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. 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*.

This study 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.[2, 3] 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 [1].
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

4. Conclusions

References

1. A., X., A., A. & I., K. Immunoglobulin heavy variable (IGHV) genes and alleles: new entities, new names and implications for research and prognostication in chronic lymphocytic leukaemia. *Immunogenetics* **67,** 61–66 (2015).
2. A., B. J. Treatment of Chronic Lymphocytic Leukemia. *The New England Journal of Medicine* **383,** 460–473 (2020).
3. Ciril, R. & Emilio, M. Chronic Lymphocytic Leukemia. *The New England Journal of Medicine* **333,** 1052–1057 (1995).