Université de Montréal

Optimizing Doxorubicin-G-CSF Chemotherapy Regimens for the Treatment of Triple-Negative Breast Cancer

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Thèse présentée à la Faculté des études supérieures
en vue de l'obtention du grade de (Maîtrise)
en sciences pharmaceutiques, option pharmacologie

29 septembre 2020

Université de Montréal

Faculté des études supérieures, Faculté de Pharmacie

Cette thèse intitulée :

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Résumé

La chimiothérapie cytotoxique reste une option de traitement de première intention pour la majorité des cancers. Un effet secondaire majeur dans les schémas chimio-thérapeutiques est la neutropénie. La thérapie prophylactique avec le facteur de stimulation des colonies de granulocytes (G-CSF), une cytokine endogène responsable de la régulation de la production de neutrophiles, est administrée en concomitance. Le moment et la dose exacts pour administrer la chimiothérapie et le G-CSF représentent des éléments cruciaux pour obtenir les résultats souhaités du traitement. En nous appuyant sur des travaux antérieurs qui optimisaient les schémas thérapeutiques du G-CSF, nous sommes basés sur une approche de pharmacologie quantitative des systèmes (QSP) pour étudier la fréquence et l'intensité de la dose dans le but de maximiser les effets anti-tumoraux de la chimiothérapie tout en minimisant la neutropénie. Dans ce travail, nous avons effectué une optimisation sur une large gamme de longueurs de cycle et de valeurs des doses de chimiothérapie afin d'identifier les meilleurs schémas en combinaison avec le G-CSF. Nos résultats suggèrent que la doxorubicine 45mg/BSA tous les 14 jours a un impact positif sur le contrôle de la croissance tumorale, et qu'il est préfèrable de retarder l'administration du G-CSF au septième jour après la chimiothérapie et de donner moins de doses pour minimiser le risque de neutropénie et le fardeau de ce médicament. Cette étude suggère des pistes possibles pour des schémas optimaux de chimiothérapie, avec le soutien prophylactique du G-CSF spécifiquement dans le contexte du cancer du sein triple négatif.

Mots-clés : Chimiothérapie cytotoxique, cancer du sein triple négatif, modélisation mathématique, G-CSF, neutropénie, croissance tumorale.

Abstract

Cytotoxic chemotherapy continues to be a first-line treatment option for the majority of cancers. A major side effect in chemotherapy regimens is neutropenia. Prophylactic therapy with granulocyte colony stimulating factor (G-CSF), an endogenous cytokine responsible for regulating neutrophil production, is administered concomitantly; the exact timing of the combination chemotherapy and G-CSF is crucial for achieving treatment results. Leveraging on previous work that optimized treatment regimens based on G-CSF timing, we developed a quantitative systems pharmacology (QSP) framework to study dose frequency and intensity of chemotherapy in order to maximize anti-tumor effects while minimizing neutropenia. In this work, we performed an optimization across a wide range of cycle lengths and dose sizes to identify the best cytotoxic chemotherapy regimens with G-CSF support. Our results suggest that doxorubicin 45mg/BSA every 14 days, has a positive impact on tumour growth control, and that to minimize the risk of neutropenia and the burden to patients it is best to delay the administration of G-CSF to day seven after chemotherapy and give fewer doses . This study suggests possible avenues for optimal chemotherapy regimens with prophylactic support of G-CSF in the context of Triple Negative Breast Cancer.

Keywords: Cytotoxic chemotherapy, TNBC, mathematical modeling, G-CSF, neutropenia, tumor growth.

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Remerciements

J'aimerais remercier d'abord ma superviseure Fahima Nekka pour soutenir ma demande à la Maîtrise en Sciences pharmaceutiques depuis que j'étais au Mexique et pour m'avoir ouvert les portes de son laboratoire. Je remercie ma co-superviseure Morgan Craig pour me montrer le monde des mathématiques et je l'admire pour m'avoir patiemment appris des concepts complexes dans une période courte. Je remercie Nathalie Letarte d'avoir accepté de faire partie de mon comité consultatif et de m'avoir répondu aux questions sur le traitement du cancer du sein.

J'apprécie l'accueil par mes collègues du laboratoire dès le premier jour à l'Université de Montréal. Merci Sara pour la visite du campus et tes conseils. Merci Paul-Antoine pour tes explications dans le cours de statistiques. Merci Florence pour me répondre aux questions de mathématiques et le soutien informatique avec Matlab. Merci Jeffrey, Abdullah et Augusto de vos conversations. Merci Imad pour m'aider à formuler des questions d'hypothèse au début de la Maîtrise. Merci Justin Le Sauteur-Robitaille de m'avoir aidé à optimiser mon code de Matlab. Je remercie tous les collaborateurs externes aussi. Je remercie surtout à Anthony Humphries et ses étudiants Shaza, Wendy, Anh Ho, Julie-Alexia et Tyler pour écouter ma présentation avant l'examen des séminaires et de m'avoir accueillie dans les réunions à McGill. Je voudrais remercier aussi Patrice Dupont pour son aide avec la revue de littérature et l'apprentissage des outils de la bibliothèque de Pharmacie. Merci beaucoup à Frédérique pour ses conseils et aussi Aymen pour avoir apporté des commentaires constructifs à mon projet. Je suis heureuse de tout l'apprentissage que mes professeurs et collègues m'ont apporté et j'apprécie beaucoup leur compagnie et leur amitié. Je souhaite que cela reste encore pour des années suivantes. Je dédie principalement cette thèse à ma famille et à mes amies au Mexique pour leur amour inconditionnel et les appels vidéo qui font ma journée. Je remercie aussi mon amie de la Licence en Pharmacie, Jennifer Bringas au Centre d'oncologie d'État ISSEMYM, Toluca pour me répondre aux questions pertinentes sur le traitement du cancer du sein ce qui a grandement enrichi l'apprentissage de l'applicabilité de mon projet de recherche.

Sincèrement,

Rosalba Vivian Paredes Bonilla

Chapter 1 – Introduction

1. The Mutational Component of Cancer Development

Cancer is the leading cause of death in Canada and is responsible for 30% of all deaths. Nearly 1 in 2 Canadians are expected to be diagnosed with cancer in their lifetime according to projected estimates of cancer in Canada in 2019 and 2020 (1,2). That said, the probability of developing cancer lies on many factors, and varies by cancer type and the intrinsic genetic factors (1). Any substance that causes cancer is known as a carcinogen. Several factors influence whether a person exposed to such carcinogens will develop cancer or not, including the amount and duration of the exposure and the individual's genetic background (3). In normal cells, hundreds of genes in a complex fashion orchestrate the process of cell division. Cells divide and create an exact copy of their genetic material. Cancer is a disease driven by mutations, located in germline cells or somatic cells. When the nucleic acid sequence is damaged in the cells that produce the next generations of cells, the resulting cells will carry mutated genetic material. Mutations are changes to the nucleic acid sequence that codes for a gene (4). Such alterations may accumulate over time and successive numbers of cells divisions. Mutations in germline cells are less frequent and are inherited, while the vast majority of cancers occur in somatic cells and cannot be inherited. Most cancer cells possess 60 or more mutations (5).

Gene mutations in cancer cells interfere with the normal instructions in a cell and can cause it to grow out of control (5,6). Normally, monitoring systems and checkpoints regulate the cell cycle, including whether it is time to advance to the next phase, or whether the cell is destined for apoptosis. A cancerous cell no longer responds to many of these signals, and divides without control (5–7). Telomeres, the specific DNA-protein structures found at both ends of each chromosome, protect the end of the genome of the chromosome from degradation, unnecessary recombination, repair, and interchromosomal fusion (8,9). It maintains genomic integrity in normal cells, preventing attack by nucleases, and their progressive shortening during successive cell divisions induces chromosomal instability (8). In the large majority of cancer cells, telomere length is maintained by telomerase, which reverses the wearing down of chromosome ends that normally happens during each cell division. Thus, telomere length and telomerase activity are crucial for cancer initiation and the survival of tumours (10).

1.1 Types of Cancer and Tumours

Cells with damaged checkpoints and uncontrol cell division are able to form masses of cells called tumours. Tumours grow and behave differently, depending on whether they are cancerous (malignant), non-cancerous (benign) or precancerous. Depending on the type of tissue in which the cancer originates and by primary site, tumours can be classified as solid or liquid tumours (i.e. blood cancers). Carcinoma and sarcoma are considered solid tumours, while lymphoma myeloma, leukemia and mix types are hematopoietic cancers. The most common types of cancers are solid tumours, such as breast cancer, colon and rectum cancer, prostate cancer, lung cancer, and melanoma of the skin (11). Cancer starts in a primary site, with the tumour being formed from epithelial tissue, connective tissue, and/or white blood cells. Tumours are known to have hypoxic cores formed by non-diving cells, and dividing cells along the periphery. As the tumour increases in size, it also starts to develop its own blood supply by forming new vessels or relying on the pre-existing vasculature. (12). Tumour cells can invade nearby tissues. Metastases are tumour cells that break away from the main tumour and travel through the blood or lymphatic system and spread to distant parts of the body to develop into new cancer cells. Metastatic cancers may be found at the same time as the primary tumour, or months, and years later and have different growth rates, depending on the type of tumour tissue (13).

1.2 Diagnosis and Staging in Tumours

The complete evaluation of a patient usually requires a thorough history and physical examination along with diagnostic testing. Many tests are needed before determining whether a person has cancer, or if another condition (such as an infection) is mimicking the symptoms of cancer (14). The diagnostic methods include laboratory tests, imaging tests, endoscopic exams, biopsies, and genetic tests. An early diagnose can save the life of a patient. Once this information is collected and analysed, and the oncologist confirms the presence of cancer, the next step would be to determine the staging.

Staging refers to the extent of the cancer in the body and classifies a cancer based on the size of the main tumour, which parts of the organ(s) have cancer, whether the cancer has spread, and where it has spread at the time of the diagnosis (15). This stage does not change over time, even if the cancer might progress. Instead, the stage helps to estimate a prognosis, to assure the patient gets the best possible treatment and to help choose a clinical trial if the patient is eligible to participate in one (17). Tumour, Node and Metastasis (TNM) staging system is the most common

staging system used for many types of solid cancers; other systems are used for cancers of the blood and immune system. This system was developed by the American Joint Committee on Cancer (AJCC) (16).

1.3 Treatments: Surgery, Radiation, Chemotherapy, and Biological Therapies

Treatment planning is based on several key factors such as the type and stage of cancer, as well as the person's age, health and lifestyle. The patient takes an important role asking questions, and expressing concerns about treatment can help make treatment a better experience. Common treatments involve surgery, chemotherapy, and radiation therapy, as well as, biological therapies. The newest treatments include immunotherapy. Treatment can be given for different reasons such as prevention, cure, control or palliative care (17–19).

Surgery is the oldest form of cancer treatment and it is often used in combination with other types of treatment. It provides the best chance to stop many types of cancer, and it also plays a part in diagnosing, staging, and supportive treatment (17,18). Radiation is a form of high energy x-ray which can destroy or prevent the spread of cancer by damaging a cancer cell's DNA. There are two primary types of radiation therapy: external and internal radiation. It is considered a local treatment, which may be used alone or with other treatments such as surgery or chemotherapy. In this vein, a combined modality therapy is when a treatment involves more than one therapy such as a combination of radiotherapy and chemotherapy (18).

Conventional cytotoxic chemotherapy or cytostatic drugs are used to destroy cancer cells primarily by interfering at the interphase and during mitosis, thus inhibiting cell division (19). A downside of chemotherapy is that it does not differentiate between highly proliferative normal cells and malignant cells (19). Cytotoxic chemotherapy can be used before surgery to shrink a tumour or after surgery to destroy any metastases that remain and to prevent the cancer from coming back. It may also be used to relieve symptoms, improve quality of life and extend life for people with advanced cancer (called palliative chemotherapy) (20). There are many chemotherapy drugs that are grouped into classes depending on their mechanism of action. Often, a combination of different classes of chemotherapy drugs is needed to attack cancer cells at different points in their growth cycle. It may also prevent resistance and help lower the chance of cancer coming back, called recurrence (19,20). The drug classes of chemotherapy include: DNA-alkylating agents, antimitotics, antitumour antibiotics, DNA-repair enzyme inhibitors, and antimetabolites (21). Alkylating agents produce their effects by binding covalently with cell constituents, impairing

DNA synthesis or inhibiting DNA replication (22). Antimitotics block the process of cell division (mitosis) so cells cannot divide and multiply. Antineoplastic antibiotic affects DNA synthesis and replication by inserting into DNA strands or by producing superoxide that cause breakage in DNA strands and prevent the tumorous or cancerous cells to divide further. DNA-repair enzyme inhibitors attack the enzymes that normally repair damage to DNA since a cancer cell dies if it cannot repair damage to DNA (20,21). Antimetabolites induce depletion in nucleotides inducing in turn an inhibition of DNA replication (23). As well, there are several subgroups for each drug class (21). Chemotherapy is generally administered orally or intravenously. The length of treatment and type of chemotherapy drug are selected according to the type of cancer and the health condition of the patient. In most cases, the national treatment guidelines provide the context for the use of chemotherapy (20). The cell cycle is a key element to understand the action of cytotoxic chemotherapy on the cycle of cancer cells (6). Cell-cycle specific chemotherapy drugs act in one or two phases of the cell cycle as marked in red below. Cell-cycle nonspecific agents are active in all phases (17–21).

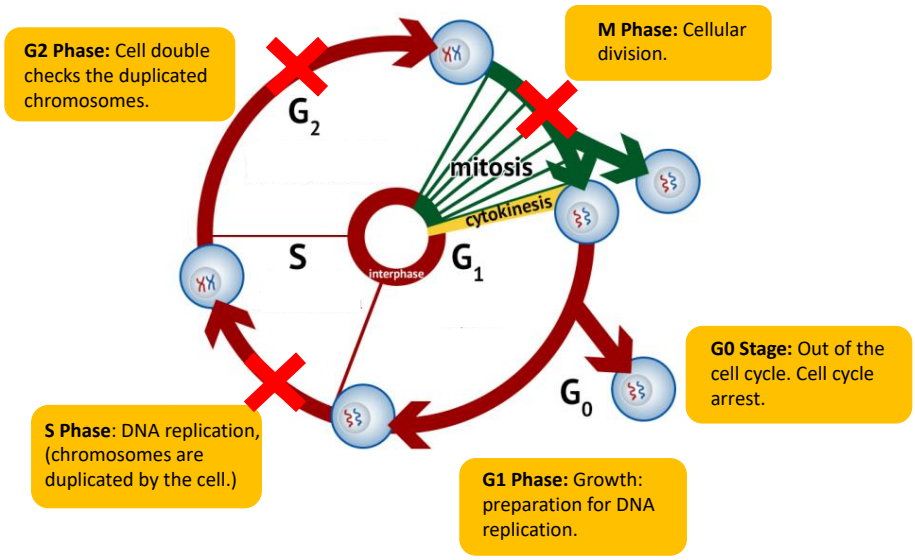

Figure 1. *The cell cycle consists of interphase and cell division. Interphase can be further divided into G0, G1, S and G2. At the interphase phases cells grow in size and cellular components are duplicated including RNA, proteins and organelles, chromosomes are duplicated, and DNA damage checkpoints prevent damaged cells from entering mitosis. Mitosis is the division of one nucleus into two genetically identical nuclei and cytokinesis is the physical separation of the cell's cytoplasm into two daughters (6) Reproduced with permission from Basis of Carcinogenesis. 1-17(2019). Copyright 2019 Springer Nature.*

Hormonal therapy is another type of therapy, which stops the cancer cells from using hormones they need for their growth, or prevents the body from producing the hormone that is causing cancer growth. Tumours that appear in the breast, prostate, endometrium (the inner lining of the uterus), and ovaries are often hormone sensitive. Hormone therapy can reduce in size or even eliminate these cancers by manipulating hormones that fuel them. It is often used following surgery, radiation therapy, or chemotherapy, but in many cases it can constitute in its own a stand-alone treatment (18). Targeted therapy use drugs to target specific molecules (such as genes or proteins) in cancer cells to stop them from growing and spreading. These drugs do not damage as many normal cells as cytotoxic chemotherapy. Immunotherapy helps to strengthen or restore the immune system's ability to fight cancer (20,21). Adjuvant therapy or supportive drugs is a term used to describe therapy given in addition to the primary cancer treatment to improve the chance of cure, prevent, manage and relieve side effects. These drugs include allopurinol, amifostine, dexrazoxane, folinic acid, mesna, ondansetron and colony-stimulating factors (CSFs, growth factors) (20). Despite the growing utilization of targeted, immunotherapeutic, and personalized approaches in cancer, most patients receive conventional cytotoxic chemotherapy as first-line treatment (17–21,24).

1.4 Cancer Treatment and Side Effects

Minimizing side effects is one of the greatest challenges in the cancer treatment. Most of the therapies mentioned above have very painful or uneasy side effects that could prevent treatment completion. Common chemotherapy and radiotherapy side effects include fatigue, nausea or vomiting, diarrhea, trouble swallowing and skin changes in radiated areas (24). During treatment, one major side effect of cytotoxic chemotherapy is neutropenia. Neutropenia is a condition that occurs when the body does not have enough neutrophils, the most abundant type of white blood cell. Neutrophils are key members of the innate immune system that protect the body against bacterial, viral, and fungal infections. Neutropenia is defined by an absolute neutrophil count (ANC) of less than 1500 cells per microliter, with severe neutropenia occurring below 500 cells per microliter (25). The production of neutrophils is regulated by endogenous colony-stimulating factors (CSFs), a class of molecules that stimulate the bone marrow hematopoietic progenitor cells to divide and generate colonies of differentiated progeny of white and red blood cells (26). Granulocyte colony-stimulating factor (G-CSF) stimulates the production of neutrophils (27). Administration of exogenous forms of G-CSF mimics the actions of endogenous G-CSF and further stimulates the production of neutrophils within the bone marrow. Some examples of

recombinant human G-CSF (rhG-CSF) include filgrastim and pegfilgrastim. However, they may also cause uneasy side effects such as thrombocytopenia, nausea, fever and bone pain (28). Therefore, a balance must be struck when administering exogenous G-CSF to patients undergoing chemotherapy to alleviate the physical and hematopoietic burden of treatment (29).

2. Breast Cancer: Genetic Bases, Incidences, Types, and Treatment Strategies

Breast cancer accounts for about 25% of all new cancer cases in women. New cases of breast cancer in 2020 estimates were 27, 700 of which 240 cases are in males and 27,400 in females according to the Canadian Cancer Statistics (2). By age, the most vulnerable group to breast cancer include women between 50 to 74 (1). Most cases of breast cancer are associated with somatic mutations in breast cells that are acquired during a person's lifetime and are not inherited. A small percentage of all breast cancers cluster in families. These hereditary cancers are due to mutations inherited in the germ line cells. Not all people who inherit mutations in these genes will ultimately develop cancer. Hereditary breast cancers tend to develop earlier in life than non-inherited (sporadic) cases, and new (primary) tumours are more likely to develop in both breasts (30). One famous example concerns the case where mutations in the BRCA1 and BRCA2 genes are inherited in an autosomal dominant pattern, which means one copy of the altered gene in each cell is sufficient to increase a person's chance of developing cancer. Although breast cancer is more common in women than in men, the mutated gene can be inherited from either the mother or the father.

The term invasive (or infiltrating) breast cancer is used to describe any type of breast cancer that has spread into surrounding breast tissue. Invasive ductal carcinoma makes up about 70-80% of all breast cancers (31). These cancers are less common but can be more serious than other types of breast cancer such as inflammatory breast cancer and triple-negative breast cancer (TNBC), an aggressive subtype accounting for about 15% of all breast cancers (32–35). TNBC refers to cancer cells lacking or having less expression of the well-known estrogen and progesterone hormonal receptors, and also do not make the protein HER/neu, a significant therapeutic target in most breast cancers (31,33,36). TNBC is more likely to affect younger women, African Americans, Hispanics, and/or those with a BRCA1 gene mutation (31,32).

2.1 Screening and Diagnosis in Breast Cancer

Screening for breast cancer is recommended for asymptomatic and symptomatic women (37). Breast cancer is generally diagnosed after an individual or their doctor finds a lump in your breast

or during screening by mammography. The doctor may recommend a hormone receptor test to measure the amount of certain proteins (hormone receptors) in cancer tissue (38). Normal breast cells have estrogen and progesterone receptors as they depend on these hormones to grow (39). However, in certain breast cancers, these receptors are continuously fueled by hormones and stimulate cells to grow. If the hormone receptor test is positive, it indicates that breast cells may have one or both of these receptors: Estrogen receptor (ER+), which means that breast cells are sensitive to estrogen, progesterone receptor (PR+) which means that breast cells are sensitive to progesterone. HER2 status testing is done on a biopsy sample taken from the primary tumour at the time of the diagnosis, along with the hormone receptor test. There are two ways to test HER2 status: by immunohistochemistry (IHC), which qualitatively measures the amount of HER2 protein in the cancer cells, and fluorescence *in situ* hybridization (FISH), which looks at the number of copies of the HER2 gene in the cancer cells. Understanding the chemical and genetic makeup of the breast cancer cells is necessary to guide treatment selection (39–42).

2.2 Treating Breast Cancer

The type of treatment depends on the TNM staging, grading of the tumour, expression or lack of expression of hormonal receptors and HER2, and overall health status at the time of diagnosis. To date, treatment of breast cancer include surgery, chemotherapy, hormonal therapy, monoclonal antibody therapy, and radiotherapy (33,37). Chemotherapy is given to shrink a large tumour before surgery (called neoadjuvant chemotherapy) when the cancer hasn't spread outside the breast or lymph nodes, to destroy cancer cells left behind after surgery and reduce the risk that the cancer will recur (called adjuvant chemotherapy), to treat cancer that comes back, and to control the symptoms of advanced breast cancer (called palliative chemotherapy). Chemotherapy is generally given at 3 weeks or sometimes 2 weeks intervals (called dose-dense regimens). Studies have shown that dose-dense regimens may further lower the risk that breast cancer will come back and improve survival (43).

The lack of hormone receptors in TNBC requires a different therapy strategy. For those with triple negative recurrent/stage IV breast cancer and germline BRCA1/2 mutations, the NCCN Panel recommends platinum agents (cisplatin and carboplatin) as preferred treatment options (37). Among preferred single agents, the NCCN Panel suggested taxanes (paclitaxel), anthracyclines (doxorubicin and liposomal doxorubicin), anti-metabolites (capecitabine and gemcitabine), microtubule inhibitors (eribulin and vinorelbine), and platinum agents (37). In this thesis, I focus

on doxorubicin (Adriamycin), an anthracycline antitumour antibiotic isolated from cultures of *Streptomyces peucetius var.caesius* that has antimitotic and cytotoxic activity against a wide spectrum of tumours (44,45). Although it is named antibiotic it is not used for this purpose.

Doxorubicin's primary mechanism of action is to intercalate within DNA base pairs, causing breakage of DNA strands and inhibition of both DNA and RNA synthesis. Doxorubicin inhibits the enzyme topoisomerase II, causing DNA damage and induction of apoptosis. When combined with iron doxorubicin also causes free radical-mediated oxidative damage to DNA, further limiting DNA synthesis (46). It is used in the treatment of a wide range of cancers including acute lymphoid leukemia, acute myeloid leukemia, Hodgkin's disease, breast cancer, and metastatic breast cancer (47). When used as a single agent, doxorubicin is administered as 60 to 75mg/m^2 IV doses over 3 to 10 minutes, day 1, every 21 days until there is an observed effect (48). In the absence of disease progression or unacceptable toxicity, doxorubicin is administered in combination with other chemotherapy drugs, with doxorubicin as a 40 to 75mg/m^2 IV dose every 21 days for 4 to 6 cycles (49,50). Lifetime cumulative doses above 550mg/m^2 are associated with an increased risk of cardiomyopathy (47).

Supportive drugs, including colony-stimulating factors, are usually administered during doxorubicin treatment to stimulate the production of white blood cells and prevent the risk of neutropenia. The initial adult dose is 5mcg/kg once a day via subcutaneous injection, short IV infusion (over 15 to 30 minutes), or continuous IV infusion. Doses may be escalated in units of 5 mcg/kg doses in each chemotherapy cycle, and treatment is continued up to 2 weeks or until the absolute neutrophil count (ANC) nadir reaches 10, 000/mm^3. Doses should be given at least 24 hours after cytotoxic chemotherapy. Transient increases in neutrophil counts are usually observed 1 or 2 days after starting treatment (28).

3. Pharmacokinetics and Pharmacodynamics

3.1 Pharmacokinetics

Pharmacokinetics (PK) describes the liberation, absorption, distribution, metabolism, and excretion (LADME) of a compound (51,52). These are not independent events and all five processes may occur simultaneously.

Liberation (L) is the process by which the drug is released from its pharmaceutical form (e.g., capsule, tablet, suppository, etc.). The most common routes of drug administration are:

injection, inhalation, sublingual, peroral, dermal, and rectal (52). Absorption (A) refers to the movement of the drug from the site of administration to the blood circulation. Bioavailability (F) is defined as the fraction (percentage) of an administered dose of unchanged medicine that reaches the blood stream (systemic circulation). The bioavailability of a drug is decreased by the first past effect: when the drug is affected by the liver before it enters to systemic circulation. Further, the ability of the compound to pass through lipid membranes also affects the bioavailability (52).

Distribution (D) is the process by which drug diffuses from the intravascular space to the extravascular space and into tissues and organs. The amount of distribution of drug compounds is determined by their physicochemical properties. The drug binding to plasma proteins (albumin) does not have a pharmacological effect; only the unbound fraction of the drug will do so. Redistribution is the transfer of a drug between the different compartments within the human body. The volume of distribution (V_D) is a parameter that reflects the extent to which a drug is distributed in the extravascular tissues rather than the plasma. A higher V_D indicates a greater amount of tissue distribution (52).

Metabolism (M), which mainly takes place in the liver, is the chemical transformation of drugs by enzymes into compounds that are easier to eliminate. There are two types of drug kinetics: zero order kinetics and first order kinetics. The zero order kinetics is when the rate of metabolism and/or elimination remains constant and is independent of the concentration of a drug (e.g., metabolism of alcohol). First order kinetics is when the rate of metabolism and/or elimination is directly proportional to the plasma concentration of the drug (applies to most drugs) (52).

Excretion (E) is the elimination of unchanged drug or metabolite from the body via renal, biliary, or pulmonary processes. Clearance (CL) is defined as the volume of plasma cleared from the drug per unit time, expressed in units of volume/time (L/hr). An equation to calculate clearance is to divide the rate of drug elimination by the plasma concentration (53,54). The Half-life ($t_{1/2}$) is the time required for the plasma concentration of a drug to be reduced by half from its initial value. After 4 half-lives, more than 90% of the drug will be eliminated. For most drugs at steady state, clearance remains constant so that drug output equals drug input. Drug clearance is influenced by age and disease (53,55).

3.2 Pharmacodynamics

Pharmacodynamics (PD) is the quantitative study of the relationship between drug exposure (dose or concentration) at the site of action and any resulting effect, namely the intensity and time course

of the effect, biochemical, physiological, toxicological responses and adverse effects (56). Every functioning molecule in an organism is a potential site of action for a drug. The drug may interact with cell membrane receptors such as G-protein coupled receptors, ion channels, and protein kinase receptors; or it may interact with intracellular receptors, enzymes, and DNA. Also the drug may not interact with receptors and only have a physical/chemical effect. There are different types of drug-receptor interactions. An agonist is a drug that has a similar effect to that of the endogenous receptor activator. An antagonist is a drug that binds to a receptor and prevents its activation. A partial agonist is a substance that has some agonistic action at a receptor but does not elicit the complete response of a true agonist. Inverse agonist binds to the same receptor as an agonist, but not to the same active site. It elicits a response that is opposite to the agonistic response and has a negative efficacy (57).

Dose-response relationships are usually described by potency (ED_{50}), maximum drug effect (E_{max}), and therapeutic range ED_{50} is the dose of a drug required to produce 50% of that drug's maximal effect (58). It is a property that is dependent on affinity but not related to efficacy. E_{max} is the maximum drug effect that can be achieved. The terms that are related to the efficacy and toxicity of cancer chemotherapy are important for the understanding of their pharmacological activity. The therapeutic range of serum drug concentrations is the range of concentrations associated with therapeutic response without side effects in the majority of patients.(52). In chemotherapy, the administered dose is based on the maximum tolerated dose (MTD) rather than dose-response. MTD represents the highest dose of a given drug that can be tolerated in absence of irreversible side effects by a population sample. Dose intensity (DI) in chemotherapy is a measure of drug dose delivered per unit of time and is expressed as (mg/m^2) per week. (59,60).

3.3 PK/PD Modelling and Quantitative Systems Pharmacology

PK/PD modelling expresses pharmacokinetic and pharmacodynamic properties with a set of mathematical expressions that describe exposure-response relationships. PK/PD models can be described by simple equations such as linear, Emax or sigmoid Emax model (61,62). Quantitative Systems Pharmacology (QSP) can be viewed as a subdiscipline of pharmacometrics that characterizes biological systems, diseases processes and drug pharmacology through mathematical computer models. In pharmacology, QSP models are typically defined by systems of ordinary differential equations (ODEs) that describe the dynamical properties of the interaction between the

drug and the biological system. QSP can be used to generate hypothesis *in silico* to aid in the design of *in vitro* or *in vivo* non-clinical and clinical experiments (63,64).

4. Tumour Growth Models

The most common mathematical models of tumour growth are deterministic in nature, and can involve a whole spectrum of details from macroscopic (empirical) to microscopic (mechanistic) levels. In this work, we have chosen a deterministic approach to model the tumour dynamics using ordinary differential equations.

4.1 Deterministic Approaches

The simplest model is the exponential model which assumes infinite tumour growth without constraints, starting from an initial value N(0) and given by the following equation at time t:

$$\frac{dN}{dt} = \alpha N$$

Where N =N(t) is the population of cancer cells (expressed as cell count, weight or tumour volume), dN/dt is the tumour growth rate, and the constant α is the growth rate. The advantage of this model is that it has only one constant (α) to estimate. Nevertheless, it is known that the availability of resources will limit tumour growth and therefore this model is not totally realistic. Typically, the individual growth rate of tumours slows over time.

The logistic model incorporates a maximum size and reaches a stable equilibrium at that point, as expressed by the following logistic model:

$$\frac{dN}{dt} = \alpha N - \frac{\alpha N^2}{K}$$

Where K is the system's carrying capacity, which is the capacity of the environment to support the population. We can put this equation in the extreme cases to understand its behaviour: when K tends to infinity, we get back to the exponential function; when K goes to 0, N tends to 0, so no further growth when the carrying capacity is limited. The advantage of this function is that it accounts for the limited resources to which the tumour has access. However, the logistic growth function has very peculiar features: for one the location of the point of inflection is exactly halfway

between the two asymptotes and secondly there is a radial symmetry in relation to that point. The inflection point is the point of the curve where the curvature changes its sign while a tangent exists (65). According to some authors the logistic model is rigid because growth rates symmetric around the point of inflection are not realized in many growth processes (66,67).

The Gompertz model is another widely used sigmoidal function originally developed to describe human mortality (61) and was eventually used to model the growth in size of entire organisms (68). This model has shown to provide the best fit for the growth of breast and lung cancer (69). It is given by the following equation:

$$\frac{dN}{dt} = \alpha N - \beta N ln N$$

Where α is the maximal growth rate and β is a parameter such that $\exp(\alpha/\beta)$ provides the maximal tumour size. The exponential component is used to describe the beginning of the tumour growth, but over the time course, the time required to double the tumour mass increases, cancerous cell growth will become slower and limited, resulting in a sigmoidal curve that is asymmetrical with the point of inflection (68,70). In practice, it is the point at which the rate of growth gets maximum value, and after that point exhibits a progressively diminishing instead of increasing growth rate (67,71).

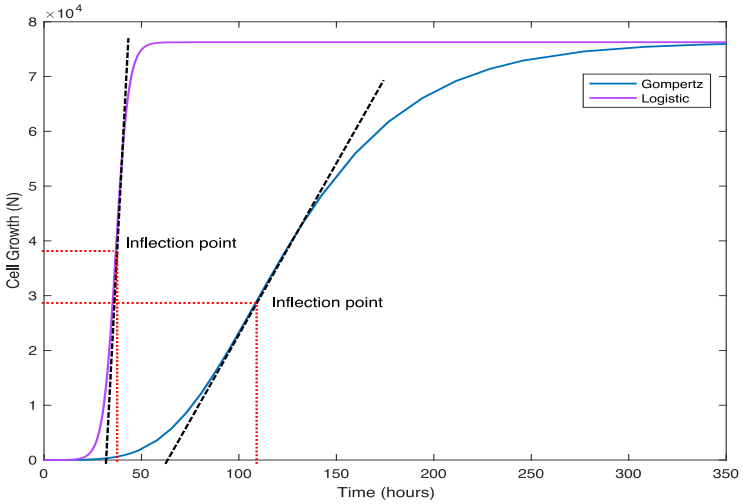

Figure 2. The Gompertz and Logistic curves and their inflection points.

The logistic model also includes two constants (α and K), but the point of inflection of Gompertz is smaller than that of the logistic and there is no symmetry involved (66,71). As it is the case of all deterministic models, the limitation of the Gompertz model is that it only describes the average behaviour of tumour growth but not the variability. It has been reported that the exponential and logistic models have failed to describe the experimental data tumour growth whereas the Gompertz model generated very good fits (69). For the purpose of our study, Gompertz growth model was considered to be the most appropriate.

4.2 Probabilistic Approaches

A stochastic process describes how a random variable (or set of random variables) changes over time and/or space (72). A stochastic process assigns a probability to each event and allows for the prediction of the probability of a certain outcome. A Markov process is a stochastic process ("memory-less") for which predictions can be made regarding future outcomes based solely on the present state of the system, with its future and past states being independent (73). In cancer, Markov chains have been used to predict the probability of a tumour to develop into metastasis. For example, the primary tumour corresponds to the position 1, and 0s elsewhere (no initial metastases). The spread of the cancer to other sites is modeled as a directed random walk on the Markov network, moving from site to site with estimated transition probabilities (74,75).

5. Models of Granulopoiesis and Myelosuppresion

Hematopoietic Stem Cells (HSCs) are immature cells found in the peripheral blood and in the bone marrow and develop into all types of blood cells (76). Granulopoiesis involves a series of maturational steps leading to the formation of granulocytes, including neutrophils, eosinophils, and basophils (77). There are different models of granulopoiesis (78–80). One of the pioneering model consists of five pools or compartments (78). The proliferative pool is assumed to be composed of two compartments, with an active compartment A and a resting compartment G_0. The maturation compartment M is where all cells mature for a fixed time, and then enter the marrow reserve compartment R. The latter compartment is like G_0, because all cells are held equivalently and can leave at random to enter the blood B. The rates α, β, and γ, particularly in this model control the release of cells along the indicated compartments and depend on the total number of cells in the system. The full representation of this neutrophil production model by S. I. Rubinow and J.L. Lebowitz, 1975 can be retrieved from reference (78).

5.1 The Friberg Model

Friberg's model consists of a semi-mechanistic PK/PD model describing the full time course of chemotherapy-induced myelosuppression through drug-specific parameters and system-related parameters, which are common to all drugs (79). The model mimics the myelopoiesis and consists of a compartment representing proliferative cells in the bone marrow, three maturation compartments with drug-insensitive cells and one compartment reflecting circulating neutrophils in the blood. The proliferative rate of the circulating neutrophils when the numbers are low is regulated with a feedback mechanism. The system-related parameters estimated are, baseline neutrophil count (ANC_0), mean transit time (MTT), and feedback factor (γ) as well as the drug effect parameters (Slope for a linear model or E_{max} and EC_{50} for an E_{max} model). The model has the structure to explore how neutropenia is related to febril neutropenia (81). The drug affected the proliferation of sensitive cells by either an inhibitory linear model or an inhibitory E_{max} model (79,81).

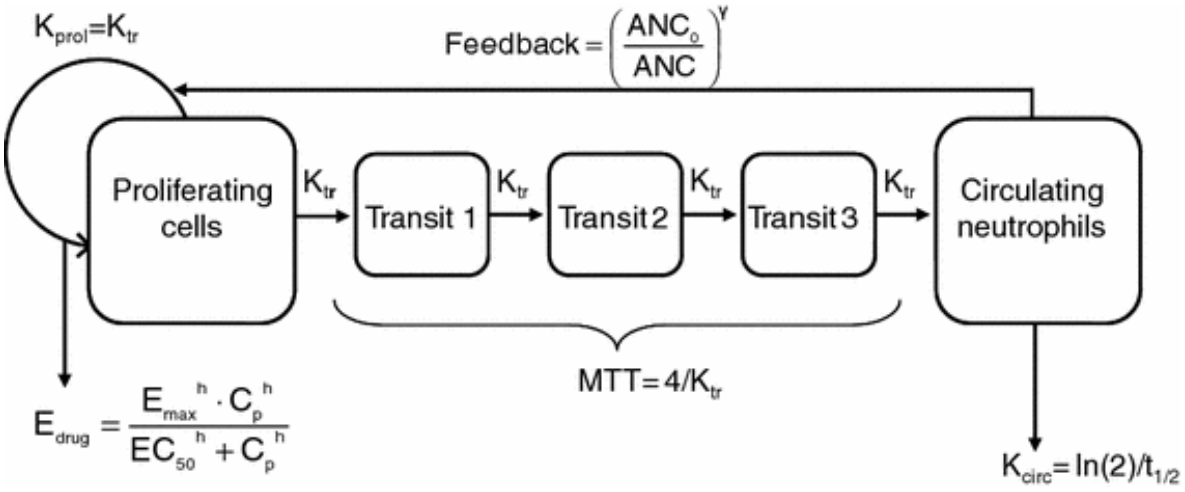

*Figure 3. The semi-mechanistic model of myelosuppression with the estimated system-related parameters (ANC_0), mean transit time (MTT), feedback factor (γ) and the drug effects parameters (E_{max} and EC_{50}), transit rate constant (K_{tr}), elimination rate constant for circulating neutrophils (K_{circ}), and $(ANCO/ANC)^{\gamma}$, f*eedback loop from the circulating neutrophils regulating the proliferative rate (81). *Reproduced with permission from Cancer Chemotherapy and Pharmacology. 69, 881-890 (2012). Copyright 2011, Springer Nature.*

Chapitre 2 – Objective and Hypothesis

Hypothesis

The QSP framework developed represents a rational way to predict optimal doses of doxorubicin-G-CSF in the context of triple negative breast cancer that can have an influence in the clinical practice.

Objective

The objective of this work is to develop a Quantitative Systems Pharmacology (QSP) framework to establish optimal cytotoxic chemotherapy regimens that maximize tumour shrinkage while minimizing the risk of neutropenia through mathematical modelling for the optimization of doxorubicin-G-CSF regimens in the context of triple negative breast cancer.

To accomplish this goal, my specific objectives were to :

- Investigate mathematical models that describe the behaviour of tumour growth using a deterministic mathematical approach.
- Integrate PK models of both doxorubicin and endogenous and exogenous G-CSF.
- Describe the PDs of tumour growth inhibition by cytotoxic chemotherapy, the myelosuppressive effects of doxorubicin on neutrophil production, and the myelostimulative effects of G-CSF on neutrophils using mechanistic mathematical models.
- Estimate parameters for tumour growth inhibition from *in vitro* studies of the effects of doxorubicin on triple negative breast cancer
- Interrogate on the optimal cycle length and dose size of doxorubicin with G-CSF support by simulating numerous scenarios using the full model.
- Develop an algorithmic approach to balance the anti-cancer and the neutropenic effects of both drugs.

Chapitre 3 – Article

"In preparation"
Optimizing Cytotoxic Chemotherapy by Maximizing Solid Tumour Shrinkage and Minimizing Neutropenia in the Context of Triple Negative Breast Cancer

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Key words: cytotoxic chemotherapy, neutropenia, granulopoiesis, mathematical modelling, triple-negative-breast cancer

Abstract

Cytotoxic chemotherapy continues to be the first line treatment option for the majority of cancers. A major side effect in chemotherapy regimens is neutropenia. Prophylactic therapy with granulocyte colony-stimulating factor (G-CSF), an endogenous cytokine responsible for neutrophil production regulation, is administered concomitant to chemotherapy. The proper timing of combined chemotherapy and G-CSF is crucial to treatment outcomes. Leveraging previous work that optimized regimens based on G-CSF timing, we developed a quantitative systems pharmacology (QSP) framework to investigate the modulation of chemotherapy dose intensity and frequency to maximize anti-tumour effects through mathematical modelling. We combined a model of tumour growth, with pharmacokinetics and pharmacodynamic models of doxorubicin and G-CSF, and a QSP model of neutrophil production to simulate prophylactic regimens against triple negative breast cancer (TNBC) with the aim of establishing schedules that best control tumour size while minimizing neutropenia. Our results suggest that cytotoxic chemotherapy with doxorubicin 45mg/BSA every 14 days, has a positive impact on tumour growth control, and that the delayed timing of G-CSF within these intensive schedules mitigates the risk of neutropenia. Importantly, we also performed multi-objective optimization within a large range of dose sizes and cycle lengths and established an algorithmic approach for designing optimal combination cytotoxic chemotherapy with G-CSF, suggesting future avenues for optimal regimens of chemotherapy with

prophylactic G-CSF support. This work demonstrates how rational considerations can positively impact on clinical decision-making in TBNC.

1. Introduction

Breast cancer accounts for about 25% of all new cancer cases in women (1). Hormone receptors estrogen (ER) and progesterone (PR) in breast cancer are both prognostic and predictive factors. Patients whose tumours are ER+PR+ are more likely to respond to antihormone therapy, and the higher the ER, the more likely the response. They have better short-term and long-term survival as a result of therapy, too. Therefore these receptors are crucial for the selection of appropriate and effective treatments with antihormone therapies such as tamoxifen, aromatase inhibitors, and selective estrogen degraders (39,40). Triple Negative Breast Cancer (TNBC), a particularly aggressive subtype, is characterized by a lack of expression in these hormonal receptors and amplification/overexpression of HER2. Only about 15% of diagnosed breast cancers are triple negative, which are more challenging to treat, and have overall worse outcomes (33–35,39).

Anti-cancer regimens generally blend a combination of drugs, typically cytotoxic chemotherapies that act to disrupt the cell cycle (19,29,82). Doxorubicin is an anthracycline that intercalates within DNA base pairs, causing breakage of DNA strands and inhibition of both DNA and RNA that is used for the treatment of TNBC (46). However, as with all cytotoxic chemotherapies, a major side effect of doxorubicin is chemotherapy-induced neutropenia, or a lack of neutrophils, the most abundant white blood cell in the body. To mitigate the risk of neutropenia, supportive granulocyte colony-stimulating factor (G-CSF) therapy is generally administered concomitantly with cytotoxic chemotherapy (29,83), typically 24 to 72 hours after chemotherapy (28). Using a mechanistic mathematical model of neutrophil production, we have recently shown that G-CSF delayed to 7 days after the administration of chemotherapy improves or completely eliminates the neutropenic status in an average patient (29,84), a finding in line with other mechanistic modelling work (82,85).

As standard-of-care protocols leveraging cytotoxic chemotherapies are not appropriate for every individual, mathematical modelling is particularly relevant in oncology as a mean to explore the treatment space with no harm to patients by allowing us to predict the impact of drug combinations (86), to delineate treatment outcomes before dosing to real humans (87), and to influence the pre-clinical decision-making process to choose the best dose and therapy schedules

(88). Leveraging our previous work in which we defined an optimized protocol for G-CSF concurrent to cytotoxic chemotherapy, here we focus on defining chemotherapy regimens that optimize the anti-cancer effect of doxorubicin and minimize the effect of neutropenia in TNBC. We combined a PK/PD model of both doxorubicin and G-CSF, our previous quantitative systems pharmacology (QSP) model of granulopoiesis (29), and a Gompertzian tumour growth model to rationalize the decision-making process with respect to defining dosing schedules that eliminate tumour bulk without exposing the patient to serious hematological side effects.

2. Methods

2.1 Tumour Growth Model

To model tumour expansion over time for both the non-drug and treatment scenarios, we used a model of Gompertzian growth. The Gompertz model is a widely used sigmoidal function, originally developed to describe human mortality, frequently applied to model tumour growth (68), given by the following equation,

$$\frac{dN}{dt} = \alpha N - \beta N \ln(\mathrm{N}), \quad (1)$$

where α is the growth rate and $\exp(\alpha/\beta)$ represents the maximal tumour size. Gompertzian growth is frequently adopted to model tumour cell growth dynamics that are initially exponential but slow over time, eventually resulting in a sigmoidal curve that is asymmetrical with respect to the point of inflection (68,70). The Gompertz model has been shown to fit closely to experimental data where other simple tumour growth models (e.g. exponential and logistic) failed (69).

2.2 Pharmacokinetic Models

2.2.1 Doxorubicin

Given its use for the treatment of TNBC, we selected doxorubicin as a representative cytotoxic chemotherapeutic agent (89). For this, we adapted a previously established three-compartment pharmacokinetic (PK) model based on previous population PK studies (90,91) given by

$$\frac{dC_1}{dt} = k_{21}C_2 + k_{31}C_3 - (k_{12} + k_{13} + k_e)C_1, \quad (2)$$

$$\frac{dC_2}{dt} = k_{12}C_1 - k_{21}C_2, \quad (3)$$

$$\frac{dC_3}{dt} = k_{13}C_1 - k_{31}C_3, \quad (4)$$

where C_1, C_2, C_3 denote drug concentrations of the drug in the central (1) and two peripheral compartments (2, 3), k_{ij} (hours^{-1}) represents the rate of transit between compartment i and j, and k_e (hours^{-1}) denotes the rate of elimination.

2.2.2 Endogenous and Exogenous G-CSF

The pharmacokinetics of G-CSF were described using our existing one-compartment model (29) (Fig. 1). This model delineates the dual routes of elimination of both endogenous and exogenous G-CSF from the plasma by internalization through the association to G-CSF receptors on the surface of neutrophils, and through linear renal elimination (84). The PKs of G-CSF is described by

$$G = \frac{k_a F(Dose_{GCSF})}{V_d} e^{-k_a t_{inj}} + G_{prod} - k_{ren}\mathrm{G}(t) - \mathrm{X}k_{int}\frac{G(t)^2}{G(t)^2 + K_D^2}\mathrm{N}(t), \quad (5)$$

which accounts for both exogenous and endogenous G-CSF pharmacokinetics. Here k_a (hours^{-1}) is the rate of subcutaneous absorption of exogenously administered G-CSF, F denotes the bioavailable fraction of exogenous drug, $Dose_{GCSF}$ (mg/BSA) denotes the exogenous dose, V_d is the volume of distribution of exogenous G-CSF (L), and $e^{-k_a t_{inj}}$ quantifies the rate of diffusion from the subcutaneous pool into circulation. G-CSF is produced endogenously and is the primary cytokine responsible for the production of neutrophils. Eq. (5) also accounts for the PKs of G-CSF in absence of exogenous administration (92), where G_{prod} represents the rate of G-CSF production (ng/ml/days), k_{ren} is the rate of G-CSF renal elimination (hours^{-1}), k_{int} is the rate of G-CSF receptor-internalization rate (hours^{-1}), K_D is the dissociation constant (ng/mL), and X is a correction factor ($X = G^{homeo}/N^{homeo}$), where G^{homeo} and N^{homeo} are the homeostatic concentrations of G-CSF and neutrophils.

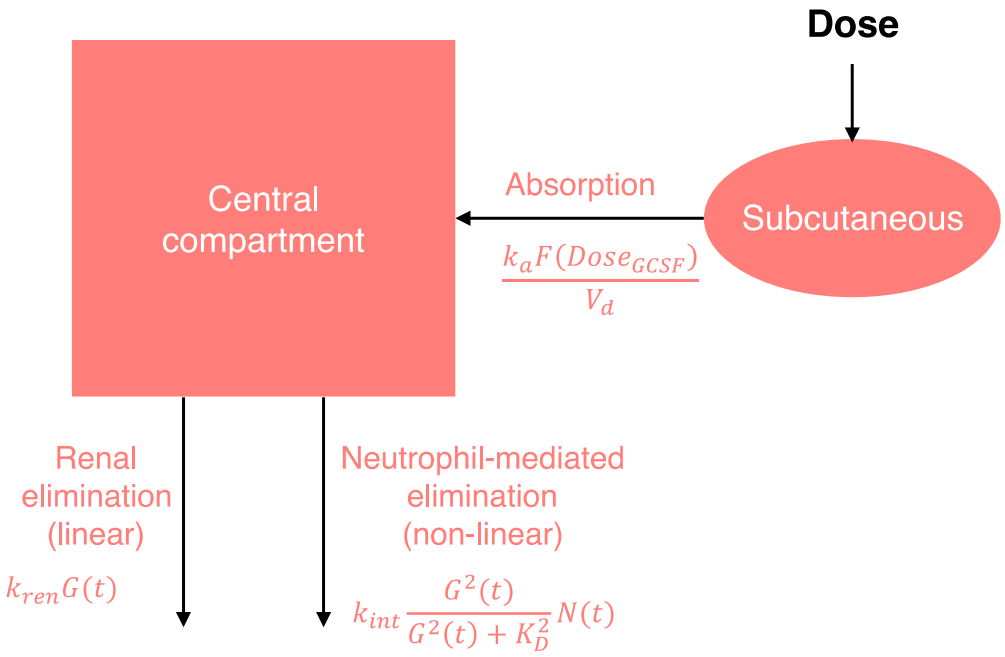

Figure 1. Schematic of the endogenous and exogenous G-CSF pharmacokinetic model. *Exogenous G-CSF is modelled as a subcutaneous administration, which diffuses from the subcutaneous pool into the blood with a rate* $k_a F(Dose_{G-CSF})/V_d$*. G-CSF elimination occurs through dual routes, primarily through neutrophil-mediated, non-linear elimination (from which the pharmacodynamic effect is exerted)* k_{int}*, and through renal, linear elimination* k_{el}*. Full details provided in Craig et al. 2015.*

2.3 Pharmacodynamics Models

2.3.1 Tumour Growth Inhibition by Cytotoxic Chemotherapy

The sigmoidal effects E_{max} model was integrated into the Gompertz model of tumour growth to represent the cytotoxic effects of the chemotherapeutic drug as

$$\frac{dV}{dt} = (\alpha V - \beta V ln V)(1 - E), \tag{6}$$

$$E = \frac{E_{max} {C_p}^h}{EC_{50} + {C_p}^h}. \tag{7}$$

Here E represents the observed effect of doxorubicin, E_{max} is the maximal effect (assumed to be 100%), EC_{50} is the plasma concentration inducing the half-maximal effect, C_p is the plasma

concentration of doxorubicin, and h is the Hill coefficient that determines the slope of the concentration-effects curve.

2.3.2 Neutrophil Production Model

We adopted our previously published QSP model from describing granulopoiesis the bone marrow beginning with the hematopoietic stem cells (HSCs) and ending with terminally differentiated circulating neutrophils (Fig. 2) (29). In this model, HSCs self-renew to maintain their population, die through apoptosis, or differentiate into the various blood lineages. Once committed to the neutrophils lineage, HSCs differentiate into progenitor cells that proliferate exponentially until they begin maturation, at which point they cease to divide. During maturation, the cells grow larger and gain receptors. At the end of the maturation process, cells are then stored in the bone marrow reservoir from which they are released into circulation. Cells in circulation then disappear through margination or apoptosis. Complete details on this model are found in (29).

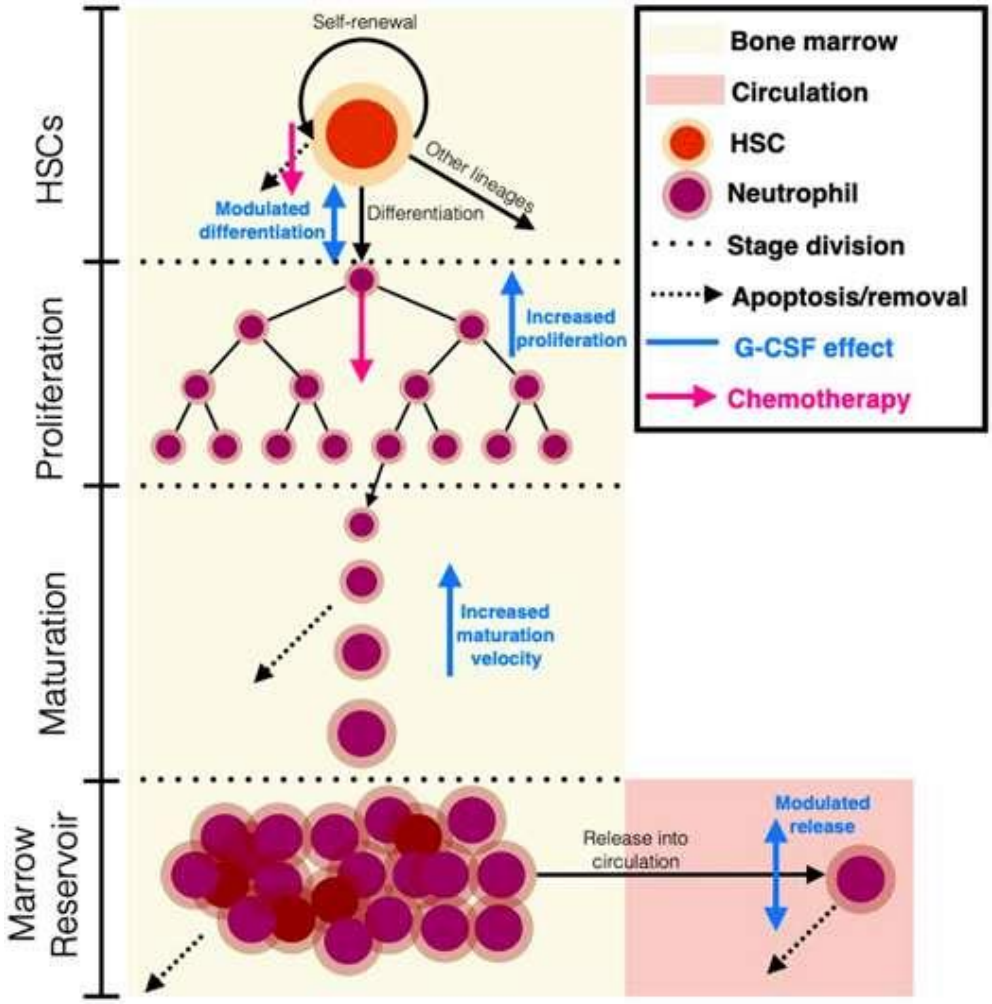

Figure 2. Schematic description of the neutrophil production model. *This model describes the process of granulopoiesis beginning at the top of the figure with the hematopoietic stem cells (HSCs) (red circle) in the bone marrow where they self-renew and die. GCSF modulates the differentiation of HSCs into neutrophils, increases their maturation velocity and proliferation. Chemotherapy inhibits differentiation of HSCs into the neutrophil lineage and inhibits neutrophil proliferation. Figure reproduced under CC BY 3.0 from Craig 2017.*

2.3.2.1 Myelosuppressive Effects of Doxorubicin on Neutrophil Production

During exposure to cytotoxic chemotherapy, granulopoiesis becomes significantly reduced because neutrophil progenitors are exposed to their antimitotic effects, ultimately causing myelosuppression. To model the myelosuppressive effects of doxorubicin, we again adopted the PD model from Craig, et al. 2015 (29). Briefly, the effects of doxorubicin on the bone marrow were modelled as a linear increase in the death rate of HSCs by,

$$\gamma_s^{chemo}\left(C_p(t)\right) = \gamma_s^{homeo} + h_s C_p(t), \tag{8}$$

where γ_s^{chemo} is the rate of apoptosis of HSCs (hours^{-1}), γ_s^{homeo} is the homeostatic rate of apoptosis in the proliferative HSCs pool (days^{-1}), and h_s is the first-order effect of chemotherapy on HSC apoptosis. When no chemotherapy is given, $C_p(t) = 0$, the expression in Eq. (8) reduces to the homeostatic rate of apoptosis. The cytotoxic effects of doxorubicin on neutrophil progenitors was modelled using a non-linear effects model given by

$$\eta_{NP}(G(t), C_p(t)) = \eta_{NP}^{chemo}\left(C_p(t)\right) + \frac{(\eta_{NP}^{max} - \eta_{NP}^{chemo}(C_p(t)))(G(t) - G^{homeo})}{G(t) - G^{homeo} + b_{NP}}, \tag{9}$$

where η_{NP} is the rate of neutrophil proliferation (days^{-1}), η_{NP}^{max} is the maximal proliferation rate of neutrophils (days^{-1}) and η_{NP}^{chemo} is the minimal proliferation rate of neutrophil progenitors during chemotherapy (days^{-1}). Here, G^{homeo} is the concentration of G-CSF at homeostasis (ng/mL). Note that the proliferation of neutrophil progenitors is simultaneously affected by chemotherapy (when administered) and exogenous/endogenous G-CSF.

2.3.2.2 Myelostimulative Effects of G-CSF on Neutrophils

G-CSF regulates neutrophil production along the entire neutrophil lineage. The G-CSF effects on the differentiation of HSCs is modelled through $\kappa_N(N)$ (days^{-1}) given by

$$K_N(N) = f_N \frac{\theta_1^{S_1}}{\theta_1^{S_1} + N^{S_1}}, \tag{10}$$

where f_N is the maximal rate of neutrophil differentiation (days^{-1}), θ_1 is the half-maximal concentration of neutrophil differentiation (10^9cells/kg), and s_1 is the Hill coefficient regulating the slope of the sigmoidal curve. The simultaneous effects of the cytotoxic agent and G-CSF in the stem cell compartment were modelled by

$$\gamma_S(G(t), C_p(t)) = \gamma_S^{min} - \frac{(\gamma_S^{min} - \gamma_S^{chemo})b_S}{G(t) - G^{homeo} + b_S}, \quad (11)$$

where γ_S is the rate of HSC apoptosis (days^{-1}), γ_S^{min} is the minimal apoptosis rate in the HSCs proliferative phase (days^{-1}), γ_S^{chemo} denotes the rate of HSC apoptosis during chemotherapy (days^{-1}), and b_S is the HSC apoptosis half-effect parameter (ng/mL). The effects of G-CSF on the proliferative rate of the neutrophils were described by Eq. (9). The effects of G-CSF on the death rate during maturation were given by

$$\gamma_{NM}(G(t)) = \gamma_{NM}^{min} - \frac{(\gamma_{NM}^{min} - \gamma_{NM}^{homeo})b_{NM}}{G(t) - G^{homeo} + b_{NM}}, \quad (12)$$

where γ_{NM} is the rate of apoptosis during maturation (days^{-1}), γ_{NM}^{min} is the minimal rate of apoptosis during maturation (days^{-1}), γ_{NM}^{homeo} is the homeostatic rate of apoptosis during maturation (days^{-1}), and b_{NM} is the half-effect parameter (ng/mL). G-CSF is known to modulate the speed of neutrophil maturation, as immature neutrophils can be observed in circulation after doses of exogenous G-CSF (93). This effect was modelled by

$$V_N(G(t)) = 1 + (V_{max} - 1)\frac{G(t) - G^{homeo}}{G(t) - G^{homeo} + b_V}, \quad (13)$$

where V_{max} denotes the maximal maturation velocity (days^{-1}) and b_V is the Michaelis-Menten parameter of maturation speed. Lastly, G-CSF modulates the rate of exit of the mature neutrophils from the neutrophil bone marrow reservoir into circulation. This effect was modelled as

$$f_{trans}(G(t)) = trans^{homeo}\frac{trans^{ratio}(G(t) - G^{homeo}) + b_G}{G(t) - G^{homeo} + b_G}, \quad (14)$$

where $trans^{homeo}$ relates the homeostatic rate of transit from the neutrophil reservoir into the circulation (days^{-1}), $trans^{ratio} = trans^{max} - trans^{homeo}$ represents the fold-difference in release from the bone marrow reservoir, and b_G the half-effect parameter of transit from the mature pool to circulation (ng/mL).

2.4 Parameter Estimation and Calibration

We performed a step-wise approach to parameter estimation by integrating various sources of data from existing literature. For the tumour growth model parameters, we digitized the data from the *in vitro* experimental study of McKenna et al., 2017 in which four different cancer cell lines from metastatic TNBC were treated with doxorubicin for 30 days to assess cell growth dynamics (89). We began by estimating the parameters in Eq. (1) from TNBC cell line MDA-MB-468 (derived from a pleural effusion metastatic site) (89). We first fit Eq. (1) to the control case in absence of the drug to estimate the growth rate α. We then fixed α and estimated the asymptotic tumour growth parameter β to data from observed *in vitro* growth after exposure to 625ng (maximum dose administered in the experiments). All parameter estimations were performed by minimizing the least squares difference between observed and predicted values using *fmincon* in MATLAB R2019a (94).

2.5 Establishing Effective and Minimally Toxic Chemotherapy Regimens

To interrogate on the optimal cycle length and dose size of doxorubicin with G-CSF support, we used our calibrated tumour growth model, fixed the G-CSF regimen to the optimal schedule (4 daily subcutaneous doses of 480mcg beginning 7 days after chemotherapy) determined by Craig et al., 2015, and simulated numerous scenarios using the full model described in Eqs. (1)-(16). To quantify the tumour fold increase (FI), we compared the initial tumour size to predictions of tumour growth volume 15 days after the last dose of chemotherapy by

$$FI = \frac{Total\ tumour\ size\ at\ the\ end\ of\ the\ last\ dose + 15\ days\ (V)}{Initial\ Tumour\ Size\ (V0)}. \qquad (15)$$

To simultaneously quantify both the positive tumour shrinkage effects of chemotherapy and the neutropenic effects, we coupled the fold increase (FI) with the fold decrease (FD) of the neutrophils given by

$$FD = {nadir}\Big/{0.22}\Big/{0.585}, \qquad (16)$$

where 0.22/0.585 the total blood neutrophil pool (29). To select optimal cycle lengths and dose sizes, we then minimized the function

$$f_{opt} = a * FD_{inv} + b * log10(FI), \quad (17)$$

where FD_{inv} is 1/FD. The logarithmic fold increase was used to provide comparable scales between FD_{inv} and $log(FI)$. Here, a and b are weights that assign a relative importance to the reduction of neutropenic effects and the tumour reduction effects of each regimen.

3. Results

3.1 Biweekly Chemotherapy Cycles Reduce Neutropenia

We fixed the G-CSF regimen as described in the methods and simulated chemotherapy cycle lengths between 7 and 28 days (Table 1). Doxorubicin dose sizes were set to be the standard 60 mg. Our results show that chemotherapy cycles beyond 14 days allow more time for tumour regrowth, from a deterministic point of view and those shorter cycles keep tumour growth better controlled (Fig. 3A), whereas neutrophils benefitted from longer treatment cycles (Fig. 3B).

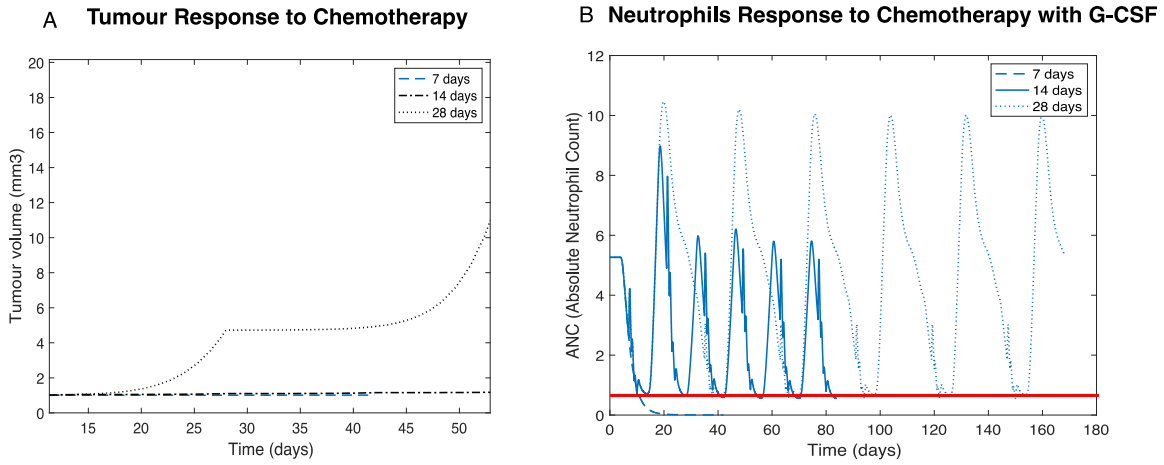

Figure 3. Effect of cycle length on tumour growth and neutropenia.. *A) Tumour dynamics for representative cycle length (7, 14, and 28 days). Doxorubicin doses were fixed to 60 mg and G-CSF was administered according to our previously determined optimal regimen (see Craig et al. 2015). B) Neutrophils response to the same representative cycle lengths.*

By varying a and b in Eq. (19), we were able to weight the relative importance of minimizing neutropenia versus maximizing tumour shrinkage, thereby providing a rational decision-making tool for clinicians. For all values of a and b that we explored, the optimal cycle lengths were 12, 13, and 14 days: 13 days if both maximing shrinkage and minimizing neutropenia are equally important, 12 days is when minimizing neutropenia was less important (Table 2).

Cycle Length	a=1, b=1	a=1, b=0,5	a=0,5, b=1
7	2181.2	2181.0	1090.7
8	1199.53	1199.40	599.91
9	522.11	521.94	261.23
10	7.41	7.19	3.93
11	2.58	2.31	1.55
12	1.94	1.62	1.28
13	1.92	1.56	1.33
14	2.00	1.58	1.42
15	2.15	1.66	1.55
16	2.41	1.86	1.75
17	2.63	2.01	1.93
18	2.77	2.08	2.08
19	2.86	2.08	2.20
20	2.95	2.08	2.35
21	3.08	2.10	2.51
22	3.25	2.17	2.7
23	3.44	2.25	2.91
24	3.65	2.34	3.13
25	3.88	2.46	3.36
26	4.17	2.64	3.61
27	4.43	2.80	3.85
28	4.64	2.92	4.05

Table 1. Optimization (f_opt) by Cycle Length. *Optimal schedules were explored by varying a and b, i.e. the relative weights of minimizing neutropenia or rapidly shrinking tumour size. As before, doxorubicin dose sizes were fixed the standard amount (60 mg) and G-CSF was administered in 480 mcg doses for 4 days starting 7 days post-chemotherapy. Smaller numbers represent the best cycle lengths and are marked in yellow.*

3.2 Lower Doxorubicin Doses Eliminate Neutropenia, Intermediate Doses Provide a Balance between Antitumour Effects and Minimal Neutropenia

Given that 14 day cycles are the most clinically-feasible and that there was little difference between the optimal 12/13 day and 14 day cycles in all scenarios we investigated (Table 1), we fixed the cycle length to 14 days and investigated the effects of varying doxorubicin dose sizes. We considered only clinically-relevant doxorubicin doses (30 mg, 45 mg, 60 mg, 75 mg, 100 mg, 125 mg), and simulated 14-day cycles at each dosing level. As expected, the tumour shrinkage effects were maximized by the largest dose, but this also came at the cost of severe neutropenic effects. We found that 45 mg and 30 mg doses were optimal when balancing tumour shrinkage and neutropenic effects. However, as seen in Table 2, 60 mg doses were not predicted to be significantly toxic (e.g. f_{opt} values of 1.24 versus 1.42 when weighting towards tumour shrinking) and provided more benefit in terms of the tumour growth effects. This finding again underlines the importance of a quantitative approach to therapy rationalization.

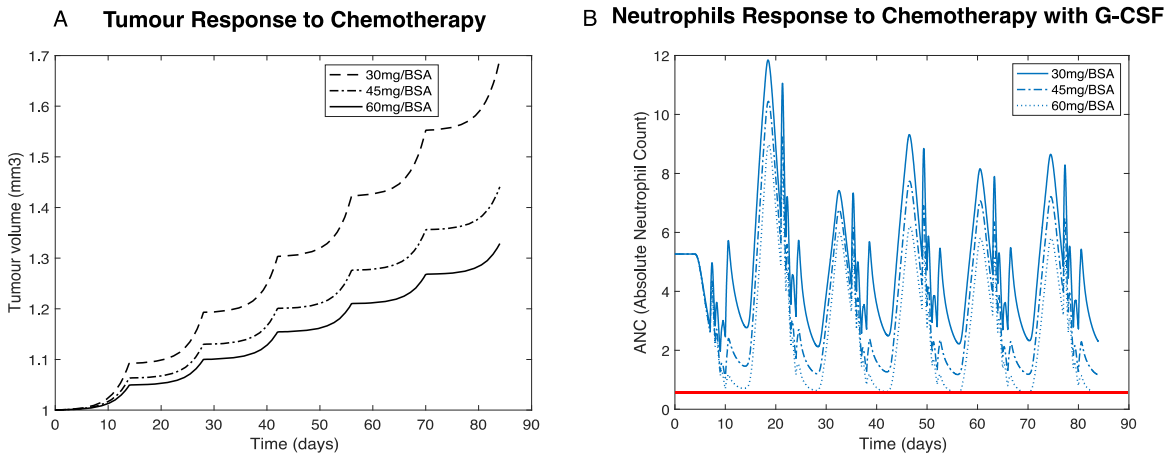

Figure 4. Effect of Dose Sizes (30, 45, and 60mg/BSA) with 14 days cycle. A) Tumour response to doxorubicin as single agent. B) Neutrophils response to Doxorubicin with G-CSF .

Dose Sizes	a=1, b=1	a=1, b=0.5	a=0.5, b=1
30	1.41	0.87	1.24
40	1.55	1.08	1.24
60	2.00	1.58	1.42
75	3.12	2.73	1.95
100	6.78	6.43	3.73
125	12.97	12.65	6.80

Table 2. Optimizing doxorubicin dose sizes*. Optimal schedules were explored by varying a and b, i.e. the relative weights of minimizing neutropenia or rapidly shrinking tumour size. According to our results from the step above, we fixed the optimal cycle length to 14days cycle for doxorubicin and G-CSF was administered in 480 mcg doses for 4 days starting 7 days post-chemotherapy. Smaller numbers represent the best cycle lengths and are marked in yellow.*

4. Discussion

Finding the right dose and regimen for each individual is the goal of precision medicine. In this work, we have leveraged a quantitative systems pharmacology approach to improve and guide therapeutic decision making for cytotoxic chemotherapy with prophylactic G-CSF support in the context of TNBC.

To explore the central question of how to best balance the tumour shrinkage effects of cytotoxic chemotherapy while reducing the neutropenia it induces, we first calibrated the Gompertz model to describe tumour growth dynamics in TNBC and coupled it with PK/PD models of doxorubicin. We next leveraged a QSP model of granulopoiesis (29) to consider the main components of a standard regimen: cycle length and dose sizes. Considering clinical feasibility and relevancy, we found that 14 day cycle lengths with dose sizes of 30 mg/m^2 and 45 mg/m^2 (and up to 60 mg/m^2) best mitigated the neutropenic risk while providing maximal tumour shrinkage benefits. Incorporating the QSP model of granulopoiesis to investigate the effects of both the chemotherapy and G-CSF in the neutrophil production pipeline was essential for us to predict the effect of doxorubicin alone, G-CSF alone, and the simultaneous effects of both drugs. This

approach enabled us to propose doxorubicin-G-CSF regimens that simultaneously maximize tumour killing while minimizing neutropenia. By exploring how the weighting of the positive and negative effects of combination doxorubicin and G-CSF affect outcomes, our results provide a rational and clinically-relevant decision-making strategy that is easily translatable to the clinic.

There are certain limitations to our work. The choice of the Gompertz model to describe tumour growth implies that cytotoxic chemotherapy effect controls tumour size but does not completely eliminate the tumour. Nonetheless, Gompertzian growth provides dose-variable carrying capacities, and initial exponential growth (therefore mimicking early tumour growth dynamics). Through model fitting, we successfully recapitulated *in vitro* growth dynamics and estimated the parameters for our PD model of tumour growth. The estimation of these constants is specific to the context of TNBC; using *in vivo* tumour growth observations would further strengthen our predictions of tumour growth dynamics. Future work should also consider other important doxorubicin side effects including cardiotoxicity. We could also explore other tumour growth models and include the development resistance to therapy to compare to the results presented here.

Despite these limitations, our approach contributes a rational scheme for determining combination chemotherapy and G-CSF regimens using doxorubicin that is easily implemented into the clinic. TNBC is an aggressive form of breast cancer that is difficult to treat. Thus, new approaches are necessary to improve outcomes. We believe that the modeling approach we present here can be a useful tool for the clinical practice, where there is a need to strike a balance between treatment aggressivity and a patient's tolerance to treatment. Ultimately, this work delineates a rational way to address prophylactic and supportive G-CSF treatment during cytotoxic chemotherapy.

5. Supplementary Information

Optimizing Cytotoxic Chemotherapy by Maximizing Solid Tumour Shrinkage and Minimizing Neutropenia in the Context of Triple Negative Breast Cancer

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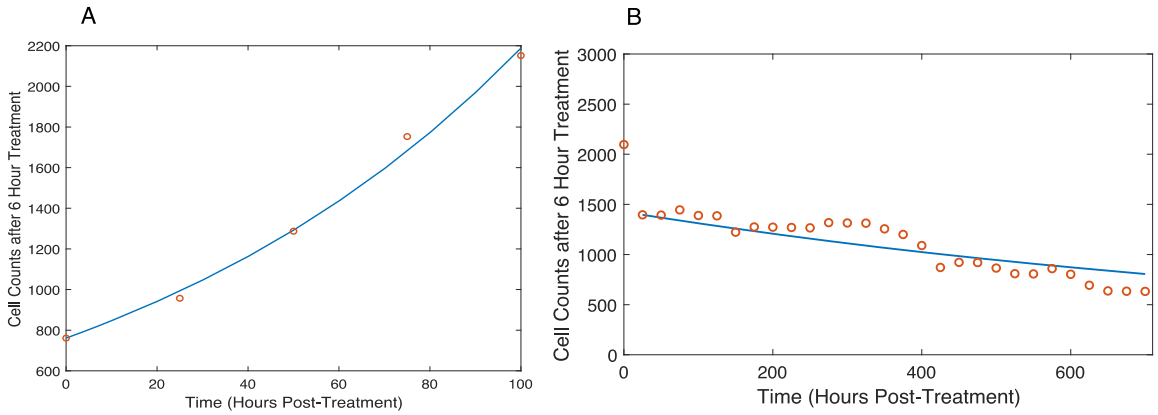

Figure S1. Fitting TNBC Cell Culture Growth Dynamics. *The effects of doxorubicin on TNBC cell line MDA-MD-468. Red circles: data digitized from McKenna, MT et al. 2017*(89)*; blue solid line: model fit A) Estimating* α *in absence of treatment (*$\alpha = 0.02265$ *(hours*$^{-1}$*)). B) Estimating* β *after exposure to 625ng of doxorubicin (*$\beta = 0.0011713$ *(cells)).*

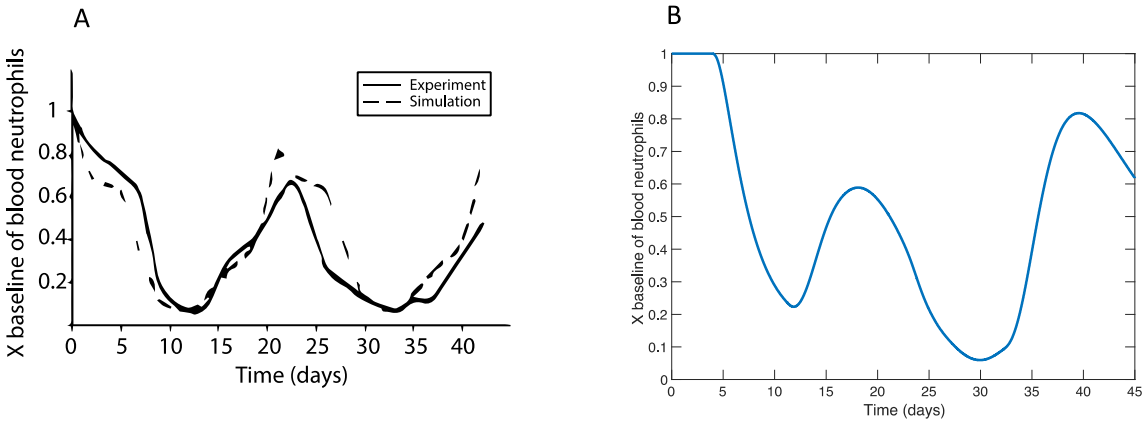

Figure S2. Neutropenic effects of doxorubicin. *A) In Vainstein et al., 2006, neutrophil counts from patients undergoing doxorubicin alone were collected and quantified as the relative fold-difference with respect to baseline. Results digitized from* (95)*. B) We calibrated the model from Craig et al. 2015* (29) *to the results in A.*

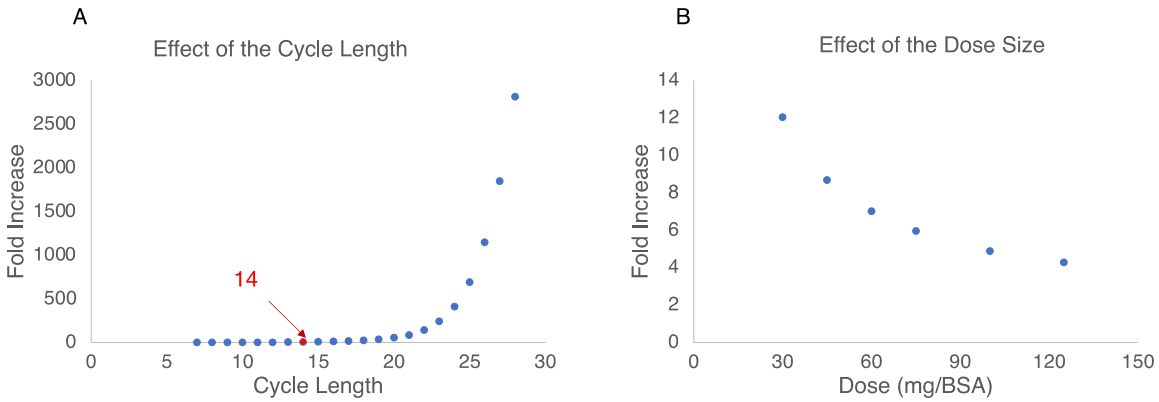

Figure S3. *A) Fold increase (FI) measurements obtained using Eq.15 in the main text for all cycle lengths (7 to 28 days), with dose sizes fixed to 60mg/BSA. B) FI measurements for all dose sizes (30, 45, 60, 75, 100, 125mg/BSA). See complete description in the Methods in the main text.*

Sensitivity analysis

We investigated the sensitivity of our predictions on parameters α and β in Eq. 19 by varying both by $\pm$ 20%. Results were then assessed by measuring the fold-difference with respect to α and β at their fixed values (see Figure S1).

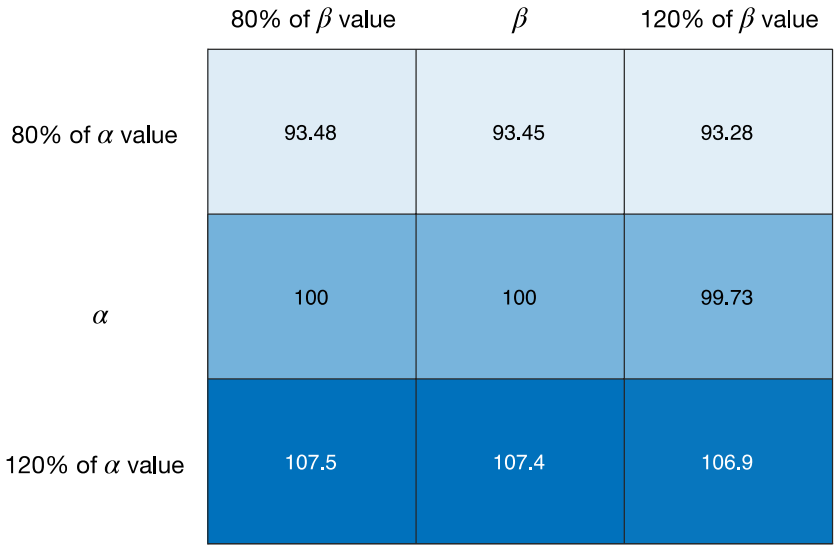

Figure S5. Sensitivity analysis of the tumour growth model. *The sensitivity of the Gompertzian tumour growth model to its parameters were explored by varying both α and β by ±20% and comparing the resulting tumour growth to the control case (where α and β values were fixed as in the main analysis).*

Chapitre 4 – Discussion

The first part of this work concerned the selection of a deterministic mathematical model of tumour growth, where determinism refers to models that only consider the average behaviour but not the variability. Among the different mathematical models, Gompertz model was selected since it proved to provide the best fits for breast and lung cancer data (69) and has the advantage to only involve two constants to be estimated (96). Then, we combined this model with the Sigmoidal Emax Model to build a pharmacodynamics (PD) model and explain the effect of the drug on the tumour growth. With this PD model, we could formulate several hypothesis. When no chemotherapy is given, the effect (E) is equal to 0, and there are tumoral cells dividing. But when chemotherapy is given and Emax is 1, the tumour cells will be affected and we expect the tumor cells to stop growing.

In the drug development process, dose-effect studies to confirm safety and efficacy of the drug are performed first on animal models and cancer cell lines. For the parameter estimation of the Gompertz model, we obtained them by digitizing the data from (89); the parameters estimations were found by minimizing the least squares difference between observed and predicted values using *fmincon* in MATLAB R2019a (94). The resulting estimated parameters are considered to be valid only for TNBC and for the context of this study. Therefore, these estimated constants should not be used to simulate dose-effect responses for other types of cancer since cancer tissue is specific to each cancer type, as well as the type of cytotoxic mechanism and potency of the drug. The parameters for the PK model of doxorubicin were obtained from (90).

In our pursuit to find the best cytotoxic chemotherapy – G-CSF regimen, we have leveraged the PK model of G-CSF and a Quantitative Systems Pharmacology (QSP) model of neutrophil production that describes the effects of cytotoxic chemotherapy and G-CSF in the bone marrow and circulating neutrophils, previously developed by Craig et al., 2015 (29). We next used our parameters estimations and PK/PD models of doxorubicin and G-CSF to simulate several doxorubicin-G-CSF regimens possibilities in MATLAB R2019a (94). We have explored the frequency and dose intensity of doxorubicin. Throughout this study, the G-CSF regimen was fixed as previously determined: 4 doses of G-CSF 480mg/m^2 starting on day 7 after chemotherapy (29). From our simulations, we concluded that 7 days cycle length of chemotherapy doses gave less time to the bone marrow to recover and the effect of G-CSF is lessened as well. Selection of a dosing

interval to sustain a desired level of effect has to take into account the time course of effect. Taking in consideration that half-life of doxorubicin is 20 – 48 hours (45,46), it would take almost 10 days to completely eliminate doxorubicin (5 half-life) in the extreme case. Therefore, cycles that are shorter than 10 days would not be good for the neutrophils, because the effects of the cytotoxic chemotherapy are still there. When we administer the biologic drug that mimics the action of endogenous G-CSF, the recovery of the neutrophils can become faster. Nevertheless, G-CSF and chemotherapy both affect the HSCs and neutrophils, but G-CSF can be dampened by high doses of chemotherapy. If dosed incorrectly this could also increase the risk of severe neutropenia.

On the side of the tumour growth, the less frequently the dose of chemotherapy is delivered, the more time we are allowing the tumour to have the chance to regrow, and therefore, spacing the doses for long time is not a choice neither. In respect to dose intensity, as could be expected, we confirmed that the highest dose in our simulations, 125mg, achieved the best anti-tumour effect, but this was disastrous for neutrophils. Our results suggest that cycle lengths of 7 days maintain the tumour within the smallest fold increase, with 14 days as the cycle that prioritizes the neutrophil counts. Neutrophil nadirs for cycles between 14 and 28 days are not significantly different, but severe neutropenic effects were observed for shorter cycles.

With these results, we have shown that a mathematical-based approach was an adequate method to select the best drug regimen. To dose doxorubicin, the importance was given to both the tumour size and neutropenia. The optimization function (f_Opt) considered the maximisation of tumour inhibition (b) and minimisation of neutropenic effects (a). Thanks to this function, we selected the best cycle length and dose intensity. In the optimization, we applied the logarithm to the fold increase of the tumour to linearize our results, and to limit the tumour volumes to a logarithmic scale base 10. As expressed in Eq.(17), we quantified the tumour growth by dividing the tumour volume at 15 days after the last dose of chemotherapy by the initial tumour volume. We also obtained the fold decrease (FD) by dividing the neutrophil nadirs by the baseline neutrophil counts. With regards to neutropenia minimization, the inverse was applied to FD. To minimize neutropenia, we expect that FD gets closer to zero. When FD tends to infinity, $FD_{inv} = \frac{1}{FD}$ tends to zero. This function allowed us to compare the different choices for determining the best one.

By implementing the optimization function, we were able to balance the effects of the cytotoxic chemotherapy and its side effects (neutropenia) while including the effects of G-CSF.

We found that 14 days cycle was optimal according to the optimization scheme. Regimens that best balanced the anti-tumour effects and still minimized neutropenia was 45mg every 14 days.

Lastly, we investigated the robustness of our estimations for the tumour growth parameters α and β through sensitivity analysis. In this study, we confirm that Gompertz model was more sensitive to changes in the α growth parameter but less so in the β parameter. Due to the fact that αN is an exponential, a fluctuation of $\pm 20\%$ is going to have a significant change on the number of cancer cells in the tumour. Nonetheless, because $\beta N ln(N)$ describes an asymptotic behaviour, a $\pm 20\%$ does not influence on the final size of the tumour size at all.

This work has its limitations that should be mentioned. The first limitation concerns the deterministic nature of the tumour growth model. Stochastic models can indeed represent better the tumour cell behaviour. However, Gompertz model is more suitable for its simplicity and the possibility to estimate its parameters in a real setting. We indeed estimated these parameters from experimental data of the dose-effect of doxorubicin on a specific TNBC cell line. The estimation of these constants is specific to the context of TNBC; using *in vivo* tumour growth observations would further strengthen our predictions of tumour growth dynamics.

Despite these limitations, our approach is relevant because we simulated numerous possibilities of cytotoxic chemotherapy regimens within the context of TNBC. Thanks to our previously optimized G-CSF regimen we were capable to balance together the anti-cancer effects of doxorubicin and the effects of G-CSF to prevent neutropenia. Finally, we achieved to select the best regimens through a numerical approach. Future work should also consider other important doxorubicin side effects including cardiotoxicity. We could also explore other tumour growth models and include the development resistance to therapy to compare to the results presented here. We believe that our modeling approach can have a real impact on the pharmaceutical industry in pre-clinical studies, where there is a necessity to translate experimental studies to drug regimens for cancer patients. TNBC is an aggressive form of breast cancer that is difficult to treat. Thus, new approaches are necessary to improve outcomes. Ultimately, we believe that the modeling approach we present here can have a real impact on clinical practices, where there is a need to strike a balance between aggressive treatment and a patient's tolerance to this treatment.

Chapitre 5 – Conclusion

In this work, we delineated a rational way to address prophylactic and supportive G-CSF treatment during cytotoxic chemotherapy with doxorubicin as single agent. We have demonstrated that mathematical and statistical approaches are important in the drug development process, especially to translate *in vitro* studies to *in vivo* human studies and to make predictions of the exact doses of a drug, that are safe and effective for the patients. We concluded that this numerical approach had the power to predict a number of cases that would have been risky to test in the clinical setting. Also, this study can guide clinicians to understand the importance of rational dosing in cytotoxic chemotherapy regimens for obtaining both positive outcomes and preventing side effects.

Future work should also consider other important doxorubicin side effects including cardiotoxicity. This aspect can be further investigated by adapting a pharmacodynamic model that describes relevant features of the heart and couple it to the effect of the drug (97). For example, the electrical properties of the heart and the changes on the rate of the heartbeat. Anthracyclines drugs such as doxorubicin can prolong the QT interval, which can lead to severe cardiac arrhythmias (98). Some authors investigated the effect of an anti-cancer drug that prolongs the QT interval, by developing a PK-PD model for translational purposes. The time course of the QT interval was described using an ordinary nonlinear differential equation with the following components: the individual heart rate correction, the circadian rhythm, and the drug effect (99,100). It has also been investigated a partial differential equation that describes the propagation of the electrical impulse of the heart to model conduction disturbances in the heart by coupling this model to the drug effect (101,102).

Références bibliographiques

1. Canadian Cancer Statistics. Health Rep. 2019;2(2):103–26.
2. Brenner DR, Weir HK, Demers AA, Ellison LF, Louzado C, Shaw A, et al. Projected estimates of cancer in Canada in 2020. Can Med Assoc J [Internet]. 2020 Mar 2;192(9):E199–205. Available from: http://www.cmaj.ca/lookup/doi/10.1503/cmaj.191292
3. National Cancer Institute. Environmental Carcinogens and Cancer Risk [Internet]. 2019 [cited 2020 Nov 24]. Available from: https://www.cancer.gov/about-cancer/causes-prevention/risk/substances/carcinogens
4. Loewe L. Genetic Mutation. Nat Educ [Internet]. 2008 [cited 2020 Mar 15];1(1):113. Available from: https://www.nature.com/scitable/topicpage/genetic-mutation-1127/
5. O'Connor C., Adams J. Unit 5: How Do Cells Know When to Divide? In: NPG Education, editor. Essentials of Cell Biology [Internet]. 2010th ed. Cambridge, MA, United States; Available from: https://www.nature.com/scitable/ebooks/essentials-of-cell-biology-14749010/122997842/
6. Murphy AE, Charnay-Sonnek F. Basis of Carcinogenesis. In: Principle of Nursing in Oncology [Internet]. 18 May 201. Springer, Cham; 2019. p. 1–17. Available from: http://link.springer.com/10.1007/978-3-319-76457-3_1
7. Jones RG, Thompson CB. Tumor suppressors and cell metabolism: a recipe for cancer growth. Genes Dev [Internet]. 2009 Mar 1;23(5):537–48. Available from: http://genesdev.cshlp.org/cgi/doi/10.1101/gad.1756509
8. Ferrier DR. DNA Structure, Replication, and Repair. In: Harvey RA, editor. Biochemistry (Lippincott's Illustrated Reviews Series) [Internet]. Sixth. Lippincott Williams & Wilkins; 2014. Available from: https://doctor2016.jumedicine.com/wp-content/uploads/sites/6/2019/01/lippincotts-biochemistry-6th-edition.pdf
9. Shammas MA. Telomeres, lifestyle, cancer, and aging. Curr Opin Clin Nutr Metab Care [Internet]. 2011 Jan;14(1):28–34. Available from: http://journals.lww.com/00075197-201101000-00006
10. Jafri MA, Ansari SA, Alqahtani MH, Shay JW. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med [Internet]. 2016 Dec 20;8(1):69. Available from: http://genomemedicine.biomedcentral.com/articles/10.1186/s13073-016-0324-x
11. U. S. National Institutes of Health NCI. SEER Training Modules, Cancer Classification [Internet]. [cited 2020 Mar 9]. Available from: https://training.seer.cancer.gov/disease/categories/classification.html
12. Vermeulen P, Pezzella F. Nonangiogenic tumor growth. In: Academic Press, editor. Tumor Vascularization [Internet]. Elsevier; 2020. p. 15–32. Available from: https://linkinghub.elsevier.com/retrieve/pii/B978012819494200002X
13. Canadian Cancer Society. Cancer Information:What is cancer? [Internet]. Types of Tumours. 2020 [cited 2020 Apr 19]. Available from: https://www.cancer.ca/en/cancer-information/cancer-101/what-is-cancer/types-of-tumours/?region=on
14. Stanford Health Care. Cancer diagnosis [Internet]. How Is Cancer Diagnosed? 2020 [cited 2020 Apr 17]. Available from: https://stanfordhealthcare.org/medical-conditions/cancer/cancer/cancer-diagnosis.html
15. Canadian Cancer Society. What is cancer? [Internet]. Staging cancer. 2017 [cited 2020 Apr 19]. Available from: https://www.cancer.ca/en/cancer-information/cancer-101/what-is-

cancer/stage-and-grade/staging/?region=on
16. Stanford Health Care. TNM Staging System - Medical Test [Internet]. TNM Staging System. 2020 [cited 2020 Apr 19]. Available from: https://stanfordhealthcare.org/medical-tests/t/tnm-staging-system.html
17. Skeel RT. Section 1. Principles of Rational Chemotherapy. Seventh. Lippincott Williams & Wilkins, editor. Handbook of Cancer Chemotherapy. Philadelphia, PA; 2007. 8 p.
18. Stanford Health Care. Cancer Treatment [Internet]. 2020 [cited 2020 Apr 25]. Available from: https://stanfordhealthcare.org/medical-conditions/cancer/cancer/cancer-treatment.html
19. Corrie PG. Cytotoxic chemotherapy: clinical aspects. Medicine (Baltimore) [Internet]. 2011 Dec;39(12):717–22. Available from: https://doi.org/10.1016/j.mpmed.2011.09.012
20. Canadian Cancer Society. Chemotherapy and other drug therapies [Internet]. 2017 [cited 2020 Apr 24]. Available from: https://www.cancer.ca/en/cancer-information/diagnosis-and-treatment/chemotherapy-and-other-drug-therapies/chemotherapy/?region=on
21. Ashkan E, Karp JE. Cancer Pharmacology: An Illustrated Manual of Anticancer Drugs. Ashkan Emadi, MD, PhD, Judith E. Karp M, editor. Springer Publishing Company, 2019; 2019. 11–113 p.
22. Warwick GP. The mechanism of action of alkylating agents. Cancer Res [Internet]. 1963 Sep;23:1315–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14070386
23. Lansiaux A. Les antimétabolites. Bull Cancer [Internet]. 2011 Nov;98(11):1263–74. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0007455115305531
24. Skeel RT. Section III. Chemotherapy of Human Cancer. In: Wilkins LW&, editor. Handbook of Cancer Chemotherapy. Seventh. 2007. p. 429.
25. Aapro MS. Bone Marrow Toxicity: White Blood Cells. In: Side Effects of Medical Cancer Therapy [Internet]. Cham: Springer International Publishing; 2018. p. 427–37. Available from: http://link.springer.com/10.1007/978-3-319-70253-7_16
26. Schrader JW. Colony-Stimulating Factors. In: Encyclopedia of Immunology [Internet]. Elsevier; 1998. p. 596–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/B0122267656001675
27. Molineux G, MaryAnn F, Arvedson T. Twenty Years of G-CSF: Clinical and Nonclinical Discoveries. Media SS& B, editor. 2012. 3–13 p.
28. Amgen Inc. NEUPOGEN® (filgrastim) Prescribing Information [Internet]. U.S.: U.S. Licence Number 1080; 1991. Available from: https://www.neupogenhcp.com/febrile-neutropenia/
29. Craig M, Humphries AR, Nekka F, Bélair J, Li J, Mackey MC. Neutrophil dynamics during concurrent chemotherapy and G-CSF administration: Mathematical modelling guides dose optimisation to minimise neutropenia. J Theor Biol [Internet]. 2015 Nov;385:77–89. Available from: http://dx.doi.org/10.1016/j.jtbi.2015.08.015
30. U.S. National Library of Medicine. Breast cancer [Internet]. 2020 [cited 2020 Apr 26]. Available from: https://ghr.nlm.nih.gov/condition/breast-cancer#genes
31. Paplomata E, O'Regan R. Breast Cancer, Including Brief Discussion of Male Breast Cancer. In: The American Cancer Society's Oncology in Practice. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2018. p. 375–96.
32. National Breast Cancer Foundation I. About Breast Cancer [Internet]. Types of Breast Cancer. 2019. Available from: https://www.nationalbreastcancer.org/types-of-breast-cancer/
33. Schwab M, editor. Triple Negative Breast Cancer. In: Encyclopedia of Cancer [Internet].

Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. p. 3784. Available from: https://doi.org/10.1007/978-3-642-16483-5_5981
34. Al-Mahmood S, Sapiezynski J, Garbuzenko OB, Minko T. Metastatic and triple-negative breast cancer: challenges and treatment options. Drug Deliv Transl Res [Internet]. 2018 Oct 5;8(5):1483–507. Available from: http://link.springer.com/10.1007/s13346-018-0551-3
35. American Cancer Society. Triple-negative Breast Cancer [Internet]. 2019 [cited 2020 Aug 5]. Available from: https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer-diagnosis/types-of-breast-cancer/triple-negative.html
36. Garrido-Castro AC, Lin NU, Polyak K. Insights into Molecular Classifications of Triple-Negative Breast Cancer: Improving Patient Selection for Treatment. DeVita V.T., Lawrence T.S. RSA, editor. Cancer Discov [Internet]. 2019 Feb;9(2):176–98. Available from: http://doi.org/10.1158/2159-8290.CD-18-1177
37. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology [Internet]. Breast Cancer. 2020 [cited 2020 Apr 27]. p. MS-67-69. Available from: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf
38. Canadian Cancer Society. Diagnosis of Breast Cancer [Internet]. 2020 [cited 2020 Apr 27]. Available from: https://www.cancer.ca/en/cancer-information/cancer-type/breast/diagnosis/?region=on
39. Stolnicu S. Prognostic and Predictive Factors in Breast Carcinoma. In: Stolnicu S, Alvarado-Cabrero I, editors. Practical Atlas of Breast Pathology [Internet]. Cham: Springer International Publishing; 2018. p. 327–56. Available from: http://link.springer.com/10.1007/978-3-319-93257-6_18
40. Henderson C. Breast Cancer: Fundamentals of Evidence-Based Disease Management. Oxford University Press, editor. 2015. 106–120 p.
41. Hoda SA. The Role of Immunohistochemistry in Breast Pathology. In: Stolnicu S, Alvarado-Cabrero I, editors. Practical Atlas of Breast Pathology [Internet]. Cham: Springer International Publishing; 2018. p. 305–26. Available from: http://link.springer.com/10.1007/978-3-319-93257-6_17
42. Biernacka A, Lerwill MF. Diagnostic Evaluation of Usual Ductal Hyperplasia and Atypical Ductal Hyperplasia. In: Stolnicu S, Alvarado-Cabrero I, editors. Practical Atlas of Breast Pathology [Internet]. Cham: Springer International Publishing; 2018. p. 205–25. Available from: http://link.springer.com/10.1007/978-3-319-93257-6_10
43. Canadian Cancer Society. Chemotherapy for breast cancer [Internet]. [cited 2020 Apr 29]. Available from: https://www.cancer.ca/en/cancer-information/cancer-type/breast/treatment/chemotherapy/?region=qc
44. Vicario GP, Novara, Penco S, Arcamone F. Daunorubicin and Doxorubicin [Internet]. Milan, Italy: U.S. Patent; 1980. Available from: https://patentimages.storage.googleapis.com/0c/48/c3/597172fcc40db0/US4211864.pdf
45. Wishart D, Knox C, Guo A, Shrivastava S, Hassanali M, Stothard P, et al. Drugbank: a comprehensive resource for in silico drug discovery and exploration. (Database issue) [Internet]. Doxorubicin. 2020 [cited 2020 Sep 8]. Available from: https://go.drugbank.com/drugs/DB00997
46. Johnson-Arbor, Kelly; Dubey R. Doxorubicin. In: StatPearls [Internet] [Internet]. Treasure Island (FL): StatPearls Publishing; 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459232/
47. Pfizer. Product monograph - Doxorubicin. 2019;(June):1–55. Available from:

https://www.pfizer.ca/sites/default/files/201908/Doxorubicin_PM_5June2019_E.pdf
48. Cancer Care Ontario. Drug Formulary / Regimens [Internet]. DOXOrubicin Regimen Breast cancer palliative. 2019. p. 1–8. Available from: https://www.cancercareontario.ca/en/drugformulary/regimens/monograph/46296
49. Cancer Care Ontario. Drug Formulary / Regimens [Internet]. AC-PACL(DD) Regimen. 2019. p. 1–10. Available from: https://www.cancercareontario.ca/en/drugformulary/regimens/regimen-info/ac-pacl-dd-patient-info
50. Cancer Care Ontario. Drug Formulary / Regimens [Internet]. DAC Regimen (Docetaxel, Doxorubicin-Cyclophosphamide). 2016. p. 1–10. Available from: https://www.cancercareontario.ca/en/drugformulary/regimens/monograph/46211
51. Kenakin TP. Chapter 9 - Pharmacokinetics. In: Kenakin TP, editor. A Pharmacology Primer [Internet]. Fifth. Elsevier; 2019 [cited 2020 Apr 26]. p. 245–93. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128139578000096
52. Lee M, Desai A. Gibaldi's Drug Delivery Systems in Pharmaceutical Care. ASHP, editor. The LADME Scheme. 2007. 12–14 p.
53. Rowland M, Tozer TM. Clinical Pharmacokinetics and Pharmacodynamics, Concepts and Applications. 4 ed. Lippincott Williams & Wilkins, editor. Baltimore MD; 2011.
54. Horde GW, Gupta V. Drug Clearance. StatPearls Publ [Internet]. 2020 [cited 2020 Nov 25]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK557758/
55. Curry SH, Whelpton R. Introduction to Drug Disposition and Pharmacokinetics. John Wiley & Sons, editor. 2017. 61,74-76.
56. Marian M, Seghezzi W. Pharmacodynamics, PK/PD Methods and Examples. In: Nonclinical Development of Novel Biologics, Biosimilars, Vaccines and Specialty Biologics [Internet]. Elsevier; 2013 [cited 2020 Apr 25]. p. 97–137. Available from: https://www.sciencedirect.com/topics/immunology-and-microbiology/pharmacodynamics
57. Golan DE. Pharmacodynamics. In: Lippincott Williams & Wilkins, editor. Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy. 2008. p. 8.
58. Curry SH, Whelpton R. Quantitative Pharmacological Relationships. In: Introduction to Drug Disposition and Pharmacokinetics [Internet]. Chichester, UK: John Wiley & Sons, Ltd; 2017. p. 162–77. Available from: http://doi.wiley.com/10.1002/9781119261087.ch8
59. Gustafson DL, Page RL. Cancer Chemotherapy. In: Withrow and MacEwen's Small Animal Clinical Oncology [Internet]. Fifth Edit. Elsevier; 2013. p. 157–79. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9781437723625000116
60. Alberto P. Dose intensity in cancer chemotherapy: definition, average relative dose intensity and effective dose intensity. Bull Cancer [Internet]. 1995;82 Suppl 1:3s-8s. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7626852
61. Meibohm B, Derendorf H. Basic concepts of pharmacokinetic/pharmacodynamic (PK/PD) modelling. Int J Clin Pharmacol Ther [Internet]. 1997 Oct;35(10):401–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9352388
62. Derendorf H, Meibohm B. Modeling of Pharmacokinetic/Pharmacodynamic (PK/PD) Relationships: Concepts and Perspectives. Pharm Res [Internet]. 1999;16:176–85. Available from: https://link.springer.com/article/10.1023%2FA%3A1011907920641#citeas
63. Leil TA, Bertz R. Quantitative Systems Pharmacology can reduce attrition and improve productivity in pharmaceutical research and development. Front Pharmacol [Internet]. 2014;5:247. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25426074

64. Derbalah A, Al-Sallami H, Hasegawa C, Gulati A, Duffull SB. A framework for simplification of quantitative systems pharmacology models in clinical pharmacology. Br J Clin Pharmacol [Internet]. 2020 Jul 4; Available from: http://www.ncbi.nlm.nih.gov/pubmed/32621550
65. Bronshtein IN, Semendyayev KA, Musiol G, Mühlig H. Geometry. In: Springer, editor. Handbook of Mathematics. 6th ed. 2015. p. 249.
66. Vieira S, Hoffmann R. Comparison of the Logistic and the Gompertz Growth Functions Considering Additive and Multiplicative Error Terms. Appl Stat [Internet]. 1977;26(2):143. Available from: https://www.jstor.org/stable/10.2307/2347021?origin=crossref
67. Pearl R, Reed LJ. On the Rate of Growth of the Population of the United States since 1790 and Its Mathematical Representation. Proc Natl Acad Sci [Internet]. 1920 Jun 1;6(6):275–88. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.6.6.275
68. Murphy H, Jaafari H, Dobrovolny HM. Differences in predictions of ODE models of tumor growth: a cautionary example. BMC Cancer [Internet]. 2016 Dec 26;16(1):163. Available from: https://bmccancer.biomedcentral.com/articles/10.1186/s12885-016-2164-x
69. Vaghi C, Rodallec A, Fanciullino R, Ciccolini J, Mochel JP, Mastri M, et al. Population modeling of tumor growth curves and the reduced Gompertz model improve prediction of the age of experimental tumors. Agur Z, editor. PLOS Comput Biol [Internet]. 2020 Feb 25;16(2):e1007178. Available from: https://dx.plos.org/10.1371/journal.pcbi.1007178
70. Bernard A, Kimko H, Mital D, Poggesi I. Mathematical modeling of tumor growth and tumor growth inhibition in oncology drug development. Expert Opin Drug Metab Toxicol [Internet]. 2012 Sep 26;8(9):1057–69. Available from: http://www.tandfonline.com/doi/full/10.1517/17425255.2012.693480
71. Goshu AT. Derivation of Inflection Points of Nonlinear Regression Curves - Implications to Statistics. Am J Theor Appl Stat [Internet]. 2013;2(6):268. Available from: http://www.sciencepublishinggroup.com/journal/paperinfo.aspx?journalid=146&doi=10.11648/j.ajtas.20130206.25
72. Altrock PM, Liu LL, Michor F. The mathematics of cancer: Integrating quantitative models. Nat Rev Cancer [Internet]. 2015;15(12):730–45. Available from: http://dx.doi.org/10.1038/nrc4029
73. Norris JR. Markov Chains [Internet]. Cambridge University Press, editor. Markov Chains: Cambridge Series in Statistical and Probabilistic Mathematics. Cambridge University Press; 1997. 170–216 p. Available from: https://www.cambridge.org/core/product/identifier/9780511810633/type/book
74. Newton PK, Mason J, Bethel K, Bazhenova LA, Nieva J, Kuhn P. A Stochastic Markov Chain Model to Describe Lung Cancer Growth and Metastasis. Ermentrout B, editor. PLoS One [Internet]. 2012 Apr 27;7(4):e34637. Available from: https://dx.plos.org/10.1371/journal.pone.0034637
75. Newton PK, Mason J, Venkatappa N, Jochelson MS, Hurt B, Nieva J, et al. Spatiotemporal progression of metastatic breast cancer: a Markov chain model highlighting the role of early metastatic sites. npj Breast Cancer [Internet]. 2015 Nov 21;1(1):15018. Available from: http://www.nature.com/articles/npjbcancer201518
76. Bethesda, MD. National Institutes of Health US. NIH Stem Cell Information Home Page [Internet]. 5. Hematopoietic Stem Cells. 2016 [cited 2020 Jun 22]. Available from: https://stemcells.nih.gov/info/2001report/chapter5.htm

77. Linden M, Ward JM, Sindhu C. Hematopoietic and Lymphoid Tissues. In: Comparative Anatomy and Histology, A mouse and Human Atlas [Internet]. Academic Press/ScienceDirect; 2012. p. 309–38. Available from: https://www.sciencedirect.com/topics/medicine-and-dentistry/granulopoiesis
78. Rubinow SI, Lebowitz JL. A Mathematical Model of Neutrophil Production and Control in Normal Man. J Math Biol [Internet]. 1975 Sep 15;1(3):187–225. Available from: http://link.springer.com/10.1007/BF01273744
79. Friberg LE, Henningsson A, Maas H, Nguyen L, Karlsson MO. Model of Chemotherapy-Induced Myelosuppression With Parameter Consistency Across Drugs. J Clin Oncol [Internet]. 2002 Dec 15;20(24):4713–21. Available from: http://ascopubs.org/doi/10.1200/JCO.2002.02.140
80. Friberg LE, Karlsson MO. Mechanistic models for myelosuppression. Invest New Drugs [Internet]. 2003 May;21(2):183–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12889739
81. Hansson EK, Friberg LE. The shape of the myelosuppression time profile is related to the probability of developing neutropenic fever in patients with docetaxel-induced grade IV neutropenia. Cancer Chemother Pharmacol [Internet]. 2012 Apr 5;69(4):881–90. Available from: http://link.springer.com/10.1007/s00280-011-1769-7
82. Scholz M, Engel C, Loeffler M. Modelling human granulopoiesis under poly-chemotherapy with G-CSF support. J Math Biol [Internet]. 2005 Apr;50(4):397–439. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15614553
83. Cancer Care Ontario. GCSF Recommendations 2016. 2016; Available from: https://www.cancercareontario.ca/en/content/cancer-care-ontario-gcsf-recommendations-2016
84. Craig M, Humphries AR, Mackey MC. A Mathematical Model of Granulopoiesis Incorporating the Negative Feedback Dynamics and Kinetics of G-CSF/Neutrophil Binding and Internalization. Bull Math Biol [Internet]. 2016 Dec 20;78(12):2304–57. Available from: http://link.springer.com/10.1007/s11538-016-0179-8
85. Vainas O, Ariad S, Amir O, Mermershtain W, Vainstein V, Kleiman M, et al. Personalising docetaxel and G-CSF schedules in cancer patients by a clinically validated computational model. Br J Cancer [Internet]. 2012;107(5):814–22. Available from: http://dx.doi.org/10.1038/bjc.2012.316
86. Kozłowska E, Färkkilä A, Vallius T, Carpén O, Kemppainen J, Grénman S, et al. Mathematical Modeling Predicts Response to Chemotherapy and Drug Combinations in Ovarian Cancer. Cancer Res [Internet]. 2018 Jul 15;78(14):4036–44. Available from: http://cancerres.aacrjournals.org/lookup/doi/10.1158/0008-5472.CAN-17-3746
87. Pillis LG de, Radunskaya AE. Best Practices in Mathematical Modeling. In: Computational Toxicology [Internet]. Humana Press, Totowa, NJ; 2012. p. 51–74. Available from: http://link.springer.com/10.1007/978-1-62703-050-2_4
88. Cassidy T, Craig M. Determinants of combination GM-CSF immunotherapy and oncolytic virotherapy success identified through in silico treatment personalization. Goldman A, editor. PLOS Comput Biol [Internet]. 2019 Nov 27;15(11):e1007495. Available from: https://dx.plos.org/10.1371/journal.pcbi.1007495
89. McKenna MT, Weis JA, Barnes SL, Tyson DR, Miga MI, Quaranta V, et al. A Predictive Mathematical Modeling Approach for the Study of Doxorubicin Treatment in Triple Negative Breast Cancer. Sci Rep [Internet]. 2017;7(1):1–14. Available from: http://dx.doi.org/10.1038/s41598-017-05902-z

90. Callies S, de Alwis DP, Wright JG, Sandler A, Burgess M, Aarons L. A population pharmacokinetic model for doxorubicin and doxorubicinol in the presence of a novel MDR modulator, zosuquidar trihydrochloride (LY335979). Cancer Chemother Pharmacol [Internet]. 2003 Feb;51(2):107–18. Available from: http://link.springer.com/10.1007/s00280-002-0542-3
91. Egbelowo OF. Nonstandard finite difference approach for solving 3-compartment pharmacokinetic models. Int j numer method biomed eng [Internet]. 2018 Sep;34(9):e3114. Available from: http://doi.wiley.com/10.1002/cnm.3114
92. Krzyzanski W, Wiczling P, Lowe P, Pigeolet E, Fink M, Berghout A, et al. Population modeling of filgrastim PK-PD in healthy adults following intravenous and subcutaneous administrations. J Clin Pharmacol [Internet]. 2010 Sep;50(9 Suppl):101S-112S. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20881223
93. Price TH, Chatta GS, Dale DC. Effect of recombinant granulocyte colony-stimulating factor on neutrophil kinetics in normal young and elderly humans. Blood [Internet]. 1996 Jul 1;88(1):335–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8704192
94. The MathWorks Inc. Matlab. Natick, Massachusetts; 2019.
95. Vainstein V, Ginosar Y, Shoham M, Ianovski A, Rabinovich A, Kogan Y, et al. Improving Cancer Therapy by Doxorubicin and Granulocyte Colony-Stimulating Factor: Insights from a Computerized Model of Human Granulopoiesis. Math Model Nat Phenom [Internet]. 2006 May 15;1(2):70–80. Available from: http://www.mmnp-journal.org/10.1051/mmnp:2008003
96. Satoh D. Model selection among growth curve models that have the same number of parameters. Cardone A, editor. Cogent Math Stat [Internet]. 2019 Sep 6;6(1). Available from: https://doi.org/10.1080/25742558.2019.1660503
97. Mégarbane B, Aslani AA, Deye N, Baud FJ. Pharmacokinetic/pharmacodynamic modeling of cardiac toxicity in human acute overdoses: utility and limitations. Expert Opin Drug Metab Toxicol [Internet]. 2008 May 18;4(5):569–79. Available from: http://www.tandfonline.com/doi/full/10.1517/17425255.4.5.569
98. Santos DS dos, Goldenberg RC dos S. Doxorubicin-Induced Cardiotoxicity: From Mechanisms to Development of Efficient Therapy. In: Cardiotoxicity [Internet]. InTech; 2018. Available from: http://www.intechopen.com/books/cardiotoxicity/doxorubicin-induced-cardiotoxicity-from-mechanisms-to-development-of-efficient-therapy
99. de Vries Schultink AHM, Suleiman AA, Schellens JHM, Beijnen JH, Huitema ADR. Pharmacodynamic modeling of adverse effects of anti-cancer drug treatment. Eur J Clin Pharmacol [Internet]. 2016 Jun 26;72(6):645–53. Available from: http://link.springer.com/10.1007/s00228-016-2030-4
100. Piotrovsky V. Pharmacokinetic-pharmacodynamic modeling in the data analysis and interpretation of drug-induced QT/QTc prolongation. AAPS J [Internet]. 2005 Sep;7(3):E609–24. Available from: http://link.springer.com/10.1208/aapsj070363
101. Michael K. Interpreting Cardiac Electrograms: From Skin to Endocardium. Demand B– B on, editor. 2017. 141 p.
102. Fernandez-Chas M, Curtis MJ, Niederer SA. Mechanism of doxorubicin cardiotoxicity evaluated by integrating multiple molecular effects into a biophysical model. Br J Pharmacol [Internet]. 2018 Mar;175(5):763–81. Available from: http://doi.wiley.com/10.1111/bph.14104