A mathematical model of T cell response predicts synergistic treatment combination in cancer immunotherapy

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Abstract
T cell responses are regulated by multiple signals including costimulation and immune checkpoints along with antigen stimulation. Recently clinical trials demonstrated that blockade of immune checkpoint signals led to dramatic clinical responses in a fraction of cancer patients. To improve the therapeutic efficacy of regimens aimed to enhance T cell responses to cancers, a predictable mathematical model is needed for designing efficient therapy. Here we provide a mathematical model to predict the net outcome of a T cell response by integrating both positive and negative signals in addition to antigen stimulation. A digital range of adjustment of each signal is formulated in our model for prediction of a final T cell response. Our model provides a rationale for synergistic treatment combination aimed to defuse resistance and maximize T cell responses against cancers.

Introduction
T cell response to antigen stimulation is a tightly controlled process. Recent clinical trials have demonstrated that unleashing T cell response to cancers could be an effective approach in treatment of human malignancies\textsuperscript{1-3}. The identification of the roles of immune checkpoint molecules in tumor immune evasion greatly contributes to the development of therapeutics aimed to block immune suppression mechanism in order to enhance antitumor T cell immunity\textsuperscript{4,5}. Although the clinical outcomes of immune checkpoint blockade are promising, the low efficiency and potential adverse effects remain as major challenges in application of this therapy to more
cancer patients. Combination therapy among different immune checkpoint targets or other therapies (i.e. chemotherapy, targeting therapy or radiotherapy, etc.) is speculated to increase the efficacy of the treatment of human cancers\textsuperscript{6,7}; however, the rationale of optimized combination is still lacking. Given the complexity in T cell responses that are regulated by a battery of signals at different stages of activation and differentiation\textsuperscript{8}, a predictable model is needed to design an effective combined therapy that would maximize the therapeutic effects of each components of a regimen. Here, we present a simple mathematical model of T cell responses that can be used to predict an outcome of a T cell response according to changes of positive or negative regulatory signals. Importantly, based on this equation, we would be able to predict that a combined therapy is essential to achieve a maximal or an enhanced T cell response. To our knowledge, this is the first mathematical model that may provide a rationale for synergistic treatment combination aimed to decrease resistance and maximize T cell responses against cancers.

\textbf{Two signal theory in shaping T cell responses}

Antigen stimulation initiates a T cell response through T cell receptor (TCR), however, the net outcome of a T cell response (activation, anergy or tolerance) to this antigen is regulated by two additional signals, i.e. costimulation (CD28) or checkpoint (CTLA-4 or PD-1, etc) that are integrated to TCR signaling pathway\textsuperscript{9}. To represent a T cell response that is initiated by antigen via TCR engagement and regulated by integrated positively or negatively signals, we present an equation as below:

\[ R = \frac{P \times T}{N \times T + 1} \]

In this equation, \( R \) is for Response; \( T \) for TCR signal; \( P \) for Positive costimulation signal; \( N \) for Negative checkpoint signal. According to this equation, we defined that when \( R=1 \), a T cell response is turned on; when \( R=0 \), a T cell response is turned off; when \( R>1 \), a T cell response is enhanced; when \( R<1 \), a T cell response is deterred or in a tolerance status. In following sections we will give several examples of different outcomes of a T cell response based on the integration of TCR signals along with positive or negative regulatory signals into this mathematical model, to see how our equation would predict a T cell response.

Besides checkpoint molecules that are directly integrated within TCR signaling pathway within primed T cells, there are other immune regulatory systems that work in parallel with TCR signals to control T cell responses. These other regulatory mechanisms include, but are not
limited to, regulatory T cells (Treg) or myeloid-derived suppressor cells (MDSCs)\textsuperscript{10}. To include these separate but inclusive regulatory systems in regulation of T cell response, we added “1” besides N to reflect these additional regulations.

**T cell response is dependent on antigen stimulation though TCR**

First of all, this equation should be able to predict the fundamental role of antigen stimulation of TCR in T cell response. Actually, in the absence of TCR stimulation and without TCR signals, i.e. T=0, then R will be 0, and there is no T cell response (Calculation 1).

\[
Calculation 1: R = \frac{P \times 0}{N \times 0 + 1} = \frac{0}{0 + 1} = 0
\]

This equation also explains why positive or negative signals \textit{alone} do not have any effects on T cell response in the absence of TCR stimulation, since when T=0, R will always be 0 whether P or N is 1 or not.

**T cell response is dependent on costimulation and regulated by immune checkpoint signals**

It has been established that a full activation (response) of T cells is dependent on the presence of costimulation, i.e. CD28 engagement\textsuperscript{11,12}. In the absence of costimulation (when P=0), R will always be 0, though there is a TCR stimulation (T=1) (Calculation 2). The outcome of calculation 2 explains T cell anergy\textsuperscript{13}, i.e. a mere TCR stimulation is not able to initiate a full T cell response.

\[
Calculation 2: R = \frac{0 \times 1}{0 \times 1 + 1} = \frac{0}{0 + 1} = 0
\]

When there is a costimulation signal (P=1), a full T cell response will be generated (R=1) (Calculation 3), as long as negative signals are absent (N=0).

\[
Calculation 3: R = \frac{1 \times 1}{0 \times 1 + 1} = \frac{1}{0 + 1} = 1
\]

If a negative signal is present (N=1), R will be 0.5 that is less than 1, indicating a deferred T cell response or a T cell tolerance (Calculation 4).
Calculation 4: \[ R = \frac{1 \times 1}{1 \times 1 + 1} = \frac{1}{2} = 0.5 < 1 \]

According to Calculation 4, a T cell tolerance is established by negative signal via immune checkpoint molecules. Calculation 4 also suggests that although both TCR stimulation and positive regulatory signals (costimulation) are present, there is no guarantee that a full T cell response can be generated due to immune regulatory mechanisms (N+1). Thus, our equation demonstrated a critical role of negative signals (immune checkpoints) in restraining the T cell response, which could be crucial in order to prevent pathology caused by any ongoing or unlimited T cell responses.

**How to break T cell tolerance and to enhance a T cell response?**

As shown in Calculation 4, the presence of negative signals or immune checkpoints significantly compromised the generation of a full T cell response initiated by TCR stimulation in the presence of costimulation. In order to enhance T cell response or break a T cell tolerance, we have to increase the strength of either costimulation or TCR stimulation. To that end, if we increase costimulation \( P \) to 2, and keep others at the same levels (\( T=1, N=1 \)), we will have \( R = 1 \) (Calculation 5), suggesting a T cell response can be restored through increase of costimulation.

This calculation is in line with an early observation that introduction of CD28 costimulation (positive signals) enhances T cell response \(^{11,12}\), or introduction of B7 molecules into tumor cells results in a strong antitumor response in vivo \(^{14}\).

Calculation 5: \[ R = \frac{2 \times 1}{1 \times 2 + 1} = \frac{2}{3} = 1 \]

However, a mere increase of TCR stimulation (let \( T=2 \)) cannot restore a T cell response in the presence of negative signals (when \( N=1 \)) (Calculation 6). This outcome may explain some preclinical and clinical observations showing strong antigenicity (e.g. high affinity antigen peptides) alone did not initiate a strong T cell response and fail to generate a protective T cell immunity.

Calculation 6: \[ R = \frac{1 \times 2}{1 \times 2 + 1} = \frac{2}{3} = 0.67 < 1 \]
Next we examined to what degree a reduction of negative signals would be required to restore or enhance a T cell response. According to calculation 7, when N is in a range of 0.1 to 0.9, R will always be less than 1, suggesting partially reduction of negative signals is not enough to restore a T cell response. As indicated from calculation 3, only a complete blockade or absence of negative signals, i.e. when N=0, a full T cell response can be achieved. This result underscores the strategy currently used in treatment of human cancer by a complete blockade of immune checkpoints (PD-1 or CTLA-4) in order to achieve objective clinical responses. Actually, the combination of anti-PD-1 and anti-CTLA-4 treatment achieved higher response rate than either alone 15.

\[
\text{Calculation 7: } R = \frac{1 \times 1}{0.1 \text{ or } 0.9 \times 1 + 1} = \frac{1}{0.1 \text{ or } 0.9 + 1} = 0.9 \text{ or } 0.5 < 1
\]

However, since a complete blockade of negative signals only can be achieved in a fraction of cancer patients, and in most situations negative signals can only be partially reduced, additional approaches are needed to restore or increase a T cell response. To that end, if negative signals are partially reduced (let N=0.5), our equation suggests a partial increase of costimulation (when \(P=1.5\)) will be able to restore a T cell response (Calculation 8).

\[
\text{Calculation 8: } R = \frac{1.5 \times 1}{0.5 \times 1 + 1} = \frac{1.5}{0.5 + 1} = 1
\]

In order to enhance T cell response (i.e. to let R>1), a double increase of costimulation (let \(P=2\)) is needed as shown in Calculation 9, if the negative signals are partially reduced (N=0.5).

\[
\text{Calculation 9: } R = \frac{2 \times 1}{0.5 \times 1 + 1} = \frac{2}{0.5 + 1} = 1.3 > 1
\]

It could be very challenging, if not impossible, to have a double increase of costimulation in order to enhance a T cell responses. For example, to have poor immunogenic tumor cells to express B7 costimulatory ligand 14, or to provide additional costimulation signals directly to T cells (e.g. 41BB stimulation) 16. Alternatively, a combination of a partially increased TCR stimulation and costimulation (\(T=1.5\); \(P=1.5\)) with a partially decreased negative signals (N=0.5) will be able to give an enhanced T cell response (R=1.29 >1) (Calculation 10). This calculation indicates that a synergistic combination can be achieved by integrating suboptimal increase of TCR and costimulation and suboptimal decrease of negative signals (e.g. partial immune checkpoint blockade) in order to enhance T cell responses.
**Calculation 10:** \[ R = \frac{1.5 \times 1.5}{0.5 \times 1.5 + 1} = \frac{2.25}{0.75 + 1} = 1.29 > 1 \]

**Discussion**

Here we present a mathematical model that can be used to predict the net outcome of a T cell response by integrating both regulatory and stimulatory signals that T cells may receive during antigen stimulation. Our equation \((R=P\times T/[N\times T+1])\) gives a digital range (0.1-0.9 or 1-2) of adjustment in each regulatory or stimulatory signal T cells may receive during antigen stimulation. As predicted from Calculation 10, a synergistic combination can be generated in integrating each signal when they can only be adjusted in a suboptimal condition due to practical limitations. The predication of our equation underscores the significance of current clinical efforts in seeking synergistic combination treatment of human cancers in order to decrease drug resistance and to increase the efficacy of cancer immunotherapy.

Our model predicts that simply increasing of TCR stimulation is not enough to increase T cell response due to the regulation of immune checkpoints. In line with this predication, objective cancer responses have not been achieved in clinical trials with several tumor antigens that have strong antigenicity. As predicted by our equation, if we combine tumor antigen peptides with immune adjuvants that are used to increase costimulation, i.e. to increase the expression of stimulatory molecules by antigen presenting cells, such tumor vaccine formulations are able to generate tumor antigen specific T cell responses. However, since some adjuvants have the potential to increase the expression of immune checkpoint molecules, the therapeutic effects of tumor antigen vaccine may be compromised due to immune regulatory mechanisms. To maximize the therapeutic effects of tumor vaccine, our calculations 9 and 10 suggest that components capable of increasing costimulation or decreasing immune checkpoint, or both, should be integrated in an optimal formulation of tumor vaccine.

The critical role of the immune checkpoint in controlling the T cell response can be significantly represented by our equation. This prediction is echoed by recent successful treatment of some human cancers with immune checkpoint blockade strategy (CTLA-4 or PD-1) that aimed to restore or enhance antitumor T cell immunity. Interestingly, to achieve more efficient reduction of negative signals as shown in our equation to reduce N value as close as possible to 0, a combined therapy of both PD-1 and CTLA-4 therapy has been approved by FDA to gain a synergy effect in treatment of metastatic melanoma. However, this combined blockade of immune checkpoints might increase the risk of enhanced adverse effects in some
patients. As predicted by our equation, adjustment of other stimulatory or regulatory signals should be considered in order to gain a safe and strong antitumor T cell response. Since the field of cancer immunotherapy has moved from an era of empirical combinations to one of rational design by considering the compatibility of each regulatory or stimulatory mechanisms, our mathematical model of T cell response can function as a rationale to design a synergistic combination that takes into count of each major factors that work together to affect the net outcome of a T cell response. As predicted by calculation 10, a synergistic combination can be achieved by integrating suboptimal adjusted stimulatory or regulatory singles in order to enhance T cell responses in cancer patients.

Our models are not designed for dose estimation or calculation in application of a particular regimen of cancer immunotherapy, rather our equation may predict a final outcome based on the signal strength a regimen may bring in. Since no defined dose-response has been established in cancer immunotherapy, i.e. highest dose is not always the optimal dose, our model can be used to calculate to what degree a signal (positive or negative) can be integrated for achieving a maximal effect in promoting T cell responses. Based on our equation, a level of signal strength can be determined (for example to set N=0.5). Accordingly, the actual dose (concentration) of a regimen (antibody used to block immune checkpoint) could be determined by selecting a dose that can lead to 50 percent reduction of negative signals. The actual effects of 50 percent reduction of negative signals in cancer treatment could be evaluated by objective biomarkers or clinical responses.

Some chemotherapy drugs cause immunogenic cell death (ICD) in tumor cells. These include a few chemotherapeutics that are currently used in the clinic, like doxorubicin, mitoxantrone, oxaliplatin, and cyclophosphamide. Accumulating clinical data indicate that the activation of adaptive immune responses induced by immunogenic cell death is associated with improved disease outcome in cancer patients. According to our model, the ICD of tumors likely contribute to the increase of T (TCR) signals by releasing more immunogenic tumor antigens, and to the increase of P (costimulation) signals by releasing a series of immunostimulatory damage-associated molecular patterns (DAMPs), so called natural adjuvants, that promotes antigen presentation and T cell priming. Including the immune checkpoint inhibitors (like anti-PD-1) that aimed to reduce N (negative) signals, a combination of chemotherapy drugs that cause ICD with PD-1 blockade would be able to achieve additive or synergistic clinical activity by coincidently increasing P and T and decreasing N as predicted by our model.
Taken together, we provide a mathematical model as a rationale for designing synergistic treatment combination aimed to defuse resistance and maximize T cell responses against cancers. Our equation indicates that a combined therapeutic formula should include approaches capable of increasing tumor antigen stimulation and costimulation, and at the same time, reducing or blocking immune checkpoint signals.

References
1. Topalian, S.L., Drake, C.G. & Pardoll, D.M. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. *Curr Opin Immunol* **24**, 207-212 (2012).
2. Sznol, M. & Chen, L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer--response. *Clin Cancer Res* **19**, 5542 (2013).
3. Pardoll, D.M. The blockade of immune checkpoints in cancer immunotherapy. *Nature reviews. Cancer* **12**, 252-264 (2012).
4. Dong, H., *et al.* Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* **8**, 793-800 (2002).
5. Dong, H. & Chen, L. B7-H1 pathway and its role in the evasion of tumor immunity. *J Mol Med* **81**, 281-287 (2003).
6. Dronca, R.S. & Dong, H. Immunomodulatory antibody therapy of cancer: the closer, the better. *Clin Cancer Res* **21**, 944-946 (2015).
7. Melero, I., *et al.* Evolving synergistic combinations of targeted immunotherapies to combat cancer. *Nature reviews. Cancer* **15**, 457-472 (2015).
8. Zang, X. & Allison, J.P. The B7 family and cancer therapy: costimulation and coinhibition. *Clin Cancer Res* **13**, 5271-5279 (2007).
9. Chen, L. & Flies, D.B. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nature reviews. Immunology* **13**, 227-242 (2013).
10. Zou, W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nature reviews. Cancer* **5**, 263-274 (2005).
11. Krummel, M.F. & Allison, J.P. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med* **182**, 459-465 (1995).
12. Linsley, P.S. & Ledbetter, J.A. The role of the CD28 receptor during T cell responses to antigen. *Annu Rev Immunol* **11**, 191-212 (1993).
13. Schwartz, R.H. T cell anergy. *Annu Rev Immunol* **21**, 305-334 (2003).
14. Chen, L., et al. Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. *Cell* 71, 1093-1102 (1992).
15. Larkin, J., et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med* 373, 23-34 (2015).
16. Ascierto, P.A., Simeone, E., Sznol, M., Fu, Y.X. & Melero, I. Clinical experiences with anti-CD137 and anti-PD1 therapeutic antibodies. *Seminars in oncology* 37, 508-516 (2010).
17. Thomas, A. & Giaccone, G. Why has active immunotherapy not worked in lung cancer? *Annals of oncology: official journal of the European Society for Medical Oncology / ESMO* (2015).
18. Mehta, N.K., Moynihan, K.D. & Irvine, D.J. Engineering New Approaches to Cancer Vaccines. *Cancer immunology research* 3, 836-843 (2015).
19. Pulko, V., et al. TLR3-stimulated dendritic cells up-regulate B7-H1 expression and influence the magnitude of CD8 T cell responses to tumor vaccination. *J Immunol* 183, 3634-3641 (2009).
20. Webster, W.S., et al. Targeting molecular and cellular inhibitory mechanisms for improvement of antitumor memory responses reactivated by tumor cell vaccine. *J Immunol* 179, 2860-2869 (2007).
21. Topalian, S.L., et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 366, 2443-2454 (2012).
22. Hamid, O., et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med* 369, 134-144 (2013).
23. Bezu, L., et al. Combinatorial strategies for the induction of immunogenic cell death. *Frontiers in immunology* 6, 187 (2015).
24. Rock, K.L., Hearn, A., Chen, C.J. & Shi, Y. Natural endogenous adjuvants. *Springer seminars in immunopathology* 26, 231-246 (2005).
25. Kepp, O., et al. Consensus guidelines for the detection of immunogenic cell death. *Oncoimmunology* 3, e955691 (2014).