Cancer Dynamics: Identification of States for Therapeutic Intervention

Amit Jangid\textsuperscript{1,2}, Md Zububair Malik\textsuperscript{1}, Ram Ramaswamy\textsuperscript{2} and R. K. Brojen Singh\textsuperscript{1}\textsuperscript{*}

\textsuperscript{1}School of Computational and Integrative Sciences, Jawaharlal Nehru University, New Delhi 110067, India.
\textsuperscript{2}Department of Chemistry, Indian Institute of Technology Delhi, New Delhi 110016, India.

We study a minimal model of the stress-driven p53 regulatory network that includes competition between active and mutant forms of the tumor-suppressor gene p53. Depending on the nature of the external stress signal, four distinct dynamical states are observed. These states can be distinguished by different dynamical properties and correspond to \textit{active}, \textit{apoptotic}, \textit{pre-malignant} and \textit{cancer} states. Transitions between any two of these states are found to be unidirectional and irreversible if the stress signal is either oscillatory or constant. When the signal decays exponentially, the apoptotic state vanishes, and for low stress the pre-malignant state is bounded by two critical points, allowing the system to transition reversibly from the active to the pre-malignant state. For significantly large stress, the range of the pre-malignant state expands and the system moves to the cancerous state which is a stable attractor. This suggests that identification of the pre-malignant state may be important both for therapeutic intervention as well as for drug discovery.

Keywords: p53-Mdm2, mutant p53, oncoprotein, stress, regulatory network, cancer dynamics.

\section*{Introduction}

The tumour suppressor gene p53, also termed as the guardian of the genome, crucially determines cell fate through various mechanisms \cite{1,2}. p53 induced different biological outcomes has been studied in detail \cite{4,5}. Activation of the p53 regulatory pathway by internal and external stress can lead to a number of different outcomes \cite{3,4}. p53 is known to be mutated in cancer, either in exonic or intronic portion of the gene due to stress \cite{3,6–10}. These mutations eventually lead to disruption in binding DNA. Hence the cell, during transformation, harbours mutated p53 that may finally develop malignancies. From a network theoretical perspective, p53 is a key hub controlling important genes as well as essential cellular functions \cite{1,11,12}. Studies on signalling networks have provided identification of many target genes for therapeutic interventions in the context of cancer \cite{1,11,12}, although identification of such genes from a dynamical perspective is still open.

Cancer is a complex disease manifested due to the interaction of nonlinear, nonadditive, and dissipative components \cite{13}. In order to understand the functionality of the cell in this state, it is required to know its behavior in the normal state and in perturbed state. Cancer dynamics has been called an emergent property that arises from these interacting components, the constituting genes, small molecules, and the fluctuating environment \cite{14}. p53 holds a central point in signal transduction pathways involving a large number of genes that respond to diverse stress signals \cite{15–18}. This reduces the risk of mutation, and prevents circumstances that can lead to cancer or other pathological states \cite{19}. Since the expression and regulation of p53 depends on its interacting partners in the regulatory pathway, its modeling often involves both negative and positive feedback mechanisms. Mathematical modeling of the p53 regulatory network can provide dynamical information and patterns in order to predict cellular mechanisms and its behavior \cite{20,21} but a challenge is to capture various cellular phases within a simplified minimal model.

p53 is maintained at low levels in the normal condition \cite{22}. Emergence of oscillatory behaviour, one such state in p53 dynamics (“active”), has been extensively studied theoretically and experimentally \cite{27–35}. DNA recovery from low dose of ionizing radiation (IR) corresponds to reversible sustained p53 oscillations with varied amplitude, whereas high dose IR induce irreversible phase leading to stable state (damped oscillations) which corresponds to apoptosis \cite{16–18,27,28}. The variability in amplitude of oscillation is found to be larger than the changes in period of oscillation both for damped and undamped conditions \cite{29}. Further, persistent DNA damage activates ATM, and ATM activates Chk2, which results into p53 oscillations to repair damaged DNA \cite{30}. However, what could be the dynamics of p53 in cancer phase is still debatable question.

Some models of p53 capture various possible dynamical states. It is well known that p53 is coupled with Mdm2 via negative feedback loop \cite{27–35}. In these studies it was observed that if negative feedback loop gets activated then DNA repair takes place, whereas, if positive feedback loop gets activated then p53 activation moves to irreversible apoptotic phase. The other regulators of p53 sometimes regulate p53 pulses, for example, inclusion of MDMX in the model system suppress p53 oscillatory amplitude, whereas, knocking out MDMX significantly enhances this amplitude \cite{32}. In recovery phase of DNA damage, the amplitude of the repetitive p53 pulses tends to lower level and become stable normal state (oscillation is minimized or vanished) \cite{33}. However, in the case of apoptosis with excess stress, this amplitude of pulse abruptly rise and moves to irreversible stable state. Similarly, the other regulators of p53 coupled with positive feedback loop (ATM, PTEN, Akt etc) sometimes can induce switching behavior in the p53 dynamical states. Even though these models could able to capture these various dynamical states (active, recovery and apoptosis) which mimic with experimental results in qualitative sense, these models could not able to capture p53 behavior in cancer phase.

Once a normal cell becomes cancerous by mutational process, this signal propagates to neighboring cells \cite{23}, thereby a competition is established between normal and cancer cells \cite{24}. The onset, development and propagation of cancer cell population in the normal cell ecology provides a new transformed physico-chemical state, which bears several similarities to first order phase

\footnotetext{*Electronic address: zubbairmalik@jnu.ac.in, ramaswamy@iitd.ac.in, brojen@jnu.ac.in

\textsuperscript{2}Electronic address: zubbairmalik@jnu.ac.in, ramaswamy@iitd.ac.in, brojen@jnu.ac.in

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A simple "competition" model for cancer is based on two types of cells, normal and cancer with population \( N_1 \) and \( N_2 \). Their dynamics in the cellular ecology can be modeled by the system of equations [26],

\[
\frac{dN_i}{dt} = R_i N_i \left[ 1 - \frac{N_i}{K_i} - C_{ij} \frac{N_j}{K_j} \right] \quad \forall \ i, j = 1, 2 \ | \ i \neq j.
\]

(1)

\( R_i \) and \( K_i \) are the intrinsic growth rate and the carrying capacity of species \( i \), and \( C_{ij} \) is the competition coefficient measuring the effect on species \( i \) by species \( j \). The equilibrium point (critical point) of the system is \( N_1 = \frac{K_1 - K_2 C_{12}}{1 - C_{12} / K_2} \) and \( N_2 = \frac{K_2 - K_1 C_{21}}{1 - C_{21} / K_1} \).

The normal phase corresponds to the condition \( \dot{N}_1 > 0 \), and \( \dot{N}_2 < 0 \) which implies that \( C_{12} \leq 0 \) and \( C_{21} \geq K_2 / K_1 \) assuming that \( N_2 << N_1 = K_1 \). Biologically this assumption reflects that in the normal phase, population of cancerous cell is small \((\approx 0)\), while population of normal cells reaches its carrying capacity. The condition \( \dot{N}_1 < 0 \), and \( \dot{N}_2 > 0 \) which implies \( C_{12} \geq 0 \) and \( C_{21} \leq K_2 / K_1 \) corresponds to cancer progression. Apart from normal and cancer phases, there are important dynamical states which may drive the cellular system into various pathological states. Since this dynamical system is function of various species, \( \dot{N}_i = F_i(N_1, N_2, \ldots, N_m) \); where \( i = 1 \ldots m \), the exact identification of critical point \((\dot{N}_i = 0)\) is difficult.

In this work, we present a minimal p53 regulatory pathway model in order to study phase transition like behavior of normal and cancer in cellular system at molecular level. We also investigate different cancer progression steps captured in the p53 dynamics and possibility of therapeutic intervention in cancer dynamics. We also discussed various key results in the normal to cancer transition in dynamical sense and observations of various stages of cancer phase.

### Materials and methods

#### Minimal p53\_A-p53\_M-DM2-ARF regulatory network model

The proposed model is a minimal regulatory network p53\_A-p53\_M-DM2-ARF under stress condition. This involves the interaction of activated p53 (p53\_A) and mutated p53 (p53\_M) along with other key regulators DM2, ARF and related molecular species as shown in FIG. 1. In the model [35] p53 induces transcription of RNA\_S and is also produced in the nucleus with a constant basal rate. After being produced, it is translated at a constant rate after proceeding in the cytoplasm, and this is followed by eventual decay. Cytoplasmic DM2 is transported to the nucleus, where it regulates p53 via negative feedback in three different ways. First, transcriptional activity by binding to the p53 transactivation domain [36], second it promotes p53 degradation [37, 38], and finally it favours the export of p53 from the nucleus to cytoplasm [39].

Oncogene activation can be incorporated in the model through either structural alterations (such as chromosomal rearrangement, mutation) or epigenetic modification (gene promoter hypomethylation) [40]. In the present model, we have assumed that a certain type of stress signal \( S \) causes structural, and epigenetic modification that result in oncogenes activation. The activated oncogene then activates ARF within the nucleus [41, 42] and since ARF is a direct inhibitor of DM2 activity by binding to the RING finger domain of DM2 this sequesters DM2 [43]. Tao, and Levine has observed that ARF blocks the nucleo-cytoplasmic shuttling of MDM2, which is essential for the ability of MDM2 to export p53 into the cytoplasm [44].

Weber and others showed that ARF binds to DM2 gene and sequesters it into the nucleolus, which in turn prevents p53 regulation by DM2, and hence leads to the activation of p53 [45]. ARF gets activated due to activation of oncoprotein Myc [45]. It is now well known that activated oncogene, such as c-Myc, leads to the promotion of mutant p53 [46], and this mutated p53 induces the expression of oncogenes [47, 48] as well as inhibits the activity of activated p53 to prevent the cell from apoptosis [49, 49, 51–53]. ARF moves from nucleus to cytoplasm to bind the DM2D and releases the p53 which is due to activation of oncogene [54].

We incorporate the regulating activity of an oncogene in the p53 network model. In a recent study, it has been shown that regulation/deregulation of c-Myc expression due to stress signal can induce mutation/s in the expression of p53 by binding to CA(C/T)GTG-containing site in the p53 promoter [55]. Hence, it has been suggested that stress induced deregulation of c-Myc expression could increase the expression of mutated p53. On the other hand, it has been observed that mutant p53 is able to regulate c-Myc expression by activating c-Myc promoter through C-terminus [47]. Further, it has been reported that p53 repress the c-Myc expression by inducing tumor suppressor miR-145 [56], because c-Myc repression by p53 is required to control the G1 cell cycle arrest [57], such that activation of c-MYC allows the functioning of mutant p53 [58]. Hence, the oncogene we have incorporated in our model is of c-Myc type which allows to interact p53\_M with oncogene and we studied the dynamical behavior of the model system which gives the similar behavior as the main model (see supplementary information). To keep the model simple we have used either hill function or direct interaction between different proteins, and parameters are estimated to observe the qualitative behavior.

### Mathematical framework of the model system

In the proposed model system (FIG. 1) can be represented by a state vector, \( \vec{X} = [x_1, x_2, \ldots, x_8]^T \), where, \( \tau \) is the transpose of the vector, and \( x_i; i = 1, 2, \ldots, 8 \) represents the concentrations of the corresponding molecular species such that \( \vec{X} = [p53\_A, p53\_M, Oncogene, RNA\_A, RNA\_C, MDM2\_C, MDM2\_Y, ARF]^T \). Then the model regulatory network is perturbed with a stress with strength \( S \) which could be irradiation (IR), molecular (or chemical toxic) fluctuations, environmental fluctuations etc. The amount of stress imparted in the model depends on the \( S \) strength, and nature of the \( S \) form introduced in the system. In this work, we have taken three different types of nature of stress \( S \), 1) constant stress form \( S = I \), 2) periodic stress...
FIG. 1: Interaction network for p53M-p53M-MDM2-ARF-Stress. Dashed arrow shows movement from nucleus to cytoplasm or vice versa, while solid arrow, and bars corresponds to activation, and inhibition on respective node. (Modified network from [35])

\[ S = I(1 + \sin(2\pi t))/T, \quad (T = 6 \text{ hrs through out the model }), \text{ and } 3) \text{ exponentially decaying stress } S = Ie^{-\lambda t}. \]

Based on the proposed model system, we arrived at the following set of coupled ordinary differential equations,

\[
\begin{align*}
\frac{dx_1}{dt} &= k_p - \left( k_1 x_7 + d_p + \gamma_{x_1} x_2 + \delta_{x_1} x_3^{n_1} x_{3M}^{n_1} + x_{3N}^{n_1} \right) x_1 \\
\frac{dx_2}{dt} &= \alpha_{x_2} + \delta_{x_2} x_1^{x_1^{n_1}} + x_3^{n_1} x_1 - \gamma_{x_2} x_7 x_2 - \delta_{x_2} x_2 \\
\frac{dx_3}{dt} &= \alpha_{x_3} + \beta_{x_3} x_1^{x_1^{n_1}} + x_3^{n_1} + \delta_{x_3} x_2^{n_2} + x_3^{n_2} - \gamma_{x_3} x_3 \\
\frac{dx_4}{dt} &= k_m + k_2 K_D^{x_1^{n_1}} + x_1^{n_1} - k_0 x_4 \\
\frac{dx_5}{dt} &= k_0 x_4 - d_{x_5} x_5 \\
\frac{dx_6}{dt} &= k_T x_5 - k_3 x_6 \\
\frac{dx_7}{dt} &= k_1 x_6 - d_{x_7} x_7 - k_3 x_7 x_8 \\
\frac{dx_8}{dt} &= k_a + \delta x_3^{n_1} + x_{3M}^{n_1} x_8 - d_a x_8 - k_3 x_7 x_8
\end{align*}
\]

Here, \( k_p \) represents the production rate of p53M, \( k_1 \) is the rate at which MDM2N ubiquitinates p53A, \( d_p \) is the degradation rate of MDM2N independent of p53A, \( \gamma_{x_1} \) is the degradation rate due to p53M inhibition. Further, \( \delta_{x_1} \) shows the rate of mutation in p53A into p53M due to pro-oncogene (oncogenic mutation), \( n_1 \) is Hill coefficient, and \( K_1 \) is the dissociation constant. \( \alpha_{x_2} \) is the production rate of p53M independent from pro-oncogene (which can be ignored), \( \delta_{x_2} \) is mutational translation rate of p53A into p53M due to pro-oncogene mutation. Now, \( \gamma_{x_2} \) is inhibition due to MDM2N (MDM2N dependent degradation), and \( \delta_{x_2} \) shows natural degradation rate of p53M (which can be ignored). \( \alpha_{x_3} \) is the production rate of pro-oncogene (ONCO) independent of stress (which can be ignored), \( \beta_{x_3} \) is the stress dependent activation rate of pro-oncogene (oncogene), \( n_2 \) Hill coefficient, \( K_2 \) is dissociation constant, \( \delta_{x_3} \) is the mutated p53M dependent activation rate, \( n_3 \) is Hill coefficient, \( K_3 \) is the dissociation constant, and \( \gamma_{x_3} \) is the natural degradation rate. \( k_m \) represents the production rate of nucleic mRNA, \( k_3 \) is the maximum production rate of nucleic mRNA, \( K_D \) represents dissociation parameter for p53M and \( k_0 \) is the transportation rate of nucleic mRNA into cytoplasm. \( d_{x_5} \) represents decay rate of mRNA into cytoplasm. \( k_T \) represents the translation rate of MDM2C, while \( k_1 \) represents nuclear localization of MDM2C. \( d_{x_7} \) is the rate of MDM2 auto ubiquitination, and \( k_3 \) is the degradation rate of MDM2N due to binding ARF to MDM2N. \( k_a \) is the production rate of ARF, \( \delta \) is the maximum activation rate of ARF due to pro-oncogene activation, \( n_4 \) is hill coefficient, \( K_4 \) is the dissociation constant, \( d_a \) is the natural degradation rate of ARF, and \( k_3 \) is the MDM2N dependent degradation of ARF.

**Results**

The system of coupled ordinary differential equations are numerically integrated using ODEINT Python. Numerical simulations are carried out for an arbitrary set of initial values for the variables, and after discarding transients the system dynamics is examined. Initial values of mutant p53, and oncogene are kept zero for each form of the new stress discussed above assuming
FIG. 2: The left column show three different form of stress discussed about. In the top row A\(_1\) (normal), A\(_2\) (apoptosis), and A\(_3\) (cancer) display the time course of p53\(_A\) (green), and p53\(_M\) (red) for constant stress of magnitude 1.00, 1.75, and 2.50 respectively (\(K=1000.0\)). B\(_1\) (normal), B\(_2\) (apoptosis), and B\(_3\) (cancer) display the time series for averaged oscillatory stress of magnitude 1.00, 1.50, and 2.50 respectively (\(K=1000.0\)). And C\(_1\) (normal), C\(_2\) (recovery from initial cancer stage), and C\(_3\) (cancer) display the time series for decaying stress of magnitude 1.00, 3.5, and 4.50 respectively (\(K=500.0\) and \(\lambda=0.05 \text{ hrs}^{-1}\)).

FIG. 3: Dynamics on the phase plane for the time series results in the FIG. 2. Green color indicates active state, blue color apoptotic, and red color cancer state. The dot shows the attractor (end point of the trajectory).

initially there is no mutant p53, and oncogene.

**Phase transition driven by Stress**

Panels A\(_1\), A\(_2\), and A\(_3\) in FIG. 2 show the time course of p53\(_A\) and p53\(_M\) for constant stress signal for three different magnitudes \(I = 1.0, \ I = 1.75, \text{ and } I = 2.50\) respectively. For small magnitude of stress signal \(S = 1\), FIG. 2 panel A\(_1\) both p53\(_A\) and p53\(_M\) dynamics show sustained oscillations in which amplitude of oscillations of activated p53 is very high, while the amplitude of mutated p53 is negligibly small. This scenario indicates the possibility of repairing damaged DNA induced by stress signal via p53-MDM2. In such situation, repetitive pulses of p53\(_A\), which dominate those of p53\(_M\) in the system, will be generated if damaged DNA is not properly repaired after delivering first pulse. Once the stress is removed, cell comes to the normal state. Hence sustained oscillations of p53\(_A\) may correspond to the repeated repair efforts of the system to fix damaged DNA.

If the magnitude of the stress signal is significantly high (\(I = 1.75\) panel A\(_2\) FIG. 2). The system attempts to repair damaged DNA by generating few pulses (five) of activated p53 (indicated by damped oscillations in p53\(_A\) and p53\(_M\) dynamics). This could be the indication that after first pulse, the system sees that the damage is not repairable, it delivers the followed pulses with smaller amplitudes, and moves to amplitude death state [59], \(A_{p53A} \rightarrow 0, \ A_{p53M} \rightarrow 0\) (when cell dies out due to apoptosis) with
p53$_A$ > p53$_M$. Then p53 pathway activates many apoptogenic genes, by delivering a constant pulse of activated p53, to kill the cell before mutated p53 gets uncontrolled over the p53$_A$ at stress condition [60, 61]. Alternatively, p53 can also trigger apoptosis by inhibiting antiapoptotic genes (surviving), thus promoting caspase activation [62]. This phase corresponds to apoptotic phase (amplitude death [59] after damped oscillations), where the concentration of p53$_A$ still dominates that of p53$_M$ in the cellular dynamics.

In the third phase p53$_A$ and p53$_M$ dynamics, for high stress (S = 2.5), are different from earlier two phases (panel A$_3$ FIG. 2). In this phase, p53$_M$ concentration grows rapidly, and is high compared to p53$_A$ in the normal phase, indicating uncontrolled behavior of p53$_A$. This dynamical behavior is qualitatively similar to the experimental observation of higher expression of mutated p53 leads to cancer [63], and in some cancers mutated p53 has dominated effect over active p53 [64]. The normal to cancer transition (NCT) is irreversible: the stress $S$ imparted to the system is able to drive the system into three distinct dynamical states in addition active, apoptosis (indicated by dominant p53$_A$, and low p53$_M$) and cancer (p53$_M$ concentration rapidly increasing behavior, and low concentration of p53$_A$ with slow decay) states (FIG. 2).

We studied the system dynamics driven by periodic stress of magnitude $I = 1.0,1.5,2.5$ (panel B$_1$, B$_2$ and B$_3$ FIG. 2 respectively). We observed three different dynamical phases, active, apoptosis, and cancer phase (panels B$_1$, B$_2$ and B$_3$ FIG. 2 respectively), which are qualitatively similar to the constant stress case (panels A$_1$, A$_2$ and A$_3$ FIG. 2 respectively). However, the behavior of p53$_A$ and p53$_M$ in FIG. 2 panel B$_2$ after successive four pulses (with decaying pulses amplitudes), we still observed small amplitude oscillations which do not die out with time which are negligible to the oscillation of active state. Increasing the magnitude, this oscillatory behavior dies out (not shown here). In the case of cancer phase, the monotonically growth of p53$_M$ is a little slower as compared to constant stress signal case indicating periodic signal helps the cell to prevent moving to either apoptosis or cancer phase.

The scenario of the behavior of the system dynamics is different in the case of exponentially decay stress. Panel C$_1$, C$_2$ and C$_3$ FIG. 2 show the time course of p53$_A$, and p53$_M$ for the magnitude $I = 1.0,3.5,4.5$. For $I = 1.0$, we observed active state with sustain oscillations (panel C$_1$ FIG. 2). Increasing the stress ($I = 3.5$), the dynamics shows that first, the stress provides a shock to the system allowing p53$_A$ moves to amplitude death [59] ($A_{p53A} \rightarrow$ constant) for small interval of time $T_{ps} \rightarrow [9.8-37]$ hrs, whereas p53$_M$ concentration is suddenly increased dominating p53$_A$ concentration during $T_{ps}$. Since p53$_{M_2}$ dominates over p53$_A$ during $T_{ps}$, this state could be considered as a premalignant signature of the system dynamics which can be termed as critical state [25]. During this short time interval ($T_{ps} \rightarrow$ finite and $A_{p53A} \rightarrow$ constant), the active state of the system is collapsed, and p53$_M$ becomes uncontrollable, and if $T_{ps} \rightarrow \infty$, then the system moves towards cancer phase. Identification of this critical state in cancer patients is very crucial for possible therapeutic intervention for preventing from cancer. After this time interval, the system regains its active state, where, p53$_A$ attains its sustain oscillation state by suppressing p53$_{M_2}$ concentration level, and then the system repairs damaged DNA. Significantly high dose of the stress signal triggers higher expression of mutated p53 protein than activated p53 which corresponds to the cancer phase. Hence, in case of exponentially decaying stress signal, we are able to observe only two phases active, and cancer phase. Dynamics on the phase plane, for the time series used in the FIG. 2, are shown in FIG. 3. Green color indicates active state (panel A$_1$, B$_1$, C$_1$, and C$_2$), blue color apoptotic (panel A$_2$, and B$_2$), and red color cancer state (panel A$_3$, B$_3$, and C$_3$). The dot shows the attractor (end point of the trajectory).

Oncogenic regulation of normal and cancer dynamics

In this section we study the cooperative impact of oncogene on the dynamics of p53$_A$ and p53$_M$ in the regulating pathway. We consider microscopic dissociation parameter $K_3$, which is an equilibrium constant that amounts to the probability per unit time to dissociate molecular complex [65]. FIG. 4 shows steady state behavior of P53$_A$, and p53$_M$ as a function of magnitude of stress ($I$) for three different values of $K_3 = 1000,500,100$. The system’s behavior and transition of the states can be studied from steady state behavior (FIG. 4). For oscillatory behavior of p53$_A$ and p53$_M$, the mean population is the average of the maxima and minima of the oscillation calculated in time window of $t = 145.82hr – 166.66hr$ (removing transients) while, in case of no oscillations, population of p53$_A$, and p53$_M$ were taken at time 166.66 hrs (end point of trajectory).

We observed different phases/states (FIG. 4 panels A$_1$, A$_2$ and A$_3$) in the dynamics of p53$_A$ and p53$_M$ driven by constant stress for three different values of $K_3 = 1000,500,100$. For small $I$ values ($I \leq 1.19$), the criteria for this was as average value of p53$_A$ reduces by 5% to its maximum averaged value in the case of without stress, both p53$_A$ and p53$_M$ exhibit oscillatory behavior (FIG. 4 panel A$_1$, $K_3 = 1000$) with the concentration of p53$_M$ maintained at minimum level as compared to that of p53$_A$. This phase may be considered as active phase of the cellular system, where, p53$_A$ delivers successive pulses to activate various genes which are involved in the pathway to repair damaged DNA. In this case, one can see that difference between $x_1$ and $x_2$ is almost constant ($\Delta x_{12} \rightarrow$ constant). Increasing the strength of the stress $I (I \rightarrow [1.19 – 2.04])$, we get that $\Delta x_{12}$ becomes variable where, p53$_A$ > p53$_M$ and $A_{p53A}, A_{p53M} \rightarrow$ constant exhibits amplitude death (cell programed death) scenario in both p53$_A$ and p53$_M$ dynamics. This state may correspond to apoptotic state (cyan area) in the system dynamics.

In apoptotic phase, the system is not able to repair damaged DNA thereby, p53$_A$ activates apoptogenic genes favoring to program cell death. It can also be observed that the concentration levels of both p53$_A$ and p53$_M$ are converged to a critical level $x_c$ as $I \rightarrow I_c = 2.04$, which is termed as critical point (FIG. 4 panel A$_1$). This critical point can be defined such as: $\lim_{l \to 1} \Delta x_{12} = 0$ and $x_1, x_2 \to x_c$. Slight increase in $I (I > I_c = 2.04)$ triggers slow dominance of p53$_M$ over p53$_A$, which is the beginning of new departure to the cancer phase. This new stage can be termed as pre-malignant regime (magenta area). Further increasing $I$, p53$_M$ is found to rapidly increased, while p53$_A$ is decreased significantly low, indicating p53$_A$ can no longer control p53$_M$ signal such that $\Delta x_{12}$ rapidly increased and then becomes stable. Hence, this phase may be considered as cancer phase (grey area) [25, 66]. In this case, critical point can be seen as the point of departure to either in apoptotic phase or cancer phase.

Decreasing the value of dissociation parameter $K_3 = 500$, we observe similar behavior in p53$_A$ and p53$_M$ dynamics (FIG. 4 panel A$_2$), but critical point can be obtained at smaller value of magnitude of stress signal, $I_c = 1.79$ and range of apoptotic
and pre-malignant state get shrunk and the range of cancer phase increased as compared to the case \( K_3 = 1000 \) (FIG. 4 panel A). For comparatively small value of dissociation parameter \( K_3 = 100, \Delta I_{c1} \approx 0 \) and \( \Delta I_{c2} \to \infty \) (FIG. 4 panel A3). In such situation, a stress state suddenly moves from active to cancer phase crossing critical point without showing the signatures of apoptotic and pre-malignant states, and then become steady \( (\Delta I_{c1} \to \text{constant}) \) both in \( p53_A \) and \( p53_M \). It may lead to first order phase transition. In case of \( c\text{-Myc} \) we did not observe pre-malignant regime in constant stress case (supplementary information, panel A1, A2, and A3 FIG. 2).

In case of periodic stress, and for same values of \( K_3 = 1000, 500, 100 \) (FIG. 4 panels B1, B2 and B3), we observed the similar pattern of four states along with critical point as we found in the case of constant stress. This results also show that all the four states can be obtained at significantly smaller values of stress signal \( I \) as compared to those of constant stress case.

We observed different scenario for exponentially decay stress. In this case, we only get three states, active, pre-malignant and cancer state for \( K_3 = 1000, 500, 100 \) and \( K_3 = 100 \) we get only two states (FIG. 4 panels C1, C2 and C3). We have also observed that there are two critical points, \( I_{c1} \) and \( I_{c2} \) \((x_{c1} > x_{c2})\) in the range \( \Delta I = I_{c1} - I_{c2} \) (wheat region, FIG. 4 panels C1 and C2). In this range \( \Delta I \), \( p53_M \) dominates over \( p53_A \) for a certain time interval \( T_{ps} \) (previous section), which is a signature of pre-malignant or critical state, which comes back to the active state after \( T_{ps} \) time interval if \( I \in [I_{c1}, I_{c2}] \), where, \( I_{c2} = 5.80 \) for \( K_3 = 1000 \) and \( I_{c2} = 3.83 \) for \( K_3 = 500 \). In the dynamical system study, the identification of this critical point/s and pre-malignant regime of any cancer type are quite important for therapeutic intervention of the cancer [25]. The reason could be if system dynamics is in this regime \( I \in [I_{c1}, I_{c2}] \), there is a possibility of repairing damaged DNA. For lower value of \( K_3 \) parameter \( (K_3 = 100) \), if \( I > I_{c1} \), the two critical points become single \( I_{c1} = I_{c2} > I_c \), and the active state directly jumps to cancer state \( (T_{ps} \to \infty) \) via \( I_c \) (FIG. 4 panels C3). These critical points can be seen as the points of departure to either in active state or cancer state. All these results indicate that the impact of oncogene is quite significant in regulating normal and cancer dynamics as well as their state transition.

**Phase transition and key to therapeutic intervention**

In this section we study the dynamical behavior of \( p53_A \) and \( p53_M \) in two parameter space driven by different stress (FIG. 5 panel A). Each point in two parameter space (Magnitude of stress, \( K_3 \)) (FIG. 5 panel A) are calculated concentrations of \( p53_A \) in the dynamics: for oscillatory dynamics each point is the average of maxima and minima obtained in the time interval [45.82, 166.66] hrs, otherwise (no oscillation) concentration are measured at time 166.66 hrs. FIG. 5 A (with constant stress) shows three distinct regimes/phases active (green region), apoptosis (yellow region) and cancer (red region). For large value of \( K_3 \), transition from active to cancer state is through apoptotic phase, while for low value of \( K_3 \), the range of apoptotic regime is so thin that slight increase in stress magnitude \( (I) \) might lead to direct cancer phase. Transition from active to apoptotic state is one directional. Figure A1, A2, A3, and A4 are the time course at different point on the heat map (FIG. 5 A).

Similar behavior was observed in the patterns of two parameter space in case of periodic stress (FIG. 5 panel B). The panels B1, B2, B3, and B4 show the corresponding time series for the parameter set \((0.5, 500.0), (1.3, 500.0), (0.96, 100.0), \) and \((2.0, 200.0)\) respectively on the heat map. It is also observed that in case of periodic stress, less magnitude of stress is required for...
In case of exponentially decay stress, we observed only two states active (green region), and cancer (red region) (FIG. 5 panels B, and C) unlike constant, and periodic stress. The significantly small yellow region as compared to active and cancer regions is observed different phase transition than constant stress.

In case of exponentially decay stress, we observed only two states active (green region), and cancer (red region) (FIG. 5 panels B, and C) unlike constant, and periodic stress. The significantly small yellow region as compared to active and cancer regions is observed different phase transition than constant stress.

The results discussed above indicate that apart from different stress, introduced in the system, there are various other factors which can drive the system to cancer state, for example, oncogene and its associated pathway/s. These factors are in fact the key to sustain the system at active state or bring back to active state from pre-malignant state by regulating these parameters and their associated pathways. Moreover, the identification of these critical point/s and pre-malignant state is very important.

Cancer recovery phase: dynamics of pre-malignant state

In this section we focus on the properties of the pre-malignant, and critical point/s, and their importance in therapeutic intervention to prevent the cancer. As we have discussed in previous sections, we could able to find only one critical point ($T_c$) for constant, and periodic stress driven system (FIG. 2, 4 and 5). In these cases the pre-malignant state is just the beginning of cancer state, and it is hard to bring back to normal state. The scenario is quite different for exponentially decay stress. Here, we study the recovery time behavior for three different set of parameters such as (magnitude of stress, and $K_3$), (magnitude of stress, and $K_4$), and (magnitude of stress, and $\lambda$) (FIG. 6). In this case, we observed two critical points $T_{c1} > 0$ and $T_{c2} > 0$. ...
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FIG. 6: The left column show decaying stress. Second column, A, B, and C, show two parameter cancer recovery behavior for the parameter set (magnitude of stress, $K_3$), (magnitude of stress, $K_4$), and (magnitude of stress, $\lambda$) respectively driven with same decaying stress. $A_1$, $A_2$, $A_3$, and $A_4$ correspond to the time course for the parameter set (3.25,700.0), (3.8, 700.0), (3.25, 500.0), and (3.8, 500.0) respectively on the heat map A. $B_1$, $B_2$, $B_3$, and $B_4$ correspond to the time course for the parameter set (3.25,300.0), (3.8, 300.0), (3.25, 1.0), and (3.8, 1.0) respectively on the heat map B ($K_3 = 500.0$). $C_1$, $C_2$, $C_3$, and $C_4$ correspond to the time course for the parameter set (3.0,0.07), (6.0,0.07), (3.0,0.03), and (6.0,0.03) respectively on the heat map C ($K_3 = 1000.0$, $K_4 = 10.0$). On the heat map green color shoes lowest recovery time, while red shows highest recovery time or no recovery (in case of cancer)

with $T_{c_2} > T_{c_1}$ separated by a time interval $T_{ps} = T_{c_2} - T_{c_1} \geq 0$ in the $p53_A$ and $p53_M$ dynamics. However, for $\text{time} < T_{c_1}$ and $\text{time} > T_{c_2}$, the system dynamics will be in pre-malignant state, where $p53_A$ dynamics showed sustain oscillatory behavior controlling $p53_M$ dynamics to maintain at minimum concentration level ($p53_M < p53_A$). This particular state is termed as pre-malignant state (discussed earlier), and is shown in FIG. 6. For certain values of the parameter set we observed that the system dynamics show a situation, $T_{ps} \rightarrow \infty$, $T_{c_1} \rightarrow T_{c_2} \rightarrow T_c$ and $p53_M > p53_A$ exhibit stable attractor, then the dynamical system becomes cancer state. In this case, we did not observed apoptotic state.

We observe that by decreasing $K_3$ and increasing the magnitude of stress $I$, $T_{ps}$ is increased, but $T_{c_1} \rightarrow \text{constant}(same)$, which is pre-malignant state (FIG. 6 panels $A_1 - A_4$) in the parameter space of $I$, and $K_3$. In such situation, there is always a possibility of bringing back into active state. However, for significantly small $K_3 \leq K_3^c$ and large $I \geq I^c$, where, $K_3^c$ and $I^c$ being critical values, we could able to observe the cancer state condition: $\lim_{(K_3 \leq K_3^c, I \geq I^c)} T_{ps} \rightarrow \infty$, $T_{c_1} \rightarrow T_{c_2} \rightarrow T_c$ and $p53_M > p53_A$ exhibiting stable attractor. Once the system reaches this phase, the dynamical process of the system becomes irreversible, and the system could not back to active state. Similar behavior and dynamical patterns can be found for set of $(I, K_3)$ in FIG. 6 panel B and $B_1 - B_4$, and for set of $(I, \lambda)$ in FIG. 6 panels C, $C_1 - C_4$, where we could see the three states distinctly.

From the perspective of dynamical system analysis, identification of these three states obtained in any type of cancer is quite important in view of prevention from that cancer. The reason could be due to the possibility of bringing back to normal condition from pre-malignant signature. Proper therapeutic intervention and drug administration needed to be done during the time $T_{ps}$ to prevent from cancer phase. It may not be able to cure the cancer once proper intervention and preventive measures are not taken up. Further, for the sake of cancer drug discovery, this pre-malignant state could be proper stage of investigation.
Discussion and Summary
A dynamical systems approach can offer fresh insights to understanding cancer progression, and therefore suggest new protocols in therapeutic intervention. Cancer can be treated in broadly in two ways by exploring dynamical behavior along with hidden patterns of cancer and associated cellular states, and second to explore proper cellular state and time for therapeutic intervention or drug discovery. In the present work we have studied a model that incorporates the dynamics of both active and mutant p53 that are driven by different forms of time-dependent stress, and have considered the impact of ARF and oncogenes through different feedback mechanisms. This simple model has four distinct final states that can be characterised by the asymptotic dynamics: these have experimental validation [29, 67] and variously correspond to active, apoptotic, pre-malignant and cancer states.

Sustained oscillations in p53\textsubscript{A} and p53\textsubscript{M} dynamics can be seen as repeated pulses that occur in the system when DNA damage is repaired. Such oscillations persist until the DNA repair is completed [33]. Stress that triggers the system to the active state is a reversible process, the dynamics reverting to normal when the stress is removed. For high stress or when there are p53\textsubscript{M} activators such as oncogene and/or ARF, the amplitude of p53\textsubscript{A} oscillation will be large enough to arrest the cell cycle. In this situation, the amplitude of p53\textsubscript{M} reaches a critical level, although lower than the amplitude of p53\textsubscript{A} [68]. Oscillatory dynamics vanishes [59] for both p53\textsubscript{A} and p53\textsubscript{M}; this is a state of amplitude death leading to a stable fixed-point attractor. This corresponds to apoptosis since the system cannot revert to oscillatory dynamics: this is an irreversible transition [25].

For large stress the production of mutant p53\textsubscript{M} becomes rapid and uncontrolled. The concentration level of p53\textsubscript{M} exceeds a critical apoptotic threshold, and this can be seen as a stress-induced premature senescence. This suppresses apoptosis and triggers cancer progression [69]. For constant or a periodic stress signal, we were able to find a condition where p53\textsubscript{A} and p53\textsubscript{M} coincide. We term this a critical point of the dynamical system, and this can be considered as leading to a new, cancer, state: mutant p53\textsubscript{M} is uncontrollable (p53\textsubscript{M})p53\textsubscript{A}). Furthermore, there is no possibility of DNA repair and the process is irreversible. However, there is a small range of stress where the concentration of mutant p53 increases slowly, compared to the monotonic increase in the cancer regime. This we term pre-malignant. For constant or periodic stress there is a single critical point and hence the system, having transitioned to the cancer state cannot revert to the normal state.

For exponentially decaying stress only three states can be observed: active, pre-malignant or cancer. There are two critical points in this case, indicating the possibility of reversing from the pre-malignant to the active state. The width of the transition region depends on the stress inducing parameters with respect to oncogene, ARF, and other mechanisms. Identification of this range of the pre-malignant state, along with critical points, is important for therapeutic intervention.

Our study provides a qualitative picture of the dynamical properties of states observed in various experiments on cellular dynamics. The present results indicate the possibility of measuring how much stress suffices to lead to cancer. It will be important to explore the role of noise in driving the dynamics to see how robust these results are to extrinsic or intrinsic stochasticity.

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Author Contributions
AJ and MZM conceived the model. AJ, MZM, RR and RKBS did analytical work. AJ, MZM, RR and RKBS did the numerical experiment and prepared the figures of the numerical results. AJ, MZM, RR and RKBS analyzed and interpreted the analytical as well as simulation results. All authors wrote and approve the final manuscript.

Additional Information
Additional information is available at https://www.preprints.org.

Conflict of Interest Statement
None declared.

Model simulation
Numerical integration were carried out using Python ODEINT.


<table>
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<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
<th>References</th>
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<td>2.</td>
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<td>3.</td>
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<tr>
<td>4.</td>
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<td>5.</td>
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<td>14.</td>
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<td>27.</td>
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