Mathematical model of BCG treatment personalization for urinary bladder carcinoma

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MicroandMacro 2015
Urinary tract: collect, store, and excrete the urine

- Bladder lined with transitional epithelium - urothelium
  - has an ability to be stretched to an exceptional degree,
  - functions as a urinary barrier,
  - lack of direct blood and lymphatic supply,
  - nutrients are delivered by diffusion.

Kidney

Pelvis and Ureter

Bladder

Urethra

urothelium
Classification of Bladder Cancers

History of the BC treatment problem

- In 1976 Dr. Alvaro Morales published a paper in the Journal of Urology that the use of intravesical vaccine Bacillus Calmette-Guerin (BCG) for the bladder cancer treatment decreased the recurrence and progression of this cancer.

- Until now BCG is the most effective treatment. Efficacy is assessed by disappearance of pathology in 50-60% of patients for 5-10 years after diagnosis.

- The effect is achieved by stimulating the immune and inflammatory response. BCG stimulates immune response that leads to the destruction of tumor cells.

- This cumulative effect can be explained, but still not completely.
Cascade of immune response induced by intravesical BCG instillation

Limitations to BCG success rates

- Although BCG is considered as the “gold standard” treatment, about half of the patients do not experience a complete response after BCG treatment. They suffer from tumor recurrence within one year, and about 80% of the patients will have recurred at 5 years.

- Therefore, there is a need to improve this protocol.
Dependence of bladder cancer incidence and mortality on age

Chisov et al., 2004
Reasons for using IL-2

- In bladder cancer patients, the presence of IL-2 in the urine after BCG treatment is correlated with a successful outcome.

- FDA has approved treatment with IL-2 injections to boost the immune system’s reaction against the tumor in other malignancies.
The cell lines involved in the BCG+IL-2 model
Mathematical model for BCG + IL-2 immunotherapy

\[
\frac{dB}{dt} = \sum_{m=0}^{N-1} b \delta(t - m \tau) - p_1 AB - p_2 BT_u - \mu_B B,
\]

\[
\frac{dA}{dt} = \gamma + \eta AB - p_1 AB - \lambda AT_u \left( \frac{I_2}{I_2 + g_I} \right) - \mu_A A,
\]

\[
\frac{dA_B}{dt} = p_1 AB - \beta A_B - \mu_{A_1} A_B,
\]

\[
\frac{dA_T}{dt} = \lambda AT_u \left( \frac{I_2}{I_2 + g_I} \right) - \beta A_T - \mu_{A_t} A_T,
\]

\[
\frac{dE_B}{dt} = \frac{\beta_B A_B I_2}{A_B + g} - p_3 T_i E_B - \mu_E E_B,
\]

\[
\frac{dE_T}{dt} = \frac{\beta_T A_T I_2}{A_T + g} - p_3 T_u E_T - \mu_E E_T,
\]

\[
\frac{dI_2}{dt} = (A_B + A_T + E_B + E_T) (q_1 - q_2 \frac{I_2}{I_2 + g_I}) + \sum_{m=0}^{N-1} i_2 \delta(t - m \tau) - \mu_{I_2} I_2,
\]

\[
\frac{dT_i}{dt} = p_2 BT_u - p_4 E_B T_i,
\]

\[
\frac{dT_u}{dt} = rT_u \left( 1 - \frac{T_u}{K} \right) - p_2 BT_u - (\lambda AT_u + \alpha E_T T_u) \left( \frac{I_2}{I_2 + g_I} \right) \left( \frac{g_T}{T_u + g_T} \right).
\]
Dynamics of tumor-Ag-activated APC (TAA-APC)

\[ \frac{dA_T}{dt} = \lambda AT_u \left( \frac{I_2}{I_2 + g_I} \right) - \beta_1 A_T - \mu A_1 A_T \]

This term is proportional to the number of non-activated APCs and uninfected tumor cells, with a rate coefficient \( \lambda \). Immature dendritic cells do not mature in the absence of inflammatory environment. We multiply this term by an IL-2 dependent term, to propose that in the absence of IL-2 the production of \( A_T \) stops while in the presence of external IL-2 the production term is close to 1.

Immature DC \( \Rightarrow \) TAA-APC: 50-75\% of \( 5 \times 10^6 \) DC within 24 h.

\[ \lambda AT_u = A_T \quad A_T = 0.5 \times 5 \times 10^6 \implies \lambda = 0.1 \times 10^{-6} \text{ day}^{-1}. \]
Dynamics of CTLs that react to tumor antigen

\[
\frac{dE_T}{dt} = \frac{\beta_T A_T I_2}{A_T + g} - p_5 T_u E_T - \mu_E E_T
\]

- **Migration term**: \( \frac{\beta_T A_T I_2}{A_T + g} \)
- **Activation term**: \( p_5 T_u E_T \)
- **Death term**: \( \mu_E E_T \)

- Tumor-effector CTLs \( E_T \) differentiate from naive T lymphocytes in lymphoid tissues and migrate to infected areas in response to signals released by TAA-APC (\( A_T \)).
- The migration element is proportional to TAA-APC and IL-2 with a maximal rate coefficient \( \beta_T \).
- Death rate coefficient: In the presence of IL-2 the effector cell median life span can be extended. Therefore, for the computer simulations we used two interchangeable values for death rate, one in the absence of IL-2, and one in the presence of IL-2.
Estimation rate of recruitment of effector cells in response to signals released by TAA-infected and activated APCs

- $\beta_T$ is a parameter that transmits the effect of $A_T$ and IL-2 to the number of effector cells.
- Kronin et al. (2001) incubated CTLs with APCs in the presence or absence of IL-2.
- This is the number of CTLs reached after 4 days of induction

\[ \text{X cells} \times 0.14 \text{ cpm/cell} = 24,000 \text{ cpm} \]
\[ \frac{171,429 - 20,000}{4} = 37,857 \text{ cells/day} \]

- IL-2 dose was 100 IU.
- The entire experiment was

\[ \beta_T = \frac{37,857}{100} \times 4 = 1514 \text{ cells/} (\text{day} \cdot I_2) \]
Estimation of death rate coefficient, \( \mu_E \): in the absence and in the presence of IL-2

- We assume that the number of CD8+ cells decays exponentially so that \( N_f = N_0 e^{-\mu t} \) where \( N_f \) = final population size and \( N_0 \) = initial population size.
- Yee et al. (2002) reported the death rates of effector cells in the presence or absence of IL-2.
- Their study shows an average of 1.47% of peripheral blood CD8+ cells at day 1 and a drop to 0.48% by day 7 in the absence of IL-2.

\[
0.0048 = 0.0147 e^{-\mu_{E1} t} \quad \text{for } t = 6 \text{ days } \Rightarrow \mu_{E1} = 0.1865 \text{ day}^{-1}
\]

- In the presence of IL-2, the corresponding rates are 1.52% at day 1 and 0.97% at day 14.

\[
0.0097 = 0.0152 e^{-\mu_{E2} t} \quad \text{for } t = 13 \text{ days } \Rightarrow \mu_{E2} = 0.0346 \text{ day}^{-1}
\]
The natural sources of $I_2$ are the activated immune APCs and CTLs with production rate $q_1$.

IL-2 external source as $i_2$, which is injected into the bladder

$I_2$ is consumed by activated APCs and CTLs. We assume that the rate of consumption is similar for both types of cells and denote its coefficient by $q_2$. 

\[
\frac{dI_2}{dt} = \frac{q_1(A_B + A_T + E_B + E_T) - q_2(A_T + A_B + E_T + E_B)I_2}{I_2 + g_1} + \sum_{m=0}^{N-1} i_2 \delta(t - m\tau) - \mu I_2
\]
Dynamics of uninfected tumor cells

\[
\frac{dT_u}{dt} = rT_u \left( 1 - \frac{T_u}{K} \right) - p_2 BT_u - (\lambda AT_u + \alpha E_T T_u) \left( \frac{I_2}{I_2 + g_I} \right) \left( \frac{g_T}{T_u + g_T} \right)
\]

- This term is to capture of \( T_u \) by APCs at rate coefficient \( \lambda \), and due to the elimination by \( E_T \) which destroy \( T_u \) at a rate coefficient \( \alpha \) (pathway B). We multiply this term by an IL-2 dependent term, that in the absence of IL-2 the production of \( A_T \) stops while in the presence of external IL-2 the production term is close to 1.

- The tumor produces a variety of mechanisms that reduce effector cell killing, then we multiply \( \frac{I_2}{I_2 + g_I} \) by \( \frac{g_T}{T_u + g_T} \), which accounts for inversely proportional reduction in killing rate, such that the term is equal to 1 and when

\[
T_u \to \infty, \quad \lim_{T_u \to \infty} \frac{g_T}{T_u + g_T} = 0
\]
Computer simulations for evaluating the efficacy of BCG protocol

Red – NR non-responders
Blue – CR complete response
Black – SR stable response
Green – PD partial disease
Simulated effect of BCG (induction) and BCG (maintenance) on 50 virtual patients

- **Red** – Tumor cells
- **Black** – BCG treatment
- **Red** – NR non-responders
- **Blue** – CR complete response
- **Black** – SD stable response
- **Green** – PR partial disease
Simulated effect of BCG+IL-2 (induction) and IL-2 (maintenance) on 50 virtual patients
Simulated effect of BCG+IL-2 (induction) and BCG+IL-2 (maintenance) on 50 virtual patients
Development of the individualized therapeutic regimen

A multi-disciplinary approach involving clinical sciences, biology and mathematical modeling may yield a real opportunity to increase disease-free survival of patients with BC in selection of i) dose, ii) frequency of BCG administration, iii) adjuvant therapy.
Biological Markers

- **Pre–BCG treatment biological markers:**
  - Multiplicity – numbers of polips
  - stage/grade and history (number of TURs before BCG).

- **Post–BCG treatment**
  - IL–6/IL–10 ratio
  - IL–2 cytokine
  - IL–8
  - IL–17

*These parameters play a role in assessing the individual risk of tumor progression and its invasiveness. There are no universally applicable predictive markers of BC until now.*
Biological Markers

- **Urinary markers:**
  - IL-6/IL-10 ratio
  - IL-2 cytokine
  - IL-8
  - IL-17

- **Biopsy:** Cell-cycle (p53; retinoblastoma protein (pRb); tumor suppressor protein p16; marker of cell proliferation Ki-67); Apoptosis (CD95; Caspase-3; Survivin; Bcl-2); Angiogenesis (MVD; VEGF); CK-20, HLA (MHC) Class I.

- **Blood markers:** TAA-cytotoxic T-Lymphocytes (TAA-CTLs): IL2/BCG treatment-related

Witjes J.A. et al., EAU Guidelines; April 2014*
Cellular Markers

- Cellular proliferation marker Ki-67 to be predictive of post-BCG tumor recurrence.

- CK20 expression was significantly correlated with recurrence-free survival (RFS).

- The correlation between tumor associated macrophages (TAMs) as a response to BCG therapy and RFS was significantly better in patients with lower TAM count.
Cytokines

- IL-2 cytokine secreted by activated T-cells and was introduced as an independent predictive parameter of BCG response. High levels of IL-2 in urine post-BCG were directly associated with an increased progression free survival (PFS).

- A time-dependent interplay between IL-2 and IL-10 levels show that repeated BCG alone may not be beneficial for the general population of BC patients.

- The IL-6/IL-10 ratio post-BCG has been evaluated in BC patients to show that if the ratio > .1 then recurrence-free survival (RFS) will be higher.

- A neutrophil chemotactic factor (IL-8) is secreted by macrophages post-BCG inducing chemotaxis of primary neutrophils and other granulocytes.
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<th>BCG Treatment Markers</th>
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<tbody>
<tr>
<td><strong>Tumor Associated Macrophages (TAM) not quantified</strong></td>
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<td>Tissue from 41 patients with BC post intravesic. BCG (increase in TAMs - tumor progression, poor prognosis)</td>
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<td>transurethral bladder biopsy, anti-CD68 mAbs (Dako, Denmark)</td>
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<td>3</td>
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<td>N/A (not studied)</td>
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<td><strong>Ki-67 (digital marker), best in combo with CK-20</strong></td>
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<td>309 pT1 BC patients from single urol. Center; adj. BCG performed; 49 mo follow-up</td>
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<td>Biopsy; mouse Ab, clone MIB-1, Dako, Germany</td>
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<td><strong>CK-20 (digital marker), best in combo with Ki-67</strong></td>
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<td>Biopsy; CK20 Ab, clone: IT-Ks 20.8 Dako, Denmark</td>
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<td>Vvoided urines from 127 subjects, cancer subjects (n = 64), non-cancer subjects (n = 63) were analyzed. The protein concns. of IL-8 assessed by ELISA. Data compared to a com. ELISA-based BCa detection assay (BTA-Trak) and urinary cytol. Area under the curve of a receiver operating characteristic (AUROC) was used to compare the performance of biomarker.</td>
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<tr>
<td>Urine; ELISA detection</td>
<td>4 (&gt;1,000 pg/mL)</td>
<td>3 (&lt;300 pg/mL)</td>
<td>2 (&lt;200 pg/mL)</td>
<td>1 (&lt;150 pg/mL)</td>
<td>0 (&lt;120 pg/mL)</td>
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<td><strong>IL-8 (REVERSE: high(er) levels as'd with better clinical outcome)</strong></td>
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<td>Basal reading of TAA-CTLs will dictate IL-2 treatment: increasing doses of s.c. IL-2 (0.25, 0.5, and 1.0 10^6 units) (Peripheral) blood</td>
<td>0/1</td>
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A summary: Pre- and post-BCG experimental biological markers for the BC mathematical model

Clinical Pathology: tumor size, stage, grade, multiplicity, CIS, number of TURs, age, gender
Genetic Polymorphisms: XPA, XPC, XPD, XPG, ERCC1, ERCC2, ERCC6, XRCC1, APEX1, IL1, IL6, IL8, FAS/FASL, TLR2, NRAMP1, PPARg, TNF-a, TGF-b
Micro RNAs: miR-9, miR-182, miR-200b
Epigenetics: methylated miR-137, miR-124-2, miR-124-3, miR-9-3, CACNA1A, PRDM2, BNIP3, TIMP3, APC, RARB, TIG1
Tumor Associated Macrophages (TAMs)
Human Leukocyte Antigen class I
Ki-67/CD20
Cytokines: IL-2, IL-6/10
Carbohydrate antigen sialyl-Tn
PODXL anti-adhesive glycoprotein
Tenascin-C
Leukocytes, dendritic cells, tumor associated macrophages (TAMs)
Human Leukocyte Antigen class I
Cytokines: IL-1, IL-2, IL-6/10, IL-8, IL-12, IL-17, IL-18
IFN-γ, TNF-β, TRAIL
Gc globulin
Hsp65
Telomerase
nNOS
NMP-22/Mcm5
P53, pRb, p21
Ki-67/CD20
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Thank You for your attention!