THE UNIVERSITY OF CHICAGO
MEDICINE & BIOLOGICAL SCIENCES

Bacillus Calmette-Guérin Immunotherapy for Bladder Cancer: Overview of an "Off-Target" Effect of BCG Immunotherapy And New Approaches

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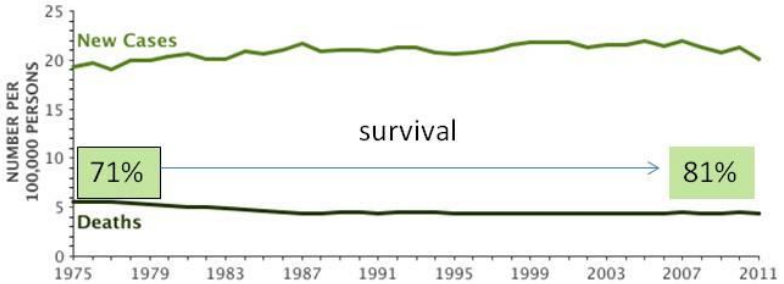
Bladder Cancer Stats - 2014

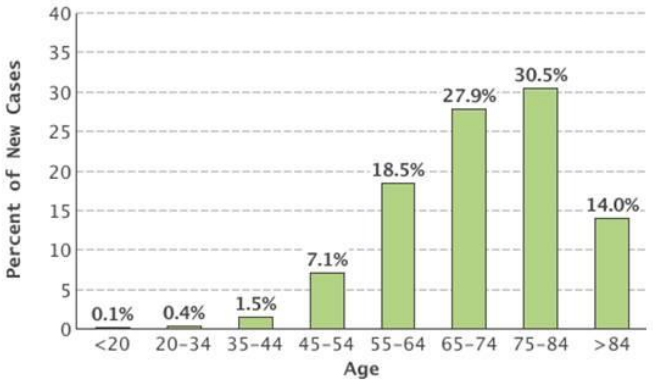
New cases in 2014
74,690
Deaths in 2014
15,580

Median age at diagnosis 73
Death 78

571,518 men and women are living with bladder cancer

Annual cost $4B

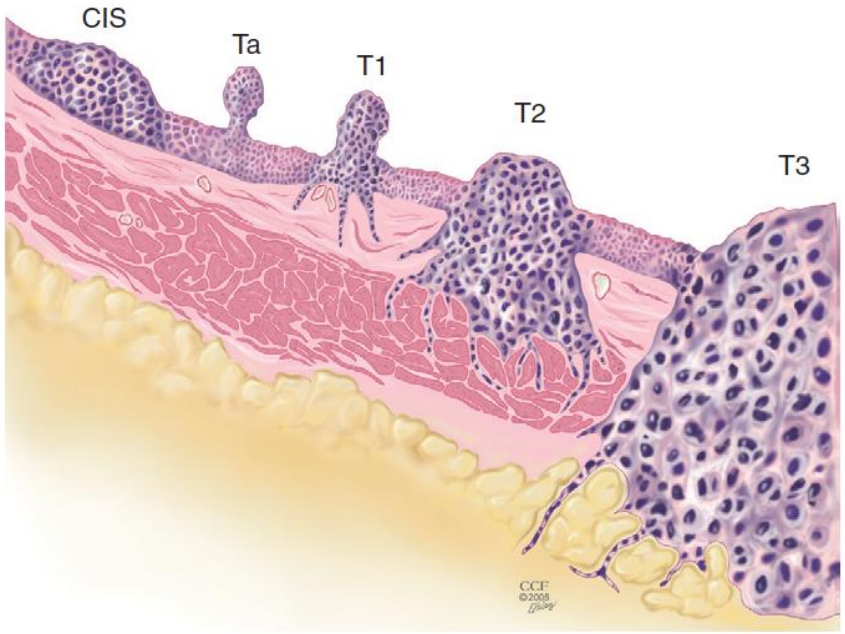




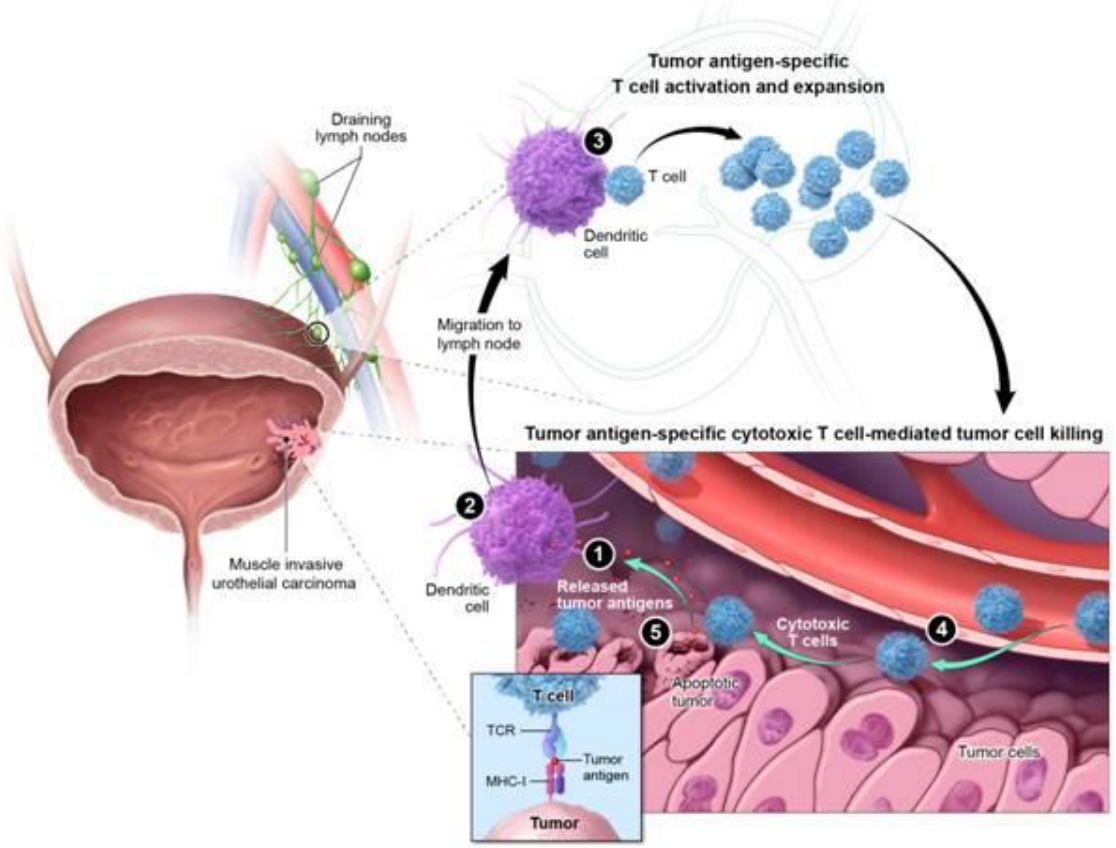
Presented By Harry Herr at 2015 Genitourinary Cancers Symposium

BCG in Practice: Bladder Cancer Staging

- Stage
  - Based on depth of penetration
  - Invasive: T2 or higher
  - Non-muscle invasive:
    - CIS
    - Ta
    - Ta
- Grade:
  - Based on microscopic features and architecture of cells
- 70% of bladder tumors are non-muscle invasive at presentation



Urothelial carcinoma-specific antitumor T-cell immunity cycle



Tumors maintain an immunosuppressive via PD-L1/PD-1 binding
Inhibiting:
T-cell migration, proliferation and secretion of cytotoxic mediators

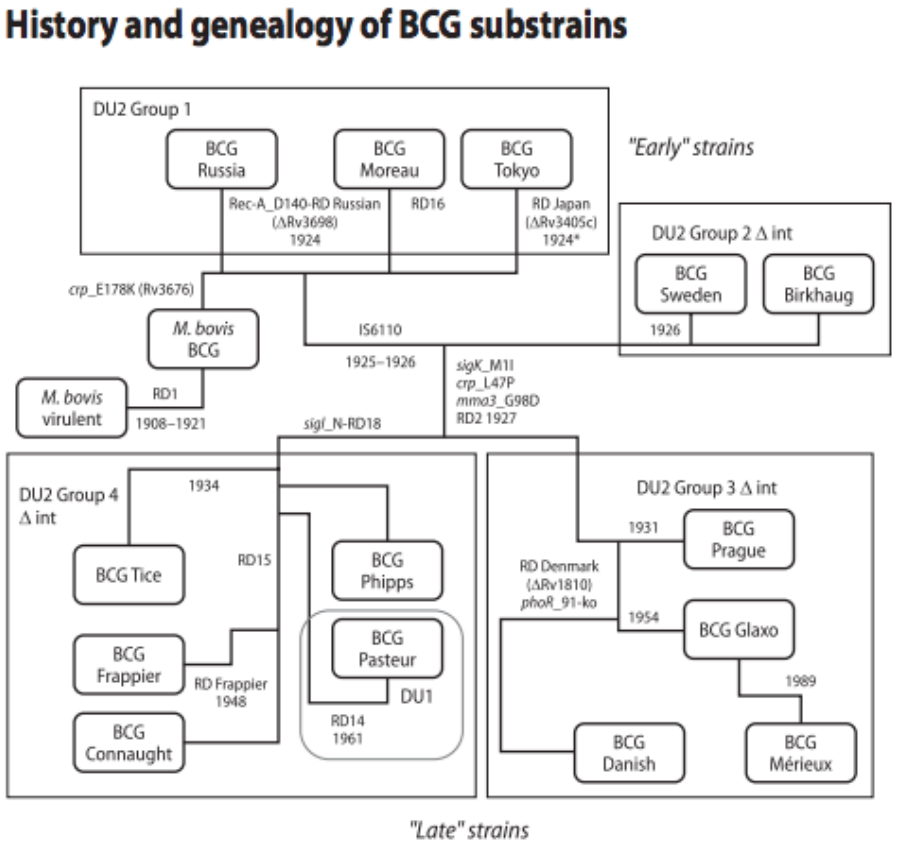
Kim JW, Tomita Y, Trepel J and Apolo AB *Current Opinion in Oncology* In Press

History of BCG: Initial Development

- Nonvirulent but genetically stable Mycobacterium bovis developed in 1921
  - Initially isolated from udder of infected cow
  - Developed by Albert Calmette (bacteriologist)and Camille Guerin(veterinarian) at the Pasteur Institute in Lille, France
  - Named Bacilus Calmette Guerin (BCG)

History of BCG: Substrains

- Several substrains developed since original strain in 1921
- Named after site of origin and/or manufacturer
- Each strain has phenotypic variation
  - ?? evidence to suggest superiority of one strain over another



WHO Technical Report Series No. 979, 2013
Annex 3: Recommendations to assure the quality, safety and efficacy of BCG vaccines.

Intravesical BCG strains

- Connaught, Tice, A. Frappier, Pasteur, Tokyo, and RIVM strains all arise from a common initial strain
- Clinical differences observed between strains
- Two approved strains in the US circled in blue

Table 2 | Comparison of BCG strains used for bladder cancer treatment

Strain	*n**	Mean CRR % (range)*	Commercial product	Weight (mg)	Recommended dose (cfu)‡	Secretion of lipid virulence factors?[61]	Secretion of MPB64/MPB70 and MPB83[76]
Moscow§	103	90.5	SII-ONCO-BCG® (Serum Institute, India)	120	$3–57\times10^8$	Yes	Present/High
Moreau RdJ	100	90	ImmunoBCG (FAP, Brazil)	80	0.04×10^8	No	Present/High
Connaught	450	79 (70–92)	Immunocyst® (Sanofi-Aventis, France)	81	$1.8–15.9\times10^8$	NT	NT
Tokyo	111	77 (63–84)	Tokyo 172 (QSMI, Thailand)	80	$0.4–0.5\times10^8$	No	Present/High
Pasteur	230	74 (40–80)	None	NA	NA	Yes	Absent/Present
Tice	277	71 (56–82)	OncoTice® (Merck, USA)	12.5	$2–8\times10^8$	Yes	Absent/Present
Glaxo	180	65 (53–88)	None	NA	NA	No	Absent/Present
A. Frappier	145	60 (39–100)	None	NA	NA	Yes	Absent/Present
S. African	13	69	None	NA	NA	NT	NT
Copenhagen	42	67	None	NA	NA	Yes	Absent/Present
Romanian	33	64	None	NA	NA	NT	NT
RIVM/1	15	60	BCG-Medac® (Medac, Germany)	80	$2–30\times10^8$	NT	NT

*Data from two studies.[4,28] ‡Based on summary of product characteristics for individual commercial products and study.[72] §Data from the manufacturer's summary. Abbreviations: CRR, complete response rate; NA, not applicable; NT, not tested.

Gan, C. *et al.* (2013) BCG immunotherapy for bladder cancer—the effects of substrain differences *Nat. Rev. Urol.*
MPB64,MPB70,MPB83 = T-cell stimulating proteins
Also, Rentsch, CA. et al. (2014) Bacillus Calmette-Guerin Strain Differences Have an Impact on Clinical Outcome in Bladder Cancer." *Eur. Urol.* (2014) 66, 677-688.

History of BCG: Vaccine for TB

- Main use for BCG → Vaccine for Tb
- 1921-1924 vaccine used to vaccinate 274 Parisian children against TB.
- WHO Expanded Programme on Immunization in 1974
  - BCG use achieved global coverage rates > 80% in countries endemic for TB
  - ~100 million children receive BCG vaccine yearly

World Health Organization

WHO Weekly Epidemiological Record, No 4, 23 January 2004.

History of BCG: Cancer

- Pearl et al, Am J Hygiene 1929:
  - First link between Tb and cancer
  - Age matched autopsy study→ noted lower frequency of cancer among patients with Tb.
- Old et al Nature 1959:
  - Demonstrated resistance of mice infected with BCG to transplantation of tumors
  - Led to discovery of tumor necrosis factor (TNF)
- Zbar et al JNCI 1971:
  - Noted suppression of tumor growth at site of tumor inoculation when if BCG infection present
  - Attributed effect to delayed hypersensitivity immunologic response to BCG

Pearl R: Cancer and tuberculosis. Am J Hygiene 1929; **9:** 97.
Old LJ, Clarke DA and Benacerraf B: Effect of bacillus Calmette-Guerin infection on transplanted tumours in the mouse. Nature 1959; **184:** 291
Zbar B, Bernstein ID and Rapp HJ: Suppression of tumor growth at the site of infection with living bacillus Calmette- Guerin. J Natl Cancer Inst 1971; **46:** 831.

History of BCG: Cancer

- Mathe et al, Lancet 1969:
  - Demonstrated remission in acute lymphoblastic leukemia in 12 of 20 patients treated with BCG
- Morten et al, Ann Surg 1974:
  - Demonstrated regression in metastatic melanoma skin lesions injected with BCG

MathéG. *et al.* Active immunotherapy for acute lymphoblastic leukaemia. *Lancet* 1, 697–699 (1969).
Morton, D. L. *et al.* BCG immunotherapy of malignant melanoma: summary of a seven-year experience. *Ann. Surg.* 180, 635–643 (1974).

History of BCG: Bladder Cancer

- Morales et al, J Urol 1976:
  - First use of intravesical BCG
  - Instilled 120 mg in 50cc of NS via urethral catheter into the bladder
  - Strain: Frappier (Montreal); packaged in vials of 6
  - Noted at least 3-6 weeks was needed to mount delayed hypersensitivity reaction
  - Side effects lasted 1 week
  - Regimen→ weekly dosing (minimize side effects) x 6 weeks (due to packaging convenience and time to mount immune response)
  - 7 of 10 patients with recurrent tumors demonstrated response (decrease recurrence and or eradication of tumor)

Morales A, Eidinger D and Bruce AW: Intracavitary bacillus Calmette-Guerin in the treatment of superficial bladder tumors. J Urol 1976; **116:** 180.

History of BCG: Bladder Cancer

- Subsequent trials have demonstrated efficacy of BCG immunotherapy in reducing recurrence and progression of bladder cancer

- Almost 40 years later, BCG continues to be recommended standard treatment of high grade non-muscle invasive bladder cancer

Mechanism of Action

- Despite ~40 years of use the exact mechanism is still not completely understood.
  - Evidence mainly from in-vitro and mouse model studies
- Requirements for effective therapy
  - Intact immune system
  - Live BCG vaccine
  - Contact of BCG with bladder cells

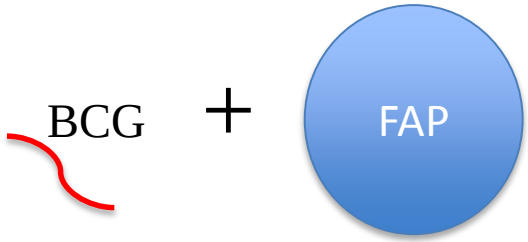
- Both urothelial cell and immune cells play a role
- Both innate and adaptive immune system contribute to anti-tumor role of BCG

Bacillus Calmette-Guérin

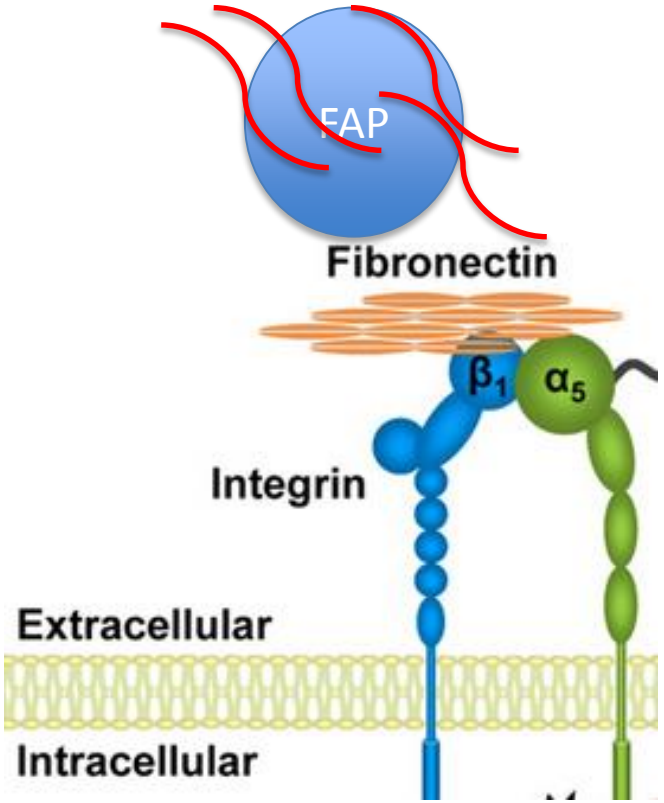
- BCG is an attenuated mycobacterium developed as a vaccine for TB
- BCG has demonstrated anti-tumor activity in several different cancers including urothelial carcinoma
- Bacilli enter lymph nodes and prime T-cell responses, but repeated instillations required
- Prior T-cell priming by parenteral immunization with BCG enhances tumor responses and correlates to clinical observations that patients with pre-existing immunity to BCG (through prior TB vaccination) have improved RFS
  - PRIME trial ongoing studying percutaneous BCG immunization prior to SOC BCG
- Rationale for combining BCG with other therapies that prime the immune system (including vaccines)

Ref: Biot, C. et al. Preexisting BCG-specific T cells improve intravesical immunotherapy for bladder cancer. Sci. Transl. Med. 4, 137ra72 (2012).

Mechanism of Action: Urothelial Cells



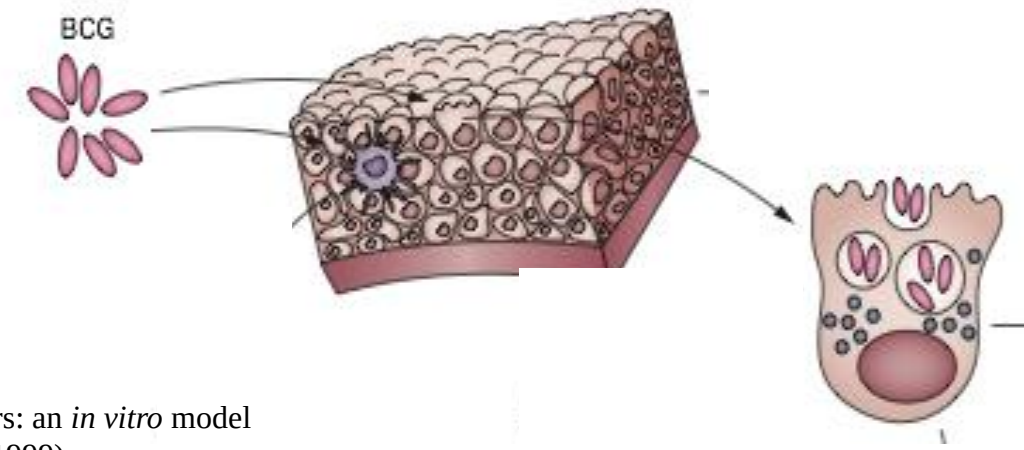
- Attachment to urothelial cells:
  - BCG binds to FAP (Fibronectin attachment protein)
  - Complex then binds to ECM
    - Fibronectin
    - Integrin alpha5 beta1



Zhao, W. *et al.* Role of a bacillus Calmette-Guerin fibronectin attachment protein in BCG-induced antitumor activity. *Int. J. Cancer* 86, 83–88 (2000)

Mechanism of Action: Urothelial Cells

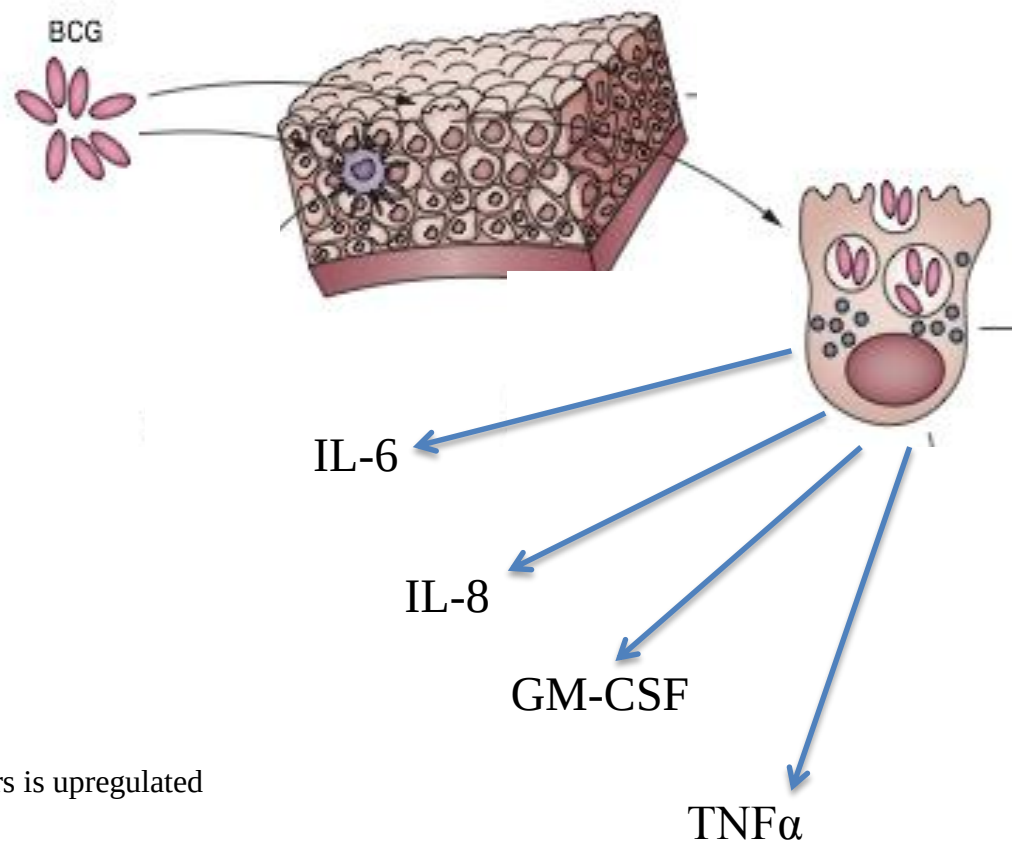
- Internalization by urothelial cells:
  - Macropinocytosis
    - May increase with oncologic aberrations (In vitro)
      - PTEN deletions
      - Ras oncogene mutations



Durek, C. *et al.* Bacillus-Calmette-Guerin (BCG) and 3D tumors: an *in vitro* model for the study of adhesion and invasion. *J. Urol.* 162, 600–605 (1999).

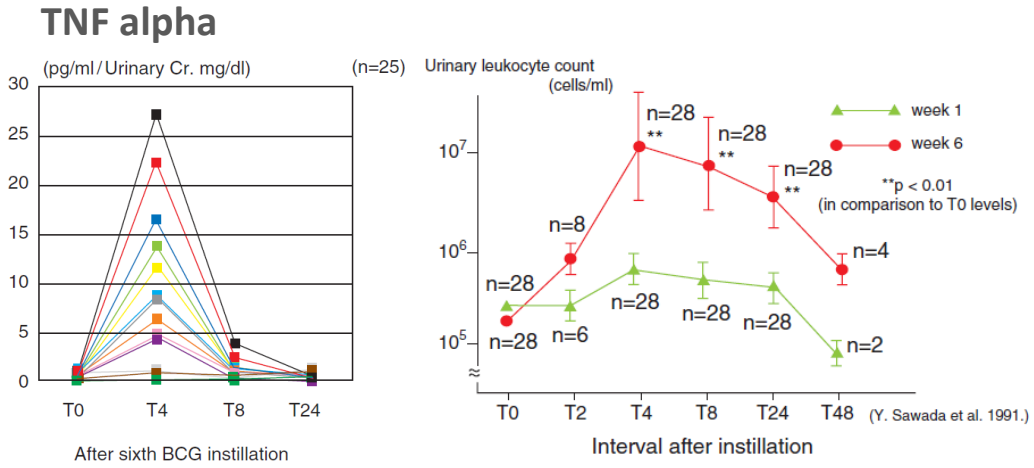
Mechanism of Action: Urothelial Cells

- Immune recruitment by bladder cells:
  - Once BCG internalized bladder cells secrete:
    - IL-6
    - IL-8
    - GM-CSF
    - TNF α
  - Infected urothelial cells can function as APC using MHC II and ICAM-1
  - Further enhances immune response

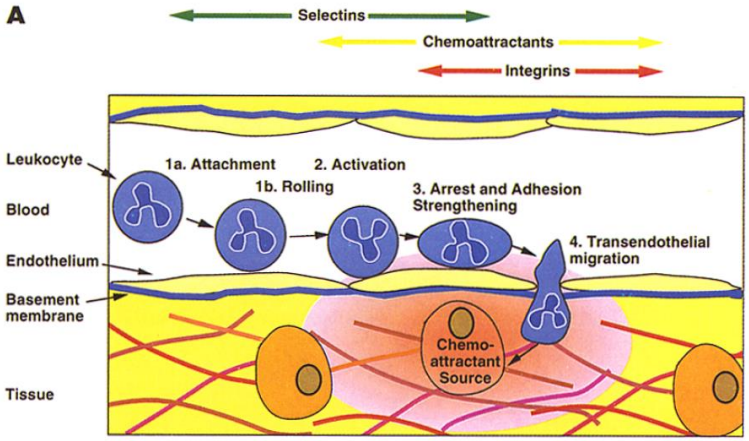


Esuvaranathan, K. *et al.* Interleukin-6 production by bladder tumors is upregulated by BCG immunotherapy. *J. Urol.* 154, 572–575 (1995).

Potential Mechanism of BCG



Shintani *et al*. Int J Urol. 2007;**14**:140-6



Springer TA. Cell. 1994;**76**:301-14

Table 2 Relationship between the urinary cytokine levels at 4 h after the sixth bacillus Calmette-Guérin (BCG) instillation and the prophylactic effects of BCG on bladder cancer (mean follow-up period = 54.1 months)

/Urinary creatinine (mg/dL)	Patients ($n = 7$) with recurrence (Mean ± SD)	Patients ($n = 13$) without recurrence (Mean ± SD)	Mann–Whitney U-test
WBC (× 105/mL)	12.60 ± 12.53	15.93 ± 17.39	$P = 0.72$
GM-CSF (pg/mL)	0.46 ± 0.55	0.32 ± 0.26	$P = 0.81$
TNF-α (pg/mL)	1.12 ± 1.53	8.65 ± 8.66	$P = 0.07$
IL-1β (pg/mL)	1.59 ± 1.82	1.81 ± 1.46	$P = 0.55$
G-CSF (pg/mL)	12.33 ± 15.20	28.28 ± 30.51	$P = 0.23$
IFN-γ (pg/mL)	0.53 ± 1.18	1.98 ± 0.68	$P = 0.89$
IL-8 (pg/mL)	0.99 ± 1.71	11.12 ± 15.85	$P = 0.20$
IL-12 (pg/mL)	0.07 ± 0.09	0.27 ± 0.60	$P = 0.53$

- BCG stimulates TNF-α
- TNF-α is associated with increased urinary lymphocytes
- ↑ TNF-α levels are significantly associated with decreased disease recurrence

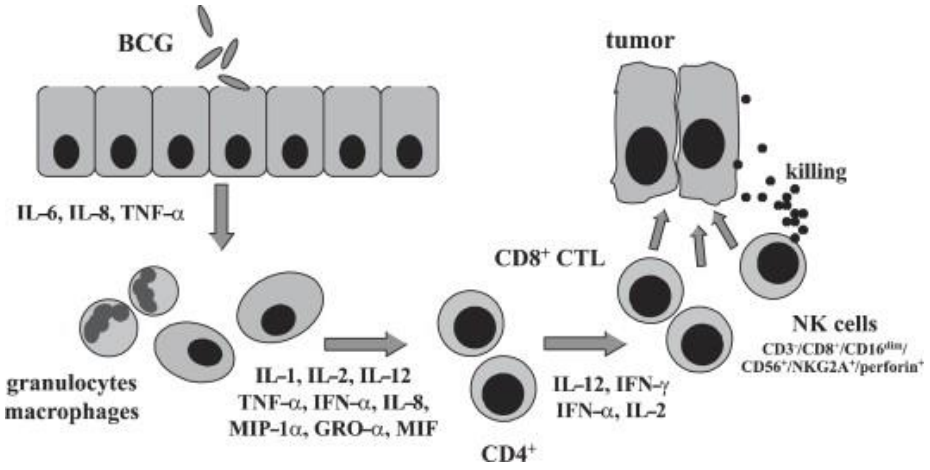
Mechanism of Action: Urothelial Cells

- Direct Cytotoxicity:
  - In-vitro exposure of BCG
    - Cell cycle arrest
    - Decreased proliferation
    - Direct cytotoxicity
      - Needs 50-100 bacteria per cell (unlikely to reach this ratio in-vivo)

Pook, S. H., Rahmat, J. N., Esuvaranathan, K. & Mahendran, R. Internalization of Mycobacterium bovis, Bacillus Calmette Guerin, by bladder cancer cells is cytotoxic. *Oncol. Rep.* 18, 1315–1320 (2007).

Mechanism of Action: Immune Cells

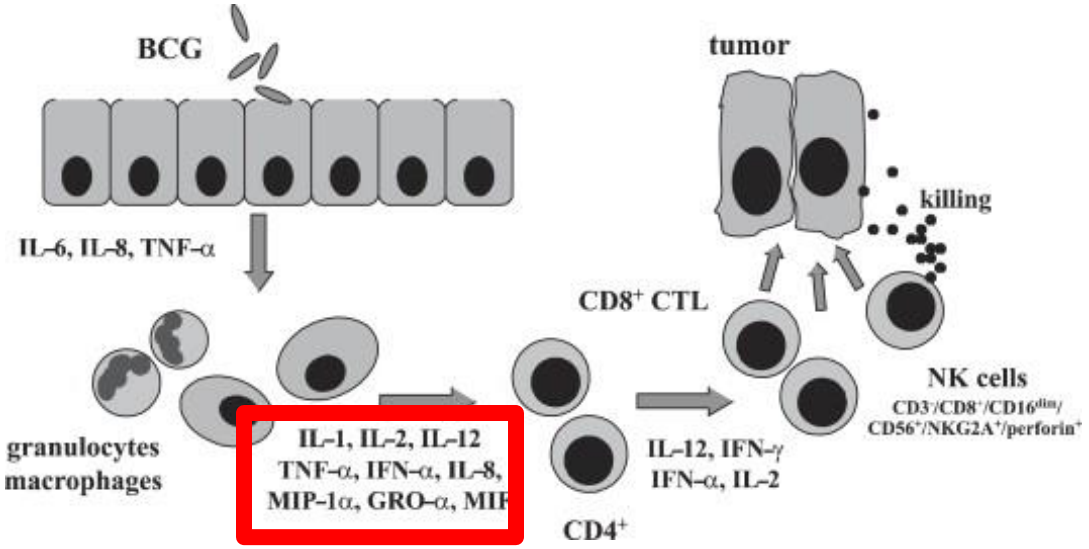
- Hours after BCG administration
  - 1) Dendritic cells
    - Process BCG antigen to enhance activation of T cell in-vitro
  - 2) Urothelial cells process BCG antigen
    - Secrete IL-6, IL-8, TNF, GM-CSF
    - MHC II and ICAM to present to CD4 T cells
  - 3) Innate immune cell recruitment
    - Initially granulocytes
    - Later monocytes and macrophages
    - Both secrete pro-inflamatory cytokines



Brandau et al. Thirty years of BCG immunotherapy for non-muscle invasive bladder cancer: A success story with room for improvement. Biomedicine & Pharmacotherapy July 2007: 61(6).

Mechanism of Action: Immune Cells

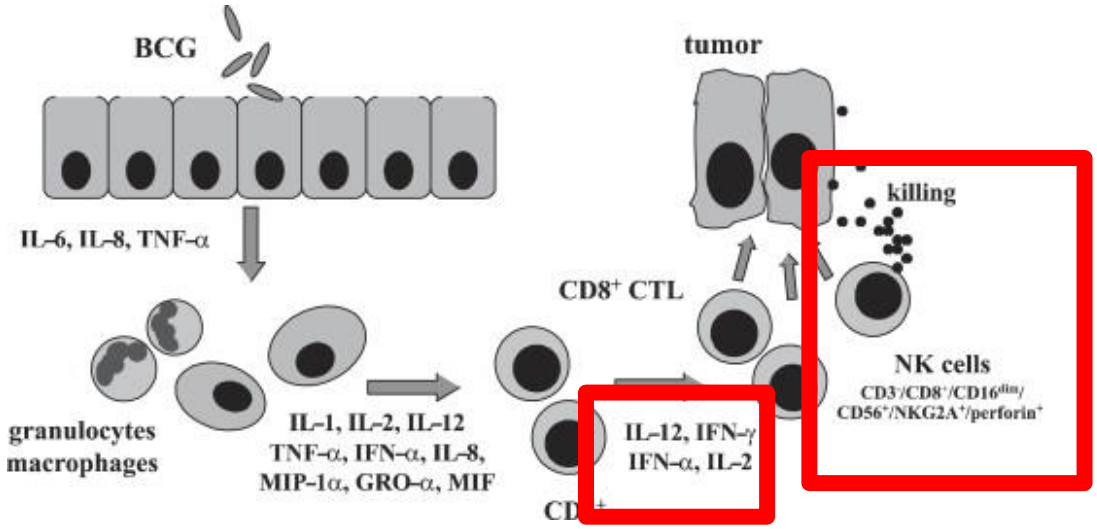
- Cytokine Release:
  - IL-1, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IL-18, TNF, IFN-γ, and granulocyte–macrophage colony-stimulating factor (GM-CSF),
  - Recruitment of lymphocytes
- CD4 cell recruitment
  - BCG therapy can result in a shift in the urinary cytokine milieu from TH2-like to **TH1-like**



Brandau et al. Thirty years of BCG immunotherapy for non-muscle invasive bladder cancer: A success story with room for improvement. Biomedicine & Pharmacotherapy July 2007: 61(6).

Mechanism of Action: Immune Cells

- Tumor cell killing:
- CD8 cells
  - Monocyte and $CD4^+$ T-cell-derived T_H1-cytokines (IL-2, IL-12, IFN-α and IFN-γ synergize) to activate cytotoxic lymphocytes
- NK-cells
  - Perforin mediated lysis
  - Distinct subset of NK cells created
    - $CD3^-/CD8^+/CD16^{dim}/CD56^+/NKG2A^+/perforin^+$ phenotype



Brandau et al. Thirty years of BCG immunotherapy for non-muscle invasive bladder cancer: A success story with room for improvement. Biomedicine & Pharmacotherapy July 2007: 61(6).

Mechanism of Action: Urothelial Summary

- Urothelial Cell Action:

Process	Evidence for role in response to BCG
Attachment of BCG to the urothelium	BCG attaches to urothelial cells through bridging of FAP and integrin α5β1 by fibronectin Blocking fibronectin can reduce BCG efficacy in the mouse model
Internalization of BCG by bladder cancer cells	Internalized BCG can be identified in urothelial cells of patients treated with BCG *In vitro*, bladder cancer cells internalize BCG, while benign urothelial cells do not Uptake of BCG by bladder cancer cells is dependent on activation of macropinocytosis by oncogenic aberrations in *PTEN* and *RAS*
Immune system recruitment by bladder cancer cells	Bladder cancer cells secrete IL-6, IL-8, GM-CSF and TNF in response to BCG *In vitro*, bladder cancer cells can act as antigen-presenting cells after exposure to and internalization of BCG
Direct cytotoxicity of BCG against bladder cancer cells	Reduced proliferation of BCG-exposed bladder cancer cells BCG internalization by bladder cancer cells can result in cell death No evidence currently supports direct cytotoxicity on bladder *in vivo*

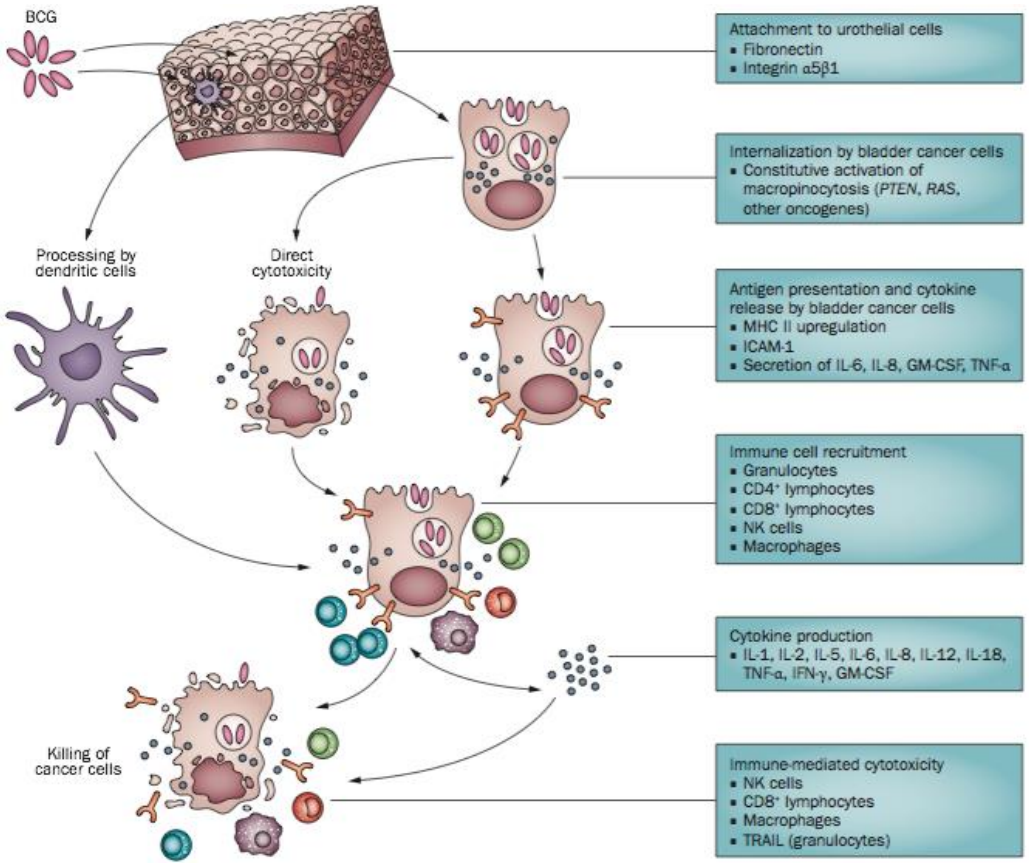
Redelman-Sidi et al. The mechanism of action of BCG therapy for bladder cancer—a current perspective. Nat. Rev. Urol. 11, 153–162 (2014).

Mechanism of Action: Summary

Immune system component	Evidence for role in response to BCG
Lymphocytes	Lymphocytes are a component of the inflammatory infiltrate in the bladders of patients treated with BCG $CD4^+$ and $CD8^+$ T cells are required for response to BCG in the mouse model
NK cells	Infiltration of NK cells in bladder wall of BCG-treated mice NK cells are cytotoxic against BCG-infected bladder cancer cells *in vitro* NK cells are required for response to BCG in the mouse model
Granulocytes	Granulocytes are the major component of the inflammatory infiltrate in the bladders of patients treated with BCG PMN are required for efficacy of BCG in the mouse model
Macrophages	Macrophages are a component of the inflammatory infiltrate in the bladders of patients treated with BCG BCG-stimulated macrophages are cytotoxic against bladder cancer cells *in vitro*
Dendritic cells	Immature dendritic cells can be found in the urine of patients treated with BCG *In vitro*, BCG-exposed dendritic cells can induce T cells to exhibit cytotoxicity against BCG-infected bladder cancer cells
Cytokines and chemokines	Massive release of cytokines and chemokines occurs in urine of patients treated with BCG BCG therapy shifts the urinary cytokine milieu from T_H2-like to T_H1-like Augmentation of a T_H1-like response can improve the efficacy of BCG in the mouse model TRAIL, an apoptosis-promoting protein, is released into the urine of patients treated with BCG, and can kill bladder cancer cells *in vitro*

Redelman-Sidi et al. The mechanism of action of BCG therapy for bladder cancer—a current perspective. Nat. Rev. Urol. 11, 153–162 (2014).

Mechanism of Action: Summary



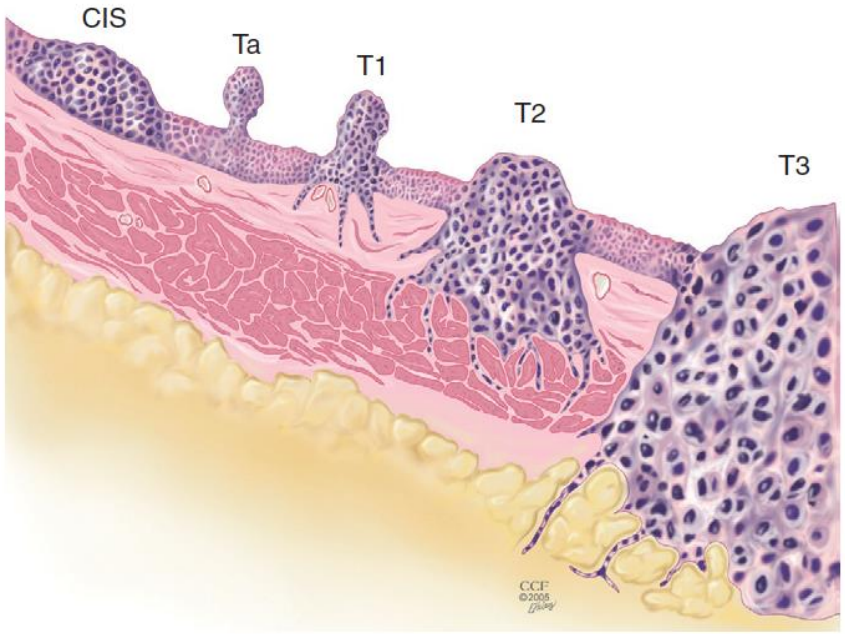
Redelman-Sidi et al. The mechanism of action of BCG therapy for bladder cancer—a current perspective. Nat. Rev. Urol. 11, 153–162 (2014).

BCG in Practice: Bladder Cancer Overview

- Bladder cancer is 4th most common cancer in men in US.
- Male to female ratio→4:1
- Median age at presentation is 70
- Smoking is the most common cause of bladder cancer
- Gross hematuria most common presenting sign

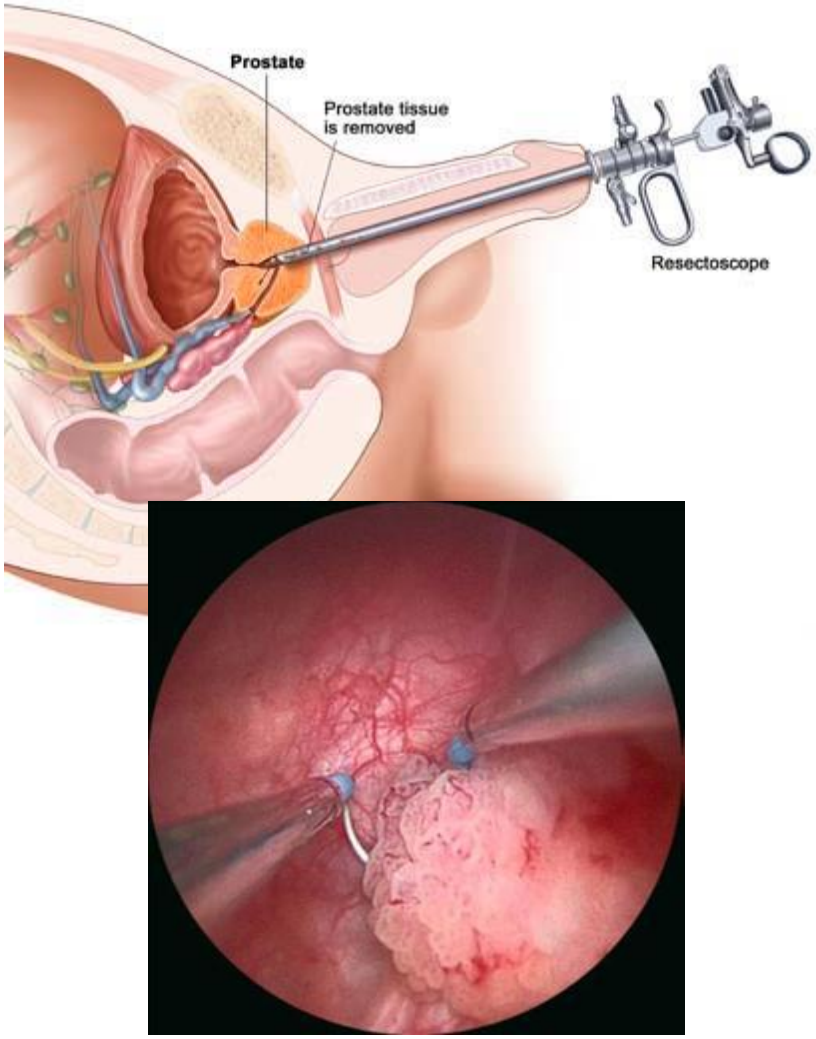
BCG in Practice: Bladder Cancer Staging

- Stage
  - Based on depth of penetration
  - Invasive: T2 or higher
  - Non-muscle invasive:
    - CIS
    - Ta
    - Ta
- Grade:
  - Based on microscopic features and architecture of cells
- 70% of bladder tumors are non-muscle invasive at presentation



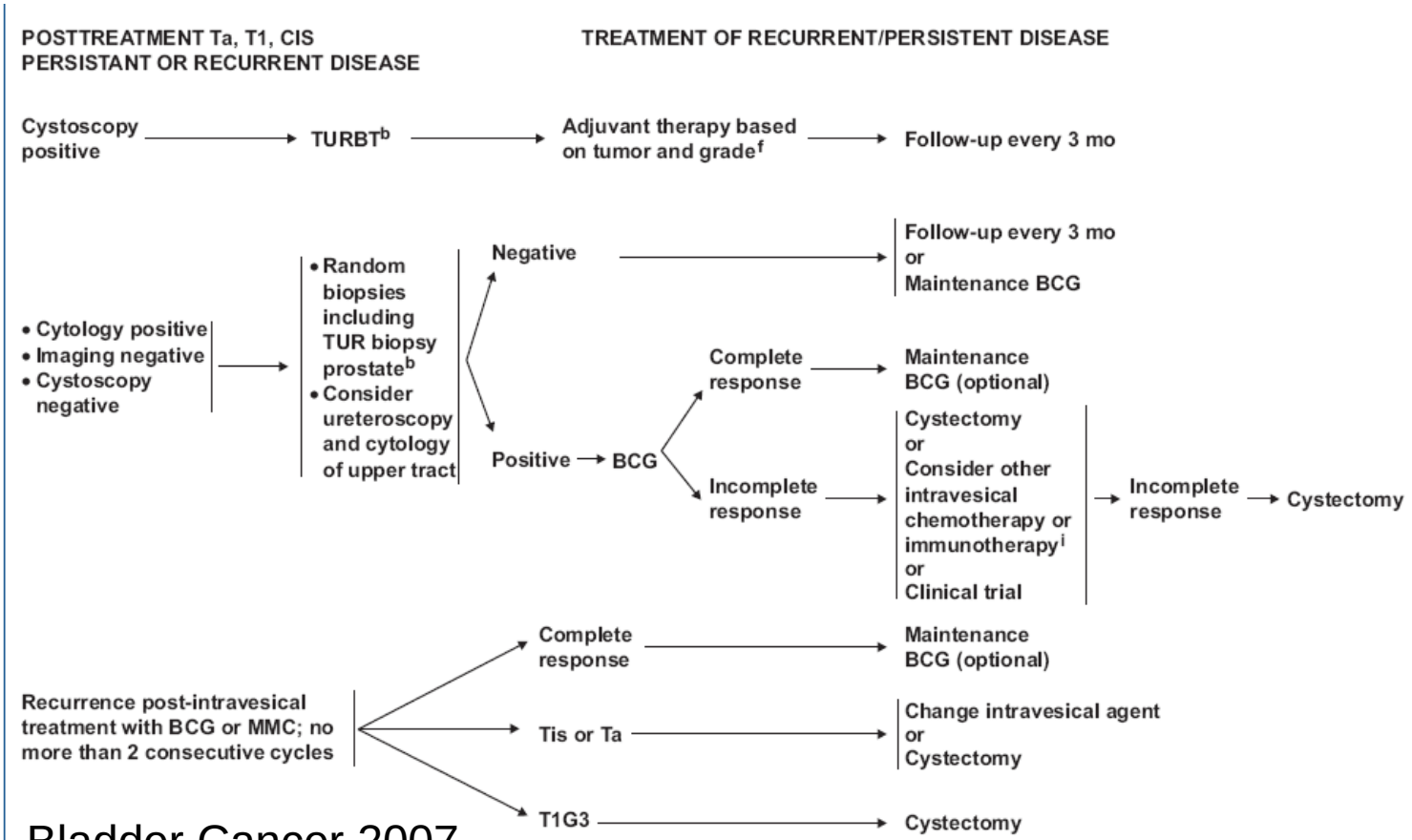
BCG in Practice: Bladder Cancer Staging

- Transurethral resection of bladder tumor (TURBT)
  - Therapeutic
  - Diagnostic
  - Prognostic



NCCN Guidelines - 2007

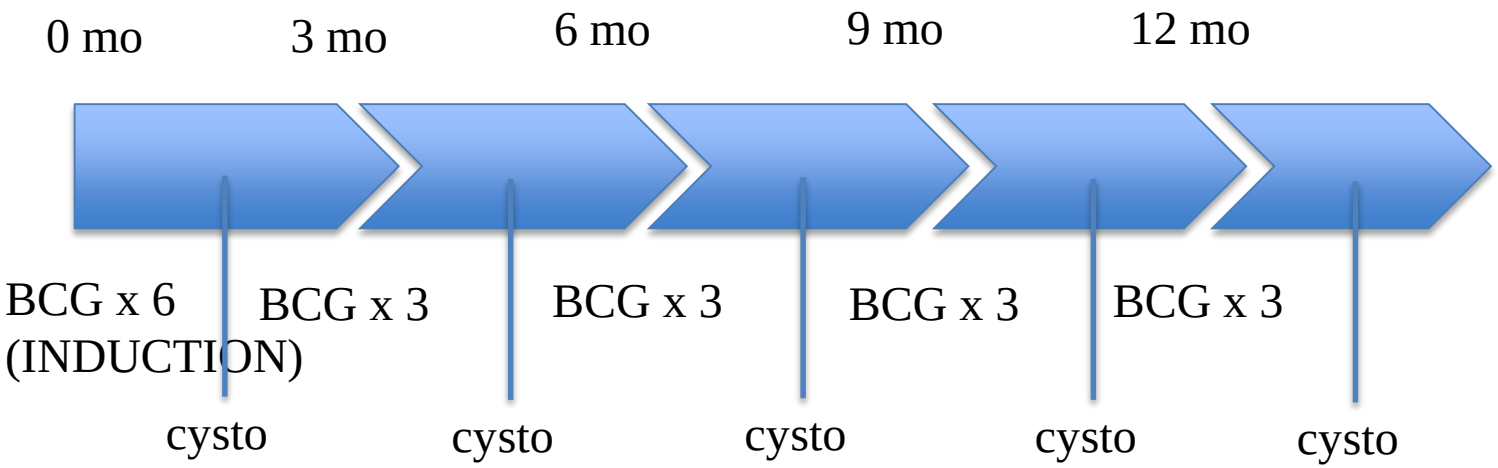
Treatment of non-muscle -invasive bladder cancer



NCCN – Bladder Cancer 2007

BCG in Practice: Indications for BCG

- Indications:
  - HG Ta
  - Any CIS
  - Any T1
- Delay administration 1-3 weeks after tumor resection to avoid systemic absorption
- BCG schedule: Induction course + maintenance



Summary of Clinical Data in the Literature

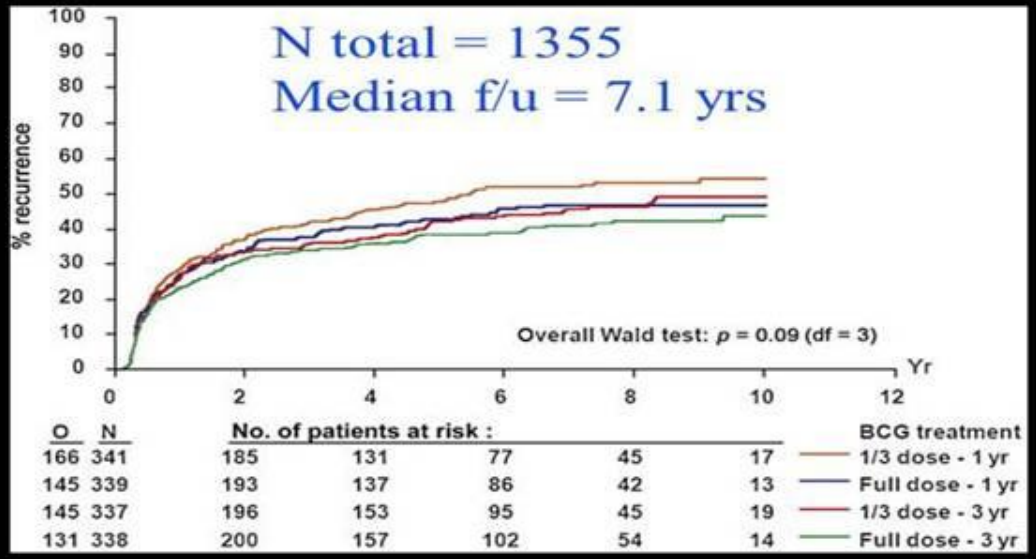
Trial	Population	CR	RFS w/ maint	RFS w/o maint	2-yr RFS rate w/ maint	2-yr RFS rate w/o maint	Adjusted* 2-yr RFS w/maint	Adjusted* 2-yr RFS w/o maint
SWOG 8507 (Lamm 2000 J.Urol) (N=384)	Ta or T1 and a) 2 tumors (primary and recurrent or 2 recurrent) w/in 1 yr b) 3 or more within 6 months c) and/or CIS	70%	77 mo	36 mo	82%	62%	48%	30%
MSK (Herr 2011 Eur. Urol) (N=806)	Ta or T1 and/or CIS; **restaging TUR required**	80%	--	--	--	73%		58%
Japan (Hinotsu 2011, BJU) (N=84)	Ta or T1 **(no CIS)** and a) ≥3 tumors at time of TURBT or b) 3rd recurrence or c) recurrence within 12 mo from previous TUR	--	--	--	93%	65%		

*Adjusted 2-yr RFS includes the induction failures in the analysis

Conclusions:

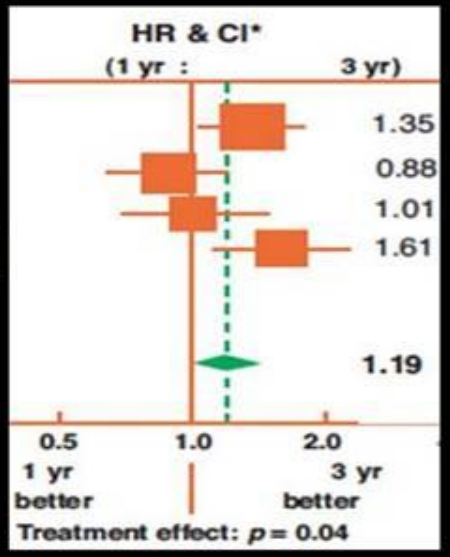
1) Maintenance BCG confers a clear benefit for patients who respond to induction BCG
2) Restaging TURBT may decrease recurrence
3) Patients with papillary disease appear to do better on maintenance than patients with CIS

BCG Dose and Duration of Maintenance



1/3 IR
FD IR
1/3 HR
FD HR

Total



- no differences in toxicity between 1/3 Dose and Full Dose (FD)
- Intermediate-risk (IR) patients should be treated with FD-1 yr (37.7%) vs FD- 3 yr (43.1%) vs 1/3 D–1 yr (55.2%) vs 1/3 D-3 yr (44.5%)
- In high-risk (HR) patients, FD-3 yr reduces recurrences as compared with FD-1 yr (33.6% vs 50%) but not progressions or deaths

Oddens, Eur Urol, 2012

MAINTENANCE BACILLUS CALMETTE-GUERIN IMMUNOTHERAPY FOR RECURRENT TA, T1 AND CARCINOMA IN SITU TRANSITIONAL CELL CARCINOMA OF THE BLADDER: A RANDOMIZED SOUTHWEST ONCOLOGY GROUP STUDY

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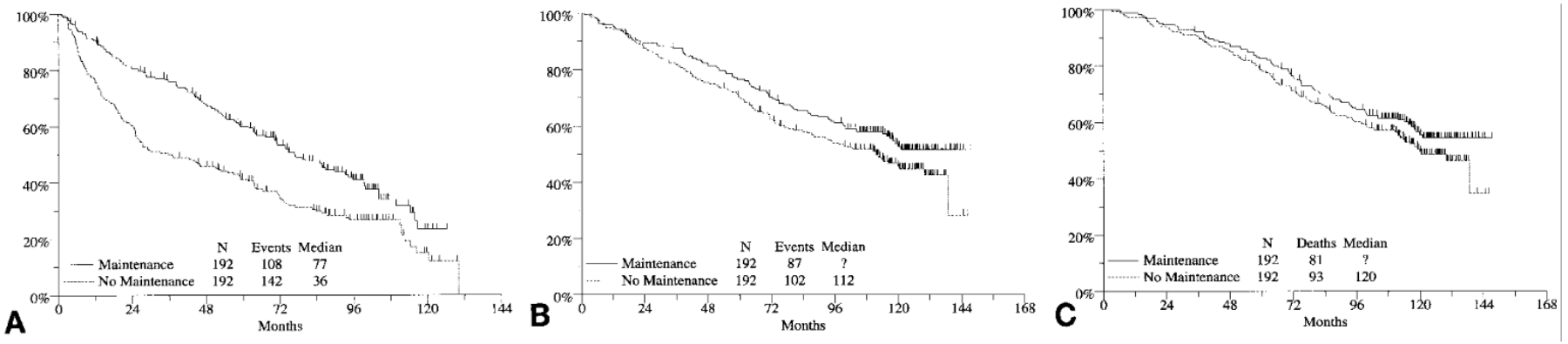


FIG. 1. Recurrence-free survival (*A*), worsening-free survival (*B*) and survival (*C*) in months by arm for eligible patients with no evidence of disease at randomization.

- Maintenance BCG was beneficial in patients with carcinoma in situ and select patients with Ta, T1 bladder cancer
- Median recurrence-free survival time was twice as long in the 3-week maintenance arm
- Patients had significantly longer worsening-free survival

BCG Therapy – Nuances

- Treatment of choice for high-grade TaT1 disease and CIS or prior chemotherapy failure in intermediate risk patients
- Risky in patients with significant immunosuppression (but low dose steroids or methotrexate OK)
- Reduced efficacy in patients >80 years old
- Provides 30-40% absolute benefit in ↓ recurrence vs. TUR and ~70% CR for CIS (~2x better than chemo)
- 50% maintain NED status for 3-4 years
- Best results are with the addition of a retreatment option for failures X1 (~50% cycle 1 then ~35% salvage w/ cycle 2)
- Should be given with at least a 1 year maintenance program for all high-grade disease *
  - Superiority over MMC only seen with BCG maintenance
  - ↓ progression (~30%) only seen with BCG maintenance
- Maintenance program only proven directly for miniseries of 3 every 3-6 months for up to 3 years (some dropoff due to intolerance)
  - Methods to improve tolerance include dose reduction, q 2 wk dosing, decreased dwell time to 30 min, brief quinolone Rx

BCG in Practice: Side effects

- Up to 90% of patients will experience side effects:
  - Fevers, fatigue, myalgias
  - Cystitis, frequency, urgency
  - Sepsis (Rare)
- Some experts suggest side effects are associated with treatment efficacy

BCG in Practice: Efficacy

- Recurrence:
  - 5 year recurrence free survival 60%
- Progression:
  - 27% reduction in progression compared to other intravesical therapy

Summary

- BCG developed from M. Bovis in 1921
- Early studies demonstrated anti-tumor effects of BCG in ALL and melanoma
- Morales et al. demonstrated efficacy in topical administration for non-muscle invasive bladder tumors in 1976
- BCG relies on complex interactions with immune cells and urothelial cells
- BCG induction + maintenance therapy is and has been the predominant therapy for non-muscle invasive bladder cancer for ~40 years

Intravesical Therapy - Future Directions

- Potential Immunotherapy
  - Check Point Inhibitors
  - Oncolytic Virus/Vaccines
  - Allogenic Tumor Cell Therapy/Vaccine

Intravesical Failures

- Chemotherapy failures - BCG
- BCG failures – controversial
  - 2nd course of BCG – more than 2 with very low success rates
  - Early cystectomy – high-risk patient (multifocal T1HG, CIS, Lymphatic/vascular invasion, Micropapillary)
  - Intravesical chemotherapy – poor results
  - Salvage intravesical therapy

The BCG Failure Problem

Intravesical therapy with BCG is used as the pre-dominant agent in N. America by >2:1 majority vs. chemotherapy even in low-intermediate grade bladder cancer

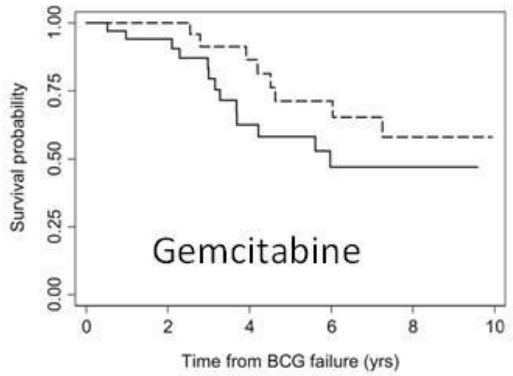
Despite superior BCG efficacy, over 50% of patients still fail

- Estimated @ incidence of ~15,000/yr or ~50,000 total in US

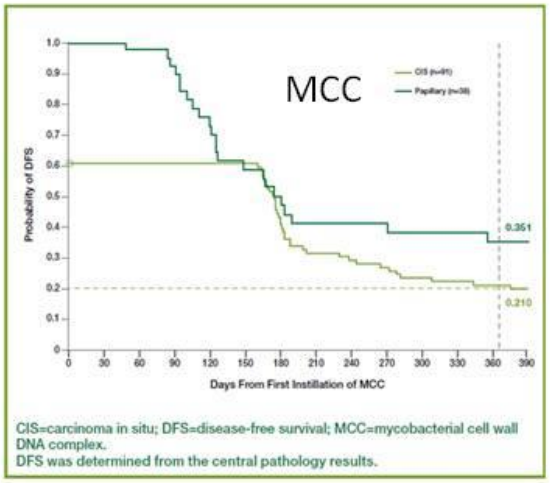
There is wide heterogeneity among BCG failures:

- Papillary vs CIS, low-grade vs. high grade, number of failures courses, time of failure, failure during maintenance
- Even the term BCG refractory has different meanings to different people

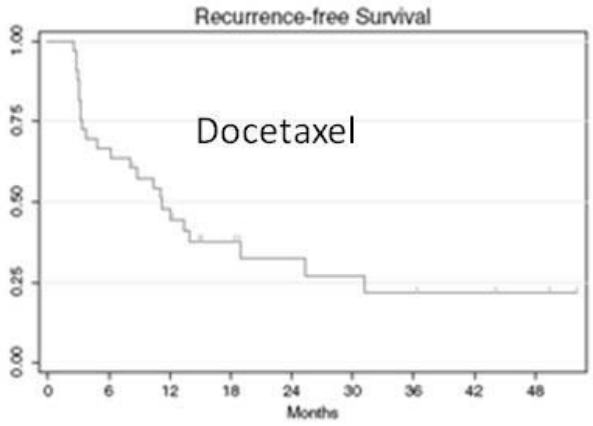
Intravesical Therapy for BCG-Failures

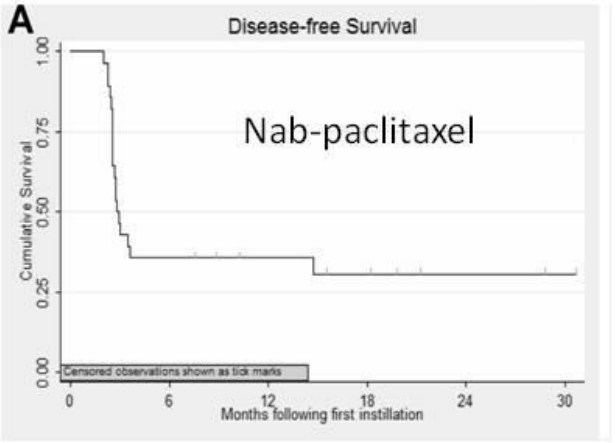


Number at risk						
Other types of BCG failure	30	27	18	12	8	4
BCG refractory	37	27	14	8	8	3



CIS=carcinoma in situ; DFS=disease-free survival; MCC=mycobacterial cell wall DNA complex.
DFS was determined from the central pathology results.

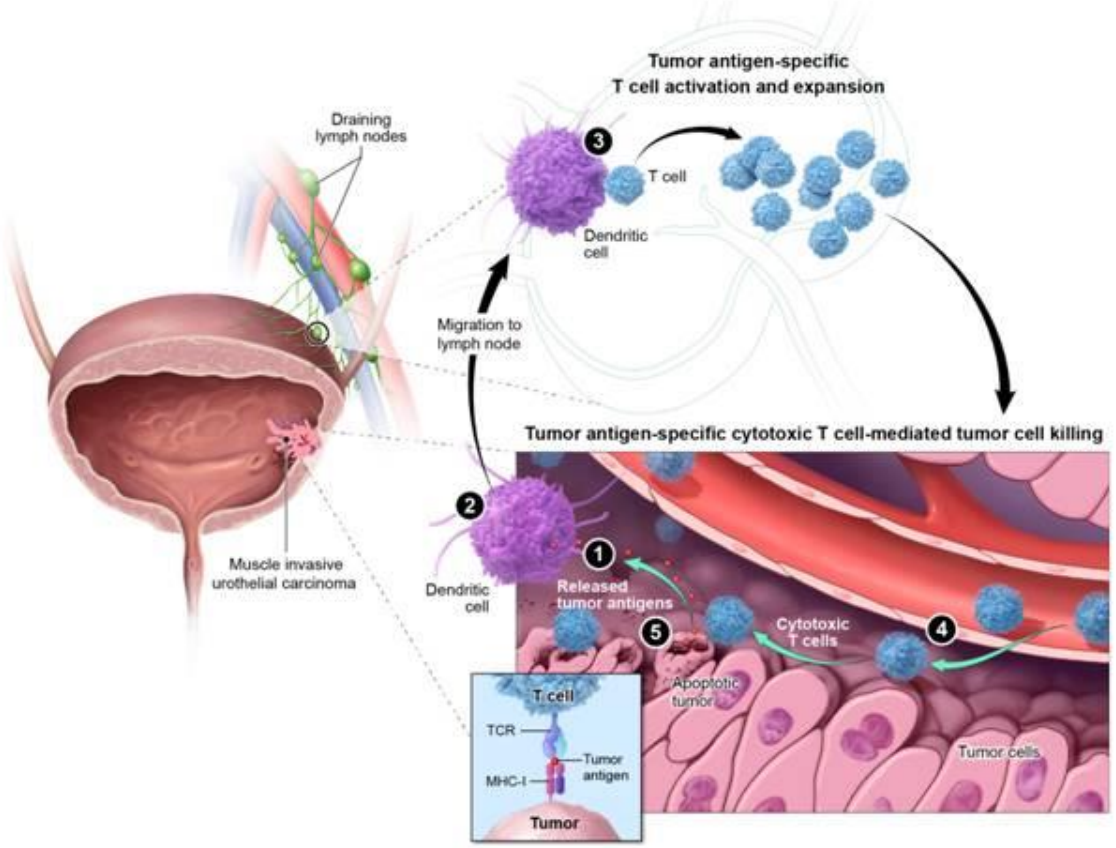




Intravesical BCG: Etiology of Failure

- UnderStaging-Inaccurate TURBT
- Large Tumor Volume
- Failure to Bind to Fibronectin
- Inadequate Immune Response-T_{Helper} Type 1
- Genetic Instability, Continued Antigenic and Mutational Drift
- PD-1, PD-L1 Mediated Immune Anergy/T Cell Exhaustion

Urothelial carcinoma-specific antitumor T-cell immunity cycle



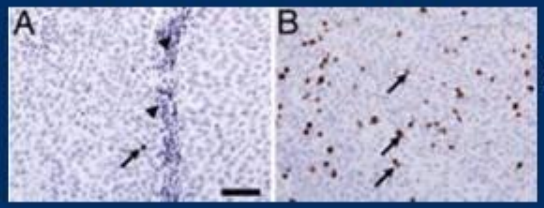
Tumors maintain an immunosuppressive via PD-L1/PD-1 binding
Inhibiting:
T-cell migration, proliferation and secretion of cytotoxic mediators

Kim JW, Tomita Y, Trepel J and Apolo AB *Current Opinion in Oncology* In Press

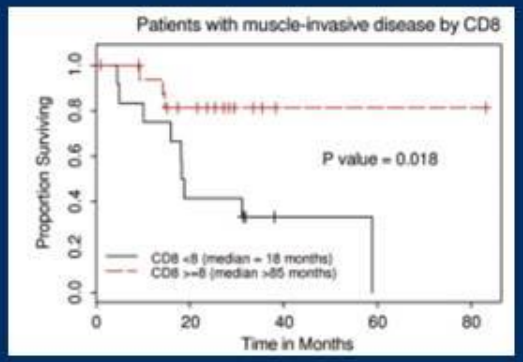
CD8 tumor-infiltrating lymphocytes are predictive of survival

Bladder Cancer

Low frequency of CD8 TILs



High frequency of CD8 TILs



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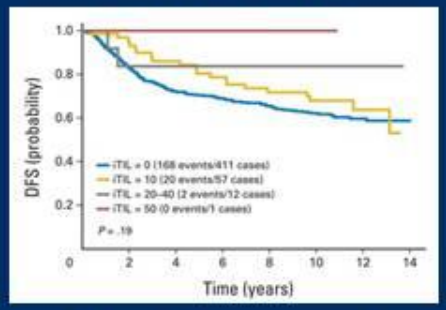
Breast Cancer



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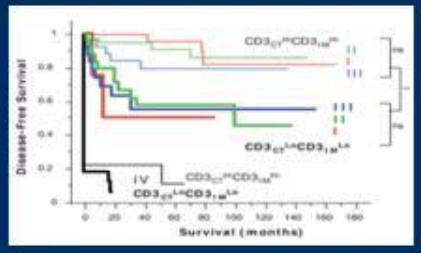
Colorectal Cancer



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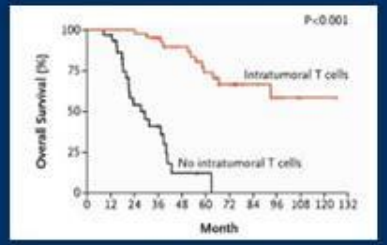
Ovarian Cancer

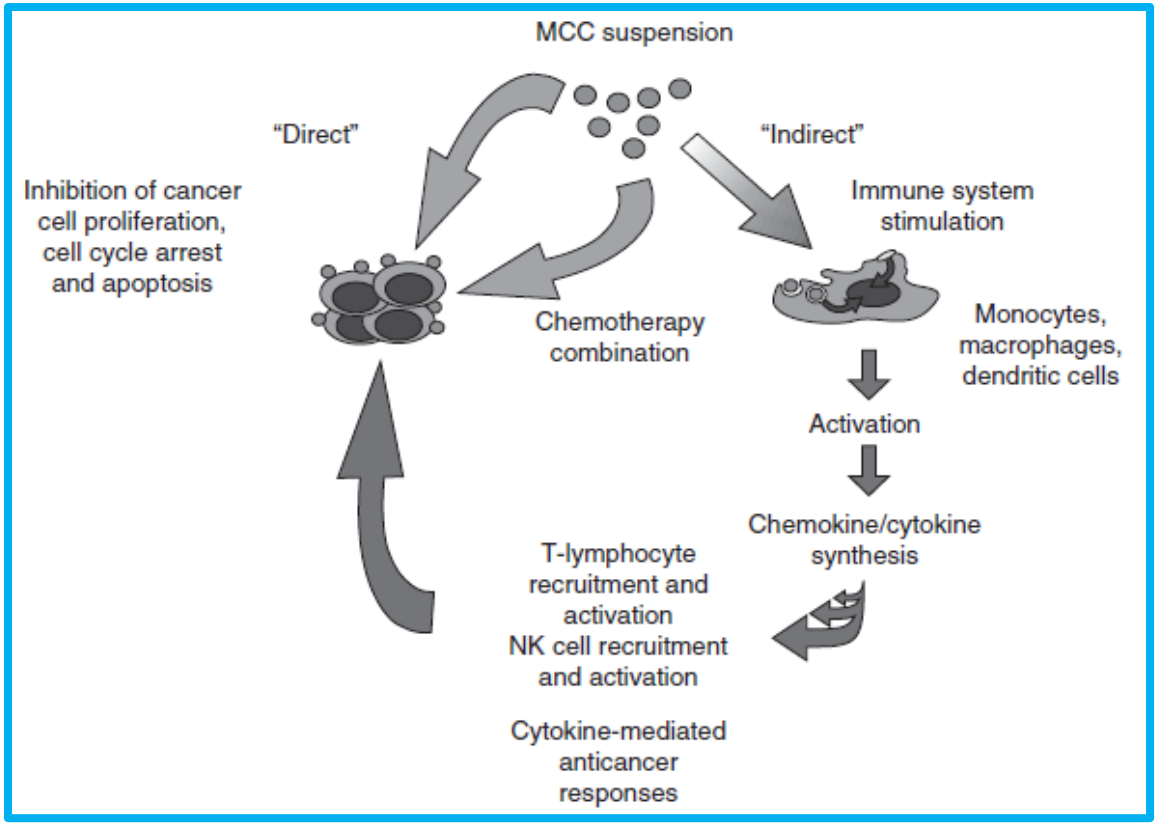


Zhang L et al. N Engl J Med 2003;348:203-213.

Presented at the **Genitourinary Cancers Symposium**

Presented by Andrea B. Apolo

MycoBacterial Cell Wall Extract

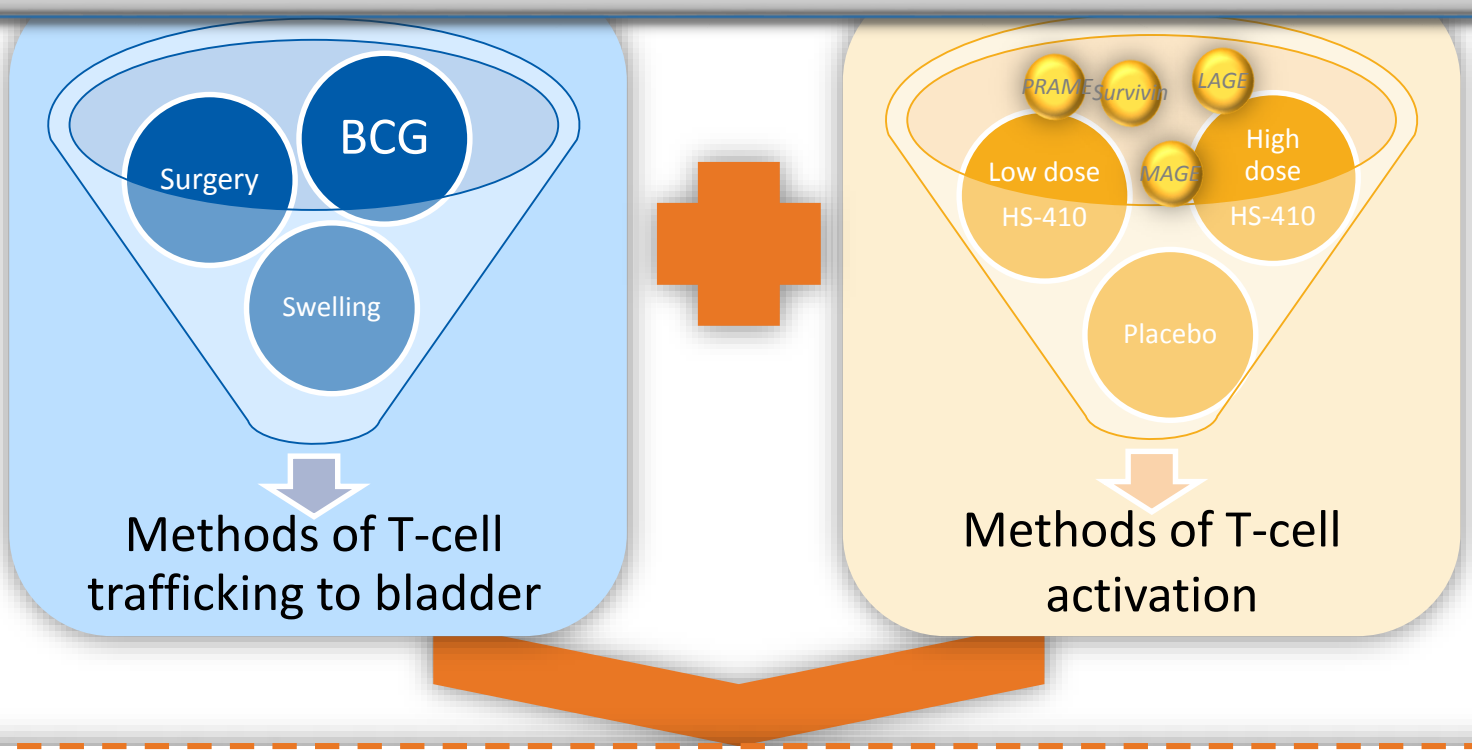


NK = natural killer

Figure kindly provided by Nigel C. Phillips of Bioniche Life Sciences Inc. and reprinted with permission © NC Phillips, 1997. NC Phillips, 2007. Filion et al. Cancer Immunol Immunother. 2000;49:325-334; Filion MC, et al. Br J Cancer. 1999;79:229-235

HS410-101 Study Rationale

T-cells in the body can be used to treat cancer. To be effective, those T-cells need to find the tumor AND be active on arrival. BCG alone has been shown to attract T-cells to the bladder – can HS-410 activate the T-cells better than BCG alone?

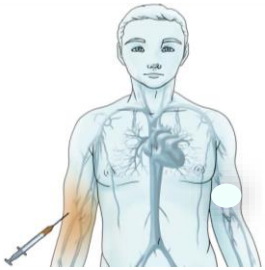


Protocol is designed to determine the best combination of T-cell trafficking and T-cell activation methods for High-risk NMIBC

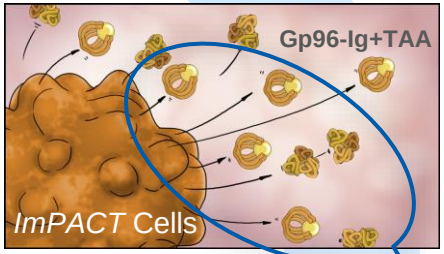
Heat Biologics

ImPACT Immunotherapy Platform

Specifically Activates Pan-Antigen CD8+ T-Cells to Kill Tumor Cells

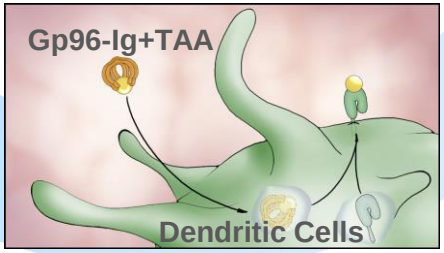


① Intradermal Injected *ImPACT®* Cells

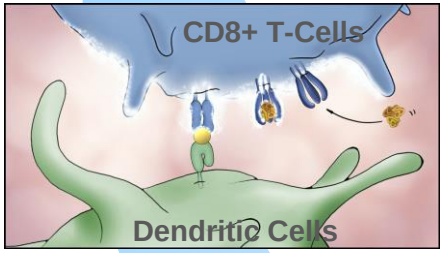


② *ImPACT* cells secrete Gp96-Ig chaperone + tumor antigens (TAA)

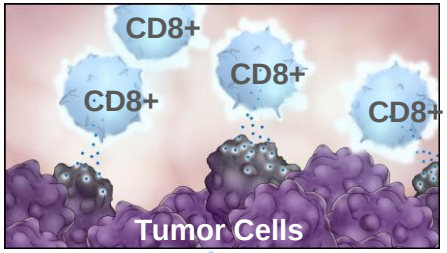
Over a dozen known UCC tumor antigens secreted, including: PRAME, Survivin, LAGE antigens, MAGE antigens…



③ Dual antigen carrier & adjuvant activates Dendritic Cells



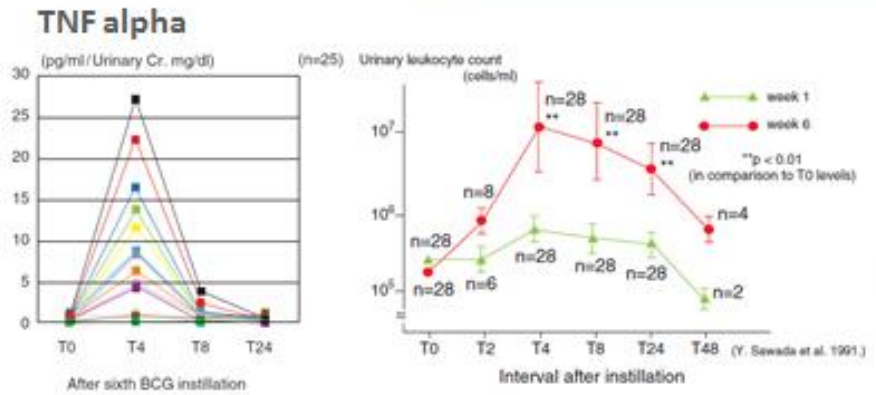
④ Selectively activate CD8+ T-Cells



⑤ CD8+ T-cells circulate & eliminate tumor cells

Heat Biologics

Potential Synergy Between BCG and HS-410



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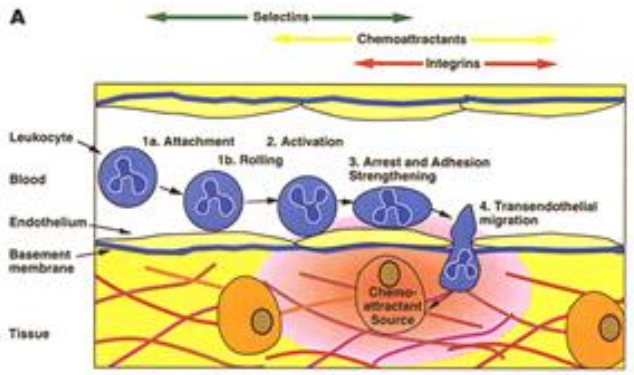

Springer TA. Cell. 1994;**76**:301-14

Table 2 Relationship between the urinary cytokine levels at 4 h after the sixth bacillus Calmette-Guérin (BCG) instillation and the prophylactic effects of BCG on bladder cancer (mean follow-up period = 54.1 months)

/Urinary creatinine (mg/dL)	Patients (n = 7) with recurrence (Mean ± SD)	Patients (n = 13) without recurrence (Mean ± SD)	Mann-Whitney U-test
WBC (× 10⁵/mL)	12.60 ± 12.53	15.93 ± 17.39	P = 0.72
GM-CSF (pg/mL)	0.46 ± 0.55	0.32 ± 0.26	P = 0.81
TNF-α (pg/mL)	1.12 ± 1.53	8.65 ± 8.66	P = 0.07
IL-1β (pg/mL)	1.59 ± 1.82	1.81 ± 1.46	P = 0.55
G-CSF (pg/mL)	12.33 ± 15.20	28.28 ± 30.51	P = 0.23
IFN-γ (pg/mL)	0.53 ± 1.18	1.98 ± 0.68	P = 0.89
IL-8 (pg/mL)	0.99 ± 1.71	11.12 ± 15.85	P = 0.20
IL-12 (pg/mL)	0.07 ± 0.09	0.27 ± 0.60	P = 0.53

- BCG stimulates TNF-α
- TNF-α is associated with increased urinary lymphocytes
- ↑ TNF-α levels are significantly associated with decreased disease recurrence

- **By increasing vascular endothelial integrin and selecting expression, BCG induced TNF-α facilitates lymphocyte infiltration into the urinary bladder. This is expected to facilitate infiltration by vaccine-stimulated CD8+ T cells.**

Heat Biologics

Evidence of CD8+ Tumor Infiltration in HS-410 Phase 1 Study