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Research article

Computational systems biology approach for permanent tumor elimination and normal tissue protection using negative biasing: Experimental validation in malignant melanoma as case study

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Supplementary

1. Equivalence between endogenous and exogenous tumor regression

Regarding exogenous tumor regression by therapeutic agents, the upper bound and values of cell lysis parameters of a particular chemotherapy drug (e.g., DNA damage producing drug as dacarbazine or temozolomide) are available from the literature [1,2], including k_M, k_A, k_K, k_B which respectively denote the DNA damage-based lysis rates of tumor cells, antitumor lymphocytes, natural-killer cells, and circulating lymphocytes (These citation numbers as [1] in this supplement refer to the bibliography at the end of this supplementary document). Figure 2 of the main paper shows the correspondence between tumor cell elimination by endogenous regression and by exogenous regression, i.e., by

spontaneous tumor remission and by therapy-induced tumor remission respectively.

Table S1. Values of the biological parameters of the tumor system.

Parameter	Value of Parameter	Description of Parameter	Reference
μ_c	1×10^{1} (per day)	Rate of decay of IL-2 concentration	[3]
γ	9×10^{-1} (per day)	Temporal decay rate of DNA blockage factor	[4]
α	7.5×10^{8} (cells per day)	Birth rate of circulating lymphocytes	[5]
β	1.2×10^{-2} (per day)	Death rate of circulating lymphocytes	[5]
k_B	6×10^{-1} (per day)	Lysis of circulating lymphocytes by DNA blockage	[2]
e	2.08×10^{-3} (per day)	Fraction of circulating lymphocytes that become NK cells	[6]
f	4.12×10^{-2} (per day)	Death rate of NK cells	[6]
p	3.42×10^{-7} (per cell per day)	Inactivation of NK cells due to tumor cells	[7]
k_K	6×10^{-1} (per day)	Lysis of NK cells by DNA blockage	[2]
g	4.91×10^{-1} (per day)	Maximum rate of NK cells recruitment by ligand-transduced tumor cells.	[8]
h	2.02×10^{7} ($cell^2$)	Steepness index of NK cells recruitment by tumor cells	[6]
d	2.34 (per day)	Saturation level of fractional tumor cell kill by CD8+ T cells, priming by ligand-transduced cell.	[8]
l	2.09 (dimensionless)	CD8+ T cell-induced tumor cell lysis	[8]
s	8.39×10^{-2} (dimensionless)	Steepness coefficient (Q) of CD8+ T cell induced tumor cell lysis.	[8]
m	2.04×10^{-1} (per day)	Death rate of CD8+ T cell.	[9]
k	3.66×10^{7} (per cell per day)	Steepness index of CD8+ Tcell recruitment, primed with ligand-transduced tumor cells.	[7]
j	2.49×10^{-2} ($cell^2$)	Max. value of CD8+ Tcell recruitment, primed with ligand-transduced tumor cells.	[7]
q	1.42×10^{-6} (per cell per day)	Inactivation rate of CD8+ Tcell induced by tumor cell	[6]
r_1	1.1×10^{-7} (per cell per day)	Generation rate of CD8+ Tcell, induced by tumor cell lysis due to NK cell	[9,10]
r_2	6.5×10^{-11} (per cell per day)	Generation rate of CD8+ Tcell, induced by interaction between tumor cell and circulating lymphocyte.	[5]
u	3×10^{-10} ($cell^2$ per day)	Regulation of CD8+ T cell by NK-cell	[5]
p_C	1.25×10^{-1} (per day)	Max. value of rate of CD8+ T cell recruitment by IL-2	[3]
g_C	2×10^{7} ($cell^2$)	Steepness index of rate of CD8+ T cell recruitment by IL-2	[3]
k_A	6×10^{-1} (per day)	Lysis of $CD8^+$ T-cells by DNA blockage	[2]
a	4.31×10^{-1} (per day)	Growth rate of tumor	[4]
b	2.17×10^{-8} (per cell)	Logistic growth of tumor (deceleration effect)	[4]
c	6.41×10^{-11} (per cell per day)	Non-ligand-transduced tumor cell lysis by NK cell.	[7]
k_M	9×10^{-1} (per day)	Lysis of tumor cells by DNA blockage	[2]

We now proceed further to delineate the threshold values of DNA damage under endogenous as spontaneous tumor regression, taking a cue from cell lethality by DNA damage or alkylation under exogenous regression by chemotherapy alkylator drugs. For instance, it is known that the maximum physiologically tolerated limit for DNA damage in a human subject of 60 kg weight is 270 mg dose of alkylator drug temozolomide, $C_6H_6N_6O_2$ [2]. This amount corresponds to 8.35×10^{20} molecules of temozolomide, since 194 grams is its molar weight (containing Avogadro's number of temozolomide molecules). On the other hand, there are 3 billion nucleotide bases in a human cell [11]. Since such alkylating drugs do methylate the guanine base, there are 0.75 billion potential target guanine molecules in a human cell.

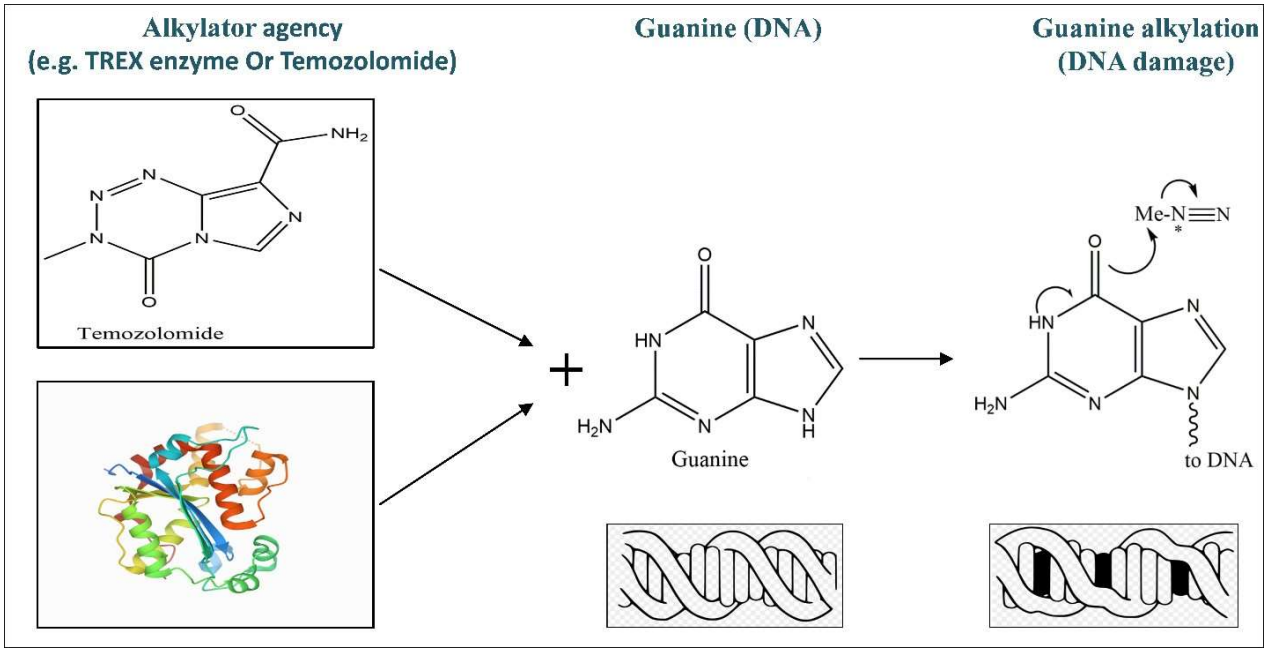

Figure S1. Estimation of DNA interference in endogenous spontaneous regression in terms of equivalent alkylation units. (the motifs are drawn in ChemDraw, the DNA helix is from Open Source, the Trex enzyme is from RCSB-PDB database).

Furthermore, one also knows that the average human body volume is 60,000 cc., however the majority of tissues are finally differentiated non-dividing form of cells at G_0 or quiescent state of cell cycle. These quiescent tissues are the nervous system, bone, muscle, skin andblood serum (with erythrocytes), totaling about 83% of body mass [12]. Indeed, such quiescent cells do not give scope of cell proliferation and DNA alkylation, hence only 17% of the body, i.e., 14,800 cc. of tissue is amenable to DNA interference or damage. Given the body's average cell density is 10^8 cells/cc [13], we find that there are 7.12×10^{20} guanine molecule targets potentially available for DNA alkylation damage.

This value of 7.12×10^{20} target guanine sites well corresponds to the aforesaid 8.35×10^{20} molecules of alkylator temozolomide molecules available for enabling the DNA damage events (this value of 8.35×10^{20} alkylator molecules well corresponds 7.12×10^{20} guanine sites targets, within about ± 5% experimental error around mean value). Hence, it can be posited that the maximally-

tolerated limit condition indicates mechanistically that the upper bound of DNA damage is 1 alkylator molecule per 1 alkylation target, i.e., 1 temozolomide methyl moiety acting univalently on 1 methylable C-6 vertex of guanine in DNA, this situation is physiologically the maximum tolerable bound in exogenous tumor regression (Figure S1). This same rationale will apply to other univalent alkylator chemotherapy drugs as dacarbazine.

2. Bounds of parameters in endogenous and exogenous tumor regression

2.1. Bounds of DNA damage

In endogenous regression, the DNA impairment is occasioned by DNA interference, as alkylation, adduct-formation and intercalation. Accordingly, for endogenous or spontaneous tumor regression, one can take the parallel physiological approach, and delineate that the upper limit of DNA damage is one DNA alkylation event per one guanine base. That is, we can take DNA alkylation as an equivalent formulation for DNA damage in endogenous regression. Thereby, it can be construed that the maximum limit ofDNA damage in endogenous tumor regression is equivalent to 7.12×10^{20} guanine molecule targets. This can be expressed as alkylation activity corresponding to 270 mg amount of imidazole alkylator temozolomide equivalent units, or alternately to 1.412 mili-equivalent units (expressing the univalently-reacting temozolomide amount in mili-equivalents). For other univalent alkylator drugs, as dacarbazine, the maximum limit DNAdamage will be similar, i.e., 1.412 mili-equivalent units. In parenthesis, one may mention that in cells, the biochemical interaction between targets, receptors, antibodies,immunomodulators etc, can be well described in equivalent amounts expressed as normalities or equivalent weights.

The aforesaid or correspondence enables one to quantitate the equivalence between (i) DNA damage due to endogenous spontaneous regression, i.e., by 7.12×10^{20} DNA molecular intercalation targets, and (ii) DNA damage due to exogenous drug-induced regression, i.e., by 1.412 mili-equivalent units or 270 mg alkylator. Thus, we have the following quantitative equivalence between endogenous and exogenous tumor regression, involving the same amount of damage of the malignant lesion.

Exogenous tumor regression (Therapy-induced tumor egression): Malignant lesion damage by 270 mg alkylator (chemotherapy temozolomide drug)	≡	Endogenous tumor regression (Spontaneous tumor regression): Malignant lesion damage by 7.12×10^{20} DNA molecular intercalation targets

From the above equivalence, one can obtain that 10^{10} DNA damage events produced by antitumor activity of endogenous spontaneous regression has the same number of damage events as produced by alkylation activity of 27 pico-gram temozolomide.

Hence, in our formulation, we can express the DNA damage of spontaneous tumor regression in terms of mg. of equivalent imidazole alkylator units, or in mili-equivalent alkylation units, and the latter parameter is taken as the units of parameter D in Eq (2) of the main text. To underscore, in

endogenous spontaneous regression, the maximal bound of DNA damage in the adult person is thus elucidated as 1.412 mili-equivalent units of alkylation (say, temozolomide-equivalents or dacarbazine-equivalents). To put in proper perspective, units in mili-equivalents are not small amounts, for instance the total amount of cuprous and cupric ion moieties in the adult human body is 1.13 mili-equivalents [12], these ions take a crucial role in protein metabolism and enzyme reactions across the body.

2.2. Bounds of antitumor factors

We hereby work out below the values of the maximum and minimum bounds of all the other antitumor entities: interleukin-2, cytotoxic T-cells (tumor-infiltrating lymphocyte), DNA alkylation, circulating lymphocytes, and natural killer cells, respectively. The values of these entities are elucidated as follows (the values are also displayed in Table S2 below):

Regarding Interleukin-2 in endogenous tumor regression, the upper limit of Interleukin-2 that can be physiologically tolerated, without harmful effects, is 7.2×10^4 i.u./kg body weight [2], which, in a therapy perspective, translates to 4.32×10^6 i.u. per average adult person (60 kg. weight). We take this value to likewise be the upper bound of interleukin-2 that can be physiologically tolerated or produced in the adult person during endogenous regression (spontaneous cancer regression). Furthermore, if there is no tumor, the immune reaction and its inflammatory cytokine (IL-2) concentration will be negligible, i.e. the lower bound of IL-2 can be taken to be 0.

On the other hand, with reference to cytotoxic T-cell population (CD8+), one knows that 20150 cells/mm^3 is the subject's maximum tolerated CD8+ T cell population [8]. So, the total number of CD8+ T-cells in the 4.5 liters of blood (whole body) is 6.05×10^{10} cells in the adult (upper bound). Likewise, as a tumor completely regresses, one knows that cytotoxic T-cell intensity reduces to negligible values [7]. The lower limit of these T-cells may be considered to be nil.

Now we come to the matter of cumulative bound of tumor infiltrating lymphocytes or cytotoxic T-cells. It transpires that in exogenous regression, the maximal bound of the injected tumor infiltrating lymphocyte or cytotoxic T-lymphocyte is 13.7×10^{10} cells cumulatively across the duration of immunotherapy [8]. Hence, we can here set this value to be the maximal bound of cytotoxic T-lymphocyte that can be physiologically tolerated or generated cumulatively in the human host during endogenous regression. Also, as per the discussion in the earlier paragraph, the minimum level of cytotoxic T-cells cumulatively can be delineated as zero.

Now we consider the limits of DNA alkylation. We have seen in the earlier sections that there is quantitative equivalence between DNA damage in endogenous spontaneous cancer regression and in exogenous therapy-induced cancer regression. The upper limit of DNA alkylation damage for both regression processes can be expressed with respect to activity of alkylator temozolomide, namely in terms of temozolomide alkylation equivalent units (tae units). It is known that [2] the maximum tolerable input of temozolomide for a human subject is 4.45 mg/kg/day. The minimum limit of DNA alkylation for tumor regression that can be tolerated by the human body is 0, which would occur if the tumor had not occurred, so that there was no need of endogenous nor exogenous tumor regression.

Thereafter, we consider the circulating lymphocyte bounds. Under active circulation, the typical

blood volume ranges from 3.5 to 4.5 liters. Individuals may have lymphocyte concentrations as high as 100×10^9 cells per liter [14]. A subject thus can have an upper bound of 4.5×10^{11} lymphocytes, using the upper bound of the blood volume. Similarly, the minimum lower bound tolerated may vary from 663–1160 lymphocyte per microliter for 6–12 months across a therapy for the patient [15]. To obtain the minimum bound, we take the lower value of cell count and lesser value of blood volume, so the minimum number of lymphocytes a subject can tolerate for up to six months is 2.32×10^9 cells.

Coming to the natural killer cells we consider as follows. The upper bound of these NK cell (with CD56/CD16 lymphocyte as a marker) is limited to 13% of the lymphocyte population [16]. So, the maximum value of the NK cell population in the individual is 5.85×10^{10} cells because the upper bound of the lymphocyte population in the preceding paragraph is 4.5×10^{11} cells. However, the lower limit of NK cells is zero in individuals with natural killer cell deficiency state, and one knows that an individual can tolerate this condition for 3.5 months before any significant infection can occur [17]. As mentioned earlier, Table S2 summarizes the information of this section.

Table S2. Limits of upper and lower bounds.

Parameters	Lower limit	Upper limit
Interleukin-2 input, per day (v_C)	0	7.2×10^4 I.U/kg/day
Cytotoxic T cells, total in person (A)	0	6.05×10^{10} cells
Tumor infiltrating lymphocytes, cumulative input over full duration (cumulative v_A)	0	13.7×10^{10} cells
DNA alkylation input (tae units), per day (v_D)	0	4.45 mg/kg/day
Circulating lymphocytes in blood, total in person (B)	2.32×10^{11} cells (max. duration: 6 months)	4.5×10^{11} cells
Natural killer cells, total in person (K)	0 (max. duration: 3.5 months)	5.85×10^{10} cells

References

1. C. M. Sakode, R. Padhi, S. Kapoor, V. P. S. Rallabandi, P. K. Roy, Multimodal therapy for complete regression of malignant melanoma using constrained nonlinear optimal dynamic inversion, *Biomed. Signal Process. Control,* **13** (2014), 198–211, https://doi.org/10.1016/j.bspc.2014.04.010
2. M. C. Perry, D. C. Doll, C. E. Freter, Chemotherapy Sourcebook, Lippincott Williams & Wilkins, Philadelphia, 2012.
3. D. Kirschner, J. C. Panetta, Modeling immunotherapy of the tumor-immune interaction, *J. Math. Biol.,* **37** (1998), 235–252. https://doi.org/10.1007/s002850050127

4. P. Calabresi, *Medical Oncology*, 2nd Eds., McGraw-Hill Professional, New York, 993.
5. L. G. de Pillis, W. Gu, A. E. Radunskaya, Mixed immunotherapy and chemotherapy of tumors: modeling, applications and biological interpretations, *J. Theor. Biol.,* **238** (2006), 841–862. https://doi.org/10.1016/j.jtbi.2005.06.037
6. V. A. Kuznetsov, I. L. Makalkin, M. A. Taylor, A. S. Perelson, Nonlinear dynamics of immunogenic tumors: Parameter estimation and global bifurcation analysis, *Bull. Math. Biol.,* **56** (1994), 295–321. https://doi.org/10.1007/BF02460644
7. A. Diefenbach, E. R. Jensen, A. M. Jamieson, D. H. Raulet, Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity, *Nature,* **413** (2001), 165–171, https://doi.org/10.1038/35093109
8. M. E. Dudley, J. R. Wunderlich, P. F. Robbins, J. C. Yang, P. Hwu, D. J. Schwartzentruber, Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes, *Science,* **298** (2002), 850–854, https://doi.org/10.1126/science.1076514
9. A. Yates, R. Callard, Cell death and the maintenance of immunological memory, *Discrete Contin. Dyn. Syst. Ser. B,* **1** (2001), 43–59. http://dx.doi.org/10.3934/dcdsb.2001.1.43
10. A. Lanzavecchia, F. Sallusto, Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells, *Science*, **290** (2000), 92–97. https://doi.org/10.1126/science.290.5489.92
11. H. F. Lodish, A. Berk, C., *Molecular Cell Biology*, 4th edition, New York, 2000.
12. J. E. Hall, A. C. Guyton, Guyton and Hall textbook of medical physiology, Saunders/Elsevier, Philadelphia, PA, 2011.
13. D. M. Ugo, Does the cell number 10(9) still really fit one gram of tumor tissue?, *Cell Cycle,* **8** (2009), 505–506, https://doi.org/10.4161/cc.8.3.7608
14. E. A. Macintyre, D. C. Linch, Lymphocytosis: Is it leukaemia and when to treat, *Postgrad. Med. J.,* **64** (1988), 42–47, https://doi.org/10.1136/pgmj.64.747.42
15. M. Jarosz, L. Hak, J. Więckiewicz, NK cells in children with acute lymphoblastic leukemia and non-Hodgkin lymphoma after cessation of intensive chemotherapy, *Cent. Eur. J. Immunol*., **34** (2009), 94–99.
16. J. E. Berrington, D. Barge, A. C. Fenton, A. J. Cant, G. P. Spickett., Lymphocyte subsets in term and significantly preterm UK infants in the first year of life analysed by single platform flow cytometry, *Clin. Exp. Immunol*., **140** (2005), 289–292. https://doi.org/10.1111/j.1365-2249.2005.02767.x
17. S. Jawahar, C. Moody, M. Chan, R. Finberg, R. Geha, T. Chatila, Natural killer (NK) cell deficiency associated with an epitope-deficient Fc receptor type IIIA (CD16-II), *Clin. Exp. Immunol.,* **103** (1996), 408–413. https://doi.org/10.1111/j.1365-2249.1996.tb08295.x

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