screening is incomplete. It does not react to tissues other than bladder.

Antibody 78.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder of other tissues. This can be classified as a group 6 antibody.

Antibody 81.48: This antibody reacts with tissues including bladder. It exhibits no specific staining patterns for bladder of other tissues. This can be classified as a group 6 antibody.

Antibody 2.51

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	363	everything
Bladder Ca 14	3/ 3	485	mucosa and endothelium
Bladder Ca 21	2/ 3	899	occasional mucosa
Bladder Ca 24	2/ 3	731	mucosa
Bladder Ca 29	1-2/ 3	645	mucosa
Bladder Ca 31	1/ 3	930	negative
Bladder Ca 32	1/ 3	1072	occasional mucosa
Bladder Ca 33	1/ 3	519	unreadable
Bladder 1	normal	680	basal ≥superficial mucosa
Bladder 2	normal	961	basal ≥superficial mucosa
Bladder 6	normal	1421	mucosa
Colon	normal	1382	epithelium
Fallopian	normal	1286	epithelium and stroma
Heart	normal	1247	faintly positive endothelium
Liver	normal	231	unreadable
Lung	normal	992	connective tissue
Ovary	normal	1325	stroma
Skin	normal	1130	negative
Small bowel	normal	1031	basal epithelium
Smooth muscle	normal	1169	endothelium
Stomach	normal	1371	everything

Antibody 2.51 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	874	some ≥ other mucosa
Bladder Ca 15	3/ 3	319	mucosa
Bladder Ca 17	2-3/ 3	297	mucosa
Bladder Ca 18	2/ 3	843	mucosa
Bladder Ca 28	2/ 3	275	mucosa
Bladder 3	normal	341	everything
Bladder 5	normal	253	mucosa and muscle

Antibody 3.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	212	unreadable
Bladder Ca 14	3/ 3	486	negative
Bladder Ca 21	2/ 3	900	negative
Bladder Ca 24	2/ 3	732	mixed positive and negative
Bladder Ca 29	1-2/ 3	646	negative
Bladder Ca 31	1/ 3	931	unreadable
Bladder Ca 32	1/ 3	763	mucosa
Bladder Ca 33	1/ 3	520	mucosa
Bladder 1	normal	681	superficial mucosa
Bladder 2	normal	962	occational superficial mucosa
Bladder 6	normal	1422	superficial ≥ basal mucosa
Colon	normal	1383	goblet cells
Fallopian	normal	1284	unreadable
Heart	normal	1245	negative
Liver	normal	69	negative
Lung	normal	993	negative
Ovary	normal	1326	negative
Skin	normal	1131	negative
Small bowel	normal	1032	unreadable
Smooth muscle	normal	1170	negative
Stomach	normal	1372	mid zone positive

Antibody 3.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 1	3/ 3	1550	negative
Bladder Ca 2	3/ 3	1658	negative
Bladder Ca 4	3/ 3	1682	occational positive mucosa
Bladder Ca 5	3/ 3	1584	mixed positive and negative
Bladder Ca 7	3/ 3	1568	negative
Bladder Ca 9	3/ 3	1576	negative
Bladder Ca 10	3/ 3	1650	mixed positive and negative
Bladder Ca 11	3/ 3	875	negative
Bladder Ca 15	3/ 3	103	mucosa
Bladder Ca 20	2/ 3	1616	mucosa some ≥ others
Bladder Ca 22	2/ 3	1624	mucosa
Bladder Ca 27	2/ 3	1560	mucosa
Bladder 3	normal	192	mucosa
Bladder 4	normal	1	occational superficial mucosa
Bladder 5	normal	143	mucosa and faintly muscle

Antibody 5.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	203	unreadable
Bladder Ca 14	3/ 3	487	negative
Bladder Ca 21	2/ 3	901	basal ≥ superficial mucosa
Bladder Ca 24	2/ 3	733	superficial ≥ basal mucosa
Bladder Ca 29	1-2/ 3	647	mucosa some ≥ others
Bladder Ca 31	1/ 3	932	unreadable
Bladder Ca 32	1/ 3	764	negative
Bladder Ca 33	1/ 3	521	unreadable
Bladder 1	normal	682	superficial ≥ basal mucosa
Bladder 2	normal	963	mucosa
Bladder 6	normal	1423	superficial ≥ basal mucosa
Colon	normal	1384	goblet cells
Fallopian	normal	1288	negative
Heart	normal	1249	negative
Liver	normal	60	negative
Lung	normal	994	negative
Ovary	normal	1327	negative
Skin	normal	1132	unreadable
Small bowel	normal	1033	negative
Smooth muscle	normal	1171	negative
Stomach	normal	1373	mid zone positive

Antibody 5.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 1	3/ 3	1551	mucosa
Bladder Ca 5	3/ 3	1585	mucosa
Bladder Ca 11	3/ 3	876	negative
Bladder Ca 15	3/ 3	94	mucosa
Bladder Ca 17	2-3/ 3	155	mucosa
Bladder Ca 18	2/ 3	845	mucosa
Bladder Ca 19	2/ 3	1543	superficial mucosa
Bladder Ca 22	2/ 3	1625	mucosa
Bladder Ca 23	2/ 3	1633	superficial ≥ basal mucosa
Bladder Ca 25	2/ 3	1593	mucosa
Bladder Ca 27	2/ 3	1561	mucosa
Bladder 3	normal	183	superficial ≥ basal mucosa
Bladder 5	normal	134	muscle

Antibody 6.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	213	unreadable
Bladder Ca 14	3/ 3	488	negative
Bladder Ca 21	2/ 3	902	mucosa
Bladder Ca 24	2/ 3	734	mucosa some ≥ others
Bladder Ca 29	1-2/ 3	648	negative
Bladder Ca 31	1/ 3	933	faintly positive
Bladder Ca 32	1/ 3	765	negative
Bladder Ca 33	1/ 3	522	unreadable
Bladder 1	normal	683	superficial mucosa
Bladder 2	normal	964	mucosa
Bladder 6	normal	1424	superficial ≥ basal mucosa
Colon	normal	1385	mucous glands
Fallopian	normal	1289	unreadable
Heart	normal	1250	negative
Liver	normal	70	negative
Lung	normal	995	negative
Ovary	normal	1328	stroma
Skin	normal	1133	sweat glands
Small bowel	normal	1034	negative
Smooth muscle	normal	1172	negative
Stomach	normal	1374	mid zone positive

Antibody 6.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	877	negative
Bladder Ca 15	3/ 3	104	mucosa
Bladder Ca 16	2-3/ 3	454	negative
Bladder Ca 17	2-3/ 3	165	mixed positive and negative
Bladder Ca 18	2/ 3	846	mucosa
Bladder 3	normal	193	mucosa
Bladder 4	normal	3	mucosa occational stroma
Bladder 5	normal	144	muscle

Antibody 6.51

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	360	unreadable
Bladder Ca 14	3/ 3	489	negative
Bladder Ca 21	2/ 3	1097	negative
Bladder Ca 24	2/ 3		
Bladder Ca 29	1-2/ 3	649	mucosa
Bladder Ca 31	1/ 3	934	negative
Bladder Ca 32	1/ 3	766	mucosa
Bladder Ca 33	1/ 3	523	negative
Bladder 1	normal	684	negative
Bladder 2	normal	965	negative
Bladder 6	normal	1425	mucosa
Colon	normal	1386	unreadable
Fallopian	normal	1290	unreadable
Heart	normal	1251	negative
Liver	normal	1114	unreadable
Lung	normal	996	negative
Ovary	normal	1329	unreadable
Skin	normal	1134	unreadable
Small bowel	normal	1073	negative
Smooth muscle	normal	1173	negative
Stomach	normal	1212	mid zone

Antibody 6.51 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	887	negative
Bladder Ca 17	2-3/ 3	294	faintly positive
Bladder Ca 18	2/ 3	847	faintly positive
Bladder Ca 28	2/ 3	272	mucosa
Bladder 5	normal	250	everything

Antibody 7.51

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	361	mucosa
Bladder Ca 14	3/ 3	490	mucosa
Bladder Ca 21	2/ 3		
Bladder Ca 29	1-2/ 3	650	mucosa
Bladder Ca 31	1/ 3		
Bladder Ca 32	1/ 3		
Bladder Ca 33	1/ 3	524	superficial ≥ basal mucosa
Bladder 1	normal		
Bladder 2	normal		
Bladder 6	normal		
Colon	normal		
Fallopian	normal		
Heart	normal		
Liver	normal	229	unreadable
Lung	normal		
Ovary	normal		
Skin	normal		
Small bowel	normal		
Smooth muscle	normal		
Stomach	normal		
	normal		

Antibody 7.51 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 15	3/ 3	317	mucosa
Bladder Ca 17	2-3/ 3	295	faintly positive
Bladder Ca 28	2/ 3	273	mucosa
Bladder 5	normal	251	mucosa

Antibody 9.51

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	364	unreadable
Bladder Ca 14	3/ 3	491	mucosa
Bladder Ca 21	2/ 3	1098	negative
Bladder Ca 24	2/ 3	736	mucosa
Bladder Ca 29	1-2/ 3	651	mucosa
Bladder Ca 31	1/ 3	935	unreadable
Bladder Ca 32	1/ 3	767	negative
Bladder Ca 33	1/ 3	525	unreadable
Bladder 1	normal	685	negative
Bladder 2	normal	966	negative
Bladder 6	normal	1426	faintly positive
Colon	normal	1387	epithelium
Fallopian	normal	1291	negative
Heart	normal	1252	negative
Liver	normal	232	faintly positive
Lung	normal	997	negative
Ovary	normal	1330	unreadable
Skin	normal	1135	negative
Small bowel	normal	1036	negative
Smooth muscle	normal	1174	negative
Stomach	normal	1376	superficial epithelium

Antibody 9.51 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	879	negative
Bladder Ca 15	3/ 3	220	mucosa
Bladder Ca 17	2-2/ 3	298	mucosa
Bladder Ca 18	2/ 3	848	mucosa
Bladder Ca 28	2/ 3	276	everything
Bladder 3	normal	342	mucosa
Bladder 5	normal	254	mucosa

Antibody 12.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	209	mucosa
Bladder Ca 14	3/ 3		
Bladder Ca 21	2/ 3		
Bladder Ca 29	1-2/ 3		
Bladder Ca 31	1/ 3		
Bladder Ca 32	1/ 3		
Bladder Ca 33	1/ 3		
Bladder 1	normal	711	basal ≥ superficial mucosa
Bladder 2	normal		
Bladder 6	normal	1452	mucosa
Colon	normal	1413	epithelium
Fallopian	normal	1317	epithelium
Heart	normal	1278	negative
Liver	normal	66	unreadable
Lung	normal	1023	alveolar
Ovary	normal	1356	faintly positive
Skin	normal	1161	negative
Small bowel	normal	1062	negative
Smooth muscle	normal	1200	negative
Stomach	normal	1481	epithelium

Antibody 12.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 15	3/ 3	100	mucosa
Bladder Ca 17	2-3/ 3	161	mucosa
Bladder 3	normal	189	mucosa
Bladder 4	normal	2	mucosa
Bladder 5	normal	140	mucosa

Antibody 14.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	198	unreadable
Bladder Ca 14	3/ 3		
Bladder Ca 21	2/ 3		
Bladder Ca 24	2/ 3		
Bladder Ca 29	1-2/ 3		
Bladder Ca 31	1/ 3		
Bladder Ca 32	1/ 3		
Bladder Ca 33	1/ 3		
Bladder 1	normal	712	negative
Bladder 2	normal		
Bladder 6	normal	1453	mucosa
Colon	normal	1414	epithelium
Fallopian	normal	1318	negative
Heart	normal	1279	negative
Liver	normal	55	unreadable
Lung	normal	1024	negative
Ovary	normal	1357	negative
Skin	normal	1162	negative
Small bowel	normal	1063	negative
Smooth muscle	normal		
Stomach	normal	1482	mid zone positive

Antibody 14.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 1	3/ 3	1546	faintly positive
Bladder Ca 4	3/ 3	1678	mucosa
Bladder Ca 5	3/ 3	1580	mucosa
Bladder Ca 7	3/ 3	1564	mucosa
Bladder Ca 9	3/ 3	1572	mucosa
Bladder Ca 10	3/ 3	1646	negative
Bladder Ca 17	2-3/ 3	150	negative
Bladder Ca 19	2/ 3	1538	negative
Bladder Ca 20	2/ 3	1612	negative
Bladder Ca 22	2/ 3	1620	negative
Bladder Ca 23	2/ 3	1628	mucosa
Bladder Ca 25	2/ 3	1588	negative
Bladder Ca 27	2/ 3	1556	faintly positive
Bladder Ca 30	1-2/ 3	78	negative
Bladder 3	normal	178	negative
Bladder 4	normal	109	negative
Bladder 5	normal	129	negative

Antibody 17.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	373	mucosa
Bladder Ca 14	3/ 3	493	negative
Bladder Ca 21	2/ 3	906	negative
Bladder Ca 24	2/ 3	738	negative
Bladder Ca 29	1-2/ 3	653	negative
Bladder Ca 31	1/ 3	937	negative
Bladder Ca 32	1/ 3	769	basal mucosa
Bladder Ca 33	1/ 3	527	unreadable
Bladder 1	normal	687	negative
Bladder 2	normal	968	superficial ≥ basal mucosa
Bladder 6	normal	1428	unreadable
Colon	normal	1389	negative
Fallopian	normal	1293	unreadable
Heart	normal	1254	negative
Liver	normal	1123	unreadable
Lung	normal	999	negative
Ovary	normal	1332	negative
Skin	normal	1137	sebaceous gland
Small bowel	normal	1038	negative
Smooth muscle	normal	1176	negative
Stomach	normal	1378	mid zone positive

Antibody 17.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 1	3/ 3	1549	negative
Bladder Ca 5	3/ 3	1583	negative
Bladder Ca 7	3/ 3	1567	mucosa
Bladder Ca 9	3/ 3	1575	negative
Bladder Ca 11	3/ 3	881	negative
Bladder Ca 15	3/ 3	93	negative
Bladder Ca 18	2/ 3	850	negative
Bladder Ca 19	2/ 3	1541	negative
Bladder Ca 22	2/ 3	1623	negative
Bladder Ca 25	2/ 3	1591	some ≥ other mucosa
Bladder Ca 27	2/ 3	1559	negative
Bladder Ca 28	2/ 3	45	some mucosa
Bladder Ca 30	1-2/ 3	80	mucosa
Bladder 5	normal	133	mucosa

Antibody 17.51

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	202	unreadable
Bladder Ca 14	3/ 3	492	negative
Bladder Ca 21	2/ 3	905	negative
Bladder Ca 24	2/ 3	737	mucosa
Bladder Ca 29	1-2/ 3	652	negative
Bladder Ca 31	1/ 3	936	negative
Bladder Ca 32	1/ 3	768	some ≥ other mucosa
Bladder Ca 33	1/ 3	526	faintly positive
Bladder 1	normal	686	negative
Bladder 2	normal	967	superficial ≥ basal mucosa
Bladder 6	normal		
Colon	normal		
Fallopian	normal	1292	negative
Heart	normal	1253	negative
Liver	normal		
Lung	normal	998	negative
Ovary	normal	1331	negative
Skin	normal	1369	faintly basal mucosa positive
Small bowel	normal	1037	epithelium
Smooth muscle	normal	1175	negative
Stomach	normal	1377	mid zone positive

Antibody 17.51 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 1	3/ 3	1548	faintly positive
Bladder Ca 2	3/ 3	1656	mucosa
Bladder Ca 3	3/ 3	1598	negative
Bladder Ca 6	3/ 3	1604	mucosa
Bladder Ca 7	3/ 3	1566	mucosa
Bladder Ca 9	3/ 3	1574	mucosa
Bladder Ca 11	3/ 3	880	negative
Bladder Ca 18	2/ 3	849	mucosa
Bladder Ca 19	2/ 3	1540	mucosa
Bladder Ca 20	2/ 3	1614	mucosa

Antibody 18.51

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	362	unreadable
Bladder Ca 14	3/ 3	494	negative
Bladder Ca 21	2/ 3		
Bladder Ca 24	2/ 3		
Bladder Ca 29	1-2/ 3	654	mucosa
Bladder Ca 31	1/ 3		
Bladder Ca 32	1/ 3		
Bladder Ca 33	1/ 3	528	mucosa
Bladder 1	normal		
Bladder 2	normal		
Bladder 6	normal		
Colon	normal		
Fallopian	normal		
Heart	normal		
Liver	normal	230	positive
Lung	normal		
Ovary	normal		
Skin	normal		
Small bowel	normal		
Smooth muscle	normal		
Stomach	normal		

Antibody 18.51 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 15	3/ 3	318	mucosa
Bladder Ca 17	2-3/ 3	296	negative
Bladder Ca 28	2/ 3	274	mucosa
Bladder Ca 3	normal	340	superficial mucosa
Bladder Ca 5	normal	252	superficial mucosa

Antibody 21.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	215	mucosa
Bladder Ca 14	3/ 3	495	negative
Bladder Ca 21	2/ 3	907	mucosa
Bladder Ca 24	2/ 3	739	mucosa
Bladder Ca 29	1-2/ 3	655	faintly positive
Bladder Ca 31	1/ 3	938	negative
Bladder Ca 32	1/ 3	770	mucosa
Bladder Ca 33	1/ 3	529	mucosa
Bladder 1	normal	688	unreadable
Bladder 2	normal	969	basal ≥ superficial mucosa
Bladder 6	normal	1429	mucosa
Colon	normal	1390	epithelium
Fallopian	normal	1294	epithelium
Heart	normal	1255	negative
Liver	normal	72	negative
Lung	normal	1000	negative
Ovary	normal	1333	unreadable
Skin	normal	1138	endothelium
Small bowel	normal	1039	negative
Smooth muscle	normal	1177	negative
Stomach	normal	1379	basal epithelium

Antibody 21.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 1	3/ 3	1547	mucosa
Bladder Ca 3	3/ 3	1597	negative
Bladder Ca 4	3/ 3	1679	some ≥ other mucosa
Bladder Ca 5	3/ 3	1581	mucosa
Bladder Ca 6	3/ 3	1605	stroma
Bladder Ca 7	3/ 3	1565	basal mucosa
Bladder Ca 9	3/ 3	1573	occational mucosa
Bladder Ca 11	3/ 3	882	negative
Bladder Ca 15	3/ 3	106	mucosa and occational stroma
Bladder Ca 17	2/ 3/ 3	167	occational mucosa
Bladder Ca 18	2/ 3	851	mucosa
Bladder Ca 19	2/ 3	1539	basal ≥ superficial mucosa
Bladder Ca 20	2/ 3	1613	negative
Bladder Ca 22	2/ 3	1621	occational mucosa
Bladder Ca 23	2/ 3	1629	mucosa
Bladder Ca 25	2/ 3	1589	mucosa
Bladder Ca 28	2/ 3	33	negative
Bladder Ca 30	1-2/ 3	85	mucosa
Bladder 3	normal	195	mucosa
Bladder 5	normal	146	mucosa

Antibody 22.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	199	mucosa
Bladder Ca 14	3/ 3	496	unreadable
Bladder Ca 21	2/ 3	1099	mucosa
Bladder Ca 24	2/ 3	740	mucosa
Bladder Ca 29	1-2/ 3	656	negative
Bladder Ca 31	1/ 3	939	mucosa
Bladder Ca 32	1/ 3	1073	mucosa
Bladder Ca 33	1/ 3	530	unreadable
Bladder 1	normal	689	mucosa
Bladder 2	normal	970	mucosa
Bladder 6	normal	1430	mucosa
Colon	normal	1391	epithelium
Fallopian	normal	1295	epithelium
Heart	normal	1256	negative
Liver	normal	56	negative
Lung	normal	1001	negative
Ovary	normal	1334	negative
Skin	normal	1370	glands
Small bowel	normal	1040	negative
Smooth muscle	normal	1178	negative
Stomach	normal	1380	superficial epithelium

Antibody 22.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	883	mucosa
Bladder Ca 15	3/ 3	90	occational mucosa
Bladder Ca 17	2-3/ 3	151	mucosa
Bladder Ca 18	2/ 3	852	mucosa
Bladder Ca 28	2/ 3	48	mucosa
Bladder Ca 30	1-2/ 3	77	mucosa
Bladder 3	normal	179	mucosa
Bladder 4	normal	110	superficial mucosa
Bladder 5	normal	130	basal ≥ superficial mucosa

Antibody 23.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	368	mucosa
Bladder Ca 14	3/ 3	497	negative
Bladder Ca 21	2/ 3	909	mucosa
Bladder Ca 24	2/ 3	741	negative
Bladder Ca 29	1-2/ 3	657	mucosa
Bladder Ca 31	1/ 3	940	negative
Bladder Ca 32	1/ 3	772	mucosa
Bladder Ca 33	1/ 3	531	unreadable
Bladder 1	normal	690	mucosa
Bladder 2	normal	971	basal ≥ superficial mucosa
Bladder 6	normal	1431	mucosa
Colon	normal	1392	epithelium
Fallopian	normal	1296	epithelium and stroma
Heart	normal	1257	negative
Liver	normal	1119	everything
Lung	normal	1002	negative
Ovary	normal	1335	stroma
Skin	normal	1140	negative
Small bowel	normal	1041	negative
Smooth muscle	normal	1179	negative
Stomach	normal	1460	epithelium

Antibody 23.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	884	faintly positive
Bladder Ca 15	3/ 3	324	mucosa
Bladder Ca 17	2 3/ 3	302	mucosa
Bladder Ca 18	2/ 3	853	faintly positive
Bladder Ca 28	2/ 3	30	mucosa
Bladder 4	normal	21	mucosa
Bladder 5	normal	258	mucosa

Antibody 24.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	207	unreadable
Bladder Ca 14	3/ 3		
Bladder Ca 21	2/ 3		
Bladder Ca 24	2/ 3		
Bladder Ca 29	1-2/ 3		
Bladder Ca 31	1/ 3		
Bladder Ca 32	1/ 3		
Bladder Ca 33	1/ 3		
Bladder 1	normal	796	basal ≥ superficial mucosa
Bladder 2	normal		
Bladder 6	normal	1454	mucosa
Colon	normal	1415	epithelium
Fallopian	normal	1319	epithelium
Heart	normal	1280	negative
Liver	normal	64	unreadable
Lung	normal	1025	negative
Ovary	normal	1358	negative
Skin	normal	1163	negative
Small bowel	normal	1064	negative
Smooth muscle	normal	1202	negative
Stomach	normal	1483	unreadable

Antibody 24.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 15	3/ 3	98	mucosa
Bladder Ca 17	2 3/ 3	159	negative
Bladder Ca 28	2/ 3	41	everything
Bladder Ca 30	1 2/ 3	119	mucosa
Bladder 3	normal	187	mucosa
Bladder 4	normal	9	mucosa
Bladder 5	normal	138	mucosa
			mucosa

Antibody 27.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	206	stroma ≥ epithelium
Bladder Ca 14	3/ 3		
Bladder Ca 21	2/ 3		
Bladder Ca 24	2/ 3		
Bladder Ca 29	1-2/ 3		
Bladder Ca 31	1/ 3		
Bladder Ca 32	1/ 3		
Bladder Ca 33	1/ 3		
Bladder 1	normal	714	basal ≥ superficial mucosa
Bladder 2	normal		
Bladder 6	normal	1455	mucosa and stroma
Colon	normal	1416	everything
Fallopian	normal	1320	everything
Heart	normal	1281	stroma
Liver	normal	63	negative
Lung	normal	1026	alveolar
Ovary	normal	1359	stroma
Skin	normal	1164	connective tissues
Small bowel	normal	1065	stroma
Smooth muscle	normal	1203	negative
Stomach	normal	1242	connective tissue

Antibody 27.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 15	3/ 3	97	mucosa and occational stroma
Bladder Ca 17	2 3/ 3	158	mucosa
Bladder Ca 28	2/ 3	51	mucosa and stroma
Bladder Ca 30	1 2/ 3	118	mucosa
Bladder 3	normal	186	mucosa and stroma
Bladder 5	normal	137	everything

Antibody 35.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	214	mucosa
Bladder Ca 14	3/ 3	498	negative
Bladder Ca 21	2/ 3	910	mucosa
Bladder Ca 24	2/ 3	742	mucosa
Bladder Ca 29	1-2/ 3	658	negative
Bladder Ca 31	1/ 3	1101	negative
Bladder Ca 32	1/ 3	773	faintly positive
Bladder Ca 33	1/ 3	532	mucosa
Bladder 1	normal	691	mucosa
Bladder 2	normal	972	unreadable
Bladder 6	normal	1432	mucosa
Colon	normal	1393	mucous cells
Fallopian	normal	1297	unreadable
Heart	normal	1258	negative
Liver	normal	71	negative
Lung	normal	1003	negative
Ovary	normal	1336	unreadable
Skin	normal	1141	negative
Small bowel	normal	1042	negative
Smooth muscle	normal	1180	negative
Stomach	normal	1461	unreadable

Antibody 35.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 8	3/ 3	793	negative
Bladder Ca 11	3/ 3	885	negative
Bladder Ca 15	3/ 3	105	mucosa
Bladder Ca 17	2 3/ 3	166	occational mucosa
Bladder Ca 18	2/ 3	854	mucosa
Bladder Ca 28	2/ 3	35	mucosa
Bladder Ca 30	1-2/ 3	84	mucosa
Bladder 3	normal	194	mucosa
Bladder 4	normal	5	mucosa
Bladder 5	normal	172	mucosa

Antibody 37.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	204	unreadable
Bladder Ca 14	3/ 3	499	endothelium and connective
Bladder Ca 21	2/ 3	911	negative
Bladder Ca 24	2/ 3	743	negative
Bladder Ca 29	1-2/ 3	659	negative
Bladder Ca 31	1/ 3	1102	negative
Bladder Ca 32	1/ 3	774	mucosa
Bladder Ca 33	1/ 3	533	unreadable
Bladder 1	normal	692	stroma
Bladder 2	normal	973	superficial mucosa
Bladder 6	normal	1433	unreadable
Colon	normal	1394	faintly positive
Fallopian	normal	1298	stroma
Heart	normal	1259	connective tissue
Liver	normal	61	unreadable
Lung	normal	1004	stroma
Ovary	normal	1337	stroma
Skin	normal	1142	unreadable
Small bowel	normal	1043	mucous
Smooth muscle	normal	1181	negative
Stomach	normal	1462	mid zone positive

Antibody 37.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	886	faintly positive
Bladder Ca 15	3/ 3	95	stroma faintly positive
Bladder Ca 17	2 3/ 3	156	mucosa
Bladder Ca 18	2/ 3	855	faintly positive
Bladder Ca 28	2/ 3	43	mucosa
Bladder Ca 30	1-2/ 3	116	superficial mucosa
Bladder 3	normal	184	everything
Bladder 4	normal	7	unreadable
Bladder 5	normal	135	mucosa

Antibody 39.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	366	superficial ≥ basal mucosa
Bladder Ca 14	3/ 3	500	negative
Bladder Ca 21	2/ 3	912	mucosa
Bladder Ca 24	2/ 3	744	superficial ≥ basal mucosa
Bladder Ca 29	1-2/ 3	660	mucosa
Bladder Ca 31	1/ 3	943	negative
Bladder Ca 32	1/ 3	1074	negative
Bladder Ca 33	1/ 3	534	unreadable
Bladder 1	normal	693	superficial ≥ basal mucosa
Bladder 2	normal	974	mucosa and stroma
Bladder 6	normal	1434	superficial ≥ basal mucosa
Colon	normal	1395	goblet cells
Fallopian	normal	1299	epithelium
Heart	normal	1260	negative
Liver	normal	1118	unreadable
Lung	normal	1005	negative
Ovary	normal	1338	unreadable
Skin	normal	1143	stroma
Small bowel	normal	1044	epithelium
Smooth muscle	normal	1182	negative
Stomach	normal	1463	epithelium

Antibody 39.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	887	negative
Bladder Ca 15	3/ 3	332	mucosa
Bladder Ca 17	2 3/ 3	300	mucosa
Bladder Ca 18	2/ 3	856	mucosa
Bladder Ca 28	2/ 3	278	everything
Bladder 3	normal	344	mucosa and occational stroma
Bladder 4	normal	20	mucosa
Bladder 5	normal	256	basal ≥ superficial mucosa

Antibody 41.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	375	unreadable
Bladder Ca 14	3/ 3		
Bladder Ca 21	2/ 3	913	negative
Bladder Ca 24	2/ 3	745	negative
Bladder Ca 29	1-2/ 3	662	negative
Bladder Ca 31	1/ 3	944	unreadable
Bladder Ca 32	1/ 3	776	negative
Bladder Ca 33	1/ 3	536	negative
Bladder 1	normal	694	negative
Bladder 2	normal	975	negative
Bladder 6	normal	1435	negative
Colon	normal	1396	negative
Fallopian	normal	1300	negative
Heart	normal	1261	negative
Liver	normal	1125	unreadable
Lung	normal	1006	negative
Ovary	normal	1339	negative
Skin	normal	1144	negative
Small bowel	normal	1045	negative
Smooth muscle	normal	1183	negative
Stomach	normal	1464	everything

Antibody 41.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	888	negative
Bladder Ca 18	2/ 3	857	mucosa
Bladder 3	normal	386	negative

Antibody 43.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	367	mucosa and endothelium
Bladder Ca 14	3/ 3	503	negative
Bladder Ca 21	2/ 3	914	mucosa
Bladder Ca 24	2/ 3	746	mucosa
Bladder Ca 29	1-2/ 3	663	mucosa
Bladder Ca 31	1/ 3	945	negative
Bladder Ca 32	1/ 3	777	mucosa
Bladder Ca 33	1/ 3	537	unreadable
Bladder 1	normal	695	mucosa
Bladder 2	normal	976	mucosa
Bladder 6	normal	1436	mucosa
Colon	normal	1397	epithelium and stroma
Fallopian	normal	1301	epithelium
Heart	normal	1262	negative
Liver	normal	235	everything
Lung	normal	1007	alveolar
Ovary	normal	1340	negative
Skin	normal	1145	unreadable
Small bowel	normal	1046	negative
Smooth muscle	normal	1184	negative
Stomach	normal	1465	mid zone

Antibody 43.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	889	mucosa
Bladder Ca 15	3/ 3	323	mucosa
Bladder Ca 17	2 3/ 3	301	mucosa
Bladder Ca 18	2/ 3	858	mucosa
Bladder Ca 25	2/ 3	32	mucosa and stroma
Bladder Ca 28	2/ 3	279	everything
Bladder 3	normal	345	mucosa
Bladder 4	normal	19	mucosa and endothelium
Bladder 5	normal	257	mucosa and endothelium

Antibody 45.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	376	superficial mucosa
Bladder Ca 14	3/ 3	504	occational mucosa
Bladder Ca 21	2/ 3	915	negative
Bladder Ca 24	2/ 3	747	negative
Bladder Ca 29	1-2/ 3	664	negative
Bladder Ca 31	1/ 3	946	negative
Bladder Ca 32	1/ 3	778	basal mucosa/ some stroma
Bladder Ca 33	1/ 3	538	mucosa
Bladder 1	normal	696	negative
Bladder 2	normal	977	negative
Bladder 6	normal	1437	negative
Colon	normal	1398	epithelium
Fallopian	normal	1302	negative
Heart	normal	1263	connective
Liver	normal	1126	everything
Lung	normal	1008	negative
Ovary	normal	1341	negative
Skin	normal	1146	negative
Small bowel	normal	1047	negative
Smooth muscle	normal	1185	negative
Stomach	normal	1466	mid zone positive

Antibody 45.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	890	negative
Bladder Ca 18	2/ 3	859	negative
Bladder 3	normal	387	mucosa/ endothelium/ stroma

Antibody 46.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3		
Bladder Ca 14	3/ 3	679	negative
Bladder Ca 21	2/ 3	916	negative
Bladder Ca 24	2/ 3	748	negative
Bladder Ca 29	1-2/ 3	665	negative
Bladder Ca 31	1/ 3	947	negative
Bladder Ca 32	1/ 3	779	negative
Bladder Ca 33	1/ 3	539	unreadable
Bladder 1	normal	697	negative
Bladder 2	normal	978	negative
Bladder 6	normal	1438	negative
Colon	normal	1399	negative
Fallopian	normal	1303	negative
Heart	normal	1264	negative
Liver	normal	54	negative
Lung	normal	1009	negative
Ovary	normal	1342	negative
Skin	normal	1147	negative
Small bowel	normal	1048	negative
Smooth muscle	normal	1186	negative
Stomach	normal	1467	negative

Antibody 46.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 1	3/ 3	1552	negative
Bladder Ca 5	3/ 3	1586	negative
Bladder Ca 7	3/ 3	1570	mucosa
Bladder Ca 9	3/ 3	1578	negative
Bladder Ca 11	3/ 3	891	negative
Bladder Ca 15	3/ 3	124	occational stroma
Bladder Ca 18	2/ 3	860	negative
Bladder Ca 19	2/ 3	1544	negative
Bladder Ca 22	2/ 3	1626	negative
Bladder Ca 23	2/ 3	1634	mucosa
Bladder Ca 25	2/ 3	1594	occational stroma
Bladder Ca 26	2/ 3	1668	negative
Bladder Ca 27	2/ 3	1562	negative
Bladder Ca 30	1 2/ 3	115	mucosa
Bladder 3	normal	177	negative
Bladder 5	normal	128	mucosa

Antibody 49.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	369	unreadable
Bladder Ca 14	3/ 3	507	unreadable
Bladder Ca 21	2/ 3	918	negative
Bladder Ca 24	2/ 3	750	negative
Bladder Ca 29	1-2/ 3	667	negative
Bladder Ca 31	1/ 3	949	unreadable
Bladder Ca 32	1/ 3	781	negative
Bladder Ca 33	1/ 3	541	unreadable
Bladder 1	normal	699	negative
Bladder 2	normal	980	negative
Bladder 6	normal	1440	negative
Colon	normal	1401	negative
Fallopian	normal	1305	negative
Heart	normal	1266	negative
Liver	normal	1368	negative
Lung	normal	1011	negative
Ovary	normal	1344	negative
Skin	normal	1149	unreadable
Small bowel	normal	1050	negative
Smooth muscle	normal	1188	negative
Stomach	normal	1469	unreadable

Antibody 49.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 1	3/ 3	1553	negative
Bladder Ca 5	3/ 3	1587	negative
Bladder Ca 7	3/ 3	1571	negative
Bladder Ca 9	3/ 3	1579	negative
Bladder Ca 11	3/ 3	893	negative
Bladder Ca 15	3/ 3	325	negative
Bladder Ca 17	2 3/ 3	303	faintly positive
Bladder Ca 18	2/ 3	862	faintly positive
Bladder Ca 19	2/ 3	1545	negative
Bladder Ca 23	2/ 3	1635	mucosa
Bladder Ca 28	2/ 3	281	faintly positive
Bladder Ca 29	1 2/ 3	667	negative
Bladder 3	normal	347	negative
Bladder 5	normal	259	negative

Antibody 49.51

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3		
Bladder Ca 14	3/ 3	506	unreadable
Bladder Ca 21	2/ 3	917	negative
Bladder Ca 24	2/ 3	749	negative
Bladder Ca 29	1-2/ 3	666	occasional mucosa
Bladder Ca 31	1/ 3	948	negative
Bladder Ca 32	1/ 3	780	basal mucosa
Bladder Ca 33	1/ 3	540	unreadable
Bladder 1	normal	698	negative
Bladder 2	normal	979	negative
Bladder 6	normal	1439	negative
Colon	normal	1400	negative
Fallopian	normal	1304	negative
Heart	normal	1265	connective
Liver	normal		
Lung	normal	1010	negative
Ovary	normal	1343	negative
Skin	normal	1148	negative
Small bowel	normal	1049	negative
Smooth muscle	normal	1187	negative
Stomach	normal	1468	negative

Antibody 49.51 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	892	negative
Bladder Ca 18	2/ 3	861	negative

Antibody 51.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	378	mucosa
Bladder Ca 14	3/ 3	508	unreadable
Bladder Ca 21	2/ 3	919	negative
Bladder Ca 24	2/ 3	751	negative
Bladder Ca 29	1-2/ 3	668	negative
Bladder Ca 31	1/ 3	950	negative
Bladder Ca 32	1/ 3	782	faintly positive
Bladder Ca 33	1/ 3	542	unreadable
Bladder 1	normal	700	negative
Bladder 2	normal	981	negative
Bladder 6	normal	1441	negative
Colon	normal	1402	faintly positive stroma
Fallopian	normal	1306	faintly positive
Heart	normal	1267	connective and endothelium
Liver	normal	1128	unreadable
Lung	normal	1012	negative
Ovary	normal	1345	negative
Skin	normal	1150	negative
Small bowel	normal	1051	negative
Smooth muscle	normal	1189	negative
Stomach	normal	1470	mid zone positive

Antibody 51.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	894	negative
Bladder Ca 18	2/ 3	1095	negative
Bladder 3	normal	389	mucosa/ endothelium/ stroma

Antibody 52.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	379	mucosa
Bladder Ca 14	3/ 3	509	mucosa and endothelium
Bladder Ca 21	2/ 3	920	negative
Bladder Ca 24	2/ 3	752	negative
Bladder Ca 29	1-2/ 3	669	negative
Bladder Ca 31	1/ 3	951	negative
Bladder Ca 32	1/ 3	783	mucosa
Bladder Ca 33	1/ 3	543	negative
Bladder 1	normal	701	negative
Bladder 2	normal	1104	negative
Bladder 6	normal	1442	faintly positive
Colon	normal	1403	negative
Fallopian	normal	1307	epithelium
Heart	normal	1268	connective and endothelium
Liver	normal	1129	stroma
Lung	normal	1013	negative
Ovary	normal	1346	negative
Skin	normal	1151	negative
Small bowel	normal	1052	negative
Smooth muscle	normal	1190	negative
Stomach	normal	1471	stroma

Antibody 52.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 8	3/ 3	794	negative
Bladder Ca 11	3/ 3	895	negative
Bladder Ca 18	2/ 3	864	negative
Bladder 3	normal	390	mucosa/ endothelium/ stroma

Antibody 55.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	200	mucosa
Bladder Ca 14	3/ 3	510	mucosa
Bladder Ca 21	2/ 3	921	mucosa
Bladder Ca 24	2/ 3	1364	mucosa
Bladder Ca 29	1-2/ 3	670	negative
Bladder Ca 31	1/ 3	952	occasional mucosa
Bladder Ca 32	1/ 3	784	negative
Bladder Ca 33	1/ 3	544	mucosa
Bladder 1	normal	702	mucosa/ occasional stroma
Bladder 2	normal	983	mucosa
Bladder 6	normal	1443	superficial ≥ basal mucosa
Colon	normal	1404	epithelium
Fallopian	normal	1308	epithelium
Heart	normal	1269	negative
Liver	normal	57	faintly positive
Lung	normal	1014	negative
Ovary	normal	1347	negative
Skin	normal	1152	negative
Small bowel	normal	1053	mucous
Smooth muscle	normal	1191	negative
Stomach	normal	1472	faintly positive epithelium

Antibody 55.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	896	negative
Bladder Ca 15	3/ 3	91	occasional mucoa
Bladder Ca 17	2 3/ 3	152	mucosa
Bladder Ca 18	2/ 3	865	mucosa
Bladder Ca 30	1 2/ 3	78	mucosa
Bladder 3	normal	180	mucosa
Bladder 4	normal	6	mucosa
Bladder 5	normal	131	mucosa and muscle

Antibody 57.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	380	mucosa
Bladder Ca 14	3/ 3	511	negative
Bladder Ca 21	2/ 3	922	negative
Bladder Ca 24	2/ 3	1071	occasional mucosa
Bladder Ca 29	1-2/ 3	671	negative
Bladder Ca 31	1/ 3	1103	negative
Bladder Ca 32	1/ 3	785	negative
Bladder Ca 33	1/ 3	545	mucosa
Bladder 1	normal	703	mucosa
Bladder 2	normal	984	endothelium
Bladder 6	normal	1444	mucosa
Colon	normal	1405	epithelium and stroma
Fallopian	normal	1309	everything
Heart	normal	1270	negative
Liver	normal	1124	everything
Lung	normal	1015	alveolar
Ovary	normal	1348	stroma
Skin	normal	1153	negative
Small bowel	normal	1054	mucous cells
Smooth muscle	normal	1192	negative
Stomach	normal	1473	negative

Antibody 57.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	897	mucosa
Bladder Ca 15	3/ 3	92	basal mucosa and stroma
Bladder Ca 17	2 3/ 3	153	negative
Bladder Ca 18	2/ 3	866	mucosa and stroma
Bladder Ca 30	1 2/ 3	79	basal ≥ superficial mucosa
Bladder 3	normal	181	mucosa and endothelium
Bladder 4	normal	16	mucosa
Bladder 5	normal	132	mucosa/ muscle/ stroma

Antibody 57.51

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3		
Bladder Ca 14	3/ 3	512	faintly positive stroma
Bladder Ca 21	2/ 3	923	negative
Bladder Ca 24	2/ 3	755	negative
Bladder Ca 29	1-2/ 3	672	negative
Bladder Ca 31	1/ 3	954	negative
Bladder Ca 32	1/ 3	1076	negative
Bladder Ca 33	1/ 3	546	negative
Bladder 1	normal	704	negative
Bladder 2	normal	985	negative
Bladder 6	normal	1445	faintly positive
Colon	normal	1406	negative
Fallopian	normal	1310	negative
Heart	normal	1271	connective
Liver	normal		
Lung	normal	1016	negative
Ovary	normal	1349	negative
Skin	normal	1154	negative
Small bowel	normal	1055	negative
Smooth muscle	normal	1193	negative
Stomach	normal	1474	mid zone positive

Antibody 57.51 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 11	3/ 3	898	negative
Bladder Ca 18	2/ 3	867	negative

Antibody 63.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	365	mucosa
Bladder Ca 14	3/ 3	513	mucosa
Bladder Ca 21	2/ 3	924	faintly positive few areas
Bladder Ca 24	2/ 3	756	mucosa some ≥ other
Bladder Ca 29	1-2/ 3	673	mucosa
Bladder Ca 31	1/ 3	955	negative
Bladder Ca 32	1/ 3	1077	mucosa
Bladder Ca 33	1/ 3	547	mucosa
Bladder 1	normal	705	mucosa
Bladder 2	normal	986	basal mucosa
Bladder 6	normal	1446	mucosa
Colon	normal	1407	epithelium
Fallopian	normal	1311	epithelium
Heart	normal	1272	negative
Liver	normal	1117	everything
Lung	normal	1017	alveolar
Ovary	normal	1350	negative
Skin	normal	1155	negative
Small bowel	normal	1056	epithelium
Smooth muscle	normal	1194	negative
Stomach	normal	1475	mid zone positive

Antibody 63.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 15	3/ 3	321	mucosa
Bladder Ca 17	2 3/ 3	299	mucosa
Bladder Ca 18	2/ 3	868	mucosa
Bladder Ca 28	2/ 3	277	mucosa
Bladder 5	normal	255	negative

Antibody 70.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	211	unreadable
Bladder Ca 14	3/ 3	514	negative
Bladder Ca 21	2/ 3	925	negative
Bladder Ca 24	2/ 3	757	negative
Bladder Ca 29	1-2/ 3	674	negative
Bladder Ca 31	1/ 3	956	negative
Bladder Ca 32	1/ 3	788	unreadable
Bladder Ca 33	1/ 3	548	negative
Bladder 1	normal	706	stroma
Bladder 2	normal	987	superficial mucosa + stroma
Bladder 6	normal	1447	superficial mucosa + stroma
Colon	normal	1408	goblet cells
Fallopian	normal	1312	stroma
Heart	normal	1273	connective
Liver	normal	1121	everything
Lung	normal	1018	negative
Ovary	normal	1351	stroma
Skin	normal	1156	epithelium
Small bowel	normal	1057	mucous
Smooth muscle	normal	1195	negative
Stomach	normal	1476	mid zone positive

Antibody 70.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 15	3/ 3	102	negative
Bladder Ca 17	2 3/ 3	163	mucosa
Bladder Ca 18	2/ 3	869	mucosa
Bladder Ca 28	2/ 3	37	mucosa
Bladder Ca 30	1 2/ 3	123	mucosa
Bladder 3	normal	191	everything
Bladder 4	normal	14	negative
Bladder 5	normal	142	mucosa + occational stroma

Antibody 72.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	208	unreadable
Bladder Ca 14	3/ 3		
Bladder Ca 21	2/ 3		
Bladder Ca 24	2/ 3		
Bladder Ca 29	1-2/ 3		
Bladder Ca 31	1/ 3		
Bladder Ca 32	1/ 3		
Bladder Ca 33	1/ 3		
Bladder 1	normal	716	mucosa
Bladder 2	normal		
Bladder 6	normal	1457	superficial ≥ basal mucosa
Colon	normal	1418	epithelium
Fallopian	normal	1322	epithelium
Heart	normal	1283	negative
Liver	normal	65	negative
Lung	normal	1028	negative
Ovary	normal	1361	stroma
Skin	normal	1166	negative
Small bowel	normal	1067	muscle and epithelium
Smooth muscle	normal	1205	negative
Stomach	normal	1486	epithelium

Antibody 72.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 15	3/ 3	99	mucosa
Bladder Ca 17	2 3/ 3	160	mucosa
Bladder Ca 30	1 2/ 3	120	mucosa
Bladder 3	normal	188	mucosa
Bladder 5	normal	139	muscle/ occational epithelium

Antibody 78.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	381	unreadable
Bladder Ca 14	3/ 3	428	negative
Bladder Ca 21	2/ 3	424	negative
Bladder Ca 24	2/ 3	433	negative
Bladder Ca 29	1-2/ 3	675	negative
Bladder Ca 31	1/ 3	438	negative
Bladder Ca 32	1/ 3	789	negative
Bladder Ca 33	1/ 3	549	mucosa
Bladder 1	normal	707	negative
Bladder 2	normal	988	negative
Bladder 6	normal	1448	mucosa
Colon	normal	1409	faintly positive
Fallopian	normal	1313	negative
Heart	normal	1274	negative
Liver	normal	1367	negative
Lung	normal	1019	negative
Ovary	normal	1352	unreadable
Skin	normal	1157	negative
Small bowel	normal	1058	negative
Smooth muscle	normal	1196	negative
Stomach	normal	1477	everything

Antibody 78.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 1	3/ 3	436	negative
Bladder Ca 10	3/ 3	446	negative
Bladder Ca 11	3/ 3	443	negative
Bladder Ca 16	2 3/ 3	425	everything
Bladder Ca 18	2/ 3	1096	negative
Bladder Ca 22	2/ 3	441	negative
Bladder Ca 28	2/ 3	440	negative
Bladder Ca 30	1 2/ 3	443	negative

Antibody 81.48

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 13	3/ 3	382	unreadabe
Bladder Ca 14	3/ 3	516	mucosa
Bladder Ca 21	2/ 3	927	mucosa
Bladder Ca 24	2/ 3	759	mucosa
Bladder Ca 29	1-2/ 3	676	mucosa
Bladder Ca 31	1/ 3	958	negative
Bladder Ca 32	1/ 3	790	mucosa
Bladder Ca 33	1/ 3	550	negative
Bladder 1	normal	708	negative
Bladder 2	normal	989	mucosa
Bladder 6	normal	1449	mucosa
Colon	normal	1410	epithelium
Fallopian	normal	1314	epithelium and stroma
Heart	normal	1275	negative
Liver	normal	1115	everything
Lung	normal	1020	negative
Ovary	normal	1353	stroma
Skin	normal	1158	negative
Small bowel	normal	1059	goblet cells
Smooth muscle	normal	1197	negative
Stomach	normal	1478	mid zone positive

Antibody 81.48 Additional Sections

TISSUE	GRADE	SLIDE #	REMARKS
Bladder Ca 18	2/ 3	871	mucosa
Bladder 3	normal	393	mucosa