Clinical efficacy of different BCG strains in the treatment of NMIBC: myth or reality

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Conflicts of Interest

• Sanofi, Merck, Janssen, Ferring, AstraZeneca, Verity
1. Why while millions of patients have been treated with BCG for prevention of NMIBC no clinical difference has been shown among studies despite the use of various strains worldwide?

2. What can we learn from BCG used as an anti-tuberculosis vaccine?

3. How can we explain the absence of difference in clinical efficacy for BCG in NMIBC throughout the globe? An insight into its mechanism of action
Tan GH, Kuk C, Zlotta AR. Are there differences among Bacillus Calmette-Guérin (BCG) strains regarding their clinical efficacy in the treatment of non-muscle invasive bladder cancer? The jury is still out but the answer is likely no.

Mycobacteria

Non-cultivable
M. leprae

Skin ulcers
M. ulcerans
M. balnei

Typical
M. tuberculosis
M. bovis
M. bovis BCG
M. microti
M. africanum
M. canetti
M. pinnipedii

Atypical (Runyon’s classification)
Based on growth rate & pigment.

Photochromogens
M. kansasii, M. marinum, M. simiae

Scotochromogens
M. scrofulaceum, M. gordonae

Non-photochromogens
M. avium-intercellulare, M. xenopi

Saprophytic
M. smegmatis
M. butricum
M. phlei

Rapid-growers
M. fortuitum, M. chelonei
History of BCG: Bladder Cancer

• Morales et al, J Urol 1976
• First use of intravesical BCG (with Martinez Pineiro, Spain 1975)
• 120 mg in 50cc of saline via urethral catheter into the bladder
• Strain: Frappier (Montreal); packaged in vials of 6
• Noted at least 3-6 weeks 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)
BCG schedule is empiric

• Schedule for BCG intravesical instillations very empiric!

• 6 weekly instillations used for 30 years but any rationale? Because indeed A Morales received six vials from the pharma company in 1976......
History and genealogy of BCG substrains

DU2 Group 1
- BCG Russia
  - Rec-A_D140-RD Russian (ΔRv3698) 1924
- BCG Moreau
  - RD16
- BCG Tokyo
  - RD Japan (ΔRv3405c) 1924*

"Early" strains

DU2 Group 2 Δ int
- BCG Sweden
- BCG Birkhaug

1926

"Late" strains

DU2 Group 4 Δ int
- BCG Tice
  - RD15
- BCG Frappier
  - RD Frappier 1948
- BCG Connaught

1934

DU2 Group 3 Δ int
- BCG Prague
  - RD Denmark (ΔRv1810)_phoR_91-ko
  - 1954
- BCG Glaxo
  - 1989
- BCG Danish
- BCG Mérieux
RD1

- Common to all BCG vaccine strains is the deletion of region of difference one (RD1) that is preserved in M. bovis and M. tuberculosis.
- Loss of this region known to have been the critical event in the attenuation of the initial M. bovis strain.

<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>Moscow®</td>
<td>103</td>
<td>90.5</td>
<td>SII-ONCO-BCG® (Serum Institute, India)</td>
<td>120</td>
<td>3–57 × 10^8</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 × 10^8</td>
<td>No</td>
<td>Present/High</td>
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<tr>
<td>Connaught</td>
<td>450</td>
<td>79 (70–92)</td>
<td>Immunocyst® (Sanofi-Aventis, France)</td>
<td>81</td>
<td>1.8–15.9 × 10^8</td>
<td>NT</td>
<td>NT</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 × 10^6</td>
<td>No</td>
<td>Present/High</td>
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<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>OncoTice® (Merck, USA)</td>
<td>12.5</td>
<td>2–8 × 10^8</td>
<td>Yes</td>
<td>Absent/ Present</td>
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<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>
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<tr>
<td>S. African</td>
<td>13</td>
<td>69</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
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<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-Medac® (Medac, Germany)</td>
<td>80</td>
<td>2–30 × 10^8</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>
CONCLUSION

• No meaningful correlations between BCG strain and survival outcomes (RFS, CSS, and OS)
Progression-free survival
NO DIFFERENCE!

Rentsch et al
Eur Urol 2014
**BCG as anti-tuberculosis vaccine**

**BCG — different strains, different vaccines?** *Behr M. The Lancet Infectious Diseases 2002: 86-92*
Protection against Mycobacterium tuberculosis infection (TB) as determined by interferon γ release assay (QuantiFERON) in children vaccinated with BCG.

A Roy et al. BMJ 2014;349:bmj.g4643
Conclusions

• Evidence from animal and human studies shows that there are significant differences in the immune response induced by different BCG vaccine strains.

• However: lack of data demonstrating the superiority of individual BCG vaccine strains!

• Pasteur 1173 P2, the Danish 1331, the Glaxo 1077 (derived from the Danish strain), the Tokyo 172-1, the Russian BCG-I, and the Moreau RDJ strains (NIBSC and WHO, 2004).

• The concentration of live particles in the vaccines ranges from 50,000 to 3 million per dose, according to the strains.

• Each strain has a different immunological profile

• There is no standardized production of BCG vaccine between manufacturers
WHY?
BCG very complex live attenuated micro-organism

- Cell wall
- Inner membrane
- Insoluble cell wall
- Glucane
- Arabinomannans
- Fatty acids
- Cytoplasm

- Culture filtrate
- AG 85 (32kd, fibronectin-binding)
- P64 (~ hsp 65)
- 22 kd
- Pst2
- Pst3
Mycobacterial (acid-fast) cell wall

- Porin
- Free lipids
- Branched and capped portion of LAM
- Mycolic acids
- Arabinan portion of LAM
- Pentaarabinosyl motifs
- LM portion of LAM
- Arabinan
- Linker
- Galactan
- Peptidoglycan
- Associated plasma-membrane proteins
- PIMs
- Polyprenyl sugars
- Plasma membrane
Cytoplasmic proteins
Cell wall proteins

Ag 85
22 kDa
PstS-2 and -3
Heat Shock proteins
ESAT-6
AG 85 complex

• Central role in synthesizing major components of the inner and outer leaflets of the mycobacterial outer membrane

• Binds Fibronectin!


IL-12 PRODUCTION

Zlotta et al, 2001
Many other parameters than the strain account for BCG efficacy in Bladder Cancer!!!!!
Toll-like receptor (TLR)

- Toll-like receptor (TLR) pathways are of particular interest in cancer immunotherapy.
- TLRs: family of receptors that bind to common components of many pathogens as well as signals released by damaged cells.
- Expressed on many innate immune cells, including dendritic cells. The most potent of all antigen presenting cells, dendritic cells play a pivotal role in bridging the innate and adaptive responses.
- TLRs present on a large portion of bladder tumors where higher TLR expression is correlated with less invasive tumors
Some individuals show a higher level of natural resistance than others to infection with certain intracellular pathogens, including *Mycobacterium bovis* BCG (BCG).

Gene encoding *Nramp* 1 (natural resistance-associated protein 1) exists in two allelic forms, differing for a point mutation.

*NRAMP1* and *hGPX1* gene polymorphisms to BCG response showed that the *NRAMP1* D543N G:G genotype displayed decreased CSS.

Gene polymorphisms that led to reduced RFS or increased recurrence risk post-BCG: *XPA, XPC, XPD, XPG, XPF, ERCC1, ERCC2, ERCC6, XRCC1, XRCC4, APEX1, GSTM1, CCNB1, PON1, and SLCO1B1*
Conclusions

• Variability in BCG strains not demonstrated to affect clinical outcome in bladder cancer
• Variability in BCG strains not demonstrated to affect efficacy in tuberculosis vaccination
• Mechanism of action of BCG in bladder cancer complex and involving many subcomponents which individually can all stimulate a robust adequate immune response
• Many other parameters than BCG strains account for the clinical efficacy of BCG
• At times of BCG shortage, differences in strains are not a concern