# In Silico Modelling, Analysis, and Control of Complex Diseases: Addressing Clinical Questions, Personalized Treatments, and Healthcare Management

**Matteo Italia and Fabio Dercole**

**Abstract** Human diseases are complex and dynamic. Understanding and controlling diseases require interdisciplinary approaches, aided by advances in digital technology, data analysis, and computational power. Specifically, in his Ph.D. Thesis, Matteo Italia has developed in silico models to study cancers, Restless Legs Syndrome (RLS), and Covid-19. The goals are to answer clinical questions, optimize treatments, and manage healthcare. For cancers, the developed models suggest that dynamic and personalized protocols can overcome drug resistance more effectively than static protocols. For neuroblastoma, the *MYCN* gene's role in treatment outcomes is explored. For melanoma, promising drug combinations are identified to overcome vemurafenib resistance. In RLS, the first mathematical model supports the hypothesis that a single neuronal generator triggers periodic leg movements, aiding disease understanding. For Covid-19, a new compartment model, including vaccination policies and protection waning, emphasizes the importance of global equitable vaccine access to mitigate the pandemic. Overall, this ensemble of works highlights the importance of a systematic computational methodology in healthcare, a sort of engineered *modus operandi* that combines data analysis, systems and control, mathematics, optimization, simulations, and coding, among others.

## 1 Introduction

Human diseases are often associated with complex and dynamical characteristics. Complexity is caused by a combination of multiple genetic, metabolic, environmental, lifestyle, and still many unknown or uncertain intertwined factors.

---

M. Italia (✉)
Mathematical Oncology Laboratory, Universidad de Castilla-La Mancha, Ciudad Real, Spain
e-mail: matteo.italia@uclm.es

F. Dercole
Department of Electronic, Information, and Bioengineering, Politecnico di Milano, Milano, Italy
e-mail: fabio.dercole@polimi.it

S. Garatti (ed.), *Special Topics in Information Technology*,
PoliMI SpringerBriefs, https://doi.org/10.1007/978-3-031-80268-3_8

Dynamism refers to striking changes in the dynamics of some bodily functions. Most diseases show the typical structural traits of complex systems, such as positive and negative feedbacks in cellular communication, high degrees of inter- and intra-cellular connections, and interactions across different spatio-temporal scales, from molecules and fast metabolic dynamics to the entire body and slower evolutionary responses. Additionally, the related dynamical processes show the typical phenomena of complex nonlinear dynamics, such as self-sustained metabolic and neural oscillations, emergent behaviours in cell populations, such as neuronal synchronization and cancer evolutionary adaptation, wave propagation of infectious contacts, and bifurcations, for example, the transition from a constant to a dynamic optimal treatment. Complexity and dynamism are thus intertwined aspects of diseases.

Understanding and controlling the evolution of a complex disease and how to optimally treat each patient are crucial challenges that benefit the contributions of engineering, mathematics, and systems and control theory in particular. There are many open questions, especially in the applications of personalized medicine. The improvement in technology, biological data collection, effective data analysis methods, and the available computational power facilitate this research mission.

This article briefly summarizes Matteo Italia's Ph.D. Thesis in Information Technology—Systems and Control at Politecnico di Milano, Milan, Italy, supervised by Professor Fabio Dercole. This work fits into a multidisciplinary context, known as in silico medicine. The general aim is to provide modelling-informed support to healthcare. To this endeavour, Italia develops and analyzes mathematical models designed to investigate the disease under study. The models, once calibrated and validated on available data, are used to address clinical questions, personalize treatments, and optimize healthcare management. This is achieved by applying an engineered *modus operandi* that includes key steps: defining the research problem, data pre-processing and analysis, model development, calibration, validation, experiment integration, simulations, result interpretation, and analysis. This approach combines various scientific disciplines, including engineering, systems and control, mathematics, and coding. Genetic, neurological, and epidemiological diseases, specifically, cancers showing fast pharmacoresistant response, the Restless Legs Syndrome (RLS), and the Covid-19 pandemic, are studied and briefly presented as follow.

The impact of improving cancer treatments is just immense. We aim to optimally overcome drug resistance investigating cell-based (CB) and population-based (PB) evolutionary models of cancer growth under chemotherapy. Section 2 faces optimal drug scheduling in a CB theoretical framework for the first time. Section 3 presents the first PB model describing neuroblastoma optimal treatments under cyclophosphamide and vincristine. Their principal result is that it is possible to steer the evolution of cancer drug resistance, transforming it into a weakness to be exploited by optimal personalized treatments. The complex relation between the *MYCN* gene and treatment outcomes defines the *MYCN* enigma in neuroblastoma. Using a gene regulatory network, Sect. 4 discusses the impact of *MYCN* regulation on apoptosis through the *ARF-MDM2-p53* signalling pathway. Finally, Sect. 5 presents how overcoming vemurafenib resistance in melanoma by adopting the SynGeNet method.

Section 6 investigates if a single neuronal generator may trigger periodic leg movements (PLMs), a key disorder of RLS-affected subjects, rather than multiple asynchronous generators (the single-generator hypothesis). Designing and calibrating the first in silico model simulating PLMs, we support the single-generator hypothesis.

Finally, Sect. 7 studies optimal vaccination strategies against Covid-19. The main message is that stopping this pandemic requires actions to increase vaccine access, and the needed increase is less severe by adopting global equitable access.

In conclusion, Italia employs an engineered *modus operandi* to tackle complex medical problems in his Thesis. For each considered disease, new mathematical models are designed, calibrated, and validated using real data, providing valuable insights. Optimal personalized treatments are proposed for several types of cancer, a specific clinical question is addressed for Restless Legs Syndrome (RLS), and vaccination control policies are suggested to combat the Covid-19 pandemic. Section 8 resumes the key results, their impacts, and possible future developments highlighting the clinical point of view.

## 2 Optimal Treatments in a Cell-Based (CB) Cancer Model Suggests Switching Among Two Alternative Drugs

Many aggressive cancers remain incurable due to their rapid development of drug resistance. We develop a CB model to describe cancer growth and evolution, assuming genetic resistance to two specific chemotherapy drugs, to investigate optimal treatments. This study extends the population-based (PB) model published by Orlando and colleagues [1], which describes the evolution of a homogeneous population of cancer cells according to a fitness landscape. The landscape delineates three distinct types of trade-offs: cells are either more, less, or equally effective when developing resistance to both drugs versus specializing in resistance to a single drug.

The CB framework allows us to account for genetic heterogeneity among cells (modeling drug resistance with two continuous phenotypes), cancer cell spatial competition (affecting cancer duplication), drug diffusion dynamics, and realistic treatment protocols (with two control variables governing drug administrations).

By calibrating our model based on Orlando et al.'s assumptions, we demonstrate that protocols alternating the two drugs minimize cancer size either by the end of the treatment or at intermediate control stages. These findings significantly contrast with those obtained using the PB model, underscoring the crucial role of spatial and genetic heterogeneities in oncology. Our study represents the first effort to identify optimal treatments within a CB framework, marking a significant step toward clinical applications.

A preliminary version of this work was presented at the ECC22 conference [2], and the complete version has been published in [3].

## 3 Optimal Personalized Treatments in a Population-Based Model of Neuroblastoma Under Chemotherapy

Neuroblastoma is one of the most common tumors in children, with over half of high-risk patients not surviving despite multi-modal therapy. A significant challenge is the one-size-fits-all approach of induction chemotherapy (rapid COJEC), which uses fixed doses in eight two-week cycles, resulting in widely varying outcomes likely due to differences in drug resistance heterogeneity [4].

We design and calibrate a population-based (PB) model using public data to describe the evolution of neuroblastoma under treatment with vincristine and cyclophosphamide, two key components of rapid COJEC, by incorporating their pharmacokinetics. We assume that cancer cells undergo three processes: growth, mutation, and drug-induced death. The rates of these processes depend on genotype, phenotype, and environment. Cancer cells can have 3 levels of genetic resistance to each drug: none, mild, and strong, resulting in 9 different clones (genotype) within the tumor. Additionally, cancer cells can reversibly adapt (phenotype) to prolonged drug exposure at the expense of growth. We develop an optimization algorithm (genetic algorithm for global search combined with Matlab *fmincon* function for local search) to minimize tumor size prior to surgery, based on distinct pre-treatment clonal compositions representing different virtual patients.

The optimal treatments leverage the varying cytotoxic properties of the 2 drugs and the competition among different clones within the tumor, suggesting that chemotherapy can be significantly enhanced by using personalized schedules. We propose that a more comprehensive multi-modal therapy approach can be improved by integrating targeted therapies specifically directed against mutations and oncogenic pathways enriched and activated by the chemotherapeutic agents. This would require the development of a decision support system informed by emerging medical technologies, such as multi-region sequencing and liquid biopsies, to estimate patient-specific tumor heterogeneity. These results, published in [5], could serve as the groundwork for establishing such a decision support system.

## 4 Addressing the *MYCN* Enigma in Neuroblastoma

This work investigates the role of the *MYCN* oncogene in determining treatment outcomes for neuroblastoma. *MYCN* amplification and overexpression are critical biomarkers associated with poor prognosis. Most patients without *MYCN* amplification respond well to therapy, but some still experience unfavorable outcomes. In these cases, tumors show low *MYCN* mRNA levels but high *MYCN* protein levels, indicating protein stabilization [6]. This complex relationship between *MYCN* and treatment outcomes defines the *MYCN* enigma.

We use an in silico approach to explore the impact of *MYCN* regulation on apoptosis through the *ARF-MDM2-p53* signaling pathway. By employing a nonlinear system of ODEs based on documented gene interactions and classical gene expression modeling [7], we conduct experiments to understand the gene regulatory network dynamics. Specifically, we examine how *MYCN* affects the expression of *p53*, a tumor suppressor gene that activates apoptosis pathways. The relative expression of *p53* and *MYCN* proteins serves as an indicator of treatment success.

Model parameters are sourced from public datasets and literature. By manipulating stressors and transcription/translation rates, we identify parameter combinations that lead to favorable or unfavorable outcomes. We analyze these combinations using the Apriori algorithm for discretely distributed parameters and visualization tools for randomly distributed parameters.

Our findings show that stressors significantly impact *p53* expression, with DNA damage stress being the most crucial. This stress promotes *p53* mRNA binding to *MDM2*, enhancing *p53* translation. The production rates of *MYCN* and *p53* are primary determinants of outcomes, with their interrelationship essential for modeling the *MYCN* enigma observed clinically.

## 5 Searching for Effective Drug Combinations to Overcome Vemurafenib Resistance in *BRAF*-Mutant Melanoma

Melanoma has become a significant global healthcare issue primarily due to excessive ultraviolet radiation exposure. The *BRAF V600E* mutation is crucial in melanoma development, driving increased proliferation and survival. Vemurafenib, a *BRAF* inhibitor, has shown promise as a first-line treatment; however, its effectiveness diminishes over time due to the development of resistance, and no effective second-line therapies are currently available.

This study explores effective drug combinations to address vemurafenib-resistant melanoma (VRM), aiming for synergistic effects. We use the SynGeNet (Synergy from Gene expression and Network mining) methods [8] to predict effective drug combinations for VRM. SynGeNet constructs a protein-protein interaction network using deferentially expressed genes in sensitive and VRM (RNA sequencing) and the public BioGRID database, applying the belief propagation algorithm. We evaluate drug combinations based on their ability of inducing gene expression profiles exhibiting anti-correlation to the subnetwork (LINCS L1000 database) and the centrality of drug targets (DrugBank and STITCH databases) within the network. The most promising combination identified is sorafenib and pioglitazone.

In vitro tests, including viability assessments and synergy analysis, guide the determination of optimal drug concentrations. These tests confirm the efficacy of the combination, which should be further validated before clinical applications.

## 6 A Model-Informed Answer to the Single-Generator Hypothesis Triggering Periodic Leg Movements (PLMs)

Here, we address a clinically-driven question about PLMs, the principal disorder of people affected by RLS. The goal is to employ numerical simulations to assess whether the complex process involving two (or more) interacting spinal or supraspinal structures responsible for generating PLMs can be effectively modeled using a single-generator approach (single generator hypothesis). Thus, we develop the first phenomenological model generating virtual or in silico PLMs.

We simplify the physiology and assumed that each leg is controlled by a single motor neuron, representative of the central nervous system (CNS) complex pathways, determining contractions of the leg muscles. The two neurons are modeled with integrate-and-fire neuron models. Each neuron receives excitatory/inhibitory inputs from the CNS physiological activity, a proportion of which equally affect both legs, while the remaining fraction is leg-specific. We calibrate this physiological activity using the control dataset (healthy subjects). For subjects showing significant PLMs, we introduce a periodic excitatory input, calibrated on RLS dataset (pathological subjects), that influence both legs representing the pathological activity.

Despite simplifying assumptions, our model-simulated data closely resemble the real polysomnographic data, displaying minimal significant differences. This provides compelling preliminary evidence supporting the single generator hypothesis for PLMs. Future model extensions will strive to develop a diagnostic and therapeutic tool that can assist healthcare specialists in making realistic forecasts and in cross-correlating and clustering data with other patient-specific information.

A preliminary and modeling oriented version of this work has been presented at the CIBB2021 conference [9], and the complete article published in [10].

## 7 Optimal Vaccination Strategy Suggests Enhancing Global Equity and Access to Covid-19 Vaccines

We adopt a model-informed approach to argue that Global Equitable Access (GEA) to Covid-19 vaccines is crucial to ending the pandemic. We develop a new epidemiological compartmental model, calibrated with public data (Our World in Data database), integrating the principal aspects of the Covid-19 pandemic: susceptible, exposed, presymptomatic, symptomatic and asymptomatic infected, quarantined, hospitalized, recovered, and death subjects; contact tracing; containment measures; vaccination campaign. Two interconnected macro-nodes describe the world, high-income (HI) and middle-to-low-income (MLI) countries, with the same epidemiological model but a different vaccine availability.

Our findings indicate that merely redistributing the currently available vaccines equitably is insufficient to stop the pandemic. However, a significant increase in vaccine production (access to vaccines) could end the pandemic within a year of vaccination and save millions of lives in MLI countries. We also explore the relationship between vaccine access and distribution among HI and MLI countries, showing that the required access to vaccines to stop the pandemic gets reduced the more global equity is used.

To estimate the socio-economic impacts, we compare simulations of the current scenario–where the virus remains potentially harmful and is likely to become endemic–with a hypothetical but realizable scenario, where the used vaccine rollouts are the peaks achieved during the 2021–2022 vaccination campaign. Our analysis shows that the global savings on vaccines in the selected scenario surpass the five-year profit of the rights holders under the current situation, justifying compensation mechanisms for necessary licensing agreements. The encouraging news is that the benefits of the selected scenario are still significant if implemented now.

The preliminary version of this work has been presented at the COMPENG 2022 conference [11], and the complete version published in [12].

## 8 Conclusion

Matteo Italia's Ph.D. Thesis focuses on in silico medicine, an interdisciplinary field gaining attention for its use of computational methods to advance medical understanding and treatment, through the lens of personalized medicine. The in silico term represents a paradigm shift in the research and applications of medicine, bringing significant transformative effects. The constructive results, achieved applying Italia's engineered *modus operandi*, are the core contributions of his Thesis. In the following, the key results, their impacts, and possible future developments are resumed, underlining both the research and clinical points of view, such as the understanding of physiological and pathological mechanisms, the evolution of a disease, treatments focusing on optimal and personalized strategies, and healthcare management.

**Cancers** are highly complex and adaptive because of high mutation rates. Indeed, chemotherapy usually kills cancer cells effectively initially, but then cancers achieve resistance. We investigate how to overcome drug resistance and treatment failures.

Designing, calibrating, and validating valuable predictive models, all the desired treatment strategies can be virtually implemented, tested, and evaluated before applying the optimal treatments to real patients. Specifically, in the theoretical framework presented in Sect. 2, we found that it is possible to exploit cancer drug resistance, steering cancer evolution towards evolutionary traps by exploiting evolutionary double binds. The optimal administration strategies are dynamic protocols that alternate between two alternative drugs (bang-bang control), obtaining substantial reductions in final tumor sizes. Moreover, Sect. 3 shows that when administering vincristine and cyclophosphamide to neuroblastoma patients, the optimal personalized treatments are based on general evolutionary principles that exploit the clonal compositions of the tumor. We discover that starting the treatment with only one drug and adding the other later, and using both drugs from the beginning but shortening the number of treatment cycles are optimal strategies for specific initial cancer compositions mimicking specific patients. The personalized optimal treatments highly outcompete standard one-size-fits-all protocols.

Section 4 addresses the *MYCN* enigma (the complex relationship between the *MYCN* gene and the treatment outcomes) in neuroblastoma treatments exploiting a calibrated gene regulatory network. Using the relative expression of *p53* (tumor suppressor gene) and *MYCN* (oncogene) as an indicator of treatment outcome, we found in silico outcomes in agreement with clinical outcomes. Moreover, we postulated that therapeutic strategies aimed at augmenting *p53* production, diminishing *MYCN* production, and facilitating the binding of the *p53* mRNA and the *MDM2* protein hold great promise to be combined with standard treatments for enhancing neuroblastoma treatment outcomes.

Analysing gene expressions and protein-protein interactions in Sect. 5, we in silico predict pioglitazone and sorafenib as effective treatment against melanoma resistant to vemurafenib, confirmed by preliminary in vitro tests. If the drug combination is approved in clinical trials, there will be available an effective second-line therapy, significantly improving melanoma treatment outcomes.

The models of Sects. 2 and 3 can be enhanced by incorporating factors like metabolism, microenvironment, multi-drug resistance, and clinical realism. For personalized medicine, patient-specific parameters such as tumor heterogeneity, drug absorption, and cancer cell growth can be identified, allowing to virtually determine the best personalized treatments. This requires a decision support system that includes all approved drugs, relevant mutations, oncogenic pathways, and the clonal composition of tumors determined through multi-region sequencing and liquid biopsies. This system will optimize therapy by customizing chemotherapy schedules and integrating other treatments like surgery and immunotherapy. Similarly, the gene regulatory network of Sect. 4 can be personalized to predict patient-specific outcomes and adjust medical interventions. Additionally, the methodology from Sect. 5 can serve as a foundation for personalized treatment decision support systems for other cancers at various stages. Using patient RNA sequencing data, patient-specific disease networks can predict effective personalized drugs. These predicted drugs can then be tested in vitro with patient-derived cancer cells before being administered to the patient.

**Restless legs syndrome (RLS)** is a common yet often overlooked condition marked by periodic leg movements (PLMs) during sleep, leading to disrupted sleep. Section 6 investigates the hypothesis that a single neuronal generator causes PLMs. The developed model, calibrated with clinical data, supports the hypothesis that a single generator could trigger PLMs. This understanding is vital for treatment decisions. The model provides a foundation for future research, potentially reducing costs, avoiding animal testing, simulating drug effects, and enhancing knowledge of RLS and PLMs by introducing new indicators and considering patient metadata.

**Covid-19** has profoundly impacted the world, with vaccine access disparities between high-income (HI) and middle-to-low-income (MLI) countries hindering pandemic control. Section 7 presents a model showing that Global Equitable Access (GEA) to vaccines is crucial. The study examines vaccination strategies by dividing the world into HI and MLI countries and varying vaccine availability and distribution.

When vaccine access is limited, prioritizing HI countries helps control infections. With moderate access, focusing on MLI countries becomes optimal. When access is sufficient, GEA is the best strategy. Enhancing vaccine production and equitable distribution is essential, although socio-economic disparities make perfect equity challenging. The model shows that achieving GEA reduces the severity of required production increases to stop the pandemic. These strategies offer significant socio-economic benefits, informing current and future pandemic responses and aiding policymakers in optimizing vaccination campaigns and resource management.

**Competing Interests** The authors have no conflicts of interest to declare that are relevant to the content of this chapter.

## References

1. Orlando, P.A., Gatenby, R.A., Brown, J.S.: Cancer treatment as a game: integrating evolutionary game theory into the optimal control of chemotherapy. Physical Biology (2012). https://doi.org/10.1088/1478-3975/9/6/065007
2. Italia, M., Dercole, F., (2022) Optimal control of two cytotoxic drug maximum tolerated dose steers and exploits cancer adaptive resistance in a cell-based framework.: European Control Conference. ECC **2022**,(2022). https://doi.org/10.23919/ECC55457.2022.9838039
3. Italia, M., Dercole, F., Lucchetti, R.: Optimal chemotherapy counteracts cancer adaptive resistance in a cell-based, spatially-extended, evolutionary model. Physical Biology (2022). https://doi.org/10.1088/1478-3975/ac509c
4. Smith, V., Foster, J.: High-Risk Neuroblastoma Treatment Review. Children (2018). https://doi.org/10.3390/children5090114
5. Italia, M., Wertheim, K.Y., Taschner-Mandl, S., Walker, D., Dercole, F.: Mathematical Model of Clonal Evolution Proposes a Personalised Multi-Modal Therapy for High-Risk Neuroblastoma. Cancers (2023). https://doi.org/10.3390/cancers15071986
6. Valentijn, L., Koster, J., Haneveld, F., Aissa, R., Sluis, P., Broekmans, M., Molenaar, J., van Nes, J., Versteeg, R.: Functional MYCN signature predicts outcome of Neuroblastoma irrespective of MYCN amplification. Proceedings of the National Academy of Sciences of the United States of America (2012). https://doi.org/10.1073/pnas.1208215109
7. Ingalls, B.: Mathematical Modeling in Systems Biology. MIT Press (2013)
8. Regan-Fendt, K.E., Xu, J., DiVincenzo, M., Duggan, M.C., Shakya, R., Na, R., Carson, W.E., III., Philip, R.O., Payne, P.R.O., Li, F.: Synergy from gene expression and network mining (SynGeNet) method predicts synergistic drug combinations for diverse melanoma genomic subtypes. Npj System Biology Application (2019). https://doi.org/10.1038/s41540-019-0085-4
9. Italia, M., Danani, A., Dercole, F., Ferri, R., Manconi, M.: The First in-silico Model of Leg Movement Activity During Sleep. Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics) (2022). https://doi.org/10.1007/978-3-031-20837-9_4
10. Italia, M., Danani, A., Dercole, F., Ferri, R., Manconi, M.: A calibrated model with a single-generator simulating polysomnographically recorded periodic leg movements. Journal of Sleep Research (2022). https://doi.org/10.1111/jsr.13567

11. Italia M, Della Rossa F, Dercole F (2022) A mathematical analysis of the socio-economic impacts of a patent waiver on COVID-19 vaccines: IEEE Workshop on Complexity in Engineering. COMPENG (2022). https://doi.org/10.1109/COMPENG50184.2022.9905430
12. Italia, M., Della Rossa, F., Dercole, F.: Model-informed health and socio-economic benefits of enhancing global equity and access to Covid-19 vaccines. Scientific Reports (2023). https://doi.org/10.1038/s41598-023-48465-y