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

Dr. Gary D. Steinberg
The Bruce and Beth White Family Professor and Director of Urologic Oncology
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

Bacillus Calmette-Guerin ("BCG") Immunotherapy for Bladder Cancer
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 Bacillus 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

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**

<table>
<thead>
<tr>
<th>Strain</th>
<th>n*</th>
<th>Mean CRR % (range)*</th>
<th>Commercial product</th>
<th>Weight (mg)</th>
<th>Recommended dose (cfu)</th>
<th>Secretion of lipid virulence factors?</th>
<th>Secretion of MPB64/MPB70 and MPB83?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moscow²</td>
<td>103</td>
<td>90.5</td>
<td>SII-ONCO-BCG²</td>
<td>120</td>
<td>3–57×10⁸</td>
<td>Yes</td>
<td>Present/High</td>
</tr>
<tr>
<td>Moreau RdJ</td>
<td>100</td>
<td>90</td>
<td>ImmunoBCG (FAP, Brazil)</td>
<td>80</td>
<td>0.04×10⁸</td>
<td>No</td>
<td>Present/High</td>
</tr>
<tr>
<td>Connaught</td>
<td>450</td>
<td>79 (70–92)</td>
<td>Immunocyst² (Sanofi-Aventis, France)</td>
<td>81</td>
<td>1.8–15.9×10⁸</td>
<td>NT</td>
<td>Present/High</td>
</tr>
<tr>
<td>Tokyo</td>
<td>111</td>
<td>77 (63–84)</td>
<td>Tokyo 172 (QSMI, Thailand)</td>
<td>80</td>
<td>0.4–0.5×10⁸</td>
<td>No</td>
<td>Present/High</td>
</tr>
<tr>
<td>Pasteur</td>
<td>230</td>
<td>74 (40–80)</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
<td>Absent/Present</td>
</tr>
<tr>
<td>Tice</td>
<td>277</td>
<td>71 (56–82)</td>
<td>OncoTice² (Merck, USA)</td>
<td>12.5</td>
<td>2–8×10⁸</td>
<td>Yes</td>
<td>Absent/Present</td>
</tr>
<tr>
<td>Glaxo</td>
<td>180</td>
<td>65 (53–88)</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
<td>Absent/Present</td>
</tr>
<tr>
<td>A. Frappier</td>
<td>145</td>
<td>60 (39–100)</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
<td>Absent/Present</td>
</tr>
<tr>
<td>S. African</td>
<td>13</td>
<td>69</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Copenhagen</td>
<td>42</td>
<td>67</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>Yes</td>
<td>Absent/Present</td>
</tr>
<tr>
<td>Romanian</td>
<td>33</td>
<td>64</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>RIVM/1</td>
<td>15</td>
<td>60</td>
<td>BCG-Medac² (Medac, Germany)</td>
<td>80</td>
<td>2–30×10⁸</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

*Data from two studies.²⁻⁴ Based on summary of product characteristics for individual commercial products and study.⁷⁻⁹ Data from the manufacturer's summary. Abbreviations: CRR, complete response rate; NA, not applicable; NT, not tested.


MPB64, MPB70, MPB83 = T-cell stimulating proteins

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

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

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

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)

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)

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

Mechanism of Action: Urothelial Cells

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

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

Potential Mechanism of BCG

BCG stimulates TNF-α

TNF-α is associated with increased urinary lymphocytes

↑ TNF-α levels are significantly associated with decreased disease recurrence

**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).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Patients (n = 7) with recurrence (Mean ± SD)</th>
<th>Patients (n = 13) without recurrence (Mean ± SD)</th>
<th>Mann-Whitney U-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x 10⁶/mL)</td>
<td>12.60 ± 12.53</td>
<td>15.93 ± 17.39</td>
<td>P = 0.72</td>
</tr>
<tr>
<td>GM-CSF (pg/mL)</td>
<td>0.46 ± 0.55</td>
<td>0.32 ± 0.26</td>
<td>P = 0.81</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>1.12 ± 1.53</td>
<td>6.65 ± 8.66</td>
<td>P = 0.07</td>
</tr>
<tr>
<td>IL-1β (pg/mL)</td>
<td>1.59 ± 1.82</td>
<td>1.81 ± 1.46</td>
<td>P = 0.55</td>
</tr>
<tr>
<td>g-CSF (pg/mL)</td>
<td>12.33 ± 15.20</td>
<td>28.28 ± 30.51</td>
<td>P = 0.23</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>0.53 ± 1.18</td>
<td>1.98 ± 0.68</td>
<td>P = 0.30</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>0.99 ± 1.71</td>
<td>11.12 ± 15.85</td>
<td>P = 0.03</td>
</tr>
<tr>
<td>IL-12 (pg/mL)</td>
<td>0.07 ± 0.09</td>
<td>0.27 ± 0.60</td>
<td>P = 0.53</td>
</tr>
</tbody>
</table>
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)

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-inflammatory cytokines

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

Mechanism of Action: Immune Cells

- Tumor cell killing:
  - CD8 cells
    - Monocyte and CD4+ T-cell-derived Th1-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+/CD16dim/CD56+/NKG2A+/perforin+ phenotype

Mechanism of Action: Urothelial Summary

- Urothelial Cell Action:

<table>
<thead>
<tr>
<th>Process</th>
<th>Evidence for role in response to BCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attachment of BCG to the urothelium</td>
<td>BCG attaches to urothelial cells through bridging of FAP and integrin α5β1 by fibronectin Blocking fibronectin can reduce BCG efficacy in the mouse model</td>
</tr>
<tr>
<td>Internalization of BCG by bladder cancer cells</td>
<td>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</td>
</tr>
<tr>
<td>Immune system recruitment by bladder cancer cells</td>
<td>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</td>
</tr>
<tr>
<td>Direct cytotoxicity of BCG against bladder cancer cells</td>
<td>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</td>
</tr>
</tbody>
</table>

# Mechanism of Action: Summary

<table>
<thead>
<tr>
<th>Immune system component</th>
<th>Evidence for role in response to BCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>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.</td>
</tr>
<tr>
<td>NK cells</td>
<td>Infiltration of NK cells in bladder wall of BCG-treated mice. NK cells are cytotoxic against BCG-infected bladder cancer cells <em>in vitro</em>. NK cells are required for response to BCG in the mouse model.</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>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.</td>
</tr>
<tr>
<td>Macrophages</td>
<td>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 <em>in vitro</em>.</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>Immature dendritic cells can be found in the urine of patients treated with BCG. <em>In vitro</em>, BCG-exposed dendritic cells can induce T cells to exhibit cytotoxicity against BCG-infected bladder cancer cells.</td>
</tr>
<tr>
<td>Cytokines and chemokines</td>
<td>Massive release of cytokines and chemokines occurs in urine of patients treated with BCG. BCG therapy shifts the urinary cytokine milieu from T\textsubscript{\text{h}}\textsubscript{2}-like to T\textsubscript{\text{h}}\textsubscript{1}-like. Augmentation of a T\textsubscript{\text{h}}\textsubscript{1}-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 <em>in vitro</em>.</td>
</tr>
</tbody>
</table>

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

Bacillus Calmette-Guerin ("BCG") Immunotherapy for Bladder Cancer
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
  - 0 mo: BCG x 6 (INDUCTION)
  - 3 mo: BCG x 3
  - 6 mo: BCG x 3
  - 9 mo: BCG x 3
  - 12 mo: BCG x 3

\[ \text{cysto} \]
## Summary of Clinical Data in the Literature

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

*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

- 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

DONALD L. LAMM,*† BREN'T A. BLUMENSTEIN, JOHN D. CRISSMAN, JAMES E. MONTIE, JAMES E. GOTTESMAN, BRUCE A. LOWE, MICHAEL F. SAROSDY,‡ ROBERT D. BOHL, H. BARTON GROSSMAN,§ THOMAS M. BECK, JOSEPH T. LEIMERT AND E. DAVID CRAWFORD||

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
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

- 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
Intravesical BCG: Etiology of Failure

- UnderStaging-Inaccurate TURBT
- Large Tumor Volume
- Failure to Bind to Fibronectin
- Inadequate Immune Response-$T_{\text{Helper}}$ 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

Breast Cancer

- High frequency of CD8 TILs

Colorectal Cancer

- Sharma et al. PNAS 2007;104:3967-3972

Ovarian Cancer

- Jérôme Galon et al. Science 2006; 313:1960-1964


Presented at the Genitourinary Cancers Symposium

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Presented by Andrea B. Apolo.

Bacillus Calmette-Guerin ("BCG")
Immunotherapy for Bladder Cancer
MycoBacterial Cell Wall Extract

NK = natural killer
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.
*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)
3. Dual antigen carrier & adjuvant activates Dendritic Cells
4. Selectively activate CD8+ T-Cells
5. CD8+ T-cells circulate & eliminate tumor cells

*Over a dozen known UCC tumor antigens secreted, including: PRAME, Survivin, LAGE antigens, MAGE antigens...*
Potential Synergy Between BCG and HS-410

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

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

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<thead>
<tr>
<th>Cytokine</th>
<th>Patients (n=7) with recurrence (Mean ± SD)</th>
<th>Patients (n=13) without recurrence (Mean ± SD)</th>
<th>Mann-Whitney U-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x 10^3/μL)</td>
<td>12.60 ± 12.53</td>
<td>15.93 ± 17.39</td>
<td>P = 0.72</td>
</tr>
<tr>
<td>GM-CSF (pg/mL)</td>
<td>0.46 ± 0.55</td>
<td>0.32 ± 0.26</td>
<td>P = 0.81</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>1.12 ± 1.53</td>
<td>8.65 ± 8.66</td>
<td>P = 0.07</td>
</tr>
<tr>
<td>IL-1p (pg/mL)</td>
<td>1.59 ± 1.82</td>
<td>1.81 ± 1.46</td>
<td>P = 0.55</td>
</tr>
<tr>
<td>G-CSF (pg/mL)</td>
<td>13.33 ± 15.20</td>
<td>28.28 ± 30.51</td>
<td>P = 0.23</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>0.52 ± 1.18</td>
<td>1.98 ± 0.48</td>
<td>P = 0.89</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.99 ± 1.71</td>
<td>11.12 ± 15.85</td>
<td>P = 0.20</td>
</tr>
<tr>
<td>IL-12D (pg/mL)</td>
<td>0.07 ± 0.09</td>
<td>0.27 ± 0.60</td>
<td>P = 0.53</td>
</tr>
</tbody>
</table>

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.

Bacillus Calmette-Guerin ("BCG") Immunotherapy for Bladder Cancer
Evidence of CD8+ Tumor Infiltration in HS-410 Phase 1 Study

<table>
<thead>
<tr>
<th></th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
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</thead>
<tbody>
<tr>
<td><strong>CD3</strong></td>
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<td></td>
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<tr>
<td>Non-Responder</td>
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<tr>
<td>25-004</td>
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<tr>
<td><strong>CD8</strong></td>
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