Predicting Response to BCG

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BCG: Since 1970s ... stuck in the past?...

Lesterhuis et al, Nature Reviews Drug Discovery, 2011

~ 1.2 Million Doses of BCG used globally for Bladder Cancer
BCG: Unsurpassed Immunotherapy

- BCG most effective therapy for NMIBC
  - Reduces recurrence, progression; prevents deaths

- However, ~30% patients fail BCG therapy
  - In non-responders, disease often progresses before curative cystectomy - decreased survival

- If we can identify non responders early, offer alternate therapy at earlier time point
Tumor Biomarkers

- Tumor P$_{53}$
  - Correlated: Saint, 2004; Lopez-Beltran, 2004; Palou, 2009
  - Not correlated: Lebret, 1998; Zlotta, 1999; Peyromaure, 2002; Esuvaranathan, 2007

- Same problem with Ki-67, Rb ....
BCG Efficacy Dependent on Optimal Schedule

- **Induction**: weekly x 6 weeks;
- **Maintenance**: 3 weekly instillations at 3 mos, 6 mos and then q 6 mos

**SWOG 8507**: Lamm et al, 2000
**EORTC 30911**: Sylvester et al, 2009
**Japan Multicenter Trial**: Hinostu et al, 2010
BCG Maintenance: Not Created Equal
Only SWOG protocol shows clear benefit

Kamat & Porten, Eur Urol, 2014
Optimal BCG
Urinary IL-2 Assay

De Reijke, J Urol, 161: 67, 1999
Repeated intravesical instillations are required to achieve robust inflammatory responses in the bladder.

Representative patient ($n = 30$)

Week 1

Week 3

‘Prime / boost’ pattern for an innate immune response??

First evidence for a recall response to secondary challenge of the innate immune system.

Slide: Albert ML, Pasteur Institute
Can the inflammatory response of patients predict response to BCG?

Can immune response biomarkers identify optimal dose, duration and schedule of BCG?
Antibody Responses to *Bacillus Calmette-Guérin* during Immunotherapy in Bladder Cancer Patients

Wendell D. Winters and Donald L. Lamm

*Departments of Microbiology [W.D.W.] and Urology [D.L.L.], University of Texas Health Science Center, San Antonio, Texas 78284*

- In contrast to the observations made in the melanoma ...
- BCG antibody levels titers did not correlate with tumor response in bladder cancer patients ....

Published in 1981
Cytokines (eg IL-8) and BCG response

Sagnak L et al, 2009
Prospective Trial: Markers of Response to Intravesical BCG

Hypotheses

- Comprehensive Panel of Cytokine response to BCG will differentiate responders from non-responders
- Innate intricacies of the immune response
- Genes which regulate inflammatory mechanisms will impact the response to BCG
- Cytogenetically abnormal cells: patterns will predict clinical tumor recurrence

PI: Kamat; NCT01007058
Study Design

Patients
(n = 125*)
- Intermediate to High Risk Disease
- Eligible for BCG Induction and Maintenance
- Followed q 3 mos; outcomes at 24 mos

Specimen Collection
- Baseline: Blood, Urine, Cells, Tissue
- Cytokines: 6\textsuperscript{th} week (I) and 3\textsuperscript{rd} week (M) of BCG
- FISH: post TUR, 6 week, 3 mos, 6 mos

Assays
- Cytokines: Luminex Platform (with Allere)
- FISH: Urovysion ® Assay; Telomeres
- SNPs: Inflammation Pathway

NCT01007058
Cytokines and BCG Response

- **Cytokine** response to BCG does differentiate responders from non-responders

  - Responders have higher levels of BCG induced cytokines at BCG - 6

  - Magnitude of induction of cytokines correlates with recurrence rate and time to recurrence

  - Complex interplay of cytokines
ΔIL-8 with 6\textsuperscript{th} BCG

\begin{center}
\begin{tikzpicture}
\begin{axis}[
width=\textwidth,
height=0.8\textwidth,
xtick={0,5000,10000},
xticklabels={0,5000,10000},
]
\addplot+[only marks] table {
0.0041
};
\end{axis}
\end{tikzpicture}
\end{center}

\textit{p} = 0.0041
ΔIL-8 with 6th BCG

p = 0.04
Risk function for ΔIL-8 with 6th BCG
AUC For Cytokine Nomogram
85.5% (95% CI: 77.9 - 93.1)
Proposed SWOG Study: T cell priming with intradermal BCG for bladder cancer

PI: Robert Svatek, MD
BCG Response: PPD mixed results

- Biot et al: 5 yr recurrence free survival
  - 75% in PPD-positive patients vs
  - 44% in PPD-negative patients [p<0.05]

- Other studies: no correlation

- Prospective randomized trial: Luftenegger
  - 156 patients: intradermal BCG + iBCG vs iBCG
  - No difference in outcome

36 new biomarkers for BCG induced bladder inflammation

1. Plasma proteins
   - IgM
   - alpha-2 Macroglobulin
   - fibrinogen
   - SHBG
   - von Willebrand Factor
   - serum amyloid P
   - apolipoprotein CIII
   - PAI-1
   - apolipoprotein H
   - α1 antitrypsin
   - C reactive protein
   - IgA
   - thyroxine binding globulin
   - BDNF
   - adiponectin

2. Stromal cell derived
   - G-CSF
   - MMP-9
   - RANTES
   - TIMP-1
   - VEGF
   - IL-1β
   - IL-6
   - IL-8
   - IP-10
   - MCP-1
   - GM-CSF
   - TNFa

3. Cellular immune response
   - EN-RAGE
   - ENA-78
   - IL-10
   - IL-16
   - IL-18
   - IL-1ra
   - IL-2
   - MIG
   - MIP-1
   - MPO

Statistically significant values, induced after 4h BCG on week 3
p < 0.05 using FDR correction for multiple analyte testing

Slide: Matthew Albert, Pasteur Institute
microRNAs and BCG response

- microRNAs such as miR-155 drives CD4+ T cells towards Th1 differentiation
- miR-29 & miR-21 may inhibit Th1 cell differentiation and and/or promote Th2 response
- BCG response prediction?

Baumjohann, Nature Reviews, 2013
Th2/Th1 ratio Predicts BCG Response

A Hematoxylin & Eosin

B Hematoxylin & Eosin

GATA 3⁺ / T-bet⁺

4.85

p = 0.001

Normal Bladder

Tis Bladder Tumor

BCG responder

BCG non responder

Nunez-Nateras R, Urol Onc, 2014
Neoadjuvant Trial in Bladder Cancer Patients
Perivascular infiltration of cells into tumor tissues with
10 mg/kg/dose of anti–CTLA-4

Carthon B C et al. Clin Cancer Res 2010;16:2861-2871
CD4+ICOS\textsuperscript{hi} T cells correlate with clinical outcome

14 melanoma: anti-CTLA-4 at 10 mg/kg/dose.

Carthon BC et al, CCR, 2010 (MDACC)
Gene Expression/Signature

- Training set 48 cT1 tumors
  - 424 and 287 genes associated with RFS and PFS, respectively.
  - cell-mediated immune response, inflammatory response, cellular growth, and proliferation.
- Validation set: 32 cT1 tumors
  - 24 genes (12 in recurrence and 12 in progression) with the highest score

Kim YJ et al, CCR, 2010
Gene Expression/Signature

Limitations: No Maintenance BCG; 13% G1; 66% G2; No re-TUR

KimYJ et al, CCR, 2010
Use of Fluorescence In Situ Hybridization to Predict Response to Bacillus Calmette-Guérin Therapy for Bladder Cancer: Results of a Prospective Trial

Ashish M. Kamat,*† Rian J. Dickstein,*‡ Fabrizio Messetti,*‡ Roosevelt Anderson,*‡ Shanna M. Pretzsch,*‡ Graciela Noguera Gonzalez,*‡ Ruth L. Katz,§ Abha Khanna,*‡ Tanweer Zaidi,*‡ Xifeng Wu,*‡ H. Barton Grossman|| and Colin P. Dinney¶

**Table 2.** Recurrence rates based on timing of first positive FISH assay

<table>
<thead>
<tr>
<th>Time + Assay Result</th>
<th>Recurrence Rate by 12 Wks (%)</th>
<th>Overall Recurrence Rate (%)</th>
<th>Overall Progression Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg FISH at baseline:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neg at 6 wks</td>
<td>5.1</td>
<td>12.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Pos at 6 wks</td>
<td>40.0</td>
<td>60.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>

**Baseline FISH**

**FISH at six weeks**

**FISH at three months**

**FISH at six months**

DOI:10.1016/j.juro.2011.10.144
Strains Matter?

Connaught BCG: decreased recurrences
BUT no maintenance used

Strains Matter?

(a) Intravesical BCG instillations

- Analysis of adaptive immune response

(b) CD8

- Mtb32309-318 Tetramers

(c) Mtb32309-318 tetramer positive cells (% CD8 T cells)

(d) CFUs/DLN

What Next?

Optimal BCG Therapy

Inducible Factors e.g. Cytokine response

Patient Factors e.g. Gene Profile

Tumor Micro Environment

miRNAs (155, 21, 29, 146a)

FoxP3, Treg, CD4+ ICOS+

TAMs, CD68; TIDCs, CD83; GATA-3+; T – Bet+

IL-6, IFN-γ, TNF-α, IL-2, IL-1b, IL-8, IL-10, IL-12(p40), IL-12(p70), IL-18 and TRAIL.
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