Industry
Biologics

Key Features
Biobina is developing safer treatment for malignant effusions and cancers of organs in the pleural and abdominal cavities and diseases. The cumulative market size exceeds $1 billion. Company's intellectual property will provide protection through 2034. Research and Development to date benefited from $6 million in philanthropic funding and seed funding from Columbia University. Biobina plans to file an IND in 2017and exit upon initiation of Phase II trials in 2020. GMP-grade material for early clinical trials will be provided by BioSeutica. Initial clinical trial focused on use of avidin construct as therapy for Malignant Pleural and Mesothelioma is expected to enroll 25 patients and last 24 months. Other abdominal and peritoneal indications (ovarian, renal, gastric, colorectal cancer) are also under consideration.

Company Resources
License Agreement between Columbia and Biobina will be executed upon funding. Patent applications provide protection through 2034.

Pre-Money Valuation:
$6,000,000

Financing Sought:
$2,500,000 – 1st round
$4,000,000 – 2nd round
$7,000,000 – 3rd round

Total External Capital Invested
$6 million in University-based funding through philanthropic sources and foundations.

Awards/Recognition
Elliott Osserman Award, Israel Cancer Research Fund
Pioneer Award, Mesothelioma Applied Research Foundation
Collaborator Award, American Cancer Society, NYC Division

Business Description: Biobina is a spin-off from Columbia University focused on early clinical development of biologic constructs for pretargeting a variety of solid tumors for radiotherapy and cytotoxic drugs. Our initial product is a patented (through 2034) construct of the egg-white protein avidin conjugated to particles of surgical grade talc as used for pleurodesis treatment of malignant pleural effusions common in lung cancer, mesothelioma, breast cancer, lymphomas, and some heart diseases and liver diseases.

The abdominal indications utilize avidin incorporated into commonly used tissue adhesives, including gelatin matrix (Gelfoam®) beads, sponge or mesh; and chemically modified fibrinogen. Either avidin-linked substance may serve as a support, sealant, clot-promoting agent, or surgical adhesive facilitating pre-targeted radio- or chemo-therapy. Contemplated uses include pre-targeted treatment of malignant pleural effusions; adjuvant surface radiotherapy targeting of abdominal malignancy during cytoreductive surgery, (ovarian, appendiceal, and peritoneal mesothelial cancer, malignant ascites, intra-abdominal sarcomas). Thus, cumulatively the thoracic and abdominal indications implemented at a conservative figure of $20,000 or more per treatment are addressing a blockbuster market. Human studies will require an IND for the parenteral (intravenous, intracavity, intratissue) use of unconjugated pure avidin as well as radiodinated biotin-avidin construct. Because technically the individual components of Biobina constructs are each safe and efficacious on their own, we expect to advance this technology rapidly to FDA approved human studies.

Furthermore, because Biobina constructs concentrate the active complex in the diseased tissue, and may employ scavenger avidin to eliminate aberrant stray radioactivity we expect to minimize toxicity in healthy tissue allowing the use of higher doses of locally applied radiotherapy. Our intent is to file for an IND for avidin through Columbia by June of 2017; enroll patients for thoracic indications in August of 2017; and file an IND application for abdominal surgical indications by mid-2018.

We will initially focus on treatment of malignant pleural mesothelioma (MPM), a disease of the lining of the lung and chest wall, with an annual incidence of 3300 cases in the US. Because this indication is an FDA-designated orphan disease, clinical development costs will be lower and the time to approval shorter. Other abdominal conditions also under consideration for initial studies are appendiceal and urinary tract malignancy We estimate the cost of development of the thoracic Phase II registration trial at under $2 million.

We aim to position Biobina constructs as a treatment that could be administered to all hospitalized patients with mesothelioma or lung malignancies that involve the pleural surfaces.

We anticipate selling Biobina or executing a co-development agreement with pharma within 4 years from funding, or at the completion of Phase 2 studies, which might well be shorter. The Company's value at that time should exceed 30 million, providing investors with 10x ROI.

Scientific Advisors

Robert Norman Taub, M.D.
Vivian and Seymour Milstein Family Prof. of Clinical Medicine Department of Medicine – Hematology/ Oncology Columbia University

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Primary Indication: Malignant pleural mesothelioma (MPM) is a rare tumor that usually forms on the tissue lining lungs and other organs. The cancer is treatable but not curable. A common cause of MPM is exposure to asbestos, and although asbestos use has decreased, the number of MPM cases is expected to rise. Pleural effusion, a condition where liquid buildup in between lung walls leads to shortness of breath, affects 95% of MPM patients. Pleurodesis is a surgical procedure where the fluid is drained and the cavity can be filled with talc particles, inflaming the surface and allowing the cavity to close and preventing recurrence of fluid buildup. Follow-up treatments include chemo-therapy to remove residual cancerous cells. However, these follow-up treatments are not selective in their targeting consequently there is a critical need for reduction of collateral toxicity that is limiting therapeutic dose. Biobina technology localizes and limits chemotherapy to areas where treatment is needed.

Secondary Indications: In the abdomen, the use of gelfoam-avidin-biotin-radioisotope coupled to gelfoam with intravenous avidin prophylaxis is projected to be useful preoperatively for stage II-III cancers of the colon, appendix, stomach, biliary tract, and kidney, which conservatively comprise at least 10% of 250,000 new patients per year. Additionally The avidin-gelfoam carrier can be appropriately modified for use in bladder and ureteral cancer combined with and during urinary diversion, (60,000 new cases per year, many may need repeated treatment).

Market size and growth: There are at least 80,000 pleural effusions diagnosed in the United States per year. Malignant pleural effusions are common (10% of diagnosed patients) among patients with lung cancer (225.000/year), breast cancer (295,000/year), lymphoma (30,000/year), leukemia (30,000/year,) and mesothelioma (30% of 3000/year), colon, appendix, stomach, biliary tract, and kidney, (10% of 250,000 diagnosed/year).

Technology: Biobina technology describes methods and kits used to target cancerous cells persisting in the pleural space after pleurodesis. Thus in the clinic talc functionalized with avidin is injected into the pleural cavity to induce pleurodesis, and a given time afterward the patient is treated with any of several biotin-conjugated radioisotopes which would precisely bombard the volume occupied by the avidin talc with intense radiation. Since biotin has an extraordinarily high affinity for avidin, the radioisotopes can now be selectively targeted to the tumor-contaminated space. Similarly, in the abdominal cavity avidin is incorporated into fibrin glue, a Gelfoam mesh or gel. To which radioactivity can be selectively directed. Biobina technology also describes more general methods using avidin-conjugated antibodies that target tumors directly and the subsequent use of biotin-labeled radioisotopes for selective killing of the tumors. These treatment methods could improve survival outcome by lowering systemic toxicity thus allowing administration of higher doses of more selective therapy.

Intellectual Property: A provisional patent application covering the use of avidin for targeted radiotherapy was filed in January, 2014 and another patent application, for clinical uses of avidin and fibrinogen or bleopmycin and talc was filed in August of 2014. The patent applications protect not only method of treatment but also administration protocols and therapeutic kits.

Regulatory Strategy: Biobina regulatory strategy is guided by the need for a safer therapy that improves organ function, cancer remission and yet minimizes toxicity to other organs. The shortest and least expensive clinical trial would involve the use of Biobina constructs as treatment for MPM. Because Biobina construct is going to be tested in clinical trials of MPM as primary therapy we are projecting a 24-month, Phase 1-2a clinical trial with 25 patients demonstrating, in the Phase 1 cohort, safety and tolerability of the construct when used for treatment of pleural effusion; and in the phase 2a cohort, effective localization of biotinylated radioisotope (Actinium 225) to avidinized talc. While potential side effects, such as immune response, are always a concern we note that avidin had been used clinically before and is not known to be immunogenic. Improved efficacy and lower toxicity would position Biobina product to become new standard of care.

Comparables - Companies Developing Treatments for MPM:
GlaxoSmithKline bought rights from Five Prime Therapeutics Inc. in 2011 in the US, EU, and Canada for FP-1039, a protein drug candidate in phase 1b testing for NSCLC with an arm for mesothelioma. GSK and Five Prime have a co-promotion option in the U.S. Five Prime received $50 million upfront and is eligible for milestone payments totaling $435 million plus royalties starting in the low double-digits and escalating into the high teens.

1. http://www.bioinvest.com/bios-go-from-first-to-worst-have-we-reached-a-bottom/

Aduro BioTech, Inc., a clinical-stage biotechnology company, announced the closing of a $55 million Series C financing in June 2014. New investor Johnson & Johnson Development Corporation (JJDC) joined the Morningside group and other new and existing investors in the transaction. Aduro plans to use the new capital to advance its lead program in metastatic pancreatic cancer and to continue clinical development in mesothelioma. In June 2014, Aduro presented data from a phase Ib trial for its therapy, CRS-207, in combination with standard chemotherapy in patients with MPM.

1. http://www.businesswire.com/news/home/20140611006115/en/Aduro-BioTech-Secures-55-Million-Series-Financing#.U-UkVWNWjo4

Topotarget bought back full rights from CuraGen for belinostat, a small molecule HDAC inhibitor being investigated for its role in the treatment of a wide range of solid tumors, including mesothelioma, in April 2008. Under the deal, CuraGen received 26.5 million new TopoTarget shares (worth $299 million) through a directed issue of shares and a commercial milestone payment of a total of $6 million, which is defined as 10% of the first $60 million of belinostat sales or partnership revenues.

1. http://investor.topotarget.com/releasedetail.cfm?releaseid=531037

Funding Strategy and Use of Proceeds: We will need approximately $2.5 million to finance the first twelve months of late preclinical and clinically focused research. Our business or operations may change in a manner that would consume available funds more rapidly than anticipated and substantial additional funding may be required to maintain operations, fund expansion, develop new or enhanced products, acquire complementary products, business or technologies or otherwise respond to competitive pressures and opportunities, such as a change in the regulatory environment or a change in preferred cancer treatment modalities.

Executive Summary: Columbia University Tech Ventures/Biobina LLC.

Avidin (AV) as a Support Device for Radioactive Y90-DOTA-Biotin instilled into the Urinary Bladder via Urethral Catheter for Treatment of Non-Muscle-Invasive (NMI) Transitional Cell Bladder Carcinoma.

Columbia University Tech Ventures.
Summary of Proposed Product Development through Phase I .

Biobina LLC, founded by Dr. Robert N. Taub MD PhD, Gleneara Bates MSW JD, and Jonathan Taub MA, MBA is a Columbia Tech Ventures spinoff focused on intracavitary treatment of cancer using drugs and isotopes. A major unmet need for such treatment is transitional cell bladder carcinoma, a designatgd orphan disease, the ninth most common neoplasm worldwide, (>270,000 newcases/yr) with 77,000 new cases and 16,000 deaths yearly in the US. 70 percent of patients present with superficial non-muscle invasive disease, about 40% of whom progress to total cystectomy and additional patients to metastatic disease. Treatment of superficial tumor with repeated intravesical BCG (best) or chemotherapy as a liquid or gel (less effective) can retard the progress of the disease, but the recurrence rate is high, and many apparently superficial tumors are later upstaged at surgery.

We believe that effective radiotherapy applied to the slightly (up to 1 cm) deeper submucosal layers of the bladder should reduce the number of potential metastatic tumor cells and extend patient survival. Our intent is to directly introduce our constructs by catheter or cystoscope. We hope to reduce the current need for 30,000 cystectomies performed in the US yearly, and to extend the progression-free survival of those with unresectable disease. Despite the need for this kind of treatment, As far as we have been able to determine, no company working in this area has developed techniques for delivering intravesical treatment that reaches to the submucosa as well. Sustained-release thixotropic gel formulations of Mitomycin C and/or an immunotherapy drug for upper urinary tract and non-muscle invasive superficial bladder cancer.These were not designed to penetrate below the tightly-constructed virtually leakproof superficial epidermis.

We have developed and patented a novel platform for delivering precise targeted radioisotope treatment for cancer that can address substantial unmet medical needs for intracavitary radiotherapy. This four component platform is a combination of Gelfoam (optional) to provide reversible attachment to tissues, covalently bound to the egg-white protein avidin, which in turn tightly binds a biotinylated chelate of radioisotopic Yttrium 90 with a k of 10E-15. The constructs and their cancer- related applications are provisionally patented until 2034.

The advantages of our Proposed Avidin-Biotinylated Y-90 treatment are that: (1) the Y90 device can penetrate 5 mm into the muscle layer; (2) The radiation dose can be calibrated for depth of penetration; (3) No novel chemical synthesis is required.

Our animal studies using freshly isolated and refrigerated porcine bladder preparations using non-radioactive construct surrogates are near completion. We will next confirm, in the same porcine model, the feasibility of clinical treatment using Indium-111 and Yttrium 90 using dosimetry developed in the in vitro microwell porcine bladder tissue explant model (described in the appendix to the proposed clinical study). We intend to indicate to the FDA that extensive animal testing to confirm safety and tolerability of Avidin will not be helpful, since data already exists for its safety after intravenous injection in man, as it does for Yttrium-90-DOTA-biotin as well.We hope to expeditiously move to a phase I trial, to confirm (1) the safety and tolerability of the projected radiation doses adequate to eradicate superficial non-muscle-invasive carcinoma in this context, and (2) that such treatment spares adjacent and distant organs and bone marrow from significant damage.

These are our projected milestones over a total 48 month period. We are seeking funds to batch manufacture and test our conjugates in rodent (optional) and pig bladder models with tracer isotopes such as Technetium 90 and Indium 111, over an initial 12 month period, during which time

Columbia University Tech Ventures.
Summary of Proposed Product Development through Phase I .

we will be seeking FDA approval and planning for a Phase I clinical trial. before initiating clinical trials of the Yttrium constructs.
Gelfoam-avidin-Y90-biotin constructs will then be tested in clinical urologic oncology collaborative trials for superficial (T1, T1S) non- muscle-invasive bladder cancer. We will carry out a Phase I/II trial with up to 28 patients demonstrating safety and tolerability of the constructs over up to month 33, and a phase II ramp-up to 60+ patients documenting time to progression compared to controls up to month 48. We anticipate that some of the Phase I patients will be eligible for Phase II evaluation, shortening the accrual time. Some of these milestones are concurrent, and may be shorter, depending on availability of funds.

Milestone	Completed by	Task Details (duration)	Respons
Preparation and characterization of radioactive constructs- stability testing	By Month 8	Preparation, testing of non-radioactive Constructs, (gels, sponge), calculation of radiation doses, calibration techniques. (Month 0-8)	Glenear Taub Collabor Medicin
Excised porcine bladder studies	By Month 12	Development of closed-system catheter techniques for placement of radioactive constructs in animals.	Glenear Taub Collabor Medicin Interven Urology
IND submission to FDA	By Month 16	(a) Device v Drug (b) Avidin-Biotinylated Y90 (concurrent)	Glenear Taub Collabor Medicin Interven Urology
Completion of Animal studies	By Month 18	Cataloging of safety, tolerability, and feasibility of construct administration (Month 6-18)	Robert N Petrukh Nuclear
IRB submission for Phase I trial in perioperative cystectomy pts	By Month 18	Vetting and review of protocol by IRB (concurrent)	Glenear Taub, C Interven
Completion of Phase I, transition to Phase II studies	By Month 30	Enrollment of 9-15 patients about to undergo cystectomy, Phase I trial of neoadjuvant Y90 radiation (Month 18-30)	Glenear Taub, C Interven
Completion of Phase II studies	By Month 48	Ramp-up of enrollment to 60 patients, (including Phase I subjects). Data evaluation. (Month 30-48)	Glenear Taub, C

Columbia University Tech Ventures.
Summary of Proposed Product Development through Phase I .

CUMC IRB#: AAA-XXXXX
Version Date:

TITLE Avidin (AV) as a Support Device for Radioactive Y90-DOTA-Biotin instilled into the Urinary Bladder via Urethral Catheter for Treatment of Non-Muscle-Invasive (NMI) Transitional Cell Bladder Carcinoma.

Coordinating Center: Columbia University Medical Center0

Consultant: Robert N. Taub, MD,PhD
161 fort Washington Avenue NY 10032
NY 10032

Regulatory Sponsor:	Columbia Technological Ventures – R. N. Taub, consultant Insert Department - Medicine Insert Address- 161 Fort Washington Avenue Ny Ny 10032 Insert Phone Number 212 305 4076
Study Agent:	Avidin
Other Agent:	Yttrium 90-DOTA-Biotin
IND Status:	**IND #: TBD** Study Possibly Exempt from IND Requirements per 21 CFR 312.2(b)

Title	Single Institution Non-Randomized Phase I Study of the Safety and Tolerability of Avidin (AV) as a Support Device for Radioactive Y90-DOTA-Biotin instilled into the Urinary Bladder via urethral catheter for Treatment of Non-Muscle-Invasive (NMI) Transitional Cell Bladder Carcinoma. .
Short Title	Avidin Optimization of local radiotherapy for superficial (NMI) Bladder Cancer.
Phase	Phase 1.
Methodology	Open label
Study Duration	24 months

Columbia University Tech Ventures.
Summary of Proposed Product Development through Phase I .

Study Center(s)	Single Center.
Objectives	To determine whether AV is safe and well tolerated as a scaffolding device to optimize localization and treatment of superficial bladder cancer with Yttrium 90 –DOTA-Biotin.
Number of Subjects	12
Diagnosis and Main Inclusion Criteria	Histologically Diagnosed transitional cell bladder carcinoma who are candidates for elective surgical total cystectomy.
Study Product, Dose, Route, Regimen	Study product: Avidin combined with Yttrium 90 –DOTA-Biotin. (A-Y90-DB) Dose: 1-2 grams Avidin containing 40MBeq/kg Y90 in 200ml Isotonic Saline pH 5.5-7.0, administered via indwelling urinary catheter. and allowed to dwell for 6.2 hrs.
Duration of administration	A-Y90-DB will be administered once, no less than one week (~25 half-lives of Yttrium-90) before contemplated total cystectomy..
Reference therapy	No standard reference therapy. Intravesical Yttrium-90 has been reportedly used (22,23,24) in the past, but not in this form, and not since the 1980s
Statistical Methodology	Conventional Phase I safety/tolerability study, 3-subject cohorts, stopped at 6 patients showing no toxicity in excess of Grade I.

1. INTRODUCTION

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Columbia University Medical Center institutional research policies and procedures.

2. STUDY OBJECTIVE

The principal objective for this single-institution open-label is to test whether intravesical instillation of the scaffold protein chicken-egg-white derived avidin (AV), firmly bound as a support for the radioactive Yttrium 90 chelator moiety tetraazacyclododecane-1,4,7,10-tetraacetic acid (Y90-DOTA), can be safely and tolerably utilized as a device for facilitating local radiotherapy of superficial non-muscle-invasive bladder cancer.

2.3 Background:

Approximately 70 percent of new urothelial (formerly called transitional cell) bladder cancer cases (76,000 per year in the US) are classified as non-muscle invasive [1]. Non-muscle invasive bladder cancer includes Ta, T1 (submucosal invasive) tumors, and Tis (carcinoma in situ [CIS]), which account for approximately 70, 20, and 10 percent of non-muscle invasive

cancers, respectively.

The rate of recurrence of non-muscle invasive bladder cancer surpasses that of all other cancers [2], and the majority of patients will experience a recurrence. Management of recurrent disease is, therefore, a critical concern in patients with non-muscle invasive bladder cancer. Determining optimal therapy, however, is complicated by the heterogeneity of disease in these patients.

Even with optimal treatment, patients with non-muscle invasive disease are at high risk of recurrence with further non-muscle invasive disease or of progression to more advanced disease.

Recurrence can occur after transurethral resection of bladder tumor (TURBT) in patients who have not received prior adjuvant intravesical therapy. Recurrence of low-grade papillary non-muscle invasive bladder cancer in this setting is the most common type of recurrence, since high-grade disease generally has been treated with intravesical Bacillus Calmette-Guerin (BCG) unless there is a contraindication.) Recurrent low-grade non-muscle invasive bladder cancer constitutes intermediate-risk disease in most cases; however, it can constitute high-risk disease if the recurrence is both large (>3 cm) and multifocal. Whether the patient previously received a single perioperative dose of intravesical chemotherapy is not usually considered important in defining this disease state. Although it is felt that recurrence rates can be mitigated by a careful TURBT with particular attention to sampling of the lamina propria, it is likely that microscopic involvement of the urothelium at the level of the lamina may be more diffuse than can be appreciated during cystoscopy, and/or that intrinsic or acquired loose attachment of bladder epitheliumto interstitial layers and to the lamina propria facilitate lateral movement of neoplastic cells beyond areas of resection, however carefully done. Recurrence can also occur in patients who have received prior adjuvant intravesical chemotherapy or BCG therapy. These are classified as "BCG failure" i.e., patients with either intermediate- or high-risk non-muscle invasive bladder cancer who received a single round of induction BCG without maintenance therapy [3], or "BCG unresponsive" (replacing the older term "BCG-refractory.") referring generally to BCG-unresponsive non-muscle invasive bladder cancer encompasses patients with persistent or recurrent high-grade (Ta/Tis or T1) disease within six months after 5 doses of BCG. Such recurrence is usually diagnosed by surveillance cystoscopy or urine cytology during surveillance after initial treatment, again pointing to reservoirs of neoplasia that may lie slightly beneath the levels routinely taken during TURBT [7,8]. However, techniques for the timely detection of recurrence need improvement. Moreover, the manufacture and supply of BCG has become less reliable and problematic [REF].

For those patients refractory to BCG or for whom BCG is not readily available, many options exist for salvage treatment, none completely satisfactory, including: recurrent, repeated TURBT; repeat courses of high or low dose BCG; BCG plus interferon alpha-2b; intravesical Valrubicin, gemcitabine, or docetaxel ; chemohyperthermia with mitomycin or epirubicin; electromotive propulsion of mitomycin; biological therapies (immune checkpoint inhibitors, mycobacterial DNA, toxic plasmids, anti EPCAM antibodies.[4,5,6] A common characteristic of these therapies is they do not directly target lamina proprial tissues, i.e., 1-2 mm below the bladder mucosal surface [9,10]; yet it is acknowledged that surgical procedures do best when this tissue layer is sampled and appropriately resected or treated. We hypothesize that application of short-range beta- emitting radioactive isotopes such as Yttrium-90 (Y90) to the bladder surface might therefore be a suitable means of treatment.

Y90 has been used since the 1960s for synovitis of the knee by direct injection into the synovial

cavity of unconjugated isotope suspended in a gel solution [11]. Conclusions reached from injection of 15 mCi or more of the isotope were that, if properly used (not for injection of the ankle joint) these injections were well tolerated and therapeutically effective in destroying inflammatory tissue and preserving joint function. More recent experience with Y90-labelled antibody directed against malignant or leukaemic lymphoid cells confirmed the relative safety and modest bone marrow toxicity, and low incidence of leukaemia and myelodysplasia of FDA-approved Y90 compared to previously used isotopes such as phosphorus 32, although rr instances of chronic cutaneous radiation ulceration have been reported for both knee and ankle. [12]. Y90 incorporated into glass microspheres 32 μm in diameter is currently designed to lodge in small capillaries and is currently used for destruction of hepatic metastases 13]. All of these extensively used substantially-dosed Yttrium 90 techniques are recognized as having satisfactory therapeutic ratios and acceptable margins of safety.

This proposed protocol focuses on the possible use of Yttrium 90 radiotherapy for treatment of superficial bladder carcinoma. The rationale for such treatment was developed in a series of preclinical experiments based upon an in vitro microtitre platform illustrated in Appendix 1 for studying the localization of yttrium 90 formulations on the inner bladder surface. We believe that it is possible to target the bladder with therapeutic doses of radiotherapy that are limited to the more superficial layers of the bladder (up to 7 mm) with Yttrium 90, (half-life 6.3 hours, median target range of 3-4 mm). The normal human non-hypertrophic bladder, when distended to a volume of 250 ml or over, is approximately 3 mm thick; the mucosal layer of even the contracted normal bladder is rarely greater than 2 mm, so that the lamina is well within the target range of Y90. It is not clear whether extreme hypertrophic folding would change the average distance between the lumen and the lamina propria, or whether a solution of isotope within the bladder would find the crevices in the bladder folds to allow penetration of radiation to the lamina propria. Both are unlikely in the absence of severe bladder hypertrophy and fibrosis. Nevertheless, as noted above, the most uniform radiation is likely to be applied to at least a partially filled bladder.

Possible prerequisites for such treatment are:

(1) A preparation of isotope which has a high molecular weight, so that it cannot readily diffuse or leak through either bladder wall or capillaries that would be exposed during application. An additional benefit would accrue if the compound were able to directly bind to bladder mucosa, so that the concentration of isotope at the surface would be higher. Our approach will be (a) to utilize commercially available clinical grade compounds of Yttrium-90 chelated with **1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid** (formula $(CH_2CH_2NCH_2CO_2H)_4$, also known as **DOTA**) which consists of a central 12-membered tetraaza (i.e., containing four nitrogen atoms) ring, capable of chelating certain metal ions such as Yttrium, and which is attached by a peptide spacer to biotin; (b) allowing this construct to bind to the therapeutically inert scaffold protein native glycosylated avidin (a 60,000 M.W. protein extracted from chicken egg whites,)and (d) optionally mixing or coupling the avidin to gelfoam or other substrates. One of the tightest biological bonds known is that between the egg white protein avidin (present in amounts of 150ug in the white of a single chicken

egg) and the endogenous vitamin biotin. (K equals 10 E -15). The biotin can be covalently linked to a spacer such as methylglycine as noted above, and from there to the compound DOTA-Y90. The molecular weight of this construct is approximately 60,000, which would effectively retard its passage through the bladder wall and other membranes. Native glycosylated avidin is a highly positively charged molecule (pI>10), and can readily bind to other large negatively charged molecules such as albumin or fibrinogen, and as we have shown, to porcine urinary bladder mucosa at pH<7.5. The strong positive charge on the avidin molecule has been shown by others to facilitate its attachment to the surfaces of free-floating neoplastic cells [25] that are conceivably present in the bladder cavity afflicted with superficial transitional cell carcinoma.

(2) A catheter system for instilling the isotope, which would likely include temporary diversion or minimization (by overnight fluid deprivation) of the urinary flow for 6-8 hours, and administration of the isotope after the bladder is emptied.
(3) Intra-treatment monitoring of the bladder volume and thickness; e.g. it might be possible to use a smaller amount of isotope and distend the bladder with a secondary balloon filled with air, if there is space all around the distending balloon of several millimeters,
(4) A three-dimensional geometrical analysis of various bladder volumes is required to arrive at the proper dose of isotope. One analysis extrapolates from prior reported experiences with intrasynovial injections of Yttrium-90 in which 15 mCi were injected in a 30 ml volume, delivering doses in a 4-6 cGy range; thus mild distention of the post-void bladder by 200ml of Y90 isotope-conjugated appropriate construct which can deliver 3000 rads (6 cGy) to the bladder surface would require four times as much, or 50-60 millicuries of Y90. Although there are few current reports of intravesical radioisotope treatments, there is scant reason to anticipate adverse consequences from such injections if appropriate precautions are taken to prevent or counteract isotope leakage. The total dose of 90Y is well within the range currently administered systemically with acceptable, usually temporary untoward effects. In this proposed treatment the majority of isotope would be removed from the body within a matter of hours.
(5) We would effect a translation of these ideas into the clinic by first safely assessing the tissue distribution of administered Indium-111-DOTA-biotin in tracer doses as a surrogate for the Y90 compound on three patients; This could be followed by a Phase I study of Fibonacci-guided escalating doses of Y90-DOTA-Biotin. The least complicated protocol would involve administering a solution of high specific activity Avidin-Biotin-DOTA-Y90 (ABDY90) in isotonic protein-free solution pH 5.5 via a Foley Catheter to overnight- fluid-deprived patients immediately after having voided, and allowing the solution to remain for up to 6 hours (one half-life), then drained and the bladder irrigated. It is intended that actual treatments not be evaluated in this Phase I tolerability study, but applicable treatments will be evaluated insubsequent IRB submitted and approved Phase I and II clinical trials.

3. INVESTIGATIONAL AGENT

Avidin is a tetrameric or dimeric [1] biotin-binding protein produced in the oviducts of birds, reptiles and amphibians and deposited in the whites of their eggs. In chicken egg white, avidin makes up approximately 0.05% of total protein (approximately 1.8 mg per egg). The tetrameric protein contains four identical subunits (homotetramer), each of which can bind to biotin (Vitamin B7, vitamin H) with a high degree of affinity and specificity. The dissociation constant of avidin is measured to be KD ≈ 10−15 M, making it one of the strongest known non-covalent bonds.[2]

In its tetrameric form, avidin is estimated to be between 66–69 kDa in size[3]. 10% of the molecular weight is attributed to carbohydrate content composed of four to five mannose and three N-acetylglucosamine residues [4] The carbohydrate moieties of avidin contain at least three unique oligosaccharide structural types that are similar in structure and composition [5]. Functional avidin is found only in raw egg, as the biotin avidity of the protein is destroyed by cooking. The natural function of avidin in eggs is not known, although it has been postulated to be made in the oviduct as a bacterial growth-inhibitor, by binding biotin the bacteria need.

Avidin was first discovered by Esmond Emerson Snell (1914–2003). The route to discovery began with the observation that chicks on a diet of raw egg-white were deficient in biotin, despite availability of the vitamin in their diet. [6] Snell later isolated the component of egg white responsible for biotin binding, and, in collaboration with Paul Gyorgy, confirmed that the isolated egg protein was the cause of biotin deficiency or “egg white injury”. After having been tentatively named avidalbumin (literally, hungry albumin) by the involved researchers at the University of Texas[7].The name of the protein was later revised to "avidin" based on its affinity for biotin (avid + biotin).[8] Since that time research on avidin- biotin binding and it possible uses for targeted therapy has expanded rapidly[9].Intravenous Avidin has been found to be an effective “clearing agent” for freely circulating irrelevant biotinylated moieties without compromising pretargeted biotinylated therapeutics access to their appropriate targets.[10]

The proposed protocol will utilize GMP Clinical grade Avidin (Pharmaceutica Inc,., British Columbia) identical to that has been directly supplied to Clinicians in the US (Sanofi-Aventis) and to Italy (Pagnelli) for 10 years or more. An IND for this material in the US was held by Sanofi Aventis from 2005-2012, during which time no adverse effects were reported related to its use.

3.1 Other Agent(s)

No other investigational agents are contemplated for this proposed study. The avidin will used in combination with the known commercially available radioisotope chelate Y90-DOTA-biotin for local radiotherapy of the cancerous bladder surface.

3.2 Preclinical Data

In unpublished preclinical data, using a novel research platform, we have found that Avidin will bind tightly to porcine bladder mucosa, and in turn will facilitate the binding of biotinylated enzyme-tagged biotin. The binding is best in protein free saline at pH of 8.0 and below, conditions easily achievable in the urinary bladder for periods of up to 6 hours (See appendix 3).

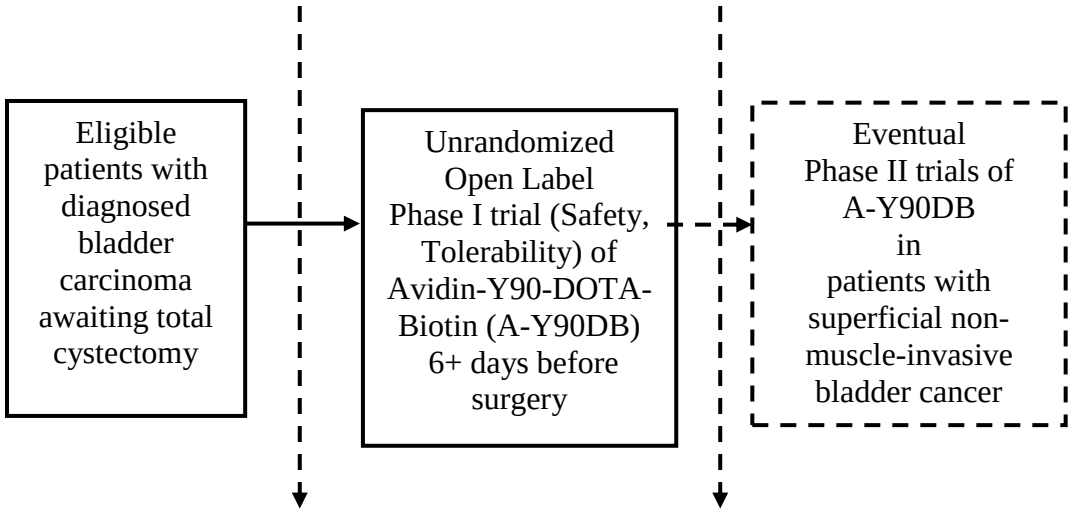
3.3 Clinical Data to Date

The underlying concept of the proposed intravesical treatment follows logically from the tissue-binding properties of Avidin [25] No clinical research evidence exists regarding this treatment, either regarding its usefulness or effectiveness. Based on parallel research using Avidin for other purposes in the US and Europe, we do not anticipate significant side effects arising during the proposed treatment

4. STUDY DESIGN

4.1 General Design

- Study Design: Phase I (safety, tolerability), fixed-dose, non-randomized, single arm, single institution study.



TREATMENT PLAN: This is tentative, and will be further developed with Interventional Radiology, Nuclear Medicine, and Urology.

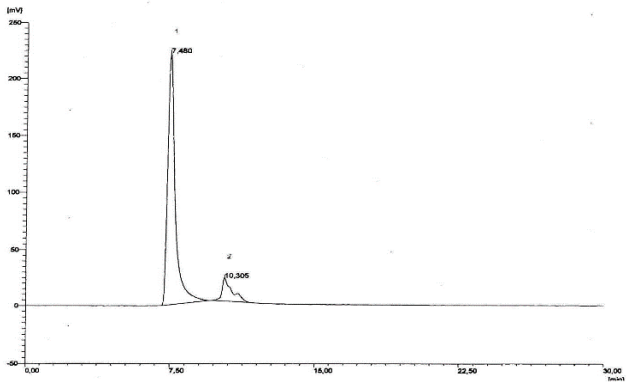
Determination of eligibility (day of treatment)- Day 0:

1. Day -1: Pt will be instructed to refrain from food or drink after midnite. (Medications may be taken with minimal liquid)

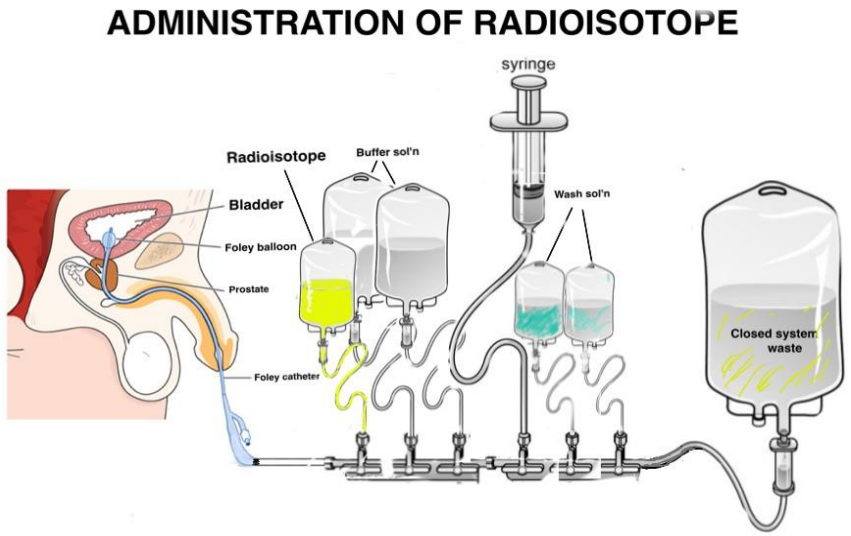
Treatment –Day -1: Preparation of A-Y90DB. (Please see "SYNTHESIS OF TOSYLATE AND MESYLATE PRECURSORS FOR ONE- STEP RADIOSYNTHESIS OF [18F] FECNT, J. Pijarowska, A. Jaron, R. Mikolajczak, Institute of Atomic Energy POLATOM –available through ResearchGate)

a.

Biotin-DOTA reagent will be obtained from Ariva Corp (formerly Macrocyclics Inc.), Plano, Texas. Twenty microliters to 100 µL carrier-free indium In-111 in 0.04 M HCl (Mallinkrodt Pharmaceutical) or Yttrium 90 -chloride in 0.05 M HCl (Perkin Elmer Corp, or Mallinkrodt Pharmaceutical) is diluted with 2 M ammonium acetate, pH 5, to a total volume of 0.25 mL, and 1 mg DOTA-biotin is added. The solution is heated for 30 minutes at 80°C in a thermal cycler followed by the addition of 25 µL 100 mM DTPA to chelate any unbound radioisotope.(The product is >99% chelated). 10mg Avidin in 250-500µl acetate buffer pH 5 is added, and the product [now >99% pure by test column: BioSep-SEC-S-2000 (sigma-aldrich) ,eluent PBS pH 6.8]



containing up to 2 millicuries of In111 or 70 millicuries of Yttrium 90 in added to 100 mg of acetate buffer in a 100 ml siliconized IV bag in a closed system which includes additional buffer-filled acetate buffer (2 X 250 ml) , normal saline (1X 1000ml) and empty IV bags attached to a single balloon or optional double-balloon Foley catheter with an optional outer condom.



b. Day 0: Intravesical Administration of Isotope.

Pt will be instructed to void. The urinary bladder will be conventionally Foley-catheterized (A) and any residual urine drained (monitored by ultrasound).

c. If tolerated, the bladder will be drained and the fluid replaced with 50 ml acetate buffer, and if tolerated, an additional 100 ml of buffer containing 10 mg Avidin coupled to Fibonacci escalating calculated doses per group of 3 enrollees of 0.1, 0.2 and 0.4 GBeq (maximal dose = 10.7 mCi kg to deliver approximately 3000 rads to the bladder surface over a 6 hour period.) of chelated Y90-DOTA-biotin, followed by additional buffer to constitute a intravesical volume of 250 ml as tolerated. Presence of adequate filling and minimal air-pockets will be assessed by ultrasound and adjusted by rotating the patient and withdrawing air and replacing with fluid, The mixture will be allowed to dwell for up to 6.2 hrs or more as tolerated. [All such manipulations to be supervised or performed by Interventional Radiology or Nuclear Medicine personnel]. Observation for immediate untoward effects (change in ultrasound-monitored bladder fluid volume, local pain, local or systemic hypersensitivity) will be carried out q 15

min X 1 hr, then q 30min X 6 hours. Blood samples will be taken q 15 min X 1 hour, then q 1 hour 6 hrs, then between 9-12 hr, 12-16 hr and 18-24 hr.

d. The radioactive material is evacuated for disposal into a specialized radioactive waste container attached to the closed system . The bladder is then flushed three times with 200 ml normal saline into a separate waste container. (In the case of Indium-111, the bladder drainage will be tested for efficacy of washout of residual radioactivity.) All subsequent urine over the ensuing 24 h is similarly disposed of.
e. Day 1-2. Pts will be kept overnight and vital signs monitored: Day 0, hourly X 4 h; then q4-6h X 24 h.
f. Pts will be instructed to report any untoward pain or hypersensitivity or other symptoms immediately.
g. Day 2-6/6-30 days. Complete blood counts with differential, metabolic panel (renal function), liver panel, and inflammatory markers will be obtained every 2 days for 6 days then bi-weekly until surgery or 30 days.
h. Skin hypersensitivity tests and serum anti-AV antibody levels at 2, 3-5, and 7-9 weeks, post treatment (See schedule) .

4.2 Dose Limiting Toxicities

Timeframe for evaluating DLTs: This is a single-dose study.
Timeframe: 2 months .

DLTs will consist of Grade 2 or higher acute hypersensitivity reactions, that do not respond fully to diphenhydramine and/or two doses of Solucortef 100mg 2 hrs apart. If 8 or more of projected 12 patients experience such reactions, the study will be closed to accrual. If no adverse grade 3 or greater reactions attributable to AV-Y90DB are observed after the first 6 patients, the study will be formally closed if it is agreed by the IRB and statistical analysis that the number is sufficient to assess to that the treatment is safe to allow its progression to a Phase II trial.

Grade II delayed hypersensitivity reactions will consist of any allergic manifestations resistant to treatment with Medrol in doses up to 60 mg daily X 1 week. If 8 or more of projected 12 patients experience such reactions, the study will be closed to accrual. If zero adverse reactions attributable to avidin are observed after the first 6 patients, the study will be formally closed if it is agreed by the IRB that the number is sufficient to determine that the treatment is safe.

4.3 Number of Patients

Total number of subjects projected for the entire study: 12 or less as agreed by IRB.
All patients treated will have been enrolled and consent obtained.

5. SUBJECT SELECTION AND WITHDRAWAL

The following eligibility criteria will be addressed:

- Disease site/type with pathologic confirmation of diagnosis: patients pathologically confirmed diagnosis of transitional cell bladder carcinoma.

- Extent of disease: involvement of bladder tissues, non metastatic, mandating total cystectomy.
- Allowable prior therapy and time limit since: All prior therapy is permissible.
- ECOG performance status: 0-2
- Allowable laboratory values with date range: All patients deemed eligible for surgery, regardless of laboratory values, if life expectancy exceeds 3 months.
- Pathology materials: A pathology review of the primary diagnosis must be available.
- Parameters: eligibility is confirmed and patient enrolled at leat 6 days before scheduled surgery. (Day0)

5.1 Inclusion Criteria

Patients must have histologically confirmed resectable primary bladder malignancy awaiting total cystectomy in whom there is the possibility of persistent residual disease addressable by local radiotherapy, or for which there is no other indicated adjuvant treatment for the current clinical situation.

Age restriction: None.

ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A).

Life expectancy of greater than 3 months.

Patients must have normal organ and marrow function as defined below: It should be determined by surgeon that organ and marrow function is sufficient to carry out total cystectomy.

6.2 Exclusion Criteria

6.2.1
Pt is unwilling or unable to undergo total cystectomy

6.2.2
History of allergic reactions such as eggs or egg products or to compounds of similar chemical or biologic composition to avidin are ineligible

6.2.3
Patients who are receiving any other investigational agents concurrently, or who have not recovered from adverse events due to investigational agents administered more than 4 weeks earlier.

6.2.4
Patients with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

6.2.5
Other appropriate exclusions that will confound interpretation of data.

6.5 Early Withdrawal of Subjects

Patients can refuse to participate at any time before treatment, and can refuse to be followed at any time afterward. In either case, the patient will be withdrawn from the study and the data relating to his-her participation will be censored.

6.5.1 When and How to Withdraw Subjects

- If at any time the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (*i.e.*, a change in diagnosis), the patient will be removed from study.
- If the patient fails to comply with the defined treatment plan and follow-up evaluations, the patient will be removed from the study.
- If the patient withdraws consent for continued participation, he/she will be removed from study.

6.5.2 Data Collection and Follow-up for Withdrawn Subjects

Even though subjects may be withdrawn prematurely from the study, we will attempt to collect as much data as possible on such subjects throughout the 60 day follow-up period for that subject. It will be a high priority to try to obtain at least survival data on such subjects by phone calls to subject, phone calls to next-of-kin if possible, and certified letters. Subjects withdrawn because of unacceptable adverse events will be followed-until resolution or stabilization of the adverse event, even beyond the 60 day period.

8.1.1 Investigational Agent(s)

Because of the possibility of adverse hypersensitivity reactions, pts will be (a) specifically asked if there is a history of allergies, specifically to egg products, and whether they regularly eat eggs or egg products (b) will be carefully observed as outlined in Section 5.1 for hypersitivity to avidin as evidenced by symptoms of itching, dyspnea, sensation of pressure, or headache; by signs of wheezing, rash, angioneurotic edema, hoarsness, or laryngeal spasm; or hypotension, altered heart rate or rhythm.

Observation will continue over a period of 60 days as in the schedule. .

Intravenous supplies for control of hypersensitivity reactions will be readily available during treatment including immediately available bedside epinephrine, solucortef, and access to the CUMC rapid response team.

Rationale for proposed starting doses:

1. Doses of 100mg have been given intravenously over 30 minutes without reports of side effects both in the US and Italy under protocol number NCT00580216 (Sanofi-Aventis, New Jersey), under IND # SSR29261. Intravenous Avidin has been shown able to deplete superfluous circulating biotinylated material which might interfere with pretargeting.

2. Safety issues:

- There have been reports of hypersensitivity to egg products; anaphylaxis has been reported in animals. There have been no other safety reports.
- There have not neen reports of hypersensitivity to intravascularly injected avidin or avidin-biotin complexes, although evidence of hypersensitivity is seen in mice after three injections.
- No reports exist of hypersensitivity to avidin after intravesical instillation.

Dose Escalation: 3 escalating doses are planned as noted above. The higher doses are justified for study because (a) the bladder is relatively impervious to systemic diffusion of isotope, and (b) the isotope will be flushed from the bladder after a 6 hour period, rather than being allowed to remain in the body. This proposed trial is: (a) to evaluate the safety and tolerability of administering sufficient doses of radiation, using Avidin as a scaffolding device, to deliver an intravesical dose of 400 MBeq/kg of Y90 to the bladder surface, somewhat higher but biologically comparable to doses currently being given intravenously for disseminated systemic treatment of hematological disease (13).

Regimen Chosen:

We feel that Yttrium radiation, unlike topically applied chemotherapy, will meaningfully penetrate and treat the mucosa of the bladder to at least a depth of 3mm. This depth is not reached by any applied topical chemotherapy.

Discuss why the risks to subjects are reasonable in relation to the anticipated benefits.

As noted above, Avidin and related substances have been used in clinical studies for over two decades, without significant side effects or risks, and the use of Y90 radiation in this context is an attractive and relatively safe option. 3 escalating doses are planned as noted above. The higher doses are justified for study because (a) the bladder is relatively impervious to systemic diffusion of isotope, and (b) the isotope will be flushed from the bladder after a 6 hour period, rather than being allowed to remain in the body. Possible adverse effect on the ureteral and

urethral orifices of the bladder are highly unlikely but theoretically possible which is why the cystectomy model has been chosen for this study.
Allergic manifestations are theoretically possible including rare thus far unreported controllable anaphylaxis, which will be carefully monitored and watched for, and prevented or treated as appropriate. In this study patients with known hypersensitivity to chicken or avian egg products will be excluded.

5.2 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 8.5 applies. The reason for study removal and the date the patient was removed will be documented in the Case Report Form.

6. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

6.1 Adverse events

Investigational Agent:
As noted earlier, there have been reports of hypersensitivity reactions to repeated intravenously injected avidin in mice . No other adverse reactions or unexpected problems have been reported in other animals or humans with this material, even after intravenous injection in doses of 100mg.

7. PHARMACEUTICAL INFORMATION:

8.1 INVESTIGATIONAL AGENT

Avidin is a tetrameric or dimeric [15] biotin-binding protein produced in the oviducts of birds, reptiles and amphibians and deposited in the whites of their eggs. In chicken egg white, avidin makes up approximately 0.05% of total protein (approximately 1.8 mg per egg). The tetrameric protein contains four identical subunits (homotetramer), each of which can bind to biotin (Vitamin B7, vitamin H) with a high degree of affinity and specificity. The dissociation constant of avidin-biotin is measured to be KD ≈ 10−15 M, making it one of the strongest known non-covalent bonds.[16]

In its tetrameric form, avidin is estimated to be between 66–69 kDa in size[17] 10% of the molecular weight is attributed to carbohydrate content composed of four to five mannose and three N-acetylglucosamine residues [18] The carbohydrate moieties of avidin contain at least three unique oligosaccharide structural types that are similar in structure and composition [19]Functional avidin is found only in raw egg, as the biotin avidity of the protein is destroyed by cooking. Intravenous Avidin has been found to be an effective “clearing agent” for freely circulating irrelevant biotinylated moieties without compromising pretargeted biotinylated therapeutics access to their appropriate targets.[20,21]

Columbia University Tech Ventures.
Summary of Proposed Product Development through Phase I .

The proposed protocol will utilize GMP Clinical grade Avidin (Pharmaceutica Inc,., British Columbia) identical to that has been directly supplied to Clinicians in the US (Sanofi-Aventis) and to Italy (Pagnelli) for 10 years or more. An IND for this material in the US was held by Sanofi Aventis from 2005-2012, during which time no adverse effects were reported related to its use.

7.1 Description

7.2 Treatment Regimen

10 ml Avidin in a solution of up 1 mg./ml will be mixed with up to 3 Gbeq Y9 -DOTA-Biotin radioactivity by nuclear medicine, with appropriate containers and precautions. This will be further diluted in 100 ml of buffered normal saline pH 5.5. The bladder will be catheterized and emptied, and refilled with the radioactive salen, and further buffere added to bring the recorded volume to not more than 250 ml. The mixure will be allowed to remain for a recorded time of six hours or longer, after which the bladder will be drained and flushed twice with 200 ml Normal saline. All drainage to be deposited in waste radioactive container. Treatment may be repeated weekly X 3 if cystectomy is not performed.

Prior and Concomitant Therapy

There are no exclusions for prior therapy assuming acceptable hematologic, renal and hepatic function, in which case exceptions may be made with IRB approval.

7.3 Packaging

The material will be dispensed in sterile glass vials for injection, each containing 10 mgms of avidin, reconstituted before use to a volume of 10 ml. for dilution as above

7.4 Blinding of Study Drug

The drug will not be blinded for this study

7.5 Receiving, Storage, Dispensing and Return

7.5.1 Receipt of Drug Supplies

Avidin will be shipped from Bioseutica BV to the CUMC Research Pharmacy who will then formulate the material for study. The final radioactive preparation will be done in the laboratory of Nuclear Medicine/Interventional Radiology.

Subject Compliance Monitoring

After treatment is completed, compliance with the study will be by scheduled followup office or hospital visits according to the included study schedule. Followup beyond 3 months per patient is not contemplated for purposes of this Phase I trial.

7.5.2 Storage

Avidin is stable at room temperature for one week, at 20 degrees C, and can be kept for 6 months or longer at 4 degrees C. Calibrated Y90-DOTA-biotin is shipped weekly from the manufacturer.

7.5.3 Dispensing of Study Drug

Regular study drug reconciliation will be performed to document drug assigned; drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

7.5.4 Return or Destruction of Study Drug

All unused drug will be destroyed at the end of this study or, if appropriate successor studies, with the agreement of the IRB.

7.6 Other Agent(s)

No other agents are planned for use in this protocol.

8. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Evluation times as noted should be within specified boundaries. Optional tests are desirable, particularly CXR or CT evaluations of adequacy of lung expansion and pleurodesis.

Columbia University Tech Ventures.
Summary of Proposed Product Development through Phase I .

	Pre-study through Day-1	Time 0-	Day 0, hourly X6 ±15min	215-235 min. (6 hr) ±60 min	12 hr ±3 hr	Day 2	Day3	Day 5-9	Day 10-16	Day 19-23	Day 40-44	Day 40-44 (off study)
Informed consent	X											
Demographics	X											
Avidin-DOTA-Biotin-Y90 prep. (Lab)	X											
Avidin-DOTA-Biotin-Y90 instill		X	X									
Avidin-DOTA-Biotin-Y90 flush				X								
Medical history	X		X	X	X	X	X	X	X	X	X	X
Physical exam	X	X			X	X	X	X	X	X	X	X
Vital signs	X		X	X	X	X	X	X	X	X	X	X
Height	X											
Weight	X					X	X			X	X	
Performance status		X					X	X	X	X	X	X
CBC w/diff, plts		X					X					X
Serum chemistry		X					O	O	O			X
Avidin Skin Testing	X							O			O	O
EKG (as indicated)		X										X
Radiologic evaluation		X				O	O	O	O	O	O	O

9. MEASUREMENT OF EFFECT

Tumor effects will not be monitored. This is a safety-tolerability study only. No antitumor treatment is being given. Nor is this study meant to evaluate the adequacy of pleurodesis, since the technique may vary from surgeon to surgeon.

The endpoint of this study will be to document any adverse events attributable to the A-B-D-Y90, in order to establish its safety for use in future studies.

If the first six patients complete A-B-D-Y90 with no toxicity greater than grade II, the study will be interrupted until two months of followup is completed for all six patients. If in the opinion of the DSMC no untoward effects are seen that are attributable to the drug, the treatment will be deemed safe.

10. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 10.0 (Adverse Events: List and Reporting Requirements). The Data Safety Monitoring Plan is described in Section 12.2.

10.1 Data Collection

The Herbert Irving Comprehensive Cancer Center has an electronic clinical trials and data management system that will be used for data collection. CRFs for the study will be built into Velos for data entry. The system has full auditing capabilities which is web-based and housed on a server in a fully HIPAA compliant server room with restricted access and video camera monitoring. All users must login with their own application username and password. Users off campus must first access the Virtual Private Network with their assigned campus username and password and then use their application credentials. Users are only able to see study information if they are indicated as study personnel in our electronic IRB system. Users are limited to access based on the role assigned in their corresponding protocol. Subject data is entered directly into the system, which (in the case of Columbia subjects) confirms the correct identity of patients via an interface with the electronic medical patient index. Staff with the appropriate IRB defined roles can run reports within the system for reporting purposes.

- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (e.g., that the subject is alive) at the end of their scheduled study period.

The subject binders will be maintained with in the CPDM offices, a secured floor within the Herbert Irving Pavilion and only the investigator and study staff will have access to the file.

10.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

10.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the

CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A".

10.4 Records Retention

Records relating to a specific research activity, including research records collected by investigators, must be maintained for at least three years after completion of the research (45 CFR 46.115(b); 21 CFR 56.115(b); 21 CFR 312.62). This minimum retention period applies whether or not any subjects were enrolled in the study.
If the research is FDA regulated, records should be retained for at least two years after approval of the investigational agent by FDA; if it is not approved, records should be retained at least two years after the study is terminated and FDA is notified (note the additional requirement below for clinical research studies);
Clinical records, including consent forms that document clinical intervention or clinical diagnostic procedure research-related procedures, must be retained in medical records by the institution for at least seven years, per CUMC and NYP policy which is based on state law.

11. STATISTICAL CONSIDERATIONS:

11.1 Study Design/Endpoints

Timeframe for evaluating DLTs: This is a single-dose study.
Timeframe: 2 months from administration of A-B-D-Y90.

DLTs will consist of Grade 2 or higher acute hypersensitivity reactions, that do not respond fully to diphenhydramine and/or two doses of Solucortef 100mg 2 hrs apart. If 8 or more of projected 12 patients experience such reactions, the study will be closed to accrual. If zero adverse reactions attributable to AV-Y90DB are observed after the first 6 patients, the study will be formally closed if it is agreed by the IRB that the number is sufficient to determine that the treatment is safe.

Grade II delayed hypersensitivity reactions will consist of any allergic manifestations resistant to treatment with Medrol in doses up to 60 mg daily X 1 week. If 8 or more of projected 12 patients experience such reactions, the study will be closed to accrual. If zero adverse reactions attributable to avidin are observed after the first 6 patients, the study will be formally closed if it is agreed by the IRB that the number is sufficient to determine that the treatment is safe.

11.2 Size/Accrual Rate

Up to 12 patients will be accrued at the rate of 0-2 per month.

11.3 Stratification Factors

None

11.4 Analysis of Secondary Endpoints

None apart from Safety-Tolerability
Timeframe for evaluating DLTs: This is a single-dose study.
Timeframe: 2 months .

DLTs will consist of Grade 2 or higher acute hypersensitivity reactions, that do not respond fully to diphenhydramine and/or two doses of Solucortef 100mg 2 hrs apart. If 8 or more of projected 12 patients experience such reactions, the study will be closed to accrual. If zero adverse reactions attributable to AV-Y90DB are observed after the first 6 patients, the study will be formally closed if it is agreed by the IRB that the number is sufficient to determine that the treatment is safe.

Grade II delayed hypersensitivity reactions will consist of any allergic manifestations resistant to treatment with Medrol in doses up to 60 mg daily X 1 week. If 8 or more of projected 12 patients experience such reactions, the study will be closed to accrual. If zero adverse reactions attributable to avidin are observed after the first 6 patients, the study will be formally closed if it is agreed by the IRB that the number is sufficient to determine that the treatment is safe.

11.5 Reporting and Exclusions

11.5.1 Evaluation of toxicity

Patient reporting, vital signs, avidin skin tests and other standard laboratory indices of hypersensitivity reactions.

11.5.2 Evaluation of response

12. STUDY FINANCES

13. PUBLICATION PLAN

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

14. REFERENCES

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Appendix 1. Microtitre Plate Bladder Testing Platform.

APPENDIX 1. We have developed a microtiter plate-based platform for performing controlled trials of different test compounds, including short-range alpha or beta emitting isotopes, simultaneously on multiple samples of explanted pleural or urinary bladder wall tissues, capable of measuring the concentration and volume of radioactive material present, determining whether there is attachment of the conjugate to bladder, assessing the depth of penetration of the radiation, and making accurate dosimetric calculations and measurements of radiation effects on bladder mucosa.

The studies are carried out in 24- well (6 quadruplicate rows) microtiter plates, each well measuring 17mm in height, and 15.3mm diameter. A circle of filter paper is optionally placed at the bottom of each well. In the present embodiment, studies are carried out with urinary bladders of 250 lb female pigs,12-15 hours after excision, immediate refrigeration, and transportation to our laboratory. The bladder is inflated with 100 ml cold normal saline, then incised longitudinally dorsally, everted and stretched over the entire face of the microtitre plate, adjusted so that the bladder thickness is approximately uniform over the entire plate, fixed against the plate surface with clips, and the elastic bladder tissue is pressed into the well, optionally until the bottom is touched (see photograph) with a hollow 12.6 outside diameter nylon plunger with an inside diameter of 6mm. giving an approximate circular exposed bladder mucosal area of ~100mm^2 with a usable chamber volume of ~ 500µl. The thickness of the bottom layer has not exceeded 2 mm. The plungers are held firmly in place by an upper plate incorporating 24 opposed hollow nylon plungers through which pipettes can add or remove liquid. In its simplest form the well is sealed tightly by redundant bladder tissue and the inside of the nylon plunger forms a rigid cylindrical well into which can be placed (a) different specific activities of liquid or gel-suspended isotope in micro or nanoparticulate form, to fill the well and directly contact the pleural or bladder mucosa; (b) Sprayed or applied Gelfoam avidin-biotin-DOTA-istope conjugates (see below) to a defined depth (e.g. 2 mm), above the pleural or bladder mucosa; (c) a gelfoam-radioisotope pledget or tile, placed to a defined depth so as to be adherent to the bladder tissue.(d) Liquid nutrient medium containing chemotherapeutic agents (e.g., mitomycin or cisplatin) (e) control medium. Multiple concentrations of each test material would be assayed by this method.

Each well would contain nutrient media, antibiotics as necessary, and be kept reasonably intact for up to 24 hours of radiation treatment, when the isotope would be washed free, biopsied or fixed as required , and optionally refrigerated for 7 or more isotope half-lives, then processed for depth of penetration of radioactivity or drugs, for or metabolic changes, and for degrees of radiation-induced DNA damage reflected by increase in DSB or SSB DNA fragments detected by electrophoresis.

(Illustrations)----------

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Pig bladder platform:

The urinary bladder is excised from a commercial pig of approximate weight 250lbs. (see Figure 1,2) The bladder is of similar size to that of humans. The bladder is inflated with 50-100 ml phosphate buffered saline (Ph 7.4 to facilitate dissection. The bladder is incised vertically on the posterior side from the dome to the trigone (f

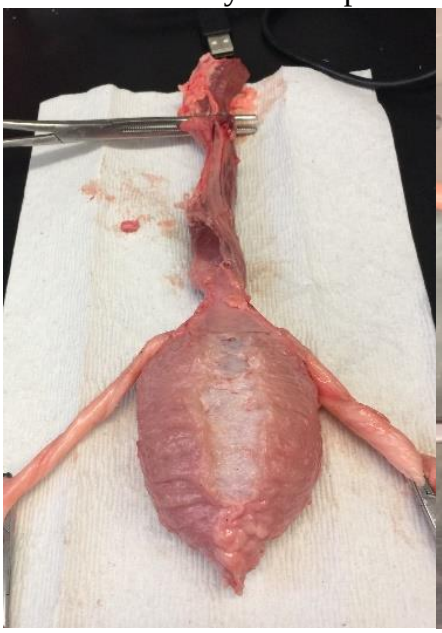


Figure 1

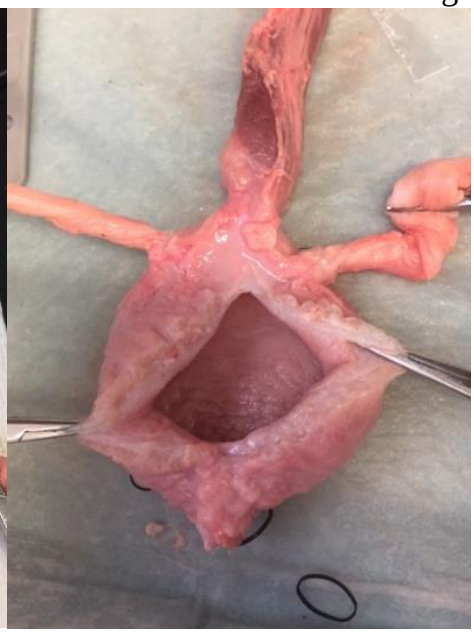


Figure 2

The bladder is then manually everted (mucosal side ooutward) and evenly stretched over a 24-well styrene microplate, into which wells, relevant filters or sensors had been previously placed. The stretched bladder is held in placed with clips. (Figure 3,4)..

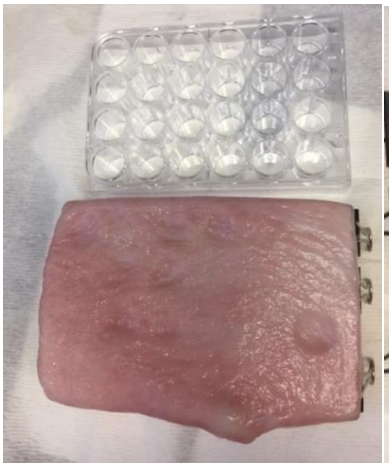


Figure 3

Figure 4—underside view

Nylon 15.3 mm spacers 20-35 mm long are then forced into each of the wells,(Figure 5) further stretching the bladder over the lower end of each spacer, forming 24 sealed cylindrical chambers each overlying a 3mm circle of stretched bladder, mucosal side up of determinable thickness (Figure 6). Microliter quantities of material relevant to each of a battery of experimental manipulations are then introduced into the wells as necessary

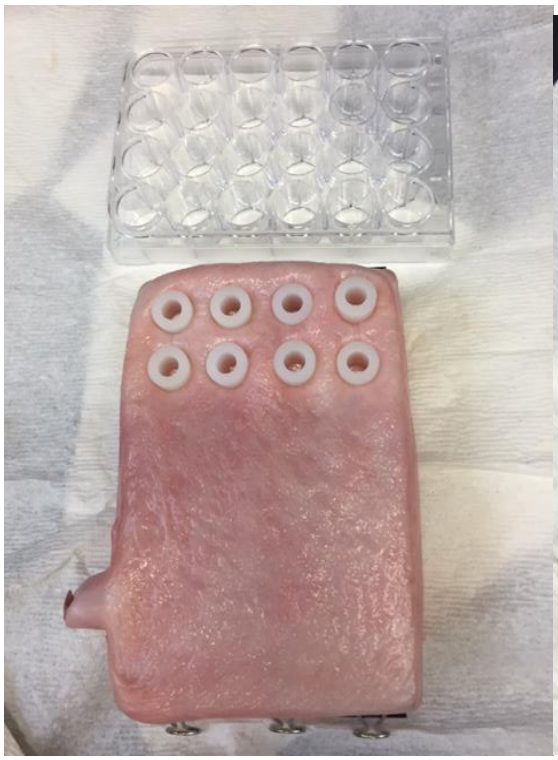


Figure 5

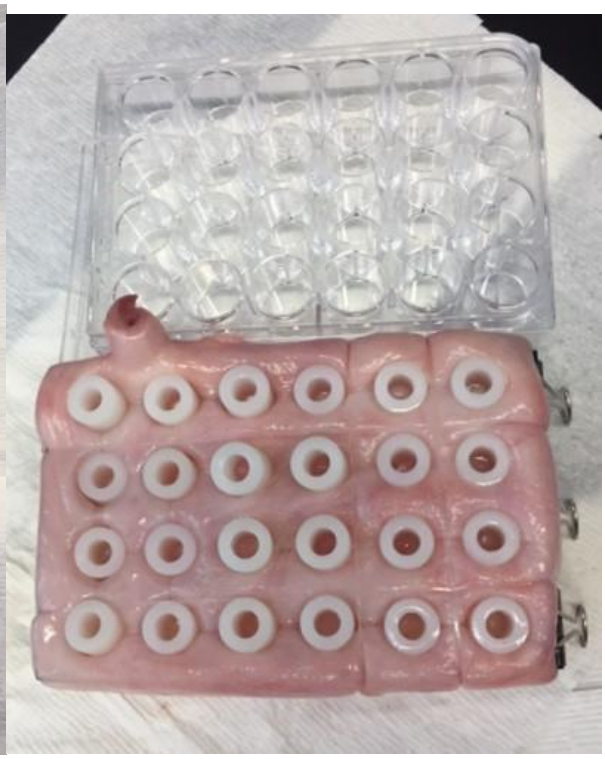
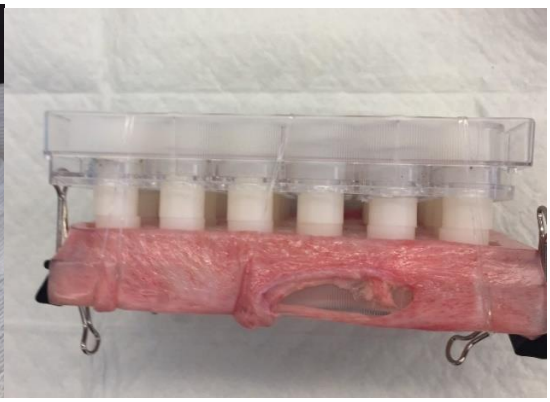
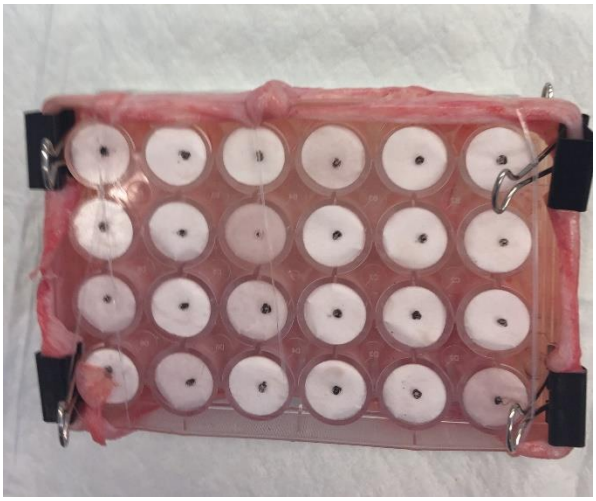
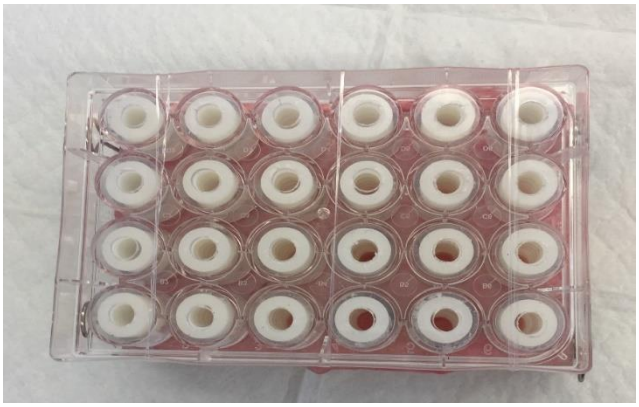


Figure 6

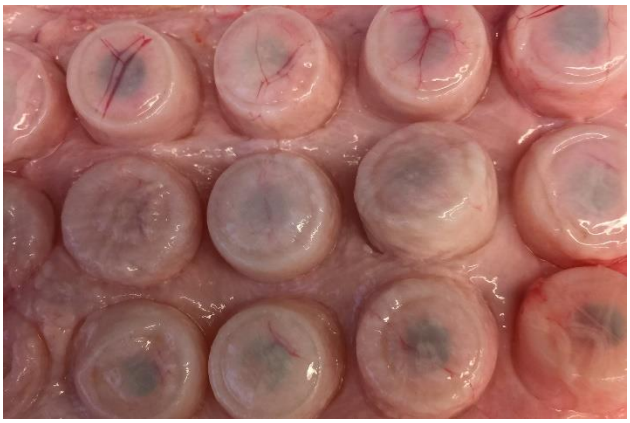


7. Left: All 24 wells are formed into chambers. 8. Right: Pressure is maintained on the nylon inserts, by inserts affixed to an upper complementary microplate held with powerful elastic bands.



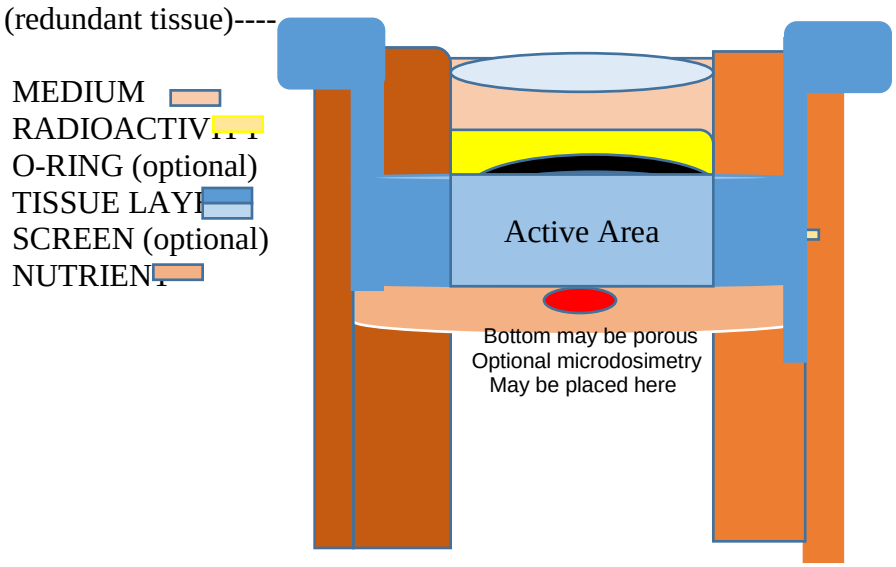


9. Left: bottom view of microplate assembly, showing a single air hole per well and a filter paper floor.
10. Right: top view of microplate assembly, note that ¼" holes in the upper plate allow introduction of reagents and wash solutions.



11. After experiment has been completed, this shows a bottom view of The shape of the individual mucosal lined experimental mucosal wells.

DIAGRAM OF MICROPLATE WELL (EXPERIMENTS 1-3)



INITIAL PLANNED EXPERIMENTS:

1. Determination of viability of bladder mucosa. 2.5 mm punch biopsies of stretched mucosa at bottom of wells from freshly delivered bladder preparations v bladder preparations frozen at -20 degrees for one week are assayed for viability using MTS(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt), and determining the reaction product colorimetrically. The MTS assay is based on the conversion of the tetrazolium salt into a coloured, aqueous soluble formazan product by mitochondrial activity of viable cells at 37°C. The amount of formazan produced by dehydrogenase enzymes is directly proportional to the number of living cells in culture and can be measured at 492 nm.

2. Attachment of horseradish peroxidase (HRP)-labeled avidin to bladder mucosa. HRP avidin (10 micrograms/ml in 100 microliters medium buffered at pH 5 and 8) , and subsequently washed X5 with fresh buffer. The residual Avidin is detected through the detection of its bound

Horseradish peroxidase (HRP), a 40,000 Dalton protein, which catalyzes the reduction of hydrogen peroxide (H_2O_2) to water (H_2O). In the presence of specific substrates, which act as hydrogen donors, the action of HRP converts colorless or nonfluorescent molecules into colored and/or fluorescent moieties. All assays will test the inside and outside of the microplate mucosa, to determine the degree of HRP leakage, if any.

3. Attachment of HRP-labeled Biotin to bladder mucosa, either alone; or after preincubation with unlabeled avidin, or as a mixture with unlabeled avidin, at Ph 5 and pH 8). The biotin will be detected using the same HRP assay as used for HRP avidin. All assays will test the inside and outside of the microplate mucosa, to determine the degree of HRP leakage, if any.

4. Experiments are performed as above, except that wecollaborate with investigators in interventional radiology and nuclear medicine. Indium-111-DOTA-biotin is substituted for HRP biotin. Yttrium will not be initially used because of the difficulty in measuring the isotope (very short range LET beta radiation, in comparison to the easily detectable gamma emission of Indium 111). All assays will test the inside and outside of the microplate mucosa, to determine the degree of Radioisotope leakage, if any. The indium assay, coupled with current knowledge of the availability, LET, and dosimetry of high specific activity 90Y should allow assessment of the isotope doses needed for effective Phase I testing.

We feel that the above experiments (the first three have been carried out—See Appendix) would be sufficient to (a) establish the feasibility of using the radioisotope for therapeutic purposes, both because it can in principle, be introduced as a liquid into the proximity of the bladder mucosa, yet will not penetrate functionally beyond the bladder to harm surrounding organs. Moreover its effects may be intensified by adherence of avidin to the negatively-charged bladder mucosal surface epithelium. Because viable bladder tissue is used in a reproducible precise platform to make these determinations, and because Indium-111, Yttrium-90, biotin and avidin have all been in use for several decades and have been generally recognized as safe, we do not at this time feel the necessity of further testing in live non-human animals or that performing such testing in non humans would add significantly to our knowledge. We hope to petition to be allowed to proceed directly to Phase I testing in patients with bladder carcinoma scheduled to undergo cystectomy, during which time detailed determinations of effective therapeutic doses could be ascertained on removed bladder specimens.
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APPENDIX 3. ANATOMY OF THE HUMAN BLADDER

It is helpful to review the **ANATOMY** of the interior of the human bladder.

The mucous membrane lining the bladder is, over the greater part of the viscous, loosely attached to the muscular coat, and becomes incresingly wrinkled or folded when the bladder is contracted: in the distended condition of the bladder the folds are effaced. Over a small triangular area, termed the **trigonum vesicæ,** immediately above and behind the internal orifice of the urethra, the mucous membrane is firmly bound to the muscular coat, and is always smooth. The anterior angle of the trigonum vesicæ is formed by the internal orifice of the urethra: its postero-lateral angles by the orifices of the ureters..

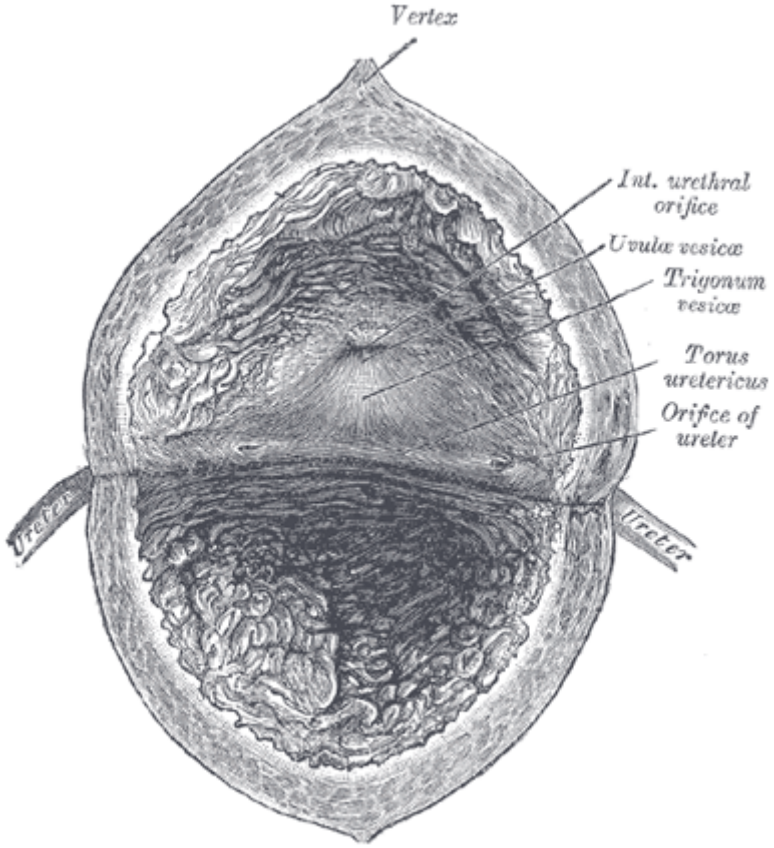

FIG. 1140– The interior of bladder.

The **orifices of the ureters** are placed at the postero-lateral angles of the trigonum vesicæ, and are usually slit-like in form. In the contracted bladder they are about 2.5 cm. apart and about the same distance from the internal urethral orifice; in the distended viscus these measurements may be increased to about 5 cm.

The **internal urethral orifice** is placed at the apex of the trigonum vesicæ, in the most dependent part of the bladder, and is usually somewhat crescentic in form; the mucous membrane immediately behind it presents a slight elevation, the **uvula vesicæ,** caused by the middle lobe of the prostate.

Structure The bladder is composed of the four coats: **serous, muscular, submucous,** and **mucous coats.**

The **serous coat** (*tunica serosa*) is a partial one, and is derived from the peritoneum. It invests the superior surface and the upper parts of the lateral surfaces, and is reflected from these on to the abdominal and pelvic walls.

The **muscular coat** (*tunica muscularis*) consists of three layers of unstriped muscular fibers: a longituinal external layer (also forming the detrusor urinae muscle) , a middle circular mostly sparse fiber layer (also forming the sphincter vesicae) , and an internal, generally longitudinal fiber layer which helps prevent reflux into the uterus when the bladder contracts .

The **submucous coat** (*tela submucosa*) consists of a layer of areolar tissue, connecting together the muscular and mucous coats, and intimately united to the latter.

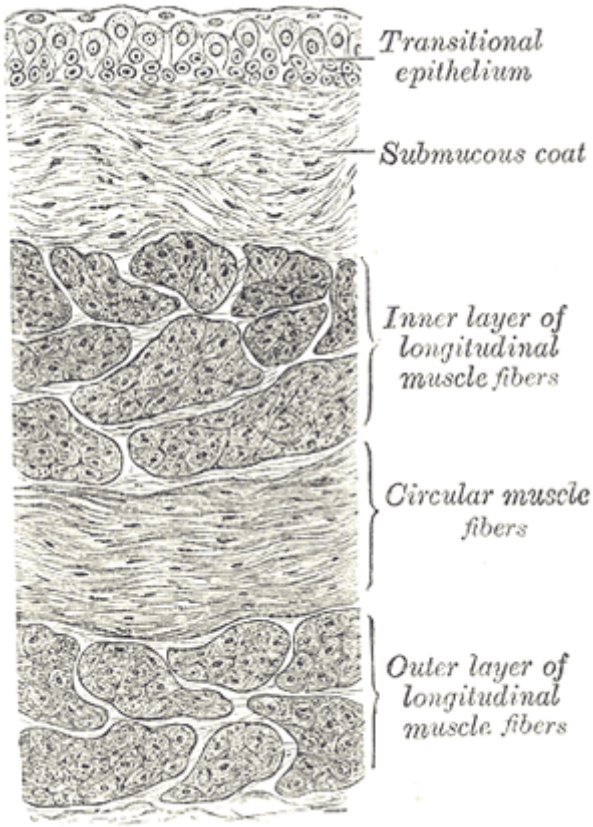

FIG. 1141– Vertical section of bladder wall.

The **mucous coat** (*tunica mucosa*) is thin, smooth, and of a pale rose color. It is continuous above through the ureters with the lining membrane of the renal tubules, and below with that of the urethra. The loose texture of the submucous layer allows the mucous coat to be thrown into folds or *rugæ* when the bladder is empty. Over the trigonum vesicæ the mucous membrane is closely attached to the muscular coat, and is not thrown into folds, but is smooth and flat. These considerations are most important to radioisotope treatment, since it suggests that the bladder should be filled to a point where the rugae have been largely obliterated. As calculated if the bladder is filled to over 250 ml, its surface area to tissue volume mandates that its total thickness cannot be more that 2-3 mm, sufficient for effective penetration of Y-90 radiation. A completely contracted bladder may well not be effectively treated. See inset.

Columbia University Tech Ventures.
Summary of Proposed Product Development through Phase I .

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