A meta-analytic approach for testing evolutionary hypotheses of acquired resistance in metastatic cancer

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ABSTRACT

A meta-analytic approach for testing evolutionary hypotheses of acquired resistance in metastatic cancer.

Nowell (1976) first proposed that unless cytotoxic cancer therapy eradicates all tumor cells, genetic or heritable variation within heterogeneous tumors will inevitably lead to the evolution of chemotherapeutic resistance through clonal selection. This evolutionary hypothesis was formalized by Goldie and Coldman (1979), who developed one of the earliest mathematical kinetic models of resistance evolution in neoplasms. Their model predicted that the likelihood of response and cure would be increased in combination vs single agent cytotoxic therapies. In a later study, Gardner (2002) developed a computational kinetic model to predict chemotherapeutic combinations, doses, and schedules most likely to result in patient response and prolonged life. This model predicts that combination therapy involving both cytotoxic and cytostatic drugs will be more effective than combination therapy involving only cytotoxic drugs. Thus far, no systematic evaluation of the Goldie and Coldman and Gardner hypotheses have been conducted in the metastatic clinical trial setting. Here I test these hypotheses using the results of over 700 phase II, III and II/III clinical trials. I show that, as predicted by Goldie and Coldman, both overall response rate and overall survival were greater in combination arms. Moreover, median duration of response – the key indicator of the rate of resistance evolution - was also greater in combination vs single agent arms. These results suggest that generally combination chemotherapy is more effective than single agent therapy for advanced solid tumors as predicted by Goldie and Coldman (1979) hypothesis and that, at least in the metastatic setting, the potential disadvantages of combination therapy with respect to accelerated resistance evolution are outweighed by the greater waiting times for resistance mutations to arise. By contrast, although combination cytotoxic and cytostatic therapy is associated with a greater average overall response rate than multi agent cytotoxic therapy, this is not the case for both median duration of response and overall survival. Hence, there is no evidence that, in contrast to the predictions of the Gardner (2002) model, combination cytotoxic and cytostatic therapy decreases the rate of resistance evolution relative to that obtaining under combination cytotoxic therapy.

Keywords: cancer, resistance evolution, chemotherapy, meta-analysis, combination therapy, monotherapy, clinical trial

RÉSUMÉ

Une approche méta-analytique pour tester des hypothèses d'évolution sur la résistance acquise dans les cancers métastatiques.

Nowell (1976) a d'abord proposé qu'à moins que la thérapie cytotoxique contre le cancer élimine toutes les cellules tumorales, les variations génétiques ou héréditaires au sein des tumeurs hétérogènes conduiront inévitablement à l'évolution de la résistance contre les agents de chimiothérapie par la sélection clonale. Cette hypothèse évolutionniste a été officialisée par Goldie et Coldman (1979), qui a développé l'un des premiers modèles cinétiques mathématiques de l'évolution de la résistance dans les néoplasmes. Leur modèle a prédit que la probabilité de réponse et la guérison serait augmenté en combinant les agents de thérapies cytotoxiques par rapport à l'utilisation d'un seul agent. Dans une étude ultérieure, Gardner (2002) a développé un modèle cinétique de calcul pour prédire les combinaisons de chimiothérapie, les doses et les horaires les plus susceptibles d'entraîner une réponse favorable du patient et d'une durée de vie prolongée. Ce modèle prédit que la combinaison impliquant à la fois les thérapies cytotoxiques et cytostatiques seront plus efficace que les combinaisons comprenant uniquement dès les thérapies cytotoxiques. Jusqu'à présent, aucune évaluation systématique des hypothèses de Goldie, Coldman et Gardner ont été menées dans le cadre d'un essai clinique métastatique. Ici, j'ai testé ces hypothèses en utilisant les résultats de plus de 700 de phase II, III et des essais cliniques II/III. Je montre que, le taux de réponse globale et la survie globale étaient plus élevés dans les combinaisons de thérapie, comme prédit par les hypothèses de Goldie et Coldman. Par ailleurs, la durée médiane de la réponse - l'indicateur clé de la vitesse d'évolution de la résistance - était également plus élevé en combinaison par rapport aux résultats lors de l'utilisation d'un seul agent. Ces agents simples suggèrent que la chimiothérapie combinée est généralement plus efficace que la monothérapie pour des tumeurs solides avancées comme prédit par l' hypothèse de Goldie et Coldman (1979), au moins en situation métastatique , les inconvénients potentiels de la thérapie combinée par rapport à l'évolution de la résistance accélérée sont compensés par de plus grands temps d'attente pour que des mutations relier à la résistance survienne. En revanche, si la combinaison de chimiothérapie cytotoxique et cytostatique est associée à un taux de réponse global moyen supérieur à un traitement cytotoxique avec multiple agents, ce n'est pas le cas pour les deux durée médiane de réponse et la survie globale. Par conséquent, il n'existe aucune preuve, contrairement aux prédictions du modèle de Gardner (2002), que la combinaison de chimiothérapie cytotoxique et cytostatique diminue le taux d'évolution de la résistance par rapport au combinaison de chimiothérapie cytotoxique.

Mots-clés: cancer, évolution de la résistance, chimiothérapie, méta-analyse, combinaison de thérapie, monothérapie, essai clinique

Table of Contents

CHAPTER 4. HYPOTHESIS 2

List of Tables

List of Figures

CHAPTER 1. INTRODUCTION

I.1 Introduction

According to the World Health Organization (WHO), cancer rates are increasing rapidly in developing countries. Some projections suggest that cancer rates may increase by 50% to 15 million new cases by the year 2020 (WHO, 2003). At present, cancer is a leading cause of death, accounting for 7.6 million deaths annually, or about 13% of all deaths in 2008 globally (WHO, 2012). Cancer treatment includes a series of interventions such as psychosocial support, surgery, radiotherapy, chemotherapy, and immunotherapy that is focused not only on disease cure, but also on prolonging life span while improving patient's quality of life (WHO, 2008).

Disease progression involves movement of tumor cells from the primary lesion to other organs via the bloodstream and/or lymphatic systems. This *metastatic* or advanced disease is often – and for many cancers, usually - fatal. Metastatic cancer may be treated with systemic therapy such as chemotherapy, biological therapy, targeted therapy, hormonal therapy; or local therapy such as surgery, radiation therapy, or a combination of both local and systemic treatments. The choice of treatment generally depends on the type of primary cancer, its size, location, and number of metastatic tumors, the patient's health, and age along with the information regarding patient's previous treatment history. Primary cancer patients usually receive *primary* treatment of surgery and/or radiation therapy followed by a course of *adjuvant* therapy, usually chemotherapy and/or hormonal therapy, for a period of fixed duration. By contrast, in the metastatic setting, patients are often on adjuvant therapy for periods of unlimited duration. Typically, an initial "first line" adjuvant therapy is administered. In case of an objective response, the patient is maintained on the therapy until there is evidence of disease progression, at that time the patient is switched to a second therapy known as "second line" treatment. This process can, in principle, continue indefinitely.

I.2. Clinical measurement of tumor response

Tumors are considered to show "innate" or "intrinsic" resistance to an adjuvant therapy if the patient shows no objective response, usually defined as objective detectable (e.g. via

imaging) reduction in total tumor mass (including both the size of individual lesion and the number of lesions in disseminated disease). In the clinical setting, a decrease in tumor size is considered to reflect an "objective" response to therapy (Rimm, 2006). Clinicians utilize this objective tumor response extensively in evaluation and guidance of cancer treatments and predictions of clinical outcomes (Therasse et al., 2000). To evaluate tumor response, evaluation of overall tumor burden at baseline (before therapy) is necessary.

There are mainly two evaluable categories of tumor response to treatment: (1) Complete Response (CR) – all target lesions have disappeared during the treatment and lymph nodes selected must return to normal size (<10mm); and (2) Partial Response (PR) – at least 50% decrease in the diameters of target lesions, taking as reference the baseline sum diameters (Therasse et al., 2000). *Overall Response Rate* is the percentage of patients showing a complete or partial response (Therasse et al., 2000), and is the most commonly used endpoint (Pazdur, 2000) in evaluation.

I.3. Acquired drug resistance

Drug resistance may be innate or acquired and may apply to a single agent or to a group of agents with the same/similar antineoplastic mechanisms of action (Chang, 2011). Some patients show so-called "innate" resistance such that their tumor shows no objective initial response to therapy. Patients whose tumor is initially sensitive to adjuvant therapy, and may therefore show objective response, may nonetheless experience disease progression after a period of time, often while on treatment. This "acquired" resistance is a major reason for treatment failure in the metastatic setting (Rivera et al., 2010; Longley and Johnston, 2005; O'Driscoll et al., 2006). The unfortunate clinical reality is that the acquired resistance eventually occurs in most metastatic tumors; this results in disease progression and, eventually, death (Gerlinger et al., 2010). Even novel therapies that have shown remarkable clinical success, such as imatinib for chronic myeloid leukemia (CML), are not immune to the problem of drug resistance especially at advanced stages of cancer (Blagosklonny, 2002; Shannon, 2002). In fact, every existing anti-cancer drug suffers from the problem of resistance (Chabner and Roberts, 2005); this problem is, therefore, the fundamental cause of most treatment failure and hence, cancer-related death.

I.4. Theories and mechanisms of acquired resistance

There is a wide range of documented mechanisms of drug resistance (Table 1.1). One particularly important mechanism is increased drug efflux that has been heavily implicated in multi-drug resistance **(**Sierra et al., 2010). An important mechanism of multidrug resistance is expression of an energy-dependent drug efflux pump P-glycoprotein (P-gp), the so-called multidrug transporter (Juliano and Ling, 1976; Ueda et al., 1987). Thus far, there are three ABC transporters (ABCR- Rim Protein, ABC1-Cholesterol transporter, and ABC transporter) that have been implicated in drug efflux and which are suspected of being potentially significant to cancer, including the *MDR*2 gene product (Borst et al., 2000), SPGP (sister of P-gp) (Childs et al., 1998), and ABC A2 (Laing et al., 1998). Other members of the ABC transporter family, in addition to the MDR (multidrug resistance genes), MRP (multidrug resistance associated proteins), and MXR (ABC transporter for anticancer drugs), may also be implicated in drug resistance (Gottesman, 2002).

Other resistance mechanisms that contribute to decreased drug penetration or tumor targeting, have been identified. These include drug sequestration in plasma**,** irregular blood flow, changes in tumor vasculature and drug diffusion in the interstitium**,** reduction of the intracellular accumulation of anti-cancer drugs by both increasing drug efflux and/or decreasing drug uptake, sequestration of drugs, alterations in drug targets (e.g. topoisomerase II) or activation of detoxifying systems (e.g. glutathione/glutathione-S transferases) (Sierra et al., 2010). Increased repair of drug-induced DNA damage, blocked apoptosis, disruptions in signaling pathways and alterations of factors involved in cell cycle control have also been implicated in both single and multidrug resistance (Filipits, 2004).

Drug resistance may also be related to changes in the tumor microenvironment (Onozuka, 2011). During therapy, drug delivery is generally poorer in hypoxic regions, where there is very limited vasculature and oxygen supply (Bhattacharya et al., 2008). Low pHe (extracellular pH) and the resulting pH gradient across the plasma membrane of tumor cells also present a barrier to drug delivery for most chemotherapeutic agents (Cairns, 2006). Additionally, high interstitial fluid pressure (IFP) in tumors can reduce the efficiency of drug delivery by reducing transcapillary fluid flow and convective transport of compounds from the bloodstream into tumor interstitium (Cairns, 2006).

I.5. Evolutionary approaches to understanding acquired resistance

To date, there are two complementary approaches to the problem of acquired resistance. The first, examples of which are described in section I.3, involves trying to ascertain the cellular, physiological or molecular mechanisms that confer resistance. A second approach has the hypothesis that whatever the mechanism, acquired resistance is in many cases an evolutionary phenomenon (Kreuzer et al., 2003; Roche-Lestienne et al., 2003; Gerlinger et al., 2010; Aktipis et al., 2011; Pepper et al., 2008). Neoplasms are genetically and epigenetically diverse populations of billions of cells (Varley et al., 2009; Park et al., 2010; Yachida et al., 2010). As drugs represent potentially strong selective agents, the potential exists for selection to drive the evolution of a tumor cell population increasingly dominated by resistant sub-clones.

Nowell (1976) first proposed that cancer progression is an essentially evolutionary process. He hypothesized that natural selection occurs in tumors in the form of clonal selection resulting from exposure to a selective agent, e.g. chemotherapy. For a therapy designed to kill tumor cells, that is, *cytotoxic* therapy, unless it eradicates all cancer cells, genetic or heritable epigenetic variation within heterogeneous tumors will inevitably lead to the evolution of chemotherapeutic resistance. Cytotoxic cancer therapies are expected to impose intense evolutionary selection pressures, thereby increasing rates of evolution (Gillies et al., 2012). Since Nowell's original formulation, considerable attention has been focused on tumorigenesis and acquired resistance as evolutionary phenomena (Kreuzer et al., 2003; Roche-Lestienne et al., 2003; Monceviciute-Eringiene, 2005; Pepper et al., 2008).

Gaining a better understanding of the evolution of resistance and identifying treatment strategies may well be important in improving patient outcomes. Application of mathematical modeling of the evolutionary dynamics of therapeutic resistance is one potentially powerful approach to the problem of resistance (Chmielecki et al., 2011; Merlo et al., 2006). Mathematical models allow systematic analysis of potential treatment strategies through variation of parameters such as drug dose, treatment timing, and combination options. Mathematical modeling can also be used to predict optimized treatment schedules based on a variety of biological end points (e.g., maximal time of disease progression, maximal rate of tumor reduction, and minimal probability of resistance, minimal tumor size, or minimal resistant cell frequency) as well as an assessment of the robustness of these

biological end points. Overall, mathematical modeling narrows down an infinite space of possible treatment strategies to a subset of strategies with the greatest potential that can then be validated in preclinical models before being introduced to patient care (Foo and Michor, 2009).

In particular, mathematical modeling approaches have explored the potential impact of chemotherapy treatment regimen of drug type, drug dose, drug combinations, and scheduling on the evolution of therapeutic resistance (Foo and Michor, 2009). In 2011, a combined experimental and mathematical modeling-based approach was applied by Mumenthaler's team (2011), to identify treatment strategies that obstruct the outgrowth of resistance in non-small cell lung cancer populations. Their mathematical model predicted about the population dynamics of mixtures of sensitive and resistant cells, thereby described how the tumor composition, initial fraction of resistant cells, and degree of selective pressure influence the time until progression of disease (Mumenthaler et al., 2011). Mathematical models for drug resistance have employed methods such as deterministic (the dynamics of a system follow a set of known rules) to stochastic (models of the future evolution are described by random events) (Lavi et al., 2012).

I.6. Evolutionary theories of acquired resistance: the evidence?

Evidence consistent with an evolutionary theory of acquired resistance comes from several sources (Table 1.2), including *in vitro*, *in vivo*, and clinical studies. Increasing number of examples of gene amplification in mammalian cells have been reported, including amplification of so called "oncogenes" in tumor cell lines as well as gene amplification resulting in the emergence of clinical chemotherapy resistance (Schimke, 1984). For some drugs, mutations are regularly found in patient's genes targeted by the drug (Schimke, 1984; Kobayashi et al., 2005) and are present in tumor samples taken before therapy (Kreuzer et al., 2003; Roche-Lestienne et al., 2003). This suggests that therapy selected resistant clones among the standing variation in the cell population during therapy (Atkipis et al., 2011).

I.7. Testing evolutionary theories of acquired resistance in the clinic

To date, there have been few, if any, direct clinical tests of any evolutionary theory of acquired resistance. Here I propose to test two such theories using data from clinical trials.

The first hypothesis is based on mathematical kinetic models of resistance evolution developed by Goldie and Coldman (1979); the second hypothesis derived from Gardner's (2002) mathematical model of the evolutionary consequences of treatment regimens involving different classes of chemotherapeutic drugs.

Goldie and Coldman (1979) developed one of the earliest mathematical kinetic models of resistance evolution in neoplasms. This model yielded two predictions of clinical significance (Goldie and Coldman, 1979), (1) that the probability of a cure decreases exponentially with the initial tumor mass (number of cells); and (2) that the likelihood of response and cure would be increased in combination vs single agent therapies. The rational for the latter is straightforward. In the Goldie and Coldman model, resistance evolves through the accumulation of resistance mutations and associated positive selection. If tumors are heterogeneous with respect to drug sensitivity, and there is little or no cross-resistance among drugs, then under combination therapy, phenotypic resistance can arise only with the accumulation of a number of different mutations in a single clone. Consequently, the waiting time for the appearance of a resistant clone in an initially sensitive tumor will be longer than under single agent therapy where comparatively fewer mutations are required for resistance. The result is that initial tumor kill is substantially higher, tumor mass is reduced much more rapidly, thereby increasing the likelihood of cure.

In the context of metastatic clinical trials, the Goldie and Coldman hypothesis makes three specific predictions: for any cancer, for any two cytotoxic drugs A and B, (1) average response rate (combined partial and complete response) should be greater in trial arms where drugs A and B are administered in combination, compared to arms where only drug A (or only drug B) is administered; (2) the overall survival should be greater in trial arms where drugs A and B are administered in combination, compared to arms where only drug A (or only drug B) is administered; and (3) as a consequence of (1) and (2), the median duration of response should be greater in trial arms where drugs A and B are administered in combination, compared to arms where only drug A (or only drug B) is administered.

All existing chemotherapeutic cancer drugs are of two types: cytotoxic or cytostatic. Cytotoxic agents are those for which exposure to the drug results in rapid cell death. Cytostatic agents, on the other hand, result in interruption of the cell cycle, thereby preventing cell division, and reducing the rate at which a clone multiplies. Most cytostatic

drugs developed to date work by some form of receptor blockade, by preventing binding of hormones such as estrogen to receptors on the tumor cell surface. Gardner (2002) developed a computational kinetic model to predict chemotherapeutic combinations, doses, and schedules most likely to result in patient response and prolonged life. A key prediction of this model is that, for a wide range of parameter conditions, combination therapy involving both cytotoxic and cytostatic drugs will be more effective than combination therapy involving only cytotoxic drugs. Here again the reason is straightforward: the comparatively intense positive selection for resistance induced by potent cytotoxic combinations is attenuated by a deceleration of the rate at which the evolutionary clock ticks through drug-induced quiescence. Intense positive selection for resistance is also induced by cytotoxic combinations, but here there is markedly less deceleration in the rate of ticking of the evolutionary clock. In the clinical context, the Gardner hypothesis makes three specific predictions: for any cancer (1) the overall response rate should be smaller in trial arms where a combination of cytotoxic drugs is administered, compared to arms where a combination of cytotoxic and cytostatic drugs is administered; (2) the median duration of response should be shorter in trial arms where a combination of cytotoxic drugs is administered, compared to arms where a combination of cytotoxic and cytostatic drugs is administered; and (3) as a consequence of (1) and (2), overall survival should be lower in trial arms where a combination of cytotoxic drugs is administered, compared to arms where a combination of cytotoxic and cytostatic drugs is administered.

Testing of Goldie and Coldman's (1979) model and Gardner's (2002) model have to date, been conducted primarily in cell culture or in animal models. Thus, no systematic evaluation of predictions of evolutionary hypotheses of acquired resistance has been conducted in the clinical trial setting yet. I proposed to do so using a meta-analytic approach whereby information from published clinical trials in the metastatic setting is extracted and analysed.

To test the predictions of both the hypotheses, two different sets of clinical trials were identified. The first set has a comparatively high degree of internal control, and includes trials with at least two arms, one in which patients received a single agent therapy (drug A or drug B only), and a second where patients received combination therapy (drugs A + B). A second and larger set of clinical trials are those having only one single agent arm, or one

combination agent arm. The sample of single and combination arms are therefore independent rather than paired as in the first analysis. Testing of the two hypotheses was associated with fitting statistical models in which the dependent variables are the various endpoints of clinical trials (overall response rate, median duration of response, and overall survival) and the independent variables are fixed effects of the factor of interest (e.g. single vs combination therapy) or other covariates that have been associated with clinical trial outcomes (type of regimen – single vs combination arm, cancer type, route of administration, toxicity, phase, number of patients in an arm and trial, number of drugs in an arm, and year of publication). In the case of the first sample, there is natural grouping induced by the fact that there are multiple arms per trial, and to capture this variability I incorporated the random effects of trial in a mixed modeling approach.

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Table 1.1. Described mechanisms of drug resistance.

Mechanism	Individual process	Citations
Cell kinetic resistance	Tumor growth	Piantadosi et al., (1983)
Pharmacokinetic resistance (including decreased drug absorption and penetration to the tumor)	Decreased drug absorption	Xia et al., (2005); Undevia et al., (2005); Atta et al., (2001)
	Excessive metabolism	Kristjansen et al., (1996); Thomas et al., (2003), Leonard et al., (2003)
	Poor drug penetration to certain sites	Thurber et al., (2011); Ludwig et al., (2006), Phillips et al., (1998)
	Blood supply of the tumor	Coleman et al., (1988); Vaupel et al., (1989);
	Drug diffusion	Kyle et al., (2007); Minchinton et al., (2006)
Cellular drug resistance	Increased drug efflux	Nambaru et al., (2011)
	Decreased drug uptake	Stavrovskaya, (2000); Szakacs et al., (2006); Simon et al., (1994)
	Sequestration of drugs	Gotink et al., (2011); Gong et al., (2003)
	Alterations in drug targets	Stavrovskaya, (2000)
	Activation of detoxifying systems	Riddick et al., (2005); Mattern et al., (2004)
	Increased repair of drug-induced DNA damage	Basu et al., (2010); Rünger et al., (2000); Tomida et al., (2002)
	Blocked apoptosis	Reed, (1999); Grossman and Altieri, (2001)
	Disruption in signaling pathways	Villanueva et al., (1998); Rodrigues et al., (2003)
	Alterations of factors involved in cell cycle regulation	Nam and Kim, (2008); White et al., (1994)

Table 1.2. Evidence supporting evolutionary theories of acquired resistance.

Study setting	Main finding	Implication	Reference
Clinical	Patients showing clinical acquired resistance have mutations known to confer resistance in genes specifically targeted by anti-cancer drugs, mutations present in tumor samples before treatment.	Therapy selects among mutations already present prior to selection.	Kreuzer et al., 2003; Roche-Lestienne et al., 2003

In Vitro	In vitro models of acquired resistance to gefitinib, obtained by exposing gefitinib-sensitive cells to increasing concentrations of the drug, led to the appearance of the same mutations identified in patients.	Possible mutations in residues conferring a high level of resistance to small molecules.	Cools et al., 2004
	Investigating the mechanisms of Danusertib acquired resistance and the ability of combination therapy to prevent or reduce emergence of resistant clones in vitro.	Combined treatment with IM and Danusertib significantly reduced the emergence of drug resistance in vitro.	Balabanov et al., 2011
In Vivo	The growth of established orthotopic tumors in severe combined immunodeficient mice was blocked for 1 month by trastuzumab, after which rapid growth resumed. These relapsing tumors were found to maintain resistance to trastuzumab.	Trastuzumab was able to block growth of tumors established from previously untreated primary tumors, two trastuzumab-resistant tumor derived cell lines tested in vivo showed only a slight, statistically insignificant, response to drug treatment or a complete lack of response, respectively, similar to that observed for the parental untransfected cell line.	du Manoir et al., 2006
	A single plate of 1 million cells and a small subcentimeter tumor in vivo can simultaneously develop multiple mechanisms of resistance.	Patients with cancers consisting of billions to trillions of cells have the capacity to simultaneously develop a wide array of resistance mechanisms.	Qi et al., 2011

CHAPTER 2. GENERAL METHODS

II.1. The University of Ottawa Metastatic Cancer Clinical Trials Database (UOMCCT)

The University of Ottawa Metastatic Cancer Clinical Trials Database was initiated in 2004, and has grown to include over 700 clinical trial records in 30 different metastatic cancer settings. In addition to the standard bibliographic information (title, authors, year of publication, journal), the database includes information on: (1) trial design, including trial phase, number of arms, number of patients in each arm, sample population, whether endpoint estimates are based on intention to treat or per protocol; (2) patient population, including sample population, patient selection criteria (performance status and number of previous treatments), etc.; (3) cancer type; (4) drug information, including drug names, toxicity, dose, intervention cycles, and administration information (frequency type, specific days, rate of dosage, cycle length, and route of drug administration); and (5) trial endpoints, including overall response rate (OR) (partial and complete), median overall survival (OS), and median duration of response (MD).

Data extraction and database populating were done primarily by undergraduate student volunteers. At the beginning of each year, the database manager produces a set of published clinical trials in the metastatic setting using the search and inclusion criteria described in section II.1.3 below. Each volunteer was given a training session in which the study objectives and metadata and data extraction protocols were described. At the conclusion of each training session, volunteers attended a workshop at which they are taken, step by step, through the process of data extraction from a published article under the guidance of the database manager. Following training, each volunteer was provided with up to five trials, with each trial being independently evaluated by at least two, sometimes three, anonymous volunteer evaluators. Using the provided metadata and protocols, students extracted information from the published trials assigned to them, entered these data into a personal copy of the database, and submitted the completed file to the database manager. For each article, the multiple data submissions from independent evaluators were then compared, and any discrepancies were resolved by the manager through direct reference to the article in question.

II.1.1. Study selection

To be included in the database, a cancer study must: (a) be either phase II or III or a combination (II/III) clinical trial in a metastatic or advanced cancer setting; (b) at least one arm of the trial must include the administration of one or more chemotherapies whose cytotoxic or cytostatic properties are well established; and (c) the article must report, at a minimum, overall response rates (OR) or median overall survival (OS) for all arms in the trial.

II.1.2. Study retrieval

In the past, literature searches have been conducted using ISI Web of Knowledge, Scholar's Portal, Google Scholar, and Medline. Since 2008, the National Centre for Biotechnology Information's (NCBI) PubMed literature survey from PubMed articles has also been employed. Searches were conducted three times a year: in September/October, January/February and May/June. All searches were saved along with the set of associated keywords and limits (if any) (Table 2.1). Search keywords included "metastatic cancer", "advanced cancer", AND "chemotherapy", "drug resistance", "acquired resistance" AND "response rate", "overall response", "median overall survival". Additional candidate studies were gathered by using more additional search phrases such as "single therapy", and "combination therapy". Also, articles listed in the bibliographies of retrieved articles that appeared (on the basis of title and abstract) to be relevant to the current study were reviewed (Fig 2.1).

II.1.3. Inclusion criteria

Selected studies: (1) must be either a phase II or III or a combination (II/III) clinical trial in a metastatic or advanced cancer setting; (2) at least one arm of the trial must include the administration of one or more chemotherapies; and (3) the article must report, at a minimum, overall response rates (OR) or median overall survival (OS) for all trial arms. Each record in the database includes information on the trial. Initial screening involved reading abstracts and titles of all articles retrieved and eliminating those that, on the basis of information in the abstract, did not meet the three inclusion criteria (136 studies, Fig 2.1). 176 studies of the

751 retrieved candidates were excluded from analysis due to lack of data on overall response rate or median survival that was not provided for all trial arms. Studies with phase I and I/II were also excluded (Fig 2.1).

II.2. Data Quality Assurance/Quality Control

An important issue relates to the quality of the data extracted by largely inexperienced student volunteers. This issue has been addressed through a set of Quality Assurance/Quality Control (QA/QC) protocols based on inter rater reliability (IRR). Under the QA/QC protocol, each article is reviewed independently by at least two independent evaluators. Each evaluator then forwards a completed copy of the data for the articles they have been assigned. In this way, multiple independent extractions from the same data source using the same metadata and extraction protocols are obtained, with the database manager then being able to check for discrepancies among raters (evaluators). For database fields for which there was no discrepancy between evaluators, the extracted data are assumed to be an accurate reflection of the information provided in the paper. In the case of discordant results, either: (a) the original evaluators were asked to verify their data entries by rechecking the original article and returning any revisions to the database manager; or (b) the database manager examined the article to ascertain the correct entry.

II.3. Datasets

The UOMCCT database includes clinical studies of some 30 different cancer types, with many being represented by only a couple of studies. Since there is ample evidence that all of the clinical endpoints considered in the study vary among different cancer types, in most cases I restricted my analysis to those types (Breast, Colorectal, Pancreatic, and Lung) where the sample of clinical trials is sufficiently large to permit reliable estimates of fitted model parameters. Specific datasets and associated sets of clinical trials are described in detail in the methods section of Chapter 3 and 4 for Hypothesis 1 (H1) and Hypothesis 2 (H2) respectively.

II.4. Dependent variables (primary endpoints) and Independent variables

II.4.1. Dependent variables (primary endpoints)

Several different endpoints are conventionally used to evaluate treatment effectiveness in cancer clinical trials. These include:

Overall Response Rate (OR): In the clinical trial setting, patients are considered to show a response if there is objective evidence (usually based on CT imaging) of at least a 25% reduction in tumor mass after therapy commences. A partial response (PR) is defined as 50% or greater decrease in the sum of the longest diameter of target lesions confirmed on two evaluations at least four weeks apart, and no increase in or appearance of new lesions (Therasse et al., 2000). Complete response (CR) means the disappearance of all clinical and radiographic evidence of disease as determined on two successive observations at least four weeks apart. For a given trial and arm, the response rate (CR, PR or OR (CR+PR)) is the proportion of patients who showed a response (Therasse et al., 2000).

Median overall survival (OS): Median overall survival is the length of time (expressed in months) by which half the subjects enrolled in the trial, in a particular arm, have died (Therasse et al., 2000). Survival is calculated from the date of enrolment in the trial to the date of death or the date when the patient was last known to be alive (Schiller et al., 2002). Median survival includes both patients who show an objective response to therapy (i.e. responders) and those who do not. But because patients who respond to chemotherapy typically live longer than those who do not (Schiller et al., 2002), the greater the response rate, the longer the median overall survival.

Median duration of response (MD): Median duration of response is the median length of time between when a patient is first considered to have shown either a partial or complete response, and the time at which a patient's disease is determined to have progressed (Therasse et al., 2000). The duration of response for a patient will depend both on, (a) the timing of the determination of response; and (b) the timing of the determination of progression. These in turn will depend upon how often radiographic imaging and associated

tests are scheduled, including how close to the start of treatment the pre-treatment imaging is conducted (Therasse et al., 2000).

II.4.2. Independent variables

I examined the effects of seven independent variables on the outcome measures (dependent variables) described in section II.4.1 above. The set of candidate predictors was selected largely on the basis of, (a) a theoretical rationale for potential effects on one or more dependent variables; or (b) empirical evidence of associations between one or more dependent variables and the candidate variable in question.

Cancer Type (CANCER): In both the primary and metastatic cancer clinical setting, patient response to therapy depends on a number of prognostic factors, including cancer type. For example, in the United States from 2005-2009, 5 year survival rates are on the order of 6% for pancreatic cancer, but 90% for breast cancer (Siegel et al., 2013). Similarly, median duration of response may be as short as 1-2 months for pancreatic cancer to 9-12 months for first-line breast cancer treatment (Weissman, 2002).

Number of patients (TOTAL.PATIENTS, ARM.PATIENTS): The number of patients enrolled in a trial (TOTAL.PATIENTS) or in a trial arm (ARM.PATIENTS) can vary dramatically, from as low as a dozen to thousands. Such variability in sample size will clearly effect the precision of any outcome estimate, but may also of itself introduce bias. Indeed, in meta-analysis of clinical trials, there is a well-known phenomenon whereby small, Phase II trials tend to report larger treatment effect sizes, which often diminish substantially – even disappear – in larger Phase III trials (Ioannidis, 2005).

Arm (ARM): An arm is a group of patients in clinical trials receiving a particular therapy (drug or drug combination and regimen). In the sample of selected trials, patients in a given arm are either given monotherapy (i.e. a single drug) or combination therapy (i.e. a combination of drugs). As the hypotheses investigated in the current study make predictions about the comparative effectiveness of mono-therapy vs combination therapy (H1, Chapter

3), or about the comparative effectiveness of different types of combination therapy (H2, Chapter 4), this variable is then of most interest in the current study.

Combination arm type (COMBINATION.TYPE): This variable is similar to ARM variable but only used in testing H2 in Chapter 4. The sample of clinical trials for H2 comprises of studies with at least one combination arm (for one sample) or two combination arms (for another sample), with patients in one arm receiving combination therapy of two or more cytotoxic drugs (CT+CT), and patients in a second arm receiving combination therapy of at least one cytotoxic and at least one cytostatic drug (CT+CS). Cytotoxic drugs can be same or different in both the CT+CT arm and CT+CS arm, hence the cytotoxic drugs in the CT+CT arms need not be the same cytotoxic drugs used in the CT+CS arms in some clinical trials.

Toxicity (TOXICITY): Cytotoxic (CT) drugs are those for which exposure to the drug results in rapid cell death (Gardner, 2002). By contrast, cytostatic (CS) drugs interrupt the cell cycle, thereby preventing cell division and reducing the rate at which a clone multiplies (Gardner, 2002). The effectiveness of cytostatic vs cytotoxic therapies has been investigated in a variety of both laboratory and clinical settings (Korn, et al., 2001). In the context of the current research, Hypothesis 2 (H2) makes specific predictions about the comparative effectiveness of combinations of cytotoxic agents vs combinations of both cytotoxic and cytostatic agents.

Phase of Clinical Trial (PHASE): Phase I clinical trials are designed to determine the metabolic and pharmacologic activity of the candidate drug in humans, dose tolerance, and preliminary information on effectiveness. Phase II trials are designed to obtain some preliminary information on the effectiveness of the candidate drug, along with establishing a short-term side-effect profile for particular indications. Phase III studies are performed after preliminary evidence suggesting effectiveness of the drug has been obtained in Phase II, and are intended to gather the additional information about effectiveness and safety that is needed to evaluate the overall benefit-risk relationship of the drug. Phase III trials are usually conducted over periods of 18 months to several years, depending on the indication. Phase IV studies evaluate the side effects, risks, and benefits of a drug over a longer period of time and

in a larger number of patients than in phase III, and are invariably after the drug has been approved and is being marketed. Some studies have a mixture of objectives, including some that would conventionally be associated with a Phase II study, as well as others that would conventionally be associated with a Phase III study. In such cases, the trial may be designated as a mixed (e.g. Phase II/III) trials. I included trial phase as a candidate predictor due to the well-known decline effect in clinical trials research. As indicated above, there are theoretical and empirical reasons for believing that effect sizes in small trials may be inflated (Ioannidis, 2008), and given that Phase II trials are generally smaller and of shorter duration than Phase III trials, there is reason for believing that phase may contribute to among-trial variation in estimates of trial outcome measures (Leff and Andrews, 2008).

Year of Publication (YEAR): There is empirical evidence that cancer treatment has improved with time. Profound improvements in outcome in metastatic colorectal cancer observed in selected patients and this improvement is associated with increased utilization of new chemotherapeutics from 1998 to 2006 and advancements in medical therapy has been shown from 2004 to 2006 (Kopetz et al., 2009).

Route of Administration (ROUTE): Route of administration refers to the way that a drug is introduced into the body. Most drug protocols specify a fixed route of administration, usually oral, intravenous, but occasionally intramuscular or intratumoral. Even with the same drug or drug combination, route of administration may influence effectiveness due to different pharmacokinetic profiles under different routes of administration (Didolkar et al., 1984; Wan et al., 1974). For example, in a colorectal cancer study, although both oral capecitabine and uracil plus tegafur have advantages over intravenous bolus 5-FU/LV in terms of toxicity, patients treated with oral uracil plus tegafur had a 22% greater risk of disease progression than patients treated with intravenous bolus 5-FU/LV (Borner et al., 2001).

Total number of Drugs (DRUGS): This variable refers to the total number of all types of drugs – cytotoxic and cytostatic, used in an arm of clinical trial. Number of various types of drugs show variation in OR and OS, hence this variable is tested in the analysis. This

variable is only used in testing H2 in Chapter 4 due to presence of *combination arm type* variable.

Number of Cytotoxic Drugs in an arm (CT): In some trials only cytotoxic drugs are administered and in others both cytotoxic and cytostatic drugs are administered in arms of the clinical trial. Hence, variation in OR and OS is possible with varying total number of cytotoxic drugs in an arm of a clinical trial. This variable is only used in testing H2 in Chapter 4 due to presence of *combination arm type* variable.

II.5. Statistical Modeling

I fitted either linear fixed effect or mixed effect models relating the various endpoints (overall response rate, median overall survival and, median duration of response) to specific fixed (arm – single vs combination, cancer type, phase of clinical trial, route of administration, year of publication, total number of patients in an arm and in a trial, total number of drugs in an arm, total number of cytotoxic drugs in an arm, and toxicity) or random (trial) effect. Two different samples were developed from included studies: 1) Sample S_1, where there are two or more observations (arms) per trial; and 2) Sample S_2, where there is only one observation (arm) per trial. For Sample S_1, the natural structure induced by trials was captured using trial as a random effect, which, at least in principle, permits inferences about the population (in this case, of clinical trials studies) from which the sample of studies is drawn (Baayen et al., 2008). Moreover, comparison of fitted models for the two independent data sets (S_1 and S_2) allow inferences concerning the generalizability and reliability of inferred effects of specific candidate predictor variables. Sample S_1 and S_2 are different in structure for both the hypotheses and those differences of dataset design and sample size are explained in the methods section of Chapter 3 and Chapter 4 for Hypothesis 1 and 2 respectively. Models were finalized by employing backward model selection method. Both fixed and mixed effects modeling was carried out using S-Plus version 8.0. The two hypotheses investigated in this study make specific predictions about expected patterns (Table 2.2). Forest and funnel plots were also developed for overall response rate in sample 1 of Hypothesis I (appendix) but not for Hypothesis II due to small sample size.

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Table 2.1. Limits for search criteria in PubMed.

PubMed Limit	Set to:
Language	English
Species	Human
Text Options	Abstract
	Links to Full Text
Subset	Cancer
Type of Article	Clinical Trial phase II
	Clinical Trial phase III

Table 2.2. Summary of hypotheses testing by statistical modeling for four cancers (Lung, Breast, Pancreatic, Colorectal). OR (overall response rate), OS (median overall survival), MD (median duration of response), are endpoints, C (combination), S (single) are arms, S1 (within control), S2 (no within control) are clinical trial samples, CT (cytotoxic), CS (cytostatic) are mode of action or toxicity of drugs in clinical trials.

Hypothesis	Predictions	Datasets	Arms	Endpoints (Dependent variables)	Models	Independent variables
1	(a) OR/OS/MD (C) > OR/OS/MD (S)	S1 (S + C)	56	OR/OS/MD	Fixed and Mixed	CANCER, ARM, YEAR, PHASE, ROUTE, TOTAL.PAT
		S2 (S/C)	54		Fixed	

						IENTS, ARM.PATIENTS, DRUGS, CT
2	(a) OR/OS/MD (CT+CS) > OR/OS/MD (CT+CT)	S1 (C + C)	3	OR/OS/MD	Fixed and Mixed	CANCER, ARM, YEAR, PHASE, ROUTE, TOTAL.PATIENTS, ARM.PATIENTS, DRUGS, CT
		S2 (C arm)	105		Fixed	

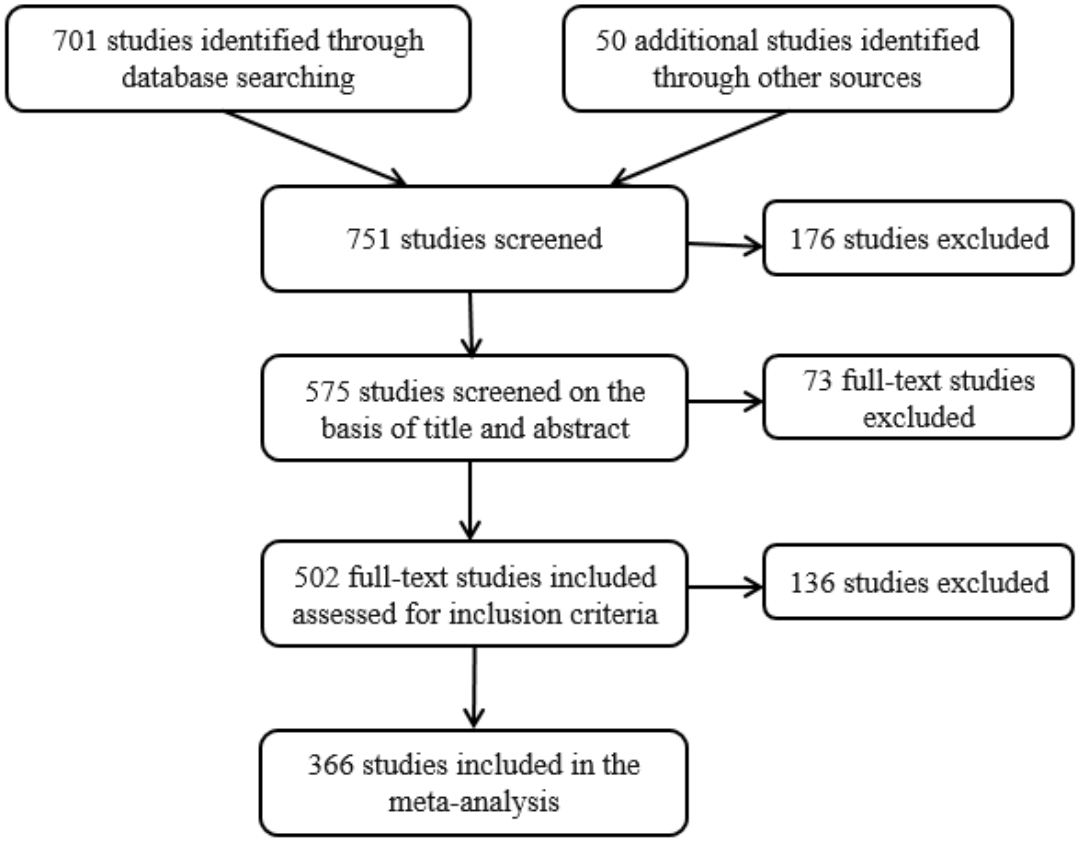

Figure 2.1. Flow chart indicating study identification based on study retrieval, screening, and eligibility according to stated inclusion criteria and those studies included in the analysis.

CHAPTER 3. HYPOTHESIS I (Goldie and Coldman, 1979)

III.1. Introduction

Drug resistance in cancer may be innate or acquired and may apply to a single agent or to a group of agents with the same/similar antineoplastic mechanisms of action (Chang, 2011). Some patients show so-called "innate" resistance such that their tumor shows no objective initial response to therapy. Patients whose tumor is initially sensitive to adjuvant therapy, and may therefore show objective response, might nonetheless experience disease progression after a period of time, often while undergoing treatment. The unfortunate clinical reality is that acquired resistance often occurs. Even novel therapies which have shown remarkable clinical success, such as imatinib for chronic myeloid leukemia (CML), are not immune to the problem of drug resistance especially at advanced stages of cancer (Blagosklonny, 2004; Shannon, 2002). In fact, every existing anti-cancer drug suffers from the problem of resistance (Chabner and Roberts, 2005); it is, therefore, the principal cause of most treatment failure and hence, cancer-related death, especially in the metastatic setting (Longley et al., 2005; O'Driscoll et al., 2006; Gerlinger et al., 2010; Rivera et al. 2010).

Nowell (1976) first proposed that cancer progression is an essentially evolutionary process. As neoplasms are genetically and epigenetically diverse populations of billions of cells (Varley et al., 2009; Park et al., 2010; Yachida et al., 2010), and as cancer drugs represent potentially strong selective agents, the potential exists for selection to drive the evolution of a tumor cell population. Under this hypothesis then, tumorigenesis and acquired resistance are viewed as largely evolutionary phenomena (Kreuzer et al., 2003; Roche-Lestienne et al., 2003; Monceviciute-Eringiene, 2005; Pepper et al., 2008). Since Nowell's original suggestion, the evolutionary theory of acquired drug resistance has been investigated using several different approaches. In principle, testing of evolutionary theories of acquired resistance should span the investigation of simplified experimental systems to longitudinal observational studies of the evolutionary dynamics of cancer in laboratory animals and in the clinical setting (Pepper et al., 2008). Direct observational studies of human neoplasms have provided some tantalizing, albeit generally weak, evidence about the role of somatic evolution in cancer (Maley et al. 2004, 2006) as well as in acquired therapeutic resistance (Curt et al., 1983; Carman et al., 1984; Horns et al., 1984; Trent et al., 1984; Visakorpi et al.,

1995; Taplin et al., 1999; Gorre et al., 2001; Roche-Lestienne and Preudhomme 2003; Wang et al., 2007; Kobayashi et al., 2005; Engelman et al., 2007; Shah et al., 2007). But direct testing of evolutionary models has, to date, been conducted primarily in cell culture or in animal models (Kirstein, 2008); still outstanding is a systematic evaluation in the clinical setting.

Goldie and Coldman (1979) developed one of the earliest mathematical models of resistance evolution in neoplasms. Their model yielded two predictions of clinical significance: (1) the probability of a cure decreases exponentially with the initial tumor mass, i.e. the number of cancer cells; and (2) the likelihood of response and cure increases in combination vs single agent therapies (monotherapies). The rationale for the latter prediction is straightforward. In the Goldie and Coldman model, resistance evolves through the accumulation of resistance mutations and associated positive selection. If tumors are initially heterogeneous with respect to drug sensitivity, and there is little or no cross-resistance among drugs, then under combination therapy, resistance can arise only with the accumulation within a single lineage of a set of different mutations that confer resistance to different drugs. Consequently, the expected waiting time for the appearance of a resistant clone in an initially sensitive tumor will be longer than under monotherapy, for which fewer mutations are required for resistance. Thus, the initial tumor kill is substantially higher under combination treatment, as a smaller proportion of tumor cells will have the full set of mutations required for resistance to all constituent drugs, and tumor mass is reduced much more rapidly than under single agent treatment. The increased time required for acquisition of the requisite set of mutations means that combination therapy should also extend survival: indeed, if the waiting times are sufficiently long, the possibility arises that the tumor will be completely eradicated before significant evolutionary change can even occur (Goldie and Coldman 1984).

Here I test the Goldie and Coldman hypothesis in the metastatic cancer clinical trial setting. In this setting, the hypothesis makes three specific predictions:

(1) For any cancer, for any two cytotoxic drugs A and B, overall response rate (that is, the proportion of patients whose tumor shows objective evidence of shrinkage upon treatment including both partial and complete response) should be greater in trial

arms where A and B are administered in combination, compared to arms where only A (or only B), is administered.

(2) For any cancer and any two cytotoxic drugs A and B, the median duration of response should be longer in trial arms where A and B are administered in combination, compared to arms where only A (or only B) is administered; and

(1) For any cancer and any two cytotoxic drugs A and B, the median overall survival should be greater in trial arms where A and B are administered in combination, compared to arms where only A (or only B) is administered, assuming that background mortality rates are the same for all arms.

III.2. Methods

III.2.1. Datasets – Sample S_1 and S_2

We identified two independent sets of clinical trials with which to test the Goldie and Coldman hypothesis. Sample S_1 is comprised of studies with at least two arms, with patients in one arm receiving single agent therapy (drug A), and patients in a second arm receiving a two-drug combination therapy (drugs A and B) (Table 1). In this set of trials, patients were randomized into arms, so that there is comparatively high within-trial control of factors other than the therapeutic regime that might influence trial outcomes. In S_1, there are total 47 studies (94 arms) that fulfil the criteria (see Chapter 2 and below) for inclusion in the analysis. A second independent set of 84 clinical trials (Sample S_2, 84 arms), comprised trials with only one arm, which might be either monotherapy (drug A only) or combination therapy (drugs A + B) (Table 3.1). Unlike sample S_1, for sample S_2 there is no within-trial control as each trial includes only a single arm. It is therefore, quite likely that the magnitude of the single vs combination effect will be reduced in Sample S_2 relative to that detected in Sample S_1 owing to lack of within-trial control.

Clinical trials with only four cancers out of 30 different types of cancers in the UOMCCT database were selected due to their sufficient sample size to test the effect of cancer type. For testing of first hypothesis, Sample S_1 and Sample S_2 datasets (Table 3.2) were developed that contains four types of cancer (Breast, Colorectal, Pancreatic, Lung) clinical trials with data for three most relevant endpoints OR, OS, and MD.

With these datasets, the hypotheses testing began with fitting fixed-effects models followed by mixed-effects models (see section III.3). In these models, the dependent variables are the various endpoints (overall response rate, median duration of response, and overall survival) whereas the independent variables are fixed effects (type of regimen – single vs combination arm, cancer type, route of administration, toxicity, phase, total patients in a trial, total patients in an arm, and year of publication) and random effects (trial as a grouping factor).

III.3. Results

Forty-seven studies satisfied the inclusion criterion for sample S_1. Virtually all reported estimates of both overall response rate and median overall survival, but around 50% studies also included estimates of median duration of response. For sample S_2, 84 studies satisfied the necessary inclusion criterion providing estimates of both overall response rate and median overall survival, but comparatively less (54 studies) providing estimates of median duration of response.

For both samples S_1 and S_2, trials included a large range of different cancers. But many cancer types had few trials which included estimates of all three endpoints (OR, OS and MD). In what follows, I therefore restricted my analysis to four cancers (lung (including both small-cell and non-small-cell), breast, pancreatic, colorectal) that had sufficient sample size ($N > 15$ trials) to permit examination of the effects of candidate predictors as well as cancer type (Table 3.2).

III.3.1. Sample S_1

I retrieved a total of 28 studies (56 arms) for which estimates of all three endpoints (OR, OS and MD) were reported. For all three endpoints (OR, OS, and MD), initial fitted models included cancer type (CANCER), trial phase (PHASE), arm (ARM), total number of patients in the trial (TOTAL.PATIENTS), number of patients in each arm (ARM.PATIENTS), and the year (YEAR) in which the trial results were published. However, for sample S_1, I was unable to investigate the effect of route of administration (ROUTE), as in all but one trial, drugs were administered intravenously.

For overall response rate, the final selected model included cancer type and arm variables. Overall, the logit of response rate varied dramatically among different cancers ($F=15.8$, $df=3, 24$, $p<0.0001$) with breast cancer showing the highest average overall response, pancreatic the lowest (Fig 3.1a). As predicted, average overall response rates were greater for combination arms than single agent arms ($F=20.3$, $df=1, 27$, $p=0.0001$; Fig 3.1b). In the original fitted model, there was no effect of PHASE ($F=0.01$, $df=1, 21$, $p=0.9$), TOTAL.PATIENTS ($F=0.61$, $df=1, 21$, $p=0.4$), ARM.PATIENTS ($F=3.29$, $df=1, 26$, $p=0.08$), and YEAR ($F=2.05$, $df=1, 21$, $p=0.1$).

For overall survival (OS), detected effects were much the same as for OR, with substantial variation among cancer types ($F=11.2$, $df=3, 24$, $p=0.0001$; Fig 3.2a) and ARM ($F=7.34$, $df=1, 27$, $p=0.01$; Fig 3.2b). Similar to OR, no effects were detected for PHASE ($F=0.37$, $df=1, 21$, $p=0.5$), TOTAL.PATIENTS ($F=3.19$, $df=1, 21$, $p=0.08$), ARM.PATIENTS ($F=0.03$, $df=1, 26$, $p=0.8$), and YEAR ($F=2.93$, $df=1, 21$, $p=0.1$).

For median duration of response (MD), the only detected effect was that of ARM ($F=6.56$, $df=1, 27$, $p=0.01$; Fig 3.3b). No effects were detected for CANCER ($F=0.42$, $df=3, 24$, $p=0.7$; Fig 3.3a), PHASE ($F=3.27$, $df=1, 21$, $p=0.08$), TOTAL.PATIENTS ($F=0.43$, $df=1, 21$, $p=0.5$), ARM.PATIENTS ($F=0.05$, $df=1, 26$, $p=0.8$), and YEAR ($F=3.24$, $df=1, 21$, $p=0.08$).

Around 50% of trials in sample S_1 reported the estimated median duration of response. Hence, if we consider trials reporting estimates of both OR and OS, but not MD, sample size increases considerably, to N = 47 (94 arms – Table 3.2).

For this larger sample (S_1 (b) – see Table 3.2), average overall response rates were again greater for combination than single agent arms ($F=33.0$, $df=1, 46$, $p<.0001$; Fig 3.4b) with, again, considerable variation among cancer types ($F=12.4$, $df=3, 43$, $p<.0001$; Fig 3.4a). As with sample S_1 (a), there was no detected effect of PHASE ($F=0.004$, $df=1, 40$, $p=0.9$), TOTAL.PATIENTS ($F=0.17$, $df=1, 40$, $p=0.6$), ARM.PATIENTS ($F=0.22$, $df=1, 45$, $p=0.6$), and YEAR ($F=0.69$, $df=1, 40$, $p=0.4$).

For overall survival, detected effects are much the same, with substantial variation among cancer types ($F=20.3$, $df=3, 43$, $p<.0001$; Fig 3.5a) and ARM ($F=17.0$, $df=1, 46$, $p=0.0002$; Fig 3.5b), but no detected effects for PHASE ($F=0.07$, $df=1, 40$, $p=0.7$),

TOTAL.PATIENTS (F=0.80, df=1, 40, p=0.3), ARM.PATIENTS (F=0.24, df=1, 45, p=0.6), and YEAR (F=7.42, df=1, 40, p=0.009).

III.3.2. Sample S_2

I retrieved 54 studies with only one arm which provided estimates of all three endpoints (OR, OS and MD). For all three endpoints (OR, OS, and MD), initial fitted models included cancer type (CANCER), arm (ARM), total number of patients in the trial (TOTAL.PATIENTS), route of administration (ROUTE), and year (YEAR) in which the trial results were published. As Sample S_2 includes only Phase 2 trials, PHASE as a candidate predictor was not investigated.

For overall response rate (OR), the final selected model for the S_2 sample included cancer type and year, but no effect of arm (F=2.28, df=1, 44, p=0.1). Overall, the logit of response rate varied dramatically among different cancers (F=11.2, df =3, 42, p<0.0001; Fig 3.6a) with breast cancer showing the highest average overall response, colorectal the lowest. There was also a small positive effect of YEAR (F=4.58, df=1, 44, p=0.03). In the original fitted model, no effects of TOTAL.PATIENTS (F=0.37, df=1, 44, p=0.5), ARM (F=2.20, df=1, 44, p=0.1; Fig 3.6b), and ROUTE (F=0.48, df=2, 43, p=0.6) were detected.

For overall survival, only cancer type showed a detectable effect (F=5.48, df=3, 42, p=0.02; Fig 3.7a). No effects were detected for TOTAL.PATIENTS (F=0.009, df=1, 44, p=0.9), ARM (F=0.96, df=1, 44, p=0.3; Fig 3.7b), ROUTE (F=2.24, df=2, 43, p=0.1), and YEAR (F=0.61, df=1, 44, p=0.4).

For median duration of response, there is only one detected effect, cancer type (F=8.63, df=3, 42, p=0.0001; Fig 3.8a). No effects were detected for ARM (F=0.20, df=1, 44, p=0.6; Fig 3.8b), TOTAL.PATIENTS (F=0.119, df=1, 44, p=0.7), ROUTE (F=1.26, df=2, 43, p=0.2), and YEAR (F=2.18, df=1, 44, p=0.1).

Similar to S_1, for S_2, I investigated a larger set of 84 trials obtained by relaxing the criterion of an MD estimate being provided. For this larger sample (S_2 (b) – see Table 3.2), effects of cancer type were detected for both OR (F=8.44, df =3, 42, p<.0001; Fig 3.9a) and OS (F=16.9, df=3, 42, p<.0001; Fig 3.10a).

For OR, an effect of ROUTE was also detected (F=6.05, df=2, 43, p=0.003), while for OS, a small effect of YEAR was detected (F=5.20, df=1, 44, p=0.02). In the original fitted model, for OR, there was no effect of TOTAL.PATIENTS (F=0.45, df=1, 44, p=0.5), ARM (F=1.90, df=1, 44, p=0.1; Fig 3.9b), and YEAR (F=3.28, df=1, 44, p=0.07).

Similarly for OS, no effects were detected in the original fitted model for ARM (F=2.27, df=1, 44, p=0.1; Fig 3.10b), TOTAL.PATIENTS (F=0.07, df=1, 44, p=0.7), and ROUTE (F=1.04, df=2, 42, p=0.3).

III.4. Discussion

The Goldie and Coldman (1979) hypothesis - translated to the metastatic cancer clinical setting - predicts that, for any two cytotoxic drugs A and B, average response rates, median duration of response and, as a result, overall survival, should be greater in combination arms than in single agent arms. Consistent with this hypothesis, I found that for the sample of studies (S_1) having more stringent internal control, that is, where the effects of other trial attributes such as, the extent of patient pre-treatment (Blackwell et al., 2010; Papaldo et al., 2006) and the types of drugs employed (Geyer et al., 2006; Martin et al., 2007) are, presumably, randomized between arms. Overall response rate, overall survival, and median duration of response were greater in combination vs single agent arms. This effect was generally not observed in the second (S_2) sample of studies, where between-trial variation, even for the same cancer type and chemotherapy regimen, is expected to be larger due to differences among trials in design and patient population attributes. This variability, which is substantially better controlled in sample S_1, will make the detection of combination vs single agent therapy more difficult.

Currently, combination chemotherapy is the standard for many advanced solid cancers including breast, colorectal, and lung (Carrick et al., 2009; Bennouna and Douillard, 2002; Milton and Miller, 2005). This is, primarily, because of the belief that combination therapy generally improves tumor response compared to that obtaining under single agent therapy. Carrick et al. (2009) showed that, based on a sample of 37 metastatic breast cancer clinical trials, time to progression (Hazard ratio = 0.78), response rate (Odds Ratio = 1.28), and overall survival (HR = 0.88) were, on average, greater under combination than single agent

therapy. Similarly, a meta-analysis of advanced non-small cell lung carcinoma including 25 randomized trials showed that combination chemotherapy was associated with a nearly two-fold increase in the objective response rate compared to single agent chemotherapy, in addition to modestly prolonging survival duration (Lilenbaum et al., 1998). More recently, in a meta-analysis of combination vs single-agent therapies in the treatment of metastatic breast cancer based on 48 trials, Carrick et al. (2009) showed a statistically significant difference in favour of combination regimens for overall survival, with no heterogeneity. Combination regimens showed a statistically significant advantage for survival over single agent. Combination regimens were also associated with significantly better time to progression and overall response rate, although heterogeneity was statistically significant in both cases. These results, as well as mine, suggest that, generally speaking, combination chemotherapy is more effective than single agent therapy for advanced solid tumors.

It is generally acknowledged that, particularly in the metastatic setting, treatment failure reflects either innate or acquired resistance (Rivera and Gomez, 2010; Moreno-Aspitia and Perez, 2009). As noted in Chapter 1, there are many different types of mechanisms that, in principle, can lead to drug resistance. If all cancer cells are susceptible to the drug, then the chemotherapy would lead to an eradication of the cancer (Wodarz and Komarova, 2005). Even if tumors are not intrinsically resistant to a specific chemotherapy treatment, the genetic and epigenetic heterogeneity of the population in the face of powerful selection imposed by cytotoxic drugs can produce cell types that are resistant to the drug (Gottesman, 2002). The proliferation of the resistant phenotype leads to resistance to the chemotherapy and results in cancer progression (Wodarz and Komarova, 2005).

Given that resistance is the major mechanism of treatment failure, then why would combination therapy reduce the likelihood of, or the rate of acquisition of, resistance? For innate resistance, the obvious explanation is that, especially in the metastatic setting, tumors are highly heterogeneous, and comprise numerous sub-clones of differing sensitivities to different drugs. If, for example, a tumor consists of cells sensitive to drug A but not B, as well as cells sensitive to drug B but not A, a combination of both drugs A and B will result in greater tumor kill than A or B alone, especially if they interact synergistically. In the clinical setting, this would present as a greater response rate as presumably such heterogeneity exists

both within and among patients. Indeed, this is the original logic underlying combination cancer therapy.

As noted above, there is substantial evidence that combination therapy results in a better/greater initial response than single agent therapy. If responders live longer than non-responders, the differential response rate under combination vs single agent therapy should translate into greater median survival. But the Goldie and Coldman hypothesis predicts that the two regimes have different implications for the evolution of resistance in initially sensitive tumors. In the Goldie and Coldman model, resistance evolves through the accumulation of resistance mutations and associated positive selection. If tumors are initially heterogeneous with respect to drug sensitivity, and there is little or no cross-resistance among drugs, then under combination therapy, resistance can arise only with the accumulation within a single lineage of a set of different mutations that confer resistance to different drugs. Consequently, the expected waiting time for the appearance of a resistant clone in an initially sensitive tumor will be longer than under single agent therapy, for which fewer mutations are required for resistance. Thus, the initial tumor kill is substantially higher under combination treatment, as a smaller proportion of tumor cells will have the full set of mutations required for resistance to all constituent drugs, and tumor mass is reduced much more rapidly than under single agent treatment. The increased time required for acquisition of the requisite set of mutations means that combination therapy should increase the median duration of response of patients that respond initially. In fact, if the waiting times are sufficiently long, the possibility arises that the tumor will be completely eradicated before significant evolutionary change can even occur (Goldie and Coldman, 1984).

The postulated evolutionary benefit of combination therapy under the Goldie and Coldman model concerns the greater expected waiting time for mutations conferring resistance to several drugs to accumulate in a single lineage. But this need not be the only difference between combination and single agent therapy. Pepper et al. (2008) note that in cancer therapy, the clinical objective is to reduce the size of the tumor as quickly as possible to achieve immediate clinical benefit. This objective in part underlies the twin concepts of a maximum tolerable dose (MTD) and the Therapeutic Index: the idea is to design killing agents for which maximum cancer cell mortality is achieved at a dose considerably lower than the dose at which the therapy is toxic to the patient. However, if heritable variation in

susceptibility to the killing agent exists in the tumor cell population, high mortality implies that only cells with very high resistance escape killing. The result is a large difference in the average value of the trait (resistance) in those cells that are killed, compared to those that survive, i.e. a large selection differential. Because evolutionary rate is proportional to the selection differential, all else being equal, therapies causing high cell mortality will increase the rate of evolution of resistance compared to those inducing lower mortality.

The implications of the positive relationship between, on the one hand, pathogen mortality induced by therapeutic agents and, on the other hand, the rate of resistance evolution, are potentially profound. As Read et al. (2011) noted, the "curious orthodoxy" of aggressive chemotherapy is ubiquitous in disease therapy, for both infectious and non-infectious diseases. Yet if indeed higher pathogen mortality increases the rate of resistance evolution, and combination therapy results in greater tumor cell mortality, resistance evolution under combination therapy will be accelerated relative to that under monotherapy. There is some empirical support for this contention. In an elegant laboratory experiment with *E. coli*, Hegreness et al. (2008) showed that, the rate of resistance evolution in combination drug treatments depended dramatically on the extent to which the associated drugs exerted synergistic vs antagonistic effects on mortality. In particular, they found accelerated resistance evolution in synergistic drug combination therapy compared to both monotherapy and antagonistic combination therapy. Thus they attribute to the larger selective advantage enjoyed by resistance mutations in synergistic vs antagonistic drug environments (Hegreness et al., 2008). A review identified that it is a requirement to understand better how to include drugs targeting synthetic lethal mechanisms (and other non-oncogene addiction drugs) within drug combinations, and to determine the extent to which they can be combined successfully with chemotherapy drugs, other targeted agents and immunotherapy without increasing toxicity (Al-Lazikani et al., 2012). Humphrey et al. (2011) reported that evidence from animal tumor models indicates that the therapeutic effects of certain drug combinations may exceed those of monotherapies. Before phase II or phase III studies of combination therapies can be initiated, rational preclinical models should guide clinical trial design and may illuminate issues such as dosing regimens (administering two drugs concomitantly or sequentially), drug interactions affecting pharmacokinetics, and interactive toxic effects (Humphrey et al., 2011). In addition, new approaches to phase I

studies of combination therapies should be considered to improve efficiency and increase our understanding of how best to test agents in combination (Hamberg et al., 2010).

As Read et al. (2011) pointed out, aggressive chemotherapy, whether in cancer or other indications, is a double-edged sword. On the one hand, it reduces the likelihood of resistance mutations arising *de novo*, of at least two reasons. First, the more aggressive the therapy, the greater the pathogen mortality, and the faster the population is reduced. As the likelihood of new mutations arising increases with population size, therapies that reduce pathogen population size quickly will, all else being equal, reduce the likelihood of resistance mutations. Second, in the content of combination therapy, the more agents in a drug cocktail, the longer the waiting time for accumulation of sufficient number of mutations to result in resistance to all constituent drugs. This is one edge of the sword. But the other edge concerns what happens given once resistance mutations arise, or if they are already present when therapy begins. In both situations, the afore-mentioned advantage of aggressive/combination therapy no longer exists. Rather, now aggressive therapy, or in the current context, the greater tumor cell mortality associated with combination therapy gives rise to stronger selection pressures. Hence, these pre-existing resistance mutations will much more rapidly sweep through the population. In so far as treatment failure in the metastatic setting reflects acquired resistance then, once resistance mutations arise, we expect the time to treatment failure to be decrease with the aggressiveness of the therapy.

My results provide clear evidence that in the metastatic setting, response rate is consistently greater under combination vs monotherapy. As response, in the clinical setting, means reduction of tumor volume on radiographic imaging, it is highly likely that this greater response rate reflects greater tumor kill, i.e. greater pathogen mortality. It also suggests that, in responders, if resistance cell phenotypes exist, they must be at comparatively low prevalence. My results also provide evidence that combination therapy results in, on average, greater duration of response than monotherapy. This suggests that, at least in the metastatic setting, that the potential disadvantages of combination therapy with respect to accelerated resistance evolution are outweighed by the greater waiting times for resistance mutations to arise. The regimes considered here reflect a conventional clinical trial protocol where a single regimen is administered for the duration of the trial if toxicity is tolerable. Other regimens are possible. One possibility is where several agents are used not in

combination, but sequentially. Such sequential regimens are attractive in the clinical setting, as demonstrated here, although combination therapy may have greater effectiveness in terms of response rate and/or duration of response, side effects are often worse (Peters et al., 1988; Miller et al., 2005; Saltz et al., 2008).

III.4.1. Developing cancer therapeutics

A clinical debate was featured at the 27th Congress of the European Society for Medical Oncology on the matter of whether combination chemotherapy should be considered standard treatment for metastatic colorectal cancer (Mocharnuk, 2002). The results of Otter and Sirotnak (1994) study reported that simultaneous administration of edatrexate with vinblastine, navelbine, or vindesine increased survival 2- to 3-fold over that obtained with single agents alone and yielded 10-40% long-term survivors, while sequential administration increased survival <2-fold over that obtained with single agents and yielded 0-20% long-term survivors. Carrick et al. (2009) study showed that in advanced disease, combination therapy was associated with significantly better time to progression and response rates. At the 6th European Breast Cancer Conference, the European School of Oncology Metastatic Breast Cancer recommends that patient- and disease-related factors should be used to choose between combination and sequential single-agent chemotherapy for metastatic breast cancer (MBC) (Cardoso et al., 2009). Additional research is needed to focus on the still unresolved issue of whether it is better to treat MBC patients sequentially with single cytotoxic agents or to treat them simultaneously with a combination of drugs (Cardoso et al., 2009). Indeed, debate continues over whether active single agents or combinations should (1) be used concurrently with an additional active drug, (2) be used in sequence, or (3) be used in combination in intermittent therapy (Mocharnuk, 2002).

In an attempt to achieve greater tumor response rates, combination chemotherapy regimens are frequently favoured over single agents for the treatment of metastatic cancer. My results, based on sample 1, suggest that combination therapy also increases the median duration of response or responders. This difference (viz., increased response rate and longer duration of response) translates into greater overall median survival. However, when both survival and toxicity are considered, it is not known whether giving more intensive chemotherapy regimens results in better health outcomes, and whether better response rates

and rates of progression free survival actually translate to better overall survival (Carrick et al., 2009). Combination chemotherapy regimens show a statistically significant advantage for survival, tumor response, and time to progression in women with metastatic breast cancer but they also produce more toxicity. An unresolved question was there for a long time that whether combination regimens are more effective than single agents given sequentially. However, more recent studies showed that combination and sequential therapy both have their place in the treatment of MBC but newer drug combinations, such as paclitaxel/trastuzumab or capecitabine/docetaxel, showed survival advantages over sequential single-agent therapy and have manageable safety profiles (Miles et al., 2002). Such combination treatments may be preferable to sequential therapy for patients requiring urgent reduction in their tumor burden. Sequential therapy allows the optimal delivery of single-drug therapy and potentially reduces the risk of toxicity, which may improve quality of life (Miles et al., 2002). Sequential therapy may be especially appropriate in elderly patients, who may be unable to tolerate the toxicity of combination therapy, or in patients with slowly growing tumors.

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Table 3.1. Characteristics of Sample S_1 and Sample S_2 trials. To be included in S_1, a hypothetical trial (Trial 1 or 2) must include both a single agent arm (A or B) and a combination arm (A+B). By contrast, trials in sample S_2 have only 1 arm, either monotherapy (Trials 1, 2) or combination therapy (Trial 3).

	Sample 1			Sample 2		
Arms	Single	Single	Combination	Single	Single	Combination
Drugs	A	B	A+B	A	B	A+B
Trial 1	X		X	X		
Trial 2		X	X		X	
Trial 3						X

Table 3.2. Sample size for four types of cancers (Breast, Colorectal, Lung, and Pancreatic).

Experimental Design	Endpoints			# Arms	# Trials
	OR	MD	OS		
Sample 1a (Within trial control)	X	X	X	56	28
Sample 1b (Within trial control)	X		X	94	47
Sample 2a (No within trial control)	X	X	X	54	54
Sample 2b (No within trial control)	X		X	84	84

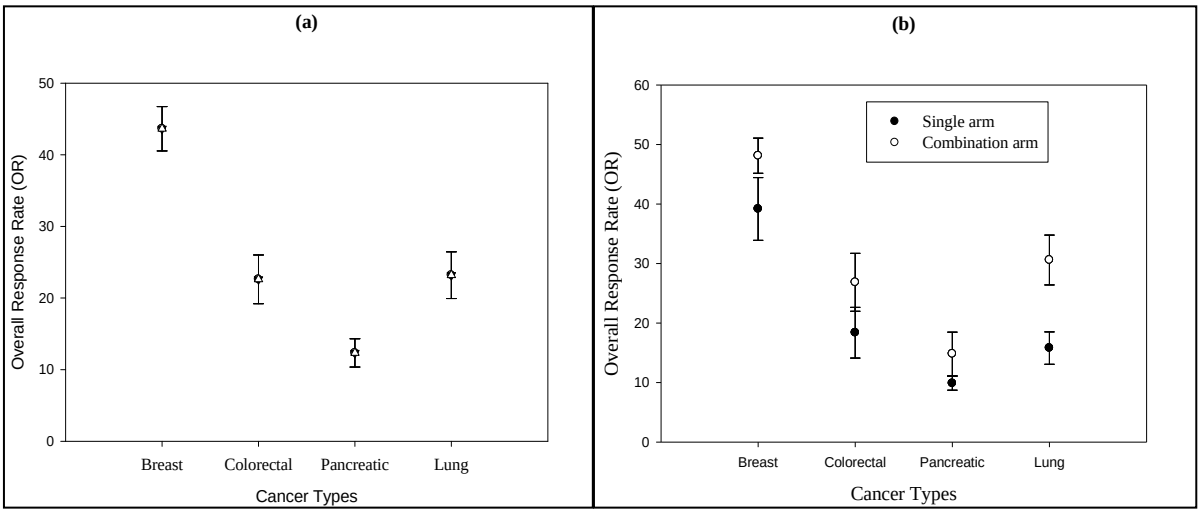

Figure 3.1. Average overall response rate (OR) and associated standard error for the four main cancer types, pooled over arm (a), and for combination and single agent arms considered separately (b). Averages are based on sample S_1a that includes the $N = 28$ studies (56 arms) that provided estimates of all three endpoints (OR, OS, MD).

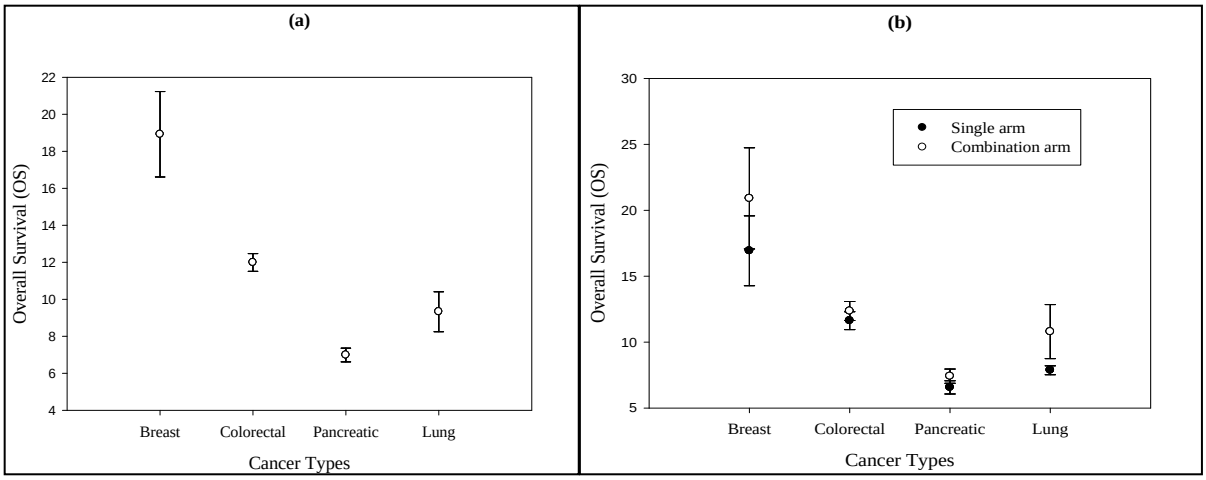

Figure 3.2. Average overall median survival (OS) in months and associated standard error of the four main cancer types, pooled over arm (a), and for combination and single agent arms

considered separately (b). Averages are based on sample S_1a that includes the $N = 28$ studies (56 arms) that provided estimates of all three endpoints (OR, OS, MD).

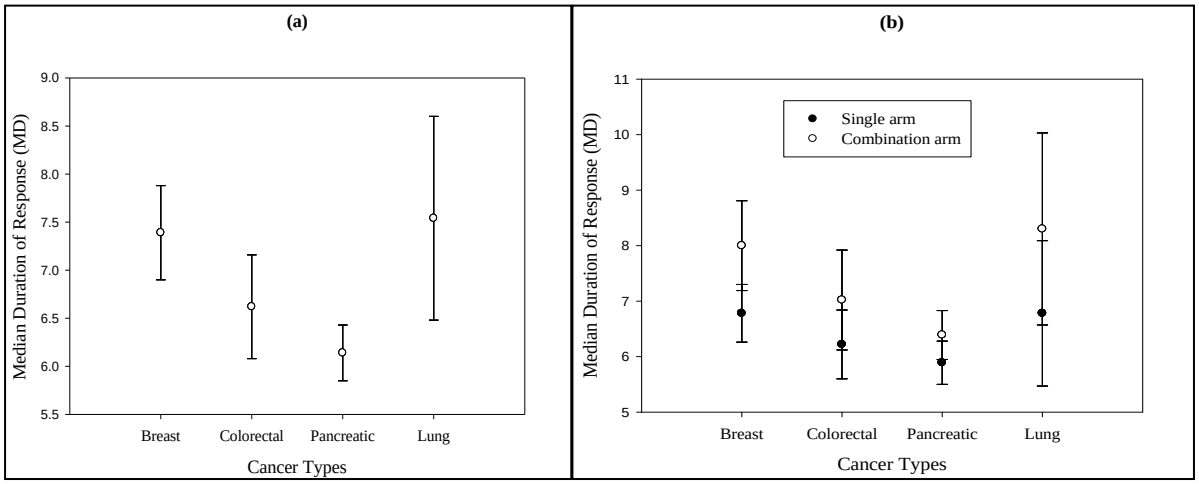

Figure 3.3. Average median duration of response (MD) in months and associated standard error of the four main cancer types, pooled over arm (a), and for combination and single agent arms considered separately (b). Averages are based on sample S_1a that includes the $N = 28$ studies (56 arms) that provided estimates of all three endpoints (OR, OS, MD).

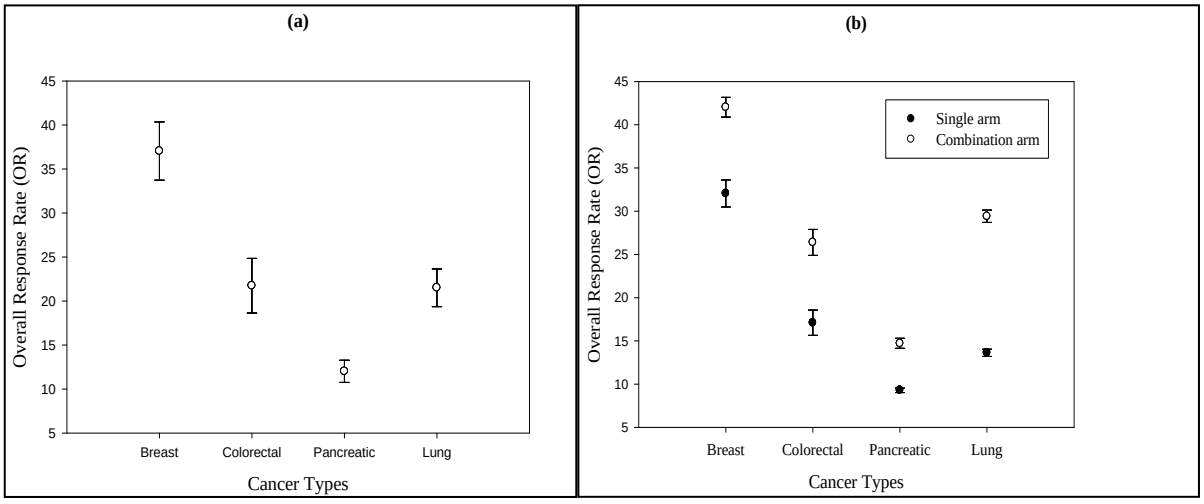

Figure 3.4. Average overall response rate (OR) and associated standard error of the four main cancer types, pooled over arm (a), and for combination and single agent arms

considered separately (b). Averages are based on sample S_1b that includes the $N = 47$ studies (94 arms) that provided estimates of two endpoints (OR, OS).

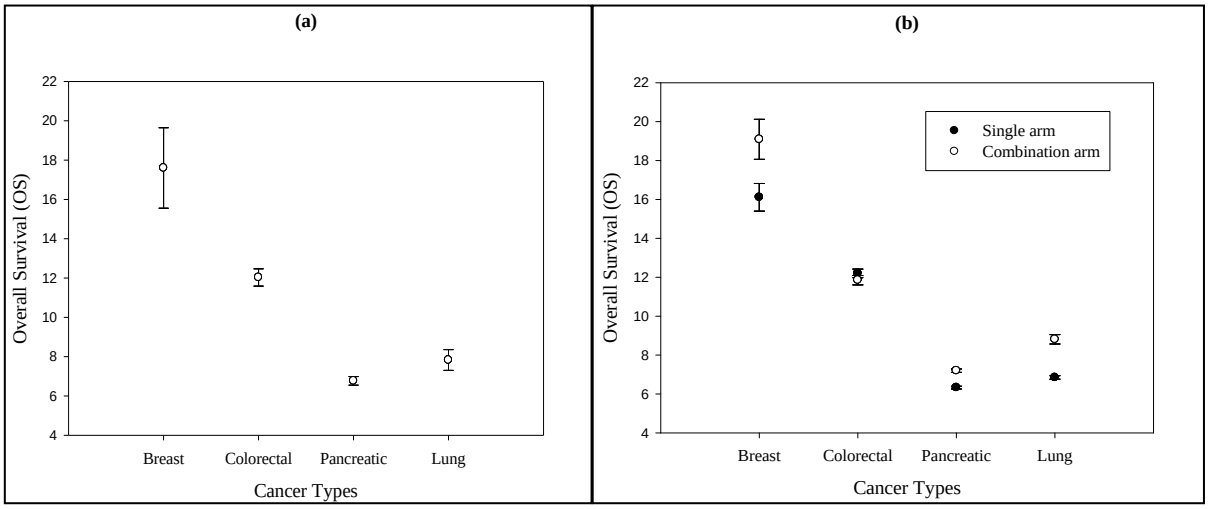

Figure 3.5. Average overall survival (OS) in months and associated standard error of the four main cancer types, pooled over arm (a), and for combination and single agent arms considered separately (b). Averages are based on sample S_1b that includes the $N = 47$ studies (94 arms) that provided estimates of two endpoints (OR, OS).

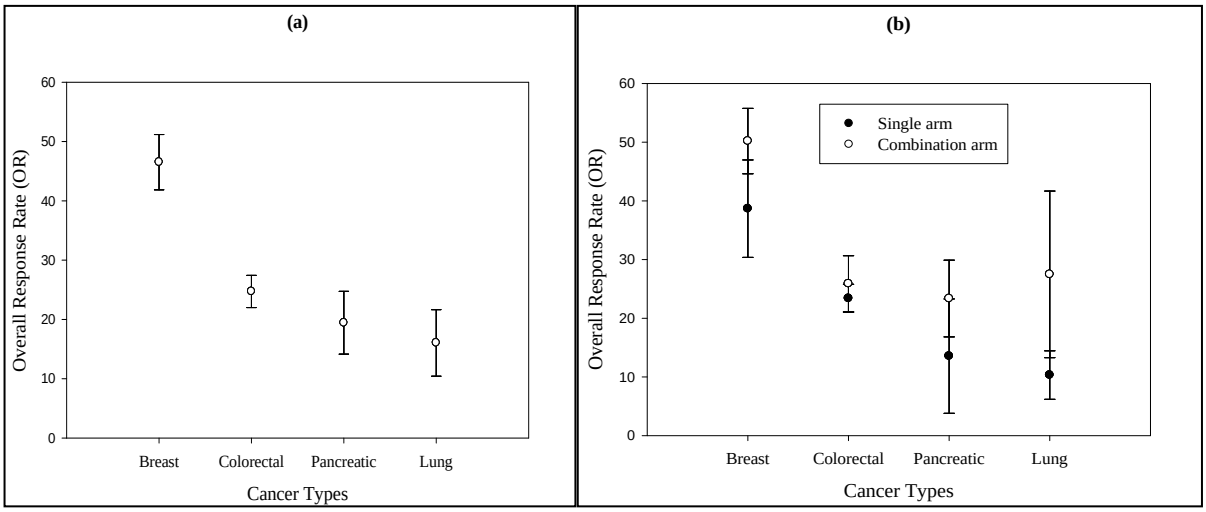

Figure 3.6. Average overall response rate (OR) and associated standard error of the four main cancer types, pooled over arm (a), and for combination and single agent arms considered separately (b). Averages are based on sample S_2a that includes the $N = 54$ studies (54 arms) that provided estimates of all three endpoints (OR, OS, MD).

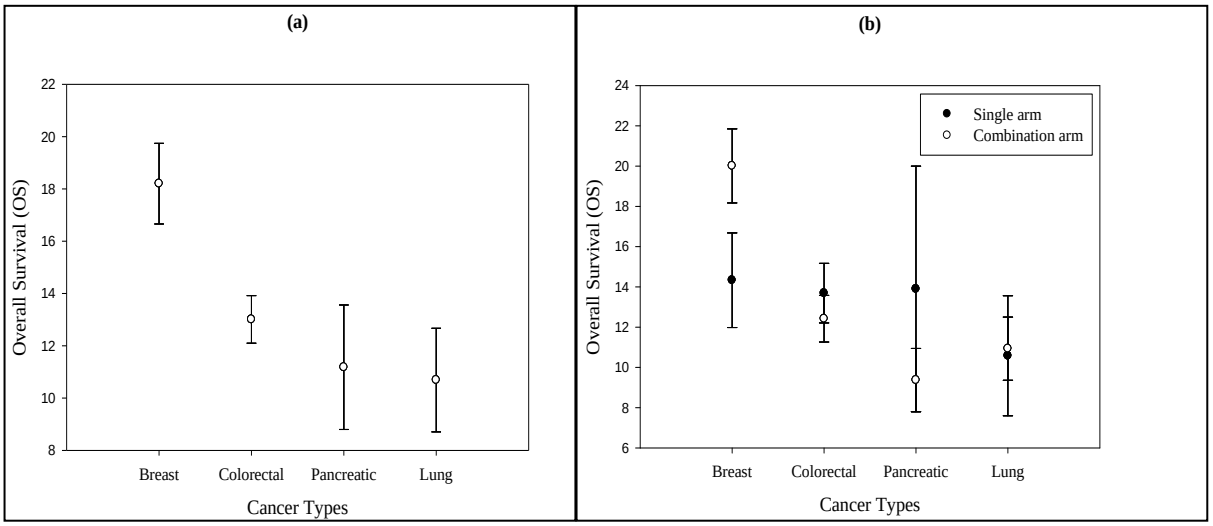

Figure 3.7. Average overall survival (OS) in months and associated standard error of the four main cancer types, pooled over arm (a), and for combination and single agent arms considered separately (b). Averages are based on sample S_2a that includes the $N = 54$ studies (54 arms) that provided estimates of all three endpoints (OR, OS, MD).

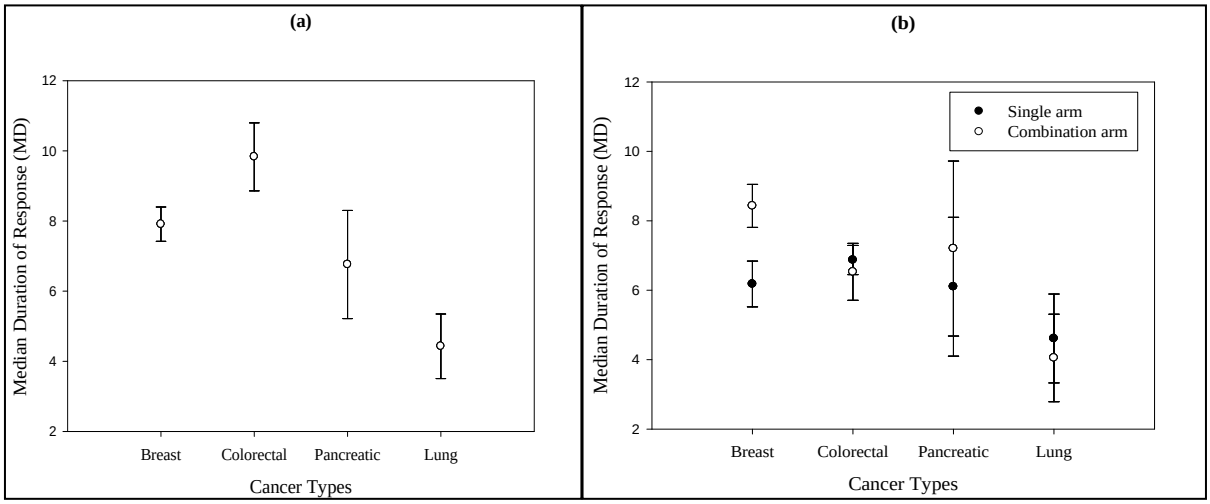

Figure 3.8. Average median duration of response (MD) in months and associated standard error of the four main cancer types, pooled over arm (a), and for combination and single agent arms considered separately (b). Averages are based on sample S_2a that includes the $N = 54$ studies (54 arms) that provided estimates of all three endpoints (OR, OS, MD).

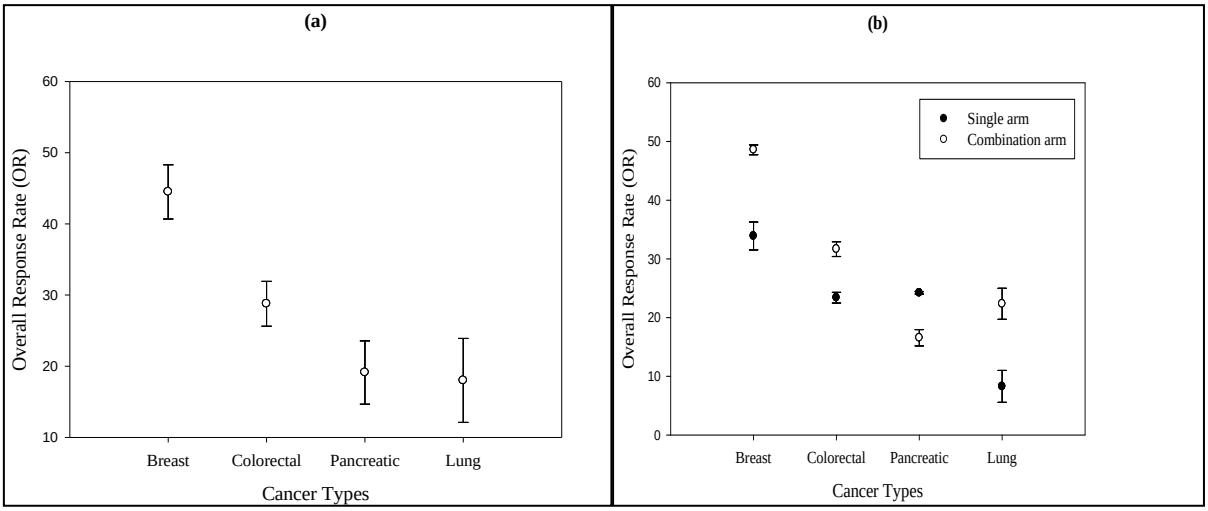

Figure 3.9. Average overall response rate (OR) and associated standard error of the four main cancer types, pooled over arm (a), and for combination and single agent arms considered separately (b). Averages are based on sample S_2b that includes the $N = 84$ studies (84 arms) that provided estimates of two endpoints (OR, OS).

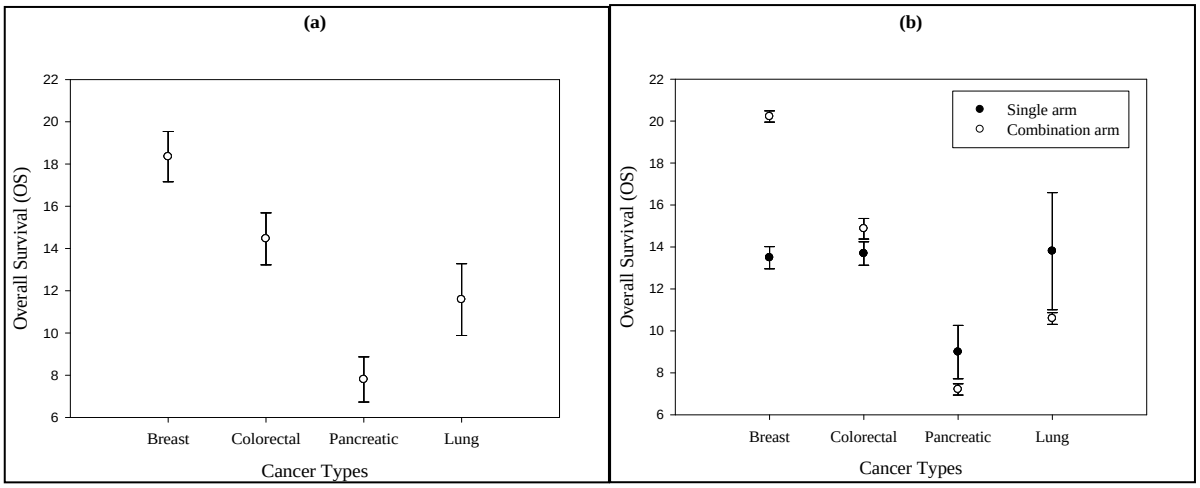

Figure 3.10. Average overall survival (OS) in months and associated standard error of the four main cancer types, pooled over arm (a), and for combination and single agent arms considered separately (b). Averages are based on sample S_2b that includes the $N = 84$ studies (84 arms) that provided estimates of two endpoints (OR, OS).

CHAPTER 4. HYPOTHESIS II (Gardner, 2002)

IV.1. Introduction

Cancer chemotherapies include both cytotoxic and cytostatic agents. Cytotoxic agents are directly toxic to cancer cells. By contrast, cytostatic drugs (Broxterman and Georgopapadakou, 2004) are those that, while not directly toxic to tumor cells, nonetheless inhibit their proliferation through interruption of the cell cycle (Blagosklonny, 2004). This classification, while convenient, is to some degree an oversimplification, in part because certain drugs, under certain conditions, may be cytotoxic, while under other conditions cytostatic (Blagosklonny, 2004). Indeed, designing assays capable of distinguishing cytotoxic from cytostatic effects both *in vitro* and *in vivo* is a challenge (Kustermann et al., 2013). For example, a nominally cytotoxic agent such as imatinib can also be cytostatic in apoptosis-resistant cells especially at low doses (Blagosklonny, 2004a). Conversely, even nominally cytostatic agents may have cytotoxic properties. For example, although sorafenib was developed as a cytostatic agent, there is evidence that it is also a cytotoxic agent, with direct toxicity resulting from DNA damage and the subsequent induction of apoptosis (Rixe and Fojo, 2007),

Most cytostatic drugs developed to date involve some type of receptor blockade, by preventing binding of hormones such as estrogen to receptors on the tumor cell surface. As such, and in contrast to cytotoxic agents, they represent a major class of so-called "targeted" therapies designed to inhibit tumor cell proliferation through the disruption of specific signalling pathways, including, for example, tretinoin (all-trans-retinoic acid), which targets the PML-RARα fusion protein in acute promyelocytic leukemia (Sun et al., 1993; Tallman et al., 1997), imatinib mesylate, which targets the BCR-ABL fusion protein in chronic myelogenous leukemia (Druker et al., 2001) and the mutated KIT receptor in gastrointestinal stromal tumors (van Oosterom et al., 2001).

While cytotoxic chemotherapy has been the mainstay of chemotherapeutic approaches to the treatment of solid cancers (Conlin and Seidman, 2008; Chonghaile et al., 2011; Park et al., 2012; Temkin and Fleming, 2009), there is increasing interest in the use of cytostatic agents. This interest is due to several potential advantages of cytostatic agents. First, as noted above, many cytostatic agents represent targeted therapies, that is, drugs that block the

growth and spread of cancer by interfering with specific molecules involved in tumor growth and progression (NCI, 2012). The use of targeted therapy has markedly changed outcomes for some diseases (Gerber, 2008). For example, imatinib has had a dramatic effect on the treatment of chronic myeloid leukemia, and rituximab, sunitinib, and trastuzumab have revolutionized the treatment of non-Hodgkin's lymphoma, renal cell carcinoma, and breast cancer, respectively (Romond et al., 2005; Moore et al., 2007; Willett et al., 2004). At least in principle, targeted drugs will interact with proteins that are specific to tumor cells or that are upregulated during malignant transformation, so under these conditions, target-based therapies have the potential of being more selective and less toxic to normal tissues (Fox et al., 2002). As a consequence, such therapies are expected to have a much more tolerable side-effect profile than more conventional broad-spectrum cytotoxic agents.

A second potential advantage of cytostatic agents concerns acquired resistance. Acquired resistance is a major cause of treatment failure, especially in the metastatic setting (Rivera et al., 2010; Longley and Johnston, 2005; O'Driscoll et al., 2006). The relatively rapid acquisition of resistance is a substantial challenge to the clinical management of advanced cancers (Lackner et al., 2012). There is, moreover, accumulating evidence that acquired resistance reflects clonal evolution and selection in response to systemic therapy (Diaz et al., 2012; Aparicio and Caldas, 2013). Unsurprisingly then, as the molecular mechanisms of resistance have begun to be elucidated, new strategies to overcome or prevent acquired resistance have begun to emerge (Lackner et al., 2012).

An important issue is, therefore, the extent to which acquired resistance can be delayed, or possibly even prevented, by the use of cytostatic agents. Kerbel et al. (1991) suggested that anti-angiogenic drugs that prevent the growth of blood vessels by targeting vascular endothelial cells, might delay the onset of resistance simply because such cells do not possess the genetic instabilities associated with cancer cells. This reduced mutation rate compared to tumor cells was predicted to dramatically increase the waiting times for resistance mutations and hence, the onset of clinical resistance. Unlike conventional cyclophosphamide (a cytotoxic agent) therapy, long term, cyclic exposure of tumor-bearing mice to the anti-angiogenic (cytostatic) drug endostatin resulted in no detectable resistance evolution over a period of almost 60 days (Boehm et al., 1997) in contrast to the rapid resistance evolution observed under cytotoxic therapy. Hosoi et al's (1998) study on

mechanism of resistance to cytostatic agent rapamycin in human cancer cells, suggests that the ability of rapamycin to inhibit c-MYC (oncogene) induction correlates with intrinsic sensitivity, whereas failure of rapamycin to inhibit induction or overexpression of c-MYC correlates with intrinsic and acquired resistance, respectively. Another study reported that the combination of BRAF or MEK1/2 inhibitors effectively transforms the cytostatic response of these inhibitors into a striking apoptotic cell death response (Sale and Cook, 2013). This not only augments the primary efficacy of BRAF and MEK1/2 inhibitors but delays the onset of acquired resistance to these agents (Sale and Cook, 2013).

In light of the general interest among clinicians and scientists in combination therapies (see Ch.3), there has, unsurprisingly, been interest in the possibility of enhancing (potentiating) the effect of cytotoxic drugs through combination with cytostatic agents. In a recent modeling study, Villasana et al. (2010) showed that when compared to treatment protocols that only consider a cytotoxic agent, the incorporation of a cytostatic drug dramatically improved the outcome and performance of the overall treatment, confirming *in silico* that the combination of a cytostatic with a cytotoxic agent improves the efficacy and efficiency of the chemotherapy (Villasana et al., 2010). Another study in non-small-cell lung cancer (NSCLC) concluded that by concurrent administration of sorafenib and vinorelbine, cisplatin or gefitinib was at least as efficacious as the individual agents alone and was well tolerated (Carter et al., 2007). These results support the inclusion of cytostatic agent sorafenib in clinical trials in NSCLC employing combinations of both cytotoxic and cytostatic agents.

Gardner (2002) developed a computational kinetic model to predict chemotherapeutic combinations, doses and schedules most likely to result in patient response and prolonged life. A key prediction of this model is that, for a wide range of parameter conditions, combination therapy involving both cytotoxic and cytostatic drugs will be more effective than combination therapy involving only cytotoxic drugs. In the Gardner model, the reason is straight forward: the comparatively intense positive selection for resistance induced by potent cytotoxic combinations is attenuated by a deceleration of the rate at which the evolutionary clock ticks through what amounts to drug-induced quiescence. Intense positive selection for resistance is also induced by cytotoxic combinations, but here there is markedly less deceleration in the rate of evolution.

Here I test Gardener's hypothesis in the metastatic cancer clinical trial setting. In this setting, the hypothesis makes three specific predictions:

(1) For any cancer, the overall response rate should be greater in trial arms where a combination of cytotoxic and cytostatic drugs is administered, compared to arms where a combination of only cytotoxic drugs is administered;

(2) For any cancer, the median duration of response should be shorter in trial arms where a combination of cytotoxic drugs is administered, compared to arms where a combination of cytotoxic and cytostatic drugs is administered; and

(3) As a consequence of (1) and (2), overall survival should be lower in trial arms where a combination of cytotoxic drugs is administered, compared to arms where a combination of cytotoxic and cytostatic drugs is administered.

IV.2. Methods

Whether a drug's effect is mainly cytotoxic or mainly cytostatic was determined with reference to the National Cancer Institute's Drug Dictionary based on identified mechanisms of action (NCI, 2013) (Table 4.1). For example, if the major source of tumor cell mortality is DNA alkylation, the drug is considered primarily cytotoxic. By contrast, if the major source of tumor cell mortality is indirect via inhibition of angiogenesis, then the drug is considered primarily cytostatic (Table 4.1).

I identified two independent sets of clinical trials to test the Gardner's hypothesis. Sample S_1 comprises studies with at least two combination arms, with patients in one arm receiving combination therapy of two or more cytotoxic drugs (CT+CT), and patients in a second arm receiving combination therapy of at least one cytotoxic and at least one cytostatic drug (CT+CS) (Table 4). Cytotoxic drugs can be same or different in both the CT+CT arm and CT+CS arm, hence the cytotoxic drugs in the CT+CT arms need not be the same cytotoxic drugs used in the CT+CS arms in some clinical trials. In this set of trials, patients were randomized into arms, so that there is comparatively high within-trial control of factors other than the therapeutic regime that might influence trial outcomes. A second independent set (Sample S_2) is comprised of trials with at least one combination arm, with patients receiving combination therapy of either cytotoxic drugs or a combination of cytotoxic and

cytostatic drugs (Table 4.2).

For each dataset, the hypothesis testing done by fitting fixed-effects models. For Sample S_1, I was unable to fit mixed-effects models due to very small sample size. In these models, the dependent variables were the various endpoints (overall response rate (OR), median duration of response (MD), and overall survival (OS)) and the fixed effects were cancer type (CANCER), combination arm type whether "CT+CT" or "CT+CS" (COMBINATION.TYPE), number of patients in the arm or trial in question (ARM.PATIENTS; TOTAL.PATIENTS), route of drug administration (ROUTE), phase of trial (PHASE), total number of all drugs used in the arm (DRUGS), number of cytotoxic drugs (CT) used in the arm, and the year (YEAR) in which the trial results were published.

IV.3. Results

In sample S_1, there were 8 trials (16 arms- see Table 4.2) for the four targeted cancers (Breast, Colorectal, Pancreatic, and Lung) that fulfil the basic criteria (see Chapter 2) for inclusion in the analysis. For Sample S_2, there were 180 trials (180 arms) for the four targeted cancers (Breast, Colorectal, Pancreatic, and Lung) (Table 4.3). Of the 8 S_1 studies, all reported estimates of both overall response rate and median overall survival, but fewer than half also included estimates of median duration of response. All 180 studies in sample S_2, provided estimates of both overall response rate and median overall survival, but comparatively few (105 or 55%) provided estimates of median duration of response (Table 4.2). For both S_1 and S_2 samples, there are two subsets of trials: (a) one which includes trials that reported OR and OS for all arms, but not MD; and (b) those that reported estimates of all three endpoints (Table 4.2, and 4.3).

IV.3.1. Sample S_1

I retrieved 8 studies (16 arms) with at least two combination arms which provided estimates of two of the three endpoints (OR and OS). Due to the small sample size, I investigated only the effect of combination arm type (CT+CT vs CS+CT).

For overall response rate (OR), the final selected model for the S_1 sample included only COMBINATION.TYPE variable ($F=2.52$, $df=1, 14$, $p=0.1$; Fig.4.1) that showed no effect of combination arm type variation of OR among the trials. Similarly, for overall

survival (OS), the final selected model for the S_1 sample included only COMBINATION.TYPE variable (F=0.29, df=1, 14, p=0.6; Fig.4.2) and no effect was detected for combination arm type on variation of OS among the studies.

IV.3.2. Sample S_2

I retrieved 105 studies (see Table 4.3) that reported estimates of all three endpoints (OR, OS and MD). Fitted fixed effects models included cancer type (CANCER), combination arm type whether "CT+CT" or "CT+CS" (COMBINATION.TYPE), number of patients in the arm or trial in question (ARM.PATIENTS; TOTAL.PATIENTS), route of drug administration (ROUTE), phase of trial (PHASE), total number of all types of drugs used in the arm (DRUGS), number of cytotoxic drugs (CT) used in the arm, and the year (YEAR) in which the trial results were published.

For overall response rate (OR), the final selected model for the S_2 sample included combination arm type, cancer type, total number of drugs in the arm, number of cytotoxic drugs in the arm, and year of publication of trial results. Overall, the response rate (OR) varied dramatically among different cancers (F=15.7, df =3, 97, p<0.0001; Fig 4.3a) with breast cancer showing the highest average overall response, pancreatic the lowest. There was also a substantial effect of COMBINATION.TYPE (F=15.4, df=1, 97, p=0.0001), with patients receiving combined cytotoxic and cytostatic therapy having higher average response rates than those receiving combination cytotoxic therapy (Fig 4.3b). In addition, overall response rate increased over time (YEAR) (F=4.91, df=1, 97, p=0.02), as well as with the number of drugs in the arm (DRUGS) (F=4.26, df=1, 97, p=0.04), and the number of cytotoxic drugs in the cocktail (CT) (F=41.1, df=1, 97, p<0.0001). No effects of PHASE (F=0.81, df=1, 92, p=0.36) and ROUTE (F=1.07, df=2, 92, p=0.34) but with a slightly significant effect of ARM.PATIENTS (F=3.53, df=1, 92, p=0.06) were detected.

For overall survival, there was again a substantial effect of CANCER (F=21.8, df=3, 98, p<0.0001; Fig 4.4a), with breast cancer again showing the longest median overall survival, pancreatic the least. In contrast to OR, however, there was no detected effect of COMBINATION.TYPE (F=0.46, df=1, 92, p=0.4; Fig 4.4b), while both PHASE (F=3.80, df=1, 98, p=0.05), and ROUTE (F=3.79, df=2,98, p=0.02) showed a detectable effects. No other effects were detected.

For median duration of response, only YEAR (F=8.76, df=1, 103, p=0.003) showed a detectable effect, with no effects detected for any other of the candidate predictor variables, including cancer type and combination arm type (Fig. 4.5a, b).

To obtain a larger set of trials, I relaxed the criterion of an estimate of median duration of response (MD). Results for OR and OS based on this sample of 180 trials were qualitatively similar to those obtaining with the smaller subset. Once again, both OR (F=11.8, df =3, 174, p<.0001; Fig 4.6a), and OS (F=44.9, df =3, 173, p<.0001; Fig 4.7a) showed substantial variation among cancer types. Average OR was similarly greater for combined cytostatic and cytotoxic therapy (F=5.5, df =1, 174, p=0.01; Fig 4.6b), but this is not the case with respect to OS (F=3.17, df=1, 172, p=0.07; Fig 4.7b).

IV.4. Discussion

Cytotoxic and cytostatic cancer drugs have different mechanisms of action, and in the quest for improved treatment, several different models have led to the prediction that combined cytostatic and cytotoxic therapy may be more effective than combination therapy involving multiple cytotoxic agents. In Gardner's (2002) kinetic model, the comparatively intense positive selection for resistance induced by potent cytotoxic combinations is attenuated by a deceleration of the rate at which the evolutionary clock ticks, through the action of cytostatic agents, so that amount to induce senescence restricts the potential for evolutionary adaptation. Alternatively, in the Villasana et al. (2010) model, the effect of a cytostatic agent is to arrest the tumor cell cycle; the resulting synchronization maximizes exposure of the tumor cell population to the killing effects of the cytotoxic agent.

My results are, in general, inconsistent with the prediction that combination therapy with cytostatic and cytotoxic agents reduces the rate of acquired resistance in metastatic cancer. While my results provide some evidence that combination cytotoxic and cytostatic therapy is associated with a greater average overall response rate than multi agent cytotoxic therapy, but this is not the case for both median duration of response and overall survival. The finding that, in my sample of studies (S_2), combination therapy involving both cytotoxic and cytostatic agents induces (on average) a higher overall response rate than combination cytotoxic therapy may simply be an artifact as a consequence of the fact that in this sample,

there is no within-study control. Unlike the case of single vs combination therapy (Ch. 3), in the metastatic setting there are very few, as yet, published "head to head" trials that explicitly investigate potential differences in effectiveness of combination cytotoxic vs combination cytotoxic and cytostatic. Even though my analysis controls for cancer type – clearly a major contributor to between-trial variation in all measurement endpoints considered in this study – there will nonetheless be substantial (uncontrolled) variation among trials in a number of factors (e.g. socioeconomic background of patient population (Braaten et al., 2009; Madison et al., 2004, Smith et al., 2013); lifestyle factors (Soerjomataram et al., 2008; Kushi et al., 2007) etc.) that are known to be associated with differences in response rate and survival rates. Systematic biases with respect to these factors in the two classes of combination therapy studies could, in principle, give rise to differences where in fact none exist. On the other hand, uncontrolled between-trial variation may also increase noise, thereby overshadowing smaller differences (e.g. in average duration of response or overall survival) between the two groups.

A second study design limitation is related to the classification of an agent as cytotoxic or cytostatic. In reality, many–possibly most–cancer drugs exert both types of effects (Devy et al., 2004). For example, the classic cancer drug paclitaxel, while exerting strong cytotoxic effects, nonetheless also can induce cytostatic effects by interfering with microtubule dynamics (Johnson et al., 1963; Altaha et al., 2002). On the other hand, the targeted therapy sorafenib, whose original mechanism of action (inhibits tumor angiogenesis, and induces tumor cell apoptosis) was clearly cytostatic (Liu et al., 2006; Rixe and Fojo, 2007), can under certain conditions produce cytotoxic effects. Cytostatic and cytotoxic modes of action have been documented for a number of drugs, including isoflavone (Polkowski et al., 2004), **e**nniatin (Dornetshuber et al., 2007), sorafenib, sunitinib, and flavopiridol (Rixe and Fojo, 2007).

In the present analysis, drugs were classified as cytotoxic or cytostatic based on their documented mode of action. Yet drugs may have different effects on cell signalling, cell cycling, apoptosis or a host of other cellular processes depending on the context, and it is entirely possible (indeed, likely) that there may exist as yet undescribed cytotoxic effects for drugs**,** I have classified as cytostatic, and *vice-versa*. Moreover, depending on how one characterizes cytostasis operationally, whether a drug is cytotoxic or cytostatic may depend

on the concentration. For example, the National Cancer Institute drug screen identifies both cytotoxic as well as cytostatic drug concentrations for traditional anticancer drugs and thousands of other compounds, if one defines cytostasis as a concentration that prevents cell growth altogether (Rixe et al., 1996).

In fact, it could be argued that all microtubule-targeting agents from vincristine to paclitaxel and, more recently, the epothilones, are inherently cytostatic (Johnson et al., 1963; Altaha et al., 2002). By interfering with microtubule dynamics, these agents arrest cells in mitosis and induce cytostasis (Rixe and Fojo, 2007). Although these agents were originally described as cytotoxic, they are in fact cytostatic, and the arrest triggers cell death (Blagosklonny et al., 1999). Thus, *in vitro* cytostasis can occur with nearly all conventional anticancer agents. Although there are fewer *in vivo* data, there is nevertheless substantial evidence that drugs conventionally regarded as cytotoxic can also be cytostatic and cause tumor growth delays in preclinical models (Szmigielska-Kaplon et al., 2002; Yachi et al., 1996). For example, sorafenib (Nexavar) is an orally active multikinase inhibitor with putative effects on tumor cell proliferation and tumor angiogenesis (Rixe and Fojo, 2007). *In vitro*, sorafenib inhibits Raf kinase, vascular endothelial growth factors receptors 1, 2, and 3, and platelet-derived growth factor receptor β (Flaherty, 2007). *In vivo* studies have shown a cytostatic effect on a large panel of tumors. Thus, although sorafenib was developed as a cytostatic agent, there is also evidence that it is also a cytotoxic agent (Rixe and Fojo, 2007). On the other hand, the patterns (or lack thereof) documented here may not reflect limitations of the study design, but rather reflect the biological and clinical reality. The consistently greater overall response rate of combination cytotoxic and cytostatic regimens within the four examined cancer types may reflect the differential innate resistance of tumors to cytotoxic vs cytostatic agents. Overall response rate is related largely to innate resistance, that is, resistance present at the outset of therapy rather than that acquired during the course of therapy. In the metastatic setting, the phenomenon of innate resistance is well-established indeed, the fact that response rates are not 100%, is adequate evidence of innate resistance.

The observed difference between the two treatments with respect to overall response rate, but not with respect to median duration of response or overall survival, means that any advantages of CT+CS combination therapy are short-lived. Response rate is mostly determined by initial tumor sensitivity and tumor kill, the greater the sensitivity, the greater

the tumor kill, and the greater (faster) reduction in tumor volume. Hence greater likelihood of a documented response results in tumor shrinkage. But as has been pointed out by a number of authors (Pepper et al., 2008; Gatenby et al., 2009; Read et al., 2011) that large tumor kill induces strong positive selection for resistance mutations. Hence, if there exists resistance mutations at the outset, or such mutations arise during therapy, they will rapidly sweep through the population. It is precisely to avoid this long(er) term disadvantage of strong positive selection that proponents of "evolutionary" approaches to cancer therapy are increasingly advocating a movement away from regimens based on "maximum tolerable dose" (Pepper et al., 2008; Read et al., 2011; Gillies et al., 2012).

The above argument suggests there may be a trade-off between short-term effectiveness in enhanced tumor kill (and therefore, one presumes, a reduced symptomology) and longer-term resistance evolution. The consequence is that one expects to see accelerated resistance evolution under therapeutic regimens that result in extensive tumor kill. In a laboratory study of antibiotic resistance, Hergeness et al. (2008) showed a negative correlation between the rate of resistance evolution and the degree of synergy between drug combinations, whether the extent of synergy was estimated from differences in bacterial growth rate at specific concentrations of both drugs compared to that obtaining in the absence of the partner. Interestingly, Hergeness et al**'s** (2008) explanation for their result was concerned not with the intensity of positive selection, but rather with the mutation rate, suggesting that drug antagonism generates sign epistasis between single-drug resistant mutants, thereby dramatically reducing the number of mutational paths to combinatory resistance. And the fewer the possible mutational paths, the more unlikely the evolution of combinatory mutations becomes (Weinreich et al., 2006). Both explanations however, call into question two elements of the "orthodoxy" of disease treatment, be it cancer or something else: aim for the maximum tolerable dose (MTD) so as to maximize pathogen mortality, and in combination therapy, avoid antagonists.

Modeling and theoretical considerations aside, what is the evidence that addition of a cytostatic agent to existing (largely cytotoxic) therapy enhance treatment effectiveness? A recent study evaluated the efficacy and tolerability of combinations of cytostatic agent sorafenib plus agents used to treat non-small-cell lung cancer (NSCLC) using preclinical models of this disease (Carter et al., 2007). This study concluded that the administration of

sorafenib and vinorelbine, cisplatin or gefitinib was at least as efficacious as the individual agents alone and was well tolerated. Park et al. (2004) showed the antiproliferative effects of the cytotoxic drugs, oxaliplatin and paclitaxel, could be greatly enhanced when combined with cytostatic agent gefitinib and could offer long-term control of gastric tumor growth and metastasis. Similarly, Ciardiello et al. (2000) demonstrated that a significantly increased survival in the combined therapy group was accompanied as compared with the groups treated with a single agent. This study identified that when combinations of lower doses of cytostatic agent ZD-1839 (Iressa) and cytotoxic drugs topotecan, raltitrexed, or paclitaxel were used, the antiproliferative effect was clearly cooperative in all cell lines examined (Ciardiello et al., 2000). But results are heterogeneous. In malignant pleural mesothelioma, conventional cytotoxic therapy, both as single agents and in combination, is largely ineffective (Favoni and Florio, 2011). Moreover, combination therapy involving both conventional cytotoxic agents (e.g. cisplatin and the taxanes) and cytotoxic antfoliates such as pemetrexed or raltitrexed show, at best, marginally increased effectiveness, with no detectable improvement associated with combinations involving cytotoxic agents and cytostatic agents such as bevacizumab (Favoni and Fiorio 2011).

In most patients with solid metastatic tumors, the cancer becomes resistant to therapy within a few months (Amado et al., 2008; Sequist et al., 2008; Gerber and Minna, 2010; Chapman et al., 2011). Understanding the evolutionary dynamics of resistance is crucial to the development of effective therapeutics, and unsurprisingly, has been the focus of experimental (Engelman et al., 2007; Corcoran et al., 2010; Bivona et al., 2011; Diaz et al., 2012; Ellis et al., 2012; Misale et al., 2012; Straussman et al., 2012; Wilson et al., 2012; Khorashad et al., 2013) and theoretical (Dewanji et al., 2005; Komarova and Wodarz, 2005; Michor et al., 2005, 2006; Haeno et al., 2007; Dingli et al., 2008; Katouli and Komarova, 2010; Lenaerts et al., 2010; Beckman et al., 2012; Bozic et al., 2012) studies. Several models (Rixe and Fojo, 2007; Villasana et al., 2010) predict that combining cytostatic and cytotoxic agents will result in better outcomes (increased response rates, increased survival) due to the reduced rate of resistance evolution. While my results provide some evidence that in the metastatic setting, combination cytostatic and cytotoxic therapy increases overall response rates compared to multi-agent cytotoxic therapy, there is no evidence of any effect on the duration of response, the clinical endpoint most closely

associated with resistance evolution.

As noted above, there are two different classes of explanations for the lack of consistency between the empirical clinical results presented here and the predictions derived from the modeling work of Gardner (2002), Villasana et al. (2010) and others. One is simply that the models are wrong, or at least incomplete. The second is that the extent to which the current study constitutes a bona fide test of model predictions depends critically on the assumption that, in the therapeutic regimens employed in the sample of trials considered here, drugs that have been classified as "cytostatic" (cytotoxic) based on documented mechanisms of action do indeed exert primarily cytostatic (cytotoxic) effects. Not only is the validity of this assumption unknown, it may well be, for all intents and purposes, unknowable, simply because while it may be possible to design assays for cytostasis and cytoxicity *in vitro*, or perhaps in non-human *in vitro* models (Rixe and Fojo, 2007), there is no guarantee that inferences regarding drug mode/mechanism of action in such model systems will apply in the clinic. Moreover, as has been pointed out by a number of authors, the cytostasis – that is, cell cycle arrest – may – and indeed, often does – trigger a cascade of apoptotic pathways resulting is cell death (i.e. cytotoxicity) (Rixe and Fojo, 2007), making the empirical distinction between the two even more problematic.

For the above reasons, I believe that from both a modeling and empirical (especially clinical) perspective, the distinction is likely not very useful, especially of the objective is to develop therapeutic agents and regimens that delay the onset of acquired resistance. A more promising approach, at least from the perspective of clinical trial research, is to use clinical trial data in the meta-analytic framework employed here (and elsewhere) to explore the effect of better-characterized classes of agents. An obvious distinction, already referred to, is conventional chemotherapies which are, for the most part, broad-spectrum, vs targeted therapies which have been "rationally" designed to target specific mutations and/or receptors in known cell-signalling pathways such as, for example cilengitide and bortezomib that bind to and inhibit the activities of the integrins as well as inhibit endothelial cell-cell interactions, endothelial cell-matrix interactions, and angiogenesis (Sawada et al., 2012). Despite the comparative novelty of such agents, there is already a nascent modeling literature (e.g. Foo and Michor, 2009; Bozic et al., 2013) that makes predictions concerning the rate of evolution of acquired resistance for targeted therapies vs other therapeutics, or under different dosing

regimens, and general results from such models (such as, for example, those explored in chapter 3 with respect to the issue of combination vs monotherapy) would seem to be more productive to explore, especially given the accelerating trend towards targeted cancer therapy (Gardner,2002; Goldie and Coldman,1979).

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Table 4.1. Anticancer drugs employed in the sample of clinical trials used in this study, along with their primary mechanisms of action and classification as either cytotoxic (CT) or cytostatic (CS).

Type of agent	Drugs	Mechanism of action	Toxicity
Alkylating agents	cyclophosphamide, ifosfamide, carmustine, dacarbazine, temozolomide, cisplatin, carboplatin, coumarin, semustine, melphalan, mitolactol, oxaliplatin, treosulfan	- DNA alkylation - Cross-linking - Formation of a reactive intermediate	Primarily cytotoxic
Antimetabolites	5-Fluorouracil, gemcitabine, methotrexate, pemetrexed, capecitabine, paclitaxel, vinorelbine, docetaxel, vincristine, vindesine, etoposide, teniposide, irinotecan, exatecan, raltitrexed, rh-endostatin, tegafur	- Incorporation into DNA as fraudulent nucleotide - Inhibition of enzymes in DNA-synthesis	Primarily cytotoxic
	folinic acid, leucovorin, levofolinic acid	- Binding of the drug's metabolite to its target enzyme - Prolonging drug activity	Primarily cytostatic
Antibiotics	doxorubicin, epirubicin, mitomycin, mitomycin-C, bleomycin, detorubicin	- Intercalation between DNA-bases - Inhibition of DNA-biosynthesis	Primarily cytotoxic
Corticosteroids	prednisone	- Kill cancer cells or slow their growth	Primarily cytostatic
Immunothearpy	interferon-alpha, corynebacterium parvum, levamisole,	- Stimulate natural immune systems to recognize - Attack cancer cells	Primarily cytostatic
Monoclonal antibodies	cetuximab, trastuzumab, bevacizumab,	- Antibodies attach themselves to tumor-specific antigens - Increasing immune response to tumor cell	Primarily cytostatic
Miscellaneous	imatinib	- Inhibition of enzymes in DNA-synthesis.	Primarily cytotoxic

	cilengitide, bortezomib	- Binds to and inhibits the activities of the integrins - Inhibiting endothelial cell-cell interactions, endothelial cell-matrix interactions, and angiogenesis	Primarily cytostatic
Hormonal agents	tamoxifen, aminoglutethimide	- Inhibitor of estrogen - Binds to estrogen-sensitive tissues - Suppresses serum levels of insulin	Primarily cytostatic
Mitotic Inhibitors	vinblastine, ixabepilone, estramustine, erlotinib, tacediline, tipifarnib	- Disturbance of spindle foundation - Arresting of mitosis during metaphase	Primarily cytotoxic
	sorafenib, lapatinib, lonidamine, marimastat		Primarily cytostatic

Table 4.2. Number of Sample S_1 trials and arms for four types of cancer (breast, colorectal, pancreatic, lung). Datasets are distinguished by the set of endpoints recorded for each trial in the set (overall response rate (OR), Median Overall Survival (OS), and Median Duration of Response (MD)).

Sample S1 (2 C arms) (CT+CT vs CT+CS) (Within trial control)				
Dataset	# Trials	# Arms	# CT+CT arm	# CT+CS arm
4 cancers OR.OS.MD	3	6	3	3
4 cancers OR.OS	8	16	8	8

Table 4.3. Number of Sample S_2 trials and arms for four types of cancer (breast, colorectal, pancreatic, lung). Datasets are distinguished by the set of endpoints recorded for each trial in the set (overall response rate (OR), Median Overall Survival (OS), and Median Duration of Response (MD)).

Sample S2 (1 C arm) (CT+CT or CT+CS) (No within trial control)				
Dataset	# Trials	# Arms	# CT+CT arm	# CT+CS arm
4 cancers OR.OS.MD	105	105	54	51
4 cancers OR.OS	180	180	99	81

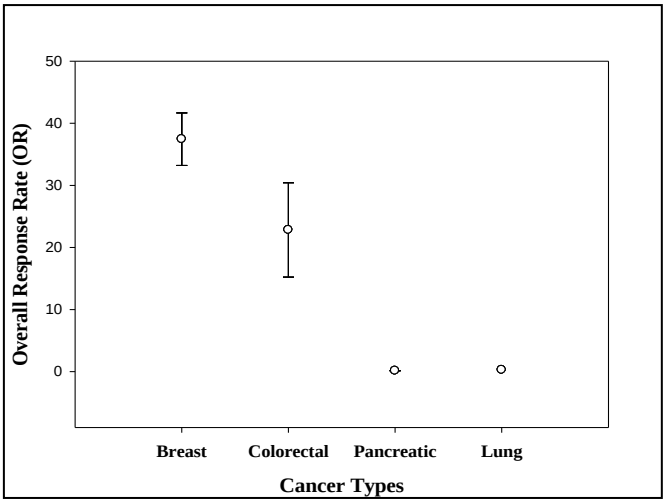

Figure 4.1. Average overall response rate (OR) and associated standard error for each of the four main cancer types (breast (*N*=4), colorectal (*N*=8), pancreatic (*N*=2), lung (*N*=2)), pooled over both type of combination therapy. Averages are based on sample S_1 that includes the *N* = 8 studies (16 arms) that provided estimates of all three endpoints (overall response (OR) and median overall survival (OS)).

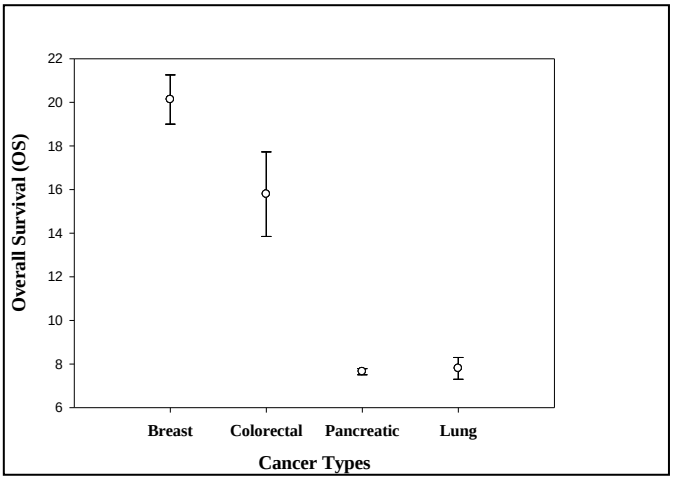

Figure 4.2. Average overall survival (OS) in months and associated standard error for each of the four main cancer types (breast (*N*=4), colorectal (*N*=8), pancreatic (*N*=2), lung (*N*=2)), pooled over both type of combination therapy. Averages are based on sample S_1 that includes the *N* = 8 studies (16 arms) that provided estimates of all three endpoints (overall response (OR) and median overall survival (OS)).

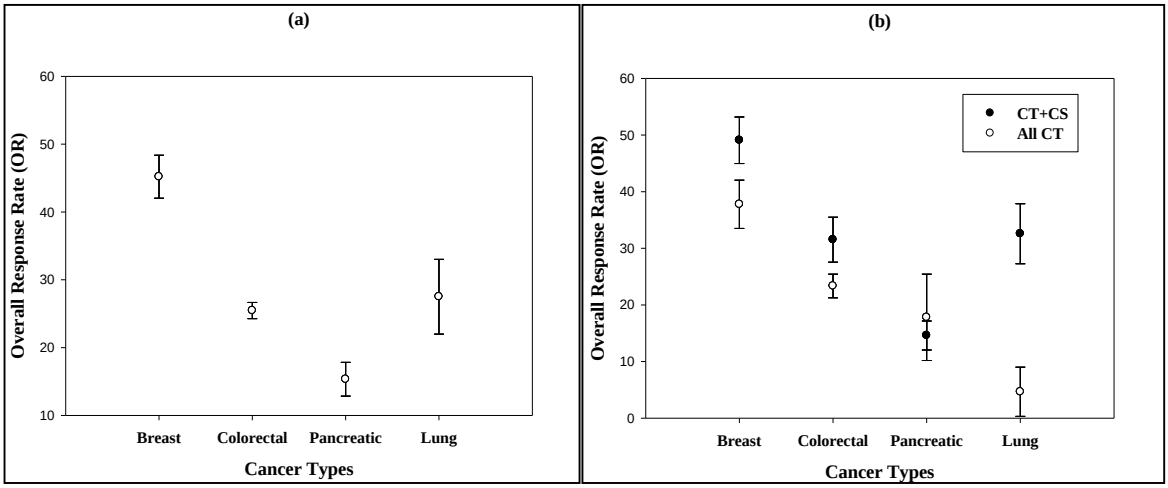

Figure 4.3. Average overall response rate (OR) and associated standard error for each of the four main cancer types (breast (*N*=35), colorectal (*N*=46), pancreatic (*N*=13), lung (*N*=11)), pooled over both type of combination therapy (a), and for combination CT+CT and CT+CS arms considered separately (b). Averages are based on sample S_2 that includes the *N* = 105 studies that provided estimates of all three endpoints (overall response (OR), median overall survival (OS), and median duration of response (MD)).

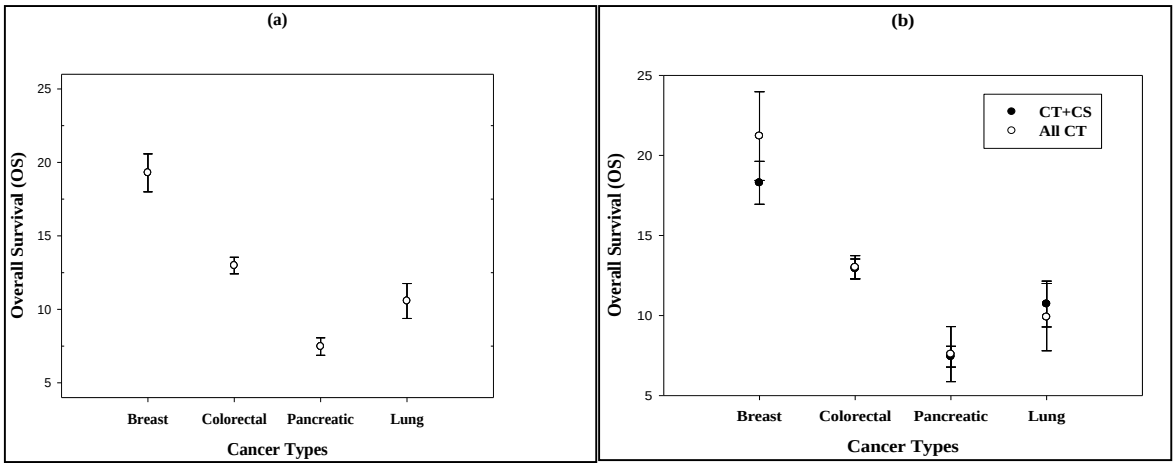

Figure 4.4. Average overall survival (OS) in months and associated standard error for each of the four main cancer types (breast (N=35), colorectal (N=46), pancreatic (N=13), and lung (N=11)), pooled over both type of combination therapy (a), and for combination CT+CT and CT+CS arms considered separately (b). Averages are based on sample S_2 that includes the N = 105 studies that provided estimates of all three endpoints (overall response (OR), median overall survival (OS), and median duration of response (MD)).

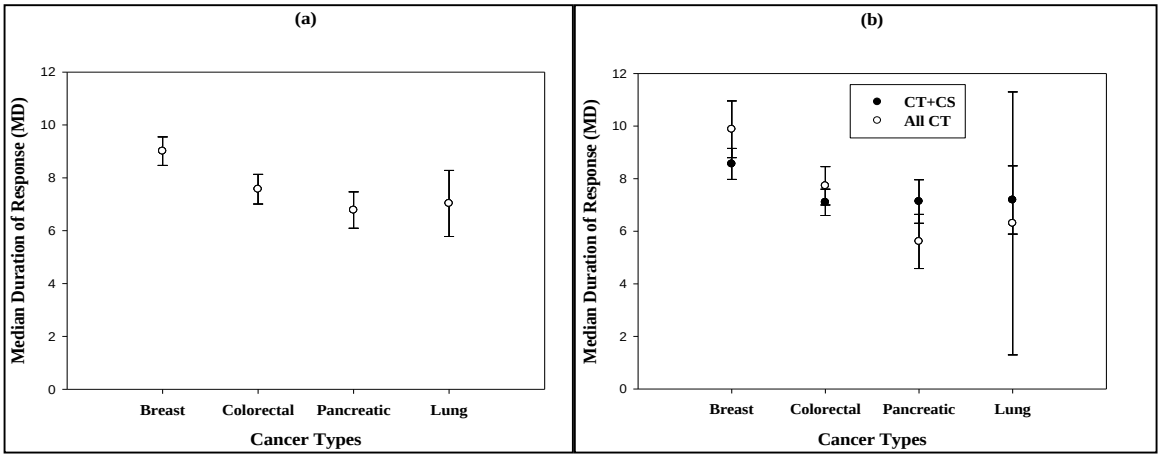

Figure 4.5. Average median duration of response (MD) in months and associated standard error for each of the four main cancer types (breast (N=35), colorectal (N=46), pancreatic (N=13), lung (N=11)), pooled over both type of combination therapy (a), and for combination CT+CT and CT+CS arms considered separately (b). Averages are based on sample S_2 that includes the N = 105 studies that provided estimates of all three endpoints (overall response (OR), median overall survival (OS), and median duration of response (MD)).

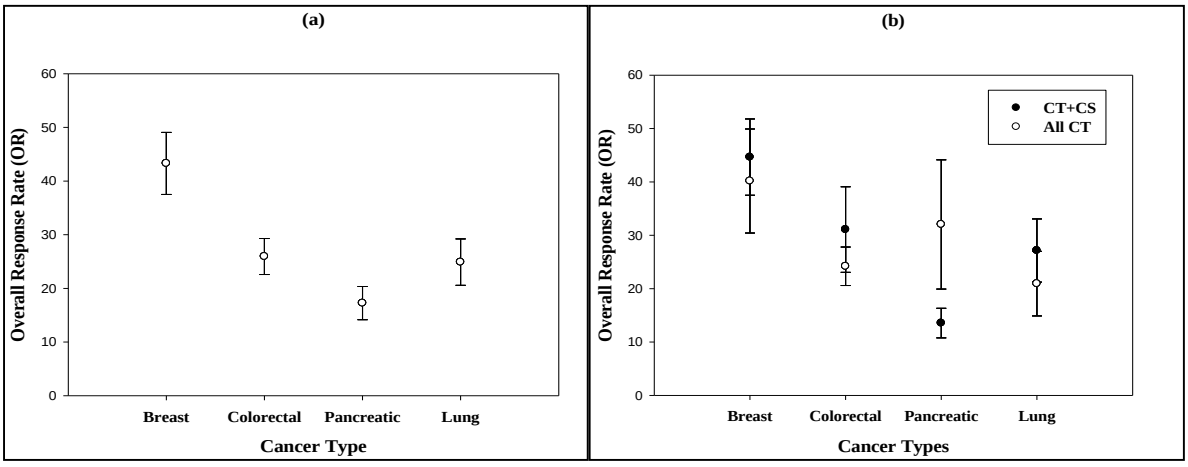

Figure 4.6. Average overall response rate (OR) and associated standard error for each of the four main cancer types (breast (*N*=56), colorectal (*N*=60), pancreatic (*N*=31), lung (*N*=33)), pooled over both type of combination therapy (a), and for combination arm CT+CT and combination arm CT+CS considered separately (b). Averages are based on sample S_2 that includes the *N* = 180 studies that provided estimates of two endpoints (overall response (OR) and median overall survival (OS)).

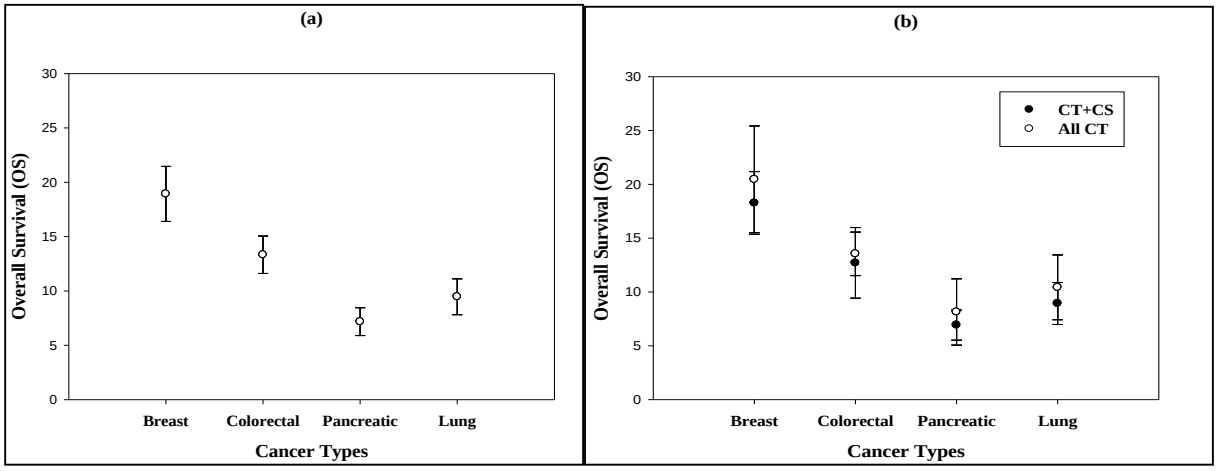

Figure 4.7. Average overall survival (OS) in months and associated standard error for each of the four main cancer types (breast (*N*=56), colorectal (*N*=60), pancreatic (*N*=31), lung (*N*=33)), pooled over both type of combination therapy (a), and for combination arm CT+CT and combination arm CT+CS considered separately (b). Averages are based on sample S_2 that includes the *N* = 180 studies that provided estimates of two endpoints (overall response (OR) and median overall survival (OS)).

Summary and Conclusions

The problem of acquired resistance is, arguably, the single largest challenge to the successful treatment of cancer, particularly in the metastatic setting. There is ample evidence, at least from pre-clinical studies, that acquired resistance arises from a Darwinian process of clonal selection in response to chemotherapy. If this is indeed the case, then successful treatment will require retarding, circumventing or co-opting the evolutionary process through the intelligent design of both cancer drugs and therapeutic regimens. To date, there have been few, if any, direct tests of any evolutionary theory of acquired resistance in the clinic. Here I tested two such theories using data from clinical trials in the metastatic setting. The first hypothesis is based on mathematical kinetic models of resistance evolution developed by Goldie and Coldman (1979) that yielded two predictions, (1) that the probability of a cure decreases exponentially with the initial tumor mass (number of cells); and (2) that the likelihood of response and cure would be increased in combination vs single agent therapies. The second hypothesis derived from Gardner's (2002) mathematical model of the evolutionary consequences of treatment regimens involving different classes of chemotherapeutic drugs. This model predicts that combination therapy involving both cytotoxic and cytostatic drugs will be more effective than combination therapy involving only cytotoxic drugs.

Tests of the two hypotheses were developed using a database of clinical trials that was initiated in 2004, and has since grown to include over 700 clinical trial records in 30 different metastatic cancer settings. This database contains information on trial design, trial phase, number of arms, number of patients in each arm, cancer type, drug information, and trial endpoints data (overall response rate, overall survival, median duration of response) for each clinical trial. Since there is ample evidence that all of the clinical endpoints considered in the study vary among different cancer types, in most cases I restricted my analysis to those cancer types (Breast, Colorectal, Pancreatic, and Lung) where the sample of clinical trials is sufficiently large to permit reliable estimates of fitted model parameters.

In the Goldie and Coldman model (1979), resistance evolves through the accumulation of resistance mutations and associated positive selection. If tumors are initially heterogeneous with respect to drug sensitivity, and there is little or no cross-resistance among drugs, then under combination therapy, resistance can arise only with the accumulation

within a single lineage of a set of different mutations that confer resistance to different drugs. In fact, if the waiting times are sufficiently long, the possibility arises that the tumor will be completely eradicated before significant evolutionary change can even occur (Goldie and Coldman 1984). The postulated evolutionary benefit of combination therapy under the Goldie and Coldman model concerns the greater expected waiting time for mutations conferring resistance to several drugs to accumulate in a single lineage.

In chapter 3, The Goldie and Coldman (1984) hypothesis - translated to the metastatic cancer clinical setting - predicts that, for any two cytotoxic drugs A and B, average response rates, median duration of response and, as a result, overall survival, should be greater in combination arms than in single agent arms. Consistent with this hypothesis, I found that for the sample of studies (S_1) having more stringent internal control, that is, where the effects of other trial attributes such as, the extent of patient pre-treatment (Blackwell et al., 2010; Papaldo et al., 2006) and the types of drugs employed (Geyer et al., 2006; Martin et al., 2007) are, presumably, randomized between arms, overall response rate, overall survival, and median duration of response were greater in combination vs single agent arms. The greater median duration of response under combination vs monotherapy observed in my study suggests that, as predicted, by Goldie and Coldman, combination therapy does slow down the rate of resistance evolution. This effect was generally not observed in the second (S_2) sample of studies which had no within-trial control and where between-trial variation, even for the same cancer type and chemotherapy regimen, is expected to be larger due to differences among trials in design and patient population attributes. This variability, which is substantially better controlled in sample S_1, will make the detection of differences between combination and monotherapy more difficult.

In chapter 4, I tested Gardener's hypothesis (2002) that predicts that for any cancer, the overall response rate, the median duration of response, and overall survival should be greater in trial arms where a combination of cytotoxic and cytostatic drugs is administered, compared to arms where a combination of only cytotoxic drugs is administered. My results are, in general, inconsistent with these predictions: although there is some evidence that combination cytotoxic and cytostatic therapy is associated with a greater average overall response rate than multi agent cytotoxic therapy, this is not the case for both median duration of response and overall survival. But unlike the study of combination vs monotherapy, there

are, to date, few studies that compare combination cytotoxic and cytostatic therapy with combination cytotoxic therapy within the same trial. Consequently, a higher overall response rate for combination cytostatic and cytotoxic therapy may simply be an artifact as a consequence of the fact that in this sample, there is no within-study control.

Even more problematic for testing the Gardner hypothesis is the fact that depending on how one characterizes cytostasis operationally, whether a drug is cytotoxic or cytostatic may depend on the concentration. I believe that from both a modeling and empirical (especially clinical) perspective, the distinction is likely not very useful, especially if the objective is to develop therapeutic agents and regimens that delay the onset of acquired resistance. A more promising approach, at least from the perspective of clinical trial research, is to use clinical trial data in the meta-analytic framework employed here (and elsewhere) to explore the effect of better-characterized classes of agents such as, for example, broad spectrum conventional chemotherapies compared to more recently developed "targeted" therapies that are directed towards specific cell signalling pathways in tumorigenesis.

APPENDIX

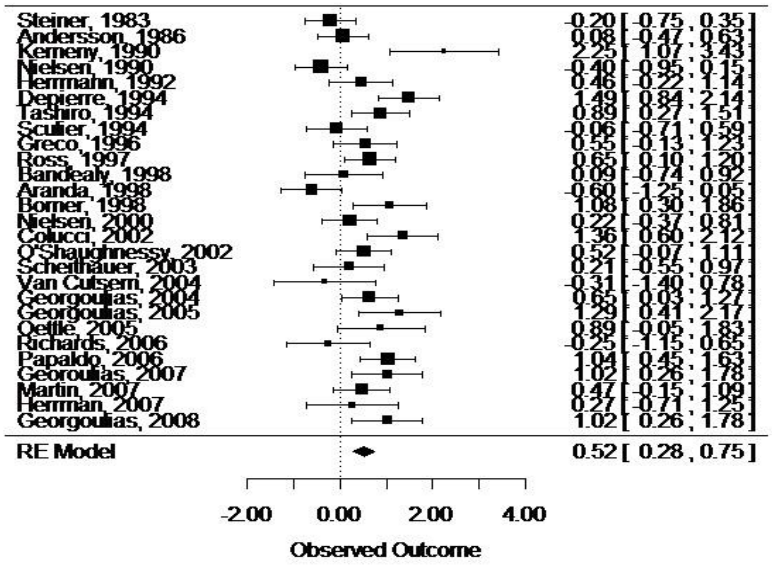

Figure 1. Forest plot for OR in sample 1 of Hypothesis I shows that most of the studies have positive value for combination arm over single arm.

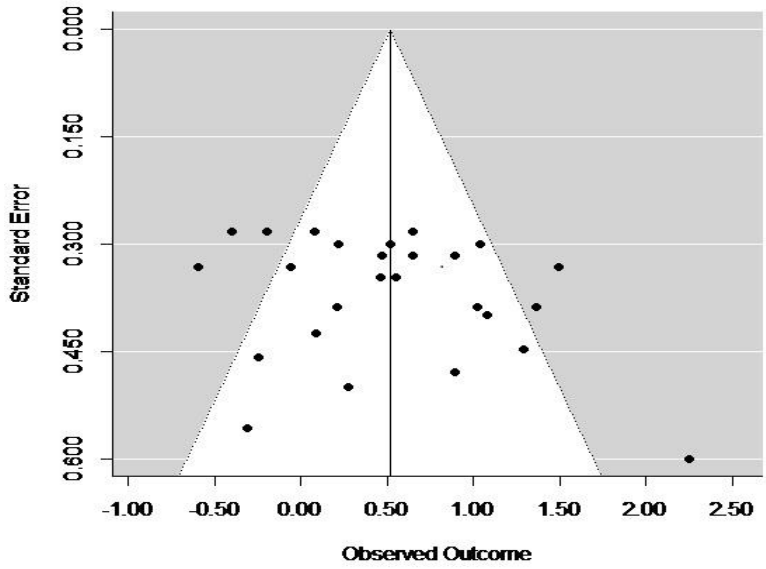

Figure 2. Funnel plot for OR in sample 1 of Hypothesis I shows that there is no publication bias due to the symmetry in treatment effects.