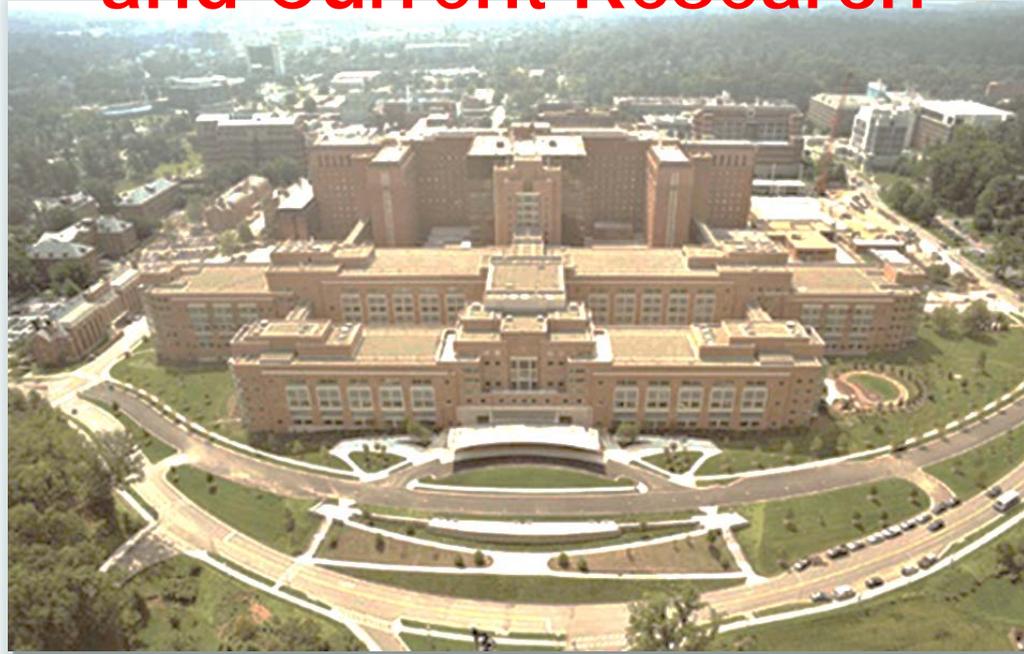


Bladder Cancer Diagnosis, Management, and Current Research



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Agenda

- Overview of Bladder Cancer
 - Epidemiology
 - Risk Factors
 - Evaluation
 - Staging
 - Grading
- Current Treatment Strategies
 - Transurethral Resection of Bladder Tumor (TURBT)
 - Intravesical Therapy
 - Radical Cystectomy
 - Chemotherapy and Radiation
 - Urinary Diversions
 - Robotic Approaches
- Current Research

Important Facts: Bladder Cancer

- 4th most common cancer in men and 12th most common cancer in women in 2014
- 74690 new cases and 15,580 deaths in 2014
- Represents 7% of all cancers and 3% of all cancer deaths
- Recurrence and routine surveillance/treatment make bladder cancer **most expensive malignancy to treat** from diagnosis to death (\$187,241/patient in 2001)
- **M:F = 3:1** (survival better in men)
- Peak incidence ages 60-70
- Majority (~93%) are urothelial cancer (transitional cell carcinoma)

Risk Factors

Exogenous

Schistosomiasis
Tobacco
Phenacetin metabolites
Cytostatics
(Cyclophosphamides)
? Sweeteners (Saccharin,
cyclamate)
Pelvic radiation
Blackfoot disease (Taiwan)
A. Fangchi (Chinese herb)

Endogenous

Chronic irritations
(catheters) /Toxins
Chronic inflammation

Industrial

Aniline dyes
Benzene derivatives
(aromatic amines)
Paints, oils, gasoline

Tryptophan metabolites

Nitrosamines

Occupations at Risk

- Dry cleaners
- **Painters**
- **Autoworkers**
- **Truck drivers**
- Paper manufacturers
- Metal workers
- Plumbers
- **Hairdressers**
- Tire and rubber workers
- **Chemical workers**
- Petroleum workers



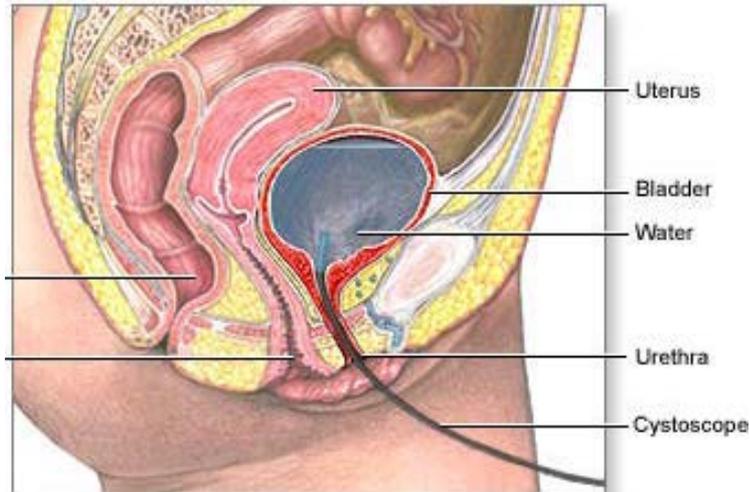
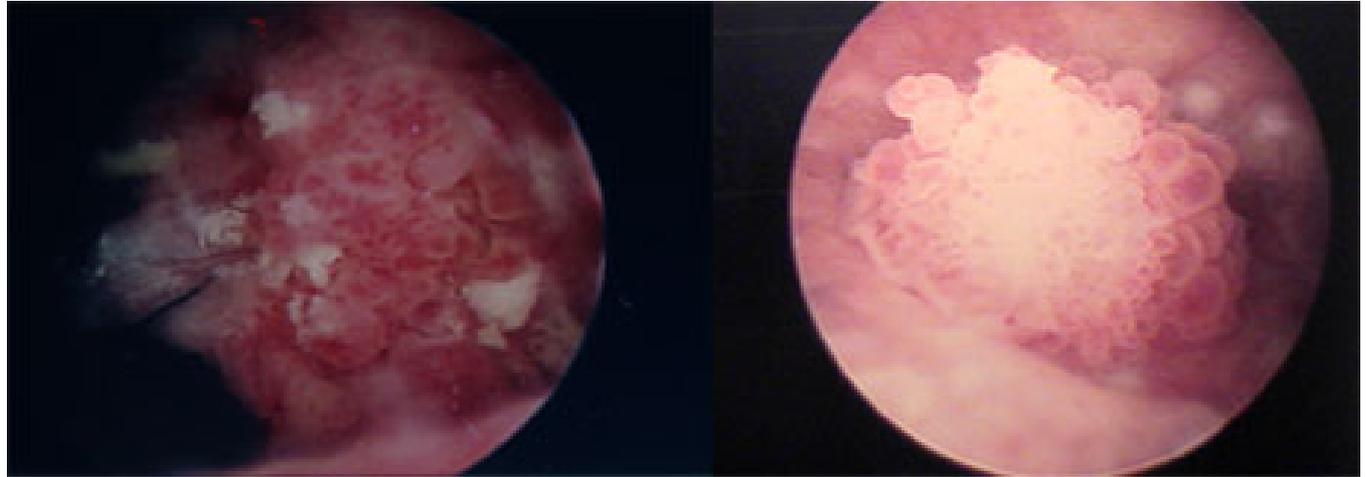
Presentation

- Gross hematuria most common
- Most commonly intermittent
 - Gross 68-97%
 - Microhematuria 11%
- Timing of hematuria
 - Initial – suggests urethral source
 - Terminal – suggests posterior urethra, bladder neck, prostate
 - Continuous – suggests bladder etiology
- Irritative voiding symptoms (especially in absence of UTI)

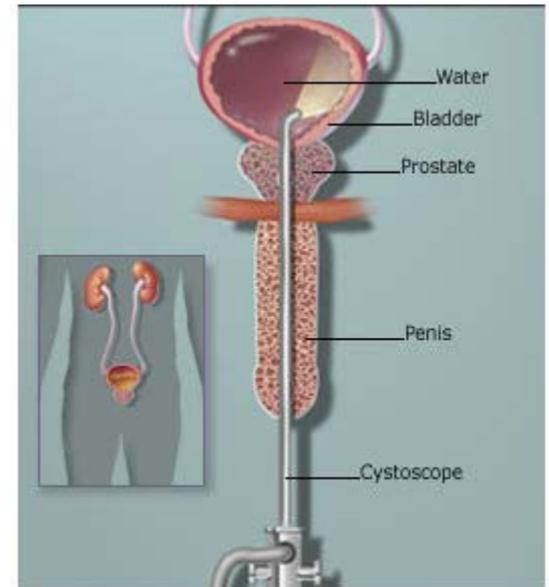
Work-up for Hematuria

- Cystoscopy
- Urinary Tumor Marker
 - Usually cytology
- Imaging
 - Renal Ultrasound and IVP traditionally
 - Now CT Urogram
 - Even MR Urogram
- Transurethral Resection of Bladder Tumor (TURBT) and Exam Under Anesthesia (EUA)

Cystoscopy



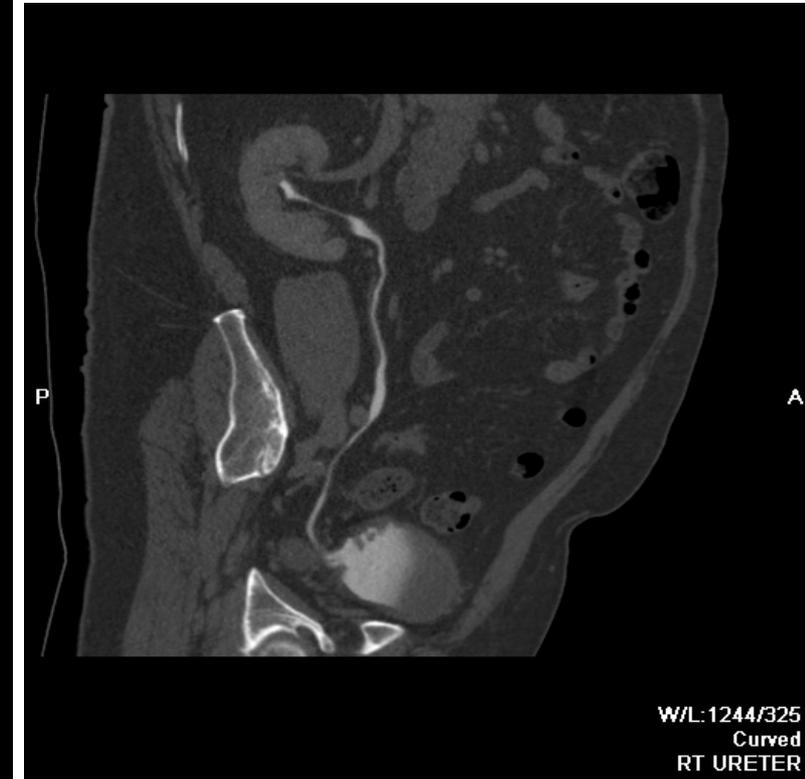
ADAM.



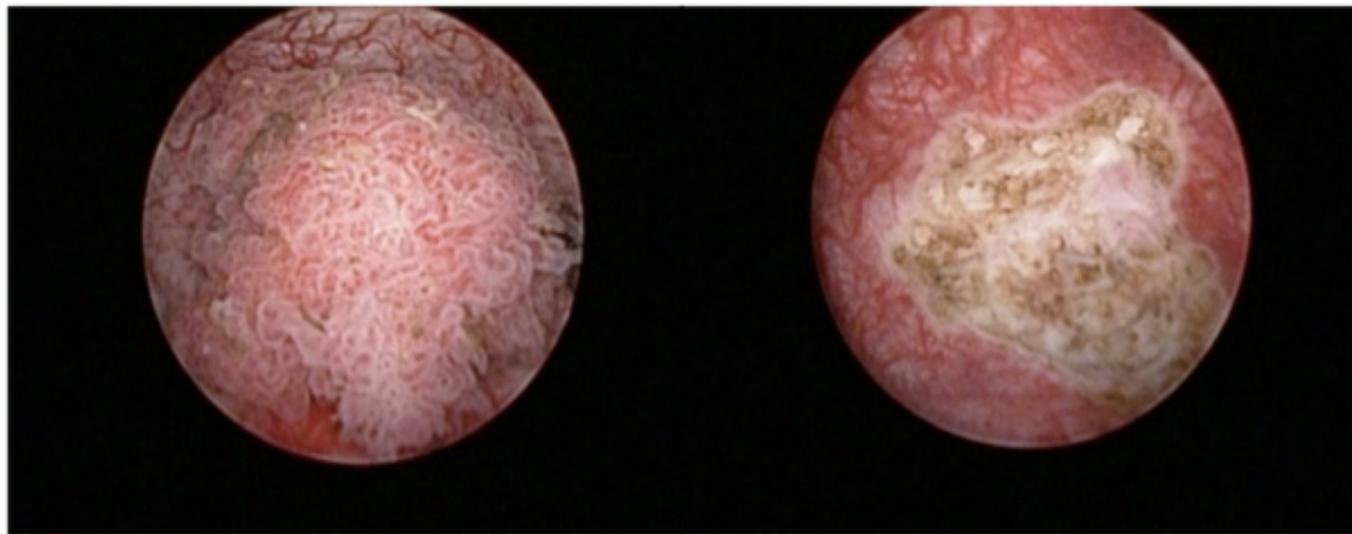
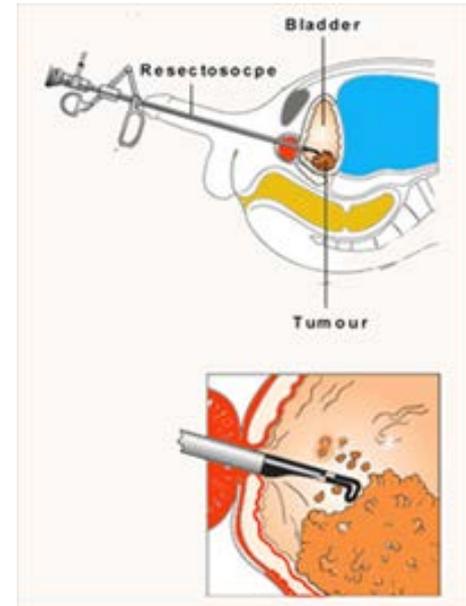
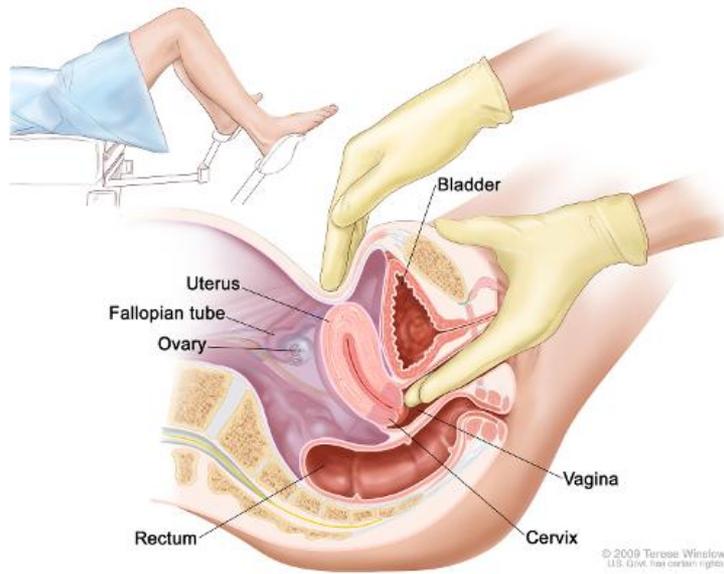
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CT Urogram – New Gold Standard

- Right ureteral tumor
- Several bladder tumors

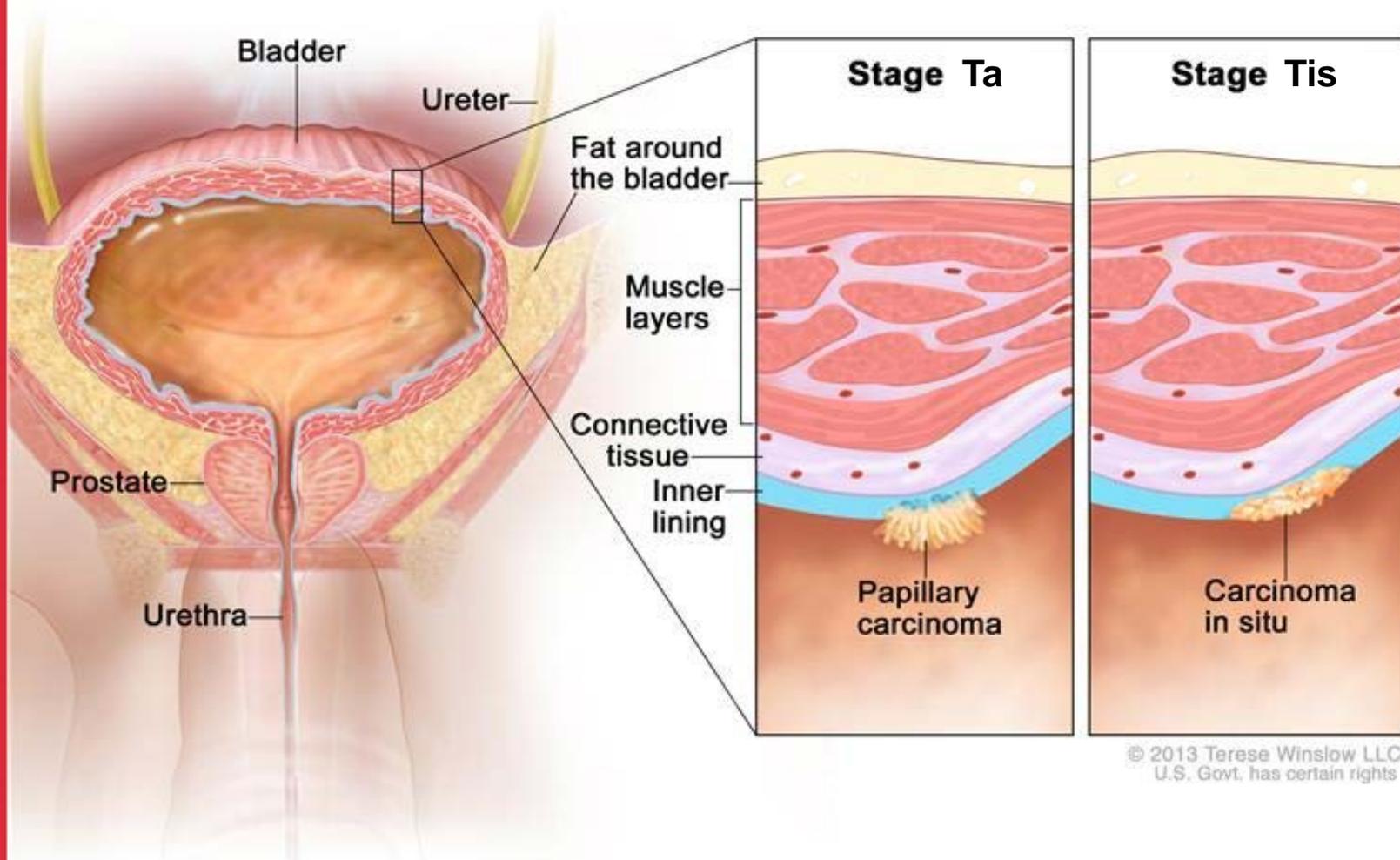


EUA and TURBT

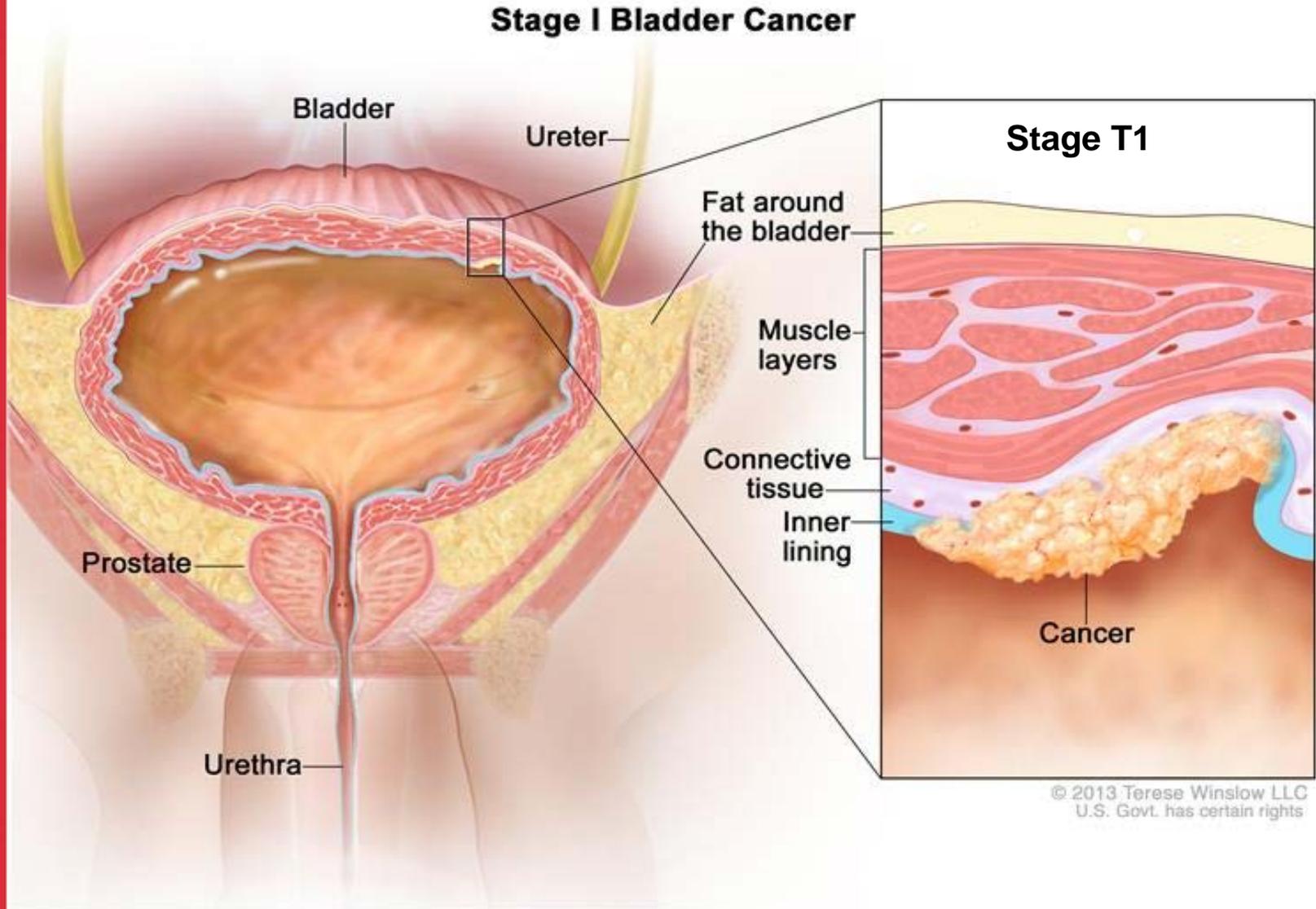


Bladder Cancer Staging

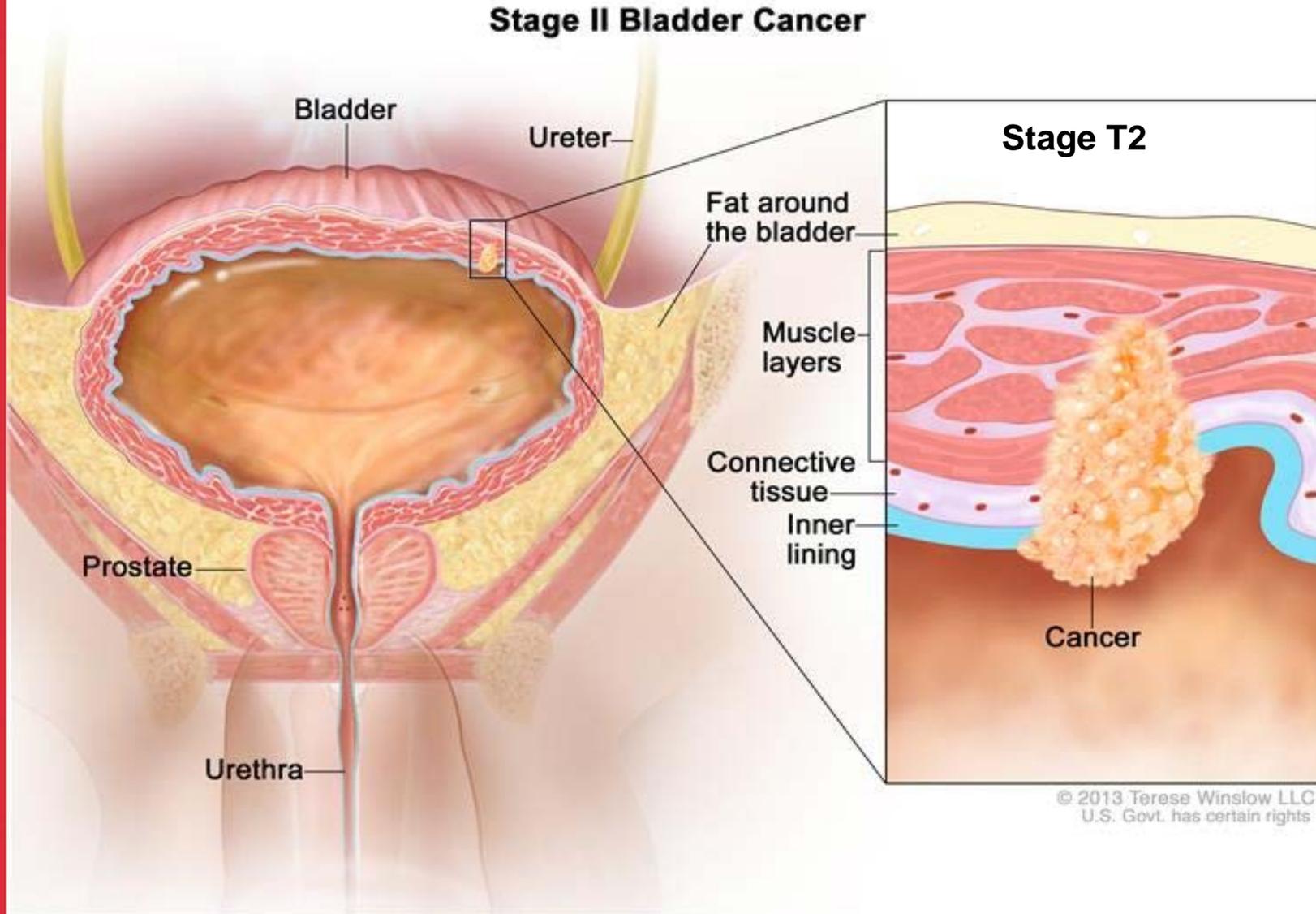
Stage 0 Bladder Cancer



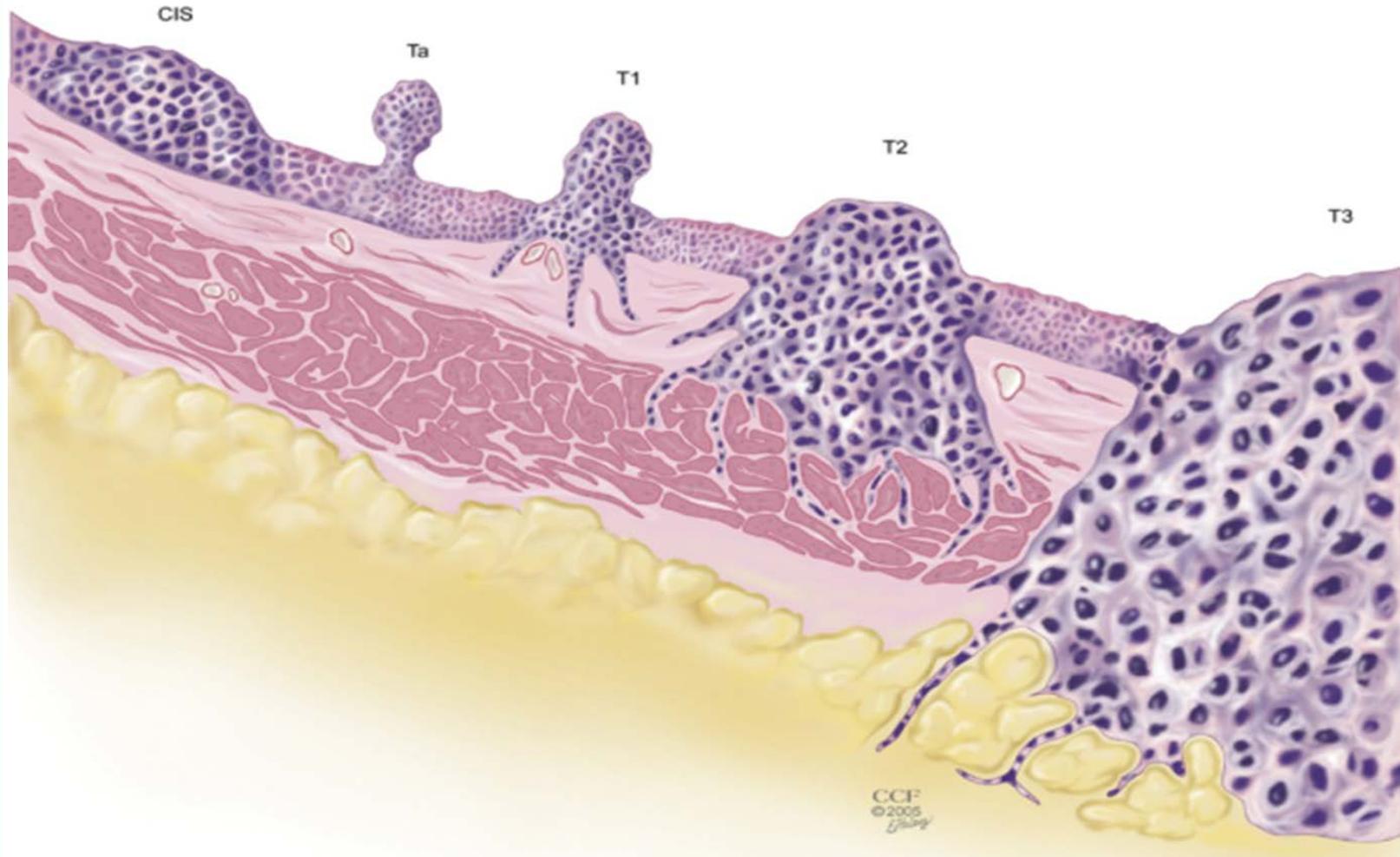
Bladder Cancer Staging



Bladder Cancer Staging



Stages of Bladder Cancer



Stage

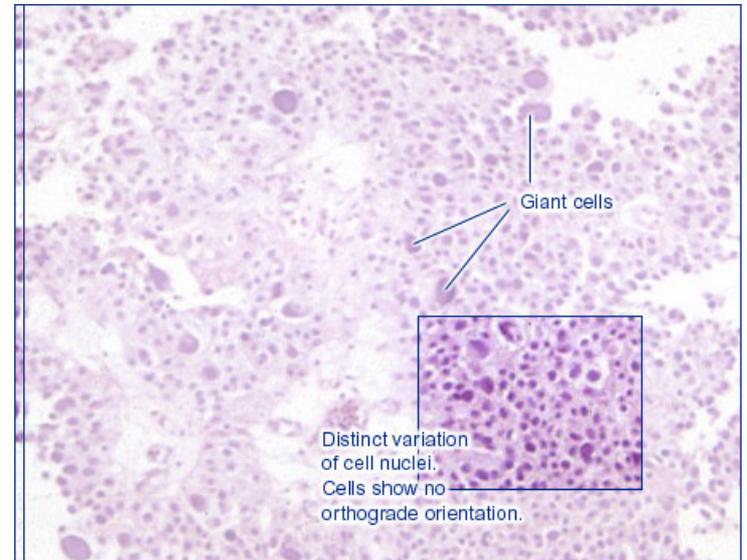
- ~70% non-muscle invasive (superficial)
 - Despite adequate therapy, 60-70% recur and 10-20% progress
 - 70% Ta and 30% T1
- ~25% muscle-invasive
 - 5 year overall survival 78% (45% with + nodes)
 - Morbidity of treatment (cystectomy +/- chemotherapy)
 - Majority present as muscle-invasive initially
- 5% metastatic disease
 - Chemotherapy produces median survival of 18 months and long-term disease-free survival in 10-15%

Grade

Low Grade



High Grade



Disease Recurrence

- Up to 70% will recur within 5 years

<u>Pathology</u>	<u>Probability of Recurrence in 5 years</u>	<u>Probability of Progression to Muscle Invasion</u>
Ta, low grade	50%	Minimal
Ta, high grade	60%	Moderate
T1, low grade (rare)	50%	Moderate
T1, high grade	50-70%	Moderate-High
Tis	50%–90%	High

Disease Progression

Estimates of disease progression in superficial bladder cancer

Tumor type ^a	% Relative frequency	% Progression	% Deaths
Noninvasive			
Papilloma	10	0–1	0
PUNLMP	20	3	0–1
Papillary cancer low grade (TaG1)	20	5–10	1–5
Papillary cancer high grade (TaG3)	30	15–40	10–25
Invasive			
Papillary cancer (T1G3)	20	30–50	33
Carcinoma in situ			
Primary	10	>50	—
Secondary	90		

Abbreviation: PUNLMP, Papillary urothelial neoplasm of low malignant potential.

^a World Health Organization/International society of Urological Pathology Consensus Classification of Superficial Bladder Cancer [9].

Data from Refs. [4,6,8,9].

Summary of Standard of Care Therapy

- Low grade, Ta or T1 disease
 - Surveillance  High Cost
High Recurrence
 - Possible intravesical therapy
- High grade (cIS, Ta, T1)
 - Repeat TURBT  High Cost
High Progression
 - Intravesical therapy
- Muscle-invasive disease (T2)  High Cost
Morbid Operation
50% Live
 - Cystectomy and urinary diversion
- Lymph Node/Distant Metastases (N+/M+)  High Cost
0% Live
 - Chemotherapy +/- radiation

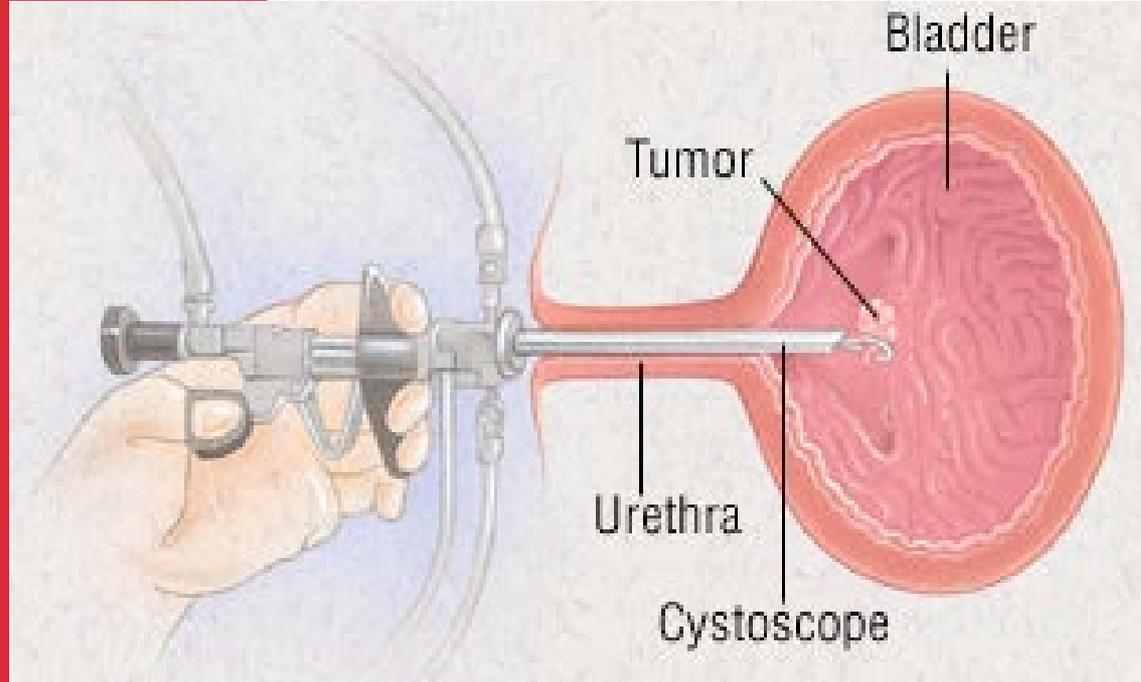
Surgical Therapies for Bladder Cancer

- Transurethral resection of bladder tumor (TURBT)
 - Intravesical Therapy
 - Radical Cystectomy
 - *** +/- Neoadjuvant Chemotherapy
 - *** Trimodal Therapy (XRT, Chemo, Surgery)
 - Urinary Diversions
 - Robotic Approaches to Bladder Surgery
 - Partial Cystectomy
 - Radical Cystectomy
- *** not to be discussed due to time

Man with Visible Blood in the Urine



TURBT



INTRAVESICAL THERAPY

Who is a Candidate for Intravesical Therapy?

- High Risk Disease
 - Multifocal disease
 - T1G3 (or all T1)
 - 70-80% recurrence rate and 30% progression
 - CIS
 - Tumors in Dome/Anterior Wall
 - High risk of progression

Intravesical Agents

Agent	Mechanism of Action
Immunomodulatory Agents	
Bacillus Calmette-Guérin (BCG)	<ul style="list-style-type: none"> • Inflammatory host response; release of cytokines • May be combined with interferons⁹⁰⁻⁹⁴
Interferons	<ul style="list-style-type: none"> • Lymphocyte activation; cytokine release; phagocyte stimulation • Antiproliferative actions • Antiangiogenic^{31,90}
Chemotherapeutic Agents	
Thiotepa	<ul style="list-style-type: none"> • Alkylating agent; cross-links nucleic acids⁹⁵
Mitomycin C	<ul style="list-style-type: none"> • Antibiotic; inhibits DNA synthesis⁷⁶⁻⁷⁸
Doxorubicin, epirubicin, valrubicin	<ul style="list-style-type: none"> • Intercalating agents; inhibits DNA synthesis^{75,96-98}
Gemcitabine	<ul style="list-style-type: none"> • Deoxycytidine analog; inhibits DNA synthesis⁹⁹⁻¹⁰³

Intravesical Agents

Agent	Mechanism of Action
Immunomodulatory Agents	
Bacillus Calmette-Guérin (BCG)	PREVENT RECURRENCE & PROGRESSION
Interferon- α	<ul style="list-style-type: none"> • Lymphocyte activation; cytokine release; phagocyte stimulation • Antiproliferative actions • Antiangiogenic^{31,90}
Chemotherapeutic Agents	
Hydroxyurea	• Alkylating agent; cross-links nucleic acids ⁹⁵
Mitomycin C	PERIOPERATIVE – DELAY RECURRENCE
Doxorubicin, epirubicin, valrubicin	BCG-REFRACTORY CIS
Gemcitabine	EXPERIMENTAL

Intravesical Therapy: Mitomycin C (MMC)

- Cross linking agent inhibits DNA synthesis and other mechanisms (alkylating agent)
- Non-cell cycle specific but sensitive in G1
- Large molecule (334 kd) – minimal systemic absorption and effects
- **Average CR: 36%; less in recurrence: 19-42%**
- Higher response in CIS (58%) than papillary lesions (43%)
- Role of maintenance therapy uncertain

Mitomycin C: Side Effects

- Chemical cystitis: up to 40% pts
- Decreased bladder capacity
- Skin rash/palmer desquamation (contact dermatitis)
- Leukopenia or bladder contraction is rare

Post-TURBT MMC

- Single post-TURBT instillation of MMC **can decrease the time to recurrence but does not affect progression (Sylvester 2004) – 39% decrease in odds recurrence compared to TURBT alone**
- Data is particularly strong for patients with a single tumor:
 - 35.8% recurrence rate compared to 65.2% recurrence for patients with multiple tumors
- EUA and AUA guidelines – give post-TURBT intravesical therapy in majority of patients who undergo solitary or multifocal papillary tumors unless contraindication

History of BCG

- BCG has anti-tumor effects
 - 1929 autopsy study – lower frequency of cancer in patients with active or healed tuberculosis (TB)
 - 1950s – Old - mice infected with BCG increased resistance to tumor transplantation
 - 1970s – Zbar – delayed hypersensitivity immunological response to BCG caused tumor inhibition. Criteria needed:
 - Close contact between BCG and tumor cells
 - Immunocompetent host capable of mounting immunological reaction to mycobacterial antigens
 - Limited tumor burden
 - Adequate numbers of viable BCG organisms

BCG and Bladder Cancer

- 1975 – deKernion – treated isolated melanoma in bladder with intravesical BCG
- 1976 – Morales – first successful use of intravesical BCG for superficial TCC
 - Devised original protocol for induction
 - 6 doses because Frappier strain packaged in 6 vials
 - 120 mg/dose because tolerated by intradermal
 - Weekly instillation because adverse effects <1 week
- 1978 – Morales treated 10 patients and BCG reduced/eradicated tumor recurrences in 7
- 2 randomized controlled trials – SWOG (Lamm) and MSKCC conducted and confirmed reduced tumor recurrences compared to TURBT alone
- 1990 – FDA approved intravesical BCG

Effectiveness of BCG

- CIS
 - 60-80% complete remission
- Residual papillary disease
 - Eradicates in 45-60% but NOT substitute for good TURBT!
- Decreases Recurrence in all 20-65% (~40%)
- Response durable in 30% at 10 years

BCG Induction Therapy

- No Established Optimal Course
- Most use 6 week course
- An additional 6 week course advantageous in:
 - CIS : 30% additional response

How to Make BCG More Tolerable

- Can decrease side effects 30-50% by one of following:
 - Decrease dose to 1/3 or less
 - Space intervals to 2 weeks instead of 1 week
 - Decrease dwell time for BCG to 30 min
 - Administer fluoroquinolone 6 and 12 h after each dose
 - Use NSAID or COX-2 Inhibitor to potentiate favorable BCG immune response

BCG Failures

- CIS and BCG
 - 7% progression rate for untreated CIS
 - 20% progression rate after CR to BCG
 - 30% recurrence rate after BCG
- Failure after one induction course for CIS
 - 30% additional response in CIS with second course
- Failure after two induction courses in NMIBC
 - 30% progression over 3-5 years
 - Only 46.7% disease-free at 3.6 years
 - 50% metastatic disease over 3-5 years
- Definitely consider alternate therapies or cystectomy
 - 81% of surveyed US urologists reluctant to recommend cystectomy even for high risk cases of BCG failure x 2
 - Any CIS, high-grade, or T1

RADICAL CYSTECTOMY

- First performed in 1887, 230 patients reported by Whitmore and Marshall in 1962 with 5 year survival rates of 21-49%
- Now 50% (78% if confined to muscle, 25% if lymph nodes involved)

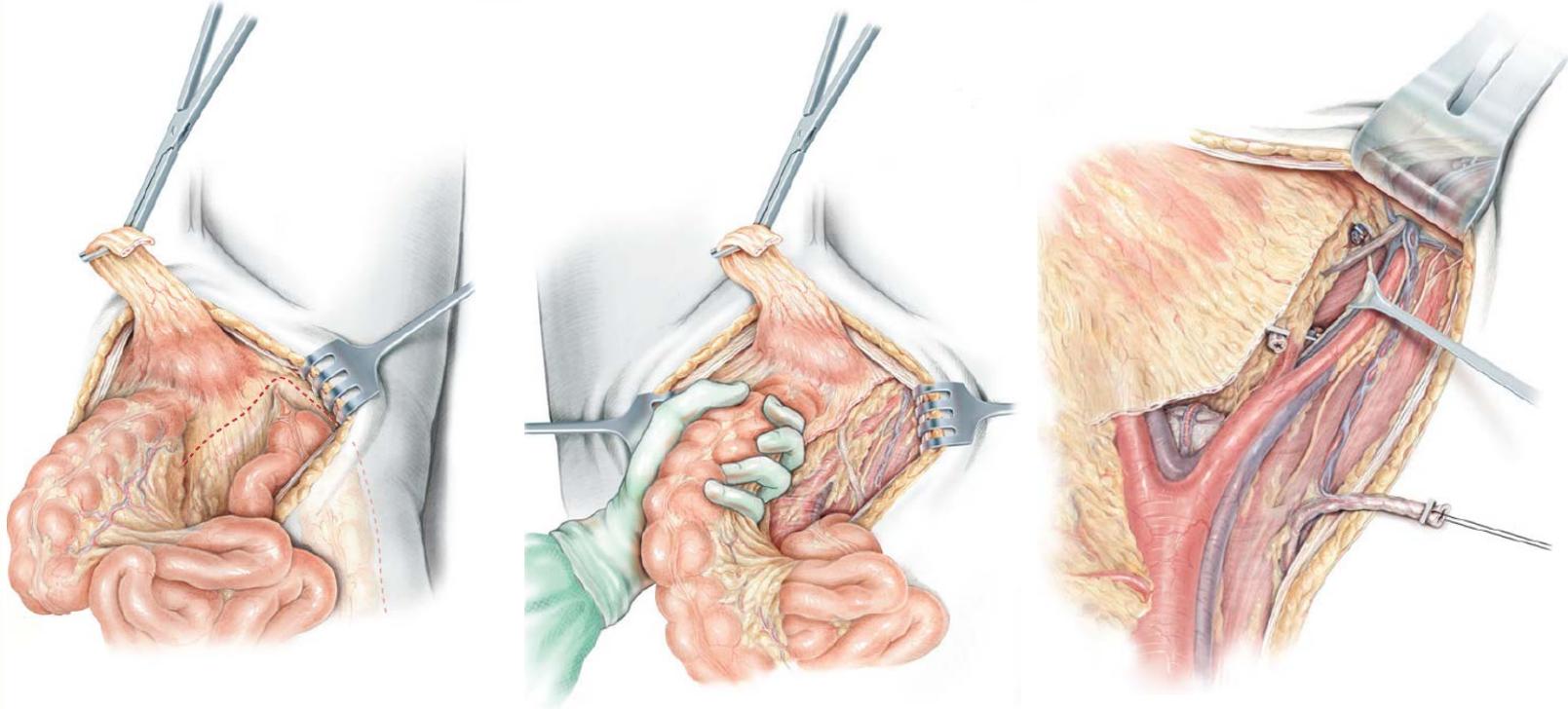
= Removal of bladder, peri-vesical fat, overlying peritoneum, pelvic lymph nodes and in:

Men - Prostate and seminal vesicles

Women - Uterus and portion of anterior vaginal wall

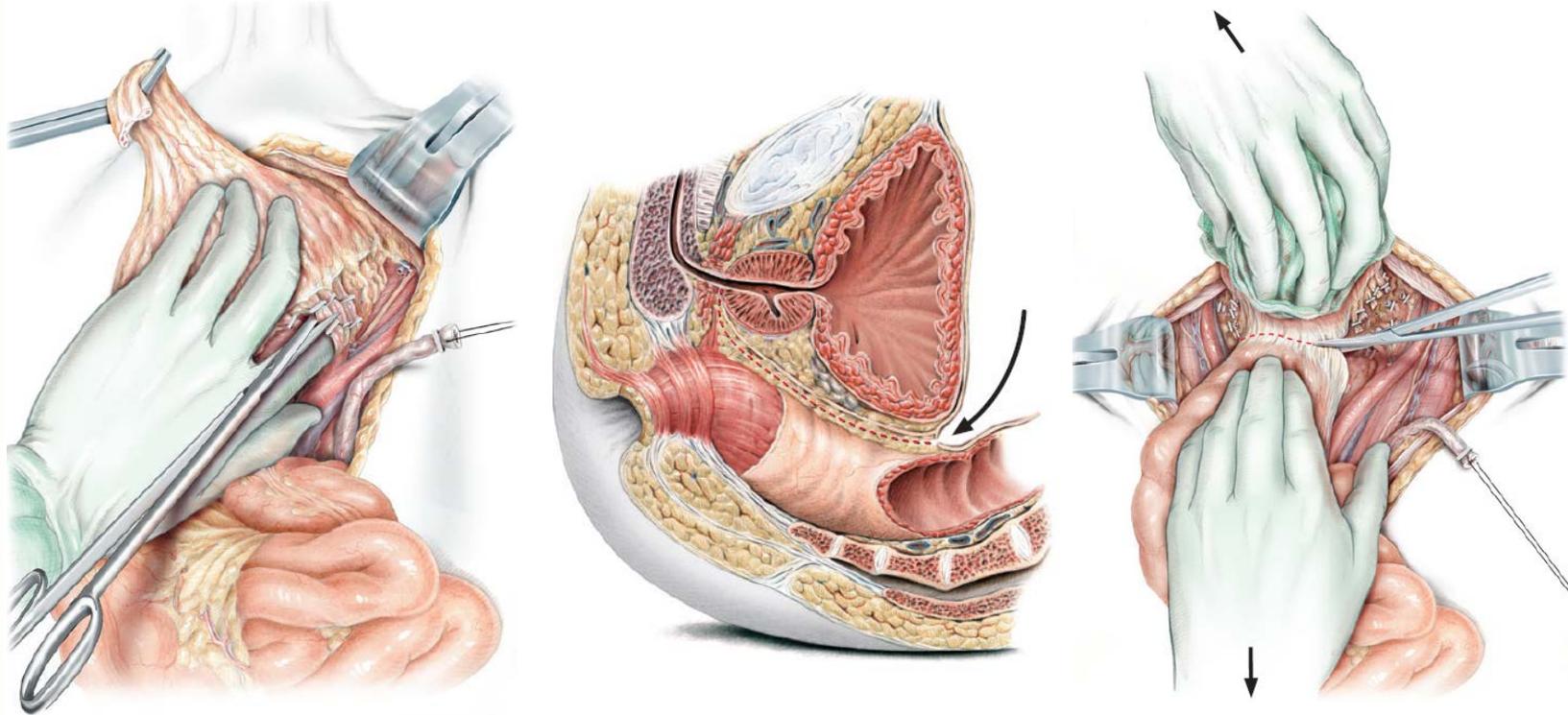
However, recent movement for genital organ sparing surgery

Bowel Mobilization and Lymphadenectomy



- Mobilize cecum and small bowel to retroperitoneal attachments (ligament of Treitz)
- Mobilize sigmoid along line of Toldt
- Pack small bowel and right colon
- Identify ureters in RP just cephalad to common iliac vessels and ligate close to bladder
- Perform meticulous lymph node dissection (either before or after cystectomy)

Division of Lateral Vascular Pedicles and Posterior Mobilization of the Bladder



- Divide lateral pedicles
- Denonvillier's fascia formed by convergence of anterior and posterior peritoneal reflections
- Divide in plane between rectum and posterior sheath of Denonvillier's fascia – “Don't go down to Brownsville!”

URINARY DIVERSION

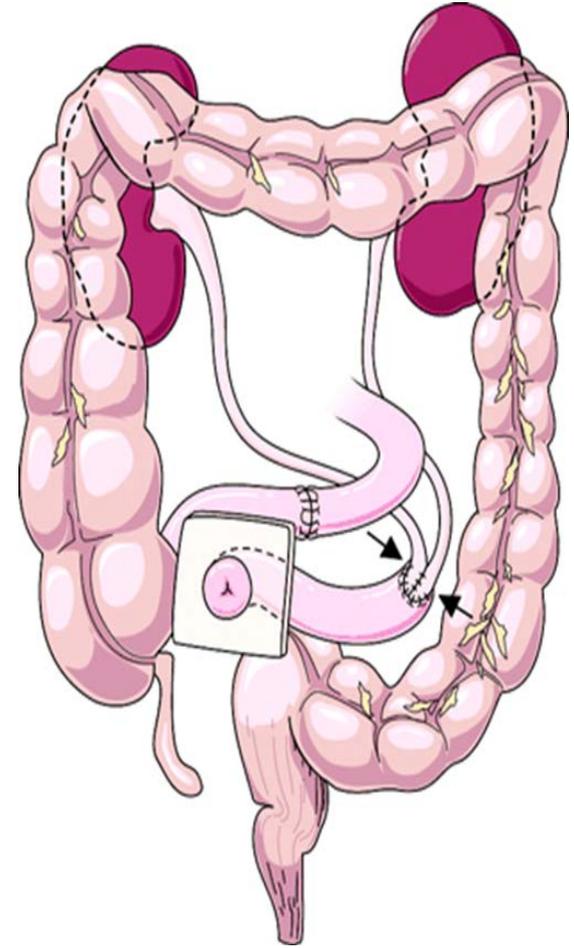
Urinary Diversion

- Bowel segment required
 - Each segment with unique metabolic consequences
- Types of Diversion
 - Conduit – non-continent “tubeless”
 - Jejunal
 - **Ileal**
 - Colon
 - Ureterostomy
 - Continent – “dry”
 - Non-orthotopic (e.g. **Indiana Pouch**)
 - Also known as continent cutaneous catheterizable pouches
 - Orthotopic (e.g. **Studer ileal neobladder**) – “can void”

Conduits (small bowel or large bowel)

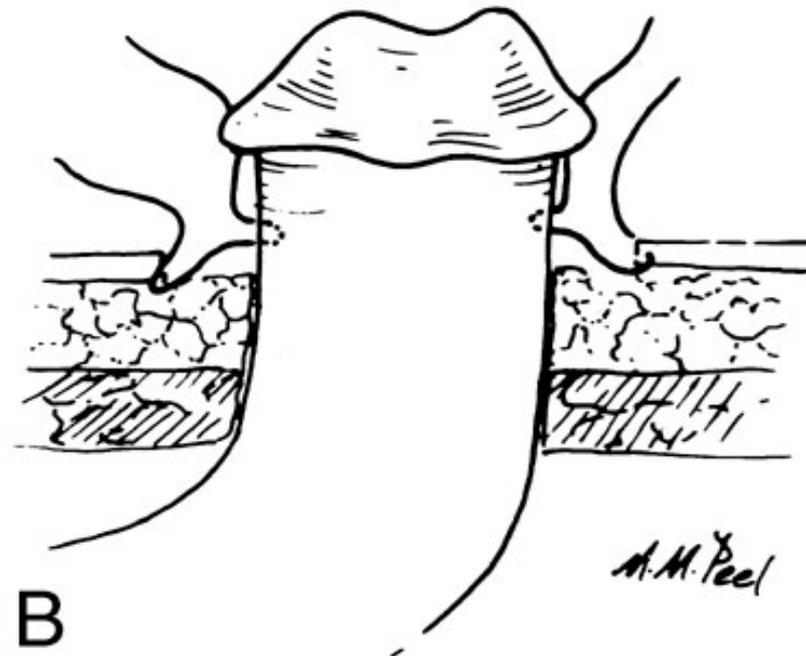
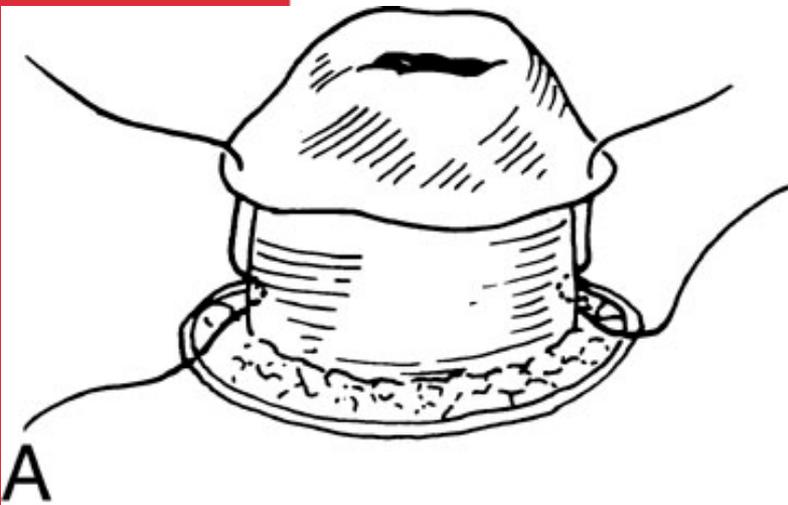
- **Advantages:**
 - Simple and quick procedure (less OR time)
 - Few inherent complications
 - Time tested – longest F/U data
 - Can compensate for short ureters
- **Disadvantages:**
 - Visible stoma
 - Negative body image
 - Need for lifelong stoma care (external appliance)
 - Anxiety of urinary leakage/odor

Ileal Conduit



Nipple Stoma:

- 4 - 6 cm of intestine is brought through the abdominal wall
- Fascial sutures are placed
- Each suture is placed in the seromuscular layer 3 cm proximal
- placed through the full thickness of the distal end of the intestine, then secured to the dermis before it is tied.

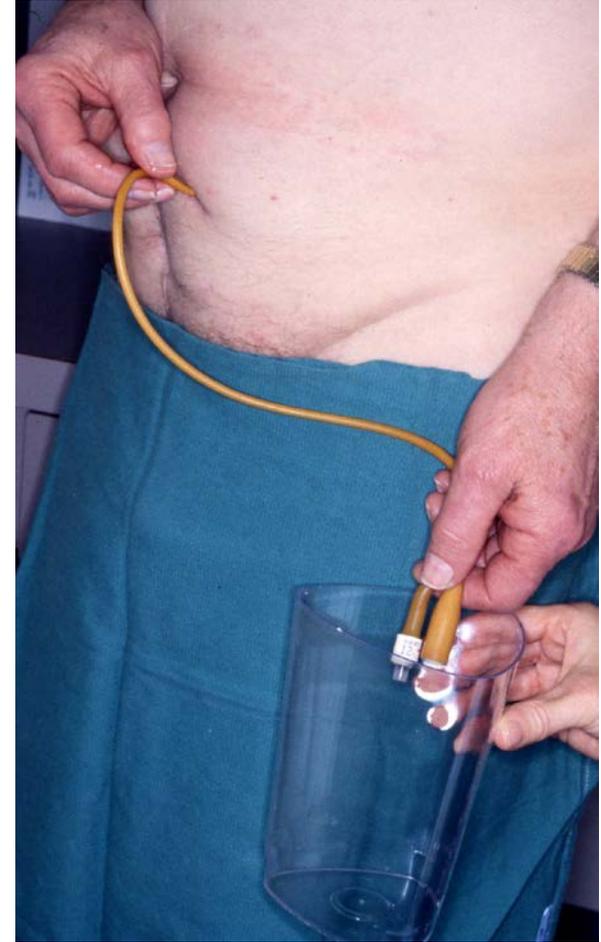
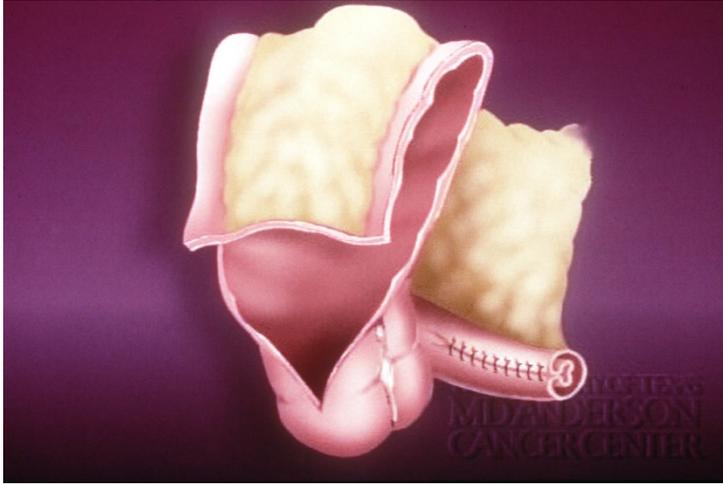


Continent Urinary Diversion

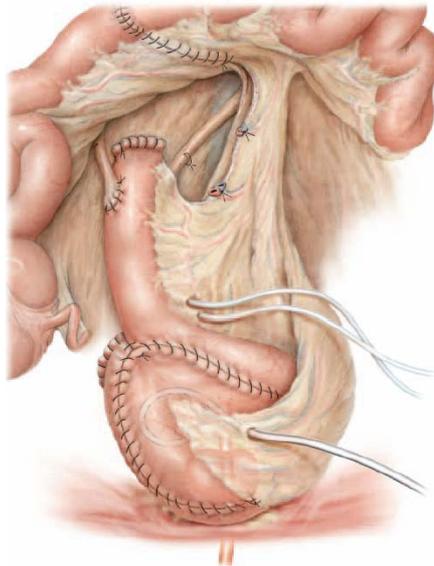
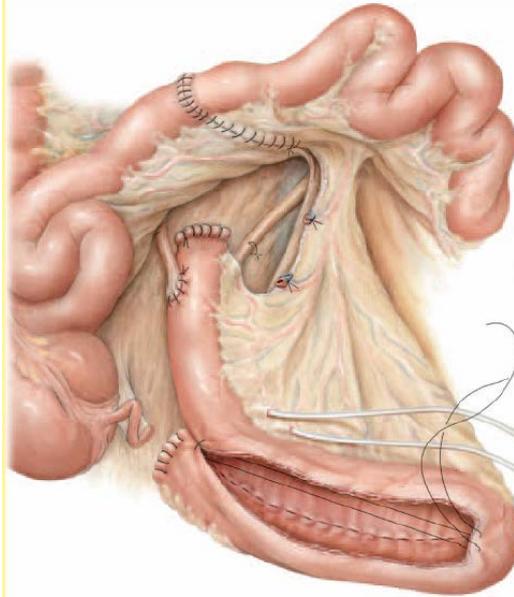
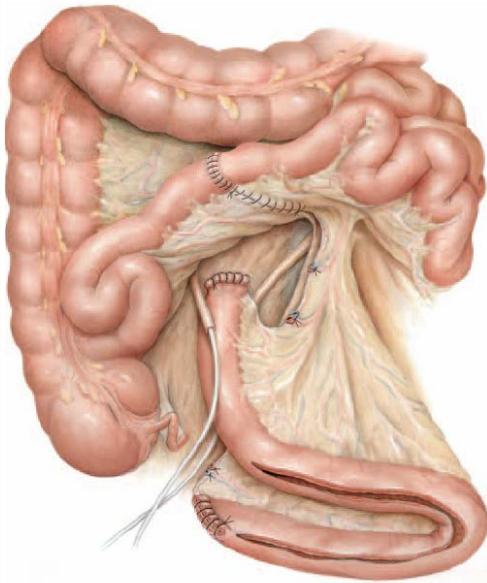
Patient Selection:

- Serum creatinine < 2
- Adequate Liver function
- Adequate bowel function
- Adequate intellectual capacity, dexterity, and mobility
 - Able and willing to perform self-catheterization
- Patient compliance (agrees to lifelong f/u)
- Absence of short gut syndrome/IBD
 - Colonoscopy prior to using any colon for diversion
- Motivated patient

Indiana Pouch



Studer Ileal Pouch

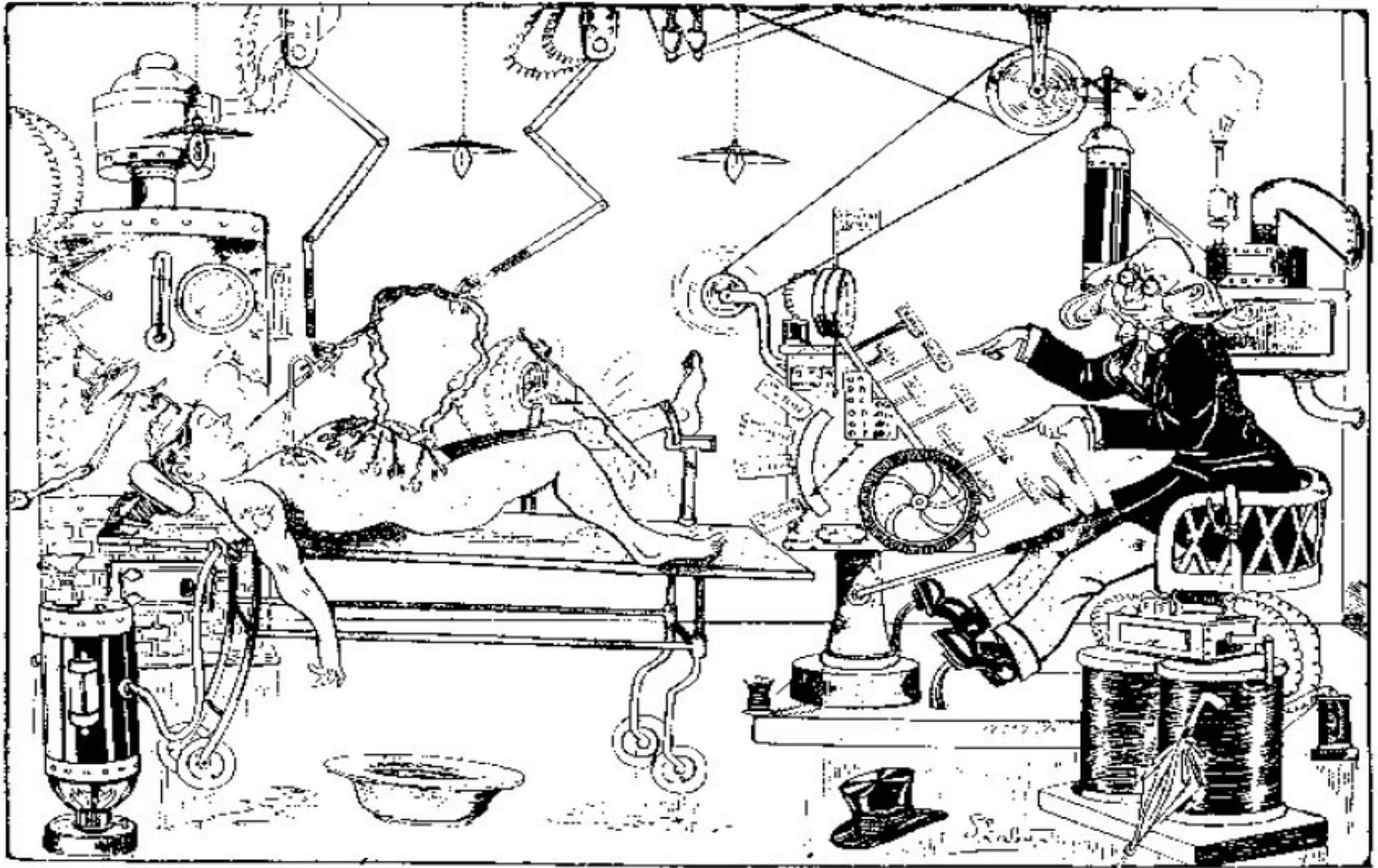


Studer Pouch

- **Pros**
 - High capacity (500cc), low pressure (<20cm H₂O)
 - 92% day continence, 80% nighttime (in Studer's hands!)
 - No ileocecal valve involved
- **Cons**
 - long ileal segment used
 - 6% metabolic disturbance, B12 and bile salt
 - 2% ureteral and 2% urethral stenosis

Neobladder - Post-op Care

- Irrigation of the neobladder regularly
- Peritoneal drainage
- Ureteral stents
- S/P tubes
- Teach the patient to void with abdominal pressure
- CIC if can't void by himself



UNE SALLE D'OPÉRATIONS EN L'AN 2000

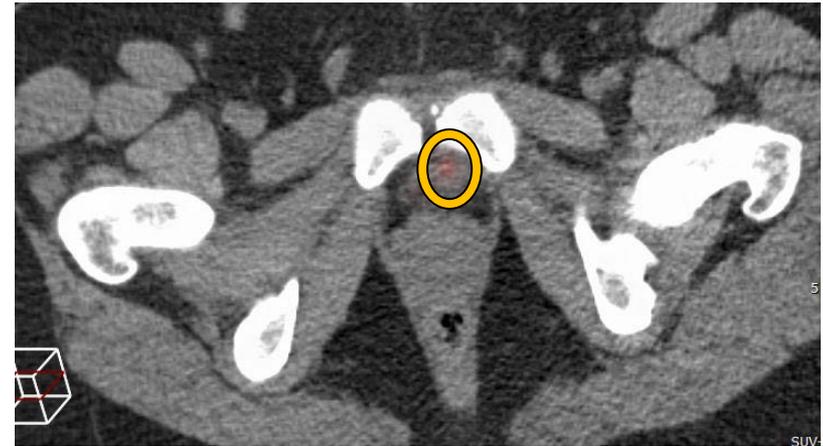
A French comic drawing from 1914 showing how the artist envisioned the operating room of year 2000



Vattikuti Urologic Institute

Robot-Assisted Laparoscopic Partial Cystectomy (RAPC)

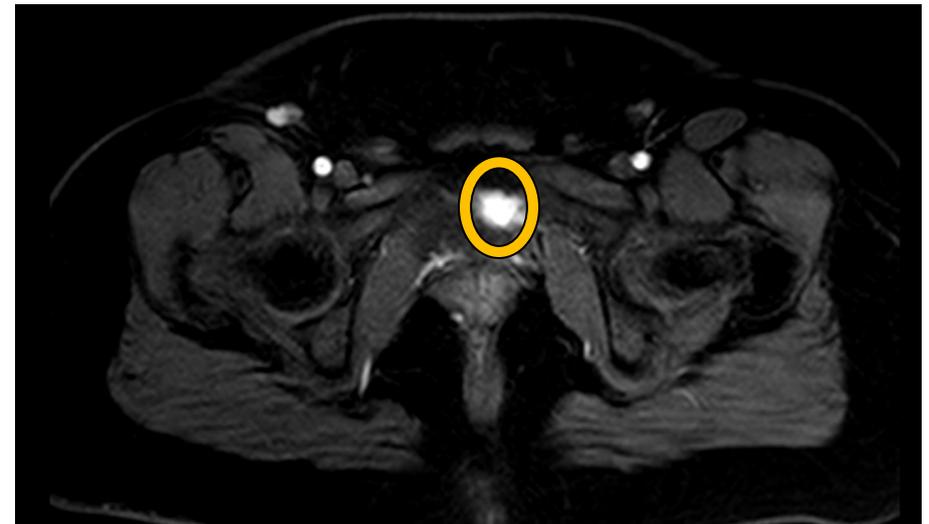
14 year old boy with history of VHL and multiple pheochromocytomas s/p bilateral adrenal surgery
Now with elevated serum catecholamines and headaches with urination



Pre-op PET/CT



Pre-op MRI



Pre-op MRI

Robot-Assisted Laparoscopic Partial Cystectomy (RAPC)

- When performed for TCC, recommend only if no CIS, negative margins possible, good resulting bladder capacity, and concomitant LN dissection

Robot-Assisted Laparoscopic Radical Cystectomy (RARC)

- Lymph node yield, OS, CSS, positive margin rate, and complications all similar to open series
- In most series, EBL, LOS, and time to bowel recovery quicker with RARC

Haber GP. BJU Int 2007

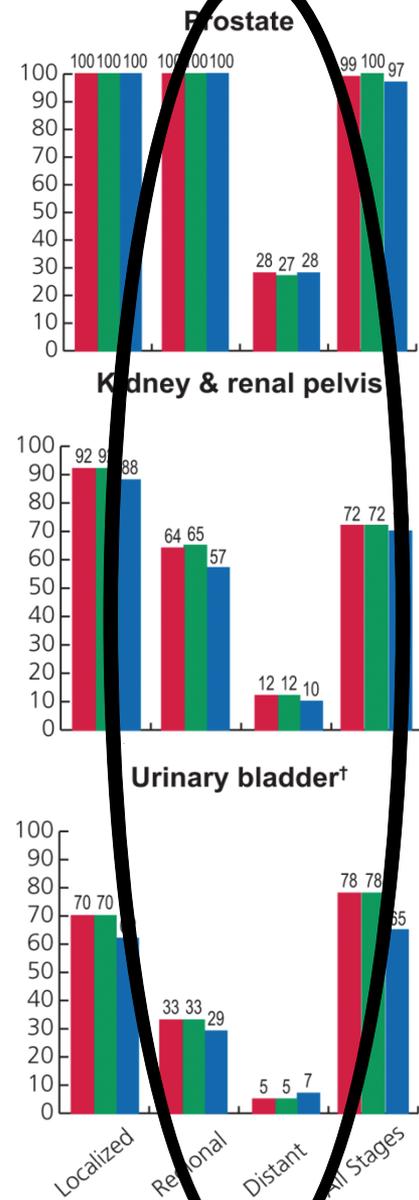
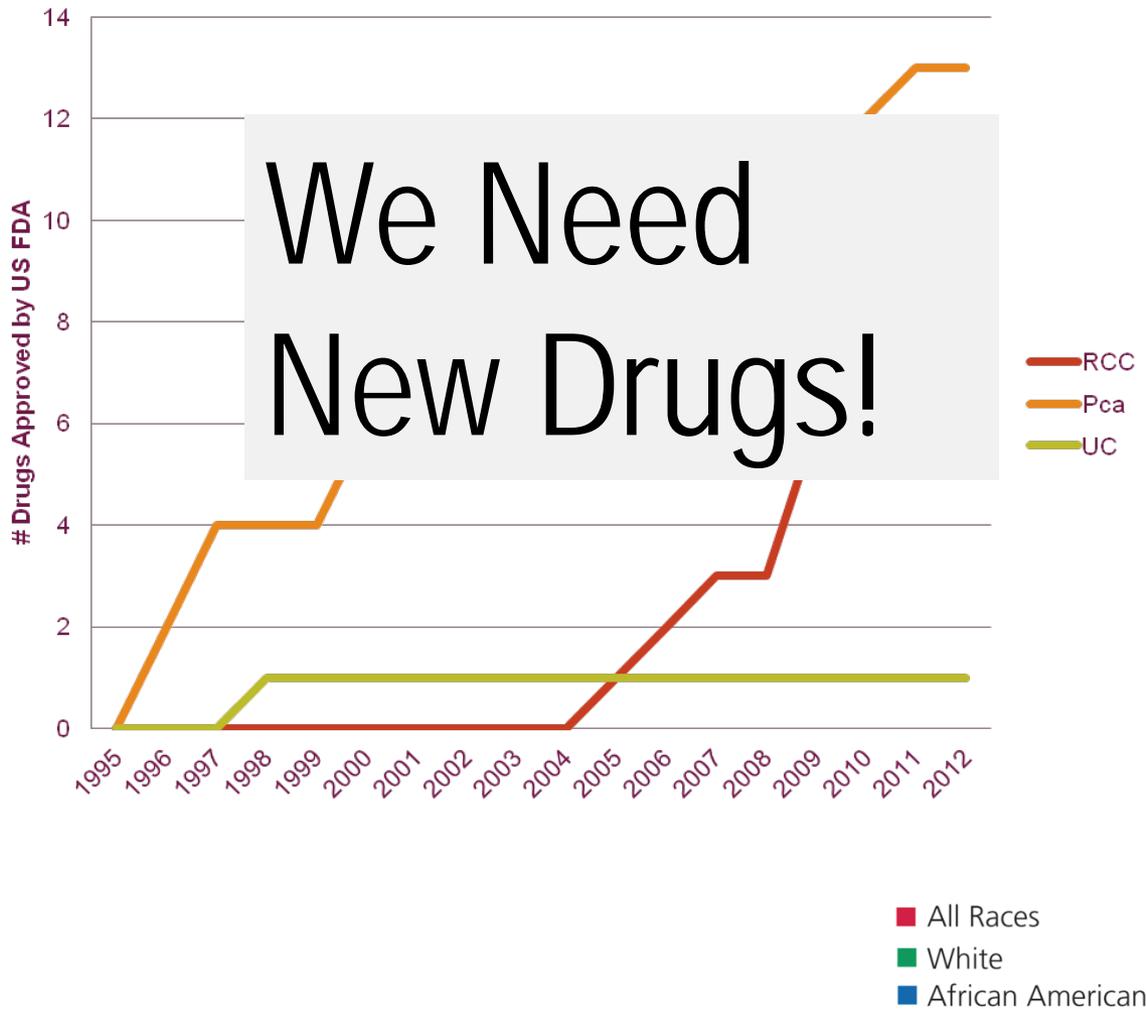
Pruthi RS. Urology 2008

Complications and Morbidity Still a Problem

- However, surgery has a 3% 90-day perioperative mortality rate
- Surgery can result in complication rate of 29-69% - most are grade I, II
- 5-year survival still ~50% for all muscle-invasive tumors at 5 years
 - Improves if final pathology favorable
 - Improvement seen with neoadjuvant chemotherapy

No Therapeutic Advance in Last Two Decades and 5-Year Survival Rates Dismal in Regional/Distant Disease

New FDA-Approved Drugs in GU Cancers



Siegel et al. CA Cancer J Clin 2014.

Galsky et al. Clinical Advances in Hematology & Oncology 2013.

PANVAC PROTOCOL

Rationale and Background

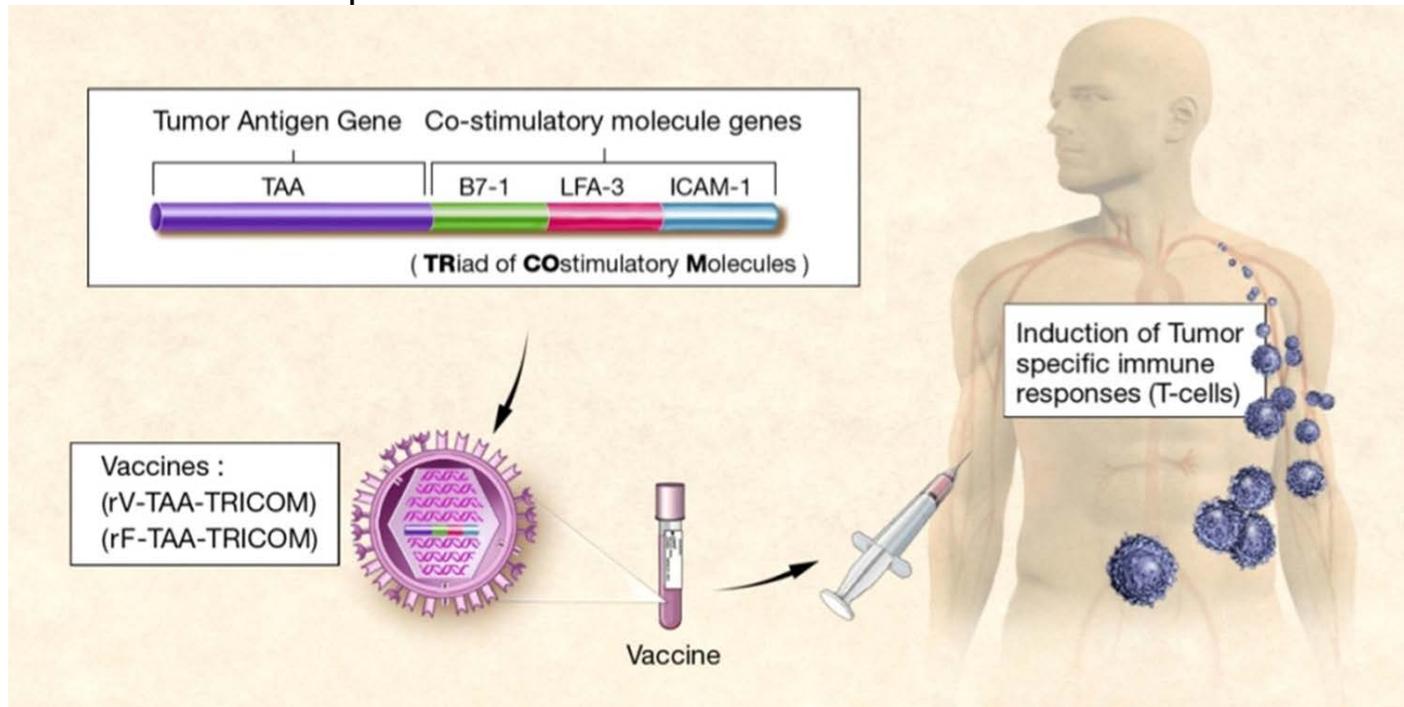
- HG NMIBC (Ta, T1, and/or CIS) is managed by BCG but still with ~35% initial failure rate after induction course in terms of progression and/or recurrence. Although 20-35% of cases that fail an initial course can benefit from a second induction course, patients best served by radical cystectomy if continue to fail to respond.
- Radical cystectomy is potentially morbid and so unmet clinical need for patients that still have NMIBC that fails to respond to BCG.

Rationale and Background

- BCG works by unclear immunologic mechanism:
 - Athymic animals only respond to BCG when T cells administered
 - BCG-induced macrophage cytotoxicity important and promoted by Th1 immune system (TNF- α , IFN- γ , IL-12, IL-18) and inhibited by Th2 immune system (IL-4, IL-10) and Tregs
 - T cell infiltration important as degree of infiltration (CD3, CD4, and CD8) with immune cells is greater in patients with a complete response to BCG

Rationale for Panvac

- Pox viral vector-based vaccine that can induce CD4 and CD8 antigen-specific immune response against MUC-1 and CEA
 - Also contains 3 co-stimulatory molecules
 - Excellent safety record in other tumors
 - Administered subcutaneously
- MUC-1 expression in up to 93% bladder tumors
- CEA expressed in 76% of HG tumors and 59% of T1 bladder tumors
- Postulate that this drug may enhance an immune response in HG tumors that have not responded to BCG



Hypotheses/Objectives

- Primary: PANVAC will augment BCG-induced cytotoxic T lymphocyte response against bladder cancer cells expressing MUC-1 and/or CEA when given with BCG and will result in greater **12 month RFS** than BCG alone in patients who failed to respond to at least 1 previous induction course of BCG
- Secondary: PANVAC+BCG will have greater **PFS** and greater **immune response** than BCG alone

Eligibility

- Adults with histologically confirmed high grade (Ta, T1, and/or CIS) UC of bladder who “failed” at least one induction course of BCG (either progressed and/or recurred)
- Patients who fail >1 induction course of BCG have been offered radical cystectomy and either refuse or are not surgical candidates for cystectomy
- ECOG PS 0-2

Schema

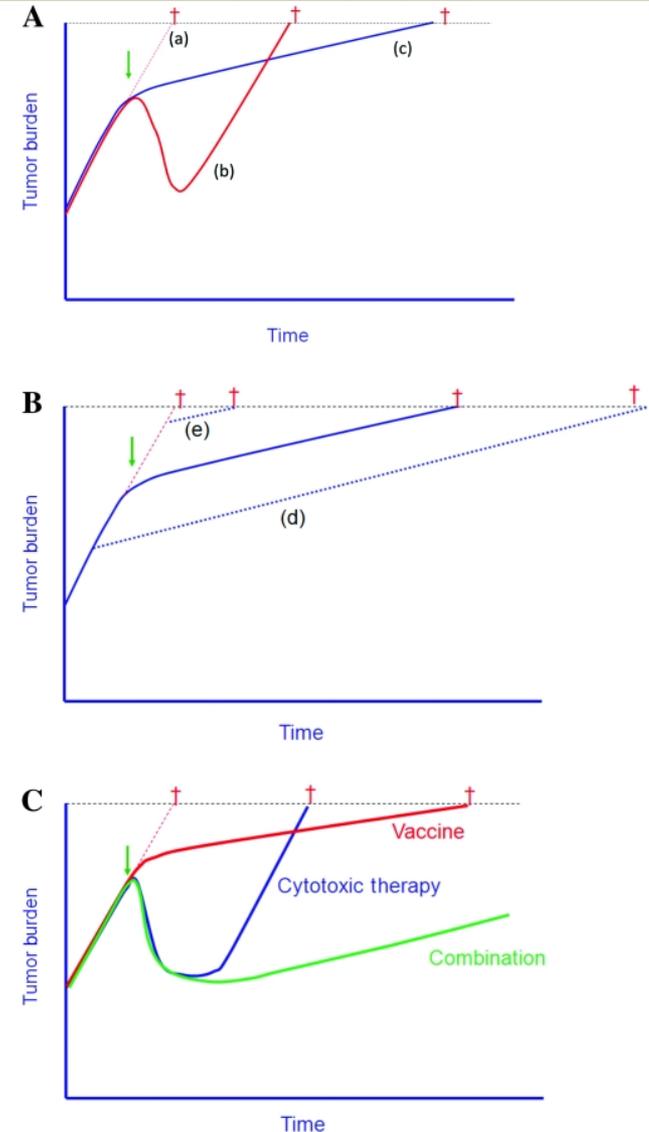


Immune Correlates

- Biopsy (compared day 0 and week 17 tissue) IHC for:
 - CEA and MUC-1
 - CD4, CD8, and Tregs (by DS for Foxp3 and CD4)
 - Myeloid derived suppressor cells (MDSC)
- PBMCs and sera at 4 time points (week 0 (prior to vaccination), week 3 (prior to BCG), week 8 (prior to last BCG), and week 17 (end of treatment):
 - Flow cytometry for 23 markers (e.g. CD4, CD8, Tregs, MDSCs, and NK)
 - In HLA-A2 allele patients, ELISPOT for CD8 T-cell responses for CEA and MUC-1 and cascade antigen Brachyury
 - If sufficient sample available, CD4 specific responses to CEA will be measured
 - Study sera for Ab to CEA
- Urine
 - Check levels of urinary cytokines at week 3 and week 5 to assess cytokine production in response to BCG and PANVAC therapy
- PPD
 - See if any correlation with immunologic response

Benefits of Immunotherapy

- Ability to work in a variety of different cancers
- No cross-resistance to chemotherapy or XRT
- Multiple killing mechanisms
- Durable responses if patient responds
 - Potential for memory and single lifelong administration

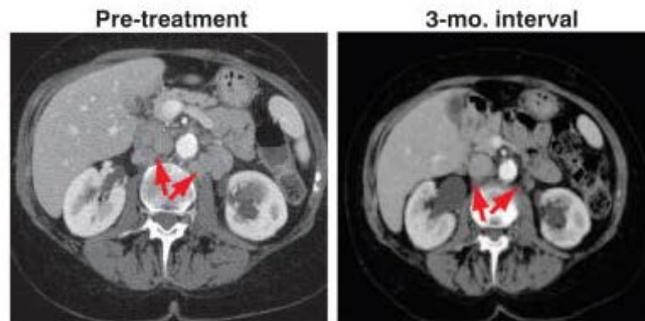


MOLECULAR TARGETED PHOTOIMMUNOTHERAPY (PIT)

Precision Targeted Therapy

PROS

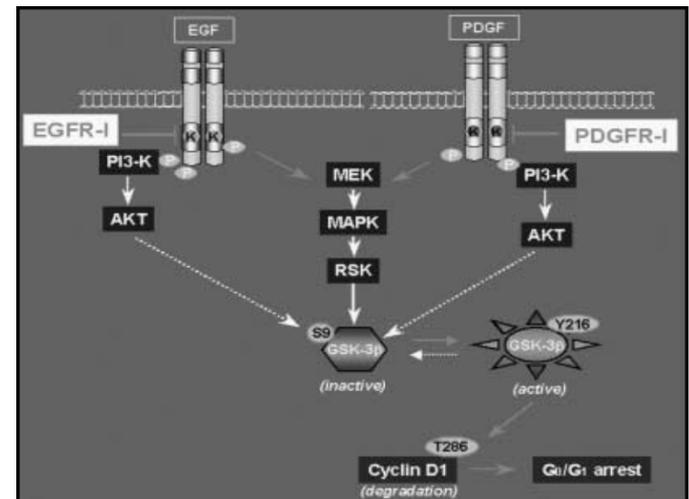
- Durable remission metastatic bladder noted after treatment with everolimus based on fs mutation in TSC1



- NY Times: young hematologist (Dr. Wartman) had ALL with FLT3 on whole-genome sequencing now in remission on sunitinib
- Certain lung cancers have an EGFR mutation making them susceptible to respond to monoclonal antibodies

CONS

- Not every patient can currently undergo whole genome sequencing
- Weak single-agent activity
- Heterogeneity in response to targeted therapy regardless of target expression
- Multiple downstream pathways



Iyer et al. *Science* 2012.
Kassouf et al. *Cancer Res* 2005.

Photodynamic Therapy (PDT) in Bladder Cancer

- PDT utilizes a photosensitizer dye which targets the tumor. It absorbs light from an external source and delivers that energy to produce cytotoxic reactive oxygen species which results in necrosis and apoptosis
- In standard photodynamic therapy, the sensitizer is instilled or injected which can lead to diffuse penetration of bladder tissue or nonspecific binding of dye
 - SIDE EFFECTS (e.g. severe bladder contractures, cutaneous photosensitization)

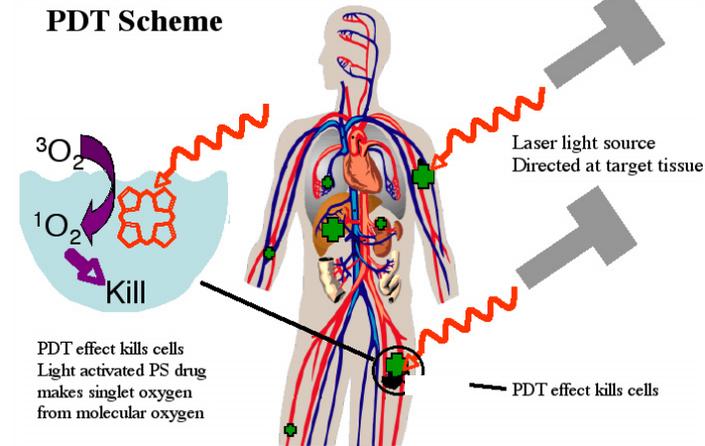
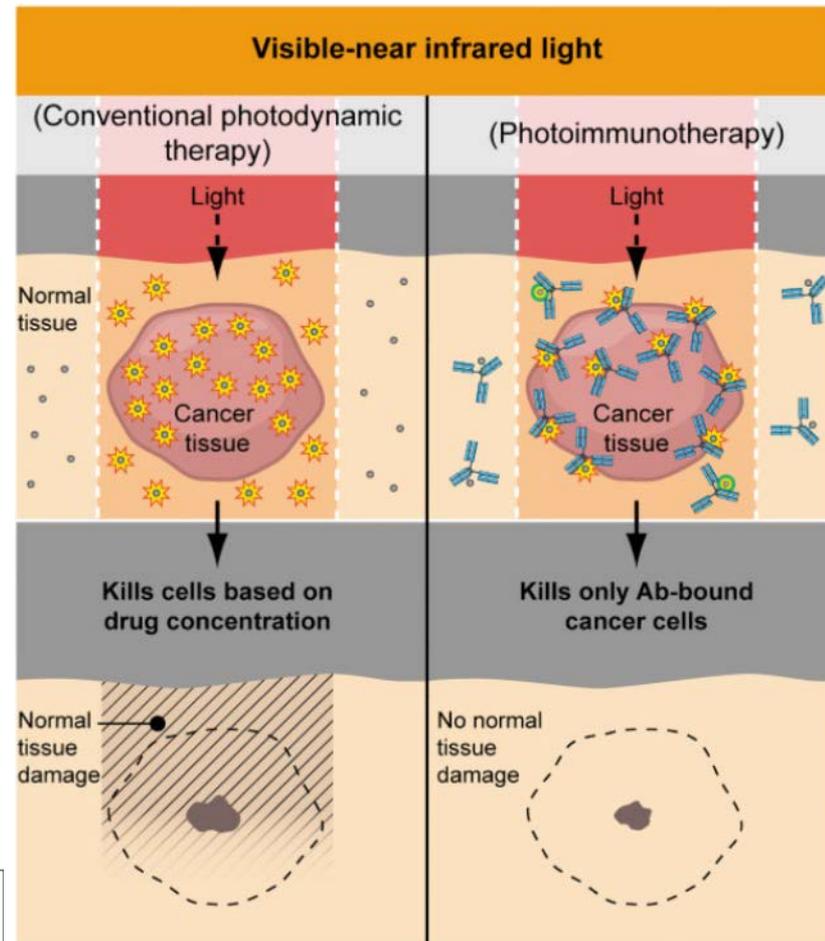


Table 5. Previous clinical studies of PDT for bladder cancer

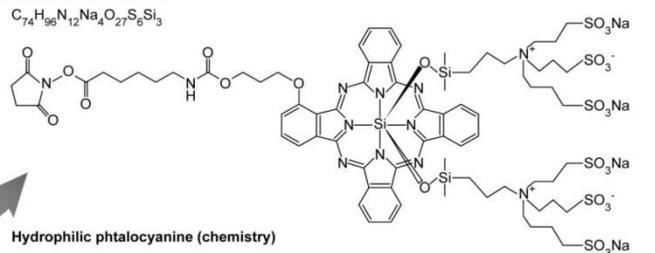
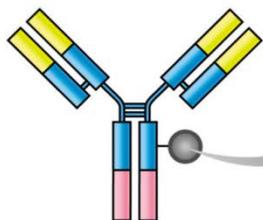
References	No. Pts	Photosensitizer	Light Dose (J/cm ²)	% Response		AEs
				Early	Late	
Nseyo et al ¹⁰	22	Photofrin II	15-20	83.30	30 (complete)	Irritating LUTS, bladder shrinkage
D'Hallewin and Baert ²⁵	18	Photofrin II	75, 100	Not applicable	60	Bladder capacity loss
Uchibayashi et al ²⁶	23	Hematoporphyrin derivative		73.50	22	Skin photosensitivity, transient bladder capacity decrease
Walther et al ²⁷	20	Photofrin II	5.1-25.6	45	20	Asymptomatic reflux, bladder contraction, fibrosis
Nseyo et al ¹⁷	58	Photofrin	10-60	75-84	53	Not applicable
Manyak and Ogan ²⁸	34	Porfimer sodium		56	44	Bladder contracture
Berger et al ²⁹	31	5-ALA	30-50	Not applicable	52 (2 yrs)	Dysuria due to urinary tract infection, hematuria
Waidelich et al ³⁰	11	5-ALA	100	Not applicable	46 (18 mos)	Transient frequency, urgency
Lee et al ¹¹	5	Fotolon	10 (intravenous), 24 (intravesical)	80 (6 mos)	60	Vesicoenteric fistula
Bader et al ²⁰	17	HAL	25 (PDT 1), 50 (PDT 2), 100 (PDT 3)	52.9 (6 mos)	11.8 (21 mos)	Irritative bladder symptoms, infection, gross hematuria
Present series	34	Radachlorin	15	90.9 (1 yr)	64.4 (2 yrs)	Irritative bladder symptoms, infection, hematuria

Molecular Targeted Photoimmunotherapy

- Photosensitizer: phthalocyanine dye, IR700, that uses near infrared light (NIR) conjugated to monoclonal antibody (mAb)
- Induces cell death after irradiating mAb-IR700-bound target cells with exposure to NIR
- Non-toxic except at thermal doses
- Hydrophilic dye will not associate with CM

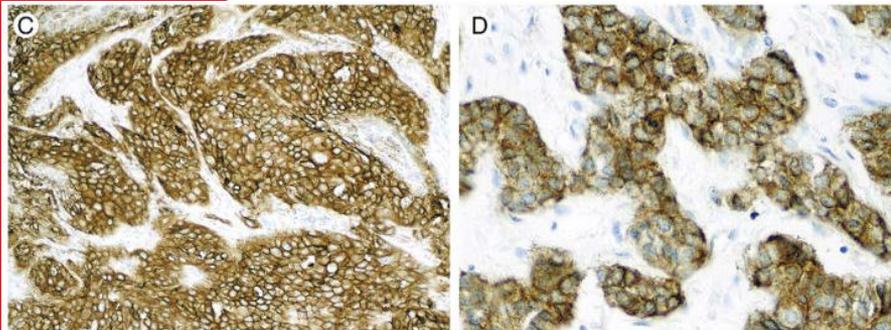
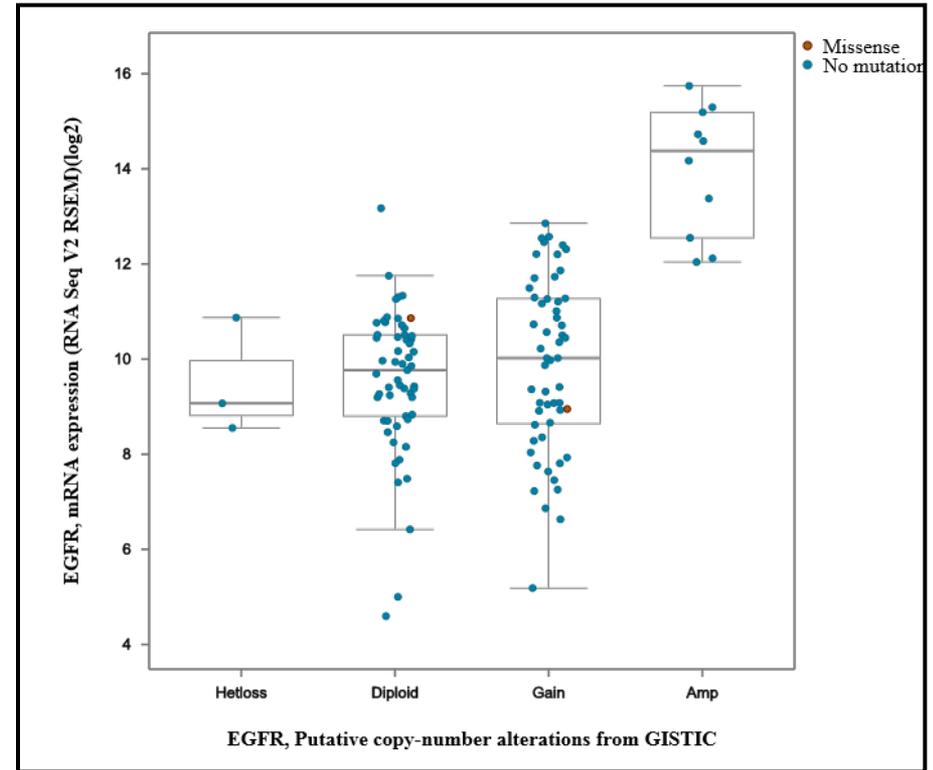


Humanized monoclonal antibody
(biology/medicine)



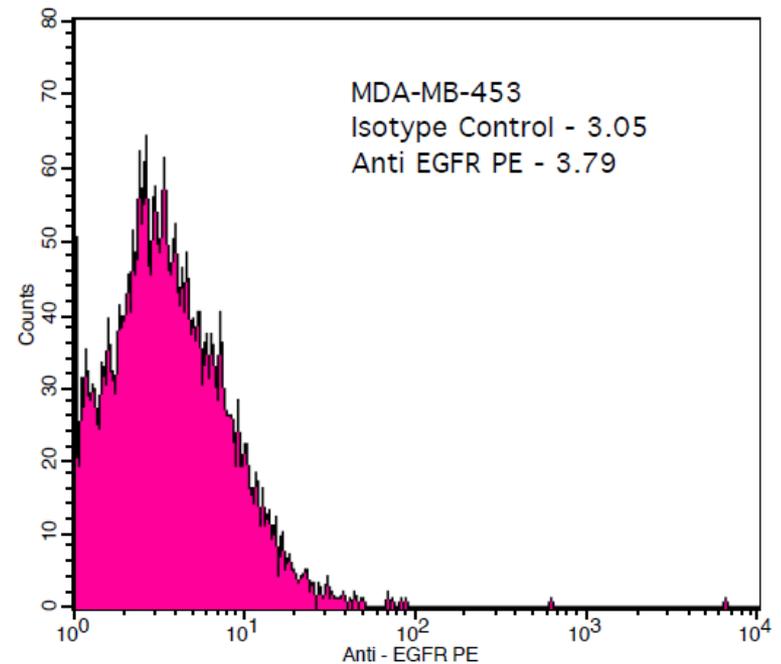
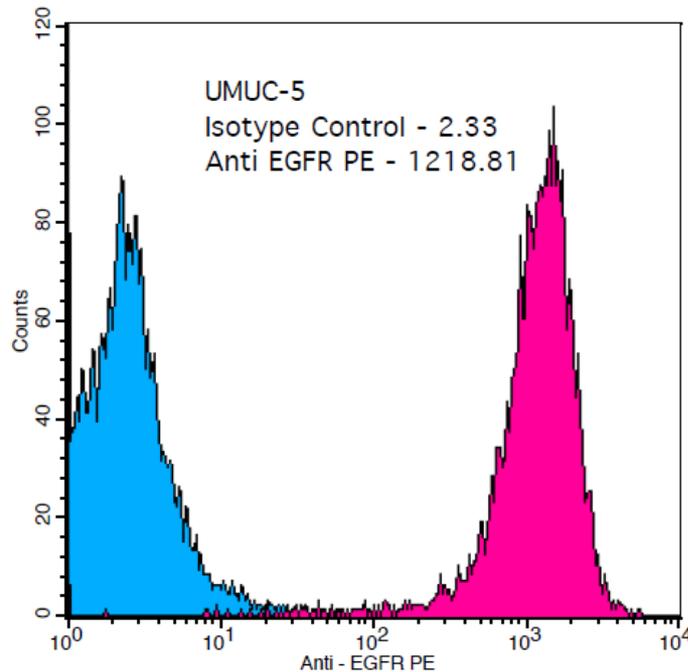
Our Target: EGFR (epidermal growth factor receptor)

- EGFR amplified in UC
- Overexpression of EGFR is an independent predictor of decreased survival and stage progression in bladder cancer

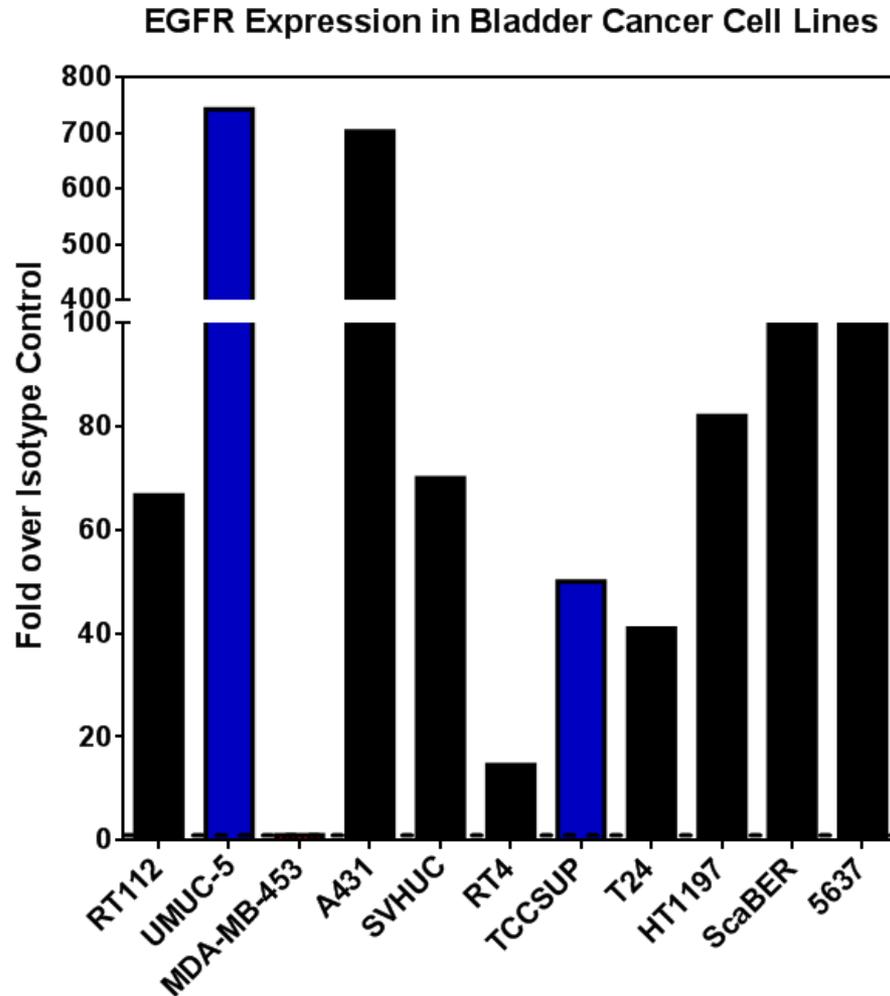


EGFR expressed in Bladder Cancer Cell Lines

Method: Expression in various Bladder Cancer cell lines analyzed using flow cytometry. Anti hEGFR - PE (Abcam) was used for these experiments. Rat IgG2a, kappa Mab-PE (Abcam) was used as a Isotype control.

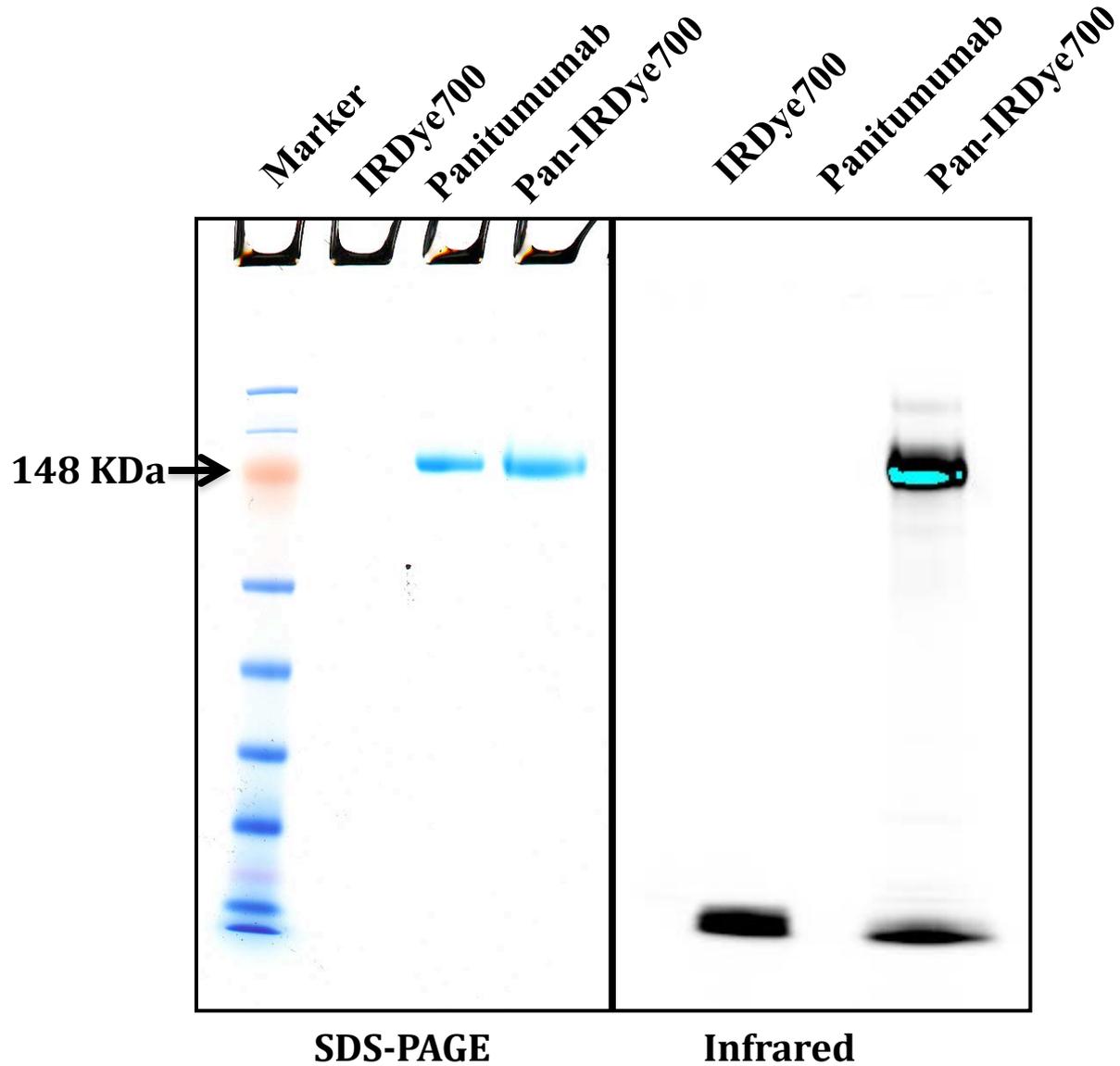


EGFR expressed in Bladder Cancer Cell Lines

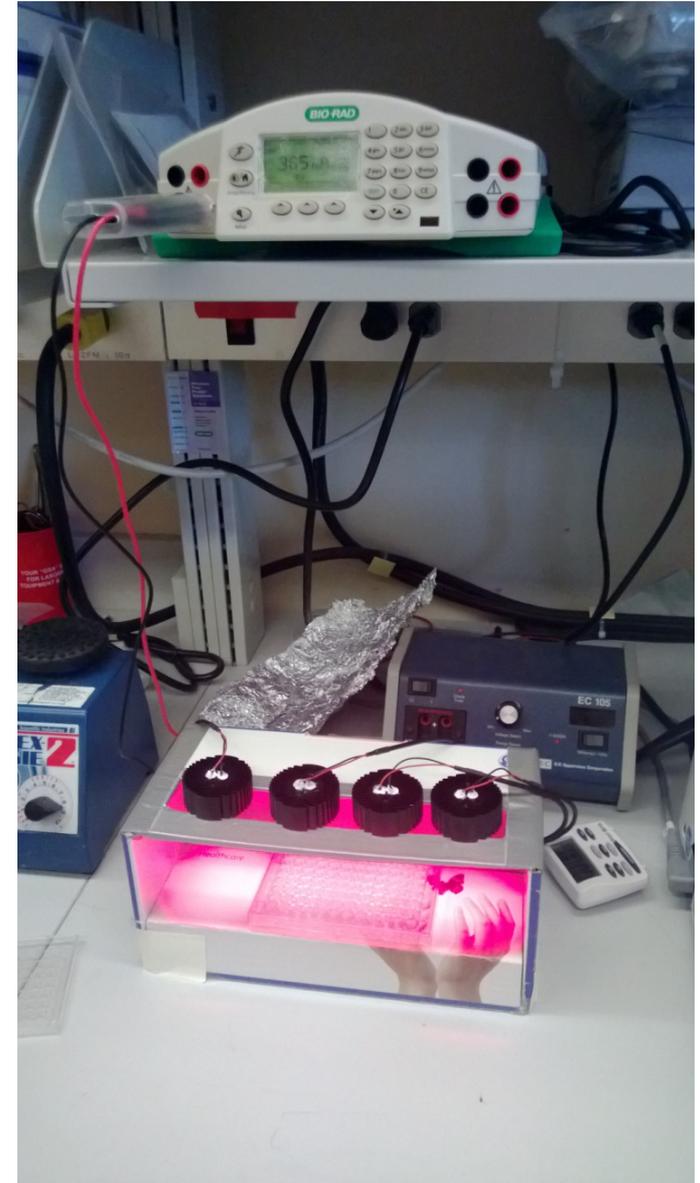
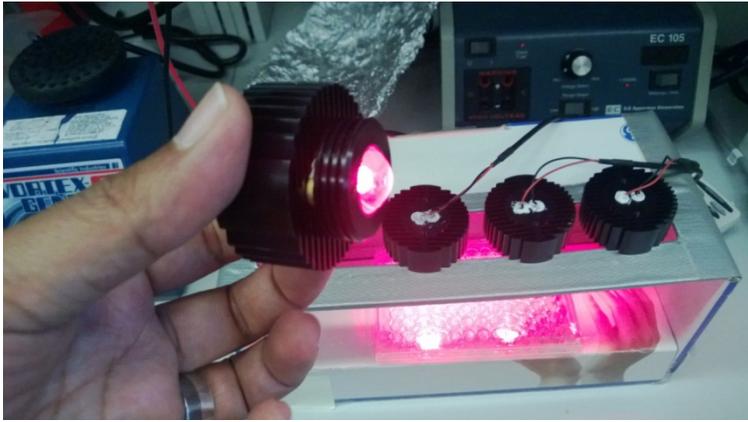
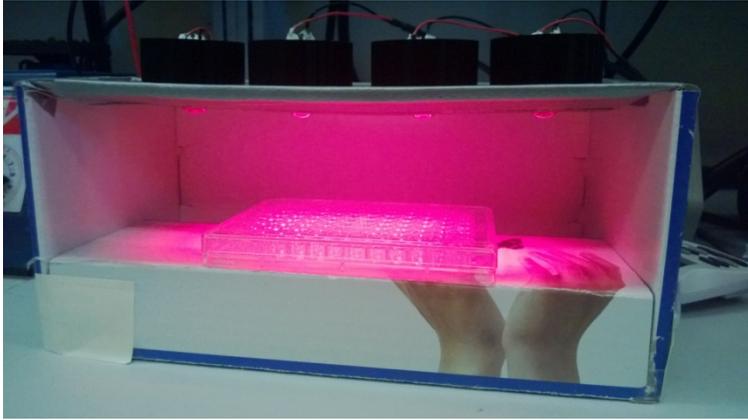


- Two cell lines UMUC-5 (high expression of EGFR) and TCCSUP (low-moderate expression of EGFR) are used for further analysis. Breast cancer cell line MDA-MB-453 or Balb-3T3 (no expression of EGFR) are used as a negative control in certain assays.

Gel Electrophoresis and Infrared Imaging of Panitumumab and Conjugated Panitumumab

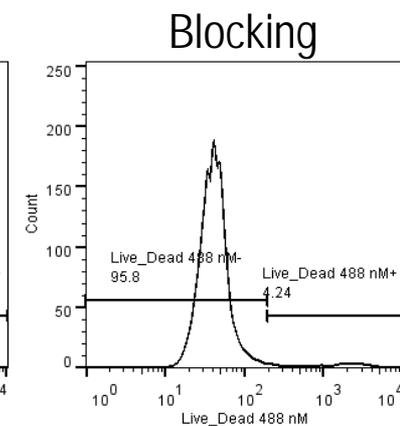
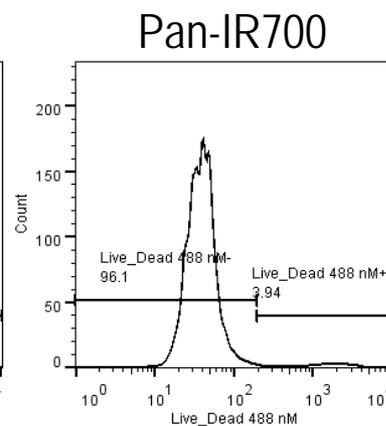
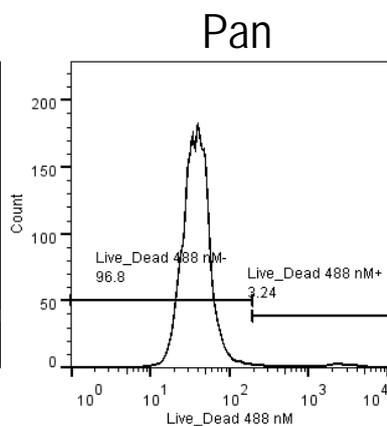
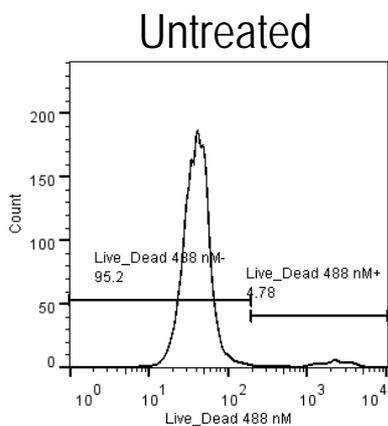


“Home-grown” Apparatus

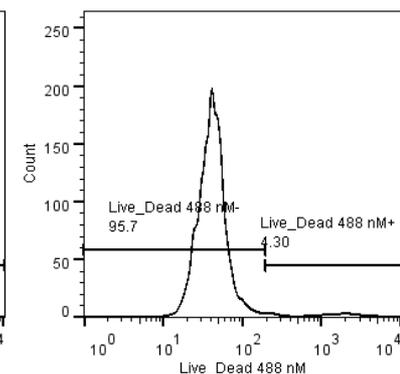
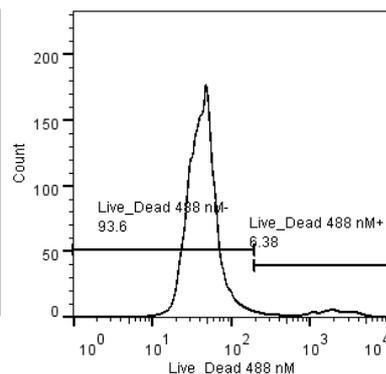
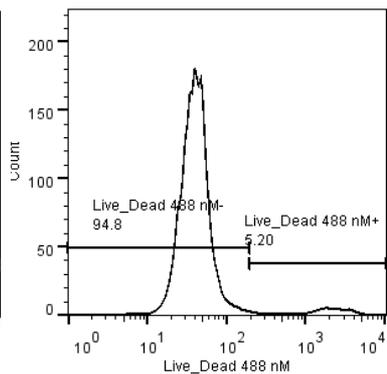
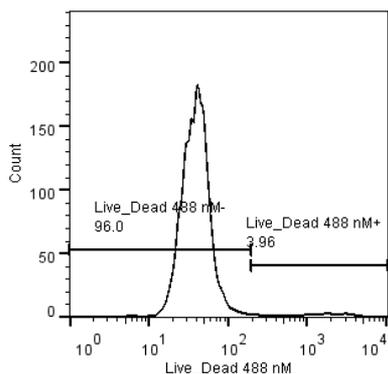


Balb 3T3 – EGFR negative control

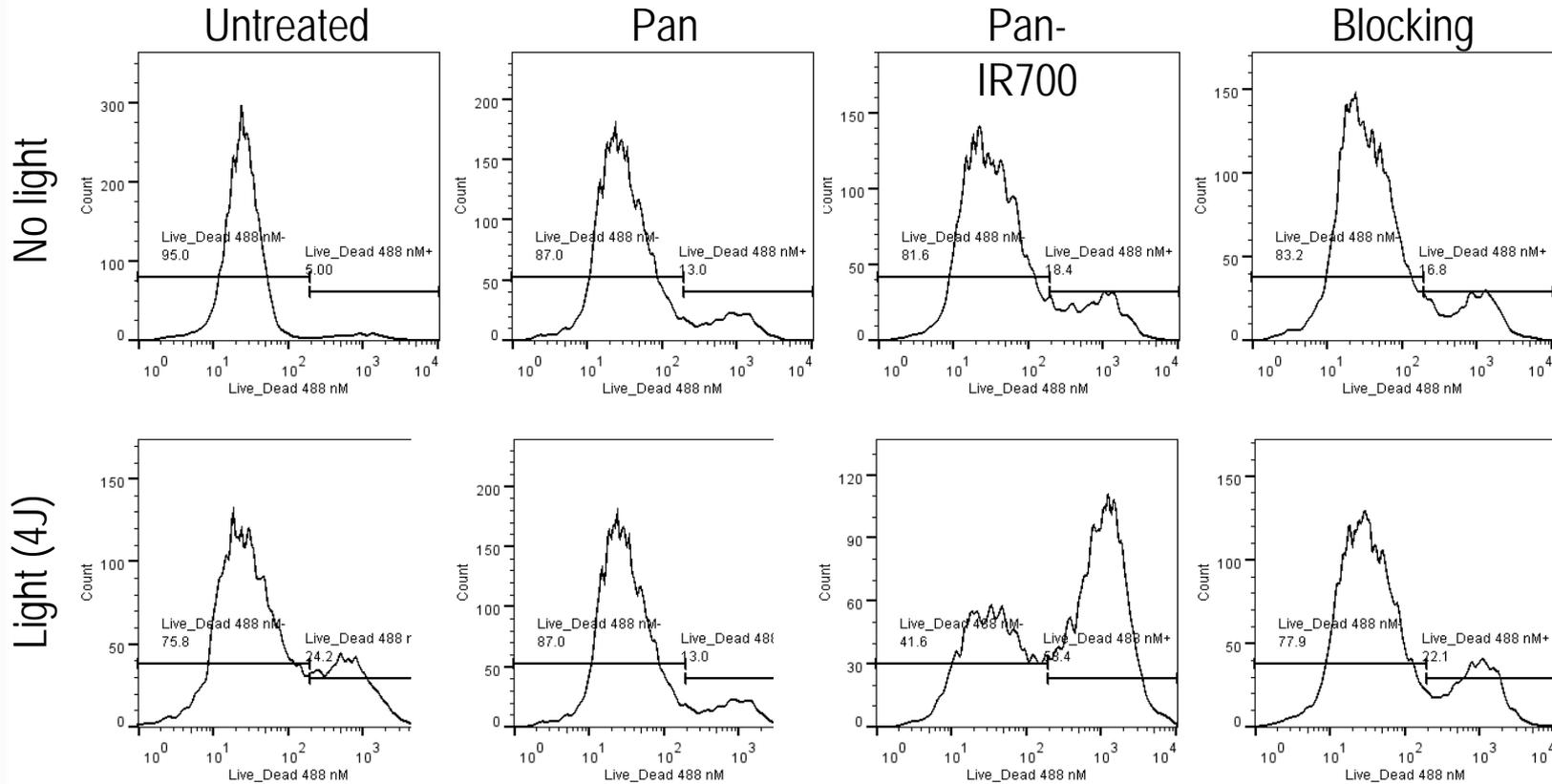
No light



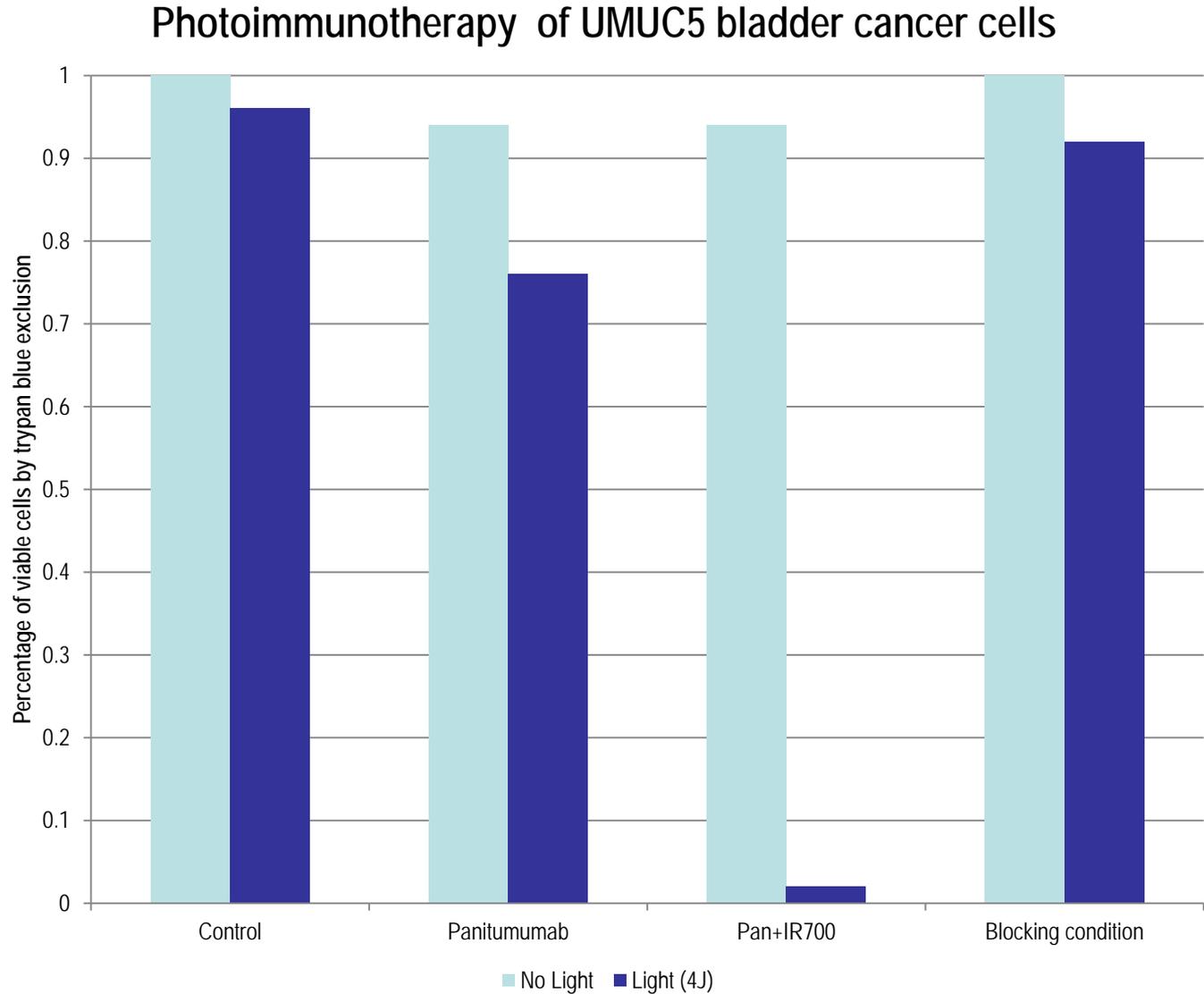
Light (4J)



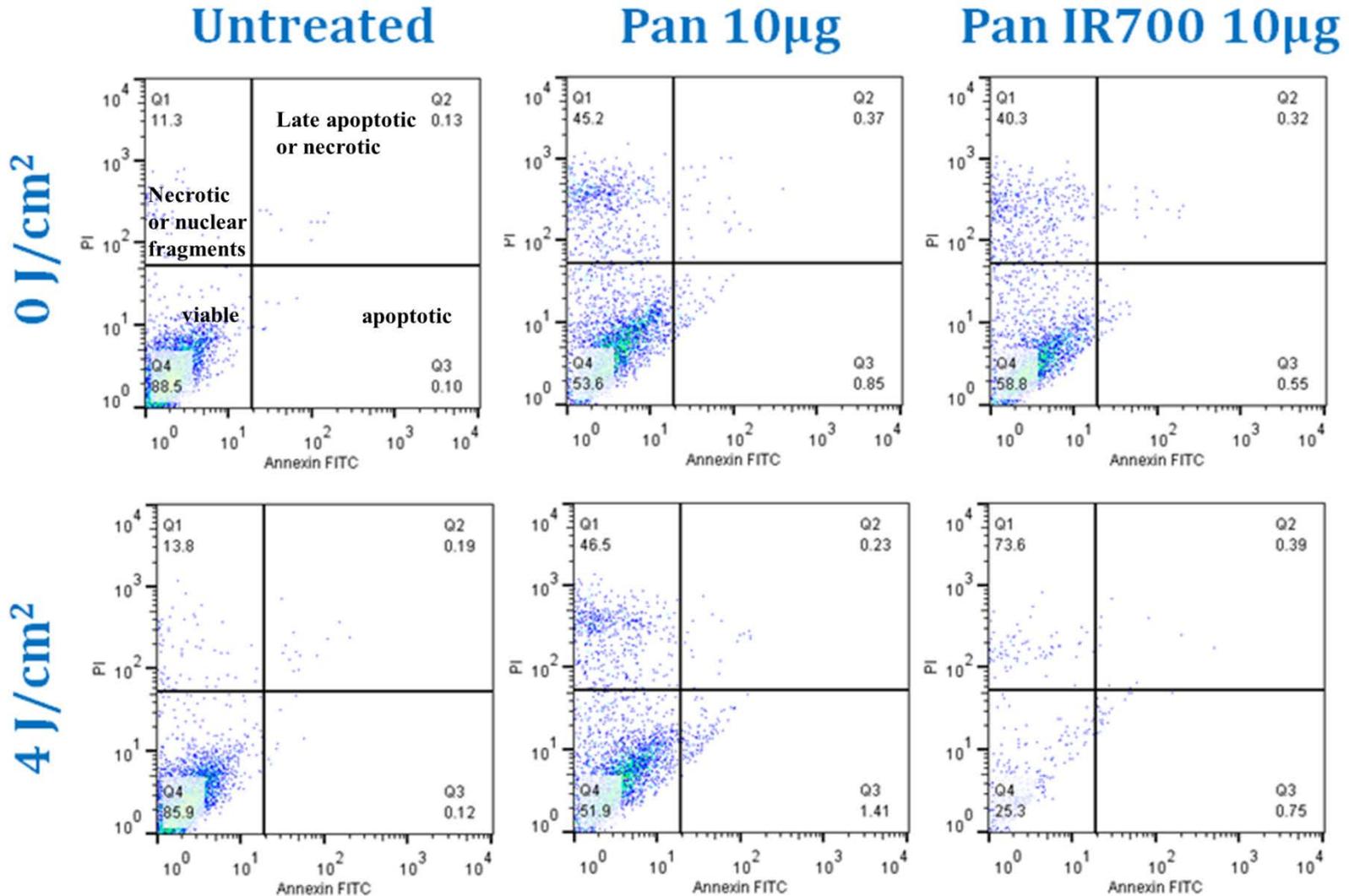
UMUC5 – EGFR ++ bladder cancer cell line



Trypan Blue Exclusion Assay



Necrosis is Potential Mechanism of Death: Annexin-V/PI Staining 60 min After PIT



Necrosis is Potential Mechanism of Death: Annexin-V/PI Staining 15 min After PIT

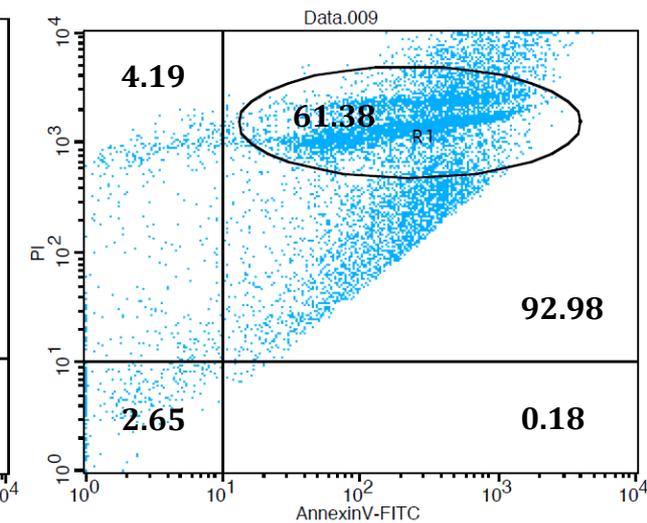
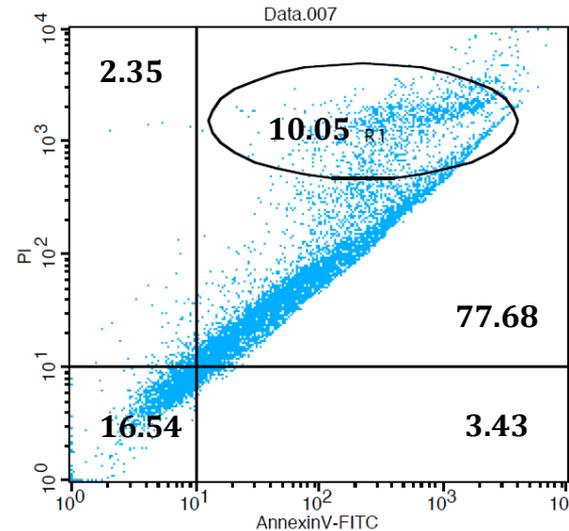
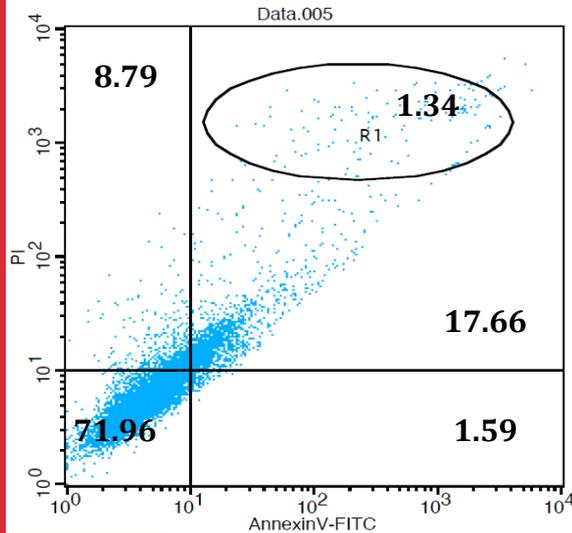
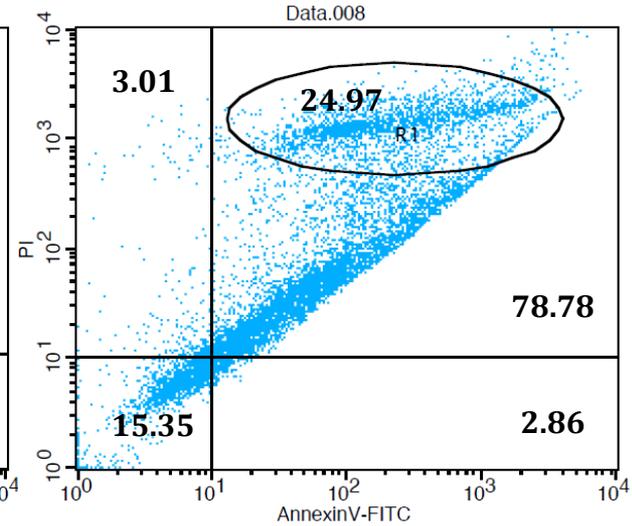
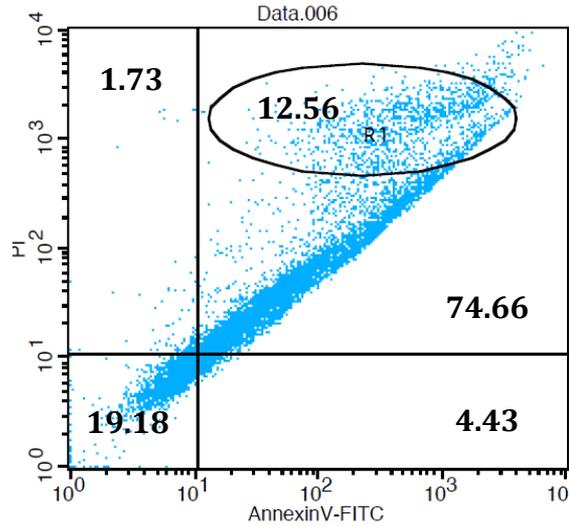
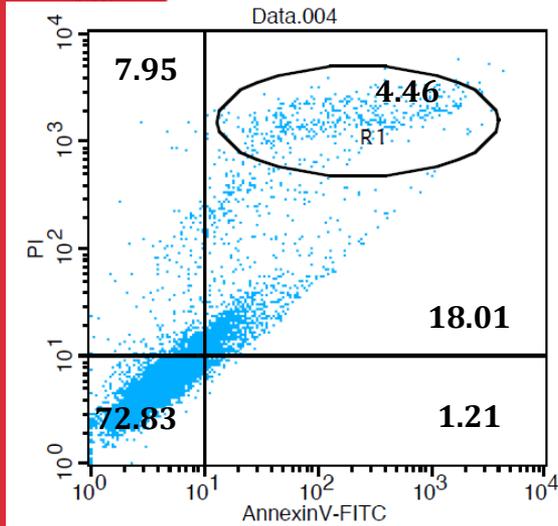
Untreated

Pan 10 μ g

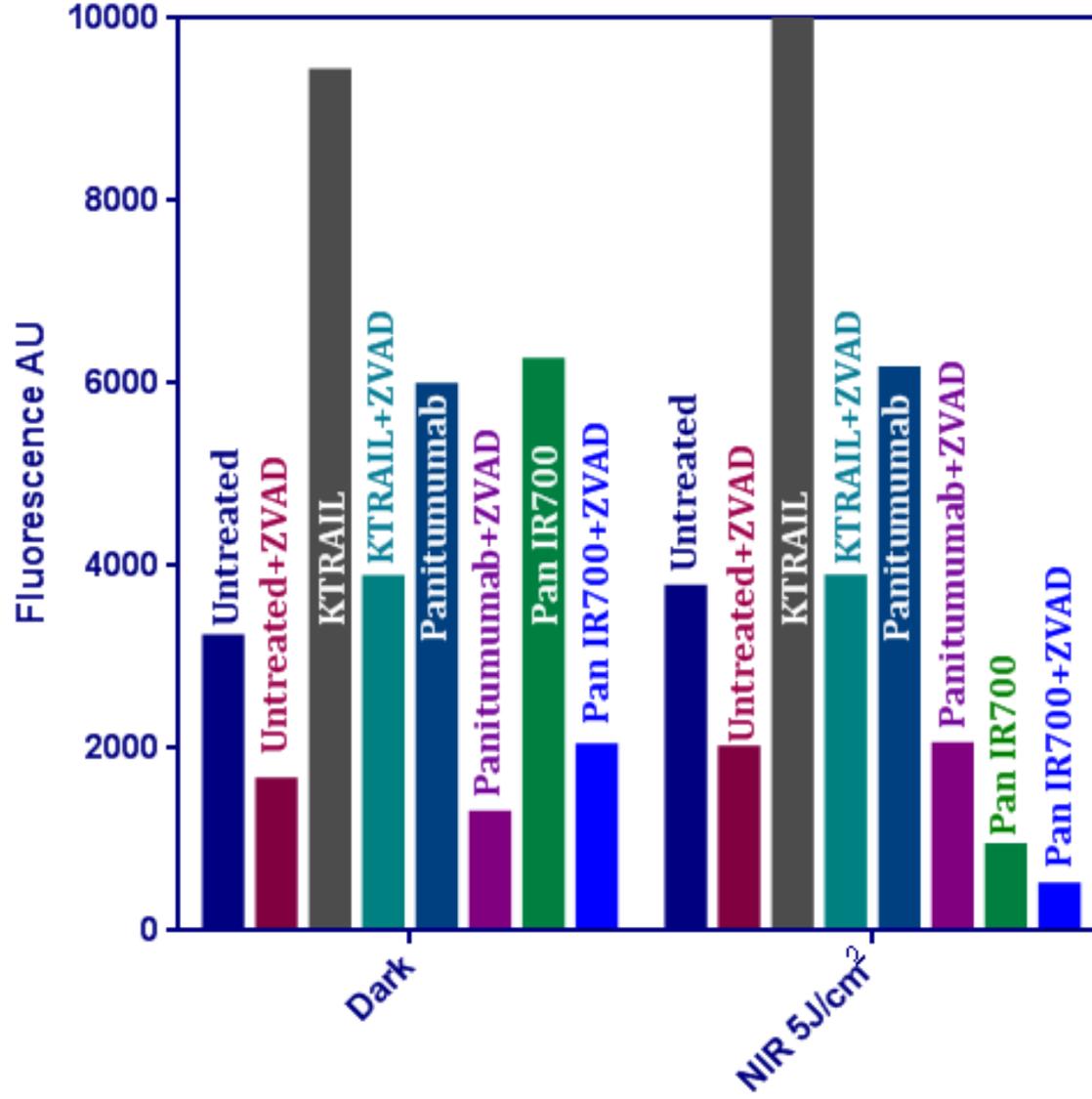
Pan IR700 10 μ g

0 J/cm²

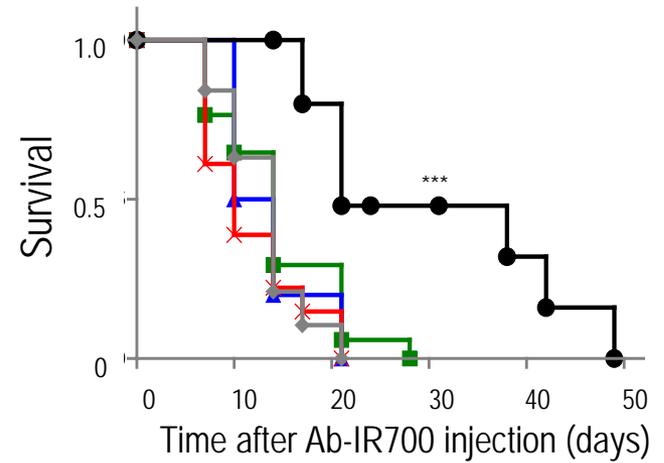
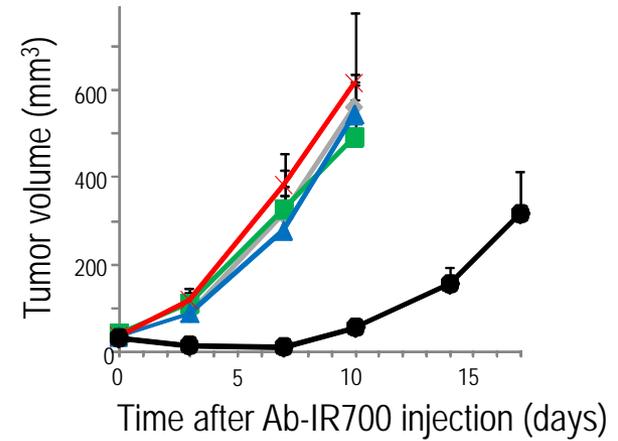
4 J/cm²



Caspase Assay: 15 min after NIR Exposure



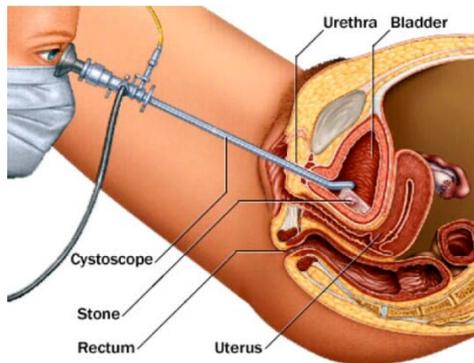
In vivo PIT effects



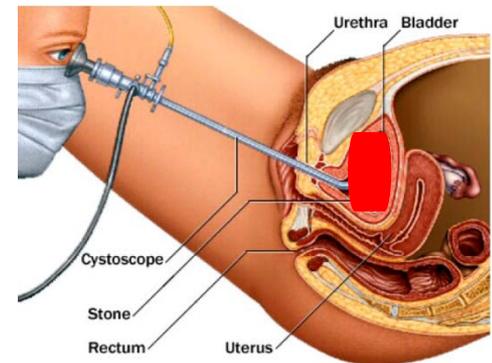
- No treatment
- Pan 300 µg iv, no PIT
- ▲— Pan-IR700 300 µg iv, no PIT
- ×— No Mab, PIT 30 J/cm²
- Pan-IR700 300 µg iv, PIT 30 J/cm²

Summary of PIT and Future Directions

- PIT platform works with EGFR based conjugate in EGFR expressing cell line
- In lines with less EGFR expression, increased energy can increase efficacy
- Mechanism is likely necrosis
- Current: Orthotopic bladder cancer model with UMUC-5
- Future: Conjugate other monoclonal antibodies targeting other receptors present in bladder cancer (FGFR3, MET, HER2, AXL) and Clinical Trial

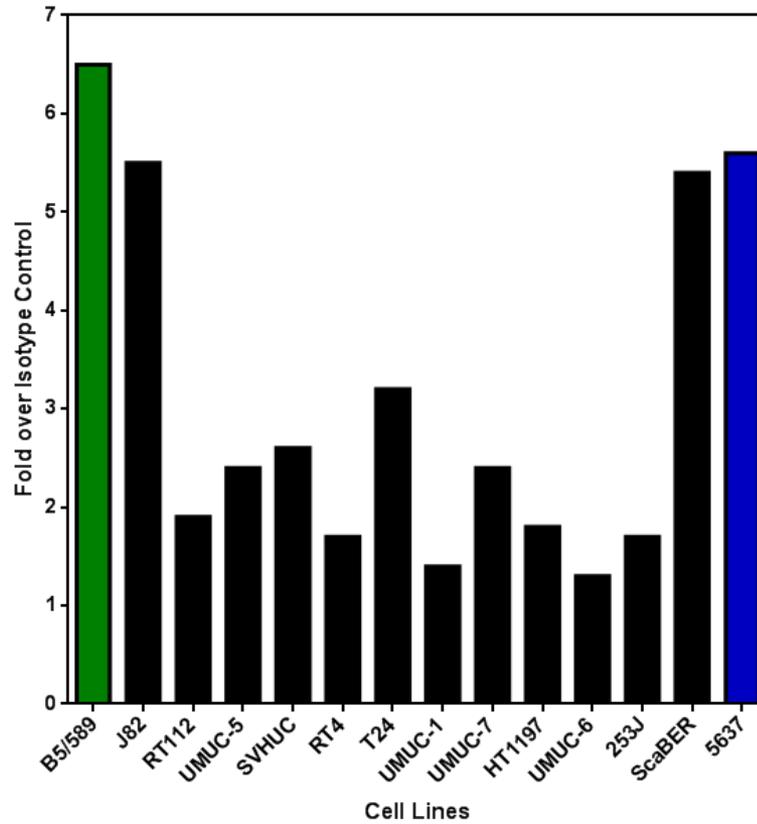


→ Characterize Tumor Surface Receptor Expression and Conjugate Target-specific mAB-IR-700 and Instill in Bladder After Resection of Tumor

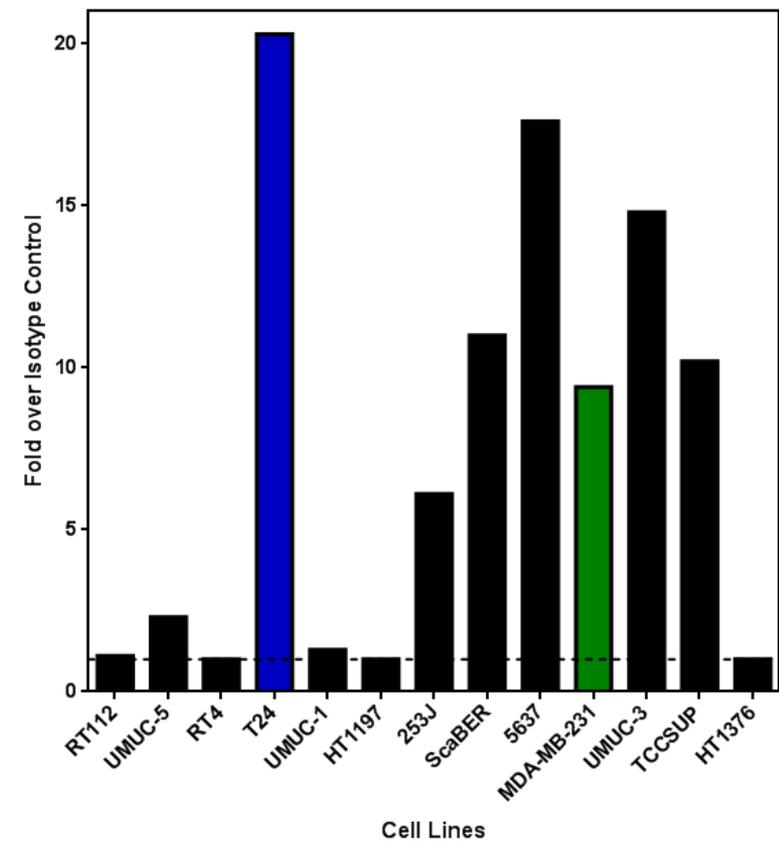


MET and AXL Expression in Cell Lines

Met Expression in Bladder Cancer Cell Lines

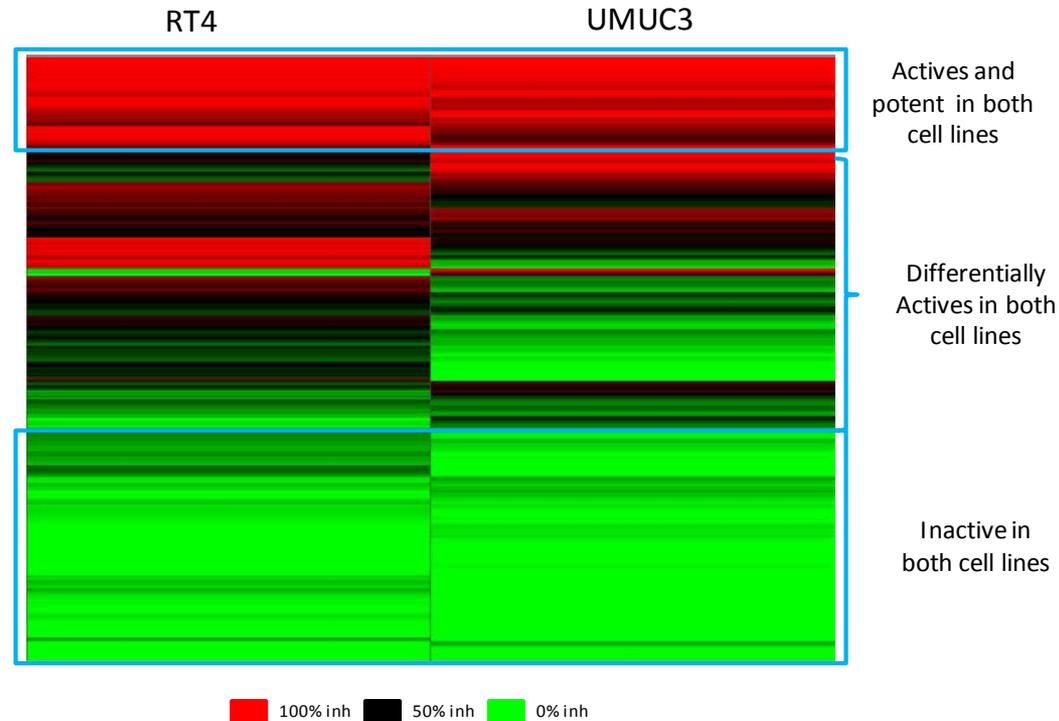


AXL Expression in Bladder Cancer Cell Lines



Other Projects

- High throughput screening of bladder cancer cell lines against 1900+ compounds



- Y-specific gene, *SMCY*, may be associated with male tumors and is associated with histone de-methylation
- IL-12/chitosan intravesical therapy

Conclusions

- Bladder cancer is common and has poor survival rates for non-localized disease
- Therapy requires surveillance, intravesical therapy, TURBT, surgery, radiation, chemotherapy
- Even though localized disease has good prognosis, recurrence is a problem making bladder cancer most expensive cancer
- Surgery evolving but still high rate of complications
- No new FDA-approved drugs in >20 years!

Conclusions

- PANVAC trial is currently open
 - 4 patients enrolled
 - Waiting for BCG to enroll more patients!
 - Greatly indebted to Day Hospital RNs for getting this trial off the ground!
- Molecular-targeted PIT has potential for effective treatment for non-localized disease and is translatable! (VPL better?)
- Other new potential intravesical agents may be identified from our screening project

Evolving Bladder Cancer Program

- Establishing a multi-disciplinary team that will see complex patients together:
 - Deborah Citrin, MD – Radiation Oncology
 - Andrea Apolo, MD – Medical Oncology
- Building a dream team:
 - Patient care coordinator
 - Nurse practitioner
 - Research nurse
 - Urologic oncology fellow
 - Data manager
 - Tissue procurement personnel

Acknowledgments

Bladder Cancer group

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 Martha Ninos
 Geri Hawks
 Donna Drake
 Robert Worrell
 Cathy Vocke

UOB Family

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Don Bottaro
Laura Schmidt
 Mehdi Mollapour
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 Carole Sourbier
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Peter Pinto
Ram Srinivasan

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 Karlena Lara-Otero

Other Collaborators

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James Gulley
Brad Wood
Jane Trepel
Craig Thomas

Molecular Imaging Group

Peter Choyke
Hisitaka Kobayashi
Kohei Sano

Bladder Multi-Disciplinary Team

Andrea Apolo
Deborah Citrin



Marston Linehan

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 Georgia Shaw

