Optimal Proliferation and Differentiation of *Chlamydia Trachomatis*

By Frederic Y. M. Wan and Germán A. Enciso

Chlamydia trachomatis is a bacterium that causes eye infection and blindness in humans. In the simplest form, it has an unusual life cycle involving two developmental forms. Within a cytoplasmic inclusion, the reticulate body (RB) repeatedly divides by binary fission and asynchronously differentiates into the infectious elementary body (EB). Upon the death of the mammalian cell that host many such inclusions, only the EB form of the bacteria survive and proceed to infect other cells. Given the bacteria's fast spreading infection, conventional wisdom would have the few initial EB turn into RB, divide and proliferate first, and then eventually start converting to maximize the terminal EB population upon host cell lysis. Several biological processes are seen as possible mechanisms for implementing such a conversion strategy. However, the optimality of an instinctual strategy with a period of proliferate without conversion prior to the onset of differentiation has never been substantiated theoretically or justified mathematically.

This paper formulates three relatively simple models that capture the essential features of the Chlamydia life cycle. When the initial infection is caused by the endocytosis of a small EB population well below the carrying capacity of the host cell, the Maximum Principle in control theory requires for these models an optimal conversion strategy that confirms and rigorously justifies the prevailing view of no conversion at the early stage of the host cell infection. However, the conventional supposition is found

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In memory of Professor David J. Benney, the authors dedicate this paper to him for his warm friendship and support as a teacher, colleague and personal friend to the first author for more than 55 years (March 7, 2017).

DOI: 10.1111/sapm.12175

STUDIES IN APPLIED MATHEMATICS 139:129–178

to be inappropriate for an initial EB (-to-RB) population near or above the carrying capacity. Previously suggested and new biological mechanisms are examined for their role in implementing the different optimal conversion strategies associated with models investigated herein.

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1. The optimal conversion of *Chlamydia trachomatis*

Chlamydia trachomatis is a bacterium that causes ocular and genital tract infections in humans [6] (also [2, 5]). In ocular infections, *C. trachomatis* is the world's leading cause of preventable blindness. This condition, known as trachoma, currently affects 84–150 million people in the world, causing blindness to 8 million people [3]. It has been targeted by the World Health Organization for elimination by 2020.

As a pathogenic bacterium, *C. trachomatis* has an unusual intracellular developmental cycle involving conversion between two chlamydial forms. Within a cytoplasmic inclusion, the reticulate body (RB) repeatedly divides by binary fission and asynchronously differentiates into the infectious elementary body (EB). Upon the death of the host cell, only the EB form of the bacteria survives and proceeds to infect other cells [1]. These developmental events have not been quantified because conventional electron microscopy only visualizes sections of the large chlamydial inclusion. Using a novel three-dimensional electron microscopy approach, a group of us in the lab of Tan and Suetterlin obtained recently quantitative data on RB and EB finding intermediates of RB replication and RB-to-EB conversion during the developmental cycle [11]. The dynamics of the developmental cycle suggest a switch from a replication-only to a conversion phase.

To gain insight to our empirical findings and some suggestions for further experimental effort, we have undertaken some mathematical modeling of the spread of Chlamydia. The report below constitutes one of the several directions of our modeling and mathematical analysis on how and how fast the infectious Chlamydia bacteria can spread. The theoretically optimal strategies for maximum spread found for different initial endocytosed EB-to-RB population sizes (and different combinations of other parameter values) are expected to provide feedback to our experimental effort.

Our report begins with a proof-of-concept study that models *C. trachomatis* cell fate with a linear growth rate for both the RB and EB populations and a known time of host cell lysis. Since the capacity to convert is not unlimited, the maximization of the model's EB population at the terminal time is a constrained optimization problem subject to the Maximum Principle of Pontryagin [4, 13, 15].

A second linear growth model allowing for proliferation and differentiation in two stages as suggested by our experimental results continues to maximize spread of infection by a bang-bang conversion strategy.

The principal results of this paper are for a nonlinear model of rate limiting growth that takes into account the space and other constraints of the host cell. For this more realistic model, the optimum conversion strategy varies significantly depending on the "initially infecting EB-to-RB population" size and other characteristics of the system (such as the natural growth rate of the RB population, the carry capacity, etc.). Multiple switching and a singular (stationary) conversion phase are now possible under suitable environment.

2. Exponential growth of *RB* cells

2.1. The model

We begin this theoretical study of *C. trachomatis* with a proof-of-concept investigation that considers the bacteria to be of two forms: RB that can proliferate inside a host mammalian cell with a rate constant α as well as convert with a "rate constant" u into elementary body (EB) that is resistant to environmental changes but does not divide. If the host cell dies, all RBs die as well because they cannot survive in the new environment. At the same time, the EB population in the host cell is released, free to attach to and enter into another host cell. Once an EB enters a new host cell, it turns into an RB and begins to replicate, repeating the life cycle as shown in Fig. 1. We abbreviate the event of the initial EB form of the bacteria entering the host cell and immediately converting into RB form as the "*initially infecting EB-to-RB population*." Of interest is the optimal rate of conversion of RB population, $R(t)$, into EB at each instant of time t to enlarge that population $E(t)$ if we want to maximize the EB population at the time of host cell's lysis.

With an initial infecting EB-to-RB population R_0, the population $R(t)$ of the reticulate bodies at a later time t is (for this proof-of-concept model) to grow exponentially in the absence of conversion to EB. When a part of the RB population is converted continuously in time at a rate uR, we have the net linear growth dynamics

$$R' = (\alpha - u)R, \qquad R(0) = R_0, \tag{1}$$

where $(\ )' = d(\ )/dt$ and the component αR is the natural linear growth rate when $u = 0$. The growth rate of the population $E(t)$ of the elementary

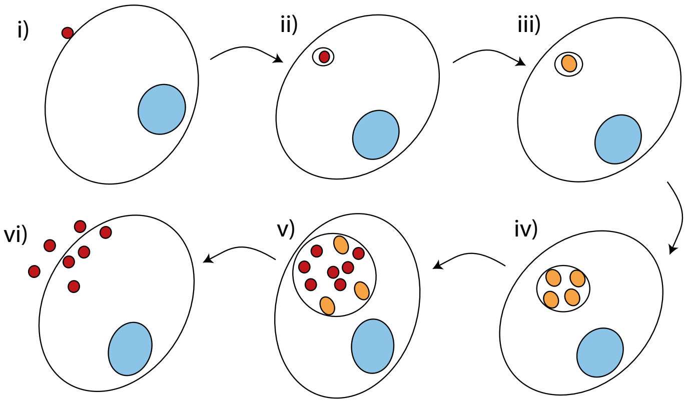

Figure 1. *Chlamydia trachomatis* life cycle.

bodies is equal to the rate of conversion uR with

$$E' = uR, \qquad E(0) = E_0. \tag{2}$$

With no loss in generality, we set $E_0 = 0$ because a positive value of E_0 only increases E but does not affect the optimal conversion rate. The EB population at the terminal time T when the host cell dies is

$$E(T) = \int_0^T uRdt. \tag{3}$$

The simplest form of the optimization problem is to choose an optimal "conversion rate constant," called the *optimal control* and denoted by $u_{op}(t)$, to maximize $E(T)$. The choice of conversion rate is limited by the obvious lower bound $u = 0$ (given that the conversion is not reversible) and a natural maximum rate constant $u_{\max}$ imposed by a limit on conversion capacity so that u is subject to the constraints

$$0 \le u(t) \le u_{\max}. \tag{4}$$

The optimization problem is to choose u to maximize the integral of uR,

$$\max_{0 \,\le\, u \,\le\, u_{\max}} \left[E(T) = \int_0^T uRdt \right], \tag{5}$$

subject to the growth dynamics (1) and the inequality constraints (4). The determination of the optimal conversion rate for a maximum $E(T)$ constitutes what is known as an *constrained optimal control problem* in engineering sciences [4, 13, 15].

Before embarking on a search for the optimal strategy for maximizing the EB population at the terminal time T, we note that such a strategy generally varies with the natural growth rate constant α, the initial infecting RB population R_0, the maximum conversion capacity $u_{\max}$, and the terminal time T. The growth rate relation (1) shows that it is the relative magnitude of the parameters α and $u_{\max}$ that affects the growth or decline of $R(t)$. If $u_{\max} < \alpha$, the RB population $R(t)$ would continue to grow even if conversion should be at a maximum rate from the start. As long as the initial RB population does not decrease at any time throughout the life span $[0, T]$ of the host cell, converting less than maximum rate would generate more RB that would not be converted before the death of the host cell. It is tempting to conclude from this observation that converting at the maximum rate possible from the start would be optimal.

On the other hand, it is well established in optimal control theory that the optimal strategy for optimal control problems with inequality constraints on the control(s) is necessarily a bang-bang control (characterized by a piecewise constant control function with a single simple jump discontinuity) when the dynamics and the performance index are both linear in the control(s). This is the case in the simple proof-of-concept model formulated above. In the subsequent development, it will be seen that the spread of bacterial infection is maximized by a bang-bang conversion strategy switching abruptly from a “replication only” phase to a “conversion only” mode (as shown in Figure 2 later). Bang-bang optimal control is not particularly unique to our problem. In addition to being ubiquitous in engineering science, it has also been found to occur in models for the life sciences. These include models on cancers chemotherapy, intestinal crypt development, genetic instability, and other linear constrained optimal control models (see [10], [12], [9], [14], and [16] and references therein). For a frequently encountered performance objective such as the time optimal problem (TOP), the solution process leading to a bang-bang control can be found in most texts on optimal control such as [4], [12], and [15]. The solution process for the models investigated herein is not of the text book variety and has to be developed individually from the information available. Furthermore, depending on the values of the system parameters such as $\alpha, u_{\max}$, T, etc., the theoretical bang-bang solution may turn out to be bang-bang only in theory. As it shall be shown near the end of this section, the switch point of the bang-bang control may be outside the solution domain.

At the other extreme, the role of the initial infecting population R_0 is rather ambiguous in the present exponential (natural RB) growth model. While the optimal control appears to be independent of R_0 for the proof-of-concept model of this section and was found to be a so also in [9], the rather complex dependence of the optimal conversion strategy on R_0 will be more clearly delineated by way of a model that incorporates the effects of a finite carrying capacity for the host cell in Section 4.

2.2. The Maximum Principle

For the solution of the optimization problem, we note that both the growth dynamics and the performance index $E(T)$ are linear in the control $u(t)$. The classical method of calculus of variations is known to be ineffective for such problems. For the more appropriate method of Maximum Principle [4, 13, 15], we form the Hamiltonian for the problem:

$$H = uR + \lambda(\alpha - u)R = uR(1 - \lambda) + \alpha R\lambda \tag{6}$$

from which we obtain the ordinary differential equation (ODE) for the adjoint function $\lambda(t)$

$$\lambda' = -\frac{\partial H}{\partial R} = -\left\{u(1 - \lambda) + \alpha\lambda\right\}, \tag{7}$$

and, from the performance index (3), the adjoint (or Euler) boundary condition

$$\lambda(T) = 0. \tag{8}$$

With

$$R' = \frac{\partial H}{\partial \lambda} = (\alpha - u)R, \tag{9}$$

the two ODE (7) and (9) form a Hamiltonian system.

The Maximum Principle then determines candidates for the optimal control $u_{op}(t)$ by the condition

$$H[u_{op}(t)] = \max_{0 \le u(t) \le u_{\max}} \{H[u]\} = \max_{0 \le u(t) \le u_{\max}} \{uR(1 - \lambda) + \alpha R\lambda\}. \tag{10}$$

The importance of the Maximum Principle is its feature that the state and adjoint functions may be taken to be known (already assuming their respective optimal solution) during the process of choosing u to maximize $H[u]$ in (10). This feature makes the maximization of (10) tractable. At the same time, it should be noted that maximizing $H[u]$ is not the same as maximizing $E(T)$ and the Maximum Principle only provides a set of necessary conditions to be satisfied by the optimal control $u_{op}(t)$. It is essentially the counterpart of the Euler differential equation(s) (DE) in the calculus of variations when there are inequality constraints on the control(s). Just as Euler DE determines a set of *extremals* to be further examined by various sufficiency criteria to determine optimality, the Maximum Principle by itself only delimits the range of potential solution candidates. It reduces to the Euler DE for the present classes of problems when the inequality constraints are not binding. As it is the case of an extremal in the calculus of variations, that any solution candidate from the Maximum Principle should maximize $E(T)$ must still be proved and we do so in all cases investigated below.

For our relatively simple model with both its performance index (3) and growth dynamics (1) linear in the control $u(t)$, it is generally expected that the optimal control is some form of bang-bang control. Nevertheless, we will deduce from the Maximum Principle *ab initio* the relevant optimal bang-bang control for our problem for two specific objectives. The method of solution for the simple model is to be subsequently extended to determine the solution of the other more complex Chlamydia models in this paper. The specific bang-bang optimal control $u_{op}(t)$ for (5) from (10) involves a switch point that varies with the values of the system parameters. The variation of the location of the switch enables us to show how the theoretically expected bang-bang control can be consistent with the strategy of maximum conversion from the start more appropriate for host cells with a sufficiently short life span T and/or a larger growth rate constant α.

2.3. *Upper corner control adjacent to terminal time*

As is the case with the conventional extremization of a function, it is natural to seek any extremizing control among those candidates that render $H[u]$ stationary so that

$$\frac{\partial H}{\partial u} = R(1 - \lambda) = 0. \tag{11}$$

With the Hamiltonian linear in the control, the stationary condition above does not involve u and therefore does not determine candidates for the optimal control that maximizes the Hamiltonian. Furthermore, with $R(t)$ positive for $t > 0$ and (by the Euler BC (8) and continuity of the adjoint function) $\lambda(t) < 1$ for some interval $(t_s, T]$, the stationary condition is not satisfied at least for that time interval adjacent to the terminal time T. To determine the optimal control $u_{op}(t)$ that maximizes $H[u]$, we note from the expression (6) that the Hamiltonian is maximized by taking u as large as possible in $(t_s, T]$, namely, $u_{op}(t) = u_{\max}$ since $R(1 - \lambda) > 0$ there.

With $u(t) = u_{\max}$ in $(t_s, T]$, the terminal value problem defined by (7) and (8) for the adjoint function λ in that interval, denoted by $\lambda_g(t)$, assumes the form

$$\lambda_g' - u_\alpha \lambda_g + u_{\max} = 0, \qquad \lambda_g(T) = 0, \tag{12}$$

where $u_\alpha = u_{\max} - \alpha$. The solution of this terminal value problem is

$$\lambda_g(t) = \frac{u_{\max}}{u_\alpha}\left\{1 - e^{-u_\alpha(T-t)}\right\} \qquad (t_s < t \leq T). \tag{13}$$

Evidently, $u_{op}(t) = u_{\max}$ as long as $\lambda_g(t) < 1$; hence, t_s is the instant nearest to T for which $\lambda_g(t) < 1$ does not hold. It follows that the switch condition

$$\lambda_g(t_s) = 1 \tag{14}$$

determines t_s. We have then the following partial result for the optimal control $u_{op}(t)$.

PROPOSITION 1. *The optimal control $u_{op}(t)$ that maximizes $H[u]$ must consist of the* ***upper corner control*** *$u_{\max}$ for an interval $(t_s, T]$ adjacent to the terminal time with t_s determined uniquely by* (14) *to be*

$$t_s = T - \frac{1}{u_{\max} - \alpha} \ln\left(\frac{u_{\max}}{\alpha}\right) < T. \tag{15}$$

Remark 1. Observe that the switch point is always $< T$ whether or not $u_{\max}$ is greater or less than α. For a sufficiently small T, t_s may be negative. In that case, the optimal control is necessarily $u_{\max}$ for all t in $[0, T]$ since $\lambda_g(t) < 1$ there. As such, the bang-bang control contains as a special case the optimal strategy of "converting at maximum rate possible from the start" under suitable circumstances.

2.4. Lower corner control at the start

Since $\lambda_g(t) > 1$ for $t < t_s$ (see (17)), the optimal control for the more typical case $t_s > 0$ is no longer the upper corner control for that earlier time. At the same time, the stationary condition $\lambda(t) = 1$ (and hence $\lambda' = 0$) cannot be met for $t < t_s$, because we would have from the adjoint DE in (7) the impossible requirement $0 = -\alpha$. We need then to seek some appropriate control option for $t < t_s$.

For the *lower corner control* $u(t) = 0 \equiv u_\ell(t)$ in that interval, the ODE for the adjoint function, now denoted by $\lambda_\ell(t)$, becomes

$$\lambda'_\ell + \alpha\lambda_\ell = 0, \tag{16}$$

with $\lambda_\ell(t_s) = \lambda_g(t_s) = 1$ by the continuity of the adjoint function. The solution for this terminal value problem is

$$\lambda_\ell(t) = e^{-\alpha(t - t_s)} \tag{17}$$

with $\lambda_\ell(t) > 1$ for $t < t_s$. Hence, the lower corner control, $u_\ell(t) = 0$, maximizes $H[u]$.

2.5. The bang-bang control

With $\lambda(t) > 1$ in $[0, t_s)$ for both extreme values (0 and u_{max}) of admissible control, it is reasonable to expect that the same is true for intermediate controls. We show presently that this is, in fact, the case. Together with the known result in the previous subsection for t in $(t_s, T]$, this leads to the following optimal control that maximizes the Hamiltonian.

PROPOSITION 2. *By the Maximum Principle, the unique optimal control that maximizes the Hamiltonian* (6) *is the bang-bang control*

$$u_{op}(t) = \begin{cases} 0 & (t < t_s) \\ u_{\max} & (t_s < t \leq T) \end{cases}, \tag{18}$$

with t_s given by (15).

Proof: We have already established that $u_{op}(t) = u_{\max}$ for $t_s < t \leq T$ with $\lambda(t_s) = 1$. For $t < t_s$, the adjoint ODE for a general (positive) control $u(t)$ may be written as

$$[e^{I(t)}\lambda]' = -u(t)e^{I(t)} < 0, \tag{19}$$

where

$$I(t) = \int_t^{t_s} [u(\tau) - \alpha]d\tau. \tag{20}$$

The terminal value problem defined by (19), (20), and $\lambda(t_s) = 1$ determines $e^{I(t)}\lambda(t)$ to be a decreasing function of time and equal to 1 at $t = t_s$. Hence, we have $e^{I(t)}\lambda(t) > 1$ and therewith $\lambda(t) > 0$ for $t < t_s$ for any $u(t)$ that satisfies the inequality constraint (4). The original form of the adjoint DE

$$\lambda' = -\{u(1-\lambda) + \alpha\lambda\}, \tag{21}$$

with $\lambda(t_s) = 1$ requires $\lambda'(t) < 0$ adjacent to but less than t_s so that $\lambda(t) > 1$ for all t in some interval (t_ℓ, t_s). In order for $\lambda(t)$ to become an increasing function for $t < t_\ell$, we must have $u(t) < 0$, which violates the inequality constraint (4). It follows that $\{1 - \lambda(t)\} < 0$ for any admissible control $u(t)$ for all t in $[0, t_s)$ and the lower corner control $u_\ell(t) = 0$ is optimal there. This proves the proposition. ■

To complete the solution, we still have to solve the initial value problem (IVP) for $R(t) \equiv R_\ell(t)$ for the interval $[0, t_s]$ (with $u = 0$) and then $R(t) \equiv R_g(t)$ for the interval $[t_s, T]$ (with $u = u_{\max}$) to get

$$R_{op}(t) = \begin{cases} R_\ell(t) = R_0 e^{\alpha t} & (0 \leq t \leq t_s) \\ R_g(t) = R_s e^{(\alpha - u_{\max})(t - t_s)} & (t_s \leq t \leq T) \end{cases}, \qquad R_s = R_0 e^{\alpha t_s}, \tag{22}$$

where continuity of the RB population at the switch point has been imposed. The two parts of Figure 2 show a typical optimal solution for the exponential growth model for $t_s > 0$ deduced from the Maximum Principle.

2.6. The optimal conversion strategy

As a theorem, the Maximum Principle only provides a set of necessary conditions for identifying candidates for the optimal control $u_{op}(t)$ that extremizes the terminal EB population. In this subsection, we complete the solution process by showing that the bang-bang control (89), in fact,

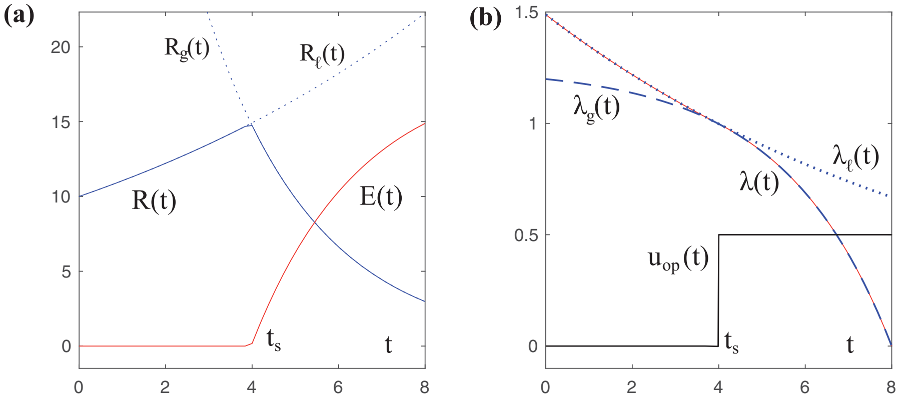

Figure 2. A typical optimal solution for the exponential growth model.

maximizes $E(T)$ (and not minimizes it). To the extent that the $u_{op}(t)$ (89) is the only candidate that meets all the necessary conditions (particularly the optimality condition (10)), we only need to show the corresponding $E(T)$,

$$[E(T)]_{u_{op}(t)} = \int_{t_s}^{T} u_{\max} R_g(t) dt = u_{\max} R_s \int_{t_s}^{T} e^{(\alpha - u_{\max})(t - t_s)} dt \tag{23}$$

$$= \frac{u_{\max} R_0}{u_{\max} - \alpha} e^{\alpha t_s} \left[1 - e^{-u_\alpha (T - t_s)}\right] \tag{24}$$

$$= R_0 e^{\alpha T} \left(\frac{\alpha}{u_{\max}}\right)^{\alpha/(u_{\max} - \alpha)}, \tag{25}$$

is a maximum and not a minimum. In other words, it suffices to show

$$[E(T)]_{u_{op}} \geq [E(T)]_{u(t)} \tag{26}$$

for some admissible control $u(t)$. This is accomplished trivially by observing $[E(T)]_{u=u_{op}} > [E(T)]_{u=0} = 0$ because no EBs are ever produced during the entire cell life span $[0, T]$ with $u = 0$ and $E(0) = 0$. For a less trivial comparison, it can be shown (but will not be proved here) that for a fixed combination of positive $R_0, \alpha, u_{\max}$, and T, the difference

$$D(T) = [E(T)]_{u=u_{op}(t)} - [E(T)]_{u=u_{\max}}, \tag{27}$$

where

$$[E(T)]_{u(t)=u_{\max}} = \frac{u_{\max} R_0}{u_{\max} - \alpha} \left[1 - e^{-u_\alpha T}\right] \geq 0, \tag{28}$$

independent of the sign of $u_\alpha = u_{\max} - \alpha$, is a nonnegative convex function of T (> 0) so that $[E(T)]_{u=u_{op}(t)}$ is a maximum.

For a sufficiently short cell life span T (and all other parameter values fixed), t_s as determined by (15) may, in fact, be negative. In that case, the optimal strategy would correspond to $u_{op}(t) = u_{\max}$, i.e., converting at $u_{\max}$ from the start. Obviously, it is not possible to implement the bang-bang control (89) for a negative switch point. In any case, with $\lambda_g(t) < 1$ for all $t \geq 0$, the upper corner control for the entire interval $[0, T]$ satisfies the optimality condition (10) and the corresponding $E[T]$ is superior to that by any other admissible control. More specifically, we have

Proposition 3. *Let* $T_0 = \ln[u_{\max}/\alpha]/u_\alpha$ *(*> 0 *independent of the sign of* u_α*). the optimal conversion strategy is to convert from the start at* $u_{\max}$ *if* $T \leq T_0$*, i.e.,* $u_{op}(t) = u_{\max}$ *for all* t *in* $[0, T]$.

Remark 2. By measuring time in units of the growth rate constant α and setting $\hat{t} = \alpha t$, $\hat{u} = u/\alpha$, we may write the threshold condition $T \leq T_0$ in the normalized form $\hat{T} \leq \hat{T}_0$ where

$$\hat{T}_0 = \frac{1}{\hat{u}_\alpha} \ln[\hat{u}_{\max}] = \frac{1}{\hat{u}_{\max} - 1} \ln[\hat{u}_{\max}] \tag{29}$$

unencumbered by the growth rate parameter α.

2.7. The bang-bang control is biologically optimal

Now that the optimality of the bang-bang control (89) has been established mathematically, we should also have a biological reason for the optimality. In our effort to arrive at a biological explanation, we should keep in mind that the performance objectives of different optimal bang-bang control are usually different. For example, the bang-bang controls of [9], [14], and [16] are for minimizing the elapsed time needed to reach some target (be it the progeny intestinal crypt cell population for a mature crypt or the malignant cancerous cell population size for carcinogenesis). The role of a bang-bang control for these well-known TOP is clearly different from our problem where the objective is to maximize the differentiated (EB) cell population size at the terminal time when the host cell lyses. Hence, whatever biological explanation for the referenced TOP cases generally does not apply to our result for maximum spread of the infectious disease.

For the purpose of maximizing the terminal EB population, suppose we convert immediately after the few EB particles having entered the host cell and transformed into the few RB particles available in the cell at the start ($t = 0$). There would be just those same few number of EB at terminal time because there would be no more RB to convert after the initial conversion. We can surely do better by allowing the few initial RB particles to divide and proliferate before conversion to get more EB at the end.

At the other extreme, we can wait and do not convert but allow the few initial RBs to divide and proliferate repeatedly. If we then convert at the last minute and at the maximum allowable rate, we would surely get more EB at terminal time ($t = T$). However, at a typical proliferation rate and a typical cell life span, it is unlikely for all accumulated RB converted just before T at the last instant. It seems clear that we could get more RB converted had we started conversion slightly earlier.

As long as the short delay to convert at the start not producing as many EB at terminal time as the result from converting a little sooner at the end, we can continue to delay conversion later. Conversely, if converting a little sooner before the end is not as productive as the result from delaying conversion near the start, we can start converting sooner near the end. When we modify the conversion times at the two ends repeatedly, increasing the one near the start and decreasing the one near the end, the resulting two terminal EB populations approach each other, possibly becoming identical at some instant inside the interval $[0, T]$.

With the performance index and the growth dynamics being linear in the control instrument, a gradual change in conversion rate with time is not an option. The switching from the not converting to converting can only be an "all or nothing" proposition and hence bang-bang. The optimality is attained by an appropriate choice of switching time t_s for the control $u_{op}(t)$ characterized by (89).

2.8. Biological mechanisms for optimal strategy

As an infectious disease, *C. trachomatis* faces an important choice during its infection of a mammalian host. Each RB bacterium can decide to proliferate (by division) to increase the RB population size, or it can convert into a sturdy EB form that survives cell lysis and is ready to infect other cells. If too many RBs convert to EB early on, the total *C. trachomatis* population size would remain moderate before the death of the host cell. Conversely, if too few cells convert into EB at the early stage, a good portion of the resulting larger RB population would remain unconverted at the end (because of the upper bound on the conversion rate) and not survive upon host cell lysis. This would leave only a smaller EB population than it could have had if conversion takes place sooner and/or at a higher rate if possible. In either case, it would seem that the terminal EB population may be made larger by adjusting the proportion of replication to conversion rate ratio.

Working under the assumption that evolutionary forces drive *C. trachomatis* infection to spread fast by maximizing the terminal EB population, the mathematics of optimal control theory has led to an optimal strategy for RB to only divide and proliferate first, and then eventually start converting. What biological mechanisms may there be for implementing such a strategy?

One prevailing hypothesis is that conversion is inhibited while infectious bacteria are in contact with the thin protective inclusion membrane surrounding an individual RB or a few of the initial batch of RB [17]. Early on in the infection process, the inclusion over a few RB is small, allowing most or all reticulate bodies to be in contact with their own inclusion membrane. As the bacterial population increases gradually, some RB form bacteria will eventually not be in contact with their inclusion membrane and these can then begin to convert. This mechanism would implement a conversion strategy of early replication and proliferation followed by late conversion.

Another mechanism recently discovered in the lab of Ming Tan and Christine Suetterlin [11] involves the size of the descendant RB. It was observed that when an RB divides, the offspring RBs do not grow to the original size of their progenitor as many other bacteria do, but instead become progressively smaller. This suggests that cell size can itself be used as a regulator for conversion. During the first five or so cell divisions after infection, the reticulate bodies are still too large to convert. If RB size does, in fact, regulate conversion, it would also lead to a delay before conversion, during which the population would only proliferate through division.

While these two and other possible mechanisms for implementing the proliferate-then-convert strategy seem plausible, the strategies themselves have never been substantiated with mathematical rigor that we have undertaken in this paper. To the extent that fast spreading of *C. trachomatis* infection requires a large terminal EB population at the time of host cell lysis, it would seem that the decision-making mechanism regulating when to convert is likely to have been shaped by evolution to maximize the terminal EB population. This latter perspective played a decisive role in the development of the optimal control type models in this paper.

3. A two-step growth and conversion model

3.1. A model more consistent with observations

The separation of *C. trachomatis* bacteria into two distinct forms, reticulate bodies and elementary bodies, was made to enable us to arrive at a relatively simple mathematical model for the purpose of illustrating the mathematics involved in the quantitative approach to one type of issues concerning *C. trachomatis* differentiation. In reality, both reticulate and elementary bodies exist in more than one form. In addition to RB and EB, at least two sets of intermediate bodies in transitional stages have been observed as the RB convert to EB [11]. A more realistic model for *C. trachomatis* differentiation and proliferation would be as shown in the schematic diagram Figure 3.

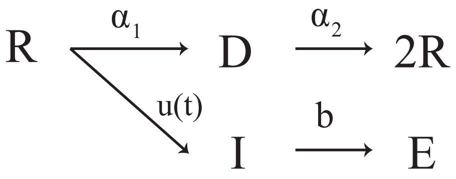

Figure 3. Two-stage proliferation and differentiation of Chlamydia.

The corresponding growth dynamics may be modeled mathematically by the following four differential equations:

$$R' = -(\alpha_1 + u)R, + 2\alpha_2 D, \ \ D' = \alpha_1 R - \alpha_2 D, \tag{30}$$

$$I' = uR - bI, \quad E' = bI, \tag{31}$$

$$R(0) = R_0, \quad D(0) = 0, \quad I(0) = 0, \quad E(0) = 0. \tag{32}$$

From the second ODE in (31), we get the total EB population at the time of host cell lysis T:

$$E(T) = \int_0^T bI(t)dt. \tag{33}$$

The determination of the optimal conversion of RB to EB to maximize the EB population at the terminal time T may be stated as the following optimal control problem:

$$\max_{0 \le u \le u_{\max}} \left[E(T) = \int_0^T bI(t)dt \right], \tag{34}$$

subject to the growth dynamics of the first three ODE, the first three initial conditions of (30)–(32) and the inequality constraints (4).

3.2. Solution by the Maximum Principle

For the solution of the optimization problem, we again apply the Maximum Principle and form the Hamiltonian of the problem:

$$H[u] = bI + \lambda_R\{-(\alpha_1 + u)R + 2\alpha_2 D\} \tag{35}$$

$$+\lambda_D\{\alpha_1 R - \alpha_2 D\} + \lambda_I\{uR - bI\} \tag{36}$$

from which we obtain the adjoint differential equations

$$\lambda_R' = -\frac{\partial H}{\partial R} = (u + \alpha_1)\lambda_R - \alpha_1\lambda_D - u\lambda_I, \tag{37}$$

$$\lambda_D' = -\frac{\partial H}{\partial D} = \alpha_2(\lambda_D - 2\lambda_R), \tag{38}$$

$$\lambda_I' = -\frac{\partial H}{\partial I} = b(\lambda_I - 1). \tag{39}$$

We also obtain from the first variation of (33) the adjoint (or Euler) boundary conditions

$$\lambda_R(T) = \lambda_D(T) = \lambda_I(T) = 0. \tag{40}$$

Again, with

$$R' = \frac{\partial H}{\partial \lambda_R}, \qquad D' = \frac{\partial H}{\partial \lambda_D}, \qquad I' = \frac{\partial H}{\partial \lambda_I}, \tag{41}$$

the six ODE (37)–(39) and (41) form a Hamiltonian system.

The Maximum Principle then determines candidates for the optimal control $u_{op}(t)$ that maximizes $E(T)$ by seeking the maximizer $u_{op}(t)$ for the Hamiltonian $H[u]$:

$$H[u_{op}(t)] = \max_{0 \le u(t) \le u_{\max}} \{H[u]\} \tag{42}$$

$$= \max_{0 \le u(t) \le u_{\max}} \{uR(\lambda_I - \lambda_R) + (....)\}, \tag{43}$$

where (.....) does not involve the control $u(t)$. As noted previously, the Maximum Principle assures us that all quantities other than the control may be taken to be those corresponding to the optimal solution when choosing the control by (42).

3.3. Upper corner control adjacent to terminal time

Unlike the simpler model of the previous section, we can no longer deduce from the Euler boundary condition $\lambda_R(T) = 0$ that the optimal conversion should be the upper corner control $u_{\max}$ in some interval $(t_s, T]$. With $1 - \lambda(t)$ in (10) replaced by $\lambda_I(t) - \lambda_R(t)$ in (42), we have $\lambda_I(T) - \lambda_R(T) = 0$ by the Euler BC (40) so that the previous argument for the upper corner control to be optimal no longer applies. Nevertheless, we prove Proposition 4 below that gives an analogous conclusion for the present problem by suitably modifying the previous method of solution.

We begin by observing that the third adjoint ODE in (39) is for $\lambda_I(t)$ alone and does not involve other adjoint functions or the control $u(t)$. Given the Euler boundary condition $\lambda_I(T) = 0$, we have for that terminal value problem the exact solution

$$\lambda_I(t) = 1 - e^{-b(T-t)} \tag{44}$$

independent of the choice of the control $u(t)$.

With both dynamics and performance index linear in the control $u(t)$, we have only the singular solution $\lambda_I(t) = \lambda_R(t)$ and the two corner controls 0 and $u_{\max}$ at our disposal for the optimal conversion strategy. We begin by eliminating the lower corner control $u_\ell(t) = 0$ for an interval adjacent to the terminal time T. For $u_\ell(t) = 0$, the terminal value problem for the corresponding adjoint functions $\lambda_{R\ell}$ and $\lambda_{D\ell}$ is the homogeneous system:

$$\lambda'_{R\ell} = \alpha_1(\lambda_{R\ell} - \lambda_{D\ell}), \qquad \lambda_{R\ell}(T) = 0,$$
$$\lambda'_{D\ell} = \alpha_2(\lambda_{D\ell} - 2\lambda_{R\ell}), \qquad \lambda_{D\ell}(T) = 0.$$

The system admits only the trivial solution

$$\lambda_{R\ell}(t) = \lambda_{D\ell}(t) = 0. \tag{45}$$

With $\lambda_I(t) - \lambda_{R\ell}(t) = 1 - e^{-b(T-t)} > 0$ for all $t < T$, the lower corner control is not optimal adjacent to the terminal time. Biologically, the lower corner control $u_\ell(t) = 0$ cannot be optimal because we can always get a larger $E(T)$ by converting in that interval as long as there is still a nonzero RB population (and $R(t)$ would be nonzero adjacent to T, given the exponential growth and decay nature of the six unknowns $R(t)$, $D(t)$, $I(t)$, $\lambda_R(t)$, $\lambda_D(t)$, and $\lambda_I(t)$).

On the other hand, for *any* nonvanishing control $u(t)$, we have from the adjoint DE (37) and (39)

$$(\lambda_I - \lambda_R)' = b(\lambda_I - 1) - \{(u + \alpha_1)\lambda_R - \alpha_1\lambda_D - u\lambda_I,\}. \tag{46}$$

In view of the Euler BC (40), we have $[(\lambda_I - \lambda_R)']_{t=T} = -b < 0$ with $[\lambda_I - \lambda_R]_{t=T} = 0$. As such, a singular solution also does not apply to any finite interval adjacent to the terminal time T and we have the following proposition.

PROPOSITION 4. *For the two-step growth model on C. trachomatis differentiation and proliferation* (30)–(32)*, we have*

$$\lambda_I(t) > \lambda_R(t), \tag{47}$$

and therewith $u_{op}(t) = u_{\max}$ *in some interval* (t_s, T) *where* $t_s < T$ *is the positive zero of the sign function* $\sigma(t) = \lambda_I(t) - \lambda_R(t)$ *nearest to T (if any exists).*

3.4. *Upper corner control not for $t < t_s$*

For the upper corner control $u_{\max}$, we designate the adjoint functions $\lambda_R(t)$ and $\lambda_D(t)$ as $\lambda_{Rg}(t)$ and $\lambda_{Dg}(t)$, respectively. We now show that t_s in

Proposition 4 is the unique zero of $\lambda_I(t) = \lambda_{Rg}(t)$ in the interval (0, T). The ODE for λ_{Rg} and λ_{Dg} may be rewritten as

$$\left[e^{-(u_{\max}+\alpha_1)t}\lambda_{Rg}\right]' = -e^{-(u_{\max}+\alpha_1)t}(\alpha_1\lambda_{Dg} + u_{\max}\lambda_I), \tag{48}$$

$$\left(e^{-\alpha_2 t}\lambda_{Dg}\right)' = -2\alpha_2 e^{-\alpha_1 t}\lambda_{Rg}. \tag{49}$$

LEMMA 1. *$\lambda_{Rg}(t)$ and $\lambda_{Dg}(t)$ must both be positive functions of t for $t < T$.*

Proof: If $\lambda_{Rg}(t)$ should be negative adjacent to the T, the ODE in (49) and the Euler BC $\lambda_{Dg}(T) = 0$ require λ_{Dg} to be negative in some interval (t_g, T). With $\lambda_I(t) \geq 0$, the ODE (48) and the associated Euler BC $\lambda_{Rg}(T) = 0$, in turn, require $\alpha_1\lambda_{Dg} < -u_{\max}\lambda_I < 0$ for $t_g < t < T$. However, this requirement cannot be met near T since $[(e^{-\alpha_2 t}\lambda_{Dg})']_{t=T} = 0$ while $\lambda_I'(T) = -b$. Hence, $\lambda_{Rg}(t)$ cannot be negative adjacent to T, contradicting the original assertion of a negative λ_{Rg} there. The lemma follows from the negativity of the right-hand side of the two ODE in (48)–(49) and the relevant two Euler BC. ■

Next, we establish the following lemma on the relative magnitude of $\lambda_{Dg}(t)$ and $\lambda_{Rg}(t)$.

LEMMA 2. *$\lambda_{Dg}(t) < \lambda_{Rg}(t)$ in some interval (t_d, T) adjacent to the terminal time T.*

Proof: Given the Euler BC in (40), we have

$$\lambda_I'(T) = -b, \qquad \lambda_{Rg}'(T) = \lambda_{Dg}'(T) = 0. \tag{50}$$

Upon differentiating the adjoint DE (37) and (38) in some small neighborhood of the terminal time that does not contain a switch of control, we also get

$$\lambda_{Dg}''(T) = 0, \qquad \lambda_{Rg}''(T) = -u_{op}\lambda_I'(T) = bu_{op} > 0, \tag{51}$$

and

$$\lambda_{Dg}'''(T) = \alpha_2\left(\lambda_{Dg}''(T) - 2\,\lambda_{Rg}''(T)\right) = -2\alpha_2 bu_{op} < 0. \tag{52}$$

It follows that

$$0 < \lambda_{Dg}(t) < \lambda_{Rg}(t) \tag{53}$$

for some interval (t_d, T). ■

It should be noted that t_d is necessarily $> t_s$ as shown by the lemma below:

LEMMA 3. *$\lambda_{R\ell}(t_s) = \lambda_{Rg}(t_s) < \lambda_{Dg}(t_s) = \lambda_{D\ell}(t_s)$.*

Proof: If $\lambda_{Rg}(t_s)$ were greater than $\lambda_{Dg}(t_s)$, then we would have

$$\lambda'_{Rg}(t_s) = \alpha_1 \left\{\lambda_{Rg}(t_s) - \lambda_{Dg}(t_s)\right\} > 0, \tag{54}$$

since $\lambda_I(t_s) = \lambda_{Rg}(t_s)$. It follows that $\lambda_{Rg}(t)$ would be an increasing function of t (while $\lambda_I(t)$ is decreasing) near t_s. However, we learned earlier that $\lambda_I > \lambda_{Rg} > \lambda_{Dg}$ for t adjacent to T (see Proposition 4 and Lemma 2). Hence, λ_{Rg} must become decreasing prior to vanishing at terminal time with $\lambda_{Rg}(t) = \lambda_I(t)$ having another zero closer to T. This contradicts the fact that t_s is the zero of $\lambda_{Rg}(t) = \lambda_I(t)$ most close to T. ■

Lemma 3 enables us to conclude from the adjoint equation for $\lambda_{Rg}(t)$

$$\begin{aligned}\lambda'_{Rg}(t_s) &= (u_{\max} + \alpha_1)\lambda_{Rg}(t_s) - \alpha_1\lambda_{Dg}(t_s) - u_{\max}\lambda_I(t_s)\\ &= \alpha_1 \left\{\lambda_{Rg}(t_s) - \lambda_{Dg}(t_s)\right\} \ < 0,\end{aligned}$$

and therewith

$$\lambda_I - \lambda_{Rg} < 0 \tag{55}$$

for $t < t_s$, given $\lambda_{Rg}(t_s) = \lambda_I(t_s)$ $(= 1 - e^{-b(T-t_s)} \approx 1)$ and $\lambda_I(t) = 1 - e^{-b(T-t)} \to 1$ from below as $t \to -\infty$. In view of (42), we conclude the following proposition.

Proposition 5. *The upper corner control $u_{\max}$ is not optimal for $t < t_s$.*

3.5. Lower corner control for $t < t_s$

Given Proposition 5 and the Hamiltonian being linear in the control $u(t)$, the lower corner control $u_\ell(t) = 0$ is expected to be optimal for $t < t_s$ because the singular solution is ruled out by (55). In order for $u_\ell = 0$ to be optimal for all t in $[0, t_s)$, we need the inequality $\lambda_{R\ell}(t) < \lambda_{D\ell}(t)$ to persist in that entire interval (as Lemma 3 only implies $\lambda_{R\ell}(t) < \lambda_{D\ell}(t)$ for some interval $(t_0, t_s]$ where t_0 may be > 0). To show this, we consider the terminal value problem for the adjoint functions $\lambda_{R\ell}(t)$ and $\lambda_{D\ell}(t)$ associated with $u = 0$

$$\lambda'_{R\ell} = \alpha_1(\lambda_{R\ell} - \lambda_{D\ell}), \qquad \lambda_{R\ell}(t_s) = \lambda_{Rg}(t_s), \tag{56}$$

$$\lambda'_{D\ell} = \alpha_2(\lambda_{D\ell} - 2\lambda_{R\ell}), \qquad \lambda_{D\ell}(t_s) = \lambda_{Dg}(t_s), \tag{57}$$

where continuity of the adjoint functions at the switch point provided the terminal conditions for the two first-order adjoint ODE. From these, we obtain the following proposition.

Proposition 6. $\lambda_{D\ell}(t_s) > \lambda_{R\ell}(t) > 0$ *for* $0 \leq t \leq t_s$.

Proof: The inequality $\lambda_{R\ell}(t) > 0$ follows from $0 < \lambda_{Rg}(t_s) = \lambda_{R\ell}(t_s) < \lambda_{D\ell}(t_s)$. As a consequence, we have $\lambda'_{R\ell}(t_s) = \alpha_1[\lambda_{R\ell} - \lambda_{D\ell}]_{t=t_s} < 0$. For

$\lambda_{D\ell}(t_s) > \lambda_{R\ell}(t_s)$, we note

$$(\lambda_{D\ell} - \lambda_{R\ell})' = (\alpha_2 + \alpha_1)(\lambda_{D\ell} - \lambda_{R\ell}) - \alpha_2 \lambda_{R\ell} \tag{58}$$

or

$$\left[e^{-(\alpha_2+\alpha_1)t} \Delta_{DR}\right]' = -\alpha_2 e^{-(\alpha_2+\alpha_1)t} \lambda_{R\ell}, \tag{59}$$

where $\Delta_{DR}(t) = \lambda_{D\ell} - \lambda_{R\ell}$. We can integrate to obtain

$$e^{-(\alpha_2+\alpha_1)t} \Delta_{DR}(t) = e^{-(\alpha_2+\alpha_1)t_s} \Delta_{DR}(t_s) + \alpha_2 \int_t^{t_s} e^{-(\alpha_2+\alpha_1)\tau} \lambda_{R\ell}(\tau) d\tau. \tag{60}$$

From this, we conclude

$$\Delta_{DR}(t) = \lambda_{D\ell} - \lambda_{R\ell} > 0 \qquad (0 \leq t < t_s), \tag{61}$$

and large compared to $be^{-b(T-t)}$ for $t < t_s$ because $\lambda_{R\ell}(\tau) > 0$ and $\Delta_{DR}(t_s) > 0$. ■

Note that $\lambda_{R\ell}$ continues to be a decreasing function of time (and increases as t decreases while λ_I asymptotes to unity as $t \to -\infty$). With

$$(\lambda_I - \lambda_{R\ell})' = -be^{-b(T-t)} - \alpha_1(\lambda_{R\ell} - \lambda_{D\ell}), \tag{62}$$

and $\lambda_{R\ell} - \lambda_{D\ell}$ is negative and large compared to $-be^{-b(T-t)}$, $\lambda_I - \lambda_{R\ell}$ is an increasing function of time for all t in $[0, t_s]$ so that $\lambda_I - \lambda_{R\ell} < 0$ and the lower corner control is optimal there. This conclusion augments the results already obtained in previous subsections to provide the complete solution of our problem as summarized in the following proposition:

PROPOSITION 7. *There exists a unique optimal control $u_{op}(t)$ that maximizes the Hamiltonian $H[u]$ for our two-step model for C. trachomatis differentiation and proliferation that is again the bang-bang control*

$$u_{op}(t) = \begin{cases} 0 & (t < t_s) \\ u_{\max} & (t_s < t \leq T), \end{cases} \tag{63}$$

with the switch point being the zero $t_s < T$ of $\lambda_I(t) = \lambda_{Rg}(t)$ where $\lambda_I(t) = 1 - e^{b(t-T)}$ and $\lambda_{Rg}(t)$ is the solution of the terminal BVP (48) *and* (40).

Proof: With the optimal control already shown to be (63) by the development preceding the proposition, we complete the proof of the proposition by noting the uniqueness of the switch point t_s given the monotonicity of $\lambda_I(t)$ and $\lambda_{Rg}(t)$. ■

Remark 3. The switch point t_s may be negative with $\lambda_I(t) > \lambda_R(t)$ for $t \geq 0$. As in the proof-of-concept model, the optimal conversion strategy would be at the maximum rate uninterrupted from the start.

Remark 4. Maximizing the Hamiltonian is not synonymous with a maximum $E(T)$. However, $u_{op}(t)$ is unique we only need to verify that the terminal EB population for the optimal control is greater than another admissible control (and it is, in comparison to that for the lower corner control).

3.6. The switch point

Unlike the proof-of-concept model of the previous section, how the system parameters $\{\alpha_k, \ b, \ u_{\max}, \ T\}$ in the present two-step conversion model contribute to the optimal conversion is much more complicated compared to the relatively simple relation (15). To get some idea how the various parameter values contribute to the location of the switch point, we recall a simplification mentioned previously about measuring time in units of a growth rate constant. The results reported below are for a normalized model that works with $\hat{t} = \alpha_2 t$ and all other quantities normalized accordingly (by dividing all other rate constants by α_2 to get $\hat{u} = u/\alpha_2$, etc., and then setting $\alpha_2 = 1$). We also choose (normalized) terminal times $\hat{T}$ long enough to illustrate certain features of the switch point t_s. In the table below and subsequent discussion, we omit all ^ from all normalized parameters, now all relative to α_2. By choosing normalized parameter values greater than, equal to, or less than 1 , the data delineate the consequences of unnormalized b, α_1, $u_{\max}$, and T relative to α_2.

The values of t_s in Table 1 were computed by solving for the adjoint functions (exactly or numerically) and finding the only zero of $\lambda_I(t_s) - \lambda_{Rg}(t_s)$. The zero exists and is unique because $\lambda_I(t)$ is monotone decreasing function and asymptotic to 1 as t decreases toward $-\infty$, while $\lambda_{Rg}(t)$ is also monotone decreasing but at a steeper rate near the start ($t = 0$) before changing to a more gentle slope when $\lambda_I(t_s)$ turns steep as t approaches T with the two graphs meeting for the Euler BC at T. A single pass algorithm has been developed for such a root finding problem but a brute force bisection algorithm is adequate for the present simple problem. Two typical solutions for the pair $\lambda_{Rg}(t)$, $\lambda_{Dg}(t)$, and $\lambda_I(t)$ are given as Figs. 4 and 5, showing the possibility of positive and negative t_s (inside or outside the interval $(0, T)$).

It is clear from the results reported in the table that the instant t_s when the bang-bang control (63) switches from lower to upper corner control increases with b when all other parameter values are held fixed. Similarly, it also increases when only $u_{\max}$ increases. In both cases, it is beneficial to wait for more proliferation when the conversion becomes more efficient as b or $u_{\max}$ increases. The consequence of increasing α_1 alone is not so monotone. As α_1 increases from 0.25, t_s first increases but attains a maximum when α_1 reaches the level of b. Combinations of parameter values

Table 1
The Switch Point t_s

		$T = 2$		$*$		$T = 5$		b
$\alpha_1 \backslash u_{\max}$	10	5.0	2.5	$*$	1.0	0.5	0.25	
	×	×	×		×	×	×	0.5
0.25	×	×	×	$*$	×	×	×	1.0
	0.39	0.21	×		1.81	0.60	×	2.5
0.50	×	×	×	$*$	1.52	0.55	×	1.0
	0.51	0.31	×		2.08	1.10	×	2.5
	×	×	×		1.34	0.47	×	0.5
1.00	0.11	×	×	$*$	1.79	0.96	×	1.0
	0.58	0.38	0.028		2.27	1.45	0.42	2.5
1.50	0.61	0.40	0.050	$*$	2.33	1.58	0.66	2.5
	×	×	×		1,57	0.80	×	0.5
2.00	0.24	0.019	×	$*$	1.93	1.21	0.34	1.0
	0.61	0.40	0.05		2.35	1.64	0.78	2.5
	.060	×	×		1.62	0.91	0.11	0.5
4.00	0.28	0.028	×	$*$	1.94	1.27	0.50	1.0
	0.60	0.36	×		2.32	1.66	0.99	2.5

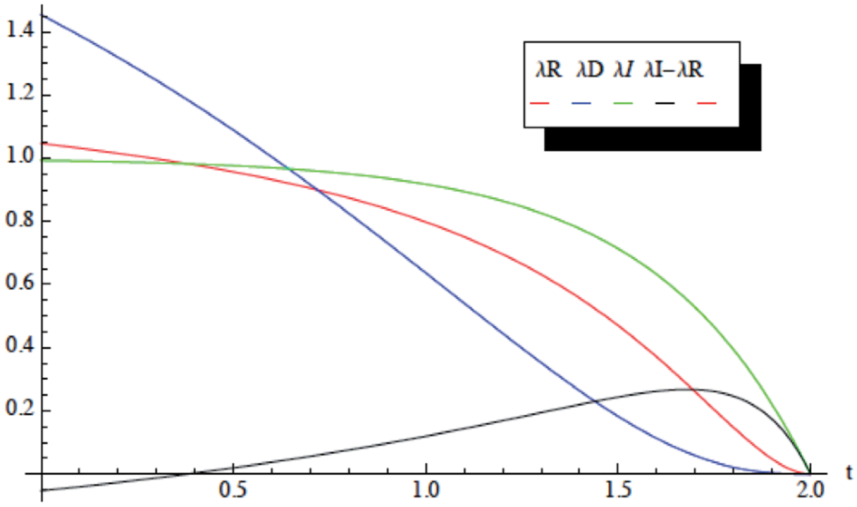

Figure 4. The adjoint functions with $\alpha_1 = 1,\ \alpha_2 = 1,\ b = 2.5,\ \ u_{\max} = 5,\ T = 2.$

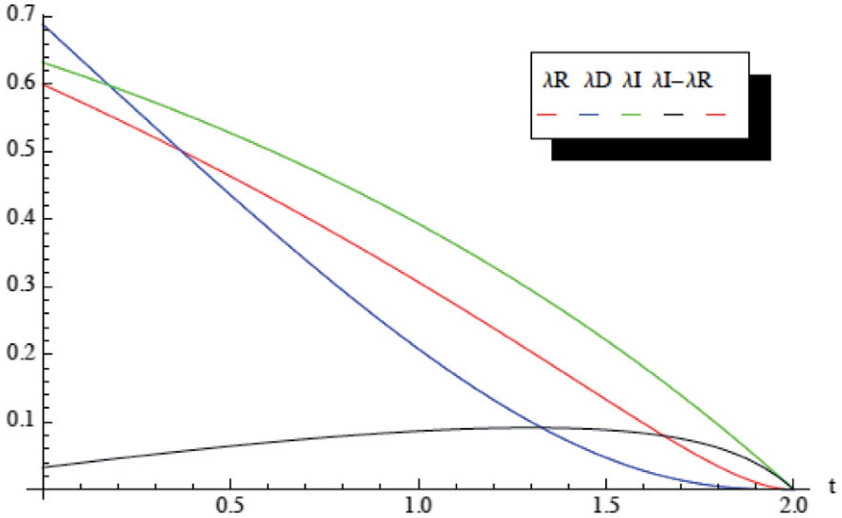

Figure 5. The adjoint functions with $\alpha_1 = 1,\ \alpha_2 = 1,\ b = 0.5, u_{\max} = 5, T = 2$.

in the table without a value for t_s are marked by "$\times''$ to indicate no root of $\lambda_I(t_s) - \lambda_R(t_s)$ in the interval $(0, T)$. The cases with no positive t_s for $T = 2$ would have a positive switch time for sufficiently larger T value (confirmed by solutions for the same cases now with $T = 3$ or 4 not shown here). It can be verified that most cases for $T = 5$ reported in the same table would not have a positive switch time if T is reduced to 2.

4. Growth with a finite carrying capacity

4.1. The model

In the proof-of-concept model, the natural growth rate of the RB population $R(t)$ was taken to be proportional to the size of the population. This limits its applicability to an initial RB population R_0 that is small compared to the carrying capacity of the host cell, a moderate growth rate constant α, and a relatively short host cell life span T. For a more realistic model, we consider here a new differentiation and proliferation model characterized by the IVP

$$R' = (\alpha - u - \beta R)R, \qquad R(0) = R_0. \tag{64}$$

The new model includes the effect of a finite carrying capacity for RB only (because EBs are much smaller in size) and reduces to the linear growth rate case if $\beta = 0$ (corresponding to the limiting case of an infinite carrying capacity). The growth rate of the EB population is kept equal to the rate of conversion uR as in (64) (not limited by some carrying capacity):

$$E' = uR, \qquad E(0) = E_0 \ (= 0). \tag{65}$$

We wish to maximize the EB population size at the terminal time T:

$$\max_{0 \leq u \leq u_{\max}} E(T) = \int_0^T uR dt. \tag{66}$$

The optimization problem as stated is to choose a control $u_{op}(t)$ to maximize the total *EB* population $E(T)$ at the time of the host cell's lysis. Again, E_0 plays no role in our model and has been set to 0.

The choice of conversion strategy is limited by the obvious lower bound $u = 0$ (given that the conversion is not reversible) and a natural maximum rate $u_{\max}$ imposed by a limited conversion capacity. Hence, $u(t)$ is subject to the constraints (4). The optimization problem is then given by (66) subject to the new growth dynamics (64) and the inequality constraints.

To reduce the number of parameters in the problem, we normalize the *RB* and *EB* populations by the carrying capacity of the *RB* population by setting $\beta R/\alpha = \hat{R}$ and $\beta E/\alpha = \hat{E}$, respectively. We also measure time in units of the growth rate constant for *RB*, effectively working with $\hat{t} = \alpha t$ (and $\hat{u} = u/\alpha$) instead of t (and u). We henceforth take the growth dynamics in the normalized form

$$R' = R(1 - u - R), \qquad R(0) = R_0, \tag{67}$$

instead of (64) (after dropping "^" from the normalized quantities).

For our previous proof-of-concept linear natural RB growth rate model, the actual (normalized) growth rate "constant" $1 - u$ does not depend on the RB population size, and correspondingly the optimal conversion strategy for maximum $E(T)$ does not depend on the initial infecting bacteria population size R_0. For the new model, the role of $1 - u$ is replaced by $1 - u - R$ so that growth (or decline) is with diminishing return. In particular, with the optimal conversion strategy now dependent on the RB population, reticulate bodies in excess of the carrying capacity may be lost due to rate limiting attrition and therefore not contributing to RB growth for a larger terminal EB population. We will delineate in the subsequent development how optimal conversion strategy varies with different ranges of R_0. Before that, we need to establish a special solution of our problem, the *singular solution*, which had no role in the simple models of previous sections.

4.2. The singular solutions

To apply the Maximum Principle, we again begin with the *Hamiltonian* for the new optimal control problem:

$$H[u] = uR + \lambda R(1 - u - R) = uR(1 - \lambda) + \lambda R(1 - R). \tag{68}$$

and generate the *adjoint differential equation* and *adjoint (Euler) end condition*

$$\lambda' = -\frac{\partial H}{\partial R} = -\lambda(1 - u - 2R) - u, \qquad \lambda(T) = 0, \tag{69}$$

for the *adjoint function* $\lambda(t)$. The principle then requires that we choose u to maximize H:

$$\max_{0 \le u \le u_{\max}} \quad H[u] = uR(1 - \lambda) + \lambda R(1 - R) \tag{70}$$

subject to the IVP for $R(t)$ in (67), the terminal value problem (69) for $\lambda(t)$, and the constraints on the control $u(t)$ (4).

To maximize H, we note that the stationary condition

$$\frac{\partial H}{\partial u} = R(1 - \lambda) = 0 \tag{71}$$

again does not determine the stationary point or *interior control* $u_i(t)$ that would normally induce an extremal $R_i(t)$ by the IVP (67) and an associated adjoint function $\lambda_i(t)$ by (69). However, the condition may be met by *singular solutions* , combinations of $R(t)$ and $\lambda(t)$ that satisfy (71) as well as their Hamiltonian system of ODE. For our model, $R(t) \equiv 0$ satisfies the stationary condition (71) but is not a singular solution because (67) requires $R(t) > 0$ for all t in $[0, T]$ for any prescribed $R_0 > 0$. The other way of satisfying (71) is $\lambda(t) = 1$ but this solution does not apply near the terminal time because the Euler BC requires $\lambda(T) = 0$. However, it may satisfy (71) away from the terminal time (as we shall see); hence,

$$\lambda_S(t) = 1 \tag{72}$$

is a possible singular solution in some subinterval away from T. For this possible singular solution, the adjoint DE requires the corresponding RB population, denoted by $R_S(t)$, to be

$$R_S(t) = \frac{1}{2} \tag{73}$$

(which cannot be met near $t = 0$ unless $R_0 = 1/2$). When (73) is met, possibly away from the initial time, the growth rate equation for the RB population requires the corresponding control to be

$$u_S(t) = \frac{1}{2}. \tag{74}$$

Regardless whether or not the singular solution (72)–(74) applies from the start or in some subintervals of $[0, T]$, the optimal strategy must switch to another control prior to the terminal time T to satisfy the Euler BC $\lambda(T) = 0$. In the next subsection, we show that the switch from the singular solution nearest to T is to the upper corner control $u_g(t) = u_{\max}$.

Whatever the appropriate combination of control components that constitutes the optimal control $u_{op}(t)$ may be, the IVP (67), the terminal value problem (69), and the optimality condition (70) must be satisfied. Together, these requirements form a set of necessary conditions for the solution of the optimization problem.

4.3. The upper corner control is optimal adjacent to T

Given $\lambda(T) = 0$, we have by continuity $1 - \lambda > 0$ for $t_s < t < T$ for some $t_s < T$. The optimality condition (70) requires that we convert as much as possible to maximize H. With the constraints imposed by (4), the optimal choice of conversion rate is by the *upper corner control* $u_{op}(t) = u_{\max}$ in $(t_s, T]$. The adjoint DE now involves $R(t)$; we need to solve the following two ODE simultaneously for R and λ with $u = u_{\max}$:

$$R_g' = R_g(1 - u_{\max} - R_g), \quad R_g(t_s) = R_s, \tag{75}$$

$$\lambda_g' = -(1 - u_{\max} - 2R_g)\lambda_g - u_{\max}, \quad \lambda_g(T) = 0, \tag{76}$$

where we have again denote the solution by $R_g(t)$and $\lambda_g(t)$, respectively. The auxiliary condition $R_g(t_s) = R_s$ augmenting the ODE for $R_g(t)$ follows from the continuity condition at the point t_s.

Note that $\lambda_g'(T) = -u_{\max} < 0$ so that $\lambda_g(t)$ is a positive decreasing function of time in some interval (t_s, T) adjacent to T. It follows that $t_s < T$ is the instant nearest T when λ_g attains the value 1, i.e.,

$$\lambda_g(t_s) = 1. \tag{77}$$

The solutions for $R_g(t)$ and $\lambda_g(t)$ for $t > t_s$ are

$$R_g(t) = \frac{u_\alpha R_s e^{-u_\alpha(t-t_s)}}{u_\alpha + R_s - R_s e^{-u_\alpha(t-t_s)}}, \quad \lambda_g(t) = u_{\max} e^{-I(t)} \int_t^T e^{I(\tau)} d\tau, \tag{78}$$

where u_α $(= u_{\max} - \alpha) = u_{\max} - 1$, R_s is the yet unknown value $R_g(t_s)$, and

$$e^{I(t)} = \frac{u_\alpha^2 e^{u_\alpha(t_s-t)}}{\left[u_\alpha + R_s - R_s e^{u_\alpha(t_s-t)}\right]^2} \tag{79}$$

with $e^{I(t_s)} = 1$. From (79), we obtain after carrying out the integration in the expression for $\lambda_g(t)$ in (78)

$$\lambda_g(t) = \frac{u_{\max}}{u_\alpha} \frac{u_\alpha + R_s - R_s e^{u_\alpha(t_s-t)}}{u_\alpha + R_s - R_s e^{u_\alpha(t_s-T)}} \left[1 - e^{u_\alpha(t-T)}\right] \tag{80}$$

for all t in $[t_s, T]$.

With (80), the switch condition (77) becomes

$$\frac{u_{\max}\left[1 - e^{u_\alpha(t_s - T)}\right]}{u_\alpha + R_s - R_s e^{u_\alpha(t_s - T)}} = 1, \tag{81}$$

which may be solved for t_s to get

$$t_s = T - \frac{1}{u_\alpha}\ln\left(\frac{u_{\max} - R_s}{1 - R_s}\right). \tag{82}$$

Observe that R_s will come from the solution in the complementary time interval $[0, t_s]$ and may be a function of t_s. The condition for determining the switch point is then a complicated nonlinear equation for t_s. Similar to the simpler proof-of-concept model, the switch point t_s could be negative if $u_{\max} \geq 1 \geq R_s$ and T is sufficiently short; it could also be undefined (when $u_{\max} < R_s < 1$). In either case, conversion at $u_{\max}$ from the start should be examined for its optimality. However, unlike the simpler models analyzed earlier, the existence and nature of a switch point t_s cannot be determined for the present improved model without first examining the complementary interval $[0, t_s)$ given that we do not yet know what R_s should be. That uncertainty notwithstanding, the Maximum Principle unequivocally singles out the upper corner control $u_{\max}$ as optimal for an interval of time adjacent to the terminal time T:

PROPOSITION 8. *The upper corner control maximizes the Hamiltonian of the optimization problem* (66) *in the interval* $(t_s, T]$.

Before turning to the optimal conversion strategy for the interval $[0, t_s)$, we note that the solution for the special case of $u_{\max} = 1$ requires a little more care but poses no obstacle to the solution of the problem. It will be addressed for the different situations below. Here, we observe instead that if $\lambda_g(t) < 1$ for all $t > 0$, then the Hamiltonian is maximized by the upper corner control for all $t \geq 0$ without switching to another admissible control (corresponding to the case $t_s < 0$ in the previous models). To the extent that a maximized Hamiltonian is not synonymous with a maximum $E(T)$, we will address the issue of a maximum $E(T)$ in a later section by the method of most rapid approach [7, 15] when it is necessary to do so.

In the next few sections, we analyze and determine the different optimal conversion strategies for all t in $[0, T]$ that maximize $E(T)$ for different ranges of R_0. Since it is most likely that only a few EBs enter and infect a host cell initially so that $R_0 \ll 1$, i.e., well below the carrying capacity of the host cell, we consider first in the next section the range $R_0 \leq 1/2$ and analyze briefly the range $R_0 > 1/2$ in the section after. Before we leave this section, we elucidate in the last subsection below the reason for a separate treatment of these two ranges of R_0.

4.4. *Lower corner control optimal only for* $R_0 \leq 1/2$

If $1 - \lambda_g(t)$ should vanish at some $t_s > 0$ and becomes nonpositive for $t < t_s$, then the Maximum Principle would require a switch to another more appropriate conversion strategy for $t < t_s$. Continuity of the adjoint function $\lambda(t)$ and the RB population $R(t)$ requires $\lambda(t_s) = 1$ for any switch from $u_{\max}$ to another admissible control. It follows that

$$[\lambda']_{t=t_s} = [-\lambda(1 - u - 2R) - u]_{t=t_s} = -[1 - 2R_s] \tag{83}$$

for any control $u(t)$ (including the two corner controls) with $R_s = R(t_s)$ for that control. Depending on the sign of $1 - 2R_s$, $\lambda'(t_s) = \lambda'_g(t_s) = \lambda'_\ell(t_s)$ may be positive, zero or negative. As such $\lambda(t)$ (for any control $u(t)$) may be increasing, stationary, or decreasing in some small neighborhood of $t \leq t_s$. Evidently, the sign of $\lambda(t) - 1$ for $t < t_s$ determines the appropriate control there. With that in mind, we have the following lemma that eliminates a switch to the lower corner control if $R_0 > 1/2$.

PROPOSITION 9. *The lower corner control is not optimal in* $[0, t_s)$ *if* $R_0 > 1/2$ *(or, for the unnormalized form of the problem, when the initial RB population is larger than half of the carrying capacity of the host cell).*

Proof: For $R_0 > 1$, we have $R'_\ell < 0$ so that $R_\ell(t)$ is monotone decreasing asymptotic to 1 from above and $R_\ell(t) > 1$ for all finite $t > 0$. With $u = 0$, the adjoint DE (69) becomes

$$\lambda'_\ell = -\lambda_\ell(1 - 2R_\ell), \quad \lambda_\ell(t_s) = 1. \tag{84}$$

The solution of this terminal value problem is

$$\lambda_\ell(t) = e^{I_s(t)}, \quad I_s(t) = \int_t^{t_s} \{1 - 2R_\ell(x)\} dx \tag{85}$$

so that $\lambda_\ell(t) < 1$ for all $t < t_s$ (with $\lambda_\ell(t_s) = 1$ and $\lambda'_\ell(t) > 0$). Since $1 - \lambda_\ell > 0$, the lower corner control is not optimal in $[0, t_s)$ if $R_0 > 1$.

For $R_0 = 1$, we have $R_\ell(t) = 1$ (since $R'_\ell(t) = 0$) and $\lambda'_\ell = \lambda_\ell$ so that $\lambda_\ell(t) = e^{t - t_s} < 1$ for $t < t_s$. With $\lambda_\ell(t) < 1$ for $t < t_s$, the lower corner control is again not optimal in $[0, t_s)$ if $R_0 = 1$.

For $1/2 < R_0 < 1$, we have from (86) $R'_\ell(0) > 0$ and $\lambda'_\ell(0) > 0$. Hence, both $R_\ell(t)$ and $\lambda_\ell(t)$ are increasing function of time with $\lambda_\ell(t_s) = 1$ and therewith $\lambda_\ell(t) < 1$ for $t < t_s$. The lower corner control is also not optimal in $[0, t_s)$ if $1/2 < R_0 < 1$. ■

By Proposition 9, the lower corner control may be appropriate for the interval $[0, t_s)$ (or a subinterval thereof) only if $R_0 \leq 1/2$. Since the infection of a healthy cell usually starts with the endocytosis of a few EB bacteria (and hence $R_0 \ll 1$), we will first focus on the range $R_0 \leq 1/2$ and determine the optimal control for maximum $E(T)$ for this range of

R_0 before turning to examine the optimal conversion strategy for the complementary range $R_0 > 1/2$.

5. Optimal conversion strategy for $R_0 \leq 1/2$

5.1. *The optimal control is bang-bang for $u_{\max} \leq 1/2$*

With the lower corner control eliminated for the range $R_0 > 1/2$, we can focus our discussion of $u_\ell(t) = 0$ for the range $R_0 \leq 1/2$. In that range, we have from (67) for $u(t) = 0$

$$R'_\ell = (1 - R_\ell)R_\ell, \;\; R_\ell(0) = R_0 \tag{86}$$

with the explicit solution

$$R_\ell(t) = \frac{R_0}{R_0 + (1 - R_0)e^{-t}} \tag{87}$$

asymptotic to 1 as $t \to \infty$. Let t_0 be the instant when $R(t)$ is at half carrying capacity. The condition $R_\ell(t_0) = 1/2$ determines t_0 to be

$$t_0 = \ln\left(\frac{1 - R_0}{R_0}\right) \tag{88}$$

with t_0 increasing from 0 to ∞ as R_0 decreases from 1/2 to 0, we need to explore the possibility of a singular solution phase, $\{R_S(t), \lambda_S(t)\} = \{1/2, 1\}$, in an interval (t_0, t_S) for some switch point $t_S > t_0$. Given $R_S(t) = 1/2$ in $[t_0, t_S]$, we have from the ODE in (67) $0 = R'_S(t) = (1 - u - 1/2)/2$ so that we must have $u(t) = u_S(t) = 1/2$ to sustain this singular solution phase. However, this requirement cannot be met if $u_{\max} < 1/2$ so that there cannot be a singular solution phase in that case.

While the singular control $u_S(t) = 1/2$ is not ruled out if $u_{\max} = 1/2$, the singular solution would persist for $t_S \leq t \leq T$ because $\{R_S(t), \lambda_S(t)\} = \{1/2, 1\}$ is also the solution of

$$\begin{aligned} R'_g &= R_g(1 - u_{\max} - R_g) = R_g(1 - 2R_g)/2, \\ \lambda'_g &= -\lambda_g(1 - u_{\max} - 2R_g) - u_{\max} = -\lambda_g(1/2 - 2R_g) - 1/2, \end{aligned}$$

with $\{R_g(t_S), \lambda_g(t_S)\} = \{R_S(t_S), \lambda_S(t_S)\} = \{1/2, 1\}$ for continuity. However, this upper corner solution (for $u_{max} = 1/2$) does not satisfy the necessary Euler BC $\lambda_g(T) = 0$. Without a singular solution phase, we have the following optimal conversion strategy for $u_{\max} \leq 1/2$.

PROPOSITION 10. *For* $R_0 \leq 1/2$ *and* $u_{\max} \leq 1/2$, *the optimal conversion strategy is by the bang-bang control*

$$u_{op}(t) = \begin{cases} 0 & (0 \leq t < t_s) \\ u_{\max} & (t_s < t \leq T), \end{cases} \tag{89}$$

with the switch point t_s *being the unique positive root of*

$$t_s = T - \frac{1}{u_\alpha} \ln\left(\frac{u_{\max}(1 - R_0) + u_\alpha R_0 e^{t_s}}{1 - R_0}\right). \tag{90}$$

The corresponding RB population and adjoint function are given by (87) *and*

$$\lambda_\ell(t) = e^{t_s - t}\left(\frac{1 - R_0 + R_0 e^t}{1 - R_0 + R_0 e^{t_s}}\right)^2, \tag{91}$$

respectively, for t *in* $[0, t_s]$ *and by the upper corner solution in* (78) *and* (80)*, denoted by* $\{R_{g0}(t), \lambda_{g0}(t)\}$*, with* $R_s = R_\ell(t_s)$.

Proof: Since a switch from the upper corner control to a singular control is not possible, there are only two possible scenarios for the optimal conversion strategy. The first is $\lambda_g(t) \leq 1$ for all t in $[0, T]$ so that $u_{op}(t) = u_{\max}$ in $[0, T]$ corresponding to the case $t_s \leq 0$. The other scenario is $\lambda_g(t_s) = 1$ and $\lambda_g(t) > 1$ for some interval $t_\ell < t < t_s$ adjacent to t_s. In the range (t_ℓ, t_s), the upper corner control is not optimal and a switch in control at t_s can only be to the lower corner control (given the singular control is not an option). The corresponding adjoint function $\lambda_\ell(t)$ is continuous at t_s and $\lambda'_\ell(t)$ is necessarily < 0 in (t_ℓ, t_s) because

$$\lambda'_\ell(t_s) = -[1 - 2R_\ell(t_s)] = -[1 - 2R_g(t_s)] = \lambda'_g(t_s) < 0. \tag{92}$$

With $R_\ell(t_s) = R_g(t_s) < 1/2$,we have by continuity $R_\ell(t) < 1/2$, $\lambda'_\ell(t) < 0$ and $\lambda_\ell(t) > 1$ in some interval (t_c, t_s). Hence, the lower corner control is optimal in (t_c, t_s). Since $R'_\ell(t) > 0$ for $R_0 \leq 1/2$, we have $R_\ell(t) < 1/2$ in $[0, t_s)$. It follows that the lower corner control is optimal in $[0, t_s)$.

The bang-bang control (89) is the only control that satisfies all the necessary conditions imposed by the Maximum Principle and $[E(T)]_{u=u_{op}(t)} > [E(T)]_{u=0} = 0$, it maximizes $E(T)$. ■

COROLLARY 1. *For* $R_0 \leq 1/2$ *and* $u_{\max} \leq 1/2$, *the optimal control* $u_{op}(t) = u_{\max}$ *for all* t *in* $[0, T]$ *maximizes* $E(T)$ *if the unique root* t_s *of* (90) *is negative. The corresponding RB population* $R_g(t)$ *and adjoint function* $\lambda_g(t)$ *are the solutions of the IVP*

$$R'_g = R_g(1 - u_{\max} - R_g), \quad R_g(0) = R_0 \tag{93}$$

and terminal value problem

$$\lambda'_g = -(1 - u_{nax} - 2R_g)\lambda_g - u_{\max}, \quad \lambda_g(T) = 0, \tag{94}$$

Table 2

$T_0(u_{\max}, R_0)$

$u_{\max}\backslash R_0$	0.01	0.05	0.10	0.15	0.20	0.25	0.4	0.5
0.10	2.66	3.27	∞	×	×	×	×	×
0.15	2.30	2/65	3.40	∞	×	×	×	×
0.20	2.06	2.31	2.75	3.54	∞	×	×	×
0.25	1.89	2,08	2.39	2,85	3,70	∞	×	×
0.30	1.75	1.91	2.15	2.48	2.97	3.88	×	×
0.35	1.64	1.77	1.97	2.23	2.58	3.10	×	×
0.40	1.55	1.66	1.83	2.04	2.31	2.68	∞	×
0.45	1.47	1.57	1.72	1.89	2.12	2.40	4.52	×
0.495	1.41	1.50	1.63	1.79	1.98	2.22	3.65	×

respectively.

Proof: The unique root t_s of (90) is the instant nearest to T when $\lambda_g(t_s) = 1$ with $\lambda_g(t) < 1$ for $t > t_s$ and $\lambda'_g(t_s) = -(1 - 2R_g(t_s)) < 0$. Hence, $u_{op}(t) = u_{\max}$ maximizes $H[u]$ and is optimal by the Maximum Principle. It is the only admissible control by that principle. It maximizes $E(T)$ since $[E(T)]_{u=u_{op}(t)} > [E(T)]_{u=0} = 0$. ■

It is evident from (90) that t_s should be positive if T is sufficiently large. More specifically, t_s should be positive if

$$T > T_0 \equiv \frac{1}{u_\alpha} \ln\left(\frac{u_{\max}(1 - R_0) + u_\alpha R_0}{1 - R_0}\right). \tag{95}$$

Table 2 provides values of T_0 for a range of $u_{\max}$ and R_0.

5.2. Subinterval of singular solution possible for $u_{\max} > 1/2$

5.2.1. The case $R_0 < 1/2$. For $u_{\max} > 1/2$ and $R_0 < 1/2$, the lower corner solution $R_\ell(t)$ again reaches the singular solution by t_0 as given by (88); it is now possible to sustain the singular solution (72)–(74) for $t > t_0$ by taking $u_{op}(t) = u_S(t) = 1/2$ given $u_{\max} > 1/2$. As the Maximum Principle requires the Euler BC $\lambda(T) = 0$ be satisfied, the optimal solution must leave the singular solution (if it should contain such a solution segment) and switch to an upper corner solution at a second switch point t_S to conform with Proposition 8. With $R_s = 1/2 = R_S(t_S)$, this upper corner solution, to be denoted by $R_{gS}(t)$ and $\lambda_{gS}(t)$, is determined by

$$R'_{gS} = R_{gS}(1 - u_{\max} - R_{gS}), \quad R_{gs}(t_S) = 1/2, \tag{96}$$

$$\lambda'_{gS} = -\lambda_{gS}(1 - u_{\max} - 2R_{gS}) - u_{\max}, \quad \lambda_{gS}(T) = 0, \tag{97}$$

with t_S determined by the continuity of the adjoint function at the switch point:

$$\lambda_{gS}(t_S) = 1. \tag{98}$$

The new upper corner solution differs from $\{R_{g0}(t), \lambda_{g0}(t)\}$ (see Proposition 10) with the latter satisfying the continuity condition $R_s = R_\ell(t_s)$. The explicit solution of the BVP (96)–(98) for $u_{\max} \neq 1$ is

$$R_{gS}(t) = \frac{u_\alpha e^{-u_\alpha(t-t_S)}}{2u_\alpha + 1 - e^{-u_\alpha(t-t_S)}}, \tag{99}$$

$$\lambda_{gS}(t) = \frac{u_{\max}}{u_\alpha}\frac{2u_\alpha + 1 - e^{u_\alpha(t_S-t)}}{2u_\alpha + 1 - e^{u_\alpha(t_S-T)}}\left[1 - e^{u_\alpha(t-T)}\right], \tag{100}$$

with

$$t_S = T - \frac{1}{u_{\max} - 1}\ln(2u_{\max} - 1). \tag{101}$$

For the special case $u_{\max} = 1$, we get by taking the limit as $u_\alpha \to 0$

$$R_{gS}(t) = \frac{1}{2 + t - t_S}, \tag{102}$$

$$\lambda_{gS}(t) = \frac{2 + t - t_S}{2 + T - t_S}(T - t), \tag{103}$$

with

$$t_S = T - 2. \tag{104}$$

For the relation between t_0 and t_S, we first observe that $t_S(T, u_{\max})$ as given by (101) does not depend on R_0, while t_0 as found in (88) depends only on R_0. As such, t_0 may be smaller than, equal to, or greater than t_S (for a prescribed combination of T and $u_{\max} > 1/2$). When $t_0 < t_S$, the gap (t_0, t_S) allows for a singular solution phase in the optimal solution of the problem; this is not true if $t_0 < t_S$. The threshold value of R_0 that separates the two optimal solution types, denoted by R_c, is when $t_0(R_0 = R_c) = t_S$. Since t_0 is a monotone decreasing function of R_0 that ranges from 0 to ∞ as R_0 decreases from 1/2 to 0, there is a unique root R_c of the equation

$$t_0(R_0) = \ln\left(\frac{1 - R_0}{R_0}\right) = t_S(T, u_{\max}). \tag{105}$$

With $t_S(T, u_{\max})$ known from (101), we have

$$R_c(T, u_{\max}) = \frac{1}{1 + e^T(2u_\alpha + 1)^{-1/u_\alpha}} \tag{106}$$

with $0 < R_c < 1/2$ for the range $R_0 < 1/2$ relevant to our discussion.

In terms of R_c, we may now differentiate the optimal control for different ranges of $R_0 < 1/2$ (for $u_{\max} > 1/2$) in the following proposition.

PROPOSITION 11. *For $R_0 < 1/2$ and $u_{\max} > 1/2$, $E(T)$ is maximized by the two-switch control*

$$u_{op}(t) = \begin{cases} 0 & (0 \le t < t_0) \\ 1/2 & (t_0 < t < t_S) \\ u_{\max} & (t_S < t \le T), \end{cases} \tag{107}$$

if $R_c < R_0$ $(< 1/2)$ and by the bang-bang control (89) *if $R_0 < R_c$, where the only switch from lower to upper corner control at t_s as t increases is given by the unique root t_{s0} of* (90) *inside the interval (t_S, t_0). The notation t_{s0} denotes a switch points from the lower corner control in $[0, t_s)$ to the upper corner control in $(t_s, T]$.*

Proof: For $R_0 \le R_c$, the bang-bang control (89) that satisfies all the necessary conditions imposed by the Maximum Principle is unique with $[E(T)]_{u=u_{op}(t)} > [E(T)]_{u=0}$. Hence, $[E(T)]_{u=u_{op}(t)}$ is maximized. In contrast, there are also admissible controls other than the two-switch control (107) in the case of $R_c < R_0 < 1/2$ that meet all the requirements of the Maximum Principle. That (107) maximizes $E(T)$ can be proved by the method of the most rapid approach (see proof of Proposition 15 and [8,22]). At this time, we only note that for $t_0 < t_S$ and the continuity requirements being met at the switch point t_0, the inclusion of a singular solution phase in $u_{op}(t)$ should be considered because it (and no other) renders the Hamiltonian stationary in (t_0, t_S):

$$\frac{\partial H}{\partial u} = R_S(t)[1 - \lambda_S(t)] = 0, \quad (t_0 < t < t_S). \tag{108}$$

That $H[u]$ is maximized can be seen from the its stationary value. For the optimal solution in the interval (t_0, t_S), we have

$$H[u_{op}(t)] = \frac{1}{4}. \tag{109}$$

Given $\lambda_S(t) = 1$, the value 1/4 is, in fact, the maximum of the remaining right-hand member of $H[u]$ $(= \lambda_S R(1 - R) = R(1 - R))$. ■

To emphasize the importance of the threshold initial population R_c, we note that an optimal conversion strategy for $R_c < R_0$ $(< 1/2)$ and $u_{\max} > 1/2$ involves a three-segment optimal control with two switches at t_0 and t_S.

The first switch point t_0 is as given by (88) and the second t_S by (101). For the first segment $[0, t_0)$, the lower corner solution, (87) and (91) (with $t_s = t_0$), applies to raise $R(t)$ to $R_S(t) = 1/2$. At the other end adjacent to T, we have the upper corner solution $\{R_{gS}, \lambda_{gS}\}\}$ as given by (99) and (100) for the interval $(t_S, T]$ to satisfy the Euler BC. These two solution segments are bridged by the singular solution $\{R_{gS}(t), \lambda_{gS}(t)\} = \{1/2, 1\}$ in the interval (t_0, t_S) that renders $H[u]$ maximum in that interval.

On the other hand, the optimal conversion strategy is given by the bang-bang control (89) if R_0 $(< 1/2) \leq R_c$. The lower corner solution is now given by (87) and (91) and the corresponding upper corner solution after switching is given by (78) and (80), respectively. The switch point t_s, which appears in both solutions for this case, is the unique positive root t_{s0} of (90). If t_s should turn out to be negative, then Corollary 1 applies with $\lambda_g(t) < 1$ for all $t \geq 0$.

At this point, we should call attention to the fact that the expression (101) for the switch point t_S (from the singular control to the upper corner control) is no longer real-valued for $u_{\max} < 1/2$. However $u_{\max} < 1/2$ is outside the range of interest for the present discussion (as it has already been treated in a previous subsection). As $u_{\max} \downarrow 1/2$ from above, the switch point $t_S \downarrow -\infty$ monotonically. It follows that for any prescribed terminal time T, there is a threshold value for $u_{\max}$ for which $t_S = 0$ so that the conversion should be at maximum rate from the start for that threshold $u_{\max}$ (and smaller).

5.2.2. The case $R_0 = 1/2$. For $R_0 = 1/2$, a stationary (singular) solution (with $\{u_S(t), R_S(t), \lambda_S(t)\} = \{1/2, 1/2, 1\}$) is possible from the start given $u_{\max} > 1/2$. Since a stationary solution that maximizes is superior (and will be proved to be so by the method of most rapid approach later), $u_{op}(t) = u_S(t) = 1/2$ $(< u_{\max})$ for $0 \leq t < t_S$ would require the upper corner solution for $t > t_S$ to satisfy the continuity condition $R_{gS}(t_S) = 1/2$ just prior to the enforcement of Proposition 8, with the switching again to take place at the last instant t_S when the required Euler BC can still be met. Hence, we have.

$$u_{op}(t) = \begin{cases} 1/2 & (0 \leq t < t_S) \\ u_{\max} & (t_S < t \leq T) \end{cases} \tag{110}$$

with the upper corner solution for $t_S < t \leq T$ again given by (99)–(101).

Note that the optimal control (110) may not be the only admissible control by the Maximum Principle. Until proven that it is not optimal, an example would be for $u_{op}(t) = u_{\max}$ for the entire host cell life span (as it also gives a positive $E(T)$).

Table 3

Variation of Switch Points and R_s with R_0 ($u_{\max} = 2$, $T = 2$)

R_0	0.1	0.15	0.20	0.25	0.289	0.30	0.35	0.40	0.45
t_0	2.197	1.735	1.386	1.099	0.901	0.847	0.619	0.406	0.201
t_{s0}	1.146	1.077	1.011	0.949	0.901	0.888	0.827	0.766	0.704
t_S	0.901	0.901	0.901	0.901	0.901	0.901	0.901	0.901	0.901
R_s	0.259	0.341	0.407	0.463	0.500	0.510	0.552	0.589	0.623

Table 4

Variation of Switch Points and R_s with R_0 ($u_{\max} = 2.5$, $T = 2$)

R_0	0.1	0.15	0.20	0.255	0.30	0.35	0.40	0.45
t_0	2.197	1.735	1.386	1.076	0.847	0.619	0.406	0.201
t_{s0}	1.448	1.384	1.322	1.076	1.205	1.147	1.088	1.028
t_S	1.076	1.076	1.076	1.076	1.076	1.076	1.076	1.076
R_s	0.320	0.413	0.484	0.500	0.588	0.629	0.669	0.696

5.3. On various threshold values

In Table 3, we report the values of the four quantities $\{t_0, t_{s0}, t_S, R_s\}$ for $u_{\max} = 2$, $T = 2$ and a range of values for R_0 in the interval (0,0.5) with the values for t_{s0} obtained numerically as the root of the switching condition (90) (obtained from (77) for $R_s = R_\ell(t_{s0})$ as given by (87)). The results in the table confirm the following:

1) the existence of a unique $R_c < 1/2$,
2) $t_S < t_0$ (thereby eliminating the possibility of a singular solution phase and leading to a bang-bang optimal control) for $R_0 < R_c$,
3) the existence of an interval (t_0, t_S) for a singular solution phase leading to the two-switch optimal control (107) for $R_0 > R_c$.

Similar calculations have also been done for other values of $u_{\max}$ and T with similar results. Table 4 reports the results for $u_{\max} = 2.5$ and $T = 2$. For this case, except for a smaller threshold value R_c (= 0.255...), how the optimal conversion strategy varies with R_0 (< 1/2) is similar to the previous case.

It should be evident from (101) that t_S is linear in T. For the two values $u_{\max}$ of Tables 3 and 4, the value of t_S for other T values is just a translational adjustment (by adding $T - 2$ to the values reported). This

Table 5
Variation of t_S and R_c with $u_{\max}$ and T

$T \setminus u_{\max}$	0.6	0.70	0.85	1.00	1.25	1.50	2.00	2.50	
2	−2.02	−1.05	−0.38	0	0.38	0.61	0.90	1.08	t_S
	0.88	0.74	0.59	0.50	0.41	0.35	0.29	0.25	R_c
4	−2.02	0.95	1.62	2	2.38	2.61	2.90	3.08	t_S
	0.51	0.28	0.17	0.12	0.09	0.07	0.05	0.04	R_c

is confirmed independently by the corresponding numerical solutions in Table 5 by comparing t_S for $T = 2$ with that of $T = 4$ for different $u_{\max}$. The nonlinear dependence of R_c on T and $u_{\max}$ can be read off the exact solution (106) and is also shown numerically for $T = 2$ and 4 and a range of $u_{\max}$ in Table 5. Note that only the portion of the results with $t_S \geq 0$ (and $R_c \leq 1/2$) in the table is relevant to the problem of this section.

We do not have an explicit solution for the switch point t_{s0} (for $R_0 < R_c$), but the following lemma provides some information about its dependence on T.

LEMMA 4. *For $R_0 < 1/2$, the switch point t_{s0} is an increasing function of the terminal time T.*

Proof: Upon differentiating (90) with respect to T, we obtain

$$\frac{dt_{s0}}{dT} = \frac{(1 - R_0)e^{-u_\alpha(T - t_{s0})}}{u_{\max}(1 - R_0 + R_0 e^{t_{s0}})} > 0. \tag{111}$$

■

How t_{s0} varies with $u_{\max}$ is a little more complicated. Upon differentiating both sides of (90) with respect to $u_\alpha = u_{\max} - 1$, we obtain the following lemma on t_{s0} being an increasing function of $u_{\max}$ independent of the sign of $u_\alpha = u_{\max} - 1$.

LEMMA 5. *For $R_0 < 1/2$ (and hence $u_{\max} > 1$ for the lower corner control to be optimal in $[0, t_s)$), the switch point t_{s0} is an increasing function of $u_{\max}$ if T is sufficiently large.*

Proof: Routine calculations from differentiating both sides of (90) with respect to $u_{\max}$ lead to

$$\frac{dt_{s0}}{du_{\max}} = \frac{1}{u_\alpha} \frac{(1 - R_0)(T - t_{s0})e^{u_\alpha(T - t_{s0})} - (1 - R_0) - R_0 e^{t_{s0}}}{(1 - R_0)e^{u_\alpha(T - t_{s0})} + R_0 e^{t_{s0}}}$$

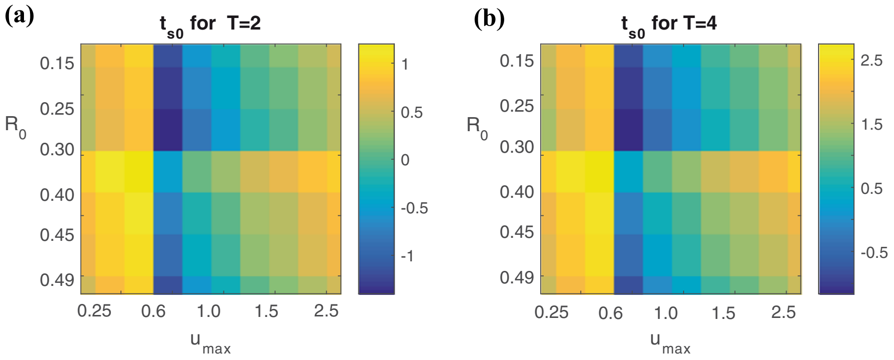

Figure 6. Variation of t_{s0} with $u_{\max}$ and R_0: (a) $T = 2$ and (b) $T = 4$.

$$\sim \begin{cases} \frac{1}{u_\alpha}(T - t_{s0}) > 0 & (u_\alpha > 0) \\ -\frac{1}{u_\alpha R_0}\left\{(1 - R_0)e^{-t_{s0}} + R_0\right\} > 0 & (u_\alpha < 0). \end{cases}$$

■

That t_{s0} is an increasing function of $u_{\max}$ is already evident from the values of t_{s0} in Tables 3 and 4 with a rather modest T magnitude of 2 (for the range $R_0 < 1/2$ where the lower corner control is optimal). Figure 6 provides additional support for this behavior for $T = 2$ and $T = 4$, respectively, for a range of $R_0 < 1/2$. It should be noted that the results for t_{s0} are relevant only to the problem for $R_0 < R_c$ with R_c given in terms of $u_{\max}$ and T by (106) (see also Table 5). Also much of the lower corner part of the two heat maps (in dark yellow) is not relevant because a singular solution phase exist in a finite subinterval in these cases. Even when there is no singular solution phase for $R_0 < R_c$, the switch point t_{s0} may also not be relevant for the problem. Cases with $t_{s0} < 0$ correspond to $\lambda_{g0}(t) < 1$ for all t in $[0, T]$ so that Corollary 1 applies throughout the host cell's life span.

5.4. *Summary for* $R_0 \leq 1/2$

The lower corner solution $\{R_\ell(t), \lambda_\ell(t)\}$ induced by the lower corner control $u_\ell(t) = 0$ has the distinct ability to increase the RB population from $R_0 \leq 1/2$ to the level of the stationary solution $\{R_S(t), \lambda_S(t)\} = \{1/2, 1\}$ at some instant $t_0 > 0$ (and even larger $R(t)$ for $t > t_0$). Once the stationary RB population 1/2 is reached, a stationary solution that maximizes is to be sustained if possible because it is superior to a corner solution. As a consequence, the optimal conversion strategy for $R_0 \leq 1/2$ will be dictated by whether the singular control can be sustained. A simple case of (the host cells) inability to have a segment of singular control is $u_{\max} \leq 1/2$ as shown in Subsection 5.1.

By Proposition 8, the optimal control $u_{op}(t)$ either cannot be $u_S(t) = 1/2$ or cannot remain a singular control and must switch to the upper corner control at some instant $t_S > t_0$ prior to the terminal time T to satisfy the required Euler BC. To the extent that the stationary solution is preferred, the switch from the singular control (if present) should be as late as possible but still enable the adjoint function to vanish at terminal time. This upper corner solution has been designated as $\{R_{gS}(t), \lambda_{gS}(t)\}$. Continuity of $R(t)$ and $\lambda(t)$ requires $\{R_{gS}(t_S), \lambda_{gS}(t_S)\} = \{1/2, 1\}$ in addition to the Euler BC $\lambda_{gS}(T) = 0$. This BVP and its solution are given by (96)–(98) and (99)–(101), respectively.

A more complete classification of possible optimal conversion strategies for $R_0 \leq 1/2$ is given in terms of a threshold initial RB population $R_c(T, u_{\max})$ defined by the conditions $t_S = t_0$ and $R_\ell(t_0) = R_{gS}(t_S) = 1/2$. Depending on the magnitude of the initial infecting population R_0 ($\leq 1/2$) relative to R_c, we have the following two qualitatively different optimal conversion strategies.

5.4.1 Case I: $0 < R_0 \leq R_c$

For this range of R_0, we have $t_S \leq t_0$. It is not possible for $u_{op}(t)$ to have a finite time interval of singular control. The optimal conversion strategy is given by the bang-bang control (89) of Proposition 10 where the switch point t_s is the unique positive root of (1) and $R_g(t_s) = (R_s =)\ R_\ell(t_s) < 1/2$. The corresponding RB population and adjoint function are given by (87) and (84) for $0 \leq t \leq t_s$ and by (78) and (80) for $t_s \leq t \leq T$. There would be no switch if the root t_s is negative and upper corner control applies throughout $[0, T]$ (see Corollary 1). (Note that the special case of $u_{\max} < 1/2$ may be regarded as $t_0 = \infty$.)

5.4.2 Case II: $R_c < R_0 < 1/2$

For this range of R_0, we have $t_0 < t_S$ and the optimal conversion strategy is given by Proposition 11 with a two-switch control (107): starting with the lower corner control $u_\ell(t) = 0$, switching to the singular control $u_S(t) = 1/2$ at t_0 as given by (88) and then to the upper corner control $u_g(t) = u_{\max}$ at the switch point t_S given by (101). The corresponding RB populations and adjoint functions are given by the lower corner solution (87) and (91) (with $t_s = t_0$) in $[0, t_0)$, by the stationary solution $\{R_S(t), \lambda_S(t)\} = \{1/2, 1\}$ in (t_0, t_S) and by the upper corner solution (99)–(101) in $(t_S, T]$.

6. Optimal conversion strategy for $R_0 > 1/2$

6.1. Upper corner control for $1 - u_{\max} \geq R_0$ $(> 1/2)$

We know from Proposition 9 that the lower corner control is not appropriate near the starting time for $R_0 > 1/2$. Neither is the singular solution because $R_0 > 1/2 \neq R_S(t)$. We must then examine the only other option of an upper corner control at the early stage of the host cell's life span, the interval $[0, t_s)$, or some subinterval of it.

Let $R_{gm}(t)$ and $\lambda_{gm}(t)$ be $R(t)$ and $\lambda(t)$ generated by $u = u_{\max}$ for t adjacent to the starting time with the change of the RB population in time governed by the IVP

$$R'_{gm} = R_{gm}(1 - u_{\max} - R_{gm}), \quad R_{gm}(0) = R_0 \ (> 1/2). \tag{112}$$

Its exact solution is

$$R_{gm}(t) = \frac{u_\alpha R_0}{(u_\alpha + R_0)e^{u_\alpha t} - R_0}. \tag{113}$$

In this section, we focus on the range $1 - u_{\max} \geq R_0$ and show that the optimal strategy is to convert at maximum rate from the start.

6.1.1. The $1 - u_{\max} = R_0$ *case.* For the very special value of $u_{\max} = 1 - R_0 < 1/2$, we see from (112) that $R_{gm}(t) = R_0$ for all t. The corresponding adjoint function that satisfies the Euler BC is determined by

$$\lambda'_{gm} = \lambda_{gm} R_0 - u_{\max}, \quad \lambda_{gm}(T) = 0, \tag{114}$$

or

$$\lambda_{gm}(t) = u_{\max}\left\{1 - e^{R_0(t-T)}\right\}. \tag{115}$$

With $u_{\max} = 1 - R_0 < 1/2$, we have $0 < \lambda_{gm}(t) < 1$ in $[0, T)$. Hence, the optimal control for this special case is $u_{op}(t) = u_{\max}$.

6.1.2. The $1 - u_{\max} > R_0$ *case.* For the broader range $u_{\max} < 1 - R_0 < 1/2$, we see from (112) that $R'_{gm}(t) > 0$ so that $R_{gm}(t) > R_0 > 1/2$ and tends to $1 - u_{\max} > R_0 > 1/2$ from below. The corresponding adjoint function that satisfies the Euler BC is determined by

$$\lambda'_{gm} = -(1 - u_{\max} - 2R_{gm})\lambda_{gm} - u_{\max}, \quad \lambda_{gm}(T) = 0. \tag{116}$$

We need only to ensure that $\lambda_{gm}(t) < 1$ for $0 \leq t < T$ for $u_{op}(t) = u_{\max}$ to be optimal. For this purpose, suppose the maximum of $\lambda_{gm}(t)$ is attained at t_m with $\lambda'_{gm}(t_m) = 0$. It follows from the adjoint ODE

$$\lambda_{gm}(t_m) = \frac{u_{\max}}{2R_{gm}(t_m) - 1 + u_{\max}} < 1 \tag{117}$$

since $R_{gm}(t_m) > 1/2$.

Altogether, these observations lead to the following optimal solution for the range $1 - u_{\max} \geq R_0$ ($> 1/2$).

Proposition 12. *For* $1 - u_{\max} \geq R_0 > 1/2$ *(or* $u_{\max} \leq 1 - R_0 < 1/2$*), the control* $u_{op}(t) = u_{\max}$ ($< 1/2$) *for all* t *in* $[0, T]$ *maximizes* $E(T)$.

Proof: Since $u_{op}(t) = u_{\max}$ is the only control that satisfies all necessary conditions for optimality required by the Maximum Principle and is superior to the lower corner control, it maximizes $E(T)$. ■

6.2. *Optimal conversion strategies for* $1 - u_{\max} < R_0$

For the complementary range $u_{\max} > 1 - R_0$ ($> 1/2$), we have from (112) $R'_{gm} < 0$ so that $R_{gm}(t) < R_0$ is a monotone decreasing function that tends to $1 - u_{\max} < R_0$ from above. As the limit population $1 - u_{\max}$ may be below $R_S(t) = 1/2$, we need to examine separately the two different possibilities of having and not having a stationary solution component for the optimal control $u_{op}(t)$.

6.2.1. The $1 - R_0 < u_{\max} \leq 1/2$ *case.* For this range of $u_{\max}$ (which is a part of the range $1 - u_{\max} < R_0$), we have ($R'_{gm} < 0$ as noted above giving) $R_{gm}(t) \downarrow 1 - u_{\max} \geq 1/2$ from above so that $R_{gm}(t) > 1/2$ for any finite t. The RB population does not reach the singular solution $R_S(t) = 1/2$. The only conversion rate not ruled out is with $u(t) = u_{\max}$ for all t in $[0, T]$. To see if it is optimal, we again examine the maximum of $\lambda_{gm}(t)$ attained at t_m with $\lambda'_{gm}(t_m) = 0$. It follows from the adjoint ODE

$$\lambda_{gm}(t_m) = \frac{u_{\max}}{2R_{gm}(t_m) - 1 + u_{\max}}. \tag{118}$$

Since $R_{gm}(t)$ tends to $1 - u_{\max}$ from above, we get from the exact expression above

$$\lambda_{gm}(t_m) < \frac{u_{\max}}{1 - u_{\max}} \leq 1, \tag{119}$$

given $0 < u_{\max} \leq 1/2$. Hence, $u_{op}(t) = u_{\max}$ is optimal by Maximum Principle and the only one that meets all the necessary conditions required by the principle. With $[E(T)]_{u_{op}(t)=u_{\max}} > [E(T)]_{u_\ell(t)=0} = 0$, we have

Proposition 13. *For* $u_{\max} \leq 1/2$ (*and* $R_0 > 1/2$)*, the control* $u_{op}(t) = u_{\max}$ *from the start is optimal and maximizes* $E(T)$.

6.2.2. The $u_{\max} > 1/2$ case. For this complementary range of $u_{\max}$, we have again $R'_{gm} < 0$ but now the monotone decreasing function $R_{gm}(t)$ tends to $1 - u_{\max} < 1/2$ from above. Hence, it is possible to have a subinterval of stationary solution, depending on the relative magnitude of the instant t_x when $R_{gm}(t)$ (see (112) and (113)) reaches $R_S(t) = 1/2$ and the instant t_S when $R_{gS}(t)$ (as previously defined by the BVP (96)–(98)) is equal to 1/2. With the exact expression (113) for $R_{gm}(t)$, the condition

$$R_{gm}(t_x) = \frac{1}{2} \tag{120}$$

defining t_x gives

$$t_x = \frac{1}{u_\alpha} \ln\left(\frac{R_0(2u_\alpha + 1)}{u_\alpha + R_0}\right). \tag{121}$$

Similar to the case of $R_c < R_0 \le 1/2$ where there would be no stationary solution component if $t_0 > t_S$, we have the following analogous proposition for $u_{\max} > 1/2$ (and $R_0 > 1/2$).

PROPOSITION 14. *For $u_{\max} > 1/2$ (and $R_0 > 1/2$), $E(T)$ is maximized by the optimal control $u_{op}(t) = u_{\max}$ for all t in $[0, T]$ if $t_x \ge t_S$.*

Proof: When $t_S \le t_x$, the maximum allowable control $u_{\max}$ is insufficient for reducing the initial RB population fast enough to get to the level of the singular solution $R_S(t) = 1/2$ before the adjoint function should leave its singular value of $\lambda_S(t) = 1$ to head toward the Euler BC $\lambda(T) = 0$ required by the Maximum Principle. In that case, the upper corner control would apply throughout the host cell's life span.

To show that it is optimal, i.e., it maximizes the Hamiltonian, we note that $R_{gm}(t)$, with $u_{op}(t) = u_{\max}$ for all t in $[0, T]$, is monotone decreasing (since $u_{\max} > 1/2$) and tends to $1 - u_{\max} < 1/2$ from above as $t \to \infty$. Concurrently, the corresponding adjoint function λ_{gm} (required to satisfy the Euler BC) is determined by (116) to be monotone decreasing in some interval adjacent to T. Suppose again $\lambda_{gm}(t)$ attains a maximum at t_m prior to declining to zero at T. In that case, $\lambda'_{gm}(t_m) = 0$ and

$$\lambda_{gm}(t_m) = \frac{u_{\max}}{2R_{gm}(t_m) - (1 - u_{\max})}. \tag{122}$$

Since $t_m < t_x$ (otherwise we would have a solution for $\{R_{gS}, \lambda_{gS}\}$ with $\lambda_{gS}(t_S) = 1$ for some $t_S > t_x$ given $\lambda_{gm}(t_m) > 1$), we must have $R_{gm}(t_m) > R_{gm}(t_x) = 1/2$. It follows that

$$\lambda_{gm}(t_m) < \frac{u_{\max}}{1 - (1 - u_{\max})} = 1 \tag{123}$$

and $u_{op}(t) = u_{\max}$ for all t in $[0, T]$ is optimal by the Maximum Principle.

Now $u_{op}(t) = u_{\max}$ is the only available optimal control. With $[E(T)]_{u_{op}(t)=u_{\max}} > [E(T)]_{u_\ell(t)=0} = 0$, $u_{op}(t) = u_{\max}$ maximizes $E(T)$. ■

On the other hand, the optimal conversion strategy would be quite different if $t_x < t_S$.

PROPOSITION 15. *For* $u_{\max} > 1/2$ *(and* $R_0 > 1/2$*), the two-switch control*

$$u_{op}(t) = \begin{cases} u_{\max} & (0 \leq t < t_x) \\ 1/2 & (t_x < t < t_S) \\ u_{\max} & (t_S < t \leq T) \end{cases} \tag{124}$$

maximizes $E(T)$ *if* $t_x < t_S$.

Proof: With t_x being the first instant for $R_{gm}(t)$ to reach $R_S(t) = 1/2$ and t_S $(> t_x)$ the latest time for $\lambda_{gS}(t)$ to leave $\lambda_S(t) = 1$ and still satisfy the Euler BC, we take $u_{op}(t)$ to be the two switch control (124) and prove below that it maximizes $E(T)$ by the method of the most rapid approach. Our goal is to show that

$$\begin{aligned} &[E(T)]_{u=u_{op}(t)} - [E(T)]_{u=u_c(t)} \\ &= \int_0^T u_{op}(t) R_{op}(t) dt - \int_0^T u_c(t) R_c(t) dt \\ &= \int_0^T \left\{ [(1-R)R - R']_{u_{op(t)}} - [(1-R)R - R']_{u_c(t)} \right\} dt > 0, \end{aligned}$$

where $u_C(t)$ is any admissible comparison control for which the corresponding $R_C(t)$ has the same initial and terminal values as shown in Fig. 7. Note that starting from the same initial population R_0, the $R_C(t)$ resulting from any comparison control (decreases with time and) can only reach the singular value $1/2$ at some instance $t_D > t_x$ (and not sooner) because $R_{op}(t)$ approaches the singular solution most rapidly with $u_{op}(t) = u_{\max} \geq u_C(t)$ for the interval $[0, t_x]$. Similarly, $R_C(t)$ can only leave the singular solution (and descend toward the terminal value $R(T)$) not later than $R_{op}(t)$ so that $t_D \leq t_{Sx}$.

The key to the our proof is to visualize the two graphs $R_{op}(t)$ and $R_C(t)$ as contours in the $R - t$ plane and the integral as line integral written as

$$\begin{aligned} &[E(T)]_{u=u_{op}(t)} - [E(T)]_{u=u_C(t)} \\ &= \int_{C_{op}} [(1-R)R dt - dR] - \int_{C_u} [(1-R)R dt - dR] \\ &= \int_{C_{op}} [G(t,R) dt + H(t,R) dR] - \int_{C_u} [G(t,R) dt + H(t,R) dR], \end{aligned}$$

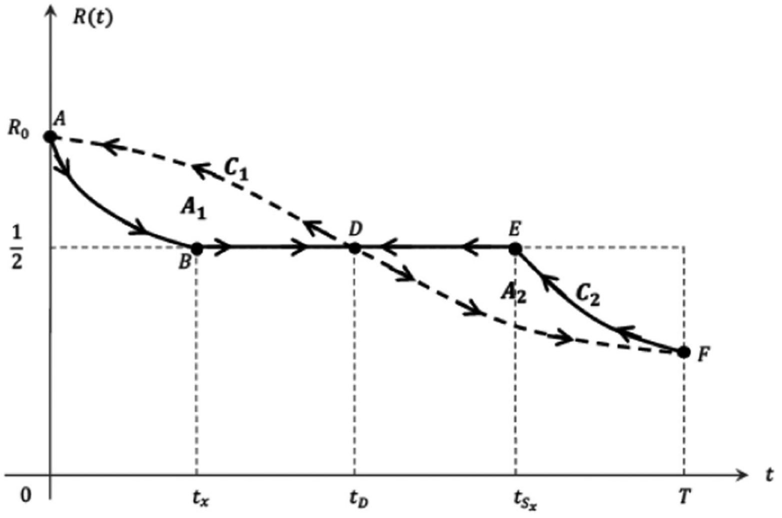

Figure 7. The optimal RB contour for the method of most rapid approach.

where $G = (1 - R)R$ and $H = -1$. The two contours C_{op} and C_u are equivalent to the union of two closed contours C_1 and C_2. We now consider each of the two closed subcontours C_1 and C_2 separately: For the loop integral around C_1, the closed contour is traversed counterclockwise from point A, to point B to the point D and back to the point A. The second loop integral around C_2 is traversed counterclockwise from point D, to E to F and back to D (see Fig. 7).

The first loop integral around C_1 may be written with the help of Green's theorem for the $t - R$ plane as

$$\begin{aligned}\oint_{C_1}[G(t,R)dt + H(t,R)dR] &= \int\int_{A_1}\left[\frac{\partial H}{\partial t} - \frac{\partial G}{\partial R}\right]dtdR\\ &= \int\int_{A_1}(2R-1)dtdR > 0\end{aligned}$$

since we have $R(t) > 1/2$ for the region enclosed by C_1. Similarly, the second loop integral around C_2

$$\begin{aligned}\oint_{C_2}[G(t,R)dt + H(t,R)dR] &= \int\int_{A_2}\left[\frac{\partial H}{\partial t} - \frac{\partial G}{\partial R}\right]dtdR\\ &= \int\int_{A_2}(2R-1)dtdR < 0\end{aligned}$$

since we have $R(t) < 1/2$ for the region enclosed by C_2. With

$$
\begin{aligned}
&\int_{C_{op}} [Gdt + HdR] - \int_{C_u} [Gdt + HdR] \\
&= \oint_{C_1} [Gdt + HdR] - \oint_{C_2} [Gdt + HdR] \\
&= \int\int_{A_1} (2R-1)dtdR - \int\int_{A_2} (2R-1)dtdR > 0,
\end{aligned}
$$

we have

$$[E(T)]_{u=u_{op}(t)} - [E(T)]_{u=u_C(t)} > 0. \tag{125}$$

The same method of proof applies to any other admissible control $u_C(t)$ with the corresponding RB population having the same two end values (see [8,22]). Hence, $E(T)$ is maximized by $u_{op}(t)$. ■

Remark 5. That any admissible control must start with the same initial RB population R_0 is dictated by the initial condition for $R(t)$. At the terminal point where $R(T)$ is not prescribed, any admissible control $u_C(t)$ should also end with the terminal RB population corresponding to the optimal control $u_{op}(t)$. Obviously, the comparison control $u_C(t)$ should not leave behind more RB than $u_{op}(t)$. At the same time, $R_C(T)$ should not be less than $R_{op}(T)$ as it would leave the singular (stationary) solution earlier than necessary to get to a lower end population. A control that leads to a shorter interval of stationary solution is inferior to one with a longer interval assuming all other requirements of the Maximum Principle are met by both.

6.3. *The relative magnitude of t_x and t_S*

Given the importance of the relative magnitude of t_x and t_S in determining the optimal conversion strategy for $u_{\max} > 1/2$ (and $R_0 > 1/2$), we establish in this subsection some quantitative measures to elucidate this relative magnitude. As in the case of relative magnitude of t_0 and t_S for $R_c < R_0 \le 1/2$, an important threshold relation for the present problem is the combination of parameter values that leads to $t_x = t_S$.

For $u(t) = u_{\max}$ ($> 1/2$), the exact solution of the IVP (112) is

$$R_{gm}(t) = \begin{cases} u_\alpha R_0 e^{-u_\alpha t}/(u_\alpha + R_0 - R_0 e^{-u_\alpha t}) & (u_{\max} \ne 1) \\ R_0/(1 + R_0 t) & (u_{\max} = 1) \end{cases}. \tag{126}$$

The condition $R_{gm}(t_x) = 1/2$ gives the following exact dependence of t_x on R_0 and $u_{\max}$:

$$t_x = \begin{cases} \ln(R_0(2u_\alpha + 1)/(u_\alpha + R_0))/u_\alpha & (u_{\max} \ne 1) \\ (2R_0 - 1)/R_0 & (u_{\max} = 1) \end{cases}. \tag{127}$$

Note that t_x is well defined and nonnegative for all $R_0 > 1/2$ (and $2u_{\max} > 1$) with

$$[t_x]_{R_0=1/2} = 0, \tag{128}$$

as it should be, while

$$[t_x]_{R_0=1} = \begin{cases} \ln((2u_{\max} - 1)/u_{\max})/u_\alpha > 0 & (u_{\max} \neq 1) \\ 1 & (u_{\max} = 1) \end{cases}. \tag{129}$$

The existence of a finite positive t_x for a range of $R_0 > 1/2$ when $u_{\max} > 1/2$ gives rise to the possibility of a switch of control to the singular control $u_S(t) = 1/2$ associated with the stationary solution $\{R_S(t), \lambda_S(t)\} = \{1/2, 1\}$. To ensure the switching is to the singular solution when it is possible to do so at t_x (in the range $u_{\max} > 1/2$), we need the corresponding adjoint function, denoted by $\lambda_{gx}(t)$, to be the solution of the terminal value problem

$$\lambda'_{gx} = -(1 - u_{\max} - 2R_{gm})\lambda_{gx} - u_{\max}, \quad \lambda_{gx}(t_x) = 1 \tag{130}$$

or

$$\lambda_{gx}(t) = \frac{u_\alpha + R_0 - R_0 e^{-u_\alpha t}}{u_\alpha + R_0 - R_0 e^{-u_\alpha t_x}} e^{u_\alpha (t - t_x)}. \tag{131}$$

While the singular (stationary) solution may be preferred, the Euler BC $\lambda(T) = 0$ requires the optimal strategy to switch to the upper corner control at an opportune time t_S to convert as much RB as possible thereafter and reduce the (shadow) worth (measured by the adjoint function λ) of any remaining RB population to zero by the time the host cell dies. For that requirement, we again work with $R_{gS}(t)$ and $\lambda_{gS}(t)$ previously defined by (99)–(101). In particular, the second switch point t_S is given by (101) to be

$$t_S = T - \frac{1}{u_\alpha} \ln(2u_\alpha + 1). \tag{132}$$

The critical relation $t_S > t_x$ needed for the two switch control (124) to be optimal is met by

$$T > \frac{1}{u_\alpha} \ln\left(\frac{R_0(2u_\alpha + 1)^2}{u_\alpha + R_0}\right) \equiv T_0. \tag{133}$$

PROPOSITION 16. *For a prescribed combination of* R_0 *and* $u_{\max}$, *the two-switch control* (124) *maximizes* $E(T)$ *if* $T > T_0$. *Proposition 13 applies if* $T \leq T_0$ *(so that* $t_x \geq t_S$*).*

For the special case of $u_{\max} = 1$, the value of T_0 may be obtained by taking the limit of (133) as $u_\alpha \to 0$ to get

$$T_0 = 2 - \frac{1}{R_0}(u_{\max} = 1). \tag{134}$$

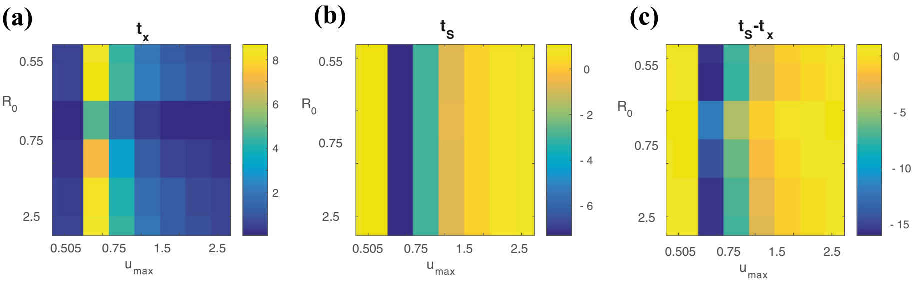

Figure 8. Heat maps for t_x and t_S with $T = 2$.

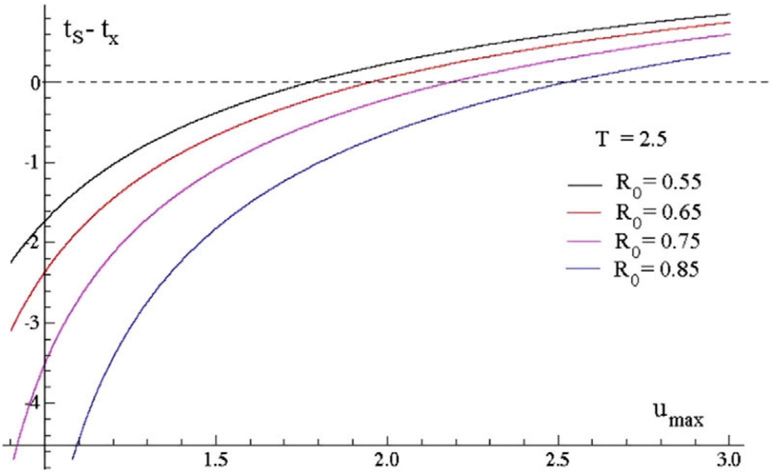

Figure 9. $t_S - t_x$ for $T = 2.5$.

Results showing how t_x and the previously calculated t_S vary with R_0 and $u_{\max}$ can be found in the two heat maps in Fig. 8.

Note that t_x as given by (120) is independent of T while the dependence of t_S on T as given by (101) is linear and additive so that its value for a different T is obtained simply by adding $T - 2$ to the relevant value in Fig. 6. Graphs of $t_S - t_x$ are plotted in Fig. 9 as functions of $u_{\max}$ for several values of R_0 with $T = 2.5$. We see from these graphs that $E(T)$ is maximized by the two-switch control (124) only for the deep blue region of the t_x heat map.

6.4. Summary of the optimal conversion strategy for $R_0 > 1/2$

For a fixed pair of $u_{\max}$ and T, the optimal conversion strategy for $R_0 > 1/2$ again depends on whether the constitution of the host cell can support a period of stationary solution that maximizes the Hamiltonian.

However, unlike $R_0 \leq 1/2$, the appropriate control depends principally on the magnitude of $u_{\max}$.

6.4.1 Case III: The $u_{\max} \leq 1/2$ *case*

For this range of $u_{\max}$, $R_{gm}(t) > 1/2$ does not reach the stationary solution for any finite t. Proposition 12 applies with the optimal control $u_{op}(t) = u_{\max}$ for all t in $[0, T]$. The resulting optimal conversion strategy is consistent with intuition when converting at maximum rate from the start still increases the RB population closer to the carrying capacity (since the normalized natural growth rate constant α $(= 1)$ exceeds the maximum conversion rate constant $u_{\max}(\leq 1)$.

6.4.2 Case IV: The $u_{\max} > 1/2$ *case*

For this complementary range of $u_{\max}$, the optimal conversion strategy depends on the relative magnitude of t_x and t_S in a way similar to the relative magnitude of t_0 and t_S.

For the range $t_x < t_S$, a two-switch control $(u_{\max} - u_S - u_{\max})$ given by (124) with a segment of stationary solution is proved to maximize $E(T)$ by the method of most rapid approach (see Proposition 15). The corresponding RB population and adjoint function defined by (126) and (131) for $0 \leq t \leq t_x$ and by (99) and (100) for $t_S \leq t \leq T$. The two switch points t_x and t_S are determined by (127) and (101), respectively. The two end segments are bridged by an interval (t_x, t_S) of stationary solution.

For the range $t_S \leq t_x$, the host cell life span is too short for conversion at maximum capacity to drive the RB population down to the singular level early enough for a segment of stationary solution. Proposition 13 applies with $u_{op}(t) = u_{\max}$ for all t in $[0, T]$ since $T \leq T_0$ for this case (see also (133)).

Remark 6. Beyond satisfying all the necessary conditions for optimality imposed by the Maximum Principle, the relevant optimal control is shown to maximize $E(T)$ in all cases some less trivially by the method of most rapid approach.

7. Concluding remarks

Given the simple exponential RB growth model of Section 2, the solution for our constrained optimization problem shows that when the host cell life span is sufficiently long, it would be in the Chlamydia bacteria's best interest (for maximum spread of infection) to delay conversion. This theoretical conclusion is consistent with intuition and conventional expectation and provides us with their mathematical justification. As significant as it may be in putting the conventional wisdom on a theoretical foundation, a more refined model with finite carrying capacity shows that the optimal strategy for achieving the same objective may not always be bang-bang of the form (89). For a relatively large initial Chlamydia population of the order of the carrying capacity (a rather rare event in practice), the optimal strategy would be to convert immediately at the maximum allowable rate.

How do the two possible biological mechanisms that seem so plausible for implementing the bang-bang control of the simpler model (see Section 2.8) play out for this alternative strategy? If only those RBs not in contact with the inclusion membrane should convert, the new strategy (of converting from the start) may be implemented immediately when the initial infecting RB population is (in rare cases) sufficiently large to have a portion of them away from the inclusion membrane (which is consistent with the theoretical requirement of a large R_0 for that strategy). In contrast, the other mechanism (that converts only after several rounds of division) would not be consistent with an immediate conversion strategy. However, it is possible that the second mechanism may be operating concurrently with the first.

Under the heading of other possible biological mechanisms not discussed explicitly earlier at the end of Section 2, one is based on the substantial difference in size between the RB and EB form of *C. trachomatis*. When a large RB population consumes all available space inside an inclusion, one way to create more space for further division would be for some RB to convert to considerably smaller EB to free up space for the remaining RB to proliferate. This “crowding” induced conversion can take place either from the start when R_0 is large or at a later stage after many rounds of division. It thus offers still another possible biological mechanism for implementing either theoretically optimal strategy.

As long as our models for the constrained maximization of the terminal EB population are linear in the “conversion rate constant” $u(t)$, there appears to be one unrealistic feature common among all possible optimal strategies from these models. When there is a switch from one temporally uniform control to another, the switch would be instantaneous and discontinuous (or bang-bang for brevity). Now an abrupt (discontinuous) change can take place when the switches are implemented by the biological mechanisms described previously and not inconsistent with the data from the experiments of [11]. For example, if conversion should take place only after many divisions of the initial infecting RB population and a new generation of RB becomes sufficiently small, then the conversion rate constant (the control) $u(t)$ would be zero during the first several divisions and switch to some finite value (instantaneously) only when a (sizable) group of later generation

RB reaches a critically small size. That is certainly a bang-bang switch because a whole generation of RB would divide and switch pretty much at the same time. In other words, the theoretical occurrence of a bang-bang control is not inconsistent with the biology of *C. trachomatis*.

What may seem problematic, however, is the fact that the various finite jump switches in the theoretical optimal strategies from these models can only be between the two corner controls (0 to $u_{\max}$) or between a corner control and the singular control (0 to $1/2$ or $1/2$ to $u_{\max}$). A switch of 0 to $1/2$ (the singular control normalized by the RB growth rate constant α) may not be realistic even if nonsmoothness in the change in conversion rate is not an issue. A casual examination of the experimental data reported in [11] seems not to support such "large" abrupt changes. In other words, the issue is not whether a bang-bang strategy is consistent with the experimental data. Not only it could be, a bang-bang strategy is also compatible with the known plausible biological processes of conversion of *C. trachomatis* discussed in earlier sections of this paper. As delineated, the issue arises from the apparent "large" magnitude (relative to the experimental data) of the conversion rate change at the switch, not its discontinuity or abruptness. In particular, a switch from 0 to $1/2$ does not appear to be supported by the experimental evidence reported in [11]. For that perceived incompatibility, a possible resolution depends on the actual biological process(es) underlying the *C. trachomatis* life cycle.

Since the models investigated do not specify the value of $u_{\max}$, they implicitly leave its specification (hence its magnitude) to the investigators based on what is known about the bacteria. The experimental data obtained in [11] are precisely what is needed for estimating $u_{\max}$. There remains the question: What if the actual $u_{\max}$ estimated from the data should result in a normalized $u_{\max}$ smaller than $1/2$? This situation has already arisen earlier in Section 5.1 and has been addressed: it would eliminate the option of a singular solution phase in the optimal strategy in that case. Altogether then, a bang-bang control with $u_{\max}$ determined from available data should not be incompatible with the findings reported in [11].

There is still the possibility that the plausible biological processes discussed herein should prove not to be responsible for the Chlamydia life cycle, and the actual biological mechanism for implementing the conversion from RB to EB involves a gradual and smooth transition from proliferation to conversion (with the discontinuous changes in the experimental data resulting from limitations of measurement techniques). In that case, a bang-bang control would be truly inappropriate and the models that led to such an optimal conversion strategy would then require modifications to incorporate features of the actual biological processes responsible for the Chlamydia life cycle (once we know what they entail). While we cannot completely eliminate this possibility, all indications from the experimental data in [11] point to an optimal strategy for maximizing the spread of the infectious *C. trachomatis* bacteria with one or more discontinuous switches (see [11] for a more detailed discussion on this assertion).

Even when a bang-bang optimal conversion strategy is consistent with the underlying biological processes for the *C. trachomatis* life cycle, the models investigated here may still need further refinement to adjust for a number of factors influencing its spread not considered in our models. These include

- the generally expected noisy environment internal to and outside the host cell that creates a natural variability in the timing and/or rate of differentiation [9];
- the mammalian inclusions for the *C. trachomatis* bacteria changing size over time leading to a *time-dependent* carrying capacity that may modify the transition from proliferation to conversion;
- the interplay between population size and the time of host cell death as a large Chlamydial population can actively cause the host to lyse (sooner) and spread the infection to other cells.

Notwithstanding model improvement to include the effect of these and other factors such as stochastic effects [8], the theoretical results from the models investigated herein form an intrinsically important cornerstone of the theoretical foundation for our understanding of the Darwinian evolution and survival of *C. trachomatis* bacteria.

Acknowledgments

The research leading to this paper was supported in part by NSF (UBM Grant) DMS-1129008 and NIH (Center Grant) P50-GM076516. The research of F.Y.M. Wan was also supported in part by NIH (Joint NSF/NIGMS Initiative Award in the Area of Mathematical Biology) R01GM067247. The research of G. A. Enciso was also supported by NIH Grant R21AI04419815.

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(Received November 29, 2016)