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Research article

Optimal strategies of oncolytic virus-bortezomib therapy via the apoptotic, necroptotic, and oncolysis signaling network

Donggu Lee[1], Aurelio A. de los Reyes V[2,3] and Yangjin Kim[1,*]

[1] Department of Mathematics, Konkuk University, Seoul, Republic of Korea

[2] Institute of Mathematics, University of the Philippines Diliman, Quezon City 1101, Philippines

[3] Biomedical Mathematics Group, Pioneer Research Center for Mathematical and Computational Sciences, Institute for Basic Science, Daejeon 34126, Republic of Korea

* **Correspondence:** Email: ahyouhappy@konkuk.ac.kr; Tel: +82024500450; Fax: +82024581952.

Supplementary: Nondimensionalization of the mathematical model and detailed analysis of the mathematical model

S.1. Nondimensionalization

S.1.1. Intracellular modules

Let variables $\bar{S}(\bar{t})$, $\bar{F}(\bar{t})$, $\bar{A}(\bar{t})$, and $\bar{R}(\bar{t})$ be activities of $I\kappa B$, $NF\kappa B$, BAX, and $RIP1$, respectively, at time $\bar{t}$. The scheme includes autocatalytic activities of $I\kappa B(\bar{S})$, $NF\kappa B(\bar{F})$, $BAX(\bar{A})$, and $RIP1(\bar{R})$, protein degradation of those key molecules, mutual inhibition between $I\kappa B$ and $NK\kappa B$ and inhibition of BAX activity by $NF\kappa B$ and $RIP1$, and activation of $RIP1$ by $NF\kappa B$ in the presence of oncolytic-virus and bortezomib. Based on biological observations, we write the phenomenological equations for the rate change of those key modules ($\bar{S}$, $\bar{F}$, $\bar{A}$, $\bar{R}$) as follows:

$$\frac{d\bar{S}}{d\bar{t}} = k_{SB}\frac{\bar{B}}{k_{12}+k_{13}[oHSV]} + \frac{k_1 k_2^2}{k_2^2+k_5\bar{F}^2} - \mu_S\bar{S}, \tag{S.1}$$

$$\frac{d\bar{F}}{d\bar{t}} = c_1 + \frac{k_3 k_4^2}{k_4^2+k_6\bar{S}^2} - \mu_F\bar{F}, \tag{S.2}$$

$$\frac{d\bar{A}}{d\bar{t}} = c_2 + \frac{k_7 k_8^2}{k_8^2+k_9\bar{F}^2} - \mu_A\bar{A}, \tag{S.3}$$

$$\frac{d\bar{R}}{d\bar{t}} = k_{10} + k_{11}[oHSV]\bar{F} - \mu_R\bar{R}, \tag{S.4}$$

where the first term in Eq (S.1) represents the signaling pathways from bortezomib to IκB in the absence and presence of OVs, k_{SB} is the signaling strength of bortezomib, B is the bortezomib level, k_{12} is a scaling factor for inhibition of the bortezomib signaling, k_{13} is the inhibition strength of bortezomib signaling by OVs, [oHSV] is a biochemical switch for oncolytic viruses with [oHSV] = $\frac{\bar{v}}{\bar{k}+\bar{v}}$ where $\bar{v}$ is the OV density, as introduced below, and k is the Hill type parameter, giving [oHSV] = 0 (1) in the absence (presence) of virus, c_1, c_2, k_{10} are the signaling pathways to the proteasome-NFκB -Bcl-2 complex, Bax, and RIP1, respectively, k_1, k_2, k_3 are the autocatalytic enhancement parameters for IκB, proteasome-NFκB-Bcl-2 complex and Bax, respectively, k_2, k_4, k_8 are the Hill-type inhibition saturation parameters from the counter part of IκB, proteasome-NFκB -Bcl-2 complex and Bax, respectively, k_5 is the inhibition strength of IκB by the proteasome-NFκB-Bcl-2 complex, k_6 is the inhibition strength of the proteasome-NFκB-Bcl-2 complex by IκB, k_9 is the inhibition strength of the Bax by the , and finally, $\mu_s, \mu_f, \mu_a, \mu_r$ are decay rates of IκB, proteasome-NFκB-Bcl-2 complex, Bax, RIP1, respectively.

By using reference values of main variables (marked in asterisk ($*$) in Table 1 in main text) and performing the following non-dimensionalization:

$$
\begin{aligned}
&t = \mu_S \bar{t}, S = \frac{\bar{S}}{S^*}, F = \frac{\bar{F}}{F^*}, A = \frac{\bar{A}}{A^*}, R = \frac{\bar{R}}{R^*}, B = \frac{\bar{B}}{B^*}, \lambda_B = \frac{k_{SB}B^*}{S^*\mu_S k_{12}},\ \alpha = \frac{k_{13}}{k_{12}},\ \sigma_1 = \frac{k_1}{S^*\mu_S},\\
&\sigma_9 = k_2,\ \sigma_4 = k_5(F^*)^2, \sigma_7 = \frac{c_1}{F^*\mu_S},\ \sigma_2 = \frac{k_3}{F^*\mu_S},\ \sigma_{10} = k_4,\ \sigma_5 = k_6(S^*)^2,\ \omega_1 = \frac{\mu_F}{\mu_S},\ \sigma_8 = \frac{c_2}{A^*\mu_S},\\
&\sigma_3 = \frac{k_7}{A^*\mu_S},\ \sigma_{11} = k_8,\ \sigma_6 = k_9(F^*)^2,\ \omega_2 = \frac{\mu_A}{\mu_S},\ \sigma_{12} = \frac{k_{10}}{R^*\mu_S},\ \sigma_{13} = \frac{k_{11}F^*}{\mu_S R^*},\ \omega_3 = \frac{\mu_R}{\mu_S},\ k = \frac{\bar{k}}{v^*},
\end{aligned}
\tag{S.5}
$$

of Eqs (S.1)–(S.4), we obtain the dimensionless equations for IκB (S), NFκB-Bcl2 (F), Bax (A), RIP1 (R) with a set of essential control parameters:

$$\frac{dS}{dt} = \lambda_B \frac{B}{1+\alpha[oHSV]} + \frac{\sigma_1\sigma_9^2}{\sigma_9^2+\sigma_4F^2} - S, \tag{S.6}$$

$$\frac{dF}{dt} = \sigma_7 + \frac{\sigma_2\sigma_{10}^2}{\sigma_{10}^2+\sigma_5S^2} - \omega_1 F, \tag{S.7}$$

$$\frac{dA}{dt} = \sigma_8 + \frac{\sigma_3\sigma_{11}^2}{\sigma_{11}^2+\sigma_6F^2} - \omega_2 A, \tag{S.8}$$

$$\frac{dR}{dt} = \sigma_{12} + \sigma_{13}[oHSV]F - \omega_3 R. \tag{S.9}$$

where [oHSV] = $\frac{v}{k+v}$ where v is the dimensionless OV density, as introduced below.

S.1.2. cancer cells & Anticancer drugs

We write the equations for the rate change of cancer, oncolytic-virus, and Bortezomib as follows:

$$\frac{d\bar{x}}{d\bar{t}} = \bar{\lambda}\bar{x}\left(1-\frac{\bar{x}}{\bar{x}_0}\right) - \bar{\beta}_1\bar{x}\bar{B}I_{apop} - \bar{\beta}_2\bar{x}\bar{v}I_{oncoly} - \bar{\beta}_3\bar{x}\bar{v}I_{necrop}, \tag{S.10}$$

$$\frac{d\bar{y}}{d\bar{t}} = -\bar{\delta}\bar{y} + \bar{\beta}_2\bar{x}\bar{v}I_{oncoly} + \bar{\beta}_3\bar{x}\bar{v}I_{necrop}, \tag{S.11}$$

$$\frac{d\bar{n}}{d\bar{t}} = \bar{\delta}\bar{y} - \bar{\mu}\bar{n}, \tag{S.12}$$

$$\frac{d\bar{v}}{dt} = \bar{u}_V + \bar{b}\bar{\delta}\bar{y}(1 + \bar{\alpha}_1\bar{B}) - \bar{\gamma}\bar{v}, \tag{S.13}$$

$$\frac{d\bar{B}}{d\bar{t}} = \bar{u}_B - (\bar{\mu}_1\bar{x} + \bar{\mu}_2\bar{y})\frac{\bar{B}}{\bar{k}_B + \bar{B}} - \bar{\mu}_B\bar{B}, \tag{S.14}$$

By using reference values of main variables and performing the following non-dimensionlization:

$$\begin{aligned} &t = \mu_S\bar{t},\ x = \frac{\bar{x}}{x^*},\ y = \frac{\bar{y}}{x^*},\ n = \frac{\bar{n}}{x^*},\ v = \frac{\bar{v}}{v^*},\ B = \frac{\bar{B}}{B^*},\ \lambda = \frac{\bar{\lambda}}{\mu_S}, x_0 = \frac{\bar{x}_0}{x^*},\ \beta_1 = \frac{B^*\bar{\beta}_1}{\mu_S},\\ &\beta_2 = \frac{v^*\bar{\beta}_2}{\mu_S},\ \beta_3 = \frac{v^*\bar{\beta}_3}{\mu_S},\ \delta = \frac{\bar{\delta}}{\mu_S},\ \mu = \frac{\bar{\mu}}{\mu_S},\ u_V = \frac{\bar{u}_V}{v^*\mu_S},\ b = \frac{\bar{b}y^*}{v^*},\ \alpha_1 = B^*\bar{\alpha}_1,\\ &\gamma = \frac{\bar{\gamma}}{\mu_S},\ u_B = \frac{\bar{u}_B}{B^*\mu_S},\ \mu_1 = \frac{x^*\bar{\mu}_1}{B^*\mu_S},\ \mu_3 = \frac{y^*\bar{\mu}_3}{B^*\mu_S},\ k_B = \frac{\bar{k}_B}{B^*},\ \mu_B = \frac{\bar{\mu}_B}{\mu_S}, \end{aligned} \tag{S.15}$$

to get the dimensionless model equations:

$$\frac{dx}{dt} = \lambda x\left(1 - \frac{x}{x_0}\right) - \beta_1 xBI_{apop} - \beta_2 xvI_{oncoly} - \beta_3 xvI_{necrop}, \tag{S.16}$$

$$\frac{dy}{dt} = -\delta y + \beta_2 xvI_{oncoly} + \beta_3 xvI_{necrop}, \tag{S.17}$$

$$\frac{dn}{dt} = \delta y - \mu n, \tag{S.18}$$

$$\frac{dv}{dt} = u_V + b\delta y(1 + \alpha_1 B) - \gamma v, \tag{S.19}$$

$$\frac{dB}{dt} = u_B - (\mu_1 x + \mu_2 y)\frac{B}{k_B + B} - \mu_B B. \tag{S.20}$$

S.2. Necessary condition of quadratic control

In our optimal control problem for ordinary differential equations, we use two controls $(u_V(t), u_B(t))$ and nine state variables $(S(t), F(t), A(t), R(t), x(t), y(t), n(t), v(t), B(t))$. The state variables satisfies a differential equations which depends on the control variable (Eqs (S.19) and (S.20)). Our optimal control problem consists of finding a piecewise continuous control and the associated state variables to minimize the given objective functional, *i.e.*,

$$J(u_V(t), u_B(t)) = \min_{u_V, u_B} \int_{t_s}^{t_f} x(t) + y(t) + C_1\frac{u_V^2(t)}{2} + C_2\frac{u_B^2(t)}{2}dt \tag{S.21}$$

$$\text{subject to Eq (S.6)–(S.9) and Eq (S.16)–(S.20)}, \quad 0 \le u_V(t) \le u_V^{max},\ \ 0 \le u_B(t) \le u_B^{max} \tag{S.22}$$

The principle technique for such an optimal control problem is to solve a set of "necessary condition" that an optimal control and corresponding state must satisfy. It is important to understand the logical difference between necessary conditions and sufficient conditions of solution sets. We can find necessary conditions from the Hamiltonian H, which is defined as follows:

$$H(t, S, F, A, R, x, y, n, v, B, u_V, u_B, \Lambda_1, \Lambda_2, \Lambda_3, \Lambda_4, \Lambda_5, \Lambda_6, \Lambda_7, \Lambda_8, \Lambda_9)$$

$$= x(t) + y(t) + C_1\frac{u_V^2(t)}{2} + C_2\frac{u_B^2(t)}{2} \tag{S.23}$$
$$+ \Lambda_1\frac{dS}{dt} + \Lambda_2\frac{dF}{dt} + \Lambda_3\frac{dA}{dt} + \Lambda_4\frac{dR}{dt} + \Lambda_5\frac{dx}{dt} + \Lambda_6\frac{dy}{dt} + \Lambda_7\frac{dn}{dt} + \Lambda_8\frac{dv}{dt} + \Lambda_9\frac{dB}{dt}$$

where $\Lambda_i (i = 1, \cdots, 9)$ is adjoint variable. Using Hamiltonian (H), we can find three conditions (optimality condition, adjoint equation, transversality condition).

The optimality conditions are

$$0 = \frac{\partial H}{\partial u_V} = C_1 u_V + \Lambda_8 \text{ at } u_V^* \;\; \Rightarrow \;\; u_V^* = -\frac{\Lambda_8}{C_1} \tag{S.24}$$

$$0 = \frac{\partial H}{\partial u_B} = C_2 u_B + \Lambda_9 \text{ at } u_B^* \;\; \Rightarrow \;\; u_B^* = -\frac{\Lambda_9}{C_2} \tag{S.25}$$

We see the problem is indeed minimization. Therefore, $\frac{\partial^2 H}{\partial u_V^2}$ and $\frac{\partial^2 H}{\partial u_B^2}$ are positive value ($C_1, C_2 > 0$)

The adjoint equations are given by

$$\Lambda_1' = -\frac{\partial H}{\partial S} = \quad -\left\{-\Lambda_1 - \Lambda 2\frac{2\sigma_2\sigma_{10}^2\sigma_5 S}{(\sigma_{10}^2 + \sigma_5 S^2)^2}\right\} \tag{S.26}$$

$$\Lambda_2' = -\frac{\partial H}{\partial F} = \quad -\left\{-\Lambda_1\frac{2\sigma_1\sigma_9^2\sigma_4 F}{(\sigma_9^2 + \sigma_4 F^2)^2} - \omega_1\Lambda_2 - \Lambda_3\frac{2\sigma_3\sigma_{11}^2\sigma_6 F}{(\sigma_{11}^2 + \sigma_6 F^2)^2} + \Lambda_4\frac{\sigma_{13} v}{k+v}\right\} \tag{S.27}$$

$$\Lambda_3' = -\frac{\partial H}{\partial A} = \quad -\{-\Lambda_3\omega_2\} \tag{S.28}$$

$$\Lambda_4' = -\frac{\partial H}{\partial R} = \quad -\{-\Lambda_4\omega_3\} \tag{S.29}$$

$$\Lambda_5' = -\frac{\partial H}{\partial x} = \quad -\Big\{1 + \Lambda_5\left(\lambda - 2\lambda\frac{x}{x_0} - \beta_1 B I_{apop} - \beta_2 v I_{oncoly} - \beta_3 v I_{necrop}\right)$$
$$+\Lambda_6\left(\beta_2 v I_{oncoly} + \beta_3 v I_{necrop}\right) - \mu_1\Lambda_9\frac{B}{k_B + B}\Big\} \tag{S.30}$$

$$\Lambda_6' = -\frac{\partial H}{\partial y} = \quad -\left\{1 - \delta\Lambda_6 + \delta\Lambda_7 + \Lambda_8 b\delta\,(1 + \alpha_1 B) - \Lambda_9\mu_2\frac{B}{k_B + B}\right\} \tag{S.31}$$

$$\Lambda_7' = -\frac{\partial H}{\partial n} = \quad -\{\mu\Lambda_7\} \tag{S.32}$$

$$\Lambda_8' = -\frac{\partial H}{\partial v} = \quad -\Big\{\Lambda_1\lambda_B\frac{B(k+v+\alpha v) - B(k+v)(1+\alpha)}{(k+v+\alpha v)^2} + \Lambda_4\sigma_{13}F\frac{k}{(k+v)^2}$$
$$+\Lambda_5(-\beta_2 x I_{oncoly} - \beta_3 x I_{necrop}) + \Lambda_6(\beta_2 x I_{oncoly} + \beta_3 x I_{necrop}) - \gamma\Lambda_8\Big\} \tag{S.33}$$

$$\Lambda_9' = -\frac{\partial H}{\partial B} = \quad -\left\{\Lambda_1\lambda_B\frac{k+v}{k+v+\alpha v} - \Lambda_5\beta_1 x I_{apop} + \Lambda_8 b\delta y\alpha_1 - \Lambda_9\left((\mu_1 x + \mu_2 y)\frac{k_B}{(k_B+B)^2} + \mu_B\right)\right\} \tag{S.34}$$

with the transversality conditions are $\Lambda_i(T) = 0$ ($i = 1, \cdots, 9$) where T is final time in our simulation period. Finally, optimality conditions are

$$u_V^*(t) = min\left(max\left(0, -\frac{\Lambda_8}{C_1}\right), u_V^{max}\right), \tag{S.35}$$

$$u_B^*(t) = min\left(max\left(0, -\frac{\Lambda_9}{C_2}\right), u_B^{max}\right). \tag{S.36}$$

S.3. Necessary condition of linear control

In our optimal control problem for ordinary differential equations, we use two controls $(u_V(t), u_B(t))$ and nine state variables $(S(t), F(t), A(t), R(t), x(t), y(t), n(t), v(t), B(t))$. The state variables satisfies a differential equations which depends on the control variable (Eqs (S.19) and (S.20)). Our optimal control problem consists of finding a linear dependent control and the associated state variables to minimize the given objective functional, *i.e.*,

$$J(u_V(t), u_B(t)) = \min_{u_V, u_B} \int_{t_s}^{t_f} x(t) + y(t) + C_1 u_V(t) + C_2 u_B(t) dt \tag{S.37}$$
$$\text{subject to Eq (S.6)–(S.9) and Eq (S.16)–(S.20)}, \quad 0 \le u_V(t) \le u_V^{max}, \;\; 0 \le u_B(t) \le u_B^{max}$$

Notice the integrand function and the right-hand side of the ordinary differential equations (Eq (S.6)–(S.9) and Eq (S.16)–(S.20)) are both linear functions of the control u_V, u_B. Therefore, the Hamiltonian is also a linear function of u_V, u_B, and can be written

$$\begin{aligned} &H(t, S, F, A, R, x, y, n, v, B, u_V, u_B, \Lambda_1, \Lambda_2, \Lambda_3, \Lambda_4, \Lambda_5, \Lambda_6, \Lambda_7, \Lambda_8, \Lambda_9) \\ &= x(t) + y(t) + C_1 u_V(t) + C_2 u_B(t) \\ &\quad + \Lambda_1 \frac{dS}{dt} + \Lambda_2 \frac{dF}{dt} + \Lambda_3 \frac{dA}{dt} + \Lambda_4 \frac{dR}{dt} + \Lambda_5 \frac{dx}{dt} + \Lambda_6 \frac{dy}{dt} + \Lambda_7 \frac{dn}{dt} + \Lambda_8 \frac{dv}{dt} + \Lambda_9 \frac{dB}{dt} \end{aligned} \tag{S.38}$$

where $\Lambda_i (i = 1, \cdots, 9)$ is adjoint variable. The necessary condition Λ_i' $(i = 1, \cdots, 9)$ are as normal. However, the optimality condition

$$\frac{\partial H}{\partial u_V} = C_1 + \Lambda_8 \tag{S.39}$$
$$\frac{\partial H}{\partial u_B} = C_2 + \Lambda_9 \tag{S.40}$$

contains no information on the control. We must try to minimize the Hamiltonian H with respect to u_V, u_B using the sign of $\frac{\partial H}{\partial u_V}$ and $\frac{\partial H}{\partial u_B}$. However, we cannot immediately find a characterization of u_V^*, u_B^*. Therefore, we define $\psi_V (= C_1 + \Lambda_8)$ and $\psi_B (= C_2 + \Lambda_9)$ called the switching function. Our characterization of u_V^* and u_B^* are

$$u_i^*(t) = \begin{cases} u_i^{max}, & \text{if } \psi_i < 0 \\ 0 \quad , & \text{if } \psi_i > 0 \end{cases} \qquad (i = V, B).$$

$u_i^*(t)$ $(i = V, B)$ is piecewise constant function, switching between only the upper and lower bound.

S.4. Comparison without and with payoff terms

In most cases, in addition to minimizing (or maximizing) terms over the entire time interval, we want to minimize the function value at a certain point in time, especially at the end of the time interval. We have seen the minimum value of the cancer cells for the entire time interval. However, out ultimate

goal is the size of cancer cells in the final time. That is, our purpose leads to the following objective function:

$$J(u_V(t), u_B(t)) = \min_{u_V, u_B} \int_{t_s}^{t_f} C_1 \frac{u_V^2(t)}{2} + C_2 \frac{u_B^2(t)}{2} dt + x(T) + y(T) \tag{S.41}$$

where $x(T)$ and $y(T)$ are a goal with respect to the final uninfected and infected cancer cell population. We call $x(T)$ and $y(T)$ a *payoff term*. The necessary conditions of the objective function may vary slightly due to the payoff terms. The detailed proof is introduced in the supplementary. In conclusion, the boundary conditions of the 5th and 6th adjoint functions corresponding to uninfected and infected cancer cells are different.

$$\begin{aligned}\Lambda_5' = -\frac{\partial H}{\partial x} = -\Big\{&\Lambda_5 \left(\lambda - 2\lambda \frac{x}{x_0} - \beta_1 B I_{apop} - \beta_2 v I_{oncoly} - \beta_3 v I_{necrop}\right) \\ &+ \Lambda_6 \left(\beta_2 v I_{oncoly} + \beta_3 v I_{necrop}\right) - \mu_1 \Lambda_9 \frac{B}{k_B + B}\Big\}\end{aligned} \tag{S.42}$$

$$\Lambda_6' = -\frac{\partial H}{\partial y} = -\left\{-\delta\Lambda_6 + \delta\Lambda_7 + \Lambda_8 b\delta\left(1 + \alpha_1 B\right) - \Lambda_9 \mu_2 \frac{B}{k_B + B}\right\} \tag{S.43}$$

$$\Lambda_5(T) = 1, \quad \Lambda_6(T) = 1 \tag{S.44}$$

In a typical optimal control problem without payoff terms, the boundary conditions of the adjoint function is always used as a transversality condition of zero at the final time, but the boundary condition is different, a shown in Eq (S.44). We do some setting to compare the results of the previous objective function Eq (S.21) and the objective function of this session Eq (S.48). The weight constant is different from the existing setting. Because it is to see the difference from the payoff terms. But for a fair comparison, we both changed to the same weight constant ($C_1 = 10^{-1}, C_2 = 2 \times 10^{-2}$). And all parameters and the upper bound of control are the same as the existing setting.

Figure S1 compares the results for Eqs (S.21) and (S.48). The upper panel (Figure S1A,B,C) and lower panel (Figure S1D,E,F) show the time courses of concentration of injection rate of anticancer drugs (bortezomib and OV), concentration of intracellular signal (NFκB, BAX, and RIP1), and cancer cell population (uninfected and infected) in absence of payoff terms and presence of payoff terms, respectively. In upper system, it is observed that a large amount of drugs are used from the beginning because the purpose is to reduce the cancer cell population at all times over time (Figure S1A). On the contrary, when the purpose is to reduce the cancer cell population at the final time, it is observed that more and more drugs are used without using a large amount at first (Figure S1D). When comparing cancer cell population for the two cases (Figure S1C,F), all of the uninfected cancer cells are infected, but the infected cancer cells remained. It remains 0.038 (end point of pink curves in Figure S1C) and 0.06 (end point of pink curves in Figure S1F), respectively, but infected cancer cells do not grow, so it does not matter much. Therefore, the most important thing to compare here is the total use of drugs ($\int_0^{30} u_B(t)dt$ and $\int_0^{30} u_V(t)dt$). In the result of Eq (S.21), the total amount of bortezomib and OV are 3.33 and 14.37, respectively ($\int_0^{30} u_B(t)dt = 3.33$, $\int_0^{30} u_V(t)dt = 14.37$). However, in the result of Eq (S.48), the total amount of bortezomib and OV are 1.59 and 7.06, respectively ($\int_0^{30} u_B(t)dt = 1.59$, $\int_0^{30} u_V(t)dt = 7.06$). That is, the use of drugs when using payoff terms for similar results decreased by more than half compared to otherwise.

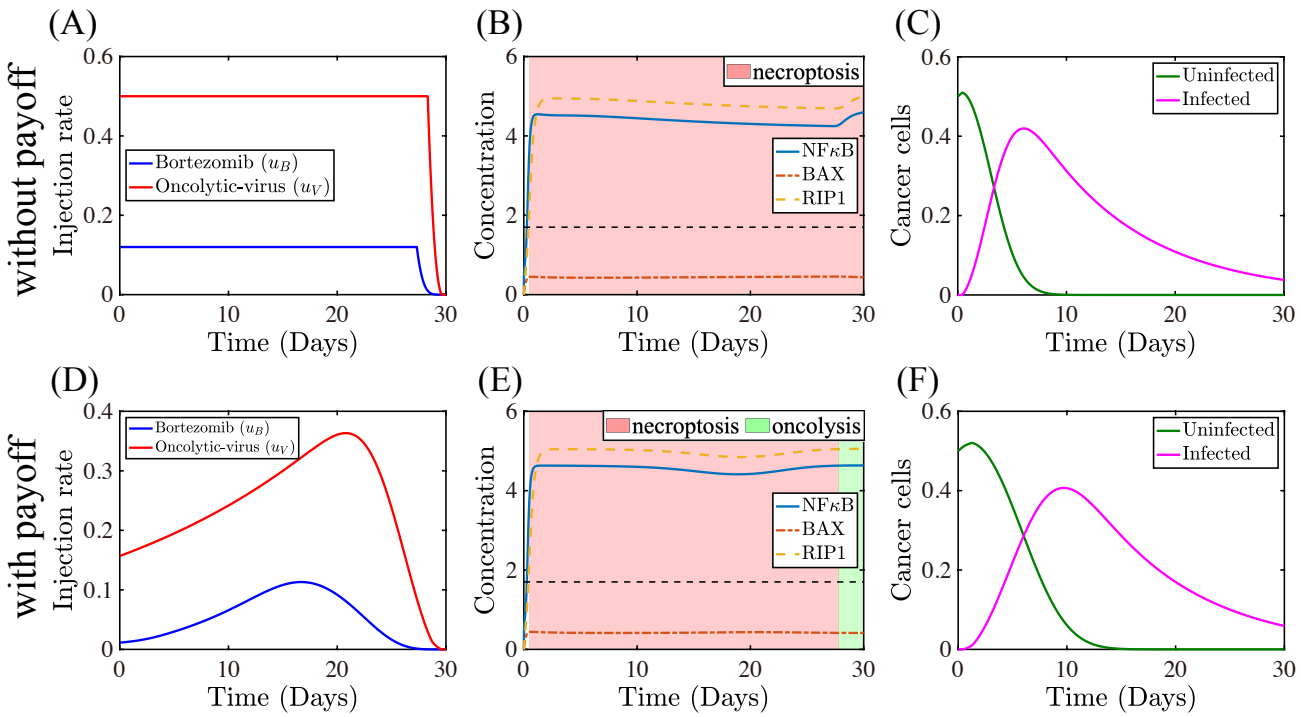

Figure S1. Comparison without and with payoff terms (A,B,C) Time courses of injection rate of OV and bortezomib, intracellular signaling (NFκB, BAX, RIP1), and cancer cell population without payoff term, respectively. (D,E,F) Time courses of injection rate of OV and bortezomib, intracellular signaling (NFκB, BAX, RIP1), and cancer cell population with payoff term, respectively.

S.5. Linear dependence on the control

The optimal control problems of singular control are difficult to solve because a complete solution cannot be derived if the Pontryagin's maximum principle is applied directly. The most common difficulty in applying Pontryagin's maximum principle arises when the objective function depends linearly on the control u_V, u_B are of the form:

$$J(u_V(t), u_B(t)) = \min_{u_V, u_B} \int_{t_s}^{t_f} x(t) + y(t) + C_1 u_V(t) + C_2 u_B(t) dt \tag{S.45}$$
$$\text{subject to Eqs (S.6)–(S.9) and (S.16)–(S.20)}, \quad 0 \le u_V(t) \le u_V^{max}, \ \ 0 \le u_B(t) \le u_B^{max}$$

Likewise, the Hamiltonian depends linearly on the control u_V, u_B are of the form:

$$\begin{aligned} &H(t, S, F, A, R, x, y, n, v, B, u_V, u_B, \Lambda_1, \Lambda_2, \Lambda_3, \Lambda_4, \Lambda_5, \Lambda_6, \Lambda_7, \Lambda_8, \Lambda_9) \\ &= x(t) + C_1 u_V(t) + C_2 u_B(t) \\ &\quad + \Lambda_1 \frac{dS}{dt} + \Lambda_2 \frac{dF}{dt} + \Lambda_3 \frac{dA}{dt} + \Lambda_4 \frac{dR}{dt} + \Lambda_5 \frac{dx}{dt} + \Lambda_6 \frac{dy}{dt} + \Lambda_7 \frac{dn}{dt} + \Lambda_8 \frac{dv}{dt} + \Lambda_9 \frac{dB}{dt}, \end{aligned} \tag{S.46}$$

and the controls (u_V, u_B) are restricted to being between a lower and an upper bound : $0 \le u_V(t) \le u_V^{max}, 0 \le u_B(t) \le u_B^{max}$. To minimize H, we need to make controls as small or as big as possible, depending on the sign of ϕ_V, ϕ_B. We introduce the calculations for ϕ_V and ϕ_B exactly in third section. In the minimizing problem, the controls are maintained upper bound of OV and bortezomib when ψ_V, ψ_B are smaller than zero, respectively. On the other hands, the controls are zero when ψ_V, ψ_B are larger than zero. However, the control is not appear zero or upper bound (u_V^{max}, u_B^{max}), because the system often appear $\psi_V = 0$ or $\psi_B = 0$. When ψ_i is zero, we have to calculate u_i^* with information of ψ_i $(i = V, B; \psi_V = C_1 + \Lambda_8 = 0, \psi_B = C_2 + \Lambda_9 = 0)$. However, the calculations of switching function of OV and bortezomib (ψ_V, ψ_B) are very difficult. In our system, there are many nonlinear term of equations (Eqs (S.6)–(S.9) and (S.16)–(S.20)). Since it cannot be solved by hand, it is inevitable to use the numerical method. Conclusionally, there is a good possibility that the control will have values other than zero and upper bound.

We investigate singular control for various objective functions. Figure S2A–D is the result of using the objective function of Eq (S.45). And Figure S2A–D is the result of the case where $t_s = 0, t_f = 30$ is set and the injection order of OV and bortezomib is not considered. Figure S2A,B shows the time courses of injection rate and concentration of OV and bortezomib, respectively. Figure S2C,D shows the time courses of intracellular signaling and cancer cell population, respectively. In this case, since they are injected together from the beginning, the nectoptosis phenotype appears and infects a large amount of uninfected cancer cells. And the second objective function as follows:

$$J_1(u_V(t)) = \min_{u_V} \int_0^{15} x(t) + y(t) + C_1 u_V(t) dt, J_2(u_B(t)) = \min_{u_B} \int_{15}^{30} x(t) + y(t) + C_2 u_B(t) dt \tag{S.47}$$
$$\text{subject to Eqs (S.6)–(S.9) and (S.16)–(S.20)}, \quad 0 \le u_V(t) \le u_V^{max}, \ \ 0 \le u_B(t) \le u_B^{max}$$

In this case, it is about the strategy of injecting OV first and bortezomib later. Figure S2E,F shows the time courses of injection rate and concentration of OV and bortezomib, respectively. Figure S2G,H shows the time courses of intracellular signaling and cancer cell population, respectively. In the second

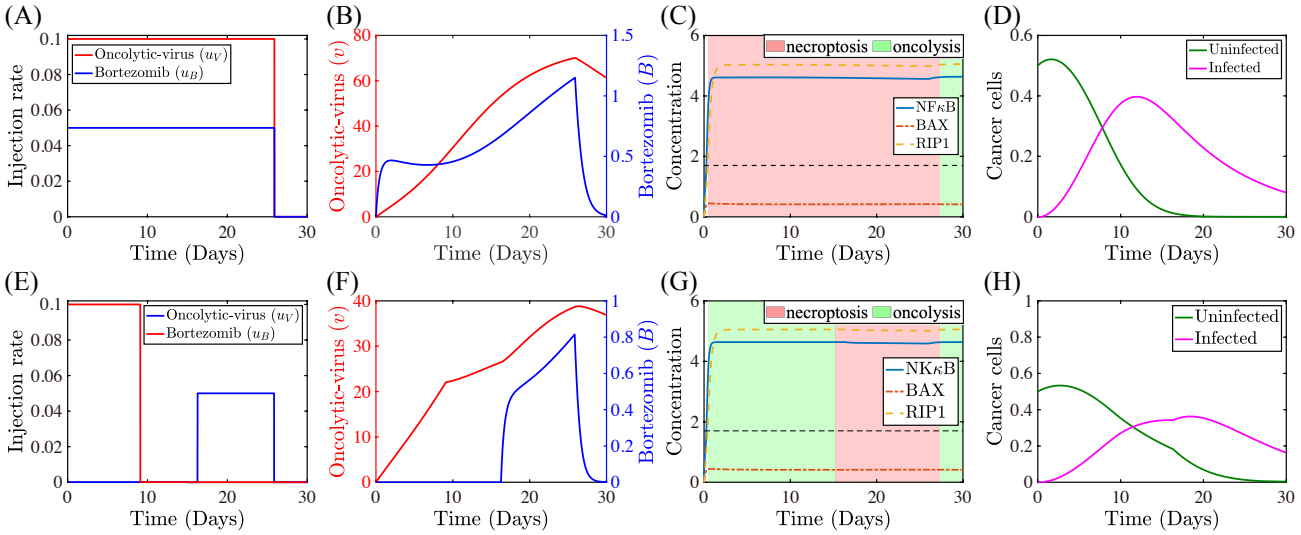

Figure S2. Dynamics of the system corresponding to two schemes of bortezomib and OV with optimal control. (A,B,C,D) Time courses of the injection rate (u_V, u_B), concentration of OV and bortezomib, intracellular signaling (NFκB,BAX,RIP1), and cancer cell population with Eq (S.45), respectively. (E,F,G,H) Time courses of the injection rate (u_V, u_B), concentration of OV and bortezomib, intracellular signaling (NFκB,BAX,RIP1), and cancer cell population with Eq (S.47), respectively.

case, OV and bortezomib are used less than half of the first case, but cancer cells show the same results at the last time. Similar to the result of the quadratic control in the main text, injecting OV first in the singular control more effective. Therefore, we will investigate at the case of injecting OV first in singular control.

In this therapeutic strategy, we have applied optimal control theory, focusing on reducing OV and bortezomib use and reducing cancer cell population. We use the new constraint term applying sigmoid function. The sigmoid function have two types: (i) terms aimed at minimizing ($y = \frac{a}{1+e^{-p(x-th_x)}}$) and (ii) terms aimed at maximizing ($y = \frac{a}{1+e^{p(P-th_P)}}$). We set weight constant of each variables and variables instead of a and P, respectively. The sigmoid function looks like a S curve. It quickly converge to two values, 0 and a, starting from the threshold of each variable called th_P. Therefore, we developed an objective function with sigmoid functions as follow:

$$J(u_B(t)) = \int_{t_s}^{t_f} C_4 u_B(t) + \frac{B_1}{1 + e^{-p(F-th_F)}} + \frac{B_2}{1 + e^{p(A-th_A)}} + B_3 F(t) - B_4 A(t) dt \tag{S.48}$$

The equation (S.48) uses only bortezomib to keep the phenotype in the apoptosis. In this case, we use two sigmoid terms: (i) minimizing of NFκB (F) and (ii) maximizing of BAX (A). If the level of BAX (A) falls to a value less than th_A, the third term in Eq (S.48) becomes B_2, which increases the weight. Conversely, if the level of BAX (A) is higher than th_A, the third term in Eq (S.48) becomes 0. Therefore, there is no need to control the BAX. Figure S3(A–D) shows the these results. Figure S3A–C shows the injection rate of bortezomib, the level of bortezomib, and the intracellular signaling (NFκB, BAX, RIP1), respectively. Bortezomib is automatically injected by sigmoid terms before NFκB and BAX exceed their respective thresholds. Therefore, the system always stay apoptosis phenotype (Figure

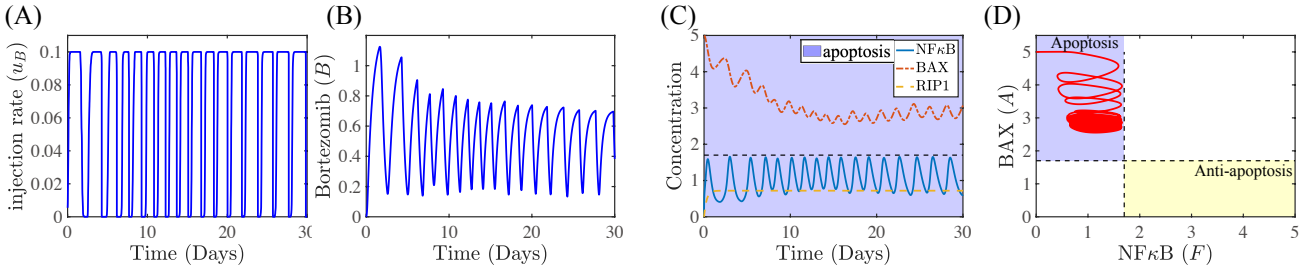

Figure S3. Dynamics of the system corresponding to two schemes of bortezomib and OV with optimal control. (A,B,C,D) Time courses of the injection rate (u_B), concentration of bortezomib, intracellular signaling (NFκB,BAX,RIP1), and solution trajectory of the NFκB (F) and BAX (A) with Eq (S.48), respectively.

S3C,D). It consistently maintains apoptosis, but necroptosis is not induced because it is not treated in combination with OV. Therefore, even if a larger amount of drugs is used for mixed treatment, cancer cells cannot be greatly reduced. This way, the result of the objective function with constraints in the mixed treatment is provided in the main text.