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A Tumor-Immune Mathematical Model of CD4+ T Helper Cell Dependent Tumor Regression by Oncogene Inactivation

Chinyere I. Nwabugwu, Kavya Rakhra, Dean W. Felsher, and David S. Paik

Abstract— **Understanding the complex dynamics between the tumor cells and the host immune system will be key to improved therapeutic strategies against cancer. We propose an ODE-based mathematical model of both the tumor and immune system and how they respond to inactivation of the driving oncogene. Our model supports experimental results showing that cellular senescence of tumor cells is dependent on CD4+ T helper cells, leading to relapse of tumors in immunocompromised hosts.**

I. Introduction

The immune system plays a major role in controlling tumor growth. AIDS and post-transplant patients with immunodeficiencies have been shown to have an increase in various types of tumors. The importance of the immune system has also been demonstrated in pre-clinical mouse models that are genetically engineered with various immunodeficiencies.

In particular, the CD4+ T cells play a significant role [1, 2]. Previously, CD8+ T killer cells were thought to be the sole direct effector cells that caused cell death. However, more recently, it has become clear that CD4+ T helper cells in the absence of CD8+ cells are able to cause significant tumor regression (by recruiting macrophages).

The focus of this work is on the inactivation of the *MYC* oncogene. The myc family of proto-oncogenes is believed to be involved in the genesis of many human malignancies [3]. Bernard Weinstein first coined the term 'oncogene addiction' in 1997 to represent the phenomenon whereby even the brief inactivation of a single oncogene may lead to sustained tumor regression providing a weakness for molecularly targeted therapy to exploit [4]. Hence it is an Achilles' heel for cancerous tumors [5]. An oncogene is a gene that is stuck in a state of constant activity and is the genesis of cancer. It has been shown that inactivation of an oncogene alone leads to tumor regression. The challenge is in the prevention of relapse of the tumor. This paper combines a model of the tumor and the immune system as it relates to oncogene inactivation.

C. N. is with the Electrical Engineering Department, Stanford University, Stanford CA 94305 USA (phone: 650-736-4183; e-mail:

K. R. was with Stanford University and is now at the Massachusetts Institute of Technology, Cambridge MA 02139 USA (e-mail: krakhra@mit.edu).

D. F. is with the Department of Medicine, Stanford University, Stanford CA 94305 USA (e-mail: dfelsher@stanford.edu).

D. P. is with the Department of Radiology, Stanford University, Stanford CA 94305 USA (e-mail: david.paik@stanford.edu).

II. Methods

Previously, we developed the model shown in Fig. 1. It is capable of representing the various cellular programs in response to oncogene inactivation. The shortcomings are that the immune system has been modeled with system parameters that have one value under an intact immune system and another value in an immunocompromised host. This allows us to replicate experimental results (e.g., the suppression of tumor relapse with an intact immune system, CD4+ cells in particular) but does not shed light on the mechanisms at play as regards the immune system.

We have started incorporating other published models of tumor-immune interactions. However, these have some shortcomings as well. For example, we have built upon the model in [6], which assumes that all interactions are mediated through effector cells (CD8+ T killer cells). However our data [1] demonstrate that CD4+ T helper cells are the critical component of tumor regression. Others have shown that the effect of CD4+ T helper cells is mediated primarily through macrophages. Another shortcoming is that most models of tumor-immune interactions model the tumor as one uniform type of cell. Our data demonstrates otherwise. In particular, tumor cells are known to go through various states such as senescence, differentiation, apoptosis, etc., leading to very different fates for each cell. Hence our work here integrates (1) our previous multi-state tumor model, (2) the immune model in [6], and (3) our changes to the immune model to account for non-CD8+ dependent pathways for tumor cell kill.

A. Tumor Model with Multiple Cellular Programs

Our model of tumor growth/regression kinetics incorporates cell intrinsic mechanisms (apoptosis, proliferative arrest, differentiation/dormancy) and immune-mediated cell extrinsic mechanisms (senescence). We have a complex model of the tumor with 'MYC on', 'MYC off', apoptosis, proliferating, differentiated, and senescent states (Fig. 1).

In our experimental model, we are able to control the expression of transgenic *MYC* using the tetracycline system. An important piece of the model comprises the 'MYC on' and 'MYC off' states, controlled in the conditional transgenic mouse model through doxycycline (dox) in the drinking water. The ability to downregulate *MYC* through dox acts as a generalized model for targeted therapeutics such as gefitinib and erlotinib. 'MYC off' tumor cells have been shown to be able to develop mechanisms to turn *MYC* back on without doxycycline through tTA, Notch, MAPK or Wnt pathways and are represented by the 'Escaped' node in the

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model [7]. This represents the eventual relapse of tumors even after treated with directed therapeutics.

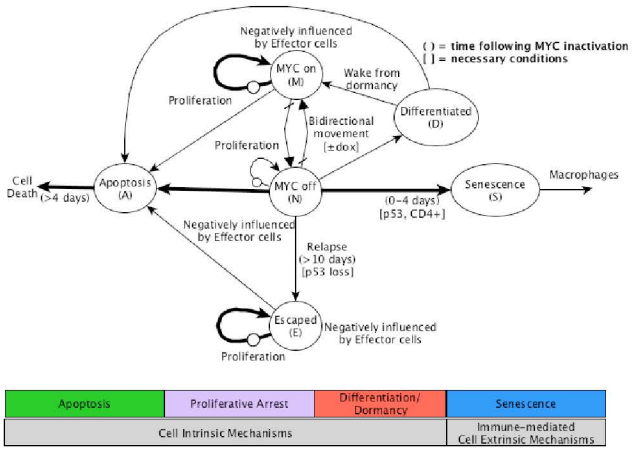

Figure 1. A diagram showing our tumor model with multiple cellular program responses to oncogene inactivation. The arrows show transition of tumor cells from one state to another. The arrows with slashes corresponding to ±dox indicate that this is an independent variable controlled experimentally. The arrows representing proliferative loops have an implicit state during proliferation representing the interphase/mitotic phase of the cell cycle.

B. *Immune System Model*

We extensively model the immune system by adapting the model from [6]. See Fig. 2.

An important difference to note in the graphical representation of this model is that it describes the positive and negative influences of the immune system on the tumor and vice-versa. The arrows signify positive influence and the dashed line negative influence. This differs from our tumor model in that the arrows represent actual movement of cells from one state to the other. Dendritic cells acting as antigen-presenting cells (APCs) can be in the unlicensed and licensed state. Dendritic cells were the chosen APCs because they are the primary mechanism for T-cell activation. T cells (CD8+ effectors, CD4+ T helpers, and Tregs) each are in an activated state before becoming fully enabled.

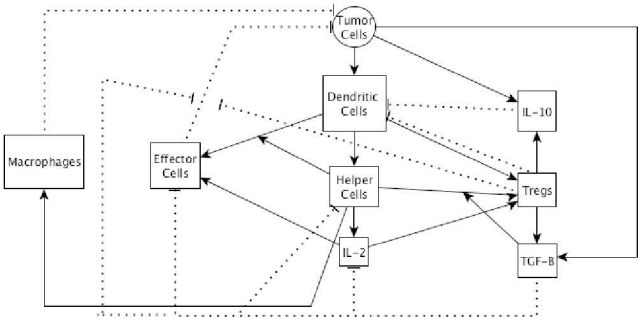

Figure 2. A diagram showing the tumor and immune system interactions. Fig. 1 expands on the "Tumor Cells" node here. The arrows show positive influences while the dotted lines show suppressive mechanisms. Adapted from [6]. We have added the mechanism for helper cells to recruit macrophages to kill senescent tumor cells.

What is novel about our application of [6] is an explicit representation of the direct role of CD4+ T helper cells through macrophages. We show that an intact immune system is necessary to completely eliminate tumors (Fig. 3).

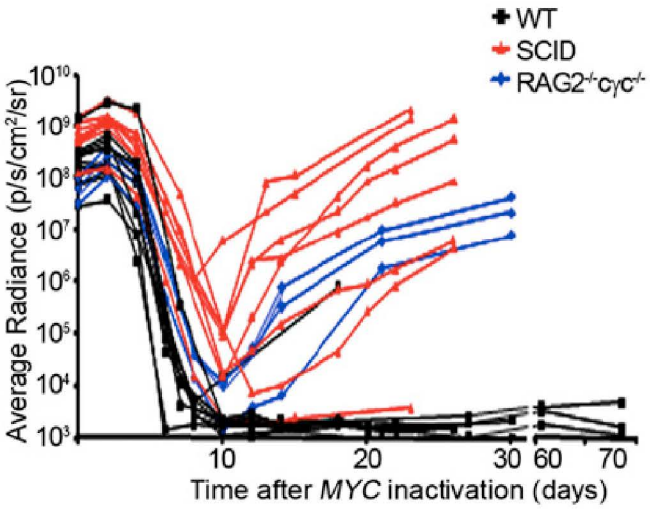

Figure 3. Tumor regression and relapse kinetics as measured by bioluminescence imaging. Wild Type (WT) are immunocompetent mice while SCID and RAG2$^{-/-}$cγc$^{-/-}$ are immunodeficient mice. Excerpted from [1]

Particularly, even in the absence of CD8+ T cells (effector cells), tumor regression can be achieved [1], which is impossible with the original model of [6]. The helper cells play a critical central role in developing immune responses by activating antigen-specific effector cells as well as stimulating the innate immune system against cancer [8]. The CD4+ T helper cells increase the rate of the transition of the tumor cells in the 'MYC off' state to 'Senescence' and macrophages mediate clearance of senescent tumor cells [9].

C. *Combined Tumor-Immune Model*

The implementation of the tumor and immune system model includes a series of ODEs (Fig. 4). Our model integrates across multiple cellular programs and across various immune cells and cytokines (15 cell types/states + 3 cytokines = 18 key model variables) in order to provide an accurate representation. Each equation represents the change in the number of tumor cells, number of immune cells or concentration of cytokines over time.

The T_X variables represent the tumor cells responding with various cellular programs where X is M when MYC is on, N when MYC is off, A when cells are undergoing apoptosis, D when cells differentiate to a phenotypically normal appearance, E for tumor cells that have escaped control, and S for senescent cells in a state of permanent proliferative arrest. This captures the heterogeneity of the tumor cells. The initial conditions were $T_M = 10^8$ and the other tumor populations were set to zero.

Our primary modification to [6] was the addition of CD4+ dependent macrophage (M) recruitment. These tumor-associated macrophages then clear senescent tumor cells.

$$\frac{dT_N}{dt} = K_{T_{N_{prolif}}} T_N - \frac{r_0 T_N^*}{\left(1 + k_2 \frac{T_N^*}{E}\right)\left(1 + k_3 \frac{R}{E}\right)\left(1 + \frac{S}{S_1}\right)} - K_{diff} T_N - K_{T_{N_{apop}}} T_N$$
$$- K_{relapse} T_N + K_{Inactivation} T_M - K_{Activation} T_N - \alpha_M H N,$$

$$\frac{dT_S}{dt} = \alpha_M H N - s T_s,$$

$$\frac{dT_D}{dt} = K_{diff} T_N - K_{wake} T_D - K_{T_{D_{apop}}} T_D,$$

$$\frac{dT_E}{dt} = K_{T_{N_{prolif}}} T_E - \frac{r_0 T_E^*}{\left(1 + k_2 \frac{T_E^*}{E}\right)\left(1 + k_3 \frac{R}{E}\right)\left(1 + \frac{S}{S_1}\right)} - K_{relapse} T_N - K_{T_{E_{apop}}} T_E,$$

$$\frac{dT_M}{dt} = K_{T_{N_{prolif}}} T_M - \frac{r_0 T_M^*}{\left(1 + k_2 \frac{T_M^*}{E}\right)\left(1 + k_3 \frac{R}{E}\right)\left(1 + \frac{S}{S_1}\right)} - K_{wake} T_D - K_{T_{M_{apop}}} T_M$$
$$- K_{Inactivation} T_M + K_{Activation} T_N,$$

$$\frac{dT_A}{dt} = K_{T_{M_{apop}}} T_M + K_{T_{N_{apop}}} T_N + K_{T_{E_{apop}}} T_E + K_{T_{D_{apop}}} T_D - K_{T_A} A(t - \tau),$$

$$\frac{dU}{dt} = \frac{a(T_M^* + T_N^* + T_E^*)}{\left(1 + \frac{I}{I_1}\right)\left(1 + \frac{R}{R_1}\right)} - \frac{\lambda U}{1 + \frac{U}{M_H}} - \delta_U U,$$

$$\frac{dD}{dt} = \frac{\lambda U}{1 + \frac{U}{M_H}} + \delta_D D,$$

$$\frac{dA_E}{dt} = \frac{\alpha_1 M_E}{1 + k_4 \frac{M_E}{D}} - \delta_A A_E - \frac{\alpha_2 A_E C}{\left(1 + \frac{S}{S_2}\right)(C_1 + C)},$$

$$\frac{dE}{dt} = \frac{\alpha_2 A_E C}{\left(1 + \frac{S}{S_2}\right)(C_1 + C)} - \delta_E E,$$

$$\frac{dA_H}{dt} = \frac{\alpha_3 M_H}{1 + k_4 \frac{M_H}{(U + D)}} - \frac{\alpha_4 A_H C}{\left(1 + \frac{S}{S_2}\right)(C_1 + C)} - \delta_A A_H,$$

$$\frac{dH}{dt} = \frac{\alpha_4 A_H C}{\left(1 + \frac{S}{S_2}\right)(C_1 + C)} - \frac{\alpha_7 H S}{S_3 + S} - \delta_H H,$$

$$\frac{dA_R}{dt} = \frac{\alpha_5 M_R}{1 + k_4 \frac{M_R}{D}} - \frac{\alpha_6 A_R C}{(C_1 + C)} - \delta_A A_R,$$

$$\frac{dR}{dt} = \frac{\alpha_6 A_R C}{(C_1 + C)} + \frac{\alpha_7 H S}{S_3 + S} - \delta_R R,$$

$$\frac{dC}{dt} = \frac{p_c A_H}{\left(1 + \frac{S}{S_4}\right)\left(1 + \frac{I}{I_2}\right)} - \frac{C}{\tau_C},$$

$$\frac{dS}{dt} = p_1 R + p_2 T - \frac{S}{\tau_S},$$

$$\frac{dI}{dt} = p_3 R + p_4 T - \frac{I}{\tau_I},$$

$$\frac{dM}{dt} = cH - M\delta_M,$$

$$\frac{dT_M^*}{dt} = \frac{T_M}{\left(1 + \left(\frac{T_M^{1-n}}{k_1}\right)^p\right)^{1/p}},$$

$$\frac{dT_N^*}{dt} = \frac{T_N}{\left(1 + \left(\frac{T_N^{1-n}}{k_1}\right)^p\right)^{1/p}},$$

$$\frac{dT_E^*}{dt} = \frac{T_E}{\left(1 + \left(\frac{T_E^{1-n}}{k_1}\right)^p\right)^{1/p}},$$

Figure 4. Set of equations represented by the model. There are 18 variables and 61 parameters in total.

Many of the system parameter values in the equations from Fig. 4, particularly those from [6] are estimated from the literature while several others are chosen by hand to match simulated tumor trajectories to empirical data.

III. Results

The simulations from our model recapitulate features in the tumor kinetics measured from *in vivo* experimental data from mouse models. We also provide an explanation for the mechanism through which the immune system brings about sustained tumor regression.

In particular, we add the effect of the CD4+ T helper cells through the macrophages. We show the population of the immune cells over time in the top plot of Fig. 5 and the concentration of the cytokines over time in the middle plot of Fig. 5. The adaptive immune system has a time constant of response of approximately 3-7 days. Macrophage recruitment approaches peak by approximately 10 days. The cytokine (TGF-β, IL-2, IL-10) response has similar dynamics.

In the presence of an intact immune system, we achieve sustained tumor regression (not shown), however, in an immune-compromised host, there is relapse (Fig. 5, bottom plot). Similar to our experimental data, the initial tumor response is proliferative arrest with the majority of tumor cell death following day 4 (after oncogene inactivation). In the absence of CD4+ cells, tumor relapse is seen by day 20-25 although the growing escaped tumor cell population begins growth almost immediately following oncogene inactivation but is not apparent until it surpasses the non-escaped tumor cell population.

TABLE I. IIMPORTANT PARAMETER VALUES

Parameter	Estimated Value (day^{-1})	Description
$K_{T_{N_{prolif}}}$	0.1	Transition coefficient from the 'MYC off' state to proliferating
K_{diff}	0.02	Transition coefficient between 'MYC off' state and differentiation state
$K_{T_{N_{apop}}}$	2	Transition coefficient between 'MYC off' and apoptosis state
$K_{relapse}$	3x10^{-8}	Transition coefficient between 'MYC off' state and Escaped state
K_{wake}	0.1	Transition coefficient between Differentiated state and 'MYC on' state
$K_{T_{E_{prolif}}}$	0.5	Transition coefficient from the Escaped state to proliferating
$K_{T_{M_{apop}}}$	0.05	Transition coefficient between 'MYC on' and apoptosis state
$K_{T_{M_{prolif}}}$	0.5	Transition coefficient from the 'MYC on' state to proliferating
$K_{T_{E_{apop}}}$	0.01	Transition coefficient between Escaped state and apoptosis state
$K_{T_{D_{apop}}}$	0.05 / 0.002 / 0.01	Transition coefficient between Differentiated state and apoptosis state (Tumor dependent: lymphoma, osteosarcoma and hepatocellular carcinoma)
$K_{Inactivation}$	0 / 2	Transition from 'MYC on' to 'MYC off' (depending on if *MYC* is on/off)
$K_{Activation}$	2 / 0	Transition from 'MYC off' to 'MYC on' (depending on if *MYC* is on/off)
α_M	1x10^{-10}	Transition coefficient between 'MYC off' state and Oncogene Inactivated Induced Senescence state
δ_M	1x10^{-5}	Cell death or deactivation of macrophages
c	1x10^{-5}	Proportionality coefficient relating macrophages to CD4+ T helper cells
s	0.8	Coefficient for removal of senescent cells due to the macrophages

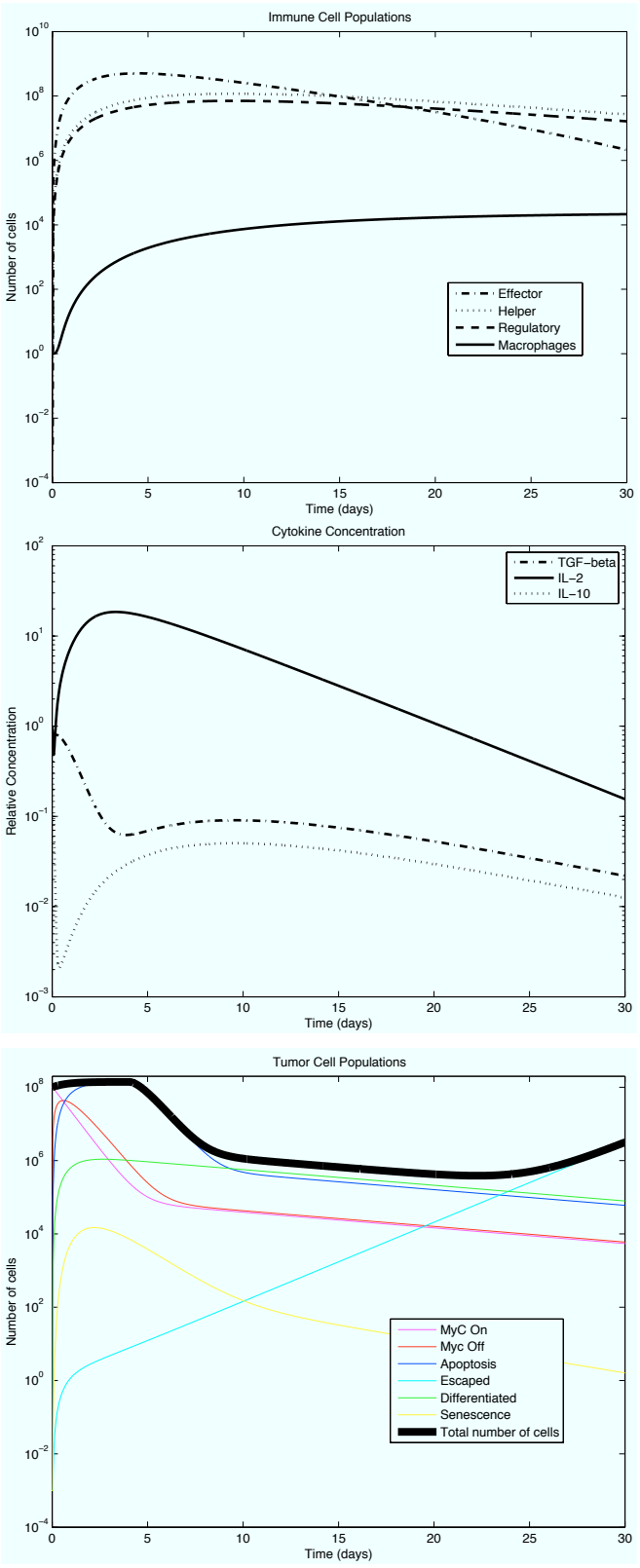

Figure 5. Simulated tumor growth kinetics with tumor cells escaping from conditional control of *MYC* due to a compromised immune system. *MYC* is inactivated at time 0. Top: Immune Cell Populations. Middle: Cytokine Populations. Bottom: Tumor Cell Populations.

IV. Conclusion

Our hybrid tumor-immune model involves the major biological features involved in the CD4+ dependent response to oncogene inactivation.

We explain the mechanisms involved in host-tumor interactions specifically as it relates to oncogene inactivation. We find that in an intact immune system (which is modeled through the specified parameters for the immune system), there is continued regression. An important factor leading to sustained regression is the action of the macrophages on senescent cells.

Our future research is directed towards a sensitivity analysis for the parameters of the model as well as further *in vivo* validation of our model on mouse models.

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References

[1] K. Rakhra, P. Bachireddy, T. Zabuawala, R. Zeiser, L. Xu, A. Kopelman, A. C. Fan, Q. Yang, L. Braunstein, E. Crosby, S. Ryeom, and D. W. Felsher, "CD4(+) T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation," *Cancer Cell,* vol. 18, pp. 485-98, Nov 16 2010.

[2] A. Corthay, D. K. Skovseth, K. U. Lundin, E. Rosjo, H. Omholt, P. O. Hofgaard, G. Haraldsen, and B. Bogen, "Primary antitumor immune response mediated by CD4+ T cells," *Immunity,* vol. 22, pp. 371-83, Mar 2005.

[3] K. M. Ryan and G. D. Birnie, "Myc oncogenes: the enigmatic family," *Biochem J,* vol. 314 (Pt 3), pp. 713-21, Mar 15 1996.

[4] I. B. Weinstein, M. Begemann, P. Zhou, E. K. Han, A. Sgambato, Y. Doki, N. Arber, M. Ciaparrone, and H. Yamamoto, "Disorders in cell circuitry associated with multistage carcinogenesis: exploitable targets for cancer prevention and therapy," *Clin Cancer Res,* vol. 3, pp. 2696-702, Dec 1997.

[5] I. B. Weinstein, "Cancer. Addiction to oncogenes--the Achilles heal of cancer," *Science,* vol. 297, pp. 63-4, Jul 5 2002.

[6] M. Robertson-Tessi, A. El-Kareh, and A. Goriely, "A mathematical model of tumor-immune interactions," *J Theor Biol,* vol. 294, pp. 56-73, Feb 7 2012.

[7] P. S. Choi, J. van Riggelen, A. J. Gentles, P. Bachireddy, K. Rakhra, S. J. Adam, S. K. Plevritis, and D. W. Felsher, "Lymphomas that recur after MYC suppression continue to exhibit oncogene addiction," *Proc Natl Acad Sci U S A,* vol. 108, pp. 17432-7, Oct 18 2011.

[8] K. L. Knutson and M. L. Disis, "Augmenting T helper cell immunity in cancer," *Curr Drug Targets Immune Endocr Metabol Disord,* vol. 5, pp. 365-71, Dec 2005.

[9] L. Hoenicke and L. Zender, "Immune surveillance of senescent cells--biological significance in cancer- and non-cancer pathologies," *Carcinogenesis,* vol. 33, pp. 1123-6, Jun 2012.