Validation of a Mathematical Model Describing the Dynamics of Chemotherapy for Chronic Lymphocytic Leukemia In Vivo

Alessia Guadagnin Pattaro[1], Giovanni Plazzotta[1], Annarita Zanon[1]

Abstract
To be added

[1] *Master's degree in Quantitative and Computational Biology, University of Trento*

1. Introduction

1.1 Chronic Lymphocytic Leukemia

In the context of personalized medicine, the field of medicine aiming at developing tailored cures for each patient, quantitative analysis can be employed to investigate whether the duration of therapy could be reduced without increasing the risk of relapse, or to compare the efficacy of different protocols[1]. In this sense, there has been an increasing use of mathematical models to study the dynamics of blood cancers under the influence of small molecule drugs and/or immunotherapy. Yet, current models have several important limitations:

1. They are built upon simulation data rather than real-life experiments.
2. They do not take into account the variability of tumor cell growth rate between patients.
3. They led to conflicting results between optimal drug doses determined by *in vitro* vs. *in vivo*.

The study presented[2] is focused on chronic lymphocytic leukemia (CLL), the most common type of blood cancer in the Western world. It involves an accumulation of lymphocytes B in secondary lymphoid organs, spleen, peripheal blood, and bone marrow.[3, 4] There is no known cause for this disease even if it is suspected to have a genetic basis. Mutations in *IGHV* (immunoglobulin heavy variable) genes are thought to help distinguishing different types of clinical behaviours of CLL[5].
The paper aims to address two issues regarding CLL treatment: the introduction of small-molecule drugs such as the *Bruton tyrosine kinase* BTK inhibitor *Ibrutinib* (Ibr) and its derivatives has transformed CLL therapy and contributed to extend the overall survival of patients. Nonetheless, patients can develop drug resistance and suffer from toxic side effects, and the disease remains incurable. Therefore, improvements in mathematical models are needed to assist clinicians in the design of more effective treatment protocols.
To build this model, the growth rate of murine A20 leukemic cells in immuno-competent Balb/c mice was determined. This allowed to formulate the logistic dynamic of these cancer cells. Then, experiments were conducted *in vivo* to compare the cytotoxicity of two drugs (the topoisomerase inhibitor *Cytarabine* Cyt and Ibr) against leukemic cells. Doses were selected by reviewing the literature on the use of these drugs in *in vivo* models. The results allowed to calculate the killing rate of A20 cells as a function of therapy. Experimental data were compared with the simulation model to validate the latter's applicability. Since this model is based on *in vivo* experiments it is more relevant to real-life situations.

2. Materials and methods

To measure the growth rate of mCherry A20 cells, 20 mice were inoculated via the tail vein with 5×10^4 logarithmic phase cells in PBS. A20 cells begin to proliferate and appear in blood in 2-3 weeks after inoculation. On Days 16 and 22 after inoculation and prior to initiation of drug therapy, blood was taken from each mouse, and mCherry A20 cells were measured. Treatments were derived by published protocols in which Cyt and Ibr had been used *in vivo*. The 20 inoculated mice were divided into 5 groups:

1. Control group, which only received PBS,
2. Cyt Low group, which received 0.12 mg/kg of Cyt for 5 days,
3. Cyt High group, which received 62.5 mg/kg of Cyt for 3 days,
4. Ibr Low group, wich recieved 9 mg/kg of Ibr in days 1-5 and 8-10, and
5. Ibr High group, which received 18 mg/kg of Ibr on days 1–5 and 8–10.

Blood was collected from all mice on Day 12 after the beginning of treatments and the frequency of A20 mCherry cells was measured using flow cytometry. The difference in frequency of these cells in the blood before and after treatment was used to calculate the leukemia growth index of each group. The difference in growth index between treated and non-treated mice was used to calculate the inhibition of cell growth as a function of treatment. Data from the *in vivo* experiments and parameters from Table 1 of paper were used to validate the model. Computer simulations were performed using fourth-order adaptive step Runge–Kutta integration, as implemented in the ODE45 subroutine of MATLAB.

3. Results and Discussion

The **growth rate *in vivo* r of A20 mCherry cells** was calculated according to

$$r = \frac{\ln N(t)/N(0)}{t} = \frac{\ln 16338/3662}{144} = 0.01\ h^{-1}$$

Where $N(0), N(t)$ are the number of cells at times 0 (16 days after inoculation) and t (22 days after inoculation).
To determine the **efficacy of Cyt and Ibr *in vivo***, five groups of mice were treated with different doses and time periods. Tail vein blood was collected on the day of initiation of treatment and two days after the last treatment. At each time point, the percent change in frequency of A20 cells in each treated mouse relative to the average frequency in the Control group was calculated. From these data, the average A20 frequency change for each treated group was obtained. The difference between the average frequency in each test group and the Control group represents the percent growth inhibition as a function of treatment. Growth inhibition due to Cyt was dose dependent (low dose 9%, high dose 58%), whereas inhibition due to Ibr was not (low and high doses about 44%). Based on previous studies and on the *in vivo* experiments, an ODE model was formulated to explain the interaction between CLL cells and chemotherapeutic drugs

$$\begin{cases} \frac{dA}{dt} = rA\left(1-\frac{A}{K}\right) - \mu_A AE - \frac{\mu_{AC}AC}{a+C} & (1) \\ \frac{dE}{dt} = -\mu_E E + \frac{pAE}{c+A} - \mu_{AE}AE - \frac{\mu_{EC}EC}{b+C} & (2) \\ \frac{dC}{dt} = \sum_{m=0}^{N-1} d\,\delta(t-m\tau) - \mu_C - \frac{\mu_{CA}CA}{a+A} & (3) \end{cases}$$

$\frac{dA}{dt}$ describes the dynamic of A20 mCherry cells. The positive term corresponds to the logistic cancer growth characterized by the coefficient r which is limited by the maximal tumor cell number K. The negative terms correspond to A20 cells being killed by effector cells with rate μ_A and to the log-kill hypothesis, with a Michaelis–Menten drug saturation response $a+C$. μ_{AC} is the death rate resulting from the action of the drug on cancer cells. $\frac{dE}{dt}$ describes the dynamic of immune effector cells. μ_E is the natural death rate of effector cells. p is the production rate of effector cells stimulated by cancer cells. c is the number of cancer cells by which the immune system response is half of its maximum. μ_{EA} is the interaction coefficient between cancer and effector cells affecting immune populations. μ_{EC} is the mortality rate due to the action of chemotherapy on effector cells and b is the drug amount for which such effect is half of its maximum. $\frac{dC}{dt}$ describes the first-order pharmacokinetics of a drug with an external source. A dose d of the drug is injected every τ hours. By modeling the injection as a shifted Dirac Delta function $\delta(tm\tau)$, the m^{th} dose raises $C(t)$ by d units at $t = m\tau$. μ_C is the deactivation rate calculated as $\mu_C = \frac{\ln 2}{t_{1/2}}$, where $t_{1/2}$ is the elimination half-life (1–3 h for Cyt and 4–6 h for Ibr). μ_{CA} is the rate at which drug molecules attack cancer cells. a represents the drug concentration producing 50% of the maximum activity in the A20 mCherry cell population. A mathematical analysis of the model (1)–(3) found that the system is characterized by three fixed points, one of which is asymptotically stable. For the **parameters** to have biological meaning, they must be positive. The initial conditions at $t = 0$ are the initial number of A20 cells $A(0) = 5 \times 10^4$ cells/mouse, the initial number of effector cells $E(0) = 2500$ cells/mouse and no drug before the treatment $C(0) = 0$. The number of drug molecules is calculated using $\frac{mN_a}{M}$, where m is the mass of the drug, N_a is Avogadro's number and M the molar mass of the drug. A **sensitivity analysis** determined the range of parameter μ_{AC} which has the most significant impact on cancer cells under the different treatments. To test the growth inhibitory effects of Cyt combined with Ibr, a simulation was ran that predicted 95% of cell growth inhibition, which represents an 85% increase in killing efficiency compared to separate treatment.

4. Conclusions

Cyt is a drug not currently used to treat CLL that is more cytotoxic to A20 cells *in vitro* than Ibr, which suggests a repurposing of this cancer drug. Indeed, this prediction was vindicated when tested in the animal model presented in this paper. A numerical simulation of the potential effect of Cyt plus Ibr on A20 cells predicted that such a combination could increase cytotoxicity and inhibit cancer cell growth by up to 95%. It would now be valuable to test this combined treatment *in vivo*, especially as these drugs have different modes of action. This model exhibits several stable states that depend on biologically related parameters: Analysis of stability shows that the free-tumor Eqm_0^* equilibrium is not stable, which means that if there are no more cancer cells and the treatment is stopped, the model is in equilibrium without growth, albeit unstable. This may also represent a state of cancer cell dormancy, an adaptive strategy used by cancer cells to overcome drug cytotoxicity. This stage may persist until complete metabolism of the drug, which would allow tumor growth to recur. The fixed point Eqm_1^* is a stable equilibrium reached when the number of cancer cells reaches its maximum. The system is not stable at Eqm_2^* equilibrium with periodical chemotherapy, which is obtained when treatment is stopped before the cancer cells are completely removed. The step

of calculating the growth rate of cancer cells *in vivo* has to be carried out for each type of cancer cell, and it's easier to perform for blood-borne cancers. Current models do not easily help personalized chemotherapy dosing, partially because tumor cell growth rates vary between patients. The validation of experimental model with simulations studies can aid in selecting an optimal range of dosages to test. Furthermore, the model can be used to simulate combination drug therapy: the model predicted that a combination of Cyt and Ibr would lead to about 95% killing of A20 cells. Such high rates of killing are not expected in clinical practice, mainly due to subsequent toxicities. This model allows to predict a potentially effective new combination of drugs. Further experiments in vivo may reveal that two drugs with different modes of action may have acceptable efficacy at a lower dosage.

References

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