Mathematical model of BCG treatment personalization for urinary bladder carcinoma

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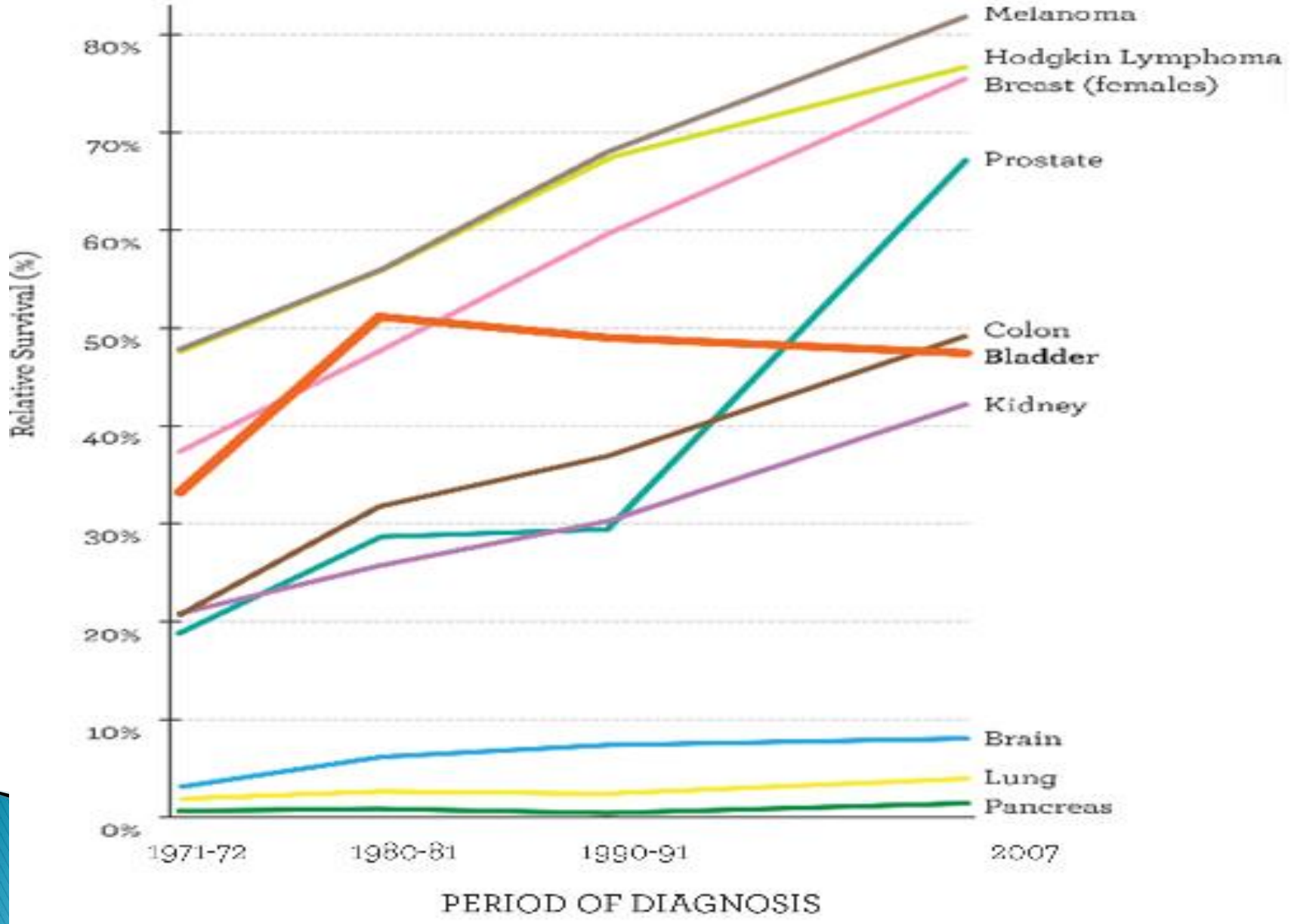
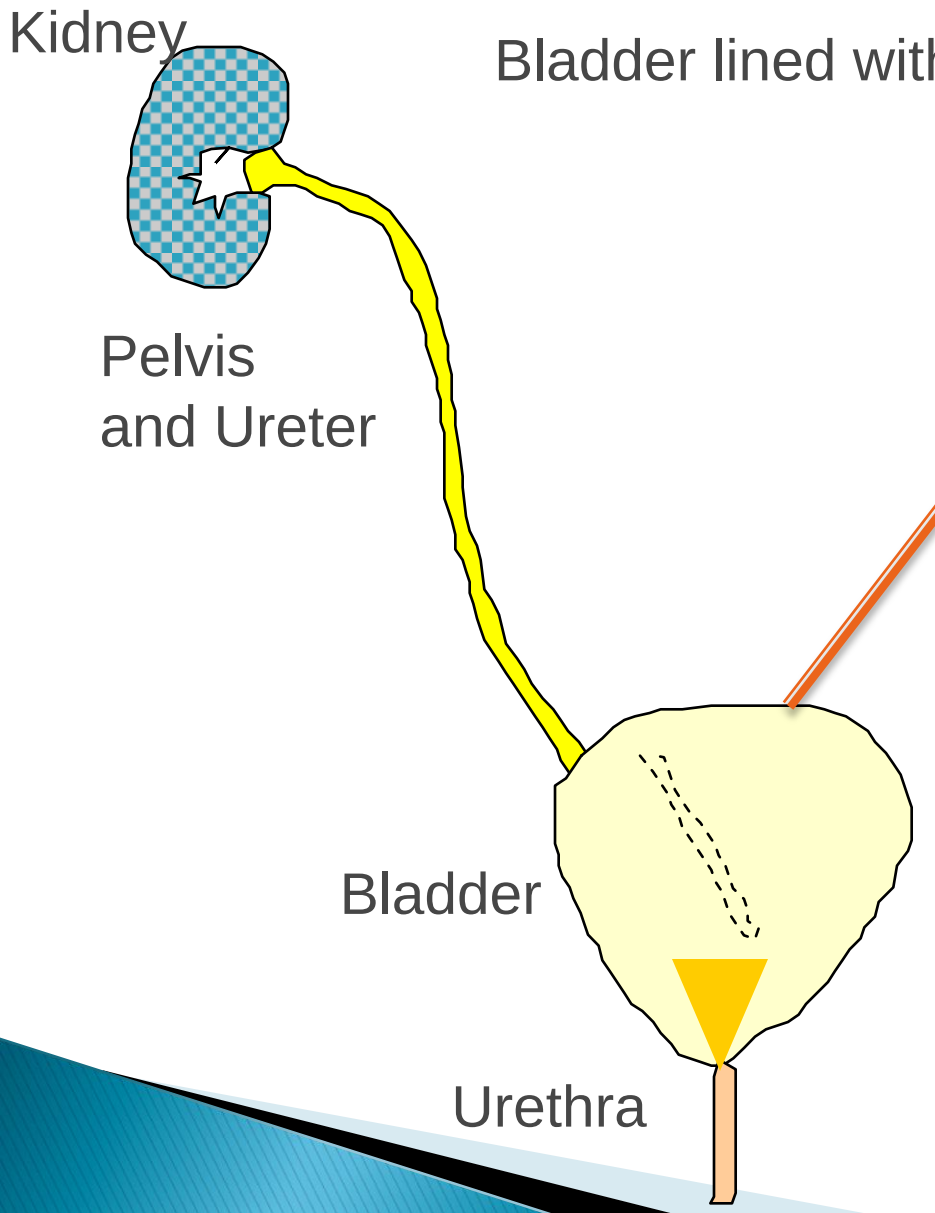
MicroandMacro 2015

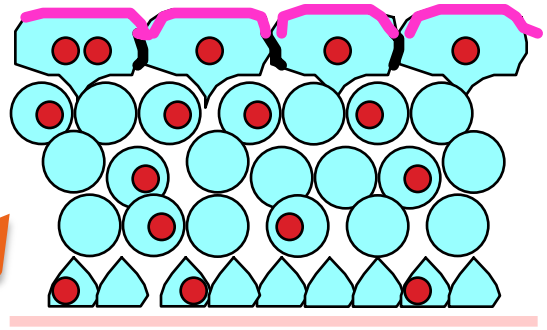
Survival rates from cancers in England over a 20 year period, *Journal of Clinical Urology, 2013*.



Urinary tract : collect, store, and excrete the urine



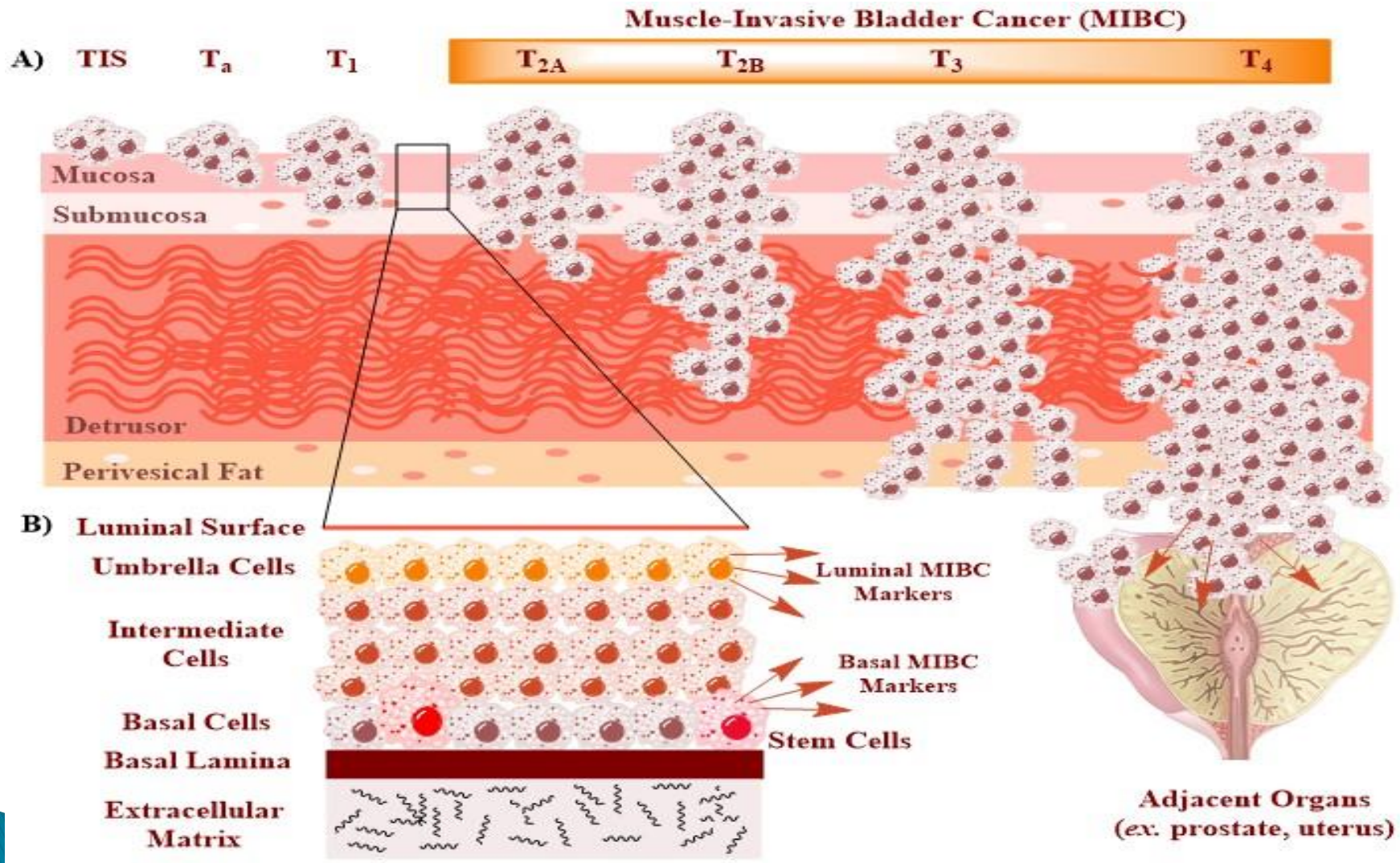
Bladder lined with transitional epithelium - urothelium



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.

Classification of Bladder Cancers



Kiselyov A., Bunimovich-Mendrazitsky S., Startsev V. 2015, *Journal of Urology.*

History of the BC treatment problem

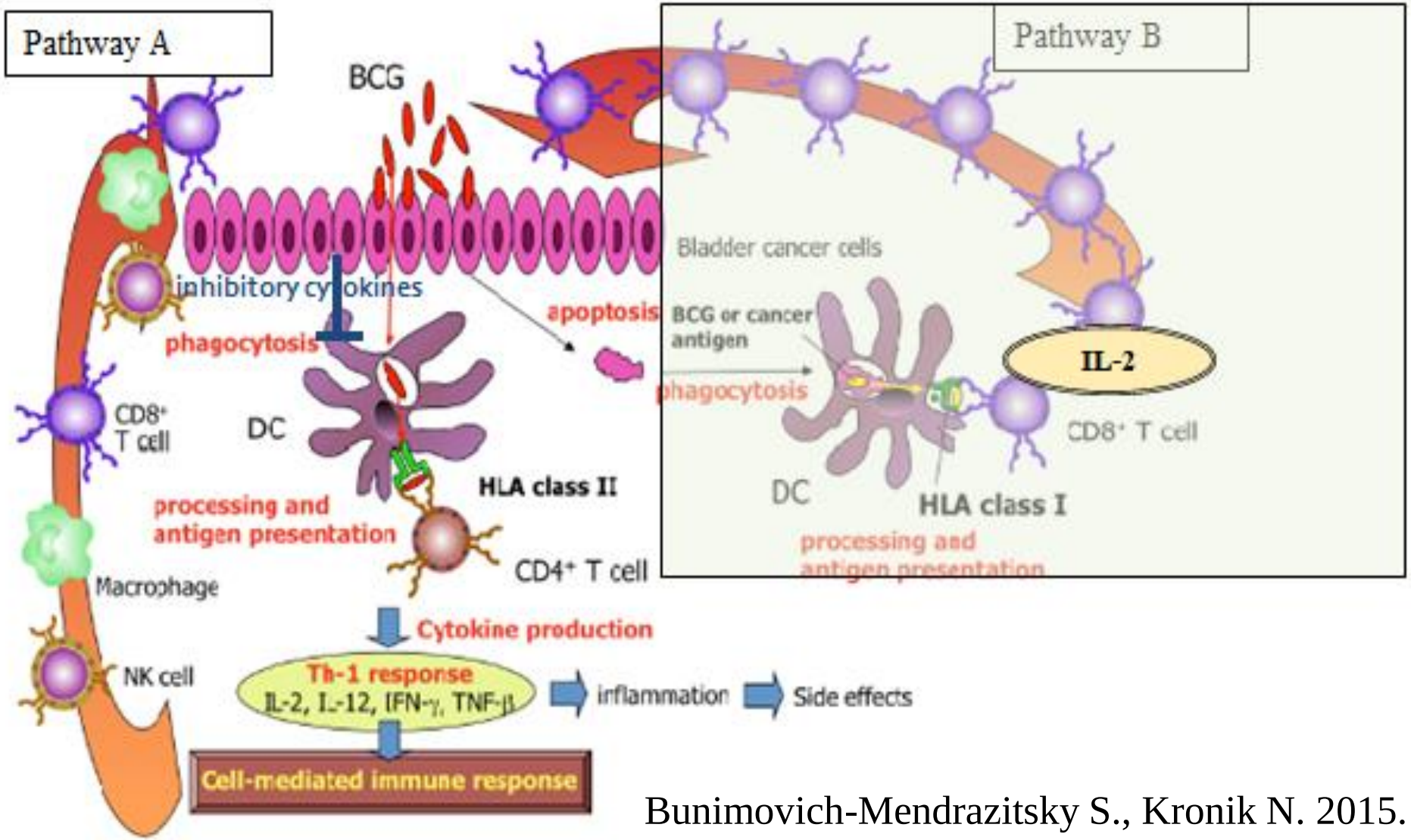
- In 1976 Dr. Alvaro Morales published a paper in the Journal of Urology that the use of intravesical vaccine Bacillus Calmette-Guerin (*BCG*) for the bladder cancer treatment decreased the recurrence and progression of this cancer.

- 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



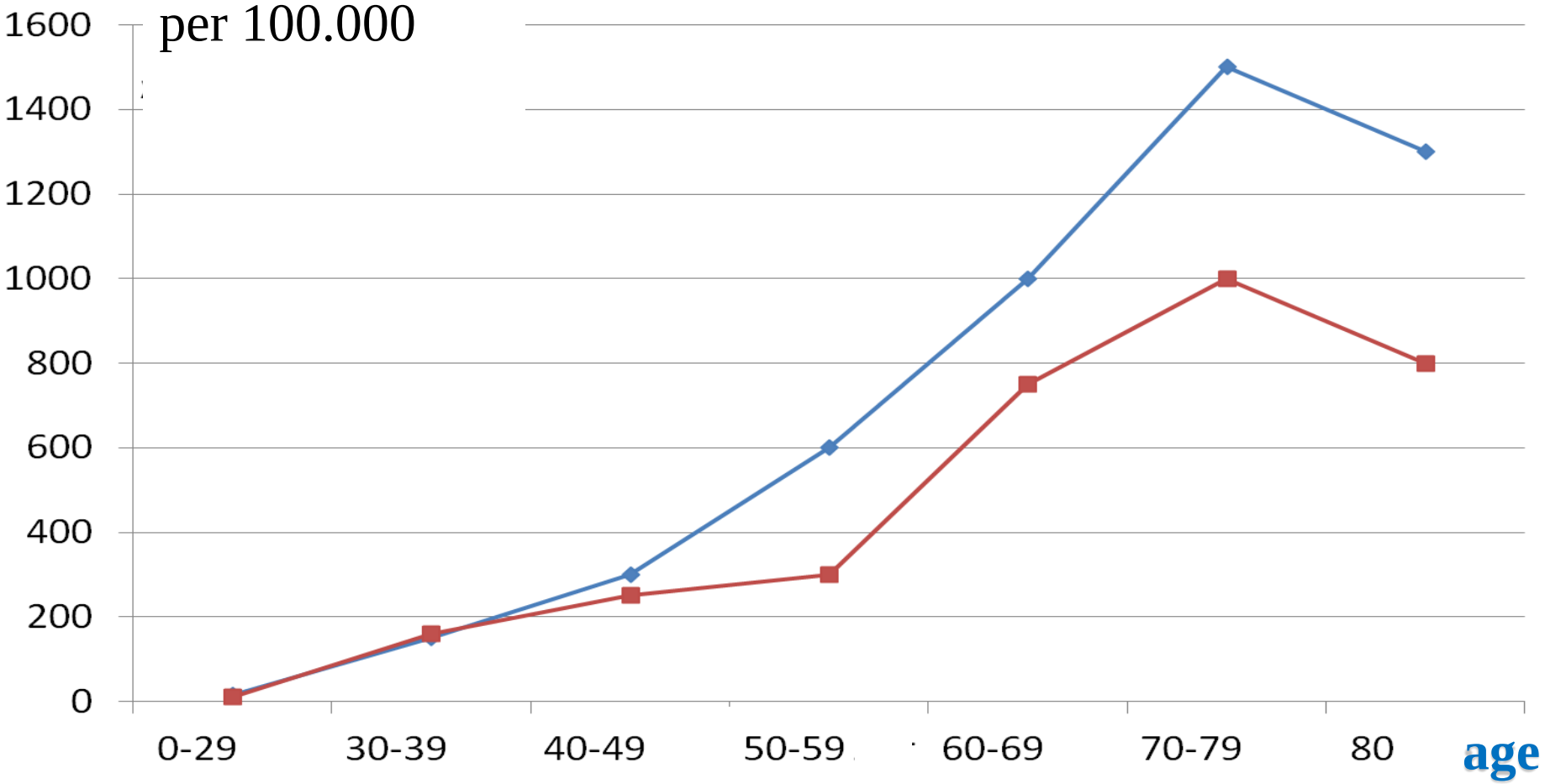
Bunimovich-Mendrazitsky S., Kronik N. 2015. *Mathematical Medicine and Biology*

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

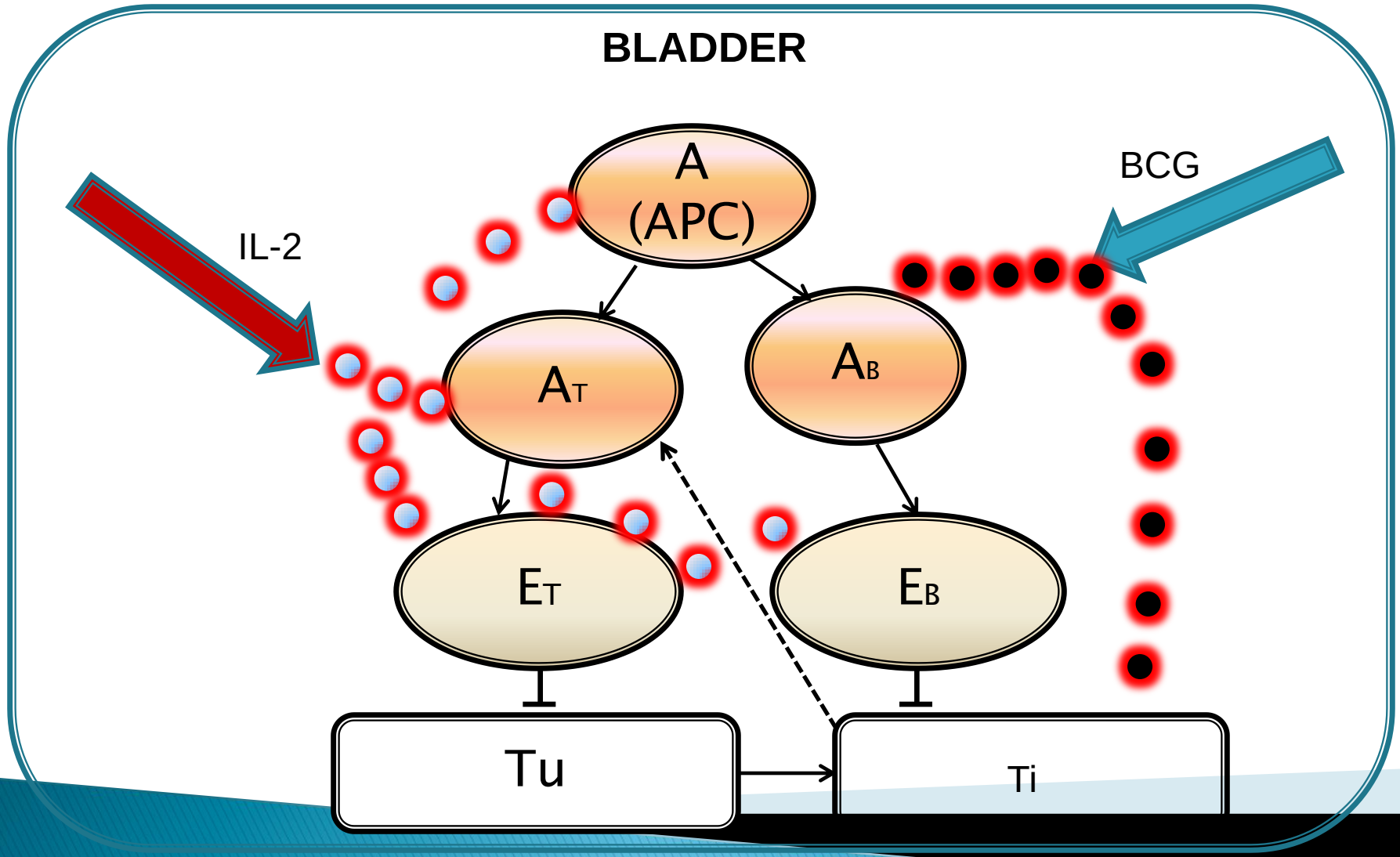


Chisov et al., 2004

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

Bunimovich-Mendrazitsky S., Kronik N. 2015.
Mathematical Medicine and Biology

Mathematical model for BCG + IL-2 immunotherapy

$$
\begin{cases}
\dfrac{dB}{dt} = \sum_{m=0}^{N-1} b\,\delta(t-m\tau) - p_1 AB - p_2 B T_u - \mu_B B, \\
\dfrac{dA}{dt} = \gamma + \eta AB - p_1 AB - \lambda\, A T_u \left(\dfrac{I_2}{I_2 + g_I}\right) - \mu_A A, \\
\dfrac{dA_B}{dt} = p_1 AB - \beta A_B - \mu_{A_1} A_B, \\
\dfrac{dA_T}{dt} = \lambda\, A T_u \left(\dfrac{I_2}{I_2 + g_I}\right) - \beta A_T - \mu_{A_1} A_T, \\
\dfrac{dE_B}{dt} = \dfrac{\beta_B A_B I_2}{A_B + g} - p_3 T_i E_B - \mu_E E_B, \\
\dfrac{dE_T}{dt} = \dfrac{\beta_T A_T I_2}{A_T + g} - p_3 T_u E_T - \mu_E E_T, \\
\dfrac{dI_2}{dt} = (A_B + A_T + E_B + E_T)(q_1 - q_2 \dfrac{I_2}{I_2 + g_I}) + \sum_{m=0}^{N-1} i_2 \delta(t-m\tau) - \mu_{I_2} I_2, \\
\dfrac{dT_i}{dt} = p_2 B T_u - p_4 E_B T_i, \\
\dfrac{dT_u}{dt} = r T_u \left(1 - \dfrac{T_u}{K}\right) - p_2 B T_u - (\lambda A T_u + \alpha E_T T_u)\left(\dfrac{I_2}{I_2 + g_I}\right)\left(\dfrac{g_T}{T_u + g_T}\right).
\end{cases}
$$

Dynamics of tumor-Ag-activated APC (TAA-APC)

$$\frac{dA_T}{dt} = \underbrace{\lambda A T_u \left(\frac{I_2}{I_2 + g_I} \right)}_{\text{production of APC activated by tumor}} - \underbrace{\beta_1 A_T}_{\text{migration TAA-APC to lymphoid tissues}} - \mu_{A_1} A_T$$

This term is proportional to the number of non-activated APCs and uninfected tumor cells, with a rate coefficient λ . 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 => TAA-APC : 50-75% of 5×10^6 DC within 24 h.

$\lambda A T_u = A_T$ $\quad A_T = 0.5 \times 5 \times 10^6 \Rightarrow$ $\quad \lambda = 0.1 \times 10^{-6}\, day^{-1}.$

Dynamics of CTLs that react to tumor antigen

$$\frac{dE_T}{dt} = \underbrace{\frac{\beta_T A_T I_2}{A_T + g}}_{\substack{migration \\ term}} - \underbrace{p_5 T_u E_T}_{\substack{in activation \\ term}} - \underbrace{\mu_E E_T}_{death\ term}$$

- 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 β_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.

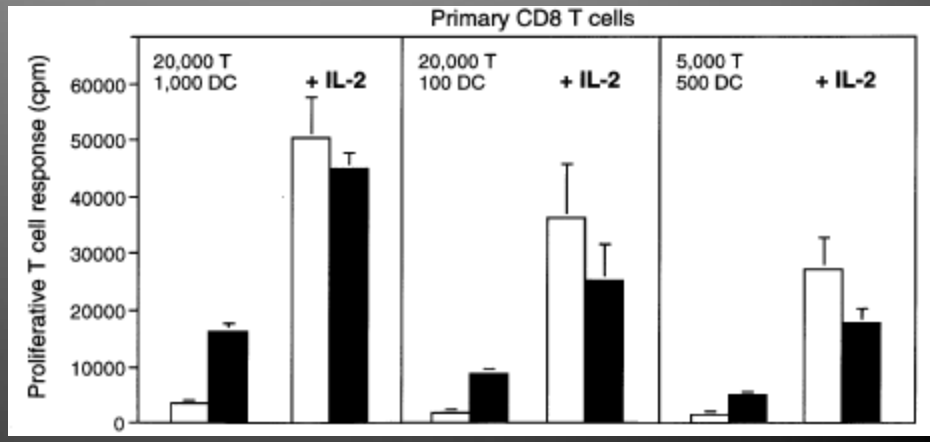
Estimation rate of recruitment of effector cells in response to signals released by TAA-infected and activated APCs

- β_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

- *X cells * 0.14 cpm/cell = 24. 000 cpm*
(171.429 – 20.000)/4 = 37.857 cells/day

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

$$\beta_T = \frac{37{,}857}{100} \times 4 = 1514\ cells/\ (day \cdot I_2)$$



Estimation of death rate coefficient, μ_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 Nf = final population size and No = 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.

$$0.0048 = 0.0147 e^{-\mu_{E1} t} \qquad for\ t = 6\ days \Rightarrow \mu_{E1} = 0.1865\ day^{-1}$$

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

$$0.0097 = 0.0152 e^{-\mu_{E2} t} \qquad for\ t = 13\ days \Rightarrow \mu_{E2} = 0.0346\ day^{-1}$$

IL-2 dynamics

$$\frac{dI_2}{dt} = \underbrace{q_1(A_B + A_T + E_B + E_T)}_{natural sourse} - \underbrace{q_2(A_T + A_B + E_T + E_B)\frac{I_2}{I_2 + g_I}}_{\sin k}$$

$$+ \underbrace{\sum_{m=0}^{N-1} i_2 \delta(t - m\tau)}_{external\ source} - \underbrace{\mu_{I_2} I_2}_{degradation}$$

- 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 .

Dynamics of uninfected tumor cells

$$\frac{dT_u}{dt} = \underbrace{rT_u\left(1-\frac{T_u}{K}\right)}_{\substack{natural tumor\\ growth}} - \underbrace{p_2 B T_u}_{\substack{tumor\\ infection\\ by BCG}} - \underbrace{(\lambda A T_u + \alpha E_T T_u)\left(\frac{I_2}{I_2+g_I}\right)\left(\frac{g_T}{T_u+g_T}\right)}_{tumor elimination by immune cells}$$

- This term is to capture of T_u by APCs at rate coefficient λ, and due to the elimination by E_T which destroy T_u at a rate coefficient α (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.

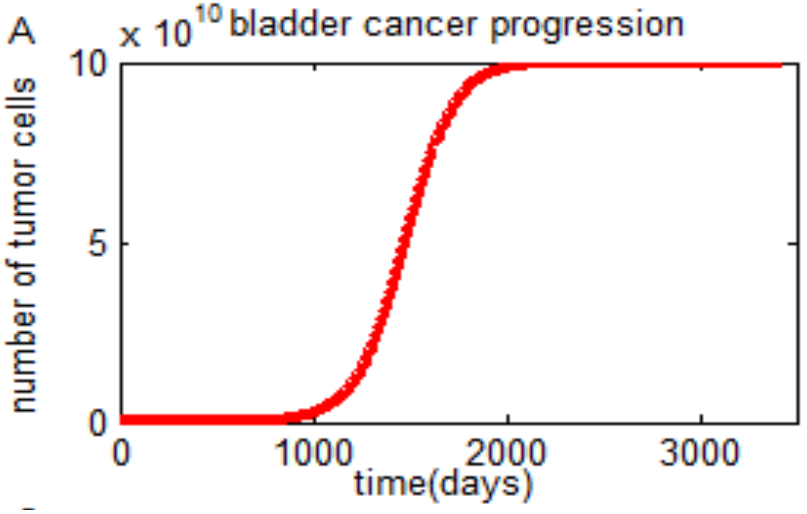
- The tumor produces a variety of mechanisms that reduce effector cell killing, then we multiply $\frac{I_2}{I_2+g_I}$ by $\frac{g_T}{T_u+g_T}$, which

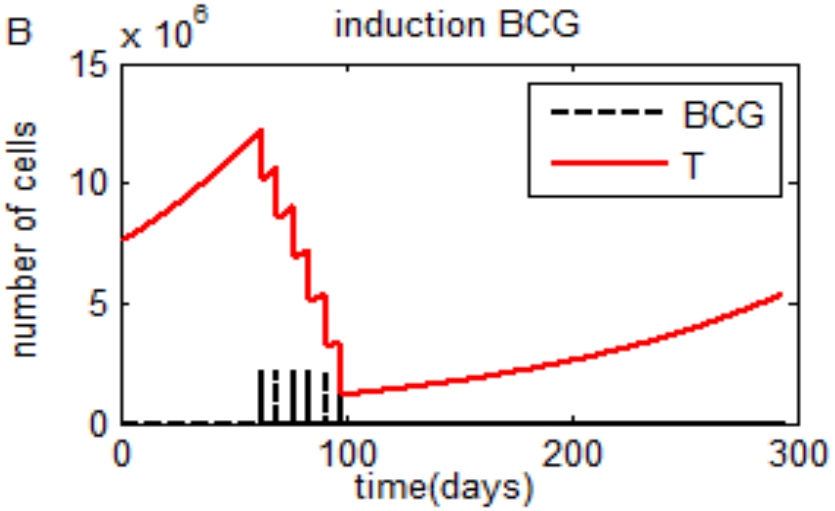
accounts for inversely proportional reduction in killing rate, such that the term is equal to 1 and when

$$T_u \to \infty, \lim_{T_u \to \infty} \frac{g_T}{T_u+g_T} = 0$$

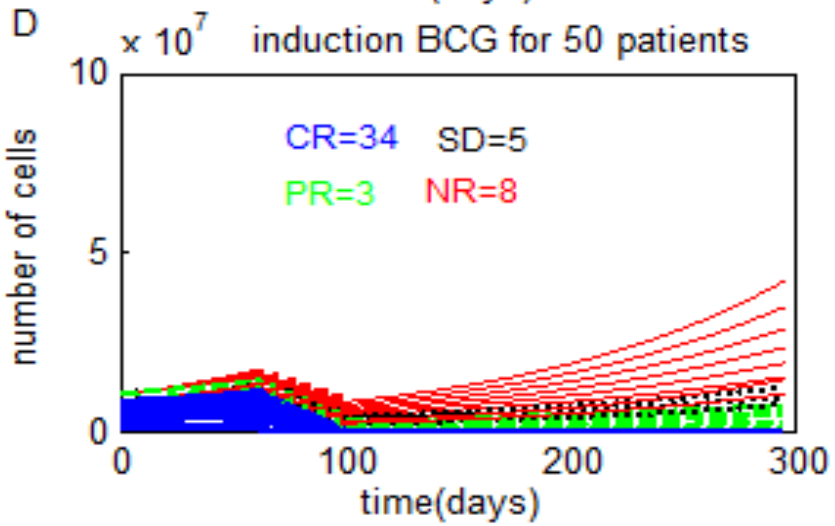
.

Computer simulations for evaluating the efficacy of BCG protocol

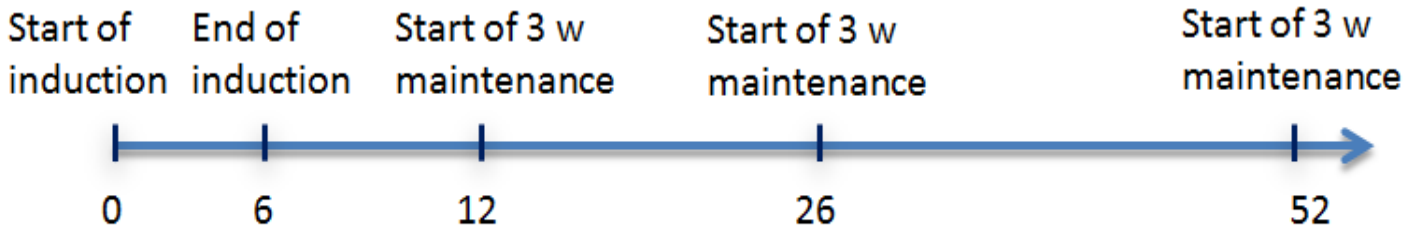


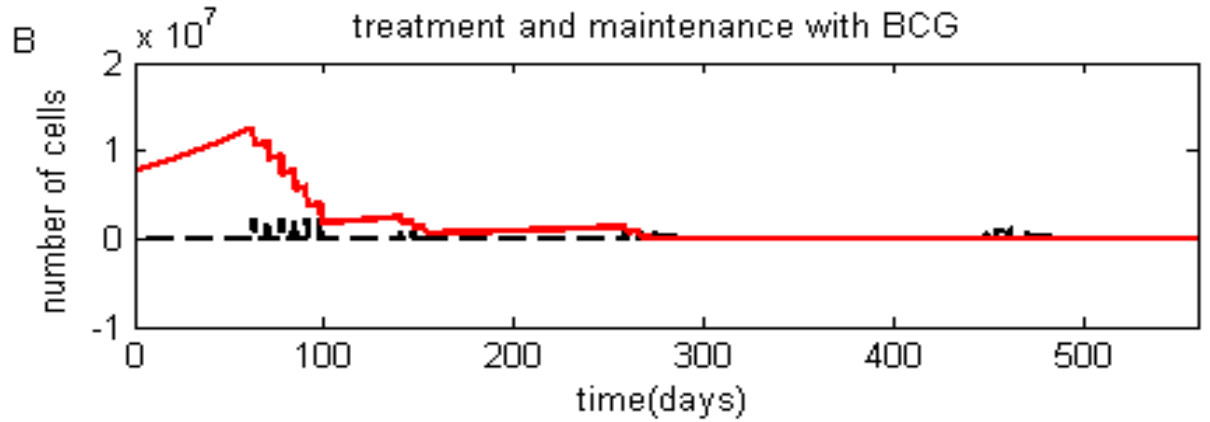


Red – NR non-responders
Blue – CR complete response
Black– SR stable response
Green– PD partial disease

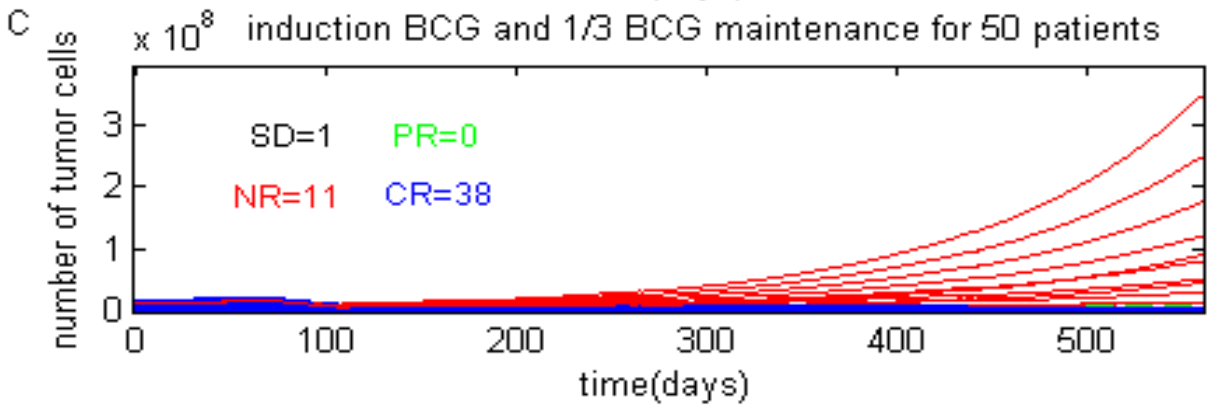


Simulated effect of BCG (induction) and BCG (maintenance) on 50 virtual patients



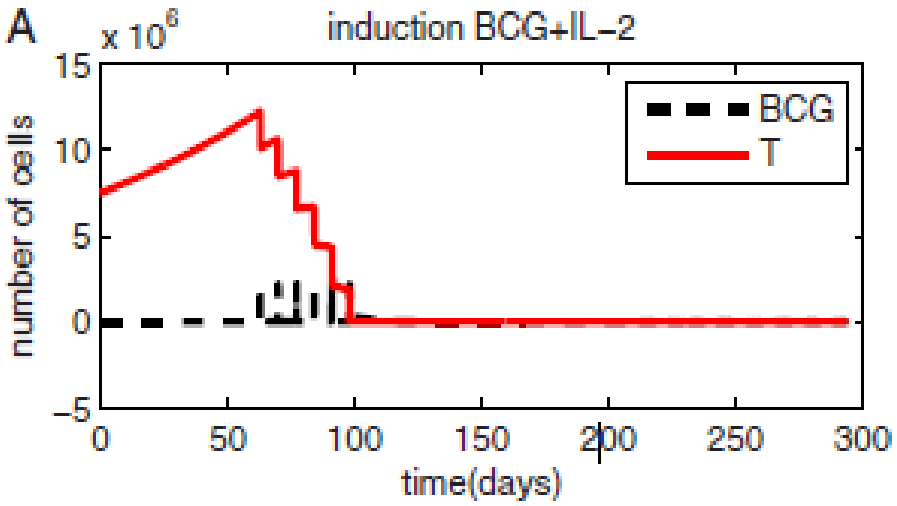


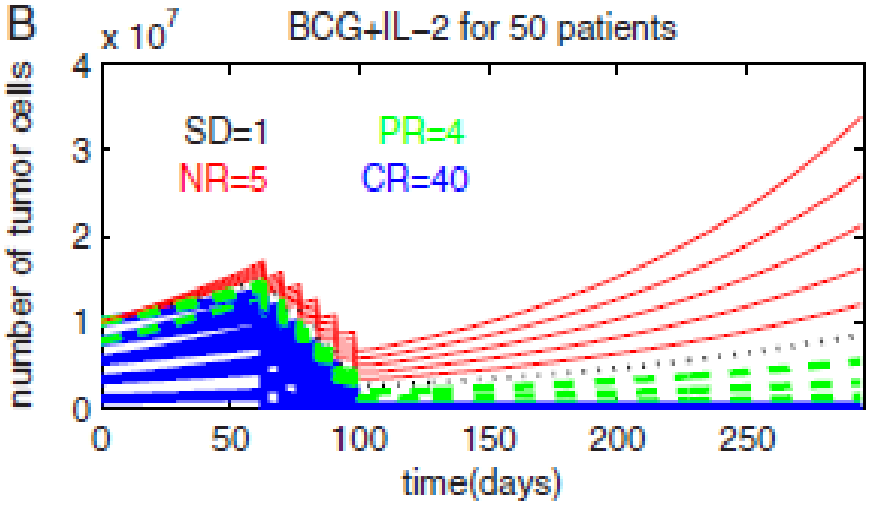
Red – Tumor cells
Black – BCG treatment

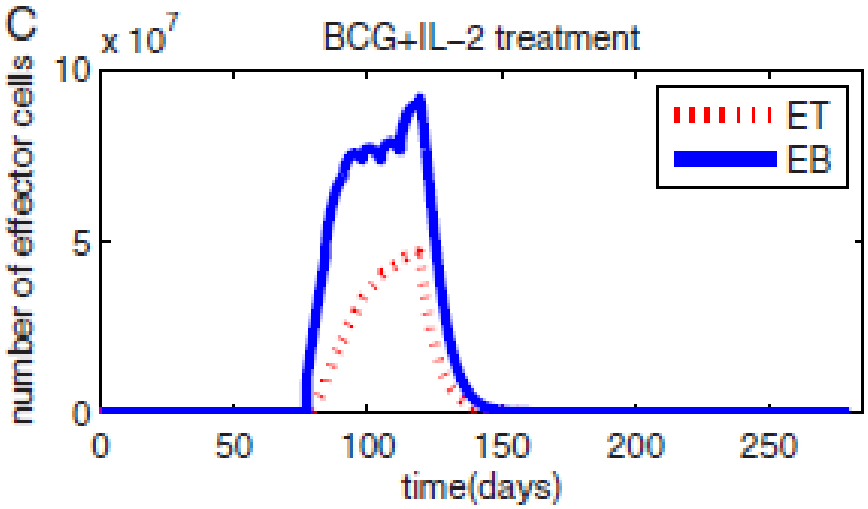


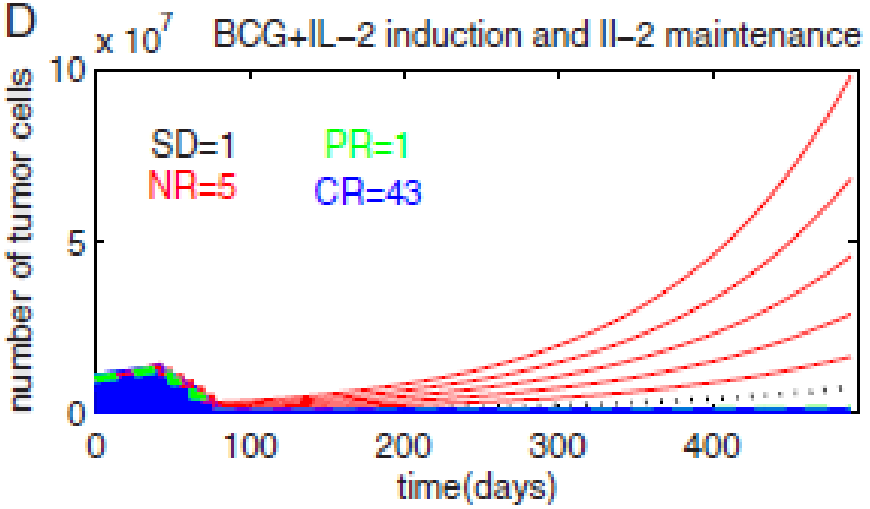
Red – NR non-responders
Blue – CR complete response
Black – SD stable response
Green – PR partial disease

Simulated effect of BCG+IL-2 (induction) and IL-2 (maintenance) on 50 virtual patients

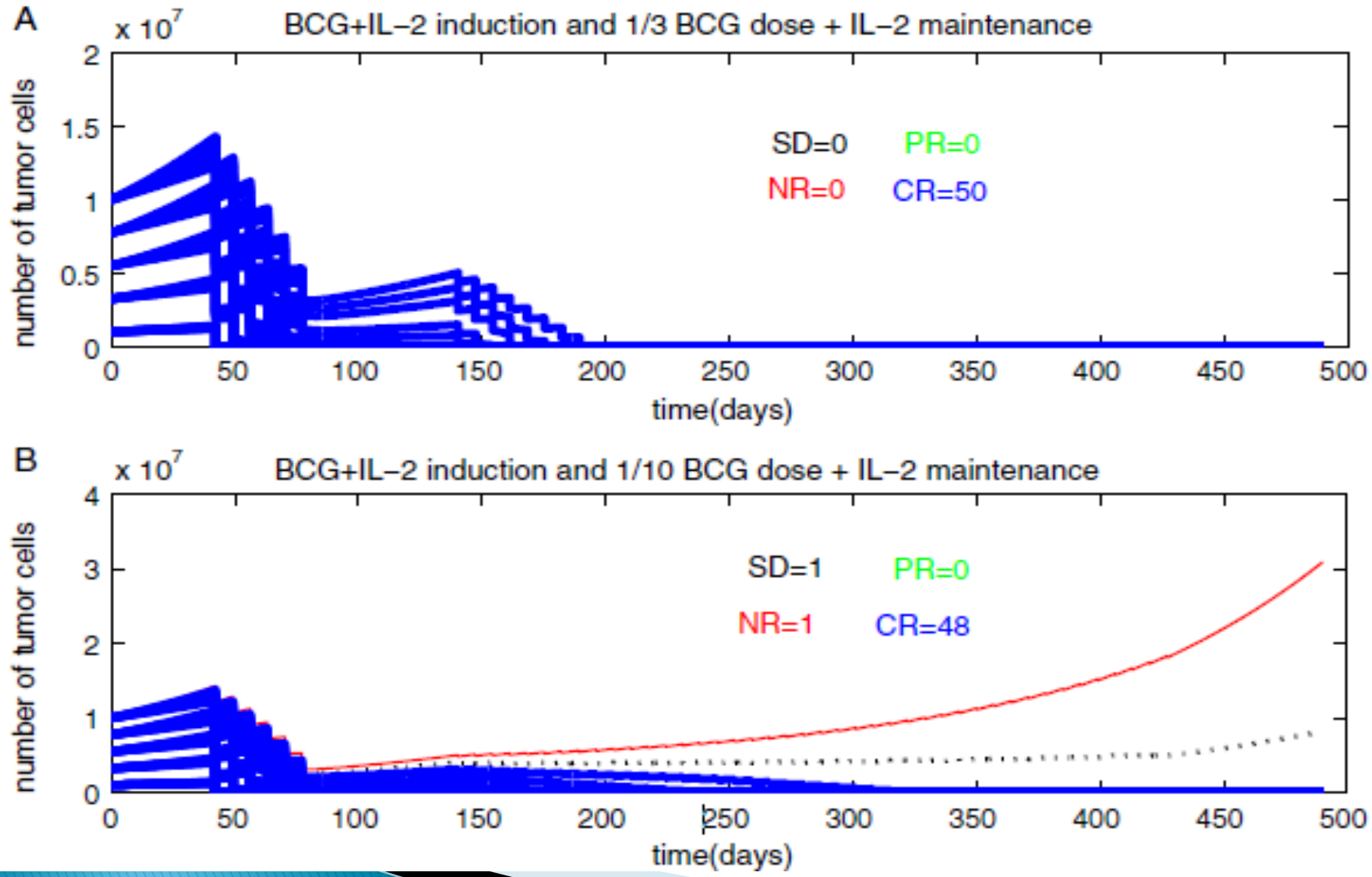








Simulated effect of BCG+IL-2 (induction) and BCG+IL-2 (maintenance) on 50 virtual patients



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

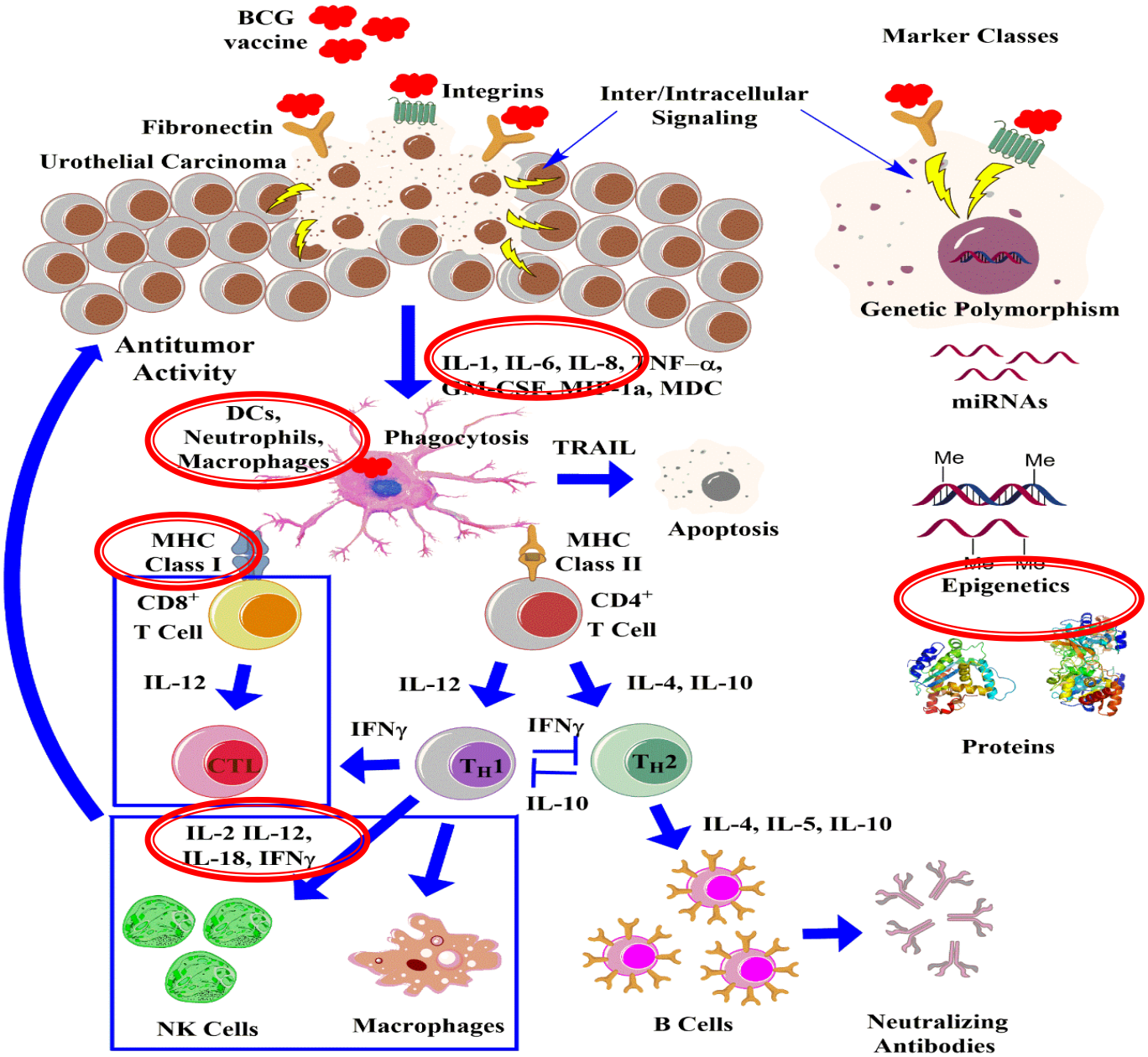
- **Urinary markers:**
  - IL-6/IL-10 ratio
  - IL-2 cytokine
  - IL-8
  - IL-17

- **Biopsy:** Cell-cycle (p53; retinoblastoma protein (pRb); tumor supressor 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

Burger M. et al., Eur. Urol. 2013; 63; 234-41
Witjes J.A. et al., EAU Guidelines; April 2014

Biomarkers to evaluate the effect of BCG



Kiselyov A. et. al. 2015

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.

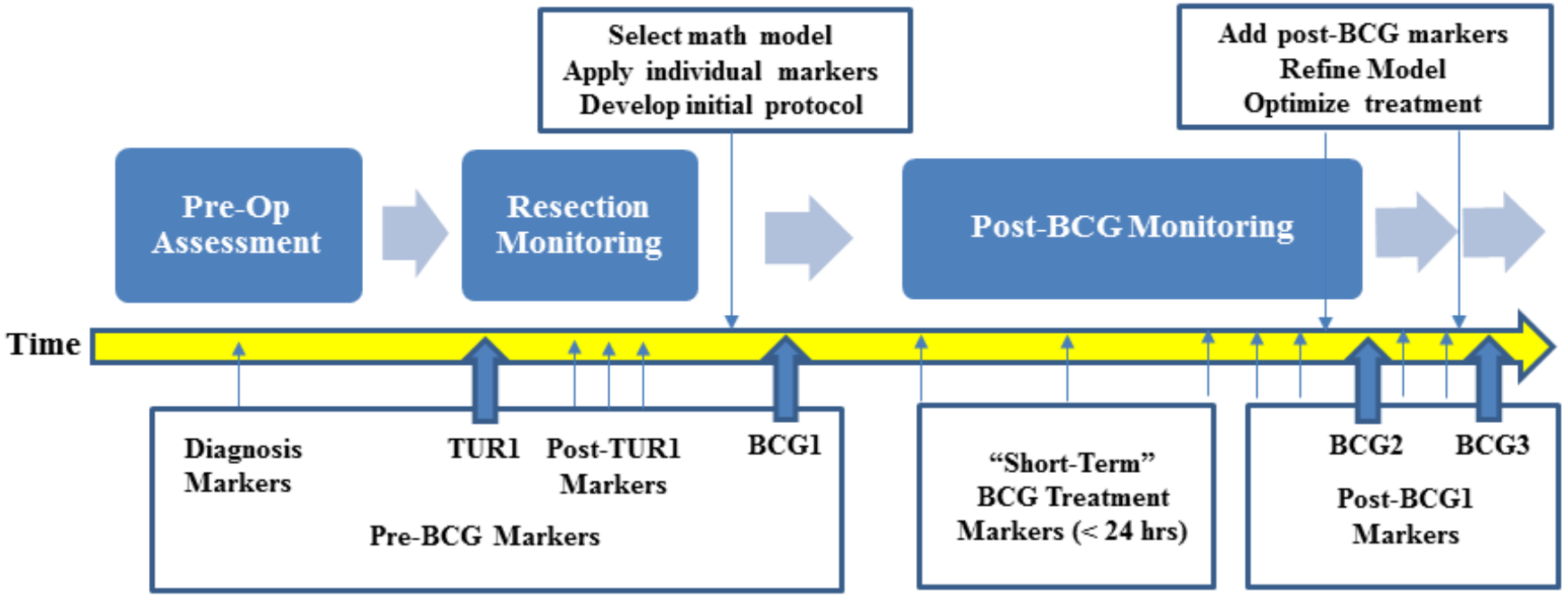
- The IL-6/IL-10 ratio post-BCG has been evaluated in BC patients to show that if the ratio >.1 then recurrence-free survival (RFS) will be higher.

- A neutrophil chemotactic factor (IL-8) is secreted by macrophages post-BCG inducing chemotaxis of primary neutrophils and other granulocytes

BCG Treatment Markers

1	Tumor Associated Macrophages (TAM) not quantified	Tissue from 41 patients with BC post intravesic. BCG (increase in TAMs - tumor progression, poor prognosis)	transurethral bladder biopsy, anti-CD68 mAbs (Dako, Denmark)	0/1	2	3	4	N/A (not studied)	**high**
2	Ki-67 (digital marker), best in combo with CK-20	309 pT1 BC patients from single urol. Center; adj. BCG performed; 49 mo follow-up	Biopsy; mouse Ab, clone MIB-1, Dako, Germany	0/1	2 (threshold Index >13)	3 (threshold index > 15-17)	4	N/A	**high**
3	CK-20 (digital marker), best in combo with Ki-67	309 pT1 BC patients from single urol. Center; adj. BCG performed; 49 mo follow-up	Biopsy; CK20 Ab, clone: IT-Ks 20.8 Dako, Denmark	0/1	2	3	4	N/A	**high**
4	IL-8 (REVERSE: high(er) levels ass'd with better clinical outcome)	Voided 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.	Urine; ELISA detection	4 (> 1,000 pg/mL)	3 (< 300 pg/mL)	2 (<200 pg/mL)	1 (<150 pg/mL)	0 (< 120 pg/mL)	**high**
5	TAA-cytotoxic T-Lymphocytes (TAA-CTLs)	Basal reac[illegible] of TAA-CTLs will dictate IL-2 treatment: increa[illegible]es of s.c. IL-2 (0.25, 0.5, and 1.0 106 u[illegible]	(Peripheral) blood	0/1	2	3	4	N/A	high

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

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
iNOS
NMP-22/Mcm5
P53, pRb, p21
Ki-67/CD20
Ezrin

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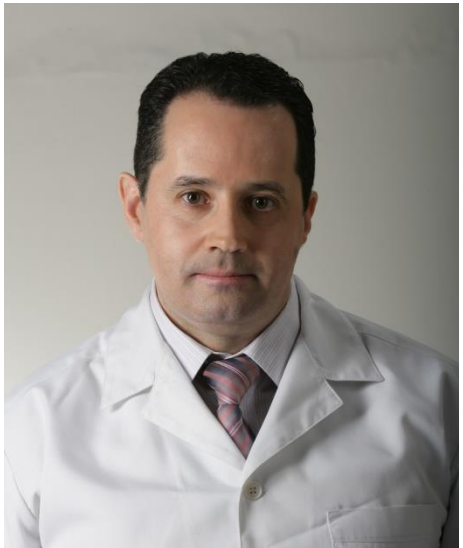

Vladimir Startsev

MD, Ph.D., MSc, Prof. of Oncology, S-Petersburg, Russia

Thank You
for your attention!